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# Interspecific Hybridisation in Poplar

R.R. Willing and L.D. Pryor

Department of Botany, Australian National University, Canberra (Australia)

Summary. Interspecific compatibility and incompatibility have been examined through the genus *Populus*. General methods of manipulation have been developed to break the incompatibility barriers. These methods are described and a hypothesis is put forward to account for the results. This proposes that at least two factors are involved, one attached to the pollen (P) and one to the stigma (S), and that the interaction of these (PS) is critically involved in the total process. Implications for future plant breeding are discussed.

Zusammenfassung. Interspezifische Verträglichkeit und Unverträglichkeit sind im Genus *Populus* untersucht worden. Wo Unverträglichkeit vorliegt, wurden Methoden entwickelt, um sie zu überwinden. Diese Methoden werden beschrieben. Die Ergebnisse führen zu einer Hypothese, in der angenommen wird, daß mindestens 2 Faktoren eine Rolle spielen. Einer ist mit dem Pollenkorn (P) verbunden, der andere mit dem Stigma (S). Ihr Zusammenspiel im Verträglichkeitsprozess ist von entscheidender Bedeutung. Schlußfolgerungen für zukünftige Züchtungsarbeiten werden erörtert.

## Introduction

The principal genera of the family *Salicaceae* are *Populus* (Poplar) and *Salix* (Willow). The former is a genus of generally large, mostly fully dioecious trees, with wind-pollinated flowers.

*Populus* is divided taxonomically into five separate sections, which are distributed naturally throughout the northern hemisphere (Rehder 1958). Table 1 shows the grouping and natural distribution of the species used in these experiments.

Altogether there are some thirty species, with numerous subspecies and many provenance differences. There are also numerous cultivars, especially as poplars are usually propagated readily by stem cuttings, readily permitting the cultivation of clones (FAO 1958).

While *Salix* extends in natural distribution to the southern hemisphere in both South America and Africa, *Populus* is confined to the northern hemisphere and no species is found naturally in the southern part of Africa, South America, Australia or New Zealand. *Populus* is widely planted throughout the world and has been introduced extensively in the southern hemisphere, especially in South Africa, Australia, Argentina and Chile (Pryor 1969), and in New Zealand (van Kraayenoord 1973).

Within each of the three sections, *Leuce*, *Aigeiros* and *Tacamahaca*, hybrids that have arisen spontaneously or by manipulation between various pairs of species are well known (Schreiner and Stout 1934; Syrach Larsen 1956) and a good many have found their way into cultivation. In addition, intersectional hybrids between species of *Ageiros* and *Tacamahaca* have arisen similarly and are widely known. For example, Henry (1914), using *P. angulata* and *P. trichocarpa* as parents, produced the widely-planted clone known as *P. "generosa"*. Schreiner and Stout (1934) produced hybrids in north-eastern USA, some of which were similar intersectional hybrids, and these have been planted in many parts of the world. The production of viable hybrids of these intersectional combinations has not required special manipulation, other than bringing pollen on to the receptive stigma. It appears that species from the Balsam and Black Poplar sections are cross-compatible in most, if not all, combinations.

The position is not so clear with the small section *Leucoides*, but if *P. ciliata* can be taken to be correctly placed in the section, then at least one species of *Leucoides (P. ciliata)* is known to be compatible with species from *Tacamahaca* and *Aigeiros*. However, there is some doubt about the systematic location of *P. ciliata* in the section *Leucoides* (FAO 1958). On the other hand, *P. lasiocarpa* is without doubt clearly characteristic of the section *Leucoides* and is known to be fully compatible with some species of *Aigeiros*.

Intersectional crosses involving species from *Leuce* and *Aigeiros*, and *Leuce* and *Tacamahaca*, have been attempted with varying success both as to the size of progeny and vigour of offspring (Stout 1934; Stettler 1968). It is evident that incompatibility between species of these sections is pronounced and, in many at-

Table 1. Location and Classification of *Populus* Species used in Experiments. Nomenclature as in FAO 1958

Genus *Populus*

| Section | *Turanga* | *Leuce* | | *Aigeiros* | *Tacamahaca* | *Leucoides* |
|---|---|---|---|---|---|---|
| Sub-Section | | *Albidae* (White) | *Trepidae* (Aspen) | (Black) | (Balsam) | |
| Geographic Location | | | | | | |
| Europe and North Africa | [ *euphratica* ] | *alba* & vars. | | *nigra* & vars. | | |
| Asia | [ *euphratica* ] | *alba* & vars. | | *nigra* & vars. | *simonii*<br>*yunnanensis* | *ciliata*<br>*lasiocarpa* |
| North America | | | *tremuloides* | *deltoides* & vars.<br>*fremontii* | *trichocarpa* | |

tempted crossings, no seed at all has been set. No records have been found of the compatibility relationships between the sections *Leuce*, *Turanga*, and *Leucoides*. Suitable material has been available to us, both from clones imported to Australia and from seedlings of species in these groups raised to the flowering stage, to permit a series of manipulations between species in these groups. In some of the species, such as *P. yunnanensis*, *P. simonii*, *P. lasiocarpa*, and *P. ciliata*, the material available for experiment has been a single clone and, to this extent, the results are rigorously valid only for that clone. Where several clones of different genotypes within one species have been available, however, the behaviour of each in compatibility relationships has been identical.

The breaking of incompatibility and the production of hybrid seedlings between the sections *Tacamahaca* and *Leuce* has been reported by Stettler (1968), using irradiated non-viable pollen which he termed "mentor" pollen. Chung and Son (1972) reported successful crossings between *Leuce* and *Tacamahaca* and also *Leuce* and *Aigeiros*, but did not disclose the methods used. Knox, Willing and Pryor (1972) described the production of hybrids between *Leuce* and *Aigeiros*, using "recognition pollen" to mediate the process. "Recognition" pollen, like "mentor" pollen, is that which is normally compatible with the female, but which has been rendered non-viable (by one of several methods) while remaining physiologically reactive so far as the compatibility response in the female is concerned.

From these facts, it seemed that pollen and stigma are both involved in the incompatibility mechanism and various treatments of either pollen or stigma were devised to determine whether incompatibility could be overcome. It has been found that material can be extracted from the surface (presumably the exine) of poplar pollen in much the same way as for *Raphanus*, as described by Dickinson and Lewis (1973). Following Echlin and Godwin (1968), they call material thus extracted "tryphine". It is thought that these extracts consist of several compounds. Whatever the precise composition, the material is readily soluble in lipid solvents such as n-hexane and there are reasons for thinking it is lipoidal (Dickinson and Lewis 1973; Whitecross 1974, pers. comm.)

This study was undertaken because of the theoretical interest in, and the practical consequences of, developing methods for breaking incompatibility barriers between poplar species. Since preliminary assessment suggested that treatment of the pollen or stigma with lipid solvents might be a way of achieving this result, the methods developed have centred mainly on the application of such solvents to pollen and stigma.

## Methods

The plant material for manipulation was prepared as described by Knox, Willing and Pryor (1972). Briefly, grafted scions carrying flower buds were prepared and placed in a greenhouse. At anthesis the female flowers, isolated in separate greenhouses, were exposed, allowing treatment of the stigmas. Pollen may be collected at the normal time of shedding and stored desiccated at - 18° for twelve months or more, as previously described (Knox, Willing and Pryor 1972). Alternatively, by manipulating temperature, the male flowers can be brought to anthesis before the female flowers are receptive so that pollen is shed, collected and treated as necessary in preparation for crossings. Pollen or stigma, or both, were treated with organic solvents, of which hexane or ethyl acetate were preferred. Pollen

Appendix I. Solvents* employed in testing incompatibility reactions in *Populus*

| | Stigmatic Treatment | Pollen Treatment |
|---|---|---|
| n-amyl acetate | + | |
| ethyl acetate | + | + |
| methyl acetate | + | |
| iso-butyl acetate | + | |
| n-butyl acetate | + | |
| acetaldehyde | + | |
| n-amyl alcohol | + | |
| n-butyl alcohol | + | |
| iso-butyl alcohol | + | |
| iso-propyl alcohol | + | |
| benzene | + | |
| carbon tetrachloride | + | |
| chloroform | + | |
| diethylether | + | + |
| petroleum ether | + | |
| n-heptane | + | |
| n-hexane | + | + |
| cyclohexane | + | + |
| iso-pentane | + | |
| pyridine | + | |
| acetone | + | |
| toluene | + | + |
| xylene | + | |
| ethylmethyl ketone | + | |
| ethanol** | + | 0 |
| methanol** | + | 0 |
| water ($H_2O$)*** | 0 | 0 |

* Anhydrous solvent
Iwanami (1972) found most of these solvents effective in storing pollen.

** Ethanol and Methanol are quickly lethal to pollen.
They will remove incompatibility if brushed on to the stigma but only in quite minute amounts since the stigmatic epidermis is easily damaged by them and they do not suit practical manipulation.

*** Water alone is completely ineffective.

from each species was used alone and also in mixtures. In *Aigeiros* poplars, the flower abscinds if there is no seed set. In species of *Leuce*, there is usually abscission too but at times this is delayed and partial development of seedless fruit occurs.

## Stigmatic Treatment

The technique of application of organic solvents is simple. Before pollination, stigmas in the receptive stage are pre-treated by very carefully brushing the stigmatic surface with a small camelhair brush, which is slightly moistened with the solvent being tested. Care must be taken to bring only small traces of the solvent into contact with the stigmatic surface, otherwise the surface tissue is "burnt" by the application and fertilization is inhibited. It has been noted that the likelihood of damaging effect varies according to the particular solvent used, and some are obviously preferable to others. After preliminary trials two solvents, n-hexane and ethyl acetate, were chosen as the most effective and suitable for large scale pollinations. (See Appendix I for complete list.) Pollination can be carried out as soon as the solvent treatment has been completed, or it can be delayed for at least some hours.

## Pollen Treatment

Pollen was treated by immersing it in the same range of organic solvents as those used in treating the stigma. Either n-hexane or ethyl acetate was found the most efficient and convenient to use in removing material from the pollen. Repeated washings of the pollen with one or other of these solvents did not affect its viability. Washing was continued until a clear supernatant was produced (when, it is presumed, the removal of soluble material from the surface of the pollen grain is complete). This usually involved three successive washings occupying about ten minutes each. After washing, the pollen was filtered and dried in a warm air stream. It remained fully viable and functional. A similar result has been obtained in several other genera, including *Lilium*, *Impatiens*, *Camellia*, and *Erythrina* (Iwanami 1972). Alternatively, the pollen/solvent suspension was stored in a deep freeze at -18°C, and the pollen remained viable for several weeks, and perhaps, would remain viable longer, as shown in other genera (Iwanami and Nakamura 1972). Pollinations were made with treated and untreated pollen, both alone and in mixture, on both treated and untreated stigmas.

Tryphine from the pollen can be isolated from the solvent as a yellowish lipoidal substance or substances, the precise characterization of which is still to be determined. Untreated pollen can be mixed with extracted "tryphine", which at room temperature is viscous, by stirring it with a small brush. This coats the surface of the pollen with the tryphine, and the coated pollen is then brushed on to a suitable stigma.

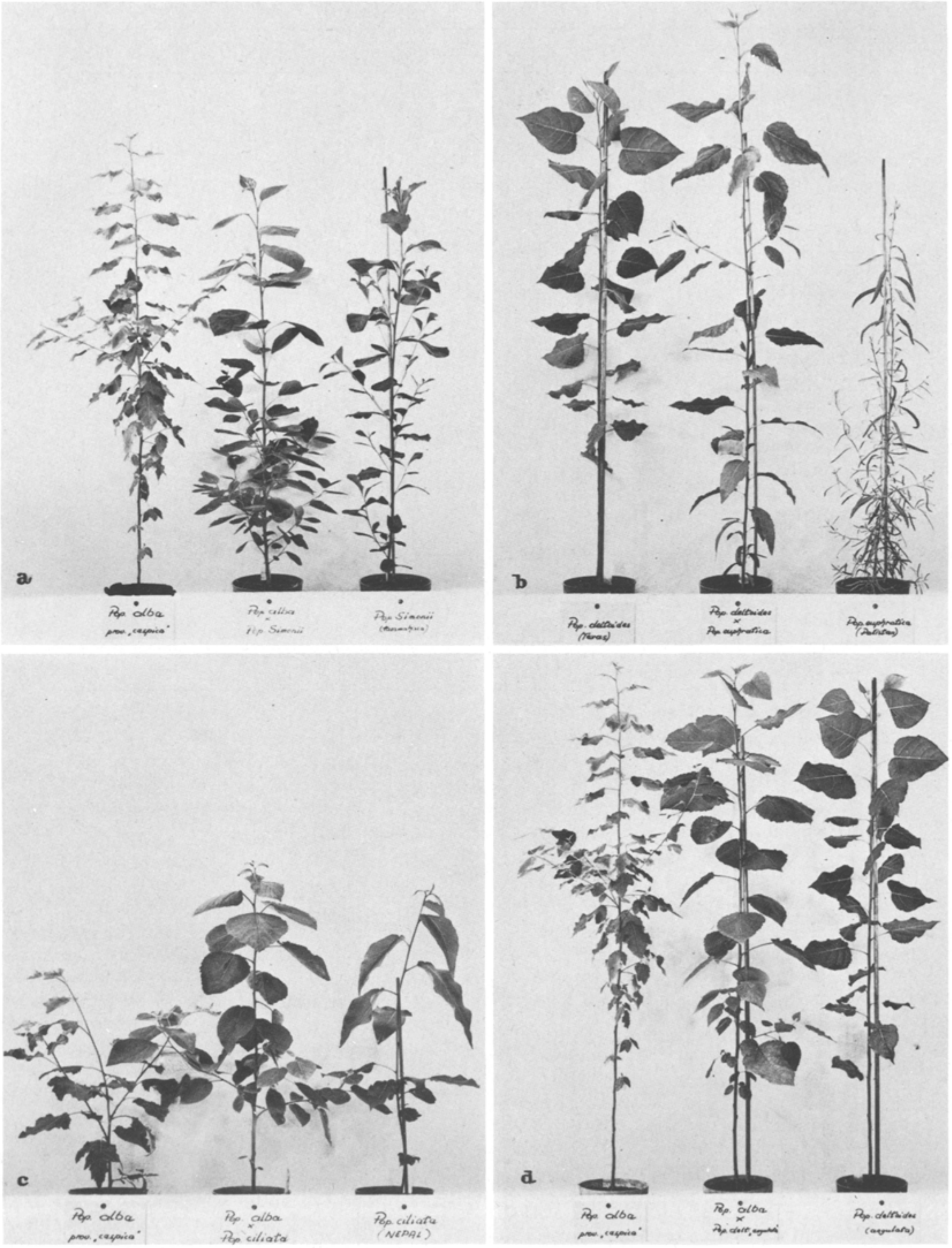

Plate 1. Successful crossings between ordinarily incompatible pairs of species.

a. Parents and hybrid of the cross P. *alba* × P. *simonii*

b. Parents and hybrid of the cross P. *deltoides* × P. *euphratica*

c. Parents and hybrid of the cross P. *alba* × P. *ciliata*

d. Parents and hybrid of the cross P. *alba* × P. *deltoides*

For use in pollen mixtures, pollen can be prepared in one of several ways; by irradiation (100,000 rads); by chemical treatment with methanol or ethanol; or by passing through several freeze-thaw cycles (Knox, Willing and Ashford 1972). Mostly the first method has been used in these experiments. Pollen which has been rendered non-viable, but which remains reactive in relation to the incompatibility mechanism, has been designated here "P factor" pollen, rather than "mentor" or "recognition" pollen.

Table 2. Number of Seed per Capsule (Mean of 10) Produced from Various Crossings, Different Pollen Mixtures and Stigmatic Treatments

| Parents - normally incompatible | | Pollen Treatments | | | Stigma Treatments | | | Compatible Cross | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (1) | | (2) | (3) | (4) | (5)** | (6) | (7) | (8)*** | | (9) |
| ♀ | ♂ | P Factor* Pollen Alone | Incompatible Pollen Alone | 1:1 P Factor/ incompatible Pollen. Mixed | Control | Hexane | Ethyl-acetate | ♀ as in Col. 1. | ♂ | |
| *deltoides* 60/141 | *alba* "Maktar" | 0 | 0 | 2.4 | 0 | 19.2 | 20.2 | *deltoides* 60/141 | *deltoides* v.*angulata* | 20.5 |
| *deltoides* 60/156 | *tremuloides* | 0 | 0 | 2.2 | 0 | 16.8 | 16.7 | *deltoides* 60/156 | *nigra* v. *italica* | 17.1 |
| *deltoides* 60/166 | *euphratica* | 0 | 0 | 1.9 | 0 | 13.0 | 12.8 | *deltoides* 60/166 | *nigra* v. *italica* | 16.6 |
| *alba* "Morocco" | *nigra* "Persistente" | 0 | 0 | 2.4 | 0 | 4.8 | 4.5 | *alba* "Morocco" | *alba* v. "Maktar" | 5.1 |
| *tremuloides* | *deltoides* v. *angulata* | 0 | 0 | 1.9 | 0 | 3.3 | - | *tremuloides* | *alba* v. *bolleana* | 3.0 |
| *alba* "Caspica" | *ciliata* | 0 | 0 | 0.8 | 0 | 1.5 | - | *alba* "Caspica" | *tremuloides* | 2.1 |
| *alba* | *simonii* | 0 | 0 | 1.4 | 0 | 1.8 | - | *alba* | *tremuloides* | 2.3 |

* P Factor pollen in these cases was prepared by $\gamma$ irradiation

** No solvent plus incompatible pollen

*** Where possible an intraspecific cross was made (2 cases) otherwise an intrasectional cross was employed. This indicates the level of seed set in a fully compatible cross.

## Evaluation of the Crosses

In each attempted cross at least five catkins, involving 30 - 50 individual flowers in each, were pollinated. The fruits in the middle part of the catkin develop better than at the base or tip, and ten fruits were selected from the largest ones in the central part of the catkin to score for seed set.

Upon ripening of the fruit, usually two to three months after pollination in *Aigeiros* poplars and a slightly shorter time in other species, seed was collected from each catkin and sown. The plants were grown for some months until they were 30 - 50 centimetres tall and displaying distinct and diagnostic foliar morphology (Plate 1). These were then scored to assess their relationship to the morphology of the parents and thus to gauge their genetic status.

## Results

Crosses between pairs of species normally found to be incompatible using various treatments are summarized in Table 2, which records, for each cross, the mean number of fertile seeds (assessed by successful germination) per capsule, based on the count from ten capsules selected as described above. It will be noticed that when *P. deltoides* was used as the female parent, the seed production per capsule was much higher than when a species of the *Leuce* group was employed. For comparison, seed production from a comparable compatible cross using the same female parent in each case is also included in Table 2, in the right hand column. This indicates particularly the number of seeds per capsule normally produced in a within-species or within-section cross. Note that seed set was obtained in the various crosses which are ordinarily imcompatible if, either (1) a pollen mixture was used which involved non-viable but reactive P-factor pollen from the compatible parent plus viable pollen of the second incompatible species, or (2) the stigma alone was solvent-treated. In the latter case very effective seed set, approaching the amount of seed obtained from a normally compatible cross, was obtained. In the former case, seed set occurred but generally in smaller amounts than normal. In the normally incompatible crosses, solvent treatment of either pollen or stigma was found to permit the production of viable seed. Hybrid status was verified by scoring the progeny for morphological characters in relation to the parents, as in crosses between *P. alba* and *P. deltoides* (Knox, Willing and Pryor 1972). This is illustrated for one such cross in Fig. 1.

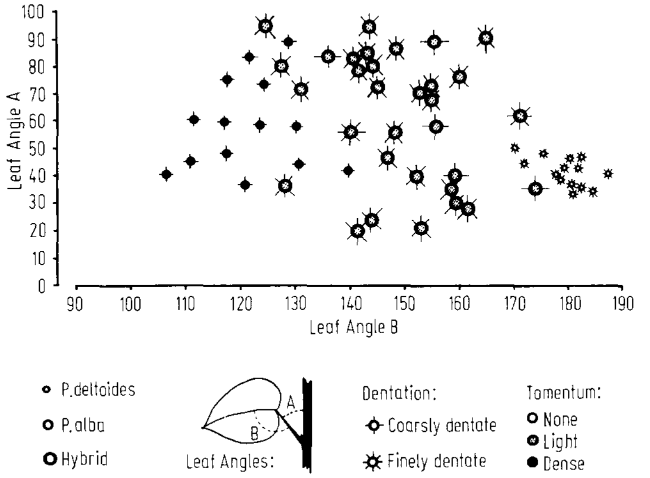

Fig.1. Scatter diagram showing morphological feature of seedlings of *P. deltoides* and *P. alba* together with those of a random sample from their $F_1$ hybrids. Some of the hybrids extend to the *P. deltoides* parent at the right and others to the *P. alba* parent at the left. The bulk are in varying degrees intermediate between the two parents

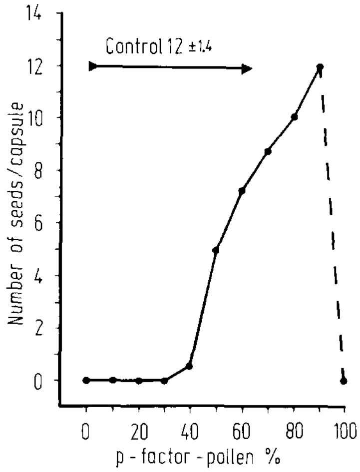

Fig.2. Effect of varying percentage of dead compatible pollen (P pollen) to live incompatible pollen on the average number of hybrid seeds formed in 10 capsules. The ♀ parent is *P. deltoides*, the dead compatible pollen (P pollen) is *P. nigra* v. *italica* and the live normally incompatible pollen is *P. alba* v. *bolleana*

Several solvents were very effective. Results of tests with various solvents (and water) are listed in Appendix I.

In pollen mixtures (Fig.2) involving both incompatible pollen and non-viable, but reactive, compatible P-factor pollen, it was found that until the percentage of non-viable treated pollen reached about 40 % in the mixture there was little or no action by the incompatible pollen. Beyond this level and up to 90 % treated pollen, the normally incompatible pollen reacted effectively and produced increasingly large amounts of seed. In the most successful responses, more than 90 % of the amount produced in a normal compatible cross was obtained by this means.

Incompatibility between *P. deltoides* v. *monilifera* and *P. alba* v. *bolleana* was also broken by washing pollen in ethyl acetate or n-hexane (Table 3), which apparently remove the tryphine. The viability of pollen remained unimpaired by such treatment, as indicated in Table 3, where the two compatible crosses, *P. deltoides* v. *monilifera* x *P. yunnanensis* and *P. deltoides* v. *monilifera* x *P. deltoides* v. *angulata* are judged to perform normally after washing.

Furthermore, when tryphine from pollen of either *P. alba* v. *bolleana* or *P. deltoides* was extracted and used to coat the surface of some other poplar species, the compatibility response was determined by that appropriate to the coating tryphine (Table 4).

It was noted also that, although many vigorously growing hybrids from intersectional crosses were produced, the progeny from normally incompatible crosses usually gave a higher percentage of weak or nonviable plants than in those which are normally compatible. As reported previously (Knox, Willing and Pryor 1972), characters such as depressed growth, loss of apical dominance, and chlorophyll defects occurred in as many as 50 % of the seedlings, depending upon the particular parents involved.

Stettler (1968) mentioned the likelihood of abnormal individuals being more frequent in "remote" hybridization, presumably since the genomes are likely to be less well matched than when species are more closely related. Thus if selection from such crosses is to be made, larger numbers must be raised than is otherwise the case. In spite of a percentage of defective seedlings, as for example in the white x black poplar crosses, some of the vigorous plants exhibited heterosis and grew faster in the nursery than did either parent. With regard to the incompatibility situation throughout the genus, the overall results are in accordance with previous work (Schreiner and Stout 1934; FAO 1958).

Of the ten possible intersectional hybrid combinations, three were found to be cross-compatible and the remaining seven were incompatible. The recorded trials (listed in Appendix II) show that, within and be-

Table 3. Effect on Seed Set of Washing Pollen in Ethylacetate

| ♀ | ♂ | Control no washing | Washed Pollen |
|---|---|---|---|
| 1. *P. deltoides* v. *monilifera* × | *P. alba* v. *bolleana* | 0 | + |
| 2. *P. deltoides* v. *monilifera* × | *P. yunnanensis* | + | + |
| 3. *P. deltoides* v. *monilifera* × | *P. deltoides* v. *angulata* | + | + |

Note 1 is normally an incompatible cross.
2 & 3 are normally compatible.
\+ indicates successful setting.

Table 4. Effect on Compatibility of Coating Pollen with Tryphine from Different Species

| Pollen Source<br>♀ | ♂ | Untreated Pollen No tryphine added (Control) | Coated Pollen Tryphine Source | | Seed Set |
|---|---|---|---|---|---|
| *P. deltoides* 60/156 | × *P. deltoides* v. *angulata* | control | | | + |
| " | × *P. deltoides* v. *angulata* | | *P. deltoides* v. *angulata* | tryphine | + |
| " | × *P. deltoides* v. *angulata* | | *P. alba* v. *bolleana* | tryphine | 0 |
| " | × *P. alba* "Maktar" | control | | | 0 |
| " | × *P. alba* "Maktar" | | *P. alba* v. *bolleana* | tryphine | 0 |
| " | × *P. alba* "Maktar" | | *P. deltoides* v. *angulata* | tryphine | + |
| " | × *P. yunnanensis* | control | | | + |
| " | × *P. yunnanensis* | | *P. deltoides* v. *angulata* | tryphine | + |
| " | × *P. yunnanensis* | | *P. alba* v. *bolleana* | tryphine | 0 |

(+ indicates successful seed set as assessed after raising plants)

tween the sections *Aigeiros* and *Tacamahaca*, there was cross compatibility and hybrids have been produced without recourse to any special manipulation other than bringing pollen on to a receptive stigma. In the section *Leucoides*, *P. ciliata* and *P. lasiocarpa* were available; the former was found to be compatible without special manipulation with both of the above sections (*Aigeiros* and *Tacamahaca*), and the latter (*P. lasiocarpa*) with *Aigeiros*. In the remaining two sections, *Leuce* and *Turanga* (four species were available in *Leuce* and one species in *Turanga*), the species were not cross-compatible with species of any other section. These relationships, as disclosed by our trials, are summarized in Fig.3. By using the manipulations of either pollen or stigma, as described above, successful seed set has resulted in all of the seven intersectional crosses which are ordinarily incompatible.

## Discussion

It has been clearly demonstrated (Knox, Willing and Ashford 1972) that when poplar pollen is moistened by water, proteins diffuse out from the pollen grain, especially at germination apertures. They recognised these proteins as hydrolases "which probably play a part in the emergence and early nutrition of the pollen tube, and in some species probably also in stigma penetration". They also pointed out that the enzymatic proteins are only a small fraction of the total substances released, and suggested "that the major part is concerned with compatibility reactions". At this stage it was considered that these proteinaceous materials were involved in incompatibility mechanisms and were mainly released from the intine. A later report (Heslop-Harrison, Knox and Heslop-Harrison 1974) showed that, in the cruciferous plant *Iberis*, the rejection response in the stigma appears to be induced by materials from the exine which are derived from the sporophyte, i.e. the female parts of the diploid plant.

In the report on *Iberis*, the authors also mention that pollen prints in agar disclose that both lipoidal "Pollenkitt" and some protein are present in the exine of mature pollen. After preliminary work on fractionation of exine protein, they stated that "the experiments do

Appendix II. Intersectional crosses in *Populus* produced in 1971-74.
In each case more than 100 plants were produced

| | Method Employed | | |
|---|---|---|---|
| | P-Pollen | Hexane on Stigma | Tryphine |
| *Aigeiros* x *Leuce* | | | |
| 1) *P. deltoides* x *P. alba* v. *bolleana* | + | + | + |
| 2) *P. deltoides* x *P. alba*"Maktar" (a) | + | + | + |
| 3) *P. deltoides* x *P. tremuloides* | + | + | |
| 4) *P. alba* x *P. deltoides* | + | + | + |
| 5) *P. tremuloides* x *P. nigra* "Persistente" (b) | + | + | |
| 6) *P. alba* "Caspica" (c) x *P. deltoides* | + | + | |
| 7) *P. alba* "Morocco" (d) x *P. nigra*"Persistente" (b) | + | + | |
| *Aigeiros* x *Tacamahaca* | | | |
| 1) *P. deltoides* v. *monilifera*(f) x *P.* "Androscoggin" (e) | compatible without treatment | | |
| 2) *P. deltoides* x *P. yunnanensis* | | | |
| 3) *P. deltoides* x *P. simonii* | | | |
| 4) *P. fremontii* x *P. yunnanensis* | | | |
| *Aigeiros* x *Leucoides* | | | |
| 1) *P. deltoides* x *P. ciliata* | compatible without treatment | | |
| 2) *P. deltoides* v. *monilifera* x *P. lasiocarpa* | | | |
| 3) *P. deltoides* 60/141 x *P. lasiocarpa* | | | |
| *Aigeiros* x *Turanga* | | | |
| 1) *P. deltoides* x *P. euphratica* | + | + | |
| 2) *P. euphratica* x *P. deltoides* | + | | + |
| *Leuce* x *Tacamahaca* | | | |
| 1) *P. alba* x *P. yunnanensis* | + | + | + |
| 2) *P. tremuloides* x *P. yunnanensis* | + | | |
| *Leuce* x *Leucoides* | | | |
| 1) *P. alba*"Morocco" (d) x *P. ciliata* | + | + | |
| *Leuce* x *Turanga* | | | |
| 1) *P. alba* x *P. euphratica* | + | | |
| 2) *P. euphratica* x *P. alba* "Maktar" (a) | + | + | |
| *Tacamahaca* x *Turanga* | | | |
| 1) *P. euphratica* x *P. simonii* | + | + | |
| 2) *P. euphratica* x *P. yunnanensis* | + | + | |
| *Turanga* x *Leucoides* | | | |
| 1) *P. euphratica* x *P. ciliata* | + | + | |
| *Leucoides* x *Tacamahaca* | | | |
| 1) *P.* "Samoui" (g) x *P. ciliata* | compatible without treatment | | |

(a) Maktar is a male clone of *Populus alba* from Maktar in Tunisia.

(b) Persistente is a male clone of semievergreen *Populus nigra* v. *italica* from Chile.

(c) Caspica is a female clone of *Populus alba* from the Caspian Sea shore in Iran.

(d) Morocco is a female clone of *Populus alba* from Morocco.

(e) Androscoggin is a male clone of *Populus maximowiczii* x *P. trichocarpa* produced by Schreiner and Stout (1934).

(f) *P. deltoides* v. *monilifera* is the name commonly applied to an ornamental female clone of *P. deltoides* introduced to Australia and New Zealand before 1900 (FAO p. 48, 1958).

(g) *P.*"Samoui" is a female clone possibly related to *P. trichocarpa*.

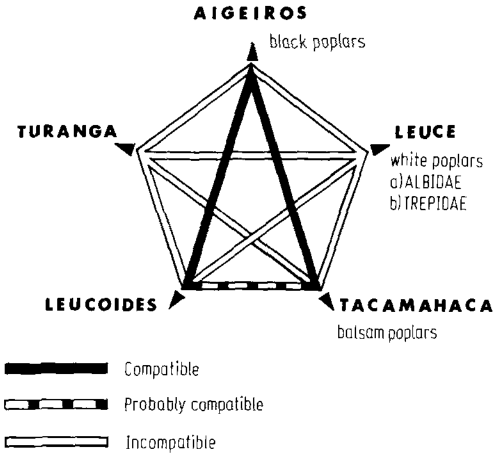

Fig.3. Illustrations of known compatibility relationships between the five sections of the Genus *Populus*

not, however, exclude the possibility that other constituents are involved, since lipid and carbohydrate moieties are also transferred to the wall (i.e. exine*) from the tapetum".

The results of the trials we have carried out suggest that n-hexane-soluble (presumably mainly lipoidal) materials from the exine play a critical role in the incompatibility reactions in poplars. This material is thought to be similar to the tryphine described for *Raphanus* by Dickinson and Lewis (1973). Thus, by extracting materials from the exine of *P. alba* v. *bolleana* with n-hexane, the incompatibility reaction with *P. deltoides* v. *monilifera* is inhibited (Table 3). It is also inhibited if the surface of the stigma is lightly brushed with n-hexane (Table 2). Similarly, if compatible pollen is coated with the solvent extract (i.e. tryphine) from an incompatible pollen, the incompatibility reaction occurs when the coated pollen is placed on a receptive stigma (Table 4). The precise composition of the solvent extract from pollen has not yet been determined. Preliminary assessment suggests that if there is protein present, it could be only in very small amounts, since no nitrogen was detectable in solvent washings using CHN analysis with a detection limit of 0.3 % for nitrogen (Hamilton and Whitecross 1975, pers. comm.). The fact that the incompatibility reaction can be prevented, by either stigma or pollen treatment, suggests that two factors (one at each location) are concerned and that the incompatibility process is inactivated by the absence of either. These might be called P (pollen) and S (stigma) factors. It seems that the S factor can be disorganized by various organic solvents, and some of the same solvents can remove the P factor from the pollen grain without impairing its viability. Further study is necessary to determine the precise biophysical and biochemical steps in the full sequence of events from the time the pollen grain lodges on the stigma until the development of callose in the stigmatic papillae. This callose development may provide the final block to fertilization (in an incompatible cross) (Dickinson and Lewis 1973; Heslop-Harrison, Knox and Heslop-Harrison 1974).

There are some other aspects of interest from these experiments. From the results using P-factor pollen, it appears there is a threshold concentration that the compatible P-factor pollen must exceed before successful seed set is obtained, and this threshold is reached when the P-factor pollen is about 40 % of the mixture (Fig.2). It appears that if the compatible pollen P-factor is present in sufficient quantity, it may nullify the reaction of the S factor that would otherwise occur if incompatible pollen alone were used, by preceding or overriding the incompatible P-factor action.

The fact that there is a threshold concentration associated with the use of P-factor pollen is not immediately explicable in the absence of more precise information on the nature of the S factor and its distribution in the stigma. There are many other aspects of the precise incompatibility mechanism which remain to be explained. Knox, Willing and Ashford (1972) reported experiments using a proteinaceous extract from whole *P. deltoides* pollen in a 1:1 Volume mix with viable *P. alba* pollen, which gave some seed set with a *P. deltoides* female.

It is evident that organic solvent treatment of the stigma almost completely removes or disorganises the S factor, since seed set is almost at the level (15 - 20 per capsule in *P. deltoides*) characteristic of a compatible cross. In comparison, the pollen extract of Knox, Willing and Ashford (1972) is far from efficient in this respect, giving only 2.7 seeds per capsule.

There is some indication from preliminary trials by one of us (R.R.W. unpub.) that a limited degree of disorganisation of the S factor can result from the application of a range of substances other than organic solvents to the stigma, and it may be that the materials involved in such responses bear little relationship to those which determine the process in the normal biological situation. These results, added to those already discussed above, lead to the conclusion that substances from both stigma and pollen are involved in the incompatibility mechanism of poplars. Just how many substances are active in what we have termed P-factor and S-factor, and what their exact chemical natures might be, are questions yet to be resolved.

## Implications in Tree Breeding

The simple and very effective manipulation by treating receptive stigmas with n-hexane provides an opportunity to mass-produce hybrids between poplar species that are ordinarily incompatible, and broadens the base for poplar breeding very greatly. This is especially important since species of aspen and white poplar have features which differ widely from those of the other groups. Some of these features are economically desirable. From the crosses so far produced, it is evident that the $F_1$ hybrids possess features which are intermediate to varying degrees between those of the parents (Plate 1). This gives excellent opportunities for the selection of clones even without further crossing. The same applies to crosses with *Turanaga*, and at least to some extent with *Leucoides*.

Improvements in vigour of growth, of form, and of disease resistance have been possible by selection within the naturally interbreeding groups (Wettstein 1952; FAO 1958), particularly aided by the removal of the geographic barriers which had separated the species of North America, Europe and Eastern Asia. This resulted in the very extensive *P. "euramericana"* suite of hybrids between the American *P. deltoides* and the Eurasian *P. nigra*, as well as those between species of *Tacamahaca* and *Aigeiros*, such as the *P. "generosa"* cross of Henry (1914).

In the latter combinations, and also those between species within *Tacamahaca*, numerous crosses were made by Schreiner and Stout (1934) using material in botanical collections. The same processes can now be repeated with crosses between the remaining groups. This has immediate Australian (and New Zealand) importance following the unintentional introduction of two species of rust, the American *Melampsora medusae* and the European *M. larici-populina*, which were both well established in Australia and New Zealand by the summer of 1973-74 (van Kraayenoord 1973; Walker, Hartigan and Bertus 1974).

Species of the section *Leuce* (such as *P. tremuloides* and *P. alba*) are immune to both these rusts, while $F_1$ hybrid families of these *Leuce* species and several *Aigeiros* species contain individuals which are immune, or highly resistant, thus broadening the base for breeding for *Melampsora* resistance. The timber of *Leuce* species is also generally of higher quality for many purposes than that of the other groups, and this feature can be expected to be incorporated at least partially in progeny from suitable combinations.

On the other hand, the aspens are mostly of high-latitude origin, and display marked photoperiodic regulation of growth rhythms. As a result they are poor performers at relatively low latitudes, even though other conditions for their growth may be favourable. While *P. alba* and *P. tremuloides* from low latitude provenances can be used in a breeding programme to circumvent this, there is now the possibility of adding as parents many more species from the Sections *Aigeiros* and *Tacamahaca* in producing interspecific hybrids.

The same applies to the poor capacity or inability of species in subsection *Trepidae* and some species of *Leucoides* to strike roots readily from stem cuttings, in contrast to the ease of rooting of most species in the genus.

In the case of the section *Turanga*, *P. euphratica* is a tree of local significance on the Indus in Pakistan, on the Upper Euphrates in Syria and Turkey, and, to some extent, on the Upper Tigris in Iraq. It has similar breeding prospects for, although this species is of moderate or poor form and rather slow growth, it is able to endure more rigorous summer climates than other species and it is likely that this feature would be expressed as a genetically determined physiological trait.

Added understanding of the incompatibility system of poplar and the development of effective methods for producing crosses between hitherto incompatible species (Appendix II), which was foreshadowed by Knox, Willing and Pryor (1972), have now become a practical reality as a result of these latest findings and the development of manipulative techniques.

It is also possible that similar methods might be applicable in some other plant groups having similar incompatibility mechanisms.

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R.R. Willing
Professor L.D. Pryor
Botany Department
The Australian National University
Box 4, P.O.
Canberra, A.C.T. 2600 (Australia)