

Interspecific Crossability Studies in Poplars

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Summary. Pollen-stigma interactions were studied with scanning electron microscopy in intraspecific and inter-sectional crosses of *Populus tremuloides* (Sect. LEUCE); *P. deltoides*, *P. nigra* (Sect. AIGEIROS); and *P. trichocarpa* (Sect. TACAMAHACA). Intraspecific variation in hydration, size of hydrated pollen grains, and tube morphology is described. Exine sculpture in *P. tremuloides* was densely spinulate or scabrate, in *P. trichocarpa* densely reticulate, in the other species somewhat intermediate. In six of seven inter-sectional cross combinations pollen-tube behavior on the stigmatic surface at 5-22 hours after pollination was the same as in conspecific matings; in *P. tremuloides* × *P. trichocarpa* crosses, the percentage of twisted and bulging tubes was significantly higher. Irradiated mentor pollen did not affect pollen-tube behavior of adjacent incompatible pollen, but increased hybrid seedling production in interspecific crosses. By contrast, hexane treatment of pollen or stigma, and the spraying of catkins with gibberellic acid or naphthalene acetic acid were ineffective. The results are discussed in relation to several mechanisms presumed to be involved in restricting gene exchange among poplar species; and in relation to Hogenboom's (1973) incongruity concept.

Key words: Interspecific hybridization – Incompatibility – Incongruity – Mentor pollen

Introduction

There has been a long sustained interest in the hybridization of poplars, largely nourished by the benefits derived from capturing heterosis and combining desirable traits, ultimately expressed in the amount and quality of wood produced. The ease with which most hybrids could be perpetuated and increased vegetatively has given further

incentive to these efforts. More recently, additional impetus has been provided by the findings that various manipulations can significantly improve crossability in combinations traditionally considered incompatible. These manipulations include:

- Mixing normal, incompatible pollen with mentor pollen, i.e. compatible pollen that has been rendered genetically inert by ionizing radiation (Stettler 1968) or one of several other treatments (Knox et al. 1972);
- Substituting mentor pollen with partially purified pollen-wall proteins extracted from compatible pollen (Knox et al. 1972);
- treating incompatible pollen with one of several solvents, thus rendering it compatible (Willing et al. 1976); and
- treating stigmas with one of several solvents, thus making them receptive for incompatible pollen (Willing et al. 1976).

Some of the manipulations, notably the mentor pollen technique, have in the meantime proved effective also in overcoming self-incompatibility in *Malus* and *Nicotiana* (see recent review by Pandey 1979). Explanations offered for the efficacy of these treatments have attributed significance to the role of: exine-bound 'recognition' proteins (Knox et al. 1972a); n-hexane soluble (presumably mainly lipoidal) materials in both pollen and stigma (Willing et al. 1976; Hamilton 1976); pollen growth promoting substances (Hamilton 1976; Pandey 1977); and substances responsible for the post-pollination growth and development of ovules, pistils and inflorescences (Stettler et al. 1976). There is no a-priori reason other than parsimony to consider alternative mechanisms as mutually exclusive. It is a common feature for congeneric species to be reproductively isolated from one another by multiple barriers (Grant 1971), and one might suspect this to be true particularly for those poplars studied belonging to different sections of the genus. In fact, it may be quite inappropriate to consider these

interspecific crossing barriers within the confining context of *incompatibility*, and more fitting to view them as different aspects of evolutionary divergence resulting in *incongruity*, as aptly recognized by Hogenboom (1973, 1975).

The present study was conducted to shed more light on pollen behavior on the stigmatic surface. An earlier survey of 28 cross combinations, making use of fluorescence microscopy, had revealed the importance of tube-growth inhibition in the stylar tissue; by contrast, only few cross combinations seemed to fail due to a lack of stigmatic penetration (Guries et al. 1976). This result seemed to question the significance attributed to the early interaction between pollen and stigma by other researchers (Knox et al. 1972; Willing et al. 1976; Hamilton 1976). It was thus decided to re-examine these phenomena at a higher resolution, with the aid of scanning electron microscopy. A further objective of the study was to test the repeatability of pollen and stigma treatments as reported successful by Willing and Pryor (1976). Major emphasis was placed on difficult-to-achieve crosses involving three of the five sections of the genus *Populus*, LEUCE Duby, AIGEIROs Duby, and TACA-MAHACA Spach.

Materials and Methods

Materials

An overview of the material used is given in Table 1.

Pollen and its Treatments

Pollen was collected from 'forced' branches in the greenhouse, from each species in separate chambers, as commonly done in

poplar breeding. The pollen was then air dried at room temperature for 24-48 hours and subsequently stored at 2-4°C in cotton-stoppered vials over Silica gel in an exsiccator. When pollen mixtures were used, equal volumes of each lot were thoroughly mixed with a glass rod. Pollination was performed by inserting attached catkins into a glass vial containing pollen and lightly dusting the flowers with the aid of a camelhair brush. The treatment of pollen with hexane, and in one experiment with ethylacetate, was done according to Willing et al. (1976) and Hamilton (1976). Special care was taken to minimize the water content in the pollen-solvent suspension (Hamilton 1976) by drying the pollen batch in a separate exsiccator for at least 22 hrs. prior to treatment; and by using the highest grade hexane (Merck No. 4367, with a max. H₂O content of 0.01 percent). In two treatments the pollen was washed in three successive changes of the solvent for ten, and five, minutes each, respectively. Subsequently the pollen was filtered and dried at room temperature. After having obtained almost invariably negative results with pollen so treated in 1977, we tried a third treatment in 1978 in which pollen was exposed to the solvent for only 5 minutes. For pollen irradiation, pollen was exposed to a Cobalt 60 source for 100 KR at the Institute for Atomic Sciences in Agriculture, ITAL, in Wageningen, Holland.

Stigmatic Treatment

Hexane treatment of stigmas was done according to Willing et al. (1976) and Hamilton (1976). In aspen, the long bracts subtending individual female flowers were removed with a forceps to better expose the stigmatic lobes. This was not necessary in the other species. Care was taken to avoid 'burning' the stigma by wiping off excessive solvent from the camelhair brush with which it was applied.

Scanning Electron Microscopy (SEM)

Pollinations performed for SEM study were done in the same way as those for seed production. Female branches were kept in the laboratory in water containers and isolated from pollen until receptive. At pollination they were transferred to a lighted growth room where they were kept at 21°C and 50% rel. humidity until examined. Precautions were taken to minimize contamination among different treatments. Their effectiveness was ascertained

Table 1. Original source of materials used in crosses

Section	Species (abbreviations used in crosses)	Female	Male
LEUCE Duby	<i>P. tremuloides</i> (TREM)	21 clones from Michigan, Ontario, Vermont, Wisconsin	16 clones from Michigan, Ontario, Vermont, Washington, Wisconsin
	<i>P. alba</i> (ALBA)	None used	11 clones from Italy and the Netherlands
AIGEIROs Duby	<i>P. deltoides</i> (DELT)	9 clones from Massa- chusetts, Michigan, Ohio	14 clones from Connecticut Illinois, Michigan, Ohio, Vermont, and Wisconsin
	<i>P. nigra</i> (NIGRA)	6 clones from the Netherlands	8 clones from Belgium and the Netherlands
TACA- HACA Spach	<i>P. trichocarpa</i> (TRICH)	5 clones from British Columbia, Idaho, Oregon, Washington	7 clones from British Columbia, Idaho and Washington

in those cases where pollens of distinct morphology were involved, as in *P. tremuloides* and *P. trichocarpa*. Most pistils for SEM study were sampled at 5-8 hrs after pollination, some at 15-22 hrs., but none later than 26 hrs. Preliminary experiments had shown that earlier samplings, even in conspecific matings, gave unreliable results due to the variable rate of pollen germination. After unsatisfactory results with several fixation and coating procedures only fresh material was used. No adverse effects were noted in the specimens in the 20-30 minutes (at 10kv) during which they were exposed to the vacuum while pollen tubes were scored. The electron microscopes used were both JEOL, JSM-U3, generating 1.5×10^{-10} Amperes at 10 kilovolt. For each treatment, 50-100 pollen tubes were assessed on each of two stigmas. No significant differences were found in pollen germination and in the relative frequency of pollen-tube types, between different stigmatic lobes of the same pistil, between stigmas from different pistils having received the same treatment, and between different samplings within the period of 5-26 hours after pollination.

Pistillate Material and its Treatment

Female branches were brought into the greenhouse in early spring. Those of *P. tremuloides* were kept in water culture (Stettler and Bawa 1971); those of *P. deltoides* were grafted as bottle grafts (Farmer and Nance 1968); those of *P. trichocarpa* and *P. nigra* were kept in both types of culture. In the case of bottle grafts, both DELT \times TRICH¹ and DELT \times NIGRA rootstocks were compared. For grafts of *P. deltoides*, the former gave higher catkin survival and faster maturation than the latter. No difference was noted between the two rootstocks for grafts of *P. trichocarpa* and *P. nigra*. Treatments with growth substances included α Naphthylacetate (NAA) at concentrations of 0.001, 0.005, and 0.01 percent, and gibberellic acid (GA₃), at 0.01 and 0.1 percent. They were prepared as sprays with a wetting agent (2 drops of 'Tween' per 1 solution) and applied to the catkins once (GA), or twice (NAA), a week to the dripping point, for 3 weeks beginning 24 hours after pollination. For collection of cotton, seed extraction, germination, raising of seedlings, and scoring of seedlings, reference is made to Koster (1968).

Experimental Design

It may be well to point out a few of the constraints with which poplar breeders have to contend in conducting breeding experiments. The convenience of having floral branches in the greenhouse, either in water culture or grafted on rootstocks, controls the environment during the actual breeding but does not cancel environmental differences experienced by the branches prior to their detachment. Since reproductive organs are laid down in the preceding summer, the impact of drought, frosts, etc. can be significant. If material, as in our case, has to be collected from different locations, this environmental variation may overshadow the genotypic effects. Once a floral branch has been detached it often behaves as a unit although different catkins on it may receive different treatments. Similarly to this branch effect there is a catkin effect governing the 20-40 pistillate flowers borne by the same ament. Thus, the ideal experiment would accommodate all treatments and control in the catkins of a given branch, with replications from different branch positions, collected from different replicates of the same clone from different locations, and repeated over several years. The major shortcoming of this design is the high probability of contamination or interaction among

catkins receiving different treatments on the same branch. We, therefore, administered the same treatment to all catkins of the same branch, used 1-2 additional branches as replicates, and considered the branch as a unit of assessment, regardless of the number of catkins it carried.

Results

I Pollen Behavior on Stigmatic Surface

1 General

Much emphasis in our study was placed on the cross combination of *P. tremuloides* and *P. trichocarpa*, in both directions, because it constituted a difficult cross on which much information was already available. Furthermore, it was of special interest since it was one of the few inter-sectional combinations in which obvious differences had been found in the surface behavior of pollen between reciprocal crosses (Guries et al. 1976). Our data are, therefore, more comprehensive on this than on other cross combinations.

The following account of results will first deal with general observations made in our microscopic investigation, and then with findings in specific crosses and treatments. Our SEM study confirmed many features of poplar pollen behavior as earlier described by Hamilton (1976). The air-borne pollen is disseminated in a dehydrated state and appears irregularly wrinkled and seemingly collapsed (Fig. 1h). It alights on a stigmatic surface that is characterized by low, convex, papillae which are devoid of copious amounts of exudate but have a 'warty' surface, i.e. irregularly spaced mounds of 1-2 μ diameter (Fig. 1d,g). The pollen then hydrates, gradually assuming a more or less spherical shape (Fig. 1a-g), and releases an exudate which cements it to the papillar surface. The pollen being inaperturate (Faegri and Iversen 1975), its tube will emerge at an unpredictable spot and will typically penetrate the cuticle close to the point of first contact. More often the penetration occurs at the contact zone of two or three adjacent papillae, but occasionally in the middle of a papilla (Fig. 1g).

The use of fresh, unfixed material in our SEM study assured us that artefacts would be minimized. On the other hand, it set constraints on the time during which specimens could be subjected to the vacuum. Poplar pollen is quite thin-walled (Erdtman 1966) and is soon weakened by the highly focused electron bombardment at high magnification. Under such conditions pollen grains soon begin to 'blister' and stigmatic papillae eventually collapse. While these constraints did not affect the identification of the types of pollen and pollen tubes, they limited photographic documentation at high magnification.

The pollens of *P. tremuloides* and *P. trichocarpa* could be reliably distinguished on the basis of exine sculpture

1 In all cross designations, the female parent is mentioned first

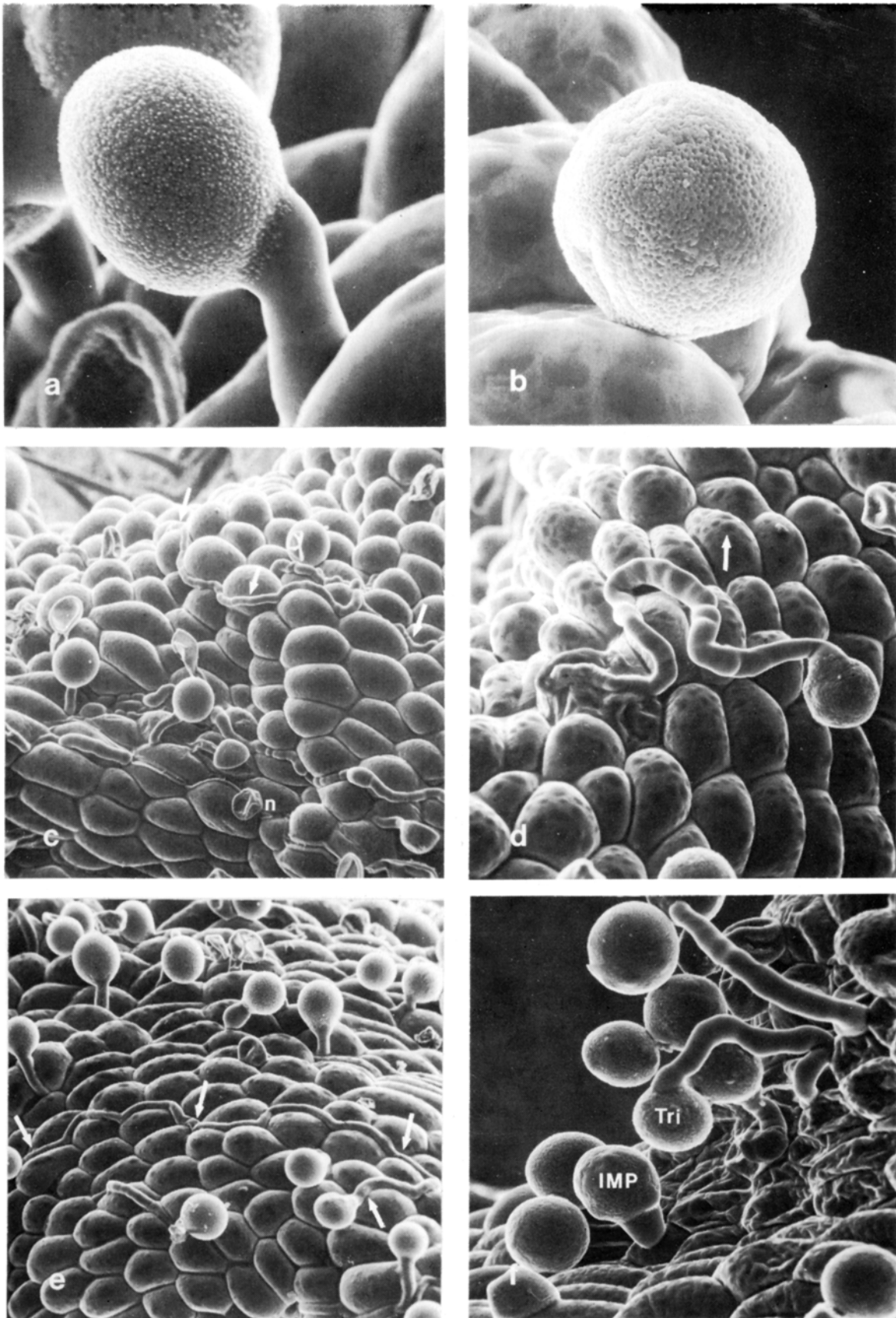


Fig. 1a-h.

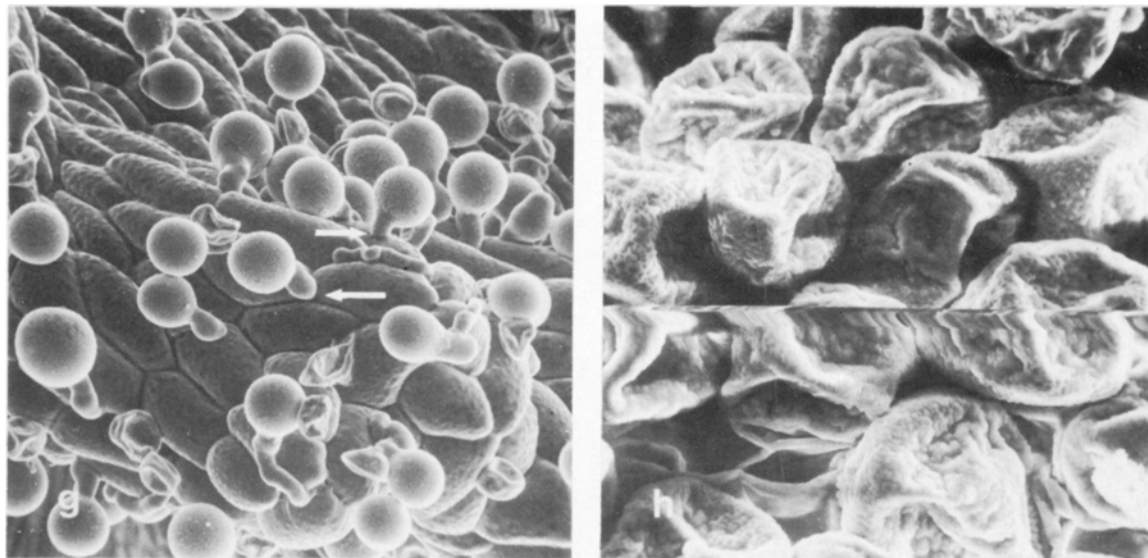


Fig. 1a-h. Scanning electron micrographs of pollen on stigmatic surface, taken at 5-20 hrs after pollination. **a** *P. tremuloides* pollen on *P. trichocarpa* stigma; note densely spinulose or scabrate sculpture and short tube, 2400X; **b** *P. trichocarpa* pollen on *P. trichocarpa* stigma; note densely reticulate sculpture, 2400X; **c** *P. tremuloides* pollen on *P. trichocarpa* stigma; note both short and extended (arrows) tubes and non-hydrated grain (n), 360X; **d** Twisted tube of *P. trichocarpa* pollen on *P. tremuloides* stigma; note 'warty' mounds on stigmatic surface; 720X. **e** *P. tremuloides* pollen with many short tubes on *P. nigra* stigma; arrows point to one continuous extended tube; 360X. **f** *P. trichocarpa* (Tri) and irradiated Mentor pollen (IMP) of *P. tremuloides* on *P. tremuloides* stigma; all twisted tubes are from Tri. 720X. **g** *P. tremuloides* pollen on *P. deltoides* stigma showing predominantly short and some twisted tubes, penetrating on top of or in-between, papillae (arrows); several non-hydrated grains, 360X. **h** Hexane treated *P. trichocarpa* pollen on *P. tremuloides* stigma; note clumping and reticulate sculpture of these non-hydrated grains, 1800X

(Fig. 1a, b); the former having a densely spinulose or scabrate, the latter a densely reticulate pattern. This provided a welcome tool to study the behavior of these pollens in mixtures on the stigma of either species, as further discussed below. Exine sculpture patterns in *P. deltoides* and *P. nigra* looked somewhat intermediate between those of the other two species but could not be consistently identified.

The size of hydrated pollen grains varied considerably but independently of species, male or female parent. Pooled data (N = 200) showed a range in diameter² from 25 to 44 microns with a mean and standard deviation of 32.1 ± 3.05 . This variation was observed in virtually every preparation and did not exhibit any obvious non-randomness in size classes and their distribution on the stigmatic surface. Often neighboring grains varied in volume by a factor of two, occasionally as much as four. Three types of pollen tubes were distinguished:

(a) Short tubes: tubes not significantly longer than the diameter of an average pollen grain and having penetrated the stigmatic surface (Fig. 1a).

(b) Twisted tubes: tubes significantly longer than the diameter of an average pollen grain, twisted and with irregular bulges, with or without sign of stigmatic penetration (Fig. 1d).

(c) Extended tubes: tubes significantly longer than the diameter of an average pollen grain, extending along the contact zone of many papillae, with or without sign of stigmatic penetration (Fig. 1c, e).

The distribution of these categories and additional observations are summarized in Table 2 and will be discussed separately for the major crosses and treatments.

2 Intraspecific Crosses

As expected, short pollen tubes were the norm in intraspecific crosses, their mean frequencies ranging from 77 to 97 percent in the four species. But in virtually every cross long tubes could also be found. In *P. tremuloides*, they were of the extended type, in the other three species, twisted.

The extended tubes of aspen were quite remarkable and, to the best of our knowledge, have not been described before in poplars. Contrary to the twisted tubes which seemed to meander aimlessly over the papillae, they were more oriented, showed fewer directional changes and typically grew along the 'valley' between adjacent papillae (Fig. 1e). Their diameter was quite constant along the length of the tube and their length exceeded that of twisted tubes by a factor of two to five. In fact, it was occasionally difficult to find the proximal or distal end of such a tube.

² Transverse diameter, i.e. perpendicular to the axis of the pollen tube; or in hydrated but ungerminated grains the diameter parallel to the plane of the stigmatic surface

Table 2. 1977/79 SEM data

Crosses (F × M)	Numbers of		Stigmas obs.	Mean % short pollen tubes	Additional Observations
	F	M			
1. Intraspecific					
TREM × TREM	2	2	13	81.0 ± 12.1	long tubes extended
TRICH × TRICH	3	3	26	77.0 ± 17.0	" " twisted
DELT × DELT	2	3	8	96.6 ± 7.0	much variation in hydration and germination among pollen lots
NIGRA × NIGRA	1	1	2	87 ^a	long tubes twisted
2. Intersectional crosses without treatment					
TREM × TRICH	3	3	31	33.0 ± 10.5	long tubes twisted
TRICH × TREM	3	4	20	81.1 ± 13.2	" " extended
DELT × TREM	3	7	10	75.0 ± 13.5	" " twisted, some extended
" × TRICH	2	1	4	74.5 ± 14.3	" " twisted
NIGRA × TREM	1	1	2	91 ^a	" " extended
" × TRICH	1	1	2	98 ^a	" " twisted
3. Intersectional crosses with treatments^b					
TREM × IMP	1	1	4	(71) ^c	most grains non hydr., low germination
TRICH × "	1	1	2	(79) ^c	
TREM × TRICH + IMP	2	2 + 1	9	n.q. ^d	many non-hydr. grains; most short tubes IMP, all long, twisted tubes TRICH.
TRICH × TREM + IMP	2	5 + 1	10	n.q. ^d	many non-hydr. grains; short tubes prevail (TREM, IMP), all long, twisted tubes IMP.
TREM × TRICH HXS	1	1	6	24	long tubes twisted
TREM × TREM "	1	1	2	77	long tubes extended
TREM × TRICH HXP	2	2	7	n.q.	} virtually no hydration or germination
TRICH × TREM HXP	3	5	16	n.q.	
" × " ETH	1	1	2	n.q.	
" × DELT HXP	1	7	2	n.q.	

^a One experiment only

^b IMP = irradiated mentor pollen HXS = hexane treated stigma HXP = Hexane treated pollen ETH = Pollen treated with Ethylacetate

^c based on less than 50 pollen tubes

^d not quantified

It was less expected to also find twisted tubes in intraspecific matings. In *P. trichocarpa* such matings occasionally had as many as 55 percent of these apparently malfunctioning tubes, showing characteristic bulges as in intersectional matings. Such tubes were not consistently associated with specific parents in either sex but were found to be more prevalent in specific male/female combinations.

3 Intersectional Crosses Without Treatments

With one exception, pollen hydration, germination, and tube growth behaved the same way in intersectional cross-combinations as in intraspecific ones. Again, much variation was found among specific genotypic combinations of a given type of cross, but neither the variance nor the mean frequency of short tubes differed significantly from the corresponding values of intraspecific matings. Extended tubes were strictly found in aspen pollen and there in every male tested. However, on *P. deltoides* stigmas, the

long tubes of aspen pollen were only of the twisted type in several genotypic combinations (Fig. 1g). Thus while the bulk of aspen pollen seemed to be capable of penetrating in short order the stigmatic surface of all species tested, a species-specific response was found in the less successful grains.

The exception to the normal surface behavior of pollen in intersectional matings was the TREM × TRICH cross. In all such matings and regardless of genotype, the frequency of short tubes was lower than in control, the difference in means being significant at the 0.1 percent level. Most pollen tubes were long and twisted although many of them would eventually penetrate. Occasional short tubes were interspersed among them and did not show selective aggregation in any area of the stigma.

4 Intersectional Crosses with Treatments

Irradiated mentor pollen (IMP) was studied only in crosses among *P. tremuloides* and *P. trichocarpa*, in both

directions. As pointed out earlier, the pollen of these two species could be reliably discriminated. In pollinations with IMP alone we generally found a normal sticking reaction but a significantly lower hydration and germination than in controls with unirradiated pollen of the same species. Most grains remained shrivelled but looked no different than non-hydrated control grains. The less than one percent that germinated showed the same types of pollen tubes in approximately the same frequencies as controls. Mixtures of foreign pollen and IMP were again studied in both cross directions. However, since TRICH \times TREM matings had shown normal pollen behavior (as explained in the previous section) little benefit was expected from the admixture of IMP in this case. By contrast, in the reciprocal cross, IMP might make the difference. We found that it did not. In several genotypic combinations, *P. trichocarpa* pollen on *P. tremuloides* stigmas grew primarily twisted tubes in spite of the presence of IMP. Most grains with short tubes were IMP, a few were *P. trichocarpa*. Even in the many instances where *P. trichocarpa* grains were adjacent to one or several hydrated IMP grains we generally found long, twisted tubes (Fig. 1f).

Stigmatic treatment with hexane, likewise, seemed to have no effect on the sticking reaction, hydration, germination, and tube growth of pollen. Again, the TREM \times TRICH cross would have been the most sensitive to detect a beneficial impact, but it showed no difference to control. Perhaps in an effort to prevent 'burning' of the stigma, our treatment had been too light. There may be indirect evidence for this since we could not detect a reduction in the waxy mounds on the papillar surface, as described by Hamilton (1976) after hexane brushing.

All chemical treatments of pollen, i.e. washing in hexane for three different periods (5 min., 3 \times 5 min., and 3 \times 10 min.) or in ethylacetate for 5 min., resulted in a reduced sticking reaction, and virtually prevented hydration and germination in every species combination and pollen batch tested. Morphologically, treated pollen looked similar to unhydrated control pollen (Fig. 1h) except that it charged up and tended to move under the electron beam.

II Seed and Seedling Production

Whereas the earlier part of our study dealt with events at the stigmatic surface, this part was concerned with the subsequent seed and seedling production, as affected by enhancing treatments. Each cross was attempted with a variety of genotypes. For experimental reasons, we chose an individual floral branch as the unit of treatment. This permitted the most effective isolation among different treatments and eliminated problems of physiological interaction particularly where sprays with growth sub-

stances were involved. However, this meant that branch effects (position and site, rootability, graft union, etc.) might be confounded with treatment effects. Furthermore, due to limited material per genotype this also reduced the number of replications to 2-3 per treatment. Since maternal genotypes within the same species behaved quite distinctly from one another with regard to flowering, capsule and catkin development, seed maturation and seed shedding, it seemed inappropriate to pool genotypes for data analysis. In other words, the nature of both the experiments and of the material defied most assumptions of conventional statistical tests. Altogether then, this part of our study may best be viewed as a survey revealing major trends, to be further examined by more straightforward experimental designs.

Although records were kept on the numbers of maturing catkins per branch as well as the numbers of seeds produced, we present here only data pertaining to the numbers of branches producing what ultimately is the object of any breeding attempt, i.e. germinated seedlings. However, we must remember that maternal genotypes differed significantly in the number of catkins and seedlings per branch; and that a lack of hybridization success in a given case may have been due to poor germination rather than the absence of hybrid seeds.

The various cross and treatment combinations are presented in Table 3 and summarized for treatments in Table 4. IMP, particularly its delayed application, was the only treatment giving a modest increase in seedling production whereas all other treatments were either equal to or worse than control. More significantly, there were cross combinations where only IMP treatment resulted in seedlings (e.g. TREM \times TRICH, TRICH \times DELT, NIGRA \times DELT). The positive influence of IMP was further reflected by a higher catkin retention (even if some of these catkins did not produce seed) and a higher seed production (even if some of these seeds did not germinate). Hexane treated pollen was ineffective except in a single branch, and this in a cross combination (DELT \times TREM) where controls were successful, too. Hexane treatment of stigmas was little more successful.

Effects of spray treatments (GA and NAA) were less noticeable in seedling production than in an increase in catkin retention and in the size and shape of capsules. For example, in DELT \times TREM crosses, capsules receiving 0.001% or 0.0001% GA, or 0.0001% NAA sprays, were significantly longer at 55 days after pollination than those of control or other treatments. They also contained more enlarged ovules. Yet, the embryos contained therein were no larger than those of DELT \times TREM control or of other treatments, none being more than half the size of DELT \times DELT embryos. In other words, the lowest concentrations of the two growth substances increased ovule and capsule growth without con-

Table 3. Summary of 1977-78 breeding trials

1. Intraspecific Crosses						
TREM × TREM 14/27(11) ^a	DELT × DELT 11/21(8)		NIGRA × NIGRA 6/15(6)		TRICH × TRICH 10/12(5)	
2. Interspecific Crosses and Treatments ^b						
TREM × TRICH	TRICH × TREM	DELT × TREM	DELT × ALBA	DELT × TRICH	TRICH × DELT	NIGRA × DELT
CONT 0/16(9)	0/9(4)	1/13(5)	2/4(3)	2/6(3)	0/12(6)	0/24(6)
IMP 3/16(9)	0/9(4)	0/4(3)	1/4(3)	1/6(3)	0/4(2)	0/6(3)
-IMP 1/16(9)	0/9(3)	1/4(3)	1/4(3)	2/6(3)	1/4(2)	3/24(6)
NAA	0/6(1)	4/33(4)		2/9(3)		0/33(6)
GA	0/4(1)	1/22(4)		0/6(3)		0/9(3)
HXS	2/18(4)	0/12(5)	0/2(2)	2/6(3)	0/12(6)	
HXP	0/10(2)	1/18(3)			0/6(2)	0/48(6)

^a 14/27(11) means: 14 branches from a total of 27 pollinated (from 11 genotypes) produced seedlings

^b Cont = control; IMP = Irrad. Mentor Pollen mixed with foreign pollen; -IMP = IMP applied 4-6 hr. after foreign pollen; NAA = NAA spray; GA = GA spray; HXS = Hexane treated stigma; HXP = hexane treated pollen

Table 4. Effectiveness of treatments for seedling production^a

	Treatments		Control ^b		Effectiveness E
	No. branches pollinated	% branches with seedlings	No. branches pollinated	% branches with seedlings	
	(1)	(2)	(3)	(4)	(E = 2/4)
IMP delayed	67	13.4	84	6.0	2.2
IMP simultan.	49	10.2	84	6.0	1.7
NAA spray ^c	81	7.4	52	5.8	1.3
Hexane treated pollen	82	1.2	58	1.7	0.7
Hexane treated stigma	50	8.0	44	11.4	0.7
GA spray ^c	41	2.4	52	5.8	0.4

^apooled data from all crosses

^bUntreated crosses involving the same parents as those in treatments

^cpooled data from all concentrations

comitant increase in embryo growth. In fact, many presumed hybrid embryos remained tiny and showed intense anthocyanin pigmentation in contrast to the evenly green color of control embryos.

Seedling phenotypes in interspecific crosses were typically intermediate between those of parental species. However, in a NIGRA × DELT cross involving delayed IMP treatment, we also recovered seedlings with maternal-species phenotypes, whose origin remained unclear. Among the several explanations, i.e. agamospermy, pollen contamination, mix-up during transplantation, the latter seemed most probable.

Discussion

Our SEM study of pollen behavior on the stigmatic surface has confirmed the observations described by

Hamilton (1976) for compatible crosses in poplars. Our study has also revealed variation at the intraspecific level that must be taken into account whenever interspecific comparisons are made. Hydrated pollen grains derived from the same tree and ending up next to one another on a stigma often varied in volume by a factor of two, occasionally by as much as four. The percentage of hydrated and of germinated grains varied, too, particularly in DELT × DELT crosses. In the TRICH × TRICH combination, and to a lesser extent in NIGRA × NIGRA, about one quarter of the germinated grains had twisted tubes (Table 2), that is, tubes one commonly associates with incompatible matings. Much of this variation seemed to be associated with the male parent or the pollen lot (i.e. probably due to handling, shipping, storage) some with peculiar male × female combinations, less with the female parent, provided the stigma was in a receptive condition. Furthermore, as long as pollen

crowding was avoided, this variation seemed to be evenly distributed over the entire adaxial surface of the stigma. Additional variation, e.g. in germination rate, may have been expressed prior to our sampling times at 5-8 hours and 15-22 hours after pollination, as shown in an earlier study (Guries et al. 1976).

Although no effort was made to identify the causes of this variation, several explanations come to mind. Grains might be at different stages of hydration, or of releasing their contents into the pollen tube; some grains might be at a higher level of ploidy, as occasionally encountered in poplars (Winton 1968) or pollen size and behavior might reflect the genotype of the gametophyte (Mulcahy 1975) and/or the anther environment in which it was formed.

In all intersectional cross combinations but one (TREM × TRICH), pollen behaved on foreign stigmas essentially the same way as on stigmas of its own species. In other words, in six of the seven cross combinations attempted, there seemed to be no barriers blocking the foreign pollen from penetrating the stigmatic surface. These results confirm at a higher level of resolution (SEM), with fewer sampling errors due to the use of fresh material, and with more genotypes tested, our earlier findings from the study of similar poplar hybridizations (Guries et al. 1976; Stettler et al. 1976). They are, however, at variance with the reports from Knox et al. 1972, Willing et al. 1976 and Hamilton 1976, in which intersectional incompatible crosses were described as typically showing twisted pollen tubes and callose deposits. Only in the seventh cross combination tested (TREM × TRICH) did we find these phenomena at a significantly higher frequency than in conspecific matings, a result which again agrees with earlier observations (Guries et al. 1976; Stettler et al. 1976).

A further finding of interest was that in intersectional crosses the presence of IMP did not significantly affect the surface behavior of the foreign pollen. Even in the one cross combination (TREM × TRICH) where the foreign pollen seemed to be inhibited at the surface and where mentor pollen might have provided some help, no change was noted in these characteristics. We were able to study this quite closely since the two types of pollen could be distinguished morphologically (Fig. 1a, b, f). As in an unaided cross, most *P. trichocarpa* grains had long, twisted tubes, some of which would eventually penetrate the stigmatic surface. By contrast almost all short tubes were from IMP grains.

Taken together, these observations give added perspective to consider the mechanisms in poplars by which the fate of a mating is decided, and how mentor pollen relates to them. Recognition, as envisioned by Knox and co-workers, places much emphasis on the pre-germination interaction between pollen and stigma. According to this

hypothesis, a recognition protein released by the exine sets the stage for germination and tube growth; this protein can be 'borrowed' from mentor pollen by admixed foreign pollen and will facilitate germination and tube growth in the latter (Knox et al. 1972). Our data described in the section above, lend little support to this hypothesis although they do not provide direct evidence to the contrary. There is no way of knowing, of course, to what extent an early recognition event may not cause a chain of reactions whose critical expression takes place at a much later stage, e.g. in the stylar tissue. All we can say is that in the period of 5-12 hours during which the majority of grains germinate, grow a tube, and penetrate the stigma in these poplars (Guries et al. 1976) no qualitative differences were noted between so-called compatible and incompatible crosses, except in the TREM × TRICH combination.

Hamilton's hypothesis proposes a stimulatory substance to be present on the stigma, presumably in the waxy mounds, and capable of promoting pollen-tube growth of any species. Compatible pollen, due to specific enzymes, is capable of efficiently degrading the wax structures, thus gaining access to the growth promoting substance. Incompatible pollen is less efficient in doing so but can profit from the breakdown-enzymes of admixed mentor pollen (Hamilton 1976). This hypothesis seems more consistent with the quantitative variation encountered in our study both within and between species. It would also be consonant with the fact that in these poplars mating success or failure seems largely dependent on the rate of pollen tube growth in the style (Guries et al. 1976).

Both hypotheses focus on surface events prior to the penetration of stigmatic papillae by the pollen tube. This had led to the notion that breeding barriers among poplars might be viewed in the context of sporophytic incompatibility (Hamilton 1976). We feel this is too restrictive a view, for two reasons. First, it ignores the interactive opportunities between male gametophyte and female reproductive apparatus from the moment of stigmatic penetration to the moment of fertilization. There is good evidence to show that IMP induces ovule and pistil growth even in the absence of developing embryos, whereas certain foreign pollen fails to do so (Stettler et al. 1976). Furthermore, this growth stimulation may not be dependent on the pollen tube reaching the ovule. Illies (1974) obtained many aspen haploids from well developed pistils after a post-pollination treatment of toluidine blue, which arrested the pollen tube in mid-style. Until the mechanisms of these post-penetration interactions have been elucidated, we prefer the term 'mentor pollen', because more encompassing, to that of 'recognition pollen' (Knox et al. 1972).

Second, and perhaps more importantly, we should not overlook the broad spectrum of isolating mechanisms

having come about as a consequence of evolutionary divergence in poplar species, particularly those in 'distant' sections. For example, *P. tremuloides* and *P. trichocarpa* are sympatric in the Pacific Northwest but have evolved separately for probably the past 25 million years (Eckenwalder 1977). In the process, they have become markedly distinct in most features of reproductive biology, from the morphology and anatomy of reproductive structures to the timing and orchestration of processes. If we further consider the obviously divergent reproductive strategies of the two species (sexual vs. asexual), their distinct demographic characteristics and ecological preferences, we may well expect hybrids among them to be difficult to obtain and many of them of low promise in the first generation, as indeed found in our earlier work (Stettler et al. 1976). Altogether then it seems much more appropriate to view the multiple crossing barriers among poplar species as a case of incongruity rather than incompatibility (Hogenboom 1973, 1975).

Finally, our repeatedly negative results with solvent treatment of pollen (hexane, ethylacetate) suggest that additional factors must be controlled before this attractive method (Willing et al. 1976) can be recommended as a tool for practical breeding. At the same time it may be well to more closely examine the delayed 'mentor' pollen application with regard to optimal timing. A sufficiently delayed application may well obviate the need for a killing or weakening treatment, such as irradiation or freeze-thawing, and as a consequence may increase the enhancement effect. Furthermore, since genotypic differences are bound to exist with regard to mentor functions, as recently shown for *Nicotiana* by Pandey (1977), and since clonal variation is generally pronounced in poplars, it may be sensible to use as mentor pollen a mixture from several clones.

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