# Spray deposition, adjuvants, and physiochemical properties affect benzobicyclon efficacy

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## Abstract

Benzobicyclon is a new pro-herbicide being evaluated in the Midsouth United States as a post-flood weed control option in rice. Applications of benzobicyclon to flooded rice are necessary for efficacious herbicide activity, but why this is so remains unknown. Two greenhouse experiments were conducted to explore how herbicide placement (foliage only, flood water only, foliage and flood water simultaneously) and adjuvants (nonionic surfactant, crop oil concentrate, and methylated seed oil [MSO]) affect herbicide activity. The first experiment focused on importance of herbicide placement. Little to no herbicidal activity (<18% visual control) was observed on two- to four-leaf barnyardgrass, Amazon sprangletop, and benzobicyclon-susceptible weedy rice with benzobicyclon treatments applied to weed foliage only. In contrast, applications made only to the flood water accounted for >82% of the weed control and biomass reduction achieved when benzobicyclon was applied to flood water and foliage simultaneously. The second experiment concentrated on adjuvant type and benzobicyclon efficacy when applied to foliage and flood water simultaneously. At 28 days after treatment, benzobicyclon alone at 371 g ai ha$^{-1}$ provided 29% and 67% control of three- to five-leaf barnyardgrass and Amazon sprangletop, respectively. The inclusion of any adjuvant significantly increased control, with MSO providing near-complete control of barnyardgrass and Amazon sprangletop. Furthermore, we used the physiochemical properties of benzobicyclon and benzobicyclon hydrolysate to derive theories to explain the complex activity of benzobicyclon observed in our study and in field trials. Benzobicyclon applications should contain an oil-based adjuvant and must be applied to flooded rice fields for optimal activity.

## Introduction

In the United States from 2006 to 2017, rice was planted on an average of 1.16 million ha, 75% of which was located in the Midsouth (Arkansas, Louisiana, Missouri, and Mississippi) (USDA-NASS 2018). The majority of rice in the Midsouth is grown in a delayed-flood rice system (Hardke 2014; Saichuk 2014). Here, rice is planted into a dry seedbed and once rice reaches the four- to six-leaf stage, a permanent flood is established. In contrast, California implements a water-seeded rice system and uses a continuous flood or establishes a flood once rice has pegged (Strand et al. 2013). In both systems, the establishment of a permanent flood is an important cultural weed management practice; however, herbicides are still needed for effective season-long weed control. The prevalent weeds in both systems are *Echinochloa* spp., *Leptochloa* spp., Cyperaceae species, ducksalad [*Heteranthera limosa* (Sw.) Willd.], weedy rice, jointvetches (*Aeschynomene* spp.), hemp sesbania [*Sesbania herbacea* (Mill.) McVaugh], and more aquatic-adapted weeds in the water-seeded systems (Norsworthy et al. 2013; Saichuk 2014; Strand et al. 2013). Although the weed species may differ between water- and dry-seeded rice, herbicide resistance is an ever-increasing problem in both systems, and new herbicide sites of action (SOA) are needed.

Benzobicyclon (3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one) is a new herbicide in the United States and will soon be marketed as a post-flood weed control option (Sandoski et al. 2014). Benzobicyclon is a pro-herbicide; in a nonenzymatic hydrolytic reaction, it is converted to the active ingredient benzobicyclon hydrolysate (Sekino 2002; Williams and Tjeerdema 2016). Benzobicyclon hydrolysate, a triketone, is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD; WSSA Group 27) (Kakidani and Hirai 2003; Sekino 2002) and is a new SOA in U.S. rice. Benzobicyclon has been commercially available in Asia since 2001, where it is used at rates from 200 to 300 g ai ha$^{-1}$ for PRE to early POST weed control in transplanted and water-seeded rice (Komatsubara et al. 2009). In the United States, benzobicylon plus halosulfuron (Butte®, Gowan, Yuma, AZ) was recently registered

in California as a slow-release granular for use in flooded rice from seeding to four-leaf rice, with benzobicyclon applied at 257 to 297 g ha⁻¹ (Anonymous 2017b). In the Midsouth, benzobicyclon is expected to be formulated as a soluble concentrate (Rogue®, Gowan) or in a water-dispersible granular premix with halosulfuron (Rogue Plus®, Gowan) at a proposed benzobicyclon use rate of 247 to 371 g ha⁻¹ (Sandoski et al. 2014).

Benzobicyclon has POST activity on several problematic and acetolactate synthase inhibitor–resistant grass, sedge, broadleaf, and aquatic weeds commonly found in rice fields, and it is especially effective on sprangletops, annual sedges, and ducksalad (Komatsubara et al. 2009; McKnight 2017; Young et al. 2018b). Flood establishment in relation to benzobicyclon application timing and flood depth is an important factor in maximizing benzobicyclon efficacy (McKnight 2017; Norsworthy et al. 2014; Young 2017). For example, in a greenhouse trial, Young et al. (2018b) showed control of one- to two-leaf Amazon sprangletop, barnyardgrass, rice flatsedge (*Cyperus iria* L.), and yellow nutsedge (*C. esculentus* L.) was improved an average of 47 percentage points to 92% when applied to a 5- or a 15-cm flood versus applications to a saturated field soil (45% control). In addition, McKnight (2017) and Norsworthy et al. (2014) found benzobicyclon was most effective when applied 24 h after the establishment of a continuous flood at pegging rice or five-leaf rice, respectively. The half-life (i.e., hydrolysis rate) of benzobicyclon in water at a pH of 7 and a temperature of 25 C is 15 h; under these conditions, the flood would need to be held for >4 d for benzobicyclon to undergo complete hydrolysis (Williams and Tjeerdema 2016). This further highlights the importance of the flood holding period in relation to application timing. Together, these trials revealed the necessity of a flood for benzobicyclon activity. However, applications in the Midsouth will be made later in the growing season; thus, there is a higher probability for benzobicyclon-containing spray droplets to come into direct contact with foliage. Therefore, research is needed to understand the uptake and translocation of benzobicyclon and the potential for benzobicyclon to be converted to the hydrolysate form at the leaf surface or within cellular content.

For POST-applied herbicides to reach their cellular target site, they must first cross multiple barriers (i.e., cuticle, cell wall, plasma membrane, and organelle membranes) and, if necessary, they must be translocated to the SOA. Most herbicides cross membranes by simple diffusion (i.e., down a concentration gradient); however, the physicochemical properties of an herbicide can significantly affect the rate at which this occurs (Sterling 1994). Two important physicochemical properties that affect herbicide uptake and translocation are the octanol/water partitioning coefficient (log $K_{ow}$) and the acid dissociation constant ($pK_a$) (Hsu and Kleier 1996; Hsu et al. 1990; Zhang et al. 2018). The log $K_{ow}$ represents the lipophilic nature of a compound, and for most herbicides, the log $K_{ow}$ value ranges from −4 to 4 (more lipophilic). In general, lipophilic herbicides diffuse across lipid barriers more freely than do more hydrophilic herbicides. Furthermore, the $pK_a$ for herbicides with an ionizable group can alter the log $K_{ow}$ (Kleier 1988). The $pK_a$ is the pH at which half of the herbicide molecules are protonated and half are negatively charged. At a pH below the $pK_a$, more of the herbicide molecules are protonated (neutral charge) and, thus, are more lipophilic. In addition to herbicide physicochemical properties, product formulations and adjuvants can improve translaminar movement (Kirkwood 1999). The objective of this investigation was 2-fold: (1) to determine the importance of benzobicyclon absorption as spray droplets versus uptake via flood water and (2) to select the appropriate adjuvant needed for optimum benzobicyclon activity.

## Materials and methods

Two separate experiments were conducted in the greenhouses located at the Altheimer Laboratory at the University of Arkansas, Fayetteville. Before the experiments, seeds of barnyardgrass, Amazon sprangletop, and weedy rice were germinated in plastic trays filled with potting mix (Sunshine premix No. 1®; Sun Gro Horticulture, Bellevue, WA). The weedy rice accessions were only used in Experiment 1. Barnyardgrass seeds were obtained from Azlin Seed Service (Leland, MS); Amazon sprangletop seeds were collected from field plots at the Rice Research and Extension Center near Stuttgart, AR; and the weedy rice seed was a subsample of multiple accessions known to be susceptible to benzobicyclon (Young et al. 2018a). Four to five seedlings at the one-leaf stage were transplanted into 7.5-L buckets (24-cm diameter) three-quarters filled with a Pembroke silt loam (fine-silty, mixed, active, mesic Mollic Paleudalfs) soil. Plant growth was maintained under of 32/22 C day/night temperature regimen and a 16-h photoperiod consisting of natural lighting supplemented with a halide (Experiment 1) or light-emitting diode (Experiment 2) lighting system. In both experiments, seedlings were flooded 1 d before the application of treatments. A 5-cm flood was established, and buckets were checked daily to ensure the appropriate flood level was maintained for 28 d after treatment (DAT). The water used in all experiments had a pH of 8.06 and an electric conductivity of 216 μS cm⁻¹.

### Benzobicyclon placement experiment

In the first experiment, benzobicyclon, at the expected field use rate of 371 g ai ha⁻¹, was applied to weed foliage only, flood water only, and both foliage and flood water. In addition, adjuvant type was tested with foliage-only treatments. The adjuvant treatments were none, nonionic surfactant (NIS; Induce, Helena Agri-Enterprises, LLC, Collierville, TN), crop oil concentrate (COC; Superb HC, WinField Solutions, LLC, Shoreview, MN), and a methylated seed oil (MSO; Leci-Tech, Loveland Products, Loveland, CO). Adjuvants were added at 1% v/v. Treatments were applied using a research track sprayer equipped with flat-fan spray nozzles calibrated to deliver 187 L ha⁻¹ at 1.6 km h⁻¹.

The experiment was conducted as a randomized complete block design with three replications and was repeated once. The weed species and size at application were barnyardgrass with two-to three-leaves (8- to 12-cm tall), Amazon sprangletop with three- to four-leaves (6- to 10-cm tall), and two-leaf weedy rice (10- to 15-cm tall). For the foliage-only treatments, activated charcoal at 0.6 g L⁻¹ was added to each bucket and stirred before and after treatment to bind benzobicyclon in the flood water. In addition, aluminum foil was used to cover large swaths of buckets with no plants to prevent droplets from reaching the flood water. In a preliminary experiment, activated charcoal in the flood water had no adverse effects on plant growth. For the flood water–only treatments, plant foliage was wrapped in aluminum foil. At 28 DAT, percent control was recorded, and plant tissue was harvested for dry weight. Dry weights represented the average grams per plant of four to five plants from each bucket and are expressed here as percent dry weight reduction from nontreated plants.

**Table 1.** Visual control and dry weight reduction of Amazon sprangletop, barnyardgrass, and weedy rice at 28 days after treatment with benzobicyclon applied at 371 g ai ha$^{-1}$ to foliage only, flood water only, and foliage and flood water simultaneously (Experiment 1).

| Spray deposition | Adjuvant[a,b] | Visual control[c] | | | Dry weight reduction | | |
|---|---|---|---|---|---|---|---|
| | | Amazon sprangletop | Barnyardgrass | Weedy rice[d] | Amazon sprangletop | Barnyard grass | Weedy rice |
| | | % | | | % | | |
| Foliage only | None | 1 D | 1 B | 2 C | 2 D | 0 C | 12 B |
| | NIS | 7 D | 1 B | 8 C | 15 C | 4 C | 18 B |
| | COC | 8 D | 3 B | 5 C | 20 C | 26 B | 13 B |
| | MSO | 17 C | 8 B | 9 C | 40 B | 33 B | 8 B |
| Flood water only | None | 59 B | 53 A | 75 B | 64 A | 67 A | 80 A |
| Foliage + flood water | None | 69 A | 54 A | 88 A | 77 A | 68 A | 78 A |

[a] Abbreviations: COC, crop oil concentrate; MSO, methylated seed oil; NIS, nonionic surfactant.
[b] The NIS, COC, and MSO were applied at 1% v/v.
[c] Mean values within a column, averaged over two trials, were separated using Fisher's protected LSD values at an α of 0.05.
[d] Weedy rice accessions were collected and prescreened for benzobicyclon susceptibility in Young et al. (2018a).

### Benzobicyclon adjuvant experiment

The second experiment focused on the efficacy of benzobicyclon (371 g ha$^{-1}$) applied with or without adjuvants to foliage and flood water simultaneously. The experimental setup was similar to the benzobicyclon placement study, except there were four replications per run and a total of three runs. The adjuvants used were the same as in Experiment 1 and the treatments were none, NIS at 0.25% v/v, and COC and MSO each at 1% v/v. A nontreated control was included. The weed species and size at application were barnyardgrass with three to four leaves (8 to 18 cm tall) and Amazon sprangletop with four to five leaves (8 to 12 cm tall). Weedy rice accessions were not used in this experiment.

Before analysis, data were tested for homoscedasticity using Bartlett's test and data were pooled across runs for each species (P > 0.05). Control data were not included in analyses. For both experiments, data were subjected to ANOVA using the MIXED procedure in SAS (SAS Institute, Cary NC) and means were separated using Fisher's protected LSD (α = 0.05).

### Results and discussion

#### Experiment 1: Benzobicyclon efficacy as a function of spray placement

Previous research has illustrated the necessity of a flood for benzobicyclon herbicide activity, but the reason for this phenomenon is not well understood. To explore this, benzobicyclon (371 g ha$^{-1}$) was selectively applied to flood water and/or plant foliage where, under our experimental setup, we assumed benzobicyclon and the hydrolysate active form were available for uptake only via the deposition method.

At 28 DAT, benzobicyclon applied simultaneously to foliage and flood water without an adjuvant provided 88%, 69%, and 54% visual control of weedy rice, Amazon sprangletop, and barnyardgrass, respectively. Similarly, plant dry weights of weedy rice, Amazon sprangletop, and barnyardgrass were reduced by 78%, 77%, and 68%, respectively, when benzobicyclon was applied simultaneously to foliage and flood water (Table 1). When benzobicyclon was sprayed on the flood water only, the percent control and biomass reduction accounted for >82% of the total efficacy observed when benzobicyclon was applied simultaneously to flood water and foliage. For example, benzobicyclon applied to foliage

and flood water simultaneously provided 69% control of 6- to 10-cm–tall Amazon sprangletop, whereas the observed control with the flood water–only treatment, although significantly different, was still 59%. In contrast, all benzobicyclon treatments applied to the foliage only, with or without adjuvants, provided <18% visual control of all weed species. However, control of all species with foliage-only treatments containing MSO was either similar to or significantly better than control obtained with foliage-only treatments with no adjuvant. The data clearly illustrate the absorption of benzobicyclon, and presumably benzobicyclon hydrolysate, through flood water is necessary and sufficient to obtain the majority of efficacy from a benzobicyclon application.

#### Experiment 2: Benefits of adjuvants with benzobicyclon

Although little POST activity was observed in Experiment 1 with foliage-only benzobicyclon treatments, the addition of an adjuvant did appear useful. A separate experiment was conducted to explore how the addition of NIS, COC, or MSO to benzobicyclon would improve weed control when applied to foliage and flood water simultaneously. Control of four- to five-leaf Amazon sprangletop and three- to four-leaf barnyardgrass with benzobicyclon alone at 371 g ha$^{-1}$ was 67% and 27%, respectively. Research has shown benzobicyclon has considerably more activity on species of sprangletop than on barnyardgrass, and our data further highlight this point (Komatsubara et al. 2009; McKnight 2017; Young et al. 2018b; CA Sandoski, personal communication). An adjuvant effect was detected in visible control estimates and dry weight reduction of Amazon sprangletop and barnyardgrass (Table 2). For Amazon sprangletop, the addition of NIS improved control to 91%, but COC and MSO were the best adjuvant options, with visual control improved to 99%. A stair-step improvement in barnyardgrass control was observed when an adjuvant was used, compared with benzobicyclon alone (27%). The addition of NIS, COC, and MSO improved barnyardgrass control to 44%, 82%, and 96%, respectively. Overall, the addition of MSO or COC to benzobicyclon provide the greatest level of control and dry weight reduction (>85%).

#### Relationship between herbicide physiochemical properties and efficacy

The translaminar movement and subsequent translocation of agrochemicals is a highly dynamic process. However, in the

**Table 2.** Visual control estimates and dry weight reduction of Amazon sprangletop and barnyardgrass at 28 days after treatment with benzobicyclon applied at 371g ai ha$^{-1}$ to foliage and flood water with or without adjuvants (Experiment 2).

| Treatment | Adjuvant[a,b] | Visual control[c] | | Dry weight reduction | |
|---|---|---|---|---|---|
| | | Amazon sprangletop | Barnyardgrass | Amazon sprangletop | Barnyardgrass |
| | | % | | % | |
| Benzobicyclon + | None | 67 C | 29 D | 69 C | 27 C |
| | NIS | 91 B | 44 C | 83 B | 61 B |
| | COC | 99 A | 82 B | 91 A | 86 A |
| | MSO | 99 A | 96 A | 93 A | 92 A |

[a] Abbreviations: COC, crop oil concentrate; MSO, methylated seed oil; NIS, nonionic surfactant.
[b] COC and MSO were applied at 1% v/v and the NIS was applied at 0.25% v/v.
[c] Mean values within a column, averaged over three trials, were separated using Fisher's protected LSD values at an α of 0.05.

**Table 3.** Physiochemical properties of benzobicyclon and benzobicyclon hydrolysate stated in the literature or derived in silico.

| Characteristic[a] | Benzobicyclon[b] | | Benzobicyclon hydrolysate | |
|---|---|---|---|---|
| | Value | Reference | Value | Reference |
| Water solubility | 0.052 mg L$^{-1}$ (pH 6) | Tomlin (2012) | 146 (pH 7) → to 6,490 (pH 11) mg L$^{-1}$ | Williams et al. (2017) |
| Log $K_{oc}$ | 3.9[b] | Advanced Chemistry Development (2019); Williams et al. (2017) | 3.0 | Williams et al. (2017) |
| Log $K_{ow}$ | 3.6[b] | Advanced Chemistry Development (2019); ChemAxon Ltd (2019); Tomlin (2012) | 1.7[b] | ChemAxon Ltd (2019); Williams et al. (2017) |
| p$K_a$ | Not ionizable | | pH 2.89 | Williams et al. (2017) |
| Log D | ~Log $K_{ow}$ | | −0.9 (pH 6.5) −1.5 (pH 7.4) | ChemAxon Ltd (2019) |

[a] Abbreviations: Log D, log of the octanol/water partitioning coefficient plus log of the acid dissociation constant; Log $K_{ow}$, octanol/water partitioning coefficient; Log $K_{oc}$, soil organic carbon/water partitioning coefficient; p$K_a$, acid dissociation constant.
[b] Average of values from multiple sources.

absence of experimentation with radiolabeled compounds, one can sufficiently predict these characteristics using the log $K_{ow}$ (i.e., lipophilicity [log P]), water solubility, and p$K_a$ values of agrochemicals (Hsu and Kleier 1996; Hsu et al. 1990; Klittich and Ray 2013; Zhang et al. 2018). Empirical or in silico–derived physiochemical properties of benzobicyclon and benzobicyclon hydrolysate were amassed from the literature and used to discuss observed herbicidal activity (Table 3).

Benzobicyclon is a highly lipophilic (log $K_{ow}$), nonpolar compound that is expected to readily penetrate the cuticle and may exhibit xylem, but no phloem mobility (Hsu and Kleier 1996; Hsu et al. 1990; Tomlin 2012; Zhang et al. 2018). In theory, this indicates benzobicyclon, if it had herbicidal properties, would act similar to a contact herbicide. However, negligible HPPD-inhibitor–like activity (i.e., bleaching) was observed in our study with foliar-only treatments, indicating benzobicyclon was not sufficiently converted to the active compound benzobicyclon hydrolysate at the leaf surface or within cellular content. It should be noted, however, that weeds in the MSO-containing treatments did exhibit noticeable necrosis at the leaf tips. In addition, benzobicyclon is predicted to have poor water solubility (0.052 mg L$^{-1}$) and may readily crystalize at the leaf surface, further inhibiting uptake and hydrolytic conversion. In contrast, benzobicyclon hydrolysate is expected to have excellent phloem mobility (ion trapping) (Hsu and Kleier 1996) and has a predicted distribution coefficient (log D; i.e., log $K_{ow}$ plus p$K_a$) value of −1.5 at pH 7.4, which is similar to that of other triketone and pyrazole HPPD-inhibiting herbicides (0.58 > log D > −1.45 at pH 7.4) (Gandy et al. 2015). However, benzobicyclon hydrolysate is ionizable (p$K_a$ 2.89) (Williams

et al. 2017) and will be negatively charged in spray solution and flood water; thus, it will have difficulty crossing the cuticle. In this study, benzobicyclon with or without adjuvants had little activity when spray droplets came in direct contact with leaf foliage, but the addition of an oil-based adjuvant, especially MSO, was necessary to optimize the activity of benzobicyclon when spray droplets came in contact with foliage and flood water.

This phenomenon is difficult to explain, but two schools of thought exist. First, and most obvious, is simply the addition of an oil-based adjuvant improved the penetration and thus diffusion of benzobicyclon across the cuticle. The addition of COC or MSO significantly improves the translaminar movement of lipophilic compounds and formulations (Beckett et al. 1992; Grossmann and Ehrhardt 2007; Miller 2017; Young and Hart 1998). Furthermore, the labels of the HPPD-inhibiting herbicides mesotrione (Anonymous 2011), tembotrione (Anonymous 2016), and topramezone (Anonymous 2017a) recommend COC or MSO as the preferred adjuvant for optimizing activity. However, benzobicyclon is a unique compound because of the slow hydrolytic conversion rate to the herbicidal active compound benzobicyclon hydrolysate (half-life approximately 15 h) (Williams and Tjeerdema 2016), and lack of systemic movement of benzobicyclon probably limits any POST activity via direct leaf uptake.

The second theory is more abstract. Benzobicyclon has poor water solubility and can readily adsorb to soil particles in soils and in flood water (log of the soil organic carbon/water partitioning coefficient approximately 3.9, Table 3). If bound to soil particles, benzobicyclon activity would be reduced because it would be unavailable for plant uptake, and conversion to benzobicyclon

hydrolysate would be significantly hindered. These physicochemical properties may help explain why McKnight (2017) and Norsworthy et al. (2014) found significantly reduced benzobicyclon activity when benzobicyclon was applied to saturated soil versus applied to flood water. Thus, we hypothesize an oil-based adjuvant will help keep the lipophilic compound benzobicyclon in suspension when applied to flood water and, additionally, will increase the probability benzobicyclon is converted to the systemic herbicide benzobicyclon hydrolysate. However, it cannot be ruled out that both theories work together to improve the overall efficacy of benzobicyclon. Regardless, benzobicyclon applications should contain an oil-based adjuvant and must be applied to flooded rice for optimal activity. Additional research is needed to replicate these results in the field and to determine crop safety to benzobicyclon plus MSO.

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