Hybrid performance and species crossability relationships in willows (*Salix*)

A. MOSSELER

Forestry Canada, Newfoundland and Labrador Region, P. O. Box 6028, St. John's, Nfld., Canada A1C 5X8

Received July 24, 1989

MOSSELER, A. 1990. Hybrid performance and species crossability relationships in willows (*Salix*). Can. J. Bot. **68**: 2329–2338.

Interspecific hybridization between *Salix amygdaloides* Anderss., *S. bebbiana* Sarg., *S. discolor* Muhl., *S. eriocephala* Michx., *S. exigua* Nutt., *S. lucida* Muhl., *S. pellita* Anderss., and *S. petiolaris* Smith revealed several forms of postzygotic inviability including seed incompatibility, seedling inviability, inferior growth performance, and reduced fertility in the F_1 hybrid progeny. The proportion of inviable and inferior progeny varied with the species combination and the specific combining ability of genotypes within each species combination. Family mean growth performance was lower in most interspecific hybrids, but several hybrid combinations produced progeny superior to the average performance of intraspecific crosses involving the same parent species. Interspecific hybrid families were also characterized by high proportions of nonflowering individuals. Viable F_1 interspecific hybrids generally suffered reduced male and female fertility, but most female F_1 hybrids produced viable open pollinated seed. The relatively high fertility and viability demonstrated by some interspecific hybrids indicates that natural interspecific gene flow between several species of subgenus *Vetrix* is possible. Crossability relationships based on F_1 hybrid viability suggest that *Salix exigua* is more closely related to species of subgenus *Vetrix* than to those of subgenus *Salix*.

MOSSELER, A. 1990. Hybrid performance and species crossability relationships in willows (*Salix*). Can. J. Bot. **68**: 2329–2338.

L'hybridation interspécifique entre les *Salix amygdaloides* Anderss., *S. bebbiana* Sarg., *S. discolor* Muhl., *S. eriocephala* Michx., *S. exigua* Nutt., *S. lucida* Muhl., *S.pellita* Anderss. et *S. petiolaris* Smith révèle plusieurs formes létales postzygotiques telles que l'incompatibilité séminale, la mort des plantules, la sous-performance de la croissance et une fertilité réduite de la progéniture hybride F_1. La proportion de la progéniture non viable ou inférieure varie avec la combinaison des espèces et le potentiel spécifique de combinaison des génotypes à l'intérieur de chaque combinaison d'espèces. La performance moyenne de croissance de la famille est plus faible dans la plupart des hybrides interspécifiques, mais plusieurs combinaisons hybrides produisent une progéniture supérieure à la performance moyenne des croisements intraspécifiques impliquant des parents de la même espèce. Les familles hybrides interspécifiques se caractérisent également par une forte proportion d'individus incapables de fleurir. Les hybrides interspécifiques de F_1 montrent une fertilité mâle ou femelle généralement réduite, mais la plupart des hybrides femelles F_1 produisent des graines viables suite à une pollinisation ouverte. La fertilité et la viabilité relativement élevées qui s'observent chez certains hybrides interspécifiques indiquent la possibilité qu'il existe un flux de gènes entre plusieurs espèces du sous-genre *Vetrix*. Les possibilités de croisement basées sur la viabilité des hybrides F_1 suggèrent que le *Salix exigua* serait plus fortement relié au sous-genre *Vertix* qu'aux espèces du sous-genre *Salix*.

[Traduit par la revue]

Introduction

The first manifestations of the genetic incompatibility that accompanies evolutionary divergence among closely related plants are observed as seed and seedling inviability and as inferior growth performance or reduced fertility in hybrid progeny (Stebbins 1950; Ayala 1978). The causes of these abnormalities are probably related to physiological or metabolic disorders resulting from genetic disharmony within the hybrid genome (Stebbins 1958). Hybrids between more closely related species generally suffer less severe expressions of hybrid inferiority or inviability (Levin 1978), suggesting that the level of crossability broadly reflects phylogenetic relationship (Jaynes 1964; Jones and Luchsinger 1979; Hans 1981). However, the relationship between crossability and phylogenetic affinity among sympatric congeners may be complicated by natural selection for reproductive isolation (selection against hybridization).

Incompatibility within the developing seed has been related to failed genetic interactions between the endosperm and the hybrid embryo in interspecific crosses (Brink and Cooper 1947; Johnston et al. 1980; Lin 1984; Munoz and Tyrene 1985; Ehlenfeldt and Hanneman 1988). Interspecific hybrid inviability and growth abnormalities expressed after germination may have a relatively simple genetic inheritance, as with heavy-metal tolerance in *Mimulus* (MacNair and Christie 1983)

and the leaf chlorosis observed in *Melilotus* (Sano and Kito 1978), or more complex modes of inheritance, as with tumour production in *Gossypium* (Philips 1976). Observations on reciprocal differences (depending on the direction of the interspecific cross) in hybrid viability have been reviewed by Stebbins (1958) and Levin (1978). Such reciprocal differences have been related to failed genetic interactions in enzyme products formed from nuclear encoded subunits of one species that fail to function properly in connection with molecular subunits encoded by the chloroplast genome of another species (Van der Meer 1974).

Reductions in fertility that often accompany interspecific hybridization may be caused either by genetic disharmony within the hybrid genome that results in a failure to differentiate floral parts or by disruptions in the processes leading up to meiosis (described as diplontic sterility by Stebbins 1958). However, the most common and best understood cause of hybrid infertility is the unequal segregation of chromosomes to the gametes resulting from failures in chromosome pairing (synapsis at metaphase) in meiosis (Swanson et al. 1981; Newell and Hymowitz 1983).

Growth and development in artificially produced interspecific hybrids among some of the more important North American willow (*Salix*: Salicaceae) species were investigated (*i*) to determine interspecific crossability relationships for a better

TABLE 1. Taxonomical classification of *Salix* species used in artificial hybridization (according to Dorn 1976)

Subgenus	Section	Species	Chromosome no. (2n)
Salix	Humboldtiana	S. amygdaloides Anderss.	38[a,c,d]
	Salicaster	S. lucida Muhl.	76[c,d]
	Longifoliae	S. exigua Nutt.	38[a,c,d]
Vetrix	Cordatae	S. eriocephala Michx.	38[c,d]
	Vetrix	S. bebbiana Sarg.	38[c]
		S. discolor Muhl.	76[a,d] 95; 114[b,c]
		S. petiolaris Smith	38[a,d]
	Vimen	S. pellita Anderss.	38[d]; 76[a]

[a]Suda and Argus (1968).

[b]Suda, unpublished data (personal communication from Dr. G. W. Argus, Vascular Plant Section, National Museum of Natural Sciences, Ottawa, Canada).

[c]Cited in Argus (1986)

[d]Love (1982).

understanding of phylogenetic relationships and for breeding purposes, (*ii*) to identify and quantify the different types of postzygotic reproductive barriers between species, and (*iii*) to explore the possibilities for natural hybridization and its potential effect on intraspecific variability in willows.

Materials and methods

Species and clones

Controlled pollinations were carried out between the following North American willow species: *Salix amygdaloides* Anderss., *S. bebbiana* Sarg., *S. discolor* Muhl., *S. eriocephala* Michx., *S. exigua* Nutt., *S. lucida* Muhl., *S. pellita* Anderss., and *S. petiolaris* Smith (Table 1). Most of the parent clones used in hybridization were collected from natural populations within a 100-km radius of Toronto, Ontario. All of the above species, with the exception of *S. pellita*, are sympatric within the collection area. The two female plants of *S. pellita* and several clones of *S. lucida* and *S. petiolaris* originated from the boreal forest ecoregion of northern Ontario.

Controlled pollinations and mating design

Controlled crosses were carried out in glasshouse compartments during the dormant season from January to April during 1983 and 1984. Dormant flower branches were potted directly into a soil medium consisting of PROMIX B (a commercial mixture of peat, vermiculite, and micronutrients) and washed mason's sand. The temperature of the glasshouse was maintained at 20 ± 5°C with a relative humidity of between 40 and 50%. Supplemental light from sodium vapour lights was used to maintain a 16-h photoperiod. Male and female plants were arranged in separate glasshouse compartments to prevent pollen contamination.

Willows are predominantly insect pollinated, and the stickiness of pollen makes it difficult to collect adequate quantities for storage. To accomplish artificial pollinations, male catkins were removed at anthesis for direct deposition of pollen on receptive female flowers. Since many of the willow species used in this study are seasonally isolated (Mosseler and Papadopol 1989), pollen anthesis and female receptivity in the different species was coordinated by staggering the planting of species over a period of several weeks to ensure a constant supply of fresh pollen as pistillate plants became receptive.

Each male catkin used in controlled pollinations was tested for *in vitro* pollen viability on a medium of 0.7% (w/v) agar, 5.0% (w/v) sucrose, and 0.001% H_3BO_4. Pollen viability was evaluated after 24 h under a dissecting microscope by a visual estimation of the proportion of pollen germinated using a five-point rating system as follows: (*i*) high — more than 66% of pollen germinated; (*ii*) medium — between 33 and 66% of pollen germinated; (*iii*) low — less than 33% of pollen germinated; (*iv*) very low — less than 5% germination;

and (*v*) nonviable — no germination. The large number of daily controlled pollinations required the use of a rapid test for screening viable pollen. Female flower receptivity in these species has been described by Mosseler (1989).

A factorial (full diallel) mating system was attempted between three pistillate and three pollen parents of each species. However, the lack of pollen parents in *S. bebbiana* and *S. pellita*, poor rooting ability in *S. bebbiana* and *S. discolor*, and the presence of only two pistillate clones of *S. pellita* did not permit a full diallel crossing scheme of nine genotype combinations per species. Every controlled cross involved a single pollen parent. No pollen mixtures were attempted.

Seed was collected from controlled crosses at the first sign of capsule dehiscence. Ten to 15 capsules were removed from each catkin to obtain the average number of seeds produced per capsule. Seed germination tests consisted of four samples of 25 seeds germinated in plastic disposable Petri dishes containing moist silica sand held in a growth chamber at 25°C. Germination counts were made within 4–8 days after germination, at which time the young germinants were approaching a height of 1 cm and were transferred to individual pots.

Assessment of hybrid viability and fertility

Hybrid seedlings were raised in a glasshouse until early to mid-August when seedling height was measured to the nearest millimetre. Height frequency distributions based on 25-mm height classes were prepared for each hybrid family to compare growth trends and patterns of variation between intra- and inter-specific families. Most families were between 90 and 120 days old at the time of measurement.

Hybrid families produced from artificial crosses made in 1983 were established in a nursery test in late May of 1984, after overwintering in cold storage. Hybrids produced during the 1984 breeding season were established in a separate, adjacent, nursery test in August 1984. Both nursery tests were established as randomized complete blocks, consisting of six blocks with four-tree plots per block, on a silty clay loam upland site that was irrigated during extended dry periods (Mosseler et al. 1988).

Height growth was measured at age 1.5 years (for hybrid families produced during the 1983 breeding season) and 2 years (for hybrids produced in 1984) after establishment in field tests. The aboveground woody biomass of hybrids produced in 1983 was harvested at ground level during the dormant season after two growing seasons to assess oven-dried biomass production. The fresh weight of the leafless, woody biomass of each plant was measured to the nearest 0.01 kg on an electronic load balance. Three of the main upright stems were randomly selected from two plants per block for the removal of a 20-cm stem section (wood and bark) from the branch centroid. Each of these stem sections was weighed to the nearest mg, dried at 105 ± 5°C for 24 h, and then reweighed for calculation of percent

TABLE 2. Proportion of successful controlled crosses (numerator) relative to the number of different genotype combinations attempted

	Species of pollen parent					
Species of female parent	SAM	DIS	ERIO	INT	LUC	PET
S. amygdaloides (SAM)	8/8	0/7	0/12	0/10	0/8	0/7
S. eriocephala (ERIO)	0/7	1/17	18/19	1/14	0/9	0/14
S. exigua (INT)	0/4	4/9	10/12	12/17	0/12	5/14
S. lucida (LUC)	0/8	0/12	0/16	0/14	15/17	0/13
S. pellita (SPEL)		4/4	0/1	0/1		
S. petiolaris (PET)	0/2	0/4	8/10	4/6	0/3	5/6

NOTE: Salix discolor (DIS) was not included as a pistillate parent because of poor rooting ability in flowering branches. Successful crosses were those producing viable interspecific F₁ hybrids.

TABLE 3. Number of seeds produced per capsule (seed set) and percent seed germination from controlled intra- and inter-specific Salix crosses

Species crossed	No. of genotype combinations	No. of seeds set per flower (mean ± SD)	% seed germination (mean ± SD)
S. exigua × exigua	10	15.5±3.89	86.5±9.05
S. exigua × discolor	3	10.5±2.41	81.2±11.87
S. exigua × eriocephala	6	15.9±3.09	89.3±3.62
S. lucida × lucida	8	17.0±3.57	80.5±15.53
S. lucida × amygdaloides	5	5.7±1.47	77.7±15.10
S. petiolaris × petiolaris	4	3.2±1.24	59.5±16.83
S. petiolaris × eriocephala	6	1.8±0.59	80.7±16.42
S. petiolaris × exigua	4	1.6±0.69	86.9±9.40

moisture content. The average moisture content for each hybrid family was used to calculate ovendry biomass.

Hybrid fertility was assessed in the 1983 hybrids after one full growing season in the nursery (spring 1985), and the 1984 hybrids were assessed in the spring of 1986 after 1.5 growing seasons in the nursery. Pollen viability was tested in vitro as described above, based on a sample of four catkins from each male hybrid. Female fertility was measured by counting seed set in 10 capsules from three catkins per pistillate hybrid. Seed germination tests on the 1983 hybrids were based on three samples of 100 seeds. The sample size for seed germination tests carried out on the 1984 hybrids was reduced to four samples of 25 seeds. The viability and fertility of the resulting open-pollinated F₂ progeny remains to be investigated.

Results and discussion

Seed maturation and germination

The success of controlled intra- and inter-specific pollination attempts (Table 2) producing viable F₁ hybrid plants and data on the mean number of seeds produced per capsule (Table 3) indicate levels of crossability between species. While reduced seed set was characteristic of most F₁ interspecific crossing attempts, this was not the case with S. exigua × eriocephala crosses, which suffered no significant reduction in seed set over intraspecific S. exigua crosses (Table 4). Reciprocal differences in crosses between S. eriocephala and S. exigua and between S. eriocephala and S. petiolaris (Table 2) probably result from the strong stigmatic barrier against foreign species pollen in S. eriocephala described by Mosseler (1989). The statistical significance of differences between intra- and inter-specific crosses (i.e., S. exigua × exigua vs. S. exigua × discolor) in number of seeds set per capsule and seed germination rates presented in Table 4 also partially reflect species cross compatibility. The general absence of significant differences in germination rate in contrast with the presence of such differences in seed set (Table 4) suggests that

seed set is a more sensitive indicator of the genetic compatibility of species.

Zygote–embryo inviability

In most cases of cross incompatibility, female catkins abscised shortly after pollination. With the exception of crosses in which S. amygdaloides was used as the pistillate parent, microscopic observations of pollination showed that catkin abscission was correlated with the failure of foreign species pollen to reach the ovules and initiate fertilization (Mosseler 1989). Catkins of S. amygdaloides were often retained to maturity following interspecific pollinations. Such catkins appeared to mature normally but released only empty seed upon capsule dehiscence. Although microscopic observations were not made to identify the sources of postzygotic inviability in interspecific crosses, microscopic observations of pollination revealed that S. lucida pollen was fully capable of fertilizing the ovules of S. amygdaloides (Mosseler 1989). Salix amygdaloides × lucida crosses always produced empty seed and appeared to be the only species combination in which fertilization was followed by zygote–embryo abortion, whereas reciprocal crosses (S. lucida × amygdaloides) produced abundant, highly germinable putative hybrid seed, but the germinants all suffered a fatal necrosis in the cotyledon stage of their development.

Hybrid viability and growth performance

Most interspecific crosses yielding mature seed also showed seed germination rates comparable to those of intraspecific crosses (Table 3). Following seed germination, the first signs of inviability in interspecific hybrids appeared as chlorotic and (or) rootless germinants. The more common abnormalities found in interspecific hybrid seedlings grown under glasshouse conditions (Table 5) were (i) dwarfed plants in which the main leader appeared to lose the apical dominance characteristic of

TABLE 4. ANOVA of number of seeds set per capsule and percent seed germination (arc-sine transformed) for controlled interspecific crosses

	Seed set per flower		Seed germination	
Source of variation	MS	$P > F$	MS	$P > F$
S. exigua × discolor	73.009** (12.799)	0.0342	0.037ns (0.051)	0.4079
S. exigua × eriocephala	0.564ns (12.884)	0.8371	0.002ns (0.034)	0.8184
S. lucida × amygdaloides	473.400*** (7.872)	0.0001	0.009ns (0.080)	0.7384
S. petiolaris × eriocephala	4.704* (0.793)	0.0409	0.252ns (0.590)	0.0727
S. petiolaris × exigua	5.611ns (1.010)	0.0565	0.368* (0.045)	0.0288

NOTE: Each ANOVA represents a set of interspecific crosses tested against intraspecific crosses involving the same pistillate species (1 degree of freedom in each comparison). Level of statistical significance is indicated as follows: ns, $P > 0.05$; *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$. Values in parentheses indicate experimental error.

TABLE 5. Abnormal morphological types observed within different interspecific hybrid combinations

Species crossed	No. of genotype combinations	Abnormal morphological types (%)				Normal plants[c]
		Twisted stems	Leaf form or colour[a]	Multiple stems[b]	Dwarf (<10 cm)	
S. bebbiana × exigua	1	29.2	20.8	18.8		37.5
S. eriocephala × discolor	1	10.2	14.3	44.9	16.3	27.1
S. eriocephala × exigua	1	19.4	23.6			55.2
S. exigua × discolor	2		50.0	22.9	65.3	24.3
S. exigua × petiolaris	4	6.4	12.9	77.0	40.5	11.1
S. exigua × eriocephala	6	0.2	6.7	27.4	51.2	29.4
S. petiolaris × eriocephala	7			43.0	17.7	42.0
S. petiolaris × exigua	2			45.6	44.4	22.3
S. pellita × discolor	4	14.5	22.5	7.6	8.0	73.0

NOTE: Percent abnormalities do not add up to 100% because some plants displayed a combination of abnormalities.
[a]Curled, wrinkled, dwarfed, or chlorotic leaves.
[b]Stems with multiple leaders and (or) profuse lateral branching.
[c]A single-stemmed plant greater than 10 cm in height with no visible deformities.

normal willow seedlings, resulting in a stunted, round bushy plant that usually failed to grow more than 10 cm in height, (ii) plants with curled or wrinkled leaves, (iii) plants with curved or twisted stems and excessive lateral branching, and (iv) plants with chlorotic or otherwise discoloured leaves.

The frequency distributions developed from early seedling height growth under glasshouse conditions (Fig. 1) present important information about the response to hybridization, demonstrating the greater within-family variation in growth performance and the high proportion of inferior progeny that often characterized interspecific crosses such as S. exigua × discolor (Figs. 1U, 1V), S. exigua × eriocephala (Figs. 1G–1I), S. exigua × petiolaris (Fig. 1L), and S. petiolaris × eriocephala (Fig. 1M). The bimodal frequency distributions shown in Figs. 1H and 1L reflect two different growth responses within the same interspecific hybrid families. Such families consisted of a group of distinctly inferior progeny and a group that showed relatively normal growth performance. Differences in growth performance observed between families sharing either a common pollen parent (Figs. 1G, 1H) or a common pistillate parent (Figs. 1G, 1J) demonstrate the important genetic effect of the specific combining ability of genotypes in interspecific hybridization. The height frequency distributions presented in Fig. 1 demonstrate the genetic incompatibility that often accompanied interspecific hybridization in willows.

Intraspecific hybrid families displayed the expected normal distribution for seedling height growth (Figs. 1A–1F, 1O–1R), although the shape of these height distributions reveals species differences in variability. Intraspecific full-sib families of S. exigua were generally more variable than full-sib families of species like S. eriocephala, S. lucida, or S. petiolaris, which usually displayed very uniform, normal distributions for seedling height growth. Among the interspecific hybrid families, S. pellita × discolor (Figs. 1S, 1T) and S. bebbiana × petiolaris (Fig. 1X) produced a remarkably uniform, vigorous F_1 progeny, with a normal distribution for seedling height growth.

After approximately 3 months of growth under glasshouse conditions, seedlings were large enough (20-30 cm in height) to be established in field tests. Survival upon outplanting in mid-August was very high, and plants normally doubled in size before growth stopped in late October.

The height growth (Table 6) and the aboveground ovendry biomass production (Table 7) experienced by interspecific hybrids were generally lower and more variable than that of intraspecific hybrid families involving similar parents. Interspecific hybrids were also characterized by lower survival (Table 6). Despite the poor average growth performance shown by most interspecific hybrid families, the best performing hybrids often exceeded the average growth performance of intraspecific hybrid families involving their parents (S. eriocephala 28 × eriocephala 24 vs. S. eriocephala 28 × dis-

NUMBER OF SEEDLINGS
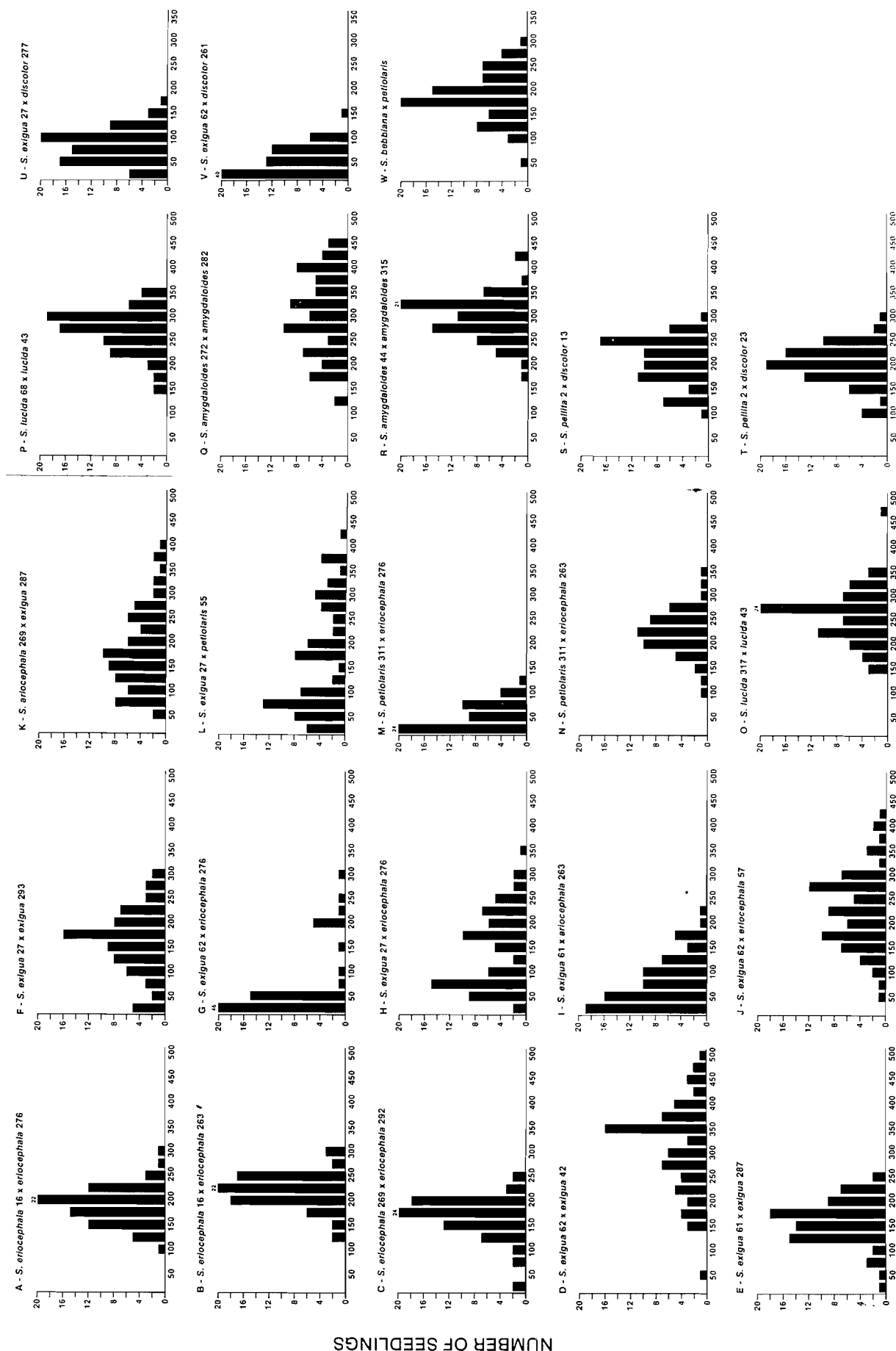
25-mm HEIGHT CLASSES

FIG. 1. Seedling height frequency distributions for F₁ hybrid families of *S. eriocephala* × eriocephala (A–C), *S. exigua* × *exigua* (D–F). *S. exigua* × *eriocephala* (G–J). *S. eriocephala* × *exigua* (K), *S. exigua* × *petiolaris* (L), *S. petiolaris* × *eriocephala* (M and N), *S. lucida* × *lucida* (O and P), *S. amygdaloides* × *amygdaloides* (Q and R), *S. pellita* × discolor (S and T), *S. exigua* × discolor (U and V), and *S. bebbiana* × *petiolaris* (W).

TABLE 6. Seedling height growth in hybrid *Salix* families

Species (numbers = genotype) combination	% survival	Height (cm) Mean ± SE	Range	C.V.
S. bebbiana 291 × *exigua* 287[a]	50	62 ± 13.8	16–163	76.8
S. bebbiana 304 × *petiolaris* 281[a]	100	190 ± 4.8	139–237	12.4
S. eriocephala 16 × *eriocephala* 24[b]	100	210 ± 5.3	170–263	12.4
S. eriocephala 28 × *eriocephala* 24[b]	96	206 ± 3.9	151–245	9.0
S. eriocephala 39 × *eriocephala* 25[b]	100	164 ± 3.2	135–201	9.5
S. eriocephala 28 × *discolor* 22[b]	83	117 ± 12.7	35–210	48.9
S. eriocephala 269 × *eriocephala* 263[a]	100	175 ± 4.2	140–220	11.9
S. eriocephala 269 × *eriocephala* 292[a]	100	154 ± 4.0	110–186	12.8
S. eriocephala 269 × *exigua* 287[a]	83	91 ± 9.3	21–137	45.7
S. exigua 61 × *exigua* 63[b]	96	225 ± 3.6	188–259	17.1
S. exigua 31 × *exigua* 66[b]	100	209 ± 4.3	168–250	10.1
S. exigua 62 × *eriocephala* 57[b]	75	116 ± 12.9	37–219	47.3
S. exigua 27 × *petiolaris* 55[b]	83	127 ± 10.2	63–188	36.1
S. exigua 62 × *petiolaris* 55[b]	71	98 ± 12.9	9–139	54.1
S. exigua 31 × *petiolaris* 55[b]	92	152 ± 10.4	43–226	32.0
S. exigua 17 × *petiolaris* 49[b]	50	157 ± 16.0	75–242	32.1
S. exigua 27 × *exigua* 66[a]	100	166 ± 4.4	125–210	12.8
S. exigua 27 × *exigua* 293[a]	100	172 ± 6.0	92–213	17.0
S. exigua 27 × *discolor* 277[a]	79	71 ± 11.7	19–159	71.3
S. exigua 27 × *eriocephala* 276[a]	92	113 ± 14.0	30–196	55.4
S. exigua 61 × *exigua* 66[a]	100	166 ± 4.6	130–214	13.3
S. exigua 61 × *exigua* 293[a]	100	134 ± 6.1	73–185	21.4
S. exigua 61 × *eriocephala* 263[a]	13	106 ± 40.6	64–187	66.2
S. exigua 61 × *eriocephala* 307[a]	46	59 ± 7.7	28–113	43.0
S. petiolaris 36 × *petiolaris* 33[b]	100	178 ± 3.2	145–210	8.7
S. petiolaris 36 × *eriocephala* 25[b]	100	171 ± 7.2	113–250	20.9
S. petiolaris 16 × *eriocephala* 57[b]	100	154 ± 4.9	110–182	15.5
S. petiolaris 04 × *eriocephala* 65[b]	92	114 ± 12.1	22–195	49.6
S. petiolaris 53 × *exigua* 42[b]	71	122 ± 10.8	36–186	36.7
S. petiolaris 311 × *petiolaris* 298[a]	96	145 ± 5.1	100–193	16.8
S. petiolaris 311 × *petiolaris* 323[a]	100	151 ± 5.1	102–200	16.5
S. petiolaris 311 × *eriocephala* 263[a]	92	56 ± 4.1	27–90	33.6
S. petiolaris 311 × *eriocephala* 292[a]	88	64 ± 6.8	28–124	48.4
S. petiolaris 311 × *eriocephala* 294[a]	67	90 ± 12.5	25–196	53.9
S. pellita 1 × *discolor* 13[b]	100	168 ± 7.0	77–229	20.5
S. pellita 1 × *discolor* 23[b]	96	149 ± 6.5	75–211	21.0
S. pellita 2 × *discolor* 8[a]	100	167 ± 6.8	89–208	19.8
S. pellita 2 × *discolor* 23[b]	100	169 ± 5.3	136–236	15.2

[a]Crosses made in 1984 and measured at age 1.5 years after establishment in field test.
[b]Crosses made in 1983 and measured at age 2.

color 22, *S. exigua* 31 × *exigua* 66 vs. *S. exigua* 31 × *petiolaris* 55, *S. exigua* 27 × *exigua* 66 × vs. *S. exigua* 27 × *eriocephala* 276, *S. petiolaris* 36 × *petiolaris* 298 vs. *S. petiolaris* 36 × *eriocephala* 25, etc.), demonstrating the potential ability of such interspecific hybrids to survive and compete effectively under natural conditions. Several interspecific crosses of *S. bebbiana* × *eriocephala*, *S. exigua* × *discolor*, *S. exigua* × *eriocephala*, *S. petiolaris* × *eriocephala*, and *S. petiolaris* × *exigua* produced too few viable progeny for inclusion in the replicated field tests but were established in unreplicated border row plots. These hybrid families showed similar trends in growth performance variation to those of the families presented in Tables 6 and 7.

Variation in ovendry biomass production (Table 7) was similar to that observed for height growth. Although more variable, the F_1 family mean ovendry biomass production in *S. petiolaris* 36 × *eriocephala* 25 approached that of the intraspecific family *S. petiolaris* 36 × *petiolaris* 33 (Table 7). Natural hybrids between these species have been identified in Ontario, and the growth performance observed in artificially produced hybrids demonstrates their potential ability to com-

pete under natural conditions. The rarity of naturally occurring hybrids between these species may be explained by differences observed in their habitat preferences. *Salix petiolaris* usually occupies seasonally inundated meadows and swamp borders, whereas *S. eriocephala* normally occupies the moist but better drained sites on floodplains, stream banks, and more elevated areas.

Interspecific hybrid families between *S. bebbiana* × *S. eriocephala* and *S. bebbiana* × *S. exigua* were extremely variable, resulting in large proportions of inviable and distinctly inferior progeny. However, several vigorous hybrid individuals of *S. bebbiana* × *eriocephala* were obtained. Among the 37 interspecific hybrid combinations assessed, the only interspecific combination producing a uniformly vigorous progeny family superior to that of the parental species came from the cross *S. bebbiana* 304 × *petiolaris* 281 (Table 7). The vigour demonstrated by this hybrid family and the phenological overlap in flowering period between the parental species (Mosseler and Papadopol 1989) suggests that natural hybridization is possible. However, to my knowledge, the existence of natural hybrids or hybrid swarms has not been described in the literature.

TABLE 7. Ovendry biomass production in hybrid *Salix* families

Species (numbers = genotype) combination	Dry weight (kg)		
	Mean ± SE	Range	C.V.
S. eriocephala 16 × *eriocephala* 24	1.04 ± 0.08	0.50–2.00	36.1
S. eriocephala 28 × *eriocephala* 24	1.12 ± 0.07	0.65–1.77	28.9
S. eriocephala 39 × *eriocephala* 25	0.58 ± 0.03	0.31–0.98	25.8
S. eriocephala 28 × *discolor* 22	0.23 ± 0.06	0.01–0.89	109.0
S. exigua 61 × *exigua* 63	0.61 ± 0.04	0.33–1.17	32.0
S. exigua 31 × *exigua* 66	0.44 ± 0.04	0.17–0.73	38.6
S. exigua 62 × *eriocephala* 57	0.18 ± 0.06	0.10–0.74	116.4
S. exigua 27 × *petiolaris* 55	0.18 ± 0.04	0.10–0.50	83.5
S. exigua 17 × *petiolaris* 49	0.26 ± 0.08	0.10–0.84	100.9
S. exigua 62 × *petiolaris* 55	0.11 ± 0.04	0.01–0.48	129.9
S. exigua 31 × *petiolaris* 55	0.22 ± 0.01	0.01–1.05	106.9
S. petiolaris 36 × *petiolaris* 33	0.52 ± 0.05	0.24–1.37	45.6
S. petiolaris 36 × *eriocephala* 25	0.50 ± 0.06	0.03–1.12	55.4
S. petiolaris 16 × *eriocephala* 57	0.31 ± 0.03	0.05–0.77	53.3
S. petiolaris 04 × *eriocephala* 65	0.35 ± 0.07	0.01–1.05	91.0
S. petiolaris 53 × *exigua* 42	0.11 ± 0.03	0.02–0.40	101.0
S. pellita 1 × *discolor* 13	0.40 ± 0.05	0.01–0.75	58.5
S. pellita 1 × *discolor* 23	0.28 ± 0.04	0.03–0.78	60.2
S. pellita 2 × *discolor* 08	0.30 ± 0.04	0.05–0.66	62.9
S. pellita 2 × *discolor* 23	0.35 ± 0.04	0.05–0.78	51.0

Viable interspecific hybrids were not obtained from any crosses involving *S. amygdaloides* or *S. lucida*. Viable hybrid seed with high germination rates was produced from most *S. lucida* × *amygdaloides* crosses that were attempted, but the young germinants, which grew normally for several days following seed germination, suffered a fatal necrosis in the cotyledon stage of their development and died shortly thereafter. Attempts to isolate an infectious causal agent on Difco prepared malt agar medium failed to identify a pathogen that could be consistently associated with the disease symptoms. This severe case of hybrid inviability appeared to result from a physiological disorder unique to *S. lucida* × *amygdaloides* crosses and was not observed in other interspecific crosses.

Interspecific crosses involving *S. eriocephala* as the pistillate parent rarely produced seeds because of the stigmatic barrier observed in this species (Mosseler 1989). The only confirmed hybrid families resulted from single crosses with *S. discolor* and *S. exigua*. Hybrid progeny were extremely variable in growth performance (Table 6) and leaf morphology.

Most of the interspecific crosses involving *S. exigua* as the pistillate parent produced abundant, viable seed. Crosses between *S. exigua* × *petiolaris* displayed a high proportion of inviable and inferior progeny during early seedling development and rarely produced progeny capable of survival under natural conditions. Crosses in which *S. discolor* or *S. eriocephala* were used as the pollen parents also produced progeny that were highly variable in growth performance (Tables 6, 7), with large proportions of inferior progeny and few viable hybrids. The same general pattern of variation in growth performance was observed in crosses of *S. petiolaris* × *eriocephala* and *S. petiolaris* × *exigua* (Tables 6, 7). These crosses resulted in reduced seed set, which nonetheless had germination rates comparable with intraspecific control crosses (Table 3). Although *S. exigua* can be artificially crossed with most of the species of subgenus *Vetrix* used in this study (Fig. 2), premating phenological isolation (Mosseler and Papadopol 1989) is probably strong enough to prevent these matings under natural conditions.

Salix pellita was highly crossable with *S. discolor* (as pollen parent) and each of the four crosses attempted in 1983 produced vigorous hybrid families with some exceptional individual hybrids (Figs. 1S, 1T; Tables 6, 7). Although a certain amount of genetic incompatibility was observed between these species in the field tests, the high proportion of vigorous progeny from these crosses and their phenological compatibility suggest that natural hybridization between these species is probable.

Hybrid fertility

Reductions in fertility often accompany interspecific hybridization (Stebbins 1950; Potts and Reid 1985; Ashton and Sandiford 1988). The lack of vigour demonstrated by many interspecific willow hybrids probably prevented some plants from reaching sexual maturity, and thus only a preliminary assessment of F_1 hybrid fertility was possible. Pollen viability in the F_1 hybrids (Table 8) ranged from high in *S. pellita* × *discolor* hybrids to very low in most hybrids involving *S. exigua* as the pistillate parent. In the latter crosses, the anthers appeared to develop normally up to the point of anthesis, when they suddenly turned brown and collapsed without shedding viable pollen. Some residual male fertility was observed in most interspecific hybrids.

Preliminary observations on open-pollinated seed set (Table 9) in catkins from F_1 interspecific hybrids demonstrated their ability to produce viable seed. Reductions in seed set observed in many of the smaller hybrid plants probably reflect their sexual immaturity as well as losses in fertility related to the effects of hybridization. Interspecific hybridization reduced fecundity in crosses involving *S. bebbiana, S. eriocephala*, and *S. exigua* while increasing seed set in many hybrids in which *S. petiolaris* was the pistillate parent. Increased seed set in *S. petiolaris* × *eriocephala* hybrids reflects the hybrid nature of a quantitative trait resulting from hybridization between two species with very different potentials for seed production per flower capsule. The number of seeds produced per capsule varied from a low of 2 in *S. petiolaris* to a high of 21 in *S. exigua*. A more meaningful statistical analysis of

TABLE 8. *In vitro* pollen viability in F_1 hybrids

Species combination	No. of genotype combinations	No. of male genotypes observed in the following pollen germination classes:				
		high	medium	low	very low	nonviable
S. bebbiana × eriocephala	1			1		
S. eriocephala × eriocephala	8	64		2		
S. eriocephala × discolor	1	1	2		1	1
S. exigua × exigua	9	88		1		
S. exigua × discolor	3	3	1	4	2	1
S. exigua × eriocephala	3		1	9	9	1
S. exigua × petiolaris	4				5	11
S. petiolaris × eriocephala	3	9	1	1		1
S. pellita × discolor	2	12	2	1		

TABLE 9. Number of open-pollinated seeds per capsule and percent seed germination (means ± SE) in the pistillate parent and several of its F_1 interspecific hybrid progeny

Pistillate parent and F_1 hybrid progeny	No. of seeds per capsule	% seed germination
S. *bebbiana* 291	4.2±0.3	90.0±4.2
BEBB 291 × ERIO 292	3.9±0.3	83.0±5.0
	3.2±0.2	73.0±8.8
BEBB 291 × INT 287	0.7±0.1	31.0
	0.2±0.1	66.6
S. *bebbiana* 304	7.4±0.5	85.0±3.4
BEBB·304 × PET 281	1.9±0.1	79.0±2.5
	2.2±0.2	81.0±1.9
	1.9±0.1	70.0±2.6
S. *eriocephala* 28	15.2±0.6	75.0±5.0
ERIO 28 × DIS 22	1.8±0.3	73.0±3.0
	0.7±0.2	39.0±8.4
S. *exigua* 27	12.5±0.7	90.0±4.8
INT 27 × DIS 277	3.3±0.2	75.0±1.9
	3.4±0.2	79.0±4.1
	7.7±0.2	72.0±4.9
S. *exigua* 62	20.9±0.7	84.0±7.1
INT 62 × DIS 277	1.9±0.3	55.0±13.3
S. *petiolaris* 36	3.2±0.2	64.0±9.8
PET 36 × ERIO 25	0.3±0.1	40.0
	2.9±0.2	68.0±5.9
	1.5±0.1	56.0±5.2
	1.5±0.1	74.0±8.4
S. *petiolaris* 4	3.3±0.2	19.0±4.1
PET 4 × ERIO 65	4.2±0.1	46.0±9.6
	5.1±0.2	23.0±3.8
	3.2±0.3	78.0±2.6
	2.0±0.3	63.0±10.2
S. *petiolaris* 311	2.0±0.1	76.0±5.9
PET 311 × ERIO 263	2.8±0.2	42.0±8.4
	3.8±0.3	70.0±4.7
	3.1±0.2	32.0±12.6
PET 311 × ERIO 276	3.6±0.2	40.0±5.6
	2.7±0.2	67.0±1.9
	4.9±0.2	62.0±3.5
PET 311 × ERIO 292	4.8±0.3	65.0±4.4
	4.6±0.3	58.0±9.4
	4.5±0.2	48.0±8.2
PET 311 × ERIO 307	3.8±0.2	83.0±2.5
	2.8±0.2	80.0±3.3
	7.0±0.2	86.0±5.0
PET 311 × INT 287[a]	2.6±0.2	74.0±7.6

NOTE: BEBB, S. *bebbiana*; DIS, S. *discolor*; ERIO, S. *eriocephala*; INT, S. *exigua*; and PET, S. *petiolaris*.

[a]Two other pistillate hybrids that were tested produced flowers but without setting viable seeds.

differences in number of seeds set (losses in fertility) resulting from interspecific hybridization must await the full sexual maturity of the hybrid plants. Nevertheless, reduced fertility is expected to result from interspecific hybridization. Despite the low seed set in many hybrids, most female hybrids retained the ability to produce some viable F_2 generation seed, indicating that advanced generation breeding and interspecific gene flow was possible. The early growth performance of the open-pollinated F_2 generation also appeared to develop normally under glasshouse conditions. However, none of the F_2 hybrid plants have yet been field tested for evidence of hybrid breakdown in subsequent generations (Connor 1983).

Conclusions

Hybridization followed by introgression of genes between species may be an important source of genetic variation for evolution (Endler and McLellan 1988) and breeding (Allard 1960). Premating barriers between willow species include seasonal (phenological) isolation (Mosseler and Papadopol 1989) and pollen–pistil incongruity (Mosseler 1989). Postzygotic barriers such as the zygote–embryo abortion, seedling inviability, inferior hybrid growth performance, and reduced fertility observed in most interspecific willow hybrids would restrict natural interspecific gene flow in *Salix*. The hybrid dysgenesis observed in artificial interspecific hybrids would result in important competitive disadvantages, and such hybrids would be selected against in nature. Nevertheless, the high viability and fertility demonstrated by some interspecific hybrids indicate that natural gene flow is possible. Even rare occurrences of such gene flow could have important ecological and evolutionary effects on within-species variability. The effects of natural interspecific gene flow on species variability and evolution in *Salix* is difficult to assess, but this possibility exists in crosses such as S. *bebbiana* × *petiolaris*, S. *pellita* × *discolor*, and S. *petiolaris* × *eriocephala*, in which reproductive barriers appear to be relatively weak. Interspecific gene flow may be responsible for the morphological variability that is observed in some willows, and the crossability relationships presented in Fig. 2 may help to explain some of this variation in future attempts to identify species and putative hybrids.

These crossability relationships (Fig. 2) expand on relationships established by Argus (1974) that demonstrate the general ease of crossability within the taxonomic subgenus and the relative strength of breeding barriers between the subgenera *Salix* and *Vetrix* (as classified by Dorn 1976). However, S. *exigua* provides an obvious exception to the expected crossability relationships based on Dorn's (1976) classification sys-

high F₁ viability
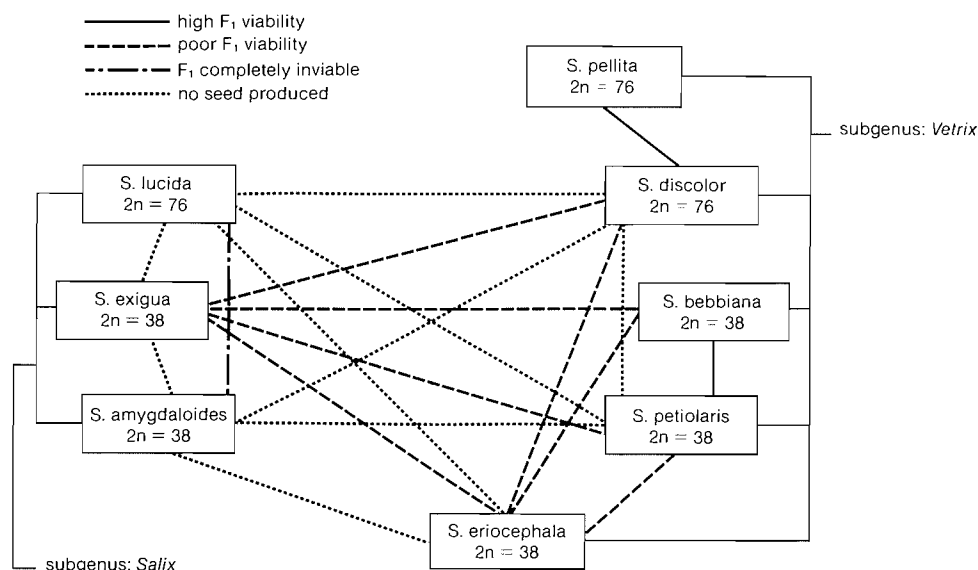poor F₁ viability
F₁ completely inviable
no seed produced

FIG. 2. Crossability relationships among eight North American willow species.

tem. The taxonomic placement of *S. exigua* within the subgenus *Salix* may need to be revised based on interspecific pollen–pistil congruity relationships (Mosseler 1989) and the viability of interspecific hybrids formed between *S. exigua* and species of subgenus *Vetrix*. Subsequent studies of variation in DNA may be helpful in clarifying phylogenetic relationships among these species.

Interest has been growing in the potential of fast-growing species of *Salix* as a source of biomass for conversion into energy and chemicals (Ranney *et al.* 1987). Interspecific hybridization may be useful in the genetic improvement and development of superior clones (Zsuffa *et al.* 1984). For the plant breeder, it is important to identify the nature of reproductive barriers because they determine the ease with which breeding barriers can be overcome through artificial means such as embryo rescue (Gill and Bajaj 1984; McCoy 1985). As a breeding method for the genetic improvement of species, interspecific hybridization has been useful for introducing genetic variation into a limited gene pool for the purpose of selecting more desireable variants (Allard 1960). With few exceptions, interspecific hybridization in *Salix* is unlikely to provide direct genetic gains in growth performance unless such genetic gains arise through the transmission of genes for disease resistance following selection from recurrent backcrosses. Preliminary observations of species differences in susceptibility to leaf diseases, such as *Melampsora* sp. (leaf rust) infections, provide some promise for the use of interspecific hybridization in resistance breeding. The high levels of fertility in many interspecific hybrids indicate that advanced generation breeding will be possible.

Acknowledgements

I gratefully acknowledge the support of my Ph.D. supervisor Louis Zsuffa; assistance from the nursery staff at the Ontario Tree Improvement and Forest Biomass Institute; valuable assistance and comments from George Argus throughout this study; and technical assistance from Ellen Mosseler and Setsuko Mosseler with various aspects of this research. Funding for this research was provided by the Canadian Forestry Service in the form of a postgraduate scholarship and by the Energy from the Forest program.

ALLARD, R. W. 1960. Principles of plant breeding. John Wiley & Sons, New York.

ARGUS, G. W. 1974. An experimental study of hybridization and pollination in *Salix* (willows). Can. J. Bot. **52**: 1613–1619.

—— 1986. The genus *Salix* (*Salicaceae*) in the southeastern United States. Syst. Bot. Monogr. **9**: 1–170.

ASHTON, D. H., and SANDIFORD, E. M. 1988. Natural hybridization between *Eucalyptus regnans* F. Muell. and *E. macrorhyncha* F. Muell. in the Cathedral Range, Victoria. Aust. J. Bot. **36**: 1–22.

AYALA, F. J. 1978. The mechanisms of evolution. Sci. Am. **239**(3): 48–61.

BRINK, R. A., and COOPER, D. C. 1947. The endosperm in seed development. Bot. Rev. **13**: 423–541.

CONNOR, H. E. 1983. *Cortaderia* (Graminae): Interspecific hybrids and the breeding systems. Heredity, **51**: 395–403.

DORN, R. D. 1976. A synopsis of American *Salix*. Can. J. Bot. **54**: 2769–2789.

EHLENFELDT, M. K., and HANNEMAN, R. E. 1988. Genetic control of endosperm balance number (EBN): three additive loci in a threshold-like system. Theor. Appl. Genet. **75**: 825–832.

ENDLER, J. A., and McLELLAN, T. 1988. The processes of evolution: toward a new synthesis. Annu. Rev. Ecol. Syst. **19**: 395–421.

GILL, M. S., and BAJAJ, Y. P. S. 1984. Interspecific hybridization in *Gossypium* through embryo culture. Euphytica, **33**: 305–311.

HANS, A. S. 1981. Compatibility and crossability studies in *Ulmus*. Silvae Genet. **30**: 149–152.

JAYNES, R. A. 1964. Interspecific crosses in the genus *Castanea*. Silvae Genet. **13**: 146–154.

JOHNSTON, S. A., DEN NIJS, T. P. M., PELOQUIN, S. J., and HANNEMAN, R. E., JR. 1980. The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. **57**: 5–9.

JONES, S. B., and LUCHSINGER, A. E. 1979. Plant systematics. McGraw-Hill Book Co., New York.

LEVIN, D. A. 1978. The origin of isolating mechanisms in flowering plants. Evol. Biol. **11**: 185–317.

LIN, B. Y. 1984. Ploidy barrier to endosperm development in maize. Genetics, **107**: 103–115.

LOVE, A. 1982. Chromosome number reports LXXIV. Taxon, **31**: 119–128.

MacNair, M. R., and Christie, P. 1983. Reproductive isolation as a pleiotropic effect of copper tolerance in *Mimmulus guttatus*. Heredity, **50**: 295–302.

McCoy, T. J. 1985. Interspecific hybridization of *Medicago sativa* L. and *M. rupestris* M. B. using ovule–embryo culture. Can. J. Genet. Cytol. **27**: 238–245.

Mosseler, A. J. 1989. Interspecific pollen–pistil incongruity in *Salix* L. Can. J. For. Res. **19**: 1161–1168.

Mosseler, A. J., and Papadopol, C. S. 1989. Seasonal isolation as a reproductive barrier among sympatric *Salix* spp. Can. J. Bot. **67**: 2563–2570.

Mosseler, A., Zsuffa, L., Stoehr, M. U., and Kenney, W. A. 1988. Variation in biomass production, moisture content, and specific gravity in some North American willows (*Salix* L.). Can. J. For. Res. **18**: 1535–1540.

Munoz, C. E., and Tyrene, P. M. 1985. Reproductive incompatibility barriers in crosses between *Vaccinium corymbosum* and *V. elliottii*. Can. J. Bot. **63**: 1987–1996.

Newell, C. A., and Hymowitz, T. 1983. Hybridization in the genus *Glycine* subgenus *Glycine* Willd. (*Leguminosae, Papilionoideae*). Am. J. Bot. **70**: 334–348.

Philips, L. L. 1976. Interspecific incompatibility in *Gossypium*. III. The genetics of tumorigenesis in hybrids of *G. gossypioides*. Can. J. Genet. Cytol. **18**: 365–369.

Potts, B. M., and Reid, J. B. 1985. Analysis of a hybrid swarm of *Eucalyptus risdonii* Hook. f. and *E. amygdalina* Labill. Aust. J. Bot. **33**: 543–562.

Ranney, J. W., Wright, L. L., and Layton, P. A. 1987. Hardwood energy crops: the technology of intensive culture. J. For. **85**: 18–28.

Sano, Y., and Kita, F. 1978. Reproductive barriers distributed in *Meliotus* species and their genetic bases. Can. J. Genet. Cytol. **20**: 275–289.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York.

—— 1958. The inviability, weakness and sterility of interspecific hybrids. Adv. Genet. **9**: 147–215.

Suda, Y., and Argus, G. W. 1968. Chromosome numbers of some North American *Salix*. Brittonia, **20**: 191–197.

Swanson, C. P., Merz, T., and Young, W. J. 1981. Cytogenetics. Prentice-Hall Inc., Englewood Cliffs, NJ.

Van der Meer, J. P. 1974. Hybrid chlorosis in interspecific crosses of *Oenothera*: polygenic inheritance of the nuclear component. Can. J. Genet. Cytol. **16**: 193–201.

Zsuffa, L., Mosseler, A., and Raj, Y. 1984. Prospects for interspecific hybridization in *Salix* for biomass production. *In* Ecology and management of forest biomass production systems. *Edited by* K. Perttu. Swed. Univ. Agric. Sci. Dep. Ecol. Environ. Res. Rep. Vol. 15. pp. 262–281.