

SHORTLEAF PINE: A SPECIES AT RISK?

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ABSTRACT.—Since the 1950s the existence of natural hybrids between shortleaf pine and loblolly pine has been recognized and reported in the literature. In a range-wide study of isoenzyme diversity in shortleaf pine, we found 16 percent of the trees from western populations were hybrids, based on the isocitrate dehydrogenase (IDH) locus. In stands thought to be pure shortleaf pine in west central Arkansas (Mt. Ida), we found 15 percent of the trees were hybrid. As a follow-up study to confirm or discount these results, we sampled native stands across Montgomery County, Arkansas, including the Mt. Ida area. These stands were mixed loblolly pine and shortleaf pine in the southeast part of the county and pure shortleaf pine in the northwest corner. In these stands we again found (1) a relatively high percentage of hybrid trees (14 percent); (2) hybrids in shortleaf pine stands beyond the natural range of loblolly pine; (3) introgression occurring in both directions; and (4) the IDH locus a reliable marker for species and hybrid determination. We are now engaged in a range-wide study of both loblolly pine and shortleaf pine to examine the cause and consequences of natural hybridization between these two species.

INTRODUCTION

Both loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) have extensive ranges across the southeastern United States. A large portion of these ranges are sympatric, allowing for possible hybridization between the two species. The probable existence of natural hybrids between loblolly pine and shortleaf pine has been a topic of discussion and concern since at least the early 1950s (Zobel 1953). Prior to the 1950s, Schreiner (1937) reported that viable artificial hybrids of shortleaf pine X loblolly pine had been produced at the Institute of Forest Genetics in California. In a later report, Little and Righter (1965) documented that this cross was first made in 1933. These artificially produced crosses demonstrated the possibility that naturally occurring hybrids might exist. These two species are normally isolated from each other by time of strobili maturity (Mergen and others 1963), but early on Zobel (1953) noted the possibility of environmentally induced overlapping maturity. The questions then became, how high is the level of hybridization across the ranges of loblolly pine and shortleaf pine, and what effect, if any, will hybridization have on the long-term integrity of each species?

Early Morphological Studies

Artificial Hybrids

Early studies of shortleaf pine X loblolly pine hybrids necessarily relied on morphological traits. Characterization of artificial hybrids showed that the F_1 trees were generally intermediate for many of the traits examined. Little and Righter (1965) described the F_1 hybrids as looking something like a loblolly pine with small cones with stout, sharp prickles, intermediate needle anatomy, and two or three needles per fascicle. Snyder and Hamaker (1978) reported shortleaf pine X loblolly pine hybrids to be distinct and intermediate using a multivariate trait value based on needle characteristics. They reported that the traits most useful in identifying individuals as hybrids were needle length, fascicle sheath length, number of rows of stomata, needle diameter, and stomata/cm. In a summary paper, Schultz (1997) reported that the hybrids tend to be intermediate to their parents for growth and survival.

Perhaps the most extensive and thorough study of artificial hybrids was conducted by Mergen and others (1965). They examined nine needle traits, five twig traits, six bud traits, three male strobili traits, and three cone traits on 40 F_1 trees grown across three locations. They compared these F_1 hybrids to an approximately similar number of open pollinated parent tree offspring at each site. They reported several interesting results, including a large environmental effect, such that mean values for traits in one environment for either parent or F_1 could overlap values for the other groups in other environments. At the same time, they noted a general tendency for intermediate values for the hybrids for most traits. They also noted, as had Little and Righter

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(1965), that for vegetative traits the hybrids tended to look more like loblolly pine than shortleaf pine. Mergen and others (1965) then examined all possible combinations of traits using pictorialized scatter diagrams (Anderson 1949) to determine which best distinguished the hybrids and the two parents. They found that by using needle length and fascicle sheath length as the two axes, and sheath type (smooth to rough), length to width ratio of the axillary scale, and twig color for the plotted points, the parents were clearly separated on the plots. They also reported that these scatter diagrams resulted in the hybrids being placed in a generally intermediate position. They concluded that in spite of large environmental influences on trait variability, the F_1 hybrids could be distinguished from the parent species using this combination of traits.

It is of interest to note that all the reported studies of artificial hybrids were of trees from the cross of shortleaf pine X loblolly pine, i.e., with shortleaf pine as the female parent. Various levels of difficulty in making the reciprocal cross have been reported, from no seed (our data, unpublished) to few (0.2 seed/flower) seed (Snyder and Squillace 1966), to not a serious problem (Richard Bryant, pers. commun. 2003). Little and Righter (1965) did report that the Institute of Forest Genetics in California produced the reciprocal cross in 1948, but apparently no information has been published concerning growing the offspring. Clearly, since loblolly pine male strobili shed pollen several weeks earlier than shortleaf pine, using shortleaf pine as the female is the easier cross, as pollen storage is not required. What is not clear is if the reciprocal cross is difficult because of logistics, or if some sort of incompatibility is involved.

Putative Hybrids

When putative hybrid individuals were found in the field, the use of morphological traits deemed useful for artificial hybrids was not as definitive. For example, when Mergen and others (1965) applied their set of traits to putative hybrids from two field populations, they were able to clearly separate the parents, but only 14 of the 62 individuals identified as putative hybrids fell in their hybrid category. They noted that the putative hybrids, although generally intermediate, tended to be similar to shortleaf pine in reproductive morphology, but resembled loblolly pine in vegetative traits. They speculated that some of the putative hybrids were backcrosses, as had Zobel (1953) in his report concerning the possible existence of natural shortleaf pine X loblolly pine hybrids.

Hicks (1973) took a statistical approach to the question of the most appropriate traits to use in identifying hybrid individuals. He measured six needle traits, three twig traits, and three cone traits, then calculated within-tree means and variances to allow estimation of sample sizes required to estimate within-tree means to within 5 percent of the original sample mean. He concluded that needle width,

axillary scale width, and cone width required prohibitively large sample sizes, while terminal bud length and number of stomatal rows showed limited variability. Of the traits he measured, he found needle length, fascicle sheath length, number of needles per fascicle, terminal bud width, cone length, and seed weight to be most useful in distinguishing shortleaf pine, loblolly pine, and their hybrids.

One of the reasons the existence of these hybrids was of interest is illustrated by the work of Abbott (1974). Based on the presence of an “atypical” loblolly selection in the Oklahoma State University seed orchard, he examined 19 loblolly and 12 shortleaf orchard selections as possible hybrids. It is generally agreed that hybrids are undesirable in seed orchards. All the trees he examined were from extreme southeast Oklahoma (i.e., McCurtain County), or adjacent counties in Oklahoma, Texas, or Arkansas. This area represents the far northwestern edge of the range of loblolly pine. Based on the work of Hicks (1973), and conversations with him, Abbott chose to measure needle length, number of needles per fascicle, cone length, number of seeds per gram, and fascicle sheath length, and then constructed a hybrid index. He found that the atypical loblolly pine was intermediate for all traits, as were three of the shortleaf pine orchard selections. He concluded that hybridization must occur relatively frequently in the sample area.

Cotton and others (1975), looking for the existence of natural hybrids, conducted a study of trees from 16 stands within a 60-mile radius of Nacogdoches, TX. They used the same traits as Abbott (1974), except terminal bud width instead of number of seeds per gram. They concluded that hybrids may exist, but at a low frequency, and attributed the existence of intermediate types to the natural range in variation found in loblolly pine and shortleaf pine. This conclusion would agree with a report by Schoenike and others (1977), who found that on the Clemson Experimental Forest, SC, putative hybrids occur at a frequency of about one in 10,000 trees.

Early Chemical and Molecular Studies

Clearly, either the frequency of hybridization is highly variable, or the use of morphological traits is limiting our ability to distinguish hybrids from their parent species. Researchers thus turned to chemical and molecular methods to attempt to resolve these questions. One of the early works in this arena was that of Hare and Switzer (1969). They conducted an analysis of seed proteins using acrylamide gel electrophoresis to compare eastern and western sources of loblolly pine to shortleaf pine. With an analysis of banding patterns, they reported that eastern loblolly pine showed 34 percent similarity to shortleaf pine while western sources of loblolly pine showed 88 percent similarity to shortleaf pine. They concluded that introgression of loblolly pine with shortleaf pine is much more frequent in western sources, such as in Oklahoma and Texas. Hare and Switzer’s (1969) results would suggest that the frequency of hybridization

is variable across the sympatric portion of these species' ranges, in particular, higher in the western part than in the eastern part.

Florence and Hicks (1980) used seed megagametophyte protein banding patterns to examine putative hybrids of these two species sampled in east Texas. Their intent was to relate hybridization to fusiform rust resistance, but in the process they also were able to use the banding patterns to support the hybrid nature of putative hybrids identified by a morphology-based hybrid index. They further suggested that introgression does occur, and probably in the direction of shortleaf pine.

Huneycutt and Askew (1989) screened both species with 20 isoenzyme systems in an attempt to identify a marker useful in distinguishing hybrids. They discovered that the isocitrate dehydrogenase (IDH) marker was monomorphic (a single allele) and monomeric (a single band) in both species, but differed between species by migration distance. They further tested the marker in the parent species and known hybrids and demonstrated that this was a simple and reliable marker to identify first-generation hybrids between loblolly pine and shortleaf pine. They also noted that the marker's utility in later generations and backcrosses would be limited by normal Mendelian segregation.

Edwards and Hamrick (1995) examined allozyme diversity in a wide-ranging sample of shortleaf pine. They screened 22 loci, including IDH, so they were able to estimate the level of hybridization in the 18 populations sampled. Of the populations they sampled, 11 were from east of the Mississippi River and seven from west of the river. They reported a generally high level of genetic similarity among all shortleaf pine populations, with one important distinction, that the eastern and western populations differed significantly in the level of hybridization to loblolly pine. The western populations had a higher percentage of hybrids (4.58 percent) than the eastern populations (1.09 percent). What is of considerable interest to us, but was not addressed, is that all but one of the western populations sampled were outside the accepted range of loblolly pine. These shortleaf pine populations are approximately 35 miles to well over 200 miles distant from the nearest natural loblolly pine populations. It is intriguing that these populations would show high levels of hybrids.

In an apparent follow-up study, Edwards and others (1997) used IDH to examine hybridization frequency in two naturally occurring, sympatric populations of loblolly pine and shortleaf pine in northern Georgia. They sampled all trees in both populations and reported 8 percent hybrids at one site and 0.4 percent hybrids at the second site. Using a chloroplast marker, they determined that shortleaf pine was the paternal parent (Wagner and others 1992) of all hybrids at both sites. They noted that all hybrid trees were juvenile, and spatially distant from mature shortleaf pines, supporting

the paternal contribution of shortleaf pine. Interestingly, they also reported that morphologically, the hybrids were easily distinguished from loblolly, but not from shortleaf, which is contrary to morphological descriptions of artificial hybrids discussed above. They recognized this discrepancy, and suggested that tree morphology may change as the hybrid trees matured, or that the hybrids they described were backcrosses. Since no mature hybrids were found, however, they discounted the second possibility. Perhaps the IDH locus is not a reliable marker.

Recent Studies at OSU

At Oklahoma State University (OSU) in the early 1990s, we initiated studies (reported by Raja and others 1997, 1998) to examine the effect of various management strategies on genetic diversity in shortleaf pine. These studies were in cooperation with the USDA Forest Service's Ecosystem Management Research on the Ozark and Ouachita National Forests. Since this work was started before the Edwards and Hamrick (1995) work was reported, we needed to conduct an isoenzyme study to characterize the shortleaf pine species to support the management study. Our range-wide study of isoenzyme diversity in shortleaf pine turned out to be very similar to that of Edwards and Hamrick (1995). We sampled six western populations and nine eastern populations, and although these populations were entirely different from the Edwards and Hamrick (1995) samples, our results, in terms of the general genetic characterization of the species, were in close agreement with their results. However, we did note that, based on the IDH locus, more than 16 percent of the trees from western populations were hybrids (they reported 4.58 percent), while eastern populations showed 4.45 percent hybrids (they reported 1.09 percent). Although three of the western populations were outside the natural range of loblolly pine, we found evidence of hybridization, on average 10 percent, as did the Edwards and Hamrick (1995) study (although their percent of hybrids was lower). We cannot explain these differences in estimated level of hybridization between the two studies, except to point out that the populations sampled were different in number of trees sampled per stand, stand locations sampled, and time of sampling (we sampled trees from the South-wide Southern Pine Seed Source Study [SSPSSS] plantings, trees from seed collected in 1951-1952). Consequently, we speculated that IDH may not be a reliable marker for determining species or degree of hybridization. We then sampled stands of shortleaf pine in the Mt. Ida, AR area for the Ecosystem Management study, and again noted a high number (15 percent) of hybrid trees based on IDH. These were trees in what were thought to be pure shortleaf pine stands, several miles north of any native loblolly pine trees or stands.

As a follow-up study (Chen and others 2004) to confirm or discount these results, we sampled native pine stands across a southeast to northwest transect of Montgomery County, AR, which includes Mt. Ida. These stands were

mixed loblolly pine/shortleaf pine in the southeast part of the county and pure shortleaf pine, up to 20 miles north of the closest known loblolly pine stands, in the northwest corner of the county. In this study we used a codominant nuclear marker and a chloroplast marker to identify hybrids and their paternity, respectively. Of the 80 trees sampled, ten (12.5 percent) were found to be heterozygous at the nuclear marker locus, i.e. hybrids. Seven of these were also confirmed to be hybrid using the IDH locus. Of the remaining three, one was not tested, and one each was homozygous for loblolly pine or shortleaf pine. We also found one tree heterozygous at the IDH locus, but not at the nuclear marker. Since we concluded that some of the hybrids were not F_1 s, a few of these genotypes would be expected. The chloroplast marker showed some of the hybrids to be of loblolly pine paternity and some of shortleaf pine paternity. Morphological data agreed with the paternity analysis in that those of shortleaf pine paternity looked more like shortleaf pine, and those of loblolly pine paternity looked more like loblolly pine. In this confirmation study, we found (1) a relatively high percentage of hybrid trees (14 percent); (2) hybrids in shortleaf pine stands beyond the natural range of loblolly pine; (3) introgression occurring in both directions; and (4) the IDH locus apparently a reliable marker for species and F_1 hybrid determination.

Current Study at OSU

The obvious next questions are: Is this level of hybridization management induced, and what effect will such levels of hybridization have on the long-term integrity of these species? If the current intensive management of loblolly pine throughout the sympatric range of these two species is in part responsible for the relatively high level of hybridization found, there are serious implications regarding shortleaf pine management. Since the USDA Forest Service, by mandate, is one of only a few organizations in the South regenerating shortleaf pine stands, and most often relies on forms of natural regeneration to do so, will the potentially overwhelming loblolly pine background pollen cloud put the future of the shortleaf species at risk? The outcomes from this research project will begin to answer these questions, and may point to management strategies designed to maintain the integrity and diversity of the shortleaf pine species. Loblolly pine will probably not be at risk because of its varied and active tree improvement and artificial regeneration programs.

Based on the research and results described above, we initiated a study to examine the cause and consequences of introgression between shortleaf pine and loblolly pine. The objectives of the research are to:

1. Estimate the level of hybridization present in today's native populations of loblolly pine and shortleaf pine.
2. Estimate the level of hybridization present in 1950s range-wide samples of loblolly pine and shortleaf pine. Samples from the SSPSSS are being used.

3. Compare levels of hybridization from objectives 1 and 2.
4. Estimate the present day level and direction of introgression occurring between these two species.
5. Compare the level of hybridization present in native shortleaf pine stands from an area of intensive loblolly pine management to that in shortleaf pine from relatively undisturbed, continuous, native, mixed shortleaf pine/loblolly pine stands.

STUDY AREAS

Field tissue (needle) collections have been made from remaining SSPSSS plantings of both shortleaf pine and loblolly pine. These trees represent seed collected in 1951 and 1952, some 54 years ago, formed at a time when man's influence, at least in reference to vast plantings of loblolly pine, was minimal. We will match these collections with collections from loblolly pine and shortleaf pine trees found currently in the "wild" on sites as close as possible to the original collection sites of the SSPSSS, at least to within the same county. This current day collection will be made from the youngest trees found on the site to represent the most recent seed fall. These collections will allow us to estimate the level of hybridization in these species at present and approximately 50 years ago.

METHODS

To meet objective four, we will use the data from the present day shortleaf pine and loblolly pine collections and subject it to an appropriate analysis (e.g., Anderson and Thompson 2002).

To meet objective five, we intend to identify two stands meeting the following set of conditions. One stand will be native shortleaf pine which has been essentially undisturbed by humans, and is surrounded by a large area (thousands of acres) also fairly undisturbed, ideally consists of mixed shortleaf pine/loblolly pine. The second stand will also be an undisturbed native shortleaf pine stand, but this stand (100 acres or so) will be surrounded by mostly planted loblolly pine (thousands of acres). From these stands we will collect seed of about 100 trees each and this seed and the resultant offspring will be screened to determine the level of hybridization. These comparison stands will both be in relatively close vicinity (the same or adjoining counties) to avoid problems associated with natural variation in hybridization levels observed in stands from across the species' native ranges (Raja and others 1997, Edwards and Hamrick 1995). Such stands have tentatively been identified.

To date we have characterized the hybrid nature of individuals using the IHD isoenzyme locus (Huneycutt and Askew 1989), a codominant nuclear ribosomal DNA marker

from the ITS-1 region and a chloroplast marker, both of which we developed (Chen and others 2004), and a number of simple sequence repeat (SSR) markers developed in Dr. Clare Williams's lab. We intend to utilize these markers in this study. We are also evaluating additional SSR markers screened at the Southern Institute of Forest Genetics in Mississippi, and we have developed a large set of amplified fragment length polymorphism (AFLP) markers to ensure a comprehensive characterization of every genotype.

RESULTS TO DATE

To develop our AFLP data set, we screened 48 primer pairs. Eighteen of these primer pairs were selected for producing multiple and clear bands. These primer pairs were then used to screen the SSPSSS shortleaf pine and loblolly pine collections. These primers produced polymorphic and monomorphic AFLP bands at 794 loci in the shortleaf pine samples and 647 loci in the loblolly pine samples. These AFLP makers were used to estimate the genetic diversity of natural shortleaf pine and loblolly pine populations sampled prior to extensive forest management.

The average heterozygosity of shortleaf pine throughout its range, west of the Mississippi River, and east of the river is 15 percent, 17 percent, and 15 percent, respectively. The heterozygosity value of the populations west of the river is a little higher than that of populations east of the river. This result agrees with the studies of Raja and others (1997) and Edwards and Hamrick (1995). The average heterozygosity of loblolly pine throughout its range, west of the Mississippi River, and east of the river is 12 percent, 12 percent, and 13 percent, respectively, based on the 647 loci. Both shortleaf pine and loblolly pine are outcrossing species, and it is not surprising to find high levels of heterozygosity in the natural populations especially given that hybridization between these two species may contribute to this high heterozygosity.

Of the numerous AFLPs produced by the 18 primer pairs, 96 were polymorphic among both the shortleaf pine and loblolly pine samples. These 96 AFLPs were produced by 17 primer pairs and should be useful in examining the hybridization level and the pedigree of hybrids, given the appropriate analysis. Preliminary analyses of these data do suggest that some of the sample trees are hybrids.

The IDH isoenzyme locus is being screened for all trees as a second indicator of trees which may be hybrids. With only some of the trees characterized to date, the IDH locus has also identified several trees as hybrids. The results from IDH and the AFLP markers will be compared to see whether these markers are reliable to distinguish shortleaf X loblolly hybrids.

By comparing the SSPSSS trees with contemporary trees from the same counties, we will be able to estimate with considerable reliability the preintensive forestry level of

hybridization in loblolly pine and shortleaf pine (1950s), and the postintensive forestry level of hybridization (2000s) across the range of the two species. Sampling of current natural regeneration in the SSPSSS counties has just begun.

By comparing seed fall from shortleaf pine in a relatively undisturbed area with that of shortleaf pine in an area essentially surrounded by loblolly pine plantings, we will also be able to compare the level of hybridization which occurs under these scenarios. These data will allow some insight into the present and potential effect that intensive management of loblolly pine is having on the genetic integrity of shortleaf pine throughout their sympatric region. The pedigree of the hybrids will also be determined, at least to the F_2 and BC_1 level, or further if the available analytical methods and software allow. These samples have not yet been collected.

DISCUSSION AND CONCLUSIONS

Since Zobel's (1953) report of suspected hybrids, the collective evidence has proven that natural hybrids between loblolly pine and shortleaf pine exist, and that the frequency of occurrence of hybrids is greater in populations west of the Mississippi River (Edwards and Hamrick 1995, Raja et al. 1997, Chen et al. 2004, among others). If the IDH locus is a reliable indicator of hybrid trees, it would appear that in general all populations of shortleaf pine west of the Mississippi River contain some hybrids. Our study demonstrating bidirectional introgression would suggest that loblolly pine populations are also affected, but to what degree is not as well documented. In retrospect, Zobel was correct in his suspicion of the existence of natural hybrids between these two pine species. We suspect he did not know what the consequences of these hybrids might or will be, nor do we; however, there are some possibilities worth discussion and further study.

It is well known that the natural ranges of loblolly and shortleaf pine overlap throughout the South (Little 1971). For example, stands in the upper west Gulf Coastal Plain in northern Louisiana and south Arkansas, such as are found in the Reynolds Research Natural Area on the Crossett Experimental Forest in Ashley County, AR, contain both species, and loblolly pine dominates these stands while shortleaf pine is a minor component of varying occurrence (Cain and Shelton 1994). However, early publications based on studies at the Crossett Experimental Forest describe the stands as "shortleaf-loblolly pine stands" (Reynolds and others 1944, Reynolds 1947), which suggests that shortleaf pine dominated the mixture in the 1930s. Unwritten marking guides for lumber company crews working in these mixed forests encouraged retention of the loblolly pine and removal of the shortleaf pine, all other things being equal, because loblolly pine grows at a slightly faster rate than shortleaf pine. But trees one would identify clearly as shortleaf pine remain common in these mixed stands. An

interesting question about these stand histories: What are the hybridization rates in shortleaf pine in the upper West Gulf Coastal Plain? Such a study might be included in the current OSU study or a follow-up study as a point of comparison with data from the pure shortleaf stands in the Ouachitas.

In this context, the ability of shortleaf pine to retain its independent identity in mixed stands on the upper west Gulf Coastal Plain suggests that it might also do so in the Ouachitas. In both situations, some attributes of shortleaf pine per se are ecologically advantageous, or were so prior to extensive forest management activity in this region. One unusual difference is shortleaf pine's ability to resprout if topkilled by fire (Mattoon 1915), which confers an advantage in establishment if fire burns through young stands containing both species. Shortleaf pine grows more slowly but endures competition longer than loblolly pine (Lawson 1990). It also is considered to be more tolerant of drought and of xeric sites than loblolly pine, which might lead to different survival probabilities for the respective species if a young mixed-species cohort experienced drought, or was established on a xeric site, or both.

It would also seem that the success of a hybrid might relate to the attributes that the hybrid inherits from its respective parents. A hybrid might grow more rapidly than a pure shortleaf pine, or might be slightly less tolerant of fire or drought than its shortleaf pine parent. One can imagine ecological circumstances that might discriminate in favor of or against the hybrid—such as a reduced ability (compared to shortleaf pine) to resprout if top-killed by fire, or a greater ability than a loblolly pine to tolerate drought. Since the most interesting silvical difference between shortleaf pine and loblolly pine is the aforementioned resprouting ability of shortleaf pine, it would be interesting to quantify this trait in hybrids. It might also be interesting to follow the percent survival of hybrids over time from seed fall, through seedling establishment to stand maturity, in stands on xeric and mesic sites, to determine if the percent of hybrids surviving increases or decreases during stand development.

The pattern of distribution of naturally-regenerated stands of shortleaf pine relative to loblolly pine plantations throughout the Ouachitas might also affect pollen distribution. Generally speaking, loblolly pine plantations in the region are on the lower slopes and valleys in the region: those lands were the acquired originally by timber companies for managing naturally regenerated shortleaf pine stands during the 1930s and 1940s because of their higher productivity than the stands on upper slopes and ridges, which remained (and still exist) in Federal ownership. Thus, on landscapes where the two species co-occur today, the loblolly pine plantations planted by those timber companies or their successors tend to be on lower slopes, whereas the shortleaf pine stands tend to be on upper slopes or ridges. Position may affect the degree to which natural introgression from the earlier shed loblolly pine pollen

cloud to receptive shortleaf pine flowers can occur, since the introgressing loblolly pine pollen would generally have to float uphill. A recent study by Dyer and Sork (2001) showed limited pollen movement in a continuous forest due to both distance and vegetative structure, but they did not address pollen movement in actively managed forests with changing canopy structure.

Finally, the major seed orchard for shortleaf pine on federal lands in the western Gulf region is located at Mount Ida, AR, in terrain not unlike that from which we collected samples for the introgression results in the recent OSU study. If the prevailing pollen cloud from the hundreds of thousands of acres of loblolly pine plantations in the region is sufficient to be considered prevalent at a landscape scale, the protective buffers that surround this seed orchard might actually provide limited to no protection against the possibility of introgression in the dominant source of genetically improved shortleaf pine seed for the mid-South. Assays of IDH in seed from that orchard or the planting stock being raised in nurseries for outplanting might be interesting, especially if a test could be produced that might be used to cull hybrids from planting stock being distributed for outplanting.

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