COAXIUM® WINTER WHEAT VARIETAL TOLERANCE TO QUIZALOFOP-P-ETHYL IN THE SOUTHERN GREAT PLAINS

By

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Abstract: CoAXium® Wheat Production System offers postemergence control of many annual grass weeds. However, crop tolerance concerns have been raised since the technology's introduction in 2017. To evaluate the response of wheat cultivars that contain the AXigen® (AX) trait to quizalofop-P-ethyl (QPE), a field study was conducted at Perkins and Tipton, Oklahoma and Hays, Kansas. Two QPE treatments, 1X rate (92 g ai ha^{-1}) and 2X rate (185 g ai ha^{-1}) were applied to cultivars at three timings: fall (three to five-leaf wheat), early spring (first hollow stem), and late spring (second node detectable) along with nontreated control. A total of six CoAXium winter wheat cultivars were tested and the 2X rate was only applied in the 2021-2022 season. For the 2020-2021 season, the highest visual injury was observed on AP18 AX at Hays (26%), Perkins (50%), and Tipton (53%) among all tested winter wheat cultivars. An early spring timing reduced wheat grain yield by 8 to 9% at Perkins and 5 to 9% at Tipton as compared to nontreated and all other timings. Similarly, the late spring application of QPE at Hays reduced grain yield by 16%, 7%, and 9% compared to nontreated, fall, and early spring timings, respectively. For the 2021-2022 growing season, the fall application of QPE at 2X rate had the highest wheat biomass reduction at Perkins (35 to 67%) across all cultivars. Biomass reduction was not detected for Crescent AX. The late spring timing of QPE resulted in 12% biomass reduction for Helix AX. Furthermore, the fall application of QPE at 2X rate reduced wheat grain yield of AP18 AX by 71% at Perkins compared to all other cultivars, and QPE rates and timings. In contrast, the late spring application of QPE at 2X rate reduced wheat grain yield of Atomic AX by 53% at Hays as compared to all other cultivars, and QPE rates and timings. Altogether, these results suggest that cultivar selection, the QPE rate and application timing, as well as environment can impact tolerance ability of CoAXium® winter wheat to QPE herbicide and should be carefully considered prior to use of the technology.

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CHAPTER I

LITERATURE REVIEW

Winter Wheat in the Southern Great Plains

In the U.S., wheat (*Triticum aestivum* L.) ranks third behind corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] in planted hectares and production (USDA 2022a). Hard red winter wheat is the most common market class of wheat grown in the U.S.. Produced mainly in the southern Great Plains region, it accounts for 57% of wheat production in the United States. In the southern Great Plains, wheat is grown not only for grain production but also is utilized for forage and in some instances for forage and grain in the same growing season, known as dual-purpose wheat. A major benefit of the dual-purpose system is that two products are grown, cattle and grain, diversifying a producer's income.

Southern Plains states Oklahoma and Kansas are key players in winter wheat forage and/or grain production. Kansas ranks first in wheat production accounting for 18% of the country's total wheat (USDA 2022c). Oklahoma ranks fourth in wheat production in the United States and is the state's most valuable cash crop (USDA 2022b). The cool, wet winter months and dry, hot summers in the region make it suitable for winter wheat production. Wheat is also a winter hardy crop and is comparable to other forage crops in terms of protein content and digestibility (Maulana 2019). The majority of a producer's income in Oklahoma comes from beef cattle, with grain following behind in second (Koscelny 1996). In Oklahoma, more than 50% of the planted hectares of winter wheat may be utilized for a dual-purpose system, while the other half is grain only (Carver 2001). In contrast, Kansas is not as dependent on cattle grazing throughout the wheat growing season.

Winter wheat for dual-purpose systems is planted in early August or September while grain only wheat is often planted in October (Carver 2011). Although, early planted dual-purpose wheat increases vegetative growth for grazing, it also increases disease and insect pressure. Another factor dual-purpose growers must manage is grain yield. Delayed planting can increase grain yield by around 18% but decreases forage production by around 68% (Maulana 2019).

Dual-purpose systems provide away for growers in the southern Plains to diversify their income; however, because of the diversity offered in products, little crop rotation occurs on much of the land despite the economic and environmental issues that can arise from monoculture systems. One of the most critical is pest pressure, including those of winter annual grasses that adapt to the similar life cycle of winter wheat (Koscelny 1996). In addition to adaptation, overuse of the same herbicide active ingredients and/or herbicide sites of action selects for herbicide resistant weed biotypes. Several common crops that winter wheat can be rotated with in the summer are soybean, cotton (*Gossypium hirsutum* L.), sesame (*Sesamum indicum* L.), corn, and grain sorghum [*Sorghum bicolor* (L.) Moench *ssp. Bicolor*] in the southern Great Plains (Armstrong 2009). When crop rotation is not utilized and conventional herbicides are no longer effective, a land manager may invest in a herbicide resistant wheat system like Clearfield® or CoAXium®. Outside of conventional wheat, these two systems may provide growers with two additional herbicides to control weed species throughout the wheat growing season. However, weed species have already developed resistance to imazamox and quizalofop-P-ethyl, herbicides used with Clearfield® and CoAXium® systems, respectively.

Economically important winter annual grass weeds in southern Great Plains winter wheat include Italian ryegrass [*Lolium perenne* L. *ssp*. *Multiforum* (Lam.) Husnot], wild oat (*Avena fatua* L.), feral rye (*Secale cereale* L.), jointed goatgrass (*Aegilops cylindrica* Host), and several *Bromus* species (*spp*.). These species are problematic in winter wheat because they share a similar growth habit and life cycle, emerging from early fall to early spring. In addition, weeds such as *A. cylindrica*, *A. fatua*, *S. cereale*, *L. multiforum*, and *Bromus ssp.* are prone to evolving resistance due to their high reproduction rate, genetic diversity, and ability to outcross (Richter 2020). Although integration of multiple weed control practices is the best long-term weed management strategy, herbicide-based weed control is often the primary short-term practice.

Herbicide Resistance & Impact of Weeds

As a result of the intense use of herbicides worldwide, resistant weed biotypes have evolved due to high selection pressure and poor herbicide stewardship. Before commercial use of herbicides, mechanical weed control such as tillage was a common weed management practice. However, intensive tillage can lead to an acceleration of soil erosion as a result of the decomposition of soil organic matter and soil structure (Arshad 1999). Additionally, soil erosion impacts water quality as runoff occurs and settles in streams or bodies of water (Uri 1998). Soil practices also can lead to physical, chemical, and biological changes and influence soil properties. Major physical properties that change with tillage may include, bulk density, water holding capacity, pore size, and aggregation. Changes in chemical properties of the soil may impact microorganism populations (Mathew 2012). As a result of the impacts of conventional tillage, many producers in the region adopted conservational or no-tillage practices to improve soil health over time.

The driving factor of conventional tillage or no-tillage practices are an increase in water infiltration, which leads to a decrease in runoff and soil erosion. Additional benefits from the practice are an accumulation of organic matter and residue on the soil surface, which helps to hold moisture and lower soil temperature. As a result, may lead to higher profits and yields for producers. Also, biodiversity improves, specifically earthworms (Derpsch 2008). Overall, as a result of conservation or no-tillage practices, soil moisture, nutrients, and structure are conserved (Mathew 2012). After the introduction of herbicides, costs associated with tillage decreased, while increasing the efficiency of weed control, resulting in higher yields. Although it resulted in higher yields, overuse of herbicides and monoculture systems increased selection pressure on resistant biotypes (Gaines 2020).

Since the first herbicide resistant weed biotype was discovered in 1957 (Delye 2022), 513 unique cases of resistance have been documented worldwide (Heap 2022a). A total of 267 species are resistant to 21 of the 31 known herbicide sites of action. Seventy-one countries have reported herbicide resistant weeds in 95 different crops. In the United States, 123 resistant weed biotypes have been documented, which is the highest recorded number of resistant biotypes for a county worldwide (Heap 2022a).

Weeds in a grain crop can cause up to 90% yield reduction. As a result, over 26 billion dollars are lost every year by weed infestations in a grain crop throughout the United States (Delye 2013). Diversified management practices or integrated management practices are economically and environmentally effective strategies to lower selection pressure and battle the evolution of herbicide resistant weed biotypes over time. Herbicide options are limited for winter annual grasses in a winter wheat due to similarities in growth habit, emergence, and maturity. However, cultural management practices such as crop rotation can drastically reduce winter annual grass species populations by breaking up adaptation cycles. Other integrated management practices to increase crop competition and minimize weed seed banks in the soil may include planting date, seeding rate, and cultivar selection (Hildebrandt 2022).

In five of the seven states in the southern region of the United States, *L. multiforum* (Italian ryegrass) is one of the top ten problematic weeds in wheat (Koepke-Hill 2011). *L. multiforum* seed contaminating wheat grain can result in 11 to 19% dockage. As its plant density increases in a field, yield loss increases (Fast 2009). Fast et al. (2009) found that wheat yield in Oklahoma was reduced 16 to 46% when plant densities increased from 0 to 30 plants/m^2 and 100 plants/m^2, respectively. In Oklahoma, *L. multiforum* was first introduced as a forage crop, but shortly thereafter became a difficult to control weed throughout the state and the surrounding southern Great Plains region. Populations of *L. multiforum* have developed resistance to 11 of the

18 Weed Science Society of America (WSSA) herbicide site of action groups (Heap 2020b). Compared to other grass species, *L. multiforum* possesses a weak dormancy and short seed longevity, so intense management can lead to a rapid decrease of the seed bank population (Collavo 2016).

Another problematic weed in the region is true cheat, *Bromus secalinus* L.. Oklahoma wheat fields heavily infested with *B. secalinus* have produced wheat grain with dockage exceeding 40% (Justice 1993). When a field is completely infested, wheat yields can be reduced anywhere from 20 to 100% depending on *B. secalinus* plant density (Driver 1993). Fast et al. (2009) reported a yield reduction of 17% when plant densities were 8 plants/m^2. Complicating this issue, some *B. secalinus* populations in Oklahoma are cross resistant to acetolactate synthase (ALS) inhibiting herbicides (Group 2) making it difficult to control in Clearfield® wheat. This scenario has left quizalopfop-P-ethyl as the only effective herbicide option for *B. secalinus* control in CoAXium® wheat. Due to these limitations, cultural strategies should be considered if land must stay in winter wheat. For example, Justice et al. (1993) observed a wheat yield increase of 12% when row spacing decreased from 23 to 7.5 cm. Other cultural practices such as, crop rotation, cultivar selection, nutrient management, planting date adjustments, and seeding rate could be effective management strategies as well. A tillage or burndown application prior to a delayed planting date allows for control of some of the active weed seed bank. However, a delay in planting is not ideal for a dual-purpose wheat system since forage production is decreased as planting date is delayed.

Other problematic winter annual grasses in winter wheat include *S. cereale, A. fatua, and A. cylindrica*. In Oklahoma wheat fields infested with *S. cereale*, yield was reduced up to 69% when plant populations were 194 plants/m^2. Additionally, yield loss of 45 and 67% occurred when respective infestations of 21 and 50 plants/m^2 of *S. cereale*, occurred in wheat fields (Fast 2009). Fast et al. (2009) categorized *S. cereale* seed as foreign material in their study. Wheat grain and *S. cereale* seed are similar in size and shape, therefore its removal from grain is difficult and costly to mechanically remove. As a result, when 0.4% foreign material is present in wheat grain, its grade is drastically reduced, and has undesirable milling and baking quality (Fast 2009).

In the early 1970s, *A. fatua* was first introduced into Oklahoma from Texas and has since spread across the state of Oklahoma and southern Great Plains region. Since this weed's introduction it has become a problematic weed in winter wheat. *A. fatua* infested wheat fields resulted in a 22 and 28% reduction in yield when respective weed populations were 30 plants/m^2 and 32 plants/m^2. Increasing the wheat population density slightly during planting decreased the negative effects that *A. fatua* had on grain yield; however, as *A. fatua* plant densities increased, yield decreased (Fast 2009).

A. cylindrica, infests over one million hectares of wheat throughout the United States, specifically the states of New Mexico, Oregon, Utah, Washington, and Wyoming (Pacific Northwest region) and Colorado, Kansas, and Oklahoma (southern Great Plains). Fast et al. (2009) categorized *A. cylindrica* seed as dockage in wheat grain. As a result, a producer receives a price reduction when the wheat grain is contaminated with *A. cylindrica* spikelets. Additionally, *A. cylindrica* has a negative impact on wheat grain by reducing the test weight. Wheat yield loss increased 18% and 21% when *A. cylindrica* plant densities were 17 plants/m^2 and 170 plants/m^2, respectively (Fast 2009).

Conventional Breeding

Wheat is an allopolyploid with 3 distinctive genomes: A, B, and D. There are two major polyploid wheat types; *Triticum aestivum* L., a hexaploid bread wheat, which contains the three genomes, and the second type, *Triticum durum* L., a tetraploid pasta wheat, which contains only the A and B genomes (Uauy 2017). The large genome size, polyploidy nature, and presence of highly repetitive DNA have complicated the use of molecular tools in wheat cultivar development. However, with new technology, wheat breeders have the ability to fully sequence wheat cultivars (Bagge 2007).

There are several different breeding techniques and methods used to breed wheat cultivars. However, the technique or method used is dependent on different factors such as the resources available, personal preference of the breeder, and the genetics of the targeted trait (Mergoum 2009). There are few herbicide tolerant wheat cultivars due to economic, practical, and political reasons (Richter 2020). All commercial wheat cultivars are non-genetically modified organisms and are traditionally bred.

To develop herbicide tolerance in wheat cultivars, plant breeders may use a technique called mutagenesis, which is a form of traditional or conventional breeding. Mutagenesis is a process in which the genetic material is changed; however, this can occur naturally, by experimental procedures in a lab, or by artificial exposure to mutagens (Shu 2009). To generate mutations, breeders can use either ionized radiation, ultraviolet light, or chemical mutagens to reach their goals (Suprasanna 2015). Ethyl methane sulfonate (EMS) or diethyl sulfate are typically adopted to induce mutations through chemical mutagenesis (Mourad 2009). Traits that can be achieved through this technique are changes in the physical form and external structure of the plant, plant function, growth, metabolism, reproductive, chemical composition within the plant, disease resistance, and most recently herbicide tolerance in wheat (Mergoum 2009). The use of this technology to induce mutations has successfully resulted in the release of over 2,700 plant cultivars with mutant traits. However, a large portion of these cultivars belong to ornamental, tuber, cereal, pulse, oil, and root crop species (Kharkwal 2009). Mutagenesis is usually paired with another technique or method to achieve different traits at one time (Mergoum 2009).

Plant breeders now have the ability to successfully use molecular markers to monitor and select for traits otherwise difficult to select conventionally. Marker-assisted selection (MAS) may be paired with other techniques such as mutagenesis (Gupta 1999). The use of said molecular markers, which are typically developed for disease resistance and agronomic traits, speeds up the selection and breeding process (Maulana 2019). Molecular markers may be used to guide selection (MAS) and to characterize germplasm in breeding populations, or to drive genetic evolution studies (Gupta 1999).

Clearfield® Technology

In 2001, the first herbicide tolerant wheat system, Clearfield®, was conventionally created by American Cyanamid, but was later transferred to BASF®. The technology was developed to support declining wheat production caused by herbicide resistant weed biotypes. The technology allows the use of PRE or POST applications of imazamox (Beyond® herbicide) for control and suppression of grass weeds, sedges, and broadleaf weeds. Problematic grass weed species in the southern Great Plains include rescuegrass (*Bromus catharticus* Vahl), *B. secalinus, L. multiforum*, *A. cylindrica*, and *S. cereale*, as well as many broadleaves. Imazamox requires uptake of the herbicide through foliage or roots for rapid translocation, providing PRE and/or POST control of many weeds. After an application of imazamox, susceptible plants may exhibit symptoms of yellowing, purpling, stunted growth, and either die or do not compete with the crop. Imazamox is a WSSA Group 2 herbicide that inhibits the acetolactate synthase (ALS) enzyme. The ALS enzyme catalyzes biosynthesis of branched-chain fatty acids, isoleucine, leucine, and valine (Anonymous 2019).

Tolerance for Clearfield® cultivars was first conferred with a single mutation in hexaploid wheat (Grey 2012). When the mutation occurs in only one genome, tolerance is limited, and stress from herbicide application and the environment causes a decrease in the recovery time of the crop. There remain only a few cultivars that confer tolerance with a single gene, which lack a robust level of tolerance at higher herbicide rates of imazamox. Factors such as expression of the gene, physiological state of the plant at time of application, and other related factors can have an effect on the tolerance to imazamox application of wheat (Grey 2012).

Due to continued need for improved technology, most contemporary Clearfield® wheat cultivars confer imazamox tolerance with two genes as part of the Clearfield® Plus Production System. This 2-gene tolerance allows for the addition of a methylated seed oil with imazamox in a tank mixture to increase the effectiveness of the herbicide and provide additional crop safety. The addition of methylated seed soil in mixture with imazamox increases the efficacy on several weed species, including *S. cereale*. Two-gene tolerance allows the cultivar to metabolize the herbicide quicker, resulting in less crop injury.

Before the introduction of Clearfield® technology in wheat, WSSA Group 2 or ALS inhibiting herbicides were readily available and used in other crops as well as wheat. However, across the United States, resistant weed biotypes were documented even before the new technology was available. In the first ten years after the technology was commercialized, several problematic weeds in winter wheat in the southern Great Plains were documented with resistance to imazamox: bushy wallflower (*Erysimum repandum* L.), flixweed [*Descurainia sophia* (L.) Webb ex Prantl], *B. japonicus*, and *B. secalinus* (Heap 2020c). Before resistant weed biotypes dominate fields, small populations often go undetected until they compromise 30% of the field's total plant population. Contamination of equipment or seed, as well as a lack of integrated weed management strategies contribute to the resistant weed biotypes that go undetected in fields (Rainbolt 2004a).

CoAXium® Technology

As herbicide resistant weed biotypes continue to evolve in winter annual grass species in wheat, a need for alternative control options arose. In 2017, a new technology called CoAXium® Wheat Production Systems, was co-launched by Albaugh® LLC, the Colorado Wheat Research Foundation, and Limagrain Cereals Seeds. The technology utilizes WSSA Group 1 herbicide, quizalofop-P-ethyl (QPE), trade name Aggressor®. Quizalofop-P-ethyl is not a new herbicide; however, its use for in-season grass weed control in wheat is new. In susceptible plants, the herbicide inhibits the acetyl CoA carboxylase (ACCase) enzyme which catalyzes lipid biosynthesis. The ACCase enzyme is responsible for the synthesis of fatty acids and the production of phospholipids, critical to cell membrane structure and function (Underwood 2016).

Quizalofop-P-ethyl is translocated in the phloem and xylem; however, translocation is slow (Anonymous 2021).

The patented trait, which confers tolerance to QPE, is referred to as AXigen®. Tolerance provided by the AXigen® trait was achieved through mutagenesis, which consisted of using EMS to induce a DNA substitute of cytosine to thymine at the 2004 location. As a result, this caused an amino acid substitution of alanine to valine in the ACCase enzyme, which makes the herbicide unable to bind, conferring tolerance. Three homoeologous loci critical to ACCase enzyme synthesis were targeted in mutagenesis. Each locus has two alleles per genome, the mutant allele and the wild-type or native allele. Two-gene herbicide tolerance in HRW wheat is currently conferred by homozygous resistance alleles present in the A and D or in the B and D genomes (Uauy 2017). Two genes provide higher tolerance levels than that of a single-gene system and should result in little to no crop injury or yield loss, whereas single-mutation lines (not commercialized) are more susceptible to crop injury and yield loss (Hildebrandt 2022). The single A genome mutation confers a higher level of tolerance to QPE compared to the B genome, whereas the single B genome mutation confers lower tolerance than the D genome (Ostlie 2015). Therefore, the paired AD mutations confer a higher tolerance than the BD mutations (Richter 2020).

The new technology allows the POST application of QPE herbicide on wheat containing the AXigen® trait. Quizalofop-P-ethyl can be applied to wheat after the four-leaf stage but before jointing, providing an additional option to control weedy annual and perennial grass species such as *S. cereale*, *A. cylindrica*, *Bromus spp*. such as *B. catharticus* and *B. secalinus*, and several other grass species (Anonymous 2021). The CoAXium® wheat systems result in 90% or greater control of susceptible *S. cereale* when QPE is applied at the recommended label rate and timing (Kumar 2021). The label states that to achieve best results, weeds should be four- to five-leaf and actively growing at time of application. Crop injury symptoms include stunting or slight yellowing caused by stress from the environment or interplant competition between the crop and weeds. However, to avoid crop injury on CoAXium® cultivars, herbicide application is not advised under cold ambient temperatures or minimum of 0 ºC predicted 5 days prior to or after application (Anonymous 2021). Bough et al. (2022) discovered that metabolism of QPE herbicide in plants in a growth chamber is delayed at a cooler temperature regime compared to a warmer temperature regime; however, above ground shoot biomass was not affected. Still, efficacy of weeds can be affected due to cooler temperatures that result in a delay in metabolism.

To prolong the use of the CoAXium® system and Aggressor® herbicide, producers are required to follow a stewardship agreement designed to delay the evolution of resistant weed biotypes. This stewardship agreement prolongs the use of the system and herbicide by requiring crop rotation, planting of certified CoAXium® seed, following the Aggressor® label for labeled rates and application timings, and implementing an integrated weed management system (Anonymous 2021). As a result, the CoAXium® wheat system should be used as a short-term tool to help manage problematic weeds.

Currently, there are 19 wheat cultivars commercially available throughout the United States that contain the AXigen® trait where QPE herbicide can be applied POST. Of these 19 cultivars, 10 are hard red winter and suitable for the southern Great Plains region. Since the introduction of CoAXium® wheat in 2017, planted hectares have become more than doubled in Oklahoma. This increase in planted hectares of CoAXium® wheat in the state was followed by complaints regarding crop tolerance. To better evaluate crop tolerance and management factors which may influence tolerance, field experiments were conducted in Oklahoma and Kansas during the 2020-21 and 2021-22 winter wheat growing seasons. My specific objective was to evaluate how the genotype relative to the AXigen® trait, QPE application timing, and QPE rate might affect herbicide tolerance across environments, either as main effects or as interactions. It was expected that some winter wheat cultivars containing the AXigen® trait would be more sensitive than others following QPE herbicide applications. Understanding cultivar tolerance to QPE is crucial as hectares planted to the trait have increased each season since the introduction of the technology. Additionally, as wheat breeders across the United States breed the AXigen® trait into local germplasm, it is critical that their releases have robust tolerance to QPE.

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CHAPTER II

COAXIUM® WINTER WHEAT VARIETAL TOLERANCE TO QUIZALOFOP-P-ETHYL IN THE SOUTHERN GREAT PLAINS

Introduction

Wheat (*Triticum aestivum* L.) ranks third behind corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] in planted and harvested hectares in the United States, resulting in a total of 18.9 million planted and 15.1 million harvest hectares for the 2021-2022 growing season (USDA 2022a). In the United States, hard red winter wheat is the most commonly grown variation of wheat, accounting for 57% of wheat production in the southern Great Plains. In this region, the system is unique in that cattle can be incorporated, and the producer can still harvest the crop for grain after grazing throughout the winter months. This is referred to as a dual-purpose system, resulting in two products for producers.

In the southern Great Plains region, Oklahoma and Kansas are crucial players in the production of wheat for grain and forage. However, the utilization of cattle in the system is more prevalent in Oklahoma as compared to Kansas. In Oklahoma, about 50% of the state's wheat production is utilized for a dual-purpose system, while the other half is grain only (Carver 2001). The weather in the region during the winter months of cool, wet conditions and hot, dry during the summer months makes it suitable for wheat production (Maulana 2019).

Due to the diversity that a winter wheat crop provides in the southern Great Plains, many producers in the region are continuous wheat growers, resulting in a monoculture system with little crop rotation. Lack of crop rotation leads to an increase in pest pressure, including winter annual grasses that adapt to life cycles similar to wheat (Koscelny 1996). Additionally, selection for herbicide resistant weed biotypes is a result of overuse of the same herbicide active ingredient or site of action (Rainbolt 2004). When conventional herbicides are no longer effective and crop rotations are no longer utilized, producers do have the option of investing in a herbicide tolerant wheat system such as Clearfield® or CoAXium®. Outside of conventional herbicides, the two systems provide an additional control option for troublesome weeds in season. However, the two systems should only serve as a short-term practice as an integration of multiple weed control practices is the best long-term management strategy (Rainbolt 2004).

Clearfield®, the first herbicide tolerant wheat system, was developed to support declining wheat production caused by herbicide resistant weed biotypes. In 2001, BASF conventionally bred the new technology, Clearfield® or Clearfield® Plus Wheat System. This technology allows the herbicide, imazamox, trade name Beyond®, to be applied PRE and/or POST for control or suppression of grassy weeds, sedges, and broadleaf weeds. Imazamox is a WSSA Group 2 herbicide which inhibits the acetolactate synthase (ALS) enzyme. However, before the introduction of Clearfield® technology in wheat, group 2 herbicides were readily available and used in wheat as well as other crops (Anonymous 2019), resulting in selection pressure for resistant biotypes long before Clearfield® wheat was commercialized.

The second herbicide tolerant wheat system was developed to tackle ALS- inhibiting herbicide resistant grass weed biotypes. The new technology, CoAXium® Wheat Production Systems, was co-launched in 2017 by Albaugh® LLC, the Colorado Wheat Research Foundation, and Limagrain Cereals Seeds. The technology allows the use ofquizalofop-P-ethyl (QPE), trade name Aggressor®, to be applied POST to AXigen® wheat cultivars to control troublesome winter annual grass species. Quizalofop-P-ethyl is a WSSA Group 1 herbicide that inhibits the acetyl CoA carboxylase (ACCase) enzyme which catalyzes lipid biosynthesis in susceptible plants (Anonymous 2021). The patented trait, AXigen®, confers tolerance to QPE through the breeding technique of mutagenesis with ethyl methane sulfonate (EMS). This technique of mutagenesis, traditional or conventional breeding, induces a DNA substitution of cytosine to thymine at the 2004 location. As a result, an amino acid substitution of alanine to valine in the ACCase enzyme occurs, conferring tolerance and making the herbicide unable to bind to the site. Use of this technique results in wheat cultivars that are traditionally or conventionally bred and aren't identified as genetically modified organisms (Richter 2020).

Although the new technology is an additional option to clean up fields infested with grass weeds, stakeholders in the state of Oklahoma started to notice crop tolerance issues in field as planted hectares to the AXigen® trait increased. To evaluate these crop tolerance concerns, field experiments were conducted in Oklahoma and Kansas during the 2020-2021 and 2021-2022 winter wheat growing seasons to better understand the herbicide tolerance of six winter wheat cultivars containing the AXigen® trait to QPE herbicide. A second objective was to evaluate herbicide tolerance of wheat cultivars containing the AXigen® trait to QPE application timings and rates. Understanding the cultivar tolerance to QPE is crucial as the planted hectares to the trait have increased each season since the technology was released. Additionally, as wheat breeders across the United States breed the AXigen® trait into local germplasms, it is critical that releases have robust tolerance to QPE.

Materials and Methods

Field experiments were conducted at Tipton (34º26'22.7"N 99º08'01.3"W; elevation of 394 m) and Perkins (35º59'16.8"N 97º02'54.1"W; elevation of 279 m), Oklahoma and Hays (38º51'11.7"N 99º19'34.4"W; elevation of 616 m), Kansas during the 2020 to 2021 and 2021 to 2022 winter wheat growing seasons. Field growing seasons are referred to the year grain harvest occurred. At the Perkins site, the soil texture was a Teller loam (fine-loamy, mixed, active, thermic Udic Argiustolls) with an average pH of 5.9 and organic matter (OM) percentage of 0.65%. At Tipton, soil texture was a Tipton loam (Fine-loamy, mixed, superactive, thermic Pachic Argiustolls) with a pH average of 5.2 and organic matter (OM) percentage of 0.9%. The

Hays site was a Roxbury silt loam (fine-silty, mixed, superactive, mesic Cumulic Haplustolls) with an average pH of 7.8 and 2.1% OM.

Wheat was drilled at a rate of 67 kg ha^{-1} using a grain drill with 18 cm row spacing at both Oklahoma sites. At the Kansas site, wheat was drilled at the same seeding rate using a 19 cm row spacing. Studies during the 2021 season were designed as a two-way factorial (wheat cultivar x application timing) and arranged in a randomized complete block design with four replications. For the 2022 season, an additional QPE rate was added resulting in a new design of a three-way factorial (wheat cultivar, application timing, and QPE rate) arranged in a randomized complete block design with four replications. Individual plots at each site were 1.2 or 3 m wide by 12.2 or 9.1 m in length. Information on wheat cultivar, planting date, herbicide application dates, and harvest date for all locations is summarized in Table 1. Information on in-season monthly maximum and minimum temperatures and rainfall are in Tables 2 and 3. Fungicide applications were applied as needed at each location after wheat dormancy in the spring. In-season herbicide applications were applied as needed for various grass and broadleaf species due to being a tolerance study.

Six CoAXium® winter wheat cultivars that contain the AXigen® trait were tested in the study for their tolerance to POST applied QPE herbicide (Aggressor®, Albaugh, LLC, 1525 NE 36th Street, Ankeny, IA 50021) at 92 g ai ha^{-1} in the fall, early spring, or late spring. An additional QPE rate of 184 g ai ha^{-1} was evaluated in the 2022 season. Winter wheat cultivars were selected based on their suitability for the southern Great Plains region, traits best adapted to each location, and variability in pedigree and genome pairing conferring tolerance to QPE. Past literature states that the 2 gene pairing of BD has a lower tolerance than AD, the single genome of B has the lowest tolerance compared to A or D, and 2 mutation lines had no to little crop injury or yield loss regardless of genome pairing, while single gene lines had crop injury and yield loss (Ostlie 2015) (Hildebrandt 2022). Cultivars included: Limagrain Cereal Seeds (LCS) Fusion AX, Crescent AX, LCS Photon AX, LCS Helix AX, and AP18 AX, all hard red winter wheat cultivars (Table 2.2

and 2.3). At Hays for the 2022 season LCS Crescent AX was replaced with LCS Atomic AX. Treatments for the 2021 season were applied at an average of three to five-leaf wheat (fall), first hollow stem (early spring), and second node detectible (late spring) between cultivars. For the 2022 season, treatments were applied at an average of three to five-leaf wheat (fall), first hollow stem to jointing (early spring), and second node detectible to flag leaf (late spring) between cultivars. Quizalofop-P-ethyl was applied at 92 g ai ha^{-1} in 2021 and 92 and 184 g ai ha^{-1} in 2022. All treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} using Turbo TeeJet 11002 nozzles, water as the carrier, and methylated seed oil at 1% vol/vol for the lower QPE rate and 2% vol/vol for the higher rate.

Visual wheat injury of each individual plot area was estimated approximately two to three weeks and four to eight weeks after each QPE application, (representing peak injury), using a scale of 0 to 100% (where 0 equaled no crop injury and 100 equaled complete crop loss). Aboveground wheat biomass was collected at peak visual injury and end-of-season, prior to harvest, from one meter of row per plot. Samples were then dried in ovens at 60 ºC for seven to ten days. Finally, the weight of dried biomass was recorded for each sample. Biomass will not be discussed for the 2021 season as there were no significant main effects or interactions.

Wheat was harvested with a Wintersteiger (Wintersteiger Inc., 4705 Amelia Earhart Drive, Salt Lake City, UT 84116) small plot combine to determine yield. A grain sub-sample was collected from each plot for assess percent moisture, test weight, and protein content. Percent moisture and test weights were determined using a DICKEY-john moisture tester model mini GAC®plus (DICKEY-john, 5200 Dickey John Road, Auburn, IL 62615). Protein content (NRI values) was determined using a Perten Da7200, NIR spectrometer (PerkinElmer Inc., 940 Winter Street, Waltham, MA 02451).

Due to significant site by treatment interactions and the addition of an additional QPE rate during the 2022 season, each site year was analyzed independently. A univariate analysis was then performed on all response variables to test for stable variance (Version 9.4, SAS Institute

Inc., SAS Campus Drive, NC). Data sets from the 2022 season were square root transformed as this transformation increased stabilization. Data sets were analyzed using generalized linear mixed procedures and treatments were separated by Fisher's Protected LSD at an α level of 0.05. In the models, fixed effects included wheat cultivar, application timing, and QPE rate while random effects included replication. Due to significant site by treatment interactions and additional QPE rate during the 2022 season, each site year was analyzed independently.

Results and Discussion (Year 1)

Peak Visual Injury

There was a cultivar by herbicide application timing interaction for peak visual injury at Hays, Perkins, and Tipton in 2021 (Table 2.6). At Hays, little injury (0 to 5%) was observed following fall and early spring applications. At the fall timing, all five cultivars responded similar to the herbicide application. However, cultivar differences were recorded following early and late spring applications. Applications were made close to target growth stage at Hays. Wheat was four- to five-leaf in the fall, at first hollow stem in the early spring, and the second node was detectable on the main stem in late spring. The highest level of visual injury (26%) was observed for cultivar AP18 AX following early and late spring applications when compared to all other cultivars. In the early spring, injury for AP18 AX was five times greater than injury for all other cultivars, resulting in an increase in percent visual injury by 21 to 25%. In late spring, Helix AX, Fusion AX, and Photon AX were similar with 16 to 18% injury. Only 3% injury was documented for Crescent AX following the late spring application, which was a similar response to fall and early spring applications.

At Perkins, little injury (4 to 9%) was observed following the fall herbicide application timing where all five cultivars responded similar to the herbicide application. However, there were similarities when evaluating herbicide application response for the early and late spring timings. Injury for AP18 AX at the early spring timing was 1.5 times greater than all other cultivars, resulting in an increase in percent visual injury of 17 to 34%. In the late spring, the highest level of visual injury (25%) also was observed for AP18 AX. Crescent AX and Fusion AX responded similar at the late spring timing with 6 and 11% injury, respectively.

At Tipton, the fall herbicide application resulted in the lowest visual injury across all tested cultivars when compared to all other timings. However, at early and late spring timings, cultivar differences emerged. AP18 AX had the highest level of injury (50%) following the early spring timing when compared to all other cultivars (10 to 42%). Also following the early spring timing, Helix AX resulted in an 8 to 32% visual crop injury compared to other cultivars. AP18 AX and Helix AX were similar with the highest levels of injury (31 to 34%) following the late spring timing. During this first year, observations followed our hypothesis that cultivars would be more sensitive following early and late spring application timings compared to fall. AP18 AX was also the most sensitive cultivar at the early spring application timing; however, at Hays AP18 AX response was similar following early and late spring applications. In contrast, Hildebrandt et al. (2022) observed little to no crop injury when QPE was applied to two mutation cultivars compared to susceptible and single mutation lines. However, Hildebrandt et al. (2022) did observe that both one and two mutation lines were more susceptible following jointing (first node detectable) or heading application timings compared to a fall application timing, dependent on year and cultivar. This observation is consistent with what we observed in our first field season.

Winter Wheat Grain Yield

For winter wheat grain yield, the main effect of QPE application timing was significant at all locations for the 2021 growing season (Table 2.7). At all three sites, there was a 6 to 9% reduction in yield following the early spring application timing compared to the nontreated control. Additionally, at Hays, there was a reduction in yield for all application timings when compared to the nontreated control. Following the late spring application, yield was reduced by 16%, 7%, and 11% when compared to the nontreated control, fall, and early spring timing, respectively. The fall and early spring timings resulted in 2165 and 2274 kg ha^{-1} grain yield, respectively. Following the fall application timing, a yield reduction of 11% was observed when compared to the nontreated control. There was also a yield reduction of 6% following the early spring timing when compared to the nontreated control. At the Perkins location, there was up to a 8 to 9% reduction in yield at the early spring application timing (4663 kg ha^{-1}) when compared to the nontreated control, fall, and late spring timings. However, the nontreated control, fall, and late spring timings resulted in 5098, 5087, and 5057 kg ha^{-1} grain yield, respectively. A similar trend was observed for the Tipton location where yield was reduced 9% following the early spring application timing compared to the fall application timing. When the early spring application timing was compared to the nontreated control and late spring timing, grain yield was reduced by 6 and 5%, respectively. Yield was similar for the nontreated control, fall, and late spring application timings. Like peak visual injury, grain yield also followed our hypothesis that cultivars would be more sensitive to early and late spring application timings.

In eastern Colorado, Hildebrandt et al. (2022) observed that single mutation lines were more susceptible to QPE applications than those of two mutations which resulted in little to no crop injury. Single mutation lines were more susceptible at the jointing (first node detectable) application timing compared to fall application timing, otherwise crop safety was better when compared to that of a susceptible cultivar. The susceptible cultivar, Hatcher, resulted in a yield reduction of 65% compared to the nontreated control, however, complete crop loss was determined at all application timings. Two mutations conferring tolerance to QPE on the A and B genomes had a reduction in yield (20 to 30%) at the jointing application timing. Incline AX (AD) had little to no crop injury or yield loss regardless of application timing. However, Fusion AX (AD) yield was reduced following the heading application timing. Additionally, the B genome confers a lower tolerance than the D genome, while the A genome confers a higher tolerance to QPE compared to the B genome. Richter (2020) found that the pairing of AD confers a higher tolerance than that of the B and D genome tolerance in cultivars containing the AXigen® trait. This was also true in our study. Cultivars that were evaluated containing the BD genomes were

Helix AX, Photon AX, and AP18 AX. These cultivars were more sensitive to QPE applications as compared to the cultivars Fusion AX and Crescent AX, containing the A and D genomes.

Results and Discussion (Year 2)

Peak Visual Injury

The cultivar by herbicide application timing and herbicide application timing by QPE rate interactions were significant at Perkins in 2022 for peak visual injury (Table 2.8). For Perkins, Hays, and Tipton, the herbicide application timing and QPE rate main effects were significant for peak visual injury (Table 2.9). At Perkins, the late spring application was made when wheat was at second node detectable to boot and resulted in the least amount of visual damage, likely because it was made past the sensitive stage of stem elongation. Conversely, the fall application resulted in the highest level of visual wheat response.

Cultivar AP18 AX resulted in the greatest level of damage (79%) following the fall application timing. Increased injury for AP18 AX compared to other cultivars was likely the result of several factors, one being growth stage at time of application. It was at three-leaf while other cultivars were at an average of four- to five-leaf. The Aggressor® herbicide label recommends to spray wheat plants containing the AXigen® trait no earlier than the four-leaf growth stage (Anonymous 2021). At the same application timing, Helix AX and Photon AX were similar with ~44% injury while Crescent AX and Fusion AX were the least injured at ~23%. Cultivars Crescent AX and Fusion AX were at four- to five-leaf when the fall timing was applied, this timing was on label and likely led to less crop injury.

However, in the early spring, injury for AP18 AX e cultivar was 1.5 times greater compared to all other cultivars. Both Helix AX and Photon AX were similar with 36% peak visual injury while little injury (8%) was observed for Crescent AX. Following the late spring application, cultivars AP18 AX, Helix AX, and Photon AX were similar with 12 to 17% injury while cultivars Fusion AX and Crescent AX resulted in the least damage (3-4%). The late spring timing was made past the target of second node detectable. Crescent AX and AP18 AX were flag leaf to boot while the other cultivars were second node detectable to majority flag leaf. Cultivar maturity characteristics and unique environmental conditions at each location led to some cultivars breaking dormancy before others during green up in the late winter or early spring.

For the application timing by QPE rate interaction at Perkins, little injury (7 to 10%) was observed following the late spring application regardless of QPE rate. However, in the fall and early spring, injury was greatest following the 2X QPE rate compared to the 1X rate. At the fall timing 2X QPE rate, injury was 51% greater than for the 1X QPE rate. A similar trend was observed for the early spring timing following the 2X QPE rate as injury increased 29% when compared to the 1X QPE rate.

All cultivars were more sensitive following the fall application timing regardless of QPE rate; however, regardless of application timing or QPE rate, Fusion AX and Crescent AX injury was 3 to 24%. Cultivars were also visually more sensitive at the fall timing following a 2X QPE rate compared to other timings and the 1X QPE rate. However, visual injury decreased as assessments were recorded past peak injury (data not shown). Supportively, Kumar et al. (2020) evaluated Fusion AX at Perkins, OK and observed 6 to 12% crop injury following a fall application of QPE at 62 to 77 g ai ha^{-1}. Although rates were lower than those used in this study, crop response was still highest following the fall application timing.

At Hays, a 7% visual injury following the late spring application timing was observed compared to the fall; however, injury following the early spring timing was similar (17%). Cultivars were close to the target growth stage for each application timing at Hays. Conversely, at Tipton, there was an increase in visual injury by 12 to 21% following the fall timing compared to all other application timings. At Tipton, the fall application timing was applied on average at three- to five-leaf wheat, the early spring timing was at our target of first hollow stem to jointing (first node detectable), and the late spring was at our target of second node detectable to boot, dependent on cultivar. Less uptake of QPE occurred for the early and late spring application timings due to limited rainfall occurring around the time of application compared to the fall timing (Table 2.5).

A similar trend was observed at Hays and Tipton where the 2X QPE rate resulted in an increase in visual injury compared to the 1X rate. At Hays, the 2X QPE rate resulted in 1.9 times greater visual injury when compared to the 1X herbicide rate. Similarly, at Tipton, when compared to the 1X herbicide rate, there was an increase in peak visual injury 1.8 times for the 2X QPE rate. It was expected that the 2X QPE rate would increase visual injury compared to the 1X QPE rate regardless of cultivar or application timings. At Hays, observations supported our hypothesis that injury would increase following the early and late spring application timings compared to the fall. Conversely, the opposite trend was observed at Perkins and Tipton.

Hildebrandt et al. (2022) observed the resistant levels of susceptible, single mutation, and two mutation wheat cultivars to QPE following various application timings and rates in eastern Colorado. The susceptible wheat cultivar, Hatcher, resulted in 100% crop injury following an early spring timing at tillering or jointing in late spring. At the jointing timing in the late spring, the single gene lines resulted in crop injury, but crop safety was improved compared to the susceptible cultivar. The two mutation cultivars, Incline AX and Fusion AX, resulted in little to no crop injury regardless of application timing or QPE rate compared to the single mutation lines. As mentioned previously, Kumar et al. (2020) evaluated Fusion AX at Perkins, OK, where following a fall application timing, crop injury (6 to 12%) was visible 35 days after application.

Wheat Biomass at Peak Visual Injury

For wheat biomass at peak visual injury, the cultivar by application timing and application timing by QPE rate interactions were significant for Perkins during the 2022 growing season (Table 2.10). There was also a cultivar by QPE rate interaction at Perkins and Tipton for the same growing season (Table 2.10). Wheat biomass was not recorded at Hays at peak visual injury in 2022. At Perkins, a 72% reduction in biomass was recorded for AP18 AX following the fall application when compared to its respective nontreated control. Cultivars Helix AX and

Photon AX responded similarly with biomass reductions of 55 and 67%, respectively. Peak visual injury followed the same trend for the fall application timing resulting in the greatest reduction of biomass for AP18 AX, Helix AX, and Photon AX. Crescent AX and Fusion AX were similar with a reduction of 6 and 35%, respectively, compared to respective nontreated controls.

Following the early spring application, a 50% reduction in biomass was recorded for AP18 AX when compared to nontreated control. Helix AX, Crescent AX, and Fusion AX were similar with 11 to 15% reduction in biomass. However, at the late spring timing a different trend emerged where the largest biomass reduction (22%) was recorded for Photon AX. Overall, the biomass was reduced the greatest following the fall application timing compared to all other timings. The late spring application did not result in reduced biomass compared to the nontreated control. However, volunteer wheat and weed pressure were high during the growing season at this location, leading to a possible increase in crop injury due to interspecific and intraspecific competition. Also at Perkins, the highest biomass reduction (62%) was observed following the 2X QPE rate at the fall timing when compared to all other timings and QPE rates. A reduction of 37% biomass following the 2X QPE rate in the late spring compared to the 1X QPE rate at the same timing.

At Tipton, Crescent AX at the 1X QPE rate and Helix AX at the 2X QPE rate resulted in the greatest biomass reductions (~24%) when compared to all other cultivars and QPE rates. Biomass was similar to AP18 AX and Fusion AX at 1X and 2X QPE rates as well as Crescent AX at the 2X QPE rate. It was expected that the 2X QPE rate would result in more crop injury leading to a decrease in wheat biomass compared to the 1X QPE rate, regardless of application timing.

End-of-season Wheat Biomass

There were application timing and QPE rate main effects for end-of-season wheat biomass at Perkins in 2022 (Table 2.11). Following an early spring timing, a 17% reduction in biomass was recorded when compared to nontreated control. However, the late spring timing resulted in a 6% decrease in biomass when compared to nontreated control and was similar to biomass produced following both the fall and early spring applications. Biomass, similar to peaky visual injury in year one, supported our hypothesis that early and late spring applications would result in increased crop response. The 2X QPE rate resulted in 16% biomass reduction when compared to its respective nontreated control. End-of-season biomass variability between sites can be attributed to weather conditions during the growing season and cultivar adaptability to the location (Tables 2.2, 2.3, and 2.5).

Winter Wheat Grain Yield

For winter wheat grain yield there was a cultivar by application timing by QPE rate interaction at Hays and Perkins in 2022 (Table 2.12). At Hays, following the 2X QPE rate in the late spring, the largest yield reduction of 53% was recorded for Atomic AX when compared to the nontreated control; however, its yield was similar to Atomic AX. Yield was also reduced 25% and 38% for Atomic AX following the 2X QPE rate at the early spring timing when compared to the 1X QPE rate at the early spring timing and the nontreated control, respectively. Although Atomic AX following the 1X QPE rate at the late spring timing was similar to 1X and 2X QPE rates at the early spring timing, a reduction of 23% in grain yield was recorded when compared to the nontreated control. Following a 2X QPE rate in the late spring, yield reductions of 24% and 33% were recorded for Helix AX when compared to the 1X QPE rate at the late spring timing and nontreated control, respectively.

A yield reduction following the 2X QPE rate was observed at all three application timings for AP18 AX when compared to the nontreated control and 1X QPE rate. However, yields were similar following the 1X and 2X QPE rates at the early spring timing. Following the 2X QPE rate at the late spring timing, 23% and 30% reductions in yield occurred for AP18 AX when compared to the 1X QPE rate at the late spring timing and nontreated control, respectively. There also was a reduction in grain yield of 27% for AP18 AX following the 2X QPE rate at the early spring timing when compared to the nontreated control. Additionally, when compared to the

1X QPE rate at the fall timing and nontreated control, grain yield reductions of 31% and 30%, respectively, occurred for AP18 AX following the 2X QPE rate at the fall timing. Regardless of application timing, there was a yield reduction for AP18 AX following the 2X QPE rate when compared to the 1X QPE rate. However, the largest yield reduction following the 2X QPE rate was observed at the late spring timing for Atomic AX.

At Perkins, differences emerged for cultivars AP18 AX, Helix AX, and Photon AX between QPE rates and application timings. Following the fall application 2X QPE rate, the largest yield reduction (71%) occurred for AP18AX when compared to the nontreated control. Reductions in yield were also recorded following the early spring timing. Following 1X and 2X QPE rates, 27 and 66% reductions in yield, respectively, were observed for AP18 AX when compared the nontreated control. However, the 1X QPE rate at the early spring timing was similar to both 1X and 2X QPE rates at the late spring timing. A yield reduction of 51% occurred following the early spring timing and a 2X QPE rate for Helix AX when compared to the nontreated control but was similar to the 2X QPE rate in the fall. Also, at the 2X QPE rate at the fall timing, yield was reduced by 38% for Helix AX when compared to the nontreated control. Following late spring timing 1X QPE rate, Helix AX yield decreased 30% when compared to the nontreated control. At the 2X QPE rate at the early spring timing, yield was reduced 48% for Photon AX when compared to the nontreated control but was similar to the 2X QPE rate at the fall timing. Following the fall timing and 2X QPE rate, Photon AX grain yield decreased 34% when compared to the nontreated control. However, Crescent AX and Fusion AX were similar at all application timings and QPE rates when compared to respective nontreated controls.

Cultivar selection contributed to crop response (peak visual injury, end-of-season biomass, and grain yield) differences that can be attributed to cultivar adaptability to each location and weather conditions throughout the growing season (data not shown). However, significant crop response as a result of cultivar by application timing and/or QPE rate interactions should not occur in a robust herbicide tolerant system, especially at herbicide and adjuvant rates that were no more than doubled. Hildebrandt et al. (2022) observed in eastern Colorado that two mutation cultivars had little to no crop injury or yield loss compared to single mutation and susceptible lines. However, lines conferring tolerance on the AB genomes had yield reductions of 20 to 30% following the jointing application timing of QPE, dependent on growing season. No yield reduction or visual injury was observed for Incline AX. However, yield for Fusion AX was reduced following QPE application at heading.

Both cultivars Incline AX and Fusion AX confer tolerance on the AD genomes. The single A genome confers a higher tolerance to QPE compared to the B genome, while the B genome confers a lower tolerance than the D genome mutation. Additionally, Richter (2020) found that the paired AD genomes confer a higher tolerance than that of the BD paired genomes. Therefore, yield differences in Hildebrandt's work suggests that crop response is more complex than just genome pairing.

In our work, crop response also couldn't be directly tied to genome pairing. For example, AP18 AX (BD) and Photon AX (AD) were often sensitive cultivars that have different genome pairings. From this work and past work completed in the Oklahoma State University Small Grains Weed Science Program, data suggests that cultivar release year may impact CoAXium® wheat response to QPE. During the 2019-2020 field season at Perkins, OK, yield of cultivar Crescent AX (AD) was decreased 31% and 57% at early and late spring timings, respectively, following QPE herbicide applications (92 g ai ha^{-1}) compared to the nontreated control (data not shown). In this work, AP18 AX was the most sensitive cultivar across all site years and was the most recently released CoAXium® cultivar used in this study.

Before Hildebrandt et al. (2022) evaluated crop safety with NIS as adjuvant work had not yet been studied using the CoAXium® system. Depending on application timing and environmental conditions around time of application, the use of an alternative adjuvant such as MSO can lead to increased crop response. On the Aggressor® label it is recommended to use NIS in the fall and MSO in the spring, due to younger wheat being more sensitive in the fall

(Anonymous 2021). Therefore, MSO applied in the fall in our study, would not be supported by the Aggressor® label and could have contributed to increased wheat injury.

Finally, besides crop tolerance, another challenge that the system will face that was not evaluated in this study is longevity. To protect the efficacy of the system, producers should follow stewardship practices that are designed to delay or lower selection pressure of susceptible weed biotypes. Stewardship practices prolongs the use of the system through the requirement of crop rotation, planting certified CoAXium® seed, following the Aggressor® herbicide label for application rates and timings, as well as implementing an integrated weed management system. However, this may be difficult for some producers in the southern Great Plains that plant wheat consecutively every year. One cannot use Aggressor® herbicide two years in a row, although, it can be used every other year (Anonymous 2021) and other WSSA Group 1 herbicides can be used in years where Aggressor® is not.

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Tables

Table 2.1. Agronomic practices at Perkins and Tipton, Oklahoma, and Hays, Kansas during the 2020-2021 and 2021-2022 winter wheat growing seasons.

Year	Location	Planting date[a]	Application dates[b]			Harvest date
			Fall	Early spring	Late spring	
2020-2021	Hays	Oct 13	Nov 18	Apr 2	Apr 23	Jul 8
2020-2021	Tipton	Nov 13	Dec 28	Mar 11	Apr 3	Jun 16
2020-2021	Perkins	Oct 7	Nov 5	Mar 16	Apr 1	Jun 15
2021-2022	Hays	Sep 30	Oct 21	Apr 5	Apr 25	Jun 29
2021-2022	Tipton	Oct 21	Nov 18	Mar 24	Apr 11	Jun 7
2021-2022	Perkins	Oct 8	Nov 4	Mar 25	Apr 14	Jun 14

[a]Winter wheat was seeded at all locations at 67 kg ha^{-1} with a drill spacing of 18 cm at Oklahoma locations and 19 cm at Hays, Kansas.

[b]Quizalofop-P-ethyl was applied to winter wheat plots in the fall (3 to 5-leaf), early spring (first hollow stem), or late spring (second node detectable).

Table 2.2. Characteristics of CoAXium® winter wheat cultivars used in field experiments during the 2020-2021 and 2021-2022 growing seasons.

Characteristics	AP18 AX	Atomic AX	Crescent AX	Fusion AX	Helix AX	Photon AX
Genomes	BD	BD	AD	AD	BD	AD
Pedigree	N/A[a]	T153*CSU donor	Bryd*Bryd	Hatcher*Bryd	T158*CSU donor	T158*CSU donor
Maturity	Medium	Early	Medium to early	Medium	Medium to early	Medium to early
First hollow stem	N/A	Intermediate	N/A	Intermediate	Intermediate	Intermediate
Yield	Excellent	Excellent	Very good	Very good	Excellent	Very good
Test weight	Very good	Good	Very good	Below average	Excellent	Excellent
Protein	N/A	N/A	Fair	N/A	N/A	Excellent
Plant height	Medium	Medium	Medium	Medium to tall	Medium to tall	Medium to tall
Seed size	N/A	Large	Small	Medium	Large	Large
Straw strength	Good	Excellent	Good	Average	Very good	Good
Shatter	Very good to excellent	Very good	N/A	Good	Very good	Very good
Winter hardiness	Very good	Very good	N/A	Very good	Very good	Very good
Drought tolerance	N/A	Excellent	Good	Excellent	Excellent	Very good

Baking quality	Acceptable	Unacceptable	Very good	Less desirable	Desirable	Desirable
Milling quality	N/A	Less desirable	Very good	Less desirrable	Acceptable	Acceptable

[a]Information was not available.

Table 2.3. Pest and disease profiles for each CoAXium® winter wheat cultivars used in field experiments during the 2020-2021 and 2021-2022 growing seasons.

Pest	AP18 AX	Atomic AX	Crescent AX	Fusion AX	Helix AX	Photon AX
Leaf rust	6[a]	2	6	8	4	6
Stripe rust	2	1	4	9	2	2
Stem rust	3	9	N/A[b]	9	1	6
Wheat streak mosaic	3	N/A	2	5	N/A	N/A
Barley yellow dwarf	5	N/A	N/A	5	N/A	N/A
Fusarium head blight	6	3	N/A	9	3	1
Tan spot	4	N/A	N/A	5	N/A	N/A
Soil borne mosaic	N/A	1	N/A	6	1	3
Powdery mildew	N/A	N/A	N/A	9	N/A	N/A
Hessian fly	5	N/A	N/A	9	N/A	N/A

[a]Rating key: 1 to 3 is resistant, 4 to 6 is intermediate, and 7 to 9 is susceptible to pest.

[b]Information was not available.

Table 2.4. Weather data at Perkins and Tipton, Oklahoma and Hays, Kansas during the 2020-2021 winter wheat growing season.

	Hays			Tipton			Perkins		
Month	Temperature °C		Rainfall[a] mm	Temperature °C		Rainfall mm	Temperature °C		Rainfall mm
	Min	Max		Min	Max		Min	Max	
October	-8[a]	24	2	----------	----------	-------------	-2	33	123
November	-13	27	23	-4	28	10	-5	26	20
December	-12	21	8	-8	23	49	-8	24	81
January	-18	19	11	-6	23	12	-8	17	57
February	-27	23	0	-22	25	8	-26	22	14
March	-11	29	113	-4	30	24	-2	27	62
April	-5	33	27	-1	31	74	-2	29	105
May	3	30	194	6	33	115	5	30	136
June	9	43	19	14	36	95	13	35	8
July	13	42	17	----------	----------	-------------	----------	------------	--------------------
Average	-7	29	41	-3	29	48	-4	27	67
Total			457			387			606

[a]All Oklahoma rainfall and temperature data was collected from the Oklahoma Mesonet (mesonet.org) and all Kansas rainfall and temperature data was collected from the Kansas Mesonet (mesonet.k-state.edu). Rainfall was determined from planting date to harvest date.

Table 2.5. Weather data at Perkins and Tipton, Oklahoma, and Hays, Kansas during the 2021-2022 winter wheat growing season.

	Hays			Tipton			Perkins		
Month	Temperature ºC		Rainfall mm	Temperature ºC		Rainfall mm	Temperature ºC		Rainfall mm
	Min	Max		Min	Max		Min	Max	
September	12[a]	22	11	-	-	-	-	-	-
October	-1	30	29	3	32	8	2	35	85
November	-9	27	5	-6	31	2	-4	26	29
December	-14	27	0	-11	29	4	-10	27	6
January	-24	19	5	-14	24	8	-15	22	7
February	-19	21	0	-14	28	31	-14	23	42
March	-14	28	30	-9	32	10	-9	27	71
April	-5	32	10	-1	37	12	-2	34	25
May	1	35	86	7	41	171	7	34	320
June	5	41	35	16	34	50	15	37	72
Average	-7	28	21	-3	32	33	-3	29	73
Total			211			296			657

[a]All Oklahoma rainfall and temperature data collected from the Oklahoma Mesonet (mesonet.org) and all Kansas rainfall and temperature data collected from the Kansas Mesonet (mesonet.k-state.edu). Rainfall was determined from planting date to harvest date.

Table 2.6. A cultivar by application timing interaction for peak percent visual wheat injury at Hays, KS and Perkins and Tipton, OK during the 2020-2021 winter wheat growing season.

	Hays			Perkins			Tipton		
	---%--								
Cultivar*application timing interaction	Fall[a]	Early Spring	Late Spring	Fall	Early Spring	Late Spring	Fall	Early Spring	Late Spring
AP18 AX	0 c[b]	26 a	26 a	9 fgh	50 a	25 c	1 f	53 a	34 c
Crescent AX	0 c	1 c	3 c	2 i	19 d	6 ghi	1 f	18 d	10 e
Fusion AX	0 c	2 c	16 b	4 hi	16 de	11 efg	1 f	11 e	21 d
Helix AX	1 c	3 c	18 b	4 hi	33 b	19 d	1 f	35 c	31 c
Photon AX	1 c	5 c	16 b	4 hi	26 c	14 def	1 f	43 b	21 d

[a]All herbicide treatments were applied with 92 g ai ha^{-1} of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume using water as the carrier. Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

Table 2.7. Application timing effect for winter wheat grain yield (kg ha^{-1}) at Hays, KS and Perkins and Tipton, OK during the 2020-2021 growing season.

	Hays	Perkins	Tipton
	------ kg ha^{-1} ------		
Application timing			
Nontreated control	2406 a[b]	5098 a	5854 a
Fall[b]	2165 b	5087 a	6035 a
Early Spring	2274 b	4663 b	5541 b
Late Spring	2034 c	5057 a	5826 a

[a]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[b]All herbicide treatments were applied with 92 g ai ha^{-1} of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume using water as the carrier. Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

Table 2.8. A cultivar by application timing and quizalofop-P-ethyl rate by application timing interactions for peak percent visual wheat injury at Perkins, OK during the 2021-2022 winter wheat growing season.

	Perkins		
	-------------------------------%-------------------------------		
Cultivar*application timing interaction	Fall[a]	Early spring	Late spring
AP18 AX	79 a[b]	53 b	17 fg
Atomic AX	----------	----------	----------
Crescent AX	22 ef	8 hi	4 i
Fusion AX	24 def	16 fg	3 i
Helix AX	42 bc	36 cd	17 fg
Photon AX	47 bc	36 cde	12 hg
Quizalofop-P-ethyl rate*application timing interaction	Fall	Early spring	Late spring
1X[c]	19 c	14 cd	7 e
2X	70 a	43 b	10 de

[a]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[c]The herbicide treatments for 2022 were applied at either 1X (92 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume or 2X rate (185 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed soil at 2% volume/volume using water as the carrier.

Table 2.9. Application timing and quizalofop-P-ethyl rate main effects for peak visual injury at Hays, KS and Tipton, OK in the 2021-2022 winter wheat growing season.

	Hays	Tipton
	-- % --	
Application timing		
Fall[a]	15 b[b]	34 a
Early spring	17 ab	22 b
Late spring	22 a	13 c
Quizalofop-P-ethyl rate		
1X[c]	13 b	16 b
2X	25 a	29 a

[a]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[c]The herbicide treatments for 2022 were applied at either 1X rate (92 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume or 2X rate (185 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed soil at 2% volume/volume using water as the carrier.

Table 2.10. Cultivar by application timing and application timing by quizalofop-P-ethyl rate interactions for winter wheat biomass (% of nontreated control) at peak visual injury at Perkins, OK during the 2021-2022 winter wheat growing season.

	Perkins		
	------------------------------------ % nontreated control -------------------------------------		
Cultivar*application timing interaction	Fall[a]	Early spring	Late spring
AP18 AX	28 g[b]	50 ef	125 ab
Helix AX	45 efg	86 cd	104 bc
Photon AX	33 fg	64 de	88 cd
Crescent AX	94 bcd	89 cd	146 a
Fusion AX	65 de	85 cd	115 abc
Quizalofop-P-ethyl rate*application timing interaction	Fall	Early spring	Late spring
1X[c]	60 c	84 b	112 a
2X	38 d	63 c	116 a

[a]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[c]The herbicide treatments for 2022 were applied at either 1X rate (92 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume or 2X rate (185 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed soil at 2% volume/volume using water as the carrier.

Table 2.11. Cultivar by quizalofop-P-ethyl rate interaction for winter wheat biomass (% of nontreated control) at peak visual injury at Tipton, OK during the 2021-2022 winter wheat growing season.

	Tipton	
	------------------------------------ % nontreated control ----------------------------------	
Cultivar*quizalofop-P-ethyl rate interaction	1X[a]	2X
AP18 AX	88 bc[b]	88 bc[c]
Helix AX	121 ab	76 c
Photon AX	140 a	109 ab
Crescent AX	76 c	95 bc
Fusion AX	87 bc	91 bc

[a]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[c]The herbicide treatments for 2022 were applied at either 1X rate (92 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume or 2X rate (185 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed soil at 2% volume/volume using water as the carrier.

Table 2.12. Application timing and quizalofop-P-ethyl rate main effects for end-of-season winter wheat biomass (% of nontreated control) at Perkins, OK during the 2021-2022 growing season.

	Perkins
	-------------------------------------- % nontreated control ---------------------------------
Application timing	
Fall[a]	102 a[b]
Early spring	83 b
Late spring	94 ab
Quizalofop-P-ethyl rate	
1X[c]	102 a
2X	84 b

[a]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[b]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

[c]The herbicide treatments for 2022 were applied at either 1X rate (92 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed oil at 1% volume/volume or 2X rate (185 g ai ha^{-1}) of quizalofop-P-ethyl plus methylated seed soil at 2% volume/volume using water as the carrier.

Table 2.13. Cultivar by application timing by quizalofop-P-ethyl rate interaction for winter wheat grain yield (kg ha^{-1}) at Hays, KS and Perkins, OK during the 2021-2022 growing season.

	Hays							Perkins						
	---------- kg ha^{-1} ----------													
Cultivar*application timing*quizalofop-P-ethyl rate interaction	Control	Fall[a]		Early spring		Late spring		Control	Fall		Early spring		Late spring	
	0X	1X[b]	2X	1X	2X	1X	2X	0X	1X	2X	1X	2X	1X	2X
AP18 AX	1920 a-d[c]	1941 b-d	1349 gh	1765 c-f	1403 f-h	1763 c-f	1359 gh	3397 a-d	3658 a-c	997 j	2507 d-h	1503 i	3114 a-e	2806 a-e
Atomic AX	1778 c-e	2158 a-c	1500 d-g	1480 e-g	1114 hi	1386 f-h	842 i	---------	------	-----	-----	-----	-----	-----
Crescent AX	---------	-----	-----	-----	-----	-----	-----	3427 a-d	3793 ab	3626 a-c	3608 a-d	3082 a-e	3215 a-e	3432 a-d
Fusion AX	1788 c-e	1930 a-d	1690 c-g	1815 cd	1762 c-f	1805 cd	1772 c-f	2835 a-f	3160 a-e	2879 a-e	2687 c-g	2092 f-i	2830 a-g	3016 a-f
Helix AX	2332 a	2076 a-c	2131 a-c	2200 ab	2054 a-c	2054 a-c	1579 d-g	3698 ab	3348 a-d	2312 e-h	3113 a-e	1841 hi	2623 c-g	2776 b-g
Photon AX	2005 a-c	2066 a-c	1724 c-g	1933 a-c	2012 a-c	2003 a-c	1855 b-d	3748 ab	3951 a	2508 d-h	3342 a-d	1982 g-i	3333 a-d	3426 a-d

[b]Herbicide applications were applied at three different timings of fall, early spring, and late spring. The fall application timing was at 3- to 5-leaf wheat, wheat was at first hollow stem for the early spring timing, and second node was detected on the main stem for the late spring timing.

[a]The herbicide treatments for 2022 were applied at either 1X rate at 92 g ai ha^{-1} of quizalofop-P-ethyl and methylated seed oil (MSO) at 1% volume/volume or 2X rate at 185 g ai ha^{-1} of quizalofop-P-ethyl and methylated seed soil MSO at 2% volume/volume using water as the carrier.

[c]Means within a column for each site year followed by a common letter were similar according to Fisher's protected LSD at $P<0.05$.

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