SUMMARY OF THE FINAL REPORT FOR THE AUSTRALIAN GOVERNMENT DEPARTMENT OF THE ENVIRONMENT AND HERITAGE

FEASIBILITY STUDY FOR GENETIC CONTROL OF CAULERPA IN SA AND NSW

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Executive Summary

Invasive populations of *C. taxifolia* remain present in South Australia and NSW, and are likely to eventually be found in Victoria and WA. Currently available approaches to controlling the plant (principally altering salinity in the field) are expensive and not easily applied in many situations. We undertook to determine the feasibility of using modern genetic approaches to developing a species-specific biocide for use against *C. taxifolia*. Following discussions with plant geneticists, we developed, tested and experimentally proved the effectiveness of a prototype biocide that was based on an enzyme critical for photosynthesis.

Comparison of genetic and amino acid sequence information for *C. taxifolia* and other plants suggests that the biocide could be customized to be species-specific, though this was not attempted in this pilot study. Public consultation indicated that if the toxin was indeed species-specific and could be delivered in such a way that it affected only the targeted area, it would be publicly acceptable and used by managers. We conclude that a species-specific biocide for *C. taxifolia* is feasible and would be used. We recommend that the next stage of biocide development be undertaken, and that it focus on three areas:

- Identification and experimental validation of a biocide that disrupts a process other than photosynthesis. We have identified an osmoregulatory enzyme as a likely candidate.
- Experimental demonstration of genus or species specificity of the biocide.
- Development of a biocide delivery system that is practical in the field, and in particular, delivery in an "Osmocote"-like pelletized form.

The next stage of biocide development should also include further consultation with the public and coastal managers, a formal hazard and risk analysis of the approach, and an exploration of regulatory issues that need to be addressed before field trials could commence. We estimate this work would take about 2 years to complete, at a cost of about \$250,000 per year. If successful, it would be followed by field trials.

1 SUMMARY AND RECOMMENDATIONS

1.1 Output assessed against specified project deliverables

1.1.1 The development of a prototype approach for delivering toxins into *C.taxifolia*.

A number of delivery approaches were tested in the laboratory, which ranged from direct injection of the prototype toxin using micro-syringes through to biolistics and direct up-take of water-soluble toxin. Biolistics and, eventually, direct injection proved effective. There is no indication to date that the prototype toxin is effective against *C. taxifolia* when and if taken up through the "roots" or stolon in water-soluble form. It is highly likely, however, that a water-soluble form that is taken up directly could be developed, given *C. taxifolia*'s demonstrated affinity for many water soluble organic compounds.

1.1.2 The compilation of a list of candidate physiological processes that can be targeted for inhibition.

A short-list of physiological processes that could be targeted for disruption was developed in consultation with scientists from CSIRO Plant Industry. Two processes in particular looked most promising – osmoregulation and photosynthesis. Suitable target genes for both processes were determined, and a prototype toxin developed and tested based on inhibition of a critical photosynthetic enzyme (phytoene desaturase – PDS). Experimental work demonstrated that a prototype toxin based on inhibiting PDS was effective against small plants, but not against larger ones, apparently due to the process mimicking natural frond loss in the plant. The results suggest strongly that a toxin based on inhibiting another process, such as osmoregulation, would be effective against an entire plant.

1.1.3 Determine likely key stakeholder acceptability of gene technology approach to controlling *C. taxifolia*.

Discussions with key stakeholders were held both in small groups and as part of the national *C. taxifolia* workshop held in Sydney in April 2004. Further discussions will be held as part of the 2004 AMSA conference, in July. Feedback to date has been broadly positive, particularly once it was understood that the biocide approach is not GM technology. The biocide would be most acceptable if it could be made species-specific and if the effect of the biocide was constrained to the immediate area of application. These concerns are consistent

with the results of our previous public consultations on approaches to managing invasive marine organisms (see Thresher & Kuris, in press). The next stages of this work should focus on testing species-specificity of the toxin (most likely one based on inhibition of osmoregulation) and developing a delivery mechanism that is effective for direct application.

1.1.4 Determine feasibility of the technology, options, time scales and resources for future development and application.

The experimental work undertaken using the prototype toxin indicates that developing a practical toxin based on inhibition of a critical physiological process is feasible. The comparison of genetic and amino acid sequences for the targeted enzyme (phytoene desaturase) indicates that at least genus-specificity is highly likely, and species-specificity probable. This would have to be confirmed once a final targeted gene was determined. Public consultation suggests that if the toxin was specific and could be limited in impact to the area targeted, it would be publicly acceptable, and used.

Options and resourcing for future work is considered below.

1.2 Options, Time Lines and Resourcing of Future Work

1.2.1 Invasive populations of *C. taxifolia* remain present in both South Australia and NSW. They are likely to eventually be found in Victoria and WA, as invasive strains of the plant are still available in the aquarium trade. The current approaches to control the plant are not easily applied to incursions in deep water and exposed environments. Even the approach currently used by NSW FRI to control shallow water incursions (burying them in salt) would be extremely expensive if applied to all known invasive populations of the plant. Essentially the same conclusions have been drawn regarding currently available options used or attempted overseas. Therefore, we conclude that there remains an urgent need for an effective and cost-effective means of eradicating invasive populations of *C. taxifolia*. Development of a species-specific biocide appears to be one of the few practical options likely to be successful.

- 1.2.2 The next stages of the development of the biocide should focus on three areas.
 - Identification and experimental validation of a biocide that kills entire plants, by targeting for inhibition a process other than photosynthesis. We have identified an osmoregulatory enzyme as a likely candidate.
 - Experimental demonstration of the degree of genus or species specificity of the biocide that can be achieved.
 - Development of a biocide delivery system that is practical in the field. Based on the facility with which the roots and stolon of *C. taxifolia* take up classes of water soluble organic substances from sediments and water, it is highly likely that an effective formulation of the biocide could be developed that could be delivered in an "Osmocote"-like pellet form. Pellets of the biocide could be sprinkled from a boat or by a diver over a patch of the plants, leading to the slow death of the patch. Alternative approaches, such as delivery of the toxin by means of a diver-held biolistics gun, are technically feasible and should also be explored, but are likely to be more expensive and less cost-effective than pellets when applied to larger patches.
- 1.2.3 This work will take about 2 years to complete, if suitably funded, following which field trials could commence. The technically most challenging component of the work is developing a pellet-able, water-soluble delivery system. We recommend that this work be undertaken by someone at the post-doctoral level, who could work across the disciplines of plant genetics and organic chemistry.
- 1.2.4 We estimate that this work would cost about \$250,000 per year for two years. Subsequent field trials would cost less than \$50,000 per year for 1-2 years, depending in part on the cost of producing suitable supplies of a pelletized toxin and the costs of getting regulatory approval for field trials. These costs should be explored during the next stage of the project.
- 1.2.5 During this stage, further public consultation should take place, and the biocide approach should be subjected to a rigorous hazard and risk analysis.

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