DEVELOPMENT OF AN *IN VITRO* ASSAY FOR RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* IN CHESTNUT

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While the story of the devastation of American chestnut (*Castanea dentata*) by chestnut blight is familiar to those who work with this tree, less well-known is the fact the another disease—Phytophthora root rot, caused by the oomycete pathogen *Phytophthora cinnamomi*—had already extirpated American chestnut from the Piedmont and Coastal Plain regions of the southeastern U.S. decades before the arrival of the chestnut blight fungus, *Cryphonectria parasitica*. The lack of resistance to *P. cinnamomi* may pose a major barrier to introducing the products of The American Chestnut Foundation's (TACF) breeding program to the southern portion of the American chestnut's original range. The integration of genes for *P. cinnamomi* resistance from Chinese chestnut into TACF's hybrid chestnuts is now proceeding, but it will take additional years of breeding and selection. Combining somatic embryogenesis-based propagation of chestnut hybrids with a reliable *in vitro* assay for resistance to *P. cinnamomi* would help to more quickly evaluate chestnut cultivars thought to carry these resistance genes—which in turn should accelerate the production of elite chestnut cultivars with resistance to both *C. parasitica* and *P. cinnamomi* for planting throughout the eastern U.S.

We tested different *in vitro* screening approaches to rapidly identify *P. cinnamomi*-resistant chestnut cultivars using embryogenic cultures, shoots, and whole plantlets of wildtype American chestnut, transgenic American chestnut carrying putative *P. cinnamomi* resistance genes, and Chinese chestnut. Inoculation of cultures of embryogenic callus grown in 48-well plates with two isolates of *P. cinnamomi* failed to distinguish between American chestnut and Chinese chestnut because embryogenic callus of both species failed to turn to the blue-black color described in previous reports for chestnut callus derived from cambium tissue; instead, callus of both species turned brown after 3-4 weeks. However, American chestnut shoots derived by axillary multiplication that were stuck into 6% water-agar gel and inoculated with *P. cinnamomi* developed obvious symptoms, including wilting and blue-black lesions on the stems, but the ultimate cause of death could not be determined because the shoots had no roots. Whole plantlets (i.e. rooted shoots) of American chestnut and Chinese chestnut grown in 2% low melting point agarose gel showed differential responses to *P. cinnamomi* inoculation. American chestnut plantlets rapidly wilted and stems blackened while Chinese chestnut plantlets remained symptom-free for at least 6 weeks; however, stem lesions eventually did appear on these plantlets. Some transgenic American chestnut plantlets expressing candidate *P. cinnamomi*-resistance genes showed evidence of limited resistance, but these still succumbed to root rot more rapidly than Chinese chestnut plantlets.

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