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Elevated Phosphorus Fertility Impact on *Cannabis sativa* 'BaOx' Growth and Nutrient Accumulation

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Abstract

Limited research exists on the fertility requirements for industrial hemp (*Cannabis sativa*) and the impact of fertility on plant growth and cannabinoids. Optimizing floral production for cannabinoid production and especially cannabidiol (CBD) production, is an economic goal for growers. Phosphorus (P) is an essential nutrient for plant growth and plays many key roles in plant growth and when deficient leads to suboptimal plant growth. Grower P fertility rate recommendations vary greatly, with suggestions of up to 196 mg·L^{-1} P over part of the production cycle. Four P fertility rates (15, 60, 120, and 180 mg·L^{-1} P) were evaluated to determine the optimal fertility for *C. sativa* on a high CBD-type cultivar 'BaOx'. Plant height, diameter, and total biomass were similar across all examined P fertility rates. Foliar P concentrations increased linearly, with the greatest P accumulation occurring in plants that received the highest fertility rate of 180 mg·L^{-1} P. Given no differences in biomass production were found, and the luxury uptake of P as fertilization rates increased, the results indicate that rates above 15 mg·L^{-1} P are not beneficial for plant growth and only add economic cost to the grower and potentially cause waste and pollution to the environment.

Keywords: *Hemp, Plant nutrition, Fertilizer rate*

1. Introduction

Limited published research articles exist on the fertility needs of floral hemp (*C. sativa*) and the impact of fertility on plant growth, floral biomass, total biomass as well as the production of secondary metabolites such as cannabinoids. Secondary metabolites utilize great amounts of the plants energy and resources to synthesize (Taura et al., 2007). Thus, these compounds are typically found in very low concentrations in the plant and synthesis depends on the plant's physiological and developmental stage (Akula and Ravishankar, 2011). When plants are nutrient stressed, growth (mass) is often inhibited to a greater extent than photosynthesis, this leads to secondary metabolite concentrations often increasing on a dry weight basis (Seigler, 1998). Limited research has been conducted on the manipulation of macronutrients and their impact on *C. sativa* plant growth and cannabinoid production. A recent publication recommended a P fertility rate of 11.25 to 15 mg·L^{-1} P for *C. sativa* (Cockson et al., 2020). However, in this study higher P rates, greater than 30 mg·L^{-1} P, were not examined. Also, in a tetrahydrocannabidiol (THC) strain, increasing P fertilization resulted in a greater bud weight and a higher THC concentration (Coffman, 1997). Although there is currently a limited number peer reviewed articles regarding optimal P rates of *C. sativa* there are numerous online horticultural industry resources that claim a higher rate of P fertility is needed to achieve optimal cannabinoid concentrations (Whipker et al., 2020). Thus, additional research is needed to examine if elevated P fertility is beneficial to plant growth metrics and cannabinoid production.

The economic concern for optimizing cannabinoid production relies on optimizing floral production. As a result, any factor that limits the floral production of hemp, such as fertility imbalance, would be a concern to growers. It is well known that plants require macro and micronutrients to ensure proper development is achieved. Although many of these essential nutrients for plants are not part of the cannabinoid structure, such as P. Phosphorus still plays many key roles in plant development and if deficient could result in less plant growth.

Phosphorus is a plant essential element and is considered a macronutrient. Phosphorus is required for numerous functions in plant, including energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism and nitrogen fixation (Niu et al., 2019). Plants require P for biomass production and secondary metabolites that are essential for growth such as adenosine triphosphate (ATP). While the under application of P can have negative effects on plant growth, an over application of P can also have adverse effects on plant growth and result in eutrophication. Also, an imbalance of P as a result of an increase in P fertility can inhibit plant uptake of zinc (Zn) resulting in Zn deficiency and can increase the availability and plant uptake of manganese (Mn) (Webb and Loneragan, 1988; Jones, 1998). Thus, applying adequate P fertility to optimize plant growth is a balance that

growers should monitor to optimize economic profits. Phosphorus toxicity in plants often occurs when P concentrations are greater than 1% of dry matter (Marschner, 2011). However, P toxicity levels have not been established for *C. sativa*. Phosphorus toxicity is less likely to occur in field settings due to tie up in the soil in comparison to containers or hydroponic production (Jones, 1998). Greenhouse growers who apply excess levels of P through the use of a NPK fertilizers should monitor foliar concentrations to avoid applying excess P which could lead to P toxicity (Jones, 1998).

The purpose of this study was to investigate the effects of elevated P fertility on the growth and subsequent floral biomass production of *C. sativa*. For growers, a fertility rate that maximizes yield while minimizing input is important.

2. Materials and Methods

High CBD hemp cultivar 'BaOx' (*C. sativa*) cuttings were obtained from 12-week-old mother stock plants. Terminal vegetative exterior canopy cuttings were taken and stuck on 14 Aug. 2020 into 13-cell foam wedge strips (dimensions: HxWxW (5x3.25x2.5 tapering to 1.5 cm)) (#87-50010, Oasis; Kent, OH). The plants were placed under a mist bench in a glass greenhouse (35.78 °N latitude with 23.9°C/18.3°C (75 and 65°F) day/night temperatures) and rooted until the first roots appeared on the outside of the plugs (~2 weeks). After root emergence, the plants were irrigated with a nurse fertilizer solution (33.4 g KNO_3, 33.4 g $Ca(NO_3)_2 \cdot 4H_2O$, 6.6 g KH_2PO_4, 13.2 g $MgSO_4 \cdot 7H_2O$ in 20L H_2O) mixed at 50 mg·L^{-1} N. After three weeks from sticking, rooted plugs were transplanted on 7 Sept. 2020 into 7.57-L plastic pots filled with a custom substrate mix to prevent P nutrient contamination that would occur by using a pH adjusted and fertilizer charged commercial substrate. The substrate was a 70:30 (v:v) mix of Canadian sphagnum peat moss (Conrad Fafard, Agawam, MA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, North Bloomfield, OH), amended with dolomitic limestone [$CaMg(CO_3)_2$ (Rockydale Agricultural, Roanoke, VA)] at 2.3 kg·m^{-3} for pH adjustment to 6.0 and wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ) at 600 g·m^{-3}. Plants were provided night interruption lighting between 22:00 and 2:00 during the vegetative stage to prevent floral initiation.

2.1 Fertilization Treatments

All fertilizers were custom blends of the following individual technical grade salts (Fisher Scientific, Pittsburgh, PA): calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$], potassium nitrate (KNO_3), monopotassium phosphate (KH_2PO_4), potassium sulfate (K_2SO_4), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), magnesium nitrate [$Mg(NO_3)_2$], monopotassium phosphate (KH_2PO_4), sodium phosphate heptahydrate ($NaH_2PO_4 \cdot 7H_2O$), iron chelate (Fe-DTPA), manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), zinc chloride heptahydrate ($ZnCl_2 \cdot 7H_2O$), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), boric acid (H_3BO_3), and sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$).

Fertilization treatments began the day of transplant. Four fertility concentrations of 15, 60, 120, and 180 mg·L^{-1} P were mixed using the previously described salts and when creating the fertilizer mixtures all other elements were held constant when possible (Table 1). Fertilizers were mixed in 100-L barrels and applied through drip irrigation as needed at every irrigation with an estimated 10% leaching fraction. The solution was delivered via pumps (model 1A; Little Giant Pump Co., Oklahoma City, OK) connected to 1.9-cm-diameter irrigation tubing fitted with circular drip emitters (Dramm USA, Manitowoc, WI). The solution and substrate pH were monitored to ensure values were within the recommended range of 5.5 to 6.5 (Whipker et al., 2019).

At the date of transplant, there were 10 single-plant replicates grown for each of the four examined fertilizer concentrations (15, 60, 120, and 180 mg·L^{-1} P). After four weeks of vegetative growth, on 12 October, night interruption lighting was curtailed to induce floral initiation.

Table 1. Macro- and micronutrient fertilizer concentration by phosphorus (P) treatment utilized in this research study.

2.2 Plant Materials

After eight weeks of floral development, plant height and diameters were measured, five plants were destructively harvested, and the most recently matured leaves were initially rinsed with deionized water, then washed in a solution of 0.5 M HCl for 1 min and again rinsed with DI water (Henry et al., 2017). The remaining shoot tissue was harvested separately, and roots were discarded.

Upon sampling, the plant tissues and the remaining above-ground plant biomass were dried at 70 °C for 96 hours, and the dry mass was weighed and recorded. After drying, leaf tissue was ground in a Foss Tecator

Cyclotec™ 1093 sample mill (Analytical Instruments, LLC; Golden Valley, MN; ≤0.5 mm sieve). The ground tissue was then placed in vials containing ~8 g of tissue and analyzed at the North Carolina Department of Agriculture & Consumer Services (NCDA) testing lab (Raleigh, NC). Plant material (0.5 g) was first rinsed in nitric acid (10 mLs of HNO_3 at 15.6N) and digested in a microwave digestion system for 30 minutes (MARS 6 Microwaves; Matthews, NC). 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). After dilution, plant mineral tissue concentration was determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) machine (Spectro Arcos EOP; Mahwah, NJ).

2.3 Statistical Analysis

Statistical analysis was conducted using SAS (version 9.4; SAS inst., Cary, NC). Plant growth metrics, leaf nutrient values, and bud weights were analyzed for differences within each data collection regarding P concentration as the explanatory variable using PROC GLM. Where the *F*-test was significant, LSD with a Tukey Kramer adjustment ($P < 0.05$) 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.

3. Results and Discussion

3.1 Growth Metrics

After twelve weeks of total growth (four weeks of vegetative growth and eight weeks of floral growth), there were no significant differences in plant height, diameter, and total above ground biomass produced for any of the examined rates (Table 2). Plants that received a P fertility rate of 60 $mg \cdot L^{-1}$ P produced the greatest total bud weight, a 20.7% increase, when compared to plants that received 120 $mg \cdot L^{-1}$ P which produced the lowest total bud weight (Table 2). Plants appeared visually similar and bud development was uniform across all fertility treatments (Fig. 1). While P toxicity symptoms can be characterized as leaf puckering and grayish-brown marginal necrosis (Cakmak and Marschner, 1986), these symptoms were not observed at any of the examined rates on *C. sativa*. Thus, greater rates than examined by this study would need to be conducted to determine P-toxicity leaf tissue symptomology.

Figure 1. Effect of phosphorus ($mg \cdot L^{-1}$) on *Cannabis sativa* 'BaOx' growth at the flower stage (after 4 weeks of vegetative growth and 8 weeks of reproductive growth).

Macronutrients ($mg \cdot L^{-1}$)						
P ($mg \cdot L^{-1}$)	N	P	K	Ca	Mg	S
15	150.1	15.1	150.0	125.5	54.2	53.9
60	150.0	60.1	150.3	128.7	72.0	53.9
120	150.2	120.2	150.2	128.7	93.1	53.9
180	150.2	180.3	150.2	128.7	45.0	53.9
Micronutrients ($mg \cdot L^{-1}$)						
	Fe	Mn	Cu	Zn	B	Mo
All P rates	4.02	0.99	0.19	0.20	0.49	0.01

Table 2. The impacts of phosphorus fertility on plant growth metrics of *Cannabis sativa* 'BaOx' at the flower stage (twelve weeks of total growth; four weeks of vegetative growth and eight weeks of floral development).

	Twelve Weeks of Growth							
P ($mg \cdot L^{-1}$)[1]	Height[2]		Diameter[2]		Total Above Ground Dry Weight[2]		Total Bud weight[2]	
	Mean	SD[3]	Mean	SD[3]	Mean	SD[3]	Mean	SD[3]
15	61.14 A	2.69	63.86 A	2.35	50.58 A	5.10	26.22 AB	1.63
60	62.78 A	2.84	62.77 A	4.47	57.82 A	4.46	28.75 A	3.30
120	63.34 A	3.40	59.34 A	4.31	49.34 A	9.31	23.82 B	3.63
180	60.16 A	1.86	61.52 A	4.00	51.06 A	5.07	26.32 AB	1.50
Significance[4]	NS		NS		NS		*	

[1] Phosphorus fertility rates based on $mg \cdot L^{-1}$.
[2] All height and diameter measurements based on cm. The diameter was calculated by taking the widest two points on a plant taken 90º 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.
[3] All standard deviation values assumed to be $\pm$ of the given value.
[4] *, **, or *** indicates statistically significant differences between sample means based on *F* test 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 $P > 0.05$. Where the *F*-test was significant, LSD with a Tukey Kramer adjustment ($P < 0.05$) was used to compare differences among means.

3.2 Foliar Nutrient Accumulation

To determine the relationship of P fertility and plant uptake, leaf tissue analysis was conducted on the most recently matured leaves (MRML) after twelve weeks of total growth. A linear relationship was observed in the accumulation of P based on fertility concentration (Table 3). Foliar P-concentrations were maximized at 180 $mg \cdot L^{-1}$ P. 'BaOx' plants grown at 180 $mg \cdot L^{-1}$ P foliar concentrations were significantly greater than plants grown at all other concentrations and accumulated 69% more P than plants grown at the lowest concentration of 15 $mg \cdot L^{-1}$ P (Table 3). While there was an increase in P accumulation, however there is not an increase in growth, thus applying a P fertility concentration greater than 15 $mg \cdot L^{-1}$ P would be considered luxury consumption for *C. sativa*. Thus, growers will not see an economic yield increase with higher P fertility.

One study conducted by Landis et al. (2019) determined that healthy and vigorous plants contained P leaf tissue concentrations of 0.31-0.44% P. Other researchers studied the impacts of P deficiency on leaf tissue P accumulation of *C. sativa* and reported that plants provided with a modified Hoagland solution accumulated 0.43% P, while plants grown without P contained 0.09% P (Cockson et al. 2019). In this study plants that received a P fertility concentration of 15 $mg \cdot L^{-1}$ P were within these ranges and contained a foliar leaf concentration of 0.42% P (Table 3). While all higher examined concentrations (60, 120, and 180 $mg \cdot L^{-1}$ P) were above the recommended ranges and these higher foliar concentrations could be considered luxury consumption and are not necessary for optimizing plant growth (Table 3).

Phosphorus toxicity in plants is reported when P concentrations are greater than 1% of dry matter (Marschner, 2011). However, even with the 180 $mg \cdot L^{-1}$ P fertility rate, plants did not accumulate P at an elevated concentration to be considered toxic. Thus, additional research would need to be conducted to determine the foliar concentration at which P-toxicity and plant growth depression occurs for *C. sativa*.

Other macro and micronutrients were also analyzed within MRML tissue samples (Table 3). 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, 1950). We tested the nutrient solutions and nutrient levels were at the target values. Additionally, with modifying nutrient fertilizer recipes, nutrient uptake nutrient availability antagonisms can occur (Bryson and Mills, 2014). Thus, increasing P could potentially increase the uptake of magnesium (Mg), and could inhibit the availability of calcium (Ca), copper (Cu), iron (Fe), potassium (K), and Zn. Magnesium concentration was lowest with 180 $mg \cdot L^{-1}$ P and reflects the lower amount of Mg used to create the fertilizer formulation (Table 1). Other elements reported to have antagonistic effects when P is increased (Cu, Fe, K, and Zn), were not negatively impacted by increasing P to 180 $mg \cdot L^{-1}$.

Although P accumulation in the MRML increased linearly and a plateau was not reached for foliar nutrient accumulation, however similarities in growth metrics was observed with no statistical difference being observed for any of the examined P fertility rates for plant height, diameter, or total biomass production. These results reinforce that P plays an important role in clone establishment and the recommended P fertility rate of 11.25 to 15 mg·L^{-1} P (Cockson et al., 2020) is adequate for promoting plant growth when compared to greater P fertility rates.

Table 3. The impacts of phosphorus fertility on the nutrient accumulation of *Cannabis sativa* 'BaOx' at the flower stage (twelve weeks of total growth; four weeks of vegetative growth and eight weeks of floral development).

	'BaOx' Nutrient Accumulation After Twelve Weeks of Growth										
P (mg·L^{-1})[1]	N[2] Mean	P[2] Mean	K[2] Mean	Ca[2] Mean	Mg[2] Mean	S[2] Mean	Fe[3] Mean	Mn[3] Mean	Zn[3] Mean	Cu[3] Mean	B[3] Mean
15	2.37 B	0.42 C	2.58 C	5.62 A	1.96 B	0.28 B	105.22 B	118.00 BC	21.84 B	2.44 C	185.60 A
60	2.18 B	0.57 B	3.63 B	4.71 B	1.95 B	0.31 B	76.58 C	114.12 C	28.46 B	2.52 BC	181.20 A
120	2.32 B	0.56 B	3.62 B	4.70 B	2.24 A	0.30 B	105.80 B	221.60 AB	44.54 A	3.20 B	200.60 A
180	2.71 A	0.71 A	4.24 A	3.91 C	1.24 C	0.36 A	150.60 A	260.00 A	50.74 A	4.64 A	176.60 A
Significance[4]	*	***	***	**	***	**	***	*	***	***	NS

[1] Phosphorus fertility concentrations based on mg · L^{-1}.
[2] All macronutrient concentrations are a percentage of leaf tissue dry weight.
[3] All micronutrient concentrations are listed as ppm or mg · kg^{-1}.
[4] *, **, 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.

4. Conclusion

Growing 'BaOx' *C. sativa* with a fertility rate of 15 mg·L^{-1} P provided similar plant height, diameter, and total above ground biomass produced in comparison to all other examined higher rates. This 15 mg·L^{-1} P rate optimized plant growth without deficiency symptoms or stunting growth due to an over or under application. Although a plateau was not reached for the foliar accumulation of P, plant growth metrics were not significantly impacted by the variation of P fertility. These results demonstrate that while P plays a large role in plant growth and development, a fertility rate of 11.25 to 15 mg·L^{-1} P recommended (Cockson et al., 2020) will provide optimal growth. Thus, growers can optimize yield and limit economic inputs without suppling the plant with greater P fertility rate that would promote luxury consumption.

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