IDENTIFICATION OF MARKERS LINKED TO *AVR1* IN *CRONARTIUM QUERCUUM* F. SP. *FUSIFORME* USING A NOVEL NEXT-GENERATION SEQUENCING APPROACH

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Fusiform rust disease, caused by infection of the fungal pathogen *Cronartium quercuum* f.sp. *fusiforme*, produces galls on stems and branches of southern pines. Stands of susceptible genotypes are often poorly stocked because stem galls weaken stems and make trees susceptible to lodging. Gene-for-gene interaction between the *Pinus taeda* resistance gene *Fr1*, and the corresponding pathogen avirulence gene *Avr1* has been documented in previous work (Wilcox et al. 1996; Kubisiak et al. 2011). Obtaining markers for avirulence loci would allow pathogen populations to be surveyed where plantations were to be established, enabling growers to plant trees with corresponding resistance genes that ensure the stand is resistant to rust. We previously showed that such markers could be identified because the gene-for-gene relationship acts as a "sieve" for avirulence alleles when a heterozygous fungal culture is inoculated on a resistant host. After inoculation of a susceptible tree (*fr1/fr1*), spores harboring either virulence or avirulence alleles (*Avr1* and *avr1*) persist and produce haploid pycniospores. In contrast, pycniospores from inoculated resistant trees (*Fr1/fr1*) only contain spores with *avr1*, selecting against the avirulence (*Avr1*) allele. Here we evaluate a restriction enzyme-based approach to identify markers linked to *Avr1*, using bulk segregant analysis to compare pycniospore DNA sequences obtained on a next generation sequencing platform (Illumina GAIIx). Reads present in spores from the susceptible host, but absent or significantly reduced in frequency in spores from the resistant host, are probably linked to *Avr1*. While the marker discovery approach does not rely on an assembled genome sequence, these markers we identify can be used as queries to interrogate the genomic sequence of *Cronartium quercuum* f.sp. *fusiforme*, as a strategy to integrate the *Avr1*-linked markers with the physical representation of the genome. This approach should lead to the identification of *Avr1* itself, and should foster an analogous strategy to quickly identify other avirulence genes in *Cronartium quercuum* f.sp. *fusiforme*.