# Screening for Resistance to *Phytophthora cinnamomi* in Hybrid Seedlings of American Chestnut[1]

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## Abstract

American chestnut (*Castanea dentata*) once was one of the primary hardwood tree species in forest ecosystems in the eastern USA. However, in the 1800s, Phytophthora root rot (PRR; also known as ink disease), caused by *Phytophthora cinnamomi*, resulted in widespread death of chestnut in the Piedmont region of southeastern states where clay soils are dominant. This was followed in the early 1900s by chestnut blight, caused by *Cryphonectria parasitica*, which almost eliminated chestnut from its primary mountain habitat. Since 1989, the American Chestnut Foundation (TACF) has been producing hybrid chestnut seedlings by crossing Chinese chestnut (*C. mollissima*) with American chestnut and then backcrossing progeny to *C. dentata* in an attempt to produce American-type chestnut trees resistant to *C. parasitica*. In recent years, hybrid seedlings planted in the field in southeastern states have died from PRR before they could be challenged by *C. parasitica*. Therefore, in 2004, we began screening hybrid seedlings for resistance to *P. cinnamomi*. In 2004 to 2006, hybrid seeds from known crosses were obtained from TACF cooperators, and seeds from *C. dentata* and *C. mollissima* were collected in the field. Seeds were stratified and then planted outside in April in replicate 568-liter plastic tubs filled with soilless container mix at a field site in Oconee Co., SC. Inoculum was produced by growing two isolates of *P. cinnamomi*, originally recovered from chestnut seedlings, on autoclaved rice grains. Seedlings were inoculated 12 to 14 weeks after planting. Inocula 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. Plants were evaluated for PRR symptoms in December when fully dormant.

Each year, seedlings started dying approximately 3 weeks after inoculation and continued to die throughout the summer months; symptoms were typical of PRR. *C. dentata* seedlings consistently were susceptible, *C. mollissima* seedlings consistently were resistant, and hybrid seedlings varied from susceptible to resistant. Resistant seedlings were planted in the field for further evaluation. Preliminary results suggest that resistance is incompletely dominant and regulated by one gene. Moreover, the genes for resistance to *P. cinnamomi* and *C. parasitica* do not appear to be linked. Screening efforts have been expanded in 2007 and will continue in coming years.

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[1] A version of this paper was presented at the Fourth Meeting of IUFRO Working Party S07.02.09, Phytophthoras in Forests and Natural Ecosystems, August 26-31, 2007, Monterey, California.

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## Introduction

American chestnut (*Castanea dentata*) once was one of the primary hardwood tree species in forest ecosystems in the eastern United States (Freinkel 2007). However, in the 1800s, Phytophthora root rot—also known as ink disease and caused by *Phytophthora cinnamomi*—resulted in widespread death of chestnut trees in the Piedmont region of the southeastern states where clay soils are dominant (Crandall and others 1945; Zentmyer 1980). This was followed in the early 1900s by chestnut blight, caused by *Cryphonectria parasitica*, which almost eliminated chestnut from its primary mountain habitat (Anagnostakis 1987; Freinkel 2007). Since 1989, The American Chestnut Foundation (TACF) has been producing hybrid chestnut seedlings by crossing Chinese chestnut (*C. mollissima*) with American chestnut and then backcrossing progeny to *C. dentata* in an attempt to produce American-type chestnut trees that are resistant to *C. parasitica* (The American Chestnut Foundation 2007). In recent years, hybrid seedlings planted in the field in several southeastern states have died before they could be challenged by *C. parasitica*. In 2003, we diagnosed Phytophthora root rot as the cause of this seedling mortality (fig 1). Therefore, we initiated a project in 2004 to screen hybrid chestnut seedlings for resistance to *P. cinnamomi*—with the objectives of identifying families with high levels of resistance and establishing a population of resistant trees for future breeding efforts.

Figure 1—Phytophthora root rot symptoms on hybrid American chestnut seedlings. **Left**, above ground: chlorosis and wilting of foliage; **right**, below ground: dead and decayed roots and a necrotic lesion advancing up from the root crown area (arrow) on the lower stem (bark removed).

## Materials and Methods

Trials began in 2004 and have been conducted each year thereafter; results from trials conducted in 2004, 2005, and 2006 are summarized here. For each trial, hybrid chestnut seeds from known crosses were obtained from TACF cooperators, and open-pollinated seeds from *C. dentata*, *C. mollissima*, and *C. pumila* (chinkapin) were collected from trees in the field. Seeds usually were received during the fall and early winter prior to planting in the spring. Seeds were mixed with moist peat and placed in a perforated plastic bag, and bags were stored in a refrigerator to stratify. In early April, when the risk of frost was minimal, germinated seeds were washed free of peat (fig. 2A) and planted outside in 568-liter plastic tubs filled with soilless container mix (Fafard 3B; Conrad Fafard, Inc., Agawam, MA) at Chestnut Return farm—a field site in Oconee Co., SC. Seeds from the same family were planted together in a row and families were replicated in multiple tubs (fig. 2B); seeds were planted in two tubs in 2004 and in six tubs in both 2005 and 2006. Plants were watered regularly throughout the growing season so that the container mix stayed moist and did not dry out. Each year, tubs were top-dressed once early in the growing season with a complete time-release fertilizer.

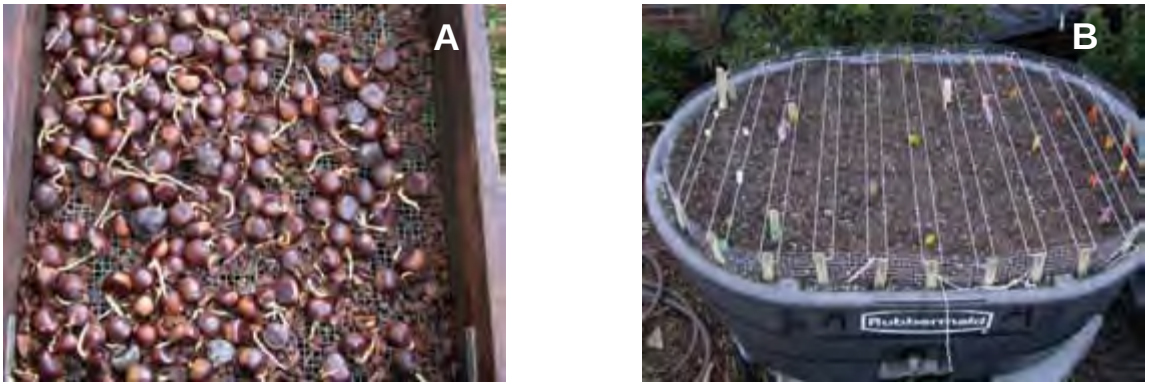


Figure 2—**A**, Germinated chestnut seeds ready for planting; **B**, plastic tub filled with container mix after planting chestnut seeds—strings identify rows and stakes delimit families.

Hybrid chestnut seedlings were inoculated 12 to 14 weeks after planting with two isolates of *P. cinnamomi* that originally had been recovered from chestnut seedlings growing at the field site. Inoculum was produced by growing each isolate axenically on autoclaved rice grains (Burns and Benson 2000) at 25°C in the dark for 10 to 14 days. Equal volumes of rice grains colonized by each isolate were combined and mixed thoroughly to produce a composite inoculum. To inoculate plants, a thin layer of rice inoculum was evenly distributed in 1- to 3-cm-deep furrows between rows of seedlings. Furrows were covered and the tubs were watered thoroughly to prevent the inoculum from desiccating. Several weeks after inoculation, the container mix in each tub was flooded once for 4 to 6 hours to promote disease development. Plants were evaluated for Phytophthora root rot symptoms in late December or early January when seedlings were fully dormant. Each plant was removed carefully from a tub—taking care to recover as much of the root system as possible. Individual plants were rated for symptom severity using a 0 to 3 scale:

0 = healthy, no visible lesions on roots
1 = lesions on at least one lateral root
2 = lesions on the tap root
3 = severe root rot, plant dead

Surviving seedlings, primarily those rated 0 or 1, were planted in an orchard at Chestnut Return farm. These plants were monitored annually for performance in the field.

## Results and Discussion

Each year, seedlings started dying approximately 3 weeks after inoculation and continued to die throughout the summer months; symptoms were typical of Phytophthora root rot (fig. 1). *C. dentata* and *C. pumila* seedlings consistently were susceptible to *P. cinnamomi* and died; *C. mollissima* seedlings consistently were resistant and grew vigorously in the infested soil. Although hybrid seedlings varied from susceptible to resistant, most were susceptible and died (1262/1693 = 75 percent; table 1). The numbers of families and hybrid seedlings evaluated increased each year, but the proportion of seedlings surviving with symptom severity scores of 0 or 1 varied depending on the genetics of the families evaluated (table 1). Over the three-year period, 43 families and almost 1700 seedlings were evaluated (table 1).

Table 1—Numbers of hybrid chestnut families and seedlings evaluated in 2004, 2005, and 2006 and the frequency of seedlings in four symptom severity (SS) classes[z]

| Year | Families (no.) | No. seedlings | | | | |
|---|---|---|---|---|---|---|
| | | Evaluated | SS = 0 | SS = 1 | SS = 2 | SS = 3 |
| 2004 | 5 | 360 | 21 | 31 | 18 | 290 |
| 2005 | 15 | 596 | 60 | 34 | 111 | 391 |
| 2006 | 23 | 737 | 8 | 24 | 123 | 581 |
| Total | 43 | 1693 | 89 | 89 | 252 | 1262 |

[z] 0 = healthy; 1 = lesions on lateral roots; 2 = lesions on tap root; 3 = plant dead

The strength of resistance in these 43 families was determined by calculating the Survival Quotient (SQ), which is expressed as percentage:

$$SQ = ([(1 \times n0) + (0.5 \times n1) + (0.25 \times n2)] / \text{total number of seedlings}) \times 100$$

where n0, n1, and n2 = no. seedlings rated 0, 1, and 2, respectively.

Of the 43 families evaluated (Table 2), 11 families (26 percent) had no survivors and a SQ of 0 percent; 18 families (42 percent) had a SQ between 0.1 and 15.0 percent; 10 families (23 percent) had a SQ between 15.1 and 30.0 percent; three families (7 percent) had a SQ between 30.1 and 40.0 percent; and one family had a SQ over 50 percent (Hyko x JB575, SQ = 56.3 percent).

Over the three-year period, 189 resistant seedlings were planted in the field for further evaluation. To date, 77 of these have survived; these 77 survivors represent

40.7 percent (77/189) of those planted in the field and 4.5 percent (77/1693) of all the hybrid seedlings evaluated. Seedlings with a symptom severity rating of 0 or 1 have survived better than those with a rating of 2. Consequently, resistance to *P. cinnamomi* was present in some of the hybrid chestnut families that had been selected for resistance to *C. parasitica*. Preliminary results suggest that resistance to *P. cinnamomi* is incompletely dominant and may be regulated by more than one gene. Moreover, the genes for resistance to *P. cinnamomi* and *C. parasitica* do not appear to be linked. Screening efforts have been expanded in 2007 and will continue in coming years.

Table 2—Strength of resistance in 43 hybrid chestnut families screened over three years, 2004 to 2006: a survival quotient was computed for each family based on the number of seedlings in each of three symptom severity classes[z]

| Family | Source of Resistance | Generation | Survival Quotient |
|---|---|---|---|
| CL-50 | Clapper | B2F2 | 0.0 |
| Andover x BX39 | Mahogany | BC3 | 0.0 |
| HP - A4 (CL248) | Clapper | B2F3 | 0.0 |
| HP - C7 (CL248) | Clapper | B2F3 | 0.0 |
| HP - L (CL248) | Clapper | B2F3 | 0.0 |
| Milliken D5 op | Clapper | B2F3 | 0.0 |
| Milliken D6 op | Clapper | B2F3 | 0.0 |
| VKN x VA307 | Clapper | B3F1 | 0.0 |
| Uxbridge KD-1 x GL356 | Graves | B3F1 | 0.0 |
| Spanish Oak Rd x IL201 | Clapper | B4F1 | 0.0 |
| VKS x CB582 | Clapper | B4F1 | 0.0 |
| Fitchburg KJ19 x BG318 | Graves | B4F1 | 0.5 |
| Swallows x TM616 | Graves | B3F1 | 0.5 |
| Pike Co Marinero x GL367 | Clapper | B3F1 | 0.6 |
| Lincoln, RI-1x QG85 | Graves | B4F1 | 1.1 |
| Fitchberg x GL96 | Clapper | BC3 | 1.2 |
| Sudbury x BE138 | Graves | BC3 | 1.4 |
| Newton CS19 x HE416 | Clapper | B4F1 | 2.4 |
| HP - B4 (CL248) | Clapper | B2F3 | 3.1 |
| rc97-107 (JoScxGR210) x opBC3 | Clapper | B3F2 | 3.2 |
| Uxbridge x GL356 | Graves | BC3 | 4.5 |
| James D-20 x Maddox MS8-12 and 9-12 | Clapper | B2F3 | 6.3 |
| rc97-m (FhSo x opAM) x opBC3 (Gr210) | Clapper | B4F1 | 6.5 |
| ob00-m (JLCe x VA307) x opBC3 | Clapper | B3F2 | 8.0 |
| ob00-025 (TRTCm x AB427) x opBC3 | Clapper | B3F2 | 9.1 |
| hu97-m (Ort x GR137) x opBC3 | Graves | B3F2 | 9.3 |
| CL-149 | Clapper | B2F2 | 10.6 |
| HP - F1 (CL198) | Clapper | B2F3 | 12.2 |
| CL-112 | Clapper | B2F2 | 12.9 |
| Milliken E7 op | Clapper | B2F3 | 17.6 |
| Frye Mtn. x B3_176 | Graves | B4F1 | 19.2 |
| ob99-199 (StT1 x GR210) x opBC3 | Clapper | B3F2 | 20.0 |
| HP - F2 (CL198) | Clapper | B2F3 | 20.6 |
| Sudbury x AB247 | Graves | BC3 | 20.7 |
| Milliken D1 op | Clapper | B2F3 | 23.3 |
| HP - F12 (CL198) | Clapper | B2F3 | 24.8 |
| CL-326 | Clapper | B2F2 | 25.0 |
| Rn88-38 x Hind | Douglas Hybrid | B2F1 | 25.0 |
| Franklin EMC x B2_214 | Mahogany | B3F1 | 29.2 |
| CL-248 | Clapper | B2F2 | 32.5 |
| Boston SB1 x JB575 | Mahogany | BC3 | 37.2 |
| Milliken Tree D1 op | Clapper | B2F3 | 37.5 |
| Hyko x JB575 | Mahogany | BC3 | 56.3 |

[z] Survival Quotient (in percent) = ([(1 × n0) + (0.5 × n1) +( 0.25 × n2)] / total number of seedlings) × 100 where n0, n1, and n2 = no. seedlings rated 0, 1, and 2, respectively

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