

THE EFFECT OF RECIPROCAL HYBRIDIZATION ON REPRODUCTION OF THE INTERSECTIONAL CROSS, *POPULUS* × *GENEROSA*

Brian J. Stanton

GreenWood Resources, P. O. Box 5905, Portland, OR 97228, USA

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ABSTRACT

Eight *Populus* × *generosa* F1 intersectional hybrid populations were created from 1993 to 2001 by reciprocal crossing of *P. deltoides* (section *Aigeiros*) and *P. trichocarpa* (section *Tacamahaca*). The *P. deltoides* × *P. trichocarpa* (DT) cross was bred using 104 *P. deltoides* females and 95 *P. trichocarpa* males. Ninety-five *P. trichocarpa* females and 81 *P. deltoides* males were used in breeding the *P. trichocarpa* × *P. deltoides* (TD) cross. Reproduction was assessed using the number of plants-per-capsule recovered by *in vitro* embryo culture. Reproduction of the *P. deltoides* females exceeded the *P. trichocarpa* females eightfold, 9.26 to 1.17 plants-per-capsule. Moreover, reproduction was more consistent for the DT cross; coefficients of variation among the *P. deltoides* and *P. trichocarpa* females were 49% and 110%, respectively. The lower and more variable level of reproduction of the *P. trichocarpa* maternal parents led to a greater reduction in the maternal effective number. Expressed as a ratio of the census number, the effective number of the *P. trichocarpa* maternal parents was reduced to .78, varying from .34 to .91 for seven of the eight populations. The effective number of the *P. deltoides* maternal parents averaged .88 and varied from .78 to 1.00 for all populations.

Keywords: *Populus deltoides*, *Populus trichocarpa*, controlled breeding, effective breeding number

INTRODUCTION

Over 20,000 hectares of hybrid poplar plantations are being managed for biomass feedstock for co-generation plants, wood chips for pulping fibers, logs for veneers and sawn wood products, and several environmental applications in the lower Columbia River valley, the mid Columbia River basin, the Willamette Valley, and elsewhere in the Pacific Northwest of North America, with growth rates approximating 28–35 cubic meters per hectare per year (STANTON *et al.* 2002). While a range of climatic and edaphic conditions require diverse management strategies, all operations rely upon the clonal propagation of highly selected interspecific hybrid cultivars to maximize yield and wood quality while maintaining resistance to pathogens, insects, cold, and wind throw. The most productive cultivars are derived from the intersectional cross between the indigenous *Populus trichocarpa* Torrey et Gray (section *Tacamahaca*) and *Populus deltoides* Bart. ex Marsh. (section *Aigeiros*) from eastern North America. Both species are dioecious and share the same basic chromosome number ($x = 19$) (SMITH 1943). They possess similar reproductive structures in terms of the numbers of stamens or pistils per flower and

flowers per inflorescence (BOES & STRAUSS 1994, NAGARAJ 1952), and hybridize naturally where their ranges overlap in Wyoming, Montana, and Idaho (ECKENWALDER 1984). Lower Mississippi River Valley provenances of *P. deltoides* are differentiated, however, from Pacific Northwest *P. trichocarpa* by a longer gestation period, approximately 12–24 weeks versus eight to ten weeks, respectively (FARMER 1966). *P. deltoides* and *P. trichocarpa* are believed to have maintained their species distinction mainly through geographic isolation (SMITH 1943, WRIGHT 1976).

HENRY (1914) first reported the artificial hybridization of *P. deltoides* and *P. trichocarpa* with the taxonomic designation *P. × generosa* Henry (synonym: *P. interamericana* Brockh.). STOUT and SCHREINER (1933) thereafter completed the first large-scale hybridization of the taxon in 1925–1927 with the production of 2,654 genotypes. Heterosis is commonplace in *P. × generosa* breeding and several productive cultivars have been released for commercial use in North America (ECKENWALDER 2001, STETTLER *et al.* 1988). Although generally considered a facile cross to make under greenhouse conditions (RAJORA & ZSUFFA 1984, ZSUFFA 1974), great variation in its success has been experienced largely

as a function of the direction of the cross. Reasonable rates of seed production are expected when *P. deltoides* is used as the female parent, whereas high rates of premature catkin abscission and/or capsule abortion are the norm when *P. trichocarpa* serves that role (T. Bradshaw, University of Washington, B. McMahon, University of Minnesota, pers. comm.). This pattern conforms to other reports of problematic hybridizations between *P. deltoides* males and females of section *Tacamahaca*, including *P. balsamifera* L. (JOENNOZ & VALLEE 1972, LARSON 1976), *P. maximowiczii* Henry (R. Hall, Iowa State Univ. pers. comm., ZSUFFA *et al.* 1999), *P. ciliata* Wall. Ex Royle (KHURANA, 1989, KHURANA & BHANWARA 1982), and *P. ussuriensis* Kom. (SU *et al.* 1999). As a consequence, various *in vitro* embryo rescue techniques are now available for breeding recalcitrant *Tacamahaca* × *Aigeiros* intersectional combinations (KOUIDER *et al.* 1984, MOFIDABADI *et al.* 1998, RAQUIN *et al.* 1993, SAVKA *et al.* 1987, THAKUR & KHOSLA 1991).

Between 1988 and 1992, attempts to cross *P. trichocarpa* females and *P. deltoides* males repeatedly failed at the same time that the reciprocal cross between *P. deltoides* females and *P. trichocarpa* males regularly produced viable seed. Based on consistent success with the intraspecific cross, *P. trichocarpa* × *P. trichocarpa* and the intrasectional cross between *P. trichocarpa* females and *P. maximowiczii* males of section *Tacamahaca*, the failure to effect the *P. trichocarpa* × *P. deltoides* cross was assumed to have been brought about by intersectional hybridization per se and not to be an artifact of the artificial propagation and breeding system being used. The magnitude of the *P.* × *generosa* reciprocal crossing effect was thereupon assessed in an applied *Populus* improvement program. For simplicity, I will hereafter use the shorthand designations DT for the *P. deltoides* × *P. trichocarpa* cross and TD for the *P. trichocarpa* × *P. deltoides* cross. Hybrid production is evaluated in terms of the number of viable plants recovered on a per capsule basis, a key component in the success of interspecific reproduction in controlled breeding programs. The effective number of *P. deltoides* and *P. trichocarpa* maternal parents in reciprocal crosses is calculated using the mean output of hybrid offspring and the variance among each kind of maternal parent. This report also contains observations on the juvenile growth and *Melampsora* leaf rust resistance of reciprocal populations of seedlings growing in a nursery.

METHODS

Eight pairs of reciprocal *P.* × *generosa* populations were developed during the period 1993–1999 and in 2001 by annual controlled pollinations of *P. deltoides* and *P. trichocarpa*. *P. trichocarpa* parents were selected phenotypes mostly from natural alluvial and some upland stands located west of the Cascade Mountains between 48° 56' N latitude (Nooksack River, Whatcom County, Washington, U.S.A.) and 42° 56' N latitude (South Umpqua River, Douglas County, Oregon, U.S.A.). The trees varied from 14 to 60 years in breast-height age at the time they were bred. The large majority of the *P. deltoides* parents came from an extensive varietal collection assembled by the U. S. D. A. Forest Service and maintained by James River Corporation at Fittler, Mississippi (COOPER 1980a, 1980b, 1980c). This collection originated from alluvial stands within the lower Mississippi River Valley between Memphis, Tennessee (35° 14' N latitude) and Baton Rouge, Louisiana (30° 36' N latitude) (FOSTER 1986). A lesser number of *P. deltoides* selections from the Texas Forest Service and Oklahoma State University was also used. These originated in provenances from the Brazos River and the Red River located approximately four to six degrees of longitude west of the Mississippi River between 35° 57' N and 30° 03' N latitudes (NELSON & TAUER 1987). *P. deltoides* parents were 14 to 22 years of age at the time of controlled crossing.

Controlled Hybridization

The standard mating design used each year employed two series of three to five disconnected, 3 × 3 factorials, one series for the DT cross type and a second for the TD cross type. Both DT and TD crosses comprised each year's population in nearly equal numbers of parents and attempted cross-pollinations. Over the eight years, the DT crosses involved 104 *P. deltoides* females and 95 *P. trichocarpa* males, whereas the TD crosses, 95 *P. trichocarpa* females and 81 *P. deltoides* males. Each year's group of female and male breeders was mostly a unique set although repeated samples were taken in some years. All crosses were conducted within a glasshouse at Camas, Washington, U.S.A. following the procedures described by STANTON and VILLAR (1996). Briefly, floral cuttings, 0.9–1.2 m in length were collected during the dormant season and stored at –2.2 °C for up to four weeks to ensure the fulfillment of chilling requirements. Staminate inflorescences of *P. trichocarpa* were forced in water culture

at 15.6 °C during the day and 4.4 °C during the night. *P. deltoides* males were forced at 23.9 °C during the day and at 10.0 °C during the night. Pollen was extracted from ripened and nearly-ripened stamens, screened through 80 mesh sieves, air dried at room temperature and humidity in a lab for eight hours followed by drying over desiccant at 23.9 °C for 24 hours, and refrigerated at 2.2 °C until used within three to five weeks. Female cuttings were propagated in a soil and aqueous culture system; a lower reservoir of deionized water was used to sustain the cuttings through floral bud burst, stigma receptivity, and early capsule development until adventitious rooting occurred in an upper soil compartment to support the developing seed crop through dehiscence. (Beginning in 1997, cuttings of *P. deltoides* were treated with a 1,000 ppm NAA/IBA commercial rooting hormone preparation for five seconds prior to potting and heating pads were used to warm soil temperatures to 24 °C to improve rates of adventitious rooting.) Pistillate buds were enclosed with glassine bags (152 × 89 × 330 mm) well before bud burst. Pollen was injected into the bags with an atomizer three times on 24-hour intervals throughout the period of stigmatic receptivity. Bags were removed 72 hours following the last pollination.

***In vitro* Propagation**

In view of the anticipated difference in reproduction of the DT and TD cross types, an *in vitro* embryo culture technique was used in rearing both types so as to establish an equitable comparison of reproduction defined as the number of plants produced on a per capsule basis. Embryo culture was initiated in *P. deltoides* at the time of capsule dehiscence, approximately 12 weeks following pollination. In *P. trichocarpa*, embryo culture was started an average of 10 weeks after pollination coincident with the onset of capsule abortion.

Capsules were disinfected in a 20% commercial hypochlorite solution and opened aseptically. There was considerable variability in the developmental stage of the ovules within the same capsule of most *P. trichocarpa* females. Upon microscopic inspection, the majority of the ovules were found to contain globular stage embryos incapable of growth in the *in vitro* system. Accordingly, embryos were excised from the integument under a magnifying lamp to identify those with discernable radicles and cotyledons that were then placed into *in vitro* culture. Embryos damaged during excision were infrequent and were recorded but not placed into *in vitro*

culture. Conversely, there was absolutely no perceptible variation among *P. deltoides* ovules; embryo maturation had progressed to an advanced stage in all cases at the time *in vitro* culture was initiated. Consequently, whole ovules were excised at random for *in vitro* culture. (Prior tests showed no difference in germination between isolated embryos and whole ovules.)

Two to four embryos were placed into 50 ml culture tubes filled with 15 ml of basic MS media modified for general *Populus* embryo culture with 60 g·l⁻¹ sucrose and 6 g·l⁻¹ agar (Tony Chen, Oregon State University, pers. comm.). Because of the large discrepancy in embryo development, well-formed embryos were excised from a maximum of 15 *P. deltoides* capsules for each DT full-sib cross and up to 100 *P. trichocarpa* capsules for each TD full-sib cross. *P. deltoides* capsules were very uniform in size and those sampled for *in vitro* culture were chosen at random. Some *P. trichocarpa* females exhibited noticeable within-catkin variation in capsule size in which case the larger ones were used. Embryo germination and growth were conducted at 24 °C under a 24-hour photoperiod using white fluorescent lights. Plants that had developed two true leaves and a branched root system were transferred to soil and acclimated under frequent misting. The number of embryos placed into culture and the number of plants recovered from *in vitro* culture were recorded by full-sib cross on a per-capsule basis and then averaged over male parents for each maternal half-sib family. The duration of *in vitro* culture prior to the transfer of DT and TD hybrid plants to soil averaged four weeks in both cases.

The impact of differential reproduction of the reciprocal crosses was assessed using the ratio of the effective number of maternal parents to their census number. This ratio quantifies the degree to which unequal reproduction among maternal parents reduces the female census number of each reciprocal population. The maternal effective number was determined for the female parentage of each cross type because earlier observations in 1988–1992 indicated that the number of viable hybrid offspring varies almost wholly by maternal genotype. The maternal effective number (N_e) was estimated for each year's reciprocal population using the following equation for dioecious species,

$$N_e = (N_o k - 2) [(\sigma_k^2 / k) + k - 1]$$

where N_o , the census number, is the number of embryogenic maternal parents, k is the mean number of recovered plants on a per capsule basis averaged across maternal half-sibs, and σ_k^2 is the variance

among maternal half-sibs in the mean number of recovered plants (SPIESS 1977).

Juvenile Seedling Performance

An effect of reciprocal hybridization on seedling phenotype was investigated in terms of height and rust resistance as observed in a nursery. One-year-old containerized plants of each year's reciprocal population were established in nurseries the year following hybridization at Westport, Oregon on the lower Columbia River floodplain (46° 08' N, 123° 22' W). The soil at Westport is deep, moderately well drained with a loam-silt loam surface overlaying a sandy loam to fine sand horizon. The site is of excellent quality for poplar culture. Each year's nursery was planted at a 0.6 m × 0.9 m spacing. DT and TD components were planted in adjoining parts of the nursery. First-year height was measured to the nearest decimeter. The degree of resistance to leaf

rust infection (*Melampsora* spp.) was scored between late September and early October of the establishment year by assignment of individual seedlings into three resistance classes based upon leaf area bearing uredia and the extent of leaf necrosis throughout the crown.

RESULTS

In vitro reproduction

There were striking and consistent differences in reproductive development between the two cross types. Every one of the embryogenic *P. deltoides* females in the DT cross throughout the eight-year study carried embryo development through capsule maturation and dehiscence. Abscission of catkin-bearing twigs in response to insubstantial adventitious rooting eliminated reproduction of six of the

Table 1. Embryo production and *in vitro* germination of *P.* × *generosa* reciprocal crosses.

Population	Maternal parents pollinated	Maternal parents yielding embryos	Paternal parents per embryogenic maternal parent	Number of mature embryos per capsule	<i>In vitro</i> germination rate (%)
DT Cross Type: <i>Populus deltoides</i> × <i>Populus trichocarpa</i>					
1993	11	11	2.7	12.52	66
1994	14	12	2.6	16.28	64
1995	12	12	1.8	17.59	56
1996	14	14	1.9	16.15	49
1997	11	9	2.3	23.25	46
1998	14	13	3.0	17.22	48
1999	12	12	3.0	27.53	42
2001	16	15	2.0	15.15	42
Totals	104	98	—	—	—
Means	—	—	2.4	18.21	52
TD Cross Type: <i>Populus trichocarpa</i> × <i>Populus deltoides</i>					
1993	11	7	3.0	2.55	58
1994	7	6	3.3	1.17	50
1995	14	14	2.9	3.31	55
1996	13	13	3.1	2.56	28
1997	14	12	2.4	1.41	37
1998	12	11	2.4	3.08	34
1999	12	10	2.3	1.83	55
2001	12	9	2.9	2.18	47
Totals	95	82	—	—	—
Means	—	—	2.8	2.26	46

104 *P. deltoides* females (5.8%); these were excluded from the analysis of reproductive output. Conversely, two major failures were observed in the reproduction of *P. trichocarpa* females in the TD cross. The most common was the abortion of most or nearly all medium-to-large size capsules prior to dehiscence. This phenomenon was exhibited by 82 of the 95 *P. trichocarpa* maternal parents (86.3 %); well-differentiated embryos and resultant viable plants were recovered from each of these by embryo culture (Table 1). A less frequent failure, restricted to 13 females (13.7%), was exhibited in inflorescences that failed to initiate fruit development or persistent catkins that produced very little or no measurable growth of their ovaries. Viable embryos were not recovered from this group and ineffective fertilization or disruption of embryogenesis at the globular stage was inferred.

The number of paternal parents contributing offspring to each maternal half-sib family was 2.4 in the DT crosses when averaged over the eight years, while a mean of 2.8 paternal parents participated in

the reproduction of the TD cross half-sib families (Table 1). This difference mainly reflects reduced adventitious rooting in *P. deltoides* especially in 1995 and 1996, and the resultant loss of cross-pollinations to some males.

The superior reproduction of the DT cross was associated with an eightfold increase in the number of well-differentiated embryos. Over the eight years, these averaged 18.21 embryos per capsule for the embryogenic *P. deltoides* maternal parents in the DT cross and 2.26 embryos per capsule for the *P. trichocarpa* maternal parents in the TD cross (Table 1). The yearly mean number of mature embryos per capsule for the *P. deltoides* maternal parents varied between 12.52 (1993) and 27.53 (1999). The same statistic for the *P. trichocarpa* maternal parents ranged between 1.17 (1994) and 3.31 (1995) embryos per capsule. Reciprocal crossing did not have as large an effect on the rate of *in vitro* germination; germination of the DT cross exceeded that of the TD cross in five of the eight years (Table 1). Averaged over the eight years, the germination rate of the

Table 2. Plant recovery and maternal effective number of *P. × generosa* reciprocal crosses.

Population	Maternal parents yielding embryos	Mean recovered plants per capsule	Maternal variance in plants per capsule	Coefficient of variation	Maternal effective number	Maternal effective ratio ¹
DT Cross Type: <i>Populus deltoides</i> × <i>Populus trichocarpa</i>						
1993	11	8.66	17.72	49	9.61	0.87
1994	12	10.42	26.52	49	10.28	0.86
1995	12	9.91	34.81	60	9.41	0.78
1996	14	8.15	17.22	51	12.10	0.86
1997	9	11.03	25.10	45	7.90	0.88
1998	13	8.11	7.06	44	12.96	1.00
1999	12	11.75	15.84	36	11.49	0.96
2001	15	6.02	11.33	56	12.80	0.85
Means	12.25	9.26	19.45	49	10.82	0.88
TD Cross Type: <i>Populus trichocarpa</i> × <i>Populus deltoides</i>						
1993	7	1.48	2.47	113	3.89	0.56
1994	6	0.85	0.28	63	12.23	2.03
1995	14	1.78	3.12	99	9.02	0.64
1996	13	0.95	2.26	159	4.43	0.34
1997	12	0.68	0.77	130	7.44	0.62
1998	11	1.47	2.46	107	6.57	0.60
1999	10	1.14	1.02	89	9.10	0.91
2001	9	1.00	1.51	123	4.65	0.52
Means	10.25	1.17	1.74	110	7.17	0.78

¹⁾ Calculated as ratio of the maternal effective number to the number of embryogenic maternal parents.

DT cross exceeded that of the TD cross 52 to 46%.

The larger number of mature embryos found in capsules of the DT cross was associated with a higher plant recovery rate consistently observed during each of the eight hybridization years (Table 2). The overall mean recovery rate of *P. deltoides* maternal parents in the DT cross surpassed the recovery rate of the *P. trichocarpa* females in the TD cross by eightfold (9.26 plants per capsule versus 1.17 plants per capsule). Even the largest annual recovery rate recorded for the TD cross during the eight years (1.78 plants per capsule, 1995) was still less than the lowest yearly mean recovery rate for the DT cross (6.02 plants per capsule, 2001) (Table 2).

The variance among maternal parent in plant recovery rates was also decidedly distinct for the two cross types. Coefficients of variation averaged 49 % for the DT cross and 110 % for the TD cross over the course of the study (Table 2). Moreover, the *P. deltoides* females never exceeded their *P. trichocarpa* counterparts in this statistic in any given year. The distribution of maternal recovery rates (converted to standard deviations and combined over years) was decidedly distinct for the two cross types, being approximately normal for the *P. deltoides* maternal parents in the DT cross and skewed toward lower values for the *P. trichocarpa* parents in the TD cross

(Figure 1). No linear correlations were observed between the standardized number of recovered plants per capsule and the latitude of the maternal provenance for either *P. trichocarpa* ($r = -.05$) or *P. deltoides* ($r = -.04$). The age of the ortets of the maternal *P. trichocarpa* parents likewise did not show any correlation with reproductive output ($r = .02$).

The combined effect of smaller and more variable plant recovery rates characterizing the *P. trichocarpa* females contributed to a larger reduction in the maternal effective number in the TD cross compared with the *P. deltoides* females in the DT cross (Table 2). The variance in reproductive output of the *P. trichocarpa* maternal parents reduced their effective number to .78 when expressed as a ratio of the census number, and varied from .34 to .91 over 7 of the 8 years. (The ratio of the maternal effective number to the census number of *P. trichocarpa* females in 1994 exceeded one due in part to a small census number of maternal parents each of whom varied little in reproduction (SPIESS 1977).) Conversely, the much more consistent reproductive output among *P. deltoides* maternal parents in the DT cross type lowered the effective number to a mean value of .88 that varied from .78 to 1.00 over the 8 years.

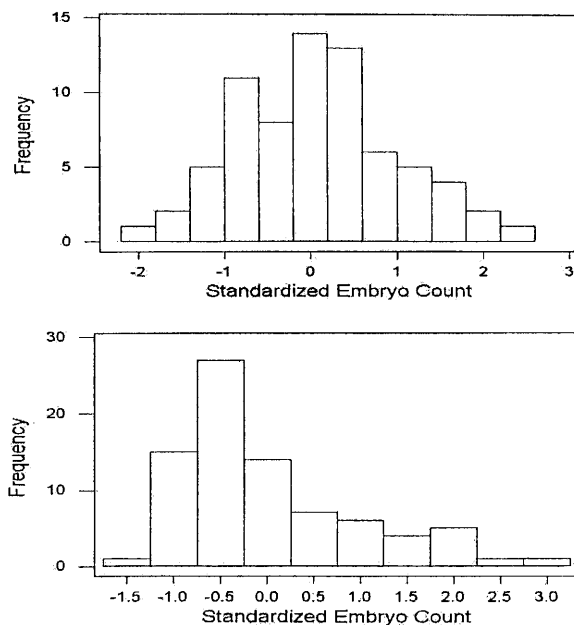


Figure 1. Frequency distribution of the standardized number of recovered plants per capsule of *P. deltoides* maternal parents in the DT (upper graph) and the TD (lower graph) *P. × generosa* cross types.

Nursery Performance

No evidence of a relationship between reciprocal hybridization was found in either height growth or leaf rust resistance variation, although appreciable

Table 3. Nursery heights of reciprocal *P. × generosa* seedlings from eight populations.

Population	DT Cross mean height (dm)	TD Cross mean height (dm)
1993	23.1	22.8
1994	22.8	25.3
1995	18.9	22.7
1996	16.5	15.9
1997	25.4	20.4
1998	14.8	12.1
1999	10.8	11.4
2001	12.5	14.5
Mean	18.1	18.1

Paired *t*-test of mean difference averaged over years, $t = -0.04$, non-significant at $p = 0.05$.

differences occurred in some years. For instance, the mean height of the DT seedlings of the 1997 and 1998 populations was 24% and 22% taller than the TD component, respectively. Conversely, the mean height of the TD cross seedlings of the 1995 and 2001 populations was 20% and 16% taller, respectively, than their DT counterparts. However, a paired t-test of the mean difference in seedling height of the reciprocal nursery populations when averaged over the eight years was not significant (Table 3). Similarly, the effect of reciprocal crossing was not reflected in the overall distribution of rust scores. While the distribution of the TD seedlings tended toward higher resistance in five of the eight populations (1993, 1994, 1995, 1998, 1999), the association between the three rust resistance categories and the two cross types for the distribution combined over all eight years showed no significant difference according to a chi-square test of homogeneity with two degrees of freedom (Table 4).

DISCUSSION

Barriers to intersectional hybridization in *Populus*

have been studied most extensively in crosses between section *Populus* and sections *Aigeiros* and *Tacamahaca* (GURIES & STETTLER 1976, KNOX *et al.* 1972, STETTLER *et al.* 1980). These barriers are predominately pre-zygotic being expressed in the retarded growth of pollen tubes (GURIES & STETTLER 1976, VILLAR *et al.* 1993). In the present study of intersectional hybridization between sections *Tacamahaca* and *Aigeiros* the main impediment was a post-zygotic one expressed mainly in premature capsule abortion by *P. trichocarpa* females. A less frequent block was the failure of some *P. trichocarpa* maternal parents to initiate capsule development. This may have been a pre-zygotic blockage arranged by low pollen germination rates as STETTLER *et al.* (1980) have reported a 24 % reduction in germination of *P. deltoides* pollen grains on *P. trichocarpa* stigmata relative to germination on *P. deltoides* stigmata. By contrast, *P. deltoides* females in no

Table 4. Distribution of reciprocal *P. ×generosa* seedling populations categorized by class of rust resistance.

Population	Cross type	High resistance (%)	Moderate resistance (%)	Low resistance (%)
1993	DT	27	31	42
	TD	16	52	32
1994	DT	7	23	70
	TD	31	36	33
1995	DT	7	36	57
	TD	11	33	56
1996	DT	3	35	62
	TD	5	30	65
1997	DT	16	51	33
	TD	3	41	56
1998	DT	15	45	40
	TD	31	41	28
1999	DT	2	27	71
	TD	8	53	39
2001	DT	7	45	48
	TD	4	30	66
Combined	DT	10	37	53
	TD	14	39	47

Chi-square test of a difference between the combined rust distribution of DT and TD cross type seedlings, $\chi^2 = 1.079$, non-significant at $p = 0.05$.

instance exhibited any symptoms indicative of either pre- or post-zygotic barriers in the DT cross. Similar phenomena are well known in reciprocal crosses between *P. deltoides* and *P. nigra* (MELCHIOR & SEITZ 1968), *P. deltoides* and *P. maximowiczii* (ZSUFFA *et al.* 1999), *P. deltoides* and *P. ciliata* (KHURANA 1989, KHURANA & BHANWARA 1982), and *P. deltoides* and *P. balsamifera* (JOENNOZ & VALLEE 1972). STETTLER *et al.* (1996) postulated the closeness with which the independent growth trajectories of individual ovules and carpels are coordinated as a potential mechanism underlying capsule abortion in *P.* × *generosa*. Normally the two processes are coincident in intraspecific crosses but may be disjointed in intersectional crosses if the growth of interspecific ovules lags behind the maturation of ovarian tissue. A timing disruption may be of greater consequence in TD crosses in view of the relatively short gestation period of the more northerly-adapted *P. trichocarpa*, relative to the longer gestation period of *P. deltoides* from lower Mississippi River Valley provenances.

The value of the current study lies in its estimation of the difference in reproductive success of reciprocal *P.* × *generosa* crosses based upon a broad sampling of female genotypes of both species replicated across eight years of hybridization. Yet, the quantification of reproduction in reciprocal crosses using the plant-to-capsule recovery rate is a preliminary assessment because: (1) There was no accounting for variation in pollen lot viability; (2) The different growing season conditions experienced by *P. trichocarpa* maternal ortets in their native habitats the year before controlled crossing during which time reproductive structures were being formed may have had a confounding effect on reproductive success; and, (3) It was assumed that between-species variation in the number of ovules per capsule had a negligible effect on plant recovery rates. These considerations notwithstanding, the trends in the magnitude of the *in vitro* recovery rates were highly repeatable among the eight years of hybridization and showed large, non-overlapping differences between the two cross types. Furthermore, the lower productivity of the TD cross is in close agreement with those few published papers that have incidentally reported on the comparative reproduction of *P.* × *generosa* reciprocal crosses (RAQUIN *et al.* 1993, STETTLER *et al.* 1980). Finally, it should be noted that the results of this study are primarily applicable to controlled breeding and improvement programs and not to the study of natural populations undergoing introgression to the extent that an artificial *in vitro* propagation system was used in studying variation in just one component – plants recovered

per capsule – of reproductive success.

Should reciprocal hybridization affect the design of *P.* × *generosa* applied improvement programs? A conservative approach would allow for the TD cross along with the DT cross type to ensure that the full range of genetic variation is encompassed in controlled hybridization, barring any poor performance of the TD cross type in commercially important traits. A distinction between TD and DT offspring was not detected in either growth or disease phenotypes in the current study. However, reciprocal crossing effects have been observed in the growth and morphology of *P. deltoides* × *P. balsamifera* (LARSSON 1976) and *P. deltoides* × *P. ciliata* populations (CHATURVEDI & RAWAT 1994), with superior performance associated with those crosses constructed with *P. deltoides* as the maternal parent. Such differences notwithstanding, programs featuring a TD component should account for greater reductions in the maternal effective number to match the selection efficiency of the DT cross type. Finally, long-term improvement programs that require estimates of hybrid breeding values for both sexes such as reciprocal recurrent selection practices will necessitate crossing in both directions (BISOFFI & GULLBERG 1996). These programs may ultimately require selection for intersectional reproductive success in *P. deltoides* and *P. trichocarpa* breeding populations as suggested by KRIEBEL (1973) and STETTLER *et al.* (1996).

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