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Clomazone; bearded sprangletop, *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. ssp. *fascicularis* (Lam.) P. M. Peterson & N. Snow; rice, *Oryza sativa*

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Bearded sprangletop (*Diplachne fusca* ssp. *fascicularis*) flooding tolerance in California rice

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Abstract

Bearded sprangletop is a problematic weed in California rice production. The objective of this research was to determine the response of two bearded sprangletop biotypes (clomazone-susceptible [S] and -resistant [R]) to flooding depth. A study was conducted in 2017 and 2018 at the California Rice Experiment Station in Biggs, CA, to evaluate the flooding tolerance of the two biotypes against 5-, 10-, and 20-cm continuous flooding depths. Plant emergence, plant height, panicles per plant, seed per panicle, 100-seed weight, and seed per plant data were collected. At the 5-cm flood depth, neither biotype was controlled, and the R biotype had 260% more emergence, produced 475% more panicles per plant, and 455% more seed per plant than the S biotype. With a 10-cm flood, only the R biotype survived flooding and produced more panicles per plant and seed per plant than any other flood depth–biotype combination evaluated. There was no emergence of either bearded sprangletop biotype at the 20-cm flood depth. Continuous flooding can still be used as a management tool to control bearded sprangletop; however, the depth of flooding appears to limit emergence of S biotypes at 5 cm and R biotypes at 10 cm, and completely inhibits growth of both biotypes at 20 cm. The results of this study indicate that clomazone-resistant bearded sprangletop is more likely to spread throughout the Sacramento Valley because this biotype can survive clomazone applications and can tolerate a standard 10-cm flood.

Introduction

Continuous flooded rice systems suppress many weed species and are commonly used in California rice production for this reason (Adair and Engler 1955; Chauhan and Johnson 2008). In California, rice is grown on approximately 200,000 ha, with water-seeded rice being the primary establishment practice since the late 1920s (Adair and Engler 1955; Pittelkow et al. 2012). Rice growers in California flood rice fields at the beginning of the growing season and pregerminated rice seed is direct seeded onto the flooded field by airplane. A flood depth of 10 to 15 cm is maintained throughout the growing season. The continuous monoculture cropping of rice has resulted in a proliferation of highly competitive weeds that are adapted to aquatic environments (Bayer et al. 1985; Brim-DeForest et al. 2017; Fischer et al. 2000). Because mechanical weed control is difficult in the flooded rice system, growers heavily depend on herbicides for weed control.

Repeated use of herbicides and flood irrigation in the California rice agroecosystem has selected for weed species that are well adapted to the system (Brim-DeForest et al. 2017). In general, grasses are considered the most difficult weeds to control because of the narrow selectivity between the grass crop and grass weeds (Carey et al. 1992). Many grass weed species are problematic in California rice production, including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], late watergrass [*E. oryzicola* (Vasinger) Vasinger], early watergrass [*E. oryzoides* (Ard.) Fritsch], and bearded sprangletop.

Bearded sprangletop is a tufted, semiaquatic grass native to California. Bearded sprangletop is a prolific seed producer (McCarty et al. 1995) and is a common weed in dry-seeded and water-seeded rice systems where the flood water level has been allowed to recede (Altop et al. 2015; Brim-DeForest et al. 2015; Hall 1978). Although other species like weedy (or red) rice (*Oryza sativa* L.) and various *Echinochloa* species are more serious competitors than bearded sprangletop, rice yield can be reduced by as much as 36% when bearded sprangletop is not controlled (Smith 1983).

Herbicide resistance in bearded sprangletop has been documented around the world (Brim-DeForest et al. 2015; Yuan et al 2019). Recently, bearded sprangletop biotypes in California were confirmed resistant to cyhalofop (Brim-DeForest et al. 2015), quizalofop (Brim-DeForest et al. 2015), thiobencarb (Brim-DeForest et al. 2015), and clomazone (Driver 2019). Because there are relatively few herbicides available that can control this species, identifying additional control methods is important to the rice industry.

Continuous flooding in rice fields has long been thought to suppress and control germination and emergence of weeds in California rice (UCANR 2012); however, anecdotal evidence from California rice producers (A. Fischer, personal communication) in recent years suggests that bearded sprangletop biotypes can establish under flood conditions. In addition, few biologic data have been published about bearded sprangletop biotypes in California, and the depth of water needed to effectively suppress or control bearded sprangletop is not clear. Altop et al. (2015) reported that bearded sprangletop biotypes in Turkey have adapted to flooded conditions through increased germination and reduced seed dormancy. Other weedy grasses, such as early and late watergrass, that occur in rice production fields have also been reported to have flooding tolerance, and control of these weeds has been supplemented by herbicide applications (Fischer et al. 2000). Therefore, the objectives for the present study were to determine the response of two bearded sprangletop biotypes to flood management and assess what effect the flooding depth in California rice has on these biotypes.

Materials and Methods

Plant Material

Seeds of two bearded sprangletop biotypes were used in this study and were collected in 2015 from rice fields in Butte (39.451185°N, 121.717131°W) and Sutter (38.938236°N, 121.808890°W) Counties in the Northern Sacramento Valley in California. Plants from the F2 generation of field-collected samples (resistant [R] biotype) and a previously characterized susceptible bearded sprangletop biotype (S biotype) from California (Driver 2019) were used in this research. The sample collected from Sutter county was resistant to clomazone. The R biotype has a GR_{50} (i.e., herbicide dose required to cause a 50% reduction in plant growth) of 3,365 g ai ha^{-1} (with a resistance index of 5-fold compared with the S biotype) (Driver 2019).

To eliminate the possibility of contamination of resistant with susceptible plants, seeds were germinated by wet chilling seed for 2 wk and then placed in a germination oven as described by Driver (2019). Then germinated seeds were transplanted to pots filled with field soil (Yolo clay loam: fine-silty, mixed, nonacid, thermic Typic Xerorthents, 1.7% organic matter). Plants were treated with clomazone at 360 g ai ha^{-1} and grown to maturity; seeds were harvested and bulked. The average daily temperature in the greenhouse was 28 C ± 5 C and the photoperiod was 16 h, supplemented by high-pressure sodium lamps yielding approximately 400 µmol m^{-2} sec^{-1} photosynthetic photon flux.

Field Study

A field trial was conducted in 2017 and 2018 at the California Rice Experiment Station in Biggs, CA (39.46°N, 121.74°W). Soils there are classified as Esquon-Neerdobe (fine, smectitic, thermic Xeric Epiaquerts and Duraquerts). Soil characteristics in the 0- to 15-cm profile include pH of 5.1, electrical conductivity of 0.35 dS m^{-1}, CEC of 32.6 cmol kg^{-1}, and 2.8% organic matter. The average minimum and maximum daily temperatures in Butte County during the growing season (May to October) in 2017 were 13.9 C and 30.9 C and in 2018 were 13.0 C and 31.5 C, respectively.

The experiment was of a randomized complete block design with four replications. A factorial treatment structure was used with factor 1 consisting of bearded sprangletop biotype and factor 2 being water depth. Polyvinyl chloride (PVC) pipe with a 78-cm diameter was used to control water depth by being placed 10 cm into the ground to seal the inside of the ring formed by the pipe. Inside each PVC ring, R or S bearded sprangletop biotypes (300 seeds $ring^{-1}$) were planted in marked rows. Rice was seeded into the plots at a rate of 80 kg ha^{-1} using the rice variety 'M206.' Rice was broadcast seeded into the plots on June 8 and June 2 in 2017 and 2018, respectively. Other pest and fertility management was conducted according to University of California recommendations (UCANR 2012). Continuous, season-long floods were established after planting at depths of 5, 10, or 20 cm. Bearded sprangletop emergence was monitored throughout the season.

To collect seed, bearded sprangletop panicles were bagged at maturity with 7.6 cm by 13.9 cm glassine bags and secured. Plant height, tiller number, panicle number, and seed production $plant^{-1}$ were determined. Data were subjected to ANOVA and means were separated using the Tukey honestly significant difference test. Statistical analysis was performed using R software (https://www.r-project.org/).

Results and Discussion

The data for the experiment were averaged across 2 yr because the year-by-treatment interaction was not significant; however, the interaction of bearded sprangletop biotype by water depth was significant. At 5-cm continuous flood, neither bearded sprangletop biotype was controlled; however, the R biotype had 260% greater emergence than the S biotype (Table 1). At 10-cm continuous flood, the S biotype was controlled, but 20% of the R biotype successfully emerged. Both bearded sprangletop biotypes evaluated in this study were controlled with a 20-cm continuous flood. In addition, rice plants were able to grow through the 20-cm flood (data not shown).

There was no difference in height between R and S biotypes at 5-cm flood depth. However, the R biotype at 10-cm flood depth had a 53% reduction in height when compared with all other flood depths (Table 1). Panicle $plant^{-1}$ production in the R biotype did not differ between 5- and 10-cm flood depths. In addition, at all flood depths evaluated, the R biotype produced more panicles $plant^{-1}$ than did the S biotype (Table 1). The R biotype produced 475% more panicles than did the S biotype at 5-cm flood depth and 425% more panicles $plant^{-1}$ at the 10-cm flood depth (Table 1).

At 5-cm flood depth, there was no difference in 100-seed weight between the S and R biotypes. However, the R biotype under a 10-cm flood produced 182% higher 100-seed weight than did the S biotype at 5 cm and 163% higher 100-seed weight when compared with the R biotype at 5-cm flood depth (Table 1). At 5-cm flood depth, the S biotype produced 455% more seed $panicle^{-1}$ than the R biotype did and 352% more seed $panicle^{-1}$ than did the R biotype at 10-cm flood depth (Table 1). However, R biotype produced more seed $plant^{-1}$ at 5- and 10-cm flood depths, due to the increase in panicles produced per plant.

This research indicated that increased water depth in rice fields may result in total or partial suppression of bearded sprangletop emergence. However, clomazone-resistant plants were able to emerge through deeper water compared with susceptible plants. These results suggest there is a fitness advantage to clomazone-resistant bearded sprangletop survival under a standard 10-cm flood, indicating that this biotype may be able to outcompete S biotypes and spread faster. In similar studies conducted with other rice weeds, a 4-cm flooding depth completely inhibited the emergence of growth of smallflower umbrella sedge (*Cyperus difformis* L.) and a 10-cm flood was sufficient to control 97% of

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Table 1. Bearded sprangletop characteristics affected by flooding depth.[a]

Biotype	Flood depth	Panicle[b]		Seed		100-Seed weight		Total weed[c]		Emergence		Plant height	
	cm	—plant^{-1}—		—panicle^{-1}—		—mg—		—seeds m^{-2}—		—%—		—cm—	
S	5	4	b	355	a	17	b	1,211	b	15	b	224	a
R	5	19	a	78	b	19	b	1,429	ab	54	a	239	a
S	10	–	–	–	–	–	–	–	–	–	–	–	–
R	10	17	a	101	b	31	a	1,711	a	17	b	105	b
S	20	–	–	–	–	–	–	–	–	–	–	–	–
R	20	–	–	–	–	–	–	–	–	–	–	–	–

[a]Data are averaged over 2 yr of the field experiment conducted at the California Rice Experiment station in 2017 and 2018.
[b]Within columns, means accompanied by the same letter do not differ according to Tukey honestly significant difference test with P = 0.05.
[c]Total seed produced per plant.

globe fringerush (*Fimbristylis littoralis* Gaudich.) (Chauhan and Johnson 2008). The University of California weed control guidelines recommend a minimum continuous flood of at least 7 cm to achieve satisfactory weed control for most rice weeds and a depth greater than 13 cm to control various *Echinochloa* species (UCANR 2012). Although control of bearded sprangletop was achieved in this study with a 20-cm flood, it is not feasible for that depth of flooding to occur on a large production scale across the Sacramento Valley. A flood depth of 20 cm would be limited by water availability and cost, the cropping system water use efficiency would decline, and the time increase to establish the flood would make management decisions more challenging while limiting the herbicides available at that water depth.

Numerous weeds that have adapted to a continuously flooded system have done so through tolerance of an anoxic environment (Benvenuti et al. 2004, Estioko et al. 2014; Kennedy et al. 1980). Various *Echinochloa* species (Chauhan and Johnson 2011; Fox et al. 1995; Fukao et al. 2003; Ismail et al. 2012; Pearce and Jackson 1991, 1992; Rumpho and Kennedy 1981; Zhang et al. 1994), Chinese sprangletop [*Dinebra chinensis* (L.) P. M. Peterson & N. Snow] (Benvenuti et al. 2004), and bearded sprangletop (Altop et al. 2015) have all been documented to germinate in completely anoxic environments. Anoxic environments are found in flooded rice fields when a continuous flood is established; however, repeated monoculture rice cropping can select for biotypes tolerant to this environment. Altop et al. (2015) reported that highly mechanized and flooded rice cultivation systems without crop rotation are likely to result in more severe bearded sprangletop infestations and select for biotypes tolerant to flooding.

Developed adaptation mechanisms for tolerating an anoxic environment is related to better tolerating oxidative stress (Kennedy et al. 1980; Pearce and Jackson 1991, 1992; Rumpho and Kennedy 1981; VanderZee and Kennedy 1981). Some of the species reported to be tolerant to flooding also are resistant to herbicides via developing a more efficient oxidative stress pathway (Fischer et al. 2000; Osuna et al. 2002; Ruiz-Santaella et al. 2006; Yasuor et al. 2008, 2010; Yun et al. 2005). To our knowledge, the most recent example of this type of herbicide resistance is the R biotype of bearded sprangletop used in this study. This biotype metabolizes clomazone via P450 oxidation to less toxic metabolites (Driver 2019). Many of the *Echinochloa* species in California rice also have this mechanism of resistance to various herbicides (Yasuor et al. 2008, 2010). Thus, it is likely that the increased efficiency of dealing with herbicide-induced oxidative stress also makes these weeds better suited to tolerating anoxic environments.

We found bearded sprangletop has adapted to flooded conditions common in most California continuously flooded rice fields. The depth of flooding appears to limit emergence of S biotypes at 5 cm and of R biotypes at 20 cm. The results of this study indicate that clomazone-resistant bearded sprangletop is more likely to spread throughout the Sacramento Valley because plants can tolerate a standard flood and survive applications of the commonly used herbicide. It is evident from this study and other research that controlling bearded sprangletop biotypes will require the use of integrated weed management. It is suggested that California rice growers rotate crops if possible, rotate herbicide modes of action, use weed-free rice seed, and increase flood levels when possible to achieve effective management of bearded sprangletop. Although a direct relationship between flooding tolerance in an anoxic environment and herbicide resistance mechanisms involving oxidative stress efficiency still needs to be established, the present results suggest research on mechanisms to mitigate oxidative stress in bearded sprangletop would be useful.

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