Fertilizers added were:

Single superphosphate	23 Kg.	Iron sulfate	12 Kg.
Sulphate of potash	46 Kg.	Urea (46 %N)	140 Kg.
Gypsum	60 Kg.	Agra magnesium, 95%	30 Kg.
Esminel	12 Kg.		

CONCLUSION

Rooted cuttings of *Clematic jackmanii* hybrids may be grown in the Sydney, Australia metropolitan area up to marketing sizes providing care is taken with all steps of the procedure mentioned and particular attention is given to the timing of taking cuttings and subsequent handling of the rooted cuttings.

POPLAR BREEDING

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Poplars have been grown in many widely different regions of the world since very early times, especially in the East and Far East. In Europe, when the North American poplars were introduced and hybridized with the European poplars, the expansion of poplar cultivation really began.

In Australia the early settlers introduced several species, growing them mainly as ornamental trees. Only very few clones were known: two clones of *Populus alba*; the Lombardy poplar, or *P. nigra* 'Italica.' (the best known poplar of them all); the Yunnan poplar, *P. yunnanensis*; and the American cottonwood, *P. deltoides*. There are no native poplars in Australia nor in the Southern Hemisphere. All poplars growing in southern Africa, South America, and New Zealand are introduced or mainpulated clones. In the 1940s some of the so-called "Schreiner hybrids" were introduced. They are known as Androscoggin poplar, Geneva poplar, Oxford poplar, and Rochester poplar. They are hybrids between species of the section AIGEIROS and TACAMAHACA, and are grown in Australia mainly as ornamentals. A little later the introduction of the well-known Italian hybrid-clones I-154, I-214, I-488 and a few others occurred to add some poplar clones which could be of interest to the wood-producing industry. In the late 1950s the match companies decided to grow their own raw-material: poplar wood for making match splints locally. It was near Grafton, N.S.W. where the first plantation for growing match wood was established. This interest in poplars stimulated the research work already underway in the Botany Department of the A.N.U. Under the direction of Prof. L.D. Pryor, work on the introduction of semi-evergreenness into cottonwoods was already in progress and, besides species and provenance introductions and evaluation, work was aimed at the production of hybrids between species of different sections within the genus.

Most poplars are very sensitive to photoperiod. Poplar species or clones derived from parents originated at high latitudes perform poorly in low latitudes. Bud-dormancy occurs early and leads to limited height and diameter growth. Those long-day clone's performance in Grafton, latitude 29°S, is unsatisfactory and makes the selection of successful clones imperative. On the other hand, poplar species or clones from low latitudes, or the semi-evergreen clones, fail to go dormant in high latitudes and are severely damaged or killed by low winter temperatures in these latitudes. The great variation in clonal behavior makes it important to select the most suitable clones for a particular latitudinal environment. The best performers in Grafton are not the clones to be grown in Victoria or Tasmania and vice versa. Of all the poplars tested in Grafton, it was the cottonwood, *P. deltoides missouriensis* (syn. var. angulata), which gave best results. We responded by two methods: raising provenances from low latitudes (Texas, Mississippi), and selecting the best performers. On the basis of form, growth rate, and disease resistance, clonal selections were made and this material is the basis of the Grafton plantation. This simple method of raising provenances from imported seed from the southern U.S., and selecting and cloning the most suitable individuals is very effective and will yield many good poplar clones in the future.

The second method used to produce poplars most suited to a particular environment is hybridization. Intrasectional hybrids are easily produced and no difficulties experienced. Our main objective in this particular programme was to try to combine semi-evergreenness with the qualities of the cottonwoods. We already had some data collected from previous experiments, crossing *P. deltoides* (Syn. *P. monilifera*) with the semi-evergreen clone *Populus nigra* 'Persistente'. This semi-evergreen poplar is, in experiments, completely day neutral. In growth cabinets, under a 6 hour day-length, growth was still continuous. At sites where poplar planting is carried out in Australia winter temperatures are endured. Budbreak and growth begin as soon as temperature and moisture conditions are favourable. In milder climates leaves are retained until the next season's leaves begin to emerge. This semi-evergreen clone is a *male* and during the growing season difficult to distinguish from *P. nigra* 'Italica'. *P. nigra* 'Persistente' has been grown in Chilė for a long time and is considered to be a mutation of *P. nigra* 'Italica'.

We knew that the segregation pattern in the F_1 produced 50% deciduous and 50% semi-evergreen types. We speculated that *semi-evergreenness* is based on one single dominant gene. The semi-evergreen parent is heterozygous, semi-evergreen being dominant to the deciduous habit, and the cottonwood homozygous recessive. A large number of hybrids with various cottonwood clones has been made; the segregation pattern was as expected and selections of evergreen segregates with good growth and form were selected and cloned. A number of clones proved very successful in Grafton and also under irrigation at Cobrawonga, Victoria. Many of these semi-evergreen clones are very well suited to shelterbelt- and windbreak stands. Unfortunately, at this time (1965), hybridizing and therefore the introduction of semi-evergreenness into other species could be done only within the section AIGEIROS, the black poplars. Despite the compatibility that exists between the AIGEIROS and TACAMAHACA sections, crosses could not be made, as at that time no female clone of the TACAMAHACA section was available in Australia. We have *P. yunnanensis* and *P. simonii* as male clones and, as already stated, *P. nigra* 'Persistente' is male also. This situation has changed. Some new clones of *P. yunnanensis* have been imported and seedlings of several provenances of *P. simonii* are in cultivation. Apart from direct crosses with the *P. n.* 'Persistente', hybridizing with semi-evergreen cottonwood hybrids, which have reached the flowering stage by now, would achieve a similar result.

In the meantime the situation concerning the possibility of producing hybrids intersectionally has changed completely. It is now possible to interbreed any two poplar clones or species from different sections and combine desired features in new hybrids.

Populus is divided taxonomically into five sections, representing some 30 species with numerous subspecies. The number of provenances and clones is endless.

The five sections are:

A. TURANGA; B. LEUCE, with 2 Subsetions: TREPIDAE and ALBIDAE; C. AIGEIROS; D. TACAMAHACA; E. LEUCOIDES.

The section TURANGA is represented by *P. euphratica* and its various subspecies. It is found in Turkestan, Iran, and Syria, where it is of economic importance; but it also occurs in Turkey, Pakistan, Palestine, Egypt, Libya, Algeria, etc. A tree of medium height, often bushy, *P. euphratica* is light and heat demanding and can tolerate brackish water. It is, among poplars, the most resistant to salinity. Its propagation is mostly done by seedlings. Softwood cuttings under high humidity strike readily but hardwood-cuttings are difficult to root. This species cannot be hybridized with any others without special manipulation. Hybrids of this species seem to be very desirable. Our hybrids made between *P. euphratica* and cottonwood and between *P. euphratica* and *P. yunnanensis* are better trees, strike from hardwood cuttings, and seem to be salt-tolerant.

The section LEUCE with its two subsections, the aspens and the true white populars, covers vast areas, extending from the polar circle to North Africa. The many species of this section are important in many countries of Europe, the Middle East, and North America. Stem cuttings of the aspens do not root, though cuttings of hybrids between aspens and the white poplars do strike quite readily. Hybrids within this section can be found quite frequently and are easily manipulated. They do not hybridize with species of other sections without special manipulation.

The section AIGEIROS are the black poplars. Here we can distinguish between two groups: the Eurasian group, represented by *P. nigra*, and the North American group by *P. deltoides*, the cottonwoods. Hybrids between these two black poplars have been extremely successful and, in Western Europe, have practically ousted the native *P. nigra*. Members of the AIGEIROS section hybridize readily with each other; they also hybridize without special manipulation with species of the TACAMAHACA section and LEUCOIDES section.

The section TACAMAHACA, the balsam poplars, is represented in North America by its two native species: *P. trichocarpa* and *P. balsamifera* (Syn.: *P. tacamahaca*). In Asia *P. suaveolens*, *P. simonii* and *P. yunnanensis* are the native species of the balsam poplars. Within the section hybrids can be readily manipulated, also with species of the AIGEIROS- and LEUCOIDES sections.

The section LEUCOIDES is represented in the Far East and one species in North America. Representatives of this section seem to be compatible with species of the sections AIGEIROS and TACAMAHACA. We have been able to produce hybrids between *P. lasiocarpa* and *P. ciliata*, representing section LEUCOIDES, and with species of the TACAMAHACA and AGEIROS sections.

We can confirm that, within the five sections, the species, subspecies, and clones can be easily hybridized and are therefore compatible. Between the possible 10 cross combinations of the five sections *only* 3 combinations are compatible. They are: AIGEIROS X TACAMAHACA, AGEIROS X LEUCOIDES, TACAMAHACA X LEUCOIDES.

The remaining 7 possible combinations are incompatible. They are: AIGERIOS X LEUCE, AIGEIROS X TURANGA,

LEUCE X LEUCOIDES, LEUCE X TACAMAHACA, TURANGA X TACAMAHACA, TURANGA X LEUCOIDES, TURANGA X LEUCE.

Intensive research over many years to find a method to overcome the incompatibility barriers has finally been successful. Now we have several methods at our disposal and can produce hybrids of any desired combination between species of any section. It is difficult to imagine all the possibilities existing now to broaden the base for poplar breeding. It will give excellent opportunities for the selection of clones improved in growth, form, disease resistance and will widen the scope immensely.

To handle a large number of crosses in the glasshouse, branches bearing the female flowerbuds were grafted on to rooted cuttings and placed in different glasshouses, according to species and intended cross. The grafts were "bottle-grafts"; the understock was rooted about 4 months prior to grafting, the grafting was done 3 to 4 weeks prior to natural flowering in the field. White poplar females give good results by placing flower bud-bearing branches in containers filled with water. The development of white poplar seed is fast, so that grafting is not necessary. Seeds are germinated immediately on ripening in flats filled with a perlite-vermiculite mixture and covered by a sheet of glass. Germination occurs within hours. Nutrient application twice weekly brings the seedlings within 3 weeks to a size ready for transplanting into a mixture of 2 parts sterilized soil, 1 part peatmoss, and 1 part sand.

The poplar species vary greatly in their flowering time and, to be able to make any intended cross, especially with males of species flowering late in the season (*P. euphratica* flowers in mid-summer), pollen from previous years has to be used.

Pollen extraction and storage: branches bearing male flowerbuds are brought into the glasshouse or laboratory, separated according to species, about 4 weeks prior to anthesis in the field and placed in water-filled containers. Anthesis occurs about 2 weeks later and pollen is released over several days. Pollen is collected daily, desiccated over silica gel for 12 to 24 hours; it has to be dry and held at -18°C. Under those conditions pollen remains viable for several years and is the source of most pollen used for the various experiments.

A pollen grain, when it comes into contact with the stigma surface, will germinate by responding to specific conditions; it is interacting with the environment of the stigma. Very complex interactions of stigma- and pollen substances are taking place to allow the pollen to germinate, the tube to penetrate the stigma surface, to grow through the stylar tissue and the micropile to release finally the nuclei and combine with the egg-nuclei to form the zygote. All these events are based on harmonious interaction of the various processes in pistil and pollen. Biochemical interactions, especially an interplay of enzymes and growth substances, coordinate events that lead to fertilization. It is understood that, if genome differences are such that these intricate and complex interactions do not occur, embryo formation cannot be achieved.

Our experiments in this field with the genus *Populus* enabled us to gain some insight into some events, which occur right at the beginning of the pistil-pollen interaction, germination and penetration. They lead us to believe that the mechanism for successful or unsuccessful interspecific interaction is based on the biochemical makeup of the tryphine of the pollen and the stigma surface. The exine products of the pollen (here called tryphine) are synthetized in the tapetum; they are transfered during the final stages of pollen maturation to the pollen grain and therefore are sporophytic in origin. Our experiments show that tryphine on its own, separated from the pollen grain, produced the same pistil-pollen reactions as complete, viable pollen grains. Hexane soluble materials from the exine (tryphine) of an incompatible pollen parent, coated on to a compatible pollen, renders this pollen incompatible, and vice versa; an incompatible pollen coated with compatible tryphine will produce reactions of a compatible pollen and produce viable embryos. The tryphine, as far as poplars are concerned, seems to be the carrier of materials responsible for the compatibility or incompatibility reactions.

To overcome the incompatibility we approached the problem in basically two ways and were able to achieve fertilization and to produce thousands of new hybrids.

A. We made up the shortage or replaced the information of the pollen carried by the tryphine by using "factor pollen" or exchanged the tryphine.

B. We disorganized or removed the inhibitor-promotor complex of the style by treating the stylar surface with certain reagents (organic solvents). Transplants of pistils and injections of pollen directly into the ovaries must be considered as modification only of this same principle.

To comply with the requirements of method A, we mixed live incompatible pollen with dead non-viable compatible pollen, termed "factor pollen" by us. We were able to obtain fertilization, the dead pollen supplying the necessary reaction materials for compatibilty and the incompatible pollen, the genetic material necessary. In such pollenmixtures it was found that, until the percentage of the nonviable (killed) pollen reached about 50% of the mixture, there was little action by the incombatible pollen. Increasing levels of treated pollen of up to 90% produced highly successful responses. More than 90% of the number of seeds produced in a normal compatible cross was obtained. "Factor-pollen" was produced in two ways:

1). Repeated freezing and thawing, about 10 times, resulted in the loss of viability with little impairment of its reaction materials.

2). Gamma irradiation was done by exposing dry pollen to a radiation dosage of 100,000 rads from a cobalt source, and very good results with such pollen mixes were obtained.

For usage, "the factor-pollen" (of the compatible type) is mixed with fresh or frozen viable incompatible pollen, ratio 9:1, and dusted on receptive stigmas, using a small water colour brush. The whole inflorescence is treated at the same time. To exchange the tryphine, the two pollen types, a compatible one, and the incompatible pollen of the species to be used for hybridization, are immersed separately in an organic solvent, such as hexane, ethyl acetate, or toluene, to extract the tryphine and, after several minutes, filtered. No pollen grains must remain in the extracts. After evaporation of the solvents a fatty substance, mainly tryphine, remains. After mixing the pollen- "fat" of the compatible pollen with the incompatible pollen, this pollen becomes compatible and pollination can proceed. It is important not to use the alcohols, ethanol or methanol. They kill the pollen outright whereas the other solvents mentioned have no detrimental effect on the viability of the pollen grains and can be used for pollen storage also.

To test theory B, the stylar incompatibility reaction has to be inhibited, so that no callose and other pollen tube growth-interfering substances may be formed and delay, and finally stop the pollen tube growth of the incompatible pollen. This can be very easily and effectively done by removing the stylar surface exudates, using a solvent such as hexane. Just before pollination the stigma surfaces in the receptive stage are carefully brushed with a camelhair brush, which is slightly moistened with the solvent. Only small traces of the solvent must come into contact with the stigmatic surface; otherwise the tissue is "burned" and fertilization inhibited. Pollination can be carried out as soon as the solvent treatment has been completed, or it can be delayed for several hours.

The fact that the incompatibility reaction can be prevented, by either stigma or pollen treatment, suggests that at least two factors (one at each location) are involved and that the incompatibility process is inactivated by the removal of either. We call them here P (pollen) and S (stigma) factors.

Developed and used by us, these methods have produced large numbers of hybrid populations which otherwise could not have come into existence. The base for poplar breeding is broadened very greatly.

Improving poplars for disease resistance becomes very important in Australia. In 1972 the poplar rust species, *Melampsora medusae*, appeared and, in the following year — 1973, *Melampsora larici-populina* became widespread. Several biotypes of these two rust races have developed and more will come into existence. Their virulence may change and, as a result, the level of resistance of the poplar clones may change also. Work done by Dr. W. Heather has helped us immensely to understand development and behavior of the *Melampsora* fungus as well as its enemy, the hyperparasites *Cladosporium* and *Eudarluca*. Breeding of rust resistant poplar clones is the basis of poplar silviculture in Australia.

The introduction of semi-evergreenness into all other clones of the remaining sections can now be easily achieved. Species and clones, difficult to strike from cuttings, but otherwise of interest and value, can be used as parents to produce progenies which root from stem cuttings. Soon we shall know if hybrids of those wide crosses can produce fertile seed. If they do, back-crosses can be made and all the hopes of the poplar breeder can come true.

THE NATURE OF CALLUS AND ITS IMPORTANCE TO THE PLANT PROPAGATOR

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Abstract. The ability of wounded plant tissue to regenerate is important for successful plant propagation. It enables a union to occur in budding and grafting, healing to occur at the base of cuttings, and allows rapid multiplication of selected plants in tissue culture techniques.

Regenerating cells from wounded tissues emanate primarily from the cambium region of the plant and they grow over the wound both radially and laterally. The overwalling of these cells is called callus and the nature of callus and its part in the rooting and unification process is explained in this paper.

With some species excess callus forms, inhibiting root primordia from emerging and preventing the formation of a viable root system on cuttings. This problem is examined with reference to the pH and nature of the propagating medium, the time of year of selection of cuttings, auxin application, wounding, trimming of callus, environment, and the type and condition of stock material. Certain recommendations are given for problems encountered with the propagation of some Australian native plants.