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Evaluation of Factors that Contribute to Injury to Quizalofop-Resistant Rice from Quizalofop Applied Postemergence

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Evaluation of Factors that Contribute to Injury to Quizalofop-Resistant Rice from Quizalofop Applied Postemergence

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ABSTRACT

Quizalofop-resistant rice technology allows for over-the-top applications of quizalofop, an acetyl-coenzyme A carboxylase-inhibiting herbicide. However, quizalofop caused significant injury to quizalofop-resistant rice in some Arkansas fields during the first year of commercialization. Experiments evaluated the effect of early-season soil moisture and nitrogen availability; pre-exposure to low rates of glyphosate and imazethapyr; planting date; and environmental conditions including, soil moisture content, air temperature, and light intensity on quizalofop-resistant rice tolerance to quizalofop applications. All experiments assessed sequential quizalofop applications made to 2-leaf followed by 5-leaf stage of rice. Sequential quizalofop applications alone and with surface irrigation or nitrogen application at the 2-leaf rice stage after the initial herbicide application caused minimal injury to quizalofop-resistant cultivars, with <10% visible injury regardless of location and rating timing if drier soil conditions persisted before quizalofop applications. Exposure to a sub-lethal rate of glyphosate or imazethapyr followed by quizalofop on the same day at the 2-leaf growth stage caused higher injury to quizalofop-resistant rice than glyphosate or imazethapyr alone at the 2-leaf growth stage. In the planting date study, variable injury levels were observed on rice in both years across planting dates depending on the environmental conditions that persisted surrounding the quizalofop application timing, with greater injury under wet and cloudy environments. Quizalofop-resistant cultivars had at least 25 percentage points greater injury, averaged over rating timings when cultivars were maintained at soil moistures of 90% or 100% of field capacity rather than at 40% or 50% of field capacity. Higher injury, ranging from 18% to 31% was observed on quizalofop-resistant cultivars maintained under low light intensity (600 µmol m^{-2} s^{-1}) compared to 5% to 14% under high light intensity (1150 µmol m^{-2} s^{-1}), persisted from 7 to 28 days after the final treatment (DAFT), averaged over quizalofop-resistant cultivars and air temperatures (20/15 C and 30/25 C day/night, respectively). Quizalofop-resistant cultivars had 5- to 21-percentage points greater injury, averaged over light intensity levels, under low temperature (20/15 C day/night) than high temperature (30/25 C day/night) conditions when evaluated at 7 DAFT. Overall, wet, cold, and cloudy environments exacerbated visual injury to quizalofop-resistant cultivars.

Nomenclature: Glyphosate; imazethapyr; quizalofop; rice, *Oryza sativa* L.

Keywords: Air temperature, environmental conditions, light intensity, quizalofop-resistant rice, rice injury, soil moisture content

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Chapter 1

General Introduction and Review of Literature

Rice Overview. Rice (*Oryza sativa* L.) is the primary staple food of more than half of the world's population. A sustainable rice production system is key to global food security (Dowling et al. 1998). A significant proportion of human society has been supported by rice for a more extended period than any other crop since it was first domesticated and cultivated between 12,000 to 6,000 years ago (Huggan 1995). As a result, rice consumption has gradually increased over the past several decades, and 507.3 million metric tons of rice were consumed globally in 2020-21 (FAS-USDA 2021). United States growers planted over 1.08 million hectares and produced 7.23 million metric tons of rice. Consumption was 4.84 million metric tons of rice domestically in 2021 (NASS –USDA 2021a; FAS-USDA 2021). The United States is the fifth largest exporter of rice, accounting for more than 6.1% of the annual volume of the global rice trade and providing a consistent and reliable supply of superior quality rice in both the long- and combined medium-and short-grain international markets (ERS – USDA 2021; FAS-USDA 2021).

The rice production region of the United States is centered substantially around the Arkansas delta region, including the Arkansas Grand Prairie, northeast Arkansas, and southeast areas of Missouri; the Mississippi River Delta has parts of Arkansas, Mississippi, Missouri, and Louisiana; the Gulf Coast of Texas and Southwest Louisiana, and the Sacramento Valley of California (McBride et al. 2018). In 2021, Arkansas, California, Louisiana, Mississippi, Missouri, and Texas rice producers planted 47%, 16%, 17%, 4%, 9%, and 7%, respectively of the total 1.08 m hectares under rice cultivation in the United States (NASS USDA 2021a). Despite the reduction in the number of rice production farms in the United States, rice production has significantly increased over the past several years. Wide-scale adoption of new technologies such as hybrid seeds, herbicide-tolerant seeds, and precision-farming equipment has increased per hectare crop yields. The adoption of innovative technologies has led to increased crop yield potential and reduced input costs, providing a competitive edge to farmers in the global rice market (Espe et al. 2016; McBride et al. 2018).

Arkansas Rice Production. In the United States, Arkansas has been the leading rice-producing state since 1973; California, Louisiana, and Texas had a nearly equal proportion of hectares under rice production with Arkansas before the 1970s. Rice cultivation increased significantly after 1967 due to the elimination of planting-area restrictions and new herbicides, fertilizers, and crop production technologies (Talbert and Burgos 2007). Arkansas accounts for approximately half of the nation's total area under rice cultivation, and the planted area was over 502,216 hectares in 2021 (NASS-USDA 2021b). The major rice-producing counties in Arkansas are located on the eastern side of the state in the Mississippi River Delta. They include Poinsett, Jackson, Lonoke, Arkansas, Cross, and Lawrence counties (Hardke 2021a).

The relatively mild temperatures and regular rainfall throughout the crop season in Arkansas are primary factors responsible for rice growth and development, resulting in favorable yields. Rice planting typically ranges from late March to early June, with harvest occurring from late August to early November each year. The majority of Arkansas rice is drill-seeded (85%), a small part, 10%, is broadcast dry seeded, and 5% is broadcast water seeded. Most rice is planted using conventional tillage, which involves fall tillage followed by spring tillage for seedbed preparation. Rice is produced mainly on silt-loam soils (50.7%), but clay and clay-loam soils also accounted for 25.5% and 20.8%, respectively, of the rice production area (Hardke 2021a, 2021b). The typical Arkansas rice production system includes flood irrigation with a permanent flood established at the 4- to 5-leaf crop stage. The presence of a continuous flood facilitates nutrient uptake and reproductive growth and results in higher yields (Beyrouty et al. 1994). The availability of better crop genetics, advanced pesticide technologies, and the adoption of innovative tools has led to significant increases in rice yield over the past several decades.

Rice Weed Control. An effective weed management program is an essential component of a rice production system. Weeds compete with rice for water, nutrients, light, and additional growth requirements. This competition results in significant reductions in rice yield, seed quality, irrigating efficiency, harvesting efficiency, and processing efficiency. Along with escalating the cost of weed management inputs, weeds also intensify insect and disease problems by serving as alternate hosts (Smith 1988). A successful weed management program requires the identification of problematic weed species, understanding the shifts in weed spectrum, utilization of proper crop technologies to mitigate the evolution herbicide-resistant weeds, and implementation of integrated weed management practices (Butts et al. 2022). The initial 4 to 6 weeks after rice emergence is critical for weed management and requires the most concentrated weed control efforts to achieve weed-free rice yields. Irrigation management is an integral part of a weed control program as it offers a unique methodology to suppress the germination of many problematic weed species and facilitates rice growth (Smith and Fox 1973). The most troublesome weeds of rice in Arkansas, rated by growers, consultants, and industry representatives in a 2020 survey that represented 40% of the total planted hectares, were barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], *Cyperus* spp., and weedy rice (*Oryza sativa* L.) (Butts et al. 2022).

Weed control is a significant input cost to rice producers. The Agricultural Resource Management Survey conducted by the United States Department of Agriculture in 2000, 2006, and 2013 showed that herbicides were utilized as an essential input in almost 95% of rice hectares in the United States. Herbicide use, measured by the number of treatments per treated acre, increased from an average of 2.83 in 2000 to 3.72 in 2013 (McBride et al. 2018). Arkansas rice producers spend about \$140 million annually on weed management with an average cost of \$266 ha^{-1} on chemical weed control and almost 81% of total herbicide cost was attributed to barnyardgrass control (Butts et al. 2022). A major obstacle to a weed control program is herbicide resistance. Problematic grass weeds in Arkansas have already developed resistance to acetyl-coenzyme A carboxylase (ACCase) inhibitors (cyhalofop, fenoxaprop); acetolactate synthase (ALS) inhibitors (bispyribac, imazamox, imazapyr, imazaquin, imazethapyr, penoxsulam, pyrithiobac-sodium); synthetic auxins (florpyrauxifen-benzyl, quinclorac); photosystem (PS) II inhibitor (propanil); and 1-deoxy-D-xylulose 5-phosphate synthase (DOXP) inhibitor (clomazone) (Barber et al. 2022; Heap 2022). The changes in the weed spectrum are a result of the evolution of resistant weeds and rice producers must re-focus their weed control objectives to manage the problematic herbicide-resistant weed populations. Best management practices that reduce the occurrence and spread of herbicide-resistant weeds include the use of herbicide-resistant rice technologies with crop rotation, and incorporating multiple herbicide sites of action (SOAs) (Norsworthy et al. 2012, 2013).

Barnyardgrass is the primary weed of rice across the globe (Bryson et al. 2009). Optimum moisture availability and temperature and high light intensity allow barnyardgrass to grow vigorously in rice fields. The presence of an efficient C4 photosynthetic pathway compared to the C3 photosynthetic pathway in rice gives barnyardgrass a competitive edge (Mitich 1990). Barnyardgrass alone can cause 70% yield losses when left uncontrolled in rice production systems (Smith 1988). Barnyardgrass is resilient and genetically diverse and has evolved resistance to bispyribac, clomazone, cyhalofop, fenoxaprop, florpyrauxifen-benzyl, imazamox, imazethapyr, penoxsulam, propanil, quinclorac, and thiobencarb in United States rice fields, making it one of the most troublesome weeds of rice (Barber et al. 2022; Heap 2022).

Weedy rice, often referred to in the literature as red rice, is a global threat to rice production. Weedy rice is substantially taller, more heavily tillered, and its rapid seedling growth and emergence provide a competitive edge over cultivated rice varieties (Delouche et al. 2007). As a result, weedy rice has been one of the most damaging weeds throughout the mid-southern United States rice cropping systems and can cause up to 82% of yield loss from season-long interference (Burgos et al. 2008; Smith 1988). In addition, the risk for herbicide resistance through transgene flow from herbicide-resistant rice cultivars to weedy rice populations is prevalent, as weedy rice and cultivated rice are closely related species (Gressel and Valverde 2009).

Herbicide Options. Propanil is a photosystem II (PSII) inhibitor and belongs to WSSA Group 5. Propanil was effective in controlling barnyardgrass, sedges, and broadleaf aquatic weeds (Smith 1965). Propanil was introduced in 1959 and was used predominantly for rice weed control for over four decades (Talbert and Burgos 2007). Around 98% of rice hectares in Arkansas were treated with propanil at least once a season by the 1990s. However, over-reliance on a single mode of action led to the selection of resistance in barnyardgrass (Carey et al. 1995).

Quinclorac was commercialized in 1992 and became available to rice producers in the mid-south shortly after barnyardgrass resistance to propanil was reported (Talbert and Burgos 2007). Quinclorac is a synthetic auxin that belongs to the WSSA Group 4. The use of quinclorac was prevalent in rice production until barnyardgrass resistance to quinclorac was reported in 1999. Propanil and quinclorac, two major herbicides used in rice production, were significantly reduced due to the evolution of multiple-resistant barnyardgrass populations (Lovelace et al. 2007).

Clomazone is a diterpene-synthesis-inhibiting herbicide and belongs to WSSA Group 13. Clomazone effectively controls grass weeds, including barnyardgrass, broadleaf signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster], Amazon sprangletop [*Diplachne panicoides* (J. Presl) McNeill)], and bearded sprangletop [*Diplachne fusca* (L.) P. Beauv.] with a preemergence application (Barber et al. 2022). In 2008, a barnyardgrass biotype was documented as clomazone-resistant in Arkansas (Heap 2022). Clomazone would have to be applied in combination with other modes of action to be useful in reducing the spread of resistance evolution (Norsworthy et al. 2009).

Rice Technologies. The evolution of resistance to multiple herbicide SOAs in grass weeds resulted in the requirement of new technology to effectively control troublesome weeds to maintain the optimum yield thresholds in Arkansas's rice production system. Herbicide-resistant (HR) rice was developed through traditional plant-breeding techniques rather than genetic transformation. Applications of specific herbicides over-the-top of rice control the targeted weeds without harming the rice. Herbicide-resistant rice, including quizalofop-resistant and imidazolinone-resistant cultivars, was planted on 39.7% of total Arkansas rice hectares in the growing season of 2020 (Hardke 2021a).

Imidazolinone-Resistant Rice. Clearfield® (CL) rice was the first herbicide-resistant rice developed for managing problematic weedy rice and other grass weeds. Louisiana State University commercially released two Clearfield rice cultivars (CL 121 and CL 141) for mid-south producers in 2002 (Croughan 2004). Clearfield rice is resistant to imidazolinone (IMI) herbicides, imazethapyr (Newpath®), and imazamox (Beyond®), allowing growers to make applications of imidazolinone herbicides without the risk of crop injury (Croughan 2004). CL rice was planted on 43% of the United States rice hectares by 2013 (McBride et al. 2018). The CL161 cultivar was available to mid-south growers in 2003 and replaced the earlier Clearfield cultivars due to having a higher tolerance to imidazolinone herbicides and higher yield potential (Sudianto et al. 2013). Additionally, FullPage® rice is imidazolinone-resistant rice commercialized in 2019 that allows for applications of imazethapyr (Preface™) and imazamox (Postscript™) (Anonymous 2019).

In Arkansas, CLL15, CLL16, CLL17, CLM04, RT 7321 FP, RT 7521 FP are the imidazolinone-resistant cultivars recently available to producers (Anonymous 2020). The gene flow between weedy rice and an imidazolinone-resistant cultivar could potentially transfer herbicide-resistant genes to weedy rice; however, the outcrossing percentage is low and results in a few hundred weedy rice plants per hectare (Burgos et al. 2008). The cross-pollination between weedy rice and imidazolinone-resistant rice cultivars is a potential threat to the sustainability of imidazolinone-resistant crop technology (Sudianto et al. 2013). Crop rotation and integration of multiple-herbicide modes of action to control troublesome weeds in imidazolinone-resistant rice is imperative to conserve the ALS-inhibiting herbicide mode of action for future crop production systems (Norsworthy et al. 2012).

In Arkansas, ground and aerial application equipment were almost equally utilized for herbicide applications. Herbicide drift was the primary concern for producers in terms of herbicide application challenges, as documented in a 2019 survey (Butts et al. 2021). Conventional rice is generally grown in a close association with imidazolinone-resistant rice and glyphosate-resistant crops, including corn, cotton, and soybeans, increasing the risk for damage to conventional rice hectares. Imazethapyr and glyphosate exposure to conventional rice at sub-lethal rates caused severe injury and reduced yield potential (Davis et al. 2011; Hensley et al. 2012).

ALS-Inhibitors. ALS-inhibiting herbicides are the members of WSSA Group 2 that inhibit acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine, and lead to plant death occurring in response to ALS-inhibition and low branched-chain amino acid production (WSSA 2021). Imazethapyr herbicide is labeled for use in the imidazolinone-resistant rice system for controlling annual grasses, sedges, and some aquatic broadleaf weeds (Barber et al. 2022). Imazethapyr provides long-residual control, persisting in the soil for a crop season, and can also potentially damage conventional rice planted in the next cropping season (Barber et al. 2022). ALS-inhibitors including, but not limited to, imazethapyr, imazamox, penoxsulam, bensulfuron, halosulfuron, thifensulfuron, prosulfuron, and bispyribac are utilized in the Arkansas rice production system (Barber et al. 2022). The over-reliance on ALS-inhibiting herbicides has resulted in resistance development in barnyardgrass, weedy rice, and *Cyperus* spp. to ALS-inhibitors (Norsworthy et al. 2013).

Quizalofop-Resistant Rice. BASF corporation commercially released the first quizalofop-resistant cultivar, PVL01 (Provisia® rice), in 2018. Provisia (BASF Corp., Research Triangle Park, NC) rice provides the grower with an enhanced crop rotational strategy to manage herbicide-resistant populations of barnyardgrass and weedy rice (Anonymous 2018). Quizalofop-resistant rice is a non-transgenic herbicide-resistant technology developed using traditional breeding techniques and allows for postemergence applications of quizalofop, an ACCase-inhibiting herbicide (Guice et al. 2015). Resistance of quizalofop-resistant rice to quizalofop is governed by a single dominant Mendelian gene, but the expression of the resistance mechanism in quizalofop-resistant rice cultivars can be environment-specific (Camacho et al. 2019, 2020). Previous research reported that quizalofop caused up to 26% injury on quizalofop-resistant rice lines resulting in slight chlorosis and necrotic symptoms but plants generally recovered from injury at later stages (Camacho et al. 2020).

Herbicide resistance in quizalofop-resistant rice is conferred by a single-point mutation, and the presence of the herbicide-resistant allele can be detected rapidly at a low cost by using normal molecular biology laboratory procedures, which suggests the potential for rapid and effective development of new quizalofop-resistant cultivars (Camacho et al. 2020; Pereira et al. 2019). Provisia® (PVL01, PVL02, and PVL03) and Max-Ace® (RTv7231 MA and RT7331 MA) are the quizalofop-resistant rice cultivars, that were commercialized by 2022 (Hines 2018, Hardke et al. 2022). Quizalofop-resistant rice was planted on 2.7% of total Arkansas rice hectares in 2020 and will potentially increase over the next growing seasons (Hardke 2021a).

ACCase-Inhibitors. Acetyl coenzyme-A carboxylase (ACCase)-inhibitors are WSSA Group 1 herbicides. The inhibition of fatty acid synthesis blocks the production of phospholipids used in building new membranes required for cell growth and leads to the death of the plant. The natural tolerance of broadleaf species to ACCase-inhibitors is because of an insensitive ACCase enzyme (WSSA 2021). ACCase-inhibiting herbicides include quizalofop, cyhalofop, and fenoxaprop for mid-south rice production and provide broad-spectrum control of weedy rice, amazon sprangletop, barnyardgrass, and other grass weeds (Barber et al. 2022). ACCase-inhibiting herbicides have a lower risk than ALS-inhibitors for evolving resistance as predicted by herbicide-resistance modeling in barnyardgrass (Bagavathiannan et al. 2014). Climatic factors influence the efficacy of ACCase-inhibiting herbicides by impacting the physiochemical processes of plants that affect herbicide absorption, translocation, and metabolism (Varanasi et al. 2015). Previous research reported that temperature, light intensity, and moisture availability influenced the response of grass species to fluazifop applications (James et al. 1984; Smeda and Putnam 1990).

Quizalofop. Quizalofop is a member of the aryloxyphenoxypropionate family that provides postemergence control of annual and perennial grass weeds. Quizalofop is a systemic herbicide that inhibits the ACCase enzyme that blocks lipid synthesis in the plant. Growth ceases soon after quizalofop application, with young and actively growing tissues affected most, followed by chlorosis and necrosis development 1 to 3 weeks after application (Shaner 2014). Quizalofop for use in the Provisia® and Max-Ace® rice systems was sold under the trade name of Provisia® and Highcard™ herbicide, respectively. Quizalofop use rate varies in quizalofop-resistant rice from 100 to 138 g ai ha^{-1} for a single application and is not to exceed 240 g ai ha^{-1} for maximum yearly application (Anonymous 2017; Anonymous 2021).

Sequential applications at the 1- to the 2-leaf stage followed by a second application at the 5-leaf stage effectively manage the most troublesome grass weeds in the quizalofop-resistant rice system, including ALS-inhibitor-resistant weeds, weedy rice, volunteer rice, and barnyardgrass (Barber et al. 2022). However, when quizalofop is applied as a tank-mixture with other herbicides, including propanil and saflufenacil, antagonism is commonly observed, resulting in poor weed control and significantly lower yields (Rustom et al. 2019). Thus, it is recommended to tank-mix quizalofop with broadleaf herbicides only in the first of the two sequential applications for broad-spectrum control of weeds (Barber et al. 2022). Sequential applications of quizalofop can be successfully used as a postemergence, broad-spectrum grass weed-control tool in quizalofop-resistant rice cropping systems with proper implementation of the stewardship program.

The need for quizalofop-resistant rice in a rice production system is of vital importance due to the competitive and resilient nature of weedy rice, barnyardgrass, and other problematic grass weeds. In 2019, several commercial fields of quizalofop-resistant rice (PVL01) in Arkansas were injured following an application of quizalofop (JK Norsworthy, personal communication). There was potential for yield loss, but the factors causing injury to quizalofop-resistant rice cultivars were not well-established yet by researchers. Therefore, this research focused on understanding the factors responsible for causing injury to quizalofop-resistant rice from quizalofop applied postemergence. It is imperative to find the potential reasons for these inconsistencies to determine the best uses of quizalofop-resistant rice technology in rice production systems.

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Chapter 2

Effects of Early-Season Soil Moisture and Nitrogen Applications on Tolerance of Quizalofop-Resistant Rice to Quizalofop

Abstract

Quizalofop-resistant rice technology allows for postemergence applications of quizalofop, an acetyl-coenzyme A carboxylase-inhibiting herbicide. Previous research reported that soil moisture availability strongly influences the response of grass species to aryloxyphenoxypropionate herbicides. Experiments were conducted at the Rice Research and Extension Center, Stuttgart, AR, and at the Pine Tree Research Station, Colt, AR, in 2021 to investigate the influence of early-season soil moisture and nitrogen (N) applications on the tolerance of quizalofop-resistant cultivars to sequential quizalofop applications. The experiment was implemented as a two-factor, randomized complete block design. The factors evaluated were cultivar (PVL02 and RTv7231 MA) and herbicide treatment [nontreated control; nontreated control fertilized with ammonium sulfate (AMS) at 112 kg ha^{-1} and surface irrigated at the 2-leaf stage; sequential quizalofop applications at 1x and 2x rates; sequential quizalofop at 1x and 2x rates, surface irrigated at the 2-leaf stage; sequential quizalofop applications at 1x and 2x rates, fertilized with AMS at 112 kg ha^{-1} then surface irrigated at the 2-leaf stage]. Sequential quizalofop applications were applied at the 2-leaf and 5-leaf stages, with 1x and 2x rates of quizalofop being 120 and 240 g ai ha^{-1}, respectively. RTv7231 MA injury was 2- to 6-percentage points higher than PVL02 at all visual injury ratings, averaged across treatment and location. Averaged over rating timings, RTv7231 MA had 9% injury when quizalofop applied sequentially at the 2x rate, fertilized with AMS, and surface irrigated compared to 4% injury after sequential quizalofop applied alone at the 2x rate. Sequential quizalofop applications

caused 1% to 9% visible injury to quizalofop-resistant cultivars, pooled over locations and rating timings. Growers may see injury to quizalofop-resistant cultivars from sequential applications of quizalofop, but the conditions under which these trials were conducted were not conducive for quizalofop injury to reduce yield.

Nomenclature: Quizalofop; quizalofop-resistant rice; rice, *Oryza sativa* L.

Introduction

Arkansas and Louisiana comprised 64% of total rice hectares planted in the United States in 2020 (NASS-USDA 2021). Quizalofop-resistant rice was grown on 15,965 hectares in Arkansas and 12,440 hectares in Louisiana in 2020 and is expected to increase rapidly in future growing seasons (Hardke 2021; Harrell 2021). Quizalofop-resistant rice technology is needed due to the continuous evolution of herbicide-resistant grass weeds (Roma-Burgos et al. 2021), including problematic weeds such as barnyardgrass (*Echinochlo*a *crus-galli* (L.) P. Beauv.), weedy rice (*Oryza sativa* L.), and sprangletop (*Diplachne spp.*) (Butts et al. 2022).

Troublesome grass weeds in United States rice fields have evolved resistance to acetyl-coenzyme A carboxylase (ACCase)-inhibitors (cyhalofop, fenoxaprop); acetolactate synthase (ALS)-inhibitors (imazethapyr, imazapyr, imazamox, imazaquin, pyrithiobac-sodium); synthetic auxins (quinclorac, florpyrauxifen-benzyl), photosystem (PS) II inhibitors (propanil); lipid synthesis inhibitors (thiobencarb); and 1-deoxy-D-xyulose 5-phosphate synthase (DOXP) inhibitors (clomazone) (Heap 2022). After grass weeds evolved resistance to six WSSA herbicide sites of action, producers were left with limited viable herbicide options. Quizalofop inhibits the ACCase enzyme involved in fatty acid synthesis, which blocks phospholipid production and restricts the formation of new membranes required for cell growth (Shaner 2014). A member of the aryloxyphenoxypropionate family of herbicides, quizalofop provides a broad-spectrum, postemergence control option for annual and perennial gramineous weeds, including weedy rice and ALS-inhibitor-resistant barnyardgrass (Guice et al. 2015).

Quizalofop-resistant rice, developed through traditional mutation breeding techniques, is a non-genetically modified technology that allows for postemergence applications of quizalofop (Guice et al. 2015). PVL02 (Horizon Ag. LLC, Memphis, TN) is a long-grain, short-stature, quizalofop-resistant rice cultivar. Commercially available to producers in 2020, PVL02 had a 10% higher yield potential when compared to PVL01 (Bruce 2019). Cultivar PVL02 was derived from the cross of Cheniere, a long-grain commercially available conventional rice cultivar, and BASF 1-5, a BASF proprietary rice line with resistance to ACCase inhibitors developed through a pedigree selection system. The BASF 1-5 line contains a mutagenized rice nucleic acid gene that encodes a plastidic ACCase enzyme responsible for ACCase herbicide tolerance, substituting an isoleucine amino acid residue for leucine amino acid residue at position 1792 in the ACCase amino acid sequence (Famoso and Linscombe 2020). PVL01 (Horizon Ag. LLC, Memphis, TN) was the first quizalofop-resistant rice cultivar also derived from crossing Cheniere cultivar and BASF1-5 line (Famoso et al. 2019). RTv7231 MA (RiceTec Inc., Alvin, TX) is a Max-Ace® rice cultivar that confers resistance to quizalofop through mutation of the G2096S gene in the carboxyl transferase domain of the ACCase coding gene (Hinga et al. 2013). RTv7231 MA and PVL02 showed a yield potential of >10,000 kg ha^{-1} and >7,500 kg ha^{-1}, respectively, when evaluated in field trials in Arkansas (Frizzell et al. 2021).

Highcard™ (ADAMA, Raleigh, NC) herbicide, a safened quizalofop formulation, is labeled for RTv7231 MA, a Max-Ace® cultivar, and commercially available in the 2022 growing season (Anonymous 2021a; Anonymous 2021b). Provisia® (BASF Corporation, Research Triangle Park, NC) herbicide formulation is a non-safened quizalofop labeled for the Provisia rice cultivars, PVL01, PVL02, and PVL03 (Anonymous 2017). Herbicide safeners grant improved crop tolerance to herbicides, and safeners to aryloxyphenoxypropionate herbicides are derived from isoxadifen-ethyl (Shen et al. 2017). Postemergence quizalofop use rate in quizalofop-resistant rice varies from 100 to 138 g ai ha^{-1} for a single application and should not exceed 240 g ai ha^{-1} for the total annual application rate (Anonymous 2017; Anonymous 2021a).

Provisia® applied postemergence caused significant injury to PVL01 and resulted in severe stand loss in some commercial fields in Arkansas during the first year of commercialization (Dr. Jason Norsworthy, personal communication). Aryloxyphenoxypropionate-resistant grain sorghum (*Sorghum bicolor* L.) was injured by quizalofop applied at the 1- to 2-leaf stage at 62 g ai ha^{-1} (Abit et al. 2012). Additionally, quizalofop caused 20% injury to the quizalofop-resistant rice cultivar, PVL01, when applied pre-flood at 120 g ai ha^{-1}, but no differences in grain yield were observed (Camacho et al. 2020). With the availability of high-yielding quizalofop-resistant cultivars and grower anticipation of adopting new crop technology to combat herbicide-resistant grass weed issues, a closer examination of the causes of injury to quizalofop-resistant rice from postemergence quizalofop applications was needed.

Soil moisture affects the efficacy of the aryloxyphenoxypropionate family of herbicides. Diclofop showed reduced control of Poaceae family plants, including yellow foxtail [*Setaria lutescens (*Weigel) Hubb.], wild oat (*Avena fatua* L.), little-seed canarygrass (*Phalaris minor* Retz.), and barnyardgrass under low soil moisture conditions (Dortenzio and Norris 1980). Maximum efficacy of diclofop applied postemergence is achieved when the field is irrigated within 1- to 2-days after application (Dortenzio and Norris 1980). Boydston (1990) reported that when fenoxaprop, fluazifop, and haloxyfop were applied postemergence to green foxtail [*Setaria viridis* (L.) Beauv.], control was reduced when moisture was withheld before and after treatment; however, green foxtail control was not reduced when irrigation was applied shortly after the herbicide treatments. Furthermore, haloxyfop provided less control of green foxtail and proso millet (*Panicum miliaceum* L.) plants growing under moisture stress conditions along with reduced herbicide retention and translocation when compared to non-stressed conditions (Kidder and Behrens 1988).

Urea is the major source of pre-flood nitrogen (N) used in the delayed-flood rice production system of the southern United States and is prone to ammonia volatilization losses. Ammonium sulfate (AMS) can be utilized, instead of urea, to increase N uptake efficiency, minimize ammonia volatilization losses, and achieve higher yield (Norman et al. 2009). Containing 210 g N kg^{-1} and 240 g sulfur (S) kg^{-1}, AMS is generally utilized as a starter N source as it is less prone to ammonia volatilization. Applied between emergence and prior to the flooding of rice, AMS can increase rice early-season vigor, canopy coverage, and grain yield (Martin 2021). Urea, hydrolyzed to ammonium carbonate by the urease enzyme, is converted into ammonia gas from the ammonium form and lost into the atmosphere rapidly under high pH and wet, warm conditions (Jones et al. 2020). Chlapecka et al. (2021) showed that AMS as a starter nitrogen fertilizer source, applied to 2-leaf rice at 112 kg ha^{-1}, increased rice grain yields by over 1000 kg ha^{-1} in the direct-seeded, delayed-flood rice production system in Arkansas. Therefore, it was hypothesized that the availability of early-season soil moisture and N applications would reduce the tolerance of quizalofop-resistant rice cultivars to quizalofop. The objective of this research was to investigate the effect of early-season soil moisture and N applications on the tolerance of quizalofop-resistant rice cultivars to sequential applications of quizalofop.

Materials and Methods

Field experiments were conducted in 2021 at the Rice Research and Extension Center (RREC), Stuttgart, AR (34.47 N 91.41 W) on a Dewitt silt-loam soil (Fine, smectitic, thermic Typic Albaqualfs) with a pH of 6.0 and 1.8% organic matter content and at the Pine Tree Research Station (PTRS), Colt, AR (35.12 N 90.93 W) on a Calloway silt loam soil (Fine-Silty, mixed, active, thermic, Aquic Flaglossudalfs) having 1.3% organic matter and a soil pH of 7.5.

The trial was implemented as a two-factor factorial, randomized complete block design with four replications. Factors were treatment and variety. The following treatments were applied: nontreated control; nontreated control, fertilized with 112 kg ha^{-1} of ammonium sulfate (AMS) and surface irrigated at the 2-leaf stage; sequential quizalofop at a 1x rate; sequential quizalofop at a 2x rate; sequential quizalofop at a 1x rate, surface irrigated at the 2-leaf stage; sequential quizalofop at a 2x rate, surface irrigated at the 2-leaf stage; sequential quizalofop at a 1x rate, fertilized with 112 kg ha^{-1} AMS and surface irrigated at the 2-leaf stage; sequential quizalofop at a 2x rate, fertilized with 112 kg ha^{-1} AMS and surface irrigated at the 2-leaf stage for a total of 8 treatments. The varieties were PVL02 and RTv7231 MA. The 1x labeled use rate of quizalofop was 120 g ai ha^{-1}, and the 2x rate was 240 g ai ha^{-1}. Sequential applications of quizalofop were initiated at the 2-leaf rice stage, followed by a second application at the 5-leaf stage before flooding. Provisia® (BASF Corporation, Research Triangle Park, NC) and Highcard™ (ADAMA, Raleigh, NC) formulations of quizalofop were used for PVL02 and RTv7231 MA cultivars, respectively. In addition, a 1% v/v crop oil concentrate (Agri-Dex®, Helena Chemical Company, Collierville, TN) was added to each herbicide treatment. Treatments including surface irrigation were irrigated at 24-hours after the 2-leaf stage for the nontreated control or at 24-hour after the initial quizalofop application.

Quizalofop-resistant cultivars were planted at the PTRS on April 19, 2021, and at the RREC on April 20, 2021. PVL02 and RTv7231 MA were planted at a seeding rate of 72 and 52 seeds m^{-1} of row, respectively, in 1.8-m wide by 5.2-m long plots. Each plot consisted of 9 drill-seeded rows on 19-cm centers. Plots were maintained weed-free using clomazone (Command 3ME, FMC Corporation, Philadelphia, PA) at 336 g ai ha^{-1} applied preemergence. Halosulfuron (Permit®, Gowan Corporation, Yuma, AZ) and quinclorac (Facet L™ herbicide, BASF

Corporation, Florham Park, NJ) were applied in combination with 1% crop oil concentrate at 40 g ai ha^{-1} and 280 g ai ha^{-1}, respectively, at 3- to 4-leaf rice stage as early postemergence herbicides. All plots were fertilized with 130 kg N ha^{-1} by using urea before flooding and maintained using the University of Arkansas Extension recommendations (Hardke et al. 2022). A permanent flood of 10-cm was established at 24 hours after the 5-leaf application and was maintained until the rice was ready to harvest. All herbicide treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha^{-1} with AIXR110015 nozzles (TeeJet® Technologies, Wheaton, IL). Sequential quizalofop treatments were applied on the following dates: 2-leaf growth stage on May 21, 2021, at the PTRS, and on May 16, 2021, at the RREC; 5-leaf growth stage on June 15, 2021, at the PTRS, and on May 31, 2021, at the RREC. All plots were drained two weeks prior to harvesting when rice reached maturity.

Crop injury was rated visually on a scale of 0 to 100, with 0 representing no injury and 100 representing complete crop death. Crop injury and small unmanned aerial system (sUAS) images were taken seven days after the initial quizalofop treatment (7 DAIT), at the 5-leaf stage application, and 7, 14, 21, 28, and 35 days after the final treatment (DAFT). A DJI Phantom quadcopter small unmanned aerial system (sUAS, DJI, Shenzhen, China) captured images for groundcover analysis. The images were subjected to Field Analyzer (Green Research Services, LLC, Fayetteville, AR) software to calculate the proportion of green pixels to determine the groundcover and then converted to relative groundcover. Relative groundcover was calculated by dividing the number of green pixels of each plot by the green pixels of the respective nontreated control plot in each block. Rice shoot density per meter row in each plot was taken 21 DAFT. Dates were recorded when rice reached the 50% heading stage for each plot. All plots were drained two weeks before harvesting and harvested using a small-plot combine to determine rough rice grain yield. Yields were adjusted to 12% moisture content. The relative groundcover, relative heading, and relative yield for treatments included quizalofop alone, and quizalofop applications followed by surface irrigation only were calculated using the nontreated control for each cultivar. Treatments that included quizalofop applications followed by AMS fertilization and surface irrigation were compared to the nontreated control fertilized with AMS and surface irrigated at the 2-leaf stage for each cultivar individually.

Statistical Analysis. All data were subjected to analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, Cary, NC). Location and block nested within locations were considered random effects. Treatment and cultivar were considered fixed effects. Beta distribution was assumed for visible injury while gamma distribution was used for relative groundcover and relative yield. Normal and Poisson distributions were assumed for relative heading and shoot density, respectively. For injury and relative groundcover response variables, rating timing was considered a repeated-measure variable that allowed for comparisons across ratings taken on the same plot over the same interval and included in the treatment structure as a fixed effect. Correlations across rating timings for the fixed effects and residuals were modeled using an autoregressive correlation structure by including TYPE = ar (1) in the RANDOM statement. An analysis of variance (ANOVA) table showing the main effects of cultivar, treatment, and rating timing and their interaction for visual injury and relative groundcover response variable is listed in Table 1. Additionally, the main effects and interactions of cultivar and treatment for relative yield are also shown in Table 1. Means were separated using Fisher's protected LSD test at $\alpha = 0.05$.

Results and Discussion

Greater injury to quizalofop-resistant cultivars was observed at 14 and 21 DAFT, and rice recovered at later ratings, averaged over cultivars (Appendix Table 1). Visual injury caused by sequential quizalofop treatments to quizalofop-resistant cultivars ranged from 1% to 6% for all evaluations, regardless of cultivar or location. Early-season soil moisture and nitrogen availability after the initial quizalofop application had minimal effect on quizalofop-resistant rice sensitivity to quizalofop as the visual injury was ≤6% regardless of cultivar and location (Appendix Table 1). The results of these trials are similar to the findings of Camacho et al. (2020), where quizalofop caused transient injury to quizalofop-resistant cultivars at a 1x or 2x rate, and minimal injury in early growth stages was insufficient to deleteriously affect yield potential. Beckett et al. (1992) showed that the addition of AMS to quizalofop did not consistently affect the herbicide efficacy on volunteer corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herrm.).

When injury ratings were pooled over treatments, quizalofop applications to the RTv7231 MA cultivar resulted in injury ratings 2- to 6-percentage points higher than PVL02 (Table 2). However, RTv7231 MA had ≤9% injury, and PVL02 had ≤3% injury, averaged over injury evaluations, respectively, regardless of treatments (Table 3). RTv7231 MA was more sensitive when initial quizalofop applications were followed by AMS fertilizer and surface irrigation than quizalofop applications alone (Table 3). Additionally, the RTv7231 MA cultivar had 5-percentage points higher injury ratings when treated with sequential quizalofop applications at the 2x rate, fertilized with AMS, and surface irrigated compared to the sequential quizalofop treatments at the 2x rate (Table 3). On PVL02, sequential quizalofop applications at the 1x or 2x rate caused 2-percentage points higher injury than sequential quizalofop applications at a 1x rate or 2x rate followed by fertilization with AMS and surface irrigated, averaged over rating timings (Table 3). The injury levels observed in this trial were slight at best and were unlikely to cause concern for a grower. Harker (1995) documented that quizalofop phytotoxicity to barley (*Hordeum vulgare* L.) was not influenced by the addition of AMS to spray solution under greenhouse conditions; however, quizalofop phytotoxicity increased to wild oat (*Avena fatua* L.) as an additive response after the addition of AMS to spray mixture. The differing response of barley and wild oat to quizalofop validated the inconsistency in quizalofop injury when applied in combination with AMS.

Groundcover of treated quizalofop-resistant cultivars started recovering relative to nontreated plots after 14 DAFT. Less than a 10-percentage point reduction in groundcover relative to the nontreated controls was observed after 14 DAFT when averaged over cultivars (Table 4). Before the 14 DAFT rating, relative groundcover was not consistent, and heterogeneity was observed across treatments, as quizalofop causes chlorosis and necrotic symptoms 1 to 3 weeks after application (Shaner 2014). At 21 DAFT, sequential quizalofop at the 1x rate had 94% relative groundcover of quizalofop-resistant rice; however, quizalofop applications at the 1x rate that were surface irrigated or fertilized with AMS then surface irrigated at the 2-leaf stage application had ≥100% relative groundcover averaged over quizalofop-resistant cultivars (Table 4). Pooled over quizalofop-resistant cultivars, sequential quizalofop alone at the 2x rate had 92% relative groundcover of rice. In comparison, ≥100% relative groundcover occurred when quizalofop was applied at the 2x rate, surface irrigated or fertilized with AMS then surface irrigated at the 2-leaf stage application when evaluated at 28 DAFT (Table 4). Averaged over treatments, greater injury to RTv7231 MA translated into a 28- to 36-percentage points reduction in groundcover compared to the nontreated control, which persisted from 7 DAIT to 7 DAFT. PVL02 had only a 16-percentage point reduction in groundcover compared to the nontreated control and with the reduction in groundcover persisting from 7 DAIT through the 5-leaf stage application (Table 5). No differences in groundcover relative to the nontreated control for PVL02 and RTv7231 MA were observed after the 5-leaf stage quizalofop application and 7 DAFT, respectively, evidence that both cultivars quickly recovered from the early-season injury caused by quizalofop (Table 5). These findings coincide with those of Godara et al. (2021), where sequential quizalofop applications at 120 g ai ha^{-1} caused greater injury and more reduction in relative groundcover of RTv7231 MA than PVL02 cultivar, but no differences in yield potential were observed.

An ANOVA table with the main effects of cultivar and treatment and their interaction for rice shoot density per meter of row at 21 DAFT and relative heading response variables is in Appendix Table 2. The PVL02 cultivar had 99 shoots m^{-1} row while RTv7231 MA had 94 shoots m^{-1} row, averaged over treatments (Appendix Table 3). PVL02 was planted at a higher seeding rate than RTv7231 MA, which contributed to the higher shoot density.

PVL02 had a delay of 1 day to reach the 50% heading stage, while no delay was observed on RTv7231 MA compared to the nontreated control for each cultivar, pooled over treatments (Appendix Table 3). Sequential quizalofop applied at the 2x rate, surface irrigated or quizalofop applied sequentially followed by fertilized with AMS, then surface irrigated at the 2-leaf stage caused a delay of 1 day to reach the 50% heading stage of rice, averaged over cultivars (Appendix Table 4). No other delays in relative heading were observed and a delay of 1 day to reach the 50% heading stage is unlikely of biological significance (Appendix Table 4). Visual injury and reduction in relative groundcover of quizalofop-resistant cultivars caused by sequential quizalofop treatment combinations were not sufficient to account for heading delay and yield reductions. Lawrence et al. (2020) observed that AMS applied at 24 kg N ha^{-1} during the 1- to 4-leaf rice growth stage did not alleviate the herbicide injury and did not impact the rice yield potential following exposure to the sub-lethal rate of paraquat. Similarly, the availability of nitrogen fertilizer and surface irrigation at the 2-leaf stage after initial quizalofop application did not facilitated the quizalofop-resistant rice recovery and yield potential.

PVL02 and RTv7231 MA have a high tolerance level to sequential quizalofop applications when dry soil conditions persist prior to quizalofop applications. Dortenzio and Norris (1980) reported that maximum efficacy of the aryloxyphenoxypropionate herbicide, diclofop, was observed when irrigation occurred within 1- to 2-days after diclofop application. In contrast to Dortenzio and Norris (1980), the availability of early-season soil moisture and N applications after quizalofop applications had a minimal effect on the tolerance level of quizalofop-resistant cultivars to quizalofop applications. Future research needs to evaluate saturated soil moisture conditions and N applied before a quizalofop application. These conditions after application did cause substantial injury to either rice cultivar, and unacceptable levels of crop injury or death have been observed in some commercial fields of quizalofop-resistant rice treated with quizalofop under saturated soil conditions. Furthermore, temperature and cloud cover different from what was experienced in these trials may play a role in the sensitivity of quizalofop-resistant rice to quizalofop herbicide.

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Appendix

Table 1. Analysis of variance for rice injury, relative groundcover, and relative yield as influenced by the effect of early-season soil moisture and nitrogen applications on quizalofop-resistant rice tolerance to quizalofop applications from the experiments conducted at the Pine Tree Research Station, Colt, AR, and the Rice the Research and Extension Center, Stuttgart, AR in 2021.[a]

	P-value		
Factor	Injury	Relative groundcover	Relative yield
Cultivar	<0.0001*	0.0056*	0.6107
Treatment	0.3337	0.0020*	0.5871
Rating timing	<0.0001*	<0.0001*	-
Cultivar × Treatment	0.001*	0.5166	0.8551
Cultivar × Rating timing	<0.0001*	0.0027*	-
Treatment × Rating timing	0.0002*	<0.0001*	-
Cultivar × Treatment × Rating timing	0.4013	0.8582	-

[a]*P*-values followed by * are significant ($\alpha = 0.05$).

Table 2. Injury to quizalofop-resistant cultivars at different rating timings averaged over treatments [sequential quizalofop applications alone and with surface irrigation or nitrogen (N) application at the 2-leaf rice stage after the initial quizalofop application] from the study conducted in 2021.[a]

Cultivar	Injury[b]													
	7 DAIT[c]		5-leaf stage		7 DAFT		14 DAFT		21 DAFT		28 DAFT		35 DAFT	
	---%---													
PVL02	3	cd	1	f	2	ef	2	c-f	2	cde	2	def	1	f
RTv7231 MA	5	ab	3	cd	7	a	7	a	8	a	4	b	3	c

[a]Data were pooled over two locations (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR).
[b]Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[c]Abbreviations: DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

Table 3. Injury to quizalofop-resistant cultivars caused by treatments averaged over rating timings (7 days after 2-leaf stage application, 5-leaf stage, 7, 14, 21, 28, and 35 days after 5-leaf stage application) in the study conducted in 2021.[a]

	Injury[b]			
Treatment[c]	PVL02		RTv7231 MA	
	---------------------------------%---------------------------------			
SQ 1x	3	cd	3	cd
SQ 1x SI	1	e	4	bc
SQ 1x AMS SI	1	e	4	bc
SQ 2x	3	cd	4	bc
SQ 2x SI	2	de	6	ab
SQ 2x AMS SI	1	e	9	a

[a]Data were pooled over two sites (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR).

[b]Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.

[c]Abbreviations: SQ, quizalofop application at the 2-leaf stage followed by the 5-leaf stage application; 1x, quizalofop at 120 g ai ha^{-1}; 2x; quizalofop at 240 g ai ha^{-1}; SI, 2-leaf stage quizalofop application followed by surface irrigation; AMS, ammonium sulfate; AMS SI, 2-leaf stage quizalofop application followed by fertilized with 112 kg ha^{-1} of ammonium sulfate and surface irrigation.

Table 4. Relative groundcover of quizalofop-resistant cultivars compared to the nontreated control at different rating timings averaged over cultivars (PVL02 and RTv7231 MA) in the field experiments conducted in 2021.[a]

	Relative groundcover[c]						
Treatment[b]	7 DAIT	5-leaf stage	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	-----------------------------	-----------------------------	-----------------------------	%	-----------------------------	-----------------------------	-----------------------------
SQ 1x	107 a-e	78 h-l	92 c-j	99 a-i	94 c-j	94 c-j	99 a-i
SQ 1x SI	58 m	63 lm	81 f-k	98 a-i	124 ab	118 abc	97 b-i
SQ 1x AMS SI	92 c-j	84 e-k	87 d-k	105 a-f	100 a-h	101 a-g	100 a-i
SQ 2x	94 c-j	74 j-m	94 c-j	98 a-i	97 b-i	92 c-j	99 a-i
SQ 2x SI	37 n	61 lm	73 j-m	87 d-k	118 abc	126 a	94 c-j
SQ 2x AMS SI	77 i-l	89 d-k	79 g-l	111 a-d	103 a-f	100 a-i	106 a-e

[a]Data were pooled over two sites (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR). Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.

[b]Abbreviations: SQ, quizalofop application at the 2-leaf stage followed by the 5-leaf stage application; 1x, quizalofop at 120 g ai ha^{-1}; 2x; quizalofop at 240 g ai ha^{-1}; SI, 2-leaf stage quizalofop application followed by surface irrigation; AMS, ammonium sulfate; AMS SI, 2-leaf stage quizalofop application followed by fertilized with 112 kg ha^{-1} of ammonium sulfate and surface irrigation; DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

[c]Relative groundcover for treatments consisted of quizalofop alone and quizalofop applications followed by surface irrigation only was calculated using the nontreated control for each cultivar while treatments included quizalofop applications followed by AMS fertilization and surface irrigation was calculated by using the nontreated control fertilized with AMS and surface irrigated for each cultivar individually.

Table 5. Relative groundcover of quizalofop-resistant cultivars compared to the nontreated control at different rating timings averaged over treatments (sequential quizalofop applications alone and with surface irrigation or nitrogen application at the 2-leaf rice stage after the initial quizalofop application) from the experiments conducted in 2021.[a]

Cultivar	Relative groundcover[c]													
	7 DAIT[b]		5-leaf stage		7 DAFT		14 DAFT		21 DAFT		28 DAFT		35 DAFT	
	---%---													
PVL02	84*	d	84*	d	96	a-d	88	cd	103	ab	106	ab	97	a-d
RTv7231 MA	64*	e	65*	e	72*	e	92	bcd	108	a	104	ab	102	abc

[a]Data were pooled over locations (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR). Means followed by same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.
[c]Relative groundcover for treatments consisted of quizalofop alone and quizalofop applications followed by surface irrigation only was calculated using the nontreated control for each cultivar while treatments included quizalofop applications followed by ammonium sulfate (AMS) fertilization and surface irrigation were compared to the nontreated control fertilized with AMS and surface irrigated for each cultivar individually.
[d]Asterisk indicates actual groundcover for the quizalofop-treated plots was less than the nontreated control within a cultivar.

Supplemental Material

Table 1. Rice injury caused by the sequential quizalofop treatments at different rating timings averaged over quizalofop-resistant cultivars (PVL02 and RTv7231 MA) from the experiments conducted in 2021.[a]

	Injury[b]						
Treatment[c]	7 DAIT	5-leaf stage	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%						
SQ 1x	2 d-l	2 h-l	2 d-l	6 a	4 a-e	3 b-j	2 e-l
SQ 1x SI	3 b-k	2 e-l	3 a-j	2 d-l	3 b-j	2 h-l	1 kl
SQ 1x AMS SI	3 a-i	1 jkl	3 a-i	2 d-l	3 b-j	2 f-l	2 i-l
SQ 2x	3 a-h	1 l	4 a-g	6 abc	6 ab	4 a-g	3 d-l
SQ 2x SI	6 abc	2 d-l	4 a-f	3 a-i	4 a-e	3 b-j	2 g-l
SQ 2x AMS SI	5 a-d	3 c-k	4 a-g	3 a-i	4 a-e	3 a-i	2 d-l

[a]Data were pooled over two locations (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR).
[b]Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[c]Abbreviations: SQ, quizalofop application at the 2-leaf stage followed by the 5-leaf stage application; 1x, quizalofop at 120 g ai ha^{-1}; 2x; quizalofop at 240 g ai ha^{-1}; SI, 2-leaf stage quizalofop application followed by surface irrigation; AMS, ammonium sulfate; AMS SI, 2-leaf stage quizalofop application followed by fertilized with 112 kg ha^{-1} of ammonium sulfate and surface irrigation; DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

Table 2. Analysis of variance for rice shoot density at 21 days after 5-leaf stage quizalofop application and relative heading as influenced by the effect of early-season soil moisture and nitrogen (N) applications on quizalofop-resistant rice tolerance to quizalofop in the study conducted at the Pine Tree Research Station, Colt, AR, and the Rice Research and Extension Center, Stuttgart, AR in 2021.

	P-value	
Factor	Shoot density	Relative heading
Cultivar	0.0089*	0.0008*
Treatment	0.3419	0.0039*
Cultivar × Treatment	0.6836	0.0751

[a]P-values followed by * are significant (α = 0.05).

Table 3. Rice shoot density per meter row and relative heading of quizalofop-resistant cultivars compared to nontreated control averaged over treatments (sequential quizalofop applications alone and with surface irrigation or nitrogen application at the 2-leaf rice stage after the initial quizalofop application) from the experiments conducted in 2021.[a]

Cultivar	Shoot density 21 DAFT[b]		Relative heading[c]	
	counts m^{-1}		d	
PVL02	99	a	1	a
RTv7231 MA	94	b	0	b

[a]Data were pooled over two sites (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR). Means followed by same letter in a column are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.

[b]Abbreviations: DAFT, days after final treatment at 5-leaf stage; d, days delay to 50% heading stage compared to the nontreated control.

[c]Relative heading for treatments consisted of quizalofop alone and quizalofop applications followed by surface irrigation only was determined using the nontreated control for each cultivar while treatments included quizalofop applications followed by ammonium sulfate (AMS) fertilization and surface irrigation were compared to the nontreated control fertilized with AMS and surface irrigated for each cultivar individually to determine 50% heading stage.

Table 4. Relative heading of quizalofop-resistant cultivars compared to nontreated control caused by different treatments averaged over cultivars (PVL02 and RTv7231 MA) in the experiments conducted in 2021.[a]

Treatment[b]	Relative heading[c]	
	d	
SQ 1x	0	bc
SQ 1x SI	0	bc
SQ 1x AMS SI	0	bc
SQ 2x	0	c
SQ 2x SI	1	a
SQ 2x AMS SI	1	ab

[a]Data were pooled over locations (Pine Tree Research Station, Colt, AR, and Rice Research and Extension Center, Stuttgart, AR). Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[b]Abbreviations: SQ, quizalofop application at the 2-leaf stage followed by the 5-leaf stage application; 1x, quizalofop at 120 g ai ha^{-1}; 2x; quizalofop at 240 g ai ha^{-1}; SI, 2-leaf stage quizalofop application followed by surface irrigation; AMS, ammonium sulfate; AMS SI, 2-leaf stage quizalofop application followed by fertilized with 112 kg ha^{-1} of ammonium sulfate and surface irrigation; d, days delay to 50% heading stage compared to the nontreated control.
[c]Relative heading for treatments consisted of quizalofop applications alone and quizalofop applications followed by surface irrigation only was determined using the nontreated control for each cultivar separately while treatments included quizalofop applications followed by AMS fertilization and surface irrigation were compared to the nontreated control fertilized with AMS and surface irrigated for each cultivar individually to determine 50% heading stage.

Chapter 3

Quizalofop-Resistant Rice Response to Quizalofop when Exposed to Low Rates of Glyphosate and Imazethapyr

Abstract

Quizalofop-resistant rice technology became commercially available for mid-southern U.S. growers in 2018. Crop injury was reported in some fields following postemergence applications of quizalofop. Glyphosate-resistant (GR) corn, cotton, soybean, and imidazolinone-resistant rice are grown near quizalofop-resistant rice. Herbicide drift from glyphosate and imazethapyr and the resulting crop injury and potential yield loss is a cause of concern for producers. Field experiments conducted near Colt, AR, and in Keiser, AR, in 2021 and evaluated whether low rates of glyphosate or imazethapyr interact with sequential quizalofop applications to exacerbate injury to quizalofop-resistant rice compared to applications of quizalofop alone. Herbicide treatments consisted of a low rate of glyphosate (90 g ae ha^{-1}) or imazethapyr (10.7 g ai ha^{-1}) applied 10, 7, 4, and 0 days before the 2-leaf growth stage of rice, and glyphosate or imazethapyr, at the same rate and timings, followed by quizalofop at 120 g ai ha^{-1} applied to 2-leaf rice. All plots treated with quizalofop received a subsequent application of the same herbicide and rate at the 5-leaf stage of rice. At 28 days after final treatment (DAFT), glyphosate followed by quizalofop the same day to 2-leaf rice caused 77% injury compared to 58% when glyphosate was applied alone, regardless of location. Glyphosate followed by quizalofop the same day reduced rough rice grain yield by 67% compared to 33% when glyphosate was applied alone to 2-leaf rice at Colt, AR. Imazethapyr followed by quizalofop the same day to 2-leaf rice caused more injury (63% and 19% injury at Colt and Keiser, AR, respectively) than imazethapyr

alone (42% and 7% injury at Colt and Keiser, AR, respectively) when evaluated at 35 DAFT. Overall, glyphosate and imazethapyr followed by quizalofop applications worsened injury compared to glyphosate, imazethapyr, and sequential quizalofop applications alone. As the interval between exposure to a low rate of glyphosate or imazethapyr and quizalofop decreases, the detrimental effect of the herbicide on quizalofop-resistant rice likewise increases.

Nomenclature: Glyphosate; imazethapyr; quizalofop; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; rice, *Oryza sativa* L.; soybean, *Glycine max* (L.) Merr.

Introduction

Arkansas is the largest rice (*Oryza sativa* L.)-producing state in the United States, accounting for approximately half of the nation's total area under rice cultivation, with over 502,225 hectares planted in 2021 (NASS-USDA 2021). Provisia® rice (BASF Corp., Research Triangle Park, NC, USA) is a non-genetically modified herbicide-resistant technology developed using traditional breeding techniques. Provisia rice allows for postemergence applications of quizalofop (Provisia, BASF Corporation, Research Triangle Park, NC) and has been commercially available since 2018. Quizalofop is an acetyl coenzyme-A carboxylase (ACCase)-inhibiting herbicide used for managing acetolactate synthase (ALS)-resistant barnyardgrass (*Echinochlo*a *crus-galli* (L.) P. Beauv.), and weedy rice (*Oryza sativa* L.), along with other annual and perennial grass weeds (Anonymous 2017; Guice et al. 2015). Provisia rice constituted 2.7% of the total rice hectares planted in Arkansas in the growing season of 2020. However, when evaluated in commercial rice trials (Frizzell et al. 2021, Hardke 2021), quizalofop-resistant rice has had lower yield potential resulting in reduced commercial adoption even though quizalofop is another tool to manage herbicide-resistant weedy rice (Roma-Burgos et al. 2021). A single-point mutation confers the resistance mechanism in quizalofop-resistant rice, and the presence of the resistant ACCase allele can be detected at low cost with standard molecular biology laboratory equipment. Therefore, rapid and effective development of new quizalofop-resistant varieties is feasible (Camacho et al. 2020; Pereira et al. 2019).

Conventional and quizalofop-resistant rice are often grown next to glyphosate-resistant (GR) corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], and imidazolinone-resistant rice, increasing the potential for injury to conventional and quizalofop-resistant rice from herbicide drift. Conventional and quizalofop-resistant rice acreage is highly susceptible to injury from glyphosate and imidazolinone herbicides due to herbicide drift, tank contamination, or misapplication. Out of 94 confirmed cases of herbicide drift onto rice in Arkansas, 35% and 14% of cases were caused by glyphosate and imazethapyr drift, respectively, on conventional rice hectares from 2010 to 2020 (personal communication, Susie Nichols of Arkansas Department of Agriculture). In a 2019 survey representing 49% of all agronomic crop hectares in Arkansas, ground and aerial application equipment were used in almost equivalent proportions for herbicide applications, and herbicide drift was the primary concern for producers in terms of herbicide application challenges (Butts et al. 2021).

Glyphosate has been the most widely used herbicide in U.S. agronomic crops since the commercialization of GR crop technology (Benbrook 2016). A broad-spectrum, non-selective herbicide, glyphosate is used preplant to control existing weeds or postemergence in genetically modified GR crops. Glyphosate inhibits enolpyruvyl shikimate-3-phosphate (EPSP) synthase, causing aromatic amino acid depletion by blocking the shikimic acid pathway (Amrhein et al. 1980; Shaner 2014). As a result of glyphosate application, growth ceases for sensitive plants, followed by chlorosis and necrosis symptomology, then plant death (Shaner 2014). Glyphosate at sub-lethal rates caused 14% injury and 32% yield reduction of conventional rice when applied at the 3- to 4-leaf stage (Davis et al. 2011). In addition, sub-lethal glyphosate rates are detrimental to rice and have been shown to cause substantial injury and yield reductions ranging from 18% to 89% when plants were exposed to a 1/8x rate at the 2- to 3-leaf to the booting rice stages (Kurtz and Street 2003). In Arkansas, a survey conducted in 2020 showed that glyphosate was used as a preplant option individually or in combination with protoporphyrinogen oxidase (PPO)-inhibitors, synthetic auxins, and diterpene biosynthesis inhibitors on 60% of total rice fields surveyed (Roma-Burgos et al. 2021). Therefore, glyphosate applied preplant could potentially cause injury to the emerged conventional and quizalofop-resistant rice fields in the early rice growth stages.

Clearfield® (HorizonAg, LLC, Memphis, TN, USA) rice is a non-transgenic, imidazolinone-resistant suite of rice cultivars developed using conventional plant-breeding approaches (Croughan 2003). Additionally, FullPage® (RiceTec Inc., Alvin, TX) rice technology was commercialized in 2019. FullPage® rice is an imidazolinone-resistant, non-genetically modified rice that allows for applications of imazethapyr (Preface™) and imazamox (Postscript™) (Anonymous 2019). The same active ingredients are enabled in Clearfield rice under the trade names Newpath and Beyond. In the imidazolinone-resistant rice system, imazethapyr is essential for preemergence (PRE) and postemergence (POST) weed control programs. Imazethapyr provides broad-spectrum weed control of sedges (*Cyperus* spp.), annual grasses, and broadleaf weeds (Barber et al. 2022). Imazethapyr obstructs the synthesis of branched-chain amino acids leucine, isoleucine, and valine by inhibiting acetolactate synthase or acetohydroxy acid synthase (Shaner 2014). Imazethapyr reduces plant growth within hours of herbicide application. The primary herbicide symptoms at 7- to 14-days after herbicide application are chlorosis in the meristematic region of the plant and necrosis throughout the plant (Shaner 2014).

Clearfield rice technology was brought to market in 2002 and quickly adopted by rice producers. By 2013, 43% of the total U.S. rice hectares were planted with imidazolinone-resistant rice cultivars (Nathan et al. 2020). In Arkansas, 37% of the total rice was planted with imidazolinone-resistant rice cultivars (30.6% and 6.4% with Clearfield and FullPage rice technology, respectively) in 2020 (Hardke 2021). In 2020, a survey conducted across Arkansas and adjacent U.S. mid-South states revealed that imazethapyr was applied as a POST option on 48% of the surveyed rice fields (Roma-Burgos et al. 2021). After the large-scale adoption of imidazolinone-resistant rice, the risk for imazethapyr pre-exposure to conventional rice fields also escalated. Lower-than-labeled rates of imazethapyr cause severe damage to conventional rice. Higher crop injury occurs in one-tiller rice, and the highest reduction in rice yield follows exposure at the boot stage (Hensley et al. 2012). In addition, an imazethapyr and imazapyr premix at low rates caused higher rice injury when applied at early rice stages than at later stages and reduced rice plant height and yield potential when applied as a late POST application (Bond et al. 2006).

Widespread glyphosate application in GR crops and imazethapyr application in imidazolinone-resistant rice can drift glyphosate and imazethapyr onto neighboring fields planted with conventional or quizalofop-resistant rice cultivars (Davis et al. 2011; Koger et al. 2005; Martin et al. 2018). The extent of injury to rice can be estimated by assessing chlorophyll content, glyphosate, or imazethapyr concentrations in plant tissue and the yield potential of treated plants (Ding et al. 2011; Ellis et al. 2003; Reddy et al. 2010). Camacho et al. (2020) have shown that quizalofop applied at 120 g ai ha^{-1} caused up to 26% injury on quizalofop-resistant rice, resulting in slight chlorosis and necrosis symptoms, but plants generally recovered from injury at later stages. Growers will likely adopt better yielding quizalofop-resistant cultivars in the coming years due to the continuous evolution of herbicide-resistant weeds in rice production systems. As this technology's adoption increases, so does the risk for sub-lethal drift of glyphosate or imazethapyr onto quizalofop-resistant rice. Therefore, it was hypothesized that glyphosate or imazethapyr would intensify the injury to quizalofop-resistant cultivars that can result from sequential quizalofop applications. The objective of this research was to determine if low rates of glyphosate and imazethapyr interact with sequential quizalofop applications to increase the risk for injury to quizalofop-resistant rice over applications of quizalofop alone.

Materials and Methods

Field experiments were conducted in the summer of 2021 on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Flaglossudalfs) having a pH of 7.8 and 1.7% organic matter at the Pine Tree Research Station (PTRS), Colt, AR (35°12’49.9” N, 90°93’12.4” W), and on a Sharkey clay loam (Very-fine, smectitic, thermic Chromic Epiaquerts) with pH of 6.7 and 1.7% organic matter at the Northeast Research and Extension Center (NEREC), Keiser, AR (35°67’65.9” N, 90°08’68.4” W). Separate experiments were conducted evaluating a low rate of glyphosate on quizalofop-resistant rice (Provisia®) and a low rate of imazethapyr on quizalofop-resistant rice.

Exposure to Low Glyphosate Rate. Glyphosate (Roundup PowerMax®, Monsanto Company, St. Louis, MO) at 90 g ae ha^{-1}, 1/12.5 of the labeled use rate in soybean, was applied 10, 7, 4, and 0 days before the 2-leaf growth stage of rice. Glyphosate at the same rate and timings was followed by quizalofop (Provisia®) at 120 g ha^{-1} applied to 2-leaf rice. Quizalofop was applied immediately following glyphosate application at the 0-day timing. All plots treated with quizalofop received a subsequent herbicide application at the same rate at the 5-leaf stage of rice. Quizalofop treatments were applied combined with 1% v/v crop oil concentrate (Agri-Dex®, Helena Chemical Company, Collierville, TN). A nontreated control and sequential quizalofop (no glyphosate or imazethapyr exposure) treatments were also included in the experiments for a total of 10 treatments. All application timings were based on the size of the rice in the nontreated control plots, and herbicide applications for the 10 days before 2-leaf growth stage were initiated when rice reached the 1-leaf growth stage. A 10-day interval was observed between the 1- and 2-leaf growth stages at both locations.

Exposure to Low Imazethapyr Rate. Imazethapyr (Newpath®, BASF Corporation, Research Triangle Park, NC) was applied at 10.7 g ai ha^{-1}, 1/10 of recommended use rate in imidazolinone-resistant rice at 10, 7, 4, and 0 days prior to 2-leaf growth stage. An imazethapyr application at the same rate and timings was followed by quizalofop (Provisia®) at the 2-leaf growth stage. Quizalofop was applied immediately after imazethapyr application at the 0-day interval to the 2-leaf rice. All plots treated with quizalofop received a subsequent quizalofop application at the 5-leaf stage. All quizalofop applications were made at the labeled rate of 120 g ai ha^{-1}. A 0.25% v/v non-ionic surfactant (Induce®, Helena Chemical Company, Collierville, TN) was added to each imazethapyr application, and 1% v/v crop oil concentrate (Agri-Dex®) was added to each quizalofop application. Experiments also included a nontreated control and sequential quizalofop (no glyphosate or imazethapyr exposure) treatment, with the initial application at the 2-leaf stage followed by another quizalofop application at the 5-leaf rice stage for a total of 10 treatments. The size of rice in nontreated control plots was utilized for application timings.

Methods Common to Both Studies. Experiments at the NEREC and PTRS were planted on May 20, 2021, and May 21, 2021, respectively. A quizalofop-resistant cultivar “PVL02” (Horizon Ag, LLC, Memphis, TN) was planted at a seeding rate of 72 seeds m^{-1} row into 1.8-m wide by 5.2-m long plots at a 1.3-cm depth. Each plot consisted of 9 drill rows spaced 19-cm apart. All plots were maintained using the University of Arkansas System Division of Agriculture’s Cooperative Extension Service’s recommended practices for proper stand establishment, fertilization, and pest management (Hardke et al. 2022). Plots were kept weed-free with labeled herbicides. Clomazone (Command 3ME, FMC Corporation, Philadelphia, PA) was applied preemergence at 560 and 336 g ai ha^{-1} at NEREC and PTRS, respectively. Halosulfuron (Permit®, Gowan Corporation, Yuma, AZ) and quinclorac (Facet L™ herbicide, BASF Corporation, Florham Park, NJ) combined with 1% crop oil concentrate were applied to 4-leaf rice at 40 g ai ha^{-1} and 280 g ai ha^{-1}, respectively. All herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 94 L ha^{-1} at 276 kPa. A four-nozzle, 1.5-m wide spray boom equipped with TeeJet® AIXR 110015 nozzles (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA) was used. Plots were fertilized with urea (46-0-0) at 350 kg ha^{-1} and 280 kg ha^{-1} at NEREC and PTRS, respectively, when rice reached the 5-leaf stage and flooded until rice reached maturity. Experimental sites were drained two weeks before harvesting.

Visual injury estimates were rated on a 0 to 100 scale, with 0 being no injury and 100 being crop death compared to the nontreated control at 7, 14, 21, 28, and 35 days after final treatment (DAFT) with glyphosate or imazethapyr alone, or glyphosate or imazethapyr followed by the 2-leaf stage quizalofop application. Overall visual injury were rated for glyphosate, imazethapyr, and quizalofop based on chlorosis, necrosis, and stunting of the rice plants and injury symptomology were not evaluated individually for each herbicide. Groundcover was assessed by taking pictures from a DJI Phantom quadcopter small unmanned aerial system (sUAS) (DJI, Shenzhen, China) on a weekly basis after the 1-leaf rice stage application. Images were analyzed using Field Analyzer (Green Research Services, LLC, Fayetteville, AR) to determine the proportion of green pixels in each image to assess the amount of groundcover reduction at 7, 14, 21, 28, and 35 DAFT. The date that each plot reached the 50% heading stage was recorded. Heading dates are reported as days relative to the nontreated control reaching 50% heading. Each plot was harvested using a small-plot combine to determine the rough grain yield, adjusted to 12% moisture. For each treatment, groundcover and yield were expressed in terms of the percentage of the corresponding nontreated control in each block.

Data Analysis. All data were analyzed using SAS, version 9.4 (SAS Institute Inc., Cary, NC), and means were subjected to analysis of variance (ANOVA) using the GLIMMIX procedure. All ANOVA results are shown in Tables 1 and 2. Experiments were implemented as a split-plot arrangement with a randomized complete block design, replicated four times. The site was considered a whole-plot factor, while herbicide treatment was considered a split-plot factor. The main effects of the site and herbicide treatment and their interaction were treated as fixed effects. Blocks nested within site and herbicide treatment nested within site were treated as random effects. Beta distribution was assumed for rice injury, and gamma distribution was used for relative groundcover and relative yield (Gbur et al. 2012). Normal distribution was used for days until heading relative to the nontreated control. Means were separated using Fisher's protected LSD test ($\alpha = 0.05$), and the Kenward-Roger degree-of-freedom approximation was utilized. For injury and groundcover response variables, rating timing was considered a repeated-measure variable that allowed for comparisons across ratings and included in the treatment structure as a fixed effect. Correlations across ratings for the fixed effects and residuals were modeled using an independence covariance structure for injury and groundcover. There was no correlation between rating timings when residuals were evaluated qualitatively (Gbur et al. 2012).

Results and Discussion

Provisia Rice Exposure to Glyphosate. Injury to rice was generally greatest when glyphosate was applied alone at the 2-leaf stage or was followed by a quizalofop application on the same day (Table 3). Averaged over locations, injury to quizalofop-resistant rice at 7 and 14 DAFT was similar for glyphosate applied alone at a 10-, 7-, 4-, and 0-day interval prior to 2-leaf stage and glyphosate at a 10-, 7-, 4-, and 0-day interval before quizalofop applied to 2-leaf rice, respectively (Table 3). At 21 DAFT, regardless of location, quizalofop application on the same day as glyphosate at the 2-leaf stage caused 15-percentage points greater injury than glyphosate alone at the 2-leaf stage (Table 3). Greater injury to rice (19-percentage points) occurred with the addition of quizalofop persisted through 35 DAFT (Table 3). Glyphosate followed by quizalofop at a 4-day interval caused a 10-percenatge point increase in injury over glyphosate applied alone four days before the 2-leaf stage when evaluated at 28 DAFT, regardless of location. There were no differences in injury between glyphosate followed by quizalofop at a 7- and 10-day interval compared to glyphosate applied alone at a 7- and 10-day interval before the 2-leaf growth stage of rice at all rating timings averaged over both locations (Table 3). The greatest injury to rice caused by glyphosate was generally observed at 14, and 21 DAFT, especially when glyphosate alone was applied to 2-leaf rice or glyphosate was followed by quizalofop on the same day (Table 3).

Glyphosate followed by quizalofop at a 0-day interval caused 88% injury compared to 69% injury caused by glyphosate applied alone at the same timing at PTRS, pooled over ratings (Table 4). No differences were observed in injury between glyphosate alone or glyphosate followed by quizalofop at the 0-day interval at NEREC, averaged over ratings. Additionally, pooled over ratings, <3% injury was observed from sequential quizalofop applications at both locations (Table 4). Higher air temperature during glyphosate application at NEREC probably caused greater injury at this location than at PTRS from glyphosate applied alone or glyphosate followed by quizalofop at a 10- and 7-day interval averaged over ratings. However, injury ratings did not differ between locations when glyphosate was applied alone at a 4-day interval compared to glyphosate applied four days prior to quizalofop, regardless of the rating dates (Table 4). Ellis et al. (2003) documented a similar finding that variations in air temperature conditions across site years affected the crop response in terms of visual injury, plant height, and yield when sub-lethal rates of glyphosate were applied to rice. In contrast, greater injury to quizalofop-resistant rice was observed after glyphosate alone or glyphosate followed by quizalofop at the 0-day interval, averaged over ratings at PTRS compared to NEREC due to saturated soil conditions during herbicide treatment. Previous research reported that higher soil moisture content increased the efficacy of the aryloxyphenoxypropionate herbicide diclofop on barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] and glyphosate on windmill grass (*Chloris truncata* R.Br.) (Dortenzio and Norris 1980; Peerzada et al. 2021).

Sequential quizalofop applications caused a <16 percentage point reduction in relative groundcover to rice compared to the nontreated control at both locations averaged across evaluations (Table 5). Glyphosate followed by quizalofop the same day caused a 6- to 47-percentage point reduction in relative groundcover compared with glyphosate alone at PTRS when evaluated at 14, 21, 28, and 35 DAFT (Table 5). At both locations, no differences in relative groundcover were observed between glyphosate followed by quizalofop at 4-, 7-, and 10-day interval and glyphosate alone applied 4, 7, and 10 days before the 2-leaf growth stage of rice at all evaluations (Table 5).

At both locations, no delay to 50% heading was seen between glyphosate followed by quizalofop applied at 10-, 7-, 4-, and 0-day intervals compared with glyphosate alone at the same timings (Table 6). Glyphosate followed by quizalofop at the 0-day interval caused a 67% yield reduction compared with a 33% reduction when glyphosate was applied alone at the same time at PTRS. However, at all other intervals between glyphosate followed by quizalofop, yields were comparable to the corresponding glyphosate alone timings at both locations. Additionally, no yield reductions resulted from sequential quizalofop applications alone at either location (Table 6). Pre-exposure of quizalofop-resistant rice to a sub-lethal glyphosate rate, to attenuate the risk for injury, needs to be avoided in a close interval with quizalofop applications. Exposure to low rates of glyphosate affects the tolerance of quizalofop-resistant rice to quizalofop applications and increases the risk for injury to the crop over individual exposure to glyphosate or quizalofop alone. Similarly, in other research, Brown et al. (2009) reported higher injury to corn when simulated sub-lethal glyphosate exposure was followed by an in-crop standard herbicide program compared to sub-lethal glyphosate exposure and in-crop herbicides alone.

Provisia Rice Exposure to Imazethapyr. Imazethapyr followed quizalofop the same day caused 12-percentage point greater injury than imazethapyr alone at NEREC when evaluated at 35 DAFT (Table 7). At PTRS, imazethapyr followed by quizalofop the same day caused 22- and 21-percentage points more injury than imazethapyr alone at 28 and 35 DAFT, respectively. Myers and Coble (1992) documented similar findings regarding weed control, where imazethapyr followed by quizalofop the same day provided higher control of fall panicum (*Panicum dichotomiflorum* Michx) than quizalofop alone or imazethapyr followed by quizalofop at 5- and 3-day intervals. No differences in injury were seen at either PTRS or NEREC between imazethapyr followed by quizalofop applied at the 4- and 7-day interval and imazethapyr applied alone at the same timings, averaged across all ratings (Table 7). At PTRS, imazethapyr followed by quizalofop at a 10-day interval caused 17- and 18-percentage points more injury than imazethapyr alone at the same timing when evaluated at 28 and 35 DAFT. Imazethapyr caused more severe injury when rice was exposed to sub-lethal rates at the 1- to 2-leaf growth stage compared to 3- to 4-leaf rice (Levy et al. 2006). A sequential application of quizalofop alone caused minimal injury (<8% at both locations regardless of evaluation) (Table 7).

No differences were found in relative groundcover between imazethapyr followed by 2-leaf quizalofop at 0-, 4-, 7-day intervals and imazethapyr applied alone at the same timings at either location (Table 8). At PTRS, imazethapyr followed by quizalofop at a 10-day interval caused a reduction in relative groundcover at 7 and 14 DAFT, ranging from 15- to 21-percentage points compared with imazethapyr alone at ten days before the 2-leaf stage (Table 8). A sequential quizalofop application never reduced relative groundcover at either location for any evaluations (Table 8). Camacho et al. (2020) observed that quizalofop at the 1x or 2x rate caused transient injury to quizalofop-resistant rice cultivars and crop injury from quizalofop applications in the vegetative stage recovered at a later growth stage and had no impact on yield potential. No differences to reach 50% heading were observed between imazethapyr followed by initial quizalofop at 0-, 4-, 7-, and 10-day intervals and imazethapyr applied alone at 0-, 4-, 7-, and 10-day intervals before 2-leaf stage at both locations (Table 9). The sub-lethal rate of imazethapyr increases the risk for injury to quizalofop-resistant rice when applied at an early growth stage (1-leaf stage) or when exposure occurs near sequential quizalofop application.

Effects of exposure of non-traited rice to sub-lethal rates of glyphosate and imazethapyr are well documented (Bond et al. 2006; Davis et al. 2011; Ellis et al. 2003; Hensley et al. 2012;

Koger et al. 2005; Kurtz and Street 2003). Pre-exposure to low glyphosate and imazethapyr rates poses more risk for injury to quizalofop-resistant rice when exposure occurs near the first quizalofop application to rice. Furthermore, pre-exposure of quizalofop-resistant rice to low rates of imazethapyr needs to be avoided as it increases damage to rice; however, no significant reduction in yield was observed after crop exposure to sub-lethal rates of imazethapyr. Therefore, the hypothesis is accepted. Sub-lethal rates of glyphosate or imazethapyr interact with quizalofop applications to increase the likelihood of injury to quizalofop-resistant rice over glyphosate, imazethapyr, and quizalofop applications alone. These experiments show the additive response in injury caused by individual herbicide exposure events; however, the severity of injury to quizalofop-resistant rice could be increased by exposure to sub-lethal rates of glyphosate or imazethapyr prior to standard herbicide applications. Future research needs to be conducted to evaluate the use of fertilizers to aid the recovery of quizalofop-resistant rice following exposure to low doses of glyphosate or imazethapyr followed by standard herbicide programs.

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Appendix

Table 1. Analysis of variance for rice injury and relative groundcover for the experiments evaluated whether low rates of glyphosate or imazethapyr interact with sequential quizalofop applications to exacerbate injury to quizalofop-resistant rice over applications of quizalofop alone at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.

	P-value[a]			
	Injury		Relative groundcover	
Factor	Glyphosate	Imazethapyr	Glyphosate	Imazethapyr
Location	0.0059*	0.0420*	0.0753	0.5027
Herbicide	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Rating timing	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × Herbicide	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × Rating timing	<0.0001*	<0.0001*	<0.0001*	0.0001*
Herbicide × Rating timing	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location × Herbicide × Rating timing	0.1298	<0.0001*	<0.0001*	0.0291*

[a]Asterisks (*) indicate significance of treatments effects.

Table 2. Analysis of variance for relative heading and relative yield of Provisia® rice experiments evaluated for whether sub-lethal rates of glyphosate or imazethapyr interact with sequential quizalofop applications to exacerbate injury to quizalofop-resistant rice over applications of quizalofop alone at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.

	Relative heading		Relative yield	
Factor	Glyphosate	Imazethapyr	Glyphosate	Imazethapyr
	----------------------------*P*-value[a]	---------------------------------------		
Location	0.0185*	0.0733	0.4888	0.8711
Herbicide	<0.0001*	<0.0001*	<0.0001*	0.1362
Location × Herbicide	0.0002*	<0.0001*	<0.0001*	0.5473

[a]Asterisks (*) indicate significance of treatments effects.

Table 3. Injury to PVL02, a quizalofop-resistant cultivar, caused by pre-exposure to glyphosate at 90 g ae ha^{-1} at different rating dates averaged over the locations (Northeast Research and Extension Center, Keiser, AR, and Pine Tree Research Station, Colt, AR) in 2021.

	Injury[a]				
Herbicide[b]	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	---------------------------------------%---------------------------------------				
G alone at 10 d	26 e-i	29 efg	21 e-l	19 f-l	17 h-n
G alone at 7 d	9 n-r	13 j-o	8 o-s	6 p-s	4 st
G alone at 4 d	17 i-n	17 g-n	17 i-n	11 l-p	10 m-q
G alone at 0 d	29 e-h	67 bc	71 b	58 cd	52 d
GFQ at 10 d	26 e-i	31 ef	17 h-n	23 e-j	18 g-m
GFQ at 7 d	12 k-p	12 k-p	4 q-t	10 m-q	5 q-t
GFQ at 4 d	23 e-j	25 e-i	25 e-i	21 e-k	15 i-o
GFQ at 0 d	33 e	78 ab	86 a	77 b	71 bc
SQ	4 rst	5 q-t	2 tu	2 u	1 u

[a]Means followed by same letter are not different based on Fisher’s protected least significant difference test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf stage quizalofop application); DAFT, days after final treatment.

Table 4. Injury to PVL02, a quizalofop-resistant cultivar, caused by pre-exposure to glyphosate at 90 g ae ha^{-1} at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021, averaged over ratings of 7, 14, 21, 28, and 35 days after final treatment.

Herbicide[b]	Injury[a] NEREC		Injury[a] PTRS	
	------------------------%		------------------------------	
G alone at 10 d	37	cd	12	efg
G alone at 7 d	12	efg	5	hi
G alone at 4 d	18	efg	11	fgh
G alone at 0 d	41	c	69	b
GFQ at 10 d	44	c	10	gh
GFQ at 7 d	15	efg	4	ij
GFQ at 4 d	23	de	20	ef
GFQ at 0 d	45	c	88	a
SQ	3	ij	2	j

[a]Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop treatment (2-leaf stage followed by 5-leaf stage quizalofop application).

Table 5. Relative rice groundcover compared to the nontreated control of PVL02 rice cultivar at different rating timings at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.

	Relative groundcover[a]									
	NEREC					PTRS				
Herbicide[b]	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	%									
G alone at 10 d	23 o-n	40 f-n	89 a-h	97 a-f	95 a-f	46 c-n	45 d-n	110 a-d	81 a-i	106 a-e
G alone at 7 d	58 a-k	74 a-i	98 a-f	98 a-f	102 a-e	82 a-i	95 a-f	136 a	87 a-i	91 a-h
G alone at 4 d	74 a-i	53 b-m	96 a-f	103 a-e	104 a-e	64 a-j	79 a-i	110 a-d	73 a-i	90 a-h
G alone at 0 d	98 a-f	26 k-o	67 a-j	97 a-f	98 a-f	71 a-i	22 mno	27 j-o	7 pq	44 e-n
GFQ at 10 d	11 op	22 no	76 a-i	93 a-g	98 a-f	65 a-j	77 a-i	117 ab	89 a-h	103 a-e
GFQ at 7 d	60 a-k	64 a-j	101 a-e	102 a-e	101 a-e	88 a-i	84 a-i	112 abc	98 a-f	94 a-f
GFQ at 4 d	87 a-i	36 i-n	99 a-e	107 a-e	105 a-e	55 b-l	38 g-n	95 a-f	81 a-i	107 a-e
GFQ at 0 d	109 a-e	37 h-n	51 b-n	99 a-e	108 a-e	100 a-e	69 a-i	4 qr	1 s	2 r
SQ	114 abc	88 a-i	99 a-e	103 a-e	103 a-e	84 a-i	88 a-i	124 ab	86 a-i	100 a-e

[a] Means followed by same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf followed by 5-leaf stage application); DAFT, days after final treatment.

Table 6. Relative heading and relative yield compared to the nontreated check of quizalofop-resistant rice cultivar, PVL02 at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.[a]

Location	Herbicide[b]	Relative heading[c]	Relative yield[d]
		d	%
NEREC			
	G alone at 10 d	5 bc	90 ab
	G alone at 7 d	1 efg	94 ab
	G alone at 4 d	3 c-f	89 ab
	G alone at 0 d	8 a	81 ab
	GFQ at 10 d	6 ab	90 ab
	GFQ at 7 d	3 def	104 ab
	GFQ at 4 d	5 bc	104 ab
	GFQ at 0 d	8 a	93 ab
	SQ	1 efg	93 ab
	Nontreated control	------	------
PTRS			
	G alone at 10 d	-1 g	91 ab
	G alone at 7 d	0 g	92 ab
	G alone at 4 d	1 fg	108 a
	G alone at 0 d	4 b-e	67 b
	GFQ at 10 d	-1 g	77 b
	GFQ at 7 d	0 g	104 a
	GFQ at 4 d	1 fg	94 ab
	GFQ at 0 d	5 bcd	33 c
	SQ	0 g	106 a
	Nontreated control	------	------

[a]Means followed by same letter within same column are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: G, glyphosate; GFQ, glyphosate followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf stage application).
[c]days delay to 50% heading stage compared to the nontreated control of PVL02 rice cultivar
[d]PVL02 cultivar yields for nontreated control plots were 9742 kg ha^{-1} and 7830 kg ha^{-1} for NEREC and PTRS, respectively.

Table 7. Injury to PVL02 rice cultivar by pre-exposure to imazethapyr at 10.7 g ai ha^{-1} different ratings averaged over experiments located at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.

	Injury[a]									
	NEREC					PTRS				
Herbicide[b]	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT	7 DAFT	14 DAFT	21 DAFT	28 DAFT	35 DAFT
	-----	-----	-----	-----	-----	%	-----	-----	-----	-----
I alone at 10 d	70 ab	70 ab	69 abc	63 a-e	48 d-l	8 y-E	13 w-C	9 x-D	25 p-w	20 r-w
I alone at 7 d	36 j-q	45 e-m	41 g-p	39 g-q	19 r-w	7 A-E	16 t-A	24 q-w	58 a-g	46 d-m
I alone at 4 d	25 n-v	25 n-v	13 w-2	3 E-H	1 H	18 s-x	24 q-w	52 b-j	48 d-k	38 i-q
I alone at 0 d	24 q-w	55 a-i	39 g-q	17 t-x	7 A-E	7 A-E	14 v-A	58 a-g	45 e-m	42 f-o
IFQ at 10 d	70 ab	71 a	70 ab	65 a-d	51 c-j	7 A-E	15 u-A	16 u-A	42 f-n	38 h-q
IFQ at 7 d	32 k-s	44 e-m	43 f-n	34 j-r	19 s-x	7 A-E	20 r-w	29 l-t	51 c-j	43 f-n
IFQ at 4 d	23 q-w	19 r-w	16 u-A	6 C-G	2 GH	17 t-y	20 r-w	69 abc	61 a-f	51 c-j
IFQ at 0 d	25 o-w	58 a-h	46 d-l	29 m-u	19 s-x	14 v-B	17 t-y	71 a	67 abc	63 a-e
SQ	8 y-E	2 GH	3 E-H	3 E-H	1 H	2 GH	2 GH	3 E-H	5 D-G	3 E-H

[a]Means followed by same lowercase and uppercase letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.

[b]Abbreviations: I, imazethapyr; IFQ, imazethapyr followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf followed by 5-leaf stage quizalofop application); DAFT, days after final treatment.

Table 8. Rice relative groundcover compared to the nontreated control of rice cultivar PVL02 at different rating timings at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.

	Relative groundcover[a]																			
	NEREC										PTRS									
Herbicide[b]	7 DAFT		14 DAFT		21 DAFT		28 DAFT		35 DAFT		7 DAFT		14 DAFT		21 DAFT		28 DAFT		35 DAFT	
	---------------------------------%---------------------------------																			
I alone at 10 d	7	s	8	rs	28	n-q	67	a-m	69	a-k	25	pq	14	qr	83	a-i	85	a-g	80	a-j
I alone at 7 d	27	opq	40	i-p	78	a-j	99	a-d	95	a-e	76	a-j	15	qr	56	b-o	51	d-o	76	a-j
I alone at 4 d	79	a-j	84	a-h	93	a-f	100	a-d	95	a-e	101	a-d	39	j-p	31	m-p	41	h-p	87	a-g
I alone at 0 d	100	a-d	73	a-k	80	a-j	105	a-d	101	a-d	102	a-d	23	pq	4	g-p	68	a-l	93	a-f
IFQ at 10 d	6	s	10	rs	33	l-p	71	a-k	80	a-j	10	rs	35	l-p	58	b-n	66	a-m	100	a-d
IFQ at 7 d	26	opq	41	g-p	79	a-j	101	a-d	100	a-d	44	f-p	23	opq	43	g-p	73	a-k	107	abc
IFQ at 4 d	99	a-d	106	a-d	104	a-d	100	a-d	109	abc	68	a-l	64	a-m	46	e-p	72	a-k	98	a-d
IFQ at 0 d	92	a-f	55	c-o	72	a-k	95	a-e	102	a-d	100	a-d	24	pq	38	j-p	60	b-n	90	a-f
SQ	115	ab	111	ab	99	a-d	100	a-d	99	a-d	81	a-i	127	a	106	a-d	105	a-d	91	a-f

[a]Means followed by same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: I, imazethapyr; IFIQ, imazethapyr followed by quizalofop; SQ, sequential quizalofop (2-leaf stage followed by 5-leaf stage quizalofop application); DAFT, days after final treatment.

Table 9. Rice relative heading compared to the nontreated control at the Northeast Research and Extension Center (NEREC), Keiser, AR, and the Pine Tree Research Station (PTRS), Colt, AR, in 2021.[a]

Location	Herbicide[b]	Relative heading[c]
		d
NEREC		
	I alone at 10 d	5 a
	I alone at 7 d	3 c-f
	I alone at 4 d	1 hi
	I alone at 0 d	4 b-e
	IFQ at 10 d	5 ab
	IFQ at 7 d	4 abc
	IFQ at 4 d	1 gh
	IFQ at 0 d	4 a-d
	SQ	0 i
	Nontreated control	--------------
PTRS		
	I alone at 10 d	2 f-h
	I alone at 7 d	3 d-h
	I alone at 4 d	3 d-h
	I alone at 0 d	3 c-f
	IFQ at 10 d	2 e-h
	IFQ at 7 d	2 e-h
	IFQ at 4 d	3 c-g
	IFQ at 0 d	3 c-f
	SQ	1 hi
	Nontreated control	--------------

[a]Means followed by same letter are not different in same column based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[b]Abbreviations: I, imazethapyr; IFQ, imazethapyr followed by quizalofop (2-leaf stage); SQ, sequential quizalofop (2-leaf stage followed by 5-leaf stage quizalofop application).
[c]days delay to 50% heading stage compared to the nontreated control of PVL02 rice cultivar.

Chapter 4

Response of Quizalofop-Resistant Rice to Sequential Quizalofop Applications Under Differential Environmental Conditions

Abstract

Quizalofop-resistant rice allows for over-the-top applications of quizalofop, an acetyl-coenzyme A carboxylase-inhibiting herbicide. However, previous reports have indicated that quizalofop applied postemergence has been shown to cause significant injury to quizalofop-resistant rice. Therefore, field experiments were conducted to evaluate the response of quizalofop-resistant rice cultivars to quizalofop applications across different planting dates. Under controlled conditions, the effects of soil moisture content, air temperature, and light intensity on quizalofop-resistant rice sensitivity to quizalofop were investigated. In the planting date experiment, variable injury levels were observed on quizalofop-resistant cultivars, depending on the impact of environmental conditions that persisted surrounding the application timings with greater injury to rice under lower solar radiation and saturated soil conditions. Quizalofop-resistant cultivars had at least 25 percentage points more injury when the soil was maintained at 90% or 100% of field capacity as PVL01, PVL02, and RTv7231 MA had ≥42%, 30%, and ≥54% injury, respectively, compared to ≤10%, ≤5%, and ≤22% injury, respectively, at 40% or 50% of field capacity, pooled over rating timing. Greater injury ranging from 18% to 31% was observed on quizalofop-resistant rice grown under low light intensity (600 µmol m^{-2} s^{-1}) compared to 5% to 14% injury under high light intensity (1150 µmol m^{-2} s^{-1}). The injury persisted from 7 to 28 days after 5-leaf stage application (DAFT), averaged over quizalofop-resistant cultivars and air temperatures (20/15 C and 30/25 C day/night, respectively). At 7 DAFT, greater injury (5- to 21-percentage points) was observed on quizalofop-resistant cultivars; PVL01, PVL02, and RTv7231 MA had 33%, 9%, and

58% injury, respectively, under 20/15 C compared to 13%, 4%, and 37% injury, respectively, under 30/25 C day/night conditions averaged over light intensities. Overall, quizalofop applications are likely to cause a greater risk for injury to quizalofop-resistant rice if applied under cool, cloudy, and moist soil conditions.

Nomenclature: ACCase, acetyl-coenzyme A carboxylase; quizalofop; rice, *Oryza sativa* L.

Introduction

Arkansas is the largest rice-producing state in the United States, accounting for 47% of the total planted US hectares under rice cultivation in 2021 (NASS-USDA 2021). Rice planting is generally initiated during the last week of March, and approximately 50% of total rice hectares are planted by April 24. Producers complete 90% of rice planting by May 21, meaning that most rice is planted from late March to mid-May (Hardke 2021a, 2021b). Quizalofop-resistant rice technology is available for Arkansas rice producers to combat troublesome herbicide-resistant grass weed species, including barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] and weedy rice (*Oryza sativa* L.) (Lancaster et al. 2018; Roma-Burgos et al. 2021). Additionally, a survey conducted in 2020 represented 40% of the total planted rice hectares in Arkansas reported high concern with problematic herbicide-resistant weeds and alternative herbicides were the second most frequent strategy utilized to control herbicide-resistant weeds including barnyardgrass, providing suitable fit for quizalofop-resistant rice technology (Butts et al. 2022). Quizalofop-resistant rice is a non-genetically modified crop technology that allows for the postemergence application of quizalofop (Guice et al. 2015). Quizalofop (WSSA Group 1) is a member of the aryloxyphenoxypropionate herbicide family and inhibits the acetyl-coenzyme A carboxylase (ACCase). Quizalofop use rates vary from 100 to 138 g ai ha^{-1} for a single application. However, the maximum usage rate should not exceed 240 g ai ha^{-1} annually in the quizalofop-resistant rice production system (Anonymous 2017). A single dominant gene governs the resistance mechanism in quizalofop-resistant rice, but the consistency of the expression of the resistance mechanism is line-specific and could vary depending on environmental conditions (Camacho et al. 2019, 2020). Quizalofop caused up to 26% injury to quizalofop-resistant rice lines after being applied at 120 g ai ha^{-1} (Camacho et al. 2020).

PVL01 (Horizon Ag. LLC, Memphis, TN) was the first quizalofop-resistant rice cultivar derived from crossing 'Cheniere,' a long-grain conventional cultivar having japonica traits, and 'BASF1-5', a quizalofop-resistant donor line having indica rice cultivar traits. The cultivar exhibited moderate resistance to lodging, 7500 kg ha^{-1} yield potential, and long-grain rice quality parameters (Famoso et al. 2019). PVL02 (Horizon Ag. LLC, Memphis, TN) is a long-grain quizalofop-resistant rice cultivar developed by crossing the Cheniere and BASF 1-5 lines. PVL02 has an increased yield potential compared to PVL01 and yielded over 8095 kg ha^{-1}. The BASF1-5 line encompasses a gene with mutagenized nucleic acid responsible for herbicide resistance, encoding a rice plastidic ACCase enzyme and substituting leucine amino acid residue with an isoleucine amino acid residue at 1792 position in rice ACCase amino acid sequence (Famoso and Linscombe 2020). The Provisia® (BASF Corporation, Research Triangle Park, NC) formulation of quizalofop is labeled for PVL01 and PVL02 cultivars (Anonymous 2017). RTv7231 MA (RiceTec Inc., Alvin, TX) cultivar was developed using conventional breeding techniques, containing a mutation at the G2096S position in the carboxyl transferase coding region of the ACCase gene, conferring quizalofop resistance (Hinga et al. 2013). The Highcard™ (ADAMA, Raleigh, NC), safened formulation of quizalofop is labeled for the RTv7231 MA cultivar (Anonymous 2021b). RTv7231 MA cultivar produced rough rice yields over 10,000 kg ha^{-1} when assessed in on-farm experiments in Arkansas (Frizzell et al. 2021). Safeners for aryloxyphenoxypropionate herbicides are derived from isoxadifen-ethyl, which improves rice tolerance to quizalofop (Shen et al. 2017). Quizalofop-resistant cultivars, PVL01, PVL02, and RTv7231 MA, were commercially available to producers in 2018, 2020, and 2022, respectively (Anonymous 2021a; Bruce 2019; Hines 2018).

Climatic factors influence the efficacy of aryloxyphenoxypropionate herbicides by affecting the physiochemical processes of target plants involving herbicide absorption, translocation, and metabolism (Varanasi et al. 2015). More specifically, soil moisture, light intensity, and air temperature are all known to influence the efficacy of ACCase inhibitors (Varanasi et al. 2015). For example, diclofop, an aryloxyphenoxypropionate herbicide, efficacy was reduced on gramineous plants, including yellow foxtail [*Setaria lutescens* (Weigel) Hubb.], wild oat (*Avena fatua* L.), little-seed canarygrass (*Phalaris minor* Retz.), and barnyardgrass when applied postemergence at low soil moisture conditions (Dortenzio and Norris 1980). Similarly, reduced control of green foxtail [*Setaria viridis* (L.) Beauv.] was observed with labeled fenoxaprop, fluazifop, and haloxyfop rates when low soil moisture conditions persisted surrounding herbicide applications (Boydston 1990). Fluazifop also exhibited reduced control of quackgrass [*Agropyron repens* (L.) Beauv.] under moisture stress conditions (Kells et al. 1984).

Fluazifop efficacy was reduced on green foxtail when air temperature increased from 18 to 30 C. The reduced efficacy was attributed to the increased herbicide absorption and volatilization from leaf surfaces at higher temperatures (Smeda and Putnam 1990). Kells et al. (1984) indicated that quackgrass plants had 26% greater absorption and extensive distribution of ^{14}C-fluazifop-butyl at 30 C compared to 20 C. Additionally, higher translocation of ^{14}C-fluazifop-butyl was observed on quackgrass exposed to non-shaded compared to shaded conditions (Kells et al. 1984). Higher absorption and translocation of ^{14}C-sethoxydim have been observed in common bermudagrass [*Cynodon dactylon* (L.) Pers.] at 35 C compared to 18 C (Wills 1984). Cyclohexanedione herbicides caused higher phytotoxicity on oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) when applied under low ultraviolet conditions, specifically during late evening or dark hours (McMullan 1996). Therefore, research was conducted to evaluate the impact of air temperature, soil moisture, light intensity, and planting environments on the sensitivity of quizalofop-resistant rice to quizalofop applications.

Materials and Methods

Planting Date Experiment. Field experiments were conducted to determine the amount of injury caused by sequential quizalofop applications to quizalofop-resistant rice cultivars across different planting dates. Experiments were conducted in 2020 and 2021 at the Rice Research and Extension Center (RREC), Stuttgart, AR, on a Dewitt silt-loam soil (Fine, smectitic, thermic Typic Albaqualfs) having 27% sand, 54% silt, and 19% clay with a pH of 5.9 and 1.9% organic matter. The experiment was implemented as a randomized complete block with a split-split plot layout with the whole plot factor being site years (2020 and 2021); the split-plot factor being the planting date as early (mid-April timing) or late (early June timing); and the split-split plot factors being a factorial treatment structure of quizalofop-resistant rice cultivars PVL01, PVL02, and RTv7231 MA; and sequential quizalofop application rate as none, 1x, and 2x in a randomized complete block design with four spatial replications. Sequential applications of quizalofop (Provisia® herbicide) were applied at the 1x rate, 120 g ai ha^{-1} and 2x rate, 240 g ai ha^{-1} in combination with 1% v/v crop oil concentrate (Agri-Dex®, Helena Chemical Company, Collierville, TN). Highcard™ herbicide, now labeled for the RTv7231 MA cultivar, was not available in the 2020 growing season; therefore, quizalofop in the form of Provisia® was the formulated product applied.

PVL01 and PVL02 were planted at 72 seeds m^{-1} row, while RTv7231 MA was planted at 52 seeds m^{-1} row. Rice was planted at a 1.3-cm depth into 5.2-m long by 1.8-m wide plots, and each plot consisted of 9 drill-seeded rows on 19-cm centers. Rice was planted on April 11, 2020, and April 14, 2021, for the early planting date and on June 3, 2020, and June 1, 2021, for the late planting date. Experiments were maintained weed-free with clomazone (Command™ herbicide, FMC Corporation, Philadelphia, PA) preemergence at 336 g ai ha^{-1}, and quinclorac (Facet L™ herbicide, BASF corporation, NC) early postemergence at 280 g ai ha^{-1} plus some hand weeding. Sequential applications of quizalofop were made at the 2- and 5-leaf growth stages of rice. Quizalofop applications at the 2-leaf growth stage were applied on May 12, 2020, and May 12, 2021, for the early planting date and on June 16, 2020, and June 16, 2021, for the late planting date. Quizalofop was applied at the 5-leaf growth stage on May 21, 2020, and May 31, 2021, for the early planting date and on June 27, 2020, and June 28, 2021, for the late planting date. Weather data, including air temperature, rainfall, and solar radiation, was assessed for a 7-day interval that spanned from three days before and three days after each quizalofop application using a weather station (Davis Instrument Corporation, Hayward, CA) at the experimental site. All herbicides were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa with a hand boom equipped with four Air Induction Extended Range (AIXR) 110015 spray nozzles (TeeJet® Technologies, Wheaton, IL). All plots were maintained using standard cultural practices and fertilized with 130 kg N ha^{-1} as urea (46-0-0) at 24 hours after the 5-leaf stage quizalofop application, prior to the establishment of permanent flood (Hardke et al. 2022).

Visual estimates of percent injury were taken on a scale of 0% to 100%, with 0% representing no injury and 100% representing plant death. Visual injury estimates were taken at the 5-leaf stage application, 14 days after 5-leaf stage application (14 DAFT), and 28 days after 5-leaf stage application (28 DAFT). Pictures of each plot were taken with a DJI Phantom quadcopter small unmanned aerial system (sUAS) (DJI, Shenzhen, China) at the 5-leaf stage application, 14 DAFT, and 28 DAFT. Images were analyzed using Field Analyzer (Green

Research Services, LLC, Fayetteville, AR) to determine the proportion of green pixels in each picture to assess the amount of groundcover. Dates were noted for each plot when rice reached the 50% heading stage. All plots were drained two weeks before harvesting, rice was harvested with a small-plot combine to quantify grain yield, and rice yields were adjusted to 12% moisture. All data were subjected to analysis of variance (ANOVA) as a randomized complete block with a split-split plot using the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The main effects and interaction of year, planting date, cultivar, and quizalofop rate were considered fixed effects. Block nested within year and planting date nested within year were considered random effects. Visual rice injury was analyzed using beta distribution, while gamma distribution was used for relative groundcover and relative yield. Normal distribution was assumed for relative heading. Means were separated using Fisher's protected LSD (P = 0.05). Analysis of variance results for all the evaluated response variables in the planting date experiment are shown in Table 1.

Soil Moisture Experiment. Greenhouse experiments were conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in the fall of 2021 to evaluate the effect of soil moisture levels on the tolerance of quizalofop-resistant rice to quizalofop applications. Two experimental runs were conducted. Each experiment was implemented as a completely randomized, two-factor factorial treatment structure with three spatial replications. The factors consisted of soil moisture levels (40%, 50%, 60%, 70%, 80%, 90%, and 100% of pore space filled with water) and quizalofop-resistant rice variety (PVL01, PVL02, and RTv7231 MA). The greenhouse was maintained at a temperature of 30 C and 25 C day and night, respectively, under a 14-hour photoperiod throughout the experiments.

The soil was a Leaf silt loam (fine, mixed, active, thermic Typic, Albaqualts) with 21% sand, 65% silt, 14% clay, pH of 6.4, and 2.3% organic matter collected from Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR. The soil was sieved and dried until it reached 0% moisture. Dried soil weighing 8000 grams was transferred into 9.1-liter buckets (24.2-cm diameter by 23.5-cm height) (Lowe's Companies, Inc., Mooresville, NC). Soil-Plant-Air-Water (SPAW) hydrology software (ARS, U.S. Department of Agriculture, SW Washington DC, USA) was used to determine the soil bulk density (1.4 g cm^{-3}) and volumetric field capacity (29.75%). The amount of water required to achieve desired moisture level was calculated utilizing the following equation:

$$W_w = FC \div BD \times ML \times W_s \quad [1]$$

where W_w is the amount of water in grams or milliliters added to each bucket for attaining the designated moisture level (ML), FC is the volumetric field capacity, BD is the matric bulk density of soil, and W_s is the weight of dried soil in grams placed in each bucket. Designated soil moisture levels were maintained every day by weighing the total weight of each bucket after germination until flooding. Quizalofop was applied sequentially at the recommended use rate, 120 g ai ha^{-1}, in combination with 1% v/v crop oil concentrate (Agri-Dex®) at the 2-leaf stage, followed by a subsequent application at the 5-leaf stage. Moisture levels were maintained until a permanent flood at a depth of 6 cm was established in each bucket at 24 hours after the 5-leaf stage application.

Air Temperature and Light Intensity Experiment. Experiments were conducted in a growth chamber (Conviron, Controlled Environments Inc., Pembina, North Dakota, USA) in the fall of 2021 and the spring of 2022 to assess the influence of air temperature and light intensity on tolerance of quizalofop-resistant rice cultivars to sequential quizalofop applications at the Crop

Science Research Center, Fayetteville, AR. The experimental design was a completely randomized, split-split plot arrangement with three spatial replications and two temporal replications. The whole-plot factor was air temperature during the 14-h photoperiod as low (20 C) and high (30 C); the split-plot factor was light intensity as low (600 µmol m^{-2} s^{-1}) and high (1150 µmol m^{-2} s^{-1}); and split-split plot factor was quizalofop-resistant rice cultivars PVL01, PVL02, and RTv7231 MA.

The growth chamber was separated into two sections with a curtain to alter the light levels on each side to achieve the designated light levels. The growth chamber was programmed to a 14-h photoperiod with 20/15 C and 30/25 C day/night temperature, respectively, for conducting two experimental runs for each air temperature level. Field soil identified as a Leaf silt loam, previously described for soil moisture level experiments, was sieved and dried until a 0% moisture level was achieved. Air-dried soil totaling 8000 g was placed in 9.1-liter buckets. Quizalofop was applied sequentially at 120 g ai ha^{-1} at the 2-leaf stage, followed by a 5-leaf stage application. A 1% v/v crop oil concentrate was added to each quizalofop application. All treatments were maintained every day at 100% field capacity after germination until all treatments were flooded at 24 hours after the 5-leaf stage application.

Methods Common to Both Controlled Condition Experiments. Quizalofop-resistant cultivars were planted at a 1.3-cm depth with eight seeds per treatment and later thinned to six plants per bucket after emergence. All treatments were maintained weed-free through hand weeding and were fertilized with 130 kg N ha^{-1} at the 5-leaf growth stage before permanent flood establishment. Provisia® formulation of quizalofop was used for PVL01 and PVL02 varieties, while Highcard™ formulation of quizalofop was utilized for RTv7231 MA variety. All quizalofop applications were made using a research track sprayer equipped with two flat-fan

TeeJet 1100067 spray nozzles (TeeJet® Technologies, Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L ha^{-1} at 1.61 km hr^{-1}.

Visual injury estimates began at seven days after 2-leaf stage initial treatment (7 DAIT), at 5-leaf stage application, and continued for 7, 14, 21, and 28 days after 5-leaf stage application (DAFT) on a scale of 0 to 100, with 0 indicating no injury and 100 indicating plant death. Plant height was measured at the 5-leaf growth stage and 28 DAFT. Pictures were taken at the 5-leaf rice stage and 28 DAFT and were analyzed using Field Analyzer (Green Research Services, LLC, Fayetteville, AR) to determine the groundcover. Aboveground biomass was harvested 28 days after the 5-leaf stage application and oven-dried for five days to constant mass to evaluate the differences in biomass accumulation among treatments.

Data Analysis for Controlled Condition Experiments. Response variables, including plant height, groundcover, and dry biomass data for each treatment, were expressed as a percentage of nontreated control. For the soil moisture level experiment, the main effects of the soil moisture level and cultivar and their interaction were fixed effects. For the growth chamber experiment, air temperature, light intensity, and cultivar main effects and their interactions were considered fixed effects. The temporal run was considered a random effect for both experiments. Beta distribution was utilized for rice visual injury, while relative groundcover, height, and biomass were analyzed with a gamma distribution. All data were subjected to ANOVA using the GLIMMIX procedure in SAS 9.4, and means were separated using Fisher's protected LSD test ($\alpha = 0.05$). For the visual injury response variable, rating timing was considered a repeated-measure variable that allowed for comparisons across ratings and included as a fixed effect in the treatment structure. Correlations across ratings for the fixed effects and residuals were modeled using an independence covariance structure, as no correlation among ratings was observed when residuals were evaluated qualitatively (Gbur et al. 2012).

Results and Discussion

Planting Date Experiment. Overall, more injury was observed on PVL01 and RTv7231 MA cultivars in 2021 compared to 2020; however, injury ratings for PVL02 did not differ between 2020 and 2021, averaged over quizalofop rates and planting dates when evaluated at the 5-leaf stage (Table 2). Higher injury to quizalofop-resistant cultivars could be attributed to greater rainfall and cloud cover during the 2-leaf quizalofop application in 2021 (Table 3). Xie et al. (1996) also reported that fenoxaprop had higher phytotoxicity on wild oat under low light intensity as aboveground biomass was reduced more under low than high light intensity. Additionally, rice planted early had 11 percentage points more injury than late planting when pooled over quizalofop-resistant cultivars, years, and quizalofop application rates at the 5-leaf stage (Table 2). More rainfall events occurred during the 2-leaf stage quizalofop applications in the early planting compared to no rainfall surrounding the 2-leaf stage application in the late planting, possibly resulting in greater injury at the 5-leaf stage (Table 3). Boydston (1990) observed that fenoxaprop, fluazifop, haloxyfop, and sethoxydim applied at labeled rates reduced green foxtail [*Setaria viridis* (L.) Beauv.] control when low soil moisture conditions persisted for ten days prior and seven days after the herbicide application compared to saturated soil conditions.

At 14 DAFT, RTv7231 MA (56% injury) had 50 percentage points greater injury than PVL01 (6% injury) and PVL02 (6% injury) cultivars, averaged over the years, planting dates, and sequential quizalofop rates (Table 2). The higher sensitivity of RTv7231 MA to quizalofop resulted in greater visual injury than other quizalofop-resistant cultivars, as Provisia® formulation (unsafened) was utilized for over-the-top applications. At 14 DAFT, with data pooled over cultivars and sequential quizalofop application rates, early planted rice had 20% injury in 2021 compared to 7% injury in 2020, which could be attributed to higher rainfall in 2021 (Tables 2 and 3). Similarly, pooled over planting dates and quizalofop-resistant cultivars, sequential quizalofop applications at the 1x rate caused 10 percentage points higher injury in 2021 at 14 DAFT. More rainfall occurred close to the quizalofop applications in 2021 than in 2020; however, no differences were observed at 2x sequential quizalofop rates between 2020 and 2021 at 14 DAFT (Tables 2 and 3).

Rice in the late planting had more injury, ranging from 22 to 25 percentage points at the 2x sequential quizalofop rate compared to the early planted rice, averaged over years and quizalofop-resistant cultivars, with injury persisting from 14 to 28 DAFT (Table 2). At 28 DAFT, RTv7231 MA had 68% injury from the 2x sequential quizalofop rate in 2020 compared to 44% in 2021 averaged over planting dates, which might be credited to the crop remaining unaffected at differential environmental conditions when higher than the labeled use rate of quizalofop was applied (Table 2). One way to overcome the negative effect of environmental conditions on the efficacy of an aryloxyphenoxypropionate herbicide is to increase the use rate (Dortenzio and Norris 1980; Kells et al. 1984).

Overall, PVL01 and PVL02 exhibited a high level of tolerance to quizalofop as no differences in injury were observed between 1x and 2x sequential quizalofop application rates; however, RTv7231 MA had a higher injury at the 2x rate compared to the 1x sequential quizalofop rate, averaged over planting dates when evaluated at 28 DAFT (Table 2). In 2020, PVL01 and RTv7231 MA had more injury when planted early than late, which was attributed to low solar radiation and wet soil conditions that persisted during the 5-leaf stage quizalofop application timing, averaged over sequential quizalofop application rates at 28 DAFT (Tables 2 and 3).

At the 5-leaf stage of rice, PVL02 had ≤28% relative groundcover when rice was planted early in 2021; however, PVL02 planted late in 2021 had no reduction in groundcover, regardless of sequential quizalofop rates (Table 4). At the 5-leaf stage, RTv7231 MA planted early had 11% relative groundcover compared to 62% relative groundcover when planted late in 2021 at the 1x rate quizalofop applications (Table 4). Overall, higher injury to quizalofop-resistant cultivars planted early resulted in a greater reduction in the relative groundcover of the crop when compared to late planting in 2021, evaluated at the 5-leaf stage. At 14 DAFT, quizalofop-resistant cultivars planted in late had 63% relative groundcover compared to 89% relative groundcover of early planted rice when quizalofop was applied at a 2x rate (Table 4). Higher rates of quizalofop might overcome the effect of environmental conditions on crop tolerance to quizalofop. Pooled over quizalofop-resistant cultivars, quizalofop rates, and planting dates, a reduction in the relative groundcover of rice was observed, ranging from 22- to 13-percentage points in 2021 compared to 2020, when groundcover was assessed at 14 and 28 DAFT (Table 4). At 28 DAFT, RTv7231 MA had 84% relative groundcover compared to PVL01 and PVL02, which had ≥97% relative groundcover averaged over years, planting dates, and sequential quizalofop rates; therefore, RTv7231 MA was observed to be more sensitive to the Provisia® formulation than PVL01 and PVL02 (Table 4).

After quizalofop applications at a 2x rate, PVL02 had a delay of two days to reach the 50% heading stage when planted late compared to early, averaged over years (Table 5). A delay of two and seven days was observed to reach the 50% heading stage of RTv7231 MA after quizalofop was applied at 1x and 2x rates, respectively, when rice was planted late (Table 5). As a result, RTv7231 MA planted late had 37% relative yield in 2020 compared to 96% relative yield in 2021, after quizalofop was applied at the 2x rate. At 28 DAFT, increased injury observed in 2020 to RTv7231 MA planted late, regardless of quizalofop rates, resulted in a reduction in yield potential in 2020 (Tables 2 and 5). Similarly, PVL02 had a 70% relative yield at the 2x rate of quizalofop applications when rice was planted late in 2021 (Table 5). In 2020, PVL01 and RTv7231 MA planted late had 77% and 37% relative yield, respectively, compared to ≥87% relative yield of cultivars planted early when quizalofop was applied at a 2x rate (Table 5). Overall, ≥84% relative yield of quizalofop-resistant cultivars was observed when quizalofop was applied sequentially at the labeled rate, regardless of planting date and years (Table 5). Research findings provide insight into the potential effects of soil moisture and solar radiation on the tolerance of quizalofop-resistant rice cultivars to quizalofop applications. The greater injury was observed under cloudy and wet soil environments. However, quizalofop-resistant cultivars recovered from injury caused by sequential quizalofop applications under differential planting environments, as a ≤16% reduction in relative yield was observed when the labeled rate of quizalofop was applied to the crop.

Soil Moisture Experiment. A significant interaction between cultivar and soil moisture level was observed for all the evaluated response variables (Table 6). Additionally, the interaction of cultivar by rating timing was significant for the visual injury response variable (Table 6). In general, greater injury to quizalofop-resistant cultivars was observed at 7 and 14 DAFT, averaged over soil moisture levels (Table 7). After the 5-leaf stage quizalofop application, the highest injury was observed on RTv7231 MA, followed by PVL01 and PVL02, regardless of rating timings, averaged over soil moisture levels (Table 7). Differences in visual injury among quizalofop-resistant cultivars were not present at the 5-leaf stage (Table 7), as quizalofop takes 1 to 3 weeks after application to cause chlorotic and necrotic symptomology (Shaner 2014). PVL01, PVL02, and RTv7231 MA had ≥42%, 30%, and ≥54% injury, respectively, after sequential quizalofop applications when the cultivars were maintained at 90% or 100% of field capacity (Table 8). However, PVL01, PVL02, and RTv7231 MA had ≤10%, ≤5%, and ≤22% injury, respectively, when quizalofop was applied sequentially to the cultivars maintained at 40% or 50% of field capacity (Table 8).

The greater injury observed on quizalofop-resistant cultivars at higher moisture levels resulted in more reduction in relative groundcover and crop height than cultivars maintained under low moisture levels. At 28 DAFT, a ≥28, ≥20, and ≥25 percentage point reduction in relative groundcover of PVL01, PVL02, and RTv7231 MA, respectively, was observed at 90% or 100% of field capacity when compared to 40% or 50% of field capacity level (Table 8). At 5-leaf rice stage, PVL01, PVL02, RTv7231 MA had at least 20, 24, and 13-percentage points reduction in relative height of rice maintained at 90% or 100% of field capacity level compared to cultivars maintained at 40% or 50% of field capacity (Table 8). However, PVL01, PVL02, and RTv7231 MA had a 24, 12, and 30-percentage points or greater reduction in rice relative height when maintained at 90% or 100% of field capacity than at 40% or 50% of field capacity, evaluated at 28 DAFT (Table 8).

PVL01, PVL02, and RTv7231 MA had ≥50%, ≥81%, and ≥31% relative biomass, respectively, at the 40% or 50% moisture level, while relative biomass was reduced to ≤19%, ≤44%, and ≤12%, respectively when quizalofop-resistant cultivars were maintained at 90% or 100% soil moisture level (Table 8). Sequential quizalofop applications caused higher injury and biomass reduction of quizalofop-resistant cultivars under high moisture soils (90% or 100% of field capacity) than in low moisture content soils (40% or 50% of field capacity). Furthermore, a significant reduction in relative groundcover and relative height of quizalofop-resistant cultivars was reported after quizalofop applications when rice was maintained at 90% or 100% of field capacity than 40% or 50% of field capacity levels. Diclofop efficacy was similarly reduced on yellow foxtail, wild oat, littleseed canarygrass (*Phalaris minor* Retz.), and barnyardgrass in other research under low soil moisture conditions and could be attributed to an alteration in metabolism within the plant (Dortenzio and Norris 1980). Similarly, fluazifop, another aryloxyphenoxyprioponate herbicide, caused greater phytotoxicity on quackgrass when applied to plants maintained under adequate moisture levels than moisture-stressed plants; however, no differences in absorption and translocation were observed in quackgrass at either moisture level (Kells et al. 1984). Overall, research demonstrated that high soil moisture contents that persisted around the time of quizalofop applications exacerbated injury to quizalofop-resistant rice, and the severity of damage to the crop could be reduced by avoiding quizalofop applications during wet soil conditions.

Air Temperature and Light Intensity Experiment. Rice injury from quizalofop applications was impacted by air temperature and light intensity (Table 9). When averaged over light intensity levels, the greater injury was observed on PVL01 (ranging from 20% to 33%) and RTv7231 MA (ranging from 37% to 62%) under low temperature when compared to cultivars maintained under high temperature. PVL01 and RTv7231 MA had rice injury ranging from 3% to 13% and 16% to 38%, respectively when assessed for visual injury from 5-leaf stage quizalofop application to 28 DAFT (Table 10). Pooled over light intensity, PVL02 had higher injury under low temperature (ranging from 6% to 9% injury) than under the high temperature (2% to 4% injury), and it persisted until 7 DAFT. However, PVL02 recovered from transient injury, and no differences in visual injury were observed at either temperature level after 7

DAFT (Table 10). Xie and others (1996) documented that fenoxaprop phytotoxicity to wild oat was reduced under high temperatures as compared to low temperatures. No differences in fenoxaprop absorption and translocation were observed; however, lower phytotoxicity to wild oat might be caused by enhanced metabolic degradation within plants at a high temperature (Xie et al. 1996). The greatest injury to quizalofop-resistant cultivars from quizalofop was observed at 7 and 14 DAFT at either temperature, regardless of light intensity levels (Table 10).

Likewise, no differences in terms of visual injury were observed between quizalofop-resistant rice grown under differential light intensity conditions prior to 7 DAFT, and the highest injury to rice was reported at 7 and 14 DAFT, averaged over quizalofop-resistant cultivars and air temperatures (Table 11). When pooled over quizalofop-resistant cultivars and air temperature levels, higher injury to rice ranging from 18% to 31% injury was observed under low light intensity compared to high light intensity (5% to 14% injury), and higher injury persisted from 7 DAFT to 28 DAFT (Table 11). Previous research also reported that high light intensity maintained for four weeks after fluazifop application caused lower phytotoxicity to couch grass (*Elymus repens* L.) than low light intensity, which could be attributed to enhanced metabolism under brighter conditions (Coupland 1986).

When averaged over temperature levels, the relative groundcover of PVL01 (77%) and RTv7231 MA (38%) was reduced under low light as compared to PVL01 (99%) and RTv7231 MA (82%) relative groundcover under high light conditions at 28 DAFT (Table 12). Furthermore, RTv7231 MA had a 37-percentage points reduction in relative groundcover under low temperature compared to high temperature, averaged over light intensity levels, when assessed at 28 DAFT (Table 12). The relative height of RTv7231 MA was reduced to 15 percentage points under low light compared to high light conditions when evaluated at the 5-leaf and pooled over temperature levels (Table 12). Similarly, the relative height of RTv7231 MA was reduced to 55 percentage points under low light and low-temperature combinations compared to the high light and low-temperature conditions at 28 DAFT. No differences were observed in relative height at either light intensity level under high-temperature conditions (Table 12). Additionally, RTv7231 MA had a 26-percentage points reduction in relative height under low light and low-temperature conditions when compared to low light and high-temperature conditions (Table 12).

No differences in relative biomass of quizalofop-resistant cultivars were observed at either light intensity level when maintained under high-temperature conditions (Table 12). However, PVL01 and RTv7231 MA had a 42- and 40 reduction in relative biomass at low light intensity compared to high light intensity, maintained under low-temperature conditions (Table 12). Likewise, no differences in relative biomass of quizalofop-resistant cultivars were observed between low and high temperatures maintained under high light intensity (Table 12). The risk of injury to quizalofop-resistant rice from quizalofop applications escalated if low air temperature and low solar radiation environment persisted at the timing of herbicide applications; however, rice recovered from transient injury if cold and cloudy conditions did not prevail simultaneously. At low light intensity, the relative biomass of PVL01 and RTv7231 MA reduced from 84% to 26% and 34% to 4%, respectively, from high to low-temperature conditions (Table 12). Quizalofop metabolism in quizalofop-resistant wheat (CoAXium™ wheat) was reduced under low temperature comapred to high temperature; however, quizalofop absorption and de-esterification of quizalofop proherbicide to quizalofop-p-acid was not reduced under low temperature (Bough et al. 2022) Overall, lower temperature and prolonged cloudy conditions increased the severity of damage to quizalofop-resistant cultivars from postemergence quizalofop applications. Clethodim efficacy on barley or oat was increased when applied in the dark or evening hours due to the absence of ultraviolet (UV) light compared to full sunlight, attributed to the susceptibility of cyclohexanedione herbicides to photodegradation by UV light during daytime (McMullan 1996).

Response of quizalofop-resistant cultivars to quizalofop was observed to be impacted by several environmental factors, including soil moisture content, light intensity, and air temperature. Research findings allow growers to mitigate the risk of injury to quizalofop-resistant cultivars from quizalofop by considering environmental conditions prior to herbicide applications. Quizalofop applications need to be avoided under cold and cloudy conditions. Furthermore, quizalofop applications onto rice under saturated soil conditions will exacerbate the severity of damage to quizalofop-resistant cultivars more than if dry conditions existed surrounding the application timings. Arkansas rice producers could quickly adopt recently released quizalofop-resistant cultivars with improved agronomic traits to address the troublesome herbicide-resistance grass weed issues. PVL03, a new Provisia cultivar, will be commercially available to rice producers in the growing season of 2022 with an increased yield and milling advantage along with blast [*Magnaporthe grisea* (TT Hebert) Barr.] and *Cercospora* resistance over the previously available Provisia cultivars (McClure 2021). Producers need to consider environmental conditions for alleviating the risk of injury to quizalofop-resistant cultivars from quizalofop applications.

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Appendix

Table 1. Analysis of variance *P*-values for rice injury, relative groundcover, relative heading, and relative yield of quizalofop-resistant cultivars for the planting date experiments conducted at the Rice Research and Extension Center, Stuttgart, AR, in 2020 and 2021.

	P-value[a]							
Factor	Injury 5-leaf stage	Injury 14 DAFT[b]	Injury 28 DAFT	RGC 5-leaf stage	RGC 14 DAFT	RGC 28 DAFT	Relative heading	Relative yield
Year	0.0008*	0.0611	0.0005*	0.0340*	0.0150*	0.0348*	0.3162	0.0842
Planting	0.0012*	0.0238*	0.0072*	0.1095	0.0106*	0.8183	0.0069*	0.0183*
Cultivar	0.0009*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0074*	0.0034*	0.0103*
Rate	0.0774	<0.0001*	<0.0001*	0.0055*	0.0050*	0.2630	<0.0001*	0.0066*
Year × Planting	0.2024	0.0023*	0.1357	0.1386	0.9298	0.2915	0.0546	0.2013
Year × Cultivar	0.0026*	0.2807	0.0347*	0.0008*	0.7037	0.7627	0.3797	0.0901
Year × Rate	0.8555	0.0025*	0.1220	0.9137	0.9577	0.4103	0.4534	0.8887
Planting × Cultivar	0.2032	0.6317	0.3831	0.0021*	0.0967	0.4576	<0.0001*	0.6000
Planting × Rate	0.8601	0.0222*	0.0012*	0.8945	0.0316*	0.1359	0.0061*	0.0200*
Cultivar × Rate	0.5350	0.8838	0.1724	0.9348	0.0004*	0.1159	0.0081*	0.2341
Year × Planting × Cultivar	0.6800	0.2881	0.0004*	0.0129*	0.4893	0.8185	0.1294	0.0080*
Year × Planting × Rate	0.3381	0.7037	0.9568	0.4143	0.2506	0.9062	0.5255	0.8573
Year × Cultivar × Rate	0.4669	0.3547	0.0194*	0.3812	0.9479	0.6215	0.6603	0.0073*
Planting × Cultivar × Rate	0.8650	0.9676	0.3772	0.0284*	0.2153	0.8686	0.0425*	0.4932
Year × Planting × Cultivar × Rate	0.8867	0.4522	0.4627	0.0235*	0.8317	0.3507	0.7152	0.0392*

[a]*P*-values followed by * are significant at α level of 0.05.

[b]Abbreviations: DAFT, days after final treatment at 5-leaf stage; RGC, Relative groundcover compared to the nontreated control.

Table 2. Injury to quizalofop-resistant rice cultivars caused by sequential quizalofop applications over different planting dates at the Rice Research and Extension Center, Stuttgart, AR, in 2020 and 2021.[a]

					Injury		
Factor	Year	Planting	Cultivar	Rate	5-leaf stage	14 DAFT[b]	28 DAFT
				g ai ha^{-1}	---------- % ----------		
Year × Planting × Cultivar	2020	Early	PVL01		13	3	4 ef
			PVL02		7	3	17 c
			RTv7231 MA		12	36	15 cd
		Late	PVL01		2	7	18 c
			PVL02		1	8	10 cde
			RTv7231 MA		1	73	69 a
	2021	Early	PVL01		17	10	4 ef
			PVL02		8	7	2 f
			RTv7231 MA		56	65	38 b
		Late	PVL01		10	7	5 def
			PVL02		4	11	5 def
			RTv7231 MA		17	46	31 b
Year × Cultivar × Rate	2020		PVL01	120	4	2	8 ef
			PVL01	240	6	11	9 ef
			PVL02	120	2	2	5 f
			PVL02	240	4	17	9 ef
			RTv7231 MA	120	3	30	16 de
			RTv7231 MA	240	4	78	68 a
	2021		PVL01	120	8	5	2 f
			PVL01	240	21	16	9 ef
			PVL02	120	6	8	3 f
			PVL02	240	6	9	3 f
			RTv7231 MA	120	32	47	26 cd
			RTv7231 MA	240	36	64	44 b
Year × Cultivar	2020		PVL01		5 c	4	9
			PVL02		3 c	5	13
			RTv7231 MA		4 c	55	39
	2021		PVL01		13 b	9	5
			PVL02		6 c	9	3
			RTv7231 MA		34 a	55	34

Table 2 (Cont.)

Factor	Year	Planting	Cultivar	Rate	Injury		
					5-leaf stage	14 DAFT[b]	28 DAFT
				g ai ha^{-1}	-------------------------------	%	-------------------------------
Planting × Rate		Early		120	12	8 c	7 b
				240	18	18 b	10 b
		Late		120	3	7 c	7 b
				240	4	40 a	35 a
Year × Rate	2020			120	3	4 c	9
				240	5	31 a	30
	2021			120	12	14 b	6
				240	17	24 a	11
Year × Planting	2020	Early			10	7 b	10
		Late			1	21 a	28
	2021	Early			22	20 a	7
		Late			9	17 a	10
Cultivar			PVL01		8	6 b	6
			PVL02		4	6 b	6
			RTv7231 MA		12	56 a	37
Planting		Early			15 a	12	8
		Late			4 b	18	17

[a]Means followed by the same letter within the same column are not different based on Fisher's protected LSD at α=0.05.
[b]Abbreviations: DAFT, days after final treatment at 5-leaf stage.

Table 3. Air temperature, rainfall, and solar radiation data were collected near the experiment site in 2020 and 2021 at the Rice Research and Extension Center, Stuttgart, AR.[a]

			Air temperature			Rainfall		Solar radiation	
Year	Planting date	Application timing	Average	Average minimum	Average maximum	Average day^{-1}	Total	Average day^{-1}	Total
			-------- C --------			------- cm -------		-------- $W\ m^{-2}$ --------	
2020	Early	2-leaf	17	12	22	0.19	1.35	236	1649
		5-leaf	21	16	26	0.36	2.54	263	1841
	Late	2-leaf	25	19	31	0	0	328	2296
		5-leaf	25	22	29	0.38	2.67	206	1442
2021	Early	2-leaf	17	12	22	0.31	2.16	221	1548
		5-leaf	20	16	25	0.85	5.94	231	1617
	Late	2-leaf	28	23	33	0	0	307	2150
		5-leaf	28	23	33	0	0.02	283	1981

[a]Weather data were recorded for a 7-day interval from 3 days prior to each application to 3 days past each quizalofop application.

Table 4. Rice relative groundcover compared to the nontreated control after sequential quizalofop applications at the Rice Research and Extension Center, Stuttgart, AR in 2020 and 2021.[a]

Factor	Year	Planting	Cultivar	Rate	Relative groundcover		
					5-leaf stage	14 DAFT[b]	28 DAFT
				g ai ha^{-1}	--- % ---		
Year × Planting × Cultivar × Rate	2020	Early	PVL01	120	106 ab	107	110
			PVL01	240	100 ab	110	106
			PVL02	120	105 ab	116	101
			PVL02	240	54 bcd	113	105
			RTv7231 MA	120	90 abc	106	99
			RTv7231 MA	240	47 bcd	76	94
		Late	PVL01	120	118 a	116	108
			PVL01	240	85 abc	103	103
			PVL02	120	99 ab	88	105
			PVL02	240	86 abc	96	104
			RTv7231 MA	120	80 abc	81	113
			RTv7231 MA	240	53 bcd	42	70
	2021	Early	PVL01	120	96 ab	83	97
			PVL01	240	61 bc	90	97
			PVL02	120	28 cde	75	73
			PVL02	240	11 e	89	95
			RTv7231 MA	120	11 e	76	81
			RTv7231 MA	240	12 e	64	74
		Late	PVL01	120	91 abc	78	106
			PVL01	240	67 bc	67	89
			PVL02	120	104 ab	76	99
			PVL02	240	104 ab	73	101
			RTv7231 MA	120	62 bc	79	80
			RTv7231 MA	240	18 de	31	73
Cultivar × Rate			PVL01	120	114	95 a	105
				240	77	91 a	98
			PVL02	120	74	87 a	94
				240	48	91 a	101
			RTv7231 MA	120	37	85 a	92
				240	27	50 b	77

Table 4 (Cont.)

Factor	Year	Planting	Cultivar	Rate	Relative groundcover 5-leaf stage	Relative groundcover 14 DAFT[b]	Relative groundcover 28 DAFT
				g ai ha^{-1}	---------- % ----------		
Planting × Rate		Early		120	52	92 a	93
				240	36	89 a	95
		Late		120	90	85 a	101
				240	60	63 b	89
Cultivar			PVL01		93	93	102 a
			PVL02		60	89	97 a
			RTv7231 MA		32	65	84 b
Year	2020				82	93 a	101 a
	2021				39	71 b	88 b

[a]Means followed by the same letter within the same column are not different based on Fisher's protected LSD at α=0.05.
[b]Abbreviations: DAFT, days after final treatment at 5-leaf stage.

Table 5. Relative heading and relative yield of quizalofop-resistant rice compared to the nontreated control for the planting date experiments conducted at the Rice Research and Extension Center, Stuttgart, AR in 2020 and 2021.[a]

Factor	Year	Planting	Cultivar	Rate	Relative heading[b]	Relative yield[c]
				g ai ha^{-1}	d	%
Year × Planting × Cultivar × Rate	2020	Early	PVL01	120	2	103 a-d
			PVL01	240	1	113 abc
			PVL02	120	0	104 a-d
			PVL02	240	2	96 a-e
			RTv7231 MA	120	0	98 a-e
			RTv7231 MA	240	2	87 b-e
		Late	PVL01	120	0	84 cde
			PVL01	240	1	77 de
			PVL02	120	0	95 a-e
			PVL02	240	1	109 a-d
			RTv7231 MA	120	2	89 b-e
			RTv7231 MA	240	8	37 f
	2021	Early	PVL01	120	0	94 a-e
			PVL01	240	1	99 a-e
			PVL02	120	-1	134 a
			PVL02	240	-1	122 abc
			RTv7231 MA	120	-1	100 a-e
			RTv7231 MA	240	-1	103 a-d
		Late	PVL01	120	0	112 a-d
			PVL01	240	2	91 b-e
			PVL02	120	2	124 ab
			PVL02	240	3	70 e
			RTv7231 MA	120	1	103 a-d
			RTv7231 MA	240	7	96 a-e
Planting × Cultivar × Rate		Early	PVL01	120	1 bcd	99
			PVL01	240	1 bcd	106
			PVL02	120	-1 d	118
			PVL02	240	0 cd	108
			RTv7231 MA	120	-1 d	99
			RTv7231 MA	240	1 bcd	94
		Late	PVL01	120	0 cd	97
			PVL01	240	1 bcd	84
			PVL02	120	1 bcd	109
			PVL02	240	2 b	88
			RTv7231 MA	120	2 b	96
			RTv7231 MA	240	7 a	59

[a]Means followed by the same letter within the same column are not different based on Fisher's protected LSD at α=0.05
[b]days delay to 50% heading stage compared to the nontreated control.
[c]PVL01, PVL02, and RTv7231 MA yielded 8929 kg ha^{-1}, 9054 kg ha^{-1}, and 11882 kg ha^{-1}, respectively in early planting interval in 2020; while PVL01, PVL02, and RTv7231 MA yielded 4970 kg ha^{-1}, 5447 kg ha^{-1}, and 11068 kg ha^{-1}, respectively in late planting interval in 2020. PVL01, PVL02, and RTv7231 MA yielded 7676 kg ha^{-1}, 7139 kg ha^{-1}, and 7344 kg ha^{-1}, respectively in early planting interval in 2021; while PVL01, PVL02, and RTv7231 MA yielded 7862 kg ha^{-1}, 6851 kg ha^{-1}, and 7986 kg ha^{-1}, respectively in late planting interval in 2021.

Table 6. Analysis of variance *P*-values for visual rice injury, relative groundcover, relative height, and relative biomass of quizalofop-resistant cultivars after sequential quizalofop applications at differing soil moisture levels for the greenhouse experiments conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in the fall of 2021.

	P-value[a]					
Factor	Rice injury	RGC 5-leaf stage	RGC 28 DAFT[b]	RH 5-leaf stage	RH 28 DAFT	Relative biomass
Cultivar	<0.0001*	0.0073*	<0.0001*	0.0023*	<0.0001*	<0.0001*
Moisture	<0.0001*	0.0002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Cultivar × Moisture	0.0164*	0.0312*	0.0044*	0.0096*	0.0134*	0.0147*
Rating timing	<0.0001*	-	-	-	-	-
Cultivar × Rating timing	<0.0001*	-	-	-	-	-
Moisture × Rating timing	0.1585	-	-	-	-	-
Cultivar × Moisture × Rating timing	0.3328	-	-	-	-	-

[a]P-values followed by * are significant at α level of 0.05.
[b]Abbreviations: RGC, relative groundcover compared to nontreated control; DAFT, days after final treatment at 5-leaf stage; RH, relative height compared to nontreated control.

Table 7. Injury to quizalofop-resistant cultivars after sequential quizalofop applications at different rating timings averaged over soil moisture levels after the greenhouse study conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in the fall of 2021.

	Injury[a]					
Cultivar	7 DAIT[b]	5-leaf stage	7 DAFT	14 DAFT	21 DAFT	28 DAFT
	--	--	-----------------	%-----------------	--	--
PVL01	12 jk	13 jk	40 e	42 de	34 f	29 g
PVL02	6 l	11 k	23 h	23 h	18 i	14 j
RTv7231 MA	12 jk	13 jk	49 bc	54 a	51 ab	46 cd

[a]Means followed by the same letter are not different based on Fisher's protected least significant difference test at $\alpha = 0.05$.
[b]Abbreviations: DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

Table 8. Rice injury, relative groundcover, relative height, and relative biomass of quizalofop-resistant cultivars at differing moisture levels following sequential quizalofop applications from the greenhouse experiments conducted at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR, in the fall of 2021.[a]

Cultivar	Soil moisture	Injury[b]		RGC 5-leaf stage[c]		RGC 28 DAFT		RH 5-leaf stage		RH 28 DAFT		Relative biomass	
		---%											
PVL01													
	40	10	ij	83	b-g	72	abc	89	bcd	97	abc	50	bcd
	50	6	jk	108	a	90	ab	99	ab	100	ab	73	abc
	60	23	fg	91	a-d	65	bcd	78	efg	96	a-d	34	def
	70	36	cde	58	h	45	def	72	f-i	87	a-e	16	hij
	80	38	cde	67	d-h	53	cde	76	e-h	81	c-f	26	e-h
	90	42	bc	62	fgh	44	def	69	h-k	73	f	19	ghi
	100	54	ab	58	h	30	fg	63	jk	55	g	9	k
PVL02													
	40	5	k	96	abc	89	ab	98	ab	99	ab	81	ab
	50	4	k	102	ab	97	a	102	a	101	a	89	a
	60	17	gh	63	e-h	67	abc	70	g-j	96	abc	41	de
	70	18	gh	83	b-g	72	abc	84	cde	99	ab	42	cde
	80	22	fg	73	c-h	74	abc	76	e-h	91	a-e	50	bcd
	90	30	def	81	b-h	62	bcd	74	f-i	83	b-f	34	def
	100	30	ef	87	b-f	69	abc	68	h-k	87	a-f	44	cde
RTv7231 MA													
	40	12	hi	89	a-e	63	bcd	94	abc	96	a-d	43	cde
	50	22	fg	68	c-h	51	cde	82	def	89	a-e	31	d-g
	60	30	def	71	c-h	39	efg	79	d-g	80	def	23	fgh
	70	37	cde	62	gh	58	cd	67	ijk	87	a-f	23	fgh
	80	41	cd	60	gh	38	efg	72	ghi	78	ef	17	hi
	90	54	ab	66	d-h	26	g	69	h-k	59	g	12	ijk
	100	57	a	64	e-h	17	h	62	k	52	g	9	jk

[a]Means followed by same letter within a column are not different based on Fisher's protected least significant difference test at α= 0.05.
[b]Injury, Injury to quizalofop-resistant cultivars averaged over rating timings (7 DAIT, 5-leaf stage, 7 DAFT, 14 DAFT, 21 DAFT, and 28 DAFT).
[c]Abbreviations: RGC, Relative groundcover compared to nontreated control; DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage; RH, relative height compared to nontreated control.

Table 9. Analysis of variance *P*-values for rice injury, relative groundcover, relative height, and relative biomass of quizalofop-resistant cultivars response to quizalofop applications at differing air temperature and light conditions from the growth chamber study conducted at the Crop Science Research Center, Fayetteville, AR.

	P-value[a]					
Factor	Rice injury	RGC 5-leaf stage[b]	RGC 28 DAFT	RH 5-leaf stage	RH 28 DAFT	Relative biomass
Temperature	0.0303*	0.1028	0.1030	0.1923	0.3542	0.0566
Light	0.0447*	0.2304	0.0211*	0.1367	0.1175	0.0620
Cultivar	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Temperature × Light	0.8088	0.1051	0.0708	0.3714	0.2925	0.1421
Temperature × Cultivar	0.0151*	0.7219	0.0015*	0.6392	0.0188*	<0.0001*
Light × Cultivar	0.5805	<0.0001*	<0.0001*	0.0037*	<0.0001*	<0.0001*
Temperature × Light × Cultivar	0.5171	0.5014	0.0951	0.5021	0.0332*	0.0042*
Rating timing	<0.0001*	-	-	-	-	-
Temperature × Rating timing	0.0787	-	-	-	-	-
Light × Rating timing	<0.0001*	-	-	-	-	-
Cultivar × Rating timing	0.0036*	-	-	-	-	-
Light × Cultivar × Rating timing	0.7166	-	-	-	-	-
Temperature × Light × Rating timing	0.7138	-	-	-	-	-
Temperature × Cultivar × Rating timing	0.0229*	-	-	-	-	-
Temperature × Light × Cultivar × Rating timing	0.1065	-	-	-	-	-

[a]*P*-values followed by * are significant at α level of 0.05.

[b]Abbreviations: RGC, Relative groundcover compared to nontreated control; DAFT, days after final treatment at 5-leaf stage; RH, relative height compared to nontreated control.

Table 10. Injury to quizalofop-resistant rice cultivars at different rating timings and temperature levels after sequential quizalofop applications pooled over light intensity levels (600 and 1150 µmol m^{-2} s^{-1}) from the experiment conducted at the Crop Science Research Center, Fayetteville, AR.

		Injury[a]					
Temperature	Cultivar	7 DAIT[b]	5-leaf stage	7 DAFT	14 DAFT	21 DAFT	28 DAFT
		%					
20 C	PVL01	13 ghi	24 def	33 c	32 cd	26 cde	20 efg
	PVL02	6 j-m	7 jkl	9 hij	8 ijk	6 j-m	4 lmn
	RTv7231 MA	17 efg	37 bc	58 a	62 a	50 b	44 b
30 C	PVL01	4 k-n	7 jkl	13 ghi	13 ghi	5 j-m	3 mn
	PVL02	2 n	3 mn	4 k-n	6 j-m	3 lmn	3 mn
	RTv7231 MA	15 fgh	21 d-g	37 bc	38 bc	21 d-g	16 efg

[a]Means followed by same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

Table 11. Rice injury at different rating timings and light intensity levels (600 and 1150 µmol m^{-2} s^{-1}) after sequential quizalofop applications, averaged over air temperature levels (20/15 C and 30/25 C day/night temperature, respectively) and quizalofop-resistant cultivars (PVL01, PVL02, and RTv7231 MA) from the experiment conducted at the Crop Science Research Center, Fayetteville, AR.

	Injury[a]											
Light Intensity	7 DAIT[b]		5-leaf stage		7 DAFT		14 DAFT		21 DAFT		28 DAFT	
	-------%										%-------	
Low	9	def	15	bc	29	a	31	a	21	b	18	bc
High	6	fg	10	cde	14	bcd	14	bcd	7	ef	5	g

[a]Means followed by the same letter are not different based on Fisher's protected least significant difference test at α = 0.05.
[b]Abbreviations: DAIT, days after initial treatment at 2-leaf stage; DAFT, days after final treatment at 5-leaf stage.

Table 12. Quizalofop-resistant rice cultivars relative groundcover, relative height, and relative biomass compared to nontreated control following sequential quizalofop applications under different temperature (20/15 C and 30/25 C day/night temperature) and light regimes (600 and 1150 µmol m^{-2} s^{-1}) from the experiment conducted at the Crop Science Research Center, Fayetteville, AR.[a]

Factor	Temperature	Light	Cultivar	RGC 5-leaf stage[b]	RGC 28 DAFT	RH 5-leaf stage	RH 28 DAFT	Relative biomass
				---------------	---------------	%	---------------	---------------
Temperature × Light × Cultivar	20 C	Low	PVL01	46	64	71	78 ab	26 d
			PVL02	91	87	91	95 ab	76 ab
			RTv7231 MA	34	23	56	43 c	4 e
		High	PVL01	68	95	91	99 ab	68 abc
			PVL02	92	92	97	102 a	91 a
			RTv7231 MA	74	72	70	98 ab	44 bcd
	30 C	Low	PVL01	82	93	85	98 ab	84 ab
			PVL02	102	98	104	99 ab	95 a
			RTv7231 MA	56	63	64	69 b	34 cd
		High	PVL01	88	103	92	96 ab	88 ab
			PVL02	81	97	95	101 ab	95 a
			RTv7231 MA	83	94	79	98 ab	76 abc
Light × Cultivar		Low	PVL01	62 c	77 b	78 bc	87	47
			PVL02	115 a	92 ab	97 a	97	85
			RTv7231 MA	44 d	38 c	59 d	54	12
		High	PVL01	77 bc	99 a	92 ab	97	78
			PVL02	86 b	95 ab	96 a	101	93
			RTv7231 MA	78 bc	82 ab	74 c	98	58
Temperature × Cultivar	20 C		PVL01	56	78 ab	80	88	42
			PVL02	91	89 ab	94	98	83
			RTv7231 MA	50	40 c	62	64	13
	30 C		PVL01	85	98 a	88	97	86
			PVL02	118	97 a	99	100	95
			RTv7231 MA	68	77 b	71	82	52

[a]Means within the same column followed by the same letter are not different based on Fisher's protected LSD at α=0.05.
[b]Abbreviations: RGC, Relative groundcover compared to nontreated control; DAFT, days after final treatment at 5-leaf stage; RH, relative height compared to nontreated control.

General Conclusions

With the ongoing evolution of herbicide-resistant grass weeds and the availability of quizalofop-resistant rice cultivars with improved yield potential, rice producer's anticipation of adopting quizalofop-resistant crop technology has increased. The experiments conducted examined the reason for inconsistencies in quizalofop-resistant rice tolerance to quizalofop applied postemergence. There was minimal impact on the tolerance of quizalofop-resistant cultivars after sequential quizalofop applications if unsaturated soil conditions persisted prior to quizalofop applications, regardless of whether the herbicide was applied alone or evaluated with differing levels of moisture or nitrogen (N) availability. Additionally, sub-lethal exposure to glyphosate and imazethapyr followed by quizalofop applications exacerbated injury over glyphosate, imazethapyr, and sequential quizalofop applied individually to quizalofop-resistant rice. Exposure to sub-lethal rates of glyphosate or imazethapyr occurring close to the timing of quizalofop application is detrimental to rice and intensified the rice injury compared to glyphosate or imazethapyr alone. Furthermore, crop injury from sequential quizalofop applications varied depending on the environmental conditions persisting at the application timings based on different planting dates. Likewise, quizalofop-resistant cultivars treated with quizalofop under wet soils, low light intensity, and low air temperature environments led to increased injury ratings. Overall, the timing of pre-exposure to sublethal rates of glyphosate or imazethapyr and environmental conditions near the time of quizalofop applications will influence the degree of injury to quizalofop-resistant rice cultivars.