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GENOTYPIC VARIATION FOR CONDENSED TANNIN PRODUCTION IN TREMBLING ASPEN (*POPULUS TREMULOIDES*, SALICACEAE) UNDER ELEVATED CO_2 AND IN HIGH- AND LOW-FERTILITY SOIL[1]

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The carbon/nutrient balance hypothesis suggests that leaf carbon to nitrogen ratios influence the synthesis of secondary compounds such as condensed tannins. We studied the effects of rising atmospheric carbon dioxide on carbon to nitrogen ratios and tannin production. Six genotypes of *Populus tremuloides* were grown under elevated and ambient CO_2 partial pressure and high- and low-fertility soil in field open-top chambers in northern lower Michigan, USA. During the second year of exposure, leaves were harvested three times (June, August, and September) and analyzed for condensed tannin concentration. The carbon/nutrient balance hypothesis was supported overall, with significantly greater leaf tannin concentration at high CO_2 and low soil fertility compared to ambient CO_2 and high soil fertility. However, some genotypes increased tannin concentration at elevated compared to ambient CO_2, while others showed no CO_2 response. Performance of lepidopteran leaf miner (*Phyllonorycter tremuloidiella*) larvae feeding on these plants varied across genotypes, CO_2, and fertility treatments. These results suggest that with rising atmospheric CO_2, plant secondary compound production may vary within species. This could have consequences for plant–herbivore and plant–microbe interactions and for the evolutionary response of this species to global climate change.

Key words: carbon dioxide; condensed tannins; global change; plant–herbivore interaction; *Populus tremuloides*; Salicaceae.

Atmospheric CO_2 is predicted to double within 100 yr (Houghton, Jenkins, and Ephraums, 1995), yet the consequences of this perturbation for plant secondary compound synthesis and plant-herbivore interactions are not fully understood (Ayres, 1993; Lindroth, 1996). The carbon/nutrient balance hypothesis (CNBH; Bryant, Chapin, and Klein, 1983; Waterman, Ross, and McKey, 1984) predicts that as green leaf carbon to nitrogen ratio (C/N) increases, as is often observed at high CO_2 (Ceulemans and Mousseau, 1994), production of carbon-rich secondary compounds such as tannins will also increase. While studies involving leaf C/N manipulation through alteration of light and/or nutrient availability have generally supported the CNBH (e.g., Larsson et al., 1986; Bryant et al., 1987; Nichols-Orians, 1991; Dudt and Shure, 1994,) results using elevated CO_2 have been more variable. For example, Lincoln and Couvet (1989) found that monoterpenes did not increase in *Mentha piperita* L. cv. Mitcham grown under elevated CO_2. In contrast, Fajer, Bowers, and Bazzaz (1992) found that *Plantago lanceolata* L. grown at 70 Pa CO_2 did not have increased carbon-based secondary compounds compared to ambient-CO_2-grown plants, and levels actually declined in some cases.

Fewer studies have examined the phytochemical response of woody plants to elevated CO_2, although results more consistently support the CNBH. In *Populus tremuloides* Michx., *Acer saccharum* Marsh. (Lindroth, Kinney, and Platz, 1993), *Betula papyrifera* Marsh. (Lindroth, Arteel, and Kinney, 1995), and *Betula pendula* Roth. (Lavola and Julkunen-Tiitto, 1994) condensed tannin concentration increased in elevated- compared to ambient-CO_2-grown plants. For example, Lindroth, Arteel, and Kinney (1995) found that tannin concentration in *B. papyrifera* doubled under twice ambient CO_2 conditions, and elevated CO_2 treatment in low-fertility soil resulted in a 60% increase in tannin concentration of *B. pendula* leaves (Lavola and Julkunen-Tiitto, 1994). Other secondary compounds showed more variable responses as the phenolic glycoside salicortin increased 55% in *P. tremuloides*, whereas ellagitannin decreased in *Quercus rubra* L. and increased in *A. saccharum* grown under elevated CO_2 (Lindroth, Kinney, and Platz, 1993). In *B. pendula*, phenolic glycoside production was variable across CO_2 treatments (Lavola and Julkunen-Tiitto, 1994).

Many plant species exhibit significant intraspecific var-

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iation in herbivore resistance, some of which is related to differences in production of defensive compounds (Lege, Smith, and Cothren, 1992; Hemming and Lindroth, 1995; Hwang and Lindroth, 1997). However, little is known about the magnitude of genetic variation within plant populations for secondary compound production under elevated CO_2. Differential genotypic responses in the synthesis of defensive compounds could have evolutionary consequences; for instance, genotypes that allocate more carbon to defense compounds may experience a selective advantage in a CO_2-enriched atmosphere (Geber and Dawson, 1993). In the only study to date examining this issue, Fajer, Bowers, and Bazzaz, (1992) found no significant differential responses of secondary compound production under elevated CO_2 among six clones of *Plantago lanceolata*. In light of documented genetic variation in other responses to elevated CO_2 (e.g., reproduction and stomatal index in *Raphanus raphanistrum* L.; Curtis, Snow, and Miller, 1994; Case, Curtis, and Snow, 1998) further study of this question is clearly warranted.

Here, we present data on genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*) grown under elevated and ambient CO_2 and two soil nutrient levels throughout one growing season. We hypothesized that, in accordance with the CNBH, plants grown under elevated CO_2 and low nutrients would have the highest levels of condensed tannin, while plants grown under ambient CO_2 and high nutrients would have the lowest levels. In addition, given high levels of clonal variation in morphology (Barnes, 1959), vegetative growth (Sakai and Burris, 1985) and secondary chemistry (Lindroth and Hwang, 1996a) within the source population and the sensitivity of tannin production by *P. tremuloides* to environmental conditions (Lindroth and Hwang, 1996b), we expected that genotypes would respond differently to elevated CO_2, with some genotypes increasing tannin production and others decreasing production or showing no change. Finally, we predicted that naturally occurring levels of lepidopteran herbivore damage would reflect treatment and genotype effects on tannin production.

MATERIALS AND METHODS

This experiment was part of a larger study of *Populus tremuloides* (Salicaceae) responses to elevated CO_2 conducted at the University of Michigan Biological Station (UMBS) in northern lower Michigan (Curtis et al., 1996). During the fall of 1993, roots from six different male genotypes (clones) were collected from Pellston Plain, Michigan, within 5 km of the elevated CO_2 study site. The rooted cuttings propagated from these samples were planted in 3 m diameter $\times$ 2.4 m tall open-top CO_2 chambers (Heagle et al., 1989) in the spring of 1994. Each chamber contained two individuals of each aspen genotype.

The chambers were positioned over open-bottom root boxes that had been filled with either low- or high-fertility soil. High-fertility soil was 100% locally derived Kalkaska series topsoil, while low-fertility soil was a mixture of 20% Kalkaska topsoil and 80% of the C horizon of a Rubicon sand (the dominant material underlying the study site). In a previous experiment using the same soil mixtures, nitrogen mineralization was significantly higher in the high-fertility treatment (348 $\mu g\ N \cdot g^{-1} \cdot d^{-1}$) than in the low-fertility treatment (45 $\mu g\ N \cdot g^{-1} \cdot d^{-1}$) (Randlett et al., 1996). Half of the chambers were maintained at ambient CO_2 (seasonal 24-h average = 35.6 Pa), and half were maintained at elevated CO_2 (seasonal 24-h average = 70.7 Pa). The experiment was arranged as a two-way randomized complete-block split-plot design with CO_2 and soil fertility crossed within each of five replicate blocks (20 chambers total). Genotype was considered a subplot within each treatment combination (the main plot).

Leaf samples were collected on three dates in 1995: 22 June, 6 August, and 13 September. In each chamber, the youngest fully expanded leaf from one individual of each genotype was clipped at the petiole, flash frozen in liquid nitrogen and stored on dry ice until it was placed in a −82°C freezer. On the last sampling date, leaves were not flash frozen, but rather were placed immediately on dry ice. After freezing, entire leaf samples minus the petioles were lyophilized and pulverized to a fine powder. Leaves also were sampled on 8 August from mature individuals of the same genotypes growing in Pellston Plain. On 29 June, leaves were sampled as above and C/N determined by CHN Analysis (Perkin-Elmer Model 2400, Perkin-Elmer Corp., Norwalk, Connecticut).

Condensed tannins were assayed by radial diffusion (Hagerman, 1987) calibrated against purified *P. tremuloides* tannin (Hagerman and Butler, 1980; R. Lindroth, personal communication, University of Wisconsin). Approximately 100 mg of leaf tissue were extracted with 70% acetone and the supernatant applied to wells within agar plates containing bovine serum albumin. The binding of protein with tannin resulted in precipitation rings whose diameter was proportional to tannin levels in the sample. In our assays, ring diameter ranged from 0.57 to 1.29 cm. Reference standards were established for each batch of agar prepared, and r^2 values for the calibration curves were always >0.98.

In 1995, *P. tremuloides* throughout the UMBS area, including chamber-grown plants, were attacked by the lepidopteran leaf miner *Phyllonorycter tremuloidiella* Braun (aspen blotch miner). This species excavates a circular section of leaf mesophyll, allowing us to measure CO_2 and soil N effects on herbivore leaf consumption and larval performance. For three of the aspen genotypes, single leaves on which the miner had reached pupal stage were collected at random from separate chambers and the leaf dry mass consumed (DW_c) was calculated by

$$DW_c = A_m(LMA_u - LMA_m)$$

where A_m was the total area of mesophyll consumed, LMA_m was the leaf mass per area of the mined tissue, and LMA_u was the leaf mass per area of an adjacent unmined section of the same leaf. Pupae were dissected from the leaf, dried, and weighed.

Overall treatment effects were analyzed using repeated-measures analysis of variance for split-plot design (Gumpertz and Browne, 1993). Within a sampling date, elevated vs. ambient response means within a fertility level were compared by least significant difference (a priori comparisons), while comparisons across fertility levels, among clones, and to mature plants were by the minimum significant range (a posteriori comparisons; Sokal and Rohlf, 1981).

RESULTS

Leaf tannin concentration was affected by both CO_2 and fertility (the main plot effects) and by genotype (the subplot effect; Table 1). At each sampling date, tannin concentration was highest in the elevated-CO_2, low-fertility treatment and lowest in the ambient-CO_2, high-fertility treatment (Table 2). This pattern of response paralleled leaf C/N (Table 3) and was consistent with predictions of the CNBH. Within a soil fertility treatment, tannin concentration was significantly higher in elevated compared to ambient-CO_2-grown plants, increasing an average of 76% across dates at high soil fertility and 22% at low soil fertility (Table 2). Within a CO_2 treatment, tannin concentration was also significantly higher in low- compared to high-fertility soil. Leaf C/N showed similar

TABLE 1. Results of repeated-measures ANOVA for tannin production in *Populus tremuloides*. Significant CO_2, fertility, and genotype effects are indicated on the between-subjects portion of the table, while significant effects through time are shown on the within-subjects portion of the table.

Source	df	MS	*F*	*P* <
A) Between Subjects				
CO_2	1	137 900	45.9	0.001
Fertility	1	135 110	45.0	0.001
CO_2 × Fertility	1	5211	1.7	0.213
Main-plot error	12	3004		
Genotype	5	47 183	22.6	0.001
CO_2 × Genotype	5	5655	2.7	0.026
Fertility × Genotype	5	2453	1.2	0.359
CO_2 × Fertility × Genotype	5	3869	1.9	0.111
Subplot error	80	2084		
B) Within Subjects				
Time × CO_2	2	1193	0.6	0.569
Time × Fertility	2	9151	4.4	0.023
Time × CO_2 × Fertility	2	1083	0.5	0.599
Main-plot error	24	2062		
Time	2	60 144	45.7	0.001
Time × Genotype	10	4302	3.3	0.001
Time × CO_2 × Genotype	10	1020	0.8	0.653
Time × Fertility × Genotype	10	2505	1.9	0.049
Time × CO_2 × Fertility × Genotype	10	1299	1.0	0.457
Subplot error	160	1316		

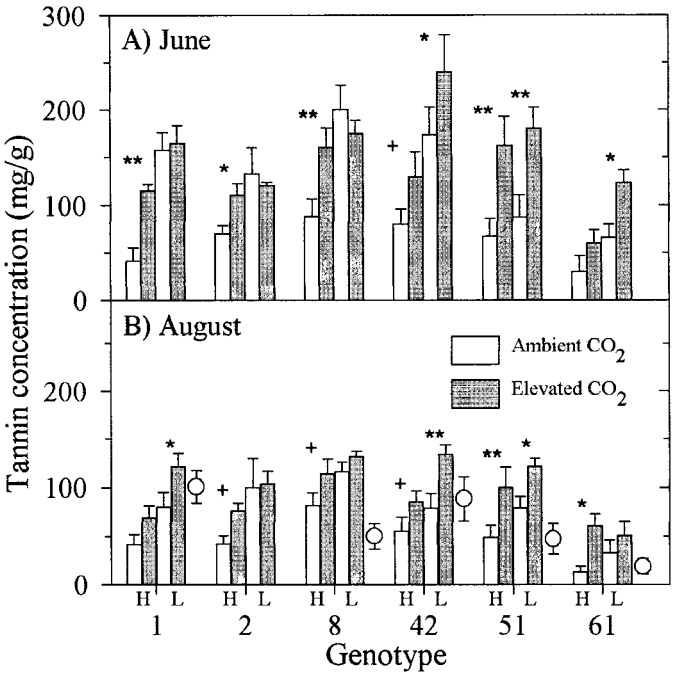

Fig. 1. Leaf tannin concentration in six genotypes of *Populus tremuloides* sampled from 2-yr-old saplings growing in field open-top chambers (bars) or from mature clones growing in Pellston Plain (open circles). Chamber-grown individuals were growing in soils of low (L) or high (H) N fertility and under ambient (open bars) or elevated (shaded bars) CO_2 levels. For clarity only June and August data are shown. Vertical bars indicate 1 SE. Significance tests were within a fertility level, where $^+ P < 0.08$, * $P < 0.05$, ** $P < 0.01$.

variation, increasing 35% due to CO_2 (across fertility treatments) and 29% due to soil fertility (across CO_2 treatments) (Table 3).

There was significant variation in leaf tannin concentration within and among aspen genotypes as a function of time, CO_2, and fertility (Table 1, Fig. 1). For clarity, only June and August data are shown in Fig. 1. September data parallel those of August. Overall, genotype 61 produced the least tannin (54.3 mg/g averaged across treatments and sampling dates), whereas genotype 8 produced the most (138.8 mg/g averaged across treatments and sampling dates). Genotypes generally responded to treatments in a manner consistent with the CNBH, with tannin concentration increasing under elevated CO_2 and low soil fertility compared to ambient CO_2 and high soil fertility, but there were notable exceptions. For example, genotypes 42, 51, and 61 showed strong responses to CO_2 under both low and high soil fertility, whereas CO_2 responses were restricted to high soil fertility in genotypes 2 and 8. This variation led to a significant CO_2 × genotype interaction for tannin concentration (Table 1). In contrast, there was no fertility × genotype interaction, indicating a consistent increase in tannins at low soil fertility across the six genotypes. There were also no significant changes in the rank order of genotypes with respect to leaf tannin concentration.

Leaf tannin concentration also varied seasonally; August levels were consistently lower than those measured in June or September (Table 2). This variation was genotype specific, however, with some genotypes showing little decline in August (e.g., genotype 2) and others showing as much as a 43% decline in this period across all treatments (e.g., genotype 42) (Fig. 1). The temporal change also was strongest at low soil fertility and was reflected in significant time × genotype and time × genotype × fertility interactions (Table 1).

There was no significant difference in leaf tannin concentration between native plants growing on the Pellston Plain (66.1 ± 14.8 mg/g) and those in our experiment grown under ambient CO_2 and low soil fertility (77.4 ± 13.2 mg/g, *t* test). Only genotype 8 differed significantly in leaf tannin between locations (Fig. 1).

TABLE 2. Effects of CO_2 and soil fertility on *Populus tremuloides* leaf tannin concentration in June, August, and September. Plants were grown for 2 yr at ambient or elevated atmospheric CO_2 and in soils of either high- or low-N availability. Mean (± 1 SE), $N = 5$.

	Condensed tannin concentration (mg/g)			
	High fertility		Low fertility	
Month	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
June	62.8 (6.22)$^{a+}$	123.1 (14.80)^{b}	136.2 (10.13)^{b}	167.4 (11.16)^{c}
August	47.0 (3.23)^{a}	84.0 (5.88)^{b}	81.1 (7.77)^{b}	90.4 (19.94)^{b}
September	80.5 (13.32)^{a}	123.4 (23.36)^{b}	112.0 (13.36)^{b}	146.1 (15.22)$^{c\tau}$

$^+$ Similar superscripts within a month indicate no significant differences at $P < 0.05$, or τ $P < 0.10$.

TABLE 3. Effects of CO_2 and soil fertility on *Populus tremuloides* leaf C and N content. Plants were grown for 2 yr at either ambient or elevated CO_2 and in soils of high- or low-N availability. Mean (± 1 SE), $N = 5$ for each treatment.

Fertility	CO_2	%C	%N	C:N ratio
High	Ambient	56.7 (0.20)^{ab}	2.9 (0.07)^{a}	20.0 (0.52)$^{a+}$
	Elevated	56.5 (0.22)^{ab}	2.2 (0.07)^{b}	26.4 (0.73)^{b}
Low	Ambient	56.2 (0.30)^{a}	2.3 (0.03)^{b}	25.3 (0.23)^{b}
	Elevated	56.9 (0.36)^{a}	1.7 (0.08)^{c}	34.5 (1.67)^{c}

[+] Different superscripts denote significant differences between treatments at $P < 0.05$.

Pupal dry mass of *P. tremuloidiella* that fed on chamber-grown plants was marginally greater in elevated compared to ambient CO_2 treatments (+8%, $P < 0.09$, Table 4), and both the amount of tissue consumed ($P < 0.01$) and pupal dry mass ($P < 0.07$) varied among the three genotypes examined. There were no effects of soil fertility on pupal mass, although larvae consumed 69% more tissue from low-fertility compared to high-fertility treatments ($P < 0.05$). Carbon dioxide treatment had no effect on the amount of tissue consumed.

TABLE 4. Leaf dry mass consumption (DW_c) and pupal dry mass (DW_p) of *Phyllonorycter tremuloidiella* feeding on *Populus tremuloides* genotypes grown at ambient or elevated CO_2 and in soils of high or low fertility. Mean (±1 SE).

Fertility	CO_2	Genotype	N	DW_c (mg)	DW_p (mg)
High	Ambient	1	5	0.87 (0.35)$^{a+}$	0.53 (0.03)^{ab}
		51	5	1.43 (0.28)^{ab}	0.50 (0.03)^{a}
		61	5	1.41 (0.22)^{ab}	0.55 (0.01)^{ab}
	Elevated	1	3	0.68 (0.28)^{a}	0.59 (0.01)^{ab}
		51	5	1.79 (0.13)^{b}	0.57 (0.03)^{ab}
		61	3	1.37 (0.11)^{ab}	0.59 (0.03)^{ab}
Low	Ambient	1	5	1.82 (0.10)^{b}	0.52 (0.02)^{ab}
		51	5	2.14 (0.15)^{b}	0.50 (0.02)^{a}
		61	5	1.97 (0.36)^{b}	0.58 (0.05)^{b}
	Elevated	1	1	2.33 —^{ab}	0.62 —^{ab}
		51	2	2.61 (1.18)^{b}	0.50 (0.01)^{ab}
		61	2	2.25 (0.38)^{b}	0.54 (0.03)^{ab}

[+] Different superscripts denote significant differences between clones at $P < 0.05$.

DISCUSSION

Our results suggest that rising atmospheric CO_2 will alter patterns of carbon allocation to leaf-condensed tannins in *P. tremuloides* in a manner consistent with the CNBH. These results are in agreement with recent work reporting increased carbon-based secondary compounds in woody species (Lindroth, Kinney, and Platz, 1993; Lavola and Julkunen-Tiitto, 1994; Lindroth, 1996), but contradict some earlier studies that showed no change in secondary compound production under elevated CO_2 (e.g., Lincoln and Couvet, 1989; Fajer, Bowers, and Bazzaz, 1992; Lincoln, Fajer, and Johnson, 1993). For example, Lincoln and Couvet (1989) found no increase in volatile monoterpenes in *Mentha piperita* cv. Mitcham grown under elevated CO_2. Likewise, the perennial herb *Plantago lanceolata* showed no significant increase in iridoid glycosides or phenylpropanoid glycosides (Fajer, Bowers, and Bazzaz, 1992). However, because tannins are immobile compounds that do not have a high turnover rate, their response to CO_2 may differ from mobile compounds such as monoterpenes. We also found that the effect of CO_2 on leaf tannins was independent of soil N fertility levels. Under conditions of low soil N mineralization, already high leaf C/N was further increased under elevated CO_2, leading to a concomitant increase in leaf tannin concentration. However, the relative response to CO_2 was greater under high soil fertility conditions, where tannin concentration almost doubled between ambient and elevated CO_2.

Consistent with the results of Hwang and Lindroth (1997), we found that leaf tannin concentration varied among aspen genotypes. However, it is particularly interesting that genotypes responded differently to increased CO_2. Some genotypes showed significant increases in tannin production, whereas others did not. Changes in tannin production may impact herbivore success, particularly when coupled with changes in plant tissue quality. Traw, Lindroth, and Bazzaz (1996) found that leaves with less foliar nitrogen and more condensed tannins had poorer gypsy moth (*Lymantria dispar* L.) larval performances. Similarly, Hwang and Lindroth (1997) reported that clonal variation in tannin production contributed to differential performance of insects feeding on those clones. Genotypes that show little or no increase in tannin production at high CO_2 may therefore be more susceptible to herbivore attack or microbial invasion, relative to genotypes showing a more marked increase in tannins. Thus, genotypes with increased tannins may have an advantage in future climates.

We also noted an overall decrease in tannin production in August compared to June and September. Several factors likely contributed to this temporal pattern. For example, the physiological age of the leaves sampled varied among dates. In September, new leaves had stopped forming and the youngest fully expanded leaves likely were more mature than corresponding leaves sampled in August. Condensed tannin concentration in *Quercus agrifolia* Nee., *Q. ilex* L., *Q. semecarpifolia* Sm., *Q. serrata* Thunb., and *Q. glauca* Thunb. increased gradually throughout the season as leaves matured (Kleiner, Montgomery, and Schultz, 1989; Mauffette and Oechel, 1989; Harinder, Dawra, and Singh, 1991), indicating that older leaves have more time to accumulate tannins and may thereby become better defended. An increase in self-shading with canopy development could have contributed to lower tannin concentration in newly expanded leaves in August compared to June, a temporal trend also observed by Auerbach and Alberts (1992). Light levels are positively correlated with foliar secondary compound conentrations in aspen (Lindroth and Hwang, 1996b).

Under elevated CO_2, leaf nitrogen content typically decreases and herbivores often respond with a compensatory increase in consumption (Lincoln and Couvet, 1989; Lindroth, Kinney, and Platz, 1993). Lincoln, Couvet, and Sionit (1986) found that larvae of the soybean looper (*Pseudoplusia includens* Walker) increased consumption of elevated-CO_2-grown *Glycine max* L., a response that was negatively correlated with leaf nitrogen concentration. Most work on the responses of herbivores to high-CO_2-grown plant tissue has been conducted with con-

trolled feeding studies under artificial growth conditions. We collected herbivory data from a naturally occurring population of *P. tremuloidiella* feeding on plants in the open-top chambers. Soil fertility clearly influenced consumption by this herbivore; 43% more dry mass was removed in low-fertility- compared to high-fertility-grown plants. However, soil fertility did not affect pupal dry mass, indicating that *P. tremuloidiella* successfully compensated for low nutritional quality through increased consumption. Elevated CO_2 effects on *P. tremuloidiella* preference and performance was less clear. There was a trend toward greater pupal dry mass following consumption of elevated-CO_2-grown tissue but no effect on the amount of tissue consumed. It is important to note that small sample sizes, particularly at high CO_2 and low soil fertility, limit our ability to draw firm conclusions about the magnitude of the CO_2 effect. Traw, Lindroth, and Bazzaz (1996) found gypsy moth pupal dry masses decreased when reared on elevated-CO_2-grown *Betula alleghaniensis* Britton leaves but did not change when reared on high-CO_2-grown *Betula populifolia* Marsh.

We found that plant genotype significantly affected both leaf dry mass consumed and leaf tissue chemistry. However, consistent with other studies, we did not find a significant correlation between leaf tannin levels and herbivore performance. Lindroth, Kinney, and Platz (1993) and Hemming and Lindroth (1995) found no relationship between condensed tannin levels and gypsy moth performance. These results suggest that variation in other defensive compounds such as phenolic glycosides or in morphological traits such as leaf toughness could have contributed to variation among clones in leaf miner preference. Auerbach and Alberts (1992) examined factors contributing to host perference in *P. tremuloideilla* and concluded that differences in phenology among *P. tremuloides, P. grandidentata*, and *P. balsamifera* could best account for observed differences in feeding on these three species. There was, however, little variation in tannin content among these potential hosts.

Open-top chamber-grown plants often differ in numerous respects from field-grown individuals and we therefore were interested in the extent to which leaf tannin levels in our experiment plants resembled those of their progenitor clones growing in a natural forest ecosystem. We found no significant difference in mean leaf tannin concentration in plants from low-fertility, ambient-CO_2 chambers compared to those growing in the low-nutrient environment of Pellston Plain. Of the five genotypes examined, only one differed in tannin level from its corresponding parent clone. In addition, the mean tannin concentration we measured in June in our low-fertility, ambient-CO_2 chamber-grown plants (136 mg/g) was within the range of tannin concentrations reported by Lindroth and Hwang (1996a) from 31 aspen clones sampled during June 1995 on the Pellston Plain (131–273 mg/g).

These results give us some confidence that our data may be useful in predicting future responses by this population to global atmospheric change. While the CNBH accurately predicted changes in leaf condensed tannin concentration, other factors such as genotype and time of year also influenced tannin production. Importantly, the differential response among genotypes to CO_2 enrichment suggests that patterns of plant secondary compound production may vary within species as atmospheric CO_2 rises, with possible consequences for plant–herbivore and plant–microbe interactions as well as the adaptive response of this species to global climate change.

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