

## Developmental Trajectories in Cottonwood Phytochemistry

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**Abstract** We examined the hypothesis that ecologically important phytochemical traits differ predictably among various developmental zones of trees (i.e., mature and juvenile zones of individual trees and juvenile ramets that sprout from roots) and that the slope of this phytochemical gradient represents a “developmental trajectory.” We focused on *Populus fremontii* (Fremont cottonwood), *P. angustifolia* (narrowleaf cottonwood), and their natural hybrids. Two major patterns emerged. First, within narrowleaf and hybrids, concentrations of important phytochemicals (condensed tannins and phenolic glycosides) differ greatly and predictably between developmental zones. Second, developmental trajectories differ greatly among these cottonwood species and their hybrids: Fremont exhibits a flat trajectory, narrowleaf a steep trajectory, and hybrids an intermediate trajectory, suggesting an additive genetic component and an ontogenetic basis to this phytochemical variation. Because diverse herbivorous species respond to the phytochemistry of their host plants, we predict that the developmental trajectories of plants play a major role in mediating ecological interactions and structuring communities, and that biodiversity in a stand of trees is determined by both interplant genetic diversity and intraplant ontogenetic diversity.

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## Introduction

Plant chemistry affects the behavior, distribution, performance, and population dynamics of herbivores (Frankel, 1959; Schultz, 1988; Bernays and Chapman, 1994; Seldal et al., 1994; Karban and Baldwin, 1997; Larsson et al., 2000; Shelton, 2000). Variation in chemistry among plants within a species results from genetic and environmental causes. Concentrations of many plant antiherbivore compounds, such as phenolic glycosides and condensed tannins, are under genetic control (Orians et al., 1993; Hwang and Lindroth, 1997; Lindroth et al., 2002), but also respond to environmental factors such as resource availability (Kinney et al., 1997; Lindroth et al., 1997; Orians et al., 2003).

Plant development can drive the distribution and/or performance of both pathogens and herbivores (Zagory and Libby, 1985; Kearsley and Whitham, 1989; Kearsley and Whitham, 1998; Karban and Thaler, 1999; Brennan and Weinbaum, 2001; Swihart and Bryant, 2001; Lawrence et al., 2003; Barrett and Agrawal, 2004) and can have community-level consequences (Waltz and Whitham, 1997). Well-characterized major cellular, physiological, and structural changes occur as plants develop (Cronk et al., 2002; Srivastava, 2002), but much less is known about the effects of development on plant chemistry (Edwards, 1982; Baldwin, 1999; Ohnmeiss and Baldwin, 2000). Some work has demonstrated higher levels of chemical defenses in juvenile shoots or ramets of the Salicaceae (Tahvanainen et al., 1985; Reichardt et al., 1990; Martinsen et al., 1998), but each of these studies was limited to a single tree species. Most other studies of the interaction of plant development and chemistry in trees have measured phytochemical concentrations in leaves of different ages within small saplings (e.g., Bingaman and Hart, 1993; Kleiner et al., 2003), as opposed to the variation that exists at any given time in mature, canopy-sized trees.

Here, we focus on plant development as a major source of phytochemical variation within individual trees. Developmental processes in plants can be generalized into two broad categories, ontogenetic and physiological or environmental (e.g., Lawson and Poethig, 1995). Ontogenetic variation arises from changes in gene expression in plant meristems (Poethig, 1990; Wiltshire et al., 1994, 1998; Jordan et al., 1999, 2000). It is widespread and results in changes in vegetative structures across whole-plant gradients (Jones, 1999). In contrast, environmental changes result from alteration of the local meristem environment by factors such as shading, water, and nutrient relations. This distinction is important, because only ontogenetically derived traits are heritable and subject to natural selection. Few studies have actually quantified the ontogenetic component of developmental traits that affects herbivores (but see Lawrence et al., 2003).

To assess developmentally based variation in plant chemistry, we studied the phytochemical variation in a hybrid swarm composed of Fremont and narrowleaf cottonwood (*Populus fremontii* and *P. angustifolia*, respectively), their F<sub>1</sub> hybrids, and complex backcrosses to narrowleaf cottonwood. Collectively, these are referred to as “cross types” (Wimp et al., 2004). Fremont cottonwood occurs at lower elevations, narrowleaf at higher elevations. The two species hybridize naturally in zones where their distributions overlap throughout the western United States (Eckenwalder, 1984), and they dominate many riparian zones in the intermountain west. The results of previous community studies performed in this hybrid system motivated several of the hypotheses of this study.

First, based on the distribution and survival of common herbivores (Kearsley and Whitham, 1989, 1998; Waltz and Whitham, 1997), we hypothesized that plant phytochemistry varies predictably within a tree across three developmental zones (juvenile ramets, juvenile zone, and mature zone). Because Waltz and Whitham (1997) showed that the juvenile zone was intermediate in its arthropod community to the mature zone and juvenile ramets, we hypothesized that it would also have intermediate phytochemistry. Second, we hypothesized that this predictable within-tree variation, which we term “developmental trajectory in phytochemistry,” has a genetic basis and would differ among cross types. Third, because different cross types support different arthropod communities in the wild and in a common garden (Wimp et al., 2005), we hypothesized that phytochemistry differs predictably among cross types (Fremont, narrowleaf, and F<sub>1</sub> and backcross hybrids). Last, because the leaf feeding beetle, *Chrysomela confluenta*, sequesters toxic phenolic glycosides and feeds preferentially on juvenile ramets, we predicted that juvenile ramets would be highest in phenolic glycosides.

This work is unique in that we studied phytochemical variation on multiple scales simultaneously: seasonal, within and among species, as well as within individuals, by using mature, canopy-sized trees. We document developmentally based phytochemical differences within trees that exist at any given time, as opposed to only the differences that occur as a result of aging of leaves at a given position in the tree through a growing season. Ultimately, because plant development affects whole arthropod communities (Waltz and Whitham, 1997), these findings could provide additional genetic and phytochemical mechanisms for understanding biodiversity, community structure, and ecosystem processes.

## Methods and Materials

### Common Garden Survey

To test the genetic basis of differences in phytochemistry among cross types, we sampled trees in a common garden established in 1991 at the Ogden Nature Center, Ogden, UT, USA. This study included 11 Fremont, 11 F<sub>1</sub> hybrid, 34 backcross hybrid, and 13 narrowleaf trees. The particular genotypes included were chosen as a representative sample of each cross type. Only leaves from the juvenile zone were sampled. Collection dates as well as sampling, chemical, and statistical methods were the same as for the field study, described below.

### Field Survey

We studied cottonwoods growing along the Weber River in the vicinity of Ogden, UT, USA, where many population, community, and ecosystem-level studies have been conducted. We sampled leaves from trees at seven sites in the 13-km-long hybrid zone as well as from the pure species zones (one Fremont site, two narrowleaf sites) on three occasions: 21–24 May, 20–23 June, and 14–18 August 2001. At the seven sites in the hybrid/overlap zone, 2–4 of the cross types co-occurred at each site. Based on restriction fragment length polymorphism marker studies that genetically characterized the species or hybrid status of individual trees (Keim et al., 1989; Martinsen et al., 2001), our collection consisted of 10 Fremont, 14 F<sub>1</sub> hybrid, 13 backcross hybrid, and 13 narrowleaf trees. These were chosen based on their genetic characterization and presence of all desired developmental zones. All trees were reproductively mature in the upper crown, but juvenile at the base. Leaves were collected

from the mature and juvenile zones of all trees. All cross types, except Fremont, reproduce vegetatively by root suckering (Schweitzer et al., 2002), and leaves were also sampled from these small (<1.5 m tall) juvenile saplings that we refer to as “juvenile ramets,” reflecting their asexual origins. Thus, for the four cross types and three possible developmental zones, eleven combinations exist. Sampling from the different developmental zones of the same trees was carried out because one of our major aims was to test for and document phytochemical differences among zones of the same trees in the field.

Leaves from the mature zone were sampled with a 6-m-long pole pruner, and the reproductive status was verified by the presence of catkins and/or their scars. Each sample consisted of 15–25 leaves taken from each zone of the tree, and these leaves were collected from around the entire circumference of the canopy within that zone. Each leaf was removed by cutting at the lamina–petiole junction, and was the fifth or middle leaf from the current year’s growth to standardize leaf age. All leaf samples were frozen between blocks of dry ice and kept under dry ice until storage at  $-20^{\circ}\text{C}$ . Leaves were lyophilized and ground to pass a 40-mesh screen on a Wiley Mill.

Nitrogen is often of critical importance to herbivores (Mattson, 1980; White, 1984), whereas condensed tannins and phenolic glycosides are the major secondary metabolites in the genus *Populus* (Palo, 1984; Lindroth et al., 1987). Nitrogen was measured by using an elemental analyzer (LECO, St. Joseph, MI, USA). After an exhaustive extraction of leaf tissue in 70% acetone with 1 mM ascorbate at  $4^{\circ}\text{C}$ , condensed tannins were determined with the acid butanol assay (Porter et al., 1986) by using tannins prepared from narrowleaf cottonwood as the standard (Hagerman and Butler, 1980; Waterman and Mole, 1994). Phenolic glycoside concentrations were determined by HPLC as described by Lindroth et al. (1993). The compounds salicortin and HCH-salicortin (hydroxy-cyclohexen-on-oyl-salicortin) are the major phenolic glycosides in this system (Rehill et al., 2005), but only Fremont cottonwood and  $F_1$  hybrids contain detectable amounts of HCH-salicortin. Given this distribution, all analyses and figures for HCH-salicortin are based on data from Fremont and  $F_1$  hybrids only. Both compounds were purified from cottonwood leaves by liquid–liquid extraction (Lindroth et al., 1986), followed by medium-pressure, “flash” chromatography (Still et al., 1978) and were used as standards. Salicortin has known antiherbivore properties (Lindroth and Hwang, 1996), and based on its structure the same is likely to be true of HCH-salicortin. Total phenolic glycosides were calculated as the sum of salicortin and HCH-salicortin. This pooled value has frequently been shown to affect herbivore preference and performance (e.g., Osier and Lindroth, 2001). All chemical measures are reported on the basis of percentage dry weight (% DW).

Data were analyzed by using repeated-measures analysis of variance [PROC MIXED, version 8, SAS (SAS Institute)] with an unstructured variance–covariance matrix and individual trees treated as subjects. Despite our explicit hypothesis for phenolic glycosides, we had no *a priori* assumptions about the trajectories of developmentally based variation for all the measured phytochemicals among all cross types. Thus, developmental zones were nested within cross type to prevent bias. Pairwise comparisons of least squares means were made with a Tukey adjustment and were considered significant when  $\alpha < 0.05$ .

Although some minor heteroscedasticity may have been present (e.g., compare Fremont vs. narrowleaf tannin concentrations and associated standard errors), we did not use any data transformations for several reasons. Valavanis (1959) indicates that for maximum likelihood methods (as used by SAS PROC MIXED), heteroscedasticity will not bias parameter estimates. Neter et al. (1996) and Montgomery (1997) both point out that as sample size increases,  $F$  tests approach permutation tests, which are distribution-free, and if total sample

size is not small,  $F$  tests can be considered in this light. Our total sample size was 420 observations for each chemical measured, with 50 test subjects (trees). With a sample size this large, we can invoke the Central Limit Theorem (Sokal and Rohlf, 1995), that the means of samples drawn from a population of any distribution will approach a normal distribution as sample size increases. Given the potential for somewhat ambiguous interpretation of transformed vs. untransformed data, we erred on the side of clarity of interpretation.

We used regression to make comparisons between cross types of the phytochemical shifts among developmental zones within trees. Because Fremont does not produce juvenile ramets, we used only juvenile and mature zone measures of condensed tannins, salicortin, and total phenolic glycosides in comparison among cross types. Each of these variables was regressed against the two developmental zones to determine the slope of the change (“developmental trajectory”), and slopes were compared. Thus, the slope indicates the direction and magnitude of the average change in concentration between the juvenile and mature zones of each cross type.

## Results

### Common Garden Survey of Phytochemical Variation

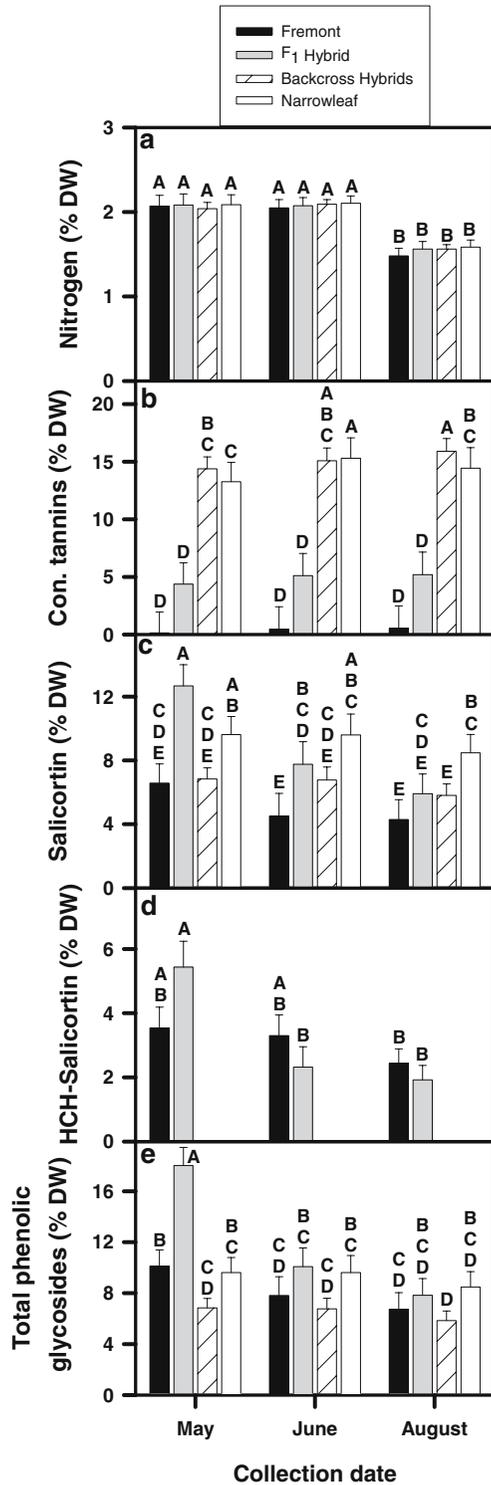
Condensed tannins, salicortin, and total phenolic glycosides in juvenile zone foliage differed significantly among cross types. Levels of seasonal variation differed widely among chemical measures and cross types.

Nitrogen concentrations were the same among cross types but did show significant seasonal variation (Table 1; Fig. 1a). Condensed tannin concentrations increased between the May and June collection dates and differed greatly among cross types (backcross = narrowleaf >  $F_1$  = Fremont). In contrast, concentrations of salicortin decreased throughout the survey (Table 1; Fig. 1c), but also differed among cross types. These differences among cross types changed during the survey, likely as a result of different seasonal changes in each type (Fig. 1c). Concentrations of HCH-salicortin decreased during the survey (Table 1; Fig. 1d), but no consistent differences existed throughout the growing season between  $F_1$  hybrids and Fremont cottonwood. Total phenolic glycoside concentrations, driven mostly by the concentrations of salicortin, decreased over the growing season (Table 1; Fig. 1e) and differed among cross types. Also, cross types differed in the amount of seasonal variation (see Fig. 1e).

**Table 1** Type III tests of effects for the repeated measures anova of concentrations of nitrogen, condensed tannins, salicortin, HCH-salicortin, and total phenolic glycosides in the common garden survey

Effect	Nitrogen		Condensed tannins		Salicortin		HCH-salicortin		Total phenolic glycosides	
	$F$	$P$	$F$	$P$	$F$	$P$	$F$	$P$	$F$	$P$
Collection date	126.45 <sub>2,65</sub>	<0.001	8.73 <sub>2,65</sub>	<0.001	29.99 <sub>2,65</sub>	<0.001	8.35 <sub>2,20</sub>	0.002	43.43 <sub>2,65</sub>	<0.001
Cross type	0.02 <sub>3,65</sub>	0.997	20.92 <sub>3,65</sub>	<0.001	2.98 <sub>3,65</sub>	0.037	0.04 <sub>1,20</sub>	0.837	5.08 <sub>3,65</sub>	0.003
Date × cross type	0.37 <sub>6,65</sub>	0.894	1.14 <sub>6,65</sub>	0.351	5.36 <sub>6,65</sub>	<0.001	3.27 <sub>2,20</sub>	0.059	9.58 <sub>6,65</sub>	0.001

**Fig. 1** Nitrogen, condensed tannin, salicortin, HCH-salicortin, and total phenolic glycoside concentrations in the common garden survey showing levels among cross types among the collection dates. HCH-salicortin was not measured in narrowleaf and backcross foliage. Each bar represents the least squares mean plus one standard error of the mean. Bars with different letters represent least squares means that are significantly different at  $\alpha = 0.05$  using a Tukey adjustment



## Field Survey: Chemistry

Statistical analysis of the effects of cross type, developmental zone, and collection date on foliar chemistry showed major response patterns, and revealed numerous higher-order interactions (e.g., cross type  $\times$  zone for a particular date; see Table 2) that occasionally varied from the dominant patterns. To facilitate clear communication of these results, we emphasize the major patterns for each chemical variable.

Nitrogen levels decreased slightly over the course of the growing season (Table 2; Fig. 2a). Concentrations of nitrogen did not differ consistently among cross types or developmental zones within each cross type (Fig. 2b).

Concentrations of condensed tannins did not differ among collection dates, but did differ among cross types and developmental zones, consistent with our hypotheses. Tannin levels varied greatly (ca. 40-fold) among cross types (Table 2; Fig. 2c). Backcross hybrid foliage had the highest concentrations, narrowleaf had intermediate concentrations, and F<sub>1</sub> and Fremont foliage both had very low concentrations (backcross > narrowleaf > F<sub>1</sub>  $\cong$  Fremont; 16.7%, 10.5%, 2.3%, and 0.4% DW, respectively).

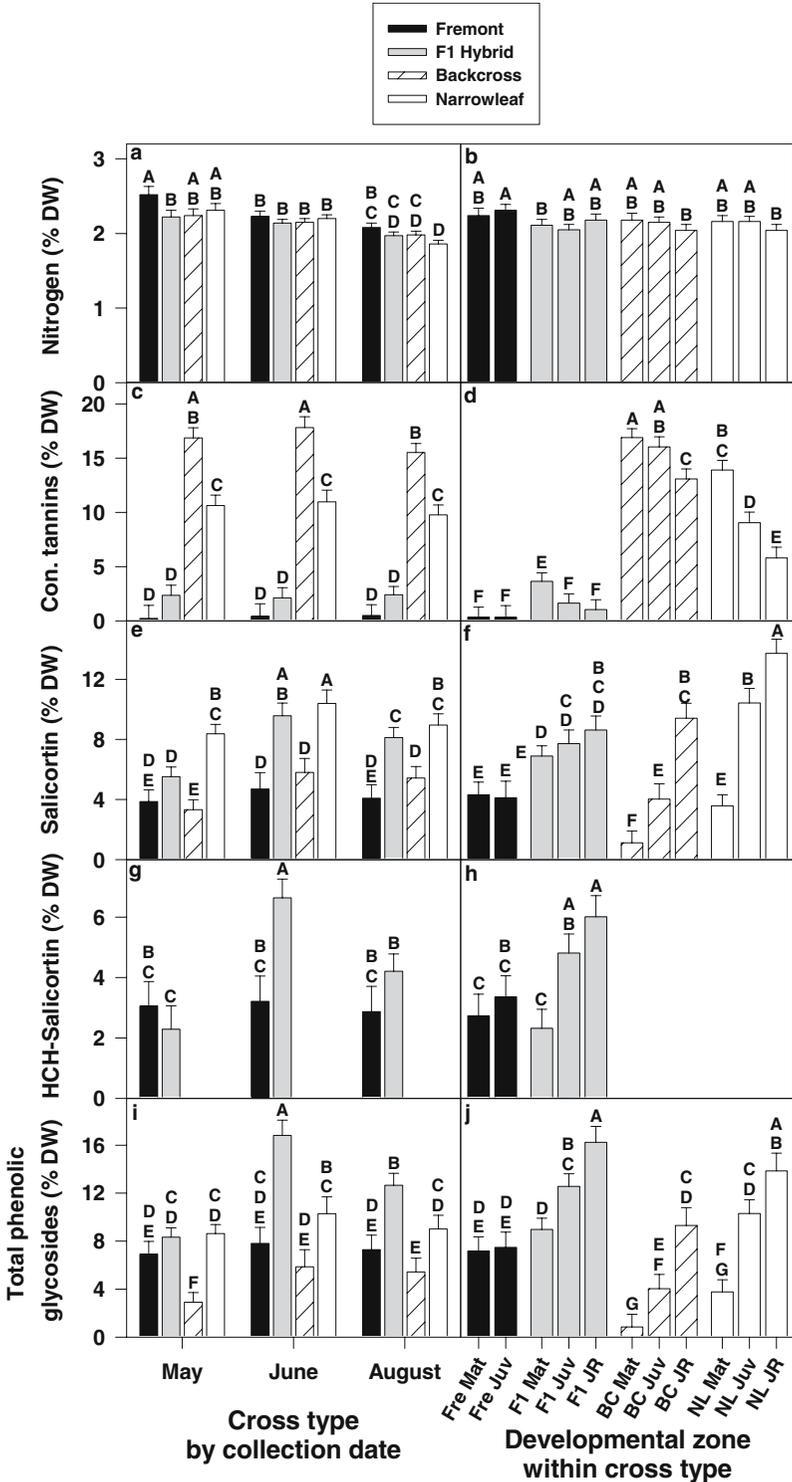
Tannin levels differed significantly among developmental zones for backcross hybrids, narrowleaf, and F<sub>1</sub> hybrids (Fig. 2d). These patterns among developmental zones within cross types changed seasonally (Fig. 3). Thus, relative to our initial prediction, the juvenile zone was frequently, but not always, intermediate in condensed tannin concentrations relative to the mature zone and juvenile ramets.

Salicortin concentrations showed strong seasonal variation, but as hypothesized, the levels also differed among cross types and within individual trees. Concentrations were lowest in May, peaked in June, and decreased to an intermediate level in August (Table 2; Figs. 2e and 4). Cross types separated into two groups: salicortin levels in both narrowleaf and F<sub>1</sub> hybrids were greater than those in backcross hybrids and Fremont (Fig. 2e).

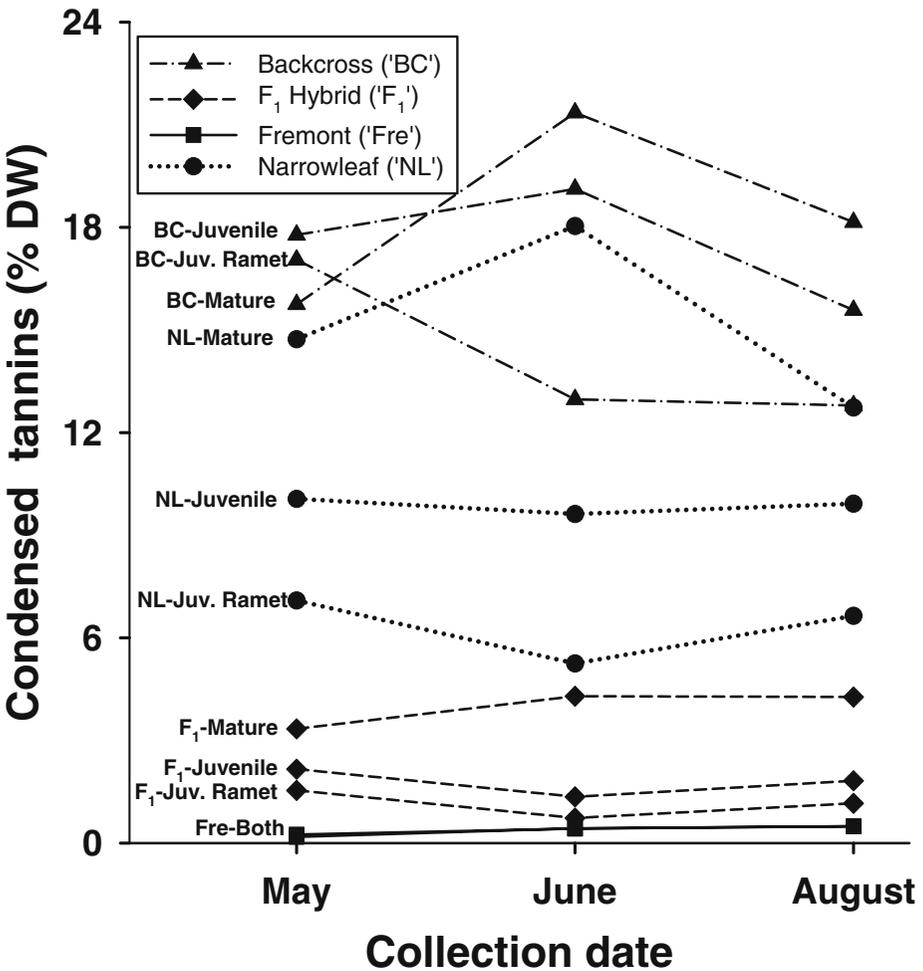
Within all cross types except Fremont, salicortin concentrations differed among developmental zones, and the patterns were generally the opposite of those for condensed tannins (Fig. 2f). For both narrowleaf and backcross hybrids, the ranks of salicortin levels among developmental zones were the same (juvenile ramets > juvenile > mature). These results correspond well with our predictions of juvenile zone intermediacy and higher

**Table 2** Type III tests of effects for the repeated measures anova of concentrations of nitrogen, condensed tannins, salicortin, HCH-salicortin, and total phenolic glycosides in the field survey

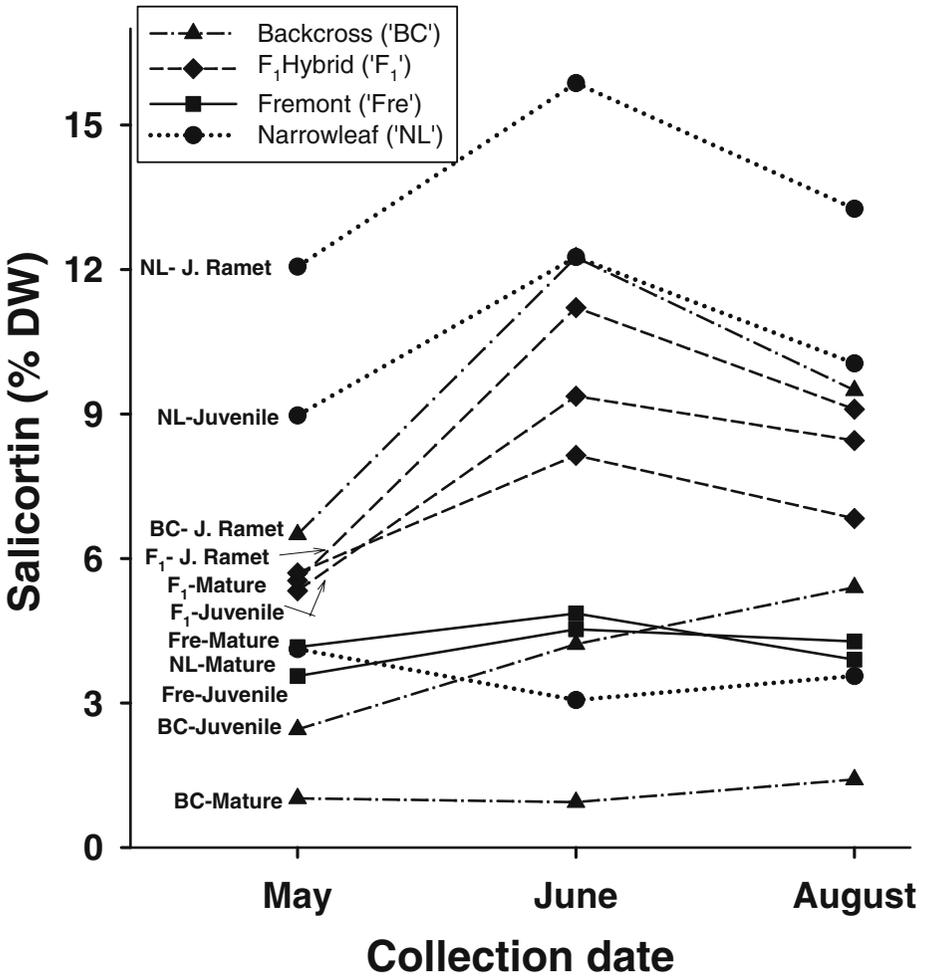
Effect	Nitrogen		Condensed tannins		Salicortin		HCH-salicortin		Total phenolic glycosides	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Collection date	77.03 <sub>2,48</sub>	<0.001	2.33 <sub>2,47</sub>	0.109	21.21 <sub>2,48</sub>	<0.001	5.74 <sub>2,38</sub>	0.007	15.39 <sub>2,48</sub>	<0.001
Cross type	1.36 <sub>3,48</sub>	0.266	83.31 <sub>3,47</sub>	<0.001	11.08 <sub>3,48</sub>	<0.001	3.71 <sub>1,24</sub>	0.066	13.41 <sub>3,48</sub>	<0.001
Date $\times$ cross type	3.08 <sub>6,48</sub>	0.013	1.46 <sub>6,47</sub>	0.212	2.47 <sub>6,48</sub>	0.037	4.25 <sub>2,38</sub>	0.022	4.33 <sub>6,48</sub>	0.001
Developmental zone (cross type)	0.97 <sub>7,48</sub>	0.464	14.09 <sub>7,47</sub>	<0.001	24.28 <sub>7,48</sub>	<0.001	6.63 <sub>3,37</sub>	0.001	20.34 <sub>7,48</sub>	<0.001
Date $\times$ developmental zone (cross type)	2.81 <sub>14,48</sub>	0.004	7.46 <sub>14,47</sub>	<0.001	4.22 <sub>14,48</sub>	<0.001	1.29 <sub>6,49</sub>	0.278	2.85 <sub>14,48</sub>	0.004



◀**Fig. 2** Nitrogen, condensed tannin, salicortin, HCH-salicortin, and total phenolic glycoside concentrations of four cross types in the field survey. HCH-salicortin was not measured in narrowleaf and backcross foliage. The left set of panels (a, c, e, g, i) highlight differences among cross types among the collection dates for all developmental zones combined. The right set of panels (b, d, f, h, j) highlight differences among developmental zones within cross types for all collection dates combined. Cross types are abbreviated as follows: NL, narrowleaf; BC, backcross; F<sub>1</sub>, F<sub>1</sub> hybrid; and Fre, Fremont. Developmental zones are abbreviated as follows: Mat, mature zone; Juv, juvenile zone; and JR, juvenile ramet. Each bar represents the least squares mean plus one standard error of the mean. Bars with different letters represent least squares means that are significantly different at  $\alpha = 0.05$  using a Tukey adjustment *within the same panel only*



**Fig. 3** Least squares means of condensed tannin concentrations for all developmental zones within cross types at the three collection dates. Cross types are abbreviated as follows: NL, narrowleaf; BC, backcross; F<sub>1</sub>, F<sub>1</sub> hybrid; and Fre, Fremont. Data for both juvenile and mature zones of Fremont cottonwood are nearly identical, hence the designation “Fre-Both” for the superimposed lines. Differences among developmental zones for narrowleaf and both hybrid cross types account for much of the variation in the system



**Fig. 4** Least squares means of salicortin concentrations for all developmental zones within cross types at the three collection dates. Cross types are abbreviated as in Fig. 3. As with condensed tannins, differences among developmental zones for narrowleaf and both hybrid cross types account for much of the variation in the system

phenolic glycoside concentrations in juvenile ramets. A similar but subtler pattern appeared in F<sub>1</sub> hybrids (juvenile ramets = juvenile, juvenile = mature, but juvenile ramets > mature). As with tannins, developmentally based differences varied among collection dates (see Fig. 4).

For salicortin concentrations, intraplant variation eclipsed interplant variation (Fig. 4). Whereas the greatest interplant variation encompassed a two-fold range (Fremont vs. narrowleaf, 4.2% vs. 9.3% DW, respectively), within narrowleaf salicortin concentrations varied over a four-fold range (3.6% vs. 13.7% DW, mature vs. juvenile ramet, respectively) and in backcross over an eight-fold range (1.1% vs. 9.4%, mature vs. juvenile ramet, respectively).

HCH-salicortin showed significant seasonal variation, which confounded differences in concentration among cross types. HCH-salicortin levels were higher in F<sub>1</sub> hybrids than Fremont in June only (Fig. 2g). Concentrations were the same between juvenile and mature foliage of Fremont, but highest in the foliage of juvenile zone and juvenile ramets of F<sub>1</sub>

hybrids, both of which differed from  $F_1$  mature foliage (Fig. 2h). Whereas HCH-salicortin concentrations among plants varied over a 1.5-fold range, and the difference was not statistically significant ( $F_1$  vs. Fremont, 4.5 vs. 3.1%, respectively), within  $F_1$  hybrids concentrations ranged over a 2.5-fold range, and the difference was statistically significant (6.2% vs. 2.5%, juvenile ramet vs. mature zone, respectively). Overall, the patterns in HCH-salicortin concentrations differed from those of condensed tannins and salicortin.

Composite concentrations of phenolic glycosides were lowest in May, peaked in June, and decreased to an intermediate level in August (Table 2; Fig. 2i), and differed significantly among cross types (Fig. 2j). Salicortin was the dominant or only phenolic glycoside contained in most foliage (ca. 66% in Fremont, 60% in  $F_1$  hybrids, and 100% in narrowleaf and backcross hybrids). Thus, the patterns of differences among developmental zones within cross types were similar to those of salicortin, as were the shifts in the patterns among months. Narrowleaf and  $F_1$  juvenile ramets had the greatest concentrations, whereas backcross and narrowleaf mature foliage both had the lowest. Also, as with salicortin and HCH-salicortin, intraplant variation driven by developmental trajectories exceeded interplant variation (contrast Fig. 2i with Fig. 2j).

#### Field Survey: Developmental Trajectories

Consistent with our prediction, developmental trajectories differed among cross types for condensed tannins and phenolic glycosides. Narrowleaf showed the greatest change in condensed tannin concentrations between juvenile and mature zones,  $F_1$  was intermediate, and backcross and Fremont showed no change (respectively,  $N = 63, 65, 74,$  and  $59$ ;  $P < 0.05$  for all pairwise differences between slopes). A slightly different pattern was found for salicortin: narrowleaf showed the greatest change, backcross was intermediate, and  $F_1$  and Fremont showed no change. Changes in total phenolic glycoside concentrations between juvenile and mature zones were greatest in narrowleaf, intermediate in hybrids, and least in Fremont. Overall, as predicted, the phytochemical trajectories of both the  $F_1$  and backcross hybrids were intermediate to those of narrowleaf and Fremont cottonwood, supporting our hypothesis of an ontogenetic basis for these patterns.

## Discussion

### Genetic and Ontogenetic Control of Phytochemistry

The data from the common garden survey argue that both condensed tannins and salicortin (and so total phenolic glycosides) are under genetic control. Because HCH-salicortin is present only in the foliage of Fremont and  $F_1$  hybrids (Rehill et al., 2005), but not narrowleaf or backcross hybrids, it, too, is under genetic control, at least at the level of presence/absence (likelihood ratio test:  $G = 71.1, P < 0.001$ ). Differences among cross types in the common garden experiment (where only juvenile zone foliage was sampled) were identical (HCH-salicortin and total phenolic glycosides) or similar (condensed tannins and salicortin) to those of the juvenile zone in the field survey. It seems unlikely that field conditions, along an altitudinal gradient of the Weber River, duplicate the conditions of the common garden. The resemblance of common garden chemistry to the juvenile zone chemistry in the field would be consistent with genetic control of these secondary plant metabolites. Thus, in aggregate, the data indicate that the major defensive chemicals in this hybrid system—condensed tannins and phenolic glycosides—vary predictably among cross

types, establishing a genetic component to the phytochemical variation. The phenotypic variation in phytochemistry found among cross types in the field survey is likely to be genetically based.

The data do not constitute direct experimental evidence for genetic control of phytochemistry in the mature zone of the canopy, because the common garden study was carried out only with juvenile zone foliage. However, any uncertainty that this might introduce to our conclusions should be considered in the following light. First, if chemistry in the mature zone is under less genetic control than chemistry in the juvenile zone for all cross types, we would have expected a less consistent pattern of changes between developmental zones within cross types, given environmental heterogeneity in the field survey. Second, most plant secondary metabolites appear to be under at least some level of genetic control (Hamilton et al., 2001). Last, even if chemistry in the mature zone is more phenotypically plastic than that in the juvenile zone, and the observed differences between juvenile and mature zones occur as a result of microenvironmental variation, the increase in phenotypic plasticity would still constitute an ontogenetic shift in the control of chemistry, because phenotypic plasticity itself can be considered a trait under genetic control (Callahan et al., 1997).

In the field survey, although the specific patterns differed among phytochemicals, the overall pattern is of predictable changes both within an individual tree and among different cross types. In total, these changes constitute evidence for ontogenetically based developmental trajectories of phytochemistry in this *Populus* system. Several lines of evidence support this conclusion. First, plant development affected phytochemistry as much or more than other variables normally emphasized in phytochemical studies, such as seasonal variation. Second, although some or all of the cross types co-occurred at most of the field sites, different cross types exhibited major differences in both their phytochemistry and the developmental trajectories of specific phytochemicals. These findings argue for a genetic basis to both phytochemistry and developmental trajectory. Third, the magnitude of change both within an individual tree and among cross types was great, e.g., up to 40-fold differences in concentration. Fourth, condensed tannins, salicortin, and HCH-salicortin show drastically different patterns of change among cross types, indicating that the changes do not occur purely because of resource availability for secondary plant metabolites. Finally, in both studies, nitrogen concentrations showed little variation, whereas the other phytochemicals varied greatly. This argues against any changes in condensed tannins or phenolic glycosides as a result of differences in available nitrogen, leading to potential protein competition with phenolic metabolites (Jones and Hartley, 1999). Because these phytochemicals are ecologically important, the developmental trajectories have the potential to affect populations, communities, and ecosystems.

### Plant Developmental Trajectory

We refer to predictable, developmentally based changes in plant traits (such as the phytochemistry we measured) as a plant's "developmental trajectory." For the sake of analysis, we concentrated on differences between the lower (juvenile) vs. upper (mature) portions of the canopy, and used the slope of the difference between these zones of the tree as a metric. The use of such a linear measure does not imply that all developmentally based changes occur in a gradual, linear fashion, or that all the traits of a given plant species change in the same manner or extent with development. Rather, this slope is intended only as a readily comprehensible means of referring to the change between developmental zones of the same cross type (if they occur), and of comparing the extent of change among cross

types. Further, we acknowledge that many developmentally based changes occur outside the scope of our measure (between juvenile and mature zones of a canopy sized tree). However, we concentrated on these developmental zones as a result of the potential major community and ecosystem consequences (most of a tree's biomass is in these zones) and for uniformity of comparison, because Fremont cottonwoods have no juvenile ramets.

We found striking differences in trajectories among cross types. Fremont cottonwood exhibits no significant trajectory, whereas narrowleaf cottonwood exhibits the steepest trajectory, and the hybrids are generally intermediate. The intermediate hybrid trajectories suggest an additive genetic mechanism to the phytochemical variation and argue that it has a genetic (i.e., ontogenetic) rather than environmental basis.

### Developmental Trajectories and Community Structure

The findings suggest a potential phytochemical basis for the distribution of two herbivores in this system with known community effects, *Pemphigus betae*, a galling aphid, and *C. confluens*, a leaf-feeding beetle. For example, for *C. confluens*, which prefers to feed on juvenile ramets (Kearsley and Whitham, 1989), tannin levels explain ca. 50% of the variation in larval weight gain (Rehill et al., unpublished data), consistent with our finding of lower tannin concentrations in juvenile ramets. Concurrently, the foliage of juvenile ramets is also highest in phenolic glycosides, which larvae sequester and convert to their own defenses (Martinsen et al., 1998).

Developmental variation in phytochemistry may be one mechanism that could cause pronounced differences in arthropod communities associated with different developmental zones of the same tree, and this leads to two predictions: First, the greater the chemical differences among developmental zones, the greater the difference in the respective communities among those zones; Second, because phytochemistry differs among developmental zones, arthropod diversity in a stand of trees is a combination of the interplant genetic diversity (Wimp et al., 2004) as well as the intraplant ontogenetic diversity (see also Kearsley and Whitham, 1989, 1998; Waltz and Whitham, 1997; Lawrence et al., 2003). At present, we know of no studies on the relationship between developmental trajectories and herbivore communities. However, for both *Quercus velutina* and *Q. alba*, condensed tannin concentrations are higher in leaves of the upper canopy vs. those of the understory, and they explain ca. 25% of the variation in leaf-chewing insect community composition (Forkner et al., 2004).

### Developmental Impacts on Ecosystem Processes

The marked increase in condensed tannin concentrations in mature foliage of narrowleaf and backcross trees, as well as the modest increase in F<sub>1</sub> hybrids, may have ecosystem consequences. Condensed tannins influence decomposition in this (Driebe and Whitham, 2000) and many other systems (Hattenschwiler and Vitousek, 2000; Hattenschwiler et al., 2003; Schweitzer et al., 2004). Increased tannin levels in the mature zone, which usually represents the majority of foliar mass in mature trees, may represent a means of controlling nitrogen cycling in the immediate environment of the tree (Northup et al., 1998; Van Breemen and Finzi, 1998; Kraus et al., 2003). Such control may be particularly advantageous to clonally propagating cross types, such as narrowleaf and backcrosses, as well as other clonal tree species (i.e., those that reproduce vegetatively), such as aspen (*Populus tremuloides*). Because leaves from ramets within a clone are more likely to fall and decompose under another member of the same clone (genotype) than nonclonal species

(i.e., an area effect), we predict that clonal species will undergo more pronounced developmental shifts in phytochemistry than will nonclonal species. Aspen has been found to have similar developmentally based chemical shifts to narrowleaf (Donaldson et al., 2006).

### Evolutionary Pressures Leading to Genetic and Ontogenetic Differentiation

The reciprocal trajectories of phenolic glycosides (decreasing with maturity) and condensed tannins (increasing with maturity) in narrowleaf, backcross, and  $F_1$  hybrids could represent a selective regime shaped by competing pressures (similar to aspen, Donaldson et al., 2006). Intense herbivory in the early life and lower portion of a tree's canopy may be balanced by the need to control nutrient cycling. This ensures adequate resources once sexual reproduction has begun. Such a pattern of competing selection pressures would be consistent with the shift from higher to lower phenolic glycosides from juvenile to mature foliage (better defended leaves in the lower canopy against herbivory) and the opposite trend for condensed tannins (thus, altering rates of decomposition and nitrogen mineralization).

The difference in the developmental trajectories of two congeneric trees (narrowleaf and Fremont cottonwoods) that have been in close proximity and/or had overlapping distributions for at least 12 million years (Eckenwalder, 1984) emphasizes that patterns of ontogenetic shifts in plant chemistry may be widespread. Phylogenetic constraints, however, seem unlikely to explain the flat trajectory of Fremont cottonwood. Selection pressures differ among environments for multiple reasons, such as the extent of herbivory, the costs of producing different defenses, and the availability of required resources such as minerals. Therefore, it seems likely that different selective regimes have led to different developmental trajectories. Developmental trajectories can be under genetic control (Wiltshire et al., 1998; Jordan et al., 1999), and variation in developmental trajectories within species may be an adaptation to environmental heterogeneity (Jordan et al., 2000).

The findings prompt a methodological caveat. Some studies of heritability compare traits of seedlings with those of mature trees. However, we have demonstrated major differences in ecologically important traits among the developmental zones of mature trees. Based on these data, if developmental trajectories of phytochemistry apply throughout the life of a tree, then the foliar chemistry of seedlings, and, therefore, resistance to natural enemies, may differ substantially from the mature zone of large trees. This may give an inaccurate representation of the genetic component of the studied traits, as others have suggested (Cornelissen et al., 2003).

Finally, given the established role of plant quality along with the potential for within-plant variation to affect herbivores (Stockhoff, 1993; Suomela and Ayres, 1994; Shelton, 2000), it is clear that developmental trajectories of phytochemistry could be important in regulating herbivore populations, structuring communities, and influencing ecosystems. More mechanistic studies are required to evaluate the community- and ecosystem-level importance of ontogenetically based chemical variation by linking the performance and distribution of keystone herbivores to plant chemistry. Such studies are a focus of continuing research with this hybrid system.

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