Effectiveness of Current Boron Application Recommendations and Practices on Peanut (*Arachishypogaea*L.) in the Virginia - Carolina Region

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ABSTRACT

Including peanut (*Arachishypogaea* L.) in crop rotations is common for eastern Virginia and the Carolinas, as it thrives in the long growing season and sandy soils. Boron (B) is widely deficient, and is more prone to leeching in sandy soils. Applied B has difficulty reaching growing points as B has reduced phloem mobility in peanuts. Current B fertilization recommendations are based on only three studies from the early ‘70s. Many changes have been made in cultivar breeding since then. This research examines if recommended B application rates and times are still necessary for optimal yield, plant health and seed quality for current cultivars. Two experiments in seven fields compared four total amounts of B applied (0, 0.3, 0.6, and 1.1 kg ha^{-1}), and application time (planting; beginning peg, R2; full seed, R6; planting and R2; planting and R6), and runner and Virginia market types, newer and obsolete cultivars, with or without B fertilization. Leaf B was elevated only directly after fertilization ($p=0.004$, $p<0.001$), and in relation to total B applied ($p<0.001$), but seed B content was unaffected. Yield was not impacted by B rate or application time. Yield was higher ($p=0.012$) for newer cultivars when B fertilized, but no different than obsolete cultivars with B. Seed from obsolete cultivars had higher ($p=0.010$) B, no difference between market types or B fertilization. Germination of all seed was ≥97%. Based on this research, it is not necessary to apply B for optimal yield, plant health and seed quality for current cultivars.

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GENERAL AUDIANCE ABSTRACT

Peanut (*Arachishypogaea* L.) is commonly part of crop rotations in eastern Virginia and the Carolinas (V-C), favoring the sandy soils and long growing season in this region. Sandy soils preferred for peanut production exacerbate an already widespread deficiency of the soil mobile micronutrient, boron (B). Most research exploring the relationship of B with peanut production was done in the 70's. Current production recommendations for peanut B fertilization are based on just three studies from the early 70's, and are being practiced, unchanged, by essentially all producers in the V-C region today. Two experiments were conducted to examine the efficacy of these recommendations. The first compared four total B rates (0, 0.3, 0.6, and 1.1 kg B ha-1) applied at five growth stage combinations (planting; beginning peg, R2; full seed, R6; planting and R2; planting and R6). The second experiment compared the response of 12 commercial cultivars that were a combination of runner and Virginia market types, obsolete and newer cultivars, when B fertilized and not. Experiments were carried out at seven fields in Virginia and North Carolina in 2015. No significant differences in yield were observed in response to rate of B fertilization, or between application times. There was no difference in seed B, except by age. Newer cultivars yielded significantly ($p=0.012$) higher than obsolete ones, but seed B content was less ($p=0.010$). For both experiments, seed germination was ≥97%. Based on this research, optimal yield and seed quality of current cultivars can be achieved without B fertilization.

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CHAPTER I: Introduction and Literature Review

Boron in Peanut

A crop produced worldwide, peanut (*Arachis hypogaea* L.) is of economic importance in many countries. The United States was the fourth largest world producer of peanuts in the 2014-2015 season with 2.35 million metric tons (USDA Foreign Agriculture Service). The major peanut producing region in the US is along the southeastern part of the country, with significant production in Texas and Oklahoma as well. Peanut has some more unique production requirements as compared to other major commodity crops grown in the US. There are micronutrients, like boron, that are of more concern in peanut production than in crops like corn.

Boron is an important micronutrient for most crops and its deficiency is more widespread across crops and acreage than for any other micronutrient (Gupta, 1993). In spite of its importance, a majority of the research done on the role of B in peanut production is from the 1960s through the 1980s. Soil characteristics, pH in particular (Gascho and Davis, 1995), and cultivar (Harris and Gilman, 1957) determine the availability of boron to be taken up and used by plants, including peanut. Boron is involved in the signaling mechanisms for rhizobia in the nitrogen fixation of legumes (Bolanos, 2004), improving overall plant growth and health. In peanut, boron deficiency causes internal kernel damage called “hollow heart” due to deforming and discoloration of the cotyledons (Harris and Gilman, 1957). It was found that peanut seed having hollow heart contained <13 mg kg^{-1} of B (Netsangtip et al., 1987). Rerkasem et al., (1987a) showed lack of boron to cause 10% increase in hollow heart of peanut kernels, but no overall decrease in yield. In an additional study, Rerkasem et al., (1987b) showed that application of 40 kg B ha^{-1} decreased the instance of hollow heart in peanut seeds in experimental fields from 34% down to no hollow heart. Damaged kernels dramatically reduce the crop quality and value (Gascho and Davis, 1995). Quality is affected by increased damaged kernel (DK) content and a potential 65% value reduction if the DK content exceeds 2.5%. In 1966, near elimination of hollow heart in Virginia had been attributed to B application (Hallock, 1966). Without sufficient B, peanut plant internodes are shortened, branches crack, and flowering time increases. More recent studies show that B fertilization increased peanut yield (Nasef et al., 2006).

Historically, the increase in overall peanut yield with B fertilization is brought into question. A 1968 study by Hartzog and Adams in Alabama showed no yield increase and almost no improvement in grade when 1.1 kg B ha^{-1} was top dressed. Again in 1973, Hartzog and Adams determined there was no yield or grade improvement when B was applied to peanuts. Another report out of Alabama (Cope et al., 1984) and also in India (Swamy and Reddy, 1983)found no increase in peanuts yields due to B application.

Boron is very mobile when available in the soil and has restricted mobility in the plant; this may significantly affect B management in peanut. For example, boron is less available on sandy soils with reduced pH and organic matter; it is water soluble and easily leached by heavy rains (Goldberg, 1997). Therefore, coarse-textured soils in humid areas are particularly prone to boron deficiency. Liming, heavy N-P-K fertilization, and dry weather also contribute to reduced B availability to the plants (Moraghan and Mascagni, 1991; Bellaloui, 2011). In the plant, B is unique among other micronutrients because it has reduced mobility in many plant species but is freely mobile in others. In peanut, some authors showed that boron has reduced phloem mobility (Raven, 1980), meaning it can be readily taken up by the roots and transported to the leaves. However, its remobilization within the plant, and redistribution from the older leaves to the growing points, may be inefficient. This is of concern if B is being foliarly applied, or if it must be taken up by the roots, and moved back down from the plant to the growing tips where the peanuts are forming. Some others demonstrated that boron can re-translocate to the pods (Campbell et al., 1975; Konsaeng et al., 2010), though possibly in a limited amount.

Boron deficiency is more common in sandy soils, and because this is where peanuts are grown, its application represents a common production practice in all peanut-growing states in the USA (Gascho and Davis, 1995). In the early 70's, Perry (1971) recommended application of 0.6 $kgha^{-1}$ B when soil boron was less than 0.18 mg kg^{-1} for the southeastern peanut growing region. Though this is what current application recommendations are based on, other studies at the same time and later reported somewhat different results. Hartzog and Adams (1971) found that soils with less than 0.07 mg B kg^{-1} did not develop hollow heart in the seeds, or reduce grade or yield.

Later in India, Jadhoa et al., (1989)found peanut yields benefitted from B fertilization when soil B was lower than 0.16 mg kg^{-1}. Soil pH is an important factor in soil available B for peanuts. For acidic soils, 0.05 mg B kg^{-1} is considered sufficient (Cox and Reid, 1964), and 0.2 mg B kg^{-1} is sufficient in alkaline soils (Hill and Morrill, 1974). These thresholds were all established in studies that used the hot water extractable B method, but the current method used by the Virginia Tech (VT) soil analysis lab is the Mehlich 1 method (Mehlich, 1953). This is one of the main labs used for soil analysis reports by peanut growers in the V-C region. No literature was found correlating results from these two methods of determining soil B concentrations. This is potentially important as producers may inadvertently now have higher or lower soil B levels in reference to the historic thresholds, simply due to a different extraction method being used to determine soil B concentrations.

In 1973, while they did not find an increase in grade or yield, Hartzog and Adams determined that boron can be applied either pre-plant in an herbicide tank mixture or sprayed on with the first or second fungicide application without damaging plant tissue. This, and Perry's (1971) B amount recommendation of 0.6 kg Bha^{-1} on sandy soils, constitute the sole research base for peanut B application in the current production guides of the V-C region. Since then, B amounts and application procedures have been practiced unchanged by peanut farmers in this region even though cultivars have dramatically changed.

Differences in B requirements between runner type cultivars varies according to an older study (Harris and Gilman, 1957). The genetic diversity among runner type peanut cultivars has dramatically increased in recent decades (Milla-Lewis et al., 2010). Another study found that runner type peanuts developed B deficiency symptoms earlier than Virginia type, and recovered more slowly (Harris, 1965). A more recent study has confirmed increases in diversity of Virginia type peanut cultivars, leading to changes in response to applied nutrients between older Gregory, and newer NC-V 11 cultivars (Jordan et al., 2010).

Perry (1971) also warned against over application due to potential B toxicity. Research in Australia showed that B over application reduced peanut yields (Blamey et al., 1981). Recently, production recommendations in South Carolina specify that B rate can be from 0.3 to 0.6 $kgha^{-1}$ when soil shows reduced B levels, though no lower threshold is given in V-C production guidelines. Visual diagnosis of B nutritional problems in peanut is difficult because symptoms usually show only at severe levels of either deficiency or toxicity. Severe B deficiency may result in deep green and mottled leaves, compacted branch terminals, cracked pods, and longer flowering period or delayed maturity (Harris and Gilman, 1957; Harris and Gilman, 1966). Boron toxicity can result in chlorosis of leaf tips and marginal necrosis (Harigopal and Rao, 1964). Peanut tissue sufficiency levels as recommended by Virginia Tech (VT) range from 25 to 60 mg B kg^{-1}, but levels can become critical if exceeding 58 mg B kg^{-1} in young leaves (Blamey et al., 1981), or 85 mg B kg-1 in youngest fully mature leaves (Gopal, 1968).

The current production guideline for boron applications on peanut across the V-C region are based solely on research in the early 70's. Across the V-C region application amounts are as suggested by Perry (1971), even though new cultivars are being grown which have higher vine production, yield and bigger kernel size than older cultivars. Crops and cropping systems that were not available in the early 70's are now being used and may have contributed to changes in soil properties with direct effect on boron soil availability. Application times are the same as those proposed by Hartzog and Adams (1973) with no regard to the different mobility of B in soil and peanut. A survey of county peanut producing champions in North Carolina showed all participants applying B (Jordan et al., 2011). A 2013 study showed that 97% of producers in VA and NC applied B annually (Morgan et al., 2014). Very seldom has B related peanut research been reported in past decades, which indicates a pressing need for new studies to evaluate B application amounts and timing in relation to peanut quality and yield in the V-C region.

NDVI Use in Peanut

Spectral imaging such as Normalized Difference Vegetative Index (NDVI) was originally developed to analyze groundcover from space (Haas et al., 1975). While it is still being used on a large scale to analyze ground covers (Frost et al., 2014, Raynolds et al., 2013), the technology is being used for other precision agricultural applications like mapping of fields for nitrogen application (Lebourgeois et al., 2012, Amaral et al., 2015, Quebrajo et al., 2015). Research has also been conducted using NDVI as an indicator of peanut maturity (Carley et al., 2008), to give an estimation of drought damage (Muthumanickam et al., 2011), peanut ground cover (Rajan et al., 2014), and other things such as crop phenotyping (Zaman-Allah et al., 2015).

All NDVI applications in agriculture rely on change in crop reflectance properties due to a presence or absence of foliage and change in the color of that foliage (Haas et al., 1975). The main factor is leaf color change due to chlorophyll and water content. The principle of NDVI is that different wavelengths of light are reflected, transmitted and absorbed in varying proportions based on the nature of the solutions in pigmented cells. For example, a change in chlorophyll content will change the way light is reflected. This can be used to quantitatively measure the change in the leaf color due to chlorophyll amount. Similarly, change in leaf water content has been strongly correlated with peanut leaf NDVI (Penuelos and Inoue, 1999). Since boron deficiency can cause a darkening of leaf color (Harris et al., 1957, Rerkasem et al., 1993), and toxicity can cause interveinal chlorosis (Gopal 1975, Harigopal et al., 1964), this gives a leaf color spectrum that may correlate to boron levels in the peanut plant. Near infrared spectroscopy has been used on other legumes to predict levels of nutrients including boron (Cozzolino and Moron, 2003). The possibility of using NDVI to predict peanut harvest date has also been investigated (Carley, et al, 2008).

Hyperspectral imaging takes measurements at many different wavelengths. In a study by Thenkabial et al.,(2000), it was shown that certain wavelengths are more useful than others in determining agricultural crop bio-information. NDVI is calculated using a visible red (R) and near infrared (NIR) wavelength in the formula *(NIR-R)/(NIR+R)*. Thenkabial et al., (2000) found the most useful bands to be in the longer wavelength region of R (650nm to 700nm), and in the NIR range of 900nm to 940nm, and additionally at the NIR wavelength of 982nm for moisture sensitivity. They recommended using twelve narrow bands for optimum sensing of crop characteristics. The Trimble® GreenSeeker Handheld (Sunnyvale, CA) measures the R wavelength of 660nm, and the NIR wavelength of 780nm for calculating NDVI (HCS-100

Technical Specifications). There is a difference of more than 100nm between the NIR wavelength read by the GreenSeeker, and the optimal NIR wavelength proposed by Thenabial et al., (2000). This could pose a difficulty in getting readings sensitive to crop characteristics like micronutrient deficiency. It is well established that nitrogen levels and drought stress can be accurately measured by certain remote sensing indices for some crops (Crain et al., 2012, Sharma et al., 2015). The major challenge is to distinguish between stress factors in a given crop. For example, in a 2007 study by El-Shikha et al., they measured several crop health indices using five wave lengths of reflectance on broccoli (*Brassica oleracea* L.). Although NDVI detected changes in plant health due to drought and low nitrogen, it was not able to distinguish between the two effects. This illustrates the problem a lot of researchers are currently working on; can remote sensing indices be developed that accurately distinguish crop stress from a single factor like micronutrient deficiency? As of yet, there has not been a set of reflectance wavelengths used to create an index for accurately detecting boron levels in peanuts.

Producers currently apply B regardless of peanut crop requirements (Morgan et al., 2014), leading to possible over application. If producers were to apply B only if the crop required it, then an accurate, quantitative, simple tool would be necessary. Taking tissue samples for nutrient analysis is far too slow, and time and labor intensive for producers to use efficiently. Since there is a leaf color change between extreme B deficiency and B toxicity, then there is the potential for small changes in leaf B level to be detected using remote sensing techniques like NDVI. The first step is establishing potential for developing a useful early detection tool of B toxicity or deficiency for peanut producers.

Germination of Peanut Seed As Affected by B Content

It is well known that plants need sufficient levels of nitrogen (N), phosphorous (P), and potassium (K) in order to grow, mature, and produce seed properly. But the role of micronutrients in this process has been much less studied (Sperotto et al., 2014). Particularly the role micronutrients play in seed development, germination, and seedling vigor (Duet al., 2009). The necessity of micronutrients to seedling vigor is shown by the increasing use and study of micronutrient seed treatments (Alhendawi et al., 2008, Almeida et al., 2015, Rehman et al., 2012). More importantly, these micronutrients should be found in adequate quantities within the seed before planting. A 2014 study by Aytac, *et al.*, showed that increased zinc (Zn) application on the crop could increase the yield and micronutrient quantities in the safflower (*Carthamus tinctorius* L.) seeds produced. Du et al. (2009), suggested that molybdenum and B should be further studied because of their critical role in increased seed production in alfalfa (*Medicago sativa* L.), a leguminous forage.

Though all growth stages are important, germination only refers to root radical emergence from a seed, this is followed by seedling growth which includes soil emergence, and seedling vigor. A study done on white clover (*Trifolium repens* L.), a legume, showed no yield differences or germination increase with higher B application, and recommended that further study was needed to determine the role B played in seed health and germination (Stoltz and Wallenhammar, 2014). Dordas et al., (2006) showed an increased yield, germination, and seedling vigor in alfalfa treated with higher B levels. Additionally, sugar beets (*Beta vulgaris* L.) treated with foliar B showed yield increases, the number of abnormal seedlings decreased, but seedling vigor remained unchanged (Dordas et al., 2007). The sugar beet study suggested that B may play a more important role in seedling vigor and development than in initial germination. Illustrating this concept; a study on rice (*Oryza sativa* L.) only showed differences in the seedling growth at five weeks old between seeds with adequate B and those without (Fugiwara and Uraguchi, 2011). Though sugar beets and rice are very different plant species than peanut, these studies bring up some potentially important areas of inquiry in the role of B in peanut seed composition, germination, and seedling development.

Boron concentration could also have an effect on peanut seed germination. A lack of boron in the peanut seed causes "hollow heart", a rotting of the cotyledons in the seed embryo as described by Harris and Gilman (1957). Peanut seed with less than 13mg B kg^{-1} can develop hollow heart (Netsangtip et al., 1987). In a study that took place in Virginia, the researcher compared peanut seed nutrient content and germination based on application of many different macro and micronutrients (Hallock, 1975). Though B was one of the micronutrient treatments, the study concluded that calcium (Ca) and potassium (K) concentrations in the peanut seeds were the determining nutrients for best germination, not B. However, a 1969 study was conducted by Gopal and Rao looking at the effects of toxic levels of B in the peanut seed. They found that peanut seed containing excessively high levels of B germinated more rapidly, and seedlings grew more quickly for up to twelve days after germination, but were chlorotic and developmentally behind the untreated seeds at the end of the twenty one day trial period. In a later study by Rerkasem et al., (1997), it was found that soybean (*Glycine max* L.) seeds, also a legume, when germinated below the deficiency threshold of B, failed to germinate or produced week plants. Seeds just above the B deficiency threshold where hollow heart occurs, produced weak and stunted plants but were highly influenced by added soil B during growth. In another study with soybean, it was found that seed with elevated B content had differing amounts of protein, oleic, and linoleic acids (Bellaloui et al., 2013), thus suggesting that B levels could influence the seed physiology of the related legume, peanut. Investigation of whether these thresholds of toxic and deficient B levels in peanut seed can occur often enough under standard production practices to damage the next generation of peanut crops through compromised seed germination is a goal of this study.

Summary

Most research investigating the impact of B levels on peanut health, yield, and seed quality has not been updated for decades. The recommendations to peanut producers in the V-C region for B application are based solely on three studies from the 1970's (Hartzog and Adams, 1973, Perry, 1971). This is in spite of conflicting research at the time over the true efficacy of B application in general peanut production, and the amounts necessary for correcting deficiencies. Soil testing procedures for determining need for B application have changed over the years, as well as peanut cultivars and cropping practices. Yet V-C production guides advise to apply B as "maintenance" in the amounts and at the times recommended by Perry (1971), Hartzog and Adams (1973). This has led to 97% of all peanut producers in the region applying B to their crops regardless of soil tests or plant needs (Morgan et al., 2014). This research aims to assess if time of B application and amounts applied as currently recommended are necessary for maintaining maximum yield and plant health. Additionally, this research is to determine if changes in cultivars used over time have made significant impact on the amount of B peanut plants need for optimal yield and developing viable seed.

If producers were to apply B only when peanuts needed, then they need a simple, quick, accurate and sensitive tool for determining if peanuts are under stress from too much or too little B. Radiometry has been successfully used to remotely sense other crop stresses like drought and N. By comparing NDVI readings of peanuts treated with different amounts of B, then it can be established if NDVI is a useful tool for peanut growers. Even if NDVI is not the best index for distinguishing B imbalance, this research can provide a basis for either pursuing other reflectance indices, or further work to determine that NDVI is not a useful tool.

Seed physiology, and seedling development are complex, but important aspects of a productive cropping system. The role some micronutrients play in this process, particularly the germination, has not been well described for peanuts. It has been established that seed with a B concentration of <13 mg kg^{-1} will develop hollow heart. This renders the seed useless, and an unsaleable contaminant of a peanut harvest. It is not known exactly how much B must be applied in the field to keep hollow heart from developing in kernels. It is also not well understood how B content that is just above the hollow heart threshold of 13 mg kg^{-1} might affect germination rates. This research will establish if under current normal field B conditions, peanut seed will develop that is at risk for hollow heart, or reduced germination.

RESEARCH OBJECTIVES

The objectives of this research were to assess current B application recommendations for optimal efficacy in peanut crop production in the V-C region by 1) comparing the tissue B content and yield when recommended, excess, and no B rates were applied, 2) determining if newer, or different market type cultivars respond differently to B fertilization, 3) if currently available NDVI sensors have potential as an early B deficiency assessment tool, and 4) if B fertilization is necessary for producing peanut seed with optimal germinability.

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CHAPTER II: Rate and Time of Boron Application

ABSTRACT

Since the 1970's, peanut producers in the Virginia-Carolina (V-C) region have been applying boron (B) by the same recommendations based on three studies. The sandy soils favored for peanut production are widely deficient in B, an essential micronutrient for proper seed development. Boron is highly mobile in soil, being easily leached, but not mobile in peanut phloem, making it difficult to get B to developing seed. To determine the need for B fertilization as currently recommended, four B rates (1.1, 0.6, 0.3, and 0 kg ha^{-1}), and five application times (planting; beginning peg, R2; full seed, R6; planting and R2; planting and R6) were compared in four fields in Virginia and North Carolina. Leaf B content was higher when recently receiving B, or the highest rate (1.1 kg ha^{-1}), but no difference is seed B content from B fertilization. All seed was above B deficiency threshold and had ≥97% germination. A Normalized Vegetative Index (NDVI) instrument was used to explore as a possible early detection tool for B fertilization of peanuts. There was no correlation between NDVI and leaf B content, or for yield due to B stress. This was difficult to confirm as there were no yield differences due to B fertilization. Based on this research, B fertilization is not necessary to achieve optimal yield, seed quality, or plant health. Additionally, NDVI is not an informative reflectance index for determining B stress in peanut crops.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) grows best in sandy soils in areas with long growing seasons. As such, it is a standard in crop rotations of eastern Virginia and the Carolinas. Like all crops, peanut requires adequate nitrogen, phosphorus, and potassium, but it also has some unique requirements in micronutrients. A critical micronutrient for peanuts, boron (B), is widely deficient in soils of the USA (Gupta, 1993). The majority of research investigating the role of B in peanut production was conducted in the 1960's through the 1980's. Several factors greatly determine the ability of peanut plants to take up and use B, mainly soil pH (Gascho and Davis, 1995), and cultivar (Harris and Gilman, 1957). Boron is water soluble, and is less available in sandy soils with low pH and organic matter (Goldberg,1997). Once B is taken up by the peanut plant, its phloem mobility is restricted, thus making redistribution from older leaves to growing points inefficient (Raven, 1980). If an insufficient amount of B is present in the peanut seed, the cotyledons of developing seed will rot, causing a condition called “hollow heart” (Harris and Gilman, 1957). Peanut seed with hollow heart are considered damaged kernels (DK), and if the percent DK exceeds 2.5 in a harvest, the overall value at market can be reduced by 65% (Balota et al., 2015). One study found that peanut seed containing less than 13 mg kg^{-1} B were likely to develop hollow heart, while seed with higher amounts of B would not (Netsangtip et al., 1987). Other plant symptoms of B deficiency include increased flowering time, cracked branches, and shortened internodes. Under some conditions B fertilization can increase yield (Nasef et al., 2006), and under other conditions it does not (Hartzog and Adams 1968 and 1973, Cope et al., 1984, Swamy and Reddy, 1983).

Boron fertilization is a common practice in the US because of the sandy soils used for peanut production (Gascho and Davis, 1995). Current B fertilization recommendations for the Virginia-Carolina (V-C) region are based on three studies. Boron fertilization of 6 kg ha^{-1} (Perry, 1971) is applied to peanut fields that have less than 0.2 mg B kg^{-1} (Hill and Morrill, 1974) at pre-plant, or sprayed with the first or second fungicide application (Hartzog and Adams, 1973). Boron fertilization has been practiced since the 1970’s, unchanged, by 97% of producers in VA and NC (Morgan et al., 2014). This is in spite of some researchers having found that soil B was sufficient for preventing hollow heart and optimal peanut yield at much lower levels like 0.16 mg kg^{-1} (Jadhoa et al., 1989), or even 0.07 mg kg^{-1} (Hartzog and Adams, 1971). Current V-C peanut production manuals do not list a lower soil B threshold, only to apply if “soil levels are reduced”. This is potentially important, as B toxicity can reduce yield (Blamey et al., 1981). Other symptoms of toxicity are chlorotic leaf tips and marginal necrosis. Virginia Tech (VT) recommends 25 to 60 mg kg^{-1} B in plant tissue to maintain healthy plant development.
There is currently now efficient way for peanut producers to determine if their fields need B fertilization. They must rely on pre-plant soil B levels, for which there is no consensus in the literature, to make application decisions. Once the crop is growing, producers can only collect tissue samples and send to a laboratory for analysis to find out if their field is developing a B deficiency due to heavy rainfall, or some other environmental factor. With the rapidly increasing use of remote sensing in agricultural production, this is a potential method for producers to

quickly, quantitatively, and cost effectively determine B fertilization needs of their peanut crops. Normalized Difference Vegetative Index (NDVI) has already been investigated for use in peanut production for determining maturity (Carley et al., 2008), an estimation of drought stress (Muthumanickam et al., 2011), percent ground cover (Rajan et al., 2014), and crop phenotyping (Zaman-Allah et al., 2015). NDVI is an index calculated by measuring visible red (R), and near infrared (NIR) canopy reflectance, then entering them in the formula (NIR-R)/(NIR+R) (Rouse 1974, Thenkabial et al., 2000). This gives a quantitative measure of "greenness". Severe B deficiency in peanuts causes dark mottling of the leaves, as well as shortened internodes (Harris et al., 1957), both of which will increase the "greenness" of the field. Severe B toxicity leads to interveinal chlorosis of the leaves and necrotic tips (Harigopal et al., 1964), both reducing the "greenness" of the field. This spectrum of leaf color due to plant B content suggests that an index like NDVI may be useful in remotely determining B stress in a peanut field.

There is widespread B application in the V-C region, discrepancies in method and efficacy of B fertilization in the literature, and changes in cultivars and production practices since the recommendations were made. Because of this, there is an urgent need to test recommended B fertilization for benefits in yield and reduction of hollow heart with modern cultivars and production practices. If it is not necessary to indiscriminately apply B to peanut fields, then producers must have guidelines and a way of determining when to apply B.

RESEARCH OBJECTIVES

The objectives of this research are to discern if current B application recommendations are still optimal for peanut crop production in the V-C region by 1) comparing if resulting tissue and seed B content and yield respond when no B, recommended, and excess rates were applied, and 2) if readily accessible NDVI sensors could potentially be used as an early B deficiency assessment tool.

MATERIALS AND METHODS

Experimental Design

In 2014, a preliminary test was done in a field at the Tidewater Agricultural Research and Extension Center (TAREC) in Suffolk, Virginia (Emporia, Eunola, Uchee. 36°39'N, 76°43'W, 66m elevation). Three B rates were applied of 0, 0.3, and 0.6 kg ha^{-1}, applied at growth stages planting, beginning peg (R2), full seed (R6), and split applied between planting and R2, planting R6. Leaf tissue samples were collected, dried and analyzed for B content five days after R2 and R6 applications. Harvested seed was also dried and analyzed for B content. Yield and damaged kernel (DK) % were measured post-harvest.

The study was planted in four locations across eastern Virginia and North Carolina in the 2015 growing season. Locations in Virginia were planted in Field 23 (Emporia, Uchee, fine sandy loam. 36°39'N, 76°44'W, 59m elevation), and Field 63 A (Emporia, Nansemond, sandy loam. 36°40'N, 76°43'W, 75m elevation) at the TAREC. Locations in North Carolina were the Peanut Belt Research Station (PBRC) in Lewiston, NC (36° 7'N, 77°10'W, 72m elevation), and the Upper Coastal Plain Research Center (UCPRC) in Rocky Mount, NC (Aycock, fine sandy loam. 35°53'N, 77°40'W, 107m elevation). The cultivar 'Bailey' was used at all locations. 'Bailey' has been on the market since 2008 (Isleib et al., 2011), and is in widespread use by most peanut producers in the Tidewater area of Virginia and North Carolina, thus making it a good standard for treatment comparisons. At all locations, the experimental design was a randomized complete block with three replications, except for Lewiston, NC which had four replications (Table 1). Plots were 10.7m in length, and 1.8m in width containing two treatment rows. There were two border rows between each plot. Planting dates were May 5thfor Fields 23 and 63 A at the TAREC, May 7th at Lewiston, NC, and May 14th at Rocky Mount, NC (Table 1). Soil samples were taken with a hand soil probe at 15, 30, 46, and 61cm at the beginning of the season, and sent to the Virginia Tech Soil Testing Lab in Blacksburg, VA. Prior to planting, lime was applied where necessary to adjust to neutral soil pH. Cultural practices were performed according to the Virginia Peanut Production Guide (http://www.ext.vt.edu). For weed control, Basagran (bentazone), Dual (metolachor), Dual Magnum (acetamide), Prowl H_2O (pendimethalin), Storm (acifluorfen + bentazon), 2-4-D, and Select Plus (clethodim) were used pre- and post-emergence. Insecticides Admire Pro (imidacloprid), Belt (flubendiamide, 1,2-

propanediol trade secret ingredient(s)), Danitol (fenpropathrin), Gramoxone (paraquat dichloride), and Orthene (acephate) were applied in furrow and during vegetation for thrips (*Thripsphysapus*) and other insect control. The fungicides Bravo (chlorothalonil), Omega (propiconazole), Proline (prothioconazole), and Provost (prothioconazole, tebuconazole) were used for disease control. Manganese, Sulfur, and Calcium were applied as recommended for high yield production (Balota et al., 2015). Optimize lift inoculant was added in-furrow for improved biological nitrogen fixation by the plants.

A summary of the seventeen treatments are presented in Table 2. Four B rates were used, 0 kgB ha^{-1}, 0.3 kgB ha^{-1}, 0.6kgB ha^{-1}, and 1.1kgB ha^{-1}. These rates were each applied at planting, beginning peg (R2), or full seed (R6) (Boote, 1982), and split applied between planting and R2, and planting and R6. Liquid B 9% was used in all applications for this experiment. Boron was applied using a backpack sprayer over both rows of the experimental plot.

Field Measurements

Twenty five days after planting (DAP) leaf tissue samples were taken from all plots at the TAREC. At least fifteen youngest fully mature leaves per plot were collected, oven dried to constant tissue moisture, ground in a home coffee grinder, and approximately 1.5g of dry powder was packed and shipped to Waypoint Analytical Lab for B tissue analysis. Tissue sample collection was repeated from the same plots five to seven days after B applications a growth stages R2 and R6. Normalized difference vegetative index (NDVI), and SPAD-chlorophyll readings were taken in all experimental plots at 25 DAP and five days after boron application at R2 and R6.

The NDVI was measured with a handheld Trimble® GreenSeeker (Trimble, GreenSeeker Handheld, Sunnyvale, CA). The GreenSeeker was hand held about 24 inches above the canopy as the plot was walked. An average of NDVI values was displayed and recorded at the end of each plot. A Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502, Konica Minolta Optics Inc., Japan) was used to confirm the NDVI readings. Three randomly sampled leaves were measured in each plot, and their values averaged. Only mature leaves near the main terminal branch were used.

After harvest, yields from each plot were weighed. Yield per hectare was calculated and adjusted to 7% standard seed moisture, which is standard moisture content for sale at market. Samples of the graded seed was then ground and sent to Waypoint Analytical for nutrient analysis.

Statistical Analysis

ANOVA was used to estimate main and interactive effects of the B rate and time of application, and location for individual growth stages using JMP software (SAS Institute Inc., Ver. 11.0, Cary, NC, 2013). Means were separated by Fisher's LSD when appropriate ($p<0.05$). Pearson's correlation coefficients were used to compare tissue B, SPAD, NDVI and yield. Non-linear regressions were made using the best fit quadratic lines to determine r and r^2 of correlations.

RESULTS

Weather

The amount of seasonal rainfall between locations was similar. The TAREC received 61.5cm, Lewiston, NC received 64.3cm, and Rocky Mount, NC received 72.6cm of precipitation over the growing season from May through October (Table 3). The average air temperature over the growing season was 23°C at all locations. Though average soil temperatures were not the same, they were similar between locations. At the TAREC soil temperature averaged 24°C, slightly lower than at Lewiston, NC (27°C), and Rocky Mount, NC (25°C) from May through October (Table 3).

Tissue and Seed Boron Content

In the 2014 preliminary experiment, there were no significant differences ($p=0.34$, 0.18, 0.27) between leaf tissue B at R2, R6, or in the harvested seed based on the rate of B applied (Table 11). There was no difference ($p=0.410$) in harvested seed B based on growth stage at which B was applied. At the R2 sampling time, leaves having just received B at R2 had significantly ($p<0.001$) higher B content based on growth stage at application. At the second sampling time (R6), there were significant ($p=0.040$) differences in leaf B content based on growth stage at application time, but there was no pattern for which treatment had higher content (Table 11)

In 2015, tissue samples were only taken from the plots at the two locations at the Virginia TAREC, Field 63 A and Field 23, for this experiment. Four total application rates were used at five application time combinations. Tissue samples were analyzed for nutrient content after all applications times, and for the harvested seed (Figure 1). Only plots receiving B at planting had received treatment at the first sampling time (25 DAP). All treatments, whether they had received B yet or not, had no statistically different amount of B in the leaf tissue. After the R2 application, treatments having just received B at R2 had significantly ($p=0.004$) higher leaf B content than those who had only received B at planting or none at all. After the R6 application time, treatments receiving all or part of their B amount at R6 had significantly ($p<0.001$) higher leaf B content than those that had only received B at planting, R2, or not at all. When the harvested seed B content was measured, there was no significant differences between plots that had received B at planting, R2, R6, split application, or no B at all. The highest amount of leaf tissue B in a sample was 272 mg kg^{-1}, and the lowest was 31 mg kg^{-1}. The highest amount of B in a seed sample was 23 mg kg^{-1}, and the lowest was 15 mg kg^{-1}; 2 mg kg^{-1} above the threshold of 13 mg kg^{-1} for developing hollow heart.

At R6 growth stage, all plots had received their full total amount of B treatment (Figure 2). Plots receiving a total of 1.1 kg B ha^{-1} over the growing season had a significantly ($p<0.001$) higher leaf B content at R6 than all other treatments. The two recommended B application amounts of 0.3 and 0.6 kg B ha^{-1} did not significantly differ in final leaf B content from the plots that received no B at all. For seeds, treatments that received 1.1 and 0.6 kg B ha^{-1} over the season still did not have significantly ($p=0.071$) higher B content than seed that received no B at all. Seed that received a total of 0.3 kg B ha^{-1} were not significantly different than any of the other treatments.

Pod Yield

For the 2014 preliminary experiment, there was no significant difference in yield or DK% based on rate of B applied (p=0.97, 0.47), or based on growth stage at application (p=0.89, 0.27) (Table 11).

In 2015, the yield was measured at all four locations. Historic research generally agreed that B application in more alkaline peanut fields was needed if pre-plant soil tests showed soil B levels below 0.2 mg kg^{-1}. Of the four fields, the two TAREC fields were at or below the threshold for B application at both 15 and 30cm soil depth. At Lewiston, NC, the soil was below the pre-plant threshold for B application for both 15 and 30cm soil depth. At Rocky Mount, NC, conversely, soil levels were above the threshold at both 15 and 30cm depth, having 0.3 mg B kg^{-1} (Figure 3). Total mean adjusted yields were compared between locations, with the two NC locations producing significantly (p<0.001) higher yields than the two VA fields. For this reason, Rocky Mount and Lewiston, NC yields were both analyzed separately from the TAREC fields.

In Virginia at the TAREC fields, there were no significant differences in yield based on B application time (Figure 4). At Lewiston, NC, where soil was below the recommended threshold for B, there were still no significant differences in yield between plots that received B at different times through the growing season (Figure 5). Rocky Mount, NC soil B levels were above the threshold for needing B application. But there were no differences (p=0.227) in yield based on B application time (Figure 6).

When yields based on B rate were compared across locations, the only significant (p<0.001) differences were between the NC locations, and the TAREC in VA (Figure 7). Within each location there were no statistically significant differences in yield due to the rate of B, however there is a general pattern 0.6 kg ha^{-1} of B producing slightly higher yields than either having no B applied, or 1.1 kg B ha^{-1}.

Plant Health Indices

Both NDVI and SPAD-chlorophyll were sampled at the same time as leaf tissue samples were taken at all four locations for comparison with leaf B content and pod yield.

There was no correlation between leaf B content and NDVI or SPAD-chlorophyll content at any growth stage (Table4). However, there was a moderately strong positive correlation between NDVI and pod yield (r^2=0.41, 0.25, 0.08, and 0.56, 0.47, 0.14) at all locations when NDVI was measured at R2 and R6 growth stages (Table 5). A quadratic fit of yield with NDVI was also observed, with a sharp yield increase when NDVI was between 0.76 and 0.88, and then plateaued when NDVI was around 0.9 (Figure 8).

DISCUSSION

Our objective was to assess current B recommendations for peanut production and determine if these are necessary for maintaining maximum yield and plant health. There was no difference in leaf tissue B between plants that received B or not at 25 DAP. At both R2 and R6 growth stages, plants that had received either part or full B application, had significantly higher leaf B content than those that had received none. This demonstrates efficacy of the B application and its availability in the young leaves within five days of application through either direct spraying or translocation from the older leaves. But little to no B was detected in newly formed leaves from the treated plants in applications older than five days. Combined, this suggests that there is limited phloem mobility for B in peanut plants.

For example, at the R2 and R6 growth stages, increased leaf B content was only detected in plants that had just received some amount of B during the most recent application. This could be because leaves forming much later after a fertilization did not benefit from earlier fertilization because B was immobile in the peanut phloem. This is in agreement with classical literature documenting B immobility in peanut.

The most telling way of discerning if B is being re-translocated via the phloem in the peanut plant is to see if foliar applied B during the season correlates to B levels in the harvested seed. Applying B at different times through the growing season had no impact on seed B content. However, the total amount of foliar B applied during the growing season did impact the harvested seed B content. Seed B content was significantly higher when at least 0.6 kg B ha^{-1} was applied, regardless of timing. Plants with greater total leaf tissue B content, also had greater B content in the seed. This suggests that some B could be re-translocated via the phloem, but only when a large enough volume of B is available. It took a rate that is higher than the currently recommended B foliar application in order to noticeably raise the seed B content. This may be because of inefficient B phloem translocation. For plants that did not receive any B during the growing season, the lowest seed B content was 15 mg kg^{-1}, distinctly above the threshold for developing hollow heart. Thus suggesting little need to apply B even if the pre-plant soil tests show B levels as low as 0.1 mg kg^{-1}.

The most important result is how the yield is affected by the foliar application of B. There was no significant yield variation due to B rate and timing of application at any of the test locations, and regardless of the pre-plant soil B levels. Both of the NC locations had significantly higher yields than the VA locations, in spite of the NC fields having both the lowest, and the highest pre-plant soil B levels. Yield differences could be due to differences in weather, management, or soil types. But there were no differences in yield even for those plots whose seed had elevated B content. There was no difference in yield at any location between plots that had received the highest rate of B, 1.1 kg ha^{-1}, and those that received none. This suggests that B had no meaningful effect on peanut yield.

If B does not need to be indiscriminately applied to peanut fields in the V-C region in order to achieve optimal yield, then producers need a tool for determining if B application is necessary. NDVI is a reflectance index based on red and near infrared light wavelengths reflected by the canopy. It has the potential to be a quick, easy, quantitative diagnostic tool of plant nutrient levels for producers, if it can be correlated to the plant nutrient content. Severe B deficiencies in peanut can lead to dark, mottled leaves and compacted branch terminals, theoretically leading to higher NDVI reflective values. This would predict an inverse relationship between actual tissue B content and NDVI. In this research no correlation between NDVI and leaf B content was found. As shown by the literature, certain wavelength ranges can be more useful than others for detection of crop nutrient levels. The NDVI sensor that was used in this research does not measure any wavelengths within those ranges. Absence of correlation can also be caused by absence of significant leaf color change due to leaf B content. While this instrument was not able to detect differences that correlated to tissue B content, other narrow band indices may be able to detect such changes. The SPAD-chlorophyll meter measures the amount of red light absorbed by the leaves due to the chlorophyll content. There was no relationship between leaf B content and SPAD in this experiment. Whether due to a lack of a broad range of B content for samples measured, or because the instruments are not sensitive to changes due to B, neither SPAD or NDIV strongly correlated to leaf B content, and therefore do not immediately present an option for producers to use in determining B application.

There are many determinant factors for peanut yield, and B does not seem to be the strongest. Curling of leaves due to drought could have been another factor that lead to lower NDVI values in some fields, confusing any possible correlation between NDVI and B excess or deficiency. It could also suggest that there is not enough color change in response to B application for NDVI to detect until such extreme deficiency and toxicity that it is usually outside the normal production experience. However, exploring other wavelengths in NDVI sensors, or multispectral sensors may yield data that can be correlated to tissue B levels in peanuts.

CONCLUSSION

There has been near universal B application in peanut production since the 1970's based on exactly the same recommendations. This experiment addressed two objectives: 1) to determine if recommended rates and timing of application are still optimal for peanut health and yield, and 2) if a commonly available GreenSeeker NDVI instrument can be effectively used to determine peanut stress due to B levels. Foliar B applications temporarily increased leaf B content during the growing season, but the increase in B did not transfer to the developing seed. This suggests that current B application methods in the V-C region are either not effective, or not necessary for providing sufficient B to developing seed. At all locations, pre-plant soil B content was at or below the recommended threshold at which B should be applied to prevent hollow heart and maximize yield. Yet, none of the untreated controls in these fields produced seed with B content below 13 mg kg^{-1}, below which hollow heart develops. Additionally, there was no difference in yield between plants that received B in any amount, and those that received none at all. Thus, because yield and seed B content were not negatively impacted by soil levels lower than the recommended threshold for application, that threshold should be reinvestigated to be set at a lower per-plant soil B level.

In order for producers to be able to only apply B when a field is under stress from lack of B, they need a quick, quantitative, and simple tool for determining B need. Because foliar symptoms of extreme B deficiency and toxicity range from dark mottling to interveinal chlorosis, there is potentially a canopy color change correlated to B stress, and thus to changes in yield due to B availability. The darker green of severely B deficient peanut leaves would give a higher NDVI value, while sufficient levels for optimum yield should give somewhat lower levels, thus making a negative correlation between NDVI canopy reflectance, and yield. There were some positive correlations between NDVI and yield, but no negative, therefore the correlation with yield is likely due to other factors like nitrogen or water availability. Given the lack of negative correlation between NDVI and yield, this is probably not the index to pursue for using remote sensing in determining stress from micronutrients like B. However, there was not a broad range of B stress exhibited in the experimental fields, therefore future experimentation should attempt to increase the range of B stress while investigating remote sensing techniques. If other wavelengths for R and NIR were used in the NDVI calculation, it is still possible that a usable index could be developed.

From this field experiment conducted evaluating B application at multiple timings with different amounts, it is likely that B has been over applied in the V-C region. The thresholds for soil B, below which B should be applied need to be lowered beyond the 0.1 mg kg^{-1} based on the data provided by this experiment. Producers should also be advised that B application under normal soil and weather conditions in the V-C region is not likely to increase yield, or reduce hollow heart, as there is not likely to be much if any hollow heart.

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CHAPTER III: Response of Multiple Peanut Cultivars to Boron Fertilization

ABSTRACT

Peanuts (*Arachis hypogaea* L.) are commonly grown as part of the crop rotation in eastern Virginia and Carolina. The sandy soils in this region are lacking in micronutrient vital to proper peanut development, boron (B). If developing peanut seed does not receive adequate B, the cotyledons will rot, causing "hollow heart" and damaging the quality of the harvest. Peanut B fertilization recommendations are based on just three studies performed in the 1970's. Since then there have been many changes through breeding of production cultivars. To investigate if B fertilization recommendations are still appropriate for modern cultivars, B fertilization was compared across a selection of runner and Virginia market types, and newer and obsolete cultivars. There was no difference in tissue B between cultivars, but seed B content was higher for obsolete versus newer cultivars. Newer cultivars produced higher (p=0.012) yields when unfertilized, but not when B was applied. The normalized difference vegetative index (NDVI) was used to investigate potential as a remote sensing technique for detecting B stress in peanuts. There was no correlation between NDVI and plant B content, seed B, or yield due to B level. Based on this research, it was concluded that newer cultivars have not changed substantially in their response to applied B, but there is no need to add B to achieve optimal yield, plant health and seed quality for modern cultivars.

INTRODUCTION

Peanut (*Arachis hypogea* L.) is commonly planted in crop rotations of eastern Virginia and the Carolinas. The long growing season, and loose, sandy soils are conducive to peanut production. Peanuts are particularly sensitive to soil boron (B) levels. Among micronutrients, B is the most widely deficient across the US (Gupta, 1993). Boron is soil mobile, and is particularly susceptible to leaching in sandy soils and after heavy rains. However, once inside the peanut plant, B is not phloem mobile (Raven, 1980). Soil pH and soil organic matter (Gasho and Davis, 1995), as well as cultivar (Harris and Gilman, 1957) have a major effect on the availability of soil B for plants to take up. When insufficient amounts of B are available during peanut seed development, the cotyledons can become deformed and rot inside the seed causing "hollow heart" (Harris and Gilman, 1957). One study found that seed with B content below 13 mg kg^{-1} could develop hollow heart, while those above did not (Netsangtip et al., 1987). Seed exhibiting hollow heart contribute to the damaged kernel (DK) percent of a harvest. If the DK content is more than 2.5%, producers could lose up to 65% at market for their harvest (Balota et al., 2015). However, most research on the impact of B on peanut production was conducted in the 1960's through the 1980's. Current peanut production guidelines for B fertilization are based on only three studies from the early 1970's. It was recommended that 0.6 kg B ha^{-1} (Perry, 1971) be applied at planting, or with the first or second fungicide application (Hartzog and Adams, 1973) when soil B is less than 0.2 mg B kg^{-1} (Hill and Morrill, 1974). Since then, B fertilization has been practiced, unchanged, by virtually all peanut producers in the Virginia- Carolina (V-C) region.

The B requirements between runner type cultivars varies (Harris and Gilman, 1957), and the genetic diversity between cultivars has greatly increased in recent years (Milla-Lewis et al., 2010). Runner type peanuts were also found to be more sensitive to B deficiency, developing symptoms earlier, and recovering more slowly than their Virginia type counterparts (Harris, 1965). Even for the Virginia types, increased genetic diversity has led to changes in response to applied nutrients between older and newer cultivars like Gregory and NC-V11 (Jordan et al., 2010).

Remote sensing is being developed and used in many aspects of agriculture. Several aspects of peanut production have been investigated using the normalized difference vegetative index (NDVI) such as maturity (Carley et al., 2008), estimating drought stress (Muthumanickam et al., 2011), ground cover (Rajan et al., 2014), and phenotyping (Zaman-Allah et al., 2015). Because severe B deficiency cases dark mottling of the leaves and compaction of internodes (Harris et al., 1957), and severe toxicity can lead to interveinal chlorosis and necrosis of the leaf tips (Harigopal et al., 1964), there is a change in leaf color due to B content. NDVI measures red (R) and near infrared (NIR) canopy reflectance and gives a measure of "greenness" using the formula (NIR-R)/(NIR+R) (Rouse, 1974). If a reflectance index like NDVI can be correlated to peanut tissue B content, then this would be a quick, quantitative way for producers to determine if a peanut field is under B stress. This research is to quantify if changes in cultivar characteristics since the 1970's has substantially changed the way peanut plants respond to B fertilization in yield, plant health, and seed B content. It also investigates if NDVI has potentially to be developed as an early detection tool for B deficiency in peanut production.

RESEARCH OBJECTIVES

Objectives of this research included assessing if current recommendations for B application are efficacious in peanut crop production in the V-C region by 1) determining if B fertilization effects newer, or different market type cultivars differently, and 2) if a commonly available NDVI sensor can potentially be used as an early B deficiency detection tool.

MATERIALS AND METHODS

Experimental Design

The peanut cultivar test was planted at the TAREC (Emporia, Uchee. 36°39'N, 76°44'W, 61m elevation), and in two producers fields in Sussex county (36°58'N, 77°14'W, 51m elevation), and in Southampton county (36°35'N, 77°12'W, 88m elevation), VA in the 2015 growing season. The test included twelve cultivars, Bailey (Isleib et al., 2008), FL07 (Gorbet and Tillman, 2006), Florunner (Norden et al., 1969), GA06G (Branch, 2006), GA13M (Branch, 2013), 07030-1-10-1, Gregory (Isleib et al., 1999), NC-V11 (Wynne et al., 1991), Sullivan (Isleib et al., 2013), TUFRun511 (2013), VA98R (Mozingo et al., 1998), and Wynne (Wynne et al., 2013). These cultivars were selected to provide a combination of six old, and six new cultivars with an equal number of runner and Virginia market type peanuts. Breeding line 07030-1-10-1, a Virginia type, was substituted for Georgia Green (1995), for which seed was not available (Table 8). Due to late planting, and poor crop stand, data will not be presented for this line. At all three locations, a randomized complete block design in three replications was used. Two row plots were planted 10.7m long, and 1.8m wide, with two border rows between each plot. Planting, digging, and harvesting dates are presented in Table 9. Soil samples were taken with a hand soil probe at 15, 30, 46, and 31cm deep in all three fields that the experiment was planted in at the beginning of the season. To maintain a neutral soil pH, lime was applied where necessary. All locations were maintained using cultural practices as presented in the Virginia Peanut Production

Guide (http://www.ext.vt.edu). Disease was controlled with Bravo (chlorothalonil), Omega (propiconazole), Proline (prothioconazole), and Provost (prothioconazole, tebuconazole). For weed control, herbicides Dual (metolachor), Dual Magnum (acetamide), Prowl H_2O (pendimethalin), Select Plus (clethodim), and Storm (acifluorfen + bentazon) were applied pre- and post- emergence. Insecticides Admire Pro (imidacloprid), Belt (flubendiamide, 1,2-propanediol trade secret ingredient(s)), Danitol (fenpropathrin), Gramoxone (paraquat dichloride), Intrro (alachlor), and Orthene (acephate), to control insects, including thrips, were applied both in furrow and during vegetation. Supplemental nutrient applications included ENC (11-8-5), Manganese, Sulfur, and calcium for optimal yield were applied as recommended (Balota et al., 2015). All fields were inoculated in-furrow with Optimize Lift (Monsanto BioAg, St. Louis, MO), to facilitate biological nitrogen fixation by plant roots. Boron was applied at full pod (R4) developmental stage using liquid B 9% in an 11.4 liter tank with a backpack sprayer over both rows in each experimental plot.

Field Measurements

Five days after boron application, plant leaves were sampled for tissue B content, Normalized Difference Vegetative Index (NDVI) and Soil Plant Analysis Development (SPAD) chlorophyll readings were taken in each plot. Leaf tissue samples were taken five to seven days after B application to allow for new leaves to mature after foliar application of B. A minimum of fifteen youngest fully mature leaves were sampled per plot, dried to a constant moisture in an oven, approximately 1.5g of dried leaves were ground in a home coffee grinder before being shipped to Waypoint Analytical Lab for B tissue analysis. A Trimble ® GreenSeeker (Sunnyvale, CA) NDVI was handheld approximately 61cm above the peanut canopy while walking the length of the plot to determine an average value for the plot. Three randomly selected mature leaves around the main terminal of plants were sampled in each plot using a SPAD-chlorophyll meter (SPAD-502, Konica Minolta Optics Inc., Japan). The SPAD values were averaged to give one value per plot.

Yields from each plot were weighed and adjusted to 7% standard seed moisture. Leaf and harvested seed samples were also sent to Waypoint Analytical for nutrient analysis.

Statistical Analysis

All statistical analysis was done using JMP software (SAS Institute Inc., Ver. 11.0, Cary, NC, 2013). ANOVA was used to estimate the main and interactive effects of B application, cultivar type, and cultivar release age. Means were separated for comparison using Fisher's LSD, when appropriate. Pearson's correlations were also used to compare tissue B, NDVI, and yield. The R^2 of correlation was determined for the best-fit quadratic line.

RESULTS

Weather

Average seasonal rainfall between the three locations varied with field 27 at the TAREC having 61.5cm, Southampton, VA had a much lower seasonal rainfall from May through October with 36.6cm, Sussex, VA had the highest rainfall with 90.7cm of rain over the growing season (Table 10). Air and soil temperatures over the season were similar (22.5°C and 24.4°C, respectively) across all locations.

Tissue and Seed Boron Content

Only one location was sampled for actual nutrient analysis of leaf tissue during the growing season and nutrient analysis of the harvested seed. Therefore location could not be analyzed as a factor. Data was collected in field 27 at the TAREC. Tissue and seed B content were only determined at the TAREC. There were no significant differences in tissue B content between newer and older peanut cultivars, runner and Virginia types, and plants that were B fertilized and those that were not (Figure 10). For the harvested seed, B content was significantly ($p=0.01$) higher in older than newer cultivars. This was not consistent with leaf tissue B, as there were no significant differences between ages. But there were no differences between Virginia and runner types, and treated or not treated with B (Figure 9). The highest leaf tissue B content sample measured was 196 mg kg^{-1}, and the lowest was 38 mg kg^{-1}. The lowest seed B content measured was 15 mg kg^{-1}, and the highest was 23 mg kg^{-1}. All leaf tissue B content was above the deficiency levels (25 mg B kg^{-1}), but some had excessive levels above the critical levels of 85 mg B kg^{-1} (Blamey et al., 1981). All seed B concentrations were above the deficiency threshold of 13mg B kg^{-1} (Netsangtip et al., 1987).

Pod Yield

At 15cm and 30cm soil depths at the TAREC, B was at or below the threshold of 0.4 mg kg^{-1} soil B recommended for sufficient peanut development. The field at Sussex, VA was below the threshold with 0.2 mg B kg^{-1} at both depths. The field at Southampton, VA was right at the threshold of 0.4 mg B kg^{-1} at both soil depths (Figure 10). There was a significant ($p<0.001$) location effect on yield, with yield in Southampton, VA being more than 1000kg ha^{-1} lower than in Sussex, VA and the TAREC (Figure 11). For all of the plots that received B fertilization, TAREC, Sussex, and Southampton locations had significantly ($p=0.007$, 0.007, and <0.001 respectively) higher yields among the newer than the older cultivars (Figure 12). Among plots that did not receive any B fertilization newer cultivars produced significantly ($p=0.003$, and 0.036 respectively) higher yields at the TAREC and Southampton, with the exception of no significant difference in yield of untreated plots at Sussex (Figure 13). Regardless of B fertilization, newer cultivars produces significantly higher yields than the older cultivars. With the exception of the new runner type grown at Southampton showing close to significant ($p=0.070$) yield decrease when B was applied, at all other locations there were no yield differences in yield between unfertilized and 0.6 kg B ha^{-1}, regardless of peanut market type, and the year of release (Tables 11 and 12).

Plant Health Indices

Normalized difference vegetative index was used the same time as leaf tissue samples were taken in order to determine a correlation between B concentration in leaf tissue and NDVI. Pearson's correlation coefficients showed no significant relationship between leaf B content and NDVI or SPAD at the R4 sampling time. There was a weak, positive correlation between SPAD and yield (Table 13). NDVI did, however, have a moderately strong positive exponential correlation with overall yield (Figure 15).

DISCUSSION

The second objective of this study was to evaluate B need in relationship to peanut market type and for newer cultivars. For this older and newer cultivars for both runner and Virginia market types were compared. At R4 growth stage, all eleven cultivars were fertilized with 0.6 kg B ha^{-1}, or left untreated. At only one location leaves were sampled for tissue analysis, but yield and plant health indices were taken at all locations. No differences were found in leaf tissue B content between fertilized and non-fertilized plants for the cultivars tested in this experiment. Some of the lack of difference could possibly be due to small sample size, especially between older and newer runner types. Because neither the older or newer cultivars had significantly higher leaf B content with B fertilization, newer cultivars may not be any better at re-translocating B than older cultivars. Soil type, weather, or management may be influencing the peanut plant's ability to take up and re-translocate B far more than the physiology of the plant itself. Interestingly, the only factor that produced significant difference in seed tissue B content, was the age of the cultivar released. This may suggest that older cultivars are actually more adept at taking B directly from the soil into the seed instead of re-translocating foliar B to the developing seed. This could also suggest that older cultivars may need less B to form mature seed. This could also be due to small sample size. Regardless of cultivar age, market type, or fertilization, the lowest seed B content was still above the 13 mg kg^{-1} threshold for developing hollow heart. This suggests that B application may not be necessary to develop seed free of hollow heart, no matter the cultivar being grown, under the current production practices when soil B is adequate.

For this experiment, all fields had a pre-plant soil B content at or below the threshold recommended for B application to achieve optimum yield. Therefore B fertilization should have shown improvements in yield across cultivars. However, for both newer and older cultivars, B fertilization did not significantly impact yield. Newer cultivars, however, did have significantly higher yields than older cultivars, as would be expected due to breeding efforts. These data are congruent with previous literature that over time, peanut cultivars have been developed with increased productivity under any set of growing conditions. Because there are no difference in leaf tissue B based on B fertilization, there must be a different determining factor for plant tissue B than B fertilization.

In order to asses if NDVI has potential as a method to detect B stress in peanut, measurements were taken at the same time as leaf tissue samples. Pearson's correlation was used to compare

NDVI and SPAD with leaf tissue B content. There was no correlation between NDVI and leaf B content. Higher NDVI values indicate more "greenness". Because of the expected darker color of peanut leaves with B deficiency, and chlorosis of peanut leaves under toxic levels of B stress, if NDVI were to correlate with peanut plant B content, it would be a negative correlation. Since there were no correlations between NDVI and leaf B content, NDVI may not be sensitive to peanut B content within a common production scenario. Conversely, there may not have been enough B stress on the peanut plants, from either too much or too little B, to create leaf color change that could be detected by a reflectance instrument. NDVI was moderately positively correlated with yield. A change in leaf reflectance had to exist, but since it was a positive correlation, it is not tissue B content. Extreme B deficiency in peanuts leads to dark green mottling of the leaves, which would increase the NDVI while decreasing yield, thus a negative correlation between NDVI and yield because of B. The correlation between NDVI and yield is most likely due to water stress, or nitrogen levels, which are the main causes of major changes in R and NIR reflectance from peanut leaves (Muthumanickam et al., 2011). Discerning between factors that strongly influence R and NIR reflectance, like water and nitrogen, and factors that have a much weaker effect on reflectance, like micronutrients, has been the goal of many researchers. This experiment did not support NDVI as a useful tool for producers to discern B stress in their peanut crops.

CONCLUSION

Boron has been applied by peanut farmers since the 1970's regardless of the plant need or soil tests, according to the same application recommendations. This experiment addressed two objectives: 1) if current peanut cultivars have changed B needs relative to cultivars commercially grown around the time the recommendations were developed, or based on market type, and 2) if NDVI could be used by producers as an early detection tool of B stress. Foliar B application had no impact on B levels in leaves or developing seed. When multiple cultivars were compared, there were no differences in seed B between runner and Virginia types. The only difference was a higher seed B content in the older cultivars. However, there were higher yields from the newer cultivars even though they had less B accumulation than the older cultivars. This suggests that the current recommendations have become obsolete, as less B accumulation in seed was necessary for obtaining improved yield.

Pre-plant soil B content was at or below the recommended threshold for most locations at which B should be applied to prevent hollow heart and maximize yield. No seed was found with a B content below the hollow heart threshold of 13 mg kg^{-1}. Therefore recommended B application practices are not necessary for reducing the hollow heart percentages. Neither is B application by current practices necessary for optimal yield across cultivar types.

If producers are going to refrain from applying B unless their fields are stressed from insufficient amounts, they must have a tool that is quick, quantitative, and sensitive to B stress. NDVI is one index of canopy reflectance that could potentially detect the change in canopy greenness from a normal healthy green, to the darker, mottled canopy under B stress. This negative correlation between NDVI and leaf canopy color should also translate into a negative correlation between NDVI and yield, as plants under extreme B deficiency also experience lower yields. NDVI negatively correlated with yield and leaf B content in this research. There were some positive correlations between NDVI and yield, which were most likely due to nitrogen or water availability, for which this particular instrument was developed. As there was not any B deficiency stress in the experimental fields, a much broader range of B availability would have be investigated to develop as usable remote sensing reflectance index. With the wavelengths measured by this instrument, and under normal field conditions in the V-C region, NDVI is not a usable tool for early determination of B deficiency stress in peanuts.

From the two field experiments conducted evaluating B application on different cultivars and at timing with different amounts, it is not likely that B application is needed in the V-C region under the soil and weather conditions in this experiment. The thresholds for soil B, below which B should be applied need to be lowered beyond the 0.1 mg kg^{-1} based on the data provided by this experiment. Producers should also be advised that B application under normal soil and weather conditions in the V-C region is not likely to increase yield, or reduce hollow heart, as there is not likely be much if any hollow heart.

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CHAPTER IV: Germination Response of Boron fertilized Peanut Seed

ABSTRACT

Peanuts (*Arachis hypogaea* L.) is a major crop of the eastern Virginia-Carolina (V-C) region. The sandy soils in the region are prone to low boron (B) a soil mobile micronutrient vital in the proper development of peanut seed. For this reason, B fertilization is a standard practice for peanut production in the V-C region. Seed with B content <13mg kg^{-1} may develop hollow heart, a rotting of the cotyledons. It is not well defined if current B fertilization practices are necessary to prevent hollow heart, or if other detrimental seed qualities could arise from excess or insufficient seed B. Seed from fields that had been B fertilized (0, 0.6, 1.1kg ha^{-1}), and with runner and Virginia market types, and newer and obsolete cultivars, were germinated in sand over ten days. Seed samples from all B fertilization treatments had ≥15mg B kg^{-1}. There were no differences in germination between fertilized and unfertilized seed, or between market types, or between newer and obsolete cultivars. Based on this research, B fertilization of peanuts in the V-C region is not necessary to maintain optimal germination of seed produced.

INTRODUCTION

Peanut (*Arachis hypogea* L.) is a regular part of crop rotations in the Virginia-Carolina (V-C) region due to the long growing season and sandy soils. Since the 1970’s it has been standard practice to fertilize peanuts with 0.6 kg ha^{-1} (Perry, 1971) boron (B) at planting or with the first or second herbicide application (Hartzog and Adams, 1973) when soil B is less than 0.2 mg kg^{-1} (Hill and Morrill, 1974). Boron is the most widely deficient micronutrient in the US (Gupta, 1993), is soil mobile, especially in sandy soils with low pH and organic matter, and is easily leached (Goldberg, 1997). But B is not highly phloem mobile in peanut plants (Raven, 1980), making them susceptible to B deficiencies in the developing seed. If there is not sufficient B in developing seed, the cotyledons can become deformed and rot, causing “hollow heart” (Harris and Gilman, 1957), a condition that contributes to the damaged kernel (DK) percent of a peanut harvest. If the DK exceeds 2.5%, the producer can be docked up to 65% of the price at market. If peanut seed has less than 13 mg B kg^{-1}, it can develop hollow heart, but if it has more, it will not (Netsangtip et al., 1987). Despite the importance of B in peanut production, most research was done in the 1960’s through the 1980’s. Additionally, very little research has been conducted investigating the effects of excess or suboptimal peanut seed B content when the seed has not developed hollow heart. Seed health can impact many stages of growth from germination, soil emergence, and seedling vigor, to mature plant productivity. The first step, however, is to investigate the effect changes in seed B content potentially has on germination. In 1975, Hallock investigated the effect on peanut seed germination of fertilization with several micronutrients, including B. Calcium was found to have an effect on germination, but B was not. Previously, Gopal and Rao (1969) determined that peanut seed with excess levels of B germinated more quickly, but the advantage was lost by the end of the twenty one day trial when these seedlings were chlorotic and developmentally behind. A study on soybean (*Glycine max* L.) seeds, another legume, with deficient B content failed to germinate or produced weak seedlings and mature plants (Rerkasem et al., 1997). Thus suggesting that even when hollow heart has not developed, there may still be difference in peanut seed germination when they contain excess or insufficient B.

MATERIALS AND METHODS

Experimental

In 2015 a germination experiment was conducted on seed from a preliminary field study investigating boron (B) fertilization recommendations for peanut production. In 2016, a germination study was again conducted using seed produced in the B application rate and time, and the response of peanut cultivars to B fertilization field studies (See Chapter II) from the 2015 growing season. This study was to determine if B fertilization, or variations in B fertilization in current Virginia-Carolina (V-C) region peanut production significantly impact the germinating ability of the seed produced.

Samples of each treatment in the effect of B application on different peanut cultivars test at the TAREC location were used. Harvested and graded seed from the 2014 preliminary field study were sampled from each treatment, and replicated in germination. Harvested and graded seed from each treatment in all three field replications from the 2015 field study were combined for a single sample of each treatment in the germination study. Seeds were germinated each year on a germinating table with temperature controlled by circulating baths (TC-502, 2009, Brookfield-temperature controller, and TC-502, 2007, Brookfield programmable controller). The steel table had 96.5cm long troughs in which to germinate seed, each trough being 9.5cm wide, and 5.5cm deep. The table had ten troughs. Each trough was divided in half to give twenty sections in which to germinate peanut seed samples at one time. Clean playground sand was used as a substrate (AOSA, 1988). Twenty six seeds were tested per sample and placed at a depth of 3cm below the surface. After seeds were placed, the substrate in each section was wetted with 17.5 ml of 0.0029% ethophone [(2-chloroethyl) phosphonic acid] in deionized (DI) water to ensure seed dormancy was broken (AOSA, 1988). Each section was then covered with more substrate to the top of the trough, and again moistened with 17.5ml DI water only. Treatments were completely randomized on the germination table. Table was covered with a solid sheet of 1.3cm styrofoam insulation before closing tightly sealing lids. Circulating baths were set to maintain a constant temperature of 25°C (AOSA, 1988). Temperature was confirmed every day by taking ten random readings of the substrate surface using a Digital Infrared Thermometer (Tool House, 1mw max output at 630-670nm, class II laser), and temperature of circulating baths was adjusted if necessary to maintain an even, constant temperature of 25°C. Number of germinated seed were counted in each section every day for ten days (AOSA, 1988). All treatments were replicated three times. Final germination percent was compared to determine significance in treatment. Mean time to germination (MTG) was also calculated according to the following formula, and compared between treatments.

$$MTG=\Sigma(N_iT_i)/\Sigma(N_i)$$

Where N_i is the number seeds that germinated that day, and T_i is the number of days since the beginning of the experiment.

Samples from the 2015 B fertilization rates and timing field experiment were germinated. Two sets of harvested seeds that had been unfertilized with B were taken from each of the four field locations. One set of seed that had been fertilized with 0.6 kg B ha^{-1} at full seed (R6) growth stage, and one sample of seed that had been fertilized with 1.1 kg B ha^{-1} at R6 were taken from each of the four field locations. Field treatment replications were combined to give one treatment sample each for each field. This gave a total of 16 samples germinated, replicated three times. Samples were arranged in the germination table the same as the previous set of samples: 26 seeds per section, 20 sections in the table, grown in a sand substrate.

RESULTS

For the germination of the 2014 preliminary field experiment of B fertilization rates and times, there were no significant (p=0.09, 0.41) difference in germination percent or MTG based on the amount of B applied to the peanut plants that produced the seed. There was also no significant (p=0.13, 0.32) difference in germination percent or MTG based on the growth stage at which the B fertilization was applied (Table 12).

In the 2015 field study on different rates and application times of B, there were no significant (p=0.071) differences in harvested seed B content. The lowest B amounts found in any seed sample were above the 13mg kg^{-1} threshold predicted for developing hollow heart. When seed from plants that had received 1.1 kg B ha^{-1}, 0.6 kg B ha^{-1}, and no B were compared, there were no significant (p=0.308) differences in final germination at ten days (Figure 16). There was also no difference (p=0.467) in overall germination based on the location where the seed was grown (Figure 16). When MTG was compared based on rate of B applied, growth stage at which it was applied, and the B content of the seed, there were no significant (p=0.57, 0.42, 0.81) difference (Table 13).

Germination of seed grown in the cultivars field study had similar results. There were still no differences (p=0.890) in germination between seed from plants that received 0.6 kg B ha^{-1}, and those from plants that received no B (Figure 17). There was also no difference (p=0.155) in germination between seed from runner type, or Virginia type plants (Figure 17). Unlike the others, there were significant (p=0.010) differences in B content of the seed from older cultivars over newer cultivars (Figure 9). This differences did no carry over into germination differences (p=0.834) between seed from newer or older cultivars (Figure 17). The MTG was compared between market type, age of cultivar release, and B content of the seed with no significant (p=0.37, 0.36, 0.59) differences (Table 13).

DISCUSION

Germination is just one step in determining the viability and health of a seed, but an important step in the case of B fertilization on peanuts. Peanut seed with B content below 13 mg kg^{-1}can develop hollow heart (Netsangtip et al., 1987), a condition where the cotyledons in the seed embryo rot and prevent germination. This acute threshold has been established for B deficiency in peanut seed, but it is unclear if there are detrimental effects of current B fertilization practices on seed germination. It was unlikely to find widespread reduction in germination due to hallow heart in this experiment as the seed B content of any treatments was never below the 13 mg B kg^{-1} threshold described by Netsangtip et al. (1987).

The first field experiment applied different rates of B fertilizations, and at different application times. Only seed from the highest application rate, 1.1 kg B ha^{-1}, lowest rate, 0 kg B ha^{-1}, and one of the middle rates of 0.6 kg B ha^{-1} were compared in the germination study. From this selection it could be determined if current B fertilization practices on 'Bailey' matter in the germination of seed produced. There were no significant differences in germination based on the amount of B received by the plant. Meaning that weather B was applied according to current practices, or not applied at all, germinability of seed produced is not effected for 'Bailey'. Because there were no differences in germination between locations, it is unlikely that environmental, management, or soil type play a major role in the germinability of peanut seed produced.

The second field experiment applied the same rate of B across different types, and ages of peanut cultivars. Especially since there were significant differences in seed B content between newer and older cultivars, it would be reasonable to suspect that the function of seeds would differ by the age of cultivar release. This was not the case, as there were no differences in germination based on age of cultivar release. This suggests that, among the cultivars investigated, even if a peanut plant is more efficient at moving B to the seed, it does not make a noticeable difference in the viability of the resulting seed. Sample size may have been a factor in the overall lack of differences seen. Samples were only 26 seeds each, and replicated three times. Because of the small sample size, if 24 seeds germinated, that was 94%, and if 25 germinated, 96%, and if 26 germinated, 100%. So the resulting germination percentages could not be very sensitive to detect slight variation in response of seed germination based on B fertilization or cultivar.

CONCLUSION

There was not great variation in seed B content initially, and none of the seed was below the 13mg B kg^{-1} threshold for developing hollow heart in this experiment. Germination is the first step in determining if small variations in peanut seed B content can impact the plants that grow from those seeds. Larger sample size and more replications would give a more precise indication of effect on germination that minor differences in seed production and B content have. From the lack of significant differences between B fertilization in seed production, or in the cultivar being fertilized, it can be concluded that under the current B application practices, it is not likely that B fertilization is necessary to ensure seed that can achieve optimal germination. This study does not investigate further developmental impacts on seedling and mature plants that minor differences in peanut seed B content could have.

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TABLES AND FIGURES

Table 1. Summary of the 2015 experiment addressing boron (B) rate and application timing: locations (Tidewater Agricultural Research and Extension Center, TAREC, Suffolk, VA; Lewiston, NC; Rocky Mount, NC), number of replications, and planting, digging, and harvesting dates, pre-plant soil B levels, and previous crop planted in experimental fields. All fields were planted in peanut cultivar 'Bailey'.

Location	**Replications**	**Plant Date**	**Digging Date**	**Harvest Date**	**Soil B 15cm (mg kg^{-1})**	**Previous Crop (2014)**
TAREC Field 23	3	5-5-15	10-7-15	10-16-15	0.2	Cotton
TAREC Field 63A	3	5-5-15	10-10-15	10-15-15	0.1	Corn
Lewiston, NC	4	5-7-15	10-13-15	11-25-15	0.1	Corn
Rocky Mount, NC	3	5-14-15	10-9-15	10-28-15	0.3	Cotton

Table 2. Summary of the 2015 experiment addressing boron (B) rate and application timing: B rate and application times in terms of growth stage (beginning peg, R2; full seed, R6), at all locations in VA and NC. All fields were planted using the cultivar 'Bailey'.

Rate (kg Bha^{-1})	**Growth Stage**
0	Planting
0.3	Planting
0.6	Planting
1	Planting
0	R2
0.3	R2
0.6	R2
1	R2
0	R6
0.3	R6
0.6	R6
1	R6
0	Planting+R2
0.3	Planting+R2
0.6	Planting+R2
1	Planting+R2
0	Planting+R6
0.3	Planting+R6
0.6	Planting+R6
1	Planting+R6

Table 3. Weather data by location (Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Lewiston, NC; Rocky Mount, NC) including average (AVG), minimum (MIN), and maximum (MAX) air temperature, and AVG soil temperature, and precipitation from May through October 2015.

Month	**AVG Air Temp**	**Max Air Temp**	**Min Air Temp**	**AVG Soil Temp**	**Rain**
		°C			cm
		TAREC, VA			
May	21	28	14	23	1.5
June	26	32	21	27	19.1
July	26	32	21	28	11.7
August	24	32	19	28	6.6
September	23	29	18	24	13.5
October	16	22	11	18	9.1
Mean/Sum	23	29	17	24	61.5
		Lewiston, NC			
May	21	28	15	25	2.7
June	26	32	21	30	7.4
July	26	32	21	30	11.2
August	25	31	20	30	11.4
September	23	28	19	26	18.8
October	16	22	11	19	12.7
Mean/Sum	23	29	18	27	64.2
		Rocky Mount, NC			
May	22	28	16	22	6.0
June	26	32	21	28	14.4
July	27	32	22	30	5.4
August	25	31	19	28	8.2
September	23	28	18	25	24.9
October	16	22	11	18	13.7
Mean/Sum	23	29	18	25	72.6

Table 4. The correlation coefficients (r) between leaf boron (B) content and Normalized Difference Vegetation Index (NDVI) and relative SPAD-chlorophyll reading relative to three B application times at Tidewater Agricultural Research and Extension Center, Suffolk, VA, in 2015. Cultivar planted was ‘Bailey’.

	Leaf B content		
(n=60)	25 DAP†	R2*	R6**
NDVI (25 DAP)	-0.148	0.151	0.163
NDVI (R2)	0.178	0.048	-0.028
NDVI (R6)	0.111	0.023	-0.049
SPAD (25 DAP)	-0.174	0.161	0.129
SPAD (R2)	-0.037	0.015	0.163
SPAD (R6)	-0.022	0.137	-0.055

† Days after planting

*Beginning pegging growth stage

**Full seed growth stage

Table 5. The correlation coefficients (r) between yield and Normalized Difference Vegetative index (NDVI), and SPAD-chlorophyll readings relative to field location in 2015. Experiment was planted using cultivar ‘Bailey’.

	Yield		
n=60	TAREC*	Lewiston**	Rocky Mount**
NDVI (25DAP)	0.0079	0.2487	0.2863
NDVI (R2)	0.5599	0.461	0.2823
NDVI (R6)	0.6251	0.6737	0.3602
SPAD (25DAP)	-0.0092	0.2457	0.079
SPAD (R2)	-0.0602	0.5117	-0.1104
SPAD (R6)	0.2254	0.6005	-0.197

*Tidewater Agricultural Research and Extension Center, Suffolk, VA

**Peanut Belt Research Station, Lewiston, NC

***Upper Coastal Plain Research Center, Rocky Mount, NC

Table 6. Summary of cultivars, market type and release year used in 2015 experiment addressing the response of different production peanut cultivars' response to B fertilization at Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Sussex county, VA, and Southampton county, VA.

Cultivar	Market Type	Release Year
FL07	Runner	2006
Florunner	Runner	1969
GA06G	Runner	2006
GA13M	Runner	2013
TUFRun511	Runner	2013
Bailey	Virginia	2008
Gregory	Virginia	1999
NC-V11	Virginia	1989
Sullivan	Virginia	2013
VA98R	Virginia	1998
Wynne	Virginia	2013
07030-1-10-1	Virginia	N/A

Table 7. Summary of the experiment addressing cultivar response to boron (B) fertilization: locations (Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Lewiston, NC; Rocky Mount, NC), number of replications, and planting, digging, harvesting dates, pre-plant soil B test, and previous crop planted in experimental field.

Location	Replications	Plant Date	Digging Date	Harvest Date	Soil B 15cm (mg kg^{-1})	Previous Crop (2014)
TAREC Field 27	3	5-13-15	10-7-15	10-26-15	0.2	Sorghum
Sussex, VA	3	5-8-15	10-7-15	10-23-15	0.1	Soybean
Southampton, VA	3	5-18-15	10-8-15	11-23-15	0.2	Cotton

Table 8. Weather data by location (Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Sussex count, VA; Southampton county, VA) including average (AVG), minimum (MIN), and maximum (MAX) air temperature, and AVG soil temperature, and precipitation from May through October 2015.

Month	**AVG Air Temp**	**Max Air Temp**	**Min Air Temp**	**AVG Soil Temp**	**Rain**
			°C		cm
		TAREC, VA			
May	21	28	14	23	1.5
June	26	32	21	27	19.1
July	26	32	21	28	11.7
August	24	32	19	28	6.6
September	23	29	18	24	13.5
October	16	22	11	18	9.1
Mean/Sum	23	29	17	24	61.5
		Sussex, VA			
May	22	28	15	23	12.5
June	25	31	20	27	30.3
July	25	31	21	27	18.1
August	24	31	18	27	5.5
September	22	29	17	24	14.6
October	15	22	9	18	9.6
Mean/Sum	22	29	17	24	90.7
		Southampton, VA			
May	21	28	15	22	4.5
June	25	31	21	26	2.2
July	26	31	21	28	7.3
August	24	31	18	28	3.2
September	22	29	18	24	10.7
October	15	22	10	17	8.6
Mean/Sum	22	29	17	24	36.6

Table 9. Comparison of yield response in 2015 between boron (B) fertilization and controls for runner and Virginia market types with newer and older release dates from all locations (Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Sussex county, VA; Southampton county, VA).

	Virginia Type				**Runner Type**			
	Newer Cultivars		Older Cultivars		Newer Cultivars		Older Cultivars	
Location	**No B Control**	**0.6 kg ha^{-1}**	**No B Control**	**0.6 kg ha^{-1}**	**No B Control**	**0.6 kg ha^{-1}**	**No B Control**	**0.6 kg ha^{-1}**
Southampton, VA	5480	5198	5489	5541	5011	4694	3909	4322
	ns		ns		ns (p=0.07)		ns	
Sussex, VA	5956	6208	6479	6609	6504	6295	5679	5848
	ns		ns		ns		ns	
TAREC 27, VA	6783	6572	5457	5384	6723	6822	6976	5939
	ns		ns		ns		ns	
All Locations	5880	5812	5489	5541	5880	5812	6079	5937
	ns		ns		ns		ns	

All yields and B application rates are reported in kg ha^{-1}.
Means separations were determined using ANOVA, and Fisher's LSD, 0.05.

Table 10. Comparison of yield response between boron (B) fertilization and controls for runner and Virginia market type cultivars by location (Tidewater Agricultural Research and Extension Center – TAREC, Suffolk, VA; Sussex county, VA; Southampton county, VA) in 2015.

	Virginia Type		**Runner Type**	
Location	**No B Control**	**0.6 kg ha^{-1} B**	**No B Control**	**0.6 kg ha^{-1} B**
Southampton, VA	5092	4926	4790	4619
	ns		ns	
Sussex, VA	6180	6380	6339	6206
	ns		ns	
TAREC 27, VA	6215	6063	6774	6646
	ns		ns	
All Locations	5818	5804	5968	5824
	ns		ns	

All yields and B application rates are reported in kg ha^{-1}.
Means separations were determined using ANOVA and Fisher's LSD, 0.05

Table 11. 2014 Field experiment at the Tidewater Research and Extension Center in Suffolk, VA, using peanut cultivar 'Bailey'. Leaf tissue boron (B) content at growth stages beginning peg (R2), and full seed (R6), harvested seed B content, yield, and damaged kernel (DK) percent based on growth stage at time of B application, and by total rate of B applied.

n=27	First Leaf Sample (R2) (mg kg^{-1})	Second Leaf Sample (R6) (mg kg^{-1})	Seed B (mg kg^{-1})	Yield (kg ha^{-1})	Damaged Kernels (%)
Growth Stage at Application					
Control	34.7b*	42.0ab*	11.7	3614	0.00
Planting and R2	60.4a	43.0b	13.6	3984	0.24
Planting and R6	42.7b	51.3ab	13.2	4885	0.05
R2	70.0a	35.5b	14.7	3220	0.07
R6	42.7b	62.0a	14.1	3951	0.14
LSD (0.05)	<0.001	0.038	ns	ns	ns
Rate of B applied					
Control	34.7	42.0	11.7	3614	0.00
0.3 kg ha^{-1}	52.0	44.2	13.8	3988	0.10
0.6 kg ha^{-1}	54.4	53.3	14.0	4029	0.14
LSD (0.05)	ns	ns	ns	ns	ns

*Column letters show significance.
Means separations were determined using ANOVA and Fisher's LSD, 0.05

TABLE 12. At the Tidewater Research and Extension Center in Suffolk, VA, a field study in the peanut cultivar 'Bailey' was planted 2014. The resulting seed used in a germination study in 2015, comparing mean time to germination (MTG), and percent germination based on the growth stage at which plants producing the seed had received B fertilization (planting, beginning peg R2, full seed R6)

n=27	MTG (Days)	Germination (%)
Growth Stage at Application		
Control	7.7	76.3
Planting and R2	7.5	89.8
Planting and R6	7.0	82.0
R2	7.5	84.0
R6	7.2	85.3
LSD (0.05)	ns	ns
Rate of B applied		
Control	7.7	76.3
0.3 kg ha^{-1}	7.3	85.3
0.6 kg ha^{-1}	7.3	85.3
LSD (0.05)	ns	ns

Means separations were determined using ANOVA and Fisher's LSD, 0.05

TABLE 13. In 2016 seed was germinated from two 2015 field experiments. The first experiment compared plant growth stages at which boron (B) fertilization was applied (at planting only or full seed, R6), and the total rate of B applied, planted in peanut cultivar 'Bailey' at locations in Suffolk, VA, and Lewiston and Rocky Mount, NC. The second experiment compared Virginia and runner market types, as well as cultivars that have been recently released (newer), and cultivars that are obsolete (older), at locations in Sussex, Southampton, and Suffolk counties in VA.

n=27	MTG (Days)
Growth Stage at Application	
Planting	6.5
R6	6.4
LSD (0.05)	ns
Rate of B applied	
Control	6.4
0.6 kg ha^{-1}	6.6
1.1 kg ha^{-1}	6.4
LSD (0.05)	ns
Market Type	
Runner	6.8
Virginia	6.5
LSD (0.05)	ns
Cultivar Release Age	
Newer	6.7
Older	6.5
LSD (0.05)	ns

Means separations were determined using ANOVA and Fisher's LSD, 0.05

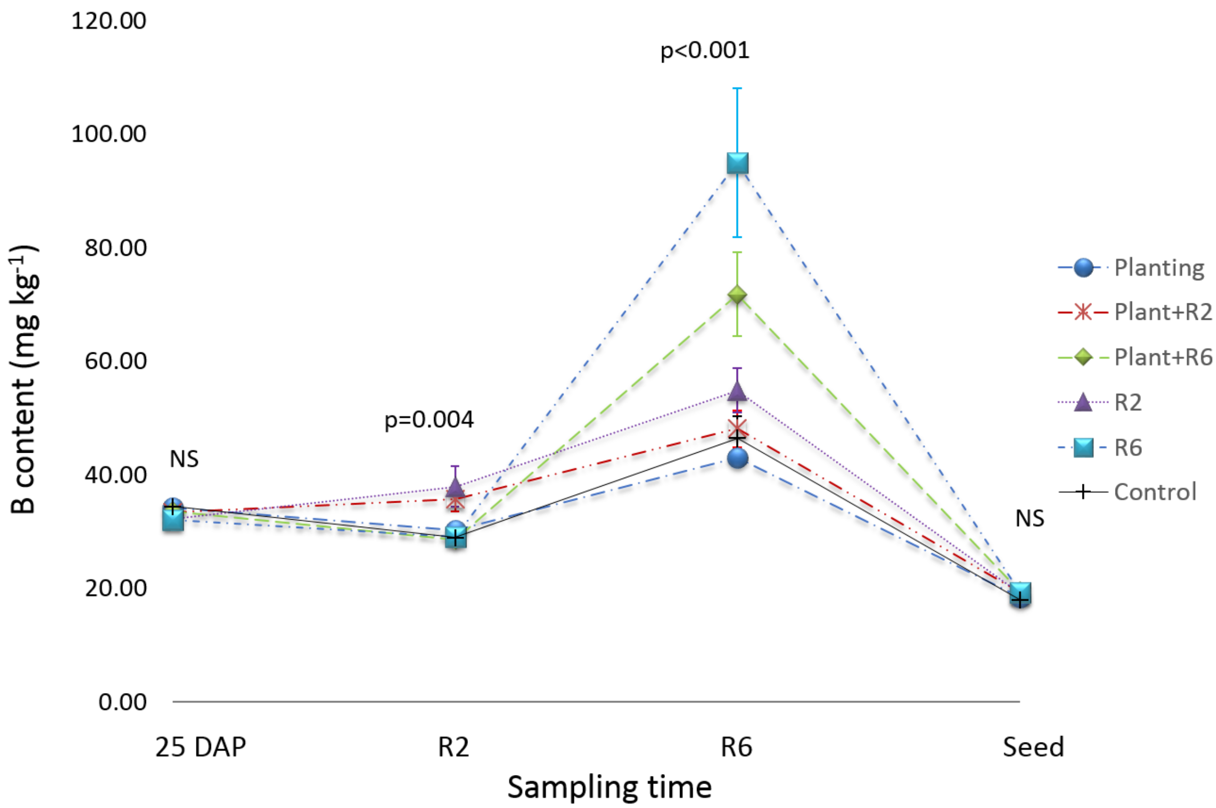

Figure 1. Leaf tissue boron (B) content at three sampling times during the 2015 growing season (25 days after planting, DAP, beginning peg, R2, and full seed, R6) and seed B content after harvest of peanut cultivar 'Bailey'. Data were from two fields at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

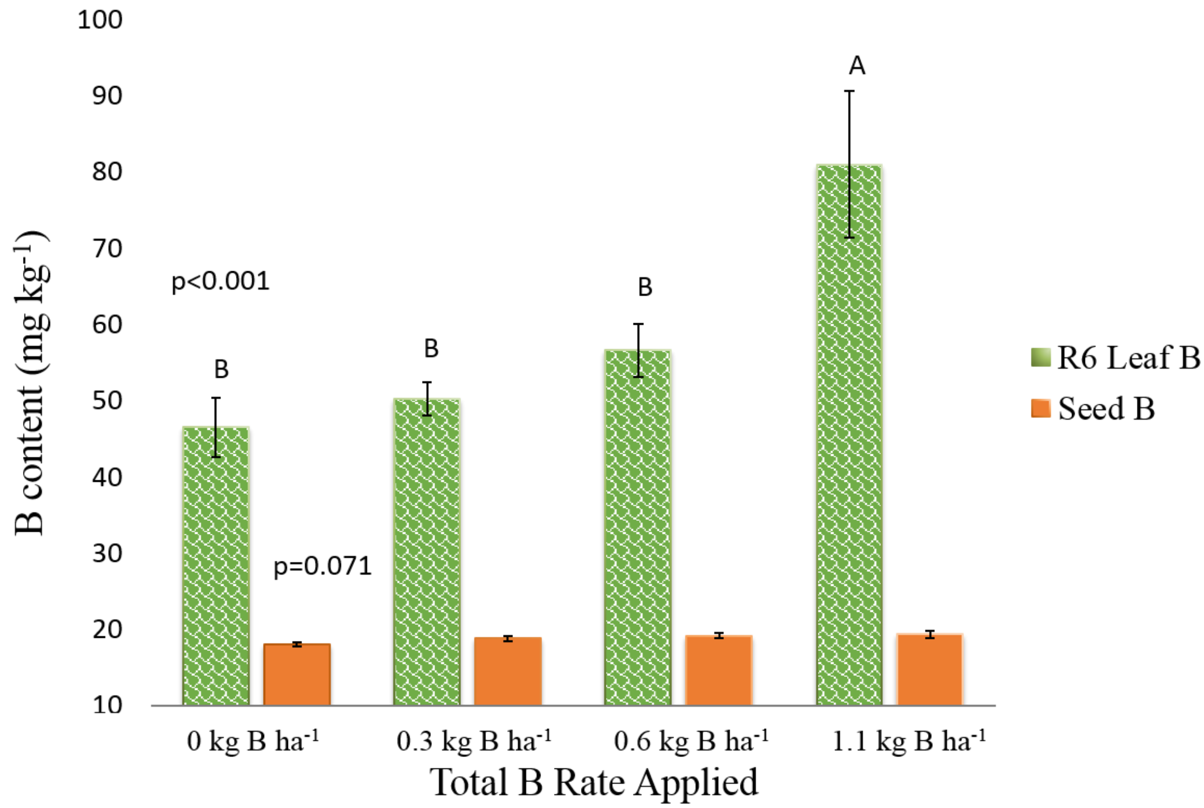

Figure 2. Leaf tissue boron (B) content at full seed (R6) growth stage, and seed B content after harvest, based on total amount of B applied over the 2015 growing season. Data were from two fields at the Tidewater Agricultural Research and Extension Center in Suffolk, VA, planted in cultivar ‘Bailey’. Means separations were determined using ANOVA and Fisher’s LSD, 0.05.

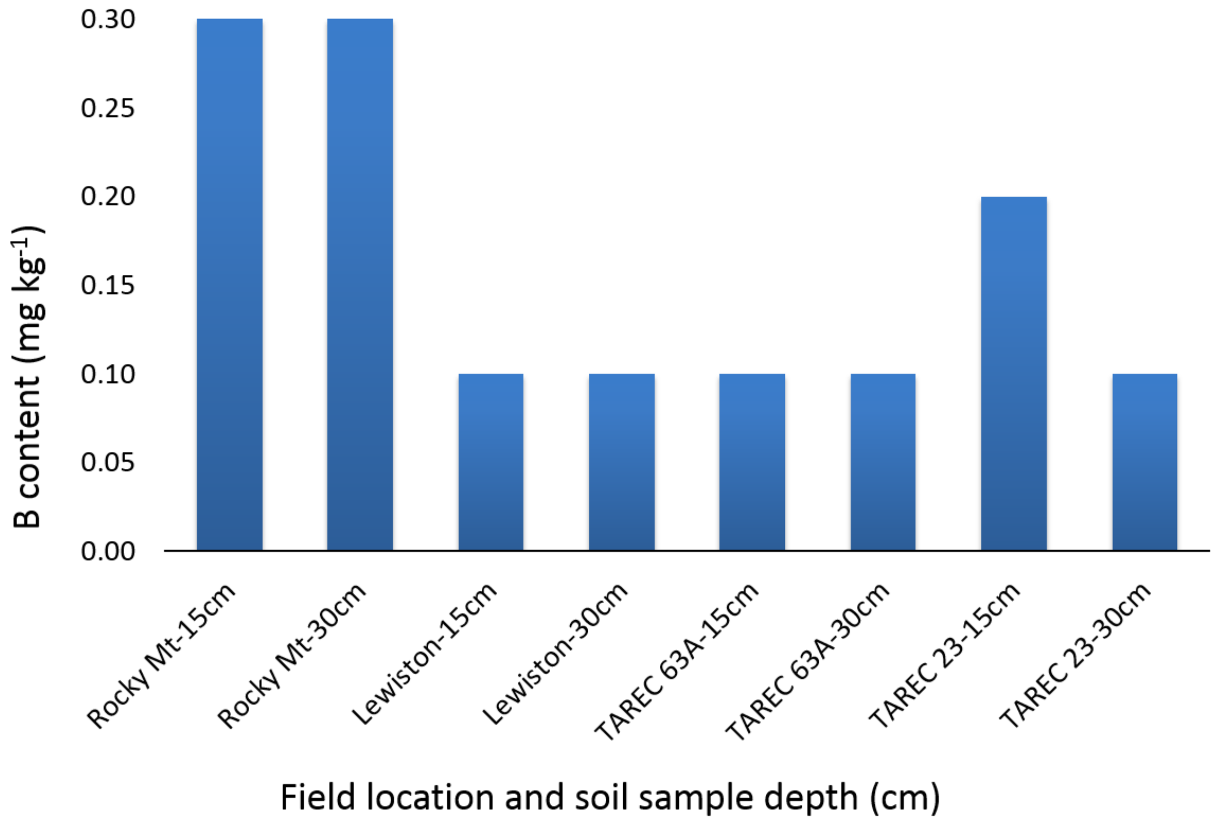

Figure 3. 2015 pre-plant soil B levels at depths of 15 and 30cm in all four experimental fields (Rocky Mount and Lewiston, NC, and Fields 63 A and 23 at the Tidewater Agricultural Research and Extension Center in Suffolk, VA).

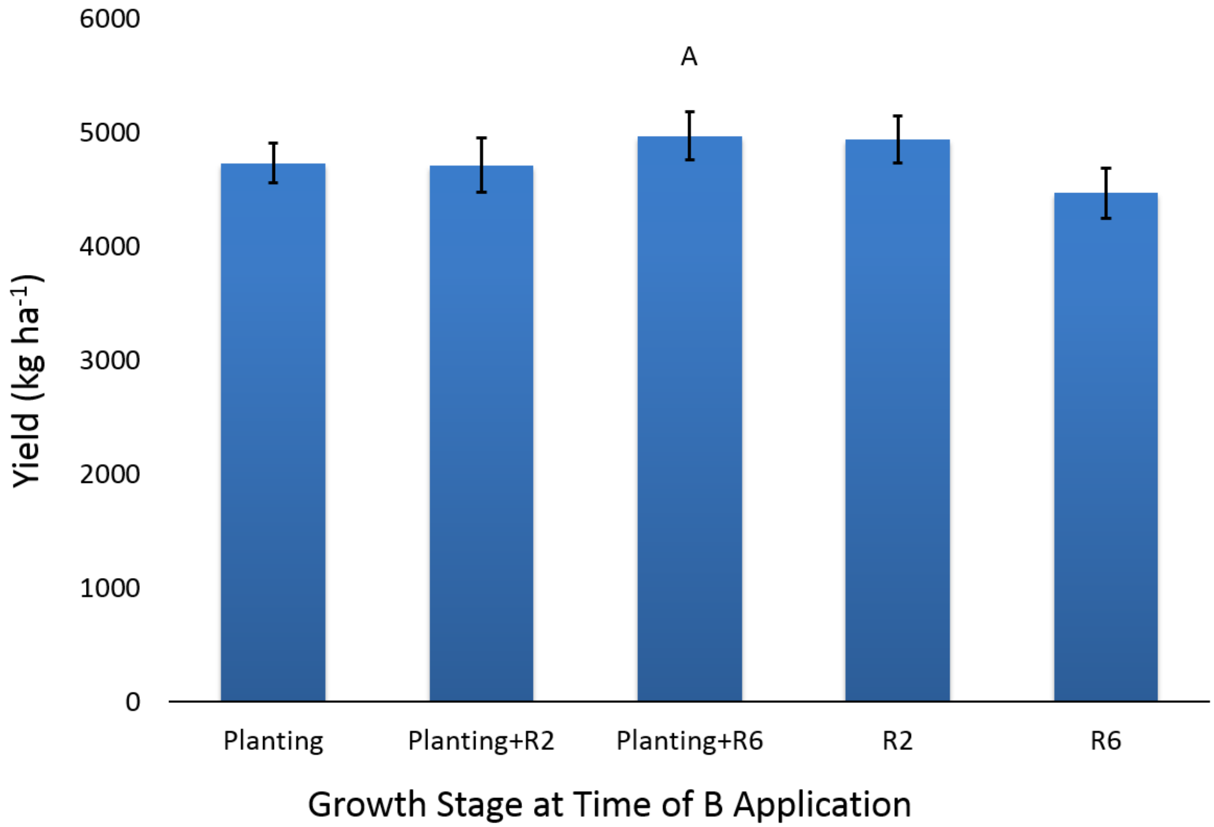

Figure 4.2015 yields based on application time of boron (B) fertilization (at planting, beginning peg, R2; and full seed, R6) from the two fields planted in peanut cultivar ‘Bailey’ at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA.

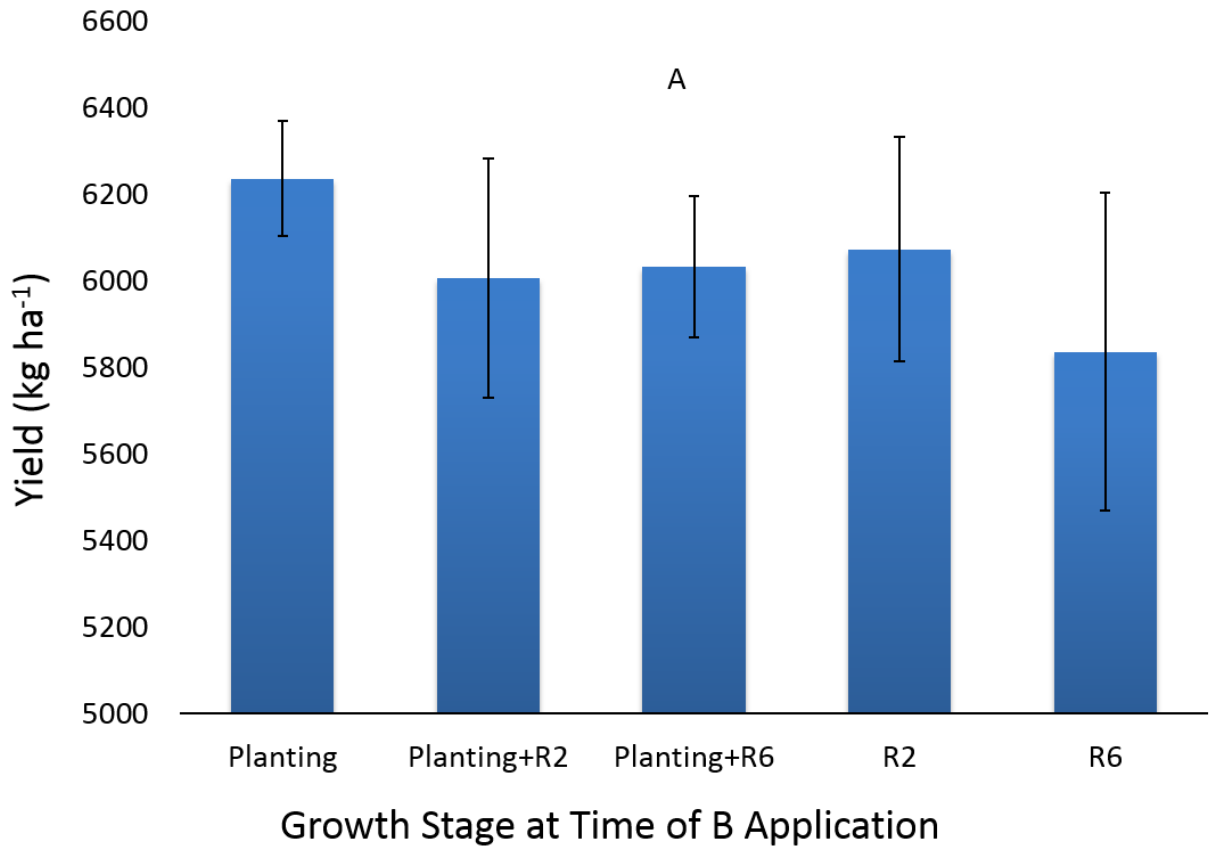

Figure 5. 2015 yields based on application time of boron (B) fertilization (at planting; beginning peg, R2; and full seed, R6) at Lewiston, NC, planted in cultivar 'Bailey'. Means separations were determined using ANOVA.

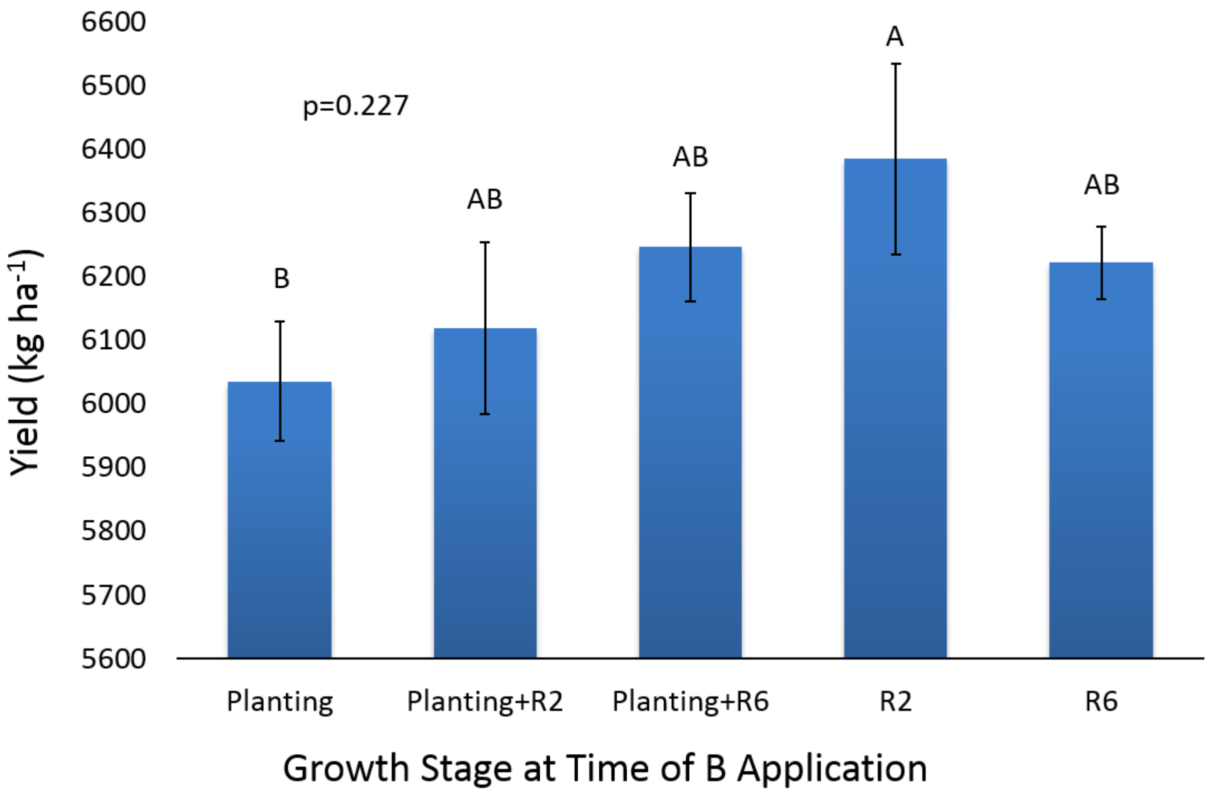

Figure 6. 2015 yields based on application time of B fertilization (at planting; beginning peg, R2; and full seed, R6) at Rocky Mount, NC, peanut cultivar was 'Bailey'. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

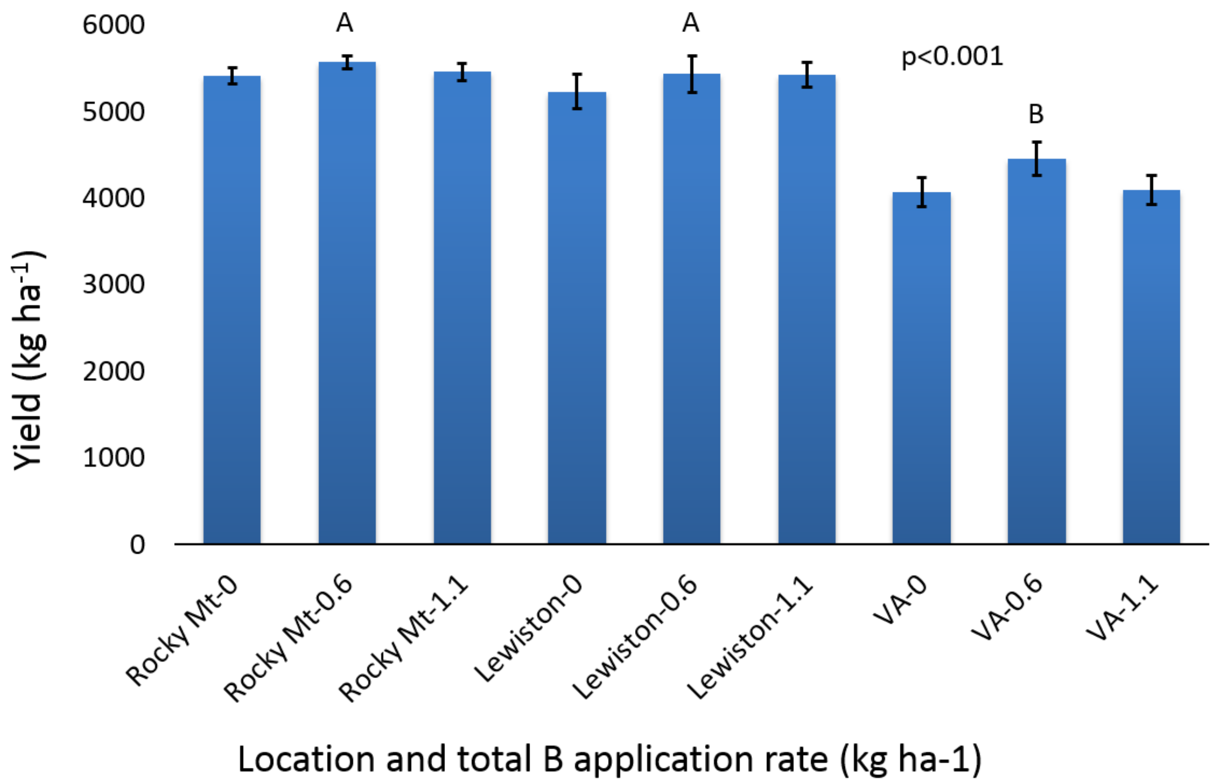

Figure 7. 2015 yields based on total application rate of B fertilization (0, 0.6, and 1.1 kg Bha^{-1}) over the growing season at Rocky Mount and Lewiston, NC, and at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. All fields were planted using peanut cultivar 'Bailey'. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

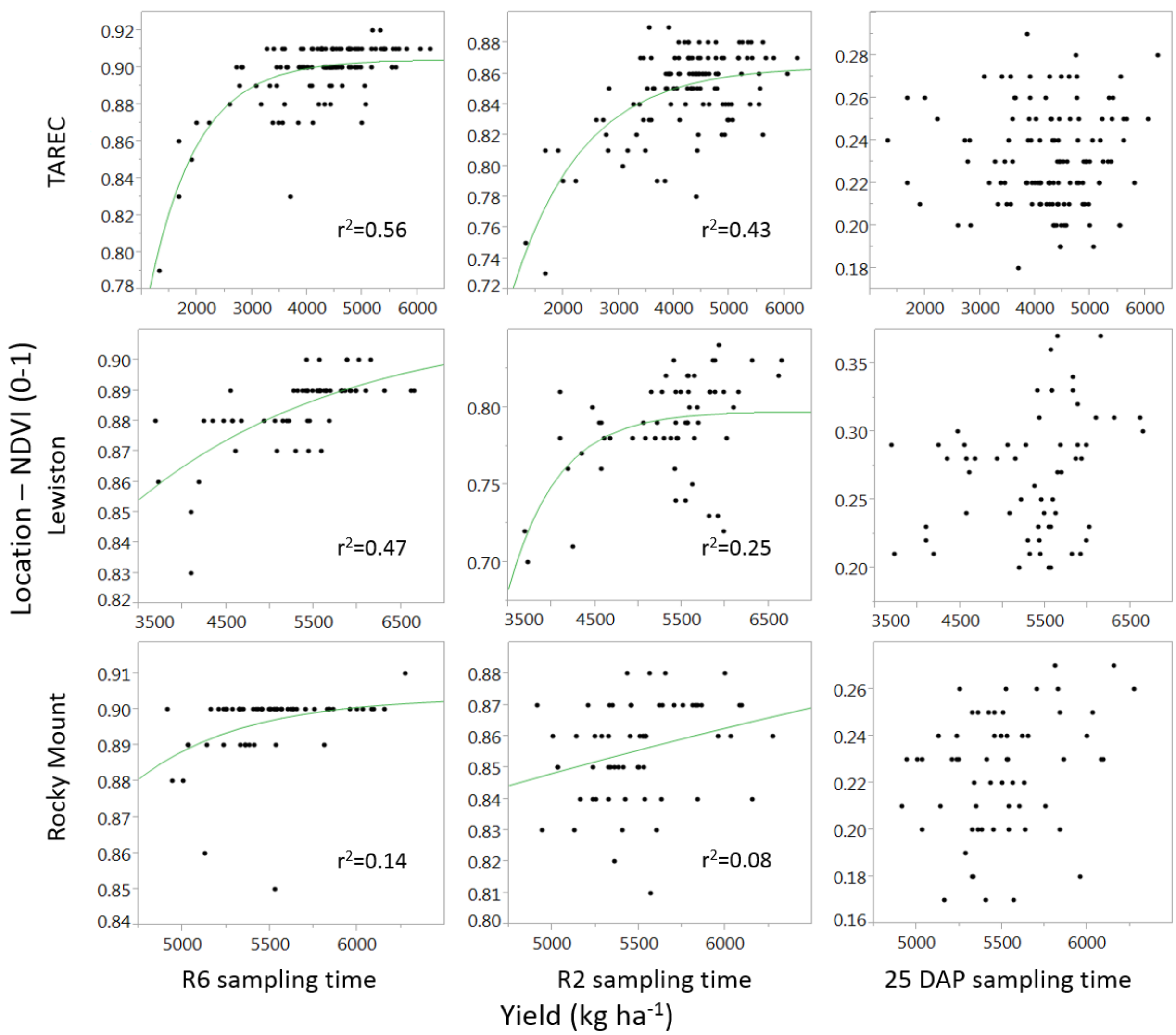

Figure 8. 2015 NDVI to yield non-linear regression of peanut leaves at three sampling times (growth stage R6, full seed, R2, beginning pod, and 25 days after planting, DAP) at Lewiston and Rocky Mount, NC, and at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. All experimental fields were planted using cultivar 'Bailey'.

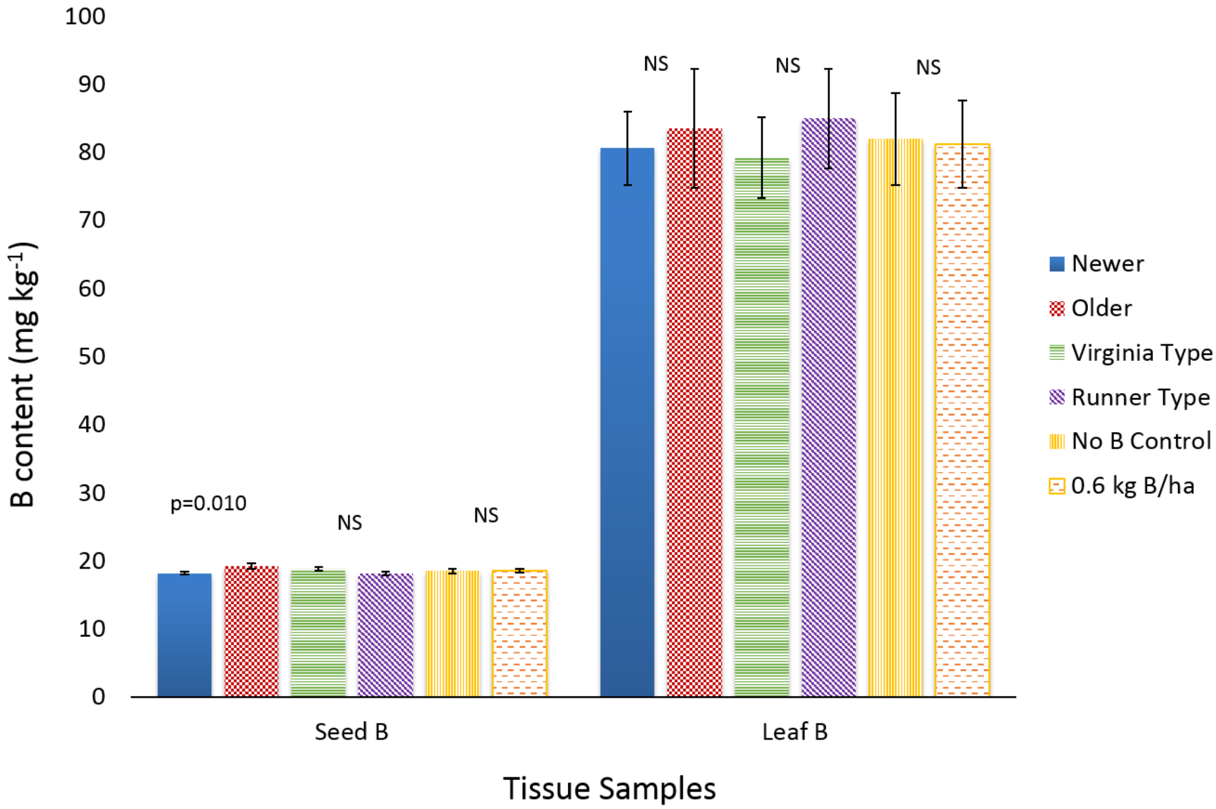

Figure 9. 2015 boron (B) content of leaves at R4 (full pod) growth stage, and of harvested seed for Older and Newer cultivars, Virginia and runner market types, and B fertilized and unfertilized plant at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

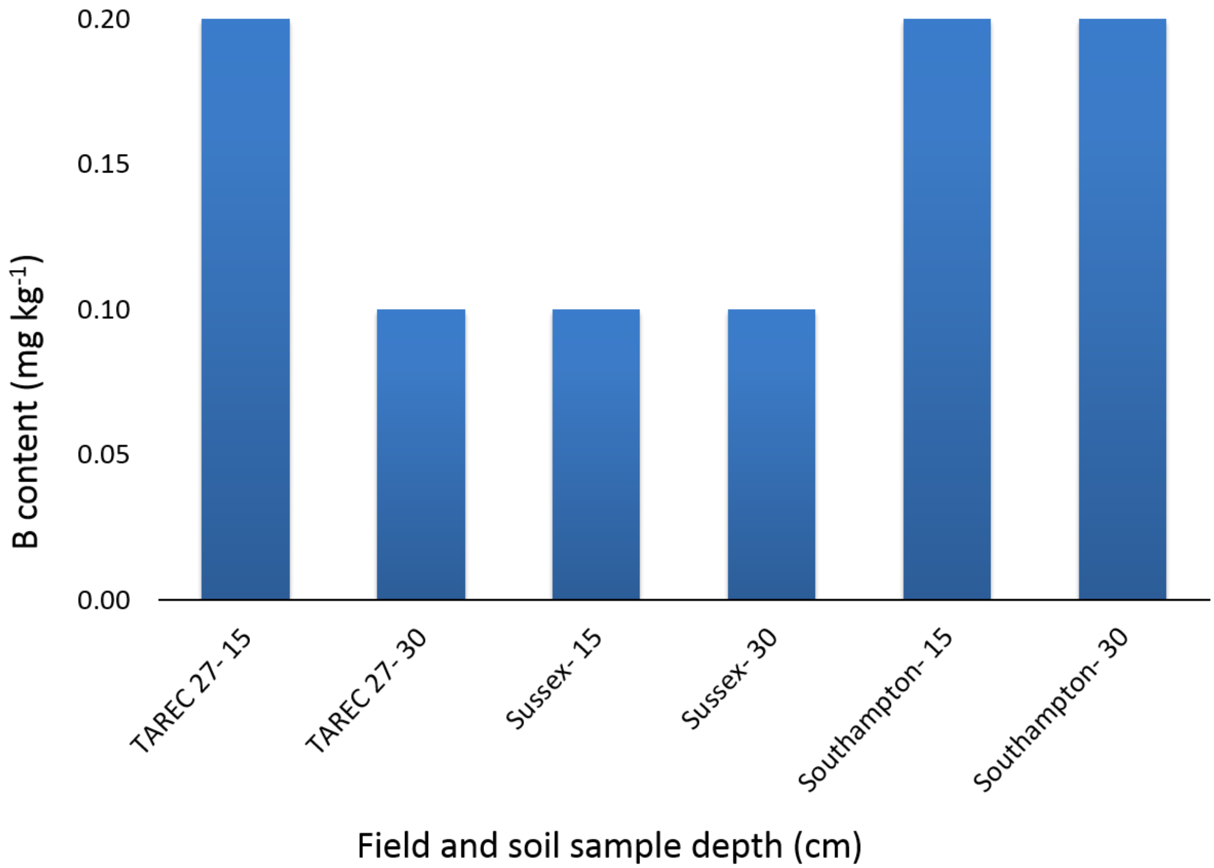

Figure 10.2015 pre-plant soil B levels at depths of 15 and 30cm in all three experimental fields (Sussex, Southampton, and the Tidewater Agricultural Research and Extension Center, VA).

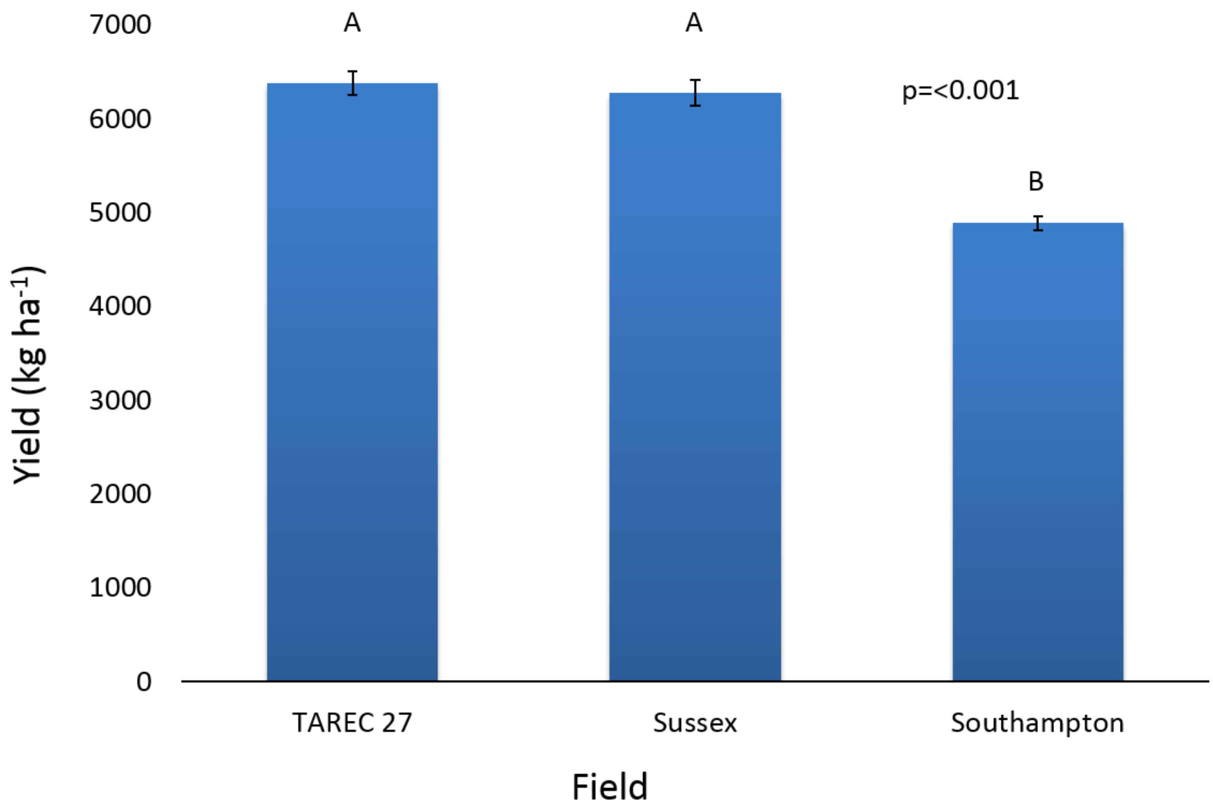

Figure 11. 2015 yield from three experimental field in Sussex, Southampton, and the Tidewater Agricultural Research and Extension Center, VA. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

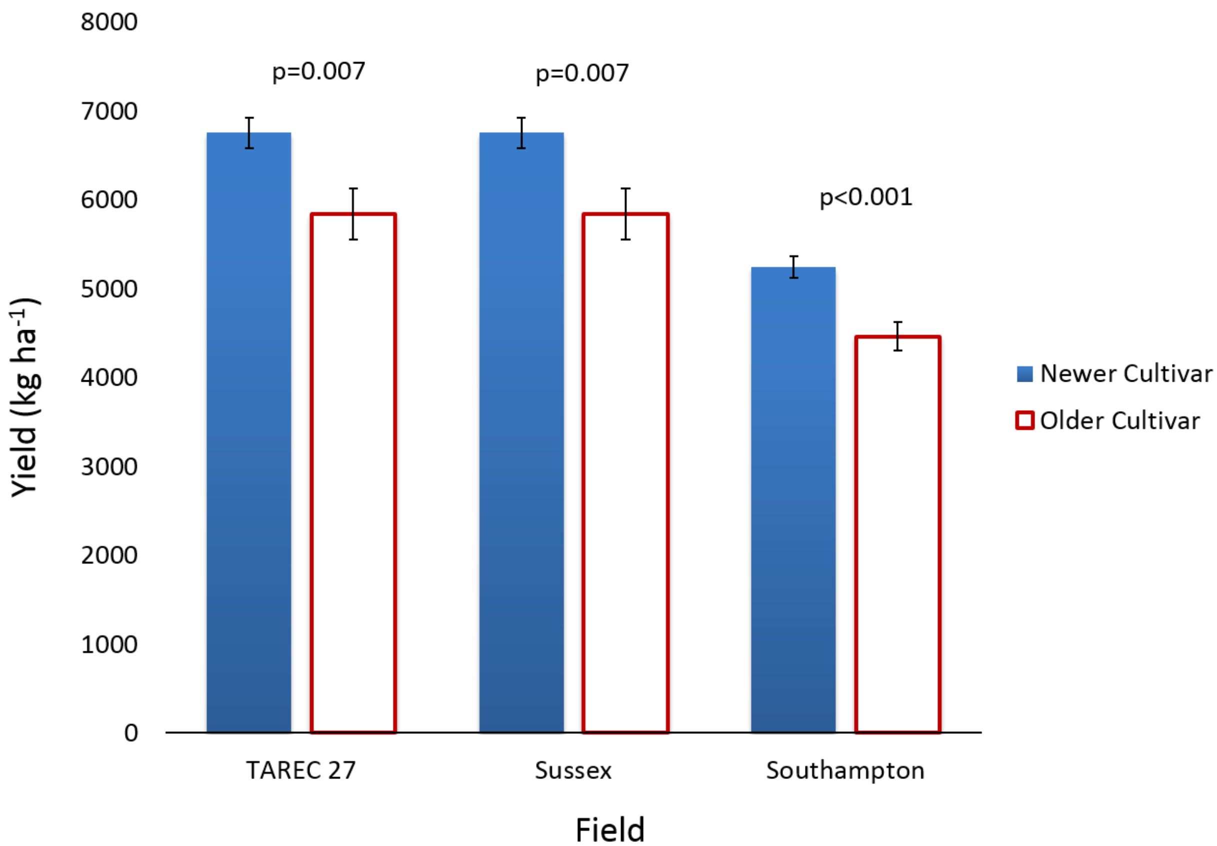

Figure 12. 2015 yield differences between older and newer cultivars when receiving no boron (B) fertilization at experimental fields in Southampton, Sussex, and the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

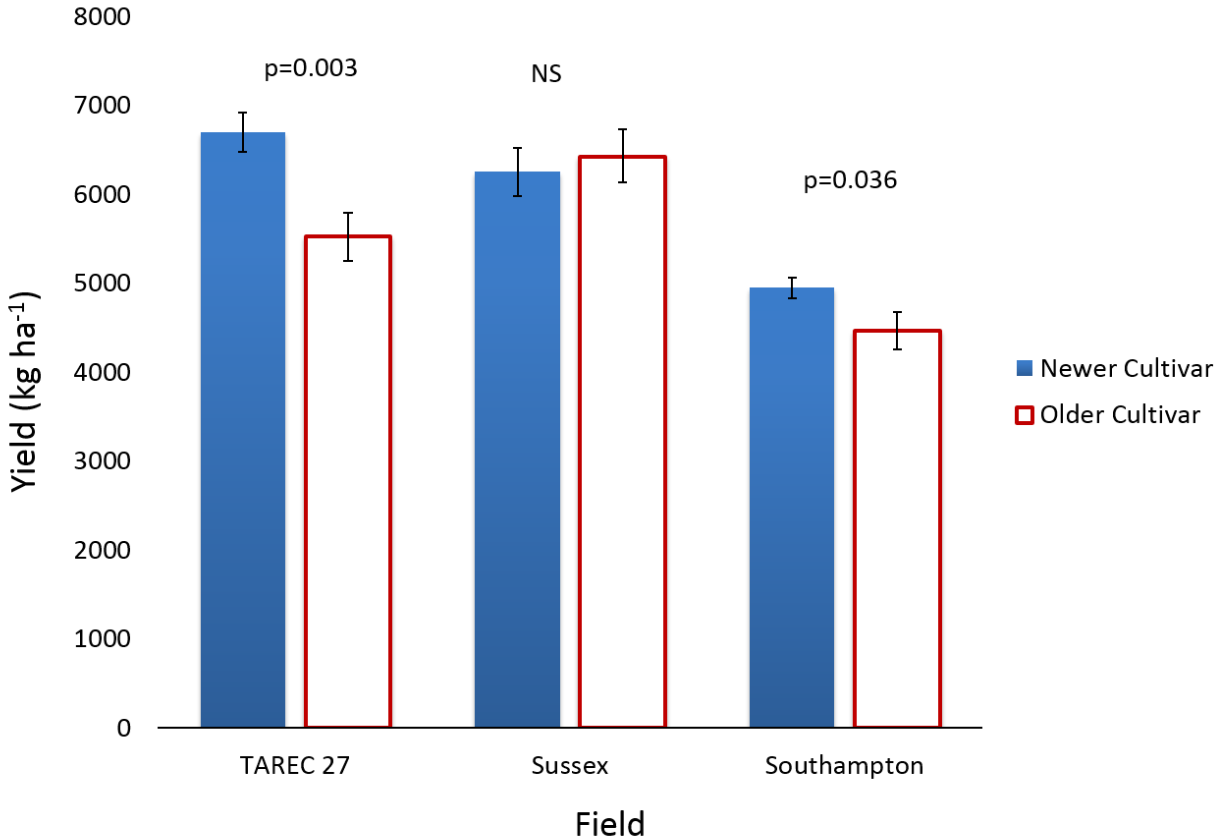

Figure 13. 2015 yield differences between older and newer cultivars when receiving any amount of boron (B) fertilization during the growing season at experimental fields in Southampton, Sussex, and the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

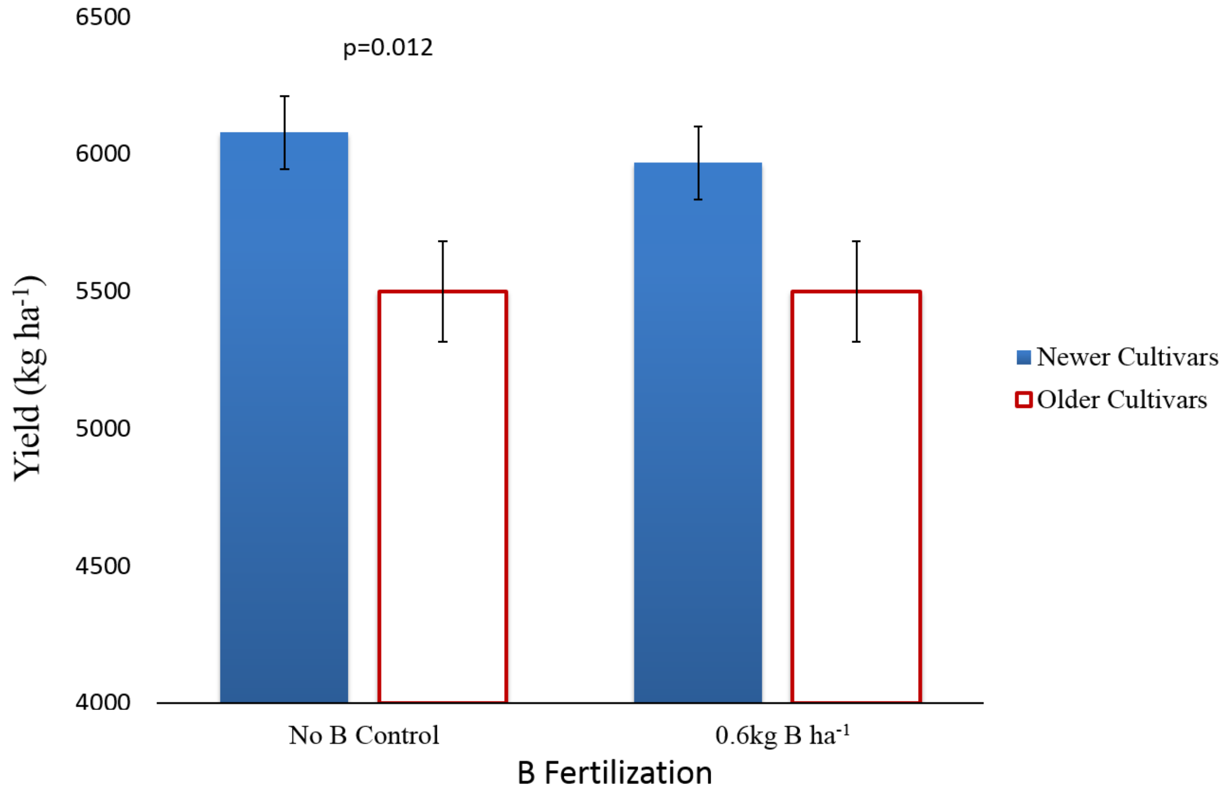

Figure 14. 2015 yield differences between older and newer cultivars when receiving no boron (B) fertilization or 0.6 kg B/ha during the growing season with all fields in Southampton, Sussex, and the Tidewater Agricultural Research and Extension Center in Suffolk, VA, combined. Means separations were determined using ANOVA and Fisher's LSD, 0.05.

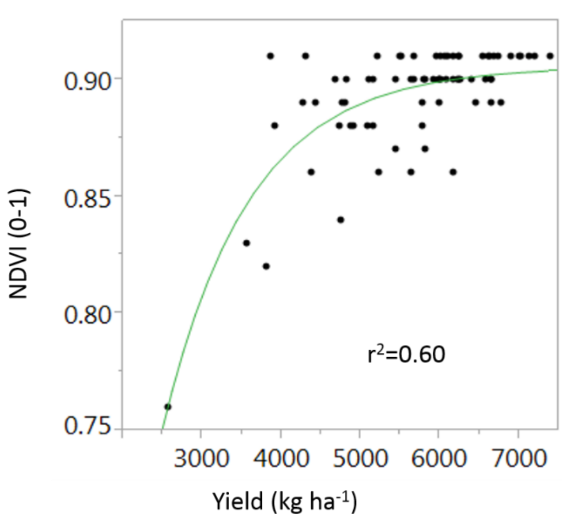

Figure 15. 2015 NDVI to yield non-linear regression of multiple peanut cultivars grown at the Tidewater Agricultural Research and Extension Center in Suffolk, VA.

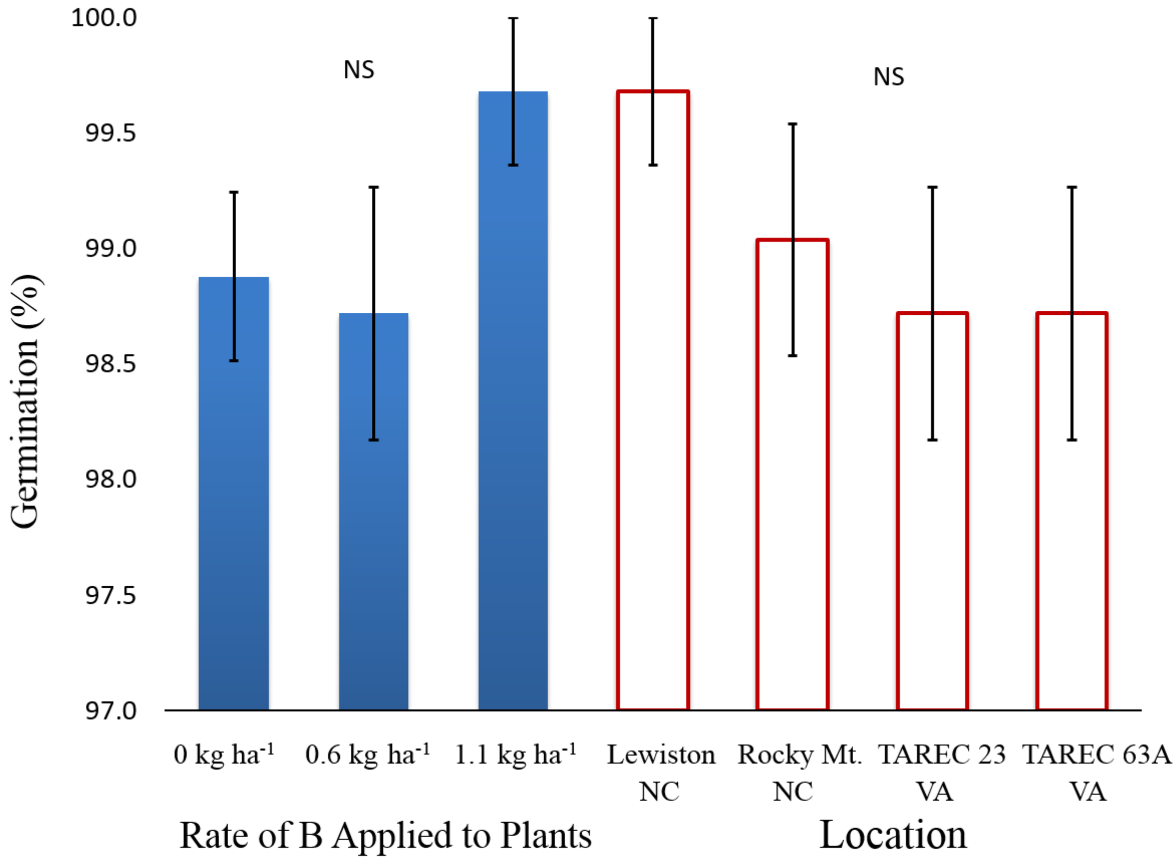

Figure 16. 2016 percent germination of peanut seed from plants that had received 0, 0.6, or 1.1 kg boron (B) ha^{-1} fertilization during the 2015 growing season, and percent germination based on location of experimental field (Lewiston and Rocky Mount, NC, and Fields 23 and 63 A at the Tidewater Agricultural Research and Extension Center in Suffolk, VA). Means separations were determined using ANOVA.

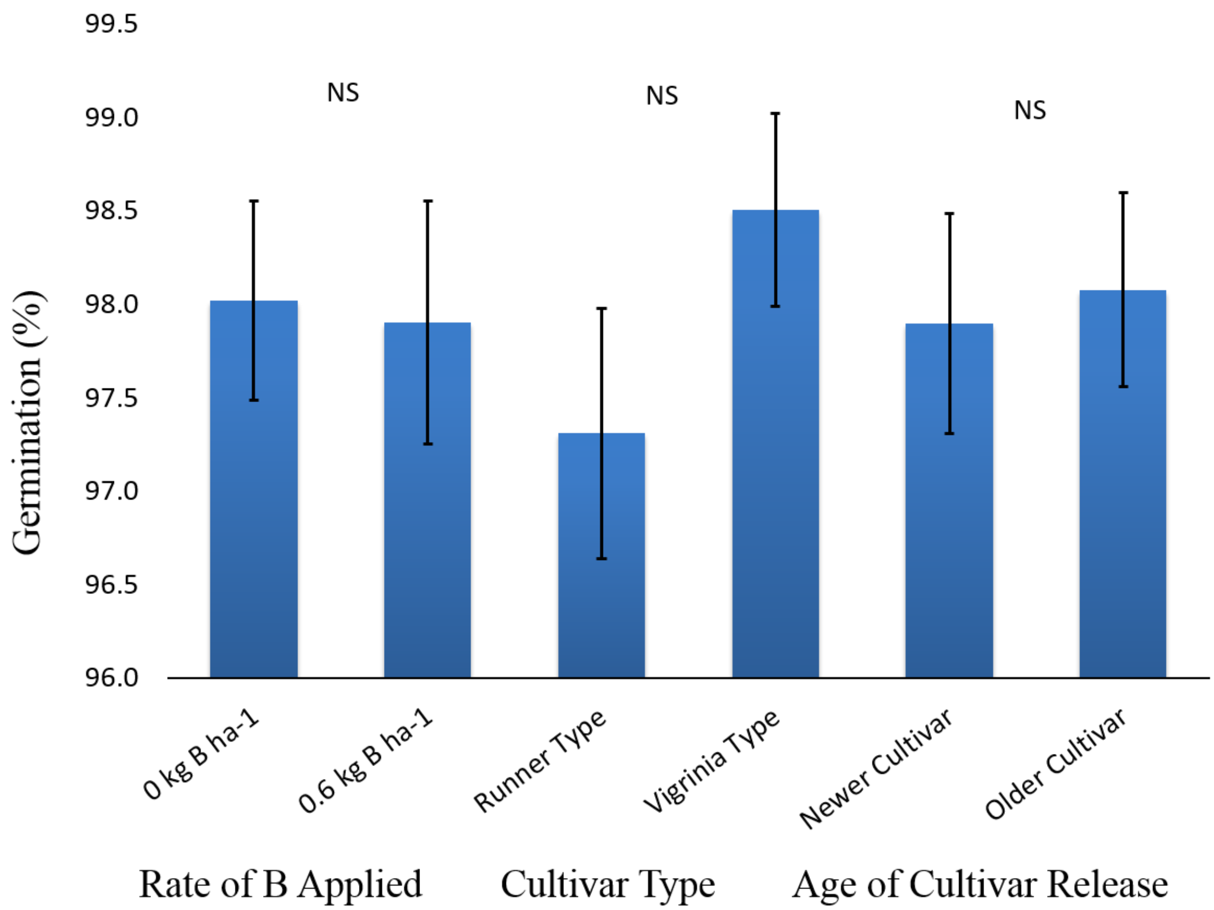

Figure 17. 2016 percent germination of peanut seed based on plants receiving boron (B) fertilization during the 2015 growing season, the market type of the peanut, and cultivar release age. Means separations were determined using ANOVA.

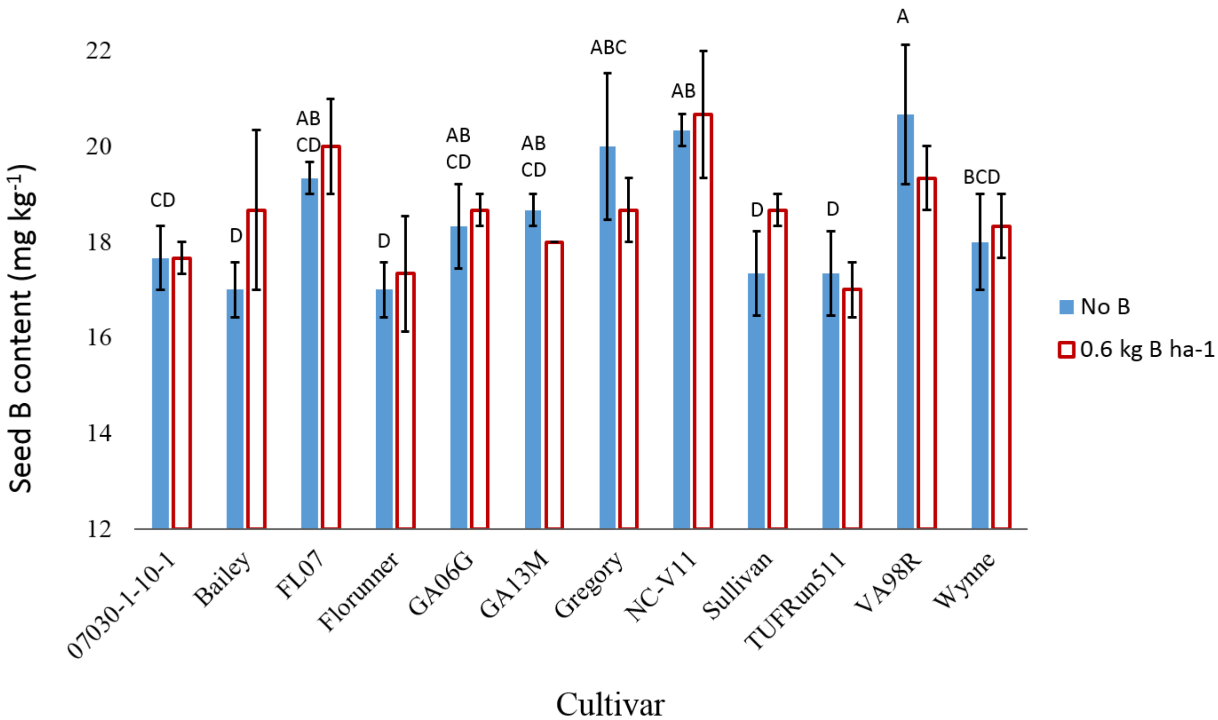

Figure 18. 2015 seed boron (B) content by cultivar based on B fertilization, from Sussex county, Southampton county, and the Tidewater Agricultural Research and Extension Center in Suffolk, VA. Means separations were determined using ANOVA, and using Fisher's LSD, 0.05.

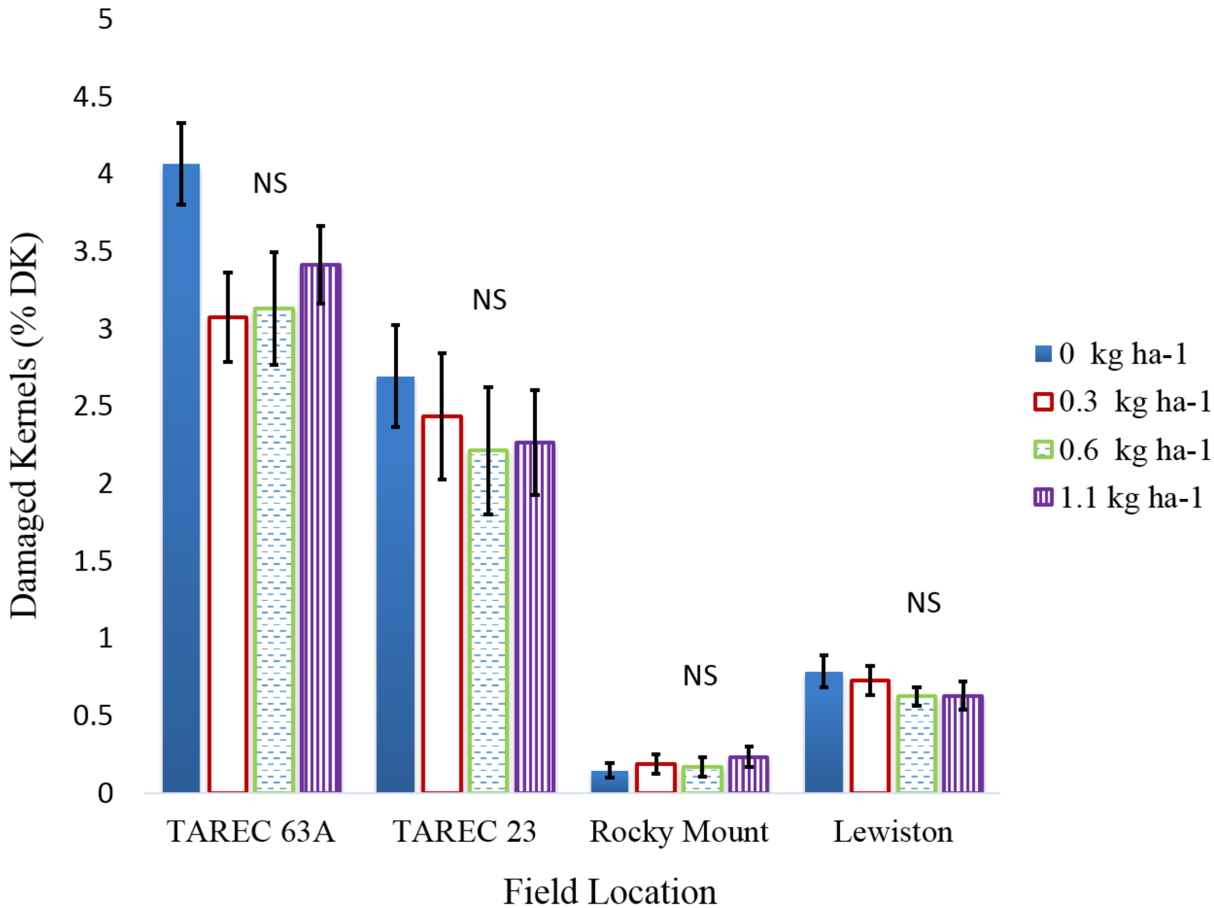

Figure 19. The percent of damaged kernels (DK) as measured in yields from cultivar 'Bailey' grown in 2015 experimental fields in Rocky Mount and Lewiston, NC, and Fields 63 A and 23 at the Tidewater Agricultural Research and Extension Center in Suffolk, VA, when fertilized with difference amounts of boron (B). Means separations were determined using ANOVA.

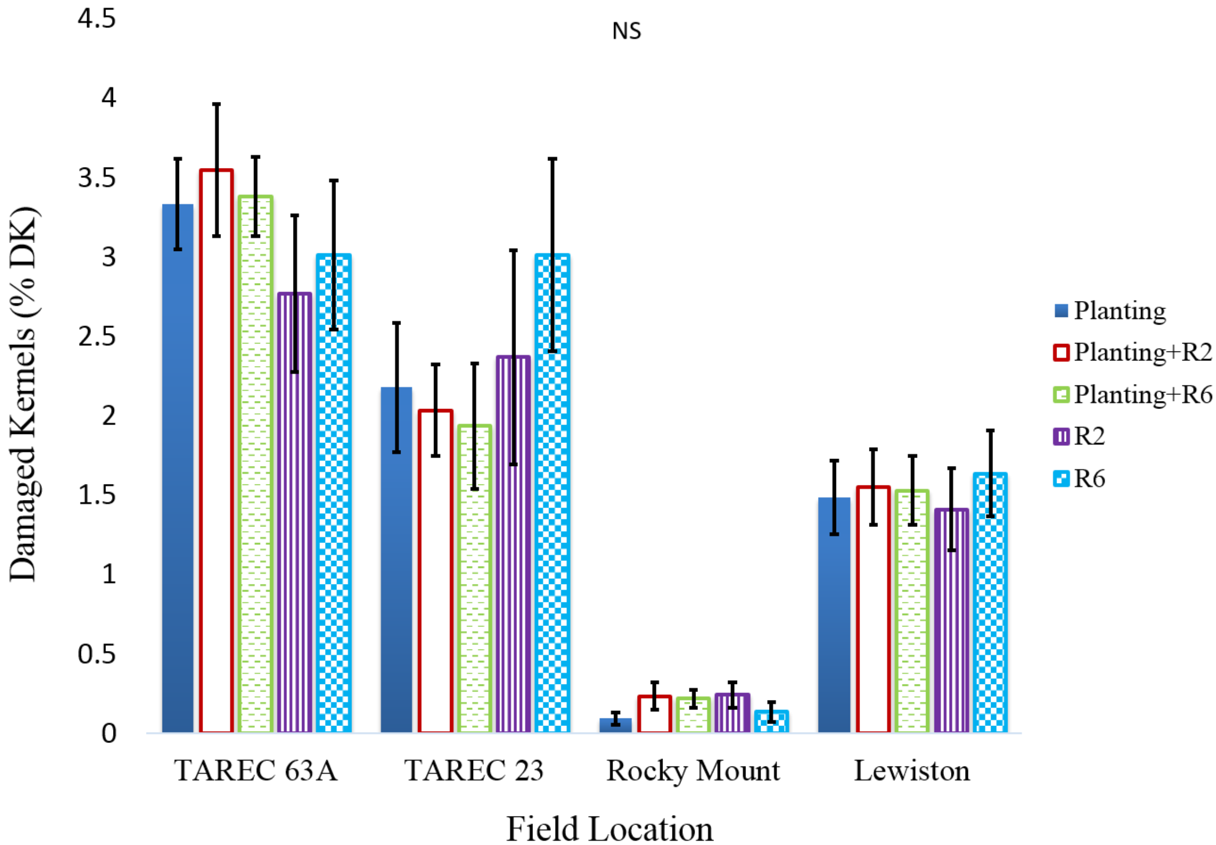

Figure 20. The percent of damaged kernels (DK) as measured in yields from cultivar 'Bailey' grown in 2015 experimental fields in Rocky Mount and Lewiston, NC, and Fields 63 A and 23 at the Tidewater Agricultural Research and Extension Center in Suffolk, VA, when boron (B) fertilization was applied at different growth stages (planting; beginning peg, R2; full seed, R6). Means separations were determined using ANOVA.

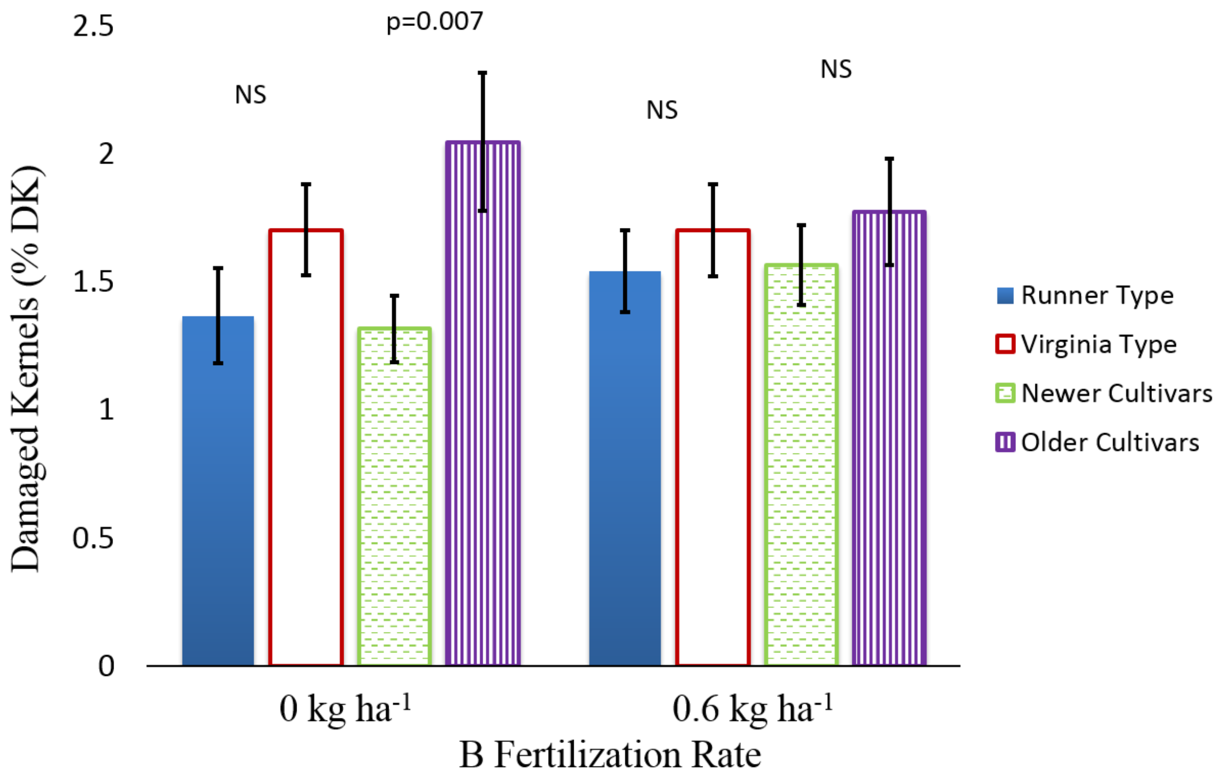

Figure 21. The percent of damaged kernels (DK) in the harvested seed as affected by the market type (Virginia, runner) of the cultivar, and the relative release date (newer, older) of cultivars. Peanuts were grown in the 2015 season at the Tidewater Agricultural Research and Extension Cent in Suffolk, VA, Sussex county, VA, and Southampton county, VA. Means separations were determined using ANOVA.

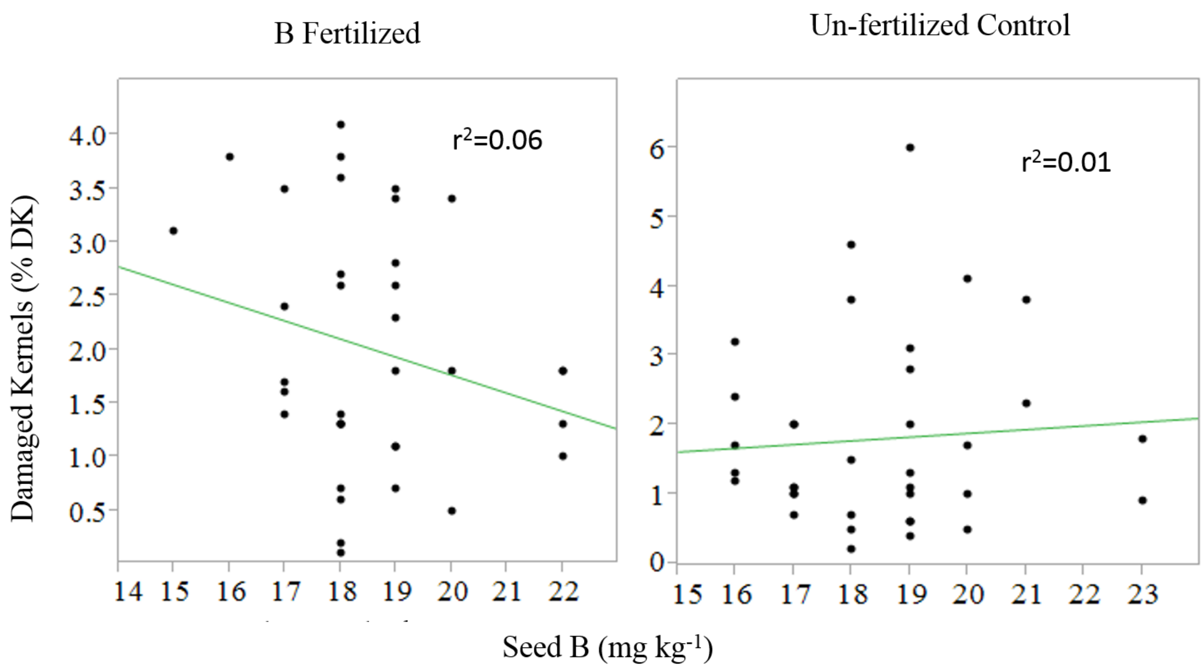

Figure 22. 2015 damaged kernel percent (DK) to harvested seed boron (B) content linear regression of peanut plants that had received B fertilization or not, grown in Sussex county, Southampton county, and at the Tidewater Agricultural Research and Extension Center in Suffolk, VA.

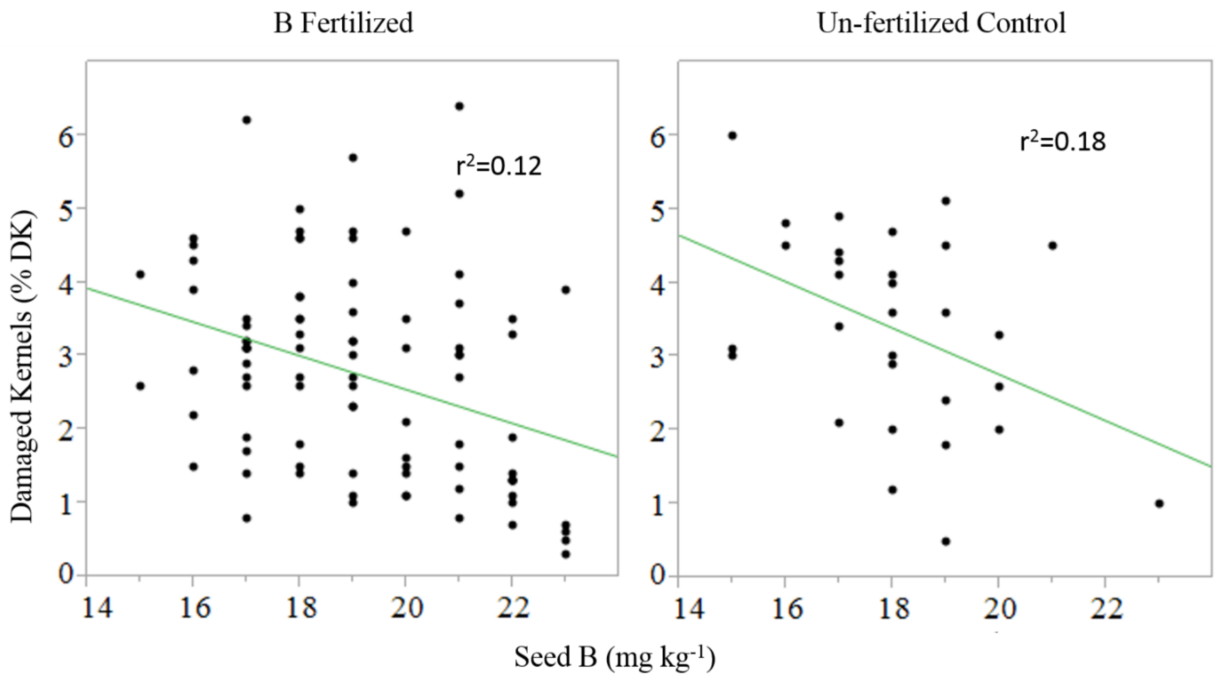

Figure 23. 2015 damaged kernel percent (DK) to harvested seed boron (B) content linear regression of peanut plants that had received B fertilization or not, grown in Lewiston, NC, Rocky Mount, NC, and at the Tidewater Agricultural Research and Extension Center in Suffolk, VA. All experimental fields were planted in the cultivar 'Bailey'.