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Characterization of Nutrient Disorders of *Cannabis sativa*

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Abstract: Essential plant nutrients are needed at crop-specific concentrations to obtain optimum growth or yield. Plant tissue (foliar) analysis is the standard method for measuring those levels in crops. Symptoms of nutrient deficiency occur when those tissue concentrations fall to a level where growth or yield is negatively impacted and can serve as a visual diagnostic tool for growers and researchers. Both nutrient deficiency symptoms and their corresponding plant tissue concentrations have not been established for cannabis. To establish nutrient concentrations when deficiency or toxicity symptoms are expressed, *Cannabis sativa* 'T1' plants were grown in silica sand culture, and control plants received a complete modified Hoagland's all-nitrate solution, whereas nutrient-deficient treatments were induced with a complete nutrient formula withholding a single nutrient. Toxicity treatments were induced by increasing the element tenfold higher than the complete nutrient formula. Plants were monitored daily and, once symptoms manifested, plant tissue analysis of all essential elements was performed by most recent mature leaf (MRML) tissue analysis, and descriptions and photographs of nutrient disorder symptomology were taken. Symptoms and progressions were tracked through initial, intermediate, and advanced stages. Information in this study can be used to diagnose nutrient disorders in *Cannabis sativa*.

Keywords: macronutrients; micronutrients; cannabis; deficiency; toxicity; fertility; symptomology; hemp; diagnostics; plant tissue analysis; CBD; THC; foliar

1. Introduction

Due to recent changes in legislation both at the federal and state levels, there has been a surge of interest in the growing, processing, selling, and using of products containing cannabidiol (CBD), derived from hemp flowers. Hemp is legally defined as *Cannabis sativa* strains with a tetrahydrocannabinol (THC) concentration no greater than 0.3% in any part of the plant (Congress, [1,2]). *Cannabis sativa* strains with a THC concentration greater than 0.3% in any part of the plant are considered marijuana. *Cannabis sativa* contains over 100 cannabinoids, which include THC and CBD. It is well known that THC has psychoactive effects. Many have reported health benefits from marijuana, which may be associated with non-THC cannabinoids, such as CBD. The broad interest in CBD is for health benefits similar to marijuana but without the psychoactive effects of THC.

Hemp has historically been grown for fiber and seed, and due to recent changes in legislation, it is being grown for flowers. Hemp grown for flowers (floral hemp) follows a horticultural production model either in a greenhouse or bedded field compared to fiber and seed hemp, which follows an agronomic production model.

There is little published research investigating fertility requirements for floral hemp. As applied research is conducted to determine nutrient rates to maximize yield and minimize inputs, as well as to develop a target range of plant sufficiency ranges to aid in nutrient management, this study provides an invaluable basis to identify nutrient deficiency in the field and to develop sufficiency ranges where nutrient corrective action can be made before visual symptoms are expressed.

The impacts of plant nutrition on plant growth and yield, as well as plant primary and secondary metabolites, are well-established (Il'in, [3]). In hemp fiber varieties, Bosca et al. [4] reported higher levels of nitrogen increased plant leaf weight and decreased leaf THC content, presumably due to THC dilution. In a marijuana strain, phosphorus treatments had greater combined leaf and flower dry weight, as well as higher THC concentration, compared to the no phosphorus treatment (Coffman and Gentner, [5,6]). Hemp producers are seeking THC levels <0.3% and high CBD concentrations (i.e., 10–20%). Given the high energy and resource requirements for plants to produce secondary plant metabolites, such as cannabinoids (Taura et al., [7,8]), it would be reasonable to assume that nutritive disorders would impact the production and quality of these metabolites.

Plant tissue analysis has been used extensively for many decades to evaluate the nutritional status of a crop. Nutrient sufficiency levels (the tissue concentration at which growth or yield is not limited) have been established for most major agricultural and horticultural plants. Because cannabis has not been widely grown legally, nutrient sufficiency ranges have not been established. The development of sufficiency ranges for the essential plant nutrients would require extensive rate studies that measure both elemental concentrations in the leaf and the yield parameters of both floral biomass and cannabinoid concentrations. Where sufficiency ranges of a crop are not available, survey ranges can be used to approximate the nutritional status of the plant. Survey ranges for Cannabis sativa in greenhouse nursery production have been published by Bryson et al. [9], and more recently, a survey of five hemp cultivars in greenhouse production, including the cultivar used in this study, by Landis et al. [10]. These tissue values are useful for cannabis growers as they aid in fertility management. This study adds to this body of knowledge as the complete fertilizer controls can serve as an additional set of survey ranges for cannabis.

The second contribution of this study results in information about nutrient deficiency levels in the leaf tissue of cannabis. Once a plant begins showing visual symptoms of impaired growth, there is a reduction in plant health or yield is implicit. In this study, when plants began showing deficiency symptoms for each nutrient, most recently, mature leaf samples were analyzed for that nutrient. This information can be used by growers and researchers to confirm the visual diagnosis with leaf concentrations.

Additionally, no visual guides of nutrient deficiencies in cannabis supported with leaf tissue analysis and documenting a progression of symptomology have been published. Tracking the specific symptomologies of various nutritional disorders over time is important because symptomologies change in appearance and location as the deficiency progresses, making correct diagnosis challenging. Therefore, this study was conducted to provide cannabis growers and researchers with descriptions of nutrient disorders, high-quality images to track the progression of these disorders, and leaf tissue nutrient concentrations associated with documented deficiency symptomology.

2. Materials and Methods

Cuttings were taken from a hemp Cannabis sativa 'T1'on 3 July 2018 (Ryes Greenhouses: Sanford, NC, USA) and stuck into 72-cell plug trays filled with a substrate mix of 80:20 (v:v) Canadian sphagnum peat moss (Conrad Fafard, Agawam, MA, USA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH, USA) amended with dolomitic lime at 8.875 kg/m^3 (Rockydale Agricultural, Roanoke, VA, USA) and wetting agent (Aquatrols, Cherry Hill, NJ, USA) at 600.3 g/m^3. After three weeks of rooting, plugs were transplanted (27 July 2018) into 15.24-cm diameter (1.76 L) plastic pots filled with acid-washed silica-sand (Millersville #2 (0.8 to 1.2 mm diameter) from Southern Products and Silica Co., Hoffman, NC, USA).

The experiment was conducted in a glass greenhouse in Raleigh, NC, USA (35°N latitude), with 24°C/20°C (D/N) temperature setpoints. Plugs were transplanted, and nutrient treatments started immediately in the automated, recirculating irrigation system made from 10.2-cm diameter PVC pipe (Charlotte Plastics, Charlotte, NC, USA), fit with 12.7-cm diameter openings to hold the pots. Control plants were grown with a complete modified Hoagland's all-nitrate solution consisting of 15 mM nitrate-nitrogen (NO_3^-), 1 mM phosphate-phosphorus ($H_2PO_4^-$), 6 mM potassium (K^+), 5 mM calcium (Ca^{2+}), 2 mM magnesium (Mg^{2+}), and 2 mM sulfate-sulfur (SO_4^{2-}) (Hoagland and Arnon, [11]); plus 72 µM iron (Fe^{2+}), 18 µM manganese (Mn^{2+}), 3 µM copper (Cu^{2+}), 3 µM zinc (Zn^{2+}), 45 µM boron (BO_3^{3-}), and 0.1 µM molybdenum (MoO_4^{2-}). Nutrient deficiencies began at transplant and were induced by withholding a single nutrient from this solution. Boron (B) and manganese (Mn) toxicities were induced by increasing the concentration tenfold higher than the complete nutrient formula. Reagent grade chemicals and deionized (DI) water (18 MΩ) were used to formulate treatment solutions. The plants were drip-irrigated with a sump-pump (model 1A, Little Giant Pump Co., Oklahoma City, OK, USA) system as needed between 6:00 and 18:00 hours. Irrigation solution drained from the pot and was captured for reuse. Nutrient solutions were replaced weekly. The experiment was terminated 9 weeks after treatments began.

Plants were tracked daily, and deficiency symptoms were photographed at the initial, intermediate, and advanced stages of symptomology. Plant anatomy terminology used to describe deficiency symptoms is given in Figure 1. Upon initial symptom development, four plants were selected for sampling. The remaining treatments, which were not symptomatic, were grown until visual symptoms appeared and then were harvested. At the onset of initial visual symptomology, whole plants (n=4) were destructively harvested, and most recent mature leaves (MRML) were subsampled and rinsed with DI water, washed in a solution of 0.5 M HCl, and again rinsed with DI water. Leaf tissue (MRML) was taken below the meristematic stem regions (apical meristem, axillary meristems, tertiary meristematic regions, etc.), and only recently matured leaves were sampled. Leaf maturity and morphology were determined based on observational leaf maturity and from indices hybridized from Heslop–Harrison and Heslop–Harrison [12] and Mediavilla et al. [13] and from established standard leaf tissue harvesting protocols (Bryson et al., [9]). The remaining plant material after MRML was harvested and was placed in a separate container for aerial tissue biomass determination. Leaf tissues and their respective remaining biomass were dried at 70 °C for 24 hours. Dried leaf tissue was ground in a mill (Thomas Wiley® Mini-Mill; Thomas Scientific, Swedesboro, NJ, USA) with a 20-mesh (1 mm) screen and analyzed for nutrient concentrations by the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Agronomic Division (NCDA&CS, [14]). Total plant dry weight (DW) was calculated by adding the oven-dry weight of the leaf tissue to the oven-dry weight of the remaining plant biomass (Table 1). Details about experimental setup, fertilizers, and design can be found in Barnes et al. [15]. Data were analyzed with SAS version 9.4 (SAS Institute, Cary, NC, USA) and subjected to analysis of variance (ANOVA) using PROC ANOVA and (GLM) PROC GLM. Where *F*-tests indicated evidence of significant differences among means, LSD (Least Significant Difference) ($P \leq 0.05$) was used.

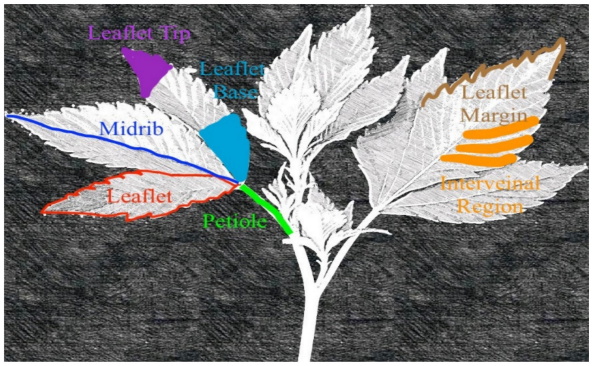

Figure 1. Sketch of a branch of *Cannabis sativa* 'T1' plant showing plant anatomy and terminology.

Table 1. Mean dry weights of *Cannabis sativa* 'T1' plants grown with a deficient macronutrient treatment compared to plants grown with a complete fertilizer.

	Dry Weight (g)					
Treatment	**–N** †	**–P**	**–K**	**–Ca**	**–S**	**–Mg**
Control	13.46	20.00	44.20	20.00	20.00	20.00
Disorder	6.77	15.44	32.27	16.63	18.01	18.01
	*** ‡	NS	**	NS	NS	NS

† Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), and magnesium (Mg). ‡ *, **, or *** indicate statistically significant differences between sample means (n=4) based on *F*-tests at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $P > 0.05$.

3. Results

3.1. Macronutrient Disorders

3.1.1. Nitrogen

Symptoms of nitrogen (N) deficiency developed 6 weeks after treatment. The plants first displayed slight stunting when compared to the control. Initially, the plants developed a slight overall yellowing or paling of the lower foliage (Figure 2). As the deficiency progressed, the yellowing became more intense and progressed up from the bottom-most leaves to the middle foliage. In advanced symptoms, the yellowing leaves became completely yellow and eventually turned necrotic and abscised.

Figure 2. Nutritional disorders of nitrogen (N), phosphorus (P), and calcium (Ca) deficiency in *Cannabis sativa* 'T1' plants. These pictures display the symptomological progression of nutritional disorders from initial, intermediate, through advanced.

Foliar N concentrations were 62% lower in the N deficient plants than in the controls. N deficient plants contained 1.62% N, while the control plants contained 4.28% N (Table 2). Foliar N concentrations in control plants were within the published greenhouse survey range of 3.30–4.76% N (Bryson et al. [9]). Plants grown in N deficient conditions produced 50% less biomass when compared to the control (Table 1).

Table 2. Foliar nutrient concentrations of *Cannabis sativa* 'T1' grown with a deficient macronutrient compared to plants grown with a complete fertilizer regime.

Treatment	–N†	–P	–K	–Ca	–Mg	–S
	Tissue nutrient concentration (% dry weight)					
Element	N	P	K	Ca	Mg	S
Complete	4.28	0.43	2.85	3.73	0.61	0.41
Disorder	1.62	0.09	0.41	0.39	0.12	0.11
	*** ‡	***	***	***	***	***
Survey Range [1]	3.30–4.76	0.24–0.49	1.83–2.35	1.47–4.42	0.4–0.81	0.17–0.26

† Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), ‡ *, **, or *** indicate statistically significant differences between sample means (n=4) based on *F*-tests at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $P > 0.05$. [1] Reference survey values from Bryson et al. (2014).

3.1.2. Phosphorus

Symptoms of phosphorus (P) deficiency developed 7 weeks after treatment. The plants first displayed slight stunting when compared to the control. Initially, the plants developed olive-green spots in an irregular spotting pattern along with the leaflets on the lower and older leaves (Figure 2). As symptoms progressed, the olive-green spots developed into larger olive-green spots that appeared sunken and almost wet in appearance with some marginal necrosis. In advanced symptoms, the yellowing leaves became severely olive spotted with large areas showing symptoms, and in severe cases, large necrotic portions developed.

Foliar P concentrations were 79% lower in the P deficient plants than in the controls. Treated deficient plants contained 0.09% P, while the control plants contained 0.43% P (Table 2). Foliar P concentrations in control plants were within the published survey range of 0.24–0.49% (Bryson et al., 2014). Plants grown in P deficient conditions did not result in statistically significant differences in biomass when compared to the control (Table 1).

3.1.3. Calcium

Symptoms of Ca deficiency developed five weeks after treatment. The plants first displayed slight stunting when compared to the control. The growing tips and newly expanding leaves showed signs of stunting and irregular growth habits (Figure 2). As the newly expanding leaves developed, the basal portion of the leaflets remained narrower and displayed a lighter green coloration when compared to the leaflet tip (Figure 2). As symptoms progressed, the yellowing at the leaflet basal portion became more intense, and the leaflets began to show symptoms of interveinal chlorosis. The new leaves that expanded showed severe stunting and marginal necrosis, resulting in leaves with irregular geometries and orientations (Figure 2). In advanced symptoms, the yellowing leaves and growing tips became necrotic. The death of the growing tip caused a proliferation of axillary shoot development to occur, resulting in a more branched architecture (Figure 2).

Foliar Ca concentrations were 90% lower in the Ca-deficient plants than in the controls. Ca-deficient plants contained 0.39% Ca, while the control plants contained 3.73% Ca (Table 2). Foliar Ca concentrations in control plants were within the published survey range of 1.47–4.42% (Bryson et al., [9]).

Plants grown in Ca-deficient conditions did not result in statistically significant differences in biomass when compared to the control (Table 1) when sampled at the onset of visual symptoms.

3.1.4. Sulfur

Symptoms of sulfur (S) deficiency developed 7 weeks after treatment. The plants first displayed a slight overall yellowing of the foliage, especially in the middle of the plant. The yellowing leaves had a more pronounced yellowing at the base of the leaflets (Figure 3). As symptoms progressed, the yellowing at the leaflet basal portion became more intense, and the yellowing intensified on newly expanding leaves (Figure 3). In advanced symptoms, the leaves became a very pale yellow in coloration, especially around the midrib and base of the leaflets (Figure 3).

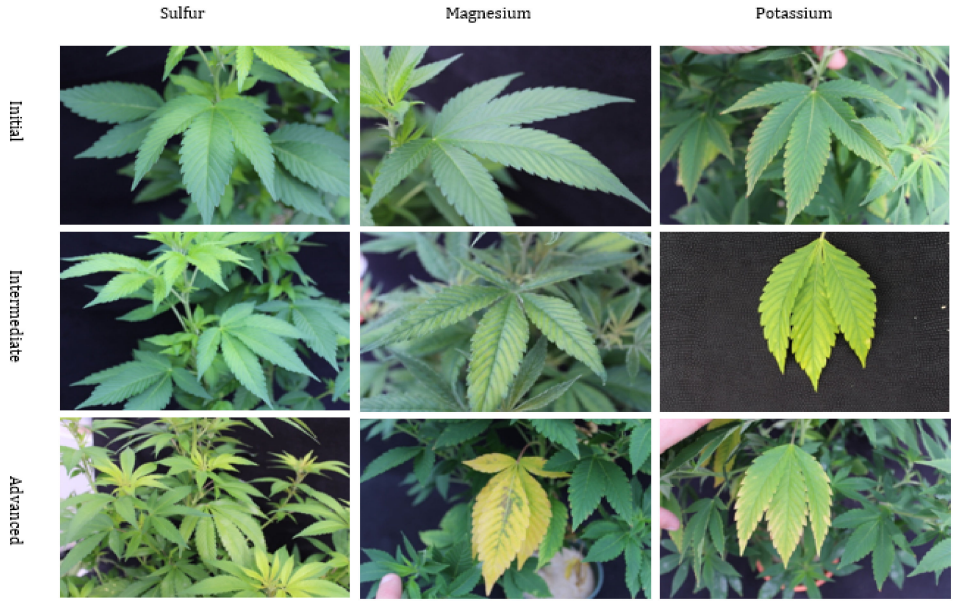

Figure 3. Nutritional disorders of sulfur (S), magnesium (Mg), and potassium (K) deficiency in *Cannabis sativa* 'T1' plants. These pictures display the symptomological progression of nutritional orders as they progress from initial, intermediate, and advanced.

Foliar S concentrations were 73% lower in the S-deficient plants than in the controls. S-deficient plants contained 0.11% S, while the control plants contained 0.41% S (Table 2). Foliar S concentrations in control plants were above the published survey range of 0.17–0.26% (Bryson et al., [9]). Plants grown in S-deficient conditions did not result in statistically significant differences in biomass when compared to the control (Table 1).

3.1.5. Magnesium

Symptoms of Mg deficiency developed 7 weeks after treatment. Initial symptoms developed with slight yellowing of the interveinal regions of the lower and older foliage (Figure 3). As symptoms progressed, the interveinal yellowing became more pronounced and intensified on the older leaves (Figure 3). In advanced symptoms, the leaves showed a very stark contrast between the green veins and the yellow interveinal regions, with some regions becoming necrotic (Figure 3).

Foliar Mg concentrations were 80% lower in the Mg-deficient plants than in the controls. Mg-deficient plants contained 0.12% Mg, while the control plants contained 0.61% (Table 2). Foliar Mg concentrations in control plants were within the published survey range of 0.40–0.81% (Bryson et al., [9]). Plants grown in Mg-deficient conditions did not result in statistically significant differences in biomass when compared to the control (Table 1).

3.1.6. Potassium

Symptoms of K deficiency developed 9 weeks after treatment. Initial symptoms developed as a yellowing of the leaf margin, especially the saw-tooth of the leaflets, on the lower and older foliage (Figure 3). As symptoms progressed, the marginal yellowing became more pronounced and intensified on the older leaves, expanding inward toward the midrib (Figure 3). In advanced symptoms, the leaflet margins yellowed, and some regions of tan necrosis developed, especially along the saw-tooth margin of the leaflets (Figure 3).

Foliar K concentrations were 86% lower in the K-deficient plants than in the controls. K-deficient plants contained 0.41% K, while the control plants contained 2.85% K (Table 2). Foliar K concentrations in control plants were above the published survey range of 1.83–2.35% (Bryson et al., [9]). Plants grown in K-deficient conditions weighed 27% less when compared to the control (Table 1).

3.2. Micronutrient Disorders

3.2.1. Manganese

Symptoms of Mn deficiency developed 6 weeks after treatment. Initially, the plants developed a bright yellow netted interveinal chlorosis on the upper and central foliage (Figure 4). This chlorotic netting initiated at the midrib of the leaflets and spread outward toward the leaf margin as symptoms progressed. In advanced symptoms, the interveinal netting became very distinct against the green veinal regions. Additionally, the interveinal regions developed small tan necrotic regions on the leaf surface (Figure 4).

Figure 4. Nutritional disorders of manganese (Mn), boron (B), and copper (Cu) deficiency in *Cannabis sativa* 'T1' plants. These pictures display the symptomological progression of nutritional orders as they progress from initial, intermediate, and advanced.

There were no statistically significant differences in the dry weights of the control plant and the Mn-deficient plants (Table 3). Despite the lack of statistical significance, foliar Mn concentrations were 74% lower in the Mn-deficient plants than in the controls. Deficiency treated plants contained 7.56 mg·kg^{-1} Mn, while the control plants contained 29.40 mg·kg^{-1} (Table 4). Foliar Mn concentrations in the control plants were slightly below the published sufficiency range of 41–93 mg·kg^{-1} Mn (Bryson et al., [9]).

Table 3. Mean dry weights of *Cannabis sativa* 'T1' plants grown with a deficient or toxic micronutrient treatment compared to plants grown with a complete fertilizer regime.

	Dry Weight (g)							
Treatment	**–B** †	**+B**	**–Cu**	**–Fe**	**–Mn**	**+Mn**	**–Mo**	**–Zn**
Control	13.46	19.99	44.20	44.20	13.46	19.99	44.20	44.20
Disorder	9.69	21.16	24.23	43.42	13.09	18.70	39.91	43.23
	* ‡	NS	**	NS	NS	NS	NS	NS

† Boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). ‡ *, **, or *** indicate statistically significant differences between sample means (n = 4) based on *F*-tests at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $P > 0.05$.

Table 4. Foliar nutrient concentrations of *Cannabis sativa* 'T1' grown with a deficient micronutrient treatment compared to plants grown with a complete fertilizer regime.

Treatment	**–B** †	**+B**	**–Cu**	**–Fe**	**–Mn**	**+Mn**	**–Mo**	**–Zn**
	Tissue nutrient concentration ($mg \cdot kg^{-1}$)							
Element	B	B	Cu	Fe	Mn	Mn	Mo	Zn
Complete	58.58	64.60	4.65	111.75	29.40	31.13	1.46	25.33
Disorder	2.46	671.75	1.41	60.1	7.56	47.88	0.06	10.7
	*** ‡	***	***	***	***	***	***	***
Survey Range [1]	56–105	56–105	5.0–7.1	100–150	41–93	41–93	0.5–1.5	24–52

† Boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). ‡ *, **, or *** indicate statistically significant differences between sample means (n = 4) based on *F*-tests at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $P > 0.05$. [1] Reference survey values from Bryson et al. (2014).

3.2.2. Manganese Toxicity

Symptoms of Mn toxicity developed 7 weeks after treatment. Initially, the plants developed a marginal yellowing of the lower leaves (Figure 5). This yellowing intensified and moved inward on the leaf surface toward the midrib. In advanced symptoms, the leaf margin became necrotic, and the leaf appeared severely chlorotic. In some cases, the symptomatic leaves abscised (Figure 5).

Figure 5. Nutritional disorders of manganese toxicity (Mn) and boron toxicity (B) in *Cannabis sativa* 'T1' plants. These pictures display the symptomological progression of nutritional orders as they progress from initial, intermediate, and advanced.

Foliar Mn concentrations were 53% higher in the Mn toxicity treatment plants than in the controls. Toxicity treated plants contained 47.88 mg·kg^{-1} Mn, while the control plants contained 31.13 mg·kg^{-1} (Table 4). Foliar Mn concentrations, in both toxicity treated and control plants, were within the published survey range of 41–93 mg·kg^{-1} (Bryson et al., [9]). While the toxicity treatments were higher, they were still within the accepted ranges for most crops. There were no statistically significant differences in the dry weights of the control plant and the Mn toxicity plants (Table 3). Higher concentrations of Mn will need to be used to further refine the threshold for toxicity symptomology.

3.2.3. Boron

Symptoms of B deficiency developed 6 weeks after treatment. Initially, the plants developed slight stunting in their growth habits when compared to the control. Upon closer inspection, the stunting proliferated from the growing tips of the B-deficient plants. The growing tips and newer foliage displayed a distorted growth pattern. The new and expanding leaflets were smaller and narrower at the leaflet base when compared to the tip (Figure 4). In advanced symptoms, the new and expanding leaves displayed severe distortion. The leaflet margins became necrotic, and the leaves distorted severely, curling inward and down as well as at odd angles from the petiole (Figure 4). In the most advanced stages, the growing tips died and became necrotic, and the whole plant showed severe wilting due to the death of the root tips and subsequent loss of root biomass.

Foliar B concentrations were 96% lower in the B-deficient plants than in the controls. Deficiency treated plants contained 2.46 mg·kg^{-1} B, while the control plants contained 58.58 mg·kg^{-1} (Table 4). Foliar B concentrations in control plants were within the published survey range of 56–105 mg·kg^{-1} B (Bryson et al., [9]). Plants grown in B-deficient conditions produced 28% less biomass when compared to the control (Table 3). This is most likely due to the death of the apical growing tip due to B-deficient conditions.

3.2.4. Boron Toxicity

Symptoms of B toxicity appeared 7 weeks after treatment. Initially, the plants developed a marginal yellowing of the lower leaves (Figure 5). This yellowing intensified along the leaf margin and moved inward toward the midrib of the leaflets. In advanced symptoms, the leaf margin turned brown and eventually became necrotic (Figure 5).

Foliar B concentrations were >10 fold higher in the B toxicity plants than in the controls. Toxicity treated plants contained 671.75 mg·kg^{-1} B, while the control plants contained 64.60 mg·kg^{-1} (Table 4). Foliar B concentrations in control plants were within the published survey range of 56–105 mg·kg^{-1} B (Bryson et al., [9]). There were no statistically significant differences in the dry weights of the control plant and the B toxicity plants (Table 3).

3.2.5. Copper

Copper deficiency manifested very late in the growth of the plants, only displaying symptomology after 9 weeks. Initially, the plants developed slight stunting in their growth habits when compared to the control. This stunting was accompanied by a slight distortion of the newer and expanding leaves, especially at the leaflet base. The base of the leaflets was narrower and displayed slight yellowing (Figure 5). In advanced symptoms, the new and expanding leaves displayed a more pronounced basal narrowing and distortion and started to exhibit marginal interveinal chlorosis (Figure 5). In the most advanced stages, the whole leaf displayed a fine interveinal chlorosis, and the leaf margin distorted, slightly curling in and downward. The new and expanding leaves also displayed a slight decrease in turgidity (Figure 5).

Foliar Cu concentrations were 70% lower in plants subjected to Cu-deficient conditions. Deficiency treated plants contained 1.41 mg·kg^{-1} Cu, while the control plants contained 4.65 mg·kg^{-1} (Table 4). Foliar Cu concentrations in control plants were slightly below the published survey range of

5–7.1 mg·kg^{-1} Cu (Bryson et al., [9]). Plants grown in Cu-deficient conditions produced 45% less biomass when compared to the control (Table 3).

3.2.6. Iron

Symptoms of upper leaf interveinal chlorosis developed on Fe-deficient plants after 9 weeks of iron deficiency treatments. The leaves were lighter in appearance compared to control plants, and symptoms spread throughout the upper half of the foliage, especially around the growing tip and newly expanding leaves (Figure 6). Symptoms of Fe stress began as a slight marginal yellowing of the leaflets, especially around the base of the leaf (Figure 6). As symptomology progressed, the upper foliage displayed interveinal chlorosis on the new and expanding leaves, while the lower and mid foliage displayed a healthy dark green coloration (Figure 6).

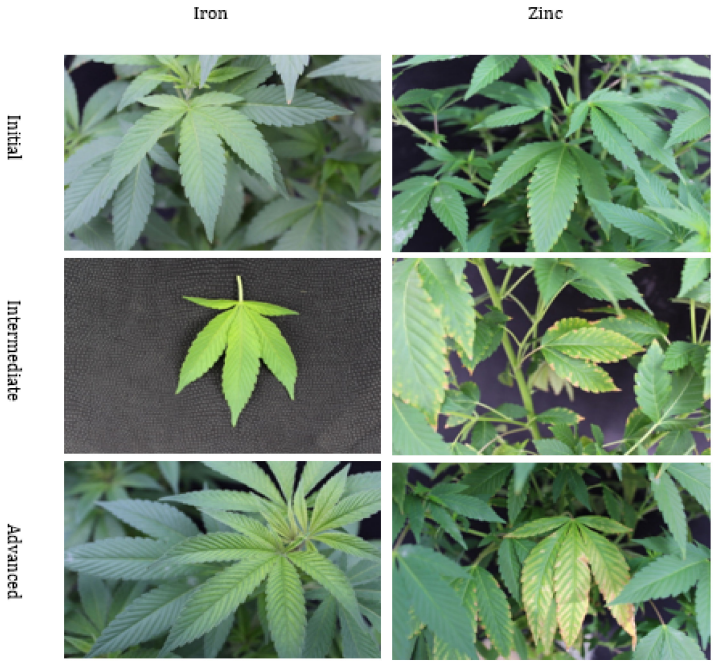

Figure 6. Nutritional disorders of iron (Fe) and zinc (Zn) deficiencies in *Cannabis sativa* 'T1' plants. These pictures display the symptomological progression of nutritional orders as they progress from initial, intermediate, and advanced.

Foliar Fe concentrations were 46% lower in plants subjected to Fe-deficient conditions when compared to the controls. Deficiency treated plants contained 60.08 mg·kg^{-1} Fe, while the control plants contained 111.75 mg·kg^{-1} (Table 4). Foliar Fe concentrations in control plants were within the published survey range of 100–150 mg·kg^{-1} (Bryson et al., [9]). Plants grown in Fe-deficient conditions produced a similar amount of biomass when compared to the control (Table 3).

3.2.7. Molybdenum

Visual symptoms did not develop on Mo-deficient plants after 9 weeks of deficiency treatments. Despite a lack of visual symptoms, foliar Mo concentrations were 96% lower in plants subjected to Mo-deficient conditions when compared to the controls. Deficiency treated plants contained 0.06 mg·kg^{-1} Mo, while the control plants contained 1.46 mg·kg^{-1} (Table 4). Foliar Mo concentrations in control plants were within the published survey range of 0.5–1.5 mg·kg^{-1} Mo (Bryson et al., [9]). Despite the statistical significance of the tissue concentrations, there was no statistical difference in dry weights of the Mo deficient plants when compared to the controls (Table 3).

3.2.8. Zinc

Zinc deficiency manifested very late in the growth of the plants, only displaying symptomology after 9 weeks. Deficiency symptoms manifested first as a marginal yellowing on the newer foliage and expanding leaves (Figure 6). This yellowing was concentrated mostly in the margin of the leaf and along the toothed portions of the leaflets. As symptoms progressed, these yellow marginal regions developed into tan irregularly shaped necrotic regions along the leaf margin (Figure 6).

Foliar Zn concentrations were 58% lower in plants subjected to Zn-deficient conditions when compared to the controls. Deficiency treated plants contained 10.70 mg·kg^{-1}, while the control plants contained 25.33 mg·kg^{-1} (Table 4). Foliar Zn concentrations in control plants were within the published survey range of 24–52 mg·kg^{-1} Zn (Bryson et al., [9]). Plants grown in Zn-deficient conditions produced similar amounts of biomass when compared to the control (Table 3). Despite the statistical significance of the Zn tissue concentrations (Table 4), there was no statistical difference in dry weights between the Zn-deficient plants and the control plants (Table 3).

4. Discussion

While some anecdotal deficiency symptoms for cannabis are available in lay publications, scientifically rigorous symptomology, particularly symptomological progression, is very limited or non-existent in the literature. In this study, most documented symptoms of deficiencies were consistent with descriptions from current literature for other plant species (Bryson et al., [9]; Barnes, [16]; Barker and Pilbeam, [17]; Gibson et al., [18]) with some exceptions. Concentrations of N, P, K, Ca, Mg, and S in the leaves when deficiency symptomology first appeared were 1.62, 0.09, 0.41, 0.39, 0.12, and 0.11%, respectively. Concentrations of B, Cu, Fe, Mn, Mo, and Zn in the leaves when deficiency symptomology first appeared were 2.46, 1.41, 60.1, 7.56, 0.06, and 10.7 mg·kg^{-1}, respectively.

Plants grown without Mo did not exhibit leaf symptomology nor less dry matter production despite leaf tissue values being 96% lower than the complete controls. While visual symptoms of Cu deficiency displayed the interveinal chlorosis in younger leaves documented in other plants, the Cu-deficient cannabis also showed an odd wilting pattern (Figure 4). This wilting in Cu-deficient hemp plants is mentioned in fiber hemp (Van der Werf, [19]). Copper is important in cell wall metabolism (Yruela, [20]). The condition which Van der Werf [19] referred to as "'gummi'-hemp" may indicate that Cu is needed in greater quantities in hemp than in other species, especially when grown for fiber. While these symptoms of wilting and lodging were in fiber hemp, which has a very vigorous vertical growth habit, the same process could be occurring in floral hemp cultivars.

Additionally, B-deficient treated plants displayed similar wilting tendencies. Upon further inspection of the roots, it was shown that the terminal growing tip had died and turned necrotic, and there were many axillary roots due to the loss of apical dominance. Boron is important in cell wall development and elongation of plant cell walls, especially in the radicle and other root meristematic regions (Hu and Brown, [21]; Whittington, [22]). The wilting seen could have been due to a lack of root mass due to an underdeveloped root system. Given all plants were irrigated for the same amount of time, it is feasible to assume that the wilting was due to a lack of water uptake because root growth was impaired.

Nutrients varied in how rapidly deficiency symptoms were visually apparent. In treatments where N, P, Ca, Mg, S, Mn, and B were withheld, deficiency symptoms were observed within 6 to 7 weeks. Conversely, where K, Zn, Cu, Fe were withheld, visual deficiencies only occurred after 9 weeks and, as previously noted, not at all in Mo. The use of visual symptomology as a diagnostic tool may be more useful for the nutrients that demonstrate symptoms sooner rather than later. However, for field and greenhouse floral hemp production, visual symptoms may express early enough in the season where rescue nutrient applications can be made. Additionally, routine MRML tissue sampling during the season will identify if nutrients are getting near the concentrations that show symptoms, thus allowing a correcting nutrient application before symptoms are expressed.

Some nutrient deficiencies had a significant suppressive effect on yield, as measured by whole plant dry weight, while withholding other nutrients did not affect total biomass. Deficiencies of N, K, B, and Cu produced significantly less crop biomass by 50, 27, 28, and 45%, respectively, as compared to the control. While all other nutrient deficiencies had less biomass, none suppressed yield at a significance level of $P > 0.05$. Above-ground biomass may not be the appropriate metric for testing critical levels in these nutrients, and other factors, such as secondary metabolite production, maybe a better indicator of the minimum nutrient concentrations for optimum production.

The development of true cannabis sufficiency ranges for each essential element requires individual rate studies coupled with measurements of both dry matter production and cannabinoid yield. Until this extensive work can be completed, survey ranges are useful tools for estimating adequate nutrient levels. In this study, the control samples served as survey ranges, which can be compared to other published survey ranges for cannabis (Bryson et al., [9]; Landis et al., [10]). Some elements in the control samples were above (K and S) or below (Cu and Mn) the reported values from Bryson et al. [9]. Other control values found in this study were within or above the listed values for Cannabis sativa 'T1' except for Mn values, which were below the listed values from Landis et al. [10]. While the ranges reported by Bryson et al. [9] are useful guides for estimating healthy nutrient levels in hemp tissue, it is important to remember that they are the survey ranges from plants grown in a production nursery of an unknown replicate number and unknown hemp cultivar and may not accurately predict optimum nutrient levels in all production systems in all cultivars. Recently, Landis et al. [10] found that significant differences in nutrient concentrations occurred among CBD cultivars, suggesting that broader target nutrient ranges may be appropriate for cannabis.

Boron toxicity was first observed at an accumulated foliar leaf tissue concentration of 671.75 $mg \cdot kg^{-1}$, and the symptoms (marginal chlorosis and necrosis in the older leaves) were consistent with B toxicity in other plants. Plants vary in their sensitivity to boron. Toxicity symptoms occur in strawberry at foliar concentrations as low as 120 $mg \cdot kg^{-1}$ (Haydon, [23]), suggesting that cannabis may be more tolerant of excess B than some crops.

5. Conclusions

This work serves as a baseline for nutritive values in the *Cannabis sativa* 'T1' cultivar, and it is recommended that the upper and lower ranges from this study, Landis et al. [10], and Bryson et al. [9] be used when evaluating leaf tissue nutrient concentrations. The nutrient disorders described in this study provide hemp growers and researchers with detailed descriptions and high-quality diagnostic images to better identify nutrient disorders. Additionally, previous works were lacking in diagnostic rigor and did not report foliar nutrient values. These nutrient values can also help growers to monitor their crops and can be used to make improved fertilization decisions. With the exception of N, K, B, and Cu, most disorders had no significant effect on overall plant dry weight at the onset of symptoms. However, if measurements of plant dry weight had also been collected at intermediate and advanced stages of symptomology, greater negative impacts on growth would likely have occurred. In addition, measurements of dry weights of floral parts at harvest and concentrations of cannabinoids from those floral could offer insight into the effects of these essential nutrients on plant growth and yield parameters other than vegetative biomass. These data illustrate the importance of recognizing nutritional disorders at an early stage to implement corrective procedures in order to optimize yield and produce a successful crop.

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