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GEORGIA PULP AND PAPER CONSORTIUM

FY97 FINAL REPORT

Project Title: Creation of Fusiform Rust Resistant Pines: Identification of Pine Genes Activated by Fusiform Rust Infection

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Summary Of Completed Work

Fusiform rust is a serious disease problem in Georgia forests; the U.S. Forest Service credits millions of dollars in losses to fusiform rust annually. The disease is caused by a fungus, *Cronartium quercuum* f. sp. *fusiforme*, and is characterized by the formation of galls (swellings) on the stems and branches of loblolly and slash pines. The long-term goal of this project is to harness pine genes activated by fusiform rust infection to create rust resistant pine trees. Our strategy is to first identify pine genes that are induced by *C. q. fusiforme* infection. The promoters controlling expression of these genes will then be attached to a gene encoding a cytotoxin (e.g. barnase, an extracellular RNA-degrading enzyme) and inserted into rust susceptible pine trees. It is hypothesized that when such transgenic trees are infected by *C. q. fusiforme*, the cytotoxin will be produced at the site of infection, neighboring pine cells will be killed, and the establishment of a fusiform rust gall will be blocked. The project funded by the GPPC during FY97 focused on the first step required for successful implementation of this strategy: the identification of pine genes induced by fusiform rust infection. This report covers progress made towards this goal and compares proposed expenditures to actual expenditures.

In our assays we compare gene transcription in galled tissue to gene transcription in ungalled tissue from the same tree (i.e. "asymptomatic" tissue). Gene transcription in galled and asymptomatic tissues is also compared to gene transcription in a second, healthy individual (i.e. a tree that has no galls). This year we identified 25 cDNA fragments that are differentially transcribed in at least 3 different sets of RNA samples. Table 1 summarizes the patterns of transcription displayed by these 25 cDNA fragments.

Table 1. Number of cDNAs Displaying Different Patterns of Transcription in Galled, Asymptomatic, and Healthy Pine Tissue

G/AH[a]	AH/G	GH/A	A/GH	G/A	GA/H
17	3	2	1	1	1

[a]The letters to the left of the slash indicate the type(s) of tissue in which the cDNA is present, while those to the right indicate the type(s) of tissue in which it is not present (G = galled, A = asymptomatic, and H = healthy). For example, "G/A" indicates that the cDNA is consistently present in G and absent in A, but its presence in H is variable.

Twelve of the 25 differentially transcribed cDNAs have been cloned and their nucleotide sequences determined. Table 2 lists the sizes of these clones and their homology (if any) to other nucleotide or protein sequences in the GenBank database. The first six clones listed in Table 2 will be analyzed further to determine their genome of origin. The last six clones in Table 2 are judged to not be worthy of further analysis either due to their apparent fungal origin or very short length.

Table 2. Cloned cDNAs -Their Size and Sequence Homologies

Clone	Transcription[a]	Size (bp)[b]	Database Homology
pDD1	AH/G	253	plant receptor-like kinase
pDD3	G/AH	273	none
pDD10	G/AH	166	none
pDD55	AH/G	139	none
pDD62	G/AH	186	none
pDD63	G/AH	167	plant heat shock protein
pDD2	G/AH	122	fungal 18s ribosomal RNA
pDD4	G/AH	408	fungal 5.8s ribosomal RNA
pDD53	G/AH	94	none
pDD56	G/AH	127	fungal 26s ribosomal RNA
pDD57	G/AH	56	none
pDD58	G/AH	56	none

[a]The letters to the left of the slash indicate the type(s) of tissue in which the cDNA is present, while those to the right indicate the type(s) of tissue in which it is not present (G = galled, A = asymptomatic, and H = healthy).

[b]bp = base pairs

Current work focuses on screening for additional differentially transcribed cDNAs, cloning and sequencing the 13 cDNAs known to be differentially transcribed, and determining the genome of origin of already cloned cDNAs. It is expected that promoters for some of the pine-derived cDNAs will be cloned during FY98.

Comparison of Proposed and Actual Budget Expenditures FY97

Budget Breakdown	Proposed Expenditures	Actual Expenditures
Operating Expenses		
Salaries and wages	28,256	23,696*
Fringe Benefits (28%)		
Supplies	15,000	19,592*
Services		
Travel	2,000	1,668
Overhead		
Equipment Operation		
Capital Expenditures		
Equipment	5,000	5,350
Facilities		
TOTAL	50,256	50,306

*Two months into this fiscal year, a research technician was hired in place of Dr. M. Steven Doggett. The salary savings were redirected and spent on supplies.

FY 1997 Final Progress Report

submitted to:

The Georgia Consortium for Technological Competitiveness in Pulp and Paper

1. Project Title

The Effects of Intensive Management on Carbon Allocation in Short-Rotation Forests

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3. Executive Summary

Objective #1: Quantify the effects of increased soil moisture and nutrient availability on fine root demography.

Final data analyses are still in progress, but we can say with some certainty that:

1. Within the control plots, the shallow fine root system is more dense than within any of the other treatments. This may be indicative of relative shifts in tree growth away from root production with the addition of fertilizer and irrigation, but we cannot yet definitively answer as to whether or not such growth may be redirected to the shoot.

2. There have been no obvious effects of irrigation, either with or without fertilizer, relative to the non-irrigated treatments. Heavy rains during most of the 1995 growing season are likely responsible for the apparent failure of irrigation too affect root productivity or dynamics.

3. Within the upper 30 cm of the soil, very fine (0.0 - 0.5 mm diameter) root length densities in irrigated plots with 1x fertilizer not significantly different than length densities in plots receiving 2x fertilizer (6382 vs. 6250 m m^2). However, very fine root biomass (<0.50 mm) is significantly greater in the 1x treatment plots (2220 vs. 1630 kg ha^{-1}). This is due primarily to higher specific root lengths (m root length per gram root mass) in the 2x fertilizer roots (3232 vs. 4013 m g^{-1}) . The biomass of larger (0.5 - 3.0 mm) roots under the 2x treatment was significantly greater than biomass in the 1x treatment in December 1995 (1860 vs. 2150 kg ha^{-1}), but not September 1996 (1930 vs. 2090 kg ha^{-1}). Coarse (3.0 - 10.0 mm) biomass did not differ between treatments at any time. The data suggest that trees are investing more carbon to short-lived fine roots in the 1x plots relative to the 2x plots. The higher specific root lengths in the 2x treatment indicate that the fine roots are on average longer and thinner than in the 1x treatment, a common response of roots to high levels of available nutrients, especially N.

Objective #2: Quantify the effects of increased soil moisture and nutrient availability on pre-coppice levels of stored labile carbon and nutrient reserves.

Our analyses of carbohydrates within the fine and coarse roots of sweetgum after one and two years of treatment show that:

1. Concentrations of total nonstructural carbohydrates (TNC) range from 5 or 6 % in the finest (<1.0 mm) roots to as much as 65% in the 3.0 - 10.0 mm roots. TNC concentrations are generally greatest in the high fertility treatment, but the differences are not significant for any of the diameter classes.

2. Soluble nonstructural carbon (SNC, or sugar) concentrations different little among treatments for all root diameter classes. The values range from 16% in the very fine roots in the 2x treatment, to less than 3% for all other size - treatment combinations.

3. For all size classes, starch concentrations were greatest in the high fertility treatment, but in no cases were the differences statistically different.

Overall, our results to date suggest that irrigation and fertilizer may be reducing very fine (<0.50 mm) root production in the high fertility treatments, in which the roots are also longer and thinner. The latter phenomenon is common in high fertility environments, and is believed to represent a morphological response designed in part to optimize uptake. Starch and TNC generally appear to be accumulating faster in the high fertility roots, perhaps because of a lack of sink strength in the apparently less-dynamics fine roots in this treatment, However, the treatment differences are not statistically significant. The extent to which lower carbohydrate concentrations in the 1x treatment may affect coppice regrowth are still unclear, and await further determinations of root productivity and turnover.

4. Deliverables

Major Milestones	Original Proposal	Actual
Monthly minirhizotron image. collection and digitization	7/1/96 - 6/30/97	7/1/96 - 6/30/97
Fine root biomass and carbohydrate sampling.	Pre-budbreak, summer, post-leaf fall and mid-winter	Pre-budbreak, summer mid-winter
Carbohydrate analyses	Immediate post-sampling	Immediate post-sampling
Summarize 2 year's minirhizotron data.	12/30/96 - 6/30/97	12/30/96 - Present
Manuscript submissions of year 1&2 minirhizotron and carbohydrate data.	4/30/97 - 6/30/97	In preparation
MS Thesis completed (Mr. Jeff Price)	6/30/97	10/31/97
Oral/poster presentation at scientific or trade meeting.	Spring/Sum 1997	Summer 1996, Spring 1997

5. Budget

State Funds	Total FY 1997	12 Month Expended
	$31,500	$31,500
Matching Funds	Original Proposal	Actual
	15,750	$14,000 (to date)