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Nursery Management Modulates Biomass Allocation and Early Field Establishment of *Pinus douglasiana* Martínez

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Abstract

Nursery decisions influence the condition of seedlings before outplanting, but their practical value depends on whether those traits remain evident during early field establishment. We evaluated *Pinus douglasiana* Martínez seedlings grown under eight combinations of container volume, fertilization, and irrigation regime. Seedlings were conditioned for six months in the nursery and then assessed after 12 months under field conditions. Container volume was the clearest source of variation. Compared with 1 L containers, 5 L containers produced seedlings with larger root collar diameter, greater shoot, root, and total biomass, and higher Dickson quality index. Total biomass was 2.2-fold greater in 5 L stock. Final survival was retained as a descriptive field variable and averaged 76.7% in 5 L treatments and 27.5% in 1 L treatments. Correlations based on treatment means associated total biomass with field diameter and descriptive survival, although these patterns were exploratory because only eight treatment means were available. Proline increased mainly under non-continuous irrigation, indicating a water-related nursery response, but it was not aligned with biomass production or first-year field performance. The treatment-level PCA separated biomass and field-growth variables from proline and lignification. These results show that larger containers improved the structural condition of *P. douglasiana* planting stock, although nursery costs, planting-site constraints, and longer monitoring should be considered before operational adoption.

Keywords: *Pinus douglasiana*; nursery management; biomass allocation; drought stress; seedling quality; root biomass; forest management

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1. Introduction

Successful forest restoration often depends on how planted seedlings cope with the first months after outplanting, when water limitation, soil constraints, transplant shock, and weak root–soil contact can sharply reduce establishment success. Root growth is central during this phase because newly planted seedlings must rapidly reconnect water uptake with transpiration demand under field conditions [1]. For this reason, seedling quality should be assessed beyond external size and should include plant attributes related to survival and growth after planting, such as root development, biomass distribution, nutrient status, and stress tolerance [2]. Mexican pines have high restoration value because Mexico holds a large diversity of *Pinus* species, with many species linked to mountain ecosystems, timber production, soil protection, and watershed regulation [3]. *Pinus douglasiana* Martínez is a native Mexican pine distributed mainly along western and southern mountain systems, including Jalisco, Michoacán, Guerrero, Oaxaca, and nearby regions, where it contributes to forest structure and has practical value for reforestation and timber-oriented restoration. Work in Jalisco has emphasized the need for growth information specific to *P. douglasiana* [4]. Evidence from Mexican pine plantations also shows that nursery quality, plant size at planting, and field conditions can influence survival and early growth [5].

Nursery practices can shape the structural and physiological attributes that seedlings take to the planting site. Container volume determines the rooting space available during nursery growth, while fertilization and irrigation regulate nutrient and water availability during nursery conditioning [6]. These nursery factors act together rather than independently because added nutrients and water are more useful when the root system has enough space to explore the substrate and transform resources into functional biomass [7]. In pines, combined fertilization and irrigation regimes can modify seedling attributes and later field performance, showing that nursery decisions may leave measurable effects after planting [8]. For field establishment, seedling size alone is less informative than the way dry matter is distributed among plant organs. Biomass allocation reflects how plants distribute resources to leaves, stems, and roots in response to environmental conditions, ontogeny, and resource availability [9]. Initial seedling characteristics can modify later growth, reserve allocation, and drought response, which means that nursery size should be interpreted together with biomass distribution and belowground development [10]. Thus, seedlings with high shoot demand but weak belowground development may appear vigorous in the nursery while remaining vulnerable after planting.

Root–shoot balance provides information that height by itself cannot capture. Height can increase under favorable nursery conditions, but tall seedlings are not necessarily better prepared for sites where water availability becomes limiting after outplanting; in contrast, root biomass, total biomass, and integrated indices such as the Dickson quality index help describe whether plant size is supported by a balanced structure [11]. Recent nursery work has shown that container size and irrigation can modify morphological and physiological traits linked to performance under dry planting conditions, reinforcing the need to evaluate seedling quality through multiple attributes rather than isolated size variables [12].

Physiological and anatomical indicators can complement biomass traits by separating structural growth from stress-related responses. Proline commonly increases in woody seedlings under drought stress, although its magnitude depends on stress duration, seed traits, and species response [13]. Stem lignification can also reflect structural adjustment to water limitation because lignin deposition contributes to xylem reinforcement, mechanical support, and drought-related tissue protection [14]. However, biochemical indicators require cautious interpretation because they do not necessarily predict field establishment directly and may vary with drought intensity and post-stress recovery [15]. Ectomycorrhizal colonization provides an additional belowground indicator because fungal associations can influence seedling water relations, root morphology, and biomass responses under drought conditions [16]; considering these variables together helps distinguish physiological response, structural adjustment, and belowground symbiotic activity.

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Container volume, fertilization, and irrigation are common management variables in forest nurseries, but their combined effects are still often interpreted through final size, survival, or isolated physiological measurements. Less attention has been given to how these practices influence dry-matter allocation, stress-related indicators, and early field response within the same nursery-to-field evaluation [17]. This point is relevant for *P. douglasiana*, because planting stock for native Mexican pines must be able to face seasonal water limitation and variable soil conditions after outplanting. This study evaluated the effects of container volume, fertilization, and irrigation regime on biomass allocation, root development, stress-related indicators, and early field response of *P. douglasiana* seedlings. We expected seedlings with greater root biomass and a more balanced allocation pattern to show better early field performance than seedlings with weaker belowground development, even when fertilization or irrigation inputs were increased. The objective was to determine whether nursery management modulates functional traits and early establishment in *P. douglasiana*, providing species-specific evidence for planting stock management under field conditions.

2. Materials and Methods

2.1. Study Area

The study was carried out in Jalisco, western Mexico, following a nursery-to-field evaluation scheme. Seedlings were first conditioned at the Valle de Ameca Forest Nursery S.P.R. de R.L., located in Ameca, Jalisco (20°33′ N, 104°03′ W; 1235 m a.s.l.). This area has a warm subhumid climate with summer rainfall, temperatures ranging from 8.5 to 32.9 °C, and mean annual precipitation of approximately 924 mm [18]. Field establishment was conducted at Las Trojes, Jocotepec, Jalisco (20°19.632′ N, 103°19.190′ W; 1840 m a.s.l.; Figure 1). The site is characterized by a transition from semiarid to warm subhumid conditions, with mean annual temperature of 18.5 °C and annual precipitation near 844 mm. Soils are classified as Vertisols, whose shrink–swell behavior and hydrological properties may affect water availability, aeration, infiltration, and early root development after planting [19,20].

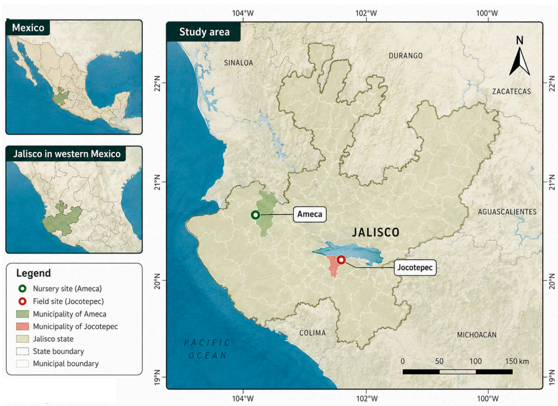

Figure 1. Geographic location of the nursery conditioning and field validation sites used to evaluate *Pinus douglasiana* seedlings in Jalisco, western Mexico. The nursery stage was conducted in Ameca, whereas field establishment was evaluated in Jocotepec. The inset maps show the position of Jalisco within Mexico and the regional location of the two study municipalities.

2.2. Plant Material and Experimental Framework

The study used *P. douglasiana* seedlings produced under operational nursery conditions before field planting. The experimental design was intended to test whether rooting space, additional nutrient supply, and irrigation frequency changed seedling structure during nursery conditioning and whether those differences were still evident after outplanting. Seedlings were first grown in polystyrene trays with 60 cavities, each with a volume of

0.165 L. At the start of the experiment, plants were 12 months old and had completed the initial production phase. They were then transferred to the experimental containers and kept under a common stabilization period before the nursery treatments began.

Three nursery factors were evaluated: container volume, fertilization, and irrigation regime. Container volume represented rooting space, fertilization represented additional nutrient input, and irrigation regime represented contrasting water supply during conditioning. These factors were selected because they can influence belowground biomass development, structural balance, and seedling response during the transition from nursery to field. Before treatment allocation, seedlings were selected for uniform size and healthy appearance. Approximately 100 seedlings per treatment were available during the nursery phase. For morphological and biomass evaluation, 12 seedlings were measured per treatment combination, giving 96 seedlings across the eight treatments. These seedlings were considered independent biological replicates for seedling-level variables. No tray or nursery block was used as the experimental unit. Seedlings were randomly assigned to treatments, and each seedling was considered the experimental unit for morphology, biomass, and quality-index variables.

For nutrient status and proline, three independent samples were analyzed per treatment. After nursery evaluation, destructive sampling, and losses during preconditioning, 70 viable seedlings per treatment were planted at the field site. Field variables were summarized from two field sampling records per treatment. Survival was calculated as a final percentage for each treatment and was therefore interpreted descriptively. The same treatment structure was followed from nursery conditioning to field assessment, allowing the analysis to evaluate whether nursery differences in biomass formation and stress-related responses remained visible during early establishment.

2.3. Nursery Conditioning and Treatment Structure

After the initial production phase, 12-month-old *P. douglasiana* seedlings were moved from 0.165 L cavities to two container volumes, 1 L and 5 L, to create contrasting rooting-space conditions. All seedlings then remained for one month under shared stabilization conditions before fertilization and irrigation treatments began.

The conditioning period lasted six months. This included one month of stabilization and five months of treatment application. The treatment structure combined two container volumes, two fertilization levels, and two irrigation regimes, resulting in eight nursery treatments. Treatment codes identified the species, container volume, fertilization condition, and irrigation regime. These treatments were selected to represent practical nursery management options. All seedlings were maintained in the same nursery area under comparable shade, substrate, and handling conditions.

Dg1FR and Dg5FR corresponded to fertilized seedlings under continuous irrigation in 1 L and 5 L containers. Dg1FSR and Dg5FSR represented fertilized seedlings under non-continuous irrigation. Dg1SFR and Dg5SFR corresponded to non-fertilized seedlings under continuous irrigation, while Dg1SFSR and Dg5SFSR represented non-fertilized seedlings under non-continuous irrigation. This coding allowed the combined effects of rooting space, nutrient addition, and water supply to be followed across nursery and field measurements.

Fertilized seedlings received an additional nutrient solution prepared with magnesium nitrate, calcium nitrate, monopotassium phosphate, potassium nitrate, urea, and Gro-green® 20-30-10 + EM (Gor Green Fertilizantes, Puebla, México). The components were dissolved in 25 L of water and applied manually twice per week during the five-month treatment period over the nursery area assigned to fertilized treatments. Each 25 L solution supplied approximately 43.9 g N, 38.0 g P_2O_5, and 44.3 g K_2O, without considering the micronutrient contribution from Gro-green® + EM. With two applications per week for

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approximately 20 weeks, the estimated macronutrient input over the fertilized nursery area was 1.76 kg N, 1.52 kg P_2O_5, and 1.77 kg K_2O. Fertilized seedlings also received 10 g plant^{-1} of Multi-micro Haifa® (Haifa Group, Haifa, Israel) directly in the substrate once per week for four months. This application served as a micronutrient complement during nursery conditioning. Non-fertilized seedlings did not receive additional foliar or substrate fertilization beyond the shared baseline substrate conditions.

Continuous irrigation was applied daily, while non-continuous irrigation was applied every 10 days. In both regimes, irrigation was supplied manually until substrate saturation, using a flow rate of 1.16 L s^{-1} for one hour. This was equivalent to 4176 L per irrigation event over the nursery area. During the five-month treatment period, estimated water input was approximately 626.4 m^3 under continuous irrigation and 62.6 m^3 under non-continuous irrigation. These values were equivalent to 1188.6 and 118.9 L m^{-2}, respectively. Gravimetric substrate moisture averaged 306% under continuous irrigation and 44% under non-continuous irrigation. Mean temperature and relative humidity during conditioning were 21 °C and 74%.

2.4. Substrate and Baseline Nursery Conditions

All seedlings received the same growing medium before the start of the conditioning treatments. The substrate was prepared with peat moss and composted pine bark at a 30:70 proportion and included controlled-release Multicote™ 24-12-6-(4) at 6 kg m^{-3}. This basal amendment supplied 24% N, 12% P_2O_5, 6% K_2O, and 4% MgO, and was considered part of the initial production condition shared by all treatments.

The substrate had 88% total porosity, 10% air-filled porosity, 78% water-holding capacity, and bulk density of 0.24 g cm^{-3}. These values describe the physical condition of the growing medium used during containerized seedling production and provide context for the evaluation of container volume and irrigation regime.

Initial chemical characterization was performed before applying the conditioning treatments. The substrate showed acidic pH, moderate electrical conductivity, high organic matter, and measurable macro- and micronutrient concentrations. These values were used only to describe the common starting environment for all seedlings. After transfer to the experimental containers, seedlings received Raizone-plus® (Fax México, Mexico City, Mexico) at 40 g in 25 L of water during the shared stabilization period. The product contains alpha-naphthylacetamide and indole-3-butyric acid. During this first month, all plants were kept under 50% shade cloth and received daily irrigation. Because this stabilization phase was common to all treatments, later differences were attributed to the assigned container volume, fertilization condition, and irrigation regime.

2.5. Field Establishment and Early Performance Assessment

After the six-month nursery conditioning phase, *P. douglasiana* seedlings were transferred to the field site at Las Trojes, Jocotepec, Jalisco, Mexico. At the field stage, 70 viable seedlings per treatment were planted, for a total of 560 seedlings across the eight nursery treatments. Before planting, competing vegetation was removed manually, and the area was fenced with barbed wire to prevent livestock damage during the first establishment year. Seedlings were planted at 4 × 4 m spacing in a staggered triangular arrangement. This layout reduced early competition among neighboring seedlings and provided enough space to evaluate survival and growth responses during the first year after outplanting. The field trial did not include formal blocks. Seedlings from the eight nursery treatments were distributed across the planting area to reduce microsite bias; therefore, no block effect was included in the statistical models.

The field site is located at 1840 m a.s.l. and is characterized by a mean annual temperature of 18.5 °C and annual precipitation near 844 mm. Soils are classified as Vertisols, with pH of 5.63, electrical conductivity of 0.06 dS m^{-1}, cation exchange capacity of 15.73 meq 100 g^{-1}, organic matter of 3.85%, and total nitrogen of 0.18%; phosphorus availability was very low, whereas calcium, magnesium, potassium, iron, manganese, copper, and zinc showed variable concentrations. These soil conditions were considered relevant because Vertisols can influence water retention, aeration, infiltration, and root development during early plantation establishment [20].

The field assessment lasted 12 months. At the end of this period, seedlings had reached 30 months of age, considering the initial nursery production stage, the six-month conditioning phase, and the first year after outplanting. The field stage was used to determine whether the biomass and stress-related traits produced during nursery conditioning were reflected in early establishment performance.

Field performance was evaluated through survival, root collar diameter, plant height, ectomycorrhizal colonization, stomatal density, and stem lignification. These variables described complementary establishment dimensions: persistence after planting, early structural growth, belowground symbiotic response, leaf-level anatomical adjustment, and structural stress expression.

Survival was calculated as (1):

$$Survival\ (\%) = \frac{Ns}{Nt} \times 100 \quad (1)$$

where Ns is the number of surviving seedlings and Nt is the total number of planted seedlings. Survival was calculated at the treatment level and was used only as a descriptive field indicator.

2.6. Nursery Biomass Partitioning and Structural Measurements

Seedling responses were evaluated at the end of the six-month conditioning phase using structural, biomass-based, and integrated quality variables. The main purpose of this evaluation was to determine whether nursery inputs modified dry matter distribution between aboveground and belowground organs before outplanting.

Root collar diameter and total height were measured as primary structural traits. Diameter was interpreted as an indicator of stem robustness and potential field stability, whereas height was considered together with biomass traits because taller seedlings are not necessarily better prepared for establishment when root development is limited [2].

A destructive sample was used to estimate aboveground and root dry biomass. Seedlings were separated into shoot and root components and oven-dried at 70 °C for 72 h until constant weight. Total dry biomass (TB) was calculated as (2):

$$TB = SB + RB \quad (2)$$

where TB is total dry biomass, SB is shoot dry biomass, and RB is root dry biomass.

The root–shoot ratio was calculated to describe biomass partitioning between belowground and aboveground structures (3):

$$RSR = \frac{RB}{SB} \quad (3)$$

where RSR is the root–shoot ratio, RB is root dry biomass, and SB is shoot dry biomass.

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The Dickson quality index (*DQI*) was included as an integrated indicator because it combines plant size, sturdiness, and biomass allocation into a single value [21]. It was calculated as (4):

$$DQI = \frac{TB}{\left(\frac{H}{D}\right) + \left(\frac{SB}{RB}\right)} \quad (4)$$

where *DQI* is the Dickson quality index, *TB* is total dry biomass, *H* is seedling height, *D* is root collar diameter, *SB* is shoot dry biomass, and *RB* is root dry biomass.

These variables were used to characterize whether nursery treatments produced seedlings with greater biomass accumulation or a more balanced distribution of dry matter. For this study, biomass partitioning was considered a central response because root investment can influence water uptake, nutrient acquisition, and early field persistence after planting.

2.7. Nutrient Profile and Stress-Related Indicators

Nutrient status was evaluated at the end of the nursery conditioning phase to determine whether the treatments modified the chemical profile of *P. douglasiana* seedlings before field establishment. Total nitrogen was determined using the semi-micro Kjeldahl method. Phosphorus, potassium, calcium, magnesium, sulfur, sodium, iron, zinc, manganese, copper, and boron were quantified after wet digestion using inductively coupled plasma atomic emission spectroscopy.

These elements were included because the database contained a broad nutritional profile, allowing nutrient responses to be interpreted together with biomass allocation and stress-related traits. This broader nutrient set was retained to support an integrated interpretation of nursery management effects on nutrient status, biomass allocation, stress expression, and early field performance.

Proline was determined following the acid-ninhydrin colorimetric method described by Bates et al. (1973) [22]. Proline accumulation is commonly associated with osmotic adjustment under drought or water-limiting conditions, although its interpretation depends on species, stress duration, and seedling developmental stage [13]. In this study, proline was interpreted as a complementary physiological indicator associated with water limitation, osmotic adjustment, or stress expression, rather than as a direct measurement of drought tolerance or seedling quality.

After 12 months in the field, ectomycorrhizal colonization was evaluated to determine whether nursery treatments influenced root symbiotic response after outplanting. Fine-root subsamples were collected from each evaluated seedling and washed with distilled water to remove soil particles. Ectomycorrhizal and non-ectomycorrhizal root tips were counted under a Fisher Scientific stereomicroscope.

Ectomycorrhizal colonization (*EC*) was calculated as (5):

$$EC\ (\%) = \frac{Nm}{Nt} \times 100 \quad (5)$$

where *EC* is ectomycorrhizal colonization, *Nm* is the number of ectomycorrhizal root tips, and *Nt* is the total number of evaluated root tips.

Stomatal density was evaluated as a leaf-level anatomical trait associated with gas exchange and water regulation. Stomatal traits influence the balance between carbon assimilation and water loss, although their response to drought or nursery conditioning may vary according to species and stress intensity [23]. Stomatal density was calculated as (6):

$$SD = \frac{Ns}{A} \quad (6)$$

where *SD* is stomatal density, *Ns* is the number of stomata, and *A* is the evaluated leaf area. Stomatal density was estimated from microscope images using a calibrated observation area. Stomata were counted within the evaluated area, and density was expressed as the number of stomata per unit area.

Stem lignification was evaluated as a structural indicator of stress-related adjustment. Lignin deposition contributes to mechanical support, xylem reinforcement, and protection against tissue collapse under water limitation [14]. The percentage of lignified stem area was calculated as (7):

$$LA\ (\%) = \frac{Al}{At} \times 100 \tag{7}$$

where *LA* is lignified stem area, *Al* is the lignified area, and *At* is the total evaluated stem area. Lignified stem area was estimated from stem cross-section images using calibrated area measurements. The lignified area and total evaluated stem area were delimited, and lignification was expressed as the percentage of lignified area relative to the total stem area. Together, these measurements allowed nursery treatments to be interpreted beyond plant size. The analysis connected nutrient profile, biomass partitioning, biochemical stress expression, and early field performance within the same experimental framework.

2.8. Statistical Analysis

Statistical procedures were organized in two stages. First, a factorial model was fitted to test container volume, fertilization, irrigation regime, and their interaction terms. These three factors were treated as fixed effects. The response variables included root dry biomass, shoot dry biomass, total dry biomass, root–shoot ratio, Dickson quality index, proline concentration, ectomycorrhizal colonization, stomatal density, and lignified stem area. For nursery traits measured at the seedling level, each seedling was used as the observational unit, and means were obtained after fitting the models. Second, the eight treatment combinations were compared directly because these codes are used throughout the tables and figures. For this comparison, a one-way ANOVA was fitted with treatment as the fixed factor. Tukey's honestly significant difference test was applied when significant differences were detected. Residual normality and homogeneity of variance were checked before interpreting parametric results. When these assumptions were not satisfied, treatment level comparisons were evaluated with non-parametric alternatives.

Survival was not included in an inferential survival model. The field record contained final survival percentages by treatment, not individual live/dead observations. Therefore, although 70 viable seedlings were planted per treatment, the available data structure did not support a binomial GLM, logistic regression, or another seedling-level survival model. Survival was retained only as a descriptive field variable. Spearman correlations were calculated using treatment means to explore associations between total biomass and selected nursery or field variables. Because this analysis used only eight treatment means, the results were treated as exploratory. Principal component analysis was also conducted with treatment means because the variables came from different sampling levels, including seedling-level measurements, nutrient and proline samples, field records, and treatment-level survival percentages. The ordination was therefore used only as a descriptive treatment-level summary. Analyses were performed in SAS 9.4, with $p < 0.05$ as the significance threshold [24].

3. Results

3.1. Nursery Treatments Modified Biomass Partitioning and Structural Development

The factorial analysis showed that container volume had the clearest effect on root collar diameter, shoot biomass, root biomass, total biomass, and Dickson quality index (Table 1). Fertilization, irrigation regime, and interaction terms affected fewer traits.

Table 1. Factorial effects of container volume, fertilization, irrigation regime, and their interactions on nursery and field traits of *Pinus douglasiana* seedlings.

Variable	Container Volume	Fertilization	Irrigation	C × F	C × I	F × I	C × F × I
Nursery root collar diameter	F = 181.64; $p < 0.001$	F = 9.91; $p = 0.002$	F = 4.79; $p = 0.031$	F = 0.30; $p = 0.584$	F = 4.02; $p = 0.048$	F = 0.12; $p = 0.731$	F = 0.06; $p = 0.804$
Nursery height	F = 2.16; $p = 0.145$	F = 2.02; $p = 0.158$	F = 0.03; $p = 0.855$	F = 1.16; $p = 0.284$	F = 0.87; $p = 0.352$	F = 1.13; $p = 0.290$	F = 1.28; $p = 0.260$
Shoot biomass	F = 345.08; $p < 0.001$	F = 21.78; $p < 0.001$	F = 7.53; $p = 0.007$	F = 7.48; $p = 0.008$	F = 0.17; $p = 0.685$	F = 0.56; $p = 0.458$	F = 0.70; $p = 0.404$
Root biomass	F = 274.00; $p < 0.001$	F = 16.78; $p < 0.001$	F = 4.01; $p = 0.048$	F = 6.25; $p = 0.014$	F = 4.75; $p = 0.032$	F = 2.75; $p = 0.101$	F = 0.69; $p = 0.407$
Total biomass	F = 430.62; $p < 0.001$	F = 2.07; $p = 0.153$	F = 8.19; $p = 0.005$	F = 0.64; $p = 0.427$	F = 0.50; $p = 0.481$	F = 1.66; $p = 0.201$	F = 0.05; $p = 0.831$
Dickson quality index	F = 378.58; $p < 0.001$	F = 3.55; $p = 0.063$	F = 6.88; $p = 0.010$	F = 1.91; $p = 0.170$	F = 3.73; $p = 0.057$	F = 1.92; $p = 0.169$	F = 0.43; $p = 0.513$
Proline	F = 426.09; $p < 0.001$	F = 345.13; $p < 0.001$	F = 12956.17; $p < 0.001$	F = 345.13; $p < 0.001$	F = 426.09; $p < 0.001$	F = 367.39; $p < 0.001$	F = 345.13; $p < 0.001$
Field root collar diameter	F = 364.31; $p < 0.001$	F = 0.05; $p = 0.822$	F = 0.20; $p = 0.670$	F = 0.58; $p = 0.469$	F = 7.56; $p = 0.025$	F = 10.83; $p = 0.011$	F = 0.00; $p = 0.953$
Field height	F = 1.68; $p = 0.231$	F = 0.52; $p = 0.493$	F = 0.06; $p = 0.809$	F = 1.24; $p = 0.298$	F = 3.15; $p = 0.114$	F = 6.37; $p = 0.036$	F = 0.97; $p = 0.353$
Ectomycorrhizal colonization	F = 150.33; $p < 0.001$	F = 14.29; $p = 0.005$	F = 35.27; $p < 0.001$	F = 0.34; $p = 0.577$	F = 3.35; $p = 0.105$	F = 3.83; $p = 0.086$	F = 92.20; $p < 0.001$
Stomatal density	F = 7.32; $p = 0.027$	F = 0.14; $p = 0.718$	F = 0.39; $p = 0.550$	F = 0.39; $p = 0.550$	F = 0.92; $p = 0.366$	F = 0.92; $p = 0.366$	F = 0.21; $p = 0.659$
Lignification	F = 0.71; $p = 0.423$	F = 16.90; $p = 0.003$	F = 11.02; $p = 0.011$	F = 21.94; $p = 0.002$	F = 3.55; $p = 0.096$	F = 10.90; $p = 0.011$	F = 1.12; $p = 0.321$

C = container volume; F = fertilization; I = irrigation regime. Survival was not included in the factorial ANOVA because it was recorded as treatment-level percentages and was interpreted descriptively.

Treatment effects were evident in the nursery phase through changes in stem robustness and dry-matter production (Figure 2A–E). Root collar diameter, shoot biomass, root biomass, total biomass, and Dickson quality index differed among treatments, whereas height showed no significant treatment response. Thus, treatment separation was expressed mainly through biomass partitioning and structural development rather than through taller seedlings. Seedlings produced in 5 L containers reached higher root collar diameter values than those produced in 1 L containers (Figure 2A). The same container-related pattern was observed for shoot, root, and total biomass (Figure 2B–D). Root biomass showed a marked contrast between container volumes, and mean total biomass in 5 L seedlings was 2.2-fold greater than in 1 L seedlings. The Dickson quality index also increased in the 5 L treatments (Figure 2E), following the same tendency observed for biomass traits. This response indicates that larger containers produced planting stock with stronger integrated structural condition before field establishment.

3.2. Nursery Treatments Modified Seedling Nutrient Status

Nursery treatments modified the macronutrient profile of *P. douglasiana* seedlings, although the response differed among elements (Table 2). Significant treatment effects were detected for N (F = 22.73, $p < 0.001$), P (F = 6.05, $p = 0.001$), K (F = 3.06, $p = 0.030$), Mg (F = 4.23, $p = 0.008$), S (F = 6.82, $p < 0.001$), and Na (F = 12.89, $p < 0.001$). Calcium did not differ among treatments (F = 1.05, $p = 0.437$). The post hoc comparison showed that N separated the 5 L non-continuous and non-fertilized treatments from the 1 L treatments and Dg5FR, whereas P, Mg, S, and Na showed more treatment-specific responses.

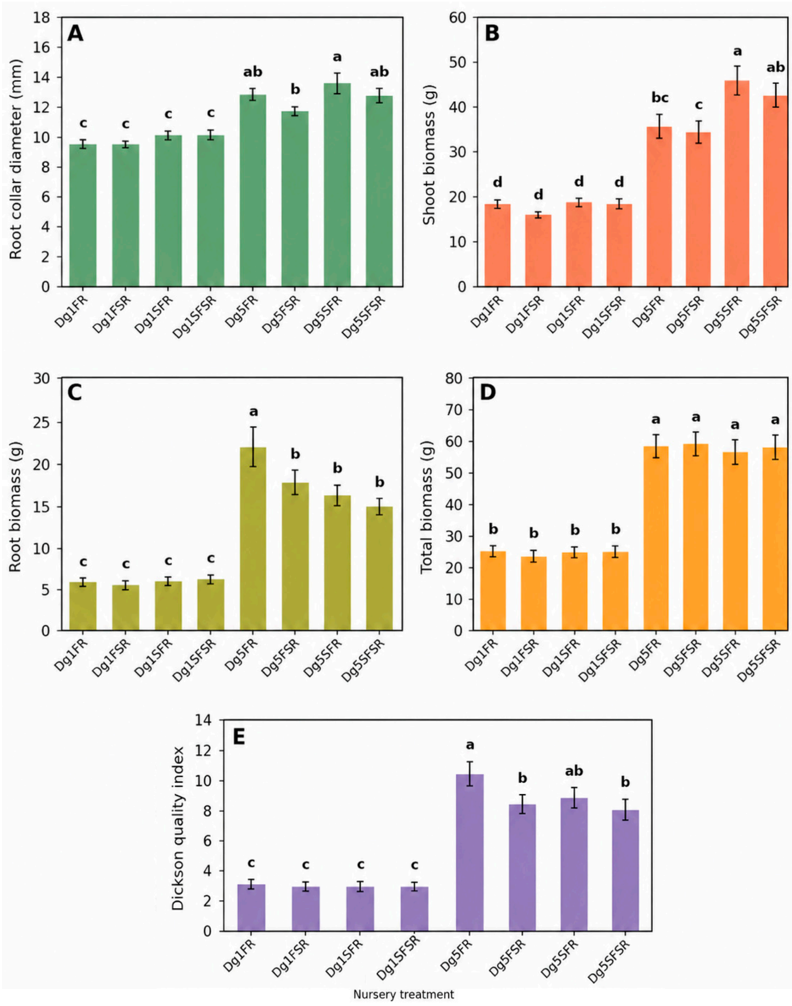

Figure 2. Effects of nursery treatments on root collar diameter (**A**), shoot biomass (**B**), root biomass (**C**), total biomass (**D**), and Dickson quality index (**E**) of *Pinus douglasiana* seedlings after six months of nursery conditioning. Bars represent means ± SE. Different letters above bars indicate significant differences among treatments according to Tukey's honestly significant difference test ($p < 0.05$). Treatments sharing at least one letter are not significantly different; for example, a treatment labeled "ab" does not differ significantly from treatments labeled "a" or "b". Treatment codes combine container volume, fertilization, and irrigation regime: 1 or 5 = container volume in liters; F = fertilized; SF = non-fertilized; R = continuous irrigation; SR = non-continuous irrigation.

Nitrogen showed the clearest macronutrient separation, with higher concentrations in several 5 L treatments, particularly under non-continuous irrigation or without additional fertilization. Phosphorus, magnesium, sulfur, and sodium showed more treatment-specific responses, whereas potassium had a significant overall treatment effect but no clear pairwise separation in Tukey's test. Calcium remained comparatively stable across treatments. The micronutrient profile also changed among treatments (Table 3). Significant effects were detected for Fe, Mn, and B, whereas Zn and Cu did not differ among treatments. Manganese showed the strongest treatment separation, while Fe and B showed more specific responses among nursery combinations.

Table 2. Macronutrient concentrations in nursery-conditioned *Pinus douglasiana* seedlings.

Treatment	N (%)	P	K	Ca	Mg	S	Na
Dg1FR	0.74 ± 0.05 b	1195.32 ± 75.28 bc	2225.11 ± 132.22 a	2048.36 ± 129.94 a	1584.61 ± 97.32 ab	906.43 ± 49.90 b	874.18 ± 65.39 bc
Dg1FSR	0.75 ± 0.04 b	1546.85 ± 21.70 ab	2799.03 ± 36.19 a	2128.76 ± 38.36 a	1847.38 ± 26.63 a	1412.85 ± 34.82 a	1299.99 ± 27.97 a
Dg1SFR	0.66 ± 0.03 b	1303.84 ± 9.11 ac	2608.46 ± 21.72 a	2028.11 ± 21.18 a	1749.86 ± 8.83 a	1119.88 ± 29.59 ab	1067.46 ± 5.51 ab
Dg1SFSR	0.52 ± 0.05 b	1228.45 ± 16.35 bc	2434.01 ± 61.19 a	1715.89 ± 32.13 a	1378.11 ± 20.84 ab	1082.44 ± 13.82 b	1114.17 ± 10.61 ab
Dg5FR	0.72 ± 0.01 b	1114.54 ± 21.96 c	2511.30 ± 61.83 a	1858.81 ± 13.06 a	1239.12 ± 12.47 b	956.41 ± 20.38 b	867.38 ± 15.19 bc
Dg5FSR	1.19 ± 0.10 a	1500.11 ± 18.46 ac	3046.73 ± 61.37 a	1909.98 ± 7.68 a	1398.73 ± 11.86 ab	980.57 ± 2.84 b	713.49 ± 13.00 c
Dg5SFR	1.10 ± 0.01 a	1231.33 ± 205.70 bc	2366.61 ± 447.72 a	1870.96 ± 329.44 a	1402.03 ± 259.41 ab	949.83 ± 157.69 b	709.06 ± 141.56 c
Dg5SFSR	1.06 ± 0.03 a	1659.09 ± 29.50 a	3005.08 ± 64.03 a	1977.26 ± 30.67 a	1482.24 ± 25.82 ab	1086.44 ± 21.21 b	898.61 ± 16.19 bc

Values are means ± SE. Different lowercase letters within each column indicate significant differences among treatments according to Tukey's honestly significant difference test ($p < 0.05$). Treatments sharing at least one letter within the same column are not significantly different; for example, a value labeled "bc" does not differ significantly from values labeled "b", "c", "ab", or "ac" when they share a common letter. Values with no letters in common are significantly different. Treatment effects were evaluated by one-way ANOVA. N is expressed as percentage; P, K, Ca, Mg, S, and Na are expressed as ppm. Treatment codes indicate species, container volume, fertilization, and irrigation regime: Dg = *P. douglasiana*; 1 or 5 = container volume in liters; F = fertilized; SF = non-fertilized; R = continuous irrigation; SR = non-continuous irrigation.

Table 3. Micronutrient concentrations in nursery-conditioned *Pinus douglasiana* seedlings.

Treatment	Fe	Zn	Mn	Cu	B
Dg1FR	76.58 ± 4.42 bc	19.80 ± 1.30 a	156.43 ± 7.97 d	2.45 ± 0.61 a	68.73 ± 4.29 ab
Dg1FSR	73.83 ± 3.88 bc	24.51 ± 3.27 a	197.36 ± 3.95 bd	2.48 ± 0.39 a	78.06 ± 1.54 ab
Dg1SFR	104.32 ± 0.80 a	44.65 ± 25.66 a	186.04 ± 0.95 cd	2.27 ± 0.14 a	71.26 ± 1.23 ab
Dg1SFSR	60.34 ± 0.82 c	16.30 ± 1.53 a	153.77 ± 4.08 d	1.77 ± 0.11 a	83.08 ± 1.90 a
Dg5FR	73.70 ± 4.83 bc	15.75 ± 1.05 a	267.18 ± 2.79 b	1.80 ± 0.19 a	78.11 ± 2.17 ab
Dg5FSR	96.64 ± 7.66 ab	24.30 ± 2.44 a	237.67 ± 0.29 bc	2.21 ± 0.04 a	82.96 ± 0.49 a
Dg5SFR	63.06 ± 8.85 c	28.80 ± 4.47 a	247.66 ± 40.13 bc	2.04 ± 0.37 a	60.88 ± 10.93 b
Dg5SFSR	74.54 ± 2.52 bc	33.56 ± 4.05 a	354.75 ± 8.72 a	2.87 ± 0.13 a	77.22 ± 2.63 ab

Values are means ± SE. Different lowercase letters within each column indicate significant differences among treatments according to Tukey's honestly significant difference test ($p < 0.05$). Treatments sharing at least one letter within the same column are not significantly different; for example, a value labeled "bc" does not differ significantly from values labeled "b", "c", "ab", or "cd" when they share a common letter. Values with no letters in common are significantly different. Treatment effects were evaluated by one-way ANOVA. All micronutrients are expressed as ppm. Treatment codes indicate species, container volume, fertilization, and irrigation regime: Dg = *P. douglasiana*; 1 or 5 = container volume in liters; F = fertilized; SF = non-fertilized; R = continuous irrigation; SR = non-continuous irrigation.

Nutrient concentrations did not mirror the biomass response exactly. The 5 L treatments produced the strongest dry mass production, but nutrient concentrations responded more specifically to fertilization and irrigation. Because nutrient contents were not calculated, these data were interpreted as concentration patterns rather than as direct evidence of nutrient accumulation. This distinction is important because tissue concentrations may be influenced by biomass dilution as seedlings increase in size.

3.3. Irrigation Regime Shaped Proline Accumulation

Proline concentration varied among nursery treatments ($F = 2173.02$, $p < 0.001$; Figure 3). The lowest values occurred in continuously irrigated seedlings, regardless of container volume or fertilization. This indicates that proline accumulation was limited when water was supplied regularly during nursery conditioning.

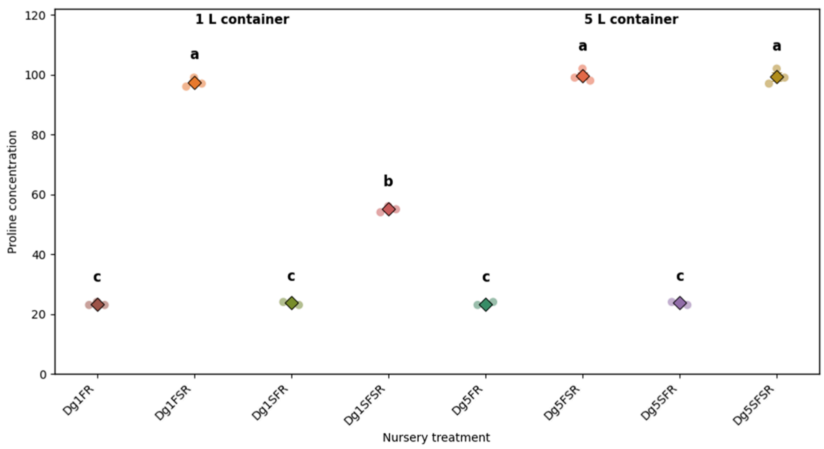

Figure 3. Treatment-level variation in proline concentration of *Pinus douglasiana* seedlings after six months of nursery conditioning. Small points represent individual observations, diamonds represent treatment means, and error bars indicate ± SE. Different letters indicate significant differences among treatments according to Tukey's honestly significant difference test ($p < 0.05$). Treatment codes combine container volume, fertilization, and irrigation regime: 1 or 5 = container volume in liters; F = fertilized; SF = non-fertilized; R = continuous irrigation; SR = non-continuous irrigation.

Non-continuous irrigation increased proline concentration, although the magnitude of this response differed among treatment combinations. Fertilized seedlings under non-continuous irrigation reached the highest proline values in both container volumes. The non-fertilized 1 L treatment under non-continuous irrigation showed an intermediate response. The main separation was therefore associated with irrigation regime. In 5 L containers, both non-continuous irrigation treatments showed high proline values, whereas continuously irrigated seedlings maintained low proline while producing more biomass. Proline accumulation was therefore more closely linked to water restriction than to biomass production.

3.4. Early Field Performance Reflected Nursery-Driven Structural Advantages

Field performance showed treatment differences mainly in root collar diameter, ectomycorrhizal colonization, and lignification (Table 4). Root collar diameter differed strongly among treatments ($F = 54.79$, $p < 0.001$). Seedlings from 5 L containers had, on average, 52.3% greater field diameter than seedlings from 1 L containers, and all 5 L treatments were placed in the highest statistical group. Field height did not differ among treatments ($F = 2.00$, $p = 0.176$), indicating that treatment separation after planting was expressed more through stem thickness than through vertical growth.

Table 4. Early field performance and functional indicators of nursery-conditioned *Pinus douglasiana* seedlings.

Treatment	Diameter (mm)	Height (cm)	Survival (%)	Ectomycorrhizal Colonization (%)	Stomatal Density	Lignification (%)
Dg1FR	8.62 ± 0.12 b	48.00 ± 5.00 a	20.00	48.36 ± 0.14 bc	10.25 ± 0.25 a	51.04 ± 2.06 bc
Dg1FSR	10.27 ± 0.40 b	59.83 ± 1.17 a	26.09	67.94 ± 0.73 a	14.75 ± 1.25 a	57.71 ± 0.35 ab
Dg1SFR	9.31 ± 0.06 b	54.67 ± 0.34 a	40.00	68.36 ± 1.34 a	12.00 ± 2.00 a	53.56 ± 0.53 abc
Dg1SFSR	9.30 ± 0.80 b	51.48 ± 4.68 a	24.00	62.46 ± 1.12 a	12.25 ± 1.25 a	56.21 ± 0.25 ab
Dg5FR	14.02 ± 0.14 a	54.30 ± 1.20 a	66.67	41.85 ± 4.96 c	15.50 ± 4.00 a	55.22 ± 1.32 abc
Dg5FSR	14.29 ± 0.26 a	54.33 ± 3.39 a	72.00	35.54 ± 3.53 cd	15.75 ± 0.25 a	60.41 ± 0.90 a
Dg5SFR	15.14 ± 0.40 a	61.50 ± 0.42 a	80.00	27.92 ± 0.59 d	17.75 ± 3.25 a	51.24 ± 2.22 bc
Dg5SFSR	13.68 ± 0.00 a	54.95 ± 3.41 a	88.00	60.12 ± 1.83 ab	16.50 ± 1.50 a	48.62 ± 0.79 c

Values are means ± SE. Different lowercase letters within each column indicate significant differences among treatments according to Tukey's honestly significant difference test ($p < 0.05$). Treatments sharing at least one letter are not significantly different; for example, values labeled "bc" do not differ significantly from values labeled "b", "c", "ab", or "cd" when they share a common letter. Treatment effects were evaluated by one-way ANOVA. Survival is presented descriptively because 70 viable seedlings were planted per treatment, but the available field record retained survival as final treatment-level percentages rather than individual binary live/dead observations.

Survival was kept as a descriptive variable because the final field record contained treatment-level percentages rather than individual live/dead data. Seventy viable seedlings were planted per treatment. After 12 months, descriptive survival averaged 76.7% in 5 L treatments and 27.5% in 1 L treatments. This result was interpreted only as field-level descriptive evidence. Ectomycorrhizal colonization differed among treatments ($F = 42.80$, $p < 0.001$), but its pattern was different from that observed for survival. The highest colonization values occurred mainly in 1 L treatments, whereas several 5 L treatments showed lower colonization despite higher descriptive survival. Stomatal density was not affected by treatment ($F = 1.47$, $p = 0.300$). Lignification differed among treatments ($F = 9.45$, $p = 0.003$), but the treatment with the highest descriptive survival did not have the highest lignified area. Field diameter provided the clearest statistical separation among treatments. Descriptive survival favored seedlings produced in 5 L containers, while height, stomatal density, ectomycorrhizal colonization, and lignification did not explain the field response by themselves.

3.5. Exploratory Associations Between Total Biomass and Nursery–Field Traits

Associations between total biomass and selected nursery–field variables were explored using treatment means (Table 5). Because the analysis was based on eight treatment means, these correlations were used only to describe treatment-level patterns.

Table 5. Associations between total biomass and nursery–field traits of *Pinus douglasiana* seedlings.

Variable	Spearman ρ	p-Value
Shoot biomass	0.952	<0.001
Root biomass	0.786	0.021
Dickson quality index	0.929	<0.001
Nursery root collar diameter	0.929	<0.001
Proline	−0.217	0.606
Field diameter	0.714	0.047
Survival	0.714	0.047
Ectomycorrhizal colonization	−0.810	0.015
Stomatal density	0.690	0.058
Lignification	−0.429	0.289

Spearman correlation coefficients were calculated using treatment means (n = 8). Significant correlations are shown at $p < 0.05$.

At the treatment-mean level, total biomass was positively associated with field diameter and descriptive survival. These relationships should be read with caution because they were calculated from only eight treatment means and do not represent individual-seedling relationships. Proline was not associated with total biomass ($\rho = -0.217$, $p = 0.606$), supporting its interpretation as a stress-related variable rather than as a direct biomass indicator. Ectomycorrhizal colonization was negatively associated with total biomass ($\rho = -0.810$, $p = 0.015$), whereas stomatal density and lignification were not significant. This pattern indicates that total biomass was more closely related to structural variables than to isolated physiological or symbiotic indicators.

3.6. PCA Separated Biomass-Related Traits from Stress Indicators

The treatment-level PCA summarized the main response patterns among nursery treatments (Figure 4). The first two axes explained 84.9% of the total variation. PC1 accounted for 69.0% and was associated mainly with shoot biomass, root biomass, total biomass, Dickson quality index, field diameter, and descriptive survival. Because the PCA was based on eight treatment means, it was interpreted as a descriptive ordination rather than as a replicate-level multivariate test. The 5 L treatments were located toward the positive side of PC1, close to the biomass and field-growth vectors. In contrast, the 1 L treatments were positioned toward the opposite side of this axis. This separation indicates that container volume was the main source of contrast in the multivariate summary.

PC2 explained 15.9% of the variation and was associated mainly with proline and lignification. Treatments under non-continuous irrigation were positioned closer to this axis, matching the higher proline response observed under restricted water supply. Thus, the PCA separated two treatment-level patterns: one related to biomass and early field structure, and another related to stress-associated variables.

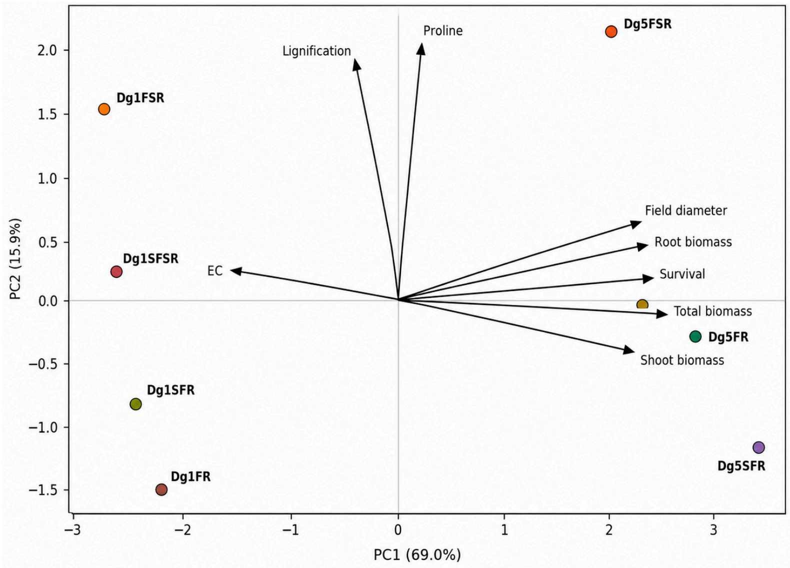

Figure 4. Multivariate response of nursery-conditioned *Pinus douglasiana* seedlings. Principal component analysis based on nursery biomass traits, proline concentration, and early field performance indicators. Points represent nursery treatments, and vectors indicate the contribution of each variable to the ordination. PC1 and PC2 explained 84.9% of the total variation.

4. Discussion

4.1. Container Volume Defined the Main Structural Response

Container volume produced the strongest structural signal in *P. douglasiana* seedlings. Plants grown in 5 L containers had greater root collar diameter, shoot biomass, root biomass, total biomass, and Dickson quality index, whereas height changed little among treatments. This pattern shows that the main nursery response occurred through dry-matter accumulation and stem robustness, not through vertical elongation.

This response agrees with the target plant concept, which emphasizes planting stock attributes related to field performance rather than external appearance alone [25]. Greater container volume may reduce physical restriction on root development and provide more growing space during nursery conditioning [26]. Although root architecture was not measured directly, the higher root biomass and Dickson quality index observed in 5 L seedlings are consistent with that interpretation. For *P. douglasiana*, container volume appears to be an important nursery decision when the goal is to produce planting stock with stronger structural condition before field establishment. Even so, larger containers should be considered with caution because they may increase substrate use, nursery space, handling effort, and transport costs.

4.2. Nutrient Responses Did Not Parallel Biomass Accumulation

Nutrient concentrations did not follow the same response observed for biomass traits. Nitrogen was higher in several 5 L treatments, but other elements varied according to the specific fertilization and irrigation combination. This distinction is important because tissue concentration is not equivalent to total nutrient uptake or nutrient content. Previous work in containerized nursery systems has also shown that irrigation and fertigation can modify seedling nutrition without producing a proportional change in morphology or biomass [27,28]. The separation between nutrient concentration and biomass production should be considered when interpreting seedling quality. In the present study, seedlings in 5 L containers showed the strongest biomass response, whereas nutrient concentrations varied depending on the element analyzed. Because nutrient contents were not estimated, the results were interpreted only as tissue concentration patterns and not as direct evidence of nutrient accumulation [29,30].

Nutrient dilution may partly explain this response. Seedlings with greater dry-matter production may distribute absorbed nutrients across more tissue, producing similar or lower concentrations even when total uptake is not necessarily lower [31]. This could explain why some non-fertilized treatments showed relatively high nutrient concentrations and why the highest biomass response was not always linked to the highest tissue concentration. For *P. douglasiana*, nutrient status helped describe the effect of nursery conditioning, but it did not fully account for biomass accumulation. Biomass production was more closely related to rooting space and structural development, whereas nutrient concentrations reflected element-specific responses to the fertilization and irrigation combinations. Micronutrients were therefore interpreted as complementary indicators of nursery nutritional status, not as direct predictors of first-year field performance [32].

4.3. Proline Reflected Irrigation-Related Stress Expression

Proline concentrations increased under non-continuous irrigation, showing that reduced irrigation frequency changed the physiological condition of *P. douglasiana* seedlings during nursery conditioning. This response agrees with studies on woody seedlings where drought commonly increases foliar proline, although the magnitude of the response depends on stress duration, species, and seedling traits. High proline values should be interpreted with caution. Depending on drought intensity, exposure time, species response, and recovery capacity, proline accumulation may indicate osmotic adjustment, stress exposure, or cellular damage rather than improved drought tolerance [15]. For this reason, proline was treated here as a stress-expression variable linked to water limitation, not as direct evidence of seedling quality or field performance.

The absence of a significant relationship between proline and total biomass supports this interpretation. Continuously irrigated seedlings produced more biomass while maintaining low proline values, whereas non-continuous irrigation treatments showed higher proline without consistently producing greater biomass. Similar conifer nursery studies have reported that reduced irrigation can change physiological behavior and stress resistance, but its effect on growth depends on the intensity and timing of water limitation [33,34]. For *P. douglasiana*, proline helped identify the physiological effect of reduced irrigation frequency during nursery conditioning. However, it did not explain early field performance by itself. This distinction is important because drought hardening may improve outplanting performance under certain conditions, but the response depends on species and site context, and excessive stress can reduce growth or planting stock quality [35].

4.4. Structural Traits Were Better Aligned with Early Field Response

The field assessment pointed more strongly to structural variables than to single physiological or symbiotic measurements. Seedlings produced in 5 L containers showed greater field diameter and higher descriptive survival, while treatment means linked total biomass with these field variables. Because survival was retained only as one percentage per treatment, this pattern should be read as descriptive evidence. This interpretation agrees with recent work indicating that planting stock evaluation benefits from combining nursery morphology, biomass traits, and field response rather than relying on one trait [36]. The association between nursery biomass and field response is relevant because early establishment depends on the capacity of planted seedlings to overcome transplant stress and resume growth. In this study, total biomass and field diameter were aligned with descriptive survival, whereas height did not separate treatments. Similar evidence indicates that nursery environment and initial seedling condition can influence transplant stress and first-summer survival when water availability becomes restrictive [37].

Ectomycorrhizal colonization showed a different pattern from biomass and descriptive survival. This result does not imply a negative effect of mycorrhizae; it only indicates that colonization percentage alone did not explain field response in this dataset; responses of conifer and Mediterranean pine seedlings to ectomycorrhizae can depend on fungal identity, soil conditions, drought, and plant competition [38,39]. The negative association between ectomycorrhizal colonization and biomass should therefore be interpreted cautiously. Smaller or more stressed seedlings may have relied more on belowground symbioses after planting, while 5 L seedlings entered the field with greater structural reserves. For *P. douglasiana*, the clearest field signal was the persistence of nursery structural condition after planting. Total biomass, root biomass, root collar diameter, and Dickson quality index were more informative than isolated physiological or symbiotic indicators for describing early field potential.

4.5. Operational Considerations for P. douglasiana Planting Stock

For nursery production of *P. douglasiana*, the main practical message is that planting stock evaluation should give more weight to root biomass, total biomass, root collar diameter, and Dickson quality index than to height alone. These traits described the nursery response more clearly and were better aligned with early field diameter and descriptive survival. Current seedling-quality frameworks also recommend evaluating nursery stock through structural traits, growing conditions, and field response rather than through a single morphological attribute [40]. The response observed in 5 L containers suggests that rooting space can be relevant for producing structurally stronger seedlings before planting. However, this recommendation should not be read as a fixed rule for all nurseries or planting sites. Larger containers require more substrate, nursery area, transport capacity, and handling effort. Their use should therefore be justified by the expected field conditions, especially where transplant stress, seasonal drought, or restrictive soils may reduce early establishment.

Root development remains a key operational criterion. Studies with deep-container systems have reported higher shoot biomass, root biomass, Dickson quality index, and post-planting root development in dry tropical forest species [41]. The present study did not measure post-planting root growth, water uptake, or nutrient acquisition, so the role of root development after outplanting should be interpreted cautiously. Even with that limitation, the nursery response indicates that reduced root restriction before planting may improve the structural condition of *P. douglasiana* seedlings. The irrigation result adds another practical point. Non-continuous irrigation increased proline, but this response was not linked to greater total biomass. Therefore, water restriction should not be treated automatically as a beneficial hardening practice. For this species, biomass formation and root development provided stronger criteria for selecting planting stock than proline accumulation alone. Nursery decisions should balance container volume, irrigation regime, production costs, and planting-site risk rather than increasing or reducing inputs without field-based justification [42].

4.6. Study Limitations and Relationship with Previous Work

Several aspects of the data structure should be considered. Field monitoring covered only the first 12 months after planting, so the results describe early establishment rather than long-term plantation performance [43]. Survival was treated descriptively because the final field record contained treatment-level percentages rather than individual live/dead observations. Correlations and PCA were also based on treatment means, which makes both analyses exploratory [44,45]. Root biomass was measured before outplanting, but post-planting root growth was not measured; therefore, the link between pre-planting root development and field performance should be interpreted as a plausible mechanism rather than direct evidence of root dynamics after planting [46].

This manuscript should be read in relation to a previously published nursery–field study on *Pinus devoniana* that evaluated similar nursery factors, including container volume, fertilization, and irrigation [47]. However, the present study is based on an independent dataset for *P. douglasiana* and addresses a different native pine species. Its contribution is centered on biomass allocation, root biomass, treatment-level relationships with early field performance, and a conservative interpretation of survival as descriptive evidence because individual live/dead records were not available. Therefore, this work provides species-specific information for *P. douglasiana* planting stock management and should not be interpreted as a duplication of the previous *P. devoniana* study.

5. Conclusions

Rooting space, represented by container volume, was the main source of variation in the nursery response of *Pinus douglasiana*. Seedlings produced in 5 L containers showed greater root biomass, total biomass, root collar diameter, and Dickson quality index than seedlings produced in 1 L containers, while height was not the main trait separating treatments. The field record after 12 months showed greater root collar diameter and higher descriptive survival percentages in seedlings from 5 L containers. Because survival was available only as treatment-level percentages, this pattern should be read as descriptive field evidence rather than as an inferential survival result.

Proline responded mainly to irrigation restriction and was not aligned with biomass production or early field performance. Thus, proline was useful for describing water-related physiological expression during nursery conditioning, but not as a direct indicator of planting stock quality. For *P. douglasiana*, biomass allocation, root biomass, root collar diameter, and Dickson quality index provided the most useful criteria for describing planting stock condition. The use of larger containers may be operationally valuable when the goal is to produce structurally stronger seedlings, but this decision should be balanced against nursery costs, handling logistics, and planting-site conditions. Future studies should include individual survival records, longer field monitoring, and direct measurements of root growth and water uptake after planting.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
DQI	Dickson quality index
EC	Ectomycorrhizal colonization
LA	Lignified stem area
PCA	Principal component analysis
RB	Root dry biomass
RSR	Root–shoot ratio
SB	Shoot dry biomass
SD	Stomatal density
SE	Standard error
TB	Total dry biomass

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