MANIPULATION OF PINE SEEDLING PHYSIOLOGY BY WATER STRESS CONDITIONING

Jon D. Johnson, John R. Seiler, and Ken L. McNabb[1]

Abstract- Seedling water stress is an important, limiting factor in the successful establishment of southern pine plantations. With the proper treatments pine seedlings can be physiologically manipulated to better withstand this planting stress. Acclimating seedlings to water stress is best accomplished by repeated, mild stressing. Presently, withholding irrigation and/or undercutting are the best cultural practices to achieve this acclimation. However, from data presented, the timing and duration between treatments is very important in eliciting and maintaining acclimation.

The physiological responses of seedlings to water stress acclimation are related to osmotic adjustment. Acclimated loblolly pine seedlings exhibited up to a 0.7 MPa shift in osmotic potential. This decrease in osmotic potential is due to an increase in cellular solute concentration; in acclimated slash pine seedlings, a conversion from starch to sugar was observed, suggesting that glucose is the primary osmoticum. The physiological effects of such osmotic adjustments included maintenance of photosynthesis during water stress, decreased transpiration and increased water use efficiency. The response of acclimated seedlings after planting was favorable; height increment after the first year was consistently larger for both acclimated slash and loblolly pine seedlings. During dry planting years, the response of acclimated seedlings would be expected to be even more dramatic.

Additional keywords: Osmotic adjustment, photosynthesis, transpiration, carbohydrate, starch, *Pinus elliottii*, *P. taeda*.

Introduction

Water is probably the most limiting environmental factor during the establishment of a southern pine stand when regenerating with bare-root seedlings. This water stress has many physiological ramifications that impact regeneration success; the most notable impacts are increased mortality and

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decreased growth of the seedlings. The physiological responses of plants to water stress have been studied extensively and have been the subject of many review articles and books (Hsaio, 1973; Kramer, 1983; Kramer and Kozlowski, 1979). The purpose of this paper is to synthesize some recent research that has dealt specifically with water stress acclimation in southern pine bare-root seedlings and its relationship to nursery practices and subsequent field performance.

Some degree of water stress develops in bare-root seedlings after planting, primarily resulting from the root system's impaired abilitiy to absorb water due to the loss of fine roots during lifting and handling, as well as deliberate root pruning prior to planting. McNabb (1985) found that 30 percent root dry weight was lost during careful hand lifting of slash pine (*Pinus elliottii* var. *elliottii* (Engelm.)). This resulted in a considerable decrease in moisture content during the first eight weeks in the field when compared to seedlings left in the nursery (fig. 1). Initially, moisture was lost from the shoots, but two weeks after planting the roots also exhibited a decrease in moisture content. The rainfall during these eight weeks (223 mm; 8.8 in) was more than adequate to maintain a favorable water balance as evidenced in the seedlings left in the nursery. Recovery of the planted seedlings required between eight and ten weeks and was probably a result of the needed initiation of new root growth. Although suberized roots have been found to absorb some water (Kramer, 1946; Chung and Kramer, 1975), new root growth is needed to re-establish soil-root contact in order to ameliorate seedling water stress. In years with less rainfall or uneven distribution, the effect of moisture stress would undoubtedly be prolonged by decreasing the rate of root growth. Southern pine seedlings are able to physiologically acclimate to such water stress and can at least partially mitigate the adverse effects of the stress.

Physiological Responses to Water Stress Acclimation

Plants exposed to sublethal water stress often acclimate to the stress by: 1) becoming tolerant of low water potentials; or 2) avoiding water stress by conserving water and maintaining water absorption at low water potentials (Clemens and Jones, 1978). The latter process seems to be more important for tree species including the southern pines (Hinckley et al. 1981) and is a function of cellular osmotic adjustment. This osmotic adjustment affects photosynthesis and transpiration, and is mediated by t he stomates (Seiler, 1984).

Osmotic adjustment, or the lowering of cell vacuole osmotic potential by the active accumulation of solutes, can prolong the avoidance of water stress by enabling the cell to absorb water and maintain a positive turgor potential (Turner and Jones, 1980) (table 1). It is positive turgor potential that drives cell enlargement and stomatal opening.

Loblolly pine (*Pinus taeda* L.) has been reported to osmotically adjust when exposed to repeated water stress. Seiler and Johnson (1985) found that one year old seedlings that were repeatedly stressed to -1.4 MPa predawn needle water potential for eight weeks exhibited an osmotic adjustment of 0.46 MPa (table 1). Similarly treated containerized seedlings conditioned for 14 weeks exhibited an osmotic adjustment of 0.74 MPa. An adjustment of greater than 0.3 MPa is probably significant enough to effect seedling survival and

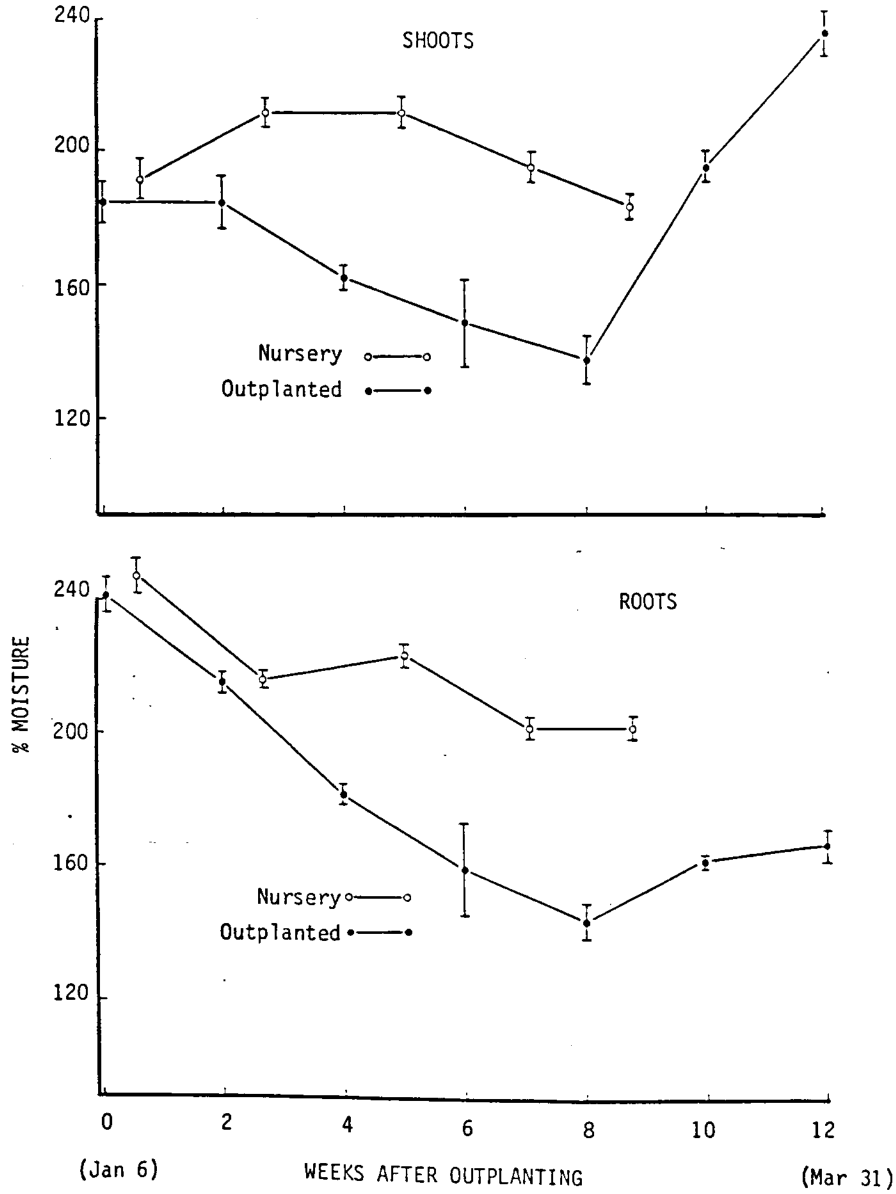

Figure 1. Changes in slash pine seedling shoot and root moisture content following planting in comparison with seedlings left in the nursery. Bars indicate the standard error of the means. (McNabb, 1985).

Table 1. Loblolly pine needle water potential components as affected by eight weeks of water stress acclimation (Seiler and Johnson, 1985).

Treatment	Water Potential	Osmotic Potential	Turgor Potential
	- - - - - - - - -	(MPa)	- - - - - - - - - -
Control	- 0.90 A	- 1.50 A	0.60 A
Acclimated	- 1.35 B	- 1.96 B	0.61 A

Means followed by the same letter within a column do not differ significantly (alpha = 0.05), n = 9.

performance. Hennessey and Dougherty (1984), however, found little osmotic adjustment in loblolly pine seedlings stressed to -0.75 MPa until four months after the treatment was initiated. At that time, osmotic potential at full turgor was 0.4 MPa lower. It is probable that this stress treatment (-0.75 MPa) was not severe enough to trigger osmotic adjustment until an overriding stress such as low temperature occurred.

Carbohydrates, organic acids, and inorganic ions can be used for osmotic regulation in plants (Kramer, 1983). In sugar maple (*Acer saccharum* Marsh.) (Parker, 1970) and black oak (*Quercus velutina* Lam.)(Parker and Patton, 1975) subjected to water stress, a simultaneous increase in sugars and decrease in starch was observed, but it was not known if these species had osmotically adjusted. Recently an investigation of carbohydrate changes in slash pine seedlings in response to repeated water stress show ed an increasing conversion of starch to sugar as duration between irrigation increased (McNabb, 1985; fig. 2). In comparison with the control treatment (standard nursery irrigation level of one inch per week) free sugars increased up to 31 percent 56 days after water was withheld. Such large changes could easily provide the needed solutes for osmotic adjustment. This is not to say that organic acids or inorganic ions are unimportant for osmotic adjustment in southern pine, but an increase of 22 mg sugar/g dry weight would be more than enough to cause a 0.4 MPa increase in the osmotic potential. Even in the least stressful treatment, excluding the control, and probably the best in terms of acclimating the seedlings (14 days since last water and an inch of water every two weeks for eight weeks), there was a 7 percent increase in free sugars or an absolute increase of 5 mg sugar/g dry weight. These data suggest that stress acclimation in southern pine seedlings does affect the carbohydrate balance and this could be involved with osmotic adjustment.

Of additional importance, extrapolated from figure 2, is the maintenance of similar total carbohydrate (starch plus sugar) concentrations across treatments. Only the most severe stress treatment, one that should not elicit an acclimation response (56 days since last water) showed a decline in total

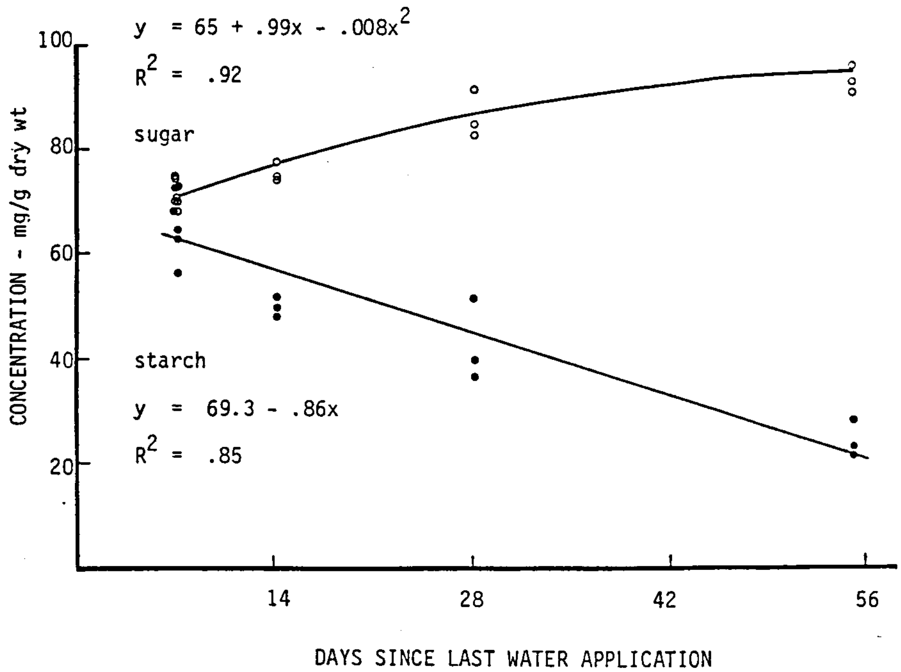

Figure 2. The sugar and starch concentration of slash pine seedlings at lifting as affected by the duration to the last irrigation. Each point represents a 20 seedling sample. (McNabb, 1985)

carbohydrate levels (117 mg/g dry weight versus 130 mg/g dry weight). This finding is important in that the acclimated seedlings did not exhibit an apparent inhibition of photosynthesis.

Acclimated loblolly pine seedlings (ones that had osmotically adjusted) were found to maintain photosynthesis when subjected to water stress (fig. 3: Seiler and Johnson, 1985). Over the entire range of needle water potentials, the acclimated seedlings exhibited a higher photosynthesis rate. Acclimation became more beneficial as stress increased below -1.6 MPa; the water potential difference at a given photosynthesis rate is virutally equal to the osmotic adjustment,i.e. 0.46 MPa. Of additional benefit to the acclimated seedlings was a decrease in stomatal conductance and transpiration (Table 2: Seiler and Johnson, 1985). With acclimation there was a 30 percent decrease in transpiration rate which is reflected in the 67 percent increase in the water use efficiency. Thus, by extrapolation, seedlings that are acclimated to water stress when planted should be able to maintain a more favorable carbohydrate and water status during the establishment period. As a result, the establishment period should be shorter because of these favorable physiological conditions and result in larger seedlings at the end of the growing season.

Table 2. Loblolly pine transpiration and water use efficiency as affected by eight weeks of water stress acclimation (Seiler and Johnson, 1985).

Treatment	Transpiration	Water Use Efficiency
	(g H_2O/g-hr)	(mg CO_2/g H_2O)
Control	0.44 A	14.1 A
Acclimated	0.31 B	23.5 B

Means followed by the same letter within a column do not differ significantly (alpha = 0.05), n = 4.

Stress Acclimation in a Nursery Setting

Given the beneficial effects of stress acclimation on seedling survival and performance discussed above, the next question becomes, how to acclimate seedlings in a nursery setting. Presently, there are two primary techniques: 1) withhold irrigation in conjunction with rain monitoring; or 2) repeatedly undercut the seedlings. The success of these techniques in acclimating seedlings to water stress depends on timing in relation to seedling phenology and duration between the treatments.

A number of studies have investigated the effects of undercutting on seedling survival and performance. Most have found that early, frequent

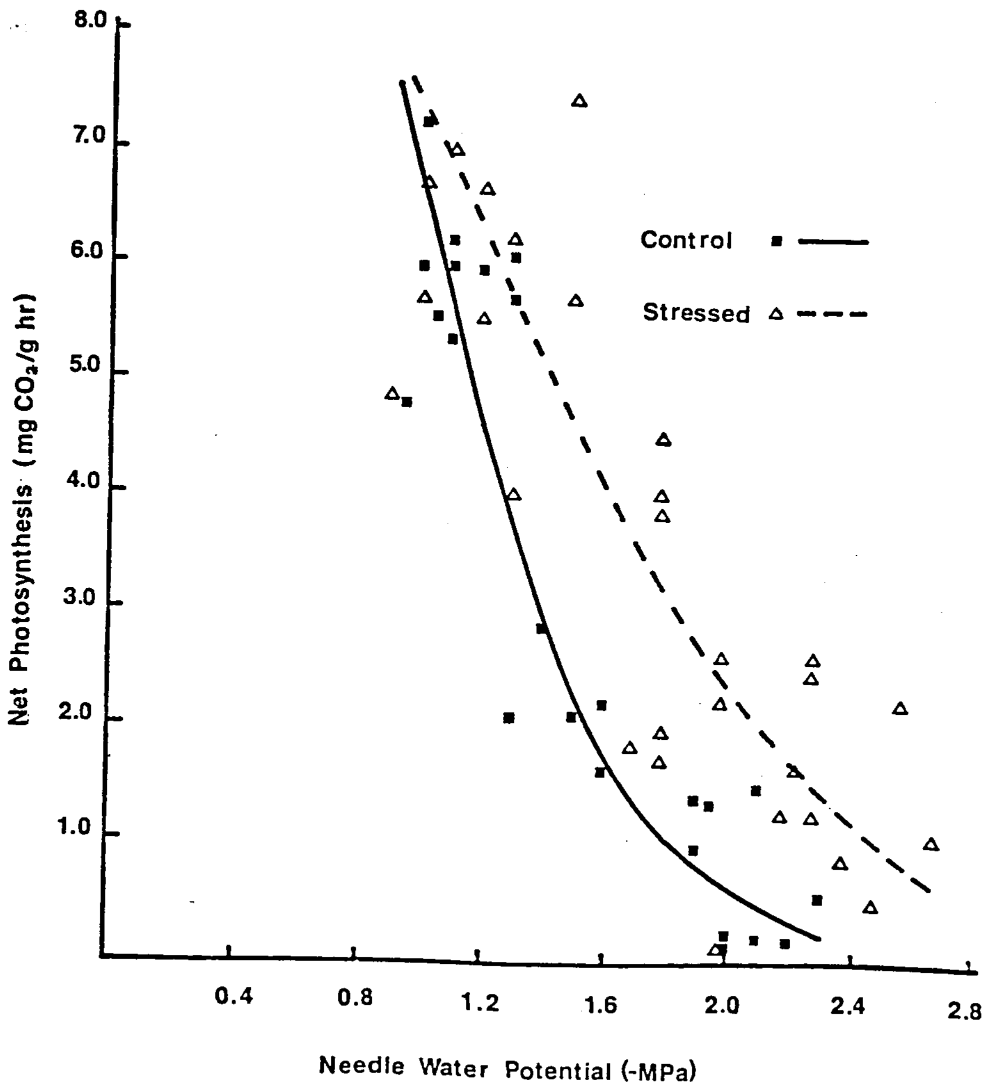

Figure 3. The response of net photosynthesis to decreasing needle water potential as affected by water stress acclimation in loblolly pine seedlings. The regression equations were:
Control - $Ln(Ps) = 2.70 - 0.82(NWP^2)$, $R^2 = 0.76$, n = 24;
Stressed - $Ln(Ps) = 2.30 - 0.38(NWP^2)$, $R^2 = 0.76$, n = 32;
(where Ps = net photosynthesis and NWP = needle water potential). (Seiler and Johnson, 1985).

undercutting is the most beneficial in that it creates repeated, mild water stress as well as desirable changes root morphology (Bacon and Bachelard, 1978; Rook, 1969; 1971). Undercutting beginning in November had no effect on the carbohydrate status of slash pine seedlings (and presumably no acclimation effect), however root growth after planting was stimulated by the most severe undercutting treatments (undercut two and four times) (McNabb, 1985). This response may have been due to wounding of the root system and a proliferation of root primordia by ethylene (Abeles, 1973; Johnson and Stumpff, 1984).

Controlling irrigation in the study by McNabb (1985) was accomplished by erecting rain exclosures and then watering by hand. Although not operationally feasible for a bare-root nursery, such a treatment could be readily applied to a containerized nursery. His findings are of particular interest to the question of seedling acclimation to water stress and the effects of duration and amount of water applied. Seedlings that received 25 mm (1 in) of water one week before lifting had similar shoot starch concentrations regardless if the seedlings had received 76 mm or 203 mm of water over the eight week treatment period (fig. 4). By plotting the data on a duration or "days to last watering" basis, the fit of the regression equations improved significantly for both shoot and root starch (fig. 5). Thus, it appears that the starch-to-sugar interconversion associated above with acclimation is very sensitive to timing of irrigation. On the positive side of this observation, irrigation could be reduced considerably without adversely affecting the seedlings' carbohydrate status while accruing the possible benefits of acclimation. However, on the negative side, it appears that an attempt to acclimate seedlings could be quickly mitigated by a rain shower just prior to lifting.

Response of Acclimated Seedlings after Planting

Field survival and performance of acclimated seedlings is the ultimate test for determining value of acclimation treatments. In fig. 6, the response of non-acclimated slash pine seedlings to planting stress is evident by large and rapid decrease in starch with a concomitant increase in sugar. These seedlings required eight weeks to recover to the levels of the nursery seedlings. Acclimated seedlings should require a shorter recovery period.

In table 3 from McNabb (1985) acclimation achieved by controlling irrigation had no affect on survival or total height after one year in the field. Acclimation did influence height increment: the 76 and 203 mm of water (both watered one week before lifting) exhibited the largest increment of 5.5 cm, whereas the 51 and 102 mm treatment (irrigated 4 and 2 weeks before lifting, respectively) showed a similar 4.5 cm increment. Only the 25 mm treatment significantly reduced height increment. Plotting these data on a duration basis showed that, with the exception of the driest treatment (56 days), all of the other treatments had a similar affect on height increment (fig. 7). Containerized loblolly pine acclimated to water stress showed a similar response in height increment with no effect on survival (Seiler and Johnson, 1984). Acclimated seedlings that had adjusted osmotically by 0.74 MPa exhibited a two centimeter (or a 13 percent increase over the control) greater height increment after one year in the field.

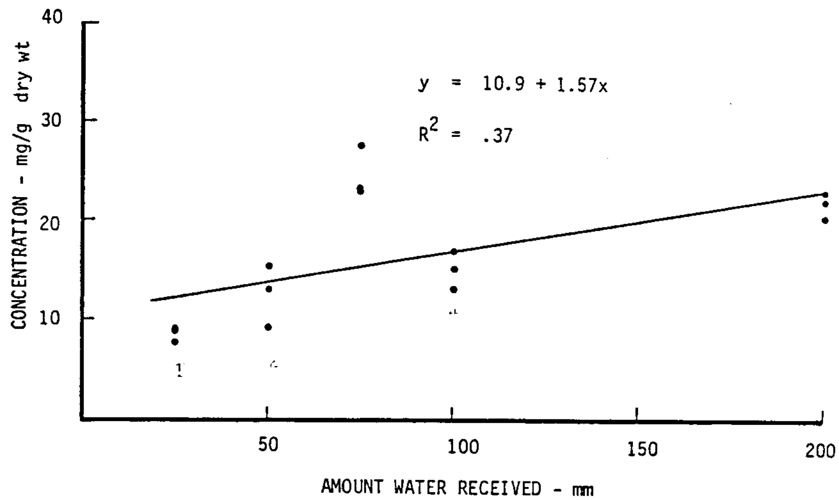

Figure 4. Slash pine shoot starch concentration at lifting as affected by the total amount of water received during the previous eight weeks. Each point represents a 20 seedling sample. (McNabb, 1985).

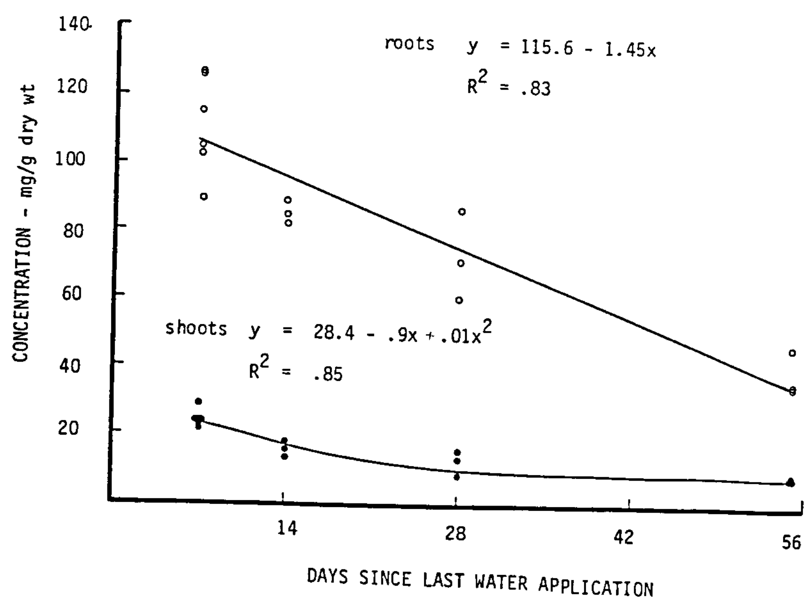

Figure 5. Slash pine shoot and root starch concentration at lifting as affected by the duration to the last irrigation. Each point represents a 20 seedling sample. (McNabb, 1985).

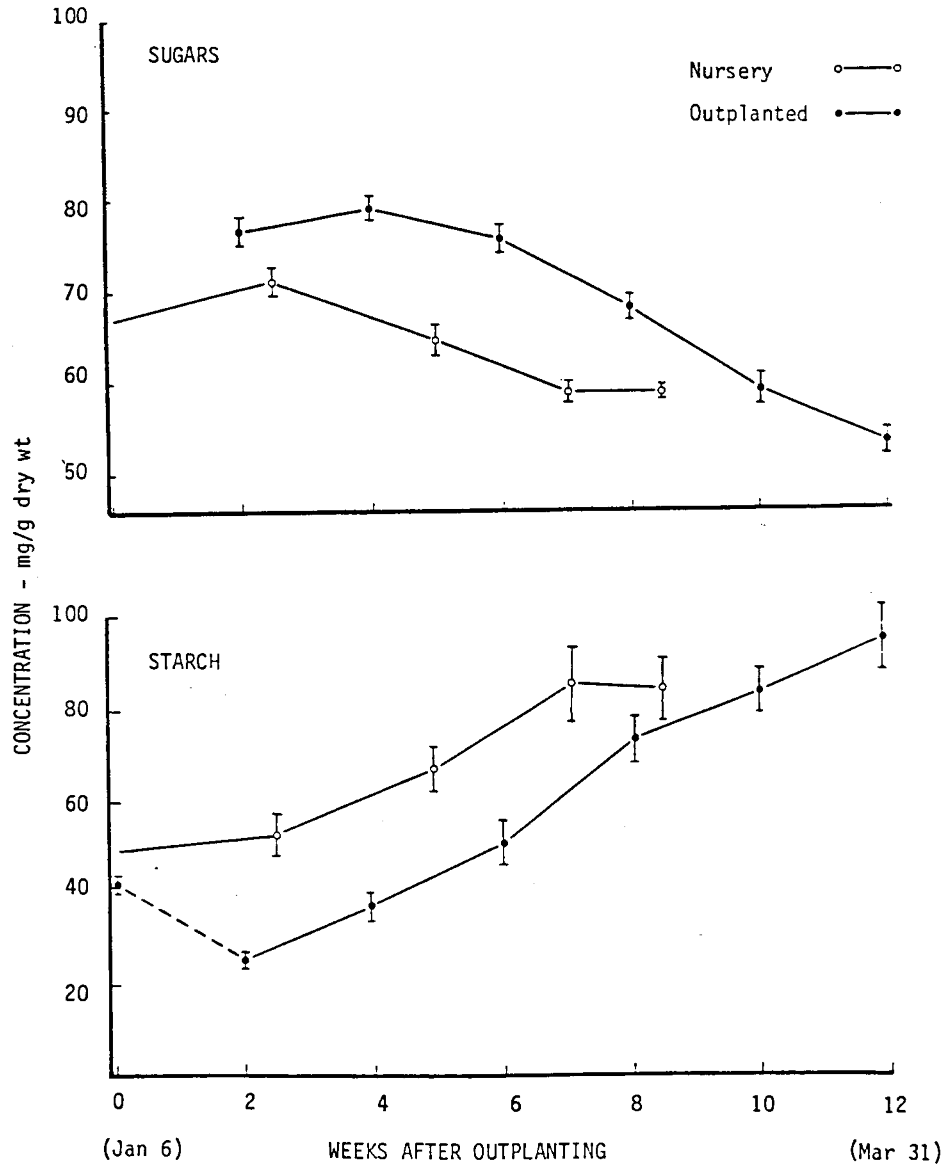

Figure 6. Changes in slash pine seedling sugar and starch concentration following planting in comparison with seedlings left in the nursery. Bars indicate standard error of the means. (McNabb, 1985).

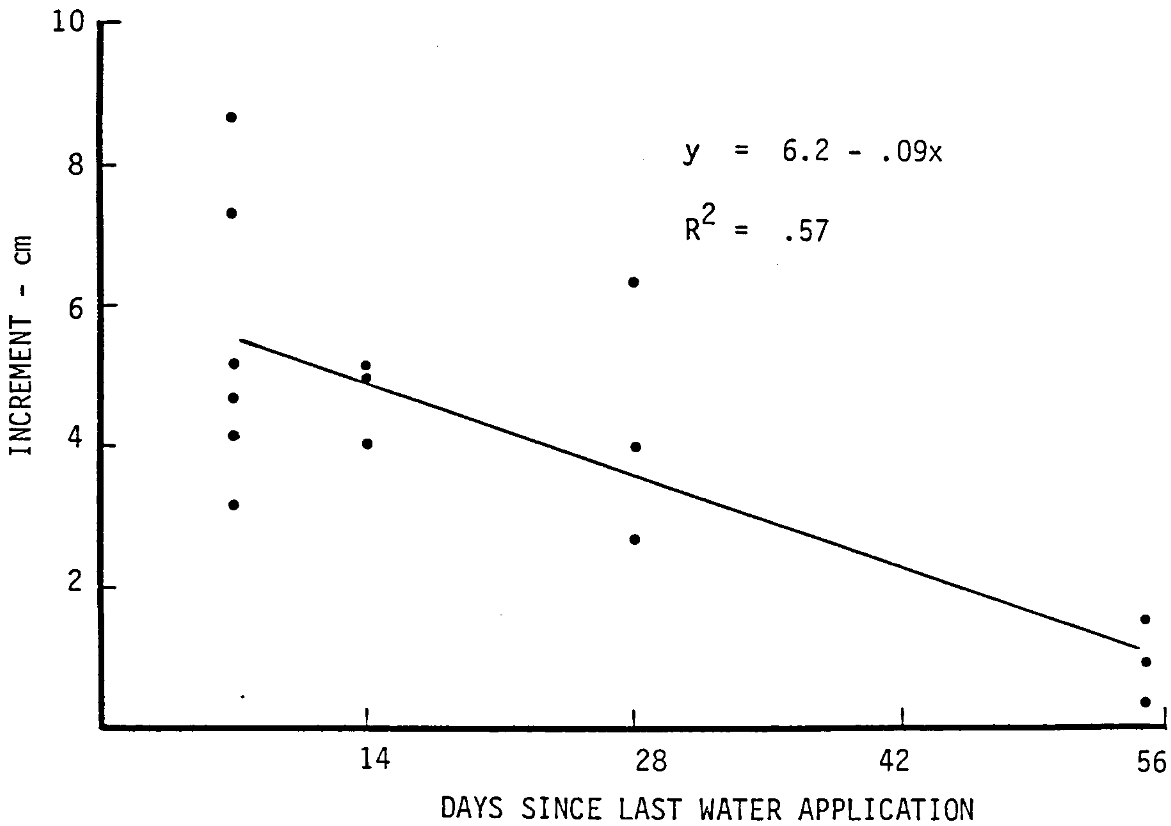

Figure 7. Slash pine seedling height increment one year after planting as affected by the duration to the last irrigation in the nursery prior to lifting. Each point represents a 50 seedling sample. (McNabb, 1985).

Table 3. Field responses of slash pine seedlings to varying amounts of irrigation in the nursery (McNabb, 1985).

	Treatment (mm of water applied)				
	25	51	76	102	203
Survival (%)	91	93	94	95	89
Total Height (cm)	29.0	33.0	33.1	32.1	32.8
Height Increment (cm)	0.9	4.4	5.6	4.7	5.4

Conclusions

The manipulation of seedling physiology by utilizing southern pines' adaptability to water stress is plausible and has been demonstrated. The primary physiological response to this stress acclimation is osmotic adjustment which impacts photosynthesis and transpiration. Acclimated seedlings demonstrated a higher sugar-to-starch ratio, a higher photosynthesis rate at a given level of water stress, a lower transpiration rate, and a higher water use efficiency. Acclimation could be achieved in the nursery by either withholding irrigation or early and repeated undercutting. Typical field response in good planting years (average or higher rainfall) included increased height increment, whereas during poor planting years, increased survival and total height could also be expected.

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