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New Leaf Nitrogen Sufficiency Ranges for Southern Highbush Blueberry

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Abstract. **Leaf analysis is a key tool for determining blueberry plant nutritional status. For leaf analysis to be useful, optimal concentration thresholds must be established. Existing thresholds, which were developed for northern highbush blueberry (NHB; *Vaccinium corymbosum*), recommend optimal blueberry leaf nitrogen (N) concentrations between 1.76% and 2.00%. It is unclear, however, whether these values accurately represent southern highbush blueberry (SHB; *V. corymbosum* interspecific hybrids) because SHB and NHB exhibit contrasting physiological responses and cultivation ranges. This study aimed to refine N sufficiency ranges for SHB, accounting for variations across production systems (deciduous and evergreen) and phenological stages (vegetative growth, flower bud initiation, bloom, and fruit development). Data from five previous experiments were analyzed by fitting Normal, Gamma, and Weibull distributions. A sufficiency range approach was then used to identify optimum leaf N thresholds for SHB. Finally, the accuracy of these thresholds was assessed with a random forest classification model that included plant, management, and environmental data. The Gamma distribution provided the best fit in three of the seven system-by-stage groups, with normal and Weibull distributions each selected in two. Refined leaf N sufficiency thresholds, derived from the best-fitting distribution model for each system by stage group, were proposed. The random forest classification supported the agronomic and physiological relevance of the proposed leaf N concentration thresholds. These results will improve nutritional status diagnostics in SHB production.**

Southern highbush blueberry (SHB; *Vaccinium corymbosum* interspecific hybrids) is a commercially important crop in regions with low chill hour accumulation globally (Lyrene 2002). In US states with mild winters, such as Florida, SHB cultivars represent nearly all new plantings and secure the state's competitive position in the early-season fresh market (Williamson and Phillips 2024). Other major US blueberry-producing states, including Georgia, have also expanded SHB acreage to meet early domestic demand and improve market timing (Scherm and Krewer 2003). Additionally, SHB production has grown significantly in countries such as Peru, Mexico, and Morocco, which are now among the top producers and exporters of fresh blueberries (Brazelton et al. 2024; US Department of Agriculture, Foreign Agricultural Service 2025). In these regions, SHB is cultivated as a deciduous or evergreen bush.

Fertilization of SHB can be challenging, especially in terms of nitrogen (N) due to crops' sensitivity to either excessive or low N availability (Phillips and Williamson 2020), as well as the shallow roots systems that can limit water and nutrient uptake (Nunez et al. 2016). Monitoring leaf N concentration is critical for accessing the nutritional status of plants and diagnosing N deficiency or excess (Reuter and Robinson 1997). Leaf N concentration data, combined with other criteria, can indicate whether N fertilization practices meet crop needs and guide fertilizer management (Bryson and Mills 1992). This is especially important for perennial fruit crops where plant nutritional status can ultimately affect yield and quality for more than one season-year (Reuter and Robinson 1997).

Existing guidelines for northern highbush blueberry (NHB, *Vaccinium corymbosum*) have evolved substantially over time and vary by region. Early studies from Oregon suggested optimal leaf N concentrations range between 1.76% and 2.10% (Hart et al. 2006), with subsequent revisions expanding this range to 1.40% to 2.20% (Strik and Davis 2023). More recent work reported optimal ranges of 1.50% to 2.00% in western Washington and 1.25% to 1.75% in eastern Washington. In Michigan, optimal N concentrations range between 1.70% and 2.10% (Lukas et al. 2022; Strik and Davis 2023). It is unclear, however, whether these values accurately represent optimal leaf N concentrations in SHB. Leaf nutrient standards must account for a range of biological and environmental factors, including species-specific growth rates, developmental stages, and nutrient uptake efficiency. In perennial horticultural crops, further complexity arises from seasonal variation, fruit load, and pruning practices (Reuter and Robinson 1997). This highlights the importance of understanding context-specific factors that influence nutrient levels and plant analysis outcomes.

Interpreting leaf nutrient concentrations relies on comparisons of measured nutrient concentrations with references derived from datasets compiled from years of observation and experimentation (Bennett 1993; Reuter and Robinson 1997). When leaf nutrient concentrations fall below the reference value for a certain nutrient, the plant is presumed to be deficient in that nutrient. A commonly used method for developing these standards in horticultural crops is the sufficiency range approach, which uses statistical distributions to define nutrient sufficiency ranges based on the observed frequency of nutrient concentrations. The sufficiency range approach accounts for the natural variation in concentrations of a certain nutrient by evaluating the fit of different distribution models and defining upper and lower sufficiency limits in the distribution (Cera et al. 2022; Mhango et al. 2021; Veazie et al. 2024a).

Machine learning techniques are also used to model plant nutrition dynamics and improve decision-making (Ennaji et al. 2023; Munir et al. 2022; Veazie et al. 2024b). In the context of nutrient diagnosis, exploratory models can be applied to evaluate the relative contribution of environmental, management, and physiological factors to plant nutritional status. This approach complements traditional sufficiency range approach methods by identifying variables that most influence nutrient classification.

The objective of this work is to use leaf N data to define leaf N concentration sufficiency ranges for SHB in a subtropical area of the southeastern Unites States, using plant tissue analysis and machine learning to support data-informed fertilization recommendations for SHB growers.

Material and Methods

Data collection. Leaf N concentration data were collected from field experiments conducted across four locations in Florida between 2021 and 2025 (Table 1). Experiments included both evergreen and deciduous production systems of SHB, evaluating various N fertilization rates ranging from 5.6 to 448.3 kg $N \cdot ha^{-1} \cdot year^{-1}$. Leaf sampling

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The data underlying this article will be shared upon request to the corresponding author.

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Table 1. Sources of southern highbush blueberry (SHB; *Vaccinium corymbosum* interspecific hybrids) leaf nitrogen (N) concentration data used in the development of the new leaf N sufficiency ranges. Data were collected from southern highbush blueberry farms in Florida between 2021 and 2025.

Location	Cultivars	Year	Data points	Notes
Commercial farms in Umatilla, FL (lat. 28° 58' 18", long. –81° 47' 6") and Archer, FL (lat. 29° 32' 51", long. –82° 29' 4")	Optimus Sentinel	2023–25	480	Study on N fertilizer rates (84 to 448 kg N ha^{-1}·yr^{-1}) of Deciduous SHB (unpublished)
UF/IFAS Plant Science Research farm in Citra, FL (lat. 29° 25' 11", long. –82° 10' 12")	Sentinel Arcadia	2022–25	910	Study on N fertilizer rates (5.6 to 448.3 kg N ha^{-1}·yr^{-1}) Evergreen SHB[i]
UF/IFAS Plant Science Research and Education Unit in Citra, FL (lat. 29° 25' 11", long. –82° 10' 12")	Farthing Emerald	2021	350	Study on N fertilizer rates (56 to 336 kg N ha^{-1}·yr^{-1}) of Deciduous SHB (unpublished)
Commercial farms in Wimauma, FL (lat. 27° 44' 2", long. –82° 14' 12") and Dade City, FL (lat. 28° 22' 34", long. –82° 20' 40")	Farthing Kestrel Endura Sentinel Ventura Albus	2022	162	Study on N fertilizer programs of Evergreen SHB (unpublished)[ii]

[i] Published study: Goldsby, L. Optimizing nitrogen rate and timing for young evergreen southern highbush blueberry. University of Florida 2024.

[ii] N fertilizer rates at commercial farms were not available.

occurred at defined phenological stages: vegetative growth (June–August), flower bud initiation (September–December), bloom (January–February) and fruit development (March–May). Samples consisted of fully expanded leaves from the current season's growth, with petioles remaining attached, and randomly distributed throughout the plant canopy. Leaf sampling was not possible during the bloom stage of deciduous SHB plants, as the crop typically blooms before fully expanded leaves are present. A total of 1902 leaf samples were analyzed over multiple years and sampling dates.

Data preprocessing. Before statistical modeling, the dataset was filtered to include only N fertilization rates within the typical commercial range for SHB in Florida, from 140 to 336 kg N·ha^{-1}·year^{-1}. Data points outside this range were excluded to reduce influence from excessively low or high rates not representative of commercial practices. Potential outliers, defined as observations below the first and above the 99th percentiles of leaf N concentration, were also excluded to minimize the influence of extreme or biologically implausible values. The final dataset consisted of 1266 observations. All leaf N concentrations are reported as percent dry weight (equivalent to g N per 100 g dry leaf tissue).

Linear mixed model. Data were fit to a linear mixed model (LMM) for quantifying the effects of production systems (deciduous vs. evergreen) and phenological stages (vegetative, flower bud initiation, bloom and fruit development) on leaf N concentrations. Fixed effects included production system, phenological stage (treated as a repeated measure), and their interaction. Random intercepts for location and year were included to account for site and temporal heterogeneity. Significance of fixed effects was assessed using type III ANOVA with Satterthwaite's approximation for denominator degrees of freedom, as implemented in the lmerTest package (Kuznetsova et al. 2017).

Distribution fitting. Data were fit to normal, gamma, and Weibull distributions (Cera et al. 2022; Mhango et al. 2021; Weibull 1951) using the MASS package (Venables and Ripley 2013) in R version 4.3.1(R Core Team 2021). Model selection was based on Akaike information criterion (AIC) value, supported by visual diagnostics (e.g., Q-Q plots and histograms). The distribution with the lowest AIC value was considered the best representation of the data among the choices available. Where two distributions had close AIC values (ΔAIC $<$2, where ΔAIC is the difference between a model's AIC and the smallest AIC among the candidate models fit to the same data), both models were considered statistically equivalent. In these cases, the final model selection was guided by visual assessment of the fitted curves relative to the observed data. The sufficiency range was based on the area between the 0.25 and 0.75 quantile interval of the best fitting distribution. "Low" and "High" ranges were defined as the 0.025 to 0.25 and 0.75 to 0.975 quantile intervals, respectively. Values below the 0.025 quantile were classified as "Deficient," and those above the 0.975 quantile were classified as "Excessive." Results were illustrated using ggplot2 in R (Wickham 2006).

Thresholds validation. The consistency of sufficiency range approach derived thresholds was assessed by implementing a random forest classification model in Python (version 3.9) to predict nutrient-status categories (deficient, low, sufficient, high, excessive) from plant, environmental, and management variables. This allowed us to assess the degree to which the sufficiency range approach categories were supported by the broader dataset, identify possible inconsistencies, and explore the relative importance of explanatory variables. Predictors were plant age, cultivar, seasonal N fertilization rate (the cumulative annual N fertilizer input), phenological stage, leaf N concentration, temperature (average air temperature per phenological stage), and rainfall (cumulative rainfall per phenological stage). Yield was not included as a predictor because previous studies with perennial crops indicate that leaf nutrient concentrations do not impact yield consistently (Arrington and DeVetter 2017; Strik et al. 2019).

Categorical predictors (cultivar, phenological stage, production system and year) were label encoded, and numerical predictors (age, N rate, temperature, rainfall and leaf N) were standardized. Temperature and rainfall data were sourced from Florida Automated Weather Network (2025). The model was configured with 100 trees (estimators), a maximum depth of 10, and a fixed random state for reproducibility. The dataset was partitioned using a stratified 70 to 30 train–test split to maintain class balance in the evaluation set. Model performance was computed on the held out test set. The relative importance of predictors was summarized using mean decrease in impurity.

Results and Discussion

Linear mixed model. Leaf N concentration was influenced by the production system, plant phenological stage, and their interaction (Table 2). The interaction indicates that stage related differences in leaf N concentration depend on production system. Leaf N concentrations vary with phenological stage due to physiological shifts in nutrient demand and remobilization (Bañados et al. 2012; Bryla et al. 2012; Bryla and Strik 2015; Carranca 2009). Recognizing these growth patterns is essential for optimizing nutrient management and understanding leaf nutrient concentrations at different phenological stages. Furthermore, major physiological and management differences between evergreen and deciduous SHB systems are likely to affect leaf N status. One key difference among

Table 2. Type III analysis of variance results from a linear mixed model evaluating the effects of production system, phenological stage, and their interaction on leaf N concentration of southern highbush blueberry plants. Random effects accounted for variability among locations and years. Significant interaction supports the development of system by stage specific sufficiency ranges. Data were collected from southern highbush blueberry farms in Florida between 2021 and 2025.

Effect	Sum Sq	Mean Sq	Num *df*	Den *df*	*F* value	Pr ($>F$)
Production system:stage	1.349	0.675	2.000	1252.086	7.215	$<$0.001
Production system	0.535	0.535	1.000	1057.391	5.727	$<$0.05
Stage	36.039	12.013	3.000	1251.528	128.475	$<$0.001

Sum Sq = sum of squares; Mean Sq = mean of squares.

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Table 3. Comparison of Akaike information criterion (AIC) values for normal, gamma, and Weibull distribution models used in the development of system by stage specific sufficiency ranges. Data were collected from southern highbush blueberry farms in Florida between 2021 and 2025.

		AIC		
System	Stage	Normal	Gamma	Weibull
Evergreen	Vegetative growth	–27.72	**–30.79**[i]	–15.13
	Flower bud initiation	**29.62**	35.81	38.34
	Bloom	152.50	154.38	**151.03**
	Fruit development	–95.37	**–95.43**	–88.27
Deciduous	Vegetative growth	69.14	**63.33**	80.90
	Flower bud initiation	**–66.91**	–64.09	–61.29
	Bloom[ii]	—	—	—
	Fruit development	359.15	366.54	**349.71**

[i] The selected models with the lowest AIC values are bolded.

[ii] Leaf sampling was not possible during bloom in deciduous SHB because leaves were not fully expanded.

production systems is that deciduous plants enter dormancy during the fall and winter, and fertilization is typically cut off during this period, whereas evergreen plants retain leaves year-round and thus require continuous N application throughout the season (Phillips et al. 2020; Reeder et al. 1998). Distinct nutrient demands and physiological processes across production systems and phenological stages justify the development of system by stage specific sufficiency ranges.

Distribution fitting and model selection. Normal, gamma, and Weibull distribution fits within each system by stage group were compared by AIC, with visual diagnostics as support (Table 3). Among the seven system by stage groups supported by the results of the linear mixed model analysis, Gamma distributions provided the best fit in three groups, Normal distributions in two, and Weibull distributions in the remaining two. In two groups, the top two models were close (ΔAIC < 2), indicating limited evidence to prefer one over the other (e.g., evergreen plants at fruit development: gamma vs. normal ΔAIC $\sim$0.06; evergreen plants at bloom: Weibull vs. normal ΔAIC $\sim$1.5). In the remaining groups, the AIC differences were $\geq$3 (moderate) to $\geq$10 (strong), favoring the selected model.

Sufficiency ranges. The best-fitting distribution models for each system-by-stage combination are shown in Fig. 1. The corresponding leaf N concentration sufficiency ranges are summarized in Table 4.

The sufficiency range of evergreen SHB during vegetative growth was estimated at 1.47% to 1.77% N, reflecting a relatively tight distribution. During flower bud initiation, the sufficiency range was slightly lower (1.26% to 1.60% N) but still fell within a similar concentration range. The sufficiency range was broader during bloom (1.45% to 1.99% N), indicating greater variability in leaf N concentrations at this stage. During fruit development, the sufficiency range was notably narrow but shifted to the upper end of the distribution, with sufficiency levels falling between 1.73% to 1.93% N.

The estimated sufficiency range for deciduous SHB was similar during vegetative growth and flower bud initiation, ranging from 1.48% to 1.88% N and 1.44% to 1.70% N, respectively. These values are closely aligned with the sufficiency range observed during vegetative growth in evergreen SHB. During fruit development, the sufficiency range broadened and shifted to the upper end of the distribution, with sufficiency levels between 1.73% and 2.40% N, suggesting increased leaf N concentrations and potentially greater variability in plant N status at this stage.

Evergreen SHB plants generally exhibited narrower and lower sufficiency range across most phenological stages compared with deciduous SHB plants, suggesting that the physiological differences or environmental conditions that allow evergreen phenology have an impact in plant N status. Deciduous plants undergo a flush of new leaves after dormancy (Fang et al. 2020a). In Florida, especially from late January to early April, these new leaves are still young and potentially contributed to the greater variability in tissue N levels observed in deciduous plants during fruit development. In contrast, evergreen plants retain their leaves year-round (Fang et al. 2020a; Phillips and Williamson 2020), supporting continuous nutrient uptake, photosynthesis, and carbohydrate partitioning, which likely led to a greater stability in leaf N concentrations.

Early studies on leaf nutrient standards for blueberry in the Pacific Northwest proposed a sufficiency range of 1.76% to 2.10% N (Hart et al. 2006). Subsequent revisions expanded this range to 1.40% to 2.20% N (Strik and Davis 2023). This sufficiency range was developed for a specific sampling window (from mid-July through mid-August) which corresponds to floral bud initiation in NHB (Lukas et al. 2022). Despite substantial differences in climate, cultivars, and management practices between NHB in the Pacific Northwest and SHB in the southeastern United States, this sufficiency range remains the primary reference for assessing the nutritional status of SHB (Phillips and Williamson 2020).

The sufficiency ranges identified in our study generally deviated from these thresholds, often exhibiting lower and narrower ranges, suggesting that the NHB standards may overestimate N requirements for SHB at certain stages. Additionally, our results suggest that stage-specific sufficiency ranges are necessary as observed variations in sufficiency ranges across stages likely reflect changes in plant growth dynamics and nutrient uptake and translocation patterns. During vegetative growth, nutrient uptake in the roots is the main source of N in the plant. At this time, vegetative meristems are the main sink for N in the plant (Fang et al. 2020b). This stable source-sink dynamic likely contributes to more stable leaf N concentrations, making this stage the most reliable period for leaf sampling and assessment of plant nutritional status. In contrast, when plants transition into reproductive growth, nutrient demands shift and there is increasing sink competition (Birkhold et al. 1992; Lambers et al. 2008), leading to different sufficiency ranges. At bloom, plants exhibit maximum competition between vegetative and reproductive sinks. Therefore, leaf nutrient concentrations can be physiologically unstable (Birkhold and Darnell 1993; Fang et al. 2017). Consequently, tissue diagnostics during these stages may not accurately reflect long-term nutritional status of the plant.

Although fruit development is a phase of high demand of nutrients, evergreen plants exhibited consistently high leaf N concentrations. This could be a result of fertilization practices or reduced vegetative growth at this stage concentrating N in the leaves (Fang et al. 2020a; Phillips et al. 2020). Additionally, evergreen and deciduous plants handle N remobilization differently. Evergreen plants translocate N from old to new leaves throughout the year, whereas deciduous plants lose their leaves at dormancy and must rely on N stored in woody tissues, which is remobilized to newly flushed leaves during the spring (Estiarte and Peñuelas; Reeder et al. 1998; Swain and Darnell 2001). These physiological differences likely contribute to the greater stability of leaf N concentration observed in evergreen compared with deciduous plants.

Model validation using random forest classification. The random forest algorithm used to assess the consistency of the sufficiency range approach derived thresholds achieved an overall classification accuracy of 95.7%. Classification performance for each system-by-stage group is summarized in Table 5, with accuracy ranging from 91.67% to 100% across all groups.

Results from the random forest model supported the validity of the sufficiency range approach for diagnosing plant nutritional status, as reflected in high classification accuracy. This supports the idea that the sufficiency range approach thresholds alone are valid and reliable enough to support nutritional status diagnostic interpretation in SHB. In instances where the model misclassified the data, the predicted categories were typically adjacent to the correct ones (e.g., data labeled as “High” predicted as “Sufficient,” or “Deficient” predicted as “Low”), indicating minor deviations rather than systematic errors. The relative importance of predictors is presented as supplementary material (Supplemental Table 1). Leaf N concentration was the most influential predictor, with 63.2% relative importance. This was expected given that categories were defined based on Leaf N concentration distributions. Other influential predictors had minor effects (<10.5% importance). The ability of the model to correctly classify sufficiency

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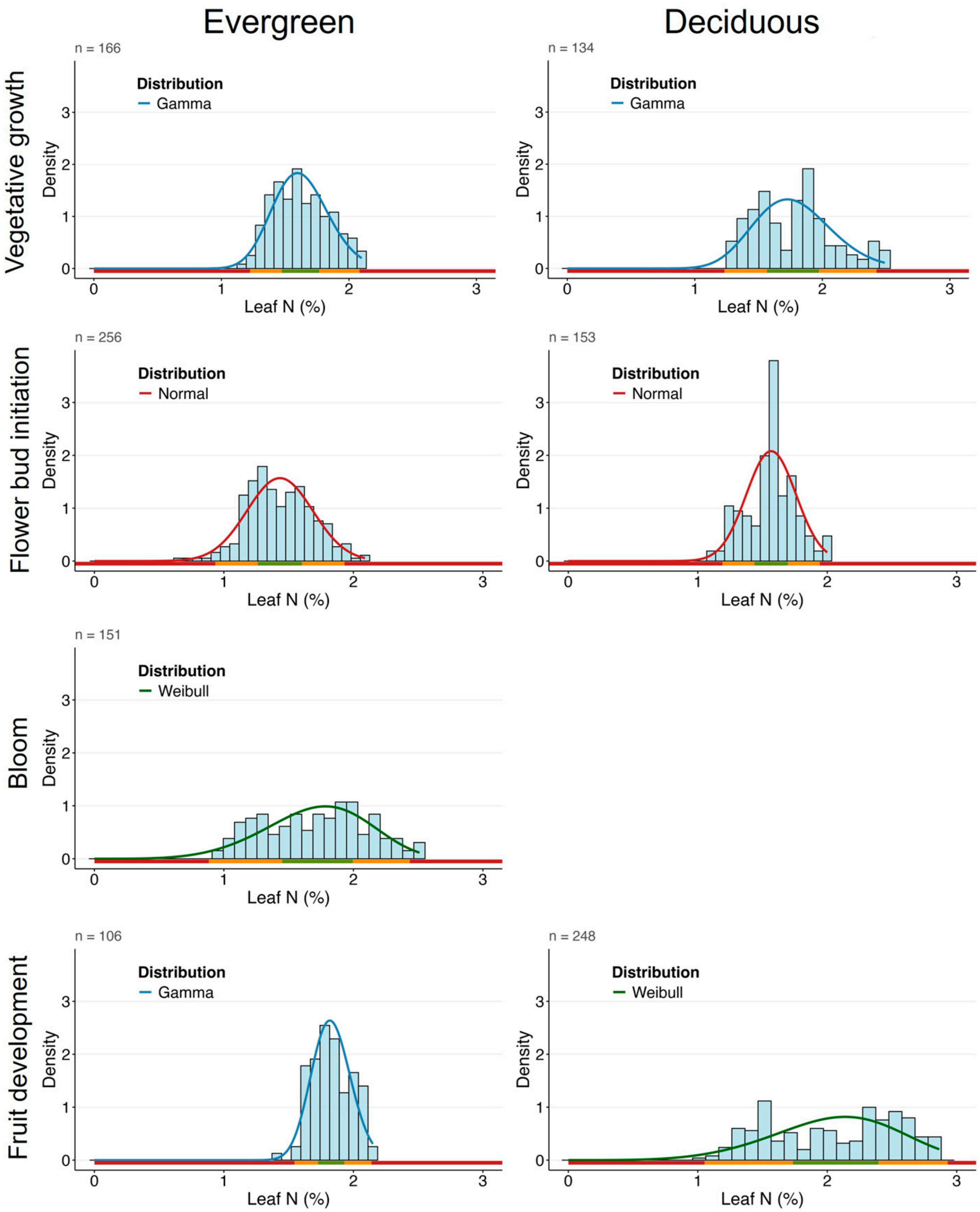

Fig. 1. Distribution of leaf nitrogen (N) concentrations in southern highbush blueberry across system by stage groups. Colored bars beneath each histogram represent interpretation ranges based on the best-fitting distribution of the group, with five transitional points (e.g., Sufficiency (green) = 0.25–0.75 quantile interval; Low/High (yellow) = 0.025–0.25 and 0.75–0.975; Deficient/Excessive (red) = <0.025 and >0.975. Data were collected from southern highbush blueberry farms in Florida between 2021 and 2025.

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Table 4. Leaf nitrogen (Leaf N, %) sufficiency ranges for southern highbush blueberry derived from the best-fitting distribution of each system by stage group. Thresholds define categories of nutritional status (deficient, low, sufficient, high, and excessive) to support diagnostic interpretation. Data were collected from southern highbush blueberry farms in Florida between 2021 and 2025.

		Leaf N (%)[i]				
System	Stage	Deficient	Low	Sufficient	High	Excessive
Evergreen	Vegetative growth	<1.22	1.22–1.47	1.47–1.77	1.77–2.08	>2.08
	Flower bud initiation	<0.93	0.93–1.26	1.26–1.60	1.60–1.93	>1.93
	Bloom	<0.88	0.88–1.45	1.45–1.99	1.99–2.43	>2.43
	Fruit development	<1.54	1.54–1.73	1.73–1.93	1.93–2.14	>2.14
Deciduous	Vegetative growth	<1.16	1.16–1.48	1.48–1.88	1.88–2.33	>2.33
	Flower Initiation	<1.19	1.19–1.44	1.44–1.70	1.70–1.94	>1.94
	Fruit development	<1.05	1.05–1.73	1.73–2.40	2.40–2.93	>2.93

[i] Values on a leaf dry mass basis.

Table 5. Summary of classification performance of nutrient sufficiency categories across each system by stage group for southern highbush blueberry. A random forest classifier using plant, environmental, and management data were used to assess the consistency and reliability of the sufficiency range approach derived categories. Data collected from southern highbush blueberry farms in Florida between 2021 and 2025.

System	Stage	Total data points[i]	Correctly classified	Classification accuracy (%)
Evergreen	Vegetative growth	54	50	92.6
	Flower bud initiation	74	69	93.2
	Bloom	49	48	98.0
	Fruit development	36	33	91.8
Deciduous	Vegetative growth	45	43	95.6
	Flower bud initiation	46	45	97.8
	Fruit development	76	76	100.0

[i] Dataset partitioned (70–30 train-test split) to maintain class balance.

categories using additional plant, management, and environmental variables provides further evidence for the agronomic and physiological relevance of the sufficiency range approach defined categories.

Conclusion

There is a constant need to refine leaf nutrient standards in horticultural crops as new cultivars are adopted and production systems evolve. Previous studies suggest optimal leaf N concentrations in blueberry range between 1.76% and 2.10%. This range was developed primarily for NHB cultivars and is not necessarily applicable to all growing regions. A robust dataset, including data from five locations, nine SHB cultivars, and multiple years, was analyzed in this study. Results from mixed linear methods supported the development of system-by-stage sufficiency ranges. New sufficiency ranges for SHB were defined. System-by-stage sufficiency ranges align with plant physiological patterns and contrast with the existing guidelines. In particular, our results suggest that current standards may be overly broad, potentially limiting their sensitivity to detect meaningful differences during critical windows for plant nutrient assessment. System-by-stage sufficiency ranges were validated through a random forest model using plant, environmental, and management data. New, crop-specific sufficiency ranges could lead to more precise management, improving fertilizer-use efficiency and environmental sustainability in SHB production. Future research should focus on linking leaf N concentrations to yield to further optimize nutrient management in SHB farms.

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