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Soil and leaf minerals and survival and growth of hybrid chestnut trees planted in forest and field plots in Connecticut

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Abstract.

There are no data in the literature on soil requirements for successful growth of chestnut trees, or on chestnut leaf minerals in relation to the mineral levels in the soil. In order to get information on this, we planted 293 two-year-old hybrid chestnut trees in two forest clear-cuts and in an old agricultural field in the center of Connecticut, USA in 2000. We tested the soils in the plots and leaf samples from the plots for minerals after one season of growth and after 12 years. The trees were seedling products of four controlled crosses. Hypovirulent (virus containing) strains of *Cryphonectria parasitica* (the cause of chestnut blight disease) were matched to the strains in the forest plots

and cankers on the native trees were treated with this biocontrol for the first four years. No biocontrol was used in the field plot. At the end of the first year there were few differences between levels of minerals in the leaves of the trees in the three plots except for calcium, which was higher in trees grown in the old field, and manganese and aluminum, which were higher in leaves from the forest plots. After 12 years there was still more calcium in leaves from the field plot than from the forest plots, and leaves from the forest plots still had much more manganese and aluminum than leaves from the field plot. After 12 years, total nitrogen was lower in the leaves from the old field than it had been initially, even though soil nitrogen levels were much higher. Growth was initially best in the forest clear-cuts, where the soil pH was lower. Survival was better, after 12 years, in the forest clear-cuts than in the old field.

The Connecticut Agricultural Experiment Station (CAES) is breeding chestnut trees for timber form and resistance to chestnut blight disease (caused by *Cryphonectria parasitica*) (Anagnostakis, 2001). The next step will be to introduce the timber hybrids into forested areas. Native-American chestnuts (*Castanea dentata*, (Marsh.) Borkh.) in the northeastern U.S. still sprout from the root collars, which are not invaded by the pathogen. The pathogen is endemic, but sprouts usually grow for a few years before they are killed back to the root collar by blight cankers. We can keep a percentage of the native sprouts alive using hypovirulent (virus containing) strains of the blight fungus for biocontrol (Anagnostakis, 1990). Natural crossing between planted trees and native

trees will result in seedlings with increased diversity and with increased resistance to chestnut blight disease.

Diller made extensive plantings of Asian and hybrid chestnuts from 1928 to 1954 in forested areas in the U.S. where the canopy trees had been girdled (Diller, 1940; 1952; Beattie and Diller, 1954). His work demonstrated that forest plantings of chestnuts could be successful, but no tests were made to document the soil characteristics of the diverse sites where he planted the chestnuts. Sparks (1997) argued that the most useful soil test for planting nut trees was for pH. He analyzed the mineral content of leaves, roots, and stems in pecan, and reported that leaf tissue analyses was the most useful for identifying deficiencies, and is the "single most reliable method for predicting nutrient needs" in pecan trees. In Smith's (2003) chapter on mineral nutrition in nut trees he said that analysis of leaf tissue is extremely useful for accessing the nutritional status of nut trees, but that there is no information on the desirable levels of minerals in the leaves of chestnut trees or on the effect of soil mineral levels on growth and survival.

Materials and Methods:

Trees

In 1997, we crossed a hybrid, which had blight resistance from a Japanese chestnut (*C. crenata* Sieb & Zucc.) with two American chestnuts, one from Connecticut (CT) and one from northern New York (NY). These offspring {*C. dentata* x (*C. dentata* x [{*C. crenata* x *C. sativa*} x *C. dentata*])} are referred to as Family 1 and Family 2. We also crossed a hybrid, which had blight resistance from a Chinese chestnut (*C.*

mollissima Bl.) with the same two American chestnuts, and these offspring {([*C. dentata* x *C. mollissima*] x *C. dentata*) x *C. dentata*}, are called Family 3 and Family 4. Seed was sown in the fall of 1997 in a light sandy loam, seedlings were dug up in late March of 2000 while they were still dormant, and all were root pruned.

Planting sites

In early April 2000, 101 trees were planted in two forest clear-cuts in Prospect, CT. One plot was on a ridge (10 trees from each family) and the second on flatter land nearby (15 trees each from families 1, 3, & 4 and 16 from family 2). Deer predation was reduced in the forest plantings by placing plastic screen tree shelters around each tree (Treessentials Co., St. Paul, MN). Shelters were left in place for three years. As a control, 192 trees (16 + 8 + 107 + 61 respectively, from the four families) were planted in an old agricultural field in Windsor, CT, which had previously been planted with tobacco. No nutrients were added to the forest soil during the course of the experiment but the field trees were side-dressed with 10-10-10 (N-P-K) fertilizer in their third spring and yearly thereafter.

Biocontrol treatments

Bark samples were taken from chestnut blight cankers in the two forest plots in 2000 and the blight fungus cultured from them in the laboratory. Pairings on agar media allowed us to sort the isolates into vegetative compatibility groups (Anagnostakis 1977). Hypovirulence viruses were moved into the strains representing the common types in each plot by pairing in the laboratory (Anagnostakis and Day 1979). These

biocontrol strains were used to treat blight cankers on the native sprouts in the forest plots for the first four years (Anagnostakis 1990). No hypovirulent strains were introduced into the field plot.

Measurements and sampling

In mid-summer 2001, 2002, and 2003 the heights of all surviving trees were measured and survival noted. The dbh of surviving trees in the forest plots was measured in 2008 and 2012, and in the field plot in 2012. Between 2008 and 2012, all the labels in Plot 2 were destroyed (possibly chewed off by animals). In 2001 soil samples were taken in an "X" pattern across all three plots and bulked by plot, and a single leaf (fully expanded leaf, third from the terminal) was taken from each tree. These data served as our "base-lines" for the plots. In 2012, soil and leaf samples were again taken from the three plots, as previously done. Leaf samples were bulked by plot and freeze-dried, and then ground for acid digestion and mineral analysis, and the soil was dried and checked for minerals and for pH.

Analyses

The mineral content (Al, B, Ca, Cu, Fe, K, Mg, Mn, N, P, S, and Zn) in the samples were analyzed by standard methods described previously (Stilwell, 1993; Sawney and Stilwell, 1994) that closely follow EPA method 3050. Briefly, about 0.5 g of dried plant tissue or soil was weighed (±0.1 mg) into 100 ml volumetric flasks and digested in 10 ml of concentrated nitric acid on a hot plate. The samples were diluted to 100 ml and the elemental analysis was carried out by inductively coupled plasma

optical emission spectroscopy (ICP-OES) using the Thermo Jarrell Ash Atom 25 (Franklin, MA, USA). The method detection limits were 2.0 mg/kg (Al, B, Ca, Cu), 4.0 mg/kg (Fe), 80 mg/kg (K), 8 mg/kg (Mg), 0.1 (Mn), 8 mg/kg (P), 8 mg/kg (S), and 0.8 mg/kg (Zn). . The nitrogen determination was carried out by combustion analysis using the Leco (St. Joseph, MI) FP-528 Protein/Nitrogen Analyzer and following manufactures procedures. The nitrogen detection limit was 300 mg/kg.

Results

The soil pH was initially much lower in the forest plot on the ridge than in the level forest plot or the old field. After 12 years, the soil pH on the ridge was closer to that in the level forest plot, and the pH of the soil in the field was much lower than initially, and similar to that in the forest plots. The average mineral contents of the soils, and the average pH values for the soils in the first and last tests are listed in Table 1. The average mineral contents of the leaves are listed in Table 2. There were few differences between levels of minerals in the leaves of the trees in the three plots at the end of the first year. Calcium level was higher in leaves from trees grown in the old field, and manganese and aluminum levels were higher in leaves from the forest plots, reflecting levels in the soils. After 12 years there was still more calcium in leaves from the field plot than from the forest plots, and more in the soil in the field plot. The 2012 leaves from the forest plots still had much more manganese and aluminum than leaves from the field plot even though there was more aluminum in the field plot soil, and similar amounts of manganese in the field and forest soils. Total nitrogen in the leaves

was slightly lower in the old field after 12 years, even though soil levels were much higher.

The average amount of height growth of trees in each family from the time of planting in the test plots to the middle of their third season (about 39 months) is shown in Table 3. Initial differences in growth between the families were significant (95% level) only in the old field (Plot 3).

In 2008, there were 19 surviving trees in Plot 1 with an average dbh of 5 cm, 28 in Plot 2 with an average dbh of 4 cm, and 24 in Plot 3. In 2012, there were 7 survivors in Plot 1 with an average dbh of 13 cm, 20 in Plot 2 with an average dbh of 7 cm, and 14 in Plot 3 with an average dbh of 15 cm. By 2012 there were no tags left on the planted trees in Plot 2, and measurements of these were simply bulked. Survival and size of individual families is listed in Table 4.

Discussion

Soil and leaf minerals

There were several differences in soil minerals between the forest plots and field plot at the end of the first season, and this may have affected initial growth in the three plots. The big difference in pH between the forest and field soil was probably a major factor in initial mineral uptake. The forest soils remained acid over the 12 years, and the field soil was very similar to the forest at the end of that time. Repeated liming for tobacco growth probably accounted for the initial higher pH in the field. The amount of soil aluminum was initially lower in the field than in the forest but was higher after 12 years. Both Kozlowski and Pallardy (1979) and Kochian (1995) say that aluminum in

acid soils (<4.7) can be very toxic to plants, but list no values. They also say that some native species exhibit resistance to the acidified forms of aluminum. The amount of soil phosphorous was higher after 12 years in the forest plots but was lower in the field, in spite of a spring side-dressings of 10-10-10 in the field plot. Potassium levels were slightly lower after 12 years in all three plots. Boron, which is considered an important micronutrient for nut trees, was lower in all plots after 12 years. Also, after 12 years, iron levels were lower in the forest plots but slightly higher in the field plot. Total nitrogen was considerably higher in the old field after 12 years, presumably due to yearly applications of fertilizer, but leaf nitrogen was decreased.

Leaf minerals were, in general, higher than reported by Sparks (1977) or Smith (2003) for pecans, by Smith (2003) for walnuts, or by Sentis et al. (2005) for hazels. Potassium levels were higher in leaves from all plots than the 3500 ppm considered toxic for pecans by Sparks (1977). Manganese levels in the leaves were higher after 12 years in all three plots. Boron levels in the leaves were higher in the forest plots after 12 years, and lower in the field plot but all were within the acceptable range for both pecans and walnuts (Sparks 1977, Smith 2003).

Tree parentage

The trees in Family 1 and Family 3 had the same CT American parent; used as the female parent for Family 1 and as the male parent for Family 3. These two families responded the same way in the three plots. They grew well initially in Plot 1 where the soil pH was lowest, and the soil was thin and rocky, and grew the least in the old field. The trees of Family 1 grew better, on average, in the first three years than the trees of

Family 3. After 12 years, survival and average dbh of Family 1 trees was greater than that of Family 3 trees in Plot 3 and the opposite was true in Plot 1.

Trees of Family 2 and Family 4 had the same NY American parent; used as the female in Family 2 and as the male parent in Family 4. There was little difference in growth of the trees in the three plots in the first three years. Low numbers of the trees of Family 2 in Plot 3 makes growth comparisons difficult, and none survived in Plots 1 or 3 after 8 years.

Tree parentage affected both survival and growth rates on the three sites. Total survival after 12 years was better in the forest plots where biocontrol strains were introduced than in the field, but greater average size was attained in the field where trees were fertilized yearly and watered if necessary, and where there was no competition from other species.

The results of this experiment will be used to choose which breeding lines to maximize for forest plantings, and which locations in the north east will be best for planting the timber-form hybrid chestnuts. The information on leaf minerals will be a base for future comparisons in the genus *Castanea*.

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Table 1. Soil pH and soil minerals listed as total mg per kg of dry soil, from two forest clear-cut plots and one old field plot. Samples were taken one year and 12 years after planting.

Mineral	**Plot 1, forest ridge**		**Plot 2, forest level**		**Plot 3, old field**	
	2001	**2012**	**2001**	**2012**	**2001**	**2012**
Aluminum	18645	13481	18935	10302	8915	14693
Calcium	235	907	235	497	767	644
Magnesium	2187	1165	1660	1464	1647	1349
Nitrogen	2818	7892	1840	11554	362	1259
Phosphorous	713	813	621	881	1123	635
Potassium	684	571	475	430	565	526
Sulfur	469	971	353	135	130	697
Boron	51	4.5	45	2.8	13	4.7
Copper	21	36	20	12	12	25
Iron	21665	13371	20770	9962	10710	15551
Manganese	106	227	121	182	206	199
Zinc	51	103	40	38	42	49
Soil pH	3.6	4.3	4.5	4.0	5.7	4.5

Table 2. Minerals in chestnut leaves bulked by plot (two forest clear-cut plots and one old field plot) are listed as total mg per kg of dry leaf tissue. Samples were taken one year and 12 years after planting.

Mineral	**Plot 1, forest ridge**		**Plot 2, forest level**		**Plot 3, old field**	
	2001	**2012**	**2001**	**2012**	**2001**	**2012**
Aluminum	715	1007	664	580	138	235
Calcium	3841	7322	4975	7589	10210	11272
Magnesium	1780	4434	2174	3221	2500	2893
Nitrogen	21343	19736	20428	20420	26582	19858
Phosphorous	1830	1676	1859	1450	3981	2120
Potassium	8788	8397	8716	9474	9647	7912
Sulfur	1549	1269	1585	1234	1796	1264
Boron	39	64	43	54	51	36
Copper	6	28	5	78	7	20
Iron	54	51	53	42	64	45
Manganese	441	1351	733	1378	418	641
Zinc	36	34	26	59	62	40

Table 3. Mean height growth in cm, and the standard error of the mean, of four families of hybrid chestnut trees after 39 months of growth in two forest clear-cut plots and in an old agricultural field, all in the central part of Connecticut, USA: Plot 1 was a forest ridge, Plot 2 was a level area near the ridge, and the field (Plot 3) had been used for many years to grow tobacco.

	Family 1	**Family 2**	**Family 3**	**Family 4**
Plot 1	131±19	107±17	114±12	89±14
Plot 2	119±14	103±10	100±14	93±13
Plot 3	110±5	(60)	86±5	98±6

Table 4. Survival and average dbh of four families of hybrid chestnut trees after 8 and 12 years of growth in two forest clear-cut plots and in an old agricultural field, all in the central part of Connecticut, USA. Plot 1 was a forest ridge, Plot 2 was a level area near the ridge, and the field (Plot 3) had been used for many years to grow tobacco. Trees in Plot 2 had no remaining tags in 2012, and individuals could not be exactly identified by map position. Therefore, all planted trees found in Plot 2 in 2012 were grouped.

Family		Plot 1		Plot 2		Plot 3	
		survivors (%)	avg. dbh, cm	survivors (%)	avg. dbh, cm	survivors (%)	avg. dbh, cm
Family 1	2008	5 (50%)	4	3 (20%)	2.5	5 (31%)	-
	2012	1 (10%)	6.2	-	-	5 (31%)	17
Family 2	2008	0	0	2 (13%)	4	0	0
	2012	0	0	-	-	0	0
Family 3	2008	7 (70%)	5.7	13 (87%)	3.3	12 (11%)	-
	2012	3 (30%)	12.2	-	-	5 (5%)	14
Family 4	2008	7 (70%)	5.3	10 (67%)	4.6	7 (12%)	-
	2012	3 (30%)	16	-	-	4 (7%)	15
All Planted Trees							
	2008	19 (48%)	5.1	28 (46%)	3.7	24 (13%)	-
	2012	7 (18%)	13	20 (33%)	6.8	14 (7%)	15