EFFECTS OF SOIL AMENDMENT TREATMENTS ON AMERICAN CHESTNUT PERFORMANCE AND PHYSIOLOGY ON AN EAST TENNESSEE SURFACE MINE[1]

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Abstract: Successful reforestation on mine sites requires the use of species adapted to harsh soil and site conditions. Research has shown that American chestnut (*Castanea dentata*) may be a suitable species due to its historical presence on xeric, nutrient limited sites, which are characteristic of many surface mines. Here we compare seedling survival and performance, through various physiological parameters, of American chestnut planted on two sites in eastern Tennessee. A seedling with high performance is identified as having greater height, greater apical elongation, greater root collar diameter, greater photosynthetic rate, and lower water stress than poorly performing seedlings. Understanding how this species responds to surface mine planting treatments will aid reforestation experts in achieving reforestation and simultaneously restoring American chestnut. This study was carried out on a mine site reclaimed using the Forestry Reclamation Approach. Two sites, containing two plots each, had similar substrates, but differed in topography and material placement. Nine treatments were applied contemporaneously during planting in a factorial arrangement: forest topsoil (sterilized and un-sterilized), Terra-Sorb (applied and not applied), and fertilizer pellets (applied and not applied). Chestnuts were direct- seeded in rows with randomly assigned treatments. The first and second year survival rates of 29 and 28% were unacceptably low for successful reforestation. Fertilizer application reduced survival, but increased both natural height and root collar diameter over the first year in surviving seedlings. Further, fertilizer increased the rate of transpiration, and resulted in a more negative water potential. Terra-Sorb reduced survival, but increased natural height and root collar diameter, most likely as a result of a lesser degree of water stress. Lastly, the inclusion of sterile soil reduced survival, but increased photosynthetic rate.

Additional key words: photosynthesis, water potential, fertilization, soil amendments, surface mine reforestation, forestry reclamation approach, ARRI

Introduction

In the recent past, forest reclamation was often overlooked on mine sites due to previous reforestation failures and belief that grass fields were less expensive to achieve (Angel et al., 2005). However, recent research shows that mine site reforestation can allow cost efficient, productive forests if performed under reclamation guidelines, utilizing the Forest Reclamation Approach (FRA) described by Burger et al. (2005). In response, mine sites utilizing reforestation as a post-mining land use are increasing (Groninger et al., 2007). As research advances, benefits are being realized. Reforestation benefits can include equal or greater productivity compared with agronomic reclamation and undisturbed forest soils (Burger et al., 2005), and erosion control (Torbert and Burger, 1994). However productive the site may be, the selected trees for reforestation must be adapted to the high light intensity and quickly draining soils that are typical of mine sites. Consequently, many projects utilize degradation-tolerant but potentially low value species such as black locust (*Robinia pseudoacacia* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) (Rathfon, et al., 2004). Therefore, a species is needed that is potentially valuable with adaptation to xeric sites which are often nutrient limited and under a high light intensity. Pine species (*Pinus* spp.) have often been used that meet these conditions. However, pine plantations don't represent the native mixed hardwood forest type of the southern Appalachians. For this reason, among others the American chestnut (*Castanea dentata* (Marsh.) Borkh.) is being considered for mine reclamation.

American chestnut was once an abundant species that dominated Eastern deciduous forests of the U.S. (Russell, 1987). Although very plastic in site requirements, American chestnut populations occurred most commonly on mid-slope sites and ridges with deep quickly draining soils for tap root penetration (Ashe, 1912; Russell, 1987). Therefore, chestnut could out-compete other tree species that were more moisture and nutrient demanding on these harsh sites (Ashe, 1912). The American chestnut has not been available in the past as a candidate for mine reclamation because of the chestnut blight. Since the blight is assumed to be more widely spread than the historic native range of the chestnut itself, there was little hope for reclamation without high chestnut mortality. In addition to the chestnut blight, another pathogen has historically threatened chestnut populations. Phytophthora root rot (*Phytophthora cinnamomi*) occurs in the same native soils that American chestnut once dominated. Phytophthora infects the host's roots,

most notably producing root rot, collar rot, branch dieback and defoliation, eventually causing mortality (Rhoades et al., 2003).

Over the past 30 years, the American Chestnut Foundation has developed an American-Chinese backcross (*Castanea dentata* x *mollissima*) which shows resistance to the blight (Hebard, 2005) while maintaining the desired morphological characteristics of pure American chestnut (Diskin et al., 2006). Further, research has shown that the high light levels on recently disturbed sites can provide rapid growth in American chestnuts (McCament and McCarthy, 2005). The Office of Surface Mining (OSM), Appalachian Regional Reforestation Initiative (ARRI), and The American Chestnut Foundation (TACF) suggest restoration of the American chestnut could coincide with the reclamation of mine sites. Particular interest is paid by these organizations to the backcrossed chestnut on mine sites due to the overlapping historic range of American chestnut with the Appalachian coal seam (introducing many surface mines as suitable planting sites), initial absence of phytophthora on recently reclaimed mine sites, the wide range of suitable habitat in which American chestnut grew, and its tolerance to slightly acidic soils. Further, the rapid initial growth exhibited by backcrossed chestnut seedlings will provide aesthetic values in conjunction with wildlife habitat and timber resources (Jacobs and Selig, 2005). Because the backcrossed chestnut is 15/16 American chestnut by lineage, growth and establishment are expected to be similar to that of the American parent.

Mine sites reclaimed using the FRA have shown a higher site index for some trees when compared to an undisturbed forest sites (Burger et al., 1998). However, the conditions on mine sites are unlike forested sites and therefore may not be suitable for all Appalachian forest species. Mine sites often have compacted overburden, which can lead to slow growth of trees (Sweigard et al., 2007). Further, water availability is often low on mine sites due to high coarse fragment content and lack of topsoil (Rodrigue and Burger, 2004). Nutritional content of the rooting medium is also a limiting factor in plant performance and mine site productivity (Kozlowski et al., 1991; Rodrigue and Burger, 2004; Walker, 2002). Lastly, the high light levels on mine sites will likely influence above-ground growth through photosynthetic response. Physiologic responses of American chestnut to mine spoil and site characteristics must be understood in order to successfully restore this species on mine sites.

The objective of this study was to determine the effect of planting treatments on the physiology and performance of American chestnut. It was hypothesized that: (1) planting treatments would have an effect on seedling survival and performance (performance is identified through height, apical meristem elongation, root collar diameter, photosynthetic rate, transpiration rate, specific leaf area and water stress); (2) treatments with the Terra-Sorb (Plant Health Care Inc., Pittsburgh, PA) amendment would differ from treatments without Terra-Sorb due to an increased water availability to the seedlings, resulting in greater water and nutrient transport, increasing seedling growth; (3) treatments with fertilizer will differ from treatments without the fertilizer amendment, as measured by growth and photosynthetic rate; (4) treatments with sterilized soil will differ from treatments with unsterilized soil in their effects on growth and photosynthetic rate.

Methods

Study site

This study site is located at Zeb Mountain, Tennessee (N 36.48972, W -84.27361, elevation ~700m) in an area mined using the cross ridge mining technique by National Coal Corporation. At this location, two sites were used with two plots within each (Fig. 1). The first site (plots A and B) conditions are characterized by a gentle slope with a northeasterly aspect and compacted subsurface material covered with uncompacted spoil piles, or end-dumps of overburden. The FRA recommends planting substrate be of the "best available material" (Burger et al., 2005). Because this was a demonstration site, the substrate material was selected to contain a relatively large proportion of brown sandstone (~50%) and small amounts of residual topsoil. Plot A was placed on the upper portion of the site, while plot B was placed on the downslope half of the plot just above a sedimentation pond. The second site (plots C and D) is characterized by a steep slope with a southeasterly aspect comprised of compacted backfill with uncompacted overburden contoured to the slope. In both cases, the uncompacted substrate was not less than 4 feet in depth. The substrate consists of a mixture of brown weathered sandstone and shale in proportions of approximately 30:70, and trace amounts of topsoil. Surface layers of substrate on plot C appeared to have larger proportion of sandstone, while plot D had a larger proportion of shale. Soil pH ranged from 6.8 to 7.2. These 4 sites represent the varied conditions existing across this reclaimed area.
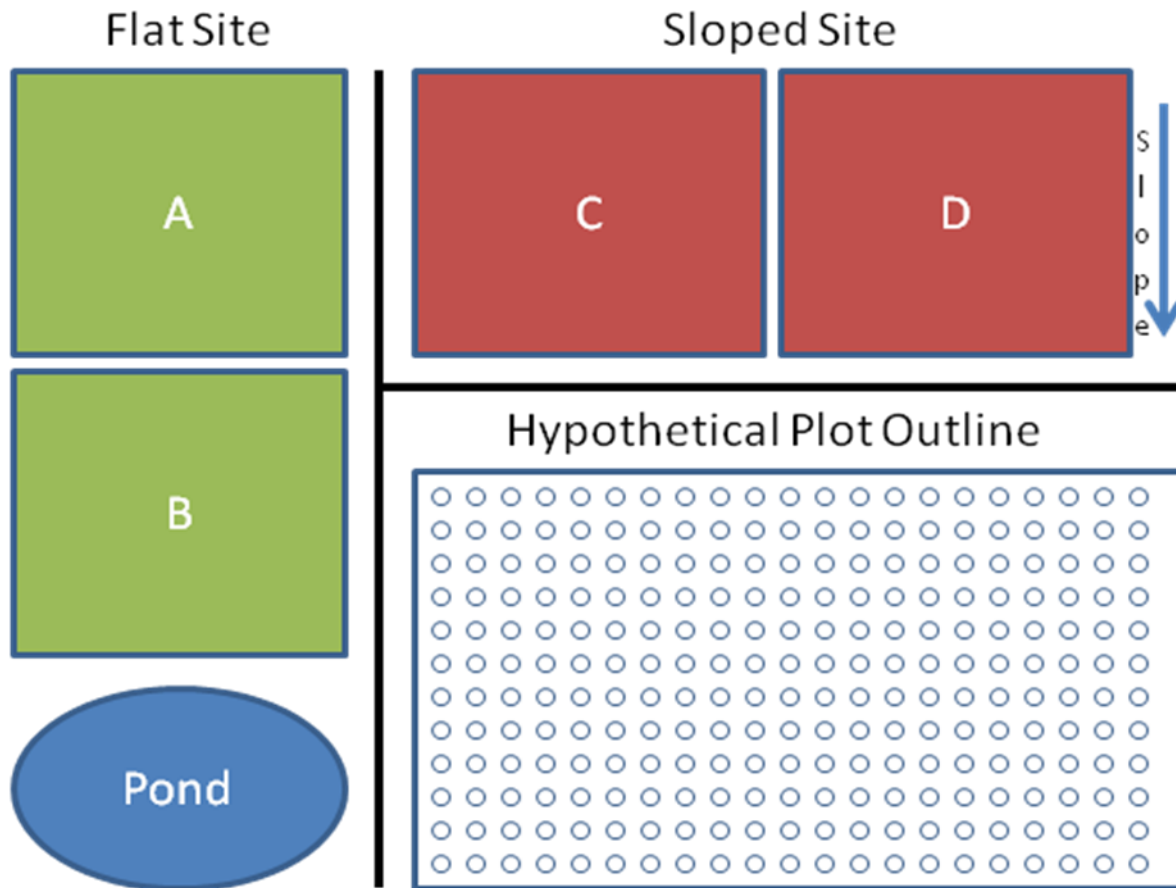
Figure 1: Mine site plot layouts for the flat site (A and B), sloped site (C and D) and a hypothetical plot outline. Plot row and column number varies between each plot. Within the hypothetical plot outline, seeds were planted at 1.5 m intervals.

Planting

Ten treatments were applied in a randomized complete block design, with blocks referring to 4 plots, A through D. Treatments were applied in a 2x2x2 factorial arrangement with factors of forest topsoil which was either sterilized or unsterilized, Terra-Sorb (Plant Health Care Inc., Pittsburgh, PA) which was applied or not applied, and fertilizer pellets (Scott's Agriform 20-10-5, The Scotts Company, Columbus, OH) which were applied or not applied (Table 1). Treatment design was created in collaboration with the American Chestnut Foundation to aid in determining best planting methods for chestnut restoration on mine sites.

Table 1: Planting treatments for American chestnut direct seeded at the mine site in May, 2008.

Treatment	½ cup Pro-Mix	½ cup Non-Sterilized Forest Topsoil	½ cup Sterilized Forest Topsoil	12 oz. hydrated Terra-Sorb	Two 10 oz. Scott's Agriform 20-10-5 fertilizer pellets
1	✓	✓		✓	✓
2	✓	✓			✓
3	✓		✓	✓	✓
4	✓	✓		✓	
5	✓		✓		✓
6	✓	✓			
7	✓		✓	✓	
8	✓		✓		

Topsoil was collected from a forested stand in the Cherokee National Forest, within the native range of the American chestnut. The stand was typical of an upland hardwood forest, dominated by chestnut oak and northern red oak. Historically, this site was host to American chestnut as evidenced by several chestnut sprouts and stumps. Topsoil from the top 3 inches of the A horizon was removed from a 10'x10' plot. Soil was stored in large plastic bins at 4°C in a walk-in cooler until it was ready for use. Sterilization was performed through moist heat autoclaving for 25 minutes at 121°C.

Chestnuts were obtained from the American Chestnut Foundation. Nuts with emergent radicles were direct-seeded in rows on plots A and B in March, 2008. Plots C and D were direct-seeded in rows in May, 2008. Treatments were randomly assigned to planting locations along rows, spaced at 1.5 meter intervals. Terra-Sorb was hydrated, and mixed with Pro-mix and forest topsoil in a 20L pail. One-half cup of this mixture was placed in a hole approximately 10 cm deep, the seed was placed on top of the mixture, and one-half cup of soil was placed on top. Where fertilizer tablets were used, they were placed at the bottom of the hole. In addition to the 20-10-5 concentrations of nitrogen, phosphorous, and potassium, the tablets also contained 2% S, 2.8% Ca, 0.5% Fe, 0.5% Mg, 0.05% Mn, 0.05% Zn, 0.05% Cu, and 0.05% B. After treatments were applied to the planting area, seeds were covered with 2.5 – 5 cm of native mine spoil. Above the planted seed was installed a 46 cm Blue-X tree shelter, secured to a wooden stake and surrounded at the base by native rocks to deter rodent herbivory.

Measurements

Survival, natural height, root collar diameter (RCD), photosynthetic rate, transpiration rate and water potential were recorded for each surviving seedling to assess growth and physiological response to treatments. Natural heights, a measure of crown height from the soil surface, were recorded from root collar to the highest live bud. Growth was monitored using natural height and RCD. Physiological responses of seedlings were measured through photosynthetic rate, transpiration rate and water potential. Growth measurements and survival were recorded at the end of growing periods in October 2008 and October 2009.

Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were recorded using a LI-COR LI-6400 Portable Photosynthesis System with Leaf Chamber Fluorometer and CO_2 Injector System attachments (LI-COR Biosciences, Lincoln, NE). These attachments allow for regulation of photosynthetically active radiation (PAR) and CO_2 concentration such that ambient conditions are extraneous from conditions within the leaf chamber. Within the leaf chamber, PAR was maintained at 1500 nm, 10% of which was blue light. Flow rate was 500 μmol, reference CO_2 was 350 μmol, and temperature was within 2 °C of ambient conditions. Photosynthetic measurements were recorded between 8:00 a.m. and 12:00 p.m., at peak photosynthetic activity for American chestnut (unpublished data), on Sept. 29 and October 9, 2009. This was near the end of the growing season, but well before seasonal declines in foliar pigments. All seedlings with leaves were measured. On each seedling, the leaf selected for photosynthetic measurements was the uppermost fully-expanded and undamaged leaf that was greater than 2 cm^2.

Pre-dawn stem water potential was used as a proxy for total plant water potential and was recorded using a PMS Scholander Pressure Chamber (PMS Instrument Company, Albany, OR) with nitrogen gas used for chamber pressurization. All seedlings with at least one lateral stem bearing at least one undamaged leaf were selected for measurements. One lateral stem that was at least 8 cm long was excised with shears, and measured immediately. Measurements were made in September, 2009.

Data Analysis

Main effects and interactions were tested using an univariate analysis of variance general linear model, using SPSS 17.0 (SPSS Inc., Chicago, IL). Prior to analysis, outliers were

identified as data points > 2 standard errors from the mean, and were not included in the model. Where significant main effects were found for survival, differences were compared using t-tests. For all other dependent variables (height, RCD, water potential, photosynthesis), where significant main effects were found, contrasts of between-subject effects were used to determine significance at an alpha level of 0.05. Where a significant interaction three-way was found between main effects, results for each treatment are presented separately, however these were not compared statistically.

Results

Mean overall seedling survival was similar in the first year (2008) (29% ± 5.4 standard error) when compared to the two-year survival (2009) (28% ± 3.7) (Fig. 2 and 3). However, in both years, n = 100. Differences in percent survival between years are due to plot-based means being used for the t-test and possible rounding errors. While some seedlings suffered mortality over the second year, others were recorded for the first time in the second growing season. In 2008, addition of fertilizer, Terra-Sorb, and the sterile soil treatment reduced survival. The greatest survival was observed for seedlings planted without fertilizer. The lowest survival was observed in seedlings planted with fertilizer. Fertilizer presence decreased survival by about 11% compared with the absence of fertilizer; Terra-Sorb decreased survival by 3% compared with the absence of Terra-Sorb; and sterilized soil reduced survival by 7% compared with non-sterilized soil (Fig. 2). This pattern extended into 2009 where fertilizer presence decreased survival by 8%, Terra-Sorb decreased survival by 1%, and sterilized soil decreased survival by 3% (Fig. 3). However, addition of Terra-Sorb and fertilizer to the soil substrate increased natural height in 2008 by 10 cm (p = 0.002) and 21.9 cm (p < 0.0001), respectively (Fig. 4). Accordingly, RCD increased by 1.5 mm with Terra-Sorb (p = 0.001) and 2.7 mm with fertilizer presence (p < 0.0001) (Fig. 5). The tallest seedlings observed were those planted with fertilizer, the shortest were those without fertilizer (Fig. 4). A similar pattern was found for RCD (Fig. 5).

In 2009, Terra-Sorb and fertilizer main effects were significant (p = 0.012, p = 0.015, respectively), with both increasing natural height by over 25% (Fig. 6). Again, greatest height occurred in seedlings planted with fertilizer. Interestingly, significant main effects on RCD were absent in this second year of growth.
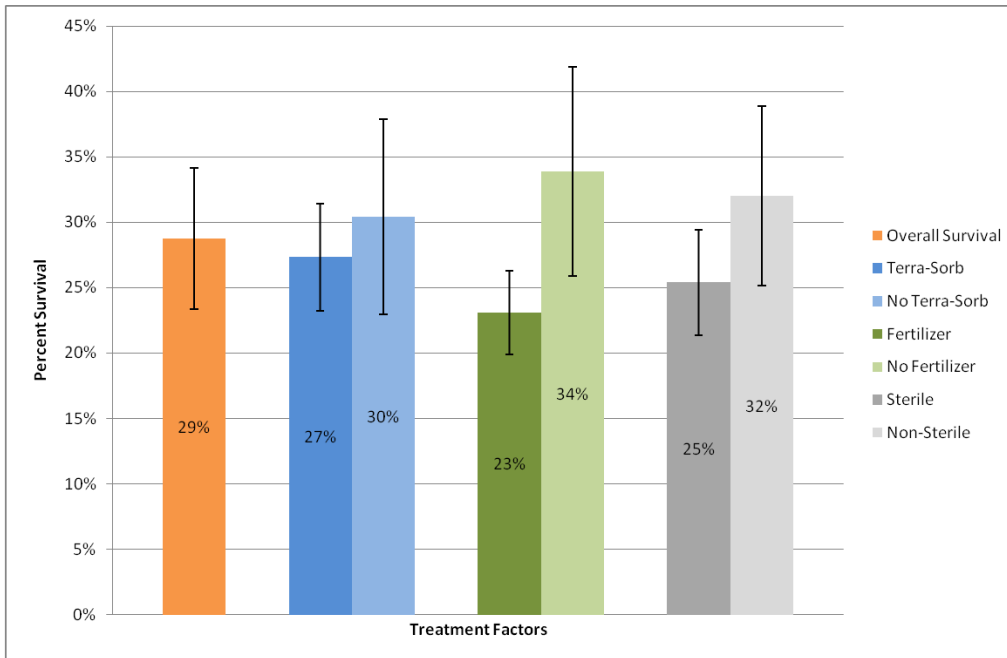
Figure 2: October 2008 survival for chestnut planted on the mine site in the spring of 2008. Means ± standard errors are shown for treatment main effects. Overall survival n = 100. In the order of treatment main effects as listed on the figure, n = 46, 54, 39, 61, 45, 55, respectively.
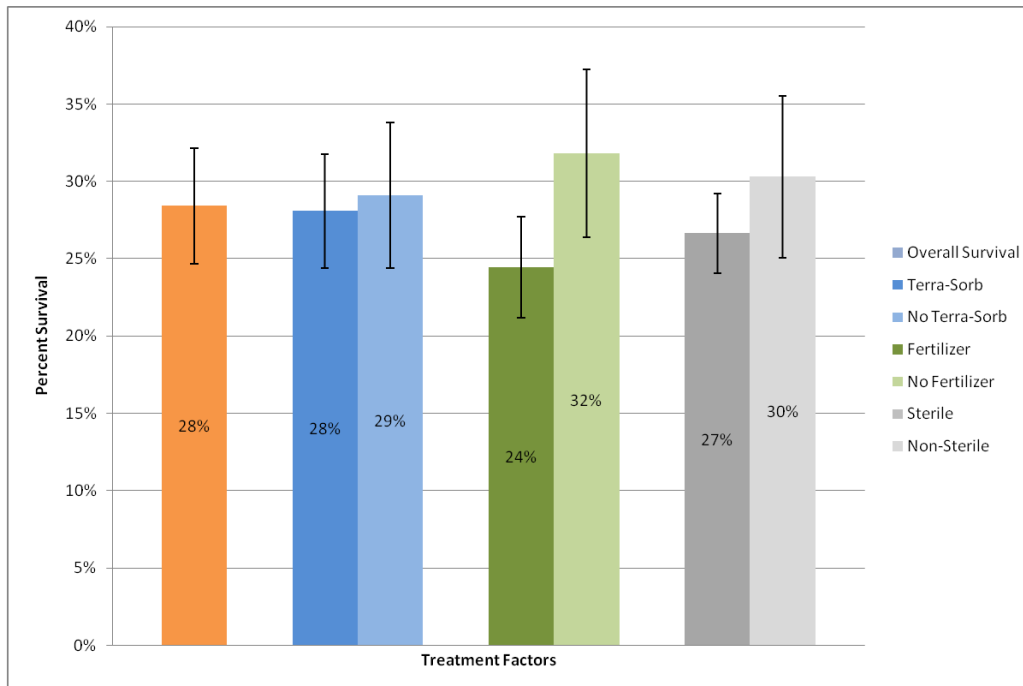

Figure 3: October 2009 survival for chestnut planted on the mine site in the spring of 2008. Means ± standard errors are shown for treatment main effects. Overall Survival n = 100. In the order of treatment main effects as listed on the figure, n = 47, 53, 42, 58, 48, 52, respectively.
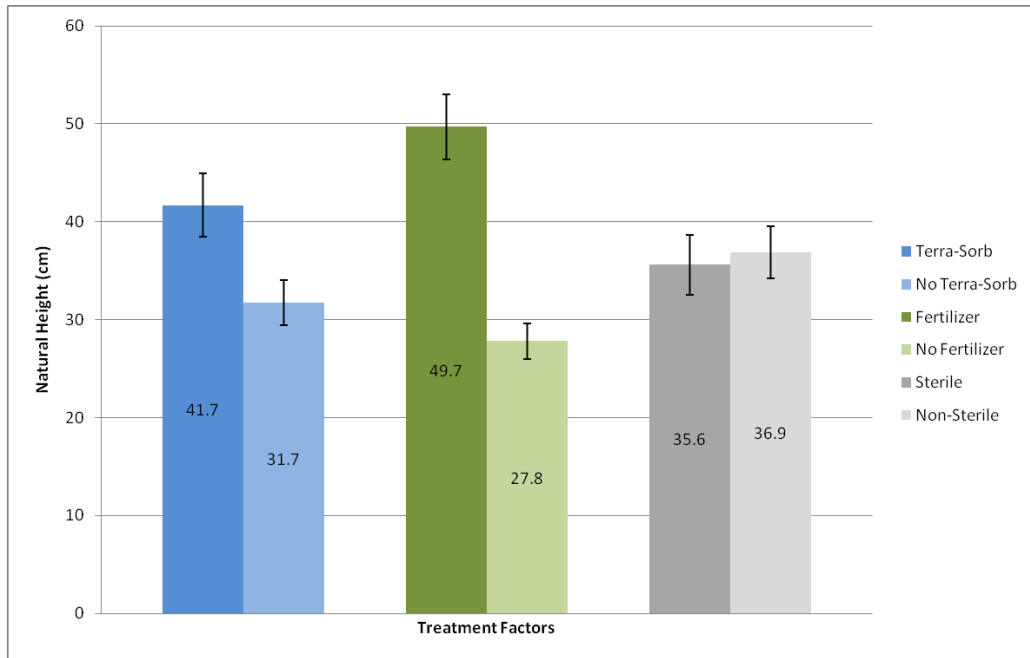
Figure 4: October 2008 natural height for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant (p = 0.012, p = 0.005, respectively). In the order of treatment main effects as listed on the figure, n = 46, 53, 39, 60, 45, 54, respectively.
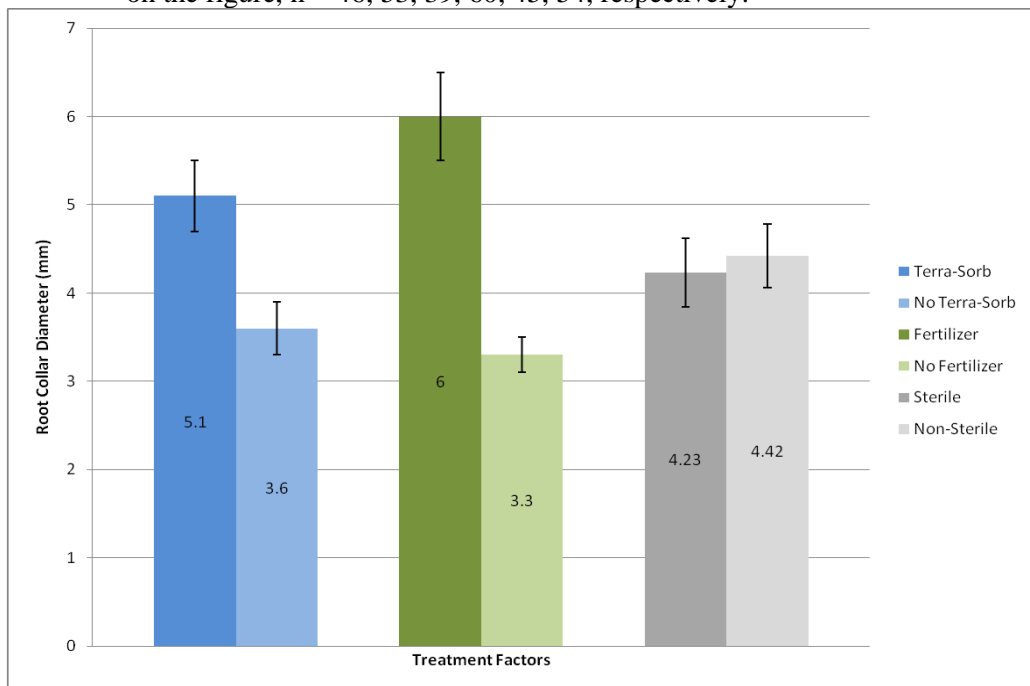

Figure 5: October 2008 RCD for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant (p = 0.001, p < 0.0001, respectively). In the order of treatment main effects as listed on the figure, n = 46, 53, 39, 60, 45, 54, respectively.
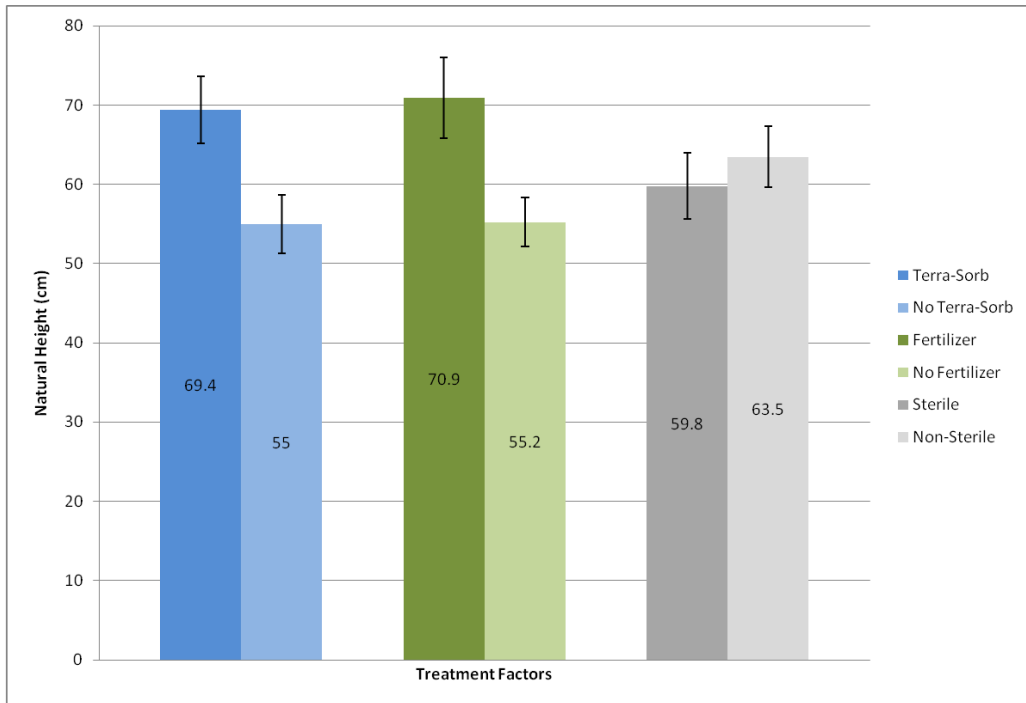
Figure 6: October 2009 natural height for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant (p = 0.001, p < 0.0001). In the order of treatment main effects as listed on the figure, n = 48, 55, 43, 60, 49, 54, respectively.

Presence of the sterile soil treatment increased photosynthetic rate by 1.24 μmol CO_2 m^{-2} s^{-1} (p = 0.017), producing the highest rate when compared to other treatment main effects (Fig. 7). However, this was not reflected in transpiration rate, which was significantly affected only by fertilizer, increasing by 0.54 mmol H_2O m^{-2} s^{-1} (p < 0.0001) (Fig. 8).

Water potential differences were significant within all three treatment main effects. The presence of Terra-Sorb and the sterile soil treatment provided less negative pre-dawn water potentials (p = 0.021, p = 0.023, respectively). However, fertilizer presence produced a more negative pre-dawn stem water potential (p = 0.015) (Fig. 9). Further, there was a significant three-way interaction between all treatment levels. The combination of the sterile soil treatment, fertilizer, and Terra-Sorb resulted in the most negative water potential at -0.93 MPa (p = 0.007). Interestingly, the treatment with the least negative water potential was not the antithesis of the previously mentioned, but the combination of the sterile soil treatment, no fertilizer, and no Terra-Sorb with a water potential of -0.45 MPa (p = 0.007) (Fig. 10).
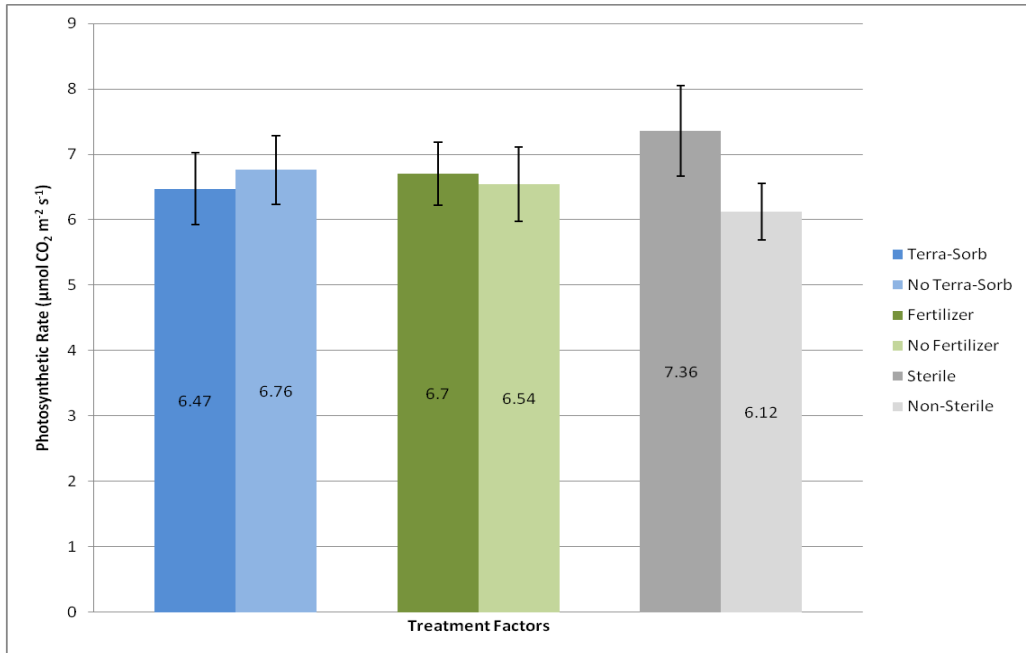
Figure 7: October 2009 photosynthetic rate for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Presence of the sterile soil treatment was the only significant treatment main effect (p = 0.017). In the order of treatment main effects as listed on the figure, n = 48, 46, 41, 53, 37, 57, respectively.
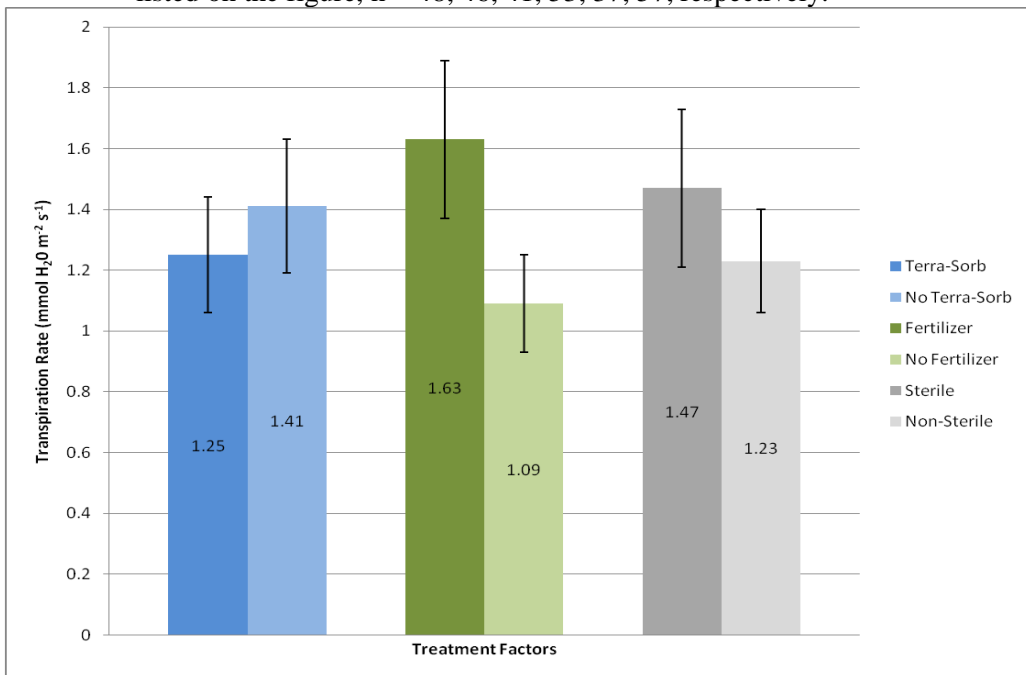

Figure 8: October 2009 transpiration rate for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Presence of fertilizer was the only significant treatment main effect (p < 0.0001). In the order of treatment main effects as listed on the figure, n = 48, 46, 41, 53, 37, 57, respectively.
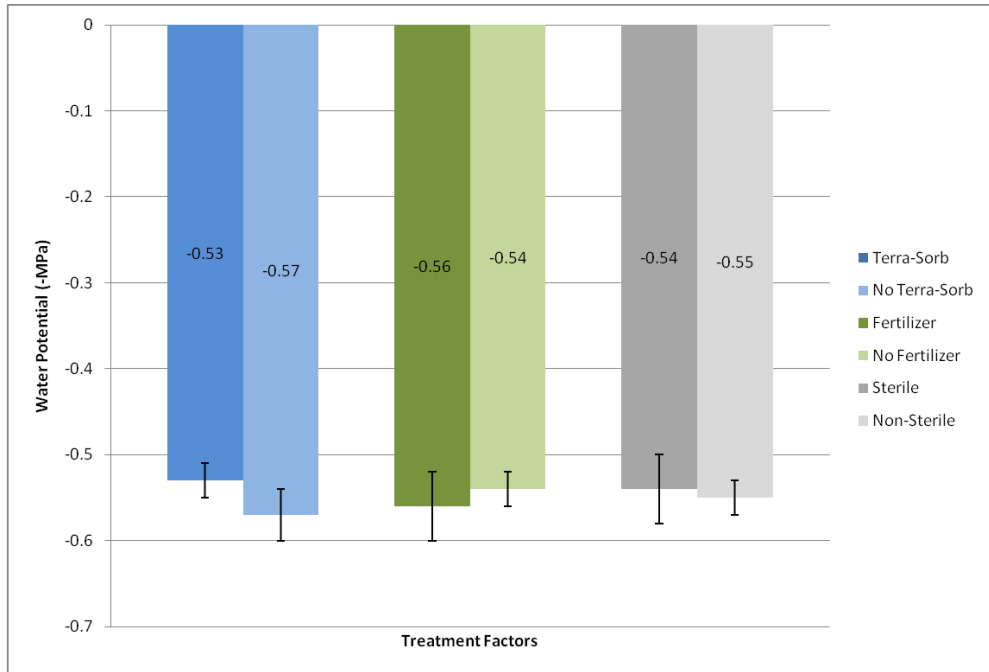
Figure 9: September 2009 pre-dawn water potential for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. In the order of treatment main effects as listed on the figure, n = 42, 36, 32, 46, 31, 47, respectively.
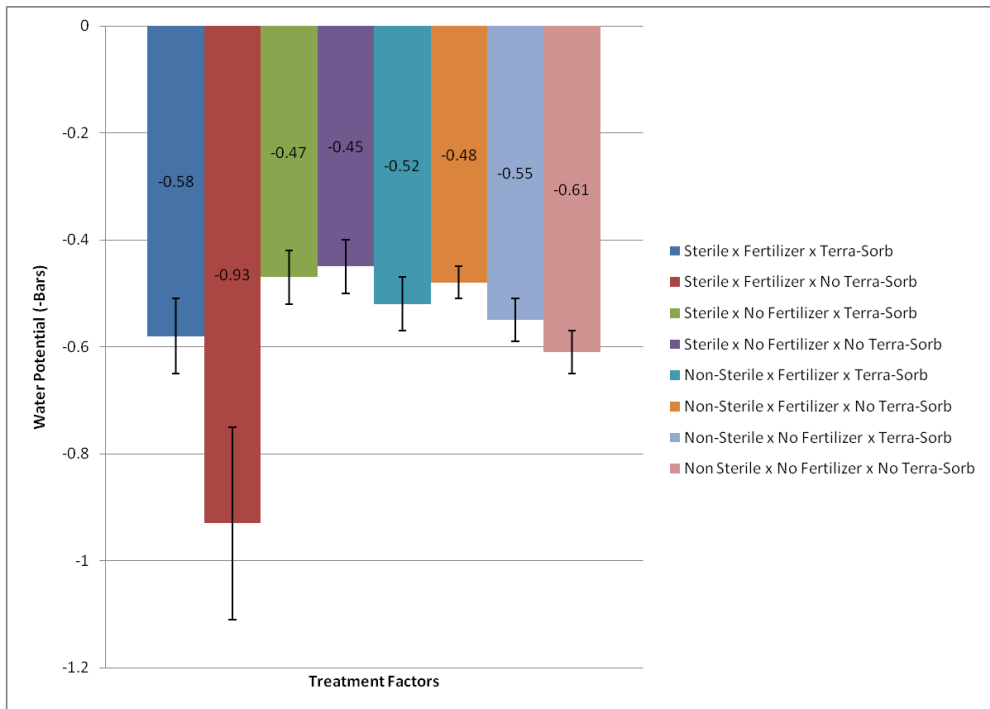

Figure 10: September 2009 pre-dawn water potential for chestnut planted at the mine site in the spring of 2008. Means ± standard errors are shown, and the three-way treatment interaction was significant at $p \leq 0.05$.

Discussion

Overall, the first and second year survival rates of 29 and 28% are unacceptably low for successful reforestation. Similar studies report direct seeding survival rates ranging from 48-84% (Fields-Johnson et al., 2010; French et al., 2008). In a side study, seeds of various genotypes were inter-planted with the pure American seeds in this experiment and survival of these genotypes averaged 70% (Miller et. al. 2009). Although un-quantified on our site, we believe that mortality due to herbivory was high. Further, the shelters used in this experiment were not vented, as were those used in Miller et al. (2009). Unvented shelters may have caused excess heating throughout the growing season, with temperatures inside the planting tube as high as 4˚C higher than ambient temperatures.

Fertilizer

Generally, fertilizer is known to increase survival of seedling transplants (Jacobs and Timmer, 2005; Kozlowski, et al., 1991). However, slow-release fertilizers have been shown to have adverse effects on survival in chestnut seedlings (Herendeen, 2007). The effect of fertilizer tablets on survival of direct-seeded chestnuts has not been clearly quantified. In this experiment, in both 2008 and 2009, fertilizer had the most negative impact on survival, when compared to other treatments. Although low-sodium slow release tablets were used, it is possible that since the seeds were planted quite near the fertilizer tablet, excessive sodium concentrations were initially reached within the rhizosphere. High levels of sodium can create Na^+ toxicity (Jacobs and Timmer, 2005), potentially reducing fertilizer uptake (Grattan and Grieve, 1999). Increased solutes in the rhizosphere can also increase osmotic potential of the soil, potentially lowering water and nutrient uptake.

Natural height increased in both years, and secondary growth in the first year, with the inclusion of fertilizer. Conversely, growth was most restricted when fertilizer was absent. Therefore, it appears that if survival occurs past the emergence stage, fertilizer will significantly increase natural height and root collar diameter, over at least the first year, on similar mine sites.

Fertilizer had no effect on photosynthetic rate at the mine site. However, there was an increase in rate of transpiration in the presence of fertilizer. This can be attributed to the flow of nutrients from the bulk soil to the root surface with water uptake. As this mass flow of nutrients reaches the root surface, the plant responds through rapid transpiration (Lambers, et al., 2008).

However, fertilizer presence may have increased water stress, as suggested by a more negative water potential, as a result of increased osmotic solutes within the soil.

Terra-Sorb

Throughout the growing seasons of 2008 and 2009, inclusion of Terra-Sorb in the soil medium played a significant role. In 2008, Terra-Sorb inclusion increased natural height and RCD growth. As hydrophilic root polymers such as Terra-Sorb can increase soil moisture and plant water availability (Al-Humaid and Moftah, 2007), enhanced growth can be expected. In 2009, natural height continued to be significantly influenced by Terra-Sorb.

Terra-Sorb was not found to significantly influence photosynthetic rate or transpiration rate. However, presence of Terra-Sorb was beneficial to the seedlings, decreasing water stress as measured by a less negative water potential. The coarse texture of the soil in this experiment makes it likely to have a low water-holding capacity. The advantage of Terra-Sorb in soil is its ability to hold many times its weight in water. As plant-available water increases with Terra-Sorb, soil water potential becomes less negative, as evidenced and supported by others (Al-Humaid and Moftah, 2007; Apostol, et al., 2009; Lambers, et al., 2008).

Soil Sterility

The inclusion of the non-sterilized native forest soil treatment increased survival in comparison with sterilized forest soil treatment. With the detrimental effects of fertilizer early in seedling establishment and survival, it is likely that in the absence of fertilizer seedlings could have relied on mycorrhizal colonization from non-sterilized soil to increase nutrient uptake. Since mine sites are often characterized as being deficient in growth-limiting nutrients (Rodrigue and Burger, 2004), the addition of the sterile soil may have provided less long-term improvement in soil nutrient status. The inclusion of native forest non-sterilized topsoil to the planting substrate could inoculate the substrate with mycorrhizae, earthworms, organic matter, and micro-organisms with which to break down that organic matter, stimulating growth (Lunt and Hedger, 2003). With presence of these organisms that begin the nutrient cycling process and build a humus layer, greater survival can be expected.

No effects of soil sterility were found in first or second year natural height or root collar diameter growth. Therefore, it seems that non-sterilized native forest soil is more important for direct-seeded tree emergence and survival than it is for tree growth on this mine site.

The increase in photosynthetic rate by presence of the sterile soil treatment may relate to American chestnut's adaptation to lowered nutrient availability on xeric sites. This finding is supported by Herendeen (2007), in which chestnut growth performance was best on nutrient poor sites. However, growth parameter results in this experiment were not supported by increased photosynthetic rate.

Conclusions

Planting treatments used in this study were shown to influence the physiology and performance of American chestnut seedlings. Overall, slow-release fertilizer application showed positive and negative effects on seedlings. Results show fertilizer presence reduced survival. However, after emergence and survival, trees planted with fertilizer generally accumulated more biomass than those without. Fertilizer had no effect on photosynthetic rate while increasing the rate of transpiration, likely due to nutrient mass flow from soil to roots which can regulate transpiration rate. Similar to fertilizer, the gel-based hydrophilic polymer Terra-Sorb used in this study produced both positive and negative effects on American chestnut performance. Survival of seeds planted was reduced in presence of Terra-Sorb. However, this was likely due to inadequate hydration of the gel before planting, which uplifted many seeds out of the ground when hydration was achieved in the soil. For surviving seedlings, both height and RCD were significantly greater after the first year in the presence of Terra-Sorb, and showed less negative water potentials. Non-sterile native forest soil increased survival when compared to the sterile soil treatment. In a system already described as nutrient limited, inclusion of sterilized soil on a mine site will not provide the increased support for seedling establishment shown by non-sterilized soil. However, no effects of soil sterility were found on growth at the mine site. Therefore, it seems that on mine sites, non-sterilized native forest soil is more important for direct-seeded tree emergence and survival than it is on growth. After survival, however, sterile soil can increase photosynthetic rate. But, this is not reflected in growth.

Results of this study may prove beneficial to reclamation specialists with an interest in using mine site reclamation in conjunction with American chestnut restoration. These sites have shown particular promise in providing suitable habitat for this species and may be potential vectors for chestnut dispersal in the future.

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