Population biology of *Cryphonectria parasitica* infected with *Cryphonectria hypovirus 1* on American chestnut trees

by

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ABSTRACT

In the early 1900's the American chestnut (*Castanea dentata* (Marsh.) Borkh.) was nearly destroyed by the introduction of the orange-pigmented, chestnut blight fungus (*Cryphonectria parasitica* (Murr.) Barr). Chestnut blight is less severe in Europe, where hypovirulent (= reduced virulence) strains of the fungus are found to be associated with healing cankers. These European hypovirulent strains are infected with a dsRNA virus, *Cryphonectria hypovirus 1* (CHV1), and have a white phenotype when grown in culture. Transmission of CHV1 in *C. parasitica* is limited by incompatibility between isolates in different vegetative compatibility (vc) types. In 1982-83, naturally formed blight cankers on American chestnut grafts, derived from large survivors, were inoculated with a mixture of four European (white) hypovirulent strains of *C. parasitica*. After 14 years the white strains were recovered throughout the inoculated grafts, which had low levels of blight damage. CHV1 had infected at least 45 new vc types, and was present in four different fungal colony morphology groups, including one type that had intermediate or partial pigmentation. However, CHV1 was unable to move throughout a single vc type within a natural canker. The objectives of this study were: 1) to determine the frequency and phenotypic diversity of CHV1-infected *C. parasitica* isolates recovered from stromata and canker tissue from natural cankers on the grafted American chestnut trees and artificially established cankers on forest American chestnuts; 2) to determine the presence or absence of CHV1 in intermediate-pigmented isolates recovered from the American chestnut research plots; 3) to investigate the roles of colony age, resistance to hypovirus infection, and functional mycelial units in the failure of CHV1 to move throughout a vc type of *C. parasitica in vitro*, and; 4) to examine the role of low temperatures and a high elevation topographic site on CHV1 survival within *C. parasitica* colonies *in vivo* and *in vitro*. The results indicated that there was no direct correlation between the amount of colony pigmentation and the presence of dsRNA. Within each of the three colony phenotype categories (pigmented, intermediate and white), several *C. parasitica* isolates tested positive for the presence of CHV1. This presence of CHV1 in intermediate isolates, coupled with the relatively large number of intermediate isolates collected from stromata on cankers, indicates that intermediate isolates may perform an important, and previously overlooked, function in biological control of chestnut blight. In this study, all CHV1 movement trials indicated that the age of the *C. parasitica* colony limited the movement of CHV1 throughout the colony. The majority of the CHV1 movement through a *C. parasitica* colony occurred between 0 and 7 days following challenge with an isogenic CHV1-infected strain. Isolation data using a lattice grid did not indicate a consistent pattern of CHV1 movement throughout a *C. parasitica* colony. Low temperatures associated with high altitude had no effect on hypovirus survival *in vivo* or *in vitro.* Additionally, no long-term *C. parasitica* resistance to CHV1 infection or movement was identified in this study. This research has identified new insights into CHV1 spread and survival that may be important in understanding the role of CHV1 in the biological control of chestnut blight.

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