

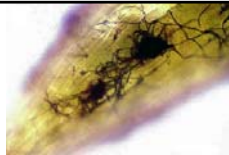
Enhanced resistance to fungal pathogens in forest trees

By Chris Larkin, Wally Wilson, Tyler Lippold and Cara Ganders

Overview

- What are the fungal pathogens and the host tree's natural adaptation for defense?
- The process of transgenic methods
- Results from this paper
- Questions and concerns that arise from this paper
 - Micorrhizal relationships
 - Other questions and concerns

The Paper



“Enhanced resistance to fungal pathogens in forest trees by genetic transformation of black spruce and hybrid poplar with a *Trichoderma harzianum* endochitinase gene”

By Andree Noel, Caroline Levasseur, Van Quy Le and Armand Seguin

Authors

- Andree Noel: Regional Commissioner of Quebec
- Caroline Levasseur: Natural Resources Canada; Biologist, Genetic Engineering of Forest Trees



Andree Noel

Authors

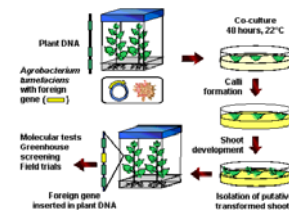
- Van Quy Le: Laval University
- Dr. Armand Seguin: Canadian Forest Service; molecular biologist



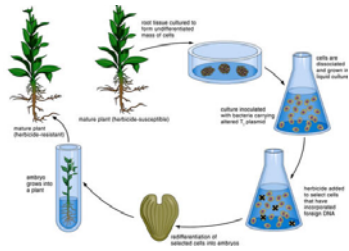
Armand Seguin

Methods

- Transformation: the addition of one or more genes to a plant's genome
- Agrobacterium-mediated transformation: transfer of DNA between bacteria and plants

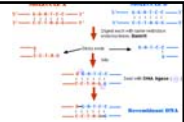


Methods Continued



Transgenic Plants: genetically engineered or modified plants

Methods continued



- Recombinant DNA: artificial DNA sequence resulting from combining two other DNA sequences in a plasmid
- Plasmid: circular, double stranded DNA molecules found in some bacteria that are capable of autonomous replication

The Research

- Inserted the endochitinase gene *ench42* from *Trichoderma harzianum* in black spruce (*Picea mariana*) and hybrid poplar (*Populus nigra* X *Populus maximowiczii*)
- Then inoculated both species with *Melampsora medusae* (poplar) and *Cylindrocladium floridanum* (spruce) and measured the resistance of each genetically modified species

Fungal Pathogens affecting spruce and poplars

- *Cylindrocladium floridanum*
(spruce root rot)



- *Melampsora medusae*
(poplar leaf rust)



Cylindrocladium floridanum

- Pathogen cycle:
 - fungal disease parasitizes at the root level
 - Tons of spores produced on infected plant parts - white powder
 - survives and over-winters in plant tissue and infested soil
 - resides in contaminated potting soil, plant material, hands, plant tools
 - once seedling roots come into contact, fungus germinates and infection occurs (Cordell et al 1989)
 - also high humidity & rainfall susceptibility to airborne spores (Leahy, 1994)
- Affects:
 - conifer and hardwood seedlings (E. white pines, black spruce, walnut, dogwoods) in tree nurseries and in forests

Cylindrocladium floridanum



Melampsora medusae

- Pathogen cycle:
 - Fungus requires 2 hosts to complete life cycle
 - Over-winters on fallen leaves and infects alternate host in spring
 - Summer is reproductive phase of fungus
 - Under and upper side of leaves have orange pustules
 - repeated heavy infection can predispose trees to damage by insects and other fungi (Forest Pests, 2004)
- Affects:
 - *P. tremuloides*, cottonwoods, many hybrid poplars (hosts)
 - Pseudotsuga mensenzii*, *Larix* spp., *Pinus* spp. (alternate hosts)

Melampsora medusae



<http://www.glf.cfs.nrcan.gc.ca/treedisease/conifer>

What about tree adaptation to these fungal pathogens?

- **ENTER:** *Trichoderma harzianum* fungus
- Natural part of the microflora of all soils and is effective at competing for nutrients, but especially effective at producing toxins against harmful pathogens (www.ncjpm.org 1998)
- *T. harzianum* is a naturally recruited microbe to fight against these pathogens, just not strong enough for the magnitude of the pathogens, such as root rot and leaf rust (Lavesseur et. al 2005)



“
Higher plants contain multiple constitutively expressed proteins for defense against infection by viruses, bacteria, and fungi. Chitinase is an antifungal agent.

”
NIST Schema Database

Chitinase (Endochitinase)

- Enzymes are complexes of proteins.
- Most all proteins can be broken down through hydrolysis, or hydrolyzed.
- Chitin is an integral component of fungal cell walls.
- Chitinase is a catalyst enzyme that hydrolyzes chitin – it (essentially) is a wrecking ball made specifically to tear apart chitin – the benefits are countless.

Natural Adaptation for Pathogen Defense

- *Trichoderma harzianum* has natural plant disease controlling abilities:
 - Fungi grows tropically toward hyphae of other fungi
 - Coils around plant pathogen
 - Degrade cell walls of target pathogen by enzyme secretion causing limited growth and activity of disease (Chet et al 2006)
 - **NOTE:** This is not a chance occurrence. *Trichoderma harzianum* recognizes signals from the host fungus

Conventional Controls for Disease

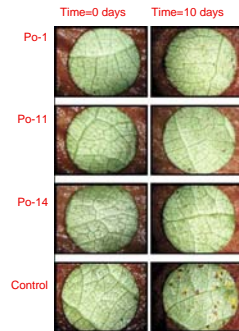
- Nurseries – strains of *Trichoderma harizanum* fungus and other chemical used in fungicide sprays
- Forests stands - no control for these pathogens are currently used (primaryindustry.nt.gov.au 1999)



<http://www.aponet.org/education/LessonsPlantPath/blackknottextfig17.htm>

The Results

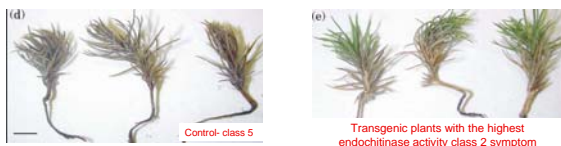
Poplar



- Compared to the control, the transgenic poplars had a reduction of *Melampsora medusae* symptoms (necrotic spots and uredial pustules)
- However, did not perform test on entire plant and article admits a need for this kind of research

The Results

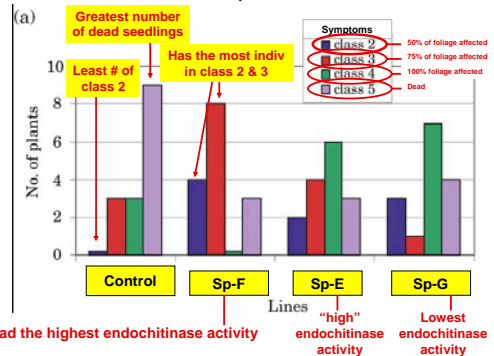
Spruce



- The plants with the highest endochitinase activity had the most resistance
- However, the affect was not as pronounced with plants with a lower level of endochitinase activity
- May be due to the fact that the in vitro testing may have accelerated disease development with high humidity levels

The Results (cont'd)

Spruce



Main purpose of the research



To find an effective way to prevent disease in forests and tree plantations

Mycorrhiza and their friends...

The unanswered question:

Since chitinase does destroy fungal cell walls, what would it do to the cell walls of mycorrhiza that are in a symbiotic relationship with a stand of transgenic spruce?"

The foodweb from the roots, upward...

- Mycorrhiza also protect tree roots from pathogens.
- Mycorrhizal hyphae do fix certain nutrients so that trees can make use of those nutrients.
- A transgenic, endochitinase-enhanced spruce tree will inadvertently (and effectively) defend itself from **all** mycorrhiza.

– *Is this a tree we want to put into our forests?*

Questions to ponder

- Logistically how would transgenic trees be introduced into natural forests (as opposed to nurseries)?
- What are the effects of the largest scope of the ecosystem?
- Genetic diversity?
- What about ethical and environmental dangers of genetic manipulation?



Plants adapt to survive within the confines of their environment; this is the foundation of genetic diversity.

Transgenic plants **can** force their environment to adapt to them...

Sources:

Gebeyaw, Mesfin, Matthew Denton, et al. Transgenic Alfalfa Secretes a Fungal Endochitinase Protein to the Rhizosphere. [USDA Agricultural Research Service](http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=154891). Retrieved May 06, 2006 from the World Wide Web: http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=154891

Larcher, W. (2003). *Physiological Plant Ecology – Ecophysiology and Stress Physiology of Functional Groups* (4th ed.). New York: Springer.

Macromolecule – endochitinase (M0W0). (1998). [NIST Schema Database](http://xpdn.nist.gov:8080/cqi-bin/query/bmcd/schema/molecule?MO_ID=M0W0). Retrieved May 06, 2006 from the World Wide Web: http://xpdn.nist.gov:8080/cqi-bin/query/bmcd/schema/molecule?MO_ID=M0W0