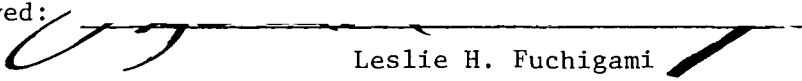


ABSTRACT OF THE THESIS OF

Cheryl Anne Cobb for the degree of Master of Science
in Horticulture presented on March 23, 1977

Title: WATER RELATIONS AND THE ROOT REGENERATION RESPONSE
OF DOUGLAS-FIR

Abstract approved:


Leslie H. Fuchigami

The effect of lifting date and root-pruning treatments on water potential, root regeneration, and shoot growth of six-month-old and three-year-old Douglas-fir seedlings bare-root transplanted into a growth chamber, and sawdust bed, respectively, was studied. Root growth in six-month-old and three-year-old seedlings was highest in February and August respectively. Survival was highest in November for seedlings of both ages. The time to first root growth, water potential four weeks after transplanting, and survival were correlated. The possibility that the maintenance of plant turgor above the critical level necessary to allow root growth is important for survival of an actively growing transplant is discussed. The effect of root-pruning on transplant water potential, root growth and survival varied with season.

In a second study six-month-old Douglas-fir seedlings were subjected to eight drying cycles in which the seedlings were allowed to dry to a -15 bar water potential before watering. Root growth, water potential, and survival of transplants were measured

under stressed and control conditions. Water potential, soil moisture content and survival of seedlings subjected to a prolonged drying period were recorded. Preconditioning treatments were effective in increasing drought resistance under stress conditions. Reductions in shoot elongation, dry matter production, and early bud set were noted. Though inadvertant watering of the transplants made conclusions impossible, survival data and data collected prior to watering indicate that root growth and survival were improved by the treatment.

Water Relations and the Root Regeneration
Response of Douglas-Fir

by

Cheryl Anne Cobb

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INTRODUCTION

In 1971 (52), ornamental research and extension representatives met with the leaders of Oregon's Nursery Industry to discuss the immediate and long range problems facing the nursery industry. The number one problem identified was the high cost of balling and bur-lapping conifers for safe shipment to market areas. Industry leaders estimated that at least 40% of their production costs were associated with this procedure. It was felt that if the trees could be bare-rooted, similar to what is done commercially for deciduous plants, shipped in light packs and rooted at destination; harvesting and shipping costs could be substantially lowered. There are other possible advantages: 1) As a result of the lower shipping costs, production could be located in ideal growing areas and the trees later shipped to potential market sites for finishing. 2) Plants could be spaced closer together at planting. 3) Cultivation and other field practices could be reduced or simplified. 4) Harvesting operations could be done during a more favorable time of the year and could easily be mechanized. 5) The movement of soil would be minimized and the bare-roots treated to reduce the spread of soil-borne disease. 6) Work with deciduous trees has shown that growth and survival of the transplants may be better in the long run.

Preliminary studies (Fuchigami, unpublished data) have demonstrated that it is possible to successfully bare-root evergreens, even some that are quite old. The roots regenerated are often numerous, fibrous,

and extremely vigorous. Unfortunately, success, as measured by subsequent growth and survival, is usually moderate to poor. This study was designed to determine the relation of water potential after transplanting to the root regeneration and establishment of bare-rooted seedlings lifted at various times of the year and root pruned to different degrees. A method for preconditioning the transplants, to increase survival was also investigated.

Root Regeneration Potential

Much effort has been spent investigating the reasons for the variable survival of transplants (34, 62, 64, 65, 66, 67, 79, 82). Collectively, these reports agree that tree vigor, phenology, care in handling and environmental conditions at the time of transplanting are all important to plant survival. The most important factor, however, appears to be the physiological readiness of the plant to regenerate roots soon after planting. Stone and Schubert (66) have used this factor to predict plant survival.

The first suggestion that the physiological condition of transplanted stock might be important in transplant survival was made by Dunning and Kirk (6), who found high seedling mortality in Ponderosa pine plantings early in the summer even when soil moisture was readily available. They concluded that the physiological condition of the seedlings, not the environment, was the limiting factor. This view was supported by Wakeley (79), who pointed out that there were no physiological differences in seedlings grown in Southeastern United States that affect field survival. Stone (62) undertook a preliminary examination of this periodic response in five coniferous species by lifting field grown plants monthly, placing them under a constant

ideal environment, and quantifying the numbers of unsuberized root tips resulting from elongation or initiation. This measurement was termed the root-regenerating potential (RRP). Major differences between species were apparent but all showed some periodicity. He concluded that there were some times of the year when plants were "physiologically unsatisfactory for planting." Ponderosa pine was examined in greater detail by Stone and Shubert (65, 66, 67). The capacity for elongation of existing roots and new root initiation was poor during the summer months, increased during the fall, peaked in late winter before bud break and decreased in early spring with the onset of bud break. Veerkamp (unpublished data), working with Douglas-fir, concurred but also found a lower peak in regeneration potential after extension growth was complete. Lavender et al. (36), Krueger and Trappe (33) and Winjum (81) found field rooting of Douglas-fir to follow much the same trend. Similar results were reported by others (38, 64, 80).

The Effect of Environment of RRP

A number of studies have dealt with the effect of root environment on RRP. Hermann (69) has found, in general, that root activity increased with temperatures up to 37°C. Merritt (42) and Stone (67) have shown that root growth responses to temperature are controlled by a basic internal periodicity.

Stone and Jenkinson (63) also observed that Ponderosa pine had a periodicity in RRP irrespective of moisture level; but as with temperature, a root growth was limited to a certain moisture range. Merritt (42) and Becker (3) concurred with this finding. Soil compaction and poor soil aeration also appear to be secondary factors

(Brown, personal communication).

Shoot environment has also been studied. Stone et al. (68) noted that the fall and winter increase in RRP was related to the onset of an effective night temperature (a value lying halfway between the daily mean and daily mean minimum for each month) of 21°C or less. A minimum exposure of 1500 hours at 10°C or less was required yearly before a peak in RRP was reached. The validity of this data has since been questioned (Jenkinson, personal communication).

Light intensity has been shown to have an effect on the root growth potential in pine and Douglas-fir (Zaerr, unpublished data). Brown and Zaerr (unpublished data) varied the light intensity from $2.4-6.9 \times 10^3$ lx in western hemlock and found no significant changes in the pattern of RRP. The effect of photoperiod on RRP has been studied by Merritt (42), who varied day length at different times of the year, and found it to be a secondary factor.

The Effect of Physiology on RRP

There has been some debate over whether or not conifer roots become dormant or have a chilling requirement (17). Lathrop and Mecklenburg (35) have found that optimum temperature for breaking root dormancy in Taxus, spp. was 2°C for seven weeks. This seems unlikely because the fall peak in root growth occurs before the chilling requirement has been fulfilled. Lavender et al. (37) have indicated that only buds appeared to have a chilling requirement and that roots have no dormancy.

The possibility that shoot dormancy has an effect on the RRP of transplants is suggested by the fact that seasonal periodicity in the RRP of transplants closely follows that of shoot rooting potential in

conifers. Recent work (Roberts, personal communication) has indicated that this is true of Douglas-fir cuttings. Lee and Hackett (38) have studied the effects of debudding on the RRP of pistachios and concluded that "the presence of physiologically non-dormant buds which provide auxin and perhaps some unknown substance(s) stimulate cambial activity and xylem differentiation." In turn, this cambial activity stimulates initiation of lateral root primordia.

It has long been known that root elongation is stimulated by low concentrations of auxin, but is inhibited by concentrations that stimulate root formation. There is some evidence that application of auxin to roots of transplanted trees sometimes improves root growth (13, 41, 70). Unfortunately, results with gymnosperms have been inconsistent (41) or negative (8). Zaerr (82) studied the effect of auxin on RRP of Ponderosa pine and concluded that it had little influence. Lee and Hackett (38) applied auxin to the decapitated shoot of pistachio; and found that the treatment amplified the RRP, but did not affect the yearly pattern.

Other investigators indicate that root-shoot competition for food reserves might account for the periodicity. Krueger and Trappe (33) investigated this phenomenon and found that total sugars in Douglas-fir roots and shoots began increasing markedly in early November and reached a peak in January that was approximately three times the summer concentration. The build-up was due primarily to two non-reducing sugars: sucrose and raffinose. Carbohydrate decreases in shoots coincided with bud break. A similar pattern was found in Noble Fir and Douglas-fir by Winjum (82) and Merritt (42), in Loblolly pine by Gilmore (11), and in Pinus strobus by Shiroya et al. (56). The

seasonal translocation of photoassimilated ^{14}C was measured in White Pine by Ursino et al. (76) and in Ponderosa pine by Ziemer (85). Maximum carbohydrate translocation to the shoots occurred just before and after bud break. Lee and Hackett (38) applied sugar solution to cut shoot tips and found an increase in RRP. They concluded that carbohydrate levels were important in root regeneration. There seems to be no correlation between carbohydrates stored in the roots and RRP (11).

Whatever the relationships, it is apparent that the plants' ability to regenerate a root system is an important factor in transplant survival. Root-soil contact is essential if water status is to return to normal and if nutrients are to be available to the plant.

Plant-Water Status and Transplant Survival

One essential factor in plant survival is the maintenance of a sufficiently high water content or turgidity to permit normal functioning of physiological processes and growth. Current theory is that most water present in plants flows as a liquid through the cell walls of the root cortex, through the cytoplasm of the endodermis, and up the xylem into the leaves. Here, it again travels along the cell walls; and finally into the stomatal pores, where it evaporates and is lost as water vapor. Gardner (9) has termed this pathway the "soil-plant-atmosphere continuum." The flow of water through this continuum is from a region of high energy status to one of low energy status. In other words, this water will flow down a gradient of potential energy (water potential) from soil to leaf to atmosphere. The rate of this flow is determined by the potential gradient and the resistance to

flow.

$$\text{Flow} = \frac{\text{Gradient}}{\text{Resistance}}$$

Evidence indicates that the flow of water through each partition of this continuum is equal, and can be expressed mathematically as:

$$T. = \frac{\psi_{\text{soil}} - \psi_{\text{root}}}{r_{\text{soil}} - r_{\text{root}}} = \frac{\psi_{\text{root}} - \psi_{\text{leaf}}}{r_{\text{root}} - r_{\text{leaf}}} = \frac{e_{\text{leaf}} - e_{\text{air}}}{r_{\text{leaf}} - r_{\text{air}}}$$

where T is defined as transpiration rate, ψ = water potential, r = resistance to flow and e = vapor pressure. It can be seen from this equation that a change in either the gradient or resistance would be felt eventually through the whole system.

A two-year old Douglas-fir seedling has the heterorhizic root system typical of most conifers - a long primary root from which extend several long first and second order and many shorter first, second, third and fourth order laterals. The ability of this root system to absorb water is balanced by an equally efficient water loss system, the open stomata. When this seedling is dug for bare-root transplanting most of the laterals are severed. Root-pruning of up to 50% of the root system often follows. Also, exposure during transplanting will at times kill many of the fine fibrous smaller roots, even when the tree is carefully handled. After harvesting, the seedlings are sometimes stored for prolonged periods of time. During storage, further drying of the roots may occur. When the seedling is replanted, the limited supply of readily available moisture in the surrounding soil will soon be exhausted; and the seedling will be forced to rely on moisture flowing from increasingly greater distances through the soil (30).

If the transpiration rate exceeds the rate of water flow through the soil, the seedling stomatal diffusion resistance will increase as a result of turgidity loss in the guard cells or of the effect of low plant water potential on growth and metabolism. If the stomates close completely, the CO_2 diffusion resistance into the chloroplasts will increase. Photosynthesis will be reduced and the energy pool for metabolic functions will become limiting. If there is incomplete stomatal closure, water potentials sufficiently low to upset metabolism will develop. In either case, the seedling will eventually die if the stress continues. If, however, the undamaged laterals can elongate rapidly, or new laterals form and grow and reach the water supply; the physiological shock resulting from transplant can be, in part, overcome.

Stress Injury and Resistance

It seems, then, that a plant's ability to withstand transplant shock is in part an ability to withstand water stress until a new root system can be regenerated. Nurserymen term those plants resistant to stress "hardy" and those susceptible "tender" or "nonhardy." In the past decade, more attention has been paid to this ability to withstand stress and quantitative methods of dealing with it explored.

When a plant is subjected to stress, it is in a state of strain. This strain can be of two types, elastic or plastic; and is manifested as a physical or chemical change in the plant. As elastic strain is reversible and not injurious in itself. The measure of an organism's ability to prevent reversible or elastic strain when exposed to an environmental stress is known as an organism's elastic resistance. In the case of plastic strain, the result is irreversible and injurious.

Plastic resistance is a measure of the organism's ability to prevent irreversible or plastic strains.

Levitt (39) has shown that plant under elastic strain can respond in three ways: 1) the elastic strain may remain constant and fully reversible without leading to other changes, 2) the elastic strain may be converted into an indirect plastic strain, and 3) the elastic strain may lead to secondary changes which induce either plastic or elastic adaptation. The first two responses are those seen in tender plants. Hardy plants respond by adapting; they find a way to exclude the strain or of tolerating it.

A plant under stress may be injured in a number of ways. The stress may cause a direct injurious plastic strain, easily recognized by the speed with which it appears (e.g. loss of turgor). Levitt calls this direct stress injury. Or, an elastic strain not harmful in itself may result. If, however, this strain is long lasting; an indirect plastic strain may occur, causing an indirect injury (e.g. loss of turgor causes a decreased rate of growth).

The third type of injury is termed secondary stress injury and is a result of secondary stress resulting from the initial stress (e.g. decreased root growth during soil water stress as a result of increased soil mechanical impedance).

There are two types of resistance a plant can develop to survive these strains: 1) Stress avoidance is stress resistance by exclusion. The plant avoids thermodynamic equilibrium with the stress by means of a chemical or physical barrier (e.g. closed stomates, thickened cuticle). 2) Stress tolerance is stress resistance through an ability to come to thermodynamic equilibrium with the stress without injury.

Response of Plants to Water Stress

The fundamental measure of plant water status is its chemical potential, expressed as energy per unit volume, and commonly called water potential (ψ). Plant ψ is effected by the hydrostatic pressure or tension, the colligative effects of solutes, and the interaction with matrices of solids and macromolecules. Hence, ψ is the algebraic sum of the component potentials arising from the effects of pressure (ψ_p), solutes (ψ_s) and matrix (ψ_m). It can be expressed mathematically as:

$$\psi = \psi_p + \psi_s + \psi_m$$

Matrix potential is very close to 0 in fully turgid plants, and in most species does not become numerically significant until much of the tissue water is lost. Therefore, in most cases, water potential can be defined as:

$$\psi = \psi_p + \psi_s$$

When a turgid plant is exposed to water stress, an initial decrease in its tissue water content will cause a sharp decrease in ψ (it will become more negative), resulting from a large decrease in ψ_p and a smaller decrease in ψ_s . If the stress continues ψ_p will decrease to an insignificant level and changes in ψ_s will account for any further reductions in ψ . Hsiao (20) notes that decreases in ψ follow a simple osmotic relationship with solution volume. As a result, the change in ψ per unit change in tissue water will decrease.

Though ψ is a useful indicator of plant water status, in that its gradients determine patterns of water transport, Hsiao (20) has indicated that absolute ψ values should not be taken as indicators of

physiological water stress. Evolutionary and physiological adaptation to environment could markedly influence the level of ψ at which water stress becomes apparent. There is also some indication that the maintenance of turgor appears to be the critical factor in maintaining growth and regulating metabolic processes in the plant (48, 49).

The response of plants to water stress has been the subject of several recent reviews (7, 20, 27, 28, 29, 31, 50, 58). These have emphasized: effects of water stress on physiology and metabolism (4, 31, 40, 58, 59, 71, 78), growth morphology and ontogeny (10, 32, 54, 59, 84), yields (59, 54) water in relation to biological macromolecules and membranes (24, 29, 54, 59), and drought resistance (39). Some of the documented effects of water stress on plants are: increases in ABA levels and ethylene production; and reductions in levels of transpiration, cytokinin levels in leaves, CO_2 assimilation in light, dark respiration, cell turgor, growth, cell wall synthesis, cell division, rate of nutrient uptake and transport, and rate of photosynthate translocation.

Preconditioning Plants to Water Stress

Even though much can be done to increase the survival of transplants by selection of healthy trees, careful handling, site preparation, and planting under good environmental conditions, a number will die as a result of desiccation (30). A number of practices aimed at increasing the plant's resistance to water stress have been used to further improve transplant survival. These methods, in theory, increase water absorption, reduce water loss, and/or allow the transplant to withstand or repair the damage resulting from the strain. The most commonly used practices have been those of undercutting and wrenching.

Undercutting consists of running a sharp blade beneath the plant bed at a predetermined depth (usually 3-4") to sever the roots of the seedlings. At regular intervals after this, a thicker blunter blade is run beneath the bed. This is termed wrenching. Any new deeply penetrating roots that have grown since the undercutting are broken off, and the seedlings are partly lifted in the soil, aerating the seedbed. Most evidence (69, 76) indicates that trees that have been regularly treated this way, in the nursery show better survival when transplanted than those that have not been "pruned." Other reports show the practice to be ineffective (72, 73) or detrimental (41, 72). These variations have been due to species, pruning method and time, or planting site differences. Rook (53) demonstrated that well-wrenched seedlings survive transplanting to dry sites better than unwrenched seedlings. He felt this was due, in part, to the capacity of the wrenched root systems to better meet the water demands of their shoots. Kozlowski and Davies (30) felt that this would confine the absorbing roots of the wrenched seedlings to a small area, resulting in little injury during lifting. Rook (53) has shown that wrenched Pinus radiata seedlings produce a finely branched compact ball of roots with increased carbohydrate levels, possibly increasing markedly their physiological readiness to regenerate new roots upon transplanting. He also noted a variation in the plant response to wrenching at different times of the year.

Another possible way of decreasing desiccation injury or death in transplants is through controlled water stress of the seedlings before transplanting, in hopes of physiologically preconditioning them to withstand the water stress they will be exposed to upon transplanting.

Though this practice is common even in large scale production (Timmis, personal communication), evidence that it is successful is almost entirely empirical. Data supporting the method do exist, but most are indirect. Numerous reports exist in the literature concerning the aftereffects of water stress on the plant. Many investigators have reported changes in transpiration, stomatal response, respiration, growth rate, leaf anatomy and internal plant water relations that linger long after moisture stress has been removed. Proponents of wrenching cite this as evidence that this prestressing does result in a useful adaptation of the plant to its environment. This belief is supported by the findings of Unterscheutz et al. (75), Hellkuist (16) and Bannister (2); working with Douglas-fir, Scots Pine and Heath, respectively. They found that seedlings from a wet or shaded environment closed stomates more rapidly than those from drier, more open habitats.

Root-Pruning and Bare-Root Transplanting

Root-pruning at the time of lifting or planting is commonly done in conjunction with bare-root transplanting. Many reasons for root-pruning have been given: 1) To facilitate handling and transplanting; 2) To obtain better field survival of seedling stock; 3) To cause development of a more compact and fibrous root system (46). Unfortunately, most of the data substantiating these claims are qualitative in nature, the time and severity of root-pruning established largely on an empirical basis and subject to controversy as to value and influence.

In normal lifting, roots are broken and cut. Schmidt (55) felt the effect of this root loss on plant survival was insignificant.

Others have noted a reduction in survival (69). It seems likely that this variation can be attributed to species variation, age, environmental and handling differences, and variation in lifting dates.

Similar variation not only in survival, but in root and shoot growth, is found in seedlings root-pruned before transplanting. An increase in survival but a decrease in height growth five years after transplanting in lifted root pruned longleaf pine was noted by McGee (47). Smith and Allen (60) found a first year reduction in height growth in pruned Douglas-fir. Mullin (45) found a combination of root- and shoot-pruning to be the most successful in increasing survival of lifted white spruce. Sutton (69) found root and shoot growth to be significantly improved by root-pruning in May-lifted stock. Similar differences were not found in Norway Spruce. Harris et al. (15) noted that the time of root pruning in Monterey Pine affected subsequent growth of the root system. Trappe (72) found distinct differences in root growth after pruning between seedlings of Pseudotsuga menziesii var. glauca and var. menziesii (Micrb.). He noted that such data indicate that "no single nursery technique can be guaranteed to produce a given form of root system on all species or varieties." Trappe and Krueger (73) indicate the need for increased research and careful observation of each species' response to transplanting and pruning, with the aim of compiling a seedling biography from which detailed pruning recommendations can be made.

Research Approach

As previously mentioned, this study was initiated in response to requests from the nursery industry for more information on bare-root transplanting of conifers. Preliminary studies with a variety of

period was also observed. Analysis of variance was used to evaluate the data.

The balance of this thesis is presented in the form of two manuscripts written for submission to the Journal of the American Society of Horticultural Science.

WATER RELATIONS AND ROOT REGENERATION
IN DOUGLAS-FIR BARE-ROOT TRANSPLANTS

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Abstract. The effect of lifting date and root-pruning treatments on water potential, root regeneration, and shoot growth of six-month-old and 3-year-old Douglas-fir seedlings bare-root transplanted into a growth chamber, and sawdust bed, respectively, was studied. Root growth in six-month-old and 3-year-old seedlings was highest in February and August respectively. Survival was highest in November for seedlings of both ages. The time to first root growth, water potential four weeks after transplanting, and survival were correlated. The possibility that the maintenance of plant turgor above the critical level necessary to allow root growth is important for survival of an actively growing transplant is discussed. The effect of root-pruning on transplant water potential, root growth and survival varied with season.

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WATER RELATIONS AND ROOT REGENERATIONS IN

DOUGLAS-FIR BARE-ROOT TRANSPLANTS

Introduction

An actively growing seedling must regenerate roots soon after transplanting if it is to survive. In lifting, a bare-root transplant loses most of its elongating laterals. Exposure during transplanting may destroy many of the remaining small roots, even with proper handling. Before planting, it is often customary to root-prune to facilitate replanting though evidence on the value of this practice is inconclusive (18, 21, 45, 72). Proponents claim a more vigorous and fibrous root system is produced, resulting in increased survival and long-term growth (76). Trappe (73) notes that the plant's response to root-pruning will vary with species, age, and physiological state of the plant.

Upon transplanting, the bare-root seedling will soon exhaust the immediately available soil moisture in its root zone. Unless the unbroken laterals elongate or new laterals form and grow (30), the plant will be forced to rely on moisture from increasingly greater distances in the soil mass. If these conditions continue, the seedling will eventually die from moisture stress. Therefore, the plant's ability to re-establish root/soil contact by elongation of existing roots and initiation of new roots is essential to transplant survival. The plant's capacity to regenerate roots is termed its root regeneration potential (RRP) (65). There is agreement that RRP in evergreens is low in summer, increases during the fall and winter, and peaks in late winter before spring bud break (64). Some have reported a second,

less prominent, peak after extension growth is completed and terminal buds are formed (Veerkamp, unpublished data; 81).

Cell enlargement is one of the plant processes most sensitive to water stress in many species. This reduction in enlargement has been related to the decrease in plant turgor pressure that usually accompanies water stress (20). Turgor provides the physical force needed for cell growth. This has been observed in the case of roots which grow in the face of a constant back pressure from the soil. It would seem, therefore, that a plant's ability to maintain cell turgor above the critical level for growth would be an important factor in transplant survival, especially at times of the year when the plant's innate RRP is low, when environmental conditions are such that the plants are active and rapidly transpiring, and when root-pruning is done after lifting.

Little is known about the importance of this critical turgor on transplant survival. Annual fluctuations in water potential have been recorded in undisturbed trees (20). Day (5) noted that November-lifted transplants did not regenerate roots and had low tissue water potentials 40 days after transplanting. A number of authors have shown that low soil water potentials inhibit root growth in transplants. The experiments presented here were designed primarily to (1) study the shoot water potential of Douglas-fir following transplanting (four weeks) at different times of the year; and (2) relate water potential to seedling rooting potential and subsequent survival. A secondary objective was to determine the effect of root-pruning on the above.

Materials and Methods

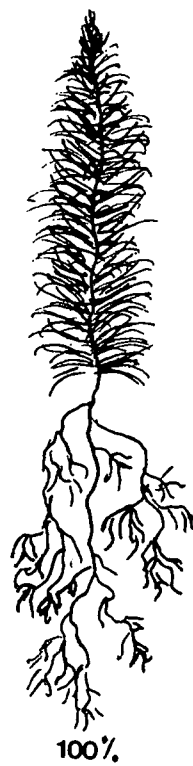
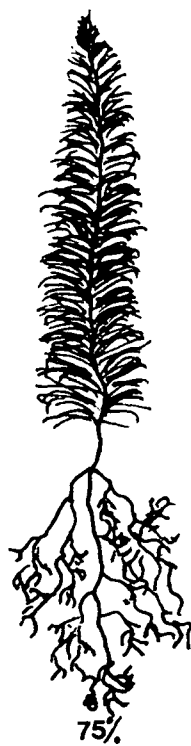
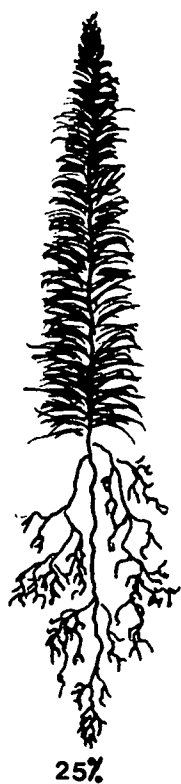
Experiment 1

Rooting and Water Potential Six-month-old greenhouse-grown Douglas-fir plug seedlings, of a uniform genetic source, grown in 250 ml Weyco plugs, were shipped to Oregon State University in June, 1975, and kept under natural conditions at the Oregon State University greenhouses. The trees were watered daily and fertilized twice a week with a dilute, complete nutrient solution during the summer months. With the onset of winter rains, watering and fertilizing were terminated. Temperature and humidity were recorded daily.

On August 4, 1975; November 17, 1975; February 2, 1976; and May 3, 1976; 126 trees were selected at random from the stock described above. On each of these dates, the number of active roots longer than one centimeter, shoot and root activity, and shoot water potential were recorded for six of the trees. Midday water potential was measured with the pressure bomb (51).

The remaining 120 trees were washed free of debris, and divided into five groups (24 per group). Each group was then root-pruned to remove approximately 0, 25, 50, 75, or 90% of their root volume below the cotyledonary scar, respectively (Fig. 1). These seedlings were then transplanted at random into plastic trays filled with washed and sifted greenhouse grade perlite, and watered to field capacity with 1/2 strength Hoagland's solution. These trays were placed into a growth chamber under a 16-hour photoperiod, temperature of $21 \pm 1^\circ\text{C}$ day and $14 \pm 1^\circ\text{C}$ night, humidity of $70 \pm 15\%$ and light intensity at plant height of 6,456 lx. Light was provided by six fluorescent and four

Figure 1. Root-pruning treatments. Percent
(by volume) of roots pruned.



incandescent bulbs. The seedlings remained under these conditions for four weeks.

At weekly intervals, six seedlings from each pruning treatment were lifted at random for determination of shoot water potential. Shoot activity and the number of new unsubsized (white) roots greater than one centimeter in length were recorded.

The data were analyzed as a completely randomized factorial design with four seasons, five pruning levels and four sampling dates.

Survival At the same times seedlings were placed in the growth chamber, 250 six-month-old Douglas-fir plug seedlings were randomly selected from the remaining seedlings described in Experiment 1. The seedlings were lifted from their containers and their roots washed free of debris; divided into five groups (50 per group) and root-pruned. They were then planted randomly into a raised sawdust bed, located in an enclosed container yard at the OSU West Greenhouses. The seedlings were watered three times daily (10 a.m., 2 p.m., 6 p.m.), for five minutes by overhead sprinklers and fertilized weekly with a dilute, complete nutrient mix during the summer.

On August 20, 1976, approximately one year after the first group was planted, the seedlings from the four lifting dates were again lifted and examined for survival, the presence of new white roots, and shoot activity. The trees were considered dead if more than 50% of the needles had browned and if root and shoot growth was absent. Data was analyzed as a completely randomized factorial with four seasons and five pruning levels.

Experiment 2

Rooting and Water Potential Three-year-old field planted Douglas-fir seedlings, of a uniform genetic source from Weyerhaeuser Jefferson Nursery were hand dug and shipped to Oregon State University in March, 1975. They were planted immediately in a raised sawdust bed located in an enclosed container yard at the OSU West Greenhouses. The trees were watered three times daily (10 a.m., 2 p.m., 6 p.m.) for five minutes and fertilized weekly with a dilute complete nutrient mix during the summer months.

On August 6, 1975; November 19, 1975; February 4, 1976; and May 5, 1976; 126 trees were selected randomly from the sawdust bed described above. Six of these were examined immediately and their number of active (white) roots, shoot water potentials and shoot elongation were recorded. Water potential was measured as in Study 1A. The remaining 120 trees were divided into five groups of equal size, their roots washed, root-pruned as previously described and transplanted into a second raised bed located close to the first one. These trees were watered as described above. The trees remained in this bed under natural conditions for four weeks.

During this time, at weekly intervals, six trees from each root-pruning treatment were selected randomly and their shoot water potentials and phenology recorded. At four weeks time, the number of new (white) roots was also recorded.

Survival On the same dates as above, three-year-old 150 Douglas-fir were lifted from the raised sawdust beds and their roots washed free of debris. They were then divided into five groups, root-pruned, and transplanted as previously described.

On August 20, 1976, approximately one year after the first group was lifted, the transplants from each lifting date were again lifted and examined for survival, the presence of new white roots and shoot elongation. Criteria for survival are described in Experiment 1.

Results

Environmental Conditions

Average monthly air temperatures varied from 18°C to 6°C, in the storage area during the course of these experiments (Table 1). Soil temperatures in the outdoor sawdust beds at a six inch depth were lowest in February and highest from July to August (Table 1).

A severe wind storm occurred approximately two weeks after the February lifting date and uprooted a number of the top heavy 90% pruned trees. Some root exposure also occurred on the other trees, in particular those 50 and 75% pruned. The trees were righted the morning after the storm.

Growth Activity of Seedlings at Time of Lifting

Root and shoot activity of undisturbed seedlings varied for each lifting date and was similar throughout the study for the six-month-old and three-year-old seedlings. In early August, when the first seedlings were lifted, shoot elongation had ceased and buds were set. The root system, however, was very active, as evidenced by large

Table 1. Climatic conditions of outdoor sawdust beds.

MONTH	AVERAGES				
	AIR TEMPERATURE DAILY MAXIMUM C°	AIR TEMPERATURE DAILY MINIMUM C°	AIR TEMPERATURE MONTHLY C°	SOIL TEMPERATURE 15cm MONTHLY C°	RELATIVE HUMIDITY 4 p.m. MONTHLY %
A	25	11	18	20	34
S	27	9	18	20	21
O	17	7	12	13	72
N	12	3	7	7	81
D	10	2	6	6	89
J	9	2	6	5	81
F	10	2	6	4	64
M	12	2	6	6	51
A	15	3	9	8	48
M	20	5	13	13	53
J	21	8	19	17	35
J	27	11	18	20	30

numbers of white root tips. By mid-November, the buds were tightly closed, but scale enlargement appeared complete, and only a few white root tips were visible, demonstrating a reduction in root growth.

When the seedlings were lifted in early February the buds were still tightly closed and no root growth was evident. Mid-March marked the onset of vegetative bud activity; and by early May, approximately 50% of the buds had burst. Root growth at this time was minimal. By late June, extension growth was complete. Bud scale initiation was complete in July.

Experiment 1 - Root Growth and Water Potential

RRP Root regeneration at four weeks time was lowest in early May (at the time of 50% bud break), increased in August and November, and was highest in February (Fig. 2). There was a significant difference in root growth on all four lifting dates.

Root regeneration response to pruning varied with the date of lifting (Fig. 3). In August and May, pruning did not significantly influence regeneration except where 90% of the roots were removed. In November, however, this relationship changed significantly. Root-pruning, except where 90% of the roots were removed, significantly increased regeneration after four weeks time. Removing 25-75% of the root system stimulated rooting. Root-pruning of February 1-lifted seedlings significantly increased new root development: removal of 25% and 50% of the root system increased regeneration. In general, root-pruning did not change the basic seasonal periodicity of RRP observed in the controls except that severe pruning (90%) resulted in very poor regeneration.

Figure 2. The number of new roots regenerated over four weeks time in six-month-old, bare-root transplant Douglas-fir seedlings. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. (x) 0% pruned, (*) 25% pruned, (.) 50% pruned, (°) 75% pruned, (■) 90% pruned. $LSD_{.05} = 12.7$.

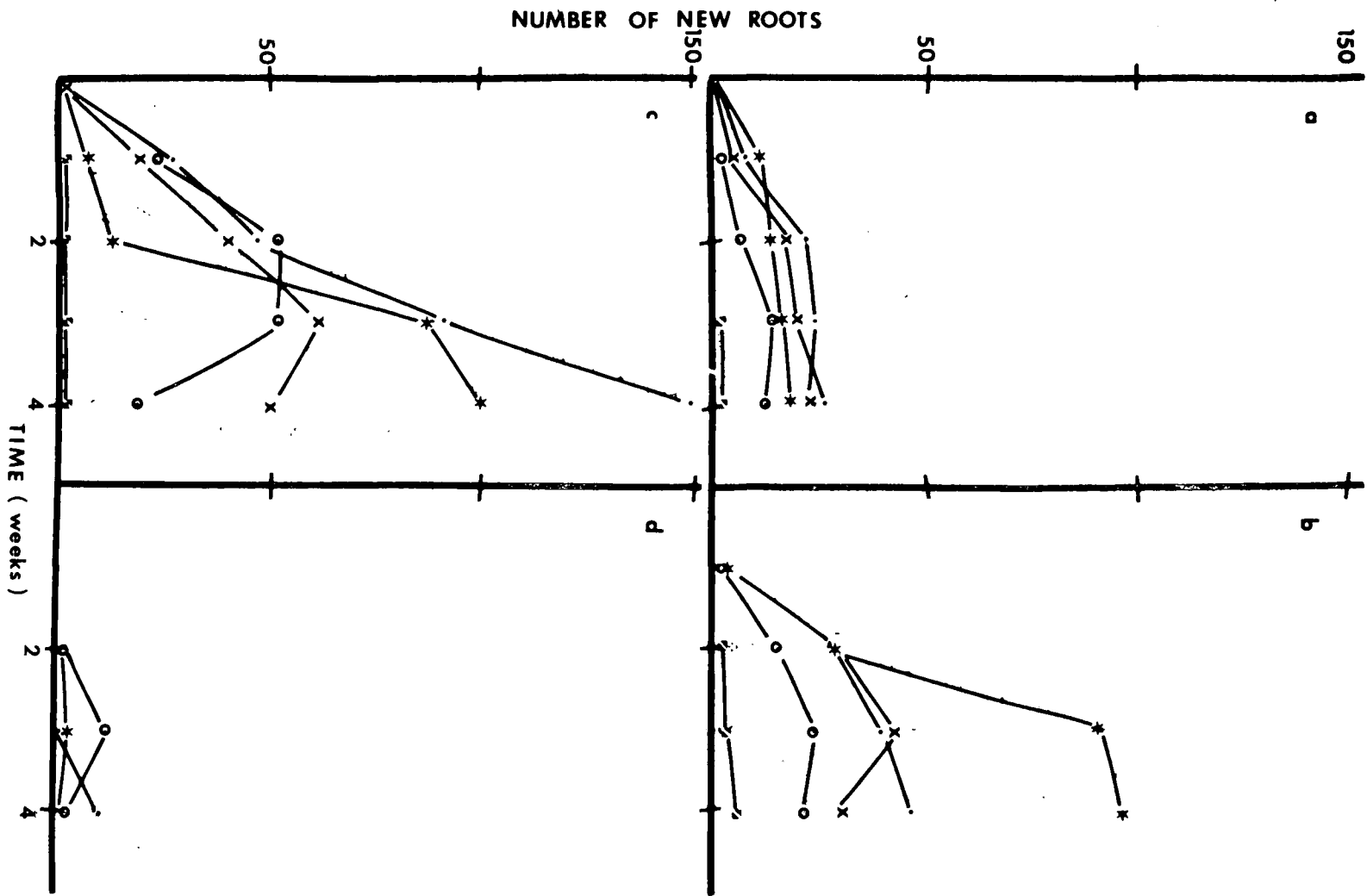
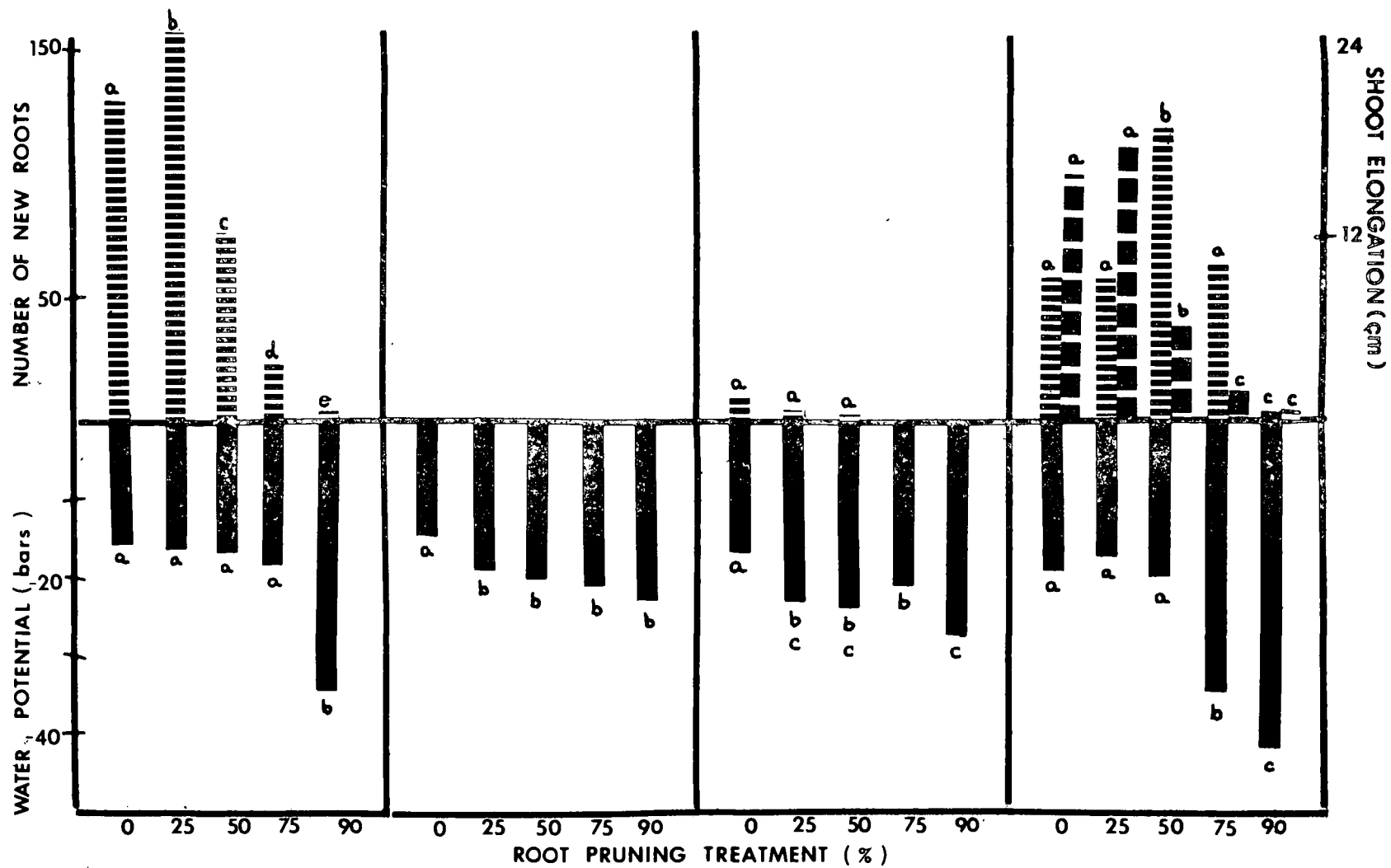


Figure 3. Average water potential (■) the number of new roots (||||) and shoot elongation (||■) at four weeks time in six-month-old, bare-root transplant Douglas-fir seedlings root-pruned to five levels. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. Means with different letters differ at 5% level by Duncans.

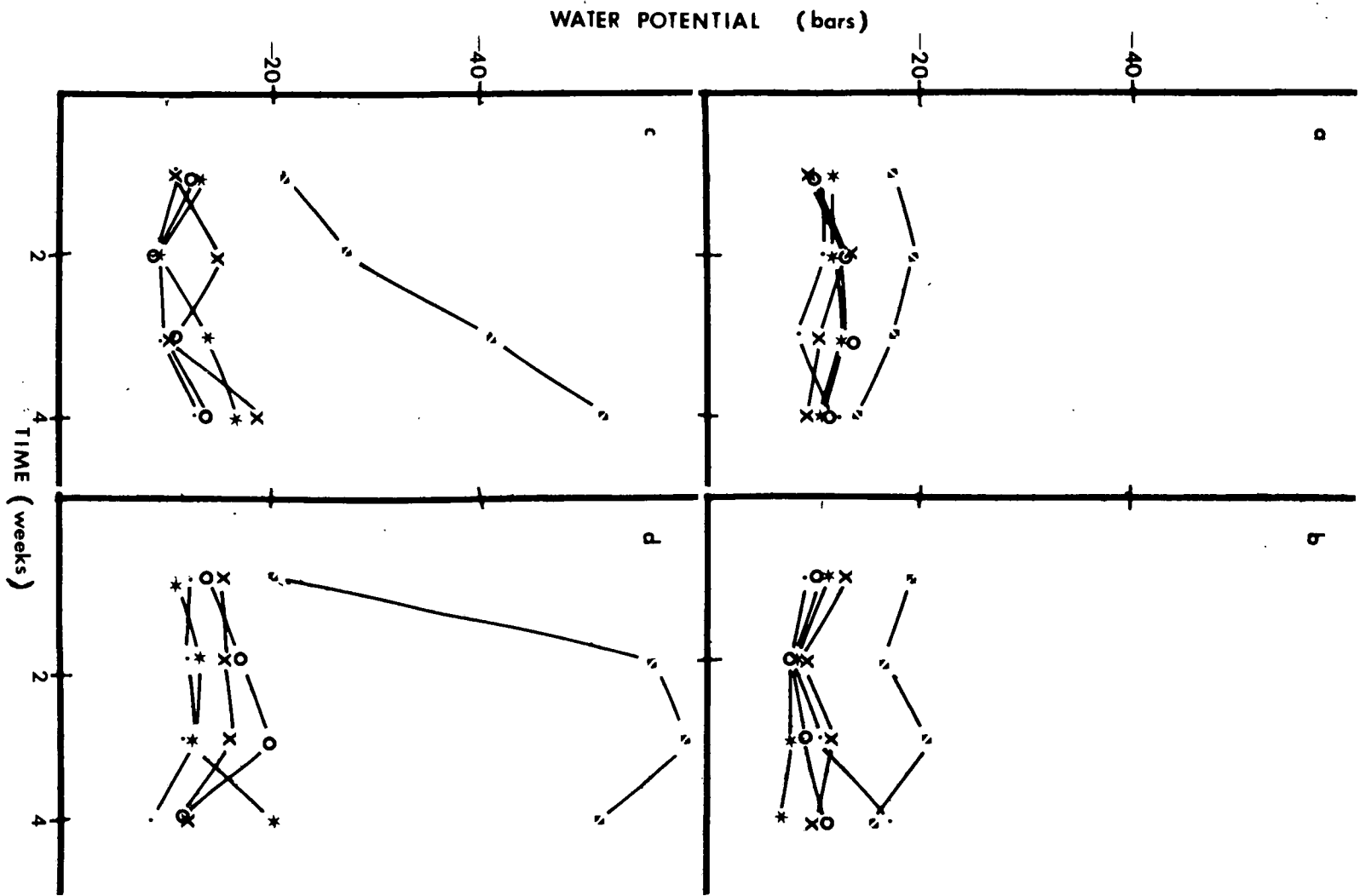


A variation in the time course of root regeneration is also apparent with season (Fig 2), and is related to survival under natural conditions ($R^2 = .678$). Seedlings lifted in August showed new root development in one week, except where 90% of the roots had been removed. November-lifted seedlings showed a similar response, except that unpruned and heavily pruned (75-90%) root systems were slower to respond, taking two weeks for new roots to develop. Seedlings lifted in February showed the most pronounced response to root-pruning with all treatments showing new root development in one week. In May, all but the most severely (90%) pruned seedlings developed roots in three weeks.

Water Potential: Water potential did not differ significantly during the four weeks following pruning treatment, except that severely pruned (90%) seedlings lifted in February and May showed highly significant fluctuations over the four week period (Fig 4). The effect of root-pruning on water potential varied only in the severely (90%) pruned seedlings, with May-lifted ones showing the most pronounced effect (Fig 3).

Shoot Growth Unpruned seedlings lifted in August and February and placed in the growth chamber showed bud break after two weeks; and in four weeks time had elongated an average of six centimeters and three centimeters, respectively (Fig. 3). Root-pruning of up to 50% of the root system had little effect on these growth responses in August-lifted trees. Heavy pruning (75 and 90%) delayed bud break and reduced extension growth. A similar delay in bud break and reduction in extension growth was seen in heavily pruned February-lifted seedlings.

Figure 4. Water potential over four weeks time in six-month-old, bare-root transplant Douglas-fir seedlings. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. (x) 0% pruned, (*) 25% pruned, (.) 50% pruned, (o) 75% pruned, (■) 90% pruned. $LSD_{.05} = 5.4$.



Fifty percent of the seedlings had burst bud when the seedlings were lifted in May. One week after transplanting, all but the most heavily pruned seedlings (75 and 90%) had burst bud. By four weeks time 0-50% pruned seedlings had elongated an average of 2-3 centimeters. Many of the heavily pruned seedlings failed to break bud and those that did showed significant reductions in extension growth. November-lifted seedlings failed to break bud.

Study 2 - Root Growth and Water Potential

Root Growth The average number of roots regenerated from unpruned 3-year-old seedlings at four weeks was minimum in November, and February, doubled in May, and was maximum in August (Fig. 5).

The effect of root-pruning on root regeneration varied with the date of lifting. Heavy (75-90%) root-pruning significantly decreased rooting in August and very severe (90%) pruning reduced rooting significantly in May. A significant increase in root regeneration was seen in May- and August-lifted seedlings with their roots pruned 50 and 25%, respectively. No roots regenerated in November-lifted trees.

Water Potential Some significant variation in water potentials during the four week period following pruning treatments is apparent, most notably in the August- and May-lifted seedlings (Fig. 6).

Root-pruning above 75 and 50% in August- and May-lifted seedlings, respectively, significantly reduced ψ_p . Reductions were also seen in the pruned November- and February-lifted seedlings (Fig. 5). A close correlation ($R^2 = .833$) was found between water potential readings at four weeks time and survival of similar outplanted seedlings.

Figure 5. Average water potential (■ ■ ■ ■), and the number of new roots (|||||) and shoot elongation (■ ■) at four weeks time in three-year-old, bare-root transplant Douglas-fir seedlings root-pruned to five levels. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. Means with different letters differ at 5% level by Duncans.

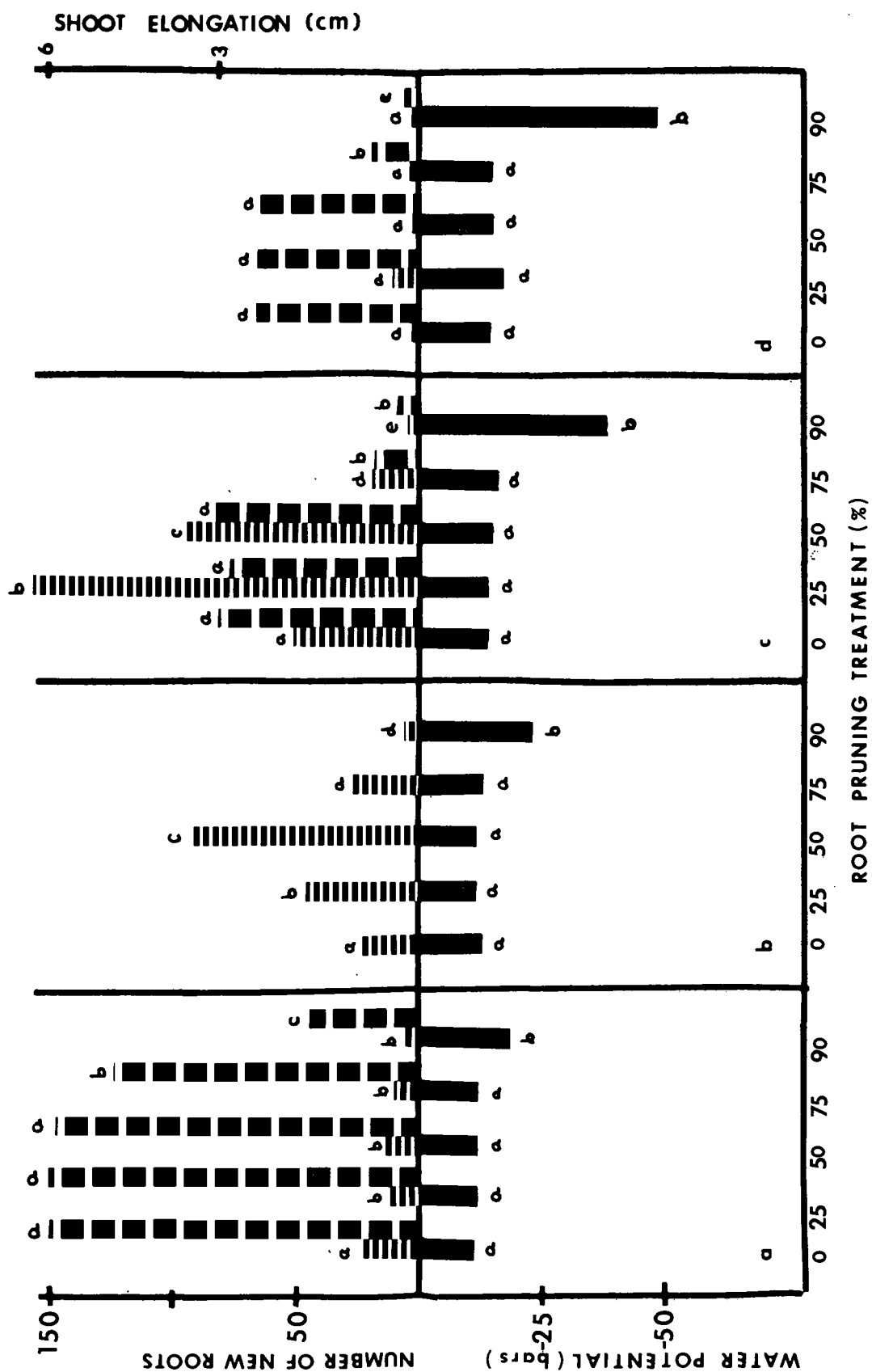
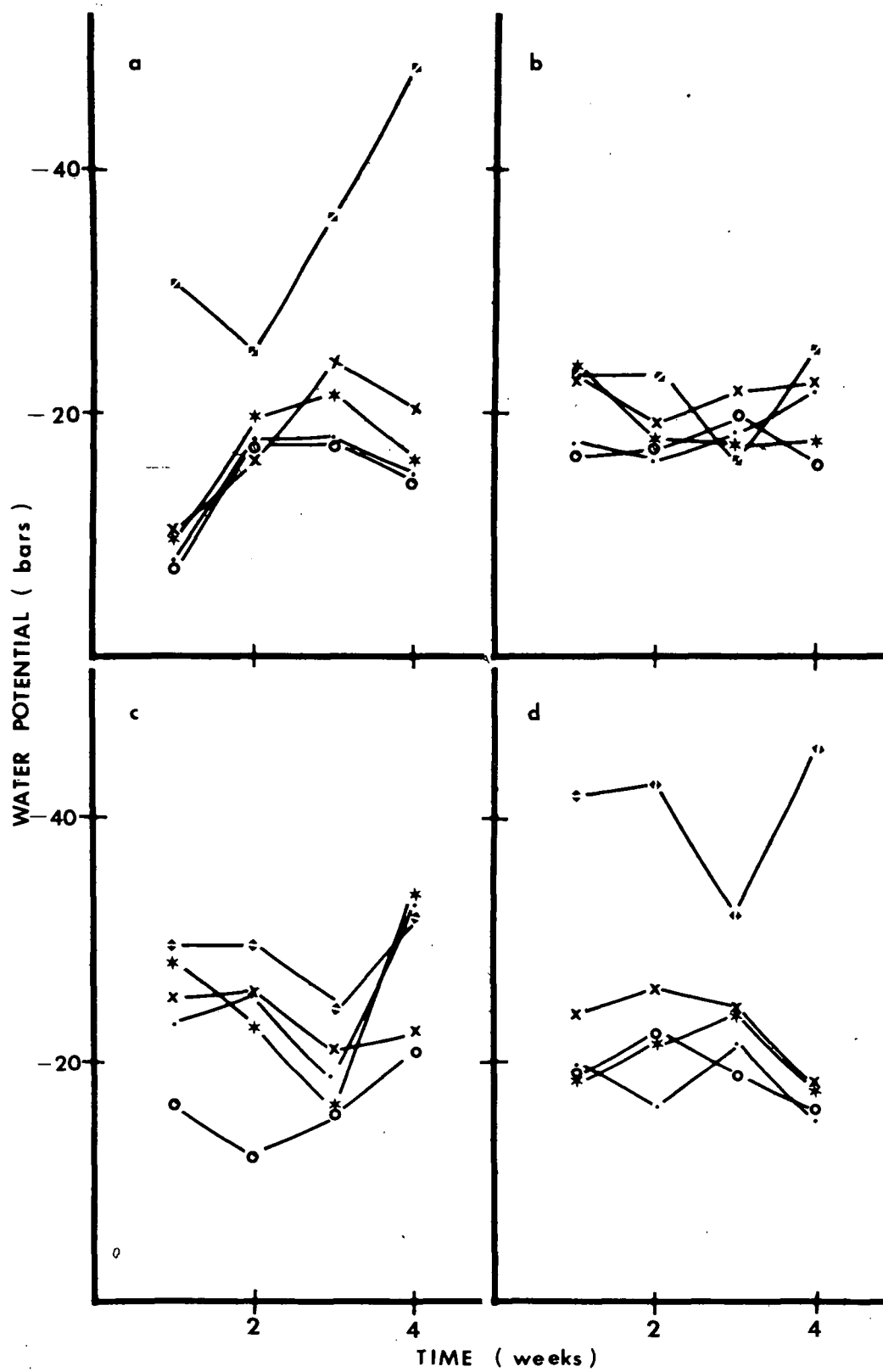


Figure 6. Water potential over four weeks time in 3-year-old bare-root transplant, Douglas-fir seedlings. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. (x) 0% pruned, (*) 25% pruned, (·) 50% pruned, (°) 75% pruned, (■) 90% pruned. $LSD_{.05} = 4.9$.



Shoot Growth Buds were not active when the trees were lifted in August, November, and February; and no bud activity was noted during the four weeks following transplanting (Fig. 5). Bud break, in May was complete in all but the 75 and 100% pruned May-lifted seedlings a week after planting. Many of these heavily pruned trees did not break bud. Extension growth at four weeks time in the 0 and 25% pruned seedlings was 16 to 18 centimeters, but was significantly less in trees more heavily pruned.

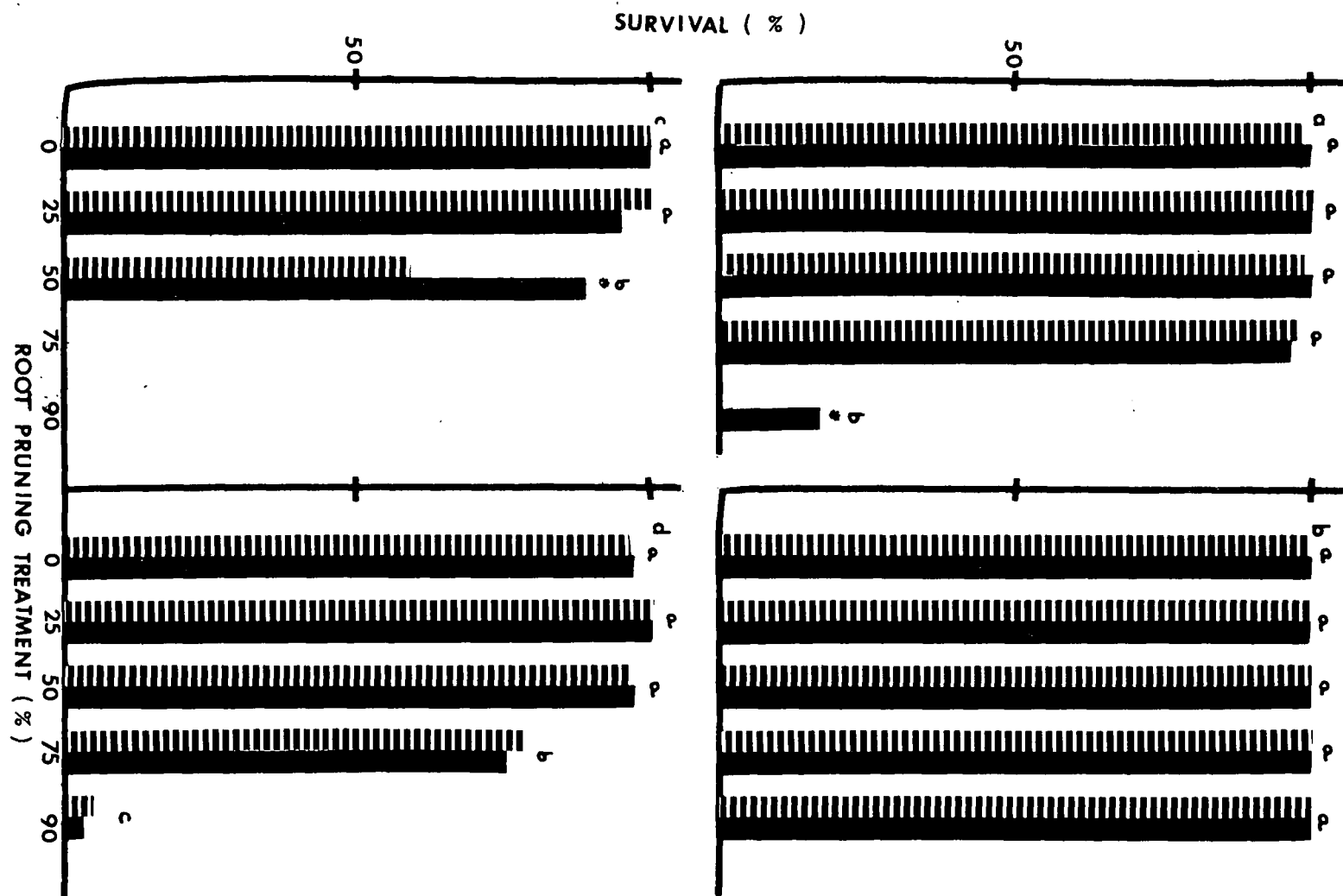
Experiment 1 and 2 - Survival

Survival Generally there were no differences in the basic pattern of survival between the two ages of seedlings, nor in the survival of the unpruned seedlings lifted at different times of the year (Fig. 7). August-lifted seedlings survived up to 75% root-pruning without reductions in survival; however, very severe (90%) root-pruning significantly reduced seedling survival. Decreases in survival in severely pruned (75 and 90%) May- and February-lifted seedlings were evident.

Discussion

The seasonal pattern of RRP found in the six-month-old seedlings transplanted into the growth chamber closely resembles that reported for Douglas-fir by Stone (64) and others (33, 81). The time course of root regeneration also varied with season, indicating a physiological variation not only in the numbers of new roots produced but also in the speed with which they developed.

Figure 7. Average survival of six-month-old (||||) and 3-year-old (■) bare-root transplant, Douglas-fir seedlings root-pruned to five levels. a) August-lifted, b) November-lifted, c) February-lifted, d) May-lifted. An * indicates six-month-old and three-year-old means differ significantly at 5% level by LSD. Means with different letters differ at 5% level by Duncans.



The seasonal pattern of new root growth in the 3-year-old seedlings transplanted into the sawdust beds was similar to those reported by others (Veerkamp, unpublished data; 33, 64, 65, 81). Soil temperature fluctuations are often offered as an explanation for this periodicity. Lavender (37) observed that both shoots and roots grew most favorably at a constant 21°C temperature and that root growth decreased as temperature decreased. Soil temperature in the sawdust bed was lowest in February and November. Root regeneration was also absent or nearly so on these dates. However, the fact that there was some growth in February but none in November cannot be explained on the basis of soil temperature alone. Others (Veerkamp, unpublished data; 42) have shown a periodicity regardless of soil temperature. The fact that the six-month-old November-lifted seedlings failed to break bud when placed under ideal growth chamber conditions indicates that these seedlings, and possibly the three-year-old seedlings, were still in rest at this time. It has been suggested that a stimulus from the bud is one factor that determines the RRP of a plant (19). If this stimulus was not released in the dormant November-lifted trees, it could account for their lower rootability than the February-lifted trees.

The effect of root-pruning on root regeneration in both the six-month-old and 3-year-old seedlings varied significantly between lifting dates. The seasonal differences in the 3-year-old transplants can no doubt be explained, in part, by environmental fluctuations. However, similar variation was noted in the six-month-old seedlings. A number of explanations are possible. No attempt was made in this

study to monitor the root-shoot ratios of unpruned or pruned seedlings. It is known that the root-shoot ratio best suited for survival of a transplant is dynamic, varying with the environment, plant vigor and age, and physiological state at the time of planting (30). Therefore, the magnitude of the effect of a standardized root-pruning treatment on the root/shoot ratio of seedlings of two different ages (with different optimum root/shoot ratios) would also vary. Veerkamp (unpublished data) noted that the ability of certain segments of the root system to regenerate new roots after transplanting varied with lifting date and with degree of root-pruning. Though no specific conclusions can be reached from his data it definitely points out the need for further work in this area.

The effect of root-pruning on tissue water potentials of both seedling types varied significantly with lifting date. In the case of the six-month-old seedlings, the differences were most significant in those heavily pruned (95%) indicating that an intact root system is not especially important for maintaining a "healthy" water potential over a short period of time, if the environment is ideal and the seedling young. The three-year-old root-pruned seedlings showed a noticeable decrease in water potential in most cases, even after light pruning. The less than ideal growing conditions and age of the seedlings, resulting in a lower shoot/root ratio, are probably explanations for the greater sensitivity of these seedlings to root-pruning.

Survival of the six-month-old and 3-year-old Douglas-fir transplants in the outdoor sawdust bed was not significantly different for the two ages (Fig. 7). The success of those severely pruned was

surprising. The survival of unpruned seedlings transplanted into forest sites varies significantly with season (62). In this study, only trees missing 75% or more of their root system showed this variation. The moist and somewhat sheltered conditions of the sawdust beds were no doubt partially responsible for the excellent survival of the unpruned trees.

The correlations found between water potential and survival of 3-year-old transplants ($R^2 = .833$) and between the time to appearance of first roots and survival of six-month-old transplants ($R^2 = .678$) support the hypothesis that a transplant's ability to withstand water stress and regenerate new roots is important for its survival. In all months except November, when the seedlings were in rest, a combination of root growth and a fairly high (20) water potential was found to give 80% survival. A study monitoring root activity and water potentials in fall and winter transplants through the period of active spring growth is needed to resolve this question. Data on variations in critical water stress with season would enable foresters and nurserymen to establish critical water stress values and handle conditions accordingly.

PRECONDITIONING OF DOUGLAS-FIR SEEDLINGS
TO DROUGHT STRESS¹

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Additional index words: drought resistance, root-growth, survival,
water potential

Abstract. Six-month-old Douglas-fir seedlings were subjected to eight drying cycles in which the seedlings were allowed to dry to a -15 bar water potential before watering. Root growth, water potential, and survival of transplants were measured under stressed and control conditions. Water potential, soil moisture content and survival of seedlings subjected to a prolonged drying period were recorded. Preconditioning treatments were effective in increasing drought resistance under stress conditions. Reductions in shoot elongation, dry matter production, and early bud set were noted. Though inadvertent watering of the transplants made conclusions impossible, survival data and data collected prior to the watering indicate that root growth and survival were improved by the treatment.

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PRECONDITIONING OF DOUGLAS-FIR SEEDLINGS

TO DROUGHT STRESS

Introduction

Desiccation is one of the most important causes of death in bare-root transplants. Preliminary studies have shown that a relation exists between xylem water potential during the first four weeks after transplanting, and transplant survival. It was suggested that maintenance of water potential above the level inhibitory to growth, until new root growth occurs, is critical for plant survival.

When a plant is bare-root transplanted, a good many of its elongating laterals, and thus water absorbing surfaces, are lost. The practice of root-pruning often reduces root surfaces even further. The transplanted seedling will soon exhaust the immediately available soil moisture. If this condition is not alleviated, transpiration in excess of water uptake will cause a sharp decrease in water potential, and turgor will be lost. Turgor pressure is a determining factor in cell initiation and growth, processes required for root extension. It would seem then, particularly at times of the year when root regeneration potential (RRP) is low and regeneration slow, that the ability to withstand moisture stress, and hence maintain turgor pressure, would be important for transplant survival.

A common method of preconditioning plants to water stress is controlled watering. Though this practice is generally accepted even in large scale production, evidence that it is successful is almost entirely empirical. Data supporting the method do exist but most are indirect and concern the aftereffects of water stress on the plant. Many investigators have reported changes in transpiration,

stomatal response, respiration, growth rate, leaf anatomy, and internal plant water relations that linger long after moisture stress has been removed (4, 10, 31, 32, 39, 40, 54, 58, 59, 71, 78, 84). Proponents of this method state this as evidence that pre-stressing does result in a useful adaptation of the plant to its environment. This position is supported by the findings of Unterscheutz et al. (75), Helkuist (16) and Bannister (2); working with Douglas-fir, Scots Pine, and Heath, respectively. They found that seedlings from a wet or shaded environment closed stomates more rapidly than those from drier, more exposed habitats.

The objectives of this study were 1) to determine whether Douglas-fir seedlings can be preconditioned to resist water stress (avoidance and tolerance) by reduced watering; 2) to determine the effects of this preconditioning treatment on shoot elongation and growth, dry matter production and plant water potential during treatment; 3) to determine whether the cultural practices of reduced watering and root pruning improve the root regeneration and survival of summer-lifted bare-root transplants.

Materials and Methods

Plant Materials

Douglas-fir seeds were sown in January, 1976, in 50 ml Weyco plugs filled with a peat-vermiculite mix (1:1, vol.), and grown in the Weyerhaeuser greenhouse in Rochester, Washington. On April 7, 1976, they were moved to the Oregon State University Greenhouse in Corvallis, Oregon. Greenhouse temperatures were 21°C day and 15°C night. Seedlings were watered daily and fertilized twice a week with a dilute complete nutrient solution. Air temperature, humidity and Belani Plate

evaporation were monitored at plant height throughout the study.

Pre-Treatment

On April 19, 1976, the seedlings were divided equally into two groups. One was maintained for nine weeks under a regime of restricted watering (stress). Seedlings in this group were watered only when the soil had dried to the point where the average midday water potential of four randomly selected seedlings was close to -15 bars. Watering, when necessary, was done carefully so that all parts of the soil plug were wetted. Seedlings were also fertilized at this time with a dilute complete nutrient solution.

The intention of this treatment was not to maintain soil water potential within narrowly defined limits (which would require frequent light watering); but to maintain an average level lower than the controls, but not injurious to the tree. Operationally such a treatment would be feasible.

The second group of seedlings was grown under the same greenhouse conditions, but with daily watering, and the same fertilization regime as above.

These treated seedlings are used in all data collections to follow.

Data Collections

Plant Height and Bud Set Heights were measured (from the top of the plug) and bud set recorded weekly for fifty seedlings (five randomly selected rows of ten) from each treatment. Measurements began on April 7 and were discontinued on July 3.

Treatment Conditions Midday plant water potential was measured on four seedlings per treatment before and after each drying cycle for

both control and stress trees. Soil was extracted from each of the four tubes, and wet and oven dry weights were recorded.

Post-Treatment Plant Weights At the end of the treatment period, the oven dry weights of the tops and roots of fifty seedlings (five randomly selected rows of ten) were recorded.

Root Growth and Water Stress After Transplanting At the end of the treatment period, 320 seedlings were selected randomly from each pre-treatment. One-half of these were root pruned to remove 50% of the root system (extracted plug cut half way up), while the root system of the second group was left intact. One half of the seedlings from both of these groups were transplanted into sand in a specially prepared high humidity chamber (12' x 4' x 6'); and the other half into sand in a dry, low humidity chamber. Each bed was divided into four blocks. Five trees from each stress treatment/pruning level class were planted per block so that there were twenty seedlings per treatment per pruning level within each block at two inch spacing. Conditions were maintained in the dry environment by reduced watering and with four household circular fans with rotating heads located in the corners of the bed. Their rotational cycle was set to give maximum coverage of the bed with the least overlap. Conditions in the moist sand environment were maintained by daily watering, and a jet-spray fogger timed to come on for one minute every fifteen minutes under a partially open transparent polyethylene tent. Air temperature, humidity, and Belani Plate evaporation were monitored over the beds.

At weekly intervals during the four week treatment, twenty seedlings from each treatment/pruning level/bed class (five from each replicate) were lifted and their xylem water potential measured with a

pressure bomb (51). All new roots were counted, then excised and oven dried and weighed.

Post Transplant Survival in Pots Two hundred forty seedlings were selected from each pre-treatment. One half of each group was root pruned to 50% (as above) and the other half left intact. These were then transplanted in 0.6 litre cylindrical pots. One half of each stress treatment/pruning level group was placed in the moist environment and one half in the dry environment previously described (groups of twenty randomized within three blocks). Seedlings in the moist environment were watered daily and those in the dry environment were watered infrequently in order to induce some drought injury after two months. Daily watering was then resumed in all treatments until it became evident which seedlings would live and which would die. Survival and bud appearance were recorded.

Drought Duration Test:Short Term Response One hundred ninety-two seedlings from each pre-treatment were watered to field capacity and placed in four blocks (32 seedlings of each treatment, with treatment positions randomized within a block) in a growth chamber at 23°C and 50% RH with a 12-hour photoperiod. For the next three weeks, seedlings were allowed to dry out. At 0, 5, 8, 12, 15, and 19 days after the last watering, five randomly selected seedlings were lifted from each treatment and block and bud appearance, water potentials, and fresh and dry weights of homogenized soil samples were recorded.

Drought Duration Test:Long Term Survival On the same occasions as described above, another three seedlings from each block and treatment were selected and their bud appearance recorded. The seedlings were watered to field capacity, transplanted into liter pots, and put

in a recovery area under greenhouse conditions. Seedlings were arranged in four blocks, as above. After two months, survival and root and shoot injury were recorded.

Analysis of variance was used to analyze the data.

Results

Environmental Conditions Temperature, humidity, and Belani Plate readings from the greenhouse and the wet and dry environments within the greenhouse are shown in Figure 8. Maximum temperatures increased during the spring and summer. Maximum temperatures in the wet environment were significantly higher than the greenhouse temperature, especially on sunny days due to the polyethylene tenting. Relative humidity decreased during the summer, and was always higher in the wet environment than in the dry environment. Belani Plate readings follow the same pattern as temperature.

Treatment Conditions The soil water retention curve for the 1:1 peat-vermiculite mixture is shown in Figure 9.

Figure 10 summarizes the treatment conditions. Eight drying cycles are illustrated. An increase in the time it took the trees to reach -15 bars ψ_w after watering can be seen in the last three cycles.

Height Growth and Bud Set

Height growth and bud set curves are shown in Figures 11 and 12. Stressed trees set buds earlier (B_{50} advanced by approximately eight days) than the control trees and showed a decrease in height growth soon after treatment began, but nevertheless continued growing.

Post-treatment Dry Weights

The average dry weights of the roots and shoots of both stressed and control trees are shown in Table 2. Dry matter production

Figure 8. Climatic conditions of greenhouse and controlled chamber environments.

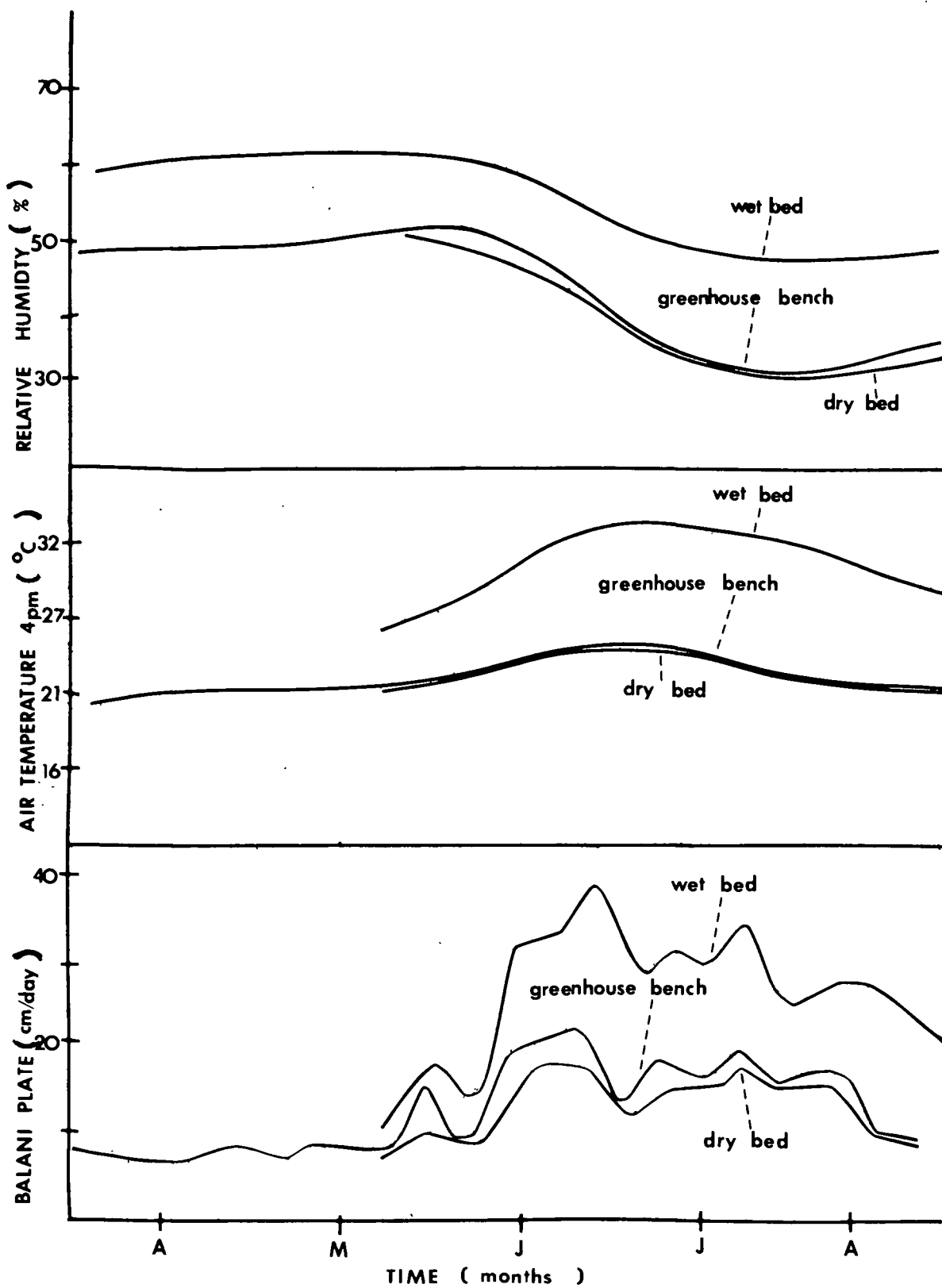


Figure 9. Soil moisture release curve for 1:1 peat vermiculite soil.

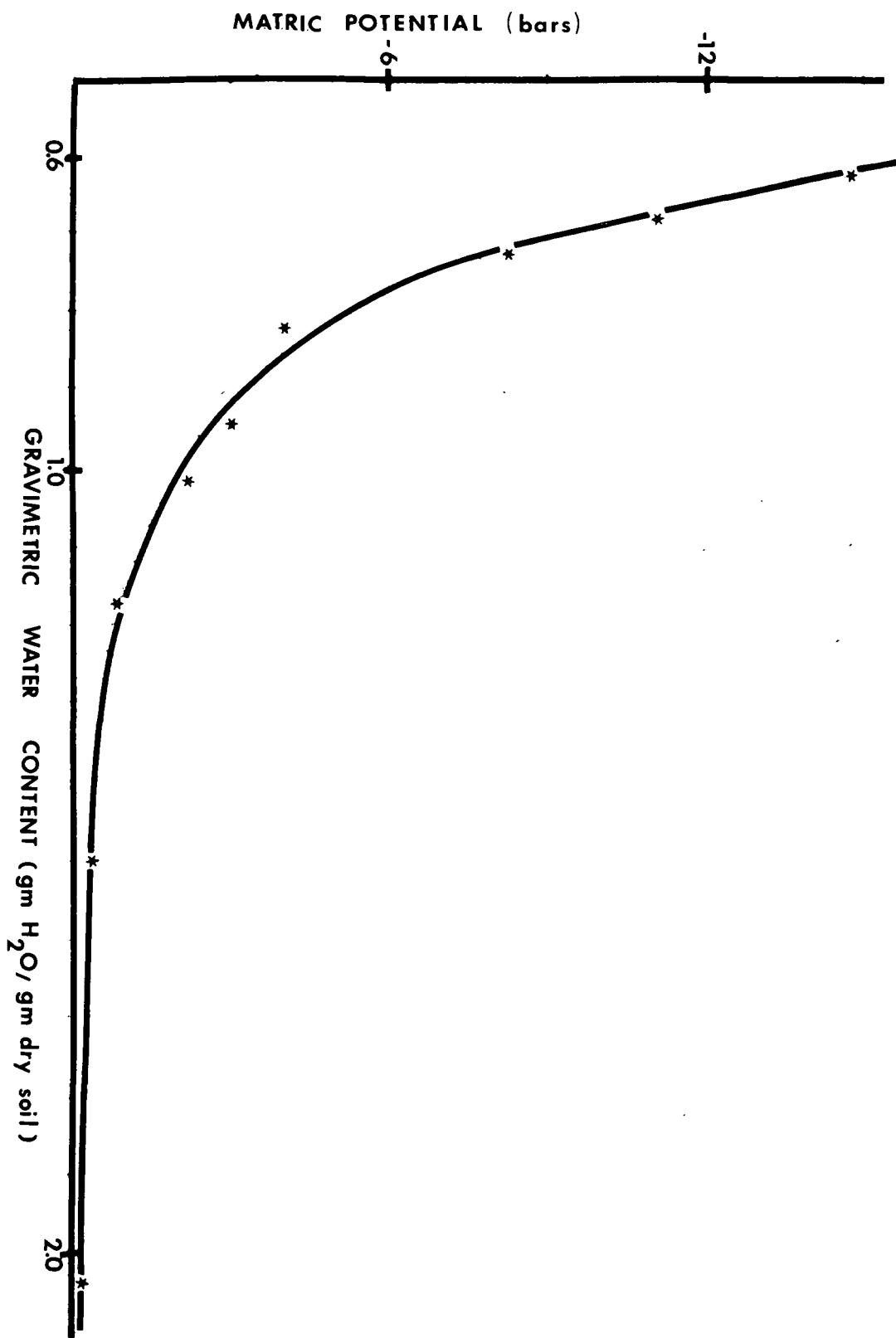


Figure 10. Treatment conditions. Eight drying cycles. Balani Plate evaporation vs. soil moisture content of a) stressed and b) control Douglas-fir seedlings.

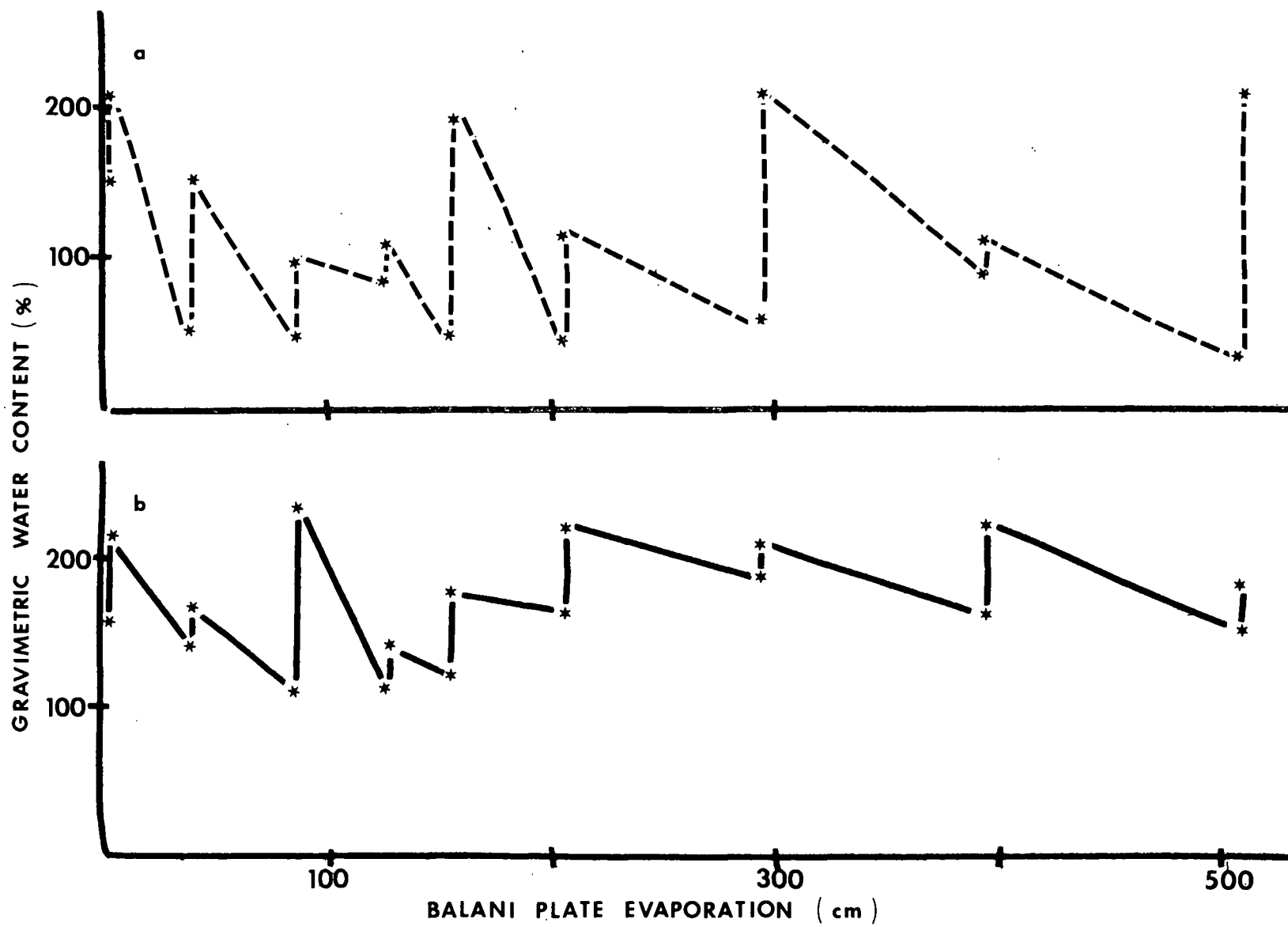


Figure 11. Height growth during treatment of control (—) and stressed (--) Douglas-fir seedlings.
LSD_{.05} = 11.2.

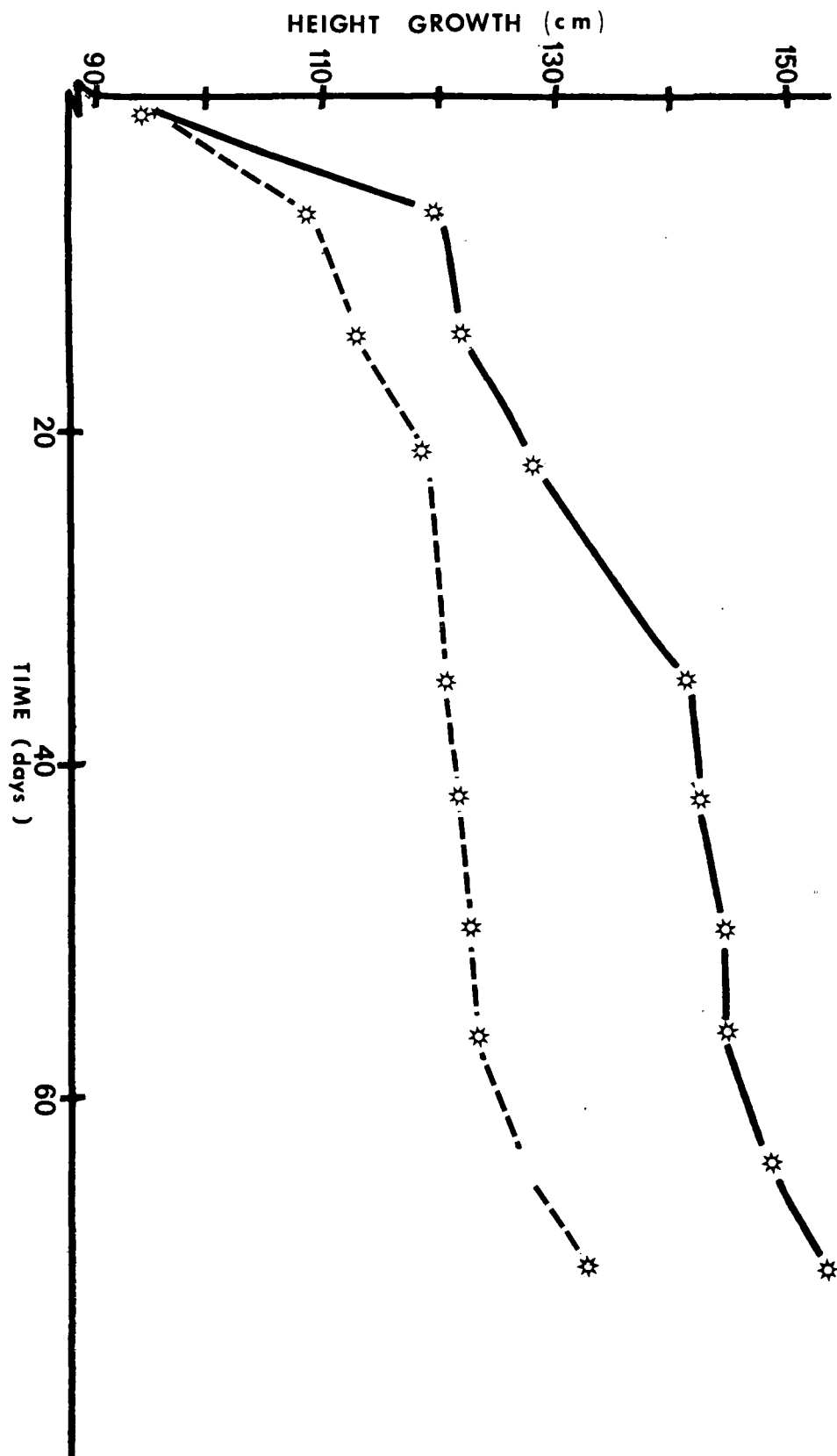


Figure 12. Bud set during treatment of control (—) and stressed (---) Douglas-fir seedlings. $LSD_{.05} = 3.2$.

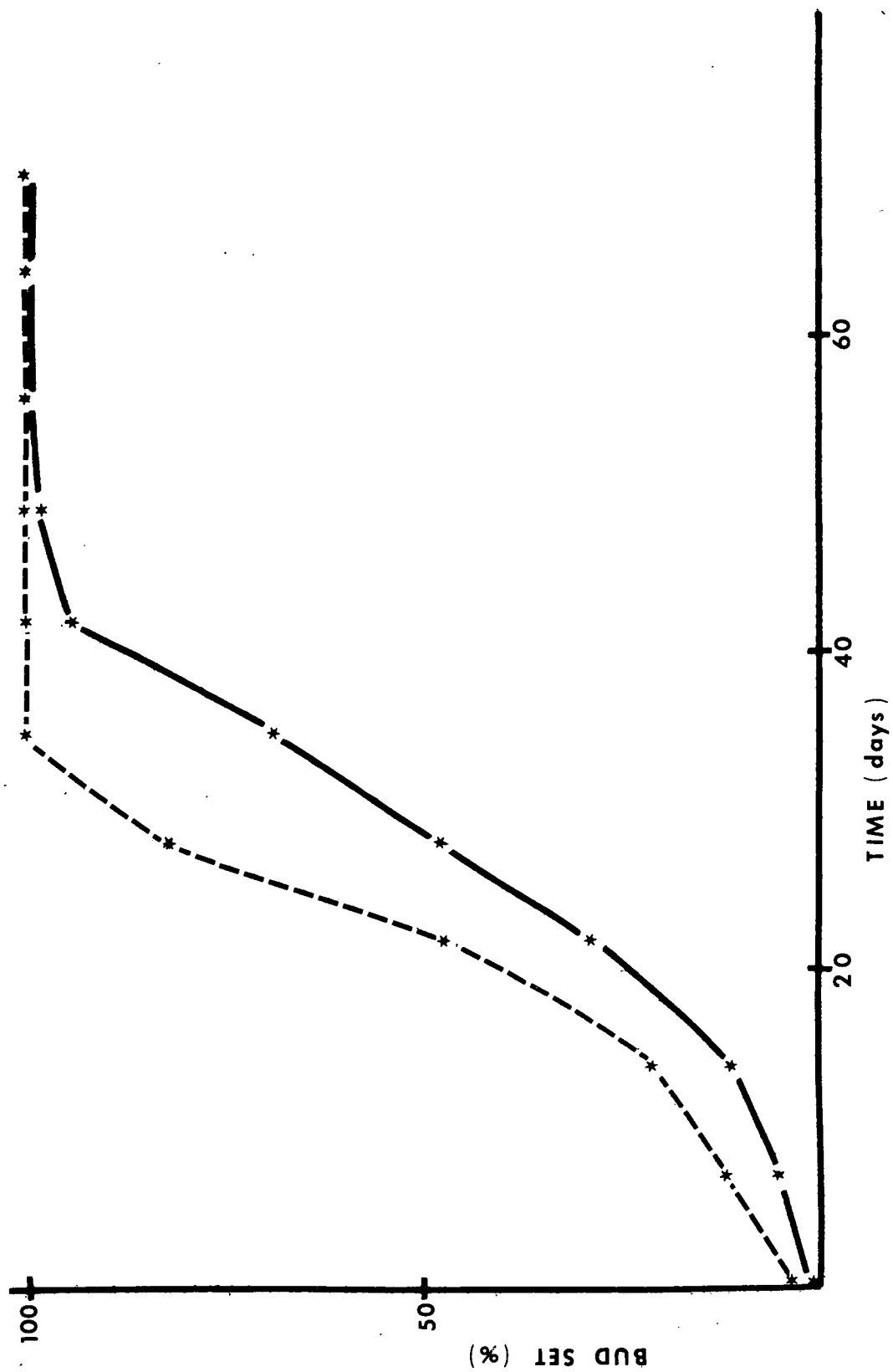


Table 2. Post treatment root and shoot dry weights and root/shoot ratios of control and stressed Douglas-fir seedlings. Means with different letters differ at the 5% level by LSD.

	CONTROL	STRESS
SHOOT	2.37 a	1.83 b
ROOT	3.91 a	1.82 b
RATIO	.71 a	1.09 b

in the pre-stressed roots and shoots was lower by 23% and 54%, respectively, in the well watered seedlings. Their shoot/root ratio was 35% higher.

Water Potential, Root Growth and Survival after Transplanting

Water was inadvertently supplied to the trees in the dry bed on the day of planting and in the middle of the fourth week. As a result, the dry bed environment was not constant during the course of the experiment, and a single analysis of variance could not be run. Data for each sampling date were treated separately.

Water Potential In general ψ increased with time in the wet environment and decreased in the dry environment. In no case did the water potential readings differ significantly between pre-stressed and control trees during the first and fourth weeks (Fig. 13). In weeks two and three, however, the pre-stressed trees showed significantly higher water potentials than the non-stressed trees. Analysis of variance has shown pre-treatment to be the most significant factor in this variation. Pruning level, bed environment and the interactions between pruning level and bed environment, and pruning level and pre-treatment were also significant. In general, water potential readings in both environments and for both levels of pruning were higher in stressed seedlings than in the controls.

Number of New Roots No consistent pattern was evident when new root growth of the pre-stressed and control trees was compared for the pruned and unpruned trees in each environment (Fig. 14). Pre-stressed trees in the dry environment did appear to have maintained an average level of root growth above that of the control trees in the same environment for the first three weeks in the bed. However, the

Figure 13. Water potential over time of control (—) and stressed (---) bare-root transplant Douglas-fir seedlings. a) wet bed, 0% pruned; b) wet bed, 50% pruned; c) dry bed, 0% pruned; d) dry bed, 50% pruned. Means with different letters differ at 5% level by LSD.

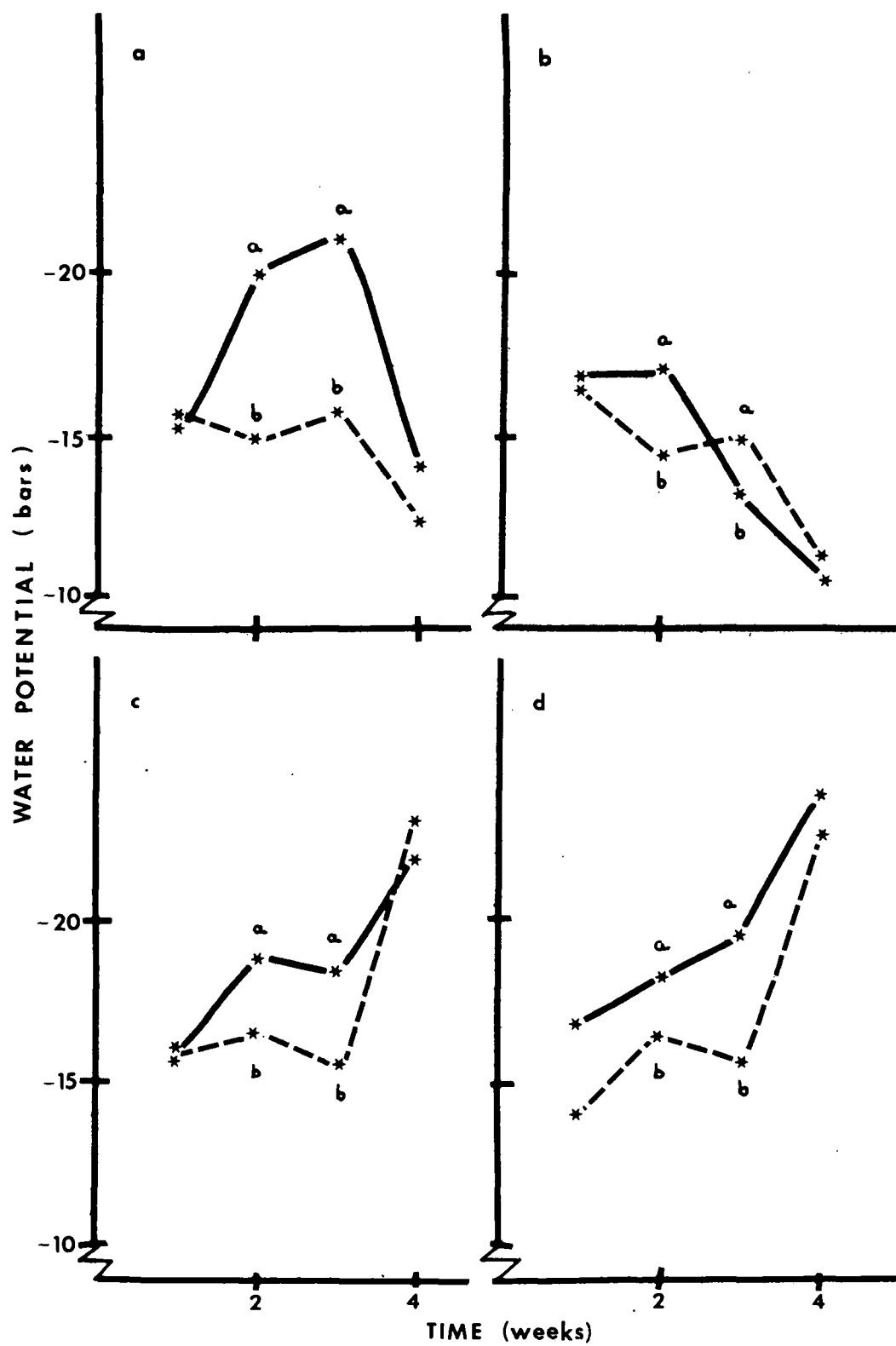
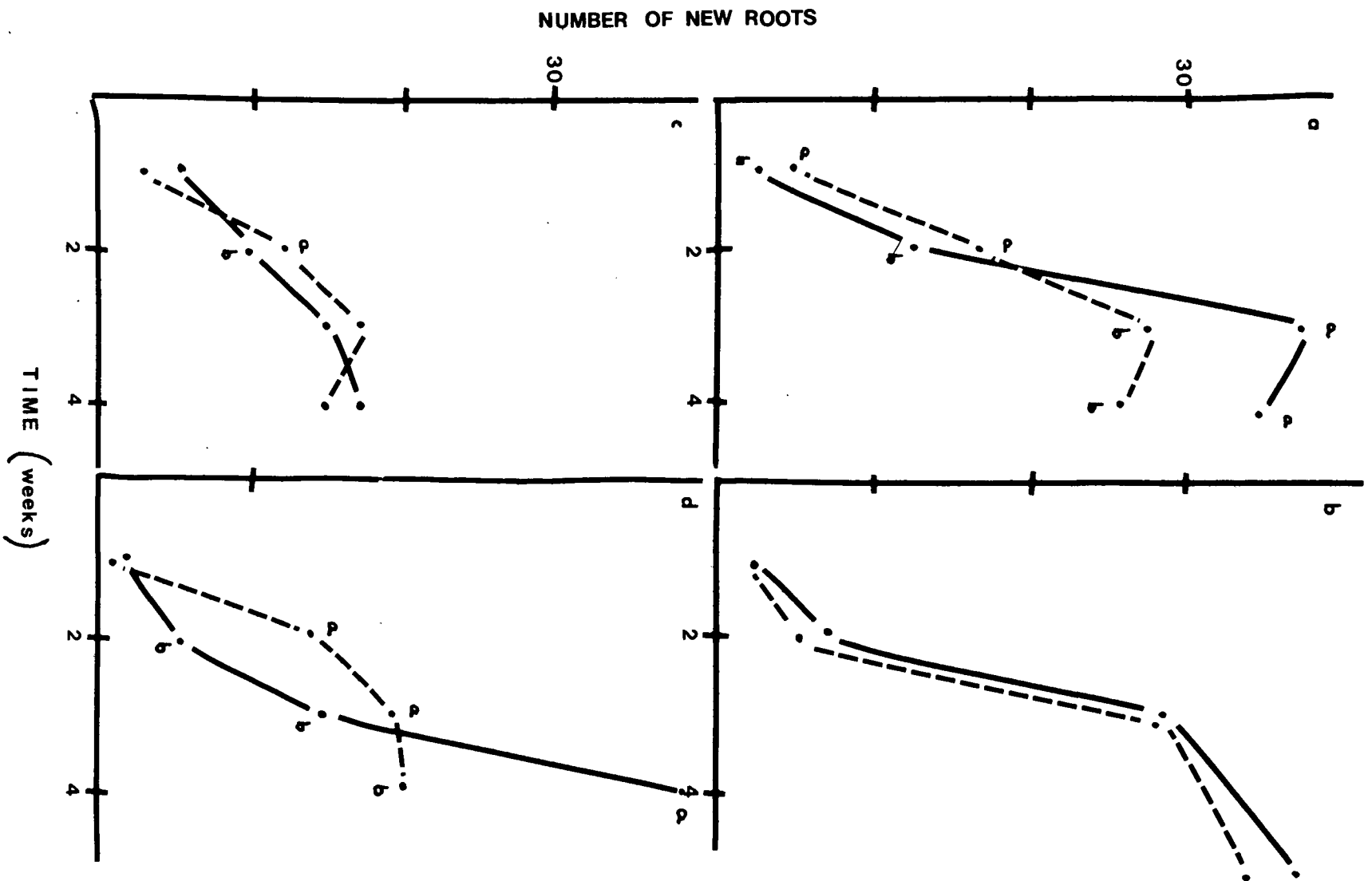


Figure 14. Number of new roots over time of control (—) and stressed (---) bare-root transplant Douglas-fir seedlings. a) wet bed, 0% pruned; b) wet bed, 50% pruned; c) dry bed, 0% pruned; d) dry bed, 50% pruned. Means with different letters differ at 5% level by LSD.



differences were not always statistically significant.

It should be noted also that in both wet and dry environments, the production of new roots appeared to be leveling out or decreasing at four weeks time. The root pruned trees, however, appeared to still be increasing their numbers of new roots.

Dry Weight of New Roots There did not appear to be a trend when comparing the dry weights of the new roots on the pre-stressed and control seedlings for each root pruning/environment combination (Fig. 15). The effect of the watering before the four weeks readings can be seen in Figure 15. Control seedlings, especially those that had been root pruned, showed a larger increase in dry matter production than the pre-treated trees.

Survival No significant differences in survival were noted between the potted pre-treated and control trees, regardless of pruning level, in the wet beds (Table 3). In contrast survival in the dry beds of the pre-treated trees were significantly higher than the control trees, regardless of pruning level.

Drought Duration Test:Short Term Responses

Water Potential Plant water potentials for both the stressed and control trees are plotted over time in Figure 16. Stressed trees showed higher water potentials on all sampling dates.

Soil Moisture Content (SMC) Soil moisture contents (bars) for both the stressed and control trees are plotted over time in Figure 16, except for the initial reading, which is lower due to the poor re-wetting of the 1:1 peat-vermiculite mix. All SMC readings for the stressed trees are higher than those for the controls.

Figure 15. Dry weight of new roots of control (—) and stressed (---) bare-root transplant Douglas-fir seedlings. a) wet bed, 0% pruned; b) wet bed, 50% pruned c) dry bed, 0% pruned d) dry bed, 50% pruned. Means with different letters differ at 5% level by LSD.

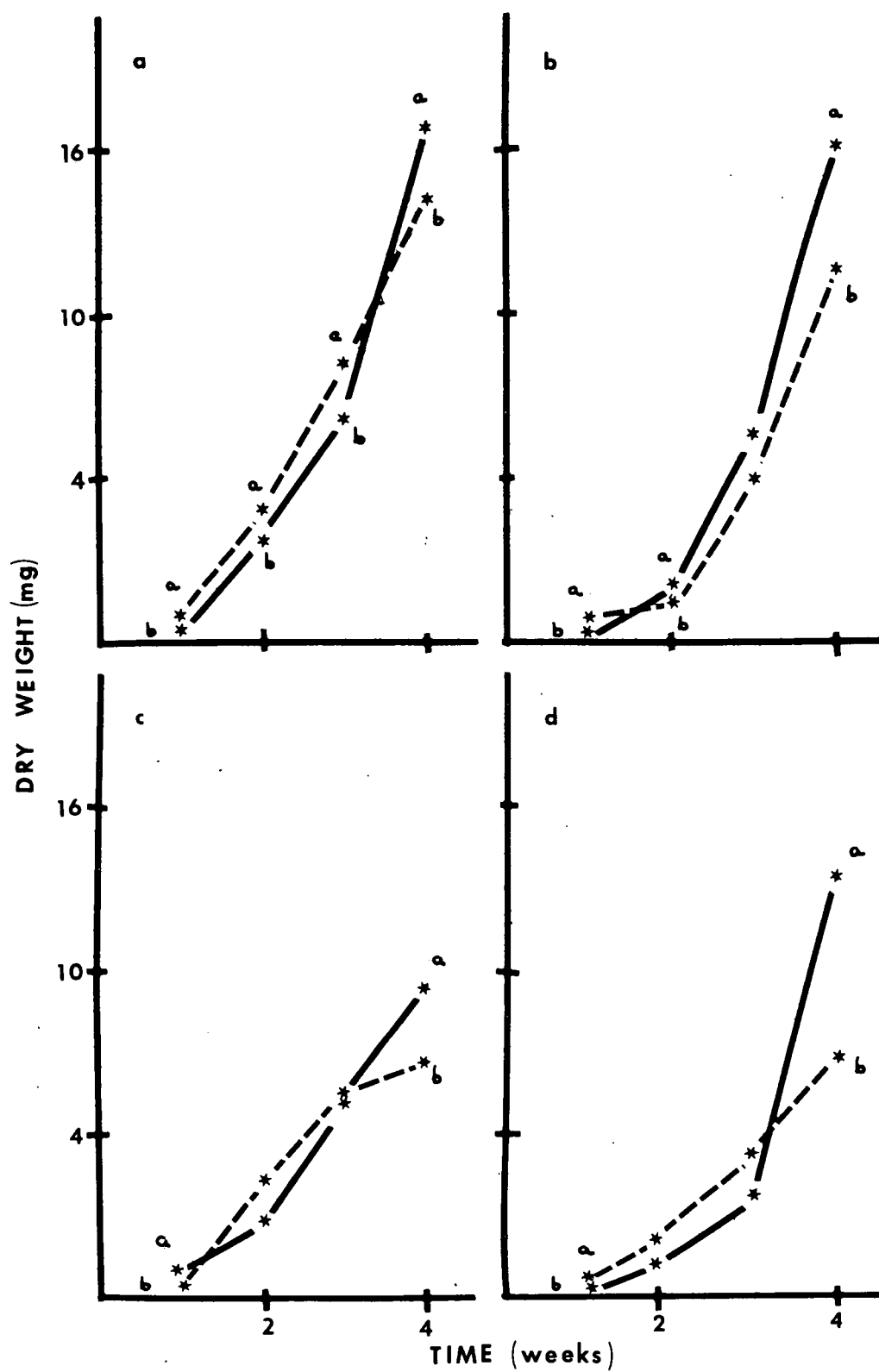
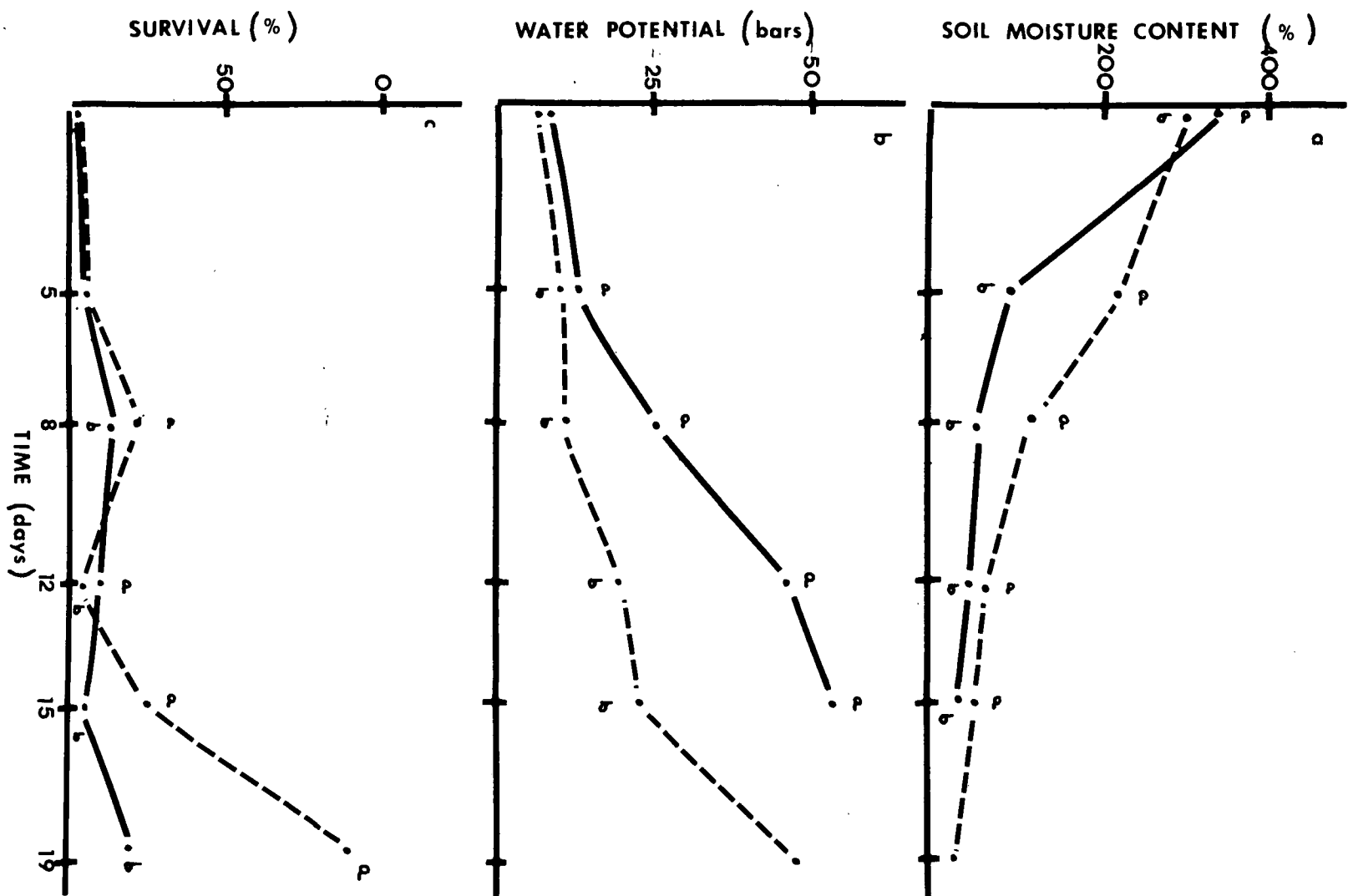


Table 3. Survival of control and stressed Douglas-fir bare-root transplant seedlings in wet and dry chambers. Means with different levels differ at 5% level by LSD.

	20% Relative Humidity		90% Relative Humidity	
	0% Pruned	50% Pruned	0% Pruned	50% Pruned
Stress	100 _a	100 _a	100 _a	100 _a
Control	83 _b	85 _b	100 _a	100 _a

Figure 16. Soil water content (a), water potential (b), and long term survival (c) of control (—) and stressed (---) Douglas-fir seedlings allowed to dry down for 0, 5, 8, 12, 15, and 19 days. Means with different letters differ at 5% level by LSD.



Survival Survival of controlled and stressed seedlings allowed to dry for 0, 5, 8, 12, 15, and 19 days is shown in Figure 16. 50% kill was seen at 17 days for the controls and at 28 days for the stressed trees.

Discussion

It is known that low soil water potentials adversely affect growth and yield in many species. As previously stated, the method used to pre-treat plants to withstand drought in this study is currently used commercially, even though very little is known concerning its effectiveness in drought-hardening trees. The data collected in this experiment indicate quite clearly pre-stressing treatments are successful in increasing plant resistance, avoidance and tolerance, to plant water stress; and in increasing its survival under stressed conditions.

Comparisons of each of the nine drying cycles (Fig. 10) show a gradual increase over time in the atmospheric evaporative demand (cm Balani Plate evaporation) required to reach the average ψ_p value of -15 bars. This observation indicates a gradual increase in plant resistance to soil moisture stress with each successive drying cycle. ψ_p data from the transplant test, though inconclusive due to the inadvertent watering, also illustrate this point. In both the wet and dry environments the pre-stressed trees had a significantly higher ψ_p potential than did those that were not stressed. Data from both the long and short duration tests also indicate that the stress treatment was successful in increasing a seedling's drought resistance. Short term ψ_p data indicate that this drought resistance consists of both drought

tolerance and avoidance. Not only are the pre-stressed trees able to avoid stress, as evidenced by their less negative ψ_p (than controls) during the drying period, but they also appear more tolerant of stress than the control plants. Control plants suffered 50% mortality when the ψ_p reached -57 bars, while stress plants reached this level at a significantly higher ψ_p (Figs. 8 and 9).

Short term soil moisture content (SMC) data further substantiates this. SMC, during the first two weeks of drying, decreased at a slower rate in the pre-stressed plugs than it did in the control trees, indicating their greater ability to avoid drought. After 19 days, the SMC readings for the stressed trees had increased and were similar to the controls. At low values the SMC curve is very steep; hence, a small change in the SMC in this portion of the curve is indicative of a large change in soil water potential (Fig. 9). Unfortunately, the soil water characteristic curve does not include the very low values observed in this study, so absolute SMC cannot be discussed here. It is probable, however, that 50% mortality would have occurred at lower soil water potential in the stressed trees (19 days) than in the controls (23 days).

In general, then, the data indicates that preconditioning can increase drought resistance; and that the effect is on both drought avoidance and drought tolerance. There is also evidence that avoidance is the more effective plant adjustment to early stages of drought stress; but as severity of stress increases, plant tolerance mechanisms become increasingly important.

The mechanisms of drought resistance are many. It is known that Douglas-fir native to dry habitats close their stomates readily in response to a stress that will have no effect on Douglas-fir whose

natural habitat is wet (3). Short term drought experiments with the same species have shown that the stomates of water stressed trees are more sensitive to water stress even after they have been re-watered (20, 75). It is likely that the ready closure of seedling stomates upon exposure to stress accounted in part for the increased drought avoidance of the pre-stressed trees. This is substantiated somewhat by the rapid reduction in growth, possibly associated with decreased turgor, reduced CO_2 absorption and thus photosynthesis is early during pre-stressing treatment.

It is also probable that preconditioning reduced cuticular transpiration. An increase in cuticular lipids has been found in drought hardened plants of a number of species (20). A distinct color difference between the control and prestressed trees was noted at the end of the nine drying cycles. The stressed trees appeared much darker than the control trees, a forest green versus a spring green.

A third, common method of drought avoidance is by decreasing the evaporative surface of the plant. This is accomplished naturally when the plant: 1) reduces its growth rate, 2) drops its leaves, or 3) grows smaller leaves. A decreased growth rate has already been mentioned. Needle drop did not appear a significant factor in this study. In similar preliminary studies with Burford Holly, leaf drop was clearly a factor. It is likely that other mechanisms are also involved in the increased drought avoidance seen in the stressed trees; but overall, Douglas-fir seems to avoid stress primarily by conserving water, a quality that classes it among Levitt's "water savers" (39).

Levitt has reviewed drought tolerance and discussed in detail the mechanisms involved, listing cell morphology, osmotic potential changes, permeability changes and enzyme modifications as some of the most important.

The effect of the stress treatment on the root growth, ψ_p , shoot elongation, and survival of seedlings transplanted into stress conditions was largely masked by the inadvertent watering at 0 and 4 weeks time. No conclusions as to the efficacy of the stress treatment can be drawn. There is some indication, however, in the pressure bomb and rooting data at 2 and 3 weeks time and in the survival data that pre-stressed seedlings did produce more new roots than the control trees and did show less mortality than the control trees planted into the dry beds. This evidence justifies further research in this area. The root pruning did not significantly affect survival of the transplants, though ANOVA of ψ_p and root number data did show pruning level to be significant on a few sampling dates. The effect was not consistent enough to confirm differences between pruned and unpruned seedlings.

Though preconditioning does appear to increase both a seedling's tolerance and avoidance to drought stress, there are still many questions unanswered. Of particular importance to the commercial grower would be the long term effects on plant vigor and growth. It was obvious in dry environment transplant beds that the control trees reacted more favorably to the inadvertent watering than did the stressed trees, possibly indicating that they were less vigorous than the non-stressed seedlings. Further work is needed to determine what planting site conditions justify the use of the lower vigor but drought resistant stock.

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