SUMMARY OF *ENDOTHIA* PARASITICA-HYPOVIRULENCE RESEARCH AT WEST VIRGINIA UNIVERSITY

W. L. MacDonald, D. F. Hindal, and W. J. Kaczmarczyk

Division of Plant and Soil Sciences
West Virginia University
Morgantown, WV 26506

ABSTRACT.--*A background and overview of the chestnut blight-hypovirulence research program at West Virginia University is given. Field studies designed to deploy, establish and test the usefulness of hypovirulent strains as a biocontrol are described. Laboratory experimentation dealing with vegetative compatibility, physiology, cytology and molecular aspects of hypovirulence also are outlined.*

West Virginia University has had a long-standing chestnut research program. Until the 1970's this research was conducted within the Horticulture Unit as part of a larger project to find improved varieties of different nut-bearing species. Several investigators over the years devoted considerable time as part of this project to collecting and out-planting seedlings from surviving American chestnuts, but none of the chestnuts resulting from these efforts showed any significant degree of resistance. While the project generated considerable statewide interest, it suffered from meager financial support and the frustrating and overwhelmingly complex problems posed by chestnut blight.

The general interest in chestnut was rekindled at the WVU Experiment Station when Jean Grente's work on hypovirulence (Grente and Berthelay-Sauret 1978) was confirmed by researchers at the Connecticut Agricultural Experiment Station (Van Alfen et al. 1975). Awareness of the impact this phenomenon could have on the plight of the American chestnut resulted in several West Virginia legislative hearings to discuss the status of the species. Eventually, some state monies were appropriated to re-examine the entire problem. The interest shown by the West Virginia Legislature probably provided the driving force behind the current federal funding. In any event, the research efforts at WVU were reborn during the late 1970's. This led to the 1978 American Chestnut Symposium held in Morgantown, West Virginia that was sponsored jointly by the West Virginia University and the U.S. Forest Service. The symposium was attended by the majority of active chestnut researchers in the United States. However, the highlights of the meeting were papers given by Grente from France and Mittempergher and Turchetti from Italy (Grente and Berthelay-Sauret 1978; Mittempergher 1978; Turchetti 1978). Their presentations provided considerable insight into the phenomenon of hypovirulence and also served to brighten the outlook for American chestnut in the United States.

In summarizing hypovirulence research at West Virginia University, it is convenient to divide the discussion into field and laboratory studies. The field experimentation has been underway for a longer period of time and seems to be an appropriate place to begin.

Field Studies

Treatment Plots. The first attempts to utilize hypovirulent strains of *Endothia parasitica* were made in the late summer of 1976. Four study plots were established in a cut-over area of the George Washington National Forest where chestnut regeneration was abundant. Cankers on living chestnut trees were treated with a French-derived hypovirulent strain (EP-43) obtained from Richard Jaynes at the Connecticut Agricultural Experiment Station. Each canker was treated by introducing hypovirulent inoculum at two points on the lateral edges of the canker. The following spring, isolations were made from the treated cankers. The colonies that resulted were examined for morphologies similar to EP-43. Even though the majority of the isolates were morphologically similar to virulent isolates, a few distinctly white colonies, similar to EP-43 were recovered. Unfortunately the rate of successful canker control was extremely low.

Work by Sandra Anagnostakis on the relationship between vegetative compatibility (v-c) and the success or failure of canker treatment led to examination of the v-c types of virulent strains that were inciting cankers within the study area (Anagnostakis 1977). The eventual objective of this effort was to match each infection with vegetatively appropriate hypovirulent inoculum. Efforts to establish reasonably clear v-c groups followed, but required several months of laboratory testing. Upon returning to the study plots in the fall of 1977 to treat cankers with the appropriate hypovirulent inoculum, we were confronted with an overwhelming number of new infections that had developed during the season. It was apparent that the progress of the disease within our study plots was too rapid for such a methodical approach to canker treatment.

In the spring of 1978, a procedure of treating cankers with mixtures of hypovirulent inoculum was adopted following the report of the success achieved with this method by Jaynes and Elliston (1980) in Connecticut. Three mixtures were used that were comprised of isolates with similar cultural morphologies and often similar potentials to invade and sporulate in host tissues. A fourth mixture, "general", was also employed, that was composed of all hypovirulent strains contained in the other mixtures as well as several North American hypovirulent components. The mixtures used were as follows:

B-type. Isolates with white colony appearance and nonpathogenic,

JR. Isolates with orange-pigmented colony appearance and nonpathogenic,

Italian. Isolates slightly orange-pigmented and moderately pathogenic,

General. All of the above isolates and four North American hypovirulent strains.

From 1978 through the fall of 1981, these mixtures were used to treat new cankers as they arose in 24 study plots at three locations in West Virginia. During the 4-year period, almost 4,000 cankers have been treated on nearly 1,000 trees. A preliminary summary of this ongoing experiment is presented as an abstract by MacDonald (this proceedings).

Challenge Study. A study was established during the summer of 1978 to determine the role of vegetative compatibility to the success or failure of canker treatment. In this study, virulent cankers were established on healthy chestnuts using isolates of known vegetative compatibility type. The resulting cankers were then challenged with hypovirulent inoculum known to be vegetatively compatible or incompatible with the virulent isolate causing the canker. Individual hypovirulent isolates and the same hypovirulent mixtures described earlier were used to treat the artificially established infections. More virulent cankers were controlled than had been anticipated from their interaction in culture. Details of this study are presented in a paper authored by Double (this proceedings).

Scratch-Wound Study. During 1979, tests designed to establish reservoirs of hypovirulent inoculum on healthy chestnut stems were initiated in an effort to demonstrate natural dissemination of hypovirulent strains. To accomplish this wounds were made by scratching the bark at ground level, 1.5 m and 3.0 m off the ground and inoculum of different hypovirulent strains introduced into the scratches. Isolations have been made from all new infections that developed on the scratched trees. The cultural morphologies of these isolates have been examined and compared to that of isolates used as inoculum. The results of this experiment have provided the first evidence of the natural dissemination of hypovirulent strains on American chestnut following their artificial introduction. A detailed summary of these efforts are reported by Willey (this proceedings).

Periodic-Inoculation Study. Interest in determining the most appropriate periods to introduce hypovirulent inoculum led to a study of the effect of month of inoculation on subsequent canker growth and sporulation. This effort provided some unexpected findings with inoculations made in December and February. Even though inoculum remained viable within the inoculation site for over 2 years, cankers did not result. Double (this proceedings) summarized these results in an abstract.

Graduate Student Field Research. From 1978-80 field studies were undertaken by Richard Baird and Ronald Willey as part of their Master of Science degrees in Plant Pathology. Both students were interested in the growth and sporulation of a variety of hypovirulent and virulent strains. Willey used living chestnut stems for his evaluation whereas Baird examined saprophytic growth on red oak, red maple, and chestnut stems inoculated after they were killed by girdling. They found significant differences in the ability of virulent and hypovirulent strains to invade, sporulate, and survive in host and nonhost tissues. Even though the dead nonhost species did not support growth and sporulation as well as living chestnut, these abilities appear to be more dependent on the isolate tested than on the host. Complete details of their work are reported in their theses (Baird 1980; Willey 1980).

Summary. Field studies are particularly time and labor intensive. In some instances, field experiments have proceeded without the scientific background

information necessary to adequately plan them. Even though some work was obviously shortsighted, the ultimate goal to establish the usefulness of hypovirulence as a biological control has not changed. The efforts have raised a variety of questions that need to be addressed. Our field efforts have been conducted entirely in cut-over areas. These areas often were chosen out of necessity because they provide the abundant numbers of chestnut stems required for experimental replication. Even though a low incidence of chestnut blight has been a prerequisite for site selection, the number of subsequent infections builds so rapidly that it is often difficult to complete planned experiments. Treatment tests, designed to simply keep trees alive, have failed largely because of the large number of new infections that develop during a single season. In short, the virulent inoculum levels are so high that cut-over areas may present the most difficult circumstances for the introduction of hypovirulent inoculum. It may be more appropriate to look for trees that are relatively isolated and thus growing where inoculum buildup would be lower.

Time is the critical factor required for the transition of a virulent *E. parasitica* population to hypovirulent. This requirement places increasing importance on the information that can be gained by carefully observing and culturally examining new infections that develop over time in areas where hypovirulent inoculum has been introduced. Field studies often have dealt with the performance of hypovirulent strains and placed relatively little emphasis on following the course of new and old infections in introduction areas. This seems to be a particularly significant point now that evidence for the natural dissemination of artificially established hypovirulent strains exists.

Laboratory Studies

Vegetative Compatibility Tests. Over the past 5 years, we have attempted to determine the vegetative compatibility of *E. parasitica* isolates obtained from new infections that develop on the American chestnut within the eight treatment plots at Parsons, West Virginia. To accomplish this, *E. parasitica* isolates were obtained from canker margins prior to treatment and paired on an agar medium with test isolates from established vegetative compatibility groups (a group contains at least 2 isolates of the same compatibility). Of 880 isolates that have been paired, 795 (90 percent) have been placed in one of 37 different groups. The balance of the isolates tested are incompatible with the test isolates and each other. Even though a large number of vegetative compatibility types has been established, 47 percent of all the isolates fall within six vegetative compatibility types. Data obtained from this effort in combination with field treatment results will be analyzed to determine if a relationship exists between the vegetative compatibility type of the *E. parasitica* strain inciting a canker and the effectiveness of the hypovirulent treatment used.

Anastomosis and Cytology Studies. Hyphal anastomoses between vegetatively compatible and incompatible virulent and hypovirulent strains of *E. parasitica* was studied by Northup. Anastomoses of the hyphae-to-peg and peg-to-peg type were observed using light and scanning electron microscopy between vegetatively compatible and incompatible types. Collapsed cells were noted at the site of anastomosis. This work is described in a thesis by Northup (1981).

Efforts to view the cytology at the point of anastomosis will be continued by Newhouse with transmission electron microscopy. His initial observations of the cytology of virulent and hypovirulent isolates provided the first micrographs of virus-like particles in hyphal tip cells of *E. parasitica* strain EP-4. An abstract describing Newhouse's et al. findings appear in these proceedings.

Physiology Studies. Physiological studies are underway to determine if defined media will provide a distinct cultural separation of virulent and hypovirulent isolates. These efforts may also provide insight into the nutritional differences between virulent and hypovirulent isolates, as well as yield information on the physiological changes induced by hypovirulence. Progress of this work is reported in a paper by Hindal in these proceedings.

Molecular Biology of *Endothia parasitica.* A mycovirus from hypovirulent strain EP-43 was partially purified and characterized by Chmelo (1981). This work is presented in a paper by Chmelo and Kaczmarczyk (this proceedings).

In another abstract, Harper-Morris (this proceedings) reports her success in detecting RNA dependent-RNA polymerase in crude virus-like particle extracts of 14-day-old cultures of strain EP-43. Additional objectives of her work are to analyze the pH, template, and optimal temperature requirement, and optimal cofactor concentration of the enzyme.

Other studies, by Wheeler, are designed to examine the base-pair ratios of individual dsRNA bands formed in polyacrylamide gels and the possible presence of unusual nucleotides. He will examine the base-pair ratios by partial hydrolysis of the dsRNA followed by high performance liquid chromatographic separation. Search for unusual nucleotides will be attempted using thin-layer chromatography plates, Hopefully, this effort will lead to a rapid method for identifying various dsRNA species.

Summary. Many aspects of the basic biology of *E. parasitica* have not been studied for over 50 years. Now we are confronted with the phenomenon of hypovirulence which further complicates our appreciation of this fungus. The phenomenon of hypovirulence and biology of *E. parasitica* lend themselves to a variety of basic studies, but none currently seems more pressing than establishing the infectivity of the virus-like particles or the dsRNA associated with hypovirulent strains. The proof of infectivity should come with further refinements in procedure. Basic studies hopefully also will provide insight into the relatedness of the dsRNA associated with European and North American hypovirulent strains. Perhaps information on the dsRNA and its similarity to that found associated with other mycoviruses can also be learned. The most challenging task ahead, however, will be to coordinate basic studies with those designed to utilize this phenomenon as a biological control in the forest,

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