CHESTNUT BLIGHT BIOLOGICAL CONTROL: A TRANSGENIC MICROBIAL APPROACH

William L.MacDonald[1], Mark L. Double[1] and Donald L.Nuss[2]
[1]West Virginia University, Morgantown, WV; [2]University of Maryland Biotechnology Institute, Rockville, MD
Contact Person: macd@wvu.edu

The early 20th century chestnut blight epidemic in North America demonstrated how destructive an invasive species can be to a susceptible host population. The result was the loss of what is estimated to be several billion American chestnut trees. Scientists of the early1900s determined the causal agent was a fungus, *Cryphonectria parasitica*, and declared there was no way to stop its invasion. This organism, a bark pathogen of Asian origin, initiates infections at wound sites. The invading fungus grows through healthy bark tissue eventually girdling the stem so that all tissues beyond the infection die. Fortunately, the American chestnut has survived by producing sprouts from root systems that have remained alive at sites that once supported remarkable stands of American chestnut. The pathogen also has survived on this population of sprouts.

In the early1930s, the same disease was discovered on European chestnuts growing in Italy. Unlike the North American epidemic, some trees survived the disease and rather than being cankered by lethal strains of the fungus the infections were swollen, more superficial and not lethal. When grown in culture strains that produced these infections had reduced pigmentation and sporulation, and resulted in small, non-lethal infections when introduced to healthy chestnut bark. A cytoplasmically-borne, double-stranded (ds)RNA that is transmissible via hyphal anastomosis has since been identified as the agent responsible for these altered traits. Strains so infected have been termed hypovirulent and the membrane-bounded dsRNAs that infect them, hypoviruses. Several dsRNA's since have been identified and characterized including ones that are associated with the recovery of American chestnut in Michigan a situation similar to what has occurred in Europe.

The phenomenon of transmissible hypovirulence has been an appealing option for disease management since its discovery. Molecular technologies now have permitted the cloning and sequence determination of several dsRNA's that have been associated with hypovirulent strains. This in turn has allowed the development of full-length infectious cDNA clones. Remarkably, transformation of virulent strains by the infectious clones produces the complete hypovirulent phenotype and also launches a cytoplasmically replicating dsRNA. Not only do these transfected strains act like the naturally occurring hypovirulent strains but they also are capable of transmitting the genetic elements responsible for hypovirulence via the sexual reproductive cycle. Because significant levels of biological control have not occurred either naturally or when hypovirulent strains have been introduced, the approach of utilizing transgenic strains appears to offer significant benefits for biological control. With naturally occurring hypovirulent strains, the formation of viable anastomoses that permit the transmission of dsRNA's is limited to strains that are closely related in their vegetative compatibility. One benefit of transgenic strains is their ability to transmit the integrated cDNA into sexual spores; this distributes the cDNA to a variety of vegetative compatibility types. There are additional benefits relative to inoculum production. Nearly all asexual spores of transgenic strains are hypovirus carriers In contrast to some naturally occurring hypovirulent strains that produce low populations of hypovirus-laden asexual spores. Similarly, when crosses between transgenic and wild-type strains occur, one half of the sexual spores carry hypovirus, a feature that does not exist when cytoplasmically-borne and wild-type strains mate.

Most research on microbial biopesticides relative to fungi focuses on the concepts of competition or antagonism where one organism is used to control another. The potential usefulness of technologies involving transgenes, that alter the virulence of economically damaging microorganisms, largely remains an untested yet potentially a useful technology. Certainly the risk of using such technologies needs to be assessed for each microorganism and its infectious agent. However, transgenes are an integral part of their host's genetics and thus the risk they pose of escape into the environment requires consideration. Clearly, any organism that benefits competitively from genetic alteration is of concern and those risks need to be quantified as best possible. With the chestnut blight/hypovirulence system, the benefits derived by enhancing the spread of detrimental hypoviruses among an exotic fungal pathogen would appear to outweigh any measurable risk.