

Drought susceptibility and recovery of transplanted *Quercus rubra* seedlings in relation to root system morphology

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Abstract

- Transplant shock, implicated by depressed seedling physiological status associated with moisture stress immediately following planting, limits early plantation establishment. Large root volume (Rv) has potential to alleviate transplant shock because of higher root growth potential and greater access to soil water.
- We investigated impacts of drought and transplant Rv on photosynthetic assimilation (A), transpiration (E), stomatal conductance (g_s), predawn leaf xylem water potential (Ψ_L), and growth of northern red oak (*Quercus rubra* L.) seedlings to explain mechanisms associated with susceptibility to transplant shock. One year-old bareroot seedlings were graded into four Rv categories and either well watered or subjected to drought consisting of low, medium, or high moisture stress by discontinuing irrigation at 22-day intervals for 3 months. Thereafter, all treatments were re-watered to examine recovery.
- Transplant shock was signified by reduced A , E , g_s , and Ψ_L , which generally increased with increasing moisture stress and Rv . Physiological status improved during recovery, though stress was still evident in seedlings exposed to medium or high moisture stress and in larger Rv seedlings. Growth declined with increasing moisture stress but was generally similar among Rv treatments, likely reflecting greater A at the whole plant level and/or reliance upon stored reserves in large Rv seedlings.
- The most effective drought avoidance mechanisms were root growth, stomatal regulation, reduced leaf area, and higher growth allocation to roots relative to shoots. Our results suggest that large initial Rv does not enhance drought avoidance during the first season after transplant in northern red oak seedlings.

Résumé – Sensibilité à la sécheresse et reprise des semis transplantés de *Quercus rubra* en relation avec la morphologie racinaire.

- Le choc de transplantation, lié à l'état physiologique déprimé des plants associé à un stress hydrique, immédiatement après la plantation, limite le début de l'installation des plants. Un important volume racinaire (Rv) a le potentiel d'atténuer le choc de transplantation en raison du fort potentiel de croissance racinaire et d'un meilleur accès à l'eau du sol.
- Nous avons étudié les impacts de la sécheresse et des transplants Rv sur l'assimilation photosynthétique (A), la transpiration (E), la conductance stomatique (g_s), le potentiel hydrique de base (Ψ_L), et la croissance des plants de chêne rouge (*Quercus rubra* L.) pour expliquer les mécanismes associés à la sensibilité à un choc de transplantation. Des plants de un an à racines nues ont été classés en quatre catégories Rv et soit bien arrosés, ou soumis à une sécheresse, consistant à un faible, moyen ou fort stress hydrique en cessant l'irrigation à 22 jours d'intervalle (27, 49 et 72 après la transplantation) pour 3 mois. Par la suite, tous les traitements ont été arrosés afin d'examiner la reprise.
- Le choc de transplantation qui généralement a augmenté avec l'augmentation du stress hydrique et Rv , a été significatif par la réduction de A , E , g_s , and Ψ_L . L'état physiologique s'est amélioré lors de la reprise, même si le stress est encore évident chez les plants exposés à un stress hydrique moyen ou élevé et chez les plants à Rv les plus importants. La croissance a diminué avec l'augmentation du stress hydrique, mais elle a été généralement similaire chez les traitements Rv , reflétant probablement une plus grande A au niveau du plant entier et / ou le recours à des réserves stockées dans les plants à Rv important.

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- Les mécanismes d'évitement de la sécheresse les plus efficaces ont été la croissance des racines, la régulation stomatique, une surface foliaire réduite et une allocation de la croissance plus élevée aux racines par rapport aux pousses. Nos résultats suggèrent que les grandes Rv initiales n'améliorent pas l'évitement de la sécheresse durant la première saison après transplantation chez les plants de chêne rouge.

1. INTRODUCTION

Northern red oak (*Quercus rubra* L.) is a dominant component of eastern hardwood forests in North America and a valuable landscape species with moderate to fast growth rates (Sander, 1990). Northern red oak grows from Cape Breton Island, Nova Scotia, Prince Edward Island, New Brunswick, Gaspé Peninsula of Quebec, to Ontario; from Minnesota south to eastern Nebraska and Oklahoma; east to Arkansas, southern Alabama, Georgia, and North Carolina (Sander, 1990). Detailed discussion on northern red oak habitat, life history, and genetics can be found in Sander (1990). Most artificial forest regeneration projects involving northern red oak use 1 + 0 or 2 + 0 bareroot seedlings for field planting (Jacobs et al., 2004b). These seedlings undergo significant transplant shock following planting, which may be associated with loss of fine root structure during nursery lifting (Struve, 1990; Struve and Joly, 1992).

Transplant shock (i.e., planting check, planting shock) is an interruption in the normal physiology of a seedling after planting that results in stunted growth or reduction of foliage, and if severe, mortality (Burdett, 1990). The major cause of transplant shock is water deficit due to restricted uptake of water at planting because of temporary impairment of seedling root function or poor initial root-soil contact (Burdett, 1990; Burdett et al., 1984). Severe planting check may limit plantation productivity by depressing photosynthetic assimilation (A) and putting planted stock at a disadvantage in competition with non-crop vegetation (Burdett, 1990; Haase and Rose, 1993). This phenomenon has accounted for plantation failures of white spruce (*Picea glauca* (Moench) Voss), Engelmann spruce (*Picea engelmannii* Parry), or a natural hybrid of these species in Canada (Vyse, 1981) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in northwestern California (Waters et al., 1991). Seedling physiological responses, such as reduced A , transpiration (E), stomatal conductance (g_s), and leaf xylem water potential (Ψ_L) implicate planting shock (Blake, 1983; Grossnickle, 1988). Plant morphological indicators of planting shock include reduced leaf area, restricted shoot growth, shortened needles, and a greater number of needles per unit of leader (Haase and Rose, 1993; Struve and Joly, 1992).

Drought resistance of plants may be divided into either drought tolerance (i.e., physiological and biochemical adaptations that permit plant function under water shortage) or drought avoidance (i.e., developmental and morphological changes that limit water shortages) (Levitt, 1972). Drought tolerance mechanisms may involve turgor maintenance (by accumulation of solutes) and/or desiccation tolerance (by protoplasmic resistance) (Jones et al., 1981). Drought avoidance permits a plant to maintain tissue water contents near optimum

levels during periods of low soil moisture availability and high atmospheric evaporative demand (Levitt, 1972). The two main drought avoidance mechanisms are stomatal closure and increased root growth relative to shoots (Johnson et al., 1984). Hence, plants may respond to drought by closing stomata to conserve tissue water, which could prevent lethal desiccation, but results in decreased A (Burdett, 1990). Alternatively, moisture stress may be alleviated by root growth, which enhances water absorption by increasing the surface area of permeable unsubsized roots and providing access to a larger volume of soil (Burdett, 1990; Sands, 1984). In addition, the expansive forces exerted by growing roots improve root-soil contact (Burdett, 1990).

It has been argued that large root volume (Rv) of bareroot seedlings at transplanting may act to confer drought avoidance that alleviates transplant shock because of greater root growth potential and access of larger Rv to soil resources (Carlson, 1986; Carlson and Miller, 1990; Haase and Rose, 1993; Jacobs et al., 2004a). However, large Rv is often correlated with large shoots, and the transpirational demand after budbreak coupled with insufficient water uptake may result in higher water deficits (Sands, 1984), which could induce transplant shock detrimental to field performance of seedlings. Consequently, seedling field survival and growth may depend on ability to mitigate internal water deficits and/or resist drought (Grossnickle, 2005; Struve and Joly, 1992), especially for northern red oak, which is generally restricted to relatively mesic sites (Sander, 1990).

Although evidence suggests that transplant shock may limit early plantation productivity (Vyse, 1981; Waters et al., 1991), relatively little research has been conducted to examine the physiological and morphological mechanisms involved (Haase and Rose, 1993). It has been demonstrated that seedlings with larger initial Rv have higher root growth potential (Carlson, 1986), tend to better tolerate transplant shock over time (Haase and Rose, 1993), and may have more rapid early growth rates (Rose et al., 1991a; 1991b; Jacobs et al., 2005). Additionally, root system morphology partly explained establishment success of some deciduous hardwood species (Struve, 1990; Jacobs et al., 2005). Therefore, a better understanding of root system development and its response to a set of limiting site conditions, such as water deficits, is essential. This knowledge can be used to help match specific seedling types to site conditions under which transplanting will have a high probability of field success. To contribute towards this understanding, we exposed seedlings with varied root volumes under controlled environmental conditions to drought ranging from well-watered to high moisture stress by periodically discontinuing irrigation at 22-day intervals for 3 months. Subsequently, all treatments were re-watered for another 2 months.

The first phase examined seedling responses to drought, while the second evaluated ability of plants to recover from induced drought. Therefore, the focus of our research was to assess the impact of drought and initial seedling Rv on plant physiological and morphological responses to explain underlying mechanisms that enable northern red oak seedlings to withstand transplant shock. This was accomplished by periodically evaluating gas exchange, water relations, and growth during the study. Specifically, our objectives were to determine (i) the influence of Rv on A , E , g_s , and Ψ_L associated with moisture stress, (ii) the physiological ability of northern red oak seedlings to recover after water stress is alleviated, and (iii) the role of increased root growth as an important mechanism to explain drought avoidance in northern red oak seedlings.

2. MATERIALS AND METHODS

2.1. Cultural conditions and experimental treatments

Bareroot northern red oak seedlings were grown under standard operational conditions, detailed in Jacobs (2003), at the Indiana Department of Natural Resources Vallonia State Tree Nursery in south-western Indiana, USA (38° 48' N, 86° 06' W). Seeds were sown in November 2000 and seedlings were grown until lifting in December 2001. Seedlings were operationally graded, packaged, and stored at 2 °C until transported to a lab at Purdue University in March 2002 for sorting. A total of 128 randomly selected seedlings were washed free of soil, tagged, and measured for stem height (root collar to base of terminal bud), stem diameter (1 cm above root collar), fresh mass, number of first-order lateral roots (FOLR; i.e. number of permanent, FOLR > 1 mm diameter at junction with taproot), and Rv using water displacement (Jacobs et al., 2005).

Seedlings were sorted into 4 Rv categories (RvC) for the study (Tab. I). On 17 May 2002, 32 seedlings from each RvC treatment were transplanted into 12.04 L pots (22.7 cm width × 39.4 cm depth). Use of relatively large containers was intended to more accurately simulate potential field conditions. Pots contained a medium of 8:5:2:2:3 (v:v:v:v:v) composted pine bark:coconut coir:bark ash:perlite:peat (Metro-Mix®560, Scotts Co., Marysville, OH, USA). Pots were placed on a greenhouse bench (mean daytime temperature 24 °C and 20 °C at night) in the Department of Horticulture Plant Growth Facility at Purdue University (40° 25' N, 86° 55' W). Following transplant, the medium in all treatments was watered to field capacity and pots were well watered for the first 27 d to induce flushing and simulate high moisture availability typical of conditions soon after spring planting. Thereafter, eight seedlings from each RvC were randomly assigned to each of four moisture stress treatments. The well-watered (WW) control treatment received irrigation to field capacity approximately every 5 d throughout the experiment. The other three treatments consisted of eliminating the aforementioned irrigation regime 27, 49, or 71 d after transplant to induce high moisture stress (HMS), medium moisture stress (MMS), and low moisture stress (LMS), respectively.

On 9 September (115 d after transplant), the medium of plants in all moisture stress treatments was re-watered to field capacity, and pots were then well watered throughout the duration of the experiment (i.e., until 15 November, 182 d after transplant) to examine drought recovery. Supplemental lighting (about 140 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for

Table I. Range, mean, and standard error (SE) of initial seedling morphological parameters sorted according to four root volume categories (RvC) ($n = 32$).

Parameter	Initial seedling data			SE
	Range		†Mean	
	Min	Max		
Root volume (cm ³)				
RvC1	7	22	17c	1.2
RvC2	23	32	27bc	0.8
RvC3	31	46	39b	1.0
RvC4	48	132	72a	7.2
Fresh mass (g)				
RvC1	10	33	24c	2.0
RvC2	28	47	37bc	1.5
RvC3	38	75	53b	2.4
RvC4	65	195	105a	10
Diameter (mm)				
RvC1	4	6	4c	0.1
RvC2	4	7	5b	0.2
RvC3	5	7	6b	0.2
RvC4	6	11	8a	0.3
Height (cm)				
RvC1	20	51	34c	2.0
RvC2	21	66	38bc	4.0
RvC3	29	87	49ab	4.0
RvC4	28	87	60a	5.0
First order lateral roots				
RvC1	1	8	5c	0.5
RvC2	3	11	7bc	0.6
RvC3	4	15	10ab	0.8
RvC4	6	19	12a	1.0

† Column means followed by different letters within RvC for each parameter differ significantly according to Tukey's HSD test $P < 0.05$.

16 h d⁻¹) was installed at time of re-watering to help maintain growth during the period of seasonal daylength decline. The experiment was arranged as a completely randomized design with factorial treatments (4 RvC × 4 moisture stress levels). Pots were re-arranged each month throughout the experiment to alleviate potential environmental variation associated with bench position.

2.2. Measurements

On 9 September, half of the seedlings (4 per treatment) were randomly selected, and harvested to examine the impact of drought on morphological development. The remaining plants were harvested at the end of the study (15 November). Seedlings were excavated from pots, washed free of media, and measured for stem height, stem diameter, fresh mass, FOLR, Rv , number of leaves, and leaf area (Model

LI-2000, Li-Cor, Inc., Lincoln, NE, USA). Seedlings were then oven-dried at 70 °C for 72 h and weighed.

Gas exchange measurements included A , E , and g_s at weekly intervals during the period 23 August (98 d after transplant and 27 d since discontinuation of LMS irrigation) through 26 September and bi-weekly from 26 September through 25 October. Gas exchange was measured using an infrared gas analyzer (Model LCA-3, Analytical Development Corporation, Hoddesdon, UK) between 1 100–1 300 h solar time. Measurements were taken during clear, sunny days. An external light source of approximately 1 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used to stabilize photosynthetically active radiation to minimize variation associated with light availability. Ambient CO_2 was drawn into the leaf chamber from a height of 3 m. Leaf temperature within the leaf chamber ranged from 26–27 °C and the sampled leaf always filled the 6.25 cm^2 leaf chamber. At each measurement, a single representative leaf was sampled from the flush prior to the current growth flush and measurements were recorded after about 1 min when readings stabilized. Measurements were conducted on five seedlings per treatment during the first two samplings, and four plants in subsequent samplings. Pre-dawn leaf xylem water potential was measured bi-weekly from 23 August until 19 September and a month thereafter. Measurements of Ψ_L were determined using a Scholander pressure chamber (Model 600, PMS Instruments, Inc., Corvallis, OR, USA) and always occurred the day following gas exchange measurements and were conducted on the same leaf used for gas exchange.

2.3. Statistical analysis

Analysis of variance (ANOVA) was conducted on all measured physiological and morphological variables using SAS and where appropriate significant means were ranked according to Waller-Duncan's multiple range tests at $\alpha = 0.05$. The Anderson and McLean's (1974) linear model was used for the ANOVA and is given as:

$$Y_{ijk} = \mu + M_i + Rv_j + MRv_{ij} + \varepsilon_{(ijk)}$$

where Y_{ijk} is seedling physiological or morphological attribute estimated from the k th replicate ($k = 1, 2, 3, 4$), from the j th RvC ($j = 1, 2, 3, 4$), and the i th moisture regime ($i = 1, 2, 3, 4$); μ = overall mean; M_i = fixed effect of the i th moisture regime; Rv_j = fixed effect of the j th RvC; followed by moisture by Rv interaction effects MRv_{ij} and ε is error associated with measured seedling physiological or morphological attribute from replicates. The ANOVA tables associated with the linear model are shown in Tables II and III.

3. RESULTS

3.1. Initial seedling morphological characteristics

Seedlings were graded into 4 RvC based on 25 percentiles of the population (Tab. I). Thus, treatments were designated as RvC1 (17 $\text{cm}^3 \pm 1.2$) (mean \pm SE), RvC2 (27 $\text{cm}^3 \pm 0.8$), RvC3 (39 $\text{cm}^3 \pm 1.0$), and RvC4 (72 $\text{cm}^3 \pm 7.2$) and differed significantly ($P < 0.0001$). Generally, seedlings with larger Rv were associated with greater plant fresh mass, stem diameter, height, and number of FOLR. For example, seedling fresh mass, diameter, and height were 338, 100, and 77% greater in

Table II. Analysis of variance associated with photosynthetic assimilation (A), transpiration rate (E), stomatal conductance (g_s), and predawn leaf xylem water potential (Ψ_L) as influenced by moisture stress (M) induced by drought and initial root volume (Rv). (Error degrees of freedom = 48).

Sampling date	Source of variation	$P > F$			
		A	E	g_s	Ψ_L^{\dagger}
23 -Aug	M	0.0001	0.0010	0.0002	0.0008
	Rv	0.0021	0.4748	0.7390	0.0001
	$M \times Rv$	0.0014	0.0061	0.0033	0.0109
4 -Sept	M	0.0001	0.0001	0.0001	0.0001
	Rv	0.0395	0.0077	0.0394	0.0001
	$M \times Rv$	0.0470	0.0418	0.0587	0.0146
12 -Sept	M	0.0001	0.0001	0.0001	–
	Rv	0.0050	0.0042	0.0295	–
	$M \times Rv$	0.1856	0.4506	0.7066	–
19 -Sept	M	0.0001	0.0001	0.0001	0.1664
	Rv	0.0052	0.0665	0.0209	0.0037
	$M \times Rv$	0.3236	0.1638	0.3418	0.6411
26 -Sept	M	0.0001	0.0001	0.0001	–
	Rv	0.0001	0.0001	0.0001	–
	$M \times Rv$	0.2272	0.0502	0.0762	–
10 -Oct	M	0.0001	0.0001	0.0001	–
	Rv	0.0001	0.0001	0.0001	–
	$M \times Rv$	0.0323	0.1379	0.0333	–
25 -Oct	M	0.0029	0.0001	0.0006	0.1540
	Rv	0.0156	0.0162	0.0109	0.0074
	$M \times Rv$	0.4221	0.9214	0.7990	0.7056

\dagger – Not determined.

RvC4 compared with RvC1 ($P < 0.0001$). Additionally, RvC4 plants had about 326% more fresh mass and were about 100% taller than RvC1 seedlings prior to transplant.

3.2. Seedling physiological responses to drought

For clarity, the experiment consisted of drought and recovery phases (Figs. 1–5 and Tab. III). The drought phase examined plant responses to moisture stress from 23 August through 9 September (i.e., 98–115 days after transplant; 27–44, 49–66, and 71–88 days after discontinuation of irrigation for the LMS, MMS, and HMS treatments, respectively). The recovery phase examined plant recovery from drought during

Table III. Analysis of variance testing effects of moisture stress (M) induced by drought and root volume (Rv) on northern red oak seedling growth response under greenhouse environments. (Error degrees of freedom = 48.)

Sampling phase	Source of variation	$P > F$						
		Root volume	Fresh mass	Diameter	Height	Lateral roots	Dry mass	# of leaves [†]
Drought	M	0.0001	0.0001	0.0001	0.1507	0.0043	0.0001	0.0061
	Rv	0.7661	0.1109	0.9834	0.0076	0.1849	0.0001	0.0001
	$M \times Rv$	0.0037	0.0006	0.1092	0.5153	0.0378	0.1339	0.2472
Recovery	M	0.0007	0.0001	0.0001	0.0001	0.6837	0.6837	–
	Rv	0.4193	0.2116	0.3102	0.0002	0.6411	0.6411	–
	$M \times Rv$	0.1396	0.0561	0.0478	0.2976	0.3813	0.3813	–

[†] – Not determined.

the period 10 September through 25 October (i.e., 116–161 days after transplant) following resumption of irrigation.

Drought induced moisture stress in seedlings and significantly affected A , E , g_s , and Ψ_L (Fig. 1, Tab. III). While WW plants maintained relatively stable Ψ_L , values declined in seedlings in the three moisture stress treatments during the drought phase (Fig. 1D). Compared with WW plants, Ψ_L was reduced (i.e., increased stress) during the drought phase by -0.4 , -0.7 , and -0.9 MPa ($P < 0.0008$) on 23 August and -1.3 , -1.5 , and -1.8 MPa ($P < 0.0001$) by 4 September in respective LMS, MMS, and HMS seedlings (Fig. 1D, Tab. II). During the recovery phase after resumption of irrigation, Ψ_L increased for all treatments on 19 September and remained relatively stable thereafter (Fig. 1D). Photosynthetic assimilation declined from 23 August to 4 September during the drought phase, increased during the recovery phase after resumption of irrigation until 26 September, and then decreased by 25 October in conformity with the phenological stage of growth decline in northern red oak (Fig. 1A). The decline in A during the drought phase intensified with increasing moisture stress ($P < 0.0001$, Fig. 1A, Tab. II). During the recovery phase, A steadily increased in LMS, MMS, and HMS treatments until 26 September, then declined thereafter. However, A in HMS seedlings never reached the level of WW seedlings during the recovery period. Trends in E (Fig. 1B) and g_s (Fig. 1C) were similar to those observed for A during the drought and recovery phases.

The general trend of decrease, increase, and then decline in A , E , and g_s found in this study indicates the relatedness of these parameters. Strong positive linear relationships were detected between g_s , A , and E in our study (i.e., g_s and E explained 86 and 91%, respectively, of the variation in A ; g_s accounted for 97% of the variation in E).

3.3. Influence of root volume on seedling physiological responses

Seedling Rv significantly affected A , E , g_s , and Ψ_L (Fig. 2, Tab. II). Plants with larger Rv experienced the highest water deficit (data pooled across moisture stress treatments) during

the drought phase. For example, RvC1 was the least stressed ($\Psi_L = -1.0$ MPa) while RvC4 was the most stressed ($\Psi_L = -3.3$ MPa) treatment by 4 September (Fig. 2D). There was a general decline in A in all RvC treatments during the drought phase, especially for RvC4 (Fig. 2A). Although all RvC treatments had similar transpiration rates on 23 August (i.e., 27, 49, and 71 d after discontinuation of irrigation for the LMS, MMS, and HMS treatments, respectively), differences became significant ($P < 0.0077$) by 4 September (Fig. 2B, Tab. II). Compared with RvC4, E was 5, 20, and 59% higher in respective RvC3, RvC2, and RvC1 treatments on 4 September during the drought phase, and 8, 38, and 31% higher on 19 September during the recovery phase (Fig. 2B). Values of E for all treatments peaked on 19 September during the recovery phase after resumption of irrigation and declined thereafter (Fig. 2B). Values for g_s also differed ($P < 0.0394$) between treatments on 4 September during the drought phase with g_s about 2 fold higher in RvC1 relative to RvC4 plants (Fig. 2C). During the recovery phase, g_s in all treatments increased on 26 September and then declined to lower levels by 25 October (Fig. 2C).

Seedling RvC by moisture stress interactions were generally significant for physiological parameters during the drought phase (i.e., 23 August through 4 September) and non-significant thereafter during the recovery phase (Tab. II). Significant interaction effects were indicated by reduced A , g_s , and E with increasing Rv under higher soil moisture stress conditions. On 23 August, for example, A was 7.96, 9.86, 8.23, and 10.16 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for respective RvC1, RvC2, RvC3, and RvC4 treatments in the WW treatment. In the water stress treatments, A declined much more rapidly for large RvC seedlings. In the LMS treatment, for example, A was 6.35, 8.82, 6.09, and 3.08 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for respective RvC1, RvC2, RvC3, and RvC4 treatments. In the MMS treatment, A was 6.31, 4.34, 6.50, and 1.66 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for respective RvC1, RvC2, RvC3, and RvC4 treatments. In the HMS treatment, A was 2.10, 3.50, 0.90, and 0.74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for respective RvC1, RvC2, RvC3, and RvC4 treatments. Similar trends were found for E , g_s , and Ψ_L for this sampling date and on 4 September (data not shown). This implies that increasing moisture stress more negatively affected performance of larger RvC seedlings.

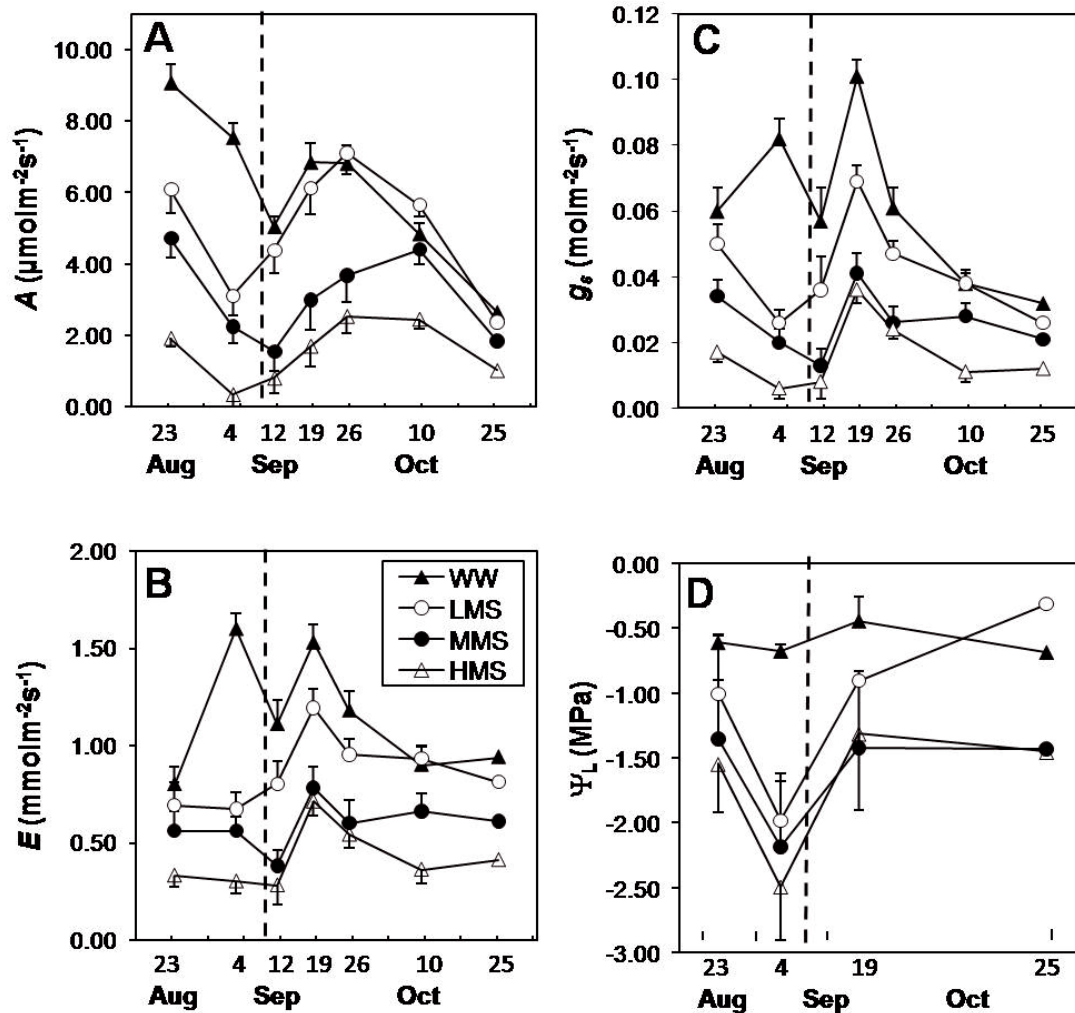


Figure 1. Variations in (A) photosynthetic assimilation (A), (B) transpiration rates (E), (C) stomatal conductance (g_s), and (D) predawn leaf xylem water potential (Ψ_L) in northern red oak seedlings grown under well watered conditions (WW), low moisture stress (LMS), medium moisture stress (MMS), and high moisture stress (HMS) as induced by drought under greenhouse environments. Bars represent standard error of the mean. The dashed line separates period during which drought was imposed from resumption of irrigation.

3.4. Effects of drought and seedling root volume on plant growth

Moisture stress reduced seedling growth except for height ($P < 0.1507$) during the drought phase (Figs. 3 and 4, Tab. III). Growth parameters remained lower following the recovery phase for plants that experienced significant water deficits. For example, plant dry mass was 110–134% greater in WW vs. HMS seedlings (Fig. 3, Tab. III). More growth was allocated to roots than shoots under increasing moisture stress, with shoot:root dry mass ranging from 1.31 (WW) to 1.10 (HMS). Compared to HMS treatments, plant fresh mass was 91, 213, and 465% (drought phase) and 37, 139, and 158% (recovery phase) higher in respective MMS, LMS, and WW plants (Fig. 3, Tab. III). Similar comparisons for R_v growth were 3, 7, and 24 times higher (drought phase) and about 2, 3, and 3 fold higher (recovery phase) in respective MMS, LMS, and

WW vs. HMS treatments. Seedling leaf area (A_L) and number of leaves were significantly reduced by drought (Fig. 4, Tab. III); the former was reduced by about 163% in HMS vs. WW plants.

Seedling R_v affected height, dry mass production, leaf area, and number of leaves at the sampling following the end of the drought phase (Figs. 4 and 5, Tab. III). For example, relative to RvC1, plant dry mass was greater by 24% in RvC2, 52% in RvC3, and 161% in RvC4 (Fig. 5). Leaf area increased from 32 to 81% and leaf numbers increased from 37 to 163% in respective RvC2 and RvC4 compared to RvC1 (Fig. 4, Tab. III). However, differences among RvC treatments were not statistically different for morphological parameters at the sampling following the recovery phase, except for height growth (Fig. 5, Tab. III).

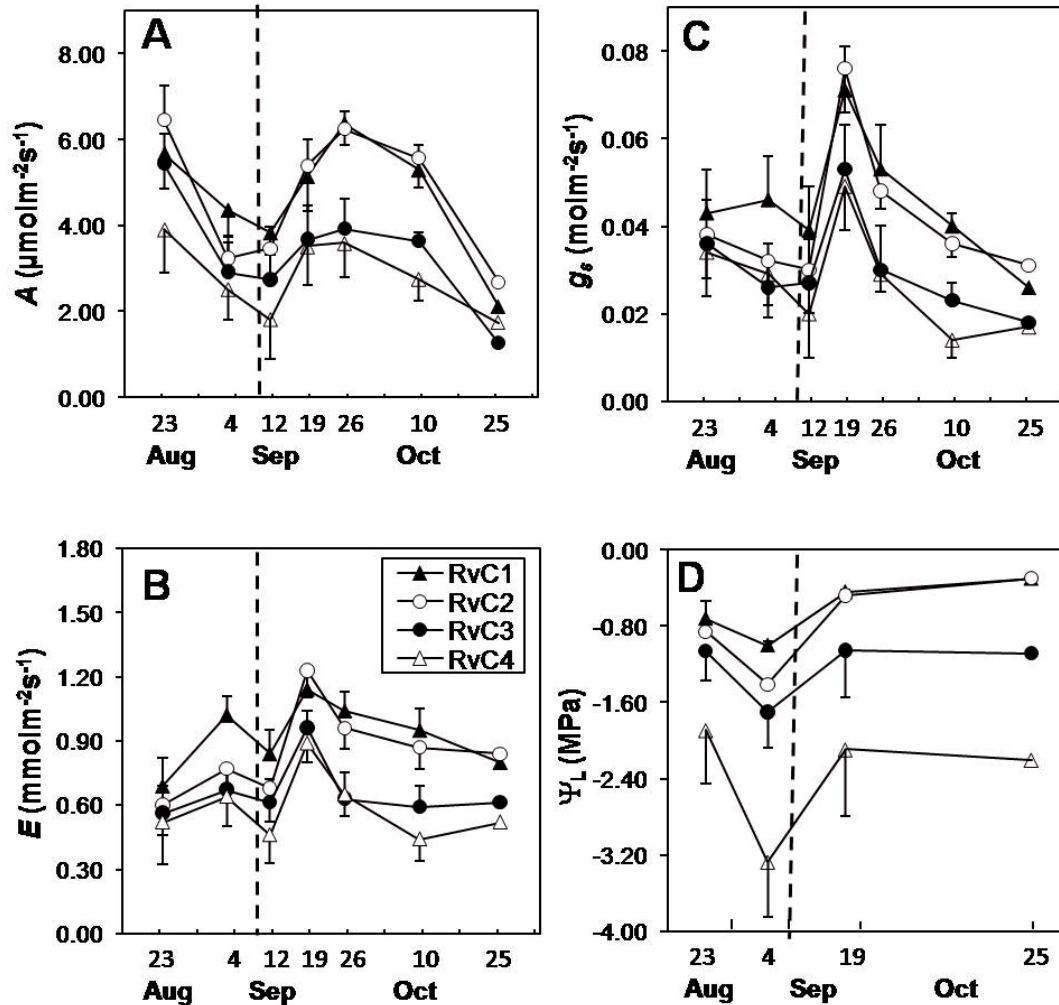


Figure 2. Variations in (A) photosynthetic assimilation (A), (B) transpiration rates (E), (C) stomatal conductance (g_s), and (D) predawn leaf xylem water potential (Ψ_L) in northern red oak seedlings as influenced by root volume category (RvC), ranging from the smallest (RvC1) to the largest (RvC4). Bars represent standard error of the mean. The dashed line separates period during which drought was imposed from resumption of irrigation.

4. DISCUSSION

4.1. Seedling physiological and morphological responses to drought

Drought induced moisture stress as indicated by lower Ψ_L in seedlings (Fig. 1). Water deficits were also associated with reduced A , E , and g_s , and growth consistent with results of others (Blake and Filho, 1988; Apostol et al., 2009). This confirms that moisture stress is an important factor causing transplant shock. These observations concur with results reported for Engelmann spruce (Burdett et al., 1984) and Douglas-fir (Haase and Rose, 1993) seedlings. Transplant shock was also indicated by reduced leaf area (Fig. 4B) consistent with results for loblolly pine (*Pinus taeda* L.) (Teskey et al., 1987), Douglas-fir (Haase and Rose, 1993), and northern red oak (Struve and Joly, 1992) seedlings. The mild water stress in WW plants following transplant indicates that there is a time

lag during which transplanted seedlings must re-establish root-soil contact for resource exploitation, and may also infer temporary impairment of root function (Burdett, 1990; Sands, 1984). This also suggests that timing planting to minimize exposure to stressful environmental conditions during the several weeks following planting will improve seedling establishment success.

Increased A with increasing g_s may be associated with improved intercellular CO_2 exchange and carbon fixation at higher g_s (Dixon et al., 1995). This may also suggest that A and g_s may be co-regulated in response to water stress (Weber and Gates, 1990). Strong positive linear relationships were detected between g_s , A , and E in our study as similarly observed for mature almond (*Prunus dulcis* (Mill.)) trees (Romero et al., 2004). In contrast, water deficits may decrease A directly by affecting the metabolic and photochemical process in the leaf or indirectly through stomatal closure leading to cessation of

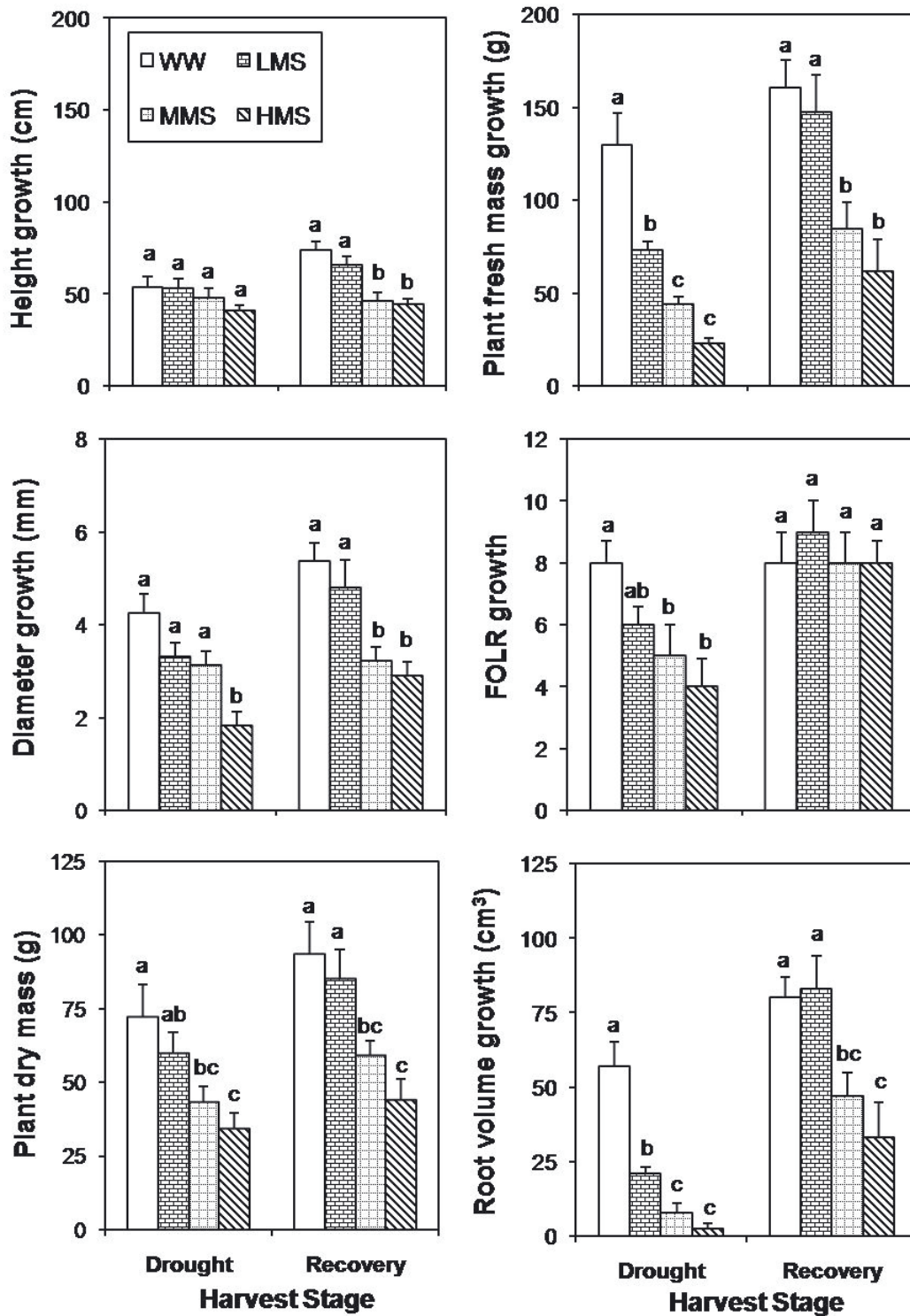


Figure 3. Northern red oak seedling growth sampled at the end of the drought and recovery (after resumption of irrigation) phases under well watered conditions (WW), low moisture stress (LMS), medium moisture stress (MMS), and high moisture stress (HMS). Bars represent standard error of the mean.

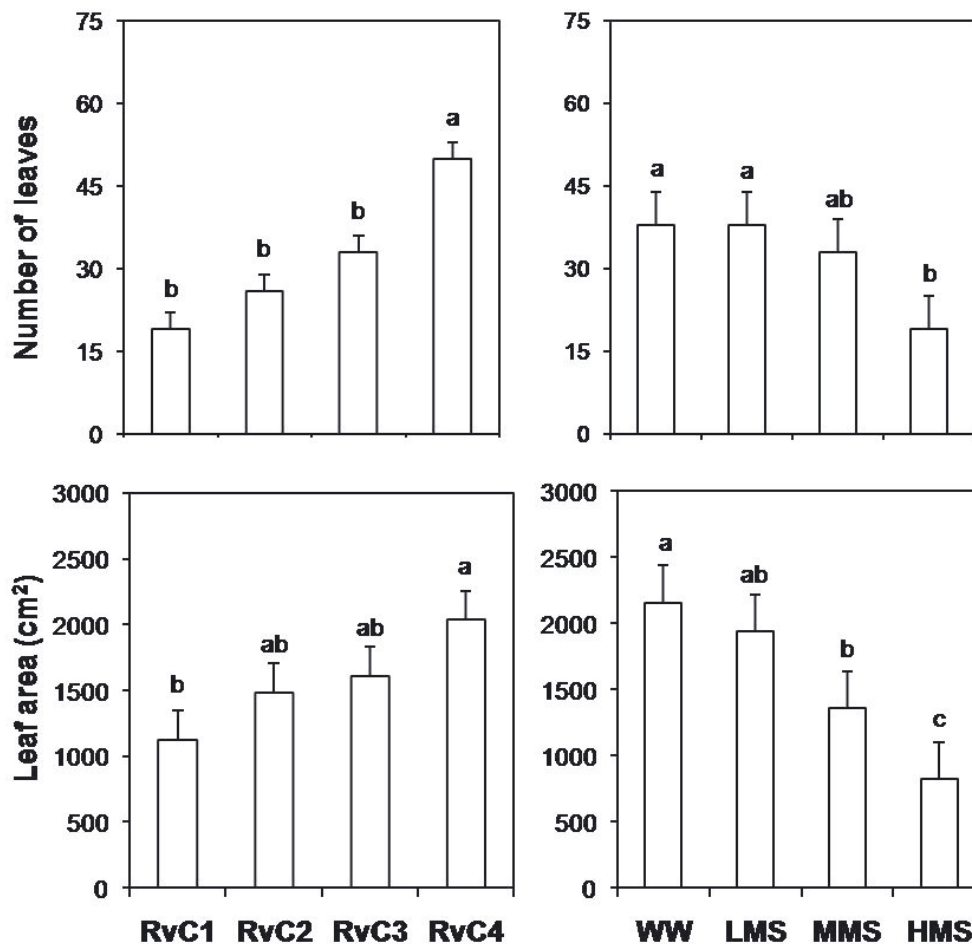


Figure 4. Northern red oak seedling leaf numbers and leaf area at the end of the drought phase as influenced by (left) root volume category (RvC), ranging from the smallest (RvC1) to largest (RvC4) and (right) grown under well watered conditions (WW), low moisture stress (LMS), medium moisture stress (MMS), and high moisture stress (HMS). Bars represent standard error of the mean.

leaf growth and consequent decreased leaf area. For instance, as Ψ_L decreases, g_s also declines (Meinzer, 1982) and A is directly related to g_s (Hinckley et al., 1978). Reductions in A and g_s in response to severe water stress were reversible (though still evident in highly stressed seedlings), suggesting that extended drought as applied in our study did not cause permanent damage (i.e., to permanent wilting point) to plant tissues. Thus, rapid (i.e., within 10 d in our study) recovery of Ψ_L and A after water deficits may reflect adaptive drought resistance in northern red oak seedlings.

4.2. Root volume alters seedling physiology and growth responses

Planting shock, caused by water or nutrient stress, may lead to root growth being limited by lack of current photosynthate or vice versa (Burdett, 1990, Grossnickle, 2000). Therefore, rapid root growth after planting is important to exploit water from surrounding soil, which will help establish a proper water balance in seedlings (Grossnickle, 2005). As demonstrated

here, increased root growth in LMS plants is one mechanism to explain the ability of these seedlings to resist transplant shock as indicated by higher Ψ_L .

Seedlings with large initial Rv have higher root growth potential (Carlson, 1986; Grossnickle, 2005), tend to better tolerate transplant shock over time (Haase and Rose, 1993), and may have more rapid early growth rates (Rose et al., 1991a; 1991b). Despite proposed advantages of planting large Rv seedlings, evidence suggests that large Rv does not mitigate drought avoidance in bareroot seedlings immediately following transplanting. No differences were found in Ψ_L at 95 or 115 d in Douglas-fir seedlings graded into different Rv classes and transplanted into pots (Haase and Rose, 1993). Similarly, Jacobs et al. (2004a) reported no significant differences in Ψ_L between RvC for Douglas-fir seedlings following field transplant. Contrary to above results, we observed that northern red oak seedlings with larger initial Rv were less able to mitigate effects of drought following transplant, as indicated by lower Ψ_L values (Fig. 2D). Furthermore, it has been shown that seedling Rv is positively correlated with leaf area (Carlson and

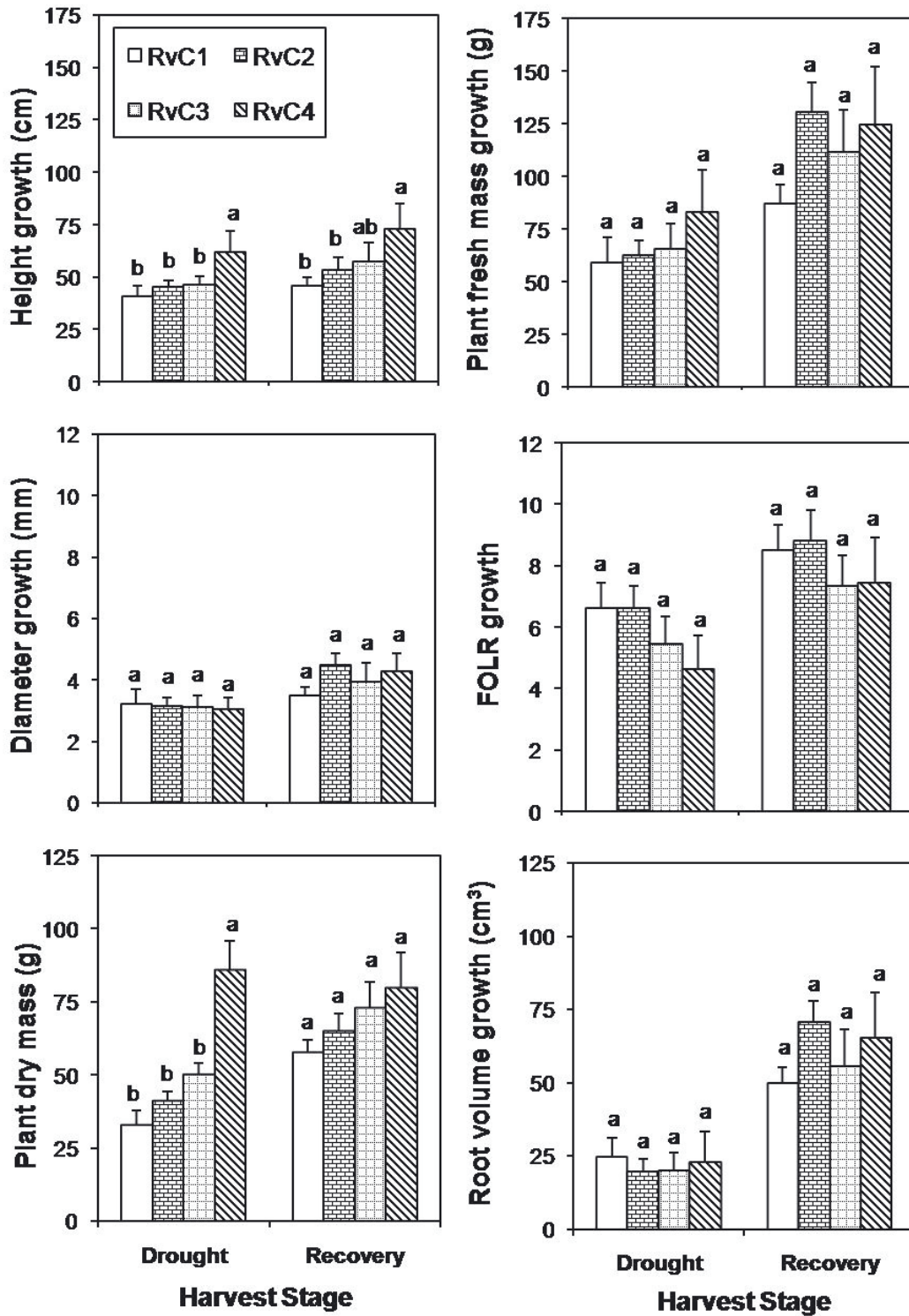


Figure 5. Northern red oak seedling growth sampled at the end of the drought and recovery (after resumption of irrigation) phases as influenced by root volume category (RvC), ranging from the smallest (RvC1) to the largest (RvC4). Bars represent standard error of the mean.

Miller, 1990). We also found that larger Rv plants were associated with greater transpiring area (Fig. 4A), which induced severe water deficits by lowering Ψ_L to -3.3 MPa in these plants (Fig. 2). Depressed A , E , and g_s with increasing Rv under drought conditions demonstrates that seedlings with larger initial Rv may be more prone to transplant shock especially on sites subject to summer drought (Blake, 1983; Grossnickle, 1988).

One mechanism to alleviate transplant shock is greater growth allocation to roots relative to shoots under stress as found in this study. Such a growth allocation strategy is a well-known adaptive mechanism by which plants respond to limitations of belowground resources (Teskey et al., 1987). In addition, reduced leaf area as observed in smaller RvC seedlings (Fig. 4) is yet another drought avoidance mechanism that alleviates transplant shock under high moisture stress (Struve and Joly, 1992). Hence, smaller transpiring leaf area as observed in RvC1 seedlings may increase survival and growth potential on droughty sites.

Large RvC seedlings with greater transpirational area may be at an earlier disadvantage on droughty sites because of anticipated increased E (Haase and Rose, 1993). However, our results do not support increased E in RvC4 seedlings (Figs. 2 and 4A). In fact, RvC4 seedlings responded to severe water deficits by limiting stomatal responses that resulted in reduced E (Fig. 2), demonstrating sensitivity of these plants to moisture stress. Furthermore, Haase and Rose (1993) contend that even if an earlier disadvantage were to occur in RvC4 plants due to higher E , the effect may be short term, during which time seedling root systems re-establish soil contact. Higher survival and growth of ponderosa pine (*Pinus ponderosa* C. Lawson) and Douglas-fir seedlings with larger initial Rv relative to those with smaller Rv after two growing seasons on harsh sites was attributed to greater access of larger root systems to limited water and nutrients (Rose et al., 1991a; 1991b). In contrast to the above scenarios, Blake and Filho (1988) reported that seedling size of *Eucalyptus* spp. was inversely related to g_s , and hence E . In our study, smaller seedlings were also associated with the highest g_s while larger seedlings had the lowest g_s (Fig. 2), consistent with these results.

Although moisture stress limited A in large RvC seedlings, growth was generally similar among RvC treatments (excepting greater height growth in RvC4 plants). Thus, greater A at the whole plant level associated with larger leaf area and numbers may be more important than A in explaining growth response in large RvC plants (Fig. 4A). This response may correspond to that of cork oak (*Quercus suber* L.) seedlings, in which increased specific leaf area associated with shading under water limiting conditions resulted in equivalent biomass production despite reductions in instantaneous photosynthetic rates (Puértolas et al., 2008). In addition, N and carbohydrate reserves are essential for new growth development before significant root uptake and photosynthesis (Salifu et al., 2008; Sloan and Jacobs, 2008). Therefore, higher internal nutrient reserves or stored carbohydrates may have contributed to growth in large plants. As a result, planting seedlings with high internal nutrient or carbohydrate reserves may improve field performance because reserves function as immediate sources

to meet ongoing growth demand until developing roots can exploit soil for growth resources. The significant moisture stress \times Rv interactions (Tab. III) reflected reduced performance of RvC4 seedlings on MMS and HMS conditions, suggesting that large stock should be targeted for sites with higher moisture availability. Strict stomatal regulation, increased root growth, root/shoot balance, and reduced leaf area appear to be some of the most effective mechanisms by which northern red oak seedlings can adapt to dry environments.

5. CONCLUSIONS

Drought induced physiological water deficit in northern red oak seedlings, which was more severe for larger RvC plants. This suggests that large initial root system size does not confer drought avoidance in bareroot northern red oak seedlings, as was similarly reported for Douglas-fir seedlings (Haase and Rose, 1993; Jacobs et al., 2004a), and that large planting stock should be targeted for sites with relatively higher soil moisture availability. Transplant shock in northern red oak seedlings was implicated by reduced Ψ_L , A , E , and growth. Rapid recovery of Ψ_L , and A after resumption of irrigation reflects adaptive drought resistance in northern red oak seedlings. Greater growth allocation to roots relative to shoots, increased root growth, reduced leaf area, and strict stomatal regulation seem to be the most effective mechanisms by which northern red oak seedlings resist moisture stress.

Controlled environment studies of seedling drought resistance have inherent limitations when extrapolating results to potential field responses. For example, root system proliferation is limited to a fixed soil volume in containers, which may alter soil-water physics. This type of experimentation under conditions free of potentially confounding ambient circumstances, however, is common in modern reforestation sciences (e.g., Haase and Rose, 1993; Apostol et al., 2009) and a necessary prerequisite to understanding mechanisms for drought resistance responses following field transplant. Use of relatively large pots (e.g., 12 L in this study) helps to better simulate field conditions, yet results must be interpreted with caution when making operational recommendations. Nevertheless, our results support those of Haase and Rose (1993) that selection of particular root system size for transplanting on sites of known moisture availability may be important to improve early growth and vigor of plantations. This knowledge is essential to help target seedlings with specific root volume to suitable planting environments for improved seedling establishment, which will enhance early plantation development.

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