A GERMPLASM COLLECTION AND EVALUATION PROGRAM FOR ALNUS GLUTINOSA

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ABSTRACT.--Collection and evaluation of Alnus glutinosa seed sources are discussed. Individual experiments involving the alder endophyte, early selection for shade and moisture stress tolerance, and breeding techniques also are discussed.

INTRODUCTION

European black alder, Alnus glutinosa (L.) Gaetrn., has a number of potential uses in the eastern United States. It can be established on high-acidity coal spoils and gives relatively good growth on such sites (Funk 1973; Plass 1977); its wood characteristics are suitable for both the wood fiber and furniture industries (Phares et al. 1975; Vurdu and Bensend 1978); and, because of its ability for symbiotic nitrogen fixation, it has shown considerable benefit in nurse crop or mixture plantings (Tarrant and Trappe 1971; Plass 1977). However, tree improvement efforts with this species have been limited. Furthermore, because much of the value of the species is dependent on its symbiotic nitrogen fixation, the task of tree improvement is likely to be more complex than for most species.

In European work, superior A. glutinosa trees and stands have been identified, and some seed-production areas (Poland, Finland), seed orchards (Sweden, West Germany), and breeding programs (Finland) have been established. In the United States of America, where A. glutinosa is an exotic species, there have been only two tree improvement projects, and they have sampled only a very small portion of the European population.

In 1963, the Forest Service, under the leadership of David Funk, established a provenance test of 15 A. glutinosa seed sources with material collected primarily from north-central Europe. The trees were planted on a strip mine area in southeastern Ohio where soil pH ranged from 4 to 5. After 7 years of growth, the best trees averaged 20.4 feet in height and 2.7 inches DBH. Seed sources from southern Germany outperformed those from northern Germany, Belgium, Denmark, or Sweden (Funk 1973).

The second project is under the auspices of the North Carolina Cooperative Hardwood Program. Several companies are cooperating, but most of the work has been done in Alabama by Gordon White of Champion International. Starting with seedling populations of unknown origin from the Illinois State Nursery, White now has some plantings 7 years old which have been producing seed for 4 years. He has selected 17 trees from these populations and used them as parents for 120 control-pollinated families that were set out as a progeny test in 1977 (Gordon White, personal communication).

Nitrogen fixation in alder root systems is due to an association with actinomycetes of the genus Frankia. Theoretical considerations (i.e., the nature of microorganisms and extrapolation from the legume/Rhizobium association), and limited observations suggest that there can be significant variation in performance due to the strain of Frankia that colonizes the roots. There also is reason to expect that mycorrhizal fungi play an active role as a third source of variation in the A. glutinosa actinorhizae system. Yet, there has been no tree improvement work to date in which sources of symbiotic variation have been deliberately controlled or exploited (Hall et al. 1978). (Actinorhizae is a term coined to denote the root system actinomycete nodule found on nonlegume nitrogen fixing plants. Its use was established at an April 1978 meeting on "Symbiotic Nitrogen Fixation in Actinomycete-Nodulated Plants" held at the Harvard Forest.)

Hence, the greatest current needs in U.S. A. glutinosa tree improvement work are to greatly increase the germplasm base available and to consider the effects of root system symbionts. A program to accomplish these goals has been established at the Iowa State University in cooperation with the NC-99 Regional Tree Improvement group and the Pennsylvania State University.

GERMPLASM COLLECTION AND TESTING

A. glutinosa has an extremely wide natural geographic range (Figure 1). In the fall of 1976, efforts were started to contact cooperators who would supply seed collections from natural or selected stands. The response was very good, and the collection now is essentially complete except for representative seed collections from East Germany, the Moscow U.S.S.R. region, and the northern Africa disjunct populations. The collection includes natural-stand material from near the Arctic circle (Norway and Finland) to the southern tip of Italy and from Ireland to the Black (U.S.S.R.) and Caspian (Iran) seas. In addition, selected stand and seed orchard seed has been received from West Germany and Poland as well as hybrid A. glutinosa X A. incana seed from Finland. (A complete list of the seed collected can be obtained from R. B. Hall.) Contrary to common belief (U.S.D.A. 1974), alder seed stores well under refrigeration, so this material should remain available for several years until depleted by research demands.

To evaluate this germplasm under U.S. conditions, a threefold program is being conducted: 1) To assess broad geographic patterns of variation, 56 natural stand collections have been chosen for a provenance study. The first planting in this study was made in central Iowa in late spring 1978. Three additional plantings are being planned for 1979, one each in northern Wisconsin, Alabama, and Pennsylvania. 2) To provide substantial information on the most promising directions to take in the improvement of this highly variable species, a second study will be conducted including several of the provenances from the first study. For each provenance, open-pollinated seed is available from five parent trees. For each parent tree, randomly selected offspring will be cloned. Hence, a partitioning of provenance, parent tree, and clonal line variation will be possible. 3) To screen the collection for useful traits such as high nitrogen-fixing capability, stress tolerance, growth rate, and form, a continuing series of germplasm evaluations will be conducted. Seed and/or seedlings can be made available to other researchers for such tests. At Penn State, a set of seed lots is being screened by Kim Steiner to assess variation in tolerance of high aluminum concentra-

Figure 1. Natural range of <u>Alnus glutinosa.</u> Compiled by R. B. Hall from information supplied by Dr. K. Browicz of the Kornik Dendrological Institute in Poland and from partial maps appearing in Jales and Suominen (1976), Koshevnikov and Plieva (1976), Yaltririk (1973), Maire (1969), and Jahandiez and Maire (1932).

tions such as would be found on some coal spoil areas. At Iowa State, a series of nursery tests were started this year and will continue until the entire collection is surveyed. The emphasis is on initial growth rate, form, and acclimatization. Promising trees will be cloned for longer-term tests.

Stock for the field tests under each of these three areas of study is being produced in containers in a greenhouse. When the seedlings are about 4 weeks old, a mixed inoculum of two European strains of A. *glutinosa* endophyte is applied so that nodulation is well established before the trees are field-planted. Little is yet known about host genotype/endophyte strain interactions, and nothing is known about long-term field survival and competition of the endophyte strains, but this procedure at least starts the trees with a uniform treatment with respect to the actinorhizal component of symbiosis. Work also is in progress by McNabb and Green at Iowa State to develop a mycorrhizal inoculum for these seedlings (Hall et al. 1978).

ALDER ENDOPHYTE STUDY

One reason that alder can survive in such harsh environments as coal spoil banks and grow vigorously under good site conditions is due to its root symbionts. The mycorrhizal fungi associated with its roots have the ability to scavenge adequate quantities of phosphate and other nutrients from deficient soils (Mejstrik and Benecke 1969). The other root symbiont important to alder, and the subject of this portion of the study, is the nodule-forming actinomycete, *Frankia alni.*

In nitrogen-deficient soils, alder seedlings without nodules or with ineffective nodules will quickly become chlorotic and die. But, when modulated by a compatible endophyte, alder can grow vigorously. It is thus important to know as much as possible about the microscopic partner in this relationship. It is particularly important for those planting black alder in this country to know if it will form effective nodules with native U.S. strains of F. *alni.* Evaluations of the genetic potential of the host plant could be seriously in error if the symbiosis is ignored.

To begin understanding potential interactions between A. *glutinosa* and its symbionts, a study has been conducted with four black alder seed sources and six actinorhizal inoculum types (Hall et al. 1978). Three of the seed sources were from Wales, The Netherlands, and Poland. The fourth seed source was red alder (A. *rubra* Bong) from Idaho.

The inoculum types were a spore (+) strain and a spore (-) strain collected in The Netherlands by Dr. Maurice Lalonde, an A. rubra-derived strain from Oregon, provided by Dr. John Gordon, and a central Iowa strain of unknown natural association. (The complete procedure of this experiment is available upon request, and full results will be published elsewhere.) At the end of 4½ weeks and again 3 months later, sample plants were harvested and checked for nodulation, chlorosis, height, survival, and nitrogen-fixing capability as assayed by acetylene-reduction assay.

Seed source significantly affected initial development of nodules and plant height (Tables 1 and 2). Seed source did not significantly affect chlorosis or survival.

Inoculum type, however, had a highly significant effect on the total weight of nodules, the number of nodules, and the average weight per nodule as well as a significant effect on the estimated amount of nitrogen fixed per gram of nodules (nodular efficiency) (Tables 3 and 4).

Table 1. Analysis of variance for number of nodules per seedling at 4½-week thinning (R^2=.92).

Source of Variation	Degrees of Freedom	Mean Square	F	Prob. F
Seed source (SS)	3	46.2	13.59	.0001
Inoculum (Inoc)	5	314.8	92.51	.0001
SS x Inoc	15	16.6	4.87	.0001
Residual	53	3.4		
Corrected total	76			

Table 2. Analysis of variance for plant height (R^2= .85) 3 months after transplanting.

Source of Variation	Degrees of Freedom	Mean Square	F	Prob. F
Seed source (SS)	3	328.5	21.1	.0001
Inoculum (Inoc)	5	665.6	42.7	.0001
SS x Inoc	15	37.9	2.4	.0085
Residual	56	15.6		
Corrected total	79			

Table 3. Probabilities of the observed differences occurring by chance from analysis of variance for several characteristics/effect of inoculum.

Parameter	Prob. F
Weight of nodules	.0003
Number of nodules	.0001
Average nodule weight	.003
Nodular efficiency	.044

Table 4. Mean nodular efficiency and plant height as affected by inoculum type.

Inoculum Type	N	uM fixed/ Gram of Nodules	N	Plant Height (cm)
Spore -	8	467 a	12	20.5 a
Spore +	11	309	14	16.3
Red alder	9	118	13	7.4
Local Iowa	Too few surviving plants for inclusion			

[a]Values connected by the same bracket are not significantly different at the .05 probability level.

Seedlings treated with the native Iowa endophyte nodulated poorly, and most plants treated with it died before the end of the experiment. Nodulation with the red alder endophyte was delayed; N-fixation efficiency and plant height growth were reduced. The two native A. glutinosa endophytes also showed interesting influences. The spore (+) strain produced a large number of nodules (an average of 12/plant at 4½ weeks, 130/plant at 4 months), but was not as effective as the spore (-) strain in terms of N-fixation efficiency and host plant height growth. The spore (-) strain produced relatively few modules (6/plant at 4½ weeks, 19/plant at 4 months), but they were more efficient in N fixation, and the host plants grew better. Additional experiments will be conducted to determine the long-term implications of these findings for field plantings and improvement programs.

The extremely poor nodulating ability of the central Iowa endophyte and the adequate nodulation, but 60% reduction in efficiency and 50% reduction in height of the plants treated with red alder endophyte, indicate that importation and testing of natural A. *glutinosa* symbionts may be very important. Also, the 40% difference in nitrogen-fixing efficiency and matching 40% difference in plant height indicate that there may also be considerable variation in efficiency between different strains of F. *alni* that naturally associate with A. *glutinosa.* This may allow for a second avenue of genetic improvement of tree performance.

EARLY SELECTION STUDY

Two particular questions have been raised with respect to A. *glutinosa* for use in plantation mixtures: What is the relative shade tolerance in the species, and under how much moisture stress can it survive? The general characteristics of the species are those of an intolerant pioneer species on moist sites, but in planted, managed stands, it is likely to have a wider amplitude of site conditions that it can tolerate.

Several forest tree species possess the ability to fix atmospheric nitrogen, but few meet the requirements of a good nurse crop (i.e., slow growth and maximum photosynthesis production under shade conditions) (Kohnke 1941). Brokau, Lowry, and Breeding (1962) suggest that *A. glutinosa* might be an excellent choice as a nurse crop to economically important species.

A survey of genetic variation in shade and moisture-stress tolerance has been initiated using latitude, elevation, and natural habitat (moist or dry) to select seed lots and using height and dry-matter production under stress conditions to select candidates from the seed lots. Twenty-four seed lots have been chosen from elevations ranging from 30 m to 335 m. The selections are distributed over 14 countries: Iran, Finland, U.S.S.R., Poland, West Germany, France, Belgium, Italy, Switzerland, Ireland, Austria, Spain, East Germany, and Greece.

An initial greenhouse study is in progress. After 7 weeks of growth from seed, measurements were taken from each plant. Then half of the plants were placed under shade cloth representing 60% shade. Half of these and half of the unshaded plants are being watered every 6 days, and the others are being watered every day. Measurements of weekly growth rates are being taken, and at the end of the study, dry-matter production of each plant will be determined.

Five seed lots selected from this initial experiment will be cloned and used in a more detailed study to determine the ability of black alder to serve as a nurse crop or as a timber crop. Nitrogen fixation rates, photosynthetic rates, and respiration rates will be determined for plants growing under three different light regimes and three moisture levels.

GRAFTING AND CONTROLLED POLLINATION STUDY

One major advantage of A. glutinosa as a subject for tree improvement work is its early flowering characteristic that should hasten breeding efforts. It is not unusual for 2- to 3-year-old seedlings to produce male and female flowers. Therefore, the extensive germplasm collection now available will offer abundant opportunities for breeding work within a few years.

Also, breeding work in Europe with black alder has shown that genetically superior trees are possible from cross as well as self pollinations (Weisgerber 1974). To be ready to capitalize on this opportunity, tests of grafted plant breeding techniques in the field and greenhouse have been started. The objectives of these tests are (1) to determine if graft incompatibilities exist in selected clones when grafted interclonally and intraclonally, (2) to determine the relative success of various grafting techniques, and (3) to collect observations on the controlled pollination and seed production of various mating combinations in the field and greenhouse. Preliminary work began in February 1978, basically to determine experimental design, uncover environmental problems that can be eliminated or controlled, practice grafting and pollination techniques, and collect initial observations on survival of the grafts.

Three A. glutinosa trees growing in an Iowa plantation were selected to provide scion material and pollen. These were chosen on the basis of available root stock in the greenhouse, amount of flowers on the trees in the field, and resistance to drought and cold damage. One other clone was chosen to use as the root stock for interclonal grafts.

Dormant flowering branches were collected from these selections to use for pollen collection and grafting. Branches with mostly male catkins were forced by placing the branches in a nutrient solution. About half of these were soaked in warm water initially, and these tended to produce pollen about 3 to 4 days earlier than the unsoaked branches.

Three graft types were used in this experiment, two veneer grafts and a double-cleft graft in which two scions were inserted into the root stock. The veneer grafts had the highest survival rates inasmuch as none of the cleft grafts survived. The older root stocks used for cleft grafts exerted too much pressure on the scions, causing damage to the tissue. Younger root stocks may be sufficiently pliable to allow use of this method.

The female flowers were grafted onto root stocks of either the same clone from which they were obtained (intraclonally) or to the specified root stock designated for interclonal grafts. Initial observations have shown no difference in mortality between inter- and intra-clonally grafted plants.

Beginning in mid-March, when the grafted flowers had reached the period of receptivity, cross- and self-pollinations were carried out using the collected pollen. For comparison, the same mating combinations were carried out on the source trees in the field. Observations are being made on strobili development, seed production, and seed quality.

In this initial phase of the work, 50 of 70 graft unions were unsuccessful. Much of this was because of inexperience with grafting, but significant moisture loss and the accumulation of phenolic compounds on the cut surfaces also were detrimental.

In future work, moisture loss will be dealt with by a number of treatments. Soaking the scions in water preceding grafting will initially add moisture to the union, and plastic and paper bags will be used to slow moisture loss from the young grafts.

The phenolic problem is an interesting one. Within seconds of making a fresh cut on nondormant material, phenolic compounds accumulate at the wound site, possibly hampering a successful graft union. Dormant root stock and antioxidants will be used in an attempt to overcome this problem.

Other areas to be explored also became evident. The initial pollen-collection technique, adapted from elm work, released too much pollen into the air of the collection room. A cleaner approach was to shake the catkins in the pollination bags until all the pollen had been released. Then, a corner of the bag was cut, and the contents poured through a screen and into a vial for storage. Methods of pollen storage are being studied to determine optimal conditions of humidity and temperature. Pollen viability, using methods outlined by Eigsti (1966), has already been tested for several storage treatments (including 22 hours of vacuum drying) with all treatments yielding some viable pollen. The use of Dr. Henry Gerhold's minibags is being considered to reduce the loss of pollination bags during high winds.

CONCLUSION

This paper reports the initial phases of a germplasm collection and utilization program for A. *glutinosa.* The program has significantly increased the base of material for tree improvement use in this country. It is expected that the symbiotic actinorhyzal system of European black alder will offer new and challenging opportunities in selection work. And the short generation time in alder suits it admirably to an intensive breeding program in which these opportunities can be realized.

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