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EFFECTS OF PAST LANDUSE AND INITIAL TREATMENT ON *CASTANEA DENTATA* SEEDLINGS

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Abstract: Efforts to impart blight-resistance to the American chestnut, *Castanea dentata,* have yielded strains with some ability to resist the disease. However, in addition to Chestnut blight, root rot caused by *Phytophthora* is a major impediment to the re-introduction of the chestnut. The degree of presence of *Phytophthora* has been associated with soil moisture and loss of forestland to agriculture. At Mammoth Cave National Park, the effects of ectomycorrhizal fungi treatments and anti-fungal treatments were tested on chestnut seedlings. In addition, the effects of placing seedlings in disturbed (*e.g*., agricultural) or undisturbed areas were analyzed. No significant difference (p=0.09) in survivability was found between disturbed and undisturbed sites. In addition, seedlings in disturbed plots grew significantly more in height than undisturbed plots (p=0.01), though with no difference in diameter (p=0.17). The use of the fungicide Ridomil gold EC^{tm} in conjunction with greater soil preparation was found to increase survivability in disturbed plots (p<0.0001), though not in undisturbed plots (p=0.76). Ridomil did not have a significant effect on growth. The effect of mycorrhizal treatments on survivability was not significant when compared to control treatments (p=0.91).

INTRODUCTION

The American chestnut, *Castanea dentata*, was once an abundant canopy tree of Eastern hardwood forests. It was highly valued by both wildlife and humans for its nuts and quality timber. The chestnut was often 25 percent of the forest or more, and grew to be one of the largest trees in its ecosystem type at up to 100 ft tall and 5 to 10 ft in diameter (Ronderos 2000). Mammoth Cave National Park is on the western border of the chestnut's native range, although recent searches have found a number of young trees and root-sprouts throughout the Highland Rim region of Kentucky and Tennessee. During the summer of 2003 a researcher found over 1000 small American chestnut trees in the Big Woods, an old growth area within Mammoth Cave National Park (Mark Depoy, Mammoth Cave National Park (pers. com,). A review of surveyed deeds between 1931 and 1937 within the area of the current park found that chestnut was noted on 27% of the deeds and that chestnuts comprised 5.1% of corner trees (Rhoades 2002).

The demise of this stately tree began when a deadly fungus was introduced into the United States near the turn of the century. The fatal blight-causing fungus, *Cryphonectria parasitica,* was discovered in New York City in 1904, and was probably introduced on ornamental chestnut trees imported from Asia. With New York as the epicenter, the fungus spread rapidly throughout the eastern United States, and by the 1950s, nearly all of the American chestnut trees in the 9 million acres of their natural forest habitat had been killed (Ronderos 2000, Smith 2000). Only a few large chestnuts and some shrubby, blight-infested trees survived the initial epidemic. The blight only top-kills chestnuts, leaving the root system intact so that many trees re-sprout after the main stem dies. Although there are many of these sprouts left within the original range, they usually do not survive long enough or remain healthy enough to begin producing seed.

After the near loss of this keystone species, the search for blight-resistant trees began, and resistant parent trees were bred together in the hopes of generating even more blight-resistant offspring. Researchers and arborists also began back-crossing American chestnuts with completely resistant Asian species to create blight-resistant hybrids. Both hybrid and 100% American chestnut breeding programs have progressed significantly toward their goals, but there is not yet a genetically pure tree that is completely resistant to the chestnut blight. Many universities and natural areas programs have begun planting the more resistant trees that are available in an attempt to reintroduce *Castanea dentata* to eastern forests, but there are many difficulties associated with this process.

One of these difficulties is caused by another harmful fungus, *Phytophthora cinnamomi*, which was noted in trees even prior to the introduction of blight (Rhoades, 2003). It is thought to be associated with levels of soil moisture and with the clearing of forest land for agricultural use. Rhoades *et al.* (2003) found that chestnut seedling mortality was highest in wet, compacted soil such as would be common in areas highly disturbed by agriculture.

In some studies, ectomycorrhizal fungi treatment has been shown to protect against root rot. Anti-fungal treatments have also shown promise in preventing the disease (Marx and Davey 1969).

In the current study at Mammoth Cave National Park (MACA), 2,000 1-year-old 100% American chestnut seedlings from the American Chestnut Cooperators Foundation were planted in various locations within the Park. We tested these trees for their growth responses to application of additional mycorrhizal fungus, fungicide treatment, and control (no treatment). We also tested for the presence of *Phytophthora* fungus within these treatment groups and analyzed a potential correlation between *Phytophthora* and tree mortality.

In this study we hypothesized that the trees would have higher survival rates and more growth in undisturbed soils, and with either fungicide or mycorrhizal treatment. We also hypothesized that tree mortality would be associated with the presence of *Phytophthora*.

METHODS

<u>Sites and Treatments</u>

We selected 20 sites within Mammoth Cave National Park for these plantings (Table 1). Plots were placed on north- or northeast-facing slopes since evidence has shown this may aid the trees in resisting chestnut blight. The spring and fall freezing and thawing associated with south- to west-facing slopes accelerates the bark-splitting that increases the chance of fungal infection. In addition, the chosen sites were well-drained with a sandstone substrate and acidic soil type. All plots were placed in areas with relatively open understories and at least some canopy opening. We placed ten plots on soils which have historically experienced disturbance of the mycorrhizal layer (*i.e.,* agriculture), and ten plots in areas with no known history of soil disturbance (including the Big Woods area).

One hundred saplings from five different parentages were planted 2 m apart in a grid pattern within each of the 20 m x 20 m plots. Each tree was marked with a metal tag with a unique identification number that was associated with its plot, specific location within plot, and family designation. We measured the height in centimeters (cm) and root crown diameter in millimeters (mm) of each tree at planting. On the plot grid, each row of 10 trees began on the downhill (northeast) end of the plot and ran upward to the top (southwest) end of the plot. After planting, vented bottles containing cotton balls soaked in coyote urine were hung at the four corners of each plot to deter deer from browsing on trees.

Table 1. Plot number, soil type, planting date, and area of plantings.

Plot	Soil Type	Date Planted	Location Description
1	undisturbed	3/21/2003	Houchens Ferry
2	disturbed	3/25/2003	Houchens Ferry
3	disturbed	3/24/2003	Houchens Ferry
4	disturbed	3/25/2003	First Creek, East
5	disturbed	3/26/2003	First Creek, East
6	undisturbed	3/27/2003	First Creek, West
7	undisturbed	3/26/2003	First Creek, West
8	disturbed	3/28/2003	First Creek, West
9	disturbed	4/1/2003	First Creek, West
10	undisturbed	4/2/2003	First Creek, West
11	disturbed	4/2/2003	Blue Spring Hollow
12	undisturbed	4/3/2003	Blue Spring Hollow
13	undisturbed	4/4/2003	Cubby Cove
14	undisturbed	4/4/2003	Cubby Cove
15	undisturbed	4/7/2003	Big Woods
16	disturbed	4/8/2003	Big Woods
17	undisturbed	4/8/2003	Big Woods
18	disturbed	4/8/2003	Big Woods
19	disturbed	4/10/2003	Big Woods
20	undisturbed	4/11/2003	Blue Spring Hollow

Within each plot, we applied three different treatment types. One-third of the trees were root-dipped in an ecto-mycorrhizal gel and wrapped in wet paper for transportation to the planting sites. This gel was prepared by mixing 53 grams of DieHard^{tm} Ecto Root dip with 113 grams of Horta-Sorb Sm^{tm} water-absorbent gel and 18.93 L of water in a 5 gallon bucket.

The fungicide treatment group was treated by watering in approximately 325 ml of a solution of 1.3 ml of Ridomil gold EC^{tm} in 11.63 L of water at planting. In order to apply the fungicide we prepared a hole, broke up the soil, and watered the solution into the hole before planting the trees.

The control group was planted with no treatment, but roots of both control and Ridomil-treated trees were wrapped in wet paper to keep the roots from drying en route to the planting sites. For efficiency of planting, these two groups were planted using a dibble or planting bar method.

In order to keep treatments from mixing, treatment groups were arranged by row within the plot, with treatment rows chosen randomly. For example, row 1 may be treated with Ridomil (R), row 2 with mycorrhizae (M), and rows 3 and 4 control (C), etc. One row, also randomly assigned, was mixed (3R, 3M, 4C) so that the number of trees with each treatment would remain consistent throughout all of the plots.

At the end of the 2003 growing season we checked the trees for survival and re-measured the height and root crown diameter of each tree.

To test for presence of *Phytophthora* and mortality associated with it, we collected 188 trees from the plots in March 2004. We collected 141 dead trees and 47 live specimens and used an ELISA test to indicate presence or absence of fungus in the genus *Phytophthora*. The test kits were ordered from the Neogen company and the tests were performed with assistance from the Biotechnology Center at Western Kentucky University.

<u>Statistical Design and Analysis</u>

We used survival, height, and diameter of the trees to assess which treatment and plot type had the greatest success. We found the change in height and diameter by simply subtracting the height and diameter measurements at planting from the 2003 season end measurements.
We tested for correlation between height change and diameter change using a Pearson correlation matrix.

A Fisher's exact two-way (similar to chi-square) test was used to test for differences in the survival of seedlings in disturbed versus undisturbed plots.

A two-way Pearson's Chi-Square test was used to test for survival of seedlings by treatment type. One-way ANOVA were used to determine whether differences existed between the different treatments and agricultural versus non-agricultural sites.

RESULTS

We found an overall survival rate of 56% for the planted seedlings. Survivorship of *C. dentata* seedlings was variable from site to site (Figure 1), with visibly higher survival rates in Cubby Cove (sites 13 and 14) and the historically chestnut-rich area of the Big Woods (sites 15-19).

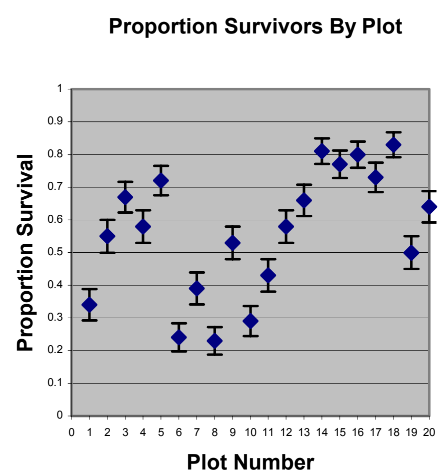

Figure 1. Survival rates for individual plots by plot number.

Percent survival by site type was 58.4% for disturbed sites and 54.5% for undisturbed sites (Table 2). Contrary to our initial hypothesis, we found no significant difference ($p=0.087$) in survival between disturbed and undisturbed sites using Fisher's exact test.

Table 2. Frequency of alive or dead trees at the disturbed and undisturbed sites. No significant difference in values, Fisher exact test ($p=0.087$).

	Disturbed	**Undisturbed**	**Total**
Alive	584	545	1129
Dead	416	455	871
Total	1000	1000	2000

To further test the differences between site types we calculated the amount of tree growth over the season and compared this between disturbed and undisturbed sites. Height difference and diameter difference were highly correlated ($r^2 = 0.695$) according to the Pearson correlation test, so because of that high correlation and the probability of error in the diameter measurements only height difference was used in the ANOVAs for difference between site types and treatments.

Using a one-way ANOVA for change in height, we compared disturbed to undisturbed sites and found that the disturbed sites had a significantly greater increase in plant height than undisturbed areas ($F=10.55$, $df=1$, $p<0.001$), as seen in Figure 2. Dead trees were measured as well, so desiccation and decay led to some negative change in height for both groups. This result also contrasted sharply with our initial hypotheses.

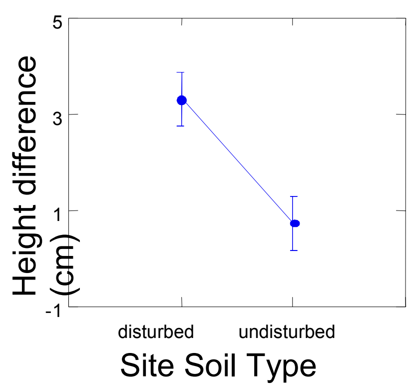

Figure 2. Height difference from planting to the end of the first growing season on disturbed (D) and undisturbed (UD) soils ($p=0.001$).

After comparing the site types to each other we separated them and tested for differences between treatments within a site type. Table 3 shows the survival rate of trees for each of the three treatments on undisturbed sites. We found no significant difference ($p=0.933$) in proportion of surviving trees for any treatment group within the undisturbed plots, indicating that neither fungicide nor mycorrhizal fungus had a strong effect on the survival of these seedlings.

Table 4 shows the same data for the disturbed plots. The only treatment associated with a significant difference in survival was the Ridomil treatment group (highlighted) with $p<0.001$. There are several possible reasons for this result related to planting method and site characteristics, which are considered in the discussion.

Table 3. Frequency of alive or dead trees at the undisturbed sites versus the three treatments. No significant difference was found using the Pearson Chi-Square Test ($p=0.933$).

	Control	**Mycorrhizal**	**Ridomil**	**Total**
Alive	184	179	182	545
Dead	157	151	147	455
Total	341	330	329	1000

Table 4. Frequency of alive or dead trees at the disturbed sites versus treatment. A significant difference in the Ridomil treatment group ($p<0.001$) is observed using the Pearson Chi-Square Test.

	Control	**Mycorrhizal**	**Ridomil**	**Total**
Alive	173	166	245	584
Dead	165	164	87	416
Total	338	330	332	1000

The last analysis we ran tested for the presence of *Phytophthora* fungus. Using the 188 collected trees, we took root scrapings per the kit directions and found an overall infection rate of 25.5%. To examine whether or not the infections were actually causing tree mortality, we compared the infection rate among the dead trees (28.4%) to that of the live trees collected (17.0%). Interestingly, there was no significant difference between the two infection rates ($chi^2 = 1.36$, $df=1$, $p=0.24$), indicating that *Phytophthora* is present within the plots but is not a major cause or tree death. We also isolated the results for dead and live trees in disturbed plots, where more infection and mortality was expected, and found the same non-significant result ($chi^2 = 1.82$, $df=1$, $p=0.177$).

DISCUSSION

There were several challenges involved in this experiment which may have had an effect on tree growth in our plots. Sites were chosen for relatively open understories, but there was still some degree of competition from other forest species, particularly the fast-growing red maples (*Acer rubrum*) and yellow-poplars (*Liriodendron tulipifera*). Some of the planted seedlings also fell prey to light browsing by deer in a few plots and leaf damage from insects in most of the plots. One challenge that limits our ability to compare treatments is the planting method. In order to apply the Ridomil properly we had to loosen the soil and water in the chemical, while the other treatment groups were slot-planted to expedite the planting process. This differential treatment may explain a great deal concerning the apparent significance of the Ridomil treatment discussed below.

We initially hypothesized that the trees would grow better in undisturbed soil sites and would survive at a higher rate with the anti-fungal treatment, but these hypotheses were not supported by the data. We found that seedlings grew more in height at the disturbed sites and that the Ridomil treatment had a significant positive effect on those sites as well. This initially led us to believe that *Phytophthora* was more of an issue in disturbed plots and that the Ridomil was mitigating that problem. When we tested for fungal presence, however, we discovered that this was not the case, since there was no significant difference in infection rates between living and dead trees including those on disturbed soils. A more probable suggestion is that the significance of the Ridomil treatment is caused by the difference in planting methods. While loosening the soil may not make a noticeable difference in light, undisturbed soils, it will have a greater impact on trees if the soil in the plot is compacted by disturbance and has higher clay content.

Although the growth was greater on disturbed soils, there was no significant difference in survival rate between site types, showing that the trees in our experiment still grew in the undisturbed areas, but a slower rate than at disturbed sites. In the Big Woods (plots 15-19), we found our highest survival rates, and this is most likely because of the site quality. Although all of the sites were chosen with the same criteria, the Big Woods area is known for the historic presence of large chestnut trees and currently holds over 1,000 natural seedlings and stump-sprouts, so more study is needed to find out exactly why *Castanea dentata* is so prevalent in this area and not in others.

We will continue to collect data on these trees as they mature, and larger trends may be discovered in future years. From this initial set of data, we see that undisturbed sites are not necessarily better habitat for chestnut plantings and that a more involved planting method and an initial fungicide treatment will aid tree growth within the first year. We also note that although *Phytophthora* fungus is present within our plots, it is does not appear to be a major cause of tree mortality within the sites we measured. We plan to continue this research by testing the soils and leaf-litter from each plot to determine more specifically what site characteristics lead to higher survival and greater growth among seedlings.

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LITERATURE CITED

Marx, D.H., and C.B. Kavey. 1969. The influence of Ectotrophic mycorrhizal fungi on the resistance of pine roots to mycorrhizae to infections by *Phytophthora cinnamomi*. Phytopathology 59:559-565.

Planting Protocols for American Chestnut Restoration Project. 2002. Mammoth Cave National Park science and resource management. Unpublished Internal Document.

Rhoades, C.C. 2002. Historic abundance of American chestnut at Mammoth Cave National Park.

Rhoades, C.C., et al. 2003. Effect of soil compaction and moisture on incidence of *Phytophthora* root rot on American chestnut (*Castanea dentata*) seedlings. For. Ecol. Manage. 184:47-54.

Ronderos, Ana. 2000. Where giants once stood: The demise of the American chestnut and efforts to bring it back. J. For. 98(2):10-11.

Smith, David. 2000. American chestnut: Ill-fated monarch of the eastern hardwood forest. J. For. 98(2):12-15.