

Defining Leaf Tissue Nutrient Standards and Nutrient Disorder Symptomology of Greenhouse-grown Spearmint (*Mentha spicata* L.)

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Keywords. deficiency, hydroponics, macronutrients, micronutrients, tissue analysis

Abstract. Spearmint (*Mentha spicata* L.) is a perennial herb popular for its distinct flavor and aroma. While traditionally grown outdoors as a field crop, controlled environment agriculture can provide an alternative method for producing spearmint. The objective of this study was to define foliar mineral nutrient interpretation ranges specific to greenhouse-grown spearmint by inducing and documenting individual nutrient disorders. Plants were cultivated in a hydroponic sand culture system and supplied with a modified Hoagland's solution, in which individual nutrients were either omitted or supplemented, while maintaining all others at consistent levels. As nutrient disorders became evident, symptoms were photographed and documented. Plant tissue was collected to measure plant dry mass and nutrient concentrations. Additional foliar tissue data were compiled with those from the present study to create a larger data set containing 315 samples. Data were analyzed to define interpretation ranges for 12 essential nutrients using a hybrid meta-analysis sufficiency range approach, which defined ranges for deficient, low, sufficient, high, and excessive tissue concentrations for each nutrient. The most representative distribution for each nutrient was selected based on the lowest Bayesian Information Criterion value. Potassium, calcium, sulfur, boron, and copper were best modeled using a normal distribution, while magnesium was best represented by a gamma distribution. A Weibull distribution provided the best fit for nitrogen, phosphorus, iron, manganese, molybdenum, and zinc. These findings provide previously unavailable resources for diagnosing nutrient disorders and evaluating foliar nutrient analysis of greenhouse-grown spearmint.

Spearmint (*Mentha spicata* L.) is a popular perennial herb with a large variety of uses due to its distinct flavor and aroma (Nau et al. 2021). There are a variety of mint (*Mentha*) species with varying ornamental uses and flavor profiles, although spearmint is one of the most common commercially cultivated species (Dole and Wilkins 2005). While spearmint is traditionally cultivated outdoors as a field crop for its essential oil, hydroponic culture may be a more water-efficient method for culinary production (Nicola et al. 2020). Spearmint has a reportedly high water requirement

(Patra and Kumar 2006), which can be more efficiently met in a controlled growing environment. Hydroponic production of spearmint is not only more water efficient, but it also has the potential to yield greater biomass and plant extracts (Surendran et al. 2017). As with conventional soil cultivation, proper nutrient management of spearmint is important to balance plant biomass and essential oil production (Brown et al. 2003).

Limited information exists for greenhouse-grown spearmint. Chrysargyris et al. (2017) evaluated N fertilizer concentrations on spearmint

growth and essential oil content and concluded that 200 mg·L⁻¹ N was sufficient for hydroponic spearmint production. An additional study recommended a P fertilizer concentration of 70 mg·L⁻¹ P, especially for essential oil production (Chrysargyris et al. 2019). While these studies provide fertilizer concentrations recommendations, they do not include corresponding tissue nutrient concentrations. Bryson and Mills (2015) reported survey ranges for field-grown spearmint based on leaf tissue samples from healthy plants, although limited scientific research exists to support these values.

Research on nutrient deficiencies in mint have focused on other species, including field-grown peppermint (*Mentha ×piperita* L.) (Arrobas et al. 2018) and greenhouse-grown Japanese mint (*Mentha arvensis*) (Janpen et al. 2019). Arrobas et al. (2018) established tissue sufficiency ranges for field and container-grown peppermint by applying varying rates of N, P, K, and B. For Japanese mint, Reuter et al. (1997) compiled adequate tissue P concentrations, as well as deficient Zn and Fe concentration based on field experiments. Additionally, Janpen et al. (2019) established that when a single nutrient was excluded, especially a macronutrient, Japanese mint plants displayed visual deficiency symptoms and less biomass. Visual symptoms were documented, but no tissue sufficiency ranges were determined. While these studies provided some general guidelines, determining comprehensive nutrient interpretation ranges specific to greenhouse-grown spearmint will provide a more accurate baseline for growers.

Spearmint lacks comprehensive leaf tissue interpretation ranges. If nutrient problems occur, the use of leaf tissue analysis aids in the diagnosis of nutrient problems (Bryson and Mills 2015). Tissue nutrient interpretation ranges have been defined for lettuce (*Lactuca sativa* L.) (Veazie et al. 2024a) and pentas (*Pentas lanceolata* Forssk.) (Veazie et al. 2024b) using a hybrid meta-analysis sufficiency range approach (SRA) (Soltanpour et al. 1995). Most leaf tissue nutrient concentration distributions tend to be skewed and normal distribution curves are less suitable for developing interpretations. Two models that account for possible skewness are the gamma and Weibull distribution curves (Cera et al. 2022; Mhango et al. 2021; Slaton et al. 2021; Weibull 1951). Evaluating multiple models allows for more accurate fitting of the data. The objectives of this study were to (1) document nutrient disorder symptomology and (2) refine leaf tissue nutrient interpretation ranges by applying this expanded SRA method to foliar tissue data from greenhouse-grown spearmint.

Materials and Methods

Plant material. Rooted spearmint plugs (Lucas Greenhouses, Monroeville, NJ, USA) were obtained on 22 May 2024. Plugs were rooted in 102-count split plug trays, where each hexagonal cell measured 4.2 cm tall × 2.9 cm wide, tapering to 1.9 cm wide at the base. Each

plug tray was placed in a plastic tray and grown indoors under fluorescent lights (Agrobrite T5 Full Spectrum; Hydrofarm, Petaluma, CA, USA), which provided $17.28 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ based on a 24-h photoperiod, and subirrigated with tap water as needed for 6 d. The plants were irrigated with deionized (DI) water to leach out potential nutrients contained in the substrate.

Expt. 1. The plugs were transplanted on 28 May 2024 into 11.5-cm-diameter (0.8 L) plastic pots containing silica-sand [Millersville #2 (0.8 to 1.2 mm diameter); Southern Products and Silica Co., Hoffman, NC, USA] and grown in a glass greenhouse in Raleigh, NC (35°N latitude), under ambient light with air temperature setpoints of 22.8°C (day) and 22.0°C (night). Fertility treatments began immediately after transplant and were applied via an automated recirculating irrigation system constructed out of a 10.2-cm-diameter polyvinylchloride pipe (Charlotte Plastics, Charlotte, NC, USA). Fertilizer solutions consisted of a modified Hoagland's solution (Hoagland and Arnon 1950) formulated with DI water and custom blends of the following individual technical grade salts (Fisher Scientific, Pittsburgh, PA, USA): calcium nitrate tetrahydrate $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$, potassium nitrate (KNO_3), monopotassium phosphate (KH_2PO_4), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), potassium chloride (KCl), calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), sodium nitrate (NaNO_3), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), sodium phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), sodium sulfate (Na_2SO_4), iron chelated with diethylenetriaminepentaacetic acid (Fe-DTPA), manganese chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), zinc chloride heptahydrate ($\text{ZnCl}_2 \cdot 7\text{H}_2\text{O}$), copper chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), boric acid (H_3BO_3), and sodium molybdate dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$). Additional details regarding fertilizer treatments, formulations, fertilizer salts, and the irrigation system are described by Barnes et al. (2012) and Veazie et al. (2022).

Expt. 1 data collection. The plants were photographed and sampled as visual deficiency or toxicity symptoms were observed, with six representative plants ($n = 6$) from each fertilizer treatment selected for sampling. Any remaining plants after sampling were retained, and advanced symptoms, if present, were also photographed and documented.

Received for publication 12 May 2025. Accepted for publication 7 Jul 2025.

Published online 8 Sep 2025.

This work was supported in part by the Specialty Crop Research Initiative, Project Award 2022-51181-38331 from the National Institute of Food and Agriculture of the US Department of Agriculture (USDA). We thank Douglas Sturtz and Mona-Lisa Banks at the USDA Agricultural Research Service for technical assistance and elemental analysis of the USDA Agricultural Research Service spearmint leaf tissue samples.

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Table 1. Sources of greenhouse-grown spearmint (*Mentha spicata* L.) leaf tissue nutrient data ($n = 315$) used to develop tissue nutrient interpretation ranges.

Source	Sample size	Sample type	Notes
North Carolina State University	165	Research	Present nutrient deficiency study
North Carolina State University	150	Research	Unpublished electrical conductivity rate study

For each plant, the most recently matured leaves were sampled for tissue analysis as recommended by Bryson and Mills (2015), and the remaining leaves and shoots were also collected to evaluate the total shoot dry mass of each sample. At 16 d after transplant (13 Jun 2024), plants in the N-, P-, K-, and S-deficient treatments and the B toxicity treatment were sampled. The Ca-, Fe-, and B-deficient treatments were sampled 21 d after transplant (18 Jun 2024), and the Mg- and Zn-deficient treatments were sampled 28 d after transplant (25 Jun 2024). All remaining deficiency treatments (Mn, Cu, and Mo) were asymptomatic and sampled upon experiment termination, 36 d after transplant (3 Jul 2024).

The most recently matured leaves were rinsed in DI water, washed in a 0.5 M HCl solution, and rinsed in DI water a second time. Immediately after sampling and washing, the tissue was dried at 70°C for 48 h in a forced-air oven, after which the dry mass for each sample was weighed and recorded. The total dry mass for each sample was calculated by adding the dry mass of the recently matured leaves to the remainder of the shoot dry mass. After drying, the leaf tissue was ground in a grinding mill (Foss Tecator Cyclotec™ 1093; Analytical Instruments, LLC; Golden Valley, MN, USA; ≤ 0.5 mm sieve). The ground tissue was placed in vials and shipped to the US Department of Agriculture, Agricultural Research Service Application Technology Research Unit (Toledo, OH, USA) for analysis as outlined by Boldt and Altland (2021). To determine foliar N concentration, ~ 2.5 mg of plant material was put into tin capsules (EA Consumables, Marlton, NJ, USA) and analyzed with a CHN analyzer (vario MICRO cube; Elementar, Hanau, Germany). To determine all other nutrient concentrations, ~ 0.25 g of plant material was combined with 5 mL of nitric acid, heated to 200°C over 20 min in a programmable microwave (MARS6; CEM Corp., Matthews, NC, USA), and held at 200°C for an additional 20 min. Once cooled, 1.5 mL of hydrogen peroxide was added, and the samples were reheated to 200°C and held for another 20 min. Once cooled a final time, 12 mL of ultra-purified water (18 M Ω -cm) was added, and the solutions were filtered (Whatman #2). Finally, a 1.3 mL aliquot of solution was diluted with 8.7 mL of 18 M Ω -cm water and analyzed using inductively coupled plasma-optical emission spectroscopy (iCAP 6300 Duo; Thermo Electron Corp., Waltham, MA, USA).

Expt. 1 data analysis. Plant dry mass and leaf nutrient concentration data were subjected to analysis of variance (ANOVA) using the

PROC ANOVA procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Since only two treatments were compared, this is statistically equivalent to a *t* test.

Expt. 2. After Expt. 1, a second aspect of the project was conducted to establish tissue nutrient interpretation ranges for each element. Due to larger sample sizes better representing the population and providing more accurate results, a more robust data set was needed. Additionally, incorporating data from varying sources reduces the bias that can occur when establishing a distribution based on data from a single research study. As a result, the tissue data obtained from Expt. 1 was compiled with data from an unpublished North Carolina State University study to establish a more robust data set with a total of 315 samples (Table 1). Excessive outliers with values greater than what is biologically feasible were removed before analysis. Each element was modeled independently using RStudio (version 2024.04.2; RStudio Team) by fitting normal, gamma, and Weibull distributions to the data. *P* values for the normal and gamma distributions were calculated based on the Shapiro-Wilk test for normality, and the *P* value for the Weibull distribution was calculated based on the Kolmogorov-Smirnov test. The Bayesian Information Criterion (BIC) was calculated for each distribution, and the optimal distribution for each element was selected based on the lowest BIC value (Table 2). The results were illustrated using ggplot2 (Wickham 2011) in RStudio. The Freedman-Diaconis rule (Freedman and Diaconis 1981) was used to determine bin width for data visualization.

Table 2. Bayesian Information Criterion (BIC) values for normal, gamma, and Weibull distribution models used to develop distribution models for each of the 12 essential elements in greenhouse-grown spearmint (*Mentha spicata* L.) using an expanded sufficiency range approach.

Element	BIC value		
	Normal	Gamma	Weibull
N	970.65	1062.84	945.18
P	100.35	103.78	90.67
K	910.65	1081.06	937.13
Ca	162.60	305.63	187.10
Mg	43.88	-79.76	-30.63
S	-462.62	-380.34	-459.16
B	2600.88	2749.26	2638.31
Cu	1719.74	1823.68	1786.00
Fe	2818.55	2845.82	1866.79
Mn	3336.31	3378.82	3335.20
Mo	1317.23	533.45	519.69
Zn	2384.83	2385.82	2377.85

The lowest BIC value is in bold type, indicating the selected model.

Table 3. Greenhouse-grown spearmint (*Mentha spicata* L.) plant dry mass as affected by nutrient deficiency or toxicity.

Treatment	Dry mass (g)												
	–N	–P	–K	–Ca	–Mg	–S	–B	+B	–Cu	–Fe	–Mn	–Mo	–Zn
Complete control ⁱ	2.06	2.06	2.06	4.96	10.53	2.06	4.96	2.06	23.01	4.96	23.00	23.01	10.53
Disorder	0.45	0.46	0.92	3.68	11.34	1.24	3.63	1.70	17.63	4.11	22.12	21.93	5.33
<i>P</i> value ⁱⁱ	***	***	***	*	NS	**	*	NS	NS	NS	NS	NS	***

ⁱ A separate set of control plants was sampled on the same dates as the corresponding nutrient treatments. Control plants for –N, –P, –K, and –S were sampled at 16 d; those for –Ca, –Fe, –B, and +B were sampled at 21 d; for –Mg and –Zn 28 d; and those for –Mn, –Cu, and –Mo were sampled at 36 d.

ⁱⁱ *, **, or *** denotes statistically significant differences between the sample means based on *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS indicates that the *F* test differences between sample means was not significant ($P > 0.05$).

Table 4. Greenhouse-grown spearmint (*Mentha spicata* L.) plant tissue nutrient concentration as affected by nutrient deficiency or toxicity.

Treatment	Tissue nutrient concentration													
	(%)						(mg·kg ^{–1})							
	N	P	K	Ca	Mg	S	–B	+B	Cu	Fe	Mn	Mo	Zn	
Complete control	5.05	0.74	4.50	1.56	0.40	0.35	62.4	64.5	3.7	95.5	51.1	1.0	25.5	
Disorder	1.12	0.07	0.51	0.20	0.07	0.09	21.1	319.5	<0.01	83.3	16.3	0.1	15.0	
<i>P</i> value ⁱ	***	***	***	***	***	***	***	***	***	*	***	***	***	***
Survey range ⁱⁱ	3.25–4.60	0.20–0.40	2.25–3.80	0.50–1.25	0.35–0.88	0.18–0.35	18–30	18–30	5–15	60–250	50–300	0.5–1.0	25–100	

ⁱ *, **, or *** denotes statistically significant differences between the sample means based on *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS indicates that the *F* test differences between sample means was not significant ($P > 0.05$).

ⁱⁱ Bryson and Mills (2015).

The sufficiency range for each macronutrient (N, P, K, Ca, Mg, and S) was defined as the range between the 0.25 and 0.75 quantiles within a 95% confidence interval. Below the sufficient range, the deficient range comprised values within the left tail (the lowest 2.5% of samples), and the low range was defined as the area between the 0.025 and the 0.25 quantiles. Above the sufficient range, the high range was defined as the area between the 0.75 and 0.975 quantiles, and the excessive range comprised values within the right tail (the highest 2.5% of samples). For micronutrients (B, Cu, Fe, Mn, Mo, and Zn), the cutoff between the deficient and low ranges was defined by the lowest 5% of samples of a 90% confidence interval (left tail). Likewise, the cutoff between the high and excessive ranges was defined by the top 5% of observations within a 90% confidence interval (right tail).

Results and Discussion

Nitrogen. Nitrogen deficiency was one of the first nutrient disorders observed, with symptoms evident within 2 weeks of transplant. The primary symptoms of N deficiency were stunted growth (Tables 3 and 4). Additionally, the lower leaves developed interveinal chlorosis, while the new growth exhibited an overall light green coloration (Fig. 1A). These symptoms, particularly stunted growth and light green foliage, are consistent with the symptoms of N deficiency reported in Japanese mint (Janpen et al. 2019). At the time of sampling, N-deficient plants had 78% less dry mass and had an N concentration 78% lower than the control plants (Tables 3 and 4). The N concentration of the N-deficient plants expectedly fell below the current recommended N leaf tissue concentration of 3.25%

to 4.60% N for field-grown spearmint (Bryson and Mills 2015), while the N concentration of the control plants was above the upper limit of the range.

Of the three evaluated models, the Weibull model best represented spearmint N foliar concentrations (Fig. 2A; Table 2). The model established a sufficiency range of 3.93%

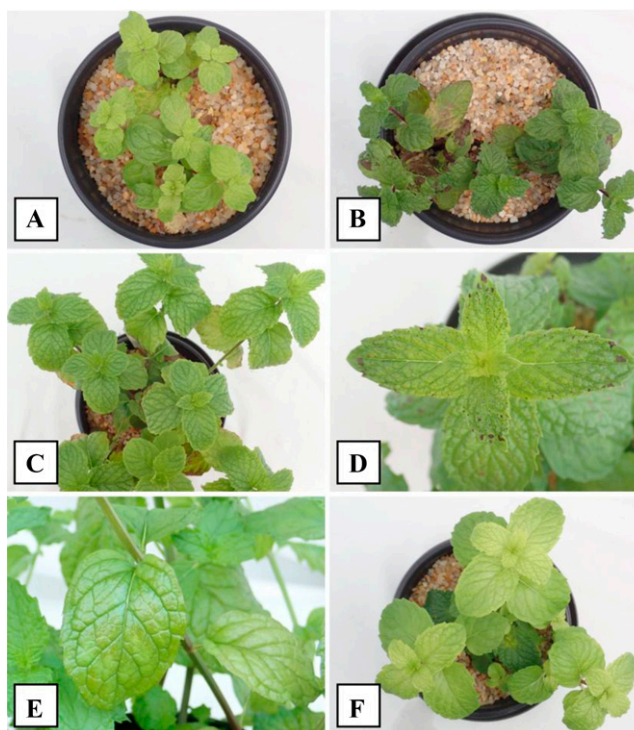
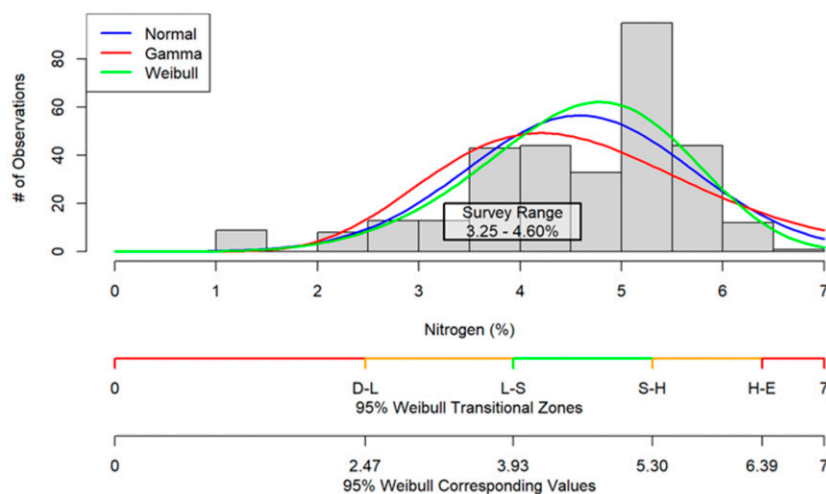


Fig. 1. Visual macronutrient deficiency symptoms of greenhouse-grown spearmint (*Mentha spicata* L.). (A) Nitrogen-deficient plants experienced minimal to no growth and developed a light green coloration that enveloped the entire plant. (B) Phosphorus-deficient plants experienced stunted growth, and the oldest foliage developed interveinal chlorosis, purpling, and necrotic spotting. (C) Potassium deficiency caused interveinal and marginal chlorosis, curling of the middle to upper foliage, and discoloration of the oldest foliage that progressed into necrosis. (D) Calcium-deficient plants developed interveinal chlorosis and necrotic speckling of the upper and middle leaves. The newest leaves also remained small, not expanding or increasing in size with maturity. (E) Magnesium deficiency occurred in the lower to middle foliage, which exhibited chlorosis and a glossy appearance. (F) Sulfur-deficient plants exhibited chlorosis of the newest growth, while the lower leaves remained green.

A Foliar Nitrogen Concentration in Spearmint: Normal, Gamma, and Weibull Distributions



B Foliar Phosphorus Concentration in Spearmint: Normal, Gamma, and Weibull Distributions

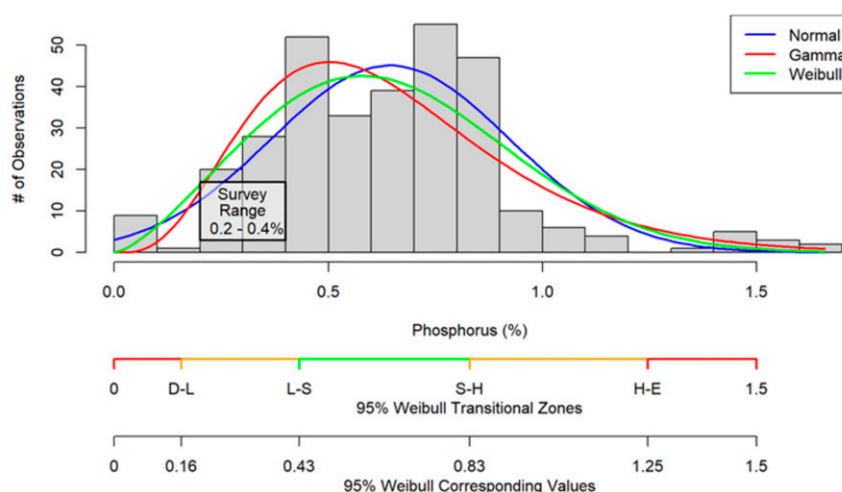


Fig. 2. Nitrogen (A) and phosphorus (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 2.5%, 25%, 75%, and 97.5% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlayed for reference.

to 5.30% N (Table 5), which shifts the recommended survey range for field-grown spearmint higher than the range for field-grown spearmint (Bryson and Mills 2015). The N concentration of the control plants, 5.05% N, fell within this new sufficiency range and

corroborated the shift upwards (Tables 3 and 4). This higher sufficiency range may reflect the larger sample size used in this study compared with the smaller sample number used by Bryson and Mills (2015) in their survey. The deficient range, <2.47% N, included the N concentration of the N-deficient plants. Nitrogen concentrations >6.39% N were classified as excessive. While N toxicity is unlikely, higher N tissue concentrations due to excessive N application will result in luxury consumption, where the plant takes up additional N without a positive effect on growth (Marschner 1995).

Phosphorus. Phosphorus deficiency, like N deficiency, also halted spearmint growth almost entirely. In addition to stunted growth, P-deficient plants exhibited faint interveinal chlorosis, purpling, and necrotic spotting of the lower leaves after 2 weeks of growth (Fig. 1B). The stems of the plants developed a deeper purple coloration than the control plants, and the oldest foliage also developed faint purple veins. As deficiency symptoms advanced, the oldest foliage became entirely necrotic and abscised. Purple coloration due to P deficiency has also been reported in Japanese mint (Janpen et al. 2019). Compared with the control plants, P-deficient plants had 78% less total dry mass and a 91% lower P tissue concentration (Tables 3 and 4). The P concentration of the deficient plants was lower than the current recommended survey range of 0.20% to 0.40% P (Bryson and Mills 2015). The control plants had a P concentration of 0.74%, which was higher than this range.

A Weibull distribution best represented the P foliar data (Fig. 2B; Table 2) and established a sufficiency range between 0.43% and 0.83% P (Table 5). This shifts the entire range of sufficiency values higher than the upper threshold of the range recommended by Bryson and Mills (2015) for field-grown crops. Due to readily available nutrients in controlled environment systems because of the predominant application of water-soluble fertilizers, greenhouse-grown plants often experience increased nutrient uptake and higher tissue concentrations compared with their field-grown counterparts (Sonneveld and Voogt 2009). The P concentration of the control plants in this study, 0.74% P, fell within the updated range. The established deficient range, <0.16% P, was corroborated by the P concentration of plants from the P deficiency treatment, which had a concentration of 0.07% P. The excessive range was established as concentrations >1.25% P.

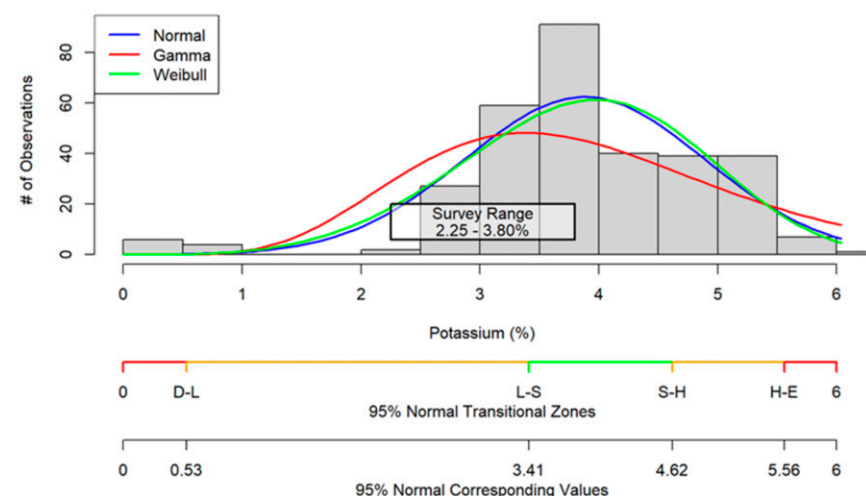
Potassium. The first symptom of K deficiency, observed 2 weeks after transplant, was brown spotting of the lower leaves and interveinal and marginal chlorosis of the upper foliage (Fig. 1C). The upper and middle foliage also exhibited downward curling. As deficiency symptoms progressed, severe chlorosis of the oldest leaves and necrotic spotting in both the upper and lower leaves was observed, which is similar to the symptoms of K deficiency reported in Japanese mint (Janpen et al. 2019). At the time of sampling, K-deficient plants had 55% less dry mass than the control plants and had a K concentration of 0.51%,

Table 5. Greenhouse-grown spearmint (*Mentha spicata* L.) nutrient distribution interpretation ranges defined from the distribution models, denoting deficient, low, sufficient, high, and excessive foliar elemental concentrations based on the sufficiency range approach using 315 samples.

Element	Unit	Nutrient ranges				
		Deficient	Low	Sufficient	High	Excessive
N	%	<2.47	2.47–3.93	3.93–5.30	5.30–6.39	>6.39
P	%	<0.16	0.16–0.43	0.43–0.83	0.83–1.25	>1.25
K	%	<0.53	0.53–3.41	3.41–4.62	4.62–5.56	>5.56
Ca	%	<0.23	0.23–1.07	1.07–1.43	1.43–1.88	>1.88
Mg	%	<0.14	0.14–0.31	0.31–0.60	0.60–1.01	>1.01
S	%	<0.10	0.10–0.32	0.32–0.48	0.48–0.59	>0.59
B	mg·kg ⁻¹	<46.7	46.7–53.8	53.8–75.0	75.0–93.8	>93.8
Cu	mg·kg ⁻¹	<0.2	0.2–4.1	4.1–9.4	9.4–13.0	>13.0
Fe	mg·kg ⁻¹	<49.7	49.7–72.2	72.2–101.4	101.4–119.9	>119.9
Mn	mg·kg ⁻¹	<34.0	34.0–70.0	70.0–135.4	135.4–187.0	>187.0
Mo	mg·kg ⁻¹	– ⁱ	<0.1	0.1–1.2	1.2–3.6	>3.6
Zn	mg·kg ⁻¹	<13.3	13.3–22.9	22.9–37.6	37.6–47.9	>47.9

ⁱConcentration was too low to report.

A Foliar Potassium Concentration in Spearmint: Normal, Gamma, and Weibull Distributions



B Foliar Calcium Concentration in Spearmint: Normal, Gamma, and Weibull Distributions

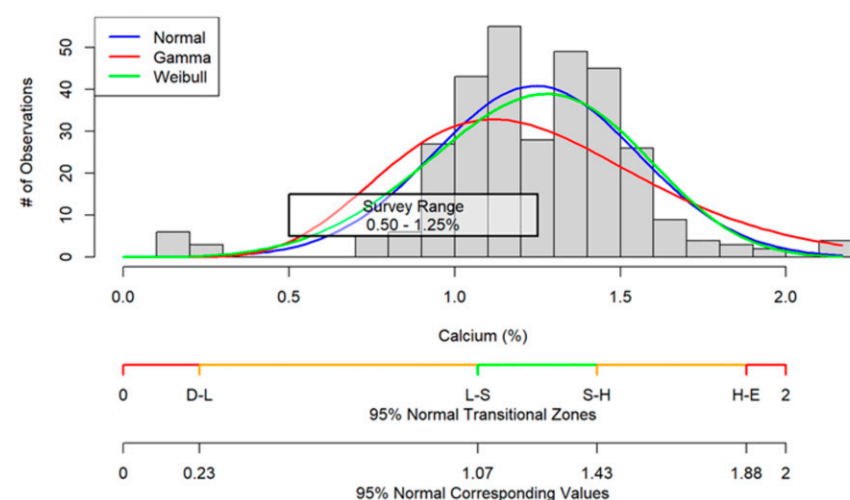


Fig. 3. Potassium (A) and calcium (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 2.5%, 25%, 75%, and 97.5% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlayed for reference.

which was 89% lower than the control plants (Tables 3 and 4). While K-deficient plants had a K concentration that expectedly fell below the range suggested by Bryson and Mills (2015), the control plants had a K concentration of 4.50% K, exceeding the recommended upper limit of 3.80%.

Of the three models evaluated, the normal distribution best fit the K foliar data (Fig. 3A; Table 2). The updated sufficiency range was defined as 3.41% to 4.62% K (Table 5), which shifts upward the recommended range of 2.25% to 3.80% K for field-grown spearmint (Bryson and Mills 2015). The control plants, which had a K concentration of 4.50% (Table 4), support the updated range. The deficiency range, $<0.53\%$ K, was also corroborated, given the K concentration of symptomatic plants that exhibited symptoms of K deficiency

were below this threshold. The boundary between the high and excessive ranges was 5.56% K. While K toxicity may not occur at high K concentrations, high K concentrations in a fertilizer solution can lead to an antagonistic effect on Mg and Ca uptake by plants and subsequent deficiency symptoms despite adequate Mg and Ca fertilization (Bryson and Mills 2015).

Calcium. Three weeks after transplant, Ca-deficient plants were visibly shorter than the control plants, and the middle and upper foliage developed interveinal chlorosis and small necrotic speckling (Fig. 1D). The newest leaves on these plants were also much smaller than the newest growth of the control plants, and they did not expand or increase in size with maturity. As symptoms advanced, the upper foliage became deeply chlorotic, and the margins of the newest growth curled

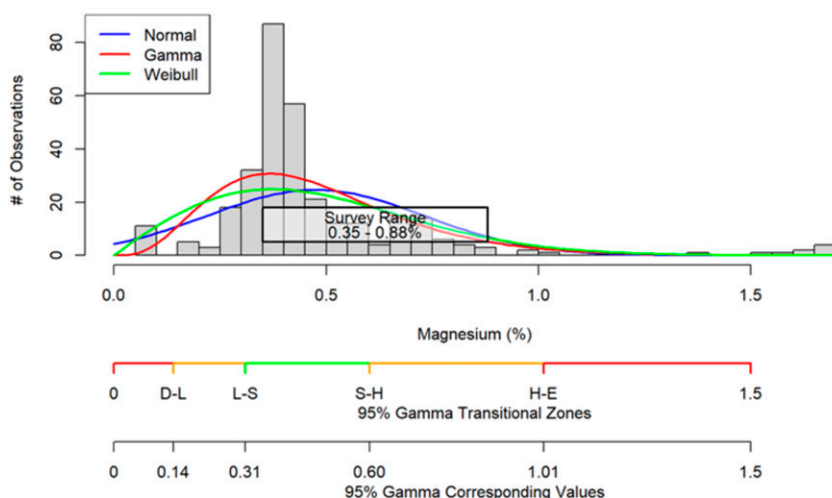
inward. Eventually, the upper stems and growing tips became entirely necrotic. While Ca-deficient plants only had 26% less dry mass than the control plants, they had an 87% lower Ca tissue concentration. The Ca concentration of deficient plants fell below the recommended range of 0.50 to 1.25% Ca suggested by Bryson and Mills (2015). The control plants, with a Ca concentration of 1.56%, were slightly above this range.

While the Ca foliar data were best represented by a normal distribution (Fig. 3; Table 2), the data followed a bimodal trend that is not characteristic of a normal distribution. This bimodal trend was due to a low number of samples between 1.20% and 1.30% Ca. This difference may be a result of the overall small sample size, as larger sample sizes tend to have less variability. According to the normal distribution, the sufficiency range for Ca was between 1.07% and 1.43% Ca (Table 5), which shifts the previously recommended range. This upward shift is consistent with the sufficiency range of 0.7% to 2.3% Ca recommended for peppermint (Arrobas et al. 2018). The Ca concentration of the control plants, 1.56% Ca (Table 4), fell above the newly defined sufficiency range. This suggests that the fertilizer solution, which provided $200 \text{ mg} \cdot \text{L}^{-1}$ Ca, may have supplied a higher Ca concentration than necessary for greenhouse-grown spearmint. The established deficiency range was $<0.23\%$ Ca, which was supported by the Ca concentration of the Ca-deficient plants. The established excessive range was $>1.88\%$ Ca. Like K, excess Ca fertilizer can induce Mg deficiency by inhibiting Mg uptake by the roots (Marschner 1995).

Magnesium. The initial symptoms of Mg deficiency, observed 4 weeks after transplant, were chlorosis of the middle and lower foliage, which also developed a glossy appearance (Fig. 1E). As symptoms progressed, chlorosis of the lower leaves intensified, and small necrotic patches began to develop. Chlorosis also spread to the upper foliage, and leaves began to curl downward. While there was no significant difference ($P > 0.05$) between the dry mass of the Mg-deficient and control plants, the Mg-deficient plants had an 83% lower Mg tissue concentration. The Mg concentration of the Mg-deficient plants fell far below the recommended survey range, which suggests 0.35% to 0.88% Mg (Bryson and Mills 2015). The control plants had a Mg concentration of 0.40%, which fell within this range.

A gamma distribution was the best fit for the Mg foliar data of the three models evaluated (Fig. 4A; Table 2). The established sufficiency range, 0.31% to 0.60% Mg (Table 5), narrowed the range previously recommended by Bryson and Mills (2015), while still including the Mg concentration of the control plants. The deficiency range, $<0.14\%$ Mg, was also supported by the Mg concentration of the Mg-deficient plants, which had a concentration of 0.07% Mg (Table 4). The excessive range for Mg tissue concentrations in spearmint was $>1.01\%$ Mg.

A Foliar Magnesium Concentration in Spearmint: Normal, Gamma, and Weibull Distributions



B Foliar Sulfur Concentration in Spearmint: Normal, Gamma, and Weibull Distributions

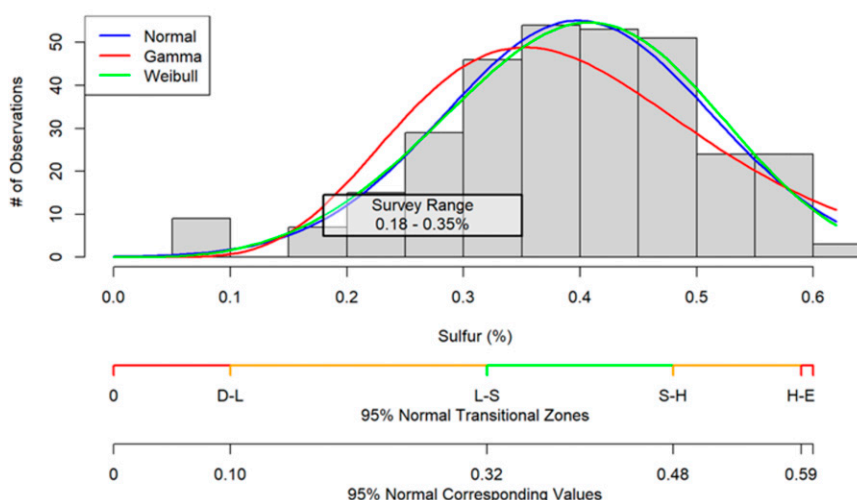


Fig. 4. Magnesium (A) and sulfur (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 2.5%, 25%, 75%, and 97.5% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlayed for reference.

Sulfur. Symptoms of S deficiency presented 2 weeks after transplant. The newest leaves of S-deficient plants were a light yellow-green color, while the lower leaves retained a healthy green coloration (Fig. 1F). In the advanced stages, the newest growth became progressively more chlorotic, eventually becoming entirely yellow. Growth was also considerably stunted, with S-deficient plants having 40% less dry mass than the control plants. The S-deficient plants had an S concentration 74% lower than the control and the mean value of 0.09% S fell below the recommended survey range, which suggests 0.18% to 0.35% S (Bryson and Mills 2015). The control plants had an S concentration of 0.35%, which fell at the upper limit of the suggested range.

A normal model best represented the S foliar data for spearmint (Fig. 4B; Table 2). The updated sufficiency range was 0.32% to 0.48% S (Table 5), which is an upward shift from the previously recommended range but still includes the S concentration of the control plants (Table 4). Concentrations <0.10% S fell within the deficiency range, and the excessive range was established as concentrations >0.59% S. While high S concentrations alone are not known to suppress plant growth, high S fertilizer applications can reduce B and Mo uptake and foliar concentrations (Bryson and Mills 2015).

Boron. Initial signs of B deficiency were prominent venation of the newest growth and thick, brittle foliage (Fig. 5A), which was evident 3 weeks after transplant. As deficiency

symptoms worsened, the upper foliage became increasingly thickened, developed a downward orientation, and the leaves curled inward at the tip. The newest growth also became chlorotic around the leaf margins. Boron-deficient plants had 27% less dry mass than the control plants and had a 66% lower B tissue concentration (Tables 3 and 4). Despite clear deficiency symptoms, the B concentration of the B-deficient plants fell within the current recommended sufficiency range of 18 to 30 mg·kg⁻¹ (Bryson and Mills 2015), while the concentration of the control plants was much higher than the upper limit of the range.

Boron toxicity was also observed 3 weeks after transplant. The primary symptom of B toxicity was chlorosis of the leaf margins, while the interior of the leaf remained green (Fig. 5B). While symptoms were most prominent on the oldest leaves, symptoms were also seen on the middle and upper foliage. The marginal chlorosis quickly became necrotic, and the leaf margins began curling upward. There was no significant difference ($P > 0.05$) in dry mass between treatments; however, the B tissue concentration of plants from the B toxicity treatment was five times the B concentration of the control plants.

Due to the exceptionally high B concentration of plants from the B toxicity treatment, these samples were excluded from the B distribution analyses to prevent a highly right skewed distribution, which would not accurately represent typical B foliar concentrations. A normal distribution had the lowest BIC value (Table 2) and best fit the B foliar data (Fig. 6A). The established sufficiency range was between 53.8 and 75.0 mg·kg⁻¹ B (Table 5), entirely shifting upward the previously recommended range, which suggested B concentrations between 18 and 30 mg·kg⁻¹ (Bryson and Mills 2015). The deficiency threshold was defined as 46.7 mg·kg⁻¹ B, which would have previously been in the sufficiency range. While this is a drastic shift upwards in recommended B tissue concentrations, the B foliar concentrations of both the control plants and B-deficient plants fall within their newly defined respective ranges, which corroborates the updated recommendations. Additionally, the previously recommended range would have determined the B concentration of the B-deficient plants as sufficient, despite plants exhibiting clear deficiency symptoms. Lastly, the threshold for the excessive range was established as >93.8 mg·kg⁻¹ B, which includes the plants that exhibited B toxicity. Boron concentrations within this range are likely to cause B toxicity and unmarketable plants.

Copper. Upon completion of the experiment, the only observed symptom of Cu deficiency was faint chlorosis of the newest growth, especially along the leaf margins (Fig. 5C). While no significant difference existed between the dry mass of the control and Cu-deficient plants ($P > 0.05$), the Cu tissue concentration of the Cu-deficient plants was below the detection threshold (<0.01 mg·kg⁻¹ Cu) (Tables 3 and 4). The Cu tissue concentration of the

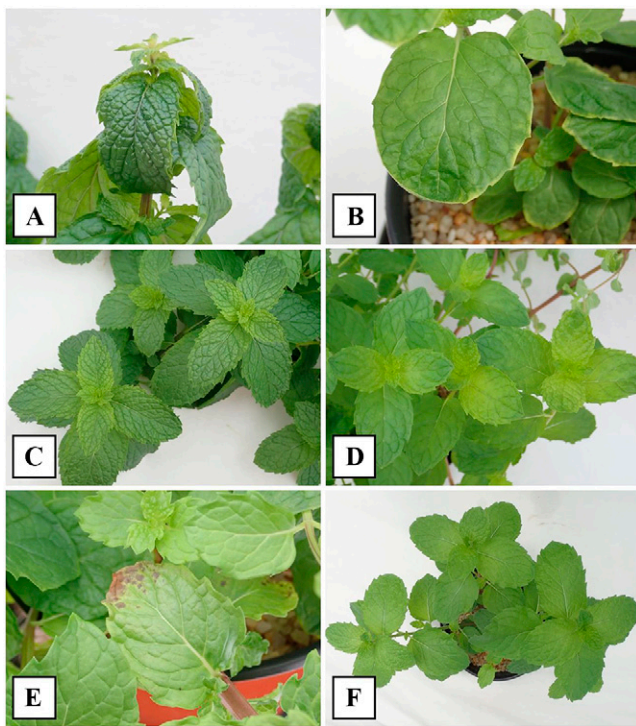


Fig. 5. Visual symptoms of micronutrient disorders greenhouse-grown spearmint (*Mentha spicata* L.). (A) Boron-deficient plants exhibited prominent venation, thickened tissue, and downward curling of newer leaves. (B) Boron toxicity caused chlorosis and curling of leaf margins in both the lower and middle foliage, which eventually became necrotic. (C) Copper deficiency caused faint chlorosis of the newest growth, particularly around the leaf margins. (D) Iron-deficient plants developed interveinal chlorosis of the upper leaves. (E) Zinc-deficient plants experienced stunted growth and purple spotting on the lower to middle foliage, which quickly became necrotic as the deficiency worsened. (F) Control plants received sufficient concentrations of all nutrients and exhibited visually healthy growth.

control plants fell below the sufficiency range of 5 to 15 mg·kg⁻¹ suggested by Bryson and Mills (2015), although no deficiency symptoms were observed.

For analysis purposes, Cu concentrations below the detection limit were assigned a concentration of 0.01 mg·kg⁻¹ Cu. A normal distribution best represented the Cu foliar data (Fig. 6B; Table 2). The established sufficiency range, 4.1 to 9.4 mg·kg⁻¹ Cu, narrows the previously recommended range. Due to the low Cu foliar concentrations observed in Expt. 1 and a lack of deficiency symptoms 36 d after transplant, a lower Cu concentration may be sufficient for greenhouse-grown spearmint. This is reflected by the updated sufficiency range. The upper value of the deficiency range was 0.2 mg·kg⁻¹ Cu, although plants grown under Cu deficiency did not exhibit deficiency symptoms despite foliar Cu concentrations of <0.01 mg·kg⁻¹. The excessive range for Cu foliar concentrations was >13.0 mg·kg⁻¹. Excess Cu should be avoided, as it can antagonize the uptake of Fe and Zn, leading to deficiencies (Bryson and Mills 2015).

Iron. The primary symptom of Fe deficiency was subtle interveinal chlorosis of the upper leaves, which intensified as the deficiency persisted (Fig. 5D). Symptoms were observed 3 weeks after transplant. These symptoms were similar to the symptoms of

Fe deficiency observed in Japanese mint (Janpen et al. 2019). No significant difference in dry mass between the Fe-deficient and the control plants was observed ($P > 0.05$), but Fe-deficient plants had a 13% lower tissue concentration than the control plants (Tables 3 and 4). Although a difference in Fe tissue concentration existed between treatments, both tissue concentrations fell within the wide sufficiency range suggested by Bryson and Mills (2015).

A Weibull distribution best represented the Fe foliar data (Fig. 7A; Table 2) and established the Fe sufficiency range between 72.2 and 101.4 mg·kg⁻¹ Fe (Table 5). This range greatly narrows the broad range recommended by Bryson and Mills (2015), which suggested a concentration between 60 and 250 mg·kg⁻¹ Fe. This updated recommendation still captured the Fe concentration of the Fe-deficient and the control plants but provides a narrower target range to prevent unnecessary overfertilization. The upper limit of the deficiency range was 49.7 mg·kg⁻¹ Fe. Despite adequate Fe application, a pH > 7.0 can make Fe unavailable for uptake and lead to low or deficient Fe tissue concentrations (Bryson and Mills 2015). This occurs most commonly in calcareous regions (Marschner 1995), where high alkalinity can occur in untreated irrigation water. The excessive range was established as concentrations >119.9

mg·kg⁻¹ Fe. While Fe toxicity in spearmint has not been reported, Japanese mint plants exhibited depressed growth and dark foliage coloration due to high Fe applications and had Fe tissue concentration of 192 mg·kg⁻¹ (Misra 1993).

Manganese. Spearmint plants grown under Mn-deficient conditions did not exhibit any deficiency symptoms during the experiment, nor was there a significant difference in dry mass between the Mn-deficient and control treatments (Tables 3 and 4). Compared with the control plants, plants grown under Mn deficiency had a 68% lower Mn tissue concentration and fell below the survey range determined by Bryson and Mills (2015); however, the Mn tissue concentration of the control plants was within this range.

While a Weibull distribution best represented Mn foliar data out of the three evaluated models (Table 2) and was used to establish Mn interpretation ranges, the data exhibited a bimodal distribution that cannot be accounted for by the chosen distributions (Fig. 7B). This bimodal distribution is likely due to the large amount of variation throughout the data set, with a high number of samples having an Mn concentration between 40 and 60 mg·kg⁻¹ Mn and between 120 and 160 mg·kg⁻¹ Mn. A larger sample size would likely decrease the variation and establish a clearer trend. Based on a Weibull distribution, the Mn sufficiency range was between these two peaks, from 70.0 to 135.4 mg·kg⁻¹ Mn (Table 5), and narrowed the previously recommended range. While this new range can provide a target Mn concentration for spearmint, tissue concentrations slightly below or above this range are unlikely to experience deficiency or toxicity symptoms. While the deficiency range was established at <34.0 mg·kg⁻¹ Mn, a lack of deficiency symptomatology suggests that spearmint is not sensitive to Mn deficiency. The lower limit of the excessive range was 187.0 mg·kg⁻¹ Mn. While Mn toxicity in spearmint has yet to be studied, high accumulation may cause undesirable visual symptoms, such as necrosis of the lower leaves, and antagonized uptake of Fe (Bryson and Mills 2015).

Molybdenum. No symptoms of Mo deficiency were observed during the growing period. The dry mass of the control plants and plants grown without Mo were not significantly different ($P > 0.05$). The Mo-deficient plants had an 88% lower Mo tissue concentration and fell below the survey range of 0.5 to 1.0 mg·kg⁻¹ Mo determined by Bryson and Mills (2015). The control plants fell just above this range with a Mo concentration of 1.01 mg·kg⁻¹.

Many samples had a Mo concentration below the detection limit and were assigned a concentration of 0.01 mg·kg⁻¹ Mo for analysis. A Weibull distribution best represented foliar Mo concentrations of the three models evaluated (Fig. 8A; Table 2). The resulting sufficiency range was 0.1 to 1.2 mg·kg⁻¹ Mo (Table 5), which is slightly broader than the previously recommended range. Because many

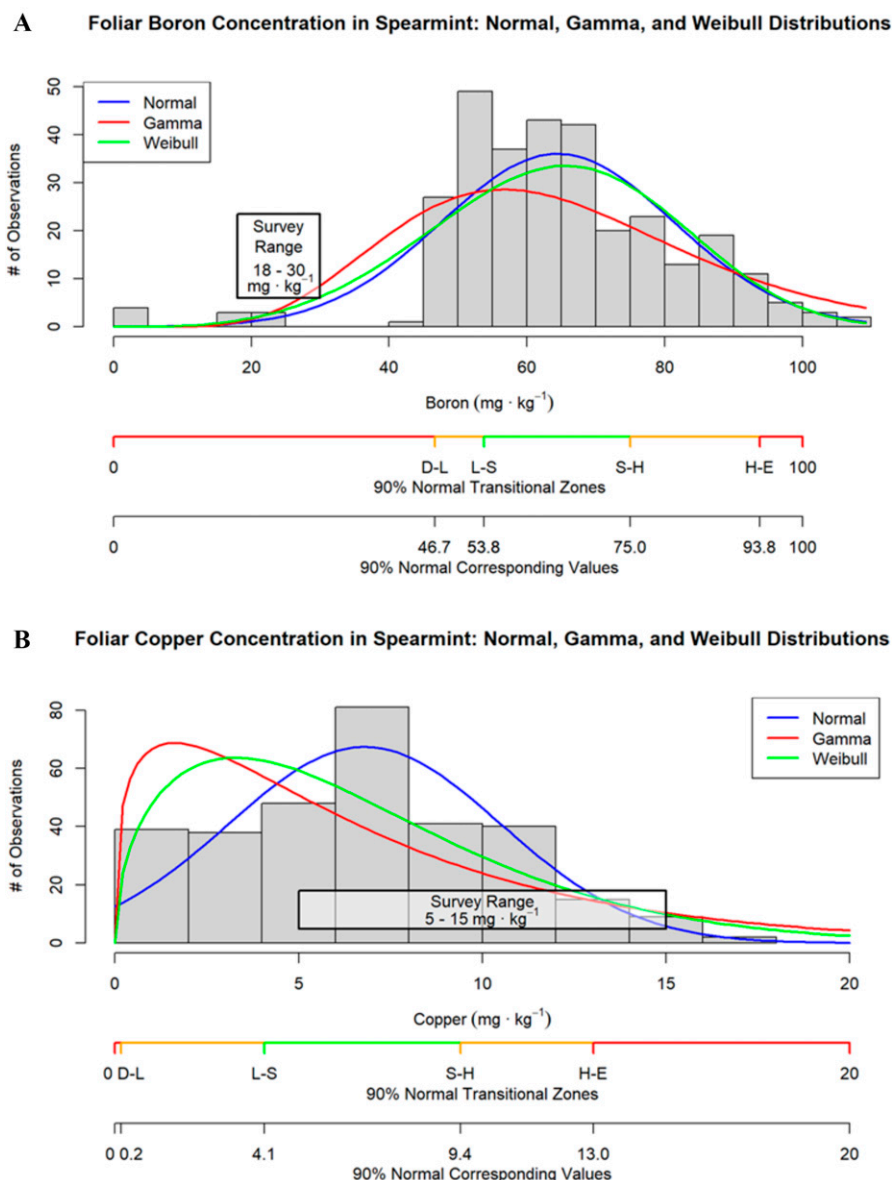


Fig. 6. Boron (A) and copper (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 5%, 25%, 75%, and 95% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlaid for reference.

samples had a very low Mo concentration and lack deficiency symptoms, a deficiency range could not be established. Instead, the deficient and low ranges were combined to establish a deficient/low range with an upper limit of $0.1 \text{ mg} \cdot \text{kg}^{-1}$ Mo. While plants from the Mo-deficient treatment had a mean Mo concentration of $0.12 \text{ mg} \cdot \text{kg}^{-1}$ Mo, all but one plant had a Mo concentration within the sufficiency range. This may explain the lack of deficiency symptomatology. Lastly, the boundary between the high and excessive ranges was $3.6 \text{ mg} \cdot \text{kg}^{-1}$ Mo. While Mo concentrations within this range are unnecessary for healthy growth, high concentrations are not likely to be problematic as Mo toxicity is uncommon in most plants (Marschner 1995).

Zinc. Four weeks after transplant, Zn-deficient plants exhibited stunted growth and purple spotting on the lower to middle leaves,

which eventually became necrotic (Fig. 5E). Plants also exhibited faint interveinal chlorosis of the upper foliage. Zinc-deficient plants had 49% less dry mass than the control plants (Fig. 5F) and had a 41% lower Zn tissue concentration (Tables 3 and 4). The suggested sufficiency range of 25 to $100 \text{ mg} \cdot \text{kg}^{-1}$ Zn presented by Bryson and Mills (2015) was corroborated by the Zn tissue concentration of the control plants, while the Zn-deficient plants fell below this range.

The Weibull model had the best BIC value (Table 2) and best represented the Zn foliar concentrations in spearmint (Fig. 8B). The updated sufficiency range was 22.9 to $37.6 \text{ mg} \cdot \text{kg}^{-1}$ Zn (Table 5), which provides a much lower upper limit compared with the range suggested by Bryson and Mills (2015) while still including the Zn concentration of the control plants. The upper boundary of the

deficient range was $13.3 \text{ mg} \cdot \text{kg}^{-1}$ Zn. The Zn-deficient plants, which exhibited deficiency symptoms and stunted growth, had a Zn concentration just above the deficiency range, at $15.0 \text{ mg} \cdot \text{kg}^{-1}$ Zn. A lower tissue concentration would likely cause more pronounced symptoms. The excessive range was established as concentrations $>47.9 \text{ mg} \cdot \text{kg}^{-1}$ Zn. While Zn toxicity is not common, excessive Zn application can antagonize the uptake of Mg and Fe, inducing deficiencies (Marschner 1995).

Conclusions

The first nutrient disorders to develop were deficiencies of N, P, K, and S, along with B toxicity. Symptoms of these nutrient disorders appeared within the first 3 weeks after transplant, suggesting that they may be the most common disorders encountered by

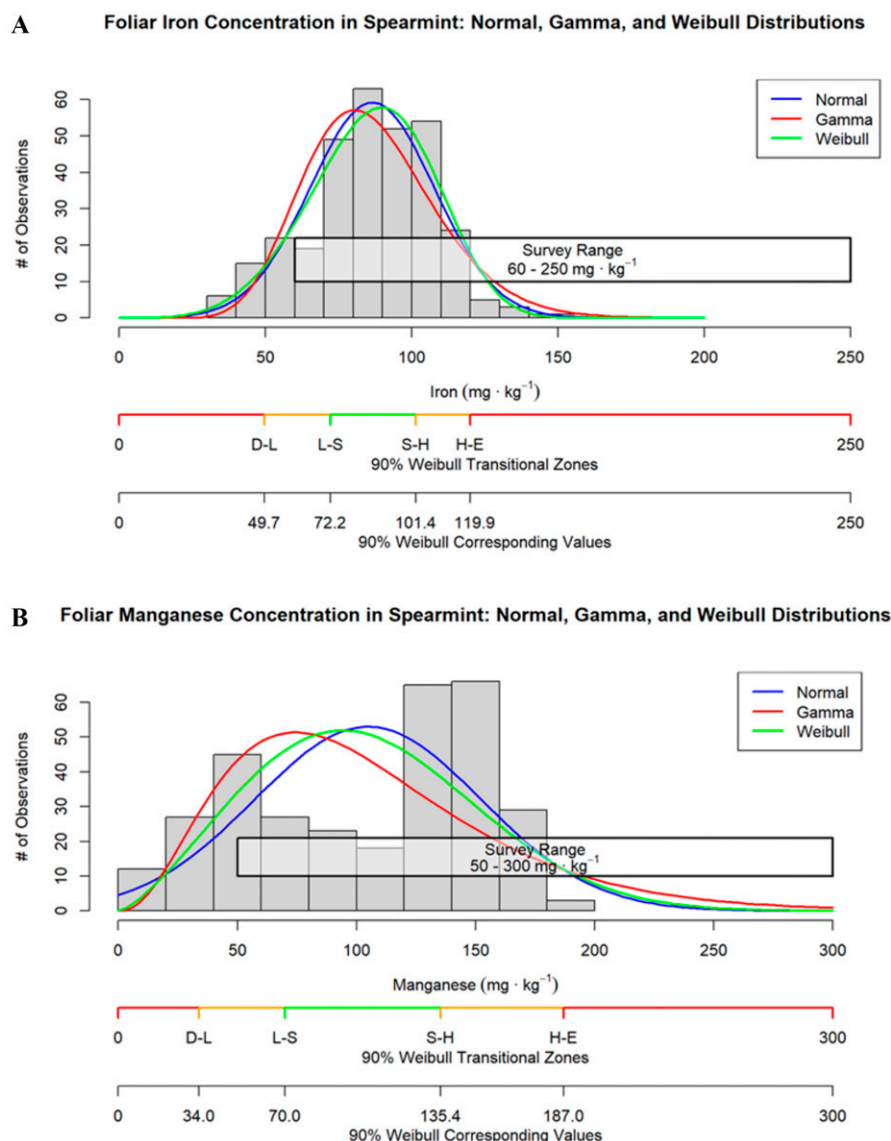


Fig. 7. Iron (A) and manganese (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 5%, 25%, 75%, and 95% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlaid for reference.

growers. Symptoms of Ca, Mg, B, Cu, Fe, and Zn deficiencies were also successfully induced, although symptoms took longer to appear. A lack of Mo deficiency symptoms was likely due to Mo tissue concentrations remaining above the deficient/low range, which may be due to trace amounts of Mo present in the plug before transplanting. For Mn, a lack of deficiency symptoms despite critically low tissue concentrations suggests spearmint is not sensitive to Mn deficiency. Overall, the documented symptomatology, descriptions, and tissue concentrations of the induced nutrient disorders will serve as a useful resource for identifying nutrient disorders in greenhouse-grown spearmint, benefiting both growers and future research.

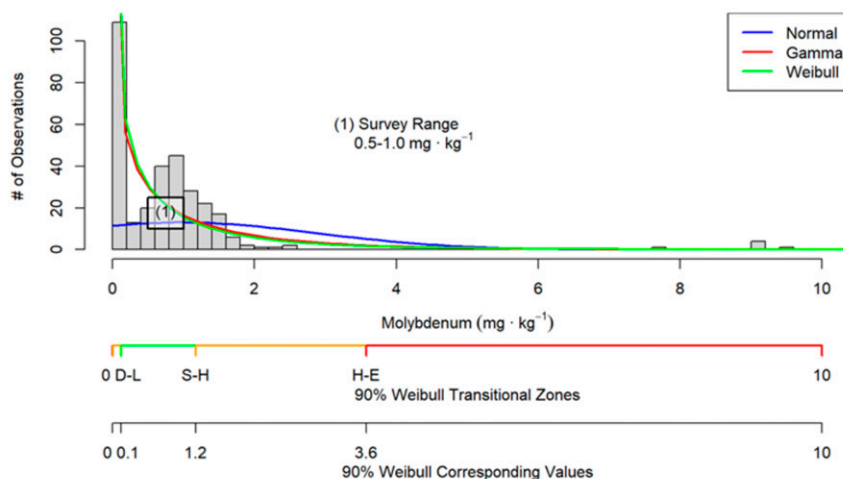
In addition to inducing and documenting nutrient disorders for 12 plant essential elements, foliar nutrient interpretation ranges specific to greenhouse-grown spearmint were established. This will provide more accurate

target values for growers to identify nutrient issues and take corrective actions. The development of these models expanded upon the SRA method to define deficient, low, high, and excessive ranges in addition to the sufficiency range. Many of the updated macronutrient sufficiency ranges, including those for P and K, are much higher than recommendations for field-grown spearmint. This is likely due to the increased availability of nutrients supplied in water-soluble fertilizers, which enhanced uptake and tissue concentrations. Further, the Ca and Mn data have bimodal distributions, which is an abnormal trend for foliar nutrient data. Likely, the small sample size ($n = 315$) and limited data sources resulted in a high amount of variation. Compiling additional tissue analysis data for greenhouse-grown spearmint and using a larger data set would likely improve the accuracy of all distributions. Despite these abnormalities, the newly developed

interpretation ranges correctly placed nutrient concentrations of the control plants in the sufficiency range and of the deficient plants in the deficient or low ranges.

While the established tissue nutrient interpretation ranges provide a guide for ensuring visually healthy spearmint crops, they do not account for the influence of fertilizer application on essential oil production. Fertilizer concentration can affect spearmint essential oil content, with N having the most significant impact (Alhasan and Hussein 2022; Amano and Belete 2022). Because essential oil is a valuable product of spearmint production, further research is necessary to evaluate the impact of the refined tissue nutrient interpretation ranges on essential oil yield. Overall, these distributions provide reference ranges for interpreting foliar analysis data and identifying nutritional disorders in greenhouse-grown spearmint.

A Foliar Molybdenum Concentration in Spearmint: Normal, Gamma, and Weibull Distributions



B Foliar Zinc Concentration in Spearmint: Normal, Gamma, and Weibull Distributions

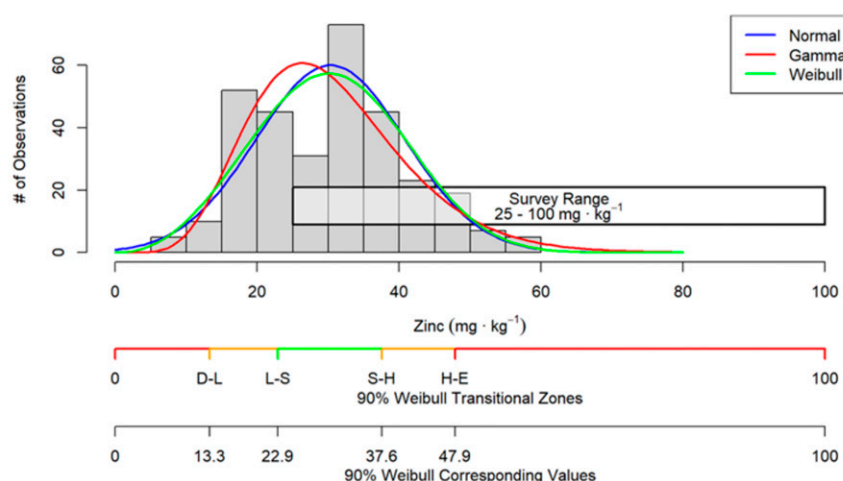


Fig. 8. Molybdenum (A) and zinc (B) foliar concentrations of greenhouse-grown spearmint (*Mentha spicata* L.) modeled using normal, gamma, and Weibull distributions. Interpretation ranges are based on the denoted distribution with four transitional points of deficient to low (D-L), low to sufficient (L-S), sufficient to high (S-H), and high to excessive (H-E), which correspond to 5%, 25%, 75%, and 95% of sample observations ($n = 315$), respectively. Survey ranges from Bryson and Mills (2015) are overlayed for reference.

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