SUMMARY OF HYPOVIRULENCE RESEARCH AT UTAH STATE UNIVERSITY

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ABSTRACT.--The *hypovirulent research program at Utah State University is directed toward understanding the biology of the hypovirulent factor and how this factor reduces the virulence of Endothia parasitica.*

Logan, Utah is a long way from the nearest American chestnut tree. Because of this distance, we in Utah will never directly enjoy the fruits of the successful control of chestnut blight. However, we feel that understanding the phenomenon of chestnut blight control by hypovirulence may lead to a wider exploitation of this phenomenon.

All of our research at Utah State University is directed toward understanding the biology of the hypovirulent factor. We are approaching this study from several different directions. Our two overall objectives are 1) to better understand the biological nature of the hypovirulent factor and 2) to understand how the hypovirulent factor reduces the virulence of *Endothia parasitica.*

Since other papers presented at this conference go into detail concerning our findings, I will only briefly mention the different approaches we are using to study these questions. One of our primary objectives is the cell-free transfer of the dsRNA associated with transmissible hypovirulence. This is a priority objective since direct proof of the role of this dsRNA in hypovirulence is needed. Our approach is to transfer the dsRNA encapsulated in liposomes into protoplasts of a virulent strain of the fungus.

In spite of the passage of many years since the demonstration that hypovirulence is cytoplasmically controlled, we are still uncertain about the biological nature of these cytoplasmic genes. As indicated above, dsRNA has been correlated with hypovirulence. Most researchers assume that the control of hypovirulence expression is determined by genes on the dsRNA. This is still speculation, however, since we have no direct evidence for this. Also we know little about how the dsRNA is packaged within the cell. On the basis of our work and that of Allan Dodds, we feel that the dsRNA is not packaged within a typical mycovirus. It appears that the dsRNA does not have a typical protein coat. The membrane-bound dsRNA containing particulate fraction that has been isolated from strain 113 is not a mycovirus, but is rather a membrane vesicle containing large amounts of the same carbohydrates that make up the fungal cell wall. This leads us to believe that the dsRNA

is packaged in cell-wall synthesizing vesicles. We do not know whether packaging of the dsRNA into these vesicles is a defense response of the fungus, or a dsRNA directed packaging phenomenon.

One way to determine the relationship of these vesicles to the dsRNA is to determine their role, if any, in dsRNA replication. A graduate student in our laboratory is doing his research on determining the site of dsRNA replication. He plans to use anti-body coupled with electron microscope techniques to determine where the replication occurs. He will particularly be seeking evidence of whether dsRNA is packaged in the vesicles at the same site that replication occurs.

The multi-segment nature of the dsRNA associated with hypovirulence is typical in that respect to mycoviruses. However, unlike mycoviruses, there is considerable strain-to-strain and intra-strain variability in the segmentation. This is unusual since differences in segmentation patterns are not consistently reflected by differences in hypovirulence phenotype. We are investigating the relationship between the dsRNA segments to determine whether they are redundant copies of each other, or whether they are each different from one another. We are using hybridization techniques for this study.

In addition to trying to better understand the nature of the hypovirulent factor, we are investigating how the presence of the dsRNA reduces the virulence of the fungus. We feel that the two most likely ways virulence is reduced are either by production of a gene product that interacts with the fungus, or by directly interacting with a virulence control site on the fungus genome. We are initiating research to test both of these hypotheses. One method we are using is to identify the translational products of the dsRNA. Another approach is to determine if there is a virulence control site on the fungus genome.

These various projects are all directed toward trying to answer the two questions raised above about hypovirulence: 1) What is its biological nature?, and 2) How does it affect the virulence of its fungal host? We currently have two people in addition to myself in our laboratory working on this project. Dr. Dane Hansen is studying the nature of the vesicles and how the dsRNA segments are related. Lee Barley, a graduate student, is studying replication of the dsRNA for his PhD research.