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Nutrient Management of Cannabis in Controlled Environments

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NUTRIENT MANAGEMENT OF CANNABIS IN CONTROLLED ENVIRONMENTS

by

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ABSTRACT

Nutrient Management of Cannabis in Controlled Environments

by

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Adequate nutrition is vital for yield and quality, but excessive fertilizer application is unsustainable, harmful to the environment, and promotes luxury uptake. In medical cannabis production nutrient management programs utilize high nutrient application with an emphasis on high P to promote yield and cannabinoid production. Another common practice in cannabis production is preharvest flushing. Preharvest flushing is thought increase quality by reducing chlorophyll and nutrient concentrations in flower. The objectives of this research were to determine the effects of excessive nutrient application and flushing on yield, cannabinoid concentration, and nutrient partitioning. We utilized closed system hydroponics to investigate the effects of two nutrient solution concentrations (electrical conductivities [EC] of 2 to 4 mS/cm), and five phosphorus (P) inputs (15, 30, 45, 60, and 90 mg/L). There was not a significant effect of EC or P input on yield or cannabinoid concentration. Yield across all treatments was 640 ± 88 g per square m (mean ± *SD*) and flower concentrations of cannabidiol (CBD_{eq}) and Δ-9 tetrahydrocannabinol (THC_{eq}) were 13.55% ± 0.85 and 0.59% ± 0.04, respectively.

Increasing nutrient input increased nutrient accumulation in the solution. At harvest, P in the recirculating solution of treatments receiving 90 mg/L P had increased to more than 300 mg/L. Phosphorus input had a significant effect on tissue P; leaf P concentrations doubled and flower P increased 70% when the P input was increased from 15 to 90 mg/L. The flushing study, composed of two experiments, examined the effects of flushing plants grown in soilless media with nitrogen (N) free solution and tap water for seven, fourteen, and twenty-one days before harvest. Visually, flushed plants were chlorotic with increasing severity as the duration of flushing increased. Tissue analysis revealed decreasing leaf N in all treatments. However, leaf N decreased more rapidly in flushed plants. At harvest leaf N was lowest in flushed plants. Flushing did not reduce flower N which was similar across all treatments. Flushing with tap water decreased yield, but total cannabinoid yield was unaffected.

(96 pages)

PUBLIC ABSTRACT

Nutrient Management of Cannabis in Controlled Environments

Julie A. Hershkowitz

Cannabis has been cultivated for millennia as a multipurpose crop for food, fiber, and medicine. Secondary metabolites called cannabinoids, including cannabidiol (CBD) and the psychoactive Δ-9 tetrahydrocannabinol (Δ-9 THC), are responsible for the medicinal properties of cannabis. The intoxicating nature of THC resulted in legislation prohibiting the possession and production of cannabis within the United States during the 20th century. As a result, research programs on cannabis production were halted at most institutions. In recent years, cannabis production has become widespread within the US as a result of increasing legalization. However, modern cannabis production nutrient management programs often include holdover practices from prohibition era clandestine production which are often based on anecdotal evidence. Practices thought to increase yield and cannabinoid production include excessive fertilizer application and preharvest fertilizer deprivation (flushing). However, increasing commercial production have increased scrutiny on the environmental impact of cannabis production.

This thesis encompasses the results of two studies pertaining to nutrient management of medical cannabis in controlled environments. The first study investigated the effects of nutrient solution concentration and phosphorus (P) supply on plant development, flower yield, cannabinoid production, nutrient use efficiency and nutrient partitioning in closed system, deepwater hydroponics. The second study examines the effects of preharvest nutrient deprivation, a practice colloquially known as flushing, on yield, cannabinoid

production and nutrient partitioning of medical cannabis. The results from the first study indicated that excessive nutrient supply did not increase flower or cannabinoid yield. Moreover, nutrient concentrations within the recirculating solution and tissue increased with increasing nutrient supply. Applied nutrients that are not utilized by the plants can enter the environment as a pollutant. In the second study preharvest nutrient deprivation did not increase flower quality or total cannabinoid yield. Moreover, a common reason used to advocate flushing is the reduction of flower tissue nitrogen (N) content, which is thought to negatively impact sensory qualities during smoking. In the leaves visual symptoms consistent with nutrient deficiency were evident within one week of initiating flushing treatments. Nutrient analysis of leaf and flower tissue showed decreasing leaf concentrations of N, but flower concentrations were unchanged.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Medical cannabis (*Cannabis sativa* L.) is grown primarily for the production of cannabinoids, which may be useful in medical and therapeutic applications. Widespread legalization and commercial production in recent years has increased the resources devoted to cannabis and the environmental impacts (Wilson et al., 2019). Excessive fertilizer application is known to be harmful to the environment, but is a common practice in medical cannabis production (Chandini et al., 2019; Savci, 2012; Ward et al., 2018). Excess fertilizer enters aquatic systems through erosion, runoff, and leaching, where high nutrient levels promote eutrophication and the death of aquatic life (Chislock et al., 2013). Furthermore, the consumption of water high in nitrate (NO_3^-) has been linked to numerous diseases in humans including birth defects, methemoglobinemia, hypertension, cancers, and thyroid disease (Chandini et al., 2019; Ward et al., 2018).

High phosphorous (P) application during flowering is thought to promote flower and cannabinoid production in medical cannabis (Bevan et al., 2021). Most agricultural P is derived from mined rock phosphate, a finite resource. At the current rate of usage, the global P demand is forecasted to exceed supply by the year 2050 (Nedelciu et al., 2020) necessitating the optimization of P application to mitigate potential food supply issues in the future (Childers et al., 2011).

A primary goal in sustainable agriculture is to optimize fertilizer use. Nutrient use efficiency (NUE) quantifies how effectively a crop utilizes fertilizer input to produce yield. Excessive fertilizer application reduces NUE by promoting nutrient loss to the environment. Excessive fertilizer application promotes luxury uptake, which is the

accumulation of nutrients in plant tissues beyond the necessary level for growth and yield (Hirel et al., 2011; Shiponi and Bernstein, 2021). Numerous studies have reported luxury uptake in cannabis.

In addition to excessive fertilizer application, flushing is another nutrient management practice thought to improve quality (Dutch Passion Blogs, 2021). In cannabis production, flushing is nutrient deprivation during the preharvest stage, that is, one to two weeks before harvest. Flushing is thought to decrease nutrient concentrations in flower. Anecdotal accounts suggests that flushing improves the organoleptic qualities of cannabis during smoking.

The objectives of these studies are to examine the effects of nutrient solution concentration, P input, and preharvest nutrient deprivation on yield, secondary metabolite production and nutrient partitioning of medical cannabis grown in controlled environments.

Botany

Cannabis is an annual, herbaceous member of the family *Cannabinaceae* (Small, 2020; McPartland, 2018). Current taxonomic classification describes cannabis as one species, *Cannabis sativa* L., with three subspecies, *Cannabis sativa.* subsp. *sativa, Cannabis sativa.* subsp. *indica, Cannabis sativa.* subsp. *ruderalis* (McPartland, 2018). Cannabis is a dioecious (occasionally monoecious), wind pollinated plant (Schilling et al., 2021). Pistillate (female) plants produce flowers in densely branched inflorescences at the apex of stems and leaf axils (Spitzer-Rimon et al., 2019). The bracts and calyxes of female flowers are densely covered in stalked, glandular trichomes, which synthesize and

store secondary metabolites like cannabinoids, terpenes, and flavonoids (Andre et al., 2016; Melzer et al., 2022; Tanney et al., 2021). Staminate (male) flowers produce four to six pendulous anthers and occur in clusters at leaf axils and stem apices (Tanney et al., 2021). Male flowers produce far fewer trichomes and secondary metabolites than female flowers (Tanney et al., 2021).

Medical cannabis is typically grown in controlled environments as a horticultural crop. Grown primarily for the production of trichome rich, female flowers, medical cannabis is propagated by feminized seed or cuttings collected from female plants. Seed-grown plants are monitored for males, which are culled. Production is divided into the vegetative stage and the flowering stage. During the vegetative stage plants are grown under long days with a photoperiod of 12 hours or more. To induce and maintain flowering the plants are transitioned to short days with a photoperiod of 12 hours or fewer.

Secondary Metabolite Production

Medical cannabis is grown for the production of secondary metabolites including cannabinoids and terpenes. Recent studies suggest that cannabinoid have potential as treatments for chronic physical and mental ailments (García-Gutiérrez et al., 2020; Pellati et al., 2018). Over 90 cannabinoids and 100 terpenes have been identified in cannabis (Andre et al. 2016). Terpenes, volatile carbon-based molecules, contribute to the fragrance and flavor of cannabis while cannabinoids are responsible for the psychoactive effects (Andre et al., 2016). The principal cannabinoids of interest are the intoxicating

delta-9-tetrahydrocannabinol (Δ^9-THC), and the nonintoxicating cannabidiol (CBD) and cannabigerol (CBG) (Andre et al., 2016).

In the United States, under the Controlled Substances Act of 1970 (CSA), cannabis is classified by the Drug Enforcement Administration (DEA) as a Schedule I controlled substance, making it federally illegal to possess cannabis or any of its derivatives (Mead, 2019). In 2018, the "Farm Bill" (US House of Representatives, 2018) was enacted, authorizing the legal production of hemp, cultivars with ≤ 0.3% THC on a weight per weight basis. The cultivation of high THC cultivars (≥ 0.3% THC, classified as marijuana) remains federally illegal. However, at the state level, programs authorizing marijuana production for medical use have been approved in 38 states and Washington, DC. Additionally, 24 states have approved the production and sale of recreational marijuana (Matthews and Hicky, 2023). Legal provisions require commercial producers to be licensed with the United States Department of Agriculture (USDA). Licenses are specific for hemp or marijuana production and hemp producers are required to prove compliance by having flower samples analyzed for cannabinoid concentration by a USDA approved laboratory. Hemp flower samples with THC concentrations ≥ 0.3% are considered noncompliant and required to be destroyed in accordance with the CSA (Yang et al., 2020).

Cannabinoid and terpene profiles are primarily under genetic control (Aizpurua-Olaizola et al., 2016; Welling et al., 2016). Numerous cultivars have been developed through selective breeding with vastly different terpene and cannabinoid profiles. Cultivars are categorized by their cannabinoid profiles into chemotypes, also called

chemovars (Tanney et al., 2021). The cultivar chemotype is evident at flower induction (Aizpurua-Olaizola et al., 2016; Welling et al., 2016). There are five chemotype classes:

Type I: THC predominant

Type II: Equal THC to CBD ratio

Type III: CBD predominant

Type IV: CBG predominant

Type V: Low or no cannabinoid content

However, cannabinoid production may be affected by environmental factors including light quantity and quality (Danziger and Bernstein, 2021a; Morello et al., 2022), drought (Park et al., 2022), and nutrient supply (Bernstein et al. 2019a; Saloner and Bernstein, 2021; Saloner and Bernstein, 2022a; Saloner and Bernstein, 2022b; Song et al., 2023). Additionally, recent studies have reported temporal and spatial variations in cannabinoid concentration (Aizpurua-Olaizola et al., 2016; Bernstein et al., 2019b; Danziger and Bernstein, 2021b; Yang et al., 2020). Cannabinoid concentrations increase over the duration of flowering, typically peaking six to seven weeks after flower induction, however, the specific period of peak cannabinoid concentration is cultivar dependent (Aizpurua-Olaizola et al., 2016; Yang et al., 2020). Plant architecture and inflorescence location also affect cannabinoid concentration and upper canopy inflorescences have higher cannabinoid concentrations than understory and intracanopy inflorescences (Bernstein et al., 2019b; Danziger and Bernstein, 2021b). However, spatial variation in cannabinoid concentrations can be mitigated by manipulating plant architecture using pruning and training techniques (Danziger and Bernstein, 2021b).

Hydroponics

In controlled environments, cannabis is grown hydroponically or in soilless mediums composed of coco coir, peat, or composted forest products (Nemati et al., 2021; Zheng 2019; Zheng, 2022). Soilless mediums like coir and peat have relatively high cation exchange capacities (CEC) and adsorb cations like ammonium (NH^{+}_{4}), potassium (K), calcium (Ca), manganese (Mn), magnesium (Mg), zinc (Zn), and copper (Cu) (Silber, 2019). This creates nutrient reserves in the media, but also reduces nutrient availability by removing cations from the soil solution.

The word hydroponics is derived from the Greek "hydro" meaning water and "ponos" meaning labor and describes the method of growing plants without soil. There are numerous hydroponic systems and techniques including deep-water, ebb-and-flow, nutrient film technique (NFT), aeroponics, and aquaponics (Sardare and Admane, 2013). A hydroponic system can be further classified by the solution management technique as open or closed. In open hydroponic systems fresh nutrient solution is used for each irrigation event and excess solution is drained off and disposed as waste (Hosseinzadeh et al., 2017). In closed systems, the nutrient solution is recycled and replenished periodically and individual ion concentrations are monitored and restored as needed (Hosseinzadeh et al., 2017). Closed systems often incorporate sterilization or filtering of the recirculated solution to mitigate pathogen infection (Sutton et al., 2006).

Roots may be suspended in nutrient solution or supported by a natural or synthetic material like perlite, mineral (rock) wool, clay pebbles, gravel, or sand (Sardare and Admane, 2013; Swain et al., 2021; Velazquez-Gonzalez et al., 2022). The definition of a hydroponic medium or system varies and some sources classify systems that utilize

soilless mediums like coir and peat as hydroponic (Savvas and Gruda, 2018). Here, hydroponics is differentiated from soilless media production by the specific, innate chemical properties of the media and to be considered a hydroponic system the media must be inert, lacking CEC and buffering capacity.

Solution Concentration and Electrical Conductivity

The total ionic concentration of a nutrient solution can be quantified by the electrical conductivity (EC; mS/cm) of the solution. In commercial production, EC is commonly used as a metric for fertilizer input with a species-dependent range of 0.8 to 4 mS/cm (Singh and Dunn, 2017). However, EC is not an accurate indicator of individual nutrient levels. Solution EC is mostly attributed to macronutrient—calcium (Ca), sulfur (S), nitrogen (N), and phosphorous (P)—concentrations with micronutrients contributing less than 1% (Bugbee, 2004). The differential uptake of nutrients can lead to imbalances in recirculating solutions, which are exacerbated by excessive concentrations in the refill solution (Bugbee, 2004). Nutrients with active uptake—N, P, K, and Mn—are quickly depleted in the recirculating solution after addition, while nutrients with intermediate or passive uptake—Ca, Mg, S, and Cu—tend to accumulate (Bugbee, 2004; Savaas et al., 1999). This can result in imbalances, precipitation, ion antagonism, and toxicity (Bugbee, 2004; Kaya et al., 2001; Savaas et al., 1999; Singh and Dunn, 2017). Additionally, the low CEC and buffering capacity of hydroponic systems increases the risk of ion toxicity, especially in closed systems (Langenfeld et al., 2022; Savvas and Gizas, 2002).

Effects of the Environment on Nutrient Uptake

Environmental parameters including light intensity, temperature, relative humidity, air movement, CO_2 concentration, water status, and the vapor pressure deficit (VPD) have direct and indirect effects on nutrient use (Lopez et al., 2021). Stomatal aperture regulates the movement of CO_2 and water vapor between the plant and the environment (Buckley, 2017). Transpiration, the loss of water through stomata, drives the mass flow of nutrients from roots to leaves (Cramer et al., 2008; Vadez et al., 2014). Water use efficiency (WUE) is the grams of biomass produced per liter of water transpired (Hatfield and Dold, 2019; Sinclair et al., 1984). Decreased transpiration increases WUE and reduces nutrient uptake (Hatfield and Dold, 2019; Sinclair et al., 1984). Carbon dioxide supplementation, a common practice in controlled environments, increases WUE by inducing stomatal closure (Chandra et al., 2008; Cramer et al., 2008; Langenfeld et al., 2022). In controlled environments, with supplemental CO_2, WUE can range from 4 to 6 g/L (Langenfeld et al., 2022). In cannabis, Chandra et al., (2008) reported a 111% increase in WUE in response to a roughly twofold increase (from 350 to 750 μM/mol; ppm) in atmospheric CO_2.

The vapor pressure deficit (VPD) also affects WUE and nutrient use. The VPD is the difference between the water vapor pressure in the atmosphere and water vapor pressure within the leaf (Vadez et al., 2014). The leaf vapor pressure is primarily a function of leaf temperature since the relative humidity within the leaf is always 100%, while the vapor pressure of the surrounding air is a function of temperature and relative humidity (Vadez et al., 2014). A low VPD (<0.3 kPa) indicates that the water content of the air is near the saturation point while a high VPD (>1.5 kPa) indicates that the air has a

large water holding potential (Vadez et al., 2014). A Low VPD decreases the rate of water loss through transpiration since the vapor pressure of the air is closer to that of the leaf interior, while a high VPD (>1.5 kPa) increases water loss (Grossiord et al., 2020; López et al., 2021; Vadez et al., 2014). Increased WUE requires a more concentrated nutrient solution to achieve the desired tissue nutrient concentrations (Langenfeld et al., 2022). The optimal nutrient solution concentration can be calculated using WUE and the desired tissue concentration.

Mass Balance

The principles of mass balance are useful for tracking nutrient use and designing efficient plant nutrition programs (Langenfeld et al., 2022). Mass balance assumes that all nutrients applied to a system are either taken up by the plant or remain in the solution (Langenfeld et al., 2022). Tissue and solution analysis are used to calculate total nutrient recovery at harvest (Langenfeld et al., 2022).

Solution Concentration and Osmotic Stress

A high ion concentration increases solution osmotic potential, reducing water potential (Luque and Bingham, 1981). Water potential quantifies how freely water can move from one place to another and the equation is: $\Psi_{total} = \Psi_s + \Psi_p + \Psi_g + \Psi_m$, where Ψ_s is the solute or osmotic potential, Ψ_p is the pressure potential, Ψ_g is the gravitational potential, and Ψ_m is the matric potential (Slatyer and Taylor, 1960). Water potential drives the Soil-Plant-Atmosphere-Continuum which is the cycle of water movement from the media through the plant to the atmosphere and back to the media (Ochsner et al.,

2019). Water always flows from high potential to low potential, and for plants to take up water from the medium, the water potential in the root must be more negative than the water potential of the media. Water potential decreases with increasing solute concentration, making it more difficult for roots to extract water, causing osmotic stress. Plants respond to osmotic stress with osmotic adjustment, a process in which plants reduce cellular water potential by accumulating solutes in root and leaf cells allowing water to continue moving from the media into the cell by osmosis (Ochsner et al., 2019; Osakabe et al., 2013). Physiological and morphological responses to osmotic stress include reductions in transpiration, photosynthesis, size, and yield (Kaya et al., 2001; Savvas et al., 1999; Singh and Dunn, 2017; Yep et al., 2020). Salinity tolerance is species specific. Walters and Currey (2018) reported the growth and morphology of three basil species was unaffected by fertigation with a solution with an EC of 4 mS/cm. Van de Sanden and Veen (1992) reported a significant reduction in vegetative growth in cucumber seedlings when the solution EC increased from 1 to 8 mS/cm. In lettuce, fresh weight decreased as EC increased from 1 to 1.8 mS/cm (Abou-Hadid et al., 1996). Studies on osmotic stress commonly utilize sodium chloride (NaCl). A pure solution containing 10 mM NaCl at 20 °C has an EC of 1 mS/cm, and a solution containing 100 mM NaCl has an EC of 9.8 mS/cm (*CRC handbook of chemistry and physics*, 1978). The fresh weight of two basil (*Ocimum basilicum* L.) cultivars irrigated with 100 mM NaCl (EC 9.8 mS/cm) decreased 50% and 75% (Barbieri et al., 2012).

Nutrient solutions with ECs that exceed recommended ranges may improve yield or quality. Sakamoto and Suzuki (2020) found that hydroponic sweet potato (*Ipomea batas* (L.) Lam) grown in a solution with an EC of 2.6 m/cm produced more biomass and

larger tuberous roots compared to lower ECs (0.8 and 1.6 m/cm). Similarly, Ding et al. (2018) found that the fresh weight of pakchoi (*Brassica campestris* L. ssp. *Chinensis*) increased with increasing EC up to 4.8 mS/cm. In tomatoes, high EC (above 4 mS/cm) improves fruit quality by increasing tissue concentrations of sugar and lycopene (Cliff et al., 2012; Lu et al., 2022; Moya et al., 2017).

Nitrogen

Nitrogen (N) is a mobile macronutrient with active uptake and usually the most limiting nutrient for plant growth (Miller and Cramer, 2005). In addition to being an integral component for the synthesis of nucleic acids, proteins, enzymes, and chlorophyll, N has also been identified as a signaling molecule in many processes, including shoot and leaf growth, branch formation, and flowering (Lin and Tsay, 2017).

Nitrogen is absorbed by roots in the form of nitrate (NO_3^-) or ammonium (NH_4^+) (Luo et al., 2020). Ammonium (NH_4^+) is not stored in plant tissues, and over application has the potential to induce phytotoxicity (Chen et al., 2004). NO_3^- does not induce toxicity and plants may hyperaccumulate NO_3^- in their tissues (Soltabayeva et al., 2018) and generally, tissue concentrations of NO_3^- correlate with rhizosphere NO_3^- concentrations (Chen et al., 2004; Devienne-Barret, 2000).

Physiological symptoms of N deficiency include decreased chlorophyll synthesis and photosynthetic parameters, resulting in restricted carbohydrate production and carbon assimilation (Saloner and Bernstein, 2021). Visual symptoms of N deficiency include general leaf chlorosis (yellowing), leaf senescence, branch stunting, and decreased yield.

Senescence, the progression of physiological and biochemical changes leading to cell degradation and death, is the terminal event of the leaf life cycle (Thomas, 2012). Generally, leaf senescence is genetically controlled, but plants may respond to abiotic stress (e.g., heat stress, nutrient deficiency, etc.) with premature senescence. Under N deficient conditions, N is remobilized from the chlorophyll and Rubisco of older leaves to support the development of new growth (Diaz et al., 2006; Masclaux-Daubresse et al., 2010; Soltabayeva et al., 2018; Zhang et al., 2017). The enzymatic catabolism of chlorophyll results in reduced chlorophyll content and leaf chlorosis (Agüera et al., 2010; Masclaux et al., 2000; Tamary et al., 2019). The reduction of leaf chlorophyll content reduces the photosynthetic rate, which, in turn, reduces carbon assimilation and growth (Agüera et al., 2010; Mu and Chen, 2021).

Phosphorus

Phosphorous is integral to energy generation, respiration, RNA and DNA synthesis, enzyme activity, signaling, and N fixation (Vance et al., 2003; White and Hammond, 2008). Phosphorous is also a structural component of phospholipids which make up cell membranes (Gutiérrez-Alanís et al., 2018).

Phosphorus deficiency triggers morphological, physiological, and metabolic changes (Hansen and Lynch, 1998; Lambers et al., 2006; Paz-Ares et al., 2021; Plaxton and Carswell, 2018; Uhde-Stone, 2017; White and Hammond, 2008). Alterations to root system architecture, including increased lateral branching and root hair production, enhance P scavenging and uptake by increasing root surface area and soil contact (Gutiérrez-Alanís et al., 2018; Niu et al., 2013; Paz-Arez et al., 2021; Péret et al., 2014;

Uhde-Stone, 2017). Increased production of root exudates containing acid phosphatases and RNases promote P solubility and the increased activity of high-affinity P transporters increase uptake (Paz-Arez et al., 2022). Stored P in tissues is remobilized by acid phosphatases and RNases and loaded into the phloem by PHO1 exporters for transport to sink tissue (Paz-Arez et al., 2022; White and Hammond, 2008).

Symptoms of P deficiency include dark green or reddish-purple foliage, stunting, premature leaf senescence, delayed maturation, and reduced flower production (Cockson et al., 2019; Hawkesford et al., 2012; White and Hammond, 2008). In cannabis, Cockson et al. (2019) reported branch stunting and mottling in older leaves which progresses to marginal chlorosis followed by necrosis.

Tissue nutrient concentrations are commonly used to gauge the adequacy of a nutrition program. For most crops, a leaf tissue P concentration of 0.25% to 0.70% is sufficient (Chakraborty and Prasad, 2021). In cannabis, Bryson and Mills (2015) report an adequate leaf tissue P concentration of 0.24% to 0.49%. Cannabis accumulates P in leaf and flower tissue, and increasing P supply increases leaf and flower P (Veazie et al., 2021; Westmoreland and Bugbee, 2022). Toxicity is rare due to the ability of plants to limit uptake by decreasing transporter activity at the root and storing excess P in the cell vacuole (Dong et al., 1999; White and Hammond, 2008).

Conclusion

In recent years, commercial medical cannabis production has increased significantly worldwide. In response, numerous cannabis nutrition studies have been

conducted. However, nutrient management practices like overfertilization and flushing are still common, despite potential environmental impacts.

Objectives and Hypotheses

The objective of these studies is to investigate the effects of common nutrient management practices on yield, cannabinoid concentration, and nutrient partitioning. The first chapter reports on the effects of P supply (15, 30, 45, 60, and 90 mg/L) and nutrient solution concentration (EC) and the second chapter examines the effects of flushing. These works will add to the growing body of knowledge concerning best management practices for cannabis nutrition in controlled environments.

We hypothesize that (1) excessive fertilizer application does not increase flower yield or cannabinoid concentration, (2) excessive fertilizer application promotes luxury uptake and nutrient waste, and (4) flushing reduces concentrations of mobile nutrients in leaf tissue, but not flower tissue as a result of remobilization.

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CHAPTER II

CANNABIS NUTRITION: ELEVATED FERTILIZER CONCENTRATION AND PHOSPHORUS INCREASE NUTRIENT ACCUMULATION IN SOLUTION BUT DO NOT INCREASE YIELD OR CANNABINOID CONCENTRATION

Abstract

Elevating fertilizer inputs above levels that are adequate for other controlled environment crops is thought to increase flower yield and cannabinoid concentration in medical cannabis cultivation. Increased legalization has heightened awareness of the environmental impact of overfertilization. Here we report the effect of increasing P supply (15, 30, 45, 60, or 90 mg/L) and nutrient solution electrical conductivity (EC; 2 and 4 mS/cm) on yield, cannabinoids, and nutrient partitioning of medical cannabis in closed-system hydroponics. Increasing EC from 2 to 4 mS cm^{-1} increased nutrient accumulation in solution, but minimally changed leaf and flower nutrient concentration. Concentrations of N, P K, S, Cu, Fe, Mn, and Mo were consistently two- to threefold higher in the flowers than in the leaves in all treatments. Reducing P in the refill solution from 90 to 15 mg/L reduced P in solution at harvest from 300 to less than 0.1 mg/L. Despite this low steady-state concentration of P in solution there was no difference in yield or quality among treatments. In tissue, leaf P doubled and flower P increased 70% when the P input increased from 15 to 90 mg/L. These data indicate cannabis tolerates high solution concentration, but excessive fertilization does not improve yield or quality.

Introduction

There is a growing awareness of the environmental impacts of medical cannabis cultivation (*Cannabis sativa* L.) as commercial production expands due to increasing legalization (Ashworth and Vizuete, 2017; Butsic and Brenner, 2016; Wilson et al., 2019; Zheng et al., 2021). Adequate nutrition is critical for optimal yield (Marschner, 2012), but excessive fertilizer application, particularly phosphorus (P), is common in medical cannabis production. High fertilizer concentration is thought to promote flower yield and secondary metabolite production (Bevan et al., 2021). However, recent studies have reported that excessive fertilization has negligible and potentially detrimental effects on yield and quality (Anderson et al., 2021; Bevan et al., 2021; Saloner and Bernstein, 2022a; Shiponi and Bernstein, 2021b; Westmoreland and Bugbee, 2022).

Controlled environments facilitate rigorous monitoring and control of nutrient and water input, and allow ideal for precision fertilizer application. The solution composition and concentration are important considerations when designing a nutrition program (Bugbee, 2004). The theoretical nutrient demand can be calculated to achieve desired tissue concentrations. Sufficiency ranges have been described for most crops, including cannabis (Bryson and Mills, 2015; Kalinowski et al., 2020; Landis et al., 2019; Marschner, 2012). The principle of mass balance, which assumes all nutrients supplied to a system are utilized by the plant or remain in solution, can be used to evaluate the efficiency of a nutrition program (Bugbee, 2004; Langenfeld et al., 2022).

The nutrient solution concentration can be quantified by the solution electrical conductivity (EC; mS/cm). EC is commonly used as a metric for fertilizer input (Singh

and Dunn, 2017), but it does not describe the concentration of specific nutrients in the recirculating solution. Fertilizer solution EC is primarily a function of macronutrient (Ca, S, N, P) concentrations with micronutrients contributing less than 1% (Bugbee, 2004). Differences in nutrient uptake can lead to imbalances in the recirculating solution, which is exacerbated by excessive supply in the refill solution (Bugbee, 2004). Nutrients with active uptake (N, P, K, Mn) are depleted quickly, while nutrients with intermediate and passive uptake (Ca) tend to accumulate (Bugbee, 2004; Savaas et al., 1999; Savvas and Gizas, 2002). Such imbalances could induce ion precipitation, antagonism, or phytotoxicity (Bugbee, 2004; Kaya et al., 2001; Singh and Dunn, 2017). Active uptake may result in low solution EC, while tissue concentrations are within the optimal range for metabolic function (Bugbee, 2004; Langenfeld et al., 2022).

Excessive nutrient application can result in luxury uptake of nutrients without a corresponding increase in yield (Chapin III et al., 1990). Luxury uptake has been observed for multiple nutrients in vegetative and flowering cannabis (Saloner et al., 2020; Saloner and Bernstein, 2022a; Shiponi and Bernstein, 2021a; Shiponi and Bernstein, 2021b; Westmoreland and Bugbee, 2022). Cannabis is particularly prone to accumulating P in leaf and flower tissue (Shiponi and Bernstein, 2021a; Shiponi and Bernstein, 2021b; Veazie et al., 2020; Westmoreland and Bugbee, 2022). Westmoreland and Bugbee (2022) reported that P concentrations increased by 35% in leaves and 11% in flowers when the P input increased from 25 to 75 mg/L.

Our objective was to quantify the effects of nutrient solution concentration (EC; 2 and 4 mS/cm) and P supply (15, 30, 45, 60, and 90 mg/L) on yield, quality, and nutrient partitioning of medical cannabis in closed system, deep-flow hydroponics.

Materials and Methods

Plant Materials

Two trials were conducted in time. For each trial 50 cuttings of the high CBD cannabis cultivar 'T1' were collected from the same mother plant, treated with rooting hormone (Hormodin® 2, Indol-3-butyric Acid 0.03%, OHP Inc., Bluffton, SC, USA), and rooted in course perlite (Hess Perlite, Malad City, ID, USA) for two weeks. Twenty-four rooted cuttings were selected for uniformity and transplanted into a 48 L (57.5 x 44.5 x 23.5 cm) hydroponic tub. One day after transplant, plants were pinched to four nodes and grown vegetatively (18/6 h, light/dark) for seven days in a greenhouse. After seven days, four plants were randomly assigned to one of six 48 L tubs filled with their respective nutrient solution treatments. The tubs were located in a walk-in grow chamber with a reproductive photoperiod (12/12 h, light/dark). After seven days of reproductive photoperiod, plants were pinched a second time to achieve two nodes per branch for a total of eight branches on each plant. Plants were harvested 56 days after the induction of reproductive growth.

Environment

Lighting was provided by full spectrum LED grow lights (Dragon Alpha, Scynce LED, Mesa, AZ). The photosynthetic photon flux density (PPFD; 400 to 700 nm) was 923 ± 102 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI 39 $mol \cdot m^{-2} \cdot d^{-1}$) at canopy height. PPFD was measured every seven days with a handheld quantum sensor (MQ-500; Apogee Instruments, Logan, UT, USA) and maintained by dimming the lights as plants grew. Temperature was 26.1 ± 1.4 / 24.2 ± 1.2 °C (day/night ± standard deviation), measured with a shielded, fan aspirated thermistor (model ST-100, Apogee Instruments Inc., Logan, UT, USA).

Relative humidity (RH) was 60 ± 9 / 51 ± 7% (day/night ± standard deviation) and the vapor pressure deficit (VPD) was 1.4 ± 0.3 / 1.5 ± 0.2 kPa (day/night ± standard deviation) measured with a temperature and RH probe (model HMP45A, Campbell Scientific Inc., Logan, UT, USA). Environmental measurements were made every ten seconds and ten-minute averages were recorded by a datalogger (model CR1000X, Campbell Scientific Inc., Logan, UT, USA).

Nutrient Solution

Nutrient solutions were formulated using the mass-balance approach described by Langenfeld et al. (2022). During vegetative growth all plants received the same nutrient solution (**Table 2-1**; 15 mg/L P; EC 2 mS/cm).

EC treatments were achieved by increasing the ion concentration of all nutrients except P (**Table 2-1**). The solution concentration is reported as the solution EC (mS/cm). Individual treatments consisted of a P input and an EC level (standard EC 2 mS/cm or high EC 4 mS/cm). P input was 15, 30, or 45 mg/L in trial one and 30, 60, or 90 mg/L in trial two (**Table 2-2**). P was supplied as KH_2PO_4 (**Table 2-2**).

Solution Refill and Monitoring

Tub solutions were replenished by hand daily to maintain a fill level of 48 L. The solution EC (Dist. 4 EC meter, HI98304, Hanna Instruments, Smithfield, RI, USA) and pH (Environmental Express® pHTestr 10 BNC pH meter, Charleston, SC, USA) was measured and recorded prior to refill each day. The pH was measured after refilling the tub solution and adjusted using HNO_3 or potassium hydroxide (KOH) to maintain a pH between 5.8 and 6.2.

Tissue and Solution Analysis

Leaf, flower, and solution samples were collected from each treatment every seven days for element analysis. Solution samples were collected prior to replenishing tub solutions. For leaf samples, the three most recently expanded leaves were collected from each plant within each treatment to obtain a fresh weight sample of ~ 10 g. A ~3 g flower sample was collected from each plant for a total fresh sample weight of ~12 grams from each treatment. Flower samples were collected from the upper inflorescences of each plant, inflorescence leaves were excluded. Tissue samples were collected from each plant in each treatment, washed with deionized water and dried at 80 °C for 48 h then ground to a fine powder with a stainless-steel grinder (KitchenAid, model BCG111OB). A leaf and flower sample from each plant within each tub was homogenized and analyzed as a single sample. All samples were submitted to the Utah State University Analytical Laboratory (USUAL, Logan, UT, USA) for mineral analysis. Solution samples were analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES) (Thermo Scientific iCAP 6300 Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Solution nitrate concentrations were analyzed with the Lachat Quikchem® 3 Channel QC8000+ Flow Injection Analyzer (QuikChem 8000; Lachat Instrument, Milwaukee, WI, USA) using Flow Injection Analysis (QuikChem® Method 10-107-04-1-C, Lachat Instruments, Milwaukee, WI, USA). To determine the tissue concentrations of Ca^{2+}, potassium (K^{+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), sulfur (S), zinc (Zn^{2+}) and iron (Fe^{3+}) 0.5 g of the powdered sample was placed in a digestion tube containing 6 mL of nitric acid (HNO_3) and digested for 10 minutes at 80 °C, then allowed to cool for two minutes before adding 2 mL of 30% hydrogen peroxide (H_2O_2). The solution was subject to an additional digestion period of one hour at 130 °C to reduce the total digest

volume to 2 to 3 mL. The digestion tube was then placed in a vortex stirrer, mixed, and cooled to room temperature before transferring the contents to a 25 mL volumetric flask. Inductively coupled plasma-optical emission spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA, USA) was used to analyze the digest and the results were reported on a dry plant basis ($mg \cdot g^{-1}$). Tissue N concentration was determined using combustion analysis (Elementar VarioMax Cube, Elementar Americas Inc., Mt. Laurel, NJ, USA).

Cannabinoid Analysis

At harvest a flower sample (~5 g) was collected from the upper inflorescences of each plant in each treatment. Samples were dried on a ventilated rack at 25 °C and 30% relative humidity for five days. Samples were ground to a fine powder using a stainless-steel grinder (KitchenAid, model BCG111OB). Flower tissue from each plant within a treatment was homogenized and analyzed as a single sample. Samples were analyzed by the Utah Department of Agriculture and Food (UDAF) Unified State Laboratory (Taylorsville, UT, USA). Cannabinoid equivalents (CBD_{eq} and THC_{eq}) were calculated following Westmoreland et al. (2021).

Plant Measurements and Harvest

At harvest height was measured from the base of the stem to the apex of the dominant inflorescence. Plants were destructively harvested by cutting the stem at the base just above the root ball. Total fresh weight was recorded for each plant. Leaves and flowers were mechanically stripped from stems using a table top bucker (High Performance Tabletop Bucker, HTAA01072, Centurion Pro Solutions Ltd, Maple Ridge, BC, CA). Inflorescence leaves were separated from flowers with a trimmer (Tabletop

Trimmer, Model KBWA-22D, Centurion Pro Solutions Ltd, Maple Ridge, BC, CA). Roots, stems, leaves, and flowers were dried in an oven at 80 °C for 48 h. Flower yield (grams per m^2) was calculated as the total oven-dried flower of each tub divided by the canopy area. Harvest index (HI) was calculated as the ratio of flower mass to total biomass (flowers, leaves, stems, and roots).

Statistical Analysis

The study was a randomized complete block design (RCBD) with time as the blocking factor. There were three P levels and two EC levels in each block, resulting in six treatments with two replicates in time. An experimental unit consisted of a tub containing a P x EC treatment. Data were normalized to the 30 mg/L treatment. Data were fitted with a simple linear model and analyzed using regression. P was treated as a continuous variable, and EC was treated as a discrete variable. Effects were considered significant at $\alpha = 0.05$. All statistical analysis were performed in RStudio (R statistical software, version 4.1.0.).

Results

Yield and Cannabinoid Concentration

There were no visual differences in plant health or inflorescence architecture among treatments. Dry flower yield was not significantly affected by P supply ($p = 0.95$) or solution EC ($p = 0.22$) (**Figure 2-1**). The average dry flower yield across all treatments was 640 ± 88 $g \cdot m^{-2}$ (mean ± SD). There was no effect of P supply ($p = 0.48$) or solution EC ($p = 0.12$) on harvest index (HI) (**Figure 2-2**). The average HI across all treatments was 48 ± 4%. There was no significant effect of solution EC or P on concentrations of

$CBD_{eq,}$ or THC_{eq}. Across all treatments, CBD_{eq} and THC_{eq} were 13.55 % ± 0.85 and 0.59 % ± 0.04, respectively (**Figure 2-3A, B**). The ratio of CBD_{eq} to THC_{eq} was similar across treatments (22.9 ± 1.3; EC p = 0.45, P p = 0.84).

Solution Nutrient Concentrations Over Time

The P input had a significant effect on the accumulation of P in the recirculating solution and inputs in excess of 30 mg/L resulted in P accumulations in both EC treatments (**Figure 2-6**). Notably, at all P levels, P accumulations were greater in the 2 mS/cm EC treatments than the 4 mS/cm EC treatments. In the 2 mS/cm EC treatments P inputs of 30, 60, and 90 mg/L resulted in solution P concentrations at harvest 44%, 242%, and 242% higher than the P input of the refill solution. In contrast, in the 4 mS/cm EC treatments P inputs of 30, 60, and 90 mg per L resulted in P concentrations only 25%, 180%, and 130% greater than P input.

Unsurprisingly, concentrations of all other ions in the recirculating solution were higher in the 4 mS/cm EC treatments (**Figure 2-4**; **Figure 2-5**). However, we observed accumulations of S (**Figure 2-4 D**), K (**Figure 2-4 B**), B (**Figure 2-5 A**), and Cu (**Figure 2-5 B**) across all treatments. Concentrations of Mn, Mo, and Zn in the recirculating solution were much lower than concentrations in the refill solution, indicating active uptake (**Figure 2-5**).

Tissue Nutrient Concentrations and Partitioning at Harvest

Leaf nutrient concentrations were within reported sufficiency ranges across all treatments throughout the duration of the study (Bryson and Mills, 2015; Cockson et al., 2019; Landis et al., 2019). The P input had significant effects on tissue P (p = <0.001). Leaf P doubled and flower P increased 70% (**Figure 2-9 B**) when the P input was

increased from 15 to 90 mg per L. Tissue K typically increased with P input ($p = 0.01$). This is probably a result of additional K supplied by KH_2PO_4, which was used to supply P (**Figure 2-9 C**; **Table 2-2**). Flower S ($p = 0.01$), leaf B ($p = 0.04$), and leaf and flower Mn ($p = 0.03$) increased with increasing P supply.

In general, flowers had higher concentrations of mobile nutrients (N, P, K, S, Mo) and leaves had higher concentrations of immobile nutrients (Ca and B). Interestingly, concentrations of Cu, Mn, and Fe were higher in the flower than in the leaves. Flower tissue concentrations of N, K, S, Fe, Mn, and Mo were roughly twofold higher and P and Cu concentrations were around threefold higher than leaf concentrations in all treatments (**Figures 2-9**, **2-10**; **Table 2-3**). Surprisingly, tissue concentrations were largely unaffected by the solution concentration (**Figures 2-9**, **2-10**).

Discussion

Yield and cannabinoid production

Numerous studies have investigated the effects of P application rate on flowering stage medical cannabis, but the optimal P varied between studies. In this study, P inputs above 15 mg/L did not improve yield or quality (Supplemental figures 1 and 3). Westmoreland and Bugbee (2022) reported that maximum yield was achieved with a P input of 25 mg/L, but, lower P inputs were not investigated. Shiponi and Bernstein (2021b) investigated a wider range of 5 to 90 mg/L P in two cultivars and found that one cultivar achieved maximum yield at 30 mg/L P, but the second cultivar had increasing yield up to 90 mg/L P. However, despite the 3-fold increase in P, yield only increased 20% (Shiponi and Bernstein, 2021b). In deep-water hydroponics, Bevan et al. (2021)

reported an optimal P input of 60 mg/L. In contrast, Cockson et al. (2020) reported that maximum fresh yield and cannabinoid production were achieved with a P input of just 11.25 mg/L. The variation in optimal P between studies could be due to environmental differences like CO_2 supplementation, light intensity, or temperature. Additionally, genetic variability could affect P requirements between cannabis cultivars (Hawkesford and Griffiths, 2019; Shenoy and Kalagudi, 2005; Shiponi and Bernstein, 2021b). Regardless, P applications exceeding 100 mg/L are excessive and unnecessary.

Excessive fertilizer application is common in medical cannabis production, but in this study doubling the fertilizer concentration to a solution EC of 4 mS/cm did not increase yield or cannabinoid concentrations. Baas and Wijnen (2023) reported no difference in THC concentrations between plants receiving fertilizer solutions with EC 1.8 mS/cm to 12 mS/cm, but flower yield decreased when the nutrient solution EC exceeded 6 mS/cm. Many crops are sensitive to high solution EC and exhibit stress responses including stunting, phytotoxicity, and yield reduction (Machado and Serralheiro, 2017; Savvas et al., 1999), but cannabis seems to tolerate higher fertilizer rates than other crops (Baas and Wijnen, 2023). In this study plant health and development were unaffected by a solution EC of 4 mS/cm. Baas and Wijnen (2023) reported that cannabis tolerated a nutrient solution EC up to 12 mS/cm with no foliar symptoms of phytotoxicity, but plants were stunted and produced less yield when fertigated with solution ECs of 6 mS/cm or higher. Notably, high solution EC does not induce visual symptoms of phytotoxicity like foliar damage in cannabis (Baas and Wijnen 2023), but this does not negate the environmental impacts of excessive fertilizer application.

Nutrients accumulated in solution

The EC of the recirculating solution increased over time, unless controlled by refilling with dilute solution, indicating that nutrient supply exceeded plant uptake. Phosphorus accumulated in the recirculating solution when P input exceeded 30 mg/L with greater accumulations corresponding to higher P input, which agrees with the findings of Westmoreland and Bugbee (2022). Interestingly, P accumulation was not evident until two weeks after the induction of flowering (Figure 3B), suggesting greater P uptake at the beginning of the flowering cycle. It is generally accepted that plant demand for external nutrient supply corresponds with the life stage (Jones et al., 2015). During the first few weeks after the start of short days cannabis exhibits a transitional period between the vegetative and reproductive stages marked by rapid stem elongation and the maturation of developing leaves, which could explain why P uptake remained high (Shiponi and Bernstein, 2021b). Nutrient uptake curves generated for sunflower (Heard and Park, 2008), lentil (Malhi et al., 2007), pea (Malhi et al., 2007), and small grains Malhi et al., 2006) demonstrate that the majority of tissue P is accumulated during the vegetative stage and bud formation with a pronounced decline in uptake during at the start of flowering. Similarly, in poinsettia ammonium (NH_4) uptake increases during the vegetative and inductive stages of flower development, but declines during anthesis (Whipker and Hammer, 1997).

Typically, P accumulations in the recirculating solution were greater in the 2 mS/cm EC treatments than in the 4 mS/cm EC treatments. In the 2 mS/cm EC treatments P inputs of 30, 60, and 90 mg/L resulted in solution P concentrations at harvest 44%, 242%, and 242% higher than the P input of the refill solution. In contrast, in the 4 mS/cm

EC treatments P inputs of 30, 60, and 90 mg per L resulted in P concentrations only 25%, 180%, and 130% greater than P input. However, tissue P was similar between EC treatments (Figures 4 and 6). This suggests that P may have precipitated in the high EC treatments possibly with Ca or S forming insoluble $Ca(PO_4)_2$ or $Ca(SO_4)_2$ in the recirculating solution.

Concentrations of Mn, Mo, and Zn were well below refill solution concentrations across all treatments, suggesting active uptake. Notably, despite the low concentrations of Mn, Mo, and Zn in the recirculating solution tissue levels were within the sufficient range for metabolic processes. Despite the antagonistic effects of high P supply on the uptake and translocation of other nutrients, particularly Zn (Zhang et al., 2021), this was not observed in the present study. In both EC treatments B, Cu, and S accumulated in the recirculating solution, which was also observed by Savvas and Gizas (2002) in closed system hydroponics. Nutrient accumulation in solution could induce ion antagonism or phytotoxicity. One method to manage nutrient accumulation and imbalances created by excessive fertilizer application is increased fertigation duration or frequency to flush accumulated salts from the media. In hydroponics, nutrient accumulations and imbalances are managed by periodically dumping and replacing the recirculating solution. However, a better management practice would be to avoid overfertilizing in the first place.

Nutrient partitioning in tissue

Nutrient distribution and remobilization within the plant is affected by the external supply, source-sink relations, life stage, and nutrient phloem mobility (Maillard et al., 2015; Ray et al., 2020). At harvest, mobile nutrient concentrations were higher in

flowers and immobile nutrients were higher in leaves, which is similar to the findings of Saloner and Bernstein (2021), Shiponi and Bernstein (2021b), Veazie et al. (2021), Westmoreland and Bugbee (2022). The transition from vegetative to reproductive growth induces the remobilization of stored nutrients from old or senescing leaves to support reproductive development (Diaz et al., 2006; Malagoli et al., 2005; Masclaux-Daubresse and Chardon, 2011; Raboy and Dickinson, 1987; Soltabayeva et al., 2018; Zhang et al., 2017). Cannabis is known to hyperaccumulate P in flowers (Shiponi and Bernstein, 2021b; Veazie et al., 2021; Westmoreland and Bugbee, 2022) and in this study, flower P was 156% higher than leaf P. P accumulation in reproductive tissues has been observed in other species and in rice the developing panicle is the primary sink for P (Julia et al., 2016). P accumulation in the floral bracts of cannabis could be storage for developing seed. In seeds, P is stored as phytic acid and time course data in soybean showed increasing phytic acid accumulation during seed development (Raboy and Dickinson, 1987). In addition to P, Cu and S also accumulated in flower. Flower Cu was 175% higher than leaf Cu across all treatments which was also reported in cannabis by Westmoreland and Bugbee (2022). Cu accumulation has also been observed in the flowers of wheat (Garnett and Graham, 2005), *Verbascum olympicum* Boiss (Güleryüz et al., 2006), and peach (Zarrouk et al., 2005). In wheat, Cu is remobilized from leaves and translocated to developing grains post anthesis (Garnett and Graham, 2005). Similarly, cannabis may accumulate Cu in floral bracts to support seed production. Sulfur concentrations in flower were 104% higher than leaf concentrations. This could be a result of an oxidative stress response triggered by high Cu concentrations. Sulfur is an essential component of the oxidative stress response and S demand increases in response

to increased Cu. Alternatively, S accumulation in floral tissue could be the result of secondary metabolite production, specifically aromatic sulfur containing compounds, which increase with flower age. Suggesting remobilization to support flower development or accumulation in floral bracts to support seed development (Dordas, 2009; Engels et al., 2012; Malagoli et al., 2005).

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TABLE 2-1. Nutrient solution composition at each EC treatment level.

Solution EC mS/cm		N	K	Ca	Mg	S	Si	Fe	Mn	Zn	B	Cu	Mo
2	mg/L	154	203	120	20	26	17	1	0.2	0.4	0.4	1	0.01
	mM	11	5.2	3	0.8	0.8	0.6	18	3	3	40	16	0.1
4	mg/L	308	407	240	40	52	34	2	0.4	0.8	0.8	2	0.02
	mM	22	10.4	6	1.6	1.6	1.2	36	6	6	80	32	0.2

Macronutrients are reported as mM micronutrients are reported in µM. Values listed for K are representative of the base solution prior to the addition of KH_2PO_4 for P treatment.

TABLE 2-2. P and K concentrations at each P treatment level.

P Treatment	P	K
	mg/L	
15	15 (0.5)	19.6 (0.5)
30	30 (1)	39 (1)
45	45 (1.5)	58.7 (1)
60	60 (2)	78 (2)
90	90 (3)	121 (3)

Values in parenthesis represent ion concentration in mM. Values listed for K represent additional K added as KH_2PO_4 for P treatment.

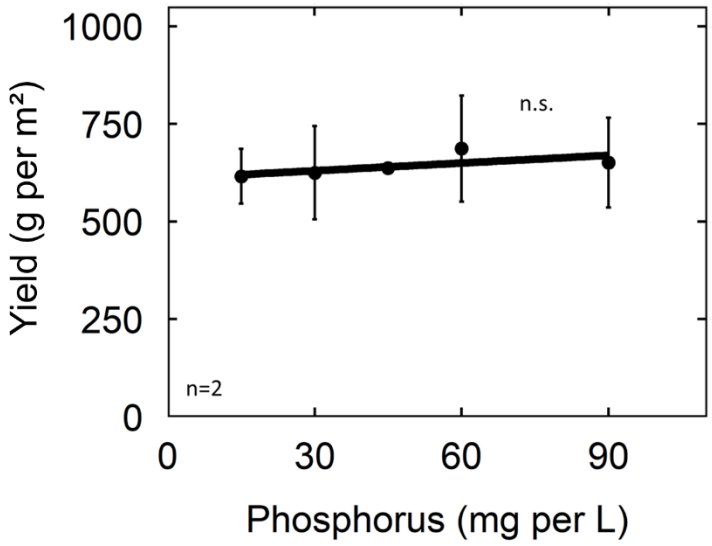

FIGURE 2-1. Effects of P input on dry flower yield. Each data point represents the mean of the two EC levels and error bars represent standard deviation from the mean. There was not a statistically significant effect of P input (p = 0.95) or EC (p = 0.22) on dry flower yield.

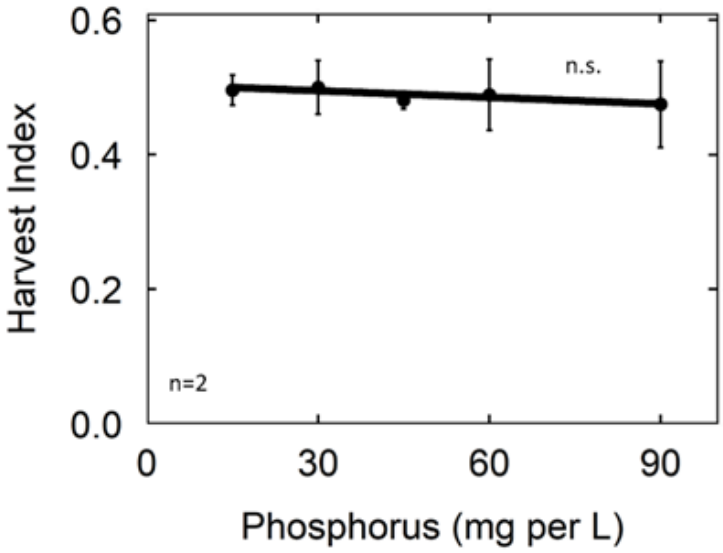

FIGURE 2-2. Effect of P input on harvest index (HI). There was no significant treatment effect of EC on harvest index so data were pooled. Each data point represents the average of the two EC levels within each P treatment and error bars represent standard deviation from the mean.

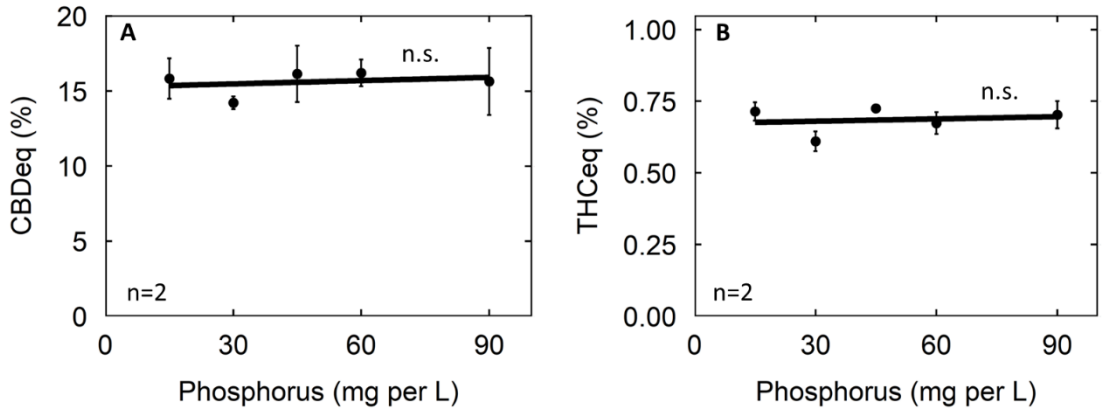

FIGURE 2-3. Effect of P input on CBDeq (A) and THCeq (B) concentrations at harvest. Solution EC treatment did not have a significant effect on cannabinoid concentrations so data were pooled. Data points represent the average of the standard and high EC treatments and error bars represent the standard deviation from the mean.

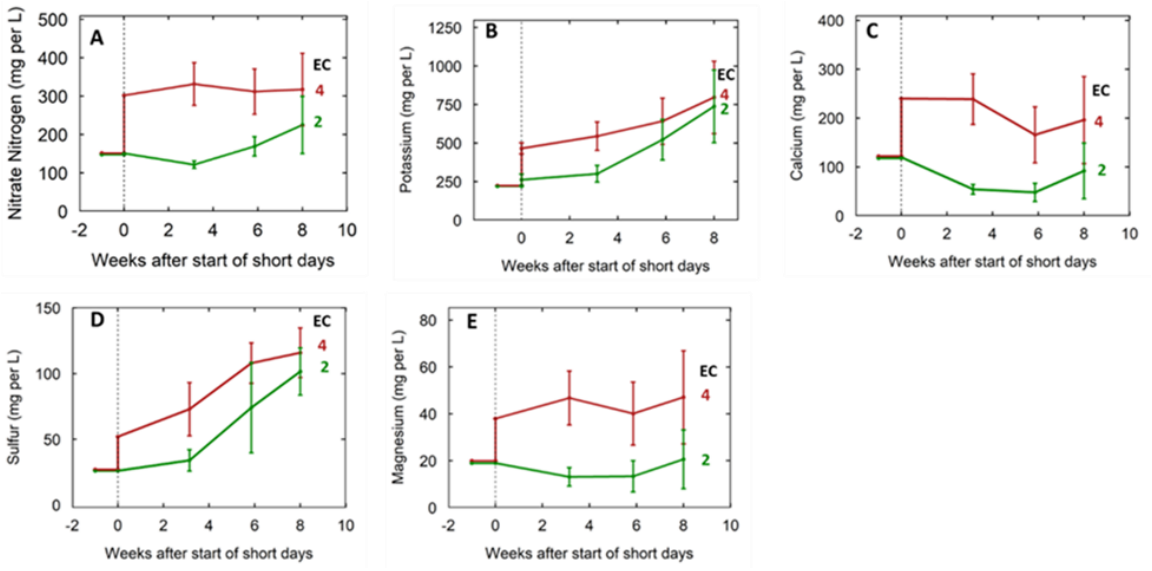

FIGURE 2-4. The effects of nutrient solution concentration (EC; mS/cm) on N **(A)**, K **(B),** Ca **(C),** S **(D)**, and Mg **(E)** concentrations in the recirculating solution over time. Each data point represents the average of the three P inputs within each EC treatment and error bars represent standard deviation from the mean (n = 3).

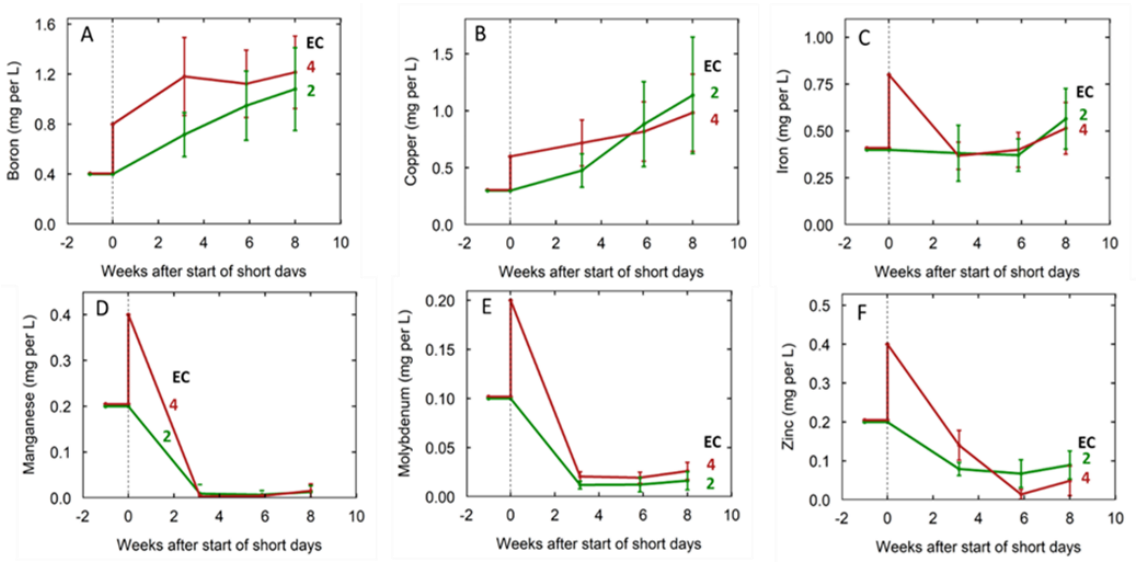

FIGURE 2-5. The effects of nutrient solution concentration (EC; mS/cm) on B **(A)**, Cu **(B),** Fe **(C),** Mn **(D),** Mo **(E)**, and Zn **(F)** concentrations in the recirculating solution over time. Each data point represents the average of the three P inputs within each EC treatment and error bars represent standard deviation from the mean (n = 3).

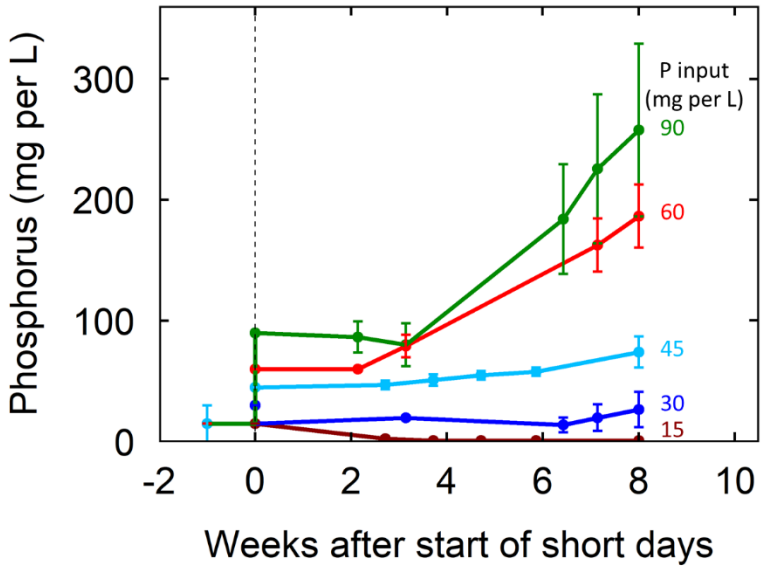

FIGURE 2-6. The effects of P input on P accumulation in the recirculating solution over time. Individual data points represent the mean of the two EC treatment levels within each P treatment. Error bars represent standard deviation from the mean (n = 2).

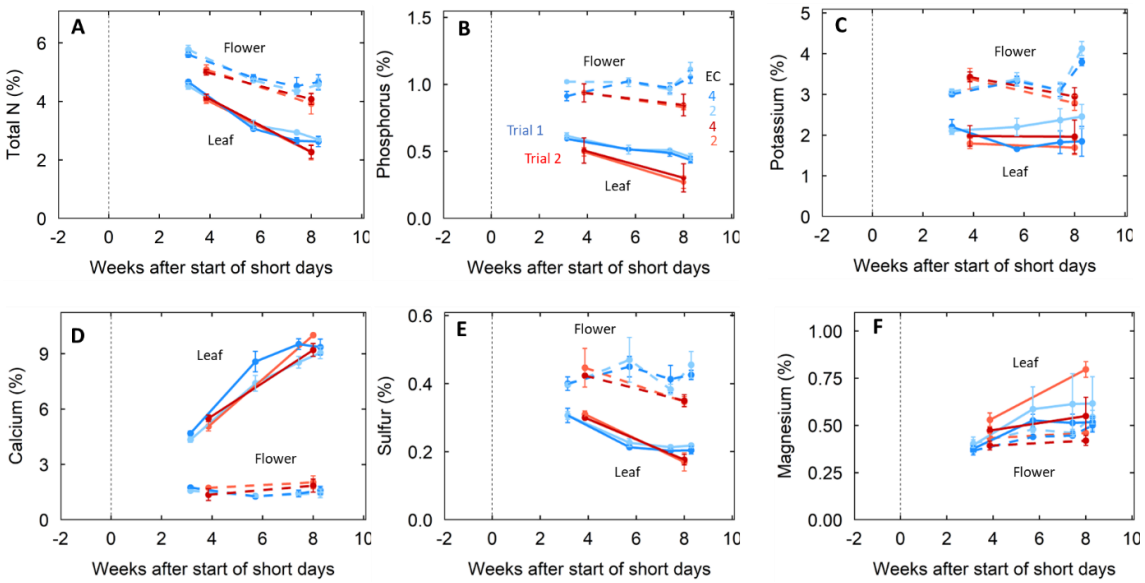

FIGURE 2-7. The effects of P input and solution concentration on N (A), P (B), K (C), Ca (D), S (E), and Mg (F) concentrations in leaf and flower tissue over time. Individual data points represent the mean of the two EC treatment levels within each P treatment and error bars represent standard deviation from the mean (n = 3).

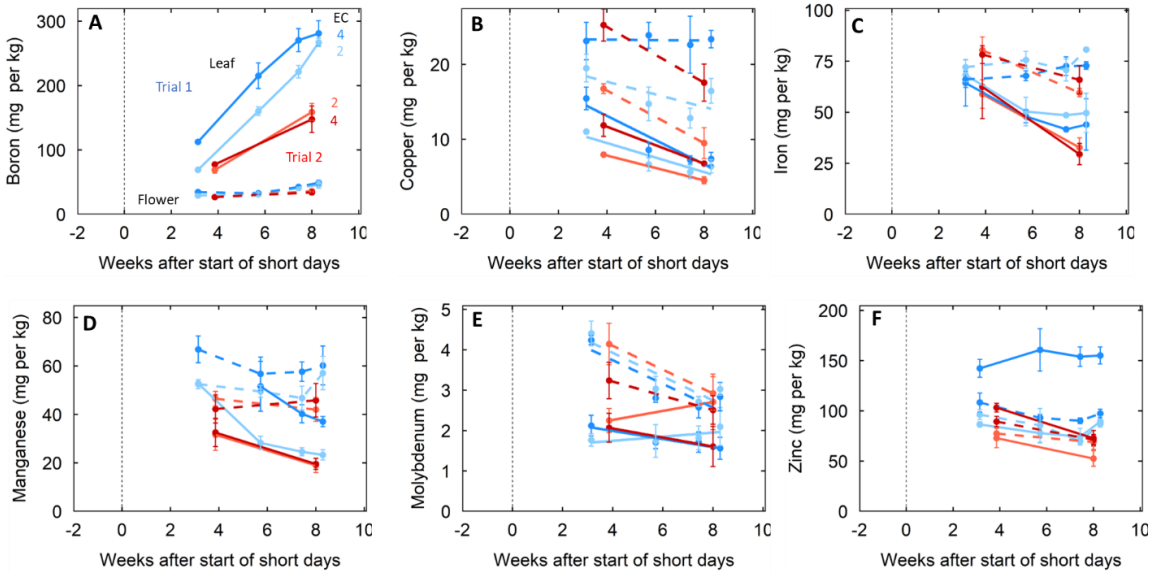

FIGURE 2-8. The effects of P input on B (A), Cu (B), Fe (C), Mn (D), Mo (E), and Zn (F) concentrations in leaf and flower tissue over time over time. Individual data points represent the mean of the two EC treatment levels within each P treatment and error bars represent standard deviation from the mean (n = 3).

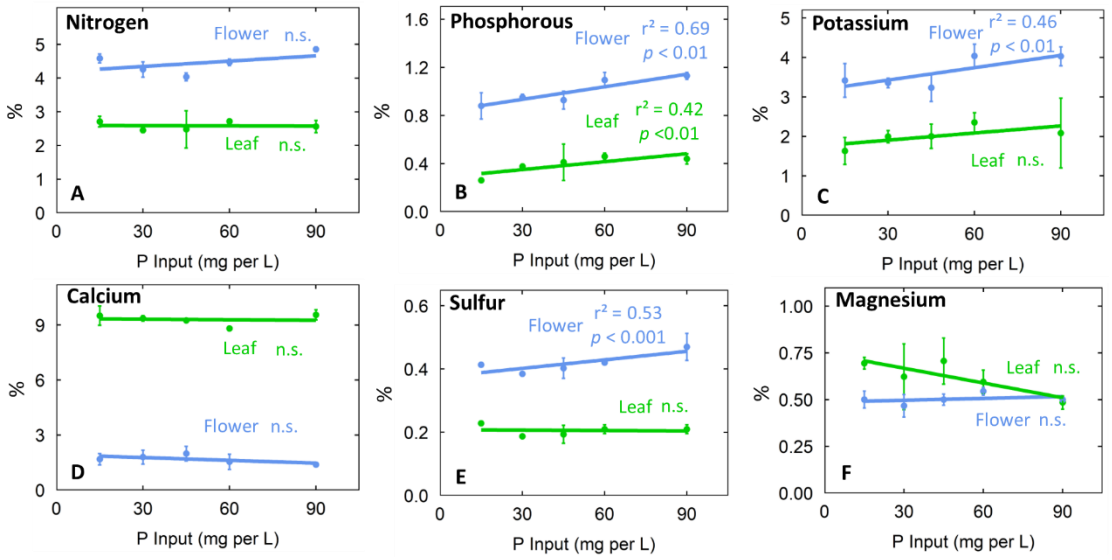

FIGURE 2-9. The effects of P input on N (A), P (B), K (C), Ca (D), S (E), and Mg (F) concentrations in flower and leaf tissue at harvest. Individual data points represent the mean of the two EC treatments within each P treatment (n = 2). Error bars represent standard deviation. Data normalized to 30 mg/L P treatments.

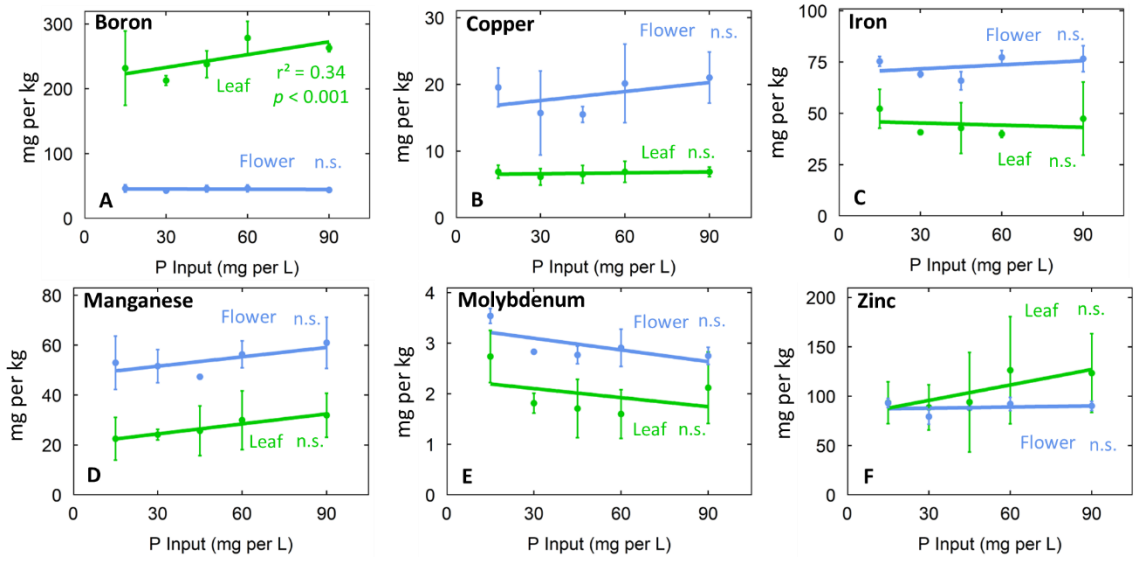

FIGURE 2-10. The effects of nutrient solution concentration (EC; mS/cm) and P input B (A), Cu (B), Fe (C), Mn (D), Mo (E), and Zn (F) concentrations in flower and leaf tissue at harvest. Individual data points represent the mean of the two EC treatments within each P treatment (n = 2). Error bars represent standard deviation. Data normalized to 30 mg/L P treatments.

TABLE 2-3. The ratio of floral nutrient concentrations to leaf concentrations at harvest. A ratio greater than 1 indicates greater concentration in leaves and less mobile nutrients (Ca and B). Values were similar among all EC and P treatments.

Ratio of flower to leaf tissue nutrient concentration

EC	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
Standard	1.8	2.8	1.7	0.2	0.6	2.1	1.7	2.3	1.2	0.2	2.4	1.3
High	1.8	2.7	1.9	0.2	0.7	2	2	2	0.8	0.2	2.9	1.7

CHAPTER III

PREHARVEST FLUSHING DOES NOT REDUCE MOBILE NUTRIENT CONCENTRATIONS IN FLOWER, BUT MAY REDUCE YIELD

Abstract

In medical cannabis production flushing is the deprivation of fertilizer during the final one to two weeks of flowering. This study examined the effects of nitrogen (N) deprivation 7, 14, and 21 days before harvest and total nutrient deprivation for 7 and 14 days before harvest on yield, cannabinoid production, and nutrient partitioning. Yield was not affected when plants were flushed by withholding N for up to 21 days, however, yield decreased under total nutrient deprivation. Leaf chlorophyll decreased with increased flushing duration. Flower concentrations of mobile nutrients were unaffected by flushing, but leaf concentrations decreased.

Introduction

Flushing is a common nutrient management practice in medical cannabis production, but few studies have examined its effects on yield, quality, or nutrient partitioning. Anecdotal evidence suggests that flushing improves the organoleptic qualities of smoked flower including flavor, throat feel, and combustion by reducing floral concentrations of chlorophyll and nutrients (Dutch Passion Blogs, 2021; Rosenthal, 2021). Dried cannabis flower is consumed through the inhalation of smoke or vapors from combusted or vaporized flower and smoke or ash color are commonly used as quality indicators by consumers, with light or white coloration signifying higher quality

(Justice and Roggen, 2021). However, Justice and Roggen (2021) postulate that ash and smoke color are a function of combustion temperature or water content. In tobacco, higher water content has been correlated with decreased combustion rate and burn temperature (Djulancic et al., 2013). Furthermore, the tobacco blend, curing method, and packing density of rolled cigarettes can affect combustion qualities and smoke constituents including increased production of harmful gases like carbon monoxide (Djulancic et al., 2013; Zha and Moldoveanu, 2004).

Flushing methods vary across growers, but generally, flushing is the reduction or complete deprivation of one or more nutrients during the final one to two weeks of flowering before harvest. Flushing is initiated by irrigating the media with pure water to obtain a leachate electrical conductivity (EC) similar to that of the input irrigation water. During the initial flush chemical solutions called finishing products may be added to irrigation. Numerous finishing products are available, but typically their purpose is to either reduce the bioavailability of nutrients in the media or to increase nutrient solubility to promote nutrient loss through leaching (Rosenthal, 2021). Following the initial flush plants are irrigated with tap water or dilute nutrient solution until harvest (Dutch Passion Blogs, 2021; Rosenthal, 2021). The premise of flushing is that nutrient deprivation forces plants to remobilize stored nutrients in order to continue growth and metabolic processes. Plants typically develop visual symptoms of nutrient deficiency during flushing like yellowing and senescence of old fan leaves (Dutch Passions Blogs, 2021). The objective of this study was to investigate the effects of flushing on yield, cannabinoid production, and nutrient partitioning in medical cannabis.

Materials and Methods

Plant Material

The study encompassed two experiments. The high CBD cannabis cultivar 'Cherry' (*Cannabis sativa* L. cv. 'Cherry') was used in experiment one and the cultivar 'T1' (*Cannabis sativa* L. cv. 'T1') was used in experiment two. The propagation protocol, environmental parameters during the experiment, and the harvest protocol were similar for both experiments.

Fifty cuttings were collected from stock plants, treated with rooting hormone (Hormodin® 2, Indol-3-butyric acid 0.03%, OHP Inc., Bluffton, SC, USA), and rooted in a soilless media composed of a 1:1 ratio of course perlite (Hess Perlite, Malad City, ID, USA) and peat (PRO-Moss TBK, Premier Horticulture Inc., Quakertown, PA, USA) for two weeks. Rooted cuttings were transplanted into 6.7 L (#2) pots filled with soilless media composed of 75% peat moss (Premier Pro-Moss TBK, Premier Horticulture Inc., Quakertown, PA, USA), 13% vermiculite (Perlite Vermiculite Packaging Industries, Inc. North Bloomfield, OH, USA), and 12% rice hulls (Riceland Foods, Inc., Stuttgart, AR, USA). Wetting agent (AquaGro® 2000 G; Aquatrols, Paulsboro, NJ, USA) was added at a rate of 0.75 g/L media and hydrated lime (Chemstar® Type S lime; Chemstar Products, Minneapolis, MN, USA) was added at a rate of 1 g per L to achieve a media pH of 5.8.

One day after transplanting, each plant was pinched to four nodes. Plants were grown vegetatively (photoperiod 18/6 h, day/night) for seven days in a greenhouse. Twenty-four plants were selected for uniformity and moved to a walk-in grow chamber with a photoperiod of 12/12 h, day/night to induce and maintain flowering. A second

pinch was conducted seven days after the induction of flowering to achieve a total of eight branches per plant.

Environment

Lighting was provided by full spectrum LED grow lights (Dragon Alpha, Scynce LED, Mesa, AZ, USA). The photosynthetic photon flux density (PPFD; 400 to 700 nm) was 969 ± 17 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI 42 $mol \cdot m^{-2} \cdot d^{-1}$). The PPFD was measured weekly at canopy level with a handheld quantum sensor (MQ-500; Apogee Instruments, Logan, UT, USA) and maintained by dimming the lights as plants grew. The temperature was 26.10 ± 1.36 °C / 24.18 ± 1.21°C (day/night ± standard deviation) measured with a shielded, fan aspirated thermistor (model ST-100, Apogee Instruments Inc., Logan, UT, USA). Relative humidity (RH) was 60 ± 8.9 %/ 50.7 ± 6.9%, day/night and vapor pressure deficit (VPD) were 1.35 ± 0.27 kPa / 1.49 ± 0.15 kPa (day/night ± standard deviation) measured with an RH and temperature sensor (model HMP45A, Campbell Scientific Inc., Logan, UT, USA). Environmental measurements were made every ten seconds and ten-minute averages were recorded by a datalogger (model CR1000X, Campbell Scientific Inc., Logan, UT, USA). Atmospheric CO_2 was supplemented to 1200 ppm.

Nutrition and Treatments

During the first four weeks of reproductive growth all plants were fertigated with a modified Utah Cannabis Solution (Bugbee and Langenfeld, 2022) containing 200 mg/L N supplied as nitrate (NO_3^-) (**Table 3-1**). All plants received the same volume at each irrigation event, however the frequency and volume applied increased as plants grew. Leachates were collected weekly to monitor electrical conductivity (EC; mS/cm) (Dist 4

EC meter, HI98304, Hanna Instruments, Smithfield, RI, USA) and pH (Environmental Express® pHTestr 10 BNC pH meter, Charleston, SC, USA).

Experiment One

Cannabis sativa L. cv. 'Cherry' were flushed with reverse osmosis water to achieve a leachate EC below 0.5 mS/cm, then irrigated with N free nutrient solution (**Table 3-2**) for 21, 14, 7, or 0 days before harvest.

Experiment Two

Cannabis sativa L. cv. 'T1' were flushed with tap water to achieve a leachate EC below 0.5 mS/cm, then irrigated with tap water for 14 days, 7 days, or 0 days before harvest.

Harvest

Plants were harvested 56 days after the induction of flowering. At harvest inflorescence diameter was measured with a digital caliper (Model CD-6″B, Mitutoyo Corporation, Kawasaki, Kanagawa, JPN). To determine flower diameter within each treatment, the diameter at the widest portion of the inflorescence was recorded for each of the eight apical inflorescences on each plant and used to calculate the mean and standard deviation.

Plants were destructively harvested by cutting the base of the stem even with the soil surface and the total fresh weight was recorded for each plant. Leaves and flowers were mechanically stripped from stems using a tabletop bucker (High Performance Tabletop Bucker, HTAA01072, Centurion Pro Solutions Ltd., Maple Ridge, BC, CA). Leaves were separated from flower using a trimmer (Tabletop Trimmer, Model KBWA-22D, Centurion Pro Solutions Ltd, Maple Ridge, BC, CA). Fresh weights were collected

for stems and flowers. Stems and flowers were dried in an oven at 80 °C for 48 h and dry weights were recorded. Fresh leaf weight was calculated by subtracting stem and flower weight from the total fresh weight for each plant. To calculate dry leaf weight a leaf sample was collected from each plant and fresh and dry weights were used to find percent water content which was then used to calculate the dry leaf mass for each plant by multiplying the percent water content by the fresh leaf weight. Flower yield (grams per m^2) was calculated as the total mass of oven dried flower from each treatment divided by the measured canopy area. The harvest index (HI) of each plant was calculated as the ratio of flower mass (g) divided by total biomass (flowers, leaves, and stems) (g).

Chlorophyll Measurements

Leaf chlorophyll concentrations were measured one day prior to harvest using a Chlorophyll Concentration Meter (MC-100 Meter; Apogee Instruments Inc., Logan, UT, USA). Measurements were collected from 10 random leaves on each plant in each treatment and each measurement was a three-point average. The average chlorophyll concertation was calculated for each treatment.

Tissue Nutrient Concentration

To determine leaf and flower elemental content, every seven days three of the most recently expanded leaves and an apical flower sample were collected from each plant within each treatment. Samples were washed with deionized water and dried at 80 °C for 48 h then ground to a fine powder with a stainless-steel grinder (KitchenAid, model BCG111OB). For each treatment a leaf and flower sample were submitted to the Utah State University Analytical Laboratory (USUAL, Logan, UT, USA) for mineral analysis. Each samples contained a homogenized blend of leaf or flower tissue from each

plant in each treatment. To determine the tissue concentrations of Ca^{2+}, potassium (K^{+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), sulfur (S), zinc (Zn^{2+}) and iron (Fe^{3+}) 0.5 g of the powdered sample was placed in a digestion tube containing 6 mL of nitric acid (HNO_3) and digested for 10 minutes at 80 °C, then allowed to cool for two minutes before adding 2 mL of 30% hydrogen peroxide (H_2O_2). The solution was subject to an additional digestion period of one hour at 130 °C to reduce the total digest volume to 2 to 3 mL. The digestion tube was then placed in a vortex stirrer, mixed, and cooled to room temperature before transferring the contents to a 25 mL volumetric flask. Inductively coupled plasma-optical emission spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA, USA) was used to analyze the digest and the results were reported on a dry plant basis (mg per g). Tissue N concentration was determined using combustion analysis (Elementar VarioMax Cube, Elementar Americas Inc., Mt. Laurel, NJ, USA).

Cannabinoid Analysis

At harvest a flower sample (~10 g) was collected from the apical inflorescences of each plant in each treatment. Samples were dried on a ventilated rack at 25 °C and 30% relative humidity for five days. Samples were ground to a fine powder in a stainless-steel grinder (KitchenAid, model BCG111OB). Flower from each plant within a treatment was homogenized and analyzed as a single sample. Samples were analyzed following the method outlined by Westmoreland et al. (2021) by the Utah Department of Agriculture and Food (UDAF) Unified State Laboratory (Taylorsville, UT, USA). Cannabinoid equivalents were calculated following the method outlined by Westmoreland et al. (2021).

Statistical Analysis

Both experiments were designed as complete randomized block designs (CRBD). Experiment one consisted of three treatments with three replicates each (n = 3). Experiment two consisted of two treatments with four replicates each (n = 4). Data were fitted with a linear model and analyzed using regression. Effects were considered significant at $\alpha = 0.05$. Data were analyzed in RStudio (R statistical software, version 4.1.0.).

Results

Yield, Inflorescence Diameter, and Cannabinoid Production

The treatment effects on yield were dependent on the flushing method. Flushing by withholding N did not significantly affect dry flower yield (**Figure 3-1**; $p = 0.3$) or inflorescence diameter (**Figure 3-4**; $p = 0.2$); dry flower yield across treatments was 571 ± 17 g/m^2 (mean ± SD). Yield decreased when plants were flushed with tap water (**Figure 3-1**; $p = <0.01$), but the inflorescence diameter was not significantly affected by the flushing treatment (**Figure 3-4**; $p = 0.07$). The severity of yield reduction when flushed with tap water was dependent on treatment duration (**Figure 3-1**).

The CBD_{eq} concentration was unaffected by flushing with N free solution, but increased slightly when flushed with tap water (**Figure 3-3**). However, there was not a statistically significant difference in total CBD_{eq} yield per plant when plants were flushed with tap water or N free solution (**Figure 3-3**). The average CBD_{eq} yield per plant across all treatments was 11 ± 1 g (mean ± SD).

Chlorophyll Concentrations and Nutrient Partitioning

In both experiments flushing induced deficiency symptoms including leaf chlorosis and senescence (**Figure 3-5**). Chlorosis severity increased with the duration of flushing treatment in both experiments. Chlorophyll concentration decreased with increasing flushing duration in both experiments (**Figure 3-6**) Tissue analysis revealed that leaf N decreased during the duration of the experiments duration while flower N remained relatively steady across all treatments. (**Figure 3-7 A**).

Discussion

Yield and Cannabinoid Production

Treatment effects on dry flower yield were dependent on the flushing method and duration. Flushing with N free solution for up to two weeks had no effect, but yield decreased when plants were flushed with tap water. In contrast, a commercial study reported no differences in yield between flushing treatments of zero, seven, or ten days, but the exact methodology of the flushing procedure was not reported (Wedryk, 2020). The lack of treatment effect on yield reported by Wedryk (2020) could be the result of nutrient stores in the media. Argo and Biernbaum (1995) reported media nutrient levels adequate for growth up to 42 days after the cessation of fertilizer application in poinsettia. In this study, complete nutrient deprivation decreased dry flower yield, this suggests that the external nutrient supply continued to contribute to growth and metabolic processes during late flowering. Generally, well fertilized plants produce more flowers and bloom over a longer period. Source-sink relations are an integral component of yield. Limitations to source tissue carbohydrate production could negatively affect sink tissue development (Smith et al., 2018). Flowers are a strong sink tissue requiring a steady

supply of nutrients and carbohydrates (Carvalho et al., 2006). It is estimated that between 50% and 80% of photoassimilated carbon produced by a mature leaf is loaded into the phloem for export to sinks (Ainsworth and Bush, 2011). Inadequate nutrient supply limits chlorophyll synthesis, photosynthesis, metabolic processes, and growth. Individual inflorescences as well as the individual flowers within an inflorescence compete for carbohydrate and mineral resources (Diggle, 1997). The development of new floral and vegetative meristems may be arrested and resources allocated to maintain existing flowers (Diggle, 1997; Steer and Hocking, 1983). In sunflower (*Helianthus annuus* L.), Steer and Hocking (1983) observed that deficient N supply slowed the rate of leaf and flower initiation.

Nutrient Partitioning

Nutrient uptake and partitioning are dependent on life stage, cultivar, and external nutrient supply (Hood et al., 1993; Ray et al., 2020). Nutrient uptake rates have been described for many crops including rose (Silberbush and Lieth, 2003), snapdragon (Hood et al., 1993), poinsettia (Whipker and Hammer, 1997), and wheat (Demotes-Mainard et al., 2001). Generally, in monocarpic plants the lifecycle is genetically predetermined and follows a basic pattern beginning with vegetative or juvenile stage then progresses to the flowering or reproductive stage followed by seed maturation and finally senescence/plant death. During vegetative growth the primary source of nutrients is from external supply from root uptake. Nutrient uptake from the root decreases and remobilization from old and senescing leaves increases. In hydroponic roses (*Rosa hybrida* L.) nutrient uptake from the solution increased during vegetative growth and decreased during bud formation (Silberbush and Lieth, 2003). In flowering stage chrysanthemum P is preferentially

partitioned to developing flowers (Henry et al., 2018). Saloner and Bernstein (2023) reported increased nutrient translocation from roots to shoots during the reproductive stage in cannabis. In snapdragons (*Antirrhinum majus* L.) nutrient uptake remained increased during the visible bud and anthesis stages, suggesting increased nutrient needs to support developing sink tissue (Hood et al., 1993). In *Phalaenopsis* orchids withholding fertilizer during flower spike initiation and bud formation reduced flower count and induced lower leaf senescence (Wang, 2000). Furthermore, stored nutrients are also remobilized from mature roots, stems, and leaves to support growth and reproductive development. Typically, when resources are adequate, remobilization is a function of growth stage or age-related senescence (Maillard et al., 2015; Ray et al., 2020). Maillard et al. (2015) observed that chlorophyll content decreases and nutrient remobilization increases with increasing leaf age. Nitrogen remobilization in maize was reported at 40% while wheat can remobilize up to 90% of leaf N to translocate to developing seeds (Maillard et al., 2015). A deficient external nutrient supply promotes premature leaf senescence and nutrient remobilization (Havé et al., 2017; Maillard et al., 2015; Ray et al., 2020; Thomas and De Villiers, 1996). In this study, the duration of nutrient deprivation corresponded with the severity of chlorosis, which was also reported by Stemeroff (2017) and Wedryk (2020). Tissue analysis revealed that flushing reduced leaf N, but flower N remained stable, indicating chlorophyll catabolism and the remobilization of leaf N for transport to flowers. Similar results were found for P, K, Ca, S, and Mg. Furthermore, there was not a significant difference in flower concentrations of mobile nutrients between flushed and non-flushed plants. External nutrient supply affects remobilization and in maize more stored N was remobilized under a deficient external N

supply (Ray et al., 2020). Similarly, Worland et al. (2017) reported that nitrate stored leaves and leaf sheaths of sorghum was rapidly depleted after N fertilization ceased with N in panicle leaf sheaths decreasing up to 85% eight days after N fertilization ceased. In this study flushing did not reduce mobile nutrient concentrations in flower tissue. Moreover, while total cannabinoid yields were unaffected, flushing could negatively impact flower yield. Future studies are needed to examine the effects of preharvest nutrient deprivation on the combustion properties and smoke constituents of combusted flower.

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Table 3-1. The nutrient solution composition during flowering. All plants received the same solution during the first six weeks of flowering in both experiments.

N	P	K	Ca	Mg	S	Si	Fe	Mn	Zn	B	Cu	Mo	Ni
						mg/L							
151 (11)	30 (1)	242 (6.2)	120 (3)	19 (0.8)	26 (0.8)	17 (0.6)	0.4 (18)	0.2 (3)	0.2 (3)	0.4 (40)	0.3 (16)	0.01 (0.1)	0.006 (0.1)

Values in bottom row represent ion concentration in mM (macronutrients) and µM (micronutrients).

Table 3-2. The composition of the N free nutrient solution used in experiment one.

N	P	K	Ca	Mg	S	Si	Fe	Mn	Zn	B	Cu	Mo	Ni
						mg/L							
0 (0)	30 (1)	242 (6.2)	120 (3)	19 (0.8)	26 (0.8)	17 (0.6)	0.4 (18)	0.2 (3)	0.2 (3)	0.4 (40)	0.3 (16)	0.01 (0.1)	0.006 (0.1)

Values in parenthesis represent ion concentration in mM (macronutrients) and µM (micronutrients).

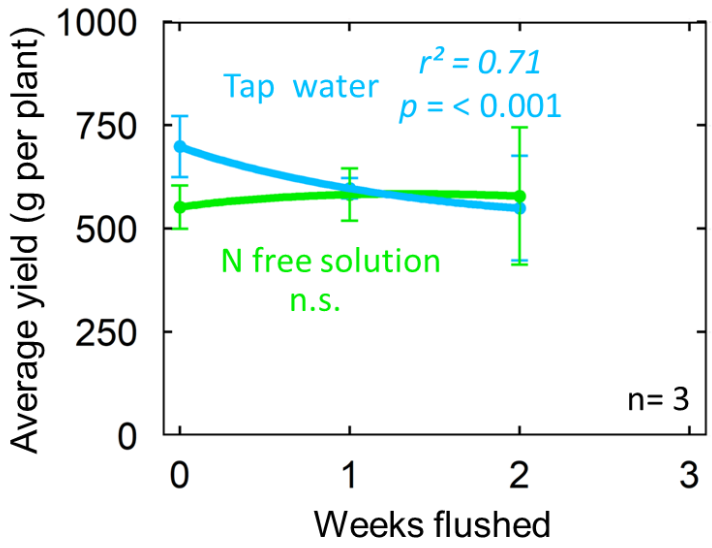

Figure 3-1. The effects of flushing on dry flower yield. Data points represent the average yield at each treatment level and error bars represent the standard deviation.

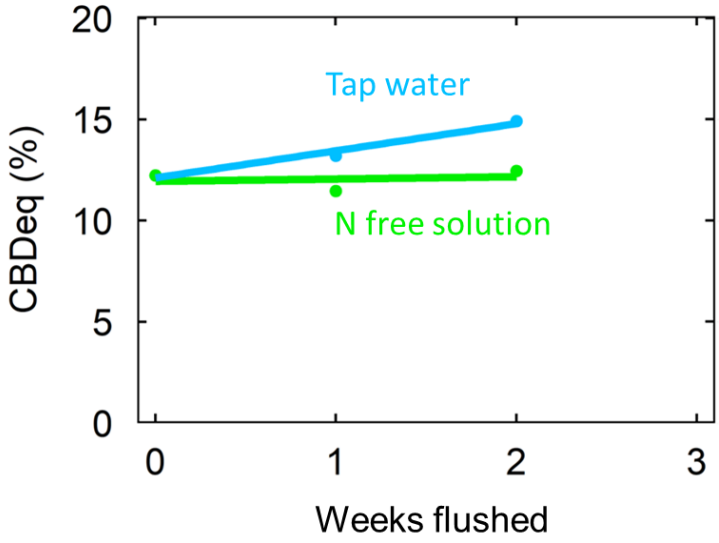

Figure 3-2. The effects of flushing on flower CBDeq concentrations. Each data point represents a single homogenized flower sample from each treatment.

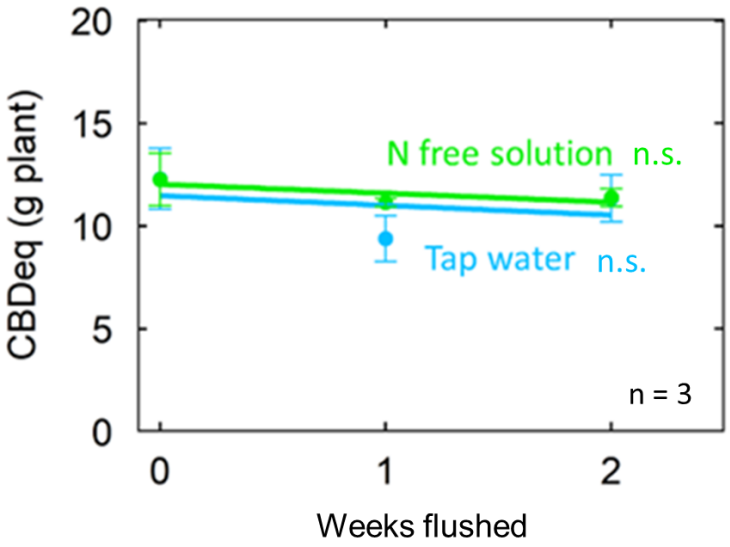

Figure 3-3. The effects of flushing on total CBDeq yield per plant. Data points represents the mean CBDeq yield per plant and error bars represent standard deviation.

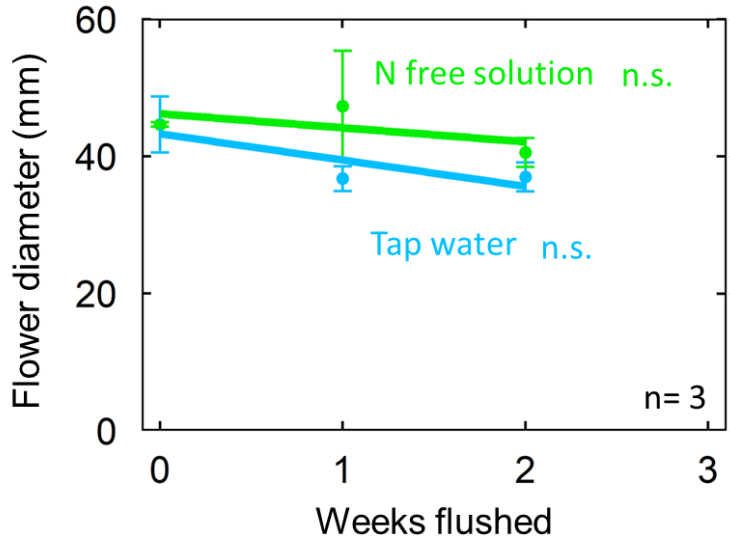

Figure 3-4. The effects of flushing on inflorescence diameter. Data points represent the average inflorescence diameter of the three plants in each treatment and error bars represent standard deviation.

Figure 3-5. The effects of flushing on leaf chlorosis.

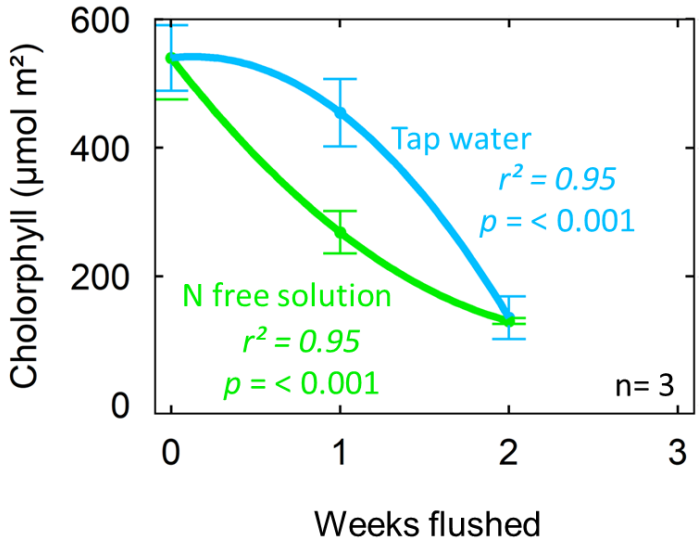

Figure 3-6. The effects of flushing on leaf chlorophyll concentration. Data points represent the average chlorophyll concentration of the three plants in each treatment and error bars represent standard deviation.

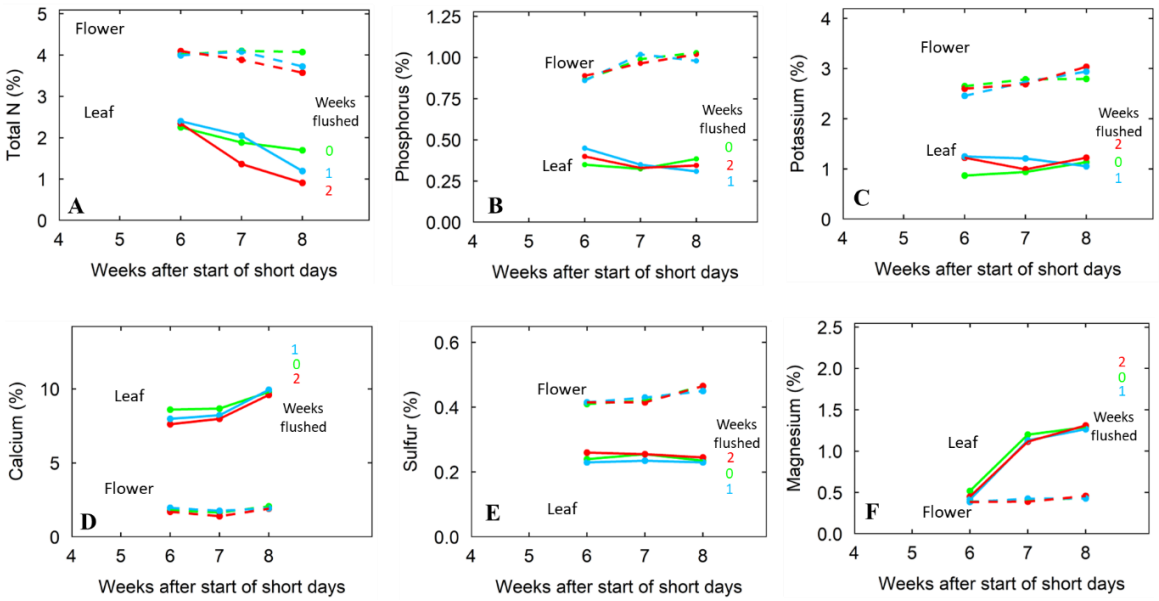

Figure 3-7. The effects of flushing on N (A), P (B), K (C), Ca (D), S (E), Mg (F) concentration in leaf and flower tissue. Flushing did not have a significant effect on tissue nutrient concentrations so data from both experiments were pooled by flushing duration. Each data point represents the average tissue concentration of six plants (n = 6).

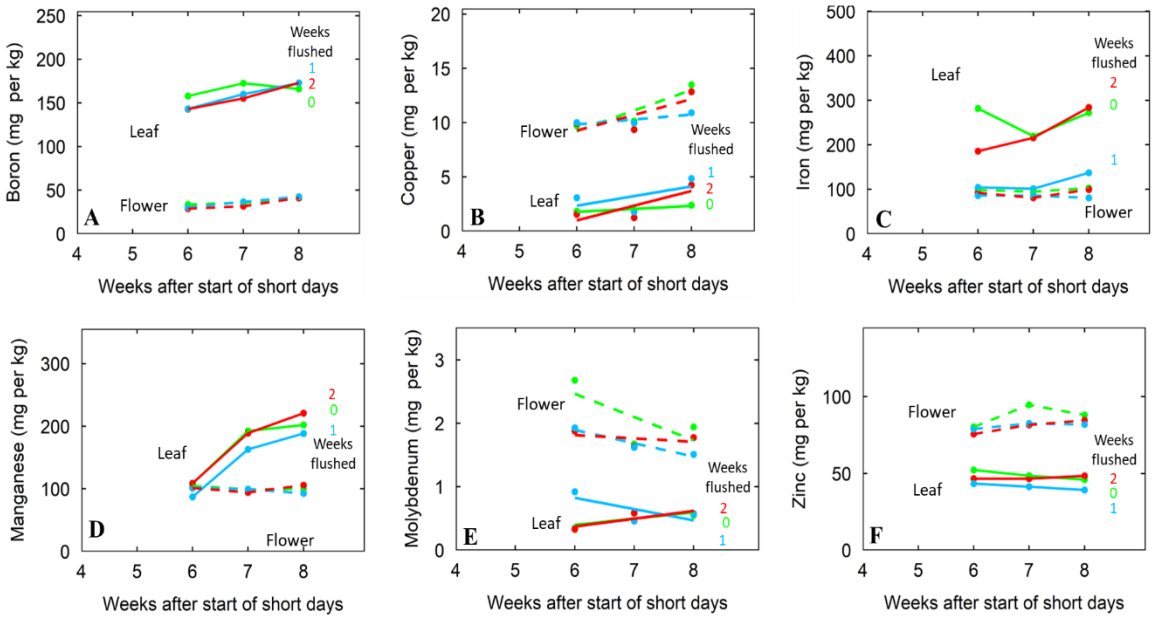

Figure 3-8. The effects of preharvest nutrient deprivation on of flushing on B (A), Cu (B), Fe (C), Mn (D), Mo (E), Zn (F) concentration in leaf and flower tissue. Flushing did not have a significant effect on tissue nutrient concentrations so data from both experiments were pooled by flushing duration. Each data point represents the average tissue concentration of six plants (n = 6).

CHAPTER IV

CONCLUSIONS

Elevated nutrient supply does not increase flower yield and cannabinoid concentration in medical cannabis. In the present study a P supply of 15 mg/L and a solution EC of 2 mS/cm was sufficient for maximum yield. Increasing the P supply in the refill solution above 30 mg/L resulted in P accumulation in solution and when P was supplied above 90 mg/L the concentration of P in the recirculating solution was nearly 300 mg/L. Mobile nutrients accumulated in flower and immobile nutrients accumulated in leaves. Flower concentrations of N, K, S, Fe, Mn, and Mo were roughly twofold higher and P and Cu concentrations were around threefold higher than leaf concentrations in all treatments. The P input affected tissue P, leaf P doubled and flower P increased 70% when the P input increased from 15 to 90 mg/L. However, the leaf tissue concentrations of all other nutrient were minimally affected by the solution concentration. Cannabis may tolerate excessive fertilizer application, but excessive fertilization does not improve yield or quality. Furthermore, excessive fertilization wastes resources and contributes to pollution.

Flushing is the practice of withholding fertilizer during the final weeks of flowering. The results of the present study indicate that flushing does not reduce the concentrations of mobile nutrients like N in flower tissue. Mobile nutrients are remobilized from leaf tissue to support flower and cannabinoid development. Importantly, the results of this study suggest that depriving plants of nutrition even at the end of the production cycle can negatively impact yield.