NEW APPROACHES TO NORTHERN RED OAK IMPROVEMENT IN INDIANA

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Abstract .-- An integrated approach to the genetic improvement of northern red oak (<u>Quercus rubra</u> L.) in Indiana is presented. The program described employs the use of a subline breeding strategy which allows for ongoing genetic improvement while minimizing the deleterious effects of inbreeding depression in advance generations. Novel uses of softwood stem cuttings in the breeding and production phases of the program will provide multiple opportunities for genetic improvement. Genetic gains will be realized in this program through the production of reforestation stock derived from clonal seed orchards and cutting propagation hedges. The advantages of using sublines and clonal propagation to improve northern red oak, and the recognition of the potential limitations associated with these approaches are discussed.

<u>Additional Keywords</u>: sublines, breeding strategy, inbreeding, clonal propagation, softwood stem cuttings, <u>Ouercus</u> <u>rubra</u> L.

INTRODUCTION

Northern red oak (<u>Ouercus rubr</u>a L.) is a fine hardwood of great importance to the Indiana wood-using industry, and has been shown to be a genetically variable tree species (Kriebel, and others, 1976; Kriebel, <u>et al</u>, 1985). The Indiana Department of Natural Resources initiated a tree improvement program for northern red oak in 1984. Coggeshall and Beineke (1986) outlined the basic components of this program which employs the use of a subline breeding strategy. This strategy will provide for continuous genetic improvement over multiple generations by minimizing the deleterious effects of inbreeding depression on genetic gains.

The potential loss of genetic gain to inbreeding depression over multiple generations has been recognized (IUFRO, 1976) as many organizations begin to develop advance generation breeding populations. Attempts to limit the deleterious effects of inbreeding depression in advance generation breeding populations of conifers have been based on the concept of subdividing the breeding population into spatially separated breeding groups (sublines), and producing outcrossed seed in orchards containing one clone from each breeding group (van Buijtenen and Lowe, 1979).

A table prepared by Beineke (1979) indicates at least 12 biological constraints that would seriously impair the progress of any oak tree improvement

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program. Predominant in this list are those factors associated with seed production that would negatively impact on breeding and orchard activities. When these factors are coupled with the potential problems associated with any multiple generation breeding program, it would appear that a northern red oak tree improvement program would be prone to failure. However, the influence of some of these negative factors could possibly be mitigated by the use of vegetative propagules in combination with a subline breeding program. This paper expands on the earlier contribution of Coggeshall and Beineke (1986) and focuses on plans for the long-term genetic improvement of northern red oak in Indiana through the utilization of stem cuttings and seeds derived from a subline breeding program.

MATERIALS AND METHODS

McKeand and Beineke (1980) outlined a mating design for black walnut (<u>Juglans nigra</u> L.) that employs the use of sublines and open-pollinated progeny testing. We have applied this strategy to northern red oak. A flow chart of the northern red oak subline breeding program is shown in Figure 1.

Subline Design :

A total of 180 selections arranged in 6 sublines, as defined by Burdon and Namkoong (1983), will be established at the Vallonia State Nursery in south central Indiana. Each subline will contain 30 clones, with each clone represented by 2 randomly placed ramets within each of 4 replications, for a total of 240 ramets per subline. To date, 131 selections have been grafted and successfully established in 5 sublines. The remaining selection and subline establishment work should be completed within 4 years.

Progeny Testing:

Progeny tests of the 180 clones in the breeding population will be established on at least 2 sites in the state. The purpose of these tests is threefold: 1) to verify the genotypic equality of the 6 sublines; 2) to identify outstanding clones in each subline for inclusion in the production seed orchards; and 3) to serve as populations in which new selections will be identified for inclusion in the second generation sublines (Figure 1).

Due to seed production problems associated with the oaks (Beineke, 1979), it is anticipated that the adequate numbers of viable seed required to establish the first generation open-pollinated progeny tests will be extremely difficult, if not impossible, to obtain in the short-run. An alternative to this situation is to multiply the scarce quantities of seed available via softwood stem cutting propagation as proposed by Burdon (1982). Our work in this area and by others (Isebrands and Crow, 1985), indicates that juvenile northern red oaks can be readily propagated by using softwood stem cuttings.

Vegetative multiplication of a fixed number of seedling offspring per family is planned, as advocated by Russell and Libby (1986). In the absence



Figure 1. Flow chart of activities in the IDNR northern red oak subline breeding program.

of large non-additive genetic effects, such clonal progeny tests should be highly efficient in evaluating parental breeding values (Burdon, 1986) and serving as populations for recurrent selections (Shaw and Hood, 1985).

Seed Orchards:

Production seed orchards will be established by utilizing the best clone(s) in each subline based on progeny test results (Figure 1). The question of how many clones to include in the orchards from each subline is debatable. At this time, it is felt that a maximum of 2 clones from each of the 6 sublines will be included in the first generation production seed orchards.

It is appropriate to question the need for production seed orchards in this program if in fact, clonal mass-propagation of superior genotypes for reforestation is contemplated. The Indiana Department of Natural Resources (IDNR) serves a wide-ranging clientele through its nursery system. The mass-production of rooted cuttings for the entire state is not considered feasible due to the varied intended uses of reforestation stock by the public and the price differentials associated with production costs. Continued production of seedling nursery stock is envisioned for the future and therefore, seed orchard establishment is assumed.

Advanced Generation Breeding:

Selection of individuals to include in the 6 second generation sublines will be distinct from the selection process for the production seed orchards (Figure 1). Thirty individuals will be selected from within the progeny tests based on combined family and within family performances for inclusion in each second generation subline. The integrity of each subline will be maintained by selecting progenies from the same groups (i.e. 30 individuals from subline 1, . . . , 30 individuals from subline 6). At this time, it is felt that a maximum of 2-3 individuals from 10-15 families could be selected per subline. The new selections will then be grafted and placed into the second generation sublines.

Subsequent matings and progeny testing will continue as in the first generation. The best clones in each second generation subline will be identified through progeny testing and placed into second generation seed orchards. Selections will be identified within the second generation progeny tests to establish the third generation sublines as before.

OPPORTUNITIES FOR USE OF STEM CUTTINGS

Several investigations of clonal propagation of oaks have been conducted (Farmer, 1965; Morgan, <u>et al</u>, 1980; Spethman, 1985; Bennett, 1987). While moderately difficult, clonal propagation of northern red oak by means of softwood stem cuttings has been successful, especially when using juvenile material (Isebrands and Crow, 1985; Teclaw and Isebrands, 1987; A. Kremer, personal communication). Mass-propagation of selected clones of <u>O. robur</u> and <u>O. patraea</u> for reforestation has in fact become a reality in Germany (Kleinschmit, 1986).

A number of opportunities are present in this northern red oak program that would take advantage of the non-additive genetic effects, increased product uniformity, and increased testing precision associated with cloning and are described below.

Progeny Testing:

The use of cuttings in progeny testing can be a beneficial tool used to improve the forward and backward selection procedures within a breeding program, while allowing for progeny tests to be established in a minimum amount of time. Clonal information within families will be obtained from the progeny tests and outstanding clones could be identified for mass-propagation (Figure 2). All seedlings used to generate the cuttings for progeny testing could hopefully be maintained in a juvenile state through hedging. These hedges could thus be rapidly utilized as a source of cutting material for reforestation once outstanding clones are identified in the progeny tests (Foster, 1986). Such a strategy will be highly efficient in realizing genetic gains (Matheson and Lindgren, 1985).

Propagation of Original Selections :

A serious drawback to cloning juvenile material while relatively simple, is the obvious lack of knowledge of their potential genetic worth. Conversely, the cloning of older, proven selections is considered extremely difficult due to maturation effects. Selected phenotypes can potentially be rejuvenated by means of serial propagation and/or hedging (Libby and Hood, 1976; Franclet, 1985). Morgan. <u>et al</u> (1980) were successful in increasing the rooting percentages of live oak (\underline{O} . <u>virginiana</u>) through serial propagation of 4 year old seedlings and suggest that further propagation could result in the successful cloning of proven mature trees.

An attempt was made to serially propagate the majority of selections in the IDNR northern red oak collection in 1987. Softwood stem cuttings were collected from potted grafts forced in a greenhouse, and stuck in jiffy pots under a mist tent. Cuttings from 45 out of 93 clones rooted after 8 weeks. While the rooting percentage was low (12 percent), 48 percent of the clones propagated did produce some rooted cuttings. The rooted cuttings represent octets that ranged from 10 to 150 + years old (3.5 to 40.0 inches d.b.h.). Attempts to re-propagate the unsuccessful clones in the IDNR collection will be made over the next 2 years. Plans call for the subsequent re-propagation of all rooted cuttings in the future and ultimate establishment of cutting propagation hedges. Clonal genetic tests will be established for all clones by using cuttings obtained from the hedges. These tests will provide information on the suitability of all clones in the IDNR collection for use as clonal reforestation material. The best clones will be identified through testing and all inferior clones will be roqued from the hedges. These roqued hedges will then be managed and maintained as a source of cutting material for reforestation (Figure 2).

Potted Breeding Orchard:

Promotion of pistillate and staminate flowering on potted northern red oak ramets will be studied. If successful, the use of a potted breeding orchard as described by Bower, et al (1986) is contemplated in order to



Figure 2. Opportunities for use of stem cuttings for testing and production purposes in the IDNR northern red oak tree improvement program.

produce limited amounts of control-pollinated seed for subsequent testing as rooted cuttings. The specific matings for the controlled crosses would be determined based on information from the progeny tests (Figure 2).

Paired matings of outstanding clones identified by progeny testing would be made by utilizing the ramets in the potted breeding orchard. Highly heterozygous progeny would result from these crosses, especially in advance generations. Cutting propagation hedges would be established to generate adequate numbers of rooted cuttings for the subsequent establishment of clonal genetic tests. Information from these tests would be used to rogue inferior clones from the cutting propagation hedges. The remaining clones in the hedges would then be managed for the production of cuttings for reforestation (Figure 2).

DISCUSSION

The establishment of a subline breeding program for northern red oak has many advantages. Division of the program into long-term (breeding) and short-term (production) components will allow for greater flexibility in acheiving genetic gains (Overton and Kang, 1985). By using a subline breeding program, inbreeding will be confined to the breeding population while heterozygous seeds are produced in the production seed orchards. It is also possible that heterosis will be exhibited by the commercial seedlings produced by this program when unrelated, inbred individuals are crossed in advanced generation production seed orchards (McKeand and Beineke, 1980).

The fixation of low frequency alleles in the breeding population will be facilitated by the use of sublines (Namkoong, 1984). Kang (1979) indicates that a breeding population size of 180 selections distributed in 6 sublines could be adequate to maintain neutral genes in the progeny population with an initial gene frequency of .01 for more than 30 generations. It is recognized however, that the use of open-pollinated progenies as sources of advance generation selections in the progeny tests is not desirable due to the potentially rapid build-up of inbreeding levels. The only alternative to this problem would be to increase the size of the breeding population. It is anticipated that additional sublines will be established and plant material exchanges facilitated through the newly formed North Central Fine Hardwood Tree Improvement Cooperative (NCFHTIC). Through such cooperation, a more optimal breeding population size - up to 20 sublines as recommended by Kang (1979) - could hopefully be established for northern red oak, thus minimizing the rate of inbreeding in advance generations.

There are multiple opportunities available in this program for the realization of genetic gains through cloning. Evaluations of the original selections, progeny test individuals, and control-pollinated seedlings in clonal tests will result in a multi-directional approach to northern red oak improvement. It is recognized that all of the opportunities for use of vegetative propagules described herein may not be realized as our experience

with this species increases. Practical problems may arise in the future that would limit the use of some of the opportunities listed. However, it is the purpose of this paper to outline the avenues we intend to follow in order to produce softwood stem cuttings of outstanding northern red oak clones for reforestation in Indiana. The production of these cuttings will allow for the retention of beneficial non-additive genetic effects, and result in increased product uniformity.

Greater genetic gains can be realized by using clonally replicated seedlings in progeny testing because estimates of both family and within family variation can be determined with higher precision (Shaw and Hood, 1985). In addition, the minimization of the time required for progeny test establishment through the use of cuttings will result in greater genetic gains per unit of time and thus increase the overall efficiency of the program (Matheson and Lindgren, 1985).

Although much work remains, initial attempts to rejuvenate the original selections in the IDNR collection are promising and the mass-production of these genotypes for reforestation use is possible. Highly desirable individuals with proven wood quality and growth characteristics could be reintroduced to Indiana'a forests, and thus arrest current dysgenic selection trends. Also, non-flowering or poor flowering genotypes in the IDNR collection could be utilized at this stage.

The exploitation of the genetic diversity present within the progeny tests will allow for genetic improvement to continue over multiple generations. An additional source of genetic diversity could also be easily created through the utilization of paired matings of clones from different sublines within the potted breeding orchard (Burdon, 1986).

Genetic recombination and selection within the breeding population will allow for the continuous accrual of genetic gains over multiple generations, even though inbreeding levels will increase within the breeding population. Outcrossing in the production seed orchards, and between superior clones in the potted breeding orchard will result in highly heterozygous genotypes being made available as either seedlings or stem cuttings. The northern red oak tree improvement program described here is flexible and multi-directional, and should provide for the continuous and rapid utilization of genetic gains over the long run.

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