

Resistance to *Phytophthora cinnamomi* Among Seedlings From Backcross Families of Hybrid American Chestnut

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American chestnut (*Castanea dentata* (Marsh.) Borkh.) once was a primary hardwood species in forests of the eastern United States. Sometime during the late 18th century, it is speculated that *Phytophthora cinnamomi*, which causes Phytophthora root rot (PRR) on many woody plant species, was introduced to the southeast region of the United States, and this pathogen spread as people moved inland from the coast (Zentmyer 1980). In the 1800s, PRR (also known as ink disease) caused extensive mortality to American chestnut trees in the southern portion of its range (Freinkel 2007, Zentmyer 1980). Then, in the early 1900s, chestnut blight, caused by *Cryphonectria parasitica*, almost eliminated American chestnut trees from eastern forests (Freinkel 2007). Since 1989, The American Chestnut Foundation (TACF) has been producing hybrid chestnut seedlings by crossing Chinese chestnut (*C. mollissima*), which is resistant to *C. parasitica*, with American chestnut and then backcrossing progeny to *C. dentata* to produce resistant American-type chestnut trees. Since 2000, hybrid seedlings planted in some locations in southeastern states have died from PRR before they could be challenged by naturally-occurring populations of *C. parasitica*. Therefore, we wanted to determine if any of the backcross trees selected for resistance to *C. parasitica* were resistant to *P. cinnamomi* as well because Chinese chestnut also is resistant to this pathogen.

Each year from 2004 to 2010, hybrid seeds from trees of known parentage were obtained from TACF cooperators, and seeds from *C. dentata* and *C. mollissima* were collected in the field. All seeds from a single cross were considered to be a family. Seeds were stratified and then planted outside in April in six or seven replicate 570 L plastic tubs filled with a soilless container mix at a field site in Oconee County, South Carolina. Each tub contained a sub-sample of seeds from each family. Inoculum consisted of two isolates of *P. cinnamomi* originally recovered from diseased chestnut seedlings growing at the study site. Isolates were grown on autoclaved rice grains or sterilized vermiculite moistened with V8 juice broth (Meadows et al. 2011, Roiger and Jeffers 1991). Seedlings were inoculated 12 to 14 weeks after planting. Inocula from the two isolates were combined, mixed thoroughly, and then evenly distributed in 1- to 3-cm-deep furrows between rows of seedlings. Seedlings were watered as needed throughout the study period, and the container mix in each tub was brought to saturation at least once while plants were actively growing to encourage disease development. Plants were evaluated in December when fully dormant. Each seedling was scored for mortality and PRR severity by examining the roots. A complete description of our methods was reported previously (Jeffers et al. 2009).

Each year, seedlings began to die approximately 3 weeks after inoculation and continued to die until the end of the growing season. *Castanea dentata* seedlings consistently were susceptible and died, and *C. mollissima* seedlings consistently were resistant and survived. In 7 years, we have tested 197 hybrid families from generations that ranged from F₁ to BC₄ (table 1). Families with seedlings resistant to *P. cinnamomi* occurred each year, but the number of resistant seedlings and PRR severity ratings varied considerably among families. Mean annual mortality for all hybrid seedling families combined was 83 percent over the 7-year period. The genes for resistance to *P. cinnamomi* and *C. parasitica* do not appear to be linked because most families were derived from backcross trees selected for blight resistance, but PRR resistance was infrequent. The pattern of inheritance of

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resistance to PRR was consistent with it being controlled by a single gene. This may facilitate introgression of PRR resistance into breeding populations of backcrossed American chestnut. The paper by Olukolu et al. in these proceedings (Genomics assisted breeding for resistance to *Phytophthora cinnamomi* in chestnut [*Castanea* sp.]) provides more detailed information on the genes controlling resistance to *P. cinnamomi* in hybrid chestnut seedlings.

Table 1—Numbers of hybrid American chestnut families screened for resistance to *Phytophthora cinnamomi* over 7 years: 2004 to 2010

Year	No. families	No. with resistance	Mortality (%)
2004	5	3	94
2005	20	9	90
2006	23	5	99
2007	41	5	80
2008	22	4	75
2009	32	8	68
2010	54	6	72

Screening of hybrid American chestnut seedlings continued in 2011 (48 families screened) and will be conducted each year for the foreseeable future as we search for resistance to PRR. In addition to hybrid seedlings, we also began to evaluate transgenic plants in 2011. These plants were developed for resistance to chestnut blight by colleagues at the State University of New York at Syracuse (College of Environmental Science and Forestry) and at the University of Georgia (Warnell School of Forestry and Natural Resources). It will be interesting to see if these plants have any resistance to *P. cinnamomi*.

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