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Title: PHYSIOLOGICAL AND ANATOMICAL STUDIES OF SEVERAL GROUPS OF DOUGLAS-FIR (PSEUDOTSUGA MENZIESII (MIRB.) FRANCO) SEEDLINGS WHICH DEMONSTRATED DIFFERENTIAL SURVIVAL POTENTIAL UNDER DROUGHT STRESS

Abstract approved: Signature redacted for privacy.
Denis P. Lavender

Several hundred two year old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were planted in an enclosure designed to permit control of soil moisture. The seedlings came from five seed sources in southern Oregon and one seed source in northwestern Oregon. Mortality, terminal and lateral budburst, and soil moisture were recorded during the growing season.

The final death count showed that the mortality of the northwest Oregon seed source (84%) differed significantly (at the 1% level) from that of four southern Oregon seed sources (ca 44%) and that the mortality of all differed significantly from that of a fifth southern Oregon source (30%).

The purpose of this study was to determine what anatomical,

morphological, and physiological characteristics were associated with survival. Root length, calcium-potassium ratio, cuticle thickness, shoot-root ratio, and root surface area are not correlated with mortality.

A relation between mortality and the amount and rate of budburst was found. The low bud activity and high mortality of the northwest Oregon seedlings may be an expression of their physiological condition. Earlier bud activity by the southern Oregon seedlings may be a response to a lower minimum temperature, and is a survival advantage in areas with severe summer drought.

Physiological and Anatomical Studies of Several Groups of Douglas-Fir (Pseudotsuga Menziesii (Mirb.) Franco) Seedlings Which Demonstrated Differential Survival Potential Under Drought Stress

by

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INTRODUCTION

Douglas-fir is one of the most valuable timber species of the Pacific Northwest. However, regenerating certain areas which previously supported productive Douglas-fir stands has proven difficult, especially on southerly and westerly slopes. Survival of planted Douglas-fir seedlings is low after such slopes are clearcut. Pharis (1966) points out that summers are hot and dry, and rapid occupation of the site by competing vegetation results in severe competition for soil moisture. He concludes that summer drought is a major factor in reducing the regeneration of this species on southerly aspects.

Douglas-fir occupies an extensive geographical area and various selection processes have operated to develop physiological types of the species. In 1937, Schreiner summed up previous studies on this subject by stating: "The investigations in connection with the problem of seed origin have shown that in many, if not all, important forest species there are rather distinct races and strains that differ in their hereditary response to a given complex of environmental conditions." Studies on photoperiodic response (Irgens-Moller, 1957), transpiration (Zavitkovski, 1964), respiration and photosynthesis (Krueger and Ferrell, 1965), and drought resistance (Ferrell and Woodard, 1966) indicate that ecotypes do exist in Douglas-fir. Isaac (1949) explains that the failure to recognize the existence of ecotypes with different adaptability to various sites has been one of the chief causes of failure in the history of artificial reforestation. Hence, survival of seedlings on dry areas will depend largely on selection of the ecotype adapted to the site. Ferrell and Woodard (1966) point out that differences in drought resistance may be found in Douglas-fir seedlings taken from north or south slopes even on a local level. This was due to a transpirational difference, giving the seedlings of the southern aspect a survival advantage. Knowledge of such survival advantages would be of great importance when planting dry local areas.

A method of recognizing drought-hardy parent trees and seedlings would be helpful in increasing the survival of Douglas-fir seedlings planted on dry, southerly slopes. This is especially a prerequisite to any tree-breeding program designed to increase survival in such areas. This investigation was conducted to determine if Douglas-fir seed from a dry area in southern Oregon produced seedlings of greater survival potential under drought stress than seed from a moist area in northwestern Oregon. Certain anatomical, physiological, and morphological characteristics were then studied to determine any direct correlation with survival potential. If characteristics could be found that are correlated with survival under drought stress, a considerable amount of time and money would be saved which would otherwise be consumed in lengthy tests of survival ability.

LITERATURE REVIEW

The study of plant survival under conditions of moisture stress has been more intensive in agricultural crops than in forest trees. However, this review will deal mainly with research conducted on coniferous species, especially Douglas-fir.

Adaptations used by plants to survive periods of moisture stress are numerous and quite varied. A given plant may have one main adaptation or it may have an interaction of many factors. Drought resistance is usually broken into two parts (Levitt, 1963; Ferrell and Woodard, 1966). One part, called "drought avoidance", deals with characteristics of the plant which enable it to prevent or postpone dehydration of its tissues. The second part involves the ability of the plant to survive dehydration of its tissues and is termed "drought tolerance". Trees that can survive drought stress include both divisions, since they have protoplasm with some ability to endure drought, combined with morphological and anatomical characteristics which postpone the formation of critical water deficits (Kramer and Kozlowski, 1960). The first section of this review will deal with drought avoidance.

Rooting characteristics play an important role in the establishment and survival of a species that must endure a seasonal dry period (Oppenheimer, 1951). Those plants which develop the most extensively branched and the most deeply penetrating root systems are best able to obtain the moisture that is required. Isaac (1949) reports an informal study of Douglas-fir seedlings grown from seed collected in both the dry east and the moist west ends of the Columbia Gorge. The seedlings were grown in fine river sand in a garden at Bridal Veil, Oregon. Greater survival was demonstrated by the seedlings of the dry site following subjection to midsummer drought. Survival was attributed to a smaller crown and a deeper, better developed root system.

Ching (1959) developed a hybrid of *Pseudotsuga menziesii* and *Pseudotsuga macrocarpa* to combine the timber qualities of the former with the drought survival qualities of the latter. He later found that the cross has a higher survival under drought stress and a more highly developed root system.[1]

Pharis (1966) discusses the poor survival of planted Douglas-fir seedlings on the south slopes of the mixed conifer zone in southwestern Oregon. He suggests that the deeper, more extensive root systems of ponderosa pine (*Pinus ponderosa* Laws.), incense-cedar (*Libocedrus decurrens* Torr.), and sugar pine (*Pinus lambertiana* Dougl.) should cause them to be more desirable for planting than

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[1] Ching, Kim K., Forest Geneticist. Personal discussion. Forest Research Lab., Oregon State University, Corvallis, Oregon. June 28, 1967.

the less drought resistant Douglas-fir.

Two Japanese researchers found that one of the most important mechanisms determining drought resistance of the conifers in their studies was root development (Satoo, 1956; Tazaki, 1960).

Ferrell and Woodard (1966) found no correlation between drought avoidance and weight of roots for Douglas-fir. However, the experimental procedures may very well have both minimized any inherent differences in root development and reduced any survival advantage accruing to seedlings with more extensive roots. With one exception, their seedlings were seven months or less in age. They were grown in pint plastic pots, and exposed to various drying conditions in the greenhouse and growth chambers. In one study, they found some correlation between the number of active root tips and drought avoidance.

The relation between root development and the size of the shoot, or absorbing surface to transpiring surface, is also important in survival. Seedlings having high ratios of shoot to root are undesirable for planting, especially on severe sites (Wilde, 1958). However, Hermann (1964) points out that if Douglas-fir seedlings have a well-developed root system, a high shoot-root ratio is not always detrimental. This conclusion was reached by checking the survival of graded seedlings. In general, however, a low shoot-root ratio has been considered to be particularly important to the survival of transplanted tree seedlings. One of the most common causes of death is an excessive water loss by transpiration before an effective absorbing surface is developed.

Zavitkovski (1964) found a significant difference in the shoot-root ratio between two Douglas-fir ecotypes. Two and three month old seedlings grown in pint plastic pots were used. Although no correlation of the ratio to survival can be determined by the study, he discusses the ecological implications of a lower shoot-root ratio. The seed source with the lower ratio also had a lower transpirational rate; these factors combined would give higher survival on dry sites.

The ability of a planted seedling to quickly send new roots into the soil mass may influence its survival. Stone (1963) points out the importance of a high "root regeneration potential" on the survival of transplanted ponderosa pine seedlings. The physiological condition of the seedlings will affect their subsequent response to transplanting; "root regeneration potential" could be used as a measure of this condition. The ability to put out new roots may also be a reflection of nursery conditions and practices. Stone (1962) further explains that Douglas-fir displays a marked seasonal periodicity in its root-regenerating potential. A seasonal variation was also found by Lavender (1964) who suggested lifting Douglas-fir seedlings from December 1 up to the time buds begin to enlarge in the spring.

The amount of water lost through the cuticle covering the epidermis is quite variable. The rate or amount of epidermal transpiration depends principally on the thickness of the cuticle which varies among species and between leaves of the same species (Kramer and Kozlowski, 1960). Satoo (1956) determined that one of the most important factors involved in his study of drought survival was the magnitude of cuticular transpiration. Kaul (1965) states that the greater drought resistance of Ilex cornuta over Rhododendron poukhanensis is apparently due to its more efficient stomatal control of transpiration and higher resistance to cuticular transpiration.

A thick layer of cutin which retards cuticular transpiration combined with stomata which close promptly when leaf moisture begins to decrease can greatly reduce water loss and prolong survival. The effectiveness of this kind of control of water loss is shown in the differences in rate of drying of detached needles. Moisture retention by the needles of Douglas-fir has been compared with various conifers by Parker (1951). He found that when the needles were left attached to cut branches and allowed to dry, Douglas-fir, western white pine (Pinus monticola Dougl.), arborvitae (Thuja plicata D. Don), grand fir (Abies grandis Lindl.), and Englemann spruce (Picea engelmanni (Parry) Engelm.) retained less needle moisture than did ponderosa pine. When the needles were removed from the branches and allowed to dry, Douglas-fir and ponderosa pine retained a higher moisture content than the remaining species. Parker (1954) found that older needles of ponderosa pine lost water more rapidly than the younger needles, possibly because of a more permeable cuticle or reduced activity of the guard cells. A reversal of this process, by absorption of water from fog, has been found in ponderosa pine and associated species (Stone, 1957) and may be a mechanism of survival.

Pharis (1966) determined that foliage moisture content may be used as an index of soil moisture stress. He transplanted two-year old seedlings of ponderosa pine, sugar pine, Douglas-fir, grand fir, and incense-cedar into pots and subjected them to drought stress. The whole plant lethal foliage moisture content was determined for foliage of various ages. The lethal level was found to differ with the age of the foliage. In a second experiment Pharis potted the previously mentioned species in pairs (two different species per pot) and subjected them to drought in September. Ponderosa pine, incense-cedar, and Douglas-fir were found to be the most drought resistant, while sugar pine was the least, and grand fir was intermediate. This was based on soil moisture content at death and also on the ability of one species to outlive another under competitive conditions.

Pharis and Ferrell (1966) have determined that when using "time to death" and "soil-moisture content at the death point" as drought hardiness tests, Douglas-fir from three coastal sources were less drought resistant than five inland sources. Lethal needle moisture contents agree with this point. With one exception, plants under well-watered conditions could be grouped as coastal or inland on the basis of their needle moisture.

Loss of water through the cuticle is minor in comparison to the large amount lost by stomatal transpiration (Kramer and Kozlowski, 1960). Transpirational differences in Douglas-fir can be enough to give one ecotype an advantage over another in survival on dry sites (Zavitkovski, 1964). In this latter study, two-month old seedlings from a dry area in Washington and a moist area in Oregon were compared. The seedlings were grown in pint plastic pots which were sealed on the bottom and top with paraffin. Transpirational loss was determined by weighing the pots. The Washington seedlings transpired less than the Oregon seedlings at every soil moisture content. As previously mentioned, Ferrell and Woodard (1966) found a transpirational difference between Douglas-fir seedlings from a south slope and seedlings from a nearby north slope.

The timing of growth may be a means of drought avoidance in nature. It has been suggested that early growth initiation and onset of dormancy in Douglas-fir seedlings of the Corvallis, Oregon area may provide a means of avoiding early summer droughts

(Irgens-Moller, 1960; Ferrell and Woodard, 1966). This was suggested because these seedlings were found to be no more drought resistant than seedlings from areas of higher precipitation, yet they came from an area having early summer drought.

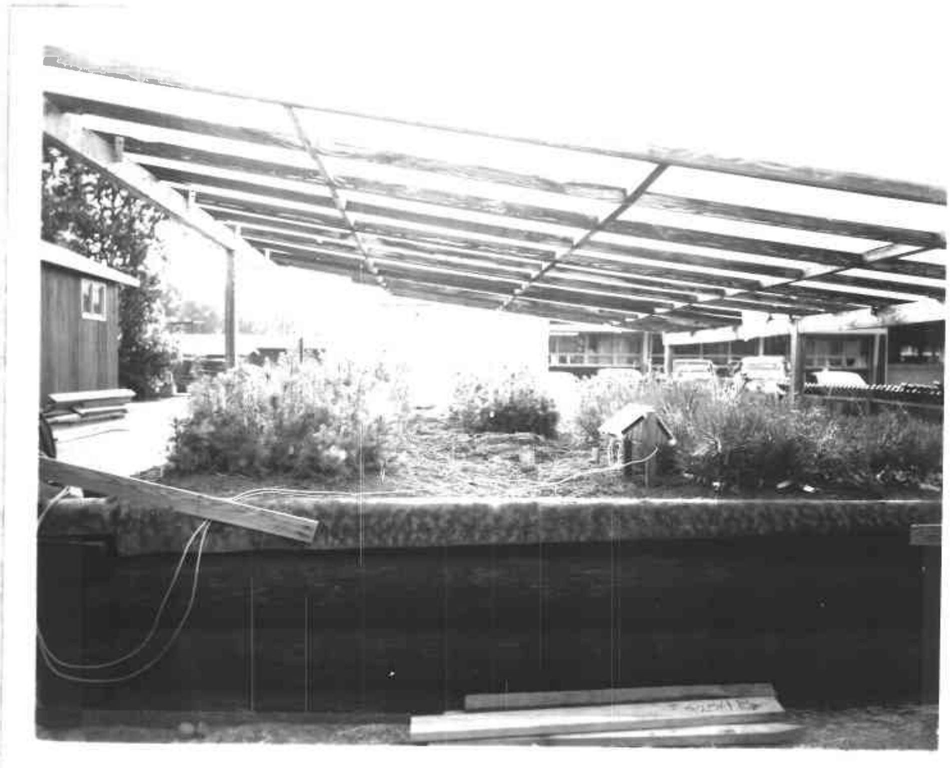
A plant may be able to postpone the day when critical water tensions arrive, but many authors point out that in the final analysis, the ability of the protoplasm to endure dehydration is the more important factor. There are many reviews of literature pertaining to protoplasmic drought resistance in plants (Levitt, 1956; Iljin, 1957; Vaadia, Raney, and Hagan, 1961; Henckel, 1964; Gates, 1964), with some disagreement among the authors on what factors are most important. An extensive amount of research has been conducted on agricultural crops, but little has been done in this area on coniferous trees.

By minimizing differences in transpiration, Ferrell and Woodard (1966) were able to determine differences in drought hardiness (tolerance) between interior and maritime sources of Douglas-fir. In their study, when the soil had reached the wilting point of sunflowers, the soil surface of the potted seedlings was covered with a light sand mulch. By this means evaporation was slowed but not halted. Since transpiration made up only 10% of the water loss, transpirational differences among seedlings were minimized. Therefore, in this technique, differences in survival were primarily a result of drought hardiness, although a small amount of drought avoidance was still present. They determined that the interior seedlings demonstrated more drought-hardiness and drought-avoidance characteristics than the maritime sources.

Kral (1965) found a correlation between the calcium-potassium ratio and the ability of various Douglas-fir provenances to withstand drought. The seedlings with the lowest ratio demonstrated a lower resistance to drought. This idea had been proposed earlier, but Levitt (1956) discredited it because it did not apply to frost hardiness and therefore should not apply to drought hardiness. He reasoned that since twigs could be removed from trees and dehardened in the laboratory without total loss or gain of elements, drought hardiness might be lost in the same way. In addition he mentions other German workers who found no correlation.

A review of the reasons for survival under drought stress demonstrates a general agreement with Parker (1956), who states that there may be contributing factors anywhere in the plant. Therefore, it is difficult to ascribe survival under conditions of moisture stress to one factor alone, since it may be operating in conjunction with less obvious factors.

Figure 1. Photographs of the enclosure permitting control of soil moisture, with a view of the north-facing side (top), and a view of the west-facing side (bottom).



MATERIAL AND METHODS

Determination of Survival Potential During Drought

One hundred and sixty-four seedlings from each of six seed sources of Douglas-fir were used in this study. Five seed sources were from a dry area near Medford, Oregon. This area is a southerly exposure with a slope approaching 30 per cent, and an elevation of about 2000 feet. The annual rainfall is approximately 20 inches. The sixth source came from the moist Tillamook Burn area in northwestern Oregon. It came from a north-facing aspect with a slope of 1 per cent and an elevation of approximately 1800 feet. In 1964 the seed from these sources was sown in parallel rows (approximately 5 feet by 100 feet) in the Oregon Forest Nursery.

In the spring of 1966 the 2-0 Douglas-fir seedlings were planted in an enclosure designed to permit control of soil moisture, but also to allow exposure of the seedlings to the natural environment (Figure 1). The seedlings for each seed source were selected from different sections of their seedbed in the nursery during the last week in February and carefully graded for uniformity by discarding the largest 25 per cent and the smallest 25 per cent of each 400 seedlings. One hundred and sixty-four replications of six seedling rows were planted in an area five feet by ten feet on the second and third of March. During the previous activities, care was taken to insure similar treatment to all seed sources. Each row contained one seedling from each of the six seed sources and was assigned to its position in the row by a table of random numbers. During the growing season, the incidence of terminal and lateral bud activity and seedling mortality were recorded every week. Soil moisture determinations were made at six locations and three depths every second week during the experimental period.[2] The soil depths were 4 inches, 12 inches, and 24 inches respectively. When the seedlings were harvested in late September, care was taken to preserve the fine roots as much as possible. Root length was recorded in centimeters for each seedling, living or dead, after which the seedlings were stored in a cold room for additional studies. During storage, the seedlings were completely enclosed in polyethylene bags.

Determination of Root Surface Area

The living seedlings of each seed source were visually segregated into four quartiles according to root size. Six seedlings from the largest size quartile and six from the smallest were arbitrarily selected for each seed source. The same procedure was followed for the dead seedlings. Within two months after harvesting,

[2]The experiment to this point was conducted by Dr. Denis P. Lavender, who made the study available to the author.

the total root area for each of the selected seedlings was determined by a method involving titration (Wilde and Voigt, 1949; Wilde, 1958). The roots were washed free of soil, placed in a container of distilled water, and then immersed in a saturated solution of boric acid (H_3BO_3) for thirty seconds. After draining for two minutes, the roots were placed in 1000 ml of distilled water, stirred, and allowed to stand for four minutes. Upon removal of the roots, a 50 ml portion was placed in an Erlenmeyer flask. After adding three drops of methyl red-bromocresol green indicator, the solution was titrated almost to the standard red end-point with .0101 N sulfuric acid. Titration was temporarily halted while distilled water was added to the solution to make the volume equal that of the standard. Then the end-point in the titration was reached and recorded. This titration value is a relative measure of the root surface area.

The standard was prepared by adding three drops of methyl red-bromocresol green indicator to a given volume of distilled water. A new standard was prepared each day titrating was done. The indicator was prepared by mixing five parts of 0.2 per cent bromocresol green solution with one part of 0.2 per cent methyl red solution, both in alcohol (Association of Official Agricultural Chemists, 1950).

Determination of Cuticle Thickness

The cuticle thickness was determined after cross-sectioning the needles and making microscopic slides. Twenty seedlings, ten which had been subjected to drought stress and ten which had remained at the nursery without stress, were used from each seed source. In early December, ten needles of the current year's growth were clipped one centimeter from the tips of terminal and lateral branches of each seedling. Both ends of each needle were excised with a sharp razor and the remaining one-quarter inch section was placed immediately in a formalin-aceto-alcohol (FAA) killing and fixing solution (Johansen, 1940). The needles were embedded using standard microtechnique methods. After a period of soaking, the embedded needles were sectioned on a rotary microtome and then allowed to dry on glass slides. The thickness of the sections varied from six to ten microns. The sections were then stained with safranin and fast green (Johansen, 1940) and mounted under a cover slip. The cuticle thickness was measured with a micrometer disk under a light microscope having a 15X eyepiece and a 43X dry objective. Comparisons were made between seed sources and between droughted and non-droughted seedlings.

Determination of Calcium-Potassium Ratio

Seedlings that had not gone through drought stress but remained at the Oregon Forest Nursery, in addition to the experimental population, were used in this part of the study. The seedlings were arbitrarily selected from different sections of the planting area, thus avoiding the effect of possible nursery soil variations.

The current year's growth of ten seedlings from each seed source was taken from the upper one-half of the crown. The branches with the foliage were placed in small paper sacks and allowed to dry in an oven at 70°C for five days. Following drying, the needles of each seed source were removed from the branches and ground in a micro-Wiley mill to pass a 40-mesh screen. This mixture was stored in screw-cap glass bottles until analysis. Two analyses were conducted for each seed source; each sample was a mixture of the foliage of ten seedlings.[3] The chemical analysis followed the procedure of Lavender and Carmichael (1966).

Determination of Shoot-Root Ratio

The shoot-root ratio was determined on the same seedlings used in the titration procedure; twelve living seedlings were used

[3] This analysis was conducted by Ralph Carmichael of the Forest Research Lab., Oregon State University, Corvallis.

from each seed source. The top was severed from the root at the root collar; then both weights were recorded to the nearest one-tenth gram. The same procedure was followed for the dead seedlings.

RESULTS AND DISCUSSION

Differences in Survival Potential of Seed Sources

The final mortality count in per cent is given in Table 1. This shows that the northwest Oregon seed source had a mortality far above that of the other five seed sources. Seed source four showed the lowest mortality of the five southern Oregon sources.

Table 1. Per cent mortality by seed source.

	Seed Source					
	1	2	3	4	5	NWO
Total Seedlings*	154	157	156	156	157	153
Survivors	86	87	88	109	91	24
Dead	68	70	68	47	66	129
% Mortality**	44.2_b	44.6_b	43.6_b	30.1_a	42.0_b	84.3_c
Subdivision of Dead Seedlings						
Budburst	41	50	35	38	44	39
No Budburst	27	20	33	9	22	90

*Some of the seedlings from each seed source were used in another problem and are not included in these results.

**Mortality per cents with different subscripts differ significantly from each other at the 1% level.

Figure 2 contains curves of the mortality and shows the rate at which death occured. The northwest Oregon seed source had the fastest rate of mortality, while seed source four had the slowest.

Statistical analysis was conducted with the data arranged in a

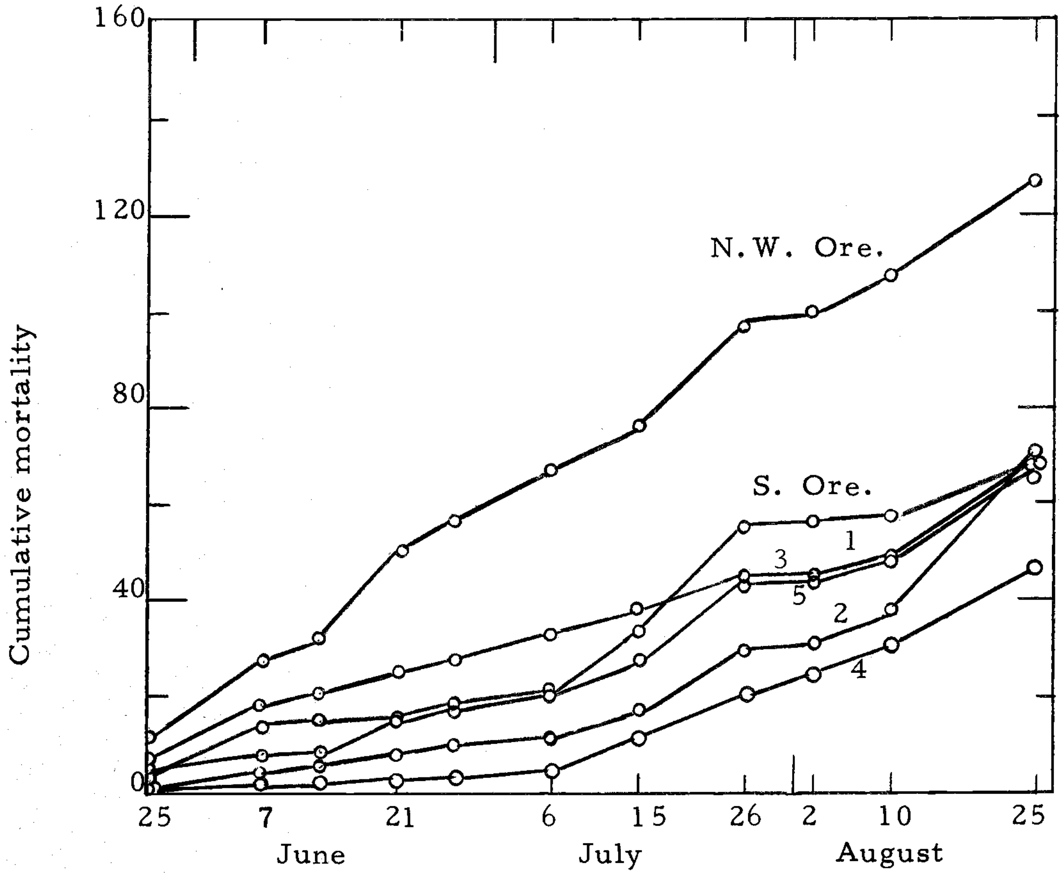


Figure 2. The rate and amount of seedling mortality by seed source during the growing season.

2 X t contingency table, using standard X^2 tests. This tested the null hypothesis that there were no differences in the true proportion surviving in the different seed sources (Cochran and Cox, 1957). The northwest Oregon seed source was highly significant (1% level) in comparison to the southern Oregon sources, as also was seed source four.

Figure 3 shows the trend of soil moisture during the growing season. Water loss from the upper four inches of soil was slow until the hot summer weather arrived, then it increased sharply. Soil moisture in this area reached a tension of 15 atmospheres by the end of May, while the 12 and 24 inch depths never reached the wilting point during the measurement period. Soil moisture per cents at 12 and 24 inch depths were almost identical, showing a fluctuating decline throughout the growing season. A rain storm terminated the study at the end of August.

The tension values for this soil were obtained through the Oregon State University Soil Physics Laboratory, and are as follows.

Soil-moisture tension	% soil moisture
0.1 atmosphere	53.0
0.3 atmosphere	46.8
5.0 atmosphere	31.5
15.0 atmosphere	26.5

As previously mentioned, root lengths were measured in centimeters for the living and dead seedlings after harvest.

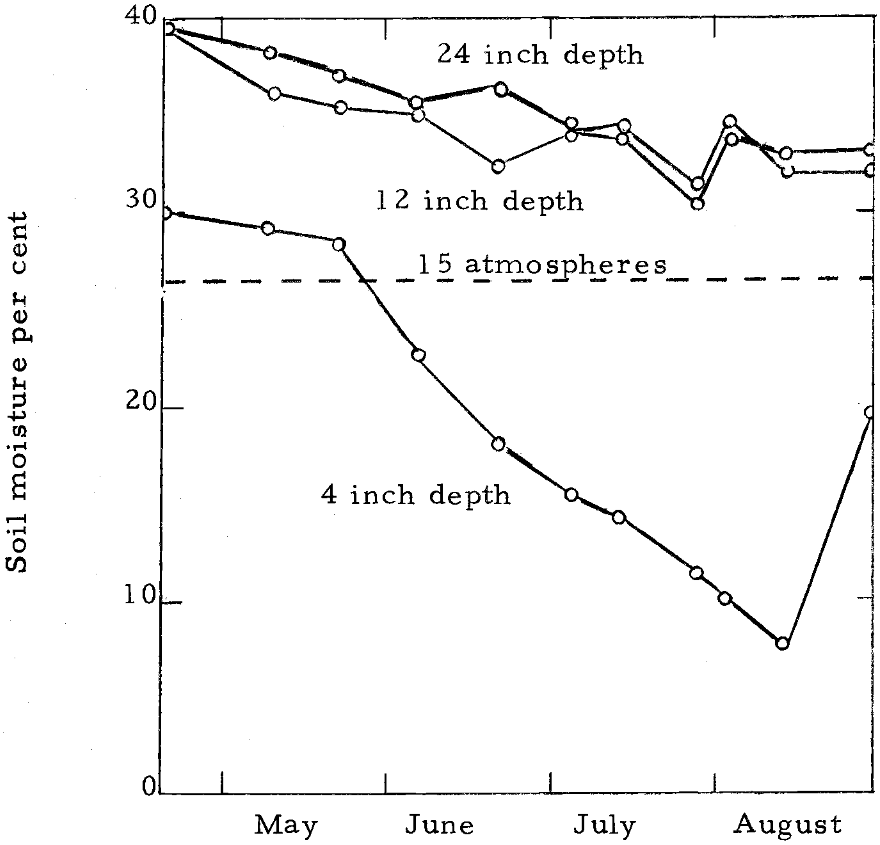


Figure 3. Trend of soil moisture in the drought enclosure during the growing season.

Table 2 gives the mean root lengths for all seedlings, alive and dead, of each seed source. It may be noted that all the roots of the dead seedlings were within the upper ten inches (25.4 cm) of soil. Therefore, they were exposed to soil moisture tensions greater than those plotted for the 12 inch level.

Table 2. Mean root lengths by seed source in cm.

	Seed Source					
	1	2	3	4	5	NWO
Live	38.46	35.53	39.77	36.21	42.67	31.33
Dead	23.05	21.85	23.49	23.87	23.04	22.41

However, the possibility exists that the roots were not completely removed from the soil due to decay or other reasons and a persistent underestimation of root length resulted.

An analysis of variance and a multiple mean comparison (Scheffe, 1953) were conducted; with one exception, no significant differences in root lengths could be found. The live roots of the northwest Oregon source were, however, significantly shorter than the live roots of seed source five at the 5 per cent level. An analysis was also conducted for the root lengths by date of mortality, however no significant differences could be found. Therefore, it can be concluded that root length played a negligible role in the differences in mortality found among the various seed sources.

Bud Activity

As previously mentioned, the incidence of terminal and lateral bud activity was recorded every week. Figures 4 and 5 present the trends of terminal and lateral budburst respectively. Lateral budburst was the same as terminal, except for a faster initiation and a higher total budburst. Seed source four of southern Oregon demonstrated the fastest rate and the highest total budburst. This source also had the highest seedling survival. The northwest Oregon seed source was opposite, having the slowest rate and the lowest total budburst. This seed source demonstrated the lowest survival of all seed sources. It was also determined that those seedlings which lacked bud activity did not survive; thus mortality was related to bud activity.

The difference in the timing of budburst may involve the minimum temperature required to break dormancy (Irgens-Moller, 1957). Some researchers feel that Douglas-fir seedlings, which have had their chilling requirements satisfied, do not break dormancy in response to long photoperiods maintained in controlled environments.[4] In southern Oregon, seedlings must break dormancy early to take advantage of the early spring rains, which are followed

[4] Personal discussion with Dr. Denis P. Lavender, Forest Research Lab., Oregon State University, Corvallis.

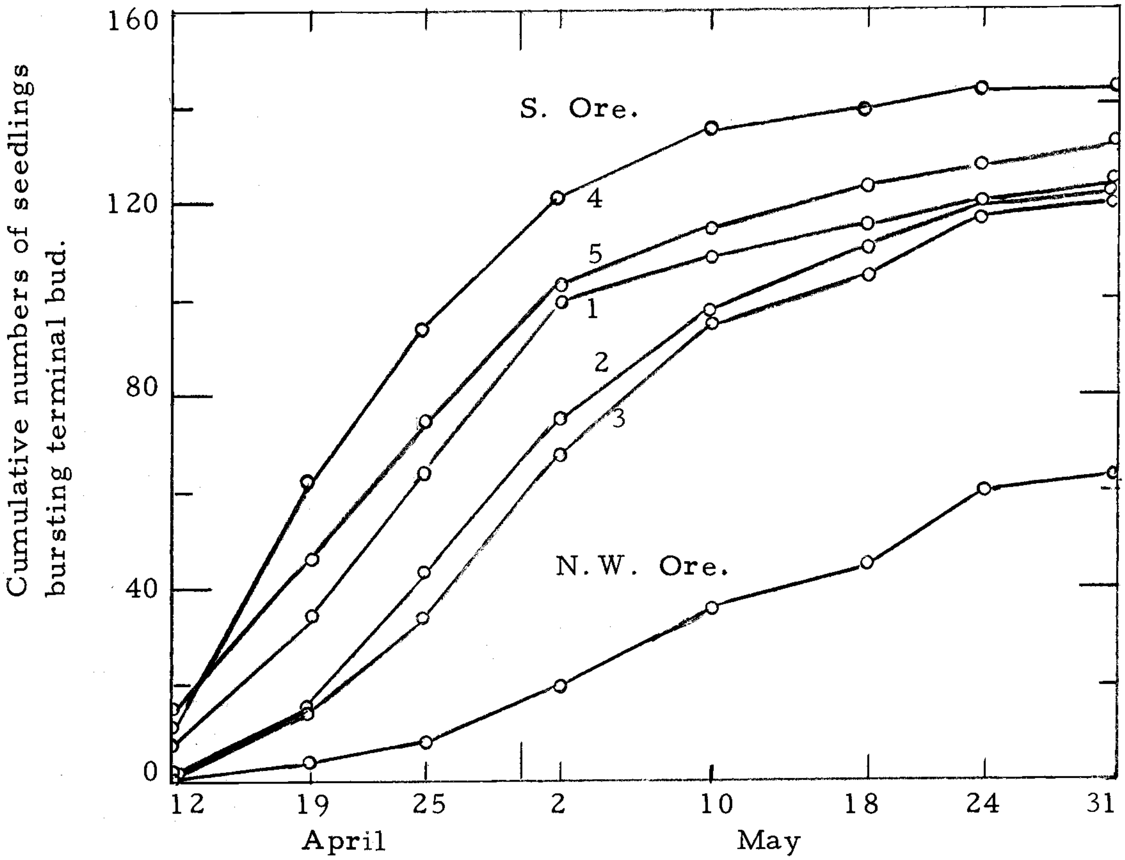


Figure 4. Rate and amount of terminal budburst by seed source and date.

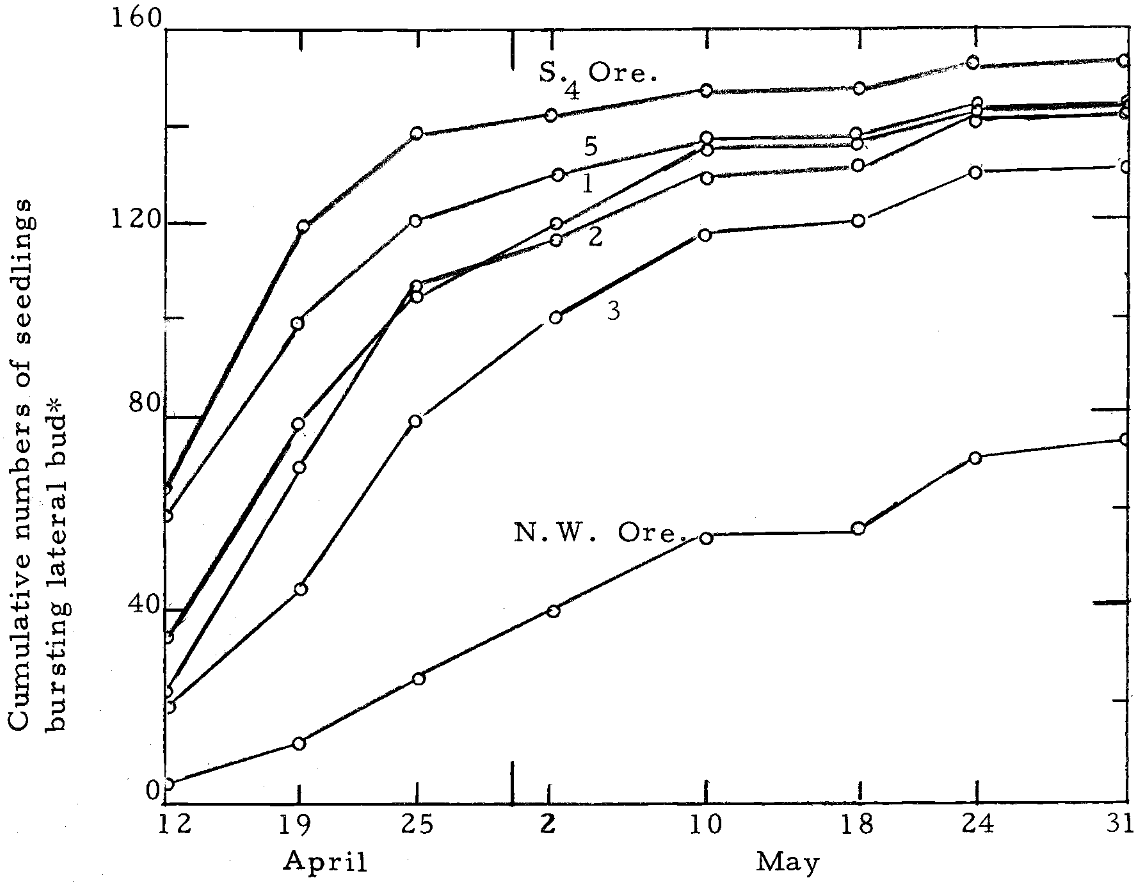


Figure 5. The rate and total amount of lateral budburst by seed source and date.

*A seedling was tallied when its first lateral bud burst.

by dry summers. According to Irgens-Moller (1960), this may be the situation for a Douglas-fir ecotype in Corvallis, Oregon. Since those trees which have slow bud activity die, there is a natural selection toward trees that respond to a lower minimum temperature. Since early bud activity may not be important for the northwestern Oregon trees to survive, there probably would be no natural selection for early growth. This may be the reason that the southern Oregon seedlings demonstrated an earlier budburst than the northwest Oregon seedlings in this study. An earlier bud activity by trees from the Medford, Oregon area was also found in the studies of Ching and Bever (1960).

Early initiation of growth may have been a factor of survival in this investigation. All southern Oregon seedlings, especially source four, began growth before soil moisture became low enough to seriously hinder their physiology. However, the later growth initiation of the northwest Oregon seedlings, which may have resulted from a higher minimum temperature requirement, came at a time when soil moisture tensions were higher. The higher tensions may have caused a critical effect on the physiology of the seedlings, which was expressed by lack of bud activity. The exact sequence of biochemical changes during the dormant period of Douglas-fir is not known; however, this sequence may very well have occurred later in the spring in seedlings from northwest Oregon. Since the soil moisture tensions may have been slightly higher during this later biochemical change, the effect on the physiology of the northwest Oregon seedlings may have been more critical.

Another consideration may be that some prior condition, not necessarily related to drought stress, was involved in mortality. In the previous consideration the seedlings were thought to be equal in vigor, since they received equal treatment in the nursery, during lifting, and at the time of planting. The critical difference was suggested to be the timing of growth initiation. However, there may have been a difference in the physiological condition of the seedlings when they were transplanted. This may be the same condition Stone found in Douglas-fir (1962) and ponderosa pine (1963) which could be measured by "root-regenerating potential". Since the ability to put forth roots is a measure of the physiological condition of the seedlings, bud activity may also be such an expression. In another study Stone (1959) found a difference in the "root-regenerating potential" of seedlings from two ponderosa pine seed sources. There may have been differences in the physiological conditions of the seed sources used in this study, especially when comparing the northwest Oregon seedlings with those from southern Oregon.

It is not known what impact seedling physiological condition has had on survival in the field. When seedlings are planted they are not checked for low bud activity or other expressions of low vigor; hence, death of seedlings is usually ascribed to some other cause. Undoubtedly much more research will be required to establish definitive criteria of seedling survival potential during all phases of reforestation programs.

Root Surface Area

The results of the root titration procedure are presented in Figure 6 (live seedlings) and in Figure 7 (dead seedlings). The titration value, a relative measure of surface area (Wilde, 1958), is plotted against the root weight. Regression equations were calculated and a correlation coefficient (r) was derived for each regression line. Covariance analysis of the data was also conducted. The correlations for the living roots from seed source one to seed source six were .96, .89, .84, .93, .98, and .80; those for the dead seedlings were .69, .97, .87, .85, .81, and .78 respectively.

It is clear that in the case of Douglas-fir of a given seed source, there is a direct relationship between root surface area and root weight. When it is desirable to pick the seedlings with the greatest absorbing surface from a seed source, those with the highest weights would be selected.

The difference in root absorbing surface among the seed sources is seen by examining the graphs. Per unit of root weight, the seed source with the greater root area would have the higher

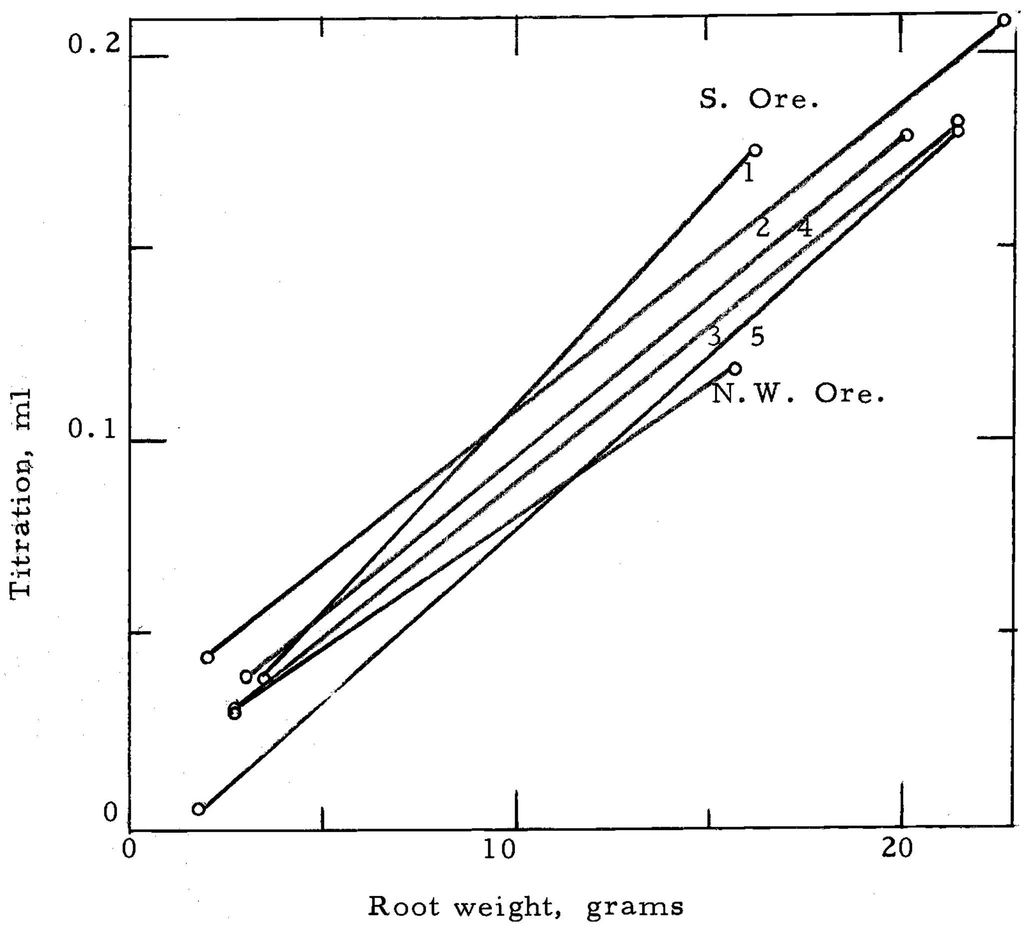


Figure 6. Regression lines of root surface area by root weight for the live seedlings of each seed source. Each line was calculated from 12 samples.

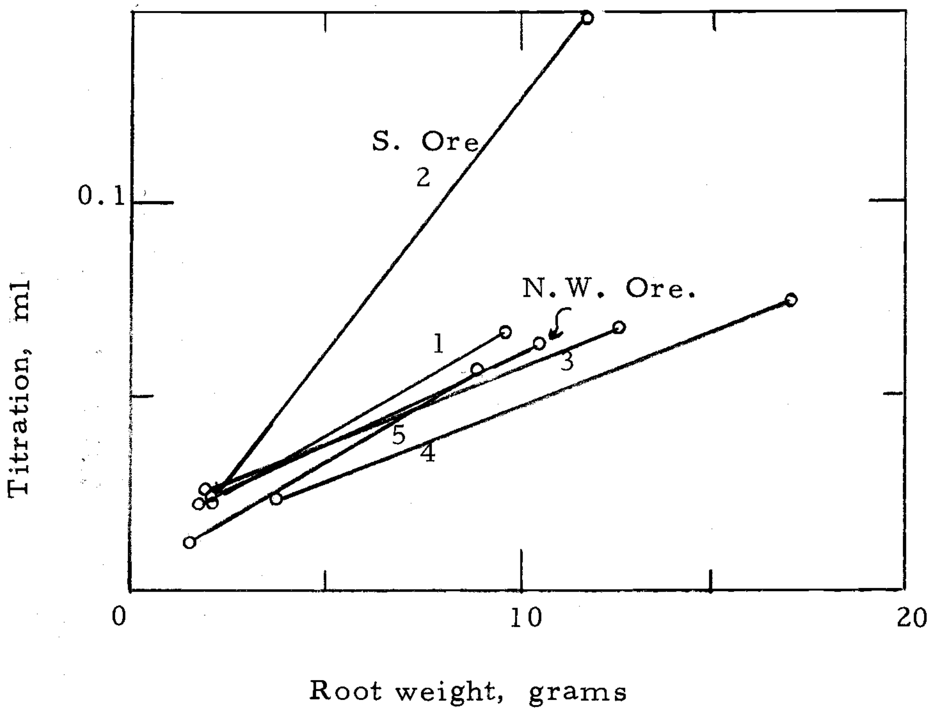


Figure 7. Regression lines of root surface area by root weight for the dead seedlings of each seed source. Each line was calculated from 12 samples.

titration value. This is best illustrated in Figure 7, where seed source two has the greatest absorbing surface, while seed source four has the least.

An analysis of covariance was conducted on the live seedlings (Figure 6) to determine differences in the regression lines as to slope and elevation. No significant differences could be found in the slope of the lines. When considering elevation, the northwest Oregon source was not significantly different from sources five, three, and four of southern Oregon. However, it was significantly lower than seed source one (1% level) and seed source two (5% level). The significance of the regression lines by covariance analysis could not be correlated with survival; thus, differences in root surface area had negligible effect on mortality in this study.

It may be noted that the roots of all seed sources appeared about equal in diameter and length. Therefore, the higher root surface area of one seed source over another per unit weight may be a reflection of greater root fibrosity.

Cuticle Thickness

A difference in cuticle thickness could not be demonstrated among the seed sources. This is in agreement with Tucker's (1966) study with two Douglas-fir seed sources. He found no significant difference in cuticle thickness between Washington and southern

Oregon seed sources grown under various environments. His seedlings were not tested for their ability to survive moisture stress, but the seedlings came from moisture regimes as extreme as those used in this study. No relationship between cuticle thickness and survival was determined in this investigation.

Calcium-Potassium Ratio

The results of the calcium-potassium analysis, when using seedlings which had not been given drought stress (Table 3), did not agree with Kral (1965). Therefore, an analysis was made of the seedlings which had been given drought stress; still no relation was found (Table 3). This last analysis was conducted some months following initial storage of the seedlings in the cold room. During the latter part of storage some of the seedlings had become infected with fungi and this may be reflected in the results for the droughted seedlings. However, some of the relationships between seed sources seem to hold in this last analysis. For example, seed source three still has the lowest ratio and seed source one holds a high position as in the non-droughted seedlings.

According to Kral, the least resistant seed source (northwest Oregon in this case) should have the lowest Ca/K ratio. This did not occur as seen in the table and as mentioned above. Seed source four did not have the highest ratio as would be expected.

Table 3. Chemical analysis* and Ca/K ratio by seed source.

Seed Source	N	P	Mg	K	Ca	Ca/K
			With Drought Stress			
1	3.04	.108	.314	.640	.490	.766
2	3.07	.125	.292	.715	.390	.545
3	3.03	.104	.267	.665	.335	.504
4	3.07	.140	.306	.555	.425	.766
5	2.95	.116	.252	.565	.345	.611
NWO	3.63	.175	.290	.880	.490	.557
			Without Drought Stress			
1	1.74	.291	.161	.800	.320	.400
2	1.81	.268	.155	.805	.245	.304
3	1.85	.245	.147	.775	.175	.226
4	1.76	.252	.144	.845	.260	.308
5	1.85	.279	.155	.800	.270	.338
NWO	2.00	.202	.148	.895	.230	.257

*Values of elements are given in per cent of oven-dry foliage weight. Each value is the average of two samples.

Elements other than calcium and potassium were also analyzed and are included in the table, although they were not used in this investigation.

Shoot-Root Ratio

An analysis of variance and a multiple mean comparison

(Scheff'e, 1953) were conducted on the shoot-root ratios. The mean ratios for the live seedlings given in Table 4 show that neither the northwest Oregon source nor seed source four are significantly different from the other southern Oregon sources. When considering the dead seedlings, seed source four has a significantly lower ratio (5% level) than seed source three. Each ratio is the mean of twelve seedlings, with the exception of the seedlings which have been dead since July 6.

Table 4. Mean shoot-root ratios by seed source.

	Seed Source					
	1	2	3	4	5	NWO
Total Live	1.030	1.248	1.274	1.048	1.252	1.191
Total Dead	.520	.581	.701*	.405*	.583	.610
Dead since July 6	.464	.566	.685*	.405*	.586	.588

*Seed source three is significantly different from four at the 5% level.

An analysis was conducted on those seedlings that had died since July 6 because part of the roots might have been lost from seelings that had died earlier. Such a root loss could influence the ratios. As can be seen from Table 4, the ratios of this third category were lowered because of a higher root value in comparison to the shoot. However, the significance was not altered from that of the total dead.

The ratios of seed source four in the last two categories are the same because there were no dead seedlings in this twelve seedling sample until after July 6. The mean ratios for the dead seedlings are lower than those of the live; the tops of the dead seedlings had lost their needles and dried out, causing the fresh weight of the roots to be higher in comparison to the tops.

The ratios of the live seedlings were not significantly different from each other; therefore, shoot-root ratio could not be correlated with mortality.

SUMMARY

This investigation was conducted to determine if Douglas-fir seed from five sources in southern Oregon produced seedlings of greater drought survival potential than a seed source from northwest Oregon. Anatomical, morphological, and physiological characteristics associated with increased survival were then studied.

In the spring of 1966, several hundred 2-0 Douglas-fir seedlings were planted in an enclosure designed to permit control of soil moisture, but still allow exposure to the natural environment. During the growing season, bud activity and mortality were recorded, and soil moisture determinations were made. The seedlings were harvested in late September and stored immediately in a cold room for further studies. The final mortality count showed that the northwest Oregon seedlings had 84 per cent mortality compared with approximately 44 per cent for four southern Oregon seed sources and 30 per cent for the fifth.

The first study was to determine if there was a difference in root area of the six seed sources and to find if this had any correlation with mortality. The total root surface was determined by a method involving titration. No correlation of root surface area to mortality could be made.

Root lengths were measured and with one exception, no

significant differences could be found. It was concluded that root length played a negligible role in the differences in mortality.

Sections of the needles were made to determine if a difference in cuticle thickness existed. The cuticle of all seed sources was approximately the same and could not be correlated with survival.

Chemical analysis to determine the calcium-potassium ratio was conducted because certain literature has concluded that more drought resistant seed sources have a higher ratio. There was no relationship found in this study.

The northwest Oregon source had a much slower rate and also the lowest total budburst. It also had the highest mortality. Seed source four of southern Oregon showed the opposite characteristics, having the fastest and highest total budburst. It demonstrated the lowest mortality of all seed sources. Lateral budburst gave the same results except for earlier initiation by all seed sources. All seedlings which did not have budburst died.

Early bud activity by the southern Oregon seed sources, may have been a survival advantage. The later activity of the northwest Oregon seed source may have subjected it to higher soil moisture tensions during budburst, which may have upset the physiological state of the seedlings, which resulted in mortality.

A physiological condition of the seedlings not related to moisture stress may also have influenced the distribution of mortality. The seedlings of the northwest Oregon source may have had a poor physiological constitution, which was expressed by lack of bud activity.

The shoot-root ratios of the dead seedlings of seed source four were significantly smaller than the ratios of seed source three. The ratios of the living seedlings showed no significant differences. Therefore, a correlation of shoot-root ratio to survival could not be made.

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