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Biochemical Systematics and Ecology 31 (2003) 233–247

www.elsevier.com/locate/biochemsysseco

The effects of plant genetic variation and soil nutrients on secondary chemistry and growth in a shrubby willow, *Salix sericea*: patterns and constraints on the evolution of resistance traits

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Received 18 April 2001; accepted 30 November 2001

Abstract

Investigators often examine the factors—genetic or environmental—that determine the concentrations of secondary chemicals and growth, but few have examined both simultaneously. We used a factorial genetic design and manipulated nutrient availability to *Salix sericea* (Salicaceae) in order to quantify: (1) genetic variation, plasticity, and genetic variation in plasticity for growth rate and the concentrations of two phenolic glycosides (salicortin and 2'-cinnamoylsalicortin), and (2) tradeoffs between secondary chemistry and growth rate, and between the two phenolic glycosides. We found genetic variation and genetic variation in plasticity for both chemicals but not for growth rate. Nutrient fertilization enhanced growth and decreased salicortin concentration. More importantly, nutrient environment affected the expression of genetic variation. Heritability was significant only in the medium (both phenolic glycosides) and in the high (2'-cinnamoylsalicortin only) fertilizer treatments, and there was significant genetic variation in plasticity. There was no evidence suggesting that selection for increased chemical concentration would occur at the expense of growth rate. Finally, it appears that selection in favor of one chemical would result in positive correlational selection for the other. Overall, the evolution of these traits might be constrained by a lack of genetic variation in some environments, but not by negative genetic correlations between the different traits. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Plant genetic variation; Soil nutrients; Phenolic glycosides; Growth rate; Evolutionary constraints; Phenotypic plasticity

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1. Introduction

The secondary chemistry of plants often affects their resistance to herbivores (e.g., Rosenthal and Berenbaum, 1992). This has led numerous investigators to examine genetic and environmental factors that determine the concentrations of secondary chemicals. Many studies have documented significant additive genetic variation in secondary chemical concentrations (e.g., Lokki et al., 1973; Ma and Bliss, 1978; Østrem, 1987; Zangerl et al., 1989; Zangerl and Berenbaum, 1990; Han and Lincoln, 1994; Orians et al., 1996; reviewed by Berenbaum and Zangerl, 1992), which indicates that herbivores could select for genotypes with enhanced production of these compounds. Other studies have shown that abiotic and biotic environmental variation causes shifts in concentration of plant chemicals (e.g., Bryant et al., 1983; Gershenzon, 1984; Herms and Mattson, 1992; Karban and Baldwin, 1997). Traits that vary as a function of environmental conditions are phenotypically plastic. For example, an increase in soil nutrient availability generally enhances growth and reduces the concentration of carbon-based secondary chemicals, like phenolics (e.g., Larsson et al., 1986; Bryant et al., 1987; Hakulinen et al., 1995).

Thus, plants can adapt to their ever-changing environment by plasticity or by selection on existing genetic variability (Schlichting and Pigliucci, 1998). Moreover, selection on phenotypic plasticity can occur (Schlichting and Pigliucci, 1998). The potential for the evolution of plasticity can be tested in quantitative genetic studies by simultaneously manipulating genetic variation and the abiotic environment, and by examining the significance of genotype-by-environment interaction in the analysis (Via, 1984; Via and Lande, 1985; Scheiner, 1993; Schlichting and Pigliucci, 1998). The presence of significant genotype by environment interaction effects (hereafter termed G×E) suggests that genotypes are specialized—they exploit environments differentially, while the absence of G×E effects suggests that genotypes are generalized—equally responsive to changes in environmental conditions (Via, 1984).

Studies simultaneously examining the effects of genotype and environment on secondary chemistry are rare (Hakulinen et al., 1995; Osier and Lindroth, 2001), even though resistance to herbivores is often determined by G×E (e.g., Maddox and Cappucino, 1986; Orians and Fritz, 1996). Hakulinen et al. (1995) present evidence that G×E affects chemical variation in plants. They found that the concentration of phenolic glycosides varied among willow clones, with nutrient fertilization, and that some clones were more responsive to fertilization than others. However, because their study used clones, which estimates only total genetic variation, it is unclear how much additive genetic variation exists for exploiting different environments (*sensu* Via, 1984).

Even if there is potential for phenotypic evolution, ecological tradeoffs may constrain evolutionary responses. The production of many secondary chemicals is costly (Baldwin et al., 1990; Han and Lincoln, 1994) and as a consequence phenotypic tradeoffs between secondary chemical production and growth are common (Bryant et al., 1983; Baldwin et al., 1990; Hakulinen et al., 1995; and reviewed by Herms and Mattson, 1992). Genetic tradeoffs exist as well (Boecklen et al., 1990; Han and

Lincoln, 1994). For example, Han and Lincoln (1994) estimate that the phenotypic and genetic cost of each gram of resin produced by *Diplacus aurantiacus* (Scrophulariaceae) is 2 and 25 g of biomass (dry weight), respectively. When such genetic tradeoffs exist, selection for increased chemical production could be constrained if there is simultaneous selection for increased growth. Tradeoffs in the production of different secondary chemicals are also common (e.g., Berenbaum et al., 1986; Berenbaum and Zangerl, 1992). For example, Berenbaum et al. (1986) showed that selection in favor of one coumarin would cause a decline in a second. Therefore, if herbivores select for different chemicals, then the evolution of increased chemical investment may be constrained.

In this study we use *Salix sericea* to examine the potential for selection of resistance traits. Like many willows, this species produces high concentrations of phenolic glycosides, which are known to deter both insect and mammalian herbivores (e.g., Tahvanainen et al., 1985; Lindroth et al., 1988). The concentrations of phenolic glycosides are determined by plant genetics (Boecklen et al., 1990; Julkunen-Tiitto and Meier, 1992; Nichols-Orians et al., 1993; Lindroth and Hwang, 1996; Orians et al., 1996; Hwang and Lindroth, 1997), and are affected by resource availability (Larsson et al., 1986; Bryant et al., 1987; Price et al., 1989; Hakulinen et al., 1995; Kinney et al., 1997; Hakulinen, 1998). However, GxE effects on chemical expression have not been examined thoroughly.

We had several goals: (1) to determine if there is genetic variation in growth rates and the production of phenolic glycosides, (2) to determine if soil nutrient availability and its interaction with genetic variation reveals plasticity and genetic variation in plasticity, respectively, for growth rates and the concentrations of phenolic glycosides; (3) to measure ecological and evolutionary tradeoffs between growth and defense (=negative phenotypic and genetic correlations); and (4) to determine if production of one phenolic glycoside is positively or negatively correlated with production of a second phenolic glycoside. We hypothesize that there exists significant genetic variation and plasticity in the production of phenolic glycosides but that GxE effects and tradeoffs could constrain evolutionary responses. Our nutrient manipulations and factorial crossing design allowed us to determine the extent of genetic variation in different environments, to calculate heritability, and to determine both phenotypic and genotypic correlations.

2. Materials and methods

2.1. Study system

Salix sericea Marshall, silky willow, is an abundant, shrubby willow (reaching 3–4 m in height) in the northeastern United States and eastern Canada (Argus, 1986). At our field site near Milford, New York, *S. sericea* produces leaves continuously from mid-May to early September and plants drop their leaves by the end of September.

The major plant secondary chemicals in this species are the phenolic glycosides

salicortin and 2'-cinnamoylsalicortin (Nichols-Orians et al., 1992). The concentration of salicortin often reaches 10% dry leaf weight while that of 2'-cinnamoylsalicortin averages about 1.5% (Orians et al., 1996). Previous studies have found significant additive genetic variation in the production of both phenolic glycosides (Nichols-Orians et al., 1993; Orians et al., 1996). Although the effects of fertilization on the concentrations of phenolic glycosides is unknown for our study system, theoretical and empirical studies suggest that concentrations will decline with increasing soil nutrient availability (e.g., Bryant et al., 1983; Larsson et al., 1986; Price et al., 1989; Herms and Mattson, 1992).

2.2. *Experimental design*

The experimental design consisted of applying a fertilizer treatment (three levels: low, medium, and high) to 2-year old plants from a factorial half-sib genetic design and measuring plant growth and chemistry. We used 2-year old plants deliberately. *S. sericea* is shade intolerant (pers. obs.) yet lives in a habitat with many fast growing competitors. Therefore we expect that rapid vertical growth is essential to initial survival, and that tradeoffs between growth and chemistry would be present since seedlings already have concentrations of phenolic glycosides similar to adults within the first growing season (Fritz et al., 2001.)

The genetic design consisted of factorial crosses among three dams and 14 sires (all were randomly chosen). Crosses were made by transferring pollen from sires to female catkins that had been covered with mesh pollination bags to prevent visitation by insect pollinators. Pollination bags were replaced and left until seed maturation, at which time, seeds were collected. Seeds from each full-sib family were planted in trays of Pro Mix™ in early summer. After a few weeks of growth, seedlings were transplanted into cell packs at the 4–5 leaf stage and later transplanted into 4L pots to overwinter. Thirty-three of the 42 full-sib families produced sufficient numbers of offspring for use in the experiment, resulting in nine missing cells in our design.

The following spring, before bud break, three cuttings (one per nutrient treatment) were made from each of five individuals from each full-sib family. Cuttings were rooted in pots in the greenhouse. In late May, cuttings were transplanted into 8 L pots in a 4:1:1 mixture of topsoil (obtained near our field site in Milford, NY), peat moss, and vermiculite, respectively. All plants were randomly placed 1 m apart in a common garden ($n=468$ plants).

Fertilization treatments began on 25 June and were applied weekly thereafter through 6 August. Each plant received 180 mL of Agway 20-20-20 (NPK) complete fertilizer at one of the following concentrations: 3.2g/L (high), 1.6 g/L (medium) and 0.8 g/L (low). These concentrations were used because the resulting growth rates encompass those of field plants. Plants were watered daily throughout the experiment.

2.3. *Plant growth rate*

We determined growth rate by measuring the growth of the three longest shoots per plant. The three marked shoots were measured on 28 June and again on 21 July.

We calculated absolute growth rate (mm/day)(AGR) by dividing the increase in shoot length by the number of intervening days. The mean AGR for each plant provides an estimate of growth rate that can then be correlated with the secondary chemistry of that plant.

2.4. *Foliar chemistry*

Leaves were collected on 21 July for analysis of plant chemistry. The first fully mature leaf from five different shoots on each plant (1 leaf/shoot) were collected to control for differences in leaf developmental age (Coleman, 1986). Leaves were kept on ice, transported to the lab and vacuum dried. Once dry, leaves were ground in a Wiley Mill (size 30 mesh) and stored in a -20°C freezer until chemical analyses were performed.

The phenolic glycosides were assayed using standard techniques (Nichols-Orians et al., 1992). Briefly, leaf powder (30 ± 3 mg) was extracted in cold MeOH (10 mg leaf powder/1 ml MeOH) with sonication for 10 min. Cold water was constantly flushed through the sonicator to prevent the samples from heating up. We centrifuged and filtered ($0.2\ \mu$ filter) each sample before placing extracts in crimp-top vials. Extracts were kept in the freezer until analysis. We quantified the concentration of glycosides with an HPLC and UV detector (274 nm). A reverse-phase NOVA-PAK C_{18} ($4\ \mu\text{m}$) column (Waters) and a gradient system of distilled water and MeOH was used. 1,3-dimethoxybenzene was used as the internal standard. Standard curves were determined for both salicortin and 2'-cinnamoylsalicortin.

2.5. *Statistical analyses*

2.5.1. *Effects of genetic variation and fertilizer treatment on plant traits*

Data were analyzed with Proc GLM (SAS, 1996) using a mixed-model ANOVA. Sire and dam effects and their interactions were random effects and fertilizer treatment was a fixed effect. Values were \log_e transformed prior to statistical analyses to improve normality and equality of variances. In mixed models, Dam and Sire effects are tested over the Sire*Dam interaction term, and Sire*Fert and Dam*Fert interactions are tested over the Sire*Dam*Fert interaction term (Neter et al., 1996). Significance of sire effects were used as tests for significant additive genetic variation. Fertilizer treatment effects were tested over the following composite error term: (Dam*Fert)+(Sire*Fert)-(Sire*Dam*Fert).

2.5.2. *Genotype \times environment interactions*

We estimated genotype \times environment interactions (=genetic variation in plasticity) with two different methods. First, we estimated significance of sire by fertilizer interactions in the ANOVA. However, some have argued that cross-environment genetic correlations are a more precise method for evaluating genetic variance across environments (Via and Lande, 1985). Therefore we also used this second method.

Expression of the same trait (e.g., salicortin) in two different environments can be considered as two different traits (Falconer, 1952). Therefore the additive genetic

correlation of the expression of these two traits estimates the extent of similar genetic control of that trait. If the additive genetic correlation across environments is +1, then they are assumed to be the same trait. That is they have the same genetic basis (Via and Lande, 1985). However, if the cross-environment genetic correlation is <1, it indicates the presence of significant genotype by environment interactions, and suggests that the phenotypes in the two environments differ in their genetic control. They may be controlled by different genes, different alleles at a locus, or by the same alleles with different effects in the different environments (Via and Lande, 1985).

Cross-environment genetic correlations were calculated using the formula from Fry (1992):

$$r_g = \frac{Cov(M_{1j}, M_{2j})}{\sqrt{Var(M_{1j})Var(M_{2j})}}, \quad (1)$$

where M_{1j} is the mean of family j in environment 1, and M_{2j} is the mean of family j in environment 2. $Var(M_{1j})$ and $Var(M_{2j})$ are corresponding variances among family means in environments 1 and 2.

We tested the hypothesis that the cross-environment genetic correlations differed significantly from +1 by estimating the 95% confidence interval using the jackknife procedure. If the upper confidence interval was less than +1, then the genetic correlation was concluded to be significantly different from 1.

2.5.3. Heritability

We calculated heritability for traits exhibiting significant additive genetic variation in the ANOVA (e.g., significant sire effects). Heritability estimates were made using analyses of each environment separately, since individual plants were cloned and placed in each environment, and therefore values determined from these plants were not independent. We used Proc Varcomp (SAS, 1996) to generate estimates of the variance components and calculated heritability as four times the sire variance divided by the total variance (Falconer, 1989).

2.5.4. Genetic and phenotypic correlations

Genetic and phenotypic correlations identify evolutionary and ecological tradeoffs between traits. Genetic correlations were calculated between salicortin and 2'-cinnamoylsalicortin within each fertilizer treatment. Genetic correlations were determined for each environment separately because clones of each genotype appeared in all environments. Genetic correlations involving AGR could not be made because estimates of sire variances for AGR in each environment were zero. We used methods recommended by Becker (1984) to calculate the value of the genetic correlation and used the Pearson correlation of sire means as an approximate test of their significance. We also calculated phenotypic correlations among salicortin, 2'-cinnamoylsalicortin, and AGR.

3. Results

3.1. Effects of genetic variation and fertilizer treatment on plant traits

There were significant genetic effects on plant traits. Sire effects were significant for salicortin and 2'-cinnamoylsalicortin (Table 1). Salicortin concentration ranged from a low of 69.9 to high of 84.5 mg/g (a 21% difference among sires), while 2'-cinnamoylsalicortin ranged from 12.3 to 18.4 mg/g dry leaf weight (a 50% difference among sires) (Fig. 1A,B).

Sire effects were also significant for salicortin and 2'-cinnamoylsalicortin within some fertilizer treatments, and estimates of heritability for salicortin and 2'-cinnamoylsalicortin varied among the treatments (Table 1B). Heritabilities range from 0.09 to 0.43 for salicortin. For salicortin, only the highest heritability, found in the medium fertilizer treatment, was significantly different from zero. In contrast, heritabilities for 2'-cinnamoylsalicortin were higher than for salicortin, ranging from 0.40 to 0.95. For this chemical, heritability increased with increased fertilizer concentration and was significant for the medium and high fertilizer concentrations. In con-

Table 1

Summary of ANOVA analysis (F, *p*-values) examining the effects of dam, sire, fertilization and interactions on absolute growth rate (mm/day), and the concentrations (mg/g dry leaf weight) of salicortin and 2'-cinnamoylsalicortin in *Salix sericea* (A.). Tests of significance (F, *p*-value) for sire effects in ANOVA and narrow-sense heritability estimates for salicortin and 2'-cinnamoylsalicortin within each fertilizer treatment (B.). Significance levels: *-*P*<0.05, **-*P*<0.01, ***-*P*<0.001

A. Summary of ANOVA analysis

Source	df _{N,D}	Error term	Absolute Growth Rate F	Salicortin F	2'-cinnamoyl salicortin F
Dam (D)	2, 17	S*D	0.45	2.30	4.41*
Sire (S)	13, 17	S*D	0.68	2.61*	3.21**
Fertilization (F)	2,	#	57.6***	37.71***	2.57
S*D	17, 34	D*S*F	4.50***	1.10	1.96**
D*F	4, 34	D*S*F	2.59*	0.21	0.80
S*F	26, 34	D*S*F	0.78	1.15	1.38
D*S*F	34, 342	Error	0.54	0.86	0.59

#-S*F+D*F-S*D*F.

B. Heritability

Treatment	Salicortin		2'-cinnamoylsalicortin	
	F	h ²	F	h ²
Low	1.78	0.09	1.79	0.40
Medium	2.64*	0.43	2.79*	0.53
High	1.18	0.15	2.82*	0.95

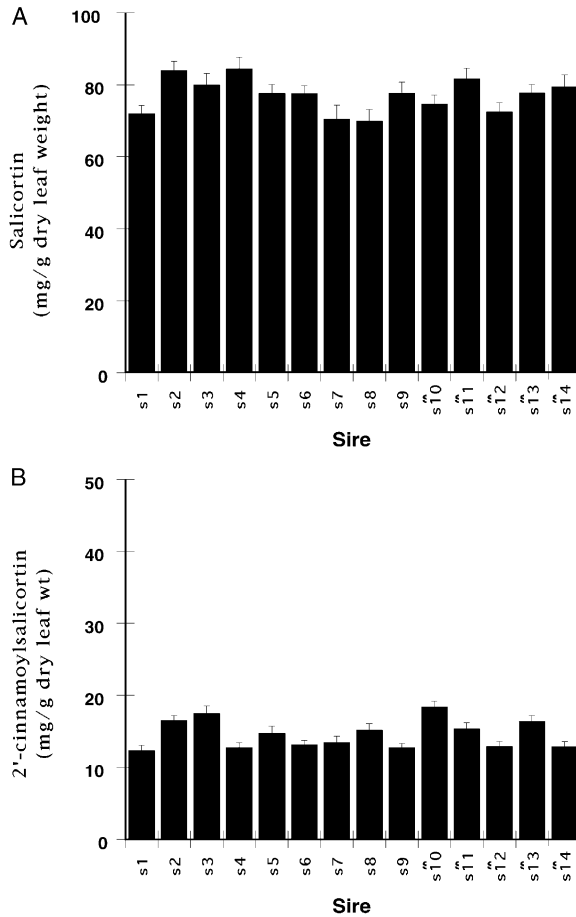


Fig. 1. Sire means (1 s.e.) for salicortin and 2'-cinnamoylsalicortin.

trast to the plant chemicals, growth rate (AGR) showed no heritable genetic variation (i.e., sire effect), but there was a significant sire-by-dam interaction term (Table 1A). The dam and sire-by-dam interaction effects were significant for 2'-cinnamoylsalicortin, but not for salicortin (Table 1A).

Fertilizer treatment caused a significant 61% increase in AGR and a significant 10% decrease in salicortin concentration (Table 1A; Fig. 2 A,B). These significant effects indicate phenotypic plasticity for these traits. The concentration of 2'-cinnamoylsalicortin was not affected by fertilizer treatment (Table 1A; Fig. 2 C) indicating the lack of phenotypic plasticity for this chemical. The significant dam-by-fertilizer effect for growth rate probably indicates differences in maternal effects and not additive genetic variation since the sire test was more robust and insignificant.

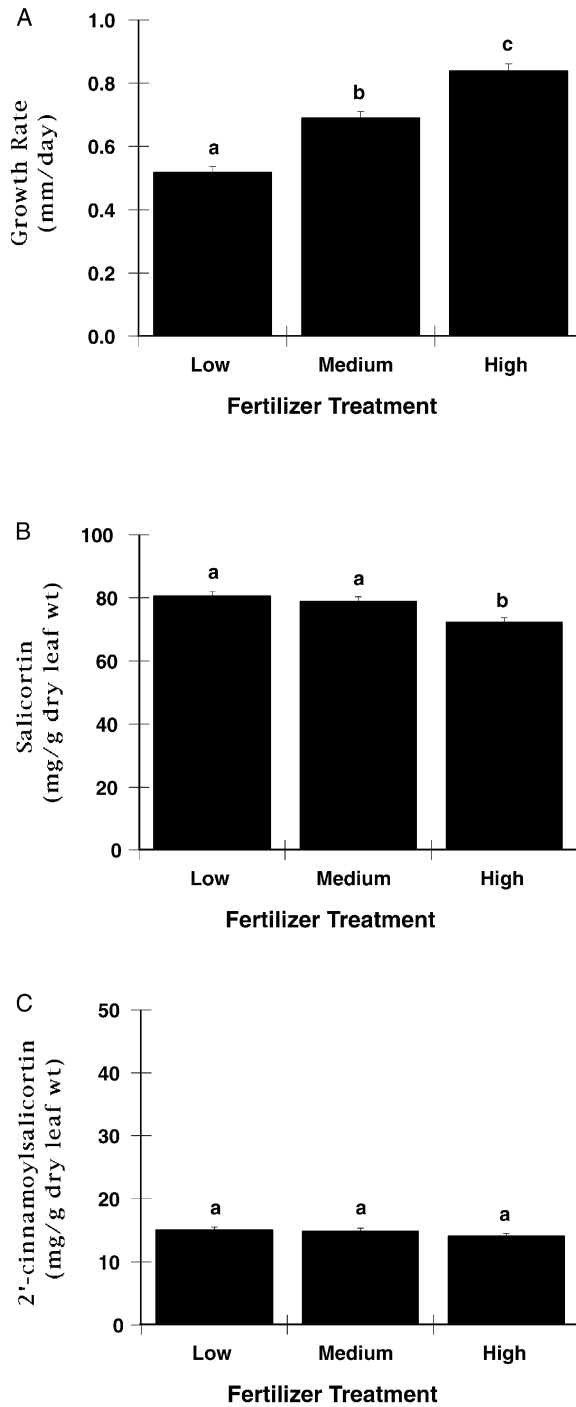


Fig. 2. The effect of fertilization on A) growth rate, B) salicortin concentration, and C) 2'-cinnamoylsalicortin concentration (mean \pm 1 s.e.)

3.2. Genotype × environment interactions

ANOVA: Using this method we found no evidence for genetic variation in phenotypic plasticity. The sire-by-fertilizer interaction effects were insignificant for AGR and for the chemicals (Table 1A).

Cross-Environment Genetic Correlation: In contrast to the ANOVA results, the cross-environment genetic correlations suggest the presence of genetic variation in phenotypic plasticity. In the medium vs high comparison the correlation was 0.23 for salicortin (Table 2B). Values of 1 indicate a lack of phenotypic plasticity and that traits are controlled by the same genes in all environments. Values less than 1.0 indicate that patterns of genetic control differ between the two environments. This pattern is also statistically significant for 2'-cinnamoylsalicortin, but the value of 0.92 suggests that the genetic differences are few. For all other pairwise comparisons (low vs medium and low vs high fertilizer), cross-environment genetic correlations were 1.0. Although all the correlations were less than 1.0 for AGR (Table 2B), the absence of significant genetic variation (Table 1) indicates there is actually a lack of genetic variation in plasticity (genotype × environment interactions).

3.3. Genetic and phenotypic correlations

There was a significant negative phenotypic correlation between AGR and salicortin concentration in the high fertilizer treatment but not in the other fertilizer treatments (Table 2A). 2'-cinnamoylsalicortin showed a significant positive correlation

Table 2

Phenotypic and genetic correlations between salicortin (SAL), 2'-cinnamoyl salicortin (CINN), absolute growth rate (AGR) and in each fertilizer treatment (A.) and cross-environment genetic correlations (\pm 95% confidence intervals) of AGR, salicortin, and 2'-cinnamoylsalicortin between fertilizer treatments (B.). Degrees of freedom for cross-environment genetic correlations are 13,13. Significance levels: †- $P < 0.1$, *- $P < 0.05$, **- $P < 0.01$, ***- $P < 0.001$

A. Within-environment genetic and phenotypic correlations

Treatment	Genetic SAL vs CINN	Phenotypic SAL vs CINN	SAL vs AGR	CINN vs AGR
Low	0.248 (12)	0.502*** (150)	-0.061 (145)	0.189* (145)
Medium	0.203† (12)	0.599*** (145)	-0.107 (139)	0.074 (139)
High	0.450† (12)	0.572*** (146)	-0.197* (139)	0.112 (139)

B. Cross-environment genetic correlations

Treatment	Salicortin	2'-cinnamoyl salicortin	AGR
Low vs Medium	1.00±0.36	1.00±0.07	-0.658
Low vs High	1.00±0.20	1.00±0.04	-0.510
Medium vs High	0.23±0.11	0.92±0.02	-0.638

with growth in the low fertilizer treatment but not in the other fertilizer treatments (Table 2A). Genetic correlations between AGR and the chemical concentrations are suggested to be zero, however, since there was no significant sire variance in AGR in any of the fertilizer treatments.

There was a significant positive phenotypic correlation between salicortin and 2'-cinnamoysalicortin in each of the fertilizer treatments (Table 2A). While the genetic correlations were also all positive in each fertilizer treatment, they were only marginally significant ($P < 0.1$) in the medium and high fertilizer treatments (Table 2A).

4. Discussion

The expression of resistance traits may be determined by genetic variation, environment (=phenotypic plasticity) and GxE interactions (e.g., Bryant et al., 1983; Berenbaum et al., 1986; Herms and Mattson, 1992; Han and Lincoln, 1994; Hakulinen et al., 1995). Significance of genetic and GxE effects suggest that selection for both increased resistance and plasticity is possible. However the evolution of increased resistance may be constrained by other factors (i.e., negative correlations between growth and defense). We found evidence for significant heritable variation in the production of two phenolic glycosides (but not in growth rate), plasticity of growth and salicortin concentration in response to fertilization, and genetic variation in plasticity. We also showed that the expression of genetic variation depends upon soil nutrients and is thus a potential constraint on the evolution of these resistance traits. We did not find evidence for other constraints.

We found significant differences in chemistry among sires. Sire means differed by as much as 21 and 50% for salicortin and 2'-cinnamoysalicortin respectively. Our estimates of heritability were 0.53 and 0.95 for 2'-cinnamoysalicortin and 0.43 for salicortin. The relative difference in heritability of these two chemicals (0.59 and 0.20) is similar to our previous work in this system (Orians et al., 1996). However, the values reported previously were lower. This difference likely reflects environmental control of heritability. In fact, we found that significance of heritability does depend upon the environment. Heritability was zero for salicortin in low and high nutrient environments, but was significant in the medium nutrient treatment. In contrast, heritability increased for 2'-cinnamoysalicortin with increasing nutrient availability. Thus nutrient environment affects the expression of genetic variation, and selection for increased production would be absent in low nutrient environments. For such selection to occur, genotypes that produce high concentrations would have to be less susceptible to herbivores. Although unknown for this system, differences among aspen clones in phenolic glycoside concentration have been found to determine their resistance to both gypsy moths and forest tent caterpillars (Hwang and Lindroth, 1997; Osier and Lindroth, 2001).

For both phenolic glycosides we found the cross-environment genetic correlations to be less than 1.0 in the medium vs high nutrient environment. This indicates that there is genetic variation in plasticity of these glycosides across these two nutrient environments. Therefore, genes affecting the traits are different or are expressed

differently in the two different environments. Most importantly, this shows that there is the potential for independent evolution of these compounds in the two nutrient environments.

Somewhat surprisingly, we did not find significant heritable genetic variation for AGR (see sire effect in Table 1). The significant Sire*Dam interaction effect for AGR indicates there may be dominant genetic variance in AGR. The lack of genetic variation in growth is consistent with our previous work (Orians and Fritz, 1996). In that study there were no additive genetic variation in the number of long shoots produced, shoot growth rates, or internode length. Although no previous studies have used quantitative genetics to estimate genetic variation in willow growth rate, several studies have found clonal differences in growth (e.g., Fowler et al., 1983; Rönnberg-Wästljung and Thorsén, 1988; Nichols-Orians et al., 1993; Houle and Simard, 1996). There are several possible explanations for the lack of genetic variation in this study. First, using clones actually estimates maternal effects. Although possible, it seems unlikely. Second, genetic differences in growth may be more evident as plants age. In previous experiments we found differences among *S. sericea* clones that had been growing for 3 years in pots (Nichols-Orians et al., 1993). In this experiment all cuttings were in their first year of growth. Finally, there may have been genetic differences in other growth traits. For example, Fowler et al. (1983) and Rönnberg-Wästljung and Thorsén (1988) focused on differences in biomass accumulation rather than shoot elongation. However, we focused on shoot elongation because we feel it is key to the survival of young willows.

The significant effects of fertilizer treatment on salicortin and AGR (Fig. 2) indicate that all these traits are plastic in their response to nutrients. As expected, growth increased while salicortin concentration decreased with increasing soil nutrient availability (Bryant et al., 1983). However, 2'-cinnamoylsalicortin did not decrease as expected, indicating the lack of plasticity in this trait. Perhaps 2'-cinnamoylsalicortin is a "dynamic" metabolite (Reichardt et al., 1991) that does not accumulate in response to low nutrient availability. Fertilization effects on concentration were not as pronounced as the genetic effects—fertilization only reduced salicortin concentration by 10%. Using aspen, Osier and Lindroth (2001) found that, although fertilization caused a decrease in phenolic glycoside concentration, herbivore performance was correlated with higher nutrient availability. In contrast, aspen clones that produced high concentrations of phenolic glycosides were less susceptible to herbivory. Thus it seems unlikely that the 10% difference in salicortin concentration we observed following fertilization is sufficient in itself to alter herbivore performance.

4.1. Evolutionary constraints

Our results provide further evidence that additive genetic variation (reviewed by Berenbaum and Zangerl, 1992) and soil nutrient availability (e.g., Bryant et al., 1983; Herms and Mattson, 1992) determine patterns of chemical variation. Here we showed that soil nutrient availability affects the expression of genetic variation, and therefore represents a potential constraint on the evolution of these resistance traits.

We also tested for the presence of negative genetic correlations between growth

rate and secondary chemical production. Several studies indicate that selection for increased chemical concentration would result in slower growing genotypes (Boecklen et al., 1990; Jing and Coley, 1990; Han and Lincoln, 1994). Despite the fact that salicortin is 7–10% dry leaf weight, we did not detect negative genetic correlations with shoot elongation in this study, or in previous studies with this system (Nichols-Orians et al., 1993; Orians et al., 1996). It is possible that other measurements of growth, or perhaps reproduction, are negatively genetically correlated with chemical concentration. Further work is required to determine if such trade-offs exist.

Finally, we determined if production of one secondary chemical occurred at the expense of the production of a second chemical (*sensu* Berenbaum et al., 1986; Zangerl et al., 1989). As we found previously (Nichols-Orians et al., 1993; Orians et al., 1996) we found no evidence of a negative genetic correlation between the two phenolic glycosides. Rather, although not quite significant ($P < 0.1$) the genetic correlations were positive. We suggest that the selection for one of the chemicals would be unlikely to affect the concentration of the other.

5. Conclusion

We have found significant heritable genetic variation in phenolic glycoside production. Phenolic glycosides are well known to deter many herbivores (e.g., Tahvanainen et al., 1985; Lindroth et al., 1988). As a consequence, herbivory could result in selection for genotypes that produce high concentrations of phenolic glycosides. Interestingly, selection in favor of genotypes with high concentrations of salicortin (or 2'-cinnamoylsalicortin) would not result in a correlational increase in the other. Finally, our results indicate that selection for either chemical would only be possible under moderate (both chemicals) or high (2'-cinnamoylsalicortin only) soil nutrient availability.

Acknowledgements

We thank Len and Eleanor Sosnowski for permitting us to conduct research on their property. Their long-term commitment to this research is greatly appreciated. Christine Branigan, Sylvan Kaufman, Nora Murphy, Anna Tom, Kelly Reape, and Michael Reed provided invaluable assistance. Durwood Marshall and Debra Palmquist provided statistical advice. We thank two anonymous reviewers for their comments on this manuscript. This research was supported by the United States Department of Agriculture (92-02081), by NSF (DEB 92-07363), and by Tufts University.

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