### CLONAL VS. HALF-SIB SEEDLING ORCHARDS FOR BLACK WALNUT

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## INTRODUCTION

Black walnut <u>(Juglans nigra</u> L.) is a highly valued species, and throughout its commercial history the highest quality trees have been harvested leaving the inferior to reproduce the next generation. Domestic consumption of walnut saw-timber in 1970 was 7.6 million board feet, while the volume of logs exported over the same period was 17.2 million board feet. Between 1963 and 1968 the inventory of black walnut decreased 10.6 percent. The average price of walnut logs has increased 3½ times in the decade 1959-68 as compared to 1949-58, and the average price of high-grade walnut lumber from 1969-71 increased approximately 20 percent (Schumann, 1971).

A tree improvement program designed to check this drain of valuable gene packages now, and to preserve them for later use is a necessity. In connection with this objective, the half-sib seedling orchard approach as proposed by the U. S. Forest Service in the Eastern Region is not adequate to provide the needed quality improvement, especially that which is needed now.

The intent of this paper is to express our views as to the breeding method which will produce the greatest quantity of genetically improved seed in the shortest time. We advocate the use of the progeny-tested clonal orchard in which the second stage begins after progeny testing. A clone bank is included.

The full-sib seedling approach is totally impractical for use with this species, due to the problems associated with the various phases of controlled pollination on mature trees.

# HALF-SIB APPROACH

The half-sib seedling approach, excluding initial mass selection, that is currently being used by the Forest Service, (Funk, 1966; Funk, 1970; Bey, Nickelson and Gerardo, 1971) can be summarized as follows:

Seed from about 100 parents, average for straightness and vigor will be collected from preset collection zones with the establishment of the zones based on 5-year genotypic variation studies (Bey, 1972). Obtaining these trees from representative locations was considered more important than selections based on minimum standards for growth and form. Only a general description, of each parent tree location will be made (Bey, Nickelson and Gerardo, 1971).

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When seed production starts, so will progeny-testing of the selected orchard trees. These tests can become the second generation seed orchard. Data from these tests will be used to determine the superior trees in the original orchard (Bey, Nickelson and Gerardo, 1971).

The justification for the half-sib seedling approach without initial mass selection has been stated as follows:

- 1. The difficulty of selecting plus-trees from wild populations because the candidates occur as single trees, and direct comparisons cannot be made. Also, they have concluded that plus-tree selection from wild populations is questionable.
- 2. Grafting is difficult and expensive.
- 3. Walnut seedlings flower as early as age 4.
- Orchards can be used for progeny tests because intensive cultural practices that are used in seed orchards are similar to standard plantation management.
- 5. Seedling orchards are easier and cheaper to establish than clonal orchards.

#### PROGENY-TESTED CLONAL ORCHARD

The progeny-tested clonal orchard, in which the second generation begins after progeny testing is discussed in the usual sense. A clone bank will be used for the preservation of superior and unusual gene packages. Genetic makeups that might be included in the clone bank are selections having disease and insect resistance, fertilizer response, desirable wood quality, and precocious flowering. A brief summary of the steps involved in the clonal approach follows.

After initial mass selection of superior phenotypes, the seed orchard and clone bank are to be established by grafting. The clone bank is located at Purdue University and will be replicated by one of our major cooperators, the Indiana Division of Forestry. Control pollination to determine combining ability is followed by progeny testing. After fullsib progeny testing, the orchard will be rogued, and at the same time a base will be established for the second-stage orchard. Through use of the clone bank, new progeny-tested selections will be ready for inclusion in the second-stage orchard. Also included, as an asset to the approach, is the possible development of two-clone orchards. The justification for the clonal approach is:

- 1. The important and necessary preservation of good genotypes.
- 2. A great opportunity for maximum genetic and profit gains.
- The asset of dealing with known genetic material and combining ability.
- 4. Grafts flower extremely early (1-2 years).
- 5. Clonal seed orchards have the benefit of maximum silvicultural effort for increased growth and seed production.
- 6. Pressures to rogue early from the seed production standpoint are not as great in a clonal orchard.
- 7. The problems with related coatings are not as serious.
- 8. The complete full-sib progeny test will be available until rotation age to be sure no mistakes were made in early roguing decisions; at the same time more than paying for its existence in quality wood production.
- 9. Improved seed will be available while progeny testing is in progress.
- 10. A clone bank combined with a seed orchard eliminates the initial need for large numbers of clones.
- 11. None of the problems associated with combining a seed orchard and a progeny test.

#### DISCUSSION

For the moment, simplicity, gain, and cost are three words we want to consider. Of course, half-sib seedling orchards are cheaper and easier to establish, but the easy way is not necessarily the best way. Also, keep in mind we are interested in profit gains, not just a cheap method.

Schreiner (1970) summarized tree breeding for different species in the U.S. It should be mentioned that the predominance of the clonal seed orchard approach is a tribute to its usefulness and practicality for species of much lesser importance than black walnut. For instance, in hardwoods there are 47 hectares in clonal orchards and 8 hectares in seedling orchards. Considering all conifers, 2239 hectares are in clonal orchards and 156 hectares are in seedling orchards (Schreiner, 1970). Of the amount that is in seedling orchards, we are not sure how much is actually half-sib in nature.

The amount of profit to be gained from clonal seed orchard programs has been estimated for P. <u>palustris</u>, P. taeda, and P. <u>elliotti</u> as being 14 percent, 18 percent, and 19 percent respectively. Early progeny tests of the southern pines show an improvement of about 15 percent in yield, and progeny tests of P. <u>elliotti</u>, 5 years old or less, indicate about 20 percent gain in growth rate from rogued clonal orchards, and comparable improvement in quality traits (Schreiner, 1970).

Table 1.-- Open-pollinated progeny tests

| Test                  | Height | Diameter |
|-----------------------|--------|----------|
| Test IAll 13 familie  | s 6.3  | 8.1      |
| Best 5 familie        | s 33.3 | 17.8     |
| Test IIAll 18 familie | s 3.6  | 1.8      |
| Best 5 familie        | s 18.9 | 14.8     |

Late flushing walnut usually avoids late spring frost damage in Indiana. It follows that late flushing trees might have superior apical dominance and straightness since they are seldom frosted back in the spring. If our selection intensity is high for apical dominance and straightness, we should also be selecting for late flushers. Compared to selections made the first 3 years, selections for apical dominance made during the last 2 years show a mean increase of 2 points on a scale of 10, on the selection system (Beineke and Lowe, 1969). The same comparison cannot be made for straightness, however, because trees getting 8 points on a scale of 10 now, would have received 9 or 10 as an early selection due to a less stringent use of our selection system at that time.

Flushing in the clone bank has occurred during a period of from 16 through 29 days. In 1972, flushing was completed in 25 days. At 12.5 days after first flush, 72 percent of the clones had not flushed. Ninety percent of those are selections made during the last 2 years. Nine clones flushed after the 'safe' date for frost, and 55 percent of those clones are later selections. Even though the flushing date range fluctuates with temperature from year to year, over the last 3 years clonal ranking remained essentially the same.

At this point the data can only be termed interesting; however, due to the affect that frost has on apical dominance and straightness, the predominance of late flushing clones in our later selections is an indication of our ability to select for those two traits.

<u>Vegetative Propagation</u> - The grafting of black walnut in our estimation is neither difficult, nor is the cost prohibitive. Yet, if the choice is to graft in the field, disappointing years will be encountered because the most important factor, climate, cannot be controlled. Although field grafting may be a slight deterrent to clone bank or seed orchard establishment, in our opinion it is not large enough to prevent the use of the clonal approach. As an alternative, controlled environment grafting in a greenhouse is successful. Years of grafting experience are not required. We have used a new man on the crew every year we have grafted, and with some practice a two-man crew can produce a greenhouse graft in 8.4 minutes. A three-man crew reduced the time to 5.4 minutes. With grafting-time rates such as these, the cost can hardly be prohibitive.

Tables 2 and 3 show our success in grafting black walnut. Other methods, including budding have been attempted but met with failure (Lowe and Beineke, 1969).

| Method | Number<br>attempted | Percent<br>success |
|--------|---------------------|--------------------|
| 1968   |                     |                    |
| Inlay  | 36                  | 83.3               |
| Cleft  | 25                  | 48.0               |
| 1969   |                     |                    |
| Inlay  | 98                  | 54.1               |
| Cleft  | 209                 | 53.1               |
| 1970   |                     |                    |
| Inlay  | 37                  | 33.0               |
| Cleft  | 192                 | 17.0               |
| 1971   |                     |                    |
| Inlay  | 57                  | 60.0               |
| Cleft  | 219                 | 34.0               |

Table 2.--Field grafting

| Table | 3. | Controlled | environment | grafting |
|-------|----|------------|-------------|----------|
|-------|----|------------|-------------|----------|

| Method                                   | Number<br>attempted | Percent<br>success    |  |
|--|---------------------|-----------------------|--|
| Growth chamber1969<br>Cleft              | 3                   | 100.0                 |  |
| Growth chamber1970<br>Cleft              | 32                  | 97.0                  |  |
| Greenhouse1972<br>Inlay<br>Cleft<br>Grav | 3<br>169<br>23      | 100.0<br>81.0<br>82.6 |  |

Combining the high success, particularly of controlled environment grafting, with low grafting time, we find it difficult to believe that grafting should deter the use of the clonal approach in black walnut.

<u>Flowering</u> - Flowering time is a most important consideration in selecting a breeding method. Including our cooperators, we know of only one walnut that has flowered at 4 years from seed. However, since the trend appears to be for quoting the time of first female flower production, we can safely say that walnut clones are capable of setting nuts the same year they are grafted.

From 82 clones with an age range of 1-4 years, and with only 4 clones at age 4, 20 percent of the clones have produced female flowers and 40 percent have produced male flowers. On the other hand, out of 136 individuals in a 10-year old plantation, 8 percent set nuts. From 850 individuals in a 5-year old plantation only 2 percent set nuts. Both plantations are located near the clone bank.

In 1972 a 125 tree half-sib seedling seed orchard in Northern Indiana at ages 9 and 10 yielded a total of only 9 trees with nuts. In 1971 only 1 out of the 125 had nuts, and the orchard had not flowered prior to 1971.

The Forest Service has turned over to the Indiana Division of Forestry a 3.5 acre half-sib seedling seed orchard which to our knowledge has not flowered in the 8-9 years of its existence. The Division of Forestry is now converting it into a clonal orchard.

We estimate that it will take seedlings 11-12 years to achieve the flowering percentage that grafts have achieved in 3.

<u>Seed Orchard/Progeny</u> Tests - One point in favor of the seed orchard/ progeny test argument is that in black walnut, both demand good sites. For the most part though, these orchards are not being placed on the best sites (Bey, Nickelson and Gerardo, 1971). Even if they were planted on the best sites, it still denies the breeder:

- 1. The possible use of irrigation and fertilization for flower stimulation.
- 2. The chance to test on various sites, in other geographic areas, or where certain disease or insect problems might occur.
- 3. Good genotypes that could possibly be discarded because of the need for early roguing.
- 4. Reliable progeny test results, because of the differential spacing after roguing, and the impossibility of avoiding competition unless initial spacing is very wide.

<u>Potpourri</u> - All predicted gains for the half-sib seedling approach, such as they are, are based on initial mass selection. The half-sib orchard approach proposed by the Forest Service leaves this out, and with it much gain.

We are convinced that inbreeding depression exists in walnut through our work with open-pollinated progeny tests. Also, we have learned through working with grafted material that black walnut is not dichogamous in all cases. With useful flower production in seedling seed orchards probably not available until about age 18; considering that the final roque does not occur until about age 26, (Table 4), in many families more than one individual will be present per plot. A number of years of seed production will occur before the final rogue. There will be no barriers to crossing between related individuals in the same plot; thus, the way is open for inbreeding depression. Bey, Hawker and Roth, (1971) discuss roguing in progeny tests, and suggest that it is possible that 40-50 percent of the trees in the better families, could be selected for second generation orchards. This not only invites inbreeding depression, but also a narrower genetic base. Little danger of inbreeding depression exists in clonal orchards since ramets will have identical genetic makeups and nondichogamous clones will be eliminated early.

In addition, what might be the problems associated with a seedling orchard that took 13-15 years to flower sufficiently, and then it was discovered that flowering times did not match, or that some of the trees were not dichogamous? The problems that might be caused from such situations include the lost time until useful seed production, combined with a decrease in genetic diversity, and another possibility for inbreeding depression. These problems could be reduced significantly with the clonal approach simply because of reduced flowering time, and clone bank utilization.

Table 4 shows that due to the length of flowering time for seedlings, the time it takes to produce seed in the second generation, which is an asset usually attributed to the seedling approach, is not greater than the clonal approach.

Establishing a large seed collection zone based strictly on climate (Bey, Mickelson and Gerardo, 1971), is not good procedure. In Indiana for example, industry has considered the North Central portion a preferred buying area because of its reputation for excellent wood quality. It would be unwise to dilute that population until more is known about the genetics of wood quality in black walnut. Again, a clone bank would be an asset in preserving this population.

In addition, if a seed collection zone was established for Southern Indiana, a large circle drawn on the map would not be appropriate. The genotype x environment interaction between the geographically diverse unglaciated uplands and good bottomland sites would be too great.

| Clonal<br>activity       | Year<br>activity<br>completed | Seedling<br>activity     | Year<br>activity<br>completed |
|--------------------------|-------------------------------|--------------------------|-------------------------------|
| Initial mass selection   | 2                             | Seed collection          | 1                             |
| Orchard and bank est.    | 4                             | Orchard establishment    | 2                             |
| Available f.s. test seed | 11                            | Initial roguing          | 6                             |
| 200 nut/tree production  | 15                            | Second roguing           | 15                            |
| Early roguing            | 18                            | Available o.p. test seed | 17                            |
| Final roguing            | 22                            | 200 nut/tree production  | 20                            |
| 200 nut/tree-second gen. | 34                            | Final roguing            | 28                            |
| Progeny test sale        | 46                            | 200 nut/tree-second gen. | 35                            |
|                          |                               | Original orchard sale    | 45                            |

Table 4.--Clonal and seedling improvement activity1

The year activity completed was derived from the author's experience from observing flowering on both seedling and grafts.

## CONCLUSIONS

The arguments used as justification for the half-sib approach which include selection, vegetative propagation, flowering, seed orchard/ progeny tests, and ease and cost of establishment are in our estimation without foundation. At the same time, it is our hope that we have shown that there not only is a need for the clonal approach but that it is not necessary to make sacrifices to satisfy that need.

# LITERATURE CITED

- Barber, J. C., and K. W. Dorman. 1964. Clonal or seedling seed orchards. Silvae Genet., 13:11-17.
- Beineke, W. F., and W. J. Lowe. 1969. A selection system for superior black walnut trees and other hardwoods. South Conf. Forest Tree Impr. Proc. 10:27-32.
- Bey, C. F. 1970. Geographic variation for seed and seedling characters in black walnut. USDA Forest Serv. Res. Note, NC-101. 4 p.
- Bey, C. F. 1971. Leaf flush in black walnut at several midwest locations. 19th N. E. Forest Tree Impr. Conf. Proc. In press.

- Bey, C. F., N. L. Hawker, and P. L. Roth. 1971. Variations in growth and form in young plantation black walnut trees. South. Conf. Forest Tree Impr. Proc. 11:120-127.
- Bey, C. F., A. S. Nickelson, and M. Gerardo. 1971. Black walnut seedling seed orchard development -- a case history. N. E. Area Nur. Conf. Cedar Rapids, Iowa. Sept. 14-16, p. 48-56.
- Bey, C. F. 1972. Genotypic variation in <u>Juglans</u> nigra---fifth-year field planting results. Unpublished report on file at Carbondale, Illinois.
- Funk, D. T. 1966. Seed Orchards. In Black Walnut Culture, N. Cent. Forest Exp. Sta. pp. 62-65.
- Funk, D. T. 1970. Genetics of black walnut <u>(Juglans nigra)</u>. USDA Forest Serv. Res. Pap., WO-10. 13 p., illus.
- Libby, W. J. 1964. Clonal selection, and an alternative seed orchard scheme. Silvae Genet., 13:32-40.
- Lowe, W. J. and W. B. Beineke. 1969. Comparing grafting techniques for black walnut. South. Conf. Forest Tree Impr. Proc. 10:231-235.
- N. C. State University Cooperative Tree Improvement and Hardwood Research Program. 1972. Genetic studies; Sixteenth annual report. p. 21-25.
- Namkoong, G., E. B. Snyder, and R. W. Stonecypher. 1966. Heritability and gain concepts for evaluating breeding systems such as seedling seed orchards. Silvae Genet., 15:76-84.
- Namkoong, G., R. C. Biesterfeldt, and J. C. Barber. 1971. Tree breeding and management decisions. J. of For., 69:138-142.
- Nikles, D. G. 1969. Breeding for high-yielding characters--growth and yield. Second World Consult. Forest Tree Breeding. FAO-FO-FTE-69-2/1.
- Schreiner, E. J. 1970. Tree breeding in U. S. forestry practice. Unasylva, 24(2-3):96-108.
- Schumann, D. R. 1971. Dimension yields from black walnut lumber. USDA Forest Serv. Res. Paper, FPL-162. 16 p.
- Shelbourne, C. J. A. 1969. Predicted genetic improvement from different breeding methods. Second World Consult. Forest Tree Breeding. FAO-F0-FrB-69-8/16.
- Squillace, A. E. 1970. Development and action programs for forest tree improvement. Unasylva, 24(2-3):63-69.
- Stern, K. and H. H. Hattemer. 1964. Problems involved in some models of selection in forest tree breeding. Silvae Genet., 13:27-32.
- Toda, R. 1964. A brief review and conclusions of the discussions on seed orchards. Silvae Genet., 13:1-4.
- Wright, J. W. 1964. Flowering age of clonal and seedling trees as a factor in choice of breeding system. Silvae Genet. 13:21-27.
- Zobel, B. J. and R. L. McElwee. 1964. Seed orchards for the production of genetically improved seed. Silvae Genet. 13(1-2):4-11.
- Zobel, B. J. 1966. Tree Improvement and economics: a neglected relationship. Proc. 6th World For. Congr.

# DISCUSSION

Funk - I'd like to make a comment and ask a question. First, regarding seed production in seedling plantations, I don't think there is any doubt that they will be slower than grafted trees, but the question is how much slower; and I don't believe we know the answer. I'll give one example that I've checked on recently. In an eight-year-old plantation, containing (nine-years-old from seed) about 250 trees, we selected 60 this summer to use in seed stimulation research and they had to have a minimum of 75 nuts to be selected as a test tree. It's on a good site, and it's the best early-seed production that I know of. I don't think that we're going to wait 15 to 18 years for seed production. The State'of Missouri is using a 15-year-old stand as a seed production area, collecting bushels of seed on what I would call a moderately good site. I agree with your figures, Chuck, for high heritability of flushing date, and I wish that you had given some for other traits. Calvin Bey and his co-workers have certainly found a high heritability for flushing, and I think it's a good place to make selections in the wild, in plantations, or anywhere else; and I think we could make gains with it pretty quick. I don't believe that you're quite accurate in stating that Bey reports relatively high narrow sense heritabilities for most major traits. If you consider growth rate to be a major trait, I don't accept the figures he gives as high narrow sense heritability; it's a matter of definition. The material that he's working with comes from a broad geographic area, and there's a big provenance component in it. If you want to put that in the numerator and consider it as part of the narrow sense heritability, then sure enough his figures get pretty big; but it doesn't fit my concept of the narrow sense heritability that is obtained from traditional mass selection. Working with any local seed collection zone, it would certainly be greatly diminished. Maybe you have some growth rate heritability data limited to the State of Indiana, but I haven't seen any.

Masters - First, concerning the 15 to 18 years from seed, Dave, I realize that we will get more seed production from certain sources than others; but the 15 to 18 year figure was based on enough seed for what we felt was a good open-pollinated progeny test. To get enough seed from all families, will require longer.

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Considering heritabilities, I accept what you had to say, for the most part. We found that our heritability estimates, mainly on growth, are similar to those Cal found. Now, if I remember correctly, most narrow-sense heritabilities I looked at were right between .4 and .5. One thing I will give you is, that in our open-pollinated progeny tests, we have progenies represented throughout the State of Indiana. This will tend to yield higher heritabilities, because of what we know about geographic variation in black walnut.

Our belief is that we can select for genetic differences by going through populations of black walnut, looking at them in the places where we can make comparisons, and comforted by what we know has been done on heritabilities in the past and from our own work. They seem to fit with what we feel is happening in these stands. We took the jump so to speak in heading in a clonal direction. Granted, we don't know all the answers, but it appears we have made a good choice. <u>Gabriel</u> - Are you using the term dichogamy in the classical sense of Sprengel or in the more broader sense of A. B. Stout? In the former, it is the relationship of time of blooming of the two sexes in hermaphroditic flowers; and in the latter, it is the relation of blooming on individual trees as well as hermaphroditic flowers. I am puzzled as to how you can have a non-dichogamous condition that would not favor selfing.

Masters - We're looking at dichogamy in terms of flowering times.

<u>Gabriel</u> - In the blooming time on individual trees or in hermaphroditic flowers?

Masters - Of individual trees.

<u>Gabriel</u> - So you're using A. B. Stout's broader designation?

<u>Masters</u> - I don't know where the term came from, but here is the way I'm using it. The pollen, if first, will be shed then female flower production begins and vice versa.

<u>Gabriel</u> - Protandry?

<u>Masters</u> - Yes.

<u>Gabriel</u> - If you have no dichogamy, neither protandry or protogyriy occurring, then there would be little difference in time of blooming and there would be the chance of male flowers pollinating females, on the same tree.

<u>Masters</u> - If you have no dichogamy this is true, but we feel that you don't find this very often in black walnut. We haven't had a chance to look into selfing in black walnut except briefly in our openpollinated progeny tests. Yet considering that it is a cross-pollinated species, probably highly heterozygous; and given what we know about its dichogamous condition, you can accept that it's possible for natural selection. to be working in favor of the heterozygote. Therefore, it's feasible for me to believe that inbreeding depression can result in those individuals that are selfed.

<u>Gabriel</u> - Do you have a dioecious condition in black walnut?

Masters - It's monoecious. Both flowers are on the same tree.

- <u>Gabriel</u> You don't have trees that have all male flowers or all female flowers on them?
- Masters No, both flowers are on the same tree.
- <u>Gabriel</u> Then there is a possibility of selfing to occur?

<u>Farmer</u> - It's been pointed out by a number of people that at this stage of hardwood breeding the important task is, perhaps, not channelizing our effort into one breeding approach, but establishing broad breeding populations. This could include an open-pollinated progeny test that would include many more individuals than you could include in your clonal breeding orchard, i.e. both clonal and seedling seed orchards. Both would be included in the same program. This would give broad breeding populations that could be used for a variety of breeding approaches later on. Assuming that we don't know whether our mass selection in the field is effective or not, for safety's sake perhaps we need to include up to 100 to 200 selections in a clonal orchard. What are you aiming at in your program?

<u>Masters</u> - Our main objective, working from the University, would not be to strive for quantities of improved seed. We're trying to gain all the knowledge that we can in order to hasten the Division of Forestry in Indiana toward their goal. We have 82 clones in the clone bank, and are striving for four to five ramets per clone. We generally add 10-15 new selections each year. Some of the earlier selections are not satisfactory and they will be reduced to one or two ramets per clone.

In the earlier years we worked on open-pollinated progeny tests in order to gain as much information as we could for that phase of the game, but now we are getting flowering in the clone bank. At first, we'll be utilizing what we feel are the better selections, testing them, and trying to get sound genetic knowledge on those first. Eventually, I'm sure, we'll want to see what potential all of them have.

I agree completely with what you had to say, Bob. We are not striving for any specific number for the clone bank. We feel it is important to keep our clone bank and the Divisions orchards as genetically broad as resources will allow.

Dorn - I'd like to make a comment concerning the selection of black

walnut. Number one; in Indiana I think it may be a little easier to find stands containing good phenotypes than in many other parts of the country, so you can be a little more critical in making selections. Number two; even though the Forest Service doesn't use comparison trees and/or set minimum standards like you do, it isn't a completely random choice. If there is a group of trees to choose from, we're going to take the best tree there, assuming it has nuts on it. You mentioned that black walnut is a species that has been high graded over the years. For that very reason we feel that we have more confidence in making selections from the second generation than we do in the trees that are standing in the forest because very likely at one time they were the poorest ones. That is why they are still there. That is one of the reasons we are not taking a grafted approach. We could be grafting what at one time were the very poorest trees; which are the survivors of many years of high grading.

<u>Masters</u> - In response, let me say that initially, we are interested in preserving what we feel might be the best of the population; before they get away from us and they get away fast; and there are obviously good individuals around. It's my opinion that the only way you are going to make gains at least equal to the clonal approach, and this is without roguing, is if narrow-sense heritabilities are extremely low. You don't have this in walnut. We need gains and we need them now.

Finally, I would like to point out that we don't select old growth individuals. A good selection system would weed these types out. We are selecting only advanced generation recombinants.

<u>Hicks</u> - I agree in principle with a lot that Chuck said, but as Don mentioned, we aren't blessed with the situation in eastern Texas that you are for comparison trees. Basically, we find walnut trees growing on old house sites. You may find one tree every ten miles as you drive down the road, and I'm sure you've seen this, too. If you collect seed from these and grow half-sib families, you'll find tremendous differences among the families, and within families, fairly consistant growth. You can call it seedling seed orchard, or whatever, but it boils down to family selection. You're not going to omit a good tree, and I don't think we are going to lose the good genotypes using family selection. Therefore, where necessary, I think a program should be geared to family selection as well as single-tree selection.

Dorn - I have a question for Don Forbes. I have noted a rather puzzling observation while looking at black cherry trees during their flowering season. I've never found a large number of bees or insects of any kind present, in contrast to other flowering trees like tulip poplar, or black locust. I've noted two or three trees in particular because they happened to be small, and have low branches that can be easily seen. On two different years, both with good seed crops on those trees, I watched these trees while in flower. I didn't see a single honeybee visiting the blossoms, and very few insects of any kind. I wondered if you have observed the same thing?

Forbes - I've seen the same situation occur, and this is what started my wondering about the selfing thing, especially in a cold weather situation. I did some control pollinations and there were no insects there, and yet there was a set. It worried me just like it puzzled you, and then I got to looking into the situation and bagged some to make sure. And I got into this situation that you saw the results of on the slides. They did self, and this brings up a question. Is this what you get exclusively in some situations?--and it's really worth considering although when you say that you haven't seen many insects, I have seen many when the temperature is right. I've pollinated with bees all over the place and flies, too; there are quite a few flies that get into the trees. But honeybees are all over the trees in Florida and in Tennessee There was one season that I was doing the work when it was cold and there were no honeybees. There was still a fair set, but it wasn't like the set that you would get if you had the type of weather that I was talking about, say about 80 F., but they were there. I didn't see any insects either, so I know that some selfing has to occur in certain trees. In Florida I bagged up into the thousands and I had only two or three flower sets -that's all. When I got up in Tennessee working in the trees, these things popped out at me. With this cold weather and no insects, I got to worrying so I bagged up some and there were cherries all over the place. I admit that I haven't been able to carry any through to maturity, but it's a ticklish situation to keep these things. Maybe they all fall off like I said. I haven't seen any go all the way through.

<u>Schreiner</u> - I have six black cherry trees on my house lot here in Durham; their seed production seems to resemble the production of your Florida trees. In the last six years they have had only two heavy seed crops; in the other years we have had wet, damp weather and they produced practically no seed. This may indicate the lack of even a medium seed set to selfing in these trees.

<u>Forbes</u> - You can imagine my surprise when it happened to me in bagging. I bagged trees in Florida to make sure there was no selfing, and then when I got up to Tennessee working with those trees and things popped out on me with this cold weather and no insects. And then I bagged up some and there were cherries all over the place, but I admit that I haven't been able to carry any through.

<u>Schreiner</u> - I have watched my trees closely; the flowers drop off before there is any indication of fruit set.

<u>Forbes</u> - On these the fruit actually set, then they dropped. This last season they were infested. I just picked up one after the other and cut it open and there was larvae in it.

<u>Gabriel</u> - You may have a case of agamocarpy in which you have fruit but no seed. You could also have something like post-zygotic abortion which could take place very soon after fertilization. But the fruit continues to develop.

<u>Forbes</u> - You saw this embryo that I showed you a picture of. Those were cherries that were almost as large as they would be at maturity, they just weren't ripe.

<u>Schmitt</u> - It occurred to me on listening to you talk if you were getting effective insect pollination, you may not be getting high percentage of cross pollination. I think it's unreasonable to assume that a bee would zip in on one raceme of one tree and take off for another tree.

Forbes - No, it's not reasonable. This has been studied and the bees are fairly localized. They tend to find a good thing and stay with it. I didn't do this work, but I have read where it has been done. I don't recall the citation, but they were suppose to be very local performers. When you find a cold situation where the insects have not been there and you have some set, you don't get nearly the total volume you would if the weather had been just right. The crop is much reduced, so we know the insects are doing some good. I wouldn't know how much of this if any is selfing. Like I've said the only ones I have worked with have dropped off for one reason or another.

Dorn - Then your conclusion, or rather your recommendation, for not using emasculation is not because of the impossibility of selfing, but because of the technical difficulty of emasculation? <u>Forbes</u> - Right. Some work has been done by just taking off the petals to reduce the attraction of the insects, but I did this and left the anthers on and got some set. So I know that the little fellows are not attracted just by the petals. And this was in the Florida situation, so I'm pretty sure that they weren't selfs. If you're worried about insect pollination, I recommend working in the greenhouse and then you know you're going to have selfing. But other than that, this bagging is something, but emasculation is worse.

<u>Schreiner</u> - I will back you up on that last statement; in 1925 I was trying to hybridize flowering cherries at the New York Botanical Garden. I do have one question on the walnut; this question also came up last year. Were you aiming at improved seed varieties or improved clonal varieties for commercial use? Are you planning to select some of your best clones and put them out as grafted, clonal varieties?

Masters - You mean plant grafted stock?

<u>Schreiner</u> - That's right because you will get some individual clones that will be better than any of the seedlings that you're going to produce from the same ortet.

Masters - That's a good question.

<u>Schreiner</u> - The point I'm trying to make is that you can get an improved clonal variety much sooner than an improved seed variety.

<u>Masters</u> - We have definitely considered this, of course, but we are not quite sure where this is going to lead, because there are still a lot of problems to be worked out with respect to handling grafted stock until they get established. I think once we do this, and I definitely think that it's coming, there's a possibility for it.

<u>Schreiner</u> - From your results I get the impression that suitable grafting methods will become available. I pointed out last year that when I was with the TVA we had a propagator who could get 90 percent survival--an old-time pecan grafter. It seems that we don't have any more nut-tree grafters left.

<u>Masters</u> - We do now. All we have to do once the graft is out, is to make sure it gets established before we let it go. If we succeed here, there won't be any problem.

<u>Forbes</u> - Are you thinking about rooting? Nobody's said anything about rooting, and this would be the answer to your question.

<u>Schreiner</u> - That would be an answer, but I think grafting with a plant like walnut would be better. What are you getting now, about six hundred dollars for a thousand board feet of walnut logs?

<u>Masters</u> - The highest price I heard last year for good quality, large diameter black walnut was six thousand dollars a thousand.

<u>Schreiner</u> - For that you could afford to pay at least two dollars per grafted ramet if you need to.

Forbes - Why not pay fifty cents for rooting?

Schreiner - Do you know anyone who has successfully rooted walnut?

Farmer - Our group at TVA's Norris Laboratory has rooted juvenile walnut as has Loy Shreve at Kansas State University. See recent issues of the <u>Plant Propagator</u> for details. The next step will be to extend these techniques to physiological mature or rejuvenated material. We are now getting 50-60 percent rooting on the average and as high as 80-100 percent with some material. Loy Shreve has reported even better average rooting percentages.

<u>Schreiner</u> - If I could root walnut that well, I most certainly would not use grafts.