CHAPTER 1
INTRODUCTION

Since its introduction into the U.S., in 1904, the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr (=*Endothia parasitica* (Murr.) P. J. and H. W. Anderson), has destroyed nearly all American chestnuts (*Castanea dentata* (Marsh.) Borkh.) within the natural range. Fortunately, the fungus does not invade the roots of trees and so American chestnuts have been able to survive predominantly as understory shrubs.

A different situation exists in Europe where many of the European chestnut trees (*Castanea sativa* Mill.) are recovering from the blight (Bazzigher et al. 1981). Evidence suggests that the survival of the European chestnut is due in part to naturally occurring hypovirulent strains of *C. parasitica* (Bissiger et al.1996). Hypovirulent strains of *C. parasitica* are characterized by low pathogenicity, reduced fruiting capacity, and the presence of double-stranded RNA (dsRNA) (Elliston 1985). The dsRNA in European hypovirulent strains has since been designated as *Cryphonectria hypovirus* 1 (CHV1) (Hillman et al. 1995). CHV1 typically produces white colony morphology in *C. parasitica* when grown in culture, whereas normal, virulent dsRNA-free strains have yellow-orange pigmented colonies; however, these changes in color are variable with regard to the hypovirulent fungal strain (Elliston 1985). Elliston (1985) characterized all the European dsRNA-containing strains he examined (including EP-43, 49, and 51, which were used to inoculate the trees to be studied in this study) as “white for at least the first 5 days after transfer, then some developed cream, yellow or light orange centers, or light orange concentric rings”.

Natural spread of hypovirulence does not exist in the U.S. to any extent, but does occur in Europe (Heineger and Ringling 1994). This occurs in part by the transmission of

dsRNA between and among strains of *C. parasitica*. Transmission has been observed *in vitro* through hyphal anastamosis between fungal strains (Anagnostakis and Day 1979). This transmission is limited, however, by incompatiblility between strains in different vegetative compatibility (VC) groups (Kuhlman and Bhattacharyya 1984). There is evidence, however, that transmission of CHV1 and other hypoviruses can overcome vegetative incompatibility and to some extent convert normal strains to hypovirulent strains. Anagnostakis (1983) demonstrated that "weekly" incompatible interactions can result in hypovirulence conversion, and other data suggest that isolates in different VC groups can be converted to hypovirulence *in vivo* if sufficient time is allowed (Robbins and Griffin 1999, Shain and Miller 1992).

An experimental plot was established in Lesesne State Forest in 1980 by grafting scions from large surviving American chestnut trees on the rootstocks of American chestnuts growing in a plantation. In 1982-1983, blight cankers on these trees were inoculated with a mixture of six pigmented, dsRNA-containing hypovirulent strains of *C. parasitica* and four white, CHV1-containing, European hypovirulent strains (Dierauf et al. 1997). The four European hypovirulent strains (EP-43, 47, 49, and 51) were composed of three VC groups (Jong and Edward 1991). These trees are now thriving and exhibit high levels of blight control (Dierauf et al. 1997). It was found that after 14 years, the white strains had spread throughout the stems and branches of the inoculated trees (Robbins and Griffin 1999). Furthermore, only one white isolate was recovered from nearby American chestnut sprouts, suggesting spread was not occurring to other trees. The situation in Lesesne is very unusual to the United States and provides an excellent opportunity to study the diversity of hypovirulent white strains in blight-controlled cankers on American

chestnut, and the characters of white strains that may be most effective in biocontrol of chestnut blight on American chestnut.

The objectives of this study were: 1) to determine the number of vegetative compatibility groups of *C. parasitica* into which CHV1 has spread on grafted American chestnut trees at Lesesne State Forest, 2) to determine the spatial pattern of the white strains of *C. parasitica* within and among cankers, outside the H-inoculated zone, on grafted American chestnut trees at the Lesesne State Forest, and 3) to determine the cultural characteristics of white strains of *C. parasitica*, which have spread at the highest frequency on grafted American chestnuts at the Lesesne State Forest.