Section 1 Abstracts: Molecular Biology of Hypovirulence

Effect of dsRNA on Gene Expression of *Cryphonectria parasitica.* N.K. Van Allen, D.H. Kim, L. Zhang and P. Kazmierczak. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA

The symptoms of *Cryphonectria parasitica* by the dsRNA derived from the French strain EP4 (EP113, EP713, UEP1) are reductions in fungal pigmentation, sporulation and virulence. Fungal growth is not affected by this dsRNA, but there is a quantitative reduction in accumulation of specific fungal proteins. The proteins that we have studied are all developmentally regulated. It is our hypothesis that the dsRNA perturbs normal regulation of specific host developmental pathways. We have identified and cloned four genes that are down-regulated in the presence of the virus (Lactase A, Cryparin, Virl and Vir2), and have deleted two of these genes *(lacA* and vir2) to determine their functions. These four cloned genes are being used as molecular markers for the effects of the virus on fungal gene expression. They also are useful for screening mutants to identify genes that may be associated with fungal pathways perturbed by the virus. Screening mutants for those with phenotypes that mimic the viral symptoms and that also reduce expression of the four marker genes has resulted in the identification of pleiotrophic mutants of the characteristics that were sought. One of these mutated genes, *Hsm* 1, has been cloned. The null mutation of *Hsm1* mimics some of the viral symptoms (pigmentation and sporulation) and quantitatively affects expression of the marker genes to the same extent as the virus. The study of mutants, such as *hsm* 1, and transcription run-on assays are being used to investigate the effects of the virus on its fungal host.

and the four ribonucleoside triphosphates being present. The reaction was insensitive to actinomycin D and alpha-amanitin. The products were primarily ssRNA molecules that corresponded to full length copies of the coding strand of the dsRNA, as indicated by hybridization to single-stranded cDNA clones of the dsRNA. The ssRNA synthesis is asymmetrical; approximately 90-95% of the products are of the plus strand while only 5-10% are of the minus strand. These data suggest that the *in vitro* reaction has both transcriptase and replicase activity, with the former being 10-20-fold more active than the latter. This RNA polymerase activity associated with host membrane vesicles is more typical of ssRNA plus-sense viruses rather than of dsRNA viruses. Current studies are directed toward identification of viral and host proteins involved in a replication complex purified from the dsRNA containing vesicles. The conserved regions of the putative RNA polymerase and RNA helicase encoded by the dsRNA of *C. parasitica* were cloned and expressed in *Escherichia coli.* The recombinant proteins were purified and used to produce polyclonal antibodies. These antibodies are being used to investigate the nature of the replication complex.

DNA Fingerprinting for Determining Genetic Relatedness within Vegetative Compatibility Groups of *Cryphonectria parasitica.* Y.-C. Liu and M.G. Milgroom, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908, USA

Genetic relatedness within and between vegetative compatibility (v-c) groups from populations of *Cryphonectria parasitica* on chestnut was analyzed using DNA fingerprinting. A DNA probe, MS5.1, was used that hybridizes to 7-12 restriction fragments in each isolate of *C. parasitica.* Isolates were collected from two populations in Michigan and one from both West Virginia and Italy. The fingerprinting patterns were highly diverse within v-c groups in the West Virginia population. The proportions of bands shared within v-c groups ranged form 0.3 to 0.9. This proportion was not different within and between v-c groups in the West Virginia population. In populations from Michigan and Italy, the diversity within v-c groups was low. The proportions of bands shared within v-c groups varied from —0.7 to 1.0 in the two Michigan populations. The proportion of bands shared within v-c groups was between 0.5 and 1.0 in Italy. However, the proportions of bands shared between v-c groups were significantly less than within v-c groups in both Michigan and Italy populations. These results show that DNA fingerprinting may identify v-c groups as clonal lineages in the Michigan and Italian populations but not in the West Virginia population.