

Evaluation of nitrate/ammonium ratios and substrate mixtures in the hardening phase of blueberry cultivation, variety Biloxi (*Vaccinium corymbosum*), under greenhouse conditions

Evaluación de relaciones nitrato/amonio y mezclas de sustratos en la fase de endurecimiento en el cultivo de arándano, variedad Biloxi (*Vaccinium corymbosum*), en invernadero

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Abstract

This research evaluated how different forms of nitrogen (nitrate and ammonium) and substrate mixtures (peat and rice husk) influence blueberry growth during its hardening-off phase in a greenhouse, with the aim of identifying the most efficient option for producing higher-quality plants. The results showed that the optimal combination was the nutrient solution with a predominance of ammonium (75%) together with a substrate with a high peat content (80%), which generated plants with greater height, greater stem thickness and up to double the biomass compared with other mixtures. The study concludes that this synergy between ammonium and peat produces more vigorous plants of better overall quality, further confirming that the Dickson Quality Index is the best indicator for evaluating the seedlings, thus offering clear technical recommendations for optimizing blueberry production under controlled conditions.

Keywords: Dickson Quality Index, nitrogen nutrition, peat, vegetative growth.

Resumen

Esta investigación evaluó cómo diferentes formas de nitrógeno (nitrato y amonio) y mezclas de sustrato (turba y cascarilla de arroz) influyen en el crecimiento del arándano durante su fase de endurecimiento en invernadero, con el objetivo de identificar la opción más eficiente para producir plantas de mejor calidad. Los resultados mostraron que la combinación óptima fue la solución nutritiva con predominio de amonio (75%) junto con un sustrato con alto contenido de turba (80%), la cual generó plantas con mayor altura, mayor grosor de tallo y hasta el doble de biomasa en comparación con otras mezclas. El estudio concluye que esta sinergia entre amonio y turba produce plantas más vigorosas y de mejor calidad integral, confirmando además que el Índice de Calidad de Dickson es el mejor indicador para evaluar las plántulas, ofreciendo así recomendaciones técnicas claras para optimizar la producción de arándano bajo condiciones controladas.

Palabras clave: crecimiento vegetativo, Índice de Calidad de Dickson, nutrición nitrogenada, turba.

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1. Introduction

The blueberry (*Vaccinium corymbosum*), belonging to the Ericaceae family, is a species native to the Northern Hemisphere that has acquired great global economic relevance, with the United States, Peru, Canada and Chile being the main traditional producers; thus demonstrating the region's enormous potential for this crop (Organización de las Naciones Unidas para la Alimentación y la Agricultura [FAO], 2025; Undurraga Díaz & Vargas Schüldes, 2013).

In this context, Ecuador has begun to introduce blueberry as a productive alternative for export, specifically the Biloxi variety from Chile, which stands out for its adaptation, vigor and productivity, requiring a minimum of 400 chill hours and producing medium-sized, firm fruit with excellent flavor (Fall Creek Farm & Nursery, 2026; Morales Alcayaga, 2017; Retamales et al., 2014 Spiers et al., 2002; Undurraga Díaz & Vargas Schüldes, 2013; Vásquez-Castillo et al., 2026).

However, the main challenge for its cultivation in Ecuador lies in the species' demanding soil requirements, which call for loose soils, an acidic pH between 4 and 5, and a high organic matter content (10 to 20%), with pH management being especially critical during the first years (Gordó, 2008).

Given the difficulty of naturally finding these soil conditions and the variability of existing soils (Cruzatty & Vollmann, 2012), Ecuadorian growers have opted for substrate-based cultivation systems, a global trend that seeks to overcome soil limitations and optimize the use of water and fertilizers (Rivière & Caron, 2001). The choice of materials and mixtures is crucial, as they must guarantee adequate physical properties such as high porosity (>85%) and a balance between moisture retention and drainage, with the incorporation of organic materials, such as peat, able to improve these characteristics (Jones et al., 2025; Ortiz-Delvasto et al., 2023; Schafer & Lerner, 2022).

However, in Ecuador these mixtures are prepared without prior physicochemical characterization, generating root development problems and water and nutritional imbalances that compromise the viability of the crop. Added to this problem is the need to optimize nitrogen nutrition during the greenhouse hardening-off phase, since it is not known how the different substrate mixtures interact with the nitrate/ammonium ratio, a key factor for this acidophilic species.

For this reason, this research seeks to evaluate how different nitrate/ammonium ratios and substrate mixtures influence blueberry growth during its greenhouse hardening-off phase, with the aim of identifying the most efficient option for producing higher-quality plants.

The study is justified by the need to generate local knowledge that makes it possible to standardize and optimize substrate cultivation, moving away from empirical practices that have caused production failures, and addressing the knowledge gap regarding the interaction between substrate and nitrogen nutrition, in order to provide technically grounded recommendations that improve the production and quality of this new crop of interest for Ecuadorian exports.

Through this study, the aim is to determine the optimal combination of substrate and nitrate/ammonium ratio that maximizes plant growth and vigor during the hardening-off phase, characterizing the agronomic behavior of the variety under different conditions in order to generate a technically grounded recommendation that reduces uncertainty in crop establishment and lays the foundation for a more efficient, competitive and sustainable local blueberry production, in line with the demands of the international market.

2. Materials and Methods

The research was carried out in the greenhouse of the Soil and Water Management Department [DMSA, *Departamento de Manejo de Suelos y Aguas* in Spanish] of the Santa Catalina Experimental Station [EESC, *Estación Experimental Santa Catalina* in Spanish] of Instituto Nacional de Investigaciones Agropecuarias [INIA], in Cutuglagua, Mejía canton, Pichincha province, at an altitude of 3,059 m a.s.l., at latitude 0° 22' 2.07" S and longitude 78° 33' 18.72" W. The greenhouse had, on average, 33.7, 6.0 and 19.85 °C as maximum, minimum and average temperature, respectively; as well as 75.6% relative humidity.

Two factors were studied in the experiment: nutrient solutions [S] and substrate mixture [M] (Table 1), from which the different treatments were formed (Table 2). The biological material consisted of clonal blueberry seedlings of the Biloxi variety. A completely randomized design [CRD] was used. The experimental unit was one plant inside a 2-liter plastic bag. Ten observations were obtained from each treatment, for a total of 50 plants in the experiment.

Regarding chemical fertilization (Tables 3 and 4), the nutrient solution was applied manually at a rate of 200 ml per plant per day for all treatments evaluated, with a weekly check of the electrical conductivity and pH of the applied solution also carried out. A chemical analysis of the irrigation water (sourced from springs or piped catchments on Atacazo hill, applied directly, without any osmotization process) was performed at the EESC Soil, Plant and Water Management Laboratory (Table 5), for which a 1 L sample was taken.

Table 1. Factors under study.

Factor under study	Description
Nutrient solutions [S]	
s1	75% nitrate and 25% ammonium
s2	25% nitrate and 75% ammonium
Substrate mixtures [M]	
m1	80% rice husk and 20% peat
m2	20% rice husk and 80% peat

Table 2. Treatments.

Treatment	Code	Nutrient solution	Substrate mixture
T1	s1m1	s1	m1
T2	s1m2	s1	m2
T3	s2m1	s2	m1
T4	s2m2	s2	m2
T5	Control	Water only	100% rice husk

Table 3. Concentration of the chemical fertilizers used.

Fertilizer	Formula	MW*	N	P	K	Ca	Mg	S
(%)								
Potassium nitrate	KNO ₃	101	13		38			
Calcium nitrate	Ca(NO ₃) ₂ ·4H ₂ O	236	15			18		
Ammonium nitrate	NO ₃ (NH ₄)	80	33					
Potassium sulfate	K ₂ SO ₄	174			41			18
Magnesium sulfate	MgSO ₄ ·7H ₂ O	246					9	13
Ammonium sulfate	(NH ₄) ₂ ·SO ₄	132	21					24
Phosphoric acid	H ₃ PO ₄	98		30				
Sulfuric acid	H ₂ SO ₄	98						32

* MW = Molecular weight. Tradecorp AZ was used for the micronutrients (5.5, 3, 0.7, 0.4, 1.4 and 0.2% Fe, Mn, Zn, Cu, B and Mo, respectively).

Response variables were evaluated over a four-month period, with records taken every 30 days from the transplant date. Plant height was determined by measuring the length of the main stem from the plant's crown to the terminal bud using a ruler, with results expressed in centimeters (Mesa Torres, 2015). The basal diameter of the main stem was recorded in millimeters using a vernier caliper, with the measurement taken at 1 cm above the plant's crown (Mesa Torres, 2015). From these data, the Robustness Index [RI], or slenderness index, was calculated using the height/diameter ratio, which is an indicator of the plants' morphological quality: low values indicate sturdier plants (thicker and of lower relative height), while high values indicate more slender plants (taller relative to their thickness), which may

be more susceptible to bending and post-transplant stress (Mollericonna Alfaro & Calle Laime, 2021). At the end of the evaluation period (four months after transplanting), biomass was obtained by separating the aerial part and the root of each plant. Each sample was placed in paper bags duly identified by treatment and observation, and placed in a forced-ventilation drying oven, Shel Lab brand, at 65 °C, for 48 hours or until constant weight was reached. The samples were then weighed on a precision balance (Shimadzu brand: Libror EB-4300S), obtaining the aerial and root dry biomass values expressed in grams per plant (g plant⁻¹), the sum of which made it possible to determine the total biomass. The aerial dry biomass/root dry biomass ratio (ADB/RDB), calculated by dividing the dry weight of the aerial part by the dry weight of the

Table 4. Ionic composition of the nutrient solutions used.

Nutrient solution	pH	EC*	Ionic species (meq L ⁻¹)							NH ₄ ⁺ /NO ₃ ⁻
		(dS m ⁻¹)	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	K ⁺	Ca ²⁺	Mg ²⁺	NH ₄ ⁺	
s1	5.0	1.00	1.50	1.25	7.25	2.31	1.37	1.82	4.50	75/25
s2	5.0	1.00	4.50	1.25	7.25	2.31	1.37	1.82	1.50	25/75

* EC = Electrical Conductivity. Micronutrients (mg L⁻¹) Fe: 1.5; Mn: 1.5; Zn: 0.15; Cu: 0.10; B: 0.6.

Table 5. Chemical analysis of irrigation water used.

Element	Mg ²⁺	Ca ²⁺	K ⁺	Na ⁺	NH ₄ ⁺	B	Cl ⁻	HCO ₃ ⁻	CO ₃ ²⁻	H ₂ PO ₄ ⁻	NO ₃ ⁻	SO ₄ ²⁻
Result (meq L ⁻¹)	0.36	0.97	0.07	0.42	0	0.01	0.48	2.19	0	0	0	0.01

root, is an important indicator of the balance between the aerial part (source of photosynthates) and the root part (absorption organ), where high values indicate a greater allocation of biomass to the aerial part, while low values indicate greater investment in roots (Mollericona Alfaro & Calle Laime, 2021). Finally, the Dickson Quality Index [DQI] was determined, which integrates multiple morphological attributes (height, diameter, biomass) into a single value, where higher values indicate better plant quality, using equation [1] (Mollericona Alfaro & Calle Laime, 2021).

$$DQI = \frac{\text{Total dry weight (g)}}{(RI + ADB/RDB)} \quad [1]$$

The respective statistical analysis was applied to the results obtained for the variables analyzed, as follows: the Shapiro-Wilks test was applied to determine normal distribution, and Levene’s test to determine homogeneity of variance. Analysis of variance [ANOVA] was calculated, determining the coefficient of variation as a percentage. Mean separation tests were applied to the treatments (Tukey at 5%) and to the factors under study (LSD) when a significant difference existed. Likewise, correlation tests (Pearson) were performed between the variables evaluated. Data analysis was carried out using the SAS statistical software, 9.4 version.

3. Results and Discussion

3.1. Plant height

According to the analysis of variance for plant height (Table 6), during the first 60 days after transplanting [DAT] no significant differences were recorded between treatments, the nutrient solution [S] and substrate mixture [M] factors, or their interaction, reflecting homogeneous growth in the initial phase. At 90

DAT differences began to appear, with a significant effect only from factor M, indicating that the composition of the growing medium was beginning to influence height. It was at 120 DAT that highly significant differences were observed for both main factors (S and M) and their interaction, showing that, by the end of the experimental period, both the nitrate/ammonium ratio and the proportion of peat and husk in the substrate, and their combination, decisively affected plant growth. The control vs. other treatments contrast showed no significance at any evaluation, although differences did exist between specific treatments at the end of the trial (Table 7); the coefficients of variation (11.48% to 20.30%) were acceptable, and the overall average height increased progressively from 11.02 cm to 26.39 cm, confirming continuous growth throughout the hardening-off phase.

The late appearance of differences, starting at 90 DAT, is consistent with the nature of blueberry, which requires an establishment period before nutritional and substrate treatments show their effect on height growth; the significance of both main factors at the end of the experiment confirms the importance of optimizing both nitrogen nutrition (NH₄⁺/NO₃⁻ ratio) and the physical characteristics of the substrate to maximize vegetative growth during the hardening-off phase (Anwar et al., 2024).

Regarding the nutrient solution factor [S] (Table 7), during the first 90 DAT no statistically significant differences were observed between s1 and s2. It was at 120 DAT that highly significant differences became established: solution s2 (25/75 NH₄⁺/NO₃⁻) reached 29.35 cm, exceeding solution s1 (24.46 cm) by 4.89 cm (approximately 20%), confirming that the predominance of ammonium in nitrogen nutrition promotes greater height growth in blueberry during the final hardening-off phase.

This result is consistent with the physiology of blueberry, an acidophilic species of the Ericaceae fa-

Table 6. Variance analysis for plant height.*

Sources of Variation	df	Mean square									
		1 DAT		30 DAT		60 DAT		90 DAT		120 DAT	
Total	49										
Treatments	4	11.20	ns	6.12	ns	2.48	ns	51.83	*	238.43	**
S (Solution)	1	12.10	ns	14.04	ns	1.71	ns	31.39	ns	239.56	**
M (Mixture)	1	14.40	ns	9.70	ns	3.19	ns	163.05	*	593.74	**
S x M	1	0.62	ns	0.07	ns	0.74	ns	11.96	ns	67.88	*
Control vs. Rest	1	17.68	ns	0.68	ns	5.72	ns	0.93	ns	52.54	ns
Error	45	5.00		4.34		3.22		14.31		15.60	
CV (%)		20.30		15.68		11.48		18.03		14.96	
Average (cm)		11.02		13.28		15.63		20.97		26.39	

* df = degrees of freedom; CV = coefficient of variation; ** = highly significant difference (p-value < 0.01); * = significant difference (p-value < 0.05); ns = not significant (p-value ≥ 0.05); DAT = days after transplanting.

Table 7. Average plant height by type of nutrient solution and by type of substrate mixture.*

Factor under study	Average height (cm)				
	1 DAT	30 DAT	60 DAT	90 DAT*	120 DAT*
Nutrient solution (S)					
s1	11.28	13.94	16.01	20.16	24.46 b
s2	10.18	12.75	15.60	21.93	29.35 a
Substrate mixture (M)					
m1	11.33	13.84	16.09	19.02 b	23.05 b
m2	10.13	12.85	15.52	23.06 a	30.76 a

* Because statistical significance was only found in these periods, the LSD test was applied; DAT = days after transplanting.

mily, which shows a natural preference for absorbing ammoniacal nitrogen $[\text{NH}_4^+]$ rather than nitric nitrogen $[\text{NO}_3^-]$, because in its native ecosystems—acidic forest and peatland soils—ammonium is the predominant form of available nitrogen; NH_4^+ absorption favors the maintenance of an acidic pH in the rhizosphere, an essential condition for the proper nutrition of this species (Espinoza-Orozco et al., 2025; Mshweshwe et al., 2025; Osorio et al., 2020).

Regarding the effect of the substrate mixture factor [M] on plant height (Table 7), during the first 60 DAT no statistically significant differences were observed between m1 (20% peat) and m2 (80% peat), indicating that both mixtures offered adequate physical conditions for initial establishment. At 90 DAT significant differences began to appear: mixture m2 reached 23.06 cm, exceeding m1 (19.02 cm) by 4.04 cm (approximately 21%), showing that the higher peat content was beginning to favor growth. This trend was consolidated at 120 DAT with highly significant differences, where m2 achieved 30.76 cm compared to 23.05 cm for m1, representing an advantage of 7.71 cm (nearly 33% higher), confirming that the higher proportion of peat in the substrate optimizes bluebe-

rry height growth during the hardening-off phase.

This result may be possible due to the physicochemical properties of peat (where the mixture with 80% peat -m2- generated the most favorable conditions for blueberry growth), owing to its high moisture retention capacity (500-800%), high cation exchange capacity (ensuring a stable moist environment and efficient nutrient availability), and its acidic character (pH 3.5-4.5), essential for micronutrient absorption, with the exception of molybdenum (Ortiz-Delvasto et al., 2023).

According to the height averages for the five treatments evaluated (Table 8), during the first 60 DAT no statistically significant differences were observed, confirming the homogeneity of the plant material at the outset. At 90 DAT significant differences began to appear, forming a top group (a) made up of T2 (s1m2) with 22.72 cm and T4 (s2m2) with 23.40 cm, both characterized by sharing the substrate with 80% peat (m2), regardless of the nutrient solution; an intermediate group (ab) made up of T3 (s2m1) and control T5; and a lower group (b) occupied by T1 (s1m1) with 17.59 cm, showing that at three months the determining factor was the substrate.

Finally, at 120 DAT the differences became significantly more pronounced, forming four clearly defined groups: T4 (s2m2) led with 31.90 cm, significantly outperforming the rest; T2 (s1m2) followed with 29.61 cm, not statistically differing from the former; at a lower level were T3 (s2m1) with 26.80 cm and control T5 with 24.34 cm; while T1 (s1m1) showed the poorest performance with 19.30 cm.

This behavior confirms that the combination of a peat-rich substrate with an ammoniacal nitrogen source optimizes blueberry height growth during the hardening-off phase. These results are in line with those reported by Anwar et al. (2024), in soilless crops, where high NH_4^+ concentrations increased vegetative growth; and by Ortiz-Delvasto et al. (2023), who found greater height in substrates with high peat content, because it favored moisture retention and cation exchange capacity.

On the other hand, it is worth noting that T5 (water only + 100% rice husk) was statistically similar to T3 (25% nitrate, 75% ammonium + 80% rice husk + 20% peat), even though the control was initially expected to show the lowest growth. This suggests that a higher proportion of ammonium did not by itself guarantee a superior response, probably because in blueberry the effect of N depends on its interaction with the substrate and on the balance between both nitrogen forms (Anwar et al., 2024; Ortiz-Delvasto et al., 2023; Xu et al., 2021). It is also possible that mixture m1, containing only 20% peat, did not offer sufficient physical and chemical conditions for ammoniacal fertilization to express a clear advantage, since peat improves water retention, cation exchange capacity and the acidity of the medium, key factors in this crop (Anwar et al., 2024; Bignami et al., 2022; Tamir et al., 2025). Therefore, the similarity between T5 and T3 reinforces that growth during hardening-off depended more on substrate quality and the $\text{NH}_4^+:\text{NO}_3^-$ balance than on the addition of N alone (Anwar et al., 2024; Arias et al., 2024; Yang et al., 2025).

3.2. Main stem diameter

The analysis of variance for main stem diameter (Table 9) showed that during the first 30 DAT there were no significant differences between treatments, indicating homogeneous development in the initial phase, with non-significant coefficients of variation (23.07% and 22.93%). At 60 DAT highly significant differences began to appear between treatments, attributable mainly to the control vs. other treatments contrast, indicating that plants with nutrient solution already exceeded the control in diameter. At 90 DAT highly significant differences became established, with significant effects from both main factors (nutrient solution S and substrate mixture M) and their interaction, showing that both nitrogen nutrition and substrate characteristics, and their combination, decisively influenced stem thickening. Finally, at 120 DAT the highly significant differences for S and M were maintained, although the interaction lost significance, suggesting independent and additive effects by the end of the cycle.

The control vs. other treatments contrast was only significant at 60 DAT, while the overall averages increased progressively from 0.68 mm to 2.12 mm, reflecting continuous thickening throughout the hardening-off phase, with coefficients of variation reaching high precision (2.56% at 90 DAT).

The significance of both main factors at the end of the experiment indicates the importance of optimizing both nitrogen nutrition ($\text{NH}_4^+/\text{NO}_3^-$ ratio) and the physical characteristics of the substrate to maximize stem thickening during the hardening-off phase (Gurrola Nieblas, 2020).

The analysis of main stem diameter by type of nutrient solution (Table 10) showed that, during the first 60 DAT, no significant differences were recorded between s1 (75% nitrate) and s2 (75% ammonium); however, at 90 DAT the differences became highly significant, with solution s2 reaching 1.96 mm, re-

Table 8. Average plant height per treatment.*

Treatment	Average height (cm)					
	1 DAT	30 DAT	60 DAT	90 DAT*	120 DAT*	
T1 (s1m1)	11.75	14.47	16.16	17.59	b	19.30 d
T2 (s1m2)	10.80	13.40	15.87	22.72	a	29.61 ab
T3 (s2m1)	10.90	13.20	16.02	20.46	ab	26.80 bc
T4 (s2m2)	9.45	12.30	15.18	23.40	a	31.90 a
T5 (Witness)						
	12.21	13.05	14.96	20.70	ab	24.34 c
T5 (Control)						

* Because statistical significance was only found in these periods, Tukey's 5% test was applied (values with the same letter are not significantly different: $p \geq 0.05$); DAT = days after transplanting.

presenting an advantage of 0.35 mm (approximately 22%) over s1 (1.61 mm). This trend was consolidated at 120 DAT, where s2 achieved 2.38 mm compared to 1.99 mm for s1, widening the difference to 0.39 mm (nearly 20% higher), confirming that the solution with a predominance of ammonium (s2: 25/75 $\text{NH}_4^+/\text{NO}_3^-$) promotes greater stem thickening in blueberry during the final hardening-off phase.

This result is consistent with the physiology of blueberry, an acidophilic species adapted to preferentially absorb nitrogen in ammoniacal form [NH_4^+], so solution s2 (75% ammonium) provided the nitrogen source most compatible with its metabolism, favoring the maintenance of an acidic pH in the rhizosphere that optimizes the availability of micronutrients such as iron, manganese and zinc, essential for vascular development and stem thickening (Bowerman, 2012).

Regarding the analysis of main stem diameter averages by type of substrate mixture (Table 10), during the first 60 DAT no significant differences were observed between m1 (20% peat) and m2 (80% peat), indicating that both mixtures offered adequate conditions for initial stem development during the establishment phase. However, at 90 DAT the differences became highly significant: mixture m2 reached 1.99 mm, exceeding m1 (1.58 mm) by 0.41 mm (approximately 26%), showing that the higher peat content was beginning to favor stem thickening. This trend was consolidated and became more pronounced at 120 DAT, where m2 achieved 2.45 mm compared to 1.93 mm for m1, widening the difference to 0.52 mm (nearly 27% higher), confirming that the mixture with 80% peat (m2) significantly optimizes blueberry stem diameter during the final hardening-off phase. This result can be explained, according to authors such as

Bowerman (2012) and Ortiz-Delvasto et al. (2023), by the physicochemical characteristics of peat, which generated the most favorable conditions for blueberry stem thickening due to its high moisture retention capacity, which ensures a constant water supply for cell division and elongation processes; its high cation exchange capacity, which allows a gradual and continuous release of nutrients essential for the formation of structural tissues; and its acidic character, which optimizes the availability of micronutrients such as iron, manganese and zinc, key for lignin synthesis; in addition, the water-air balance, by combining low bulk density with high porosity, favors greater stem growth.

According to the analysis of main stem diameter averages for the five treatments evaluated (Table 11), during the first 30 DAT no statistically significant differences were observed. At 60 DAT significant differences began to appear, with an initially unexpected result standing out: control T5 (water + 100% rice husk) showed the largest diameter (1.32 mm), possibly due to lower osmotic tension; however, a key trend became evident: treatments with the 75% ammonium solution (s2) (T4 and T3) formed the intermediate group and began to differ from those with 75% nitrate (s1) (T1 and T2), which occupied the lower group, indicating that the ammoniacal nitrogen source was already exerting a favorable effect regardless of the substrate. At 90 DAT the differences became more pronounced, with a clear advantage consolidating for T4 (s2m2) at 2.20 mm. Finally, at 120 DAT the differences consolidated into two well-defined groups: T4 (s2m2) led with 2.64 mm in the top group (a), significantly outperforming the rest; T2 (2.26 mm) and T3 (2.13 mm) were in an intermediate

Table 9. Analysis of variance for the main stem diameter variable.*

Sources of Variation	df	Mean square				
		1 DAT	30 DAT	60 DAT	90 DAT	120 DAT
Total	49					
Treatments	4	0.04 ns	0.03 ns	0.18 **	0.78 **	1.25 **
S (Solution)	1	0.07 ns	0.01 ns	0.19 ns	1.20 **	1.52 *
M (Mixture)	1	0.06 ns	0.03 ns	0.08 ns	1.73 **	2.69 **
S x M	1	0.01 ns	0.03 ns	0.09 ns	0.05 *	0.01 ns
Control vs. Rest	1	0.02 ns	0.06 ns	0.37 **	0.13 ns	0.81 ns
Error	45	0.02	0.04	0.04	0.13	0.29
CV (%)		23.07	22.93	19.42	2.56	25.35
Average (mm)		0.68	0.95	1.14	1.75	2.12

* df = degrees of freedom; CV = coefficient of variation; ** = highly significant difference (p-value < 0.01); * = significant difference (p-value < 0.05); ns = not significant (p-value ≥ 0.05); DAT = days after transplanting.

Table 10. Average for the main stem diameter by type of nutrient solution and by type of substrate mixture.*

Factor under study	Average main stem diameter (mm)						
	1 DAT	30 DAT	60 DAT	90 DAT*	120 DAT*		
Nutrient solution (S)							
s1	0.72	0.92	1.03	1.61	b	1.99	b
s2	0.63	0.94	1.18	1.96	a	2.38	a
Substrate mixture (M)							
m1	0.72	0.90	1.06	1.58	b	1.93	b
m2	0.64	0.96	1.15	1.99	a	2.45	a

* Because statistical significance was only found in these periods, the LSD test was applied; DAT = days after transplanting.

Table 11. Average for the main stem diameter per treatment.*

Treatment	Average main stem diameter (mm)							
	1 DAT	30 DAT	60 DAT*	90 DAT*	120 DAT*			
T1 (s1m1)	0.77	0.92	1.04	b	1.44	b	1.73	b
T2 (s1m2)	0.67	0.92	1.03	b	1.78	ab	2.26	ab
T3 (s2m1)	0.66	0.89	1.08	ab	1.71	b	2.13	ab
T4 (s2m2)	0.61	1.00	1.27	ab	2.20	a	2.64	a
T5 (Witness)	0.73	1.03	1.32	a	1.66	b	1.87	b
T5 (Control)								

* Because statistical significance was only found in these periods, Tukey's 5% test was applied (values with the same letter are not significantly different: $p \geq 0.05$); DAT = days after transplanting.

position (ab), not statistically differing from T4 or from the lower group made up of T1 (1.73 mm) and T5 (1.87 mm). This result (at 120 DAT) indicates that stem diameter responded to the interaction between the form of N and substrate composition. The higher value in T4 (75% ammonium + 80% peat) suggests that a high fraction of NH_4^+ was more effective when combined with a substrate with a higher peat content, capable of offering better water retention, greater cation exchange capacity and a pH more suitable for blueberry (Bignami et al., 2022; Tamir et al., 2021; Tamir et al., 2025). In contrast, T2 and T3 showed an intermediate response, indicating that favoring only the nitrogen source or only the substrate was not enough to maximize stem thickening. Thus, the data confirm that, during hardening-off, blueberry structural growth depended more on the appropriate combination of higher peat content and ammonium predominance than on each factor separately (Anwar et al., 2024; Arias et al., 2024; Bryla et al., 2025).

In summary, this behavior confirms that the synergy between an ammoniacal nitrogen source and a substrate with a high peat content optimizes blueberry stem thickening during the final hardening-off phase. These results are in line with those obtained by Espinoza-Orozco et al. (2025), where NH_4^+ increased biomass and thickening under saline conditions. The

effect of peat is consistent with studies indicating that it improved water and nutrient availability, emphasizing its role in structural tissues (Bowerman, 2012; Ortiz-Delvasto et al., 2023).

3.3. Robustness Index [RI]

According to the analysis of variance for RI (Table 12), during the first 30 DAT no statistically significant differences were observed between treatments, with relatively high coefficients of variation (27.08% and 30.18%) typical of the establishment stage. At 60 DAT highly significant differences were recorded between treatments, where the nutrient solution factor [S] showed a significant effect and the control vs. other treatments contrast was also highly significant, indicating that the nitrate/ammonium ratio was beginning to influence plant robustness and that the control clearly differed from the treatments with nutrient solution; however, the substrate factor [M] and its interaction with the solution did not reach significance. At 90 and 120 DAT the differences between treatments ceased to be significant, both for the main factors and for their interaction, showing a convergence toward the end of the experiment where the plants tended to balance their height/diameter ratio, probably because height growth and stem thickening occurred pro-

portionately across all treatments. The coefficients of variation ranged between 22.60% and 30.18%, acceptable values for this morphological index, while the overall RI average showed a decreasing trend from 16.68 to 12.87, indicating that stem diameter increased proportionally more than height over time, resulting in sturdier plants by the end of the experimental period.

Regarding the analysis of robustness index averages by type of nutrient solution (Table 13), during the first 30 DAT no statistically significant differences were observed between s1 (75% nitrate) and s2 (75% ammonium). However, at 60 DAT significant differences were recorded: plants with s1 showed an RI of 16.31 (more slender morphology), while those with s2 reached 13.70, representing a 19% reduction and showing a more robust architecture favored by the predominance of ammonium. In the final evaluations (90 and 120 DAT), the differences ceased to be statistically significant: at 90 days, s1 recorded 13.02 and s2 a numerically lower value of 11.40; at 120 days, both treatments reached practically identical va-

lues (12.90 for s1 and 13.00 for s2). This convergence indicates that, although the ammonium solution promoted greater robustness at intermediate stages, by the end the plants of both treatments balanced their height/diameter ratio during the hardening-off phase. This temporal behavior can be explained from blueberry physiology through three key processes. Initially, at 60 days after transplanting, the greater robustness induced by the solution with a predominance of ammonium (s2) reflects an early response in which rhizosphere acidification, derived from NH_4^+ absorption, optimizes micronutrient availability and stimulates accelerated stem thickening relative to height growth (Tamir et al., 2021; Yuan-Yuan et al., 2021). Toward the final evaluations (90-120 DAT), a morphological compensation occurs (Yuan-Yuan et al., 2021). This convergence is due to a morphological homeostasis mechanism typical of the species, which tends to restore balanced structural proportions toward the end of the growth cycle, regardless of the temporary alterations induced by the nutritional treatments (Cárdenas-Navarro et al., 2024; Cárdenas-Navarro et al.,

Table 12. Analysis of variance for the robustness index variable.*

Sources of Variation	df	Mean square									
		1 ddt		30 ddt		60 ddt		90 ddt		120 ddt	
Total	49										
Treatments	4	2.04	ns	41.13	ns	53.79	**	9.12	ns	2.16	ns
S (Solution)	1	0.00	ns	60.12	ns	67.70	*	26.32	ns	0.08	ns
M (Mixture)	1	0.30	ns	62.45	ns	37.36	ns	2.76	ns	0.48	ns
S x M	1	5.49	ns	0.55	ns	13.97	ns	5.92	ns	7.80	ns
Control vs. Rest	1	2.39	ns	41.42	ns	96.13	**	1.50	ns	0.28	ns
Error	45	20.40		19.81		12.19		7.72		10.36	
CV (%)		27.08		30.18		24.40		22.60		24.78	
Average		16.68		14.74		14.31		12.29		12.87	

* df = degrees of freedom; CV = coefficient of variation; ** = highly significant difference (p-value < 0.01); * = significant difference (p-value < 0.05); ns = not significant (p-value ≥ 0.05); DAT = days after transplanting.

Table 13. Average robustness index by type of nutrient solution and by type of substrate mixture.*

Factor under study	Average robustness index					
	1 DAT	30 DAT	60 DAT*	90 DAT	120 DAT	
Nutrient solution (S)						
s1	16.57	16.43	16.31 a	13.02	12.90	
s2	16.57	13.98	13.70 b	11.40	13.00	
Substrate mixture (M)						
m1	16.66	16.45	15.97	12.47	13.06	
m2	16.48	13.95	14.04	11.95	12.84	

* Because statistical significance was only found in these periods, Tukey's 5% test was applied (values with the same letter are not significantly different: p ≥ 0.05); DAT = days after transplanting.

2025; Yuan-Yuan et al., 2021).

The analysis of robustness index averages by type of substrate mixture (Table 13) shows that no statistically significant differences were observed between m1 (20% peat) and m2 (80% peat) at any evaluation time (1, 30, 60, 90 and 120 DAT). This indicates that, although the substrate mixtures had significant effects on height and diameter individually, the relationship between the two variables (RI) remained similar between both substrates throughout the experiment; mixture m2 produces larger plants in absolute terms, with greater height and diameter, while the fact that the height/diameter ratio remains similar indicates that the plant’s basic architecture is not compromised by the use of mixtures with lower peat content.

The analysis of robustness index averages by treatment (Table 14) indicates that during the first 30 DAT no statistically significant differences were observed between treatments. At 60 DAT significant differences were recorded, forming two opposing groups: the most slender plants (higher RI) corresponded to the top group (a), made up of T1 (s1m1) with 16.68 and T2 (s1m2) with 15.93, both characterized by the 75% nitrate solution (s1); in contrast, the sturdiest plants (lower RI) formed the bottom group (b), with T4 (s2m2) reaching 12.15 and control T5 at 11.54. In the final evaluations (90 and 120 DAT), the differences ceased to be significant, with a clear convergence of values observed: at 90 days, although T4 kept the lowest RI (10.75) and T1 and T2 the highest (up to 13.15), the range narrowed to 2.4 units; at 120 days, all treatments converged into an even smaller range of 1.1 units, with values between 12.45 (T4) and 13.55 (T3), reflecting that, regardless of the initial differences, plants tend to balance their height-diameter ratio toward the end of the cycle, reaching a proportionally similar morphology. These results agree with the study by Yuan-Yuan et al. (2021), where the treatments applied balanced height/diameter later on. The

disappearance of significant differences in the robustness index at 90 and 120 days after transplanting can be explained by a morphological convergence typical of the hardening-off stage, where blueberry plants tend to stabilize their height-diameter ratio, acting as a homeostatic attractor that prevails over the initial differences induced by the treatments (Rojas Mego, 2024). This final uniformity is due to coordinated and compensatory growth, since the treatments that promoted greater height (such as T4) also proportionally stimulated stem thickening, keeping the ratio within similar margins (Peñuelas Montoya et al., 2024). Time reduced the differences between treatments until they became non-significant, reflecting a progressive growth adjustment (Peñuelas Montoya et al., 2024; Rojas Mego, 2024).

3.4. Aerial dry biomass [ADB], root dry biomass [RDB], total dry biomass [TDB], ADB/RDB ratio and Dickson Quality Index [DQI]

The analysis of variance for the biomass and quality variables evaluated at 120 DAT (Table 15) revealed that the treatments had highly significant effects on all variables.

The nutrient solution factor [S] showed highly significant effects on ADB, TDB, the ADB/RDB ratio and DQI, but was not significant for root dry biomass [RDB], suggesting that root development was more influenced by the physical characteristics of the substrate than by the form of nitrogen. The substrate mixture factor [M] showed highly significant effects on all variables (ADB, RDB, TDB, ADB/RDB and DQI), confirming that the proportion of peat versus rice husk affects all aspects of growth and quality. The S x M interaction was significant only for ADB and ADB/RDB, indicating that the factors acted synergistically in biomass partitioning toward the ae-

Table 14. Average robustness index per treatment.*

Treatment	Average robustness index				
	1 DAT	30 DAT	60 DAT*	90 DAT	120 DAT
T1 (s1m1)	16.29	17.56	16.68 a	12.90	12.57
T2 (s1m2)	16.85	15.30	15.93 ab	13.15	13.24
T3 (s2m1)	17.03	15.35	15.26 ab	12.05	13.55
T4 (s2m2)	16.12	12.61	12.15 b	10.75	12.45
T5 (Witness)	17.12	12.93	11.54 b	12.65	13.14
T5 (Control)					

* Because statistical significance was only found in these periods, Tukey’s 5% test was applied (values with the same letter are not significantly different: p ≥ 0.05); DAT = days after transplanting.

Table 15. Analysis of variance for the biomass and quality variables.*

Sources of Variation	df	Mean squares									
		BSA		BSR		BST		ADB/RDB		DQI	
Total	49										
Treatments	4	44.04	**	0.78	**	56.01	**	15.26	**	0.14	**
S (Solution)	1	29.73	**	0.10	ns	33.34	**	25.48	**	0.06	**
M (Mixture)	1	131.44	**	2.44	**	169.74	**	27.50	**	0.44	**
S x M	1	6.31	*	0.04	ns	7.39	ns	7.80	*	0.01	ns
Control vs. Rest	1	8.69	*	0.54	*	13.58	*	0.26	ns	0.04	*
Error	45	1.58		0.09		2.02		1.94		0.01	
CV (%)		27.44		29.16		25.29		31.39		26.06	
Average		4.58 g		1.03 g		5.62 g		4.44		0.32	

* df = degrees of freedom; CV = coefficient of variation; ** = highly significant difference (p-value < 0.01); * = significant difference (p-value < 0.05); ns = not significant (p-value ≥ 0.05); DAT = days after transplanting.” followed by “** ADB = aerial dry biomass, RDB = root dry biomass, TDB = total dry biomass, DQI = Dickson Quality Index.”

rial part, while for RDB, TDB and DQI the effects were mainly additive (independent). The control vs. other treatments contrast showed significant differences for ADB, RDB, TDB and DQI, but not for the ADB/RDB ratio, showing that the nutrient solutions increased biomass production and quality without altering the relative partitioning between the aerial part and the root. The coefficients of variation (25.29% to 31.39%) were acceptable for this type of variable. The LSD test for the biomass and quality variables evaluated (Table 16), in relation to factor S, shows that the solution with a predominance of ammonium (s2: 25% NO_3^- / 75% NH_4^+) is superior to the solution with a predominance of nitrate (s1: 75% NO_3^- / 25% NH_4^+) for the production of blueberry plants during the hardening-off phase. Plants grown with s2 showed significantly greater aerial biomass (44% increase), total biomass (37% increase), ADB/RDB ratio (44% increase) and Dickson Quality Index (27% increase). Although root biomass showed no significant differences, the superiority in the other variables, particularly in DQI, which integrates multiple quality attributes, confirms that the solution with 75% ammonium produces plants of better overall quality.

The observed results have clear physiological bases that explain the better performance of the 75% ammonium solution (s2). First, blueberry, as an acidophilic species, is evolutionarily adapted to acidic soils where ammonium [NH_4^+] predominates over nitrate [NO_3^-], which translates into greater efficiency in its absorption and assimilation (Yuan-Yuan et al., 2021; Zhang et al., 2022). From an energy standpoint, ammonium requires a lower metabolic cost, since it can be incorporated directly into amino acids via the enzymes glutamine synthetase and glutamate synthase, unlike nitrate, which must be reduced in a process

that consumes NADH and reducing power, freeing up resources for greater biomass production (Langenfeld y Bugbee, 2025; Zhang et al., 2022). In addition, NH_4^+ absorption induces the excretion of protons (H^+) into the rhizosphere, acidifying the environment and favoring the availability of micronutrients such as iron, manganese, zinc and copper, essential for photosynthesis and enzymatic activity (Tamir et al., 2021; Yang et al., 2025). The nitrogen form modulates the hormonal balance: ammonium stimulates cytokinins and aerial growth, while nitrate promotes auxins and root development (De la Peña et al., 2022; Langenfeld & Bugbee, 2025; Yang et al., 2025). This would explain the greater investment in aerial biomass with ammonium (s2), although without significant differences in roots.

On the other hand, the LSD test for the biomass and quality variables studied, in relation to factor M (Table 16), indicates that the substrate mixture with 80% peat and 20% rice husk (m2) is superior to the mixture with 20% peat and 80% rice husk (m1) for the production of blueberry plants during the hardening-off phase. Plants grown in m2 showed: more than double the aerial biomass (122% increase), 60% more root biomass, more than double the total biomass (108% increase), 46% higher ADB/RDB ratio, and 91% better Dickson Quality Index. These differences are explained by the properties of peat: high moisture retention capacity, high cation exchange capacity, an acidic pH suitable for the species, and physical stability (Bowerman, 2012; Ortiz-Delvasto et al., 2023). Tukey’s 5% test for the biomass and quality variables analyzed by treatment (Table 17) shows that treatments with substrate m2 (80% peat) are superior to those with substrate m1 (20% peat) in all the biomass and quality variables evaluated. The combina-

Table 16. DMS test for the biomass and quality variables by type of nutrient solution and by type of substrate mixture.

Factor under study	Average									
	ADB (g)		RDB (g)		TDB (g)		ADB/RDB		DQI	
Nutrient solution (S)										
s1	3.93	b	1.04		4.97	b	3.61	b	0.30	b
s2	5.65	a	1.14		6.80	a	5.20	a	0.38	a
Substrate mixture (M)										
m1	2.98	b	0.84	b	3.82	b	3.58	b	0.23	b
m2	6.61	a	1.34	a	7.94	a	5.23	a	0.44	a

tion T4 (s2m2: 25/75 solution + 80% peat substrate) produces the best results in all variables, with aerial biomass (7.07 g), root biomass (1.36 g), total biomass (8.43 g) and DQI (0.47) values significantly higher than the other treatments, except for T2, with which it forms a homogeneous group. Treatment T2 (s1m2) is a viable alternative, with no statistical differences from T4, provided that substrate m2 is used. Treatment T1 (s1m1) should be avoided, since it produces the worst plant quality, with biomass and DQI values even lower than the unfertilized control. The control (T5), although it produces plants without fertilization (with 100% rice husk substrate), only reaches moderate levels of biomass and quality, far below those achieved with the optimal combinations.

The superiority of T4 is due to the synergy between peat, which provides acidity, moisture and cation exchange capacity (Ortiz-Delvasto et al., 2023), and the 75% ammonium, which takes advantage of blueberry's preference and acidifies the rhizosphere (Yang et al., 2025; Zhang et al., 2022). This combination produces plants with more than double the biomass and a quality index 2.6 times higher than the worst combination. These results are in line with those obtained by Espinoza-Orozco et al. (2025) and Yang et al. (2025), where NH_4^+ favored biomass by acidifying and releasing Fe/Mn; and with those of Ortiz-Delvasto et al. (2023), where peat optimized roots and assimilates.

3.5. Correlation analysis of variables

The Pearson correlation coefficients, and their respective significance levels for all the variables evaluated at the end of the experiment (Table 18), reveal a pattern of correlations that has important implications for evaluating plant quality in blueberry during the hardening-off phase.

Strong to very strong positive correlations exist between most of the morphological variables (PH, MSD) and biomass variables (ADB, RDB, TDB), as

well as with DQI. This indicates that height growth, stem thickening and biomass accumulation occur in a coordinated and proportionate manner. DQI is confirmed as the best integrative quality indicator, with strong to very strong correlations with all variables except RI. Its near-perfect correlation with TDB ($r = 0.92$) and very strong correlation with ADB ($r = 0.90$) and RDB ($r = 0.81$) validate it as an excellent tool for assessing the plants' overall quality. RI does not correlate significantly with any biomass variable or with DQI, indicating that it should not be used as the sole quality indicator. A plant can be sturdy (low RI) but have little biomass, or slender (high RI) but have a lot of biomass and good overall quality. PH and MSD are good predictors of quality, with strong correlations with DQI (0.78 and 0.72, respectively), which justifies their use as quick indicators in field evaluations. Aerial biomass (ADB) is an excellent predictor of total biomass ($r = 0.99$) and of DQI ($r = 0.90$), suggesting that, in the absence of the possibility of calculating DQI, ADB can be a good substitute indicator.

These findings have important practical implications for evaluating plant quality in blueberry nurseries: it is recommended to use DQI as the main quality indicator, complemented with height and diameter measurements, in agreement with the studies by Osorio-Espinoza et al. (2025). RI, although useful for describing morphology, should not be the sole quality criterion, and the strong correlation between ADB and RDB ($r = 0.71$) indicates that the plants are developing balanced aerial and root systems, which is favorable for their subsequent field performance.

4. Conclusions

Both the nitrate/ammonium ratio and the substrate mixture significantly influenced the vegetative growth of blueberry, with the optimal combination being treatment T4 (s2m2), characterized by a nutrient solution with a predominance of ammonium

Table 17. Tukey's test (5%) for the biomass and quality variables per treatment.

Treatment	Average*									
	ADB (g)		RDB (g)		TDB (g)		ADB/RDB		DQI	
T1 (s1m1)	1.72	c	0.76	b	2.48	c	2.34	b	0.18	c
T2 (s1m2)	6.14	a	1.32	a	7.46	a	4.88	a	0.41	a
T3 (s2m1)	4.24	b	0.93	b	5.17	b	4.82	a	0.29	b
T4 (s2m2)	7.07	a	1.36	a	8.43	a	5.59	a	0.47	a
T5 (Witness)										
T5 (Control)	3.75	b	0.83	b	4.58	b	4.59	a	0.26	bc

* Values with the same letter are not significantly different ($p \geq 0.05$).

Table 18. Pearson test correlation for evaluated variables.

Variable 1	Variable 2	Pearson*	p-value**
PH	MSD	0.62	<0.0001
PH	RI	0.1	0.5107
PH	ADB	0.9	<0.0001
PH	RDB	0.6	<0.0001
PH	TDB	0.89	<0.0001
PH	ADB/RDB	0.62	<0.0001
PH	DQI	0.78	<0.0001
MSD	RI	-0.65	<0.0001
MSD	ADB	0.57	<0.0001
MSD	RDB	0.28	0.0482
MSD	TDB	0.55	<0.0001
MSD	ADB/RDB	0.48	0.0004
MSD	DQI	0.72	<0.0001
RI	ADB	0.07	0.6274
RI	RDB	0.17	0.2503
RI	TDB	0.09	0.5458
RI	ADB/RDB	-0.06	0.6968
RI	DQI	-0.21	0.134
ADB	RDB	0.71	<0.0001
ADB	TDB	0.99	<0.0001
ADB	ADB/RDB	0.64	<0.0001
ADB	DQI	0.9	<0.0001
RDB	TDB	0.78	<0.0001
RDB	ADB/RDB	-0.04	0.7686
RDB	DQI	0.81	<0.0001
TDB	ADB/RDB	0.56	<0.0001
TDB	DQI	0.92	<0.0001
ADB/RDB	DQI	0.39	0.0047

* Correlation coefficient between the corresponding variables: a coefficient equal to zero indicates a lack of association, while the closer it is to 1 or -1, the greater the strength of the association (Coyne & Thompson, 2006).

** Probability associated with the hypothesis test for null correlation (no correlation) (Balzarini et al., 2008).

(25% NO₃⁻ / 75% NH₄⁺) and a substrate with a high peat content (80%), especially from 90 days after transplanting onward, establishing itself as the best option for producing vigorous plants.

The plant's overall quality, evaluated using DQI, was significantly higher in treatment T4, which combined the ammoniacal nitrogen source (s2) with the peat-rich mixture (m2), since it produced the highest accumulations of aerial, root and total dry biomass, with values more than double those of the least efficient

combinations.

The correlation analysis showed that DQI was, in this study, the best integrative indicator of plant quality, presenting very strong correlations with total dry biomass and with height; in contrast, RI showed no significant correlation with biomass or DQI, suggesting that it should not be used as the sole criterion for determining seedling quality; while aerial dry biomass was confirmed as an excellent predictor of overall quality.

Contributor roles

- Damian Alejandro Orellana Zuleta: conceptualization, investigation, methodology, resources, formal analysis, visualization, writing – original draft.
- Víctor Julio Moreno Izquierdo: software, visualization, writing – review & editing.
- Yamil Everaldo Cartagena Ayala: conceptualization, investigation, methodology, data curation, validation, writing – review & editing.
- Dafne Isaac Serrano Pérez: validation, writing – review & editing.

Data availability

The data will be available on request.

Use of Artificial Intelligence

During the preparation of this manuscript the authors used SciSpace for writing purposes, at 8%. After using this tool/service/software, the authors reviewed and edited the content as needed, to avoid bias, errors, or fabrication of references, and take full responsibility for the content of the published article.

Ethical implications

The authors declare that there are no ethical implications.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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