Pre-fertilization Barriers to Hybridization in the Poplars[1])

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Introduction

Artificial hybridization has played a lengthy and important role in the domestication of the poplars. Contributing factors include the convenience of reproductive manipulation, the short time required for superior hybrids to be recognized, and the ease with which successful cultivars can be propagated. While the breeder's intuition played a major factor in early hybridization efforts, attempts at capturing heterosis or combining desirable traits have become increasingly systematic and during the past 40 years have resulted in thousands of hybrid cultivars being tested on an extensive scale in many parts of the world (e.g. Stout and Schreiner, 1933; Heimburger 1940, 1968; Hyun and Hong, 1959; Zufa, 1968; Steenackers, 1969; Chung and Son, 1972). However, the transfer of genes among certain poplar species, particularly those belonging to different sections of the genus, is constrained by barriers whose site and action are still unknown in all but a few cases (reviewed in Zsuffa, 1975).

The observation by Stettler, 1968; Stettler and Bawa, 1971, that pollen mixes of killed compatible (= "mentor") pollen and foreign pollen facilitated wide crosses between poplars not normally possible, provided the stimulus for renewed research on breeding barriers in the genus. Stettler's work has been repeated with other poplar species (Zufa, 1968; Knox, Willing and Pryor, 1972) with varying degrees of success depending upon the species used and the methodology employed. Attempts to explain the mentor pollen phenomenon in the poplars have focused upon the roles of pollen-wall proteins (Knox, Willing and Pryor, 1972) and stigmatic compounds, particularly lipids (Whitecross and Willing, 1975; Willing and Pryor 1976), as well as the promotion of catkin rentention following pollination (Stettler and Guries, 1976).

In this paper we report results from a survey of post-pollination phenomena for a number of poplar crosses involving seven species from three different sections of the genus. We have examined pollen germination, pollen-tube growth, and micropylar penetration for twenty-eight crossing combinations to identify the site(s) and strength of possible barriers to hybridization. Related data on catkin retention, embryo development, seed production, and on the effects of manipulating mentor-foreign pollen mixes, are reported elsewhere (Stettler and Guries, 1976).

Materials and Methods

The species used in this study are listed in Table *1.* Female branches of P. tremuloides and P. trichocarpa were collected from wild trees in King and Kittitas Cos., Washington; female branches of P. alba and P. deltoides were collected on the University of Washington campus, Seattle, Washington. Pollen of P. alba and P. trichocarpa was collected from trees in Seattle, Washingon; pollen of the remaining species was provided by L. Zsuffa, Ministry of Natural Resources, Ontario, Canada, and D. Einspahr, Institute of Paper Chemistry, Appleton, Wisconsin, receipt of which is gratefully acknowledged. Female branches were maintained in water culture as previously described (Stettler and Bawa, 1971). Flowers were usually receptive 3–5 days after being brought into the greenhouse. Pollination was performed by inserting attached catkins into a small glass vial containing pollen and lightly dusting the flowers using a camels-hair brush.

Table 1. — Populus species used in this study

Section	Male Parent	Female Parent
"Leuce" Duby.	P. alba *v.* Bolleana Lauche P. tremuloides Michx.	P. alba L. P. tremuloides
"Aigeiros" Duby.	P. deltoides Marsh. P. nigra *v. italica* Muenchh.	P. deltoides
"Tacamahaca" Spach.	P. *balsamifera* Muenchh. P. *Maximowiczii* Henry. P. trichocarpa Hook.	P. trichocarpa

Pistils were sampled at 2, 4, 8, 12, and 16 hours after pollination for pollen germination and pollen-tube growth analyses, and at 3 or 6 days after pollination for micropylar penetration analysis. Pistils were fixed in FAA and stored at —20 °C until analyzed. A large number of unpollinated catkins interspersed among the treated ones were also sampled at 24 hours after pollination to detect pollen contamination. It was generally found to be negligible. Pistils were cleared in 40% NaOH for 24 hours followed by several water washes. Staining was according to Martin (1959) except that the concentration of aniline blue was 0.01%. Fluorescence microscopy was used with a Zeiss microscope equipped with a Zeiss UG-1 excitation filter and 47, -65 barrier filters. The UV source was an Osram HBO 200 w mercury vapor lamp. Pollen grains and tubes appeared bright yellow-green against a blue-grey background.

Results

Pollen germination

Pollen germination varied among crosses, but all pollen species showed good to excellent germination in at least one crossing combination (Table 2), indicating that all pollens were viable. A grain was considered germinated if the pollen-tube length was at least four times the diameter of the pollen grain.

In crosses where P. trichocarpa and P. deltoides served as female parents, "Aigeiros" and "Tacamahaca' species pollen, and P. alba pollen, germinated rapidly, but P. tremuloides pollen germination was somewhat delayed. However, by 12 hours after pollination all combinations involving P. deltoides and P. trichocarpa females showed sufficient pollen germination to fertilize all available ovules. In some combinations, the large numbers of overlain pollen tubes obscured many grains and prevented precise counts (Fig. *1).* The estimates reported in these instances are, we feel, conservative, but preclude standard statistical

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Table 2. — Average number of germinated gains and one S.D. at 2 and 12 hrs. after pollination[1].

		♂ *delt*	*nigra*	*bals*	*Max.*	*trich*	*alba*	*trem*
♀ *delt*	2 hr	18.5 ± 7.6	78.9 ±22.3	128.3 ±59.2	43.6 ±18.2	9.6 ± 4.6	14.5 ± 6.6	0.0
	12 hr	250— 400	400— 500	>500	>500	400— 500	150— 200	42.1 ±23.5
trich	2 hr	8.0 ± 2.3	50.1 ±27.0	150— 200	150— 200	15.4 ± 7.6	22.4 ±10.9	0.7 ± 0.8
	12 hr	90.6 ±50.2	170.2 ±71.8	300— 400	400— 500	154.2 ±72.7	112.4 ±33.2	120.8 ±49.2
alba	2 hr	1.4 ± 1.3	1.5 ± 1.6	4.5 ± 2.8	1.8 ± 1.8	0.9 ± 1.0	3.9 ± 1.8	1.5 ± 1.2
	12 hr	58.4 ±32.7	40.4 ±16.2	63.2 ±27.2	38.4 ±16.1	33.8 ±28.5	34.8 ± 5.5	32.0 ±18.2
trem	2 hr	0.0	0.0	3.1 ± 5.1	0.0	0.1 ± 0.3	21.8 ± 6.3	0.4 ± 0.5
	12 hr	2.2 ± 5.6	13.9 ± 8.7	6.0 ± 5.9	0.7 ± 1.0	3.3 ± 6.0	119.4 ±68.5	23.8 ±13.4

[1] Based upon counts from 10 pistils per cross.

analysis of data. However, where differences among species did exist by 12 hours after pollination, they were usually quite obvious.

Where *P. alba* served as female parent, there were no significant differences among pollen species for numbers of germinated grains by 12 hours after pollinations $F_s = 1.58 < F_{.05(6,28)} = 2.45$). However, in crosses were *P. tremuloides* served as female parent, "Aigeiros" and "Tacamahaca" pollens showed a marked germination inhibition and only "Leuce" species pollen germinated well. Even by 12 hours after pollination, the numbers of germinated grains of most species were quite small *(Table 2)*. The smaller numbers of grains recorded on *P. alba* and *P. tremuloides* stigmas even for compatible crosses are primarily due to their smaller surface area relative to *P. deltoides* and *P. trichocarpa* stigmas.

In view of the small number of genotypes represented in this study (typically 1—3 per species), and the marked variation both within and between species in terms of germination success (as evidenced by the means and standard deviations in *Table 2)*, analysis beyond recognizing general trends seems unwarranted. Pollen germination appears to be a serious barrier to hybridization only in *P. tremuloides* when "Aigeiros" and "Tacamahaca" poplars serve as pollen parents. Delays in pollen germination *(e.g. P. trichocarpa* × *P. tremuloides)* may be a problem in certain instances.

Pollen-tube growth

Pollen tubes became polarized toward the style immediately on penetrating the stigma surface *(Fig. 2)*. They were easy to observe in the stigmatic tissue, but could not be followed in the style because of the concentration of vascular tissue of approximately the same color. However, once into the ovary individual tubes could again be followed in most cases.

In crosses where *P. deltoides* and *P. trichocarpa* served as female parents, there were small though significant differences in Pollen-tube growth rates among "Aigeiros" and "Tacamahaca" pollen tubes, but pollen tubes of *P. alba* and *P. tremuloides* were clearly inhibited *(Figs. 3—4; Table 3)*. The inter-specific differences observed among the "Aigeiros" and "Tacamahaca" poplars are likely to be no greater than intra-specific differences, and genotypic variation similar to that observed for pines (Barnes, Bingham and Squillace, 1962) and corn (Mulcahy, 1971; Gorla *et al.*, 1975) may account for such differences. In any event, it seems obvious that among the species tested the "Leuce" poplars and the "Aigeiros" and "Tacamahaca" poplars form two distinct groups with respect to pollen-tube growth rates.

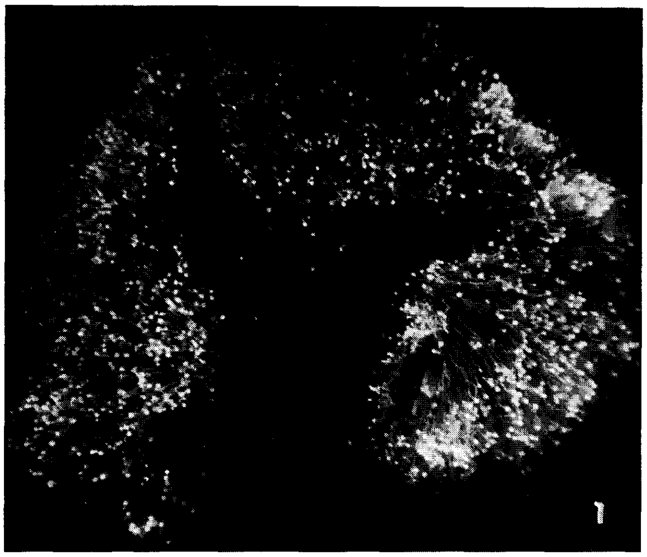
Where *P. alba* served as female parent, *P. tremuloides* pollen tubes exhibited the best growth and by 16 hours after pollination the longest tubes had grown into the style, making accurate measurements impossible *(Fig. 5)*. Pollen tubes of *P. alba* and *P. nigra* grew somewhat more slowly in *P. alba* stigmas, while tubes of *P. deltoides* and the "Tacamahaca" poplars were approximately one half as long as those of *P. tremuloides* by 12 hours after pollination. Differences among species were significant *(Table 4)* with the relative rankings of means being approximately the reverse of the situation in *P. trichocarpa.*

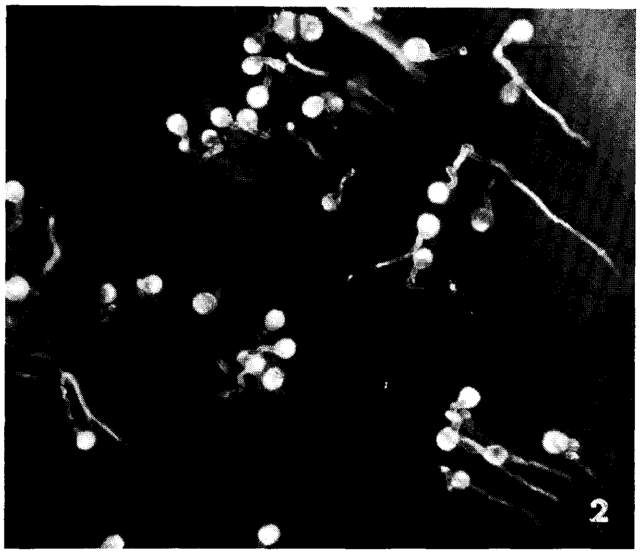
Except in rare instances, only pollen tubes of *P. tremuloides, P. alba* and *P. nigra* penetrated the stigma surface when *P. tremuloides* served as female parent. By 16 hours after pollination, pollen tubes of *P. alba* and *P. nigra* were approximately one-half as long as those of *P. tremuloides* *(Fig. 6; Table 5)*. Although small numbers of pollen grains of *P. deltoides* and the "Tacamahaca" poplars did germinate on *P. tremuloides* stigmas *(Table 2)*, they typically failed to penetrate the stigma surface and grew in a twisting fashion *(Fig. 7;* compare with *Fig. 2)*. Frequently, sites of callose deposition were observed at the point of pollen-tube contact with the stigma *(Fig. 8)*. Such callose deposits are characteristic of pollinations in which pollen tubes fail to penetrate the stigma surface (Dickinson and Lewis, 1973: Knox, 1973) and were not observed in any other species used as female parent. This finding suggests a type of stigmatic barrier peculiar to *P. tremuloides* among the species examined.

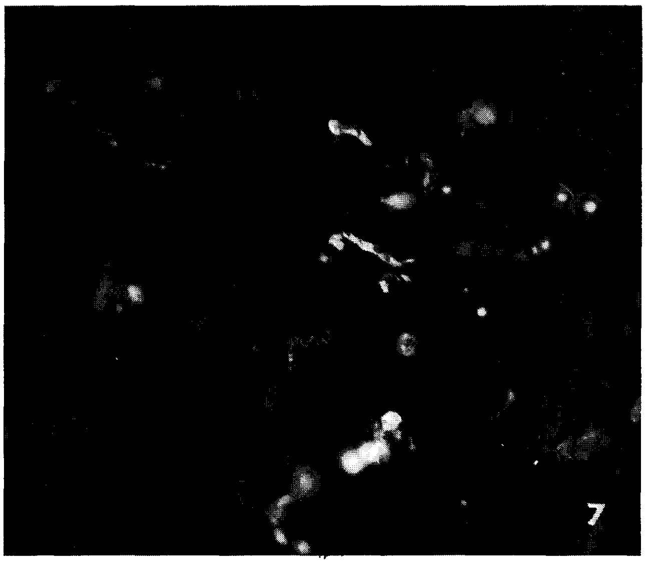
In general, differences in pollen-tube growth followed sectional lines, but some exceptions were noted. Inhibition of pollen-tube growth appears then to be a moderately strong incompatibility barrier in certain poplar crosses. It manifests itself at two different sites; *i.e.* at the stigmatic surface, as in the *P. tremuloides* × "Tacamahaca" crosses; or in the stigmatic tissue, as in the *P. trichocarpa* × "Leuce" crosses.

Micropylar penetrations.

Pistil squashes *(P. alba* and *P. tremuloides)* or excised ovary contents *(P. trichocarpa* and *P. deltoides)* were ex-







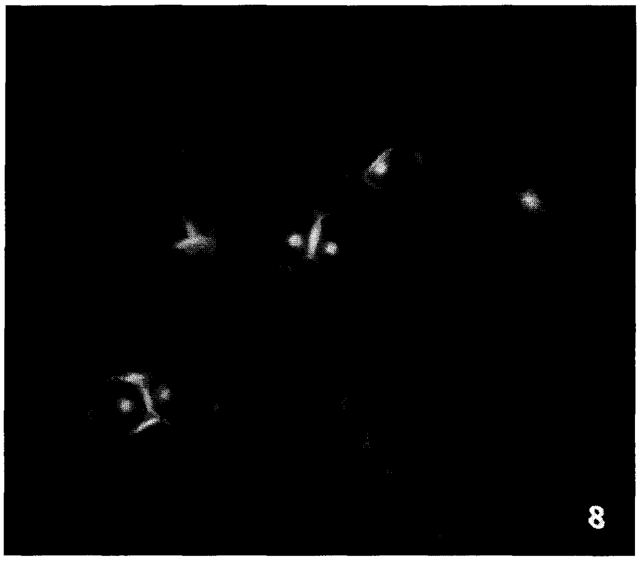


Figure 1. — Pollen tubes of *P. nigra* in *P. deltoides* stigmas 12 hours after pollination (20×). Note the large number of overlain grains and tubes. — *Figure 2.* — Pollen tubes of *P. balsamifera* in *P. deltoides* stigmas 4 hours after pollination (80×). — *Figure 7.* — Pollen tubes of *P. balsamifera* on stigma of *P. tremuloides* 16 hours after pollination (80×). — *Figure 8.* — Sites of callose deposition in stigmatic cells of *P. tremuloides* following pollination by *P. Maximowiczii* (200×). The pollen tubes failed to penetrate the stigma and were washed away in clearing. — *Figure 9.* — Micropylar penetration of *P. trichocarpa* ovule following pollination by *P. trichocarpa* (200×).

amined for numbers of ovules having a pollen tube growing into the micropyle. While this in itself is not evidence that fertilizations occurred, it does indicate that pollen tubes were able to deliver gametes to the ovule and fertilizations were therefore possible. Fragmentation of pollen tubes and differences in tissue translucence following clearing hindered the observations, and it is likely that our data underestimate the numbers of penetrations that actually occurred. However, as these data generally paralleled the results of the pollen-tube growth analysis, any bias introduced by these techniques affected all treatments to the same degree. A typical micropylar penetration is shown in *Fig. 9*.

With the exception of the cross *P. trichocarpa* × *P. nigra* no differences were apparent in the number of penetrations observed among "Aigeiros" and "Tacamahaca" pollens in *P. trichocarpa* pistils *(Table 6)*. Numbers of penetrations observed for *P. alba* and *P. tremuloides* pollens were much reduced or zero. A similar pattern of inter-specific differences was also observed in *P. deltoides* ovules, although the overall distribution was less clear-cut due to the smaller sample.

Pollen tubes penetrating the micropyle were observed for all seven pollen species in *P. alba* pistils, but *P. tremuloides* pollen tubes were clearly superior to the other poplars in this regard *(Table 6)*. This finding indicates that although differences in incompatibility reactions do exist in *P. alba*, no absolute barrier to crossing exists prior to micropylar penetration for the pollen species examined.

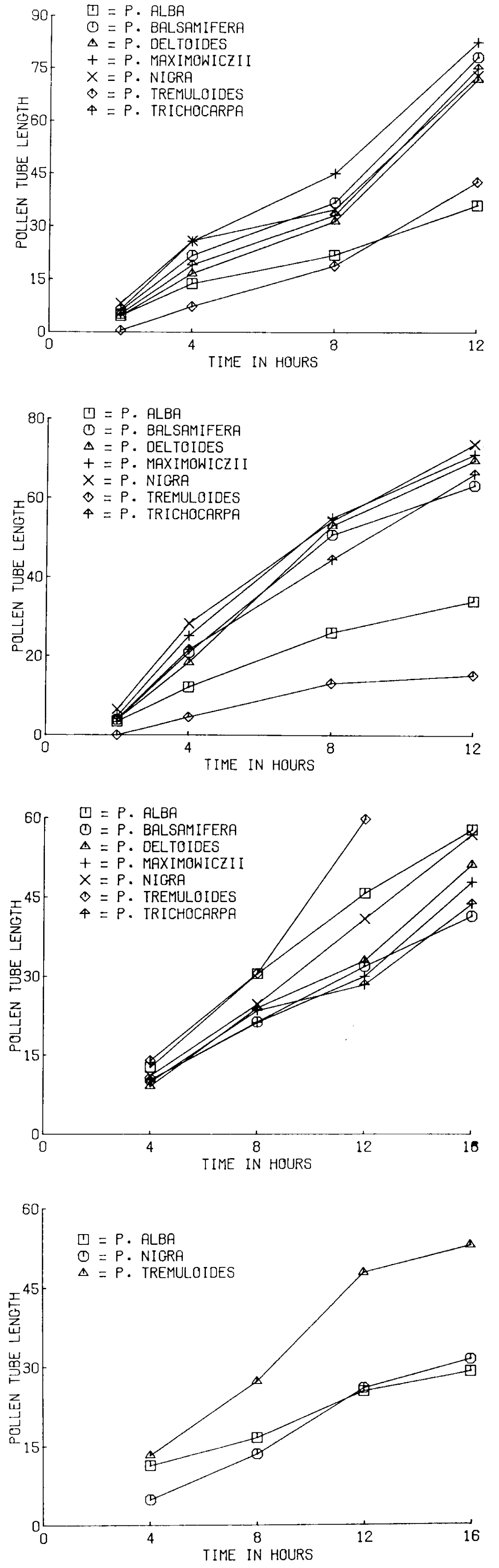
The numbers of micropylar penetrations recorded for *P. tremuloides* flowers were almost precisely those expected based upon pollen-tube growth data if no differential barriers to fertilization are present in the ovary and ovules *(Table 6)*. A single pollen tube of *P. deltoides* did ultimately effect a penetration in *P. tremuloides* indicating that such events do occasionally occur.

Since large differences in pollen-tube growth had been observed, the failure to detect micropylar penetrations among certain crosses at 3 days after pollination could simply be due to slower pollen-tube growth. Therefore, in a subsequent experiment, pistils were sampled 6 days after control pollination of *P. trichocarpa* and *P. tremuloides* catkins on trees. Analysis of this material indicated that micropylar penetrations did occur in the cross *P. trichocarpa* × *P. tremuloides* and reciprocal, but with a low frequency *(Table 7)*. The reduced penetration frequency in the *P. trichocarpa* × *P. tremuloides* cross relative to that in a compatible cross is most likely due to the slower tube growth of *P. tremuloides* pollen in the foreign stigma *(Fig. 3)*. More surprising were the positive results in the reciprocal cross since pollen tubes of *P. trichocarpa* had been found earlier not to penetrate the stigmatic surface but to remain short and twisted. Nevertheless, the frequency of ovule penetration was very low, suggesting that such instances were rare events perhaps due to a damaged stigma.

During the course of this study some 8,000 ovules were examined and more than 500 micropylar penetrations recorded. Although large numbers of pollen tubes were frequently present in the ovary, in no instance was more than one pollen tube seen to enter the same micropyle.

Discussion

Results obtained from intra- and inter-specific crosses showed that several pre-fertilization barriers can restrict crossing among poplar species and that different barriers



Figs. 3—6. — Pollen-tube growth following intra- and inter-specific pollination of *Populus* flowers. Means are based upon measurement of 5 long tubes from each of 10 pistils per cross. 95% confidence intervals for means are found in *Tables 3—5*. *Fig. 3. P. trichocarpa* as female parent. *Fig. 4 P. deltoides* as female parent. *Fig. 5. P. alba* as female parent. *Fig. 6. P. tremuloides* as female parent.

Table 3. — Student-Newman-Keuls test for differences in mean pollen-tube length 12 hours after pollination. Data were transformed to common logarithms to stabilize variances and are presented here with 95% confidence intervals after back-transformation. Means underscored by the same line are not significantly different at the .05 level.

P. trichocarpa as female parent

	$\bar{Y}_1$	$\bar{Y}_2$	$\bar{Y}_3$	$\bar{Y}_4$	$\bar{Y}_5$	$\bar{Y}_6$	$\bar{Y}_7$
♂	*alba*	*trem*	*delt*	*nigra*	*trich*	*bals*	*Max*
μ	34.82	42.20	70.80	72.36	74.08	77.64	81.87
95% C.I.	30.80— 39.34	40.43— 44.05	67.32— 74.46	69.84— 74.99	70.86— 77.45	75.04— 80.35	79.00— 84.82

P. deltoides as female parent

	$\bar{Y}_1$	$\bar{Y}_2$	$\bar{Y}_3$	$\bar{Y}_4$	$\bar{Y}_5$	$\bar{Y}_6$	$\bar{Y}_7$
♂	*trem*	*alba*	*bals*	*trich*	*delt*	*Max*	*nigra*
μ	15.45	34.00	63.26	66.25	69.54	71.09	73.67
95% C.I.	14.25— 16.76	32.97— 35.08	61.29— 65.28	63.74— 68.65	66.56— 72.70	68.98— 73.27	71.52— 75.91

Means based upon lengths of 10 pollen tubes from each of 5 pistils (100 units = 1.5 mm).

Table 4. — Student-Newman-Keuls test for differences in mean pollen-tube length 12 hours after pollination of *P. alba* flowers. Data were transformed to common logarithms to stabilize variances and are presented where with 95% confidence intervals after back transformation. Means underscored by the same line are not significantly different at the .05 level.

	$\bar{Y}_1$	$\bar{Y}_2$	$\bar{Y}_3$	$\bar{Y}_4$	$\bar{Y}_5$	$\bar{Y}_6$	$\bar{Y}_7$
♂	*trich*	*Max*	*bals*	*delt*	*nigra*	*alba*	*trem*
μ	28.40	30.03	31.89	32.95	40.91	45.70	59.68
95% C.I.	25.07— 32.16	27.05— 33.33	29.64— 34.30	29.91— 36.29	36.50— 45.84	42.53— 49.09	53.85— 66.13

Means based upon lengths of 10 pollen tubes from each of 5 pistils (100 units = 1.5 mm).

Table 5. — Student-Newman-Keuls test for differences in mean pollen-tube length 16 hours after pollination of *P. tremuloides* flowers. Data were transformed to common logarithms to stabilize variances and are presented here with 95% confidence intervals after back-transformation. Means underscored by the same line are not significantly different at the .05 level.

	$\bar{Y}_1$	$\bar{Y}_2$	$\bar{Y}_3$
0	*alba*	*nigra*	*trem*
μ	27.50	29.55	51.83
95% C.I.	25.13— 30.10	26.82— 32.56	49.06— 54.67

Means based upon lengths of 10 pollen tubes from each of 5 pistils (100 units = 1.5 mm).

may operate not only in different species combinations but even in reciprocal crosses of the same species pairs.

Pollen germination appears to be a significant barrier only when *P. tremuloides* is used as female parent as none of the "Tacamahaca" poplars, nor *P. deltoides,* showed appreciable germination on aspen stigmas even 16 hours after pollination. However, the subsequent finding that a low level of micropylar penetrations occurred in *P. tremuloides* × *P. trichocarpa* crosses indicates that this barrier is occasionally breached, for reasons that are still unknown.

P. tremuloides also differed from the other poplars in exhibiting a delayed pollen germination even on compatibles stigmas. Previous reports for in vivo aspen pollen germination of 2—6 hours (Winton, 1968; Illies, 1975) are in agreement with observations made during this study. In other reports on poplar pollen germination *in vivo,* Mel-

Table 6. — Numbers of ovules examined and micropylar penetrations recorded 3 days after intra- and inter-specific pollinations[1]).

♀		♂ *delt*	*nigra*	*bals*	*Max.*	*trich*	*alba*	*trem*
delt	ovules	119	134	116	123	131	126	138
	Penetrations	1	2	0	8	4	0	0
trich	ovules	313	307	282	240	259	272	290
	Penetrations	15	3	13	10	12	1	0
alba	ovules	40	32	48	52	40	40	40
	Penetrations	6	1	5	5	2	5	13
trem	ovules	94	103	119	122	105	91	110
	Penetrations	1	13	0	0	0	11	40

[1]) Based upon counts from 10 pistils per cross.

Table 7. — Numbers of ovules examined and micropylar penetrations recorded 6 days after intra- and inter-specific pollinations.

		♂ *trichocarpa*	*tremuloides*
♀ *trich*	ovules	905	850
	Penetrations	201	46
trem	ovules	1250	1250
	Penetrations	5	105

CHIOR and SEITZ (1968) found no pollen germination barrier in any crossing combination of *P. deltoides* and *P. nigra.* KNOX, WILLING and ASHEFORD (1972) found that for the cross *P. deltoides* × *P. deltoides* pollen germinated rapidly with many tubes reaching the style within 90 minutes, while for the cross *P. deltoides* × *P. alba,* pollen germinated in this time but tubes seldom penetrated the stigma. For the poplars examined in this study, including these same crosses, pollen grains were seldom seen to germinate in less than 1—2 hours and typically required 8—12 hours for the fastest growing tubes to reach the style.

Pollen-tube growth posed a significant barrier to hybridization, particularly for the "Tacamahaca" × "Leuce" and the "Aigeiros" × "Leuce" crosses. The slow growth rates exhibited in these crosses could restrict hybridization in several ways: 1) slow-growing tubes may never reach the ovules; 2) these tubes may reach the ovules, but the ovules are no longer receptive; 3) the stimulus required for catkin retention may not be provided, or then may be provided too late or in too small an amount. In most crosses where pollen-tube growth is inhibited it is apparent that those micropylar penetrations (and corresponding fertilizations) that do occur are either considerably delayed or greatly reduced in number, or both. The growth of functionally compatible pollen tubes seems to be of critical importance in at least some poplars in determining crossing success. In the absence of compatible pollen-tube growth, *P. trichocarpa* catkins abscise within two weeks of pollination. Catkin retention in this case can be promoted by heavily irradiated maternal species pollen which produces a pollen-tube but sets no seed, but not by "Leuce" species pollen which also produces a pollen tube but ultimately results in premature catkin abscission (STETTLER and GURIES, 1976). A similar situation has recently been reported for *Passiflora* species in which flowers abscise following interspecific crosses despite pollen germination and pollen-tube growth. The incompatibility, apparently due to the failure of the foreign pollen to provide or stimulate hormonal growth substances, was corrected by the exogenous application of such substances or by treating one of three stigmas per flower with compatible pollen and the other two with foreign pollen. (PAYAN and MARTIN, 1975).

The frequency of micropylar penetrations seemed to validate the observations made on pollen-tube growth. In no case were large numbers of penetrations found when pollen-tube growth was inhibited. Whether this inhibition alone accounts for the difficulty in crossing many of the poplars, or whether additional post-fertilization barriers also restrict crossing is unknown at present.

Investigations of incompatibility barriers in angiosperm tree species have been infrequent (reviewed in HAGMAN, 1975; and KRIEBEL, 1975), but results from the best-studied cases involving *Alnus* and *Betula* have indicated that reduced pollen-tube growth is the principle factor in hybridization failure.

A summary of the results of pollen germination, pollen-tube growth, and micropylar penetration is provided in *Fig. 10.* These poplars fall into two groups largely along taxonomic lines with the balsam poplars at one extreme and aspen at the other. *P. alba,* while more closely related to *P. tremuloides,* appears somewhat intermediate with respect to the characters studied here. It seems possible then, that *P. alba* could act as an intermediary in attempts to exchange genes between the balsam poplars and aspen. The overall scheme is remarkably close to the crossability series aspen — white polar — cotton wood — balsam poplar originally proposed by HEIMBURGER (1940) in which he suggested that "genetic affinities" restrict crossing to members of adjacent pairs in the series.

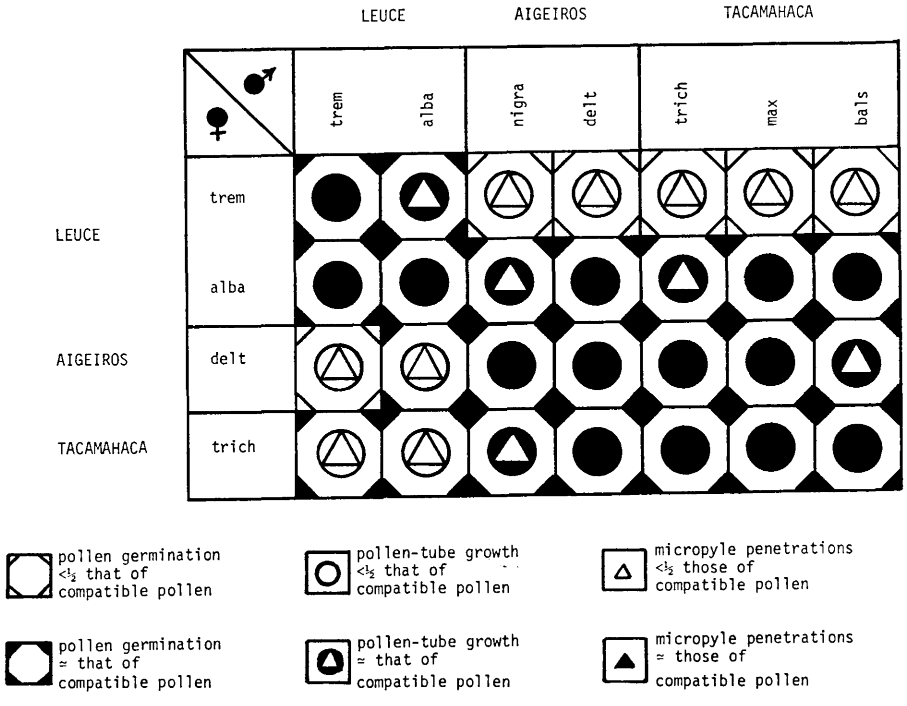


Figure 10. — Summary of results for pollen germination, pollen-tube growth, and micropylar penetration for selected *Populus* species.

Several techniques recently developed to overcome cross-incompatibility barriers in poplars have met with varied success and interpretation. The admixing of irradiated compatible "mentor" pollen (Stettler, 1968; Stettler and Bawa, 1971) has been repeatedly effective in facilitating intersectional crosses (Zufa, 1968; Knox, Willing and Pryor, 1972; Willing and Pryor, 1976; Stettler and Guries, 1976). Its success has been attributed to a special class of pollen proteins which engage in a recognition reaction at the stigma surface and which can be "borrowed" by in compatible pollen in a pollen mix (Knox, Willing, and Asheford, 1972). These researchers also developed improvements in the preparation of mentor, or "recognition" pollen, which markedly increased hybrid seed yield.

More recently, attention has shifted to the role of stigmatic compounds in such a recognition phenomenon. Whitecross and Willing (1975) and Willing and Pryor (1976) found that a pre-pollination treatment of stigmas with lipid solvents *(e.g.* hexane, or ether) permitted crosses among selected species from all five sections of the genus with resultant seed sets as high as in compatible crosses. The results were interpreted to suggest that two factors, one associated with the pollen, the other with the stigma, were concerned with the incompatibility reaction and that the incompatibility process is inactivated by the absence of either (Willing and Pryor, 1976). Unfortunately, no data were provided on pollen-tube growth or on the phenomena associated with the incompatible control crosses that might have shed light on the ontogenetic stages at which the barriers manifested themselves. Additional studies utilizing *P. trichocarpa* as female parent have been conducted which indicate that irradiated mentor pollen may act as an indirect facilitator of hybridization via the promotion of fruit development, rather than as a direct provider of recognition substances (Stettler and Guries, 1976).

It appears that at least 3 factors are involved in cross-incompatibility in poplars; two of them associated with recognition factors residing in the pollen and stigma respectively, and a third associated with the promotion of fruit development. The relative importance of the three factors seems to vary in different cross-combinations, but the mode of interaction among them is still unknown.

Summary

A systematic examination of 28 crossing combinations was conducted for several *Populus* species *(P. trichocarpa, P. balsamifera, P. Maximowiczii,* all section *Tacamahaca; P. deltoides* and *P. nigra v. italica,* section *Aigeiros; P. alba* and *P. tremuloides,* section *Leuce)* to identify the site(s) and strength of possible barriers to hybridization. Results from studies of pollen germination, pollen-tube growth, and micropyle penetration indicated that several pre-fertilization barriers operate to impair or prohibit crossing between certain species pairs.

Pollen germination appeared to be a serious barrier to hybridization only in *P. tremuloides* when *Aigeiros* or *Tacamahaca* poplars served as pollen parents. Pollen tubes of *Aigeiros* and *Tacamahaca* species pollen rarely penetrated the stigma of *P. tremuloides,* growing instead in a twisting fashion on the surface.

Inhibition of pollen-tube growth was a common barrier in *Tacamahaca* by *Leuce* and *Aigeiros* by *Leuce* crosses, as well as in *P. alba* when *Aigeiros* or *Tacamahaca* poplars served as pollen parents. Data on micropylar penetrations in general paralleled the results of the pollen tube studies. With respect to crossability barriers, these poplars fall into two groups largely along taxonomic lines with the balsam poplars at one extreme and aspen at the other.

Key words: Populus spp., crossability, incompatibility barriers.

Zusammenfassung

Es wurden 28 Kreuzungskombinationen zwischen Pappelarten verschiedener Sektionen auf sog. Kreuzungsbarrieren hin untersucht. Hierbei ergab die Untersuchung der Pollenkeimung, des Pollenschlauchwachstums und des Pollenschlaucheintritts in die Mikropyle, daß zwischen gewissen Arten Unverträglichkeiten vorliegen. So scheint die Pollenkeimung z. B. bei Kreuzungen mit *Populus tremuloides* als Mutterbaum nur dann gehemmt zu sein, wenn *Aigeiros* oder *Tacamahaca*-Arten als Vaterbäume fungieren. Die Pollenschläuche von *Aigeiros* und *Tacamahaca*-Arten dringen nur selten in die Narbe von *P. tremuloides* ein. Eine Hemmung des Pollenschlauchwachstums war ganz allgemein als Kreuzungsbarriere bei *Tacamahaca*- × *Leuce*-Arten und *Aigeiros*- × *Leuce*-Arten zu beobachten, ebenso bei *Populus alba* × *Aigeiros*- oder *Tacamahaca*-Arten als Vaterbäumen. Das Durchdringen der Mikropyle ist als etwa paralleles Kriterium zu den Ergebnissen aus den Pollenschlauchuntersuchungen zu sehen. Was insgesamt gesehen das Auftreten von sog. Kreuzungsbarrieren betrifft, hat sich herausgestellt, daß solche im weitesten Sinne zwischen den taxonomischen Linien, Balsampappeln auf der einen Seite und Aspen auf der anderen Seite, zu beobachten waren.

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Einige Wuchs- und Holzeigenschaften bei einer Kreuzung Populus alba x Populus grandidentata im Vergleich mit dem Schwarzpappelbastard 'Robusta'

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1 Einleitung

Seit langem werden innerhalb der Pappelsektion Leuce (Weiß- und Zitterpappeln) Züchungsarbeiten durchgeführt. Bereits vor dem 2. Weltkrieg hat v. Wettstein mit Arten der Sektion Leuce gearbeitet. In Deutschland haben sich nach dem Kriege mehrere Stellen im Rahmen der Pappelforschung auch mit den Weißpappeln und Aspen befaßt (Hattemer *u. a.* 1967, Melchior *u. a.* 1966, Seitz 1963, v. Schönborn 1965). In Baden-Württemberg hat Schlenker bald nach dem Kriege ein umfangreiches Züchtungsprogramm mit Pappeln begonnen, wobei der Schwerpunkt bei der Sektion Leuce lag (Schlenker 1953, Heimmburger 1958).

Als recht vielversprechend erwiesen sich dabei Kreuzungen zwischen der europäischen Silberpappel *(Populus alba* L.) und einer nordamerikanischen Aspenart *(Populus grandidentata* Michx.). Die beiden im folgenden auch als alba und grand. bezeichneten Arten sind miteinander leicht zu kreuzen, und die Kreuzungspopulationen zeigen ein rasches Jugendwachstum, gute Formeigenschaften und gute Bewurzelungsfähigkeit bei Verwendung von Holzstecklingen (Heimburger 1968, Johnson 1946, Kopecky 1969, Zufa 1971). Bei Artkreuzungen entstehen jedoch selbst bei Wahl von Kreuzungspartnern mit guter Kombinationseignung in der Regel so heterogene Nachkommenschaften, daß ein großer Teil der Hybridsämlinge für eine wirtschaftliche Verwendung nicht geeignet ist (Heimburger 1968). Wegen der guten Bewurzelungsfähigkeit besteht bei Kreuzungen *alba* × *grand.* jedoch die Möglichkeit der Auslese einzelner guter Pflanzen mit anschließender Vegetativvermehrung. Zur Klärung der Frage, welche Variation in Sämlingsnachkommenschaften dieser Kreuzung zu erwarten ist und welche genetischen Gewinne durch Auslese und Vegetativvermehrung möglich erscheinen, wurden vorliegende orientierende Untersuchungen an Holzproben von Sämlingen einer Kreuzung *alba* × *grand.* und von aus Stecklingen herangezogenen Bäumen von *P.* × *euramericana* cv. 'Robusta', nachfolgend als Robusta bezeichnet, durchgeführt.

Als Merkmale, die an den Holzproben zu erheben waren, wurde neben der Jahrringbreite die Dichte des Holzes gewählt, da sie sowohl die in der Volumeneinheit erzeugte reine Holzsubstanz angibt, als auch ein komplexes Holzmerkmal darstellt, das Rückschlüsse auf die innere Holzqualität und die Holzverwendungsmöglichkeiten zuläßt (für weitergehende Literatur hierzu sei auf Kennedy 1968 und Marcet 1965 verwiesen).

2 Material und Versuchsdurchführung

2.1 *Ausgangsmaterial und Feldversuchsanlage*

Die Kreuzung *P. alba* × *P. grandidentata* wurde von Schlenker 1952 in Stuttgart-Weilimdorf durchgeführt. Als Mutter diente ein *alba*-Klon, der unter der Bezeichnung „Dillingen Nr. 27" geführt wird. Der Pollen stammt aus einer Direktsendung von Prof. Heimburger in Kanada. Ob es sich dabei um Pollen eines einzigen Baumes handelt (was wahrscheinlich ist), oder um ein Pollengemisch, ist nicht bekannt. Bei der aus dieser Kreuzung hervorgegangenen F_1-Population (Weilimdorf Nr. 5229) handelt es sich demnach mindestens um Halbgeschwister, wenn nicht um Vollgeschwister.

Mit den Sämlingspflanzen der F_1-Generation wurden in verschiedenen Teilen von Baden-Württemberg „Beobachtungsflächen" angelegt, um die Standortstoleranz der Neuzüchtungen zu testen. Diese Flächen wurden meist nicht als Versuchsflächen im strengen Sinne angelegt, sondern die Neuzüchtungen wurden je nach verfügbarer Flächengröße und Geländeform block- oder reihenweise zusammen mit bekannten Altsorten angepflanzt.

Das hier untersuchte Material stammt von der Beobachtungsfläche „Eschelhof" im Forstbezirk Unterweissach (jetzt Fbz. Backnang). Die Auspflanzung der zweijährigen Sämlinge erfolgte 1954 im Verband 5 × 5 m. Als Vergleichssorte wurden im Block daneben ebenfalls zweijährige Pflanzen der Schwarzpappelsorte 'Robusta' gepflanzt.

Der Standort ist eine flache Talsenke mit nährstoffreichem, grundfrischem kolluvialem Aueboden (lehmiger Sand) im Bereich des Stubensandsteins (mittl. Keuper). Die Fläche liegt im inneren Schwäbisch-Fränkischen Wald (Regionalgesellschaft: paenemontaner Buchen-Tannen-Wald) in 470 m Meereshöhe. Die Jahresdurchschnittstemperatur beträgt ca. 7,8° C, die jährlichen Niederschläge im Durchschnitt ca 1000 mm.

2.2 *Holzuntersuchungen*

Nach einer Durchforstung in der Versuchsanlage im Jahre 1973 bot sich die Gelegenheit, einige Holzmerkmale

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