varies from miniature size plants like 'Ginko Craig' and 'Golden Tiara' that can easily be established in 7.5-cm pots, to very large eyed plants like 'Frances Williams' that is best replanted in pots larger than 10 cm. However, we have found that some cultivars seem to increase eye production when their root systems are restricted in smaller pots. *Hosta* is one of the easiest perennials to divide at almost any time during the growing season. Again, when divided after leaves are fully expanded, reduce leaf area, and shade.

Tricyrtis, an increasingly popular shade-tolerant perennial, has an interesting way of propagating itself in our climate. The crown of potted plants will usually winter kill leaving uninjured roots. Shoots will then form on these roots and begin growth. Separating new shoots from the medium in the spring may yield 50 or more new plants from a 10-cm pot.

Tissue Culture. Some propagators chose tissue culture to rapidly increase new varieties or to reduce disease incidence. Although *Hemerocallis* and *Hosta* have been widely propagated from tissue culture, some caution must be exercised. Some cultivars are very uniform, but others may be very variable. Many types of *Hemerocallis* 'Stella de Oro' are now in the trade because they have originated from tissue culture. Variegated hostas are even more of a problem. *Hosta* 'Patriot' is a relatively new and popular sport of 'Francee' that was introduced by John Machem, a long-time I.P.P.S. member. Some less reliable tissue-culture labs have shipped many 'Patriot' propagules that have not been rogued properly to unsuspecting U.S.A. nurseries. The best way to avoid this problem is to purchase propagules from reputable labs and to know what the cultivar should look like.

Although sales of perennials are booming, knowledge about propagation methods is just beginning to become general knowledge. This is due, in part, to the large number of taxa involved, but also to some proprietary knowledge which is now being shared. As methods and knowledge about perennial propagation become more available, even more of these popular plants will become available to the general public rather than residing in botanical gardens or in the gardens of the gardening elite.

Cultivar Integrity in Australian Tree Production

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In Australian tree production, many of the issues concerned with producing high-quality trees have been addressed. Root system research has lead to the production of trees that successfully establish post-transplanting. Also, tree cultivars of non-Australian origin are quickly imported by the major propagating companies, and are made available to the market for testing and sales, once quarantine requirements are met. This leaves three areas of deficiency in Australian tree production:

1) Formative pruning and the development of tree canopies to best suit the end use.

2) Trialing of taxa to ascertain their suitability for differing Australian landscape situations.

3) Making certain that the cultivars we are using are the cultivar they purport to be.

With trees, it is especially important that horticulturists plant known cultivars. Estimates indicate that street trees require expenditure of approximately $10,000 over an amenity life of 25 years (pers. comm, G.M. Moore, May 1996). This expenditure can be decreased greatly if the trees planted have functional qualities that will require less pruning, dead-wooding, and line clearance work over their lives. Some of these issues can be solved by appropriate formative pruning, but most of this expenditure can be decreased by planting selections known to have ideal form with little dead wood. Fruit drop in street trees also requires money inputs in removal; planting non-fruiting selections can eliminate these costs.

The use of sub-optimal cultivars can be readily shown. *Gleditsia triacanthos* 'Shademaster' is known to be a non-fruiting form of the honey locust that has improved form. Trees planted in Camberwell Victoria as 'Shademaster' bear fruit annually. Trees planted throughout southeastern Australia as *Pyrus ussuriensis* have been shown to be a poorly formed type of *P. calleryana*. Planting trees of mistaken identity will add to their cost: with the *Gleditsia* there are unsightly fruit to remove; the poorly formed *Pyrus* will need to be removed before they split.

These mistakes show that Australian horticulturists should be planting asexually propagated trees where the propagation material is taken only from superior (or elite) stockplants showing selected characteristics. To most horticulturists, this means that they should be planting cultivars. Unfortunately, "cultivar" can mean almost anything: "..a horticultural variety or race that has originated and persisted under cultivation..." (Bailey and Bailey, 1976). Since this term is so ambiguous, tree growers should know that the stock they grow goes beyond being a cultivar, and is asexually propagated from a known stockplant. These selections are best be known as "clones".

There are a number of reasons why appropriate clonal material has not been used. The horticultural community intends to use clonal material, but frequently this does not happen. Many times, the errors occur because:

- The importation process for tree cultivars is problematic. Shipping plants to Australia is a low priority to many international growers, and, if Plant Breeders Rights cannot be granted on these plants, it is of little financial value to the exporting nursery. A lack of care occasionally occurs with exporting nurseries. Later, these plants are kept in quarantine facilities for many months, or for many years, and labels can easily be lost or switched. Finally, budded plants can die back to their bud unions after methyl bromide treatment; this can lead to the understock being propagated as the selected cultivar.
- Incorrect names can often be used. We believe that one of the problems with the ornamental pear species in Australia arises since both *P. calleryana* and *P. ussuriensis* are frequently given the common name of "Manchurian pear". This can lead to translational errors and using the wrong name for the wrong plant.
- Understock material is sometimes used as propagation material instead of the clone budded. When budding is the accepted propagation method, this can lead to significant spread of an erroneous selection.

- Anecdotal information indicates that trees looking similar to a clonal cultivar are sold under the clonal name by some growers. Appearance of plants is not a sufficient basis to use the clonal name; physiological/functional differences are not obvious and the plant may perform quite differently from the clone it resembles.

Identifying the trees to clonal level can be extremely difficult. In assessing the putative *Pyrus ussuriensis*, using morphologic characteristics was adequate to prove that they were *P. calleryana* of unknown origin (Kellow and Will, 1995). Analysing individual clones to test for differences is normally more difficult and most often is successful using chemotaxonomic techniques.

There are three general classes of chemotaxonomic techniques that are of significant use to the tree grower to verify the clonal identity of a named plant; isozyme analysis, restriction-fragment-length-polymorphism analysis, and techniques that use polymerase chain-reaction technology. All of these techniques analyse the genetic constituents of the plant in some ways. Although great advances using PCR and RFLP mapping have been made in identifying plant clones (see the work of Thomas et al., 1994, identifying grapevines at The University of Adelaide), isozyme analysis should be considered the most suitable method for clonal identification. This recommendation is given because:

1) There is published reference to hundreds of genera in which this technique has been used successfully.
2) It requires moderately inexpensive laboratory equipment and chemicals. A well-organised laboratory can be established for approximately $20,000.
3) The technique can be used quickly, and results can be obtained within hours.

ISOZYME ELECTROPHORESIS TECHNIQUES FOR CLONAL IDENTIFICATION

Isozyme electrophoresis techniques have been used for over 20 years. Initial use was restricted to basic biological medical sciences, but the technique is now widely used in routine plant analysis. Isozyme analysis is based on the movement of proteins in a size-restricted matrix under voltage, then stained to show a specific protein type. Biologically, the system works for identification of individuals, since each individual produces proteins that are slightly different from each other and are therefore unique. Once these unique proteins are extracted from an individual, exposed to electrical charge within a size-restricted matrix, and are stained to become visible, they can be compared with other differing or similar individuals. Isozyme analysis produces a protein "fingerprint" of an individual that will remain constant given unchanging techniques of assessment.

Isozyme analysis is not a new technique, and has been used to "fingerprint" many plant clones. In the I.P.P.S., the first discussion of this technique occurred in 1985, with a paper discussing the identification of apple clones (Larsen et al., 1985). Isozyme analysis has been used to describe plant clones ranging from raspberries (Cousineau and Donnelly, 1992) to turf grasses (Vermeulen et al., 1991). Tree growers should find papers on *Acer rubrum* clones (Tobolski and Kemery, 1992) and *Taxus* clones (Greer et al., 1993) of special interest.

In work done at Burnley College identifying the *Corylus avellana* clones currently grown in Australia, we have further developed the isozyme technique to make it quicker and easier without losing any quality of results. In improving the work of Ahamad et al. (1987), we have developed an isozyme electrophoresis system that

can distinguish more than 35 clones. This system uses a minimum of tissue, approximately 50 mg is harvested from 2-year-old stem material. This stem material need be only about 1 cm in length, and it can be analysed during any season. Using a relatively simple extraction buffer, we have obtained zymograms (patterns on polyacrylamide gels that are the "fingerprints") showing 3 or 4 regions of variability using a peroxidase staining system.

This simplified method allows for extremely rapid screening of plants to assess clonal identity. It takes approximately 4 h total time, with 3 h of labour to analyse 40 samples. Further, we have found that the method is suitable for other crops and other staining systems (e.g. *Guichenotia* spp. using aryl esterase stains). Because this technique is efficient, it should bring costs of isozyme studies to a level at which tree growers can afford to use them. A detailed description of the technique will be available later in 1996 (Griffiths and Will).

Isozyme electrophoresis techniques are not perfect. Proteins for cultivar analysis must be selected carefully to eliminate the possibility of artifacts appearing because of plant vigour/health, tissue age, and time of year. Further, some enzyme stains may not be suitable as they may not show variable (polymorphic) forms of protein, thus making the individuals apparently identical. Finally, isozyme electrophoresis techniques can have limited application, as they are protein fingerprints, not exact maps of DNA or other genetic material. For this reason, isozyme analysis without further protein purification cannot link a specific trait possessed by the plant with a specific protein (Chen, 1991). If these possible problems are avoided, isozyme electrophoresis techniques are still valid for quick and cost-effective clonal identification.

CONCLUSION

Tree growers should be using chemotaxonomic methods, possibly including isozyme electrophoresis techniques, to guarantee the use of clonal stock. As plant quality continues to improve, correctly identifying the tree cultivar to clonal level will become more important. Isozyme electrophoresis is a valid technique for clonal tree identification that is accurate, of reasonable cost, and can be done quickly without the use of large amounts of plant tissue.

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