Biochemical Chances Associated With Stock Storage

By Frank J. Baron

Every section of the country seems to have something to brag about. For a long time California foresters have bragged about having the toughest problems in reforestation. Now we think we have something more constructive to discuss.

The concept of complete site preparation has given a shot in the arm to reforestation in California. Planting has increased and the increase has emphasized the need for more and better nursery stock. As a consequence research is asking "What is good nursery stock?" "Can planting stock be stored safely until the planting areas are ready?"

These questions are vital to the whole concept of artificial regeneration of forests. We still don't have all the answers. However, cooperative studies by E. C. Stone of the University of California and G. H. Schubert of the Pacific Southwest Forest and Range Experiment Station in Berkeley over the past half dozen years show why conflicting results have attended grading and storing of nursery stock. The November 1959 issue of the Journal of Forestry contains an article by Stone and Schubert which summarizes results of many investigations.

Most of the early studies were concerned with physical factors temperature, humidity, and packing material--the "outside" factors. Recently the "inside" factors, that is, physiological conditions within the plants, have become apparent. Often these are so strong they overshadow completely the effects of purely physical factors. These "inside" factors are, of course, not visible. In other words, you may be planting essentially "dead" trees although they look healthy. Although most of the work of Stone and Schubert has been with ponderosa pine, indications are that the results are applicable to other species.

It isn't possible to discuss storage of planting stock without considering the concept of physiological grading of nursery stock. The December 1959 issue of Forest Science contains an article by. Stone and Schubert which shows seasonal variability in root production. This means that certain times of the year are conducive to planting failure even with fresh-lifted stock which is not stored.

How have we determined seedling vigor? Much earlier research used survival after field outplanting as the check. This, of course, is the ultimate criterion, but it is slow and subject to many unknown and uncontrolled variations. Stone and Schubert contend that survival is directly related to root development, and they check the performance of seedlings and transplants in greenhouse tests. Their studies show that the ability to regenerate roots promptly after planting is the key to success. Root-regeneration can be either can be either extensions of existing roots or actually new roots.

One series of tests used fall-lifted stock (1-1 ponderosa pine from 4 different seed zones in the Sierras) grown at the Forest Service's Mount Shasta nursery. Beginning in mid-September, trees were lifted twice a month through December 1. These were then subjected to 4 storage periods (0, 1, 2, 3 months) before planting. The rate of root development was shown to be strongly affected by soil temperature. Therefore the roots were grown in soil maintained at 20° C. The survival and root development were rated after 1 month.

Results indicate the importance of time of lifting. Survival increased as lifting was delayed until November 1. After that survival was essentially unaffected by lifting date until December 1. Each month of storage reduced survival at any lifting date. There was also a variation among the seed zones. The lateral root development parallels the survival pattern, but indicates even more strongly than mere survival the effects of lifting date. In other words, until November 1, the

trees had pot reached a state of "physiological hardening" to prepare them for either immediate planting or the rigors of cold storage.

The next year, California State Division of Forestry nurseries at Ben Lomond, near Santa Cruz, and Parlin Fork, near Fort Bragg, were included in the study. These results were summarized by Schubert in a file report September 17, 1959. Again, these tests involved fall lifting only, -with (2-0) ponderosa pine. Only 2 storage periods were used (3 and 6 months).

Results ,with Mount Shasta stock compared favorably- with the previous year's test. That is, November 1 was the break-off date. Before then survival and root growth was unsatisfactory after either 3 or 6 months of cold storage. Results with the coast nurseries were not conclusive. At both state nurseries there seemed to be a continuing rise in survival and root production even after December 1. The seed zone differences were not consistent in these tests. Differences between nurseries were much greater than between zones at each nursery.

Last year two more nurseries were included in the study; the new Forest Service, nursery at Placerville, and the State nursery at Magalia. Test plots were hand-sown at these 5 locations with seed from 4 different zones. From October to December, and from March to May, sample seedlings. (1-0) were lifted at 2--week intervals. At Ben Lomond lifting continued throughout the winter.

Results have not yet been completely analyzed. However, preliminary checks indicate 'results similar to those for older stock. Survival and root production of trees lifted before November 1 fell much below that of trees lifted later in the season. The longer the storage period, the later should :the stock be lifted. Moreover, the cutoff date for spring lifting appears determinable from this study. There seems to be considerable variation among nurseries in this regard.

Climatic differences between nurseries are being investigated by installing thermographs at each nursery. It seems probable that any date in one year will not compare exactly with the same date in another year, when considering the physiology of the seedlings. By accumulating this information over several years, we hope to be able to predict at what time lifting will result in the most satisfactory storage.

Other sources of "inside" information include .the results of biochemical analyses of plant material. Henry Hellmers of the Pacific Southwest Forest- and Range Experiment Station has been using the laboratory facilities at California Institute of Technology in Pasadena to this end. His work has been confined to one-year-old Jeffrey pine seedlings, stored either at the Mt. Shasta nursery or in the laboratory cold rooms. Three aspects were tested: water, enzymes, and starch. Four parts of the plants were analyzed: roots, stems, needles, and buds..

Four types of storage techniques were involved:

1. Left in the ground (control)
2. In shipping bales (regular. pack)
3. In pliofilm bags (bag pack)
4. In tubs of water (tub pack)

The analyses were made at four different times:

1. When freshly lifted
2. Stored three months
3. Stored six months
4. At the planting site

Results indicated great variability with respect to water content. However, the differences within treatments were as great as between them. More correlation was noted with respect to enzymatic activity. Intensity of staining with tetrazoleum salts was used to detect the presence of dehydrogenases which are enzymes necessary for life. Activity of these enzymes was highest in freshly lifted trees, even when removed from under snow. The roots lost their enzymes after six months storage, but the buds retained some activity.

The most significant results were obtained from starch analyses. Iodine was used to stain and indicate the presence of this reserve food. Freshly lifted trees had the greatest amounts, with the root cortex in highest concentrations. The rays of root and stem were next highest. In the regular pack and tub pack no starch remained in the stems after 6 months cold storage, and only traces remained in the roots. In the bag pack there was significantly more in both roots and stems, but still much less than in the freshly lifted stock.

Future problems in Hellmers' line of investigation are to determine (1) how starch is used, and (2) if starch is depleted in storage, the possibilities of replenishing it before planting.

You can see from the various studies described here the way **in** which basic research can assist in everyday problems. Even if the *answers* don't arrive immediately, we are better able to ask the right questions. We intend to continue investigations along the lines suggested by the earlier studies of Stone and Schubert, but amplified by the additional information that is becoming available.

Description of Slides Shown b Frank Baron

"The idea is to show the relationship between survival and root production in time of lifting and length of storage. The darkest bars represent the trees that were stored for three months **in** decreasing sequence, 2, 1, and 0 months of storage, the dates at which the trees were lifted from the nursery **on** the bottom. You can see that

without any storage, there wasn't too much difference in the survival as predicated by the time of lifting for the nursery; but as you increase the length of storage, the survival fell off particularly in the first two lifts. It wasn't until after the middle of October that you reached what would be considered an acceptable level of survival. It more or less leveled off after that time. So if you're just lumping fall lifting together and didn't specify the date of lifting, you could get any combination of those results of length of storage or time of lifting. This, of course, is just one seed zonee in the state. You're probably familiar with the seed zone delineation in California. This again is the survival, the percentage of trees that were alive after only one month at approximately. 20 C in these greenhouse water baths. The next slide shows the same idea, but in terms of the root production of the seedlings.. Now you see that even for those early lifts without any storage the amount of root production was very low and you just couldn't consider that those trees would do very much if they wouldn't produce roots. They might be alive for a short time but not for very long. Then again, as you get past mid-October and into November the survival jumps up considerably. Even here the storage wasn't as good as we'd like to have it over a three-month storage period. These again are 1-1 trees, ponderosa pine from seed zone 3. Now the next slide will show you some of the results that we had in the next year. I have some mimeographed material I should have passed out before, I guess.

"We have three nurseries now. The two preceding charts covered merely the Mt. Shasta area. Now in the study reported here the number of nurseries was increased by including two of the division of California nurseries, at Parlin Fork and Ben Lomond. Both these nurseries are along the coast, incidentally, and have considerably different growing cycles than the Mt. Shasta Nursery.' I also included five seed zones, one more than we had in the other study. The four charts that are of interest are Tables 1, 2, 3 and 4 in this blue line report. Two periods of storage were involved, 3 months and 6 months, and the criteria are as listed in that blue line report. You'll notice that the Mt. Shasta Nursery results are quite **similar to those** we have for the 1-1. The number of roots and the survival and the number of seedlings with elongated roots increased considerably after November, 1. In looking at page 4, Table 1, Mt. Shasta Nursery you'll see that after November '1 the survival was 96 percent or better and the number of roots were about 2,000 plus or minus a few. Now, looking at the Ben Lomond Nursery you're see that the number of seedlings alive continues to increase all the way from October 1 to December 1. The same thing held for the Parlin Fork Nursery, so we actually didn't get the complete picture on what was happening there. It seems as though the later you lifted, the better the survival. On Table 2 you have the same thing for six-months storage, and essentially the picture remains the same except that even for the Mt. Shasta Nursery the later you lift it, right up to December 1, the better would be your survival and the greater would be the amount of roots you produce. So, it seems to be tied up very strongly with the date of lifting and the time of storage. You

will also find on the front page of this blue line report a summary of the publications that has been the result of these particular studies.

"What I wanted to show you on the next series of slides was the type of seedlings that we're dealing with. These are 2-0. We've got considerable variability between the nurseries, but we didn't get much consistency in the responses of the seedlings from different seed zones. There was no consistent difference. This happens to be the seedlings representing seedlings of five different seed zones grown at the Parlin Fork Nursery. Just for information, you can see zones 1, 2, 3, 4 and 5 starting at the left. There isn't too much difference in the size of the seedlings and there wasn't too much difference in the survival or the root production. I've gone through the past, and now we're on the current phase of our study. In addition to having five seed zones, of course we wanted to have five seed zones but one of the seed zones got lost in shipping so we wound up with four, we have five nurseries now. We now have the nursery at Placerville as well as Carl's Mt. Shasta Nursery and three of the state nurseries.

"Also, on top of that we have two age classes. We have run the 1-0 seedlings this year, and we expect to run the 2-0's beginning with the fall of this year. These are the 1-0's from the Mt. Shasta Nursery with the seed zones 1, 2, 4 and 5. Here you can see - there seems to be a size difference between 1 and 5 on one side, on the small side, and 2 and 4 on the large side. That seems to be rather consistent, and you could see it even looking at the beds themselves. The next slide is, I believe, from the Galien Nursery. Again you see the 2 and 4 are larger than the 1 and 5. But we're just now analyzing the data on these studies. We haven't been able to detect any consistent difference in their survival or root production, but there does seem to be some size difference.. Zones 2 and 4, incidentally, are the lower elevations on the west slope of the Sierras. Zone 1 is the east side type. The northeast part of California and Zone 5 is a higher elevation on the west slope south of Lake Tahoe. There's an increase in size differential as you come south. Mt. Shasta is the most northerly, while Galia is next, and then Placerville and the two coast nurseries. I just wanted to show you the consistent differences in size at different nurseries. You might put on the next slide. This is, I believe, Placerville. Again, you see they're getting up there pretty good size. These are just one growing season. They were planted in April, I believe, and harvested last fall, about November.

(Colored slides were shown. They are hard to interpret without seeing them.)

"1 also have a report from Henry Hellmers who has done some preliminary work with the study of biochemical changes in the nursery

stock during storage. It's very preliminary, but its also extremely interesting. He started in 1957-58 with a study on Jeffrey pine, just Used zone 4, and I believe it was 1-0 seedlings. These were grown at the Mt. Shasta Nursery. He stored them. under various conditions and analyzed for three main factors, water, content, enzymatic activity, and the food reserves in the plant tissue itself. He analyzed the plants by-individuaI components, needles, stems, cross sections, roots, and buds. He also analyzed the stock when stored in the ground, .or left in the beds which were the con trol. He also put them in the standard bales which he called regular pack. .He put them in tubs of water which he called a tub pack and plastic bags , called the bag pack. So he had these four methods; and he tested these four components of the seedlings , needles, stems, roots and buds at four different times, when t hey were lifted, after three months of storage, just prior to shipping, then actually at the planting site itself . He stored them,. not only at Mt. Shasta, but also at the Cal-Tech facilities .at Pasadena, and- the results were rather consistent. .As for wafter Content, he found he got no correlation at all with the survival of the. trees which he had outplanted on the Sequoia National Forest. The variability within treatment was much greater than any variability between them, and he couldn't get any correlation at all on just water content of the tissue itself. He got a little bit better :results with enzyme studies. He used tetrazolium chloride which was a dye that essentially measuresthe reducing power of the tissue. Suppostedly, it's closely relatedto an enzyme system called dehydrogenization. It's like a basal metabolism test on seedlings. With this type of study we .found that we got the. highest activity right the freshly. dug stock. This applied. even if you had to dig the trees out of the nursery from under a blanket of *show*. They had a very, very high enzymatic activity as measured by tetrazolium chloride. He got lesser and lesser amounts as they were stored. He found also that the roots became completely depleted of any enzymatic activity over this six-month period, but the buds themselves never completely lost all activity. There was always some left in the bud. The third category of tests was on the reserve foods. He limited these primarily to the study of starch in the tissue which he detected by iodine stains. This is a rough measure of how much available energy *is* present in the trees. In other words, what kind of working capital is present at any particular time? Well, again, he found that he had the highest starch content in the freshly dug seedlings. He found also that the cortex of the root had the greatest amount of starch, although there was also some in the rays of both the roots and the stem. This declined as the trees were stored over increasingly longer periods of time. When we measured them in the spring, either as they were taken immediately from storage or at the time they were planted a *few* weeks later at the planting site, the results were as follows:

> For a regular pack they found that the starch was present. only in traces, and this was only in the roots. There was none that he could detect in the stem. In the tub pack there was slightly more; there was a little bit

more in the roots but still it was not in the stem. The trees that were stored in these pliofilm bags were considerably better than the other two methods of storage. There was some starch present both in the roots and in the stems, but it didn't compare at all with the freshly dug in that there was much more in the seedlings when they were dug fresh. There was more in the roots than there was in the stems. All this was extremely interesting to him. I haven't had a chance to talk to him recently about what he intends to do, but he posed two questions he wanted to investigate further: "Is the starch actually used up during the storage period?" Where he just measured the starch itself and it disappeared, he didn't know whether perhaps the starch was converted to some other form, such as sugar. So he hopes to continue studies to find out if there are other forms of energy to which this starch is converted. However, if the starch is used up and the reserves become depleted, he is interested in answering another question: "Could this loss be replenished somehow before the trees are planted to build up so•called working capital on the seedlings to withstand the shock of transplanting?" Another thing that he found out was the interesting work in these water relations--the fact that water content itself isn't a complete picture. Since he didn't get very good results with his water content studies, he noticed that other studies have shown that the turgidity aspects of water were more important than just the water content itself. In other words, how much water pressure was present? Was it a percentage of water with respect to dry matter? What sort of turgid pressure was it exerting? Well, that's about the size of it right now.

"You see various lines of activity going on with both the basic research and just studying plant tissues as such. The other aspect of it **is**, "How are these results applicable to problems that we're facing today?" I'm inclined to think that both methods are useful and can't say that you should study either one to the exclusion of the other. Its opening up a lot of new lines of thinking, and I'm certainly glad I'm connected with the project. Thank you."