challenge that remains for Plant Pathologists is to develop the capacity to integrate this information with existing knowledge of the biochemistry, physiology, ecology, and epidemiology of the host-parasite interactions such that it can be applied to improve crop production. These improvements may be realized through better diagnostic tools, targeted modifications in host plants to enhance durable resistance, and superior predictive models of disease development to guide disease management practices. In this presentation, I will suggest a vision of how the advances of genomics will influence plant health in the future, providing illustrative examples from various research approaches.

PL-3

Fungal Hypoviruses and Their Role in the Biological Control of Plant Diseases

Neal K. Van Alfen
Professor and Dean
College of Agricultural and Environmental Sciences, University of California, Davis, CA 95616, USA.

Biological control of fungal diseases of plants is a concept that has been embraced with great enthusiasm. It implies that management strategies can be developed that do not rely on regular, costly applications of fungicides. Even more importantly, it provides a strategy to manage fungi that cannot be controlled using fungicides or other known methods. Unfortunately, there are relatively few examples of successful biological controls that have been demonstrated for fungal diseases of plants. This lack of success has resulted in a waning of effort toward finding biological solutions to plant disease problems. I believe that this retreat from seeking biological management strategies for fungal diseases of plants is premature.

Significant effort has been expended toward seeking biological controls for insect pests, in part, because of the known problems associated with insecticide use. Plant pathologists have never had the array of chemicals available to control plant diseases that have been developed for insect control, thus fungicide use remains low compared with insecticide use world-wide. We are also less aware of the detrimental effects of fungicide use on natural ecosystems than are entomologists in their knowledge of the effects of insecticides, so we expend much less effort than do entomologists in looking for alternatives to pesticides. The effort of the entomologists to find biological controls has been rewarded with numerous successful examples. There is reason to assume that similar efforts to find biological controls of fungi would be met with similar success.

A strategy for biological control of fungi that holds great promise is the use of mycoviruses. As with all known organisms, fungi harbor viruses; some of these have potential in biological control strategies. The most well characterized viruses of fungi that have potential for biological control are the hypoviruses of the chestnut (*Castanea spp.*) blight fungus, *Cryphonectria parasitica.* This group of viruses is a successful naturally occurring biological control for chestnut blight under some circumstances. We do not yet fully understand all of the circumstances that contribute to the success or failure of this biological control.

This type of mycovirus causes its host, *C. parasitica*, to remain in a juvenile state of growth, i.e. to perturb normal developmental processes of the fungus, the results of which are low virulence (hypovirulence), poor asexual and sexual sporulation. Saprophytic growth of the fungus is not affected by the virus, so in culture the fungus grows normally but does not sporulate or develop the normal pigmentation associated with development. Molecular studies of virus-infected strains of the fungus suggested that a number of genes are transcriptionally down and up-regulated in virus infected strains. Evidence has also been presented that normal cell-signaling processes are perturbed in virus infected strains.

My laboratory has been seeking to understand how the virus is able to perturb developmental processes of this fungus. Our approach has been to examine groups of genes, and their products, that are down-regulated in virus-infected strains to seek commonalities among these perturbed genes. A number of genes were selected based on their differential expression for further characterization. Genes were cloned and their products identified. In most cases, the genes were deleted to study their role in the biology of the fungus. Among the genes identified were the fungal sex pheromones, a cell-surface hydrophobin, and a laccase. The pheromones and hydrophobin are involved in fungal sporulation, and are thus developmentally regulated.

A number of the gene products characterized had common endoprotease recognition sites, suggesting that the preproproteins encoded by these genes were processed in the same way during secretion. The recognition sites were the same as those reported for the conserved kex2 endoprotease of *Saccaromyces cerevisiae.* This endoprotease is known to be involved in the processing a secretion of the alpha pheromone of yeast; a pheromone precursor gene with similar processing sites and signals was found to be down-regulated in virus-infected strains of *C. parasitica.*

These results led us to investigate the secretion of one of the kex2-processed proteins in both virus-infected and healthy strains of the fungus. Our results showed that the secretion of the cell-surface hydrophobin, cryparin, is perturbed in the virus-infected strain. Secretion is much slower when the virus is present.

Isolation of the components of the vesicular secretory system of *C. parasitica* showed that the virus genome is found in association with trans-golgi vesicles. The virus lacks a capsid but is able to replicate in association with these fungal vesicles. The trans-golgi vesicles were much more abundant in virus infected strains than they were in uninfected strains. The vesicle fraction that contains the virus vesicles also was enriched for clathrin and an associated adaptor protein. Based on these results, we hypothesize that the virus utilizes a vesicle fraction for its replication and movement that is normally involved in secretion of

proteins important to fungal development. We suggest that capture of these vesicles by the virus perturbs the normal function of these vesicles and thus the secretion of these proteins. We also hypothesize that a build-up of these vesicles results in a regulatory response to reduce transcription of these proteins important to development, resulting in the observed symptom of poor development in virus infected fungi.

It is important to understand the molecular biology of the viruses that affect fungi since they represent new types of viruses. All fungal viruses characterized to date lack an infectivity cycle. It is unclear why all viruses that infect fungi should only be transmitted through spores or hyphal anastomosis. This lack of documentation of infectivity probably reflects the dearth of our knowledge of fungal viruses rather than a true biological phenomenon. Within *C. parasitica* viruses from a number of different families have been found, so it is clear that we are only in our infancy of understanding the biological diversity and importance of viruses in the ecology of fungi. There are probably many types of fungal viruses that will be found useful for biological control, but they must first be sought and then studied if we are to make progress in learning how to use them for this purpose. One of the inherent advantages of using viruses to control fungi is that they move within the thallus of the fungus, potentially allowing control of those fungi that are deeply buried within hosts, or hidden within the soil. These are the fungi that are very difficult to control by any known means.