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Research on the biology of fusiform rust in the southeastern United States

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Summary

The incidence of fusiform rust throughout the southeastern United States has continued to be one of the major forest disease problems in the southeastern United States. In the past, much of the research has concentrated on field studies with provenance selection and genetic breeding of pine families to increase resistance in the host. In the last ten years, there has been an increased interest in the actual biology of the fusiform rust fungus. Three areas of research on-going in the south are overlapping the areas of molecular, cellular, and population biology. In order to control the disease, a combination of host genetics will be valuable, but so will a biological understanding of the survival mechanisms and ecology of *Cronartium* species. In the last decade, research has started in the southeastern United States with collaborations between federal and state agencies concentrated on the biology of fusiform rust.

Key words: *Cronartium quercuum* f. sp. *fusiforme*, *Pinus elliottii*, *Pinus taeda*

1 Introduction

Fusiform rust incidence has increased over time with the intensive culture of loblolly (*Pinus elliottii* Engelm. var. *elliottii*) and slash (*P. taeda* L.) pines. At present, the annual losses to fusiform rust have been estimated at $35 million in the five southern states of Florida, Georgia, South Carolina, North Carolina, and Virginia, with significant losses occurring in Alabama, Mississippi, and east Texas as well (Schmidt 1998). The incidence of fusiform rust from the causal fungal pathogen *Cronartium quercuum* (Berk.) Miy. ex Shirai f. sp. *fusiforme* has increased as land use patterns have changed in the southeastern United States (Eye et al. 1997). Former agricultural lands used for the production of crops, such as cotton and tobacco, have been increasingly planted for forestry crops since the turn of the century. Current estimates of slash and loblolly pine acreage in the southern United States is 47.9 million acres (Starkey *et al.* 1997). Although a recent study of forest inventory data for four states (Mississippi, North Carolina, South Carolina, and Virginia) has shown a downward trend in rust infection, there are few long-term studies assessing whether it is management changes or improved genetics that may be influencing changes on long-term performance. Although many years and much federal and state funding have been spent on examining provenance studies and the incidence of rust, far less research has actually been done on what conditions and genetics favor or

slow fusiform rust spread in the field, and on the biology, ecology, and genetics of the organism itself.

2 Population biology

Three areas of research concentration in the southern United States have been in the population, molecular, and cellular definition of resistance responses. Initial population studies on the C. *q. fusiforme* pathogen were undertaken in the USDA, Forest Service, Southern Institute of Forest Genetics in Mississippi. Pycnial samples were collected over a wide range of the southern and southeastern United States to determine patterns of genetic differentiation among and within field populations using RAPD markers. In the results of this initial research, there appeared to be some genetic differences in populations east and west of the Mississippi River (Hamelin et al. 1994). There appeared to be more variation in certain regions, and research was extended to a more comprehensive sampling of pycnial samples. A larger collection has been taken of pycnial populations on twenty or more loblolly pines at 25 geographic locations. throughout the southern and southeastern United States. This study was joined by the researchers at the USDA Forest Service in Athens, Georgia, and the Daniel B. Warnell School of Forest Resources at the University of Georgia. At present, the Southern Institute Forest Genetics has identified 21 potential markers that are polymorphic and showed consistent bands. These markers were identified by Doudrick *et al.* (1993), and correspond to RAPD markers that either have been found in genetic maps constructed for a segregating population of C. *q. fusiforme* infecting loblolly pine or were unlinked to map loci. The goals are to examine the amount of relatedness among the different geographical locations of the populations (Roberds et *al.* 1997). This research will provide a broader picture of the genetic variability present in the pathogen population. Samples were taken to measure variability within small natural stands and between stands. Sampling these sites over time will present a temporal view of the pathogen's population changes in natural stands.

3 Molecular biology

Differential display of cDNAs is being used to unravel the interaction between C. *q. fusiforme* and pine. Researchers in Sarah Covert's Lab at the University of Georgia are examining the differential transcription of genes in healthy and C. *q. fusiforme* infected pine (Covert *et al.* 1997). Their research goals are two-fold. First, they want to improve our basic understanding of how C. *q. fusiforme* causes galls to form on pine trees. Ultimately, they want to use the information gained from these basic studies to design genetic engineering strategies that will create resistant pines. The structural and biochemical characteristics of fusiform rust galls all indicate that C. *q. fusiforme* is interfering with the normal growth and development of pine xylem. Their working hypothesis is that the fungus causes at least some of these effects by altering pine gene expression at the level of transcription. To determine if pine genes in galls exhibit different patterns of transcription than pine genes in healthy tissue, and to identify particular genes that are altered in their transcription, they am using the polymerase chain reaction (PCR)-based

technique known as differential display. They chose this technique in part because it will allow them to clone and characterize the affected genes. It also will allow them to identify fungal transcripts that may play a role in gall formation. The infected and healthy seedlings used in this study are from a rust susceptible seed lot and are provided by Carol Young at the USDA, Forest Service, Rust Screening Center, Asheville, NC. For the differential display experiments, each infected tree is paired with a healthy tree, and RNA is extracted from three different types of tissue: galled, asymptomatic, and heathy. Each RNA sample is reverse transcribed to produce cDNA. A subset of each cDNA sample is then amplified in the PCR by a variable combination of arbitrary and/or oligo-dT anchored primers. The resulting products are separated by size on a polyacrylamide gel. After the differential display reactions, the differentially transcribed cDNAs are cut from the acrylamide gel, reamplified, and cloned. The clones are sequenced and compared to sequences filed at GenBank, at the National Center for Biotechnology Information.

To date, Dr. Covert's lab has cloned 22 interesting cDNA fragments that appear to be differentially transcribed in galled and healthy pine tissues. Their current work focuses on verifying the differential transcription of the genes encoding these clones and determining if they are from the fungal or the pine genome. In the near future, they plan to select a few of these clones for more detailed analysis in an effort to determine what role, if any, they play in gall formation.

4 Cellular biology

The final area of concentration is in cellular responses of early events in germination and infection. In work by Wilcox *et* al. (1996), in which genomic mapping was used to identify a region of the loblolly pine genome that determines resistance to fusiform rust, random amplified polymorphic DNA (RAPD) markers used for mapping were obtained by genotyping haploid megagametophytes. The genotype of the megagametophyte is identical to the contribution of the seed parent to the diploid seedling. The seedling was then transplanted and grown. In this case, we know the genetics of the individual seedling. Working with progeny sets inoculated with C. *q. fusiforme*, segregating RAPD markers amplified from megagametophyte DNA were used to construct genomic maps for the parent families. Genomic maps were then used to detect associations between markers and symptom types in a factorial experiment where full-sib progeny were screened in the greenhouse against multiple pathogen isolates and scored for disease resistance. They found the resistance locus due to its very high association with field resistance. Having this advanced knowledge of whether a seedling carries a major gene for resistance to a known pathogen isolate allows us to plan some future cellular studies looking at how seedlings of known resistance respond at the early stages of inoculation and infection. The question that we would like to ask with our research is whether there are early resistance and susceptible responses that can be characterized when genotypes of seedling and fungal sources are held constant.

5 Guidelines for the future

Defining fusiform rust biology is an intricately woven act because we are trying to understand the biology of this organism foremost without the overlapping complexities contributed by its obligate host. After understanding the biology of the organism, our next area of interest is that of deployment. Wilcox et al. (1996) provided an open window to begin sorting the resistance genes in families. Since we have made breakthroughs on being able to designate a host as having or carrying a major gene for resistance (Wilcox et al. 1996), we can begin to set up a stable of tester plants. In the future we would like to forecast the deployment of known genotypes to monitor pathogen virulence.

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References

Covert, S.F., Warren, J. & Zwart, A. 1997. Differential gene transcription in galled and asymptomatic tissues of loblolly pine infected with *Cronartium quercuum* f. sp. *fusiforme*. TAPPI Proceedings. Biological Sciences Symposium. San Francisco, CA. p. 93-98.

Doudrick, R.L., Nance, W.L., Nelson, C.D., Snow, G.A. & Hamelin, R.C. 1993. Detection of DNA polymorphisms in a single urediniospore derived culture of *Cronartium quercuum* f. sp. *fusiforme*. Phytopathology 83: 388-392.

Hamelin, R.C., Doudrick, R.L. & Nance, W.A. 1994. Genetic diversity in *Cronartium quercuum* f. sp. *fusiforme* in loblolly pines in the southern U.S. Current Genetics 26: 359-363.

Pye, J.M., Wagner, J.E., Holmes, T.P. & Cubbage, F.W. 1997. Positive returns from investment in fusifortn rust research. USDA, Forest Service, Southern Research Station, Research Paper SRS-4.55 p.

Roberds, J.H., Kubisiak, T.L., Spaine, P.C., Covert, S.F. & Doudrick, R.L. 1997. Selection of RAPD markers for investigation of genetic population structure in fusiform rust fungus infecting loblolly pine. In: Proceedings of the 24th biennial Southern forest tree improvement conference. Orlando, Florida, June 9–12, 1997. p. 293-298.

Schmidt, R.A. 1998. Fusiform rust disease of southern pines: biology, ecology and management. University of Florida. Agricultural Experiment Station. 14 p.

Starkey, D.A., Anderson, R.L., Young, C.H., Cost, N.D., Vissage, J.S., May, D.M. & Yockey, E.K. 1997. Monitoring incidence of fusiform rust in the south and change over time. USDA, Forest Service, Southern Region Forest Protection, Protection Report R8-PR 30.

Wilcox, P.L., Amerson, H.V., Sederoff, R.R., O'Malley, D. & Kuhlman, E.G. 1996. Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. Proceedings of the National Academy of Sciences 93: 3859-3864.