Section 1 Abstracts: Molecular Biology of Hypovirulence

RNA Polymerase Associated with Double-Stranded RNA of *Cryphonectria parasitica.* Tzion Fahima, Pam Kazmierczak, Yarning Wu and Neal Van Allen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA

Double-stranded RNA containing vesicles isolated from hypovirulent strain EP113 of *Cryphonectria parasitica* possessed RNA-dependent RNA polymerase activity that was absent from comparable preparations of the dsRNA-free strain EP155. The incorporation of [^{32}P]UTP into RNA was proportional to the concentration of dsRNA-containing vesicles. Activity was dependant upon Mg + ,

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.