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Establishment, Sex Structure and Breeding System of an Exotic Riparian Willow, Salix ×rubens

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ABSTRACT.—Several Eurasian tree willows (*Salix* spp.) have become naturalized in riparian areas outside of their native range. *Salix* ×*rubens* is a Eurasian willow that is conspicuous along streams in the high plains of Colorado. We examined establishment of seedlings and cuttings, the sex structure and the breeding system of *S.* ×*rubens*. An experiment was conducted on establishment and growth of seedlings and cuttings under a range of hydrologic conditions. Seedlings became established under all conditions except when flooded, although many fewer seedlings became established where soil surface conditions were relatively dry. Cuttings became established under all experimental conditions, but most frequently where soil moisture was highest. The sex structure of *S.* ×*rubens* was determined along several streams in the Colorado high plains. Of 2175 trees surveyed, >99% (2172) were female. *Salix* ×*rubens* produce viable seed apparently as a result of hybridization with another Eurasian willow, *S. alba* var. *vitellina*. *Salix* ×*rubens* often reproduces vegetatively, which, combined with low hybrid seedling survival in the field, may explain the unusual sex structure. *Salix* ×*rubens* will likely continue to spread vegetatively in high plains riparian areas, and the potential for spread through hybridization could increase if males of compatible *Salix* spp. are planted near extant *S.* ×*rubens*.

INTRODUCTION

Invasions of plant communities by exotic species have received considerable attention from ecologists and conservation biologists (*cf.*, Mooney and Drake, 1986; Drake *et al.*, 1989). Because of frequent natural and human-induced disturbance, riparian and wetland ecosystems are frequently susceptible to invasions by nonindigenous plants (Robinson, 1965; Olson and Knopf, 1986; Thompson *et al.*, 1987). *Salix* ×*rubens* Schrank (*S. fragilis* L. × *S. alba* L.) is a tree willow native to parts of Eurasia (Meikle, 1984). Several Eurasian willows, including *S.* ×*rubens*, *S. alba* (and related cultivars and varieties), *S. fragilis* and *S. babylonica* L. have been introduced to many areas outside of their native ranges including North America, New Zealand and South Africa, where they have since become naturalized along watercourses (Hough, 1907; Schopmeyer, 1974; Hathaway, 1976; Argus, 1986; Albee *et al.*, 1988; Weber, 1990; Henderson, 1991).

Salix ×*rubens* (fide G. W. Argus) and *S. alba* L. var. *vitellina* (L.) Koch were likely intro-

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duced to Colorado by settlers in the late 1800s or early 1900s. Local floras refer to *S.* ×*rubens* as *S. fragilis* (Weber, 1990). Presently, *S.* ×*rubens* and (to a much lesser extent) *S. alba* var. *vitellina* dominate riparian zones near towns and homesteads where they were planted, but are less abundant elsewhere. This pattern of occurrence has been previously described for *S. fragilis* × *S. alba* (Dwelley, 1980). This distribution suggests that these species, especially *S.* ×*rubens*, are slowly invading riparian communities in Colorado; however, reasons for this slow invasion are not understood.

The degree to which a species successfully colonizes and occupies sites outside its native range is dependent, in part, on the species' reproductive characteristics (Baker, 1974). In the present study, we examine the modes of reproduction of *Salix* ×*rubens* in Colorado to better understand its pattern of occurrence and to provide a basis for formulating effective management strategies for riparian ecosystems where *S.* ×*rubens* occurs. Specifically, we examine (1) establishment of seedlings and cuttings, (2) the sex structure, and (3) the breeding system of *S.* ×*rubens*.

METHODS

Experimental facility and design.—Two experiments, one examining establishment and growth of vegetative cuttings of *Salix* ×*rubens*, and one examining establishment and growth of seedlings of *S.* ×*rubens*, were conducted outdoors near Fort Collins, Colorado. The experimental apparatus included 15 steel tanks, 122 × 92 cm (diam × depth), each containing three, 30 × 100 cm (diam × depth) planters made of PVC pipe. The treatments in both experiments were hydrologic regimes and were applied to tanks. Three replicates of five hydrologic treatments were completely randomized. Seeds and vegetative cuttings were separately planted in one planter per tank (*see below*). The third planter in each tank was used for soil sampling. An experimental unit was a planter containing either seedlings or cuttings.

Hydrologic regimes.—The following treatments were imposed (Fig. 1):

(1) water table 5 cm above the surface from 15 April until 15 July 1992 followed by a drawdown of 0.4 cm/day for the remainder of the growing season;
(2) water table rising from 15 cm below the surface on 15 April to 2 cm below the surface on 20 May through 27 May, followed by a drawdown of 0.2 cm/day for the remainder of the growing season;
(3) water table rising from 32 cm below the surface on 15 April to 2 cm below the surface on 10 June through 17 June, followed by a drawdown rate of 0.7 cm/day for the remainder of the growing season;
(4) water table rising from 43 cm below the surface on 15 April to 15 cm below the surface on 10 June to 17 June, followed by a 1 cm/day drawdown rate until 14 July. On 15 July, the water table was raised to 5 cm above the surface, drawndown by 2 cm/day until 22 July, 1 cm/day from 23 July to 5 August, and 0.4 cm/day for the remainder of the growing season and
(5) water table rising from 51 cm below the surface on 15 April to 23 cm below the surface on 27 May to 17 June, followed by a 0.2 cm/day drawdown until 15 July, and a 0.1 cm/day drawdown rate until 19 August. The water table was not altered after 19 August and remained 34 cm below the surface.

These hydrologic regimes represent a range of conditions that might reasonably exist along regulated stream reaches in the high plains of Colorado. They are based on U.S. Geological Survey stream gauge data from April through October for the years 1988, 1990 and 1991, on regulated reaches of Boulder Creek (upstream reservoir) and the Cache la

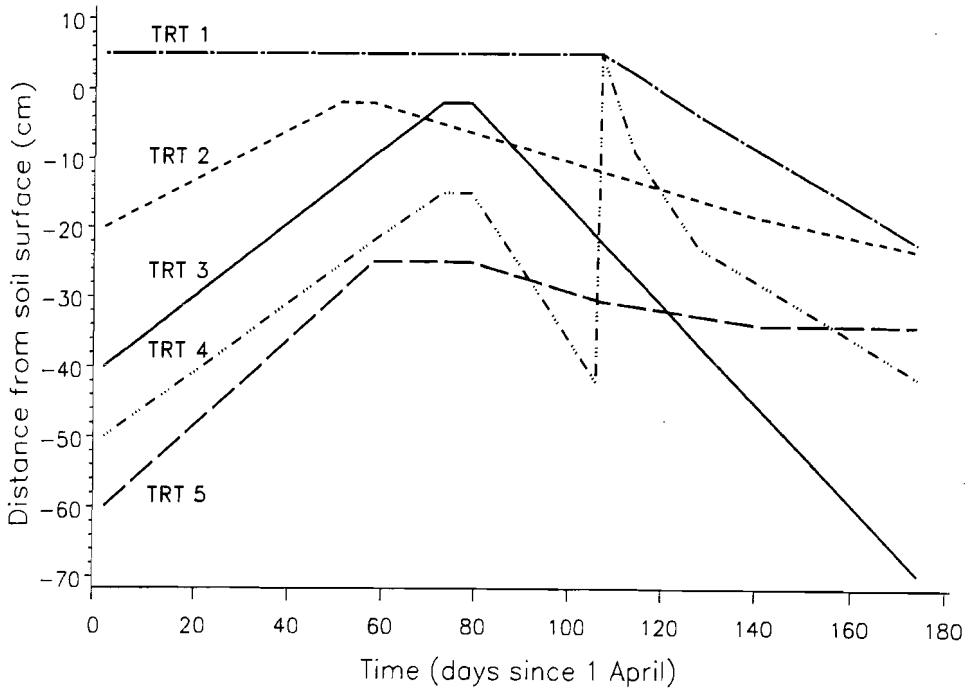

FIG. 1.—Distance of water table from soil surface in five hydrologic treatments over 180-day period. Cuttings were planted on day 15. Seeds were planted on days 48, 57 and 62

Poudre River (upstream diversions). In determining the hydrologic regimes, we also considered results from a similar experiment and did not impose conditions that were likely to result in 100% mortality (Segelquist *et al.*, 1993). We expected the hydrologic conditions in treatment 2 to enhance *Salix* ×*rubens* seedling establishment, because the hydrograph peak corresponded to *S.* ×*rubens* seed dispersal.

Water levels in the tanks were manipulated to simulate the five hydrologic regimes. The depth of the flooded tanks (treatments 2 and 4) was extended by wrapping galvanized steel flashing around the top of the tank for the period of inundation. Water levels were controlled by adding water (drawn from the Cache la Poudre River) to the tanks, or by draining water from faucets attached near the bottom of each tank. A series of holes 1.3 cm in diam were drilled into the lower 10 cm of each planter to allow water exchange. Otherwise, the planters were exposed to ambient climatic conditions.

Substrate properties.—Planters were filled to 92 cm with coarse sand mined from the floodplain of the Cache la Poudre River near Fort Collins, Colorado. Similar sandy substrates typically occur on river bars where species in the Salicaceae become established (Wilson, 1970; Ostercamp and Costa, 1987). Before the experiment, the sand substrate was analyzed to determine textural class, pH, salinity and concentrations of micronutrients as in Segelquist *et al.* (1993). The experimental substrate was composed of approximately 6% gravel (>2000 μm), 78% sand (>300–2000 μm), 16% fine sand (>75–300 μm), and less than 1% silt and clay. The pH ranged from 8.0 to 8.5. Based on separate analyses of Ca, Mg, Na, K and B, the sodium adsorption ratio (SAR) calculated for the sample with a pH of 8.5 was

only 1.2, indicating that salinity (Na) was not excessive for any substrate sample. For all samples, NO_3-N and P were ≤2 ppm, K was ≤15 ppm, and organic matter was <0.1 ppm.

Vertical moisture distributions in planters were estimated in order to examine the relationship between soil moisture and successful rooting of cuttings. In the laboratory, five planters were placed in each of two tanks. The same coarse sand used in the establishment experiments was air-dried at room temperature for 48 h and placed in the 10 planters to 10 different depths: 19.5, 24.5, 30, 38.5, 50, 60, 68.5, 81, 89.5 and 97 cm. The tanks were filled to 10 cm with water, and this level was checked and maintained on a daily basis. One week after the planters and tanks were filled, soil moisture was gravimetrically measured at 2-cm increments throughout each of the 10 planters.

Establishment and growth of seedlings and cuttings.—Seeds of *Salix* ×*rubens* were collected on two dates in 1992 (15 and 29 May), which spanned the natural seed dispersal period. On each date, 20–40 catkins nearing dehiscence were collected from each of 10–15 *S.* ×*rubens* trees along the Cache la Poudre River in Fort Collins, Colorado. These catkins were stored in a paper sack at room temperature for 24 h, which enabled most of the capsules to open. Seeds were separated from cotton by sifting collected material through a series of soil screens and stored in sealed plastic cups at 3 C until planted, which has been previously shown to preserve viability for 3–4 mo in *Salix* spp. (Zasada and Densmore, 1977). Seeds were sown in the same planter in each tank on three dates (18 May, 25 seeds; 27 May, 50 seeds; and 1 June, 25 seeds). Seeds collected on 15 May were sown on 18 May and 27 May, and seeds collected on 29 May were sown on 1 June.

To examine the effect of soil moisture on germination, a sample of the top 1 cm of sediment was taken from the supplemental planter in each tank at the time of planting, and the percent water was determined gravimetrically. To test the viability of planted seed, five replicates of 20 seeds were placed on moistened filter paper in petri dishes in an environmental chamber at 20 C and the number of successful germinants was counted after 72 h. A successful germinant had expanded cotyledons and an elongated hypocotyl.

The number of germinants in each planter was counted 72–96 h after planting (22 May, 30 May and 5 June). The number of living seedlings in each planter was counted four times during the growing season (18 June, 9 July, 7 August and 8 September). The number of cuttings with living leaves was counted on the following dates: 4 May, 20 May, 18 June, 9 July, 6 August and 8 September.

In March 1992, 1-yr-old twigs were collected from 15 *Salix* ×*rubens* trees along the Cache la Poudre River in Fort Collins, Colorado, and stored in plastic bags at 3 C. On 15 April, the twigs were cut to a length of 20 cm, and 20 twigs were planted to a depth of 10 cm in each of the 15 planters.

Above- and belowground portions of all seedlings and cuttings were harvested in mid-September. Eighty percent of the seedlings were recovered. In the laboratory, root and shoot lengths of every recovered seedling and cutting were measured, and roots and shoots were separated, oven-dried at 60 C for 72 h and weighed.

Data analysis.—Because most of the response variables exhibited nonhomogeneous variances that did not improve after various transformations, standard analysis of variance tests were not conducted. Instead, multiresponse permutation procedures for one-way completely randomized designs (MRPP) were used to evaluate the significance of treatment differences. MRPP do not require assumptions about normality or homogeneous variances, and use Euclidean distances, which are more congruous with the data space than standard linear models. The MRPP standardized test statistic (T), based on Euclidean distances, detects both shifts in central tendency and differences in dispersion (Biondini *et al.,* 1988; Zimmerman *et al.,* 1985). Seedling analyses other than end-of-season survival were restricted to

TABLE 1.—Number of male and female *Salix* ×*rubens* and *S. alba* var. *vitellina* along several Colorado watercourses

Watercourse	Reach length (km)	*S.* ×*rubens*		*S. alba*	
		♀	♂	♀	♂
Cache la Poudre R.	6.5	1065	3	1	56
Boulder Cr.	2.3	662	0	0	3
Cherry Cr.[a]	4.0	260	0	0	74
Plum Cr.[b]	1.6	185	0	1	15
Totals	14.4	2172	3	2	148

[a] Some trees along Cherry Cr. had shed their catkins at the time of this survey; hence, the numbers recorded here are probably low. Also, we observed apparent hybrids along Cherry Cr.

[b] We observed 28 apparent hybrids along Plum Cr.

the four treatments in which seedlings survived. Individual seedlings or cuttings were treated as subsamples. Growth response values were averaged within planters to produce a single value per experimental unit per response variable (sample size = 3 per treatment per experiment). A significance level of $P \leq 0.05$ was used in all tests.

Sex structure.—In late May and early June 1991, the sex of each flowering *Salix* ×*rubens* and *S. alba* var. *vitellina* individual was noted along selected reaches of four streams in Colorado: (1) Cache la Poudre River in the city of Fort Collins (40°34′45″N, 105°2′30″W); (2) Boulder Creek in the city of Boulder (40°1′15″N, 105°13′45″W); (3) Cherry Creek in the city of Denver (39°43′0″N, 104°58′30″W); (4) Plum Creek near the town of Sedalia (39°26′15″N, 104°58′45″W). Reach lengths varied from 1.6–6.5 km (Table 1). Individuals were defined as a single stem or collection of stems arising from a common base. All adult trees observed were flowering at the time of the surveys except along Cherry Creek, where some trees had already shed their catkins.

Breeding system study.—Results of the sex-ratio surveys suggest that seed set in female *Salix* ×*rubens* trees was likely a result of either agamospermy or hybridization with *S. alba* var. *vitellina*. To distinguish between these two possible breeding systems, seed set was compared in *S.* ×*rubens* trees growing in two contrasting situations: (1) female *S.* ×*rubens* trees growing at least 1.6 km from any male *S. alba* var. *vitellina* tree ("isolated"); and (2) female *S.* ×*rubens* growing adjacent to a male *S. alba* var. *vitellina* ("adjacent"). Five trees were selected from each of the above two situations (10 trees). From each tree, five catkins were selected containing several capsules that appeared to be near dehiscence. Three of these capsules were randomly selected from each catkin (total of 150 capsules), the contents of each capsule were removed, and filled seeds were counted. A Wilcoxon rank sum test was used to determine whether seed set per tree was significantly different ($P \leq 0.05$) for the two growing situations.

RESULTS

Establishment and growth of seedlings and cuttings.—Laboratory viabilities of seeds planted on 18 May, 27 May and 1 June 1992 were 72 ± 2.5, 68 ± 2.5, and 68 ± 3.7%, respectively (mean ± SE). Most viable seeds germinated within 24 h. Ninety-six h following the 18 May planting, we observed germinants in all replicates of treatments 1, 2 and 3, but none in treatments 4 and 5, where surface soil moisture was less than 1% (Table 2). Seventy-two h following the 27 May planting we again observed seedlings in all replicates of treatments

TABLE 2.—Seed germination in experimental planters 3 days following planting, and percent water of top 1 cm of planter substrate at time of sowing for three planting dates. Values are for each replicate

Planting date	Treatment	No. of germinants in the 3 reps/trt 72–96 h after planting	Percent water of top 1 cm soil on planting date
18 May	1	11, 4, 11	20, 20, 20
	2	9, 3, 4	18, 20, 20
	3	1, 6, 5	9, 8, 6
	4	0, 0, 0	<1, <1, <1
	5	0, 0, 0	<1, <1, <1
27 May	1	7, 9, 6	20, 20, 20
	2	6, 3, 6	20, 19, 20
	3	3, 4, 4	10, –, 13
	4	0, 0, 1	6, 5, 6
	5	0, 0, 0	5, 6, 4
1 June	1	—[a]	—[a]
	2	16, 5, 10	20, 20, 20
	3	12, 24, 18	14, 16, 18
	4	4, 5, 2	7, 6, 7
	5	1, 2, 1	6, 6, 5

[a] No census taken because of difficulty in observing seedlings in algal build-up

1, 2 and 3; however, there were generally fewer seedlings in these treatments compared with the previous count (Table 2). We observed one seedling in one replicate of treatment 4, where soil moisture in the top 1 cm had increased to 6% before planting. Ninety-six h following the 1 June planting, all replicates of all treatments contained seedlings (Table 2).

Because none of the seedlings in treatment 1 (flooded) had roots contacting the soil surface, and because some of these seedlings were enveloped by algae and therefore difficult to accurately count, we discontinued censusing this treatment after 30 May. We observed no living seedlings in treatment 1 after dropping the water level in mid-July. The number of living seedlings in treatments 2 and 3 declined significantly between censuses on 5 June and 18 June, but then remained essentially stable for the remainder of the growing season (Fig. 2). Numbers of living seedlings in treatments 4 and 5 were essentially stable following the 5 June census (Fig. 2). Fluctuations after 18 June were minor and inconsistent for all treatments, with the exception of observed mortality in one of the replicates of treatment 3 near the end of the growing season. The minor fluctuations in seedling counts were probably a result of periodically failing to see one or two individuals. Treatment differences in numbers of living seedlings at the end of the season were statistically significant both when including treatment 1 (where survival was zero for all replicates; $P = 0.005$) and when omitting treatment 1 ($P = 0.04$).

No statistically significant differences existed among treatments in any of the four measured growth variables for seedlings (Table 3). Despite the lack of statistical significance, an examination of the data suggests some differences between plants in treatments 3 and 5. Plants in treatment 3, which had the most surviving seedlings, had the highest mean values for shoot height and both biomass variables. For each of these three variables, a treatment 3 replicate had the highest measured value (of 11 values). Plants in treatment 5, which had the fewest surviving seedlings, had the lowest mean value for shoot height, root

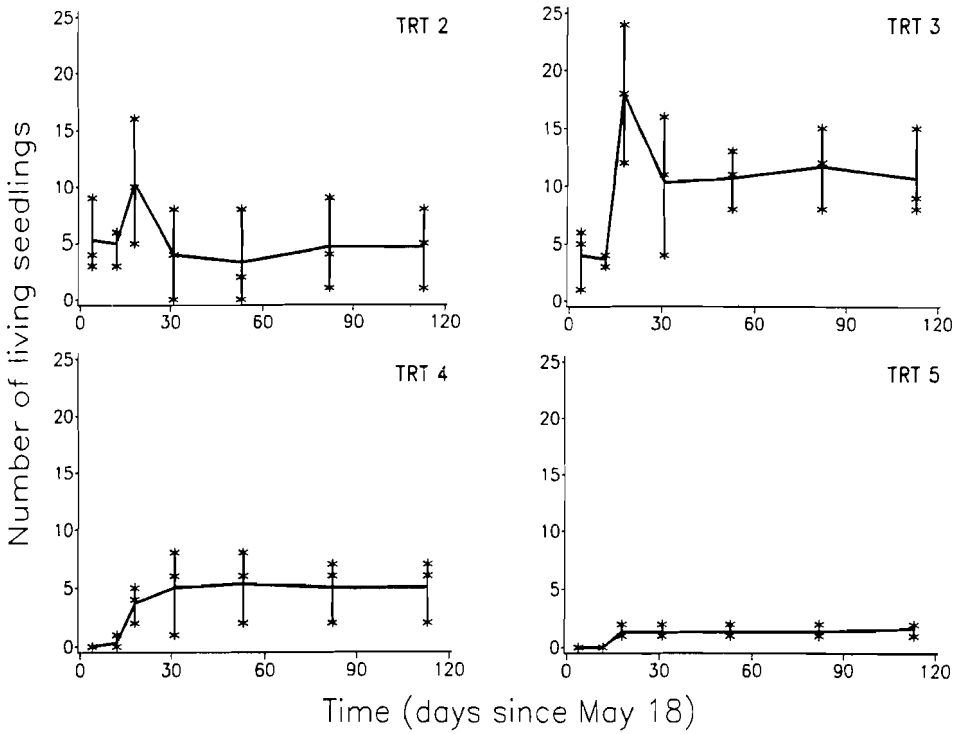

FIG. 2.—Mean numbers of living willow seedlings throughout the growing season in the four hydrologic treatments with survivors. Asterisks represent replicate values. Solid lines indicate treatment means. Twenty-five seeds were planted on day 0, 50 seeds on day 9, and 25 seeds on day 14. After day 14, numbers are equivalent to percentages

TABLE 3.—Mean growth of seedlings of *Salix* ×*rubens* at the end of the growing season and significance of hydrologic treatment differences. Means for each replicate are given in parentheses below the respective treatment mean. Probability values are from multi-response permutation procedures with four treatments

Variable	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Significance of treatment effect (P)
Seedling shoot height (mm)	7.2 (7.1, 9.0, 5.3)	7.3 (7.8, 10.1, 3.9)	6.4 (7.6, 8.5, 3.0)	4.2 (3.0, 5.5)	0.71
Seedling root length (mm)	204 (166, 302, 146)	177 (170, 180, 179)	166 (148, 192, 158)	135 (147, 122)	0.22
Per-plant shoot biomass (mg)	3.0 (2.6, 4.0, 2.5)	5.7 (6.4, 8.6, 2.2)	2.5 (2.6, 2.8, 2.0)	2.2 (2.0, 2.5)	0.12
Per-plant root biomass (mg)	5.1 (2.9, 9.0, 3.3)	7.5 (6.2, 12.9, 3.3)	3.1 (2.6, 2.8, 4.0)	4.2 (6.0, 2.5)	0.67

TABLE 4.—Mean growth of cuttings of *Salix* × *rubens* at the end of the growing season and significance of hydrologic treatment differences. The high and low replicate means are given in parentheses below the respective treatment mean. Probability values are from multi-response permutation procedures with five treatments

Variable	Treatment 1	2	3	4	5	Significance of treatment effect (P)
Percent plants established	100 (100, 100)	100 (100, 100)	95 (90, 100)	73 (55, 95)	38 (15, 55)	0.003
Root length (mm)	348 (332, 358)	399 (380, 432)	481 (365, 596)	676 (424, 827)	608 (551, 648)	0.01
Per-plant root dry weight (mg)	389 (354, 428)	574 (560, 600)	504 (469, 554)	580 (502, 635)	1034 (748, 1562)	0.0002
New shoot length (mm)	68 (66, 70)	81 (75, 91)	78 (74, 84)	74 (69, 79)	90 (75, 115)	0.17
Per-plant new stem dry weight (mg)	99 (93, 108)	131 (124, 139)	130 (129, 131)	118 (111, 126)	130 (98, 171)	0.02
Per-plant leaf dry weight (mg)	162 (147, 186)	216 (199, 241)	217 (186, 241)	212 (185, 237)	254 (146, 408)	0.36

length and per-plant shoot biomass. For all four growth variables, a treatment 5 replicate had the lowest measured value.

Treatment differences in percentage of cuttings established at the end of the season were highly significant (Table 4). Cuttings in treatments 1, 2 and 3 all had very high survival and low variability among replicates, whereas treatment 4 had lower survival and much higher variability, and treatment 5 had the lowest survival and high variability. After 20 May, the number of living cuttings remained essentially stable in all treatments. Establishment of cuttings was greatest when soil moisture at 5 cm (one-half the depth to which cuttings were planted) was between 20% (saturated) and 11.5%, though high rates of establishment also occurred from 5 to 11.5% soil moisture. Limited establishment occurred when soil moisture was below 5% at 5 cm.

Treatment differences in root length, per-plant root biomass and new stem dry weight of cuttings were statistically significant (Table 4). Plants in treatment 1 (flooded) had notably lower mean values for both root growth variables, and plants in treatment 2 generally had shorter roots than in treatments 3, 4 and 5. Treatment 1 also had the lowest mean values for new shoot length and per-plant new stem and leaf dry weights. Cuttings in one of the treatment 5 replicates had consistently high values for the four dry weight variables measured. Examination of the data suggests that per-plant biomass in treatment 5 is density-dependent: values of all dry weight variables increased with decreasing density. The planter in which only three cuttings rooted and survived had the highest values of any replicate of any treatment. However, per-plant root biomass values for the treatment 5 replicates with nine and 11 plants were still higher than values from any other treatment, suggesting a treatment effect. P-values changed, but general significance ($\alpha = 0.05$) of treatment effects did not change for any growth variable when including or excluding treatment 5.

Sex structure.—Only three of 2175 *Salix* × *rubens* individuals were male, and only two of 150 *S. alba* var. *vitellina* were female (Table 1). In addition, several apparent hybrids were

observed at Plum Creek and Cherry Creek. Assessment of hybridity was based on branch and leaf morphology.

Breeding system study.—Seed set was significantly greater ($P = 0.03$) for *Salix* ×*rubens* trees growing adjacent to *S. alba* var. *vitellina* than for isolated *S.* ×*rubens*. Only three seeds (all from one tree) were set in the 75 capsules from isolated *S.* ×*rubens*, whereas 170 seeds (seed set in every tree) were set in the 75 capsules from *S.* ×*rubens* growing near *S. alba* var. *vitellina*. Mean number of filled seeds per capsule ranged from 0.80–3.47 in the adjacent trees, and from 0.0–0.20 in the isolated trees.

DISCUSSION

Establishment and growth of seedlings and cuttings.—Our experimental results indicate that *Salix* ×*rubens* seed can germinate and 1st-yr seedlings can survive under a variety of hydrologic conditions found along regulated western U.S. watercourses. Substantial seedling mortality from 1 June to 18 June in treatments 2 and 3 may have occurred because the extremely moist conditions in these treatments produced a substrate in which the germinants were essentially floating in water. This medium appeared to make proper orientation and subsequent anchoring difficult for germinants. Further, the lack of substrate stability may have enhanced the potential for high-intensity thunderstorms to dislodge or disorient germinants, or to cover them with soil splash (Engstrom, 1948). Segelquist *et al.* (1993) noted that plains cottonwood mortality and reduced growth in the same substrate used in this study were associated with soil moisture conditions below 2%. The generally high percentage of survivors following 18 June in all planters except one treatment 3 replicate may have been due to availability of moisture at relatively shallow depths.

A much higher percentage of cuttings than seedlings became established, which might be expected given the larger amount of stored carbohydrates in cuttings, and the known propensity of cuttings of *Salix* spp. to root easily (Chmelar, 1974; Densmore and Zasada, 1978). Cuttings became established most frequently where soil moisture and the water table were high, and less frequently where soil moisture and the water table were lower. Conroy and Svejcar (1991) obtained similar results in their study of 2nd-yr survival of cuttings of *S. geyeriana*.

Significant differences between treatments in growth responses of cuttings appeared to be largely due to uniformly lower growth in treatment 1, which may be attributable to lower oxidation-reduction potentials during the period of flooding. Anaerobiosis has been shown to adversely affect shoot growth, leaf initiation and expansion, cambial growth, and root growth in many tree species (Kozlowski *et al.*, 1991). Differences in root lengths of cuttings appear to be associated with differences in early season water table depth. Mahoney and Rood (1992) suggest that roots of riparian polars will elongate to maintain contact with a deep water table, whereas roots growing where the water table is shallow and constant tend to be relatively short. Lieffers and Rothwell (1986) showed that rooting depth in black spruce (*Picea mariana*) and tamarack (*Larix laricina*) is generally correlated with depth to water table. The water table was deepest during the 1st mo in treatments 4 and 5, and root lengths were the longest. In treatments 1 and 2 the water table was very shallow, and root lengths were the shortest. Roots in treatment 3, which had the deepest and most rapidly declining water level from late July through the end of the growing season, were intermediate in length, suggesting that root lengths are more associated with early than late season water tables.

Sex structure.—One explanation for the nearly complete absence of male *Salix* ×*rubens* is that hybrid seedlings are establishing relatively frequently under natural conditions, and that their sex is genetically or environmentally determined. Female-biased sex ratios in

populations of *Salix* spp. have been reported, although the cause of the bias is not always understood (Alliende and Harper, 1989). Mosseler (1989) observed many biased sex ratios in interspecific hybrids of *Salix* spp., including several that were highly female skewed. Deviations from a 1:1 male-female sex ratio in natural populations of dioecious species have been attributed to environmental conditions in two ways. First, sex-related ecological adaptations may lead to differential mortality among the sexes (Freeman *et al.*, 1976; Grant and Mitton, 1979; Crawford and Balfour, 1983). Second, sex expression may be labile in response to environmental conditions (Freeman *et al.*, 1980). Environmental sex determination has not, however, been reported to result in nearly as great a difference between the numbers of males and females as we found.

A more likely explanation for the paucity of males is that *Salix* ×*rubens* is reproducing vegetatively and that the occasional males are actually hybrids with *S. alba* var. *vitellina*. Unisexual populations of *Salix* species have been reported previously and sometimes have been attributed to exclusively vegetative reproduction of the parent stock. In a Virginia drainage, Argus (1986) noted a unisexual stand of *S. lucida* Muhlenberg which apparently resulted from the introduction and subsequent spread of one or a few clones. In New Zealand, all *S. fragilis* (a parent of *S.* ×*rubens*) stems are male due to the planting and subsequent vegetative spread of male parent stock (Hathaway, 1976), and in southern Africa, only female *S. babylonica* and *S. fragilis* occur for the same reason (Immelman, 1987; Henderson, 1991). In Great Britain, three of four *S. fragilis* segregates described by Meikle (1992) are unisexual. The scarcity of male *S. fragilis* in North America (possibly *S.* ×*rubens*) was previously noted by Harrington (1964) and Vines (1977).

Breeding system.—Viable seed in Colorado *Salix* ×*rubens* could be produced in at least three ways: (1) via self-fertilization from hermaphroditic flowers; (2) apomictically (agamospermy) and (3) via hybridization with *S. alba* var. *vitellina* (backcrossing). Hermaphroditic flowers are known to occur on some willows (Mosseler and Zsuffa, 1989); however, we have not observed hermaphroditic flowers on *S.* ×*rubens* trees in Colorado. Sacchi and Price (1988) found no apomictic seed production in two populations of *S. lasiolepis* Benth. in Arizona, and in a review of apomixis in the angiosperms, Nygren (1967) did not report agamospermy in any taxa in the Salicaceae. On the other hand, the ability of many *Salix* species to hybridize has long been recognized (*e.g.*, Ikeno, 1922; Nilsson, 1954; Argus, 1974; Meikle, 1975; Mosseler, 1989). Hybridization, backcrossing and introgression between *S. fragilis* and *S. alba* have been previously reported (Meikle, 1975; Rechinger, 1992).

The results of our breeding system study suggest that pollen must be present for viable seeds to be produced in *Salix* ×*rubens*. Agamospermy cannot be ruled out because some species require pollen in order to set seed agamospermously (Richards, 1986). However, given the lack of recorded agamospermy and the prevalence of recorded hybridization in *Salix* (above), viable seed set is most likely the result of hybridization between *S.* ×*rubens* and *S. alba* var. *vitellina*.

Synthesis and conclusions.—Observations on the distribution of *Salix* ×*rubens* suggest that vegetative reproduction predominates. In Colorado, *S.* ×*rubens* and *S. alba* var. *vitellina* individuals that have not been planted are often located immediately adjacent to a channel, where seedlings would have been removed by scouring. We have observed very few individuals long distances from existing mature trees. Populations of these species are almost always located in and downstream of cities, towns and homesteads. If seedlings were establishing frequently, individuals would be expected to occur far from existing trees, both up- and downstream, given the long-range dispersibility of *Salix* spp. seeds.

Our observations and results suggest that *Salix* ×*rubens* in Colorado reproduces largely from fallen branches and twigs that take root. The closely related *S. fragilis* is known to

reproduce in this manner (*e.g.*, Werthner, 1935; Polunin, 1976; Mitchell, 1985). At Boulder Creek, we identified many seedling-sized *S.* ×*rubens* as twig sprouts, whereas we identified none as germinants (pers. observ.). We have excavated several *S.* ×*rubens* individuals which were of vegetative origin at Plum Creek and the Cache la Poudre River. Furthermore, the *S.* ×*rubens* cuttings grown in our establishment experiment had generally high rates of successful rooting, survival and growth.

If reproduction is predominantly vegetative, then high levels of hybrid seedling mortality must occur. Seedlings of *Salix* ×*rubens* are rare in Colorado even where this species dominates the overstory (Friedman, 1993). Establishment failure could result from the fact that *S.* ×*rubens* disperses seed prior to peak flows in streams of Colorado's high plains. River stage commonly increases in mid-June as a result of snowmelt runoff and high intensity thunderstorms, whereas *S.* ×*rubens* generally disperses seed in mid-May to early June. On the banks of the Cache la Poudre River, we observed 100% mortality of 1st-yr seedlings growing that apparently resulted from inundation, scouring, and flotsam deposits following *S.* ×*rubens* seed germination (pers. observ.). Similarly, we observed many seedling-sized *S.* ×*rubens* that suffered top dieback or outright mortality as a result of high stream flows at Boulder Creek (pers. observ.). These sources of mortality highlight the importance of a seed dispersal period that coincides with the peak or declining limb of the local hydrograph.

Seedling establishment could also be inhibited by the potential genetic inferiority and consequent low competitive ability of the hybrid seedlings (Stace, 1975). In a study of interspecific *Salix* hybrids, Mosseler (1989) observed several abnormal morphological types, including dwarfed plants and plants with twisted stems, abnormal leaf form or color, or multiple stems. We observed seedlings in the field and in the experimental planters with twisted stems, which could contribute to poor orientation and anchoring. These growth characteristics inhibited seedlings from attaining substantial height growth, which in turn could make them more susceptible to soil splash and direct displacement during thunderstorms. For other *Salix* species, 100% seedling mortality has been attributed to insufficient soil moisture, as related to precipitation or alluvial groundwater (McBride and Strahan, 1984; Sacchi and Price, 1992).

Assuming rare seed reproduction and occasional vegetative reproduction, *Salix* ×*rubens* should continue to be a significant component of the overstory in high plains riparian areas. *Salix* ×*rubens* trees resprout readily from stumps following natural (*e.g.*, beaver) or human destruction (*e.g.*, cutting). Therefore, any potential efforts to eradicate these trees would probably require chemical as well as mechanical means. Barring fatal insect or disease problems, the ability to resprout essentially ensures a tree's survival to the lifespan of its root crown. If vegetative reproduction continues to be predominant, *S.* ×*rubens* should continue to spread slowly, resulting in significant changes in high plains riparian woodlands over the long term. Vegetative reproduction should favor *S.* ×*rubens* over native species along regulated streams where the timing, magnitude and frequency of flooding have been altered and consequently inhibited seed reproduction of native species (Scott *et al.*, 1993).

Further planting of certain exotic willows should be avoided if conservation of native flora is a primary management goal. Staminate *Salix alba* var. *vitellina* and *S. pentandra* L. (laurel leaf willow), a species that can cross with *S. fragilis* or *S. alba* (Meikle, 1984), are sold in Colorado nurseries. Planting staminate individuals of either of these two species near extant *S.* ×*rubens* stands or individuals would likely increase hybrid seed set and promote the spread of hybrids, presuming a low rate of hybrid seedling establishment. Staminate *S.* ×*rubens* and *S. fragilis* are not currently sold in Colorado nurseries. Introduction of either of these taxa has the potential to drastically increase sexual reproduction.

Our study suggests three promising areas for future work on the ecology of *Salix* ×*rubens*

and *S. alba* var. *vitellina*. Electrophoretic studies comparing *S.* ×*rubens* × *S. alba* var. *vitellina* seedlings from controlled crosses and from the field would better determine the breeding system responsible for seed production. Electrophoretic studies on adults in the field could then be conducted to determine the relative importance of vegetative and sexual reproduction for *S.* ×*rubens* and *S. alba* var. *vitellina*. Detailed field studies designed to examine seedling recruitment and vegetative reproduction would provide a crucial corollary to the experimental work presented here. Finally, studies of the age-class distribution of these species over a wide geographic range are needed to gain a better understanding of the extent and rate of spread. These kinds of studies would further our knowledge of this common but overlooked species, thereby enabling riparian land stewards to make wise decisions regarding its future management.

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