2017

USING EXOGENOUS HORMONE APPLICATION TO SUPPRESS AXILLARY SHOOT DEVELOPMENT IN TOBACCO

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Digital Object Identifier: https://doi.org/10.13023/ETD.2017.075

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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Lexington, KY

2017

ABSTRACT OF THESIS

USING EXOGENOUS HORMONE APPLICATION TO SUPRESS AXILLARY SHOOT DEVELOPMENT IN TOBACCO

The variability in the number of basal axillary shoots (ground suckers) among all tobacco (Nicotiana tabacum L.) varieties, has increased since the hydroponic transplant production system became the standard. The larger root ball of hydroponically produced transplants compared to traditionally produced transplants potentially generates a difference in the ratio of auxin (inhibits axillary shoot formation) to cytokinin (promotes lateral branching), that induces basal axillary shoot development. Starting in 2014, studies were conducted to investigate whether the addition of synthetic auxins or cytokinins to hydroponic transplant production could prevent ground sucker formation. Different tobacco cultivars, with high or low ground sucker potential, were evaluated in extensive dilution trials using the synthetic auxin 1-Naphthalene acetic acid (NAA) or cytokinin 6-Benzylaminopurine (BA). Initial results indicated that a 2nM concentration of NAA significantly reduced axillary shoots in the known ground sucker producer, compared to the control. From these results, NAA and BA was added to the hydroponic transplant solution at concentrations in the range of 2-50 nM for the 2015 field trials; however, these studies failed to confirm the preliminary greenhouse findings. Nonetheless, it was clear from the 2015 field results that the varieties used are genetically different from one another in ground sucker potential. Following the 2015 field study, an additional greenhouse experiment using higher concentrations of NAA showed that a 1µM NAA hydroponic solution reduced ground sucker number. From this, a second set of field studies were conducted in 2016 using the higher rates of NAA. Burley variety TN86 and dark variety KTD6 (known ground sucker producers) were used in the 2016 studies. Five hormone treatments (NAA 500-5000 nM and an untreated control) and five tray (128, 200, 242, 288, and 338 cells per tray) were evaluated. Tray size was added to determine if increasing transplant root ball size was correlated with an increase in ground suckers. Although statistically significant differences were found among treatments applied to both TN86 and KTD6, there was no treatment that consistently reduced ground sucker numbers. No notable trend in ground sucker number indicates that an increase in the root ball size of tobacco transplants produced in the float bed system compared to traditionally produced transplants is not the sole cause of increased ground sucker number. In conclusion, from the results of the entire study, it is apparent that an exogenous auxin application (within the conditions used in this study) will not consistently or predictably suppress ground sucker development in tobacco; perhaps the only consistency in the data is how inconsistent ground sucker formation is from environment to environment, and variety to variety.

KEYWORDS: Tobacco, Auxin, Cytokinin, Apical Dominance, Ground Suckers

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March 14, 2017

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Chapter One: Introduction

Axillary shoots arising from the base of the plant prior to topping, often colloquially referred to as 'ground suckers' among tobacco farmers, were rarely a problem when tobacco transplants were grown in traditional plant beds. However, axillary shoots have become a significant problem since the introduction of hydroponic transplant production, which is the current industry standard (Pearce et al, 1999). Axillary shoots that develop at the root shoot junction (basal axillary shoots) were normally absent in the majority of tobacco varieties when transplants were produced using traditional practices; however, basal axillary shoots were a problem in a few varieties during suboptimal growing seasons (Palmer, 2007).

In the modern hydroponic or 'float bed' transplant production system all varieties exhibit the tendency to produce basal axillary shoots, especially in the presence of environmental stress (Bailey, 2007; Palmer, 2007; Maksymowicz and Palmer, 1997). Hydroponic transplant production produces a more vigorous root system (Pearce et al, 1999; Maksymowicz and Palmer, 1997). Roots are the location of cytokinin (CK) biosynthesis; and, a larger root mass physically alters the amount of cytokinin available for uptake into the foliar region of the plant body due to increased CK biosynthesis. Augmented CK concentration leads to a suboptimal auxin to cytokinin ratio. Auxin and cytokinin are antagonistic plant growth regulators (PGRs) responsible for regulating branching (Muller and Leyser, 2011; Kieber, 2002). An imbalance in the ratio of these phytohormones will ultimately cause undesired effects. In addition, the ratio of these two hormones is influenced by temporal and spatial factors that determine environmental ques (Cline, 1996; Sato and Mori, 2001). In optimal growing conditions, axillary shoot

development is repressed in most varieties after apical dominance is established in the field following transplanting; as a result, basal axillary shoots rarely grow beyond 6-8 inches in length and are of no real consequence. However, the repression of basal axillary shoots after transplanting is not guaranteed, and varies significantly with variety and growing season (Moore, 2010; Bailey, 2007). If the development of basal axillary shoots, due to a suboptimal endogenous auxin to cytokinin ratio caused by genetics and/or physical characteristics, is not prevented the ground suckers can reach considerable size, and decrease the yield potential of the primary stalk and add significant difficulty during harvest (Bailey, 2007; Moore, 2010). Ground suckers can often reach a size large enough that growers harvest them along with the primary plant to prevent yield reduction. Although this method prevents a substantial diminution in biomass, the cured leaf arising from axillary shoots typically does not meet the quality standards demanded by tobacco companies and is not purchased.

To my knowledge, no research has been conducted on the effectiveness of potential plant growth regulator (PGR) management treatments that may minimize or eliminate basal axillary shoot development during the production of tobacco transplants. Tobacco scientists do not have a clear understanding of the mechanism behind basal axillary shoot development, but have speculated that both physical injury to the apical meristem, a larger root mass, and genetic differences among varieties that lead to different auxin and cytokinin concentrations are possible culprits. Regardless of the initial instigate, a suboptimal auxin to cytokinin ratio is likely the causal agent of axillary shoot development.

What causes the suboptimal auxin to cytokinin ratio responsible for basal axillary shoot development? An obvious answer to this question is physical damage to the shoot apex, the location of auxin biosynthesis in a healthy plant. If the apical meristem is intact and functioning properly, why do some tobacco varieties readily produce basal axillary shoots? The answer to this question is less clear, but genetic variation among varieties that alter auxin and cytokinin perception is an intriguing prospect. Another practical basis could be the larger root mass and correlative increased cytokinin concentration instigated by hydroponic transplant production.

Numerous studies have assessed sucker control and the factors involved such as MH residue levels, chemical controls, application methods, and yield differences, but none have tested methods to prevent ground sucker formation in cultivated tobacco. Research in other species has shown that both apical and basal axillary shoot initiation can be manipulated by modifying endogenous hormone ratios. Specifically, the auxin to cytokinin ratio is known to have a profound effect on axillary and basal shoot development in many different plant species. With that in mind a safe, and effective ground sucker control agent may already be available.

The objectives of this study were to: 1) Identify a plant growth regulator (PGR) for use in the hydroponic transplant production system to completely suppress ground suckers in burley and dark tobacco. 2) Determine the effect different exogenous hormone sources (cytokinins and auxins) have on ground sucker formation in burley tobacco. 3) Quantify inherent differences in the number of ground suckers produced by different tobacco varieties.

Chapter Two: Literature Review

Auxin

The Greek word ‘αυξα΄νω’, which means ‘to grow’ when translated into English, gives us ‘auxin’: the name of a small class of molecules derived from the amino acid tryptophan with the ability to induce dramatic growth responses in plants. Auxin is a group of phytohormones (plant hormones), known to regulate axillary bud dormancy, but how is not completely understood (Muller and Leyser, 2011). The classical theory is that auxin produced in the shoot apical meristem is actively transported basipetally in the polar auxin transport stream (PATS) promoting axillary bud dormancy. In addition to promoting axillary bud dormancy, auxin has been implicated as a major factor regulating most of the quantitative growth that occurs throughout the life cycle of a plant (Teale et al., 2006).

Extensive research has been conducted over the past century looking at the effect of auxin on axillary shoot development and whole plant architecture. Results from an experiment conducted by Thimann et al. in 1934 showed decapitation (removal of the shoot apical meristem) of *Vicia* spp. plants promotes axillary shoot growth. In contrast, application of exogenous auxin to the decapitated plant suppressed axillary shoot growth, indicating that auxin produced in the shoot apex has a major role in the regulation of axillary bud dormancy. However, direct application of auxin to axillary buds present on the decapitated plants was alone not sufficient to prevent bud break (Thimann et al., 1934).

Hall and Hillman observed in *Phaseolus vulgaris* L., that radiolabeled indole-3-acetic acid (IAA), the most abundant naturally occurring auxin, is not translocated to the

axillary buds when applied to a decapitated plant; however, axillary shoot suppression is still achieved. In addition, 2, 4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin incapable of basipetal movement in plants, did not prevent axillary shoot formation when applied apically to decapitated *Helianthus annuus* L. plants (Brown et al., 1979). These results, when coupled with the inability of IAA to move upwards from the stem into the bud, indicate that auxin does not directly prevent axillary shoot formation, but its basipetal polar transport plays a significant role (Sachs and Thimann, 1967; Booker et al., 2003). There is increasing evidence to suggest a close-fitting correlation between auxin transport out of a bud and the breaking of dormancy (Balla et al., 2011). Thus, the PATS coming from the apex of an intact plant lowers the sink strength of the stem and prevents auxin canalization out of a dormant bud preventing bud break. In addition, it has been shown in pea plants that auxin export out of dormant buds occurs rapidly after removal of the shoot apex. Furthermore, auxin export ceases upon exogenous auxin application to the decapitated plant.

Sachs proposed the auxin canalization hypothesis in 1981, to explain the many developmental processes dependent on auxin transport gradients. Auxin transport between a source and sink leads to the basipetal polarization and upregulation of active auxin transporters (Sachs, 2000). In addition, research has demonstrated that in dormant axillary buds PIN1 (a major auxin transport protein) is non-polar localized, contrary to active axillary buds in which PIN1 is expressed to a higher degree and in a polar localized fashion. This establishes a connection between the bud and the PATS in the main stem (Balla et al., 2011). The canalization hypothesis elucidates how auxin moving in the main stem indirectly suppresses bud break, by providing an understanding of how

the PATS maintains a low auxin sink strength in the main stem and prevents rapid auxin export out of buds in an intact plant.

Numerous proteins are involved in the active transport of auxin molecules across cells and tissues (Lomax et al., 1995). Specialized, plasma membrane localized, influx and efflux proteins mediate the transport of auxin, and allow for the formation of gradients, minima, and maxima that have crucial roles in differential plant growth, such as organogenesis and different tropic responses. The establishment of auxin gradients via active auxin efflux and influx modulates basal transcription of auxin sensitive genes, and plays a crucial role in determining the fate of the cell; e.g. suppression or activation of elongation growth (Kepinski and Leyser, 2005).

Rubery and Sheldrake, and Helen and Goldsmith, arrived independently at the same hypothesis for an auxin transport model. The chemiosmotic model of auxin transport is based on knowledge that the protonation state of weak acids is determined by the pH of the environment. Considering that the cell wall is acidic, a significant portion of auxin would be in a protonated state (IAAH). The protonated form of IAA can passively diffuse across membranes, however within a cell the cytosol is neutral and IAA occurs primarily in the anionic form (IAA-). The anionic form of IAA cannot passively diffuse across membranes, and therefore the chemiosmotic auxin transport model proposed the existence of plasma membrane auxin efflux carriers that actively move auxin out of a cell.

It was later confirmed that plasma membrane localized auxin transporters exist, and that there are three distinct groups. The three main categories of transmembrane

proteins responsible for active auxin transport are as follows: 1.) AUX1/LIKE AUX1 (auxin influx permeases) 2.) ABCB transporters (auxin efflux transporters) 3.) PIN proteins (auxin efflux carriers) (Finet and Jaillais, 2012). The previously mentioned auxin transport proteins are all known to play important roles in auxin fluxes *in vivo*, but the PIN proteins have received the bulk of the attention. A primary reason for this is the polar localization of PIN proteins correlates with the directionality of auxin movement. Although the direction of auxin movement cannot be directly visualized in plants, the polar localization of PIN proteins coordinated at the tissue level provides a means to determine the apparent directionality of auxin transport. Using PIN polar localization to estimate the direction of auxin movement is backed up with experimental data and simulations (Band et al., 2014). However, this is not to say that PIN localization is static. PIN protein localization can rapidly change in response to developmental and environmental ques.

PIN localization and its subsequent effect on the PATS results in changes in the basal transcription levels of auxin sensitive genes. Recent research has shown that auxin plays a significant role in the inhibition of shoot branching via targeted degradation of a family of transcription factors that act as suppressors of multiple auxin sensitive genes. This family of proteins, known as the Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) family, plays an important role in axillary bud development and dormancy (Leyser, 2010). The plant hormone auxin interacts directly with the Aux/IAAs and the TRANSPORT INHIBITOR RESPONSE1 (TIR1) and/or related proteins that belong to the AUXIN SINGNALING F-BOX PROTEIN (AFB) family, stabilizing the interaction between Aux/IAA and TIR1 proteins (Tan et al., 2007).

TIR1 and other AFBs are SCF-type protein-ubiquitin ligases, when active (in the presence of auxin) polyubiquitinate Aux/IAAs marking them for degradation by the 26S proteasome (Leyser, 2010). The transcription level of thousands of genes is influenced either directly or indirectly by the degradation of the Aux/IAA suppressors. Genes that contain auxin response elements are direct targets of auxin response factors (ARFs), which are transcription factors that dimerize with one another in order to activate transcription. In the absence of auxin, Aux/IAA suppressor proteins can from heterodimers with ARFs bound to DNA preventing transcription. Upon degradation of Aux/IAA the ARF bound to DNA can form a homodimer with a free ARF and promote transcription.

Genes under this type of direct auxin regulation, interestingly include the Aux/IAA genes (Leyser, 2010). This feedback loop regulates and replenishes the auxin suppressor proteins (Aux/IAA), in order to stop auxin responses once the auxin signal has stopped.

The auxin pathway is under intense regulation, and the slightest alteration of the endogenous levels of this biochemical produce pronounced effects. (Dun et al, 2006; Leyser, 2010) With this in mind, amending the auxin concentration in tobacco during the transplant production phase could theoretically prevent lateral buds from forming, and thus prevent the development of basal axillary shoots.

Cytokinin

Another PGR involved in the regulation of branching, is cytokinin (CK), which is believed to be primarily produced in the roots. However, research has shown that CK biosynthesis does in fact occur in aerial portions of higher plant species (Nordstrom, 2003). The most abundant naturally occurring CKs in higher plants are adenine derivatives with either an isoprene or an aromatic side chain substituted at the N^6 terminus, and include: isopentyladenine (iP), zeatin (Z), and dihydrozeatin (DZ) (Werner et al., 2001; Miller et al., 1955). The hormones are biologically active in their free base forms, while inactive conjugated forms are capable of transport, and storage due to protection from degradation. Cytokinins (CKs) were first identified in the 1950s due to their involvement in plant cell division; in addition, CKs also play a prominent role in crop productivity, root/shoot development, transduction of nutritional signals, senescence, photomorphogenic growth, and many other aspects of plant morphogenesis (Sakakibara, 2006; Kieber, 2002; Miller et al., 1955).

CKs are thought to antagonize auxin in the regulation of axillary bud growth and apical dominance (Muller and Leyser, 2011). On the other hand, research has shown that auxin can actually have a stimulatory effect on CK biosynthesis, depending on the plant organ. In *Arabidopsis thaliana,* auxin has been shown to upregulate the gene expression of *AtIPT5 and AtIPT7,* genes that encode important enzymes that catalyze the initial reaction in CK biosynthesis, in the roots (Sakakibara, 2006; Miyawaki et al., 2004). Conversely, *CYP735A1* and *CYP735A2,* genes that encode important enzymes that catalyze one of the early reactions in CK biosynthesis, transcript accumulation decreases in the presence of auxin in *A. thaliana* roots, but increases in the presence of CK.

Interestingly, CK actually down regulates *AtIPT1, AtIPT3, AtIPT5,* and *AtIPT7* transcription, and thus its own biosynthesis in roots (Sakakibara, 2006). From this it's obvious that the interdependency of auxin and cytokinin on one another's biosynthesis and activity may elucidate the basis for the diverse morphological responses of higher plants to different environmental and biological ques.

Historic exogenous hormone application studies have provided evidence to support the notion that the cytokinin to auxin ratio directly affects the growth of axillary shoots. For example, in excised *A. thaliana* stems with a single attached axillary bud, basally supplied cytokinin is sufficient to promote bud break and subsequent axillary shoot development even when auxin is applied apically (Muller and Leyser, 2011; Chatfield et al., 2000). Contrary to the observation that direct application of auxin to axillary buds present on the decapitated plants was alone not sufficient to prevent bud break (Thimann et al., 1934), direct application of cytokinin to axillary buds on intact plants promotes bud outgrowth (Wickson and Thimann, 1958). This provides further evidence of the antagonistic effect of cytokinin on auxin, in regards to axillary shoot development.

Apical Dominance

The growth habit of Tobacco, not unlike many other plants, is heavily influenced by activity of the primary shoot apical meristem (SAM). The SAM, which develops during embryogenesis, together with the activity of additional axillary meristems (AXM), that are subsequently formed after seed germination, regulate plant architecture (Kerstetter and Hake, 1997). The SAM provides the main stalk of the plant. The overall plant is then modified by the development and elongation of the AXM (referred to as

"suckers" in tobacco). The formation of suckers generally involves two developmental stages: the formation of AXM in the leaf axils and the growth of axillary buds. In many plant species, including *N. tabacum*, the growth of axillary buds is inhibited by the SAM, via hormonal sensing and signaling. This phenomenon is referred to as apical dominance, and in earlier literature 'correlative inhibition' and 'paradormancy'.

Apical dominance is defined broadly as "the inhibitory control of the shoot apex over the outgrowth of lateral buds" (Cline, 1997; Napoli et al., 1999; Sato and Mori, 2001). However, with that general definition in mind it is important to recognize that there can be considerable variation in the level of apical dominance exhibited from species to species. In addition, there is much debate centered around the mechanism by which apical dominance is promoted and lateral shoot development suppressed. Still, there are three reoccurring hypotheses that indicate a central role for the plant hormone auxin in controlling apical dominance.

The classic hypothesis focuses on the actual amount of endogenous auxin (which has an inhibitory effect on the growth of axillary buds), and indicates that auxin content directly effects the movement and activity of a secondary messenger such as cytokinin (which promotes axillary bud outgrowth) to suppress bud break (Phillips, 1975; Tamas, 1995; Napoli et al., 1999). This is contrary to other beliefs that the ratio of these two hormones rather than the absolute level of either hormone control axillary bud dormancy (Sato and Mori, 2001).

However, strong evidence collected from various decapitation studies supports the classic theory. It has been shown in pea and other legumes, that upon decapitation

(removal of the primary auxin source) there is an increase in cytokinin biosynthesis in the stem and transport into axillary buds (Tanaka et al., 2006). Tanaka and company also demonstrated that this increase in cytokinin biosynthesis, and subsequent transport and lateral bud development could be prevented by applying auxin apically to counteract the absence of an endogenous auxin source.

The auxin transport hypothesis, contrary to the classical theory, indicates that auxin movement via the PATS not the actual auxin content exerts regulatory control on lateral bud dormancy (Leyser, 2005). Based on this theory in order for bud outgrowth to occur, the growing lateral shoot tip must become an auxin source strong enough to export auxin into the PATS (Dun et al., 2006; Leyser, 2005). However, in intact plants where axillary shoot development is completely suppressed it is proposed that the basipetal PATS is at maximum capacity, preventing auxin export from the lateral buds and promoting dormancy.

This hypothesis when coupled with the canalization hypothesis elucidates how auxin moving in the main stem could indirectly suppress bud break, by providing an explanation of how the basipetal PATS maintains a low auxin sink strength in the main stem and prevents rapid auxin export out of buds in an intact plant. Interestingly, this indicates that auxin may have a dual role in the maintenance of apical dominance, i.e. its polar transport out of buds maintains development of axillary shoots while its basipetal movement from the apical meristem promotes axillary bud dormancy.

The bud transition hypothesis states that an axillary bud goes through different developmental processes with varying degrees of auxin sensitivity. This hypothesis

describes three developmental stages at which a lateral bud can exist (Cline, 1997; Napoli et al., 1999; Sato and Mori, 2001). The first is deemed the dormant stage, the second is the transition stage, and the third is the sustained growth stage. Dormancy, broadly defined, is "the temporary suspension of visible growth of any plant structure containing a meristem" (Lang, 1987; Sato and Mori, 2001). Axillary bud dormancy can be broken in a number of different ways, including physical damage to the apical meristem, and other environmental and developmental cues. Whenever dormancy is broken, the axillary bud typically develops into an actively growing shoot serving as a plant survival mechanism (Sato and Mori, 2001). Essentially dormant axillary buds act as a reservoir of meristems with the potential to replace the primary shoot if it becomes damaged.

However, according to the bud transition hypothesis it is possible for a dormant bud to receive a que that will break dormancy, enter the transition stage, and then return to a dormant state (negligible growth). Stafstrom and Sussex showed that the transcription level of a ribosomal protein gene (*rp*L27) increased after decapitation in pea plants, but prior to lateral bud outgrowth. Afterwards, it was observed that some of these buds halted growth and reentered a dormant state, indicating that a stage must exist in-between dormancy and sustained growth. The existence of a transition stage is backed up with measurements taken on buds that halted growth, showing a decrease in the transcription level of the *rp*L27 protein (Stafstrom and Sussex, 1992).

From these observations and others, it appears that buds in the transition stage or stages are more receptive to auxin signaling that promotes axillary shoot growth than a dormant bud, but are still less receptive than buds in the sustained growth stage. Numerous factors are believed to influence the developmental stage of a bud and the

level of auxin sensitivity; such as, the age of the plant (maturity), the location of the bud on the plant (node number), genetics, light and temperature (Dun et al., 2006; Stafstrom, 1995).

In addition, the assumption that the position of an axillary bud; i.e., the node on which it is located, effects the responsiveness to auxin indicates that axillary bud position may exert some level of control on axillary shoot development. In 1989, Weberling categorized plants into three distinct morphological zones: the enrichment zone (site of floral initiation), the inhibition zone (an area adjacent to the enrichment zone where little axillary bud outgrowth occurs), and the innovation zone (the basal region of the plant where buds can either remain dormant or develop into axillary shoots phenotypically similar to the primary stem). Since Weberling's characterization, other researchers have proposed that these distinct plant regions influence the receptivity of axillary buds to different signals; e.g., cytokinin and auxin (King and Van Staden, 1988; Morris et al., 2005), indicating that node position exerts some control on the development of axillary shoots.

Appeal of Preventing Basal Axillary Shoots in Tobacco

Numerous studies have shown that axillary shoot growth is detrimental to final cured leaf yield and quality in both burley and dark tobacco varieties (Gaines, 1959; Seltmann and Nichols, 1983). In addition, optimal leaf area, dry matter accumulation, and nicotine biosynthesis occur when actively growing axillary shoots are suppressed (Atkinson and Sims, 1973). There are effective chemical options available to control sucker formation after a tobacco crop is topped, but none available to prevent/control

ground sucker formation (axillary shoots that develop soon after transplanting at the base of the plant before the loss of the apical meristem).

Topping is a process in tobacco production, where after flowering the terminal panicle inflorescence (apical meristem) is removed. Removal of the inflorescence partitions photosynthate into leaf rather than seed production, increasing final cured leaf yield and quality (Seltmann 1970; Garvin 1980; Steffens and Seltmann 1982; Clapp and Seltmann 1983; Meyer et al. 1987; Gorman et al. 1989). After the apical meristem is removed, the primary auxin source is no longer present and axillary bud dormancy is broken promoting axillary shoot growth. However, this lateral shoot growth can be suppressed with chemicals that retard lateral shoot growth, and compensate for the auxin/cytokinin imbalance created by the physical removal of the shoot apex.

Although current chemicals provide adequate control of axillary shoots that arise after topping, ground sucker suppression cannot be achieved via the same method. Ground sucker formation occurs prior to removal of the inflorescence, often times in the absence of any physical damage to the shoot apex. Basal shoot formation, in the presence of an intact shoot apex, is likely due to the complexity of maintaining apical dominance.

Along with physical damage to the apical meristem, environmental and developmental ques regulate axillary shoot development. It is possible that certain environmental and/or developmental signals can undermine the inhibitory effect of the shoot apex, and break the dormancy of a basal axillary bud, although the apical meristem is intact at the time of ground sucker formation. For this reason, a preventative rather than curative treatment, applied before transplanting, that maintains an auxin to cytokinin ratio

favorable for axillary shoot suppression would be one logical solution.

Current Sucker Control Methods

Producers have access to three categories of chemicals labeled for use in tobacco sucker control: 1) Contacts, or chemicals that destroy axillary shoots after they come into direct contact with the leaf axil. 2) Local systemics, or chemicals that must come into close contact with the leaf axil and are then translocated to surrounding tissues inhibiting cell division and subsequent sucker growth. 3) Systemics, or chemicals that are absorbed by plant tissue (leaves, leaf axils, and surrounding areas) and translocated to the axillary bud where they inhibit cell division and subsequent sucker growth.

All three categories of sucker control chemicals labeled for use in tobacco can adequately control axillary shoot growth that occurs after topping, but have no effect on ground sucker growth. In addition, contacts, local systemics, and systemics are typically only effective in controlling axillary shoots less than one inch in size (Bailey et al., 2009). Problematic ground suckers are always larger than one inch by the time any post topping sucker control measures are taken, and therefore are unaffected. Furthermore, ground suckers that form before topping, and have not been manually removed, have already reduced final cured leaf yield and quality, before any post topping sucker control measures are taken.

Moreover, previous research has shown that the chemicals used for sucker suppression after topping can cause damage and other detrimental effects whenever applied to young leaves (Aycock and McKee, 1975; Mylonas and Pangos, 1978). The adverse effects that current sucker control chemistries (contacts, local systemics, and

systemics) have on young plants effectively eliminates the potential of early applications aimed at ground sucker prevention. Thus, an alternative approach to ground sucker prevention is necessary.

Transplant Production

The most common tobacco transplant production system in the United States is the 'float bed' system (Davis and Nielson, 1999). It is a hydroponic system, first introduced by Speedling Inc. in the 1980s, in which tobacco transplants are grown in polystyrene trays containing a soilless growth media, and are floated on a nutrient solution. The float bed system takes approximately 8 weeks to produce usable transplants from seed. This system is advantageous over the traditional plant bed system; i.e., plants produced using the float bed system are more vigorous and have a much larger root mass (Davis and Nielson, 1999).

The quality of water used to create the nutrient is usually not of concern, unless there are high levels of bicarbonate and boron, or low calcium levels. In situations where water quality does not meet standards, an alternative source may be necessary to avoid detrimental growth effects.

Typically, media types used in the United States for this production system are peat-based, and come in different combinations that include vermiculite and perlite. It is important to avoid very coarse textured media (media containing > 50% perlite), in order to prevent dry cells. Media that is comprised entirely of peat have been shown to underperform a medium that combines peat with either vermiculite, perlite, or both.

Numerous studies have shown that a two-step fertilization system provides successful transplant production (Jones et al., 1992). The first application occurs either at the time of seeding or shortly after, and is followed several weeks later by the second application. The goal is to maintain a nutrient solution that is approximately 100 ppm N.

Clipping (removal of leaf material) tobacco seedlings is another unique characteristic of the float bed transplant production system. Clipping typically occurs at 3-5 day intervals once the plant has reached approximately 4-5 cm. At each clipping interval, 1-2.5 cm can safely be removed, in order to provide transplants of a uniform size appropriate for use with a mechanical transplanter. It is easy to injure the apical bud during this process, and therefore clipping is considered to be a contributing factor to basal axillary shoot development. Clipping also lowers the ratio of aerial to terrestrial plant material, which could potentially alter the inherent auxin to cytokinin ratios and promote basal shoot growth.

Chapter Three: Materials and Methods

In vitro Evaluation of the Effect of Auxin and Cytokinin on Tobacco Seedlings

To initiate the research project, *in* vitro experiments were performed on tobacco varieties selected for differences in inherent capacity for basal axillary shoot development to evaluate the effect of exogenous hormone treatments (different concentrations of auxin and cytokinin) on plant growth. Selected cultivars were burley Hybrid 403, a low basal axillary shoot producer, and burley TN 86, a high basal axillary shoot producer. Two synthetic hormone sources were used in this experiment, 1-Napthaleneacetic acid (NAA; a synthetic auxin) and 6-Benzylaminopurine (BA; a synthetic cytokinin). The initial hormone treatments were: 10, 50, and 200 nM BA; and 200, 1000, and 5000 nM NAA.

Seeds of the two chosen varieties were surface sterilized and then plated onto half strength Murashige and Skoog media. To surface sterilize the seeds, they were first placed in 1 ml of 70% ethanol, mixed and then allowed to incubate for 5 minutes. The seeds were then removed from the ethanol and placed in 1 ml of a 50% commercial bleach solution, and mixed and allowed to incubate. After 20 minutes, the bleach solution was removed and the sterilized seeds were washed three times with 1 ml of autoclaved distilled water. To prepare half strength MS media, 2.215 g/L of Murashige and Skoog Basal Medium with vitamins (PhytoTechnology Laboratories; Product ID# M519) was dissolved in distilled water, along with 1% sucrose per liter with the pH adjusted to 5.7 with 2M KOH. Agar (0.8% per liter) and the medium solution were added to an autoclave-safe bottle and autoclaved for 20 min at 120^{o} C. The solution was mixed after removal from the autoclave to ensure a homogenized solid medium once poured and

allowed to cool. After approximately one hour (temperature of the media ~ 55^{o}C), in a sterile cabinet 50 ml was poured into each plate and allowed to solidify. For hormone treated plates 50 ml was added first to a 50 ml Falcon tube containing the appropriate hormone solution, mixed, and then poured into a 50 ml square plate.

Seeds were plated onto half strength Murashige and Skoog media and allowed to germinate. Once uniform size was achieved, plantlets were transferred to new 50 ml square plates containing a hormone treatment, or no hormone treatment to serve as controls. Each hormone treated and control plate had four TN 86 and four H 403 seedlings of approximately uniform size. The square plates were oriented vertically, and arranged in a completely randomized design in a controlled environment growth chamber set to 30^{o}C and 16 hrs of light provided by both fluorescent and incandescent bulbs.

After three days, the root tip of each seedling was marked to serve as a reference point. After an additional week, data were collected to determine the effect of the hormone treatments on growth and development. Root length (the distance between the initial reference point and the root tip at time of measurement), chlorophyll content, and fresh weight of roots were parameters chosen to determine whether or not the plants appear to be developing normally compared to control plates. Root length was measured using ImageJ to process plate pictures, chlorophyll content was determined using an Opti-Sciences CCM-300 chlorophyll content meter, and root fresh weight was collected using a standard scientific digital scale. This process identified three levels of each hormone that did not severely impact growth and development *in vitro*.

Greenhouse Evaluation of Tobacco Transplants Produced in Presence of PGRs

The effect of exogenous hormone application on plant growth and axillary bud development were evaluated in two greenhouse studies, with the first study conducted in 2014 and the second in 2015. In 2014, TN 86 and H403 seedlings were grown in a controlled greenhouse environment on the University of Kentucky campus in Lexington, Kentucky. Temperature at tray level was maintained at 20 to 24° C. Natural day length was extended to 16 h with high pressure sodium lamps delivering a photosynthetically active radiation (PAR) at seedling canopy level of 300 μmol m^{-2} sec^{-1}. In all greenhouse experiments 242 cell polystyrene trays were filled with peat-vermiculite soilless substrate (Carolina Mix), and a single tobacco seed was placed in each cell. Seedlings remained in the float beds, under the management practices described in the 2013-2014 Kentucky and Tennessee Tobacco production guide, until they reached a size suitable for transplanting. The plants were clipped (removal of 1-2.5 cm of excess leaf tissue to promote vigorous stems and prevent diseases) at five day intervals starting whenever plants reached 4-5 cm from bud to root/shoot junction.

In the 2014 greenhouse study, all trays were placed in individual float beds containing 3.78541 L of water, 7.8 g of 20-10-20 water soluble fertilizer, and pest management chemicals suggested in the 2013-2014 Kentucky and Tennessee Tobacco production guide, and arranged in a completely randomized design (CRD) with two replications. Three application times were evaluated using 2, 10, and 50 nM BA and 2, 10, and 50 nM NAA, and untreated controls applied at either 2, 4 and 8 weeks post seeding, giving a total of 42 trays. After approximately 8 weeks post seeding (enough time for seedlings to reach a size suitable for mechanical transplanting), five plants from

each tray were transferred into individual 3 L plastic pots arranged in a completely randomized design. Each pot was filled with a peat-vermiculite substrate (Pro Mix), and each individual tobacco plant per pot was allowed to grow for an additional two weeks (the typical length of time required for noticeable basal axillary shoot development to occur). After two weeks in individual pots, data for leaf number, stem length (cm), and total number of ground suckers were collected for each plant, hormone concentration, application time combination.

In 2015, after the first field evaluation, another greenhouse experiment was conducted using the initial NAA treatments used in the *in vitro* experiments and modified BA treatments. The experiment had a total of seven hormone treatments: NAA 200 nM, 1μM, 5μM, BA 50 nM, 250 nM, 1.250 μM, and an untreated control. All trays were placed in individual float beds containing 3.78541 L of water, 7.8 g of 20-10-20 water soluble fertilizer, and pest management chemicals suggested in the 2013-2014 Kentucky and Tennessee Tobacco production guide, and arranged in a completely randomized design (CRD) with two replications. Two application times were used: a onetime application of the hormone treatment at two weeks post seeding, and a reoccurring application of the same hormone treatment at every watering. For the later application time, when additional water was needed all water was removed from each individual float bed, and then a new water/hormone/nutrient/pesticide solution was added to bring the float bed back to the appropriate volume. A total of 26 trays were used during this experiment.

At approximately 8 weeks post seeding (enough time for seedlings to reach a size suitable for mechanical transplanting), five plants from each tray were transferred into

individual 3 L plastic pots arranged in a completely randomized design. Each pot was filled with a peat-vermiculite substrate (Pro Mix), and each individual tobacco plant per pot was allowed to grow for an additional two weeks (the typical length of time required for noticeable basal axillary shoot development to occur). After two weeks in individual pots, data for total basal axillary shoot number were collected.

Quantification of Basal Axillary Shoots on Field Grown Tobacco

In 2015, H403 and TN86 seedlings were grown in controlled greenhouse environments on the University of Kentucky campus in Lexington, Kentucky (one set of 28 trays for Lexington and one set of 28 trays for Versailles), and the University of Tennessee Research and Education Center in Greeneville, Tennessee (one set of 28 trays for Greeneville). Temperature at tray level was maintained at 20 to 24° C. Natural day length was extended to 16 h with high pressure sodium lamps delivering a photosynthetically active radiation (PAR) at seedling canopy level of 300 μmol m^{-2} sec^{-1}. In all field experiments 242 cell polystyrene trays were filled with peat-vermiculite soilless substrate (Carolina Mix), and a single tobacco seed was placed in each cell. Seedlings remained in the float beds until they reached a size suitable for transplanting as described in the 2013-2014 Kentucky and Tennessee Tobacco production guide, and where clipped (removal of 1-2.5 cm of excess leaf tissue to promote vigorous stems and prevent diseases) at five day intervals starting whenever plants reached 4-5 cm from bud to root/shoot junction.

In the 2015 field season, matching hormone treatment trays; i.e., H 403/2 nM NAA/Application at 2 weeks post seeding and TN 86/2 nM NAA/Application at 2 weeks

post seeding, were placed in individual float beds containing 7.57082 L of water, 15.6 g of 20-10-20 water soluble fertilizer, and pest management chemicals suggested in the 2013-2014 Kentucky and Tennessee Tobacco production guide. Trays for field evaluation in Lexington were seeded on March 30th 2015; trays for Versailles and Greeneville were seeded on April 6th. All float beds were arranged in a completely randomized design (CRD). Seven hormone treatments (2, 10, and 50 nM NAA and 2, 10, and 50 nM BA, and an untreated control), and two application times (2 and 4 weeks post seeding) were evaluated giving a total of 28 trays per location.

Three locations were used for the 2015 field evaluations: The University of Kentucky C. Oran Little Research Center in Versailles, Kentucky, The University of Kentucky Spindletop Farm in Lexington, Kentucky, and The University of Tennessee Research and Education Center at Greeneville, Tennessee. The field layout was identical at each location. The experimental design was a split-split plot with three replications at each location (variety as the whole plot, application time as a sub plot, and hormone treatment as a sub-sub plot). After approximately 8 weeks in the float system plants were taken from each location/hormone/application tray and transplanted into a field. Tobacco plants were transplanted on May 21st 2015 in Lexington, May 22nd in Greenville, and June 5th in Versailles. Due to an unusually wet year, sucker data was not collected until approximately 50 days post transplanting, rather than 14 days post transplanting like in the greenhouse experiments, at each of the three locations.

In the 2016 field season, field evaluations incorporated a dark tobacco variety (KTD6), a high basal axillary shoot producer. One burley variety (TN86) was also used. However, cytokinin (BA) treatments were not administered during this experiment, and

application time was fixed at 2 weeks post seeding. In addition, tray size (number and size of cells per tray) was added as an additional treatment.

In 2016, TN86 and KTD6 seedlings were grown in controlled greenhouse environments on the University of Kentucky campus in Lexington, Kentucky (one set of trays for Lexington and one set of trays for Versailles), and the University of Tennessee Research and Education Center in Greeneville, Tennessee (one set of trays for Greeneville). Temperature at tray level was maintained at 20 to 24° C. Natural day length was extended to 16 h with high pressure sodium lamps delivering a photosynthetically active radiation (PAR) at seedling canopy level of 300 μmol m^{-2} sec^{-1}. In all field experiments 242 cell polystyrene trays were filled with peat-vermiculite soilless substrate (Carolina Mix), and a single tobacco seed was placed in each cell. Seedlings remained in the float beds until they reached a size suitable for transplanting as described in the 2013-2014 Kentucky and Tennessee Tobacco production guide, and where clipped (removal of 1-2.5 cm of excess leaf tissue to promote vigorous stems and prevent diseases) at five day intervals starting whenever plants reached 4-5 cm from bud to root/shoot junction.

Matching hormone treatment trays; i.e., TN 86/500 nM NAA/tray size 128-338 and KTD6/500 nM NAA/tray size 128-338, were placed in individual float beds containing 189.271 L of water, 95.2545 g of 20-10-20 water soluble fertilizer, and pest management chemicals suggested in the 2013-2014 Kentucky and Tennessee Tobacco production guide. The control trays were placed in individual float beds containing 378.541 L of water, 190.509 g of 20-10-20 water soluble fertilizer, and pest management chemicals suggested in the 2013-2014 Kentucky and Tennessee production guide. Trays for field evaluation in Lexington were seeded on March 28th 2016, Versailles on March

29th and Greeneville on March 29th. All float beds were arranged in a completely randomized design (CRD). Five hormone treatments (500 nM, 1000 nM, 3000 nM, 5000 nM and an untreated control), and five tray sizes (128, 200, 242, 288, and 338) were evaluated per location.

Three locations were used for the 2016 field evaluations: The University of Kentucky C. Oran Little Research Center in Versailles, Kentucky, The University of Kentucky Spindletop Farm in Lexington, Kentucky, and The University of Tennessee Research and Education Center at Greeneville, Tennessee. The field layout was identical for each experiment (burley and dark studies) at each location. The experimental design was a randomized complete block (RCBD) with three replications at each location. After approximately 8 weeks in the float system plants were taken from each location/hormone/tray size combination and transplanted into a field. Tobacco plants were transplanted on June 9th 2016 in Lexington, June 9th in Greenville, and June 9th in Versailles. In order to match time of measurement to 2015, 2016 sucker data was not collected until approximately 50 days post transplanting, rather than 14 days post seeding like in the greenhouse experiments, for both experiments at each of the three locations.

Chapter Four: Results and Discussion

In vitro Evaluation of the Effect of Auxin and Cytokinin on Tobacco Seedlings

In vitro experiments were performed on tobacco varieties selected for differences in inherent capacity for basal axillary shoot development to evaluate the effect of exogenous hormone treatments (different concentrations of auxin and cytokinin) on plant growth. Selected cultivars were burley Hybrid 403, a low basal axillary shoot producer, and burley TN 86, a high basal axillary shoot producer. Two synthetic hormone sources were used in this experiment, 1-Napthaleneacetic acid (NAA; a synthetic auxin) and 6-Benzylaminopurine (BA; a synthetic cytokinin). The initial hormone treatments were: 10, 50, and 200 nM BA; and 200, 1000, and 5000 nM NAA.

Seeds of the two chosen varieties were surface sterilized and then plated onto half strength Murashige and Skoog media. Seeds were then allowed to germinate, and once uniform size was achieved, four seedlings of each variety were transferred to new 50 ml square plates containing a hormone treatment, or no hormone treatment to serve as controls. The square plates were oriented vertically, and arranged in a completely randomized design in a controlled environment growth chamber set to 30^{o}C and 16 hrs of light provided by both fluorescent and incandescent bulbs. After three days, the root tip of each seedling was marked to serve as a reference point. After an additional week, data was collected to determine the effect of the hormone treatments on growth and development.

The ANOVA for the initial *in vitro* root length experiment is shown in Table 1. There were two significant main effects impacting *in vitro* root length during the

Table 1 Analysis of Variance for initial *in vitro* root length experiment.

In vitro Root Length			
Source of Variation	F Value	Pr > F	Significance
Variety	0.07	0.7919	ns
Hormone	63.25	<0.0001	s
Concentration	6.04	0.0033	s
Variety*Hormone	0.73	0.3942	ns
Variety*Concentration	0.11	0.8950	ns
Hormone*Concentration	2.05	0.1337	ns

*ns denotes no significance and s denotes significance at α=0.05.

initial experiments. Both hormone type and concentration had significant effects on *in vitro* root length.

A Dunnett's Test was conducted to determine if the effect of the two hormone types on *in vitro* root growth differed from the control. Figure 1 shows that both BA and NAA, averaged over variety and concentration, differ from the control. Additionally, Figure 1 shows the results of a Tukey's Honest Significant Difference Test (HSD) comparing all possible pairs of hormone type and control. Each bar marked with a different letter are significantly different per Tukey's HSD at α=0.05. These initial results are like other findings in maize that showed a decrease in root elongation when indole-3-acetic acid (IAA), a naturally occurring auxin, was applied exogenously to the roots in solution (Pilet and Saugy, 1987).

A decrease in root length to the magnitude observed in Figure 1 was initially a promising finding. One of the first theories we derived to explain the variability observed among burley tobacco varieties in terms of ground sucker formation was that the large physical size of the root ball produced via the hydroponic transplant production system disrupts the ratio of endogenous auxin to cytokinin and promotes bud break. With this in mind, it is easy to imagine that a decrease in root ball size due to exogenous auxin application could potentially solve basal axillary shoot development. However, a decrease in root growth to this magnitude is detrimental to the survival of the plant, and was of no real use.

Figure 2 displays the results of a Dunnett's Test conducted to determine if any of the hormone concentrations differed from the control. It was concluded that all the

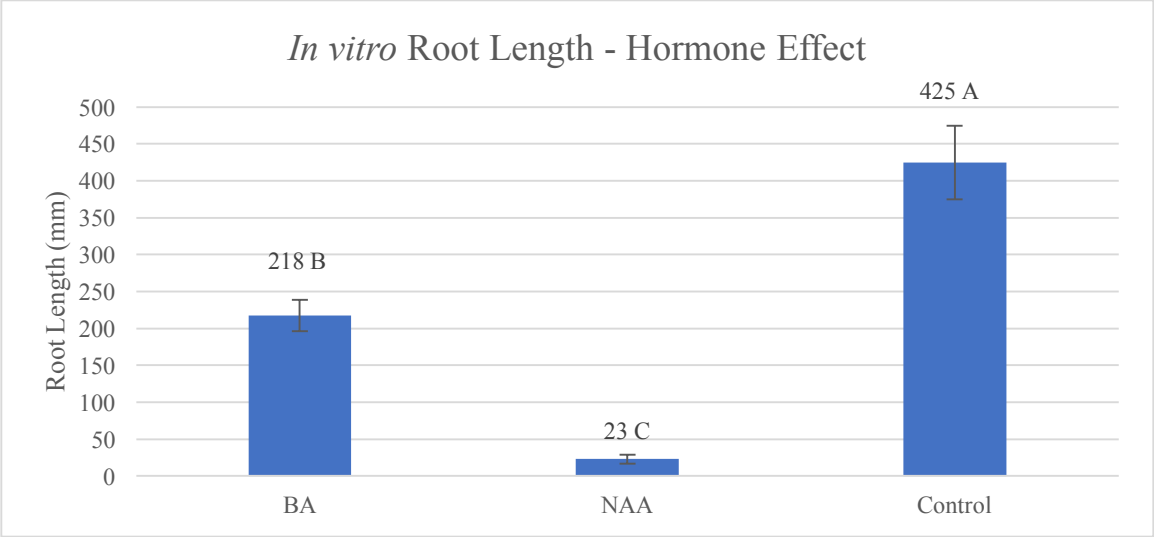

Figure 1 The effect of hormone type on *in vitro* root length averaged over variety and concentration for initial lab experiment.

* The means for BA and NAA are statistically different than the Control according to Dunnett's Test at α=0.05; in addition, means marked with different letters are statistically different than each other according to Tukey's Honest Significant Difference Test at α=0.05.

*Standard error of the mean is displayed as the error bar at the apex of each mean bar.

*Numeric values on the apex of each mean bar represent mean values.

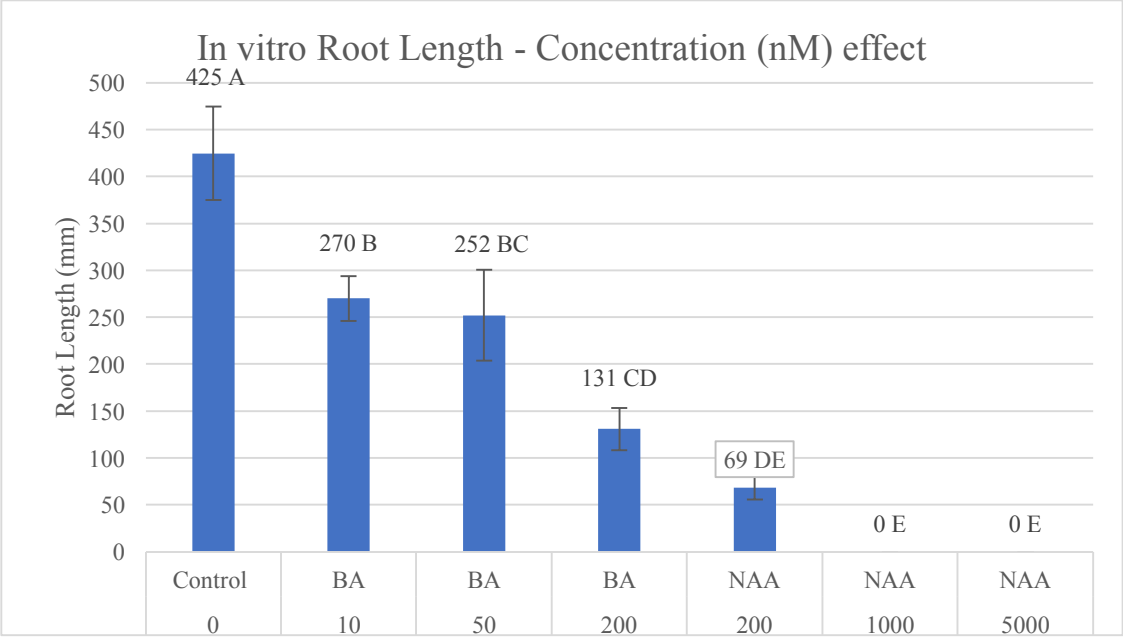

Figure 2 The effect of hormone concentration on *in vitro* root length averaged over variety for initial lab experiment.

* All hormone concentration means are statistically different than the control according to Dunnett's Test at α=0.05; in addition, all means marked with a different letter are statistically different than one another according to Tukey's Honest Significant Difference Test at α=0.05.

*Standard error of the mean is displayed as the error bar at the apex of each mean bar.

*Numeric values on the apex of each mean bar represent mean values.

hormone concentrations differed from the control concentration of 0. The results of a Tukey's HSD are also shown in Figure 2. Like hormone type, hormone concentration also showed significant effects on root length. Interestingly, the low and high BA concentrations inhibited root length more than the intermediate, whereas NAA inhibited root length to an increasing degree as concentration increased. Much like what Pilet and Saugy observed using exogenous IAA in maize, 5000 nM NAA dramatically reduced root elongation, and in the case of burley tobacco completely prevented any further root elongation *in vitro.*

In addition, it has been shown that exogenous application of BA to rice seedlings reduced root elongation, but increased lateral root growth (Liu et al, 2000). These findings are similar to what we observed in burley tobacco, albeit at much lower concentrations than were used by Liu et al. From this information, we decided that the initial concentrations we used were too high for both hormone types.

From the information gathered in the initial *in vitro* root length experiment, we decided that the concentrations we used were too high for both hormone types. Therefore, a second round of *in vitro* evaluations at lower than the initial concentrations were conducted using the same method as the previous experiment. The ANOVA for the second *in vitro* root length experiment is shown below in Table 2. There were three significant main effects impacting *in vitro* root length during the second experiment. Variety, hormone type, and concentration had significant effects on *in vitro* root length.

Figure 3 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another. This is

Table 2 Analysis of Variance for the second *in vitro* root length experiment.

In vitro Root Length			
Source of Variation	F Value	Pr > F	Significance
Variety	19.66	<0.0001	s
Hormone	25.89	<0.0001	s
Concentration	15.74	<0.0001	s
Variety*Hormone	0.34	0.5631	ns
Variety*Concentration	0.16	0.9200	ns
Hormone*Concentration	1.72	0.1722	ns

*ns denotes no significance and s denotes significance at α=0.05.

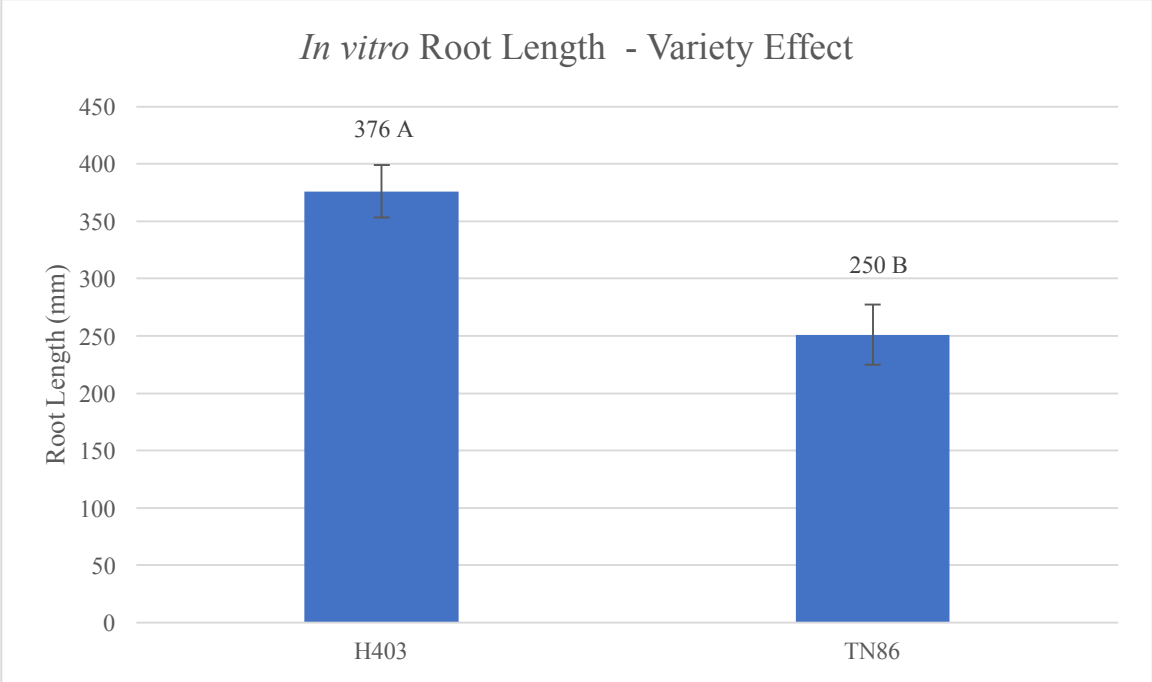

Figure 3 The effect of variety on *in vitro* root length averaged over hormone type and concentration for second experiment.
* The means marked with different letters are statistically different than each other according to Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

contrary to what was observed in the initial experiments using much higher concentrations, and indicates that there are genetic differences among the two varieties for exogenous hormone sensitivity. In hind sight, it is apparent that the genetic component is most likely responsible for the statistically significant differences between the two varieties for mean *in vitro* root length observed here, and mean ground sucker number observed in later greenhouse and field experiments.

A Dunnett's Test was conducted to determine if the effect of the two hormone types on *in vitro* root growth differed from the control. Figure 4 shows that BA, but not NAA, differed from the control. Additionally, Figure 4 shows the results of a Tukey's Honest Significant Difference Test, which determined significant differences between all possible pairwise comparisons. From this information, it was concluded that NAA, when averaged over variety and concentration used in this lab experiment, would be a suitable range to test in a greenhouse environment. It was also noted that the phenotype produced by BA, when averaged over variety and the concentrations used in this lab experiment, was different than the control. This indicates that there is evidence to suggest this range of BA concentrations could not be used as an alternative hormone treatment to determine if exogenously applied cytokinin would further disrupt the native auxin to cytokinin ratio, promote bud break, and provide proof of concept (that is, a shift in the native auxin to cytokinin ratio, as a result of the hydroponic transplant production system, is responsible for basal axillary shoot formation), without dramatically altering desirable growth characteristics. However, chlorophyll content and root weight measurements were taken to provide another parameter to determine phenotypic differences between BA treatments and a control.

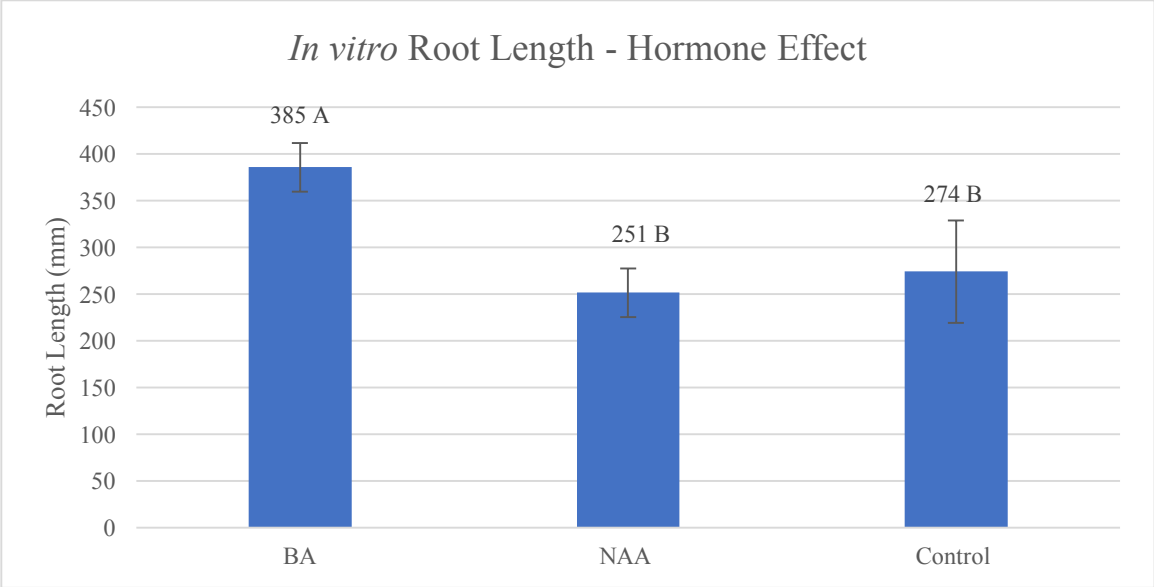

Figure 4 The effect of hormone type on *in vitro* root length averaged over variety and concentration for second experiment.
* The mean for BA is statistically different than the mean for Control; however NAA is not statistically different than the control according to Dunnett's Test at α=0.05. In addition, means marked with different letters are statistically different than each other according to Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

Figure 5 displays the results of a Dunnett's Test conducted to determine if any of the hormone concentrations differed from the control. It was concluded that all the hormone concentrations did not differ from the control concentration of 0, except for BA 2 and 50 nM. The results of a Tukey's Honest Significant Difference Test is also shown in Figure 5. From this information, we successfully identified a NAA range (2-250 nM) that was not significantly different from the control, and a BA range that produced roots longer on average than the control (this is contrary to what was observed at the initial concentrations). From this, we determined a NAA range (2-50 nM) to use in greenhouse evaluations to determine the effect of exogenous NAA on *in vivo* ground sucker number. More information needed to be collected on BA to make a similar decision.

In addition to root length, Chlorophyll Fluorescence Ratio (CFR) data was taken during the second *in vitro* experiment, and used as another measure to determine if the chosen hormones and concentrations significantly impacted normal plant growth and development (chlorophyll content/leaf color). The ANOVA for *in vitro* CFR is shown in Table 3. There were three significant main effects impacting *in vitro* CFR. Variety, hormone type, and hormone concentration had significant effects on *in vitro* CFR. This is consistent with what we observed for *in vitro* root length.

Figure 6 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another for CFR. This is consistent with what was observed for *in vitro* root length; i.e., there is evidence that indicates genetic differences among the two varieties for exogenous hormone sensitivity.

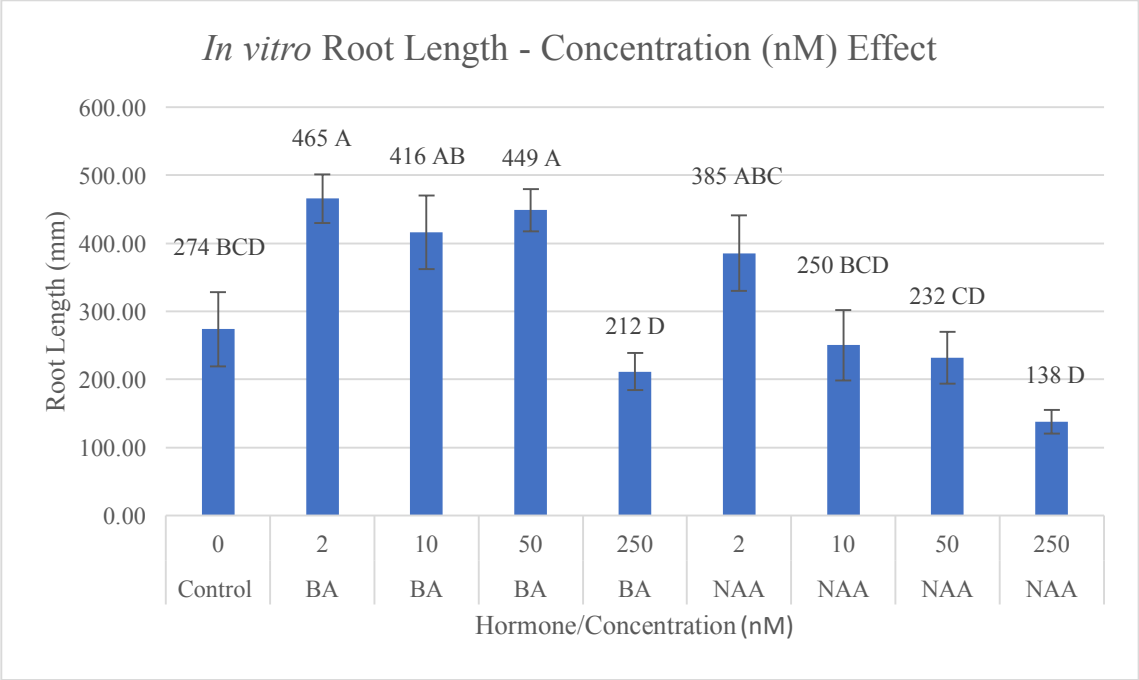

Figure 5 The effect of hormone concentration on *in vitro* root length averaged over variety and hormone type for 2nd experiment.
* BA 2 and 50 nM concentration means are statistically different than the control according to Dunnett's Test at α=0.05; in addition, all means marked with a different letter are statistically different than one another according to Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

Table 3 Analysis of Variance for *in vitro* CFR experiment.

In vitro Chlorophyll Fluorescence Ratio (CFR)			
Source of Variation	F Value	Pr > F	Significance
Variety	5.98	0.0176	s
Hormone	12.06	0.0010	s
Concentration	14.45	<.0001	s
Variety*Hormone	2.68	0.1069	ns
Variety*Concentration	0.18	0.9122	ns
Hormone*Concentration	2.69	0.0549	ns

*ns denotes no significance and s denotes significance at α=0.05.

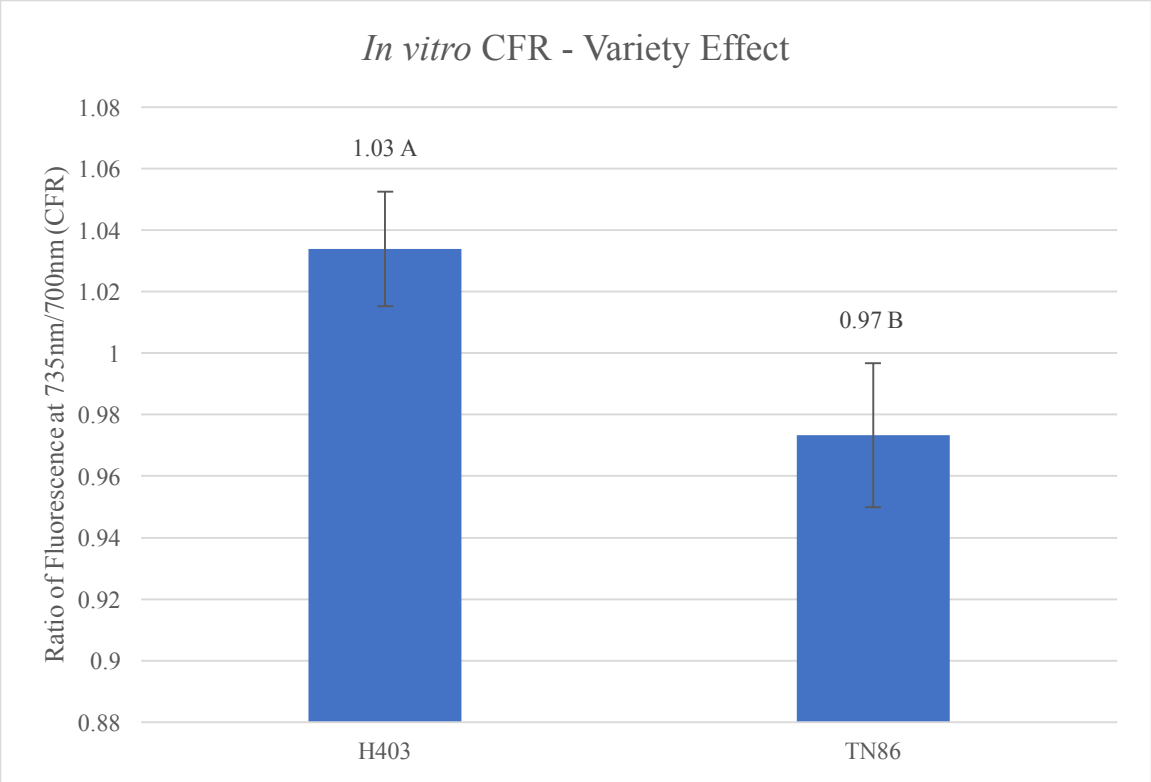

Figure 6 The effect of variety on *in vitro* Chlorophyll Fluorescence Ratio (CFR) averaged over hormone type and concentration.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.

*Standard error of the mean is displayed as the error bar at the apex of each mean bar.

*Numeric values on the apex of each mean bar represent mean values.

Once again, the importance of this observed genetic difference among the two varieties became more apparent during the *in vivo* experiments.

A Dunnett's Test was conducted to determine if the effect of the two hormone types on *in vitro* CFR differed from the control. Neither BA nor NAA differed from the control (Figure 7). Additionally, Figure 7 shows the results of a Tukey's Honest Significant Difference Test comparing all possible pairs. From this information, it was concluded that NAA, when averaged over variety and concentration used in this lab experiment, would be a suitable range to test in a greenhouse environment. It was also noted that the phenotype produced by BA, when averaged over variety and the concentrations used in this lab experiment, was not different than the control. This is contrary to what was observed for *in vitro* root length, where BA was found to be different than the control. However, when considering our original hypothesis, that an increase in root ball size due to the hydroponic transplant production system causes an imbalance in the auxin cytokinin ratio, a treatment that increases root length (exogenous BA) could provide a proof of concept. From this information, it was determined that BA, averaged over variety and concentrations, would be a suitable range (2-50 nM) to test in a greenhouse situation.

Figure 8 displays the results of a Dunnett's Test conducted to determine if any of the hormone concentrations differed from the control. It was concluded that none of the hormone concentrations differed from the control concentration of 0, except NAA 250 nM. The results of a Tukey's Honest Significant Difference Test is also shown in Figure 8. These results were not unexpected, NAA at higher concentrations has herbicidal effects similar to 2,4-D, another synthetic auxin molecule. In this case, NAA 250 nM

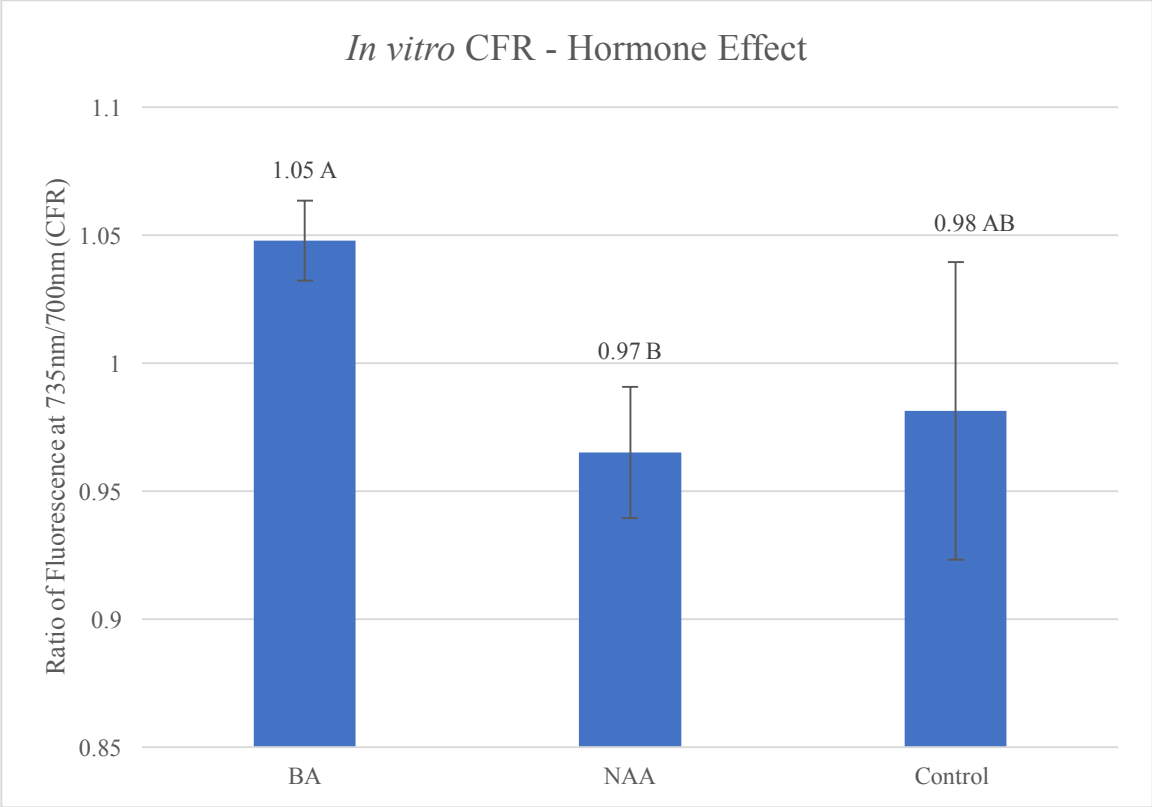

Figure 7 The effect of hormone type on *in vitro* Chlorophyll Fluorescence Ratio (CFR) averaged over variety and concentration.
* Neither the mean for BA or NAA is statistically different than the mean for Control, per Dunnett's Test at α=0.05. In addition, means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

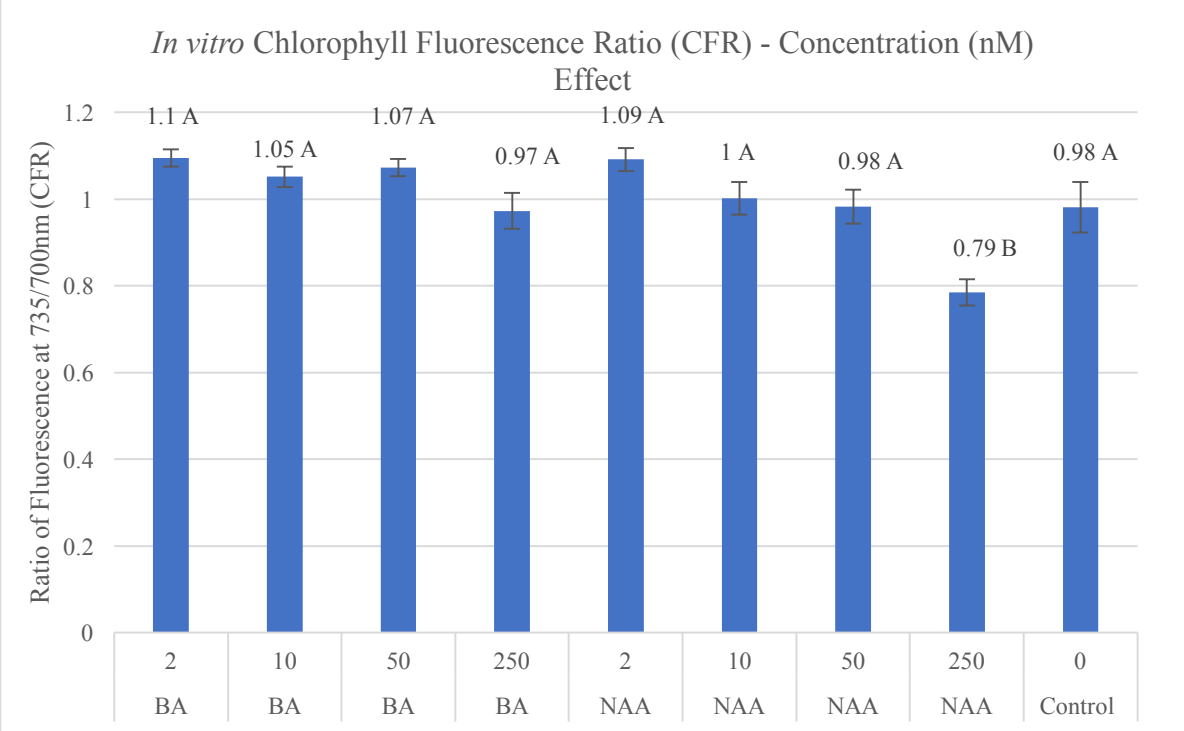

Figure 8 The effect of hormone concentration on *in vitro* CFR averaged over variety and hormone type.

* The NAA 250 nM concentration mean is statistically different from the control according to Dunnett's Test at α=0.05; in addition, all means marked with a different letter are statistically different than one another according to Tukey's Honest Significant Difference Test at α=0.05.

*Standard error of the mean is displayed as the error bar at the apex of each mean bar.

*Numeric values on the apex of each mean bar represent mean values.

produced plants that had more chlorotic leaves than the control or any other hormone treatments. For this reason, 250 nM NAA was not used as a treatment in subsequent greenhouse evaluations.

In addition to root length and CFR, root fresh weight data was taken during the second *in vitro* experiment and used as another measure to determine if the chosen hormones and concentrations significantly impacted normal plant growth and development (root mass). The ANOVA for *in vitro* root fresh weight is shown in Table 4. There was one significant main effect impacting *in vitro* root fresh weight. Variety had a significant effect on *in vitro* root fresh weight; however hormone type and concentration did not.

Figure 9 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another for *in vitro* root fresh weight. This is consistent with what was observed for *in vitro* root length and CFR; i.e., there is evidence that indicates genetic differences among the two varieties for exogenous hormone sensitivity. Furthermore, the importance of this observed genetic difference among the two varieties became more apparent during the *in vivo* experiments.

Summary of *In vitro* Findings

It was determined that the initial hormone concentrations (BA 10-200 nM and NAA 200-5000 nM) were too high; i.e., detrimental effects on plant growth were observed. Next, a different set of hormone concentrations were tested (BA 2-250 nM and NAA 2-250 nM). From the information gathered in that experiment, it was determined that NAA 2-50 nM and BA 2-50 nM would be suitable concentration ranges to test in the

Table 4 Analysis of Variance for *in vitro* root fresh weight (g).

In vitro root fresh weight (g)			
Source of Variation	F Value	Pr > F	Significance
Variety	7.74	0.0073	s
Hormone	1.25	0.2676	ns
Concentration	0.41	0.7442	ns
Variety*Hormone	0.00	0.9814	ns
Variety*Concentration	0.02	0.9956	ns
Hormone*Concentration	1.75	0.1672	ns

*ns denotes no significance and s denotes significance at α=0.05.

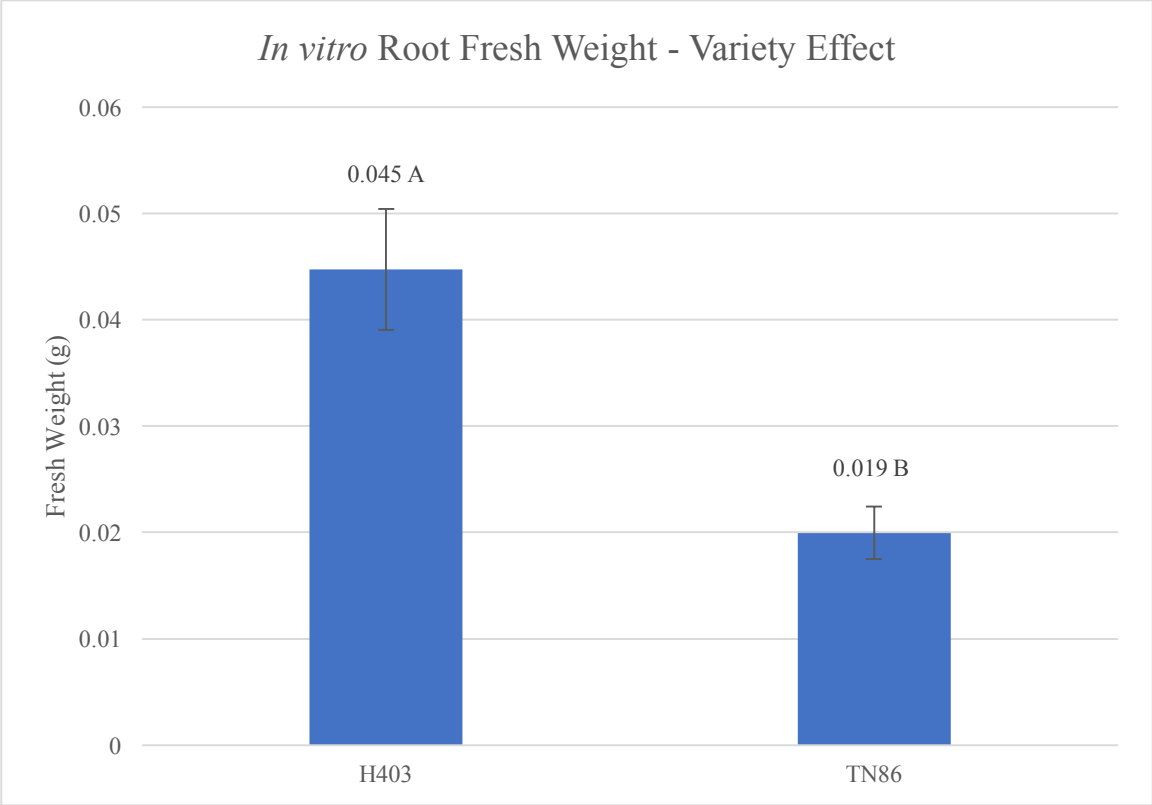

Figure 9 The effect of variety on *in vitro* root fresh weight averaged over hormone type and concentration.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

hydroponic tobacco transplant production system in a controlled greenhouse environment.

Greenhouse Evaluation of the Effect of Exogenous NAA and BA on Tobacco

For the greenhouse experiments that took place in 2014, TN86 and H403 seedlings were grown in a controlled greenhouse environment on the University of Kentucky campus in Lexington, Kentucky. Three application times were evaluated using 2, 10, and 50 nM BA and 2, 10, and 50 nM NAA, and untreated controls; the treatments were applied at either 2, 4 or 8 weeks post seeding. All trays were placed in individual float beds, and arranged in a completely randomized design (CRD) with two replications. All measurements were made 14 days post transplanting. Data collected from each application time were analyzed as separate experiments.

The ANOVA for greenhouse leaf number when the hormones were applied two weeks post seeding is shown in Table 5. There were no significant main effects impacting greenhouse leaf number for the initial experiments where hormone treatments were applied two weeks post seeding. This indicates that whenever any of the chosen hormone treatments were applied two weeks post seeding, leaf number was not affected. These results are desirable, considering the goal of this research was the identification of hormone treatments that do not negatively affect current suitable agronomic traits, but could potentially reduce basal axillary shoot (ground sucker) number in field conditions.

The ANOVA for greenhouse stem length (cm) when the hormones were applied two weeks post seeding is shown in Table 6. There were three significant main effects, and one significant interaction impacting greenhouse stem length. Variety had a significant effect on greenhouse stem length; additionally there was a significant

Table 5 Analysis of Variance for Greenhouse Leaf Number – Hormone treatments applied two weeks post seeding.

Greenhouse Leaf Number - Application Two Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	0.02	0.8969	ns
Hormone	3.58	0.0607	ns
Concentration	2.85	0.0615	ns
Variety*Hormone	0.4	0.5294	ns
Variety*Concentration	1.34	0.2650	ns
Hormone*Concentration	2.17	0.1186	ns

*ns denotes no significance and s denotes significance at α=0.05.

Table 6 Analysis of Variance for Greenhouse Stem Length (cm) – Hormone treatments applied two weeks post seeding.

Greenhouse Stem Length (cm) - Application Two Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	29.59	<0.0001	s
Hormone	16.81	<0.0001	s
Concentration	11.45	<0.0001	s
Variety*Hormone	0.28	0.5969	ns
Variety*Concentration	3.05	0.0508	ns
Hormone*Concentration	38.89	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*A significant interaction trumps the individual main effects.

interaction between hormone type and concentration. This indicates that whenever any of the chosen hormone treatments were applied two weeks post seeding, stem length was affected. These results are undesirable, considering the goal of this research was the identification of hormone treatments that do not negatively affect current suitable agronomic traits, but could potentially reduce basal axillary shoot number in field conditions.

Figure 10 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another for greenhouse stem length. This is consistent with what was observed for *in vitro* root length; i.e., there is evidence that indicates genetic differences among the two varieties for exogenous hormone sensitivity.

Figure 11 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if any of the hormone by concentration interactions were statistically different. It was concluded that BA 10 and 50 nM, NAA 2 and 10 nM, and the untreated control are statistically the same. In other words, BA 2 nM and NAA 50 nM stunted plant stature in both varieties. This is both contrary to and supported by findings by Sachs in a classic 1965 paper, where it was observed that exogenous auxin does not promote stem elongation. Here, we observed that auxin had no effect on stem elongation at the low and intermediate concentrations, but retarded stem elongation at the 50 nM concentration. Interestingly, BA 2 nM produced plants with stem lengths statistically different than 50 nM NAA, but consistently shorter than the control plants.

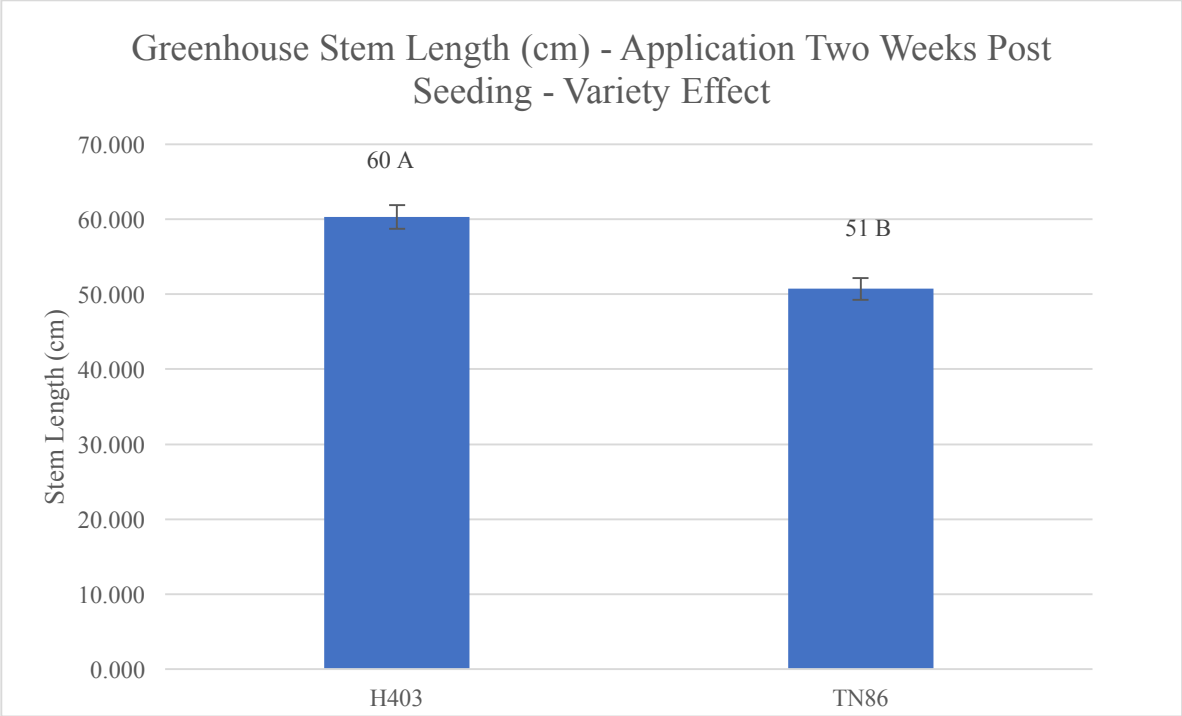

Figure 10 The effect of variety on greenhouse stem length (cm) averaged over hormone type and concentration.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

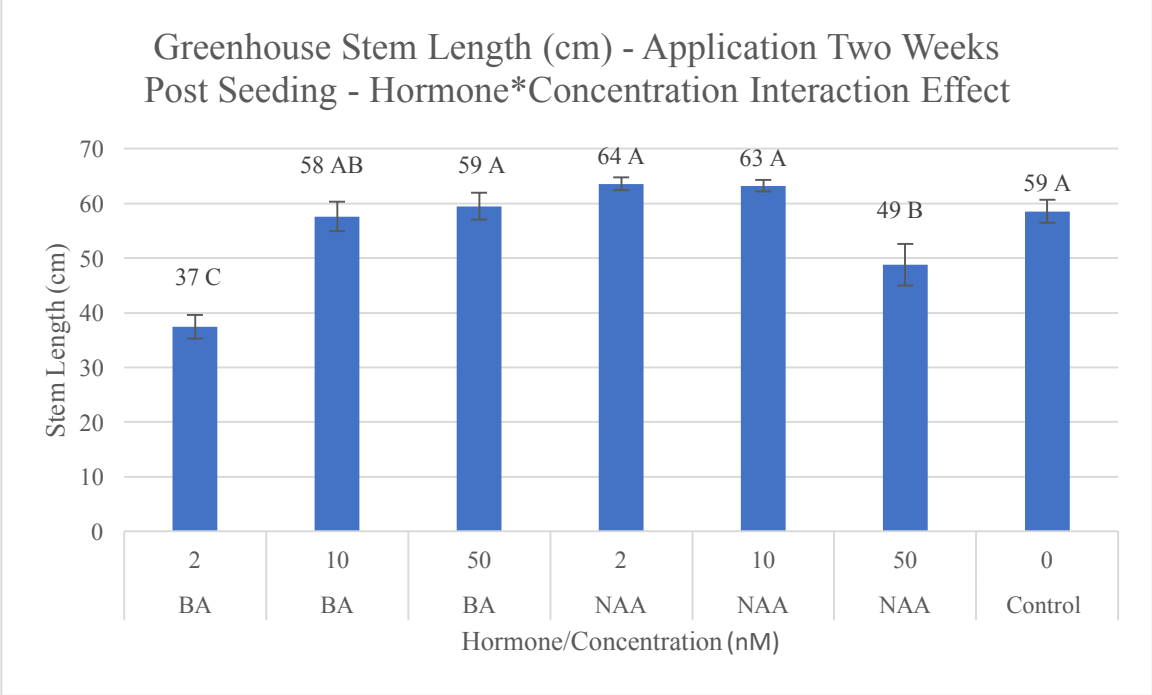

Figure 11 The effect of hormone*concentration interaction applied two weeks post seeding on greenhouse stem length (cm) averaged over variety.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

The ANOVA for greenhouse basal axillary shoot number when the hormones were applied two weeks post seeding is shown in Table 7. There were three significant main effects impacting greenhouse ground sucker number. Variety, hormone type, and hormone concentration had significant effects on greenhouse ground sucker number.

Figure 12 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another for greenhouse ground sucker number. Once again, the two varieties show a markedly different response to the treatments, and an inherent difference for observed ground sucker number in the control plants. It is worth noting here that a significant difference among varieties was potentially the only true consistency we observed in all experiments.

Figure 13 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if any of the hormone types were statistically different. It was concluded that BA, NAA, and the untreated control are statistically the same. This is not consistent with our *in vitro* stem length findings. In that experiment, BA produced plants that were significantly different than the control plants; in this experiment, BA produced plants that were statistically the same as the control. In other words, BA produced plants with different stem lengths than the control, but the same amount of basal axillary shoots. However, it is consistent with our *in vitro* findings for stem length when NAA is the hormone applied. In that experiment, NAA produced an effect not different from the control, which is what we observed here for ground sucker number. Nevertheless, this is undesirable. Unfortunately, this series of greenhouse experiments was not successful in

Table 7 Analysis of Variance for Greenhouse Basal Axillary Shoot (Ground Sucker) number – Hormone treatments applied two weeks post seeding.

Greenhouse Ground Sucker Number - Application Two Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	161.38	<0.0001	s
Hormone	6.88	0.0098	s
Concentration	6.94	0.0014	s
Variety*Hormone	2.27	0.1347	ns
Variety*Concentration	0.56	0.5744	ns
Hormone*Concentration	0.37	0.6927	ns

*ns denotes no significance and s denotes significance at α=0.05.

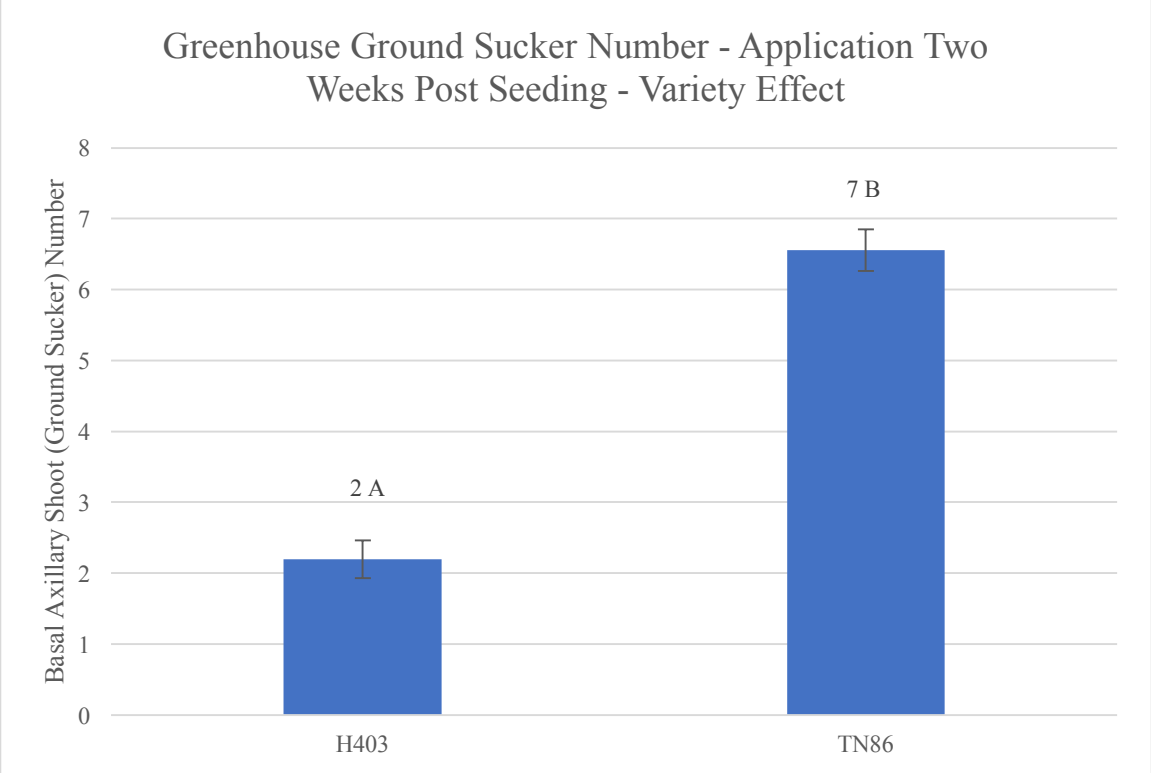

Figure 12 The effect of variety on greenhouse ground sucker number averaged over hormone type and concentration.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

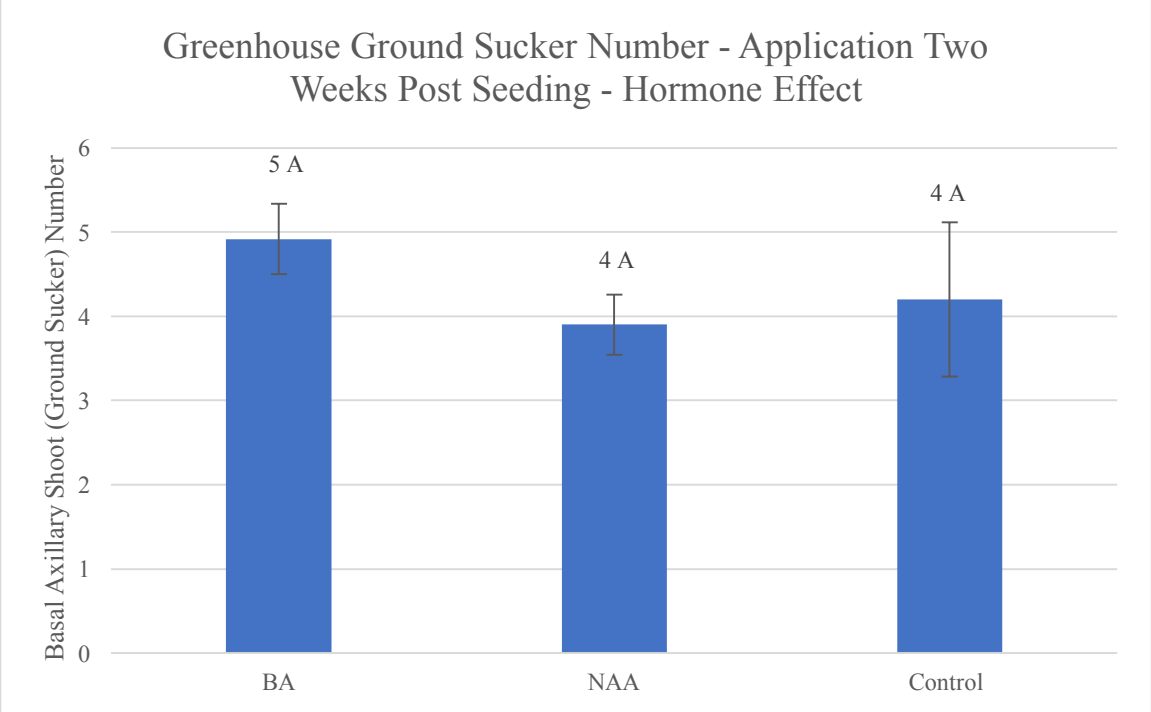

Figure 13 The effect of hormone type when applied two weeks post seeding on greenhouse ground sucker number averaged over variety and concentration.
*Means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

identifying a hormone treatment that produced an effect not different than the control for every measurement except total ground sucker number.

Figure 14 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if any of the hormone concentrations differed from one another. From this figure, one can see that none of the hormone concentrations differed from the control; however 2 nM NAA treated plants had the lowest number of basal axillary shoots and was statistically different from the intermediate and high BA concentrations. According to the classical apical dominance hypothesis in its simplest form, the observed results are what one would expect; NAA should lower total ground sucker number, and BA should increase total ground sucker number. However, this was not observed across concentrations.

The data from the four weeks post seeding application time will be presented following the same format as above. The ANOVA for greenhouse leaf number when the hormones were applied four weeks post seeding is shown in Table 8. There was one significant main effect impacting greenhouse leaf number when hormone treatments were applied four weeks post seeding. Concentration affected leaf number whenever hormones were applied four weeks post seeding. These results are undesirable, considering the goal of this research was the identification of hormone treatments that do not negatively affect current suitable agronomic traits, but could potentially reduce basal axillary shoot number under field conditions. However, the overall model term is insignificant (Table 8) and therefore trumps the individual significance of concentration. This is comparable to what was observed whenever the hormones were applied two

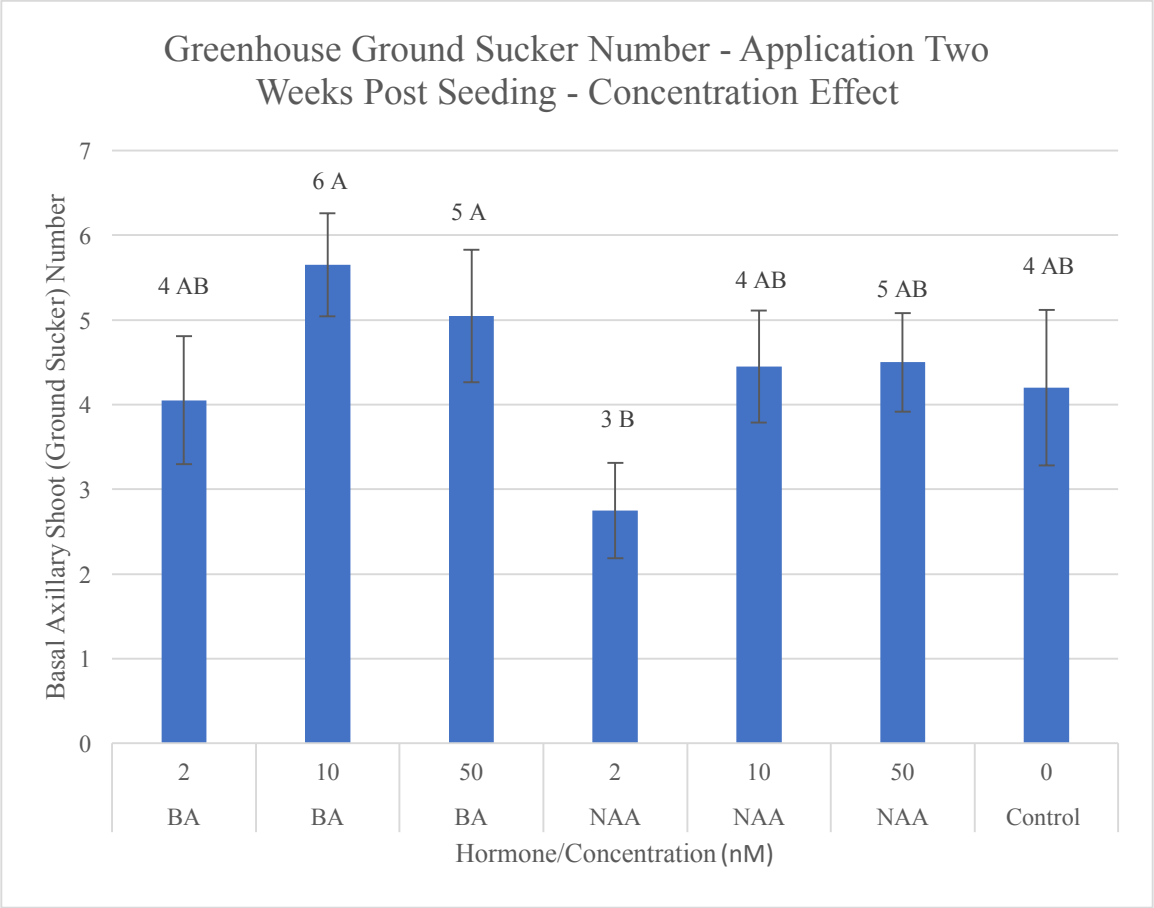

Figure 14 The effect of hormone concentration applied two weeks post seeding on greenhouse ground sucker number averaged over variety.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

Table 8 Complete Analysis of Variance for Greenhouse Leaf Number – Hormone treatments applied four weeks post seeding.

Greenhouse Leaf Number - Application Four Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	0.10	0.7468	ns
Hormone	0.74	0.3922	ns
Concentration	4.24	0.0164	s
Variety*Hormone	2.73	0.1011	ns
Variety*Concentration	0.80	0.4523	ns
Hormone*Concentration	0.45	0.6391	ns
Model	1.41	0.1761	ns
Error			

*ns denotes no significance and s denotes significance at α=0.05.
*overall model is nonsignificant

weeks post seeding; i.e., leaf number was not affected by the treatments when applied four weeks post seeding.

The ANOVA for greenhouse stem length (cm) when the hormones were applied four weeks post seeding is shown in Table 9. When tested at α=0.05, there were no significant main effects impacting greenhouse stem length when hormones were applied four weeks post seeding. This is contrary to what was observed when hormones where added to the float water at two weeks post seeding. It appears that whenever more time elapses from seeding to hormone application there is less of an effect on obvious agronomic traits, such as stem length (cm).

The ANOVA for greenhouse basal axillary shoot number when the hormones were applied four weeks post seeding is shown in Table 10. There was one significant main effect impacting greenhouse ground sucker number. Variety had a significant effect on greenhouse ground sucker number when the hormones where applied four weeks post seeding. This is consistent with what was observed in previous experiments; i.e., variety has a big effect. These two varieties were chosen based on the assumption of being unrelated, and exhibiting different propensities to form basal axillary shoots. This assumption is clearly validated when the previous results are compiled and considered.

Figure 15 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if the two varieties selected for the experiment were statistically different. It was concluded that the two varieties differed from one another for greenhouse ground sucker number whenever hormones were applied four weeks post seeding. Once again, the two varieties show a markedly different response to the treatments, and an inherent difference for observed ground sucker number in the control

Table 9 Analysis of Variance for Greenhouse Stem Length (cm) – Hormone treatments applied four weeks post seeding.

Greenhouse Stem Length (cm) - Application Four Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	1.79	0.1839	ns
Hormone	1.62	0.2060	ns
Concentration	2.36	0.0988	ns
Variety*Hormone	0.85	0.3575	ns
Variety*Concentration	1.03	0.3592	ns
Hormone*Concentration	0.48	0.6193	ns

*ns denotes no significance and s denotes significance at α=0.05.

Table 10 Analysis of Variance for Greenhouse Basal Axillary Shoot (Ground Sucker) number – Hormone treatments applied four weeks post seeding.

Greenhouse Ground Sucker Number - Application Four Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	105.1	<0.0001	s
Hormone	0.25	0.6152	ns
Concentration	0.25	0.7762	ns
Variety*Hormone	0.05	0.8294	ns
Variety*Concentration	1.04	0.3559	ns
Hormone*Concentration	0.22	0.8006	ns

*ns denotes no significance and s denotes significance at α=0.05.

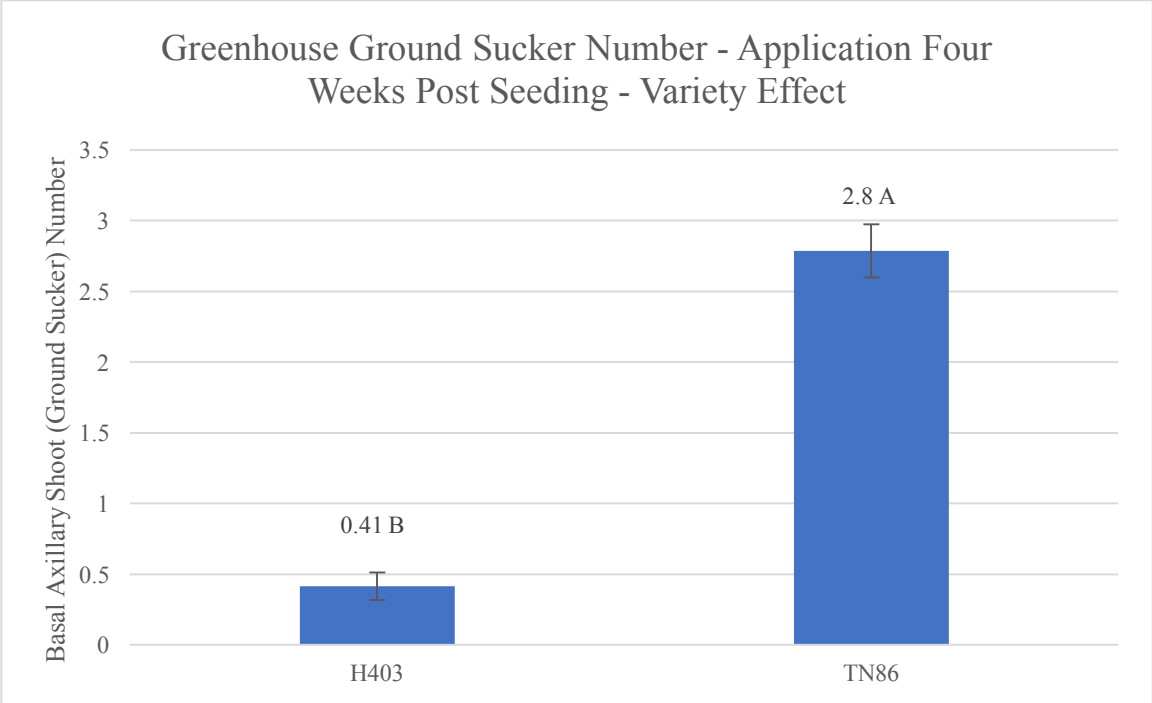

Figure 15 The effect of variety on greenhouse ground sucker number averaged over hormone type and concentration.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

plants. At this point, it became apparent that basal axillary shoot development is under intense genetic control, and that the two varieties used in the experiment have two very different genetic identities (in terms of the genes involved in basal axillary shoot development). In hind sight, identifying and altering the genetic action behind basal axillary shoot development would likely be the most successful method to completely suppress ground sucker formation in cultivated tobacco.

The data from the eight weeks post seeding application time will be presented using the same format as with the two and four weeks post seeding application times. The ANOVA for greenhouse leaf number when the hormones were applied eight weeks post seeding is shown in Table 11. There was one significant main effect impacting greenhouse leaf number when hormone treatments were applied eight weeks post seeding. Variety affected leaf number whenever hormones were applied eight weeks post seeding. These results are undesirable, considering the goal of this research was the identification of hormone treatments that do not negatively affect current suitable agronomic traits, but could potentially reduce basal axillary shoot number in field conditions. However, the overall model term is insignificant (Table 11) and therefore trumps the individual significance of variety. This is comparable to what was observed whenever the hormones were applied two or four weeks post seeding; i.e., leaf number was not affected by the treatments.

The ANOVA for greenhouse stem length when the hormones were applied eight weeks post seeding is shown in Table 12. There were two significant main effects, and one significant interaction impacting greenhouse stem length. Hormone and concentration had significant effects on greenhouse stem length; additionally there was a

Table 11 Complete Analysis of Variance for Greenhouse Leaf Number – Hormone treatments applied eight weeks post seeding.

Greenhouse Leaf Number - Application Eight Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	4.78	0.0306	s
Hormone	2.05	0.1546	ns
Concentration	0.11	0.8996	ns
Variety*Hormone	1.08	0.2997	ns
Variety*Concentration	1.23	0.2950	ns
Hormone*Concentration	0.28	0.7534	ns
Model	1.49	0.1414	ns
Error			

*ns denotes no significance and s denotes significance at α=0.05.
*overall model is nonsignificant

Table 12 Analysis of Variance for Greenhouse Stem Length (cm) – Hormone treatments applied eight weeks post seeding.

Greenhouse Stem Length (cm) - Application Eight Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	0	0.99	ns
Hormone	7.4	0.0074	s
Concentration	5.23	0.0066	s
Variety*Hormone	0.03	0.8585	ns
Variety*Concentration	0.5	0.6096	ns
Hormone*Concentration	4.81	0.0097	s

*ns denotes no significance and s denotes significance at α=0.05.
*A significant interaction trumps the individual main effects.

significant interaction between hormone type and concentration. Variety had no effect, which contradicts what was observed for greenhouse stem length whenever the hormones were added two weeks post seeding. This information indicates that eight weeks post seeding is not too late to apply the hormone treatments and still see a significant effect, which directly contradicts what was observed for the four weeks post seeding application time. The four week application time produced no significant differences in stem length.

Figure 16 displays the results of a Tukey's Honest Significant Difference Test conducted to determine if any of the hormone type/concentration interactions were statistically different. Contrary to what was previously observed BA 50 nM significantly stunted plants compared to the control, otherwise these results are consistent with what was observed for the two weeks post seeding application.

The ANOVA for greenhouse basal axillary shoot number when the hormones were applied eight weeks post seeding is shown below in Table 13. There were three significant main effects (Variety, Hormone Type, and Hormone Concentration), and one significant interaction (Variety*Hormone) impacting greenhouse ground sucker number.

Figure 17 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among variety/hormone type combinations selected for the experiment. It was concluded that the effect of hormone type differed between the two varieties. Additionally, it appears that NAA treatments effectively reduced sucker number in TN86, and did not increase sucker number in H403. These results were promising and indicate that NAA applied eight weeks post seeding could potentially reduce basal axillary shoot number in varieties with a tendency to produce

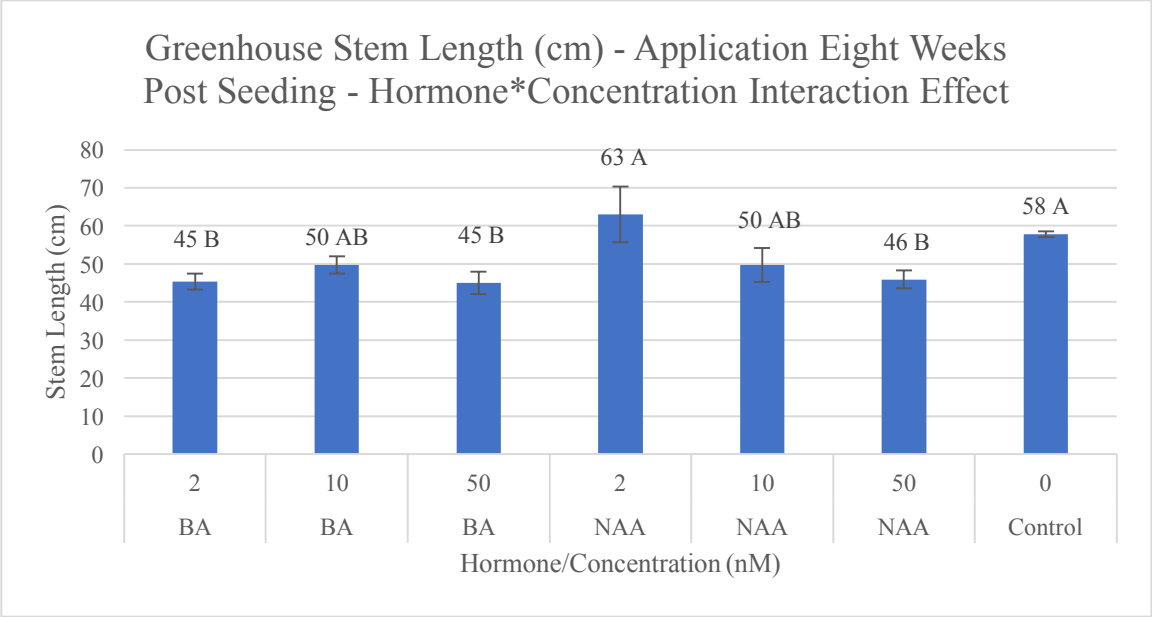

Figure 16 The effect of hormone*concentration interaction applied eight weeks post seeding on greenhouse stem length (cm) averaged over variety.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

Table 13 Analysis of Variance for Greenhouse Basal Axillary Shoot (Ground Sucker) number – Hormone treatments applied eight weeks post seeding.

Greenhouse Ground Sucker Number - Application Eight Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Variety	100.37	<0.0001	s
Hormone	12.88	0.0005	s
Concentration	4.69	0.0109	s
Variety*Hormone	6.35	0.013	s
Variety*Concentration	0.86	0.4245	ns
Hormone*Concentration	0.44	0.6431	ns

*ns denotes no significance and s denotes significance at α=0.05.

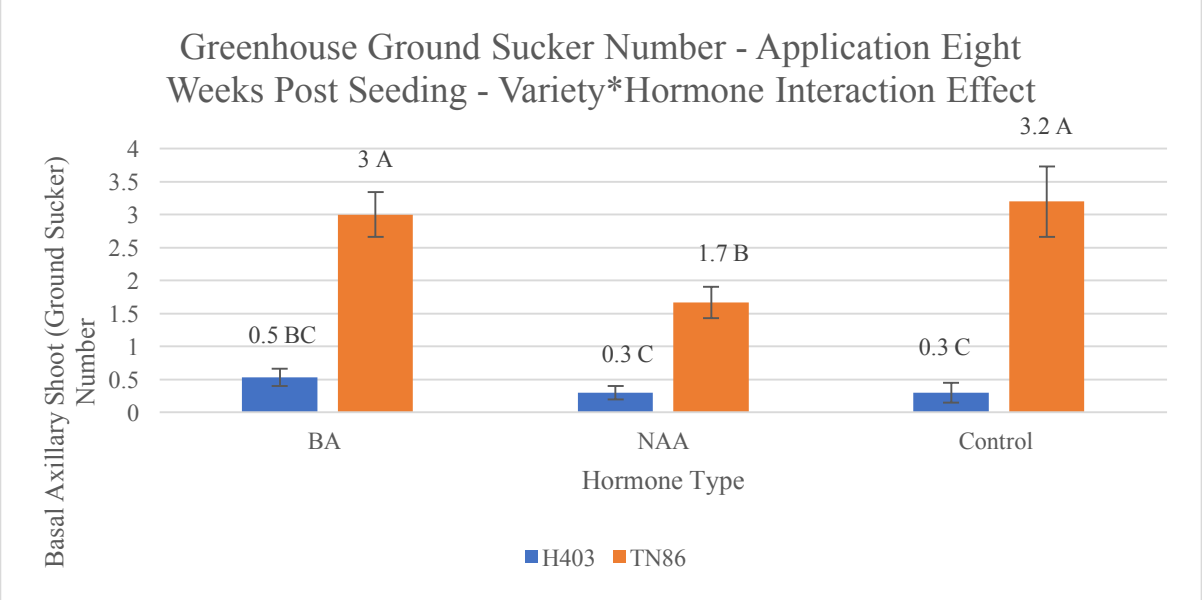

Figure 17 The effect of variety*hormone interaction when applied eight weeks post seeding on greenhouse basal axillary shoot number averaged over concentration.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

ground suckers, while not affecting ground sucker number in varieties without the tendency to produce basal axillary shoots.

Figure 18 displays the results of a Tukey's Honest Significant Difference Test, which show no concentrations to be significantly different from the control plants and directly contradicts what was observed for the variety by hormone interaction.

Summary of Initial Greenhouse Evaluations

From the information gathered in the first set of greenhouse experiments, it was concluded that the 2-50 nM concentration range would be appropriate for both hormones to test in the 2015 field evaluations. It was also determined that the two and four weeks post seeding application times were the most appropriate for the 2015 field evaluations. Two weeks post seeding showed the most promising results in terms of a reduction in ground sucker number, and four weeks had no significance. In other words, two weeks post seeding appeared to be the most likely application time to reduce ground sucker number in an *in vivo* setting. However, even though no significant effects were noted for the four weeks post application time, a decision was made to see if treatment effects could be detected in the field. If it worked in the field, that would be desirable; if it didn't work, it would provide hidden replications for the evaluation of the inherent difference in ground sucker formation between the two varieties in a field situation.

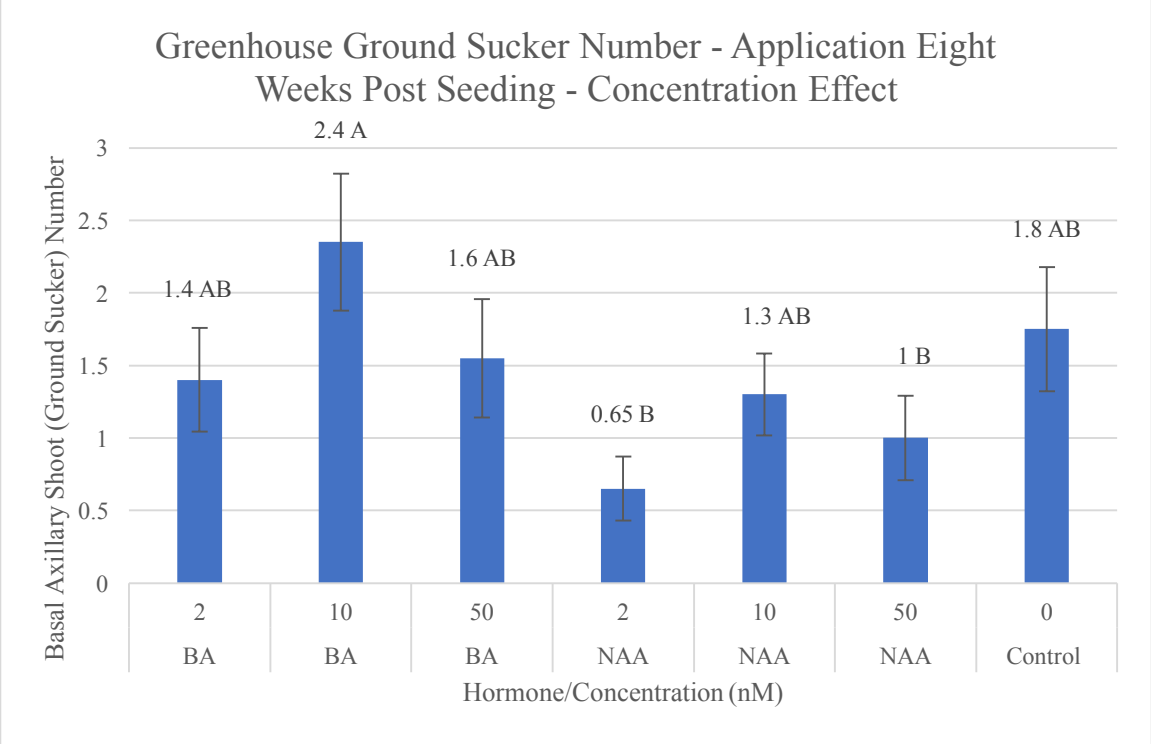

Figure 18 The effect of hormone concentration applied eight weeks post seeding on greenhouse ground sucker number averaged over variety.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.

2015 Field Evaluation of the Effect of Exogenous NAA and BA on Tobacco

In 2015, H403 and TN86 seedlings were grown in controlled greenhouse environments on the University of Kentucky campus in Lexington, Kentucky (one set of plants for Lexington and one set of plants for Versailles/Woodford Co.), and the University of Tennessee Research and Education Center in Greeneville, Tennessee (one set plants for Greeneville). Seven hormone treatments (2, 10, and 50 nM NAA and 2, 10, and 50 nM BA, and an untreated control), and two application times (2 and 4 weeks post seeding) were evaluated using a split-split plot design replicated three times per location. At each of the three locations, sucker data were collected approximately 50 days post transplanting, rather than 14 days post transplanting interval used in the greenhouse experiments. From the results of the preliminary studies, it was concluded that the two varieties (TN86 and H403) behave inherently differently in terms of ground sucker formation, and in their response to exogenous auxin and cytokinin application. In addition, the three locations used for the field evaluations were chosen to provide different growing environments, which could result in different levels of ground sucker pressure. For these reasons the 2015 field data were analyzed separately for each variety/location combination.

The data indicated there were no significant main effects impacting ground sucker number for H403 at Greenville. The ANOVA for H403 field basal axillary shoot number at Greeneville is shown in Table 14. This was not unexpected. Previous greenhouse results showed that H403 did not respond as dramatically as TN86 to exogenous hormone application, and are supported by this set of field data.

Table 14 Analysis of Variance for H403 at Greeneville Basal Axillary Shoot (Ground Sucker) number.

Greeneville H403 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	2.25	0.1344	ns
Hormone	1.47	0.2254	ns
Application*Hormone	0.41	0.5208	ns
Concentration	1.12	0.3405	ns
Application*Concentration	1.43	0.2324	ns
Hormone*Concentration	2.56	0.0543	ns
Application*Hormone*Concentration	2.18	0.0895	ns

*ns denotes no significance and s denotes significance at α=0.05.

The ANOVA for TN86 field basal axillary shoot number at Greeneville is shown in Table 15. There was one significant main effect (Concentration) and one significant interaction (Application*Concentration). Due to the interaction between application time and concentration, this combination of treatments was analyzed and presented together. Figure 19 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among Application*Concentration combinations selected for the experiment. For the two weeks post seeding application time, no concentrations used in the experiment were statistically different than the control concentration of zero for field ground sucker number averaged over hormone type, when tested at α=0.05. For the four weeks post seeding application time, 2 and 10 nM had significantly lower field ground sucker numbers averaged over hormone type than the control or the 50 nM treatments.

At Lexington, there was one significant main effect (Hormone Type) impacting ground sucker number for H403, and two significant interaction effects (Application*Concentration and Application*Hormone*Concentration) (Table 16). For TN 86, there were no significant main effects impacting ground sucker number at Lexington; however there were two significant interactions (Application*Concentration and Application*Hormone*Concentration) (Table17). Figure 20 displays the results of a Tukey's Honest Significant Difference test conducted at α=0.05. There is evidence to suggest that there are differences in mean ground sucker values among different treatments for each variety. However, there is no useful trend.

Table 15 Analysis of Variance for TN86 at Greeneville Basal Axillary Shoot (Ground Sucker) number.

Greeneville TN86 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	0.33	0.5649	ns
Hormone	2.69	0.1018	ns
Application*Hormone	0.16	0.6902	ns
Concentration	2.73	0.0432	s
Application*Concentration	10.01	<0.0001	s
Hormone*Concentration	0.42	0.7419	ns
Application*Hormone*Concentration	0.44	0.7233	ns

*ns denotes no significance and s denotes significance at α=0.05.

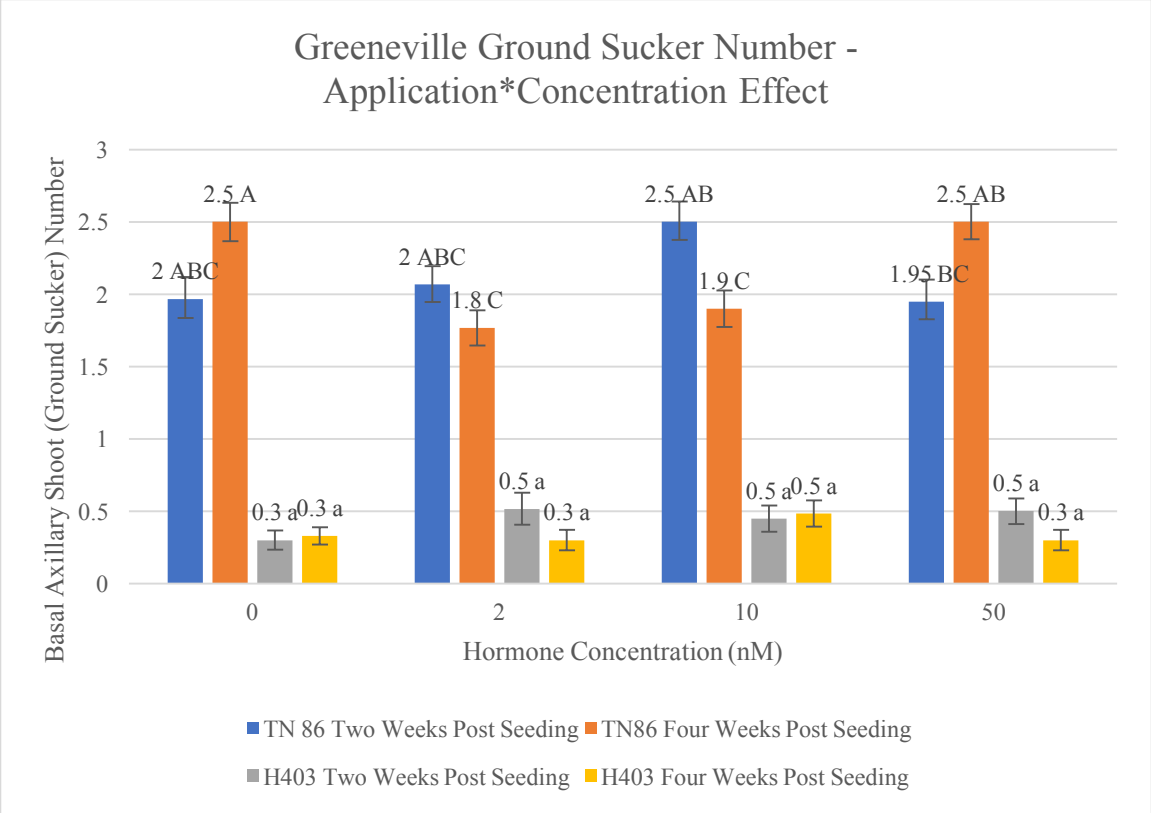

Figure 19 The effect of application*concentration interaction at Greeneville field ground sucker number averaged over hormone type.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*Note that uppercase letters represent comparisons made for TN86, and lowercase letters represent comparisons made for H403.

Table 16 Analysis of Variance for H403 at Lexington Basal Axillary Shoot (Ground Sucker) number.

Lexington H403 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	0.03	0.8549	ns
Hormone	13.39	0.0003	s
Application*Hormone	0.54	0.4647	ns
Concentration	2.33	0.0735	ns
Application*Concentration	15.45	<0.0001	s
Hormone*Concentration	2.97	0.0317	ns
Application*Hormone*Concentration	6.09	0.0005	s

*ns denotes no significance and s denotes significance at α=0.05.

Table 17 Analysis of Variance for TN86 at Lexington Basal Axillary Shoot (Ground Sucker) number.

Lexington TN86 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	0.55	0.4589	ns
Hormone	0	1	ns
Application*Hormone	0.98	0.3234	ns
Concentration	0.95	0.4179	ns
Application*Concentration	4.31	0.0052	s
Hormone*Concentration	0.64	0.5888	ns
Application*Hormone*Concentration	3.9	0.009	s

*ns denotes no significance and s denotes significance at α=0.05

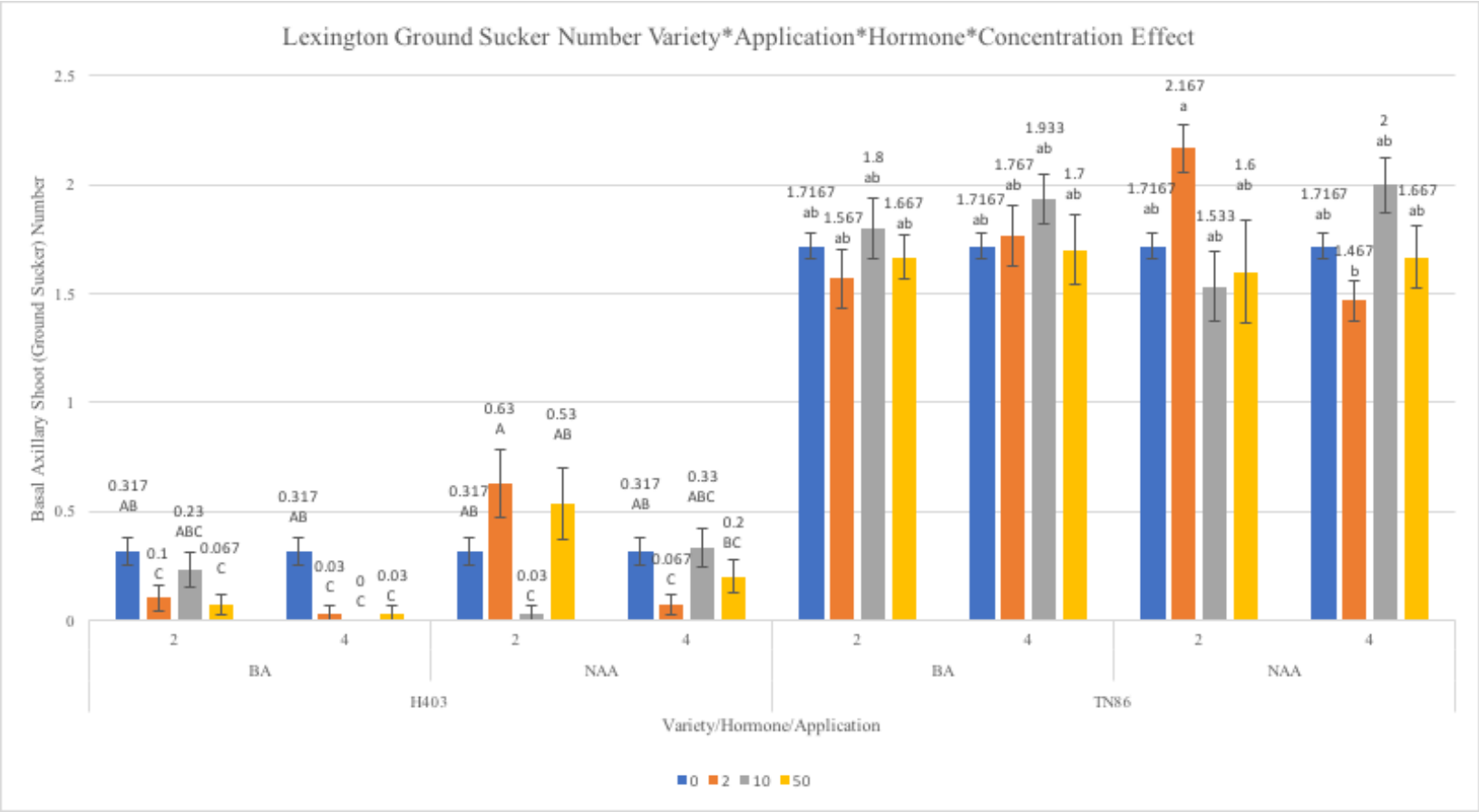

Figure 20 The effect of application*hormone*concentration interaction on H403 and TN86 at Lexington field ground sucker number.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Note that uppercase letters represent comparisons made for H403, and lowercase letters represent comparisons made for TN86.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values

At Woodford County, there was one significant effect impacting ground sucker number for H403, however the overall model is nonsignificant and trumps the apparent significance of concentration (Table 18). This is consistent with what we observed for H403 in Greeneville. For TN86, there were no significant main effects at the Woodford County site; however there was one significant interaction (Application*Concentration) (Table 19). Figure 21 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among Application*Concentration combinations applied to TN86 at Woodford Co.

2015 Field Summary

From the 2015 field results, it was determined that the hormone concentrations chosen for field evaluation were not within a high enough range to produce meaningful reductions in basal axillary shoot number in either variety. However, it was very clear from the 2015 field results that the two varieties (H403 and TN86) are genetically different from one another with regard to their propensity to develop ground suckers. It was also apparent that basal axillary shoot number is under intense genetic regulation in both varieties, and that a simple one time application of a hormone solution (at least within the range tested in 2015) will not suppress axillary shoot formation in tobacco. In addition, it is obvious that only certain varieties exhibit a problematic propensity to form basal axillary shoots. H403 is not one of these varieties, thus no further hormone evaluations were performed on this variety. From the information compiled in the 2015 field season, we decided to try the initial lab concentrations in a greenhouse setting to gauge what would happen if we used the *in vivo* concentrations in the field.

Table 18 Analysis of Variance for H403 at Woodford Co. Basal Axillary Shoot (Ground Sucker) number.

Woodford Co. H403 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	0.19	0.6606	ns
Hormone	0.77	0.38	ns
Application*Hormone	0	1	ns
Concentration	3.67	0.0124	s
Application*Concentration	1.35	0.2571	ns
Hormone*Concentration	0.64	0.5874	ns
Application*Hormone*Concentration	1.67	0.172	ns
Model	1.45	0.1128	ns
Error			

*ns denotes no significance and s denotes significance at α=0.05.
*overall model was nonsignificant

Table 19 Analysis of Variance for TN86 at Woodford Co. Basal Axillary Shoot (Ground Sucker) number.

Woodford Co. TN86 Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Application Time	0.05	0.4395	ns
Hormone	1.71	0.0891	ns
Application*Hormone	2.01	0.0867	ns
Concentration	0.46	0.619	ns
Application*Concentration	5.21	0.0019	s
Hormone*Concentration	1.15	0.1459	ns
Application*Hormone*Concentration	0.64	0.4763	ns

*ns denotes no significance and s denotes significance at α=0.05.

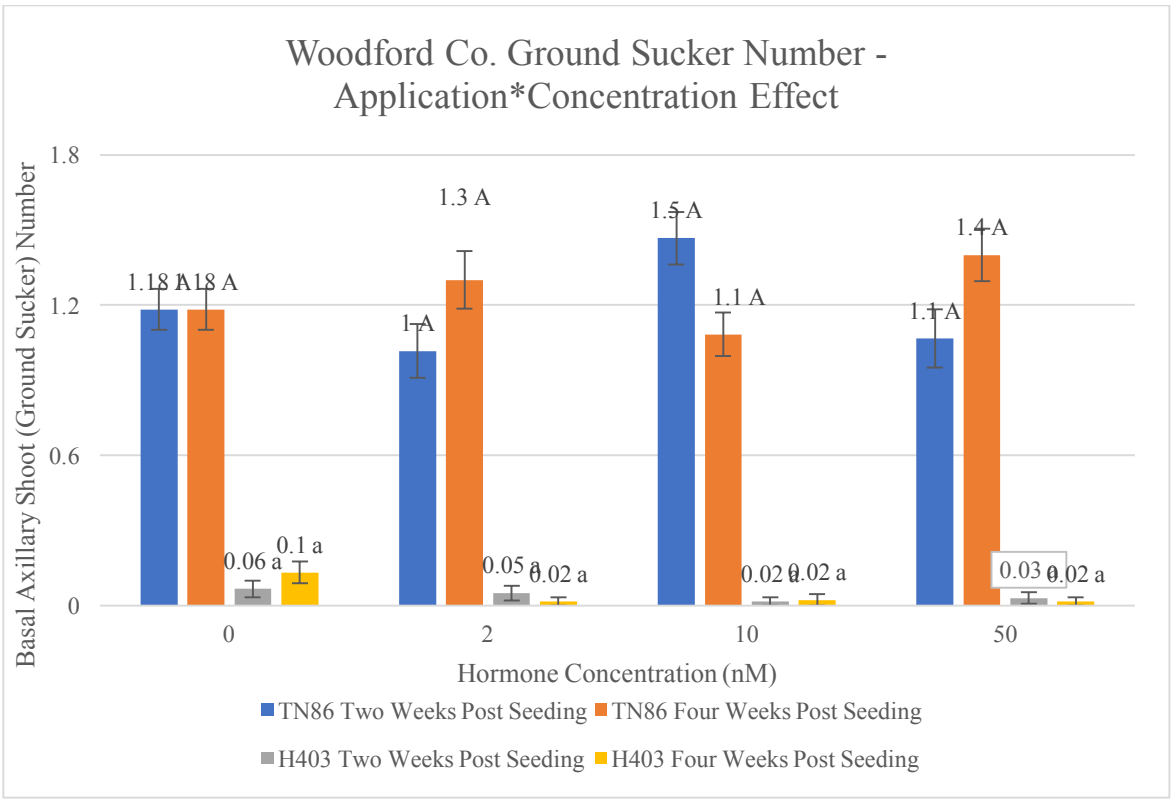

Figure 21 The effect of application*concentration interaction at Woodford Co. field ground sucker number averaged over hormone type.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.
*Standard error of the mean is displayed as the error bar at the apex of each mean bar.
*Numeric values on the apex of each mean bar represent mean values.
*Note that uppercase letters represent comparisons made for TN86, and lowercase letters represent comparisons made for H403.

Greenhouse Evaluation of the Effect of Initial Lab Concentrations on TN86

After the first field evaluation in 2015, another greenhouse experiment was conducted using TN86, the initial NAA treatments used in the *in vitro* experiments and modified BA treatments. The experiment had a total of seven hormone treatments: NAA 200 nM, 1μM, 5μM; BA 50 nM, 250 nM, 1.250 μM; and an untreated control arranged in a Completely Randomized Design with two replications. Two application times were used: a onetime application of the hormone treatment at two weeks post seeding, and a reoccurring application of the same hormone treatment at every watering. For the later application time, all water was removed from each individual float bed, and then a new water/hormone/nutrient/pesticide solution was added to bring the float bed back to the appropriate volume.

The ANOVA for greenhouse stem length (cm) when the initial lab hormone concentrations were applied two weeks post seeding is shown in Table 20. There were two significant main effects, and one significant interaction impacting greenhouse stem length. Hormone and concentration had significant effects on greenhouse stem length; additionally there was a significant interaction between hormone type and concentration.

Figure 22 displays the results of a Tukey's HSD conducted to determine significant differences among hormone*concentration combinations. It was concluded that NAA 5 μM was significantly different than all other hormone*concentration combinations. Evidence suggests all hormone*concentration combinations, other than NAA 5 μM, are statistically the same for stem length (cm). These results are inconsistent with results from previous experiments that measured stem length, but considering that all treatments did not negatively affect stem length these results are desirable.

Table 20 Analysis of Variance for Greenhouse Stem Length (cm) – Initial lab hormone treatments applied two weeks post seeding.

Greenhouse Stem Length (cm) - Initial Lab Concentrations Application Two Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Hormone	14.65	0.0003	s
Concentration	27.62	<0.0001	s
Hormone*Concentration	19.27	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*A significant interaction trumps the individual main effects.

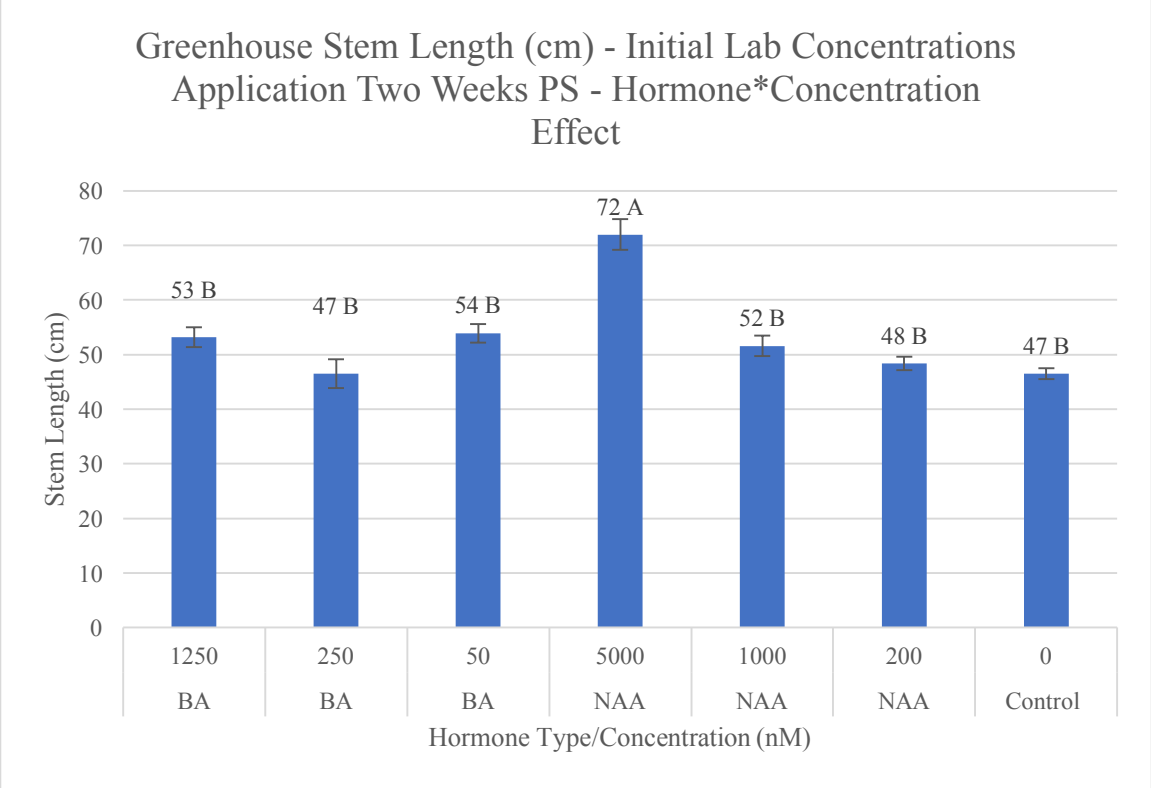

Figure 22 The effect of initial lab hormone*concentration interaction when applied two weeks post seeding on greenhouse stem length for TN86.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

The ANOVA for greenhouse basal axillary shoot number when the initial lab hormones and concentrations were applied two weeks post seeding is shown in Table 21. There were two significant main effects (Hormone and Concentration) impacting greenhouse ground sucker number. Figure 23 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among hormone types selected for the experiment. NAA was statistically different than both BA and the control for greenhouse ground sucker number. Figure 24 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among hormone concentrations selected for the experiment. From these results, it was concluded that the hormone concentrations initially evaluated in the laboratory studies should be tested in a field situation. It appears that although this range of concentrations was detrimental to growth in an *in vitro* environment, they do not pose the same problem when scaled up to the hydroponic transplant production system. Thus, it would be appropriate to test these concentrations in a field setting, especially when coupled with findings displayed in Figure 24.

Next, the ANOVA for greenhouse stem length (cm) when the initial lab hormone concentrations were applied at each watering post seeding is shown in Table 22. There were two significant main effects, and one significant interaction impacting greenhouse stem length. Hormone and concentration had significant effects on greenhouse stem length; additionally, there was a significant interaction between hormone type and concentration.

Figure 25 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among hormone*concentration

Table 21 Analysis of Variance for Greenhouse Basal Axillary Shoot (Ground Sucker) number – Initial lab hormone treatments applied two weeks post seeding.

Greenhouse Ground Sucker Number - Initial Lab Concentrations Applied Two Weeks Post Seeding			
Source of Variation	F Value	Pr > F	Significance
Hormone	120.86	<0.0001	s
Concentration	9.88	0.0002	s
Hormone*Concentration	2.93	0.0608	ns

*ns denotes no significance and s denotes significance at α=0.05.

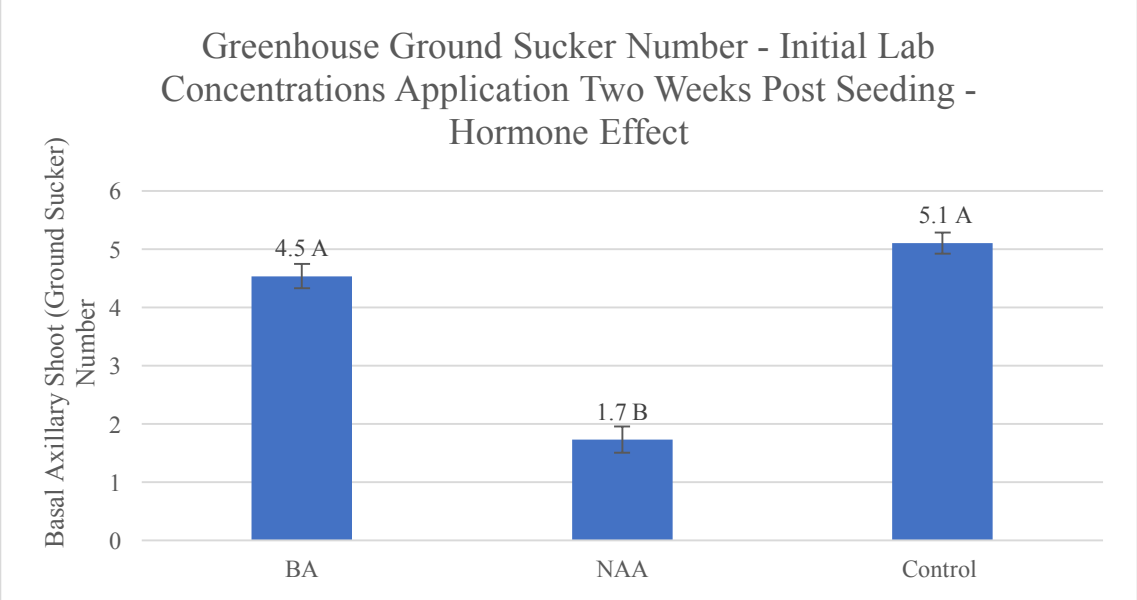

Figure 23 The effect of hormone type applied two weeks post seeding on greenhouse ground sucker number averaged over initial lab concentrations.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

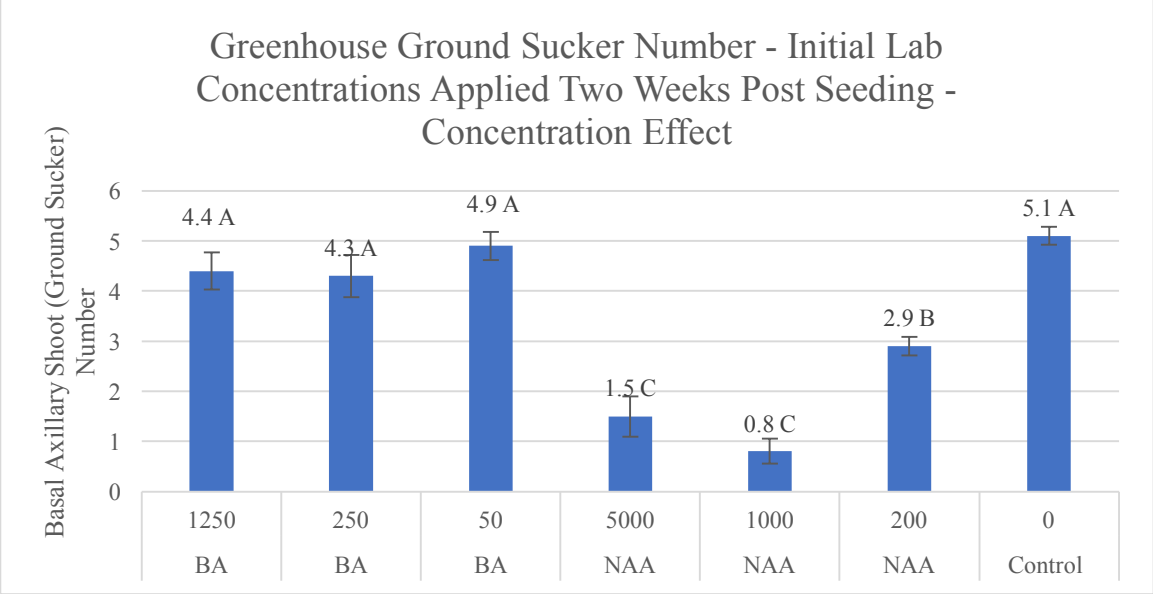

Figure 24 The effect of initial lab concentration when applied two weeks post seeding on greenhouse basal axillary shoot number for TN86.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05.

*Standard error of the mean is displayed as the error bar at the apex of each mean bar.

*Numeric values on the apex of each mean bar represent mean values.

Table 22 Analysis of Variance for Greenhouse Stem Length (cm) – Initial lab hormone treatments applied each watering post seeding.

Greenhouse Stem Length (cm) - Initial Lab Concentrations Application Each Watering			
Source of Variation	F Value	Pr > F	Significance
Hormone	37.52	<0.0001	s
Concentration	81.51	<0.0001	s
Hormone*Concentration	68.39	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*A significant interaction trumps the individual main effects.

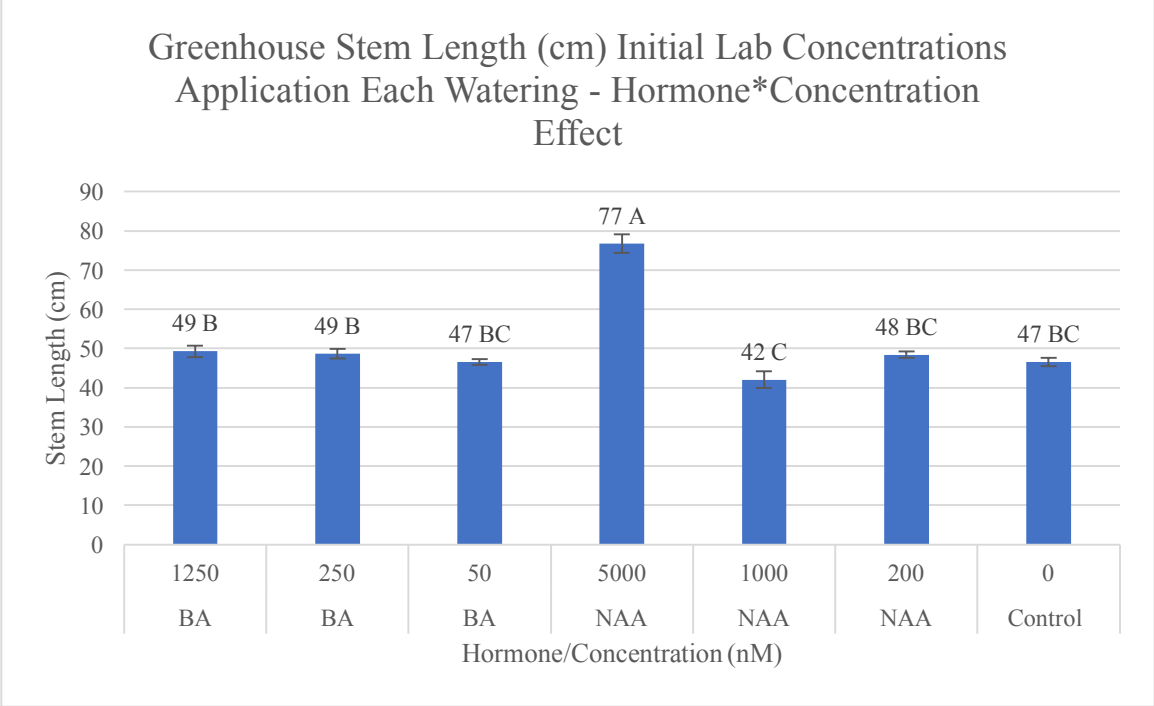

Figure 25 The effect of initial lab hormone*concentration interaction when applied each watering post seeding on greenhouse stem length for TN86.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

combinations selected for the experiment. It was concluded that NAA 5 μM was significantly different than all other hormone*concentration combinations. Evidence suggests all hormone*concentration combinations, other than NAA 5 μM, are statistically the same for stem length (cm), when tested at α=0.05. These results are nearly identical to what we observed in the same experiment where hormones were applied a single time rather than each watering.

The ANOVA for greenhouse basal axillary shoot number when the initial lab hormones and concentrations were applied at each watering post seeding is shown in Table 23. There was one significant main effect (Hormone) impacting greenhouse ground sucker number. Figure 26 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among hormone types selected for the experiment. NAA was statistically different than both BA and the control for greenhouse ground sucker number. This is consistent with what we found using the same concentrations, but with a onetime application. Figure 27 displays the nonsignificant effect of concentration on basal axillary shoot number when hormones where added at each watering post seeding.

Table 23 Analysis of Variance for Greenhouse Basal Axillary Shoot (Ground Sucker) number – Initial lab hormone treatments applied each watering post seeding.

Greenhouse Ground Sucker Number - Initial Lab Concentrations Application Each Watering			
Source of Variation	F Value	Pr > F	Significance
Hormone	49.93	<0.0001	s
Concentration	0.4	0.6709	ns
Hormone*Concentration	0.4	0.6709	ns

*ns denotes no significance and s denotes significance at α=0.05.

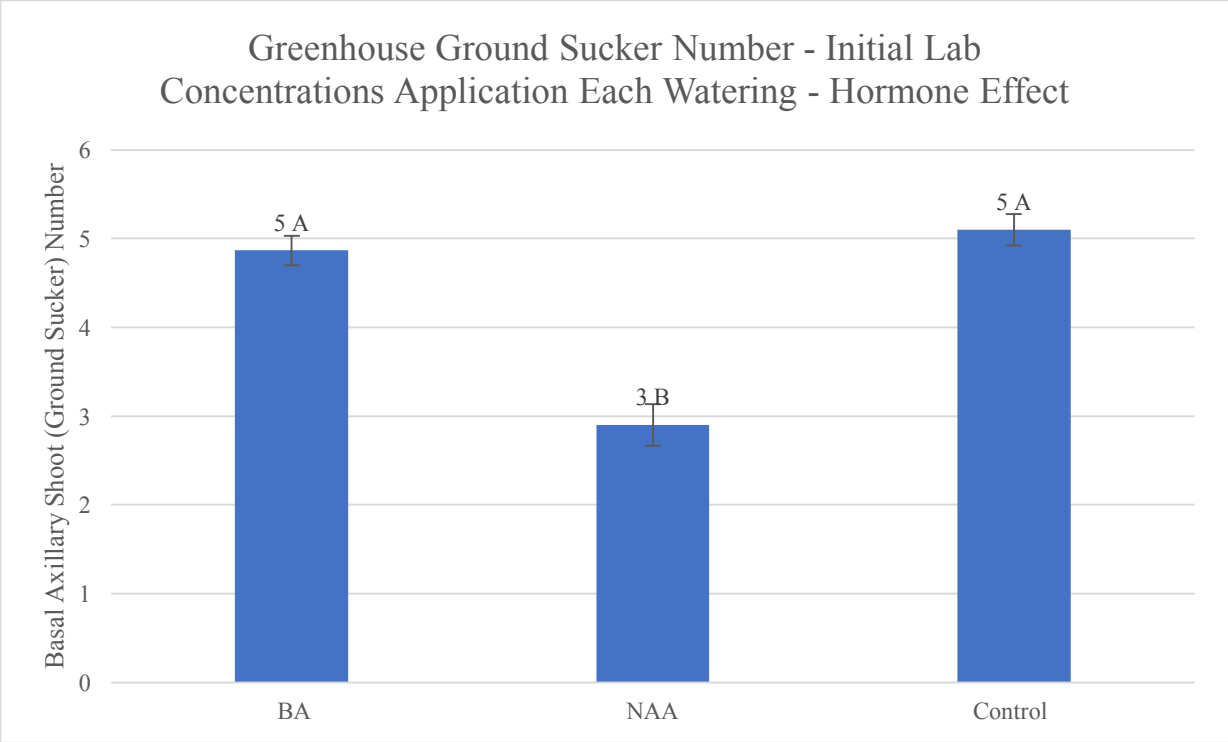

Figure 26 The effect of hormone type applied each watering post seeding on greenhouse ground sucker number averaged over initial lab concentrations.
* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

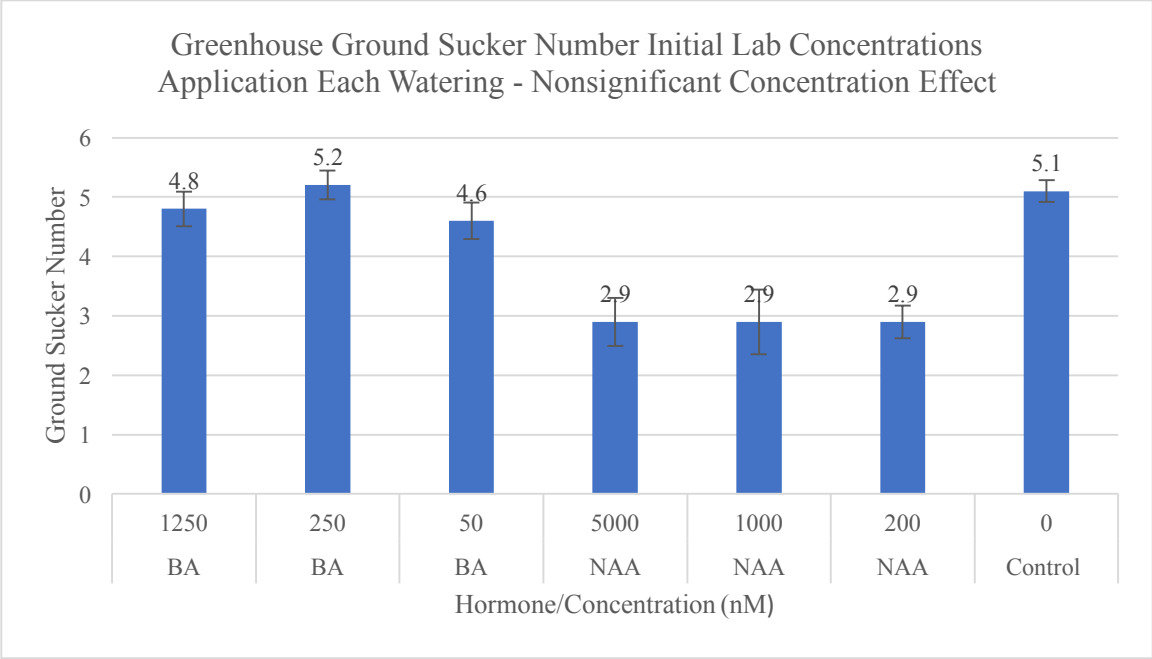

Figure 27 Nonsignificant effect of initial lab hormone*concentration interaction when applied each watering post seeding on greenhouse basal axillary shoot number for TN86. * The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

Summary of Second Greenhouse Evaluation

Results from the second set of greenhouse evaluations suggested that a onetime application of NAA at the initial lab concentrations, applied two weeks after seeding, produced significant results and should be tested under field conditions during the 2016 field season. This would be a direct field replication of the 2nd greenhouse evaluations. A decision was made to also include tray/cell size used to produce transplant for the field as another variable. This was done to quantify differences in ground sucker formation among plants with different root ball sizes. Since ground suckers are also problematic in dark tobacco, a dark tobacco experiment was also conducted in the 2016 field season using the same experimental design and hormone treatments as the 2016 burley field evaluations.

2016 Field Evaluation of the Effect of Exogenous Auxin and Tray Size on Tobacco

From the results of the 2015 field studies, it was concluded that TN86 and H403 behave differently in terms of ground sucker formation, and exogenous hormone sensitivity. Furthermore, H403 displayed a low propensity to form basal axillary shoots. For this reason, H403 was not used in the 2016 field trials. In the 2016 field season, field evaluations incorporated a dark tobacco variety (KTD6), a high basal axillary shoot producer. One burley variety (TN86) was also used. However, cytokinin (BA) treatments were not evaluated during the 2016 filed experiment, and application time of NAA treatments was fixed at 2 weeks post seeding. In addition, tray size (number and size of cells per tray) was added as an additional treatment. Five hormone treatments (NAA 500 nM, 1000 nM, 3000 nM, 5000 nM and an untreated control), and five tray sizes (128, 200, 242, 288, and 338 cells per tray) were evaluated per location. The experimental

design was a randomized complete block (RCBD) with three replications at each location. The same three locations were used in 2016 as in 2015 to provide differing environments that would possibly provide differing levels of ground sucker pressure. For these reasons the 2016 field data was analyzed separately for each variety/location combination.

At the Greeneville location, there were two significant main effects (NAA Concentration and Tray Size), and one significant interaction (Concentration*Tray) impacting ground sucker number for TN86 in 2016 (Table 24). Figure 28 displays the results of a Tukey's HSD conducted to determine significant differences among Concentration*Tray combinations applied to TN86 at Greeneville in 2016. Although statistically significant differences were found among treatments, there was no treatment that produced consistently low ground sucker numbers.

Similar results were observed at Greeneville for dark variety KT D6. There were two significant main effects (NAA Concentration and Tray Size), and one significant interaction (Concentration*Tray) impacting ground sucker number for KTD6 at Greenville in 2016 (Table 25). This is identical to what was observed for TN86 at the same location, and indicates that each tray size and hormone concentration combination behaves differently. Figure 29 displays the results of a Tukey's HSD conducted to determine significant differences among Concentration*Tray combinations for KTD6 at Greeneville 2016. These results are inconsistent with those for TN86, and indicate an outside factor not accounted for is driving basal axillary shoot formation. Much like TN86, there were several statistically significant differences found among treatments

Table 24 Analysis of Variance for TN86 at Greeneville 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Greeneville Burley Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	10.88	<0.0001	s
NAA Concentration	64.34	<0.0001	s
Tray Size	4.06	0.0028	s
Concentration*Tray	13.93	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*block is significant, but not an effect of interest.

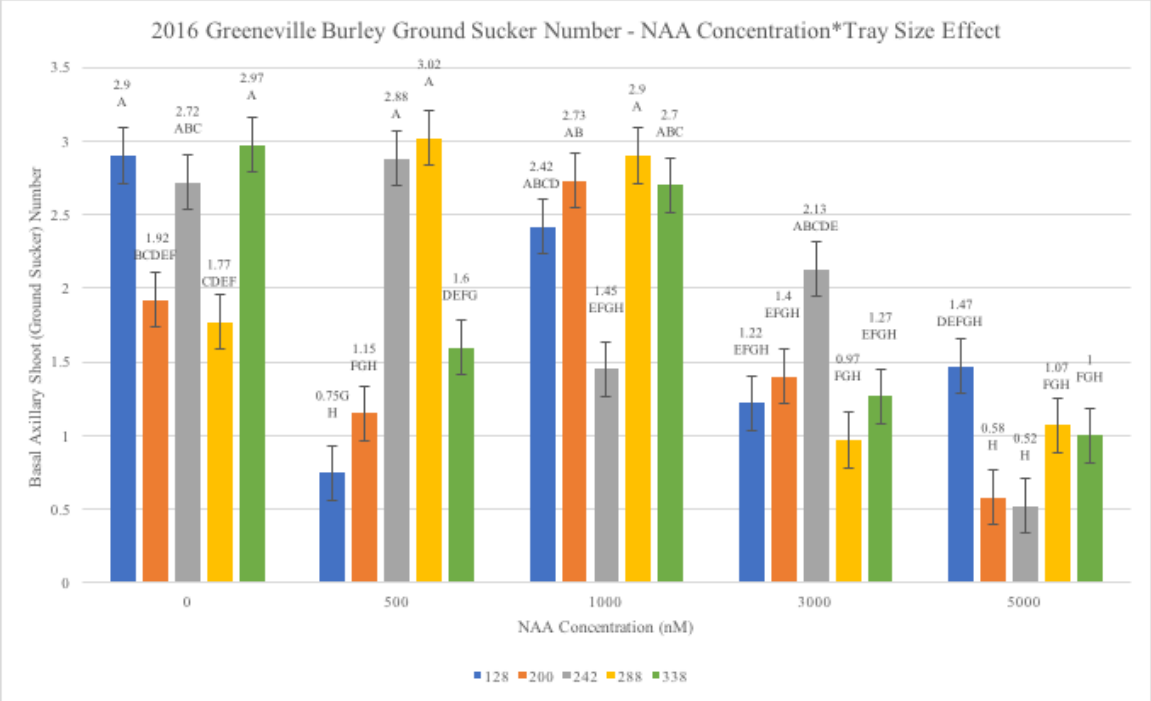

Figure 28 The effect of concentration*tray interaction on TN86 at Greeneville 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

Table 25 Analysis of Variance for KTD6 at Greeneville 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Greeneville Dark Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	15.47	<0.0001	s
NAA Concentration	15.68	<0.0001	s
Tray Size	6.51	<0.0001	s
Concentration*Tray	6.08	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*block is significant, but not an effect of interest.

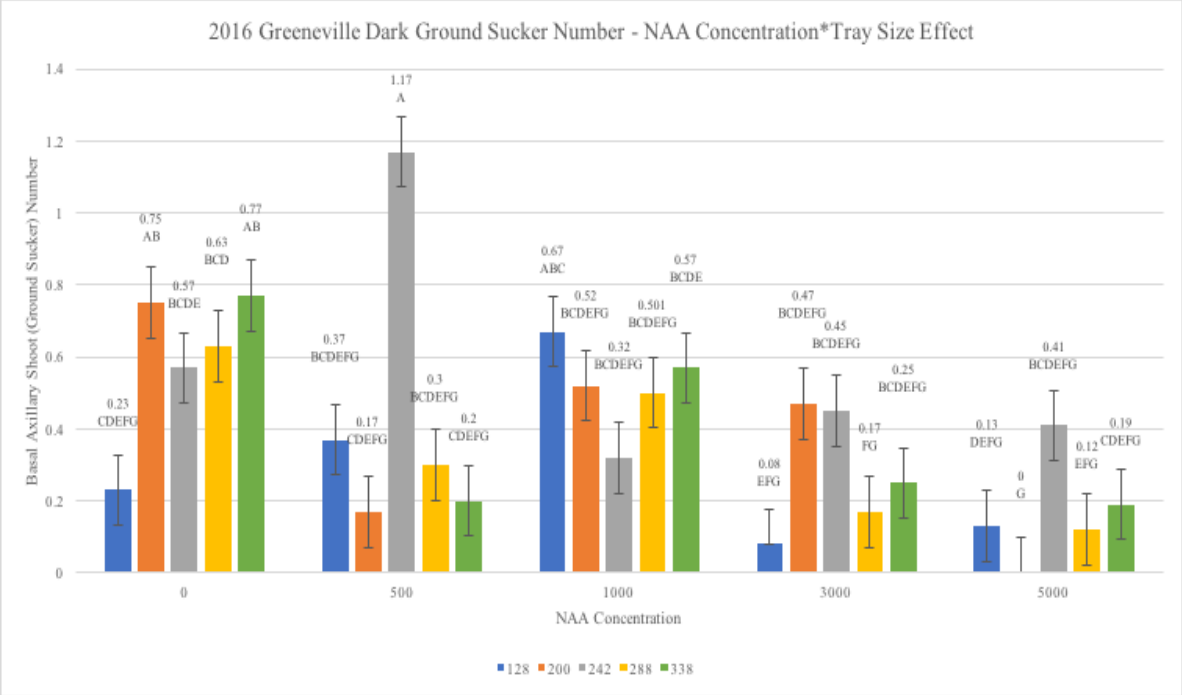

Figure 29 The effect of concentration*tray interaction on KTD6 at Greeneville 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

applied to KTD6; however, there was no single treatment that produced consistent ground sucker numbers.

At Lexington, there were two significant main effects (NAA Concentration and Tray Size), and one significant interaction (Concentration*Tray) impacting ground sucker number for TN86 in 2016 (Table 26). This is identical to what was observed for TN86 and KTD6 at the Greeneville location, and indicates that each tray size and hormone concentration combination behaves differently. Figure 30 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among Concentration*Tray combinations applied to TN86. Again, these results are similar to what was observed in Greeneville in 2016.

For KT D6, there were there were also two significant main effects (NAA Concentration and Tray Size), and one significant interaction (Concentration*Tray) impacting ground sucker number for KTD6 at Lexington (Table 27). This is identical to what was observed for TN86 and KTD6 at the Greeneville location, and TN86 in Lexington. Figure 31 displays the results of a Tukey's Honest Significant Difference Test conducted to determine significant differences among Concentration*Tray combinations applied to KTD6. These results are similar to what was observed in Greeneville in 2016. That is, there is no notable trend in ground sucker number across tray size*NAA concentration combinations. Perhaps the only consistency in the data from both field seasons is how inconsistent ground sucker formation is from year to year, location to location, and variety to variety.

Table 26 Analysis of Variance for TN86 at Lexington 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Lexington Burley Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	2.21	0.1105	ns
NAA Concentration	30.27	<0.0001	s
Tray Size	11.12	<0.0001	s
Concentration*Tray	1.72	0.0369	s

*ns denotes no significance and s denotes significance at α=0.05.

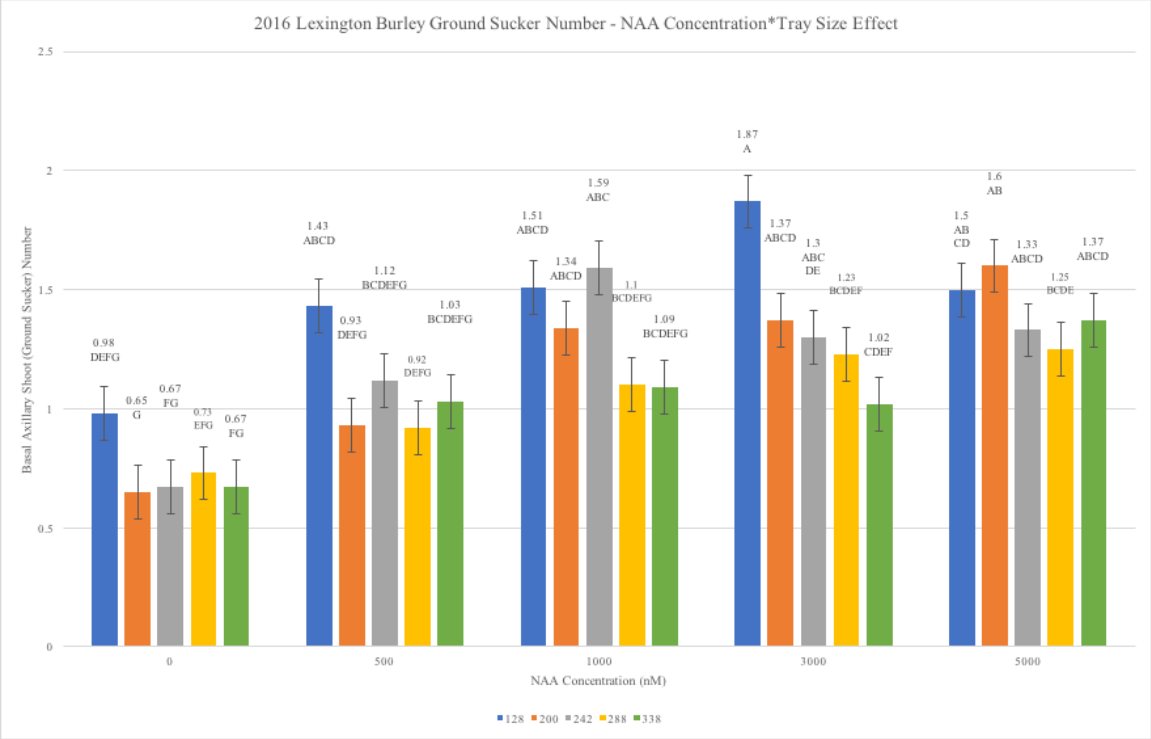

Figure 30 The effect of concentration*tray interaction on TN86 at Lexington 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

Table 27 Analysis of Variance for KTD6 at Lexington 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Lexington Dark Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	12.88	<0.0001	s
NAA Concentration	4.6	0.0011	s
Tray Size	5.08	0.0005	s
Concentration*Tray	3.57	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*block is significant, but not an effect of interest.

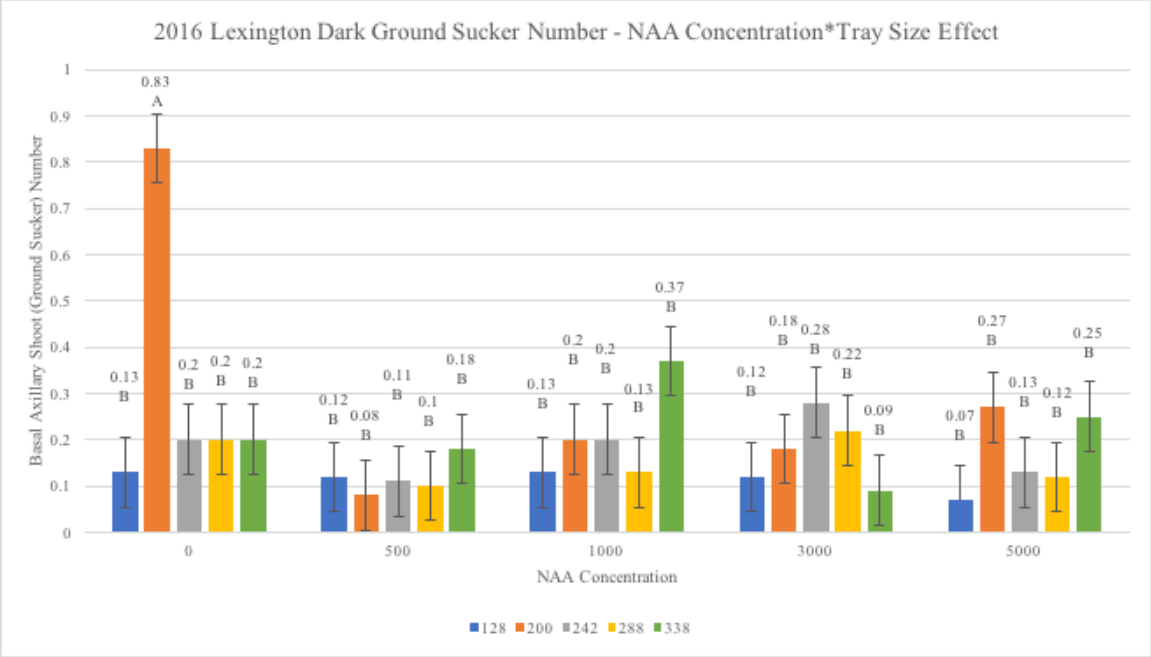

Figure 31 The effect of concentration*tray interaction on KTD6 at Lexington 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

Results for both TN86 and KTD6 at the Woodford County location were very similar to those observed at Greeneville and Lexington in 2016. There were two significant main effects (NAA Concentration and Tray Size), and one significant interaction (Concentration*Tray) impacting ground sucker number for both TN86 (Table 28) and KTD6 (Table 29). This is identical to what was observed for TN86 and KTD6 at both the Greeneville and Lexington locations. Figures 32 and 33 displays the results of a Tukey's HSD conducted to determine significant differences among Concentration*Tray combinations applied to TN86 and KTd6, respectively. Again, these results are very similar to what was observed in Greeneville and Lexington. This is not to say that the results mirror those observed at the other two locations, but that all locations are incredibly variable and no consistent trend in ground sucker number was present across tray size*NAA concentrations. Once again, the only consistency in the data from both field seasons is how inconsistent ground sucker formation is from year to year, location to location, and variety to variety.

Table 28 Analysis of Variance for TN86 at Woodford Co. 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Woodford Co. Burley Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	29.92	<0.0001	s
NAA Concentration	10.67	<0.0001	s
Tray Size	18.23	<0.0001	s
Concentration*Tray	2.17	0.0046	s

*ns denotes no significance and s denotes significance at α=0.05.
*block is significant, but not an effect of interest.

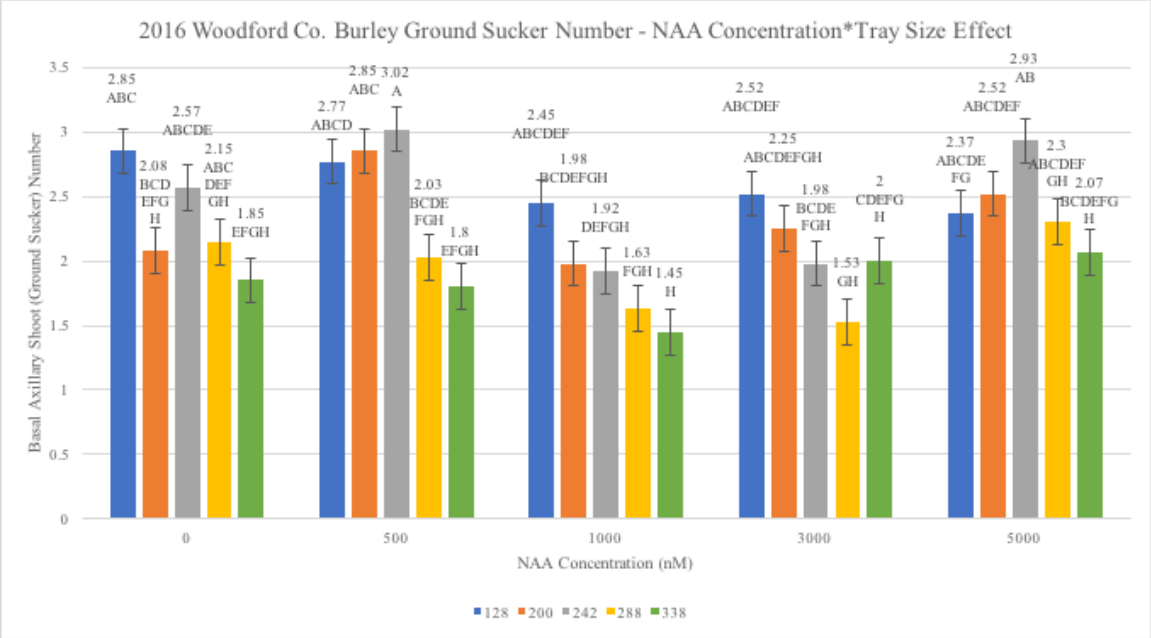

Figure 32 The effect of concentration*tray interaction on TN86 at Woodford Co. 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

Table 29 Analysis of Variance for KTD6 at Woodford Co. 2016 Basal Axillary Shoot (Ground Sucker) number.

2016 Woodford Co. Dark Ground Sucker Number			
Source of Variation	F Value	Pr > F	Significance
Block	8.72	0.0002	s
NAA Concentration	4.64	0.0010	s
Tray Size	4.92	0.0006	s
Concentration*Tray	10.54	<0.0001	s

*ns denotes no significance and s denotes significance at α=0.05.
*block is significant, but not an effect of interest.

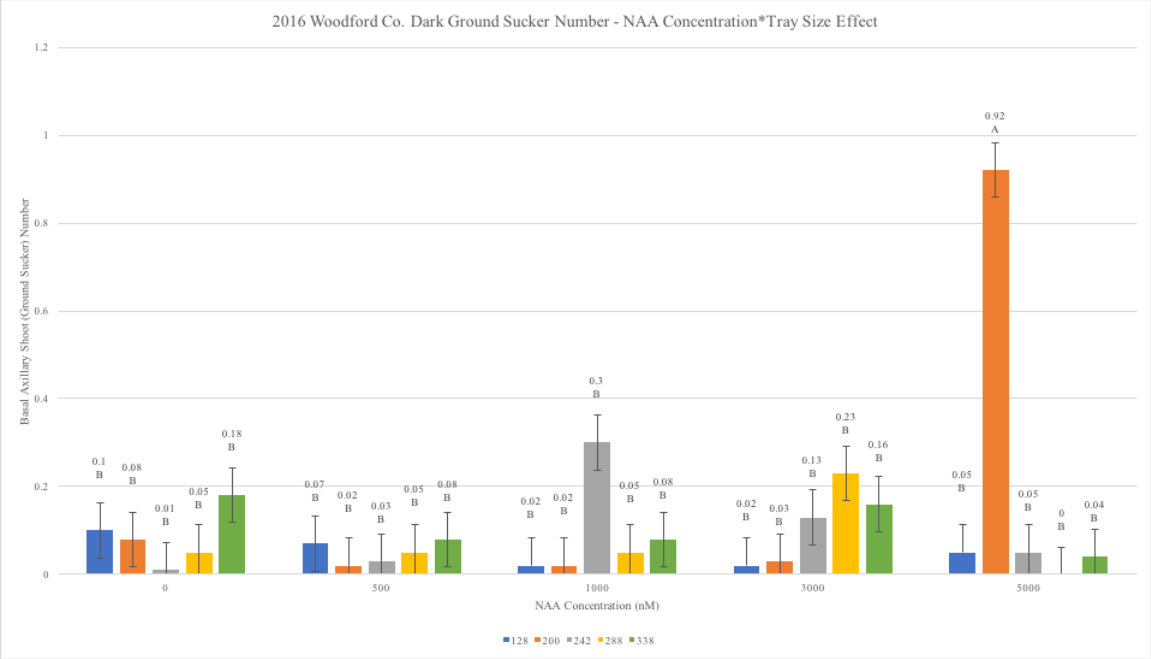

Figure 33 The effect of concentration*tray interaction on KTD6 at Woodford Co. 2016 field ground sucker number.

* The means marked with different letters are statistically different than each other per Tukey's Honest Significant Difference Test at α=0.05. Standard error of the mean is displayed as the error bar at the apex of each mean bar. Numeric values on the apex of each mean bar represent mean values.

*legend displays tray size

2016 Field Summary

Although several statistically significant differences were found among treatments applied to both TN86 and KTD6, there was no single tray size or NAA concentration that produced consistent ground sucker numbers. No notable trend in ground sucker number across treatments indicates that the initial hypothesis (that an increase in the root ball size of tobacco transplants produced in the float bed system compared to traditionally produced transplants is the cause of an increase in ground sucker number) was incorrect. Perhaps the only consistency in the data from both field seasons is how inconsistent ground sucker formation is from year to year, location to location, and variety to variety. In conclusion, from the results from all locations in 2015 and 2016, it is apparent that an exogenous auxin application (within the concentration ranges, application methods, and environmental conditions used in this study) will not consistently and predictably suppress basal axillary shoot development in tobacco.

Chapter Five: Summary and Conclusions

From the results of the *in vitro* experiments, it was determined that NAA 2-50 nM and BA 2-50 nM would be suitable concentrations to test in the hydroponic tobacco transplant production system in a controlled greenhouse environment. This decision was based on evidence that this range of hormone concentrations for both BA and NAA did not produced deleterious morphological effects at the seedling stage of growth. In hind sight, it is obvious that this type of *in vitro* environment does not adequately represent the float bed environment in which tobacco transplants are produced, and later experiments were conducted. One possibility is that the float bed system is less efficient at providing the hormone in solution to the plant for uptake, compared to the *in vitro* environment present in a petri dish. This is one explanation for why severe growth defects were observed at the initial high hormone concentrations *in vitro*, but the same deleterious effects on growth were not observed at the same concentrations *in vivo* (greenhouse).

The first set of greenhouse studies were conducted at the University of Kentucky campus in Lexington, Kentucky. From the information gathered in that set of greenhouse experiments, it was concluded that application two weeks and four weeks post seeding and the 2-50 nM concentration range would be appropriate for both hormones to test in the 2015 field evaluations . This decision was based on the identification of a NAA treatment that significantly reduced ground sucker number in the high basal axillary shoot producer, and did not increase ground sucker formation in the low producer. However, these results appear to be misleading when the field evaluation results are included in the discussion. In the greenhouse, ground sucker pressure appears to be much more intense than ground sucker pressure present in any of the field environments used in this study.

This could be due to a number of different factors that include: differences in water regimen, amount of space and substrate available for root expansion, temperature, humidity, and other physical stresses compared to a field situation. Due to an increase in sucker pressure and subsequent ground sucker development, the exogenous hormone applications could have had a greater effect on greenhouse grown potted plants than on the same plants grown in a more realistic field situation.

After the data from the 2015 field season were compiled and analyzed, it was obvious that the hormones and concentrations used did not produce meaningful reductions in basal axillary shoot number in any variety on a field scale. Once again, this is likely due to greater sucker pressure in the greenhouse, thus more ground suckers. In other words, the endogenous auxin to cytokinin ratio is likely different in greenhouse grown plants than field grown plants. One way to validate or refute this claim would be to measure endogenous hormone levels. If that is the case then results observed in the greenhouse would not be accurate indicators of what to expect in a field situation.

It was, however, very obvious from the 2015 field results that the low ground sucker producers and high producers are genetically different from one another in terms of propensity to develop ground suckers. In addition, it is obvious that only certain varieties exhibit a problematic propensity to form basal axillary shoots. H403 is not one of these varieties, thus no further hormone evaluations were performed on this variety. From the information compiled in the 2015 field season, It was decided to try the initial lab concentrations in a greenhouse setting to gauge what would happen if the *in vivo* concentrations were used in the field. The severe growth defects observed at these concentrations *in vivo* were not observed at the same concentrations in the second set of

greenhouse environments. One possibility is that the float bed system is less efficient at providing the hormone in solution to the plant for uptake, compared to the *in vitro* environment present in a petri dish.

From what we observed in the second set of greenhouse evaluations, it was determined that a onetime application at the initial lab concentrations (NAA 200-5000 nM) produced significant results and would be sufficient to test in the 2016 field season. However, based on what was learned in from the 2015 field studies, it was not certain that the greenhouse results would be reproducible in a field situation. A decision was made that only one hormone application time, two weeks post seeding, would be evaluated in the field using the initial lab concentrations for NAA only. No BA treatments were evaluated in the 2016 field season. This was a direct field replication of the 2nd greenhouse evaluations. A decision was alsomade to incorporate tray size as another treatment. This was done to quantify differences in ground sucker formation among plants with different root ball sizes. A dark tobacco experiment was also conducted in the 2016 field season using the same experimental design and hormone treatments as the 2016 burley field evaluations.

Although several statistically significant differences were found among treatments applied to both TN86 and KTD6, there was no single tray size or NAA concentration that produced consistent ground sucker numbers. No notable trend in ground sucker number across tray size indicated that the initial hypothesis (that an increase in the root ball size of tobacco transplants produced in the float bed system compared to traditionally produced transplants is the cause of an increase in ground sucker number) was incorrect. If the hypothesis had been correct, then as tray cell size increased, ground sucker number

should have increased across hormone concentration and variety. This was not the case, and indicates that it is not a simple physical difference in root mass that leads to the great variability in ground sucker number found commonly in the hydroponic transplant production system.

In addition, our hypothesis that augmenting the endogenous auxin to cytokinin ratio by adding exogenous hormones to the float bed solution would prevent ground sucker development was also invalidated. A consistent increase or decrease in ground sucker formation was not observed for any of the exogenous hormones used in any of the field experiments. If hormones could be added to the float water to successfully repress ground sucker development, then a consistent increase or decrease in grounds sucker number would have been identified, especially considering the wide range of concentrations used in this study. This was not the case. One obvious explanation for this could be that we used the wrong application method. Auxin was the focus of our research as the most likely hormone to easily suppress basal axillary shoot development. It is well known that this class of hormones is typically translocated basipetally rather than acropetally. Our experiment relied solely on acropetal translocation of both hormones, and therefore may have had no chance of being successful from the start. However, it was important to test the method we used in this study due to its relative ease compared to foliar application of the hormones, and that it would be a management practice easily adopted by producers.

In conclusion, perhaps the only consistency in the data from both field seasons is how inconsistent ground sucker formation is from year to year, location to location, and variety to variety. From the results of the entire study, it is apparent that an exogenous

auxin application (within the concentration ranges, application methods, and environmental conditions we used) will not suppress basal axillary shoot development in tobacco. Moving forward, it would be ideal to first evaluate and quantify basal axillary shoot formation in all widely grown modern commercial varieties, to determine whether there is a substantial need for a ground sucker prevention agent. A second step would be to determine if differing ratios of endogenous auxins and cytokinins existed among tobacco varieties, and if differences were identified, whether the ratios were correlated to an increased propensity to form ground suckers. If such a correlation was identified, then a more genetic/plant breeding oriented approach would likely prove more effective.

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2. Carmack, W.J., Miller, R.D., and Smalle, J.A. 2016. Using Exogenous Hormone Application to Suppress Axillary Shoot Development in Tobacco. University of Kentucky Department of Plant and Soil Science Student Mini-Symposium. Lexington, KY.
3. Carmack, W.J., Miller, R.D., and Smalle, J.A. 2016. Using Exogenous Hormone Application to Suppress Axillary Shoot Development in Tobacco. Tobacco Worker's Conference. Nashville, TN.