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Genetics of breeding characters with possible effects on biomass production in *Salix viminalis* (L.)

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Abstract Genetic variation in number of sylleptic shoots, wood density, dry matter content, flower production, budbreak, growth cessation, insect susceptibility and rust susceptibility and the relationships of these characters with other growth characters were studied. Forty of sixty-four families in an 8×8 factorial crossing with *Salix viminalis* were used. Fourteen plants from each family were planted in the framework of a nutrient experiment with optimal and suboptimal nutrient availability and in a sandy soil. The analyses showed high additive genetic variances for all characters studied. Characters measured in both nutrient environments did not show any genotype × environment interaction. Both in the nutrient and in the sand environment insect susceptibility was negatively correlated to different growth characters. This was also the case for rust susceptibility in the sand environment. The highest negative correlation was found between number of days to budburst and weight, indicating the importance of early budburst for production. Ideas on important characteristics for an *Salix* ideotype are discussed.

Key words Variation · Genetic correlations · Path analysis · Ideotype · *Salix viminalis*

Introduction

The construction of an ideotype, i.e. a model tree of a desirable phenotype that will be high producing at a specific site, is one way to summarize the overall physiology of the plant (Dickman et al. 1994). The ideotype concept has been more widely used by crop breeders than by forest tree breeders, with the notable exceptions of the Finnish conifer ideotype and poplar ideotype (see review by Dickman et al. 1994). Stettler et al. (1992) suggested the use of ideotypes as one possibility for breeding in short-rotation forestry. Bisoffi and Gullberg (1996) suggested testing the possibility of creating ideotypes by selection in some of the sub-populations of a long-term breeding population. An important piece of information in this process is to know how the traits are related. Can characters be changed independently of each other or are there genetic constraints that cause correlated changes in the other characters?

The aim of the study presented here was to analyse a set of characters that could be relevant in the definition of a *S. viminalis* L. ideotype. These characters are basically of two kinds: (1) production-related characters that directly affect carbon allocation and wood quality and (2) adaptive traits that must be changed in order to adapt the plants to biotic and abiotic stress. In the first group, we have sylleptic shoots, wood density, dry matter content and flower production. In the second, we consider budbreak and growth cessation, on one hand, and susceptibility to gall midge and leaf rust on the other.

A genetic analysis of these characters both independently and in relation to growth traits such as height, diameter, shoot number and weight, will help our understanding of how they should be selected for and integrated into an ideotype.

Nutrient availability is one of the important factors in a high-producing *Salix*-crop (Ingestad and Ågren 1984), and an interaction between genotypes and contrasting levels of nutrient availability will identify rank changes between genotypes from one nutrient availability level to another. This is also useful information when attempting to identify an ideotype for *S. viminalis*.

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More specifically, the purpose of the present investigation was to study the above-mentioned characters with respect to: (1) genetic variation, (2) genotype × nutrient interaction and (3) genetic correlations to growth traits.

Materials and methods

Plant material and measurements

An 8 × 8 factorial crossing was created in 1987, and 40 of the 64 families were used in this study (Rönnberg-Wästljung et al. 1994). The parents were clones of *S. viminalis* L. collected in Sweden and 1 clone originating from Holland. Fourteen seed plants were propagated from each family to be planted in two pairs of contrasting environments. One pair of environments consisted of field sites where the soil types differed, one being sand and the other heavy clay. The other pair of environments consisted of two contrasting levels of nutrient availability, high and low, that were created in a sand box with inert sand. All environments were located south of Uppsala (59°48′, 17°39′). The experiments were planted in an incomplete block design (Rönnberg-Wästljung et al. 1994). For this study, only measurements from material grown in the sand environment and in the nutrient experiment were used. In the sand environment, twelve clones per family, 480 clones in total, were planted, and in the nutrient environments 6 clones per family, for a total of 240 clones, were planted. A more detailed description of the material and the environments is given in Rönnberg-Wästljung et al. (1994).

The material was planted in the sand environment in 1988 and harvested after the first growing season and then after 3 and 5 years. Measurements in the sand environment used for this study are summarized in Table 1. At the second harvest, samples from each plant in four of the eight replicates were taken to determine the dry matter content. The weight equilibrium of the shoot was determined, and a sample of 0.4 m with this point in the middle was taken. The fresh weight of the sample was measured, and after the sample had been dried at 104°C for 3 days the dry matter content was estimated by dividing the dry weight by the fresh weight. Two growing seasons after the first harvest the material in the sand environment became infected by rust fungi (*Melampsora* sp.) for the first time. The rust intensity was studied (Gullberg and Ryttman 1993). On one of the most infected leaves of the plant, the degree of the rust colonies was quantified using a scale of 0 to 5, where 5 indicates that most of the leaf is covered with rust. Five of the eight replicates were measured. In the fall, 1 year after the second harvest, susceptibility to the gall midge (*Dasineura marginemtorquens)* was recorded. A scale of 0 to 5 was used where 0 = no infection, 1 = a few galls, 2 = less than 50% of the leaves infected, 3 = more than 50% of the leaves infected, 4 = a few leaves with no infection and 5 = all leaves infected. In the following spring the material was classified for number of flowers on the highest shoot. The classification was made on the 1-year-old part of the shoot; 1 equals 10% flowers of that part, and 2 equals 20% and so on.

The nutrient experiment was harvested each year for 3 years. Measurements used in this study are summarized in Table 1. During the first growing season, height of the longest shoot was measured each week. Growth cessation of the plants was estimated as the height growth after September 1st (1988) in relation to total height growth. During the first growing season, the material was also classified into four groups according to number of sylleptic shoots. The number of leaves on the highest shoot that were attacked by gall midge (*D. marginemtorquens*) was also recorded during the first growing season. Since the height of the tallest shoot was measured, the number of leaves attacked by gall midge per decimeter could be estimated. At the second harvest, wood density of the plant was measured. A sample of about 7 cm was taken from the stem, the bark was removed, the volume was determined by water displacement in a trough, and the dry weight of the sample was taken after drying at 104°C for 24 h. In the spring of the third growing season, the number of days to budburst was recorded in the low nutrient environment. The plants were checked each day and the buds were considered as broken at a specific stage. Number of days from the first assessment to when the buds were broken gave the number of days to budburst.

Table 1 Characters included in the study and year of measurement

Measured character	Environment[a]		
	L	H	Sand
Sylleptic shoots	88	88	–
Density	89	89	–
Dry matter	–	–	91
Flowers	–	–	93
Budburst	90	–	–
Growth cessation	88	88	–
Insect damage	88	88	92
Rust damage	–	–	90
Weight	88, 89, 90	88, 89	91, 93
Shoots	88, 89, 90	88, 89	91, 93
Height	88	88	91
Diameter	88	88	91

[a] H, High nutrient environment; L, low nutrient environment; Sand, sand environment

Statistics and estimation of genetic parameters

Analyses of variance were made using the procedure GLM in SAS (SAS Institute 1989), and variance components were estimated with the REML method in the SAS procedure VARCOMP. The linear model, here called model 1, was:

$$y_{jklnp} = \mu + r_j + b_{k(j)} + m_l + f_n + mf_{ln} + c_{p(ln)} + e_{jklnp} \quad (1)$$

where

y_{jklnp} = observed value of a given character for an individual plant
μ = overall mean
r_j = effect of replicate j, j = 1, 2, … , r; r = 5 or 8, fixed effect
$b_{k(j)}$ = effect of block k within replicate j, k = 1, 2, … , b; b = 6 or 12, fixed effect
$m_l \sim N(0, \sigma^2_m)$ = effect of male l, l = 1, 2, … , 8, random effect
$f_n \sim N(0, \sigma^2_f)$ = effect of female n, n = 1, 2, … , 8, random effect
$mf_{ln} \sim N(0, \sigma^2_{mf})$ = interaction effect between male l and female n, random effect
$c_{p(ln)} \sim N(0, \sigma^2_c)$ = effect of clone p within family, p = 1, 2, … , c, c = 6 or 12, random effect
$e_{ijklnp} \sim N(0, \sigma^2_e)$ = random error.

All random effects were assumed to be statistically independent and, as indicated above, normally distributed. Since the division into replicate and block within replicate was done to reduce the experimental error, replicate and block within replicate were considered as fixed.

Theoretically, the variance components from model 1 have the following genetic composition (Becker 1984):

$$\sigma^2_f = \sigma^2_m = 1/4\ \sigma^2_a + 1/16\ \sigma^2_{aa} + \ldots \quad (2)$$

$$\sigma^2_{mf} = 1/4\ \sigma^2_d + 1/8\ \sigma^2_{aa} + 1/8\ \sigma^2_{ad} + 1/16\ \sigma^2_{dd} + \ldots \quad (3)$$

$$\sigma^2_c = 1/2\ \sigma^2_a + 3/4\ \sigma^2_d + 3/4\ \sigma^2_{aa} + 7/8\ \sigma^2_{ad} + 15/16\ \sigma^2_{dd} + \ldots \quad (4)$$

where
σ_a^2 = additive genetic variance
σ_d^2 = dominance genetic variance
σ_{aa}^2, σ_{ad}^2, σ_{dd}^2 = epistatic genetic variances (σ_i^2).

From Eqs. 2, 3 and 4 the genetic parameters can be estimated as:

$$\hat{\sigma}_a^2 = 2(\hat{\sigma}_f^2 + \hat{\sigma}_m^2) \quad (5)$$

where the expected value (E) of $\hat{\sigma}_a^2 = \sigma_a^2 + 1/4\ \sigma_{aa}^2 + \ldots$ or

$$\hat{\sigma}_a^2 = 2(\hat{\sigma}_c^2) - 6(\hat{\sigma}_{mf}^2) \quad (6)$$

where

$$E(\hat{\sigma}_a^2) = \sigma_a^2 + 3/4\ \sigma_{aa}^2 + \sigma_{ad}^2 + 24/16\ \sigma_{dd}^2 + \ldots$$

$$\hat{\sigma}_d^2 = 4(\hat{\sigma}_{fm}^2)$$

where

$$E(\hat{\sigma}_d^2) = \sigma_d^2 + 1/2\ \sigma_{aa}^2 + 1/2\ \sigma_{ad}^2 + 1/4\ \sigma_{dd}^2 + \ldots$$

$$\hat{\sigma}_i^2 = \hat{\sigma}_{c(fm)}^2 - (\hat{\sigma}_f^2 + \hat{\sigma}_m^2) - 3\hat{\sigma}_{fm}^2 \quad (7)$$

where

$$E(\hat{\sigma}_i^2) = 1/4\ \sigma_{aa}^2 + 1/2\ \sigma_{ad}^2 + 3/4\ \sigma_{dd}^2 + \ldots$$

Formula 7 gives an estimate of a linear combination of a fraction of the epistasis effects, but it contains interactions from two to multi loci.

The narrow-sense heritability (h^2) can be estimated as:

$$\hat{h}^2 = \hat{\sigma}_a^2/\hat{\sigma}_p^2$$

where $\hat{\sigma}_a^2$ can be estimated from formula (5) or (6) and
where $\hat{\sigma}_p^2$ = the sum of all variance components in model 1.

All variables, including the variables in discrete classes, were exposed to analyses of variance. The residual plots from each analysis were examined and considered acceptable. Mean values, over all replicates, were estimated for characters measured in discrete classes as a way to make the variables continuous. Analyses of variance on mean values were also performed but disregarded. The additive variance did not change when estimating on mean values but the heritability estimates changed since the phenotypic variance is lower with mean values. Due to the risk of overestimating the heritabilities when using mean values and since the residual plots were acceptable using individual values, all analyses were done on individual values.

To study the genotype × environment interaction in the characters that were measured in the two nutrient environments an expanded linear model, here called model 2, was used:

$$y_{ijklnp} = \mu + s_i + r_{j(i)} + b_{k(ij)} + m_l + sm_{il} + f_n + sf_{in} + mf_{ln} + smf_{iln} + c_{p(ln)} + sc_{ip(ln)} + e_{ijklnp} \quad (8)$$

where
y_{ijklnp} = observed value of a given character for an individual plant
μ = overall mean
s_i = effect of environment i, i = 1,2, fixed effect
$r_{j(i)}$ = effect of replicate j within environment i, j = 1, 2, … , r; r = 5, fixed effect
$b_{k(ij)}$ = effect of block k within environment i and replicate j, k = 1, 2 … , b; b = 6, fixed effect
$sm_{il} \sim N(0, \sigma_{sm}^2)$ = interaction effect of environment i and male l, random effect
$sf_{in} \sim N(0, \sigma_{sf}^2)$ = interaction effect of environment i and female n, random effect
$smf_{iln} \sim N(0, \sigma_{smf}^2)$ = interaction effect of environment i, male l and female n, random effect
$sc_{ip(ln)} \sim N(0, \sigma_{sc}^2)$ = interaction effect between environment i and clone p within family, random effect
$e_{ijklnp} \sim N(0, \sigma_e^2)$ = random error.
Some of the effects are already defined below model 1.

To obtain a covariance component $\sigma(x, y)$ of the characters x and y, we used model 1 for x, y and x + y. As $\sigma^2(x + y) = \sigma^2(x) + \sigma^2(y) + 2\sigma(x, y)$, some rearrangements give

$$\sigma(x, y) = 0.5\ [\sigma^2(x + y) - \sigma^2(x) - \sigma^2(y)] \quad (9)$$

to be used for estimating $\rho = \sigma(x, y)/\sqrt{[\sigma^2(x)\ \sigma^2(y)]}$.

The estimates of covariance and variance components were obtained by using Eqs. 1 and 9. Before Eq. 1 was applied, the phenotypic values x and y were standardized (mean = 0, SD = 100) to avoid severe rounding error effects when using Eq. 9.

The additive genetic correlation was estimated by first adding female and male components separately, i. e.:

$$r_a = [\sigma_m(x, y) + \sigma_f(x, y)]/\sqrt{[(\sigma_m^2(x) + \sigma_f^2(x))\ (\sigma_m^2(y) + \sigma_f^2(y))]}.$$

The standard error of the correlations was estimated according to Falconer (1981, p 285):

$$\sigma_{(ra)} = [(1 - r_a^2)/\sqrt{2}] * \sqrt{[(\sigma(h_x^2)\ \sigma(h_y^2))/(h_x^2\ h_y^2)]}.$$

Path analyses were conducted to get more information out of the interrelationships between the characters and their influence on weight. The path coefficients (p) were estimated as the partial regression coefficients (Sokal and Rohlf 1995). The correlation between the characters and the resultant character (weight) is the direct effect of the character (path coefficient) plus all indirect effects of the character on the resultant character via other paths. The path analysis was also done on standardized values.

Results

Variance components estimated for the nutrient environments reveal large genetic effects for growth cessation and insect susceptibility (Table 2). The number of sylleptic shoots had somewhat smaller genetic effects, as did budburst, while density had the smallest genetic effects, especially in the high nutrient environment (Table 2). Number of flowers estimated in the sand environment showed large father effects compared with the mother effect (Table 3).

Genetic variances and heritabilities for the characters analysed are presented in Table 4. Two different ways of estimating heritability are presented, depending on how the additive genetic variance was calculated. The largest differences between the two estimates are for sylleptic shoots in the high nutrient availability and for insect susceptibility and rust susceptibility in the sand environment. When calculating the additive genetic variance from the father and the mother variance, we found that most of the genetic variance is additive, that is, 80–100% of the total genetic variance for all characters, except budburst and rust susceptibility for which 69% and 63%, respectively, of the total variance was additive. Number of flowers and insect susceptibility show the highest heritabilities among these characters when estimated from the variances of the father and mother, while insect susceptibility and rust susceptibility in the sand environment have the highest heritability values when estimated from the clonal variance.

The environment, i.e. nutrient availability, had a significant effect (when analysed with model 2) on all

Table 2 Variance components (%) and significance for characters measured in the nutrient environments

Variance component	Character[a]								
	Syll (H)	Syll (L)	Gc (H)	Gc (L)	Den (H)	Den (L)	Ins (H)	Ins (L)	Bb (L)
Rep	–***	–***	–***	–***	–***	–***	–***	–***	–***
Block (Rep)	–**	–**	–***	–***	–***	–***	–***	–***	–***
Father = F	6**	6**	12***	11***	2*	3**	14***	10***	4**
Mother = M	8***	7***	12***	10***	8***	7***	21***	16***	7***
F × M	0^{ns}	1^{ns}	1^{ns}	1^{ns}	0^{ns}	0^{ns}	1^{ns}	1^{ns}	2^{ns}
Clone (F × M)	24***	19***	32***	31***	8***	14***	29***	30***	15***
Error	62	67	43	46	82	76	35	43	72
Error variance[b] (abs. value)	0.392	0.340	4.94×10^{-4}	5.32×10^{-4}	9.73×10^{-4}	9.02×10^{-4}	0.123	0.162	3.08

F-ratio significant at *5% level, **1%level, ***0.1%; ^{ns} = non-significant
[a] Syll, Number of sylleptic shoots; Gc, growth cessation; Den, density; Ins, insect susceptibility Bb, bud burst; (H), high nutrient availability, (L), low nutrient availability
[b] Error variance is given as the absolute value

Table 3 Variance components (%) and significance for characters measured in the sand environment

Variance component	Character[a]			
	Fl	Dm	Ins	Rust
Rep	–***	–***	–***	–***
Block (Rep)	–***	–***	–***	–***
Father = F	23***	4***	11***	12***
Mother = M	3*	18***	14***	10***
F × M	2*	2^{ns}	0^{ns}	0^{ns}
Clone (F × M)	36***	28***	35***	45***
Error	36	48	40	33
Error variance[b] (abs. value)	2.543	1.81×10^{-4}	0.206	0.301

F-ratio significant at *5% level, **1%level, ***0.1% level; ^{ns} = non-significant
[a] Fl, Number of flowers; Dm, dry matter; Ins, insect damage; Rust, rust susceptibility
[b] The error variance is given as the absolute value

Table 4 Estimates and standard errors of genetic parameters. Percentage of total genetic variance

Character	h^2_{cl}[a]	SE	h^2_{fm}[a]	SE	$\hat{\sigma}^2_a$[b]	SE	%	$\hat{\sigma}^2_d$[b]	SE	%	$\hat{\sigma}^2_i$[b]	SE	%
Sylleptic shoots (H)[c]	0.49	0.07	0.28	0.12	0.18	0.08	75	0	0	0	0.06	0.04	25
Sylleptic shoots (L)[c]	0.34	0.13	0.27	0.12	0.13	0.06	81	0.01	0.04	6	0.02	0.04	12
Density (H)	0.17	0.05	0.18	0.10	21.6^{d}	11.3^{d}	100	0	0	0	-0.88^{d}	6.5^{d}	0
Density (L)	0.28	0.06	0.20	0.10	24.0^{d}	11.8^{d}	83	0	0	0	4.9^{d}	7.0^{d}	17
Dry matter (S)[c]	0.46	0.11	0.44	0.21	16.6^{d}	7.9^{d}	86	2.4^{d}	2.1^{d}	13	0.2^{d}	4.3^{d}	1
Flowers (S)	0.58	0.12	0.51	0.26	3.64	1.9	80	0.64	0.45	14	0.25	1.0	6
Budburst (L)	0.15	0.15	0.22	0.11	0.94	0.47	69	0.43	0.37	31	−0.16	0.38	0
Growth cessation (H)	0.60	0.18	0.48	0.21	55.3^{d}	23.8^{d}	85	2.9^{d}	10.1^{d}	5	6.6^{d}	15.1^{d}	10
Growth cessation (L)	0.57	0.18	0.44	0.19	50.1^{d}	21.9^{d}	81	3.6^{d}	10.3^{d}	6	7.9	14.4^{d}	13
Insect damage (H)	0.52	0.16	0.71	0.29	0.25	0.10	96	0.01	0.03	4	−0.03	0.06	0
Insect damage (L)	0.56	0.17	0.52	0.22	0.20	0.09	92	0.01	0.03	4	0.008	0.05	4
Insect damage (S)	0.71	0.05	0.49	0.20	0.25	0.10	81	0	0	0	0.06	0.05	19
Rust (S)	0.90	0.12	0.43	0.18	0.39	0.16	63	0.006	0.05	1	0.22	0.09	36

[a] h^2_{cl} is calculated with the additive genetic variance estimated with formula 6, and h^2_{fm} is calculated with the additive genetic variance estimated with formula 5
[b] $\hat{\sigma}^2_a$, $\hat{\sigma}^2_d$, $\hat{\sigma}^2_i$ = additive, dominance and epistatic genetic variation, respectively. The additive genetic variance is estimated with formula 5
[c] H, High nutrient environment; L, low nutrient environment; S, sand environment
[d] Numbers should be multiplied by 10^{-5}

variables except insect susceptibility, while the interactions between nutrient environments and genotypes were non-significant in most cases (Table 5).

The genetic correlations between density, dry matter content, insect susceptibility, growth cessation, budburst, amount of sylleptic shoots, rust, number of flowers, on one hand, and different growth traits on the other are shown for each environment in Table 6. Many of the estimates have standard errors larger than the estimate and could be regarded as being

Table 5 Variance components (%) and significance for characters measured in the nutrient environments and evaluated with model 2

Variance component	Character			
	Sylleptic shoots	Growth cessation	Density	Insect damage
Nutrient = N (fixed effect)	–***	–**	–***	–^{ns}
Rep (N) (fixed effect)	–***	–***	–***	–***
Block (Rep × N) (fixed effect)	–***	–***	–***	–***
Father = F	6**	12***	1^{ns}	12***
N × F	0^{ns}	0^{ns}	1*	0^{ns}
Mother = M	7***	9***	8***	18***
N × M	0^{ns}	0^{ns}	0^{ns}	0*
F × M	1^{ns}	0^{ns}	0^{ns}	1^{ns}
N × F × M	0^{ns}	0^{ns}	0^{ns}	0^{ns}
Clone (F × M)	18***	21***	9***	30***
N × Clone (F × M)	4**	6***	2^{ns}	0^{ns}
Error	64	52	79	39
Error variance[a] (abs. value)	0.366	9.02×10^{-4}	93.8×10^{-5}	0.142

F-ratio significant at *5% level, **1% level, ***0.1% level; ^{ns} = non-significant
[a]The error variance is given as the absolute value

nonsignificant. Estimates of genetic correlations often have large standard errors due to the fact that they are a combination of three different estimated variance/covariance components.

In many cases rust susceptibility in the sand environment and insect susceptibility in the low nutrient environment have a significant genetic correlation with growth traits. The number of days to bud burst also shows a significant negative correlation with weight, especially in year 1990.

Path analyses (Tables 7–9) show the direct and the indirect effects of the characters on weight. In the sand environment, the growth characters height, diameter and number of shoots each show a high direct effect on weight. The negative genetic correlations between the characters rust susceptibility, insect susceptibility, number of flowers and weight could each be divided into a positive direct effect and larger negative indirect effects (Table 7). The positive correlation between dry matter and weight has a negative direct effect; this negative direct effect is compensated for especially by a high positive indirect effect via diameter (Table 7). In the high nutrient environment the negative correlation between sylleptic shoots and weight has a large negative direct effect on weight, while in the low nutrient environment this correlation is less negative as well as the direct effect between sylleptic shoots and weight. For insect susceptibility the negative correlation and also the negative direct effect on weight is higher in the low nutrient environment than in the high nutrient environment (Tables 8, 9).

Discussion

When interpreting the results, it must be kept in mind that they could be affected by the performance of single parents since the crossing scheme involves only eight females and eight males. Linkage disequilibrium was found in some of the populations from which the parents derived (Lascoux et al. 1996), and this could influence some of the correlations. A linkage disequilibrium also increases the epistatic variation (Cockerham 1956). However, since a significant epistatic variation was only found for rust susceptibility we can disregard linkage disequilibrium as a possible cause for the genetic correlations for all characters except rust susceptibility.

Genetic variation

In contrast to growth traits such as weight and number of shoots, where dominance or epistasis can be a considerable part of the genetic variance (Rönnberg-Wästljung and Gullberg 1994), most of the genetic variance is additive for the characters studied.

The two methods that we have used to estimate additive variance show somewhat different results (Table 4). One reason for the latter is that the estimate in which the clonal variance is used is more biased with epistasis than the estimate that uses the variances of the father and mother. For characters where epistatic variance is of importance, the additive variance as well as the heritability will be higher when estimating it from the clonal variance than when estimating it from the variances of the father and mother. Rust susceptibility is the character in this study that differs most between the two types of heritability estimates. It is also the character with the highest epistatic variance. Estimating the additive genetic variance from the clonal variance though gives a more exact estimate with lower standard errors due to higher degrees of freedom for the clonal variance than when using the variances of the father and mother.

Table 6 Genetic correlations and standard errors (in parentheses) in three different environments

Character[a]	w91	h91	d91	s91	w93	s93		
Sand								
Dry matter (91)	0.35 (0.30)	0.56 (0.22)	0.70 (0.16)	− 0.08 (0.32)				
Number of flowers (93)	− 0.46 (0.27)	− 0.23 (0.32)	0.03 (0.33)	− 0.47 (0.27)	− 0.17 (0.33)	− 0.42 (0.29)		
Insect damage (92)	− 0.31 (0.30)	− 0.56 (0.22)	− 0.51 (0.23)	0.14 (0.32)	− 0.40 (0.27)	0.05 (0.33)		
Rust (90)	− 0.48 (0.25)	− 0.67 (0.16)	− 0.86 (0.08)	0.30 (0.28)	− 0.74 (0.14)	0.05 (0.31)		
Character[a]	w88	h88	d88	s88	w89	s89	w90	s90
High nutrient								
Sylleptic shoots (88)	− 0.53 (0.26)	− 0.42 (0.27)	− 0.30 (0.32)	− 0.31 (0.35)				
Density (89)	− 0.07 (0.40)	0.23 (0.34)	− 0.35 (0.34)	− 0.62 (0.26)	− 0.10 (0.39)	− 0.30 (0.32)		
Growth cessation (88)	0.05 (0.36)	0.04 (0.33)	0.08 (0.35)	− 0.56 (0.26)				
Insect damage (88)	− 0.17 (0.34)	− 0.38 (0.27)	− 0.19 (0.33)	0.56 (0.25)				
Low nutrient								
Sylleptic shoots (88)	− 0.30 (0.32)	− 0.46 (0.28)	− 0.18 (0.32)	− 0.21 (0.42)				
Density (89)	0.20 (0.36)	0.45 (0.29)	0.04 (0.35)	− 0.28 (0.43)	0.36 (0.37)	− 0.47 (0.28)		
Bud burst (90)	− 0.29 (0.34)	− 0.43 (0.30)	− 0.30 (0.32)	0.35 (0.41)	− 0.66 (0.23)	0.46 (0.29)	− 0.90 (0.07)	0.10 (0.50)
Growth cessation (88)	0.35 (0.31)	0.20 (0.33)	0.29 (0.30)	0.07 (0.43)				
Insect damage (88)	− 0.55 (0.24)	− 0.55 (0.24)	− 0.57 (0.22)	0.46 (0.34)				

[a] The character is expressed as a letter and the year of measurement. W, Weight; h, height; d, diameter; s, number of shoots

Density has the lowest heritability, of all the characters, 0.17/0.18, which is close to the overall heritability value observed for growth characters (Rönnberg-Wästljung et al. 1994). The other wood quality character reported here, dry matter content, shows a heritability of 0.44/0.46. From the genetic point of view it is more rewarding and gives a higher genetic response to select and breed for higher dry matter content than for density. The broad-sense heritability estimates for moisture content and specific gravity for American willows reported by Lin and Zsuffa (1993a) varied between 0.26 and 0.62 for moisture content and between 0.42 and 0.62 for specific gravity, depending on the computation method. Specific gravity (measured in g/cm^3), which is possibly similar to our density character, although not described in detail in their paper (Lin and Zsuffa 1993a), showed a higher heritability than ours. One explanation is that the estimates from Lin and Zsuffa (1993a) are broad-sense heritabilities which should give higher estimates than our estimates of narrow-sense heritability.

Insect susceptibility together with rust susceptibility shows the highest heritabilities of the characters studied. Our estimate of insect susceptibility was consistent with one obtained previously for both nutrient environments (Strong et al. 1993). Strong et al. (1993) also showed how the density of the gallmidge population changed the heritability.

Gullberg and Ryttman (1993) found estimates of heritability for rust intensity between 0.41 and 0.63, depending on the model used and if female or male variance was used to estimate the additive variance. The authors concluded that there was considerable additive genetic variation for rust resistance and that selection for field resistance was possible. In our present study we also show a significant epistatic variance that can be taken into consideration in the selection.

Genotype × nutrient interaction

The genotype × nutrient interaction is only significant in a few cases for the characters discussed above. This is in contrast to the growth characters reported earlier (Rönnberg-Wästljung and Gullberg 1994) where genotype × environment interactions could have a considerable effect both when soil properties and nutrient availability were different. Still, nutrient availability influences the characters density, growth cessation and number of sylleptic shoots, so the plants have a lower density, later growth cessation and more sylleptic shoots in the high nutrient environment. However, this does not cause an interaction between genotype and environment. Insect susceptibility is not influenced by the nutrient availability, which is also reported elsewhere (Strong et al. 1993).

Table 7 Path analyses in the sand environment on weight

Variable[a]	Correlated weight	Direct effect	Indirect effect							
			h	d	s	Rust	Insect	Flowers	Dry matter	RSQ[b]
Height	0.322	0.630	–	0.931	− 0.682	− 0.148	− 0.063	− 0.0548	− 0.290	0.986
Diameter	0.609	1.018	0.576	–	− 0.384	− 0.189	− 0.059	0.0066	− 0.360	
Number of shoots	0.479	1.168	− 0.368	− 0.335	–	0.067	0.015	− 0.112	0.044	
Rust	− 0.475	0.221	− 0.423	− 0.871	0.354	–	0.064	− 0.0004	0.180	
Insect	− 0.307	0.114	− 0.349	− 0.522	0.152	0.123	–	− 0.0128	0.186	
Flowers	− 0.457	0.239	− 0.145	0.028	− 0.546	− 0.0004	− 0.0062	–	− 0.027	
Dry matter	0.350	−0.513	0.356	0.713	− 0.099	− 0.0774	−0.0416	0.0126	–	

[a] h, Height of highest shoot; d, diameter of highest shoot; s, number of shoots; rust, rust susceptibility; insect, degree of insect damage; flowers, number of flowers on the highest shoot
[b] RSQ = r-square

Table 8 Path analyses in the high nutrient environment on weight

Variable[a]	Correlated weight	Direct effect	Indirect effect						
			h	d	s	Syll	gc	Insect	RSQ[b]
Height	0.765	− 0.054	–	0.454	0.046	0.213	0.118	− 0.011	0.872
Diameter	0.888	0.810	− 0.030	–	− 0.063	0.152	0.025	− 0.006	
Number of shoots	0.466	− 0.183	0.014	0.281	–	0.153	0.185	0.017	
Sylleptic shoots	− 0.532	− 0.504	0.023	− 0.245	0.055	–	0.142	− 0.004	
Growth cessation	0.048	0.328	− 0.019	0.062	− 0.103	− 0.217	–	− 0.002	
Insect damage	− 0.172	0.030	0.021	− 0.156	− 0.102	0.064	− 0.028	–	

[a] h, Height of highest shoot; d, diameter of highest shoot; s, number of shoots; syll, amount of sylleptic shoots; gc, growth cessation; insect, degree of insect damage
[b] RSQ = r-square

Table 9 Path analyses in the low nutrient environment on weight

Variable[a]	Correlated weight	Direct effect	Indirect effect						
			h	d	s	Syll	gc	Insect	RSQ[b]
Height	0.877	0.151	–	0.286	0.0774	0.0903	0.0365	0.236	0.914
Diameter	0.850	0.375	0.115	–	0.0275	0.0354	0.0534	0.243	
Number of shoots	0.283	0.367	0.032	0.028	–	0.400	0.0122	− 0.196	
Sylleptic shoots	− 0.292	− 0.199	− 0.044	− 0.067	− 0.0729	–	0.0517	0.063	
Growth cessation	0.353	0.179	0.031	0.112	0.025	− 0.052	–	0.063	
Insect damage	− 0.555	− 0.429	− 0.083	− 0.212	0.167	0.029	− 0.0265	–	

[a] h, Height of highest shoot; d, diameter of highest shoot; s, number of shoots; syll, amount of sylleptic shoots; gc, growth cessation; insect, degree of insect damage
[b] RSQ = r-square

Genetic correlations and path analyses

Sylleptic shoots

Allocation studies in *Populus* have shown that a large number of sylleptic shoots have a positive effect on growth (Ceulemans 1990; Hinckley et al. 1992; Isebrands 1982). Higher growth may be due to an early development of leaves on the sylleptic shoots, which gives an advantage with respect to leaf area for these clones. Also, more of the carbon produced in the sylleptic shoots was exported to the main stem than carbon produced in proleptic shoots.

In contrast to the *Populus* studies, no significant genetic correlations between amount of sylleptic shoots and other growth traits could be detected in this study, with the exception of weight in the high nutrient environment, which showed a negative genetic correlation with number of sylleptic shoots. Based on the findings in *Populus*, a positive correlation between sylleptic shoots and growth could be expected in *Salix*, and indeed the phenotypic correlation between

number of sylleptic shoots and weight is positive (not shown).

Theoretically, the contradiction in the genetic and phenotypic estimate of the correlation is possible since the phenotypic correlation is dependent both on the genetic correlation and the environmental correlation (Falconer 1991, p 283). If the heritabilities are low and the environmental correlation is of an opposite sign and larger than the genetic correlation, there could be large differences between phenotypic and genetic correlations.

An alternative, but not less likely explanation, is that sylleptic shoots might not have similar functions in *Salix* and *Populus*. In *Salix* the sylleptic shoots are quite small and usually fall off at the end of the growing season, whereas sylleptic shoots in *Populus* are large and permanent.

The genetic correlation between syllepsis and weight in the low and high nutrient environments is most certainly influenced by environment, as shown by the different values observed. Changes in correlations between growth traits due to environment have been demonstrated earlier by Rönnberg-Wästljung and Gullberg (1996). The path analysis shows that the negative genetic correlation in the high nutrient environment is dependent both on the strong negative direct effect on weight but also on the indirect negative effect via diameter. In the low nutrient environment it is mainly the direct effect that causes the negative correlation since the indirect effects are very weak.

Wood quality

Dry matter content in the plant shows a positive genetic correlation with height and diameter, while density shows a negative correlation with number of shoots in the high nutrient environment. In studies with *Salix eriocephala* (Lin and Zsuffa 1993b), similar results were obtained with respect to dry matter (presented as moisture content), but there were negative genetic correlations between specific gravity on the one hand and height and diameter on the other hand. The positive genetic correlation between dry matter and weight can be divided into a considerable negative direct effect and large positive indirect effects via diameter and height.

Phenology

There is a 10-day difference between plants with early budburst and plants with late budburst. The strong negative genetic correlation between number of days to budburst and weight indicates that just a few days difference in budburst is of great importance for the total weight of the plant. The study by von Fircks (1994), where there were differences in bud break of 29 days between clones of *S. viminalis*, suggests that there are large differences in the productivity of the clones.

An early start in the spring seems to be of greater importance for growth than late growth cessation, since no correlation was found between growth cessation and weight, height or diameter. An early development of leaves gives an advantage in total leaf area and photosynthetic capacity. Studies with *Salix* have shown higher leaf area (Stadenberg et al. 1994) and higher photosynthetic ability (MacDonald et al. 1984) to have a positive effect on growth. The only correlation found for growth cessation with growth traits was a negative correlation with number of shoots.

We believed that the presence of many flowers on the shoot would also slow down development of leaf area since leaves are developed after the flowers. This does not seems to be the case since no strong (negative) genetic correlation could be found with the growth traits.

Insect susceptibility

The genetic correlations between gall midge susceptibility and diameter and height of the plant are negative both in the sand and in the low nutrient environment. In the low nutrient environment, there is also a negative correlation between weight and gall midge susceptibility. Results from Glynn et al. (1996) show a reduction in the growth of susceptible plants due to gall midge infestation. Completely resistant clones, possibly caused by a different mechanism than the incomplete resistance studied in this analysis, also showed a reduction in growth if they were exposed to the gall midge. This growth reduction was explained as the price the plant paid to produce a defence. In our study, the differences in the correlation between the high and low nutrient environment might be explained by the high nutrient availability to the plants. The production of a defence or an attack of the gall midge is no longer a cost for the plant when there is high nutrient availability. The path analysis also reveals that it is only in the low nutrient environment that a direct negative effect on weight due to insect susceptibility can be seen.

Rust susceptibility

A negative genetic correlation between rust susceptibility and height, diameter and weight (one of the years), was found in the sand environment. A negative correlation was what we expected since also in this case it is reasonable to expect a cost for establishing a defence against the fungi. In a study by Rajora et al. (1994) with *Populus*, rust resistance was negatively correlated to growth in the first year in the nursery, but in the second year the resistance showed a positive correlation to growth, as it also did in the fourth year of growth in the

field. The path analysis in this study shows that the direct effect of rust on weight is positive and that the high indirect effects via height and diameter are the main contributors to the negative correlation between rust susceptibility and weight.

A reduction in growth in *Populus* due to rust susceptibility has been shown by Spiers (1976), Wang and van der Kamp (1992) and Widin and Schipper (1976). In some of the studies, diameter growth was mostly affected by the rust infection (Spiers 1976; Widin and Schipper 1976), while others showed a total reduction of biomass due to the rust. Thus, Wang and van der Kamp (1992) showed yield losses of 25% to more than 50% depending on the disease severity. In *Salix*, indications of growth loss due to rust infection were demonstrated by Rönnberg-Wästljung and Thorsén (1988). In a stand that was supposed to be planted with 1 *S. viminalis* clone, at least 2 clones were identified and they differed in rust susceptibility (Verwijst 1990). The uninfected clone had a larger size and higher weight than the infected clone, and Verwijst (1990) also found differences in size-weight relationships when he compared infected and non-infected shoots.

Conclusions

All of the characters included in this study show relatively high heritabilities, and most of the genetic variance is additive, thereby providing an opportunity for the improvement of the characters via a programme with recurrent selection. The only character that shows a significant effect of interaction between loci, i.e. epistatic genetic variation, is rust susceptibility. This suggests that clonal selection may also improve the resistance towards rust infection.

The presence of strong genetic correlations indicate that the correlated response has to be taken into account when selecting for some of the characters. The strong negative genetic correlation between number of days to flush and weight shows that a selection for early budburst can increase the production. On the other-hand, an early budburst could result in frost damage some years, so a balance between the risk of frost damage and higher production has to be considered. The negative correlation between insect and rust susceptibility and growth characters shows the necessity of selecting for resistance. A higher dry matter content is also valuable since it positively influences height and diameter, while few sylleptic shoots is desirable due to its negative correlation with weight in the high nutrient environment.

The relationships between the characters reported here could be used in the construction of an ideotype for *Salix viminalis*. Some characteristics of an *S. viminalis* ideotype could be: high resistance towards rust fungi and gall midges, few sylleptic shoots, early budbreak (depending of the frequency of early frosts) and high dry matter content. The ideotype should not be regarded as stable and fixed since genetic correlation has been proven to change between environments (Rönnberg-Wästljung and Gullberg 1996). The need for several ideotypes depends on how representative different environments will be in the large-scale cultivation of *Salix*. A breeding programme that provides the flexibility to select in different directions towards separate ideotypes solves some of these problems (Gullberg 1993; Bisoffi and Gullberg 1996)

The characters that were measured in the two nutrient environments did not show a large amount of genotype × environment interaction, indicating that with respect to utilization of nutrients and for these particular characters it may be sufficient to select in a single environment. For other characters such as weight and number of shoots genotype × environment interaction has been shown (Rönnberg-Wästljung et al. 1994).

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