

EVALUATION OF A PLANT NITROGEN
RECOMMENDATION MOBILE PHONE
APPLICATION IN THE GREENHOUSE AND USE OF
OPTICAL SENSORS IN THE LANDSCAPE

By

LANCE SWEARENGIN

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Thesis Approved:

Dr. Bruce Dunn

Thesis Adviser

Dr. Brian Arnall

Dr. Carla Goad

Dr. Justin Moss

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Abstract: Use of a mobile phone application for iPhone that provides nutritional recommendations from optical sensors may give growers a quick reference for determining and effectively adjusting a crops nutritional status under a greenhouse production schedule but has not been tested. *Stachys* 'Helene Von Stein' and *Verbena* 'Homestead Purple' were supplemented with (0, 5, 10, 15, 20, and 25 g) of a 16N-3.9P-10K controlled release fertilizer (CRF) and tested up to 42 days after treatment (DAT). *Hibiscus* 'Aphrodite' and *Clethra* 'Hummingbird' were supplemented with (0, 10, 20, 30, 40, and 50 g) of the same 15N-3.9P-10K CRF. Plants were evaluated at 42 DAT on growth and plant quality measures including plant height, plant width, flower number, and dry weight. 'Helene Von Stein' and 'Aphrodite' responded favorably to the fertilizer recommendations provided by the mobile phone application as the treatment corrections produced favorable dry weights and flower numbers that were comparable to the highest performing fertilizer levels. A less substantial response was observed in the recommended treatment corrections for 'Homestead Purple' and 'Hummingbird' as the recommendations failed to produce dry weights and flower numbers in the corrected treatments that were similar to highest performing fertilizer levels. An accompanying field study was constructed to examine SPAD and atLEAF chlorophyll leaf sensor values on ornamental landscape plant materials grown under field conditions. Plants consisted of *Forsythia* 'Lynwood Gold', *Hibiscus* 'Lavender Chiffon', and *Salvia* 'May Night'. 'Lavender Chiffon' values were not different from each other across all testing dates with the exception of the last testing date in October, and 'Lynwood Gold' sensor values were only observed to be different from each other in the July and October testing dates. 'May Night' values were not different from each other for each testing date with the exception of the last testing date in October. Leaf nitrogen values for 'Lavender Chiffon and 'Lynwood Gold' showed a decreasing trend, while 'May Night' showed a stable trend over the course of the study. Significant environmental conditions played a substantial role in the field study results as record drought conditions plagued the region and supplemental irrigation was not used in the study. Significant correlations were observed between the SPAD and atLEAF sensor values in all species studied.

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CHAPTER I

REVIEW OF LITERATURE

Nitrogen is an integral component of leaf chlorophyll and is an essential element regarding plant growth and crop nutrition (Muchecheti et al., 2016). Plant roots take up nitrogen from the soil in the forms of ammonium and nitrate (Muñoz-Huerta et al., 2013). These two forms of nitrogen are then assimilated into amino acids, which form the building blocks of plant proteins (LeDuc and Rothstein, 2010). Proteins are integrated into organic molecules needed by the meristematic regions of plants for growth (Marschner, 2012). Nitrogen is needed by plants in the largest quantity of any other plant nutrient due to its composition in chlorophyll, which allows for the absorption of light energy needed to power photosynthesis furthering plant growth and yield (Chang and Robinson, 2003). Nitrogen management tools used to determine crop fertility can significantly increase crop management allowing for improved yield and sustainability, alleviating instances of crop disease, and environmental problems associated with the over application of fertilizers (Casa et al., 2014).

Advanced methods used to determine greenhouse crop nutrient status rely primarily on non-destructive measurements by chlorophyll optical sensors such as the soil plant analysis development (SPAD) and the atLEAF chlorophyll leaf sensors (Zhu et al., 2012). These sensors have been used by researchers to accurately estimate the nitrogen value of agricultural crops and demonstrate the ability to rapidly disseminate valuable information in determining the fertility needs of significant greenhouse and field crop species (Vesali et al., 2015). Determining the amount of chlorophyll contained in plant leaves provides a strong indication to the overall plant health, vigor, and fertility of greenhouse crops (Steele et al., 2008). Research has shown that there is a strong correlation between the chlorophyll content of plant leaves and leaf nitrogen, and as a result of measuring chlorophyll content the plant nitrogen status can be obtained (Tewari et al., 2013). The SPAD and atLEAF chlorophyll leaf sensors measure the chlorophyll content of plant leaves, which positively correlates with leaf nitrogen, thereby providing a leaf nitrogen estimation which further estimates a crops fertility (Jinwen et al., 2011). Research has also shown that the SPAD and atLEAF chlorophyll leaf sensor values correlate with each other (Basyouni et al., 2015; Dunn and Goad, 2015; Huang and Peng, 2004).

Chlorophyll leaf sensors give greenhouse production growers the ability to use non-destructive means that rapidly and accurately estimate the nutrient status of a crop in a timely manner. The over application of fertilizers can inflate production costs and reduce the quality of ground and surface waters causing waste and pollution (Wang et al., 2012). Both the SPAD and atLEAF chlorophyll leaf sensors use non-destructive methods to sample plant leaf chlorophyll through two light emitting diodes. One diode transmits

light in the red LED wavelength, and one transmits light in an infrared LED wavelength (Minolta Camera Co. Ltd., 1989). A sensor value is provided that is proportional to the optical density measured, which is the difference between the measured ratios of these wavelengths of light emitting through the leaf surface in sequence. Figure 1.1 shows the spectral absorbance of chlorophyll in living leaf tissue and the specific wavelength associated with chlorophyll a and chlorophyll b. The SPAD sensors peak chlorophyll absorbance is measured by the red LED wavelength at 650 nm, while other non-chlorophyll cellular components such as cell walls are measured by the infrared wavelength at 940 nm (Bauerle et al., 2003; Monje and Bugbee, 1992). The atLEAF sensors peak chlorophyll absorbance is measured by the red LED wavelength at 660 nm, while other non-chlorophyll cellular components are measured by the infrared wavelength at 940 nm (Zhu et al., 2012). A microprocessor calculates a value that is proportional to the relative optical density which is based on the ratio between the red LED and infrared LED wavelengths (Minolta Camera Co. Ltd., 1989). The SPAD and atLEAF sensor values provide a precise indication as to the relative greenness of plant material through the difference in the absorbance of chlorophyll (Monje and Bugbee, 1992). For plant leaves at different stages of development and overall pigment content, the chlorophyll fluorescence at 685 nm and 735 nm was found to be virtually linearly proportional to the chlorophyll content in leaves of beech trees (*Fagus sylvatica* L.), elm trees (*Ulmus minor* Miller), and a wild vine called Virginia creeper (*Parthenocissus tricuspidata* L.) (Gitelson et al., 1998). This research is significant as the chlorophyll leaf sensors can detect the overall fertility of a plant, and by establishing methods to

determine the overall fertility of a growing crop may further precision farming methods associated with field and greenhouse crop production.

The science of chlorophyll leaf sensor technology has been studied since the formulation of the invention in 1963 in Osaka, Japan (Minolta Camera Co. Ltd., 1989; Wang et al., 2004). The SPAD sensor was initially developed to monitor the fertilization requirements of rice (*Oryza sativa* L.), but since then has diverged to include other important agricultural crops such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), potato (*Solanum tuberosum* L.), and other valuable ornamental landscape plants such as perennial and annual crop species (Asano et al., 1986; Chang and Robinson, 2003; Coelho et al., 2012; Loh et al., 2002; Uddling et al., 2007; Waskom et al., 1996; Wood et al., 1993; Zheng et al., 2015). Many private researchers and agricultural institutions such as universities and private companies have studied chlorophyll leaf sensor technology for use in greenhouse crop production, field crop production, and plant nutrient research, to increase precise nutrient management for production (Chang and Robinson, 2003; Loh et al., 2002). Potted plants and annual landscape crops such as poinsettia (*Euphorbia pulcherrima* L. (Willd. ex Klotzsch), ornamental cabbage (*Brassica oleracea* L.), geranium (*Pelargonium x hortorum* L.H. Bailey), vinca (*Catharanthus roseus* L. (G. Don), and zinnia (*Zinnia elegans* L.) have been studied to quantify crop nitrogen status using chlorophyll leaf sensors thereby increasing crop productivity and plant product quality (Altland et al., 2002; Basyouni et al., 2015; Dunn and Goad, 2015; Khan et al., 2004; Wang et al., 2012).

Many studies have shown that there is a correlation between the chlorophyll content of plant leaves and the chlorophyll leaf sensor values, but controversy regarding

the reliability of these sensors to accurately estimate the foliar nitrogen content of selected crops remains (Wang et al., 2004). Leaf chlorophyll sensor readings and extractable leaf chlorophyll were studied in several cultivars of maple (*Acer rubrum* L.) showing that the correlation between the two was poor (Sibley et al., 1996). Other scientific investigations show a positive correlation between chlorophyll leaf sensor values and extractable leaf chlorophyll on hardwood forest specimens, ornamental landscape plants, and fruit trees (Chang and Robinson, 2003). Waskom et al. (1996) found that the biological differences in corn hybrids showed an inconsistency in the relationship between the sensor values and the total extractable leaf nitrogen content of leaves at different stages in the crop production cycle. This was due to many factors including the time of tassel initiation and time of sampling within the crop production cycle (Waskom et al., 1996). Waskom et al. (1996) also showed that crop yield predictions were largely dependent on the time of sampling within the crop production cycle illustrating that field crops are significantly more challenging to evaluate using chlorophyll leaf sensors. Inconsistencies among studies suggest that field variables such as environmental conditions and cultural practices play heavily on chlorophyll leaf sensor evaluation regarding field crops (Johnson, 1993; Sibley et al., 1996; Wang et al., 2004). Greenhouse crop variables are less of an issue regarding chlorophyll leaf sensor diagnostic methods because greenhouse crops are grown in a controlled environment thereby reducing variation in the crops performance and eliminating variables that would skew the correlation between the leaf sensor values and leaf nitrogen content (Chang and Robinson, 2003; Johnson, 1993; Sibley et al., 1996; Wang et al., 2004).

There are many ways to determine the amount of nutrition available to plants such as taking leaf samples and submitting them for plant tissue analysis (Bauerle et al., 2003; Richardson et al., 2002). This costly process takes time for growers to get the sample results back making leaf and soil samples costly and time consuming for growers (Casa et al., 2014; Sibley et al., 1996). Production growers encounter increased costs associated with the over application of essential nutrients, and runoff can damage the environment by contaminating ground and surface waters (Djumaeva et al., 2012; Jinwen et al., 2011). The use of chlorophyll leaf sensors in crop production proves to be a useful tool for production facilities saving time and money, while also promoting a clean and pollution free environment (Bullock and Anderson, 1998; Hawkins et al., 2007; Zheng et al., 2015). The soil plant analysis development (SPAD) sensor is the most widely used chlorophyll leaf sensor, and a newer chlorophyll leaf sensor called the atLEAF sensor is a cheaper alternative to the SPAD sensor (Novichonok et al., 2016) (Figures 1.2 and 1.3). Leaf chlorophyll content analyzed using both of these chlorophyll leaf sensors can lead to improved crop management practices as both sensors have been found to correlate with each other and also leaf nitrogen (Vesali et al., 2015).

Using the SPAD and atLEAF sensor values to estimate the average nitrogen content and fertility of specific greenhouse crop species depends on the threshold of optimum fertility for each greenhouse crop species and cultivar (Mizusaki et al., 2013; Zheng et al., 2015). Fertility needs for different greenhouse crop species can vary widely based on cultivar, season, and where the crop is in its growth cycle (Novichonok et al., 2016). Evaluation of the threshold of optimum fertility for a particular crop species can provide for the precise application of nitrogen by providing for an optimum nutritional

index that correlates with the chlorophyll leaf sensor values (Mizusaki et al., 2013). This may result in the most favorable notification of a greenhouse crop species overall health and maximum yield regarding fruiting, flowering, and foliage characteristics, which are the desirable qualities sought after in the retail market and nursery industry (Zheng et al., 2015). The development of an index of optimum fertility for greenhouse and field crop species used in conjunction with the SPAD and atLEAF sensors to determine leaf chlorophyll content may provide the opportunity for greenhouse growers to improve efficiency and reduce production costs and waste benefiting production growers and the environment (Cortazar et al., 2015; Mizusaki et al., 2013; Zheng et al., 2015).

Determining the fertility needs of a crop based on the SPAD sensor values requires knowledge of the crops nutrient threshold at which below a specific SPAD sensor value the crop would respond favorably to the application of fertilizer (Zheng et al., 2015). Making sense of the chlorophyll leaf sensor values requires a look into the threshold of proper nutrition for each specific crop species or cultivar. Threshold SPAD and atLEAF values for each crop must then be established at which the sensor values correspond with crop nitrogen status. By using the upper and lower threshold values of a specific crop, a proper fertilizer recommendation can be utilized to increase crop production, yield, and production efficiency (Zheng et al., 2015). Crop yield is directly related to the amount of nutrients taken up by a crop, and the optimization of nitrogen inputs is important for lowering production costs, maximizing crop yield, and decreasing the environmental impact of production facilities (Teoh et al., 2012). Precise fertilizer applications may significantly improve the ability of growers to control these important production variables.

It is commonly observed that the amount of fertilizer required to produce a significant yield is often over estimated (Bullock and Anderson, 1998). Chlorophyll leaf sensor values and leaf chlorophyll content are species or cultivar specific and are affected by environmental growth conditions (Mizusaki et al., 2013). Chlorophyll leaf sensor measurements vary based on the non-uniform chlorophyll distribution within plant leaves and this variability is seen in different plant species and across cultivars (Parry et al., 2014; Monje and Bugbee, 1992). Therefore, individual thresholds are needed to predict the fertilizer recommendations for each crop species to increase production efficiency and yield (Mizusaki et al., 2013). The SPAD sensor has been shown to detect nitrogen deficiency in crops such as corn, rice, potato (*Solanum tuberosum* L.), and cauliflower (*Brassica oleracea* L.), and after supplemental fertilizer was applied to the crops, based on the SPAD sensor readings, crop yield losses were prevented by correcting the crops negative nutritional status (Altland et al., 2002).

Sampling procedures using chlorophyll leaf sensors require accuracy to adequately provide a uniform estimation of the chlorophyll concentration of a crop (Mickelbart, 2010). The relationship between absolute leaf chlorophyll concentration and the optical absorbance of leaf chlorophyll shows a non-linear curve that differs among recent studies due to the variation in experimental techniques used to sample leaves and estimate the leaf nitrogen concentration from chlorophyll leaf sensor values (Parry et al., 2014). Accurate sensor readings depend on the exact morphological location of the sample taken on the individual leaf blade. Samples taken laterally to the margin of the individual plant leaf blade, while avoiding the leaf apex, leaf base, and midrib of the leaf surface area provide a more accurate representative of leaf chlorophyll content (Dunn and

Goad, 2015). This accuracy also depends on which leaves the readings are taken from on the plant overall. Readings taken on leaves from the mid portion of the overall plant, while avoiding the upper and lower portion of the plant, and avoiding leaves that are chlorotic or damaged, can provide for a largely more accurate reading as seen in ornamental cabbage (Dunn and Goad, 2015).

Chlorophyll leaf sensors values have been shown to correlate with leaf nitrogen and can measure the fertility of a crop at a certain period of time, but cannot adequately provide insight into the future fertility needs of the crops (Bullock and Anderson, 1998). Environmental variables such as the water status of the crop and plant growth stage have shown to skew the accuracy of sensor readings (Bauerle et al., 2003). Sensor readings are often correlated to leaf nitrogen, but a standard estimate of number of leaves sampled, number of plants used to represent a crop, and leaf sampling location on the leaf and plant overall require a standard procedure to account for the variability between the results of scientific studies (Bonneville and Fyles, 2006; Bullock and Anderson, 1998; Dunn and Goad, 2015; Mickelbart, 2010).

Chlorophyll leaf sensors have traditionally been used by researchers as a tool to estimate the nitrogen content and fertility status of agricultural crops (Vesali et al., 2015). Nitrogen management in crop production is a significant component of crop nutrient management and precision farming practices (Zheng et al., 2015). Crop nutrient studies regarding the SPAD chlorophyll meter have primarily centered on rice production, but in recent years it has branched out many other useful agricultural crops such as corn, wheat, potato, pecan (*Carya illinoensis* (Wang. K. Koch), and even more recently horticultural crops such as poinsettia, geranium, zinnia, chrysanthemum (*Chrysanthemum x*

morifolium L.), ornamental cabbage (*Brassica oleracea* var. *capitata* L.), and ornamental kale (*Brassica oleracea* var. *acephala* L.) (Basyouni et al., 2015; Bullock and Anderson, 1998; Dunn and Goad, 2015; Khan et al., 2004; Wang et al., 2012; Zheng et al., 2015). The benefits of such studies rely primarily on endeavors created to establish a threshold of proper nutritional status for individual plant species. These thresholds exhibit optimum fertility for a specific crop species and or cultivar that can aid growers in alleviating crop disease and pest issues early in the production process (Casa et al., 2014; Cortazar et al., 2015; Novichonok et al., 2016; Jinwen et al., 2011). Development of a mobile phone application that can provide fertilizer recommendations based on these thresholds and is related to the relationship between chlorophyll leaf sensor values and the nitrogen content of a crop is a valuable diagnostic tool that production growers can use to decrease the financial costs associated with crop fertilization, while quickly improving production procedures that will help alleviate nutrient runoff into ground and surface waters (Cortazar et al., 2015; Vesali et al., 2015). Mobile phone applications that are used in coordination with chlorophyll leaf sensors are increasing in use and have been established for smartphone platforms such as android and iPhone (Vesali et al., 2015).

The future of leaf sensor technology lies in the optical sensor realm of research initiatives and the development of an application to help growers make sense of the sensor values (Cortazar et al., 2015; Vesali et al., 2015). Remote sensing techniques and digital imaging of field crops rely primarily on ground based remote sensing, air borne remote sensing, and satellite based remote sensing techniques (Taskos et al., 2015; Tewari et al., 2013). Currently, growers use visible cues and destructive leaf nitrogen testing, along with tests of the crops pH and electrical conductivity (EC), to determine the

nutritional activity taking place within a crop. The development of chlorophyll leaf sensors and a companion mobile phone application based on proper fertility thresholds, provides the potential to test crops in a timely manner, giving a precise estimate to the amount of nutrition available to a specific crop species, using non-destructive methods.

Researchers have developed a mobile phone application to assist growers in the use of chlorophyll leaf sensor technology (Figure 1.4). This mobile phone application provides a fertilizer recommendation that is associated with the nutrient status of a specific crop species or cultivar using the input of SPAD, atLEAF, or nitrogen leaf sample values. The mobile phone application then provides a nitrogen recommendation for a crop species by notifying the user if the crop requires an adjustment to align the crops nutritional status within acceptable limits. Newer mobile phone applications have been developed for android devices such as the Smart-SPAD application which uses contact imaging by a smartphone in conjunction with the SPAD chlorophyll leaf sensor technology (Vesali et al., 2015). The development of a mobile phone application to correlate SPAD and atLEAF values with specific plant species and or cultivar fertilizer recommendations will give production growers vital insight into the nutritional status of specific crops regarding their plant nutrient status whether values are sufficient, deficient, or particularly high in fertilizer concentration. In theory, the chlorophyll leaf sensor values are directly correlated to the plant nitrogen status; and the plant nitrogen recommendations application for iPhone delivers fertilizer recommendations that provide for proper fertilizer application rates for growers that result in optimum plant growth and form for specific plant species providing uniform and healthy ornamental and agricultural crop yields.

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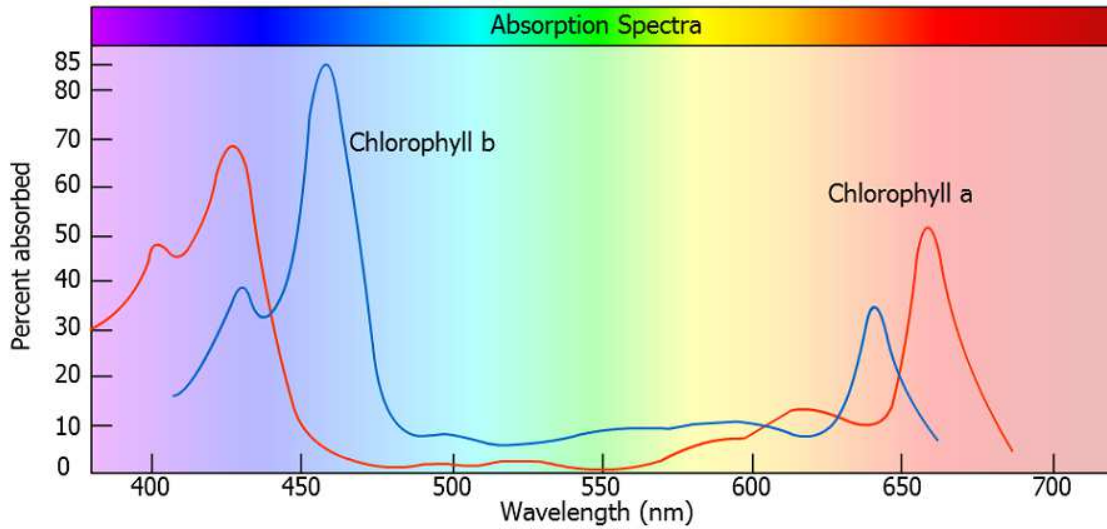


Figure 1.1. The figure shows the spectral absorbance of chlorophyll in living leaves. Chlorophyll has absorbance peaks in two distinct regions: the blue region (400 nm to 500 nm) and the red region (600 nm to 700 nm), with no transmission in the near infra-red (NIR) region. Taking advantage of this fact, scientists designed sensors that emit light in the red region and the NIR region. By comparing the reflectance or the absorbance of these transmittances at the two wavelengths, a value is generated that represents green vegetation of the sample (Basyouni and Dunn, 2013).



Figure 1.2. The SPAD-502 chlorophyll leaf sensor.



Figure 1.3. The atLEAF chlorophyll leaf sensor.

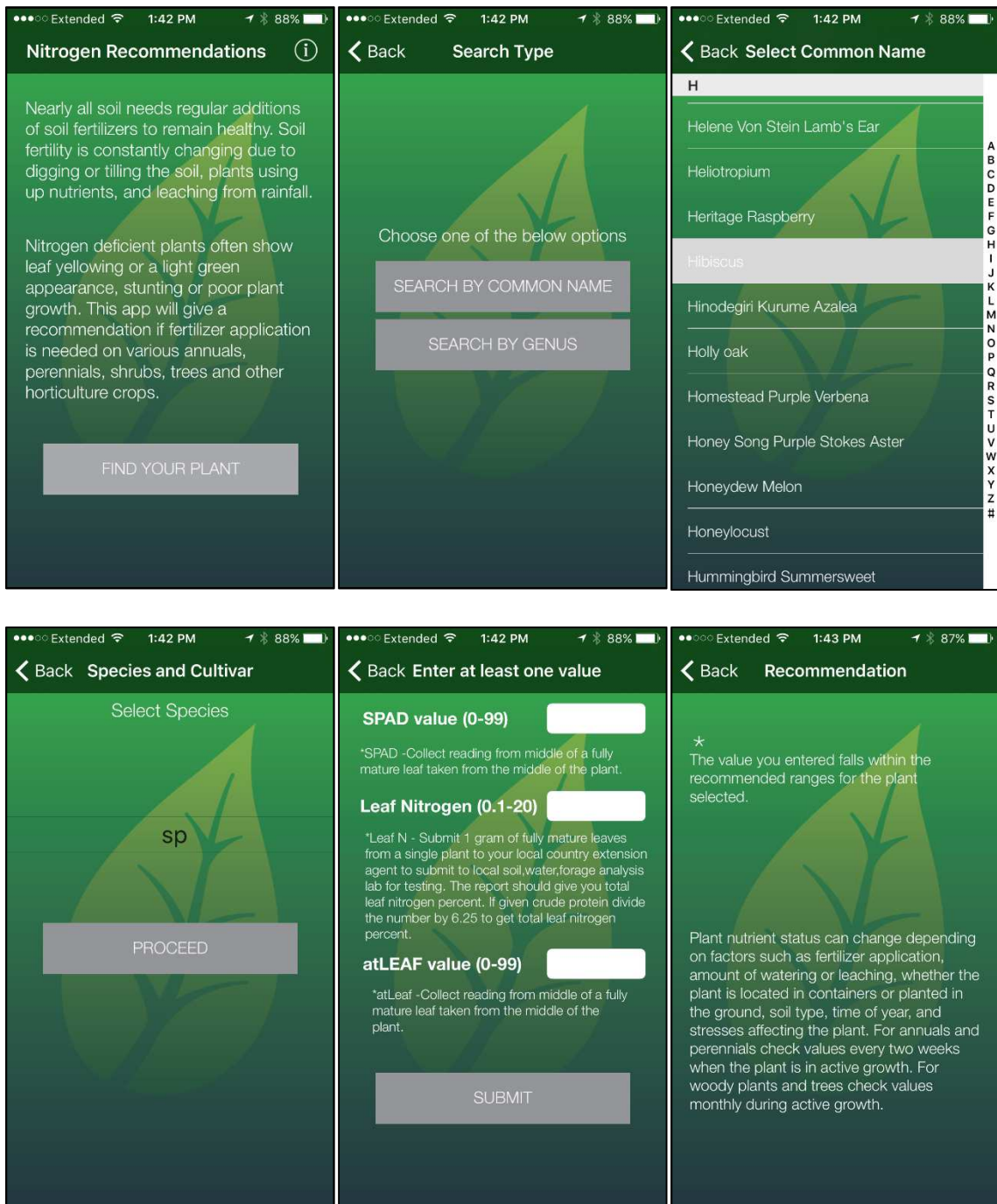


Figure 1.4. The plant nitrogen recommendations mobile phone application for iPhone is shown in screenshots. (Upper Left) Introductory screen detailing information about the application. (Upper Middle) Detailed plant search by common or scientific name. (Upper Right) List of common names. (Lower Left) Species or cultivar selection. (Lower Middle) SPAD, atLEAF, and leaf nitrogen value input screen. (Lower Right) Plant nutrient status recommendation.

CHAPTER II

GREENHOUSE STUDY

ABSTRACT

The purpose of this study is to evaluate the efficiency of a plant nitrogen recommendations mobile phone application in regards to the ability to make a fertilizer recommendation to correct nutritional deficiencies during production in the greenhouse. The greenhouse study was comprised of four species of common greenhouse crops consisting of two perennial herbaceous species *Stachys* ‘Helene Von Stein’ and *Verbena* ‘Homestead Purple’, and two perennial woody species *Hibiscus* ‘Aphrodite’ and *Clethra* ‘Hummingbird’. Herbaceous cultivars were supplemented with 0, 5, 10, 15, 20, and 25 g of 16N-3.9P-10K controlled release fertilizer (CRF). The woody cultivars were supplemented with 0, 10, 20, 30, 40, and 50 g of the same 16N-3.9P-10K CRF. SPAD and atLEAF chlorophyll leaf sensor readings were recorded for seven consecutive weeks. Additional fertilizer was applied at a rate of 20 g for the herbaceous cultivars and 30 g for the woody cultivars. Supplemental fertilizer was applied when the plant nitrogen mobile application suggested that the crops required additional fertilizer establishing a treatment correction group for each cultivar. End measures of plant height, width, flower number, and dry weight were recorded and compared for correlation to sensor values and leaf

nitrogen. At 7 days after treatment (DAT), fertilizer corrections were applied to the 0 g treatment level of *Stachys* ‘Helene Von Stein’ based on the fertilizer recommendations of the plant nitrogen mobile application. ‘Helene Von Stein’ produced marketable landscape plant materials at the end of the study as the treatment correction groups 0 (+20) for SPAD and atLEAF recovered using the application recommendation based on increased dry weight and flower number comparable to the top performing fertilizer levels end measures. ‘Homestead Purple’ was less responsive in the treatment correction as the nitrogen mobile application gave this fertilizer recommendation too late in the production process to correct the negative nutritional status of the SPAD and atLEAF 0 (+20) treatment groups. At 14 DAT fertilizer corrections were applied to the 0 g treatment level of *Hibiscus* ‘Aphrodite’, and at 21 DAT a fertilizer correction was applied to the 0 g *Clethra* ‘Hummingbird’ treatment level. ‘Aphrodite’ responded quickly to the treatment correction for the 0 (+30) treatment level in both the SPAD and atLEAF groups producing marketable landscape plant materials at the end of the study based on increased flower number and dry weight comparable to the top performing fertilizer level end measures. ‘Hummingbird’ was less responsive in the treatment correction for the 0 (+30) corrected treatment levels due to the fact that the nitrogen mobile application gave this fertilizer recommendation too late in the production process to correct the negative nutritional status of the 0 g treatment level. Significant correlations were observed among the SPAD and atLEAF sensors and with nitrogen rate.

INTRODUCTION

Determining the nutritional status of greenhouse production crops can be difficult and time consuming due to the existing methods of soil and plant analysis available to production growers (Wang et al., 2012). Oftentimes a crop can be nutrient deficient before visible signs appear, and many times it may be too late to make corrections regarding the nutrient status of a crop particularly under short production schedules (Dunn and Goad, 2015). Elements of greenhouse production such as excessive fertilization and constant leaching by automated irrigation systems can be detrimental to the environment and leave crops depleted of significant nutrients needed for optimum crop yield and uniformity (Wang et al., 2012). Optimization of nitrogen fertilization regarding greenhouse production has become the object of research due to its environmental and economic impact on production facilities and the environment (Muñoz-Huerta et al., 2013). Increasing awareness of ground and surface water pollution by production facilities may lead to more stringent and prohibitive regulation of fertilizers by governmental agencies in the future promoting production growers to investigate more efficient crop nutritional status analysis techniques (Hawkins et al., 2007).

Fundamental strategies that growers use to test the nutritional status of greenhouse crops include the diagnosis of soil pH and electrical conductivity (EC), as well as the laboratory analysis of soil and leaf samples (Basyouni and Dunn, 2013). These efforts are relatively accurate, but can be time consuming and increase production

costs thus limiting the ability to correct crop nutrient status in a timely and cost effective manner (Loh et al., 2002). Modern technological advances in chlorophyll leaf sensor technology such as the soil plant analysis development (SPAD) and atLEAF chlorophyll leaf sensors provide insight into the nutritional status of greenhouse crops rapidly and through non-destructive means (Wang et al., 2012). The development of chlorophyll leaf sensors advance the availability of growers to determine the relative amount of leaf chlorophyll in intact plant leaves through non-destructive means, but it may not give the grower a baseline to judge the sensor values against regarding the specific plant species under production (Kapotis et al., 2002). Currently the best way to use the sensor values is to correlate them with destructive chlorophyll leaf measurements such as foliar leaf nitrogen analysis (Kapotis et al., 2002). This is due to the fact that there is a strong correlation observed between the chlorophyll leaf sensors and leaf nitrogen (Patane and Vibhute, 2014).

Nitrogen is the leading essential elemental nutrient required for plant growth and development as this nutrient plays a major role in the process of photosynthesis which produces chlorophyll, the primary photosynthetic pigment in higher plants (Bullock and Anderson, 1998). The vitality of greenhouse production depends predominantly on fertilization techniques requiring nitrogen in the largest quantity of any other plant macronutrient. The photosynthetic process is an important aspect of plant physiology and crop production due to the fact that it determines leaf chlorophyll content, plant size, crop yield, uniformity, and transpiration rate (Basyouni and Dunn, 2013). Application of nitrogen based fertilizers during critical phases of plant growth and development can

dramatically enhance crop output and uniformity in considerable ways by producing marketable ornamental landscape plant materials (Coelho et al., 2012).

Diagnosing leaf chlorophyll content gives insight into the nutritional status of a particular crop based on the photosynthetic activity taking place in leaf tissues, because there is an exponential relationship between leaf chlorophyll content and the SPAD and atLEAF values provided by the sensors (Patane and Vibhute, 2014; Wood et al., 1993). There is a strong correlation between leaf nitrogen concentrations and the photosynthetic activity taking place in the chloroplasts of plant mesophyll tissues (Zakeri et al., 2014). This is due to the fact that 75% of leaf nitrogen accumulated in the chloroplasts of leaf mesophyll tissues is used in the production of the photosynthetic pigments making up chlorophyll (van den Berg and Perkins, 2004). The photosynthetic pigments chlorophyll a and chlorophyll b are instrumental in converting light energy into stored chemical energy that is used in the primary production processes associated with plant growth and development (Steele et al., 2008). Associations can be observed in the amount of chlorophyll present in plant leaf tissues and the vitality of a crops nutritional status for the reason that this gives an indication to the rate of photosynthetic activity taking place within the plant. Therefore, diagnosing leaf chlorophyll content using SPAD and atLEAF chlorophyll sensors indicates the amount of photosynthetic activity taking place within a plant, which strongly correlates with the available nitrogen and nutritional status of a crop overall (Zakeri et al., 2014).

Using SPAD and atLEAF chlorophyll leaf sensors to determine crop nutritional status may prove to also be a useful tool for growers if there is a positive correlation made between the chlorophyll leaf sensor values and the fertilizer recommendations

made by the plant nitrogen mobile application. The development of the plant nitrogen recommendations mobile application may provide greenhouse production growers a relatively precise indication to the nutritional status of specific plant species allowing them to maintain proper crop nutrition at critical growth stages by applying precise fertilizer concentrations. The mobile application was developed for use on mature plant materials to determine the overall nutritional status of plant materials in the landscape. Production growers can use SPAD and atLEAF readings or leaf nitrogen values providing a balanced representative value to input into the mobile application to obtain a recommendation regarding crop fertility and to accentuate fertilization techniques. Using the input of nutritional information on a specific plant species; the mobile application can make an estimation using the collected SPAD and atLEAF values. The plant nitrogen mobile application may also prove to be a useful reference tool in production facilities allowing growers to determine the nutritional status of a crop species and specific cultivars thereby allowing them to use precision in the application of fertilizers and promoting the formulation of an accurate nutritional regimen. The objective of this study was to evaluate the plant nitrogen recommendations mobile phone applications ability to detect nitrogen deficiencies in herbaceous and woody ornamental landscape plant materials started as plugs and bare root plant specimens, respectively, and grown out in a regular greenhouse production cycle to determine the effectiveness of the fertilizer recommendations in correcting the negative nutritional status of greenhouse grown crops.

MATERIALS AND METHODS

Experiment One. Plant Material and Experimental Methods for 'Helene Von Stein' and 'Homestead Purple'. On 10 January 2014, 250 rooted cuttings each (4 to 8 leaves) of lamb's ear (*Stachys byzantina* L.) 'Helene Von Stein', and verbena (*Verbena canadensis* L.) 'Homestead Purple' were obtained from Greenleaf Nursery Inc. (Parkview, OK). All 500 rooted cuttings were put in the greenhouse until transplantation into pots 4 d later using a single cutting. A sample of the potting media and tap water were analyzed, and both showed an initial total nitrogen content of $<0.5 \text{ mg}\cdot\text{L}^{-1}$. On 14 January 2014, the rooted cuttings were transplanted into standard 15.24 cm diameter pots and filled with approximately 20.84 kg of Metro Mix 380 media (Sun Gro Horticulture, Bellevue, WA) per pot. On 15 January 2014, six different rates of 16N-3.9P-10K (Osmocote® Plus, Everris Dublin Co., Marysville, OH) controlled release fertilizer (CRF) were added to the surface of the pots. Fertilizer rates were applied at 0, 5, 10, 15, 20, and 25 g. Fertilizer rates were selected to establish a low 5 g fertilizer rate to a high 25 g fertilizer rate for evaluation. The pots were drip irrigated at a rate that allowed media saturation and approximately 25% leaching. Pots were irrigated with tap water through drip emitters and were grown in the Department of Horticulture and Landscape Architecture Research Greenhouses at Stillwater, OK under natural photoperiods. Greenhouse growing condition temperatures were set at 22°C/17°C day/night with a photosynthetic photon flux density (PPFD) range of 400-700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 1200 HR.

Experimental Design. Three groups were created per cultivar consisting of six fertilizer levels within each group. The three groups established were a SPAD group, an

atLEAF group, and a control group. Each group contained 11 plants per fertilizer rate with 10 being tested using the sensors and one extra plant per fertilizer rate used to evaluate leaf nitrogen samples. Leaf nitrogen samples were taken by collecting 10 leaves per plant for each fertilizer rate. Leaf samples were analyzed at the end of the study for total nitrogen content (g kg^{-1} DM) by the Soil Water and Forage Analytical Laboratory (SWFAL) at Oklahoma State University, using a LECO TruSpec Carbon and Nitrogen Analyzer (LECO Corporation, St. Joseph, MI). Leachate was collected from five pots per treatment level for both cultivars every week to evaluate the pH and electrical conductivity (EC) using the Pour-Thru extraction method (Whipker et al., 2001).

Methods for Collecting Data. The experiment consisted of six fertilizer rates, replicated 33 times with single pot replications, with a total of 198 pots per cultivar, 66 pots per group. Fertilizer rates and cultivars were assigned to pots in a completely randomized design (CRD). SPAD and atLEAF sensors were used on the control groups of each cultivar testing 10 plants per fertilizer level by sampling leaves from the middle portion of the plant starting at 7 DAT. The sensor samples were taken from the middle of the leaf avoiding the midrib, leaf base, and leaf apex. The SPAD and atLEAF values were each averaged across the 10 plants in each fertilizer rate to obtain a value to input into the plant nitrogen mobile phone application for each specific cultivar. Upon receiving a recommendation to add additional fertilizer from the plant nitrogen mobile phone application an additional 20 g of controlled release fertilizer was added to the corresponding treatment level along with the SPAD and atLEAF groups, respectively. These treatment corrections were then tracked through the end of the study at 42 days after treatment (DAT) to evaluate the recommendations of the plant nitrogen mobile

phone application regarding the marketable quality of the treatment correction groups. End measures were collected on plant height, plant width, and dry weight, for each treatment level and the treatment corrections. Flower number was recorded for 'Homestead Purple'. Height was recorded by measuring the plant from the base of the soil to the upper apex of the plant. Width was recorded by taking two perpendicular measurements across the plant and averaging the two measurements. Dry weight was calculated by cutting the plant off at the base of the soil and drying in a plant dryer for 72 hours at 49°C.

Experiment Two. Plant Material and Experimental Methods for 'Aphrodite' and 'Hummingbird'. On 10 January 2014, 250 rooted cuttings each (4 to 8 leaves) of rose of Sharon (*Hibiscus syriacus* L.) 'Aphrodite', and summersweet (*Clethra alnifolia* L.) 'Hummingbird' were obtained from Greenleaf Nursery Inc. (Parkview, OK). All 500 rooted cuttings were put in the greenhouse until being transplanted into pots 4 d later with a single cutting. On 14 January 2014, the rooted cuttings were transplanted into standard 20 cm diameter pots and filled with approximately 1.05 kg of Metro Mix 902 media (Sun Gro Horticulture, Bellevue, WA). On 15 January 2014, 6 different rates of 16N-3.9P-10K (Osmocote® Plus, Everris Dublin Co., Marysville, OH) CRF were added to the surface of the pots. Treatments were 0, 10, 20, 30, 40, and 50 g. Fertilizer rates were selected to establish deficient 10 g fertilizer rates to excessive 50 g fertilizer rates for evaluation. Upon receiving a recommendation to add additional fertilizer from the plant nitrogen mobile phone application an additional 30 g of controlled release fertilizer (CRF) was added to the corresponding treatment level and SPAD and atLEAF groups, respectively.

Methods for Collecting Data. The methods for recording the SPAD and atLEAF sensor readings and end measurements for plant height, plant width, dry weight, and flower number were similar to Experiment One for each treatment level and the treatment correction levels. Flower number was recorded for ‘Aphrodite’ and ‘Hummingbird’.

Statistical Analysis. The sensor response variables were measured weekly for 6 weeks and generalized linear mixed models methods were used for the repeated measures analysis. For the end measure responses mixed models methods were used since unequal variance were evident among the treatment levels. Pearson correlations were computed with levels of significance $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Tukey pairwise comparisons of significant effects were performed, all tests were conducted at the 0.05 level of significance. All data were analyzed using SAS 9.4.

RESULTS

Experiment One. Effects of Fertilizer Treatment Levels on SPAD and atLEAF Sensor Values of Stachys ‘Helene Von Stein’. *Stachys ‘Helene Von Stein’* SPAD sensor values increased from 0 DAT through 14 DAT in the 10 and 15 g treatment levels with the exception of the 5, 20, and 25 g treatment levels which continued to increase through 21 DAT (Table 2.1). The 0 g treatment level increased through 7 DAT where the critical value of (38.4) was reached and then decreased thereafter (Table 2.1). Upon reaching this critical value the SPAD 0 (+20) corrected treatment level was initiated (Table 2.1). The SPAD 0 (+20) corrected treatment level started at 14 DAT with a SPAD value of

(37.0) and increased through 42 DAT ending at (51.0), which was not different than the 25 g treatment level (Table 2.1). The greatest SPAD sensor value of (51.7) was seen in the 25 g treatment level at 35 DAT and was not different from the 15 and 20 g treatment levels or the SPAD 0 (+20) corrected treatment level (Table 2.1).

The SPAD 0 (+20) corrected treatment level sensor values were less than all fertilizer treatment levels with the exception of the 0 g treatment level at 14 DAT (Table 2.1). At 21 DAT the SPAD 0 (+20) corrected treatment level was less than the 20 and 25 g treatment levels and was not significantly different from all other treatment levels except for the 0 g treatment which showed to decline from 14 DAT (Table 2.1). At 28 and 35 DAT the SPAD 0 (+20) corrected treatment level was not significantly different from the 15, 20, and 25 g treatment levels (Table 2.1). At 42 DAT, the SPAD 0 (+20) corrected treatment level value of (51.0) was observed to be significantly different from all treatment levels except the 25 g treatment level (Table 2.1).

Stachys 'Helene Von Stein' atLEAF sensor values increased from 0 DAT through 14 DAT for all treatment levels with the exception of the 0 and 25 g treatment levels, which increased through 7 DAT (Table 2.1). The 0 g treatment level sensor values decreased from 7 DAT through 42 DAT, and the 25 g treatment level increased through 7 DAT, decrease slightly over 14 and 21 DAT, and then increased through 42 DAT (Table 2.1). The 0 g treatment level reached the critical value of (46.2) at 7 DAT and upon this observation the atLEAF 0 (+20) corrected treatment level was initiated (Table 2.1). The atLEAF 0 (+20) corrected treatment level started at 14 DAT with an atLEAF value of (44.6) and increased through 35 DAT then decreased at 42 DAT (Table 2.1). The greatest atLEAF sensor value of (58.7) was observed at 35 DAT in the atLEAF 0 (+20)

corrected treatment level and was not significantly different from the 25 g treatment level at 35 DAT (Table 2.1). At 42 DAT the atLEAF 0 (+20) corrected treatment level showed a value of (54.6) and was significantly different from all fertilizer treatment levels with the exception of the 20 and 25 g treatment levels (Table 2.1).

At 14 DAT, the atLEAF 0 (+20) corrected treatment level was significantly less than all other treatment levels with the exception of the 0 g treatment level (Table 2.1). At 21 DAT the atLEAF 0 (+20) corrected treatment level was only different from the 0 and 20 g fertilizer treatments (Table 2.1). At 28 DAT the atLEAF 0 (+20) corrected treatment level was shown to be greater than all treatment levels with the exception of the 20 g treatment level (Table 2.1). At 35 DAT the atLEAF 0 (+20) corrected treatment level was not different from the 25 g treatment level, but was different from all other treatment levels (Table 2.1). At 42 DAT the ending value of (54.6) for the atLEAF 0 (+20) corrected treatment level was not significantly different from the 20 and 25 g treatment levels (Table 2.1).

The greatest pH value for ‘Helene Von Stein’ was observed in the 5 g treatment level at 21 DAT with a reading of (7.2) and was different from all other treatment levels at this date (Table 2.2). The greatest pH values in the 0 (+20) SPAD and atLEAF corrected treatment levels were observed at 14 DAT both with a value of (6.9) (Table 2.2). The greatest EC value was observed in the 25 g treatment level (1951 S) at 7 DAT and was not different from all other treatment levels at that testing date (Table 2.2). The greatest EC value in the 0 (+20) SPAD corrected treatment level was observed at 21 DAT with a value of (1036 S) and was not different from all treatment levels with the exception of the 0, 5, and 25 g treatment level (Table 2.2).

End Measures and Plant Characteristics of Stachys 'Helene Von Stein'. The greatest leaf nitrogen value for *Stachys 'Helene Von Stein'* was seen in the SPAD 0 (+20) corrected treatment level with a value of (2.31) g·kg⁻¹ DM (Table 2.3). The greatest plant height was seen in the 25 g treatment level showing a final height measurement of (21.08 cm) and was not different from the 10, 15, and 20 g or the atLEAF 0 (+20) corrected treatment level (Table 2.3). The atLEAF 0 (+20) corrected treatment level was not significantly different for plant height from all treatment levels with the exception of the 0 g treatment level at 42 DAT (Table 2.3). The greatest plant width was observed in the SPAD 0 (+20) corrected treatment level with a value of (49.91 cm), and was significantly different from all other treatment levels with the exception of the 20 g, 25 g, and the 0 (+20) atLEAF corrected treatment level (Table 2.3). The greatest shoot dry weight was observed in the 25 g treatment level showing a weight of (65.66 g) and was not different from the SPAD 0 (+20) and atLEAF 0 (+20) corrected treatment levels and the 15 and 20 g treatment levels (Table 2.3). Correlations were observed between fertilizer rate, SPAD, atLEAF as well as plant height and width (Table 2.4). The greatest correlations were seen between the SPAD and atLEAF sensor values (0.995), plant height and plant width (0.995), and between the atLEAF sensor values and plant width (0.994), the atLEAF sensor values and plant height (0.991), and fertilizer rate with the SPAD (0.906) and the atLEAF (0.895) sensors (Table 2.4).

Experiment One. Effects of Fertilizer Treatment Levels on SPAD and atLEAF Sensor Values of Verbena 'Homestead Purple'. *Verbena 'Homestead Purple'* SPAD sensor values increased from 0 DAT through 7 DAT in the 15 g treatment level (Table 2.5). The 5 and 20 g treatment levels continued to increase through 14 DAT (Table 2.5).

The 25 g treatment level SPAD sensor value increased through 14 DAT, and the 0 g treatment level SPAD sensor value decreased starting at 7 DAT (Table 2.5). The critical value for the SPAD 0 g treatment level of *Verbena* ‘Homestead Purple’ was never reached in conjunction with the plant nitrogen mobile application recommendations and therefore no treatment correction was initiated (Table 2.5). The highest SPAD sensor value of (51.0) was observed at 35 DAT in the 20 g treatment level and was different from all treatment levels with the exception of the 25 g treatment level (Table 2.5).

Verbena ‘Homestead Purple’ atLEAF sensor values increased from 0 DAT through 7 DAT for all treatment levels with the exception of the 0 and 15 g treatment levels (Table 2.5). The 0 g treatment level decreased from 7 DAT through 14 DAT (Table 2.5). The 25 g treatment level increased through 7 DAT and then decreased through 28 DAT (Table 2.5). It then increased at 35 DAT and then decreased through 42 DAT (Table 2.5). The critical value for the atLEAF 0 g treatment level of *Verbena* ‘Homestead Purple’ was never reached in conjunction with the mobile application recommendations and therefore no treatment correction was initiated (Table 2.5). The greatest atLEAF sensor value of (57.0) was observed at 35 DAT in the 25 g treatment level and was not significantly different from the 20 g treatment level but was different from all other treatment levels (Table 2.5).

The greatest pH value for ‘Homestead Purple’ was observed in the 5 g treatment level at 42 DAT with a reading of (7.3) and was not different from all other treatment levels with the exception of the 20 and 25 g treatment levels at this date (Table 2.6). The greatest EC value was observed in the 20 g treatment level (2208 S) at 28 DAT and was

different from all other treatment levels with the exception of the 25 g treatment level at that testing date (Table 2.6).

End Measures and Plant Characteristics of Verbena 'Homestead Purple'. The greatest plant height was seen in the 25 g treatment level showing a final height measurement of (20.32 cm) which was different from all other treatment levels (Table 2.7). The 5 g treatment level for height was not significantly different from the SPAD 0 (+20), atLEAF 0 (+20), and the 10 g treatment levels (Table 2.7). The greatest measurement for plant width was observed in the 10 g treatment level (97.53 cm) and was different from all treatment levels with the exception of the 25 g treatment level (83.31 cm) (Table 2.7). Greatest shoot dry weight was observed in the 25 g treatment level with a measurement of (50.14 g) and was not significantly different from the 10, 15, and 20 g treatment levels (Table 2.7). Greatest flower number was observed in the 25 g treatment level (119) and was not different from the 15 and 20 g treatment levels with flower numbers of (73) and (87), respectively (Table 2.7). The greatest leaf nitrogen (2.51) g·kg⁻¹ DM was observed in the 15 g treatment level (Table 2.7). Correlations were observed between nitrogen rate, SPAD, atLEAF, dry weight, and flower number (Table 2.8). The greatest correlations were seen between nitrogen rate and flower number (0.980), dry weight and flower number (0.960), dry weight and nitrogen rate (0.959) and between dry weight and the SPAD sensor (0.931) (Table 2.8). Nitrogen rate was correlated with the SPAD sensor (0.813) and the atLEAF sensor (0.812) (Table 2.8).

Experiment Two. Effects of Fertilizer Treatment Levels on SPAD and atLEAF Sensor Values of Hibiscus 'Aphrodite'. *Hibiscus 'Aphrodite'* SPAD sensor values increased from 0 DAT through 42 DAT in all treatment levels with the exception of the 0

and 20 g treatment levels (Table 2.9). The 0 g treatment level increased through 7 DAT, and the 20 g treatment level increased through 35 DAT (Table 2.9). The greatest SPAD sensor value of (80.9) observed in the 50 g treatment level at 42 DAT was not different from the 10 and 40 g treatment levels, but was different from all other treatment levels (Table 2.9).

At 14 DAT the critical value of (32.7) was reached in the 0 g treatment level at which time the SPAD 0 (+30) corrected treatment level was initiated (Table 2.9). The SPAD 0 (+30) corrected treatment level started at 21 DAT with a SPAD value of (37.1) which increased through 42 DAT with an ending value of (64.8) (Table 2.9). The SPAD 0 (+30) corrected treatment level was significantly different from all other treatment levels at 21, 28, and 35 DAT (Table 2.9). At 42 DAT the SPAD 0 (+30) corrected treatment level was not significantly different from the 10, 20, and 30 g treatment levels but was significantly different from the 0, 40, and 50 g treatment levels (Table 2.9).

The *Hibiscus* 'Aphrodite' atLEAF sensor values increased from 0 DAT through 42 DAT for all treatment levels with the exception of the 0, 10, and 20 g treatment levels (Table 2.9). The 0 g treatment level sensor values increased until 14 DAT when the critical value of (44.6) was reached and the atLEAF 0 (+30) corrected treatment level was initiated (Table 2.9). The greatest atLEAF sensor values were observed in the 30 and 50 g treatment levels at 42 DAT with values of (74.4) and (77.5), respectively (Table 2.9). The greatest atLEAF sensor value overall of (77.5) was not significantly different from the 30 and 40 g treatment levels, and was significantly different from all other treatment levels at 42 DAT (Table 2.9).

The atLEAF 0 (+30) corrected treatment level was initiated at 21 DAT with a value of (44.2) which increased through 42 DAT with an ending value of (65.2) (Table 2.7). At 21 DAT the atLEAF 0 (+30) corrected treatment level was less than all other treatment levels with the exception of the 0 g treatment level with a value of (44.2) (Table 2.7). At 28 DAT and 35 DAT the 0 (+30) corrected treatment level was different from all other treatment levels with a value of (55.5) (Table 2.7). At 35 DAT the atLEAF 0 (+30) corrected treatment level was different from the 0 g treatment level with a value of (60.0) (Table 2.7). At 42 DAT the atLEAF 0 (+30) corrected treatment level ended with a value of (65.2) which was different from all treatment levels with the exception of the 10 and 20 g treatment levels (Table 2.7). The greatest sensor value in the atLEAF 0 (+30) corrected treatment level was observed at 42 DAT with an ending value of (65.2) and was not different from the 10 and 20 g treatment levels (Table 2.7).

The greatest pH value for 'Aphrodite' was observed in the 0 g treatment level at 35 DAT with a reading of (7.1) and was different from all other treatment levels at this date with the exception of the 10 g treatment level (Table 2.10). The greatest pH values in the 0 (+30) SPAD and atLEAF corrected treatment levels were observed at 21 DAT both with values of (6.8) and (6.9), respectively and were different from all other treatment levels with the exception of the 0 and 10 g treatment levels (Table 2.10). The greatest EC value was observed in the 50 g treatment level (2630 S) at 28 DAT and was different from all other treatment levels at that testing date (Table 2.10). The greatest EC value in the 0 (+30) SPAD corrected treatment level was observed at 21 DAT with a value of (923 S) and was different from all treatment levels with the exception of the 40 and 50 g treatment levels (Table 2.10). The greatest EC value in the 0 (+30) atLEAF

corrected treatment level was observed at 42 DAT with a value of (1410 S) and was different from all treatment levels with the exception of the 30 and 50 g treatment levels (Table 2.10).

End Measures and Plant Characteristics of Hibiscus 'Aphrodite'. The greatest leaf nitrogen values for *Hibiscus 'Aphrodite'* were observed in the atLEAF 0 (+30) corrected treatment with a value of (6.68) g·kg⁻¹ DM (Table 2.11). The greatest plant height measurement was observed in the 30 g treatment level at (25.90 cm) but was not different from all other treatment levels (Table 2.11). The greatest measurement of plant width was observed in the 20 g treatment level at (12.57 cm) and was not different from all other treatment levels (Table 2.11). The greatest measurement of shoot dry weight was observed in the 30 g treatment level at (10.2 g) and was not significantly different from all other treatment levels (Table 2.11). The greatest number of flowers was observed in the 20 g treatment level at (1.36) and was not different from all other treatment levels with the exception of the 0 g treatment level (Table 2.11). The greatest correlations were observed between the SPAD and atLEAF sensor values (0.987) and correlations between height and width (0.860) were also observed (Table 2.12).

Experiment Two. Effects of Fertilizer Treatment Levels on SPAD and atLEAF Sensor Values of Clethra 'Hummingbird'. *Clethra 'Hummingbird'* SPAD sensor values increased from 21 DAT through 42 DAT in all treatment levels with the exception of the 0 g and SPAD 0 (+30) corrected treatment level (Table 2.13). The greatest SPAD sensor value was observed in the 30 g treatment level at 42 DAT with a value of (44.3) and was not different from the 20, 40, and 50 g treatment levels (Table 2.13). At 35 DAT the greatest SPAD sensor value was observed in the 50 g treatment level with a value of

(38.5) and was not different from the 30 and 40 g treatment levels (Table 2.13). At 24 DAT the greatest SPAD sensor value was observed in the 20 g treatment level with a value of (28.7) and was not significantly different from all other treatment levels (Table 2.13). At 21 DAT the greatest SPAD sensor value was observed in the 50 g treatment level with a value of (30.0) and was not different from all other treatment levels (Table 2.13).

The SPAD 0 (+30) corrected treatment level was initiated at 21 DAT when the critical value of (25.9) was reached in the 0 g treatment level (Table 2.13). At 24 DAT the SPAD 0 (+30) corrected treatment level was not significantly different from all other treatment levels with a value of (26.0) (Table 2.13). At 35 DAT the SPAD 0 (+30) corrected treatment level decreased from the previous week with an observed value of (25.3) which was significantly different from all other treatment levels with the exception of the 0 g treatment level (Table 2.13). At 42 DAT the SPAD 0 (+30) corrected treatment level ended with a value of (25.0) that was observed to be significantly different from all other treatment levels (Table 2.13).

Clethra 'Hummingbird' atLEAF sensor values increased from 21 DAT through 42 DAT in all treatment levels with the exception of the 0 g, 10 g, and the atLEAF 0 (+30) corrected treatment level (Table 2.13). The atLEAF sensor values in the 0 g treatment level decreased from 21 through 42 DAT (Table 2.13). The 10 g treatment level started with a value of (37.8) at 21 DAT and then decreased to (33.1) at 24 DAT then it continued to increase through 42 DAT (Table 2.13). The atLEAF 0 (+30) corrected treatment level increased from 24 to 35 DAT and then decreased at 42 DAT with an ending value of (29.9) (Table 2.13).

The atLEAF 0 (+30) corrected treatment level was initiated at 21 DAT when the critical value of (33.1) was reached in the 0 g treatment level (Table 2.13). At 24 DAT an atLEAF 0 (+30) corrected treatment level sensor value of (32.9) was observed and was not different from all other treatment levels (Table 2.13). At 35 DAT the atLEAF 0 (+30) corrected treatment level value was (33.9) and was different from all other treatment levels with the exception of the 0 g treatment level (Table 2.13). At 42 DAT the atLEAF 0 (+30) corrected treatment level value was (29.9) and was different from all other treatment levels with the exception of the 0 g treatment level (Table 2.13).

The greatest pH value for 'Hummingbird' was observed in the 0 g treatment level at 42 DAT with a reading of (7.1) and was different from all other treatment levels at this date with the exception of the 10 g treatment level (Table 2.14). The greatest EC value was observed in the 50 g treatment level (1253 S) at 42 DAT and was different from all other treatment levels at that testing date with the exception of the 20 and 40 g treatment levels (Table 2.14).

End Measures and Plant Characteristics of Clethra 'Hummingbird'. The greatest leaf nitrogen values for *Clethra* 'Hummingbird' were observed in the 40 g treatment level with a value of (3.36) g·kg⁻¹ DM (Table 2.15). The greatest measurements of plant height (41.14 cm), plant width (34.03 cm), and shoot dry weight (16.04 g) were not significantly different from all treatment levels with the exception of the SPAD 0 (+30) and atLEAF 0 (+30) corrected treatment levels and the 0 g treatment level (Table 2.15). Flower number was not significantly different from all other treatment levels across all treatment dates (Table 2.15). The greatest correlations were seen between the plant width and dry weight (0.992) (Table 2.16). There was also a correlation between the atLEAF

sensor and dry weight (0.932) (Table 2.16). The SPAD and atLEAF sensors were highly correlated (0.945), and the SPAD sensor was highly correlated with plant width (0.935) (Table 2.16). Nitrogen rate was correlated with the atLEAF sensor (0.823) and the SPAD sensor showed a weak correlation to nitrogen rate (0.741) (Table 2.16).

DISCUSSION

Stachys 'Helene Von Stein'. The SPAD values ranged from (30.6) in the 0 g treatment to (51.7) in the 25 g treatment, and the atLEAF values ranged from (37.9) in the 0 g treatment to (58.7) in the 0 (+20) atLEAF corrected treatment showing that the sensor values increased with greater nitrogen rates, which is in agreement with what other studies have found (Wang et al., 2004; Wang et al., 2012; Zhu et al., 2012) (Table 2.1) (Figure 2.1). The SPAD 0 (+20) corrected treatment level started at 14 DAT with a SPAD value of (37.0) and increased through 42 DAT ending at (51.0), which was not different than the 25 g treatment level (Table 2.1). This coincides with what Basyouni et al. (2015) found regarding corrected treatments in poinsettia (*Euphorbia pulcherrima* L. (Willd. ex Klotzsch) finding that corrected treatments could be used to correct nitrogen deficiencies during production. At 42 DAT, the SPAD 0 (+20) corrected treatment level sensor value of (51.0) was different from all treatment levels except the 25 g treatment level, and the atLEAF 0 (+20) corrected treatment level sensor value of (54.6), which was different from all treatment levels with the exception of the 20 g and 25 g treatment levels (Table 2.1) (Figure 2.2 – 2.3). The corrected treatment levels of SPAD and atLEAF both

produced higher quality plant materials based on the increased shoot dry weight measurements by using the mobile application recommendation to add fertilizer at 14 DAT and resulted in shoot dry weight values similar to the 20 and 25 g treatment levels (Table 2.3). This agrees with what Basyouni et al. (2015) found in potted poinsettia, and what Khoddamzadeh and Dunn (2016) found in garden mums chrysanthemum (*Dendranthema x grandiflorum* Ramant.).

Plant height was observed to be greatest in the 25 g treatment level (21.08 cm) and was not different from the 15 g treatment level and the 0 (+20) atLEAF treatment level with both showing the same measurement (18.54 cm) (Table 2.3). This is this is not in agreement with what Dunn et al. (2015) found in blanket flower (*Gaillardia aristata* ‘Arizona Apricot’), but is in agreement with what Dunn et al. (2016) found regarding ornamental kale (*Brassica oleracea* L. ‘Nagoya Red’). Dunn et al. (2015) found that height was not influenced by nitrogen rate in ‘Arizona Apricot’, and this difference may be due to the fact that the methods used for ‘Helene Von Stein’ applied significantly higher nitrogen rates ranging from 5 to 25 g while the ‘Arizona Apricot’ study used 4 to 12 g nitrogen rates. Gaillardia as a native plant may require less fertilizer when grown in field applications, but Sowmyamala and Nagaraju, (2013) found that increased nitrogen rates influences all parameters of growth in gaillardia including increased flowering, plant height, and decreased the time to first date of flowering. Dunn et al. (2016) did find that ‘Nagoya Red’ was influenced by greater nitrogen rates with coincides with what was found in ‘Helene Von Stein’. This may be due to the fact that ‘Helene Von Stein’ and ‘Nagoya Red’ are grown for their foliage and not for their flowering attributes which

agrees with what Wang et al. (2004) found in different cultivars of peace lily (*Spathiphyllum* sp. Schott).

The greatest plant width was observed in the SPAD and atLEAF 0 (+20) corrected treatment levels and were not different from the 20 and 25 g treatment level (Table 2.3). This was in agreement with Dunn et al. (2015) as the greater nitrogen rates produced the greatest plant widths in ‘Arizona Apricot’ (Table 2.3). The greatest shoot dry weight was seen in the 25 g treatment level and was not different from the SPAD and atLEAF corrected treatment levels which agrees with what Basyouni et al. (2015) found in poinsettia, as well as what Khoddamzadeh and Dunn (2016) found in chrysanthemum, and Wang et al. (2012) found in geranium (*Pelargonium x hortorum* L.H. Bailey) (Table 2.3).

Correlations were observed between fertilizer rate, SPAD, atLEAF as well as plant height and width (Table 2.4). Correlations compare with other crops such as what Bullock and Anderson (1998) found in corn (*Zea mays* L.) as correlations were observed between nitrogen rate and both sensors, and between the sensors, respectively. Plant quality and salability of ‘Helene Von Stein’ relies largely on dry weight and size, which reflects in the corrected treatment levels for SPAD and atLEAF at 42 DAT (Figure 2.2 – 2.3). Adding 20 g of fertilizer to the crop at 7 DAT may save fertilizer and produce marketable plant material regarding shoot dry weight comparable to adding 10 or 15 g of fertilizer at the start of the production process (Table 2.3) (Figure 2.4). This shows that using the SPAD and atLEAF sensors in conjunction with the mobile application may save fertilizer used in the production process helping growers to use precision in fertilizer applications based on the end measures for plant dry weight for this cultivar.

Verbena 'Homestead Purple'. The corrected treatment levels for SPAD and atLEAF were not initiated by the mobile application recommendation and the only positive growth is seen in the 15 and 20 g treatment levels (Figure 2.5). The critical value for 'Homestead Purple' was never reached in conjunction with the mobile application, and therefore a corrected treatment was never established. Altland et al. (2002) found that SPAD sensors were able to detect a nitrogen deficiency in vinca (*Catharanthus roseus* L. (G. Don) and that supplemental nitrogen applications were able to prevent yield losses during crop production. The variability in leaf type on 'Homestead Purple' may play a role in the inconsistency seen in the sensor readings leading to the critical value not being reached in conjunction with the mobile application. Dunn and Goad, (2015) and Mickelbart, (2010), found that precision in evaluating the nitrogen status of a crop using the sensors required careful consideration of the leaf sampling procedure and collection practices. Dunn and Goad, (2015) also found that the atLEAF sensor position during sampling also affected sensor values. The variability and small size of the 'Homestead Purple' leaves at juvenile stages of growth may have caused variability in the sensor readings, which prevented the critical value being reached in conjunction with the mobile application. Wang et al. (2012) found that the fertility requirements of ornamental plants growing in a greenhouse environment varied between plant age and type. This finding could support what was found in 'Homestead Purple' as the mobile application did not recommend fertilizer in the early stages of the crop due to the variation in leaf type and as a result of plant age.

Plant quality measures for 'Homestead Purple' showed that the greatest plant height (20.32 cm) was observed in the 25 g treatment, and the greatest plant width was

observed in the 10 g treatment level (97.53 cm) (Table 2.7). Verbena is grown for its groundcover like habit which make a plant width more desirable than plant height. Flower number would also be a marketable and desirable characteristic for ‘Homestead Purple’. Overall, the 15 g treatment level had the best visual characteristics and 15 g of fertilizer added at the beginning of the production cycle would have produce desirable plant material for market over the greatest fertilizer levels (Figure 2.7). Flower number was highly correlated with nitrogen rate and flower number and dry weight also showed a significant correlation coinciding with what others have found regarding geranium (*Pelargonium x hortorum* L.H. Bailey) (Wang et al., 2012) (Table 2.8).

Hibiscus ‘Aphrodite’. ‘Aphrodite’ did recover using the nitrogen mobile application fertilizer recommendations in a timely manner as the SPAD and atLEAF critical values were reached at 14 DAT providing for enough time to correct the negative nutrient status of the crop (Table 2.9) (Figure 2.6). This coincides with what others have found regarding treatment corrections (Basyouni et al., 2015; Bullock and Anderson, 1998; Khoddamzadeh and Dunn, 2016). The SPAD corrected treatment group at 42 DAT was not significantly different from the 10, 20, and 30 g treatment level, and the atLEAF corrected treatment group at 42 DAT was not significantly different from the 10 and 20 g treatment levels (Table 2.9) (Figure 2.6). The corrected treatment application levels were determined using the high application rate provided by the fertilizer manufacturer. The 40 and 50 g treatment levels were high in nitrogen application, which can be seen in the stunted growth and less than sufficient flowering (Figure 2.7). The nitrogen mobile application gave an appropriate fertilizer recommendation, but the SPAD (5.75 g) and the atLEAF (6.20 g) dry weights were not significantly different from all

other treatment levels due to the elevated fertilizer rates resulting in no significant treatment affects (Table 2.11). The 30 g treatment level performed best with fertilizer application at the beginning of the crop cycle regarding plant height, dry weight, and flower number (Table 2.11) (Figure 2.6). Adding fertilizer at 21 DAT did not allow the corrected treatment levels to measure up with the 30 g treatment level end measures (Table 2.11) (Figure 2.6). Correlations were observed between the SPAD and atLEAF sensors and this coincides with other studies (Bullock and Anderson, 1998) (Table 2.12).

Clethra 'Hummingbird'. 'Hummingbird' did not recover using the nitrogen mobile application fertilizer recommendations. The critical values for SPAD and atLEAF were reached at 21 DAT for the 0 g treatment correction groups (Table 2.13) (Figure 2.8). The SPAD and atLEAF corrected treatment groups at 42 DAT are both significantly different from all other treatment levels (Table 2.13) (Figure 2.8). The higher treatment levels did not respond to the nitrogen mobile application recommendations as the fertilizer treatment levels were elevated, but did produce marketable plant materials (Figure 2.9). The mobile application gave an appropriate fertilizer recommendation regarding the critical value, but the SPAD (3.87 g) and atLEAF (3.76 g) dry weights were significantly different from all treatment levels due to the application recommendation at 28 DAT, which is half way into the crop schedule (Table 2.15). Adding fertilizer at 28 DAT did not allow the corrected treatment groups to catch up with the performing fertilizer treatment of 30 g (Table 2.13) (Figures 2.8-2.9). Significant correlations were observed between nitrogen rate and the SPAD and atLEAF sensors coinciding with what was found in the Bullock and Anderson (1998) study, but

Khoddamzadeh and Dunn (2016) found that the SPAD and atLEAF sensors were not correlated with each other in chrysanthemum (Table 2.16).

The 15 g treatment level of ‘Helene Von Stein’ showed the greatest response measures regarding height and shoot dry weight as both parameters were not different from the corrected treatment levels to which 20 g was added (Table 2.3). It is recommended that adding 15 g of fertilizer at the beginning of the crop cycle would produce marketable plant materials over adding 20 g of fertilizer regarding the growth responses seen at 42 DAT (Table 2.3). The response of ‘Homestead Purple’ showed that the 15 g treatment level produced favorable plant material regarding shoot dry weight and flower number similar to the higher fertilizer treatment levels (Table 2.7).

Recommendations of adding 15 g of fertilizer would produce the same growth responses as adding 20 g of fertilizer at the beginning of the crop cycle (Table 2.7). ‘Aphrodite’ showed a greater response in height, width, shoot dry weight, and flower number in the 10 g treatment level and was not different from the highest fertilizer treatment levels (Table 2.11). Adding 10 g of fertilizer produced an increased growth response regarding these parameters over adding 30 g of fertilizer (Table 2.11). ‘Hummingbird’ showed an increase in height, width, shoot dry weight, and flower number in the 10 g treatment level similar to the higher treatment levels (Table 2.15). Adding 10 g of fertilizer would produce plant materials similar to the treatment levels with increased fertilizer levels (Table 2.15). Chlorophyll leaf sensors are valuable tools that can determine the nitrogen status of a growing crop at a specific point in time, but accurate predictions for future nitrogen applications must be made on a cautionary basis taking into account the environmental growth conditions, type of plant material, and sampling methods.

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Table 2.1. SPAD and atLEAF measurements on *Stachys* ‘Helene Von Stein’ with different rates of fertilizer at seven dates after treatment (DAT) using 16N-3.9P-10K controlled release fertilizer.

Fertilizer rate (g)	0	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
SPAD							
0	37.7a ^z	38.4c	37.5c	36.2e	33.9d	33.5d	30.6f
0 (+20)^y	--	-- ^x	37.0c	46.4cd	50.1a	50.1a	51.0a
5	41.6a	42.7b	43.4b	44.3d	43.3c	43.3c	41.2e
10	40.5a	45.9ab	47.1a	46.3cd	45.2bc	45.8bc	43.5de
15	40.9a	46.9a	49.0a	47.2bc	48.1ab	48.2ab	45.5cd
20	40.4a	45.8ab	47.2a	50.2ab	48.5a	48.6ab	47.9bc
25	38.7a	48.5a	50.2a	50.8a	50.6a	51.7a	48.6ab
atLEAF							
0	49.4a ^z	46.2c	44.5c	43.4d	39.8f	40.9e	37.9d
0 (+20)^y	--	-- ^x	44.6c	53.6bc	57.0a	58.7a	54.6a
5	49.6a	51.4b	52.6b	51.5c	49.7e	49.4d	47.7c
10	50.2a	54.1ab	54.9ab	54.6ab	51.7de	52.7c	50.8bc
15	49.9a	55.3a	56.0a	54.4ab	52.9cd	55.9b	51.8b
20	45.8b	54.2ab	56.8a	56.3a	55.3abc	54.7bc	53.1ab
25	48.4ab	55.6a	54.9ab	53.8bc	54.5bc	56.2ab	55.0a

^zAverage means (n=10) within a column for each sensor with the same letter are not significantly different at the 5% level.

^yIndicates 20 g added to create a corrected treatment group.

^xCorrected treatment group started at 7 DAT with average values of SPAD (38.4) and atLEAF (46.2).

Table 2.2. pH and EC measurements on *Stachys* ‘Helene Von Stein’ with different rates of fertilizer at six dates after treatment (DAT) of 16N-3.9P-10K.

Fertilizer rate (g)	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
pH						
0	6.9a ^z	7.1a	7.0ab	7.1a	6.8ab	6.8abc
0 (+20)SPAD^y	-- ^x	6.9a	6.7bc	6.8abc	6.9ab	6.9ab
0(+20)atLEAF^y	-- ^x	6.9a	6.5c	6.5c	6.5bc	6.7bc
5	6.7ab	6.8ab	7.2a	7.1a	7.0a	7.0a
10	6.6bc	6.8ab	6.9bc	7.1a	6.9a	6.9ab
15	6.4c	6.8ab	6.7bc	7.0a	6.8ab	6.8abc
20	6.0d	6.5bc	6.4c	6.9ab	6.8ab	6.9abc
25	5.80d	6.1c	6.6c	6.6bc	6.3c	6.6c
EC (S)						
0	477a ^z	536b	510b	428bc	468b	544a
0 (+20)SPAD^y	-- ^x	477b	1036a	739ab	892a	480a
0(+20)atLEAF^y	-- ^x	477b	699ab	865a	674ab	701a
5	591a	512b	482b	357bc	453b	418a
10	948a	526b	604ab	286c	578ab	676a
15	1114a	599b	629ab	663ab	553ab	545a
20	1390a	1077a	790ab	570abc	552ab	447a
25	1951a	1381a	518b	511abc	547ab	481a

^zAverage means (n=5) within a column with the same letter are not significantly different at the 5% level.

^yIndicates 20 g added to create a corrected treatment group for SPAD and atLEAF.

^xCorrected treatment group started at 7 DAT.

Table 2.3. Response of *Stachys* ‘Helene Von Stein’ to six fertilizer rates 42 days after initial fertilizer treatment with SPAD and atLEAF correction groups included.

Fertilizer rate (g)	Height (cm)	Width (cm)	Shoot dry weight (g)	Leaf N ^z (g·kg ⁻¹ DM)
0	6.85c ^y	18.98d	7.86d	0.98
0 (+20)SPAD^x	17.27b	49.91a	57.44ab	2.31
0 (+20)atLEAF^x	18.54ab	48.26a	60.94a	2.15
5	15.74b	38.22c	29.00c	1.38
10	18.03ab	42.73b	47.76b	1.66
15	18.54ab	42.79b	57.50ab	1.86
20	18.28ab	44.76ab	59.91a	2.04
25	21.08a	48.00a	65.66a	2.13

^zLeaf nitrogen content from 10 mature leaves and no petioles from one plant per treatment.

^yThe average means (n=10) within a column with the same letter are not significantly different at P < 0.05.

^xIndicates 20 g added to create a corrected treatment group for SPAD and atLEAF at 7 DAT.

Table 2.4. Pearson correlation (r) matrix for fertilizer rate, sensor readings, height, width, and dry weight of *Stachys* ‘Helene Von Stein’ at 42 DAT.

	SPAD	atLEAF	Height	Width	Dry weight
Fertilizer rate	0.906*	0.895*	0.849*	0.844*	0.945**
SPAD		0.995***	0.977**	0.985**	0.971**
atLEAF			0.991***	0.994***	0.970**
Height				0.995***	0.944**
Width					0.941**

*, **, ***, representing correlation coefficient (r) significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

Table 2.5. SPAD and atLEAF measurements on *Verbena* ‘Homestead Purple’ with different rates of fertilizer at seven dates after treatment (DAT) using 16N-3.9P-10K controlled release fertilizer.

Fertilizer rate (g)	0	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
SPAD							
0	41.5ab ^z	40.4b	38.5b	37.1b	33.0c	33.3d	30.8d
5	40.0ab	42.8ab	46.6a	40.7ab	40.1b	41.7c	41.2bc
10	44.9a	42.7ab	43.4ab	42.5ab	41.2b	43.8c	43.1b
15	38.2b	45.7a	44.3a	41.9ab	43.9ab	45.5bc	43.4b
20	43.8a	44.5a	47.0a	45.3a	48.2a	51.0a	50.0a
25	40.6ab	46.7a	46.2a	44.0a	47.3a	49.0ab	44.7b
atLEAF							
0	49.5ab ^z	47.6d	44.9c	46.4b	43.0c	38.1c	39.3e
5	49.1ab	49.8cd	49.3b	51.8a	48.0b	50.2b	50.2bcd
10	46.0b	52.1abcd	54.2a	48.5ab	47.9b	50.0b	51.5abc
15	52.5a	50.2bcd	53.4ab	52.1a	51.8ab	50.5b	47.9cd
20	50.1ab	54.9ab	53.5ab	52.5a	53.2a	53.4ab	52.0abc
25	49.2ab	55.2a	55.1a	53.4a	49.6ab	57.0a	53.3a

^zAverage means (n=10) within a column for each sensor with the same letter are not significantly different at the 5% level.

Table 2.6. pH and EC measurements on *Verbena* ‘Homestead Purple’ with different rates of fertilizer at six dates after treatment (DAT) of 16N-3.9P-10K.

Fertilizer rate (g)	14 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
	pH					
0	6.9a ^z	7.0a	6.9a	7.0a	7.0a	7.2a
5	6.8a	7.0a	7.1ab	7.2a	7.0a	7.3a
10	6.4b	6.6b	6.5bcd	6.9ab	6.7b	7.0a
15	6.2b	6.4c	6.7bc	6.8b	6.7b	7.0a
20	5.9b	6.3c	6.3d	6.2c	6.6b	6.8b
25	5.9b	6.1c	6.4cd	6.0c	6.5b	6.8ab
	EC (S)					
0	461b ^z	514b	500b	498c	500a	505a
5	613b	521b	504b	481c	489a	527a
10	1027ab	685ab	696ab	454c	526a	514a
15	1694a	810ab	492b	531bc	489a	525a
20	1822a	1556a	1778a	2208a	530a	888a
25	2012a	1848a	887ab	1314abc	719a	683a

^zAverage means (n=5) within a column with the same letter are not significantly different at the 5% level.

Table 2.7. Response of *Verbena* ‘Homestead Purple’ to six fertilizer rates 42 days after initial fertilizer treatment (DAT) with SPAD and atLEAF correction groups included.

Fertilizer rate (g)	Height (cm)	Width (cm)	Shoot dry weight (g)	Flower number	Leaf N ^z (g·kg ⁻¹ DM)
0	9.14d ^y	31.75f	4.98d	2d	2.00
5	17.27bc	49.53de	24.92bc	47bc	2.46
10	12.95cd	97.53a	32.66ab	60b	1.68
15	7.11d	62.23cd	40.24ab	73a	2.51
20	8.38d	69.85bc	48.16a	87a	2.32
25	20.32a	83.31ab	50.14a	119a	2.17

^zLeaf nitrogen content from 10 mature leaves and no petioles from one plant per treatment.

^yThe average means (n=10) within a column with the same letter are not significantly different at P < 0.05.

Table 2.8. Pearson correlation (r) matrix for fertilizer rate, sensor readings, height, width, dry weight, and flower number of *Verbena* ‘Homestead Purple’ at 42 DAT.

	SPAD	atLEAF	Height	Width	Dry weight	Flower number
Fertilizer rate	0.813*	0.812*	0.204	0.644	0.959**	0.980***
SPAD		0.840*	0.124	0.677	0.931**	0.813*
atLEAF			-0.00042	0.796	0.875*	0.895*
Height				-0.001	0.265	0.202
Width					0.710	0.692
Dry weight						0.960**

*, **, ***, representing correlation coefficient (r) significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

Table 2.9. SPAD and atLEAF measurements on *Hibiscus* ‘Aphrodite’ with different rates of fertilizer at seven dates after treatment (DAT) using 16N-3.9P-10K controlled release fertilizer.

Fertilizer rate (g)	0	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
SPAD							
0	34.9b ^z	36.5c	32.7c	32.1d	25.2c	24.9c	21.1d
0 (+30)^y	--	--	-- ^x	37.1c	51.0b	53.2b	64.8c
10	38.6ab	45.3ab	54.0ab	59.7ab	64.5a	68.9a	72.2abc
20	37.4ab	45.7ab	52.2b	64.1a	66.9a	71.0a	67.6c
30	39.8a	46.1ab	54.7ab	60.1ab	66.8a	69.6a	71.0bc
40	37.7ab	42.4b	54.9ab	59.3b	66.8a	69.8a	79.5ab
50	40.6a	48.0a	58.4a	60.0ab	65.3a	71.6a	80.9a
atLEAF							
0	44.3a ^z	46.1b	44.6b	40.2c	38.8c	32.0c	32.2d
0 (+30)^y	--	--	-- ^x	44.2c	55.5b	60.0b	65.2c
10	48.1a	50.6ab	60.0a	60.7b	68.7a	67.3a	70.0bc
20	46.2a	51.9a	60.7a	65.3ab	65.1a	68.9a	68.4bc
30	49.4a	49.8ab	63.8a	68.6a	69.2a	70.2a	74.4ab
40	45.3a	51.8a	59.6a	65.5ab	66.4a	69.5a	72.7ab
50	49.5a	49.2ab	61.3a	66.5a	68.3a	72.6a	77.5a

^zAverage means (n=10) within a column for each sensor with the same letter are not significantly different at the 5% level.

^yIndicates 30 g added to create a corrected treatment group.

^xCorrected treatment group started at 14 DAT with average values of SPAD (32.7) and atLEAF (44.6).

Table 2.10. pH and EC measurements on *Hibiscus* ‘Aphrodite’ with different rates of fertilizer at five dates after treatment (DAT) of 16N-3.9P-10K.

Fertilizer rate (g)	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
	pH				
0	6.9a ^z	7.0a	6.9a	7.1a	7.0a
0(+30)SPAD^y	--	6.8a ^x	6.7a	6.6bc	6.5c
0(+30)atLEAF^y	--	6.9a ^x	6.6b	6.5bc	6.6c
10	7.0a	6.8a	6.6b	7.0a	7.0a
20	6.8b	6.7bc	6.4c	6.7b	6.8b
30	6.9ab	6.7bc	6.4c	6.6bc	6.6c
40	6.9a	6.7bc	6.2d	6.5c	6.4d
50	6.9ab	6.6c	6.2d	6.5c	6.3d
	EC (S)				
0	476a ^z	491b	480e	477c	498b
0(+30)SPAD^y	--	923b ^x	856d	763bc	875b
0(+30)atLEAF^y	--	872b ^x	1101cd	344d	1410a
10	489a	869b	969d	581ba	552b
20	489a	1457b	1400cd	697bc	792b
30	486a	1862ab	1842bc	733abc	918ab
40	503a	1887a	2182b	802ab	872b
50	493a	2220a	2630a	1112a	1339a

^zAverage means (n=5) within a column with the same letter are not significantly different at the 5% level.

^yIndicates 30 g added to create a corrected treatment group for SPAD and atLEAF.

^xCorrected treatment groups for SPAD and atLEAF started at 14 DAT.

Table 2.11. Response of *Hibiscus* ‘Aphrodite’ to six fertilizer rates 42 days after initial fertilizer treatment (DAT) with SPAD and atLEAF correction groups included.

Fertilizer rate (g)	Height (cm)	Width (cm)	Shoot dry weight (g)	Flower number	Leaf N ^z (g·kg ⁻¹ DM)
0	19.81a ^y	9.65ab	2.20a	0.00b	2.17
0 (+30)SPAD^x	22.86a	9.77ab	5.75a	0.60a	5.78
0 (+30)atLEAF^x	21.59a	10.92ab	6.20a	0.53a	6.68
10	21.84a	11.43ab	5.76a	1.30a	4.66
20	23.62a	12.57a	7.52a	1.36a	6.15
30	25.90a	11.81ab	10.20a	1.13a	6.05
40	20.32a	8.255ab	5.84a	1.28a	6.08
50	12.27a	7.874b	5.41a	0.64a	6.25

^zLeaf nitrogen content from 10 mature leaves and no petioles from one plant per treatment.

^yThe average means (n=10) within a column with the same letter are not significantly different at P < 0.05.

^xIndicates 30 g added to create a corrected treatment group for SPAD and atLEAF at 14 DAT.

Table 2.12. Pearson correlation (r) matrix for fertilizer rate, sensor readings, height, width, dry weight, and flower number of *Hibiscus* ‘Aphrodite’ at 42 DAT.

	SPAD	atLEAF	Height	Width	Dry weight	Flower number
Fertilizer rate	0.777	0.765	0.263	0.523	0.384	0.298
SPAD		0.987***	0.072	0.075	0.620	0.784
atLEAF			0.1712	0.017	0.715	0.776
Height				0.860*	0.769	0.504
Width					0.534	0.427
Dry weight						0.697

*, **, ***, representing correlation coefficient (r) significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.

Table 2.13. SPAD and atLEAF measurements on *Clethra* ‘Hummingbird’ with different rates of fertilizer at four dates after treatment (DAT) of 16N-3.9P-10K controlled release fertilizer.

Fertilizer rate (g)	21 DAT	28 DAT	35 DAT	42 DAT
SPAD				
0	25.9ab ^z	25.0a	24.3c	22.7d
0 (+30)^y	-- ^x	26.0a	25.3c	25.0c
10	25.9bc	26.5a	33.5b	37.2b
20	26.6bc	28.7a	33.5b	43.4a
30	25.5bc	26.9a	37.6a	44.3a
40	27.7bc	28.4a	38.3a	42.7a
50	30.0ab	28.6a	38.5a	41.8ab
atLEAF				
0	33.1ab ^z	32.0a	29.1d	28.7c
0 (+30)^y	-- ^x	32.9a	33.9d	29.9c
10	37.8a	33.1a	39.5c	44.9b
20	33.3ab	35.5a	39.5c	45.6ab
30	33.2ab	33.7a	40.9bc	48.0ab
40	32.8b	37.5a	43.2abc	47.6ab
50	33.9ab	35.8a	44.4ab	50.6a

^zAverage means (n=10) within a column for each sensor with the same letter are not significantly different at the 5% level. *Clethra* was unavailable for testing until 21 DAT.

^yIndicates 30 g added to create a corrected treatment group.

^xCorrected treatment group started at 21 DAT with average values of SPAD (25.9) and atLEAF (33.1).

Table 2.14. pH and EC measurements on *Clethra* ‘Hummingbird’ with different rates of fertilizer at three dates after treatment (DAT) of 16N-3.9P-10K.

Fertilizer rate (g)	21 DAT	24 DAT	42 DAT
	pH		
0	6.9a ^z	--	7.1a
0(+30)SPAD^y	--	6.6a	--
0(+30)atLEAF^y	--	6.5b	--
10	7.0a	--	6.9ab
20	6.7b	--	6.8b
30	6.6b	--	6.5c
40	6.7b	--	6.2d
50	6.6b	--	6.3d
	EC (S)		
0	485c ^z	--	501c
0(+30)SPAD^y	--	763a	--
0(+30)atLEAF^y	--	344b	--
10	669b	--	611b
20	1122ab	--	1133ab
30	1215a	--	866b
40	1128a	--	981ab
50	1085ab	--	1253a

^zAverage means (n=5) are presented starting at 21 DAT. Means within a column with the same letter are not significantly different at the 5% level.

^yIndicates 30 g added to create a corrected treatment group for SPAD and atLEAF at 21 DAT.

Table 2.15. Response of *Clethra* ‘Hummingbird’ to six fertilizer rates 42 days after initial fertilizer treatment with SPAD and atLEAF correction groups included.

Fertilizer rate (g)	Height (cm)	Width (cm)	Shoot dry weight (g)	Flower number	Leaf N ^z (g·kg ⁻¹ DM)
0	28.44b ^y	13.71b	4.33b	2a	1.08
0(+30)SPAD^x	29.21b	12.82b	3.87b	2a	1.10
0(+30)atLEAF^x	27.94b	11.43b	3.76b	2a	1.16
10	39.37a	29.59a	13.45a	2a	3.05
20	34.29ab	29.21a	14.49a	2a	3.00
30	33.52ab	32.63a	15.42a	2a	3.20
40	41.14a	34.03a	16.04a	2a	3.36
50	30.48ab	28.06a	13.36a	2a	3.34

^zLeaf nitrogen content from 10 mature leaves and no petioles from one plant per treatment.

^yThe average means (n=10) within a column with the same letter are not significantly different at P < 0.05.

^xIndicates 30 g added to create a corrected treatment group for SPAD and atLEAF at 21 DAT.

Table 2.16. Pearson correlation (r) matrix for fertilizer rate, sensor readings, height, width, dry weight, and flower number of *Clethra* ‘Hummingbird’ at 42 DAT.

	SPAD	atLEAF	Height	Width	Dry weight	Flower number
Fertilizer rate	0.741 ^z	0.823 [*]	0.159	0.648	0.668	0.851 [*]
SPAD		0.945 ^{**}	0.463	0.935 ^{**}	0.966 ^{**}	0.625
atLEAF			0.455	0.912 [*]	0.932 ^{**}	0.634
Height				0.718	0.667	0.355
Width					0.992 ^{***}	0.572
Dry weight						0.595

^{*}, ^{**}, ^{***}, representing correlation coefficient (r) significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively.



Figure 2.1. Results of applying 0, 5, 10, 15, 20, and 25 g (left to right) of 16N-3.9P-10K controlled release fertilizer to *Stachys* 'Helene Von Stein' at 42 DAT.



Figure 2.2. SPAD *Stachys* 'Helene Von Stein' corrected treatment group shown at 42 DAT with 20 g of 16N-3.9P-10K controlled release fertilizer added at 7 DAT.



Figure 2.3. atLEAF *Stachys* 'Helene Von Stein' corrected treatment group shown at 42 DAT with 20 g of 16N-3.9P-10K controlled release fertilizer added at 7 DAT.

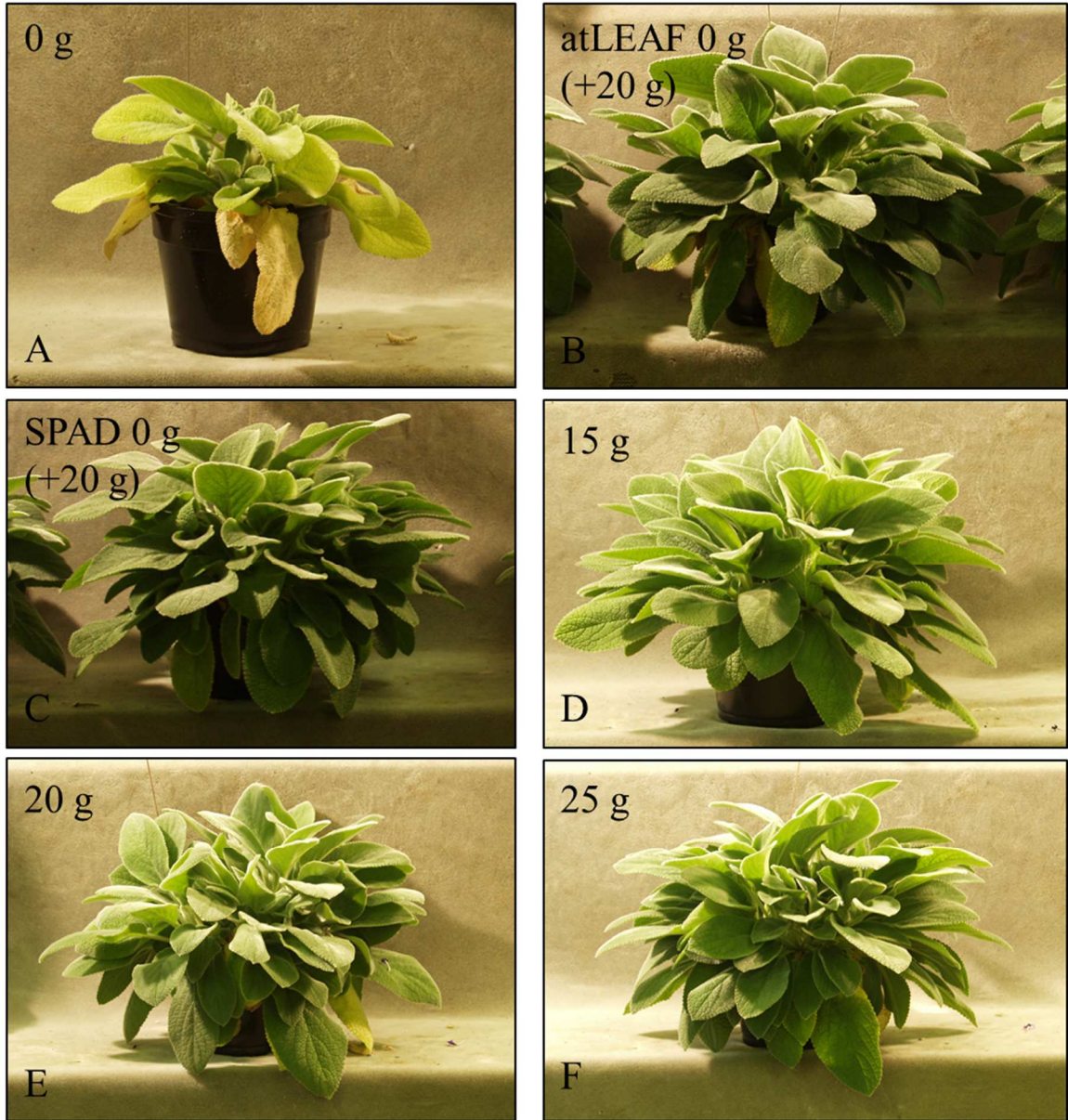


Figure 2.4. *Stachys 'Helene Von Stein'* shown at 42 DAT with the applied treatments of 16N-3.9P-10K controlled release fertilizer. (A) 0 g treatment level. (B) atLEAF 0 (+20) treatment level. (C) SPAD 0 (+20) treatment level. (D) 15 g treatment level. (E) 20 g treatment level. (F) 25 g treatment level.



Figure 2.5. *Verbena* 'Homestead Purple' shown at 42 DAT with the applied treatments of 16N-3.9P-10K controlled release fertilizer. (A) SPAD 0 (+20) treatment level. (B) atLEAF 0 (+20) treatment level. (C) 15 g treatment level. (D) 20 g treatment level.

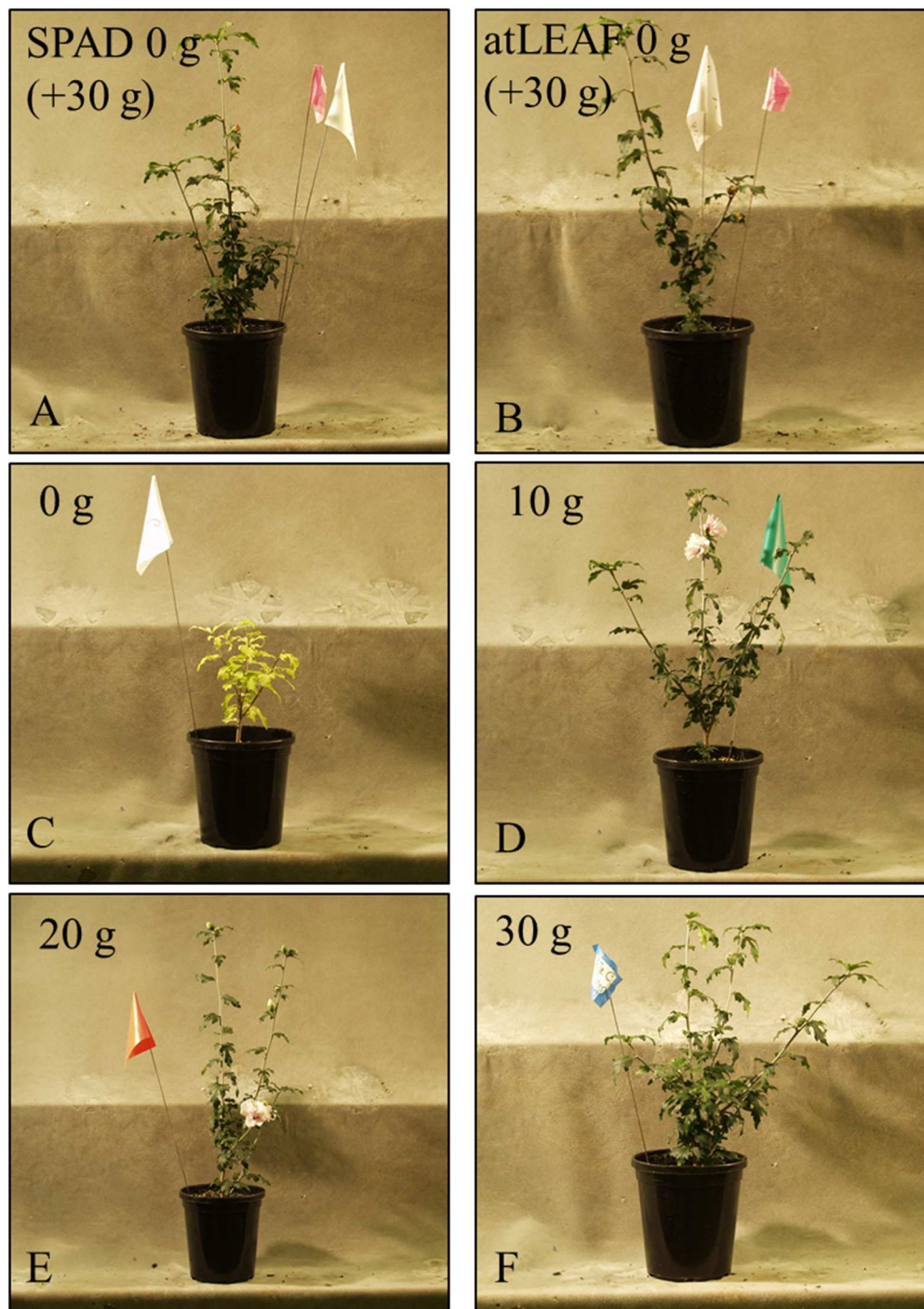


Figure 2.6. *Hibiscus* 'Aphrodite' shown at 42 DAT with the applied treatments of 16N-3.9P-10K controlled release fertilizer. (A) SPAD 0 (+30) treatment level. (B) atLEAF 0 (+30) treatment level. (C) 0 g treatment level. (D) 10 g treatment level. (E) 20 g treatment level. (F) 30 g treatment level.



Figure 2.7. *Hibiscus* 'Aphrodite' at 42 DAT. (A) 40 g treatment level. (B) 50 g treatment level.

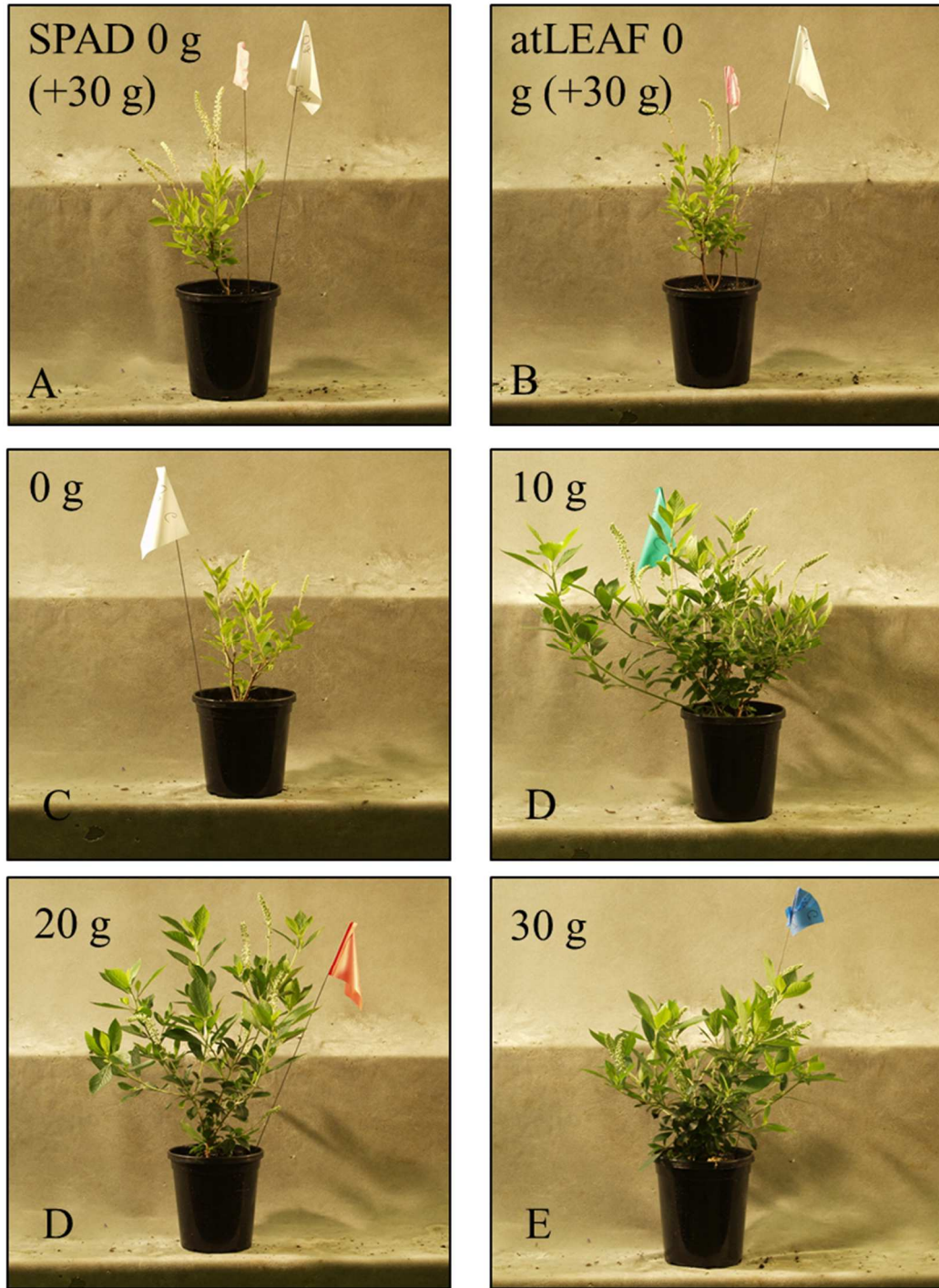


Figure 2.8. *Clethra* 'Hummingbird' shown at 42 DAT with the applied treatments of 16N-3.9P-10K controlled release fertilizer. (A) SPAD 0 (+30) treatment level. (B) atLEAF 0 (+30) treatment level. (C) 0 g treatment level. (D) 20 g treatment level. (E) 30 g treatment level. (F) 40 g treatment level.



Figure 2.9. *Clethra* 'Hummingbird' at 42 DAT. (A) 40 g treatment level. (B) 50 g treatment level.

CHAPTER III

FIELD STUDY

ABSTRACT

Field production methods used to determine crop nutrient status have long relied on costly and time consuming destructive leaf nitrogen laboratory testing. Modern advances in chlorophyll leaf sensor technology using the SPAD and atLEAF chlorophyll leaf sensors allow for a quick and responsive diagnostic method of crop fertility analysis regarding field grown crops. This field study was constructed to examine SPAD and atLEAF chlorophyll leaf sensor values on ornamental landscape plant material grown under field conditions. Plants consisted of two woody perennial plant species *Forsythia* ‘Lynwood Gold’, *Hibiscus* ‘Lavender Chiffon’, and one herbaceous perennial *Salvia* ‘May Night’. One gallon potted plants were planted in the field at the Oklahoma State University Botanical Garden in Stillwater, Oklahoma in a completely randomized design (CRD). SPAD and atLEAF sensor readings were sampled biweekly and plant leaf nitrogen samples were taken monthly. Results show that there was a correlation between the SPAD and atLEAF sensor values, but the values were not positively correlated with leaf nitrogen concentration for all three cultivars tested due to the number of leaf nitrogen samples taken over the course of the study. ‘Lavender Chiffon’ SPAD and atLEAF

values were not different from each other across all testing dates with the exception of the last October testing date, and leaf nitrogen showed a decreasing trend over the course of the season. ‘Lynwood Gold’ SPAD and atLEAF values were observed to be different from each other in both July testing dates at the beginning of the study and at the last October testing date at the end of the study. Leaf nitrogen for ‘Lynwood Gold’ also showed a decreasing trend over the course of the season. ‘May Night’ SPAD and atLEAF values were not different for each testing date with the exception of the last October testing date at the end of the study. Leaf nitrogen for ‘May Night’ showed a stable trend throughout the course of the season.

INTRODUCTION

Crop yield is directly linked to the precise application of fertilizers containing nitrogen which is required in the largest quantity by crops and is the most mobile and dynamic nutrient accentuating plant growth (Teoh et al., 2012). Optimization of nitrogen applications regarding field crop production should be synchronized with crop demand, where precise timing is crucial and can increase crop yield while mitigating losses of nitrogen from cropping systems (Busato et al., 2010). Over application of nitrogen can increase production costs and negatively impact the environment due to nutrient runoff and nitrification of ground and surface waters (Busato et al., 2010). Growers can run into increased costs associated with the fertilization process due to over application of essential nutrients in the field (Wang et al., 2012). Poor crop performance due to poor

fertilization methods has been linked to economic losses and can substantially decrease crop yield, in addition excessive fertilizer applications are oftentimes unnecessary posing a potential risk to human, livestock, and surrounding environmental waters (Wood et al., 1993). The use of chlorophyll optical sensors in field crop production proves to be a useful tool as production facilities can save time and money, while also promoting a clean and pollution free environment (Bullock and Anderson, 1998).

The amount of chlorophyll present in plant leaf tissue provides insight into the amount of photosynthesis that is taking place within the plant, which can correlate with a field crops nutritional status (Gitelson et al., 1999). Diagnosing leaf chlorophyll content gives insight into the nutritional status of a particular crop based on the photosynthetic activity taking place in leaf tissues, due to the fact that there is an exponential relationship between leaf chlorophyll content and the SPAD and atLEAF values provided by the sensor (Patane and Vibhute, 2014; Wood et al., 1993). There is a strong correlation between leaf nitrogen concentrations and the photosynthetic activity taking place in the chloroplasts of plant mesophyll tissues (Zakeri et al., 2014). This is due to the fact that 75% of leaf nitrogen accumulated in the chloroplasts of leaf mesophyll tissues is used in the production of the photosynthetic pigments of chlorophyll (van den Berg and Perkins, 2004). The photosynthetic pigments chlorophyll a and chlorophyll b are instrumental in converting light energy into stored chemical energy that is used in the primary production processes associated with plant growth and development (Steele et al., 2008).

Associations can be observed in the amount of chlorophyll present in leaf tissues and the vitality of crops nutritional status and ultimate yield for the reason that leaf chlorophyll content gives an indication to the rate of photosynthetic activity taking place within the

plant (Muchecheti et al., 2016; Uddling et al., 2007). Therefore, diagnosing leaf chlorophyll content using SPAD and atLEAF chlorophyll sensors indicates the amount of photosynthetic activity taking place within a crop, which strongly correlates with the vitality and nutritional status of a crop overall (Zakeri et al., 2014). When used as a diagnostic tool, chlorophyll leaf sensors can increase crop yield and productivity by assisting production growers in using precise management techniques to estimate the proper amount and timing of nitrogen applications (Bullock and Anderson, 1998; Busato et al., 2010; Chang and Robinson, 2003; Casa et al., 2014).

One significant feature of the relationship between chlorophyll leaf sensor values and leaf chlorophyll content is that the sensor values are species or cultivar specific and are affected by environmental and geographical growth conditions such as available water, drought and the availability of essential nutrients in the soil (Djumaeva et al., 2012; Mizusaki et al., 2013). Therefore, different thresholds of diagnostic use regarding nitrogen applications need to be established that are species dependent regarding field crops (Mizusaki et al., 2013; Ruiz-Espinoza et al., 2010). There is a large magnitude and scope of field crop research using chlorophyll leaf sensors that range from analytical techniques used to determine specific nutrient thresholds that are species and cultivar specific to determining future crop yield by recommending precise nitrogen applications in crop species such as rice (*Oryza sativa* L.), potato (*Solanum tuberosum* L.), corn (*Zea mays* L.), grape (*Vitis vinifera* L.), wheat (*Triticum aestivum* L.), and other beneficial agricultural crops (Bullock and Anderson, 1998; Busato et al., 2010; Casa et al., 2014; Coelho et al., 2012; Hawkins et al., 2007; Huang and Peng, 2004; Jinwen et al., 2011; Monje and Bugbee, 1992; Ruiz-Espinoza et al., 2010; Steele et al., 2008; Waskom et al.,

1996; Wood et al., 1993; Zakeri et al., 2014; Zheng et al., 2015; Zhu et al., 2012). Field studies on rice have been numerous and calculations of sensor values regarding leaf nitrogen have shown a strong correlation between the two (Casa et al., 2014). Rice grain yield was found to be positively correlated with the chlorophyll leaf sensor values and leaf nitrogen content, and environmental conditions such as high temperatures at specific growing stages affected crop yield and the soluble sugar content of rice (Yang et al., 2014; Yang et al., 2016).

Other important horticultural field crop research concentrating on ornamental landscape and native trees species have yielded valuable information regarding the use of chlorophyll leaf sensor and the fertility requirements of tree species such as maples (*Acer saccharum* Marsh.), figs (*Ficus benjamina* L.), cottonwood (*Populus deltoides* W. Bartram), and fruit as well as other hardwood trees (Asano et al., 1986; Bauerle et al., 2003; Djumaeva et al., 2012; LeDuc and Rothstein, 2010; Loh et al., 2002; Mickelbart, 2010; Novichonok et al., 2016; Richardson et al., 2002; van den Berg and Perkins, 2004). The use of chlorophyll leaf sensors in crop production is widely used, and the formulation of precise analytical techniques to determine crop nutrient status have been evaluated to define the best practice in sampling methods for large and diverse crop species (Mickelbart, 2010).

Accurate sampling methods using chlorophyll leaf sensors is crucial to obtain worthy data that can be used to translate and diagnose crop nutrient status and this aspect of research has been widely studied and evaluated in the literature (Dunn and Goad, 2015; Jinwen et al., 2011; Mizusaki et al., 2013; Novichonok et al. 2016; Yonglin Qin et al., 2012). Results of these studies have confirmed that the morphological position of

sampling on the leaf blade and the sampling location on the plant overall have had an influence on the accuracy and precision of chlorophyll leaf sensor readings (Dunn and Goad, 2015; Ling et al., 2011; Mickelbart, 2010). Chlorophyll leaf sensor readings taken in the middle of the leaf blade and avoiding the midrib, petiole, leaf base, and leaf tip provide a more accurate representation of leaf chlorophyll content overall in cabbage (*Brassica oleracea* L.) (Dunn and Goad, 2015). Furthermore, the sampling location in the plant canopy can also lead to mixed results. Canopy samples should be taken from the middle of the overall plant avoiding the upper and lower portions of the canopy as well as leaves that are underdeveloped or in senescence regarding leaf age (Mickelbart, 2010). The number of leaf samples per plant, number of plants sampled per crop, and the geographical area sampled in the field are other considerations that should be taken into account to obtain an accurate sample of a crops nutrient status.

Chlorophyll leaf sensors have been found to be a useful diagnostic tool for determining the nutrient status of agricultural field crops, but the limitation of diagnosing the nutrient status of woody plant species is inherent due to the perennial nature and adaptive tolerance of nutritional variance seen in woody species over agricultural field food crops (Johnson, 1993; Loh, 2002; Sibley et al., 1996). Tree plantation and forest management requires knowledge of the foliar nitrogen content to successfully assess and correct a negative nutritional status and prevent tree pest and plant disease (Djumaeva et al., 2012). Accurate representations of a tree plantation or a field crops nutrient status using laboratory methods can be time consuming and costly which present problems with adequately providing nutrients in a timely manner as field conditions can change rapidly for the time it takes to get results back from the laboratory (Patane and Vibhute, 2014).

Remote sensing techniques and digital imaging of field crops rely primarily on ground based remote sensing, air borne remote sensing, and satellite based remote sensing techniques (Taskos et al., 2015, Tewari et al., 2013).

Assessment of the performance of the SPAD and atLEAF chlorophyll leaf sensor values in the field and tracking how the values changes over the course of time is significant, but as Wood et al. (1996) has shown, determining the fertility of field crops depends largely on the species, cultivar, geographic location, and environmental conditions the crop is grown under. The specific conditions of the growing crop coupled with the specific requirements of the crop species provides different results in the sensor values. Therefore, to determine crop nutritional status using chlorophyll leaf sensors in the field an index of the specific crop species nutritional thresholds must be completed for each crop species (Monje and Bugbee, 1992; Wood et al., 1996; Zhu et al., 2012). These specific nutritional thresholds will help growers determine the nutritional status of a field crop, and this precise information for each crop will help alleviate crop losses and excessive fertilization providing purpose for and increasing the reliability of the sensor values provided by chlorophyll leaf sensor technology. The objective of this experiment was to evaluate how the SPAD and atLEAF sensor readings collected on woody and herbaceous ornamental landscape plant materials change under field grown conditions, over the course of the growing season, with readings being taken during the spring and summer months.

MATERIALS AND METHODS

Plant Material and Experimental Methods in the Field. One gallon potted plants of rose of sharon (*Hibiscus syriacus* L.) ‘Lavender Chiffon’, forsythia (*Forsythia x intermedia* Zabel) ‘Lynwood Gold’, and meadow sage (*Salvia nemorosa* L.) ‘May Night’ were planted in March 2009 at the Oklahoma State University Botanical Garden in Stillwater, Oklahoma in a completely randomized design (CRD). Plants were purchased from Greenleaf Nursery Company and planted in March of 2009. Soil samples of the planting area were analyzed in March of 2009 and showed a pH of (6.7) with an organic matter composition of 3.8 %. The nutrient content of the soil sample shows a deficiency in nitrogen and phosphorus, and all other macronutrients in the sample are within the sufficiency range. Micronutrient concentration in the sample shows that the soil is high in iron (45.8%) and zinc (8.38%) with boron (0.97%) and copper (1.26%) in adequate measures. Plant specimens did not receive supplemental irrigation throughout the duration of the study, and weeds were removed monthly to avoid competition in the testing area.

Experimental Design and Methods for Collecting Data. SPAD and atLEAF chlorophyll leaf sensor samples were taken biweekly starting in July 2013 and ending in October 2013. Leaf samples were collected from each plant by taking random leaf samples from the middle portion of each plant specimen, and 10 plants per cultivar were sampled. Sensor readings were taken from the middle portion of the leaf blade by avoiding the midrib, leaf base, and leaf apex from ten plants per species. Plant leaf nitrogen samples were taken monthly by collecting 10 leaves from one plant per species

to observe the leaf nitrogen value. Sensor readings for each specific plant were averaged providing an overall sensor value for each plant species.

Statistical Analysis. Statistical data was analyzed using SAS 9.4 software. The sensor response variables were analyzed using generalized linear mixed models methods for the repeated measures analysis. Tukey pairwise comparisons of significant effects were performed, and all tests were conducted at the 0.05 level of significance. Correlations were analyzed using the PROC CORR procedure, and PROC GLIMMIX was used to calculate the least square means and compute the trend analysis.

RESULTS AND DISCUSSION

Effects of Cultivars and Leaf Nitrogen on SPAD and atLEAF Sensor Values. The greatest SPAD sensor value for *Hibiscus* ‘Lavender Chiffon’ was observed at the first September testing date with an averaged value of (45.5), which was not different than any other date with the exception of the last October testing date showing a value of (37.7) (Table 3.1). The greatest atLEAF sensor value of (53.4) was observed at the first testing date for August, which was not different from any other date with the exception of the second October testing date showing a value of (48.2) (Table 3.1). The SPAD and atLEAF values for ‘Lavender Chiffon’ were not different from each other at all testing dates through the growing season with the exception of the last testing date showing a stable trend regarding the values (Table 3.1). This coincides with what Bullock and Anderson (1998) found in field grown corn as there was a stable trend in the sensor

values across the growing season. The decrease in sensor values at the last testing date of October corresponded with leaf senescence and the advent of the fall season. The highest leaf nitrogen sample of (2.7) g·kg⁻¹ DM was observed at the beginning of the study on the first July testing date (Table 3.2). After dropping to (1.6) g·kg⁻¹ DM at the first August testing date, the leaf nitrogen increased to (2.2) g·kg⁻¹ DM at the first September testing date, and then remained stable ending at (2.0) g·kg⁻¹ DM at the first testing date in October (Table 3.2). The lowest leaf nitrogen reading of (1.6) g·kg⁻¹ DM corresponds with the least amount of rainfall observed during the 2013 drought and agrees with what Bauerle et al. (2003) found regarding chlorophyll content, as chlorophyll is a sensitive indicator of plant stress which was observed regarding this leaf nitrogen value. The SPAD and atLEAF sensors showed a strong correlation with each other, but there was a weak correlation between the sensors and leaf nitrogen due to the number of leaf nitrogen testing dates (Table 3.1). Hardin et al. (2012) also found a weak correlation between the SPAD sensor and leaf nitrogen values for field grown Pecan (*Carya illinoensis* (Wang) K. Koch).

The greatest SPAD sensor value for *Forsythia* ‘Lynwood Gold’ was observed at the second September testing date with an averaged value of (52.5), which was different from all other values across testing dates (Table 3.3). The greatest atLEAF average sensor value of (62.9) was also observed in the second testing date for September and was also different from all other values across all testing dates (Table 3.3). The increase observed in the sensor values from the second August testing date to the greatest values seen in the second September testing date coincides with increased leaf production after flowering which was also seen in other field species such as the various maple (*Acer*

rubrum L.) cultivars studied by Sibley et al. (1996). The greatest leaf nitrogen value of (2.1) g·kg⁻¹ DM was observed at the first July 2013 testing date and continued to decline over the rest of the testing dates ending at (1.5) g·kg⁻¹ DM at the last testing date in October 2013 (Table 3.4). The SPAD and atLEAF sensors showed a correlation with each other, but there was a weak correlation between the sensors and leaf nitrogen due to the number of leaf nitrogen testing dates (Table 3.1). Numerous studies have shown that leaf nitrogen and sensor value correlation may change from season to season in field grown crops (Chang and Robinson, 2003; Eguchi et al., 2006; Mizusaki et al., 2013; Nielsen et al., 1995). Environmental variables such as the water status of the crop and plant growth stage have also shown to skew the accuracy of sensor readings (Bauerle et al., 2003). Sensor readings are often correlated to leaf nitrogen, but a standard estimate of number of leaves sampled, number of plants used to represent a crop, and leaf sampling location on the leaf and plant overall require a standard procedure to account for the variability between the results of scientific studies (Bonneville and Fyles, 2006; Bullock and Anderson, 1998; Dunn and Goad, 2015; Mickelbart, 2010).

The greatest SPAD sensor value for *Salvia* ‘May Night’ was observed in the first September testing date with a value of (39.9), which was significantly different from all other values across all testing dates (Table 3.5). The greatest atLEAF sensor value was also observed in the first September testing date with a value of (43.9), which was also significantly different from all other values across all testing dates (Table 3.5). The greatest leaf nitrogen value of (3.1) g·kg⁻¹ DM was also observed at the first July testing date and continued to remain stable through all testing dates ending with a value of (3.0) g·kg⁻¹ DM at the last October testing date (Table 3.6). The SPAD and atLEAF sensors

have correlated with each other, but there was a weak correlation between the sensors and leaf nitrogen (Table 3.1). This could be due to the number of leaf nitrogen samples taken over the course of the study (Table 3.1). Waskom et al. (1996) found that the biological differences in corn hybrids showed a significant inconsistency in the relationship between the sensor values and the total extractable leaf nitrogen content of leaves at different stages in the crop production cycle. Waskom et al. (1996) also showed that crop yield predictions were largely dependent on the time of sampling within the crop production cycle illustrating that field crops are significantly more challenging to evaluate using chlorophyll leaf sensors. These inconsistencies among studies suggest that field variables such as environmental conditions and cultural practices play heavily on chlorophyll leaf sensor evaluation regarding field crops (Johnson, 1993; Monje and Bugbee, 1992; Sibley et al., 1996; Wang et al., 2004). Carter and Knapp, (2001) found that plant stress in response to environmental factors such as drought, competition, dehydration, and the variability of leaf chlorophyll composition in the leaves can play a significant role in the response of the sensor values and cause reduces and variable leaf nitrogen values. The variability observed in the sensor responses and leaf nitrogen values can be directly related to the environmental stresses caused by substantial dehydration, drought stress, and competition (Carter and Knapp, 2001). Significant environmental conditions played a substantial role in the field study results as record drought conditions plagued the region and supplemental irrigation was not used in the study (Carter and Knapp, 2001). Future research should identify the nutrient sufficiency ranges for field crops used in conjunction with sensor values so that mobile reference applications may collaborate effectively.

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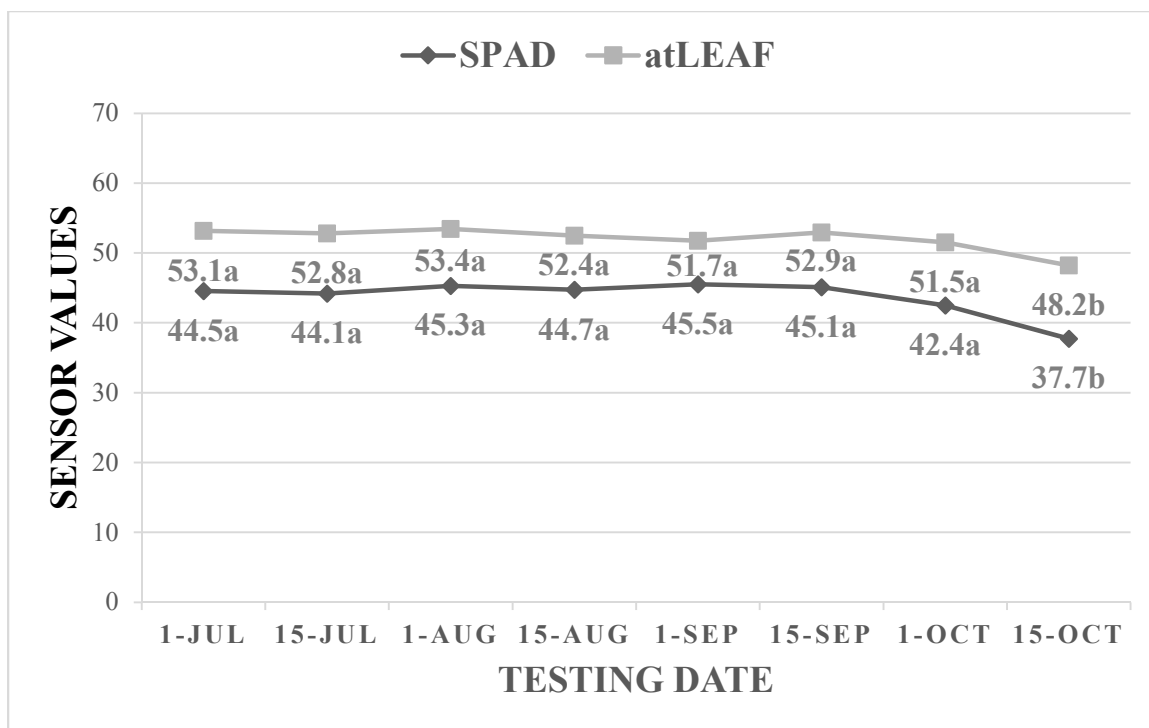


Figure 3.1. SPAD and atLEAF sensor values for *Hibiscus* ‘Lavender Chiffon’ taken from July 2013 to October 2013. The average means for each testing date are represented (n=10), and means for each sensor with the same letter are not significantly different at $P < 0.05$.

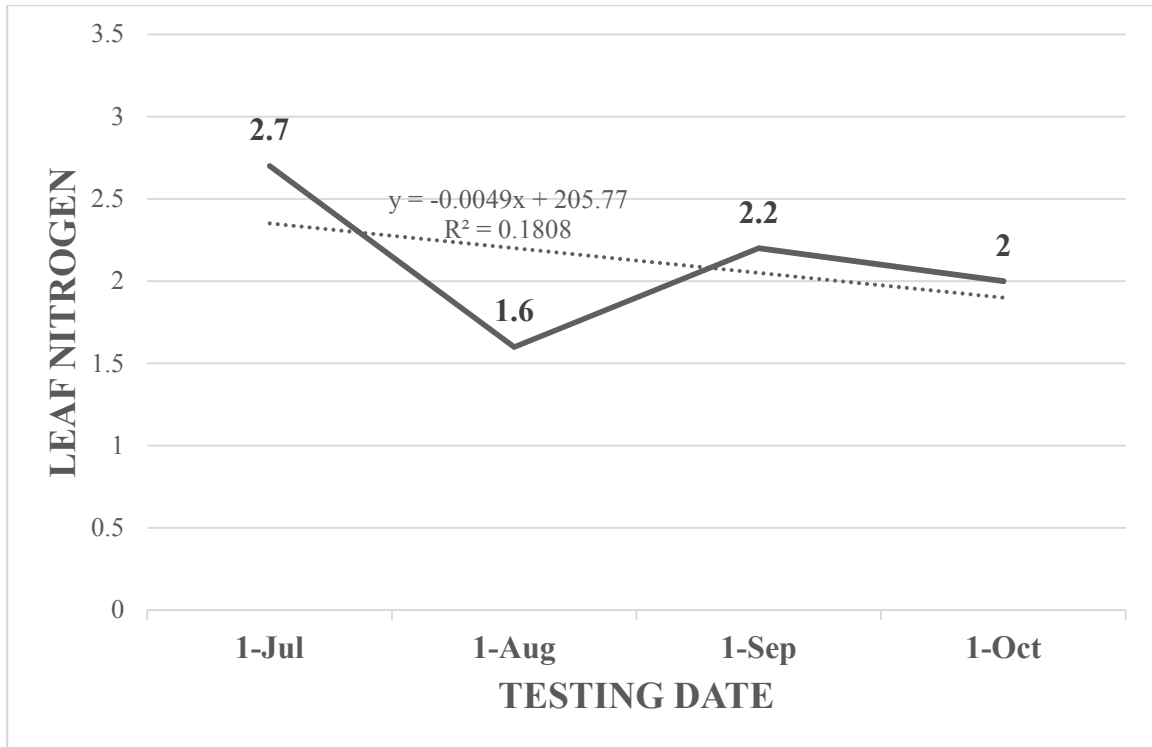


Figure 3.2. Leaf nitrogen values n=(4) for *Hibiscus* 'Lavender Chiffon' taken from July 2013 to October 2013.

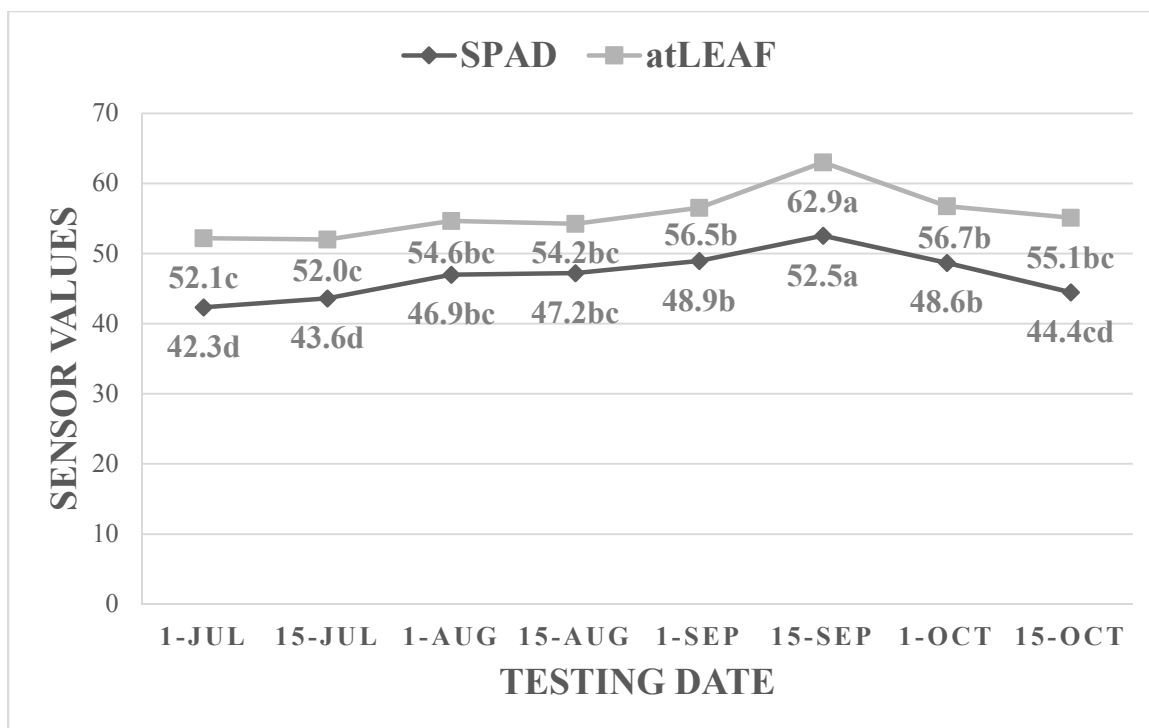


Figure 3.3. SPAD and atLEAF sensor values for *Forsythia* ‘Lynwood Gold’ taken from July 2013 to October 2013. The average means for each testing date are represented (n=10), and means for each sensor with the same letter are not significantly different at $P < 0.05$.

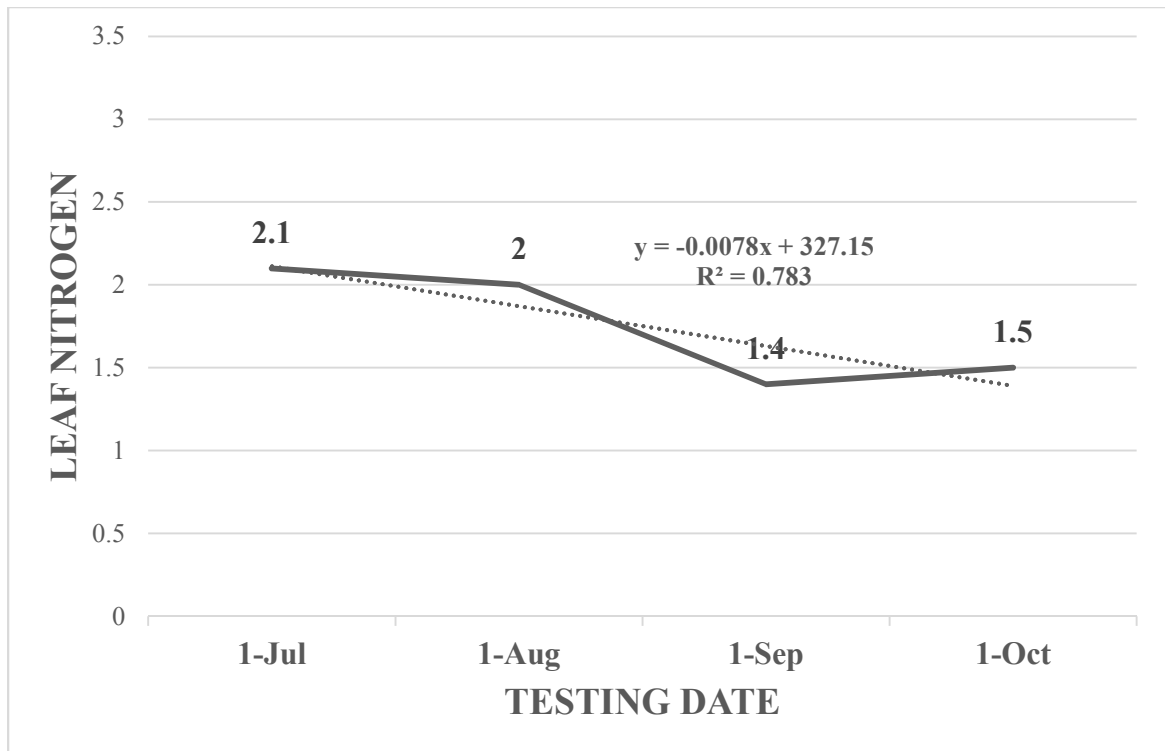


Figure 3.4. Leaf nitrogen values n=(4) for *Forsythia* 'Lynwood Gold' taken from July 2013 to October 2013.

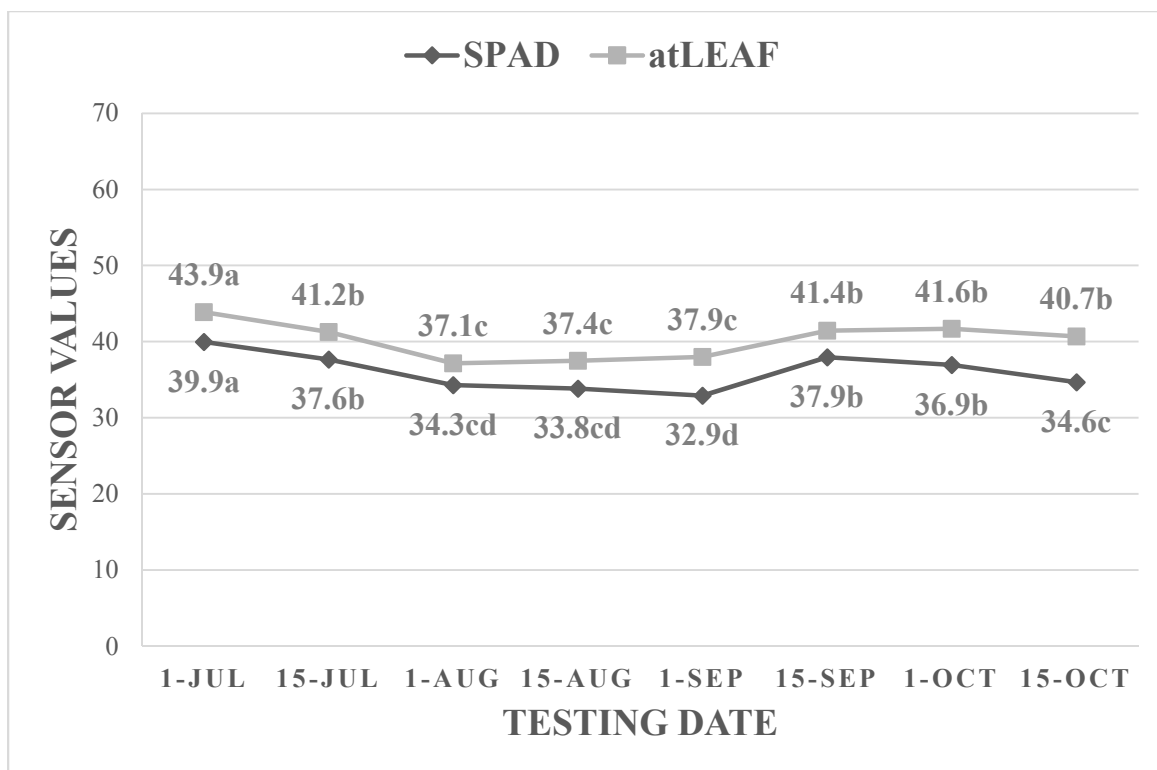


Figure 3.5. SPAD and atLEAF sensor values for *Salvia* ‘May Night’ taken from July 2013 to October 2013. The average means for each testing date are represented (n=10), and means for each sensor with the same letter are not significantly different at $P < 0.05$.

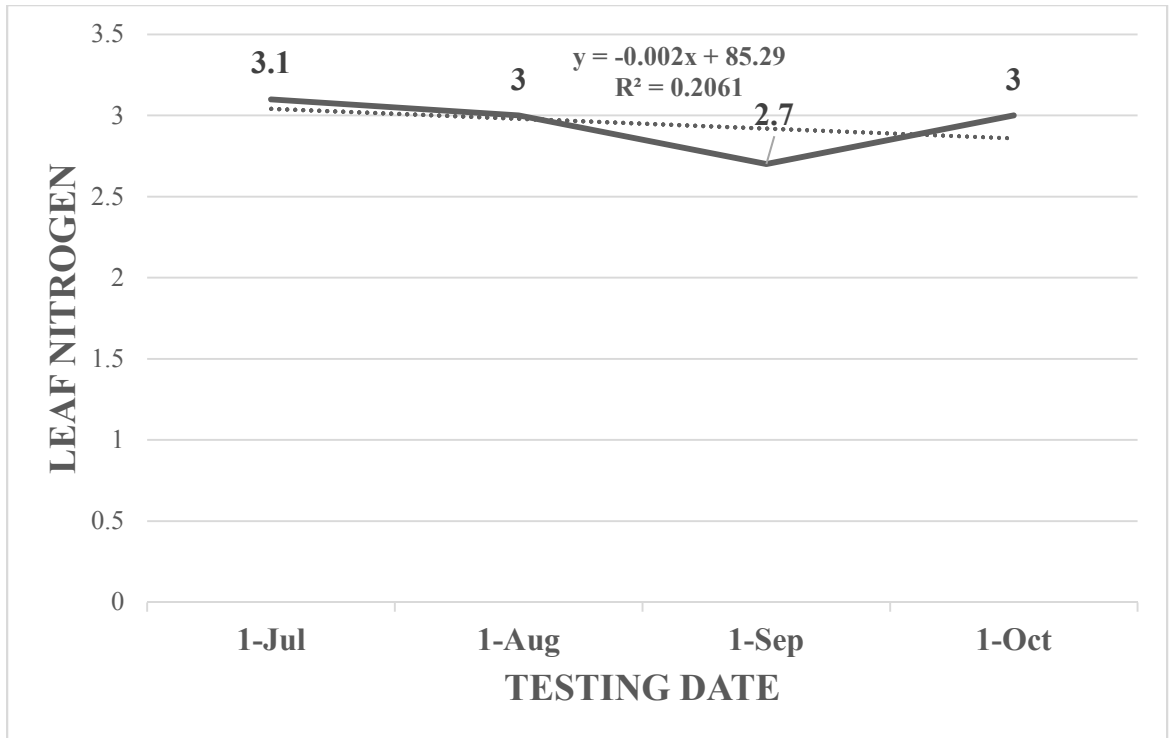


Figure 3.6. Leaf nitrogen values n=(4) for *Salvia* 'May Night' taken from July 2013 to October 2013.

Table 3.1. Pearson correlation (r) matrix and significance levels (p) for SPAD and atLEAF sensor values in the field with leaf nitrogen.

<i>Hibiscus</i>		
	atLEAF	Leaf N
SPAD	0.925 ^{***z}	-0.026
atLEAF		-0.034
<i>Forsythia</i>		
	atLEAF	Leaf N
SPAD	0.750 ^{***}	0.216
atLEAF		0.263
<i>Salvia</i>		
	atLEAF	Leaf N
SPAD	0.910 ^{***}	0.369
atLEAF		0.636

^z*, **, ***, Representing correlation coefficient (r) significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. (n=110).

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VITA

Lance Swearingin

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF A PLANT NITROGEN RECOMMENDATION MOBILE PHONE APPLICATION IN THE GREENHOUSE AND USE OF OPTICAL SENSORS IN THE LANDSCAPE

Major Field: Horticulture

Biographical:

Education:

Completed the requirements for the Master of Science in Horticulture at Oklahoma State University, Stillwater, Oklahoma in December, 2016.

Completed the requirements for the Bachelor of Science in Horticulture at Oklahoma State University, Stillwater, Oklahoma in May, 2013.

Experience:

Department of Horticulture and Landscape Architecture Teaching Assistant, August 2013 to August 2015. Oklahoma State University Herbarium Undergraduate Fellowship in Botany, January 2012 to May 2012. Student Curator of the Bellamy Parks Jansen Botanical Art Collection at the Oklahoma State University, Edmon Low Library, December 2012 to May 2013. Horticultural Curator of the botanical collection at the Oklahoma City Zoological Park and Botanical Garden, Oklahoma City, Oklahoma, December 2014 to present.

Professional Memberships: Association of Zoological Horticulture board member and education committee chair. Professional member of the American Public Garden Association. Professional member of Botanical Garden Conservation International. Professional member of the Oklahoma City Garden Council.