21. ROOTING AND GRAFTING OF SLASH PINE 1/

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Asexual propagation is an important phase in a forest tree improvement program. In the forest genetics program with slash and longleaf pines at Lake City, Florida, this method has been used to multiply and perpetuate desirable germ plasm, estimate the genotype of selected trees, induce early flowering, and establish seed orchards for superior forest tree seed. In this report, I hope to give you a thumbnail sketch of some of the problems we were confronted with, report on the results of our experiments, and describe workable rooting and grafting methods which have been field tested.

At first I would like to talk about vegetative propagation by rooting.

Type of Cutting

Our findings with slash pine cuttings on the effect of age of tree on rooting are in agreement with those of other species. More than 90 percent of slash pine cuttings from very young trees root but cuttings collected from older trees root poorly and with much difficulty. The dividing line between extensive rooting and poor rooting lies between 2-year-old trees and 3-year-old trees.

Season of Collection

The first studies with slash pine cuttings were conducted in outdoor propagation beds. Since 1943, about all of the experiments were carried out in the greenhouse during the fall and winter months for lack of environmental control during the summer in both greenhouse and field. Effect of season of collection on the ability to survive in the propagation beds was quite varied. In one experiment cuttings were collected during the period from August till December. At the beginning of the dormant stage survival was low but increased steadily until the middle of November when a sharp drop occurred. Cuttings collected in the early part of February started growth shortly after they were planted in the warm propagation beds. From this it appears that late October and early November is the best time to collect the slash pine cuttings.

1/ The work described in this report was carried out at the Lake City, Florida, Research Center of the Southeastern Forest Experiment Station, while the author was in charge of its forest tree improvement program.

One difficulty with these experiments is the fact that season and environment in the greenhouse are confounded. This makes it difficult to isolate either factor.

Treatment

Cuttings have been prepared in a number of ways, but no method of physical treatment was superior to that of planting the cuttings right after removal from the tree. Etiolating the shoots prior to their removal from the tree, along with wounding the base, gave poor results. With some species, etiolation is beneficial. When strips of brown paper were wrapped around the upper part of the shoot, the overall effect was depressing. It lowered the starch content within the cuttings and the tight paper wrap provided poor aeration around the needles.

Good response was recorded when the basal parts of the cuttings were wounded before they were treated with chemicals. This resulted in a well-developed root system on some of the cuttings.

Storing the cuttings for a few weeks in a pre-callusing box prior to chemical treatment is beneficial with some plants but it was found undesirable for slash pine. On most of the cuttings which were stored in sphagnum moss at 82 ^{o} F. for a two-week period callus had started to form at the base. Callus growth, however, did not cover the entire bases and the fungi destroyed the parenchymatous tissue around the base and subsequent survival of these cuttings was poor.

Several of the most promising growth regulators were used in conjunction with vitamins and sucrose. Up till now over 300 different treatments have been tested. Various methods of application were tested, including treatment under vacuum. Past results with cuttings from mature slash pine trees have not definitely established the beneficial effect of chemical treatments. None of the treatments or treatment combinations gave consistently better results; many times results in the following years contradicted conclusions of the earlier studies. Cuttings have rooted under more than 50 types of treatments. These conditions ranged from no treatment to the most complex chemical treatment with growth regulators, vitamins, nutrients, sugar, and fungicide. However, chemical treatment of slash pine cuttings from very young plants was beneficial. Supplementing the nutritional level, in the cuttings, by spraying the foliage with nutrients and sucrose, had a depressing effect. This treatment favored a large-number of fungi which destroyed the meristematic tissue of the cuttings.

Throughout the experiments with cuttings, the detrimental effects of microorganisms was evident. Applications of ferbam, streptomycin, santobrite, or Phygon XL to the propagation beds helped to check fungal activities.

Slash pine cuttings require a humid environment during the rooting period. Best results were obtained when cuttings were sprayed at eight to 10-minute intervals during the daylight hours. Neither placing the cuttings with their bases in water, nor the use of a closed propagation frame was suitable for rooting. When the bases were submerged, they rooted as a result of poor aeration. Closed propagation frames in which the said medium was kept moist at all times and where the foliage was sprayed daily with water were a complete failure. The needles dried out within a few weeks after collection. When cuttings are rooted in open propagation beds and sprayed intermittently, direct insolation had a detrimental effect on survival, but good results were obtained in shaded beds.

Addition of vermiculite to a sand rooting medium increased the average level of rooting. Of 15 rooting media tested, a 50-50 mixture of sand and vermiculite proved to be best. Apparently good aeration along with adequate moisture-holding capacity are necessary characteristics of a good rooting medium. The medium was maintained at a temperature between 80 to 85 F.

Air-Layering

One of the important factors which contribute to the failure of root development in slash pine cuttings is the shock which the cuttings sustain when they are removed from the tree. A large amount of reserve carbohydrates and other nutrients are needed to form the callus tissue and to develop the roots. Once a cutting is severed from the tree, no further nutrients can be translocated from the stem to the cutting. Then the cutting has to rely on the nutrients stored before it was severed. In air-layering on the other hand, roots are induced to form on the branches while the are still a part of the parent plant. Although no previous attempt to air-layer pine trees in America was reported it was felt that this method might bring about the production of rooted material from the refractory species which are difficult to propagate by cuttings.

In our experiment where sphagnum moss was used as rooting material and a polyethylene film as wrapping material the effects of time of application and effect of indolebutyric acid were studied. To study the effects of indolebutyric acid treatment, two branches growing from the same whorl were used. The environmental conditions for the two groups were equivalent except for the factor specifically under test.

The results on adventitious root induction are presented in this table where the data have been assembled by time of year and by chemical treatment. The time span for the first root formation is also given. The average number of roots developed for all attempts, including the uprooted branches, was 5.58 for the treated branches whereas only 1.46 were produced by the untreated air-layers. The difference between the number of branches which rooted and number of roots produced per rooted branch indicated a beneficial effect of chemical treatment which was significant at the one percent level. Average rooting percentage was 84.6 for the treated branches whereas only 50.0 percent of the untreated branches rooted. The same chemical treatment was lethal when it was used on slash pine cuttings. The branches which were air-layered during October produced the first roots after 23 weeks. These branches became semi-dormant soon after girdling and root initiation probably took place at the start of the next growing season.

From our experience with slash pine we found that the best time to apply the air-layers is during May, June, and July. Sturdy, vigorous, straight shoots in the upper part of the crown should be selected. Before making the girdle, the needles are stripped for a distance of about eight-inches. The best place to prepare the girdle is some 10-inches from the tip of the branch, preferably in the current year's growth. On very vigorous shoots of trees below 10-years of age a one-inch girdle should be used. On older trees a 1/2-inch girdle will be satisfactory. Be sure that all the bark and cambium is removed. The best results were obtained when a 1.2 percent mixture of indolebutyric acid in talcum powder was used as a rooting powder. The upper rim of the girdle should be treated with a liberal dose of the powder. We found that the indolebutyric acid treatment stimulated the formation of well-balanced root systems.

As a rooting medium, moist sphagnum moss was used. When the sphagnum moss was collected in a dry state we soaked it in water for several hours before using. Excess water was squeezed out. After a handfull of moist sphagnum moss is packed around the treated girdle, the air-layer is covered with a plastic sheet, 9 by 10 inches in size. Commercially available air-layering sheets gave very satisfactory results. When successful, the roots will form within six to eight weeks, but the marcotte should be left on the tree for at least 12 weeks so that the roots become firmer and develop side branches. The white root tips will become visible through the plastic wrapping. After the roots are well formed, the branch is clipped off about four to five inches below the girdle, and is potted in loose soil and kept shaded during the initial period. The new plant needs frequent watering. Also, fertilizing with a soluble fertilizer will stimulate active root growth. Potted plants can be outplanted into the field after roots are well developed.

Besides being able to take advantage of beneficial effects of chemicals, air-layers can be obtained without the use of extensive physical installations. Also, air-layered branches in general have a better balanced root system than cuttings.

Grafting

Another method to propagate plants asexually is by grafting. various types and modifications of standard horticultural grafting methods have been tried with pines. It appears that special techniques have to be developed for the various species occurring in different climatic regions. A study was devised with dormant slash pine scions to determine the limiting factors. Three types of grafts--cleft graft, veneer graft, and bottle grafts--were kept under four environmental conditions. Greenhouse-open bench, greenhouse sweatbox, field-partial overhead shade, and field-partial overhead shade covered by individual humidity chambers.

Best overall results were obtained with bottle grafts (Figure 1).

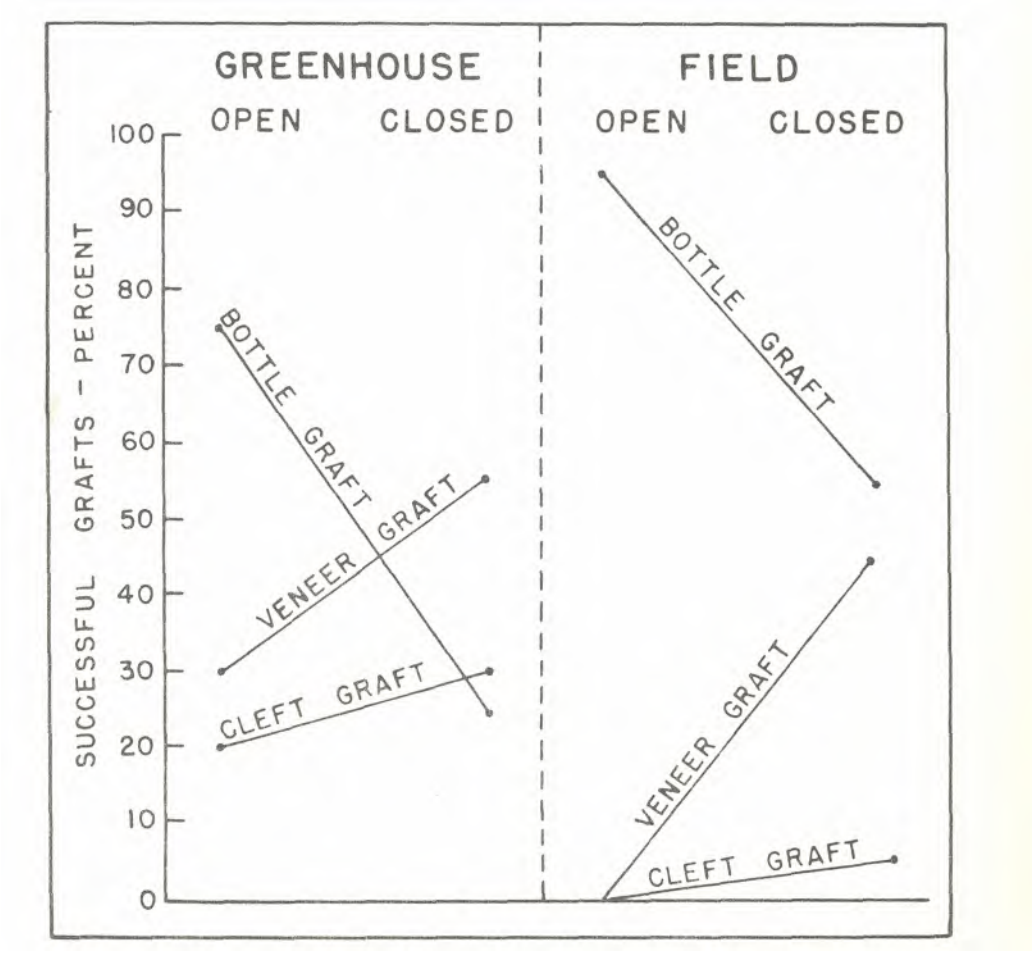

Figure 1.--Relative success of three different grafting methods in four different environments.

Placing both veneer and cleft grafts under sweat-box conditions increased their successful unions, while bottle grafts kept under these conditions had a smaller number of successful unions than those kept in the open. The average temperature in the sweat-boxes in the greenhouse and in the field was always considerably higher than under open conditions. This higher temperature was not depressing enough to completely offset the beneficial effect of the high humidity for the cleft and veneer grafts, but it had a significantly depressing action on the bottle grafts which were not dependent upon high humidities. Under open field conditions where the relative humidity was comparatively low, the only successful unions were obtained with bottle grafts. Veneer and cleft grafts failed completely and most scions had dried out and died after 10 days.

However, when the veneer and cleft grafts were placed in an atmosphere with a high relative humidity, successful grafts were obtained. This would indicate that transpiration was reduced to the point where it did not desiccate the tissue and allowed the scion and stock to form a union.

Pines in general do not develop root pressures under normal conditions, and this fact appears to be one of the main reasons for the low number of successful cleft graft unions in the dormant stage. The only successful cleft grafts were obtained with plants where either a small living limb was below the cut surface or where an epicormic branch was formed on the remaining stub shortly after grafting. Evidently some green foliage is required to act as a pump in supplying water and nutrients from the roots of the stock to the scion.

The bottle-graft method under open field conditions has been successfully used to establish seed orchards for superior forest tree seed.

Grafting during the succulent stage of the growing season presented few problems if the scions were covered by a polyethylene film and kept in full shade during the :initial knitting period. A large percentage of successful unions was obtained when stock and scion were in a succulent condition. We found the method suitable for grafting directly on stock growing in the field, and on potted plants in a lath-house or greenhouse. The best time to graft with succulent material is during the later part of April, May, June, or the early part of July. Grafting can be started as soon as the leader has added several inches of new growth, even though not all the needles of the new growth have ruptured the sheath.

As in any type of grafting, the outcome greatly depends upon the condition of the stock. The stock plants should be healthy and in a vigorous growing condition. When potted plants are used, best results will be obtained if they have been in the container for at least six months prior to grafting.

A cleft graft method for succulent grafts is best. After the union is tied and sealed with grafting wax, the graft is covered with a plastic bag. Polyethylene plastic bags of various sizes can be purchased in hardware stores. They are sold as packaging material for deep-freezers. For field grafting the scion is shaded by placing a Kraft paper bag over the plastic bag. Holes should be cut for ventilation in the side of the paper bag which faces north.

Heteroplastic micrografts were made to induce early flowering. One- to four-month-old seedlings were grafted successfully on slash pine, ponderosa pine, pitch pine, eastern white pine, Douglas-fir, white spruce, and Norway spruce. Grafts outside the pine family on Chamaecyparis survived for eight months. No flowers showed after one year. A high anthocyanin content in the needles of heteroplastic grafts was pronounced. Anthocyanin forms in slash pine seedlings during the winter months when they were grown at a low level of nitrogen; also high starch concentrations cause an early and strong anthocyanin formation. The rusty color of some of the scions probably indicated a high C/N ratio in the foliage. If this was the case, the nutritional factors for abundant flowering were favorable, but the factor or factors which tip the balance to transform vegetative growth to flower formation were not present.

Cross sections of graft unions during the formation stages were examined to find out whether past failures in grafting slash pine scions failed because of inherent incompatibilities or because of faulty grafting techniques. The study did not reveal any incompstibilities and showed that parenchymatous cells of medullary rays, phloem, cortex, and cambium, participated in bridging the space between stock and scion tissues. The stock contributed the greatest part of the wound tissue, but the scion took part in callus formation. A continuous bridge between respective anatomical parts of the graft partners was apparent after six weeks.

To sum up the experiments on rooting one can say that advances have been made in this field but a great deal of additional research needs to be done. For grafting, I feel that the methods as tested will be adequate to carry a slash pine tree improvement program through to the grafted seed orchard stage.

(This paper was illustrated by kodachrome slides in addition to Figure 1 above.)