

Water availability alters the relative performance of *Salix sericea*, *Salix eriocephala*, and their F₁ hybrids

Colin M. Orians, Daniel I. Bolnick, Bernadette M. Roche, Robert S. Fritz, and Ted Floyd

Abstract: Seedlings of *Salix eriocephala* Michx., *Salix sericea* Marsh., and their F₁ hybrids were tested for their growth under contrasting water conditions. One-year old seedlings were grown in pots and subjected to one of two experiments, a scheduled-watering experiment (watered every 1, 4, 8, or 12 days) or a dry-down experiment (no watering after an initial period without water limitation). In the scheduled-watering experiment, short-term growth of hybrids was suppressed relative to the parents. However, subsequent watering allowed hybrids to recover. By the end of the 48-day experiments, hybrids exhibited heterosis across all treatments. In the dry-down experiment, hybrids again were highly susceptible to the onset of drought conditions. From these results, we hypothesize that temporal and spatial variability in water availability could determine the survivorship of hybrids and the frequency of introgression; high water availability may enhance the relative performance of hybrids, while low water availability may limit hybrids.

Key words: Salicaceae, willows, hybridization, water relations, performance, heterosis.

Résumé : Les auteurs ont étudié la croissance de plantules des *Salix eriocephala* Michx. et *Salix sericea* Marsh. ainsi que leurs hybrides F₁, sous différents régimes hydriques. Ils ont cultivés des plants d'un an en pots et les ont soumis à un de deux types de traitements, soit l'irrigation périodique (arrosés tous les 1, 4, 8 ou 12 jours), ou soit le dessèchement (aucun arrosage après une période sans limiter l'eau). Avec les arrosages périodiques, la croissance à court terme des hybrides est inférieure à celle des parents. Cependant, les arrosages subséquents permettent aux hybrides de se reprendre. À la fin des 48 jours d'expérimentation, les hybrides montrent de l'hétérosis dans tous les traitements. Dans l'expérience de dessèchement progressif, les hybrides sont également susceptibles à l'apparition des conditions sèches. À partir de ces résultats, les auteurs formulent l'hypothèse que la variabilité de la disponibilité de l'eau dans le temps et l'espace pourrait déterminer la survie des hybrides et la fréquence de l'introgression; une grande disponibilité de l'eau peut augmenter la performance relative des hybrides, alors que le manque d'eau peut limiter les hybrides.

Mots clés : Salicaceae, saules, hybridation, relations hydriques, performance, hétérosis.

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Introduction

The ecological and evolutionary consequences of hybridization are diverse (Anderson 1953; Keim et al. 1989; Campbell et al. 1993; Arnold and Hodges 1995; Rieseberg

1995; Arnold 1997). If hybrids are fertile and survive to reproduce, hybridization may lead to speciation or may facilitate introgression, the transfer of genes between species. In light of the importance of hybridization, numerous recent studies have evaluated the relative resistance of hybrids and their parental taxa to biotic and abiotic factors (e.g., Whitham 1989; Strauss 1994; Arnold and Hodges 1995). These and other studies have shown that the relative resistance of hybrids to stress depends upon the inheritance and expression of numerous morphological, chemical, and physiological traits in hybrids (Rieseberg 1995; Arnold and Hodges 1995; Grant and Grant 1996).

Traditionally, the expression of traits in hybrid taxa was thought to be intermediate (Rieseberg 1995). However, recent studies indicate a diversity of patterns. Expression of a trait may be intermediate (additive), similar to one (dominance) or both parents (no difference), less than or greater than either parent (an extreme phenotype), or completely absent (Rieseberg and Ellstrand 1993; Fritz et al. 1994). Furthermore, environmental conditions can alter the expression of particular traits such that the relative performance of hy-

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brids may vary across environments (Arnold and Hodges 1995; Arnold 1997; Emms and Arnold 1997; Wang et al. 1997). For example, Wang et al. (1997) found that the performance of hybrid sagebrush was superior within the hybrid zone but inferior within the parental habitats.

Hybridization is extremely common among willows (Argus 1986; Thibault 1998). We have been exploring the consequences of hybridization between two willow species (*Salix sericea* Marsh. and *Salix eriocephala* Michx.) and have found that the expression of phenotypic traits in their hybrids is highly variable (Fritz et al. 1994; Orians and Fritz 1995) and can change with environment (Fritz et al. 1996; Orians and Floyd 1997). Recently we have begun to focus on the relative resistance of *S. sericea*, *S. eriocephala*, and their hybrids to variation in water availability. Willows and other members of the family Salicaceae are water-demanding species, and water availability has been shown to be a major determinant of growth and survival in this plant family (Price and Clancy 1986; Barros and Neill 1987; Bishop and Chapin 1989; Dawson and Bliss 1989a, 1989b; Sacchi and Price 1992; Liu and Dickmann 1993). For example, Sacchi and Price (1992) observed that low water availability was the primary cause of mortality during the first 2 years of growth in *Salix lasiolepis* Benth. However, willow species differ in their tolerance to water deficits (Dawson 1990). In our system, *S. eriocephala* is most common along rivers, while *S. sericea* is more common in marsh habitats. In areas where the two species co-occur, *S. sericea* is more common in the drier areas (personal observation). Since these species appear to differ in their tolerance to water deficits, the resistance of hybrids to water stress relative to the parental taxa would be expected to follow one of three patterns described earlier: intermediate, similar to one, or different from both of the parental taxa. Preliminary observations indicated that hybrids were more susceptible to drought than either parental taxa, indicating that water availability may be an important determinant of hybrid growth and survival. This study was designed to quantify the relative performance of *S. eriocephala*, *S. sericea*, and their F₁ hybrids to varying levels of water availability. We hypothesized that the growth of all three taxa would be similar when water availability is high, but that the growth of hybrids would be suppressed more than that of either parent under drought conditions.

Materials and methods

The system

Salix sericea is a 0.5–4.0 m high shrub that occurs predominantly in swamps in northeastern Canada and eastern United States (Argus 1986). *Salix eriocephala* is a 0.5–6.0 m high shrub that frequently occurs along streams in northeastern Canada and eastern United States (Argus 1986). The species often co-occur, and hybridization between the two is common (Argus 1974, 1986; Mosseler and Papadopol 1989). Genetic analysis of one population near Cooperstown, N.Y., demonstrated that, although introgression is occurring, the high frequency of pure parental genotypes may reflect hybrid disadvantage (Fritz et al. 1994).

To test whether water availability limits the performance of hybrids in this system, we performed two greenhouse experiments. We measured the growth of the three taxa (*S. sericea*, *S. eriocephala*, and their hybrids) under four different watering reg-

imes, and we tested how quickly the different taxa responded to the onset of drought.

Plants for the greenhouse experiments

We performed controlled crosses between *S. sericea* and *S. eriocephala* individuals that were known to have pure parental genotypes (from RAPD analysis by S.J. Brunsfeld, unpublished data) to yield six families of each taxon, pure *S. sericea*, pure *S. eriocephala*, and F₁ hybrid seedlings. Seedlings were germinated and grown in pots for one summer at our field site in New York. In the fall the dormant seedlings were transported to Williams College in Williamstown, Mass., where they overwintered.

The next year these 1-year-old seedlings were used in our experiments. We were not interested in testing the effects of family within each taxon, so we used the families as replicates; one sibling from each of the six different families was randomly assigned to each water treatment (described below). In May, the plants were transplanted into 20-L pots containing soil, peat, and vermiculite (3:1:1) and 30 g of slow-release Osmocote 14:14:14 N:P:K fertilizer (Grace Sierra Horticultural Products Co., Mirkpitas, Calif.). An initial fertilizer dose of 100 mL of fertilizer solution (2.5 g/L of water, Agway Sol-U-Green 20:20:20 N:P:K with micronutrients) was given to each seedling. This level of fertilizer addition resulted in growth rates typical of young plants. Plants were watered daily and kept in an open greenhouse on the roof of the Thompson Biology Laboratory at Williams College, Williamstown, Mass. Herbivores were largely absent on the roof, but any that were found were removed immediately. Analysis of photosynthetically active radiation (PAR) with a quantum sensor on a LI-COR 6200 portable photosynthesis meter indicated that the greenhouse did not unduly reduce PAR (1443 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in full sun, 1082 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the greenhouse). These willows reach photosynthetic saturation between 800 and 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (G. Meyer, unpublished data).

Scheduled-watering experiment

One plant of each family was assigned to each of four water treatments: high (HW), medium (MW), low (LW), and drought (DW) (6 replicates or families \times 3 taxa \times 4 treatments = 72 plants). Plants were arranged in an 8 \times 9 randomized block. We maintained a high water supply for 1.5 months and began the drought treatment on 13 June (day 0). The HW plants received 1 L each day; MW received 3 L every 4 days; LW received 3 L every 8 days; and DW plants were given 3 L every 12 days. We watered plants in a single pulse. The treatments continued for 48 days. We allowed plants to wilt, rather than deviate from watering schedules, since recovery from wilting is a component of drought responses. Our sample size for each treatment was only six, so our design is a conservative test and leaves open the possibility that significant differences could not be detected because of low statistical power. Nevertheless, striking differences were observed.

Water parameters

On days 24 and 48, we measured relative leaf water content (RWC) (Turner and Begg 1981) and predawn stem water potential (Ψ) (Hsiao 1973; Turner 1981; Kramer 1983) prior to watering. RWC was measured by cutting leaves (two per plant) at the petiole with a razor, immediately inserting them into a floral water pick containing distilled deionized water (Turner 1981; T. Whitlow, unpublished data). Leaves were then weighed within 1 min on a Mettler Balance to get fresh weight (W_f), reinserted in the water pick, and allowed to rehydrate at 5°C in the dark for 24 h. We reweighed the leaves to get saturated weight (W_s), and then dried the leaves at 85°C until dry and weighed them again (W_d). RWC values were calculated as

$$[1] \quad \text{RWC} = 100 \times \frac{(W_f - W_d)}{(W_s - W_d)}$$

Table 1. Significance of water treatment, plant taxon, and water \times taxon interaction effects for stem water potential and leaf relative water content (RWC) (using two-factor ANCOVAs with the initial number of leaves per plant as a covariate) in the scheduled-watering experiment.

Plant trait	Water effect		Taxon effect		Water \times taxon	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Day 24						
Water potential (MPa)	31.59	<0.001	1.79	0.18	1.00	0.43
Leaf RWC (%)	6.36	<0.001	0.07	0.93	0.22	0.97
Day 48						
Water potential (MPa)	23.25	<0.001	0.90	0.44	0.65	0.69
Leaf RWC (%)	6.85	<0.001	0.53	0.59	0.62	0.71

Predawn stem water potential was measured with a PMS pressure chamber model 610 (PMS Instrument Co., Corvallis, Oreg.) (Cleary and Zaerr 1986). A single stem from each plant was cut at the third leaf below the first fully expanded leaf. Stem removal was unlikely to have affected our results for two reasons. First, the willows are shrubby and each plant had many shoots (>30; see Table 3). Second, removal only occurred once during the experiment (day 24), and the second measurement was not taken until the end of the experiment. The chamber was pressurized at a rate of 0.1 MPa/10 s to minimize error. The effects of water treatment, plant taxon, and their interaction on RWC and water potential were analyzed with two-way ANCOVAs (type III sums of squares) with total leaf numbers as the covariate (SAS Institute Inc. 1989). The total number of leaves was used because plant size is positively correlated with water use rates and stress levels. Note that there was no difference among the taxa in the number of leaves per plant at the beginning of the experiment ($p = 0.21$). The Tukey–Kramer studentized range test was used to determine which taxa or treatments were contributing to the significant results (SAS Institute Inc. 1989).

The effects of these treatments on the relative availability of water in the potting soil was determined with a Lincoln Soil Moisture Meter (P.O. Box 67274, Lincoln, Neb.) (0–10 scale, with 10 equal to saturation). The probe was inserted to a depth of 10 cm at three points in each pot after calibration in a pot containing our potting soil saturated to field capacity.

Growth parameters

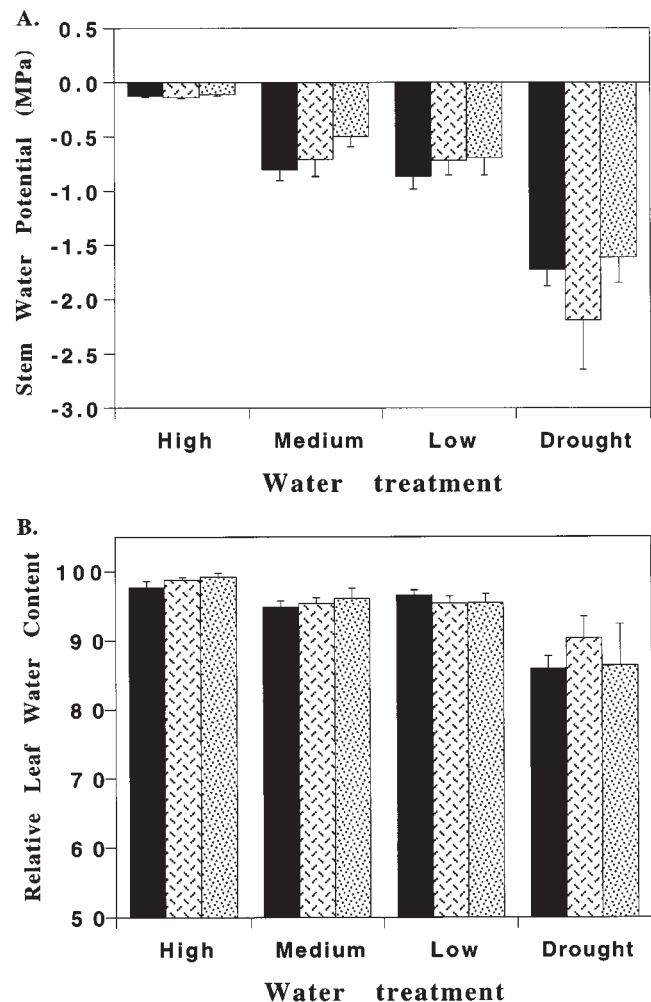
At the beginning and at the end of the experiment we counted the total number of leaves, the total number of shoots, and the total length of all shoots for each plant. At the very end, we harvested, dried and weighed the leaves, stems, and roots of each plant. Hypotheses were tested using two-way ANOVAs, with water treatment and taxon as the main effects, and differences among treatment means were determined using the Tukey–Kramer means comparison test.

During the experiment, on days 0, 12, 26, 36, and 46, we recorded stem length, the number of nodes, the number of leaves, and the number of lateral shoots on three randomly marked stems per plant and calculated stem growth rates, leaf senescence (number of leafless nodes divided by the total number of nodes), and lateral shoot production. We ran two-factor repeated measures MANCOVAs for the growth response that were repeatedly measured (SAS Institute Inc. 1989). Again, initial plant size (total number of leaves) was used as a covariate to control for size effects.

Dry-down experiment

One replicate plant of each of the families was assigned to the dry-down experiment (6 replicates \times 3 taxa = 18 plants). We regularly watered the 18 dry-down plants until 11 July and then terminated watering. Before the start of the dry-down, we counted the

Fig. 1. Mean (SE) stem water potential (A) and leaf relative water content (B) on day 24 for *S. eriocephala* (■), F₁ hybrids (▨), and *S. sericea* (▩) at each water treatment in the scheduled-watering experiment. Each value is the mean of six plants.



total number of leaves and stems, and measured the lengths of all stems on each plant.

After termination of watering, soil moisture and leaf expansion were monitored daily, and RWC and water potential were recorded regularly (see details below). Every day, we used the Lincoln Soil Moisture Meter to measure the relative availability of water. The probe was inserted to a depth of 10 cm at three points in each pot after calibration.

Table 2. Significance of water treatment, plant taxon, and water × taxon interaction effects on stem elongation, leaf senescence, and number of lateral shoots produced during the scheduled-watering experiment.

Plant trait	Water effect		Taxon effect		Water × taxon	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Stem elongation	54.51	<0.001	12.17	<0.001	2.75	0.02
Leaf senescence	58.79	<0.001	6.59	0.002	3.20	0.01
No. of lateral shoots	3.35	0.03	3.34	0.04	1.38	0.29

Note: Significance was determined with two-factor repeated measure MANCOVAs (with the initial number of leaves used as a covariate). Senescence was calculated as the number of nodes without leaves/total number of nodes.

Leaf expansion rate was measured to determine if the onset of drought differentially affected the three taxa. Two immature leaves on each of five stems per plant were marked, and leaf length was measured daily. When leaves neared maturity, markers were moved to younger leaves to insure that reduced expansion rates were a function of stress rather than maturity. No further measurements were taken once all the leaves wilted.

We measured predawn water potential and RWC as described earlier. Water potential and RWC values were measured on dry-down days 1, 4, 6, 8, 11, 14, 18 and 1, 4, 5, 6, 9, 11, 14, 15, respectively. Only a single measurement was made on each plant on each day, and because of the shrubby nature of these plants (≥30 stems per plant), relatively little area was removed.

Soil moisture, leaf water potential, RWC, and leaf expansion rates were analyzed with a two-factor repeated measure MANCOVA (SAS Institute Inc. 1989). Taxon, treatment, and taxon × treatment interaction effects were tested using starting numbers of leaves as a covariate. Differences among the means for each taxa were determined using the Tukey–Kramer means comparison test.

Results

Scheduled-watering experiment

Water parameters

There were no pretreatment differences in leaf RWC and stem water potential (*p* > 0.20). Water treatments significantly affected water potential and RWC at day 24 and 48 in all taxa, but there were no taxon effects or water × taxon interaction effects (Table 1). Up until day 24, plant water status was highest in the HW treatment and lowest in the DW treatment (Figs. 1A and 1B) (*p* < 0.05). There was no difference between the MW and LW treatments. At this point in the experiment, the growth of DW plants was severely affected (see results below). Interestingly, at the end of the experiment (day 48), mean water potential (MPa) was lower for the LW and MW than the HW and DW plants (*p* < 0.05) (HW: -0.19 ± 0.03 (mean ± SE); MW: -1.68 ± 0.21; LW: -1.63 ± 0.18; DW: -0.83 ± 0.13). This indicates that, once plants were watered on day 24, DW plants did not experience such severe water stress again. As before, there was no difference between LW and MW treatments (*p* < 0.05). Relative soil moisture at the end of the experiment also suggests that water was not as limiting for the DW plants (HW: 8.16 ± 0.53; MW: 1.47 ± 0.64; LW: 1.37 ± 0.61; DW: 4.63 ± 0.72; note that a value of 10 is saturated, and 0 is dry).

Plant growth

The growth of the marked stems during the first half of the experiment was determined by water treatment, taxon,

Fig. 2. Mean (SE) stem elongation for *S. eriocephala* (■), F₁ hybrids (▨), and *S. sericea* (▩) at each water treatment during the first 26 days of the scheduled-watering experiment. Three shoots were measured per plant and averaged; each value is the mean of six plants. Negative values reflect death and loss of meristems, resulting in decreased stem length.

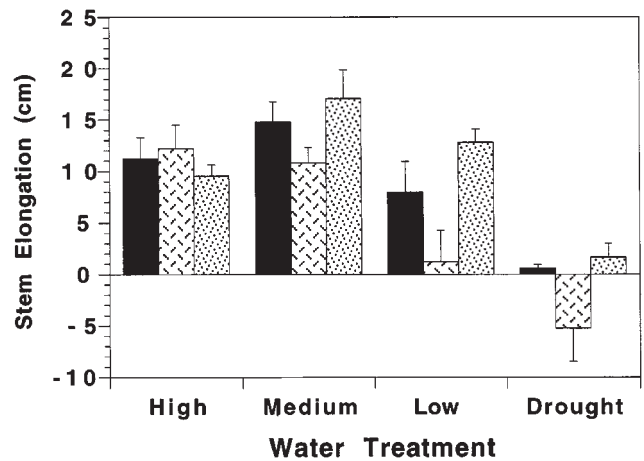
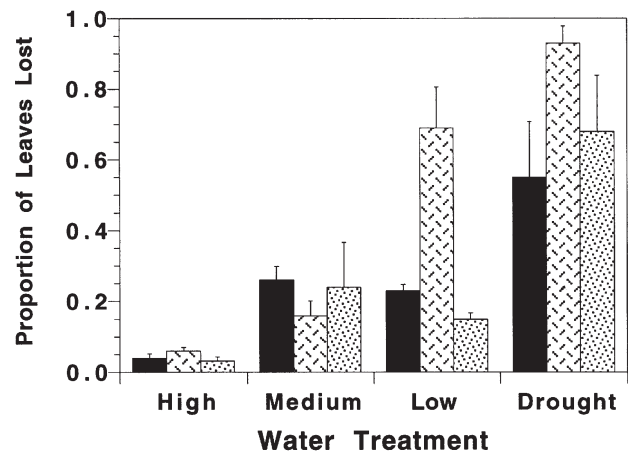


Fig. 3. Mean (SE) cumulative leaf senescence (the proportion of nodes on marked stems that had lost their leaf) for *S. eriocephala* (■), F₁ hybrids (▨), and *S. sericea* (▩) during the first 26 days of the scheduled-watering experiment. Three shoots were censused per plant and averaged, and each value is the mean of six plants.



and their interaction (Table 2, Fig. 2). Hybrids were more susceptible to conditions of limited water availability. Tukey–Kramer diagnostics indicated that, in the LW and DW treatments, the elongation of stems was significantly lower for the hybrids (*p* < 0.05). Stem elongation in hybrids

Table 3. Means (SE) and significance of water treatment, plant taxon, and water \times taxon (W \times T) interaction effects (ANOVA) on plant size parameters measured at the end of the scheduled-watering experiment.

Plant trait	Treatment	<i>S. eriocephala</i>	Hybrid	<i>S. sericea</i>		Significance		
						Water	Taxon	W \times T
Total no. of leaves	HW _a	339 (59)	713 (118)	510 (55)	<i>F</i>	5.44	5.59	0.76
	MW _a	436 (72)	581 (140)	486 (82)	<i>p</i>	0.002	0.01	0.61
	LW _b	288 (93)	431 (66)	464 (34)				
	DW _b	245 (32)	363 (101)	218 (73)				
Total shoot length (cm)	HW _{ab}	865 (177)	1472 (235)	1124 (137)	<i>F</i>	15.85	4.54	1.13
	MW _a	1326 (102)	1397 (161)	1344 (185)	<i>p</i>	<0.001	0.01	0.36
	LW _b	935 (102)	1154 (60)	1062 (76)				
	DW _c	635 (41)	753 (94)	590 (72)				
Total no. of shoots	HW	29.8 (9.2) _a	53.2 (2.1) _{ab}	36.5 (4.3) _a	<i>F</i>	3.45	4.44	2.55
	MW	66.7 (12.5) _a	52.5 (11.4) _b	44.6 (7.3) _a	<i>p</i>	0.02	0.02	0.03
	LW	48.0 (11.3) _a	89.5 (10.3) _a	45.0 (6.9) _a				
	DW	44.5 (2.4) _a	44.2 (6.4) _b	40.3 (6.3) _a				

Note: For total number of leaves and total shoot length, different letters indicate significantly different water treatment effects across all taxa using Tukey–Kramer tests ($p < 0.05$). Because of the significant interaction effect for total number of shoots, different letters indicate significant differences among water treatments for each taxon. Significance of taxon effects is presented in the text. HW, high water; MW, medium water; LW, low water; DW, drought.

Table 4. Means (SE) and effect significance (ANOVA) of overall plant size parameters as measured at the end of the scheduled-watering experiment.

Plant trait	Treatment	<i>S. eriocephala</i>	Hybrid	<i>S. sericea</i>		Significance		
						Water	Taxon	W \times T
Stem mass (g)	HW _a	46.3 (8.6)	47.9 (11.6)	30.1 (4.2)	<i>F</i>	23.32	0.012	1.38
	MW _a	45.0 (1.5)	36.6 (5.0)	38.8 (5.7)	<i>P</i>	<0.001	0.89	0.24
	LW _b	18.1 (3.0)	17.8 (1.0)	19.7 (3.0)				
	DW _b	11.3 (1.1)	16.6 (7.0)	8.5 (0.8)				
Leaf Mass (g)	HW _a	33.5 (4.1)	38.0 (7.0)	26.2 (2.3)	<i>F</i>	27.24	0.84	0.96
	MW _a	31.2 (3.5)	28.1 (4.3)	28.6 (2.4)	<i>p</i>	<0.001	0.44	0.46
	LW _b	14.2 (2.9)	14.0 (1.9)	16.1 (1.5)				
	DW _b	9.5 (0.8)	12.9 (5.4)	7.0 (0.8)				
Root mass (g)	HW _a	58.3 (14.0)	63.6 (19.9)	39.6 (8.5)	<i>F</i>	11.68	0.03	0.86
	MW _b	34.2 (1.7)	33.6 (6.6)	33.6 (5.2)	<i>p</i>	<0.001	0.97	0.53
	LW _c	19.0 (2.5)	11.8 (2.6)	9.6 (1.3)				
	DW _c	7.1 (0.7)	5.9 (0.5)	5.8 (1.1)				

Note: Different letters indicate significantly different water treatment effects across all taxa using Tukey–Kramer tests ($p < 0.05$). Significance of taxon effects presented in the text. HW, high water; MW, medium water; LW, low water; DW, drought.

Table 5. Means (SE) and significance of taxon effect on initial plant size (ANOVA) at the start of the dry-down experiment for the three taxa.

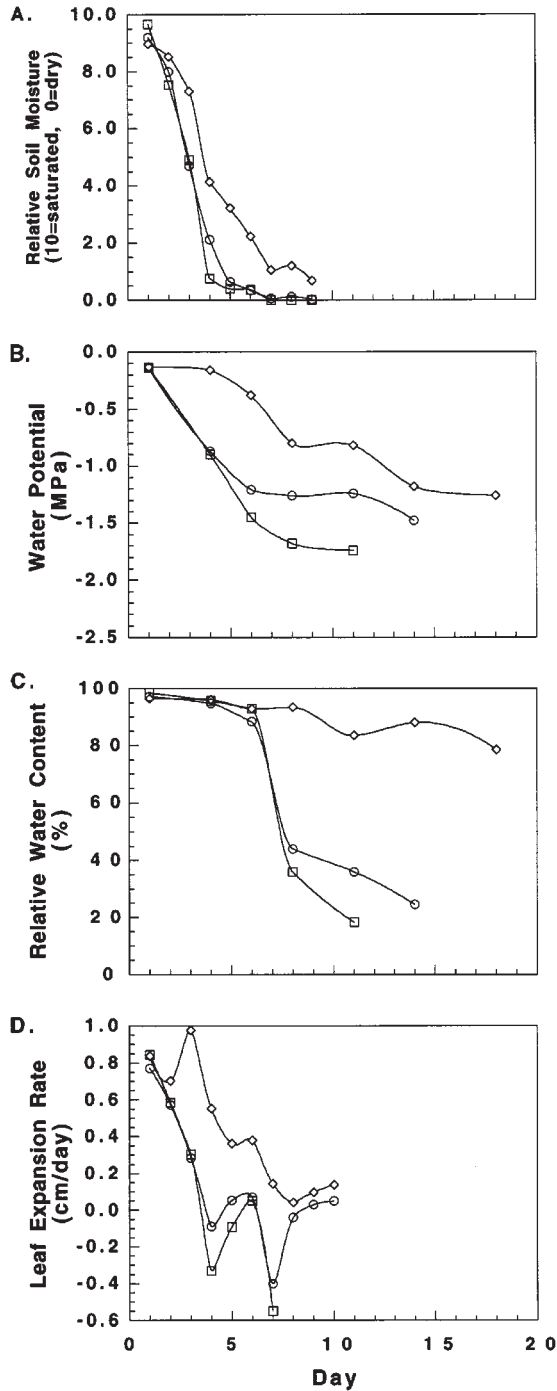
Plant trait	<i>S. eriocephala</i>	Hybrid	<i>S. sericea</i>	Significance	
				<i>F</i>	<i>p</i>
Starting no. of leaves	391 (48) _b	573 (60) _a	314 (36) _b	7.55	0.005
Total stem length (m)	1.01 (0.1) _b	1.26 (0.1) _a	0.65 (0.1) _c	5.03	0.02
No. of stems	29.5 (3.2) _b	45.3 (3.9) _a	30.7 (4.3) _b	4.64	0.02

Note: Different letters indicate significantly different taxon effects using Tukey–Kramer tests ($p < 0.05$).

was effectively halted under LW, and net stem loss occurred under DW because of the death of the apical meristems. No differences among the taxa were observed in the HW and MW treatments. Because of the loss of apical dominance and extensive production of lateral shoots midway through the experiment reduced stem elongation, we are not presenting the data on stem elongation after this point.

Leaf senescence was also determined by water, taxon, and their interaction. Overall, hybrids were also more likely than the parental species to lose leaves when water stressed (Table 2, Fig. 3). Tukey–Kramer diagnostic tests indicated that hybrids in the HW and MW treatments were comparable to *S. sericea* and *S. eriocephala* ($p > 0.5$), whereas hybrids in the LW and DW treatments lost a greater proportion of their

Fig. 4. Soil moisture (A), stem water potential (B), relative leaf water content (C), and leaf expansion rates (D) for *S. eriocephala* (○), *S. sericea* (◇), and F₁ hybrids (□) in the dry-down experiment. Watering ceased on day 0, and parameters were monitored as the plants deteriorated. Curves for each taxa end when leaf senescence, desiccation, or plant death precluded further measurement.



leaf area ($p < 0.05$). Hybrids lost approximately 70% and 95% of their leaves while parental taxa lost only 20 and 60% in the LW and DW treatments, respectively.

The production of lateral shoots off of the marked stems was determined by water treatment and taxon (Table 2).

More lateral shoots were produced in plants in the lower water treatments ($p < 0.05$) and hybrids produced more than the other taxa ($p < 0.05$). All three taxa averaged between one and two side-shoots per marked stem in the HW and MW treatments. At LW, *S. eriocephala*, hybrids, and *S. sericea* averaged 3.3 ± 0.9 , 12.7 ± 3.9 , and 8.9 ± 1.8 lateral shoots, respectively. At DW, *S. eriocephala*, hybrids, and *S. sericea* averaged 5.2 ± 0.9 , 7.9 ± 1.2 , and 5.4 ± 1.1 lateral shoots per stem.

Water stress dramatically reduced the growth and biomass accumulation of all three taxa (Tables 3 and 4). In general, leaf production, total shoot length, and biomass production was high in the HW and MW treatments and low in the LW and DW treatments. There was also a significant taxon effect on total leaf production, total shoot length, and total number of shoots (Table 3). In contrast to the stem elongation results described above, hybrids, at the end of the experiment, showed no signs of reduced growth relative to the parental taxa. In fact, there was evidence of heterosis (hybrid vigor). Hybrids had significantly more leaves ($p < 0.05$), significantly greater total stem length ($p < 0.05$), and often a greater number of shoots than either parent across all treatments (Table 3). The higher production of lateral shoots by hybrids (as reported above) and the high number shoots (Table 3) may have contributed to the enhanced growth at the whole-plant level. However, there was no effect of plant taxon or a plant taxon \times water treatment interaction on the accumulation of stem, leaf, or root mass (Table 4).

Dry-down experiment

Initial size

At the start of the experiment (11 July), hybrids had significantly more leaves, greater total stem length, and more stems (Table 5). Up until 11 July the plants received identical amounts of water and fertilizer. This suggests that hybrids grew faster than the two parental taxa and confirms the observation of heterosis in our scheduled watering experiment.

Response to acute water deficits

During dry-down there was a rapid decline in relative soil moisture, followed by a more gradual decline in stem water potential, leaf RWC, and leaf expansion rate (Fig. 4, Table 6). These measurements were also affected by taxon (all measurements) and taxon \times day interaction effects (relative soil moisture and RWC only) (Table 6). *Salix sericea* maintained higher stem water potential and leaf expansion rates than the other two taxa ($p < 0.05$). By day 4 there was extensive wilting of leaves on hybrid plants, as indicated by the negative leaf expansion rate (Fig. 4D). For relative soil moisture and RWC, there was no difference at the beginning but by day 3 (relative soil moisture) and day 8 (RWC), *S. sericea* differed ($p < 0.05$) (Figs. 4A and 4C). In summary, pots with *S. sericea* retained soil moisture longer than other taxa (Fig. 4A), and *S. sericea* maintained high stem water potentials and leaf RWC for a longer period of time (Figs. 4B and 4C) and retained positive leaf growth longer (Fig. 4D). In all taxa, leaf expansion ceased at stem water potential levels of about -0.7 MPa.

Table 6. Statistical effects of two-factor repeated-measure MANCOVAs (with starting number of leaves as the covariate), testing day, taxon, and day \times taxon interaction effects on soil moisture, stem water potential, leaf relative water content (RWC), and leaf expansion rate in the dry-down experiment.

Plant trait	Day effect		Taxon effect		Day \times taxon	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Soil moisture	162.40	<0.001	27.17	<0.001	2.01	0.02
Stem water potential	9.33	<0.001	10.79	<0.001	1.34	0.24
RWC	20.71	<0.001	13.59	<0.001	4.82	<0.001
Leaf expansion rate	14.94	<0.001	4.73	0.01	1.35	0.18

Discussion

As hypothesized, both experiments suggest that hybrids are highly susceptible to the onset of drought. In the scheduled-watering experiment, hybrids in the DW treatment had reduced stem growth rates, higher leaf senescence rates and slightly lower stem water potential (Figs. 1–3). In the dry-down experiment, the water status and leaf expansion rates of both hybrids and *S. eriocephala* quickly declined and leaf expansion of hybrids was negative by day 4, and no measurements could be taken after day 7 (Fig. 4).

Contrary to our hypothesis, if water availability increases following drought-induced mortality of the apical meristems or in the absence of drought, hybrids actually exhibit heterosis. By the end of the scheduled-watering experiment and at the beginning of the dry-down experiment, hybrids had more leaves, greater stem length, and more stems (Tables 3 and 5). Thus, hybrids produce more stems constitutively, suggesting reduced apical dominance. Furthermore, when drought kills the apical meristems, the induction of additional lateral shoots appears to allow hybrids to compensate for the initial drought-induced damage. Interestingly, hybrids did not have greater biomass at the end (Table 5). Perhaps the constant production of lateral shoots by hybrids means that their shoots are on average younger and less dense, or perhaps hybrids maximize vertical growth and invest less in woody tissue. This could help hybrids compete for light. However, the lack of strong apical dominance in hybrids would appear to counteract this potential benefit.

Obviously, if drought conditions are alleviated (as they were in our first experiment), hybrids are capable of rapid recovery. The production of lateral shoots appears critical to their recovery and even allows them to exhibit heterosis in the DW treatment. What would happen if the drought conditions were more sustained or more frequent? We suspect that the enhanced susceptibility of the hybrids to the onset of drought would lead to reduced growth and higher mortality. If so, we would expect hybrids to be more common in wetter habitats. We have preliminary evidence indicating this is the case, but further study is required.

Although the reason that hybrids are more susceptible to the onset of drought is unknown, preliminary results indicate that hybrids are relatively poor at regulating water. Unlike *S. sericea*, hybrids are poor regulators of their stomata (M. Holbrook, unpublished data). In response to gradual dry-down, *S. eriocephala* appears to maintain actively growing meristems by shedding the lower leaves on a stem (personal observation). Hybrids, in contrast, tend to lose their meristems. This was evident in the scheduled-watering

experiment; the hybrids had negative stem growth, while *S. eriocephala* did not. Furthermore, based on preliminary data, hybrids appear more susceptible to cavitation than either parental taxa (M. Holbrook, personal observation). This may explain why the apical meristems of hybrids died so readily in the DW treatment. Further investigation is necessary to isolate the importance of stomatal control, selected leaf drop (senescence), and cavitation on the relative resistance of these taxa to water stress.

Implications

Hybrids are clearly distinct from their parental taxa in their performance under varying levels of water availability. These differences may have several longer term fitness consequences that affect hybrid population dynamics and introgression. First, meristem loss may reduce the competitive ability of the hybrids. A bushy growth form is less likely to reach the canopy than a single stem. This would be particularly harmful for shade-intolerant species such as willows. Second, high senescence rates reduce carbohydrate supplies for growth. When carbohydrates are limited, the concentrations of secondary chemicals may go down, making plants more vulnerable to herbivores and pathogens (Du Toit et al. 1990; Kruger and Manion 1994). Third, the carbohydrate demand from regrowth may deplete reserves needed to overwinter and to initiate leaf and flower production the next growing season (Kigel 1980; Crawley 1983; Dickson and Isebrands 1991). Although germination is often cited as the growth stage with the highest mortality (White 1980; Argus 1986; Bishop and Chapin 1989; Raven 1992; Sacchi and Price 1992), first-winter survival is also crucial (Douglas 1995). Douglas (1995) found 89% overwinter mortality in *Salix setchelliana* Ball seedlings and suggested that a lack of carbohydrate reserves contributed to the high mortality.

We believe that light competition, increased vulnerability to enemies, and overwinter survivorship could limit the survival and reproduction of hybrids previously exposed to drought stress. Additional studies are needed to test whether these factors operate to reduce hybrid performance. If they do, any reductions in hybrid fitness will lower the frequency of backcrossing and slow introgression. In the absence of sustained drought stress, it is clear that hybrids exhibit heterosis, and this could increase the rate of both backcrossing and introgression. Perhaps introgression will be more common in wetter habitats, especially in sites that vary little in water availability from year to year.

Arnold and Hodges (1995) argue that studies of hybridization should focus on the performance of hybrids across an

environmental gradient. Until recently, such studies were rare (Emms and Arnold 1997; Wang et al. 1997). Both Emms and Arnold (1997) and Wang et al. (1997) demonstrated the importance of accounting for environmental conditions. For example, Emms and Arnold (1997) found that the performance of hybrids was similar to the parents in most habitats but superior in others. Here we have shown that water availability differentially affects the performance of hybrid and parental willows. Hybrids exhibit heterosis when water is not limiting, but when water is limiting their relative performance may be lower, a result consistent with the bounded hybrid superiority model of hybrid performance (Moore 1977). Clearly, as others have shown, the performance of hybrids must be evaluated in an environmental context. Environmentally dependent heterosis and suppression may affect the survival of hybrids and rate of introgression.

The availability of water is one of the most important determinants of the distribution of plant species (Hsiao 1973; Pickett and Bazzaz 1976; Grace 1993). The water relations of hybrids may be similar to or distinct from the parental species, so that their performance depends upon water availability. If so, spatial and temporal variation in water availability may often control the distribution of hybrids and the patterns of introgression.

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