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Impact of Phosphorus on *Cannabis sativa* Reproduction, Cannabinoids, and Terpenes

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Abstract: Many abiotic factors, such as mineral nutrients—including phosphorus (P)—fertility, can impact the yield and growth of *Cannabis sativa*. Given the economic portion of *C. sativa* is the inflorescence, the restriction of P fertility could impact floral development and quality could be detrimental. This study sought to track the impacts of varying P concentrations (3.75, 7.50, 11.25, 15.0, 22.50, and 30.0 mg·L^{-1}) utilizing a modified Hoagland's solution. This experiment examined plant height, diameter, leaf tissue mineral nutrient concentrations, and final fresh flower bud weight as well as floral quality metrics, such as cannabinoids and terpenes. The results demonstrated that during different life stages (vegetative, pre-flowering, flowering), P concentrations impact *C. sativa* growth and development and yield. Regarding the cannabinoid pools, results varied for the individual cannabinoid types. For the acid pools, increasing fertility concentrations above 11.25 mg·L^{-1} P did not result in any increase in cannabinoid concentrations. These results indicate that, if a crop is being produced under greenhouse conditions, specifically for cannabinoid production, an excessive P supply did not result in higher cannabinoid production. However, plants grown with a higher rate of P fertility (30.0 mg·L^{-1}) had greater plant width and may result in more buds per plant.

Keywords: macronutrients; cannabis; hemp; cannabinoids; terpenes; life-cycle; phosphorus (P); cannabidiol (CBD); tetrahydrocannabinol (THC); foliar

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1. Introduction

United States federal and state laws in recent years have resulted in changes in legal restrictions and definitions of *Cannabis sativa*. Additionally, the consumer and markets have observed 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 *C. sativa* strains with a tetrahydrocannabinol (THC) concentration no greater than 0.3% in any part of the plant (Congress [1,2]). *C. sativa* strains with a THC concentration greater than 0.3% in any part of the plant are considered marijuana and have varying restrictions in legality, distribution, and consumption. High CBD *C. sativa* (hemp) contains over 100 cannabinoids, which include THC, CBD, and cannabigerol (CBG), in varying forms. It is well known that THC has psychoactive effects and has thus resulted in its controversial legal standing. Many health benefits from certain cannabinoids, such as CBD, have been reported. The benefits of CBD have gained popular support due to these health discoveries and the non-psychoactive effects that drug-type cannabis can produce due to low levels of THC.

Hemp has historically been grown for its fiber and seed. The changes in legislation, have recently resulted in a market for flowers and flower derived distillates. 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.

Due to the previously illicit nature of *C sativa*, there is little published research investigating the cultural and production needs of this crop. One of the most limiting factors to obtaining optimal yield is mineral nutrient supply. Given the economic portion of industrial hemp can be the floral material or seeds, phosphorus (P), and other fertilizers are vital for proper plant development and the production of primary and secondary metabolites (Il'in [3]). It is also known that nitrogen (N) fertility plays an important part in the production of THC in *C. sativa* (Bócsa et al. [4]). Very little work has been done in optimal nutrient needs for *C. sativa* in protected environments. The impacts of N, P, and potassium (K) were studied in greenhouse produced *C. sativa*; however, these results only explored a few widely distributed interval concentrations and were completed in soil culture rather than a soilless substrate (Coffman et al. [5]). Critical leaf tissue nutrient ranges have been established for all macro and micronutrients (Cockson et al. [6]). This work found that, with a modified Hoagland's solution regime, *C. sativa* "T-1" plants accumulated 0.43% P in most recently matured leaf tissue with regard to foliage dry weight. Plants grown without P contained 0.09% leaf tissue concentrations. Additionally, a survey of multiple *C. sativa* high CBD cultivars reported an average leaf tissue value of 0.35% P with vegetative mother stock plants (Landis et al. [7]). Work completed by Heard et al. [8] showed that 56 days after planting, macronutrient concentrations in floral portions of fiber varieties started increasing. This study also indicated that seed formation resulted in a huge demand for P resources, and floral material required large N and K resources as well.

These works reported that severe limits to growth and yield are encountered when P resources were limited. From prior work, the general survey range for leaf tissue fertility levels were reported, and the lower deficiency ranges were present for *C. sativa* plants grown in the greenhouse (Landis et al. [7]). Nutrient and accumulation and partitioning were tracked with fiber varieties in the field (Heard et al. [8]). However, no research has been completed on the optimal P concentration for greenhouse-grown hemp. To this end, this study examined the optimal fertility concentrations for were tested utilizing six concentrations. Leaf tissue accumulation, plant growth metrics, and the impact of these P concentrations on the cannabinoid and terpene profiles were measured. Thus, this work seeks to refine the optimal concentrations of P fertility and the impacts that this nutrient can have on the yield of economic portions such as cannabinoids and terpenes in greenhouse production.

2. Materials and Methods

Tip cuttings from *C. sativa* ('BaOx'), a high cannabidiol (CBD) cultivar, mother stock were obtained on 24 July 2019 from tertiary branches from six-month-old mother stock plants. The cuttings were propagated in 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 g/m^3. Cuttings were rooted and grown in a glass greenhouse (35° N Latitude; Raleigh, NC, USA) with average temperatures of 25.8 and 21.3 °C (78.4 and 70.3 °F) day and night, respectively. While rooting, plants were moved to a white plastic covered rooting chamber (1.52 mL × 5.49 mL) with mist nodules (placed every meter in pairs along the length of the bench) running for 4 s every minute and then a week later every two minutes for 6 s. After four weeks of propagation, the plugs were transplanted into 11 L (3 gallon) pots filled with substrate mixed with 80:20 peat perlite (*v:v*) amended with the same parameters as the substrate recipe above (Henry [9]).

The plants were completely randomized (randomized block design) among three benches and subjected to six P (varying from 3.75, 7.50, 11.25, 15.0, 22.50, to 30.0 mg·L^{-1}) concentrations supplied during each irrigation. Macronutrients and micronutrients followed the recipe established by Henry et al. [10] with fertility N and K being held at 150 mg·L^{-1}. Data collection occurred four times throughout the experiment corresponding to the three distinct life stages of *C. sativa* (vegetative,

pre-flowering, and flowering) as well as transplant and harvest. Ten replicates were harvested from each P concentration treatment at each sample date. In *C. sativa*, the use of night interruption or daylight extension can be utilized to keep plants in a vegetative state (Whipker et al. [11]). The critical night length (CNL) in cannabis is thought to be 9–10 h of darkness with 14–15 h of light, though this will vary by cultivar (Whipker et al. [11]). The pre-flowering stage is considered to have been initiated once the CNL is achieved which will result in an incremental change in the plant toward a reproductive stage, or shortly after (1–2 weeks) the induction of the conditions necessary to flower. The final stage of *C. sativa* for this experiment was the flowering stage. This stage was quantified at 8 weeks after the induction of the CNL. Each of the above stages represented a milestone for data collection.

In addition to the above three life stages, incremental data collection points before the first (at 8 weeks after transplant) and after the third data (pre-flowering at 4 weeks after the induction of the CNL) collection points. The first group of data was taken 13 September 2019 and recorded plant growth by measuring plant height from the substrate surface and taking two canopy diameter measurements each at the widest point of the foliage and at 90° from each other. Additionally, substrate pH and electrical conductivity (EC) were measured using a meter (Hanna Instruments: Model 9813-6, Woonsocket, RI) utilizing the PourThru method (Cavins et al. [12]). These data were used to ensure fertility and pH conditions were within acceptable limits and were not deviating, and to calculate a plant growth index. Plants were also treated on the 13th September with a pre-charge of $MgSO_4 \bullet 7\,H_2O$ (239.65 g in 100 L^{-1}) supplied at 500 mL per pot to ensure adequate magnesium (Mg) was provided.

The second round of data (the vegetative stage of *C. sativa*) was taken on 10 October 2019 and recorded plant growth described in the method above. Additionally, whole plants (n = 10) were destructively harvested, and most recently mature leaves were subsampled and rinsed with DI water, washed in a solution of 0.5 M HCl, and again rinsed with DI water. The remaining above ground plant material was placed in a separate container for biomass determination.

The root balls of the above plants were also quantified for root biomass production. This was accomplished utilizing a root washing protocol in which a light stream of water was moved longitudinally down the root ball to wash substrate away from the roots. After the entire circumference of the root ball had been washed, the root ball was subjected to a light agitation submerged in a bucket of water for three minutes. The above protocol was repeated for a minimum of eight times or until the root ball was free of the substrate.

Roots and shoots were separated and dried at 70 °C for 72 h. 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. Total shoot 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. Leaf tissue analysis was completed by the North Carolina Department of Agriculture and Consumer Services (NCDA) testing lab (Raleigh, NC, USA). Ground tissue samples were placed in vials containing ~8 g of tissue and delivered for analysis. Plant material (0.5 g) was treated with nitric acid (10 mLs of HNO_3 at 15.6 N) and was then digested in a microwave digestion system for 30 min (MARS 6 Microwaves, Matthews, NC, USA). After microwave digestion, the plant material was diluted with 50 mLs of deionized water and then vacuum filtered through acid-washed paper (Laboratory Filtration Group, Houston, TX, USA). After dilution, plant mineral tissue concentration was determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) instrument (Spectro Arcos EOP, Mahwah, NJ, USA). More detailed descriptions, calibrations, and procedures can be found at the NCDA [13].

The third round of data (the pre-flowering stage of *C. sativa*) was taken on 7 November 2019. This data collection mirrored the first set of data and took plant growth, pH, and EC measurements as described above. Plants again received an application of $MgSO_4 \bullet 7\,H_2O$ (239.65 g/100 L) supplied at 500 mL per pot.

The final round of data collection (the flowering stage of *C. sativa*) occurred on 5 December 2019. Data were collected on plant growth metrics as well as flower bud collection. Buds (n = 5) were

collected by taking the shoot apical bud (cola bud), four-terminal axillary buds, and four interior canopy buds (Figure 1). Fresh weights were taken of the composite bud samples. Details on bud harvest protocol can be found in the Supplementary Materials (Figures S1–S4).

Figure 1. A reproductive portion of a mature *C. sativa* "BaOx" plant at 8 weeks of reproductive growth after induction of the critical night length (CNL). This portion of the plant consists of the upmost three secondary branches and the shoot apical meristem (SAM) floral raceme (shown in the singular grey oval). The secondary axillary terminal racemes are observed as the two floral structures circled in black. Finally, the three blue circled floral structures are the interior buds. Note that all floral structures are determinate at the nodes.

Upon bud harvest after 8 weeks of CNL induction and reproductive growth, the different bud morphologies and types were harvested for more information please reference the supplemental material and references [14–16] or supplementary citation [1–3]. Buds were then analyzed for cannabinoids and terpenes (Avazyme Inc., Durham, NC, USA). Upon arrival, buds were lyophilized, ground, and a 2 g (1.98–2.02 g) sub-sample from the composite buds obtained. Analysis for cannabinoids was accomplished through high pressure liquid chromatography (SHIMADZU 8050 and 8040 Triple Quadrupole UHPLC/MS/MS analysis; Austin, TX, USA). Exact testing methods are unavailable given that Avazyme is a private company and their testing methods are proprietary.

C. sativa has multiple different cannabinoids and molecular types within each cannabinoid. The active forms of the cannabinoids are cannabigerol (CBG), cannabidiol (CBD), cannabichromene (CBC), and **Δ^9**-tetrahydrocannabinol (Δ^9 THC). These forms are typically considered active given they have been decarboxylated. The other forms are the acid pools of the above cannabinoids which need to be decarboxylated to become the active form (cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), cannabichromenic acid (CBCA), and cannabigerolic acid (CBGA) (Brighenti et al. [17] and Welling et al. [18]). Additional cannabinoids and forms exist but are not reported here, given that their concentrations were either too low to detect, were not tested for, or were present in the same concentrations regardless of P treatment (cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV)). Total CBD and THC were calculated by the following equations (0.877 represents the compensatory mass loss of the carboxyl group upon decarboxylation of the THCA and CBDA molecules):

$$\Delta^9\ \text{THC} + (0.877 \times \text{THCA}) = \text{Total THC};$$

$$\text{CBD} + (0.877 \times \text{CBDA}) = \text{Total CBD}.$$

Finally, terpenes were tested by Avazyme to determine if any differences in concentration were detected based on treatments (α-pinene, myrcene, limonene, eucalyptol, geraniol, and β-caryophyllene). No differences were seen in the 3-carene, cymene, and terpinolene and thus they were not reported given they were either all similar in concentration or were below the analytical threshold to determine any changes.

Statistical analysis was carried out using SAS (version 9.4; SAS inst., Cary, NC, USA). Plant growth metrics, leaf nutrient values, and bud weights were analyzed for differences within each data collection set regarding P concentration as the explanatory variable using PROC GLM. Where the *F*-test was significant, LSD with a Tukey–Kramer adjustment ($p \leq 0.05$) was used to compare differences among means. Deviations in plant metrics, total plant dry weights, leaf tissue values, and bud weights were calculated on a percentage basis from the controls.

Additionally, regression models treated P as the y variable, and the concentration of fertility as the x variable. Each concentration was also subjected to PROC NLIN to determine if the regression model was non-linear and to calculate the P concentration at which the plateau occurred. Equations for regression and non-linear analysis can be found in Henry et al. [9,10]. Regression models were compared, and the polynomial or non-linear model, which resulted in the greatest statistical significance (α = 0.05, 0.01, 0.001) and the greatest r^2 values, were selected if a regression model was determined to be necessary when compared to the GLM results.

3. Results

3.1. Vegetative

3.1.1. Vegetative Stage Plant Growth Metrics (Data Collection 1)

The first group of data was collected 4 weeks after transplant. Given that the plants were still young and developing, no differences were observed in plant height or plant diameter regarding P concentration (Table 1).

Table 1. The impacts of phosphorus (P) fertility on plant growth metrics of *C. sativa* “BaOx” after four weeks of vegetative growth.

P Fertility Concentrations ^{z}	Four Weeks of Growth			
	Height ^{y}		Diameter ^{y}	
	Mean	SD ^{x}	Mean	SD
3.75	40.49 A	7.29	32.28 A	8.91
7.50	41.63 A	10.19	30.21 A	10.30
11.25	42.32 A	6.31	34.00 A	9.15
15.0	45.60 A	3.50	39.22 A	5.64
22.50	48.09 A	5.63	38.35 A	6.59
30.0	47.31 A	4.26	36.32 A	5.29
Significance ^{w}	NS		NS	

^{z} P fertility concentrations based on mg·L^{-1} (ppm). ^{y} All height and diameter measurements based on cm. Diameter was calculated by taking the widest two points on a plant taken 90° from each other. These numbers were then added together and divided by 2 to obtain the diameter measurement. ^{x} All standard deviation values assumed to be ± of the given value. ^{w} *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

3.1.2. Vegetative Stage Plant Growth Metrics (Data Collection 2)

Despite the lack of differences observed in the growth metrics at four weeks into the vegetative growth cycle, statistically significant differences occurred with biomass production, plant height, and diameters after eight weeks of vegetative growth (Tables 1 and 2).

Table 2. The impacts of phosphorus (P) fertility on plant growth metrics of *C. sativa* "BaOx" after eight weeks of vegetative growth.

P Concentrations ^{z}	Height ^{y}		Diameter ^{y}		Total Above Ground Dry Weight ^{y}		Total Below Ground Dry Weight ^{y}		Root to Shoot Ratio ^{x}	
	Mean	SD ^{w}	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.75	63.60 C	3.93	35.30 C	5.51	12.30 B	1.60	4.10 A	1.22	0.34 A	0.12
7.50	70.50 B	4.49	38.25 CB	4.49	20.84 AB	1.49	6.86 A	2.01	0.33 A	0.10
11.25	77.90 A	1.98	51.29 A	5.92	30.66 A	2.98	7.02 A	0.28	0.23 A	0.03
15.0	73.12 AB	2.50	44.40 AB	3.62	24.68 A	6.56	8.06 A	2.79	0.35 A	0.19
22.50	75.78 AB	2.43	49.56 A	2.71	30.70 A	10.43	8.72 A	4.02	0.28 A	0.07
30.0	74.24 AB	2.16	43.58 ABC	3.80	26.18 A	4.25	9.44 A	4.02	0.35 A	0.10
Significance ^{v}	***		***		***		NS		NS	

^{z} P fertility concentrations based on mg·L^{-1} (ppm). ^{y} All height and diameter measurements based on cm. Diameter was calculated by taking the widest two points on a plant taken 90° from each other. These numbers were then added together and divided by 2 to get the diameter measurement. All dry weights were in grams and taken based on oven dried material. ^{x} Root to shoot ratio calculated by dividing the root dry weight by the above ground dry weight. ^{w} All standard deviation values assumed to be ± of the given value. ^{v} *, **, or *** Indicates statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

The shortest plant height corresponded to the lowest P concentration, was statistically shorter than all other concentrations and was 18.3% shorter than the tallest plant grown under 11.25 mg·L^{-1} P (Figure 2). At the lowest three levels of P fertility (3.75, 7.50, and 11.25 mg·L^{-1}), plant heights increased as P fertility increased. After the 11.25 mg·L^{-1} P threshold was reached, there was no statistical difference observed among the higher concentrations (15.0, 22.50, and 30.0 mg·L^{-1}). This would indicate that, in the vegetative state, an increase in P fertility will increase plant height linearly until the 11.25 mg·L^{-1} threshold. After this threshold is reached, no increase in plant height will be observed with increasing fertility. The resultant height at which P fertility plateaued was 75.1 cm when the model was subjected to a non-linear analysis.

Figure 2. Eight-week-old vegetative plants across all P fertility concentrations. Note the decrease in branching and height at the lowest fertility treatments (3.75 and 7.50 mg·L^{-1}).

The plant diameters followed a quadratic trend when correlating changes correlating to P concentrations (Table 2). The trend showed the vertex at the 11.25 $mg{\cdot}L^{-1}$ P concentration with the 3.75 and 30.0 $mg{\cdot}L^{-1}$ P concentrations being statistically similar and comprising the lower rates when compared to the vertex. The greatest plant diameter was observed at 11.25 $mg{\cdot}L^{-1}$ P and the smallest was observed at the lowest P concentration (3.75 $mg{\cdot}L^{-1}$).

3.1.3. Vegetative Stage Plant Growth below Ground Metrics (Data Collection 2)

Differences were observed in plant growth and development regarding above ground plant portions. Given plants were grown in a substrate with a confined rooting environment in pots, and given the correlation between P and root growth, the root biomass production was analyzed. No differences were observed in root biomass production and P concentration (Table 2). Of note, the standard deviations of the root biomass at the higher P concentrations were almost four times higher than those of the lowest concentration. Due to the statistically insignificant correlation between P concentration and root biomass production, the root to shoot ratio was also statistically insignificant (Table 2).

3.1.4. Vegetative Stage Plant Leaf Tissue Accumulation Metrics (Data Collection 2)

To quantify the relationship between P fertility and plant uptake, leaf tissue analysis was conducted on the most recently mature leaves after eight weeks of vegetative plant growth. A strong quadratic relationship was observed in the accumulation of P based on fertility concentration (Figure 3 and Table 3). The lower concentrations (3.75, 11.25, and 15.0 $mg{\cdot}L^{-1}$ P) were all statistically similar with the 7.50 $mg{\cdot}L^{-1}$ concentration showing the lowest accumulation of leaf tissue P. Both higher concentrations (22.5 and 30.0 $mg{\cdot}L^{-1}$ P) resulted in leaf tissue P concentrations which were 2.5 to 3 times greater than the lowest measured leaf tissue concentration (7.50 $mg{\cdot}L^{-1}$ P) concentrations, respectively. The lowest P fertility rate of 3.75 $mg{\cdot}L^{-1}$ P contained 0.28% P. It is thought to be due to the lack of vegetative growth (dry weight) which resulted in the accumulated P in the plant to be higher in concentration in the more compact plant's leaf tissue. The relationship between P concentration and leaf tissue P was determined to be linear and can be modeled by the equation below, where P = the leaf tissue P, and x = the $mg{\cdot}L^{-1}$ P provided.

$$P = 0.2603 - (0.0013(x)) + \left(1.338e - 5\left(x^2\right)\right).$$

The above equation had an adjusted r^2 value of 0.776 (Figure 3). This model should be used with caution when higher levels of P are provided, given an upper range was not observed in this model given there was no leaf tissue plateau in P concentration and given variability which occurs among and even within *C. sativa* cultivars (Cockson et al. [15]).

In addition to P, other macro and micronutrients were also analyzed within leaf tissue samples (Tables 3 and 4). These elements exhibited differences in accumulation or trends regarding increases in P fertility. When altering nutrients within a modified Hoagland's solution, sometimes ionic antagonisms or precipitation can occur if the ions within the solution are drastically altered (Hoagland and Arnon [19]). Additionally, when modifying nutrient concentrations complications in plant uptake, nutrient uptake, and nutrient availability can occur (Bryson and Mills [20]). Hence, increasing P could potentially increase the uptake of magnesium, and could inhibit the availability of calcium, copper, iron, K, and zinc.

Thus, the interactions of the abovementioned elements were analyzed to ensure the nutrient solution alterations did not result in unintended impacts on fertility. Many elements exhibit a decrease in concentration with increasing P, apart from K which stayed constant at all levels. The increases in P concentrations resulted in larger biomass at the higher concentrations which may account for the decreasing trend in concentration observed in the elements due to a dilution effect of the nutrients (Tables 2 and 3).

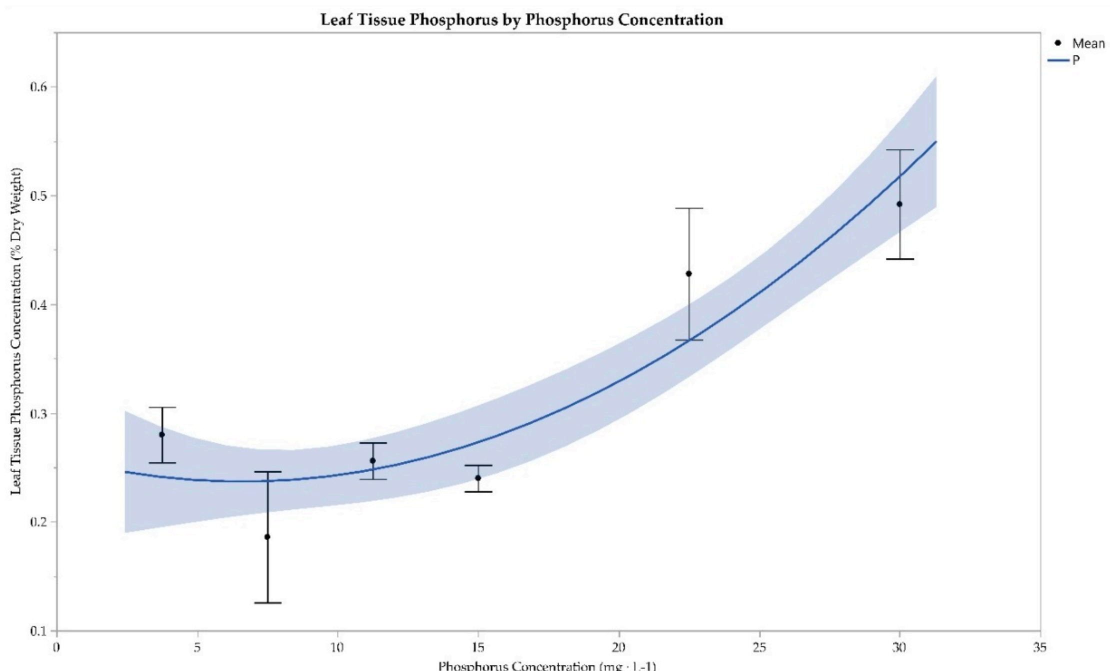

Figure 3. The impacts of phosphorus (P) fertility on P accumulation in leaf tissue of *C. sativa* "BaOx" after 8 weeks of vegetative growth. Graph displays means for each group of plants (n = 10) with error bars representing standard deviations of data and the shaded blue representing the confidence region for the quadratic line of fit.

Table 3. The impacts of phosphorus (P) fertility on macronutrient accumulation in leaf tissue of *C. sativa* "BaOx" after eight weeks of vegetative growth.

P Fertility Concentrations ^{z}	Nitrogen ^{y}		Phosphorus ^{y}		Potassium ^{y}		Calcium ^{y}		Magnesium ^{y}		Sulfur ^{y}	
	Mean	SD ^{x}	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.75	4.61 A	0.13	0.28 C	0.03	2.71 A	0.09	4.05 A	0.11	1.26 A	0.07	0.34 AB	0.01
7.50	4.07 C	0.34	0.17 D	0.05	2.80 A	0.19	3.39 B	0.32	1.07 BC	0.08	0.32 BC	0.02
11.25	4.48 AB	0.22	0.26 C	0.03	2.82 A	0.17	2.85 C	0.15	0.98 C	0.06	0.35 A	0.02
15.0	3.57 D	0.31	0.23 C	0.03	2.65 A	0.20	3.24 B	0.44	1.12 B	0.12	0.28 D	0.01
22.50	4.10 C	0.29	0.42 B	0.05	2.63 A	0.13	2.76 C	0.32	0.97 C	0.06	0.31 C	0.01
30.0	4.23 BC	0.33	0.51 A	0.06	2.80 A	0.17	2.86 C	0.20	0.99 C	0.09	0.31 C	0.03
Significance ^{w}	***		***		NS		***		***		***	

^{z} P fertility concentrations based on mg·L^{-1}. ^{y} All macronutrient concentrations are a percentage of leaf tissue dry weight. ^{x} All standard deviation (SD) values assumed to be ± of the given value. ^{w} *, **, or *** indicate statistically significant differences between sample means based on F-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the F-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

Table 4. The impacts of phosphorus (P) fertility on micronutrient accumulation in leaf tissue of *C. sativa* "BaOx" after eight weeks of vegetative growth.

P Fertility Concentrations ^{z}	Iron ^{y}		Manganese ^{y}		Zinc ^{y}		Copper ^{y}		Boron ^{y}	
	Mean	SD ^{x}	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.75	93.85 AB	6.45	159.50 A	35.29	43.33 A	8.44	5.04A	0.96	99.29 A	11.64
7.50	101.82 AB	10.00	102.44 B	35.37	42.96 A	6.76	3.88 BC	0.44	87.65 B	10.61
11.25	94.51 AB	3.02	78.61 B	19.54	41.28 AB	4.22	4.22 AB	0.50	72.13 C	7.94
15.0	89.01 B	6.71	70.67 B	17.57	40.53 ABC	4.18	2.91 C	0.78	63.17 CD	6.82
22.50	76.30 C	3.72	80.74 B	13.75	33.86 C	3.23	3.48 BC	0.73	60.43 D	4.19
30.0	72.20 C	5.57	82.05 B	31.67	34.39 BC	3.24	3.10 C	0.88	57.13 D	3.88
Significance ^{w}	***		***		***		***		***	

^{z} P fertility concentrations based on mg·L^{-1}. ^{y} All micronutrient concentrations are listed as ppm or mg·kg^{-1}. ^{x} All standard deviation (SD) values assumed to be ± of the given value. ^{w} *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

3.2. Pre-Flowering

Pre-Flowering Stage Plant Growth Metrics (Data Collection 3)

Statistical differences were observed in both pre-flowering height and diameter. Plants displayed an increase in plant height with increasing P concentrations, with the greatest plant height being observed at the highest concentrations. The shortest plant height was observed at the lowest concentration and was 18.7% smaller than the largest plants (Table 5).

Table 5. The impacts of phosphorus (P) fertility on plant growth metrics of *C. sativa* "BaOx" after four weeks of the induction of the critical night length (CNL).

P Fertility Concentrations ^{z}	Twelve Weeks Growth			
	Height ^{y}		Diameter ^{y}	
	Mean	SD ^{x}	Mean	SD
3.75	78.10 B	6.47	59.85 C	6.91
7.50	86.33 AB	9.95	53.04 C	6.10
11.25	84.94 AB	2.03	62.09 BC	6.24
15.0	86.76 AB	6.62	64.52 BC	7.80
22.50	94.76 A	7.42	87.64 A	12.49
30.0	96.04 A	4.50	77.10 A	10.21
Significance ^{w}	**		***	

^{z} P fertility concentrations based on mg·L^{-1} (ppm). ^{y} All height and diameter measurements based on cm. Diameter was calculated by taking the widest two points on a plant taken 90° from each other. These numbers were then added together and divided by 2 to obtain the diameter measurement. All dry weights were in grams and taken based on oven dried material. ^{x} All standard deviation values assumed to be ± of the given value. ^{w} *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

Plant diameter increased with an increase in P concentration with the smallest plant diameter being observed at the 7.50 mg·L^{-1} concentration, and the greatest at the 22.50 mg·L^{-1} concentration. Plant diameters were all similar up to the 15.0 mg·L^{-1} concentration, after which no increase was observed in the higher concentrations (Table 5).

3.3. Flowering

3.3.1. Flowering Stage Plant Growth Metrics (Data Collection 4)

Significant differences were observed in both plant height and diameter with regard to P concentration after 8 weeks (Table 6). Plants increased in both height and diameter as P concentration increased. The greatest plant height was observed at the highest P concentration and resulted in a 22.16% increase in plant height when compared to the lowest concentration (3.75 mg·L^{-1}). Similarly, the greatest plant diameters followed a similar trend with the two highest concentrations (22.50 and 30.00 mg·L^{-1} P) being statistically similar and the highest concentration producing the greatest plant diameter. The lowest P concentration resulted in plants that were 31.7% less in diameter than the highest concentration (Table 6).

Table 6. The impacts of phosphorus (P) fertility on plant growth metrics of *C. sativa* "BaOx" after eight weeks of critical night length (CNL) induction.

P Fertility Concentrations ^{z}	Height ^{y}		Diameter ^{y}		Total above Ground Dry Weight ^{y}		Bud Fresh Weight ^{x}	
	Mean	**SD ^{w}**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
3.75	79.78 C	4.57	62.54 C	9.11	92.23 BC	25.06	32.51 B	4.85
7.50	79.44 C	6.33	65.25 C	5.02	74.60 C	18.97	29.21 B	5.28
11.25	84.46 BC	4.00	73.64 B	2.15	104.17 BC	13.22	37.96 AB	4.23
15.0	86.97 B	2.67	79.94 B	4.53	116.81 B	19.79	39.41 AB	6.35
22.50	94.81 A	5.03	90.425 A	5.33	163.95 A	23.55	43.05 A	13.35
30.0	97.46 A	2.80	91.65 A	4.97	167.98 A	26.03	39.12 AB	5.33
Significance ^{v}	***		***		***		**	

^{z} P fertility concentrations based on mg·L^{-1}. ^{y} All height and diameter measurements based on cm. Diameter was calculated by taking the widest two points on a plant taken 90° from each other. These numbers were then added together and divided by 2 to obtain the diameter measurement. All dry weights were in grams and taken based on oven-dried material. ^{x} Bud fresh weights (g) taken at the time of harvest. Weights include the terminal shoot apical bud, three axillary terminal buds, and three interior buds. ^{w} All standard deviation values assumed to be ± of the given value. ^{v} *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

3.3.2. Bud Fresh Weights

After 8 weeks of floral development, differences were observed in the production of floral material per plant. Plants at the higher P concentrations (22.5 and 30.0 mg·L^{-1}) were more vigorously branched and had wider diameters. Given hemp plants will produce inflorescences at each node, the more nodes per branching structure results in greater floral biomass per plant.

When the terminal apical bud, three-terminal secondary branch buds, and three interior branch buds were harvested, differences were observed in floral production based on P concentration (Table 6). The greatest floral fresh weight was observed at the 22.50 mg·L^{-1} P concentration, which was statistically greater than 3.75 and 7.50 mg·L^{-1} P, although it was statistically similar to the weights observed in the upper four concentrations (11.25, 15.0, and 30.0 mg·L^{-1} P). The lowest bud fresh weight was observed

at the 7.50 mg·L^{-1} P concentration. The 22.50 mg·L^{-1} P concentration resulted in bud fresh weights which were 47.4% greater than the 7.50 mg·L^{-1} P concentration (Table 6).

3.3.3. Cannabinoids

After 8 weeks of the induction of the CNL, differences were observed in both the active and acidic pools of cannabinoids in the floral material sampled. A trend was seen within the acid form of the cannabinoids (CBDA, CBGA, THCA, and total CBD and THC) (Tables 7 and 8). All acid cannabinoid pools were optimized reaching maximum concentrations at the 11.25 mg·L^{-1} P concentration of P and after this rate was reached, an increase in P concentration did not result in statically greater or lesser acid pool cannabinoid levels. This held true for CBDA, CBGA, THCA, as well as total THC, CBD, and total cannabinoids (Tables 7 and 8).

For the active cannabinoid pools, (Δ^9 THC, CBG, CBD, and CBC) all pools showed a plateau in concentration above which any increase in P concentration resulted in statistically similar concentrations. For Δ^9 THC, the highest concentration was seen at 15.0 mg·L^{-1} P above which point, all concentrations were statistically similar. Additionally, the lowest concentration of Δ^9 THC occurred at the lowest P fertility rate (Table 7).

For CBG, the plateau occurred at the 7.50 mg·L^{-1} P concentration. After this concentration, an increase in P fertility did not result in an increase or decrease in the concentration of CBG, given that all values above this point were statistically similar (Table 7).

Both CBD and CBC resulted in a plateau in bud concentration at 11.25 mg·L^{-1} P. After the aforementioned rate, increasing P concentrations did not result in any increase in CBD or CBC given they were all statistically similar (Table 7).

Table 7. The impacts of phosphorus (P) fertility on active cannabinoid pools of *C. sativa* "BaOx" floral material after eight weeks of critical night length (CNL) induction.

P Fertility Concentration ^{z}	Δ^9 THC ^{y}		CBG ^{y}		CBD ^{y}		CBC ^{y}	
	Mean	SD ^{x}	Mean	SD	Mean	SD	Mean	SD
3.75	0.10 A	>0.01	0.71 A	0.14	0.73 A	0.28	0.14 A	0.04
7.50	0.12 AB	0.02	1.04 B	0.24	1.03 A	0.28	0.20 AB	0.05
11.25	0.22 AB	0.06	1.15 B	0.07	2.05 B	0.50	0.32 B	0.07
15.0	0.25 C	0.08	1.12 B	0.10	2.37 B	0.65	0.33 B	0.08
22.50	0.26 C	0.09	0.98 B	0.14	2.30 B	0.70	0.34 B	0.11
30.0	0.24 C	0.03	0.99 B	0.08	2.20 B	0.24	0.31 B	0.04
Significance ^{w}	***		***		***		***	

^{z} P fertility concentrations based on mg·L^{-1}. ^{y} Abbreviations are as follows: Delta 9 Tetrahydrocannabinol (Δ^9 THC), Cannabigerol (CBG), Cannabidiol (CBD), Cannabichromene (CBC). Any variance in the above cannabinoids (CBDA, CBGA, THCA, CBCA, etc.) indicates the acid form of the molecule. The acidic version of the molecule is present in larger quantities in the plant and is converted to the non-acid forms through decarboxylation. Total CBD and THC are calculated on a concentration basis of mg·g^{-1} of a composite sample which had been lyophilized (1.98–2.02 g). All values are expressed in terms of concentration (mg·g^{-1}) of 2 g oven dried composite weight. ^{x} All standard deviation values assumed to be ± of the given value. ^{w} *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

Table 8. The impacts of phosphorus (P) fertility on acid cannabinoid pools, total THC, total CBD, and total cannabinoids of *C. sativa* "BaOx" floral material after eight weeks of critical night length (CNL) induction.

P Fertility Concentration [z]	CBDA [y]		CBGA [y]		THCA [y]		Total THC [y]		Total CBD [y]		Total Cannabinoids [y]	
	Mean	SD [x]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.75	94.06 A	18.70	3.06 A	0.59	3.71 A	0.72	3.32 A	0.67	83.22A	16.58	102.50 A	20.15
7.50	129.36 AB	29.41	4.05 AB	1.12	5.07 AB	1.15	4.56 AB	1.02	114.48 AB	25.97	140.86 AB	31.97
11.25	157.41 B	11.67	5.44 B	0.44	6.18 B	0.47	5.64 B	0.44	140.09 B	10.49	172.77 B	12.91
15.0	151.35 B	14.51	4.94 B	0.62	5.89 B	0.57	5.42 B	0.57	135.11 B	13.33	166.26 B	16.41
22.50	144.08 B	21.05	4.38 B	1.00	5.66 B	0.85	5.22 B	0.83	128.66 B	19.15	158.00 B	23.84
30.0	140.11 B	9.67	4.88 B	0.21	5.49 B	0.38	5.06 B	0.33	125.08 B	8.41	154.23 B	9.99
Significance [w]	***		***		***		***		***		***	

[z] P fertility concentrations based on mg·L^{-1}. [y] Abbreviations are as follows: Delta 9 Tetrahydrocannabinol (Δ^9 THC), Cannabigerol (CBG), Cannabidiol (CBD), Cannabichromene (CBC). Any variance in the above cannabinoids (CBDA, CBGA, THCA, CBCA, etc.) indicates the acid form of the molecule. The acidic version of the molecule is present in larger quantities in the plant and is converted to the non-acid forms through decarboxylation. Total CBD and THC are calculated on a concentration basis of mg·g^{-1} of a composite sample which had been lyophilized (1.98–2.02 g). The "Total" column indicates the concentration of cannabinoids calculated by the equations listed in the materials and methods. All values are expressed in terms of concentration (mg·g^{-1}) of 2 g oven dried composite weight. [x] All standard deviation values assumed to be ± of the given value. [w] *, **, or *** indicate statistically significant differences between sample mean based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

3.3.4. Terpenes

Except for geraniol, all terpenes were statistically similar regardless of P rate. For geraniol, the two lowest P concentrations (3.75 and 7.50 mg·L^{-1}) resulted in statistically higher concentrations of geraniol than all the higher P concentrations (Table 9).

Table 9. The impacts of phosphorus (P) fertility on the terpene concentration of *C. sativa* "BaOx" floral material after eight weeks of critical night length (CNL) induction.

P Fertility Concentration [z]	α-Pinene [y]		Myrcene [y]		Limonene [y]		Eucalyptol [y]		Geraniol [y]		β-Caryophyllene [y]	
	Mean	SD [x]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.75	0.03 A	>0.01	1.60 A	0.34	0.15 A	0.05	0.02 A	>0.01	0.04 A	0.01	0.34 A	0.10
7.50	0.04 A	>0.01	1.02 A	0.12	0.10 A	>0.01	0.01 A	>0.01	0.03 A	0.01	0.35 A	0.04
11.25	0.04 A	0.03	1.22 A	0.65	0.13 A	0.10	0.02 A	>0.01	0.01 B	>0.01	0.38 A	0.04
15.0	0.03 A	>0.01	1.04 A	0.30	0.11 A	0.05	0.01 A	>0.01	0.01 B	>0.01	0.35 A	0.05
22.50	0.03 A	>0.01	1.19 A	0.23	0.13 A	0.03	0.02 A	>0.01	0.01 B	>0.01	0.35 A	0.08
30.0	0.03 A	>0.01	1.09 A	0.28	0.11 A	0.02	0.01 A	>0.01	0.01 B	>0.01	0.32 A	0.06
Significance [w]	NS		NS		NS		NS		*		NS	

[z] P fertility concentrations based on mg·L^{-1}. [y] Total terpenes are calculated on a concentration basis of mg·g^{-1} of a composite sample which had been lyophilized (1.98–2.02 g). All values are expressed in terms of concentration (mg·g^{-1}) of 2 g oven dried composite weight. [x] All standard deviation values assumed to be ± of the given value. [w] *, **, or *** indicate statistically significant differences between sample means based on *F*-test (proc GLM) at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the *F*-test difference between sample means was $p > 0.05$. Values with the same letter indicate a lack of statistical significance while values with different letters indicate statistically significant results.

4. Discussion

4.1. Vegetative Plant Stage

The lack of statistical differences observed in both the height and diameter of the *C. sativa* plants is not surprising, given the earliness of the data collection date. Cannabis is known to be impacted by external factors, such as caliper size, regarding rooting success and vigor of vegetative cuttings (Cockson et al. [21]). These rooting challenges could readily deplete the available resources within the cuttings and result in a decrease in rooting vigor at transplant. This is primarily a result of P resources being involved in root growth and formation (Cockson et al. [21]). Additionally, high CBD cultivars do not have as vigorous a stem biomass production that some *C. sativa* types exhibit, such as those grown for fiber (Heard et al. [8]). Additionally, the lack of statistical differences observed after four weeks of vegetative growth could also indicate that the necessary biomass and nutrient accumulation was not yet impacting growth metrics. This can be seen in Table 1 where differences occurred in plant height and diameter regarding P concentrations, given the plants had longer to grow and the P limitations could be detected.

After eight weeks of vegetative growth, differences were observed in the heights and diameters of *C sativa* plants correlating to P concentration, showing that the lowest P rate resulted in both shorter and narrower plants. However, this difference was only observed between the lowest concentration and the remaining concentrations. This may indicate that a P concentration below 7.50 mg·L^{-1} P may be close to the lower critical level of fertility for hemp during the vegetative stage. Further disparities in growth metrics (plant height and width) were observed at the later stages (pre-flowering and flowering); however, to maintain early vegetative growth, P fertility concentrations below 7.50 mg·L^{-1} may be adequate.

When we compare the leaf tissue values of the lower fertility levels of P, these values were similar to or within the values observed in Bryson and Mills [20] of 0.24–0.49% leaf tissue P. Additionally, the values observed in a survey of hemp plants grown in greenhouse conditions in North Carolina demonstrated that only the upper two P concentrations (22.50 and 30.0 mg·L^{-1}) were within the survey values explored (Landis et al. [7]). This adds credence to the idea that *C. sativa* may need less P resources in the vegetative stage. This trend of lower P fertility needed by crops was observed in the work by Henry et al. [10], who looked at six different species of floricultural crops. This work demonstrated that most species explored received adequate P fertility below the 15.0 mg·L^{-1} P concentration in a greenhouse growth setting.

Additionally, the lower P concentrations may not have produced divergent results, due to the role P plays in plant growth and development. For most plants, the optimal range of P concentration within the plant ranges from 0.3–0.5% plant matter (Marschner [22]). Within plants, P plays an important part in many enzymatic functions within plants by acting as a product, a substrate, or an activating or inhibitory component. Many of these enzymatic functions are directly related to metabolite and secondary metabolic pathways or have direct impacts on the energy available to these pathways (Fredeen et al. [23]). Thus, the impacts of P deficiency would most likely be observed later in the *C. sativa* lifecycle when the developing floral sinks start drawing upon the energy resources within the plant. This has been observed anecdotally in various *C. sativa* operations and visits to grower operations by the authors. Plants which appear normal and healthy in the vegetative stage will produce deficiency symptoms shortly after the induction of short days and the production of floral materials. Furthermore, this was also observed in our experiment, that even the highest concentration of P fertility resulted in some visual symptoms of nutritive stress (Figure 4).

Figure 4. Comparison of a vegetative stage (left) vs. reproductive stage (right) *C. sativa* "BaOx" plant both grown at 30.0 mg·L^{-1} P fertility. Notice specifically that the foliage leaves at the base of every node showing signs of olive green yellowing (right) which is indicative of P deficiency. These symptomologies were categorized in another work and thus are indicative of P deficiency (Cockson et al. [6]).

Thus, the importance of P fertility in the enzymatic pathways and processes, and as a storage source for energy within plants, may indicate that P stress from lower fertility levels may be observed more in the reproductive stage rather than the vegetative stages.

Additionally, even at the highest P fertility concentration, no plateau in leaf tissue accumulation was observed. This may indicate that the upper threshold (adequate and/or luxury uptake) for P fertility within *C. sativa* may be above 30.0 mg·L^{-1}.

4.2. Pre-Flowering Plant Stage

Upon the induction of the CNL, and the consequent initiation of the reproductive phase of *C. sativa*, the plant height and diameters increased though the trends within the P concentrations held relatively constant. For example, the two highest P concentrations (22.50 and 30.0 mg·L^{-1}) resulted in an increase in plant height of 21.3% and 23.0% and an increase in plant diameter of 46.4% and 28.8%, respectively, as compared with the 3.75 mg·L^{-1} P concentration (Table 5). These results indicate that the determinant growth habit of *C. sativa* upon the initiation of the reproductive phase will result in greater lateral expansion compared to horizontal expansion. This may be due to the conical architecture and shape of the *C. sativa* "BaOx" (Figure 4). The differences in bud weight distribution and architecture will vary by *C. sativa* cultivar (Figure 5).

Figure 5. Six *C. sativa* plants of different cultivars (left to right: "Cherry Wine", "Stout", "Sweetened", "Ethephon", "BaOx", and "Suver Haze") exhibiting differences in flowering shape, plant architecture, and branching after eight weeks of flowering stage growth under short day conditions.

4.3. Flowering Plant Stage

The final data taken after 16 weeks of growth, which corresponded to 8 weeks of reproductive growth, may result in the most accurate life stage regarding the impacts of P fertility on plant height and diameter given the determinant nature of *C. sativa*. A small increase in either plant height or diameter was observed between the pre-flowering and flowering stage regarding plant height, regardless of P concentration (Tables 5 and 6). However, plants continued to expand laterally, and final data collection revealed that, at the two highest P concentrations, plants were almost as tall as they were wide (Table 6). Additionally, the greatest total biomass production was observed at the two highest P concentrations (Table 6). These results indicate that, upon the completion of the reproductive stage, the greatest production of vertical, lateral, and biomass was observed at the two highest P concentrations.

While the plant growth metrics were optimized at the two highest concentrations, the fresh bud weight was optimized at the second-highest P concentration (22.50 mg·L^{-1}). This may indicate that a higher P concentration may help to optimize bud fresh weight after 8 weeks of reproductive growth but may not display benefit during the vegetative stage. This may be largely due to the changes in sources and sinks and growth habit (indeterminate during the vegetative stage and determinate at the reproductive stage) given that P is important in the storage of energy within the plant (Table 6).

After the CNL induction, and eight weeks of in floral growth, differences were observed in the cannabinoid profile regarding P concentration. Within the active pool, a plateau was seen at the 11.25 mg·L^{-1} P rate. This indicates that, after a certain P concentration is reached, increasing fertility does not increase the acid pools of cannabinoids in the terminal, terminal axillary, or interior fluorescence of *C. sativa*. This trend held true for the total CBD, THC, and total cannabinoids pools as well. These results may indicate that P fertility has a profound impact on cannabinoid synthesis up to a certain concentration, after which no increase is seen, despite more P resources being available at luxury consumption (Tables 7 and 8).

These results also indicated that the active cannabinoid pools (CBD, CBC, CBG, and Δ^9 THC) within *C. sativa* all show increases up to the 11.25 mg·L^{-1} P concentration, regarding P concentrations. These results also indicate that all active pools are present in some concentration as background levels in floral material. This may be due to oxidative stress, water stress, or heat events causing the acid pool to spontaneously decarboxylate. These results may be useful in refining better standards for testing for floral material, given there is a systemic background concentration of all decarboxylated cannabinoids. This may prove especially helpful regarding Δ^9 THC, given that an average concentration of 0.25 mg·g^{-1} was detected at all fertility rates and in all floral materials above 15.00 mg·L^{-1} P (Table 7).

These results may indicate that, given the biosynthetic pathways of phytocannabinoids, that certain levels of P resource scarcity impact these by downregulating their products. The biosynthesis of phytocanabionids was recently characterized but is being elucidated further to establish the nuances of the pathways regarding other more well-known systems (Sirikantaramas et al. [24]). This work showed that cannabinoid precursors result from two paths; namely, the polyketide pathway which produces olivetolic acid (OLA) and the plastidal 2-C-methyl-D-erythritol 4-phophate (MEP) pathway, which results in geranyl diphosphate (DPP). In the synthesis of GPP, the many intermediary steps result in structural components or active sites and energy resources which all contain P (Fellermeier and Zenk [25], Gagne et al. [26]). Within this pathway, limiting the available P resources would have a large impact on plant energy molecules, such as adenosine triphosphate (ATP) as well as limiting P resources to the MEP pathway itself. Thus, the limiting of these P resources seems to be below the 11.25 mg·L^{-1} concentration, and above this value, no greater increase in the acid pools of the cannabinoids tested occurred.

Terpenes showed no discernable trend of increase or decrease regarding P concentration except for the terpene geraniol (Table 9). This terpene is an important terpene alcohol and is considered a fragrance terpene (Backtorp et al. [27], Chen and Viljoen [28]). Geraniol is bosynthethetically derived from geranyl diphosphate (GPP). In turn, GPP is synthesized from isopentenyl disphosphate (IPP) through the acetate–mevolanate or the plastidic non-mevolonate pathway which involves the pyruvate/triose-phsophate) pathway (Mahmoud and Croteau [29], Bohlmann et al. [30], Chen and Viljoen [28]). Given both the precursor energy molecules (pyruvate/triose-phosphate) and the GPP molecule, involving molecules which have P as a structural component, theoretically, an increase in P would result in an increase in these pathways and the subsequent terpene. However, the inverse was seen with higher geraniol values seen at the lower P concentrations, and the higher concentrations resulting in lower geraniol values. Given geraniol can increase in production during times of stress, these results may indicate that low P concentrations may cause an increase in geraniol due to nutritive stress (Chen and Viljoen [28]).

5. Conclusions

These results indicate that *C. sativa* has different fertility requirements based on the life stage and the end goal of production. For example, if a grower is producing mother stock plants for vegetatively propagated cuttings, plants will remain vegetative throughout their lifecycle. Thus, a concentration of 11.25 mg·L^{-1} P or greater may be adequate for this operation.

If *C. sativa* plants are to be grown for the florescence and/or cannabinoids or terpenes either for the fresh flower market or a distillate market, a P concentration above 11.25 mg·L^{-1} is preferred. While a P concentration of 22.5 mg·L^{-1} resulted in the greatest bud fresh weight when compared to the lowest two concentrations, it did not result in any greater increase in the active or acid cannabinoid pools. Additionally, higher P rates above 22.5 mg·L^{-1} did result in greater lateral production and consequently more nodes to produce the economic portion (floral material). Thus, a follow-up study should be completed to see if the increase in lateral nodes and floral material would result in a greater whole plant yield in floral material, despite the higher concentration of P resources not resulting in greater cannabinoid production in said flowers. Thus, for production in a cannabinoid or distillate market, a P fertility concentration of 11.25 mg·L^{-1} would be adequate, while for fresh

market production, a P fertility concentration may be greater (22.5 mg·L^{-1}) to account for more visually appealing floral material.

Additionally, these results indicate that the luxury consumption level for *C. sativa* regarding plant growth metrics and leaf tissue accumulation was not reached, given that no leveling off or plateauing of leaf tissue P was observed. This may indicate that *C. sativa* requires higher levels of P fertility to reach the uppermost limit of resource accumulation in the leaf tissue. Higher levels of P fertility concentrations should be explored to elucidate the uppermost levels of P resources the plant can acquire in the leaf tissue. Additional screening should be completed with other cultivars to quantify different P fertility needs more accurately for other types of *C. sativa*, given that a wide variety of plant architectures exists within *C. sativa* (Figure 5). Furthermore, the sampling of different plant parts (petioles, stems, roots etc.) for mineral nutrient concentration overtime would help illuminate the accumulation and reallocation of mineral resources within *C. sativa* over its life stages.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/21/7875/s1, Figure S1: The terminal growing tip with four nodes (four blue circles) of a four-week-old vegetative *C. sativa* 'BaOx' plant. Figure S2: This two-week-old *C. sativa* 'BaOx' plant has been induced with long nights and is starting to develop reproductive structures. Figure S3: The eight-week-old *C. sativa* 'BaOx' plant terminal apical florescence is displaying changes in leaflet morphology. Figure S4: Eight-week-old reproductive secondary branch from a *C. sativa* 'BaOx' plant. Note how the internode length decreases (see black arrows) as the plant grows toward its branch terminus.

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