

Environmental Fate Studies Relating to the Use of *Chondrostereum purpureum* as a Bioherbicide

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Abstract

Use of the pathogenic fungus *Chondrostereum purpureum* (Pers. Ex Fr.) Pouzar as a natural means of controlling vegetative regrowth from stumps of native hardwoods is receiving increased attention in Canada (Gosselin 1996, Dumal *et al.* 1997, Shamoun and Hintz 1998). The technique involves treating freshly cut stumps with the mycelium of indigenous strains of this ubiquitous fungus. However, the deployment of fungal plant pathogens with wide host-ranges may have certain undesirable environmental side effects. These negative impacts may be linked, among others, to the introduction of new genetic material to local genepools and to increased post-treatment spore load. In addressing both of these topics, we assessed: i) the extent of genetic variability among Canadian populations of *C. purpureum* both within and between different ecozones, and ii) the possible post-treatment effect of an increased spore load on surrounding vegetation.

To study the first point, a Québec collection of 43 isolates of *C. purpureum*, from 14 different host species in 3 forest zones, and 92 isolates sampled from four Canadian geographical regions were analyzed using random amplified polymorphic DNA (RAPD) (Williams *et al.* 1990). For the latter collection, samples were grouped into four populations each corresponding to a Canadian ecozone (ecozones 1, 2, 4 and 5) as defined in the Canadian registration guidelines for microbial pest control agents (Anonymous 1998). This grouping is based on geological and ecological characteristics. For the Québec population, RAPD profiles of all primer-template combinations (7 primers) were visually compared. Variability was initially assessed within and between different collection sites, for samples from a given host species. The genetic variation among samples isolated from different host species was then assessed, regardless of collection site. For Canadian populations, 22 polymorphic amplicons generated by 4 primers were analysed. Genetic diversity estimates (Nei 1973) and an analysis of molecular variance (AMOVA) (Huff *et al.* 1994) were used to investigate population structure.

Finally, the occurrence of *C. purpureum* sporophores was assessed along eight 1.6km transects radiating from 4 treatment sites. Transects had sampling plots at 200m or 400m intervals. Over a 3-year period, over 10,000 trees, including paper birch, the most susceptible species, were surveyed. In addition, the presence of the fungus was examined in tissue samples from 220 stumps trees (paper birch, aspen and pin cherry) cut when environmental conditions were favorable for spore release from treatment sites. RAPD markers diagnostic of the deployed strains were also developed to determine if detected infections were attributable to the treatment (Gosselin 1999).

C. purpureum was found to be a highly heterogeneous pathogen with a continuously distributed population across Canada ($G_{St} = 0.048$). A maximum of 0.3% of nontarget

trees examined bore *C. purpureum* sporophores; a level corresponding to natural occurrence of the fungus. All sporophores were associated with either injured or recently killed trees, or with fresh stumps. A higher mean level of disease (15%) occurred on the 220 stumps sampled, infection levels varied from 0 to 45% between plots. However, RAPD analysis showed that over 85% of the infections detected were attributable to naturally occurring inoculum while less than 2.3% of the stumps contained deployed strains. The occurrence of infection was not related to host species or to distance from treated site.

These results underscore the importance of considering the population structure in the process of its registration and provide further knowledge to ascertain that the deployment of *C. purpureum* as a bioherbicide may provide considerable benefits with minimal risks to the environment.

Acknowledgments

The authors gratefully acknowledge the funding provided by Hydro-Québec.

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