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Chemical defense over decadal scales: Ontogenetic allocation trajectories and consequences for fitness in a foundation tree species

Olivia L. Cope[1] | Eric L. Kruger[2] | Kennedy F. Rubert-Nason[3] | Richard L. Lindroth[3]

[1]Department of Integrative Biology, University of Wisconsin-Madison, Madison, WI, USA
[2]Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA
[3]Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA

Correspondence
Olivia L. Cope
Email: ocope@wisc.edu

Present address
Kennedy F. Rubert-Nason, Department of Natural and Behavioral Sciences, University of Maine, Fort Kent, ME, USA

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Abstract

1. Expression of herbivore defense traits can change dramatically during the course of plant development. Little is known, however, about the degree of genetic or sexual variation in these ontogenetic defense trajectories or whether the trajectories themselves are adaptive, especially in long-lived species.
2. We used a 13-year dataset of chemical defense traits, growth and survivorship from a common garden of trembling aspen (*Populus tremuloides*) genotypes to document long-term defense trajectories and their relationship to tree fitness during juvenile and early mature stages.
3. Overall, concentrations of the two principal classes of aspen defense compounds (salicinoid phenolic glycosides [SPGs] and condensed tannins [CTs]) decreased to differing degrees in foliage of juvenile trees and then remained relatively constant in maturity. Initial values, juvenile rates of change and average mature values all exhibited significant genetic variation for both SPGs and CTs.
4. Relationships between defense trajectory parameters and metrics of tree fitness (growth and survivorship) depended on compound type and tree sex. Females with higher-allocation SPG trajectories (high initial juvenile concentrations, slow juvenile declines, high mature concentrations) grew more slowly relative to females with lower-allocation trajectories. In males, higher-allocation SPG trajectories had a lesser effect on growth but were associated with reduced mortality. Juvenile CT trajectories were not correlated with tree fitness, but average CT concentration in maturity was positively related to growth in females.
5. These results suggest that ontogenetic defense trajectories are adaptive and subject to natural selection. Genotypic variation and ontogeny shape tree defensive chemistry, both independently and interactively. These patterns of defense expression have the potential to structure trophic interactions and the genetic composition of forests in both space and time.

KEYWORDS
genotypic variation, ontogeny, phytochemistry, plant defense, *Populus tremuloides*, salicinoids, sexual dimorphism, tannins

1 | INTRODUCTION

Allocation to herbivore defense changes both quantitatively and qualitatively throughout plant development (Barton & Koricheva, 2010). The null model explaining these trajectories is ontogenetic drift, in which changes in defense are selectively neutral by-products of changes in plant size (Barton & Boege, 2017). However, given that the costs and benefits of herbivore defense also change as plants develop (Barton & Boege, 2017; Boege & Marquis, 2005; Orians, Hochwender, Fritz, & Snäll, 2010), natural selection may act on ontogenetic trajectories of defense allocation. For example, defenses may be especially costly for establishing juveniles (Bryant & Julkunen-Tiitto, 1995; Orians et al., 2010) or reproductive females (Barrett & Hough, 2012) due to competing resource demands specific to those ontogenetic stages. The degree of genetic and sexual variation in defense trajectories, and whether that variation correlates with fitness outcomes (i.e. is under natural selection), is largely unknown (Barton & Boege, 2017). Longitudinal evidence for the ontogenetic patterns themselves is lacking in all but a few species, especially in long-lived plants such as trees (Barton & Koricheva, 2010; Nissinen et al., 2018), because obtaining it requires long-term studies over many years.

Herein, we summarize results of a study on trembling aspen (*Populus tremuloides* Michx.) that, to our knowledge, is the first decadal-scale longitudinal assessment of herbivore defense dynamics in trees. Aspen has a well-characterized suite of herbivore defenses: the main foliar chemical defenses are salicinoid phenolic glycosides (SPGs) and condensed tannins (CTs), each of which can make up more than 20% of leaf dry mass (Donaldson, Stevens, Barnhill, & Lindroth, 2006; Lindroth & St. Clair, 2013). SPGs are effective defenses against the major lepidopteran and some mammalian herbivores of aspen and exhibit minimal induction (Boeckler, Gershenzon, & Unsicker, 2011; Lindroth & St. Clair, 2013). CTs, on the other hand, are generally ineffective in reducing lepidopteran or mammalian herbivory, but are inducible and are negatively correlated with the performance of chrysomelid beetles and aphids (Barbehenn & Constabel, 2011; Donaldson & Lindroth, 2004; Lindroth & St. Clair, 2013; Rubert-Nason, Couture, Gryzmala, Townsend, & Lindroth, 2017). CTs may also confer tolerance to herbivory, as they can facilitate nutrient uptake by trees following defoliation (Madritch & Lindroth, 2015).

Aspen exhibits tremendous phenotypic variation in many traits, including both constitutive and induced levels of chemical defenses (Keefover-Ring, Rubert-Nason, Bennett, & Lindroth, 2015; Rubert-Nason, Couture, Major, Constabel, & Lindroth, 2015; Stevens & Lindroth, 2005). This variation is underpinned by significant heritable genetic variation (Donaldson & Lindroth, 2007; Kanaga, Ryel, Mock, & Pfrender, 2008). In general, defense induction in aspen is minimal compared with the high levels of genotypic variation in phytochemistry (Rubert-Nason et al., 2015). Aspen is also a dioecious species. While sexual dimorphism is not prominent in most aspen populations, there is evidence, albeit somewhat equivocal, that females may be faster growing yet more sensitive to the costs of defense (Cole, Stevens, Anderson, & Lindroth, 2016; Sakai & Burris, 1985; Stevens & Esser, 2009).

Allocation to herbivore defense can be costly in terms of plant growth, and the magnitude of costs can be shaped by other factors, such as resource availability (Koricheva, 2002; Züst & Agrawal, 2017). In aspen, costs of defense are environmentally mediated and variable among defense compounds (Cole et al., 2016; Donaldson, Kruger, & Lindroth, 2006; Hwang & Lindroth, 1997; Osier & Lindroth, 2006). Ontogenetic changes in allocation to different defenses may underlie the diversity of findings with respect to defense costs. A cross-sectional survey of aspen trees of different age classes found that SPG concentrations are highest in young trees and decrease exponentially with tree age, whereas CT concentrations are low in young trees and high but stable in older trees (Donaldson, Stevens, et al., 2006).

Throughout our 13-year longitudinal study of aspen, we annually measured levels of foliar chemical defense and growth of twelve genotypes (six male, six female) in a common garden located in southern Wisconsin, U.S.A. Measurements during that period spanned ontogenetic stages from young saplings to reproductively mature trees. We predicted that (a) trajectories of herbivore defense would mirror those observed in previous cross-sectional studies, with decreasing concentrations of SPGs and increasing concentrations of CTs over time; (b) trajectories of defense would vary among aspen genotypes; (c) genotypic variation in defense trajectories would correlate with tree fitness in terms of growth and mortality; and (d) lifetime fitness impacts would be greatest on female trees and for defense allocation at early ontogenetic stages.

2 | MATERIALS AND METHODS

2.1 | Genotype propagation and study site

Root material was originally collected from twelve trembling aspen clones located in forested areas across four counties in south-central Wisconsin, USA. The clones were identified as unique genotypes via microsatellite marker analysis (16 loci; C. Cole and R. Lindroth, *unpublished data*). Adventitious shoots growing from the roots were placed into tissue culture for micropropagation (Donaldson, 2005). Replicate ramets from the micropropagation procedure were transplanted into pots containing a peat-based growing medium in late winter 2001 and grown in a greenhouse. They were then outplanted to individual 5-L pots (containing a 40–40–20 mixture of topsoil, sand and perlite supplemented with 20 g slow-release 14:14:14 N-P-K fertilizer) and moved outside in late spring 2001. Potted trees were watered as needed for the 2001 growing season. In late April, 2002, the 1-year-old saplings were outplanted to a common garden, located at the University of Wisconsin's Arlington Agricultural Research Station (43.3°N 89.3°W). The garden was underlain by a relatively fertile, silt loam soil (Plano silt loam, mesic Typic Argiudoll), and had previously been used for corn (maize) production. The site was ploughed and disked prior to planting. One sapling per clone was planted at 3 m × 3 m spacing in each of 15 replicate blocks, in a randomized complete block design with a perimeter of non-experimental trees. Individual trees were surrounded by weed barrier fabric (1.3 m × 1.3 m), and the plot was mowed routinely, until canopy closure, to control growth of herbaceous vegetation.

2.2 | Measurement of fitness proxies

We used growth and mortality as surrogate measures of plant fitness, because both parameters are practical to measure in trees and relevant to studies of trade-offs and selection over time (Crone, 2001; Younginger, Sirová, Cruzan, & Ballhorn, 2017; Züst & Agrawal, 2017), and because reproduction cannot be measured in juvenile trees. Tree size was measured each spring from 2002 to 2016. All yearly measurements were made on a subset of trees, with approximately five replicates per genotype in 2002–2003 (trees aged 1–2 years) and 10 replicates per genotype in 2004–2016 (trees aged 3–15 years). We measured tree height (*H*) with meter sticks for trees <2 m and with telescoping height poles for taller trees. From 2002 to 2004, we measured stem diameter (*D*) at 10 cm above the soil surface, whereas from 2005 to 2016, we measured *D* at breast height (1.4 m). Our principal metric of tree size was the index D^2H (Causton, 1985), which is widely used as a proxy for biomass in aspen and other *Populus* species (Stevens, Kruger, & Lindroth, 2008). In prior work involving the same 12 aspen genotypes (E.L. Kruger, L. Holeski, K. Keefover-Ring, R. Lindroth, *unpublished data*) D^2H was correlated very closely with woody shoot (stem plus branch) biomass (across all genotypes, $R^2 = .98$, $p < .0001$, RMSE < 1% of average shoot mass, $n = 432$). Tree growth was calculated as the increase in D^2H between 2002 and 2016 (trees aged 1–15 years). Since roughly half of the study trees were not measured for D^2H in 2002, we used genotype means for 2002 D^2H in growth calculations. Volume index differences among individuals were minor in 2002 (mean $D^2H = 8.65 \times 10^{-5}$ m^3, standard error 0.58×10^{-5} m^3), and most of the variation that did exist was among genotypes ($F_{1,11} = 8.22$, $p < .001$). Tree mortality was surveyed in summer 2016, and trees were defined as dead if their crowns were leafless. Genotypes were sexed at maturity by floral morphology.

2.3 | Leaf chemical analyses

We analysed the chemistry of leaves collected in mid-summer from 2003 to 2015 (trees aged 2–14 years). Defoliation occurred at low, ambient levels for the duration of the study. Approximately 15–30 leaves were collected haphazardly from throughout the crown of each tree; telescoping pole pruners (12 m) were used to access leaves on taller trees. Leaf samples were vacuum dried, and then ball-mill ground. From 2003 to 2009, phytochemistry was evaluated for each sample using a combination of chromatography and colorimetric assays; from 2010 to 2015, all phytochemistry was evaluated using near infrared reflectance spectroscopy (NIRS) referenced to laboratory-assayed values from a subset of samples (as described in Rubert-Nason et al., 2013).

Laboratory analysis methods for SPGs changed over the course of the study as instrumentation improved. From 2002 to 2009, SPG concentrations were analysed by high performance thin layer chromatography (HPTLC; Lindroth, Kinney, & Platz, 1993); from 2010 to 2015, they were analysed by ultra-high performance liquid chromatography (UHPLC; Rubert-Nason, Keefover-Ring, & Lindroth, 2018). Comparison of the HPTLC and UPLC methods using an independent technical standard of aspen leaf tissue revealed that the two methods produced identical results ($t_{92} = .12$, $p = .91$; Table S2). Compounds included in total SPG calculations were the primary aspen SPGs salicortin and tremulacin, with trace amounts of salicin and tremuloidin (mean 0.15% leaf dry mass combined for salicin and tremuloidin) included when those compounds were measurable by UHPLC in later years. Salicortin, tremulacin and tremuloidin analytical standards were purified from *P. tremuloides* foliage (Rubert-Nason et al., 2018); salicin analytical standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). CT concentrations were analysed using the acid butanol method (Porter, Hrstich, & Chan, 1985) with standardization against CTs purified from *P. tremuloides* foliage (Hagerman & Butler, 1989). Total foliar nitrogen concentration (% dry mass) was analysed using an elemental analyzer (2002–2007; LECO Corporation, St. Joseph, MI, USA, 2008–2015: Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | Data analyses

Trajectories of herbivore defense allocation were defined for two ontogenetic periods: "juvenile" and "mature". In aspen, the transition from juvenile to mature is not easily discernible and likely varies among individuals and genotypes. Therefore, for comparisons of defense trajectories during the two developmental stages, we selected windows of time during which all trees were clearly juvenile or clearly mature and excluded transitional years. The juvenile period was defined as ages 2–6 years (2003–2007), a window in which trees did not flower. The mature period was defined as ages 9–14 years (2010–2015), a period during which all genotypes but one were recorded as flowering. Although aspen trees can live many for many decades, cross-sectional data from 15- to 50-year-old trees show minimal changes in phytochemical traits for trees in that age group (Donaldson, Stevens, et al., 2006).

To quantify ontogenetic defense trajectories during the juvenile stage, we modelled defense allocation as a function of age for each individual tree. Both plant age and size are approximations of plant ontogeny (Barton & Boege, 2017). We chose to calculate trajectories of change as a function of age, as opposed to a function of size, because ontogeny in terms of reproductive maturity is not always correlated with size in the case of trees (Santos-del-Blanco, Bonser, Valladares, Chambel, & Climent, 2013; Thomas, 1996) and flowering occurred simultaneously for trees and genotypes at both ends of the size (D^2H) range in this study.

Slopes and intercepts of age-based trends in SPG and CT concentrations were generated for individual trees. CT trajectories were linear but SPG concentrations declined exponentially, so were log-transformed before calculating slopes. Temporal patterns were similar among the most abundant individual SPG compounds measured (Figure S1), so we proceeded with the analysis using pooled SPG data (i.e. [salicortin] + [tremulacin] + [salicin] + [tremuloidin]). Because slopes of change in SPGs and CTs over time were not significantly different from zero during the mature stage (p > .98), we calculated average SPG and CT concentrations during that time period for each individual.

We tested the significance of genotype and sex as determinants of variation in the growth and defense traits of individual trees using ANOVA. To estimate clonal repeatability (similar to broad-sense heritability, but for clonally derived plants; Falconer, 1989) for each trait, we divided genotypic variance by the sum of model variances, both of which were extracted from linear mixed models with genotype as a random effect (Barker, Holeski, & Lindroth, 2018). In addition, we tested the temporal consistency of genotypic SPG, CT and D^2H values using Kendall's coefficient of concordance (W, Parsons, Bockheim, & Lindroth, 2008). W measures the consistency with which genotype means for a trait are ranked over time, ranging from 0 (not consistent) to 1 (perfectly consistent).

We used measures of concentration (mass per leaf dry mass) for all chemical traits included in our analyses. We recognize that concentration is not a perfect proxy for plant allocation to chemical defense and that use of graphical vector analysis (GVA) can improve interpretation of changes in phytochemistry (Koricheva, 1999). Unfortunately, we did not record total mass per leaf during the first five years of the study, so constructing GVA plots for the juvenile stage was not possible. The GVA for the mature stage (when we were able to calculate chemical content per leaf, Figure S2) confirms that neither SPGs nor CTs changed significantly in concentration or content between ages 9 and 14 years.

To explore the adaptive value of defense trajectories, we created linear models relating defense trajectory parameters to genotype-mean tree growth (change in D^2H from age 1 to 15 years) and cumulative per cent mortality (also through age 15) calculated per genotype. These mortality models of course do not identify the causes of tree death, but do indicate whether patterns exist in the relationships between genotype traits and mortality rates. We tested the independent and interactive effects of defense trajectory parameters and sex on these fitness metrics using type III ANOVA. Although initial size can have a substantial effect on subsequent growth (Kaelke, Kruger, & Reich, 2001), that was not the case in this study ($F_{1,41}$ = 0.32, p = .578), so initial size was not included as a covariate in growth models. Model assumptions of linearity, constant variance and normality were checked by visualizing both raw data and model residuals; tree growth was log-transformed and genotype mortality was square-root-transformed to adhere to model assumptions. All analyses were performed in R version 3.4.2 (R Core Team, Vienna, Austria).

3 | RESULTS

Over 14 years, average tree volume index (D^2H) increased nearly 5,200-fold (Figure 1). Volume growth (change in D^2H from age 1–15 years) varied 35-fold among individual trees and 13-fold among genotypes ($F_{11,74}$ = 20.08, p < .001; Table S1), with a clonal repeatability estimate of 0.84 (Table 1). Rank order of genotype D^2H means was highly consistent over time (W = .999, p < .001). Variation in volume growth was distributed fairly uniformly among eleven of the twelve genotypes, whereas the twelfth was highly divergent, as its mean D^2H growth was more than double that of the next largest genotype (Table S1). Volume growth did not differ significantly between sexes ($F_{1,84}$ = 1.96, p = .165). Cumulative per cent mortality, calculated per genotype, varied widely (0%–67%; Table S1) but was not sexually dimorphic ($F_{1,10}$ = 0.98, p = .346).

Across individual trees, initial SPG concentrations ranged from 4% to 25%. Foliar levels (% dry mass) of SPGs declined exponentially with age during the juvenile stage (trees aged 2–6 years) but exhibited no significant age-dependent trends during the mature stage (trees aged 9–14 years; Figure 2a). Slopes of year-to-year change in log-transformed SPG concentrations during the juvenile stage ranged from −0.7 to 0.2, with an average of −0.2 (SE = .016). SPG concentrations averaged for each individual over the mature stage ranged from 1% to 10% dry mass.

As with SPGs, foliar CT concentrations exhibited declines with age during the juvenile stage (2–6 years) but no age-dependent trends in the mature stage (Figure 2b). Initial CT concentrations were

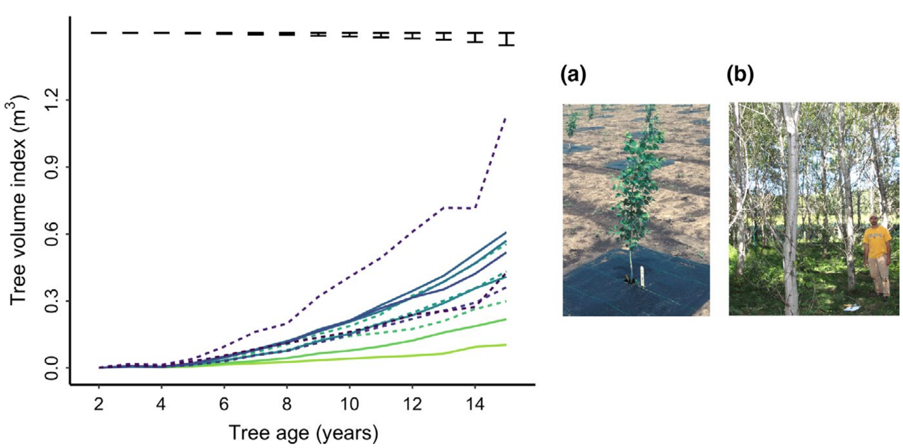

FIGURE 1 Average tree size (volume index, calculated as D^2H) of twelve aspen genotypes over 14 years (trees aged 2–15 years, 2003–2016). Females are shown in solid lines, males in dashed lines. Error bars at top of figure represent means of genotype SEs in m^3 for each year. Photographs show the study trees at age 2 years (a) and 12 years (b)

TABLE 1 Clonal repeatability estimates (H^2) for growth and defense traits

Trait	H^2
Tree growth (D^2H, m^3), age 2–15 years	0.84
Initial juvenile SPGs (% d.m.), age 2 years	0.64
Rate of juvenile SPG change, age 2–6 years	0.74
Average mature SPGs (% d.m.), age 9–14 years	0.87
Initial juvenile CTs (% d.m.), age 2 years	0.86
Rate of juvenile CT change, age 2–6 years	0.52
Average mature CTs (% d.m.), age 9–14 years	0.80
Nitrogen (% d.m.), age 2–14 years	0.58

4%–24%. During the juvenile stage, CT levels exhibited linear rates of change ranging from −3.8 to 2.1% per year, with an average slope of −0.8% per year (*SE* = .11% per year). CT concentrations averaged over the mature stage for each individual ranged from 6% to 18%.

Each of the parameters characterizing ontogenetic trajectories of SPGs and CTs (initial juvenile level, juvenile trend slope, mature average) showed significant genotypic variation ($p \leq .001$; Table S1). Clonal repeatability estimates for these traits were quite high, ranging from 0.52 to 0.87 (Table 1). Although slopes of change in chemical traits varied among genotypes, the overall rank order of genotype-mean SPG and CT concentrations remained fairly consistent over time, especially for SPGs (SPGs: $W = .751$, $p < .001$; CTs: $W = .324$, $p < .001$). Sexual dimorphism was significant for only two defense parameters: on average, juvenile CT slopes were 123% more negative in females relative to males ($t_{107} = 2.8$, $p = .01$) and mature SPG averages were 27% higher in males relative to females ($t_{107} = 2.4$, $p = .02$).

Foliar nitrogen concentrations decreased slightly, on average, from 2.9% to 2.3% dry mass over 13 years (Figure S1). Mean nitrogen concentrations varied significantly but modestly among genotypes ($F_{1,98} = 14.69$, $p < .001$) and, from a relative standpoint, were 3% higher in females than in males ($F_{1,108} = 4.86$, $p = .03$).

Tree growth was correlated with all three SPG trajectory parameters (initial juvenile level, juvenile trend slope, mature average) and with the mature average for CT concentration. Most of the relationships between growth and defense were sex specific. A significant trade-off between initial juvenile SPGs and growth was evident in female but not male trees (Figure 3a). However, slow declines in juvenile SPGs (represented by less-negative rates of change of log-transformed juvenile SPGs, i.e., *x*-values towards the right in Figure 3b) and higher mature SPGs (Figure 3c) were correlated with reduced growth independent of sex. Higher CT concentrations in maturity were correlated with faster growth in female trees only (Figure 3d).

Variation among genotypes in cumulative tree mortality (%) was significantly correlated with variation in juvenile SPG parameters and marginally correlated with mature defense parameters. These relationships were also sex specific. Female genotypes with higher initial SPGs, slower (less negative) rates of juvenile SPG decline and higher mature SPGs experienced more mortality than females with SPG allocation trajectories that started lower, declined more quickly or ended lower (Figure 4a–c). On the other hand, male genotypes with higher-allocation SPG trajectories experienced *less* mortality than males with lower-allocation trajectories (Figure 4a–c). Mature CTs had a contrasting relationship with mortality: females with higher mature CT allocation experienced less mortality (Figure 4d).

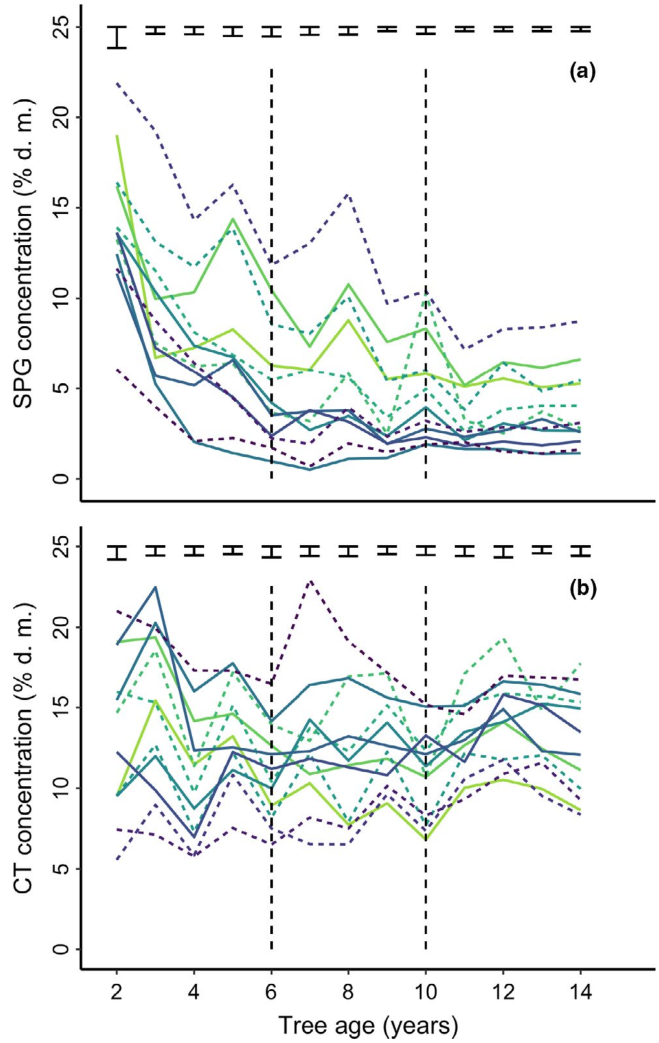

FIGURE 2 13-year trajectories in concentrations of foliar chemical defenses for twelve aspen genotypes. Females are shown in solid lines, males in dashed lines. Panel a: salicinoid phenolic glycosides (SPGs); panel b: condensed tannins (CTs). Error bars at top of figure represent means of genotype *SEs* in per cent dry mass for each year. Vertical lines indicate the end of the juvenile stage (age 6 years) and the beginning of the mature stage (age 10 years)

4 | DISCUSSION

In this first-ever decadal study of plant growth and defense, we characterize long-term ontogenetic trajectories of herbivore defense allocation in aspen that are both genetically variable and correlated with fitness outcomes. These results suggest that trajectories of defense are adaptive and subject to natural selection.

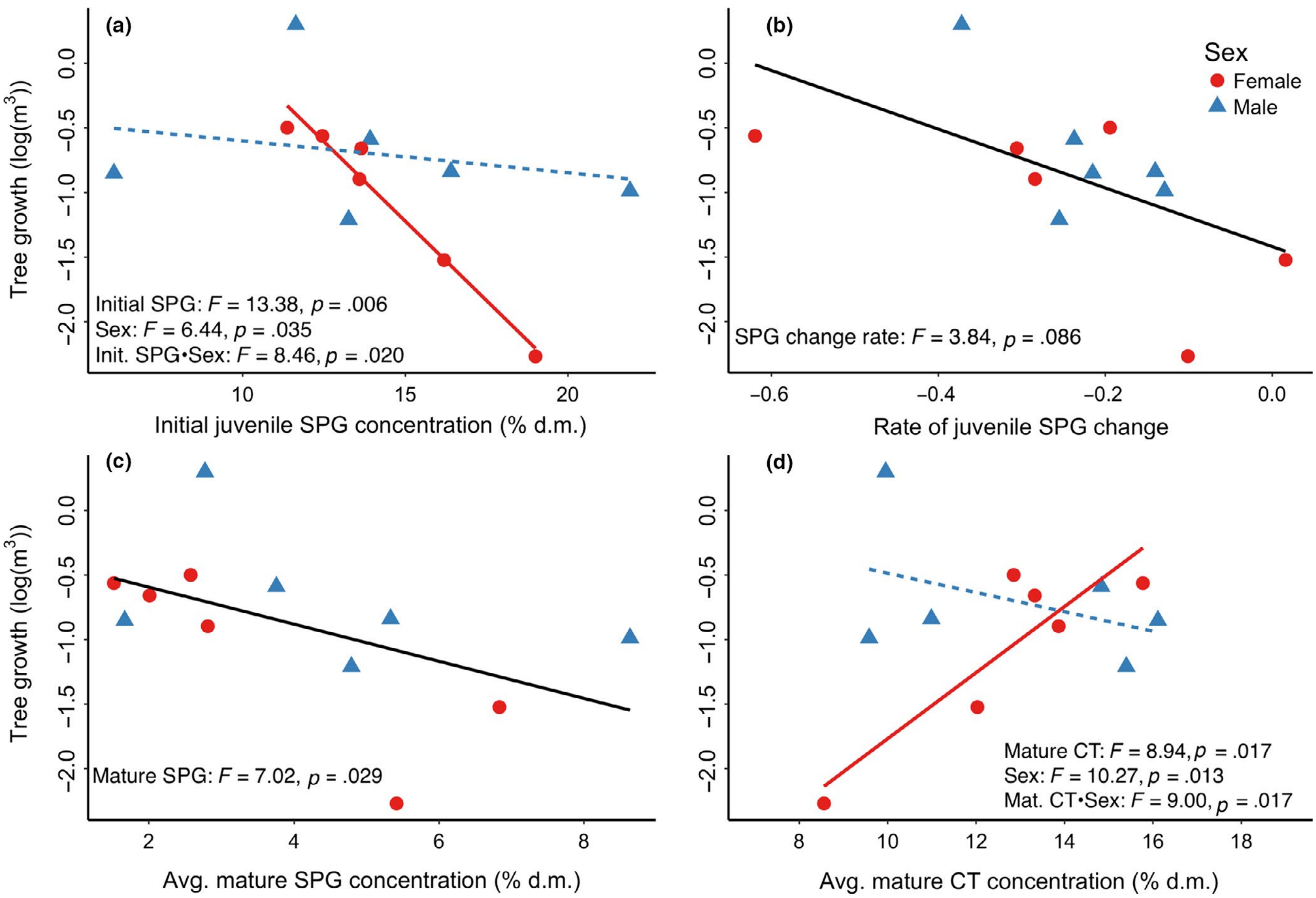

FIGURE 3 Relationships between genotype-level growth and defense-trajectory parameters for the 12 aspen genotypes. Panels show sex-specific plots of volume growth (D^2H change from age 1–15 years) against initial concentrations of salicinoid phenolic glycosides (SPG at age 2 years; panel a), rates of change in juvenile SPG concentrations (trees aged 2–6 years; panel b) and average mature concentrations of SPGs and CTs (trees aged 9–14 years; panels c–d). Note that in all panels, D^2H change is log-transformed. SPG change rates (panel b) are slopes over time of log-transformed concentration values; rates vary from very negative (steep exponential declines in SPGs) through zero (no change) to slightly positive (slight increases in SPGs). ANOVA outputs included for statistical relationships where $p \leq .10$

Trends of defense allocation in this study largely mirror those characterized in past cross-sectional studies of aspen, as expected from our first prediction. The pattern of exponential decline in juvenile SPGs is consistent with previous studies of aspen SPGs (Donaldson, Stevens, et al., 2006), other low-molecular-weight phenolics in birch (Wam, Stolter, & Nybakken, 2017), and salicinoids in European aspen (Nissinen et al., 2017). Beyond genotypic variation, this ontogenetic decline may be the largest source of spatiotemporal variation in SPGs, which are not greatly influenced by environmental factors (Lindroth & St. Clair, 2013). Previous characterizations of CTs in trembling aspen documented a strong increase in concentration up to 2 years of age, and minimal change thereafter (Donaldson, Stevens, et al., 2006). Accordingly, the minimal changes in CTs observed in this study likely stem from the fact that we began our measurements with two-year-old saplings rather than seedlings. On an individual or genotype level, CTs changed significantly in the juvenile stage, and the direction of change for most genotypes was negative, but overall trends in CT concentrations were relatively flat throughout the entire study period.

Observed ontogenetic trends in herbivore defense allocation likely arose due to changes in the costs and benefits of various types of defense. For example, the initially high levels and subsequent declines in aspen SPGs with age may be related to defense against, and eventual escape from, damage by ground-dwelling mammals (Bryant, 1981; Bryant & Julkunen-Tiitto, 1995; Koricheva & Barton, 2012; Lastra, Kenkel, & Daayf, 2017). Notably, other species of Salicaceae that do not outgrow mammalian herbivory maintain high levels of SPGs that do not exhibit ontogenetic declines (Nissinen et al., 2018).

All parameters characterizing ontogenetic trajectories of defense allocation were genetically variable in both juvenile and mature stages, consistent with our second prediction. Sexual dimorphism was much less extensive than genotypic variation: males and females differed significantly in only two parameters describing defense trajectories. Male trees had higher mature average SPGs and less-negative juvenile rates of change in CTs. These two results contradict a meta-analysis across plants that found lower defense allocation in males than females (Cornelissen & Stiling, 2005). However,

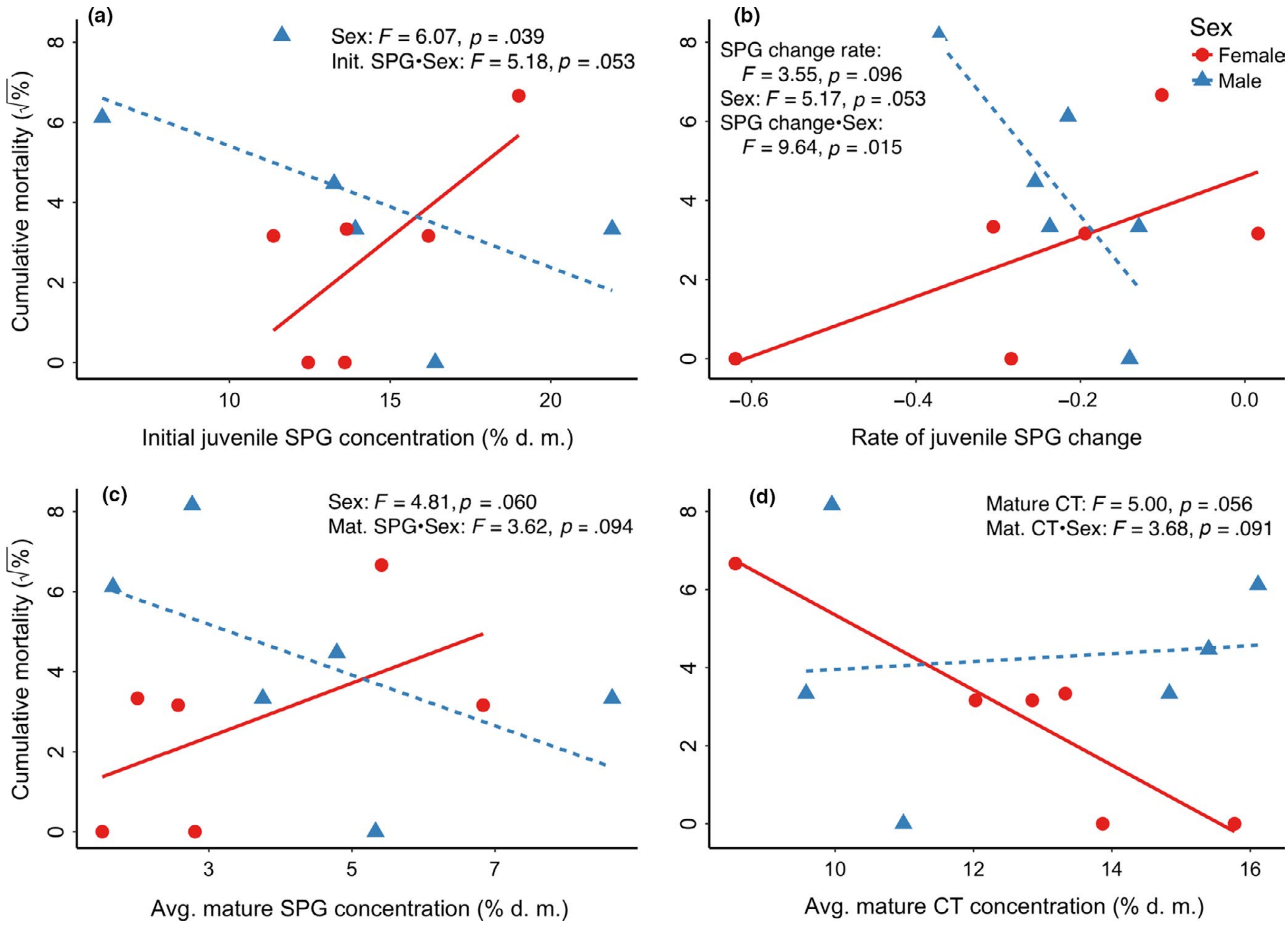

FIGURE 4 Relationships between genotype-level mortality and mean defense-trajectory parameters for the 12 aspen genotypes. Panels show sex-specific plots of cumulative mortality (%, through age 15 years) against initial concentrations of salicinoid phenolic glycosides (SPG at age 2 years; panel a), rates of change in juvenile SPG concentrations (trees aged 2–6 years; panel b) and average mature concentrations of SPGs and CTs (trees aged 9–14 years; panels c–d). In all panels, % mortality is square-root-transformed. SPG change rates are slopes over time of log-transformed concentration values; rates vary from very negative (steep exponential declines in SPGs) through zero (no change) to slightly positive (slight increases in SPGs). ANOVA outputs included for statistical relationships where $p \leq .10$

the relative lack of sexual dimorphism in concentrations of chemical defense compounds in our study mirrors the minimal evidence of sex differences in defense concentrations found in many previous studies of *Populus* (McKown et al., 2017; Randriamanana et al., 2014; Stevens & Esser, 2009) and other dioecious woody plants (Nell et al., 2018; Stark & Martz, 2018). The sole other long-term study of tree defense to date (Nissinen et al., 2018), involving dioecious *Salix myrsinifolia*, also reported greater genotypic variation than sexual dimorphism in foliar defense compounds.

Variation in trajectories of herbivore defense appears to impact aspen fitness over the long term, in agreement with our third prediction and in contrast to the null model of ontogenetic drift (Barton & Boege, 2017). Significant relationships between tree fitness metrics (growth, survival) and defense trajectory parameters indicate that the latter have adaptive value. In contrast, foliar nitrogen did not vary widely among genotypes in this study, nor did it correlate with tree fitness. Nitrogen (measured as a proxy for protein) is nutritionally important for insects and can attenuate the impact of SPGs on herbivore performance (Hemming & Lindroth, 2000; Lindroth & Bloomer, 1991). The lack of genotypic variation in foliar nitrogen in our study indicates that secondary chemistry, rather than protein, is likely to be the main driver of genotypic variation in insect herbivory.

Interestingly, most of the fitness impacts of defense trajectories were sexually dimorphic. Female aspen trees were more sensitive to growth-defense trade-offs than males with respect to SPG parameters. These results support our fourth prediction and the well-established notion that females are more sensitive to defense costs because of their higher reproductive investment (Barrett & Hough, 2012; Sakai & Burris, 1985), even though overall growth here was not sexually dimorphic. CTs had less pronounced trajectories of defense and fewer impacts on tree fitness than did SPGs; fitness outcomes were not correlated with the initial concentrations or age-based trends of CTs during the juvenile stage, but high CT concentrations in maturity were associated with increased growth and survivorship in female trees.

Previous studies of relationships between size and chemical defense in mature aspen also revealed that size was more strongly correlated with defense allocation in female trees (Cole et al., 2016; Stevens & Esser, 2009), though the directionality of growth effects differed in these studies: females' size was negatively correlated with CTs but positively correlated or uncorrelated with SPGs. This difference in patterns could be a consequence of examining trait correlations within a single year in previous studies (Cole et al., 2016; Stevens & Esser, 2009) versus across many years in our study. In addition, environmental variation strongly influences both growth and CT concentrations in aspen (Barker, Holeski, & Lindroth, 2019; Osier & Lindroth, 2006) so the relationship between growth and CTs is context-specific and thus may vary among studies.

The question arises as to why we would detect a growth-defense trade-off for one type of defense compound (SPGs) and not for another (CTs)? The answer may lie with the different biochemical properties and modes of action of SPGs and CTs. Low-molecular weight phenolics like SPGs may undergo high turnover in leaf tissue (Massad et al., 2014), whereas that does not appear to be the case for CTs (Hättenschwiler & Vitousek, 2000). Thus, SPGs may be more costly per unit concentration because they require continual re-investment of assimilated carbon. In addition, differing modes of action likely contribute to different selection pressures for the two types of compounds. SPGs confer resistance against diverse herbivores (Lindroth & St. Clair, 2013). SPGs have also been implicated in pathogen resistance (Kruger & Manion, 1994), which may partly explain the positive relationship between SPGs and survivorship in male genotypes. In contrast, the roles of CTs in conferring resistance is less clear, but the compounds may increase tolerance in defoliated trees by enhancing N uptake from litter and herbivore frass (Madritch & Lindroth, 2015). Finally, CT expression is highly plastic and responsive to resource availability, which may attenuate costs compared with more genetically determined, resource-independent SPG expression (Barker et al., 2019; Donaldson & Lindroth, 2007; Osier & Lindroth, 2006). The modest negative correlation between SPGs and CTs in mature trees ($F_{1,108}$ = 53.5, p < .001, r = −.58; data not shown) does, however, complicate comparisons of results for the two defenses.

Correlations between tree fitness and SPG trajectory parameters were significant in both juvenile and mature ontogenetic stages, and effect sizes were not greater for juvenile defense allocation as posited in our fourth prediction. These results indicate that, even though opportunity costs are presumably higher for juvenile defense investment (Barton & Boege, 2017), the strength of selective pressure acting on variation in juvenile defense trajectories may not differ from that acting on variation in mature defense.

Genotypic variation in, and fitness consequences of, ontogenetic trajectories of defense indicate that these phytochemical changes are not simply a neutral by-product of development but are in fact adaptive and subject to natural selection. Because variation in defense trajectories throughout tree lifetimes may differentially affect fitness of tree genotypes, trajectories should be considered as important contributors to overall defense phenotypes not only at the individual level, but also at the population level (Barton & Boege, 2017). Plant defense traits—especially those of foundation species—play a role in structuring communities of herbivores, detritivores and their natural enemies (Barker et al., 2018; Carmona & Johnson, 2016; Kanaga et al., 2009; Poelman, van Loon, & Dicke, 2008) and influence ecosystem processes such as terrestrial and aquatic nutrient cycling (Hättenschwiler & Vitousek, 2000; Madritch, Greene, & Lindroth, 2009; Schweitzer et al., 2008). Thus, selection on ontogenetic trajectories of defense has implications for both functional and structural composition of forest ecosystems through space and time. Additionally, sexual dimorphism in the sensitivity of tree fitness to variation in defense trajectories could affect sex ratios of aspen under different environmental conditions. The relationships we found between fitness and juvenile SPG trajectories are particularly relevant to aspen grown commercially for bioenergy, which is typically harvested in the late juvenile stage (Tullus, Rytter, Tullus, Weih, & Tullus, 2012; Weih, 2004).

Ontogenetic variation in defense is a nontrivial component of overall heterogeneity in plant quality (Koricheva & Barton, 2012). Improved understanding of ontogenetic defense variation in trees, especially in foundation species such as aspen, will help resolve patterns of foliar defense composition and associated ecological processes at the scale of whole stands and forests. Genotypic variation in chemical defense creates spatial mosaics of ecosystem function in aspen landscapes (Madritch et al., 2009, 2014) and may also be the bellwether of a tree population's capacity to tolerate and/or adapt to future pest disturbances (Six, Vergobbi, & Cutter, 2018). Moreover, because forests are typically also mosaics of different-aged successional patches (Trumbore, Brando, & Hartmann, 2016), ontogenetic variation and its interactions with genotypic composition have the potential to shape ecosystem functioning at broad spatiotemporal scales. Landscape-level variation in aspen chemistry and its impacts on associated organisms and ecosystem functions may thus be a consequence of combined genotypic and ontogenetic variation.

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AUTHORS' CONTRIBUTIONS

R.L.L. conceived the study design, acquired funding and established the experimental garden; R.L.L., K.F.R.-N. and O.L.C. collected the data; O.L.C. and E.L.K. analysed the data; O.L.C. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.104d626 (Cope, Kruger, Rubert-Nason, & Lindroth, 2019).

ORCID

Olivia L. Cope https://orcid.org/0000-0002-5559-8164

Eric L. Kruger https://orcid.org/0000-0003-2463-9231

Richard L. Lindroth https://orcid.org/0000-0003-4587-7255

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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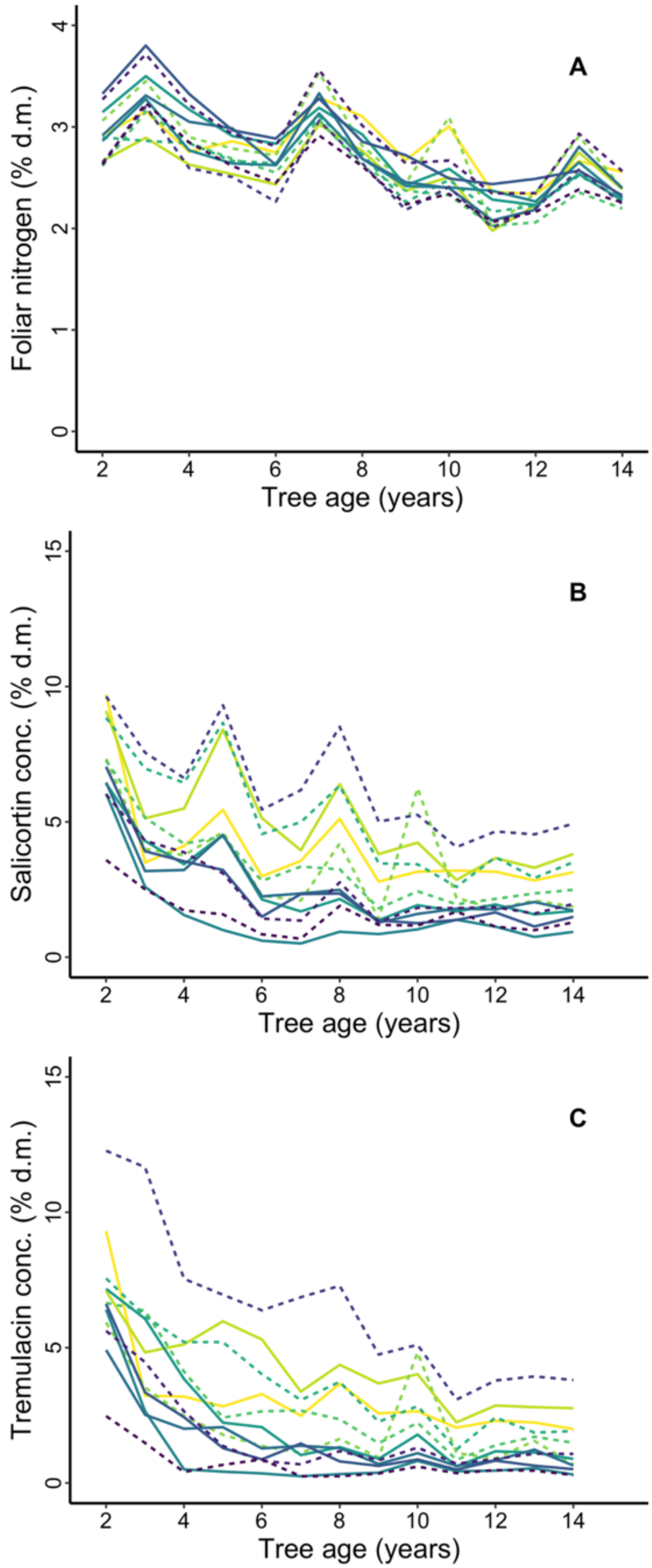

Fig. S1: 13-year trends in foliar concentration (% dry mass) of nitrogen (A) and the two principal aspen SPG compounds, salicortin (B) and tremulacin (C), for twelve aspen genotypes (females: solid lines, males: dashed lines). The spike in foliar nitrogen at age 7 is likely due to substantial runoff from an adjacent, upslope fertilized cornfield during a heavy rainfall event.

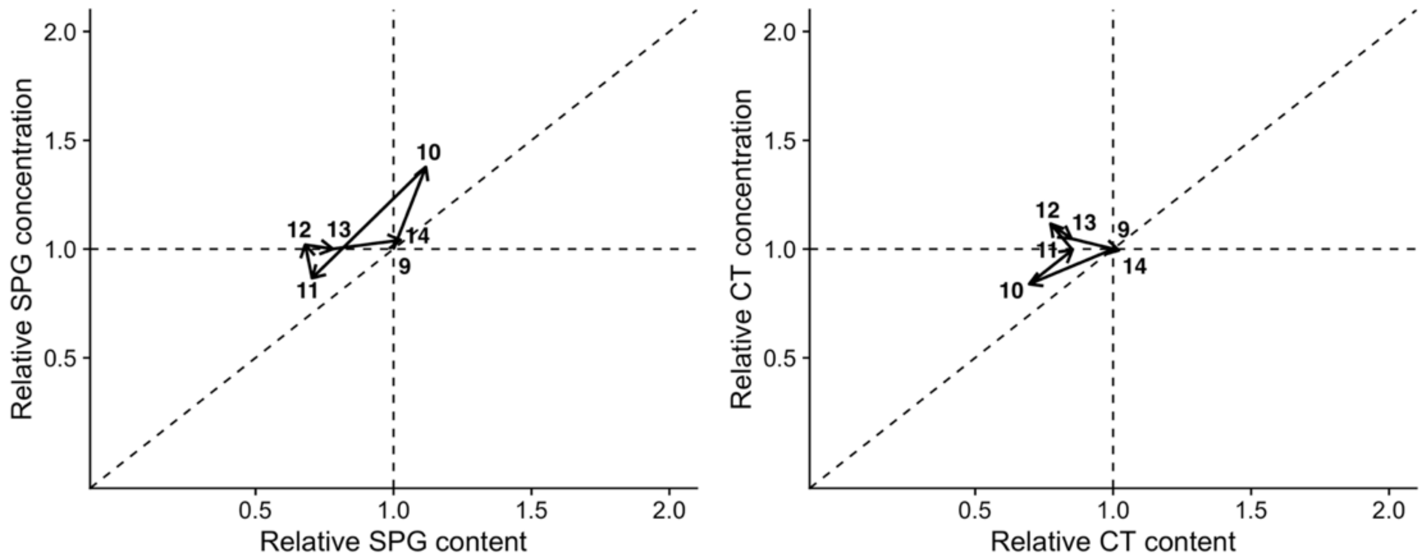

Fig. S2: Graphical Vector Analyses (GVAs) of global-mean SPGs and CTs during the mature phase (trees aged 9-14 years). Y axis is relative concentration (mg/mg leaf), X axis is relative content (mg/leaf). These axes are anchored on the mean values for 9-year-old trees, and each vector arrow represents the change in mean phytochemical allocation between years. Number labels are tree ages.

Table S1: Genotype means (with SEs in parentheses) for tree growth, mortality, and foliar chemical traits, including initial juvenile levels, juvenile rates of change, and mature averages for SPGs and CTs, as well as overall averages for nitrogen.

		Tree volume growth (D^2H, m^3)	**Cumulative mortality (%)**	**Initial juvenile SPGs (% d.m.)**	**Rate of juvenile log(SPG) change per year**	**Mature SPGs (% d.m.)**	**Initial juvenile CTs (% d.m.)**	**Rate of juvenile CT change per year**	**Mature CTs (% d.m.)**	**Nitrogen (% d.m.)**
Genotype	**Sex**	**Age: 2-15 y**	**Age: 2-15 y**	**Age: 2 y**	**Age: 2-6 y**	**Age: 9-14 y**	**Age: 2 y**	**Age: 2-6 y**	**Age: 9-14 y**	**Age: 2-14 y**
Dan 1	F	0.10 (0.02)	40.0 (0.2)	19.0 (1.7)	-0.10 (0.04)	5.9 (0.5)	9.5 (0.8)	-1.01 (0.26)	8.6 (0.6)	2.82 (0.05)
Dan 2	F	0.22 (0.03)	40.0 (0.1)	16.2 (1.9)	0.02 (0.03)	6.8 (0.2)	19.1 (1.5)	-2.02 (0.48)	12.0 (0.4)	2.57 (0.05)
PG 1	M	0.30 (0.03)	20.0 (0.1)	13.3 (0.4)	-0.26 (0.02)	4.8 (0.7)	14.7 (1.1)	-0.53 (0.24)	15.4 (0.8)	2.82 (0.05)
PG 2	M	0.56 (0.04)	11.1 (0.1)	13.9 (2.1)	-0.24 (0.02)	3.8 (0.2)	16.0 (0.9)	-1.04 (0.16)	14.8 (0.2)	2.53 (0.04)
PG 3	M	0.43 (0.06)	0.0 (0.0)	16.4 (1.0)	-0.14 (0.03)	5.3 (0.2)	9.6 (0.8)	-0.54 (0.20)	11.0 (0.3)	2.58 (0.04)
PI 12	F	0.41 (0.05)	0.0 (0.0)	13.6 (0.8)	-0.28 (0.02)	2.8 (0.1)	9.5 (0.9)	-0.15 (0.16)	13.9 (0.4)	2.75 (0.03)
PI 3	F	0.57 (0.05)	0.0 (0.0)	12.5 (0.7)	-0.62 (0.03)	1.5 (0.1)	15.5 (0.4)	-1.16 (0.38)	15.8 (0.3)	2.64 (0.03)
Sau 1	F	0.61 (0.02)	10.0 (0.1)	11.4 (0.7)	-0.19 (0.01)	2.6 (0.1)	18.9 (1.0)	-2.59 (0.19)	12.9 (0.3)	2.70 (0.04)
Sau 2	F	0.52 (0.02)	11.1 (0.1)	13.7 (2.1)	-0.31 (0.03)	2.0 (0.1)	12.3 (0.5)	0.44 (0.29)	13.3 (0.3)	2.87 (0.03)
Sau 3	M	0.37 (0.04)	11.1 (0.1)	21.9 (1.1)	-0.13 (0.02)	8.6 (0.2)	5.6 (0.9)	0.32 (0.30)	9.6 (0.3)	2.50 (0.03)
Wau 1	M	1.35 (0.22)	66.7 (0.2)	11.6 (0.6)	-0.37 (0.02)	2.8 (0.1)	7.5 (0.3)	-0.10 (0.15)	10.0 (0.2)	2.93 (0.04)
Wau 2	M	0.43 (0.10)	37.5 (0.2)	6.1 (0.9)	-0.22 (0.06)	1.7 (0.1)	21.0 (0.6)	-1.12 (0.19)	16.1 (0.4)	2.52 (0.05)

Table S2: SPG concentration data from a common technical standard (aspen leaf tissue) run simultaneously with SPG analyses for this study. HPTLC was used from 2004-2009 and UHPLC was used from 2010-2015. Cumulative means were calculated across all replicates within years for each method (HPTLC and UHPLC). A t-test was performed across all replicates and confirmed that values from the two analytical methods do not significantly differ ($t_{92} = 0.12$, $p = 0.91$).

Method	Year	Number of replicates	SPGs in leaf tech. standard (% d.m.,)	SPGs in leaf tech. standard (% d.m., cumulative mean)
HPTLC	2004	1	10.02	8.85
HPTLC	2005	1	9.48	
HPTLC	2006	5	8.83	
HPTLC	2007	11	9.09	
HPTLC	2008	18	8.66	
HPTLC	2009	9	8.72	
UHPLC	2010	14	8.19	8.82
UHPLC	2011	16	8.51	
UHPLC	2012	5	11.31	
UHPLC	2013	6	9.51	
UHPLC	2014	9	8.64	
UHPLC	2015	3	8.46	