Center for
Advanced Forestry Systems

CAFS Project 14-49 (Original 08-01) / FPC RW20
Study Plan:
Silviculture of Varietal Loblolly Pine (*Pinus taeda* L.) Plantations:
Impacts of Spacing and Silvicultural Treatments on Varieties with Differing Crown Ideotypes

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INTRODUCTION AND JUSTIFICATION

Forests are vital to the world's ecological, social, and economic health. Forests provide a major part of the earth's oxygen and store a substantial portion of its carbon. Forests provide habitats for much of the world's plants, animals, and microorganisms—and recreational opportunities for its people. Today, wood from forests is a major economic commodity, serving as the raw material for building materials, paper, packaging, and fuel wood. In the future, wood from forests will also provide much of the raw material for a wide range of new bio-based fuels and materials.

Because of the continued increase in the world's populations, demand for forest products is increasing while large amounts of forest land are being degraded or lost to other land uses such as urbanization (Wear and Greis 2002). In addition, timber harvests are being restricted in native forests in many parts of the world. The use of intensively managed plantations for wood production must increase to meet the increasing demand for wood and fiber, while still conserving large areas of native forests (Sedjo and Botkin 1997). Moreover, the important role of forests in sequestering carbon and providing a feedstock for biofuels and biomaterials is gaining in importance. Recreation, habitat, and clean water have become increasingly important services to society provided by forests. To maintain economically viable, wood-based industries, it is necessary to develop and incorporate technological advances into forest management. Increasingly, the paradigm of forest management is to manage different tracts of forestland for distinct purposes (Hunter 1990). Thus, the most sensitive habitats are preserved, whereas other forests may be managed to produce clean water, recreational opportunities, or maximize wood production for various uses from biomass to sawtimber.

Productivity of southern pine plantations has increased substantially during the last 50 years due to improved silvicultural practices, including genetic improvement, site preparation, weed control and fertilization (Fox et al. 2007a). Despite the large gains in productivity resulting from previous forestry research, growth rates in many pine plantations in the southern U.S. average less than 10 m^3/ha/year—substantially lower than forest plantations in many other parts of the world. Fortunately, theoretical models (Landsberg et al. 2001; Sampson and Allen 1999), empirical field trials (Amateis et al. 2000; Jokela et al. 2000; Borders and Bailey 2001), and operational experience show that these growth rates are well below what is possible. It is clear that low levels of leaf area are resulting in poor productivity (Vose and Allen 1988; Albaugh et al. 1998). Historically, water availability, whether too little or too much, had been considered the principal site resource limiting productivity; however, recent research indicates that chronically low levels of available soil nutrients, principally nitrogen (N) and phosphorus (P), are as important as water in many areas (Albaugh et al. 1998, 2004; Sampson and Allen 1999). Low soil nutrient levels reduce leaf area development, growth efficiency, and stand volume production. Forest fertilization is therefore needed in most stands to achieve high rates of production (Fox et al. 2007).

Forest fertilization is a widespread silvicultural practice in the southern U.S., where over 1.2 million acres (486,000 ha) of pine plantations were fertilized with P or N+P in 2005 (Albaugh et al. 2007). In forestry, N is commonly applied as urea or diammonium phosphate. Phosphorus is applied as either triple super phosphate or diammonium phosphate. The cumulative growth response of loblolly pine plantations following mid-rotation fertilization with N+P is approximately 450 ft^3 /acre (31 m^3/ha) over eight years (Fox et al. 2007b). The financial returns from forest fertilization can be financially attractive. Depending on factors such as fertilizer cost and the value of the timber produced, rates of return in excess of 15% can be obtained following forest fertilization.

A considerable amount of research has been conducted on the impacts of planting density on growth and stand development of loblolly pine plantations (Harms and Loyd, 1981). Wide spacing tends to increase the diameter growth of the trees, but reduces total biomass production. Therefore, the appropriate planting density depends on the management objectives and the products that are desired. Management regimes focused on sawtimber production will generally use a wider initial spacing while regimes focused on biomass production will generally utilize narrow initial spacing. However, intensive silvicultural management practices, including deployment of advanced genotypes, fertilization and weed control, tend to accelerate stand development and when employed may change the appropriate spacing of plantations.

Deployment of genetically improved forest trees is standard practice throughout most of the world. To date, management decisions have been fairly simple as to where to plant open-pollinated (OP) families and how to manage them with appropriate silvicultural systems. An integrated research approach is needed to quantify how nutrient availability and genetics interact to affect stand growth and development. As silvicultural inputs become more intensive and tree improvement produces more intensively selected and less genetically heterogeneous full-sib families or clones, there is a greater need to understand how elite genotypes respond to silvicultural manipulations (Li et al. 1991). Smart deployment of genotypes will depend on familiarity with the material, because some genotypes will likely be interactive and others will not (Figure 1). Additionally, how specific genotypes respond to specific silvicultural manipulations will have to be better understood for landowners to benefit from future genetic gains and improved silvicultural systems.

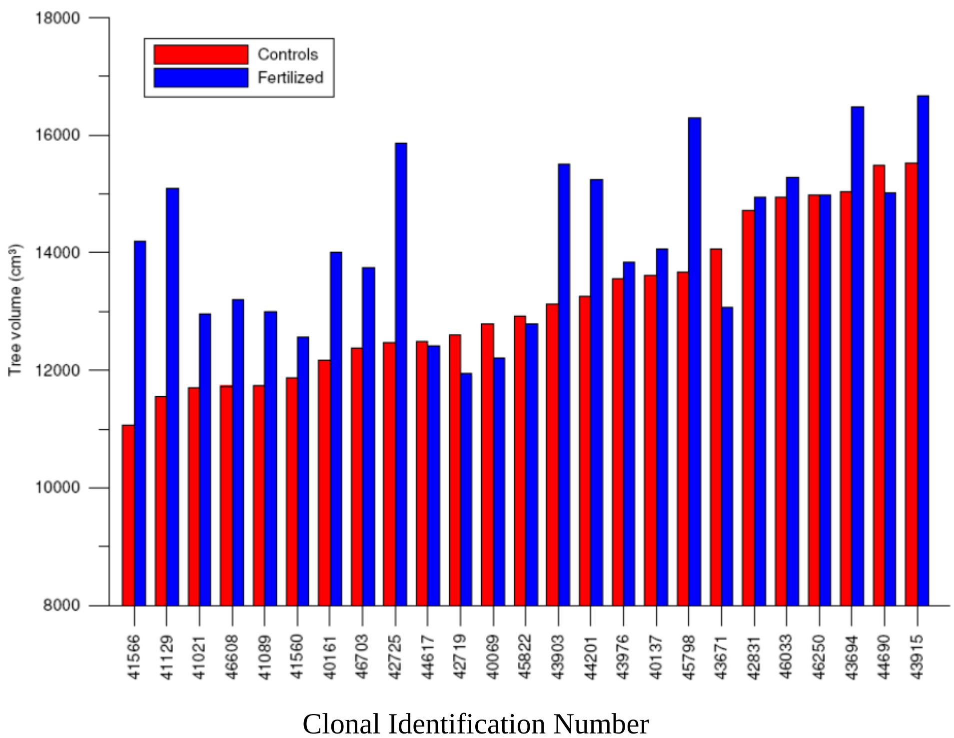


Figure 1. Variation in growth of clonal loblolly pine following fertilization in the Virginia Piedmont.

Clonal forestry holds the greatest promise to increase the productivity of forest plantations in the near term (Wright and Dougherty 2007). Clonal forestry relies on vegetative propagation to mass-produce identical copies of selected individual trees that possess improved genetic potential (Gleed et al. 1995). Clonal Eucalyptus plantations are widely planted in the southern hemisphere and have dramatically improved productivity (Arnold 1995). Growth rates exceeding 100 m^3/ha/year have been documented in intensively managed clonal eucalyptus plantations in Brazil (Evans 1992, Stape et al. 2004). In addition, clones with specific wood properties have been developed to optimize pulp production. The technology to mass-produce clones of southern pines is still under development. However, recent advances in somatic embryogenesis for loblolly pine now permit large numbers of clonal loblolly pine to be produced and deployed. Based on results from clonal plantations in other parts of the world, it should be possible to increase productivity in much of the southern U.S. by at least 50%—i.e., by deploying appropriate clones to specific soil types, and then implementing integrated, intensive silvicultural regimes. Mean annual increments exceeding 30 m^3/ha/yr may be attainable. However, this increase in growth will only occur when clones managed intensively (Figure 2). One important attribute attributed to clonal plantations in addition to improved growth is an increase in stand uniformity. Increased uniformity is important because it enables foresters to prescribe treatments and harvest stands more precisely. However, it is clear that increased uniformity of clonal plantations only occurs when they are intensively managed and resource limitations are eliminated. Use of clones in poorly managed stands will not by itself substantially increase plantation growth or uniformity (Figure 2).

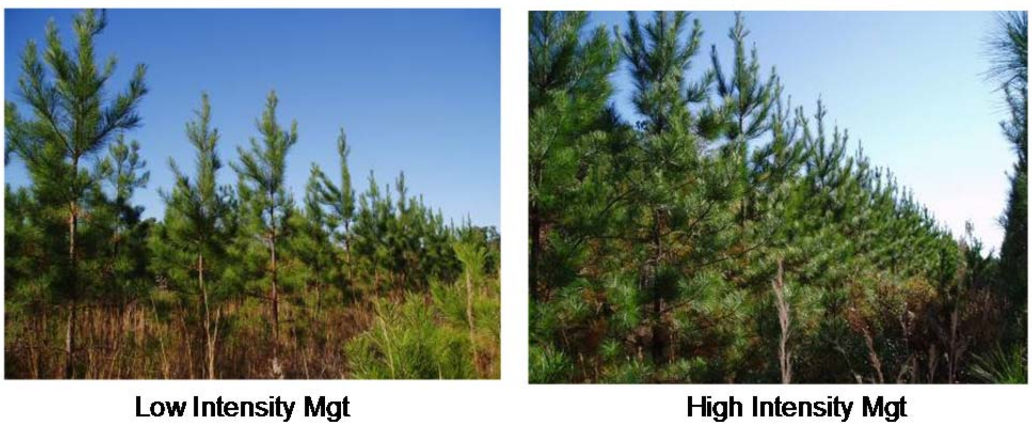


Figure 2. Impact of silviculture intensity on growth and uniformity of clonal loblolly pine planted in South Carolina.

As silvicultural inputs become more intensive and tree improvement produces more rigorously selected and less genetically heterogeneous clones, a better understanding of how specific genotypes respond to specific silvicultural manipulations is needed by industry to benefit from future genetic gains and the development of improved silvicultural systems (Li et al. 1991). It is more likely that genotype by environment (GxE) interactions will become significant when clones are deployed in intensively managed plantations (Sierra-Lucero et al. 2003). These interactions must be quantified in order to develop forest management regimes that optimize growth and financial returns for each clone that is deployed (Figure 1).

In a clonal forestry program, large numbers of candidate clones must be developed and screened to determine those that are suitable for deploying in forest plantations (Gleed 1995). This will be on ongoing process because as new clones are developed, they must be evaluated. This presents a problem because it is difficult to efficiently evaluate the growth response of large numbers of individual clones to the wide variety of silvicultural treatments that may be used.

The concept of a crown ideoptype in forest trees was developed by Dickmann (1985). An ideotype is tree with a consistent set of properties or characteristics that will tend to respond to management practices in a uniform and consistent manner. In forestry, the ideotype is usually defined by the crown characteristics of the tree and includes properties such as branch size, branch angle, number of branches and tendency to self prune (Martin et al. 2001). Trees with a "crop ideotype" tend to have narrow crowns and small branches and grow well without excessively competing for site resources with other similar trees. In contrast, the "competition ideotype" has a large crown and aggressively expands its crown to exploit site resources to the detriment of its neighbors. The appropriate ideotype to plant in a specific stand depends on the management objectives and practices. For example, a tree with a dense, narrow crown trees may be desired in closely spaced plantations where biomass production is the management objective. In contrast, wide, large crown trees may be desired in a plantation managed at low densities for sawtimber productions. If clones can be classified into a limited number of crown ideotypes, it might be possible to evaluate the growth response of each crown ideotype to various silvicultural treatments. It would then be possible to accurately predict growth responses of newly developed clones to silviculture treatments with minimal additional empirical testing.

Productivity of loblolly pine plantations established in the southern hemisphere countries such as Brazil, Argentina and Uruguay is substantially greater than in the southern United States (Evans 1992, Cubbage et al. 2007). Growth rates in excess of 10 to 15 tons/acre/year (20 to 30 m^3/ha/yr) are common. Although growth of the very best managed loblolly pine plantations in the southern United States can approach this rate of productivity (Borders and Bailey 2001), productivity in most plantations is less than 10 tons/acre/year (20 m^3/ha/yr). A number of theories have been proposed to explain the differences in productivity between intensively managed plantations in the southern U.S. and those in South America including climatic differences such as 12 month growing seasons coupled with low night time temperatures during the summer, more productive soils that have few nutrient limitations, and lack of insect and disease problems. Process models have been widely used to explore differences in productivity across regions (Sampson and Allen 1999, Landsberg et al. 2001). However, there have been few empirical trials established that directly compare growth and productivity of loblolly pine using similar genetics and silvicultural practices. Dramatic increases in our understanding of the factors affecting growth and productivity of loblolly pine may be possible if uniform empirical field trials were established using the same clones in various regions of the southern United States and South America that have different climatic and edaphic properties.

OBJECTIVES

The main objectives of this study are to:

1) Evaluate the crown ideotype approach to clonal testing in loblolly pine to determine if there is a consistent growth response to silvicultural treatments among clones that are classified into the same crown ideotypes;
2) Determine impacts of increasing genetic uniformity, from open pollinated to control pollinated to clone, on growth and uniformity of loblolly pine plantations, and the occurrence of GxE interactions;
3) Compare growth response, carbon allocation patterns (above and below ground), and ecophysiological processes both at the individual tree and stand level of clones of loblolly pine when managed under different silvicultural management intensities and planted at different initial spacings designed to optimize biomass production, mixed products, or sawtimber production;

4) Test if simpler Nelder's design trials can be used to accelerate Spacing x Clone ideotype behavior characterization;
5) Compare the effects of different climatic and edaphic conditions and silvicultural regimes on growth and ecophysiology of loblolly pine varieties.

MATERIALS AND METHODS

Three complimentary experimental designs will be used in this study of varietal silviculture. This will include 1) Single tree plots with multiple varieties similar to conventional progeny tests; 2) Block plots of single varieties with individual plots planted at different densities; and 3) A Nelder design with different variets. This will enable us to evaluate the growth response of different loblolly pine varieties (open pollinated, control pollinated and clones) to different planting densities and different silvicultural regimes that include a variety of stand density, weed control, and fertilization treatments. Each of the varieties in the study will be classified into a crown ideoptype to determine if all clones of a specific crown ideotype respond in a similar manner.

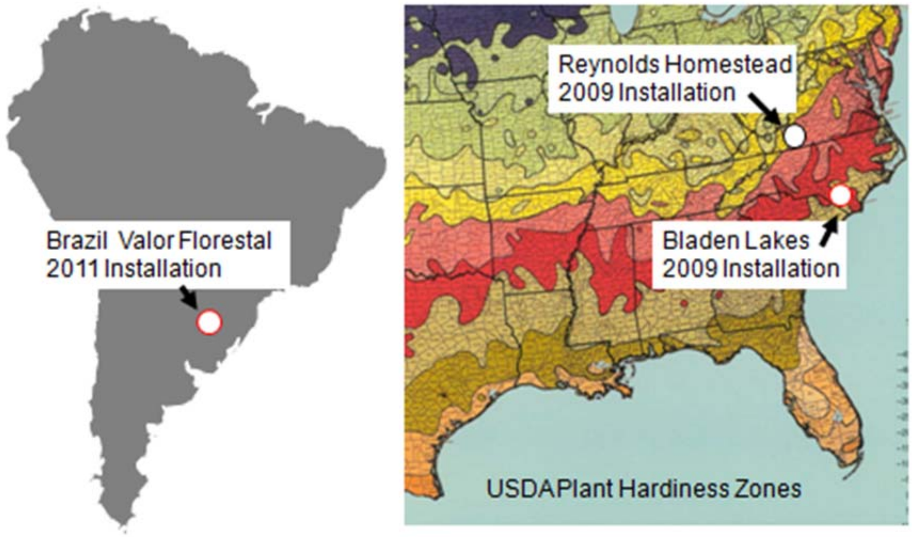
Study Sites

The study is currently planned to be installed at three locations (Figure 3). Two sites were established in 2009 in the southeastern United States. An additional sites will be established in 2010 in Brazil in the state of Santa Catarina or Parana. These sites will provide a range of climatic and edaphic conditions that affect growth efficiency of potential productivity of loblolly pine in the South (Figure 4). Additional sites may be established depending on interest of industrial collaborators and financial resources. For example, a study site in southern Louisiana would provide a higher productivity site to contrast the study site proposed in Oklahoma (Figure 4).

The first site in located the Coastal Plain of North Carolina at Bladen Lakes State Forest. The Coastal Plain sources of loblolly pine included in the study are considered well adapted to the climatic conditions at this site. The study site is approximately 51 acres in size. The soils at the site are somewhat poorly to poorly drained Ultisols mapped as the Rains series (Fine-loamy, siliceous, semiactive, thermic Typic Paleaquults). The previous stand was a loblolly pine plantation that was harvested in 2007.

The second study site will be located in the Piedmont of Virginia at the Virginia Tech Reynolds Homestead Research Center. Questions exist about the adaptability of the Coastal Plain sources of loblolly pine at this site because of colder winter temperatures and greater potential for ice and snow damage. Consequently, Piedmont sources are generally recommended for this site, although coastal sources are successfully planted here. The soils at the site are mapped as well-drained Fairview Series (Fine, kaolinitic, mesic Typic Kanhapludults). The site supported a mixture of loblolly pine, pitch *(Pinus rigida)* x loblolly pine hybrid, and white pine (*Pinus strobus* L.) plantations and natural Virginia pine (*Pinus virginiana*) stands that were harvested in 2007 and 2008.

The third study site will be located in Brazil in Parana State. This site will represent the upper end of productivity for loblolly pine in the world. The study planted at this site will include the six genotypes planted in the United States and a local landrase of loblolly pine that is adapted to the conditions in Brazil. The soils at this site are well drained



Brazil CAFS's site ➔ On google maps ➔ **Rio Negrinho**, Santa Catarina, Brazil

Figure 3. Location of clonal silviculture studies at the Reynolds Homestead Research Center in the Virginia Piedmont and at Bladen Lakes State Forest in the North Carolina Coastal Plain installed in 2009. Location of the Brazil installation in Santa Catarina State planted in 2011.

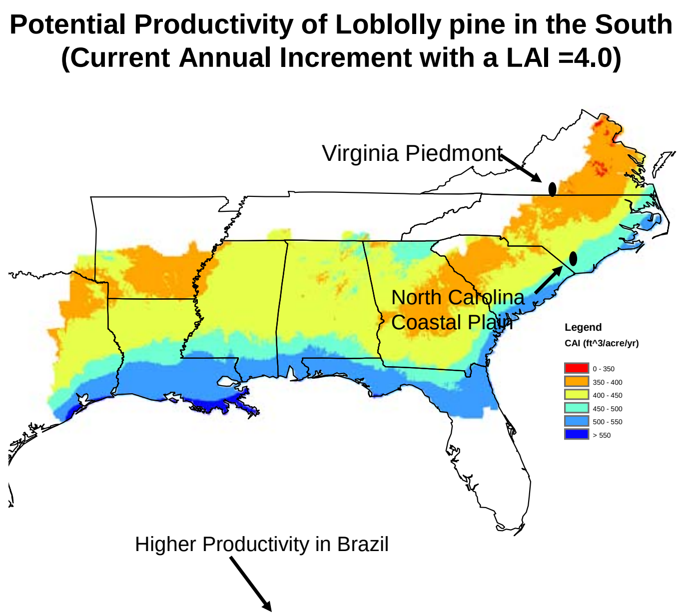


Figure 4. Potential Productivity of loblolly pine in the southern United States in relation to the location of study sites for the NSF Variety x Silviculture Study. Sites selected for the study include a range of potential productivity and growth efficiencies.

Block Plot Study Design and Treatments

The study design in the two sites in the US is a split split-plot with 4 replications at VA and 3 replications at NC (Fig 7a and 7b). Two levels of silviculture (operational and intensive) were the whole plot treatments, six genotypes (4 clonal varieties, 1 OP family and 1 CMP family) were the split-plot treatments, and three initial planting densities (617, 1235 and 1852 trees/ha) were the split split-plot treatments (Figure 1). The total number of plots was 144 at VA (2 silviculture treatments x 6 genotypes x 3 planting spacing x 4 replicates), and 108 at NC (2 silviculture treatments x 6 genotypes x 3 planting spacing x 3 replicates). There are no buffer rows between plots with different spacing or varieties. The buffer between whole plots with different silvicultural treatment was at least 20 m to avoid cross-over of the fertilization effect in the adjacent whole plots. The analysis of variance table for this design with 4 replications (Gomez and Gomez 1984) is summarized in Table 1.

Table 1. Analysis of Variance for NSF Varietal Silviculture Block Plot Study

Source	Degree of Freedom
Block (b=4)	**b-1=3**
Silvicultural Level (s=2)	**s-1=1**
Error A	**(b-1)(s-1)=3**
Genetic Variety (g=6)	**(g-1)=5**
Genetic Variety x Silviculture Level	**(g-1)(s-1)=5**
Error B	**s(b-1)(g-1)=30**
Planting Spacing (p=3)	**p-1=2**
Planting Spacing x Silviculture Level	**(p-1)(s-1)=2**
Planting Spacing x Genetic Variety	**(p-1)(g-1)=10**
Silviculture Level x Planting Spacing x Genetic Variety	**(s-1)(p-1)(g-1)=10**

Error C	**Sg(b-1)(s-1)=72**
Total	**(bsgp)-1=143**

The study design in Brazil was also a split split-plot with 3 blocks, but it was an incomplete block because of a limited number of seedlings from the individual families and clones were available (Fig 7c). Two levels of silviculture (operational and intensive) were the whole plot treatments. The split plot treatment in the intensive silviculture included 7 genotypes, the six used in the US and 1 locally adapted OP family. The split plot treatment in the operational plots only included 4 genetic entries: the locally adapted OP family, and 3 of the clones (C1, C2, C3). The OP Three initial planting densities (617, 1235 and 1852 trees/ha) were the split split-plot treatments (Figure 7c). The total number of plots was 99 at Brazil. There are no buffer rows between plots with different spacing or varieties. The buffer between whole plots with different silvicultural treatment was at least 20 m to avoid cross-over of the fertilization effect in the adjacent whole plots.

Main Plot Treatment: Silvicultural Intensity

Two levels of silvicultural intensity will be established as the main plots. The first level of silviculture will follow good operational practices in each location established for the study. Second level of silviculture will be designed to achieve near maximum growth for the existing soil and climatic conditions at each site. These two levels of silviculture will vary at the locations as follows:

Reynolds Homestead Research Center(VA)

Operational Practices: At the study site at the Reynolds Homestead Research Center in the Virginia Piedmont this will include chemical site preparation followed by burning. Site preparation at VA included an aerial application of mixed solution of Accord XRTII plus (9.3 L/ha), Milestone VM plus (9.3 L/ha), and Chopper (1.46 L/ha). The site was broadcast burned in November 2008. Banded herbaceous weed control will be used during the first growing season. The operational silvicultural treatment was a banded weed control after planting, with a mix of Arsenal AC (292 ml/ha) and Oust XP (146 ml/ha). At VA, in the third growing season, a solution of Escort (55 ml/ha) was applied to control blackberry (*Rubus* spp.). After the 4th growing season the rows between each plot were mowed to again control the blackberry. A second application of Escort was applied at 1 oz/acre during the spring of the 5th growing season. The need for additional hardwood control will be evaluated annually based on the current recommendations of the Virginia Department of Forestry based on the “free to grow” status of the plantation. Additional hardwood control will be applied if needed.

Intensive Practices: The same initial chemical site preparation and burning regime used in the operational treatment will be used in the intensive treatment. This will be followed by broadcast herbaceous weed control during the first two years. Directed sprays of herbicide will be used to eliminate all hardwood competition from these plots. The intensive silvicultural treatments consisted of a broadcast weed control with a mix of Arsenal AC (292 ml/ha), Oust XP (146 ml/ha) and Escort (18 ml/ha) in the first growing season; Arsenal AC (292 ml/ha) and Oust XP (146 ml/ha) in the second growing season; and Escort (55 ml/ha) in the third growing season. Tip moth control was applied after planting using PTM insecticide (BASF Corporation) (1.5 ml/tree) in the intensive treatment. The intensive treatment was fertilized after planting with 93 g/tree of nitrogen and 10 g/tree of phosphorus in the form of Arborite coated urea fertilizer, which was spread around the base of each individual seedling. After the 4th growing season the rows between each plot were mowed to again control the blackberry. A second application of Escort was applied at 1 oz/acre during the spring of the 5th growing season. The intensive treatment was fertilized with 200 lb/acre N and 20 lb/ac P in the form of Arborite Coated Urea fertilizer during the early spring at the start of the 6th growing season.

Bladen Lakes State Forest (NC)

Operational Practices: Site preparation at NC included a chemical application of Chopper (2.33 L/ha), Krenite (11.6 L/ha), and Garlon (1.53 L/ha), followed by V-blade bedding on 3.66 m centers using a Savannah bedding plow (Savannah Global Solutions, Savannah, GA). A banded herbaceous weed control treatment will be applied after planting. Tthe operational silvicultural treatment was a banded weed control after planting, with a mix of Arsenal AC (292 ml/ha) and Oust XP (146 ml/ha).

Intensive Practices. The site will be chemically site prepared as above. Residual slash piles will be raked as needed and the site will be V-blade bedded on 12-ft centers using a Savannah bedder. The intensive silvicultural treatments consisted of a broadcast weed control with a mix of Arsenal AC (292 ml/ha), Oust XP (146 ml/ha) and Escort (18 ml/ha) in the first growing season; Arsenal AC (292 ml/ha) and Oust XP (146 ml/ha) in the second growing season; and Escort (55 ml/ha) in the third growing season. Tip moth control was applied after planting using PTM insecticide (BASF Corporation) (1.5 ml/tree) in the intensive treatment. The intensive treatment was fertilized after planting with 93 g/tree of nitrogen and 10 g/tree of phosphorus in the form of Arborite coated urea fertilizer, which was spread around the base of each individual seedling. The intensive treatment was fertilized with 200 lb/acre N and 20 lb/ac P in the form of Arborite Coated Urea fertilizer during the early spring at the start of the 6th growing season.

Santa Catarina State, (Brazi)

Operational Practices: At the study site in Brazil, the operation practices will be the normal practices used by Renova will include chemical site preparation and additional weed control with glyphosate that is used to establish operational plantations.

Intensive Practices. At the study site in Brazil, the intensive practices will be the normal practices used by Renova will include chemical site preparation and additional weed control with glyphosate to main complete weed control until crown closure. The intensive treatment was fertilized with 150 kg/ha of triple superphosephate during the first growing season after planting.

Split Plot Treatment: Genetic Entries

Six different genetic entries will be used as the subplots. This will include one open-pollinated family, one control pollinated family and four different clones. The clones represent a range in crown ideotypes with two having moderately wide crown and two having broad crowns. The specific genetic entries provided by Arborgen for the block plot installations in 2009 are listed in Table 2.

Table 2. Genetic sources, family ID and predicted crown ideotypes of the genetic sources proposed for the block plot plantings.

Genetic Source	Color of Plot Flags in Field	Arborgen Family or Variety ID	Crown Ideotype
Family 1 - Open Pollinated (OP)	White	AG89S	-
Family 2 - Mass Control Pollinated (MCP)	Pink	AGM20	-
Variety 1	Red	PM212	Moderate +
Variety 2	Yellow	NQ26	Broad
Variety 3	Blue	GE34	Narrow
Variety 4	Green	PT1056	Broad
Family 3 Open Pollinated Land Race (Brazil Only)	Grey	Rigesa Fam	

Table 3. Parents of the six genotypes assessed that were provided by Arborgen. Genotypes C1, C2, C3, and C4 correspond to the varieties; OP; open pollinated family; CMP; control mass pollinated family. A, B, C, D and E are arbitrary codes representing the pedigree of the parents. Family 3 is an open-pollinated family developed by Rigesa in Brazil and is a land race adapted to southern Brazil.

		Mother		
		A	B	D
Father	A			C1
	B			C4
	C	C2	CMP	
	E			C3
	?	OP		

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Split-Split Plot Treatment: Planting Spacing

Three different planting densities will be employed in this study. The lowest planting density will be 250 trees/acre (617 trees/ha) which is designed as a sawtimber regime where diameter growth is maximized. The medium planting density will be 500 trees/acre (1235 trees/ha) which is designed as a mixed product regime that includes both pulp and sawtimber production objectives. The high planting density will be 750 trees/acre (1852 trees/ha) designed as a biomass or pulpwood regime.

The spacing between planting rows in all three planting densities will be 12 ft. Therefore, to achieve the desired planting densities, the distance between trees within the row will vary (Figure 5). The spacing for the low density of 250 tree/acre (617 trees/ha) will be 12 ft x 14.5 ft (3.66 m x 4.42 m). The spacing for the medium density of 500 tree/acre (1235 trees/ha) will be 12 ft x 7.26 ft (3.66 m x 2.21 m). The spacing for the high density of 750 trees/acre (1852 trees/ha) will be 12 ft x 4.84 ft (3.7 m x 1.47 m)

At the end of the first growing season, seedlings that died were replanted with extra seedlings from the same genotype that were grown in containers. This was done to maintain the target stand density, so that the effect of mortality inter-tree competition was minimized. The location of each tree replanted was recorded.

Plot Layout and Planting

Each individual plot will include 81 trees in a 9 row by 9 planting space configuration at Reynolds (Figure 5). Because of space limitation, on block at Reynolds was established as a 7 x 9 configuration and all blocks at Bladen lakes were a 7 x 9 configuration. Because of the different within row spacing, the size of the plots for each of the three spacings will vary (Figure 5) There will be no buffer trees between the individual plots within a main plot. However, the arrangement will permit a 5 x 5 tree internal plot to be established that is not influence by the tree**s** in the adjacent plot. This design will permit limited destructive sampling of clones located outside the core 5 x 5 tree central plot. This design will also enable studies of below-ground ecophysiological processes on an individual clone in an area where all the roots will be from trees of the same clone.

Each main plot will contain the six subplots with different genetic entries in the US (Figure 6) and 7 genetic entries in the intensive plots and 4 genetic entries in the operational plots in Brazil. There will be a one to two row buffer around each main plot. The buffer between different silvicultural main plots will be larger so that fertilizer treatments do not affect the adjacent main plot. Based on the desired planting densities, each main plot including the two row buffer will be 372 ft x 498 ft (113.39 m x 151.79 m). (Figure 6). Thus each main plot will be approximately 4.25 acres (1.72 ha). Consequently, each full block will require at least 8.5 acres (3.44 ha) and the complete experiment with 4 replications will require a minimum of 34 acres (13.77 ha).

Each planting spot will be marked with a pin flag. The pin flags will be color coded with a separate color representing each clone, OP or CMP genetic entry (Table 2, Figure 7). Seedlings will be hand planted during February – March 2009 in the US and in July/August 2011 in Brazil.

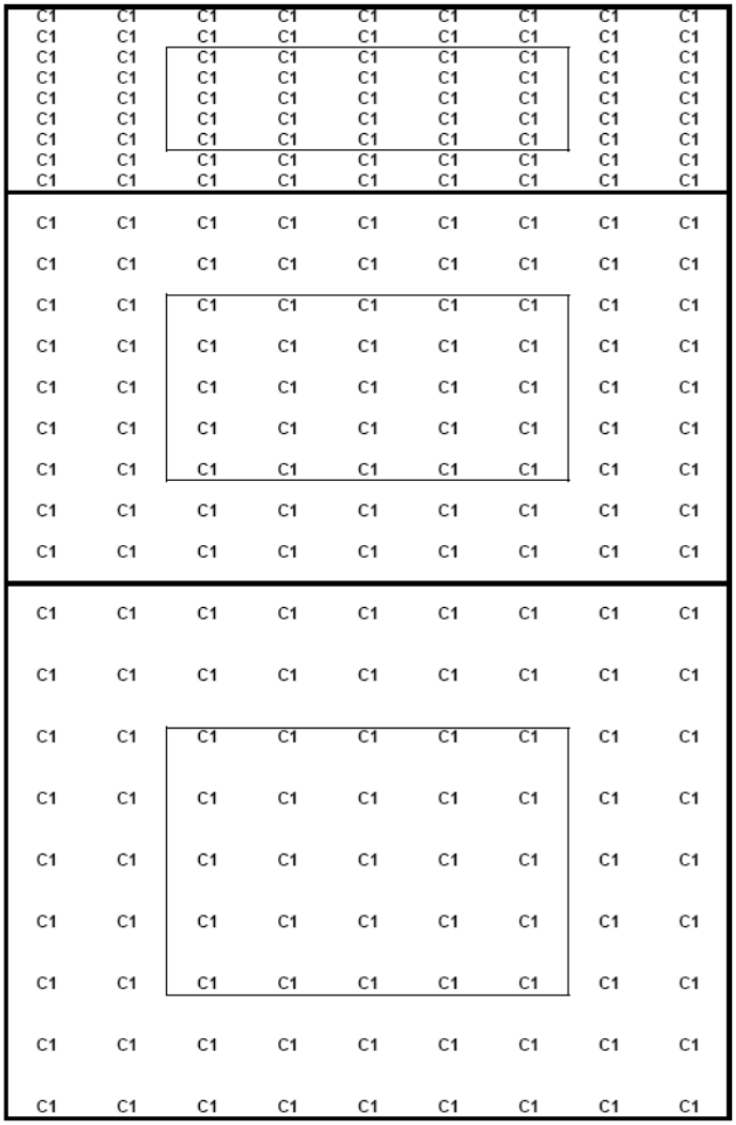


Figure 5. Layout of individual 9 tree x 9 tree plots for one clone at three spacings: 250 trees/acre = 12 ft x 14.5 ft; 500 trees/acre = 12 ft x 7.26 ft; 750 trees/acre = 12 ft x 4.84 ft. Bold lines enclose the 81 tree (9 x9) plot. Light lines enclose a 25 tree (5 x 5) interior plot.

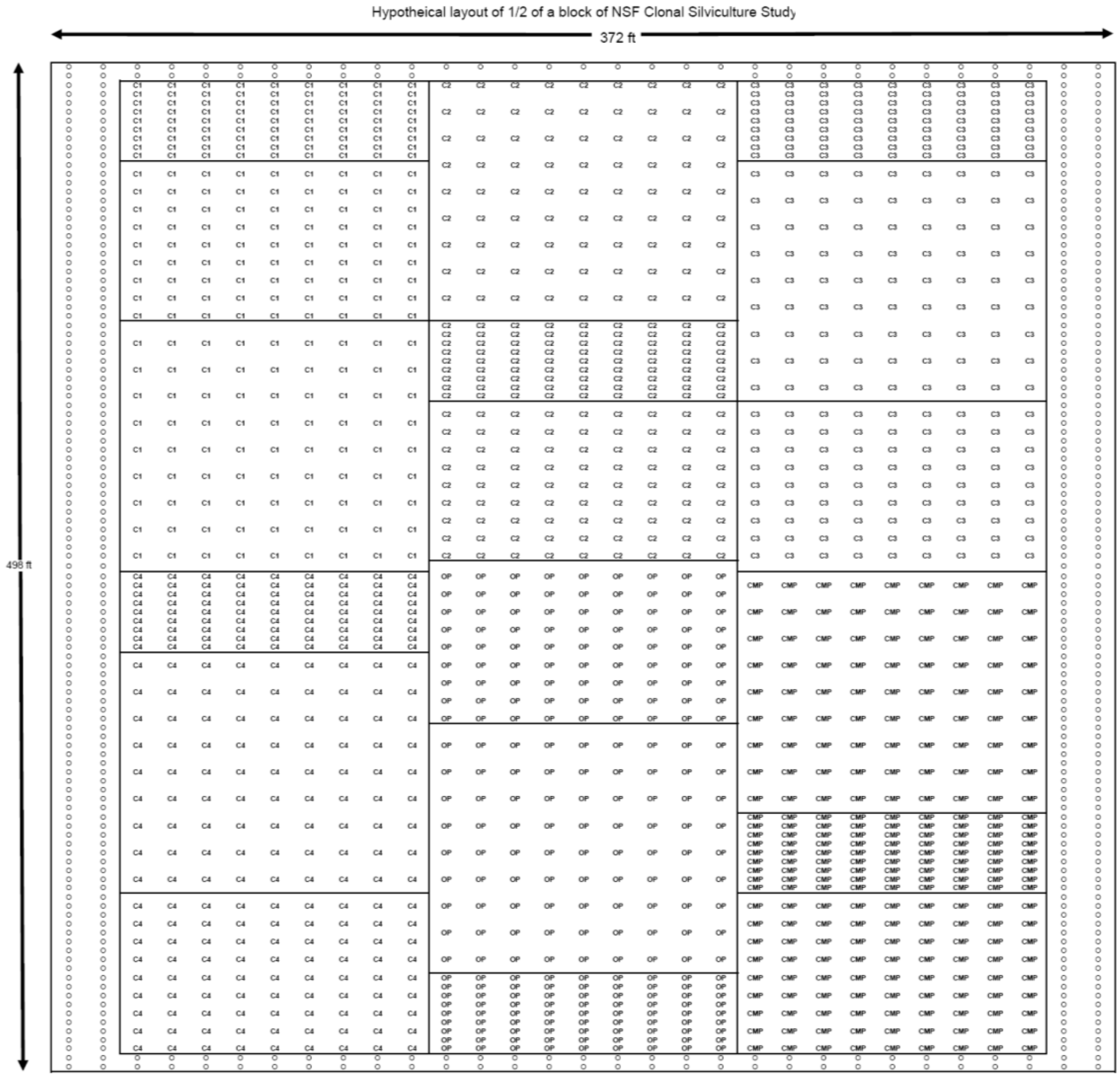


Figure 6. Hypothetical layout of three different spacing (250trees/acre; 500 trees/acre and 750 trees/acre) and six genetic entries (OP = open pollinated family; CMP = control pollinated family; C = clone) at one level of silvicultural treatments. This represents ½ of a full block.

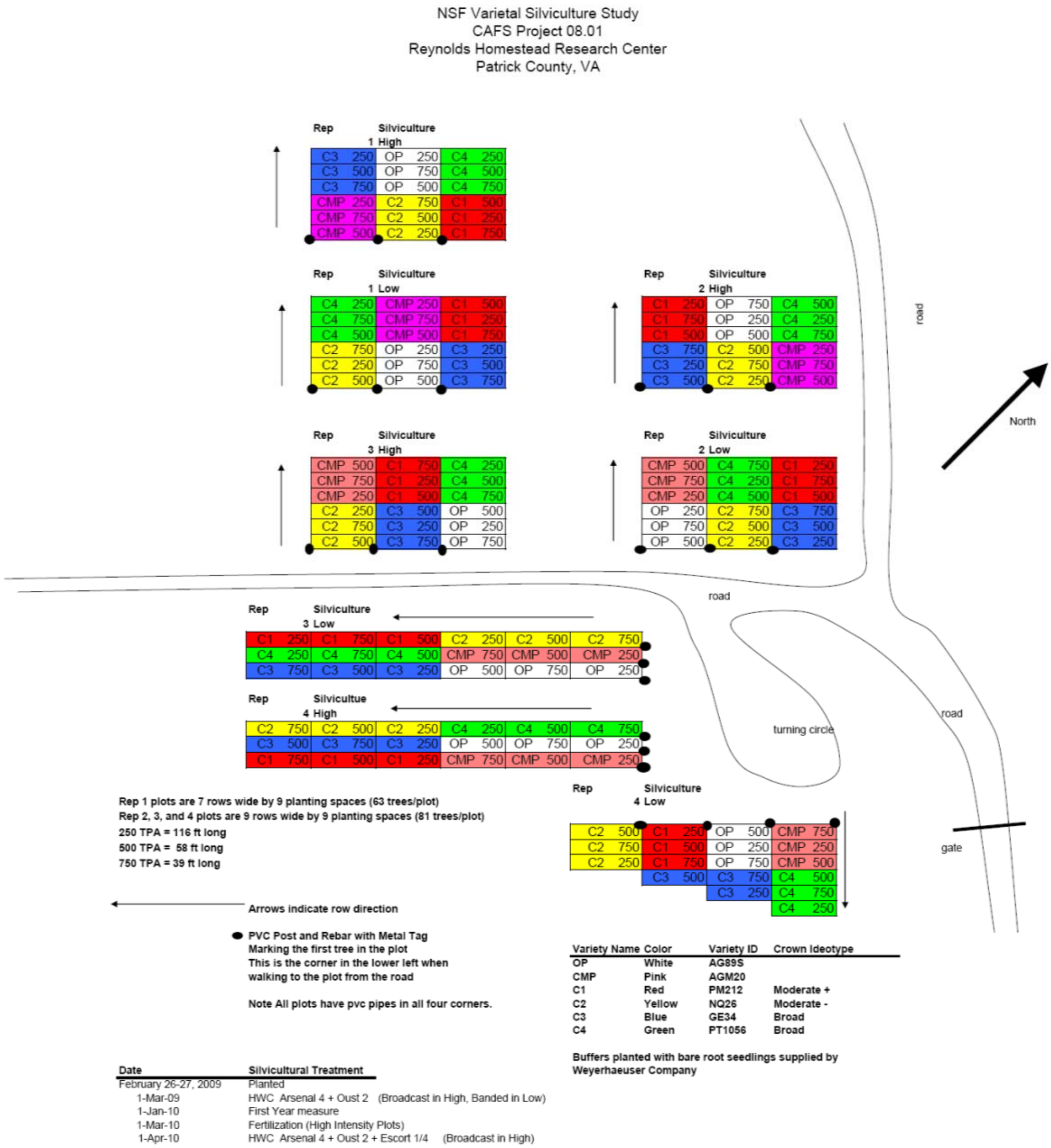


Figure 7a. Study layout and clone allocation for the installation at Reynolds Homestead Research Center. Each genetic entry is assigned a color and each planting spot in each plot is marked with a wire stake flag with that color. The color associated with each genetic entry is listed in Table 2.

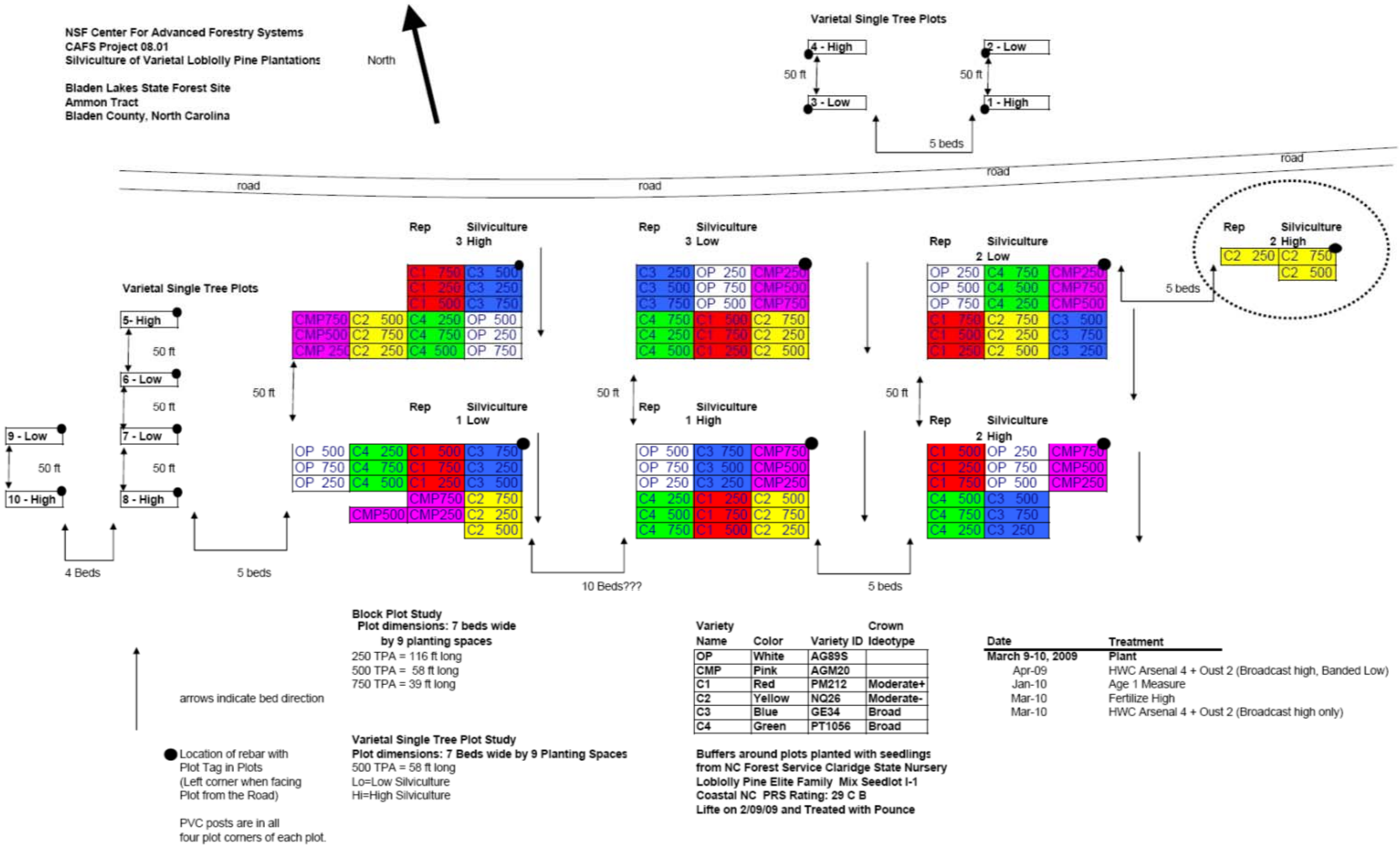


Figure 7b. Study layout and clone allocation for the installation at Bladen Lakes State Forest. Each genetic entry is assigned a color and each planting spot in each plot is marked with a wire stake flag with that color. The color associated with each genetic entry is listed in Table 2.

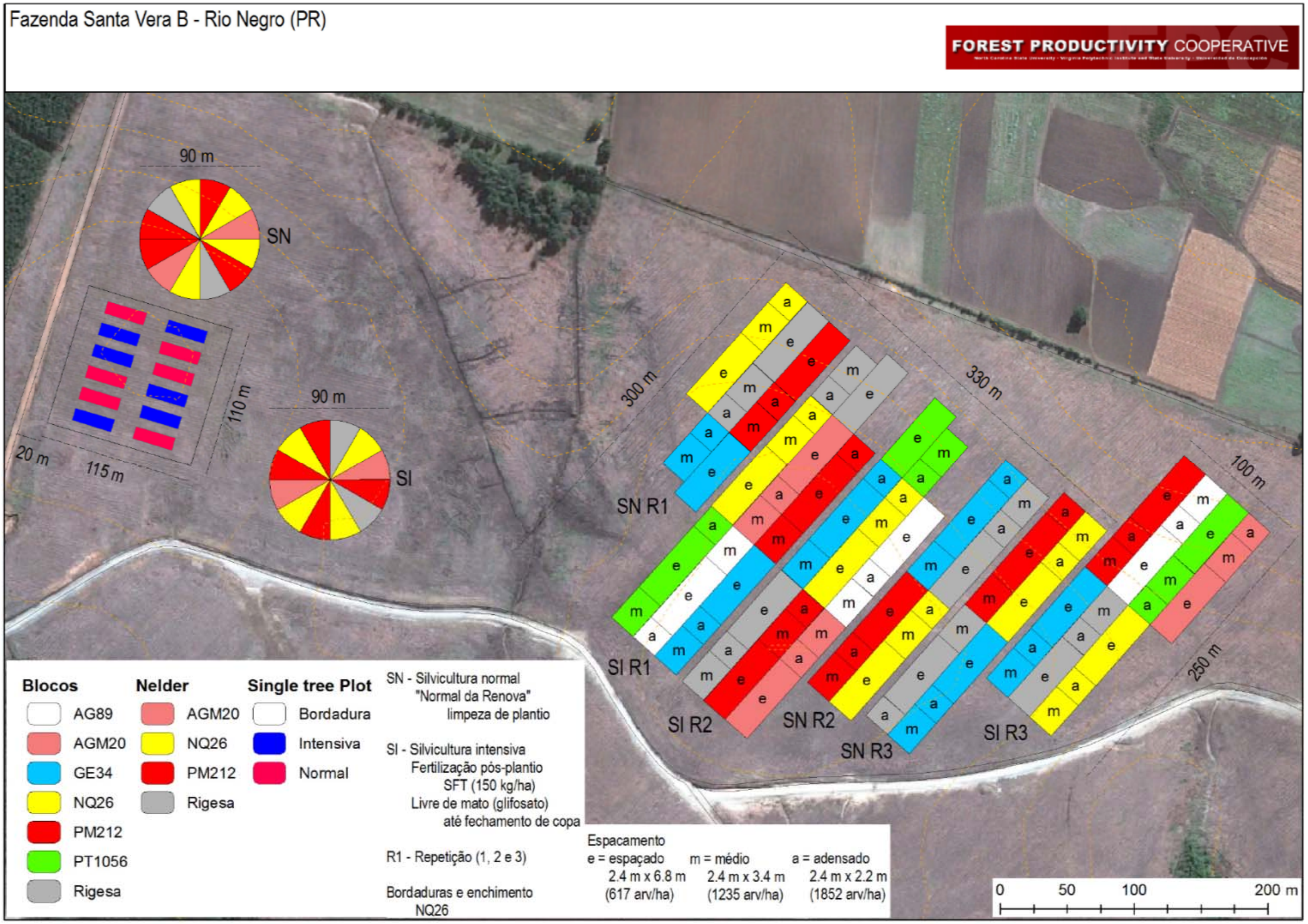


Figure 7c. Study layout and clone allocation for the installation at Santa Catarina State in Brazil. Each genetic entry is assigned a color and each planting spot in each plot is marked with a wire stake flag with that color. The color associated with each genetic entry is listed in Table 2.

Nelder Study Design and Treatments

The Nelder Design is an efficient way to evaluate a wider range of initial planting densities in forest plantation with different genetic materials (Namkoong 1965). Trees are planted in a "spoke and wheel" manner. As the distance from the center point of the wheel increases, growing space allocated to each seedling increases. This translates into an effectively wider and wider spacing of each seedling (Figure 8).

A Nelder design will be installed as a companion study to the block plot planting at each of the study sites (Table 3). There will be 36 spokes in each Nelder. Trees will be planted on the spokes of the Nelder wheel at distances ranging from 17 ft to 143 ft (5.18 m to 43.59 m) from the center point. The equivalent spacings in the Nelder design will range from 2890 trees/acre (7142 trees/ha) to 92 trees/acre (227 trees/ha) (Figure 8, Stape 1995).

The six varieties used in the block plot planting will be included in each Nelder. A single variety will be planted in three adjacent spokes on the Nelder wheel, forming a "pie wedge" of each variety (Figure 9). Each variety will be replicated twice in each individual Nelder. Two Nelder wheels will be planted at each site which will provide 4 replications of each variety at the range in spacing (Figure 9).

The silviculture applied in the Nelder design will duplicate the intensive practices used at each location (see above).

Table 3. Proposed Nelder desing to compliment block plot.

Spacing	Distance from Center (ft)	Stocking (stem/ac)	Distance from Center (m)	Stocking (trees/ha)
Internal buffer	17	-	5.30	-
Spacing 1	21	2890	6.42	7142
Spacing 2	26	1974	7.78	4878
Spacing 3	31	1344	9.42	3322
Spacing 4	37	917	11.41	2267
Spacing 5	45	625	13.82	1545
Spacing 6	55	426	16.74	1052
Spacing 7	67	290	20.27	717
Spacing 8	81	198	24.55	489
Spacing 9	98	135	29.73	333
Spacing 10	118	92	36.00	227
External buffer	143	-	43.60	-

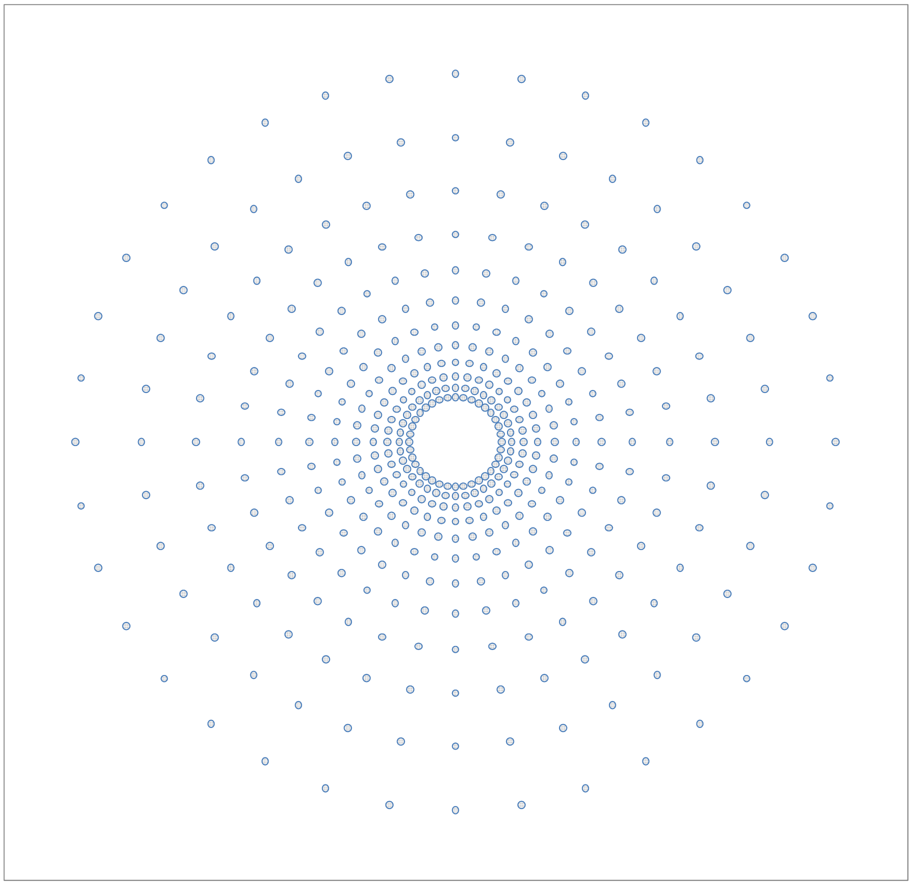


Figure 8 Typical Nelder design used to evaluate multiple initial planting spacings.

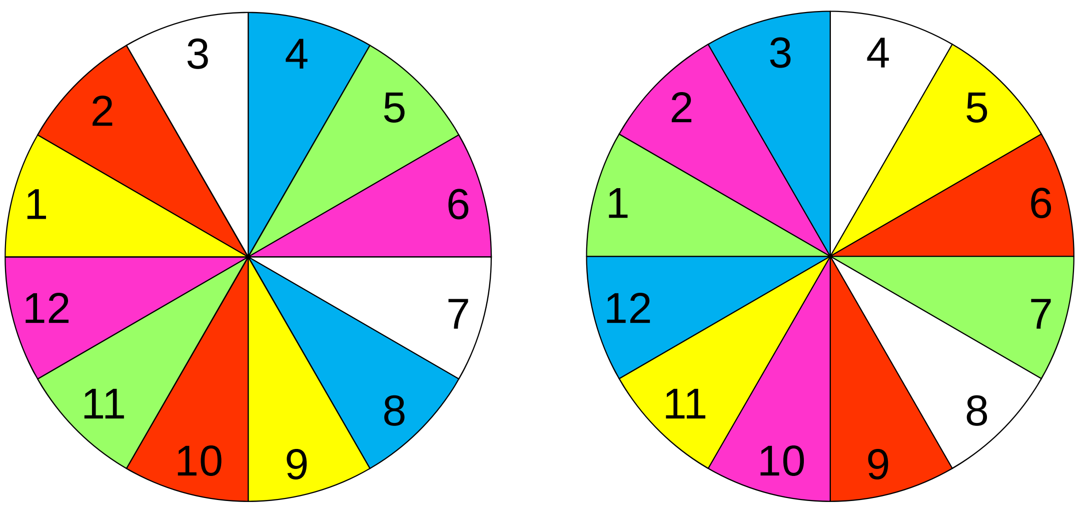


Figure 9 Two Nelder’s Wheels providing 4 replications of each genetic material.

Single-Tree Plot Study Design and Treatments

Modern tree improvement progeny tests typically utilize single-tree plots because these plots have higher statistical precision (White et al. 2007). They produce better population parameter estimates and better rankings of parents, families and clones. This is because 1) it is generally possible to include a larger number of varieties with more replications for a given amount of effort; and 2) block size is usually smaller so soils and environmental conditions within blocks are more homogeneous. Because varietal development programs must test large numbers of varieties to identify individuals that are suitable for operational deployment, single tree plots are the best way to efficiently test a large number of candidate varieties. Above-ground ecophysiological parameters, such as photosynthesis and respirations, can also be measured in single tree plots. However, below-ground processes can not be reliably measured because roots from different varieties occupy the same soil volume.

In this study, we are only able to test a limited number of varieties in the block plots and the Nelder designs. This is problematic because the inference base on different varieties will be small. Therefore, in an effort to increase the number of varieties that are evaluated in this study, a series of single-tree plots will be established at some of the study locations.

Single tree plots will be established utilizing one spacing (500 trees/acre) but will include the two levels of silvicultural treatments used in the block plots. The six varieties used in the block plots and the Nelder design will be included in the single-tree plots. The number of varieties included in the single tree plots will be based on their availability. 63 varieties (including checks) were included in the study at the Bladen Lakes State Forest site established in 2009. The varieties included at the site in Bladen Lakes State Forest are listed in table 4.

Table 4. Varieties and checks provided by Arborgen for use in the single-tree plot study at Bladen Lakes State Forest in 2009. A total of 63 varieties and checks were included in the study. This includes the six varieties used in the block plots and the Nelder plantings.

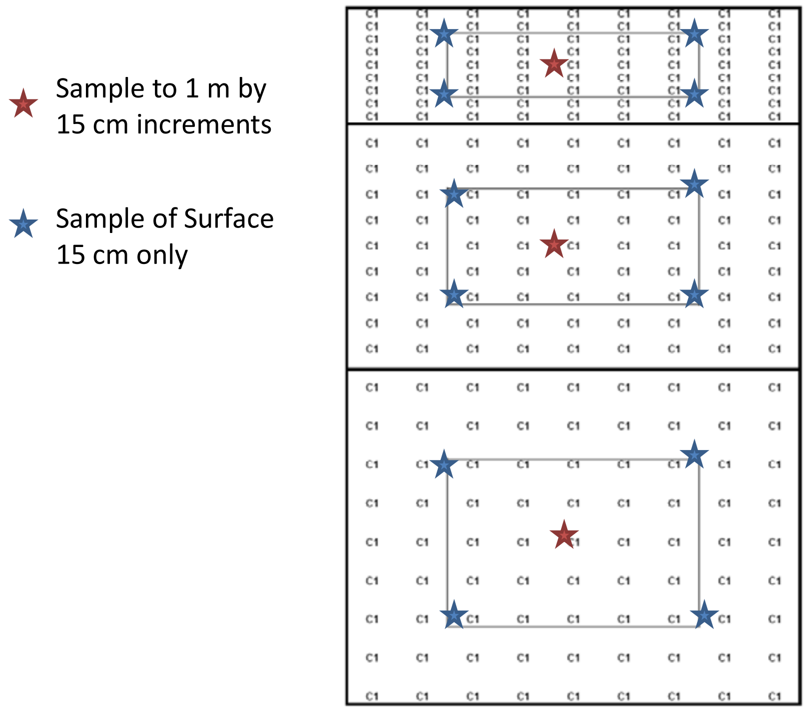
#	Line ID	Variety ID	#	Line ID	Variety ID
1	01NQ0026	NQ26	33	06BT0057	M506
2	01PM0212	PM212	34	06BT0187	M517
3	06BK0004	M4	35	06BT0326	M527
4	06BK0011	M11	36	06BT0337	M538
5	06BK0014	M14	37	06BT0363	M564
6	06BK0021	M21	38	06BT0492	M583
7	06BK0382	M26	39	06BT0495	M586
8	06BK0387	M31	40	06BT0514	M605
9	06BK0400	M44	41	06BT0527	M618
10	06BK2573	M59	42	06BT0529	M620
11	06BK2720	M81	43	06BT0741	M640
12	06BK2722	M83	44	06BT0745	M644
13	06BL0130	M98	45	06BT0751	M650
14	06BL0537	M145	46	7-56 OP	7-56 OP
15	06BL0549	M157	47	96GE0034	GE34
16	06BM0152	M223	48	AG-89 S	AG-89 S
17	06BM0153	M224	49	AGM-20	AGM-20
18	06BM0208	M253	50	ATS Mix	ATS Mix
19	06BM0211	M256	51	I1563498	A10
20	06BM0231	M276	52	I1563516	A28

21	06BM0254	M299	53	I1573604	A77
22	06BM0277	M322	54	I1573611	A84
23	06BM0278	M323	55	I1573620	A93
24	06BM0451	M346	56	I1603914	A205
25	06BM0459	M354	57	I1624000	A279
26	06BM0717	M400	58	I1624026	A305
27	06BM0774	M412	59	I1624032	A311
28	06BM0779	M417 S	60	MW CKA	MW CKA
29	06BS0173	M430	61	MW CKI	MW CKI
30	06BS0181	M438	62	PT1056	PT1056
31	06BS0323	M464	63	WY CK4	WY CK4
32	06BS0664	M483			

At the Bladen Lakes State Forest Site established in 2009, the single-tree plots containing 63 varieties were located adjacent to the block plots (Figure 7b) Ten replications of each 63-tree plots were established. The plots were paired so that half the plots will receive the operational silvicultural treatments and the other half receive the intensive treatment used in the block plot study. Thus, there will be 5 replications of each silvicultural treatment (Figure 7b)

Appendix 1. Soil Sampling Protocol

RW 20 Soil Sampling Protocol



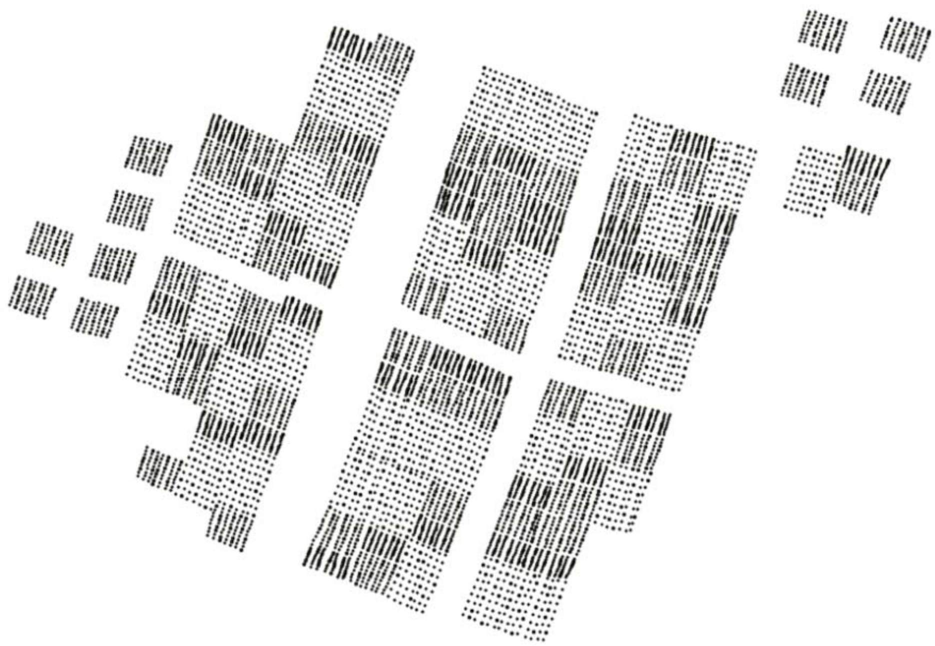
Soil samples to characterize each site were collected in each plot as follows:

1) Samples of the surface 15 cm of mineral soil were collected at 5 locations in each plot. Samples were kept separate and analyzes separated.
2) Samples were collected at 15 cm depth increments to a depth of 1 m at the center of each plot. Individual samples at each depth increment were analyzed separately.

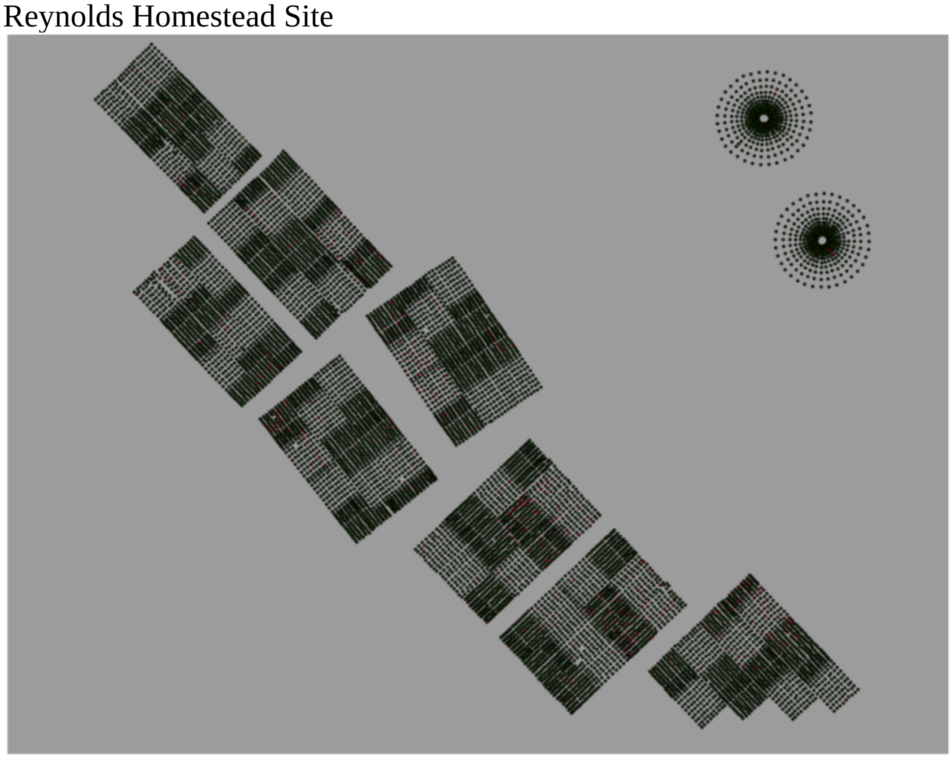
Appendix 2 GPS Location of Individual trees.

The location of each tree in all plots was determined to approximately 2 cm accuracy at the study sties Reynolds Homestead and Bladen Lakes State Forests.

Bladen Lakes Site



Reynolds Homestead Site



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