Optimizing American chestnut seedling root morphology

Final Report

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Long term project goal: To determine the optimal handling of American chestnut nuts to produce 1-0 bare root nursery stock with well-developed root systems to maximize survival and growth following out-planting
Short term goal 1: To identify the effect of radicle damage on root morphology of American chestnut seedlings following one year of growth in the nursery
Short term goal 2: To compare the overall morphologies of fall- versus spring-planted chestnuts.
Short term goal 3: To determine the effects of both variables on first-season survival and growth of seedlings planted in the field.

Background and Rationale

Since the American chestnut, *Castanea dentata,* was all but eliminated from North America by the imported chestnut blight in the early to middle of the 20th century, botanists, foresters, and citizen-scientists have been working to reintroduce this native tree into its former range. Although the mission has not yet been accomplished, breeding programs and advances in biotechnology have brought the goal within striking distance. Large numbers of seedlings for regeneration will be required for both research and reforestation. Unfortunately, American chestnut disappeared as a member of the dominant/ co-dominant portion of the Eastern deciduous forest canopy before a body of knowledge concerning regeneration and silviculture could be amassed. As a result, information concerning traits as basic as shade tolerance and nutrient requirements is either totally lacking or conflicting. Particularly disturbing is the lack of consensus on standards for seedling quality (Schlarbaum et al. 2006) and the best way to produce and plant those seedlings.

Trees for artificial regeneration, especially in the Eastern United States, are commonly planted as 1-0 nursery stock. The seeds are sown into nursery beds either in the fall or spring, and are lifted the following winter for planting purposes (one year in the nursery bed, zero years in a transplant bed). The seedlings are shipped bare root, with one of several media (peat moss, gel, etc.) on the roots to provide moisture. This type of hardwood planting stock should be stored cold after lifting from the nursery bed, and ideally should be planted before bud break in the spring. Although bare root nursery stock is not the only method used to produce seedlings for forest research and reforestation, it has many advantages which lend itself to hardwood regeneration in general. When 1-0 nursery stock is lifted from the nursery bed, there is the opportunity to examine the seedlings and cull those whose growth rate and morphology indicate inferior growth potential. Even from the same mother tree, offspring will exhibit a great deal of inherent variation in out-planting fitness, separate from any cultural effect (Kormanik 1986; Kormanik et al. 1997, 2000). Bare root culture would allow the opportunity for grading standards based on morphology, including the very important root form, to be developed in the future.

As opposed to containerized seedlings, the roots of bare root hardwood seedlings integrate more readily with the native soil, instead of staying largely within the potting mix-filled planting hole (Schulte and Whitcomb 1975). Clearly, given the size of a growing American chestnut tree, the root architecture of the plant must be sufficient to prevent tree toppling down the road, a problem which has plagued some conifer plantations derived from containerized stock (Burdett, et al. 1986, Halter et al. 1993). While no long-term studies have been conducted comparing the survival and growth rates of bare root, containerized, and direct-seeded chestnut plantings, one short-term study (Phelps et al. 2006) concluded that initial survival rates were not different for containerized and bare root American chestnut nursery stock, while direct seeded plants were not able to effectively compete with on-site vegetation. When the cost is factored into the reforestation equation, bare root is certainly an attractive option, since containerized hardwood plantings cost about 4.5 times more than bare root plantings (Sweeney et al. 2002).

Every tree species, or at least every tree genus, has unique properties which require consideration by the people charged with producing advanced regeneration. For American chestnut, the challenge begins with its recalcitrant seed. The nuts are highly perishable, and not given to long-term storage (Woodroof 1963). In *Seeds of Woody Plants in the United States* (1974), Sanders recommends collecting the mature nuts at least every other day, curing them at 60^{o}F to 70^{o}F for one to seven days, then either sowing them immediately into a nursery bed or storing them in moist medium at 30^{o} F to 36^{o} F with a relative humidity of 70% for spring planting in the nursery. Although American chestnut's propensity to germinate while in cold storage was noted, Sanders made no recommendations on handling the germinated nuts during sowing. Quality differences between fall and spring planted seedlings were not explored. The American Chestnut Foundation's recommendation on pre-germinated nuts is to: "not be concerned" about inadvertent radicle damage (TACF 2002). In fact the Handbook states "...some growers suggest that breaking off the radicle (or initial root) yields a more vigorous root system." The American Chestnut Cooperators' Foundation, on the other hand, stresses the need to handle taproots gently when planting germinated nuts or transplanting seedlings from the pot to the field (www.ppws.vt.edu/griffin/grow.html). Neither publication cites references to support their recommendations.

The handling of nuts, especially germinated ones, becomes a critical issue for reforestation. First, mechanical planters, like those used for oaks and other large-seeded species, were not designed to gently handle acorns or nuts whose radicles have already emerged. Second, and more critically, is the question of how radicle damage affects the root morphology of American chestnut. Do the changes to root morphology, if any, make the seedling more or less difficult to lift and plant? Even more importantly, how do these alterations affect out-planting survival and growth, both in the short and long term? Finally, from the perspective of our lab's work with American chestnut embryogenic cultures, understanding the importance of the radicle is critical to how we will go about producing and handling our somatic seedlings.

Preliminary study

To begin to gain a better understanding of the situation, we conducted a preliminary study beginning in fall 2007. Chestnuts were germinated in the greenhouse (Fig. 1) and planted at the Georgia Forestry Commission's Flint River Nursery. Half of the nuts were planted gently, with their radicles intact, while half of the nut had their radicles deliberately broken, such as would typically happen when handling germinating nuts. The American chestnuts were handled identically to all other hardwoods in the field, according to published protocol (Kormanik et al. 1994). In March 2008 (Fig. 2) the seedlings were lifted, and their roots examined for differences in morphology. The differences in the two treatments were dramatic. Seedlings from nuts whose radicles had been broken did not develop a taproot at all. Instead, a flat, shallow root system was evident in every nut that received this treatment (Fig. 3). Seedlings from nuts which were carefully planted so as to preserve the radicle produced deep root systems, with strong laterals emerging along the length of the taproot (Fig. 4). Subsequently, these seedlings were out-planted in North Georgia at the UGA Mountain Research and Education Center at Blairsville, GA. They are currently being grown without irrigation, and will be monitored for performance. This study is still in progress and is only preliminary, lacking sufficiently large sample size for adequate statistical analyses. However, the dramatic difference in treatments, with the potential effect on out-planting performance, strongly suggests a need for a more extensive study.

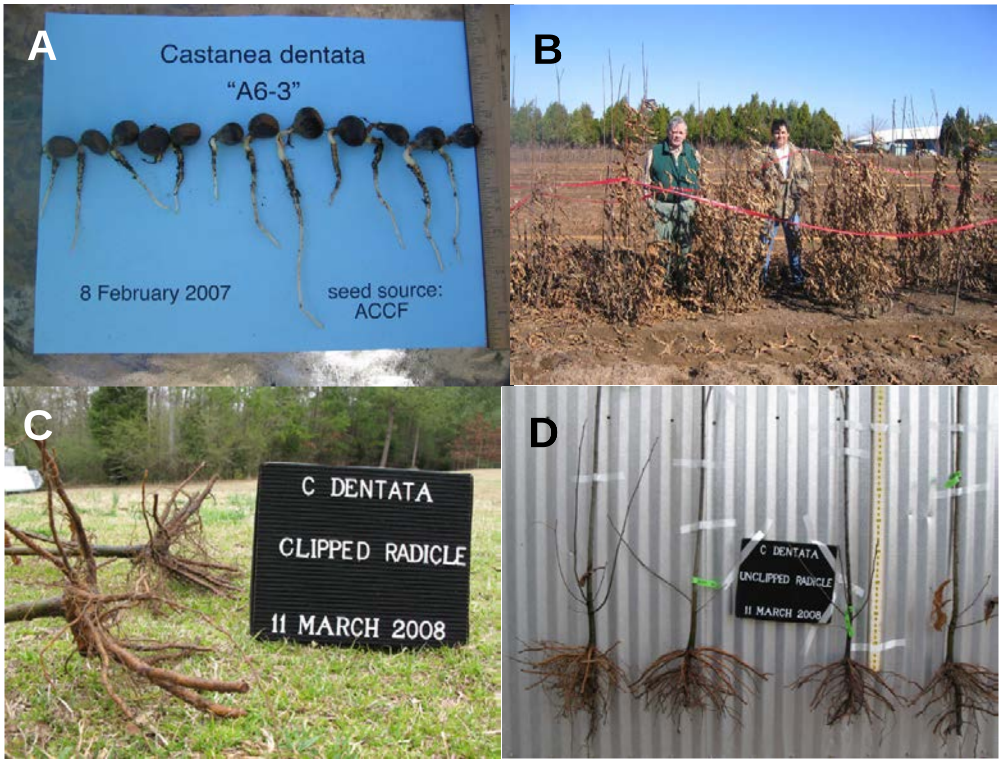

Figure 1. Preliminary test of radicle-clipping treatment. **A.** Germinated American chestnuts prior so planting in the nursery. **B.** 1-0 American chestnut seedlings in the nursery just prior to lifting in March 2008. **C.** Damaging the radicle prior to planting results in a flat root system morphology totally lacking a taproot. **D.** Nuts planted with intact radicles develop a deep root system with strong laterals.

Year 1

Open pollinated nuts from four American chestnut mother trees (Bell Hollow, Plummer S, Plummer 1I and Plummer 4) were obtained from Fred Hebard at TACF. On 13 January 2010, the first of four field treatments was planted at the Georgia Forestry Commission's Flint Nursery, located in Dooley County, Georgia. These chestnuts were planted whole, before the radicles had emerged from the seeds. The seeds were covered with pine bark, and kept moist to prevent desiccation before germination (Fig 1).

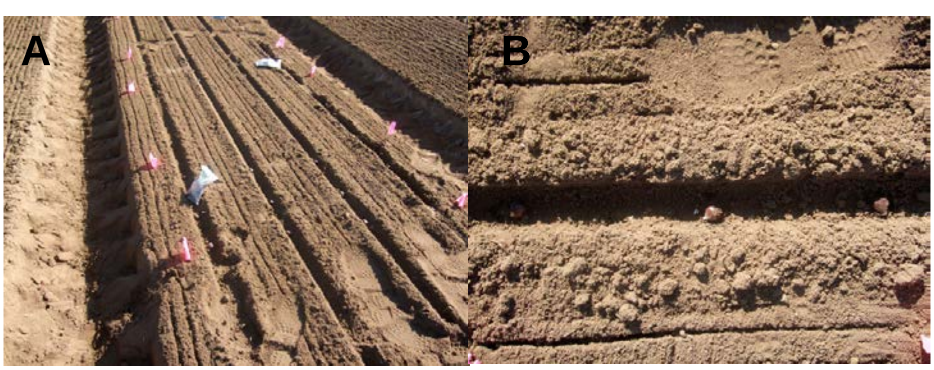

Fig. 1. A-D. Planting American chestnuts at the Georgia Forestry Commission's Flint Nursery, 13 January 2010.

On 26 January 2010, nuts from two mother trees (Plummer S and Plummer 1I) were planted in pots in a greenhouse at the University of Georgia Warnell School of Forestry and Natural Resources' Whitehall Forest. By 5 February, the radicles were long enough to apply treatments. Four treatments were applied: (1) radicle meristem removed, (2) radicle cut midway between the tip and the base, (3) radicle cut at the base and (4) radicle intact (Fig. 2). The chestnuts were carefully planted in pots, and are being grown in the greenhouse until the lateral and adventitious roots emerge. Histological sections will be taken from the different treatments to see whether all the new lateral roots emerge from the pericycle, or if radicle damage causes adventitious root development that arises from other cell types.

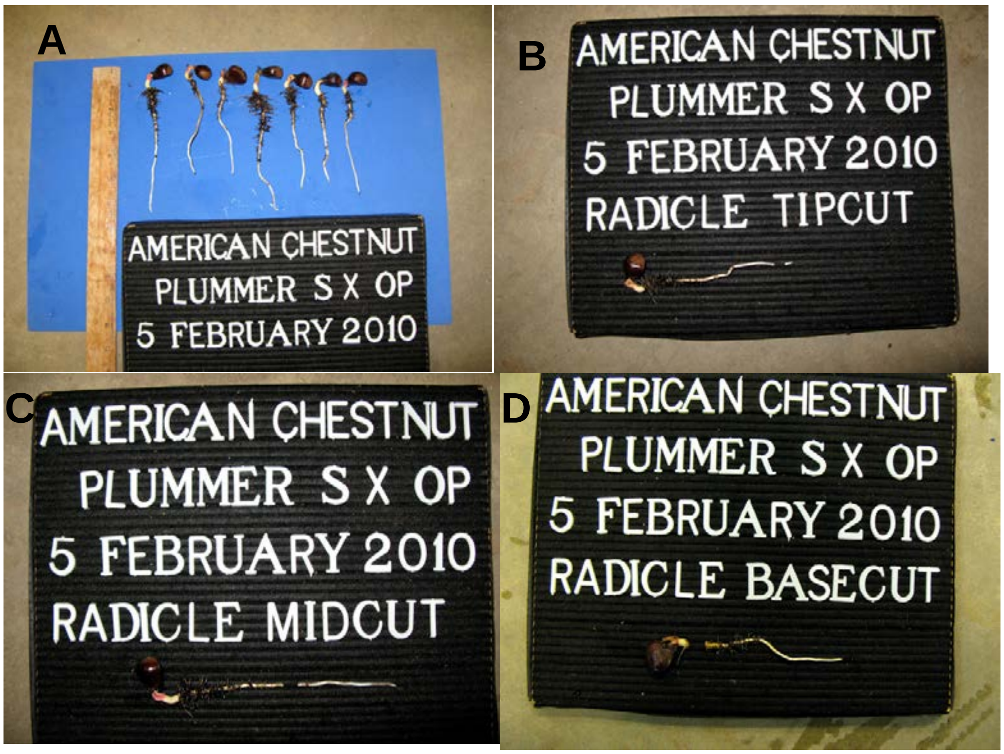

Fig. 2. Four radicle treatments were applied to germinated nuts prior to planting for future histological examination of root system.

On 25 March 2010, the remaining three treatments were installed at Flint Nursery. Most of the remaining chestnuts had, by this time, germinated in refrigerated storage. For each mother tree, three treatments were applied before planting in the nursery bed: (1) radicle intact, (2) radicle tip cut and (3) radicle base cut. The chestnuts were carefully planted so no further damage was done to the radicles (Fig. 3A). By this date, the first field treatment, planted on 13 January 2010, had both root strike and top growth. Most of the seedlings in this treatment had between four and six true leaves (Figs. 3B, 3C).

Fig. 3. Spring at Flint Nursery. (A) Planting the last three treatments at Flint River Nursery. B and C. Winter planted American chestnuts, up and growing.

The histology work on the American chestnut roots will be completed this year. The chestnut seedlings planted at Flint Nursery will be grown according the standard protocol applied to all hardwoods at that nursery. In late winter 2011, these chestnuts will be lifted, and root morphology evaluated. They will be out-planted, and data on the effect of radicle damage on survival and growth of chestnut seedlings will be collected and analyzed. Results of this study will give those interested in growing American chestnuts, from nursery personnel to hobbyists, more information on how to best produce chestnut seedlings for reforestation.