Irrigation Management Strategies for Medical Cannabis in Controlled Environments

By

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A post-approval (re)-examination of the raw data discovered unrecoverable errors in the handling and reporting of the water potential data, with further issues regarding the nature of the treatment differences. The general errors are:

1) Upon further review of the raw data logs it was discovered that the Control and Mild Stress treatments were in fact the same (ref. page 38). Both treatments had the same number of irrigation events (i.e. total water applied was the same) in all three trials. The irrigation events were offset by 1-day but they both occurred at 2-day intervals.

    a. Resolution – Any observed differences between the control and mild stress treatment should be disregarded. Detected differences in agronomic and metabolite production between the Control and Mild Stress treatments can only be attributed to random chance and variation in the growth facility, not a response to distinct irrigation regimes. Affected Figures – Disregard Control and Mild Stress treatment effects in Figures 4.11 (page 54) and 4.13 (page 55)

2) Raw data was not properly filtered to remove erroneous readings (e.g., positive values of plant water potential; non-sinusoidal or flat line response) resulting from instrument fouling and condensation within the sensing chamber. The inclusion of erroneous data resulted in skewed relationships between cumulative plant water potential and cumulative vapour pressure deficit.

    a. Resolution – Disregard the relationships depicted in Figures 4.1 (page 44), 4.2 (page 45), 4.3 (page 46), 4.4 (page 47), 4.5 (page 48), 4.6 (page 49), 4.7 (page 50), 4.8 (page 51), and 4.9 (page 52).

3) The apparent two-phase relationship between CWP and CVP shown in Figure 4.1 (page 44) and subsequently highlighted and independently analysed in Figures 4.2 (page 45) and 4.3 (page 46), is the result of missing data (logger failure) during the middle phase of the first growth cycle; the missing data was not properly accommodated resulting in an apparent separation of the response relationship between early and late growth periods. The subsequent analysis of flower cycles 2 and 3 included an analysis of this artifact-based separation; as such, Figures 4.5 (page 48), 4.6 (page 49), 4.8 (page 51), and 4.9 (page 52) are not meaningful as there is no data to suggest or support treating the data as two distinct groups.

Based on the above errors, any conclusions outlined in section 6.1 (page 62-65) should also be disregarded.

ABSTRACT

Irrigation Management Strategies for Medical Cannabis in Controlled Environments

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Medical cannabis production is a new industry in Canada and represents a challenge for the production of a repeatable and standardized product for medical use. A reliable and reproducible environmental control strategy can contribute significantly to meeting this challenge. Irrigation management and control of plant water status is one of the key environmental control elements. To assess the effects of various irrigation management strategies this study deployed *in situ* stem psychrometers to measure the water status of plants. As a routine feedback device for irrigation control these devices are not ideal for large-scale production so correlation with the key environment variable representing the aerial demand for moisture (vapour pressure deficit) was assessed. By establishing a relationship between cumulative water potential (cWP) and cumulative vapour pressure deficit (cVPD) an irrigation management strategy that predicted plant water status based on measurements of cVPD could be employed. Three treatments; control (irrigation events every 1-2 days), mild-stress (irrigation events every 2 days), and moderate-stress (irrigation events every 3 days) were tested. The effects of flushing were also investigated to determine whether it had the intended effect of reducing nutrient concentrations within the dried bud. Through the use of psychrometers, water status (cWP) thresholds were correlated with humidity (cVPD) thresholds and reduced irrigation frequency resulting in water use reductions up to 45.7% which had negligible impacts on yield and cannabinoid profile. Flushing was found to be ineffective in removing any significant amount of nutrient from the bud.

DEDICATION

Dedicated to my parents, brother and sister, and my pets. Without all of your support I definitely would not have been able to complete this research and thesis. Thank-you for helping me move twice, borrow your car for 8 months, giving support and kind words when stressed out, and all of the home-shopping.

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Friends and family – I really appreciate that you all listen to me talk endlessly about cannabis and not get too upset. You have helped me through the tough times and the good times, I couldn't have done this without your support and care.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

THC – Tetrahydrocannabinol

CBD – Cannabidiol

Ψ – Water Potential

RH – Relative Humidity

VPD – Vapour Pressure Deficit

pH – Potential of Hydrogen

T_p – Turgor Pressure

Π – Osmotic Potential

T – Matric Potential

g – Gravity constant

SVP – Saturated Vapour Pressure

CMH – Ceramic Metal Halide

RO – Reverse Osmosis

HPS – High Pressure Sodium

WBD – Wet Bulb Depression

CWBD – Corrected Wet Bulb Depression

cWP – Cumulative Water Potential

cVPD – Cumulative Vapour Pressure Deficit

HPLC – High-Pressure Liquid Chromatography

GC-MSD – Gas Chromatography – Mass Spectrometry

ANOVA – Analysis of Variance

SE – Standard Error

CHAPTER 1 – INTRODUCTION

Cannabis is a medicinal plant that is now legal to use for medical applications in Canada (SOR/2016-206). When considering pharmacological applications of cannabis, it is important to have consistent levels of medically active plant secondary metabolites present in the flower to ensure proper dosing and assessment of treatment outcomes by the prescribing doctor (Flores-Sanchez, Josefina, & Verpoorte, 2008; Pertwee, 2014). Tightly controlled plant production environments (i.e., growth chambers) that provide a consistent growth environment for the developing plants go a long way to ensure that patients are receiving a standardized product.

Current production methods employed at most cannabis production facilities include the use of automated irrigation and environment control systems. Used correctly, these systems can provide environmental challenges, such as induction of mild drought stress that could influence the production of plant secondary metabolites for the benefit of the end consumer/patient. Controlled or moderate drought stress has been demonstrated to influence the production of certain secondary metabolites (Kleinwächter & Selmar, 2015). In terms of medicinal plants such as cannabis, this alteration in secondary metabolism could lead to greater percentages of active compounds. Currently, there are very few data available for cannabis with respect to drought induced metabolite modifications, so the duration and intensity of drought stress required to appropriately modify the production of secondary metabolites is not known.

Measuring water status in plants can be accomplished using a variety of technologies including the pressure bomb (Scholander, 1965) and psychrometers (Dixon and Tyree, 1984) or indirectly with soil moisture probes. The pressure bomb requires excision of plant material for testing and cannot be used for continuous automated monitoring. Soil moisture probes can be used to take continuous measurements; however, significant variability is often observed

due to sensor placement (within soil column) and heterogeneity of the growth substrate (Ehlers & Goss, 2003). Psychrometers can be used to continuously and non-destructively measure whole-plant water potential (Tran, 2014). Water potential gradients drive water movement from roots to leaves in all plants. Automating this measurement at a single location at the base of the stem and with appropriate temporal resolution (15 min) provides virtually continuous assessment of the variation in this key physiological variable in a plant's response to environmental conditions. Water potential is a powerful measurement in that it integrates a plant's response to all environmental conditions to assess plant water status. Integrating water potential over time provides a means to quantify plant responses to environmental management such as irrigation scheduling. The opportunity to exploit this measurement approach in the feedback control of environmental variables is most obvious in managing irrigation by identifying thresholds of cumulative water stress beyond which irrigation is initiated. This repeated integration of water potential as a tool for scheduling irrigation based on plant need could help ensure a consistent and standard medicinal crop production.

Flushing is currently standard common practice used by many cannabis producers. This involves irrigating the plants with nutrient free water for the last two weeks before harvest. The belief is that this method of irrigation will reduce the concentration of nutrients within the bud. This reduction is then believed to have impacts on the final taste of the product when consumed. Regardless of the anecdotal impacts on taste, there is little evidence that flushing the root zone during the final phase of production would result in a net export of nutrients from the maturing bud.

This study was performed to assess the impact of controlled drought stress on the yield and composition of medicinal compounds in cannabis flowers. The main objectives were:

1) Devise an experimental protocol for the application of the stem psychrometer to measuring water potential in cannabis.
2) Correlate cumulative water potential measurements with key environment variables (esp. cumulative vapour pressure deficit) to predict plant water status and devise appropriate irrigation management strategies.
3) Assess the impact of controlled drought stress on the final flower yield.
4) Assess the impact of controlled drought stress on the composition of secondary metabolites/medicinal compounds in the flower
5) Assess the impact of flushing on the nutrient composition of the harvested flower buds.

CHAPTER 2 – LITERATURE REVIEW

2.1 CANNABIS REGULATIONS

Cannabis is in a group of plants found, both naturally and as a cultivated crop, throughout the world (Farag & Kayser, 2015b; Mechoulam, 1970; Pertwee, 2014). It has been cultivated for thousands of years as a source of hemp fiber and for its psychoactive and medicinal properties (Farag & Kayser, 2015b; Farquhar-Smith & Paul, 2002). Cannabis has also been used medicinally dating back approximately 5000 years (Lemberger, 1980; Pertwee, 2014). Even with all these different uses, cannabis remains a prohibited substance throughout most of the world (Graham, 2002; Pertwee, 2014). Canada was the first nation to implement a national policy for the use and paid supply of medicinal cannabis to patients provided by licensed cannabis producers (Graham, 2002). This policy has allowed patients to access the cannabis cultivar that works best for them in addressing their specific medical condition (Graham, 2002).

2.2 CANNABIS TAXONOMY

The specific taxonomic classification of *Cannabis spp*. is still debated with differing views on whether there exists one, two, or three species of *Cannabis*. According to Emboden (1974), naturally occurring, location-specific differences (ecotypes) between *Cannabis* plants justifies polytypic genus designation that includes; *Cannabis sativa, Cannabis indica,* and *Cannabis ruderalis* (Emboden, 1974). Other researchers have claimed that *Cannabis* is monotypic based on cannabinoid data from crossing *Cannabis sativa* L. and *Cannabis ruderalis,* which showed no discernible difference between any of the progeny tested (Beutler & Marderosian, 1978). More recent cannabinoid chemical composition studies also suggest that cannabis is a monotypic genus (Elzinga, Fischedick, Podkolinski, & Raber, 2015). Small

and Cronquist (1976) suggest that there are two different types of cannabis; wild type, and domesticated type. This type classification is based on the general characteristic of cannabis plants found both in nature and under cultivation. Wild type plants have limited intoxicant potential and are mainly used as a fibre source while domesticated varieties have much stronger intoxicant potential and are used mainly as inebriants (Small & Cronquist, 1976). Throughout this thesis, the term 'cannabis' will be used *collectively* for all types/species/lines etc., of cannabis.

2.3 Medical Applications of Cannabis

Cannabis is an annual, dioecious plant that produces a range of medically active compounds used in the treatment of a wide range of human health conditions. Historically, cannabis has been prescribed as an anticonvulsive (preventing or arresting seizures), analgesic (pain relief), anxiolytic (reduce anxiety), and antitussive (prevent or relieve coughing) medication (Aizpurua-Olaizola et al., 2016; Farquhar-Smith & Paul, 2002; Iversen, 2003; Lemberger, 1980; Pertwee, 2014). More recent research suggest that the phytochemical complement present in cannabis may also have clinical applications as an antinociceptive (reducing sensitivity to painful stimuli), cardiovascular intervention (heart or blood treatment), immunosuppressive (suppressing immune response), antiemetic (treatment for nausea), appetite stimulation, antineoplastic (cancer treatment), antimicrobial, anti-inflammatory, neuroprotective/antioxidant (protection from toxic substances in brain), with further potential for treating psychiatric conditions (Aizpurua-Olaizola et al., 2016; Flores-Sanchez et al., 2008; Pertwee, 2014). Patients prescribed cannabis can ingest the product in several ways depending on personal preference and the medical condition being treated. Patients can: 1) absorb the active compounds through the digestive tract via ingestion of extracts added to food or taken in capsule form, 2) inhale vaporized extracts or partially

combusted cannabis buds or extracts (smoke), or 3) through oral mucosal membrane absorption via cannabis extract drops (Mechoulam, 1970; Pertwee, 2014).

Cannabis has been shown to have multiple medically relevant applications; however, there are known detrimental effects, particularly with respect to psychological disorders (Hao, Gu, & Xiao, 2015; Pertwee, 2014). Cannabis has been shown to exacerbate pre-existing psychiatric conditions in some users (Hao et al., 2015; Pertwee, 2014), while potentially leading to earlier onset in others. Pertwee (2014) demonstrated that patients with schizophrenia that had a history of cannabis use, tended to experience their first psychotic episode at an earlier age than those that did not have a history of cannabis use. The use of cannabis before the age of 18, during which the human brain is still developing, has been shown to negatively influence brain development (Hao et al., 2015). Cannabis use is also a possible risk factor for cardiovascular issues in all users regardless of age or physical health due to an increase in blood pressure typically experienced during use (Hao et al., 2015; Pertwee, 2014).

Cannabis produces two primary phytochemical compounds that exhibit some medically beneficial properties; cannabidiol (CBD) and tetrahydrocannabinol (THC) (Andre, Hausman, & Guerriero, 2016; Flores-Sanchez et al., 2008; Mechoulam, 1970; Pertwee, 2014). In addition to these primary compounds (comprising approximately 20-30% of secondary metabolites in medical cannabis plants), there are numerous other metabolites/metabolite classes present in cannabis including other cannabinoids, phytocannabinoids, flavonoids, stilbenoids, terpenoids, alkaloids and lignans (Andre et al., 2016; Croteau, Kutchan, & Lewis, 2000; Flores-Sanchez et al., 2008; Humphrey & Beale, 2006; Mechoulam, 1970). Currently, 480 different secondary metabolites have been identified in cannabis. The absolute and relative amounts of these compounds vary depending on the cultivar and horticultural production methods (Caplan et al. 2017; Flores-Sanchez et al., 2008; Pertwee, 2014). In terms

of plant ecology and evolution in their native habitats, secondary metabolites perform critical functions such as herbivory prevention (herbivore toxicity; palatability), or as an attractant for predators of herbivores (Croteau et al., 2000; Farag & Kayser, 2015b; Humphrey & Beale, 2006). Conversely, some secondary metabolites contribute to seed distribution by encouraging foraging (due to flavour) by herbivores leading to seed ingestion and dispersal during later defecation (Croteau et al., 2000; Farag & Kayser, 2015b; Humphrey & Beale, 2006).

The medicinal compounds found in cannabis are thought to exert their effects primarily through interactions with cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2) receptors in the brain and immune cells respectively (Farquhar-Smith & Paul, 2002; Iversen, 2003). The CB_1 receptors are located throughout the brain, with particularly high levels occurring in the hippocampus (Farquhar-Smith & Paul, 2002; Iversen, 2003; Wilson & Nicoll, 2001). The hippocampus is the part of the brain associated with memory formation and spatial memory (Iversen, 2003; Wilson & Nicoll, 2001). The high concentration of CB_1 receptors in the hippocampus is evidenced by cannabinoid effects on memory and spatial awareness (Farquhar-Smith & Paul, 2002; Iversen, 2003; Wilson & Nicoll, 2001). In contrast to the cerebral localization of CB1 receptors, CB_2 receptors are predominant on immune cells and in organs containing high quantities of immune cells such as tonsils (lymphatic tissue) and the spleen (Farquhar-Smith & Paul, 2002). Due to the location of these receptors on immune cells there may be effects on pain and inflammation from neuroimmune interactions; however, this needs further investigation to determine the impacts (Farquhar-Smith & Paul, 2002).

2.4 Current Cannabis Production Methods

Cannabis is a dioecious species, although hermaphroditic flowers can occur under some circumstances (Cervantes, 2006; Pertwee, 2014). The female plant produces the flower (common vernacular, 'bud') from which the majority of the active compounds are extracted or consumed. Male individuals do not produce a flower containing the secondary metabolites of interest and only serve the function of pollinating the female plants (Cervantes, 2006; Pertwee, 2014). By growing female plants without allowing male plants to pollenate them, seeds are not produced within the flower. Cannabis producers focus on the female plants, as the main goal is production of the flower without any seeds present to facilitate ease of intake or drug delivery (Cervantes, 2006; Farag & Kayser, 2015b; Flores-Sanchez et al., 2008; Pertwee, 2014).

Clonal plant propagation practices follow the same general procedures at most cannabis production facilities. Briefly, stock plants, or "mothers", are kept vegetative by maintaining an 18-hour photoperiod and environment conditions mimicking spring weather with temperatures typically around 20˚C and relative humidity around 70% while providing vegetative-specific nutrient regimes during irrigation events so that numerous cuttings can be excised and stimulated to root (e.g., using rooting hormone). The results are viable genetic clones that can be grown to maturity and their flowers harvested (Cervantes, 2006; Hansen, 1986; Pertwee, 2014). Multiple cultivars, termed "strains" are kept at all times so that multiple products can be offered to patients. Clones are maintained in propagation chambers for approximately 14 days to allow sufficient root development. After the cuttings have established roots they are transplanted to larger pots and are moved to a larger growth chamber for the vegetative phase of growth. For cannabis plants in the vegetative growth phase, an 18-hour photoperiod is used to maintain vegetative growth along with environment conditions that more closely replicate the final environmental conditions (i.e., 24˚C and 65%

relative humidity (RH)) that will be encountered in the flowering stage of growth so the plants will be better adapted to the final environment before entering the final growth chamber. The clones are kept vegetative for approximately 15-20 days, depending on cultivar and growers, or until they have developed sufficient biomass to support flowering. Flowering is the final and longest growth stage in cannabis production. This takes approximately 55-65 days, depending on the cultivar and environmental conditions, and is considered the most important part of production as the final product, the cannabis flower, is initiated, develops, and matures during this phase. During this time the growers will adjust environment conditions, irrigation schedules, as well as nutrient applications to produce the highest quality flower possible with consistent levels of cannabinoids, pleasant odour and taste, and impressive appearance (Cervantes, 2006; Farag & Kayser, 2015b; Pertwee, 2014).

Current irrigation scheduling in cannabis production generally involves a subjective assessment of need by the grower, commonly achieved through frequent inspections of the crop and rooting substrate. This method is subjective and does not take into account the actual water status of the plants. Growers have used soil moisture content as a measure for irrigation; however, the measurements from these devices are influenced by sensor location within the pot/media and growth media composition, necessitating sensor calibration for specific growth media/systems (Ehlers & Goss, 2003). An average soil moisture content for a representative sample of the entire crop can be used as an irrigation trigger although there are many other important factors that need to be considered to develop proper irrigation scheduling (Ehlers & Goss, 2003).

Irrigation for large-scale cannabis producers is generally done using automated systems. The water is applied either using spigots, drip irrigation, or flood tables (Pertwee, 2014). These methods of irrigation have the benefit of ease of use but they can often result in uneven wetting of the growth substrate (Ehlers & Goss, 2003; Pertwee, 2014). Uneven soil moisture

will skew the measurements made with soil moisture sensors, which can lead to over or under-application of irrigation water (Pertwee, 2014). Using a visual inspection method and inadequate soil moisture readings to schedule irrigation events makes it difficult for the grower to maintain the plants at a consistent water status, which in turn can lead to variation in secondary metabolite production (Flores-Sanchez et al., 2008).

Flushing is a practice used by many cannabis producers to have an impact on the taste of the flower. This is accomplished by irrigating with non-fertigated water for the last two weeks of the flower cycle. It is believed that this method of irrigation will reduce the amount of nutrients found in the flower (Cervantes, 2006). There has been no evidence provided to support this claim, so testing will need to be completed to determine its validity.

2.5 Production Issues

A significant issue, or suite of issues, facing the cannabis industry is the lack of systematic production strategies based on scientifically valid production studies. The reason behind this paucity of scientific research is that up until recently cannabis has been a prohibited substance in most developed nations. This prohibition greatly restricted the volume and scope of research on the species (Mechoulam, 1970; Pertwee, 2014). An example of this prohibition-induced knowledge gap is the relatively poor characterization of the phytochemical complement of cannabis (Beutler & Marderosian, 1978; Elzinga et al., 2015; Emboden, 1974; Small & Cronquist, 1976), although this is rapidly changing as legalization advances in developed countries such as Canada. Prohibition era research, as well as emerging research, also suffers from a lack of consistency in the plant samples used in the research. Large variability arises between and within studies as a result of cannabis preparation methods (Cervantes, 2006; Coffman & Gentner, 1979; Farag & Kayser, 2015a,

2015b; Flores-Sanchez et al., 2008; Mechoulam, 1970; Pertwee, 2014). Currently, individual medical cannabis producers employ a wide range and combination of production strategies, from different growth environments and root substrates, through to the methods used to cure the final product or extract the medicinal compounds (Mechoulam, 1970; Pertwee, 2014). Further, each producer has their own genetic line or lines, which further adds to the inconsistencies and variation in available studies (Mechoulam, 1970; Pertwee, 2014). Using standardized production methods along with consistent storage and sample preparation, the cannabis samples used for critical scientific research will greatly improve the quality and consistency of scientific findings.

Growth procedures for cannabis vary widely based on the source of information/protocols. Since cannabis remains illegal or is a highly-controlled substance in most developed countries, there are very few reliable scientific sources of information for establishing growth parameters. Much of the information available specifically for cannabis is found in online forums where [typically] anonymous users share information. Very few of these forums contain any scientifically derived procedures and are mostly anecdotal in nature. This lack of information makes production difficult when growers are relying on anecdotal evidence, that may or may not be applicable to their production system, instead of proven scientific sources (Pertwee, 2014). Applying rigorous scientific research methods to the development of production strategies is dispelling much of the anecdotal production doctrine prevalent in the medicinal cannabis industry (Mechoulam, 1970; Pertwee, 2014).

2.6 Cannabinoid Production and Standardization

Concentrations of secondary metabolites in plants are affected by water supply, nutrients, humidity, temperature, CO_2 concentration, air movement, plant structure, and light intensity,

quality, and distribution (Caplan et al, 2017; Jia, Sun, Li, Li, & Chen, 2015; Kleinwächter & Selmar, 2015; A. Pirzad, Alyari, Shakiba, Zhetab-Salmasi, & Mohammadi, 2006; Alireza Pirzad et al., 2011; Singh-Sangwan, Abad Farooqi, & Singh Sangwan, 1994). These factors affect secondary metabolite production, particularly when they are outside of optimal ranges, through stress responses to changes in growth environment that cause issues for the plant (Flores-Sanchez et al., 2008). As a plant exhibits or is exposed to a physical, chemical or biological stressor, it will respond by altering the activity of various metabolic pathways in order to adjust and accommodate the stress condition. In cannabis for example, a common response to drought stress is an increase in the production of secondary metabolites related to that stress, such as abscisic acid (Flores-Sanchez et al., 2008; Pertwee, 2014; Wang & Irving, 2011). Abscisic acid plays key metabolic and developmental roles and has been shown to influence the storage of certain compounds that can increase herbivore palatability, thereby ensuring seed distribution before the parent organism succumbs to the water stress (Flores-Sanchez et al., 2008; Pertwee, 2014; Wang & Irving, 2011). Water availability has a strong effect on physiological activity including the production of secondary metabolites. It has been shown that plants grown under mild to moderate drought stress produced the highest levels of desirable secondary metabolites in medicinal and spice plants (Kleinwächter & Selmar, 2015). On the other hand, the lowest secondary metabolite yields were found in both optimally (for biomass production) irrigated and high drought stress groups (Jia et al., 2015; Kleinwächter & Selmar, 2015; Alireza Pirzad et al., 2011; Singh-Sangwan et al., 1994). Given the dynamic relationship between plant water status and the phytochemical makeup of the final product, cannabis nurseries are faced with an interesting dilemma in terms of irrigation. The goal is to produce a plant with the greatest yield while also having a standardized and enhanced phytochemical balance. This will require the design of specialized irrigation protocols that allow the plant to exhibit a controlled but mild drought stress before

an irrigation event is triggered. These systems would then, in theory, enhance secondary metabolite production without causing any appreciable reductions in productivity. Development of these irrigation protocols requires a method to measure the effects of irrigation regimes on the actual water status of the plant to understand how different amounts of water will affect the production of secondary plant metabolites and yield.

2.7 Growth Environment

2.7.1 Temperature

The temperature at which cannabis is grown will have a substantial influence on growth and development. Temperature is generally kept around 24˚C for most cultivars during the propagation and vegetative phases of growth (Cervantes, 2006; Farag & Kayser, 2015b; Pertwee, 2014). As the plants enter the flowering phase, the temperature is generally lowered to approximately 18˚C as this is the temperature better representing fall weather, which is the time of the year that cannabis would flower in its native range (Farag & Kayser, 2015b; Pertwee, 2014).

2.7.2 Humidity and Vapour Pressure Deficit (VPD)

The amount of moisture (water vapour pressure) in the air is a major governing parameter in plant growth. In indoor controlled growth environments, the aerial moisture content is measured as humidity (relative or absolute), vapour pressure deficit (VPD), or both (Ehlers & Goss, 2003; Pertwee, 2014; “Understanding and Using VPD,” 2009). Vapour pressure deficit is the main driving force determining the concentration gradient that governs the rate of water loss from the surface of leaves (assuming open stomates) to the surrounding air. The measurement is the difference (or deficit) between the vapour pressure caused by

aerial moisture and the vapour pressure at the moisture saturation point (Ehlers & Goss, 2003). One of the main impacts of VPD within growth environments is the modulation of the plant stomata (Ehlers & Goss, 2003; Pallardy & Kozlowski, 1979) with stomatal response being strongly correlated with VPD (Pallardy & Kozlowski, 1979). Maintaining a steady and appropriate VPD in a growth environment will allow the plant to maintain open stomata throughout all light periods for no reductions in CO_2 uptake (maximize net carbon fixation) (Ehlers & Goss, 2003; "Understanding and Using VPD," 2009).

2.7.3 Insect Pests

Indoor growth facilities will always have to manage insects within the growth areas. There are numerous pests that could be present within a facility including; aphids, fungus gnats, mites, thrips, and whiteflies (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006). For cannabis production, the main pests present are fungus gnats, springtails, and spider mites (Cervantes, 2006; Pertwee, 2014). These pests are very common in almost any indoor growth environment including greenhouses and indoor production facilities (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006).

Proper management of plant pests is critical in any plant production system, but even more so in medicinal plant production where control measures are more limited(Health Canada, 2017) Since the end use of cannabis is medical treatment, Health Canada has established necessary restrictions on the types of pest control options accessible by growers. Physical and/or pheromone pest traps such as yellow sticky cards and diatomaceous earth are permitted (Cervantes, 2006; McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006; Pertwee, 2014). Another control method is the use of biological

control agents, such as known predators or pathogens of the specific pest species (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006). If the previous options are insufficient then it may be necessary to apply chemical control agents, of which there are many; however, the cannabis industry is limited to those approved by Health Canada or by any other certification body (e.g., organic) that the producer is governed by (Health Canada, 2017). Pesticides can be very effective when used properly, the issue is that plants can react adversely to the application of certain pesticides and it can take some time for recovery (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006; Pertwee, 2014). This could cause a reduction in growth for some time after application of the pesticide causing a loss of final yield. Chemical pesticide use requires a cost-benefit analysis to determine whether pesticide application is worth the risk at the time of application.

2.7.4 Bacterial and fungal infections

Bacterial and fungal infections can be difficult to control in growth environments. Pathogen spread can occur through the air, from pests, via infected growth media, or from cultivation practices through horticultural personnel moving between growth chambers and using contaminated tools and instruments (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006). With so many different modes of infection, controlled environment growth facilities must have strong controls in place to prevent pathogen entry. Once a pathogen is inside a facility, it can replicate quickly if controls are not in place to reduce or stop the spread. In cannabis production, the most common pathogens are powdery mildew (mainly *Erysiphales* spp.) and Septoria (*Septoria lycopersici*). These infections can cause issues ranging from nuisance through to complete crop failure (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006; Pertwee, 2014). When these

infections are spotted, they must be dealt with immediately to reduce the chance of transferring the infection to other plants.

Pathogen control is achieved via several mechanisms. The first and most common method of control is to maintain proper environment conditions; conditions unfavorable to the development and spread of pathogens. For the most common infections in cannabis, such as powdery mildew, this is accomplished by keeping the humidity and temperature low enough so that there is no layer of moisture formed on the plant, and also by keeping the environment clean and sanitary (McPartland et al., 2000; "Nova Scotia Greenhouse Pest Control Training Manual," 2006). Bacterial and fungal pathogens have a much better chance of establishing themselves when the environment is moist and warm with little air movement ("Nova Scotia Greenhouse Pest Control Training Manual," 2006). The second method of control is the use of bactericidal and fungicidal chemicals. This is an effective method of control when the infection has become too large to control by hand. Spraying can have detrimental effects on plants by causing minor tissue damage that could affect the final yield. Most of these sprays function by applying an alkaline layer of water on the leaf which will destroy fungal spores and bacteria that are contacted. If there is a buildup of alkaline material on a plant leaf it could cause damage to the cuticle and damage to internal leaf structures. The choice of chemical control agent needs to be determined by conducting a cost-benefit analysis to see if the spray makes sense to control the infection. In the cannabis industry there is a limited list of options for pesticides as Health Canada has set standards for the chemical control agents that are found to not cause any detrimental effects to humans when ingested (Health Canada, 2017).

2.7.5 Growth media and nutrients

The substrate in which plants are grown has a major influence on overall plant productivity. Soil moisture or moisture holding capacity is an important component of the growth media, and has a significant influence on nutrient availability and water uptake, and therefore on photosynthesis and plant growth (Cervantes, 2006; Ehlers & Goss, 2003; Pertwee, 2014). When the soil moisture is too low, the plant's ability to take up nutrients is limited as a result of low water availability and reduced access to nutrients stored in the media (Ehlers & Goss, 2003). When drought conditions prevail, stomata close to conserve water. This in turn reduces the amount of CO_2 entering the leaves for use in photosynthesis (Ehlers & Goss, 2003). When there are very low levels of CO_2 available, the plant will have to reduce or even stop photosynthesis. Stomata will often close, thereby reducing photosynthesis, well before visual (wilting) symptoms of water stress occur. In order to ensure maximum production, it is important to maintain proper soil moisture based on quantitative and predictive metrics such as soil moisture (Cervantes, 2006; McPartland et al., 2000; Pertwee, 2014). Soil pH also plays a direct role in nutrient availability in the root zone. When the soil pH is either too high (too basic) or too low (too acidic), the roots are unable to take up certain nutrients due to pH lockout as shown below in Figure 2.1 (Cervantes, 2006; Ehlers & Goss, 2003; Pertwee, 2014). For cannabis, the ideal soil pH has been shown to be approximately 6.5 as this allows for the plant to have access to all available nutrients without any pH lockout; however, this will differ between cannabis cultivars as each will have different requirements for soil pH (Cervantes, 2006; McPartland et al., 2000; Pertwee, 2014).

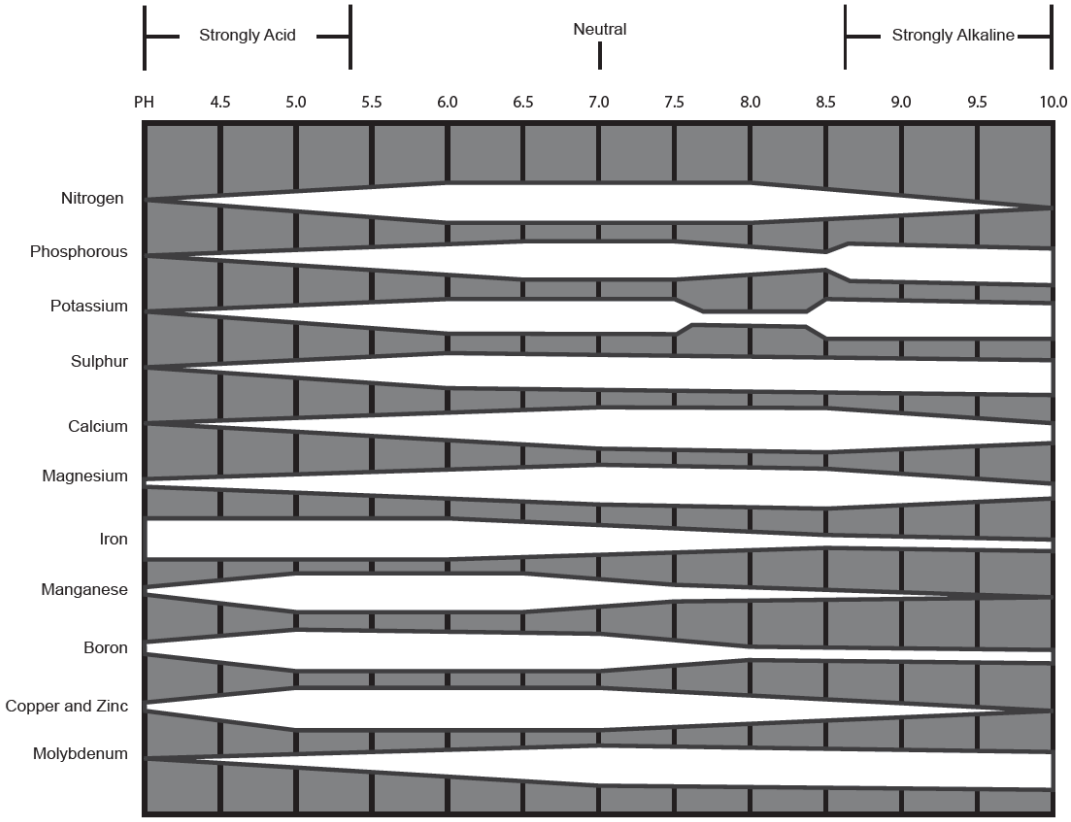

Figure 2.1 - Nutrient Availability According to Soil pH (Adapted from McPartland et al., 2000)

Soil pH has strong effects on nutrient availability. When the pH is too low (acidic), or too high (alkaline), the plant will not be able to take up sufficient amounts of many of the main required nutrients. The availability of metals such as Iron, Manganese, and Zinc is increased when the soil pH is acidic and reduced when alkaline. A neutral, or slightly acidic soil pH is ideal for most plants as this is the zone that provides the greatest availability of nutrients for plants (McPartland et al., 2000).

In the medical cannabis industry, numerous growth substrates are in use, including organic mixes of peat moss, vermiculite, and manure through to hydroponic growth systems using rock wool or other inert media (Cervantes, 2006; Pertwee, 2014). Each different growth media will require different amounts of nutrients and pH adjustment since these systems will interact differently with the roots of the plants. This causes issues for growers when attempting to compare nutrient feed rates between growth media and across different systems (Cervantes, 2006; Pertwee, 2014).

2.7.6 Light intensity, quality, and photoperiod

Light is the source of energy that a plant uses for growth and production through photosynthesis. This is one of the most important factors in any controlled environment growth system and needs to be tuned in concert with other environment parameters. Light intensity is the amount of light and is usually measured in $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Cervantes, 2006; McPartland et al., 2000; Pertwee, 2014). For all plants, there is a light intensity threshold beyond which the plant's photosynthetic apparatus is saturated. Past this point any extra amount of light is detrimental for the plant as this extra light energy needs to be dissipated through processes such as the xanthophyll cycle (Pertwee, 2014). Light quality refers to the spectral composition (i.e., wavelengths; colours) delivered by a given light source. To produce a healthy and standardized crop, the light needs to be uniform and tuned according to the needs of the plant being grown (Cervantes, 2006; Pertwee, 2014). Photoperiod is the amount of time that light is provided to plants during each day. For cannabis, the photoperiod used in vegetative growth is 18h of light with 6h of darkness. In flowering stage, the photoperiod is 12h as it is a short-day plant (Cervantes, 2006; Farag & Kayser, 2015a, 2015b; McPartland et al., 2000; Pertwee, 2014).

Light intensity, quality, and photoperiod all have an impact on the water status of the plant. Applying more light to a plant will cause increased transpiration and thus decrease (make more negative) the water potential of the plant (i.e. more water stress). To maintain a predictable and desirable water potential diurnal rhythm in the plant, all light properties need to be fine-tuned specifically to the crop being grown.

2.8 Plant Water Status

2.8.1 Water Potential and Plant Water Status

Water potential (Ψ) responds to all the environmental variables previously described, to give a measure of the forces governing the movement of water through the plant over time. The movement of water through a plant exists within a continuum from soil to roots to leaves to atmosphere. This gradient can be impacted by many different environmental factors such as lighting, carbon dioxide, soil composition, nutrients and water content, temperature and relative humidity. Changes in any of these factors will have an impact on Ψ, which can then result in variations in plant productivity and quality. This is a powerful measurement to use in plant production, specifically for standardization and improvement of production methods, because any changes or modifications in environmental management strategies will show up in the Ψ measurements. For a crop such as cannabis that is being produced under stable or consistent conditions within a controlled growth facility, the monitoring of plant water status can be an indicator of the environmental control system functionality or of disturbances within the production system.

2.8.2 Measurement Techniques: Soil Moisture

Measuring soil moisture is a common method used to determine when to irrigate. Soil moisture sensors give a reading of volumetric water content (Ehlers & Goss, 2003). This is a common measurement used in many greenhouses and indoor production facilities to help quantify how much water is present in the root zone and allows for irrigation to be scheduled or even triggered when the soil moisture content is too low. Using soil water content as the only measurement for irrigation can potentially lead to over or under-irrigation because the soil moisture does not necessarily indicate the water relations of the plant and the plant may not require any additional water when the soil moisture seems to be low (Ehlers & Goss,

2003). The consistency of this measurement also depends on the uniformity of the growth media within the pot as well as the location of the sensor during measurements (Ehlers & Goss, 2003). Using this measurement in conjunction with other measurements such as aerial humidity, temperature, and light intensity can provide a more reliable indicator for when to irrigate but is not a direct indicator of the actual water potential of the plant.

2.8.3 Measurement Techniques: Pressure Chamber

The pressure chamber is a plant water status measuring device described by and deployed in field experiments by Scholander *et al.* in 1965. This device is used to measure the water potential of an excised piece of plant material (Dixon, Grace, & Tyree, 1984; Dixon & Tyree, 1984; Scholander, *et al*, 1965). These devices are portable, robust, allow fast measurements, and don't require complex equipment for proper use and data collection; however, with the development of more modern water potential measuring devices, the pressure bomb has seen reduced use in water relations studies (Boyer, 1995; Ehlers & Goss, 2003; Scholander et al., 1965).

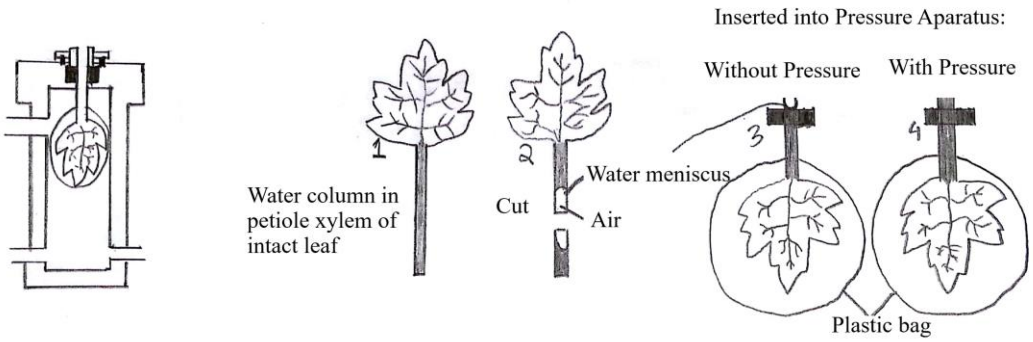

Figure 2.2 - Pressure Chamber (Adapted from Ehlers & Goss, 2003)

The pressure chamber is used by inserting an excised part of a plant into the apparatus with the xylem exposed. Pressure is this increased within the chamber until water becomes present at the exposed xylem. The pressure required to do this is the water potential of the excised part of plant measured in megapascals (MPa) (Ehlers & Goss, 2003).

The main disadvantage of this technology is the requirement to excise sample tissue from the plant before use in the chamber (Dixon et al., 1984; Dixon & Tyree, 1984; Ehlers & Goss, 2003; Jones, 2004; Scholander et al., 1965). Removing tissue, typically from the periphery of the plant, it is unclear how readings made on this tissue are representative of the entire plant. It also removes the ability to test the same piece of tissue at different times of the day to determine any changes throughout the diurnal pattern of the plant. This also means that the pressure bomb is unable to take whole plant Ψ measurements since the entire plant would have to be cut down to be used in the device (Dixon & Tyree, 1984; Ehlers & Goss, 2003; Scholander et al., 1965).The second disadvantage of this technology is automation or the lack thereof (Ehlers & Goss, 2003; Jones, 2004, 2007). To use a plant water device for irrigation scheduling, the ability to automate measurements while not requiring the removal of plant material is required to provide the most standardized and consistent measurements.

2.8.4 Measurement Techniques: Psychrometers

An accurate instrument for measuring the effect of irrigation on plant Ψ is the *in situ* stem psychrometer (Dixon & Downey, 2015; Dixon et al., 1984; Edwards & Dixon, 1995a, 1995b; Robinson, *et al*, 2007; Robinson, *et al* 2009). The overall Ψ in a plant is governed by gravity, pressure, osmosis, and capillary action, as summarized in Equation 2.1.

$$\Psi = \mathrm{T_p} - \Pi - T - g \tag{2.1}$$

Where:

Ψ = Total Water Potential (MPa)

$\mathrm{T_p}$ = Turgor Pressure (MPa)

Π = Osmotic Potential (MPa)

T = Matric Potential (MPa)

g = Gravity (MPa) (Negligible for cannabis since plant height is approximately 1m)

Turgor pressure and osmotic potential are generally the largest contributors to water potential and largely represent the measurement with the stem psychrometer. Turgor pressure is the pressure that pushes the plasma membrane against the cell wall as water moves into the cell, largely due to osmotic gradients. Osmotic potential results from the dynamic solute gradient that exists between the plant cell and its extracellular surroundings (Dixon & Downey, 2015). Matric potential is the energy required to remove water from a porous medium to overcome capillary and absorptive forces and generally has a minor impact on total water potential relative to turgor pressure and osmotic potential. The effect of gravity is only noticeable for very tall plants such as trees (Dixon & Downey, 2015).

To measure plant water potential, the PSY1 stem psychrometer will be used. This psychrometer is a device that is attached to exposed xylem tissue on the stem of a plant to

measure plant water potential (Dixon & Downey, 2015; Dixon & Tyree, 1984). It can be used in field conditions and has been previously shown to be accurate and reliable (Dixon & Downey, 2015; Dixon *et al*., 1984; Edwards & Dixon, 1995a, 1995b, Robinson *et al*., 2007, 2009; Tran, 2014). The PSY1 stem psychrometer functions by measuring the vapour pressure in a tiny chamber attached to and in equilibrium with the water conducting tissue of a plant (Figure 2.3) (Dixon and Tyree, 1984). The deployment of three thermocouples in the instrument measure the absolute temperature of the chamber body (BT thermocouple) and the differential temperature between the Peltier cooled psychrometric measuring junction (C thermocouple) and the evaporating surface of the plant tissue (S thermocouple). This precise and accurate assessment of the thermal environment of the instrument allows equally precise and accurate measurements of vapour pressure in equilibrium with the forces (ie. water potential) on the liquid water in the plant.

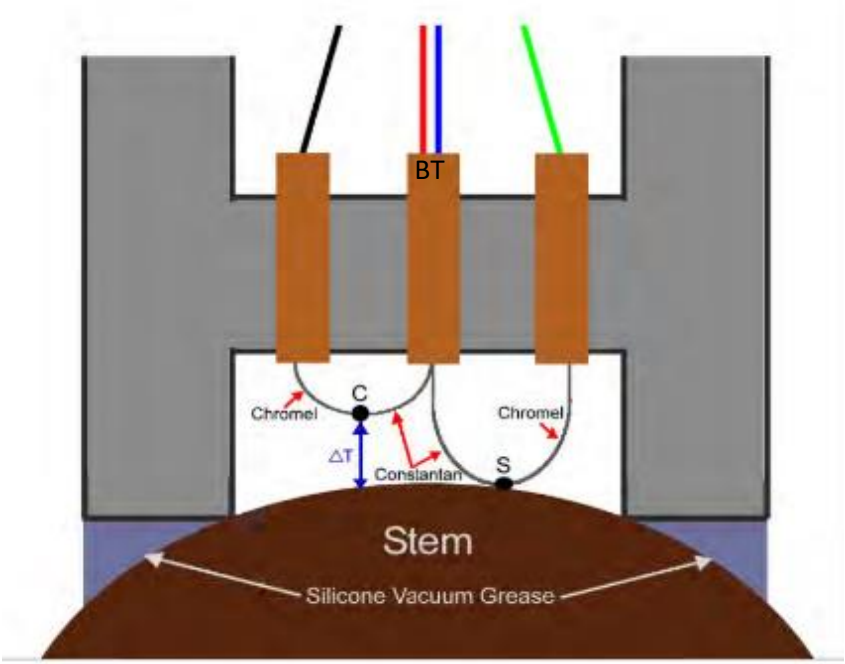

Figure 2.3 - PSY1 Stem Psychrometer Chamber and Thermocouples (Dixon & Downey, 2015)

The psychrometer is attached to the stem of a plant over a section of exposed xylem. Silicone grease is applied to the sides of the psychrometer to ensure an air-tight seal. The two thermocouples are both attached to solid copper posts in the psychrometer body. The s-thermocouple (sample thermocouple) extends out such that it contacts the exposed xylem of the plant. The c-thermocouple (Peltier cooled measuring thermocouple) measures the wet bulb depression following Peltier cooling and condensation of chamber moisture on the thermocouple. Measurements are corrected for both ambient chamber temperature (BT thermocouple) and the error inducing temperature gradient between the evaporating surface of the plant tissue and the measuring thermocouple (Dixon & Tyree, 1984).

The use of psychrometers to measure the effects of irrigation on other plants has been previously investigated (Dixon et al., 1984; Edwards & Dixon, 1995a, 1995b, Robinson et al., 2007, 2009; Tran, 2014). Using water potential as a feedback control variable to develop irrigation protocols and enhance and standardize the production of secondary plant metabolites in cannabis has not been attempted. By employing psychrometers to measure the water potential response to different irrigation frequencies, while concurrently measuring the associated yield and secondary metabolite dynamics, this study seeks to determine the relationship between plant water potential drought thresholds and yield and secondary plant metabolite/medicinal compound content. In addition, concurrent measurements of VPD are expected to establish a predictable relationship between water status and aerial humidity that could be exploited in irrigation management.

To practically improve irrigation practices for a production facility, the use of psychrometers as a source of feedback control data is not suitable. The setup and maintenance of these devices requires extensive training and data handling to achieve interpretable results (Dixon & Downey, 2015; Shackel, 1984; Tran, 2014). It is impractical to expect growers to deploy these research tools on a large scale to apply irrigation management strategies. An objective of this study was to establish a predictable relationship between psychrometer measured plant water status, expressed as cumulative water potential over time, and the key environment variable in determining environmental demand for moisture from the plant, the aerial vapour pressure deficit (VPD), expressed as cumulative VPD.

Vapour pressure deficit (VPD) is the difference between the current moisture content of the air and the moisture content required for the air to be saturated (Ehlers & Goss, 2003; Tran, 2014; "Understanding and Using VPD," 2009) as shown in Equation 2.2.

$$VPD = \left(\frac{100-RH}{100}\right) \times SVP \qquad (2.2)$$

Where:

VPD = Vapour Pressure Deficit (MPa)

RH = Relative Humidity (%)

SVP = Saturated Vapour Pressure (MPa)

Vapour pressure deficit is an important influencing variable in the rate of water loss from a leaf. Regulation of gas exchange, including water loss, is an important plant function governed by stomata (Pallardy & Kozlowski, 1979; "Understanding and Using VPD," 2009). The opening and closing of stomata is, in part, modulated by VPD. For example, in an environment with little water availability in the root zone, as VPD increases, there is less water available both in the air and in the root zone causing the plant to respond by closing the

stomata by removing water from the guard cells (Pallardy & Kozlowski, 1979; Tran, 2014; "Understanding and Using VPD," 2009). This allows the plant to protect itself from excess transpiration during excess heat, drought stress, or low humidity. By maintaining appropriate environmental conditions, plants can keep stomata open throughout the day, allowing continued gas exchange to support photosynthesis (Pallardy & Kozlowski, 1979; Tran, 2014; "Understanding and Using VPD," 2009). The general relationship between VPD and water potential is known (Dixon & Tyree, 1984). Species specific relationships, within this broader understanding, have been developed and used to determine cumulative VPD thresholds that could be used to control irrigation scheduling (Tran, 2014). Correlating cumulative plant water potential with cumulative VPD thresholds could allow the grower to irrigate based on plant needs rather than a standard daily interval or other subjective irrigation schedule protocol.

2.9 SUMMARY

Production of a standardized cannabis crop in a controlled environment requires fine-tuned control of all environment conditions and inputs, including irrigation. Current irrigation practices do not account for actual plant requirements, as conveyed by plant water potential. Automated stem psychrometers allow the water status of the cannabis plant to be monitored in near real time and can be used to standardize water application. The use of cumulative VPD correlated with cumulative Ψ will allow for an irrigation management strategy that predicts actual plant water status with greater reliability and accuracy than any other approach. The resulting implications for yield and quality of the crop are the subject of this study.

CHAPTER 3 – METHODS

3.0 SITE DESCRIPTION

Research was conducted at ABcann Medicinals Incorporated., a federally licensed medicinal cannabis production facility, located in Napanee, ON. This facility produces cannabis indoors in controlled growth chambers designed by Controlled Environments Limited (Winnipeg, MB, Canada) in collaboration with the University of Guelph's Controlled Environment Systems Research Facility (CESRF). ABcann has separate growth chamber facilities for mother plants, propagation, vegetative growth, and flower production. Each of these chambers are briefly described below.

3.1 MOTHER ROOM

The growth chamber used for vegetative growth of the stock plants (mother plants) was a Conviron GH-630 (Controlled Environments Ltd., Winnipeg, MB, Canada). This chamber was developed specifically for ABcann Medicinals by Conviron in design consultation with the University of Guelph's Controlled Environment Systems research group. Mother plants were grown in 12.5L Air Pots (Caledonian Tree Co., Prestonpans, Scotland) that are 275mm tall and have a diameter of 306mm including many holes on the sides of the pot to prevent root wrapping and allow better airflow to the root zone. Environmental conditions in the mother room are summarized in Table 3.1. Ceramic metal halide (CMH) bulbs (Philips Lighting, Markham, ON, Canada) were used for lighting (18-hr photoperiod) in the mother room. All environment parameters were controlled by ARGUS Controls systems (ARGUS Control Systems Ltd., Surrey, BC, Canada). The stock plants were flushed with 4L of conditioned reverse osmosis (RO) water 4 and 2 days before taking vegetative cuttings for propagation (cloning). Conditioned water is RO water that has had a

metered amount of Calcium (Ca) and Magnesium (Mg) added to provide plants with consistent water quality.

Days	Temperature (Day and Night) (˚C)	Relative Humidity (%)	CO2 (ppm)	Light (µmol/m^2/s)
All	20	70	600	180

Table 3.1- Environment Condition Setpoints in Mother Room

Environment condition stepoints for the mother room are shown. Measurements are taken from the ARGUS measurement aspirators within the room located just above the plant canopy. The environment does not change in the mother room to maintain vegetative growth of all stock plants for the production of future vegetative cuttings for propagation.

3.2 PROPAGATION

All growth/production experiments were conducted using the same propagation procedure and genetic line (cv. Wappa). Cuttings were excised from stock plants by selecting approximately 13cm sections of vegetative stem tissue with two fully expanded leaves from the lower half of the stock plant and cutting it at a 45˚ angle. Cut ends were dipped in 0.2% Indole-3-butyric acid (IBA) gel (EZ-GRO Inc, Kingston, ON, Canada) and stuck in 28-cell trays (I.T.M.L. Inc., Brantford, ON, Canada) with 5.72cm wide, 5.72cm high peat-based pots (Jiffy Products N.B. Ltd., NB, Canada) containing Pro-Mix PG Organic substrate (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada). The substrate was pre-soaked in a solution of 'Spurt' organic fertilizer (2.0N–0.0P–0.83K; EZ-GRO Inc., Kingston, ON, Canada) at a rate of 5.0 ml·L^{-1} in reverse osmosis (RO) water. Finally, the fully expanded leaves were cut by removing approximately 30% of the leaf tips. The trays were then placed in a propagation chamber for 14 days under an 18-hr photoperiod (Conviron ATC60, Controlled Environments Ltd., Winnipeg, MB, Canada; see table 3.2 for environmental condition setpoints). After 14 days, the cuttings were moved to the vegetative growth chamber.

Days	Temperature (Day) (˚C)	Temperature (Night) (˚C)	Relative Humidity (%)	Light (µmol/m^2/s)
1 to 6	24	24	95	50
7 to 10	24	24	80	80
11 to14	24	24	60	115

Table 3.2 - Environment Condition Setpoints for Propagation of Vegetative Cuttings

Environment conditions in the propagation chambers are shown throughout the 14-day cycle. These conditions were selected to encourage root development in the vegetative cuttings. Measurements are taken from the ARGUS control system which is measured through multiple sensors located within the chamber.

3.3 Vegetative Growth

Cuttings were re-potted into 10.2 cm plastic (poly-propylene) pots and placed into fifteen-cell trays (I.T.M.L. Inc., Brantford, ON, Canada) filled with Pro Mix Organik Plus Growing Media (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) that had been pre-wetted with conditioned RO water. Vegetative growth was completed in a Conviron MTPC192 growth chamber (Controlled Environments Ltd., Winnipeg, MB, Canada) for a further 20 days. Environmental condition setpoints maintained throughout the vegetative cycle are shown in table 3.3. Chamber lighting was provided by fluorescent lights (Philips Lighting, Markham, ON, Canada) with an 18-hour photoperiod to maintain vegetative growth. After 20 days, the plants were fully rooted and had grown to an average height of approximately 25cm. At this stage clones were repotted to 9.5L pots containing Pro Mix Organik Plus Growing Media (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) and transferred to one of two bloom rooms (Conviron GH-1400 growth chamber; Controlled Environments Ltd., Winnipeg, MB, Canada) for the final flowering phase of growth.

Days	Temperature (Day) (˚C)	Temperature (Night) (˚C)	Relative Humidity (%)	CO2 (ppm)	Light (µmol/m^2/s)
1 to 3	24	24	80	500	100
4 to 5	24	24	80	500	200
6 to 10	24	24	75	600	300
11 to 12	24	22	70	700	400
13 to 20	24	22	70	800	400

Table 3.3 - Environment Condition Setpoints for Vegetative Growth

Environment condition setpoints for vegetative growth are shown for the 20-day cycle. The plants were kept in the vegetative growth room until they had established roots and grown to approximately 25 cm in height. At this point they were large enough to be re-potted into the final pot size and enter the flowering cycle. Measurements are taken from the ARGUS control system which is measured through multiple sensors located within the chamber.

3.4 Flowering Growth

Flowering takes 56-days to complete for the 'Wappa' cultivar in this production facility. The environment condition setpoints used during all flowering cycles is shown in Table 3.4. Three flowering cycles were completed for this research, all cycles follower the same environmental setpoint schedule. For the third flower cycle, there were technical issues within the chamber causing a higher temperature, CO_2 concentration, and lower light levels for the last third of the cycle.

Days	Temperature (Day) (˚C)	Temperature (Night) (˚C)	Relative Humidity (%)	CO2 (ppm)	Light (µmol/m^2/s)
1 to 2	24	22	75	600	400
3 to 6	24	22	75	600	620
7 to 10	22	20	70	600	620
11 to 44	20	18	65	600	620
45 to 46	18	16	60	600	620
47 to 49	18	14	60	600	620
50 to 56	18	12	60	600	620

Table 3.4 - Environment Condition Setpoints for Flowering Growth

Environment condition setpoints in the flowering room for the 56-day flowering cycle. The temperature and RH are slowly reduced throughout the cycle while the CO2 concentration and light intensity remain steady. Measurements are taken from the ARGUS control system which is measured through multiple sensors located within the chamber.

After the 56-day flowering cycle, the plants were harvested by cutting the main stem approximately 3cm above the soil surface with garden shears. Before any further processing,

the plants were photographed and the whole plant fresh mass was measured. The plants were then transported to the processing room in large plastic containers for further preparation before drying. The plants had all their large leaves that are easily accessed with minimal touching of the bud removed and then were hung upside-down on metal racks as whole plants and placed into the drying room (Conviron MTPS216 drying chamber; Controlled Environments Ltd., Winnipeg, MB, Canada).

3.5 Drying and Curing

Cannabis needs to be properly dried after harvest to avoid the formation of mould and to begin the curing process. Relative humidity in the drying room was maintained at 45% and temperature was maintained at 18˚C for the entire drying process to remove enough moisture from the flower to avoid mould formation and to begin the curing process.

The curing of cannabis flowers was achieved via storage of the final product in controlled relative humidity (62%) containers to avoid mould formation and allow for the product to be stored until sale. To maintain this relative humidity, all samples were stored along with a RH control packet (Boveda Inc., Minnetonka, MN, USA) to maintain a RH of 62%. All cannabis samples were stored for 21 days before being shipped out for laboratory testing to mimic commercial practices of product storage before sale.

3.6 Psychrometer Deployment and Maintenance

3.6.1. Calibration

Before psychrometers were connected to the plants, they were calibrated. Each psychrometer required a specific calibration equation to account for individual differences between psychrometers to give accurate, repeatable, and comparable measurements. This was

performed using six different sodium chloride (NaCl) molal solutions and testing filter paper at 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 molality (mol/kg) concentrations. The psychrometers have indentations inside the cover for test filter paper to be used in calibrations (Figure 3.3). A thin layer of Clearco Silicone Grease 2012 NSF 61(Clearco Products Co., Inc., Willow Grove, PA, USA) was added to provide an airtight seal between the cover and the psychrometer chamber for calibration. The filter paper was saturated with one of the six calibration solutions and quickly placed inside the test chamber, which was then connected to the main psychrometer body. The psychrometers were then placed into a temperature controlled chamber at 20˚C ± 0.2˚C to maintain a consistent and accurate chamber temperature to facilitate equilibration. Each calibration generated a reading of Wet Bulb Depression (WBD), which was then corrected by using an empirical formula to find Corrected Wet Bulb Depression (CWBD) (Dixon & Downey, 2015). A reading was taken for each of the six NaCl solution concentrations to allow for a calibration curve to be established. This curve showed how accurate the psychrometer is by comparing the known WP of the NaCl solutions against the CWBD to create an equation showing the relationship between WP and CWBD for each psychrometer and gives a r^2 value showing how well the equation described the data collected from each psychrometer. Values from the calibration curve were then used in the calculation of WP for each psychrometer.

Figure 3.1 – Filter paper disc placed into psychrometer calibration indentation. The filter paper discs are used to calibrate each psychrometer for accurate measurements

3.6.2 Measurements of Water Potential on Plants

A section of the water conducting tissue (xylem) was exposed by removing the cuticle with a razor blade in order to expose the water conducting tissue and apply the psychrometer to the stem of the plant, as shown in Figure 3.2.

Figure 3.2 - Exposed xylem tissue (water conducting tissue) to which a stem psychrometer will be attached

The psychrometer was then attached to the stem of the plant using a custom in-house clamp (Figure 3.3). Clearco Silicone Grease 2012 NSF 61(Clearco Products Co., Inc., Willow Grove, PA, USA) was applied around the psychrometer mounting point to create a seal between the xylem and the psychrometer chamber as shown in Figure 3.3.

Figure 3.3 - Installed stem psychrometer. Psychrometer face is placed against exposed xylem and clamped in place.

Insulation was added around the psychrometer using polyester batting (Figure 3.4 A) and heavy-duty aluminium foil (Figure 3.4 B). This insulation was used to maintain a stable temperature for each psychrometer installation as temperature gradients can lead to errors in measurements.

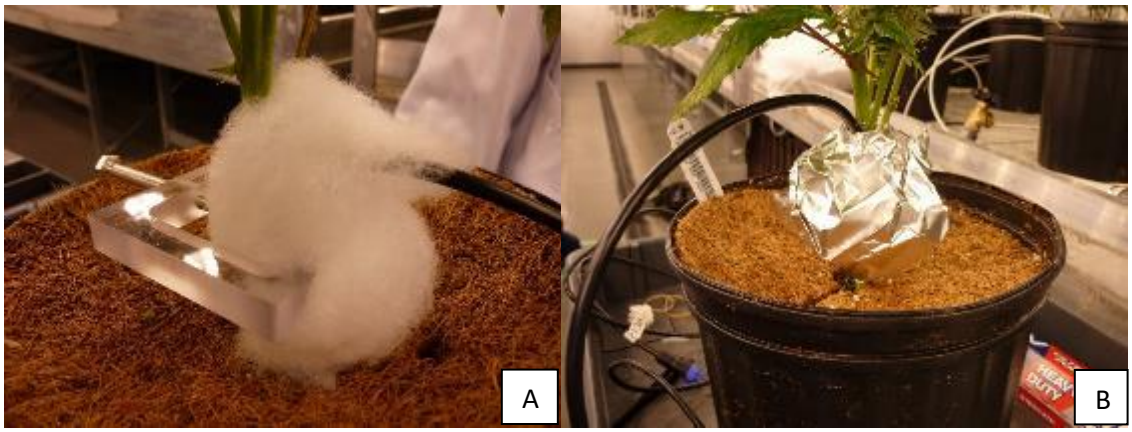

Figure 3.4 – (A) Polyester Batting as Insulation on Psychrometer, and (B) Heavy-Duty Aluminium Foil on Psychrometer

Psychrometers required routine maintenance to continue making accurate measurements. The three main issues encountered with psychrometer applications were; silicone grease entering the thermocouple chamber of the psychrometer, condensation within the thermocouple chamber, or a physical movement of the psychrometer causing debris to enter the thermocouple chamber. To correct these problems, cleaning of the psychrometer thermocouple chamber was occasionally needed. This was accomplished using chloroform to

dissolve any silicone within the chamber. The chamber was then cleaned with RO water to remove any residual from the chloroform. The two thermocouples often required readjustment so that they were in the proper position to take accurate and repeatable measurements. After any significant psychrometer maintenance, a recalibration was usually required to ensure that the calibration equation was still accurate for that psychrometer to function properly with the datalogger.

3.7 Datalogger Use and Maintenance

The datalogger used for measurements with the stem psychrometers was the Campbell Scientific CR7X (Campbell Scientific Canada Corp., Edmonton, AB, Canada). This datalogger has been modified in-house at the CESRF at the University of Guelph to work with the stem psychrometers. The CR7, as configured, can control and log up to 24 psychrometers. This device was placed in a large Rubbermaid plastic container (Newell Brands Inc., Atlanta, Ga., USA) underneath one of the benches in the flowering chamber, allowing all psychrometer cables to reach it (Figure 3.5).

Figure 3.5 – CR7X Datalogger with Psychrometer Cables Attached and Laptop for Remote Access Stored in Large Plastic Container Underneath Flowering Bench in Flowering Chamber

The CR7 datalogger was programmed to take measurements from all twenty-four psychrometers serially every fifteen minutes. The data was stored on the CR7 and needed to be downloaded daily to avoid overwriting previous psychrometer readings. A small laptop remained tethered to the CR7 inside the plastic container to allow remote access from another computer using TeamViewer 11 software (TeamViewer Inc., Göppingen, Germany). The data was then added to a Microsoft Excel (Microsoft Corp., Redmond, Washington, USA) spreadsheet where calculations were performed to determine the WP for each psychrometer reading.

3.8 Cumulative Water Potential and VPD

Cumulative WP and VPD were both calculated by integrating all measurements taken while the lights were on. Each treatment had all WP and VPD readings averaged across all psychrometers before integrating the values. The VPD values used were logged to, and retrieved from, the ARGUS control system. A relationship between cumulative WP (cWP)

and cumulative VPD (cVPD) was determined by regressing cWP against cVPD for each treatment. In the event this relationship proves to be predictable, the environmental variable (cVPD) will then be used to estimate the plant water status and related irrigation management strategies.

3.9 Flushing of Cannabis Plants

At the end of the flowering cycle, two weeks prior to harvest, the effects of flushing of the root zone with clear water were assessed with respect to water relations and nutrient content of buds. Flushing occurred over the last two weeks of production when the nutrient solution was replaced with clear water during routine irrigation events. Another flushing method was to apply an additional 10L of water without any fertilizer to the plant over two irrigation events at the start of the two-week period of clear water irrigation. To assess the relative effects of various irrigation management/flushing treatments among the plants under the experimental protocols in this study, the following treatments were monitored for plant water potential during the treatments and subsequent bud nutrient content was determined on 3 separate experiments with each treatment:

1) Control Treatment with 10L initial flush completed twice and non-fertigated water until harvest
2) Control Treatment without 10L initial flush and non-fertigated water until harvest
3) Mild-Stress Treatment with non-fertigated water until harvest
4) Mild-Stress Treatment with fertigated water until harvest
5) Moderate-Stress Treatment with non-fertigated water until harvest
6) Moderate-Stress Treatment with fertigated water until harvest

The first irrigation treatment was the ‘control’ which was the standard irrigation procedure used in the facility. This treatment has irrigation events every 2 to 3 days. The second irrigation treatment termed ‘mild-stress’ was an irrigation event applied every 2 days, and the third irrigation treatment termed ‘moderate-stress’ was an irrigation event applied every 3 days.

3.10 Cannabinoid and Terpene Analysis

Cannabinoid and terpene testing was completed at RPC Labs (Fredericton, NB, Canada). Samples were collected by removing the cannabis flower from the top of the main stem for consistency of samples. All samples were shipped to RPC 21 days after completing the drying process. To complete these tests, 10 g of dried cannabis flower was required with 5 g for cannabinoid and 5 g for terpene testing. This testing for cannabinoids was performed using High-Pressure Liquid Chromatography (HPLC) following SOP: OAS-SV21. The terpene testing was performed using Gas Chromatography – Mass Spectrometry (GC-MSD).

3.11 Plant Tissue Analysis for Nutrient Composition

Plant tissue analysis for nutrient composition was completed at A&L Labs located in London, ON, Canada. Samples were collected using the same method as described for cannabinoid and terpene analysis. Testing required 5 g of dried cannabis flower for analysis. Plant tissue was dried and ground prior to analysis. Metals in plant tissue were digested with nitric and hydrochloric acids on a hot block then analyzed by ICP-AES (Thermo Scientific iCAP8500). A Leco Analyzer was used for the combustion of sample and thermal conductivity detection of nitrogen for total nitrogen analysis. Acetic acid extraction and

cadmium reduction – colourimetric detection using a Lachat QuikChem 8500 FIA analyzer was used for nitrate analysis.

3.12 Experimental Design and Statistical Analysis

The experiment was set up as a Randomized Complete Block Design (RCBD) with four blocks, each containing two plants for each of the three irrigation treatments (Figure 3.6). Since it was not possible to trigger irrigation through cWP measurements or an automated system, the irrigation events were applied based on a daily schedule and were applied by hand. Each irrigation event for all treatments had 2L of water applied while following the nutrient guidelines used by the facility.

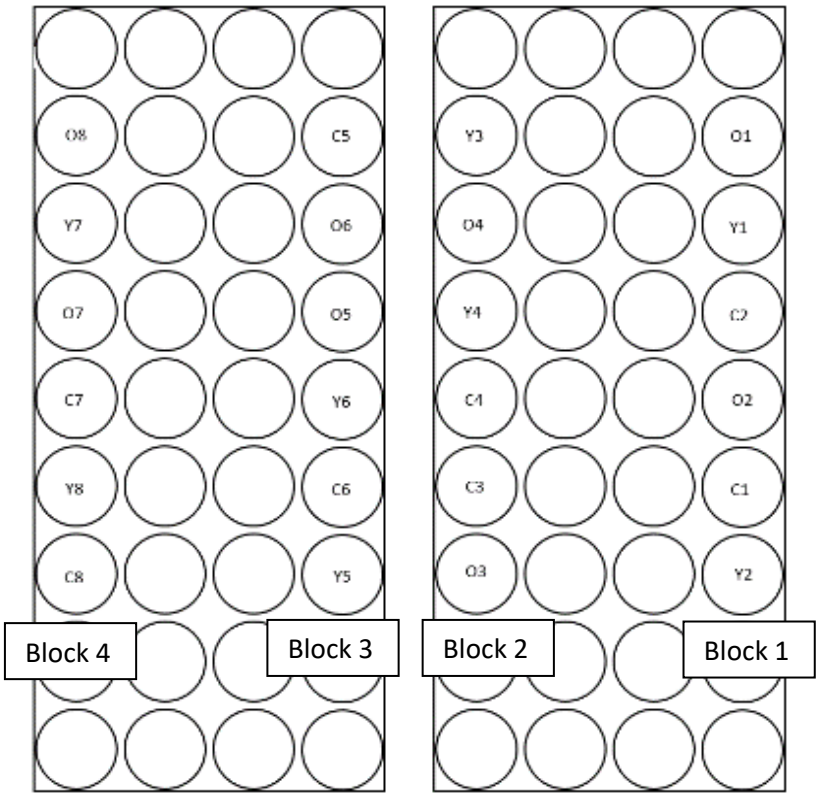

Figure 3.6 – Bench Layout for Flower Cycle 1.

Each block was located along the sides of the benches for easy access to the plants for psychrometer maintenance and irrigation. All Cycles were in the same locations with random arrangements of plants within each block. The codes shown in the figure represent plants from each of the treatments with C1-C8 being the control treatment, Y1-Y8 being the mild-stress treatment, and O1-O8 being the moderate-stress treatment.

Blocks were arranged along the perimeter of two benches to allow access to the psychrometers for routine maintenance (Figure 3.7 A). When the benches were pushed together there was a large canopy creating similar environmental conditions for all plants (Figure 3.7 B).

Figure 3.7 – (A) Side view of two benches showing the blocks along the side of the tables, and (B) canopy view of the space between two benches. This shows that there is a continuous canopy when benches are pushed together.

Once the plants were placed within their blocks, the psychrometer cables were then attached to the sides of the benches using tape to avoid any strain/tension on the psychrometer installation site. The cables were then organized and routed along the back of the table to keep them off the floor and out of the way of facility workers. All cables were then routed to the datalogger which was located beneath one of the benches.

Statistical analysis was performed using R Software (Version: 3.4.0, R Development Core Team (2008)) using a fixed effects one-way Analysis of Variance (ANOVA) analysis. For each of the three flower cycles, an ANOVA analysis was performed to find whether there was a statistically significant difference between treatments. In flower cycle 2, block 1 was removed from the analysis as there were three missing units from this block. A Post-hoc test for significant differences between treatments was performed using Tukey's Honest Significant Difference test. Linear models were made for each regression line showing the relationship between cWP and cVPD. Differences in slope were determined by examining the significance ($p<0.05$) of the interaction between the variables.

CHAPTER 4 - RESULTS

4.1 CUMULATIVE WATER POTENTIAL AND VAPOUR PRESSURE DEFICIT RELATIONSHIPS

Figures 4.1 – 4.9 represent the relationship between cWP and cVPD for all treatments throughout the three flower cycles. The entire flower cycle data are shown in Figures 4.1, 4.4, and 4.7. Figures 4.2, 4.5, and 4.8 include data from the first-half of each flower cycle. Figures 4.3, 4.6, and 4.9 include data from the second-half of each flower cycle. Both the whole-cycle, first-half, and second-half of the cycle are presented separately to better illustrate the differences in data spread. Responses for each treatment are shown in different colours with control being blue, mild-stress being green, and moderate-stress being orange. The data show a linear equation for each treatment that describes the relationship between cWP measured with the stem psychrometers and cVPD measured with the ARGUS control systems. In all three cycles, these relationships show that as cVPD increased, cWP became more negative.

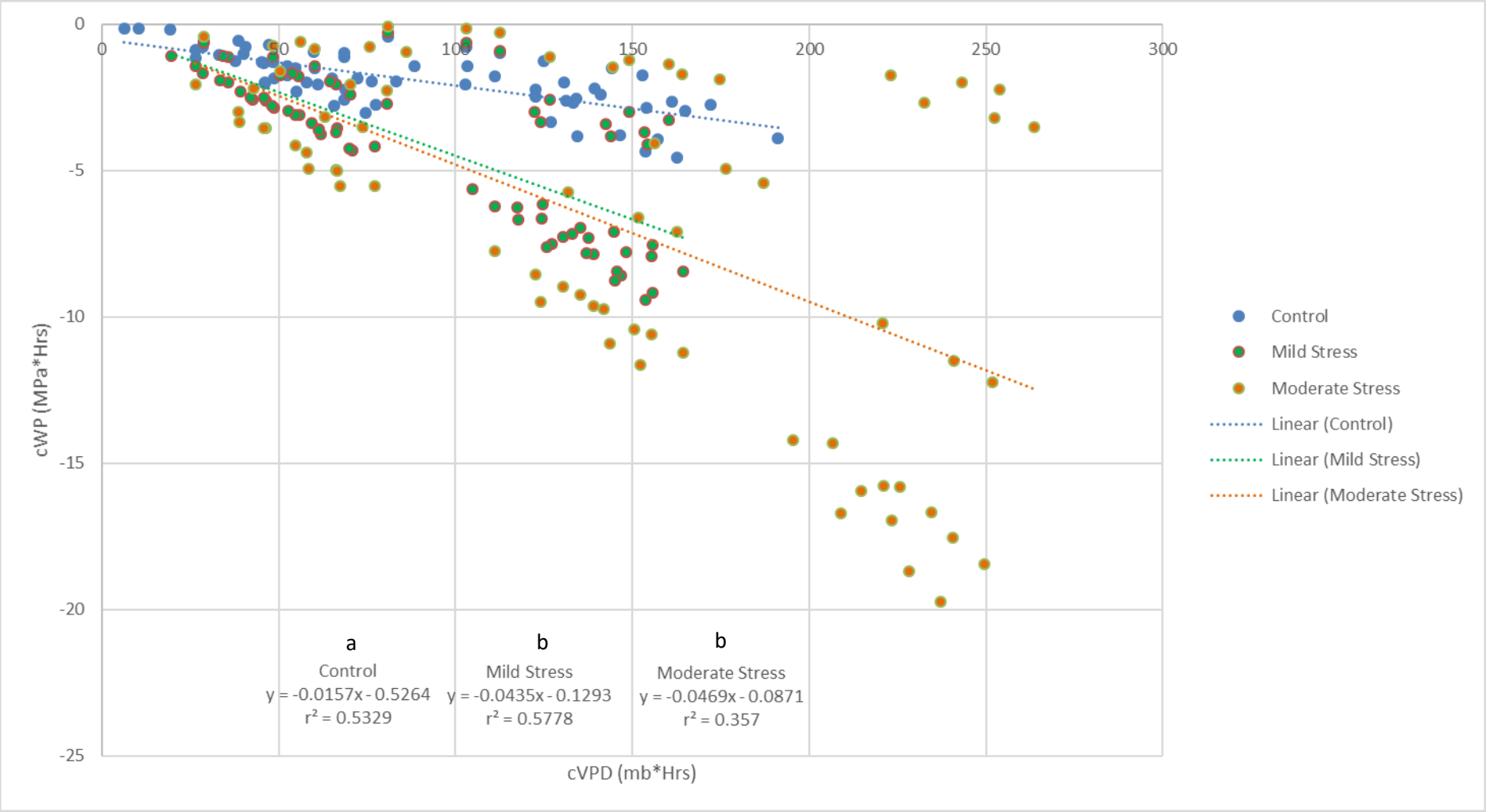

Figure 4.1 – Whole cycle data for cWP vs cVPD for flower cycle 1. This figure shows cWP vs cVPD for the entirety of flower cycle 1 for each treatment. Each day from the cycle has 3 points represented on the graph. This shows that when the entire cycle of data is included there is a separation of the data with two groups having different slopes. The linear equations are derived for each treatment with r^2 representing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

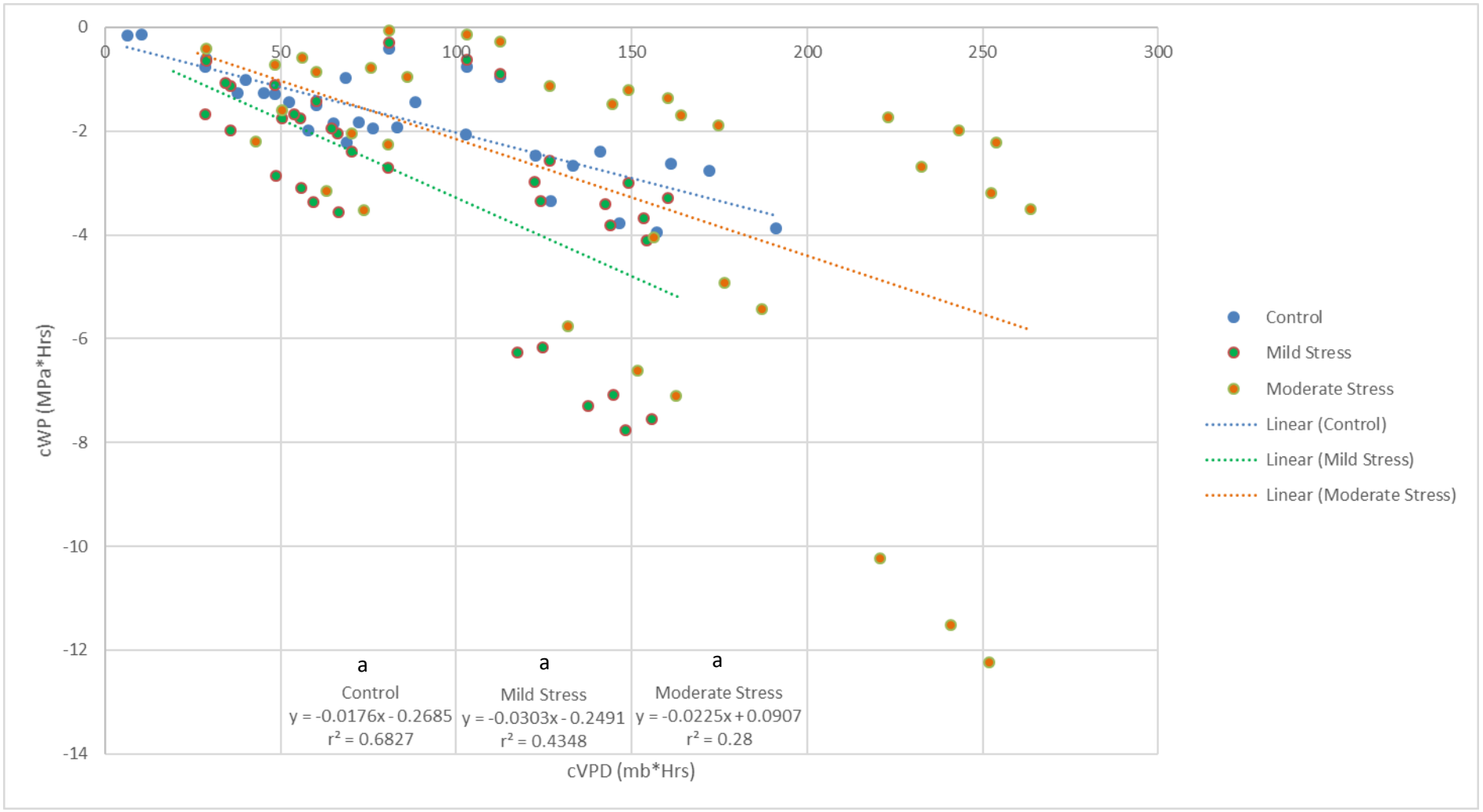

Figure 4.2 – First half of cycle data for flower cycle 1. This figure shows cWP vs cVPD for the first half of flower cycle 1 for each treatment. Each day from the cycle has 3 points represented on the graph. The data from the first half shows much more spread than in the rest of the cycle. The r^2 value is much lower than the second half relationships as well representing how the linear equation is not reliable for fitting the data at the early stages of flowering. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

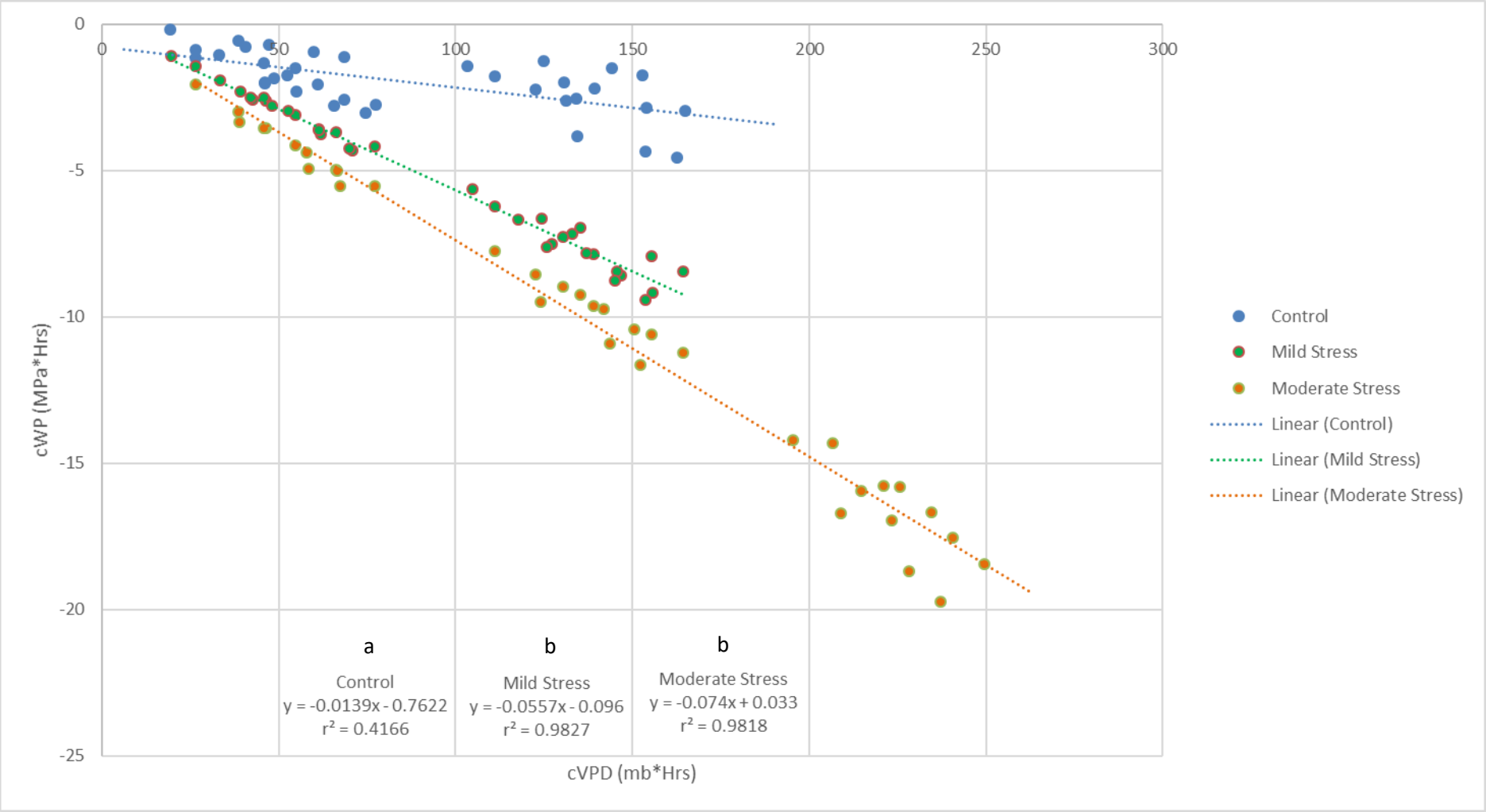

Figure 4.3 – Second half of cycle data for cWP vs CVPD for flower cycle 1. This figure shows cWP vs cVPD for the second half of flower cycle 1 for each treatment. Each day from the cycle has 3 points represented on the graph. When using only the second half of the flower data the data is no longer split into two different groups as is visible in Figure 4.1 for the whole cycle data and has higher r^2 values. The linear equations are derived for each treatment with r^2 representing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

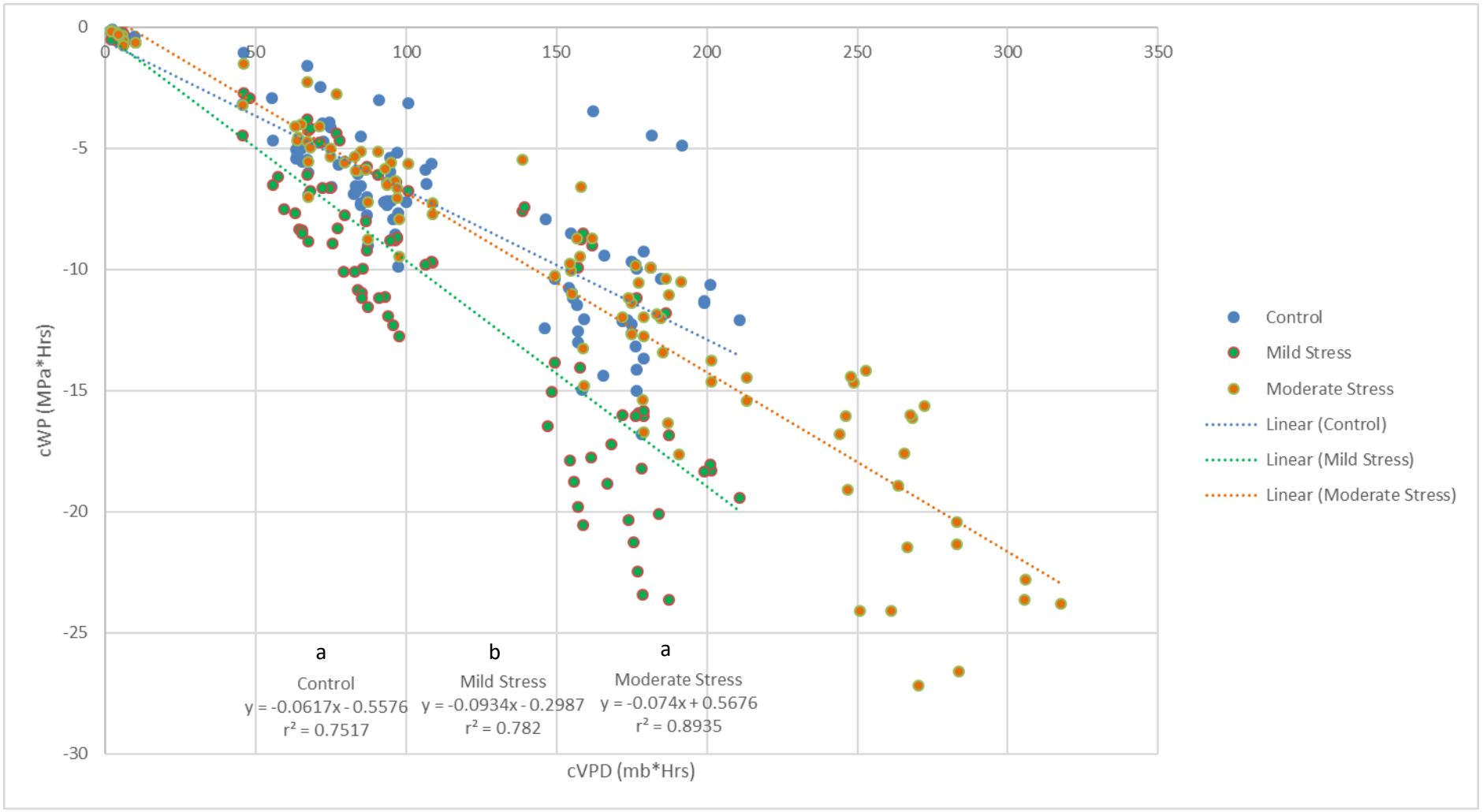

Figure 4.4 – Whole cycle data for cWP vs cVPD for flower cycle 2. This figure shows cWP vs cVPD for the entirety of flower cycle 2 for each treatment. Each day from the cycle has 3 points represented on the graph. Similar to flower cycle 1, when using data from the entire cycle there a separation of the data with two groups having different slopes. The second flower cycle experienced more negative cWP due to the fact that there was increased airflow within the chamber. The linear equations are derived for each treatment with r^2 showing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

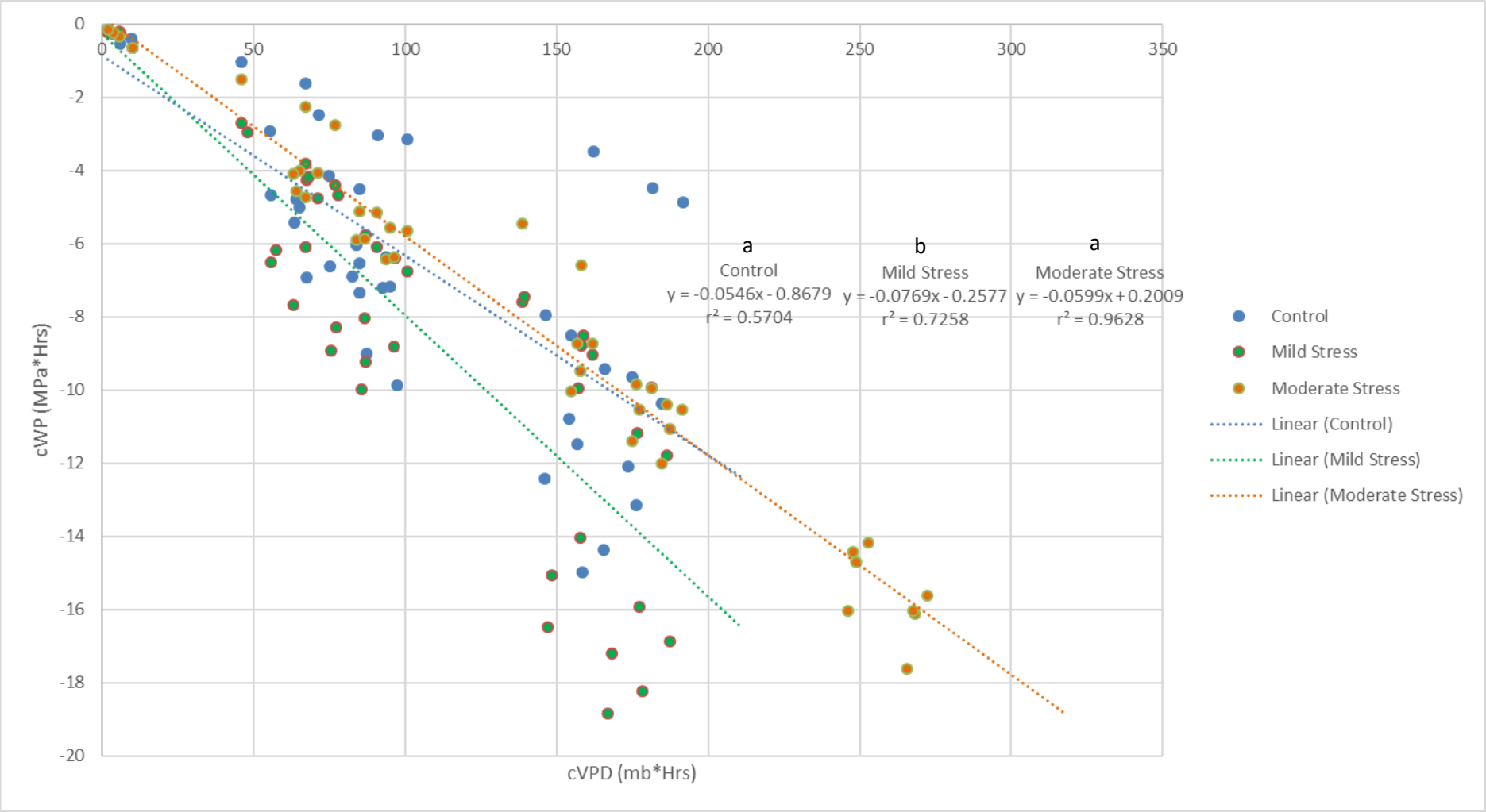

Figure 4.5 – First half of cycle data for cWP vs CVPD for flower cycle 2. This figure shows cWP vs cVPD for the first half of flower cycle 2 for each treatment. Each day from the cycle has 3 points represented on the graph. This data shows a larger spread than the rest of the cycle. The r^2 values are lower except for the moderate-stress treatment. The data for the moderate-stress shows two distinct groups from the first half to the second half showing a difference in growth stages. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

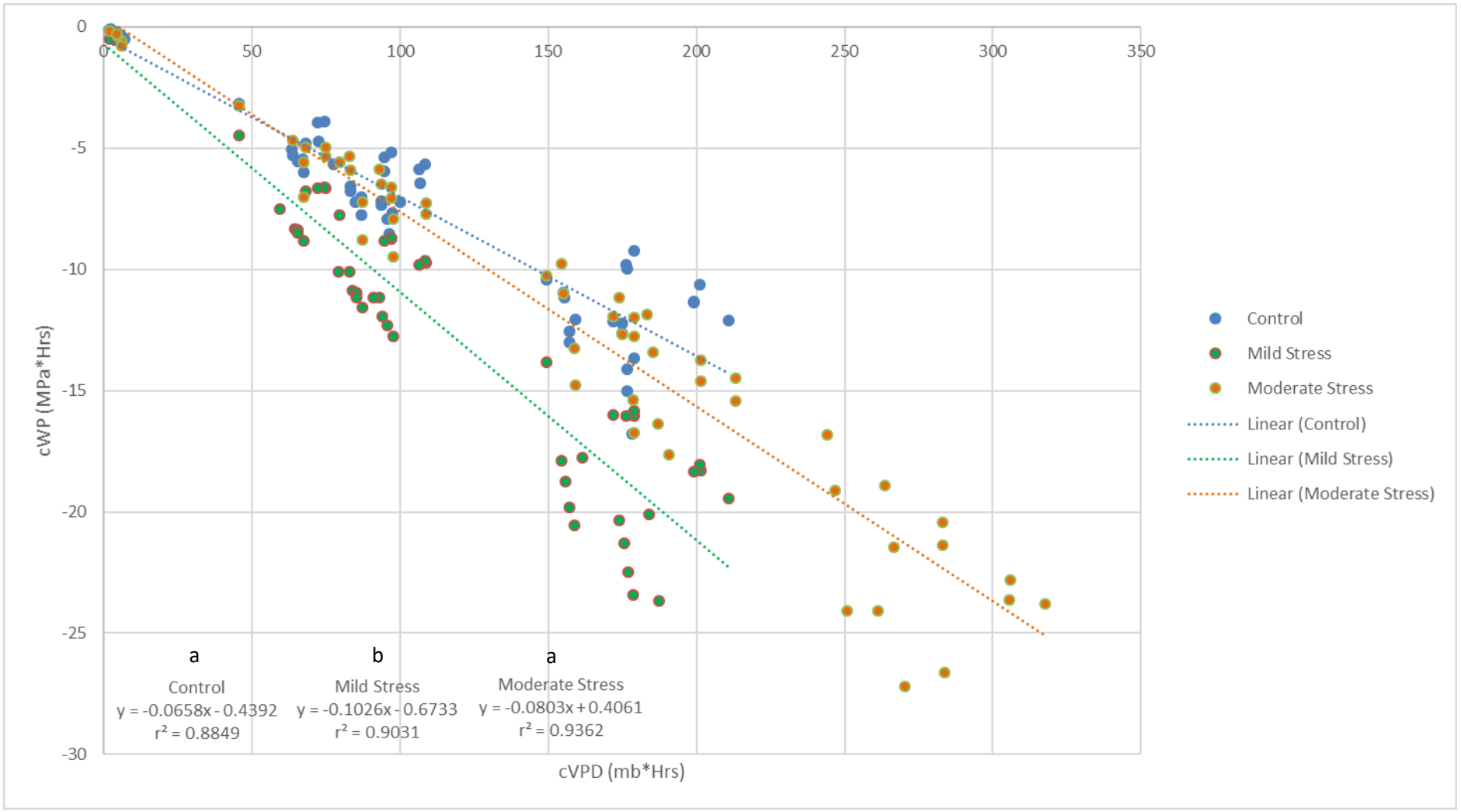

Figure 4.6 – Second half of cycle data for cWP vs CVPD for flower cycle 2. This figure shows cWP vs cVPD for the second half of flower cycle 2 for each treatment. Each day from the cycle has 3 points represented on the graph. When using only the second half of the flower data, the data is not as spread out and has higher r^2 values. The linear equations are derived for each treatment with r^2 representing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

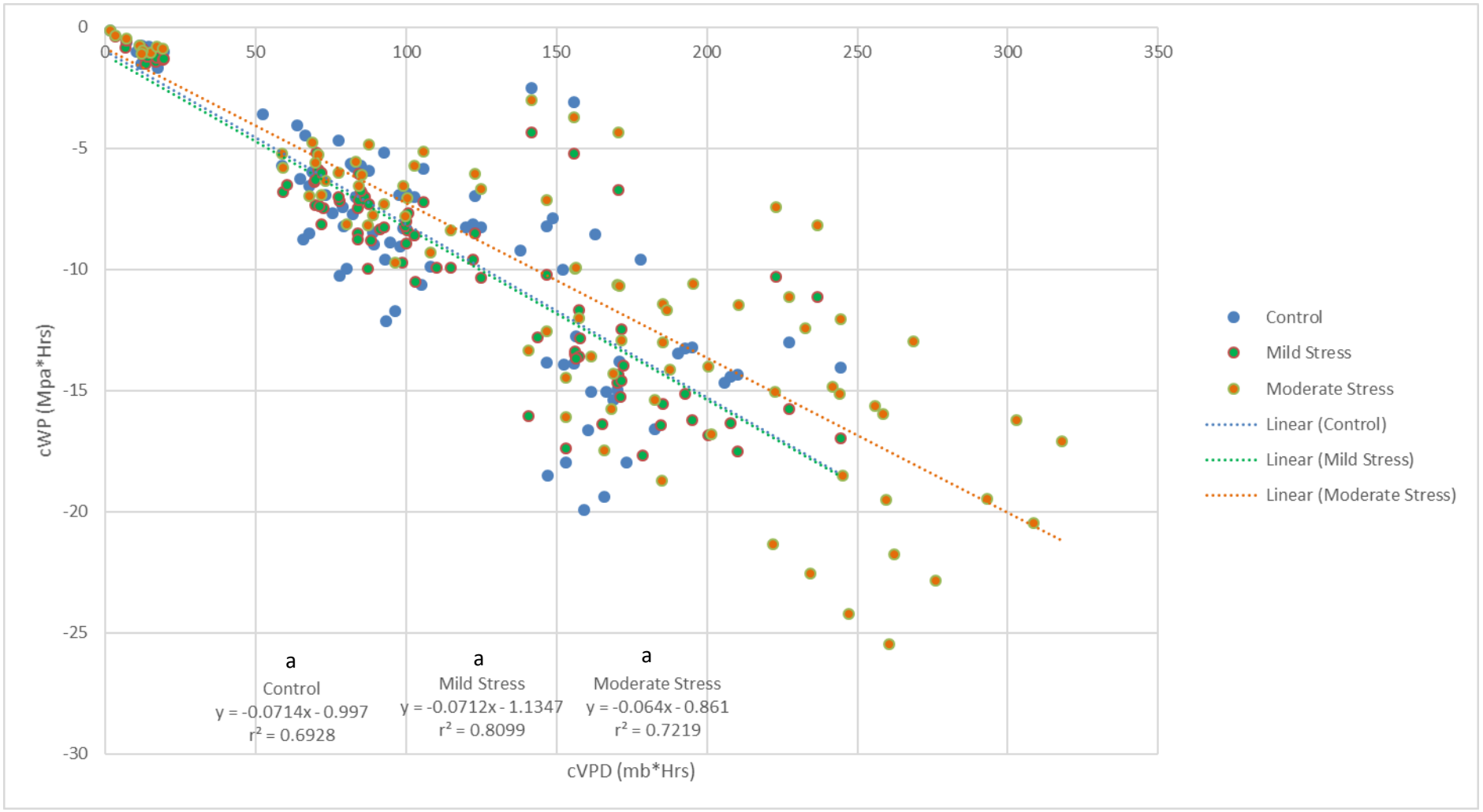

Figure 4.7 - Whole cycle data for cWP vs cVPD for flower cycle 3. This figure shows cWP vs cVPD for the entirety of flower cycle 3 for each treatment. Each day from the cycle has 3 points represented on the graph. Similar to flower cycle 1 and 2, when using data from the entire cycle the data is spread out. The second and third flower cycles experienced more negative cWP due to the fact that there was increased airflow within the chamber causing increased transpiration through the leaves. The linear equations are derived for each treatment with r^2 representing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

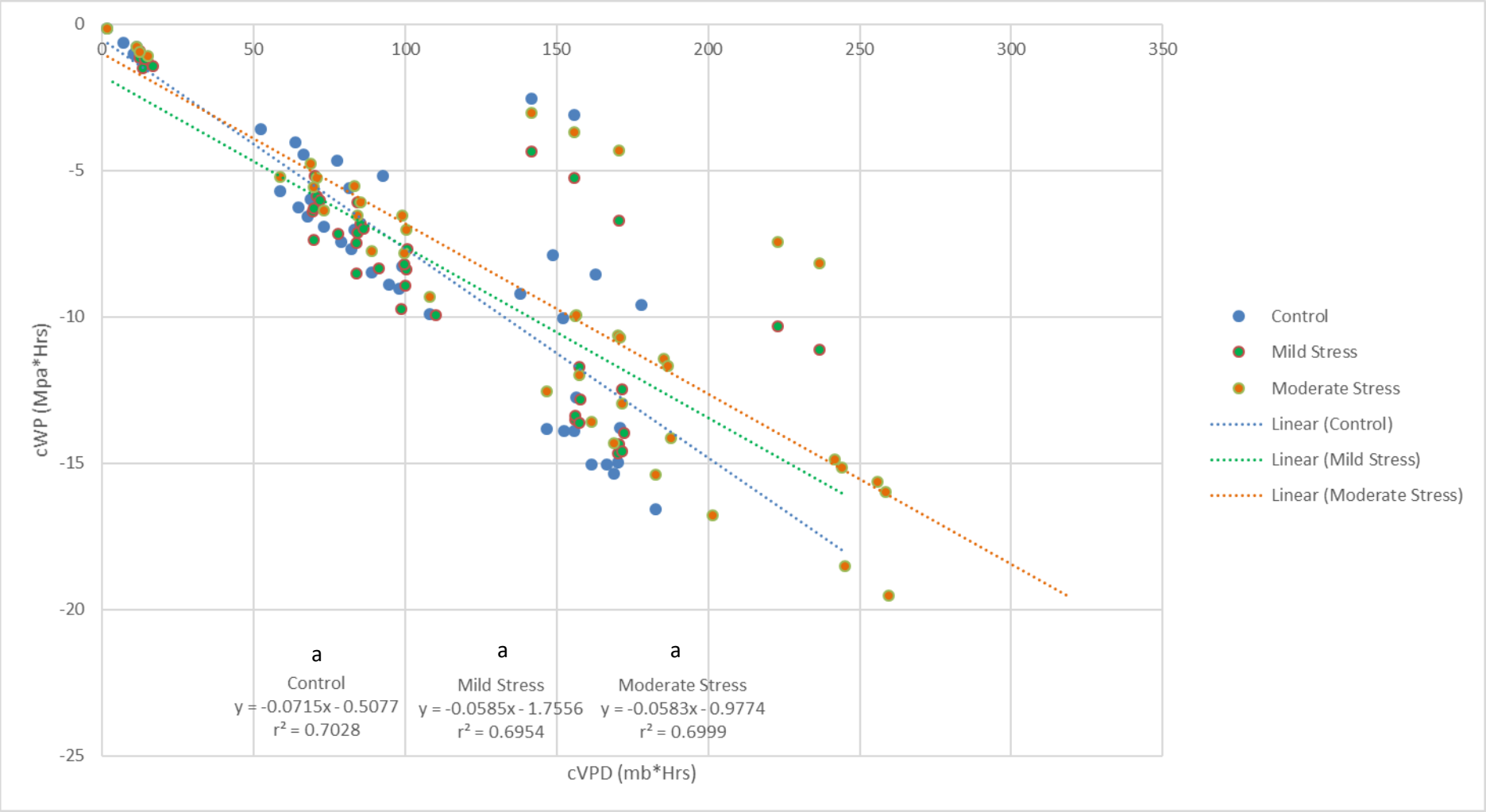

Figure 4.8 - First half of cycle data for cWP vs CVPD for flower cycle 3. This figure shows cWP vs cVPD for the first half of flower cycle 3 for each treatment. Each day from the cycle has 3 points represented on the graph. The data from the first half of flower cycle 3 shows that some of the points that are the furthest outliers are from the first half of the cycle. The r^2 values have decreased a slight amount when compared to the whole cycle data (Figure 4.7) showing less reliability of the linear relationship during the first half of the cycle. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

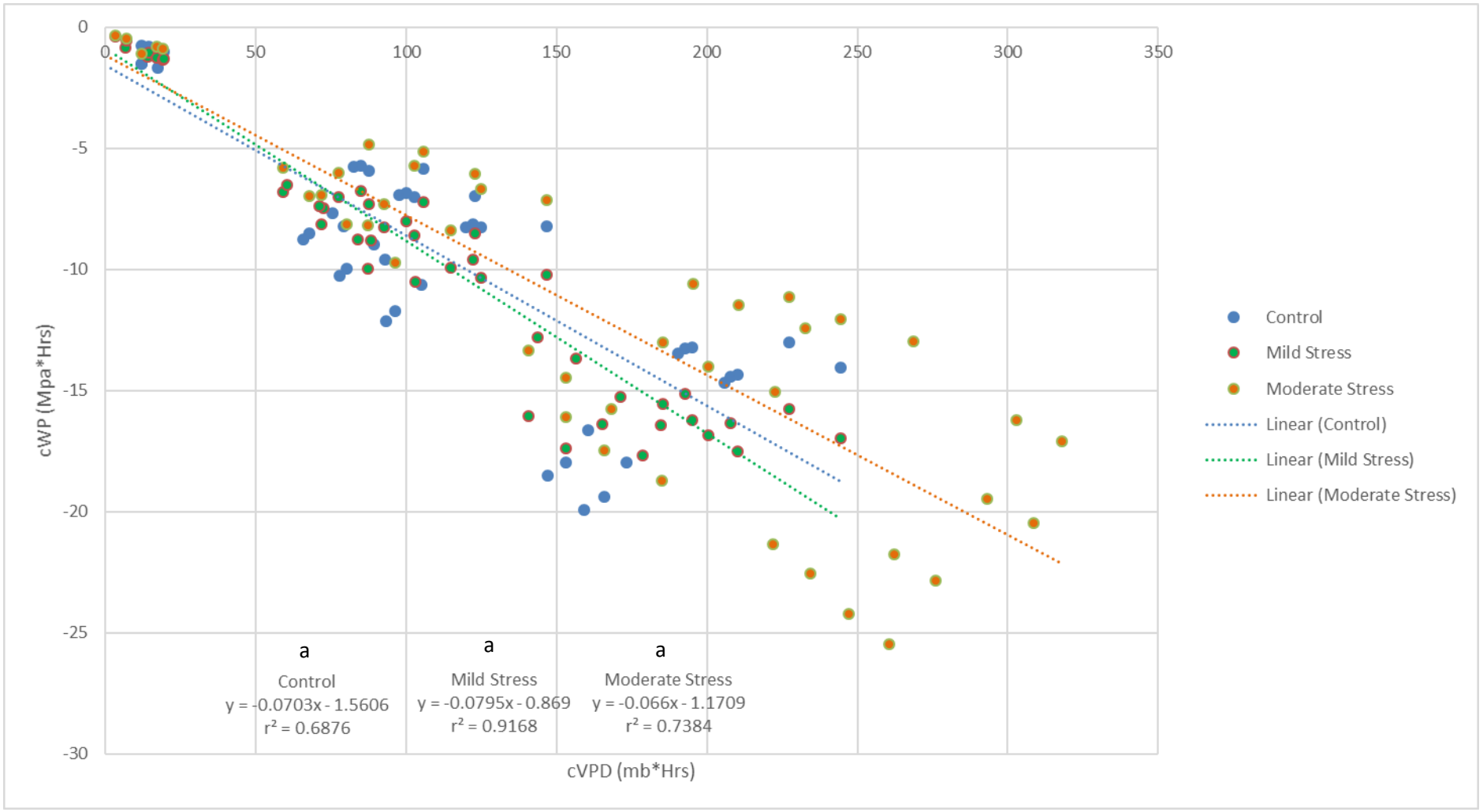

Figure 4.9 – Second half of cycle data for cWP vs CVPD for flower cycle 3. This figure shows cWP vs cVPD for the second half of flower cycle 3 for each treatment. Each day from the cycle has 3 points represented on the graph. When using only the second half of the flower data, the spread of data is reduced and the linear equations have higher r^2 values. The linear equations are derived for each treatment with r^2 representing how well the model fits the data. Letters indicate the difference between the slopes of the best fit lines analyzed with ANOVA at a significance of $p<0.05$.

4.2 Dry Yield from Treatments

Figures 4.10 – 4.12 represent the final dry yield from the plants for each treatment. Figure 4.10 represents the whole plant dry yield in grams per plant while Figure 4.11 and 4.12 represent the bud dry yield in grams per plant. Measurements for each treatment are shown in different colours with control being blue, mild-stress being green, and moderate-stress being orange. Error bars represent the standard error (+/- SE) for each treatment with significance indicators located above. All measurements were taken after the same amount of time of drying after harvest.

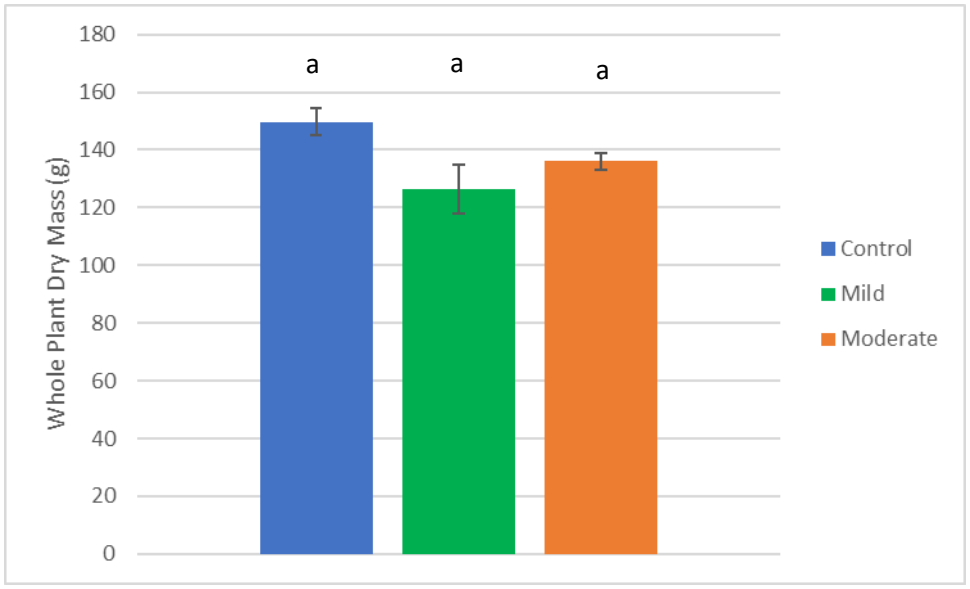

Figure 4.10 – Whole Plant Dry Yield for Flower Cycle 1. The whole plant dry mass was measured after the drying period by weighting the entire plant. All plant yields were not significantly different between the three treatments. Error bars represent the standard error (+/- SE). Letters indicate the difference between the whole plant dry mass between the treatments analyzed with ANOVA at a significance of p<0.05.

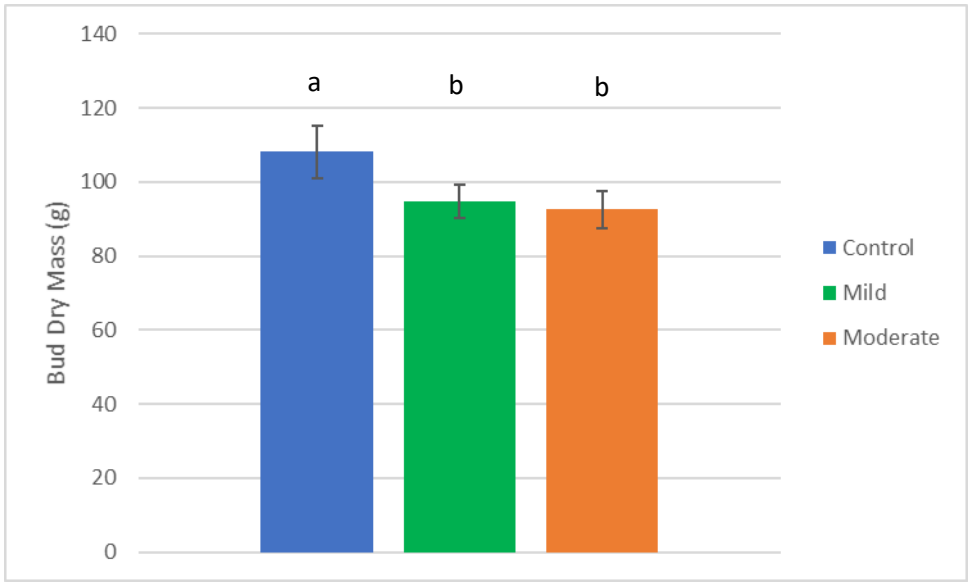

Figure 4.11 – Bud Dry Yield for Flower Cycle 2. The dried bud mass was measured after the drying period by removing all bud from the plant. The control treatment was significantly different from the mild and moderate stress treatments. Error bars represent the standard error (+/- SE). Letters indicate the difference between the bud dry mass between the treatments analyzed with ANOVA at a significance of p<0.05.

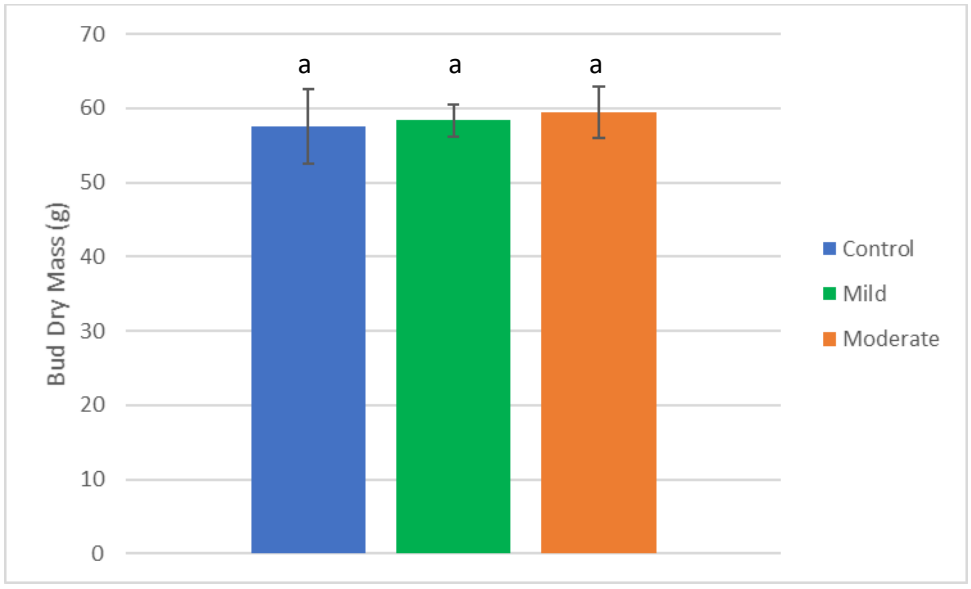

Figure 4.12 – Bud Dry Yield for Flower Cycle 3. The dried bud mass was measured after the drying period by removing all bud from the plant. All bud dry yields were not significantly different between the three treatments. Error bars represent the standard error (+/- SE). Letters indicate the difference between the bud dry mass between the treatments analyzed with ANOVA at a significance of p<0.05.

4.3 Cannabinoid Production from Treatments

Figures 4.13 – 4.15 represent the percent THC by mass from the buds for each treatment. Measurements for each treatment are shown in different colours with control being blue, mild-stress being green, and moderate-stress being orange. Error bars represent the standard error (+/- SE) for each treatment with significance indicators located above. All measurements were taken after the same amount of time of drying in the drying room and being stored in sealed bottles with 62% humidity control packets.

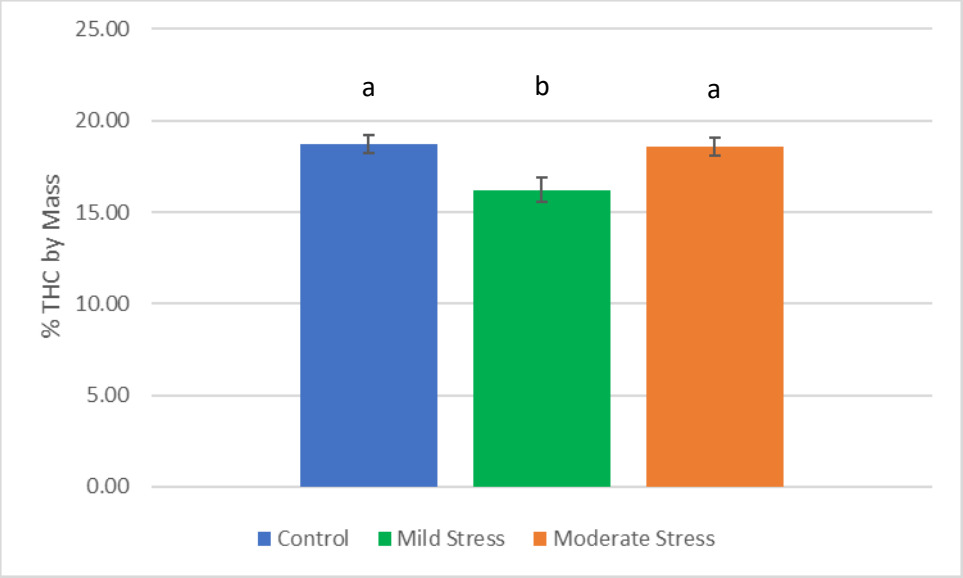

Figure 4.13 – THC Percent by Mass for Flower Cycle 1. The THC % was significantly different in the mild-stress treatment. Error bars represent the standard error (+/- SE). Letters indicate the difference between the percent THC by mass between the treatments analyzed with ANOVA at a significance of $p<0.05$.

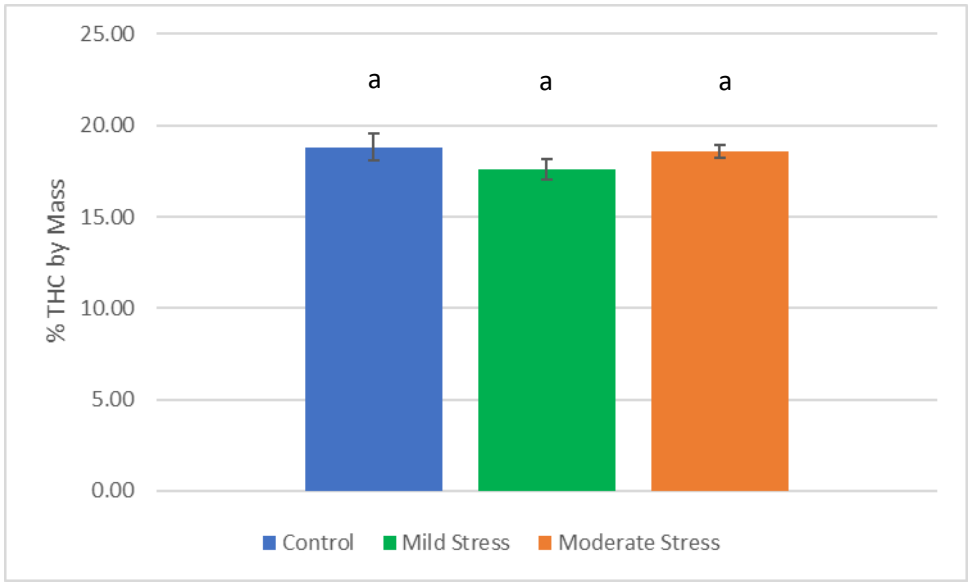

Figure 4.14 – THC Percent by Mass for Flower Cycle 2. All THC % were not significantly different for all three treatments. Error bars represent the standard error (+/- SE). Letters indicate the difference between the percent THC by mass between the treatments analyzed with ANOVA at a significance of p<0.05.

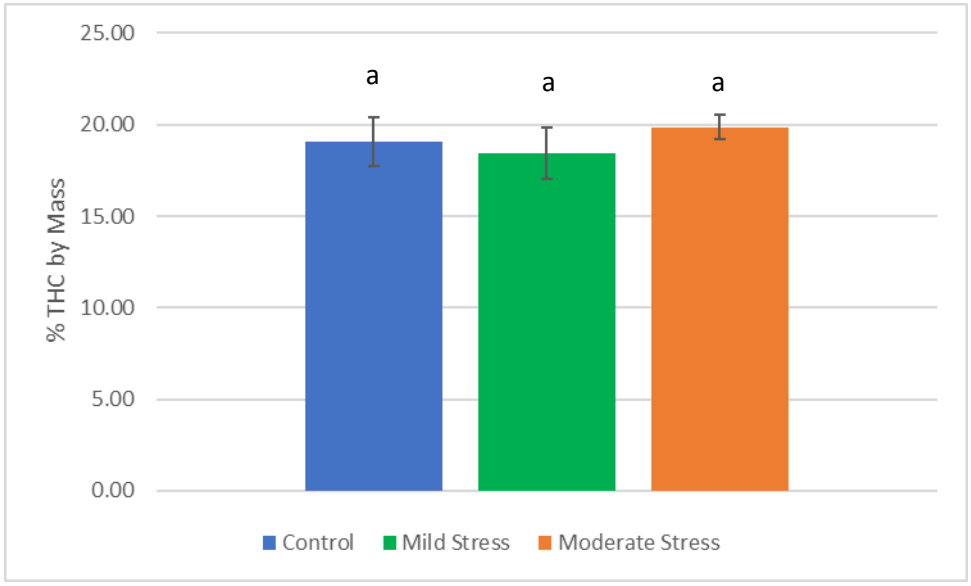

Figure 4.15 – THC Percent by Mass for Flower Cycle 3. All THC % were not significantly different for all three treatments. Error bars represent the standard error (+/- SE). Letters indicate the difference between the percent THC by mass between the treatments analyzed with ANOVA at a significance of p<0.05.

4.4 Flushing Elemental Analysis Data from Dried Buds

Figures 4.16 – 4.18 represent the nutrient concentrations by mass of the major nutrients within the dried bud after flushing as described in section 3.9. Flower cycle 1 contains 5 treatments while flower cycle 2 and 3 both contain 6 treatments since the control treatment without the 10L two-time initial flush was not applied in time. Error bars represent the standard error (+/- SE) for each treatment. There were no significant differences between any of the treatments within each flower cycle.

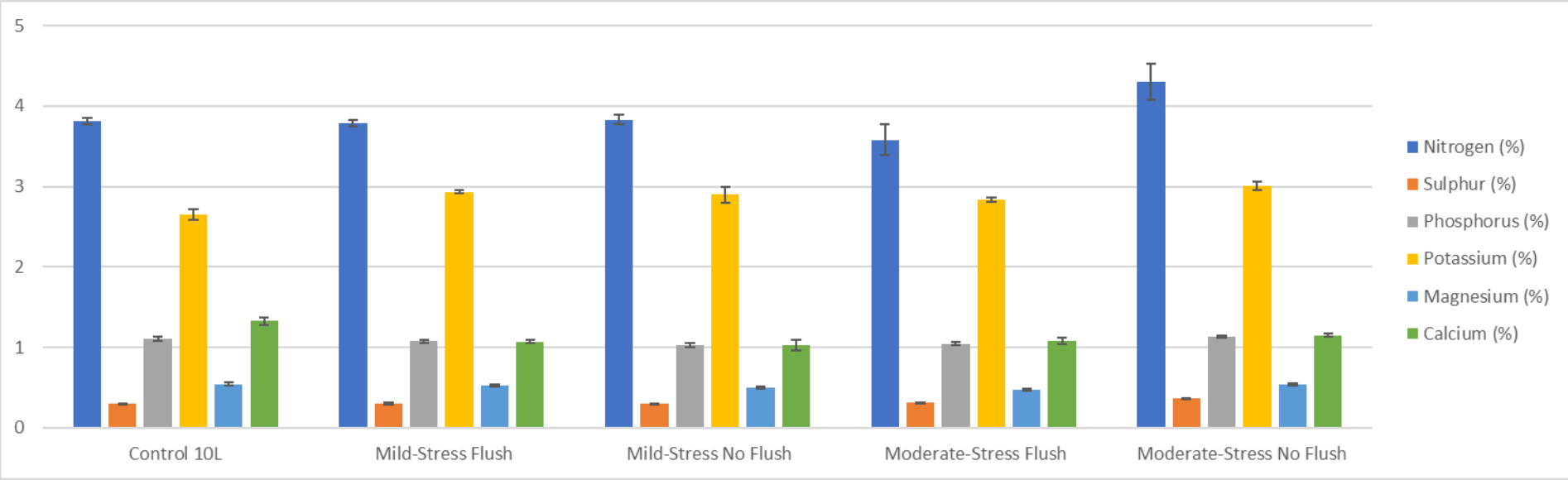

Figure 4.16 – Flower cycle 1 elemental analysis of bud after flushing. Shows the percent concentration by mass of the nutrients within the dried bud. Error bars represent the standard error (+/- SE). There are no significant differences between any of the treatments analyzed using ANOVA at significance of $p<0.05$.

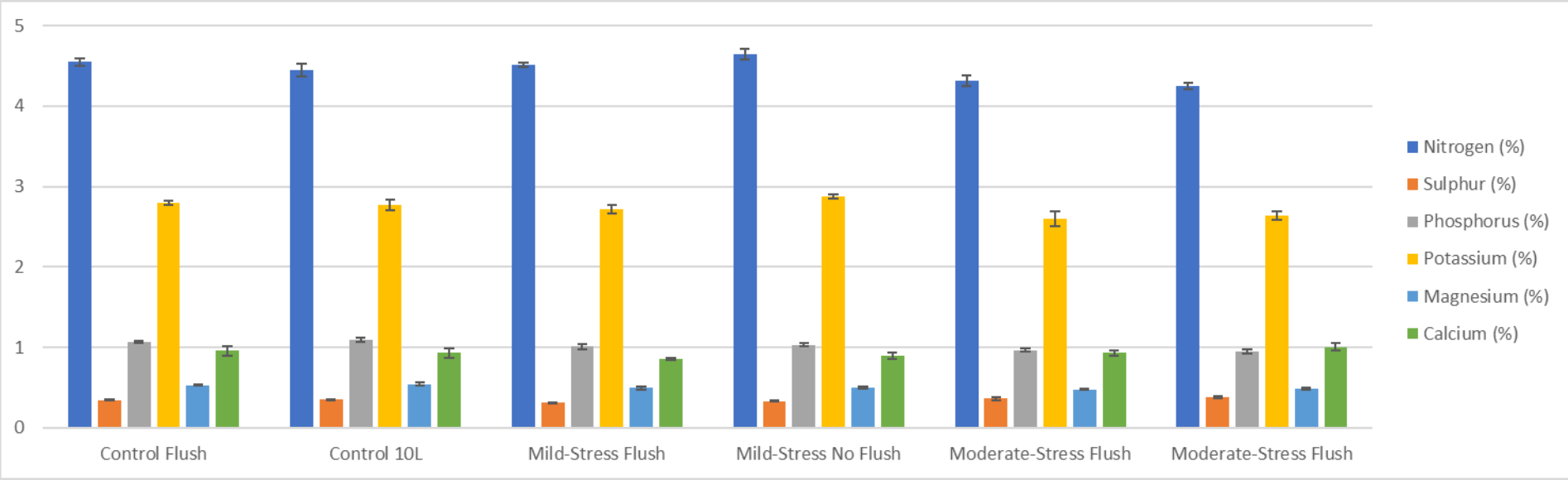

Figure 4.17 – Flower cycle 2 elemental analysis of bud after flushing. Shows the percent concentration by mass of the nutrients within the dried bud. Error bars represent the standard error (+/- SE). There are no significant differences between any of the treatments analyzed using ANOVA at significance of $p<0.05$.

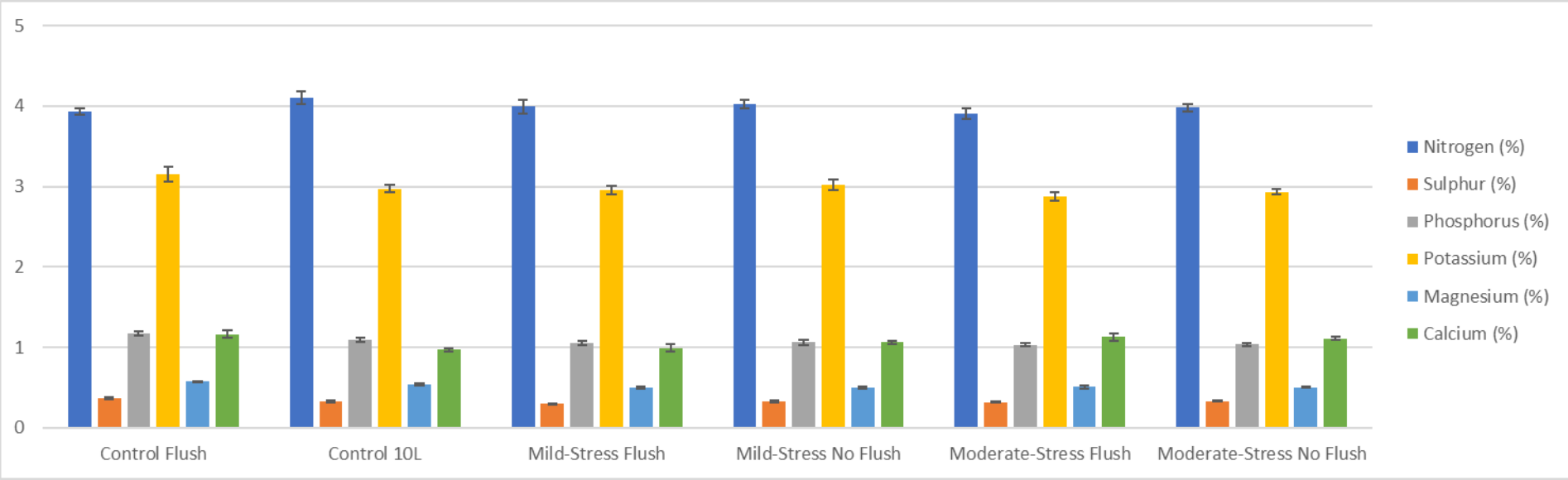

Figure 4.18 – Flower cycle 3 elemental analysis of bud after flushing. Shows the percent concentration by mass of the nutrients within the dried bud. Error bars represent the standard error (+/- SE). There are no significant differences between any of the treatments analyzed using ANOVA at significance of $p<0.05$.

4.5 Water Reduction from Treatments

To quantify how much water was saved by reducing irrigation frequency, the data were compared with the control treatment with the 10L flush as the baseline. The 10L flush treatment was applied twice to that treatment at the beginning of the flushing period causing an additional 16L of water to be used during the flush period compared to all the other treatments since the other treatments only received 2L of water during these two irrigation events. The data are show in Table 4.19.

Treatment	Volume (L) applied/Irrigation	# of Irrigation Events	Added Flush Amount (L)	TOTAL L/Plant	% Reduction
Control with 10L Flush	2	27	16	70	0
Control with Clear Water Irrigation	2	27	0	54	22.9
Mild-Stress with Clear Water Irrigation	2	26	0	52	25.7
Mild-Stress with Fertigation	2	26	0	52	25.7
Moderate-Stress Clear Water Irrigation	2	19	0	38	45.7
Moderate-Stress with Fertigation	2	19	0	38	45.7

Table 4.19 – Water reduction from treatments. This data shows that the moderate-stress treatment was able to reduce water use by over 45% while the mild-stress treatment was able to reduce use by 25%. Removing the 10L initial flush was able to reduce water use by 22.9%.

CHAPTER 6 – DISCUSSION

This study was conducted to determine the impact of controlled drought stress on the yield and composition of medicinal cannabis. The use of irrigation thresholds measured in cWP and cVPD were used to create a system of measurement that was based on plant water relations that can then be used to irrigate plants to yield a controlled drought stress. The impacts of flushing were investigated to determine whether this anecdotal practice is effective in its main goal of reducing nutrient concentrations within the dried bud.

6.1 CUMULATIVE WATER POTENTIAL AND VAPOUR PRESSURE DEFICIT RELATIONSHIPS: SETTING IRRIGATION THRESHOLDS AND IMPACTS ON PRODUCTION

The relationship between cWP and cVPD was very strong within the controlled growth chambers employed in this study when compared with similar studies that were conducted outdoors. This is because the environment conditions within the chamber were consistent, being tightly maintained by the environmental control system. When using this relationship in an outdoor growth system, the fluctuation of the environment conditions would yield a more variable VPD making this relationship more difficult to interpret. The ideal environment parameter to match with cWP is cVPD since they are both part of the water status continuum for plants where cWP is a measure of the actual force that the plant exerts to pull water from the root zone and cVPD measures the environmental demand for water in the air. These two measurements are strongly linked when growing plants within a controlled indoor system so cVPD should theoretically have the strongest relation to cWP.

When observing the relationship between cWP and cVPD for the entire cycle, there was an apparent inflection point as shown in figures 4.1, 4.4, and 4.7. This inflection resulted in a low value for r^2 suggesting that cVPD was not a reliable measure to predict cWP when

looking at the entire data set for the cycles. To better understand the water relations of the crop, the two apparent growth phases were separated to examine the separation in more detail and to determine if the respective irrigation management strategies could more reliably be predicted by the cWP vs cVPD relationships. The first half of the flower cycle is when the plants establish their final vegetative structures such as stems and leaves that will be used as support for the bud and provide photosynthetic capacity for growth. During the first phase of each cycle, the plants were still somewhat vegetative which resulted in more varied water potential readings. Approximately half-way through the flower cycle, the plants had entered full reproductive (flowering) development; they were no longer adding any more vegetative structures and maintained a consistent size. The second-half of the growth cycle consisted of bud formation and development. Since the plants were no longer in vegetative growth and were mainly forming bud, the water status stabilized. The r^2 value for each linear model representing these two phases of the flowering stage increased during the second half, indicating that cVPD better described the cWP measurements during this phase. This showed that making a relationship between cWP and cVPD was much more reliable when the plant being measured was not undergoing rapid vegetative growth, however the relationship during vegetative growth was still quite strong.

Developing irrigation thresholds using this relationship can have large implications on the final dry yield of the bud. Total dry bud yield per plant is the most important production metric in commercial cannabis production. Flower cycles 1 and 3 (figures 4.10 and 4.11) showed no significant difference between the dry yields of the 3 irrigation treatments, but in cycle 2, the control treatment had statistically significantly higher yield than the other treatments as shown in figure 4.12. In this cycle, the control treatment also had the largest SE for its average dry mass. This difference between treatments could be explained by the differences in air handling within the chamber along with different cultivars being grown on

the same bench causing varying airflow for the test plants. There was also a large decrease of dry yield of approximately 40g per plant from flower cycle 2 to 3. The main difference between these cycles was the environmental conditions. In the third flower cycle, the temperature, CO_2 concentration, and light intensities were changed near the end of the cycle. The temperature and CO_2 concentration were increased while the light intensity was decreased. This change, along with the impact of modified air handling could have caused the decrease in yield. The two different cycles had different cultivars of plants being grown alongside the test plants which could cause a difference in air movement due to different canopy structures.

THC concentration is another important factor in commercial cannabis production as the potency of the product has implications for the product's use in medical treatments. Through controlled irrigation thresholds, it may be possible to influence the productivity of the plant to increase concentrations of target cannabinoids. Flower cycles 2 and 3 showed no significant differences in THC concentration between treatments, but in flower cycle 1 the mild-stress treatment did have statistically significant lower levels of THC. In this flower cycle, the mild-stress treatment did exhibit a larger SE in both THC concentration and dry yield compared to the other treatments. After completing the data collection, it was noticed that the plants never got too drought stressed since the cWP measurements for the mild-stress treatment never reached lower than approximately -10MPa*hrs as shown in figure 4.1. When comparing to the other two flower cycles, the cWP measurements for the mild-stress treatment in these cycles reached lower than -20Mpa*hrs as shown in figures 4.4 and 4.7. This is most likely due to the increased air movement in the chamber for these cycles once the new overhead fans were installed. Since the mild stress plants in cycle 1 were able to exhibit a higher Ψ, this could be the reason for them to have different THC concentrations from the other treatments in this cycle.

A controlled irrigation threshold for cannabis production can also be used as an economic tool by the producer. By reducing the irrigation frequency there were large reductions in water use with little impact on overall yield and THC concentrations. Reducing irrigation frequency from the control treatment of irrigation approximately every 2 days with a two-time 10L flush, to the moderate-stress treatment of irrigation every 3 days without the flushing, there was a 45.7% reduction in water use. Another option for reducing water use is to examine flushing practices. In these experiments, removing the two-time 10L flush reduced water use in the same treatment without the two-time 10L flush by 22.9%. With the non-significant impacts on final dry yield and THC concentrations, it makes sense for cannabis producers to investigate flushing practices further and to reduce irrigation frequency to save input costs. An additional consideration is the impact on energy use in the environment control strategy. A significant reduction in water use for irrigation will lead to a significant reduction in humidity with which the air handling system must contend.

The use of cWP thresholds in cannabis production is not ideal due to the setup and maintenance of stem psychrometers. With the strong relationship between cWP and cVPD due to the repeatable and consistent environment provided within the controlled growth chamber, an irrigation threshold could be replaced by simple timing, such as irrigation every 3 days. This threshold was established through experimental work but since the VPD was so consistent, it is possible for growers to adhere to these thresholds just by following a consistent irrigation schedule.

6.2 Flushing Nutrients from Growth Media

The practice of flushing is a current common industry practice but there is no evidence in published literature of its effectiveness in reducing nutrient concentrations within

the bud or even whether or not this is a desirable result. After testing the nutrient concentrations from each treatment from three separate experiments, there were no significant differences in nutrient levels between any treatments within each experiment. This result showed that the intended purpose of flushing to reduce nutrient concentrations within the bud has no effect. These data show that for the last two weeks of the flower cycle for cannabis, it was possible to use no fertilizer water for irrigation with no significant impact on yield while saving input costs on fertilizer.

6.3 Future Study

With the use of irrigation thresholds measured in cWP or cVPD there are many other areas of research that can be studied. Specifically, for cannabis, there is the potential to investigate the impacts of further drought stress on the yield and concentrations of cannabinoids. This experiment did not push the plants too far with respect to water stress, and through the use of higher thresholds there could be increased production of certain cannabinoids that would be the target of use for certain medical treatments. Drought stresses could also be used to change the overall composition of cannabinoids produced and could allow producers to create irrigation thresholds for specific cannabis cultivars to produce target cannabinoids. This research can also be applied to any other medicinal plant so that producers can create irrigation thresholds that produce higher levels of medicinal compounds. It could also be used in the production of herbal plants to increase the production of target secondary metabolites to create plants with the desired physical characteristics such as a desirable culinary compound within a basil plant.

Irrigation thresholds could be set up for specific growth phases of the plant. This research showed that there were separate growth phases shown in the cWP vs cVPD

relationships, so thresholds designed specifically for each growth phase could be developed based on plant needs and requirements during these phases to enhance growth.

CHAPTER 7 – CONCLUSION

Irrigation management has many impacts on the final product of cannabis. By implementing irrigation control strategies using cWP and/or cVPD thresholds, there can be a reduction in water and fertilizer use to achieve the same yield and THC concentration in cannabis. Irrigation practices can also be finely tuned to the specific cultivar through further study to provide the exact amount of water required to achieve the desired final product. This reduction can also save a producer money through reduced water and fertilizer use.

The relationship between cWP and cVPD can be used to create irrigation thresholds to be used in production facilities for other crops. For cannabis, the first half of the flower cycle was rapid vegetative growth causing the reduced accuracy of the cWP and cVPD relationship. The second half of the flower cycle has a very strong relationship between cWP and cVPD since the plants are only producing flower and no longer undergoing vegetative growth. Measuring Ψ in a large-scale cannabis production facility with the use of stem psychrometers is not ideal, so the use of cVPD as a feedback variable for initiating irrigation can apply to the entire crop and be controlled using automated systems such as ARGUS. VPD is already measured in most production facilities, so the adaption of cVPD thresholds will be easy to implement. To apply this method of irrigation scheduling to other cannabis cultivars or other plants, a relationship between cWP and cVPD will need to be established to ensure proper water relations are met within the crop but the general principles demonstrated here indicated that reduced water use, consistent irrigation frequency measured through

cVPD and no flushing of the root zone are elements of a desirable irrigation management strategy for cannabis.

LITERATURE CITED

Aizpurua-Olaizola, O., Soydaner, U., Öztürk, E., Schibano, D., Simsir, Y., Navarro, P., … Usobiaga, A. (2016). Evolution of the Cannabinoid and Terpene Content during the Growth of *Cannabis sativa* Plants from Different Chemotypes. *Journal of Natural Products*, *79*(2), 324–331. http://doi.org/10.1021/acs.jnatprod.5b00949

Al-Amad, I., & Qrunfleh, M. (2016). Effect of Babylon weeping willow (*Salix babylonica* L.) extracts on rooting of stem cuttings of olive (*Olea europaea* L.) "Nabali." *Acta Horticulturae*, (1130), 391–396. http://doi.org/10.17660/ActaHortic.2016.1130.58

Al-Saqri, F., & Alderson, P. G. (1996). Effects of IBA, cutting type and rooting media on rooting of *Rosa centifolia*. *Journal of Horticultural Science*, *71*(5), 729–737. http://doi.org/10.1080/14620316.1996.11515453

Altamura, M. (1996). Root histogenesis in herbaceous and woody explants cultured in vitro. A critical review. *Agronomie*, *16*(10), 589–602. http://doi.org/10.1051/agro:19961001

Alves, E. C., Guimarães, J. E. R., Franco, C. K. B., & Martins, A. B. G. (2016). Number of leaflets on rooting of lychee herbaceous cuttings. *Ciência Rural*, *46*(6), 1003–1006.

Aminah, H., Dick, J. M., & Grace, J. (1997). Rooting of *Shorea leprosula* stem cuttings decreases with increasing leaf area. *Forest Ecology and Management*, *91*(2–3), 247–254. http://doi.org/10.1016/S0378-1127(96)03857-1

Andre, C. M., Hausman, J.-F., & Guerriero, G. (2016). Cannabis sativa: The Plant of the Thousand and One Molecules. *Frontiers in Plant Science*, *7*(February), 1–17. http://doi.org/10.3389/fpls.2016.00019

Arena, M. J., Schwarz, O. J., & Witte, W. T. (1997). Experiments with Locust and Willow Diffusates on Rooting Cuttings. *HortScience*, *32*(4), 590.

Bagheri, M., & Mansouri, H. (2015). Effect of Induced Polyploidy on Some Biochemical Parameters in Cannabis sativa L. *Applied Biochemistry and Biotechnology*, *175*(5), 2366–2375. http://doi.org/10.1007/s12010-014-1435-8

Beutler, J. A., & Marderosian, A. H. (1978). Chemotaxonomy of Cannabis I. Crossbreeding between Cannabis sativa and C. ruderalis, with analysis of cannabinoid content. *Economic Botany*, *32*(4), 387–394. http://doi.org/10.1007/BF02907934

Boyer, J. S. (1995). *Measuring the Water Status of Status of Plants and Soils*. San Diego, California: Academic Press Inc.

Caplan, D., Dixon, M., & Zheng, Y. (2017). Optimal Rate of Organic Fertilizer during the Vegetative-stage for Cannabis Grown in Two Coir-based Substrates. *HortScience*,*52*(9), 1307-1312. doi:10.21273/hortsci11903-17

Cervantes, J. (2006). *Marijuana horticulture: The indoor/outdoor medical grower's bible*. Vancouver, WA: Van Patten Publishing.

Chen, J., Kang, S., Du, T., Guo, P., Qiu, R., Chen, R., & Gu, F. (2014). Modelling relations of tomato yield and fruit quality with water deficit at different growth stages under greenhouse condition. *Agricultural Water Management*, *146*, 131–148. http://doi.org/10.1016/j.agwat.2014.07.026

Clarke, R. C. (1981). *Marijuana Botany: An Advanced Study, the Propagation and Breeding of Distinctive Cannabis* (illustrate). Oakland, CA: Ronin Publishing.

Coffey, W. L. P., Gordon, R. J., & Dixon, M. (1997). Patterns of stem water potential in field grown potatoes using stem psychrometers. *Potato Research*, *40*, 35–46.

Coffman, C. B., & Gentner, W. A. (1979). Greenhouse propagation of cannabis sativa l. by vegetative cuttings. *Economic Botany*, *33*(2), 124–127.

Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). Secondary Metabolites. In *Biochemistry Molecular Biology of Plants* (Vol. 7, pp. 1250–1318). American Society of Plant Physiologists. http://doi.org/10.1016/j.phytochem.2011.10.011

Davis, T. D., & Potter, J. R. (1989). Relations between carbohydrate, water status and adventitious root formation in leafy pea cuttings rooted under various levels of atmospheric CO_2 and relative humidity. *Physiologia Plantarum*, *77*(2), 185–190. http://doi.org/10.1111/j.1399-3054.1989.tb04967.x

Dixon, M., & Downey, A. (2015). PSY1 Stem Psychrometer Manual. *Statewide Agricultural Land Use Baseline 2015*.

Dixon, M., Grace, J., & Tyree, M. T. (1984). Concurrent measurements of stem density, leaf and stem water potential, stomatal conductance and cavitation on a sapling of Thuja occidentalis L. *Plant, Cell and Environment*, *7*, 615–618. http://doi.org/10.1111/1365-3040.ep11592146

Dixon, M., & Tyree, M. T. (1984). A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. *Plant, Cell and Environment*, *7*(9), 693–697. http://doi.org/10.1111/1365-3040.ep11572454

Edwards, D. R., & Dixon, M. (1995). Mechanisms of Drought Response in Thuja-Occidentalis L. II. Post-conditioning water stress and stress relief. *Tree Physiology*, *15*(2), 129–133. Retrieved from #

Edwards, D. R., & Dixon, M. (1995). Mechanisms of Drought Response in Thuja-Occidentalis L. I. Water-Stress Conditioning and Osmotic Adjustment. *Tree Physiology*, *15*(2), 121–127. Retrieved from #

Ehlers, W., & Goss, M. (2003). *Water Dynamics in Plant Production*. CABI Publishing.

Elzinga, S., Fischedick, J., Podkolinski, R., & Raber, J. (2015). Cannabinoids and Terpenes as Chemotaxonomic Markers in Cannabis. *Natural Products Chemistry & Research*, *3*(4). http://doi.org/10.4172/2329-6836.1000181

Emboden, W. A. (1974). Cannabis - A Polytipic Genus. *Economic Botany*, *28*, 304–310.

Farag, S., & Kayser, O. (2015). Cultivation and breeding of *Cannabis sativa* L. for preparation of standardized extracts for medicinal purposes. In *Medicinal and aromatic plants of the world* (Vol. 1, pp. 165–186). Dordrecht: Springer Netherlands. http://doi.org/10.1007/978-94-017-9810-5

Farag, S., & Kayser, O. (2015). *Medicinal and Aromatic Plants of the Middle-East*. (A. Mathe, Ed.). Budapest, Hungary: Springer. http://doi.org/10.1007/978-94-017-9810-5

Farquhar-Smith, & Paul, W. (2002). Pain and cannabinoids: science and evidence. *Pain Reviews*. http://doi.org/10.1191/0968130202pr188ra

Flores-Sanchez, Josefina, I., & Verpoorte, R. (2008). Secondary metabolism in cannabis. *Phytochemistry Reviews*, *7*(3), 615–639. http://doi.org/10.1007/s11101-008-9094-4

Gesto, M. D. V, Vazquez, A., & Vieitez, E. (1977). Rooting Substances in Water Extracts of Castanea sativa and Salix viminalis. *Physiologia Plantarum*, *40*(4), 265–268. http://doi.org/10.1111/j.1399-3054.1977.tb04070.x

Graham, D. S. (2002). Medical marijuana: Canada's regulations, pharmacology, and social policy. *Canadian Pharmacists Journal*, *137*(902), 23–27.

Haapala, T. (2004). *Establishment and Use of Juvenility for Plant Propagation in Sterile and Non-Sterile Conditions*. *Plant Breeding*. http://doi.org/952-10-1915-8

Hackett, W. P. (1970). The influence of auxin, catechol and methanolic tissue extracts on root initiation in aseptically cultured shoot apices of the juvenile and adult forms of Hedera helix. *Journal of the American Society of Horticultural Science*, *95*, 398–402.

Haissig, B. E. (1974). Influences of auxins and auxin synergists on adventitious root primordium initiation and development. *New Zealand Journal of Forestry Science*, *4*(2), 311–323.

Hansen, J. (1986). Influence of Cutting Position and Stem Length on Rooting of Leaf-Bud Cuttings of Schefflera Arboricola. *Scientia Horticulturae*, *28*, 177–186.

Hao, D. C., Gu, X. J., & Xiao, P. G. (2015). *Medicinal Plants: Chemistry, Biology and Omics*. *Medicinal Plants: Chemistry, Biology and Omics*. Elsevier Ltd. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-84942795559&partnerID=40&md5=0f077e72b53adf30f61c2bbd2ff3f9a6

Hao, D. C., Gu, X. J., Xiao, P. G., & Peng, Y. (2013). *Chemical and biological research of Clematis medicinal resources*. *Chinese Science Bulletin* (Vol. 58). http://doi.org/10.1007/s11434-012-5628-7

Hartmann, H., Davies, F., & Geneve, R. (2002). *Hartmann and Kester's plant propagation: Principles and practices* (7th ed.). Upper Saddle River, NJ: Prentice Hall.

Health Canada. *Access to Cannabis for Medical Purposes Regulations*. (2017).

Humphrey, A., & Beale, M. (2006). Terpenes. In *Plant Secondary Metabolites Occurrence, Structure and Role in the Human Diet* (pp. 47–106). Blackwell Publishing Ltd. http://doi.org/10.1002/9780470988558

Husen, A., & Pal, M. (2006). Variation in shoot anatomy and rooting behaviour of stem cuttings in relation to age of donor plants in teak (Tectona grandis Linn. f.). *New Forests*, *31*(1), 57–73. http://doi.org/10.1007/s11056-004-6794-5

Iversen, L. (2003). Cannabis and the brain. *Brain*, *126*(6), 1252–1270. http://doi.org/10.1093/brain/awg143

Jia, X., Sun, C., Li, G., Li, G., & Chen, G. (2015). Effects of progressive drought stress on the physiology, antioxidative enzymes and secondary metabolites of Radix Astragali. *Acta Physiologiae Plantarum*, *37*(12), 1–14. http://doi.org/10.1007/s11738-015-2015-4

Johnson, R. W., Dixon, M., & Lee, D. R. (1992). Water relations of the tomato during fruit growth. *Plant, Cell & Environment*, *15*(8), 947–953. http://doi.org/10.1111/j.1365-3040.1992.tb01027.x

Jones, H. G. (2004). Irrigation scheduling: Advantages and pitfalls of plant-based methods. *Journal of Experimental Botany*, *55*(407), 2427–2436. http://doi.org/10.1093/jxb/erh213

Jones, H. G. (2007). Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany*, *58*(2), 119–130. http://doi.org/10.1093/jxb/erl118

Kawase, M. (1970). Root-promoting Substances in Salix alba. *Physiologia Plantarum*, *23*(1), 159–170. http://doi.org/10.1111/j.1399-3054.1970.tb06404.x

Kawase, M. (1964). Centrifugation, Rhizocaline and Rooting in Salix alba L. *Physiologia Plantarum*, *17*(4), 855–865. http://doi.org/10.1111/j.1399-3054.1964.tb08212.x

Kleinwächter, M., & Selmar, D. (2015). New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. *Agronomy for Sustainable Development*, *35*(1), 121–131. http://doi.org/10.1007/s13593-014-0260-3

Kuzdzal, S., Clifford, R., Winkler, P., & Bankert, W. (2016). A Closer Look At Cannabis Testing Analytical and Measuring Instruments. *C&En*.

Lang, A., & Thorpe, M. (1986). Water potential, translocation and assimilate partitioning. *Journal of Experimental Botany*, *37*(4), 495–503. http://doi.org/10.1093/jxb/37.4.495

Lata, H., Chandra, S., Khan, I., & ElSohly, M. (2011). Molecular analysis of genetic fidelity in Cannabis sativa L. plants grown from synthetic (encapsulated) seeds following in vitro storage. *Biotechnology Letters*, *33*(12), 2503–8. http://doi.org/10.1007/s10529-011-0712-7

Lata, H., Chandra, S., Khan, I. a., & Elsohly, M. a. (2009). Propagation through alginate encapsulation of axillary buds of Cannabis sativa L. - An important medicinal plant.

Physiology and Molecular Biology of Plants, *15*(1), 79–86. http://doi.org/10.1007/s12298-009-0008-8

Lata, H., Chandra, S., Khan, I., & ElSohly, M. A. (2009). Thidiazuron-induced high-frequency direct shoot organogenesis of Cannabis sativa L. *In Vitro Cellular and Developmental Biology - Plant*, *45*(1), 12–19. http://doi.org/10.1007/s11627-008-9167-5

Leakey, R. R. B., & Coutts, M. P. (1989). The dynamics of rooting in Triplochiton scleroxylon cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. *Tree Physiology*, *5*(1), 135–146. http://doi.org/10.1093/treephys/5.1.135

Lemberger, L. (1980). Potential therapeutic usefulness of marihuana. *Ann.Rev.Pharmacol.Toxicol.*, *20*, 151–172.

Lisson, S., & Mendham, N. (1998). Response of fibre hemp (Cannabis sativa L.) to varying irrigation regimes. *Journal of the International Hemp Association*, *5*(1), 9–15.

Machida, H., Ooishi, A., Hosoi, T., Komatsu, H., & Kamota, F. (1977). Studies on Photosynthesis in Cuttings during Propagation. *Journal of the Japanese Society for Horticultural Science*, *46*(2), 274–282. http://doi.org/10.2503/jjshs.46.274

Majeed, M., Khan, M. A., & Mughal, A. H. (2009). Vegetative propagation of aesculus indica through stem cuttings treated with plant growth regulators. *Journal of Forestry Research*, *20*(2), 171–173. http://doi.org/10.1007/s11676-009-0031-1

McPartland, J. M., Clarke, R. C., & Watson, D. P. (2000). Hemp Diseases and Pests Management and Biological Control. New York: CABI Publishing.

Mechoulam, R. (1970). Marihuana Chemistry. *Science*, *168*(3936), 1159–1166.

Morgan, D. L., & McWilliams, E. L. (1976). Juvenility as a factor in propagating Quercus. *Acta Hort.*, *56*, 263–268.

Nakawuka, P. (2013). Effect of deficit irrigation on yield, quality and grower returns of native spearmint and hops in Washington State, (May).

Ofori, D. A., Newton, A. C., Leakey, R. R. B., & Grace, J. (1996). Vegetative propagation of Milicia excelsa by leafy stem cuttings: Effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management*, *84*(1–3), 39–48. http://doi.org/10.1016/0378-1127(96)03737-1

Pallardy, S. G., & Kozlowski, T. T. (1979). Stomatal response of populus clones to light intensity and vapor pressure deficit. *Plant Physiology*, *64*(1), 112–114. http://doi.org/10.1104/pp.64.1.112

Pertwee, R. G. (2014). Handbook of cannabis, 781. http://doi.org/10.1017/CBO9781107415324.004

Pirzad, A., Alyari, H., Shakiba, M. R., Zhetab-Salmasi, S., & Mohammadi, A. (2006). Essential Oil Content and Composition of German Chamomile (Matricaria chamomilla L.) at Different Irrigation Regimes. *Journal of Agronomy*, *5*(3), 451–455. http://doi.org/10.3923/ja.2006.451.455

Pirzad, A., Shakiba, M. R., Zehtab-Salmasi, S., Mohammadi, S. A., Sharifi, R. S., & Hassani, A. (2011). Effects of Irrigation Regime and Plant Density on Essential Oil Composition of German Chamomile (*Matricaria chamomilla*). *Journal of Herbs, Spices & Medicinal Plants*, *17*(2), 107–118. http://doi.org/10.1080/10496475.2011.584824

Potter, D. J. (2009). *The propagation, characterisation and optimisation of Cannabis sativa L. as a phytopharmaceutical*. King's College London, London, PhD Diss.

Potter, D. J., & Duncombe, P. (2012). The Effect of Electrical Lighting Power and Irradiance on Indoor-Grown Cannabis Potency and Yield. *Journal of Forensic Sciences*, *57*(3), 618–622. http://doi.org/10.1111/j.1556-4029.2011.02024.x

Radovich, T. J. K., Kleinhenz, M. D., & Streeter, J. G. (2005). Irrigation Timing Relative to Head Development Infl uences Yield Components, Sugar Levels, and Glucosinolate Concentrations in Cabbage. *Journal of the American Society for Horticultural Science*, *130*(6), 943–949.

Rakshit, A., Singh, H. B., & Sen, A. (Eds.). (2015). *Nutrient use efficiency: From basics to advances*. *Nutrient Use Efficiency: From Basics to Advance*. Springer. http://doi.org/10.1007/978-81-322-2169-2

Raviv, M., & Blom, T. J. (2001). The effect of water availability and quality on photosynthesis and productivity of soilless-grown cut roses. *Scientia Horticulturae*, *88*(4), 257–276. http://doi.org/10.1016/S0304-4238(00)00239-9

Reichman, S. M. (2002). *The Responses of Plants to Metal Toxicity: A review focusing on Copper , Manganese and Zinc*. *Environment*. Retrieved from http://www.plantstress.com/articles/toxicity_i/Metal_toxicity.pdf

Robinson, S., Dixon, M., & Zheng, Y. (2007). Vascular blockage in cut roses in a suspension of Pseudomonas fluorescens. *Journal of Horticultural Science and Biotechnology*, *82*(5), 808–814. http://doi.org/10.1080/14620316.2007.11512310

Robinson, S., Graham, T., Dixon, M., & Zheng, Y. (2009). Aqueous ozone can extend vase-life in cut rose. *Journal of Horticultural Science and Biotechnology*, *84*(1), 97–101. http://doi.org/10.1080/14620316.2009.11512487

Saffari, M., & Saffari, V. R. (2012). Effects of media and indole butyric acid (IBA) concentrations on hopbush (Dodoneae viscosa L) cuttings in green house. *Annals of Forest Research*.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. a, & Hammel, H. T. (1965). Sap pressure in vascular plants. *Science*, *148*(3668), 339–46. http://doi.org/10.1126/science.148.3668.339

Schreiber, L. R., & Kawase, M. (1975). Rooting of cuttings from tops and stumps of American elm. *HortScience*, *10*, 615.

Shackel, K. A. (1984). Theoretical and experimental errors for in situ measurements of plant water potential. *Plant Physiology*, *75*(3), 766–772.

Shackel, K. A., Ahmadi, H., Biasi, W., Buchner, R., Goldhamer, D., Gurusinghe, S., … Yeager, J. (1997). Plant water status as an index of irrigation need in deciduous fruit trees. *HortTechnology*.

Singh-Sangwan, N., Abad Farooqi, a H., & Singh Sangwan, R. (1994). Effect of drought stress on growth and essential oil metabolism in lemongrasses. *New Phytologist*, *128*(1), 173–179. http://doi.org/10.1111/j.1469-8137.1994.tb04000.x

Small, E., & Cronquist, A. (1976). A Practical and Natural Taxonomy for Cannabis Author(s): Ernest Small and Arthur Cronquist Published by: International Association for Plant Taxonomy (IAPT) Stable URL : http://www.jstor.org/stable/1220524 . *Taxon*, *25*(4), 405–435.

Soundy, P., Mpati, K. W., Elsa, S. du T., Mudau, F. N., & Araya, H. T. (2008). Influence of cutting position, medium, hormone and season on rooting of fever tea (Lippia javanica L.) stem cuttings. *Medicinal and Aromatic Plant Science and Biotechnology*, *2*(2), 114–116.

Tran, N. (2014). Relating Cumulative Water Potential to Concurrent Vapour Pressure Deficit, (1). Retrieved from http://www.ictinternational.com/casestudies/relating-cumulative-water-potential-to-concurrent-vapour-pressure-deficit/

Turner, N. C. (1988). Measurement of plant water status by the pressure chamber technique. *Irrigation Science*, *9*(4), 289–308. http://doi.org/10.1007/BF00296704

Veierskov, B. (1988). Relations between carbohydrates and adventitious root formation. In T. D. Davis, B. E. Haissig, & N. Sankhla (Eds.), *Adventitious root formation in cuttings* (pp. 70–78). Portland, OR.

Wang, Y. H., & Irving, H. R. (2011). Developing a model of plant hormone interactions. *Plant Signaling & Behavior*, *6*(4), 494–500. http://doi.org/10.4161/psb.6.4.14558

Wilson, R. I., & Nicoll, R. a. (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*, *410*(6828), 588–592. http://doi.org/10.1038/35069076

Nova Scotia Greenhouse Pest Control Training Manual. (2006).

Understanding and Using VPD. (2009), (January). Retrieved from http://www.arguscontrols.com/resources/VPD_Application_Note.pdf