4. Haramaki, S. 1971. Tissue culture of Gloxinia. *Proc. Int. Plant Prop. Soc. 21:* 442-449.
5. Knudson, L. 1946. A nutrient solution for the germination of orchid seed. *Amer. Orch. Soc. Bull.* Oct. 1946.
6. Murashige, T. Principles of *in vitro* culture. 1966, *Proc. Int. Plant Prop. Soc. 16: 80-87.*
7. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant. 15:* 473-497.
8. Wimber, D.E. 1963. Clonal multiplcation of Cymbidiums through tissue culture of shoot meristems. *Amer. Orch. Soc. Bull. 32:* 105-107.

THE PRODUCTION OF INTERSPECIFIC HYBRIDS BETWEEN INCOMPATIBLE PAIRS OF EUCALYPTS

L.D. PRYOR and R.R. WILLING

Botany Department
Australian National University
Canberra, Australian Capital Territory

ABSTRACT. A method of breaking interspecific incompatibility barriers between pairs of species in different sections of the genus *Populus* has been found to function in a similar way between pairs of species in some groups of *Eucalyptus*.

INTRODUCTION

Breeding incompatibility is a phenomenon frequently found in flowering plants. It is well known within species and is commonly expressed by the inability of individuals to self fertilize successfully or the inability of some groups of individuals in a species to be fertilized successfully by some other groups within the same species. In a similar way groups of species within larger genera often fail to cross even when other barriers to interbreeding such as geographic isolation or lack of synchronous flowering are removed. Recently considerable attention has been given to the precise mechanisms involved since histochemical and electron microscopic methods have become available to allow much more precise determination of the processes than was previously possible (4). These studies to some extent have already indicated means of approaching the problem of breaking interspecific incompatibility with the consequent opening of ways to extend interspecific breeking in various genera. This allows the consequent production of some forms which may have greater potential utility than those existing.

Work has been carried out with poplar which has lead to the development of techniques for breaking interspecific incompatibility between reproductively isolated species in that genus (5) and it has been found possible to apply the same methods to some extent in eucalyptus.

BREAKING INTERSPECIFIC INCOMPATIBILITY IN POPLAR

From the manipulative point of view, poplar has some distinct advantages. The plants are dioecious and in most species flowers of each sex are produced in separate catkins on separate trees. They can readily be transferred to the glasshouse as grafted scions and, after pollination, the fruit ripens usually in eight to ten weeks. Afterwards upon extraction and sowing, seed germinates in a few hours and seedlings large enough to display distinctive morphological features are obtained in another eight to twelve weeks making evident the results of intended hybridization at that time.

From a series of experiments it was found that one method of breaking interspecific incompatibility was by treating a receptive stigma very lightly with an organic solvent such as hexane before applying otherwise incompatible poplar pollen to the stigma. Good seed set was then obtained (Willing and Pryor unpublished). The precise details of the changes associated with a successful outcome in the situation are still being elucidated but, in the meantime, it is evident that the technique has some application in eucalypts and may perhaps have still wider implications.

APPLICATION IN EUCALYPTUS

Eucalyptus is a very large genus (2) with several distinct subgeneric groups, some of which certain authors (1, 3) suggest might be ranked as genera rather than subgenera.

The largest of these subgeneric groups, *Symphyomyrtus*, is divided into eleven sections of which one, *Adnataria*, contains a group of species which are considerably different from those in the remaining ten sections (8).

From the knowledge of spontaneously occuring hybrids found rarely in the field, and of some attempted crossings by manipulation, it had been evident that species of *Adnataria* cannot readily interbreed with other sections of *Symphyomyrtus*. Indeed very few cases of such crosses are known in spite of the fact that in numerous field situations species from pairs of sections in *Symphyomyrtus*, one of which is *Adnataria*, grow side by side and reach anthesis often at the same time. On a number of occasions manipulated crossings have failed as well (Willing and Pryor unpublished). There are no hybrids of any sort known between the subgeneric groups in the genus but because there were a few cases known between species of the section *Adnataria* and species of other sections of *Symphyomyrtus*, it was thought that if there were to be any positive effect of the technique, it might be most readily indicated in these combinations which were known at times to produce sparingly an occasional hybrid.

The result has been quite dramatic in that substantial numbers of seed have been set in capsules following stigma treatment lightly with hexane. In particular, a number of hybrids have been obtained between *E. grandis (Symphyomyrtus,* section *Transversaria)* and *E. leucoxylon (Symphyomyrtus,* section *Adnataria)* and also between *E. pulverulenta (Symphyomyrtus,* section *Maidenaria)* and *E. leucoxylon* as well as between *E. pulverulenta* and *E. melliodora (Symphyomyrtus,* section *Adnataria).*

The assessment of the seedlings by such crossings as hybrid is usually possible in the cotyledonary stage. Seedlings of the parental types are raised at the same time so that a visual comparison of relatively trivial young seedling characters of the parents and the putative hybrids can be made. At a later state, when they have grown to some extent, additional morphological characters are readily available in various seedling features, which can be used to test whether the plants so raised are truly hybrid or not (6).

In the case of *E. grandis* and *E. leucoxylon* there is a distinct difference in the leaf venation at about the tenth leaf pair and a comparison of the plants produced from crossings with the two parental types indicates a degree of intermediacy between the parents. In the case of *E. pulverulenta* and *E. leucoxylon* hydridity is similarly indicated by the intermediate juvenile leaf shape which is known to be a sensitive indicator of hybrid status. (7).

As with crosses in poplar between species from different sections which are ordinarily incompatible it is found that even in the limited numbers so far raised that in crosses between species of *Adnataria* and species in other sections of *Symphyomyrtus* that there is a rather higher proportion of poorly viable plants in the progenies than is the case in non-hybrid seedling populations of a particular species or in the progeny from interspecific crossings between pairs of species which are ordinarily cross compatible. This suggests that the matching of the genomes in the case of the wider crosses is less close than in the crossings between more closely related species and therefore to obtain adequate numbers of hybrids from which a selection of vigorously growing individuals can be made will entail raising larger populations than in the case where the relationship between the parental species is closer and more of the progeny are fully viable.

Trials have been carried out between species of the various eucalypt subgeneric groups but none has yet reached the stage of producing seedlings. It may be that the method employed is successful only with species which are related to the extent that those of *Adnataria* are to the rest of *Symphyomyrtus.*

Nevertheless there is no way of forecasting whether species pairs taken from different subgenera will hybridize if so manipu-

lated, and investigation of the genus in this way is well merited. Such investigation will serve two widely different interests. Firstly, the possibility of producing plant material with new and valuable characters which, when propagated, will be found of practical use. Secondly, the evidence of interbreeding capability when a particular method is used to remove an incompatibility barrier may allow an added assessment of affinity between species beyond that which is indicated on morphological grounds.

Within the genus there are a number of species which, as far as it is known, are reproductively isolated. Two of these, *E. deglupta* and *E. microcorys* have been placed in separate sections in Symphyomyrtus but they are species which are unusual in the genus as a whole and may perhaps merit ranking as monotypic subgenera. If, by manipulation in the way described, they hybridize successfully with some other species of *Symphyomyrtus* in the way the species of *Adnataria* do this would support the notion that they should remain as sections of that subgenus rather than being assigned a higher rank.

SUMMARY

Interspecific breeding incompatibility between pairs of species from different sections of the genus *Populus* has been removed by various means including lightly treating receptive stigmas with an organic solvent before pollination. This same method has been found effective in producing successful crossings readily between pairs of species of *Eucalyptus*, one from the section *Adnataria* of the subgenus *Symphyomyrtus* and the second from other sections of that subgenus. Without such manipulation, crosses of this type fail or, at the most, produce progeny sparingly.

LITERATURE CITED

1. Andrews, E.C. 1913. The development of the natural order *Myrtaceae*. *Proc. Linn. Soc. N.S.W. 38:* 529-568.
2. Blakely, W.F. A Key to the Eucalypts. Third edition 1965. Forestry and Timber Bureau, Canberra.
3. Carr, D.J. and Carr, Stella G.M. 1962. Natural groups within the genus *Eucalyptus*. In "The Evolution of Living Organisms". (Symposium). Royal Society of Victoria: Melbourne.
4. Knox, R.B., Heslop-Harrison, J. and Reed, C. 1970. Localization of antigens associated with the pollen grain wall by immunofluorescence. *Nature*, Lond. 225, 1066-1068.
5. Knox, R.B., Willing, R.R. and Pryor, L.D. 1972. Interspecific hybridization in poplars using recognition pollen. *Silvae Genetica* 21, 65-69.
6. Pryor, L.D. 1955. A hybrid swarm between *Eucalyptus odorata* Behr and *Eucalyptus leucoxylon* F. Muell. *Trans. Roy. Soc. S. Aust. 78:* 92-96.
7. Pryor, L.D. 1956. The identity of *Eucalyptus subviridis* Maiden and Blakely. *Proc. Linn. Soc. N.S.W.* 81: 101-104.
8. Pryor, L.D. and Johnston, L.A.S. 1971. A Classification of the Eucalypts. The Australian National University Press, Canberra.