HOW DO INVASIVE WILLOWS MOVE ACROSS THE AUSTRALIAN LANDSCAPE?

Willows (*Salix* spp., Salicaceae) are a group of deciduous northern hemisphere trees and multi-stemmed shrubs that are typically found in riparian or other moist to wet habitats near permanent water sources. Attractive trees or shrubs, a number of willow species (and species hybrids) are available as horticultural cultivars (*e.g.*, weeping willows and pussy willows) and consequently have been spread well beyond their indigenous habitats, including to Australia. Currently there are a minimum of 20 tree willows (*e.g.*, *S. babylonica* or 'weeping willow' and *S. nigra* or 'black willow') and shrub willows (*e.g.*, *S. viminalis* or 'common osier' and *S. cinerea* or 'pussy willow') that have naturalised in rural riparian areas in Australia or have been planted for erosion control.

Figure 1. Young willows establishing along the edge of the Buckland River in the Australian State of Victoria

Unfortunately, the riparian adaptations of most willow species make them aggressive weeds outside their native habitats (Fig. 1). *Salix* species are either dioecious or monoecious and wind pollinated. The small seeds are distributed within catchments by water and among catchments by wind dispersal. They also proliferate vegetatively when stem fragments lodge and develop new roots. While the optimum conditions (continuously wet bare sediment) for seed germination are not common and the seed is short-lived (2-6 weeks), the large number of seeds produced, their rapid germination (24-48 hours), rapid growth, and early sexual maturity (2 to 3 years for some species) makes most of the willows aggressive weeds in Australian riparian habitats.

Initially the spread of willows in Australia was limited by the fact that many of the dioecious species were introduced as single sex clones. Unfortunately willow species hybridise readily. When different sexes were introduced for various species, this early barrier was breached by interspecific hybridisation; with the result that many naturalised willow populations in Australia are of hybrid origin.

The environmental damage caused by willows accrues from the displacement of native species, the reduction of water flow and the alteration of watercourses as the roots invade the stream beds and a reduction in oxygen levels when the fall of leaves in the autumn overloads streams with organic matter. Current estimates suggest that the current infestations only occupy 5% of the suitable habitat and that, without active and ongoing control, the current infestations in Victoria, Tasmania, New South Wales, and the Australian Capital Territory will spread rapidly and widely.

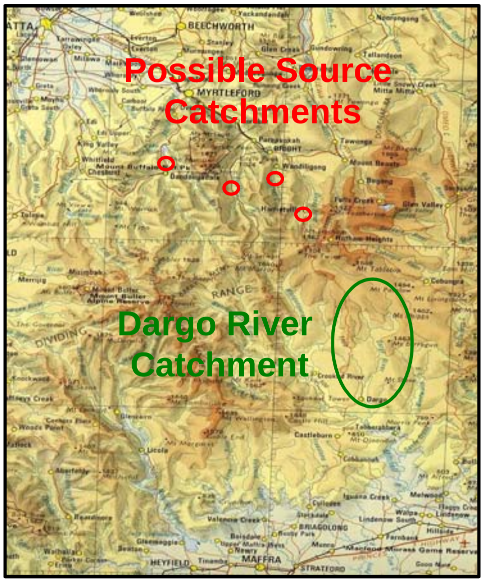

Figure 2. Geographic location of catchments in which Pussy Willow plants will be sampled for genetic analyses.

While almost all willow types naturalised in Australia are aggressive weeds, the willow that is the focus of this research project, the 'pussy willow' or 'grey sallow' (*S. cinerea*), is particularly aggressive. Both male and female clones of these multi-stemmed dioecious shrubs have naturalised, and the seeds of this species can germinate under a wider range of conditions than those of the other willows. The standard intervention strategy for willows is currently being applied to *S. cinerea* in the Dargo River catchment in East Gippsland, Victoria (Fig. 2). In brief, this involves starting the eradication process in the headwaters and moving downstream to eliminate the possibility of re-establishment of plants from plant fragments floating downstream. This sensible and practical approach could be compromised, however, if wind-borne seed is arriving from other catchments and re-infesting cleared zones. On ground observations indicate that seedling recruitment is occurring. While some of this is probably within catchment recruitment, the geographic proximity of populations in neighboring catchments means that the possibility that seeds are being blown in from other catchments cannot be excluded, and thus effective eradication efforts would require a multiple catchment strategy. Successful and efficient eradication in one catchment therefore now requires identifying the neighboring catchments that are contributing immigrant seeds to the area of interest so that a regional (or stepping stone) rather than a single catchment (linear) control strategy can be implemented.

Resolution of this issue is critical for the long term success of willow eradication efforts in eastern Gippsland. If immigrant seeds are arriving from other catchments subsequent to eradication, continual monitoring and recurrent eradication will be necessary. **The fundamental scientific and environmental management question that needs to be addressed is, "on what geographical scale must willow eradication and post-eradication monitoring be conducted to ensure that these efforts will be effective over the long term."**

To answer this question it is necessary to determine the extent and pattern of seed immigration across catchments in eastern Gippsland. Using standard ecological techniques to observe patterns of seed dispersal directly by either marking seeds with dyes, or using radiating transects of seed traps, is impossible over the scales involved and given the number of potential source populations within these complex landscapes. Instead, this study aims to evaluate whether molecular genetic markers can be used to unambiguously identify a seedling's source directly based on its relative genetic similarity to known candidate populations. If successful this would provide a methodological framework that can be used in the future to quantify spatial patterns of seed dispersal across riparian landscapes in detail, allowing determination of biologically appropriate scales for willow management. This will result in significant improvements in best management practices for willow control in Australia.

Analytical and Technical Background

Identifying the extent of seedling immigration and the pattern of dispersal across catchments with molecular markers is theoretically simple. Using molecular markers, the reproductively mature trees in each of the potential source populations and those of the target catchment are genotyped to identify markers unique to each of the populations. If each population is genetically unique, then the seedling genotypes indicate their origins (Fig. 3). This is an analytical procedure called 'assignment testing'. In practical terms, successful application of assignment testing depends on: 1) the ability to genotype representative samples of all relevant populations and; 2) high levels of genetic discrimination among the possible source populations.

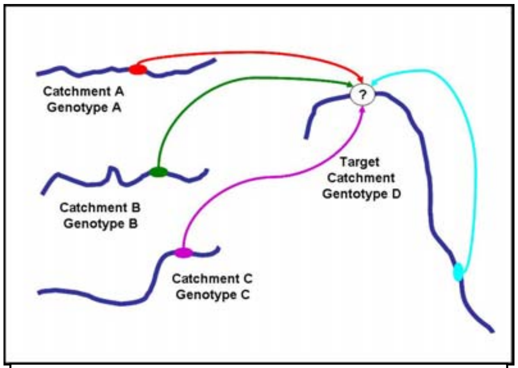

Figure 3. A diagrammatic summary of possible willow immigration across catchments. If each of the potential source populations is genetically unique, the genotypes of the immigrant seedlings reveal their geographic origins.

Assignment testing determines the relative probabilities that each of the potential source populations has produced a given seedling. Thus there will always be a population that is determined to be most likely even if the real source population has not been sampled. This limitation is easily overcome by good field surveys and sampling.

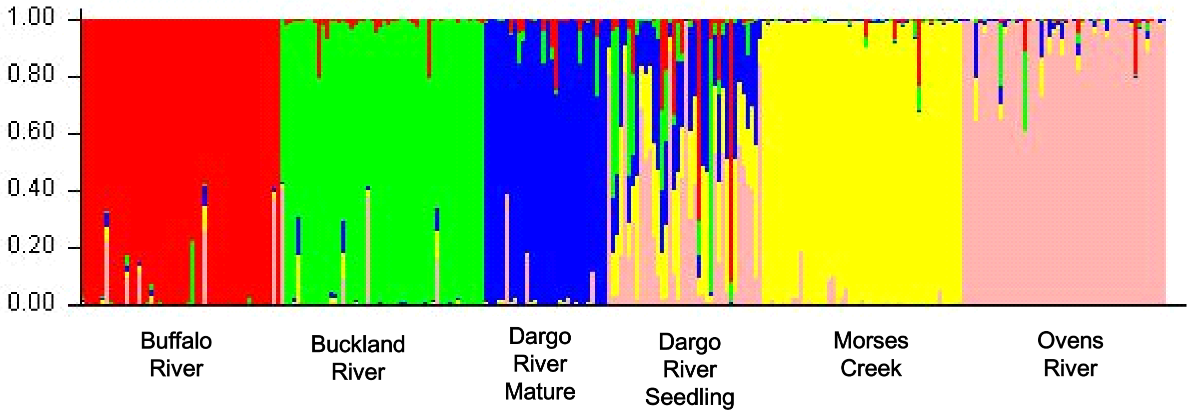

Figure 4. In a preliminary assignment analysis, the probability that individual Dargo River seedlings originate from within the Dargo River catchment or from one of the four possible source catchments is indicated by the color. This preliminary analysis suggests that Morses Creek and the Ovens River are the primary external genetic contributors to the Dargo River seedlings.

The level of genetic discrimination among the potential source populations is a more critical issue. The source populations must be genetically differentiated if assignment testing is to be applied successfully. The ideal situation occurs when each population carries genetic markers that are unambiguously unique to that population (private alleles). If each population has a unique maker then assignment testing is absolute rather than probabilistic. (If a seedling has Marker A and Marker A is only found in Population Z then population Z must be the source of the seedling.)

More commonly, populations are differentiated by unique frequencies of shared markers rather than unique markers. In this more typical situation, assignment testing nominates the most likely source of genotypes by examining multiple markers. (If a seedling has Markers A, B, and D, and those markers occur at high frequency in only one potential source population then that population is the most likely source of the seedling.) The more markers that are examined the greater the level of discrimination and statistical surety – that is the more power the technique has to unambiguously 'assign' seedlings to sources. The number of markers that are required depends on the level and pattern of genetic discrimination among the potential source populations.

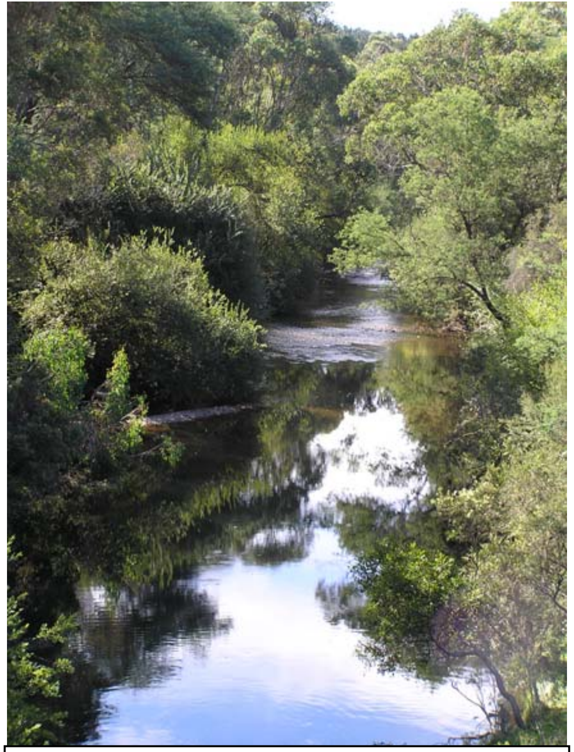

Figure 5. Buffalo River (Victoria, Australia)

Fundamentally then the ability to apply assignment testing successfully to the questions posed in the project brief will depend on the level of genetic diversity among the *S. cinerea* populations naturalised in eastern Gippsland and, more importantly, how this genetic diversity is structured among populations. If the genetic discrimination among populations is high then robust assignments will be possible with a small number of markers. Intermediate levels of population discrimination will require more markers. If there is little population discrimination (few markers are polymorphic or marker frequencies are similar in all populations) then assignment testing may not be feasible. For this reason, pilot studies as described in this project brief are absolutely critical before committing to large scale projects.

There are no publicly available data on the levels and distribution of genetic diversity among naturalised willow populations in Australia. Therefore the first step is to undertake a small pilot project to ascertain the technical and analytical feasibility of using assignment testing to track willow immigration across catchments in eastern Gippsland. The task is to evaluate whether there are sufficient levels of molecular genetic differentiation among putative source populations relative to a designated recipient catchment to permit statistically rigorous identification of immigrant seedlings over scales that are useful to inform on-ground willow management activities.