Final Report

Consequences of Landscape Patterns on the Genetic Composition
of Remnant Hardwood Stands in the Southeast:
A Pilot Study

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Objectives

The objectives of this project were two-fold. One goal was to establish a pilot study that would generate preliminary genetic data for a tree species in fragmented hardwood stands on the Savannah River Site (SRS). It was anticipated that this data would permit assessment of the feasibility of long-term genetic research for which external funding support could be generated. A second objective was to initiate studies that addressed fundamental questions of how landscape structure, in conjunction with the population dynamics and reproductive characteristics of the tree species, influences genetic structure and long-term viability of hardwood forest stands at SRS and in similar southeastern landscapes.

Rationale

Fragmentation of plant habitats can result in small, genetically isolated populations. Spatial isolation and small population size may have several consequences, including reduced reproduction, increased inbreeding and the stochastic loss of genetic variability. Such losses of genetic and genotypic diversity can reduce plant fitness and may diminish population viability. Deleterious genetic effects resulting from small population sizes can be ameliorated by gene flow via pollen and seed into fragmented populations. Despite the widespread and ongoing fragmentation of natural populations, the genetic consequences of fragmentation have been empirically examined for only a few plant species, and even fewer temperate hardwood species. To our knowledge, no studies have examined gene flow in temperate hardwoods in the context of landscape structure.

Proposed strategy

The initial strategy for this project was to a) identify a hardwood species that was amenable for the studies from a genetic standpoint, b) identify and delimit suitable study sites within varying landscapes on SRS, c) obtain multilocus allozyme genotypes from maternal trees, from potential pollen donor trees and from progeny within the delimited study sites, and d) estimate “maternal” reproduction of trees within the study sites. The genetic data would permit estimation of gene flow into study sites, a critical measure for assessment of the long-term viability of forest fragments. Estimates of relative “male” fertility per tree would also be obtained from the genetic data. Outcrossing rates, as well as biparental inbreeding (mating amongst relatives) were to be estimated. For most forest trees, inbreeding (either selfing or mating among relatives) is accompanied by inbreeding depression, a decline in viability and fecundity of progeny. To predict the long-term trajectories of forest fragments, estimates of $N_e m$ (the effective number of migrants per generation) were anticipated, based on rates of pollen migration and measures of relative male and female fertility. $N_e m$ predicts the influence of genetic drift (leading to a loss of genetic diversity) on populations.

Preliminary studies

In the fall of 1997, when this study was initially funded, three oak (*Quercus*) species were chosen as candidate species for the project [*Q. alba* L. (white oak); *Q. stellata* Wang (post oak); and *Q. rubra* (red oak)]. Leaf samples were collected from 24 individuals of each of these

species from several SRS sites to determine their suitability for genetic analysis. A battery of preliminary electrophoretic trials were run to determine which electrode/gel buffer combinations and enzyme stains produced the best results for each species. At the conclusion of these trial runs, *Q. alba* was chosen for further study, as it appeared to have sufficient genetic diversity for the proposed studies. Furthermore, U.S. Forest Service reports (J. Blake, pers. comm.) indicated that white oaks had experienced a significant decline in SRS forests. Survey data from 1700's and 1800's indicated that white oaks were once the dominant component of hardwood forests at SRS, comprising about 12% of these forests. Recent data, however, indicates that they presently represent less than one percent of the remnant hardwood forests, and they are not regenerating.

The study species: *Quercus alba* L., White Oak (Fagaceae)

White oak is one of the most important hardwood trees. It is widespread across eastern North America, ranging from southern Canada to northern Florida (Elias 1987). It grows in a variety of soils, but does best in deep, rich, well-drained loamy soils. White oak is seldom found in pure stands, but it is generally in mixed hardwood stands, most often with other oaks and hickories (Elias 1987, Rogers 1990).

White oak is a large, long-lived, slow-growing tree that often reaches 24 to 30 m in height and 91 to 122 cm dbh. Individual trees as tall as 46 m, as large as 244 cm dbh, and as old as 600 yrs have been recorded. When open-grown, the species is characterized by a stocky bole, with a wide-spreading crown. Forest grown trees develop a tall straight trunk with a compact crown (Rogers 1990).

White oak can mature in as little as 20 years if open-grown (Young and Young 1992), but forest trees take as long as 50 to 200 years to become reproductive (Rogers 1990). The species is monoecious, with separate male and female flowers found on the same individual. Flowers appear in the spring from late March to late May (depending on latitude) at about the same time leaves appear. Staminate flowers appear first, with pistillate flowers appearing five to ten days later. Pollen dissemination (by wind) is usually completed within three days, barring wet weather. High abscission rates of female flowers are common and may be related to weather during pollination, ovule development and fertilization (Rogers 1990). Dry winds and freezing weather are detrimental to flower development and pollen shedding (Rogers 1990). Acorn maturity is reached approximately 120 days after pollination, with acorn drop occurring about 25 days later in September and October (Rogers 1990).

Data on acorn production is somewhat contradictory. According to Elias (1987) white oak is "generally a prolific seed producer, with large crops produced every four to six years". Young and Young (1992) list the seed-bearing interval for the species at four to ten years.

A more detailed accounting is given by Rogers (1990). He states that "White oak can produce seeds prolifically, but good acorn crops are irregular and occur only every four to ten years. Sometimes several years may pass without a crop. Acorn yields range from 0 to 500,000 acorns per hectare. This great variation in acorn production exists not only among isolated stands of oaks but also among individual trees within stands and from year to year....Individual white oak trees tend to have either very good or very poor seed crops and are noticeably consistent in seed production from year to year....Several studies have shown that only a small portion of the total mature acorn crop (sometimes only 18 percent) is sound and fully developed; the remainder is damaged or destroyed by animals and insects".

Acorns are an important but inconsistent food source for 180 species of birds and mammals, including whitetail deer, squirrels, turkeys, raccoons, mice, quail, crows and woodpeckers (Rogers 1990). Deer also browse on twigs and foliage of seedlings and young saplings. Weevils of the genera *Curculio* and *Conotrachelus* cause most insect acorn damage (Rogers 1990). However, two Cynipid moths (*Melissopus latiferranus*) and *Valentinia glandulella*) also damage acorns. In years of light acorn production, crops are often completely destroyed (Rogers 1990, Young and Young 1992).

Sound white oak acorns germinate upon falling to the ground. Germination is severely limited by flooding, and bottomland sites are likely to experience recruitment only during periods of drought (Rogers 1990; B. Bongarten, School of Forestry, UGA, Athens, GA, pers. comm.).

Field surveys, Mapped sites and Collection of adult leaf tissue

Using habitat- and forest-type maps provided by US Forest Service, field surveys were undertaken to identify suitable *Quercus alba* sites for genetic analyses. These surveys were initiated in 1997, and continued in 1998 and 1999, with less emphasis in later years. Ideally, for the genetic studies of gene flow into forest fragments that were originally envisioned, the identification of fairly isolated forest fragments with no more than five to twelve reproductive *Q. alba* trees was desirable. Despite extensive searches such sites were not located on SRS. All “remnant” *Q. alba* sites (both upland and bottomland) that were identified harbored many more trees, and extended over considerable distances. Although this altered the experimental designs that were initially anticipated, estimates of gene flow and $N_e m$ were still feasible. One isolated tree and two semi-isolated mature trees were identified.

Adult *Q. alba* trees were mapped at twelve sites, several of which were closely adjacent to one another. Each adult white oak was permanently marked with a numbered aluminum tag. Diameter at breast height was recorded for each tagged tree. Mapped sites included one population on Boggy Gut Road that was mapped in three sections. This was a fairly extensive, but scattered, population of white oak. Two sites were on Tyler Bridge Road, one on either side of the road. One of these sites was adjacent to a University of Florida seed study, the other may have been an old homesite. Two sites were mapped on Road F, one on either side of the highway (one site was mapped in two sections). Two additional mapped sites were off Road C and Road 8-1 (another site adjacent to a University of Florida seed study). A bottomland population of *Q. alba* was mapped on either side of Road A-17-2, at the southern end of SRS. Maps of the *Q. alba* sites with tagged trees, as well as data sheets with coordinates of the mapped trees, are in the Appendix of this report. These twelve sites and several others (including two unmapped sites on Road F, a roadside site on A-17, and a site near Crackerneck Wildlife Management Area (as well as the three semi-isolated trees) were monitored yearly for acorn production.

It was never the intent to obtain detailed genetic data from each of the twelve mapped sites for this pilot project. However, when it became clear that white oak reproduction was limited and extremely spotty at best, we decided to hedge our bets, and map trees at a number of different locales. In essence, we were going to let the trees (by their reproduction) delimit the study areas and the experimental design. This was in contrast to our original plan, which was to carefully select and map a few study sites that were as close to ideal for the goals of the project as possible.

In anticipation of identifying several of the mapped sites that would “work” by virtue of

having sufficient acorn production, extensive efforts were made to obtain leaf samples for genetic analysis from all the mapped trees at the twelve sites during the summers of 1998 and 1999. Leaf samples are necessary to genetically characterize potential pollen donors at some radius around an acorn-bearing tree. It was anticipated that leaves would not be amenable for genetic analyses at the time of acorn drop in the fall when “viable” study sites would be chosen. Some leaf samples were obtained using a 30 foot pole pruner. However, the leaves of many trees could not be reached with a pruner. For these a shotgun was employed to bring leaf samples to the ground. Again, the intent was not to genetically analyze all samples, but to have the samples available for analyses once sites with sufficient acorn production were identified.

Acorns were searched for and collected during the 1998, 1999 and 2000 fall seasons, but seed crops were extremely light. Acorns were difficult to find, despite extensive on-the-ground searches through leaf litter. Many acorns that fell during these years were very small, and most were infected with weevils (presumably *Curculio* and *Conotrachelus* species; Rogers 1990, Young and Young 1992). Although we doubted the viability of many seeds because of their size, and because many had their caps still attached, and many had weevil holes (sometimes embryos are still viable) all collected acorns were planted in these years. Germination was so poor that the number of seedlings obtained in these years did not warrant genetic analyses; the data would have been insufficient to calculate any useful genetic parameters. The number of acorns collected, the number of trees sampled, and the percentage germination were not recorded on a yearly basis, since we anticipated a season with a “good acorn crop” that would permit the successful completion of the study before the grant was terminated.

Allozyme analyses of field collections

Following two years of limited acorn reproduction, it became clear that the lack of reproduction might affect the successful completion of the initial goals of this project. In order to obtain some genetic information on *Q. alba* trees, eight *Q. alba* sites (Table 1) were sampled (as above) in July 2000 to obtain genetic diversity data for the species. Forty-eight adult trees were sampled from each of the eight sites for a total of 384 individuals. Leaf samples were kept on ice until enzymes were extracted in the laboratory at the University of Georgia.

Leaf samples were crushed using a mortar and pestle under liquid nitrogen and with clean ocean sand. An extraction buffer (Mitton et al. 1979) was added to solubilize and stabilize the enzymes. Enzymes were absorbed onto paper chromatography wicks that were stored in microtest plates at -70 C until needed for analysis.

Allozymes were resolved using standard starch gel electrophoretic procedures. Based on the preliminary trials, we used four gel-electrode combinations and 11 enzyme stains to resolve 20 allozyme loci on 12 % starch gels. Gel and electrode buffer recipes were from Table 1 in Soltis et al (1983), except where noted. The enzymes stained (and loci resolved) on System 8 were fluorescent esterase (*Fe-1*, -2 and -3) and peroxidase (*Per-1* and -2); on System 6 they were menadione reductase (*Mnr-1*), phosphoglucosomerase (*Pgi-1* and -2) and triose phosphate isomerase (*Tpi-1*, -2 and -3). Amino acid transferase (*Aat-2*), and leucine amino transferase (*Lap-1*) were stained on System 10. A morpholine-citrate buffer (pH 6.25; Conkle et al. 1982) was used for 6-phosphoglucosomate dehydrogenase (*6-Pdgh-1* and -2), isocitrate dehydrogenase (*Idh-2*), UTP-glucose-1-phosphate (*Ugpp-1*), malate dehydrogenase (*Mdh-1* and -2) and shikimate dehydrogenase (*Skdh-1*). Stain recipes were modified from Soltis et al. (1983), except

for amino acid transferase (Cheliak and Pitel 1984) and UTP-1-glucose-phosphate (Manchenko 1994).

2001 Acorn Collections – A Changed Strategy

A new statistical methodology (Two- Gener) to obtain estimates of gene movement was published by Smouse et al in 2001. The rationale for developing this methodology was that paternity analyses, which have been traditionally used to obtain direct estimates of gene flow, and which we were planning to use, have numerous short-comings. Paternity analyses rely on carefully delimited, mapped study sites that contain relatively few individuals. They are also heavily reliant on obtaining a battery of highly polymorphic loci. In addition, estimates of gene flow derived from these studies are extremely labor intensive. Moreover, the estimates often assume that pollen movement is independent of environmental context (i.e., the influence of the landscape on gene movement is not taken into account). This has been due, in part, to the necessity of sampling a few, relatively isolated individuals, and also to the labor-intensive nature of obtaining estimates for just a few sites. To address these concerns, Smouse and his colleagues developed a two-generational (parent-offspring) approach to the study of genetic structure that allowed quantification of heterogeneity among the male gamete pools sampled by maternal trees scattered across the landscape. The technique permits estimation of mean pollination distance, the effective number of pollen donors per maternal individual, and effective neighborhood size. A major attraction of the Smouse et al. technique for the purposes of this study was that acorns could be sampled (and would *best* be sampled) from individuals scattered across the landscape, rather than from individuals within carefully delimited study sites with few individuals. Given the difficulty of finding white oaks with sufficient reproduction for traditional analyses, we hoped that this different sampling and statistical technique would allow us to address questions posed in the proposal, albeit in a different way. Smouse et al. are presently continuing to refine their methodology; they are using simulations and empirical data to determine the minimal sampling effort required to obtain accurate estimates of the desired genetic parameters. In 2001, however, it was thought that as few as 25 progeny sampled from about 20 trees scattered across a landscape would be sufficient to obtain good statistical estimates of a number of informative genetic parameters (barring any questions about how landscape features altered gene movement). As data has become available Smouse (pers. comm.) has modified his recommendations, suggesting an increase in the number of trees sampled and a decrease in the number of progeny sampled per tree.

In fall of 2001, we again collected acorns from white oaks on SRS. We were hoping to find enough acorns to apply the statistical analyses of Smouse et al. to obtain data similar to that originally proposed. Acorn collections in 2001 were limited to acorns of large size with detached caps, since previous years' plantings documented lack of viability of small acorns and those with caps still attached. A total of 1580 acorns were collected from 16 SRS trees (Table 2). Dozens of trees were searched for acorns; searches were not limited to previously mapped sites since individuals could be widely scattered for the Smouse et al. (2001) procedure. A few trees were producing massive numbers of acorns, but most had very limited reproduction. Acorns were tested for viability using a float test, which simply consists of placing acorns in water and discarding all those that float (Young and Young 1992). Some good acorns may be sacrificed in this procedure, but its use avoids planting many inviable seeds. The percentage of acorns that did not float (i.e., were likely to be viable) ranged from zero to 91% per tree (Table 2). Even

some of the acorns that did not float were later found to be infested with weevils. After the float test only eight trees had sufficient acorns to warrant planting (Table 3). Acorns were planted immediately after being tested for viability. Germination rates for these eight trees ranged from 58 to 93% (Table 3).

In February 2002, 273 seedlings from these trees were crushed under liquid nitrogen with clean ocean sand using a mortar and pestle. Allozyme analyses followed methods listed above. The seedling genetic data were used (see below) to estimate genetic diversity, and to obtain an estimate of the mating system. Insufficient maternal trees and progeny from these collections precluded use of Smouse's Two-Gener Analysis for the estimation of the effective number of pollen donors per year and neighborhood size.

Genetic data analyses

Genetic diversity and structure

Genetic diversity statistics were calculated for the eight populations collected in 2000 and for the seedlings germinated in 2001 using a Fortran program developed by M. D. Loveless and A. Schnabel. The following measures of genetic diversity (Hedrick 1985) were calculated: the percentage polymorphic loci (P), the mean number of alleles per locus (A) and per polymorphic locus (AP), the effective number of alleles per locus (A_e), observed heterozygosity (H_o) and expected heterozygosity (H_e). These values are reported for the eight sites and for progeny of each tree germinated in 2001 (parameters subscripted with a p in each case) and for the species (designated with an s subscript) as described in Hamrick and Godt (1989). Wright's inbreeding coefficient (F) was calculated to estimate divergence from Hardy-Weinberg expectations of genotype frequencies (Wright 1922). Chi-square tests were employed to test whether the F values differed significantly from expectations (Li and Horvitz 1953). Overall deviations from Hardy-Weinberg expectations were estimated using Wright's F_{IS} statistic (Wright 1965).

To describe genetic structure, the proportion of total genetic diversity found among populations (G_{ST}) was calculated from the equation $G_{ST} = (H_T - H_S)/H_T$ where H_T is total genetic diversity at polymorphic loci and H_S is the mean genetic diversity within populations (Nei 1973, 1977). G_{ST} values range from 0.0, when alleles are found in equal frequencies within populations, to 1.0, when populations are monomorphic for different alleles.

Nei's genetic identity and distance measures (Nei 1972) were calculated for each pair of sites and for the progeny of each tree pair to estimate divergence. Two indirect estimates of gene flow were made. The number of migrants per generation (Nm) was estimated from the mean frequency of "private alleles" (i.e., those alleles restricted to a single population) using the methodology of Slatkin (1985) and Barton and Slatkin (1986). Estimates of gene flow were also made using Wright's (1931) equation [$Nm = (1 - G_{ST})/4G_{ST}$], which estimates gene flow based on overall allele frequency differences. Hereafter, $Nm(W)$ refers to gene flow estimated from G_{ST} values, whereas $Nm(S)$ refers to gene flow estimates based on "private allele" frequencies.

Mating system estimates

Mating system parameters, including the mean single locus outcrossing estimate (t_s), the multilocus outcrossing rate (t_m) and the correlation of outcrossed paternity within progeny arrays (r_p) were estimated using K. Ritland's multilocus mating system program (Ritland 1990, 2002;

available at <http://genetics.forestry.ubs.ca/ritland/programs>). Genetic data from the seedlings of the eight trees (collected in 2001) provided the basis for these estimates. The mixed mating model (Brown and Allard 1970) used in these estimates has the following assumptions: 1) each fertilization is a random outcrossing event with probability t , or a self-fertilization, with probability $s = 1 - t$; 2) the likelihood of an outcross event is independent of maternal genotype, 3) outcross pollen frequencies are homogeneous among maternal plants; and 4) selection has not occurred between fertilization and assessment of progeny genotypes. A further assumption of the multilocus outcrossing estimate is that no association exists between alleles at different loci (Shaw et al. 1981). Although violations of the assumptions are common, most departures result in relatively minor effects (Brown 1989).

Results

Genetic diversity and structure

2000 – The eight SRS site collections

Fourteen of the 20 loci resolved in preliminary trials were scoreable. Enzyme expression of the collected leaf samples was extremely poor, presumably due to the drought and the extremely high temperatures (leaves were collected in July). Eight (57%) of the 14 loci were polymorphic ($P_s = 57.1$), with an average of 3.13 alleles per polymorphic locus (AP_s ; Table 1). The average number of alleles per locus (A_s) was 2.21. Genetic diversity for the species (H_{es}) was 0.187.

Within sites, 54.1% of the loci were polymorphic, on average (P_p ; Table 1), with a mean of 2.44 alleles per polymorphic locus (AP_p) and 1.77 alleles per locus (A_p). Mean expected heterozygosity (H_{ep}) was 0.178 within sites.

Total genetic diversity (H_T) was 0.327, with most of the variation found within populations ($H_s = 0.311$). The percentage of total genetic variation found among populations (G_{ST}) was 4.2%.

Estimates of gene flow ranged from $Nm(S) = 5.51$ (based on four “private” alleles) to $Nm(W) = 5.71$. Nei’s (1972) genetic identities between population pairs ranged from 0.967 to 0.994, with a mean of 0.983 ($S.D. = 0.008$).

Mean observed heterozygosity within sites was 0.183, while mean expected heterozygosity was 0.187. Although five of the 50 loci tested for deviations from Hardy-Weinberg were statistically significant, the overall F_{IS} value was 0.0003, indicating that the populations were randomly mating.

2001 – Seedling data

In contrast to the field collected adult tissue, all of the enzymes resolved in preliminary trials were scoreable. Sixteen of the 20 loci (80%) resolved were polymorphic (P_s) with two to four alleles per locus (mean = 2.5; Table 4). *Pgi-1*, *Tpi-1*, *Tpi-2* and *Mdh-1* were monomorphic for all seedlings. Eight of the sixteen polymorphic loci were major contributors ($H_T > 0.36$) to genetic diversity. These were *Fe-1*, *Pgi-2*, *Tpi-1*, *Aat-2*, *Lap-1*, *Idh-2*, *Ugpp-1* and *Skdh-1* (Table 5). Genetic diversity (H_{ep}) for the progeny of the trees ranged from 0.133 to 0.216, with a mean of 0.176 (Table 4). For the species genetic diversity (H_{es}) was 0.195 (Table 4).

A small percentage (9.6%) of the genetic diversity found was distributed among the progeny of the trees (Table 5). The progeny of any given tree had, on average, 90.4% of the genetic

diversity within them. On a per locus basis, G_{ST} values ranged from 0.007 (*Per-2*) to 0.450 (*Pgd-1*).

Mean observed heterozygosity among the progeny sets was 0.203, whereas mean expected heterozygosity was 0.176. Significant deviations from Hardy-Weinberg expectations were found for 12 of 80 tests; all were negative, indicating an excess of heterozygotes. The overall fixation index (F_{IS}) was low (-0.098), and negative, again indicating a slight heterozygote excess overall.

The lack of divergence among progeny was indicated by the low G_{ST} values and high genetic identities. Genetic identities (I ; Nei 1972) between sets of progeny pairs ranged from 0.937 to 0.994, with an overall mean of 0.960 ($S.D.$ = 0.016). Estimates of gene flow based on the genetic structure data were $Nm(W)$ = 2.35, and $Nm(S)$ = 3.53 (based on six “private” alleles).

Mating system estimates

Sixteen polymorphic loci were used for these estimates. Variance estimates for the outcrossing rates were obtained from 100 bootstraps. The multilocus outcrossing estimate (t_m) for *Q. alba* was 1.02 (SD = 0.05), whereas the mean single locus outcrossing estimate (t_s) was 0.970 (SD = 0.06). The difference between the multilocus outcrossing rate and the mean single outcrossing rate (i.e., the rate of biparental inbreeding) was 0.05 (SD = 0.04). The correlation of outcrossed progeny within progeny arrays ($= r_p$, the probability that a randomly chosen pair of progeny from the same array are full sibs) was 0.262.

Discussion

Genetic diversity and structure, Mating system

We have included genetic diversity estimates from the eight surveyed sites, and from the progeny of eight trees for comparative purposes. Although more loci were resolved in the seedlings than in the adult leaf tissue (20 vs 14), a greater number of sites and trees were sampled for the adult study. Genetic diversity values are strikingly similar, despite the differences in loci scored. For example, mean percentage of polymorphic loci was 54.1 for the adults, and 50.6 for the progeny, while the mean number of alleles per polymorphic loci was 2.44 for the adults and 2.23 for the progeny. Gene diversity was 0.178 for the adults surveyed and 0.176 in the progeny data set. Where the data sets diverged somewhat was in the calculation of species values. A higher proportion of polymorphic loci were found in the progeny data set (this may be related to the greater number of resolved loci), but the adult data set had a greater number of alleles per polymorphic loci (perhaps due to more geographically widespread sampling). Species' genetic diversity found in the two studies was fairly similar (H_{es} = 0.195 for the progeny and 0.187 for the adults). The progeny data set, though limited in geographic scope, may provide a somewhat more accurate picture of the genetic diversity overall since more loci were resolved. It is used in the comparisons that follow.

White oak has relatively high levels of genetic diversity. This was not unexpected, since it has been shown that among plants trees tend to have the highest level of genetic diversity (Hamrick 1978). Allozyme reviews have shown that two factors best predict genetic diversity found within a species (Hamrick and Godt 1989). These are the breeding system, with outcrossed, wind-pollinated species having the highest genetic diversity, and geographic range,

with widespread species having the most genetic diversity. White oak has a combination of traits, including woodiness, wind-pollination, an outcrossed mating system and a large geographic range, that favor high genetic diversity.

Despite our limited geographic sampling, genetic diversity found in this study compares favorably with that found for other outcrossing, wind-pollinated trees (Table 6). For example, mean P_s was 69.1 for 128 studies of trees with similar breeding characteristics (Hamrick et al. 1992) and 80.0 for white oak. Hamrick et al. (1992) reported mean $H_{es} = 0.173$ for these 128 studies, whereas $H_{es} = 0.195$ for *Q. alba*. Genetic diversity statistics found for *Q. alba* in this study also compared favorably with two previously published studies of oaks (Table 6; Manos and Fairbrothers 1987, Guttman and Weight 1989). On the basis of these studies, there is little evidence that *Q. alba* on SRS has lost a substantial portion of its genetic diversity, despite a substantial decline in numbers, and fragmentation of the hardwood forests on the site. Since many of the extant trees may pre-date fragmentation, and population sizes remain large this is not surprising.

The genetic survey data provided no evidence of inbreeding within *Q. alba* on SRS. The F_{IS} value was 0.0003 for the adult trees, and -0.0098 for the seedlings, strongly suggesting that the species is randomly mating. Positive F_{IS} values suggest inbreeding within populations, whereas negative values indicate a heterozygote excess (which could be caused by heterosis, or negative assortative mating). An F_{IS} value of zero indicates random mating.

The seedling data set was used to estimate the mating system of the species. This may not have been entirely valid since some of the maternal trees occurred at some distance from one another and may not be interbreeding. Nonetheless, since wind-dispersed pollen is carried over long distances, the populations are likely to be in genetic contact. Our multilocus estimate of the outcrossing rate ($t_m = 1.02$, $S.D. = 0.05$) suggests that *Q. alba* is 100% outcrossed. The biparental inbreeding estimate (0.05) was so low as to be insignificant. In sum, we found no evidence that inbreeding is a factor in these populations. This clearly pertains to one of the questions posed by this study – that is, could forest fragmentation be leading to high levels of inbreeding, and resultant inbreeding depression? Inbreeding could certainly contribute to low recruitment in tree species, which are usually highly outcrossed. We found no evidence that this is currently the case in *Q. alba* forest fragments on SRS. It is, of course, feasible that inbreeding depression (particularly in years of adverse environmental conditions) may have affected the viability of acorns before assay. The mixed mating model assumes that this is not the case. This assumption could be tested through pollination experiments, but this would be very difficult for *Q. alba*, due to its stature when mature and its mode of pollination.

The correlation of outcrossed paternity within progeny arrays (r_p) was 0.262. The effective number of pollen donors (N_{ep}) per tree is equal to $1/r_p$. Hence, for our data set, the effective number of pollen donors per tree was 3.82.

Very little genetic structure was evident among *Q. alba* on SRS. Genetic identities among sites and among sets of progeny were uniformly high (mean $I = 0.98$ for the adults and 0.96 for the progeny). The proportion of genetic variation found among sites (G_{ST}) was 4.2%, and 9.6% among progeny sets. (A higher G_{ST} was expected for the progeny since that data included a maternal effect). G_{ST} values are expected to increase over time, and genetic identities to decrease, if forest fragments are genetically isolated and genetic drift predominates. Because many *Q. alba* trees on SRS probably pre-date fragmentation and since the study sites maintained large numbers of trees, we did not expect to see a significant effect of genetic drift.

Similar levels of genetic structure have been found for other wind-pollinated trees. Hamrick

et al. (1992) found a mean G_{ST} of 7.7% in a review of 146 studies of outcrossing, wind-pollinated trees, intermediate between the two values reported in this study. A mean $G_{ST} = 8.5\%$ was found for 48 studies of trees within the Fagaceae (Hamrick and Godt 1996). We expect that estimates of genetic divergence would be larger if we had sampled across *Q. alba*'s geographic range.

Estimates of gene flow based on genetic structure are based on historical levels of gene flow, and may not reflect current levels. Nonetheless, they provide useful comparative data. Gene flow rates of $Nm > 1$ migrant per generation are generally considered necessary to counter the effects of genetic drift (Wright 1931). Our genetic data suggest that levels of gene flow in *Q. alba* have been much higher than this, ranging from 2.35 to 5.71. Such high rates of gene flow are typical of wind-pollinated tree species at the spatial scale sampled (Hamrick and Godt 1989).

White oak reproduction

The paucity of white oaks producing acorns during the timeframe of this grant precluded accomplishing the stated goals of the project. While a few trees seemed to produce massive numbers of acorns, most surveyed trees produced no or very few viable acorns during the years of this study. Although we anticipated white oak would have mast years, we nonetheless anticipated acquiring enough acorns from sufficient numbers of mapped trees during at least one of the four years to complete our goals. During a conversation about white oak reproduction early in the project (1998) with Dr. Bruce Bongarten (currently Associate Dean, School of Forestry at UGA), Dr. Bongarten indicated that although white oak had mast years, it still tended to have a low level of reproduction on a yearly basis. We anticipated, therefore, especially when using the methodology of Smouse et al. (2001), to find sufficient acorns from scattered trees for genetic analyses. So, what are we to make of the low white oak reproduction that we encountered?

There are a number of factors that may have resulted in low seed production. One factor was the prolonged (four year) and severe drought experienced by the Southeast. Many plants were severely stressed during this extended period of low water availability. Another weather factor was the occurrence of late spring frosts after warm weather during several years of the study. In 1998, Dr. Bongarten (pers. comm.) suggested that these may have affected white oak reproduction during that year; Rogers (1990) also indicates that cold weather and frosts during flowering may have an adverse effect on acorn production.

It may also be that the interval between mast years is quite long, and that white oak reproduction on a widespread scale in any given period of time is low. Rogers (1990) indicates that seed-bearing intervals may be up to ten years. He also indicates that some trees are more consistent acorn producers than others; we saw evidence of this idiosyncrasy in our study.

While it is possible that a fraction of the trees that we were surveying had actually not reached reproductive age, quite a number of trees on our surveyed sites were massive, stately trees over 100 yrs old. We strongly suspect that these trees must have mast years when they produce thousands of acorns. Relatively little seedling recruitment was apparent at many sites, although there were exceptions. After a good seed year one might expect a carpet of seedlings underneath and around the maternal tree. The low recruitment is consistent with low acorn production.

A combination of factors, including the drought, late frosts and the underlying idiosyncratic

reproductive behavior of white oak trees, are likely to have contributed to our inability to find sufficient viable seed from enough trees to accomplish the goals of this project. Other ongoing seed studies at SRS may provide insight into the temporal variation in white oak reproduction across sites and trees and provide guidance for any future studies that require white oak acorns.

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Table 1. Genetic diversity¹ within eight *Quercus alba* sites on SRS.

Site	P	AP	A	A_e	H_{obs} (SD)	H_e (SD)
Nature Trail	50.0	2.57	1.79	1.34	0.190 (0.046)	0.182 (0.059)
Boggy Gut	57.1	2.38	1.79	1.27	0.150 (0.044)	0.156 (0.052)
RdA172	53.9	2.14	1.62	1.31	0.204 (0.048)	0.180 (0.057)
SwgumSite	57.1	2.50	1.86	1.34	0.181 (0.046)	0.181 (0.060)
RdEl	57.1	2.38	1.79	1.32	0.176 (0.048)	0.177 (0.059)
Rd8-1	50.0	2.71	1.86	1.30	0.183 (0.047)	0.170 (0.056)
RdF	57.1	2.25	1.71	1.36	0.192 (0.047)	0.194 (0.060)
TylerBridge	50.0	2.57	1.79	1.34	0.188 (0.043)	0.187 (0.060)
Mean:	54.1	2.44	1.77	1.32	0.183	0.178
$S.D.$:	4.7	0.19	0.08	0.03	0.016	0.020
Species	57.1	3.13	2.21	1.34	-----	0.187

¹ P is the percent polymorphic loci, AP is the mean number of alleles per polymorphic locus, A is the mean number of alleles per locus, A_e is the effective number of alleles, H_{obs} is observed heterozygosity, H_e is gene diversity, or expected heterozygosity. $S.D.$ is the standard deviation.

Table 2. *Quercus alba* acorns collected by tree in 2001, and percentage of "viable" acorns using the "float test" (Young and Young 1992).

Tree	"Viable"	Total Collected	Percent "Viable"
421	31	67	46
435	0	15	0
451	2	69	3
606	0	47	0
415	3	18	17
444	8	58	14
538	0	41	0
625	73	145	50
PF	34	164	21
452	4	23	17
606	0	34	0
605	130	370	35
501	313	344	91
698	22	37	59
396	104	127	82
602	0	17	0
26	4	38	11
CRFD ¹	8	152	5
78-D ¹	18	199	9
Totals	754	1931	
Mean (SD)			25.6 (28.7)

¹ These trees were two isolated off-site trees that are listed for comparison.

Table 3. Germination percentages¹ for *Quercus alba* and sample sizes for genetic analyses.

Tree	Acorns Planted	Acorns Germinated	% Germination	Allozyme Sample Size
625	73	50	69	48
396	104	76	73	56
605	130	87	67	73
421	31	18	58	18
501	99	92	93	60
50	35	28	80	28
698	22	17	77	16
PF	33	24	73	22
Mean:	66	49	74	40

¹ All acorns that germinated were counted (even though some seedlings were not viable).

Table 4. Genetic diversity¹ within the progeny of eight *Quercus alba*.

Tree	<i>P</i>	<i>AP</i>	<i>A</i>	<i>A_e</i>	<i>H_{obs}</i> (<i>SD</i>)	<i>H_e</i> (<i>SD</i>)
625	45.0	2.22	1.55	1.33	0.187 (0.046)	0.181 (0.051)
396	50.0	2.20	1.60	1.22	0.153 (0.040)	0.136 (0.040)
421	47.4	2.22	1.58	1.37	0.213 (0.072)	0.186 (0.053)
50	50.0	2.20	1.60	1.35	0.227 (0.060)	0.191 (0.051)
501	50.0	2.40	1.70	1.39	0.244 (0.041)	0.200 (0.055)
698	52.6	2.00	1.53	1.21	0.169 (0.069)	0.133 (0.042)
PF	50.0	2.20	1.60	1.41	0.239 (0.073)	0.216 (0.053)
605	60.0	2.42	1.85	1.29	0.192 (0.037)	0.166 (0.046)
Mean	50.6	2.23	1.63	1.32	0.203	0.176
<i>SD</i>	4.0	0.13	0.10	0.07	0.020	0.017
Species values	80.0	2.50	2.20	1.36	-----	0.195

¹ *P* is the percent polymorphic loci, *AP* is the mean number of alleles per polymorphic locus, *A* is the mean number of alleles per locus, *A_e* is the effective number of alleles, *H_{obs}* is observed heterozygosity, *H_e* is gene diversity, or expected heterozygosity. *S.D.* is the standard deviation.

Table 5. Nei's (1973, 1977) genetic diversity statistics¹ for the polymorphic loci for *Quercus alba* seedlings.

Locus	H_T	H_S	G_{ST}
<i>Fe-1</i>	0.494	0.421	0.148
<i>Fe-2</i>	0.046	0.043	0.046
<i>Fe-3</i>	0.013	0.013	0.025
<i>Per-1</i>	0.019	0.018	0.035
<i>Per-2</i>	0.003	0.003	0.007
<i>Mnr-1</i>	0.019	0.018	0.025
<i>Pgi-2</i>	0.399	0.354	0.112
<i>Tpi-1</i>	0.505	0.458	0.091
<i>Aat-2</i>	0.365	0.309	0.153
<i>Lap-1</i>	0.520	0.475	0.086
<i>6Pgd-1</i>	0.082	0.045	0.450
<i>6Pgd-2</i>	0.046	0.041	0.081
<i>Idh-2</i>	0.426	0.377	0.116
<i>Ugpp-1</i>	0.457	0.430	0.057
<i>Mdh-2</i>	0.003	0.003	0.016
<i>Skdh-1</i>	0.497	0.452	0.088
Mean:	0.243	0.217	0.096

¹ H_T is the total genetic diversity, H_S is genetic diversity found within the progeny of a given tree and G_{ST} is the proportion of genetic diversity found among progeny of different trees.

Table 6. Comparison of genetic diversity found in *Q. alba* seedlings and other studies.

	Number of		P_s	A_s	A_{cs}	H_{cs}	P_p	A_p	A_{ep}	H_{ep}
	Pops	Loci								
This study (seedlings)	—	20	80.0	2.20	1.36	0.195	50.6	1.63	1.32	0.176
Manos and Fairbrothers ¹	2	16	62.5	2.05	----	----	28.2	1.45	1.11	0.096
Guttman and Weight ²	1	18	66.7	2.30	1.38	0.276	-----	-----	----	----
Fagaceae ³	--	--	65.3	----	----	0.198	-----	-----	----	----
Outcrossing, wind-pollinated trees ⁴	--	--	69.1	2.31	1.23	0.173	53.0	1.84	1.21	0.154

¹1987²1989³Based on 48 studies, Hamrick and Godt, 1996⁴Based on 128 studies, Hamrick et al. 1992

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Appendix One. The following are maps of 12 *Quercus alba* sites at the Savannah River Site where mature trees have been tagged. Each dot represents a tree. Data sheets with the x,y coordinates of mapped trees accompany the maps.

Road 8.1

	-1- plant #	-2- x	-3- y
1	471.00	0.00	0.00
2	472.00	-0.69	0.72
3	473.00	-1.12	10.64
4	474.00	-8.56	12.68
5	475.00	7.17	-24.99
6	476.00	42.24	-33.09
7	477.00	63.61	-17.06
8	478.00	32.67	17.37
9	479.00	43.30	25.00
10	480.00	31.40	40.19
11	481.00	31.45	34.93
12	482.00	21.25	31.50
13	483.00	16.43	30.90
14	484.00	15.73	43.23
15	486.00	21.57	-10.52
16	487.00	26.29	16.43
17	488.00	28.67	20.08
18	489.00	11.07	32.15
19	490.00	1.71	48.97
20	491.00	-3.11	56.42
21	492.00	-17.23	60.02
22	493.00	-24.84	76.34
23	494.00	-28.62	102.52
24	495.00	-31.02	57.59
25	496.00	-32.99	57.25
26	525.00	-26.24	66.33
27	470.00	-48.07	24.55

Road C

Road C

-1-   -2-   -3-
 Tree No   X (m) 757   Y (m) 757

1	757.00	0.00	0.00
2	758.00	-15.65	-20.76
3	759.00	-18.58	-16.73
4	760.00	-4.39	-14.34
5	761.00	2.78	-15.76
6	762.00	2.08	-9.78
7	763.00	2.97	-2.68
8	764.00	24.25	-14.00
9	765.00	26.20	-6.53
10	766.00	23.64	-4.17
11	767.00	30.00	-0.52
12	768.00	19.90	13.42
13	769.00	39.28	17.49
14	770.00	9.65	33.64
15	771.00	10.82	35.38
16	772.00	17.75	38.06
17	773.00	20.42	51.81
18	774.00	11.89	51.87
19	775.00	21.24	77.91
20	776.00	23.85	76.58
21	777.00	27.95	69.45
22	778.00	8.72	63.94
23	779.00	2.05	71.72
24	780.00	0.98	82.60
25	781.00	8.29	83.22
26	782.00	7.46	85.46
27	783.00	8.54	91.00
28	784.00	17.43	91.02
29	785.00	16.78	84.82
30	786.00	22.16	89.64
31	787.00	20.84	91.32
32	788.00	26.64	84.39
33	789.00	43.95	98.31
34	790.00	54.04	98.43
35	791.00	62.66	84.91
36	792.00	48.13	107.42
37	793.00	56.52	112.86
38	794.00	70.06	117.18
39	795.00	65.11	124.39
40	796.00	65.35	102.15
41	797.00	23.97	-1.26

Road A17-2

-1-   -2-   -3-
 Tree no.   o mid-roa   To 700

1	700.00	5.00	0.00
2	699.00	9.50	18.70
3	698.00	13.20	19.80
4	697.00	-18.90	16.80
5	696.00	9.40	49.00
6	695.00	8.60	57.00
7	694.00	9.20	66.00
8	693.00	11.40	75.00
9	692.00	23.00	81.00
10	691.00	20.10	89.00
11	690.00	25.00	89.00
12	689.00	28.50	90.00
13	688.00	21.70	92.00
14	687.00	15.70	92.00
15	686.00	23.30	93.00
16	685.00	27.80	98.00
17	684.00	59.40	100.00
18	683.00	67.90	106.00
19	682.00	9.10	133.00
20	681.00	21.50	141.00
21	680.00	18.60	150.00
22	679.00	-6.60	172.00
23	678.00	-8.50	190.00
24	677.00	-12.80	232.00
25	676.00	18.80	227.00
26	675.00	21.10	227.00
27	674.00	19.20	223.00
28	673.00	15.10	241.00
29	672.00	15.20	255.00
30	671.00	8.80	259.00
31	670.00	29.00	88.00

Boggy Gut 1

Boggy Gut 1

	-1- Tree No	-2- m	-3- angle	-4- X (m)	-5- Y (m)
1	701.00	0.00	0.00	0.00	0.00
2	702.00	12.20	22.00	4.57	11.31
3	703.00	20.20	356.00	-1.41	20.15
4	704.00	8.50	300.00	-7.36	4.25
5	705.00	16.80	289.00	-15.88	5.47
6	706.00	12.40	263.00	-12.31	-1.51
7	707.00	15.40	263.00	-15.29	-1.88
8	708.00	23.90	271.00	-23.90	0.42
9	709.00	26.30	263.00	-26.10	-3.21
10	710.00	41.60	282.00	-40.69	8.65
11	711.00	24.90	241.00	-21.78	-12.07
12	712.00	26.00	233.00	-20.76	-15.65
13	713.00	20.30	239.00	-17.40	-10.46
14	714.00	23.00	224.00	-15.98	-16.54
15	715.00	15.20	217.00	-9.15	-12.14
16	716.00	20.65	187.00	-2.52	-20.50
17	717.00	10.20	83.00	10.12	1.24
18	718.00	51.20	61.00	44.78	24.82
19	719.00	51.20	58.00	43.42	27.13
20	720.00	46.00	58.00	39.01	24.38
21	721.00	55.30	58.00	46.90	29.30
22	722.00	50.80	65.00	46.04	21.47
23	723.00	39.00	90.00	39.00	2.39e-15
24	724.00	42.30	88.00	42.27	1.48
25	725.00	41.50	94.00	41.40	-2.89
26	726.00	45.00	103.00	43.85	-10.12
27	727.00	64.70	94.00	64.54	-4.51
28	728.00	46.70	88.00	46.67	1.63
29	729.00	49.00	114.00	44.76	-19.93
30	730.00	60.40	112.00	56.00	-22.63
31	731.00	46.00	327.00	-25.05	38.58
32	732.00				
33	733.00				
34	734.00				
35	735.00	31.00	233.00	-24.76	-18.66
36	736.00	33.00	234.00	-26.70	-19.40
37	737.00	34.00	234.00	-27.51	-19.98
38	738.00	42.00	226.00	-30.21	-29.18
39	739.00	47.00	225.00	-33.23	-33.23
40	740.00	47.00	228.00	-34.93	-31.45
41	741.00	43.00	231.00	-33.42	-27.06
42	742.00	48.00	224.00	-33.34	-34.53
43	743.00	48.00	220.00	-30.85	-36.77
44	744.00	53.00	213.00	-28.87	-44.45
45	745.00	60.00	213.00	-32.68	-50.32
46	746.00	64.00	203.00	-25.01	-58.91
47	747.00	68.60	201.00	-24.58	-64.04
48	748.00	73.00	202.00	-27.35	-67.68
49	749.00	38.00	186.00	-3.97	-37.79
50	750.00	41.00	197.00	-11.99	-39.21

Boggy Gut 1

	-1- Tree No	-2- m	-3- angle	-4- X (m)	-5- Y (m)
51	751.00	44.00	199.00	-14.32	-41.60
52	752.00	49.00	198.00	-15.14	-46.60
53	753.00	49.00	191.00	-9.35	-48.10
54	754.00	47.00	190.00	-8.16	-46.29
55	755.00	42.00	193.00	-9.45	-40.92
56	756.00	42.00	183.00	-2.20	-41.94

Boggy Gut 2

Boggy Gut 2

	-1- plant #	-2- x (m)	-3- y (m)
1	305.00	0.00	0.00
2	306.00	31.52	12.74
3	307.00	37.09	14.98
4	308.00	-9.51	-14.09
5	309.00	-0.31	-18.00
6	525.00	-12.31	-15.76
7	526.00	-19.09	-19.09
8	527.00	-13.97	0.98
9	528.00	-48.52	-6.82
10	529.00	-60.85	-4.26
11	530.00	-60.85	-14.26
12	531.00	-70.38	-9.76
13	532.00	-71.74	-5.79
14	533.00	-64.96	-20.75
15	534.00	-70.50	-24.03
16	535.00	-54.44	-46.96
17	536.00	49.67	23.15
18	537.00	46.04	35.08
19	538.00	66.26	45.20
20	539.00	52.36	39.58
21	540.00	52.50	25.54
22	541.00	64.69	63.13
23	542.00	58.26	45.34
24	543.00	56.52	47.45
25	544.00	47.75	49.47
26	545.00	76.17	43.80
27	546.00	34.27	32.76
28	547.00	37.90	51.23
29	548.00	38.42	42.27
30	549.00	27.10	57.76
31	550.00	23.41	59.65
32	551.00	30.44	30.63
33	552.00	23.85	32.55
34	553.00	27.44	34.76
35	554.00	23.32	34.64
36	555.00	19.35	21.79
37	556.00	15.74	20.31
38	557.00	4.12	31.78
39	558.00	4.94	26.05
40	559.00	3.22	17.95
41	560.00	-10.47	30.44
42	561.00	-7.08	40.48
43	562.00	-6.24	57.10
44	563.00	-37.75	0.45
45	564.00	-43.64	7.36
46	565.00	-45.38	5.79
47	566.00	-75.46	-4.94
48	567.00	-71.86	-73.80
49	568.00	-65.08	-89.66

Boggy Gut 3

	-1- Plant #	-2- x (m)	-3- y (m)
1	332.00	0.00	0.00
2	333.00	-7.43	1.58
3	334.00	-10.34	-8.68
4	335.00	1.31	-24.97
5	336.00	9.34	-5.61
6	550.00	-11.02	-51.84

Road F1

	-1- plant #	-2- x (m)	-3- y (m)
1	601.00	0.00	0.00
2	602.00	-18.79	-6.84
3	603.00	-29.23	-13.02
4	604.00	-44.99	0.79
5	605.00	-53.56	-19.50
6	606.00	-33.46	-38.49
7	607.00	-37.55	-51.88
8	608.00	-14.67	-45.33
9	609.00	-55.67	-57.13
10	610.00	-37.83	-53.31
11	610+	-64.19	-62.50
12	611.00	-46.05	-55.53
13	612.00	-42.53	-58.92
14	613.00	-43.18	-63.85
15	613+	-49.10	-61.39
16	614.00	-44.51	-65.46
17	615.00	-42.24	-68.47
18	616.00	-29.08	-54.34
19	617.00	-27.69	-56.26
20	618.00	-31.72	-63.61
21	619.00	-31.72	-64.95
22	620.00	-29.77	-63.98
23	621.00	-29.77	-63.98
24	622.00	-30.66	-74.19
25	623.00	14.62	-6.51
26	624.00	29.13	-10.60
27	625.00	25.68	-4.07
28	626.00	-0.63	-10.14
29	627.00	18.79	6.84
30	628.00	34.18	30.78
31	629.00	49.74	40.28
32	630.00	-21.82	39.36
33	631.00	-38.02	27.63
34	632.00	-40.90	13.29
35	633.00	-1.67	-45.56
36	634.00	11.34	-65.65
37	635.00	29.97	-56.46
38	636.00	36.76	-58.15
39	637.00	21.20	-48.47
40	638.00	-2.83	-53.93
41	639.00	3.21	-45.89
42	640.00	2.41	-45.94
43	641.00	5.51e-15	-45.00
44	Unmarked	-9.37	23.18
45	642.00	4.78e-15	-39.00
46	643.00	-9.32	-34.77
47	644.00	-5.62	-24.36
48	645.00	12.74	-31.52
49	646.00	22.27	-29.55
50	647.00	55.79	-4.88

Road F2

	-1- plant #	-2- x (m)	-3- y (m)
1	648.00	0.00	0.00
2	649.00	-13.58	3.39
3	650.00	-31.82	3.34
4	651.00	-40.38	7.12
5	652.00	-54.79	15.71
6	653.00	-61.58	27.96
7	654.00	-60.33	31.79
8	655.00	-87.84	68.28
9	656.00	-86.47	72.04
10	657.00	-82.48	75.05
11	658.00	-88.70	74.05
12	659.00	-93.51	85.29
13	660.00	-93.28	85.41
14	1B	-89.50	82.62
15	400.00	-76.58	102.48
16	485.00	-33.13	34.17

Road F3

	-1- plant #	-2- x (m)	-3- y (m)
1	661.00	0.00	0.00
2	662.00	11.37	-6.30
3	663.00	6.78	4.24
4	664.00	-1.63	-2.52
5	665.00	-29.96	-1.57
6	666.00	-34.66	3.06
7	667.00	-41.96	-0.19
8	668.00	-36.65	3.13
9	669.00	-26.89	1.13

Tyler Bridge 1

Tyler Bridge 1

	-1- plant #	-2- x (m)	-3- y (m)
1	408.00	0.00	0.00
2	409.00	-1.34	-12.73
3	410.00	10.56	-8.25
4	411.00	18.17	-5.56
5	412.00	30.91	8.28
6	413.00	29.44	17.00
7	414.00	33.43	20.09
8	415.00	34.67	27.09
9	416.00	44.70	14.52
10	417.00	0.35	20.00
11	418.00	-4.11	16.50
12	419.00	-14.91	21.30
13	420.00	-13.11	9.18
14	421.00	-24.59	46.50
15	422.00	-21.44	47.50
16	423.00	-19.25	45.92
17	424.00	-36.34	62.68
18	425.00	-24.59	59.10
19	426.00	-17.75	65.30
20	427.00	-10.22	77.32
21	428.00	0.24	83.31
22	429.00	-15.17	123.46
23	430.00	-29.67	97.26
24	431.00	-22.41	87.31
25	432.00	-32.34	-16.48
26	433.00	-39.15	-18.26
27	434.00	-60.01	1.89

Tyler Bridge 2

Tyler Bridge 2

	-1- Tree #	-2- x (m)	-3- y (m)
1	435.00	0.00	0.00
2	436.00	-1.16	8.22
3	437.00	0.27	15.70
4	438.00	-2.72	25.86
5	439.00	12.16	45.40
6	440.00	-50.72	5.33
7	441.00	-71.94	35.64
8	442.00	-33.26	-7.07
9	443.00	-7.61	-16.31
10	444.00	-9.12	-26.47
11	445.00	30.32	-6.45
12	446.00	42.34	5.58
13	497.00	48.92	47.24
14	447.00	88.97	-32.18
15	448.00	118.52	-37.39
16	449.00	127.12	-24.08
17	450.00	96.49	21.29

Tyler Bridge 3

Tyler Bridge 3

	-1- plant #	-2- x (m)	-3- y (m)
1	451.00	0.00	0.00
2	452.00	2.58	-5.53
3	453.00	26.67	4.22
4	454.00	-21.84	15.87
5	455.00	-20.00	0.00
6	456.00	-22.99	0.80
7	457.00	-24.47	-11.41
8	458.00	-34.44	-16.06
9	459.00	-22.35	-24.82
10	460.00	-23.72	-28.27
11	461.00	-34.70	-30.96
12	462.00	-40.49	-40.32
13	463.00	-49.53	-33.42
14	464.00	-49.69	-39.54
15	465.00	-48.44	-40.31
16	429.00	-28.87	-55.67