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Evaluation of Benzobicyclon for use in Midsouthern Rice (*Oryza sativa*) Systems

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Crop, Soil, and Environmental Science

by

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University of Arkansas  
Bachelor of Science in Crop Science, 2014

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This thesis is approved for recommendation to the Graduate Council

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## Abstract

A new herbicide site of action (SOA) is needed by rice (*Oryza sativa* L.) producers in the Midsouth for the control of problematic and herbicide-resistant weeds. Currently, six problematic weeds are relevant to Midsouthern rice producers because of resistance to at least one SOA, if not more. Gowan Company is in the process of commercializing benzobicyclon for use in Midsouthern rice systems. Benzobicyclon, a Group 27 post-flood herbicide, controls a broad spectrum of aquatics, broadleaves, grasses, and sedges, including those currently resistant to Group 2 herbicides. This will be the first 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide commercially available in US rice production. Since, benzobicyclon is still under development, it is important to evaluate this herbicide in a drill-seeded rice production system. Therefore, experiments were conducted across Arkansas to determine rice cultivar tolerance, weed spectrum controlled, rate optimization, compatible tank-mix partners, and rotational crop safety. It is recommended to apply benzobicyclon into a continuous flood system. *Japonica* cultivars exhibited excellent crop safety to benzobicyclon, while *indica* cultivars showed high levels of sensitivity to the herbicide. Benzobicyclon effectively controlled Amazon sprangletop (*Leptochloa panicoides* J. Presl), ducksalad (*Heteranthera limosa* Sw.), California arrowhead (*Sagittaria latifolia* Willd.), *indica* rice, hemp sesbania (*Sesbania herbacea* Mill.), northern jointvetch (*Aeschynomene virginica* L.), red sprangletop (*Leptochloa chinensis* L.), rice flatsedge (*Cyperus iria* L.), and smallflower umbrella sedge (*Cyperus difformis* L.). The efficacy and spectrum of control of benzobicyclon is increased when applied with tank-mix partners such as bispyribac, cyhalofop, halosulfuron, imazamox, penoxsulam, and propanil. Cotton, grain sorghum, soybean, and sunflower can safely be planted in rotation with a drill-seeded rice crop

that has been treated with benzobicyclon post-flood without concerns of crop yield loss. The findings of this research suggest that benzobicyclon has a strong fit in midsouthern rice systems.

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## Chapter 1

### General Introduction

Rice (*Oryza sativa* L.) is commercially grown throughout many parts of the world, providing the primary food source for over half of the world's population. Because of the high calorie content of rice, it is highly consumed in developing countries (ERS 2017). The United States is globally known as a producer of high-quality rice even though the U.S. only produces approximately 2% of the world's rice. Currently, the U.S. is responsible for approximately 10% of the world's rice trade (ERS 2017).

Two species of cultivated rice are available today including: *Oryza sativa* L., the Asian rice, and *Oryza glaberrima* Stued., the African rice. Rice is classified into three categories by subspecies, stature, and grain length. There are four major subspecies of rice that are produced worldwide: *indica*, *japonica*, aromatic, and glutinous. The type of rice grown in an area is dependent upon climate, adapted cultivars, weed control needs, and market preference (ERS 2017). Each *japonica* subspecies in the U.S. comprises a different stature - short or standard statured and semi-dwarf. Arkansas is known for producing a tropical japonica, while California produces a temperate japonica. The first commercial hybrid rice cultivar was created by RiceTec, Inc. from *indica* and *japonica* subspecies (Hardy 2003). Shortly after the introduction of hybrid cultivars, BASF Corporation introduced its Clearfield® rice cultivars in 2002. Hybrid rice represented more than half of Arkansas rice acreage in 2013, which aided Arkansas in being ranked 3<sup>rd</sup> in the U.S. for average yield.

The grain length determines factors such as price, consumer and domestic uses, and distribution (ERS 2017). Rice is classified by three grain lengths: short, medium, and long (ERS

2017). Arkansas primarily produces long-grain and medium-grain rice both of which are available in hybrid and inbred cultivars and of *japonica* background. Hybrid rice cultivars were created by crossing two different parents. Rice resistant to the imidazolinone herbicides was created through the process of natural selection (Croughan et al. 1999). This imidazolinone-resistant rice, also known as Clearfield® rice, allows imazethapyr and imazamox to be applied for control of troublesome weeds like weedy rice and problematic, herbicide-resistant weeds like barnyardgrass (Hardke 2013).

The Arkansas Grand Prairie, Mississippi delta, Gulf Coast, and the Sacramento Valley of California are the four main regions that account for U.S. rice production (ERS 2017). These regions share capabilities for flooded irrigation that allow for rice production. Arkansas leads the U.S. in acreage planted, acreage harvested, and total production of rice (Hardke 2016). In 2015, Arkansas rice producers accounted for 50% of the total amount of U.S. rice produced. The state also planted 529,000 ha of rice in 2015, which represented 49% of the total acres planted in the U.S. (Hardke 2016).

**Southern Rice Production Practices.** Rice is known as a high input crop compared to other row crops such as corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). This is associated with the high operating costs due to increased fuel, fertilizer, and irrigation expenses because of the flooded rice production system (ERS 2017). In addition, weed control cost can regularly exceed \$247.00 ha<sup>-1</sup> due to the complex weeds in rice and severity of grass pressure (RC Scott, personal communication).

The majority of U.S. rice is produced on a silt loam soil and undergoes conventional tillage methods. The silt loam soil texture is ideal for facilitating a flooded irrigation system

(Hardke 2013). The normal regime includes tillage in the fall and tillage for seedbed preparation in the spring. Rice is normally drill-seeded; however, 20% of Arkansas rice is broadcast-seeded by either dry-seeding or water-seeding (Hardke 2013). The majority of Arkansas rice is grown in a delayed flood system. This demands an ample supply of water for optimum production. The flooded production system allows increased management of diseases, weeds, nutrients, and insects (Hardke 2013).

A one-year crop rotation plan with soybean is recommended in Arkansas (Hardke 2013). Soybean acts as a beneficial rotational crop due to the availability of herbicides offering different sites of action (SOA) to control weedy rice, barnyardgrass, and sedges. The monoculture rice system, in addition to the over-use and over-reliance on certain rice herbicides, has led to many hectares becoming infested with herbicide-resistant species. Herbicides used in soybean often have different SOA from those in rice. In rice production systems where weedy rice is the most common problematic weed species, a one-year rice followed by two-year soybean crop rotation can be implemented to break the cycle of weedy rice seed production, thus decreasing the amount of seed returning to the soil seedbank (Smith 1981). In addition to increased weed control, a soybean rotation system can implement no-till practices, which can reduce operating costs and increase crop yield for both rice and soybean.

**Rice Weed Control.** Weeds must be controlled in rice production to prevent yield loss and to assure quality grain is produced (Smith 1968). Weeds directly compete with rice for light, water, nutrients, and physical space (Smith 1988). In order to effectively manage weeds in rice, it is important to understand how and when they are most competitive (Hardke 2013). It is well known that weeds are most competitive when simultaneously emerging with the crop (Knake and Slife 1965). To achieve maximum weed control, it is most beneficial to follow a season-

long weed management program that incorporates tillage, use of high quality seed, an optimum planting date, adequate stand establishment, and proper irrigation management (Norsworthy et al. 2012; Odera and Rainbolt 2005).

A consistent, strong stand of rice is the first goal in a successful weed management program. In recent years, seeding rates have decreased significantly in hybrid rice and conventional rice in part due to advances in seed treatments (Hardke et al. 2013). High quality, certified seed should be selected for planting. A burn-down herbicide application in addition to field cultivation close to planting is a standard practice. Early herbicide applications and field cultivation help ensure a well-prepared, weed-free seedbed is available for the drill seeding of rice (Odera and Rainbolt 2005). The flooded rice production system allows for unique weed control opportunities such as control by flood depth. Proper management of irrigation throughout the growing season is important because flood depth has a significant impact on weed seedling emergence (Chauhan 2012).

Almost all fields receive a postemergence (POST) herbicide application immediately prior to flooding at the 4- to 6-leaf growth stage of rice. This application is to control weeds that have escaped previous control measures and hopefully ensure that the field is free of weeds for the remainder of the growing season. Unfortunately, lack of timely flooding, weed size at application, and environmental conditions during application are just a few factors that result in escaped weeds that must be controlled post-flood.

**Problematic Weeds in Southern Rice Systems.** There are currently 250 documented cases of herbicide-resistant weed species in the world (Heap 2017). Six of the 250 resistant species are relevant to Arkansas rice production including: barnyardgrass (*Echinochloa crus-galli* (L.)

Beauv.), Palmer amaranth [*Amaranthus palmeri* (S.) Wats.], weedy rice (*Oryza sativa* L.), yellow nutsedge (*Cyperus esculentus* L.), smallflower umbrella sedge (*Cyperus difformis* L.), and rice flatsedge (*Cyperus iria* L.). In addition to the six herbicide-resistant species in Arkansas rice production, sprangletops (*Leptochloa* spp.) and northern jointvetch (*Aeschynomene virginica* L.), are also among the most troublesome weeds of Arkansas rice (Norsworthy et al. 2013). Many of these weed species are semi-aquatic or aquatic and thrive in rice production systems. Weeds having a C<sub>4</sub> photosynthetic pathway such as barnyardgrass are much more competitive than less efficient C<sub>3</sub> rice plants (Smith 1988).

Barnyardgrass thrives in fertile, wet soils, which makes it highly competitive with rice (Holm et al. 1977). Bagavathiannan et al. (2011) reported barnyardgrass seed production can reach up to 39,000 seed plant<sup>-1</sup>. The emergence period of barnyardgrass in relation to rice emergence significantly impacts barnyardgrass seed produced (Bagavathiannan et al. 2010). The fibrous roots of barnyardgrass and rice often overlap and compete for nitrogen. The high availability of nitrogen fertilizers throughout the rice growing season adds to the competitiveness.

The competitive characteristics and widespread resistance of barnyardgrass to rice herbicides make it difficult to control for southern producers. In a recent survey across the Midsouth, barnyardgrass was identified as the most problematic weed in rice production (Norsworthy et al. 2013). Currently, populations of barnyardgrass exist in Arkansas having resistance to propanil, quinclorac, clomazone, and several acetolactate synthase (ALS) herbicides (Heap 2017). Over-reliance on propanil and quinclorac resulted in multiple resistance to herbicides becoming common. Hence, a new SOA is desperately needed for barnyardgrass control in rice.

Like barnyardgrass, sprangletops (Amazon and bearded) have a C<sub>4</sub> photosynthetic pathway giving them a competitive edge over rice. Bearded and Amazon sprangletop are most competitive with rice early in the growing season. Sprangletop plants are not as competitive as barnyardgrass as reported in multispecies weed interference studies (Smith 1988). Smith (1988) reported that sprangletop species have finer stems, narrower leaves, and short stature. However, when barnyardgrass and sprangletops exist in the same system they act synergistically in regards of yield loss. Barnyardgrass and sprangletops cause a greater yield loss than barnyardgrass alone (Smith 1988). Smith (1983) reported that bearded sprangletop causes the greatest yield reductions, when at high densities. Furthermore, sprangletop at densities of 108 plants m<sup>-2</sup> can reduce yields 36% (Smith 1983).

Consultants in the Midsouth consider sprangletops among the top five problematic weeds in southern rice production (Norsworthy et al. 2013). Except for two populations from Louisiana that are resistant to cyhalofop and fenoxaprop, there are no known populations of herbicide-resistant sprangletops in the southern U.S. (JK Norsworthy, personal communication). Although sprangletops can be partially controlled with propanil, acetyl CoA-carboxylase (ACCase)-, and ALS-inhibiting herbicides applied POST in rice, populations continue to increase likely because of the ineffectiveness of quinclorac on this weed and the fact that it often germinates later in the season. Late emergence allows for escape of preemergence (PRE) herbicides such as clomazone, thiobencarb, and pendimethalin that have often dissipated (RC Scott, personal communication). A new SOA that is highly effective in controlling sprangletops would be well received by southern rice producers. Such a herbicide would reduce selection for resistance to the herbicides that are currently being used to manage this weed.

Yellow nutsedge, rice flatsedge, and smallflower umbrella sedge populations continue to increase in Midsouth rice, likely because of the ineffectiveness of clomazone and quinclorac on these weeds and the fact that clomazone is applied as a PRE or delayed PRE application to almost all rice in Arkansas (Norsworthy et al. 2013). The sedges share the same C<sub>4</sub> photosynthetic pathway making them more competitive and more photosynthetically efficient than rice (Smith 1988). These sedges have a solid triangular stem, which differentiates them from grasses which possess a round, hollow-stem (Bagavathiannan et al. 2014). Reproductive and morphological characteristics can be used to differentiate the sedges from one another. Seeds are produced by rice flatsedge and smallflower umbrella sedge whereas yellow nutsedge predominately reproduces by underground tubers. The ability to propagate through rhizomes and tubers makes yellow nutsedge a prolific and aggressive competitor having rapid growth in rice production systems (Nelson and Renner 2002). The propagative reproductive nature of yellow nutsedge makes it more difficult to control due the ability of underground tubers to resprout after herbicide applications. The underground structures act as storage organs allowing the weed to proliferate in a range of environmental conditions (Nelson and Renner 2002).

Sedges are an increasingly problematic weed species for Arkansas rice producers as documentation of ALS-resistant populations increase (Norsworthy et al. 2013). Yellow nutsedge, rice flatsedge, and smallflower umbrella sedge have all selected for resistance to the various ALS herbicides (Heap 2017). Sedge control demands a systematic approach including early POST and pre-flood or early post-flood herbicide applications that incorporate three different SOA (Bagavathiannan et al. 2014).

Northern jointvetch is a broadleaf weed that competes with rice, and its seed affects the grade and quality of rice. A recent survey reported that northern jointvetch was the most

problematic broadleaf weed in Arkansas rice production (Norsworthy et al. 2007). Its ability to reach heights of 1.5 m results in shading of shorter stature rice, and most of its impact on rice is through shading (Smith 1988). There are few control options for northern jointvetch late in the growing season, which has led to some consultants suggesting greater research emphasis on post-flood control of this and other broadleaf weeds (Norsworthy et al. 2007).

Weedy rice, also known as red rice, is similar to commercial rice in its taxonomic and physiological traits making it a difficult weed to control in cultivated rice. Controlling weedy rice in cultivated rice with traditional rice herbicides is often unsuccessful. BASF released imidazolinone-resistant Clearfield rice in 2002. Imidazolinone herbicides were applied to imidazolinone-resistant rice providing control of weedy rice. The imidazolinone herbicides also provided control of barnyardgrass, other grass weeds, and many problematic broadleaf weeds (Hardke 2013). The imidazolinone-resistant rice system is widely used in the Midsouth, and at one point, accounted for more than 60% of the planted rice acreage in the U.S. (Burgos et al. 2014). Overuse, poor stewardship, and outcrossing of imidazolinone-resistant rice from 2002 to 2008 eventually led to ALS-resistant weedy rice populations in Arkansas (Burgos et al. 2008)

There is much phenotypic and genetic variation of weedy rice across the U.S. The varying phenotypic characteristics include: hull color, pericarp color, presence or absence of awn, awn length, flag leaf length, panicle length, seed shattering, and flowering timing (Burgos et al. 2014). Hull color can be one of the identifying characteristics of weedy rice as the color can vary from straw to black. The variation in color of weedy rice represents the phenotypic diversification of the populations. Weedy rice also varies in tissue color throughout the season from light green to dark green.



Propanil was introduced to rice production in 1959 as a broad-spectrum herbicide to control barnyardgrass and other problematic weeds. The repeated use of propanil for more than 30 years led to the evolution of herbicide-resistant barnyardgrass populations (Baltazar and Smith 1994). The first herbicide-resistant barnyardgrass population was discovered in 1989 on a rice farm in Poinsett County, Arkansas. Propanil-resistant barnyardgrass was found to survive applications of propanil at twice the labeled rate. Further research in field experiments led to the conclusion that increasing the rate to four times the labeled rate would still not be an effective option for resistant barnyardgrass (Baltazar and Smith 1994).

Quinclorac, a novel SOA for rice in the late 1990's, quickly became the solution for controlling propanil-resistant barnyardgrass. Quinclorac was applied as either a PRE or POST herbicide and was often tank mixed with propanil (Talbert and Burgos 2007). Overuse and over reliance on this herbicide also quickly led to resistance, which was documented in 1999 in Craighead County, Arkansas (Lovelace 2003). Today, the occurrence of barnyardgrass populations having multiple resistance to propanil and quinclorac is common throughout Arkansas (Norsworthy et al. 2012). Clomazone and eventually the ALS herbicides imazethapyr and imazamox, among others, became commonplace for barnyardgrass control in Arkansas. As a result of use of a single effective SOA along with a lack of proper rice rotation with soybean in some fields, the eventual occurrence of resistance to clomazone and ALS-inhibiting herbicides was inevitable. Barnyardgrass in Arkansas now encompasses resistance to four different SOA making it the most problematic weed in rice. The excessive use of ALS herbicides has also led to the evolution of ALS-resistant weedy rice, rice flatsedge, smallflower umbrella sedge, and yellow nutsedge as well (Norsworthy et al. 2013). Rice producers must protect and preserve the

efficacy of herbicides that are currently effective, and a new SOA is needed to diversify current weed control programs.

Herbicides are the foundation of successful weed control programs in almost all crops globally. In Midsouth rice, herbicides must continue to be an effective and affordable solution for controlling weeds if rice is to continue as a profitable crop in this region. The preservation of the available SOA is of utmost importance to ensuring the longevity of these herbicides. Across all cropping systems, there are numerous herbicide target sites; however, over 50% of the current herbicides inhibit only three of the 18 known target sites. The photosystem II, ALS, and protoporphyrinogen IX oxidase target sites have been targeted in corn, cotton, soybean, rice, and other crop herbicides (Cole et al. 2000). Over reliance on herbicides that target these sites suggest that a new SOA is needed in rice to control problematic weeds and reduce selection pressure on the limited remaining SOA.

There has not been a new SOA commercialized in agronomic crops since the release of sulcotrione, a 4-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitor, in 1991 and the trend for new discovery has slowed considerably (Rüegg et al. 2007). The HPPD SOA must be incorporated into a rice grower's current weed management plan to guard the long-term utility of the technology by delaying or avoiding the chances for the evolution of resistance (Duke 2012).

Historically HPPD herbicides have required high application rates when used in rice production (Matsui et al. 1983). High application rates are often costlier, especially when applied aerially. There are three main families of HPPD-inhibitors, the pyrazolones, triketones, and diketonitriles. Benzbicyclon is a new herbicide that has been created from the triketone HPPD-inhibiting herbicide family, which will provide a new SOA in rice production (Almsick 2009).

Benzobicyclon, [3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[[3.2.1]oct-2-en-4-one], is an HPPD-inhibiting herbicide that causes bleaching symptoms and controls many annual grasses, sedges, and broadleaf weeds in flooded rice fields (Komatsubara et al. 2009). SDS Biotech K.K. discovered the herbicide, which led to its development in paddy rice and eventual commercialization in Japan in 2001. The metabolic effects associated with HPPD herbicides like benzobicyclon are due to the inhibition of the homogentisate products plastoquinone and  $\alpha$ -tocopherol biosynthesis. Secondary effects of HPPD herbicides associated with lethality include the inactivation of phytoene desaturase, absence of chloroplasts development, lipid peroxidation, and tyrosine accumulation (Cole et al. 2000).

Benzobicyclon must be metabolized before having herbicidal activity (Komatsubara et al. 2009). Komatsubara et al. (2009) documented the metabolized form of benzobicyclon, benzobicyclon hydrolysate, shows potent inhibition of HPPD. The metabolic properties of benzobicyclon result in slow conversion to the active molecule (Komatsubara et al. 2009). The fate of the herbicide can be effected when the herbicide is in the solution phase (plant available) (Miller and Westra 1998). The water solubility concentration of benzobicyclon is  $0.05 \text{ mg L}^{-1}$  and the adsorption coefficient ( $K_{oc}$ ) is 1,104; hence, the herbicide has low susceptibility to movement by leaching (Curran 2001; Kegley et al. 2014). Therefore, it is important to maintain a constant concentration of benzobicyclon in the water to maximize plant uptake.

*Japonica* rice cultivars have shown excellent safety to benzobicyclon (Sandoski et al. 2014). Bleaching is the primary symptom in weeds following an application of benzobicyclon (Almsick 2009; Komatsubara et al. 2009). These symptoms may appear on new growth as early as one week of POST applications and are followed by necrosis and eventual death of sensitive species; however, when Norsworthy et al. (2014) made PRE applications symptoms developed

slower than what would be expected of traditional preemergence herbicides in rice (Komatsubara et al. 2009).

Benzobicyclon has shown excellent control of bulrush (*Scirpus juncooides*) in Japan paddy rice fields. Benzobicyclon at 200 to 300 g ai ha<sup>-1</sup> have been used to control sulfonylurea herbicide-resistant bulrush (Komatsubara et al. 2009). Gowan Company has come to agreement with SDS Biotech K.K. to proceed with development of benzobicyclon for use in U.S. rice production systems as a POST application. Davis et al. (2013) reported promising control of several important annual grass and broadleaf rice weeds in Arkansas with POST applications of benzobicyclon. Application rates of benzobicyclon up to 371 g ha<sup>-1</sup> were evaluated for control of Amazon sprangletop, barnyardgrass, duckweed, and hemp sesbania. The application timing of benzobicyclon in relation to flood timing has shown different levels of weed control. Norsworthy et al. (2014) documented that application timing of benzobicyclon immediately after the flood provided a higher level of weed control. The importance of flood depth was evident as benzobicyclon has a low water solubility and may be shoot absorbed. As flood depth increased, less herbicide was needed to obtain effective control of the evaluated weeds, thus efficacy improved (Davis et al. 2013; Norsworthy et al. 2014).

To improve control of herbicide-resistant weed populations and other problematic weeds, different herbicide SOA and tank-mix combinations are being evaluated. McKnight et al. (2014) reported increased control of sedges and broadleaf weeds when benzobicyclon was tank-mixed with imazethapyr. Imazethapyr at 105 g ha<sup>-1</sup> followed by 70 g ha<sup>-1</sup> was evaluated with and without benzobicyclon at 246 g ha<sup>-1</sup>. Control of yellow nutsedge and duckweed increased when benzobicyclon was mixed with imazethapyr (McKnight et al. 2014).

Recently benzobicyclon has controlled ALS-weedy rice in a greenhouse and field setting at two different locations in Arkansas (Young et al. 2016). These populations vary in phenotypic characteristics and susceptibility to ALS-herbicides and benzobicyclon. For these reasons, benzobicyclon shows promise to control multiple herbicide-resistant weeds in the Midsouth, offering producers a new MOA in their current herbicide programs.

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## Chapter 2

### Optimizing Benzobicyclon Efficacy in Rice

Due to the repetitive use of the same herbicide sites of action (SOA) in rice, many weeds have evolved resistance. Growers need to integrate multiple SOA by tank-mixing herbicides for effective weed control. Benzobicyclon is a new post-flood herbicide for U.S. rice that is expected to be commercially available in 2018. As a Group 27 herbicide, benzobicyclon will offer a new SOA to rice producers in the U.S. Research evaluating benzobicyclon in combination with post-flood herbicides is needed. Three field experiments were conducted in 2015 and 2016 across Arkansas at the University of Arkansas Agricultural Research and Extension Center located in Fayetteville, the Pine Tree Research Station near Colt, AR, the Rice Research and Extension Center near Stuttgart, AR, and at the University of Arkansas Pine Bluff Farm near Lonoke, AR to evaluate benzobicyclon efficacy based on weed size at application, flood depth, weed spectrum, tank-mixes with post-flood herbicides, and use rate. The greatest efficacy on barnyardgrass with benzobicyclon occurred when applied at the early timing in a 15-cm flood depth 2 weeks after treatment. Control with benzobicyclon was greater at 371 g ai ha<sup>-1</sup> compared to 247 g ha<sup>-1</sup>. Complete control of Amazon sprangletop and acetolactate synthase-resistant rice flatsedge were achieved with rates as low as 247 g ha<sup>-1</sup> at the early timing 3 weeks after treatment at both the 5- and 15-cm flood depth. The addition of benzobicyclon at 247 g ha<sup>-1</sup> to post-flood herbicides generally increased barnyardgrass and sprangletop spp. control. Benzobicyclon at 247 g ha<sup>-1</sup> added to halosulfuron at 53 g ha<sup>-1</sup> increased barnyardgrass control. The addition of benzobicyclon at 247 g ha<sup>-1</sup> to other post-flood herbicides such as halosulfuron at 53 g ha<sup>-1</sup>, imazamox at 45 g ha<sup>-1</sup>, and cyhalofop 280 g ha<sup>-1</sup> increased red sprangletop and

Amazon sprangletop control (>90%) at 4 weeks after treatment. The addition of benzobicyclon to post-flood herbicides will broaden and improve spectrum of weed control in U.S. rice.

**Nomenclature:** Benzobicyclon; cyhalofop, imazamox, halosulfuron, Amazon sprangletop, *Leptochloa panicoides* J. Presl; barnyardgrass, *Echinochloa crus-galli* L. Beauv.; red sprangletop, *Leptochloa panicea* (Retz.) Ohwi; rice flatsedge, *Cyperus iria* L.; rice, *Oryza sativa* L.

**Key words:** efficacy, herbicide resistance, flood depth, site of action, target size.

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## Introduction

Rice growers in the midsouthern U.S. needed a solution for weedy rice and barnyardgrass control in the early 2000's; hence, imidazolinone-resistant rice was launched in 2002, enabling the use of imazethapyr and imazamox within the crop (Hardke 2013). These herbicides quickly became the foundation of many weed control programs in rice. By 2008, imidazolinone-resistant cultivars accounted for 40% of the rice acreage in Arkansas (Wilson and Runsick 2008). The acreage steadily increased from the time of its introduction until 2011 when imidazolinone-resistant rice accounted for 69% of the Arkansas rice acreage (Hardke and Wilson 2012). Thereafter, a steady decline in the imidazolinone-resistant rice acreage occurred partly due to the evolution of imidazolinone-resistant weedy rice and barnyardgrass populations (Heap 2016). In a 2012 survey of Midsouth crop consultants, barnyardgrass and weedy rice were the first and third most important weeds of rice (Norsworthy et al. 2013). Imazethapyr and imazamox are no longer an effective option for producers with infestations of these imidazolinone-resistant weeds.

Imidazolinone herbicides are no longer an effective option for barnyardgrass and weedy rice control for many rice producers in the midsouthern U.S. (Hardke 2013; Norsworthy et al. 2012). Additionally, over reliance of acetolactate synthase (ALS)-inhibiting herbicides has led to yellow nutsedge (*Cyperus esculentus* L.), smallflower umbrella sedge (*Cyperus difformis* L.), rice flatsedge (*Cyperus iria* L.), and several aquatic weeds evolving resistance to this site of action (SOA) (Heap 2016). Annual sedges continue to become more common and problematic in Arkansas rice because of ALS resistance and extensive use of clomazone early in the growing season (Bagavathiannan et al. 2014; Norsworthy et al. 2007, 2008; Scott et al. 2014).

Prior to ALS resistance, herbicides such as halosulfuron, imazethapyr, imazamox, penoxsulam, and bispyribac were used frequently to control most problematic weeds of rice (Masson et al. 2001; Scott et al. 2016; Wilson et al. 2014). Propanil, a photosystem II inhibitor (WSSA Group 7), was once exclusively relied on for postemergence control of barnyardgrass. Over reliance on this herbicide led to evolution of propanil-resistant barnyardgrass (Carey et al. 1995; Heap 2016). Today, barnyardgrass in Arkansas has evolved resistance to acetyl CoA carboxylase inhibitors (WSSA Group 1), ALS inhibitors (WSSA Group 2), quinclorac (WSSA Group 4), propanil (WSSA Group 7), and clomazone (WSSA Group 13) (Heap 2016). It is imperative growers preserve the effective herbicide SOAs that are available for use today.

One of several strategies to mitigate the evolution of herbicide resistance is the use of multiple effective SOA over the course of a growing season (Norsworthy et al. 2012). Program approaches to weed control are more effective and create the opportunity to overlap applications allowing for broadened and improved weed control. The integration of multiple SOA into a herbicide program reduces the risk for resistance. For example, Beckie (2006) reported that weed populations can evolve resistance to ALS-inhibiting herbicides in as few as five applications in the absence of multiple SOA.

Producers often prefer to tank-mix herbicides because it can reduce application costs and potentially broaden the spectrum of weed control (Hydrick and Shaw 1994). For example, Norsworthy et al. (1998) showed that improved levels of propanil-resistant barnyardgrass control can be achieved when tank-mixing thiobencarb with propanil over propanil alone. Likewise, tank mixing can improve control of broadleaf species such as hemp sesbania (*Sesbania herbacea* Mill.). Norsworthy et al. (2010) reported increased (>95%) hemp sesbania control with the addition of propanil to triclopyr, 2,4-D, aciflurofen, carfentrazone, penoxsulam, quinclorac,

halosulfuron, bentazon, and bispyribac over applying propanil alone. Using a program approach while utilizing full-recommended rates in tank-mix combination with multiple effective herbicide SOA will offer a grower the most sustainable and effective weed control program.

Growers in Asia have used benzobicyclon, a 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting (WSSA Group 27) post-flood herbicide, with much success for controlling ALS-resistant sedge (*Cyperus* spp.) populations in paddy-rice (Komatsubara et al. 2009; Sekino et al. 2008). Benzobicyclon provides broad-spectrum control of many aquatic, broadleaf, sedge, and grass weeds of rice (Davis et al. 2013; Norsworthy et al. 2014; Sandoski et al. 2014). Benzobicyclon was recently registered in U.S. rice production (Anonymous 2017). It is the first HPPD-inhibiting herbicide for use in U.S. rice production. Benzobicyclon is expected to be rapidly adopted in the Midsouth because ALS-resistant sedge populations are becoming more difficult to control with currently available herbicides (C. Sandoski, personal communication).

Adding benzobicyclon to current weed control programs will incorporate a new SOA improving and broadening weed spectrum. A new SOA will reduce the selection pressure on currently labeled post-flood herbicides (Norsworthy et al. 2012). Benzobicyclon will likely improve weed control when tank-mixed with post-flood herbicides, especially halosulfuron (Young et al. 2016). Northern jointvetch (*Aeschynomene virginica* L.) and barnyardgrass control with benzobicyclon were improved by the addition of halosulfuron (Young et al. 2016). Furthermore, California arrowhead (*Sagittaria latifolia* Willd.), duckweed (*Heteranthera limosa* Sw.), and ALS-resistant smallflower umbrella sedge were completely controlled (100%) by benzobicyclon alone while halosulfuron provided unacceptable control of these weeds (Young et al. 2016).

The effectiveness of a herbicide, including a tank-mix, is largely dependent on herbicide rate, weed size at application, and flood depth at application (Davis et al. 2013; Lee and Oliver 1982). Davis et al. (2013) reported that rate and flood depth are critical to success with applications of benzobicyclon. Weed size is another important consideration when making late season post-flood herbicide applications. For example, Chauhan et al. (2012) reported greater control when making postemergence applications of bispyribac, fenoxoprop plus ethoxysulfuron, or penoxsulam plus cyhalofop to 4-leaf compared to 8-leaf barnyardgrass.

The objectives of this research were to 1) determine if weed control with benzobicyclon is influenced by weed size and flood depth at application, 2) evaluate the efficacy of benzobicyclon and halosulfuron alone versus a tank-mixture, and 3) determine what tank-mix partners with benzobicyclon would broaden and improve weed control over benzobicyclon alone.

## **Materials and Methods**

**Influence of Weed Size, Species, and Flooding Depth on Benzobicyclon.** A controlled field experiment was conducted in 2015 at the University of Arkansas Agricultural Research and Extension Center located in Fayetteville, Arkansas to characterize benzobicyclon efficacy as influenced by flooding depth and rate. The weeds evaluated included Amazon sprangletop and yellow nutsedge. The experimental design was completely randomized with three replications having a two-factor factorial treatment structure. The factors consisted of three flooding depths (saturated, 5-cm, and 15-cm) and two application rates (none and benzobicyclon at 247 g ai ha<sup>-1</sup>).

The experiment was repeated at the same site in Fayetteville, Arkansas in 2016 with an additional factor (application timing) and two additional weed species (ALS-resistant rice

flatsedge and barnyardgrass). The experimental design was completely randomized with three replications having a three-factor factorial treatment structure. The factors consisted of two application timings (early: target 2-leaf weeds and late: target 6-leaf weeds), three flooding depths (saturated, 5-cm, and 15-cm), and two application rates (none and benzobicyclon at 247 g ai ha<sup>-1</sup>).

For both years, a Pembroke silt loam (fine-silty, mixed, active, mesic Mollic Paleudalfs) soil was placed into tubs (61-cm x 47-cm x 40-cm) to an approximate 22-cm depth and then seeded to two weed species per tub in the field, except for yellow nutsedge for which tubers were planted. Tubs were used to ensure that the proper flood depth was maintained for the duration of the experiment. Height, leaf number, and density of each weed species at application are provided in Table 2.1. Immediately prior to herbicide treatment, the flooding depths were established and maintained throughout the experiment by daily watering to the desired depth. The water level was marked inside each tub to the corresponding depth to ensure each tub maintained and received the proper flooding depth. During rainfall events, the tubs were covered to prevent increased flood depths. Benzobicyclon was applied using a CO<sub>2</sub>-pressurized backpack sprayer consisting of a three-nozzle, handheld boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703) calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa.

**Benzobicyclon plus Halosulfuron Tank-Mix.** A field experiment was conducted in 2015 and 2016 at the Rice Research and Extension Center near Stuttgart, Arkansas (hereafter referred to as Stuttgart) to assess halosulfuron and benzobicyclon alone and as a tank-mix on common weeds of midsouthern U.S. rice. The experimental design was a randomized complete block with six treatments and an untreated control with three replications. The treatments included a low (35 g ai ha<sup>-1</sup>) and high (53 g ha<sup>-1</sup>) of halosulfuron, a low (247 g ha<sup>-1</sup>) and high (371 g ha<sup>-1</sup>) rate of



benzobicyclon, a low rate of both benzobicyclon ( $247 \text{ g ha}^{-1}$ ) and halosulfuron ( $35 \text{ g ha}^{-1}$ ), and a high rate of both benzobicyclon ( $371 \text{ g ha}^{-1}$ ) and halosulfuron ( $53 \text{ g ha}^{-1}$ ). Individual bays were used to prevent benzobicyclon movement among treatments. This is the only way to ensure that non-benzobicyclon plots were not contaminated by the herbicide. The bays measured 3.0 by 45.7 m levee to levee. Two experimental plots were planted within one bay. The plots measured 1.8 by 22.8 m and were planted with a 9-row cone drill on May 7, 2015 and April 25, 2016. CL111 was planted at 66 seed  $\text{m}^{-1}$  of row in 2015 and 2016, and a 1.5 m alley was created between plots. Maintenance applications of imazethapyr at  $35 \text{ g ai ha}^{-1}$  (1/2X rate) were made to 2- to 3-leaf rice followed by propanil at  $1,680 \text{ g ai ha}^{-1}$  (1/3X rate) at the 4- to 5-leaf rice growth stage to suppress weeds while ensuring the presence of some weeds after establishment of the permanent flood. Herbicide treatments were made on June 23, 2015 and June 9, 2016. Treatments were applied post-flood using a  $\text{CO}_2$ -pressurized backpack sprayer consisting of a four-nozzle, handheld boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703), calibrated to deliver  $143 \text{ L ha}^{-1}$  at 276 kPa. Two passes were made covering the entire plot and the water surface area adjacent to the plot. The test required 11 individual bays, with the flood maintained at an approximate 7-cm depth throughout the season and nitrogen ( $168 \text{ kg ha}^{-1}$ ) was applied immediately prior to flooding to simulate normal rice culture.

**Tank-mixing Benzobicyclon with Post-flood Rice Herbicides.** A field experiment was conducted in 2016 at the University of Arkansas Pine Bluff Farm near Lonoke, Arkansas (hereafter referred to as Lonoke) and at the Pine Tree Research Station near Colt, Arkansas (hereafter referred to as Pine Tree) to assess commonly used rice herbicides and benzobicyclon alone and as a tank-mix on common weeds of midsouthern U.S. rice. The experiment was a split-plot where the main plot was a randomized complete block with a split of treatments

containing benzobicyclon and not containing benzobicyclon with four replications. The experiment consisted of a main plot of benzobicyclon rate (none vs. 247 g ai ha<sup>-1</sup>) and a subplot of other rice herbicides, including penoxsulam at 35 g ai ha<sup>-1</sup>, bispyribac at 23 g ai ha<sup>-1</sup>, halosulfuron 53 at g ai ha<sup>-1</sup>, imazamox 45 at g ai ha<sup>-1</sup>, cyhalofop 280 at g ai ha<sup>-1</sup>, saflufenacil 25 at g ai ha<sup>-1</sup>, carfentrazone 18 at g ai ha<sup>-1</sup>, propanil at 3,360 g ai ha<sup>-1</sup>, bentazon at 840 g ai ha<sup>-1</sup>, halosulfuron plus thifensulfuron at 35 and 5 g ai ha<sup>-1</sup>, and a none treatment, respectively. Eight individual bays were used to prevent benzobicyclon movement among treatments (Davis et al. 2013; McKnight et al. 2014). The bays measured 5 by 67 m levee to levee. Eleven experimental plots measuring 1.8 by 5.2 m were contained in each bay, consisting of nine rice rows on an 18-cm spacing with a 1 m alley between plots. CL111 was planted at 66 seed m<sup>-1</sup> of row on May 14 at Pine Tree and on May 19 at Lonoke.

To provide suppression of early-season weeds while ensuring the presence of some weeds after establishment of the permanent flood, the Lonoke location received a low rate of clomazone (64 g ai ha<sup>-1</sup>) immediately after planting followed by propanil (1,680 g ai ha<sup>-1</sup>) at the 2- to 3-leaf rice growth stage. No maintenance herbicide applications were made at the Pine Tree location because the field site had a historically lower weed density and weeds emerged later than at Stuttgart. The treatments were made as a tank-mix, simultaneously in the bays that contained benzobicyclon. The bays that did not contain benzobicyclon allowed for individual applications of the subplot herbicides. All treatments were applied post-flood using a CO<sub>2</sub>-pressurized backpack sprayer consisting of a handheld four-nozzle boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703) calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. Two passes of the boom were made to cover the entire plot and water surface area adjacent to the plot. Each treatment was replicated four times, and the test required eight

individual bays. The flood was maintained at approximately a 7-cm depth throughout the season and nitrogen ( $168 \text{ kg ha}^{-1}$ ) was applied immediately prior to flooding to simulate normal rice culture.

**Assessments.** For the benzobicyclon trial in the tubs, herbicide efficacy was assessed at 2 and 3 weeks after treatment. Ratings were based on a scale of 0 to 100%, with 0 being no control relative to the saturated, nontreated check and 100% being complete control of the evaluated weed species. Aboveground biomass was collected the day of the final assessment to determine relative biomass reduction of the experimental treatments compared to the saturated, nontreated check. All biomass was oven-dried at  $66^\circ\text{C}$  for 7 days and weighed.

In the trial evaluating benzobicyclon and halosulfuron alone and as a tank-mixture, weed control was rated 3, 5, and 9 weeks after treatment (WAT) relative to the nontreated control using the method described earlier. The trial evaluating tank-mixing post-flood herbicides with benzobicyclon was rated for weed control at 2, 4, and 6 WAT. For both trials, experimental plots were machine harvested at crop maturity to determine rough rice yield at an adjusted moisture of 12%.

**Statistical Analyses.** For the tub study data in 2015, the weed control data were transformed by  $(\log(x+1))$ , analyzed, and then back-transformed. In 2015, the data collected for Amazon sprangletop and yellow nutsedge, weed control means and standard errors were reported because the assumptions for analysis of variance (ANOVA) were not met. Each assessment date was analyzed separately. The biomass data underwent transformation  $(\log(x+1))$  and were subjected to ANOVA. The treatment means were separated using Fisher's protected LSD ( $\alpha=0.05$ ). Mean

separation was based on the transformed data, but back-transformed means were presented for reporting results.

In 2016, weed control and biomass reduction data were transformed if needed and were subjected to ANOVA and means separated using Fisher's protected LSD ( $\alpha=0.05$ ). Data transformations were performed by  $(\log(x+1))$ . Mean separation was based on the transformed data but back-transformed means were presented in the percentage form for reporting results. Each assessment date was analyzed separately.

For the benzobicyclon plus halosulfuron trial, there was no significant year effect; therefore, data were combined over years. All weed control and yield data were subjected ANOVA and means separated using Fisher's protected LSD ( $\alpha=0.05$ ). Each assessment date was analyzed separately. The yield was analyzed separately because of the different environmental conditions in 2015 versus 2016. In the trial evaluating tank-mixing benzobicyclon with other post-flood herbicides, locations were analyzed separately due to varying weed densities, sizes, and spectrum at the locations. For the tank-mixing of benzobicyclon with post-flood herbicide trial, all weed control and yield data were subjected ANOVA and means separated using Fisher's protected LSD ( $\alpha=0.05$ ). For the weed control, each assessment date was analyzed separately. All data were analyzed using JMP statistical software (JMP, Version 12.1 SAS Institute Inc., Cary, NC).

## **Results and Discussion**

**Influence of Weed Size, Species, and Flooding Depth on Benzobicyclon.** For 2015, the importance of flood depth and weed species on benzobicyclon efficacy can be seen in Tables 2.2. The results of this experiment indicate benzobicyclon should only be used in a continuous flood

environment based on the efficacy observed as a function of flood depth. For the saturated treatment, benzobicyclon controlled Amazon sprangletop by only 69% at 3 WAT (Table 2.2). This level of weed control would be deemed unacceptable in a commercial production system; hence, the need for maintaining a flooded environment within the field.

Based on these data, benzobicyclon was effective in controlling Amazon sprangletop (>89%) at 3 WAT at the 5 and 15 cm flood depths (Table 2.2). For the two weeds evaluated in 2015, flooding increased the sensitivity of Amazon sprangletop to benzobicyclon. Based on these results, benzobicyclon alone does not appear to provide effective yellow nutsedge control, and hence should be combined with other herbicides if either of these weeds are of appreciable density in a commercial field. Furthermore, observation from this experiment also led to the conclusion that benzobicyclon is a slow acting herbicide that may need as many as four weeks after application to achieve maximum control. The slowness of the herbicidal activity may be partly attributed to its SOA, but more importantly the fact that benzobicyclon must be converted to the benzobicyclon hydrolysate to become herbicidally active may have the greatest impact on the delay in activity (Williams and Tjeerdema 2016). For example, yellow nutsedge control increased 27 percentage points from 2 WAT to 3 WAT at the 15 cm flood depth (Table 2.2). Nevertheless, the level of control would have likely increased, for both species, as time progressed after the assessment at 3 weeks. Therefore, it is important for growers to establish and maintain a permanent flood with a minimum depth of 5 cm when applying benzobicyclon.

In 2016, a third factor, application timing was added to the experiment to determine if efficacy was influenced by size of the targeted weed species at application in addition to flood depth and rate (Tables 2.3). Amazon sprangletop was completely controlled by 2 WAT when applied at the early timing for both the 5 cm and 15 cm flood depth. However, there was a 28%

reduction in control at 2 WAT for the 15-cm flood depth when applied at the late timing. The importance of herbicide application timing is displayed by observing the differences in control at 2 WAT for the different timings. Similarly, a greater reduction (55%) of control is observed on a more tolerant species, barnyardgrass, when comparing the early and late timings 2 WAT at the 15 cm flood depth (Table 2.3). For effective management of barnyardgrass, applicators must be timely when treating with benzobicyclon and even then, effective control may not be obtained based on these results. Targeting weeds at the proper size is imperative for growers to have effective herbicide applications. Weed size must be incorporated into integrated weed management strategies for consultants and growers. The size of the weed at application is an important best management practice for reducing selection for the evolution of herbicide resistance and for the proper stewardship of the herbicide (Norsworthy et al. 2012).

In addition, it is important to note that benzobicyclon is an effective herbicide option for control of rice flatsedge, including ALS-resistant biotypes (Table 2.3). Benzobicyclon, a Group 27 herbicide, will offer growers a new SOA for hard-to-control annual sedge species. The importance of rice flatsedge is increasing as it continues to evolve resistance to the ALS-inhibiting herbicides and becomes widespread. The importance of weed size at application is also observed in the yellow nutsedge results as control increased 35 percentage points when applied at the early timing versus the late timing at 2 WAT (Table 2.3). The dry weight reduction of all species is documented in Table 2.4. Applications of benzobicyclon at both timings and all flood depths resulted in 100% dry weight reduction of Amazon sprangletop in 2016 (Table 2.4). For rice flatsedge, early applications containing benzobicyclon resulted in (>99%) dry weight reduction in 2016. Additionally, clomazone, which is ineffective in providing sedge control,

continues to be applied to almost every hectare at planting, sometimes alone, placing continued selection on POST-applied herbicides (Norsworthy et al. 2014; Bagavathiannan et al. 2014).

**Benzobicyclon plus Halosulfuron Tank-Mix.** Weed heights and densities at application are shown in Table 2.5. All benzobicyclon-containing treatments provided a high level of control (>97%) of ALS-resistant smallflower umbrella sedge, ducksalad, and California arrowhead at all assessment dates (data not shown). Benzobicyclon, a Group 27 herbicide, will offer producers a new, alternative SOA for the control of problematic ALS-resistant smallflower umbrella sedge. Barnyardgrass was never effectively controlled (>80%) by benzobicyclon alone or in combination with halosulfuron (Table 2.6), which may have been partly due to its large size at application (Table 2.5). Hence, it is important to emphasize the need to make timely applications and avoid salvage situations, especially when barnyardgrass is present in the field.

High levels (>90%) of hemp sesbania control occurred 3 WAT with the high rate of benzobicyclon alone and both rates of benzobicyclon plus halosulfuron (Table 2.6). The overall importance of adding benzobicyclon to halosulfuron can be documented by observing the yield of the high rate combination treatment of benzobicyclon plus halosulfuron (Table 2.7). The high rate combination treatment yielded higher than the other treatments. The yield also indicates the crop safety shown by CL111 to the high rate tank-mix of benzobicyclon plus halosulfuron. Therefore, it is concluded that benzobicyclon plus halosulfuron broadens and improves control of late season problematic weeds and increases the likelihood for growers to see higher yields when weeds are present in rice after establishment of the permanent flood.

**Tank-mixing Benzobicyclon with Post-flood Herbicides.** Weed heights and densities at time of application are listed in Table 2.8. Tank-mixing herbicides will often increase and broaden

control. The addition of benzobicyclon to penoxsulam, bispyribac, halosulfuron, saflufenacil, carfentrazone, propanil, bentazon, and halosulfuron plus thifensulfuron significantly increased control of Amazon sprangletop at Lonoke at 6 WAT (Table 2.9). For barnyardgrass, the only improvement in control was the addition of benzobicyclon to saflufenacil, resulting in control improving from 9% with saflufenacil alone to 64% with the tank-mixture 4 WAT (Table 2.9). The tank-mix of bispyribac-sodium plus benzobicyclon provided >90% control of barnyardgrass across all evaluations at Lonoke. For the graminicides, the addition of benzobicyclon to the tank generally increased control of broadleaf weeds, as expected, like hemp sesbania and northern jointvetch at Lonoke (Table 2.10). Benzobicyclon has excellent activity on northern jointvetch as seen 6 WAT with control of all benzobicyclon treatments being  $\geq 99\%$  at Lonoke (Table 2.10).

At Pine Tree 6 WAT, benzobicyclon improved control of halosulfuron, imazamox, saflufenacil, carfentrazone, and bentazon (Table 2.11). Benzobicyclon improved control of red sprangletop when added to nearly all of the herbicides 4 and 6 WAT at Pine Tree. Few differences in control among treatments were observed for Asiatic dayflower and yellow nutsedge (Table 2.12). The addition of benzobicyclon to cyhalofop, penoxsulam, propanil, and halosulfuron plus thifensulfuron improved Asiatic dayflower control 4 WAT at Pine Tree.

The rough rice yields indicate that the majority of the herbicides applied with benzobicyclon provided adequate weed control so that yield was not affected (Table 2.13). A yield loss was documented at Lonoke when benzobicyclon was applied with bispyribac and cyhalofop. Similarly, a yield loss was documented at Pine Tree when benzobicyclon was applied with bentazon. However, for the treatments that underwent a yield loss it was only at one of the two locations. This suggests these treatments should be re-evaluated. The yields at Pine Tree were generally higher than Lonoke because of the uptake of the pre-flood fertilizer. At Pine Tree, it



was applied and flooded in a timely fashion. At Lonoke, the pre-flood fertilizer was applied and did not receive a flood until nearly a week later. This suggest some of the pre-flood urea fertilizer likely volatilized as ammonia and was not taken up as efficiently by the crop. This research indicates that benzobicyclon is an effective post-flood herbicide that has a broad spectrum of activity. Increased weed control is expected when benzobicyclon is tank-mixed with most other post-flood rice herbicides. The ability to apply a Group 27 herbicide, like benzobicyclon, will give growers another option to control late season escapes.

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Table 2.1. Leaf number, height, and density of weed species at time of application for the benzobicyclon efficacy experiment in 2015 and 2016.<sup>a</sup>

Weed species	Application timing <sup>b</sup>											
	2015						2016					
	Early			Late			Early			Late		
	Leaf	Height	Density	Leaf	Height	Density	Leaf	Height	Density	Leaf	Height	Density
	no.	cm	plants tub <sup>-1</sup>	no.	cm	plants tub <sup>-1</sup>	no.	cm	plants tub <sup>-1</sup>	no.	cm	plants tub <sup>-1</sup>
Amazon sprangletop	1-3	13	16	3-5	23	17	1-2	13	11	3-5	22	13
Barnyardgrass		NP			NP		1-2	14	9	5-6	33	10
Rice flatsedge		NP			NP		1-3	13	13	4-5	23	10
Yellow nutsedge	2-4	18	15	5-6	33	9	1-2	18	10	5-6	33	11

<sup>a</sup> “NP” Indicates not present. Barnyardgrass and acetolactate synthase-resistant rice flatsedge were not included in the 2015 experiment due to lack of germination.

<sup>b</sup> “Leaf number” indicated by the number of true leaves present on the plant at application.

Table 2.2. Post-flood Amazon sprangletop control 2 and 3 weeks after treatment (WAT) as influenced by the interaction of benzobicyclon rate and flood depth in 2015.

Treatments		Amazon sprangletop			Yellow nutsedge		
Benzobicyclon <sup>c</sup>	Flood depth <sup>d</sup>	Control		Dry weight reduction	Control		Dry weight reduction
		2 WAT <sup>b</sup>	3 WAT		2 WAT	3 WAT	
g ai ha <sup>-1</sup>		-----%-----		% of nontreated	-----%-----		% of nontreated
0 (none)	Sat	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	5 cm	0 (0)	0 (0)	-4 (3)	0 (0)	0 (0)	-57 (11)
	15 cm	0 (0)	0 (0)	-5 (7)	20 (7)	0 (0)	-21 (14)
247	Sat	50 (15)	69 (6)	74 (4)	25 (10)	68 (6)	50 (16)
	5 cm	65 (12)	95 (5)	75 (4)	25 (7)	74 (7)	13 (22)
	15 cm	71 (12)	89 (7)	73 (4)	38 (5)	65 (10)	25 (16)

<sup>a</sup> Each assessment was date was analyzed separately. Means followed by the standard error in parentheses

<sup>b</sup> WAT' abbreviates weeks after treatment

<sup>c</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>d</sup> Abbreviation: Sat, saturated

Table 2.3. Post-flood control of problematic weeds 2 and 3 WAT as influenced by rate, application timing, and flood depth in 2016.

Treatments			Control <sup>a</sup>							
			Amazon sprangletop		Barnyardgrass		Rice flatsedge		Yellow nutsedge	
Benzobicyclon <sup>c</sup>	Application timing	Flood depth <sup>d</sup>	2 WAT <sup>b</sup>	3 WAT	2 WAT	3 WAT	2 WAT	3 WAT	2 WAT	3 WAT
g ai ha <sup>-1</sup>			-----%							
0 (none)	Early	Sat	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		5 cm	0 (0)	3 (3)	0 (0)	7 (3)	0 (0)	7 (3)	0 (0)	3 (2)
		15 cm	67 (2)	58 (9)	38 (14)	42 (11)	67 (7)	72 (4)	0 (0)	12 (4)
	Late	Sat	0 (0)	0 (0)	15 (0)	0 (0)	13 (13)	0 (0)	0 (0)	0 (0)
		5 cm	2 (2)	0 (0)	18 (4)	0 (0)	5 (3)	0 (0)	7 (4)	0 (0)
		15 cm	18 (7)	5 (5)	22 (7)	7 (7)	20 (12)	0 (0)	13 (9)	0 (0)
	Early	Sat	40 (6)	55 (13)	17 (9)	23 (7)	37 (15)	57 (14)	28 (4)	45 (12)
		5 cm	100 (0)	100 (0)	88 (12)	93 (7)	68 (6)	100 (0)	52 (7)	62 (4)
		15 cm	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	73 (4)	80 (6)
247	Late	Sat	63 (17)	62 (19)	28 (6)	25 (13)	68 (6)	47 (7)	32 (2)	23 (7)
		5 cm	88 (4)	100 (0)	20 (6)	18 (8)	88 (12)	91 (8)	17 (7)	50 (6)
		15 cm	72 (10)	90 (10)	45 (13)	30 (13)	80 (10)	90 (5)	38 (16)	53 (7)

<sup>a</sup> Each assessment was date was analyzed separately. Means followed by the standard error in parentheses

<sup>b</sup> WAT' abbreviates weeks after treatment

<sup>c</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>d</sup> Abbreviation: Sat, saturated

Table 2.4. Dry weight reduction of problematic weeds as influenced by the interaction of benzobicyclon rate, application timing, and flood depth in 2016.

Treatments			Dry weight reduction <sup>a</sup>			
Benzobicyclon <sup>b</sup>	Application timing	Flood depth	Amazon sprangletop	Barnyardgrass	Rice flatsedge	Yellow nutsedge
g ai ha <sup>-1</sup>			------%-----			
40	0 (none)	Sat <sup>d</sup>	--	--	--	--
		5 cm	77 (17)	12 (16)	56 (22)	-14 (11)
		15 cm	18 (23)	59 (18)	39 (29)	5 (22)
		Late	55 (55)	-18 (25)	1 (35)	-15 (12)
	247	5 cm	-31 (40)	-13 (29)	73 (18)	-40 (32)
		15 cm	24 (22)	13 (14)	-7 (10)	-16 (22)
		Early	100 (0)	61 (18)	99 (1)	58 (21)
		5 cm	100 (0)	58 (23)	100 (0)	32 (15)
		15 cm	100 (0)	100 (0)	100 (0)	67 (6)
		Late	100 (0)	33 (13)	26 (74)	-26 (22)
		5 cm	100 (0)	-20 (26)	100 (0)	-3 (7)
		15 cm	100 (0)	61 (16)	97 (3)	61 (19)

<sup>a</sup> Each assessment was date was analyzed separately. Means followed by the standard error in parentheses

<sup>b</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>c</sup> The 'No Benzobicyclon X Early X Saturated' treatment was the untreated control and not included in the analysis

<sup>d</sup> Abbreviation: Sat, saturated



Table 2.5. Density and height of weed species present in the experiment evaluating benzobicyclon plus halosulfuron in 2015 and 2016.<sup>a</sup>

Weed species	2015		2016	
	Density	Height	Density	Height
	plants m <sup>-2</sup>	cm	plants m <sup>-2</sup>	cm
Smallflower umbrella sedge	2	15	NP	
Barnyardgrass	6	28	9	25
Hemp sesbania	5	29	9	31
California arrowhead	NP		NP	
Ducksalad	NP		NP	

<sup>a</sup> Abbreviation: NP, weed not present at time of application

Table 2.6. Post-flood control of barnyardgrass and hemp sesbania with applications of benzobicyclon and halosulfuron averaged over 2015 and 2016 at Stuttgart, AR.

Herbicide <sup>b</sup>	Rate g ai ha <sup>-1</sup>	Control <sup>a</sup>					
		Barnyardgrass			Hemp sesbania		
		3 WAT <sup>c</sup>	5 WAT	9 WAT	3 WAT	5 WAT	9 WAT
		-----%					
Halosulfuron (low)	35	13 b	0 b	0 c	48 b	100 a	98 a
Halosulfuron (high)	53	20 b	0 b	0 c	48 b	99 a	98 a
Benzobicyclon (low)	247	47 a	40 a	43 b	76 ab	90 a	84 b
Benzobicyclon (high)	371	57 a	46 a	56 ab	90 a	88 a	96 a
Benzobicyclon (low) + halosulfuron (low)	247 + 35	51 a	58 a	63 a	94 a	96 a	99 a
Benzobicyclon (high) + halosulfuron (high)	371 + 53	49 a	60 a	73 a	99 a	98 a	98 a

<sup>a</sup> Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ )

<sup>b</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>c</sup> WAT, weeks after treatment

Table 2.7. Rough rice yield as influenced by weed control from applications of benzobicyclon and/or halosulfuron averaged across 2015 and 2016 at Stuttgart.

Herbicide	Rate	Rough rice yield <sup>a,b</sup>
	g ai ha <sup>-1</sup>	kg ha <sup>-1</sup>
Halosulfuron (low)	35	6190 d
Halosulfuron (high)	53	6590 cd
Benzobicyclon (low)	247	7700 b
Benzobicyclon (high)	371	7380 b
Benzobicyclon (low) + halosulfuron (low)	247 + 35	7000 bc
Benzobicyclon (high) + halosulfuron (high)	371 + 53	8880 a

<sup>a</sup> Rough rice machine harvested on September 14, 2015 and August 31, 2016.

<sup>b</sup> Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD ( $\alpha = 0.05$ )

Table 2.8. Density and height of weed species present in the tank-mix field experiment in 2016 at Lonoke and Pine Tree.<sup>a</sup>

Weed species	Lonoke		Pine Tree	
	Density	Height	Density	Height
	plants m <sup>-2</sup>	cm	plants m <sup>-2</sup>	cm
Barnyardgrass	7	30	8	36
Amazon sprangletop	3	25	NP	
Hemp sesbania	3	30	NP	
Northern jointvetch	1	18	NP	
Red sprangletop	NP		4	19
Asiatic dayflower	NP		6	13
Yellow nutsedge	NP		8	30

<sup>a</sup> Abbreviation “NP” indicates weed not present at location or time of application

Table 2.9. Post-flood control of barnyardgrass and Amazon sprangletop as influenced by benzobicyclon rate and additive herbicide at Lonoke in 2016.

Herbicide <sup>a</sup>	Additive rate g ai ha <sup>-1</sup>	Benzobicyclon rate g ai ha <sup>-1</sup>	Control				
			Barnyardgrass			Amazon sprangletop	
			2 WAT <sup>b</sup>	4 WAT	6 WAT	4 WAT	6 WAT
			-----%-----				
Penoxsulam	35	0 (none)	64	53	24	80	69
Penoxsulam + benzobicyclon	35	247	64	40	28	73	100*
Bispyribac	23	0	90	88	84	86	70
Bispyribac + benzobicyclon	23	247	97	90	90	99	100*
Halosulfuron	53	0	33	31	16	49	54
Halosulfuron + benzobicyclon	53	247	44	43	29	94*	100*
Imazamox	45	0	45	45	31	78	77
Imazamox + benzobicyclon	45	247	64	71	54	96*	100*
Cyhalofop	280	0	88	85	80	88	91
Cyhalofop + benzobicyclon	280	247	78	85	61	96	100
Saflufenacil	25	0	25	9	5	79	66
Saflufenacil + benzobicyclon	25	247	47	64*	37	95	100*
Carfentrazone	18	0	20	26	9	65	75
Carfentrazone + benzobicyclon	18	247	30	30	24	96*	100*
Propanil	3360	0	38	80	35	61	76
Propanil + benzobicyclon	3360	247	28	83	26	97*	100*
Bentazon	840	0	28	39	15	61	60*
Bentazon + benzobicyclon	840	247	18	23	20	96*	99
Halosulfuron + thifensulfuron	35 + 5	0	55	56	26	63	71*
Halosulfuron + thifensulfuron + benzobicyclon	35 + 5	247	50	53	26	95*	100

<sup>a</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>b</sup> Abbreviations: WAT, weeks after treatment

\* Indicates a statistical improvement of efficacy with the addition of benzobicyclon according to Fisher's protected LSD ( $\alpha = 0.05$ ). Each assessment date was analyzed separately.

Table 2.10. Post-flood broadleaf weed control as influenced by benzobicyclon rate and additive herbicide at Lonoke in 2016.

Herbicide <sup>a</sup>	Additive rate	Benzobicyclon rate	Control			
			Hemp sesbania	Northern jointvetch		
			4 WAT <sup>b</sup>	2 WAT	4 WAT	6 WAT
	g ai ha <sup>-1</sup>	g ai ha <sup>-1</sup>	-----%-----			
Penoxsulam	35	0 (none)	83	65	83	80
Penoxsulam + benzobicyclon	35	247	98	96*	97	100*
Bispyribac	23	0	70	84	88	85
Bispyribac + benzobicyclon	23	247	91	81	93	100
Halosulfuron	53	0	73	60	71	84
Halosulfuron + benzobicyclon	53	247	90	85*	94*	100
Imazamox	45	0	71	74	81	48
Imazamox + benzobicyclon	45	247	94*	84	93	100*
Cyhalofop	280	0	78	21	24	35
Cyhalofop + benzobicyclon	280	247	96	91*	91*	100*
Saflufenacil	25	0	84	86	86	90
Saflufenacil + benzobicyclon	25	247	91	89	94	100
Carfentrazone	18	0	81	75	81	92
Carfentrazone + benzobicyclon	18	247	93	70	94	99
Propanil	3360	0	67	73	76	80
Propanil + benzobicyclon	3360	247	90*	88	93	99*
Bentazon	840	0	69	65	70	59
Bentazon + benzobicyclon	840	247	95*	68	96*	100*
Halosulfuron + thifensulfuron	35 + 5	0	73	74	78	89
Halosulfuron + thifensulfuron + benzobicyclon	35 + 5	247	90	85	90	100

<sup>a</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>b</sup> Abbreviations: WAT, weeks after treatment

\* Indicates a statistical improvement of efficacy with the addition of benzobicyclon according to Fisher's protected LSD ( $\alpha = 0.05$ ). Each assessment date was analyzed separately.

Table 2.11. Post-flood control of barnyardgrass and red sprangletop as influenced by benzobicyclon rate and additive herbicide at Pine Tree in 2016.

Herbicide <sup>a</sup>	Additive rate	Benzobicyclon rate	Control					
			Barnyardgrass			Red sprangletop		
			2 WAT <sup>b</sup>	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
	g ai ha <sup>-1</sup>	g ai ha <sup>-1</sup>	-----%-----					
Penoxsulam	35	0 (none)	83	84	75	51	53	41
Penoxsulam + benzobicyclon	35	247	85	86	83	86*	91*	91*
Bispyribac	23	0	79	88	83	79	78	79
Bispyribac + benzobicyclon	23	247	81	86	81	84	88*	94*
Halosulfuron	53	0	0	0	0	0	0	0
Halosulfuron + benzobicyclon	53	247	81*	82*	68*	88*	90*	91*
Imazamox	45	0	80	85	60	74	78	71
Imazamox + benzobicyclon	45	247	91	93	89*	90*	91*	96*
Cyhalofop	280	0	83	85	61	85	85	84
Cyhalofop + benzobicyclon	280	247	83	85	75	93	91	92
Saflufenacil	25	0	38	38	9	13	19	18
Saflufenacil + benzobicyclon	25	247	68*	68*	34*	85*	89	92*
Carfentrazone	18	0	0	0	0	0	0	0
Carfentrazone + benzobicyclon	18	247	78*	89*	53*	85*	88*	78*
Propanil	3360	0	79	80	78	76	79	89
Propanil + benzobicyclon	3360	247	85	83	61	88*	91*	94
Bentazon	840	0	0	0	0	0	0	0
Bentazon + benzobicyclon	840	247	70*	71*	50*	90*	89*	92*
Halosulfuron + thifensulfuron	35 + 5	0	19	21	20	3*	1	15
Halosulfuron + thifensulfuron + benzobicyclon	35 + 5	247	70*	66*	41	79	80*	81*

<sup>a</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>b</sup> Abbreviations: WAT, weeks after treatment

\* Indicates a statistical improvement of efficacy with the addition of benzobicyclon according to Fisher's protected LSD ( $\alpha = 0.05$ ). Each assessment date was analyzed separately.

Table 2.12. Post-flood control of Asiatic dayflower and yellow nutsedge as influenced by benzobicyclon rate and additive herbicide at Pine Tree in 2016.

Herbicide <sup>a</sup>	Additive rate	Benzobicyclon rate <sup>a</sup>	Control			
			Asiatic dayflower		Yellow nutsedge	
			2 WAT <sup>b</sup>	4 WAT	2 WAT	4 WAT
	g ai ha <sup>-1</sup>	g ai ha <sup>-1</sup>	-----%			
Penoxsulam	35	0 (none)	90	0*	79*	0*
Penoxsulam + benzobicyclon	35	247	91	89	91	89
Bispyribac	23	0	76	74	84*	89
Bispyribac + benzobicyclon	23	247	80	84	98	99
Halosulfuron	53	0	80	79	86	90
Halosulfuron + benzobicyclon	53	247	86	84	96	96
Imazamox	45	0	84	81	0*	85
Imazamox + benzobicyclon	45	247	81	79	93	95
Cyhalofop	280	0	0*	0*	0*	0*
Cyhalofop + benzobicyclon	280	247	81	81	91	93
Saflufenacil	25	0	88	86	85	88
Saflufenacil + benzobicyclon	25	247	90	90	91	94
Carfentrazone	18	0	89	85	79	86
Carfentrazone + benzobicyclon	18	247	83	83	88	90
Propanil	3360	0	71	71*	83*	86
Propanil + benzobicyclon	3360	247	83	86	96	98
Bentazon	840	0	74	78	88	90
Bentazon + benzobicyclon	840	247	81	83	98	99
Halosulfuron + thifensulfuron	35 + 5	0	66	65*	78*	85
Halosulfuron + thifensulfuron + benzobicyclon	35 + 5	247	79	81	90	94

<sup>a</sup> Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

<sup>b</sup> Abbreviations: WAT, weeks after treatment

\* Indicates a statistical improvement of efficacy with the addition of benzobicyclon according to Fisher's protected LSD ( $\alpha = 0.05$ ). Each assessment date was analyzed separately.



Table 2.13. Rough rice yield as influenced by benzobicyclon rate and additive herbicide applications made post-flood in 2016 at Lonoke and Pine Tree. Post-flood applications made with the addition of 1% (v/v) crop oil concentrate

Herbicide	Additive rate g ai ha <sup>-1</sup>	Benzobicyclon rate g ai ha <sup>-1</sup>	Rough rice yield <sup>a</sup>	
			Lonoke	Pine Tree
			----kg ha <sup>-1</sup> ----	
None		0 (none)	4660	6230
None + benzobiyclon		247	5600*	5800
Penoxsulam	35	0	5810	7020
Penoxsulam + benzobicyclon	35	247	5390	7170
Bispyribac	23	0	6800	9180*
Bispyribac + benzobicyclon	23	247	6860	6450
Halosulfuron	53	0	6110	7760
Halosulfuron + benzobicyclon	53	247	5560	7830
Imazamox	45	0	6470	7650
Imazamox + benzobicyclon	45	247	7060*	7000
Cyhalofop	280	0	8160*	6690
Cyhalofop + benzobicyclon	280	247	5560	7910*
Saflufenacil	25	0	5240	6230
Saflufenacil + benzobicyclon	25	247	6630*	5910
Carfentrazone	18	0	5800	5140
Carfentrazone + benzobicyclon	18	247	6280	7530*
Propanil	3360	0	6230	6920
Propanil + benzobicyclon	3360	247	5670	8800*
Bentazon	840	0	5750*	6680
Bentazon + benzobicyclon	840	247	5120	7630
Halosulfuron + thifensulfuron	35 + 5	0	5820	6980
Halosulfuron + thifensulfuron + benzobicyclon	35 + 5	247	5910	7060

<sup>a</sup> Rough rice machine harvested on September 26, 2016 and October 4, 2016 at Lonoke and Pine Tree, respectively

\* Indicates a statistical difference in yield with the addition of benzobicyclon according to Fisher's protected LSD ( $\alpha = 0.05$ ). Each assessment date was analyzed separately. The untreated control and the benzobicyclon alone treatment where not included in the analysis

## Chapter 3

### Tolerance of Southern U.S. Rice Cultivars to Benzobicyclon

Benzobicyclon is the first 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide pursued for commercial registration in U.S. rice production. Benzobicyclon will be applied post-flood for control of aquatics, broadleaves, grasses, and sedges. Two studies were conducted to investigate the response of rice cultivars to four post-flood application timings and cultivar tolerance to benzobicyclon. A study was conducted in 2015 and 2016 to evaluate the response of eight rice cultivars to post-flood application timings of benzobicyclon at 494 g ai ha<sup>-1</sup> (proposed 2X rate). ‘Caffey’, ‘CL151’, ‘CLXL745’, ‘Jupiter’, ‘LaKast’, ‘Mermentau’, ‘Roy J’, and ‘XL753’ were evaluated in response to applications of benzobicyclon. The highest level of visible injury was observed in LaKast at 7% in 2015. No visible injury was detected among other cultivars either year at 2 weeks after treatment. In 2015 and 2016, less than a four-day delay to reach 50% heading occurred across all cultivars. Jupiter, XL753, LaKast, CLXL745, and Roy J showed the greatest delay in maturity in either year. Rough rice yield was not affected by any of the post-flood application timings of benzobicyclon. A second study was conducted in 2016 at three locations throughout Arkansas to investigate the tolerance of 19 *japonica* (inbred and hybrid) and two *indica* inbred cultivars to a premix containing benzobicyclon at 494 g ai ha<sup>-1</sup> and halosulfuron at 72 g ai ha<sup>-1</sup> applied 1 week after flooding. The *japonica* cultivars showed excellent crop safety to applications of benzobicyclon while the *indica* cultivars, Rondo and Purple Marker, showed severe phytotoxicity. Benzobicyclon only caused a delay in heading of less than 2 days to the *japonica* cultivars. The *indica* cultivars had a severe delay in heading. Rough rice yield of the *japonica* cultivars was not affected by benzobicyclon while yields of both *indica* cultivars was negatively affected. This research shows that benzobicyclon can safely

be applied to drill-seeded *japonica* inbred and hybrid cultivars in a post-flood application without concerns for crop injury. Benzobicyclon should not be used on *indica* cultivars as it will cause severe injury, delayed heading, and yield loss.

**Nomenclature:** Benzobicyclon; rice, *Oryza sativa* L.

**Key words:** Benzobicyclon, cultivar, HPPD, post-flood, rice, timing, tolerance

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## Introduction

Rice plays a significant role in feeding an increasing population. Efforts are constantly being made to improve yields and the nutritional content of rice. In this process, many conventional and imidazolinone-resistant (Clearfield®) inbred and hybrid cultivars have been developed and are available to growers. The majority of the cultivated rice planted in the United States, including conventional and herbicide-resistant cultivars, is of a *japonica* genetic origin, rather than an *indica* as used in other regions of the world (Burgos et al. 2014). Currently, several rice cultivars are resistant to imidazolinone herbicides, now BASF Corporation (Research Triangle Park, North Carolina) is in the process of commercializing a cultivar resistant to quizalofop, an acetyl CoA carboxylase (ACCase)-inhibiting herbicide (Lancaster et al. 2015). Conventional lines of rice are sensitive to quizalofop and imidazolinone herbicides (Meier 2012; Street and Snipes 1987).

These herbicide-resistant rice cultivars play a crucial role in a successful weed management program. The imidazolinone herbicides control a broad spectrum of problematic weeds including barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and many broadleaf weeds. It is imperative that growers integrate more than one site of action (SOA) into weed management programs, otherwise over reliance on one herbicide SOA can lead to resistance (Norsworthy et al. 2007, 2012, 2013). The over reliance and poor stewardship of the imidazolinone herbicides in imidazolinone-resistant rice led to herbicide resistance in multiple weed species, including barnyardgrass and weedy rice (*Oryza sativa* L.) (Burgos et al. 2008, 2014; Heap 2016; Sudianto et al 2013). The imidazolinone-resistant rice hectares rapidly increased from 2002 to 2008 which led to some cultivated imidazolinone-resistant rice outcrossing with weedy rice. Now growers

are faced with controlling acetolactate synthase (ALS)-resistant weeds in rice systems with limited herbicide options.

Benzobicyclon is a 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide that will control a broad spectrum of grasses, aquatics, broadleaves, and sedges, including those resistant to ALS-inhibiting herbicides (Komatsubara 2009). However, benzobicyclon must be hydrolyzed before having herbicidal activity. Benzobicyclon hydrolysate shows potent inhibition of HPPD making the hydrolyzed form of benzobicyclon an effective herbicide. In Korea, transplanted *japonica* rice cultivars have shown excellent safety to benzobicyclon while *indica* rice cultivars have shown severe phytotoxicity (Kwon et al. 2012). Bleaching of leaf tissue is the primary symptom in weeds following an application of benzobicyclon (Almsick 2009; Komatsubara et al. 2009). These symptoms may appear on new growth as early as one week after postemergence (POST) application and are followed by necrosis and eventual death of sensitive species.

SDS Biotech K.K. discovered the herbicide benzobicyclon which led to its development in paddy rice and eventual commercialization in Japan in 2001 (Komatsubara et al. 2012). Benzobicyclon has shown excellent control of rock bulrush (*Scirpus juncooides* Roxb.) in Japanese rice fields. Benzobicyclon at 200 to 300 g ai ha<sup>-1</sup> has been used to control sulfonylurea-resistant rock bulrush (Komatsubara et al. 2009). Benzobicyclon is being developed for use in U.S. rice production systems as a post-flood application (C. Sandoski, personal communication). This will be the first HPPD-inhibiting herbicide commercially available in U.S. rice production. Acetolactate synthase (ALS)-resistant rice flatsedge (*Cyperus iria* L.), yellow nutsedge (*Cyperus esculentus* L.), and smallflower umbrella sedge (*Cyperus difformis* L.) are becoming increasingly problematic for Arkansas growers and consultants (Norsworthy et al. 2013; Scott et al. 2016).

The increasing frequency of ALS-resistant rice flatsedge in Arkansas rice fields is expected to lead to use of benzobicyclon across vast acreage once labeled (C. Sandoski, personal communication).

An important consideration is the production differences across the geographic regions in which rice is produced. Benzobicyclon has effectively been used in production systems that utilize a transplanted cultivar into a rice paddy (Komatsubara et al. 2012; Senkino et al. 2008). Brazzle et al. (2014) documented that benzobicyclon has a potential fit in the water-seeded rice production systems in California. It is important to evaluate benzobicyclon in a drill-seeded production system, which is the predominant means of growing rice in the midsouthern U.S.

It is known that there are differences in sensitivity to HPPD-inhibiting herbicides among *japonica*, *indica*, and *japonica X indica* rice cultivars (Kim et al. 2012; Kwon et al 2012). Korean scientists evaluated 26 rice cultivars of varying backgrounds including *japonica*, *indica*, and *japonica X indica* cultivars to the HPPD-inhibiting herbicides mesotrione, benzobicyclon, and tefuryltrione (Kwon et al. 2012). These herbicides were applied at various timings and doses onto transplanted cultivars. Kwon et al. (2012) observed that benzobicyclon was more injurious to *japonica X indica* crosses than *japonica* cultivars. Injury observed included phytotoxicity, necrosis, detached leaves, and bleaching. These symptoms may appear on new growth as early as one week after a POST application and are sometimes followed by necrosis and eventual death of sensitive cultivars.

Kwon et al. (2012) reported that the highest levels of injury occurred when benzobicyclon was applied at 5 days after transplanting on the high yielding *japonica X indica*-type cultivars. The Hyangmibyeo-1 and Dasanbyeon cultivars, both *japonica X indica*-type

cultivars, were severely injured 7 days after application (DAA) of benzobicyclon at 400 g ai ha<sup>-1</sup>. Of the *japonica*-type cultivars, Sinseonchalbyeon showed the highest injury at 7 DAA; albeit, injury was much less than that observed on cultivars having some *indica* background (Kwon et al. 2012). Based on these results, it is hypothesized that the commonly grown *japonica* cultivars grown in the U.S. should exhibit greater tolerance to benzobicyclon compared to the lesser grown *indica* cultivars.

Before a new herbicide SOA can be commercially marketed, extensive varietal testing must be conducted to determine if it is safe to use. Previous literature shows that injury can occur on specific cultivars when saflufenacil is applied POST. Montgomery et al. (2014) documented that CLXL745 sustained the highest level of injury (13%) of five inbred cultivars when saflufenacil at 50 g ai ha<sup>-1</sup> was applied. CLXL745 exhibited more injury than inbred, long-grain cultivars CL151 and Cheniere. Other cultivars of inbred, medium-grain had greater tolerance, exhibiting  $\leq 10\%$  injury. However, research has shown that no differences in rice tolerance occurred when twice the labeled rate of penoxsulam was applied to 10 different rice cultivars (Bond et al. 2007). It is unknown at this time whether hybrids and inbred conventional medium and long-grain, drill-seeded rice cultivars will respond negatively to benzobicyclon applied post-flood. Hence, the objective of this research was to determine if benzobicyclon could safely be applied to different cultivars across multiple post-flood timings and to determine if *indica* cultivars are more sensitive to benzobicyclon than *japonica* cultivars.

## **Materials and Methods**

**Tolerance to Post-flood Timing.** A field experiment was conducted in 2015 and 2016 at the Rice Research and Extension Center near Stuttgart, Arkansas on a Dewitt silt loam soil (fine,

smectitic, thermic Typic Albaqualfs) to evaluate the tolerance of eight rice cultivars to benzobicyclon as a function of application timing. The experimental design was a randomized complete block design with a split-plot treatment structure, with a main plot of application timing and a subplot of rice cultivar with 4 replications. Each replication consisted of 4 bays with one timing randomly associated with each bay. This experiment required 16 bays. Individual bays were created for this experiment to prevent the bays treated with benzobicyclon from contaminating the nontreated bays. The bays measured 4.9 by 48.8 m levee to levee. Eight rice plots measuring 1.8 by 5.2 m were planted in each bay with a 9-row cone drill allowing for a 1 m alley between plots on May 7, 2015 and May 14, 2016. Rice seeding rates for the inbred and hybrid cultivars varied from 61 to 40 seed m<sup>-1</sup> of row, respectively.

The bays were kept weed-free using labeled herbicides based on University of Arkansas recommendations (Scott et al. 2016). The main plot was treated with benzobicyclon at 494 g ha<sup>-1</sup> at 1, 4, or 5 weeks after flooding (WAF) along with a nontreated control in each replication. Benzobicyclon was applied using a CO<sub>2</sub>-pressurized backpack sprayer consisting of a handheld boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703), calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. The boom consisted of 5 nozzles, and two passes per bay were made covering the entire water surface area. The subplot included eight rice cultivars comprised of medium and long-grain cultivars commonly grown in Arkansas: Jupiter, Caffey, CL151, LaKast, XL753, CLXL745, Mermentau, and Roy J. Each treatment combination of timing and cultivar was replicated four times. The flood was maintained at approximately an 8-cm depth throughout the season.

***Japonica* vs. *Indica* Rice Tolerance.** In 2016, an experiment was conducted at three locations across Arkansas: University of Arkansas Pine Bluff Farm near Lonoke on an Immanuel silt loam



(fine-silty, mixed, active, thermic Oxyaquic Glossudalfs), Rice Research and Extension Center near Stuttgart on a Dewitt silt loam soil (fine, smectitic, thermic Typic Albaqualfs), and Southeast Research and Extension Center near Rohwer on a Sharkey silty clay soil (very-fine, smectitic, thermic Chromic Epiaquerts).

This experiment was setup as a randomized complete block design with a split-plot treatment structure, with a main plot of herbicide rate and a subplot of rice cultivar. Individual bays were created for this experiment to ensure a permanent flood. The bays measured 42.6 m long and 9.1 m wide from center of levee to center of levee. Twenty-one plots measuring 1.8 by 5.2 m were planted in each bay with a 9-row cone drill allowing for a 1 m alley between plots on May 13, 14, and 19 at Rohwer, Stuttgart, and Lonoke, respectively. Rice seeding rates of the inbred and hybrid cultivars were 61 and 40 seed m<sup>-1</sup>, respectively.

The experiment was kept weed-free through use of herbicides and occasional hand weeding. The two main plot treatments consisted of a premix containing benzobicyclon at 494 g ha<sup>-1</sup> and halosulfuron at 72 g ha<sup>-1</sup> applied 1 week after permanent flood establishment and a nontreated control. Benzobicyclon was applied using a CO<sub>2</sub>-pressurized backpack sprayer consisting of a handheld boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703), calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. The boom consisted of 6 nozzles, and three passes per bay were made covering the entire water surface area. The subplot included 21 cultivars that are described in Table 3.1. Each treatment combination of herbicide rate and cultivar was replicated four times, and the test required eight individual bays. The flood was maintained at approximately an 8-cm depth throughout the season.

**Assessments.** For both experiments, rice tolerance was visually rated every 14 d after treatment (DAT) on a scale of 0 to 100%, with 0 being no injury and 100% being death of the crop. Ratings were taken 2, 4, and 6 weeks after every application timing and based on comparison with the nontreated control (NTC) for the tolerance to post-flood timing experiment. The *japonica* vs. *indica* rice tolerance test was rated 2, 3, 5, and 6 weeks after application. Number of days to 50% heading was visually assessed in each plot and reported relative to the nontreated control for each cultivar. Plots were machine harvested at crop maturity to determine rough rice yield at an adjusted 12% moisture.

**Statistical Analyses.** All data were analyzed using JMP statistical software (JMP, Version 12.1 SAS Institute Inc., Cary, NC). In the timing experiment, years are presented separately because of a significant treatment by year interaction. Data were analyzed within each cultivar, year, and assessment to evaluate if timing had an impact on delayed heading or rough rice yield. Delay in heading could not be subjected to ANOVA because many of the means had delays of zero, meaning the data did not meet the assumption of homogeneity of variance. Therefore, means are reported followed by the standard error. Yield data were subjected to ANOVA and means separated using Fisher's protected LSD ( $\alpha=0.05$ ).

## **Results and Discussion**

**Tolerance to Post-flood Timing.** In 2015, LaKast had minimal injury at each of the timings. Injury of 5% was observed 57 DAA at the 1 WAF (V7-V10 tillering) timing, 7% injury 34 DAA at the 4 WAF timing, and 6% injury 27 DAA at the 5 WAF timing on LaKast (data not shown). The minimal injury on LaKast was not observed in 2016. No injury was observed in 2016 to any of the eight rice cultivars 2 weeks after treatment (WAT) for each of the benzobicyclon

applications (data not shown). The minimal injury that appeared on LaKast was mild chlorosis located at the water line on the shoot of the cultivar. Chlorosis or necrosis were never observed in the other rice cultivars from time of application through rice harvest. The cultivars in this study were of *japonica* background, which likely contributed to the high level of tolerance observed in this experiment (Hardke 2013). However, Kwon et al. (2012) documented 50 to 80% injury to five *japonica*  $\times$  *indica* lines following benzobicyclon at 400 g ai ha<sup>-1</sup> in a Korean rice system. Similar to the results reported here, Kwon et al. (2012) observed minimal injury (0-10%) on the 18 *japonica* lines. With LaKast only showing minimal injury, it appears that benzobicyclon at 494 g ai ha<sup>-1</sup> applied 1 WAF (tillering), 4 WAF, or 5 WAF will not affect agronomic performance of the rice cultivars evaluated.

In 2015 and 2016, no more than a 4-day delay was observed across all cultivars to reach 50% heading (Table 3.2). Jupiter, Mermentau, LaKast, CLXL745, and Roy J showed delays greatest delay in heading in either year. Roy J was delayed 3 and less than 2 days by the application (2X the anticipated rate) in 2015 and 2016 at 1 WAF (Table 3.2). LaKast, Mermentau and CLXL745 were delayed less than 4 days, when benzobicyclon was applied 5 WAF in 2016. There was no delay to 50% heading greater than 2 days in either year when benzobicyclon was applied 4 WAF (Table 3.2). The observed delays in 2016, although minimal, could be due to environmental conditions. The summer of 2016 had increased temperatures and dew point during the pollination period, which could have ultimately had an impact on yield (J. Hardke, personal communication). The ability to harvest the rice would likely not be affected in a delay of  $\leq 3$  d to reach 50% heading (Bond et al. 2012).

Rough rice yields differed among individual cultivars, as expected because medium grain cultivars often yield lower than long-grain cultivars (Hardke 2013). Yield loss occurred for at

least one of the three application timings compared to the nontreated control for XL753 in 2015 and CL151, Jupiter, and LaKast in 2016 (Table 3.3). Otherwise there was no apparent impact of benzobicyclon application timing on yield loss of the cultivars evaluated, leading to the conclusion that benzobicyclon can safely and effectively be applied up to 5 WAF without crop injury, delayed heading, or yield loss.

***Japonica* vs. *Indica* Rice Tolerance.** No injury to any of the 19 *japonica* cultivars was observed at any of the locations following the 1 WAF application of benzobicyclon plus halosulfuron (data not shown). This was expected as *japonica* cultivars in Asia were reported to have excellent safety to benzobicyclon (Kwon et al. 2012). The *indica* cultivars, Purple Marker and Rondo, suffered severe injury, including high levels of chlorosis 2 WAT. Rondo had more injury than Purple Marker at 2 WAT (Table 3.4). Rondo had 66% injury and Purple Marker 59% at 2 WAT, with injury increasing as the season progressed. By 6 WAT, Purple Marker and Rondo were injured 98 and 96%, respectively. It is unlikely that Rondo and Purple Marker have the HIS1 gene, resulting in them being susceptible to benzobicyclon (Kato et al. 2015). Based on these results, it appears that *indica*-type cultivars are highly sensitive to benzobicyclon and should not be used in a cropping system in conjunction with benzobicyclon.

The *japonica* cultivars experienced minimal heading delays as the highest delay recorded was <2 days (Table 3.5). Likewise, there was no reduction in grain yield relative to the respective nontreated control for any of the *japonica* cultivars when treated with benzobicyclon at 494 g ai ha<sup>-1</sup> plus halosulfuron at 72 g ai ha<sup>-1</sup> applied 1 WAF (Table 3.5). Conversely, of the few Purple Marker and Rondo plants that were severely injured but did survive following treatment with benzobicyclon, these plants had not reached 50% heading by the final heading

date assessment. The severe injury from benzobicyclon to the *indica* cultivars resulted in 86 to 98% reduction in grain yield.

Results from this experiment support findings of the previous post-flood timing experiment in that benzobicyclon can safely be applied post-flood to all *japonica*-type cultivars studied without concerns for injury, a substantial delay in heading, and reductions in grain yield. Screenings for tolerance to benzobicyclon as new cultivars are commercialized should continue because any presence of *indica*-type germplasm in the background of a cultivar could result in significant damage to the crop following a benzobicyclon application. Without the presence of a functioning HIS1 gene, injury can occur in *indica* rice. Based on the previously published efficacy data on benzobicyclon (McKnight et al. 2014; Norsworthy et al. 2014; Sandoski et al. 2014), the herbicide should provide midsouthern U.S. growers a new tool to control a wide assortment of weeds post-flood in rice with minimal risk for injury to the crop.

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Table 3.1. Brief description of cultivars evaluated for tolerance to benzobicyclon.

Cultivar	Inbred or hybrid	Medium or long grain	<i>Japonica</i> or <i>indica</i>	Conventional or imidazolinone-resistant
CL111	Ib	L	J	IR
CL151	Ib	L	J	IR
CL153	Ib	L	J	IR
CL163	Ib	L	J	IR
CL172	Ib	L	J	IR
CL272	Ib	M	J	IR
CLXL4534	H	L	J	IR
CLXL729	H	L	J	IR
CLXL745	H	L	J	IR
CLXP766	H	L	J	IR
Diamond	Ib	L	J	C
Gemini 214 CL	Ib	L	J	IR
Jupiter	Ib	M	J	C
LaKast	Ib	L	J	C
Mermentau	Ib	L	J	C
Purple Marker	Ib	L	I	C
Rondo	Ib	L	I	C
Roy J	Ib	L	J	C
Thad	Ib	L	J	C
Titan	Ib	M	J	C
XL753	H	L	J	C

<sup>a</sup> Abbreviations: Ib, inbred; H, hybrid; M, medium; L, long; J, *japonica*; I, *indica*; C, conventional; IR, imidazolinone-resistant

Table 3.2. Days delay to 50% heading of eight rice cultivars in response to post-flood applications of benzobicyclon applied at 494 g ai ha<sup>-1</sup> plus 1% (v/v) crop oil concentrate in 2015 and 2016.

Cultivar	Timing <sup>a</sup>	Days delayed in heading <sup>b</sup>			
		2015 <sup>c</sup>		2016 <sup>d</sup>	
		-----d-----			
Caffey	1 WAF	-	-	1	(1.00)
	4 WAF	<1	(0.25)	1	(0.95)
	5 WAF	-	-	1	(0.41)
CL151	1 WAF	1	(1.41)	-	-
	4 WAF	<1	(0.48)	<1	(0.25)
	5 WAF	1	(0.41)	-	-
CLXL745	1 WAF	<1	(0.29)	-	-
	4 WAF	-	-	1	(0.48)
	5 WAF	<1	(0.29)	4	(0.96)
Jupiter	1 WAF	<1	(0.25)	1	(0.29)
	4 WAF	<1	(0.48)	1	(1.00)
	5 WAF	<1	(0.48)	3	(0.85)
LaKast	1 WAF	<1	(0.48)	4	(1.65)
	4 WAF	2	(0.48)	<1	(0.25)
	5 WAF	-	-	1	(1.00)
Mermentau	1 WAF	1	(0.75)	2	(1.50)
	4 WAF	<1	(0.48)	2	(1.50)
	5 WAF	-	-	4	(1.65)
Roy J	1 WAF	3	(1.41)	3	(0.95)
	4 WAF	-	-	<1	(0.29)
	5 WAF	<1	(0.75)	<1	(0.25)
XL753	1 WAF	<1	(0.50)	-	-
	4 WAF	-	-	-	-
	5 WAF	-	-	-	-

<sup>a</sup> Abbreviations: WAF, weeks after flood.

<sup>b</sup> Symbol: <, indicates less than. Standard error of mean reported within a cultivar.

<sup>c</sup> Days until 50% heading on nontreated cultivars in 2015: Caffey - 71; CL151 - 65; CLXL745 - 66; Jupiter - 67; LaKast - 69; Mermentau - 65; Roy J - 78; XL753 - 68.

<sup>d</sup> Days until 50% heading on nontreated cultivars in 2016: Caffey - 91; CL151 - 84; CLXL745 - 76; Jupiter - 87; LaKast - 85; Mermentau - 86; Roy J - 95; XL753 - 84.

Table 3.3. Rough rice yield of eight rice cultivars in response to post-flood applications of benzobicyclon at 494 g ai ha<sup>-1</sup> plus 1% (v/v) crop oil concentrate in 2015 and 2016.

Cultivar	Timing <sup>a</sup>	Rough rice yield <sup>c</sup>	
		2015	2016
		-----kg ha <sup>-1</sup> -----	
Caffey	None <sup>b</sup>	7750 (477)	6930 (464)
	1 WAF	6860 (477)	6550 (464)
	4 WAF	6820 (477)	6340 (464)
	5 WAF	7460 (477)	6550 (464)
CL151	None	9050 (726)	8030 a
	1 WAF	8210 (726)	7260 a
	4 WAF	8710 (726)	7510 a
	5 WAF	8760 (726)	5430 b
CLXL745	None	13120 (564)	9930 (626)
	1 WAF	11950 (564)	11900 (626)
	4 WAF	13190 (564)	11300 (626)
	5 WAF	12500 (564)	10300 (626)
Jupiter	None	5750 (567)	7230 a
	1 WAF	6500 (567)	6740 ab
	4 WAF	6980 (567)	7250 a
	5 WAF	5840 (567)	5670 b
LaKast	None	10970 (639)	8600 a
	1 WAF	9000 (639)	6500 b
	4 WAF	9100 (639)	8270 ab
	5 WAF	8970 (639)	9025 a
Mermentau	None	6450 (737)	8710 (585)
	1 WAF	8960 (737)	7970 (585)
	4 WAF	8680 (737)	8130 (585)
	5 WAF	9430 (737)	7230 (585)
Roy J	None	8950 (789)	6050 (368)
	1 WAF	9260 (789)	5830 (368)
	4 WAF	8610 (789)	6440 (368)
	5 WAF	9210 (789)	6750 (368)
XL753	None	10990 a	11200 (649)
	1 WAF	8900 b	10880 (649)
	4 WAF	11100 a	11560 (649)
	5 WAF	11730 a	12220 (649)

<sup>a</sup>Abbreviations: WAF, weeks after flood.

<sup>b</sup>None: No benzobicyclon was applied to this cultivar.

<sup>c</sup>Means within a cultivar and column with the same lowercase letters are not different according to Fisher's protected LSD ( $\alpha=0.05$ ). Standard error of mean reported for those cultivars for which no application timing effect occurred.

Table 3.4. Visible injury of *indica* cultivars used evaluating the tolerance to a premix containing benzobicyclon at 494 g ai ha<sup>-1</sup> and halosulfuron at 72 g ha<sup>-1</sup> plus 1% (v/v) crop oil concentrate at Lonoke, Rohwer, and Stuttgart. All of the *japonica* cultivars had zero injury (data not shown).

Cultivar	Injury			
	2 WAT <sup>a,b</sup>	3 WAT	5 WAT	6 WAT
	-----%-----			
Purple Marker	59 b	79	94	98
Rondo	66 a	78	90	96

<sup>a</sup> Abbreviations: WAT, weeks after treatment.

<sup>b</sup> Means within a column with different lowercase letters are indicate a statistical difference according to Fisher's protected LSD ( $\alpha=0.05$ ). Absence of letters indicates the ANOVA did not show statistical significance at  $\alpha=0.05$ .

Table 3.5. Response of 21 rice cultivars to a 1 week after flood application of a premix containing benzobicyclon<sup>a</sup> at 494 g ai ha<sup>-1</sup> and halosulfuron at 72 g ha<sup>-1</sup> plus 1% (v/v) crop oil concentrate at Lonoke, Rohwer, and Stuttgart.

Cultivar	Herbicide	Days delayed in heading <sup>cd</sup>		Rough rice yield
		-----d-----		kg ha <sup>-1</sup>
CL111	None <sup>b</sup>	-	-	6730
	Treated	-	-	6620
CL151	None	-	-	5890
	Treated	-	-	5200
CL153	None	-	-	6740
	Treated	-	-	6680
CL163	None	-	-	5990
	Treated	1	(0.77)	5330
CL172	None	-	-	4920
	Treated	<1	(0.13)	4360
CL272	None	-	-	6640
	Treated	<1	(0.26)	5780
CLXL4534	None	-	-	8570
	Treated	1	(0.45)	8880
CLXL729	None	-	-	8690
	Treated	<1	(0.52)	7650
CLXL745	None	-	-	8620
	Treated	1	(0.45)	8250
CLXP766	None	-	-	9020
	Treated	<1	(0.52)	8420
Diamond	None	-	-	7080
	Treated	<1	(0.26)	5920
Gemini 214 CL	None	-	-	8420
	Treated	1	(0.52)	8600
Jupiter	None	-	-	5360
	Treated	2	(0.26)	4640

Table 3.5 cont.

Table 5.5 cont.

Cultivar	Herbicide	Days delayed in heading		Rough rice yield
		-----d-----		kg ha <sup>-1</sup>
LaKast	None	-	-	6350
	Treated	-	-	6270
Mermentau	None	-	-	6120
	Treated	-	-	5970
Purple Marker	None	-	-	3070
	Treated	NR	NR	430*
Rondo	None	-	-	6540
	Treated	NR	NR	130*
Roy J	None	-	-	6010
	Treated	-	-	5550
Thad	None	-	-	5920
	Treated	2	(0.93)	5980
Titan	None	-	-	5930
	Treated	<1	(0.26)	6400
XL753	None	-	-	9060
	Treated	<1	(0.26)	8460

<sup>a</sup> Benzobicyclon plus halosulfuron contained 1% (v/v) crop oil concentrate.

<sup>b</sup> Symbol: <, indicates less than. None: No benzobicyclon was applied to this cultivar.

<sup>c</sup> Standard error of mean reported for those nonsignificant means.

<sup>d</sup> Zero days delayed to reach 50% heading denoted by “-”. *Indica* cultivars did not reach 50% heading by the final heading evaluation and are denoted by “NR”.

\* Denotes a significant difference relative to its respective nontreated according to Fisher's protected LSD ( $\alpha=0.05$ ).

## Chapter 4

### Benzobicyclon as a Post-flood Option for Weedy Rice Control

With increasing selection pressure on currently registered herbicides, a new site of action is needed in rice production for the control of problematic weeds. A new herbicide, benzobicyclon, is being developed by Gowan Company. Benzobicyclon, a WSSA Group 27 herbicide, controls a broad spectrum of grasses, aquatics, broadleaves, and sedges, including those currently resistant to WSSA Group 2 herbicides. This will be the first 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide commercially available in U.S. rice production. An observation of benzobicyclon controlling weedy rice was made in the summer of 2015. This prompted a greenhouse evaluation in the spring followed by field evaluation in the summer of 2016 to determine the efficacy of benzobicyclon on weedy rice accessions collected across Arkansas, Mississippi, and southeast Missouri. A total of 100 accessions were screened in the greenhouse and field. Percent mortality was determined in the greenhouse and percent control was recorded in the field. Benzobicyclon at 371 g ai ha<sup>-1</sup> caused at least 80% mortality of 22 accessions in the greenhouse and at least 80% control of 30 accessions in the field. For most accessions, plants within the accession varied in response to benzobicyclon. Based on these results, the sensitivity of weedy rice to benzobicyclon varies across the Midsouth, and it may provide an additional control option for weedy rice in some fields.

**Nomenclature:** Benzobicyclon; weedy rice, *Oryza sativa* L.; rice, *Oryza sativa* L.

**Key words:** herbicide resistance, phenotypic characteristics, screening, survey, tolerance.



## Introduction

Rice is a wild grass from Asia that was cultivated into a staple crop over thousands of years. This wild grass was slowly domesticated to meet the needs of the early farmers in ancient times (Kovach et al. 2007). Today, there are two known major subspecies of Asian rice grown: *japonica* and *indica*. *Japonica* and *indica* rice represent the deepest genetic differentiation within weedy rice (Kovach et al. 2007). These two groups are sometimes grown in the same geographical regions despite several morphological and physiological differences. *Japonica* and *indica* have been distinguished based on morphological characteristics including: grain shape, apiculus, hair length, leaf color, or through sensitivity to potassium chlorate (Oka 1988). Although there is much distinction between morphological characteristics, many of the phenotypic traits are shared by both *japonica* and *indica* groups. Hull color, presence or absence of awn, and pericarp color are some of the phenotypic traits shared by both groups of rice (Burgos et al. 2014; Kovach et al. 2007;).

Wild types of rice, also known as weedy rice, possess the same characteristics as the cultivated varieties, and if an infestation occurs, it can be detrimental to the cultivated crop. Because of the extensive similarities to cultivated rice, weedy rice is a threat to global rice production (Delouche et al. 2007). Weedy rice is highly competitive and difficult to control in a cultivated rice system, and in some cases, it can lead to total crop failure (Burgos et al. 2006; Dieatta et al. 1985). Gealy et al. (2015) reported that U.S. weedy rice populations can be divided phenotypically and evolutionarily into two distinct groups: strawhull (awnless) and black hull (awned).

The genetic and phenotypic diverse populations of weedy rice are distributed throughout the rice-producing areas in the Midsouth. Weedy rice grows taller, produces more biomass and

tillers, and outcompetes cultivated rice for nutrients (Burgos et al. 2006; Estorninos et al. 2005). Burgos et al. (2008) reported that weedy rice caused an average of \$275 ha<sup>-1</sup> in losses in Arkansas. Weedy rice reduces yield and has the capability to reduce grain quality because of seed contamination (Ottis et al. 2005). These characteristics in addition to herbicide-resistant cases have distinguished it as one of most problematic weeds in midsouthern U.S. cultivated rice.

The first documented case of herbicide-resistant weedy rice located in Arkansas was in 2002 (Heap 2016). The weedy rice was found to be resistant to acetolactate synthase (ALS)-inhibiting (WSSA Group 2) imidazolinone herbicides. Imidazolinone-resistant weedy rice has been widespread over the southern U.S. rice-producing area because of a high percentage of planted cultivars possess the imidazolinone resistance trait (Clearfield®) (Burgos et al. 2008, 2014). Since development and commercialization in 2002, imidazolinone-resistant rice has made up the majority of the planted acreage in Arkansas up until 2014 (Burgos et al. 2008; Hardke 2013, 2015). The technology enabled growers to make applications of imazethapyr to control weedy rice infesting cultivated fields (Norsworthy et al. 2013). Although a successful, effective technology, total control was not always achieved because of various environmental, biological, and herbicide-application circumstances (Burgos et al. 2008). The genetic and phenotypic similarities to the cultivated rice led to the outcrossing of the herbicide-resistant trait to weedy rice.

Strategies like crop rotation, use of certified seed, applications of selective grass herbicides, and other methods have been implemented by growers and recommended by consultants to control weedy rice infested fields (Burgos et al. 2008). Weedy rice can be controlled through crop rotation with the common rotation being soybean. Rotation to soybean allows for the use of graminicides or glyphosate to control weedy rice outside of a rice crop. This

reduces the amount of seed going back into the soil seedbank (Burgos et al. 2008). Arkansas established a zero tolerance law for certified seed, prohibiting Arkansas rice seed to not have more than 15 weedy rice seed per 45 g infestations of weedy rice contained in it (AMS 2017). Other weed management practices include spot applications of glyphosate to rice or hand removal of plants; however, these practices are only feasible on infestations of low populations (Burgos et al. 2008).

A need for new sites of action (SOA) to control imidazolinone-resistant weedy rice exists in the Midsouth. The effective imidazolinone-resistant rice system that made up a majority of the acreage in 2013 has since declined to 46% of the acreage in 2015 (Hardke 2013, 2015). This decline is partially attributed to the failure of imazethapyr to control weedy rice (Norsworthy et al. 2013). Producers that have fields infested with herbicide-resistant, off-type rice now have no effective herbicide options for control.

Benzobicyclon, a 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide, is used in Asian rice for control of acetolactate synthase (ALS)-resistant sedges (*Cyperus* spp.) and rushes (*Scirpus* spp.) (Komatsubara et al. 2009; Sekino et al. 2008). It provides broad-spectrum control of many aquatics, broadleaves, sedges, and some grasses (Sandoski et al. 2014). If commercialized in the U.S., it will be the first herbicide targeting the HPPD-inhibiting SOA for use in rice. Widespread use of benzobicyclon is expected as ALS-resistant sedge populations are becoming more difficult to control in the Midsouth (C. Sandoski, personal communication).

There are known differences in sensitivity to HPPD herbicides among *japonica*, *indica*, and *japonica* x *indica* rice cultivars. Korean scientists evaluated 26 rice cultivars of varying backgrounds including: *japonica*, *indica*, and *japonica* x *indica* cultivars to the HPPD herbicides mesotrione, benzobicyclon, and tefuryltrione (Kwon et al. 2012). These herbicides were applied

at various timings and doses. Kwon et al. (2012) documented the *indica* and *japonica* x *indica* crosses showed greater injury than the *japonica* cultivars. Injury observed included phytotoxicity, necrosis, detached leaf, and bleaching. These symptoms may appear on new growth as early as one week of postemergence applications followed by necrosis and eventual death of sensitive species. Kwon et al. (2012) documented that the highest levels of phytotoxicity were on the high yielding, *japonica* x *indica*-type cultivars.

Because benzobicyclon has shown the ability to control various types of *indica* and *japonica* x *indica* cultivars, it exhibits potential for use in southern U.S. rice systems for control of weedy rice. If benzobicyclon can be successfully implemented into U.S. rice systems, it would provide a new SOA for potentially controlling weedy rice in conventional and imidazolinone-resistant rice systems.

Since the first documented case of ALS-resistant weedy rice in Arkansas, the number of resistant cases has increased across other rice-producing states in the Midsouth (Burgos et al 2008; Heap 2016). Weed surveys have been a successful tool for estimating weed flora within a geographical region (Johnson 2013). With resistance confirmed in weedy rice to the imidazolinone herbicides and genetic and phenotypic trait differences confirmed among weedy rice across the Midsouth, the potential to control weedy rice with benzobicyclon may exist.

In 2015, an observation was made in a field study conducted at the Rice Research and Extension Center near Stuttgart, Arkansas and at the Pine Tree Research Station near Colt, Arkansas. At both locations, bays treated post-flood with benzobicyclon at 247 or 494 g ai ha<sup>-1</sup> had a high level of weedy rice control relative to bays not containing benzobicyclon. This prompted a weedy rice survey across the Midsouth to assess sensitivity to benzobicyclon. The

objective of this research was to survey the weedy rice accessions in Arkansas, Missouri, and Mississippi for sensitivity to benzobicyclon and determine if any phenotypic characteristic would correlate with response to the herbicide.

## **Materials and Methods**

**Collection and Plant Materials.** Following the observation of benzobicyclon controlling weedy rice at 247 and 494 g ai ha<sup>-1</sup>, weedy rice panicles were collected from 88 rice fields in Arkansas, 5 in Mississippi, and 7 in Missouri in the summer of 2015 (Table 4.1; Figure 4.1). If a field contained only 1 weedy rice plant, then approximately 5 panicles were collected. However, if a field contained multiple plants, 25 to 35 panicles were collected. A handheld global positioning system was used to record the coordinates for each sampling site. Accessions were designated as AR (Arkansas), MO (Missouri), or MS (Mississippi) and assigned a number ranging from 1 to 100 (Table 4.1 and Figure 4.1).

Samples were collected in rice fields suggested by local crop consultants, county extension agents, and growers. For all samples collected, it was not known at the time of collection from which herbicides the plants had escaped control. The AR accessions were collected during rice harvest from August 17 to 21, 2015. Each accession was evaluated for hull color (straw, black, or mixed) and presence or absence of an awn (Table 4.1 and Table 4.2). Any accession characterized as ‘mixed’ contained both straw and black hull accessions. The accessions were stored at room temperature for 5 months prior to the greenhouse experiment.

**Benzobicyclon Tolerance in the Greenhouse.** First, the 100 accessions were screened for resistance to imazethapyr. For each accession, approximately 7 to 8 seeds were planted in a pot with a 12 cm diameter. Plants were thinned to 5 per pot after emergence. Each accession had a

total of 4 pots and 20 plants. The screening had two replications in time. The results revealed 63% of the weedy rice accessions were resistant to the ALS-inhibiting herbicide imazethapyr at 105 g ai ha<sup>-1</sup> (data not shown).

Benzobicyclon tolerance was investigated at the Altheimer Laboratory in Fayetteville, Arkansas in 2016. The experiment was conducted twice in the greenhouse in a randomized complete block design with two replications per accession per treatment and 25 plants per replication for a total of 50 plants per treatment. The seeds were sown into individual rows in a Pembroke silt loam (fine-silty, mixed, active, mesic Mollic Paleudalfs) soil that was 6 cm deep in stainless steel containers measuring 123-cm in length and 51-cm in width. Twenty-five accessions were planted in rows 2.5-cm apart in each container. After emergence, accessions were reduced to 25 plants per row. Due to poor germination some rows did not have 25 plants emerge; therefore, the percentage mortality was calculated off the total number of plants that emerged. The containers were kept in a greenhouse under conditions of 32/22 ± 3 C day/night temperatures with a 16-h photoperiod consisting of natural lighting supplemented by a metal halide lighting system. All containers were irrigated on a daily basis to ensure healthy plant growth.

The herbicide treatment was applied to 2- to 3-leaf weedy rice plants that were approximately 14- to 16-cm tall. Treatments were made after flooding the containers to a permanent 5- to 7-cm flood depth. It is important to maintain a permanent flood for the continued activity of benzobicyclon (McKnight et al. 2014). Benzobicyclon was applied post-flood at 371 g ai ha<sup>-1</sup> plus 1% v/v crop oil concentrate (COC) (Agri-Dex, Helena Chemical Co., West Helena, AR 72390) with a CO<sub>2</sub>-pressurized backpack sprayer consisting of a four-nozzle, handheld boom equipped with 110015 AIXR nozzles (Teejet Technologies, Springfield, IL

62703) calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. Beginning 14 days after application, dead plants were recorded and removed from treated containers three times weekly for up to six weeks after application.

**Benzobicyclon Tolerance in the Field.** A field study was conducted in 2016 at the Pine Tree Research Station near Colt, Arkansas to evaluate tolerance of the same 100 weedy rice accessions to benzobicyclon (Table 4.1; Figure 4.1). The experiment was conducted in a randomized complete block design with two replications per accession per treatment. Each replication was contained within an individual bay (18 m by 24 m). A 10-row drill was used to create furrows within the test site, and each weedy rice accession sown by hand on June 9, 2016 into a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs). Approximately 60 to 80 seed m<sup>-1</sup> of row were sown in 4.5 m rows into the first, fifth, and tenth furrow with each pass of the drill through the field, resulting in a 70 to 85 cm spacing between rows. Additionally, commercial *japonica* cultivars, CL111 and Roy J, were sown as standards for comparison. At the 1- to 2-leaf rice growth stage, flags were used to mark a portion of each strip that contained 50 individual weedy rice or rice plants. The intent of marking 50 plants was to assess mortality similar to greenhouse evaluations after treatment.

The flood was established in both bays simultaneously by multiple inlet irrigation 1 week prior to the herbicide treatment. The flood was maintained at a 6- to 8-cm depth for the duration of the experiment and nitrogen (168 kg ha<sup>-1</sup>) was applied immediately prior to flooding to simulate normal rice culture. Maintenance herbicide applications were applied to ensure the trial was kept weed free. At planting, clomazone was applied at 360 g ai ha<sup>-1</sup> and prior to flooding fenoxaprop and halosulfuron were applied at 122 g ai ha<sup>-1</sup> and 53 g ai ha<sup>-1</sup> plus 1% v/v COC, respectively. Benzobicyclon was applied one week after flooding to each bay from levee to levee

at 371 g ha<sup>-1</sup> plus COC at 1% v/v. Most accessions and the commercial rice cultivars had four leaves at application. By three weeks after treatment, it was impossible to access mortality because carcasses had decayed or moved throughout the bay. Additionally, the profuse tillering of some tolerant accessions made it difficult to differentiate individual plants. Hence, the flagged portion of each plot was visibly rated for control on a 0 to 100% scale, with 0 being no control and 100% being complete plant death. As expected, the commercial cultivars were non-responsive to benzobicyclon; hence, these served as controls during the evaluation.

**Statistical Analysis.** Using PROC GLIMMIX, a logit analysis using a randomized complete block design with two blocks and 100 treatments was conducted in SAS 9.4 (SAS Institute Inc. Cary, NC 27513). Treatments which were 100% and 0% mortality were not included in the analysis. The results from the logit scale were back transformed to a proportion and results presented as percent control. CL111 and Roy J did not have a negative response and were not reported. A frequency distribution table was created in JMP 12.1 (SAS Institute Inc. Cary, NC 27513) to describe the relationship of hull color and presence or absence of awn. A one-way analysis of variance (ANOVA) was performed in JMP 12.1 where the treatments were hull color and the response was percent mortality. A separate one-way analysis of variance (ANOVA) was performed where the treatments were presence or absence of awns and the response was percent mortality. A map was constructed to show the spatial distribution of the percent mortality.

## **Results and Discussion**

**Benzobicyclon Tolerance in the Greenhouse.** The mortality of weedy rice accessions collected from across the Midsouth varied in sensitivity to benzobicyclon at 371 g ha<sup>-1</sup> (Table 4.2; Figure 4.1). Based on the greenhouse data, 22 of 100 accessions had  $\geq 80\%$  mortality. There were 10



accessions completely controlled (100% mortality); however, 37 accessions exhibited a high level of tolerance to benzobicyclon based on  $\leq 20\%$  mortality. Roy J and CL111 showed excellent safety to benzobicyclon (0% injury). These findings are similar to those of Kwon et al. (2012) where rice cultivars in Asia varied in sensitivity to benzobicyclon based on genetic origin. In this research, Kwon et al. (2012) found increased tolerance of the *japonica* rice cultivars to be greater than those of *indica* origin. The findings of Kwon et al. (2012) and the benzobicyclon patent (Kato et al. 2015) reinforce the results found in the 100 weedy rice accessions collected across the Midsouth, with likely the presence or absence of the *HIS1* gene within an accession and among accessions being a major contributor to differences in sensitivity to the herbicide (Table 4.3). Based on these results, there appears to be much variation in response of weedy rice accessions in the Midsouth to benzobicyclon. Because of the differing response among accessions, it is likely that the weedy rice in the Midsouth is from both *indica* and *japonica* origins, and there may be some accessions comprised of *indica*  $\times$  *japonica* crosses.

The phenotypic characteristics of weedy rice are shown in a frequency distribution table (Table 4.1). Of the 100 weedy rice accessions, 47% did not have an awn. The majority of the straw hull accessions (81%) were awnless. Conversely, 96% of the black hull accessions had the presence of an awn. These findings are similar to those found by Burgos et al. (2014) and Gealy et al. (2015) when assessing the phenotypic characteristics of weedy rice accessions across the Midsouth. The majority of weedy rice in the Midsouth is straw hull in color and does not bear awns, while those that are black hull in color will most likely have an awn. The findings of Gealy et al. (2009) indicate that straw hull weedy rice accessions without awns are more closely associated with *indica* than *japonica* lines.

The one-way ANOVA between awn presence or absence and percentage mortality revealed a marginal statistical difference ( $p=0.0147$ ) (data not shown). The same analysis conducted between hull color (straw, black, or mixed) and percentage mortality, the lack of a relationship between these two variables was indicated by the  $p\text{-value} = 0.1362$  (data not shown). Therefore, a grower will be unable to assess hull color during the fall and successfully determine whether the weedy rice in the subsequent rice crop will be sensitive to benzobicyclon. However, these findings suggest that weedy rice plants bearing awns are more likely to be tolerant to benzobicyclon. Nevertheless, the phenotypic characteristics of weedy rice including the presence or absence of awns and/or hull color are not sufficiently reliable to determine sensitivity or tolerance to benzobicyclon.

Evidence suggests that the sensitivity or tolerance to benzobicyclon is based on the genetic background of the weedy rice (Kato et al. 2015). Kato et al. (2015) reported that the *HIS1*-gene located on the 2<sup>nd</sup> chromosome of rice has been identified as conferring tolerance to HPPD inhibitors. The *HIS1* gene is expressed mainly in shoots of rice, where uptake of benzobicyclon is believed to be greatest in a flooded rice culture. Additionally, the homologous gene (*HSL1*) is located on the 6<sup>th</sup> chromosome of rice and is believed to be expressed at low levels and be partially responsible for tolerance to HPPD inhibitors. These two genes are the determining factors for the susceptibility to HPPD inhibitors such as benzobicyclon.

**Benzobicyclon Tolerance in the Field.** Sensitivity to benzobicyclon was documented in the field in addition to the greenhouse. Benzobicyclon controlled ( $\geq 80\%$ ) 30 of the 100 accessions in the field (Table 4.3). Of the 100 accessions evaluated, seven were completely controlled in the field (100%). However, 34 of the accessions had minimal sensitivity to benzobicyclon based on control ratings  $\leq 20\%$ . Benzobicyclon efficacy appeared slightly higher in the field than in

greenhouse, but overall greenhouse and field results closely matched (Tables 4.3). The increased activity of benzobicyclon could be due to environmental conditions such as temperature, relative humidity, or pH of water. The parent molecule, benzobicyclon, is a pro-herbicide that must be hydrolyzed to benzobicyclon hydrolysate to exhibit herbicidal activity (Komatsubara et al. 2009; Williams and Tjeerdema 2016). Williams and Tjeerdema (2016) documented that the conversion of benzobicyclon to benzobicyclon hydrolysate increases as a function of temperature and pH of water. Johnson and Young (2002) confirmed another triketone herbicide, mesotrione, has increased activity on certain weed species as temperature and relative humidity increases. Mesotrione efficacy increased seven-fold on large crabgrass at 32 C compared to 18 C. Large crabgrass was also two-fold more susceptible to mesotrione at 85% relative humidity compared to 30% relative humidity (Johnson and Young 2002). The results of the percent control data suggest similarities to the research conducted by Williams et al. (2002) and Johnson and Young (2002).

Some of the weedy rice accessions varied in percentage mortality and control because seed were obtained from multiple plants at most collection sites, with sometimes there being differences in phenological characteristics for a particular collection site. Therefore, weedy rice accessions within a field may vary in genetic background, with some being highly susceptible to benzobicyclon. Hence, benzobicyclon may offer some rice growers an option for controlling weedy rice in the absence of a herbicide trait. Sensitivity of the 100 weedy rice accessions to benzobicyclon is likely based on genetic origin. Regardless of the sensitivity possessed by some of the accessions to benzobicyclon, there is too much genetic diversity in the weedy rice populations in the Midsouth to deem benzobicyclon as an effective post-flood herbicide option for control of weedy rice (Figure 4.1). However, benzobicyclon will offer a new SOA to

producers and provide broad-spectrum control of many aquatics, grasses, broadleaves, and sedges (Norsworthy et al. 2014; Sandoski et al. 2014). For those producers that apply benzobicyclon, the opportunity for control of weedy rice is merely an added benefit.

A need for a rapid assay to determine the susceptibility of weedy rice to benzobicyclon is needed if this herbicide is to be used successfully for control of this weed. Such an assay would need to be simple and mobile to enable applicators to test and treat sensitive fields of weedy rice with benzobicyclon. If this was possible producers would have a new SOA for the control of weedy rice.

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Table 4.1. Weedy rice accessions listed by county, hull color, awn, and GPS coordinates from which they were collected.

Accession	County	Hull color	Awn	Latitude	Longitude
				°N	°W
AR1	Desha	Straw	No	33.60267	-91.41006
AR2	Chicot	Straw	No	33.54211	-91.38511
AR3	Chicot	Straw	No	33.53917	-91.35831
AR4	Desha	Straw	No	34.18541	-91.88524
AR5	Desha	Straw	Yes	33.83537	-91.40168
AR6	Desha	Straw	Yes	33.85178	-91.40222
AR7	Chicot	Straw	Yes	33.48016	-91.38602
AR8	Chicot	Straw	No	33.41890	-91.38939
AR9	Ashley	Straw	No	33.28428	-91.46906
AR10	Ashley	Mixed	Yes	33.18478	-91.59997
AR11	Ashley	Black	Yes	33.19108	-91.59586
AR12	Ashley	Straw	No	33.29553	-91.46339
AR13	Drew	Straw	No	33.74517	-91.57603
AR14	Drew	Straw	No	33.76775	-91.43571
AR15	Lincoln	Straw	No	33.96905	-91.67650
AR16	Jefferson	Black	Yes	33.88644	-91.34083
AR17	Jefferson	Straw	No	34.28598	-91.99814
AR18	Jefferson	Straw	No	34.30961	-91.96481
AR19	Jefferson	Black	Yes	34.38548	-91.78567
AR20	Jefferson	Straw	Yes	34.41339	-91.83607
AR21	Jefferson	Black	Yes	34.43131	-91.81256
AR22	Arkansas	Straw	No	34.38503	-91.43515
AR23	Arkansas	Black	Yes	34.38519	-91.49369
AR24	Lonoke	Black	Yes	34.54444	-91.70622
AR25	Lonoke	Black	Yes	34.48600	-91.94350
AR26	Lonoke	Mixed	Yes	34.49136	-91.93056
AR27	Monroe	Black	Yes	34.72772	-91.23555
AR28	Monroe	Black	Yes	34.73078	-91.25690
AR29	Monroe	Straw	No	34.70319	-91.14719
AR30	Lee	Straw	No	34.74097	-91.04922
AR31	Lee	Black	Yes	34.76851	-91.03602
AR32	Lee	Black	Yes	34.78219	-91.00197
AR33	St. Francis	Straw	Yes	35.03553	-90.40144
AR34	St. Francis	Mixed	Yes	35.05767	-90.40355
AR35	St. Francis	Mixed	Yes	35.06924	-90.40372
AR36	St. Francis	Mixed	Yes	35.11191	-90.98546



Table 4.1 cont.

Accession	County	Hull color	Awn	Latitude	Longitude
				°N	°W
AR37	Cross	Straw	No	35.15991	-91.01144
AR38	Cross	Mixed	Yes	35.19938	-90.93515
AR39	Cross	Black	Yes	35.20080	-90.86839
AR40	Cross	Mixed	Yes	35.26697	-90.61833
AR41	Crittenden	Straw	No	35.16505	-90.35226
AR42	Crittenden	Black	No	35.16870	-90.34130
AR43	Crittenden	Straw	No	35.35262	-90.29897
AR44	Mississippi	Straw	No	35.68588	-90.08599
AR45	Mississippi	Mixed	Yes	35.78501	-90.07886
AR46	Mississippi	Straw	No	35.80241	-90.03580
AR47	Mississippi	Mixed	Yes	35.61186	-90.38516
AR48	Poinsett	Straw	Yes	35.55233	-90.84455
AR49	Poinsett	Straw	No	35.51875	-90.84566
AR50	Poinsett	Straw	No	35.61455	-90.90422
AR51	Poinsett	Straw	No	35.62738	-90.88055
AR52	Craighead	Black	Yes	35.80290	-90.89263
AR53	Craighead	Mixed	Yes	35.89308	-90.95206
AR54	Randolf	Straw	No	35.23433	-91.86869
AR55	Greene	Straw	No	36.24136	-90.74122
AR56	Clay	Straw	No	36.32819	-90.58522
AR57	Clay	Straw	No	36.38547	-90.59191
AR58	Jackson	Mixed	Yes	35.80213	-91.13598
AR59	White	Straw	No	35.07538	-91.73469
AR60	White	Straw	No	35.07130	-91.73508
AR61	White	Black	Yes	35.17377	-91.71921
AR62	Crittenden	Black	Yes	35.16735	-90.36708
AR63	Greene	Straw	No	36.15576	-90.68562
AR64	Greene	Straw	No	36.04436	-90.72133
AR65	Greene	Straw	Yes	36.13543	-90.66741
AR66	Greene	Straw	No	36.10147	-90.37663
AR67	Greene	Straw	No	36.0430.53	-90.71832
AR68	Greene	Straw	No	36.10563	-90.68680
AR69	Lonoke	Straw	No	34.58044	-91.73327
AR70	Lonoke	Straw	Yes	34.61441	-91.77616
AR71	Lonoke	Straw	Yes	34.57025	-91.71447
AR72	Lonoke	Straw	No	34.60463	-91.70352
MO73	New Madrid	Mixed	Yes	36.55500	-89.65250
MO74	Stoddard	Straw	Yes	36.71500	-89.74250

Table 4.1 cont.

Accession	County	Hull color	Awn	Latitude	Longitude
				°N	°W
MO75	Pemiscot	Black	Yes	36.34305	-89.88805
MO76	Butler	Black	Yes	36.63472	-90.47472
MO77	New Madrid	Mixed	Yes	36.43028	-89.79972
MO78	Pemiscot	Black	Yes	36.17444	-89.89666
MO79	Butler	Black	Yes	36.60083	-90.20055
MS80	Washington	Straw	Yes	33.88341	-91.35830
MS81	Washington	Black	Yes	33.28755	-90.93371
MS82	Bolivar	Straw	No	33.75436	-90.69319
MS83	Tunica	Black	Yes	33.14010	-90.75185
MS84	Sunflower	Straw	No	33.85758	-90.52939
AR85	Greene	Straw	No	36.06821	-90.69009
AR86	Greene	Straw	No	36.21985	-90.61732
AR87	Greene	Black	Yes	36.16392	-90.69972
AR88	Greene	Black	Yes	36.15877	-90.84504
AR89	Greene	Black	Yes	36.22981	-90.71716
AR90	Greene	Black	Yes	36.18514	-90.67325
AR91	Greene	Mixed	Yes	36.13263	-90.72615
AR92	Greene	Straw	No	35.99387	-90.46820
AR93	Lawrence	Straw	No	36.00052	-91.05013
AR94	Lawrence	Mixed	Yes	35.93349	-90.93851
AR95	Lawrence	Black	Yes	35.95822	-90.90048
AR96	Poinsett	Straw	No	35.55822	-90.35540
AR97	Arkansas	Black	Yes	34.27977	-91.57418
AR98	Ashley	Straw	No	33.19108	-91.59596
AR99	Desha	Mixed	YES	34.70795	-90.34079
AR100	Craighead	Mixed	Yes	35.80400	-90.87926

<sup>a</sup> Accession state of origin indicated by ‘AR’ (Arkansas), ‘MO’ (Missouri), and ‘MS’ (Mississippi).

<sup>b</sup> Hull color ‘Mixed’ indicates any accession that had multiple black, straw, gold, brown, or gray colored samples within the accession.

<sup>c</sup> Presence or absence of an awn indicated by ‘Yes’ or ‘No’.

Table 4.2. Responses of 100 sensitive weedy rice accessions following a post-flood application of benzobicyclon at 371 g ai ha<sup>-1</sup>. <sup>a,b,c</sup>

Accession <sup>d,e</sup>	Mortality	SE of Mortality	Control	SE of Control
	%		%	
AR1	64	2.4	21	0.4
AR2	86	1.2	76	0.3
AR3	25	1.9	25	0.3
AR4	89	1.3	21	0.4
AR5	72	1.9	75	0.3
AR6	62	2.3	63	0.4
AR7	25	2	28	0.3
AR8	100	-	94	0.2
AR9	100	-	20	0.3
AR10	43	2.3	18	0.3
AR11	89	1.3	20	0.3
AR12	0	-	18	0.3
AR13	84	1.3	97	0.2
AR14	57	2.4	100	-
AR15	100	-	90	0.3
AR16	50	2.5	10	0.2
AR17	100	-	80	0.3
AR18	86	1.2	100	-
AR19	59	2.5	18	0.3
AR20	37	2.2	25	0.3
AR21	5	0.5	16	0.4
AR22	78	1.7	87	0.2
AR23	59	2.9	49	0.5
AR24	39	2.3	8	0.2
AR25	27	1.9	5	0.2
AR26	44	2.3	36	0.5
AR27	78	1.7	35	0.4
AR28	29	2.1	13	0.2
AR29	95	0.6	85	0.3
AR30	100	-	97	0.1
AR31	76	1.7	45	0.4
AR32	100	-	100	-
AR33	69	2	45	0.4
AR34	100	-	75	0.3
AR35	84	1.3	83	0.3
AR36	31	2.2	75	0.3
AR37	57	2.4	100	-
AR38	23	1.7	35	0.4
AR39	83	1.9	15	0.3

Table 4.2. cont.

Accession	Mortality	SE of Mortality	Control	SE of Control
	%		%	
AR40	0	-	25	0.3
AR41	9	1.1	95	0.2
AR42	0	-	25	0.3
AR43	84	1.3	90	0.3
AR44	62	2.2	28	0.3
AR45	81	1.5	100	-
AR46	100	-	98	0.1
AR47	67	2.1	35	0.4
AR48	65	2.2	99	0.1
AR49	57	2.4	96	0.1
AR50	100	-	88	0.2
AR51	62	2.3	90	0.2
AR52	100	-	89	0.2
AR53	39	3	8	0.2
AR54	57	2.3	97	0.2
AR55	2	0.2	5	0.2
AR56	23	1.7	23	0.3
AR57	13	1.1	63	0.4
AR58	13	1.1	35	0.4
AR59	43	2.3	75	0.3
AR60	64	2.1	94	0.2
AR61	39	2.3	18	0.3
AR62	28	1.9	100	-
AR63	9	0.9	90	0.3
AR64	83	1.6	100	-
AR65	27	1.8	16	0.4
AR66	6	0.6	30	0.3
AR67	2	0.2	40	0.4
AR68	19	1.5	93	0.2
AR69	0	-	21	0.4
AR70	4	0.4	68	0.3
AR71	5	0.5	58	0.4
AR72	8	0.7	50	0.4
MO73	25	1.7	10	0.3
MO74	0	-	10	0.2
MO75	25	2	49	0.5
MO76	17	1.3	50	0.4
MO77	9	0.8	5	0.2
MO78	31	2.2	10	0.2
MO79	9	1.1	71	0.5
MS80	0	-	20	0.3

Table 4.2. cont.

Accession	Mortality	SE of Mortality	Control	SE of Control
	%		%	
MS81	5	0.5	10	0.2
MS82	8	0.7	10	0.3
MS83	0	-	20	0.3
MS84	25	2	99	0.1
AR85	3	0.3	5	0.2
AR86	2	0.2	0	-
AR87	8	0.7	8	0.2
AR88	39	2.3	10	0.2
AR89	5	0.5	23	0.3
AR90	13	1.1	43	0.4
AR91	4	0.4	5	0.2
AR92	38	2.2	10	0.3
AR93	100	-	90	0.2
AR94	5	0.5	5	0.2
AR95	7	0.7	20	0.3
AR96	2	0.2	10	0.2
AR97	4	0.4	10	0.2
AR98	2	0.2	45	0.4
AR99	0	-	20	0.3
AR100	7	0.7	18	0.3

<sup>a</sup>Accession state of origin indicated by ‘AR’ (Arkansas), ‘MO’ (Missouri), and ‘MS’ (Mississippi).

<sup>b</sup> Post-flood application made at 2- to 3-leaf with the addition of crop oil concentrate at 1% v/v.

<sup>c</sup> Accessions evaluated at 6 wk after post-flood applications in the greenhouse.

<sup>d</sup> The accessions of 100 or 0%, mortality or control, were not included in the analysis; therefore, “-” indicates a non-calculated standard error; “SE” indicates standard error

<sup>e</sup> Due to poor germination some rows did not have 25 plants emerge per replication; therefore, the percentage mortality was calculated from the total number of plants that emerged.

Table 4.3. Frequency distribution to describe the phenotypic characteristics of 100 weedy rice accessions collected from across the Midsouth.

Hull color <sup>a</sup>	Awned	
	No	Yes
	----no. of accessions <sup>b</sup> ----	
Straw	46	11
Black	1	25
Mixed	0	17

<sup>a</sup> Hull color designated as 'Mixed' indicates those accessions that contained both 'Straw' and 'Black' hull colors

<sup>b</sup> A total of 100 accessions of weedy rice collected from Arkansas, Mississippi, and Missouri

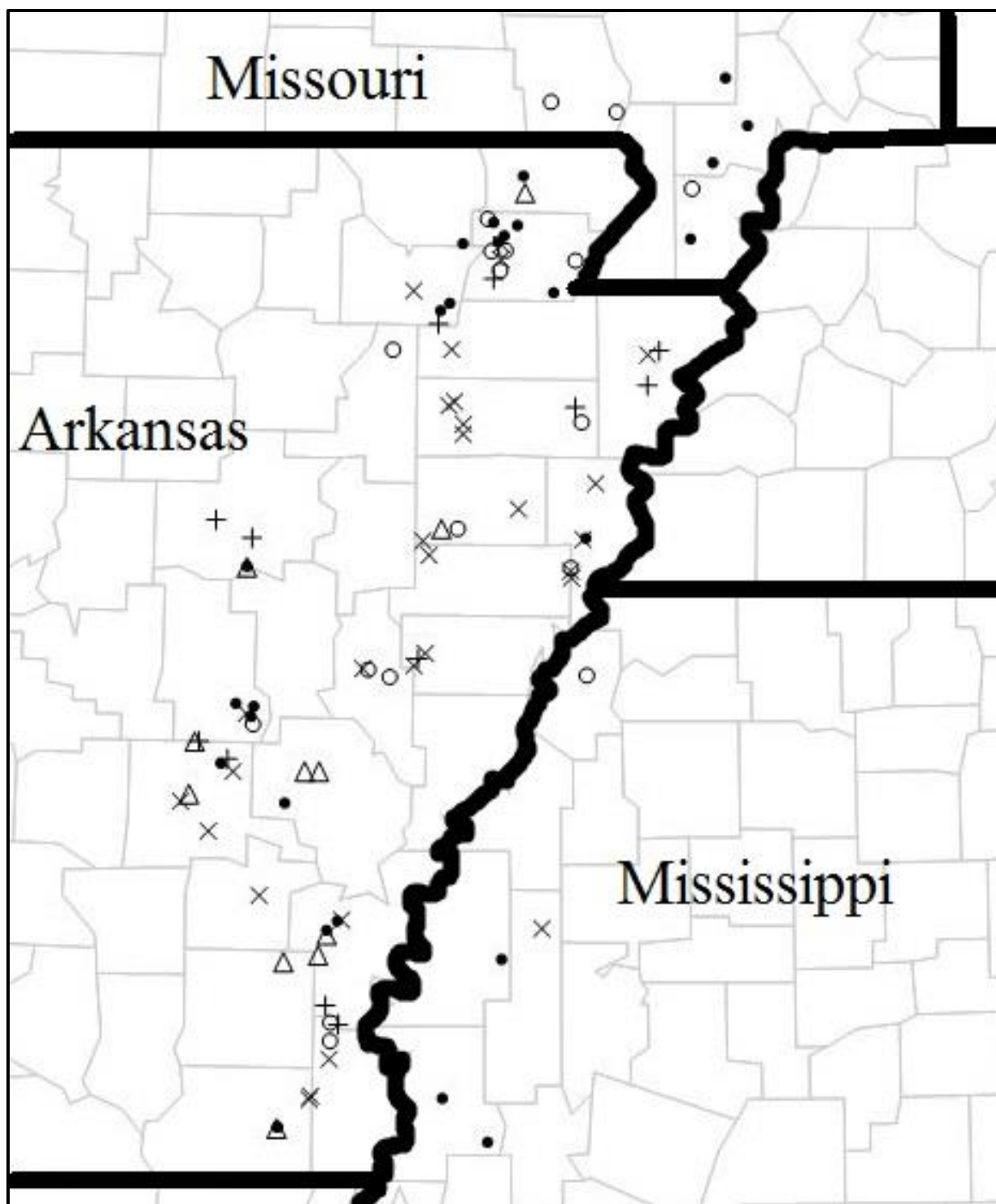


Figure 4.1 A county map of Arkansas, Mississippi, and Missouri illustrating the percent mortality of 100 weedy rice accessions to benzobicyclon at 371 g ai ha<sup>-1</sup> applied post-flood. Markers indicate percent mortality '●' 0-20%, '○' 21-40%, 'Δ' 41-60%, '+' 61-80%, and 'X' 81-100%.

## Chapter 5

### Potential for Benzobicyclon Carryover from Rice to Rotational Crops

Two field studies were conducted from 2015 to 2016 on a Calloway and Dewitt silt loam soil near Pine Tree and Stuttgart, Arkansas, respectively. The objective was to evaluate the potential for a benzobicyclon application to carryover from rice and cause damage to corn, cotton, grain sorghum, soybean, and sunflower planted the subsequent year. No injury or stand reduction occurred for any crop following applications of benzobicyclon at 247 and 494 g ai ha<sup>-1</sup> at both locations. Grain sorghum had a 29% height reduction at Pine Tree 12 days after planting (DAP); however, no differences in height occurred at Stuttgart, and by 40 DAP, all heights were similar between treated and nontreated plots at both locations. Plant height of corn, cotton, soybean, and sunflower was not reduced in treated plots. At Pine Tree, a 53% and 56% reduction in corn yield resulted from benzobicyclon at 247 and 494 g ha<sup>-1</sup>, respectively, whereas cotton, grain sorghum, and soybean yields were not affected by benzobicyclon residues. Yields of all crops at Stuttgart were not negatively affected by benzobicyclon application the previous year. This research shows that benzobicyclon applied to rice can be rotated the subsequent growing season to cotton and soybean without fear of injuring these crops or causing a negative impact on crop yield.

**Nomenclature:** Benzobicyclon; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; grain sorghum, *Sorghum bicolor* L. Moench; rice, *Oryza sativa* L.; soybean, *Glycine max* L. Merr.; sunflower, *Helianthus annuus* L.

**Key words:** Benzobicyclon, carryover, crop injury, crop rotation, silt loam, yield loss

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## Introduction

The persistence of herbicides in the soil is an important consideration in crop production because of the potential for injury to the crop or subsequent crops. Herbicide persistence is dependent upon multiple factors such as rainfall, soil texture, microbial activity, tillage, and rate of herbicide applied (Banks et al. 1979; Loux and Reese 1993; Renner et al. 1988). Growers need to take into consideration these factors when developing an integrated weed management plan.

Crop rotation is often used for control of weedy rice and other problematic weeds in rice. It enables producers to improve weed control and increase yields (Beaty 1982). Rotating rice to soybean or grain sorghum and applying the recommended herbicides allows for control of weedy rice and other hard-to-control weeds within the crop. However, some of the common rice herbicides have the potential for carryover to the subsequent crop. Herbicides like imazethapyr are known to carryover and be injurious to subsequently planted crops such as corn (Curran et al. 1991). Alister et al. (2005) reported chili pepper (*Capsicum annuum* L.), sugarbeet (*Beta vulgaris* L.), and tomato (*Solanum lycopersicum* L.) to be highly sensitive to imazethapyr 300 days after application (DAA). Other crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are known to need intercropping periods up to 540 DAA of the imidazolinone herbicides due to persistence in the soil.

The high potential for carryover does not mean these herbicides cannot be utilized in a crop rotation to a sensitive crop. The process of natural selection was used to create a rice cultivar having resistance to the imidazolinone herbicides (Sudianto et al. 2013). This technology, also known as Clearfield® rice, allows for the application of imazethapyr and imazamox in crop for control of troublesome weeds like weedy rice (*Oryza sativa* L.) and

problematic herbicide-resistant weeds like barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) (Hardke 2013). In 2008, imidazolinone-resistant rice accounted for 40% of the rice acreage in Arkansas (Wilson and Runsick 2009). The acreage of imidazolinone-resistant rice steadily increased from the time of its introduction until 2011 as producers relied on the effective imidazolinone herbicides to control weedy rice and barnyardgrass. Imidazolinone-resistant rice accounted for the 69% of the rice acreage in Arkansas in 2011 (Hardke and Wilson 2012).

In recent years, the acreage of the imidazolinone-resistant rice has steadily declined in part because of weedy rice and barnyardgrass resistance to imazethapyr and imazamox. Hardke et al. (2015) reported imidazolinone-resistant rice accounted for only 44% of Arkansas' rice acreage in 2015. The imidazolinone herbicides are no longer an effective option for producers with infestations of these imidazolinone-resistant weeds.

Due to the repetitive use of the imidazolinone herbicides and the lack of effective weed control options for rice producers in the Midsouth, there is a need for a new site of action (SOA) to implement effective strategies for weed management in rice (Norsworthy et al. 2007). Producers have exhausted the imidazolinone-resistant rice system in efforts to control problematic weeds. Hard-to-control weed species that have evolved resistance to the imidazolinone herbicides used in rice include barnyardgrass, rice flatsedge (*Cyperus iria* L.), yellow nutsedge (*Cyperus esculentus* L.), smallflower umbrella sedge (*Cyperus difformis* L.), and weedy rice (Heap 2016; Norsworthy et al. 2007; Norsworthy et al. 2013). An integral part of most successful weed control programs is crop rotation, and rice producers in Arkansas commonly make use of this practice by rotating rice with soybean and other crops (Riar et al. 2013).

Benzobicyclon is a novel 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide used in paddy-rice systems in Asia (Komatusbara et al. 2009). Benzobicyclon shows broad-spectrum activity on aquatic, grass, sedge, and broadleaf weeds without causing phytotoxicity to *japonica* rice varieties (Komatsubara et al. 2009; Kwon et al. 2012). Komatsubara et al. (2009) and Sekino et al. (2008) reported herbicidal activity on rock bulrush (*Scirpus juncoides* Roxb.) was not affected by soil texture. Benzobicyclon applied at 200 to 300 g ai ha<sup>-1</sup> is known to have residual activity for eight weeks on rock bulrush (Sekino et al. 2008). Sekino et al. (2008) reported benzobicyclon only moving 1- to 2-cm in the soil profile. These results imply a minimal concern for mobility in the soil. Benzobicyclon is a pro-herbicide which requires water to convert benzobicyclon to its herbicidal form, benzobicyclon hydrolysate (Komatsubara et al. 2009; Williams and Tjeerdema 2016). However, benzobicyclon hydrolysate has some leaching potential due to its aqueous solubility of 7.26 mg L<sup>-1</sup> (Williams and Tjeerdema 2016). However, the parent molecule, benzobicyclon, has a low leaching potential with a K<sub>oc</sub> of 1,104 (Kegley et al. 2017). Similarly, another triketone herbicide, mesotrione, has a high leaching potential with a K<sub>oc</sub> of 80 (Kegley et al. 2017). Conversion of benzobicyclon to benzobicyclon hydrolysate can be affected by the pH of water (Williams and Tjeerdema 2016). However, benzobicyclon has not been tested in Arkansas rice systems, and sensitivity to other Arkansas row crops is not known.

As a comparison, mesotrione is a commonly applied HPPD-inhibiting herbicide used in corn and other crops (Riddle et al. 2013). Mesotrione is registered for preemergence (PRE) and postemergence (POST) use for broadleaf weed control. Because of the effective residual activity of mesotrione, it can sometimes cause injury to sensitive crops grown in rotation 1 year after application (Riddle et al. 2013). The current mesotrione label has recropping intervals of 4

months for wheat and up to 10 months for soybean (Anonymous 2015). Specialty crops such as sugarbeet and cucurbits (*Cucurbitaceae*) have a recropping interval of 18 months (Anonymous 2015).

Rice producers in the Midsouth are in need of a new SOA for weed management and herbicide resistance management in rice (Norsworthy et al. 2007, 2012, 2013). If labeled, benzobicyclon will be applied post-flood in rice (C. Sandoski, personal communication). Until recently, no HPPD-inhibiting herbicides have been labeled for use in rice (Scott et al. 2016). Therefore, it is important to understand the potential for carryover of benzobicyclon in rice to subsequent crops. Hence, the objective of this experiment was to determine if benzobicyclon used in a rice system would be detrimental to corn, cotton, grain sorghum, soybean, and sunflower planted the subsequent growing season.

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## **Materials and Methods**

A field experiment was initiated in 2015 at two locations in Arkansas and concluded the subsequent year (2016). The experimental sites were located at the Pine Tree Research Station (PTRS) near Colt, AR, on a Calloway silt loam soil (fine-silty, mixed, active thermic Aquic Fraglossudalfs) (hereafter referred to as Pine Tree) and the Rice Research and Extension Center (RREC) near Stuttgart, AR, on a Dewitt silt loam (Fine, smectitic, thermic Typic Albaqualfs) (hereafter referred to as Stuttgart).

This experiment was conducted as a randomized complete block design with four replications and three experimental treatments. In 2015 at both locations, the Clearfield® 111 rice cultivar was drill-seeded in 19-cm wide rows at a rate of 66 seed m<sup>-1</sup> of row. Plots 4 m wide by 50 m long and 6 m wide by 50 m long were established at Stuttgart and Pine Tree, respectively.

Each experimental plot was self-contained within individual bays, which allowed for separation of the benzobicyclon treatments applied in 2015. The plots would later be planted to corn, cotton, grain sorghum, soybean, and sunflower in 2016.

The following treatments were applied in the summer of 2015: benzobicyclon after permanent flood was established at 247 and 494 g ai ha<sup>-1</sup> in addition to a nontreated. The treatments were applied with a CO<sub>2</sub>-pressurized backpack sprayer and handheld boom. At Pine Tree, the handheld boom contained six 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703) on 48 cm spacing and was calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. Treatments at Stuttgart were made with a handheld boom that consisted of five 110015 AIXR nozzles (Teejet Technologies, Springfield, IL 62703) on 48 cm spacing and was calibrated to deliver 143 L ha<sup>-1</sup> at 276 kPa. At both locations, spray applications were made down each side of the plot to ensure the total surface area of the water was treated. Treatments were applied in 2015 on June 23 at Stuttgart and June 24 at Pine Tree. Weeds were controlled at Stuttgart using halosulfuron at 35 g ai ha<sup>-1</sup>, 2,4-D amine at 533 g ae ha<sup>-1</sup>, and fenoxaprop at 800 g ai ha<sup>-1</sup> prior to flood. Weeds were controlled at Pine Tree using clomazone at 336 g ai ha<sup>-1</sup> and quinclorac at 276 g ae ha<sup>-1</sup> at planting followed by halosulfuron at 35 g ha<sup>-1</sup> and 2,4-D amine at 533 g ha<sup>-1</sup>. The 2015 crop served solely as an opportunity to apply the benzobicyclon herbicide treatments post-flood and evaluate the potential for carryover to subsequent crops.

Immediately following the rice harvest in 2015 the crop residue was burned which is a typical practice for rice in this region. In 2016, cultivars of corn, cotton, grain sorghum, soybean, and sunflower were planted directly into the plots from the previous year without use of tillage prior to planting. All crops were planted at the same seeding rates at each location; however, cultivars varied by location due to availability of seed (Table 5.1). The cultivars of each crop are

listed in Table 5.1. Soil samples were collected to a 10-cm depth at the time of planting to quantify soil pH, organic matter content, and soil texture at each location (Table 5.2). Rainfall and irrigation amounts were recorded over the 2-year period (Table 5.3). The crops planted the year following rice were visually evaluated for injury at 2, 4, 6, and 12 weeks after planting (WAP). Visual injury was assessed on a scale of 0 to 100%, with 0 being no injury and 100% being crop death. Crop stands were measured by counting emerged plants in 2 m of row per plot at 2 WAP. Crop heights were measured at 12 and 40 days after planting. Each crop, except sunflower due to bird damage and lodging, was harvested with a small-plot combine (Massey Ferguson 8, AGCO, Duluth, GA 30096) to test for yield loss as a result of benzobicyclon applications made the previous year. Plots were kept weed-free throughout the 2016 growing season using standard herbicide programs for each crop (Scott et al. 2016).

— All data were analyzed by ANOVA using JMP Pro Version 12.1.0 (SAS Institute Inc. Cary, NC 27513). Because of differences in rainfall between locations as well as differences in soil characteristics, all data were analyzed separately by location. All means were separated using Fisher's protected LSD ( $\alpha=0.05$ ), and benzobicyclon treatments were directly compared to the nontreated.

## **Results and Discussion**

An anticipated 1X and 2X rate of benzobicyclon was compared to a nontreated control to evaluate the potential for benzobicyclon to carryover to five crops that are sometimes grown in rotation with rice. Of the five crops evaluated, soybean would be the most commonly rotated crop with rice (Hardke and Wilson 2012; Hardke 2013; 2015). Although, Pine Tree and Stuttgart both had a silt loam soil, the pH and organic matter varied by location (Table 5.2). Pine

Tree had a higher soil pH (8.3) and higher organic matter content (1.9%) than Stuttgart (Table 5.2). There is no published literature on the influence of soil pH or organic matter on persistence of benzobicyclon or benzobicyclon hydrolysate in soil; albeit, most herbicides tend to be more persistent as organic matter content increases, lowering the risk for carryover to subsequent crops (Rauch et al. 2007). Additionally, benzobicyclon activity in water increases as a function of pH; hence, it seems likely that the risk for persistence would be greatest at Pine Tree where the soil pH was greater than that at Stuttgart (Williams and Tjeerdema 2016).

The benzobicyclon treatments in 2015 were made into a continuous flooded system. Rainfall and irrigation events over the 2-year period are presented in Table 5.3. The irrigation events in the summer of 2016 consisted of approximately a 10-cm flush monthly at both locations. Rainfall and irrigation can impact the persistence of herbicides in the soil ultimately causing carryover injury to the subsequent crops (Hill 2015). The minimal differences of rainfall and irrigation amounts recorded at Pine Tree and Stuttgart suggest that soil moisture is not a contributing factor for benzobicyclon hydrolysate carryover.

**Visible Injury and Crop Density.** No visible injury, which would typically be in the form of chlorosis or bleaching (Komatsubara et al. 2009; Norsworthy et al. 2014; Sekino 2008), was observed for any of the crops at any time during the growing season (data not shown). Likewise, there were no differences in crop density for any of the crops at either location (Table 5.4). Based on a rate titration experiment with soil-applied benzobicyclon in the greenhouse, it is known that low rates of the herbicide can cause injury to most of the crops evaluated in the field (J.K. Norsworthy, personal communication). The lack of visual symptoms on these crops would indicate that the concentration of benzobicyclon hydrolysate to be lower than level needed to induce an observable response by the crop soon after planting. Based on these results, it would

not be likely to observe early-season damage to corn, cotton, grain sorghum, soybean, or sunflower planted the year following a post-flood benzobicyclon application to rice.

**Crop Height.** No significant differences in crop height occurred among benzobicyclon treatments within a crop at Stuttgart or at Pine Tree, except for a significant effect of crop height occurring for grain sorghum 12 DAP (Table 5.5). Benzobicyclon at 494 g ai ha<sup>-1</sup> applied to rice the previous growing season resulted in a grain sorghum height of 5 cm at 12 DAP compared to 7-cm tall plants in the absence of benzobicyclon, a 29% reduction. The reduction in crop height may have been associated with the higher soil pH (8.3) at Pine Tree, but it seems likely that chlorosis or bleaching should have been linked with a height reduction in grain sorghum. By 40 DAP, grain sorghum heights in all benzobicyclon treatments were similar. None of the benzobicyclon treatments had a deleterious effect on corn, cotton, soybean, or sunflower plant height at Pine Tree.

**Grain Yield.** Surprisingly, there was a significant effect of benzobicyclon rate for corn yield at Pine Tree (Table 5.6). Corn treated with either rate of benzobicyclon produced grain yields less than those in the nontreated control. Corn yields at Pine Tree were reduced 53% and 56% by benzobicyclon at 247 and 494 g ha<sup>-1</sup>, respectively (Table 5.6). In previously conducted benzobicyclon carryover research, a late-season application (mid-August) of benzobicyclon at 494 g ha<sup>-1</sup> to a flooded, noncropped silt loam soil did result in carryover to corn planted the subsequent year (J.K. Norsworthy and C.A. Sandoski, unpublished data). In the previous trial that did not contain rice, benzobicyclon and benzobicyclon hydrolysate were present in the upper 10-cm of soil at a concentration of 4.51 ppb and 5.05 ppb, respectively, approximately 12 months after application.



There were no differences in grain yield among benzobicyclon treatments for any of the other crops evaluated at Pine Tree as well as all crops at Stuttgart (Table 5.6). It should be noted that grain yield of sunflower was not obtainable because of bird damage and severe lodging of the plants.

**Practical Implications.** Crop rotation is an important practice used by many rice growers that strive to build a strong integrated weed management program (Riar et al. 2013). Crop rotation can be practiced effectively if a tolerant crop is planted in rotation with the herbicide program using in the subsequent crop or the persistence of the herbicide is such that it allows other sensitive crops to be grown in rotation without great risk for injury the following crop. The incorporation of benzobicyclon into a weed control program in Midsouth rice will offer producers a new SOA for use in the crop, and there appears to be little risk for damage to soybean, the most frequently rotated crop with rice. Rotation from rice to cotton and sunflower, mainly grown for recreational sportsman activities, is sometimes practiced, and based on results from this research, the risk for carryover to these crops would also be low. With a reduction in early-season grain sorghum height at Pine Tree along with grain yield reduction in corn, it is believed that additional research may be needed on these crops before sufficient confidence would exist to allow for rice treated with benzobicyclon to be rotated to corn or grain sorghum the subsequent growing season, especially on soils having high pH. Furthermore, future research should aim at better understanding the role of soil pH on the persistence of benzobicyclon and its active metabolite, benzobicyclon hydrolysate.

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Table 5.1. Planting dates and seeding rates of the five crops planted in Pine Tree and Stuttgart, AR.

Location	Crop	Cultivar	Planting date	Row spacing	seeding rate 1000 seed ha <sup>-1</sup>
Pine Tree	Corn	DeKalb 60-67RIB	May 11, 2016	76	110
	Cotton	Stoneville 4946	May 11, 2016	76	150
	Grain sorghum	Pioneer 84P80	May 11, 2016	76	250
	Soybean	Asgrow 46X6	May 11, 2016	76	370
	Sunflower	Non-oilseed feed-grade	May 11, 2016	76	330
Stuttgart	Corn	DeKalb 60-67RIB	May 13, 2016	91	86
	Cotton	Delta Pine 1518B2XF	May 13, 2016	91	120
	Grain sorghum	Pioneer 84P80	May 13, 2016	91	175
	Soybean	Progeny 4814LLS	May 13, 2016	91	290
	Sunflower	Non-oilseed feed-grade	May 13, 2016	91	330

<sup>a</sup> Unknown germination of the non-oilseed, feed-grade sunflower seed required a high seeding rate

Table 5.2. Physical and chemical characteristics of the soils at Pine Tree and Stuttgart.

Location	Soil series	Family	Texture	pH	Organic matter	Sand	Silt	Clay
						----- % -----		
Pine Tree	Calloway	Aquic Fraglossudalfs	Silt loam	8.3	1.9	12.1	69.9	18.0
Stuttgart	Dewitt	Typic Albaqualfs	Silt loam	6.0	1.8	8.4	71.4	20.2

Table 5.3. Rainfall amounts and irrigation events throughout the year following benzobicyclon applications at Pine Tree and Stuttgart.<sup>a,b</sup>

Month/Year	Pine Tree		Stuttgart	
	Irrigation	Rainfall	Irrigation	Rainfall
	----- cm -----			
June/2015	19.0	3.0	15.0	7.5
July/2015	61.0	3.5	64.0	5.9
August/2015	11.0	3.0	10.0	0.2
September/2015 <sup>c</sup>	0.0	1.3	0.0	0.3
October/2015	0.0	3.2	0.0	8.8
November/2015	0.0	10.2	0.0	22.8
December/2015	0.0	7.0	0.0	6.9
January/2016	0.0	6.7	0.0	3.8
February/2016	0.0	6.5	0.0	8.4
March/2016	0.0	35.7	0.0	34.7
April/2016	0.0	15.8	0.0	8.4
May/2016	0.0	9.6	0.0	17.1
June/2016	10.1	3.7	10.1	6.0
July/2016	10.1	6.4	10.1	8.8
August/2016	0.0	9.0	0.0	15.2

<sup>a</sup> Treatments applied by location: June 23, 2015 (Stuttgart) and June 24, 2015 (Pine Tree)

<sup>b</sup> Flush-type irrigation used for all crops in 2016 at both locations

<sup>c</sup> Rough rice harvested by location: September 14, 2015 (Stuttgart) and September 16, 2015 (Pine Tree)

Table 5.4. Crop stand counts at 14 day after planting five crops following a post-flood application of two rates of benzobicyclon the previous season at Pine Tree and Stuttgart, AR.<sup>a</sup>

Location	Rate	Crop stand counts				
		Corn	Cotton	Grain sorghum	Soybean	Sunflower
	g ai ha <sup>-1</sup>	----- plants m <sup>-1</sup> row -----				
Pine Tree	nontreated	8	9	17	27	20
	247	8	11	19	25	24
	494	8	11	16	26	22
Stuttgart	nontreated	6	8	18	17	27
	247	7	9	19	17	23
	494	7	6	19	17	25

<sup>a</sup> No differences occurred in crop stands among benzobicyclon rates within a crop and location for any of the evaluated crops.

Table 5.5. Heights of five crops planted the subsequent growing season following a post-flood application of two rates of benzobicyclon and a nontreated on a silt loam soil at Pine Tree and Stuttgart, AR.

Location <sup>a</sup>	Rate g ai ha <sup>-1</sup>	Crop height <sup>a</sup>									
		Corn		Cotton		Grain sorghum		Soybean		Sunflower	
		12 DAP <sup>b</sup>	40 DAP	12 DAP	40 DAP	12 DAP	40 DAP	12 DAP	40 DAP	12 DAP	40 DAP
		----- cm -----									
Pine Tree	nontreated	11	72	3	23	7 a	66	5	13 <sup>c</sup>	4	37
	247	10	77	3	25	6 ab	75	4	11	4	36
	494	11	79	4	27	5 b	75	5	13	4	33
Stuttgart	nontreated	8	85	4	21	5	59	5	31	4	44
	247	8	75	2	23	5	60	5	41	4	43
	494	8	82	1	24	5	63	6	32	5	44

<sup>a</sup> For a specific location, means within a column followed by the same lowercase letter are not statistically different based on Fisher's protected LSD (0.05). Absence of letters indicates the ANOVA did not show statistical significance at  $\alpha=0.05$ .

<sup>b</sup> Abbreviation: DAP, days after planting

<sup>c</sup> Soybean heights at Pine Tree at 40 DAP were lower than expected due to white-tailed deer (*Odocoileus virginianus*) feeding on the terminal of the plants.



Table 5.6. Grain yield of four crops planted the subsequent growing season following a post-flood application of two rates of benzobicyclon and a nontreated on a silt loam soil at Pine Tree and Stuttgart, AR.

Location <sup>a</sup>	Rate g ai ha <sup>-1</sup>	Grain yield <sup>b</sup>			
		Corn	Cotton	Grain sorghum	Soybean
		----- kg ha <sup>-1</sup> -----			
Pine Tree	nontreated	6330 a	1330	2020	3340
	247	3000 b	1540	3650	3930
	494	2760 b	1150	3060	3200
Stuttgart	nontreated	10650	1990	5500	6300
	247	10300	2070	4820	6170
	494	10040	2130	5090	6010

<sup>a</sup> For a specific location, means within a column followed by the same lowercase letter are not statistically different based on Fisher's protected LSD (0.05). Absence of letters indicates the ANOVA did not show statistical significance at  $\alpha=0.05$ .

<sup>b</sup> Sunflower was not harvested due to severe bird damage at both locations.

## General Conclusion

Benzobicyclon, a Group 27 post-flood herbicide, will offer producers in the Midsouth a new herbicide site of action (SOA) in rice (*Oryza sativa* L.) for the control of problematic and herbicide-resistant weeds. Benzobicyclon controls a broad spectrum of aquatics, broadleaves, grasses, and sedges, including those currently resistant to Group 2 herbicides. This will be the first 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide commercially available in US rice production. This research validates that benzobicyclon is an effective post-flood herbicide for use in a drill-seeded rice production system. It is recommended to apply benzobicyclon into a permanent flood. The results from these experiments indicate that *japonica* rice cultivars exhibited excellent crop safety, while *indica* cultivars showed elevated levels of sensitivity to benzobicyclon. Benzobicyclon effectively controlled Amazon sprangletop, ducksalad, California arrowhead, *indica* rice, hemp sesbania, northern jointvetch, red sprangletop, ALS-resistant rice flatsedge, and ALS-resistant smallflower umbrella sedge. These aquatic, broadleaf, grass, and sedge species can be most problematic post-flood. Benzobicyclon will be an effective tool for producers to control these species late in the season. The efficacy and spectrum of control of benzobicyclon is increased when applied with tank-mix partners such as bispyribac, cyhalofop, halosulfuron, imazamox, penoxsulam, and propanil. Rotational crops such as cotton, grain sorghum, soybean, and sunflower can safely be planted in rotation with drill-seeded rice that has been treated with benzobicyclon post-flood without concerns of crop yield loss. Benzobicyclon will offer producers a new, alternative SOA to treat problematic and ALS-resistant weed populations. The findings of this research verify that benzobicyclon will be effective in drill-seeded rice systems and will directly improve and influence current weed control practices in the Midsouth.