I. EVALUATION OF SEED TREATMENTS AND NITROGEN ON SOYBEAN NODULATION AND GRAIN YIELD

II. INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS ON WINTER WHEAT GRAIN YIELD AND QUALITY

By

ELIZABETH MARY EICKHOFF

Bachelor of Science in Agronomy & Agricultural Business
Northwest Missouri State University
Maryville, MO
2017

Master of Science in Plant and Soil Sciences
Oklahoma State University
Stillwater, OK
2018

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2021

I. EVALUATION OF SEED TREATMENTS AND NITROGEN ON SOYBEAN NODULATION AND GRAIN YIELD

II. INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS ON WINTER WHEAT GRAIN YIELD AND QUALITY

Dissertation Approved:

Dr. William R. Raun

Dissertation Adviser

Dr. Brian Arnall

Dr. Hailin Zhang

Dr. Paul Weckler

Name: ELIZABETH MARY EICKHOFF

Date of Degree: MAY, 2021

Title of Study: I. EVALUATION OF SEED TREATMENTS AND NITROGEN ON
SOYBEAN NODULATION AND GRAIN YIELD
II. INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS
ON WINTER WHEAT GRAIN YIELD AND QUALITY

Major Field: SOIL SCIENCE

Abstract: Soybeans *(Glycine max)* are known to form symbiotic relationships with soil microbes and conduct biological nitrogen fixation (BNF). However, only 50-60% of the crop's nitrogen (N) requirement is met through this process. Previous work has suggested that the use of seed inoculants and molybdenum (Mo) can improve the plant's ability to fix nitrogen (N) thus potentially improving grain yield. Therefore, the objective of this study was to evaluate different seed treatments as well as fertilizer N applications and their effect on soybean nodulation, tissue N concentration, grain yield, and protein and oil concentration. The seed treatments evaluated include a standard inoculant rate, double inoculant rate, Mo and combinations of all products in addition to an at-planting N application of 45 kg N ha^{-1}. It was noted that nodule counts were improved at most locations when a seed treatment was applied. Alternatively, nodule counts commonly significantly decreased when N was applied at planting. Significant increases in grain yield were observed in 4 of the 7 site years when inoculants were used, particularly in higher yielding environments. Similar findings were noted with seed protein. Although, applying N at planting commonly decreased nodule counts, grain yield was not largely impacted. In conclusion, higher rates of seed inoculants can increase plant nodule counts and boost grain yield in certain environments and perhaps provide additional profitability for producers.

Winter wheat (*Triticum aestivum*) is extensively grown throughout the state of Oklahoma. Producers commonly apply nitrogen (N) and phosphorus (P) fertilizers each season; however, pre-plant N and P interactions are not well understood. The objective of this study was to evaluate the effects of N, P and their interactions on winter wheat grain yield, grain N, and N uptake. To evaluate these effects, Experiment 702 was established in 1996 at the Cimarron Valley Research Station near Perkins, OK. This experiment was designed as a randomized complete block design with a complete factorial treatment structure consisting of four pre-plant N rates (0, 56, 112, 168 kg ha^{-1}) and three P rates (0, 14.8, 29.6 kg P ha^{-1}). Twenty years of yield data from this trial were analyzed in addition to grain N and N uptake when data was available. Analysis was first conducted separately for each year and then under different environmental grain yield and in-season precipitation (ISP) parameters. Results indicated that N and P interactions were more common among high yielding environments.

TABLE OF CONTENTS

Chapter Page

I. EVALUATION OF SEED TREATMENTS AND NITROGEN ON SOYBEAN NODULATION AND GRAIN YIELD 1

ABSTRACT..1
INTRODUCTION 2
REVIEW OF LITERATURE 5
  Soybean Inoculation..5
  Molybdenum 7
  Nitrogen Management in Soybeans 9
OBJECTIVE 11
MATERIALS AND METHODS...12
  Treatment Structure and Experimental Design.................................12
  Trial Establishment Methods 13
  Data Collection 14
  Statistical Analysis...16
RESULTS 16
  2019 Bixby Irrigated 16
  2019 Lahoma 19
  2019 Lake Carl Blackwell Irrigated..21
  2020 Bixby Irrigated 24
  2020 Haskell 27
  2020 Lake Carl Blackwell Dryland 30
  2020 Lake Carl Blackwell Irrigated..33
DISSCUSSION...36
  Nodule Counts 36
  Grain Yield..38
  Seed Protein Concentration...40
  Seed Oil Concentration 41
  Tissue N Concentration...42
CONCLUSIONS..43
TABLES 44
FIGURES...64

Chapter Page

II. INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS ON WINTER WHEAT GRAIN YIELD AND QUALITY....71

ABSTRACT....71
INTRODUCTION....72
REVIEW OF LITERATURE....74
  Function of Nitrogen....74
  Nitrogen Placement....75
  Nitrogen Rates....76
  Function of Phosphorus....77
  Phosphorus Placement....78
  Phosphorus Rates....80
  Use of Long-term Studies to Quantify Environmental Effects....81
OBJECTIVE....82
MATERIALS AND METHODS....82
  Treatment Structure and Experimental Design....82
  Trial Establishment and Data Collection....83
  Statistical Analysis....85
RESULTS....86
  Grain Yield....86
  Grain N Concentration....93
  Nitrogen Uptake....96
DISSCUSSION....99
  Grain Yield by Year....99
  Grain Yield among Environmental Groups....101
  Grain N Concentration among Environmental Groups....104
  Nitrogen Uptake among Environmental Groups....105
CONCLUSIONS....106
TABLES....108
FIGURES....118

REFERENCES....129
APPENDICIES....140

LIST OF TABLES

Table Page

1. Treatment structure as applied at Bixby, Lahoma, Lake Carl Blackwell, and Haskell during 2019 and 2020. 44
2. Pre-plant soil test characteristics (0-15 cm) at Bixby, Lake Carl Blackwell, Lahoma, and Haskell, OK. 2019 and 2020. 45
3. Location descriptions, soil series, and previous cropping history of experiment sites within Oklahoma, 2019 and 2020 46
4. Field activities by trial location, 2019 and 2020 47
5. Total monthly rainfall at each site-year 48
6. Average monthly ambient temperature at each site-year. 49
7. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, 2019. 50
8. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2019. 51
9. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lahoma, OK, 2019. 52
10. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lahoma, OK, 2019. 53
11. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2019 54
12. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2019. 55
13. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2020 56

Table Page

14. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2020 57
15. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Haskell, OK, 2020. 58
16. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Haskell, OK, 2020....59
17. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Dryland, 2020. 60
18. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Dryland, 2020....61
19. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2020....62
20. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2020 63
21. Treatment Structure as applied each season, Experiment 702, Perkins, OK....108
22. Planting date, harvest date, cultivar, cultivar acid soil tolerance, grain yield, and grain N data availability for each year, Experiment 702, Perkins, OK (1998-2020)....109
23. Data groupings as defined by environmental yield and total in-season precipitation received. Experiment 702, Perkins, OK (1998-2020) 110
24. Grain yield main effect analysis by year for P rate, N rate, and N*P interactions and cultivar used. Experiment 702, Perkins, OK (1998-2020) 111
25. Grain yield main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).. 112
26. Treatment structure and treatment means for grain yield, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020). 113
27. Grain N concentration main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020)....114
28. Treatment structure and treatment means for grain N concentration, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020). 115
29. N uptake main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020)....116

Table Page

30. Treatment structure and treatment means for N uptake, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020)....117
31. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. All years combined (1998-2020)....141
32. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. Conventional Tillage (1998-2007)....142
33. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. No-Tillage (2008-2020)....143
34. Eigen-values of the correlation matrix and the proportion of variance explained by the 9 largest principal components across all years combined (1998-2020). Twenty-seven weather parameters included totaling 683 observations 145
35. Primary loadings that comprise the 9 largest principal components. (1998-2020)....146
36. ANOVA of the fitted linear model for grain yield containing 9 principal components. All years combined (1998-2020)....147
37. Eigen-values of the correlation matrix and the proportion of variance explained by the 7 largest principal components while under conventional tillage (1998-2007). Twenty-seven weather parameters included totaling 323 observations. 149
38. Primary loadings that comprise the 7 largest principal components. Conventional tillage (1998-2007)....150
39. ANOVA of the fitted linear model for grain yield containing 7 principal components. Conventional tillage (1998-2007)....151
40. Eigen-values of the correlation matrix and the proportion of variance explained by the 7 largest principal components after transition to no-tillage (2008-2020). Twenty- seven variables included totaling 360 observations. 153
41. Primary loadings that comprise the 7 largest PC after transition to no-tillage (2008-2020)....154
42. ANOVA of the fitted linear model for grain yield containing 7 PCs. No-tillage (2008-2020) 155

LIST OF FIGURES

Figure Page

1. Soybean root architecture as described 64
2. The average number of nodules per plant, by at-planting N rate (0, 45 kg N ha^{-1}) for each site year. 65
3. The average number of nodules per plant for the check, single inoculant, double inoculant, and molybdenum seed treatments for each site year (Trt 1-4) 66
4. Average number of nodules per plant by seed treatment averaged across all locations. 67
5. Average grain yield by at-planting N rate (0, 45 kg N ha^{-1}) for each site year 68
6. The average grain yield for the check, single inoculant, double inoculant, and molybdenum seed treatments for each site year (Trt 1-4) 69
7. Grain yield for each seed treatment averaged across all locations. 70
8. Pre-plant soil test pH, P, and K for growing seasons 2018-19 and 2020-21 118
9. Relationship between total in-season precipitation received and environmental mean grain yield for Experiment 702, Perkins, OK while under conventional tillage (1998-2007) 119
10. Relationship between total in-season precipitation received and environmental mean grain yield after transition to no-till management, Experiment 702, Perkins, OK (2008-2020) 120
11. Relationship between environmental yield and time, Experiment 702, Perkins, OK (1998-2020) 121
12. Grain yield as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007) 122
13. Grain yield as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020). 123
14. Grain N concentration (%) as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007) 124

Figure Page

15. Grain N concentration (%) as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020). 125
16. Nitrogen uptake as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007)..126
17. Nitrogen uptake as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020)...127
18. Visual P difference in the field 128
19. Phosphorus stratification in the top 6 inches of soil of selected treatments in Experiment 702, Perkins, OK.. 140
20. Variance and magnitude of explanatory variables across all years combined (1998-2020). Relative importance of each principal component in explaining the variance of the weather variables included. 144
21. Variance and magnitude of explanatory variables with the conventional tillage dataset (1998-2007). Relative importance of each principal component in explaining the variance of the weather variables included 148
22. Variance and magnitude of explanatory variables after transition to no-tillage (2008-2020). Relative importance of each principal component in explaining the variance of the weather variables included 152

CHAPTER I

EVALUATION OF SEED TREATMENTS AND NITROGEN ON SOYBEAN NODULATION AND GRAIN YIELD

ABSTRACT

Soybean is a staple oil seed crop that is extensively grown. Soybeans are known to form symbiotic relationships with soil microbes and conduct biological nitrogen fixation (BNF). However, only 50-60% of the crop's nitrogen (N) requirement is met through this process. Previous work has suggested that the use of seed inoculants and molybdenum (Mo) can improve the plant's ability to fix nitrogen (N) thus potentially improving grain yield. Therefore, the objective of this study was to evaluate different seed treatments as well as fertilizer N applications and their effect on soybean nodulation, tissue N concentration, grain yield, and protein and oil concentration. The seed treatments evaluated included a standard inoculant rate, double inoculant rate, Mo and combinations of all products in addition to an at-planting N application of 45 kg N ha^{-1}. Data were collected during the 2019 and 2020 growing seasons across five locations within Oklahoma. It was noted that nodule counts were improved at most locations when a seed treatment was applied. Alternatively, nodule counts commonly significantly decreased when N was applied at planting. Significant increases in grain yield were observed in 4 of the 7 site years when inoculants were used, particularly in higher yielding environments. Similar findings were noted with seed protein. Although, applying N at planting commonly decreased nodule counts, grain yield was not largely impacted. In conclusion, higher rates of seed inoculants can increase plant nodule counts and boost grain yield in certain environments and perhaps provide additional profitability for producers.

INTRODUCTION

Legumes comprise a large portion of agriculture production worldwide. In 2014, it was estimated that around 230 million hectares were planted to legume crops across the world (Stagnari et al., 2017). This estimate, however, only includes the top 15 species grown. To put this in perspective, that same year, approximately 221 million hectares were planted to wheat alone across the globe (Stagnari et al., 2017). Furthermore, global grain legume production more than doubled from the 1980s to the mid-2000s (148 million tons to 310 million tons) (Gowda, 2009). Within the legume family, soybean remains the most prominently grown oil seed. In 2018, over 124 million hectares of soybeans were harvested globally producing over 348 million tonnes (FAO, 2018). The United States and Brazil are the leading producers in soybean production, with 35.6 and 34.7 million hectares harvested in 2018, respectively (FAO, 2018).

With a growing world population, legume crops have gained more attention. Soybeans serve as a critical source of food, oil, and protein (Pagano and Miransari, 2016). They are also a primary food source in many developing countries and are largely consumed by vegetarians because of their high nutritional value (Nedumaran et al., 2015). Legumes can offer solutions to a growing food security issue and potentially lessen agriculture’s environmental impact with their ability to fix nitrogen (N). The benefits of implementing legume crops include but are not limited to: reduced N input, improved soil quality from crop rotation practices, better weed control, and reduced greenhouse gas emissions.

Since soybeans are the primary oil seed crop grown globally, research is continually focused on improving the production of this crop. Scientists have been investigating methods to increase soybean yields by improving its N fixation ability. Biological nitrogen fixation (BNF) is essentially the capture of unlimited atmospheric nitrogen gas (N_2) to form ammonia (NH_3) (Gresshoff et al., 2015). Bacteria, collectively known as Rhizobia, form a symbiotic relationship with legume crops, including soybean, to aid in BNF (Biswas and Gresshoff, 2014). Pagano and Miransari (2016) stated that inoculants' proper use is one of the major factors affecting soybean production today. As a method to improve N fixation in soybean, the use of bacteria inoculants has been evaluated overtime. Seed inoculation and foliar inoculations have proven to be successful in increasing the grain yield of soybean (Moretti et al., 2018). However, when large data sets are analyzed in various regions, it has been noted that variable results exist (Leggett et al., 2017). Region specific evaluations of inoculant methods, rates, and products are needed to achieve more concrete recommendations for soybean producers.

The use of bacteria inoculants is just one method to boost N fixation. The micronutrient molybdenum (Mo) also plays a critical role in ensuring the plant’s BNF process. Very small amounts of Mo are needed for proper plant function; however, its nutrient deficiencies can prove to be problematic and reduce yields (Kaiser et al., 2005). Because of Mo’s vital role in N fixation, research has looked at the application of this nutrient in various forms and its effect on nodule formation and ultimately grain yield.

Previously published work has evaluated different methods of Mo application in soybean, such as seed treatment, soil, and foliar applications. Treating soybean seed with Mo seems to be the superior method to alleviating Mo deficiencies and is a more economical option according to Parker and Harris (1962) and Sedberry (1973).

Soybeans can fix 50-70% of their N requirement (Iowa State University, 2008). With an N gap remaining, questions have been raised as to whether soybeans would benefit from small fertilizer N applications. The primary concern with pre-plant N applications in soybeans is the risk of reducing nodulation. Therefore, studies have suggested N applications during the reproductive growth stages can be more beneficial and boost grain yield (Wesley et al., 1998). However, other research has demonstrated that soybean N response is highly dependent on the environmental conditions, and other management factors must be considered when making N management decisions (Mourtizinis et al., 2018; Wesley et al., 1998).

Furthermore, improving N fixation in soybean alone would have a substantial impact on the agriculture industry. In 2014, BNF was valued at 18.5 million US dollars (Islam and Adjesiwor, 2017). Eighty-one percent of that value was attributed to soybean production alone, as it is the most widely grown legume crop. Improving BNF by even just one percent can save thousands of dollars. Moreover, improving legume BNF efficiency can potentially decrease N fertilization by 1,547 thousand metric tons (Tauer, 1989) lessening agriculture's environmental impact. To provide for a growing world population and move towards more sustainable agriculture production systems, increasing soybean grain production, and oil and protein concentration is a vital area of study.

LITERATURE REVIEW

Soybean Inoculation

Seed inoculation has been noted to improve N_2 fixation in soybeans in addition to other legume crops. Soybean inoculation response is often more prevalent in fields where soybeans have not been grown, or native bacteria populations are not largely present (Mueller, 2015). A symbiotic relationship between soybean plants and certain bacteria strains exists and is responsible for the N_2 fixation process. Several environmental factors can reduce N_2 fixation and cause poor nodulation. Such factors include temperature, acidity, moisture, and soil nutrients such as N, phosphorus, calcium, and Mo (Hardarson, 1993).

It has been suggested that increased amounts of *Bradyrhizobial* strains, which are more effective in N_2 fixation compared to other strains, in the seed zone can increase N_2 fixation (Mueller, 2015). In Brazil, a study was conducted to determine whether adding additional inoculants to soybeans via seed and foliar methods could improve grain yields. The significance of this work was that the soils already contained high populations of native bacteria. It was discovered that seed inoculations increased grain yield by 28% compared to a non-inoculated seed. Additionally, throughout the season, foliar inoculant applications increased nodulation and grain yields by 2-7%, indicating that soybeans can continue to form nodules throughout the season with additional inoculations regardless of native bacteria levels (Moretti et al., 2018). Other literature has further noted increases in soybean yield by inoculating seed each year (Conley and Christmas, 2006; Beuerlein, 2005).

Furthermore, water stress has been known to limit plant growth and, particularly, reduce N_2 fixation in legume crops. A research study conducted in Iran evaluated the impact of drought and seed inoculation on soybean yield components. The authors concluded that water stress reduced bacteria activity, pod number, seeds per pod, seed weight, and seed yield (Sadeghipour and Abbasi, 2012). Maximum yields were achieved when optimum water and 20 kg N ha^{-1} were applied as a starter in addition to using seed inoculation with a mix of bacteria strains (Sadeghipour and Abbasi, 2012). This indicates that N and seed inoculation may offer methods to reduce water stress impacts. Additional work from Italy investigated the effect of cover and seed inoculation methods on soybean yield and quality. In this experiment, "cover inoculation" is a practice of inoculating plants at the V3 growth stage via irrigation water. Cover inoculation resulted in increased nitrogenase activity during reproductive growth stages and increased seed protein 6.7-16% and seed production by 10.5-16.1% (Ciafardini and Barbieri, 1987).

Literature reporting on the benefits of soybean inoculation using *Bradyrhizobium japonicum* in both Argentina and the United States noted variable responses between the two regions. Over 150 trials were used in the analysis for each country, with most of them already having *B. japonicum* strains present in the soil. Yield increases from inoculation were greater in Argentina than in the United States, 190 kg ha^{-1} and 60 kg ha^{-1}, respectively (Leggett et al., 2017). Additionally, inoculation treatments in the US were more effective in high pH soils, although Argentina showed greater benefits in low pH soils. It was further noted that inoculation of late planted soybean crops in the US resulted in the most positive response. In Argentina, soybean inoculation seemed to have the greatest effect in soils without a previous soybean cropping history (Leggett et al., 2017). The results of this comprehensive study demonstrate that various factors play a key role in the effectiveness of soybean seed inoculations.

Molybdenum

Molybdenum is considered one of the eight micronutrients required for plant growth (Sedberry, 1973). Estimates show that only 0.5 ppm of Mo within plant tissue is ample for healthy soybean growth (Rasnake, 1982). Even with micro amounts needed of this nutrient, Mo is essential to carrying out several biochemical processes within plants. Molybdenum is a primary component of several molybdoenzymes that are responsible for carrying out N reduction and assimilation, N fixation, sulfur metabolism, and other biochemical reactions (Kaiser et al., 2005). Since Mo is key to plant N health, deficiencies of this element often result in higher nitrate levels within plant tissue and lower yields (Kaiser et al., 2005). Furthermore, plants commonly exhibit stunted growth and low chlorophyll content when Mo is lacking (Marschner, 1995). Deficiencies are most generally seen in legume crops but have been observed in other horticulture and agriculture crops as well (Sedberry, 1973).

Agricultural soils often have less than 4 mg kg^{-1} of Mo; however, greater amounts can be found (Reisenauer, 1965). Other literature states that Mo exists in the soil lithosphere in quantities of 2-3 mg kg^{-1} but can reach levels as high as 300 mg kg^{-1} in soils with higher organic matter (Kaiser et al., 2005). Both mass flow and diffusion processes assist in making Mo available to the plant, where mass flow is the primary movement method in soils where more than 0.004 mg kg^{-1} Mo is present (Lavy and Barber, 1964).

Often the majority of soil Mo is plant unavailable and is highly dependent upon soil pH levels. In contrast to other micronutrients, Mo is more plant available in alkaline soils than acidic soils (Kaiser et al., 2005). Mo is known to adsorb to positively charged metal oxides at a pH level of 4-5 (Kaiser et al., 2005). With that, liming the soil has shown to be beneficial in improving Mo plant availability. The average soil pH across Oklahoma was found to be 6.0 with a range of 5.0 to 7.0 with 300 grid-sampled fields (Manuchehri and Arnall, 2018). Since soil Mo is often plant unavailable within this range, there is a need to investigate methods of improving Mo availability in Oklahoma soils to improve N fixation levels of soybeans.

Applying Mo fertilizer in soybeans has been briefly investigated overtime. Previous literature has noted various successes with seed treatments as well as soil and foliar applications of Mo in soybean. In Georgia, Parker and Harris, 1962 noted that soybean seed yield, leaf N, seed protein, and seed weight increased from applying Mo or lime in moderately acid soils. The authors further stated that an application of 0.22 kg ha^{-1} of Mo improved yields equivalent to applying two tons of lime. It was also discovered that seed treatment of Mo was superior to foliar applications in this study.

Research in Louisiana also investigated different methods of applying Mo as either foliar, soil, or seed applied. Sodium molybdate, which contains 39.65% Mo, was the source in all application methods. Applying a rate of 36.5 mL ha^{-1} (0.5 oz ac^{-1}) as a seed treatment resulted in plant toxicity at a few locations and was then reduced to 18.3 mL ha^{-1} (0.25 oz ac^{-1}). The authors concluded that all three Mo application methods were equally effective in correcting Mo deficiency in acid soils. However, the seed treatment method was more economically feasible due to the lower rates of Mo needed (Sedberry, 1973).

Nitrogen Management in Soybeans

It is estimated that soybeans can fix 50-70% of their N requirement (Iowa State University, 2008). A review publication which recently reviewed the results of 637 data sets investigating the impact of N fertilization of soybeans on N_2 fixation, N uptake, and grain yield concluded that soybean BNF met 50-60% of the crop N demand (Salvagiotti et al., 2008). Based on these estimates, an N gap of 30-50% remains. This has triggered researchers to investigate methods that can potentially close this gap and lead to higher soybean yields.

During the reproductive growth stages, N demand is at its peak for soybeans which has attracted interest in making N applications at this stage. Response to N applications in soybeans thus far have been highly variable, according to previous literature. Work from the University of Missouri showed minimal to no yield response to applied N fertilizer (Scharf and Wiebold, 2003). This conclusion was drawn after 48 experiments were conducted over a wide range of environments where N was applied either as pre-plant or at pod initiation. However, studies across Kansas found N applications at the R3 growth stage to improve grain yield by 463 kg ha^{-1} (6.9 bu ac^{-1}) on average in 6 of the 8 sites evaluated (Wesley et al., 1998). The two non-responsive sites were lower yielding, leading the authors to conclude N applications during the late season in soybeans are more successful in high yielding environments that are often irrigated versus low yielding environments.

A meta-analysis conducted by Salvagiotti et al. (2018), noted that a negative exponential relationship exists between N_2 fixation and fertilizer N rate applied when N is placed at 0-20 cm in depth. To reduce this negative effect, it is suggested to use a slow-release N source, such as polymer-coated urea, to limit adverse plant nodulation effects. Applying N during pod filling stages could be beneficial according to this review to meet the increased crop demand. This was further emphasized in other research where it was found that fewer nodules formed when 56 kg N ha^{-1} was applied pre-plant as ammonium sulfate. However, when N applications are made later in the season, applying up to 112 kg N ha^{-1} had limited, if any effect on the number of nodules (Beard and Hoover, 1971). Based on the authors' findings, it is apparent that the timing of N application can have a significant effect on nodule formation.

Previous literature concerning ammonium nitrate applications discovered that soybean nodulation was more affected by uniform incorporation of ammonium nitrate than deep soil applications (Harper and Cooper, 1971). In this growth chamber experiment, it was noted that nodule weight and hemoglobin content were reduced due to N application; however, nodule number was not as affected. Results from this work suggest that the placement of fertilizer N in soybeans plays a large factor in whether nodulation development is negatively impacted.

In another review, the analysis of 207 environments of soybean N studies across the United States showed that N management decisions had a small impact on grain yield (Mourtizinis et al., 2018). It was noted that higher N rates increased the average environmental yield, although 93% of environment-specific-N-rate responses were not significant. It was further discussed that large yield variability among environments with the same N rate was due to environmental differences and other management practices rather than the N rate itself. The authors further highlighted that other practices such as irrigation and seeding rates need to be taken into consideration when making N management decisions in soybean production.

A final consideration concerning N applications in soybeans is that of the sulfur (S) to N ratio. When soils are S deficient, often the efficiency of N applications are reduced (Fazili et al., 2008). Sulfur is a key nutrient in oil seed crops and is often required in higher amounts compared to cereal grains (Jamal et al., 2010). Work by Wehmeyer, 2020 evaluated the effect of additional N and S applications on soybean grain yield and yield components. The author notes applying additional S did not yield significant increases in grain yield, protein, or oil concentration. However, the locations utilized in this study were noted to have sufficient soil S present thus, limiting fertilizer response. Assessment of soil S levels in the present study are of interest to further evaluate N response in soybean production.

OBJECTIVE

The objective of this study was to evaluate Mo and inoculant seed treatments in addition to fertilizer N applications and their effect on soybean *(Glycine max)* nodulation, tissue N concentration, grain yield, and grain protein and oil concentration.

MATERIALS AND METHODS

Treatment Structure and Experimental Design

This experiment was conducted over two growing seasons (2019 and 2020) over four different locations across Oklahoma (Lahoma, Lake Carl Blackwell, Bixby, and Haskell) including both irrigated and dryland conditions. A complete factorial treatment structure of twelve treatments with one extra treatment was implemented in a randomized complete block design with four replications. The treatments were tested individually and in combination of each seed treatment, including Mo, inoculant, and a double inoculant rate (Table 1). Additionally, an at-planting application of N as urea was evaluated in combination with each seed treatment. The Mo seed treatment product used was 10% Moly Liquid by WinField United applied at 142 g per 45 kg of seed (5 oz/ 100 lbs of seed). This is the recommended rate given on the product label. Exceed Superior Legume Inoculant for soybeans was used for seed inoculation at a rate of 142 and 284 g per 45 kg seed (5 % 10oz per 100 lbs of seed). The rate of 142 g was the product label recommendation while the 284 was simply double the recommended rate. The inoculant product used peat as a carrier material and was weighed out and applied to each batch of seed corresponding to the specified treatments. Moly Liquid was measured using a syringe and then applied to each batch of seed. Each seed treatment was mixed in an individual bucket by hand and vigorously shaken and rolled to promote maximum product retention. Seed was treated on or within 2-3 days of planting for each location to ensure inoculant viability. The broadcast N treatments both at-planting and mid-season were weighed out and applied by hand per plot at a rate of 45 kg of N ha^{-1}.

Trial Establishment Methods

Pre-plant composite soil samples were taken each season at 0-15 cm in depth by replication. Approximately 18 soil cores were taken per replication and mixed to obtain the composite soil sample. Soil samples were then oven dried at 65˚C for 24 hours. After drying, soil samples were then rolled to ensure fineness and passed through a 1 mm sieve. Once taken to the lab, samples were analyzed for soil test NO_3^-, NH_4^+, P, and K prior to planting to ensure P and K were not limiting. If soil test levels of P and K were lower than 80% sufficient, the appropriate fertilizer was applied to ensure 100% sufficiency to limit outside nutrient deficiency factors. However, at the 2020 Bixby and Haskell locations, soil P and K levels were not adjusted due to time constraints. Additional soil test nutrient concentrations reported were Ca, Mg, SO_4^-, Zn, Fe, and Mo. Soil levels of Mo were however, below the detection limit of 0.04 ppm when extracted using DTPA and analyzed using an inductively coupled plasma mass spectrometer (ICP). Total C and N were also determined using dry combustion techniques with a LECO CN828 instrument where 200 mg of soil was combusted at 950ºC. Total C results were then used to calculate percent soil organic matter (OM%). Soil test results for each location are reported in Table 2. Additionally, descriptions of soil type for each location were obtained using the NRCS Soil Survey database and previous cropping history of locations were recorded for data interpretation (Table 3).

Field plot sizes were 3.04 x 6.09 m^2 with 6.09 m alleys between each replication. However, Bixby and Lahoma trials during 2019 had plot sizes of 3.04 x 9.14 m^2. Plots were planted using a four row MaxEmerge planter at a population of 251, 940 seeds ha^{-1}. Row spacing was set at 76.2 cm. In between planting each seed treatment combination, the planter was emptied and cleaned using compressed air and damp cloths to prevent cross contamination of seed treatments. The indeterminate soybean variety P46A57BX was used both seasons, which is equipped with Roundup Ready 2 Xtend technology. This variety is well suited for dryland conditions and double crop acres, which are common throughout Oklahoma. It has near excellent ratings for shattering and harvest standability and a larger canopy cover ideal for wide row spacing. Appropriate herbicide and insecticide applications were made as needed throughout the season.

Data Collection

Dependent variables used for trial evaluation include primary root and lateral root nodule counts, plant tissue N analysis, grain yield, and seed protein and oil concentrations. Plant root nodule counts were taken at the R1-R2 growth stage when soybean nodule formation peaks and the plant's N demand is greatest (Lofton & Arnall, 2017). Five plants were sampled from the outer two rows of each plot. Plants were dug up with care to minimize unintentional nodule removal and gently washed with water. Nodules were then removed by hand and counted. This nodule estimation method is similar to that employed by Solomon et al. (2012) and Carciochi et al. (2019). As with other dicot plant species, the root system of soybeans is classified as an allorhizic system (Osmont et al., 2007). This system consists of a primary root and lateral roots, as shown in Figure 1 (Fenta et al., 2011). When counting, nodules were labeled as either "primary root" or "lateral root" nodules. This is a significant differentiation as literature states primary root nodules are more influenced by seed treatments, whereas lateral root nodules are largely influenced by the free-living bacteria present within the soil (Stanton, 2011). A primary root and lateral root nodule average was calculated from the five sampled plants and used for statistical analysis. Total nodule count was also included in which the average primary root and lateral root nodule counts were summed.

Additionally, plant tissue samples were gathered during the R1-R2 growth stage. Fifteen trifoliates were taken from the new plant growth of each plot using the outer two rows as not to disturb the center two rows that are used for grain yield estimation. Tissue samples were dried at 65˚C for 6 hours and ground. Collected samples were then analyzed for N content using dry combustion methods. The Leco CN 828 model was used to determine tissue N %. In this method, 100 mg of each sample was combusted at 950ºC to estimate total N concentration.

At maturity, grain was harvested from the center two rows using a Kincaid 8XP plot combine. Yield reported was normalized to 13% moisture. Seed protein and oil concentrations were then obtained using NIR spectroscopy methods. The results of TRT 13 were not fully evaluated for root nodule counts during 2019 and 2020 because counts were made prior to a mid-season application. Therefore, nodule results could be attributed to seed treatment only. Additionally, tissue N results for TRT 13 in 2019 were not fully evaluated because samples were taken prior to fertilizer application, meaning results were due to seed treatment only. Table 4 outlines the description and timeline of all field activities.

Additionally, weather data for each site-year was obtained from the Oklahoma Mesonet. The Mesonet sites of Bixby, Haskell, Lake Carl Blackwell, and Lahoma were all used for their respective locations in this study. Total monthly rainfall and average monthly temperature at each site year was reported to aid in the holistic analysis of this experiment (Tables 5 & 6).

Statistical Analysis

Data analysis was performed using SAS 9.4 software (SAS Institute, Cary, NC). A generalized linear procedure (Proc GLM) was used to generate the least squares means of each treatment for each variable. Due to year and location factors having a significant influence, each site-year was analyzed separately. The GLM main effect model included Mo rate, inoculant rate, and at-planting N rate as fixed effects while replication was noted as a random effect. Using a replication and treatment model, SED values were then calculated and reported in the data tables. This model was also used for single-degree-of-freedom contrast comparisons among treatments. R Studio software was used to create data visuals (R Core Team, 2021).

RESULTS

2019 Bixby Irrigated

The 2019 trial at Bixby was planted on June 13th (Table 4). Planting at this location was delayed due to high rainfall in May (331 mm). The total amount of rain received at this location from May-October was 1003 mm (39 in) (Table 5). Average monthly air temperature for this period was 23˚C (73˚F) (Table 6). This site was under a linear pivot irrigation system that was used when plants exhibited signs of moisture stress. Soil type at this location was classified as a Wynona silty clay loam with 0-1 percent slope and occasionally flooded (NRCS, 2021). The average soil pH at this site was 6.7 (Table 2), and was previously planted to soybeans the prior season (Table 3) Treatment means and statistical results are noted in Table 7 for this location while the interaction and main effect model results are noted in Table 8.

Nodule counts at Bixby in 2019 were collected on July 23rd at the R2 growth stage. Primary root nodule counts ranged from 5.7 to 7.8 nodules per plant. The lowest number of nodules was noted where a double inoculation rate was used along with an application of N at planting (TRT 10) with the check treatment close behind (5.8 nodules). In contrast analysis, there were no significant differences noted between seed treatments.

Lateral root nodule counts ranged from 3.0 to 7.4. The lowest number of lateral nodules occurred in TRT 12, while the highest number was noted in TRT 13. Treatment 12 included both a Mo and double inoculant rate with an N application at planting, whereas TRT 13 only used a Mo and single inoculant seed treatment (mid-season N had not yet been applied). Contrasts between specific seed treatments did not show significance for lateral root nodules. However, it was noted that in treatments where N was applied at planting, lateral root nodule counts were significantly decreased (α=0.01).

Grain yield at this site year ranged from 2.5 to 2.9 Mg ha^{-1}. Differences in grain yield were not significant for any treatment (α=0.05). The highest yield was observed in TRTS 4 and 13, which consisted of an inoculant seed treatment only and then a Mo seed treatment with single inoculation and a mid-season N application. The lowest yields were recorded in TRTS 2, 7, and 9. These treatments consisted of Mo seed treatment only, Mo and double inoculant, and inoculant with an at planting N application. Interactions and main effects of independent variables were not significant for grain yield.

Seed protein concentration ranged from 32.4 to 33 percent at Bixby in 2019. The lowest protein concentration was seen in TRTS 8 and 13. These treatments included a Mo and at-planting N application and a Mo and single inoculant with a mid-season N application, respectively. Applying a seed treatment of any kind at this site significantly decreased seed protein concentration compared to the check (α=0.05). Contrast comparisons indicated that the Mo seed treatment specifically, decreased seed protein compared to the check and single inoculant rate treatment (α=0.05). Even though the low inoculant rate improved seed protein compared to Mo, it was not significantly different from the check. When analyzed as a main effect, Mo decreased seed protein overall (α=0.01). Seed oil ranged from 19.7 to 20.2 percent. The lowest oil concentration was found in TRT 10, where a double rate of inoculant was applied with an at-planting N application. Significant differences among treatments were not noted for seed oil concentration.

Tissue samples were collected on July 23rd, and mid-season N application was applied this day as well. Tissue N % ranged from 4.2 to 4.7 %. Applying a seed treatment overall did significantly improve tissue N concentration (α=0.05). Further analysis using single-degree-of-freedom contrasts indicated that the Mo seed treatment alone improved tissue N % significantly compared to the check (α=0.01) by 0.5%. However, the use of a Mo seed treatment did not improve tissue N concentration compared to inoculant use. Additionally, using a low inoculant rate significantly improved tissue N concentration, whereas a double inoculant rate did not (α=0.05). As a main effect, applying Mo significantly increased tissue N percentage as well (α=0.05).

2019 Lahoma

The Lahoma site was planted on May 14th representing a fuller season growth period (Table 4). The total amount of rain received from May to October was 787 mm (31 in) (Table 5). Average monthly air temperature during this same period was 23˚C (73˚F) (Table 6). Soil type at Lahoma was classified as a Grant silt loam with 1-3 percent slopes (NRCS, 2021). Pre-plant composite soil sample analysis results are noted in Table 2. The average soil pH at the trial site was 6.5, optimum for soybean growth. This trial was planted into fallow ground and did have a crop history of soybeans within the past two years (Table 3). Treatment means and statistical results are noted in Table 9 while interaction and main effect model results are noted in Table 10.

Nodule counts at this site were taken on June 27th (Table 4). Primary root average nodule counts per plant ranged from 5.8 in the check treatment to 9.4 with a double inoculant rate. The use of Mo or inoculants at this site did not have a significant impact on the primary root nodule number overall (α=0.05). However, applying the higher inoculant rate significantly increased primary root nodules compared to the check alone (α=0.05).

Lateral root nodule counts ranged from 2.9 to 9.6 nodules per plant. In this location, applying N broadcast at planting significantly reduced the number of lateral root nodules present. No differences were seen in lateral nodule counts across seed treatments in the main effect model. However, when comparing Mo to the check or double inoculant rate, Mo significantly improved lateral nodulation by 6.1 and 2.8 nodules, respectively. The application of N as a main effect was also noted to substantially decrease lateral root nodules and total nodule count (α=0.01) (α=0.05).

Grain yield at Lahoma in 2019 ranged from 2.1 to 4.2 Mg ha^{-1}. The highest yield was recorded in TRT 13, resulting from both a Mo and inoculant seed treatment with a mid-season N application. The lowest yield was noted in TRT 9 that consisted of a single inoculant seed treatment and an at-planting N application. The use of a seed treatment did lead to higher yields on average but was not significant. Additionally, applying N at planting did not significantly impact yield. Interaction of main effects nor main effect evaluations of any independent variable was significant for grain yield. Heavy insect pressure at this location did contribute to some yield loss. Green stem was noted at this site due to insect damage and therefore a harvest aid was used prior to grain collection. Considering these outside factors, grain yield results were variable at this site.

Seed protein concentration at this location ranged from 34.3 to 36.5%. Treatment 9 resulted in the lowest seed protein concentration and had the lowest grain yield. The highest seed protein was observed in TRT 10, where only a double inoculant rate was used in addition to an at-planting N application. Applying Mo, an inoculant, or N did not significantly influence grain protein in the main effect model. Where N was applied, however, protein concentrations were slightly higher compared to not applying N. Evaluation of main effect interactions showed significance with inoculant*N at planting (α=0.05). The main effect of Mo was not significant.

Differences in seed oil concentration were not apparent across treatments for this site year. Oil concentration ranged from 14.1 to 18.1%. Treatment 10 resulted in the lowest oil concentration where a double inoculant rate was applied with an at-planting N application. Treatment 7 resulted in the highest oil percentage where a Mo and double rate inoculant seed treatment was applied. Results from specific treatment contrasts did not show significance for seed oil. Main effect interaction evaluations noted significant interactions between Mo and inoculant as well as inoculant and N at planting (α=0.05).

Tissue samples were collected on June 27th. Tissue N concentration was lowest at 4.2%. This result was noted in the check treatment and the double inoculant rate with N applied at planting. Using a Mo seed treatment alone resulted in the highest tissue N concentration of 4.7%. Applying a seed treatment of any kind did not improve tissue N significantly compared to the check (α=0.05). Furthermore, the use of an inoculant or applying N at planting did not have an impact on tissue N concentration. Again, the timing of N application comparison could not be evaluated due to the early collection of tissue samples. The main effect analysis noted that Mo significantly increased tissue N overall while applying N at planting tended to decrease tissue N (α=0.01).

2019 Lake Carl Blackwell Irrigated

The Lake Carl Blackwell site was planted on May 15th. The total amount of rain during May to October was 975 mm (38 in) (Table 5). Average monthly air temperature during this time was 23˚C (73˚F) (Table 6). Although linear irrigation was available at this location, limited irrigation was used at this site due to the high rainfall amounts received. Soil type at LCB was classified as a Port-Oscar complex with 0-1 percent slopes and occasionally flooded (NRCS, 2021). Pre-plant composite soil sample analysis results are outlined in Table 2. The average soil pH at this location was 5.75. This location was fallow the prior year and had been out of soybeans for at least three years (Table 3). Treatment means and statistical results are noted in Table 11 while interaction and main effect model results are noted in Table 12.

Nodule counts at this site were recorded on June 28th. Primary root nodule counts ranged from an average of 0.5 to 3.8 nodules per plant. The lowest count was recorded in TRT 13, where a Mo and low inoculant rate was applied (mid-season N had not yet been applied). The highest number of nodules was found in TRT 10 with a high inoculant rate and an at-planting N application. Significant differences were not noted in primary root nodule numbers for the contrasts evaluated. An interaction was noted between Mo and inoculant use for primary root nodule counts but was not significant for lateral or total nodule counts (α=0.05).

Lateral root nodule counts ranged from 1.2 to 5.3 nodules per plant. The check treatment resulted in the lowest number of lateral nodules, whereas TRT 3, a single inoculant rate, resulted in the greatest number of nodules. It was noted that the use of a single inoculant significantly increased nodule number on lateral roots compared to the check (α=0.05). However, the use of a double rate did not largely differ from the check or single inoculant rate. Neither Mo nor applying N at planting impacted the number of lateral root nodules observed.

Grain yield at this location was relatively higher compared to the other sites during 2019. The yield achieved ranged from 3.2 to 5.1 Mg ha^{-1}. Treatment 5 resulted in the lowest yield, which consisted of only an at-planting N application. The highest yield was found in TRT 10 where a double inoculant rate was applied with a broadcast N application at planting. Treatment 4 was close behind at 5.0 Mg ha^{-1}, which included a double inoculant rate only. Applying a high inoculant rate compared to the check significantly increased grain yield from 3.4 to 5.0 Mg ha^{-1} (α=0.05). Adding 45 kg N ha^{-1} at planting to that treatment increased yield slightly more reaching 5.1 Mg ha^{-1}. However, when comparing a single inoculant rate to a double rate, significance was not observed. In main effect analysis, the use of an inoculant did significantly increase grain yield overall (α=0.01). Additionally, heavy insect infestations throughout the growing season contributed to some extent of yield loss. Appropriate insecticide applications were made as needed, however damage still did occur. Upon harvest it was noted that several pods did not fully mature and suffered from green bean syndrome. Grain scores were taken to estimate yield damage. Scores indicated that damage was mostly uniform across treatments.

Seed protein at this location showed similar findings to grain yield. Protein concentration ranged from 34.7 to 36.4%. The check treatment as well as TRT 5 resulted in the lowest protein concentration. The highest concentration was observed in TRT 4, where a double rate of inoculant was applied. Applying a double rate significantly improved seed protein compared to the check treatment however, a single rate did not (α=0.05). However, differences were not significant when comparing the high and low inoculant rates against one another. Furthermore, as a main effect, the use of an inoculant increased seed protein overall ($\alpha=0.05$).

Seed oil concentration was similar to that of the other locations in 2019 ranging from 15.4 to 17.2 %. The check treatment noted the lowest oil concentration, whereas applying a double inoculant rate alone resulted in the highest oil concentration. However, no significant differences between any treatments were noted at this location for seed oil concentration.

Tissue samples were taken on June 28th. Tissue N concentration ranged from 4.0 to 4.6% N. The lowest N concentration was noted in TRT 13, including both a Mo and single inoculant seed treatment (mid-season N was applied that same day). The highest N percentage was observed in TRTS 8 and 9, including a Mo and at-planting N application and then a double inoculant and at-planting N application, respectively. Significant differences were not found for any contrast comparison concerning tissue N concentration.

2020 Bixby Irrigated

The Bixby site was planted on June 19th. The total amount of rain during May to October was 697 mm (27 in) (Table 5). The average monthly air temperature was 22˚C (72˚F) (Table 6). This site was also under supplemental irrigation like the prior year, which was used when plants exhibited signs of moisture stress. Soil type at this location was classified as a Wynona silty clay loam with 0-1 percent slope and occasionally flooded (NRCS, 2021). The trial in 2020 was located next to the trial in 2019, but it was not in the exact same location. Pre-plant composite soil sample analysis results are outlined in Table 2. The average soil pH at this location was optimal at 7.16. The previous crop planted at this site was soybeans (Table 3). Treatment means and statistical results are noted in Table 13 while interaction and main effect model results can be found in Table 14.

Nodule counts for this location were taken on August 4th. Primary root nodule counts were relatively higher compared to other site-years in this experiment ranging from 19.6 to 24.4 nodules per plant. The lowest nodule counts were found in TRTS 3 and 9 where a single inoculant rate was applied and a single inoculant rate with N applied at planting, respectively. Treatment 6, which consisted of a Mo and single inoculant rate, resulted in the highest number of primary root nodules. Results at this location indicated that combining a Mo seed treatment with a single inoculant rate significantly improved primary root nodule numbers compared to applying either of those seed treatments alone. However, applying Mo or any rate of inoculant alone did not significantly differ from the check.

Lateral root nodule counts were similar to primary root nodule numbers at this site ranging from 17.1 to 27.7 average nodules per plant. The lowest number of lateral root nodules was found in the check treatment, while the greatest number of nodules was recorded in TRT 4. Treatment 4 consisted of a double inoculant rate. Again, similar to the primary root nodule results Mo, inoculation, nor the application of N had a significant effect as a whole on the number of lateral root nodules (α=0.05). A significant difference was noted between the check treatment and high inoculant rate, which resulted in an increase of 10.6 nodules (α=0.05).

Grain yield at Bixby during 2020 was slightly higher compared to the 2019 growing season with a range of 2.8 to 3.8 Mg ha^{-1}. The check resulted in the lowest yield, whereas TRT 13 resulted in the greatest yield, which included a Mo and single inoculant rate with a mid-season N application. Contrast analysis did not result in any significant findings. When comparing a single or double inoculant rate to the check, yield was not largely improved. Furthermore, although TRT 13 resulted in the highest yield, it was not significantly different than TRT 11 when N was applied at planting (11 vs. 13). Again, in the main effect model analysis, it was found that the use of an inoculant improved grain yield overall ($\alpha=0.05$).

Evaluation of grain quality yielded few significant differences as well. The range of seed protein concentration varied slightly from 36.2 to 36.8%. The lowest seed protein was found in TRT 8, which included a Mo seed treatment and an at planting N application. The highest protein concentration was found in TRTS 3, 4, and 7. All of these treatments used an inoculant and did not include an N application. It was noted that treatments containing a seed treatment with an N application at planting had significantly less protein than seed treatments that did not include an application of N ($\alpha=0.05$).

Seed oil concentration ranged from 22 to 22.5% at this site. The lowest oil concentration was noted in TRT 4, which also had the highest seed protein. Treatment 9 recorded the highest oil percentage while it also had a lower protein concentration. The use of a seed treatment or application of N did not affect seed oil results at this location. No significant differences were found between specific treatment contrasts.

Tissue N samples at this location were collected on August 11th after mid-season fertilizer N was applied the week before. Tissue N concentration ranged from 4.7 to 5.2. The lowest N concentration was noted in TRTS 10 and 11, which both contained an N application at planting. The highest N percentage in tissue was in the check treatment. The use of an inoculant or Mo seed treatment had little effect on this variable. However, N application at planting significantly decreased N concentration in tissue samples (α=0.05). It was further noted that timing of N application was significant, indicating that mid-season N applications result in higher tissue N concentration compared to N applications at planting. This could largely be due to the timing of tissue sampling. Similarly, in main effect analysis, applying N at planting significantly decreased tissue N concentration (α=0.05).

2020 Haskell

The Haskell location was planted on June 4th. The total amount of rain during May to October was 700mm (28in) (Table 5). The average monthly air temperature was noted at 22˚C (72˚F) (Table 6). Soil type at this location is classified as a Taloka silt loam with 0-1 percent slopes (NRCS, 2021). Pre-plant composite soil sample analysis results are outlined in Table 2. The average soil pH at this location was near optimal at 6.95. This location was notably low in soil K and Mg content with an average of 38.6 and 8.0 mg kg^{-1}, respectively. Out of all site years evaluated, this site year had the highest Fe content as well with 49.9 mg kg^{-1}. The previous crop of this location was winter wheat which was still standing at the time of planting. With this additional ground cover, soybeans were planted into high soil moisture. Furrow closure was not 100%, but crop stands were not affected. It should also be noted that the Mo + single inoculant rate treatments were planted with only three rows per plot due to a planting error. With that in mind, the center two rows were not always available for harvest. However, nodule counts and tissue samples were still not taken from the rows used for grain yield estimation. Treatment means and statistical results are noted in Table 15 while interaction and main effect model results are noted in Table 16.

Nodule counts were taken on July 24th. Primary root nodule counts ranged from 5.5 to 15.3 nodules per plant. The lowest primary root nodule counts were recorded when a Mo and single inoculant rate plus an application of N at planting was used (TRT 11). The highest primary root nodule count was noted where a single rate of inoculant was used, and N at planting was applied. The check treatment also had a high count of primary root nodules with 15. No response was seen in nodule formation for the Mo or inoculant seed treatments. Additionally, applying N at planting did not seem to affect nodule formation at this location.

Lateral root nodules showed a greater range among treatments varying from 8.4 to 22.2 nodules per plant. As seen in primary root nodules results, TRT 11 had the lowest number of lateral root nodules. The highest number of lateral root nodules was seen with the use of a single rate of inoculant and an at-planting N application (TRT 9). No significant differences were noted between treatments.

Grain yield at Haskell ranged from 2.3 to 3.3 Mg ha^{-1}. Yield levels at this location were relatively low and were potentially limited by K and Mg deficiencies. The lowest yield was noted in TRT 10 with a double inoculant rate and an at-planting N application. The highest yield was seen in TRT 8, which used a Mo and at-planting N application. Although the highest yield resulted from the Mo seed treatment, Mo did not have a significant main effect overall (α=0.05). Analysis from specific treatment contrasts indicated that the use of inoculation at any rate did significantly reduce yield compared to the check treatment. This was further confirmed in main effect analysis (α=0.05). Additionally, the application of N at this site did not impact final grain yield.

Seed quality analysis also yielded little differences. Protein concentration was slightly higher than Bixby, ranging from 37.2 to 38.9 %. Treatment 13 containing a Mo and single inoculant treatment with a mid-season N application resulted in the lowest protein concentration. Contrastingly, TRT 3, which had a double inoculant rate, resulted in the highest seed protein percentage. Differences between treatments were not significant.

Seed oil concentration also did not show significant differences between treatments. Results ranged from 21.5 to 22.7 %. Treatment 5 which only included an at-planting N application, recorded the lowest oil concentration. The highest oil concentration was noted when a Mo and double inoculant rate was applied in addition to an at-planting N application (TRT 12).

Tissue N concentration was consistent across treatments, excluding TRT 12. The range of tissue N % was 3.6 to 6.8. The lowest tissue N % was found in TRTS 8 and 10 consisting of a Mo seed treatment, and high inoculant rate in addition to applying N at planting, respectively. The highest tissue N concentration was observed in TRT 12, which essentially combined TRTS 8 and 10. This concentration is particularly high and does raise concern to validity. Use of Mo and inoculant seed treatments or applying N at planting did not significantly influence tissue N. However, applying N mid-season did increase tissue N concentration compared to an at-planting application. This is likely due to the timing of tissue samples taken.

2020 LCB Dryland

The Lake Carl Blackwell dryland location was planted on May 6th. The total amount of rain during the months of May through October was 498 mm (20in) (Table 5). The average monthly air temperature was 22˚C (72˚F) (Table 6). Soil type at this location was classified as a Port-Oscar complex with 0-1 percent slopes and occasionally flooded (NRCS, 2021). Pre-plant composite soil sample analysis results are outlined in Table 2. The average soil pH at this site was optimal at 7.26. The previous year cropping history was a fallow system and this field did not have a history of soybeans for at least 3 years. Treatment means and contrast results are noted in Table 17 while interaction and main effect model results are noted in Table 18.

Nodule counts were taken on July 1st. Nodule counts at this location were much less compared to Bixby and Haskell. Primary root nodule counts ranged from 1.6 to 5.0 nodules per plant. The highest number of primary root nodules was observed in TRT 4, which contained a double inoculant rate. The lowest number of primary root nodules occurred without a seed treatment with N applied at planting. Based on contrast analysis, using a single or double inoculant rate was not different from the check. However, a decrease in nodules when N was applied was noted (α=0.01). In further analysis regarding main effects, it was found that applying an inoculant significantly increased primary root nodule counts while applying N at planting reduced primary root nodule counts (α=0.01).

Lateral root nodules were also notably less at this location compared to Bixby and Haskell. Nodule counts of these roots ranged from 1.0 to 3.5 nodules per plant. The lowest nodule count occurred in TRT 12, which had both Mo and inoculant seed treatment in addition to an at-planting N application. Treatment 13 contained a Mo and single inoculant treatment in addition to a mid-season N application and resulted in the greatest number of lateral root nodules. However, because the mid-season N was not applied until after nodule counts were taken, this result can be related to seed treatment applied only. It is also noted that TRT 6 contained the same seed treatments and resulted in 2.9 lateral nodules per plant. Significant effects were not observed amongst of the contrast comparisons. However, main effect analysis indicated that applying N at planting noticeably decreased lateral root nodule numbers (α=0.01). The same result was found when evaluating total nodule counts (α=0.01).

Grain yield ranged from 1.9 to 4.9 Mg ha^{-1}. Shattering was noted at this site upon harvest. Very dry harvest conditions led to small yield losses as plots were mechanically harvested. The lowest yield was present in TRT 8, which included a Mo seed treatment and N application at planting. The highest yield was achieved with a double inoculation rate. An important note is that this treatment also had the most primary root nodules. Neither seed treatment nor N application had a significant impact on grain yield.

Furthermore, through specific treatment contrasts, no differences were found between treatments.

Seed protein concentration at this site varied from 35.5 to 37.0 %. The lowest protein concentration occurred in TRT 5, where only an N application at planting was made without a seed treatment. The highest protein concentration was recorded in both the check treatment and TRT 6. This treatment consisted of a Mo and double inoculant rate. Seed treatment did not significantly impact seed protein but applying N at planting significantly decreased protein concentration overall (α=0.05). When comparing the check treatment to applying N at planting alone, protein concentration significantly decreased by 2.5% (α=0.01). Similarly, in main effect analysis, applying N at planting reduced seed protein overall (α=0.05). The timing of N application was also found to be significant, whereas applying N mid-season improved protein concentration compared to an at-planting N application (α=0.05).

Seed oil concentration varied slightly from 21.1 to 22.5 %. The lowest oil concentration observed was in TRTS 4 and 5, where a double rate of inoculant and an N application at planting was applied, respectively. The greatest oil concentration was achieved when both a Mo and single inoculant seed treatment was used. Contrast nor main effect analysis resulted in any significant differences.

Tissue N concentration for this location ranged from 4.2 to 4.7 nodules per plant. Treatment 7, which included a Mo and double inoculant rate application, resulted in the lowest N concentration, whereas a double inoculant rate with N applied at planting resulted in the highest percentage (TRT10). Little differences in tissue N concentration existed, leading to no significant effects of the treatment components evaluated.

2020 Lake Carl Blackwell Irrigated

The Lake Carl Blackwell irrigated location was planted on May 6th. The total amount of rain during the months of May through October was 498 mm (20in) (Table 5). The average monthly air temperature was 22˚C (72˚F) (Table 6). Due to irrigation system issues and normal average rainfall received, this site was never irrigated this year. Soil type at this location was classified as a Port-Oscar complex with 0-1 percent slopes and occasionally flooded (NRCS, 2021). Pre-plant composite soil sample analysis results are outlined in Table 2. The average soil pH at this location was 5.67, notably less than the dryland site. This site was fallow prior to this planting season and was out of soybeans for at least 3 years (Table 3). Treatment means and statistical results are noted in Table 19 while interaction and main effect model results are noted in Table 20.

Nodule counts were taken on July 1st. The number of nodules per plant at this site was lower compared to the LCB dryland site. This location also showed the greatest response to seed treatments out of all site years. Primary root nodule count ranged from 0.2 to 7.4 nodules per plant. The lowest nodule count was found in the check treatment, while the highest recorded number was with a Mo and double inoculant rate seed treatment. In single contrast comparisons, applying a seed treatment of any kind significantly improved nodule number (α=0.0001). Applying Mo alone was not significantly different from the check. However, the use of a single or double rate of inoculant greatly improved nodule numbers compared to both the check and Mo treatment. Although, when comparing the two inoculant rates, there was no significant benefit to using a higher rate (α=0.05). When both a Mo and single or double rate of inoculant was used, nodule numbers were significantly greater than when either of the products or rates were applied alone.

For lateral root nodules, average nodules per plant ranged from 0.7 to 7.8. The check treatment had the lowest number of nodules, whereas TRT 6 that used both a Mo and single inoculant rate resulted in the highest number of nodules. Significance was not noted in lateral root nodules for the main effects at this location. Applying a seed treatment vs. not applying one was not significant. Lateral root nodule numbers were significantly reduced, when N was applied at planting vs. no application (α=0.01). As was found in primary root nodule analysis, combining Mo and either a single or double rate of inoculant improved lateral nodule numbers compared to applying any of those treatments alone (α=0.05). It was also noted that Mo and N at planting had a significant interaction (α=0.05).

Grain yield results at this site were higher compared to the dryland site. Yield ranged from 3.0 to 4.8 Mg ha^{-1}. The lowest grain yield was noted in TRT 8, where a Mo and N application was applied. The highest grain yield was achieved using a single rate inoculant with an N application (TRT 9). The use of a seed treatment, or N application did not significantly impact grain yield through contrast analysis. However, as a main effect, using an inoculant did significantly increase grain yield overall (α=0.05).

Seed protein concentration at this location ranged from 35.1 to 38.2 %. The lowest protein concentration occurred in TRT 8, consisting of a Mo seed treatment and applying N at planting. Seed protein was greatest in TRTS 7 and 13. These treatments consisted of a Mo and double rate inoculant with an N application at planting and a Mo and single rate inoculant with an N application mid-season. Applying a seed treatment generally improved seed protein compared to using untreated seed (α=0.05). When comparing specific treatments, neither the single nor double inoculant rate significantly improved seed protein compared to the check. However, in main effect analysis, it was noted that the use of an inoculant significantly improved seed protein concentration overall (α=0.01).

Seed oil concentration ranged between 21.1 and 23.2 %. The lowest oil concentration was found in TRT 6, which contained a Mo and single inoculant rate. Alternatively, this same treatment had one of the higher protein concentrations. The highest oil concentration was seen in TRT 8 with a Mo and at planting N application. It was noted that applying a seed treatment of any kind significantly decreased seed oil concentration (α=0.01). Of the seed treatments, the individual treatments of Mo, single inoculant, and double inoculant were all considerably lower in oil concentration compared to the check (α=0.01) (α=0.05) (α=0.05). When comparing the seed treatments to one another, however, no significance was found. Through main effect analysis, it was further noted that the use of an inoculant significantly decreased seed oil overall (α=0.05).

Tissue N concentration at this location varied from 4.3 to 5.1 %. The lowest N concentration was found when using a Mo seed treatment only. When a Mo treatment was used combined with a single inoculant rate and an at-planting N application, the highest tissue N concentration was achieved. At this location, both a single and double inoculant rate resulted in substantially greater tissue N concentration than using Mo (α=0.05) (α=0.01). However, neither inoculant rate significantly differed from the check treatment. It was also noted that applying N with a seed treatment vs. not applying greatly improved tissue N concentration (α=0.05). This was also illustrated in main effect analysis (α=0.01). Moreover, the use of an inoculant as a main effect was noted to increase tissue N concentration (α=0.05).

DISCUSSION

Nodule Counts

Primary root nodule counts, exhibited a limited seed treatment response across all locations. Using an inoculant significantly increased primary root nodule formation in one out of the seven site-years. Although there was a limited significant response, a general trend was noted that using an inoculant resulted in more primary root nodules compared to the check in six out of the seven site years. Additionally, the Mo seed treatment did not significantly affect primary root nodule formation at any location. However, in four out of the five site years the addition of Mo did numerically increase primary root nodule counts compared to the check.

Lateral root nodule numbers were influenced by the application of N as opposed to seed treatment effects. Three out of the seven site years noted significant decreases in lateral root nodules when N was applied at planting. Moreover, all site years documented a decrease in lateral root nodules where N was applied at planting. Contrastingly, inoculant and Mo treatments rarely had a significant effect. This finding is consistent with previous work, which also noted that seed treatments influence primary root nodules, whereas lateral root nodules are more influenced by native soil microbes (Stanton, 2011).

Of the main effects evaluated in this study, it was noted that the application of N at planting was the variable that significantly impacted the total nodule number per plant. A decrease in total nodule number was noted across all site years when N was applied at planting compared to no application while three out of the seven site years were significant (Figure 2). This finding is consistent with several other studies. McCoy et al. (2018) noted a 52% decrease in the number of nodules per plant when applying 45 or 135 kg N ha^{-1} at the V4 growth stage compared to the check. Furthermore, Beard and Hoover (1971) noted a linear decrease in root nodule number as applied N rate increased. Kaschuk et al. (2016) also noted a linear decrease in soybean nodule number with increasing N rates.

Neither Mo nor inoculant, when evaluated as a main effect, had a significant influence on total nodule count. However, at various locations, Mo, a single inoculant rate, or a double inoculant rate significantly increased total nodule numbers compared to the check treatment (Figure 3). It was apparent that applying a seed treatment in general, did not significantly increase nodule counts. Still, it was noted that higher nodule counts were observed when a seed treatment was applied compared to the check, numerically and were largely influenced by location. Results illustrated in Figure 4 note that when all site years are combined, the double inoculant rate resulted in the greatest number of nodules per plant. Carciochi et al. (2019) also found limited significant response to different inoculation methods (seed and soil applications) in soils that commonly had a history of soybean production. Furthermore, the authors noted common nodule count levels of 30-60 nodules per plant dependent on inoculation treatment. These numbers are very similar to our present findings suggesting that native soil microbial communities were present and sufficient enough to aid in the BNF process.

Moreover, when observing results of total nodule counts from a soil properties point of view, it was noted that locations with a pH of 7 or greater did result in higher nodule counts compared to locations with a pH below 7, except the LCB irrigated location in 2020. This is commonly due to the lower survival rate of bacterial strains within acidic soil conditions (Graham, 1992). The previous history of soybeans also was noted to influence nodulation. Bixby was noted to have the highest nodule counts during 2020. This location was previously planted to soybeans the prior year. Additionally, Lake Carl Blackwell was noted to have the least number of nodules per plant in 2019 and 2020 and had not had a history of soybeans for at least 3 years. Locations that had a more recent history of soybeans seemed to produce more nodules compared to sites that had been out of a soybean rotation for 3 or more years.

Grain Yield

A wide range of yield environments was evaluated in this study, including irrigated and dryland environments throughout different northern Oklahoma regions. Average environmental grain yields ranged from 2.68 to 4.29 Mg ha^{-1}. The application of

N was not noted to significantly affect grain yield at any location (Figure 5). Similar work conducted by Albareda et al. (2009) evaluated inoculating soybean seed as well as the impact of applying 50 kg N ha^{-1} during the reproductive growth stages. The findings of this study indicated that applying N later in the season had no additional benefit compared to seed inoculations alone. Furthermore, similar results were found by Kaschunk et al. (2016) that evaluated both basal and top dressing of N in soybean. Moreover, Wesley et al. (1998) theorized that yield benefits of N applications in soybeans are more common in high yielding environments. Due to certain weather conditions of the southern Great Plains, the environment of the present study may have restricted optimal plant growth and, therefore, limited yield response. It also should be noted that the Bixby location had higher levels of soil N present in the soil, which can limit fertilizer response. This does not explain results from the other locations with inherently low soil N content; although, a lack of yield response in deficient N soils has been noted in previous work. A study conducted in Iowa which consisted of converting a grass pasture to soybean production, noted that applying N did not significantly improve soybean yield (Diaz et al., 2009). Soil tests in that experiment were noted at 4.3 mg kg^{-1} of NO_3^-, very similar to our present study, and encompassed N rates ranging from 0 to 280 kg N ha^{-1}. Furthermore, recall that P and K levels at the LCB location both years were adjusted to meet 100% sufficiency if needed, whereas the other locations were not. This likely had little influence on overall results due to the fact that P and K were not limiting at this location as indicated by soil test results. However, a K deficiency was noted at Haskell visually during the season and confirmed in pre-plant soil test results (Table 2). Previous literature has indicated that lower rates of P and K can be used with inoculation to improve micronutrient and macronutrient uptake of soybeans (Nyoki and Ndakidemi, 2018). It is uncertain how this deficiency impacted grain yield results at this site.

When comparing treatments that included a seed treatment in general to those that did not, no significant increase in yield was recorded. When comparing all three single seed treatments to the check, an increase in grain yield was only noted when applying a double inoculant rate at the LCB irrigated site in 2019 (Figure 6). Moreover, it was noted that irrigated environments tended to have a greater yield. In those environments, Lake Carl Blackwell and Bixby, the use of inoculant when analyzed as the main effect was seen to significantly increase yield (α=0.05). These site years with notable differences in yield due to inoculant use, however, did not have a significant increase in nodule number per plant. The Haskell location in 2020 noted a significant decrease in yield where an inoculant was used. This may be due to challenges with the native soil microbial communities or other soil properties. Again, previous cropping history of the present sites could aid in explaining why limited yield response was seen. All locations did have a previous history of soybeans within the past five years. Work by Leggett et al. (2017) noted that in Argentina, yield response due to seed inoculation was more common in fields without a prior history of soybean production. When combining all site years, grain yield recorded was highest with the Mo and then double inoculant rate, respectively (Figure 7).

Seed Protein Concentration

Results of seed protein were highly variable across all site years. The LCB irrigated locations in 2019 and 2020, which had significant increases in yield due to inoculation, showed significant increases in protein concentration as well due to inoculation. However, other locations showed no significance. This could indicate that higher yielding environments are more likely to exhibit a boost in protein concentration when seeds are inoculated compared to lower yielding environments. The Mo seed treatment did not have a significant effect on seed protein except at Bixby in 2019. At this location, Mo negatively impacted grain protein. Another location, LCB dryland in 2020, found that the application of N at planting decreased seed protein. Overall, the results of this two-year study indicate a minimal significant influence of seed treatment or N application on seed protein of soybeans.

It should be further noted that seed quality or concentration parameters are highly influenced by environmental factors. A meta-analysis conducted by Rotundo and Westgate, 2009 expressed that protein and oil concentration of soybean is affected by water and temperature stress. Soybean protein concentration was found to be less impacted than oil concentration by water stress. Often higher temperature environments resulted in greater protein concentration. This could explain why higher protein concentrations were noted within the present Oklahoma environment of this study.

Seed Oil Concentration

Average seed oil concentration across locations was consistent, excluding the Lahoma and LCB location in 2019, which exhibited notably lower oil percentages at 16.6

and 16.5%, respectively. Both of these locations experienced water stress conditions during the month of July which could attribute to this result. As noted before, water stress can significantly decrease oil concentration of soybeans (Rotundo and Westgate, 2009). The main effects of Mo and N application at planting were not significant at any location. The use of an inoculant was found to significantly decrease seed oil concentration at the LCB irrigated location in 2020. At this location, seed protein was significantly increased by inoculating the seed. Previous literature has noted that soybean protein and oil concentration exhibit a negative linear relationship (Hymowitz et al., 1972). This is consistent with our results in that locations that resulted in higher seed protein concentrations generally exhibited lower oil concentration.

Tissue N Concentration

Variable results were noted across site-years for tissue N. It was noted that the application of N at planting significantly decreased tissue N concentration in two out of the seven site-years and increased tissue N concentration at one location. Furthermore, Mo was noted to significantly increase tissue N concentration at two of the seven locations in 2019. Being that tissue samples were only taken once during the growing season, quantifying the impact of N applications on tissue N in soybeans is challenging. Furthermore, measuring total plant biomass N may have offered a better estimation of plant N concentration as opposed to only tissue analysis. However, this was not included in the objectives of this study.

CONCLUSIONS

The use of a Mo seed treatment did not significantly improve nodulation nor grain yield of soybean within this study conducted. However, the use of an inoculant increased nodule numbers at most locations, with the higher rate resulting in the highest counts. When higher grain yields were achieved, the use of an inoculant was seen to significantly increase yield compared to treatments without inoculation. Similar findings were noted with seed protein as well. Furthermore, applying 45 kg N ha^{-1} reduced the average number of nodules per plant more commonly in dryland environments than irrigated conditions. The application of N at planting, regardless of the effect on nodulation, did not significantly impact grain yield. Nodule formation response to the use of an inoculant was inconsistent, indicating potential differences in native soil microbial communities.

In conclusion, evaluation of soybean inoculants among different environments will continue to be important in order to better understand management influences on BNF. Yield boosts from inoculants may only be seen in high yielding years but applying seed inoculants will minimize the risk of nodulation failure. Moreover, due to limiting environmental conditions, applying 45 kg N ha^{-1} is not recommended based on the lack of yield response whereas applying N as opposed to inoculation methods had no added benefit. It is hypothesized that BNF can meet the needs of a soybean crop in this environment based on the findings of this experiment.

TABLES

Table 1. Treatment structure as applied at Bixby, Lahoma, Lake Carl Blackwell, and Haskell during 2019 and 2020.

Treatment	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})
1	0	0	0	0
2	142	0	0	0
3	0	142	0	0
4	0	284	0	0
5	0	0	45	0
6	142	142	0	0
7	142	284	0	0
8	142	0	45	0
9	0	142	45	0
10	0	284	45	0
11	142	142	45	0
12	142	284	45	0
13	142	142	0	45

Table 2. Pre-plant soil test characteristics (0-15 cm) at Bixby, Lake Carl Blackwell, Lahoma, and Haskell, OK. 2019 and 2020.

Year	Location	pH	NH_4^+-N	NO_3^--N	P	K	SO_4^{2-}-S	Ca	Mg	Mo	Zn	Fe	TN	OM
			mg kg^{-1}										%	%
2019	Bixby	6.7	1.9	15.6	41.2	114.7	3.1	776.0	166.9	<0.04	0.7	16.9	0.05	1.33
2020	Bixby	6.9	12.0	23.4	50.7	121.7	3.7	1083.6	217.4	<0.04	0.8	17.1	0.06	1.39
2019	LCB Irrigated	5.8	4.5	1.3	16.4	116.9	7.8	952.3	270.9	<0.04	0.5	34.4	0.06	1.46
2020	LCB Irrigated	5.7	3.1	5.3	33.0	119.0	6.8	765.1	236.9	<0.04	0.7	39.5	0.07	1.64
2020	LCB Dryland	7.3	3.7	4.5	73.0	118.8	6.1	1233.9	415.4	<0.04	0.8	27.5	0.09	1.98
2019	Lahoma	6.5	4.5	0.5	30.0	194.6	5.0	1505.6	313.5	<0.04	0.2	13.6	0.07	1.66
2020	Haskell	7.2	3.0	3.7	34.7	38.6	3.7	1045.0	88.0	<0.04	0.5	49.9	0.08	1.71

pH – 1:1 soil:water; NH_4^+-N and NO_3^--N – 2M KCL extract, P and K – Mehlich III extraction, SO_4^{2-}-S -$CaH_4P_2O_8$ extract, Mo, Zn, and Fe-DTPA extract, TN and TC – dry combustion

Table 3. Location descriptions, soil series, and previous cropping history of experiment sites within Oklahoma 2019 and 2020.

Year	County	Site Name	Coordinates	Soil Series	Previous Crop	Years out of Soybean
2019	Tulsa	Bixby	35.964072, -95.863105	Wynona silty clay loam	Soybean	0
2019	Major	Lahoma	36.388220, -98.109942	Grant silt loam	Fallow	1+
2019	Payne	LCB Irrigated	36.148110, -97.289036	Port-Oscar complex	Fallow	3+
2020	Tulsa	Bixby	35.963978, -95.861855	Wynona silty clay loam	Soybean	0
2020	Muskogee	Haskell	35.741097, -95.634694	Taloka silt loam	Wheat	1+
2020	Payne	LCB Irrigated	36.149800, -97.289164	Port-Oscar complex	Fallow	3+
2020	Payne	LCB Dryland	36.149532, -97.288577	Port-Oscar complex	Fallow	3+

Table 4. Field activities by trial location, 2019 and 2020.

	2019			**2020**			
	Bixby	Lahoma	LCB Irrigated	Bixby	Haskell	LCB Irrigated	LCB Dryland
Planting Date	6/13/2019	5/14/2019	5/15/2019	6/19/2020	6/4/2020	5/6/2020	5/6/2020
At-planting Fertilizer	6/13/2019	5/14/2019	5/15/2019	6/19/2020	6/4/2020	5/6/2020	5/6/2020
Nodule Count	7/23/2019	6/27/2019	6/28/2019	8/4/2020	7/24/2020	7/1/2020	7/1/2020
Mid-Season Fertilizer	7/23/2019	7/11/2019	7/11/2019	8/4/2020	7/24/2020	7/2/2020	7/2/2020
Tissue Sampling	7/23/2019	6/27/2019	6/28/2019	8/10/2020	8/4/2020	7/7/2020	7/7/2020
Harvest Date	11/5/2019	10/17/2019	10/16/2019	10/16/2020	10/16/2020	10/1/2020	10/1/2020

Table 5. Total monthly rainfall at each site-year.

Year	Location	Apr	May	June	July	Aug	Sept	Oct	Nov
		mm							
2019	Bixby	123.2	331.5	106.9	101.1	172.2	97.3	193.9	121.7
2019	Lahoma	96.7	320.3	167.4	7.4	177.0	56.9	57.7	30.5
2019	LCB Irrigated	111.0	413.5	102.6	33.3	208.0	163.6	53.6	58.7
2020	Bixby	89.4	150.9	16.3	242.1	101.9	58.7	127.5	27.4
2020	Haskell	86.9	277.1	26.7	145.0	104.9	74.4	71.4	20.6
2020	LCB Irrigated/ Dryland	20.8	62.2	57.9	152.7	51.9	49.0	125.0	26.9

Table 6. Average monthly ambient temperature at each site-year.

Year	Location	Apr	May	June	July	Aug	Sept	Oct	Nov
					˚C				
2019	Bixby	17.0	20.4	24.5	26.7	26.8	25.9	13.9	7.7
2019	Lahoma	14.7	18.2	24.3	27.4	27.2	25.8	12.6	6.6
2019	LCB Irrigated	15.7	19.2	24.1	26.9	27.0	25.8	13.0	6.9
2020	Bixby	14.6	19.2	26.1	27.3	25.3	20.9	14.1	11.9
2020	Haskell	14.2	18.9	25.6	27.0	25.1	20.8	13.7	11.5
2020	LCB Irrigated/ Dryland	14.1	18.8	26.1	27.3	25.2	20.3	13.6	10.8

Table 7. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2019.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	5.8	5.7	11.4	2.7	33.0	19.9	4.2
2	142	0	0	0	7.3	6.1	13.4	2.5	32.5	20.0	4.7
3	0	142	0	0	7.1	7.5	14.6	2.6	33.0	20.1	4.5
4	0	284	0	0	6.1	5.4	11.5	2.9	32.6	20.2	4.3
5	0	0	45	0	4.6	5.9	10.5	2.6	32.7	20.0	4.3
6	142	142	0	0	6.6	5.7	12.2	2.7	32.5	20.1	4.4
7	142	284	0	0	7.1	6.1	13.2	2.5	32.6	20.1	4.4
8	142	0	45	0	6.2	3.9	10.0	2.8	32.4	20.1	4.6
9	0	142	45	0	5.9	4.2	10.1	2.5	32.9	19.8	4.2
10	0	284	45	0	5.7	2.9	8.5	2.8	32.8	19.7	4.5
11	142	142	45	0	6.6	6.6	13.2	2.6	32.5	20.2	4.5
12	142	284	45	0	6.5	3.0	9.4	2.8	32.7	20.2	4.5
13	142	142	0	45	7.8	7.4	15.2	2.9	32.4	20.0	4.6
SED					1.3	1.3	2.1	0.2	0.2	0.2	0.2
CV					28	34	25	11	1	1	5
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	*	ns	*
Mo vs Check, Mo vs Inoc, Mo vs Double Inoc					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	*,*,ns	ns,ns,ns	**,ns,ns
Inoc vs Check, Inoc vs. Double Inoc, Double Inoc vs Check					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	*,ns,ns
Mo & Double Inoc Alone vs combined (2,4 vs 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs combined (2,3 vs 6)					ns	ns	ns	ns	ns	ns	ns
Urea Application vs Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					ns	**	**	ns	ns	ns	ns
Timing of N Application (Trt 11 vs 13)					--------	--------	--------	ns	ns	ns	---------

SED – Standard error difference between two equally replicated means, CV – coefficient of variation,
ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 8. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2019.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	ns	ns	ns	ns	ns	ns	ns
Mo*N at Planting	ns	ns	ns	ns	ns	*	ns
Inoculant*N at Planting	ns	ns	ns	ns	ns	ns	ns
Main Effects							
Mo	ns	ns	ns	ns	**	-----	*
Inoculant	ns	ns	ns	ns	ns	ns	ns
N at Planting	ns	**	**	ns	ns	-----	ns

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 9. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lahoma, OK, 2019.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	5.8	5.6	11.4	3.2	35.1	16.5	4.8
2	142	0	0	0	8.0	9.6	17.5	3.5	35.4	17.4	5.0
3	0	142	0	0	8.0	7.4	15.4	3.5	35.5	16.6	5.0
4	0	284	0	0	9.4	5.4	14.7	3.2	35.1	16.6	5.0
5	0	0	45	0	5.9	2.9	8.8	2.7	34.6	17.9	4.7
6	142	142	0	0	6.1	6.1	12.2	3.3	35.8	15.2	5.0
7	142	284	0	0	6.9	8.4	15.3	3.8	35.2	18.1	5.0
8	142	0	45	0	5.3	3.6	8.9	2.8	35.3	17.8	5.0
9	0	142	45	0	6.7	4.4	11.1	2.1	34.3	18.0	4.7
10	0	284	45	0	9.3	5.7	15.0	3.4	36.5	14.1	4.9
11	142	142	45	0	8.5	5.2	13.7	3.8	35.8	15.3	4.9
12	142	284	45	0	6.9	6.0	12.9	3.3	35.5	15.7	4.9
13	142	142	0	45	8.9	8.1	17.0	4.2	35.3	17.5	5.1
SED					1.6	1.9	2.9	0.5	0.5	1.2	0.1
CV					31	45	31	22	2	10	4
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	ns	ns	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,ns,ns	*,ns,*	*,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					ns,ns,*	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Mo & Double Inoc Alone vs combined (2,4 vs. 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs combined (2,3 vs. 6)					ns	ns	ns	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					ns	**	*	ns	ns	ns	*
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	ns	ns	----------

SED – Standard error difference between two equally replicated means, CV – coefficient of variation, ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 10. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lahoma, OK, 2019.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	ns	ns	ns	ns	ns	*	ns
Mo*N at Planting	ns	ns	ns	ns	ns	ns	ns
Inoculant*N at Planting	ns	ns	ns	ns	*	*	ns
Main Effects							
Mo	ns	ns	ns	ns	ns	-----	*
Inoculant	ns	ns	ns	ns	-----	-----	ns
N at Planting	ns	**	*	ns	-----	-----	*

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 11. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2019.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	0.7	1.2	1.9	3.4	34.7	15.4	4.1
2	142	0	0	0	1.9	2.5	4.4	4.4	35.1	16.9	4.2
3	0	142	0	0	3.2	5.3	8.4	3.9	36.0	15.9	4.4
4	0	284	0	0	2.8	4.8	7.6	5.0	36.4	17.2	4.2
5	0	0	45	0	1.1	2.0	3.1	3.2	34.7	16.1	4.4
6	142	142	0	0	0.9	3.6	4.5	4.6	35.8	16.5	4.4
7	142	284	0	0	1.9	3.8	5.7	4.4	35.4	16.2	4.3
8	142	0	45	0	2.3	2.7	5.0	3.9	35.7	16.2	4.6
9	0	142	45	0	3.2	3.5	6.7	4.7	35.7	16.6	4.4
10	0	284	45	0	3.8	4.0	7.8	5.1	36.2	17.1	4.6
11	142	142	45	0	0.6	2.8	3.4	4.2	35.8	16.7	4.2
12	142	284	45	0	0.8	2.7	3.5	4.9	35.6	17.0	4.3
13	142	142	0	45	0.5	1.6	2.1	4.1	35.0	16.6	4.0
SED					1.5	1.8	3.1	0.7	0.7	0.9	0.2
CV					116	83	90	23	3	8	8
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	ns	ns	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					ns,ns,ns	*,ns,ns	ns,ns,ns	ns,ns,*	ns,ns,*	ns,ns,ns	ns,ns,ns
Mo & Double Inoc Alone vs. Combined (2,4 vs. 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs. Combined (2,3 vs. 6)					ns	ns	ns	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					ns	ns	ns	ns	ns	ns	ns
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	ns	ns	---------

SED – Standard error difference between two equally replicated means, CV – coefficient of variation,
ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 12. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated, 2019.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	*	ns	ns	ns	ns	ns	ns
Mo*N at Planting	ns	ns	ns	ns	ns	ns	ns
Inoculant*N at Planting	ns	ns	ns	ns	ns	ns	ns
Main Effects							
Mo	-----	ns	ns	ns	ns	ns	ns
Inoculant	-----	ns	ns	**	*	ns	ns
N at Planting	ns	ns	ns	ns	ns	ns	ns

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 13. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2020.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At Planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	21.2	17.1	38.2	2.8	36.5	22.4	5.1
2	142	0	0	0	19.7	21.9	41.5	3.1	36.5	22.3	4.9
3	0	142	0	0	19.6	24.0	43.6	2.9	36.8	22.3	5.1
4	0	284	0	0	22.8	27.7	50.4	3.4	36.8	22.0	5.0
5	0	0	45	0	20.6	20.6	41.2	3.0	36.6	22.3	5.0
6	142	142	0	0	24.4	21.1	45.4	2.9	36.6	22.3	5.1
7	142	284	0	0	20.4	19.4	39.8	3.4	36.8	22.3	5.1
8	142	0	45	0	18.0	17.7	35.6	3.3	36.2	22.4	5.1
9	0	142	45	0	19.6	18.1	37.7	3.2	36.4	22.5	4.8
10	0	284	45	0	22.0	24.7	46.7	3.7	36.6	22.1	4.7
11	142	142	45	0	22.7	19.4	42.1	3.0	36.6	22.2	4.7
12	142	284	45	0	20.1	17.9	38.0	3.6	36.3	22.4	4.9
13	142	142	0	45	21.4	20.2	41.6	3.2	36.7	22.4	5.1
SED					2.6	4.2	5.5	0.4	0.3	0.3	0.2
CV					17	28	19	19	1	2	5
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	ns	ns	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					ns,ns,ns	ns,ns,*	ns,ns,*	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Mo & Double Inoc Alone vs Combined (2,4 vs. 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs. Combined (2,3 vs. 6)					*	ns	ns	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					ns	ns	ns	ns	*	ns	ns
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	ns	ns	*

SED – Standard error difference between two equally replicated means, CV – coefficient of variation,
ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 14. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Bixby, OK, Irrigated, 2020.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	*	ns	ns	ns	ns	ns	ns
Mo*N at Planting	ns	ns	ns	ns	ns	ns	ns
Inoculant*N at Planting	ns	ns	ns	ns	ns	ns	ns
Main Effects							
Mo	ns	ns	ns	ns	ns	ns	ns
Inoculant	ns	ns	ns	*	ns	ns	ns
N at Planting	ns	ns	ns	ns	ns	ns	*

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 15. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Haskell, OK, 2020.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	15.0	14.5	29.5	3.0	38.2	22.2	3.7
2	142	0	0	0	12.4	11.3	23.7	3.1	37.8	22.4	3.9
3	0	142	0	0	13.4	10.7	24.1	2.6	38.2	22.3	3.7
4	0	284	0	0	14.4	20.4	34.8	2.8	38.9	21.8	3.7
5	0	0	45	0	13.2	11.4	24.6	3.1	38.6	21.5	3.7
6	142	142	0	0	13.0	10.3	23.6	2.7	37.8	22.6	3.9
7	142	284	0	0	12.8	13.1	25.9	2.7	38.0	22.1	3.8
8	142	0	45	0	13.7	13.5	27.1	3.3	38.2	22.3	3.6
9	0	142	45	0	15.3	22.2	37.5	2.9	37.6	22.3	3.7
10	0	284	45	0	11.9	10.8	22.6	2.3	38.4	22.2	3.6
11	142	142	45	0	5.5	8.4	14.2	2.8	38.1	22.1	3.7
12	142	284	45	0	12.1	13.8	25.8	2.9	37.9	22.7	6.8
13	142	142	0	45	11.1	11.4	22.4	2.9	37.2	22.6	4.0
SED					3.3	5.2	7.5	0.3	0.8	0.5	0.1
CV					37	7	41	16	3	3	5
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	ns	ns	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Mo & Double Inoc Alone vs. Combined (2,4 vs. 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs. Combined (2,3 vs. 6)					ns	ns	ns	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					ns	ns	ns	ns	ns	ns	ns
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	ns	ns	*

SED – Standard error difference between two equally replicated means, CV – coefficient of variation, ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 16. Interactions and main effects primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Haskell, OK, 2020.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	ns	ns	ns	ns	ns	ns	ns
Mo*N at Planting	ns	ns	ns	ns	ns	ns	ns
Inoculant* N at Planting	ns	ns	ns	ns	ns	ns	ns
Main Effects							
Mo	ns	ns	ns	ns	ns	ns	ns
Inoculant	ns	ns	ns	*	ns	ns	ns
N at Planting	ns	ns	ns	ns	ns	ns	ns

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 17. Treatment structure, treatment means, and single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Dryland, 2020.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	3.2	3.2	6.4	2.8	37.0	21.5	4.4
2	142	0	0	0	3.0	2.5	5.5	3.0	36.2	22.1	4.5
3	0	142	0	0	4.8	3.2	8.0	3.4	36.9	21.9	4.5
4	0	284	0	0	5.0	1.8	6.8	1.8	36.6	21.1	4.4
5	0	0	45	0	1.6	1.5	3.1	3.1	35.5	22.1	4.3
6	142	142	0	0	4.7	2.9	7.6	3.3	36.3	22.5	4.4
7	142	284	0	0	4.6	1.9	6.5	2.3	37.0	21.5	4.2
8	142	0	45	0	1.9	1.5	3.3	1.9	36.9	21.4	4.4
9	0	142	45	0	2.9	2.5	5.4	2.9	36.6	21.8	4.6
10	0	284	45	0	4.7	2.4	7.0	2.7	36.5	21.9	4.7
11	142	142	45	0	2.8	1.2	3.9	2.4	35.6	21.9	4.3
12	142	284	45	0	2.3	1.0	3.2	2.0	36.9	21.4	4.5
13	142	142	0	45	3.9	3.5	7.4	2.9	36.8	21.5	4.4
SED					1.1	0.9	1.6	0.9	0.5	0.6	0.2
CV					45	59	40	48	2	4	6
Contrasts											
Applying a Seed Trt vs. Check					ns	ns	ns	ns	ns	ns	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns
Mo & Double Inoc Alone vs. Combined (2,4 vs. 7)					ns	ns	ns	ns	ns	ns	ns
Mo & Inoc Alone vs. Combined (2,3 vs. 6)					ns	ns	ns	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	*	ns	**	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					**	ns	**	ns	ns	ns	ns
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	*	ns	ns

SED – Standard error difference between two equally replicated means, CV – coefficient of variation, ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 18. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Dryland, 2020.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	ns	ns	ns	ns	ns	ns	ns
Mo*N at Planting	ns	ns	ns	ns	ns	ns	ns
Inoculant* N at Planting	ns	ns	ns	ns	ns	ns	ns
Main Effects							
Mo	ns	ns	ns	ns	ns	ns	ns
Inoculant	**	ns	ns	ns	ns	ns	ns
N at Planting	**	**	**	ns	*	ns	ns

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 19. Treatment structure, treatment means, single-degree-of-freedom contrasts for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated 2020.

Trt	Mo Rate (g 45 kg^{-1} seed)	Inoculant Rate (g 45 kg^{-1} seed)	At-planting N Rate (kg N ha^{-1})	Mid-Season N Rate (kg N ha^{-1})	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
1	0	0	0	0	0.2	0.7	1.0	4.3	35.7	22.8	4.6
2	142	0	0	0	1.6	2.3	3.9	3.8	37.2	21.7	4.3
3	0	142	0	0	5.8	3.7	9.5	4.3	37.4	21.8	4.7
4	0	284	0	0	4.2	2.5	6.7	4.2	37.4	21.8	4.9
5	0	0	45	0	0.9	4.5	5.3	3.8	36.1	22.2	4.9
6	142	142	0	0	6.8	7.8	14.5	4.0	38.1	21.1	4.8
7	142	284	0	0	7.4	6.1	13.4	4.3	38.2	21.5	4.9
8	142	0	45	0	1.1	1.2	2.3	3.0	35.1	23.2	4.9
9	0	142	45	0	4.2	2.6	6.8	4.8	37.4	22.1	5.0
10	0	284	45	0	2.7	1.7	4.4	4.0	37.4	21.9	4.9
11	142	142	45	0	3.4	2.4	5.8	4.1	37.2	22.1	5.1
12	142	284	45	0	2.2	1.0	3.1	4.4	36.8	22.4	4.9
13	142	142	0	45	7.1	6.0	13.1	4.0	38.2	21.5	4.9
SED					1.1	2.1	2.8	0.5	0.9	0.4	0.2
CV					42	3	4	17	4	3	6
Contrasts											
Applying a Seed Trt vs. Check					***	ns	**	ns	*	**	ns
Mo vs. Check, Mo vs. Inoc, Mo vs. Double Inoc					ns,**,*	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	**,ns,ns	ns,*,**
Inoc vs. Check, Inoc vs. Double Inoc, Double Inoc vs. Check					***,ns,**	ns,ns,ns	**,ns,*	ns,ns,ns	ns,ns,ns	*,ns,*	ns,ns,ns
Mo & Double Inoc Alone vs. Combined (2,4 vs. 7)					***	*	**	ns	ns	ns	ns
Mo & Inoc Alone vs. Combined (2,3 vs. 6)					**	*	**	ns	ns	ns	ns
Urea Application vs. Check					ns	ns	ns	ns	ns	ns	ns
Seed Trt with Urea Application vs. Seed Trt Alone					***	**	**	ns	ns	**	**
Timing of N Application (Trt 11 vs. 13)					--------	--------	--------	ns	ns	ns	ns

SED – Standard error difference between two equally replicated means, CV – coefficient of variation,
ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

Table 20. Interactions and main effects for primary root nodules, lateral root nodules, total nodules, grain yield, seed protein, seed oil, and tissue N concentration, Lake Carl Blackwell, OK, Irrigated 2020.

	Primary Root Nodule Count	Lateral Root Nodule Count	Total Nodule Count	Grain Yield (Mg ha^{-1})	Seed Protein (%)	Seed Oil (%)	Tissue N (%)
Interactions							
Mo*Inoculant	ns	ns	ns	ns	ns	ns	ns
Mo*N at Planting	*	*	**	ns	*	**	ns
Inoculant* N at Planting	**	ns	*	ns	ns	ns	ns
Main Effects							
Mo	-----	-----	-----	ns	-----	-----	ns
Inoculant	-----	ns	-----	*	**	*	*
N at Planting	-----	-----	-----	ns	-----	-----	**

ns, *,**,***, not significant, significant at the 0.05, 0.01, 0.0001 probability levels respectively.

FIGURES

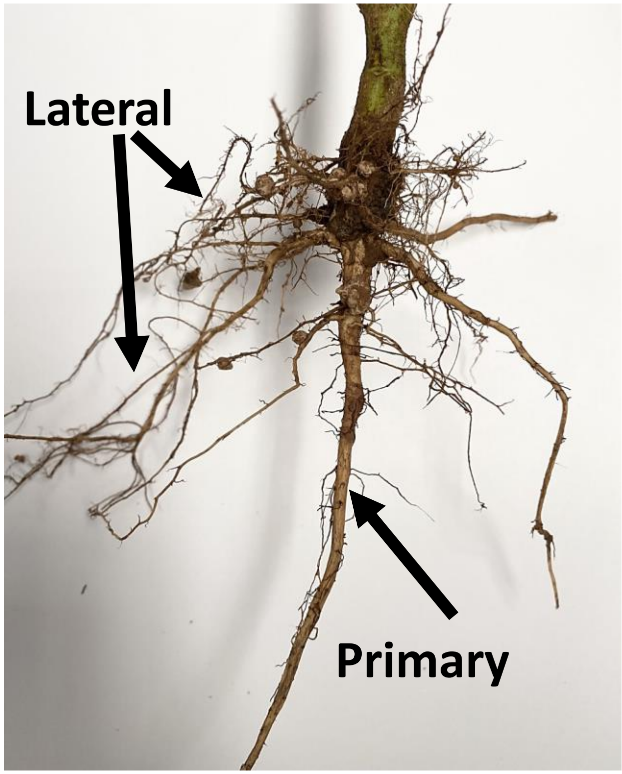

Figure 1. Soybean root architecture as described.

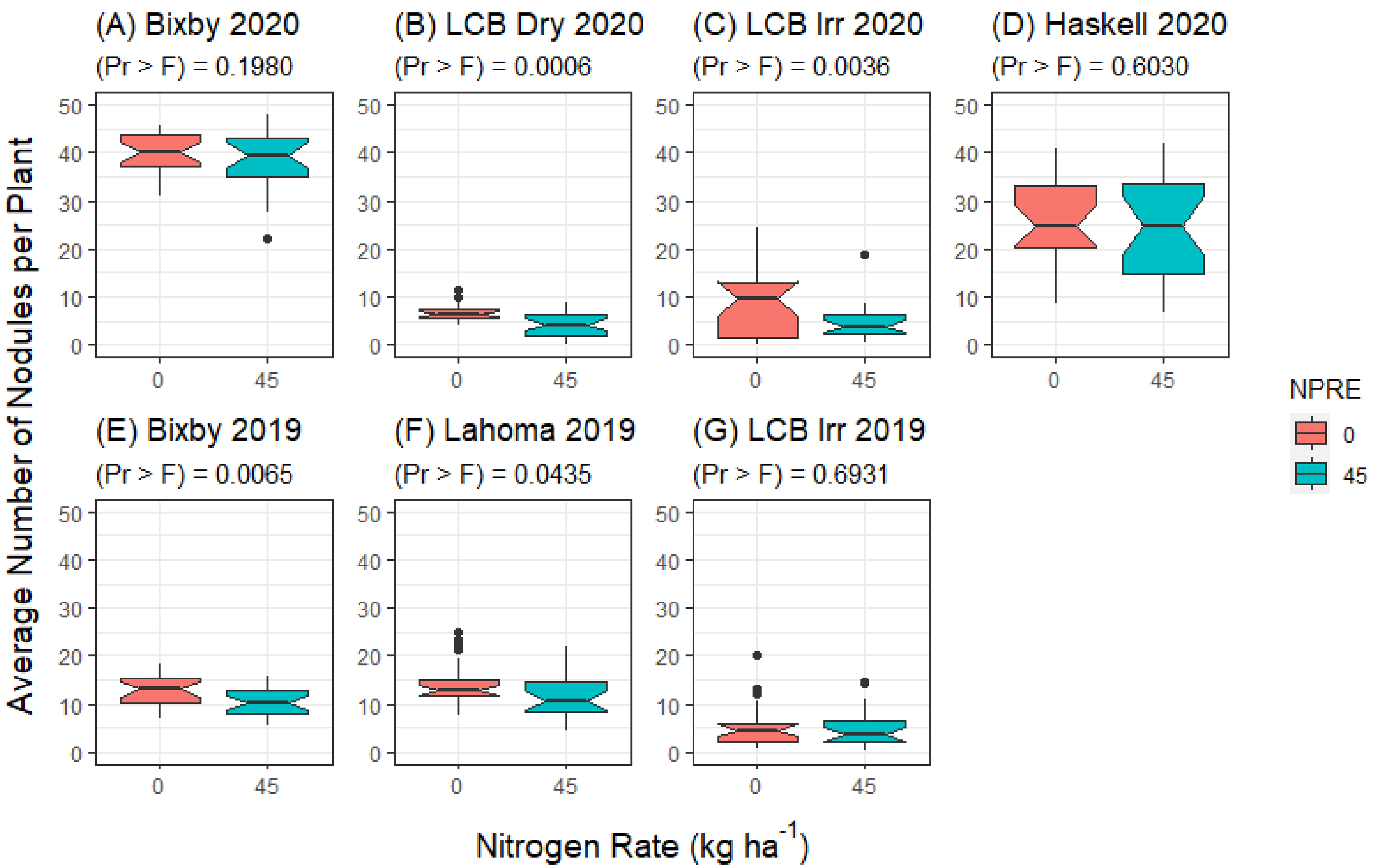

Figure 2. The average number of nodules per plant, by planting N rate (0, 45 kg N ha^{-1}) for each site year. Four of the seven site years noted significant decreases in nodule numbers per plant when 45 kg N ha^{-1} was applied at planting.

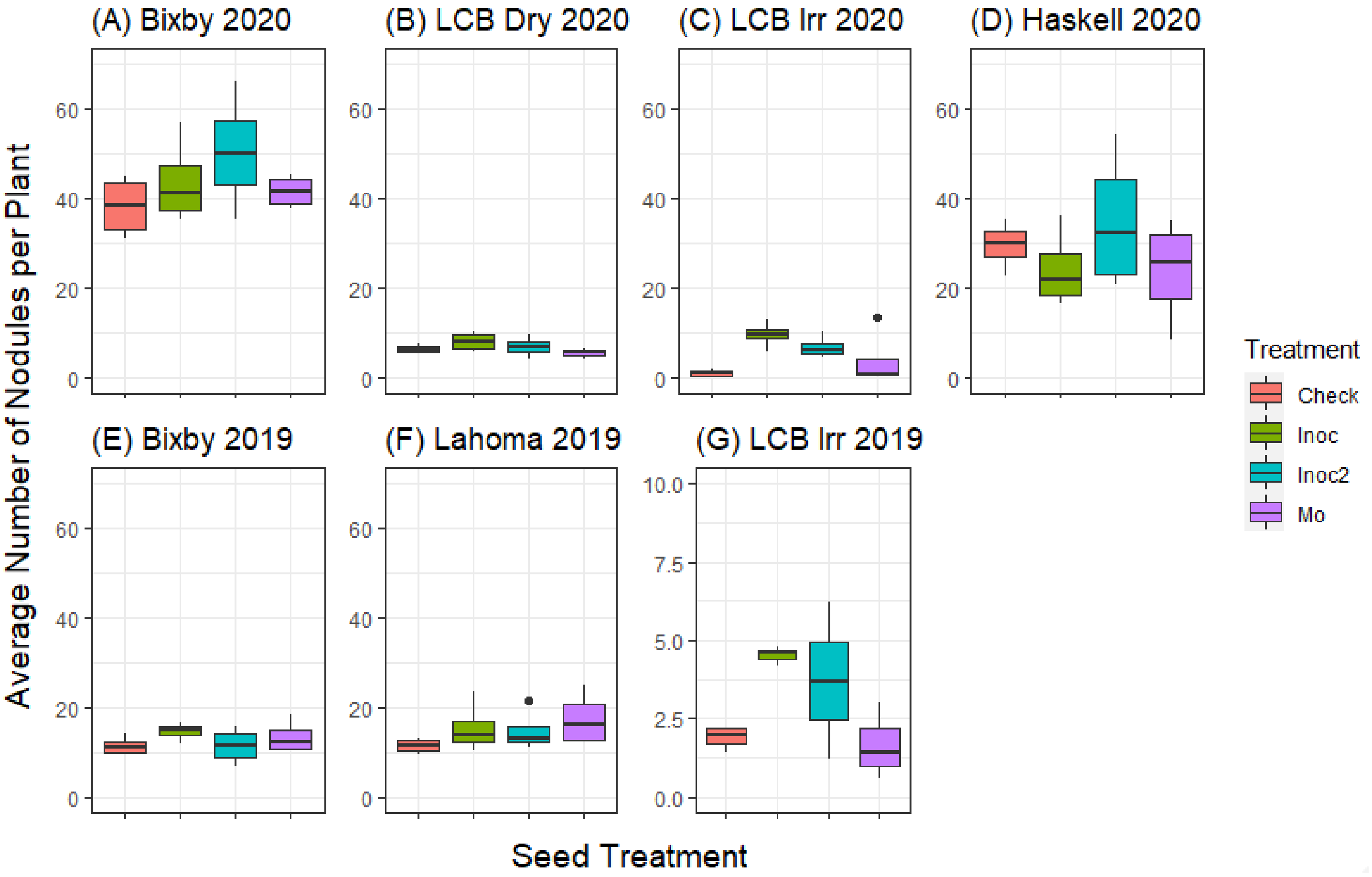

Figure 3. The average number of nodules per plant for the check, single inoculant, double inoculant, and molybdenum seed treatments for each site year (TRTS 1-4). In general, the use of a seed treatment increased total plant nodulation. The LCB location did not have a prior history of soybeans for at least 3 years and was noted to have the least number of nodules per plant.

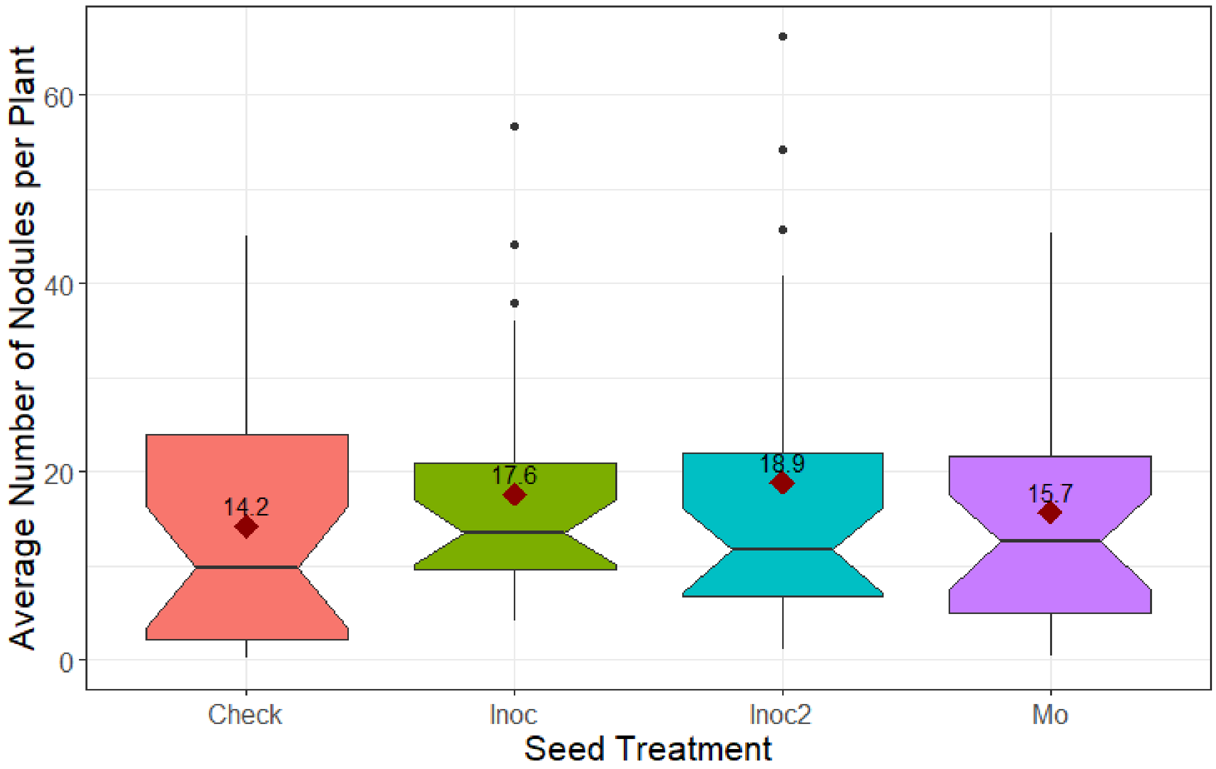

Figure 4. Average number of nodules per plant by seed treatment averaged across all locations. A double inoculant rate commonly resulted in the greatest number of nodules per plant.

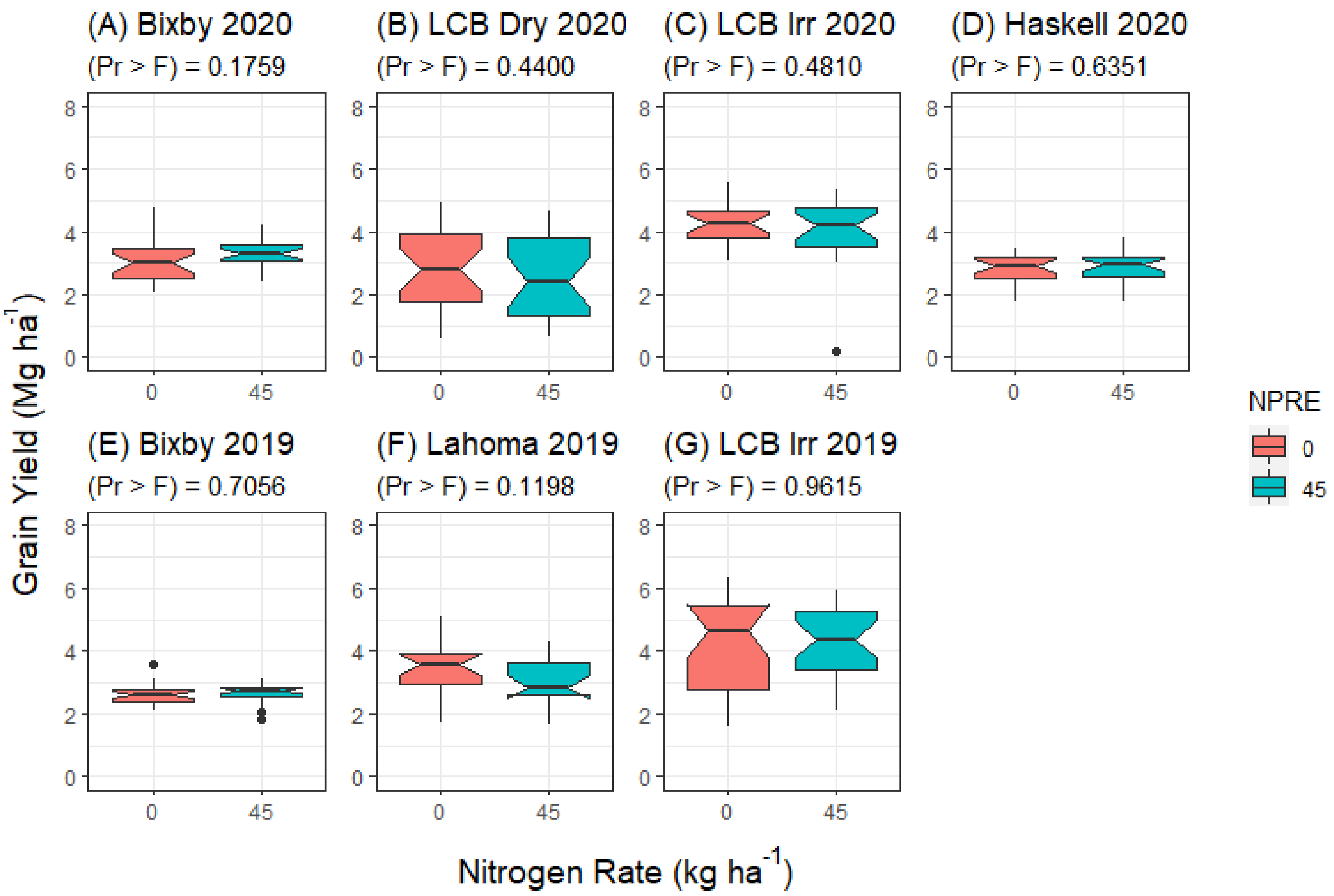

Figure 5. Average grain yield by planting N rate (0, 45 kg N ha^{-1}) for each site year. Applying N at planting did not significantly affect grain yield, although it was common to see a decrease in grain yield when N was applied.

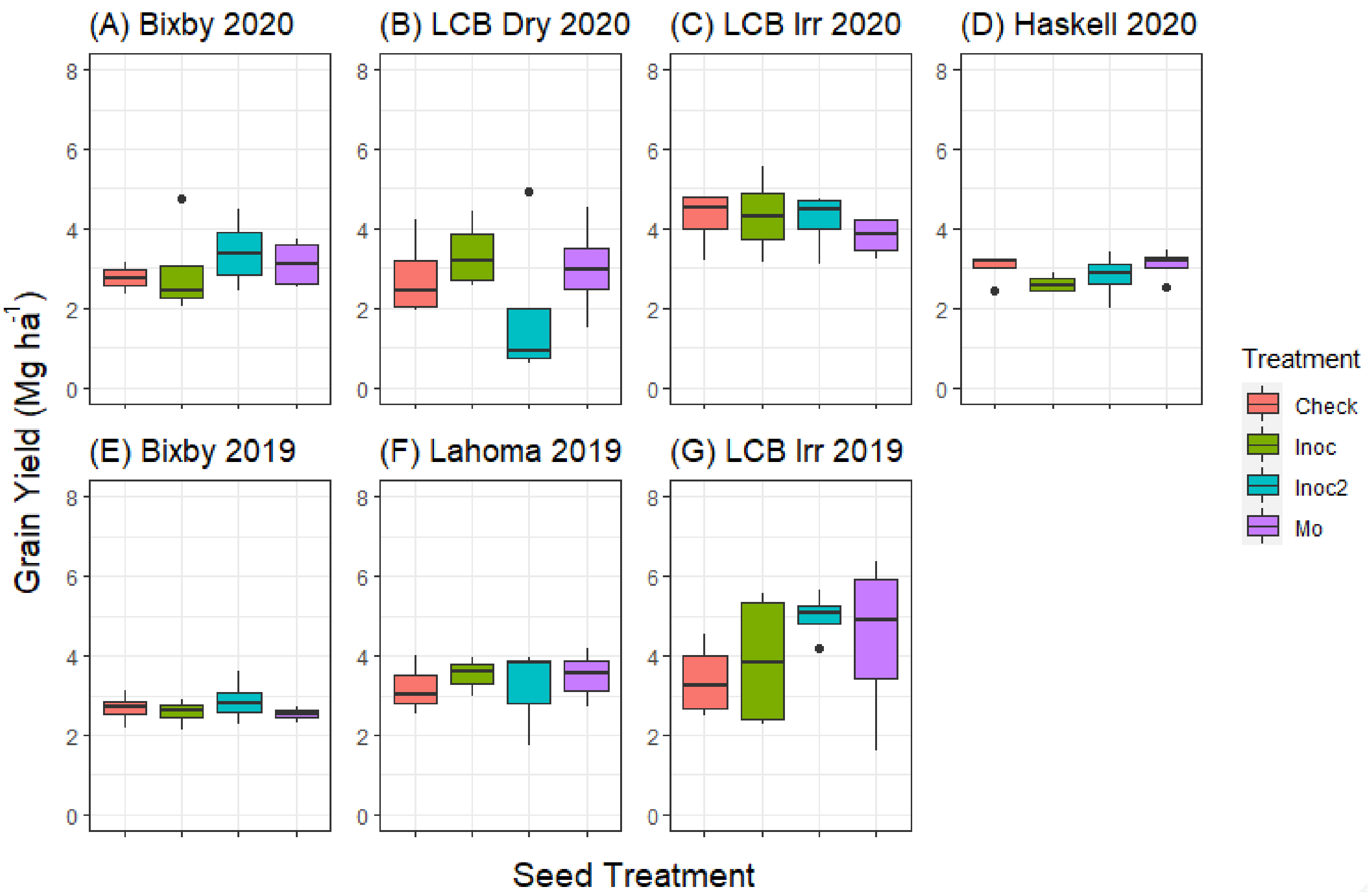

Figure 6. The average grain yield for the check, single inoculant, double inoculant, and molybdenum seed treatments for each site year (TRTS 1-4). The use of inoculant or molybdenum seed treatment did not significantly improve grain yield.

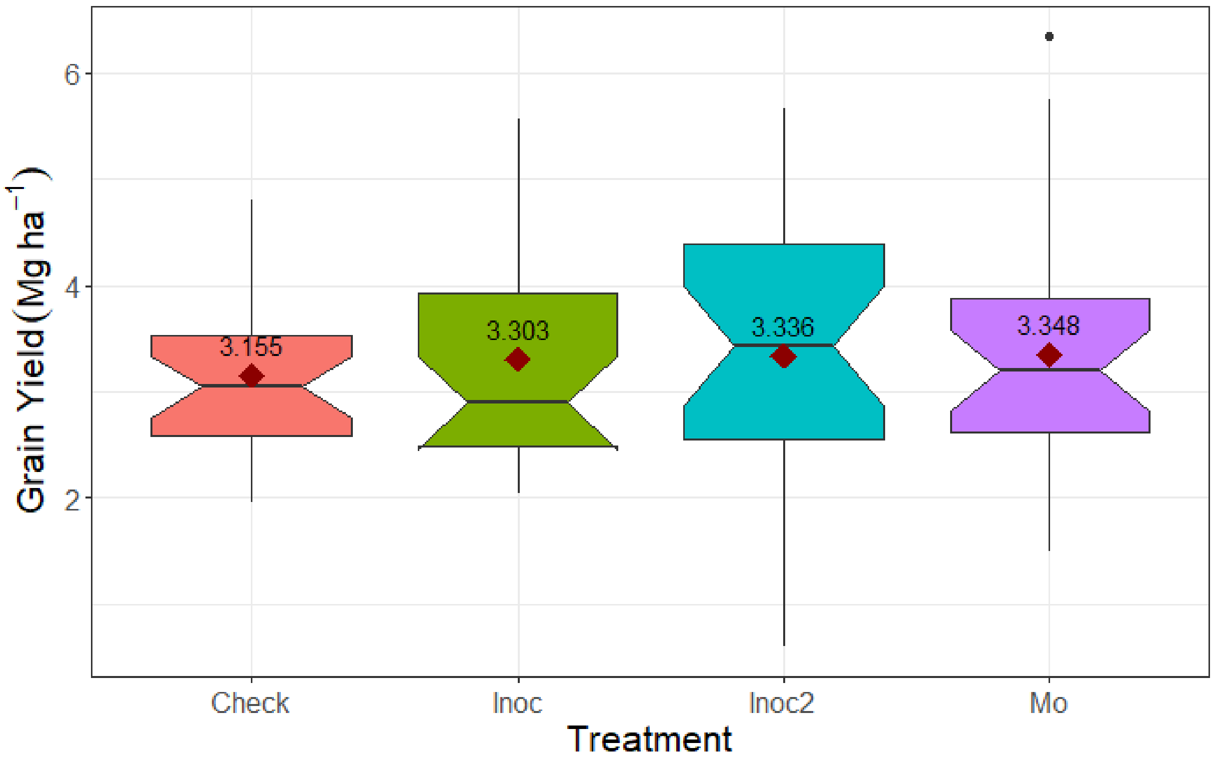

Figure 7. Grain yield for each seed treatment averaged across all locations. The double rate of inoculant and Mo seed treatments resulted in greater yields on average.

CHAPTER II

INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS ON WINTER WHEAT GRAIN YIELD AND QUALITY

ABSTRACT

Winter wheat (*Triticum aestivum*) is extensively grown throughout the state of Oklahoma. Producers commonly apply nitrogen (N) and phosphorus (P) fertilizers each season; however, pre-plant N and P interactions are not well understood. The objective of this study was to evaluate the effects of N, P, and their interactions on winter wheat grain yield, grain N, and N uptake. To evaluate these effects, Experiment 702 was established in 1996 at the Cimarron Valley Research Station near Perkins, OK. This experiment was designed as a randomized complete block design with a complete factorial treatment structure consisting of four pre-plant N rates (0, 56, 112, 168 kg ha^{-1}) and three P rates (0, 14.8, 29.6 kg P ha^{-1}). Twenty years of yield data from this trial were analyzed in addition to grain N and N uptake when data was available. Analysis was first conducted separately for each year and then under different environmental grain yield and in-season precipitation (ISP) parameters. It was noted that P response was more apparent in the

initial harvest years or when more susceptible cultivars to acid soil were used, whereas N rate had a significant impact each year. Furthermore, N and P interactions were more commonly observed in high yielding conditions. It was evident that greater grain N concentrations were noted with the high N rate. Moreover, N uptake was often greater among high yielding environments. Including additional environmental parameters amongst data groups of long-term experiments could provide greater insight into N and P interactions in winter wheat.

INTRODUCTION

Wheat is one of the oldest cultivated crops in the world. Together, wheat, corn, and rice provide 60 percent of the total plant-based calories humans consume worldwide (Environmental Literacy Council). Moreover, winter wheat production constitutes a large portion of crop production acres across the southern Great Plains. In Oklahoma alone, 1.6 to 2.4 million hectares are planted each year (OSU, 2018). In 2018, 1.9 million Mg were produced, generating approximately $360 million in revenue (Marshall, 2018). This names winter wheat Oklahoma's number one cash crop.

Not only is winter wheat a staple food source for a growing population, it is also a vital forage source for cattle producers. The southern Great Plains region is especially noted for dual-purpose winter wheat production systems. In this system, producers graze out wheat fields during their vegetative stage and then pull cattle off in time for the crop to fully mature. High forage yields in early growth stages are often desirable to meet this dual-purpose need and contribute to higher grain yield at maturity. Nitrogen (N) and phosphorus (P) are two critical nutrients needed to meet these goals. It is well noted throughout literature that N and P fertilization is vital to crop performance (Campbell et al., 1995). It is further estimated that 30-50% of crop yields achieved are due to N and P applications (Nelson, 1990). This signifies that substantial yield losses can occur if N or P is limiting.

With the importance of N and P applications well documented, the search for optimum N and P rates in winter wheat production is of high priority. Numerous studies have investigated different rates, timings, and placement methods of applying both N and P to achieve maximum yield. However, a definitive answer remains unknown. This is primarily due to the need for site-specific management of these nutrients to achieve optimum results due to highly variable environments. With high environmental variability in terms of rainfall, soil type or texture, and other soil characteristics, region specific recommendations must be made with these factors in mind. However, achieving optimum grain yield is not the only goal. Limiting environmental impact from N and P leaching or runoff is a priority as well. Further research is needed to refine the optimum N and P rates for winter wheat production while considering temporal environmental factors. Environment often plays a key role in the response of nutrient applications. The United States southern Great Plains is known as the largest area of winter wheat cropland receiving low precipitation amounts worldwide Fischer et al. (2014), therefore, analysis of N and P influences on grain yield under different ISP conditions is valuable. Such interpretations concerning environment interactions with fertilizer N and P can only be truly achieved by analyzing long-term fertility experiments.

REVIEW OF LITERATURE

Function of Nitrogen

Nitrogen is considered one of the most crucial elements required for plant growth. In the form of nitrate, and ammonium Tisdale et al. (1985), plants absorb N. This element is essential for protein formation and is a large component of chlorophyll, which is essential for plant photosynthesis (Tisdale et al. 1985). Nitrogen presents several management challenges with its highly mobile nature in the soil and susceptibility to loss pathways. The N cycle itself is a very dynamic process with numerous environmental factors in play. Often 10-30% of N applied in crop production systems is lost through leaching alone (Meisinger and Delgado, 2002). Surface applications of N via urea also have a high risk of loss due to volatilization.

World nitrogen use efficiency (NUE) for cereal crops was documented at 33% (Raun and Johnson, 1999). Since that initial value was determined, advancements have been made in further refining N management practices in agriculture. More recent literature has stated that the world NUE for cereal crops now stands at 35% (Omara et al., 2019). Furthermore, the authors document that the United States has an NUE of 41%. Although these numbers represent a move in the right direction, the need for continuous research in N management is needed to further secure our world’s food supply and better our environment.

Nitrogen Placement

Several methods of N placement are available to winter wheat producers (Bryant-Schlobohm et al., 2020). The optimum placement method has been a heavily researched topic. Such methods evaluated over time include broadcast, foliar applications, banding, and banding with the seed. A farmer is often limited in the method of application he or she implements due to equipment availability. Furthermore, the timing of N application and the source can play a significant role in the success of a placement method.

Varying results concerning placement methods of N have been observed. One study evaluated banding urea in the fall compared to applying ammonium nitrate in the spring in a winter wheat cropping system. It was found that the fall banded applications resulted in the same or higher grain yield compared to spring applications. Furthermore, the authors note that grain yield results were more related to the NO_3-N levels in the top 60 cm of the soil profile (Karamoanos et al., 2003). Broadcasting methods are often a common practice for winter wheat N applications. Although this can be a convenient method for producers, Blackshaw (2004) cautions that this method can contribute to greater weed competition. His work demonstrated that surface banding or point-injected N were preferable over broadcast N as they contributed to greater grain yields and a reduced field weed seed bank.

Urea ammonium nitrate (UAN) is a common N form applied as a foliar spray. The spring application of UAN is beneficial to yield levels and fertilizer recovery efficiency Dhillon et al. (2020b), whereas the method of delivery (flood nozzles vs. streamer nozzles) does not have a large impact (Edwards et al., 2009). It was also noted that March N applications showed a greater increase in grain yield compared to February applications. An additional experiment noted that foliar applications of N using UAN late season were noted to have little effect on grain yield; however, increases in grain N concentrations were noted (Woolfolk et al. 2002).

Nitrogen Rates

Nitrogen rates needed for winter wheat production are highly variable. Numerous factors affect the optimum N rate, which changes each growing season due to environmental variability. The previous crop planted, yield goal and environmental rainfall are just a few of the factors that influence N needs. The need for optimizing in-season N rates has been well documented in previous literature. Work by Flowers et al. (2004) demonstrated that reduction of N inputs upwards of 48.6% could be achieved by using in-season N rate optimization. This is critical not only for optimizing production and profitability but also for limiting environmental impacts.

Several different methods detailing the optimum N rate during any given growing season are published in research. These methods are always evolving. Two commonly used methods are the sensor-based and the yield goal estimation methods (Raun et al., 2017). Recommendations from Oklahoma State University suggest that producers use the sensor-based method where Normalized Difference Vegetative Index (NDVI) readings are taken from a standard fertilizer rate in the field and compared to an N Rich Strip NDVI value (Raun et al., 2001). The N-rich strip is simply a pass through the field where N is not a limiting factor, generally two times the farmer’s standard N application. By entering these two NDVI values along with the Growing Degree Days (GDD) and yield goal of the field, OSU's online calculator will give producers a recommended mid-season N rate. This calculator is a product of using data from several long-term wheat fertility trials over many years to produce a viable algorithm capable of making in-season yield estimates. Further research has noted the advantage of using quantitative GDD > 0 to be more reliable than subjective morphological scales for making in-season fertilizer application decisions (Dhillon et al., 2020a).

Other N rate recommendation methods only incorporate a simplified equation where yield goal estimation is used, and adjustments for soil test nitrate along with N supplied from the previous crop are made (Kaiser, 2018). This calculation method does not account for environmental parameters during each specific growing season that can often be quantified through NDVI measurements. This hinders optimum production achievement as N needs vary from year to year due to the environment. Walsh et al. (2012) demonstrated how incorporating soil moisture data into in-season yield prediction models can improve its accuracy. This experiment was also conducted over eight years, noting the importance of temporal analysis when working to optimize N rate recommendations. Furthermore, a recent study noted in-season estimate of yield (INSEY), pre-plant N rate, mean air temperature, and total rainfall from September to December, led to an improved final grain yield prediction in winter wheat (Aula et al., 2021).

Function of Phosphorus

Phosphorus is a critical nutrient for plant growth. It is considered the second macronutrient required following N (Tisdale et al., 1985). Phosphorus plays a significant role in energy storage and transfer within plants and is a critical component of ATP and ADP (Tisdale et al., 1985). It is noted that P promotes root and vegetative growth, which can lead to greater grain and forage yields. Phosphorus is primarily taken up in the form $H_2PO_4^-$ but can also be utilized by the plant in the form of HPO_4^{2-} (Tisdale et al., 1985).

Soils are commonly abundant with P; however, only a small portion of this reserve is readily available to plants in soil solution (Clarkson and Grignon, 1991). Numerous soil and environmental factors can affect the availability of P. Of those factors, P concentration in soil solution and P-buffer capacity are often the most influential (Marschner, 1986). Phosphorus is also known to quickly react with other soil nutrients such as calcium, iron, and aluminum and become plant unavailable (Lindsay et al., 1989). Because of P's immobile nature and affinity to bind with other soil elements, placement of P fertilizer is often a topic of study.

Even though P is immobile in the soil, it is still at risk for surface runoff when over applied. This can lead to pollution of waterways. Zhang et al. (2006) demonstrated the importance of site-specific management of P in winter wheat production systems to reduce runoff potential. Additionally, it has been documented that the world P use efficiency (PUE) is estimated at only 16% in cereal grains (Dhillon et al., 2017). At this remarkably low value, P management methods must be continually evaluated.

Phosphorus Placement

Correct nutrient placement is vital to increasing crop yields. Nutrients must be placed in such a way that they are made most available to the plant. However, the best fertilizer placement method can vary greatly depending upon soil characteristics, weather, crop type, and nutrient source. In recent years, moving from conventional tillage systems to no-till operations has triggered more research questions concerning the proper application method of P when soil incorporation is not an option. A P placement study conducted over three years in Texas evaluated the influence of P applications on both forage and grain yield. It was found that applying 20 kg P ha^{-1} increased soil test levels of P by up to threefold at the surface level (Sij et al., 2007). Furthermore, this application of P increased forage production by 18 to 54%, and grain yields improved by 27 to 29% compared to no application of P (Sij et al., 2007). They further noted that broadcast P applications were equally as good as or better than injected applications to boost forage growth. Additional work in Colorado found that the method of placement for P had little effect on wheat grain yield when rates applied were to alleviate a P deficiency already known in the soil (Halvorson and Havlin, 1992). Other studies have shown that broadcast applications of P are often just as effective as dual placement methods in no-tillage systems (Raun et al., 1987; Westerman and Edlund, 1985).

Research has also investigated the effect of applying P with the seed. A few studies have shown that seed placed, and side banded P applications in winter wheat responded remarkably similar in grain yield (Campbell et al., 1996; Karamanos et al., 2003). In other cases, it has been noted that seed applied P resulted in higher grain yields compared to banding P next to the furrow in situations where planting is delayed (Sander and Eghball, 1999). It is suggested that with the seed applications of P give plant roots better access to this nutrient in the early season, when it is most critical for tiller development. Similarly, Fiedler et al. (1989) found that P applications with the seed resulted in a larger grain yield increase per kilogram of applied P than that of broadcast applications.

Previous work has also investigated the usefulness and efficiency of foliar applications of P as a method to alleviate mid-season deficiencies and boost yields. Work by Mosali et al. (2006) showed that applications of foliar P at Feekes 7 increased grain yield compared to treatments without foliar P. A greenhouse study evaluated the impact of foliar N and P applications on winter wheat; however, results indicated that grain yield was increased when both foliar N and P were applied. Yield also increased with foliar N alone, and foliar P by itself had no impact (Alston, 1979). Optimal nutrient management practices are not only concerned with placement methods but application rates as well.

Phosphorus Rates

Phosphorus deficiencies are common across the Great Plains, causing yield losses to occur. In cereal crops, a deficiency of P can cause a reduction in tillers produced and a reduced number of grains per tiller (Grant et al., 1999). Since P is an immobile nutrient in the soil, fertilizer P recommendations are based on soil test P levels rather than yield goal. Soil test index levels can be used to calculate the percent sufficiency of P, which can then be used to calculate a proper P fertilizer rate recommendation. Information from Oklahoma State University indicates that a P soil test index of 65 and above is considered 100% sufficient for small grain crops. Soil test index levels of 0-10 are less than 50%

sufficient and require 29-39 kg ha^{-1} of P (Zhang et al., 2017). Standard recommendations for P requirements of winter wheat are 0.12 to 0.14 kg of P per bushel of grain, dependent upon soil test results (Stewart, 2020).

The timing of the availability of P to plants is critical as well. Research demonstrating the importance of P for early season plant growth dates back to the 1920s. Preliminary culture wheat studies by Gericke demonstrated that early season deficiencies in P greatly limited plant growth but limitations of P after four weeks did not reduce yield (Gericke, 1924). More recent work has found similar results to these pioneer studies. It has been noted that reductions in P availability in barley within the first 24 days of growth caused reductions in plant tillers (Green et al., 1973). Furthermore, consistent findings occurred in winter and spring wheat as maximum tiller, and secondary root production was observed when P was readily supplied during the first 5 weeks of growth (Boatwright and Viets, 1966).

Use of Long-term Studies to Quantify Environmental Effects

In order to fully grasp N and P interactions with the environment, the analysis of long-term studies is critical. Barnett et al. (1995) explained that long-term experiments increase in value as they can often serve as the database of various sustainable agriculture parameters. Furthermore, Camara et al. (2003) stated that long-term agriculture experiments might be the only way to determine management practice sustainability and shed light on wider crop production trends. Such management practices often include the evaluation of tillage practices. A similar approach can also be used to estimate the sustainability of monoculture wheat production under continuous N and P fertilization. Evaluation of environmental parameters such as rainfall, temperature, and soil moisture on N and P response overtime can provide producers with more defined N and P recommendations.

OBJECTIVE

The objective of this research is to evaluate N and P interactions in winter wheat overtime and their influence on grain yield, grain N, and N uptake. An additional objective of this analysis was to assess the influence of N and P rates on grain yield, grain N, and N uptake under different environmental grain yield and in-season precipitation parameters.

MATERIALS AND METHODS

Treatment Structure and Experimental Design

A long-term experiment was established in 1996 at the Cimarron Valley Research Station located in Perkins, OK. A complete factorial arrangement of treatments was implemented in a randomized complete block design with three replications (Table 21). Plot sizes were set at 3.05 x 9.14 m with a 9.14 m alley in between each replication. This trial consists of twelve treatments of differing combinations of pre-plant N and P rates. Nitrogen rates include 0, 56, 112, and 168 kg N ha^{-1} (0, 50, 100, 150 lbs N ac^{-1}). Phosphorus rates included 0, 14.8, and 29.6 kg P ha^{-1} (0, 30, and 60 lbs P_2O_5 ac^{-1}).

Nitrogen was applied as a pre-plant broadcast application using urea (46-0-0). Phosphorus was also applied as a pre-plant broadcast application using triple super phosphate (0-46-0). A ground-driven barber spreader was used for broadcast applications.

The soil type at this location is considered a Teller fine sandy loam, fine-loamy, mixed, thermic, Udic Argiustoll soil (USDA, 2020). At the initiation of this experiment in 1996, a composite soil sample was taken at 0-15 cm depth. The soil pH was recorded at 5.95, organic carbon 5.34 g kg^{-1}, and total N was observed at 0.50 g kg^{-1}. Total phosphorus (P) and potassium (K) levels were 8.99 and 133 mg kg^{-1}, respectively. This trial was under conventional tillage practices from 1996 to 2007 and was transitioned to no-till management after 2007. It was prudent that this transition was made to promote conservation tillage practices within the region further and provide relevant data for extension recommendations.

Trial Establishment and Data Collection

The analysis of this study includes twenty years of compiled data, although years are not continuous. Each season, pre-plant soil samples were taken at a depth of 0-15 cm for nutrient analysis. Fifteen to eighteen soil cores were pulled from each plot to obtain the composite soil sample. Nutrient analysis included soil test NO_3^--N, P, K, TC, and soil pH. The data records for soil analysis, however, were incomplete. Therefore, soil test values were not available for each growing season dating back to experiment initiation. For data interpretation, soil sample analysis at the initiation of this experiment (1996) and the 2018-19 and 2020-21 growing season values are included for soil pH, and soil test P and K and can be found in Figure 8. As illustrated in the figure, an apparent decline in soil pH has occurred overtime and is more prevalent in the plots with higher N rates. This is expected as continual application of N acidifies the soil overtime (Schroder et al., 2011). Additionally, soil test P values have increased overtime in treatments where P has been continuously applied. Soil test K values on the other hand have declined overtime, especially at the higher N rate treatments due to higher grain yields that resulted in greater removal rates. However, current soil test K results indicated that most plots are still relatively close to 100% sufficiency for winter wheat production.

Planting occurred each season during October, with a few seasons planted in early November (Table 22). The planting rate was approximately 89.6 kg seed ha^{-1} drilled in with a Great Plains 3.04 m wide drill. At crop maturity, approximately 16.7 m^{2} were harvested per plot to determine final grain yield. The plots were mechanically harvested using a Kincaid 8XP plot combine, and grain yield was recorded using a Harvest Master yield monitor. Grain yield reported was adjusted to 12.5% moisture. Field equipment used as described relates more to recent production seasons, although similar equipment would have been used in the prior years. Across the duration of this experiment, yield data was not obtained in 2008, 2016, and 2018. Outside influencing factors such as weed or disease pressure resulted in insufficient data or prevented harvest, and therefore those years were not included in this analysis.

Grain samples were collected at harvest each year by plot. After harvest, samples were oven-dried at 65ºC for 24 hours. Samples were then ground using a milling grinder and further refined through a rolling process. In this process, the sample is placed in a small glass bottle containing three metal rods and closed at the end. Bottles are then placed in tubes and laid upon a rolling machine. The machine runs for 24 hours, continually rotating the bottles, allowing the metal pins to further grind the grain sample until proper fineness is reached. To determine grain N content, 150 mg of each sample was weighed for dry combustion N analysis methods. A Leco CN628 was used to determine grain N analysis. In this method, the 150 mg sample is combusted at 950ºC, and the released N_2 is quantified. Records for grain N content overtime were incomplete; thus analysis results of this variable is limited to twelve years of data. Nitrogen uptake was also calculated for each season when grain N was available (Table 22). Nitrogen uptake was calculated as:

$$\text{N uptake (kg N)} = \frac{\text{Grain N \%}}{100} \times \text{Grain Yield (kg ha}^{-1}\text{)}$$

When performing a meta-analysis on multiple years of data, creating meaningful year groupings is of interest to better interpret overall environmental effects. For this analysis, years were grouped based on the following factors: tillage practice, total ISP, and environmental mean grain yield. Figures 9 and 10 illustrate how each of these groups were formed. Under each tillage practice, the grouping quadrant boundaries as illustrated were created based on average environmental grain yield overall and average total ISP received for the years included. Those years that fell within each boundary were then combined and analyzed for subsequent main effect and contrast analysis for each dependent variable of interest. Descriptions of each combined data group are outlined in Table 23.

Statistical Analysis

Data analysis was performed using SAS 9.4 software (SAS Institute, Cary, NC). Initial analysis included main effect analysis of P and N rate and their interaction on grain yield alone for each year separately due to year being a significant factor. A generalized linear procedure (PROC GLM) was then used to generate treatment means and evaluate main and interaction effects of each year grouping. The model included N rate, P rate, and N rate by P rate interaction as fixed effects, while year and replication were random effects. This procedure was also used for single-degree-of-freedom contrast comparisons to evaluate linear and quadratic trends among N, P, and N by P interactions. A replication and treatment model was then used separately to determine specific differences among treatments. In this model, the fixed effect was treatment, while year and rep(year) were noted as random effects. Specific contrast comparisons among treatments were then analyzed and interpreted. RStudio software was also used for data visualization (R Core Team, 2021).

RESULTS

Grain Yield

Evaluation of main effect analysis for grain yield was of interest to evaluate the impact of P and N applications overtime. In Table 24, it is noted that the main effect of P rate was significant in the first three years of this experiment. Since then, limited response to P has been noted. Alternatively, N rate as a main effect was significant in 15 out of the 20 years. Limited P and N interactions were present but were noted in 2004, 2005, 2006, 2010, 2015, and 2019. It is important to consider that wheat cultivars used evolve with time and carry improved genetic characteristics that can impact both N and P response and grain yield. As described in Table 22, the cultivar used has changed overtime and notably they differ in their acid soil tolerance rating (Marburger et al., 2018; Zhang et al., 2000). The cultivars Tonkawa, Deliver, and IBA had the lowest acid soil tolerance ratings meaning they were highly susceptible to acid soil conditions or Al^+ toxicity. The years where a significant P rate effect were noted did commonly coincide with the use of Tonkawa and Deliver but not IBA (Table 24). However, in 2010 and 2011 when Deliver was sown, both a positive and negative P rate effect was noted, respectively, which is curious.

The general relationship between grain yield over time was further evaluated. In Figure 11, an increasing trend in environmental grain yield was apparent while under conventional tillage until 2006. Furthermore, the yield obtained in 2007 was extremely low. With limited field notes, it is hypothesized that outside environmental constraints contributed to this result. Thus, this year was marked as an outlier. A strong positive relationship between environmental grain yield and time was noted under conventional tillage with an R^2 value of 0.42 which could be representative of improved genetics evolving overtime.

Upon transition to no-till management, lower environmental grain yields were present in the initial years due to planting challenges. A strong positive relationship between grain yield and time was present under no-till management with an R^2 value of 0.72 (Figure 11). Grain yields overtime in more recent years have exhibited larger variability from year to year. Perhaps this variability was representative of increasing environmental change. Raun et al. (2019) stated that the randomness of a biological system increases with time. The authors further recognized that optimum fertilizer rates are largely different for each year as influenced by the environment. To better grasp the influence of rainfall and environmental yield on the N and P rate effect, each year was then grouped into a category based on these parameters and evaluated for grain yield, grain N, and N uptake.

Conventional Tillage

Group 1a: High yield, low rainfall environment

Group 1a encompassed the years of 2002, 2003, 2004, and 2005. In this grouping, 400 to 500 mm of rain was received for each growing season evaluated. Grain yield in group 1a averaged above 2.5 Mg ha^{-1}. In the analysis of main effects, a Year*P rate*N rate interaction was observed (Table 25). It was further noted that a highly significant (p=0.0041) quadratic trend was noted in grain yield for the N rate. This trend can be loosely observed in Figure 12. The highest grain yield was noted in TRT 12, which included the highest P rate (29.6 kg P ha^{-1}) and the highest N rate (168 kg N ha^{-1}). From single-degree-of-freedom contrasts, it was observed that applying N in this environment significantly improved yield compared to no application. However, differences across N rates applied were not significant, indicating a limited N response to higher N rates (Table 26).

Group 1c: Low yield, low rainfall environment

Group 1c encompassed the years 2000, 2001, and 2006. In this group, 250 to 450 mm of rainfall was received, and the environmental mean grain yield was less than 2.5 Mg ha^{-1}. In the analysis of main effects, the significant highest order interaction was Year*P rate (p=0.0029). It was further noted that the N rate alone was highly significant (p=0.0005). In this environment, an N*P interaction, however, was not significant. Trend analysis confirmed that a linear trend in grain yield was apparent for P rate, while a quadratic trend was present for N rate (Table 25). Figure 12 indicates that the linear trend across P rates was negative. An increasing trend in grain yield was present as N rate increased, which seemed to level off at a rate of 112 kg N ha^{-1} (Figure 12). The highest grain yield was noted in TRT 8, which contained a P rate of 14.8 kg P ha^{-1} and an N rate of 112 kg N ha^{-1} (Table 26). Further analysis among treatment levels indicated that the P rate of 29.6 consistently decreased grain yield compared to the P check (p=0.0066). Applying a P rate of 14.8 did not significantly differ when compared to the check (p=0.9627). It was further noted that N boosted grain yield compared to the check. However differences among rates were not apparent (Table 26).

Group 1d: Low yield, high rainfall environment

Group 1d encompassed the years of 1998 and 1999, the initial start of this experiment. During these years, roughly 700 mm of rainfall was received in season, and environmental grain yield averaged less than 2 Mg ha^{-1}. Interactions for year, P rate, or N rate were not significant in this group. However, both the P and N rates alone had a highly significant effect (Table 25). Contrast comparisons noted that applying 29.6 kg P resulted in statistically higher grain yield compared to a 0 and 14.8 kg rate (Table 26).

Additionally, among N rates applying 168 kg N compared to 112 was not largely different. Moreover, a significant linear relationship across both N and P rates was present ($p=<0.0001$). This positive trend was widely apparent in Figure 12, which illustrates grain yield increasing across P and N rate combinations. The highest grain yield was achieved in TRT 9, which contained a P and N rate of 29.6 and 112 kg ha^{-1}.

No-Till Management

Group 2a: High yield, low rainfall environment

Group 2a encompassed the years 2014 and 2017. Within this group, trials received between 250 and 350 mm of ISP. Environmental grain yield averaged just above 1.4 Mg ha^{-1}. Although this yield would not be considered high at all, this group will still be referred to as high yielding due to the grouping methods. This group achieved higher average yields relative to the other groups under no-till management.

In this high-yielding, low rainfall environment, the N rate had the largest impact on grain yield and varied between years. A significant Year*N rate interaction was noted in this group ($p=0.0005$) (Table 25). Alternatively, the P rate did not have a large impact on achieved grain yield. Given the years included in this grouping, a limited P response would be expected as soil P builds up overtime with continual applications. A quadratic trend in grain yield was noted across N rates, however, no significant trends were identified across P rates. Figure 13 notes that higher yield levels occurred at higher N rates, while yield across P rates remained constant, especially at higher N rates. The highest grain yield in this grouped data set was observed in TRT 8 where 14.8 and 112 kg P and N were applied, respectively. Treatment 12 was close behind, which incorporated P and N rates of 29.6 and 168 kg ha^{-1}. Although higher yields were seen under higher N rates, contrast comparisons among N rates indicated those increases with N rates above 56 kg N ha^{-1} were not significant (Table 26). Furthermore, significant increases in grain yield compared to the check were not evident until applying 112 kg N ha^{-1}.

Group 2b: High yield, high rainfall environment

Group 2b encompassed the years 2010, 2015, and 2020. The year 2019 was omitted from this group since it received unusually high ISP. The average total rainfall for each year in this group was approximately 478 to 550 mm. Environmental grain yield had a larger range from 1.5 to nearly 3.5 Mg ha^{-1}. Both Year and N rate had a significant effect on grain yield, and the interaction of these terms was significant (p=<0.0001). The interaction between N and P rate was also significant. In trend analysis, it was confirmed that a linear trend in grain yield was present for the interaction of P and N rate (p=<0.0001). In Figure 13, this linear trend can be observed as N rates increase; however, trends across P rates were less apparent. The highest grain yield in this environment group was achieved in TRT 12 where 29.6 and 168 kg of N and P were applied, respectively (Table 26). Contrast comparisons indicated that yield levels obtained at an N rate of 168 were not significantly greater than 112 (p=0.4190). The application of 112 kg N ha^{-1} was suitable enough for this high rainfall environment.

Group 2c: Low yield, low rainfall environment

Group 2c contained only one year, 2011. This year was characterized by approximately 360 mm of rainfall and had an environmental mean yield of less than 1

Mg ha^{-1}, representing very low yield levels. Main effect analysis results indicated both a significant P and N rate effect, and the interaction of these variables was not significant (Table 25). Furthermore, linear trends across both N and P rates were confirmed. It was also noted that the linear trend of the interaction of N and P was significant ($p=<0.0001$). Going further into the data set, such trends are visible in Figure 13. As both N and P rates increase, the linear trend in yield is found to be negative. Contrast comparisons indicated that applying N and P significantly decreased grain yield (Table 26). Higher levels of rainfall for this season may have lessened the response to N. The highest grain yield was achieved in the check treatment indicating no positive response to N or P fertilization during this season.

Group 2d: Low yield, high rainfall environment

Group 2d encompassed the years 2009, 2012, and 2013. In-season precipitation received during these years ranged between 500 and 550 mm. Environmental yields were characterized as low, ranging from 0.04 to just above 1 Mg ha^{-1}. In this data group, the Year*N rate was significant ($p=<0.0001$). P rate nor an N*P interaction had a significant effect on grain yield in this environment (Table 25). A linear trend for N rate was also confirmed, however, no significant trends were present across P rates. The positive linear trend in grain yield across N rates can be identified in Figure 13. While increasing yields were seen as N rate increased, yield level did not largely vary among P rates, which was further confirmed in contrast comparisons (Table 26). The highest grain yield was noted in TRT 11, which consisted of 14.8 kg P ha^{-1} and 168 kg N ha^{-1}. Comparisons among N rates indicated that yields achieved with the high rate of N were not significantly greater than yields achieved when applying 112 kg (p=0.1334). However, yields were statistically greater with the high N rate compared to 56 kg N (p=0.0095).

Grain N Concentration

Conventional Tillage

Group 1a: High yield, low rainfall environment

Data included in group 1a included the years 2002 and 2003; data were not available for 2004 or 2005. Differences in grain N were present among treatments for group 1a. The interaction of the main effects year, P rate, and N rate were not significant. However, it was noted that both year and N rate had a significant impact on grain N (p=0.0023 and p=<0.0001). Phosphorus rate did not have a significant effect on grain N in this selected environment. The N rate response was found to fit a quadratic trend (Table 27). Figure 14 illustrates grain N concentration by combinations of N and P rates. Within each N rate, differences in grain N were not seen across P rates. The highest grain N content was observed in TRT 12, which contained both the high P and N rates. It was further noted that grain N % achieved with a rate of 168 kg N was significantly greater than all other N rates evaluated (Table 28).

Group 1c: Low yield, low rainfall environment

Data from 2000 and 2006 were included in this group; 2001 data was not available. Group 1c observed a significant three-way interaction of Year*Prate*Nrate (p=0.0414) (Table 27). Higher N rates did lead to higher grain N concentration and was evident in Figure 14. Furthermore, a significant positive linear trend in grain N content across increasing N rates was confirmed and is evident in Figure 14 (p=<0.0001). The highest grain N concentration was noted in TRT 10, which had an N rate of 168 kg N ha^{-1} without applying P. Among N rates, the high rate of 168 kg N ha^{-1} resulted in significantly higher grain N levels than lower rates (Table 28).

Group 1d: Low yield, high rainfall environment

Group 1d noted slightly lower grain N concentrations compared to 1c, which was a low rainfall environment. In this data group, Year*N rate interaction was significant (Table 27). Additionally, the P rate alone greatly impacted grain N achieved (p=0.0049). A negative linear trend in grain N content was present as P rate increased (p=0.0058). Furthermore, a positive linear trend in grain N concentration was noted as N rate increased (p=<0.0001). Data presented in Figure 14 further illustrated these relationships. Recall that this group contained the earliest years of this experiment. It is interesting to see a linear increase in grain yield over P rates among this group, followed by a linear decrease in grain N concentration. Perhaps the crop put more energy on grain yield rather than grain protein when applications of P were made.

No-Till Management

Group 2a: High yield, low rainfall environment

Grain N content was only available for 2017 within this group. Therefore, the results described here relate to only one year of data. For this environment, N rate was highly significant (p=<0.0001) (Table 27). Phosphorus rate did not significantly affect grain N concentration at the $\alpha=0.05$ level but was close behind (p=0.0661). The interaction of N and P was not significant for grain N. It was further noted that grain N concentration increased linearly as the N rate increased ($p=<0.0001$). Data represented in Figure 15 for this environmental group illustrates the strong linear relationship between grain N and N rate. However, consistent trends among P rates were not apparent. The highest grain N concentration was noted in TRT 10, which contained no application of P and 168 kg N ha^{-1}. Contrast analysis among N rates indicated that grain N % was highest at an N rate of 168 kg (Table 28).

Group 2b: High yield, high rainfall environment

Grain N results for group 2b include data from 2015 and 2020. Data from 2010 was not available. A significant Year*P rate and Year*N rate interaction was noted for this environment (Table 27). However, the P rate*N rate interaction was not. It was further noted that a linear trend in grain N was seen as the N rate increased ($p=<0.0001$). A linear decreasing trend among P rates was also noted ($p=<0.0001$). Moreover, a linear trend of N rate*P rate was nearly significant at the $\alpha=0.05$ level with a p-value of 0.0571. Graphical representation of this data in Figure 15 highlights this nearly significant interaction trend. It is visually apparent that an antagonistic interaction between P rate and N rate exists for grain N concentration in this environment. The highest grain N concentration was achieved without a P application and an N rate of 168 kg N ha^{-1}. Consistent with other group analyses, an N rate of 168 resulted in significantly greater grain N % (Table 28).

Group 2d: Low yield, high rainfall environment

In the environmental group of 2d, a significant Year*N rate interaction was noted (p=0.0128). P rate did not have a large impact on grain N % under this environment. Trend analysis indicated a significant quadratic trend across the N rate (Table 27). Increased variability in this data set is noted in Figure 15. A quadratic trend was observed where grain N concentration was highest at the rate of 112 kg N ha^{-1} and then slightly decreased as higher rates of N were applied. The highest grain N concentration was present in treatment 10 with an N rate of 168 kg N ha^{-1} alone. Contrast analysis indicated that grain N concentration was not statistically different between the N rates of 112 and 168 kg N ha^{-1} (p=0.4517) (Table 28).

Nitrogen Uptake

Conventional Tillage

Group 1a: High yield, low rainfall environment

The years 2002 and 2003 were included to represent this group. A significant Year*N rate interaction was present for N uptake in this low rainfall environment. Phosphorus rate was not noted to influence N uptake alone, and interactions were also not significant. Trend analysis indicated a significant quadratic trend across N rates (Table 29). Furthermore, a significant linear trend for the P rate*N rate interaction term was observed (p = 0.0413) and was illustrated in Figure 16. It is evident that N uptake steadily increased across N rates and leveled off at the rate of 112 kg N ha^{-1}. Differences in P response are also seen within N rates and vary among them. Nitrogen uptake was greatest for TRT 12, which incorporated both the highest N and P rates (Table 30). Analysis across N rates in contrast comparisons indicated that 168 kg N significantly increased N uptake compared to 56 kg. However, it had no added benefit over 112 kg N ha^{-1}.

Group 1c: Low yield, low rainfall environment

In group 1c, a Year*P rate interaction was noted (p=0.0030). The effect of the N rate was also highly significant (p=<0.0001). Through contrast analysis, a linear trend in N uptake was noted across P rates. Furthermore, a quadratic trend was noted across N rates (Table 29). The interaction of N and P was not significant. Figure 16 indicates that the linear trend across P rates was negative. The greatest N uptake occurred with the high N rate and 14.8 kg of P ha^{-1} (Table 30). Contrast analysis among P rates indicated a significant decrease in N uptake when comparing 0 to 29.6 kg P (p=0.0496). Among N rates, N uptake was greatest at the highest N rate but was not significantly different than 112 kg N (p=0.4112).

Group 1d: Low yield, high rainfall environment

Nitrogen uptake in group 1d was significantly influenced by year, P rate, and N rate individually (Table 29). The interactions of these main effect terms were not significant. A strong positive linear trend occurred in N uptake as both N and P rates increased (Figure 16). The highest N uptake was achieved with 29.6 kg P ha^{-1} and 168 kg N ha^{-1}. Among P rates, the rate of 29.6 resulted in the greatest N uptake (p=<0.0001, p=0.0166). Since this group consists of the first two years of this trial, it was evident that P was needed to boost N uptake by advancing early-season establishment and biomass production. Furthermore, the high N rate resulted in significantly higher N uptake out of all N rates evaluated (Table 30).

No-Till Management

Group 2a: High yield, low rainfall environment

Due to data availability constraints, this group only included one year of data to evaluate N uptake. During the 2017 harvest season, a significant N rate effect was observed ($p=<0.0001$). Phosphorus rate, however, did not have any influence (Table 29). Additionally, trend analysis indicated a significant linear trend across N rates for N uptake, which has been a common finding throughout this study. Data visualized in Figure 17 illustrated this positive linear trend as well. It is important to note that a quadratic trend was nearly significant and would be at the $\alpha=0.1$ level. The greatest N uptake was noted for TRT 8, which contained a P rate of 14.8 and an N rate of 112 kg N ha^{-1}. The high N and P rate treatment, however, was close behind. Contrast analysis among N rates indicated that the highest N uptake in this environment at 112 kg N was not significantly different from 168 kg N ($p=0.09549$) (Table 30).

2b: High yield, high rainfall environment

Data from 2015 and 2020 were included in this environmental group. A significant Year*P rate and Year*N rate interaction was observed within this environment, further emphasizing the effect of environment on N uptake (Table 29). An interaction was also present between P and N rate ($p=0.0227$). Trend analysis further indicated a significant quadratic trend for N uptake across N rates. Moreover, the linear trend for N rate*P rate was significant (p=0.0013). Higher levels of N uptake occurred in this high-yielding, high rainfall environment compared to other environmental groups under the no-till management system. P rate had more influence within the high N rate than lower N rates (Figure 17). The greatest N uptake occurred where the highest N and P rates were applied. However, differences between the two N rates (112 and 168) were not significant (Table 30).

Group 2d: Low yield, high rainfall environment

Nitrogen uptake across treatments in group 2d varied greatly. The interaction between year and N rate was highly significant (p=<0.0001). Furthermore, a linear trend for N uptake was noted across N rates (p=0.0001). Figure 17 further illustrated this positive linear trend in N uptake as the N rate increases. The highest N uptake within each N rate occurred at a P rate of 14.8 kg P ha^{-1} except with the 0 N treatments. The highest N uptake among treatments was noted in TRT 11, which contained the highest N rate and a P rate of 14.8 kg P ha^{-1}. Contrast analysis among P rates indicated no significant differences, while it was noted 168 kg N resulted in a statistically greater amount of N uptake (Table 30).

DISCUSSION

Grain Yield by Year

Nitrogen rate consistently impacted grain yield nearly every year illustrating the importance of N management in winter wheat production. Phosphorus rate on the other hand, had a more limited impact and was restricted to certain growing seasons. Environmental characteristics as we know vary greatly year to year and influence N and P response. Cultivar genetics is also a key component of the environment and can have an influencing effect overtime, especially when evaluating long-term experiments. Wheat cultivars used evolve with time and carry improved genetic characteristics that can impact both N and P response and grain yield. As described in Table 24, the cultivar used has changed overtime and notably they differ in their acid soil tolerance rating. This rating can have an influence on P response. As mentioned earlier, P readily binds with Al+ and Fe which are present in larger concentrations at low pH levels thus limiting P availability in acidic soils. Cultivars that have a higher tolerance rating often possess certain morphological or physiological characteristics that assist in accessing P in these conditions. The cultivars Tonkawa, Deliver, and IBA had the lowest acid soil tolerance ratings meaning they were highly susceptible to acid soil conditions or Al^+ toxicity (Marburger et al., 2018; Zhang et al., 2000). The years where a significant P rate effect was noted did commonly coincide with the use of Tonkawa and Deliver. However, in 2010 and 2011 when Deliver was sown, both a positive and negative P rate effect was noted, respectively which is curious. In growing seasons where more tolerant varieties were grown (Jagger, Duster, Double Stop), a significant overall P rate effect was not seen. These more tolerant cultivars could perhaps have more developed characteristics to access or uptake soil P in the present acid soil conditions. Penn et al. (2015) evaluated various winter wheat varieties commonly used in the southern Great Plains to determine their P use efficiency under acid and calcareous soil conditions. The authors' concluded cultivars differed in their ability to extract P under the different soil conditions (acid, calcareous: low P, higher P) while others were able to perform similarly under both conditions. These results suggested that certain cultivars were more P uptake efficient while others had increased P utilization that contributed to their overall P use efficiency. Similar work by Maske et al. (2000) evaluated wheat cultivars and their P uptake and P utilization efficiency under acid and calcareous soil conditions. Results indicated P uptake was able to explain 71-100% of grain yield variation under acidic soil conditions while P utilization explained 60-63% under calcareous soil conditions. Although the cultivar used in each of these years is obviously not the sole reasoning for the observed P rate effect, it certainly offers a potential reasoning. Cultivar genetic components can also have an impact on N management overtime as more high yielding cultivars are developed and refined. In fact, work by Aula et al. (2019) evaluated the effect of cultivar used over time in two similar long-term winter wheat fertility trials within Oklahoma. The authors' found a mean annual grain yield increase overtime of 12 and 30 kg N ha^{-1} for two experiments respectively when grown under adequate N management. The trend in grain yield overtime as illustrated in Figure 12 is also similar in that overtime, an increasing trend in grain yield was observed. However, this represents the mean environmental grain yield which averages across all N rates as opposed to a high N rate alone.

Grain Yield among Environmental Groups

Across the duration of this long-term experiment, differences in grain yield were apparent within the change of using tillage. As noted earlier, a sharp decline in grain yield was observed upon transition to no-till management and since then has been gaining ground. This decline in yield was not only attributed to management transition, however. Field notes indicate that compacted soil and dry conditions led to sowing challenges. It was also noted that weed control challenges were present during the initial trial years under no-till management. Despite these outside challenges, literature does report that yield declines upon transition to no-till management can occur. Pittelkow et al. (2015) conducted a meta-analysis concerning the effect of transitioning from conventional tillage to no-till practices and found a 2.6% decrease in yield for wheat production.

Additionally, it is necessary to consider the implications of no-till management on nutrient stratification particularly that of P in this case. In the treatments where P was applied broadcast on the surface, there is the potential for P stratification in the top few inches of the soil. This has been demonstrated by unpublished work, Souza et al. (2020), which evaluated three long term winter wheat fertility experiments within Oklahoma, one of which was Experiment 702. The researchers noted that the majority of P was confined to the top two inches of soil in those treatments that have received the higher P rate continually. As much as 150 mg kg^{-1} was found in the top inch while only 50 mg kg^{-1} was present in the 6th inch down. With such stratification in the soil profile, roots may have limited access to P as the primary method of uptake is through root contact. As the plant matures and extends root systems beyond the upper topsoil inches, less P uptake would occur in those root zones. Moreover, P is noted to have a very low diffusion coefficient thus limiting its movement to other root zone areas as the P diffusion gradient changes from active plant uptake (Clarkson, 1981). Under the no-till environments of this study, this could offer a possible explanation as to why limited response was observed to P applications. The plant may not have been able to access the surface applied P due to intense stratification. Nutrient stratification within the soil then presents questions as to how best to manage these soil profiles. Other published literature has suggested the use of limited tillage to reduce P stratification (Garcia et al., 2007). Although, DeLaune and Sij, (2012) noted increases in P runoff when no-till soils were occasionally tilled. Schwab et al. (2006) offered another alternative to alleviate P stratification in no-till soils by subsurface applying P which resulted in greater P uptake and grain yield of corn and sorghum.

Results of the present study also indicated that ISP seemed to have a negative relationship with grain yield under conventional tillage management. Similar results were indicated by Stone and Schlegel (2006), where ISP was related to grain yield of winter wheat and sorghum. The authors noted a greater yield response to water supply in no-till management compared to conventional tillage. It has also been commonly demonstrated that no-till management often leads to more efficient water use (Peng et al., 2019; Habbib et al., 2020).

A significant N and P interaction was only noted in high yielding environments of the year groupings evaluated. Consistent results were noted by Lollato et al. (2019), where wheat yield responded to P in high yielding environments. Under conventional tillage, this interaction took place in years considered to have lower rainfall, while under no-till, this was seen in high rainfall conditions. Grant et al., (1984) observed N and P interactions amongst both conventional and no-till management. The author notes that winter survival of the wheat crop was stronger amongst plots that had been fertilized with P and N as opposed to N alone. In the present study, it was visually apparent in early growth stages that plots that received P and N noted greater biomass growth that may contribute to stronger survivability (Figure 18).

An interaction between year and N rate was almost always seen for grain yield results regardless of environment group. This illustrates N’s highly unpredictable nature and that N response greatly depends on multiple environmental conditions. Grain yield resulting from 112 or 168 kg N was not significantly different under each environment. A similar quadratic trend in grain yield was reported by Woodward and Bly (1998). When comparing the pre-plant or split applied N rates of 110 and 165 kg N ha^{-1}, no benefit was found in yield at the higher rate, and split applied applications resulted in lower grain yields at the highest N rate.

Among all environmental groups, year was still a significant factor indicating that additional environmental conditions outside of ISP played a key role in grain yield response to N and P fertilization. Furthermore, group 1d noted a larger P effect out of the conventional tillage groups evaluated. This group contained the early years of this experiment; thus, a P response was expected. Figure 8 illustrated how soil test P has increased overtime with continual applications of P thus limiting a P response in the more recent seasons. Additionally, it was noted that a decrease in soil pH has occurred with the continual application of N overtime.

Grain N Concentration among Environmental Groups

Grain N concentration was highly influenced by the year within each environmental group. A year by N rate interaction was commonly noted, except for low rainfall environments under conventional tillage. It was again noted that P rate was more of a factor in the initial years of the experiment, but negatively influenced grain N concentration. Our results are consistent with the literature, where P has been found to decrease grain N (Lollato et al., 2019). Moreover, N by P interactions were not present for grain N concentration achieved. Consistent across all environments except group 2d, the highest N rate resulted in significantly higher grain N. Wood and Bly (1998) further noted the highest grain protein contents in winter wheat when applying a high rate of 165 kg N ha^{-1}. Phosphorus rate was not a significant factor in grain N concentration observed overall. However, in contrast to comparisons between P rates, it was often noted that P decreased grain N although consistent trends were not observed. Moreover, previous work has also noted N and P co-applications to have an insignificant effect on grain N concentration (Duncan et al., 2018).

Nitrogen Uptake among Environmental Groups

Nitrogen uptake was also highly influenced by the year among each environmental group. It was commonly noted that higher N rates resulted in greater N uptake, although the highest N rate was not significantly different from 110 kg N ha^{-1}. Phosphorus rate was rarely noted to significantly affect any environmental group outside of 1d, which contained the initial years of this experiment. However, the low P (14.8 kg) rate seemed to result in the greatest N uptake within each N rate level, indicating the synergistic effect of co-applications. Literature compiled by Duncan et al. (2018) described the overall importance of nutrient interactions or co-application of macronutrients and their overall NUE impact. In their review, it was noted that co-applications of N and P compared to N+K or N+S had the greatest effect on grain recovery of fertilizer N. Similar results were noted by Lollato et al. (2019), where they also increased N removal and NUE with co-applications of P.

CONCLUSIONS

By grouping years based on environmental grain yield and ISP, this work attempted to better quantify N and P interactions among different environmental conditions. Although this analysis incorporated certain environmental parameters, several other environmental conditions were not accounted for. This was evident as the main effect of the year within each group was nearly always significant. Due to data unavailability and lacking data for group 1b, full interpretation of N and P responses under different yield and ISP levels was limited. Nonetheless, certain trends were apparent. It was noted that a significant P rate effect on grain yield was more pronounced in the early years of this long-term trial and often coincided with years where non-acid soil tolerant cultivars were used. When analyzed among environmental groups, the application of 29.6 kg P ha^{-1} often decreased grain yield compared to the check whereas the lower rate (14.8 kg P) often increased grain yield. Furthermore, under each environment, applying 168 kg N did not have a significant benefit in grain yield compared to 112 kg N. Grain N however, consistently observed a significant increase when applying 168 kg N. Moreover, high yield environments regardless of tillage practice noted greater N uptake although differences between 112 and 168 kg N were still not significant. Furthermore, P applications were still noted to increase N uptake and often grain yield in general. Long-term experiments will continue to be of value to better grasp the impact of environmental characteristics on crop production and fertilizer response. This work clearly illustrates that factors outside of ISP in dryland environments have a critical influence on production levels achieved and N and P response; thus, including more parameters could allow for a more robust interpretation.

TABLES

Table 21. Treatment structure as applied each season, Experiment 702, Perkins, OK.

Treatment	Pre-Plant P kg P ha^{-1}	Pre-Plant N kg N ha^{-1}
1	0	0
2	14.8	0
3	29.6	0
4	0	56
5	14.8	56
6	29.6	56
7	0	112
8	14.8	112
9	29.6	112
10	0	168
11	14.8	168
12	29.6	168

Table 22. Planting date, harvest date, cultivar, cultivar acid soil tolerance, grain yield, and grain N data availability for each year, Experiment 702, Perkins, OK (1998-2020).

Year	Planting Date	Harvest Date	Cultivar	Cultivar Acid Soil Tolerance	Grain Yield	Grain N	N Uptake
1998	*Na*	*Na*	Tonkawa	1	+	+	+
1999	*Na*	6/10/1999			+	+	+
2000	10/8/1999	5/30/2000			+	+	+
2001	11/17/2000	6/7/2001			+		
2002	10/16/2001	6/11/2002	Jagger	1	+	+	+
2003	*Na*	5/31/2003			+	+	+
2004	*Na*	*Na*	Jagger	1	+		
2005	10/18/2004	6/7/2005	Jagger	1	+		
2006	10/11/2005	*Na*	Jagger	1	+	+	+
2007	*Na*	6/6/2007	Jagger	1	+		
				Transitioned to No-Till Management			
2008	10/20/2007	*Na*	Fannin	1			
2009	10/21/2008	6/19/2009	Duster	1	+	+	+
2010	11/5/2009	7/1/2010	Deliver	5	+		
2011	10/11/2010	6/9/2011	Deliver	5	+		
2012	10/13/2011	6/12/2012	Centerfield	2	+	+	+
2013	10/8/2012	6/13/2013	Centerfield	2	+	+	+
2014	10/23/2013	6/16/2014	Double Stop	1	+		
2015	10/21/2014	6/11/2015	IBA	5	+	+	+
2016				Fallow Period			
2017	10/1/2016	6/9/2017	IBA	5	+	+	+
2018	*Grain not harvested*		Double Stop	1			
2019	10/11/2018	6/11/2019	Bentley	2	+	+	+
2020	10/6/2019	6/8/2020	Double Stop	1	+	+	+

Na = data not available, + data for specified variable was included in analysis,
Acid Soil Tolerance scale: 1 = most tolerant : 5 = least tolerant

Table 23. Data groupings as defined by environmental yield and total in-season precipitation received. Experiment 702, Perkins, OK (1998-2020).

Group	Tillage	Environment		Group Description	Years Included
		Yield ($Mg\ ha^{-1}$)	Rainfall (mm)		
1a	Conventional	≥ 2.50	≤ 520	High yield, low rainfall	2002, 2003, 2004, 2005
1b	Conventional	≥ 2.50	≥ 520	High yield, high rainfall	none
1c	Conventional	≤ 2.50	≤ 520	Low yield, low rainfall	2000, 2001, 2006
1d	Conventional	≤ 2.50	≥ 520	Low yield, high rainfall	1998, 1999, 2007*
2a	No-Till	≥ 1.40	≤ 478	High yield, low rainfall	2014, 2017
2b	No-Till	≥ 1.40	≥ 478	High yield, high rainfall	2010, 2015, 2019*, 2020
2c	No-Till	≤ 1.40	≤ 478	Low yield, low rainfall	2011
2d	No-Till	≤ 1.40	≥ 478	Low yield, high rainfall	2009, 2012, 2013

*2007 and 2019 were not representative of the data set grouping and were not included in group analysis.

Table 24. Grain yield main effect analysis by year for P rate, N rate, and N*P interactions and cultivar used. Experiment 702, Perkins, OK (1998-2020).

Year	Cultivar	Cultivar Acid Soil Tolerance	Main Effect		
			P Rate	N Rate	P Rate * N Rate
1998	Tonkawa	1	*	***	ns
1999			**	***	ns
2000			**	*	ns
2001			ns	ns	ns
2002	Jagger	1	ns	ns	ns
2003			ns	***	ns
2004	Jagger	1	ns	***	**
2005	Jagger	1	ns	**	*
2006	Jagger	1	ns	***	**
2007	Jagger	1	ns	ns	ns
2008	*Not Harvested*				
2009	Duster	1	ns	***	ns
2010	Deliver	5	**	***	**
2011	Deliver	5	*	**	ns
2012	Centerfield	2	ns	ns	ns
2013	Centerfield	2	ns	***	ns
2014	Double Stop	1	ns	ns	ns
2015	IBA	5	ns	***	*
2016	*Fallow Period*				
2017	IBA	5	ns	***	ns
2018	*Not Harvested*				
2019	Bentley	2	***	***	***
2020	Double Stop	1	ns	***	ns

* significant at alpha = 0.05, ** significant at alpha=0.01, *** significant at alpha = 0.001, ns = not significant, Acid Soil Tolerance scale: 1 = most tolerant : 5 = least tolerant

Table 25. Grain yield main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

	1a	1b	1c	1d	2a	2b	2c	2d
	Grain Yield (Mg ha^{-1})							
Main Effect Model	*p-value*							
Year	<0.0001	*Na*	0.0152	<0.0001	0.0013	<0.001	*Na*	<0.0001
Rep(Year)	0.2173	*Na*	0.0235	0.0244	<0.0001	0.3492	0.0045	0.6577
P Rate	0.6903	*Na*	0.0046	0.0003	0.7735	0.006	0.0283	0.2638
N Rate	<0.0001	*Na*	0.0005	<0.0001	0.0006	<0.0001	0.0025	<0.0001
P Rate * N Rate	0.0038	*Na*	0.0698	0.8647	0.9665	<0.0001	0.9430	0.3690
Year*Prate	0.9757	*Na*	0.0029	0.7773	0.3848	0.4661	*Na*	0.4665
Year*Nrate	<0.0001	*Na*	0.0985	0.2859	0.0005	<0.0001	*Na*	<0.0001
Year*Prate*Nrate	0.0041	*Na*	0.5355	0.9212	0.8372	0.2341	*Na*	0.6035
Trend Analysis Contrasts								
P Rate Linear	0.5062	*Na*	0.0049	<0.0001	0.4767	0.0002	0.0106	0.6807
N Rate Linear	<0.0001	*Na*	0.0655	<0.0001	0.0002	<0.0001	0.0003	<0.0001
N Rate Quadratic	0.0041	*Na*	0.0004	0.0288	0.0577	<0.0001	0.2165	0.0892
Prate*Nrate Linear	0.5289	*Na*	0.9580	0.6296	0.4603	<0.0001	<0.0001	0.2457

Table 26. Treatment structure and treatment means for grain yield, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

Treatment	P Rate (kg P ha^{-1})	N Rate (kg N ha^{-1})	1a	1b	1c	1d	2a	2b	2c	2d
			Grain Yield (Mg ha^{-1})							
1	0	0	3.08	*Na*	2.18	0.77	1.51	2.01	1.28	0.57
2	14.8	0	2.47	*Na*	1.66	0.83	1.42	1.69	1.10	0.50
3	29.6	0	3.13	*Na*	1.64	1.00	1.43	1.79	1.16	0.51
4	0	56	3.43	*Na*	2.28	1.01	1.61	2.58	1.08	0.83
5	14.8	56	3.56	*Na*	2.45	1.31	1.70	2.59	0.87	0.99
6	29.6	56	3.41	*Na*	2.23	1.51	1.77	2.65	0.86	0.68
7	0	112	3.33	*Na*	2.28	1.44	1.94	2.67	0.92	0.94
8	14.8	112	3.62	*Na*	2.53	1.60	2.00	3.17	0.78	0.93
9	29.6	112	3.33	*Na*	1.93	1.90	1.95	3.08	0.59	0.93
10	0	168	3.43	*Na*	2.23	1.50	1.78	2.44	0.96	0.95
11	14.8	168	3.58	*Na*	2.30	1.83	1.86	3.01	0.67	1.17
12	29.6	168	3.65	*Na*	1.78	1.87	1.99	3.24	0.54	1.07
SED			0.44	*Na*	0.40	0.24	0.33	0.29	0.20	0.26
CV			16	*Na*	24	21	23	14	27	38

Treatment Contrasts	1a	1b	1c	1d	2a	2b	2c	2d
	p-value							
P Rate								
0 vs 14.8	0.9076	*Na*	0.9627	0.0144	0.7741	0.0233	0.0497	0.3258
0 vs 29.6	0.5792	*Na*	0.0066	<0.0001	0.5278	0.0017	0.0106	0.7292
14.8 vs 29.6	0.5027	*Na*	0.0066	0.0437	0.7299	0.3599	0.4815	0.1852
N Rate								
0 vs 56	<0.0001	*Na*	0.0004	<0.0001	0.0775	<0.0001	0.0448	0.0006
0 vs 112	<0.0001	*Na*	0.0020	<0.0001	0.0004	<0.0001	0.0015	<0.0001
0 vs 168	<0.0001	*Na*	0.0308	<0.0001	0.0028	<0.0001	0.0007	<0.0001
56 vs 112	0.7545	*Na*	0.5558	0.0004	0.0543	0.0002	0.1470	0.2593
56 vs 168	0.4879	*Na*	0.1339	<0.0001	0.1904	0.0028	0.0806	0.0095
112 vs 168	0.3149	*Na*	0.3451	0.3949	0.5246	0.4190	0.7457	0.1334

SED = Standard Error Difference, CV = Coefficient of Variation

Table 27. Grain N concentration main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

	1a	1b	1c	1d	2a	2b	2c	2d
	Grain N %							
Main Effect Model	*p-value*							
Year	0.0023	*Na*	<0.0001	0.0004	*Na*	0.0058	*Na*	<0.0001
Rep(Year)	0.7478	*Na*	0.8580	0.7759	0.1125	<0.0001	*Na*	0.0282
P Rate	0.5558	*Na*	0.098	0.0049	0.0661	0.0001	*Na*	0.2597
N Rate	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
P Rate * N Rate	0.0758	*Na*	0.1366	0.1891	0.7120	0.1131	*Na*	0.6789
Year*Prate	0.6035	*Na*	0.7472	0.2434	*Na*	0.0150	*Na*	0.6671
Year*Nrate	0.3909	*Na*	0.4990	0.0161	*Na*	0.016	*Na*	0.0128
Year*Prate*Nrate	0.8060	*Na*	0.0414	0.8661	*Na*	0.9335	*Na*	0.9959
Trend Analysis Contrasts								
P Rate Linear	0.2909	*Na*	0.2415	0.0058	0.4837	<0.0001	*Na*	0.1628
N Rate Linear	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
N Rate Quadratic	0.0096	*Na*	0.1826	0.0620	0.2612	0.8240	*Na*	0.0393
Prate*Nrate Linear	0.0964	*Na*	0.5030	0.4310	0.3018	0.0571	*Na*	0.3879

Table 28. Treatment structure and treatment means for grain N concentration, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

Treatment	P Rate (kg P ha^{-1})	N Rate (kg N ha^{-1})	1a	1b	1c	1d	2a	2b	2c	2d
			Grain N %							
1	0	0	2.06	*Na*	2.12	2.15	1.55	1.69	*Na*	2.14
2	14.8	0	1.92	*Na*	1.92	2.09	1.53	1.71	*Na*	2.07
3	29.6	0	1.94	*Na*	2.16	2.08	1.57	1.69	*Na*	2.02
4	0	56	2.22	*Na*	2.11	2.34	1.63	1.86	*Na*	2.20
5	14.8	56	2.56	*Na*	2.32	2.07	1.58	1.79	*Na*	2.28
6	29.6	56	2.34	*Na*	2.28	2.03	1.59	1.73	*Na*	2.19
7	0	112	2.61	*Na*	2.45	2.51	1.70	2.08	*Na*	2.46
8	14.8	112	2.56	*Na*	2.23	2.34	1.65	1.90	*Na*	2.50
9	29.6	112	2.65	*Na*	2.58	2.24	1.72	1.91	*Na*	2.45
10	0	168	2.66	*Na*	2.74	2.60	1.86	2.14	*Na*	2.53
11	14.8	168	2.69	*Na*	2.72	2.45	1.73	2.05	*Na*	2.49
12	29.6	168	2.88	*Na*	2.67	2.64	1.78	2.01	*Na*	2.49
SED			0.16	*Na*	0.18	0.16	0.05	0.08	*Na*	0.12
CV			8	*Na*	9	8	4	5	*Na*	6
Treatment Contrasts			*p-value*							
P Rate										
0 vs 14.8			0.4561	*Na*	0.3359	0.0047	0.0245	0.0041	*Na*	0.9613
0 vs 29.6			0.2722	*Na*	0.2675	0.0079	0.4837	0.0002	*Na*	0.1638
14.8 vs 29.6			0.7212	*Na*	0.0426	0.8478	0.1026	0.3502	*Na*	0.1498
N Rate										
0 vs 56			<0.0001	*Na*	0.0418	0.6010	0.1038	0.0046	*Na*	0.0004
0 vs 112			<0.0001	*Na*	<0.0001	0.0002	<0.0001	<0.0001	*Na*	<0.0001
0 vs 168			<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
56 vs 112			0.0010	*Na*	0.0114	0.0012	0.0046	<0.0001	*Na*	<0.0001
56 vs 168			<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
112 vs 168			0.0458	*Na*	0.0002	0.0030	0.0031	0.0021	*Na*	0.4517

SED = Standard Error Difference, CV = Coefficient of Variation

Table 29. N uptake main effect model and contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

	1a	1b	1c	1d	2a	2b	2c	2d
	N Uptake (kg N ha^{-1})							
Main Effect Model	*p-value*							
Year	0.0005	*Na*	0.3979	<0.0001	*Na*	<0.0001	*Na*	<0.0001
Rep (Year)	0.7393	*Na*	0.0832	0.0022	0.0001	0.0212	*Na*	0.4736
P Rate	0.2144	*Na*	0.0807	0.0003	0.8604	0.7682	*Na*	0.1975
N Rate	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
P Rate * N Rate	0.1437	*Na*	0.2267	0.8598	0.9794	0.0227	*Na*	0.4308
Year*Prate	0.9848	*Na*	0.0030	0.8146	*Na*	0.0143	*Na*	0.3060
Year*Nrate	0.0237	*Na*	0.7238	0.3011	*Na*	<0.0001	*Na*	<0.0001
Year*Prate*Nrate	0.9235	*Na*	0.2367	0.7464	*Na*	0.1249	*Na*	0.5615
Trend Analysis Contrasts								
P Rate Linear	0.0815	*Na*	0.0316	<0.0001	0.7146	0.4706	*Na*	0.4787
N Rate Linear	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
N Rate Quadratic	0.0092	*Na*	0.0218	0.1082	0.0669	<0.0001	*Na*	0.1811
Prate*Nrate Linear	0.0413	*Na*	0.5947	0.2004	0.7487	0.0013	*Na*	0.3985

Table 30. Treatment structure and treatment means for N uptake, SED, CV, and single-degree-of-freedom contrast analysis results for each year group. Experiment 702, Perkins, OK (1998-2020).

Treatment	P Rate (kg P ha^{-1})	N Rate (kg N ha^{-1})	1a	1b	1c	1d	2a	2b	2c	2d
			N Uptake (kg N ha^{-1})							
1	0	0	61.28	*Na*	41.69	16.31	13.84	41.73	*Na*	12.79
2	14.8	0	47.11	*Na*	27.71	17.44	16.50	36.31	*Na*	10.82
3	29.6	0	54.99	*Na*	29.47	20.65	16.11	37.12	*Na*	11.16
4	0	56	68.89	*Na*	45.79	23.25	22.42	57.69	*Na*	18.70
5	14.8	56	78.74	*Na*	55.93	27.00	24.79	55.08	*Na*	23.23
6	29.6	56	75.57	*Na*	48.90	30.73	26.09	53.49	*Na*	15.65
7	0	112	73.40	*Na*	57.41	35.33	34.91	68.16	*Na*	24.05
8	14.8	112	82.77	*Na*	53.90	37.44	35.71	68.49	*Na*	23.76
9	29.6	112	83.33	*Na*	50.48	42.30	31.43	68.83	*Na*	23.06
10	0	168	76.99	*Na*	62.02	36.82	33.08	58.85	*Na*	26.05
11	14.8	168	85.46	*Na*	62.71	43.63	33.49	69.61	*Na*	30.30
12	29.6	168	91.10	*Na*	47.68	47.80	34.92	72.71	*Na*	27.49
SED			9.81	.	10.69	4.57	5.81	7.18	.	6.52
CV			16	.	27	18	26	15	.	39

Treatment Contrasts	*p-value*							
P Rate								
0 vs 14.8	0.3339	*Na*	0.6072	0.0373	0.5963	0.7647	*Na*	0.3890
0 vs 29.6	0.0838	*Na*	0.0496	<0.0001	0.7146	0.5756	*Na*	0.5756
14.8 vs 29.6	0.4353	*Na*	0.1487	0.0166	0.8688	0.7940	*Na*	0.1569
N Rate								
0 vs 56	<0.0001	*Na*	0.0004	<0.0001	0.0140	<0.0001	*Na*	0.0007
0 vs 112	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
0 vs 168	<0.0001	*Na*	<0.0001	<0.0001	<0.0001	<0.0001	*Na*	<0.0001
56 vs 112	0.1807	*Na*	0.3542	<0.0001	0.0092	<0.0001	*Na*	0.0445
56 vs 168	0.0145	*Na*	0.0892	<0.0001	0.0104	0.0002	*Na*	0.0001
112 vs 168	0.2474	*Na*	0.4112	0.0221	0.9549	0.6266	*Na*	0.0498

SED = Standard Error Difference, CV = Coefficient of Variation

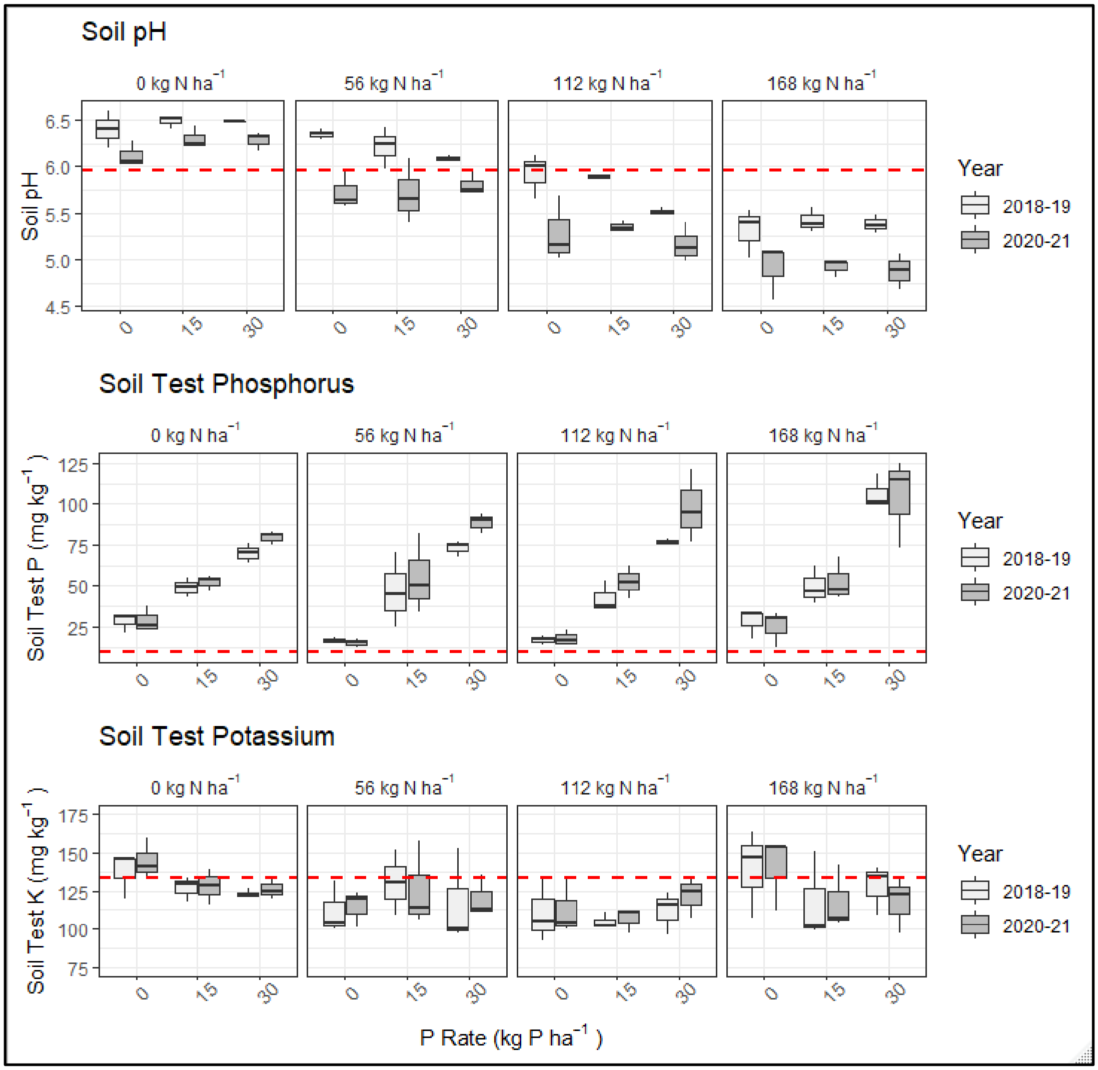

Figure 8. Pre-plant soil test pH, P, and K for growing seasons 2018-19 and 2020-21. The dashed red line indicates initial soil test values in 1996. Soil pH levels are notably decreasing where continuous N is applied. A build-up in soil test P is apparent, especially among high P rates. Furthermore, a slight drawn down in soil K levels were commonly noted across treatments overtime as K has not been applied.

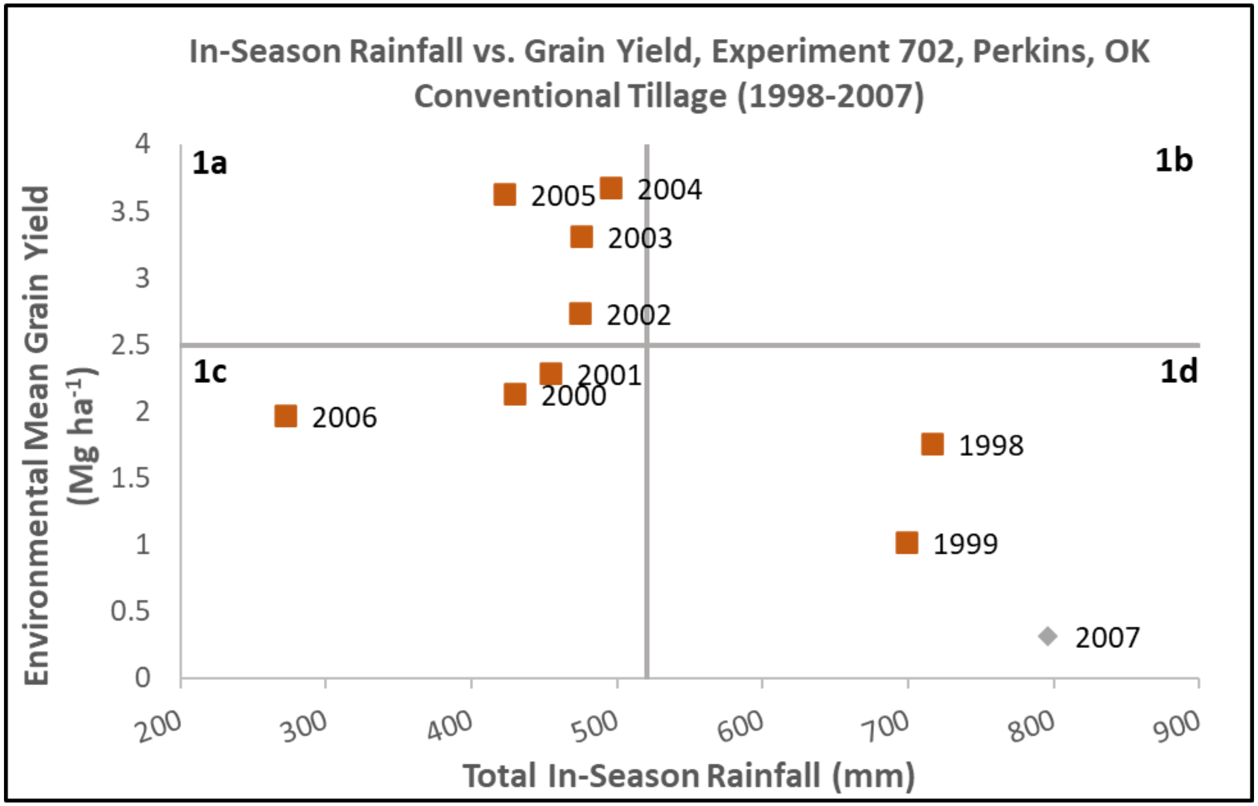

Figure 9. Relationship between total in-season precipitation received and environmental mean grain yield for Experiment 702, Perkins, OK while under conventional tillage (1998-2007). This was used to group years by similar rainfall and yield level. Each quadrant (1a, 1b, 1c, 1d) represents an environmental grouping used for further analysis. The year 2007 was not representative of the 1d quadrant and was therefore not included in subsequent group analysis.

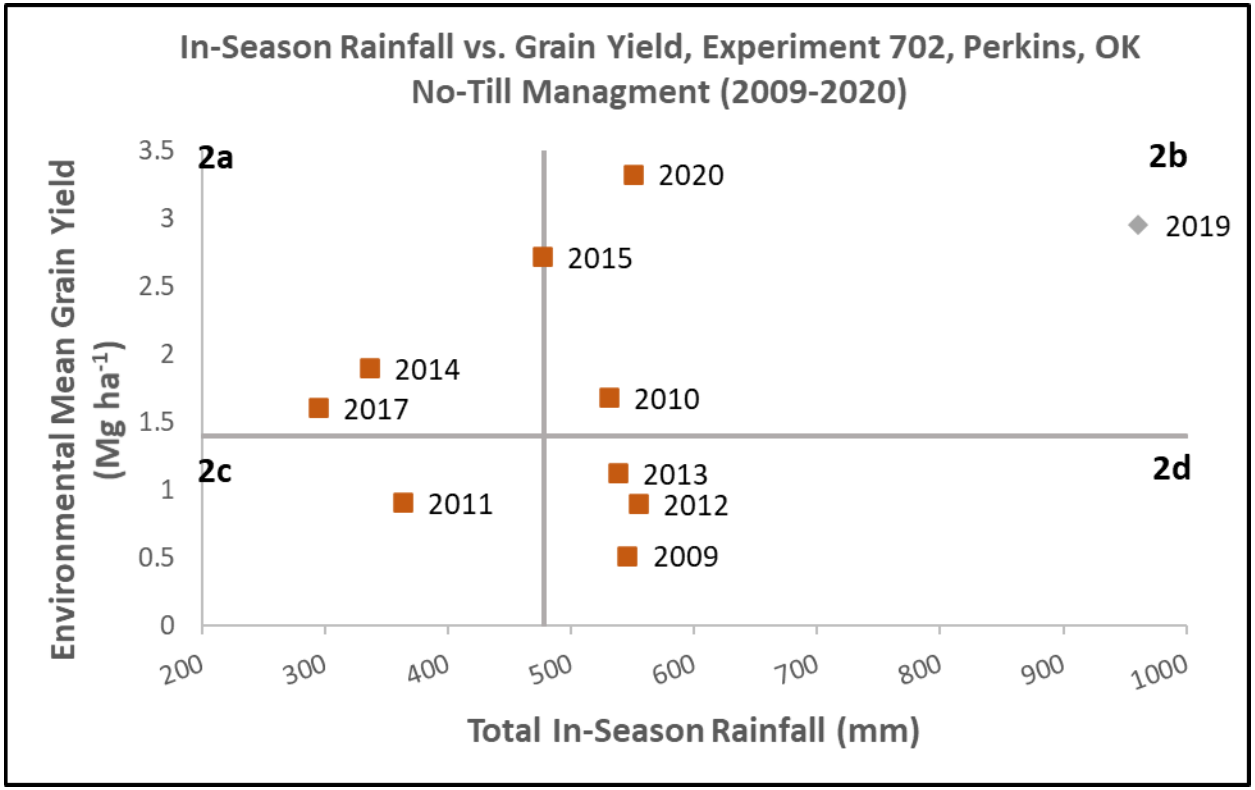

Figure 10. Relationship between total in-season precipitation received and environmental mean grain yield for Experiment 702, Perkins, OK after transition to no-till management (2008-2020). This was used to group years by similar rainfall and yield level. Each quadrant (2a, 2b, 2c, 2d) represents an environmental grouping used for further analysis. The year 2019 was not representative of the 2b quadrant and was therefore not included in subsequent group analysis.

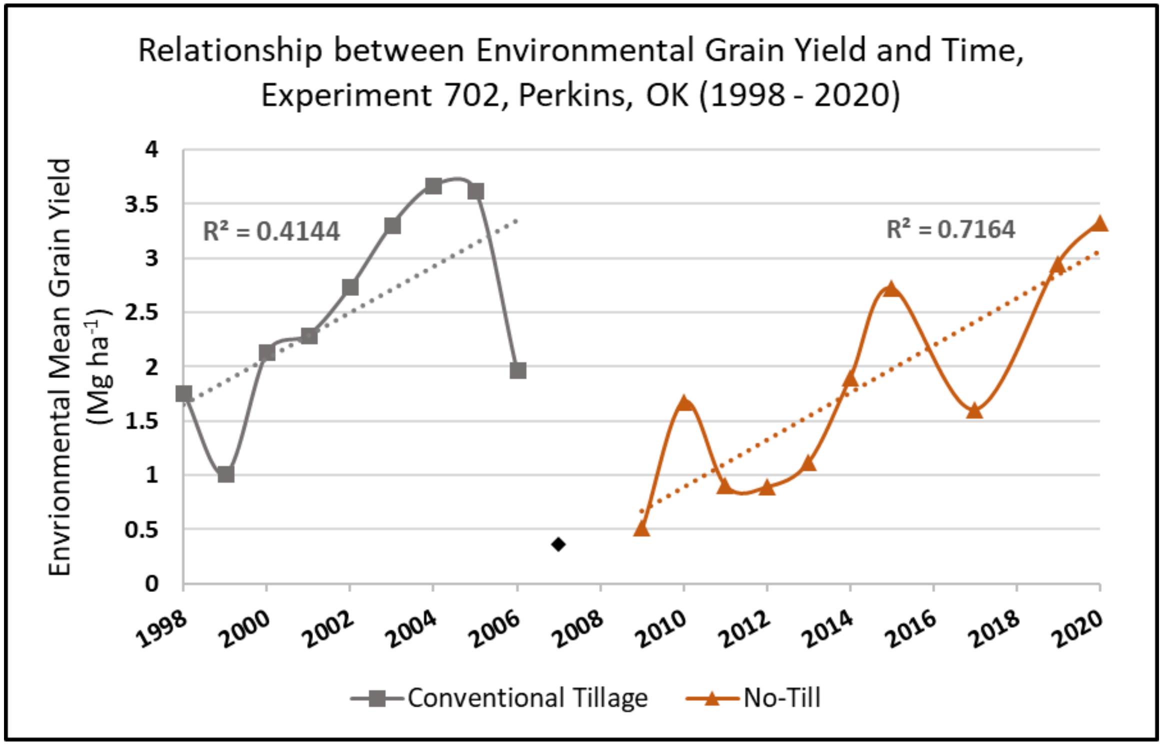

Figure 11. Relationship between environmental yield and time, Experiment 702, Perkins, OK (1998-2020). Under each tillage practice, an increasing trend in grain yield was apparent.

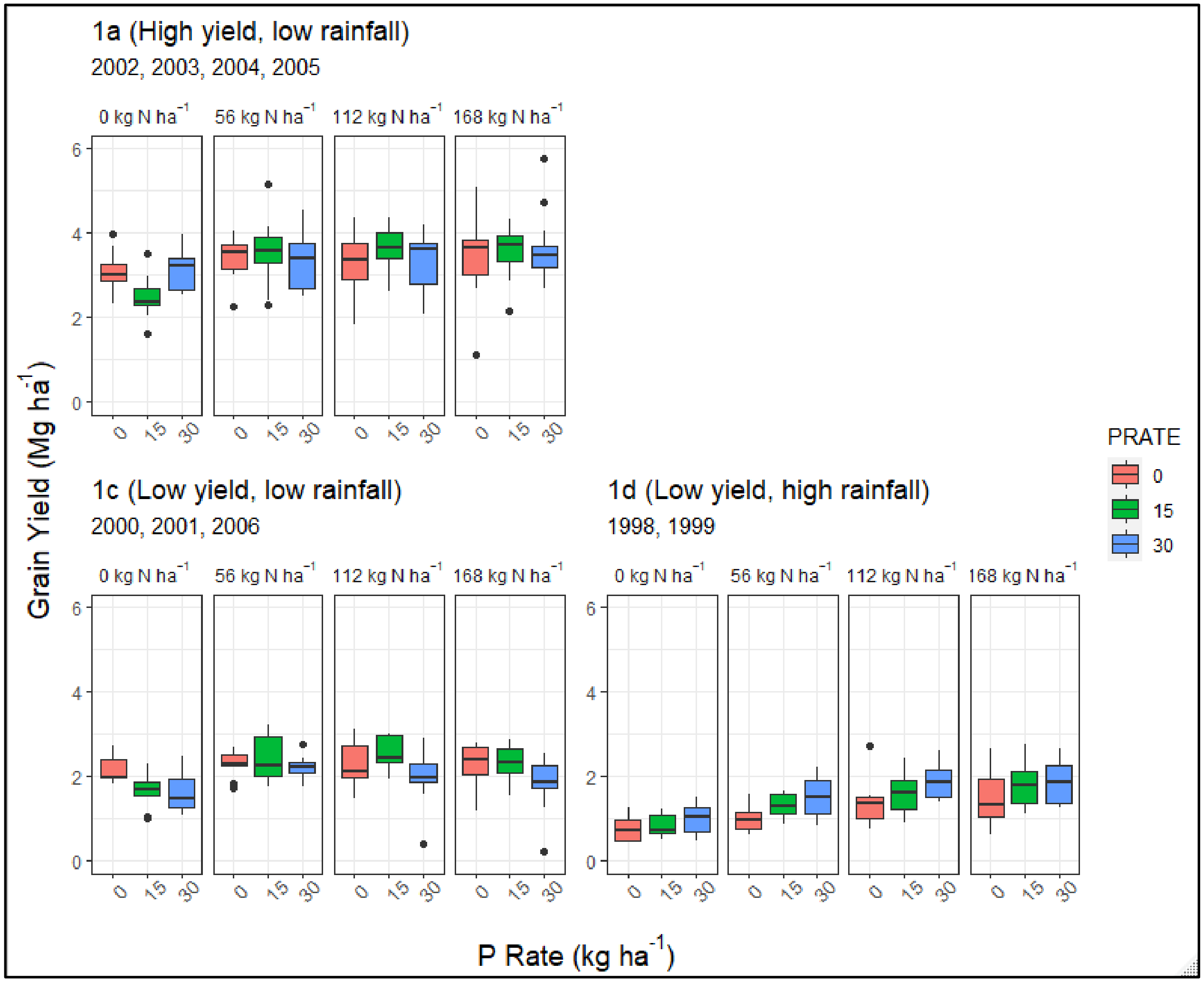

Figure 12. Grain yield as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007).

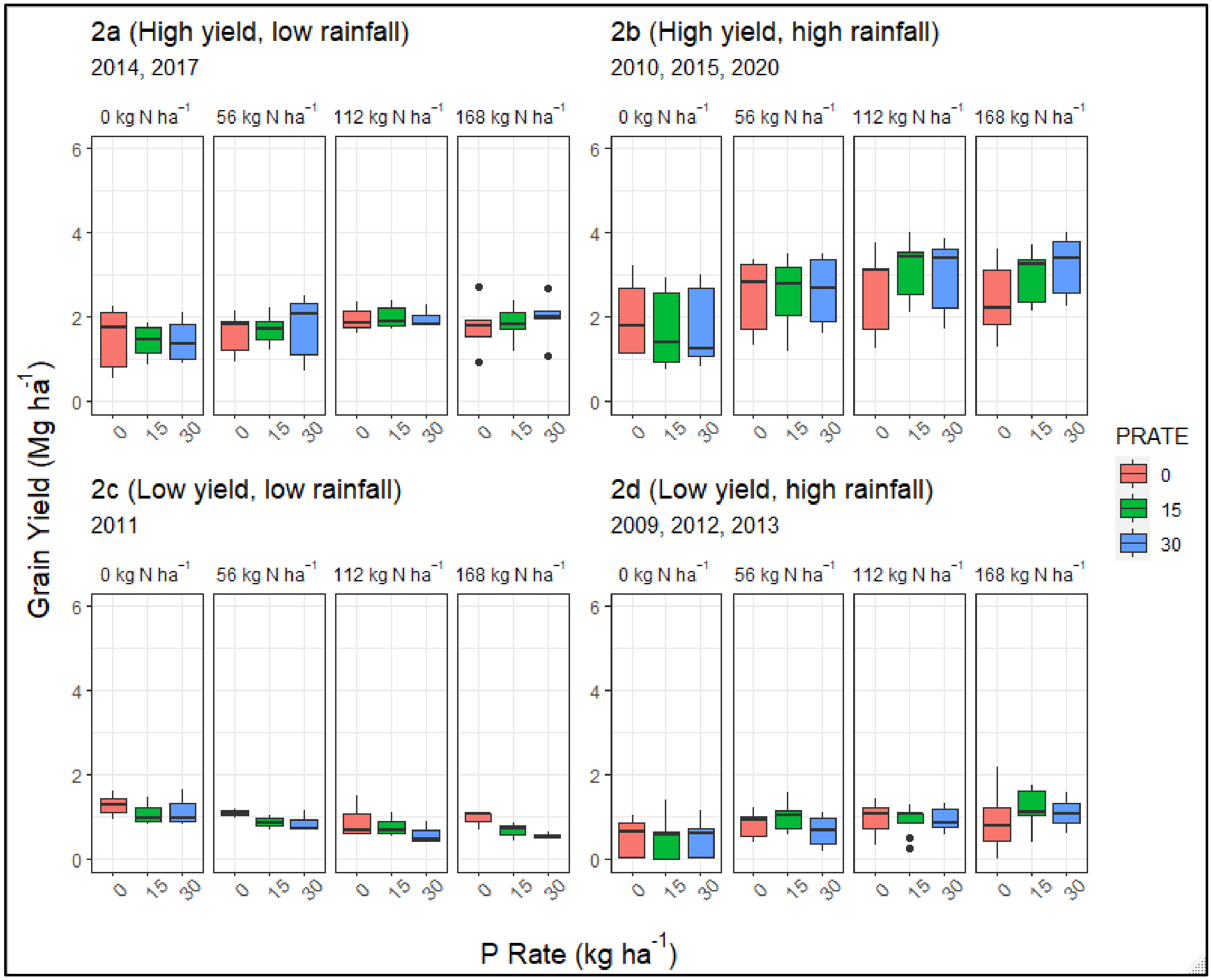

Figure 13. Grain yield as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020).

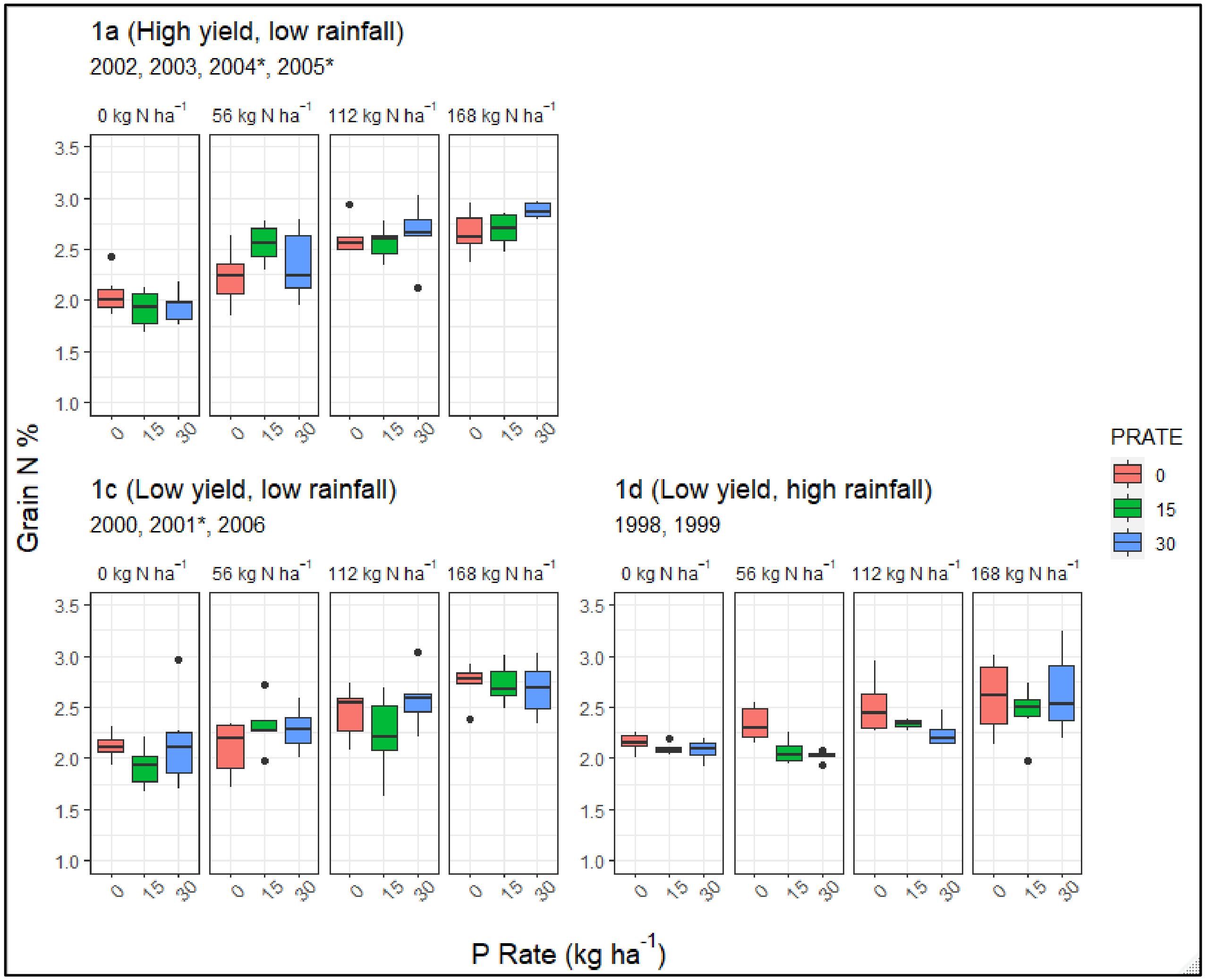

Figure 14. Grain N concentration (%) as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007). * next to the year indicates data was not available for that season.

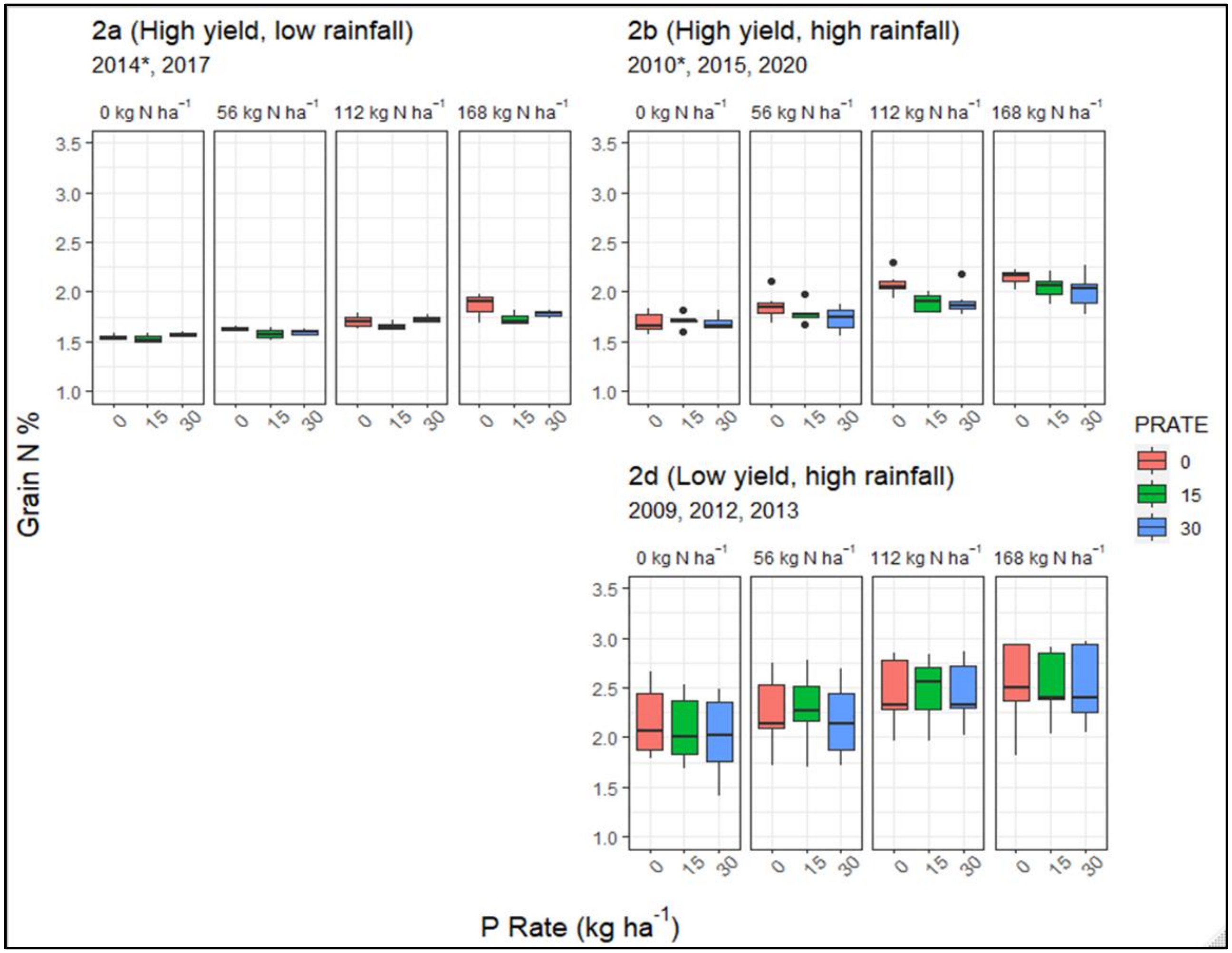

Figure 15. Grain N concentration (%) as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020). * next to the year indicates data was not available for that season.

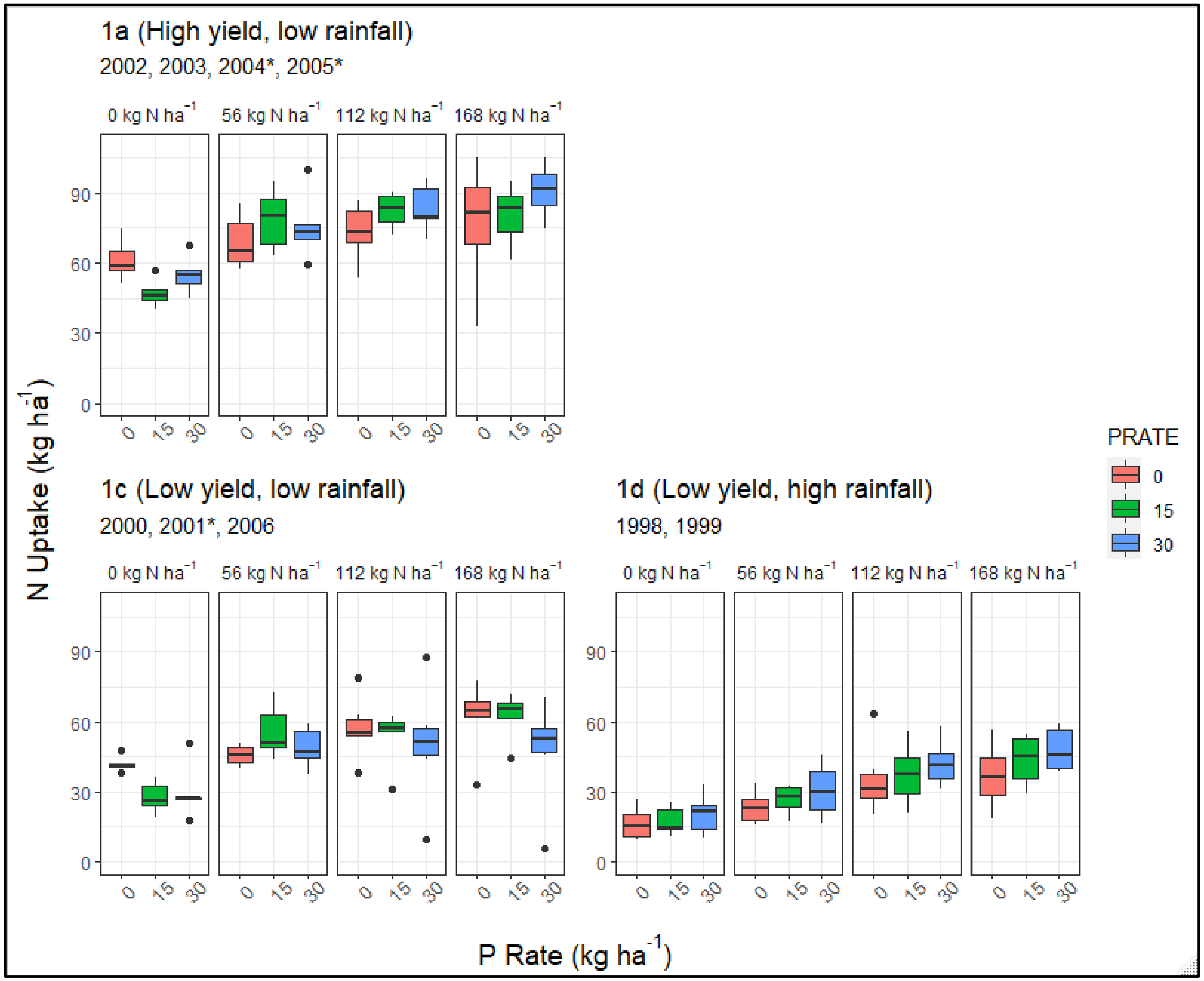

Figure 16. Nitrogen uptake as influenced by N and P rate for each environmental year grouping while under conventional tillage, Experiment 702, Perkins, OK (1998-2007). * next to the year indicates data was not available for that season.

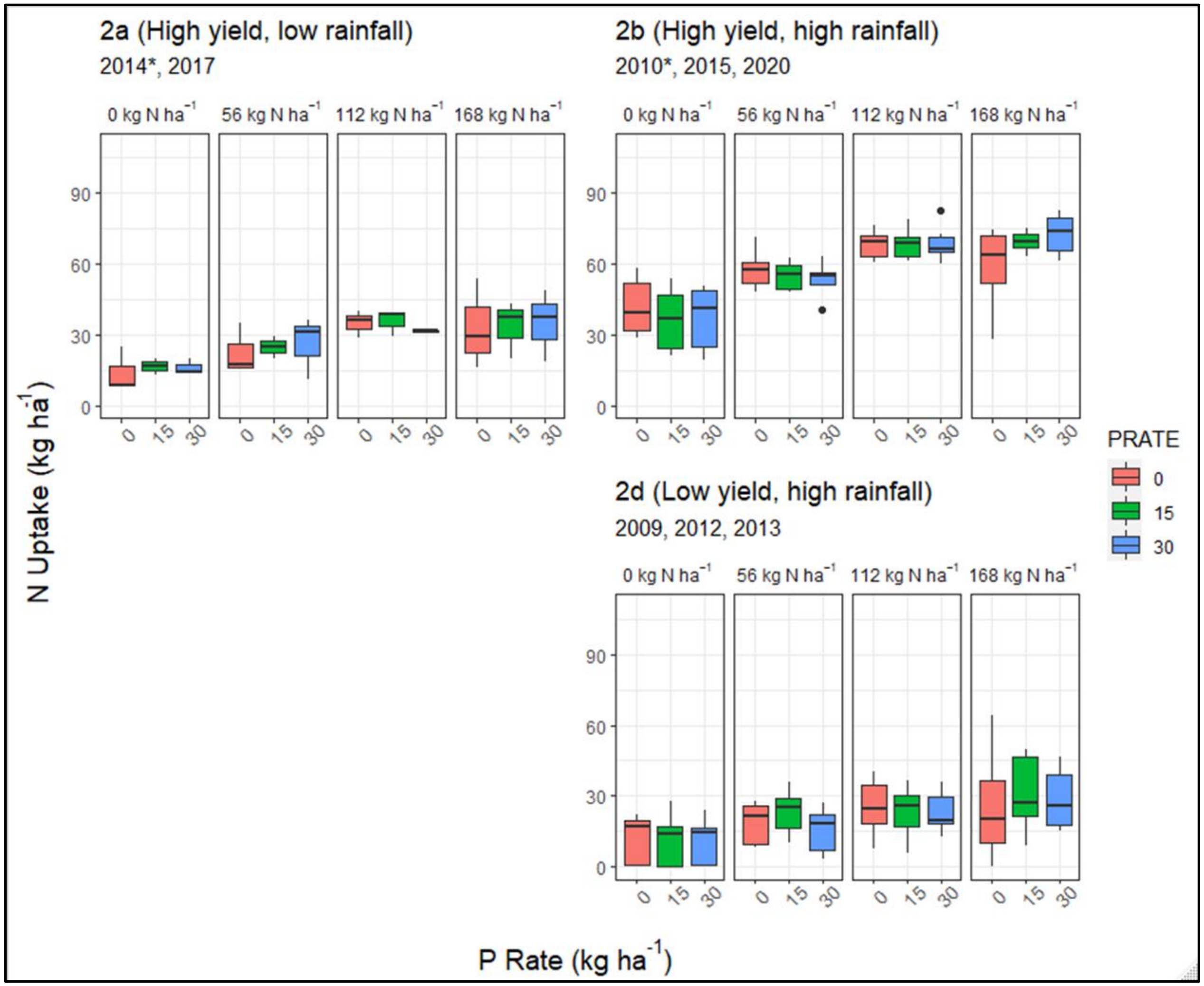

Figure 17. Nitrogen uptake as influenced by N and P rate for each environmental year grouping after transition to no-till management, Experiment 702, Perkins, OK (2008-2020). * next to the year indicates data was not available for that season.

Figure 18. Image was taken in January of 2019. Both plots received 112 kg N ha^{-1} while the left did not receive P, and the right plot received 29.6 kg P ha^{-1}. Early season growth benefited from additional P applications even though soil test P values were high.

REFERENCES

Albareda, M., Rodríguez-Navarro, D. N. and Temprano, F. J., (2009). Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Research*, *113*(3), 352-356.

Alston, A. M. (1979). Effects of soil water content and foliar fertilization with nitrogen and phosphorus in late season on the yield and composition of wheat. *Australian Journal of Agricultural Research*, 30(4), 577-585.

Aula, L., Omara, P., Eickhoff, E., Oyebiyi, F., Dhillon, J. S., and Raun, W. R. (2020). Effect of winter wheat cultivar on grain yield trend under different nitrogen management. *Agrosystems, Geosciences & Environment*, *3*(1), e20017.

Aula, L., Omara, P., Nambi, E., Oyebiyi, F. B., Dhillon, J., Eickhoff, E., Carpenter, J, and Raun, W. R. (2021). Active Optical Sensor Measurements and Weather Variables for Predicting Winter Wheat Yield. *Agronomy Journal*. https://doi.org/10.1002/agj2.20620

Barnett, V., Payne, R. W., and Steiner, R. (1995). Agricultural sustainability: economic, environmental, and statistical considerations/edited by Vic Barnett, Roger Payne, and Roy Steiner.

Beard, B. H., and Hoover, R. M. (1971). Effect of Nitrogen on Nodulation and Yield of Irrigated Soybeans 1. *Agronomy Journal*, 63(5), 815-816.

Beuerlein, J. (2004). Ohio inoculation study. Available at agcrops.osu.edu/research/2004/04inoc% 20report11. pdf (verified 10 July 2008). *Agronomic Crops Network*, Ohio State Univ., Columbus.

Biswas, B., and Gresshoff, P. M. (2014). The role of symbiotic nitrogen fixation in sustainable production of biofuels. *International Journal of Molecular Sciences*, 15(5), 7380-7397.

Blackshaw, R. E. (2004). Application method of nitrogen fertilizer affects weed growth and competition with winter wheat. *Weed Biology and Management*, 4(2), 103-113.

Boatwright, G. O., and Viets, F.G., Jr. (1966). Phosphorus absorption during various growth stages of spring wheat and intermediate wheatgrass. *Agronomy Journal.* 58: 185–188.

Bryant- Schlobohm, R., Dhillon, J., Wehmeyer, G. B., and Raun, W. R., (2020). Wheat grain yield and nitrogen uptake as influenced by fertilizer placement depth. *Agrosystems, Geosciences & Environment* 3:1: e20025.

Camara, K. M., W.A. Payne, and P.E. Rasmussen. (2003). Long-term effects of tillage, nitrogen, and rainfall on winter wheat yields in the Pacific Northwest. *Agronomy Journal*, 95(4), 828-835.

Campbell, C. A., Mcleod, J. G., Selles, F., Zentner, R. P., and Vera, C. (1996). Phosphorus and nitrogen rate and placement for winter wheat grown on chemical fallow in a Brown soil. *Canadian Journal of Plant Science*. 76: 237–243.

Campbell, C.A., Myers, R. J. K., and Curtin, D. (1995). Managing nitrogen for sustainable crop production. *Fert. Res*. 42:277-296

Carciochi, W. D., Rosso, L. H. M., Secchi, M. A., Torres, A. R., Naeve, S., Casteel, S. N. ... and Ciampitti, I. A. (2019). Soybean yield, biological N 2 fixation and seed composition responses to additional inoculation in the United States. *Scientific Reports*, 9(1), 1-10.

Ciafardini, G., and Barbieri, C. (1987). Effects of cover inoculation of soybean on nodulation, nitrogen fixation, and yield. *Agronomy Journal*, 79(4), 645-648.

Clarkson, D. T. (1981). Nutrient interception and transport by root systems. *Physiological processes limiting plant productivity*, 307-330.

Clarkson, D. T., and Grignon, C. (1991). The phosphate transport system and its regulation in roots. In: C. Johansen et al., editors, Phosphorus nutrition of grain legumes in the semi-arid tropics. *ICRISAT*, Patancheru, India. p. 49– 61.

Conley, S., and Christmas, E. (2006) Utilizing inoculant in a corn-soybean rotation. *Purdue Ext. Bull.* SPS-100-W. Purdue Univ., West Lafayette, IN

DeLaune, P. B., and J. W. Sij. (2012). Impact of tillage on runoff in long term no-till wheat systems. *Soil and Tillage Research* 124: 32-35.

Dhillon, J. S., Figueiredo, B. M., Eickhoff, E. M., and Raun, W. R. (2020a). Applied use of growing degree days to refine optimum times for nitrogen stress sensing in winter wheat. *Agronomy Journal* 112: 537-549.

Dhillon, J., Eickhoff, E., Aula, L., Omara, P., Wehmeyer, G., Nambi, E., Oyebiyi, F., Carpenter, T., and Raun, W. R. (2020b). Nitrogen management impact on winter wheat grain yield and estimated plant nitrogen loss. *Agronomy Journal* 112: 564-577.

Dhillon, J., Torres, G., Driver, E., Figueiredo, B., and Raun, W. R. (2017). World phosphorus use efficiency in cereal crops. *Agronomy Journal,* 109(4), 1670-1677.

Edwards, J. T., Arnall, D. B., and Zhang, H. (2009). Nitrogen fertilizer timing and source affect hard red winter wheat yield, but application method does not. *Crop Management*, 8(1), 0-0.

FAO, (2018). Food and Agriculture Organization of the United Nations. FAOSTAT statistical database.

Fazli, I.S., Jamal, A., Ahmad, S., Masoodi, M., Khan, J. S., Abdin, M. Z. (2008). Interactive effect of sulfur and nitrogen on nitrogen accumulation and harvest in oilseed crops differing in nitrogen assimilation potential. *Journal of Plant Nutrition* 31: 1203-1220

Fenta, B. A., Schluter, U., Garcia, B. M., DuPlessis, M., Foyer, C. H., and Kunert, K. J. (2011). Identification and application of phenotypic and molecular markers for abiotic stress tolerance in soybean. Soybean—Genetics and Novel Techniques for Yield Enhancement, 181-200.

Fiedler, R. J., Sander, D. H., and Peterson, G. A. (1989). Fertilizer phosphorus recommendations for winter wheat in terms of method of phosphorus application, soil pH, and yield goal. *Soil Science Society of America Journal*, 53(4), 1282-1287.

Fischer, R. A., Byerlee, D., Edmeades, G. O. (2014). Crop yields and global food security: will yield increase continue to feed the world? ACIAR Monograph No. 158. Australian Centre for International Research: Canberra. xxii + 634pp.

Flowers, M., Weisz, R., Heiniger, R., Osmond, D., and Crozier, C. (2004). In-Season optimization and site-specific nitrogen management for soft red winter wheat. *Agronomy Journal*. 96: 124-134.

Garcia, J. P., Wortmann, C. S., Mamo, M., Drijber, R., and Tarkalson, D. (2007). One-time tillage of no-till: Effects on nutrients, mycorrhizae, and phosphorus uptake. *Agronomy Journal*, *99*(4), 1093-1103.

Gericke, W. F. (1924). The beneficial effect to wheat growth due to depletion of available phosphorus in the culture media. *Science,* 60: 297–98.

Gowda, C. L. L., Rao, P. P., and Bhagavatula, S. (2009). Global trends in production and trade of major grain legumes.

Graham, P. H. (1992). Stress tolerance in Rhizobium and Bradyrhizobium, and nodulation under adverse soil conditions. *Canadian Journal of Microbiology*, 38(6), 475-484.

Green, D. G., Ferguson, W. S., and Warder, F. G. (1973). Accumulation of toxic levels of phosphorus in the leaves of phosphorus-deficient barley. *Canadian Journal of Plant Science*. 53: 241–246.

Gresshoff, P. M., Hayashi, S., Biswas, B., Mirzaei, S., Indrasumunar, A., Reid, D., Samuel, S., Tollenaere, A., van Hameren, B., Hastwell, A., Scott, P., and Ferguson, B. (2015). The value of biodiversity in legume symbiotic nitrogen fixation and nodulation for biofuel and food production. *Journal of Plant Physiology,* 172, 128-136.

Habbib, H., Hirel, B., Spicher, F., Dubois, F., and Tétu, T. (2020). In Winter Wheat (Triticum Aestivum L.), No-Till Improves Photosynthetic Nitrogen and Water-Use Efficiency. *Journal of Crop Science and Biotechnology*, 23(1), 39-46.

Halvorson, A. D., and Havlin, J. L. (1992). No-till winter wheat response to phosphorus placement and rate. *Soil Science Society of America Journal*, 56(5), 1635-1639.

Hardarson, G. Methods for enhancing symbiotic nitrogen fixation. Plant Soil 152, 1–17 (1993). https://doi.org/10.1007/BF00016329

Harper, J. E., and Cooper, R. L. (1971). Nodulation Response of Soybeans (Glycine max L. Merr.) to Application Rate and Placement of Combined Nitrogen 1. *Crop Science*, 11(3), 438-440.

Hungria, M. (2018). Can additional inoculations increase soybean nodulation and grain yield? *Agronomy Journal*, 110(2), 715-721.

Hymowitz, T., Collins, F. I., Panczner, J., and Walker, W. M. (1972). Relationship between the content of oil, protein, and sugar in soybean seed 1. *Agronomy Journal*, 64(5), 613-616.

Illston, B. G., Basara, J. B., Fiebrich, C. A., Crawford, K. C., Hunt, E., Fisher, D. K., Elliot, R., and Humes, K. (2008). Mesoscale monitoring of soil moisture across a statewide network. *Journal Atmospheric Ocean. Technology*. 25, 67–182.

Islam, M. A., and Adjesiwor, A. T. (2017). Nitrogen Fixation and Transfer in Agricultural Production Systems. Nitrogen in Agriculture-Updates, Amanullah and Shah Fahad, *IntechOpen*, DOI:10.5772/intechopen.71766.

Jamal, A., Moon, Y. S., and Zainul Abdin, M. (2010). Sulphur-a general overview and interaction with nitrogen. *Australian Journal of Crop Science*, 4(7), 523.

Kaiser, D., (2018). Wheat Fertilizer Recommendations. University of Minnesota Extension.

Karamanos, R. E., Stonehouse, T. A., and Flore, N. A., (2003). Response of winter wheat to nitrogen and phosphate fertilizer placement and time of application. *Canadian Journal of Plant Science*, 83(3), 483-488.

Kaschuk, G., Nogueira, M. A., De Luca, M. J., and Hungria, M. (2016). Response of determinate and indeterminate soybean cultivars to basal and topdressing N fertilization compared to sole inoculation with Bradyrhizobium. *Field Crops Research*, 195, 21-27.

Lavy, T. L., and Barber, S. A. (1964). Movement of molybdenum in the soil and its effect on availability to the plant. *Soil Science Society of America Journal*, 28(1), 93-97.

Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., and Leister, T. (2017). Soybean response to inoculation with Bradyrhizobium japonicum in the United States and Argentina. *Agronomy Journal*, 109(3), 1031-1038.

Lindsay, W., Vlek, P., and Chien, S. (1989). Phosphate minerals. Minerals in Soil Environment. 2: 1089– 1130.

Lofton, J., and Arnall, B. (2017). Understanding Soybean Nodulation and Inoculation. *Oklahoma State University. Extension Publication*. https://extension.okstate.edu/fact-sheets/understanding-soybean-nodulation-and-inoculation.html

Lollato, R. P., Figueiredo, B. M., Dhillon, J. S., Arnall, D. B., and Raun, W. R. (2019). Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: a synthesis of long-term experiments. *Field Crops Research* 236: 42-57.

Manske, G. G. B., Ortiz-Monasterio, J. I., Van Ginkel, M., Gonzalez, R. M., Fischer, R. A., Rajaram, S., and Vlek, P. L. G. (2001). Importance of P uptake efficiency versus P utilization for wheat yield in acid and calcareous soils in Mexico. *European Journal of Agronomy*, *14*(4), 261-274.

Marburger, D., Hunger, B., Carver, B., and Royer, T., (2018). Wheat Variety Comparison. *Oklahoma Cooperative Extension Service*. PSS-2142

Marschner, H. (1995). Mineral nutrition of higher plants. 2nd ed. Academic Press, San Diego, CA

Marshall, T., (2018). Oklahoma Wheat Review. USDA, Oklahoma Field Office.

McCoy, J. M., Kaur, G., Golden, B. R., Orlowski, J. M., Cook, D. R., Bond, J. A., and Cox, M. S. (2018). Nitrogen fertilization of soybean affects root growth and nodulation on two soil types in Mississippi. *Communications in Soil Science and Plant Analysis*, 49(2), 181-187.

Meisinger, J. J., and Delgado, J. A. (2002). Principles for managing nitrogen leaching. *Journal of Soil and Water Conservation*, 57(6), 485-498.

Moretti, L. G., Lazarini, E., Bossolani, J. W., Parente, T. L., Caioni, S., Araujo, R. S., and Hungria, M. (2018). Can additional inoculations increase soybean nodulation and grain yield? *Agronomy Journal*, 110(2), 715-721.

Mosali, J., Desta, K., Teal, R. K., Freeman, K. W., Martin, K. L., Lawles, J. W., and Raun, W. R. (2006). Effect of foliar application of phosphorus on winter wheat grain yield, phosphorus uptake, and use efficiency. *Journal of Plant Nutrition*, 29(12), 2147-2163.

Mourtzinis, S., Kaur, G., Orlowski, J. M., Shapiro, C. A., Lee, C. D., Wortmann, C., Holshouser, D., Nafziger, E., Kandel, H., Niekamp, J., Ross, W. J., Lofton, J., Vonk, J., Roozeboom, K., Thelen, K., Lindsey, L., Staton, M., Naeve, S., ... and Conley, S. (2018). Soybean response to nitrogen application across the United States: A synthesis-analysis. *Field Crops Research*, 215, 74-82.

Mueller, N., Elmore, R., and Shapiro, C. (2015). Soybean Inoculation: When, Where, and Why. *Crop Watch*. Accessed: June 29, 2020. https://cropwatch.unl.edu/soybean-inoculation-when-where-and-why

N.a. (2008). Seed inoculation. Integrated Crop Management. Iowa State University. Accessed June 24, 2020: https://crops.extension.iastate.edu/soybean/production_seedinoc.html

N.a. (2015). Crops. The Environmental Literacy Council. Accessed May 20, 2020. https://enviroliteracy.org/food/crops/

Nedumaran, S., Abinaya, P., Jyosthnaa, P., Shraavya, B., Parthasarathy, R., and Bantilan, C. (2015). Grain Legumes Production, Consumption and Trade Trends in Developing Countries. Working Paper Series No 60. ICRISAT Research Program, Markets, Institutions and Policies. Patancheru 502 324, Telangana, India: International Crops Research Institute for the Semi-Arid Tropics. 64 pp

Nelson, L. B. (1990). History of the U.S. fertilizer industry. Muscle Shoals, AL: Tennessee Valley Authority.

Nyoki, D., and Ndakidemi, P. A. (2018). Rhizobium inoculation reduces P and K fertilization requirement in corn-soybean intercropping. Rhizosphere, 5, 51-56.Oklahoma Mesonet. Daily data retrieval. University of Oklahoma. https://www.mesonet.org/index.php/weather/category/past_data_files. Accessed 15 Nov (2020)

Omara, P., Aula, L., Oyebiyi, F., and Raun, W. R. (2019). World cereal nitrogen use efficiency trends: Review and current knowledge. *Agrosystems, Geosciences & Environment*, 2(1).

Osmont, K.S., Sibout, R., and Hardtke, C. S. (2007). Hidden branches: Developments in root system architecture. *Annual Review of Plant Biology*, Vol. 58, (June 2007), pp. 93-113, ISSN 1543-5008

OSU, 2018. Wheat. Accessed May 20, 2020. http://wheat.okstate.edu/

Pagano, M. C., and Miransari, M. (2016). The importance of soybean production worldwide. Abiotic and Biotic Stresses in Soybean Production (pp. 1-26). Academic Press.

Parker, M. B., and Harris, H. B. (1962). Soybean Response to Molybdenum and Lime and the Relationship between Yield and Chemical Composition 1. *Agronomy Journal*, 54(6), 480-483.

Peng, Z., Wang, L., Xie, J., Li, L., Coulter, J. A., Zhang, R., ... and Choudhary, S. (2019). Conservation Tillage Increases Water Use Efficiency of Spring Wheat by Optimizing Water Transfer in a Semi-Arid Environment. *Agronomy*, 9(10), 583.

Penn, C. J., Bell, P. R., Carver, B., Arnall, D. B., and Klatt, A. (2015). Comparison of Phosphorus Use Efficiency Among Various Winter Wheat Accessions Grown in Acid and Calcareous Soils. *Journal of plant nutrition*, *38*(14), 2279-2293.

Pittelkow, C. M., Linquist, B. A., Lundy, M. E., Liang, M., Van Groenigen, K. J., Lee, J. ... and Van Kessel, C. (2015). When does no-till yield more? A global meta-analysis. *Field Crops Research*, 183, 156-168.

R Core Team. (2021). R: *A language and environment for statistical computing* (Version R-4.0.3) Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rasnake, M. "Use of Molybdenum for Soybean Production" (1982). *Soil Science News and Views*. 84. https://uknowledge.uky.edu/pss_views/84

Raun, W. R., and Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, 91(3), 357-363.

Raun, W. R., Dhillon, J., Aula, L., Eickhoff, E., Wehmeyer, G., Figueiredo, B., ... and Fornah, A. (2019). Unpredictable nature of environment on nitrogen supply and demand. *Agronomy Journal*, 111(6), 2786-2791.

Raun, W. R., Figueiredo, B., Dhillon, J., Fornah, A., Bushong, J., Zhang, H., and Taylor, R. (2017). Can yield goals be predicted?." *Agronomy Journal* 109: 2389-2395.

Raun, W. R., Olson, R. A., Sander, D. H., and Westerman, R. L. (1987). Alternative procedure for total phosphorus determination in plant tissue. *Communications in Soil Science and Plant Analysis*, 18(5), 543-557.

Raun, W. R., Solie, J., May, J., Zhang, H., Kelly, J., Taylor, R., Arnall, B., Ortiz-Monasterio, I. (na). Nitrogen Rich Strips for wheat, corn, and other crops. *Oklahoma State University Extension*.

Reisenauer, H. M. (1965). Molybdenum. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 9, 1050-1058.

Rotundo, J. L., and Westgate, M. E. (2009). Meta-analysis of environmental effects on soybean seed composition. *Field Crops Research*, 110(2), 147-156.

Ruiz Diaz, D. A., Pedersen, P. and Sawyer, J. E. (2009). Soybean response to inoculation and nitrogen application following long-term grass pasture. *Crop Science*, 49(3), 1058-1062.

Sadeghipour, O., and Abbasi, S. (2012). Soybean response to drought and seed inoculation. *World Applied Sciences Journal*, 17(1), 55-60.

Salvagiotti, F., Cassman, K. G., Specht, J. E., Walters, D. T., Weiss, A., and Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, 108(1), 1-13.

Sander, D. H., and Eghball, B. (1999). Planting date and phosphorus fertilizer placement effects on winter wheat. *Agronomy Journal*, 91(4), 707-712.

SAS Institute. (2011). *SAS/STAT ® User's guide.* Cary, NC: SAS Institute.

Scharf, P. C., and Wiebold, W. J. (2003). Soybean yield responds minimally to nitrogen applications in Missouri. *Crop Management*, 2(1), 1-6.

Schroder, J. L., Zhang, H., Girma, K., Raun, W. R., Penn, C. J., and Payton, M. E. (2011). Soil acidification from long-term use of nitrogen fertilizers on winter wheat. *Soil Science Society of America Journal*, *75*(3), 957-964.

Sedberry, J. E., "Molybdenum investigations with soybeans in Louisiana" (1973). LSU Agricultural Experiment Station Reports. 499. http://digitalcommons.lsu.edu/agexp/499

Sij, J. W., Pinchak, W. E., Malinowski, D. P., Robinson, D. L., Bevers, S. J., Baughman, T. A., and Gill, R. J. (2007). Yield and economic responses to phosphorus fertilizer placement in dual-use and grain-only wheat production systems. *Agronomy Journal*, 99(3), 773-778.

Solomon, T., Pant, L. M., and Angaw, T. (2012). Effects of inoculation by Bradyrhizobium japonicum strains on nodulation, nitrogen fixation, and yield of soybean (Glycine max L. Merill) varieties on nitisols of Bako, Western Ethiopia. *International Scholarly Research Notices*, 2012.

Stagnari, F., Maggio, A., Galieni, A., and Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture*. 4, 2. https://doi.org/10.1186/s40538-016-0085-1

Stanton, M. (2011). Evaluating Soybean Nodulation. Michigan State University. Accessed June 29, 2020: https://www.canr.msu.edu/news/evaluating_soybean_nodulation#:~:text=There%20should%20be%208%20to,the%20location%20of%20the%20nodules.

Stewart, W. M, (n.a). Winter Wheat Fertilization. Mosaic. https://www.cropnutrition.com/resource-library/winter-wheat-fertilization (Accessed June 15, 2020)

Tauer, L. W. (1989). Economic impact of future biological nitrogen fixation technologies on United States agriculture. *Plant and Soil*, 119, 261-270.

Thao, T. Y., Singleton, P. W., and Herridge, D. (2002). Inoculation responses of soybean and liquid inoculants as an alternative to peat-based inoculants. Proceedings of the ACIAR Proceedings 109e on Inoculants and Nitrogen Fixation of Legumes in Vietnam, 67-74.

Tisdale, S. L., Nelson, W. L., and Beaton, J. B. (1985). Soil Fertility and Fertilizers. New York: Macmillan.

USDA., Web Soil Survey. 2020. Available at: https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (Accessed: February 8, 2020)

Walsh, O. S., Klatt, A. R., Solie, J. B., Godsey, C. D. and Raun, W. R. (2013). Use of soil moisture data for refined GreenSeeker sensor-based nitrogen recommendations in winter wheat (Triticum aestivum L.). *Precision Agriculture*, 14(3), 343-356.

Wehmeyer, G. (2020) Dissertation: Optimum Sulfur Application for Soybean (Glycine max) in Oklahoma.

Wesley, T. L., Lamond, R. E., Martin, V. L., and Duncan, S. R. (1998). Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. Journal of Production Agriculture, 11(3), 331-336.

Westerman, R. L., and Edlund, M. G. (1985). Deep Placement Effects of Nitrogen and Phosphorus Grain Yield, Nutrient Uptake, and Forage Quality of Winter Wheat 1. *Agronomy Journal*, 77(5), 803-809.

Woodard, H. J., and Bly, A. (1998). Relationship of nitrogen management to winter wheat yield and grain protein in South Dakota. *Journal of Plant Nutrition*, 21(2), 217-233.

Woolfolk, C. W., Raun, W. R., Johnson, G. V., Thomason, W. E., Mullen, R. W., Wynn, K. J., and Freeman, K. W. (2002). Influence of late-season foliar nitrogen applications on yield and grain nitrogen in winter wheat. *Agronomy Journal*, 94(3), 429-434.

Zhang, H., Krenzer, G., Johnson, (2000). Liming Raises Soil pH and Increases Winter Forage Yields. *Production Technology*. PT 2000-10

Zhang, H., Schroder, J. L., Davis, R. L., Wang, J. J., Payton, M. E., Thomason, W. E., ... and Raun, W. R. (2006). Phosphorus loss in runoff from long-term continuous wheat fertility trials. Soil Science Society of America Journal, 70(1), 163-171.

Zhang, H., Raun, W. R., and Arnall, B. (2017). OSU Soil Test Interpretations. Oklahoma State University. https://extension.okstate.edu/fact-sheets/osu-soil-test-interpretations.html

APPENDICIES

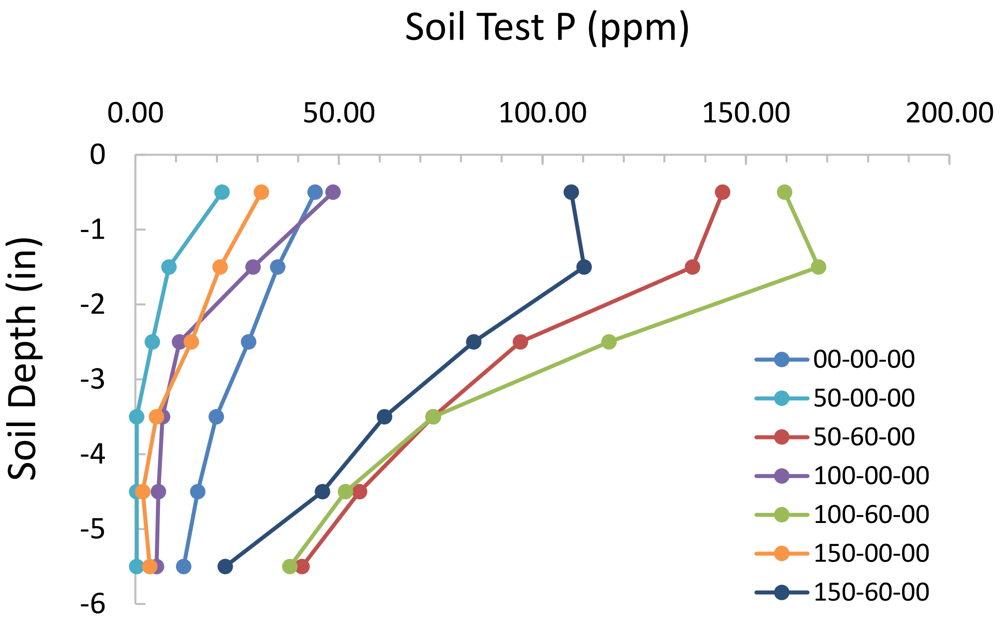

Figure 19. Phosphorus stratification in the top 6 inches of soil of selected treatments in Experiment 702, Perkins, OK. Legend indicates N, P, K fertilizer applied in lbs of N and P_2O_5 ac^{-1}. (unpublished: Souza et al., 2020)

Table 31. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. All years combined (1998-2020).

Model: Yield = PC1 + PC2 +PC3 +PC4

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.	Primary Loading	Loading Value
(Intercept)	2.0054	0.04121	48.667	< 2e-16	***		
Comp.1	0.0121	0.02879	0.42	0.674475	ns	Rain Dec	0.582
Comp.2	0.12486	0.03265	3.824	0.000143	***	Rain Nov	0.510
Comp.3	0.11533	0.03445	3.348	0.00086	***	Rain Jan	0.535
Comp.4	0.36583	0.03696	9.899	< 2e-16	***	Rain Apr	-0.700
				RSE	1.077		
				Multiple R^2	0.1546		
				Adj R^2	0.1496		
				p-value	< 2.2e-16		

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

Table 32. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. Conventional Tillage (1998-2007).

Model: Yield = PC1 + PC2 +PC3 +PC4

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.	Primary Loading	Loading Value
(Intercept)	2.27895	0.03846	59.256	< 2e-16	***		
Comp.1	0.42888	0.02217	19.349	< 2e-16	***	Rain Nov	0.442
Comp.2	-0.07969	0.02841	-2.805	0.00535	**	Rain Jun	-0.504
Comp.3	-0.43432	0.0306	-14.194	< 2e-16	***	Rain Oct	0.443
Comp.4	0.37353	0.03514	10.629	< 2e-16	***	Rain Dec	0.554
				RSE	0.6912		
				Multiple R^2	0.6866		
				Adj R^2	0.6827		
				p-value	< 2.2e-16		

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

Table 33. ANOVA of the fitted linear model for grain yield containing 4 PC with their primary loading. Analysis contains monthly rainfall only. No-Tillage (2008-2020).

Model: Yield = PC1 + PC2 +PC3 +PC4

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.	Primary Loading	Loading Value
(Intercept)	1.75997	0.03938	44.689	< 2e-16	***		
Comp.1	0.2704	0.02474	10.929	< 2e-16	***	Rain Nov	-0.522
Comp.2	0.15573	0.02747	5.669	2.98E-08	***	Rain Jan	0.493
Comp.3	0.42335	0.02983	14.191	< 2e-16	***	Rain Mar	0.497
Comp.4	0.10113	0.03706	2.729	0.00666	**	Rain Dec	-0.598
				RSE	0.7472		
				Multiple R^2	0.5038		
				Adj R^2	0.4982		
				p-value	< 2.2e-16		

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

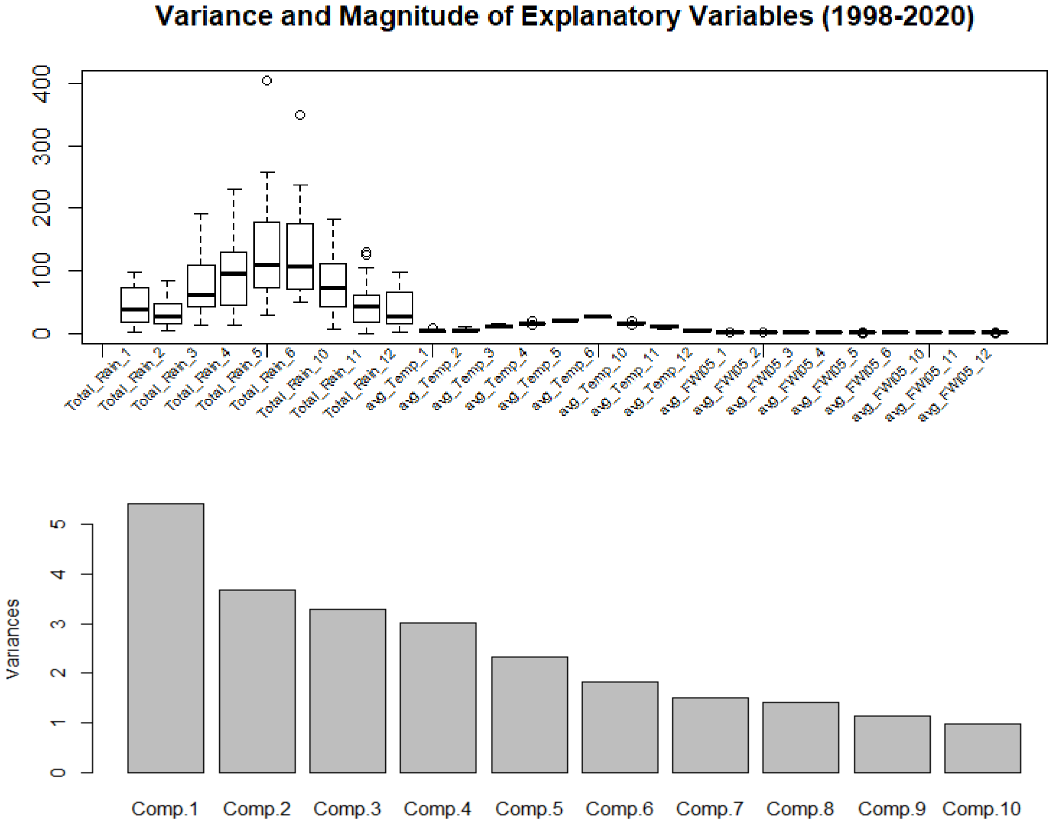

Figure 20. Variance and magnitude of explanatory variables across all years combined (1998-2020). Relative importance of each principal component in explaining the variance of the weather variables included.

Table 34. Eigen-values of the correlation matrix and the proportion of variance explained by the 9 largest principal components across all years combined (1998-2020). Twenty-seven weather parameters included totaling 683 observations.

Component	Eigen Vector	Standard Deviation	Proportion of Variance	Cumulative Proportion
PRIN 1	5.40	2.32	0.20	0.20
PRIN 2	3.68	1.92	0.14	0.34
PRIN 3	3.28	1.81	0.12	0.46
PRIN 4	3.01	1.73	0.11	0.57
PRIN 5	2.32	1.52	0.09	0.66
PRIN 6	1.82	1.35	0.07	0.72
PRIN 7	1.50	1.22	0.06	0.78
PRIN 8	1.41	1.19	0.05	0.83
PRIN 9	1.13	1.06	0.04	0.87

Table 35. Primary loadings that comprise the 9 largest principal components. (1998-2020).

	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6	PRIN 7	PRIN 8	PRIN 9
Loading 1	FWI Jan 0.392	Rain May 0.350	Temp Jun -0.421	Temp Feb 0.392	Rain Nov -0.413	Rain Apr -0.403	FWI Oct 0.438	Rain Feb -0.373	Temp Apr 0.416
Loading 2	FWI Dec 0.370	FWI May 0.347	Rain Jun 0.386	Rain Jan 0.343	FWI Jun -0.410	Temp Dec 0.368	Rain Jan 0.428	Temp Apr -0.370	Temp Oct 0.349
Loading 3	FWI Nov 0.312	Temp May -0.341	Rain Mar 0.306	Rain Nov 0.326		Rain Feb 0.351	FWI Mar -0.305		Rain Mar -0.339

Table 36. ANOVA of the fitted linear model for grain yield containing 9 principal components. All years combined (1998-2020).

Model: Yield = PC1 + PC2 +PC3 +PC4 +PC5 +PC6 +PC7 +PC8 +PC9

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.
(Intercept)	2.0054	0.02753	72.838	< 2e-16	***
Comp.1	0.12519	0.01185	10.567	< 2e-16	***
Comp.2	-0.12614	0.01436	-8.787	< 2e-16	***
Comp.3	0.06062	0.01521	3.985	7.48E-05	***
Comp.4	-0.0161	0.01587	-1.014	0.311	ns
Comp.5	0.1021	0.01806	5.653	2.33E-08	***
Comp.6	0.33682	0.02042	16.497	< 2e-16	***
Comp.7	0.39092	0.0225	17.376	< 2e-16	***
Comp.8	0.1581	0.0232	6.815	2.10E-11	***
Comp.9	0.42118	0.02587	16.283	< 2e-16	***
				RSE	0.7195
				Multiple R^2	0.6254
				Adj R^2	0.6204
				p-value	< 2.2e-16

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

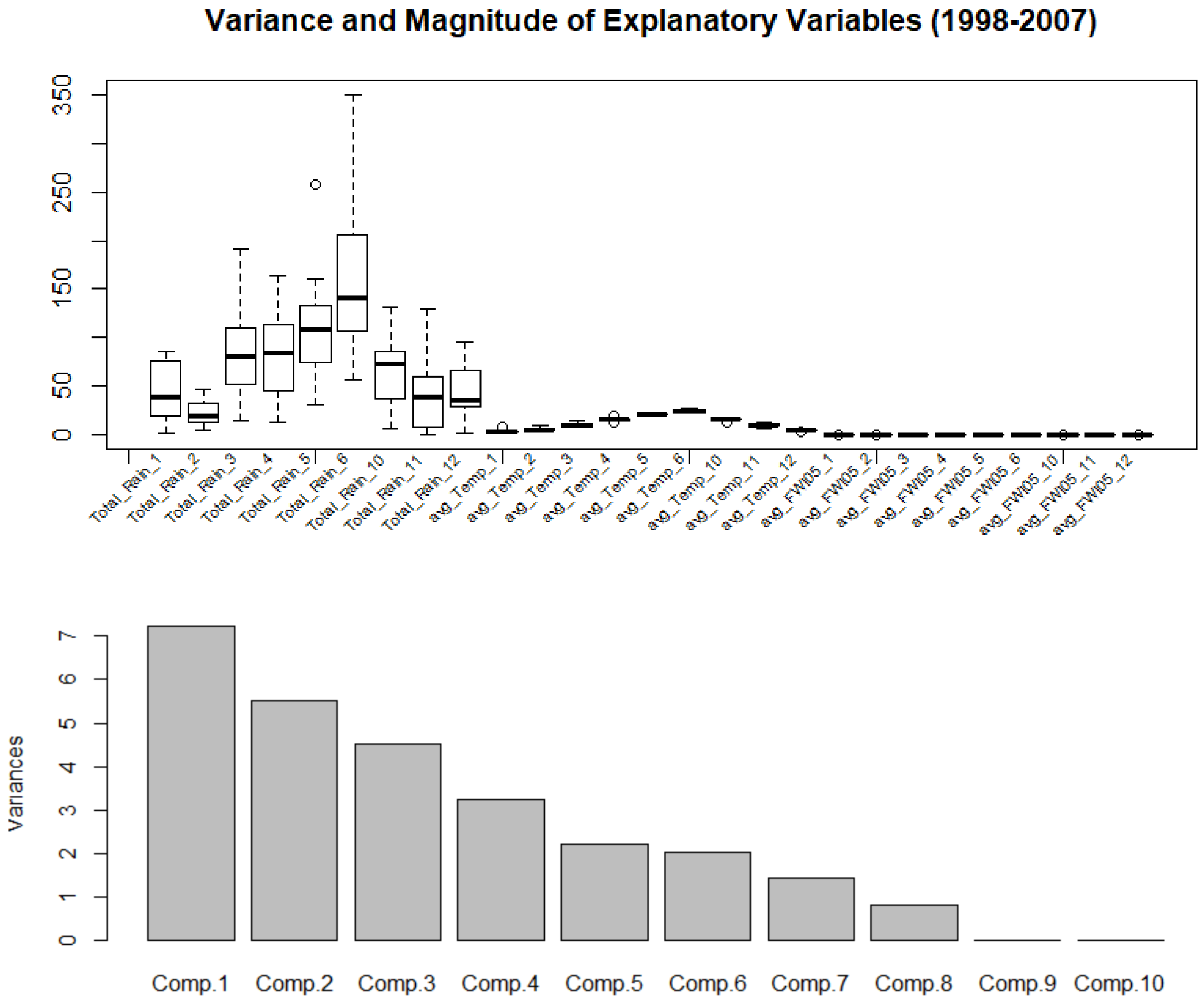

Figure 21. Variance and magnitude of explanatory variables with the conventional tillage dataset (1998-2007). Relative importance of each principal component in explaining the variance of the weather variables included.

Table 37. Eigen-values of the correlation matrix and the proportion of variance explained by the 7 largest principal components while under conventional tillage (1998-2007). Twenty-seven weather parameters included totaling 323 observations.

Component	Eigen Vector	Standard Deviation	Proportion of Variance	Cumulative Proportion
PRIN 1	7.22	2.69	0.27	0.27
PRIN 2	5.52	2.35	0.20	0.47
PRIN 3	4.52	2.13	0.17	0.64
PRIN 4	3.24	1.80	0.12	0.76
PRIN 5	2.21	1.49	0.08	0.84
PRIN 6	2.03	1.42	0.08	0.92
PRIN 7	1.45	1.20	0.05	0.97

Table 38. Primary loadings that comprise the 7 largest principal components. Conventional tillage (1998-2007).

	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6	PRIN 7
Loading 1	FWI Dec 0.359	Rain Nov 0.322	Temp Dec -0.346	Rain Apr 0.417	Temp Mar 0.388	Temp May 0.495	Rain Jan 0.516
Loading 2	Temp Jan -0.354		Rain Mar 0.328	Temp Feb 0.395	FWI Oct 0.355	Temp Oct 0.461	Rain Oct -0.434
Loading 3	FWI Feb 0.340		Temp Nov -0.312	Temp Nov 0.341	FWI Mar -0.345	Rain May -0.314	Temp Jun 0.388

Table 39. ANOVA of the fitted linear model for grain yield containing 7 principal components. Conventional tillage (1998-2007).

Model: Yield = PC1 + PC2 +PC3 +PC4 +PC5 +PC6 +PC7

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.
(Intercept)	2.27895	0.0365	62.444	< 2e-16	***
Comp.1	-0.02072	0.01358	-1.526	0.128	ns
Comp.2	0.26962	0.01554	17.354	< 2e-16	***
Comp.3	-0.15029	0.01716	-8.759	< 2e-16	***
Comp.4	-0.30126	0.02028	-14.857	< 2e-16	***
Comp.5	-0.34099	0.02454	-13.898	< 2e-16	***
Comp.6	-0.10417	0.02562	-4.066	6.05E-05	***
Comp.7	-0.03252	0.03034	-1.072	0.285	ns
				RSE	0.6559
				Multiple R^2	0.7204
				Adj R^2	0.7142
				p-value	< 2.2e-16

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

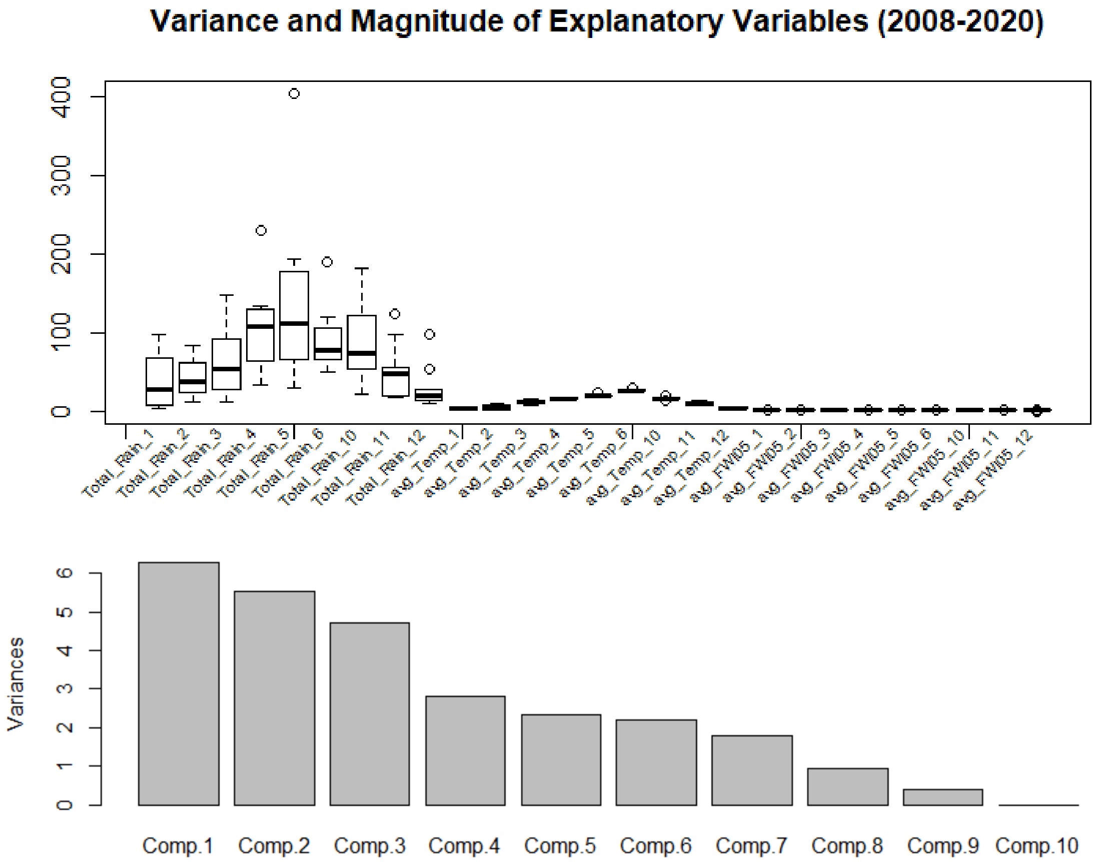

Figure 22. Variance and magnitude of explanatory variables after transition to no-tillage (2008-2020). Relative importance of each principal component in explaining the variance of the weather variables included.

Table 40. Eigen-values of the correlation matrix and the proportion of variance explained by the 7 largest principal components after transition to no-tillage (2008-2020). Twenty- seven variables included totaling 360 observations.

Component	Eigen Vector	Standard Deviation	Proportion of Variance	Cumulative Proportion
PRIN 1	6.27	2.50	0.23	0.23
PRIN 2	5.55	2.36	0.21	0.44
PRIN 3	4.71	2.17	0.17	0.61
PRIN 4	2.80	1.67	0.10	0.72
PRIN 5	2.33	1.53	0.09	0.80
PRIN 6	2.20	1.48	0.08	0.88
PRIN 7	1.80	1.34	0.07	0.95

Table 41. Primary loadings that comprise the 7 largest PC after transition to no-tillage (2008-2020).

	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6	PRIN 7
Loading 1	FWI Dec -0.358	Rain Mar 0.376	Rain May 0.390	Temp Jun -0.478	Rain Feb 0.506	Temp Apr -0.405	Temp Dec -0.465
Loading 2	FWI Jan -0.347	Temp Mar 0.319		FWI Oct 0.349	FWI May -0.361	FWI Jun -0.396	Temp Nov 0.357
Loading 3	FWI Nov -0.319	FWI Jun -0.307		Temp May 0.332	Temp Oct -0.335	Rain Dec -0.367	Rain Apr 0.306

Table 42. ANOVA of the fitted linear model for grain yield containing 7 PCs. No-tillage (2008-2020).

Model: Yield = PC1 + PC2 +PC3 +PC4 +PC5 +PC6 +PC7

	Estimate	Std. Error	t value	Pr(>|t|)	Sign.
(Intercept)	1.75997	0.03511	50.123	< 2e-16	***
Comp.1	-0.18805	0.01402	-13.41	< 2e-16	***
Comp.2	0.12659	0.01491	8.491	5.82E-16	***
Comp.3	0.23421	0.01618	14.473	< 2e-16	***
Comp.4	0.08794	0.02097	4.193	3.49E-05	***
Comp.5	-0.11464	0.023	-4.985	9.75E-07	***
Comp.6	0.15583	0.02369	6.578	1.73E-10	***
Comp.7	-0.02436	0.02621	-0.929	0.353	ns
				RSE	0.6662
				Multiple R^2	0.6089
				Adj R^2	0.6011
				p-value	< 2.2e-16

*** significance at alpha =0.001, ns = not significant at alpha = 0.05

VITA

Elizabeth Mary Eickhoff

Candidate for the Degree of

Doctor of Philosophy

Dissertation: I. EVALUATION OF SEED TREATMENTS AND NITROGEN ON SOYBEAN NODULATION AND GRAIN YIELD

II. INFLUENCE OF PRE-PLANT NITROGEN AND PHOSPHORUS APPLICATIONS ON WINTER WHEAT GRAIN YIELD AND QUALITY

Major Field: Soil Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Soil Science at Oklahoma State University, Stillwater, Oklahoma in May, 2021.

Completed the requirements for the Master of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in 2018.

Completed the requirements for the Bachelor of Science in Agronomy and Agricultural Business at Northwest Missouri State University, Maryville, Missouri in 2017.

Professional Memberships:

American Society of Agronomy, 2017
Crop Science Society of America, 2017
Soil Science Society of America, 2017