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## Research Article

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# Weed control and rice response from clomazone applied at different timings in a water-seeded system

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**Abstract**

Clomazone is a widely used herbicide in California water-seeded rice for control of bearded sprangletop and watergrass. Generally, clomazone is applied to a flooded rice field at day of rice seeding. However, interest exists among growers to delay the clomazone application. Weather variability may encourage growers to practice Leathers' method. Leathers' method is the practice of draining the field 1 to 2 d after air seeding to encourage better and more uniform seedling establishment, then reflooding back to a 10- to 15-cm flood 4 to 7 d later. Therefore the objective of this study was to evaluate grass weed control and rice response at four rates of clomazone, applied at two timings: at day of seeding (DOS) in a continuous 10-cm flood and after Leathers' method. This study was conducted in 2019 and 2020 at the Rice Experiment Station in Biggs, CA. In 2019, there were no difference across clomazone rates on control of bearded sprangletop independent of application timing used; however, in 2020, bearded sprangletop control with clomazone applied after Leathers' method was 70% to 71% across clomazone rate by 60 d after treatment (DAT), compared to 92% to 97% in the DOS applications. Watergrass control was 100% in 2019 across clomazone rate and application timing. However, in 2020, watergrass control was greater at the DOS application at 54% to 71%. Clomazone applied at the 0.7 kg ha<sup>-1</sup> Leathers' method resulted in 84% bleaching by 14 DAT and was similar across all Leathers' method clomazone applications and the 0.7 kg ha<sup>-1</sup> DOS application. There was no rice grain yield difference among all clomazone-treated plots, with the exception of the 0.7 kg ha<sup>-1</sup> Leathers' method interaction with the DOS applications.

**Introduction**

The majority of California's rice production is in the Sacramento Valley on approximately 200,000 ha. Medium-grain varieties make up more than 90% of the cultivated rice hectares (Cal Rice 2020). The rice crop reached a value of nearly US\$900 million in 2019, making it a major agricultural commodity in California (CDFA 2020). Growers in California primarily plant rice in a water-seeded system, where rice is pre-germinated and air-seeded onto fields with a 10- to 15-cm standing flood. The water-seeded production system was first introduced for weed suppression and established an efficient agronomic method that led to overall high rice productivity with environmentally benign approaches in preserving water quality and wildlife habitat (Hill et al. 2006).

Bearded sprangletop is a semiaquatic annual grass commonly found in seasonal wetland areas (Altop et al. 2015). Both water-seeded and dry-seeded rice fields are excellent environments for this grass species (Driver et al. 2020a; Smith 1983). Bearded sprangletop, barnyard-grass [*Echinochloa crus-galli* (L.) P. Beauv.], early watergrass [*Echinochloa oryzoides* (Ard.) Frisch], and late watergrass [*Echinochloa phyllopogon* (Stapf) Koso-Pol.] are weedy grasses of the California rice agroecosystem that have adapted well to the permanently flooded cropping conditions, making these grasses particularly problematic weeds. When compared to broad-leaves and sedges, weedy grasses are the major predictors of yield loss in California rice fields (Brim-DeForest et al. 2017). Bearded sprangletop and watergrasses have shown to reduce rice yields up to 59% in unmanaged fields (Smith 1983; Gibson et al. 2002).

Herbicides continue to be a major tool for weed management in rice (Gibson et al. 2002; Hill et al. 2006). Unfortunately, the majority of herbicides that control watergrass are not effective on bearded sprangletop (Osca 2013). Currently California water-seeded rice growers have only four herbicides labeled for bearded sprangletop control, which include clomazone, thiobencarb, benzobicyclon, and cyhalofop-butyl, of which only cyhalofop-butyl is a postemergence herbicide (Driver et al. 2020b). A survey conducted by the University of California has recorded resistance to thiobencarb and cyhalofop-butyl in several bearded sprangletop populations (UC Rice 2016). Driver et al. (2020b) reported that bearded sprangletop resistance to clomazone in California

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rice fields is minimal, indicating the potential for continued use of clomazone to control bearded sprangletop. The authors suggested that growers' complaints about lack of bearded sprangletop control with clomazone may be attributed to bearded sprangletop escaping the clomazone application due to later weed emergence or mistimed application, demonstrating a need to study the effects of clomazone application timings in the water-seeded system.

Clomazone has been an important herbicide for control of bearded sprangletop and watergrass in California rice (Driver et al. 2020b). A microencapsulated granule of clomazone is specifically formulated for flooded California rice culture; the herbicide label specifies a day of seeding (DOS) application to a flooded rice field and a 14-d water-holding period (Anonymous 2003).

Clomazone is a pro-herbicide that metabolizes to the active herbicide 5-keto clomazone in susceptible plants, causing bleaching, a white appearance on the leaves, by inhibiting the formation of photosynthetic pigments. The 5-keto clomazone disrupts the enzyme 1-deoxy-D-xylulose-5-phosphate synthase at the first step of isoprenoid synthesis in the greater carotenoid synthesis pathway (Ferhatoglu and Barrett 2006). Carotenoids play a role in protecting the chlorophyll from photooxidation; when inhibited, degradation of chlorophyll and membranes occurs from singlet oxygen and triplet chlorophyll, leading to eventual plant death (Hess 2000). Clomazone can also cause bleaching on rice but does not result in reduced grain yields when used according to the manufacturer's label (Jordan et al. 1998; Zhang et al. 2005).

Several California rice growers practice an early drain for increased stand establishment in the water-seeded system, called Leathers' method (Williams et al. 1990; Brim-DeForest et al. 2017). Leathers' method is the practice of completely draining the field 1 to 2 d after air seeding, then reflooding back to 10 to 15 cm after 4 to 7 d (Williams et al. 1990). This method can be helpful when there is poor seedling establishment due to weather, windborne seedling drift, or predation. Rice root development is encouraged with Leathers' method by providing an aerobic environment for the rice seedlings, but the method also encourages greater weed pressure because weed seeds can germinate more easily in that environment. If practicing Leathers' method, a clomazone application is made after the drain to allow for the proper water-holding period. However, research to examine the activity of clomazone application timing in water-seeded rice is lacking. Therefore the objective of this research was to study the grass weed and rice response to clomazone applied at DOS and after Leathers' method.

## Materials and Methods

The study was conducted in the 2019 and 2020 growing seasons at the Rice Experiment Station in Biggs, CA (39.46°N, 121.74°W). Soils at the study site are characterized as Esquon-Neerdobe (fine, smectitic, thermic Xeric Epiaquerts and Duraquerts), silty clay with a pH of 5.1 and 2.8% organic matter. In both years, a pass with a single offset stubble disk was done after the previous year's harvest, then the field was flooded to 10 cm during the winter months and drained in early spring. Field preparation in spring consisted of one pass with a chisel plow and two passes with a single-offset disk, followed by a land plane to smooth the soil surface. Then a corrugated roller was used to pack the soil and eliminate large clods on the soil surface. Seeds of rice cultivar 'M-206' were pre-germinated by placing the seeds in steel bins and filling with water until all seeds were covered. A 5% sodium hypochlorite solution was added to the water for the first hour for disease control, then the water was

drained and bins refilled with only water for the remaining 24 h. The seed was then drained until dry for up to 5 h and seeded by aircraft at a rate of 135 kg ha<sup>-1</sup> onto the field with 10-cm standing water. Standard agronomic and pest management practices were followed based on the University of California rice production guidelines (UCANR 2018). Seeding dates were June 13, 2019, and May 28, 2020, respectively.

The experimental design was a split-plot design, where application timing was the main plot and the herbicide rates were the sub-plots. The field was split by a 6-m-wide levee into two sections, to control water management in the main plots; on one side, the flood was continuously present, and on the other side, Leathers' method was implemented. On each side, there were four replications of the herbicide rates set as randomized complete blocks. Each plot was surrounded by 2.2-m-wide levees to prevent contamination from adjacent treatments; plot sizes were 3 m wide × 6 m long.

Clomazone (CERANO<sup>®</sup> 5 MEG, Wilbur-Ellis Co. LLC, Fresno, CA, USA) was broadcast by hand at 0, 0.5, 0.6, and 0.7 kg ai ha<sup>-1</sup> at two application timings. The clomazone timings for the main plots were day of seeding (DOS) in continuous flood plots and 7 d after seeding (DAS) in Leathers' method plots. In Leathers' method, the water was lowered to the soil level 2 DAS, and plots were reflooded to 10 cm after 5 d. Additional herbicides were applied later in the season for broadleaf and sedge weed control in treated plots, which included smallflower umbrella sedge (*Cyperus difformis* L.), ricefield bulrush [*Schoenoplectus mucronatus* (L.) Palla], duck salad [*Heteranthera limosa* (Sw.) Willd.], and redstem (*Ammannia* spp.). The treatments included penoxsulam (Granite<sup>®</sup> GR, Corteva Agriscience, Wilmington, DE, USA) applied at 35 g ai ha<sup>-1</sup> at 23 DAS and 25 DAS in 2019 and 2020, respectively, followed by an herbicide mixture of propanil (SUPERWHAM!<sup>®</sup> CA, RiceCo LLC, Memphis, TN, USA) applied at 4,500 g ai ha<sup>-1</sup> and triclopyr (Grandstand<sup>®</sup> CA, Corteva Agriscience) applied at 420 g ai ha<sup>-1</sup> at 38 DAS and 30 DAS in 2019 and 2020, respectively.

Visual ratings for bearded sprangletop and watergrass species control were conducted at 14, 28, 40, and 60 d after treatment (DAT) and were based on a percentage scale, ranging from 0 (no control) to 100 (full control or no weeds observed in the plot). Watergrass species were grouped together for ease of identification; however, barnyardgrass, early watergrass, and late watergrass were commonly found in the study field. Visual rice injury ratings were conducted 14 and 60 DAT for bleaching injury on a scale ranging from 0 (no injury) to 100 (plant death). Plant height was recorded at 110 DAT by measuring from the soil to the extended panicle. In 2019, grain yield was harvested with a specialized small-plot combine (SPC40, Almaco, Nevada, IA, USA), and in 2020, grain yield was hand-harvested from two 1 m<sup>2</sup> quadrats in each plot and mechanically threshed (Large Vogel Plot Thresher, Almaco). Rice grain yield for both years was adjusted to 14% moisture.

Data were analyzed with analysis of variance, and means were separated using Tukey's honestly significant difference at a significance level of  $\alpha = 0.05$  using R (R Development Core Team 2021). The LME4 (Bate et al. 2015) and LMERTEST (Kuznetsova et al. 2017) packages were used to fit the linear models. The EMMEANS package (Lenth 2020) was used to estimate marginal means along with the MULTCOMP package to generate multiple comparisons among means (Hothorn et al. 2008). Treatment × year interactions were observed for the weed control data; therefore these data were analyzed and presented individually by year. Weed control data were analyzed with the herbicide rates, application timings, and

**Table 1.** Weed control from clomazone applied at day of seeding and after Leathers' method in water-seeded rice in 2019 and 2020.<sup>a,b,c</sup>

Application timing	Clomazone rate	2019						2020					
		Bearded sprangletop			Watergrasses			Bearded sprangletop			Watergrasses		
		28 DAT	40 DAT	60 DAT	14 DAT	40 DAT	60 DAT	28 DAT	40 DAT	60 DAT	14 DAT	40 DAT	60 DAT
	kg ha <sup>-1</sup>							%					
Day of seeding	0.5	100 ab	100 a	100 a	81 b	100 a	100 a	93 a	93 ab	95 a	71 ab	36 b	54 ab
	0.6	100 ab	100 a	100 a	90 ab	100 a	100 a	91 a	95 a	97 a	81 a	69 a	69 a
	0.7	100 ab	100 a	100 a	86 ab	100 a	100 a	95 a	95 a	92 a	81 a	70 a	71 a
Leathers' method	0.5	100 ab	100 a	100 a	79 b	100 a	100 a	70 b	80 b	71 b	40 c	25 b	21 c
	0.6	99 b	99 a	100 a	81 b	100 a	100 a	76 b	85 ab	71 b	50 bc	30 b	24 bc
	0.7	100 ab	100 a	100 a	95 a	100 a	100 a	75 b	86 ab	70 b	49 bc	33 b	24 bc

<sup>a</sup>Abbreviation: DAT, days after treatment.

<sup>b</sup>Floodwater was lowered to the soil level 2 d after seeding and reflooded to 10 cm 5 d later, followed by the clomazone applications.

<sup>c</sup>Means accompanied by the same letter in each column do not significantly differ with Tukey's at  $\alpha = 0.05$ .

evaluations as fixed factors and replication as a random factor. There was no significant treatment  $\times$  year interaction for rice injury and yield data; therefore the two years' data were combined. The herbicide rates and application timing were considered fixed factors, while year and replication were considered random factors.

## Results and Discussion

### Weed Control

Weed community composition varied each year; in 2019, a lower watergrass pressure was present than in 2020. In 2019, the nontreated control plots had a relatively low abundance of watergrass cover averaging 10%, while the sedges and broadleaves had on average 70% relative cover by 28 DAT. In 2020, the nontreated control plots had a much greater relative abundance of watergrass cover, averaging as 76%, while the sedges and broadleaves had on average 30% by 28 DAT (data not shown). These differences are not surprising; Brim-DeForest et al. (2017) observed similar seasonal variation in weed population composition. The authors recorded a higher proportion of broadleaves in 2013 and a higher proportion of watergrass in 2014 in the same California rice field. Similarly, Lundy et al. (2014) observed annual variations in weed composition due to differences in temperature and soil moisture at the start of the growing season.

In 2019, there was no differences across the clomazone rate and application timing for bearded sprangletop control. Control was 100% by 60 DAT across all treated levels and application timings; however, in 2020, interaction was observed across application timings (Table 1). The applications after Leathers' method decreased bearded sprangletop control to 70% across all clomazone treatments by 60 DAT in 2020, compared to 92% to 97% at the DOS applications that year (Table 1). Osca (2013) found that a shallow flood depth early in the season favors a greater bearded sprangletop emergence, which is consistent with the findings from this study in 2020. Leathers' method may have encouraged more rapid bearded sprangletop growth by the time of the application, leading to decreased control. Altop et al. (2015) reported that bearded sprangletop emergence was greater in continuous flood conditions. Altop et al. concluded that various Turkish populations of bearded sprangletop had adapted to flooded conditions due to reduced

dormancy and greater rates of germination. Similarly, Driver et al. (2020a) found that California bearded sprangletop biotypes resistant to clomazone may have a fitness advantage, as they were able to emerge successfully from 20-cm flood depth, unlike susceptible biotypes, which were suppressed at a 5-cm flood depth. Driver et al. suggest that to achieve greater overall suppression of bearded sprangletop, a 7- to 13-cm continuous flood is recommended. The results of this study are inconclusive in suggesting which application timing provides greater bearded sprangletop control due to differences each year, but it provides evidence to suggest that emergence time and quantity can fluctuate from year to year. The lower control levels observed in 2020 may be due to the greater observed pressure of bearded sprangletop, as well as its ability to emerge later than other grasses (Driver et al. 2019). Clomazone rate appears to have no effect in control of bearded sprangletop (Table 1). Similarly, Driver et al. (2020b) found that susceptible bearded sprangletop biotypes were fully controlled at clomazone rates as low as 0.2 kg ha<sup>-1</sup>.

In 2019, there was interaction across clomazone rate and application timing for watergrass control at 14 DAT only; by 40 DAT, watergrass species were not observed in the majority of all treated plots (Table 1). With lower grass pressure in 2019, the watergrass present may have been outcompeted by the rice plants, resulting in much greater control with the combination of the clomazone treatment and crop cover (Gibson et al. 2002). Caton et al. (1998) used a model that demonstrated that later planting dates can lead to greater rice stand densities and assist with weed suppression. In the present study, rice was seeded 17 d later in 2019 when compared with 2020.

In 2020, an interaction occurred for watergrass control across application timing, but no interaction across clomazone rate was observed (Table 1). Zhang et al. (2005) observed increases in controlling barnyardgrass with increased clomazone rates from 0.2 to 1.1 kg ha<sup>-1</sup> in a drill-seeded system; however, Webster et al. (1999) observed that barnyardgrass control was not dependent on the clomazone rate or application timing in a drill-seeded flooded culture. The Leathers' method applications resulted in 21% to 24% watergrass control, compared to 54% to 71% at the DOS applications (Table 1). Williams et al. (1990) and Brim-DeForest et al. (2017) recorded a higher watergrass composition later in the season in fields where Leathers' method was implemented, suggesting reduced watergrass control when Leathers' method is applied.

**Table 2.** Rice injury as affected by clomazone applied at four rates at day of seeding and after Leathers' method in water-seeded rice in 2019 and 2020.<sup>a,b,c,d</sup>

Application timing	Clomazone rate kg ha <sup>-1</sup>	Bleaching	
		14 DAT	40 DAT
		%	
Day of seeding	0.0	0 d	0 a
	0.5	20 cd	0 a
	0.6	42 bc	0 a
	0.7	66 ab	0 a
Leathers' method	0.0	0 d	0 a
	0.5	62 ab	0 a
	0.6	57 ab	0 a
	0.7	84 a	0 a

<sup>a</sup>Abbreviation: DAT, days after treatment.

<sup>b</sup>Floodwater was lowered to the soil level 2 d after seeding and reflooded to 10 cm 5 d later, followed by the clomazone applications.

<sup>c</sup>Means accompanied by the same letter in each column do not significantly differ with Tukey's at  $\alpha = 0.05$ .

<sup>d</sup>Data are averaged over both study years.

### Rice Response

Rice injury was evident with all clomazone treatments by 14 DAT. There was interaction across clomazone rate and application timing. The high rate of 0.7 kg ha<sup>-1</sup> demonstrated greater bleaching when compared to the low rate of 0.5 kg ha<sup>-1</sup> at the DOS application, but this was not observed with the Leathers' method application. The 0.5 kg ha<sup>-1</sup> rate at the Leathers' method application was no different than the 0.7 kg ha<sup>-1</sup> rate at the DOS application (Table 2). Zhang et al. (2005) and Bollich et al. (2000) also reported significant rice bleaching at higher clomazone rates. Zhang et al. (2005) reported that clomazone applied as a delayed preemergence treatment at 4 d after a surface irrigation caused greater rice injury than with clomazone applied as a preemergence in drill-seeded rice. Jordan et al. (1998) also observed more rice injury from clomazone applied in a drill-seeded system than in a water-seeded system. The authors attributed this response to a higher absorption of clomazone by the drill-seeded rice when water was introduced 24 h after clomazone application to promote rice germination, whereas in the water-seeded system, the rice had reduced absorption of clomazone due to the continuous flood. In agreement, Bollich et al. (2000) observed greater bleaching in drill-seeded rice than in water-seeded rice and suggested that it was due to a greater concentration of clomazone in the water, which caused the rice to germinate in the drill-seeded system. In this study, rice plants may have imbibed greater concentrations of herbicide because of the lower flood levels in Leathers' method, leading to greater injury.

By 40 DAT, no bleaching was observed in any plots. Zhang et al. (2005) reported similar decline in bleaching symptoms. In addition, Webster et al. (1999) observed no bleaching symptoms approximately 20 d after clomazone application. Rice absorbs similar amounts of clomazone as susceptible grass species but does not readily metabolize the clomazone molecule to its active form, leading to greater ability to recover from clomazone symptoms (Tenbrook and Tjeerdema 2006). Consequently, there were no differences in rice plant heights among treatments (data not shown).

Combined-year yields demonstrated interaction across clomazone rate and application timing. Rice treated with clomazone at DOS had greater yields than the nontreated control, while rice treated with clomazone after Leathers' method did

**Table 3.** Rice grain yield as affected by clomazone applied at four rates at day of seeding and after Leathers' method in water-seeded rice in 2019 and 2020.<sup>a,b,c</sup>

Application timing	Clomazone rate kg ha <sup>-1</sup>	Yield
Day of seeding	0.0	2,620 b
	0.5	5,710 a
	0.6	5,910 a
	0.7	6,030 a
Leathers' method	0.0	2,500 b
	0.5	4,050 ab
	0.6	4,130 ab
	0.7	3,440 b

<sup>a</sup>Floodwater was lowered to the soil level 2 d after seeding and reflooded to 10 cm 5 d later, followed by the clomazone applications.

<sup>b</sup>Means accompanied by the same letter in each column do not significantly differ with Tukey's at  $\alpha = 0.05$ .

<sup>c</sup>Data are averaged over both study years.

not yield greater than the nontreated control. Still, all treated plots in this study observed no differences in yield, except from the interaction of the 0.7 kg ha<sup>-1</sup> rate at the Leathers' method application with the DOS applications (Table 3). An increased weed pressure can be encouraged with Leathers' method and attributed to some of the yield reduction (Brim-DeForest et al. 2017). For the most part, the results of this study coincide with the results of Jordan et al. (1998), Webster et al. (1999), Mudge et al. (2005), and Zhang et al. (2005), which demonstrated that rice grain yield was not negatively impacted by clomazone application. Previous research has demonstrated rice varieties to have differential tolerance to clomazone. Long-grain varieties appear to be more clomazone tolerant, whereas medium-grain varieties obtain greater injury, but both varieties maintain their yield potentials (Zhang et al. 2004). However, Bollich et al. (2000) recorded a potential reduction in yield from a long-grain variety when clomazone rates were 0.8 to 2.2 kg ha<sup>-1</sup>. Short-grain varieties were shown to suffer yield reduction after a clomazone application (Mudge et al. 2005). The results from this study demonstrate the potential for yield reduction in medium-grain rice with a clomazone rate of 0.7 kg ha<sup>-1</sup>, if applied after use of Leathers' method.

In conclusion, neither clomazone application timing provided greater grass weed control. Weed community composition can differ significantly year to year, and use of Leathers' method may encourage an increase in grass weed pressure (Brim-DeForest et al. 2017). Nevertheless, Leathers' method remains useful to some growers to encourage rice seedling establishment, but it may not be appropriate in extremely weedy fields. Rice bleaching is evident with increasing clomazone rates and was observed with greater severity after Leathers' method yet largely did not impact rice grain yield. The 0.7 kg ha<sup>-1</sup> rate of clomazone is still important to use to avoid greater development of clomazone-resistant weeds.

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