
Molecular Markers for Monitoring Mycoherbicides

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The indigenous fungus *Chondrostereum purpureum* is a promising biological control agent for forest weeds in reforestation sites and utility company rights-of-way. Applied as a stump treatment, *C. purpureum* suppresses vegetative resprouting of several deciduous tree species. To support the registration of this biocontrol, we are investigating several aspects of this system, including: the population diversity and dynamics of the fungus, the environmental fate and persistence of specific biocontrol isolates, the progression of biocontrol by monitoring infection within treated stumps, and the genetic stability of the formulated biocontrol product. Molecular genetic markers have been developed to discriminate *C. purpureum* from other fungi and to identify specific applied isolates. A unique genetic marker has been developed from a sequence-characterized amplified region (SCAR) of the *C. purpureum* rDNA intergenic spacer (IGS) that is species-specific. Another SCAR primer set was designed which amplifies repetitive polymorphic DNA from *C. purpureum*, allowing individual genotypes to be distinguished. These PCR markers may be used to detect and identify biocontrol isolates of *C. purpureum*. Our investigations of the nature of the repeated marker and specific examples of applications of genetic markers to the estimation of infection frequency and environmental fate of *C. purpureum* in field trials will be discussed.

Molecular Characterisation of *Phloeospora mimosae-pigrae* Isolates from Different Locations in Central and South America

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In 1998 five new isolates of *Phloeospora mimosae-pigrae* were imported into Australia from Ecuador, Venezuela, Cuba, and Brazil to assess their potential for biological control of *Mimosa pigra*. The genetic relatedness of the five new isolates and the original introduced isolate from Mexico was studied at the molecular level. Different extrac-

tion methods were tested to identify a reliable method for the extraction of DNA from mycelium of *P. mimosae-pigrae*. Fungal DNA was successfully extracted (Drenth & Govers, 1994). The primers ITS5 (White *et al.*, 1990) and P3 (Kusaba & Tsuge, 1995) were used to amplify the nuclear rDNA region containing the two internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA of the *P. mimosae-pigrae* isolates in the polymerase chain reaction (PCR) assay. The PCR was performed in 50 μ l reactions. Three to five μ l of the PCR assay was then subjected to preparative electrophoresis in 2% agarose gels. The amplification yielded a single visible DNA product. Restriction Fragment Length Polymorphism (RFLP) analysis was used to distinguish the different isolates amplified in the PCR assay. The isolates gave different patterns when the PCR product was digested with the restriction enzyme *Mse I*. In order to further analyse differences between isolates, the ribosomal region amplified in the PCR is being sequenced. Nursery trials have commenced to compare the morphology and germination potential of the isolates and to determine whether these biological characteristics can be linked to genetic markers detected at the molecular level.

Post-Release Spread of Musk Thistle Rust Monitored from Virginia to California Using DNA Sequence Information

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The rust pathogen *Puccinia carduorum*, originally collected in Turkey in 1978, was studied in Virginia for control of musk thistle (*Carduus thoermeri*) in a series of field experiments from 1987-90. The pathogen has been spreading by natural dispersal mechanisms across the U.S. on musk thistle since the studies. This spread had previously been confirmed as far west as Wyoming, as determined by DNA sequence analysis of ribosomal Internal Transcribed Spacer 2 (ITS2) DNA sequences from *P. carduorum* urediniospore samples. A region of sequence identity in the ITS2 of *P. carduorum* permits us to distinguish the pathogen from a morphologically similar strain of *P. carduorum* which is indigenous to California, but only pathogenic on the closely related slenderflower thistle (*C. tenuiflorus*). Teliospores of *P. carduorum* collected from musk thistle at a site near Mt. Shasta, California, in 1998 were found to contain the same ITS2 sequence as that from the foreign isolate originally studied in Virginia, confirming the spread of the pathogen across the entire U.S.

The Use of Molecular Techniques in the Classical Biological Control Programme against an Invasive *Ligustrum* Species in La Réunion

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An introduced species of *Ligustrum* has recently become a major threat to forest ecosystems in the Indian Ocean island of La Réunion. It arrived from Mauritius where this exotic species had already had a serious impact on the remnant native vegetation. Molecular techniques were utilized alongside traditional techniques and historical research to elucidate its taxonomy and determine its exact area of origin. Dried leaf samples were collected during natural enemy surveys in Sri Lanka and India (the Western Ghats, Assam and Meghalaya) and subjected to RFLP and RAPD analysis. RFLP studies were employed to compare samples from India with those from Sri Lanka and the results showed that there were distinct differences and that the samples from the population introduced into La Réunion matched exactly those from Sri Lanka. This confirmed an earlier identification of the weed as *Ligustrum robustum* subsp. *walkeri* or Ceylon privet. RAPD analysis revealed that the levels of variation in both Sri Lankan and La Réunion populations were similar and suggested that more than one introduction had been made. These results are supported by comparisons between the natural enemies collected from each region. Literature searches suggest that the likely source of the introduction was an exchange of plant material between botanical gardens during the colonial era. As a result, collection efforts were concentrated in Sri Lanka. However, a paucity of co-evolved specialist natural enemies suggests that this subspecies may have been isolated from the original centre of diversity of *L. robustum* (and of the genus) in Eastern Asia at an early stage. Thus, a potential new encounter from the mainland could hold the key to the success of the classical biocontrol programme against this weed. Further surveys in S. China and Vietnam are planned which may reveal potential biocontrol agents not only for *L. robustum* but also for other invasive *Ligustrum* species.

