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<u>Abstract</u>--\_The first year's growth and yield of space planted seedlings of bermudagrass, <u>Cynodon</u> spp., and bahiagrass, <u>Paspalum</u> <u>notatum</u>, was positively correlated (r=+. 34 to +.43) with later average yields when seedlings establishment and management was kept as uniform as possible. Being able to propagate superior genotypes of bermudagrass vegetatively has greatly facilitated the improvement of this species. Recurrent restricted phenotypic selection has been 4 times as efficient as mass selection in improving forage yields of Pensacola bahiagrass.

<u>Additional keywords:</u> Bermudagrass, <u>Cynodon</u> spp., bahiagrass, <u>Paspalum notatum</u>, correlation coefficients.

Increasing growth and yield is one of the major objectives of the forage crop breeder. Although the ultimate objective is increased yield of meat or milk from the animals consuming the forage, it is usually realized by increasing the growth rate and yield of the forage consumed. Thus the forage breeder is always concerned with increasing yield. To do this, he must be able to predict and measure yield controlled by growth rate.

Since April 30, 1936, we have been trying to increase the yield of perennial cross pollinated grasses. The two grasses with which we have worked more than others are bermudagrass, <u>Cynodon</u> spp. and bahiagrass, <u>Paspalum notatum</u>. Commercial vegetative propagation of superior bermudagrass hybrids such as Coastal has greatly facilitated the genetic improvement of this grass. Although bahiagrass can be propagated vegetatively, its much slower establishment rate from sprigs has made seed propagation its only commercial method of increase to date. Our experience improving yields of these two grasses may be helpful to the breeders of trees and it is to this end that this paper is written.

## BREEDING BETTER BERMUDAGRASSES (1)

Many years ago, H. K. Hays, with years of plant breeding experience said, "Use the best parents you can find. They usually have the best offspring". Our experience confirms this statement. But how do you find them?

Coastal bermudagrass is the best of 5000 F hybrids between two excellent parents (2). J. L. Stephens saw a superior bermudagrass plant in a cotton patch on the Coastal Plain Experiment Station, increased it, compared it with a common bermuda check and named it Tift bermudagrass. An unknown person in South Africa used his eyes to select the other parent for Coastal. Both parents were grown in single plots along with other bermudagrasses in a uniform environment in our introduction garden. By observing them throughout the season and comparing their entire season's growth in the fall, we were able to detect growth rate differences that would have been difficult to detect on a short-time basis. We could also note resistance to diseases, absence of seed heads and other characteristics desired in the new variety.

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The plant breeder hybridizes to combine desired characters and to obtain hybrid vigor. We knew nothing about the parents for Coastal bermudagrass except that they possessed the characteristics desired and appeared to yield as well or better than any other bermudagrass in our nursery. Variation we had observed in common bermudagrass suggested that these parents were heterozygous. We could have spent several years testing all of our potential parents. We believed, however, that we would be able to propagate bermudagrass vegetatively and that we needed only to create and isolate a superior plant. It seemed more efficient; therefore, to start our breeding program by studying a large population of hybrids from these parents. If the parents were heterozygous, as assumed, each hybrid would carry a different set of gametes and one or more might possess the hybrid vigor necessary to increase yield. Because the parents came from different parts of the world and were probably unrelated, we expected them to have a good chance of producing the high yielding F 1 hybrid desired.

The hybrid seeds produced in the summer of 1937 were planted at uniform rates in flats of soil in the greenhouse in December and were transplanted to 2-inch clay pots in February. In April, 1938, they were set in the field on 5-foot centers in a sandy soil without fertilizer and were kept free of weeds throughout the season. A visual yield rating was made June 13, 1938, and height and diameter measurements, heading dates, and disease resistance ratings were made throughout the summer. In the fall, using the plant appearance at that time as much as our copious notes, we selected 128 plants (the range of types and the larger plants) and started three 4-inch pots of each in the greenhouse. Total dry matter produced by these potted plants cut at 3-week intervals during the winter failed to correlate with the total seasonal production of these selections in replicated field plots in 1940. These potted plants (one in the center of each 4 x 24 foot plot), were used to establish this clipping test with 3 replications in 1939. Weeds were controlled and most plots were completely sodded over by the fall of 1939.

A visual yield rating made June 13, 1938 less than 3 months after the 2" potted seedlings were set in the field correlated with the total 1940 yield (r= +.34, P=.05), suggesting that superior yield could be recognized early in some plants. However, several years of measuring yield in two replicated tests and other criteria were required to prove that Coastal was superior to many other bermudagrass hybrids produced **in** 1937.

As we have continued our bermudagrass breeding work releasing Suwannee, Midland, Coastcross-1, and Tifton 44, we have concluded that:

- 1. Coastal bermudagrass is an excellent parent transmitting many of its superior traits to its offspring.
- Superior bermudagrass parent plants are usually heterozygous, giving highly variable offspring when crossed with Coastal or any other parent. Usually, several hundred F hybrids of any cross must be screened to give one plant significantly more productive than Coastal.
- 3. By keeping everything as uniform as possible from the time the seeds are planted in the greenhouse until they are spaced on 9 x 9 foot centers in a uniform deep sand soil, we believe it is possible to select the highest yielding 5% of the plants visually at the end of the first season.

4. Yield data collected from a replicated clipping test conducted for 3 years will usually separate the best from the better clones and will indicate how it compares with its parents and other check varieties. Occasionally outstanding hybrids can be detected at the end of the first year in a replicated clipping test.

## BREEDING PENSACOLA BAHIAGRASS

Pensacola bahiagrass is a stoloniferous perennial that can spread 12 to 18 inches per year. It is a sexual diploid that is presently propagated by seed. Most plants are highly self-incompatable and may, therefore, be considered F hybrids. The variability between individual plants is substantial. We are presently using two plant breeding procedures to increase the forage yield of this grass. They are recurrent restricted phenotypic selection, (a more efficient form of mass selection) and the production of commercial F1 hybrids. Seed for such hybrids will be produced by harvesting all seed from isolated fields vegetatively planted to alternate strips of two self-sterile cross-fertile clones that give high yielding hybrids. A brief description of each follows.

Recurrent restricted phenotypic selection (RRPS) is based on early research that proved that yields of spaced plants cut at the end of one season were positively correlated with 5-year yields of the same plants when compared in a vegetatively planted replicated clipping test conducted for 5 years (r=+.43 and +.39) (3). RRPS begun in 1960 initially involved spaced plant populations of at least 1000 plants evaluated with green plant yields taken in two consecutive seasons. Using the total forage yield for two years, we selected the five top yielding plants in each 25 spaced plant (5 x5) block. Two culms with roots attached ready to flower the next day were taken from each selection, were placed in buckets of water and were grouped together in a space less than 1 meter in diameter in the laboratory by a north window. Each morning thereafter as they flowered, they were agitated to insure complete intermating between the 200 selections. When mature, seeds from these culms were harvested and used to start plants in the greenhouse for the next spaced-plant nursery. The 25-plant grid selection technique helped to reduce soil heterogeneity effects and the polycross procedure allowed selection on both the male and the female side and thus doubled the progress expected from mass selection that takes open pollinated seed from the field for the next cycle.

As we advanced through successive cycles of RRPS, we observed that most of the plants collected after yields or yield ratings were taken in the second year were the same that would have been selected at the end of the first year. We observed further, that, if great care was exercised to keep everything uniform, we could have selected visually in July, most of the plants that yielded more when cut in October. It appeared possible, therefore, to double our efficiency again by getting one cycle per year instead of one cycle every two or more years.

For the last two years, we have successfully completed one cycle per year as follows:

1. The soil in the field for space planting is fumigated in late March with methyl bromide to control weeds. Bahiagrass seedlings grow better after this treatment than following the use of other herbicides.

- Following a spring growth rating that reflects winter injury, we study all date including actual yields taken on the previous year's spaced plant nursery and discard the seedling progenies from the poorest one-sixth of the maternal plants.
- 3. The remaining 166 progeny are given accession numbers ranging from 1 to 166. Each of six plants from each selection is placed in a separate one-half pound kraft bag numbered with its accession number. These are then placed in six different garbage cans so that one plant of each accession occurs in each can.
- 4. In mid April as these potted plants are set in the field (4) (each garbage can making a separate replication), their accession number is recorded in a graph-paper planting plan. Such a planting and such records allow an analysis of the performance of the 6-plant progeny of each selection and permits one to trace the pedigree of any seedling.
- 5. By mid-July when all the better plants are producing flowering culms, the five better plants in each 5 x5 grid are checked on a graph-paper field plan.
- 6. The following morning, we collect two culms ready to flower from each selection, tag them with a row and plant number and group them together in the laboratory polycross previously described.
- 7. Each morning a tent made from paper suspended over the polycross is agitated to create a thorough mixing of the pollen produced. As soon as anthesis is complete, the tent is removed to give the culms access to the indirect light from large north windows.
- When mature, the seeds from each selection are harvested, threshed, and blown to separate empty florets and chaff from florets containing caryopses.
- 9. In early-December, we plant in the greenhouse, 125 caryopses from each selection in 18-inch rows spaced 2 inches apart in flats of steam sterilized soil.
- 10. In mid-January, we transplant the seven most vigorous plants from each row into 2" clay pots of steam sterilized soil. These are set in sand on a greenhouse bench where they grow into large vigorous plants by mid-April.
- 11. All records including winter injury and spring growth are then studied and the poorest one-sixth of the progenies are discarded.
- 12. By mid-April the roots of each plant have formed a mat inside the 2-inch pot that holds the soil intact as the plants are pulled from the pots and put into the one-half pound kraft bags for field planting.

The original seed lots and seed from the space planting of each cycle of improvement have been kept in cold storage to permit a measure of progress achieved. Studies of replicated space plant plots from each cycle indicate that RRPS has produced higher yielding single plants as well as populations with higher mean yields. Through cycle 6 there has been a straight line increase in population yield of 2.5%/year in seeded plots and 8.7%/year in spaced plantings.

Our Pensacola bahiagrass breeding program directed toward the development of commercial F  $_{_1}$  hybrids assumes the following:

- 2. Following a spring growth rating that reflects winter injury, we study all date including actual yields taken on the previous year's spaced plant nursery and discard the seedling progenies from the poorest one-sixth of the maternal plants.
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Our Pensacola bahiagrass breeding program directed toward the development of commercial F  $_{\rm l}$  hybrids assumes the following:

- 1. Self-sterile cross-fertile clones can be found that will give high yielding  $F_1$ hybrids. We have clones that qualify and produce  $F_1$  hybrids that have yielded 30% more than the check (commercial Pensacola Bahia) in replicated clipped plots over a 3-year test period.
- 2. It will be possible to establish commercial seed production fields with alternate strips of such a pair of clones planted vegetatively. We have established small pilot fields, setting clonal material with vegetable planters. We believe we can greatly reduce the hand labor required for such plantings by using the Bermuda King bermudagrass sprig digger to harvest sprigs and the Bermuda King fairway planter to plant them. More research to confirm preliminary tests is required.
- 3. Herbicides can be used to keep seeds falling to the ground from developing plants until the sprigs are sodded over. Seeds that fall to the ground in well established bahiagrass sod rarely produce plants.
- 4. Once established such seed fields should produce hybrid seed of the perennial Pensacola bahiagrass for many years by merely combining all seed produced in the field. Seed harvested from small pilot fields confirm this conclusion.

Breeding in this commercial hybrid program consists of finding excellent clones that seed well and give the highest yielding F hybrids when crossed. Because all clones are single crosses, 2-clone single <sup>1</sup>crosses are in effect double crosses. Single crosses can be easily produced by placing together in 3 x 14 inch glassing bags culms from two parents that will flower at the same time, putting them in water in the laboratory and shaking them daily until anthesis is complete. Following this procedure we produced seed for a partial diallel involving eight superior clones selected from RRPS cycle 4.

In December, these seeds were planted in flats of soil and 60 seedlings from each cross were transplanted to 2-inch clay pots as in the RRPS breeding procedure. In mid-April 4 x 14 foot plots replicated five times were established by planting in methyl bromide treated soil, 12 of these potted seedlings one foot apart in the center of each plot. By the end of the first year, these plants had spread to make a solid sod strip at least 18 inches wide. By July of the second year, the width of the sod exceeded the two foot wide strip cut for forage yield determinations. Forage yields were usually taken 3 times in the first year and 4 times in succeeding years. Average annual yields of the 29 single crosses in this test ranged from 9,547 to 15, 930 pounds/acre of dry matter. The top yielding hybrid produced 31% more dry matter than the check.

Four of the clones included in a replicated plot test and cut four times per year gave average annual yields ranging from 14,882 to 17,987 lbs/A. The mean yields of the single crosses involving these four clones correlated well with the clone yields (r=+.89).

To date we have obtained three-year dry matter yields from replicated plots of 41 selected Pensacola bahiagrass clones. These have given average annual yields ranging from 11,888 to 17,987 lb/A of dry matter. Seed yields for these clones has ranged from 391 to 1188 lb/A, indicating that the choice of clones for commercial hybrid seed production can greatly influence the cost of the hybrid seed produced.

These 41 clones have been test crossed with two of our best clones and the crosses have been compared in replicated yield trials (5 replications) for 2 years. Correlation coefficients between these 2-year yields and the 3-year yields of the 41 clones were r=+. 365 and +.374 P=.01, respectively. We had hoped that they would be higher.

In this hybrid program, the clones must be evaluated for seed yield and other traits. The positive correlations though small, indicate that the higher yielding clones will tend to give higher yielding two-clone hybrids. However, the small size of the correlation coefficients indicate that two-clone hybrids must be yield tested to find the best one.

In conclusion, we would like to suggest that breeding cross-pollinated trees may not differ greatly from breeding cross-pollinated grasses. More time per generation (from seed to seed) will be required for most, if not all tree species and probably more years for evaluation will be needed. I believe the genetic principles will be essentially the same.

Just as there has been a great advantage in being able to propagate a superior genotype of bermudagrass vegetatively, there should be a similar, perhaps even greater, advantage for the vegetative propagation of forest trees. Breeders of fruit and ornamental trees have demonstrated the need for and the value of vegetative propagation with their crops. Most of the offspring from crosses between two superior heterozygous clones of bermudagrass or bahiagrass have yielded less than their parents. I would expect most of the offspring of two superior heterozygous clones of a forest tree species to have a slower growth rate than their parents. After 20 to 30 years, the difference in the yield of wood between the superior vegetatively propagated genotype and the seedlings from two superior clones could make vegetative propagation profitable. Certainly the breeder of superior forest tree varieties will be able to make greater progress in less time at lower cost if practical methods of vegetative propagation can be found. We have found significant differences in the ease with which bahiagrass genotypes can be propagated vegetatively. I would expect a similar relationship in forest tree genotypes.

Regardless of the manner in which the end product of forest tree breeding is used, the breeder and the industry will profit from research designed to reduce the time required to grow one generation. When we started breeding pearl millet, we grew one generation a year. When we added greenhouses we could grow two. Using short days, high temperatures, and seed treatments to break dormancy, we can now grow four generations per year. For some breeding objectives, particularly introducing resistance genes to protect a variety from a disease, learning to grow four generations a year was a very significant plant breeding advance.

Our experience with grasses suggests that the relative growth rate of a tree can be estimated early in its life cycle. The problem will be the development of a uniform screening system that will subject every genotype in a population to the same, or very similar, environmental pressure. The need to maximize uniformity throughout the screening procedure is apparent. The gain in efficiency that could most certainly result from such early screening for growth rate will certainly warrant a substantial investment in research designed to develop such a screen. Finally I would like to suggest that every fascet of your current forest tree breeding program can be made more efficient. For many year, we, me and my entire staff, have been reminding ourselves that "We haven't found the best way to do anything yet". This repeated reminder has significantly improved the efficiency of our plant breeding research and I believe it can improve yours.

## LITERATURE CITED

- Burton, Glenn W. 1947. Breeding bermudagrass for the southeastern states. Jour. of Amer. Soc. of Agron. 39:551-569.
- Burton, Glenn W. 1954. Coastal bermudagrass. Ga. Coastal Plain Exp. Sta. Bull. NS 2. Sept.
- Burton, Glenn W. 1974. Recurrent restricted phenotypic selection increases forage yields of Pensacola bahiagrass. Crop Sci. 14:831-835.
- Burton, Glenn W. 1976. Precise back-saving transplanter for small potted plants. Crop Sci. 16(5):732-734.