THE PERFORMANCE OF WINTER WHEAT VARIETIES WITH AND WITHOUT THE ALMT1 GENE IN ACID SOILS

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Abstract: Aluminum (Al) tolerant wheat cultivars are often utilized in the southern Great Plains to damper the yield limiting impacts of Al toxicity in acidic soils. The tolerance is mainly facilitated by Al-activated malate transporter 1 (ALMT1) gene, which increases anion permeability of wheat roots and exudes malate in the presence of Al. However, no study has explored the phenotypic incongruities of those closely related genotypes with ALMT1(+) or without ALMT1(-)]. Moreover, there is currently no quantitative ranking of Al tolerance for newly released winter wheat varieties in forage and grain dual-purpose (DP) management systems. This field scale study consisting of two locations in central Oklahoma was established to determine the Al tolerance of eight parentally related but gene divergent winter wheat varieties [(Duster (+), Lonerider (+), OK14319 (+), Jagger (+), Iba (-), Gallagher (-), Spirit Rider (-), Smith's Gold (-)]. The design structure was a split-plot in a randomized complete block with a two-way treatment (6 x 8). Main plots were amended with alum/hydrated lime to reach the following target soil pH: 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0. Soil samples were collected at two months after planting in order to determine soil pH and Al saturation (Al_{sat}). Each variety was hand clipped during December to determine fall forage yield. Grain was harvested in June to measure grain yield, wheat protein concentration, test weight and wheat moisture content of each variety. Results varied between study years and locations. Significant differences were found between the relative forage yields of ALMT1 (-) and (+) genotypes groups at Stillwater and Chickasha in Year 1 ($p = 0.0042$ and $p = 0.0440$, respectively); however, differences were not significant in Year 2 ($p = 0.7228$ and $p = 0.7792$, respectively). No significant differences were found between relative grain yields of genotype groups at Chickasha ($Al_{sat} \leq 8\%$) or Stillwater ($Al_{sat} \leq 38\%$) in Year 1 ($p = 0.9172$ and $p = 0.2102$, respectively) or Year 2 ($p = 0.2106$ and $p = 0.2684$, respectively). Notwithstanding genotype group affiliation, significant differences were found among varieties in their response to Al concentration and soil acidity. Similarly, the productivity of genotype groups in this study varied between years and was not wholly dependent on the presence or absence of the ALMT1 gene. Additionally, varieties differed in their yearly and environmental responses despite close parental relationships. Nevertheless, the utilization of acid tolerant winter wheat varieties has the potential to significantly reduce yield loss under acidic soil conditions with high Al concentrations. The findings in this study should equip researchers and producers with the necessary knowledge to reduce yield losses when traditional methods of soil acidity amelioration such as liming are not feasible.

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CHAPTER I

INTRODUCTION

Today, agriculture is a ubiquitous and essential global industry, comprised of hundreds of domesticated plant species, all with the purpose of providing sustenance for the world. Wheat (*Triticum aestivum* L.) is a major dietary staple on nearly every continent in the world; in fact, it is arguably one of the most vital crops in global agriculture. Wheat is grown on more land than any of the three major cereals: wheat, rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (Mayer, 2014; FAOSTAT, 2014). During the 2015-2016 growing season, over 220.4 million hectares of wheat were harvested, which paralleled a worldwide production of 749.4 million tonnes (FAO, 2018a). The United States is fourth in the world in wheat production, following India, China and the European Union (USDA, 2018a). Approximately 47.37 million tonnes of wheat were produced in the U.S. in 2017.

As of 2016, wheat production in Oklahoma ranked 5th in the U.S., following Washington, Montana, North Dakota, and Kansas (USDA 2017a). In 2016, Oklahoma produced a near record breaking 3.71 million tonnes of wheat on 1.42 million hectares. As it stands, Oklahoma is responsible for 6% of the total wheat production in the United States (USDA, 2017b). In 2017, Oklahoma fell short of those yields, producing 2.68 million tons of wheat on 1.17 million hectares. In terms of winter wheat, Oklahoma is ranked 2nd in the U.S. Oklahoma yielded an average of 2.22 tonnes ha^{-1} in 2017, compared to 2.62 tonnes ha^{-1} in 2016 (USDA, 2017a).

While a vast amount of the wheat grown in the state is for grain, 40% to 60% of Oklahoma's wheat crop is dual-purpose (DP) (Edwards and Horn, 2017). In the DP production system, winter wheat is grazed (forage) by livestock until first hollow stem, which occurs between February and March (Zhang et al., 2017; Carver et al., 2001). Dual purpose wheat allows producers the opportunity to experience higher profitability, enhanced operational efficacy, and optimal utilization of the crop throughout the growing season. Due to warm temperatures and higher average rainfall in comparison to the rest of the Great Plains, the southern Great Plains is ideal for DP wheat. According to the National Climate Assessment, average annual precipitation is greater than 127 cm in Oklahoma, while most of the Great Plains averages 76.2 cm and lower. However, because of the east-west gradient, distribution of average precipitation varies across the southern Great Plains. According to the Oklahoma Climatological Survey, over a 30-year period the Panhandle experiences an average annual precipitation of ~50.8 cm, while southeastern Oklahoma stands between 114.3 and 127 cm per year. Forage is strongly impacted by acidic soil conditions (soil pH < 5.5). As pH drops below 5.5, Al containing materials begin to dissolve into exchangeable aluminum (Al^{+3}). This acidity driven increase in Al^{+3} contributes to one of the greatest antagonists in Oklahoma crop production, Al toxicity (Zhang & Raun, 2006; Kariuki et al., 2007; Lollato, 2012, Lollato et al., 2019). Al toxicity occurs when high concentrations of Al ions overwhelm the edaphic environment, resulting in poor stand establishment and growth retardation (Cronan et al., 1989; Kariuki et al., 2007). The expulsion of Al species and hydrogen ions (H^{+}) is crucial to the productivity of the soil, forage biomass, and grain yields.

In Oklahoma, some counties report a soil pH < 5.5 on nearly 50% of their farmland (Figure 1). The 1994 to 1999 soil survey indicated that 39 percent of central Oklahoma wheat fields had pH values less than 5.5 (Zhang, 2001). Despite counties such as Payne, Pawnee, and Osage whom have realized an average soil pH increase of 0.3 since the 2009-2013 survey, counties such as Garfield have decreased by nearly the same amount (Zhang, 2018).

Additionally, the 2014-2017 survey results for all four counties show more likeness to their 2004-2008 survey results, where soil pH was lower for all four counties, than to their 2009-2013 survey results.

Traditionally, lime application (pelletized or agricultural) to the top 15 cm has been used to amend soil acidity and combat Al toxicity. However, the long-term investment (5-7 years) and cost of purchasing and applying lime may not be ideal for many Oklahoma producers (Lollato, 2012), especially those who lease land for farming. Therefore, a low soil pH tolerant cultivar is considered one of the most practical and economically amicable guards against the yield-decreasing effects of Al toxicity. Studies have recognized the ability of Al tolerant wheat cultivars to reduce the implications of Al toxicity on wheat forage and grain yields (Johnson et al., 1997; Carver et al., 2001; Kariuki et al., 2007; Lollato, 2012; Limon-Ortega and Martinez-Cruz, 2014; Lollato et al., 2016; Lollato et al., 2019); however, no study has explored the expression of the Al tolerance gene in wheat, ALMT1, in closely related wheat varieties within differing environments. Moreover, the role of the edaphic environment in inducing the phenotypic expression of Al tolerance remains to be studied. Additionally, there is a knowledge gap involving the influence of the ALMT1 gene on the preservation of forage and grain yields under a DP management system. For example, Kariuki et al. (2007) presented Al tolerance rankings for widely used wheat varieties for forage and grain yields; presently there is a need for a new ranking system that involves newly released varieties. Given grandiose eminence of DP wheat within Oklahoma and the dire misfortunes bestowed upon producers via soil acidity and aluminum toxicity, the objective of this study is to assess the effects of soil acidity and Al concentration on the forage yields of eight closely related wheat varieties with (+) or without (-) the ALMT1 gene.

CHAPTER II

LITERATURE REVIEW

2.1 Wheat in global agriculture

Today, agriculture is a ubiquitous and essential global industry, comprised of hundreds of domesticated plant species, all with the purpose of providing sustenance for the world. Wheat (*Triticum aestivum* L.) is a major dietary staple on nearly every continent in the world; in fact, it is arguably one of the most vital crops in global agriculture. Wheat is grown on more land than any of the three major cereals: wheat, rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (Mayer, 2014; FAOSTAT, 2014). During the 2015-2016 growing season, over 220.4 million hectares of wheat were harvested, which paralleled a worldwide production of 749.4 million tonnes (FAO, 2018a).

2.1.1 United States wheat production

The United States is fourth in the world in wheat production, following India, China and the European Union (USDA, 2018a). Approximately 47.37 million tonnes of wheat was produced in the U.S. in 2017. The U.S. exports 50% of its wheat crop and accrues a revenue of $9 billion through global marketing (USDA, 2018b). According to the USDA Small Grains 2017 Summary, 73% of the wheat produced in 2017 was winter wheat. Furthermore, 60% of that was hard winter wheat. Though winter wheat occupies a majority of the market, other classes such as spring wheats (hard, white, soft, hard, and durum) represent the remaining 27% of market volume.

Hard winter wheat is grown heavily across the central and southern Great Plains, including Oklahoma (FAO, 2017a).

2.1.2 Oklahoma wheat production

As of 2016, wheat production in Oklahoma ranked 5th in the U.S., following Washington, Montana, North Dakota, and Kansas (USDA 2017a). In 2016, Oklahoma produced a near record breaking 3.71 million tonnes of wheat on 1.42 million hectares. As it stands, Oklahoma is responsible for 6% of the total wheat production in the United States (USDA, 2017b). In 2017, Oklahoma fell short of those yields, producing 2.68 million tons of wheat on 1.17 million hectares. In terms of winter wheat, Oklahoma is ranked 2nd in the U.S, following Kansas. Oklahoma yielded an average of 2.22 tonnes ha^{-1} in 2017, compared to 2.62 tonnes ha^{-1} in 2016 (USDA, 2017a). Approximately 97.3% was hard winter wheat, with 0.7% reported as soft winter wheat. The remaining 2.2% of the wheat planted was of an unknown class and variety (USDA, 2017a). Soft winter wheat is a specialty wheat used for Asian noodles and lighter, softer breads and pastries. Soft winter wheat has low moisture and high extraction rates (>55%), meaning more brand and germ remain in the flour post milling. While a vast amount of the wheat grown in the state is for grain, 40% to 60% of Oklahoma's wheat crop is dual-purpose (DP) (Edwards and Horn, 2017). In the DP production system, winter wheat is grazed by livestock until first hollow stem (jointing), which occurs between February and March (Zhang et al., 2017; Carver et al., 2001). Following jointing, cattle grazing is terminated in efforts to promote the maximization of grain production. Grazing even 2 weeks past jointing has been proven to decrease grain yields by upwards of 10%, and an additional 10% for each week following (Fieser et al., 2006). While the economic constraints of the wheat market are difficult to influence, agronomical pressures such as those associated with aluminum (Al) toxicity in acidic soils can be addressed. Aluminum toxicity occurs when high concentrations of Al ions overwhelm the edaphic environment, resulting in

poor stand establishment and growth retardation (Cronan et al., 1989; Kariuki et al., 2007). The expulsion of Al species and hydrogen ions (H^+) is crucial to the productivity of the soil, forage biomass, and grain yields.

2.1.3 Genetics of wheat

Wheat contains 21 pairs of chromosomes and is allopolyploid (composed of more than two genomes). Homologous sets (pair of maternal and paternal chromosomes) of seven chromosomes are found in its three sub-genomes: A, B, and D (2n =6x = 42, AABBDD) (IWGSC, 2014). Al tolerance in wheat is attributed to the expression of the gene on chromosome 4D called ALMT1 (Carter & Froese, 2016). Through long-term evaluations, plant breeders have succeeded in identifying a locus conferring Al-tolerant/Al-resistant mechanisms in wheat (Aggarwal et al., 2015). Genetic markers are biological tags used to keep track of a genetic locus from generation to generation. Tools such as marker-assisted breeding (MAS) coupled with polymerase chain reaction (PCR) and electrophoresis allow breeders to identify genetic differences between cultivars (Jiang, 2013). Breeders use these differences (base deletion, insertion and substitution) to screen plants for the desired marker allele(s) and select for the locus. In the presence of trivalent aluminum (Al^{+3}), malate (an organic acid released through anion channels that chelates Al^{+3}) and K^+ (potassium ions) are ejected from the root tips of Al tolerant cultivars (Ryan et al., 1997). The accompanied release of K^+ is accredited to stabilization of intracellular pH and electroneutrality of the root apex (Ryan et al., 1997; Osawa and Matsumoto, 2002). According to Carter & Froese (2016), an Al tolerant cultivar is one of the most attainable and least economically daunting defenses against yield loss brought on by soil acidity. Kariuki et al. (2007) collected quantitative data regarding the Al-tolerance ranking of several winter wheat cultivars and concluded that the Al-tolerance ranking order showed little variation between forage and grain yields: 2137 > Jagalene = Ok101 > Jagger = 2174 ≥ Ok102 > Custer =AP502CL for

grain yield; and 2137 > Ok101 = Jagalene = Jagger > 2174 = Ok102 > Custer = AP502CL for forage yield. Since the disclosure of these findings, other studies have expatiated on the intensified decrease of forage yield in comparison to grain yield under Al toxic soil conditions (Johnson et al., 1997; Lollato et al., 2012). These studies also indicate that forage production is more sensitive to low pH than grain yield. For example, Lollato et al. (2016) observed that the minimum soil pH required to acquire >5% symptomatic forage biomass for Ruby Lee and Duster was 5.9. However, in terms of grain, the minimum soil pH for maximizing grain yield was 5.8 for Ruby Lee and 4.8 for Duster. Wheat progression and growth stages, such as tiller formation, are hindered at low soil pH. However, tiller formation does not directly correspond to grain yield due to the genetic robustness surrounding its expression. Consequently, this results in a drastic difference between grain yields and forage yields under low pH conditions. For example, when soil pH was 6.5 and 4.5, forage yields for the wheat variety Custer were 2095 kg ha^{-1} and 145 kg ha^{-1}, respectively, and grain yields for the same variety were 2094 and 220 kg ha^{-1}, respectively. Therefore, the percent increase in yields were 1340% for forage and 840% for grain with the optimum pH. Nevertheless, there are discrepancies between the responses to Al toxicity amongst various wheat groupings. Such differences are not explained by identified loci such as ALMT1, suggesting that Al tolerance is not monogenic in wheat and not limited to one source (Carter & Froese, 2016). Moreover, this was further evidenced by Johnson et al. (1997) concluding that Al tolerance is greatly influenced by the edaphic environment.

a. Al toxicity and dual-purpose wheat

Despite higher rainfall and favorable climatic conditions, Al toxicity stands as an antagonist to the Oklahoma dual purpose wheat industry. According to Lollato et al. (2012), Al toxicity damages root growth in wheat and reduces plant biomass and grain yields. Al^{+3} enters root apices and obstructs root growth in wheat by blocking cell division, which limits the amount

of nutrients and water the plant can uptake, especially in the vegetative stage (Johnson et al., 1997; Acevedo et al., 2006; Kariuki et al., 2007; Panda et al., 2009, Lollato et al., 2019). Underdeveloped roots have a dramatic impact on the vegetative stage because, unlike grain, forage does not develop later in the season (Idupulapati et al., 2016). Consequently, Al toxicity is most harmful in the forage production phase of DP wheat system. Additionally, soil properties also play a vital role in the effects of Al toxicity on wheat yields. Parameters such as soil texture, cation exchange capacity, and base saturation greatly influence the interaction between plants and exchangeable aluminum. For example, croplands with equivalent soil pH values may reveal contrary values in Al concentrations, and expectantly, different yields in grain and forage. In a study by Johnson et al. (1997), TAM 105, the most acidic soil pH sensitive genotype in their study, performed markedly better under limed environmental conditions. Under low pH conditions, the average forage yields for two locations were 33 kg ha^{-1} (Lahoma) and 62 kg ha^{-1} (Stillwater). However, averaged across four limed environments TAM 105 showcased a forage yield of 442 kg ha^{-1}, a yield increase of 613% and 1239%, respectively. Moreover, the tolerant cultivar, 2180, produced the most forage under low pH conditions (172 kg ha^{-1}), while only producing ~ 236% more forage under limed conditions (406 kg ha^{-1}). Similarly, in the pH gradient study conducted by Kariuki et al. (2007), the authors concluded that forage yield is more sensitive to Al toxicity than grain yield in winter wheat. Furthermore, using the varieties Ruby Lee, Duster, TAM 203, and 2174, Lollato et al. (2016), reported similar findings. In their pH gradient study, Lollato et al. (2016) concluded that the pH threshold for maximizing forage yield (5.5-6.0) is higher than that required for maximizing grain yield (4.8-5.8). Even in the most tolerant variety, 2174, maximum yields were only maintained down to a pH of 5.5 for forage and 4.8 for grain. This supports the claims of researchers such as Kariuki et al. (2007) that forage yield is more sensitive to Al toxicity than grain yield. The insinuations of these claims involving Al toxicity are most relevant to DP wheat farmers.

b. Al-tolerant wheat and Oklahoma soils

The acidification of Oklahoma soils over the last few decades has become a serious encumberment to maximizing forage and grain yields across the state (Kariuki et al., 2007). Continuous harvesting of crops has stripped the soil of essential basic ions, and profuse use of nitrogen fertilizer has resulted in a surge in soil acidity across Oklahoma (Zhang & Raun, 2006). As pH drops below 5.5, Al containing materials begin to dissolve. This overwhelming presence of Al contributes to one of the greatest antagonists in Oklahoma crop production, Al toxicity (Zhang & Raun, 2006; Kariuki et al., 2007; Lollato, 2012; Lollato et al., 2019). Traditionally, lime application (pelletized or agricultural) to the top 15 cm has been used to ameliorate acidity caused by fertilizers and combat Al toxicity. However, the long-term investment (5-7 years) and cost of purchasing and applying lime may not be ideal for many Oklahoma producers (Lollato, 2012), especially those who lease land for farming. Therefore, a low soil pH tolerant cultivar is considered one of the most practical and economically amicable guards against the yield-decreasing effects of Al toxicity. Although there is an Al-tolerance ranking of winter wheat cultivars in existence (Edwards et al., 2012), these rankings do not consider the absence or presence of the ALMT1 gene. For example, Gallagher was ranked excellent (1 on a scale of 1 to 5) in Al tolerance despite not having the ALMT1 gene, while Iba, its ALTM1 gene absent counterpart, was ranked poorly in Al tolerance (4 on a scale of 1 to 5). Moreover, both varieties share an ALMT1 positive parental line (Duster) yet differ vastly in their response to Al toxicity. According to Ma et al. (2005), there are quantitative trait loci (QTL) outside of the ALMT1 promoter region that attribute around 50% of the phenotypic differences observed for Al tolerance. Similarly, there is a knowledge gap involving the influence of the ALMT1 gene on the preservation of forage and grain yields under a DP management system.

2.1.4 Soil properties, environmental conditions, and wheat production

There are numerous burdens in wheat production, and the state of the soil works to either alleviate or generate those difficulties (Chien et al., 2008; Zhang et al., 1998). Presently, there exist numerous solutions to mend crop yield under both inclement soil and prevalent environmental conditions. Unfortunately, unfavorable soil and climatic conditions are not a rare phenomenon, and cropping lands are growing more unproductive (Schroder et al., 2011). Moreover, as the number of unproductive soils increase, the ability to produce enough food for the growing population is challenged (USDA, 2018b; Zhang & Raun, 2006). Soil unproductivity is perpetuated by a plethora of issues, such as diminution of soil fertility, increased erosion, increased soil acidity, severe drought and climatic changes (USDA, 2018b; Zhang et al., 2017; Williams et al., 2009; Schlenker & Roberts, 2009; Kariuki et al, 2007). The promise of increased population pressure may only expedite the dilapidation of the world's productive soil (Abdulaha-Al Baquy et al., 2017). Cracking the mystery around the effects of intensive agricultural management strategies will influence the efficacy of food produced. Additionally, characterization of the outcomes of these management strategies within different soil types have had limited exploration (Abdulaha-Al Baquy et al., 2017). This lack of exploration includes the effects of acidic soils on isogenic crops within different soil types and properties.

2.2 Soil classification system

Soil physical properties such as structure, texture, and color are crucial indicators of soil quality. When evaluated appropriately, physical properties provide agronomical insight on the arability of a soil. For example, water-holding capacity (WHC), which is influenced by porosity, is an indicator of how much water is plant available. Soil texture and structure directly influence soil parameters such as WHC, compaction, and porosity. Thus, classification of soils, such as those in Oklahoma, are essential for overcoming obstacles and maximizing crop production. In

1975, a comprehensive soil classification system was developed by soil scientists, and it has been continuously classified into 12 soil orders.

2.2.1 Soil orders in Oklahoma

Oklahoma soils are diverse in composition and exist under the variable climatic conditions of the transition zone (Qiao et al., 2014). Farmers in Oklahoma plant close to 1.8 million hectares of winter wheat a year (FAOSTAT, 2017), across seven of the twelve soil orders: inceptisols, entisols, ultisols, alfisols, mollisols, aridisols, vertisols (NRCS, 2019). Inceptisols are soils with a moderate degree of development and contain no clay accumulation. Inceptisols are scattered and sparser than other soil orders in Oklahoma, with the lot in the west central part of the state. Entisols are spread across the state of Oklahoma and possesses little to no development. These soils closely resemble the soil parent material, which implies that these soils have experienced a lower degree of cultivation and agricultural disturbance. Ultisols are highly weathered soils with high concentrations of translocated clays. Ultisols are relatively acidic and can be found in the outlying eastern portion of Oklahoma. Alfisols, while less weathered and acidic than Ultisols, contain a clay enriched sub-soil with an affinity to aluminum and iron. Mollisols, which are found both in central Oklahoma, as well as the northern and southern margins, are highly fertile with high base saturation (BS). Aridisols in Oklahoma are limited to the Panhandle region. Aridisols possesses accumulations of salt, gypsum, or carbonates. Vertisols occupy small sections of Oklahoma in diagonally from southwest to northeast, with a majority located in the eastern part of the state on the southern margins. Vertisols are clay-rich soils that swell or crack depending on soil moisture levels (Carter & Gregory, 2008). Since 1999, Oklahoma State University's Soil, Water & Forage Analytical Laboratory has engendered soil pH surveys derived from Oklahoma cropland soil sample data. Since then, every 4 to 5 years, summaries are released for all agricultural soil samples within the state of Oklahoma (Zhang,

2013). These surveys have identified the percentage of farmland soils that possessed a soil pH <5.5. In 2008, 23.6% of the crops planted in Oklahoma were planted in acidic soils (<5.5) (Zhang, 2009). Similarly, the summary from 2009 to 2013 included over 61,000 samples (Figure 1) and suggested that 23.8% of the total Oklahoma crop was planted in acidic soils (<5.5) (Zhang, 2014). Some counties have nearly 50% of farmland with soil pH below 5.5 as shown in Figure 1. Therefore, soil acidification is an alarming encumberment to crop production in this region. The 1994 to 1999 soil survey indicated that 39 percent of central Oklahoma wheat fields had pH values less than 5.5 (Zhang, 2001). Despite acid soil conditions being more prevalent in eastern Oklahoma, farmers in this region have proven better equipped to regulate their soil acidity (Zhang & Raun, 2006). However, this differs from central and western Oklahoma, which show that acidification is increasingly worsening over time (Zhang & Raun, 2006). Numerous studies have shown that the soil acidification within the Great Plains region is a result of continuous crop production and robust application of ammoniacal nitrogen (N) fertilizers (Johnson et al., 1997; Schroder et al., 2011; Butchee et al., 2012; Sutradhar et al., 2014; Reeves & Liebig, 2016).

2.3 Effect of N fertilization on soil pH

In a study by Schroder et al. (2011), long-term plots initiated in 1970 were used to compare the impact of N fertilizers on Al saturation (Al_{sat}) in soils with continuous winter wheat production, using application rates of 34, 68, 136, and 272 kg N ha^{-1} (38 to 302% of the agronomic recommendation), and an untreated check (0 N). The degree at which an ammoniacal fertilizer acidifies a soil varies upon the N source, according to Chien et al. (2008). However, Schroder et al. (200l) showed that despite the source, increasing N fertilizer rate from 0 to 272 kg ha^{-1} was inversely correlated with soil pH. Similarly, under long-term application of ammoniacal N fertilizers, soil pH continued to negatively correlate with N fertilizer rates (0 to 272 kg ha^{-1}). They also concluded that across all experiments the soil pH decrease shifted from a linear

decrease to a quadratic decrease as time became greater. This spectacle was assumed to be a result of pH buffering by exchangeable aluminum species between a soil pH of 4 and 5. Further investigation is required to determine the cause for this time dependent shift from linear to quadratic decreases in soil pH. Nitrogen fertilization acted as an acidifying agent and intensely lowered soil pH in the upper 15 cm of the soil profile. Although it is true that N fertilizers are not acidic, their contributions to soil have been proven to be acid forming. The addition of N fertilizers (ammonium nitrate [NH_4NO_3], urea [NH_2CONH_2], and anhydrous ammonia [NH_3]) generate soil acidity as the result of the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-); this process generates H^+ which lowers soil pH. When N fertilizer that contains NH_4^+ is converted to NO_3^- through this process called nitrification (which is driven by oxidizing soil microbes), two moles of H^+ are generated for every mole of nitrate (Kochian et al., 2005; Schroder et al., 2011; Xiao et al., 2014). The increase in soil acidity constitutes an intense boost in reactive Al concentrations. Several studies have explored and quantified the detrimental effect of high Al concentration in low pH soils (<5.0) on crop yields (Kochian et al., 2005; Kariuki et al., 2007; Zhang et al., 2011; Butchee et al., 2012; Lollato et al., 2012; Sutradhar et al., 2014). As soil pH decreases, reactive Al species increase and become toxic to plant roots (Kariuki et al., 2007; Aggarwal et al., 2015)

2.4 Al accumulation in acid soils

Al has been deemed the most abundent of the earth metals and represents 8.2% of the earth's weight (Barbalace, 2011). Overall, within the earth's crust, Al ranks third amongst all chemical elements, following oxygen and silicon (Aggarwal et al., 2015). For most crops, induction of toxicity symptoms requires that Al^{+3} exist in minimal concentrations of 10 to 20 ppm. Al concentrations of this level are hard to attain unless perpetuated by acidifying fertilizers and lime indebtedness (Bear, 1957). The critical pH for most plant species grown within an acidic

edaphic environment is <5.5. Critical pH is the value at which crop production is negatively impacted or the point at which the presence of Al becomes toxic (Kariuki et al., 2007). Although this varies from crop to crop, most crops will sustain damage below their critical pH level. Distressingly, it is estimated that nearly 50% of the world's suitable cropping land is deemed acidic (Kochian et al., 2005).

2.4.1 Formation of exchangeable Al in acid soils

Knowing what Al species are present at certain soil pH level is important, especially for crops such as wheat. Al species impact wheat roots in the following order from least to greatest: $AlF_2^+ < AlF^{2+} < Al^{+3} < Al_{13}$ (Mossor-Pietraszewska, 2001). Despite Al_{13} being most toxic to wheat roots, it is polynuclear and highly unstable (Ramgareeb et al., 2004). Consequently, Al_{13} effortlessly precipitates out of the solution as gibbsite ($Al(OH)_3$). Thus, the bulk of Al toxicity research is centered around the mononuclear form, Al^{+3}. As pH lowers, there is an increase in the hydrolysis of Al oxides, and the amount of positively charged Al species. Trace amounts of Al^{+3} arise in the soil when the pH approaches 6.0. The presence of Al^{+3} is negligible in many soils until the pH falls beneath 5.5. However, in crops such as wheat, Al^{+3} is rarely a problem until the soil pH is <5.0. However, the amount of soluble Al increases dramatically in nearly all soils as the soil pH drops below pH 5.0 (Kariuki et al., 2007; Lollato et al., 2019). As base cations are desorbed from cation exchange sites, Al^{+3} is absorbed. Under the acidic soil conditions, absorbed Al^{+3} (exchangeable acidity) will reach equilibrium with solution Al^{+3} (active acidity) (Tang et al., 2007). As soil pH lowers, the dissolving of Al compounds, such as $Al(OH)^0_3$ causes a reaction with H^+ ions which releases aluminum Al^{+3} (hydrolysis). Since there are three H^+ expended for every aluminum ion, the process decreases pH at a very slow rate in comparison to other acid soil reactions. However, the transition from a non-active solid Al compound to a solution available form (Al^{+3}) can become toxic to plants.

pH < 5

$$Al^{+3} + H_20 = Al(OH)^{+2} + H^+$$

pH 5 – 6.5

$$Al(OH)^{+2} + H_20 = Al(OH)_2^{+} + H^+$$

$$Al(OH)_2^{+} + H_20 = Al(OH)^0_3 + H^+$$

Overall Buffering Reaction pH < 5.5

$$Al(OH)_3 + 3H^+ = Al^{+3} + 3H_2O$$

Additionally, as the nitrification of NH_4^+ renders H^+, the pH is lowered, and more Al oxides are hydrolyzed. Eventually, the cation exchange sites are dominated by Al^{+3}. Additionally, Fe oxides can present additional issues under very acidic soil conditions. The presence of soluble Fe results in a series of nutrient disorders and multiple deficiencies of other nutrients such as potassium. However, Fe oxides will only hydrolyze after all the Al oxides have reacted and dissolved (Havlin et al., 2013). There is need for exploration surrounding the presence of Fe oxides and their effects on Oklahoma's acid soils.

2.4.2 Exchangeable Al, Al_{sat} and soil pH

According to Kariuki et al. (2007), evaluation of exchangeable Al using an Al_{sat} value illustrated an inverse exponential relationship between Al_{sat} and soil pH. In their pH gradient study, a regression analysis linking yields and Al_{sat} revealed that compared to other ranges, cultivar yields varied most when Al_{sat} was greater than 30%. However, cultivars with a more formidable Al tolerance suffered much smaller yield losses than their less tolerant counter parts

when Al_{sat} increased from less than to greater than 30%. Similarly, the study reported that a sizeable diminution in soil acidity resulted in a relatively small increase in the yields of tolerant cultivars. For example, when Al_{sat} was 40%, 20%, and 0%, forage yields for the wheat variety Ok101 were 961 kg ha^{-1}, 1643 kg ha^{-1}, and 2325 kg ha^{-1}, respectively. Conversely, when Al_{sat} was 40%, 20%, and 0%, forage yields for the wheat variety AP502CL were 191 kg ha^{-1}, 833 kg ha^{-1}, and 1475 kg ha^{-1}, respectively. The authors reported that the percent increase in forage yields when Al_{sat} was decreased from 40% to 0%, were 141% in Ok101 and 672% in AP502CL. Additionally, Kariuki et al. (2007) discovered a similar relationship between grain yields and Al_{sat}. Analysis of those same cultivars indicated that when Al_{sat} was 40%, 20%, and 0%, grain yields for the wheat variety Ok101 were 1387 kg ha^{-1}, 1673 kg ha^{-1}, and 1959 kg ha^{-1}, respectively. Conversely, Al_{sat} was 40%, 20%, and 0%, grain yields for the wheat variety AP502CL were 166 kg ha^{-1}, 940 kg ha^{-1}, and 1714 kg ha^{-1}, respectively. The authors stated that the percent increase in grain yields when Al_{sat} was decreased from 40% to 0%, were 41% in Ok101 and 932% in AP502CL. The lesser Al tolerant cultivar, AP502CL, suffered higher percent yield losses than the more tolerant cultivar, Ok101. Understanding how Al species and Al_{sat} are characterized and quantified in the soils is crucial to conquering the issue of acidic soils.

2.4.3 Al_{KCl} and Al_{sat} in low-pH soils

There are two predominant ways of measuring Al levels in the soil, potassium chloride extractable Al (Al_{KCl}) and Al_{sat}. Both methods observe an increase in value as soil pH decreases. However, Al_{KCl} is the concentration of Al^{+3} in solution and on the exchange sites and Al_{sat} is the relative abundance of Al^{+3} in relation to all exchangeable cations. As a non-buffered salt solution, KCl removes exchangeable Al^{+3}, as well as a negligible amount of non-exchangeable Al from soil exchange sites. This process constitutes as the Al_{KCl} extraction method, which is an integral part of the Al_{sat} methodology. Al_{sat} determines the Al concentration relative to the total amount of

exchangeable cations, mainly calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) (Kariuki et al., 2007). Al_{sat} is the amount of KCl-extractable aluminum divided by the total amount of extractable bases plus the amount of KCl-extractable aluminum. It is well known that crops grown in soils with adequate BS will buffer against Al toxicity; therefore, Al_{sat} may be superior to Al_{KCl} in assessing Al toxicity. Kariuki et al. (2007) found that like the relationship between Al concentration and pH, changes in Al_{sat} were more distinct at lower pH values than at higher pH values. The study emphasized that an increase in pH from 5.5 to 6.0 revealed a decrease in Al_{sat} from 7.3% to 2.2%, while a pH increase of 5.0 to 5.5 showed a decrease in Al_{sat} from 23.5% to 7.3%. It was concluded that the more intense the soil acidity, the greater the level of Al solubilization, resulting in an overall increase in Al_{sat}. Therefore, the method that will be utilized for measuring Al levels of the soil in this experiment will be Al_{sat}.

2.4.4 Management of soil acidity

Presently, there are several strategies producers use to ameliorate soil acidity and augment a soil's agricultural affinity. These methods include liming soils (predominately with calcium carbonate), banding phosphorus, using genetically Al tolerant cultivars, and applying plant residues (Aggarwal et al., 2015).

a. Liming

Liming is probably the most effective, elastic, and economical counter-measurement against soil acidity, lasting between five to eight years per application based on soil test recommendations (Lollato et al., 2012). Moreover, various authors have explicated the long-term residual effect of liming of highly weathered soils such as Oxisols and Ultisols (Alleoni et al., 2003; Tiritan et al., 2016; Rheinheimer et al., 2018). Rheinheimer et al. (2018) reported that at 12 and 18 years following a surface lime reapplication (3.6 Mg lime ha^{-1}) on a sandy ultisols under no-till, the soil pH, BS, and exchangeable cations differed from the control at depths up to 25 cm.

Additionally, potential acidity ($H^+ + Al^{+3}$), exchangeable Al and Al_{sat} were higher in the first 17 cm of the control. Therefore, such findings support the supremacy of lime as a soil acidity neutralizer. Calcium carbonate and calcium oxides work to neutralize pH through a variety of chemical reactions:

$$CaCO_3 + 2H^+ \rightleftarrows Ca^{+2} + CO_2 + H_2O$$

$$CaCO_3 + 2H_2O \rightleftarrows Ca(OH)_2 + H_2CO_3$$

$$Ca(OH)_2 + 2H^+ \rightleftarrows Ca^{+2} + H_2O$$

Calcium Carbonate Equivalent (CCE) is used to determine the amount of lime needed to neutralize both reserve and active acidity in the soil. This is a value determined by the purity of liming material in comparison to pure $CaCO_3$ (neutralizing value of 100%) (Havlin et al., 2013).

Effective Calcium Carbonate Equivalent (ECCE) is CCE in combination with the fineness of the lime particles. The higher the fineness factor, the greater the ECCE of the liming material (Chan, 2016). Unfortunately, liming is not as easily justified on small family farms, which are abundant in developing countries. Additionally, liming is less effective in ameliorating soil acidity deeper than 6 inches or in the subsoil (Aggarwal et al., 2015). Subsoil pH is challenging to adjust and may require more frequent and higher rates of lime in order to have an effect. The challenge for producers is that the cost of lime can only be justified through a marginal profit increase. Various researchers have reported on the economic gains and drawbacks of differing soil amelioration strategies (Edwards et al., 2013; Lollato, 2012; Brorsen et al., 2011; Epplin et al., 2002; Boman et al., 1992). There is much to consider when assessing the economics of lime requirements in low pH soils. For example, cost of lime influences the optimal N rate and timing of applications. Researchers have determined that splitting N into seasonal applications considerably decreased soil acidification, thus reducing the lime requirement (Brorsen et al.,

2011). In the United States, around 10% of the farmland is leased or branded for ownership transfer (USDA, 2016). Most of this farmland is on short lease to other farmers. According to Lollato (2012), lime cost cannot be justified economically in the first year of application. Farmers are often reluctant to invest in lime for low pH soil amelioration, because the benefits are reaped in the years following application. Consequently, a large percentage of wheat producers in Oklahoma address acidic soil conditions through the cheaper, short term yet less effective, practice of banding of phosphorus (P) fertilizer.

b. Phosphorus banding

Orthophosphates act as transient anti-acidic agents when applied to acidic soil that contain Al^{+3}. Banding phosphorus (P) eliminates the active acidity near the application region by complexing with plant harming Al^{+3}. For this reason, plant harming Al may be reduced for one growing season in soils where P is banded. According to Epplin et al. (2002), where lime was estimated to be \$0.02 kg^{-1} ECCE in the year of application, it was economical to omit liming and to apply 73 kg ha^{-1} DAP (18-46-0) in-furrow. It was founded that over a five-year period the most economical method was a combination of lime and DAP in-furrow applications. Banding P fertilizer radically increases the concentration of plant available P in the soil solution during wheat emergence (Sloan et al., 1995). Orthophosphates (HPO_4^{-2} and $H_2PO_4^-$) react with H^+ and Al^{+3} ions and consequently produce insoluble complexes that precipitate Al out of the soil solution. The $H_2PO_4^-$ orthophosphate is more abundant in lower pH soils than HPO_4^{-2}, which deprotonates in pH >7.2. Precipitation of these P complexes result in less Al toxicity and improved forage and grain yields in wheat. At 4.7< pH <5.5, approximately 67 kg ha^{-1} of P_2O_5 is required to reduce the amount of plant harming Al to concentrations that allow for maximization of forage yields (Boman et al., 1992). However, the phosphorus is usually depleted within a few months following the application (Allen et al., 1992), and the practice needs to be repeated

annually. Utilization of P fertilizer bands to ameliorate soil acidity is economical for leased land operations or when lime costs are high and only as a temporary counter-measure (Boman et al., 1992).

c. Organic residues

Additionally, plant residues, especially legumes such as vetch, have been shown to counter soil acidity and lower Al concentration in soils (Xiao et al., 2014). In a study by Tang et al. (2007), it was found that additions of feedlot manure and poultry litter increased soil pH, increased crop biomass, and reduced the amount of exchangeable Al in the soil. This study concluded that animal manures could potentially reduce Al toxicity in acidic soils but require a comprehensive field evaluation. Organic molecules and base cations are released during decomposition of residues. Organic molecules react to produce insoluble complexes of Al and other metals. Soil pH is increased by the presence of base cations, decarboxylation of plant residue and the production of NH_4^+. Once soil pH is increased, Al is precipitated out of the solution. According to Xiao et al. (2014), increased vetch applications caused an increase in soil pH and inorganic N (NO_3^-, NH_4^+), while reducing exchangeable Al in the soil through complexation. However, these reductions were transient and highly dependent on the initial pH of the soil. Vetch application was also less effective in ameliorating acidic soils of higher pH (those between 4.40 and 6.74). This short-lived change in soil pH was strongly dependent not only on the initial pH but soil characteristics as well (buffering capacity, soil type, microorganisms, etc.) (Xiao et al., 2014).

d. Acid soil tolerant cultivars

In Oklahoma, it is crucial that acid-soil tolerant varieties both limit the effects of Al toxicity and function as high yield DP crops. Johnson et al. (1997) discovered that under limed conditions, forage and grain yields did not differ between Al-tolerant and -intolerant varieties.

Furthermore, total forage production did not differ under acid soil conditions, despite differing early-season phenotypes. Al-tolerant varieties failed to outperform intolerant varieties until Al_{sat} surpassed 30%. Similar results regarding cultivar response to Al_{sat} >30% were reported by Kariuki et al. (2007). DP management is being practiced on nearly 8 million acres in Oklahoma, southern Kansas, and Texas. Understanding the implications that soil acidity and Al toxicity have on the forage and grain yields of current wheat varieties is imperative to the profitability and longevity of the Oklahoma wheat market.

2.5 Inducing Al toxicity in low pH soils for experimentation

While crops like wheat grow best in soil with pH between the range of 5.5-7.5, a variety of crops (blueberries, sweet potatoes, etc.) prefer soils with pH <4.5. In some cases, soil is treated with sulfur-based compounds such as aluminum sulfate (alum) or elemental sulfur to lower soil pH values. Through alum application, aluminum oxides, H^+ ions, and sulfate (SO_4^{-2}) are introduced to the soil solution.

$$A1_2(SO_4)_3 + 6H_20 \rightleftarrows 2A1(OH)_3 + 2H^+ + SO_4^{-2}$$

However, SO_4^{-2} is not the acidifying agent, the presence of SO_4^{-2} is favored as it is a plant available macronutrient. Indeed, it is the increase of H^+ activity that raises soil solution acidity. As pH lowers, aluminum oxides undergo a series of dissociations, resulting in the increase of exchangeable Al^{+3} in soil solution and on exchange sites.

2.5.1 Crop based response to Al toxicity

Most studies on the performance of crops in acid soils were conducted at a single low pH soil. For example, Lollato et al. 2012 conducted a pH study involving the low soil pH sensitive cultivar, Fuller. In this study the objective was to assess the effects of various low soil pH amelioration products (agricultural lime, pelletized lime, phosphate fertilizer) on the soil pH,

winter wheat yields (forage and grain) as well as, spatial distribution. The soil pH for the control ranged from 4.73 to 4.90 with extractable Al_{KCl} concentrations ranging between 54.7 mg kg^{-1} to 20.1 mg kg^{-1}. While other studies, such as Kariuki et al. (2007) and Lollato et al. (2019), evaluated crop performances under a range of pH from very acidic to slightly basic.

a. The effect of soil acidity on other crops

In an experiment by Butchee et al. (2012), the effects of soil pH on grain sorghum production were evaluated by lowering soil pH in certain parts of the field using alum ($A1_2(SO_4)_3$) and increasing soil pH in other parts of the field through the application of hydrated lime ($Ca(OH)_2$). This resulted in a pH gradient ranging between 4.0-7.0, where sorghum parameters and yield could be quantified. Butchee et al. (2012) reported that plant mortality increased with decreasing soil pH with a critical soil pH of 5.42. Stand counts at the beginning of the season were greater than the number of heads counted at harvest. Suggesting that soil acidity had the greatest impact on plant vitality during the season than on stand establishment. They reported a relationship between decrease in plant counts and soil pH. Although sorghum grain grows best in soils with pH between 5.5 and 7.0 experienced losses between emergence and harvest, the number of plants significantly decreased when soil pH was less than 4.4. In another soil pH gradient intensive study, manipulation of soil pH through application of alum and hydrated lime was also practiced in sunflower production. In this study, Sutradhar et al. (2014) aimed to determine the critical soil pH and aluminum concentration for sunflower. The study reported critical soil pH, Al_{KCl} and, Al_{sat} differed between soil types. For example, at pH values near 4.6, the Al concentration at Haskell, Lahoma and Perkins were 35.6 mg kg^{-1}, 58.4 mg kg^{-1} and 96.0 mg kg^{-1}, respectively (Sutradhar et al., 2014). Signifying that inherent differences among soil types greatly influence the extent at which low pH diminishes crop yields.

While many studies have evaluated the deleterious impact of Al toxicity on hard red winter wheat grain and forage yield, no researcher has presented a publication that explores the relationship between closely related genotypes that vary in Al tolerance [ALMT1 (+) or (-)] and the edaphic environment. Moreover, the role of the edaphic environment in inducing the phenotypic expression of Al tolerance. Given the grandiose eminence of DP wheat within Oklahoma and the dire misfortunes bestowed upon producers via soil acidity and aluminum toxicity, the objective of this study is to assess the effects of soil acidity and Al concentration on the forage yield and grain yields of eight closely related wheat varieties with (+) or without (-) the ALMT1 gene.

CHAPTER III

MATERIALS AND METHODS

3.1. Study Sites.

The field experiments were conducted over a two-year period during the 2017-18 and 2018-19 growing seasons at two locations. The first location was the Efaw Research Site near Stillwater, OK (36°8'4"N, 97°6'13"W), an Easpur loam (Fine-loamy, mixed, superactive, thermic Fluventic Haplustolls). The other location was at the South-Central Research Station in Chickasha, OK (35°2'46"N, 97°54'40"W), a Dale silt loam (Fine-silty, mixed, superactive, thermic Pachic Haplustolls).

3.2. Treatments and Experimental Design.

The experimental design was a randomized complete block with treatments in a split-plot arrangement with four replications The main plots consisted of six targeted soil pH values (4.0, 4.5, 5.0, 5.5, 6.0, 7.0). Main plots were measured 6 m by 6 m. Subplots consisted of eight wheat varieties (Duster, Iba, Gallagher, Spirit Rider, Lonerider, Smith's Gold, OK14319, and Jagger). Four of the eight varieties (Lonerider, Jagger, OK14319, Duster) carry the ALMT1 gene related to aluminum toxicity tolerance in wheat (Table 1). The remaining four varieties (Spirit Rider, Smith’s Gold, Iba, and Gallagher) were reported to be absent of the gene entirely (B. Carver, personal communication). Each subplot consisted of eight rows spaced 15.24 cm apart and 3 m in length.

3.3. Soil pH Assessment

A soil sample, consisting of 12 to 15 soil cores to a depth of 15 cm, was collected from each main plot prior to planting. These samples were tested to acquire the resident soil pH and buffering index (BI) of the plot, and major plant available nutrients. Soil pH was measured by using a glass (H^+ sensitive) electrode and reference electrode on a soil/water mixture with a ratio of 1:1 (Miller and Kissel, 2010). Soil samples were tested for residual nitrogen (nitrate-N), which was used in calculating the amount of N required for sufficient crop fertility. Plant available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted simultaneously by Mehlich 3 (M3) solution (composed of EDTA, NH_4NO_3, NH_4F, acetic acid and nitric acid) at the soil/solution ratio of 1:10 (Pittman et al., 2004). P, K, Ca, and Mg in M3 extract were quantified by an inductively coupled plasma (ICP) spectrometer.

3.4. Adjusting soil pH using alum or lime prior to planting wheat

Alum (UNIVAR Manufacturing, New Wilmington, Pennsylvania) and hydrated lime (Peteline & Son, Rapid City, South Dakota) was used in each main plot to attain one of the six target soil pH values based on the initial pH (Table 2 and Table 3). A response curve developed from a laboratory experiment in 2009 was used to determine the amount of material needed to reach a given target pH of the selected soils (D. B. Arnall, personal communication, August 9, 2017; Lollato et al., 2019). This response curve was developed through the collection of a large field sample from both Stillwater and Chickasha. Several subsamples weighing 0.5 kg were treated with alum or hydrated lime. The samples were regularly moistened over a four-month period. Following this period of wetting and drying, the relationship between amendments and soil pH of each subsample was assessed and graphed. These pH values were used to create a response curve and site-specific equations for acquiring a target pH through specified applications of alum and hydrated lime (Lollato et al., 2019; Sutradhar et al., 2014).

3.5. Field Methodology

All plots were established using conventional tillage prior to planting. Seed were sown at each location during mid-September at a 135 kg ha^{-1} rate using a Wintersteiger (Hege small-plot, conventional drill). Inherent top soil NO_3-N, plant available P, and K values were obtained through pre-planting soil test. During Year 1, no P and K fertilizer was banded during planting; however, urea (46-0-0) was broadcasted at 146 kg ha^{-1} during jointing (March 2018). During Year 2, fertilizers were applied prior to planting (soil nutrients were increased to levels req. for 4035 kg ha^{-1} grain yields). Urea was applied pre-plant at 118 kg ha^{-1} and 147 kg ha^{-1} at Chickasha and Stillwater, respectively. Additionally, Muriate of potash (MOP) was applied pre-plant at 70 kg ha^{-1} at Stillwater. Due to insufficient soil P, Diammonium phosphate (DAP) was applied in-furrow at Chickasha at 56 kg ha^{-1}. Plots were harvested for grain 6 June 2018 and 12 June 2019 at Stillwater,and 4 June 2018 and 11 June 2019 at Chickasha with a small plot combine (Wintersteiger). Grain protein concentration (GPC) was measured with near-infrared reflectance spectroscopy using a Perten DA 7200 (Perten Instruments Inc., Springfield, IL). Pesticides were applied following Feekes 5 in terms of amount of product per hectare. In Year 1, 1148 g ha^{-1} of Axial XL, 140 g ha^{-1} Powerflex HL, 21 g ha^{-1} Finesse, and 2100 ml ha^{-1} MCPA Ester was applied to Stillwater and Chickasha. In Year 2, 140 g ha^{-1} Powerflex HL, 938 ml ha^{-1} MCPA, 21 kg/ha Finesse, and 910 g ha^{-1} Nexicor at both locations.

3.6. Vegetative Development Evaluations

Three months after planting, forage samples were hand clipped down to the soil surface, above the crown of the plant. Forage samples consisted of two, 1 m row segments from each plot. After all forage samples were collected, the entire study was mowed to a height of 6 cm to simulate grazing. All samples were dried at 85° C for three to four weeks prior to forage weighing (Table 4). Simulated grazing was initiated at the same time as forage clippings using a

1.5 m deck, self-propelled mower with grass catcher attachment (Hustler 260K). Plots were mowed to a regrowth threshold of 5 cm as described by Edwards & Butchee (2013) (Table 4). Quasi-gene analysis was used to explore the ALMT1 gene effect in the background of Duster. Quasi-gene analysis only included observations at Stillwater and was facilitated by limiting genotype groups to include only those varieties related to Duster; thus, Jagger and Spirit rider observations were excluded. Genotype groups were redefined as quasi gene (QG) genotype groups and were as follows: ALMT1(-) [Gallagher, Smith's Gold, Iba] and ALMT1(+) [Duster, OK14319, Lonerider]. The objective of this analysis was to determine whether the quasi-gene (QG) groups and the original total-gene (TG) genotype groups, which consisted of all eight varieties, differed in their yield responses to Al_{sat}. Intercepts and slopes of both QG and TG groups were compared based on statistical significance of corresponding parameters using a single degree of freedom test (Table 6).

3.7. Extractable Aluminum and Al Saturation Determination

Sixty days following planting, soil samples were collected and analyzed in the same manner as presented in Section 3.3, with the addition of extractable Al and Al_{sat} analysis. Two grams of soil samples were weighed and placed into a 60mL plastic cup. Using a bottle-top dispenser, 20mL of 1.0 M KCl (74g KCl in 1 L DI water) was added to each cup. The cups were covered and placed on a reciprocal shaker for 30 minutes. Extracts were captured using folded Whatman #1 filter paper and a labelled 103mL plastic cup. The amount of Al extracted with 1M KCl was quantified using an inductively coupled plasma spectrometry (ICP).

The equation below, proposed by Sumner and Miller (1996), were used to determine the effective cation exchange capacity (ECEC) of each soil analyzed with ICP-OES:

$$\text{ECEC } (\frac{meq}{100g}) = [\text{Na}] + [\text{K}] + [\text{Ca}] + [\text{Mg}] + [\text{Al}_{\text{KCl}}] \quad [1]$$

The values for the basic cations (Ca^{+2}, Mg^{+2}, K^{+}) were obtained by using the M3 extraction mentioned in the above sections. The meq weights were determined by dividing the atomic weight of the element by its valence charge (e.g. Ca is 40/2 = 20 mg/meq; Mg is 24/2 = 12 mg/meq; K is 39/1 = 39 mg/meq; Al is 30/3 = 10 mg/meq).

Al_{sat} was calculated using the following equation:

$$\% Al_{sat} = (Al_{KCl}/ECEC) \text{ X } 100 \quad [2]$$

3.8. Statistical Analysis

Soil and plant data were analyzed using PC SAS Version 9.4/JMP Pro 13 (SAS Institute, 2017a; SAS Institute, 2017b). Locations were not combined due to differences in aluminum concentration of edaphic environments. The Levene's test was performed for variety and gene groups [ALMT1(+)/ALMT1(-)] to assess homoscedasticity of variance. Study years within each location were not combined as a result of heterodasticity of variance attributed to spatio-temporal influences on genotypic responses. Mixed model procedures were performed using JMP Pro 13 with random effects: rep and rep x acidity; fixed effects: variety/gene group, acidity, and variety/gene group x acidity. Effect levels were as follows: acidity [low, moderate, high] where, low : <5, moderate: $5 \leq pH \leq 5.8$, and high: > 5.8; gene group [ALMT1(+) and ALMT1(-)]; variety [(Duster (+), Lonerider (+), OK14319 (+), Jagger (+), Iba (-), Gallagher (-), Spirit Rider (-), Smith's Gold (-)]. Effects were compared by pairwise comparisons of least square means using Tukey-Kramer HSD test for varieties and student t-test for gene groups. Relative yield was used to compare difference between the gene groups, as applicable. Relative yield was determined by expressing the yield of all six observations of any variety in a single rep, as a percentage of the highest yielding observation of the six observations of that variety within that rep.

Threshold pH and yield plateaus for varieties and gene groups were characterized using segmented linear-plateau (LP) response models using the PROC NLIN in SAS Version 9.4 as detailed by Antonangelo et al. (2019). Models were considered admissible only when NLIN convergence criterion was both successfully met and significant ($p < 0.05$). Linear regression was performed on varieties and gene groups to unveil their relationships with Al concentration. Dummy variable regression with an indicator term in the model was used to compare linear model parameters. Where slopes were considered equal, ANCOVA, using Al_{sat} as a continuous predictor variable ($p < .0001$), was used to compare intercepts. Previous studies have utilized segmented plateaus and simple linear regression to characterize genotypic responses to soil pH and Al_{sat} (Lollato et al., 2019; M Abdulaha-Al Baquy et al., 2017; Sutradhar et al., 2014; Kariuki et al., 2007; Costa et al., 2003; Wise, 2002).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Soil pH, Exchangeable Aluminum, and Aluminum Saturation

4.1.1 Stillwater, OK

During Year 1, soil pH ranged from 4.5 to 7.1, while 1 M KCl extractable Al concentrations (Al_{KCl}) and Al saturation (Al_{sat}) varied from 0 to 184 mg kg^{-1} and from 0 to 39%, respectively (Table 5). Additionally, the average difference between the target soil pH and actual soil pH of the 24 main plots was ±0.4, with 12 out of 24 being within ±0.4. In Year 2, soil pH ranged from 4.3 to 6.5, while Al_{KCl} concentrations and Al_{sat} varied from 0 to 165 mg kg^{-1} and from 0 to 35%, respectively (Table 5). Additional amendments were added in Year 2, thus lowering the average difference between target soil pH and actual soil pH of the 24 main plots to ±0.2, with 23 out of 24 being within ±0.4 (Table 3). This is in accordance with Lollato et al. (2019) whom reported that 81% to 88% of their soil pH measured main plots were within ±0.4 of their target pH. A statistically significant inverse exponential relationship was found between Al_{KCl} ($p < 0.001$, $r^2 = 0.92$) and Al_{sat} ($p < 0.001$, $r^2 = 0.92$), when expressed as dependent variables with soil pH as the independent variable; hence, small changes in soil pH < 5.5 resulted in considerable increases across both Al_{KCl} and Al_{sat} (Figure 2).

4.1.2 Chickasha, OK

During Year 1, soil pH ranged from 5 to 7.1, yet Al_{KCl} and Al_{sat} were low, varying between 0 to 57 mg kg^{-1} and from 0 to 8.14%, respectively. In Year 2, despite additional amendments being added to lower the pH of target main plots (4 and 4.5) at double estimated rate, only slight increases in soil acidity were observed with soil pH ranging from 4.9 to 6.7 (Table 5). Similarly, Al_{KCl} and Al_{sat} were still lower than Stillwater, varying between 0 to 66 mg kg^{-1} and from 0 to 10.05%, respectively; thus, a significant relationship was not found between soil pH and Al_{sat}. However, the trend of the response was similar to Stillwater, in that small changes in soil pH < 5.5 resulted in considerable increases across Al_{sat} (Table 3). This is contrary to findings by Lollato et al. (2019) whom at the same location reported a significant relationship between soil pH and Al_{sat} where, small changes in soil pH < 5 resulted in considerable increases across Al_{sat}. Additionally, Al_{KCl} and Al_{sat} ranged from 0 to 64 and 0 to 7.8%, respectively. Two possible reasons for this divergence could be as follows: 1) changes in reserve acidity and base saturation (BS); and 2) a larger set of data points due to a longer study duration. Inherent differences among edaphic environments can noticeably impact the amount of Al that is solubilized at similar soil pH (Sutradhar et al., 2014). The Dale soil series described at Chickasha is characterized by a neutral Ap horizon (0 to 18 cm), with slightly acid to moderately alkaline reactions (NRCS, 2019); thus, with the expectation of leaching and eluviation, long term experimental additions of acidic cations could in theory, produce a slightly acidic sampling depth, similar to the Easpur soil series at Stillwater and the Konawa soil series (Kariuki et al., 2007). We hypothesize that unlike Konawa and Easpur soil series, the growth rate associated with Al concentrations and soil pH with the Dale series at Chickasha lacked the high Al concentrations required to support the scale parameters of the exponential model. For example, the relationship between soil buffer index (BI) and Al_{sat} was linear at Stillwater ($p < 0.0001$), but cubic at Chickasha ($p < 0.0001$); thus, a BI of 6.5 corresponded with 38% Al_{sat} at Stillwater, while at

Chickasha that same BI corresponded with 9% Al_{sat} (Figure 4 and Figure 5). Notwithstanding similar soil texture and varying parent material, the divergence in soil buffering of these locations could be attributed towards differing operational fractions of Al, and higher organically bound Al and dissolved organic carbon (DOC), which would effectively decrease the Al_{KCl} fraction in soil pH < 5.93 (Wang et al., 2015; Antonangelo et al., 2016). Moreover, Lollato et al. (2019) collected observations over three years opposed to two years, which could influence the optimization of the growth and scale parameters of the exponential model. Thus, these observations, accompanied with differing Al concentrations, supported the decisions to defer primarily to Stillwater in answering our research question.

4.2 Weather Conditions

At Stillwater, between planting and forage clipping, daily average rainfall varied from 2.86 to 1.95 mm in Year 1 and Year 2, respectively; while at Chickasha, daily average rainfall varied from 2.39 to 3.31 mm, respectively. Total rainfall at Chickasha was 50% higher in Year 2 (305 mm) than Year 1 (203 mm); thus, forage yields (kg ha^{-1}) were 25% higher in Year 2 ($p < .0001$) (Figure 6). Although total rainfall at Stillwater was 36% higher in Year 1 (261 mm) than Year 2 (166 mm), there was less than a half of a percent difference in forage yields (kg ha^{-1}) between study years ($p = 0.86$) (Figure 7). Additionally, Grain yields (kg ha^{-1}) were 23% and 37% higher in Year 2 at Chickasha and Stillwater, respectively (Figure 6 and 7). There are three possible explanations: 1) rainfall frequency; 2) in-furrow fertilizer application of DAP; and 3) genotype group response to spatio-temporal fluctuations. The amount of consecutive days without rainfall was about three times more intense in Year 1 than Year 2 at both locations. There was virtually no rainfall between late-October and mid-December (Figure 8 and Figure 9). Additionally, DAP has been shown to influence forage and grain yields in soils with low soil pH; moreover, when applied at 73 to 151 kg ha^{-1}, DAP has been shown to induce a tradeoff between forage and grain yields of both susceptible and tolerant varieties (Kaitibie et al., 2002). Thus, the forage yield

increases observed at Chickasha could be a result of rainfall or fertilizer application at the expense of grain yields. However, it would be difficult to discern whether grain yields at Chickasha were negatively impacted by in-furrow application of DAP as yields were over 25% higher in Year 2 for both forage and grain. Finally, ALMT1(-) [Gallagher, Smith's Gold, Spirit Rider, Iba] and ALMT1(+) [Duster, OK14319, Lonerider, Jagger] genotype groups responded differently during Year 1 and Year 2. Despite similarities in forage yields (kg ha^{-1}) between years at Stillwater, the variances of relative forage yield (RFY) by genotype group were unequal in Year 1 ($p = 0.0093$) and equal in Year 2 ($p = 0.4383$). Additionally, RFY of ALMT1(+) was 10% higher than ALMT1(-) in Year 1 ($p = 0.0103$); and ALMT1(+) was about 1% lower than ALMT1(-) in Year 2 ($p = 0.8469$) (Figure 10 and Figure 11). Further analysis revealed that Year 2 RFYs were significantly lower for ALMT1(+) ($p = 0.0009$) and only slightly lower for ALMT1(-) ($p = 0.6591$) when compared to Year 1. Moreover, during that same year at Chickasha, RFYs were slightly higher ($p = 0.1205$) for ALMT1(+) and significantly higher for ALMT1(-) ($p = 0.0052$) in comparison to Year 1. Furthermore, during Year 2, variances were smaller for ALMT1(-) at Chickasha ($p = 0.0033$) and larger for ALMT1(+) at Stillwater ($p = 0.0035$) (Data not shown). Coefficient of variations of genotypes groups for RFY ranged from 16-25 and 33-36 for ALMT1(-); and 24-35 and 17-19 for ALMT1(+), at Stillwater and Chickasha, respectively. These observations suggest that gene effect in this study was not static, implying that the response of individual varieties within genotype groups was a function of yearly-ecological dependent, spatio-temporal fluctuations (Washmon et al., 2002).

4.3 Gene Group Specific Responses of Forage and Grain to Acidity

4.3.1 Relative Forage Yield

In Year 1, relative forage yield (RFY) differed between the ALMT1 (-) and ALMT1 (+) genotype groups at Stillwater ($p = 0.0042$) and Chickasha ($p = 0.0440$), with ALMT1 (+) yielding higher at both locations. At Stillwater, RFYs were significantly lower at higher acidity, where,

low = moderate > high ($p = 0.0057$); however, at Chickasha, differences between acidity levels were not statistically significant ($p = 0.9285$). In terms of acidity levels, results were similar at Chickasha in Year 2 ($p = 0.8857$), but RFY did not differ between ALMT1 (-) and ALMT1 (+) genotype groups ($p = 0.7792$). During Year 2 at Stillwater, RFYs were different across all acidity levels, where, low > moderate > high ($p = 0.0057$); yet, RFY did not differ between ALMT1 (-) and ALMT1 (+) genotype groups ($p = 0.7228$). No statistically significant interaction existed between genotype group and acidity during Year 1 or Year 2 at Chickasha ($p > 0.3818$) or Stillwater ($p > 0.2145$). Means comparison of genotype group/acidity levels were conducted by year and location. Although no statistically significant difference was found at Chickasha for either year, notable differences were observed during Year 1 and Year 2 at Stillwater (Figure 12, Figure 13, Figure 19, and Figure 20). The lack of a difference between genotype groups in Year 2 at Chickasha, despite an increase in soil acidity and a significant difference between groups in Year 1, suggests that the differences observed between genotype groups in Year 1 were not attributed to soil pH and Al concentrations alone. Evidences suggests that the variation in gene group response to acidity, similar to other studies, could be accredited to the edaphic environment and differences individual winter wheat varieties outside of the ALMT1 gene (Lollato et al., 2019; Kariuki et al., 2007; Johnson et al., 1997).

4.3.2 Relative Grain Yield

In Year 1, relative grain yield (RGY) did not differ between the ALMT1(-) and ALMT1(+) genotype groups at Stillwater ($p = 0.2102$) or Chickasha ($p = 0.9172$). At Stillwater, RGYs yield decreased as acidity increased, where, low ≥ moderate ≥ high ($p = 0.0255$); however, at Chickasha, RGYs differences between acidity levels were not statistically significant ($p = 0.7418$). In Year 2, RGYs did not differ between the ALMT1(-) and ALMT1 (+) genotype groups at Stillwater ($p = 0.2684$) or Chickasha ($p = 0.2106$). Similarly, differences between acidity levels were not statistically significant at Stillwater ($p = 0.0958$) or Chickasha ($p =$

0.2633) (Data not shown). This provides additional support for the suggestion that the gene group forage and grain yields responses are contingent upon year dependent environmental-plant-edaphic dynamics.

4.3.3 Forage Yield and Grain Yield

4.3.3.1 Stillwater

Segmented linear-plateau (LP) response models were used to characterize forage and grain yields of genotype groups and individual varieties (Table 7 and Table 8) as a function of soil pH at Stillwater for Year 1 and Year 2. Previous studies have utilized the slope of linear models as the sensitivity of wheat genotypes to soil acidity; the greater the slope, the more sensitive that genotype to acidity (Kariuki et al., 2007). Previous studies have acknowledged that forage and grain yields are especially sensitive when Al_{sat} is greater than 30% (Kariuki et al., 2007; Lollato et al., 2019). In this study, Al saturation intensity was used as a categorical visual aide with effect levels low, moderate, high, where, low Al_{sat} <12%, moderate 12% < Al_{sat} < 30%, high Al_{sat} >30%. During Year 1, LP slopes of ALMT1(-) were nearly identical for grain and forage; while, slopes of ALMT1(+) showed that forage was nearly six times more sensitive to acidity than grain (Figure 14). In comparison, the slopes of the LP response models for forage indicated that ALMT1(+) was three times more tolerant than ALMT1(-). Grain yield LP response models for ALMT1(+) and ALMT1(-) were nearly identical except for their slopes which showed ALMT1(+) to be 76% more tolerant to acidity than ALMT1(-). Conversely, during Year 2, ALMT1(+) forage was less tolerant to soil acidity than ALMT1(-) (Figure 15). The same was true for grain yields which revealed ALMT1(-) to be slightly more tolerant to acidity than ALMT1(+) (Figure 15). Additionally, ALMT1(+) successfully converged with the LP model for forage, whereas ALMT1(-) forage yields did not plateau. This reversal in dominant genotype group was supported by the linear responses of grain and forage yields to Al_{sat} (Figure 16 and Figure 17).

Slopes of genotype groups for forage and grain yields were considered equal for Year 1 (p = 0.9887 and p = 0.0844, respectively) and Year 2 (p = 0.6374 and p = 0.2331). Although slopes were equal, intercepts differed significantly between genotype groups for forage during Year 1 (p = <.0001) and Year 2 (p = <.0049), but did not differ significantly for grain in Year 1 (p = 0.0581) or Year 2 (p = 0.4101) (Table 6). Thus, the anticipated efficacy of the ALMT1 gene in acidic conditions was not consistent in this study. These findings suggest that the tolerance of these closely related acid salt tolerant varieties to acidity and Al toxicity might be benefitted by the ALMT1 gene but is certainly not beholden to it.

4.3.3.2 Chickasha

At Chickasha, tables and graphs were not used to show data as Al concentration were not ideal; however, our findings were reported and explored in this section. There was no evidence of significant linear relationships between soil pH and forage yields for the ALMT1(-) group, ALMT1(+) group, or individual varieties in Year 1 (p = 0.6366, p = 0.5489, and p = > 0.2999, respectively) or Year 2 (p = 0.7788, p = 0.6283, and p = > 0.4811, respectively). Surprisingly, the relationship between Al_{sat} and forage yield was also not significant during Year 1 (p = > 0.3841, p = 0.0569, and p = > 0.1337, respectively) or Year 2 (p = 0.3592, p = > 0.0629, and p = > 0.1438, respectively). In terms of grain yield in Year 1, ALMT1(-) and ALMT1(+) showed significant positive linear relationships between Al_{sat} and grain yield (p = 0.0021 and p = 0.0105); however, there was no evidence of a significant linear relationship between soil pH and grain yield for both genotype groups (p = 0.0906 and p = 0.2465, respectively). In Year 2, there was no evidence of significant linear relationships between Al_{sat} and grain yields for the ALMT1(-) or ALMT1(+) groups (p = 0.1996 and p = 0.6437). Additionally, despite the absence of a significant linear relationship between soil pH and grain yield for the ALMT1(+) group (p = 0.2890), a significant positive inverse linear relationship existed for the ALMT1(-) group (p = 0.0243). In Year 1, the trend between Al_{sat} and grain yield was positive for all varieties, yet Duster, OK14319, and Iba

were the only three varieties that showed significant increases. In Year 2, there was no evidence of significant linear relationships between soil pH and grain yields ($p = > 0.10$) or Al_{sat} and grain yields ($p = > 0.1206$). The increase in grain yields despite an increase in Al_{sat} supports previous findings that the benefits of tolerant varieties grain yield may not be realized when $Al_{sat} < 12\%$ (Johnson et al.,1997; Wise, 2002; Kariuki et al., 2007; Schroder et al., 2011; Lollato et al., 2019). Moreover, during Year 1, Iba uniquely showed that increases in pH significantly decreased grain yield ($p = 0.0025$) while, noting an increase in grain yield as Al_{sat} increased ($p = 0.0319$). These observations suggest that Al concentrations at Chickasha were not high enough to negatively impact the wheat yields of even the most susceptible varieties (Iba), while highlighting potential influences from ancillary experimental factors on the response of individual varieties to soil acidity and increasing Al concentrations. Whether these factors be environmental or otherwise, the discrepancies within genotypic responses may not be wholly ascribed to the ALMT1 gene.

4.4 Quasi-Gene Group Analysis

Forage yields did not differ in terms of the statistical significance of corresponding parameters of either slopes or intercepts during Year 1 or Year 2. Slopes for grain yields did not differ; however, intercepts for grain yield did differ in terms of statistical significance of corresponding parameters during Year 1 (QG, $p = < 0.001$; TG, $p = 0.0581$). In Year 2, grain yield did not differ in terms of statistical significance of corresponding parameters for slopes or intercepts. Furthermore, the QG analysis suggests that within the background of Duster, ALMT1(+) gene grain yield did significantly differ in its mean response when Al_{sat} is zero. Meaning, in terms of grain yield, varieties in the background of Duster that were ALMT1(+) performed noticeably better than those that were ALMT1(-) at a neutral pH. However, unlike difference in slope, which could be attributed to sensitivity, dissimilarity between intercepts may be better ascribed to factors such as environmental fluctuations, seed vitality, and yield potential of individual varieties. The variably transient nature of such factors can be illustrated through genotypic

comparisons across growing seasons. For example, with the presence of ALMT1(+) gene, Duster is considered to be superior in DP management systems and is used as a standard for comparison of other varieties (Edwards et al., 2012). When compared to the ALMT1(+) variety Jagger, Duster had an intercept that was 27% higher for grain yield during Year 1, and barely 1% higher in Year 2 (Table 10). Thus, these observations were considered to be ancillary to the ALMT1 gene effect. Although slopes did not differ in corresponding significance, QG slopes for both genotype groups showed greater sensivity to Al_{sat} than TG slopes for forage and grain in Year 1. However, during Year 2 slope dominance segrated by gene group for both forage and grain. The forage and grain QG slopes for ALMT1(+) were more tolerant than their TG forage slopes, while the forage and grain QG slopes for ALMT1(-) were less tolerant than their TG forage slopes (Table 11). The difference in the distance between the linear responses of genotypes groups was more pronounced in the TG genotype groups than the QG genotype groups. Thus, it is implied that the ALMT1 gene provides an advantage to varieties in the background of Duster.

4.5 Grain Protein Concentration

Strong positive correlations between grain protein concentration (GPC) and Al_{sat} were found during both study years for the ALMT1(-) and ALMT1(+) genotype groups ($p = \leq .0001$). Despite a 3% lower overall average GPC in Year 2, genotype groups showed consistency in the statistical significance of their individual linear responses,and dominance of ALMT1(+) over ALMT1(-) in average percent protein in Year 1 and Year 2 (Figure 18). Further analysis indicated that all winter wheat varieties, with the exception of Duster ($p = 0.3210$), showed significant positive linear relationships between Al_{sat} and GPC in Year 1. The same was true of these winter wheat varieties in Year 2, with the exception of Duster ($p = 0.1130$) and Spirit Rider ($p = 0.1904$) (Table 12). Grain protein levels are a function of genotype, N fertilization, environmental, and spatio-temporal influences (Limon-Ortega and Martinez-Cruz, 2014). Thus, perceiving the first two factors to be fairly constant, it is likely that the higher GPC observed in Year 1 at Stillwater

was a consequence of truncated rainfall at the expense of higher grain yields. Moreover, the duly noted increases in GPC across increasing Al_{sat} could be attributed towards two phenomena: 1) enhanced grain storage protein composition (Yu et al., 2018) as a result of increased sulfur content from the application of aluminum sulfate [Alum; $Al_2(SO_4)_3$] (Limon-Ortega and Martinez-Cruz, 2014); and 2) inverse yield-protein relationships (Terman et al., 1969). Various studies have shown increased protein biosynthesis as a derivative of the application of sulfur containing amendments (e.g. ammonium sulfate [$(NH_4)_2SO_4$]) (Woolfolk et al., 2002; Limon-Ortega and Martinez-Cruz, 2014; Yu et al., 2018; Tao et al., 2018). It is probable that clay deposits of translocated sulfate provided readily available sulfur to deep roots during antithesis (Feekes 10.5) and grain filling (Feekes 11) (Large, 1954) allowing for increased GPC across growing seasons. Additionally, it is more likely that the lower GPC observed in Year 2 was a derivative of higher rainfall which resulted in increased grain yields, thus decreasing GPC (Terman et al., 1969).

CHAPTER V

CONCLUSION

This study evaluated the performance of winter wheat varieties with (+) and without (-) the aluminum tolerance gene (ALMT1) under varying soil pH and aluminum saturation (Al_{sat}) conditions at two locations in two growing seasons. Overall, as Al_{sat} increased, yields of both ALMT1(-) and ALMT1(+) genotypes decreased. Consistent with other studies, ALMT1(-) and ALMT1(+) forage yields were found to be more sensitive to Al toxicity than grain yield. Grain protein content illustrated a significant positive linear relationship with Al_{sat}, which we hypothesize to be a consequence of increased sulfur bioavailability in lieu of subsequent Alum applications and the reduction of grain yields. Surprisingly, ALMT1(-) and ALMT1(+) genotype groups did not express consistent differences in their responses to Al_{sat}; thus, an incontestable ranking system was not possible. However, QG analysis did show that under increasing Al_{sat}, slopes of ALMT1(-) were consistently greater than ALMT1(+), suggesting the significance of the ALMT1(+) gene in the background of Duster. Moreover, this study did reveal that in terms of individual variety response to acidity and Al toxicity, Duster, Spirit Rider and Gallagher, respectively, were the most tolerant and consistent across years. Spirit Rider and Gallagher are considered ALMT1(-), while having theoretical proportions of alleles from Duster respectively equal to 0% and 50% based on pedigree (Table 1). However, Jagger, which is ALMT1(+) and 0% related to Duster, did not show consistent responses to acidity and Al toxicity, ranking 4th in Year 1, and 7th in Year 2. This suggests that Al tolerance among commonly grown hard winter wheat

cultivars is not wholly dependent on the presence or absence of the ALMT1 gene. Furthermore, this study supported findings in favor of the utilization of acid-salt tolerant varieties as a management practice in wheat production systems with acidic soils. For example, Iba, the only susceptible (acid-salt "intolerant") variety in this study differed drastically in response to Al_{sat}, showing a slope significantly different from all other varieties in Year 2. Our study revealed that ALMT1(+) and ALMT1(-) responses differed between edaphic environments. While our conclusions for Al tolerance were derived completely from observations at Stillwater, noteworthy differences in the inherent soil characteristics of the Dale (Chickasha) and Easpur (Stillwater) soil series promotes a research question involving the impact of the distribution of Al fractions of semi-analogous soil taxonomic series on cropping systems with acidic soils. Moreover, the effect of varying spatial-temporal conditions and edaphic environments on the expression of Al tolerance in closely related acid tolerant wheat varieties was evident in this study. Thus, there is provision for future exploration involving the identification of ancillary factors that confer acid tolerance in wheat, and long-term responses of gene groups to acidic soils across a myriad of edaphic environments.

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APPENDICES

Table 1. Varieties by presence of ALMT1(+) or absence of ALMT1 (-) and their relative percentages of Duster.

Variety	ALMT1	%Duster
Duster	(+)	100
OK14319	(+)	50
Lonerider	(+)	25
Jagger	(+)	0
Gallagher	(-)	50
Smith's Gold	(-)	25
Spirit Rider	(-)	0
Iba	(-)	50

Table 2. Soil pH, target soil pH, and aluminum sulfate (Alum) and calcium hydroxide (Ag lime) amendment rates for each main plot for Year 1 and Year 2 at Stillwater.

		Year 1			Year 2		
Target pH	Main Plot	Soil pH 1 (8/09/2017)	Amendment Rate (kg/plot)	Soil pH 2 (11/9/2017)	Soil pH 3 (6/17/2018)	Amendment Rate (kg/plot)	Soil pH 4 (11/20/2018)
4	105	4.3	3.3 (Alum)	4.6	4.5	8.1 (Alum)	4.3
	205	4.4	4.3 (Alum)	4.6	4.6	9.8 (Alum)	4.4
	301	4.4	4.3 (Alum)	4.6	4.4	6.5 (Alum)	4.4
	401	4.1	1.1 (Alum)	4.5	4.4	6.5 (Alum)	4.3
4.5	106	4.6	1.1 (Alum)	5.3	5	8.1 (Alum)	4.6
	203	4.4	0.7 (Ag lime)	5	4.9	6.5 (Alum)	4.6
	304	4.6	1.1(Alum)	5.1	4.9	6.5 (Alum)	4.9
	402	4.9	4.3 (Alum)	5	4.9	6.5 (Alum)	4.4
5	101	4.8	1.4 (Ag lime)	5.7	5.5	8.1 (Alum)	4.6
	202	5.2	2.2 (Alum)	5.1	5	*	5
	303	4.7	2.0 (Ag lime)	5.4	5.3	*	5.3
	403	4.6	2.7 (Ag lime)	5.5	5.2	*	5.5
5.5	103	5.4	0.7 (Ag lime)	5.6	5.6	*	5.6
	201	4.9	4.1 (Ag lime)	6.1	6.2	11.3 (Alum)	5.1
	302	5	3.4 (Ag lime)	5.8	5.6	*	5.6
	404	5	3.4 (Ag lime)	5.4	5.7	*	5.5
6	102	5.4	4.1 (Ag lime)	6.2	6.2	*	6.2
	204	5.8	1.4 (Ag lime)	5.5	5.8	*	5.8
	306	5.3	4.8 (Ag lime)	6.5	6.5	8.1 (Alum)	5.8
	405	4.9	7.5 (Ag lime)	5.8	5.6	2.7 (Ag lime)	5.8
7	104	6.1	6.1 (Ag lime)	6.7	7.1	*	7.1
	206	6.4	4.1 (Ag lime)	6.8	6.8	*	6.8
	305	6.5	3.4 (Ag lime)	7.1	6.9	*	6.9
	406	6	3.4 (Ag lime)	7.1	6.8	*	6.8

*No amendment was applied. Amendments rates for year 2 were x 1.5 the soil curve estimation.

Table 3. Exchangeable Al and Al saturation (Al_{sat}) for each main plot for Year 1 and Year 2 at Stillwater.

Target pH	Main Plot	----------Year 1----------		----------Year 2----------			
		Al ppm 1 (11/9/2017)	Al_{sat} 1 (11/9/2017)	Al ppm 2 (6/17/2018)	Al_{sat} 2 (6/17/2018)	Al ppm 3 (11/20/2018)	Al_{sat} 3 (11/20/2018)
4	105	148	28%	141	28%	147	28%
	205	120	23%	128	25%	150	30%
	301	130	28%	178	35%	131	28%
	401	184	39%	138	30%	165	35%
4.5	106	51	10%	67	14%	93	18%
	203	63	12%	84	16%	92	19%
	304	68	13%	82	16%	74	14%
	402	71	14%	94	16%	104	14%
5	101	14	2%	26	5%	78	15%
	202	42	8%	46	9%	44	8%
	303	28	5%	35	7%	47	9%
	403	28	5%	36	7%	61	12%
5.5	103	14	2%	8	1%	6	1%
	201	0	0%	0	0%	26	5%
	302	9	2%	9	2%	33	7%
	404	17	3%	6	1%	26	5%
6	102	0	0%	0	0%	3	0%
	204	21	4%	2	0%	21	4%
	306	0	0%	0	0%	4	1%
	405	10	2%	7	1%	4	1%
7	104	0	0%	10	1%	0	0%
	206	0	0%	0	0%	3	1%
	305	0	0%	0	0%	0	0%
	406	0	0%	0	0%	0	0%

Table 4. Dates for simulated grazing treatments, Canopeo, forage heights, clipping data collection, forage weighing, and grain harvest at Stillwater and Chickasha, OK for Year 1 and Year 2.

Year	Grazing Simulation		Canopeo & forage height	Forage Clipping	Forage weighing	Grain harvest
			Stillwater			
2017-2018	18-Dec-17	16-Feb-18	11-Dec-17	18-Dec-17	11-Jan-18	4-Jun-18
2018-2019	7-Dec-18	*	6-Dec-18	6-Dec-18	13-Dec-18	12-Jun-19
			Chickasha			
2017-2018	10-Jan-17	15-Feb-18	18-Dec-17	19-Dec-17	16-Jan-18	6-Jun-18
2018-2019	19-Dec-18	*	19-Dec-18	19-Dec-18	1-Jan-18	11-Jun-19

*Neither location received a second grazing simulation during the 2018-2019 growing season.

Table 5. Ranges for soil pH, Al saturation and exchangeable Al (mg/kg) for Stillwater and Chickasha for Year 1 and Year 2.

Location	Year	Soil pH	Al Saturation (Alsat)	Al (mg/kg)
Stillwater (STW)	1	4.5 – 7.1	38.77% - 0%	184 - 0
	2	4.3 – 6.5	34.90% - 0%	165 - 0
Chickasha (CHK)	1	5 – 7.1	8.14% - 0%	57 - 0
	2	4.9 – 6.7	10.05% - 0%	66 - 0

Table 6. Comparison of slopes and intercepts of ALMT1 (-) and ALMT1 (+) genotypes for quasi-gene grouped (QG) and total gene grouped (TG) varieties surrounding forage yields and grain yields; considering Al saturation (Al_{sat}) as a continuous predictor variable at Stillwater in Year 1 and Year 2. $\alpha = 0.05$.

Year 1											
Group	Yield Type	Slopes of ALMT1 genotype groups significantly different?	N	df	F Ratio	Prob > F	Intercepts of ALMT1 genotype groups significantly different?	N	df	F Ratio	Prob > F
Total Gene (TG)	Forage (kg/ha)	No	182	1	0.0002	0.9887	Yes	182	1	76.9393	<.0001
	Grain (kg/ha)	No	182	1	3.4478	0.0844	No	182	1	3.6376	0.0581
Quasi-Gene (QG)	Forage (kg/ha)	No	136	1	0.0378	0.8462	Yes	136	1	62.2091	<.0001
	Grain (kg/ha)	No	136	1	2.8578	0.0966	Yes	136	1	23.3418	<.0001
Year 2											
Group	Yield Type	Slopes of ALMT1 genotype groups significantly different?	N	df	F Ratio	Prob > F	Intercepts of ALMT1 genotype groups significantly different?	N	df	F Ratio	Prob > F
Total Gene (TG)	Forage (kg/ha)	No	184	1	0.2229	0.6374	Yes	184	1	8.1143	0.0048
	Grain (kg/ha)	No	183	1	1.4317	0.2331	No	183	1	0.3142	0.4101
Quasi-Gene (QG)	Forage (kg/ha)	No	139	1	1.3885	0.2407	Yes	139	1	6.7597	0.0104
	Grain (kg/ha)	No	138	1	1.9120	0.1690	No	138	1	0.7647	0.7647

Table 7. Segmented linear and linear regression models of forage yield by soil pH for individual varieties at Stillwater for Year 1 and Year 2.

Year 1								
		Plateau?	Plateau Forage (kg/ha)	Threshold pH	Linear equation	R^2	F Value	p-value
ALMT1 (+) VARIETIES	Duster	No	*	*	y = -132.66x + 2644.99	0.03	0.26	0.77
	Jagger	Yes	1892.48	5.03	y = 1491.54x - 5614.5 (x < 5.03)	0.31	4.73	0.02
	Lonerider	Yes	2120.09	4.72	y = 5303.94 - 22939.2 (x < 4.72)	0.34	5.16	0.0155
	OK14319	Yes	2050.5	4.71	y = 5107.11x - 22004.3 (x < 4.71)	0.38	5.7	0.012
ALMT1 (-) VARIETIES	Gallagher	Yes	1555.38	6.55	y = 338.05x - 656.17 (x < 6.55)	0.45	8.28	0.0016
	Iba	Yes	960.62	5.19	y = 1231.99x - 5436.6 (x < 5.19)	0.32	4.63	0.0229
	Smith's Gold	Yes	1944.42	5.76	y = 685.87x - 2009.71 (x < 5.76)	0.27	4.06	0.0322
	Spirit Rider	Yes	1433.51	4.77	y = 2121.59x - 8690.85 (X < 4.77)	0.25	3.16	0.065
Year 2								
		Plateau?	Plateau Forage (kg/ha)	Threshold pH	Linear equation	R^2	F Value	p-value
ALMT1 (+) VARIETIES	Duster	No	*	*	y = 350.70x - 185.40	0.26	3.44	0.0521
	Jagger	Yes	1491.54	5.8	y = 766.05x - 2194.61 (x < 5.8)	0.54	11.7	0.02
	Lonerider	No	*	*	y = 380x - 700	0.28	4.04	0.0335
	OK14319	Yes	1747.36	5.83	y = 682.59x + 2232.4 (x < 5.83)	0.43	7.8	0.012
ALMT1 (-) VARIETIES	Gallagher	No	*	*	y = 400x - 600	0.41	7.185	0.0042
	Iba	No	*	*	y = 789.76x - 2209.94	0.58	12.9	<.0001
	Smith's Gold	No	*	*	y = 434.34x - 840.39	0.42	7.87	0.0028
	Spirit Rider	No	*	*	y = 405.77x - 196.30	0.28	3.67	0.0448

Table 8. Segmented linear and linear regression models of grains yield by soil pH for individual varieties at Stillwater for Year 1 and Year 2.

Year 1

		Plateau?	Plateau Forage (kg/ha)	Threshold pH	Linear equation	R^2	F Value	p-value
ALMT1 (+) VARIETIES	Duster	No	*	*	y = 1236x - 2994	0.13	1.50	0.2458
	Jagger	No	*	*	y = 257x + 953	0.19	2.52	0.1044
	Lonerider	Yes	3153	6.33	y = 355x + 905 (x < 6.33)	0.22	2.92	0.0758
	OK14319	No	*	*	y = 367x + 976	0.16	1.95	0.1673
ALMT1 (-) VARIETIES	Gallagher	No	*	*	y = 288x + 799	0.18	2.27	0.1277
	Iba	No	*	*	y = 754x -1882	0.54	12.5	0.0003
	Smith's Gold	Yes	3208	5.05	y = 2331x - 8571 (x < 5.05)	0.56	13.6	0.0002
	Spirit Rider	Yes	3151	4.67	y = 8927x - 38512	0.58	14.7	<.0001

Year 2

		Plateau?	Plateau Forage (kg/ha)	Threshold pH	Linear equation	R^2	F Value	p-value
ALMT1 (+) VARIETIES	Duster	No	*	*	y = -218x + 4880	0.09	0.96	0.4006
	Jagger	No	*	*	y = -263x + 5253	0.11	1.26	0.3055
	Lonerider	No	*	*	y = -196x + 4667	0.04	0.45	0.6458
	OK14319	No	*	*	y = -302x + 5269	0.25	3.40	0.0554
ALMT1 (-) VARIETIES	Gallagher	No	*	*	y = -367x + 5771	0.27	3.89	0.0363
	Iba	Yes	3366	4.45	y =10345x – 42702 (x < 4.45)	0.36	5.13	0.0172
	Smith's Gold	No	*	*	y = -256x + 5201	0.08	0.95	0.4041
	Spirit Rider	No	*	*	y = -271x + 5750	0.75	0.77	0.4762

*Unrealized data point

Table 9. Slopes, intercepts and coefficients of determination (r^2), and significance of Al saturation as predictor variable (p-value) for forage yield during Year 1 and Year 2 at Stillwater.

		Year 1				Year 2			
Genotype	Variety	Intercept	Slope ($kg\ ha^{-1}\ Al_{sat}^{-1}$)	R^2	Prob > F	Intercept	Slope ($kg\ ha^{-1}\ Al_{sat}^{-1}$)	R^2	Prob > F
ALMT1 (+) VARIETIES	Duster	2019a	-17.0a	0.08	0.2043	1824abcd	-17.0a	0.14	0.0844
	Jagger	1959a	-22.3a	0.27	0.0092	2102abc	-36.3ab	0.46	0.0004
	Lonerider	2228a	-30.5a	0.37	0.002	1480d	23.0a	0.31	0.0056
	OK14319	2135a	-25.7a	0.39	0.0026	1597d	-30.7ab	0.33	0.004
ALMT1 (-) VARIETIES	Gallagher	1395c	-20.6a	0.43	0.0005	1712bcd	-20.7a	0.22	0.0193
	Iba	1036d	-27.5a	0.37	0.0028	2476ab	-51.4c	0.53	0.0001
	Smith's Gold	1941ab	-32.7a	0.38	0.0014	1745cd	-28.4ab	0.36	0.0019
	Spirit Rider	1512bc	-18.0a	0.35	0.0036	2136a	-18.1a	0.1	0.1476
	Meanβ	1778	-24	0.33	0.0283	1884	-28	0.31	0.0329

ϕ Values with the same letter within the same row are not statistically significant ($p<.05$)
ψ ANCOVA was used to compare intercepts; slopes compared using dummy variable regression with interaction terms
β Mean values were obtained by averaging outputs of all varieties within a column.

Table 10. Slopes, intercepts and coefficients of determination (r^2), and significance of Al saturation as predictor variable (p-value) for grain yield during Year 1 and Year 2 at Stillwater.

Genotype	Variety	Year 1				Year 2			
		Intercept	Slope (kg ha^{-1} Al_{sat}^{-1})	R^2	Prob > F	Intercept	Slope (kg ha^{-1} Al_{sat}^{-1})	R^2	Prob > F
ALMT1 (+) VARIETIES	Duster	3205a	-9.0a	0.03	0.4542	3751ab	0.04ab	<.0001	0.9967
	Jagger	2530b	-16.1a	0.15	0.0659	3707ab	6.69a	0.01	0.5867
	Lonerider	3019a	-23.0a	0.31	0.0057	3517bc	11.4a	0.02	0.4781
	OK14319	3146a	-19.7a	0.37	0.0035	3514bc	15.6a	0.14	0.0825
ALMT1 (-) VARIETIES	Gallagher	2570b	-19.4a	0.17	0.0451	3678ab	15.5	0.1	0.1424
	Iba	2569b	-43.6a	0.57	<.0001	3535c	-32.9b	0.2	0.0416
	Smith's Gold	3301a	-34.9a	0.45	0.0003	3833ab	2.1ab	0.001	0.8797
	Spirit Rider	3272a	-28.9a	0.48	0.0004	4225a	9.1a	0.02	0.5843
	Meanβ	2952	-24	0.29	0.0722	3720	3.0	0.06	0.474

ϕ Values with the same letter within the same row are not statistically significant (p<.05)

ψ ANCOVA was used to compare intercepts; slopes compared using dummy variable regression with interaction terms

β Mean values were obtained by averaging outputs of all varieties within a column.

Table 11. Linear regression models of ALMT1 (-) and ALMT1 (+) genotypes for quasi-gene grouped (QG) and total gene grouped (TG) varieties surrounding forage yields and grain yields; considering Al saturation (Alsat) as a continuous predictor variable at Stillwater in Year 1 and Year 2.

Group	Yield Type	Year 1							
		ALMT1(+)				ALMT1(-)			
		Linear Equation	N	R^2	P-value	Linear Equation	N	R^2	P-value
Total Gene (TG)	Forage (kg/ha)	= 2084 – 2412x	90	0.24	<.0001	= 1474 - 2403x	92	0.23	<.0001
	Grain (kg/ha)	= 2968 – 1768x	90	0.13	0.0006	= 2922 – 3045x	92	0.26	<.0001
Quasi-Gene (QG)	Forage (kg/ha)	= 2129 – 2466x	66	0.24	<.0001	= 1464 – 2623x	70	0.23	<.0001
	Grain (kg/ha)	= 3124 – 1784x	66	0.24	0.0008	= 2816 – 3147x	70	0.27	<.0001
Group	Yield Type	Year 2							
		ALMT1(+)				ALMT1(-)			
		Linear Equation	N	R^2	P-value	Linear Equation	N	R^2	P-value
Total Gene (TG)	Forage (kg/ha)	= 1741 – 2592x	92	0.24	<.0001	= 1998 – 2933x	92	0.25	<.0001
	Grain (kg/ha)	= 3617 + 868x	92	0.02	0.1410	= 3834 – 317x	91	0.002	0.6934
Quasi-Gene (QG)	Forage (kg/ha)	= 1628 – 2286x	69	0.2	<.0001	= 1949 - 3220x	70	0.31	<.0001
	Grain (kg/ha)	= 3589 + 923x	69	0.3	0.1764	= 3698 – 578x	69	0.007	0.4976

Table 12. Linear regression models of grain protein concentration (GPC) by Al saturation (Al_{sat}) for ALMT1(-) and ALMT1(+) genotype groups and individual winter wheat varieties.

Year 1				
	Linear Equation	R^2	F Value	p-value
ALMT1(+) Group	GPC = 0.06x + 0.14	0.16	16.60	0.0001
Duster	GPC = 0.03x + 0.128	0.05	1.04	0.3210
Jagger	GPC = 0.07x + 0.144	0.22	6.19	0.0209
Lonerider	GPC = 0.07x + 0.133	0.36	11.6	0.0027
OK14319	GPC = 0.05x + 0.136	0.21	5.09	0.0361
ALMT1(-) Group	GPC = 0.06x + 0.13	0.29	36.17	<.0001
Gallagher	GPC = 0.06x + 0.132	0.32	10.22	0.0042
Iba	GPC = 0.05x + 0.127	0.33	10.0	0.0049
Smith's Gold	GPC = 0.04x + 0.125	0.19	4.94	0.0374
Spirit Rider	GPC = 0.07 + 0.128	0.46	16.9	0.0005
Year 2				
	Linear Equation	R^2	F Value	p-value
ALMT1(+) Group	GPC = 0.3x + 0.1	0.24	28.40	<.0001
Duster	GPC = 0.02x + 0.099	0.12	2.74	0.1130
Jagger	GPC = 0.03x + 0.098	0.36	12.52	0.0018
Lonerider	GPC = 0.05x + 0.097	0.39	13.39	0.0015
OK14319	GPC = 0.03x + 0.103	0.23	6.36	0.0198
ALMT1(-) Group	GPC = 0.03x + 0.09	0.24	27.7	<.0001
Gallagher	GPC = 0.05x + 0.092	0.49	20.75	0.0002
Iba	GPC = 0.04x + 0.096	0.45	15.70	0.0008
Smith's Gold	GPC = 0.03x + 0.098	0.26	7.93	0.0101
Spirit Rider	GPC = 0.02x +0.089	0.08	1.84	0.1904

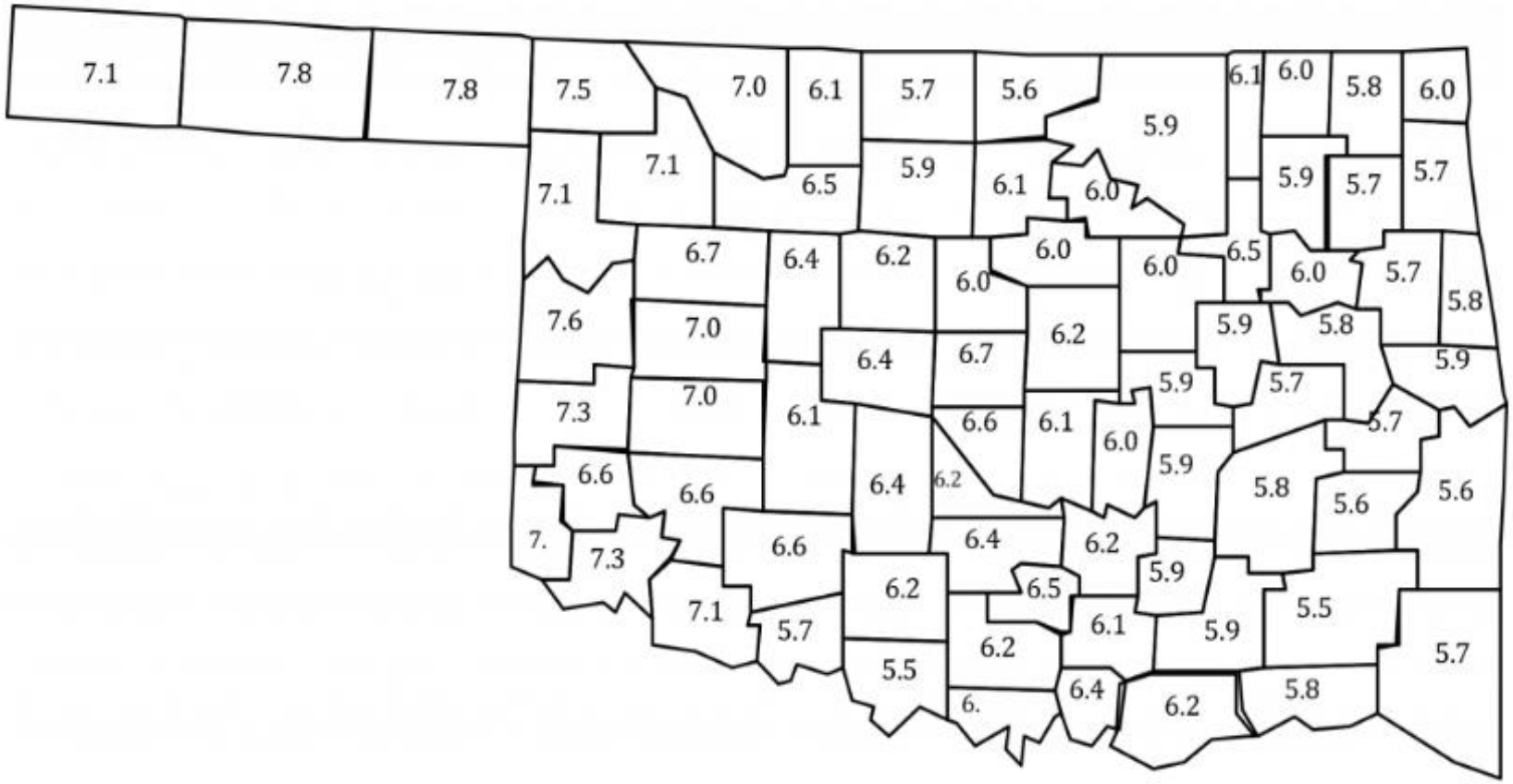

Figure 1. Median soil pH values for Oklahoma counties obtained from soil samples tested between 2014-2017.

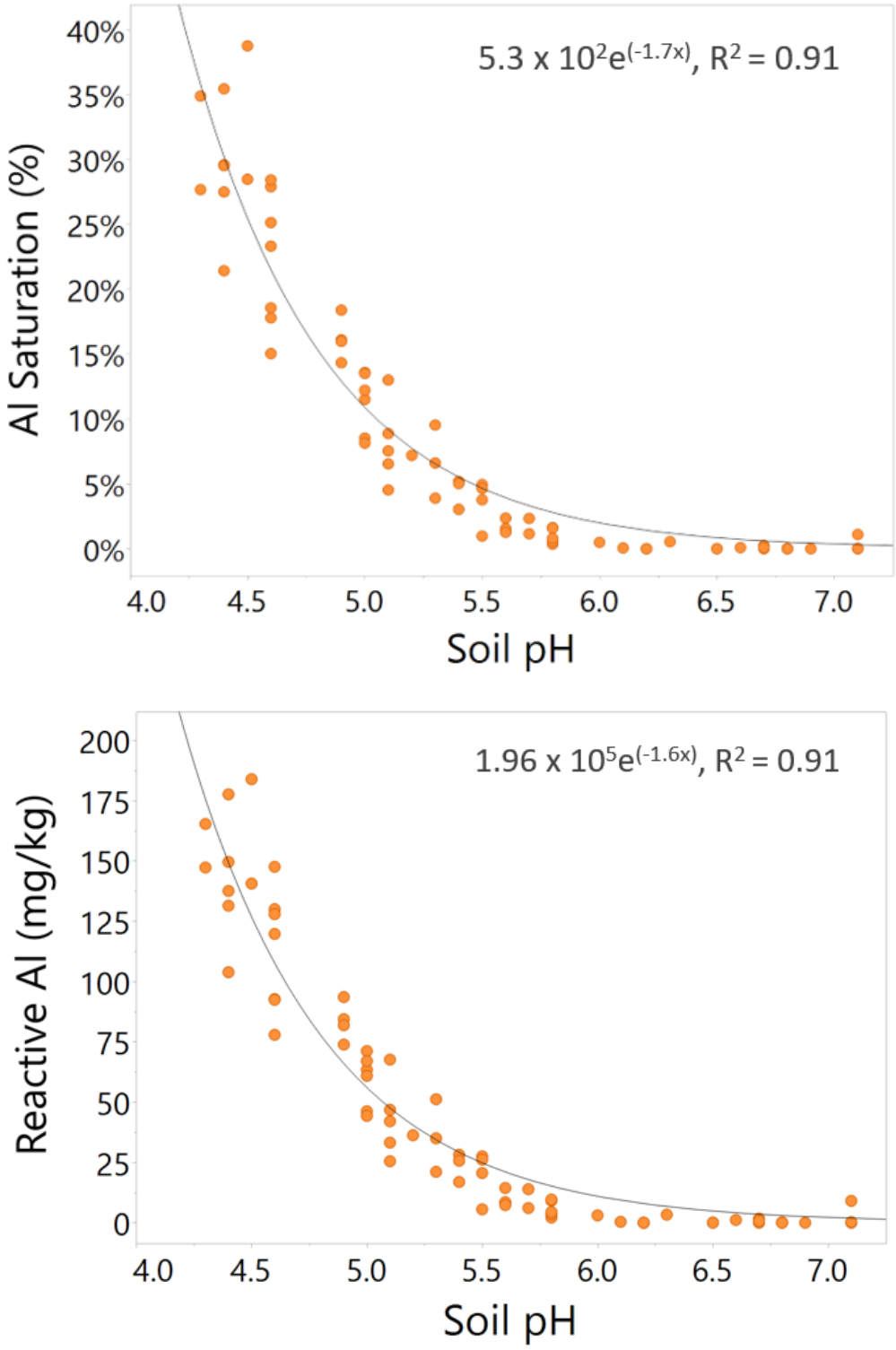

Figure 2. Relationship of soil pH between percent Al saturation (Al_{sat}) and exchangeable Al (reactive Al) by soil pH for a Easpur loam soil at Stillwater, OK (Year 1 and Year 2 combined). $p < 0.0001$.

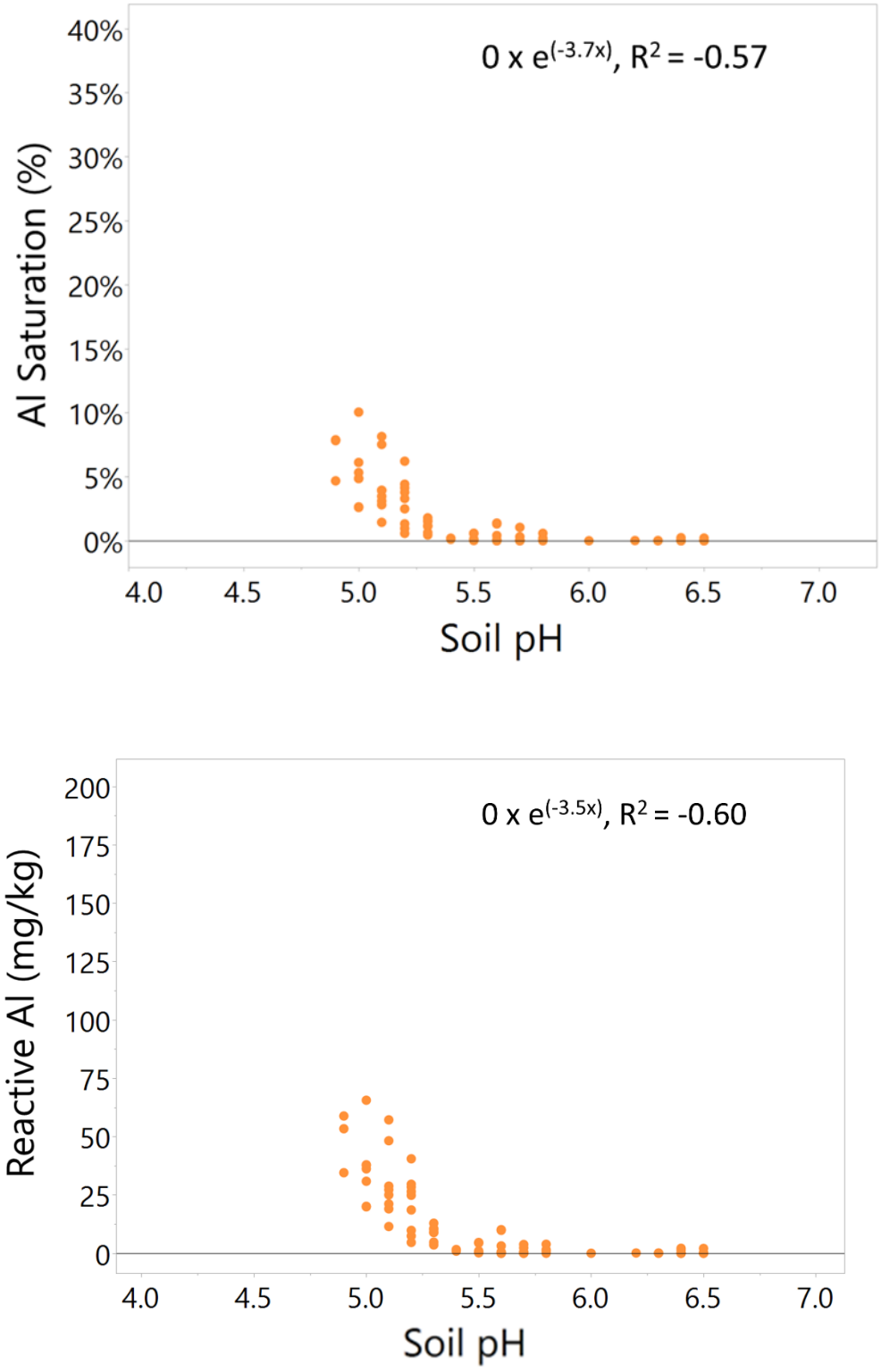

Figure 3. Relationship of soil pH between percent Al saturation (Al_{sat}) and exchangeable Al (reactive Al) for a Dale silt loam soil at Chickasha, OK (Year 1 and Year 2 combined). Chickasha was shown to lack a significant exponential relationship with Al concentrations.

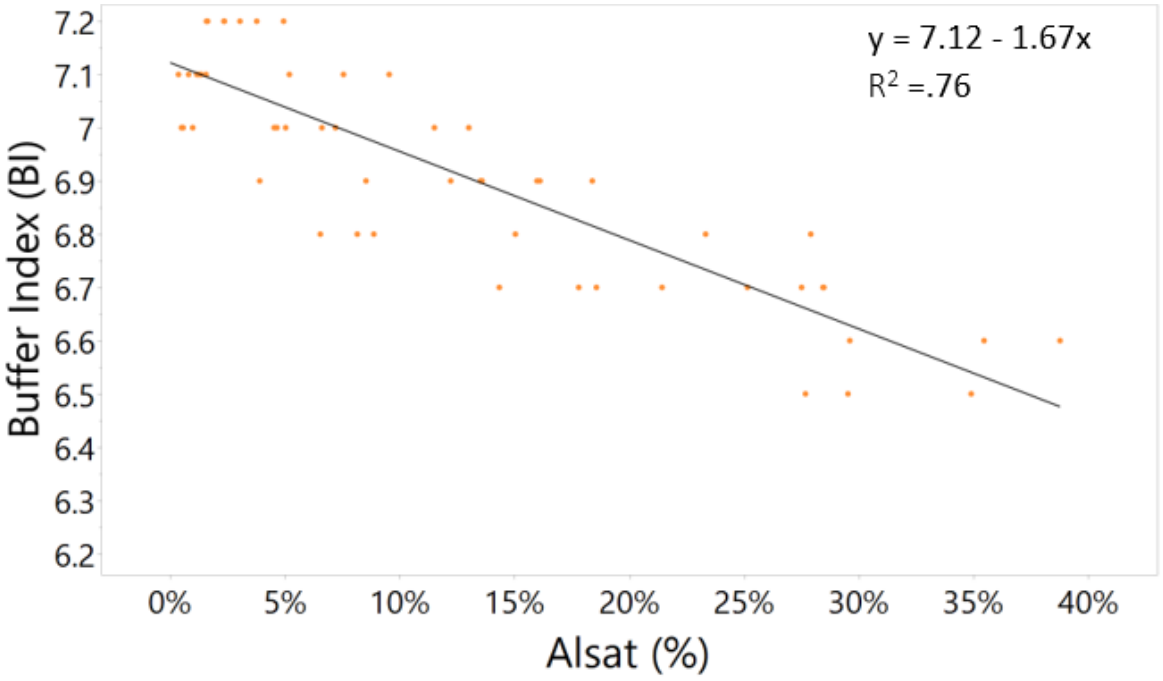

Figure 4. Linear relationship between buffer index (BI) and Al saturation (Al_{sat}) at Stillwater, OK (Year 1 and Year 2 combined).

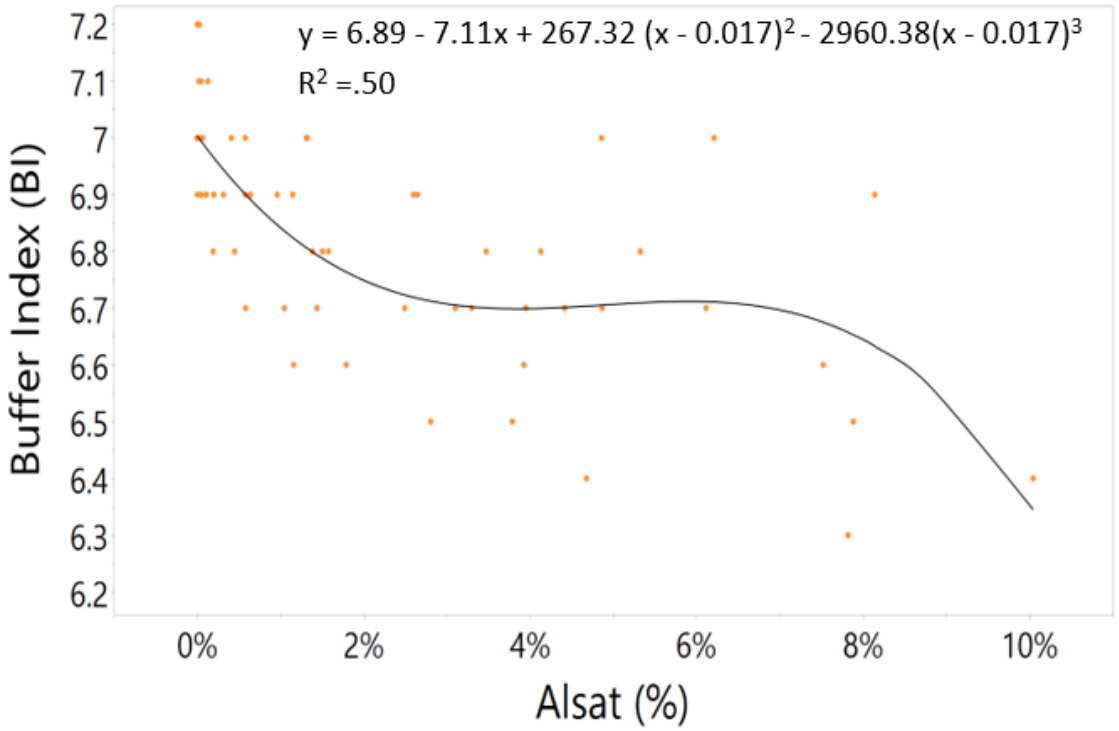

Figure 5. Cubic relationship between buffer index (BI) and Al saturation (Al_{sat}) at Chickasha, OK

(Year 1 and Year 2 combined).

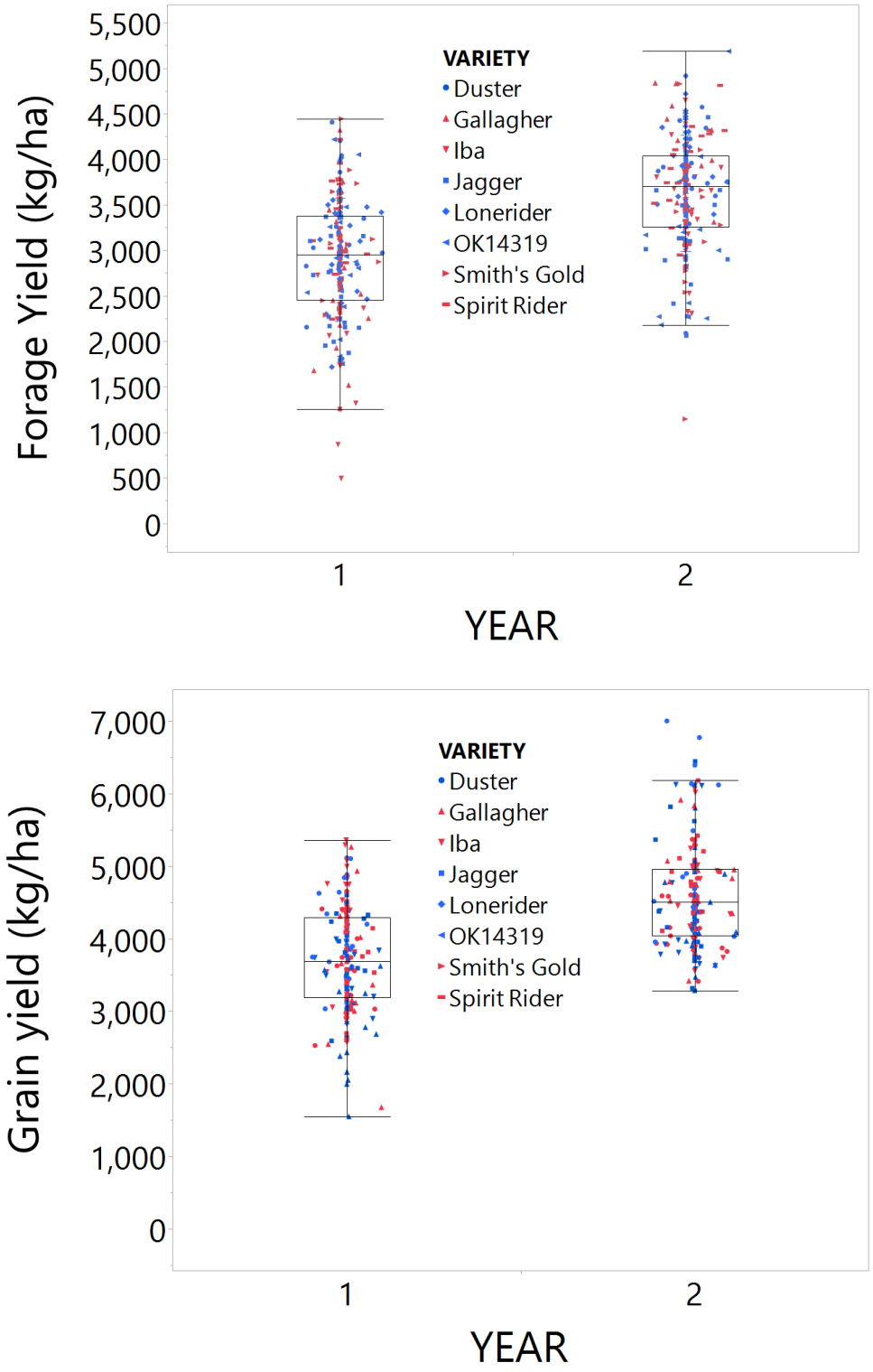

Figure 6. Boxplot comparison of forage yields and grain yields during Year 1 and Year 2 at Chickasha, OK. $p < .0001$. Blue = ALMT1(+) and Red = ALMT1(-).

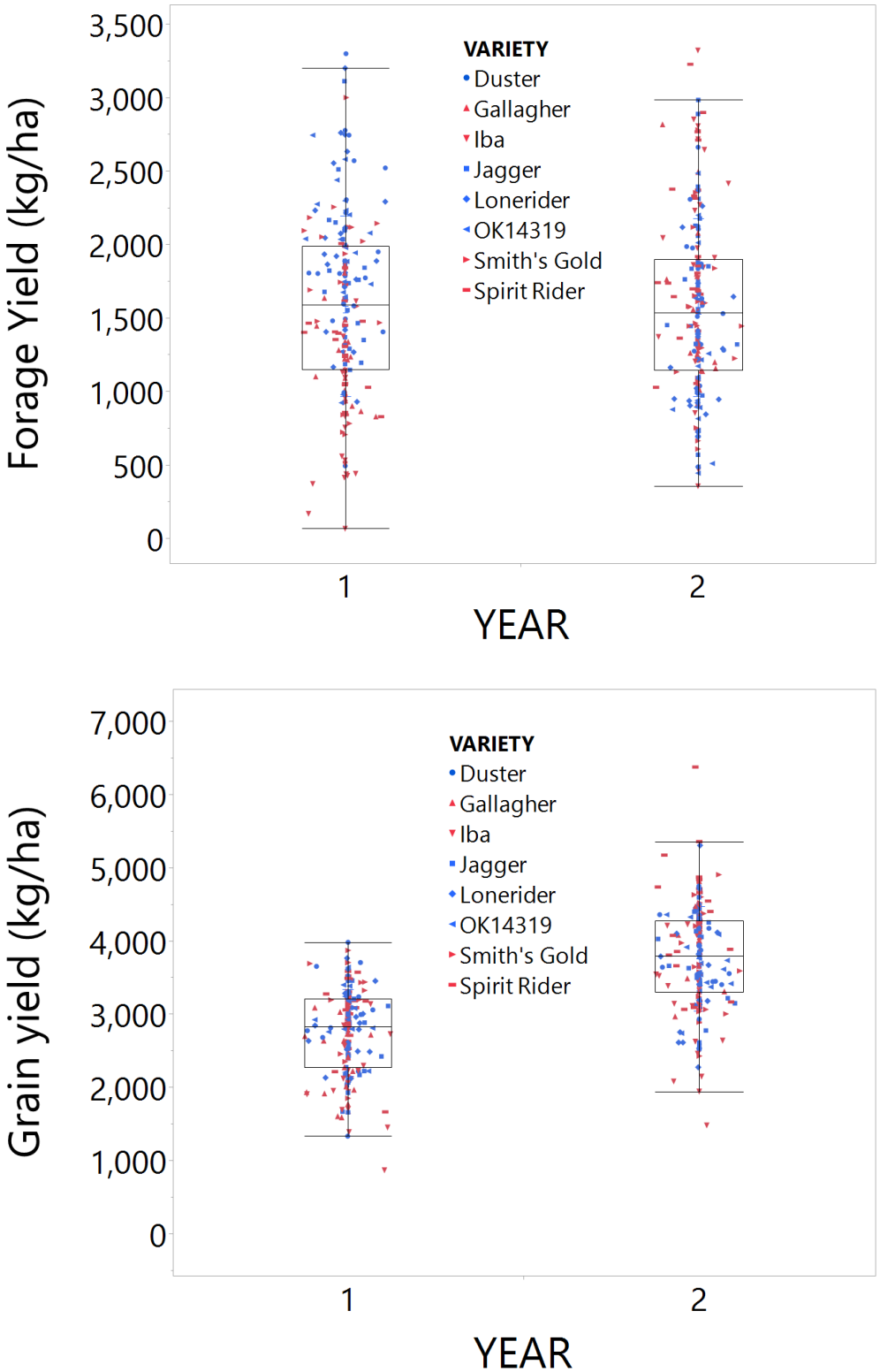

Figure 7. Boxplot comparison of forage yields (p = 0.86) and grain yields (p <.0001) during Year 1 and Year 2 at Stillwater, OK. Unequal variances for grain yield; Levene test (p = 0.0413). Blue = ALMT1(+) and Red = ALMT1(-).

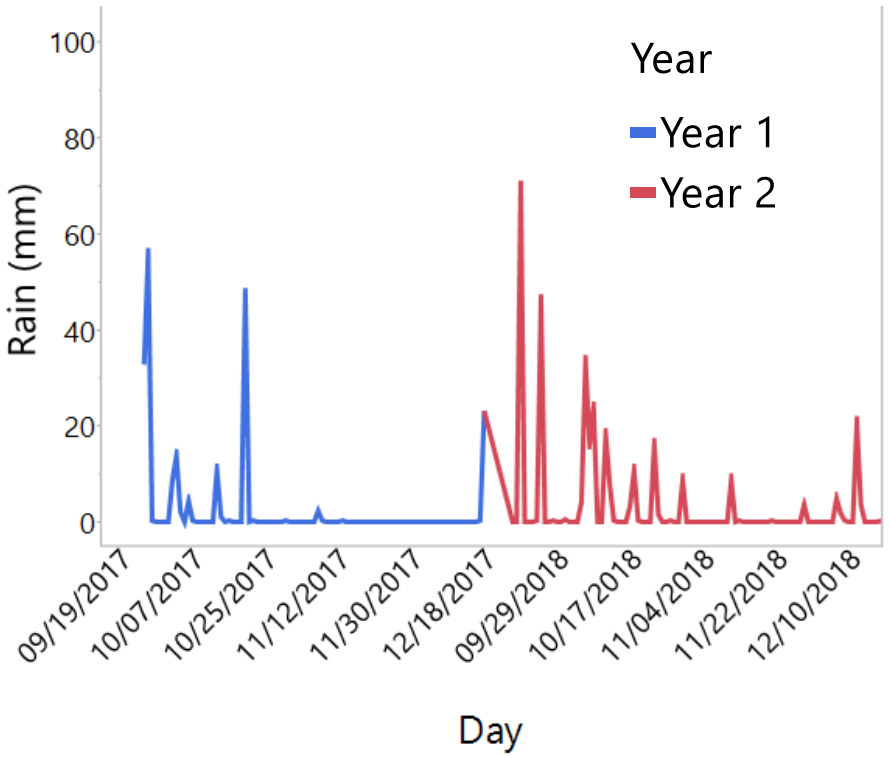

Figure 8. Rainfall frequency and amoiunt in Stillwater, OK (Year 1 and Year 2).

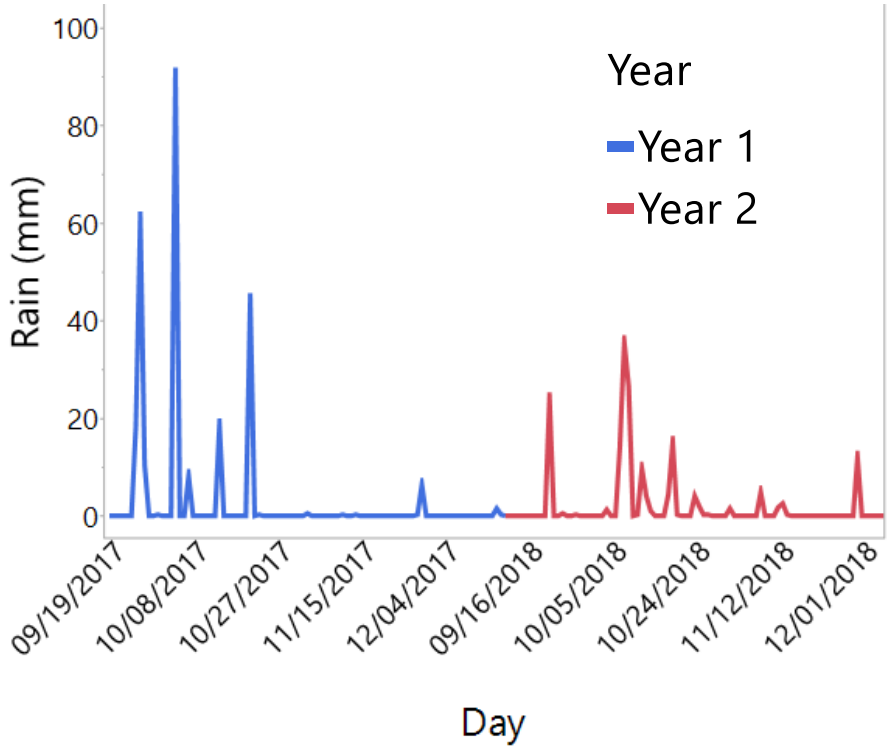

Figure 9. Rainfall frequency and amount in Chickasha, OK (Year 1 and Year 2).

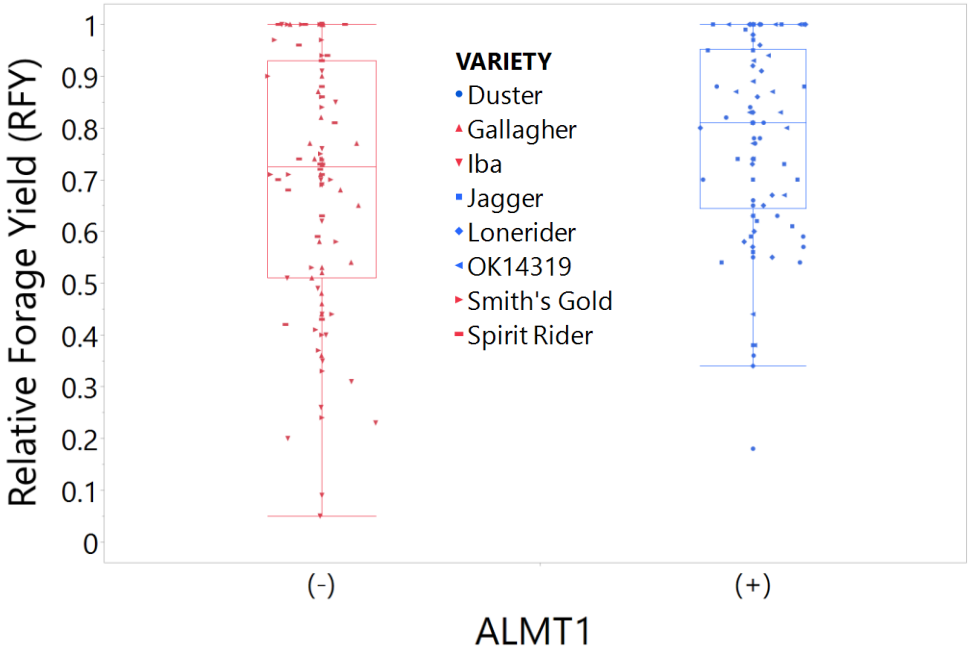

Figure 10. Boxplot comparison of relative forage yields (RFY) for genotype groups [ALMT1(+) and ALMT1(-)] during Year 1 at Stillwater, OK. p = 0.0103. Unequal variances; Levene test (p = 0.0093). Blue = ALMT1(+) and Red = ALMT1(-).

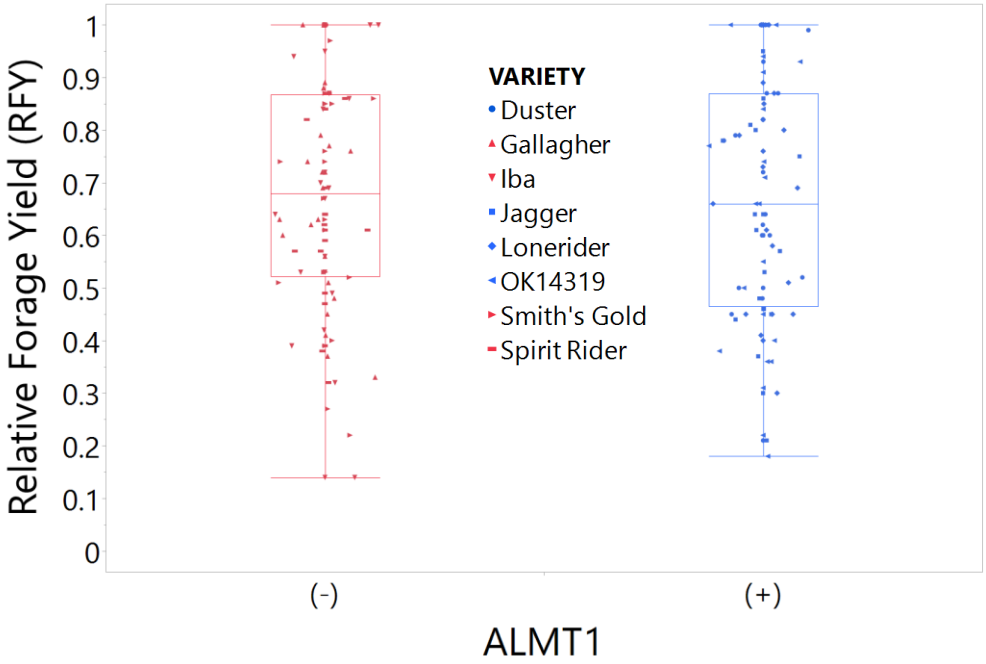

Figure 11. Boxplot comparison of relative forage yields (RFY) for genotype groups [ALMT1(+) and ALMT1(-)] during Year 2 at Stillwater, OK. p = 0.8469. Equal variances; Levene test (p = 0.8469). Blue = ALMT1(+) and Red = ALMT1(-).

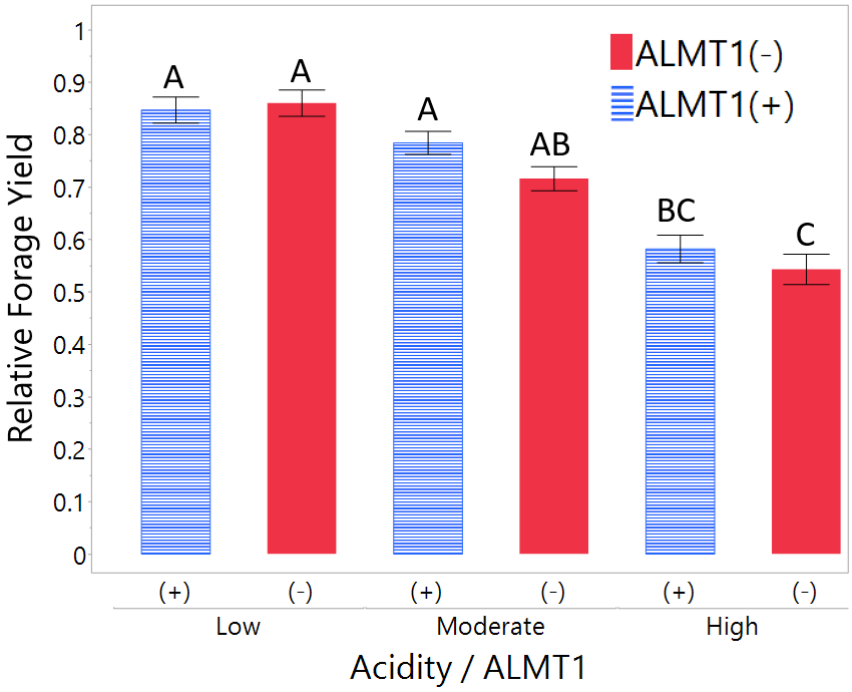

Figure 12. Effects of acidity and genotype group [ALMT1(+) and ALMT1(-)] on relative forage yield (RFY) in Year 1 at Stillwater, OK. Each error bar is constructed 1 standard error from the mean. Levels not connected by same letter are significantly different. Low: pH<5, Moderate: 5<pH<5.8, High: pH>5.8.

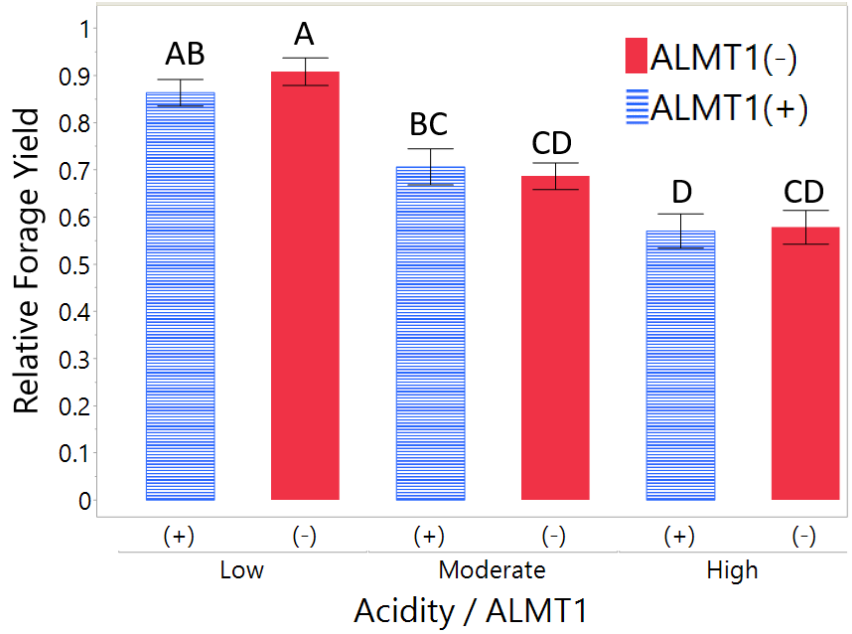

Figure 13. Effects of acidity and genotype group [ALMT1(+) and ALMT1(-)] on relative forage yield (RFY) in Year 2 at Stillwater, OK. Each error bar is constructed 1 standard error from the mean. Levels not connected by same letter are significantly different. Low: pH<5, Moderate: 5<pH<5.8, High: pH>5.8.

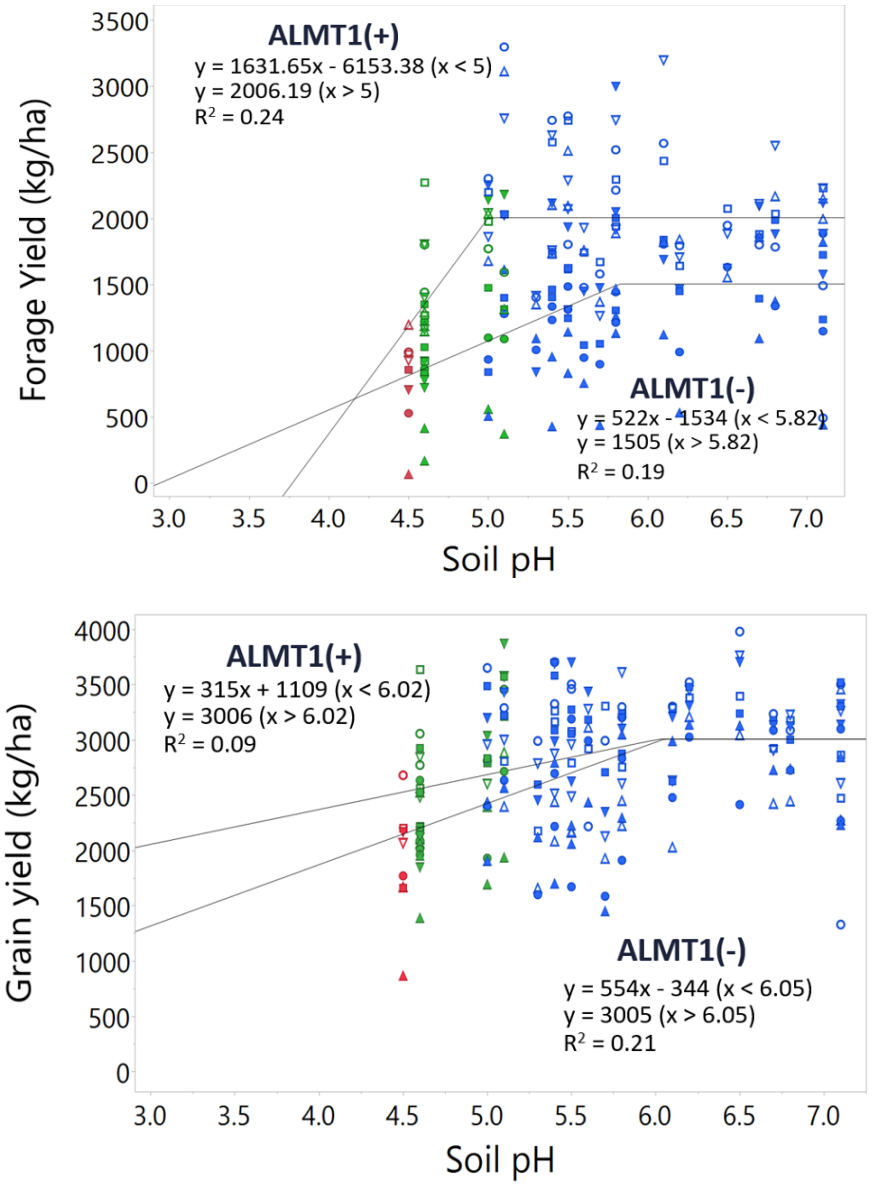

Figure 14. Effect of soil pH on the forage and grain yields of genotype groups in Year 1 at Stillwater, OK. $p < .0001$. Low = Al_{sat}<12%, Moderate = 12%<Al_{sat}<30%, High = Al_{sat}>30%.

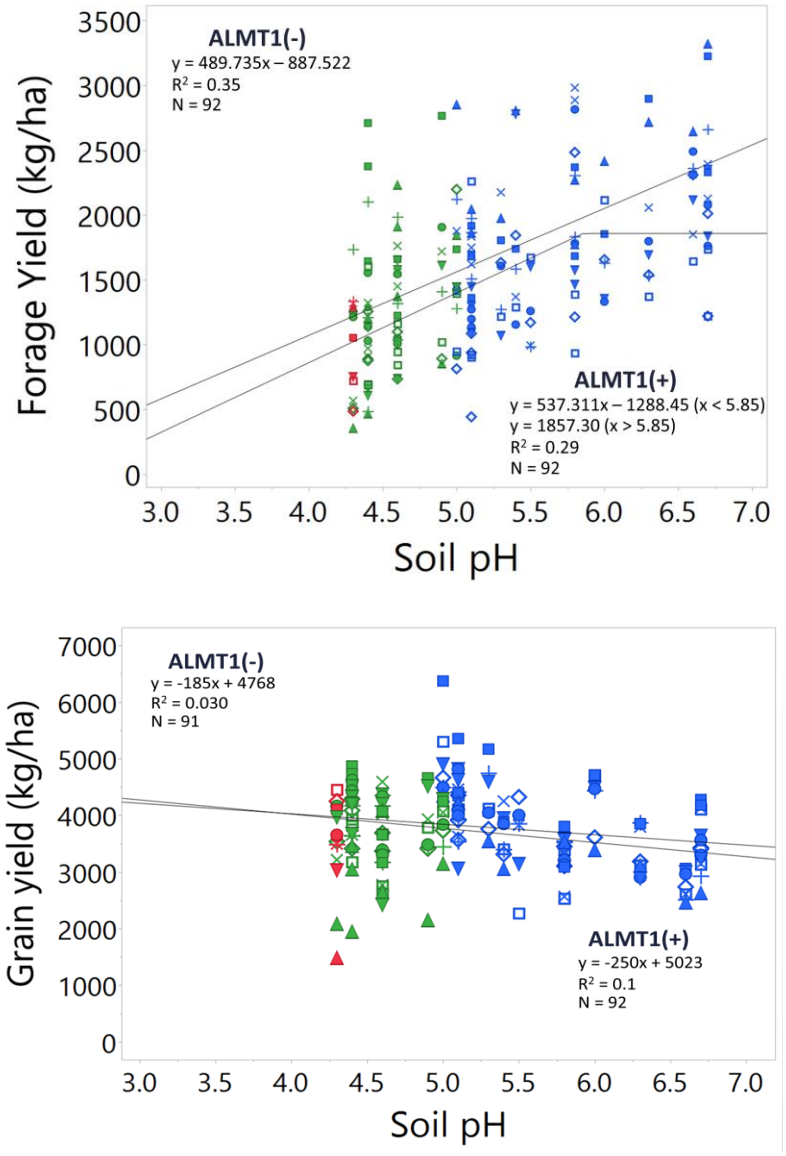

Figure 15. Effect of soil pH on the forage and grain yields of genotype groups in Year 2 at Stillwater, OK. Genotype group ALMT1(+) forage yields reached a point of no change (plateau) and was less tolerant ALMT1(-) genotype group. Similarly, Genotype group ALMT1(+) was less tolerant for grain yields. $p < .0001$; ALMT1(-) grain yield response, $p = .0991$. Low: Al_{sat}<12%, Moderate: 12%<Al_{sat}<30%, High: Al_{sat}>30%.

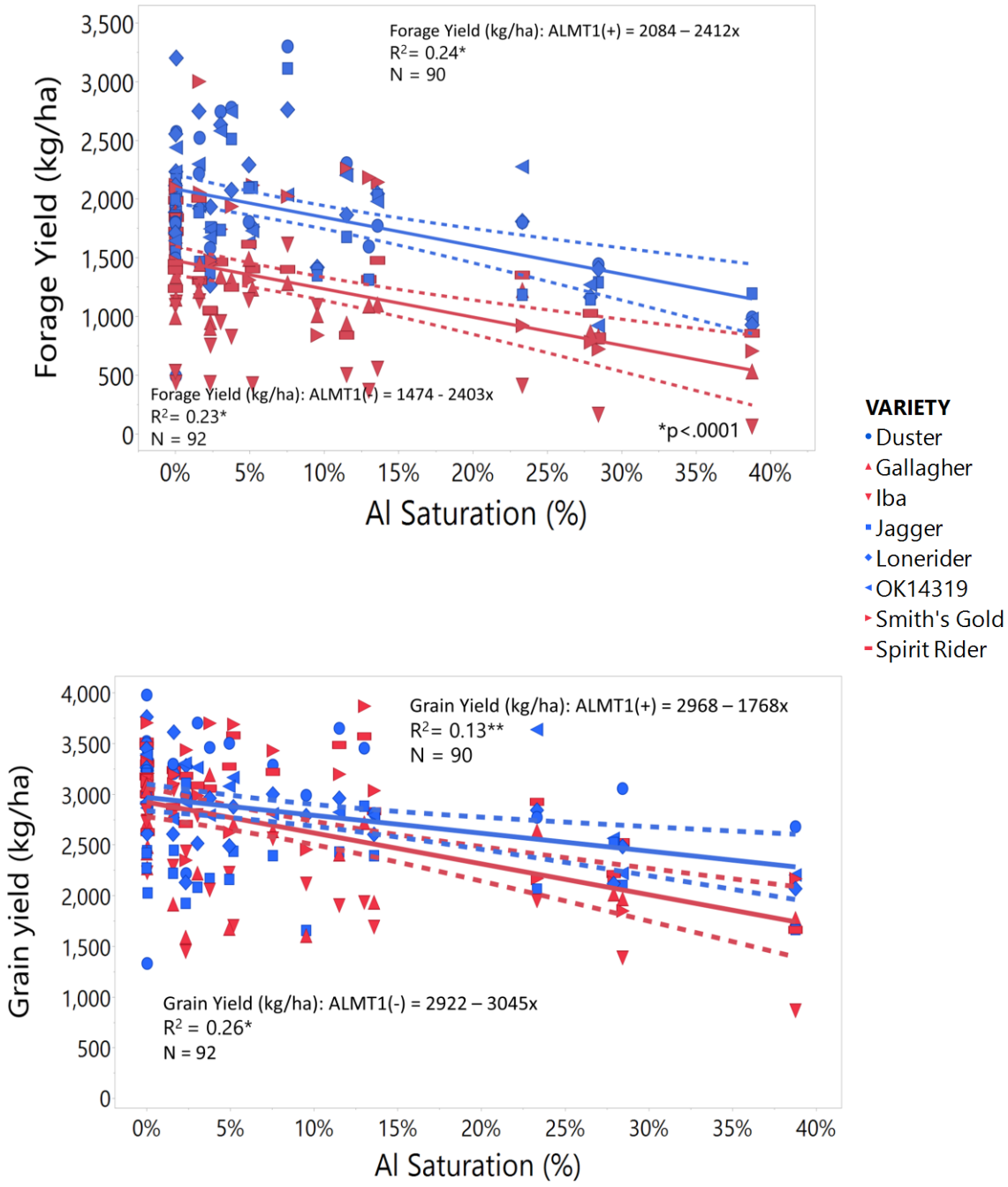

Figure 16. Effects of Al saturation on forage and grain yields of genotype groups in Year 1 at Stillwater, OK. ** = p = 0.0006, * = p < .0001. Blue = ALMT1(+) and Red = ALMT1(-).

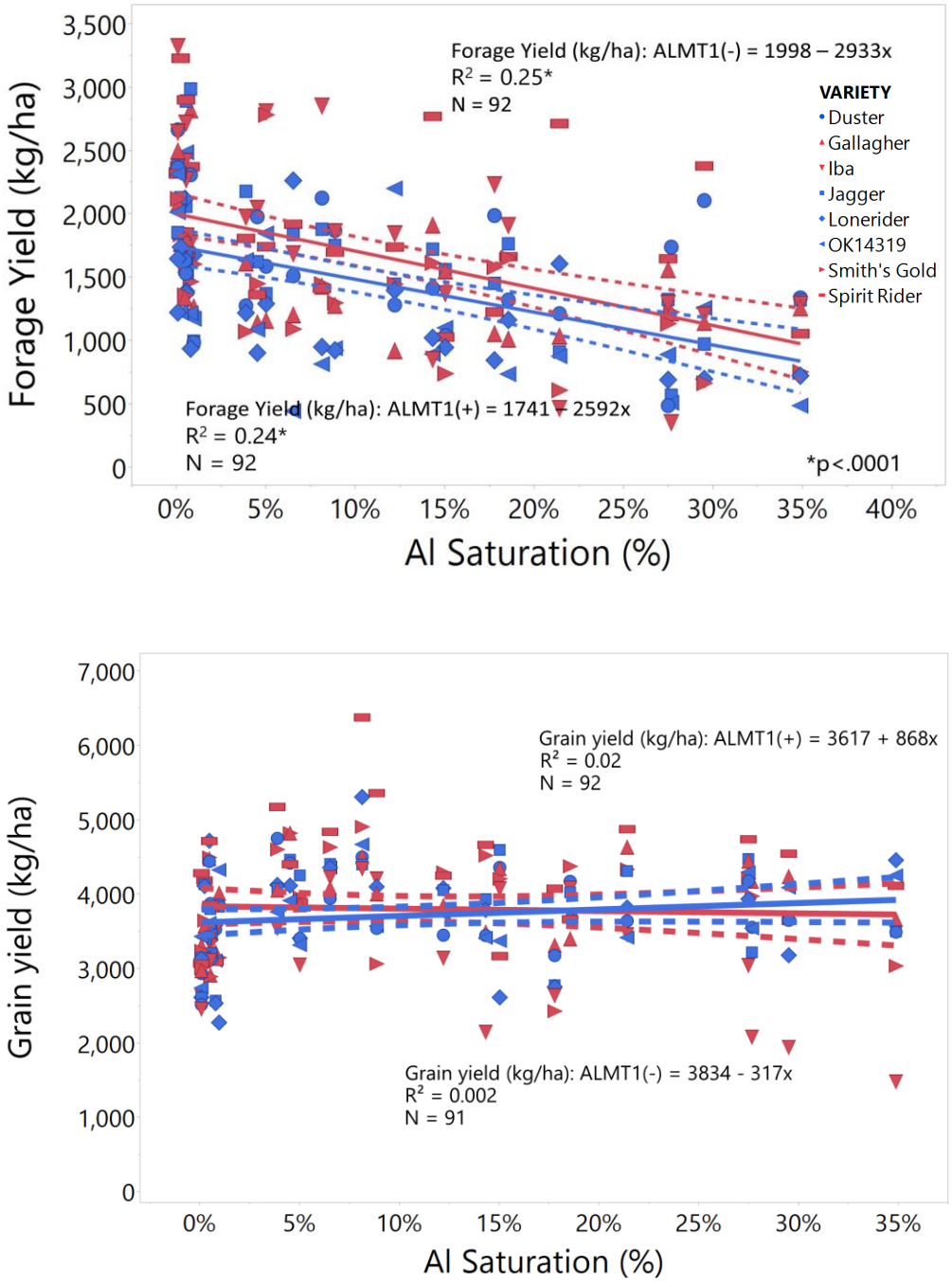

Figure 17. Effects of Al saturation on the forage and grain yields of genotype groups in Year 2 at Stillwater, OK. Blue = ALMT1(+) and Red = ALMT1(-). $p < .0001$; ALMT1(-) grain yield response, $p = 0.6934$; ALMT1(+) grain yield response, $p = 0.1410$.

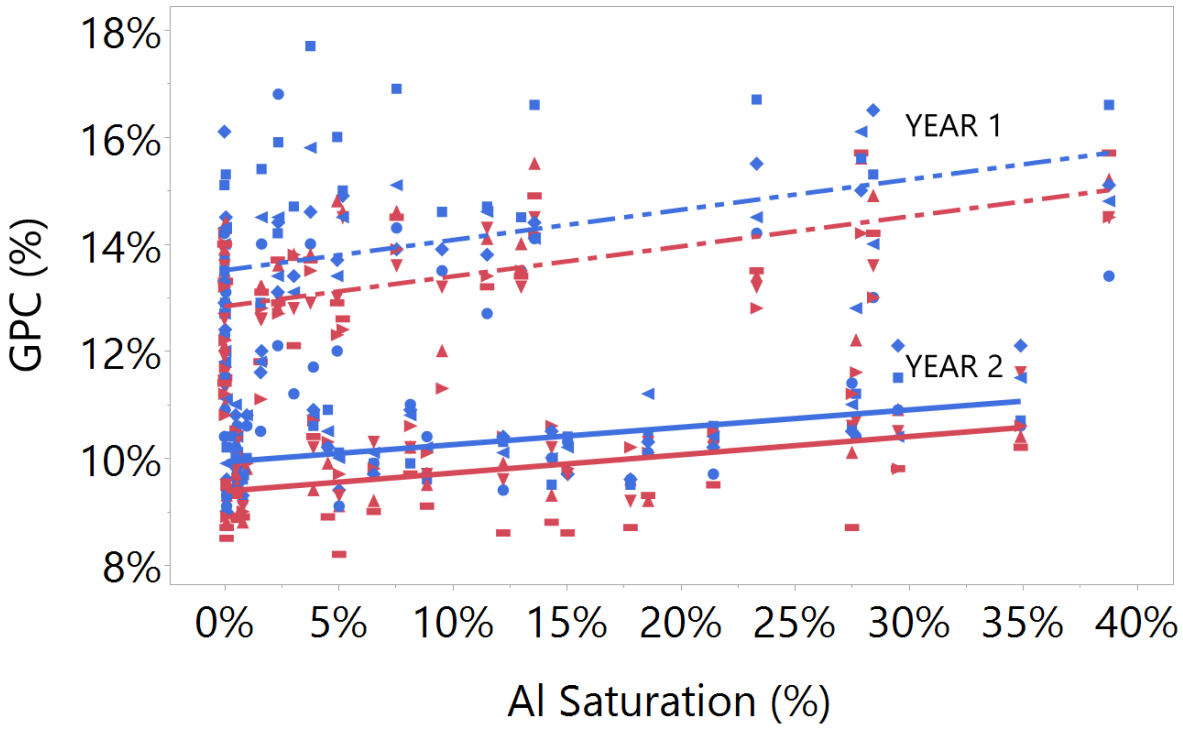

Figure 18. Positive linear relationship between Al saturation and grain protein concentration (GPC) for genotype groups ALMT1(-) and ALMT1(+) during Year 1 and Year 2 at Stillwater, OK. p <.0001. Blue = ALMT1(+) and Red = ALMT1(-).

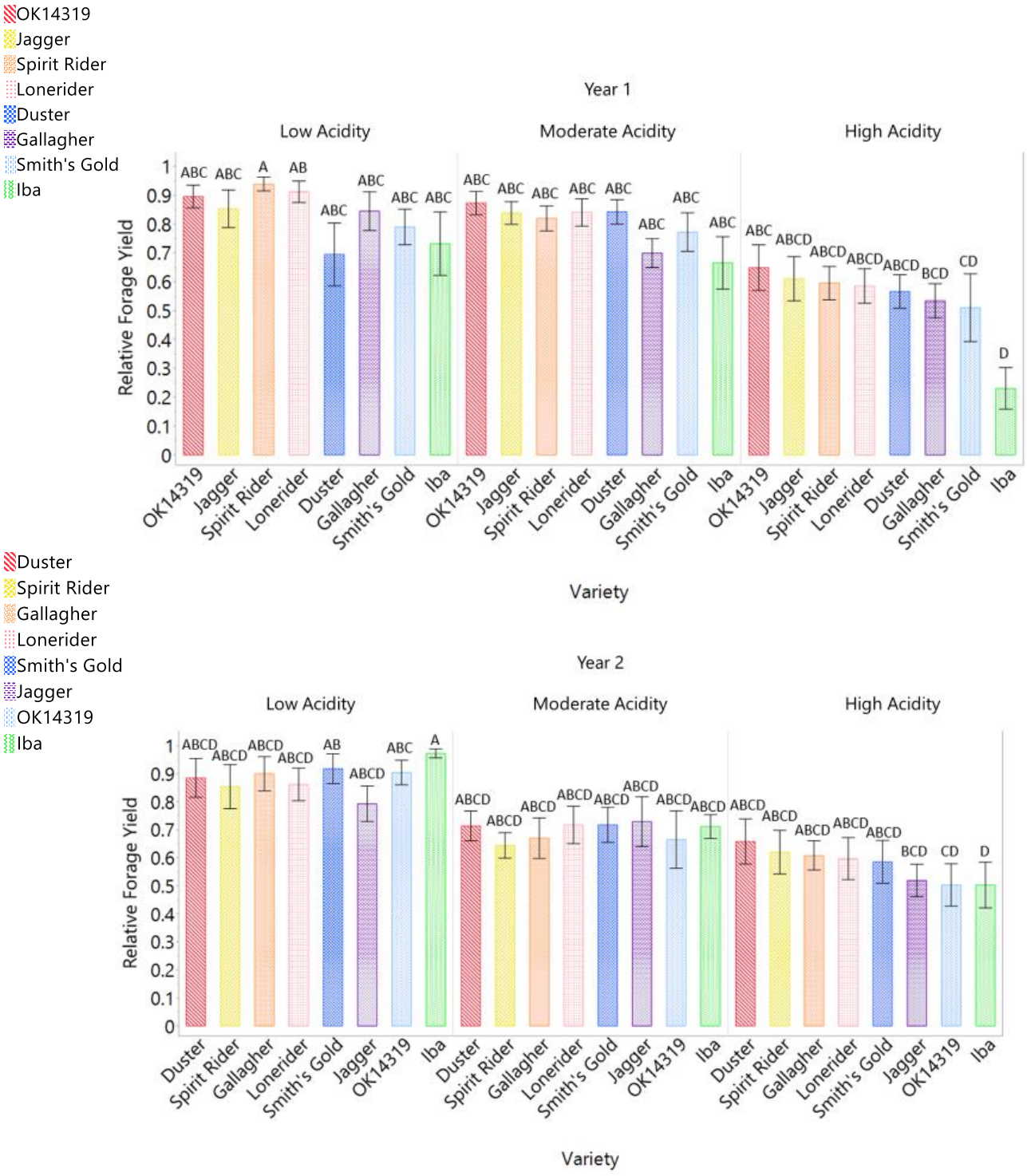

Figure 19. Effects of acidity and variety on relative forage yield in Year 1 and Year 2 at Stillwater, OK. Each error bar is constructed 1 standard error from the mean. Levels not connected by same letter are significantly different. Low: pH<5, Moderate: 5<pH<5.8, High: pH>5.8.

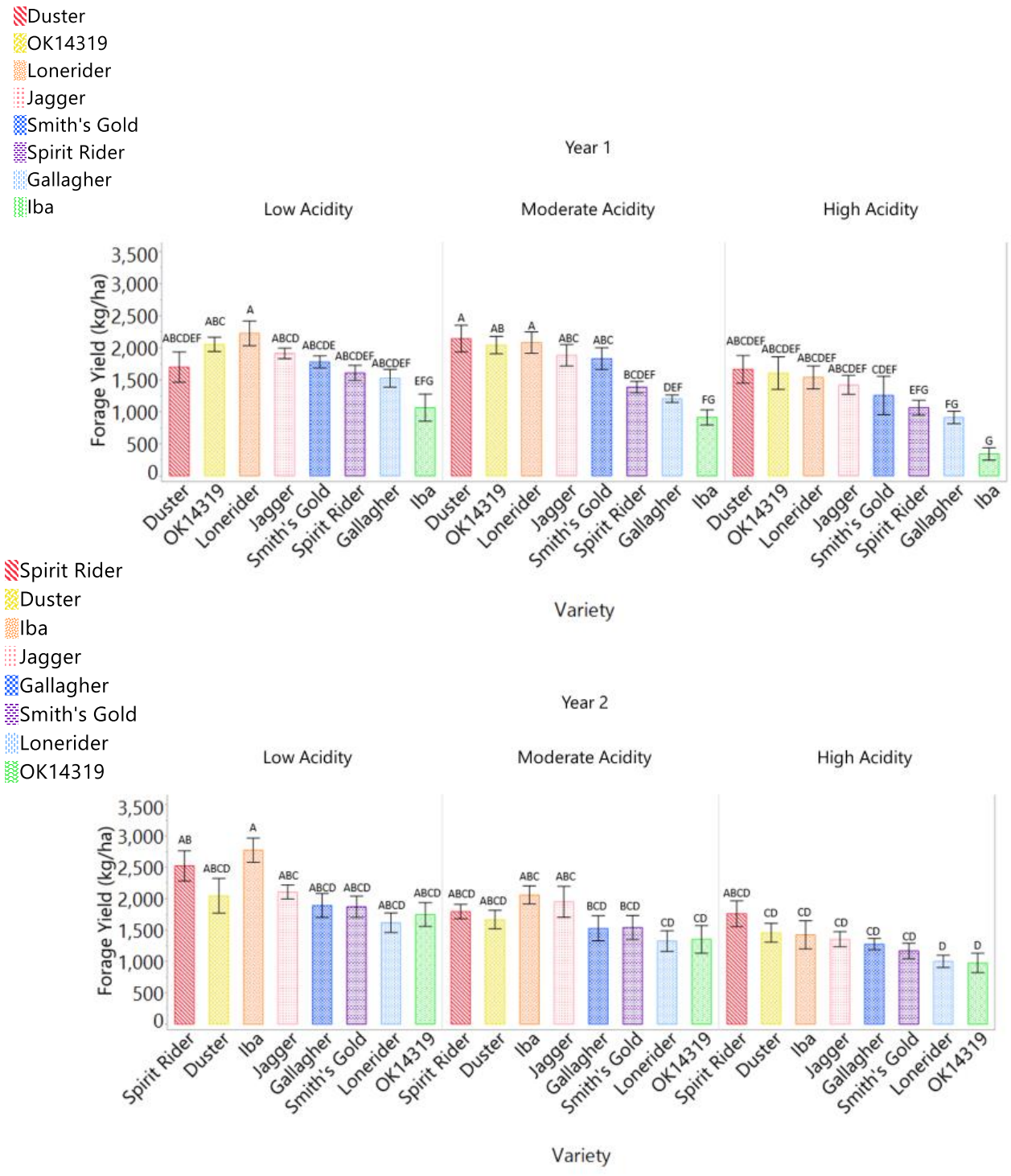

Figure 20. Effects of acidity and variety on forage yield (kg ha^{-1}) in Year 1 and Year 2 at Stillwater, OK. Each error bar is constructed 1 standard error from the mean. Levels not connected by same letter are significantly different. Low: pH<5, Moderate: 5<pH<5.8, High: pH>5.8.

VITA

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