

## ABSTRACT

SMITH, JAMES TURNER. Paclobutrazol Drench Efficacy in Soilless Substrates Containing Wood and Pine Bark, and Vegetative Hemp Growth in Peat and Coir-Based Substrates. (Under the direction of Dr. Brian Jackson).

Previous research has reported that pine bark as a horticultural substrate can reduce the efficacy of drench applied plant growth regulators (PGR). There is however no understanding or evaluation of the effect of pine bark age on substrate interaction with PGRs. Due to the physical and chemical changes that occur during aging, the efficacy of PGRs in substrates containing pine bark may be affected by the age of the pine bark used. To address this question, paclobutrazol drench applications of 0, 1, 2, and 4 mg of active ingredient were applied to 'Antigua Yellow' marigold (*Tagetes erecta*) grown in plastic containers filled with a peat: pine bark: perlite substrate, in ratios of either 40:40:20 or 60:20:20 (v:v:v). Bark substrates obtained and used in this study were 0, 3, 6, 9, and 12 months old. A general trend of less growth control with older bark was observed, with the interaction of age and PGR concentration affecting width and growth indices in mixes containing 40% bark, and only dry weight in mixes containing 20% bark. The results of this experiment indicate older bark has a greater influence on PGR drench applied efficacy. Consequentially, growers utilizing aged bark will need to increase PGR drench applied concentrations to account for the reduction in growth control.

The commercialization and increased use of wood fiber is a recent trend in production horticulture. Prior research has evaluated the effects of wood materials on PGR efficacy but the influence of a fibrous wood material like pine tree substrate (PTS) has not yet been examined. Fibrous wood substrates can have higher surface area than other wood substrates and therefore may influence PGR efficacy, two experiments were conducted to address this question. Paclobutrazol drench applications of 0, 1, 2, and 4 mg of active ingredient were applied to

‘Perfection Yellow’ marigold (*Tagetes erecta*) and ‘Pacino Gold’ sunflower (*Helianthus annuus*) grown in plastic containers filled with a peat and PTS blend in the ratios of 90:10, 80:20, 70:30, and 60:40, plus a peat: perlite blend of 80:20 which was included as a control. In the first study, no interaction was found between substrate type and PGR rate on any growth parameters of either species. In the second experiment the substrate and PGR interaction did not affect sunflower but, was found to affect height, diameter, and growth index of marigold. Although an interaction of substrate and PGR was found to affect marigold in the repeat experiment, visual differences were minimal. These data suggest growers utilizing 40% or less PTS in peat-based substrates will not need to adjust paclobutrazol drench applied concentrations from established rates in peat: perlite mixes.

Another recent trend in production horticulture is the continued de-regulation and increased production of cannabis (*Cannabis sativa* L.). Little scientific literature exists on cultivation techniques for cannabis in container production environments; and there is almost none concerning horticultural substrate requirements. Substrates are one of the most important factors in maximizing crop production in controlled environments. To gain a more thorough understanding of cannabis cultivation in container culture, two studies were conducted to evaluate vegetative growth in a variety of horticultural substrates. Four cultivars were grown in peat-based and coir-based mixes containing either 0, 15, 30, or 45% perlite by volume. Additionally, to evaluate a range of commercially available mixes, plants were grown in 20 mixes marketed to the cannabis industry. Aside from coir-based mixes with higher levels of perlite, growth measurements were similar in all substrates tested. These experiments suggest cannabis can be grown successfully in a range of horticultural substrates.

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Paclobutrazol Drench Efficacy in Soilless Substrates Containing Wood and Pine Bark, and  
Vegetative Hemp Growth in Peat and Coir-Based Substrates.

by  
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## **DEDICATION**

To my family, for which I am profoundly grateful.

## **BIOGRAPHY**

Turner Smith grew up in western North Carolina. He completed a Bachelor of Science in Ecology and Environmental Biology at Appalachian State University in 2012. Upon completion of his undergraduate degree, he moved to Estes Park, Colorado. Here he interned for Rocky Mountain National Park's greenhouse, supporting the park's vegetation management plan. He was then hired as a member of the native vegetation restoration crew. Turner continued his career with the National Park Service by working two seasons in Devils Tower, Wyoming at Devils Tower National Monument, on the exotic invasive plant management crew. Between his seasons at Devils Tower, Turner worked as a horticultural technician at The North Carolina Arboretum in Asheville, North Carolina. After four years of working seasonally in Colorado, Wyoming, and North Carolina, Turner decided to further his education by pursuing a Master of Science degree in horticulture.

## **ACKNOWLEDGMENTS**

I would like to thank my family for their unconditional support, and all the peers, professors, and professionals that have helped me navigate these last two years of learning, research, and professional development.

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# CHAPTER 1

## Literature Review

### Introduction

The United States Department of Agriculture reported the value of sales, for tax purposes, of horticultural specialty crops (horticultural specialty crops includes floriculture crops, nursery stock, propagative materials, sod, vegetable transplants, food crops grown under protection, and vegetable and flower seeds) in 2014 was \$13.8 billion (Vilsack and Reilly, 2015). The bulk of this industry is dependent on container production for part, if not all, of a respective crop's lifespan. Containers filled with soilless substrates offer many advantages over in-ground environments. Soilless substrates are lighter, meaning containers can be moved easily and transported more inexpensively. Soilless substrates can allow precision application of costly inputs, such as fertilizers, plant growth regulators (PGRs), pesticides, and water and prevent excessive runoff (Fields et al., 2014a; Fields et al., 2014b; Fonteno, 1993; Fonteno et al., 2013). Physical properties such as total porosity, container capacity, air space, and bulk density, can be altered to provide an even distribution of air, water, and nutrients suiting the needs of different crops. Substrates are far more than a random assortment of organic and inorganic materials in a pot, they are highly engineered tools providing an optimized environment for root growth and overall crop health. These characteristics, and many more, have led growers to become increasingly dependent on substrate science, especially when producing high value crops.

### Sphagnum Peat Moss

Peat moss is the primary organic substrate used for container-grown plants in the United States today, with most of this material originating in Canada. Peat used for horticultural

practices is composed primarily of three sphagnum moss species, *Sphagnum angustifolium* (C. Jens. ex Russ.), *S. magellanicum* Brid., and *S. fuscum* (Schimp.) Klinggr. (Rippy and Nelson, 2007), which accumulate in the acidic, waterlogged conditions of bogs and wetlands in cooler climates (Handreck and Black, 2010). The partial decomposition of sphagnum moss in bogs is responsible for the beneficial physical properties peat imparts to soilless substrates. Sugars are decomposed and lignin, the relatively stable organic polymer responsible for cell wall rigidity, is left behind (Handreck and Black, 2010). The preservation of cellular structures is responsible for the highly porous nature of peat, and low bulk density. This provides a high container capacity (ability to hold water) while also providing a high level of air space (Handreck and Black, 2010). The high cation exchange capacity (CEC), dependent on species composition (Rippy and Nelson, 2007), allows nutrients to be readily available to plants. Low initial pH, resulting from the acidic conditions of bogs, allows growers to adjust pH to suit the needs of their crops.

### **Pine Bark**

In the southeastern U.S. mixes containing aged pine bark, peat and sand make up the majority of container substrates (Boyer et. al. 2006). In the nursery industry, when utilized as a primary component, pine bark often comprises 75 to 100% of the container substrate by volume (Lu et al., 2006). The primary species of pine bark used in this region are Loblolly pine (*Pinus taeda* L.), shortleaf pine (*Pinus echinata* Mill.), slash pine (*Pinus elliottii* Engelm.), and longleaf pine (*Pinus palustris* Mill.) (Bunt, 1988). Much of the pine bark used for soilless substrates is a byproduct of forestry industries such as paper, pulp and lumber (Bunt, 1988). Like peat, pine bark can provide optimal ranges of physical properties for plant growth.

Environmental, mechanical, and processing and handling methods can all influence pine bark characteristics (Jackson, 2014), meaning pine bark producers can engineer products to better suit the needs of different growers. Milling and screening to produce a range of particle sizes, and thus physical properties, is one of the methods employed by pine bark producers. Another technique to alter physical and chemical properties is aging. Although more time and resources must be invested in the aging processes for turning and wetting pine bark piles (Bilderback, 2002), much of the pine bark used in container substrates is aged after processing. Aged pine bark has been associated with higher container capacity and water retention compared to fresh bark (Harrelson et al., 2004; Bilderback, 2002), resulting in higher quality plants. Composting of pine bark is also used to create a more favorable substrate component. The composting process is similar to aging, pine bark is left in piles to age and periodically turned and wetted, but a nitrogen source such as urea is also added (Davis et al., 1992). The nitrogen source serves to increase microbial populations that accelerate the decomposition of the bark.

### **Alternative Substrates**

In recent years, the future availability and affordability of peat moss has become uncertain. Most of the peat moss used in the U. S. is imported from Canada. The transportation costs of this substrate component are an important consideration for growers. The harvest of peat moss is also weather dependent, and if the weather is overly wet during the harvest season it can cause shortages in supply. There have been recent social concerns about the environmental impacts of peat moss harvesting as well (Canadian Sphagnum Peat Moss Association, 2014). The supply of aged pine bark for container substrates has also become uncertain. Pine bark is increasingly being used as fuel sources, leading to a rise in its price. A shift in forestry practices

has also affected pine bark availability. The use of mobile in-field equipment that processes trees into “clean chips” discards bark at the harvesting site (Boyer et. al. 2006). These uncertainties in availability, cost, and public opinion have necessitated the exploration for new alternative substrates. During the last twenty years, extensive research has been devoted to the evaluation of physical and chemical characteristics of alternative substrates.

### **Wood Substrates**

One class of alternative substrates growing in popularity are wood materials. Wood substrate components were first utilized in Europe in the 1980’s, and later adopted in the United States following the work of Laiche and Nash (1986) comparing pine bark with and without wood, and pine tree chips. Several wood products have been researched in the U.S. including, clean chip residual, red cedar chips, WholeTree, and pine tree substrates. Wood products are readily available and can nearly reduce transportation costs due to their proximity to nursery operations.

Clean chip residual, or CCR is generated as a waste product from equipment that produces “clean chips” for pulp mills. Clean chip residual composed of roughly 50% wood, 40% bark, and 10% needles (Boyer et al. 2006) and is often used as a fuel source or disposed of at the harvest site. When amended with peat, CCR has been found to be a suitable substrate for production of annuals (Boyer et al., 2008a) and perennials (Boyer et al., 2008b) though CCR has been reported to have issues with high air space and low container capacity (Boyer et al., 2008a). Retarded plant growth reported by Boyer et al., (2008b) seemed to indicate that the physical properties of the substrate were the limiting factors, rather than nitrogen immobilization; an issue associated with wood substrate materials (Jackson and Wright 2007; Jackson et al., 2008;

Jackson et al., 2009a; Wright et al., 2008). The addition of peat moss to both PB and CCR reduced air space, increased water holding capacity, leading to a more favorable environment.

Red cedar chips are a byproduct of the manufacture of cedar oil, used in the perfume industry (Vandiver et al., 2013). Red cedar chips have been reported to be a suitable component of pine bark-based mixes (Carmichael et al., 2012; Edwards et al., 2012; Starr et al., 2013) and peat-perlite mixes (Vandiver et al. 2013) though sometimes resulting in retarded growth. Like CCR, less growth in red cedar mixes has been attributed to increased air space and reduced container capacity (Carmichael et al., 2012).

Another alternative substrate that has been studied is WholeTree. WholeTree is composed of all the above ground portions of pine trees (wood, cambium, bark, needles, cone) harvested during the thinning process. Whole tree is a manufactured product and because of this, issues associated with physical properties of other substrate components can be overcome. A consistency of quality can be achieved that many alternative substrates lack (Fain et al., 2008). Differences in milling and screening can alter particle size and shape, resulting in more suitable air space and container capacity. Aging of WholeTree further increases suitability of physical properties by reducing air space and increasing container capacity compared to fresh WholeTree (Gaches et al., 2011).

Pine tree substrates (Jackson and Wright, 2007) are yet another manufactured alternative substrate. The trunks of loblolly pine trees are chipped and ran through a hammermill when they are about 15 years old (Jackson et al., 2010). Pine tree substrates can potentially offer a higher level of consistency than WholeTree substrates because a higher level of control of the stock feed, chipped pine logs vs. whole trees, can be achieved.

Work by Wright and Browder (2005), Jackson and Wright (2007), and Jackson et al. (2010) have all demonstrated the feasibility of growing horticultural crops in pine tree substrates. Pine tree substrates have received much attention in the last decade with studies being conducted on nitrogen immobilization and required fertilizer rates (Jackson and Wright 2007; Jackson et al., 2008; Jackson et al., 2009a; Wright et al., 2008), use in long term crops (Jackson et al., 2009b), hydrophysical properties (Fields et al., 2014b), irrigation management (Riley et al., 2014), and liming requirements (Jackson et al., 2009c).

### **Coconut Coir**

Coconut coir is another alternative substrate that has risen in popularity. Coconut coir refers to the processed husk from the fruit of *Cocos nucifera* L. A byproduct of the production of coconut oil and fruit, coconut husk is ground to separate pith from long fibers which can be used for the manufacture of rope and rugs (Abad et al., 2005; Evans et al., 1996). The remaining pith, or coconut coir dust, and remaining fibers have proven to be a suitable soilless substrate component due to favorable physical and chemical properties. Coir has been widely marketed to the cannabis industry as an alternative to peat. Caplan et al., (2017b) reported that coir-based mixes are one of the primary substrates used by cannabis growers in North America.

### **Plant Growth Regulators**

There is an increasing demand to produce near identical crops on strict production schedules, in today's horticulture industry. The application of PGRs to control excessive growth, and increase marketability, of containerized greenhouse crops is common practice in the floriculture industry. Plant growth regulators are either naturally occurring plant hormones,

responsible for development, or synthetic compounds that mimic or inhibit the action of these hormones (Barrett, 2001). The primary benefit of a PGRs is to allow the production of compact, uniform crops, that can be more tightly spaced in the growing area. Some PGRs also provide additional benefits beyond growth control. Ancymidol, chlormequat chloride, daminozide, flurprimidol, and uniconazole induce higher chlorophyll content (greener leaves), reduced water stress, and aid in disease suppression (Whipker, 2017a).

One of the most common application methods of PGRs are substrate drenches (Currey et al., 2010; Dasoju et al., 1998b; Owen et al., 2016). Although there are many other methods to deliver PGR including liner or bulb dips (Blanchard and Runkle, 2007), foliar sprays (Whipker and McCall, 2000), and spike applications (Barrett et al., 1994), substrate drenches have advantages over these methods. Paclobutrazol, of the triazole chemical class is most effective when applied to roots and shoots. Plant growth regulators of this chemical class do not readily transport through phloem and so must be applied in a way that allows them to reach the xylem stream (Barret, 2001). Drenches allow a precise and uniform level of control unattainable by methods like foliar sprays and can provide longer lasting effects (Bonaminio and Larson, 1978). Application directly to the substrate, not only prevents risk of drift associated with foliar sprays, but also ensures active ingredient stays where it is intended rather than entering recirculated irrigation water (Whipker, 2017b).

### **Plant Growth Regulators in Pine Bark**

One of the concerns with drench applications is adsorption of active ingredient by organic substrate components. With pine bark being one of the most widely used organic substrate components used in ornamental production and several studies have investigated it's

influence on PGR efficacy. Tschabold et al., (1975) was one of the first to report on pine bark and PGR interactions. It was found that drench applications of ancymidol to *Chrysanthemum morifolium* were less effective when substrates contained greater amounts of pine bark. Bioassays were then performed on columns of substrates with and without pine bark. It was reported ancymidol remained available to plants in both, but the top 5 cm of the pine bark substrate column retained 70% of the active ingredient while the column without pine bark had an even distribution. Barrett (1982) also investigated the relationship of ancymidol as well as two other experimental PGRs (PP333, and EL-500), with pine bark substrates and saw a similar trend of reduced efficacy. It was suggested the most important factor in reduced efficacy was hydrophobic attraction between non-polar portions of PGR molecules and bark.

Other studies examining the efficacy of PGR in substrates containing pine bark have reported similar negative trends in plant growth (Bonaminio and Larson, 1978; Million et al., 1998a; Million et al., 1998b; Million et al., 1999; Newman et al., 1993; Quarrels and Newman, 1994), but pine bark can be a highly variable product. Environmental, mechanical, and processing and handling methods can all influence pine bark characteristics (Jackson, 2014), meaning several factors could be responsible for magnitude of reduced efficacy. One of these factors is bark age. Much of the pine bark used in container substrates is aged after processing, with no industry standards for a specific length of time, and little scientific literature describing changes during the aging process. In a review of pine bark substrate literature by Kaderabek (2017) it was reported that out of over 80 articles reviewed only 10 listed the specific age of pine bark used. Of those, only 4 addressed physical and chemical changes of pine bark during the aging process.

With pine bark being a very common substrate component and age of pine bark possibly differing between suppliers, more scientific data is needed to describe differences between ages and management practices that can maximize effectiveness of the substrate at these different ages. No information has been reported on the effects of pine bark of known ages on PGR efficacy.

### **Plant Growth Regulators in Alternative Substrates**

Plant Growth Regulator efficacy has also been examined in alternative substrates such as coconut coir, parboiled rice hulls, and wood. Coconut coir was reported to have similar influence on paclobutrazol activity compared to peat (Million et al., 1998a). Dasoju et al. (1998a) found paclobutrazol to have similar activity in coir as in peat at 2 mg active ingredient, but increased activity in coir when concentration was increased to 4 mg. Dasoju et al. (1998a) suggested paclobutrazol may have higher activity at higher concentrations in coir-based substrates, compared to peat-based substrates. Parboiled rice hulls blended as a substrate component ratio of (v/v) 20% were reported to have no influence on PGR efficacy by Currey et al. (2010). Two studies have examined PGR drench efficacy in substrates containing wood Felipe et al. (2013) and Owen et al. (2016). Felipe et al. (2013) compared efficacy of drench applied paclobutrazol in mixes composed of 50:50 peat: distilled cedar chips, 50:50 peat: wholetree, 50:50 peat: pine bark, and 75:25 peat: perlite. A significant interaction between PGR rate and substrate was reported for shoot dry weight of impatiens and petunias, but no interaction was reported for plant width. Owen et al. (2016) formulated peat-based substrates using either perlite or pine wood chips (PWC) at (v/v) ratios between 10 and 30%. It was reported that PWC had no effect on paclobutrazol efficacy when applied as a drench. Although pine wood chips were reported to

have no influence on paclobutrazol efficacy (Owen et al. 2016), and distilled cedar and Wholetree had mixed results (Felipe et al. 2013) there is a possibility other pine wood materials may influence paclobutrazol differently. Fibrous wood substrate components such as PTS have had increased commercialization and marketing compared to PWC. These fibrous wood materials may have a higher surface area than PWC so it is uncertain if they interact with PGRs differently.

### **Research Objectives**

Since pine bark age can differ between suppliers, and substrate drenches are one of the most widespread application methods of PGR, research is needed to gain a more thorough understanding of pine bark and PGR interactions. It is the objective of this research to evaluate the effect of pine bark age and percent (vol.) on the efficacy of paclobutrazol substrate drenches.

Another objective of this research is to evaluate the effect of PTS amendment on paclobutrazol substrate drenches. The growing popularity of fibrous wood substrates, and lack of scientific data explaining how these substrates may affect PGR substrate drenches presents a challenge to the efficient use of these tools for crop management. This objective was addressed by measuring efficacy of paclobutrazol in peat-based substrate blends containing different ratios of PTS amendment.

### **Container Production of Hemp**

*Cannabis sativa* L. is one of the earliest examples of cultivated plants, with domestication possibly occurring 8,500 years ago (Small and Cronquist, 1976; Small and Marcus, 2002). The economic importance and domestication efforts by man have focused on the seed, fiber, and

flowers. Fiber has been utilized for cordage and cloth, seeds have served as a source of oil, as well as food, and flowers have been selected for resin with intoxicant or medicinal properties (Schultes, 1970; Clarke and Merlin, 2017). According to Small and Cronquist (1976) cannabis cultivated for medicinal resin falls under two taxon, *C. sativa* subs. *sativa* and *C. sativa* subsp. *indica* (Lam.) (Hillig, 2005; McPartland and Guy, 2017; McPartland, 2018). These taxa are known commonly as hemp and marijuana, respectively. Subspecies *sativa* is differentiated by containing < 0.3% tetrahydrocannabinol (THC) by dry weight, and includes cultivars cultivated for cannabidiol (CBD), fiber and seed. Subspecies *indica* contains > 0.3% THC and is cultivated for its intoxicant properties.

Today, the seed and fiber cultivars of hemp are grown solely as field crops. Hemp cultivars cultivated for flower with high levels of cannabinoids, primarily CBD, are also grown in the field, but several factors lend CBD hemp to be treated as a horticultural crop, instead of a field crop (Febles, 2018). Difficulty of mechanizing harvest and challenges of producing consistent, uniform, high quality flowers contribute to CBD hemp being grown indoors as a horticultural crop. The photoperiodic nature of cannabis also enables several cropping cycles to be grown in a year using controlled environments.

In 2017, CBD hemp accounted for 23% of all U.S. hemp-based product sales; \$190 million, of an \$820 million industry (Hemp Bus. J., 2018). It is estimated the U.S. hemp industry will reach a market value of \$1 billion dollars in 2018 (Hemp Bus. J., 2018). A large portion of this growth can be attributed to increased production of hemp-derived CBD, food, personal care and industrial products (Hemp Bus. J., 2018).

Little scientific literature exists on cultivation techniques for cannabis in container production environments; and there is almost none concerning substrate requirements. To our

knowledge, Caplan et al. (2017a, b) in their work on optimal rates of organic fertilizer for cannabis growth, was the only studies that have reported physical properties of substrates used in cannabis cultivation. The data reported in the two studies however, was limited to four commercially available coir-based substrate blends. Other research regarding cultivation of cannabis provide only vague description of substrates used, peat/perlite mixture (Potter and Duncombe, 2012), 4:1 mix of composted bark and coarse washed river sand (Lisson et al., 2000), 3 soil-leaf: 1 mold-Kureha: 1 compost (Yoshimatsu et al., 2004), or no description of substrates used, soil-filled pots (Coffman and Gentner, 1979), grown in pots (Potter, 2014).

## **Research Objectives**

Due to the importance of substrates in container production and the lack of scientific literature describing substrate effect on cannabis growth, the objective of this research was to evaluate substrates with varying physical properties and their effect on cannabis vegetative growth. Two of the most common substrate components in mixes marketed to the cannabis industry are peat and coir (Caplan et al., 2017a). For this reason, we chose to focus on peat and coir, alone and with increasing ratios of perlite. An additional objective was to quantify the physical properties of a range of commercially available substrates marketed to the cannabis industry and evaluate vegetative growth of cannabis in these mixes.

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## **CHAPTER II**

### **Paclobutrazol Drench Activity Reduced in Substrates Containing Aged Pine Bark Compared to Fresh Bark**

## Abstract

Previous research has reported that pine bark as a horticultural substrate can reduce the efficacy of drench applied plant growth regulators (PGR). There is however no understanding or evaluation of the effect of pine bark age on substrate interaction with PGRs. Physical and chemical characteristics of pine bark change significantly over time during the aging or composting process. Therefore, the efficacy of PGRs in substrates containing pine bark may be affected by the age of the pine bark used. To address this question, paclobutrazol drench applications of 0, 1, 2, and 4 mg of active ingredient were applied to ‘Antigua Yellow’ marigold (*Tagetes erecta*) grown in plastic containers filled with a peat: pine bark: perlite substrate, in ratios of either 40:40:20 or 60:20:20 (v:v:v). The pine bark of various ages was obtained from a commercial bark supplier. Bark substrates obtained and used in this study were 0, 3, 6, 9, and 12 months old. After six weeks, plant height, growth index, and dry weights were recorded. A general trend of less growth control with older bark was observed, with the interaction of age and PGR concentration affecting width and growth indices in mixes containing 40% bark, and only dry weight in mixes containing 20% bark. The results of this experiment indicate older bark has a greater influence on PGR drench applied efficacy, likely due to physical changes in the aging process, compared to fresh bark. Consequentially, growers utilizing aged bark will need to increase PGR drench applied concentrations to account for the reduction in growth control.

## **Introduction**

The application of plant growth regulators (PGRs) to control excessive growth, and increase marketability, of containerized greenhouse crops is common practice in the floriculture industry. Plant growth regulators are either naturally occurring plant hormones, responsible for development, or synthetic compounds that mimic or inhibit the action of these hormones (Barrett, 2001). The primary benefit of PGRs is to allow the production of compact, uniform crops, that can be more tightly spaced in the growing area. Some PGRs also provide additional benefits beyond growth control. Ancymidol, chlormequat chloride, daminozide, flurprimidol, and paclobutrazol induce higher chlorophyll content (greener leaves), reduced water stress, and aid in disease suppression (Whipker, 2017a).

One of the most common application methods of PGRs are substrate drenches (Currey et al., 2010; Dasoju et al., 1998; Owen et al., 2016). Although there are many other methods to deliver PGR including liner soaks (Blanchard and Runkle, 2007), bulb dips (Krug et al., 2006), foliar sprays (Whipker and McCall, 2000), and spike applications (Barrett et al., 1994), substrate drenches have advantages over these methods. Paclobutrazol, of the triazole chemical class is most effective when applied to roots and shoots. Plant growth regulators of this chemical class do not readily transport through phloem and so must be applied in a way that allows them to reach the xylem stream (Barret, 2001). Drenches allow a precise and uniform level of control unattainable by methods like foliar sprays and can provide longer lasting effects (Bonaminio and Larson, 1978). Application directly to the substrate, not only prevents risk of drift associated with foliar sprays, but also ensures active ingredient stays where it is intended rather than entering recirculated irrigation water (Whipker, 2017b).

Substrate drenches can have drawbacks. One of the concerns with drench applications is adsorption of active ingredient by organic substrate components. Pine bark is one of the most widely used organic substrate components used in ornamental production and several studies have investigated its influence on PGR efficacy. Tschabold et al., (1975) was one of the first to report on pine bark and PGR interactions. It was found that drench applications of ancymidol to *Chrysanthemum morifolium* were less effective when substrates contained greater amounts of pine bark. Bioassays were then performed on columns of substrates (30.48cm in height and 6.4cm in diameter) with and without pine bark. It was reported ancymidol remained available to plants in both, but the top 5cm of the pine bark substrate column retained 70% of the active ingredient while the column without pine bark had an even distribution. Barrett (1982) also investigated the relationship of ancymidol as well as two other experimental PGRs (PP333, and EL-500), with pine bark substrates and reported a similar trend of reduced efficacy. It was suggested the most important factor in reduced efficacy was hydrophobic attraction between non-polar portions of PGR molecules and bark.

Other studies examining the efficacy of PGR in substrates containing pine bark have reported similar results with pine bark presence leading to a reduction in growth control (Bonaminio and Larson, 1978; Million et al., 1998a; Million et al., 1998b; Million et al., 1999; Newman et al., 1993; Quarrels and Newman, 1994), but pine bark can be a highly variable product. Environmental, mechanical, and processing and handling methods can all influence pine bark characteristics (Jackson, 2014), meaning several factors could be responsible for magnitude of reduced efficacy. One of these factors is bark age. Much of the pine bark used in container substrates is aged after processing, with no industry standards for a specific length of time, and little scientific literature describing changes during the aging process. In a review of pine bark

substrate literature by Kaderabek (2017) it was reported that out of over 80 articles reviewed only 10 listed the specific age of pine bark used. Of those, only four addressed physical and chemical changes of pine bark during the aging process.

With pine bark being a common substrate component and age of pine bark possibly differing among suppliers, more scientific data is needed to describe differences between ages and management practices that can maximize effectiveness of the substrate at these different ages. Although studies have investigated different variables that may influence PGR efficacy in pine bark substrates, level and particle size (Quarrels and Newman, 1994), and fresh versus composted pine bark (Million et al., 1998b), no information has been reported on the effects of pine bark age on PGR efficacy. Since pine bark age can differ among suppliers, and substrate drenches are one of the most widespread application methods of PGR, the objective of this study was to evaluate the effect of pine bark percent and age on the efficacy of paclobutrazol substrate drenches.

## **Materials and Methods**

A 12-month study (Kaderabek, 2017) was implemented on 20 Aug. 2015 at a commercial PB supplier (TH Blue Inc, Eagle Springs, NC). Longleaf pine bark (*Pinus palustris* Mill.) from two lumber companies, Jordan Lumber Company (Mt. Gilead, NC) and Troy Lumber Company (Troy, NC) was obtained the week of 17 Aug. 2015. The bark was passed through a hammer-mill (grinder make; and screened (Powerscreen Chieftan, Dungannon, UK) into three fractions: nuggets, mini nuggets, and fines. Fines were passed through a 1.27 cm screen. The < 1.27 cm fraction was placed in three piles of approximately 191 m<sup>3</sup> each, with dimensions of

approximately 16.8 m x 10.1 m x 3.2 m. Piles were turned every four to six weeks using a front-end loader.

The piles were sampled at the time of installation (fresh bark) and every month after turning, depending on weather and turning constraints, for a period of 12 months. Only samples collected at months 0 (fresh bark), 3, 6, 9, and 12 were used for the present study. Sampling and sample storage procedures were based on the methods outlined in the US Composting Council's Test Methods for the Examination of Composting and Compost (Thompson, 2002). Samples used were a composite mix of three replications of three locations (top, middle and bottom) of each pile. Once collected, samples were sealed in 4 mil poly bags, and stored indoors at a temperature of 4°C to minimize any moisture or microbial changes during storage.

On 21 Sept. 2017 aged pine bark was hand blended with Canadian sphagnum peat (Sun Gro Horticulture, Agawam, MA), at ratios of 60:20 and 40:40% (v:v) peat: pine bark, and amended with perlite (Carolina Perlite Co., Gold Hill, NC) at 20% (v). Substrate blends were fluffed and wetted by hand to a moisture content of 50% before adding 200 mesh dolomitic limestone (Mississippi Lime Company, Vicksburg, MS) at a rate of 2.97 kg/m<sup>3</sup>. Substrate blends were then sealed in bags to equilibrate overnight. Substrate blends contained either 60:20:20 (v:v:v) or 40:40:20 (v:v:v) peat: pine bark: perlite. A control substrate blend of 80:20 (v:v) peat: perlite with a lime rate of 2.97 kg/m<sup>3</sup> was also included. Substrate pH values were determined on 22 Sept. by the 2:1 method (2 deionized water: 1 substrate) using a pH/EC meter (HI 9813-6; Hanna Instruments, Woonsocket, RI). All substrate blend pH values fell between 5.3 and 5.8. The physical properties (total porosity, container capacity, air space, and bulk density) of all substrate blends were determined using the North Carolina State University (NCSU) Porometer method (Table 1) (Fonteno et al., 2005).

'Antigua Yellow' African Marigold 288 plugs, acquired from a commercial horticulture company (Ball Horticultural Company, West Chicago, IL), were transplanted into 12.7cm azalea containers (855 mL) on 23 Sept. Plants were hand watered with clear water and automatic drip irrigation was installed. Plants were fertilized twice a day at each watering with 150 mg·L<sup>-1</sup> nitrogen (N) from 17N-5P-17K-3Ca-1Mg water soluble fertilizer (Greencare Fertilizers Inc., Kankakee, IL) containing 4% ammoniacal (NH<sub>4</sub>)-N and 12.8% nitrate (NO<sub>3</sub>)-N, 5% phosphate (P<sub>2</sub>O<sub>5</sub>), 17% potassium (K<sub>2</sub>O), 3% calcium, and 1% magnesium.

On 12 Oct., 19 days after planting, 3 fl oz of solution containing either 0, 1, 2, or 4 mg of active ingredient (a.i.) per container paclobutrazol (Piccolo 10XC; Fine Americas Inc., Walnut Creek, CA) was applied to each container as a substrate drench. To prevent potential loss of active ingredient, trays were placed under each container prior to application. The experiment was a completely randomized design with 6 replications of 5 substrates x 4 PGR concentration combinations.

Beginning 27 Oct. and continuing until 10 Nov. all visible buds were hand pinched to maximize differences in vegetative growth among treatments. On 27 Nov. when all plants had reached anthesis, height (from substrate surface to the top of the bloom) was measured for each plant, along with two canopy width measurements (width at widest point, then the container was rotated 90° and the second width measurement was taken). Plants were then harvested at the substrate level and dried in an oven at 75°C for 5 days before dry weights were recorded.

Data was subjected to means separation using the PROC GLIMMIX procedure (version 9.4; SAS Institute, Cary, NC). PROC REG and PROC GLM were then used to determine the best fit linear or quadratic model for PGR rates.

## Results and Discussion

Physical properties of all substrate blends were similar (Table 1.1) except for the mix containing 40% fresh bark. Air space in the 40% fresh bark substrate was 28.4%, nearly 36% greater than the substrate with the next highest air space (20% fresh bark at 20.9% air space). Container capacity was 52.5%, a 14% decrease from the substrate with the next lowest container capacity (20% fresh bark at 61.4% container capacity). These differences in air space and container capacity were also observed by Harrelson et al., (2004) in their work involving nitrogen and irrigation requirements of fresh versus aged pine substrates. The increased air space was likely due to the larger particle size, and reduced percentage of fines, associated with fresh pine bark (Bilderback, 2002; Bilderback et al., 2013). The reduction of container capacity could be explained by the lack of micropores associated with fresh bark, to hold water (Bilderback et al., 2005).

In mixes containing 40% pine bark (Fig. 1.1), plant width and growth index were influenced by an interaction between pine bark age and PGR concentration ( $p = 0.0205$ , and  $0.0069$ , respectively) (Fig. 1.2, 1.3). Dry weight and height were both influenced by the main effects of pine bark age and PGR concentration with both parameters having  $p$ -values  $< 0.0001$  for both main effects (Table 1.2, and Fig. 1.2, 1.3). The trend of smaller plant dry weights in fresh bark that was observed in this work has been described before. Harrelson et al. (2004) reported plants grown in fresh bark had lower dry weights compared to plants grown in aged bark. These results also reiterate work done by Laiche (1974), which demonstrated fresh bark resulting in generally acceptable plant quality, but not always the best when compared to old or composted bark. In mixes containing 20% pine bark (Table 1.3.) the only growth measurement affected by an interaction of pine bark age, and PGR concentration was dry weight ( $p = 0.0459$ ).

Height, width, and growth index were only affected by PGR concentration with all parameters having p-values <0.0001.

The effect of pine bark age on plant growth with increased bark percentage is likely due to the fresh bark treatment. Pine bark aged three months and greater, regardless of volume, had little effect on air space and container capacity which can alter irrigation requirements. Both reduced plant measurements in fresh bark and the slight trend of reduced PGR efficacy in substrates with older bark could be related to percentage of fine particles. The bioassays conducted by Tschabold et al. (1975) showed that ancymidol was restricted to the top portions of the columns containing pine bark. With the increase in fine particles associated with aged bark, this increased surface area could potentially hold higher concentrations of PGR in the top portions of containers, out of reach from roots in lower portions. This effect of particle size would also agree with Quarrels and Newman, (1994) who reported poinsettias were less affected by uniconazole when grown in mixes containing bark with smaller grinds.

## **Conclusions**

Although not always significant, there was a general trend of reduced growth control with increasing age of pine bark. These effects were more pronounced when 40% bark was used compared to 20% bark. This data indicates aged bark may cause a greater reduction in drench applied PGR efficacy (reduced growth control) compared to fresh bark. This reduction in growth control is likely the result of finer particle sizes, associated with aged bark, preventing active ingredient from evenly distributing throughout the substrate. Specific age of pine bark used in substrates is an important characteristic for growers to be aware of. If a substrate is composed of

40% aged pine bark growers will need to account for this in their PGR program by increasing the concentration that would normally be used in a peat: perlite mix or a mix containing fresh bark.

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Table 1.1. Physical properties of substrate blends containing 60:20:20 or 40:40:20 peat: pine bark of known ages: perlite by volume.<sup>z</sup>

Substrate Blends	Total porosity <sup>y</sup> (% vol)	Container capacity <sup>x</sup> (% vol)	Air space <sup>w</sup> (% vol)	Bulk density <sup>v</sup> (g·cm <sup>-1</sup> )
Control <sup>s</sup>	83.8 abcd	64.4 bcd	19.5 b	0.10 f
0 Month 20%	82.3 de	61.4 d	20.9 b	0.11 de
0 Month 40%	80.9 e	52.5 e	28.4 a	0.13 bc
3 Month 20%	84.7 abcd	64.0 bcd	20.7 b	0.11 ef
3 Month 40%	83.2 cde	63.4 bcd	19.8 b	0.14 ab
6 Month 20%	86.3 a	69.8 a	16.5 b	0.12 cd
6 Month 40%	82.4 de	62.7 cd	19.7 b	0.14 ab
9 Month 20%	85.4 abc	67.3 ab	18.1 b	0.11 de
9 Month 40%	83.3 bcde	67.0 abc	16.3 b	0.14 ab
12 Month 20%	85.8 ab	70.2 a	15.6 b	0.12 de
12 Month 40%	83.9 abcd	67.5 ab	16.4 b	0.14 a

<sup>z</sup> Physical property data represent the mean (n=3). Analysis performed using the North Carolina State University Porometer method (Fonteno et al. 1995).

<sup>y</sup> Total porosity is equal to container capacity + air space.

<sup>x</sup> Air space is the volume of water drained from the sample ÷ volume of the sample.

<sup>w</sup> Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

<sup>v</sup> Bulk density after forced-air drying at 105°C for 48 h; 1g·cm<sup>-1</sup> = 0.5780 oz/inch<sup>3</sup>.

<sup>s</sup> Substrates were formulated with 60, or 40% sphagnum peat/ 20, or 40% pine bark and 20% perlite.

Table 1.2. Growth measurements of marigold (*Tagetes erecta*) grown in substrates composed of 40:40:20 peat: aged pine bark: perlite (v:v:v), with substrate drench applied paclobutrazol concentrations of 0, 1, 2, and 4 mg A.I. per container.<sup>z</sup>

Pine bark age (months)	Conc of A.I. <sup>y</sup> (mg)	Growth index (GI) <sup>x</sup> (cm)	Plant width <sup>w</sup> (cm)	Dry weight <sup>v</sup> (cm)
Peat:Perlite <sup>s</sup>	0	17.33	18.60	6.80
	1	17.11	17.91	5.96
	2	15.58	16.46	4.49
	4	14.69	15.04	4.47
0 <sup>r</sup>	0	15.34 c-f	16.25 a-e	4.64 fgh
	1	15.72 a-f	16.79 a-d	4.77 e-h
	2	14.67 efg	15.38 cde	3.88 ghi
	4	13.06 g	13.71 e	2.79 i
3	0	17.31 abc	18.50 a	6.14 b-f
	1	15.70 a-f	16.54 a-d	5.53 efg
	2	15.36 c-f	16.67 a-d	4.89 e-h
	4	14.08 fg	15.00 de	3.74 hi
6	0	17.70 a	18.75 a	7.51 ab
	1	17.42 ab	18.46 a	7.46 abc
	2	15.50 b-f	15.58 b-e	5.70 def
	4	14.97 d-g	15.71 b-e	5.42 e-h
9	0	16.17 a-e	17.08 a-d	7.23 a-d
	1	17.17 abc	18.17 ab	7.29 a-d
	2	16.36 a-e	16.88 a-d	6.37 a-e
	4	14.97 d-g	15.38 cde	5.78 c-f
12	0	17.19 abc	18.17 ab	7.90 a
	1	16.72 a-d	17.79 abc	7.23 a-d
	2	17.20 abc	17.83 abc	7.98 a
	4	16.14 a-e	17.13 a-d	6.41 a-e

<sup>z</sup>Tukey-Kramer grouping for least square means of growth index, plant width, and dry weight for pine bark age by PGR rate. Data subjected to PROCGLIMMIX (SAS 9.4, Cary, NC).

<sup>y</sup> Substrate drench applied paclobutrazol, mg of A.I. per container.

<sup>x</sup> GI = (Height from substrate to tallest point + diameter at widest point + second diameter 90° turn from first) / 3.  
Pine bark age\*PGR p = 0.0069

<sup>w</sup> Plant width = (diameter at widest point + second diameter 90° turn from first) ÷ 2  
Pine bark age\*PGR p = 0.0205

<sup>v</sup> Dry weight of all above ground plant parts after forced-air drying at 75°C for 72 h.  
Pine bark age p < 0.0001, PGR p < 0.0001, pine bark age\*PGR p = 0.0957

<sup>s</sup> Peat:Perlite control (80:20 peat: perlite by vol.)

<sup>r</sup> Mixes composed of 40:40:20 peat: bark: perlite by vol.

Table 1.3. Growth measurements of marigold (*Tagetes erecta*) grown in substrates composed of 40:20:20 peat: aged pine bark: perlite (v:v:v), with substrate drench applied paclobutrazol treatments of 0, 1, 2, and 4 mg A.I. per container.<sup>z</sup>

Pine bark age (months)	Conc of A.I. <sup>y</sup> (mg)	Growth index (GI) <sup>x</sup> (cm)	Plant width <sup>w</sup> (cm)	Dry weight <sup>v</sup> (cm)
Peat:Perlite <sup>s</sup>	0	17.33	18.60	6.80
	1	17.11	17.91	5.96
	2	15.58	16.46	4.49
	4	14.69	15.04	4.47
0 <sup>r</sup>	0	17.47 a	18.25 ab	6.61 c-f
	1	16.72 a-d	17.75 abc	5.70 d-h
	2	15.42 cde	16.63 b-e	4.06 hi
	4	13.92 e	14.33 f	3.19 i
3	0	18.03 a	19.13 a	6.87 b-e
	1	15.67 b-e	16.54 b-f	4.50 ghi
	2	15.56 cde	16.25 b-f	4.87 f-i
	4	14.89 de	15.33 def	4.26 hi
6	0	18.33 a	19.75 a	8.93 a
	1	17.25 abc	18.29 ab	7.25 a-e
	2	15.50 cde	16.42 b-f	6.20 c-g
	4	14.53 e	15.21 ef	5.61 d-h
9	0	17.31 abc	18.13 ab	7.87 abc
	1	17.50 abc	18.17 ab	7.27 a-e
	2	15.75 b-e	16.79 b-e	6.06 d-g
	4	14.58 e	15.04 ef	5.52 e-h
12	0	17.94 a	19.42 a	8.45 ab
	1	16.53 a-d	17.54 a-d	7.38 a-d
	2	15.08 de	15.79 c-f	5.49 e-h
	4	14.36 e	14.96 ef	4.83 f-i

<sup>z</sup> Tukey-Kramer grouping for least square means of growth index, plant width, and dry weight for pine bark age by PGR rate. Data subjected to PROCGLIMMIX (SAS 9.4, Cary, NC).

<sup>y</sup> Substrate drench applied paclobutrazol in mg A.I. per container.

<sup>x</sup> GI = (Height from substrate to tallest point + diameter at widest point + second diameter 90° turn from first) ÷ 3.

Pine bark p = 0.2368, PGR p < 0.0001, pine bark age\*PGR p=0.0833

<sup>w</sup> Plant width = (diameter at widest point + second diameter 90° turn from first) ÷ 2

Pine bark age p = 0.228, PGR p < 0.0001, pine bark age\*PGR 0.0737

<sup>v</sup> Dry weight of all above ground plant parts after forced-air drying at 75°C for 72 h.

Pine bark age\*PGR 0.0459

<sup>s</sup> Peat:Perlite control (80:20 peat: perlite by vol.)

<sup>r</sup> Mixes composed of 60:20:20 peat: bark: perlite by vol.



Figure 1.1. Marigold (*Tagetes erecta*) grown in substrates (from left to right) composed of 80:20 peat: perlite and 40:40:20 peat: aged pine bark: perlite (v:v:v), with bark ages of 0, 3, 6, 9, and 12 months, treated with substrate drench applied paclobutrazol concentration of 4 mg A.I. per container.

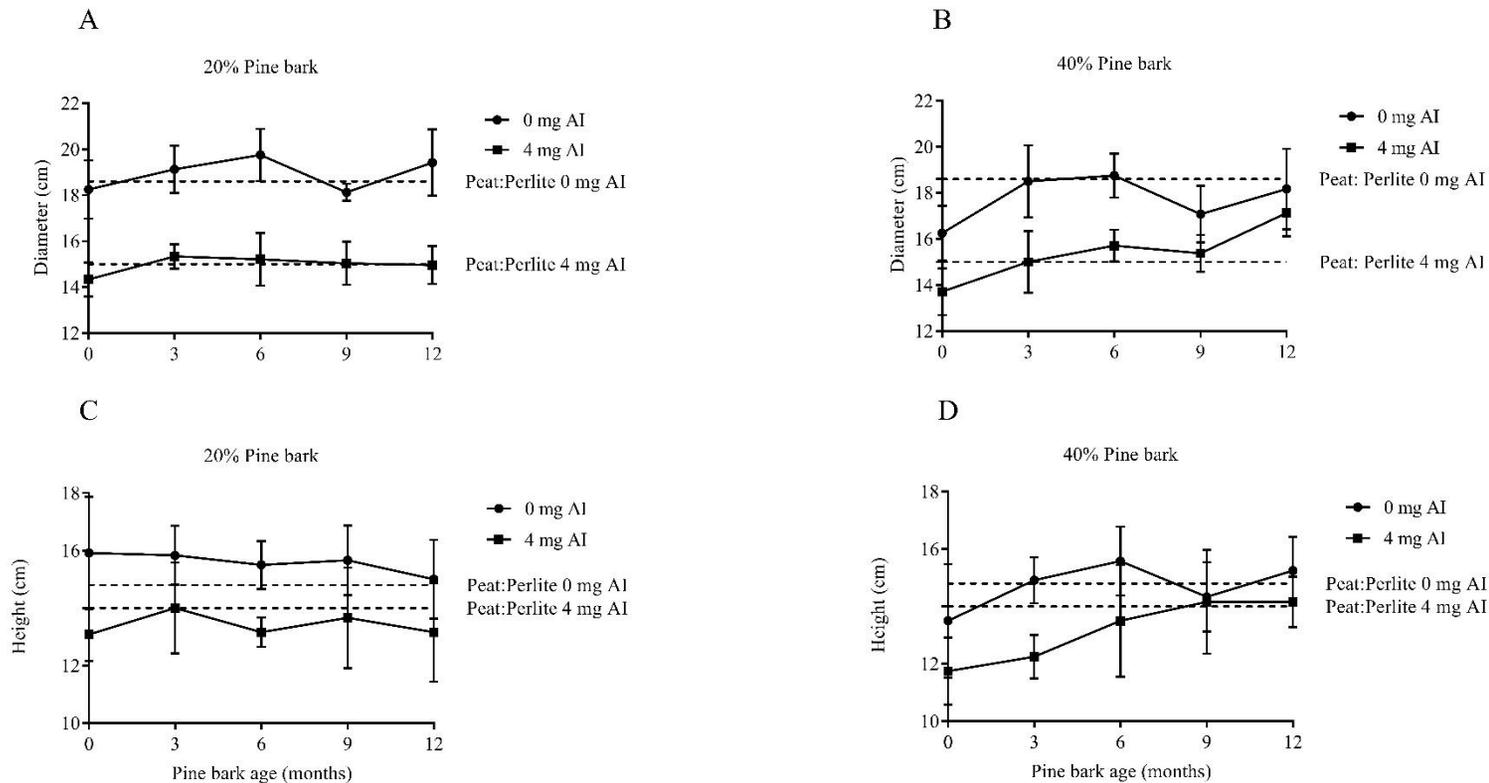


Figure 1.2. Diameter and height measurements of marigold (*Tagetes erecta*) grown in substrates composed of 60:20:20, and 40:40:20 peat: aged pine bark: perlite (v:v:v), as well as a peat: perlite control substrate, with drench applied paclobutrazol concentrations of 0, and 4 mg A.I. per container.<sup>z</sup>

Data were subjected to analysis of variance by general linear model procedures and regression analysis.

<sup>NS</sup>, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively; L = linear, Q = quadratic.

(A) 20% bark at 0 mg a.i.:  $y = 0.044x + 18.67$  L<sup>NS</sup>, Q<sup>NS</sup>; 20% bark at 4 mg a.i.:  $y = 0.032x + 14.78$  L<sup>NS</sup>, Q<sup>NS</sup> (B) 40% bark at 0 mg a.i.:  $y = -0.03373x^2 + 0.4853x + 16.6595$  L<sup>NS</sup>, Q<sup>NS</sup>; 40% bark at 4 mg a.i.:  $y = 0.24028x + 13.94167$  L<sup>\*\*\*</sup>, Q<sup>NS</sup>. (C) 20% bark at 0 mg a.i.: L<sup>NS</sup>, Q<sup>NS</sup>; 20% bark at 4 mg a.i.: L<sup>NS</sup>, Q<sup>NS</sup>. (D) 40% bark at 0 mg a.i.: L<sup>NS</sup>, Q<sup>NS</sup>, 40% bark at 4 mg a.i.:  $y = 11.82 + 0.225x$  L<sup>\*\*</sup>, Q<sup>NS</sup>.

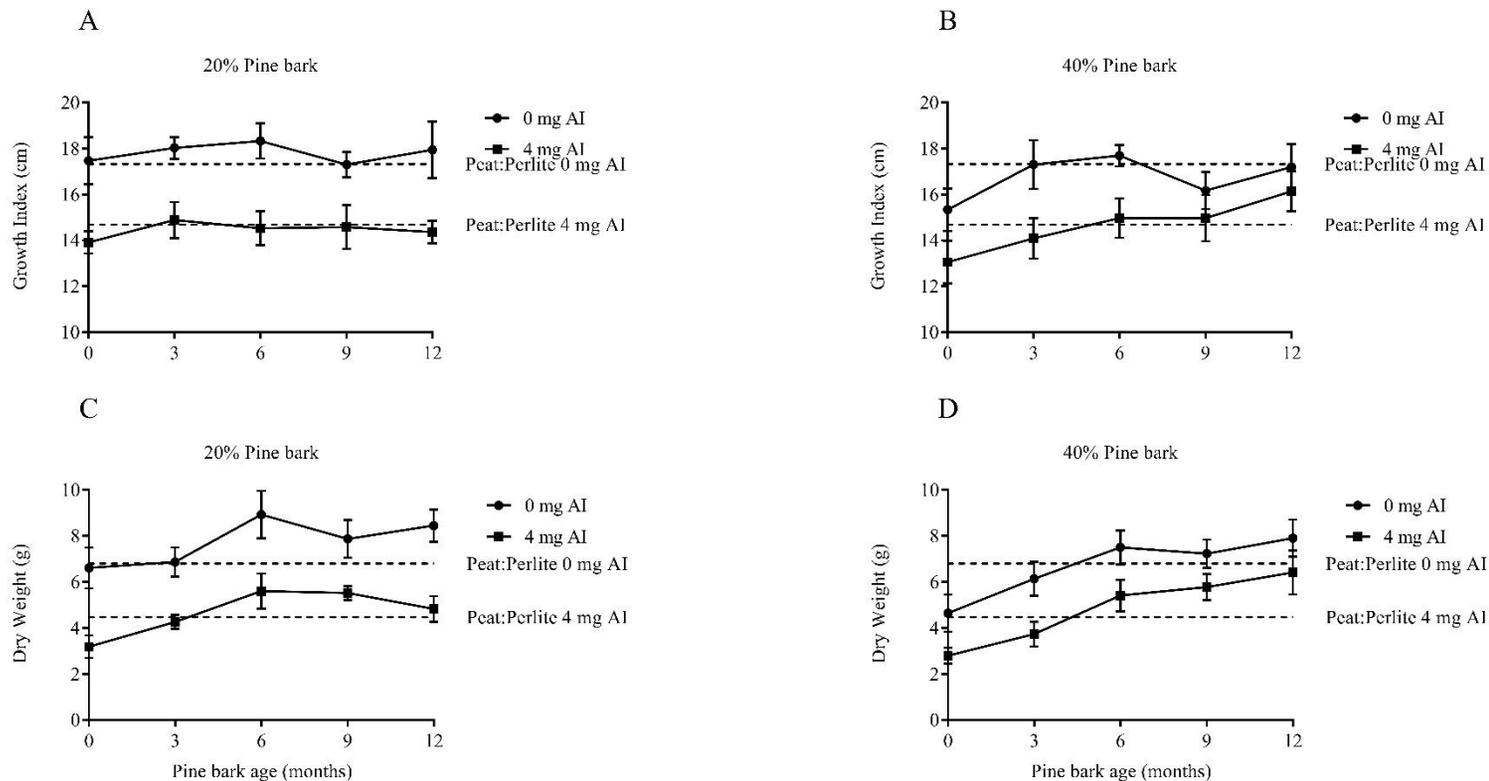


Figure 1.3. Growth index [(height + widest width + perpendicular width) ÷ 3] and dry weight measurements of marigold (*Tagetes erecta*) grown in substrates composed of 60:20:20, and 40:40:20 peat: aged pine bark: perlite (v:v:v), as well as a peat: perlite control substrate, with drench applied paclobutrazol concentrations of 0, and 4 mg A.I. per container.<sup>z</sup>

Data were subjected to analysis of variance by general linear model procedures and regression analysis.

<sup>NS</sup>, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively; L = linear, Q = quadratic.

(A) 20% bark at 0 mg a.i.:  $y = 0.007x + 17.77$  L<sup>NS</sup>, Q<sup>NS</sup>; 20% bark at 4 mg a.i.:  $y = -0.01565x^2 + 0.207x + 14.06$  L<sup>NS</sup>, Q<sup>NS</sup> (B) 40% bark at 0 mg a.i.:  $y = -0.0302x^2 + 0.4485x + 15.6786$  L<sup>NS</sup>, Q<sup>\*\*</sup>; 40% bark at 4 mg a.i.:  $y = 0.23519x + 13.2333$  L<sup>\*\*</sup>, Q<sup>NS</sup>. (C) 20% bark at 0 mg a.i.:  $y = 0.156x + 6.81$  L<sup>\*\*\*</sup>, Q<sup>NS</sup>; 20% bark at 4 mg a.i.:  $y = -0.0393x^2 + 0.6235x + 3.064$  L<sup>\*</sup>, Q<sup>\*\*\*</sup> (D) 40% bark at 0 mg a.i.:  $y = -0.02604x^2 + 0.56626x + 4.69186$  L<sup>\*\*\*</sup>, Q<sup>\*\*</sup>; 40% bark at 4 mg a.i.:  $y = 0.30939x + 2.97$  L<sup>\*\*\*</sup>, Q<sup>NS</sup>.

## **CHAPTER III**

### **Paclobutrazol Drench Activity not Affected by Sphagnum Peat-based Blends Containing**

#### **Pine Tree Substrate**

## Abstract

Previous research has reported horticultural substrates containing pine bark can reduce the efficacy of drench applied plant growth regulators (PGRs), and there is concern other organic materials may also negatively influence PGRs. Prior research evaluated the effects of wood materials on PGR efficacy but the influence of a fibrous wood material like pine tree substrate (PTS) has not yet been examined. Fibrous wood substrates can have higher surface area than other wood substrates and therefore may influence PGR efficacy, therefore two experiments were conducted to address this question. Paclobutrazol drench applications of 0, 1, 2, and 4 mg of active ingredient were applied to ‘Perfection Yellow’ marigold (*Tagetes erecta*) and ‘Pacino Gold’ sunflower (*Helianthus annuus*) grown in plastic containers filled with a peat and PTS blend in the ratios of 90:10, 80:20, 70:30, and 60:40, plus a peat: perlite blend of 80:20 which was included as a control. Height, diameter, growth index and dry weight were recorded at anthesis for marigold. Height, diameter, and growth index were also measured at anthesis for sunflower, but dry weights were recorded once all plants had reached anthesis. In the first study, no interaction was found between substrate type and PGR rate on any growth parameters of either species. In the second experiment the substrate and PGR interaction did not affect sunflower but, was found to affect height, diameter, and growth index of marigold. Although an interaction of substrate and PGR was found to affect marigold in experiment 2, visual differences were minimal. These data suggest growers utilizing 40% or less PTS in peat-based substrates will not need to adjust paclobutrazol drench applied concentrations from established rates in peat: perlite mixes.

## **Introduction**

The use of plant growth regulators (PGRs) to produce uniform, compact plants in greenhouse environments is common practice in the floriculture industry. The primary benefit of PGRs is to control excessive growth allowing tighter spacing and increased yield per growing area. Plant growth regulators which provide these benefits include ancymidol, chlormequat chloride, daminozide, flurprimidol, and paclobutrazol (Whipker, 2017a). Additional benefits of PGR use include higher chlorophyll content (greener leaves), reduced water stress, and the potential for increased disease suppression (Whipker, 2017a).

A variety of methods are utilized by the floriculture industry to apply PGRs, including liner soaks (Blanchard and Runkle, 2007), bulb dips (Krug et al., 2006), foliar sprays (Whipker and McCall, 2000), spike applications (Barrett et al., 1994), and substrate drenches (Currey et al., 2016; Dasoju et al., 1998b; Owen et al., 2016). One of the most popular application methods employed by growers are substrate drenches. The triazole chemical class does not readily travel through phloem, but when applied to roots and shoots, the active ingredient can enter the xylem stream and reach the growing tip (Barrett, 2001). Drenches also provide high precision of control with longer lasting effects (Bonaminion and Larson, 1978). An additional benefit is reduced risk of drift and buildup of active ingredient in re-circulated irrigation water (Million et al., 1999b; Whipker, 2017b).

A drawback of drench applications is the potential adsorption of active ingredient by organic substrate components. Aside from vermiculite, perlite, and sand, most of the substrate components used today are comprised of organic materials. For this reason, many researchers have examined PGR efficacy in a range of horticultural substrates. A study by Tschabold et al. (1975) reported drench applications of ancymidol to substrates containing pine bark were less

effective in controlling growth of *Chrysanthemum morifolium*. Reduced efficacy of PGRs in substrates containing pine bark has also been reported by other studies (Barrett, 1982; Bonaminio and Larson, 1978; Million et al., 1998a; Million et al., 1998b; Million et al., 1999a; Newman et al., 1993; Quarrels and Newman, 1994). Plant growth regulator efficacy has also been examined in substrates containing coconut coir, and parboiled rice hulls. Coconut coir was reported to have similar influence on paclobutrazol activity compared to peat (Barrett et al., 1998a). Dasoju et al. (1998a) found paclobutrazol to have similar activity in coir as in peat at 2 mg active ingredient, but increased activity (greater control of growth) when concentration was increased to 4 mg in coir. Parboiled rice hulls blended as a substrate component ratio of (v/v) 20% were reported to have no influence on PGR efficacy by Currey et al. (2010). Two studies examining PGR drench efficacy in substrates containing wood are Felipe et al. (2013) and Owen et al. (2016). Felipe et al. (2013) compared efficacy of drench applied paclobutrazol in mixes composed of 50:50 peat: distilled cedar chips, 50:50 peat: whole tree, 50:50 peat: pine bark, and 75:25 peat: perlite. A significant interaction between PGR rate and substrate was reported for shoot dry weight of impatiens and petunias, but no interaction was reported for plant width. Owen et al. (2016) formulated peat-based substrates using either perlite or pine wood chips (PWC) at (v/v) ratios between 10 and 30%. It was reported that PWC had no effect on paclobutrazol efficacy when applied as a drench.

Wood materials, specifically pine tree substrates, are growing in popularity (Jackson and Wright, 2007). Wood substrate components were first utilized in Europe in the 1980's, and later adopted in the United States following the work of Laiche and Nash (1986) comparing pine bark with and without wood, and pine tree chips. Work by Wright and Browder (2005), Jackson and Wright (2007), and Jackson et al. (2010) further demonstrated the feasibility of growing

horticultural crops in pine tree substrates (PTS). Even though extensive research has been conducted over the last twenty years concerning pine tree substrates, little is known about how these substrate components will influence PGR efficacy.

Pine wood chips were reported to have no influence on paclobutrazol efficacy (Owen et al. 2016), and distilled cedar and wholetree had mixed results (Felipe et al. 2013) but there is a possibility other pine wood materials may influence paclobutrazol differently. Fibrous wood substrate components such as PTS have had increased commercialization and marketing compared to PWC. These fibrous wood materials may have a higher surface area than PWC so it is uncertain if they interact with PGRs differently. Due to the growing popularity of wood fiber, wide use of PGR substrate drenches, and lack of scientific literature explaining how wood fiber may affect these substrate drenches, it was the objective of this study to examine the interaction of the two. This objective was addressed by measuring efficacy of paclobutrazol in peat-based substrate blends containing different ratios of PTS amendment.

## **Materials and Methods**

Freshly shredded (processed same day) (1300 V-Mill; Morbark LLC, Winn, MI) Loblolly pine (*Pinus taeda* L.) wood was acquired from Parker Bark (3295 US-117, Rose Hill, NC 28458) on 23 Feb. 2018 (Fig. 2.1). Bark was not removed before shredding. Shredded wood was stored in a covered polypropylene bulk bag outdoors until 16 Mar. before being hammermilled (Model 35; Meadows Mills, North Wilkesboro, NC) at 3000 rpm through a 6.35mm screen to create PTS (Fig. 2.1).

### *Experiment 1.*

Seeds of 'Perfection Yellow' African marigold (*Tagetes erecta* L.), and 'Pacino Gold' sunflower (*Helianthus annuus* L.) were sown on 21 Feb. 2018, into a 288 cell plug tray, and 1203 cell packs [8.0 x 4.0 x 5.5 cm (length x width x height); ITML Horticultural Products, Middlefield, OH] respectively, using Sunshine® mix #1 (Sun Gro Horticulture, Agawam, MA).

On 17 Mar. Canadian sphagnum peat (Sun Gro Horticulture, Agawam, MA), was fluffed and wetted by hand before being amended with the hammermilled PTS at 90:10, 80:20, 70:30, and 60:40 (v:v) respectively. A substrate of Canadian sphagnum peat and perlite (Carolina Perlite Co., Gold Hill, NC) at ratio 80:20 (v:v), was also used in this study as a control. All substrates were hand wetted to a moisture content of 50% before 200 mesh dolomitic limestone was added at a rate of 2.37 kg/m<sup>3</sup> (Mississippi Lime Company, Vicksburg, MS). Blends were then bagged and allowed to equilibrate overnight. Substrate pH values were determined using the 2:1 (2 deionized water and 1 substrate) method using a pH/EC meter (HI 9813-6; Hanna Instruments, Woonsocket, RI). On 18 Mar. an additional 0.89 kg/m<sup>3</sup> 200 mesh dolomitic limestone was added to all blends to further adjust pH. After the final pH adjustment substrate blends had pH values between 5.1 and 5.6. Physical properties including total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD) of all substrate blends were determined on three replicate samples of each substrate treatment using the North Carolina State University (NCSU) Porometer method (Fonteno et al., 1995).

Marigold and sunflower plugs were transplanted into 12.7cm (855mL) and 15.2cm (1330mL) azalea containers, respectively on 19 Mar. Plants were hand watered with tap water and automatic drip irrigation was installed. Plants were fertilized at each watering with 150 mg·L<sup>-1</sup> nitrogen (N) from 16.8N-5P-17K-3Ca-1Mg water soluble fertilizer (Greencare

Fertilizers Inc., Kankakee, IL) containing 4% ammoniacal (NH<sub>4</sub>)-N and 12.8% nitrate (NO<sub>3</sub>)-N, 5% phosphate (P<sub>2</sub>O<sub>5</sub>), 17% potassium (K<sub>2</sub>O), 3% calcium, and 1% magnesium.

On 6 April, 18 days after planting, 3 fl oz of solution containing either 0, 1, 2, or 4 mg of active ingredient (a.i.) per container paclobutrazol (Piccolo 10XC; Fine Americas Inc., Walnut Creek, CA) was applied to each container as a substrate drench. Sunflowers received 4 fl oz of solution with the same dosages of either 0, 1, 2, or 4 mg a.i. To prevent potential loss of active ingredient, plastic trays were placed under each container prior to application. The experiment was a completely randomized design with 6 replications of 5 substrates x 4 PGR concentration combinations.

On 16 May, 6 weeks after PGR application, canopy height and two canopy width measurements were taken for marigold. Plants were then harvested at the substrate level and allowed to dry in an oven at 75°C for 5 days, before dry weights were recorded.

Beginning 30 April, and continuing until 15 May, sunflower height and two canopy width measurements were taken on the date of anthesis for each respective plant. On 15 May, after all plants had reached anthesis, plants were harvested at the substrate level and dried in an oven at 75°C for 5 days, before dry weights were recorded.

Data was subjected to means separation using the GLIMMIX procedure (version 9.4; SAS Institute, Cary, NC). PROC REG and PROC GLM were then used to determine the best fit linear or quadratic model for PGR rates.

### *Experiment 2.*

Except where indicated, procedures used in Expt. 2 were the same as Expt. 1. Seeds of ‘Perfection Yellow’ marigold and ‘Pacino Gold’ sunflower were sown on 1 June into a 288 cell plug tray, and 1203 cell packs, respectively.

The same shredded pine wood acquired on 23 Feb. was used as the PTS parent material for experiment two. Although this material was stored outdoors and aged compared to experiment one, it was decided these variables would be minimal compared to acquiring new shredded pine wood. Fresh shredded pine wood would come from different trees of unknown ages, locations, and microclimates, all of which could have affected the end PTS product. The shredded wood was hammermilled at 3000 rpm through a 6.35mm screen on 20 June. All substrate blends were hand wetted to a moisture content of 50% before 200-mesh lime was added at a rate of 3.26kg/m<sup>3</sup>, and then allowed to equilibrate in bags overnight.

Marigold and sunflowers were transplanted on 22 June. On July 5, 13 days after transplanting, substrate drench PGR applications were performed. Beginning 23 July, and continuing until 29 July, sunflower measurements were taken on the date of anthesis for each respective plant. On 29 July, after all plants had reached anthesis, plants were harvested. Marigolds were measured and harvested on 6 Aug. 32 days after PGR application.

## **Results and Discussion**

### *Experiment 1.*

There were few differences in physical properties of substrates tested (Table 2.1). Total porosity was increased by the addition of PTS compared to the peat: perlite control. The CC, AS,

and BD of the PTS mixes however, were the same as the control excluding the 40% PTS, which had lower CC, higher AS, and higher BD. The similarities in physical properties indicate the lower ratio PTS mixes will act the same as the control.

No interaction was found between substrate treatment and PGR rate for any growth parameter of either species. For this reason, effects of substrate and PGR are reported independently. Plant growth regulator rate had the greatest effect on the growth parameters measured compared to substrate treatment. A linear relationship was found between PGR rate and height and PGR rate and GI for marigold (Fig. 2.2). All other growth parameter for both marigold and sunflower had quadratic relationships with PGR rate, with larger rates resulting in small growth measurements (Fig. 2.2,2.3).

Substrate treatment affected plant height, width, GI, and dry weight of marigold (Table 2.2). The general trend observed was an increase in respective growth parameter with the increase of PTS. Although not significant, the average height, width, GI and dry weight of marigold grown in 30% PTS were greater than all other substrate treatments. Substrate treatment effects were less pronounced in sunflower, with differences only occurring in height and GI (Table 2.2). The general trend of 30% PTS having larger plant growth parameters was also observed in sunflower but the maximum difference between greatest height and GI were 3.51cm and 2.21cm, respectively.

### *Experiment 2.*

An interaction between substrate and PGR was found to affect height ( $p = 0.0004$ ), GI ( $p = 0.0056$ ) and dry weight ( $p = 0.0283$ ) of marigold. Although this result contrasts with the results from experiment 1, it should be noted the only differences in means separation between substrate

treatments for any given PGR rate were found at 4 mg and 10% PTS for GI and at 4 mg and 30% PTS for height. The 4 mg, 10% PTS growth index was smaller than all other PTS treatments but the same as the peat: perlite control. The 4 mg, 30% PTS for height was larger than all other 4 mg substrate treatments, with the other PTS treatments being the same as the peat: perlite control. Visual differences between treatments were minimal (Fig. 2.4). For dry weight, all PTS treatments performed the same as the control, there were no differences between substrate treatments for any given PGR treatment in the means separation. Even though there was a significant interaction between substrate and PGR rate for marigold growth parameters, no clear pattern was recognizable. Further complicating a clear explanation for differences between experiment 1 and 2 is the lack of interaction between substrate and PGR for sunflower height, width, GI and dry weight. For this reason, it seems the more practical data for real world application are the main effects of PGR rate and substrate treatment.

Although substrate treatments did affect growth parameters of marigold and sunflower, PGR rate had the most visible effect. Quadratic relationships were found between PGR rate and all growth measurements for both species, except marigold height which had a linear trend (Fig. 2.5, 2.6). As expected, 0 mg of active ingredient produced the largest growth measurements and 4 mg produced the smallest for both species.

Substrate affected height, width, and growth index of marigold (Table 2.2). Marigold height followed the same trend as experiment 1 with 30% PTS having the plants with the greatest height. The greatest width and GI however, were found in the 40% PTS treatment. Substrate treatment affected width, and dry weight of sunflower (Table 2.2). The 30% PTS treatment had plants with the greatest width, but the peat: perlite control had plants with the greatest dry weight.

## Conclusions

In experiment 1 there was no interaction of substrate and PGR for any growth measurement of either species. Although experiment 2 did have a significant interaction for height, diameter, and growth index of marigold there were very few visual differences that would indicate this would have significance to a commercial grower. The decrease in time to anthesis from 68 to 37 days for sunflowers and 69 to 45 days for marigolds indicates that another variable, such as light levels, may have influenced growth. Experiment 1 was conducted in March, April, and May while experiment 2 was conducted in June and July. The increased light levels during the summer months (Korczynski et al., 2002) may have exaggerated differences in growth parameters measured.

Overall, these data suggest growers do not need to adjust paclobutrazol substrate drench concentrations to account for the use of 10, 20, 30, or 40% (by vol.) PTS in peat-based substrates. Interactions observed in experiment 2 were visually insignificant and likely influenced by other factors not measured in this study. Altering current substrate drench applied PGR rates to account for the presence of fibrous wood materials is not necessary.

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Table 2.1. Physical properties of 4 peat-based mixes containing either 10, 20, 30, or 40% fibrous pine tree substrate by vol. and a control mix composed of 80% peat 20% perlite by vol.<sup>z</sup>.

Substrate Blends	Total porosity <sup>y</sup> (% vol)	Container capacity <sup>x</sup> (% vol)	Air space <sup>w</sup> (% vol)	Bulk density <sup>v</sup> (g·cm <sup>-1</sup> )
80:20 Peat: Perlite	85.1c	69.2a	15.9b	0.10b
90:10 Peat: PTS	89.6a	72.3a	17.3b	0.10b
80:20 Peat: PTS	88.7ab	70.3a	18.3b	0.10b
70:30 Peat: PTS	88.2b	68.2a	20.0b	0.11b
60:40 Peat: PTS	88.2b	63.5b	24.7a	0.12a

<sup>z</sup>Physical property data represent the mean (n=3). Analysis performed using the North Carolina State University Porometer method (Fonteno et al. 1995).

<sup>y</sup>Total porosity is equal to container capacity + air space.

<sup>x</sup>Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

<sup>w</sup>Air space is the volume of water drained from the sample ÷ volume of the sample.

<sup>v</sup>Bulk density after forced-air drying at 105°C for 48 h; 1g·cm<sup>-1</sup> = 0.5780 oz/inch<sup>3</sup>.

Table 2.2. Plant height, width, growth index and dry weight for marigold (*Tagetes erecta*) and sunflower (*Helianthus annuus*) grown in 90:10, 80:20, 70:30, and 60:40 peat: pine tree substrate (PTS) (v:v), as well as 80:20 peat: perlite, six weeks after a substrate drench applied paclobutrazol treatment at concentration 0, 1, 2, or 4 mg per container.

Substrate Blends	Marigold exp. 1				Marigold exp. 2			
	Height <sup>z</sup> (cm)	Width <sup>y</sup> (cm)	Growth index <sup>x</sup> (cm)	Dry weight <sup>w</sup> (g)	Height	Width	Growth index	Dry weight
80:20 Peat: Perlite <sup>y</sup>	24.65c	27.58b	26.61c	14.04b	40.61c	32.19b	35.00b	10.23NS
90:10 Peat: PTS <sup>t</sup>	26.59b	30.87a	29.44b	17.12a	40.43c	32.03b	34.83b	10.17
80:20 Peat: PTS	26.90ab	30.80a	29.50b	17.67a	41.10bc	33.33ab	35.92ab	10.65
70:30 Peat: PTS	28.44a	32.91a	31.42a	18.41a	44.18a	33.20ab	36.86a	10.72
60:40 Peat: PTS	27.51ab	32.02a	30.52ab	17.81a	43.23ab	34.62a	37.49a	11.00

Substrate Blends	Sunflower exp. 1				Sunflower exp. 2			
	Height	Width	Growth index	Dry Weight	Height	Width	Growth index	Dry weight
80:20 Peat: Perlite	41.55b	36.89NS	38.44b	22.74NS	39.62NS	38.01b	38.55NS	10.78a
90:10 Peat: PTS	41.00b	38.02	39.01ab	23.30	40.40	39.42ab	39.75	10.55ab
80:20 Peat: PTS	42.18ab	37.72	39.21ab	23.58	40.86	38.99ab	39.61	10.17ab
70:30 Peat: PTS	44.51a	38.72	40.65a	24.08	39.57	39.85a	9.75	9.89ab
60:40 Peat: PTS	42.75ab	38.25	39.75ab	22.47	39.55	38.99ab	39.18	9.85b

<sup>z</sup> Height is measured from substrate level to tallest point on plant

<sup>y</sup> Diameter is the average of the width at widest point, and the width when turned 90° from the widest point.

<sup>x</sup> Growth index is (height + diameter 1 + diameter 2) ÷ 3.

<sup>w</sup> Dry weight is the mass of all above-ground plant material after forced-air drying at 75°C for 72 h.

<sup>y</sup> Control substrate was formulated with 80% peat and 20% perlite by vol.

<sup>t</sup> Substrates were formulated with peat with 10, 20, 30, or 40% fibrous pine tree substrate by vol.

NS Not significant ( $\alpha > 0.05$ )



Figure 2.1. Shredded pine wood (left), and pine tree substrate (right).

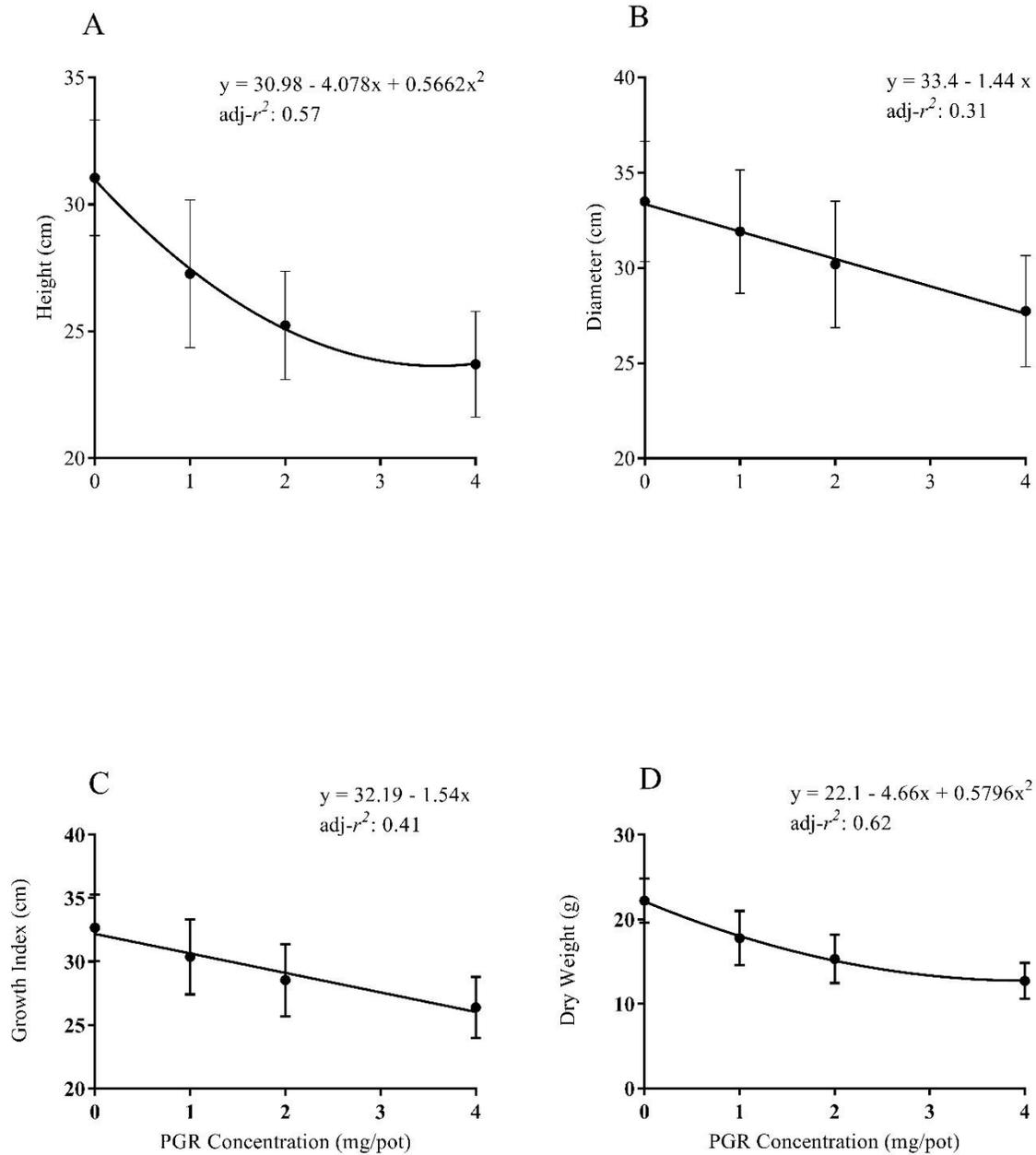


Figure 2.2. Height, diameter, growth index, and dry weight of marigold (*Tagetes erecta*) grown in 90:10, 80:20, 70:30, and 60:40 peat:pine tree substrate (PTS) (v:v), as well as 80:20 peat:perlite, six weeks after substrate drench applied paclobutrazol at concentration 0, 1, 2, or 4 mg per container. Data were subjected to analysis of variance by general linear model procedures and regression analysis.

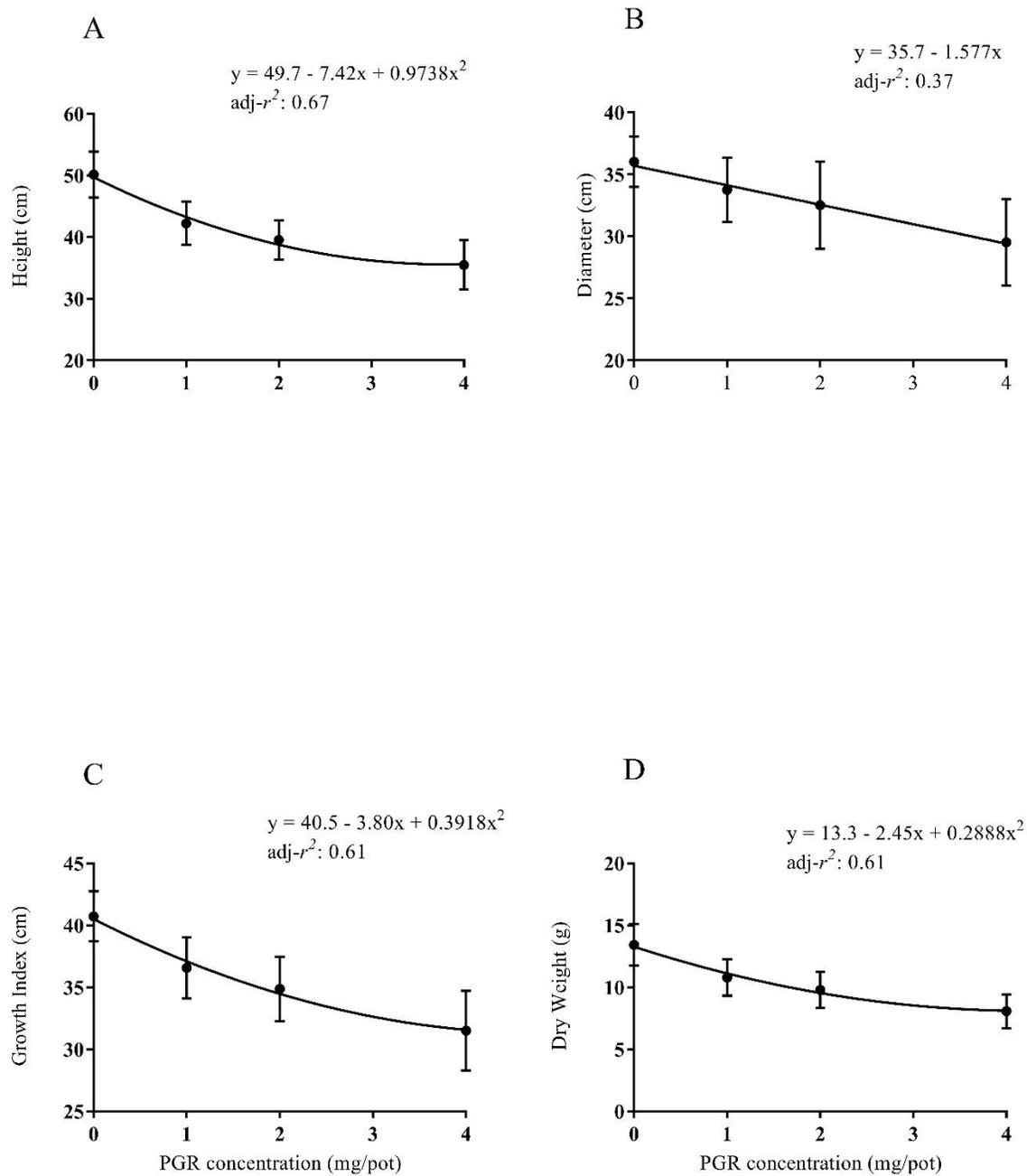


Figure 2.3. Height, diameter, growth index, and dry weight of marigold (*Tagetes erecta*) grown in 90:10, 80:20, 70:30, and 60:40 peat:pine tree substrate (PTS) (v:v), as well as 80:20 peat:perlite, 32 days after substrate drench applied paclobutrazol at concentration 0, 1, 2, or 4 mg per container. Data were subjected to analysis of variance by general linear model procedures and regression analysis.



Figure 2.4. From left to right, marigold (*Tagetes erecta*) grown in 80:20 peat: perlite, and 90:10, 80:20, 70:30, 60:40 peat: pine tree substrate blends (v:v), treated with substrate drench applied paclobutrazol rates (from top to bottom) of 0, 1, 2, and 4 mg active ingredient per container.

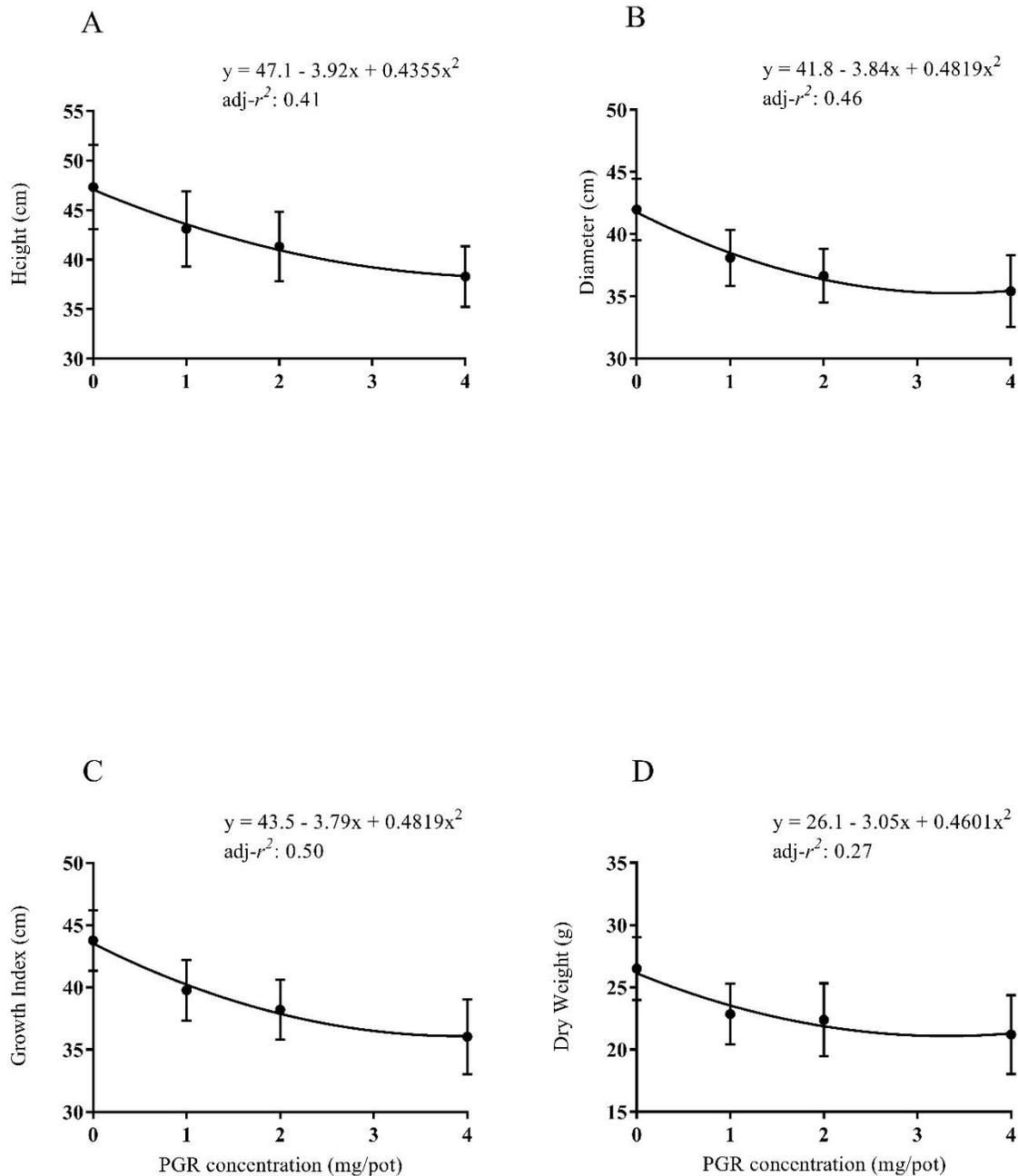


Figure 2.5. Height, diameter, and growth index of sunflower (*Helianthus annuus*) grown in 90:10, 80:20, 70:30, and 60:40 peat:pine tree substrate (PTS) (v:v), as well as 80:20 peat: perlite, at date of anthesis following a substrate drench applied paclobutrazol treatment at concentration 0, 1, 2, or 4 mg per container. Sunflowers were harvested, and dry weights recorded six weeks after paclobutrazol treatment. Data were subjected to analysis of variance by general linear model procedures and regression analysis.

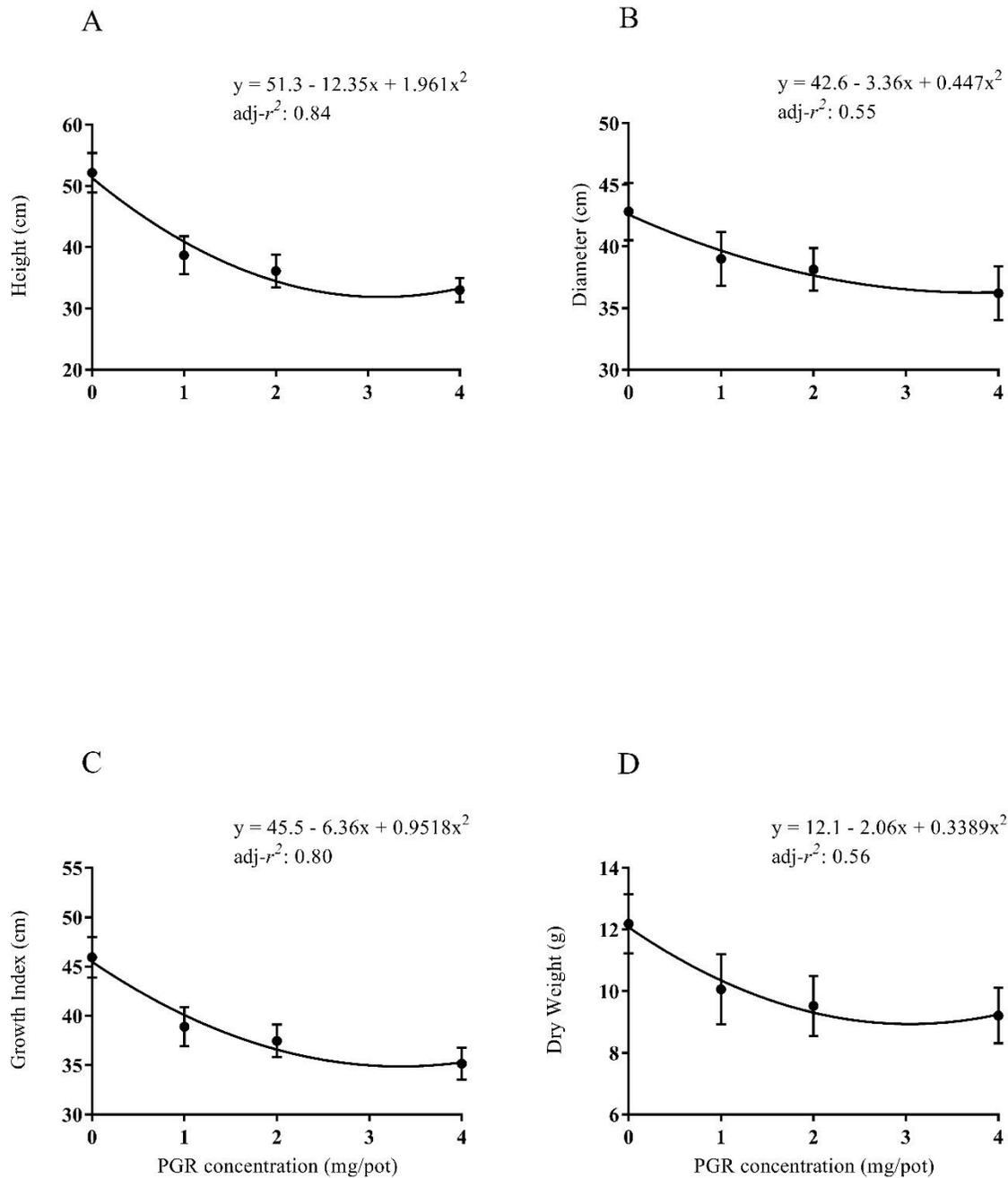


Figure 2.6. Height, diameter, and growth index of sunflower (*Helianthus annuus*) grown in 90:10, 80:20, 70:30, and 60:40 peat:pine tree substrate (PTS) (v:v), as well as 80:20 peat: perlite, at date of anthesis following a substrate drench applied paclobutrazol treatment at concentration 0, 1, 2, or 4 mg per container. Sunflowers were harvested, and dry weights recorded 24 days after paclobutrazol treatment. Data were subjected to analysis of variance by general linear model procedures and regression analysis.

## **CHAPTER IV**

### **Industrial Hemp Vegetative Growth Affected by Substrate Composition**

## **Abstract**

Little scientific literature exists on cultivation techniques for cannabis (*Cannabis sativa* L.) in container production environments; and there is almost none concerning horticultural substrate requirements. Substrates are one of the most important factors in maximizing crop production in controlled environments and the efficient use of inputs such as water and fertilizer. Due to the growing economic importance of hemp in the United States, and the need for a more thorough understanding of its cultivation in container culture, two studies were conducted to evaluate vegetative growth in a variety of horticultural substrates. Four cultivars, ‘Cherrywine’, ‘Baax’, ‘T1’, and ‘Sweetened’ were grown in peat-based and coir-based mixes containing either 0, 15, 30, or 45% perlite by volume. Six weeks after transplant, height, diameter, growth index and dry weight were measured. Additionally, to evaluate a range of commercially available mixes, ‘Cherrywine’, and ‘Baax’ were grown in 20 mixes marketed to the cannabis industry. These plants were harvested and dry weights measured at four weeks after transplant. Aside from coir-based mixes with higher levels of perlite, growth measurements were similar in all substrates tested. Physical properties of all 28 mixes were determined using the NCSU Porometer method, and all were found to be within or close to recommended ranges for most horticultural crops. These experiments suggest cannabis can be grown successfully in a range of horticultural substrates.

## Introduction

*Cannabis sativa* L. is one of the earliest examples of cultivated plants, with domestication possibly occurring 8,500 years ago (Small and Cronquist, 1976; Small and Marcus, 2002). The economic importance and domestication efforts by man have focused on the seed, fiber, and flowers. Fiber has been utilized for cordage and cloth, seeds have served as a source of oil, as well as food, and flowers have been selected for resin with intoxicant or medicinal properties (Schultes, 1970; Clarke and Merlin, 2017). According to Small and Cronquist (1976) cannabis cultivated for medicinal resin falls under two taxon, *C. sativa* subs. *sativa* and *C. sativa* subsp. *indica* (Lam.) (Hillig, 2005; McPartland and Guy, 2017; McPartland, 2018). These taxa are known commonly as hemp and marijuana, respectively. Subspecies *sativa* is differentiated by containing < 0.3% tetrahydrocannabinol (THC) by dry weight, and includes cultivars cultivated for cannabidiol (CBD), fiber and seed. Subspecies *indica* contains > 0.3% THC and is cultivated for its intoxicant properties.

Today, the seed and fiber cultivars of hemp are grown solely as field crops. Hemp cultivars cultivated for flower with high levels of cannabinoids, primarily CBD, are also grown in the field, but several factors lend CBD hemp to be treated as a horticultural crop, instead of a field crop (Febles, 2018). Difficulty of mechanizing harvest and challenges of producing consistent, uniform, high quality flowers contribute to CBD hemp being grown indoors as a horticultural crop. The photoperiodic nature of cannabis also enables several cropping cycles to be grown in a year using controlled environments.

In 2017, CBD hemp accounted for 23% of all U.S. hemp-based product sales; \$190 million, of an \$820 million industry (Hemp Bus. J., 2018). It is estimated the U.S. hemp industry will reach a market value of \$1 billion dollars in 2018 (Hemp Bus. J., 2018). A large portion of

this growth can be attributed to increased production of hemp-derived CBD, food, personal care and industrial products (Hemp Bus. J., 2018). As of 26 June 2018, North Carolina's contribution to this industry includes 348 licensed hemp growers with 4,548 acres (1840.51 hectares) licensed and 1,630,485 sq. ft. (151,477 m<sup>2</sup>) greenhouse space licensed for production (NC Ind. Hemp Comm., 2018a). There are also at least 121 licensed processors as of Aug. 2018 (NC Ind. Hemp Comm., 2018b). In 2017, North Carolina was listed as number 8 out of the top 10 hemp producing states in the U.S. (Nichols, 2018).

In North Carolina's emerging hemp industry, most greenhouse production of CBD hemp is focused on the vegetative stage. Stock plants are held under long days to prevent flowering, and cuttings are periodically harvested and rooted to be transplanted in the field. Vegetative propagation of CBD hemp is preferred over sowing seed to reduce potential variation in cannabinoid content and ensure that only female plants are grown. If a single plant in a grower's crop tests over 0.3% THC the entire crop must be destroyed (NC Gen. Assembly, 2015). In 2017, 10 of the 121 growers in North Carolina had their crops destroyed because they exceeded this threshold (Hart, 2017). With environmental impacts on cannabinoid content not yet fully understood, and the risk of financial loss so high, a standardization of cultivation practices such as substrates used, or fertility programs may also limit variables that are currently unknown.

Substrates are one of the most important factors in maximizing crop production in controlled environments. To determine the most effective substrate for a specific growing environment, it is necessary to understand the substrate's physical properties. Substrates possessing the ideal total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD) for a given crop can provide an even distribution of air, water and nutrients to the root environment. Irrigation events and nutrient inputs can be minimized, avoiding excessive runoff,

and allowing for more profitable container production (Fields et al., 2014a; Fields et al., 2014b; Fonteno, 1993; Fonteno et al., 2013).

Little scientific literature exists on cultivation techniques for cannabis in container production environments; and there is almost none concerning substrate requirements. To our knowledge, Caplan et al. (2017a, b) in their work on optimal rates of organic fertilizer for cannabis growth, are the only studies that have reported physical properties of substrates used in cannabis cultivation. The data reported in the two studies however, was limited to four commercially available coir-based substrate blends. Other research regarding cultivation of cannabis provide only vague description of substrates used, peat/perlite mixture (Potter and Duncombe, 2012), 4:1 mix of composted bark and coarse washed river sand (Lisson et al., 2000), 3 soil-leaf: 1 mold-Kureha: 1 compost (Yoshimatsu et al., 2004), or no description of substrates used, soil-filled pots (Coffman and Gentner, 1979), grown in pots (Potter, 2014).

Due to the importance of substrates in container production and the lack of scientific literature describing substrate effect on cannabis growth, the objective of this research was to evaluate substrates with varying physical properties and their effect on cannabis vegetative growth. Two of the most common substrate components in mixes marketed to the cannabis industry are peat and coir (Caplan et al., 2017a). For this reason, we chose to focus on peat and coir, alone and with increasing ratios of perlite. An additional objective was to quantify the physical properties of a range of commercially available substrates marketed to the cannabis industry and evaluate vegetative growth of cannabis in these mixes.

## Materials and Methods

Hemp cuttings from cultivars ‘Boax’ and ‘T-1’ were collected from Triangle Hemp (Durham, NC) and stuck on 12 June. Cuttings of ‘Cherrywine’, and ‘Sweetened’ were collected from Broadway Hemp (Sanford, NC) on 13 June. All cuttings were three nodes in length and had at least 3 fully expanded, uncut leaves. Cuttings were stuck in 72 cell plug flats [3.6 x 6 cm (diameter x height)] containing Sunshine® mix #1 (Sun Gro Horticulture, Agawam, MA), with one node below the substrate surface, and placed under mist. The mist bench was covered with opaque white plastic. Four hours of night interruption, between 22:00 and 2:00 was initiated on 22 June (4, 40W incandescent bulbs per 1.52 x 6.1m bench) following the summer solstice on 21 June to prevent flowering as daylength decreased. Cuttings were propagated and grown in a glass greenhouse at 35°N latitude in Raleigh, NC, USA.

### *Experiment 1.*

On 10 July, four peat-based (Lambert Peat Moss, Rivière-Ouelle, Québec) and four coir-based (RainSoil, Las Vegas, NV) substrate blends were mixed using a soil mixer (Model 12101; Bouldin & Lawson, McMinnville, TN). Peat was fluffed and wetted by hand to a moisture content of 50% before being added to the soil mixer. Coir bricks were submerged in water until they could be fluffed by hand, and then added to the soil mixer. Both peat and coir-based substrates had perlite (Carolina Perlite Co., Gold Hill, NC) added at ratios of 0, 15, 30, and 45% by volume. All mixes were amended with wetting agent (Aquatrols, Paulsboro, NJ) at a rate of 0.607kg/m<sup>3</sup>. Peat mixes containing 0, 15, 30, and 45% perlite had rates of 7.06, 6.18, 6.18, and 5.357kg/m<sup>3</sup> of dolomitic limestone (Rockydale Quarries Corp., Roanoke, VA) added, respectively. Liming rates were similar to rates used in other published research concerning

substrates (Henry et al., 2018) and decreased as peat level decreased. The physical properties (TP, CC, AS, and BD) of all substrate blends were determined using the North Carolina State University (NCSU) Porometer method established by Fonteno et al. (1995).

Rooted cuttings were transplanted 12 July into a 5.68L plastic blow molded nursery pots (C600; Nursery Supplies Inc., Kissimmee, FL). Plants were hand watered with clear water and drip irrigation was installed. Drip irrigation was applied as needed and plants were fertilized at each watering with 150 mg·L<sup>-1</sup> nitrogen (N) from 13N -0.86 Phosphorous (P) -10.8 Potassium (K) water soluble fertilizer (Excel® 13-2-13, The Scotts Co., Marysville, OH). Night interruption continued at the same rate as was used for cuttings, 4 hours (between 22:00 and 02:00) of incandescent 40W bulbs, spaced four per 1.52 x 6.1m bench. Plants were grown in a poly greenhouse at 35°N latitude in Raleigh, NC, USA, and arranged in a randomized block design grouped by perlite percentages. Blocks were arranged by perlite percentages to allow treatments with similar irrigation requirements to be grouped accordingly. The experiment consisted of 6 replications x 4 cultivars x 8 substrate treatments.

PourThrus were conducted 30 July and 17 Aug on 3 replications of each substrate treatment to monitor pH and electrical conductivity. Plants were measured on 22 Aug, 6 weeks after transplant. Measurements included height (from substrate surface to the tallest point on the plant along with two canopy width measurements (width at widest point, then the container was rotated 90° and the second width measurement was taken). The average of these three measurements were used to determine the growth index (GI) of plants. Plants were then harvested at the substrate level and dried in an oven at 75°C for seven days before dry weights were recorded.

### *Experiment 2.*

Except where indicated, procedures used in Expt. 2 were the same as Expt. 1. Rooted cuttings of *Cannabis sativa* L. ‘Cherrywine’ and ‘Baox’ were transplanted on 12 July into 7.57 L nursery containers filled with 20 commercially available substrate blends from five horticultural substrate companies. On 9 Aug., 4 weeks after transplant, 3 replicates were harvested at substrate level and oven dried before recording dry weights. An additional 3 replicates were harvested on 22 Aug. but data was compromised due to a lab oven malfunction.

## **Results and Discussion**

### *Experiment 1.*

Total porosity, CC, AS, and BD values (Table 3.1) were similar for all treatments. There was however a slight decline in total porosity as perlite percentage increased. The peat and coir used for these experiments were already highly porous with TP values of 88.78 and 95.55% respectively. Peat can have highly variable physical properties and has been reported to have TP values ranging from 72 to 84% by Hanan (2017) and 94.2% by Abad et al. (2005). Abad et al. (2005) reported on physical properties of coir samples from six different countries and found a TP range of 94.1 to 98.3%. Adding small particles, in this case perlite, to a coarse medium with high porosity will reduce the total volume of the material. Fine particles fill large pore spaces reducing TP (Hanan, 2017). To further increase the TP of the substrate blends, the perlite used would require a higher TP than the peat or coir alone.

Although there are no recommended ranges for substrate physical properties to maximize Cannabis growth, or universally accepted standards in general, the physical properties observed were similar to the suggested ranges for many container-grown horticultural crops in commercial

environments (Bilderback et al., 2005; Yeager et al., 1997). Container capacity was higher (70% to 74%) than the recommended range of 45 to 65% in some treatments but did not coincide with the smaller growth parameters observed.

Two interactions were found to affect GI of the cannabis cultivars grown. Type of substrate (peat or coir) by percentage of perlite (Table 3.2), and type of substrate by cultivar (Table 3.3). Three out of the four cultivars (Cherry Wine, Baox, T1) had larger GI when grown in peat compared to coir. Peat with 0 or 30% perlite had plants with the largest GI, while coir with 45% perlite were the smallest. There was little difference in GI overall between substrate treatments, except for coir with 45% perlite.

For dry weights, the interaction of substrate type and percentage of perlite had an effect (Table 3.2). Cultivar also had an effect, with ‘Sweetened’ and ‘Baox’ having larger dry weights and ‘Cherry Wine’ and ‘T1’ having smaller dry weights (data not shown). All peat blends and coir with 0% perlite had plants with similar, greater dry weights, coir with 30 and 45% perlite had plants with the smallest dry weight. Other than coir with 45% perlite, there was little difference between dry weight among treatments. Figure 3.1 illustrates similarities between all peat blends and 100% coir for ‘Cherry Wine’.

Other than slight negative trends in GI and dry weight for plants grown in coir with increasing levels of perlite, substrate blend treatments appeared to have little effect on plant growth. PourThru pH and EC values were similar for all substrate blends (data not shown), indicating that neither played a role in growth differences. These data suggest peat and coir-based substrates possessing TP, CC, AS, and BD values within suggested ranges of most other horticultural crops (Bilderback et al., 2005; Yeager et al., 1997) will have minimal effect on GI and dry weight of vegetative cannabis.

### *Experiment 2.*

Physical properties for all commercial mixes were similar (Table 3.4). Air space was within the range of 10 to 30% for all commercial mixes. Total porosity with an average of 87%, was at the higher end of the recommended range of 50 to 85%. Container capacity was also slightly higher with an average of 66% with the recommended range being 45 to 65% (Bilderback et al., 2005; Yeager et al., 1997). Dry weight values at 4 weeks after transplant were similar, with minimal visual differences (Table 3.5). Mixes that produced the plants with the largest dry weights were 5 and 6 (16.5 and 16.3g, respectively) and mixes that produced the plants with the lowest dry weights were 9 and 11 (8.6 and 7.1g, respectively).

Although there was some variation (around 10 to 20% maximum) of physical properties tested in the substrates studied, dry weights were relatively unaffected. These data suggest cannabis can be grown successfully in a variety of commercial substrate mixes.

### **Conclusions**

Except for coir-based substrates with higher levels of perlite, both experiments showed little difference in growth measurements between substrates used. Mixes used in experiment 1 and experiment 2 had varying ratios of substrate components but physical properties all fell within or near recommended ranges for nursery container crops. These data suggest mixes designed to optimize cannabis vegetative growth should possess physical property ranges generally accepted as suitable for most other horticultural crops.

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Table 3.1. Physical properties of peat based and coir-based substrate blends containing 0, 15, 30, or 45% perlite by vol<sup>z</sup>.

Substrate Blends	Total porosity <sup>y</sup> (% vol)	Container capacity <sup>x</sup> (% vol)	Air space <sup>w</sup> (% vol)	Bulk density <sup>v</sup> (g·cm <sup>-1</sup> )
100:0 Peat: Perlite <sup>s</sup>	88.78c	64.23c	24.56a	0.11a
85:15 Peat: Perlite	85.40d	62.57cd	22.83ab	0.11a
70:30 Peat: Perlite	81.88e	60.30d	21.58abc	0.11a
55:45 Peat: Perlite	81.73e	60.43d	21.29bc	0.12a
100:0 Coir: Perlite	95.55a	74.45a	21.10bc	0.07c
85:15 Coir: Perlite	92.54b	73.74ab	18.80cd	0.08c
70:30 Coir: Perlite	87.30c	70.32b	16.98d	0.09b
55:45 Coir: Perlite	83.81d	63.47cd	20.34bc	0.11a

<sup>z</sup> Physical property data represent the mean (n=3). Analysis performed using the North Carolina State University Porometer method (Fonteno et al. 1995).

Lettering represents a Tukey-Kramer grouping for least square means of the respective physical property for each substrate.

<sup>y</sup> Total porosity is equal to container capacity + air space.

<sup>x</sup> Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

<sup>w</sup> Air space is the volume of water drained from the sample ÷ volume of the sample.

<sup>v</sup> Bulk density after forced-air drying at 105°C for 48 h; 1g·cm<sup>-1</sup> = 0.5780 oz/inch<sup>3</sup>.

<sup>s</sup> Substrates were formulated with peat or coir amended with 0, 15, 30, or 45% perlite by vol.

Table 3.2. Growth index (GI) and dry weight measurements of *Cannabis sativa* L. cultivars ‘Cherry Wine’, ‘Baox’, ‘Sweetened’ and ‘T1’ in peat and coir based substrates containing 0, 15, 30, and 45% perlite by vol.<sup>z</sup>

Substrate Blends	GI (cm) <sup>y</sup>	Dry weight (g) <sup>x</sup>
100:0 Peat: Perlite <sup>s</sup>	75.71ab	31.33ab
85:15 Peat: Perlite	71.91bc	28.58 ab
70:30 Peat: Perlite	78.28a	32.74 a
55:45 Peat: Perlite	71.77bc	26.82 ab
100:0 Coir: Perlite	71.66bc	27.03ab
85:15 Coir: Perlite	68.11c	23.84bc
70:30 Coir: Perlite	68.86c	19.81cd
55:45 Coir: Perlite	60.86d	14.00d

<sup>z</sup> Tukey-Kramer grouping for least square means of growth index (GI) and dry weight for substrate by perlite (% vol).

<sup>y</sup> GI = (Height from substrate to tallest point + diameter at widest point + second diameter 90° turn from first) / 3.

Substrate\*Perlite percentage interaction P-value = 0.0015

<sup>x</sup> Dry weight of all above ground plant parts after forced-air drying at 75°C for 72h.

Substrate\*Perlite percentage interaction p-value = 0.0027

<sup>s</sup> Substrates were formulated with peat or coir amended with 0, 15, 30, or 45% perlite by vol.

Table 3.3. Growth index and dry weight measurements of *Cannabis sativa* L. cultivars ‘Cherry Wine’, ‘Baox’, ‘Sweetened’ and ‘T1’ in peat and coir based substrates.<sup>z</sup>

Substrate*Cultivar	Growth index (GI) <sup>y</sup> (cm)	Dry Weight <sup>x</sup> (g)
Peat: Cherry Wine <sup>w</sup>	81.84a	26.99b
Peat: Baox	79.27ab	N/A <sup>v</sup>
Peat: Sweetened	75.93bc	35.35a
Peat: T1	60.63e	N/A
Coir: Cherry Wine	71.86cd	17.73c
Coir: Baox	70.53d	N/A
Coir: Sweetened	72.49cd	26.57b
Coir: T1	54.61f	N/A

<sup>z</sup> Tukey-Kramer grouping for least square means of growth index (GI) and dry weight for substrate by cultivar.

<sup>y</sup> GI = (Height from substrate to tallest point + diameter at widest point + second diameter 90° turn from first) / 3. Substrate\*Cultivar p-value = 0.0268

<sup>x</sup> Dry weight of all above ground plant parts after forced-air drying at 75°C for 72 h. Substrate\*Cultivar p-value = 0.8881

<sup>w</sup> All ratios of perlite included in peat and coir least square means grouping.

<sup>v</sup> Data not available due to lab oven malfunction.

Table 3.4. Physical properties of 20 commercially available substrate mixes marketed to the cannabis industry<sup>z</sup>.

Substrate Blends	Total porosity <sup>y</sup> (% vol)	Container capacity <sup>x</sup> (% vol)	Air space <sup>w</sup> (% vol)	Bulk density <sup>v</sup> (g·cm <sup>-1</sup> )
Mix 1	89.32abc	60.07hi	29.26a	0.09de
Mix 2	84.76gh	64.14e-g	20.62cde	0.10c
Mix 3	88.20cd	67.48b-e	20.72cde	0.09d
Mix 4	91.14ab	68.12bcd	23.02bc	0.09d
Mix 5	88.76bc	68.80bc	19.95cdef	0.08e
Mix 6	91.69a	68.38bc	23.31bc	0.09d
Mix 7	89.16bc	71.13ab	18.04defgh	0.08e
Mix 8	88.72bc	70.97ab	17.75efgh	0.08e
Mix 9	87.36cdef	73.55a	13.81h	0.09cd
Mix 10	85.89defg	66.32cdef	19.57cdef	0.09d
Mix 11	84.99fgh	63.59fgh	21.39cde	0.09d
Mix 12	87.68cde	72.90a	14.77gh	0.09cd
Mix 13	87.69cde	65.34cdefg	22.35bcd	0.09d
Mix 14	84.42gh	68.59bc	15.83fgh	0.10c
Mix 15	85.19fgh	58.71i	26.48ab	0.10c
Mix 16	85.75defg	71.17ab	14.58gh	0.09d
Mix 17	87.96cde	60.05hi	27.91a	0.10c
Mix 18	85.08fgh	62.25ghi	22.83bc	0.13b
Mix 19	82.83h	64.41defg	18.42defg	0.15a
Mix 20	85.69efg	70.63ab	15.06gh	0.09d

<sup>z</sup> Physical property data represent the mean (n=3). Analysis performed using the North Carolina State University Porometer method (Fonteno et al. 1995). Lettering represents a Tukey-Kramer grouping for least square means of the respective physical property for each substrate.

<sup>y</sup> Total porosity is equal to container capacity + air space.

<sup>x</sup> Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

<sup>w</sup> Air space is the volume of water drained from the sample ÷ volume of the sample.

<sup>v</sup> Bulk density after forced-air drying at 105°C for 48 h; 1g·cm<sup>-1</sup> = 0.5780 oz/inch<sup>3</sup>.

Table 3.5. Dry weight at four weeks after transplant for *Cannabis sativa* L. grown in 20 commercially available substrate mixes.<sup>z</sup>

Substrate Blends	Dry Weight (g) <sup>x</sup>
Mix 1	10.13bcd
Mix 2	10.66abcd
Mix 3	12.63abcd
Mix 4	14.85ab
Mix 5	16.50a
Mix 6	16.28a
Mix 7	13.53abc
Mix 8	12.80abcd
Mix 9	8.56cd
Mix 10	11.38abcd
Mix 11	7.08d
Mix 12	12.63abcd
Mix 13	13.25abc
Mix 14	13.88abc
Mix 15	10.10bcd
Mix 16	12.50abcd
Mix 17	12.33abcd
Mix 18	11.73abcd
Mix 19	11.76abcd
Mix 20	12.61abcd

<sup>z</sup> Tukey-Kramer grouping for least square means of dry weight for each substrate.

<sup>x</sup> Dry weight of all above ground plant parts after forced-air drying at 75°C for 72h.



Figure 3.1. *Cannabis sativa* L. cultivar 'Cherry Wine' grown in (from left to right) 100:0, 85:15, 70:30, 55:45 peat: perlite and 100:0 coir: perlite (v:v).