Title
Identifying biological limitations of early season phenological stages of weedy rice (Oryza sativa f. spontanea Roshev.) and implications for management in California rice cropping systems

Author
Galvin, Liberty Baker

Publication Date
2022

Peer reviewed|Thesis/dissertation

Identifying biological limitations of early season phenological stages of weedy rice (*Oryza sativa* f. *spontanea* Roshev.) and implications for management in California rice cropping systems

By

LIBERTY BAKER GALVIN
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Horticulture and Agronomy

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

____
Kassim Al-Khatib, Chair

____
Bradley D. Hanson

____
Cameron Pittelkow

Committee in Charge

2022

Liberty Baker Galvin

September 2022

Horticulture and Agronomy, Weed Science

Identifying biological limitations of early season phenological stages of weedy rice (*Oryza sativa* f. *spontanea* Roshev.) and implications for management in California rice cropping systems

Abstract

California weedy rice accessions (*Oryza sativa* f. *spontanea* Roshev.) were new pests as of 2016 with limited documentation of what influenced their phenological development. It is imperative to control weed species early in their life cycle to limit competition with crop plants, however, weedy rice is visibly undetectable before panicle development in rice fields due to the phenotypic similarities between these *Oryza sativa* relatives. Thus, research was needed to determine if viable weedy rice control strategies from other regions, e.g., deep flooding, would be applicable to California accessions. We hypothesized that California weedy rice accessions 1, 2, 3, 4, and 5 would behave in unique ways compared to other rice growing regions of the world due to the wet-direct seeding techniques, continuous annual flooding, and monoculture-rice agronomic practices common in California. Greenhouse, field, and laboratory studies were conducted to study early-season developmental stages of California weedy rice accessions (i.e., germination, emergence), and how these phases influence the efficacy of weed management methodologies. One management option is the stale seedbed methodology, which is dependent on maximizing seed germination of the target weed

species. California weedy rice seed as well as M-206, a common rice cultivar used in California, were germinated under a range of temperature and moisture conditions. Results demonstrate that seeds are greatly influenced by moisture availability and are unlikely to germinate to the maximum extent in the presence of moisture stress. Many weedy, wild, and cultivated *Oryza sativa* seeds have a lower germination threshold temperature of 10°C; accession 5 seeds have a similar lower-threshold temperature, while accession 1, 2, and 3 have a lower germination threshold of 15°C. All weedy accessions have higher germination threshold temperature compared with M-206. Controlled seedling emergence studies verified our assumptions that California weedy rice accessions are likely well-adapted to flooded conditions. Weedy rice seedlings buried in the soil at either 1.3 or 2.5 cm were able to successfully emerge through the soil as well as 15 cm of flood waters. Unlike accessions from regions where drill-seeding or hand transplanting methods are utilized, California weedy rice seedlings are not suppressed with deep flooding alone. Seedlings did not emerge from the soil when buried at or below 5 cm, while seeds buried at a shallower depth of 1.3 cm resulted in maximum seedling emergence. These results can be coupled with the germination temperature and moisture parameters to maximize seedling emergence in the field. This allows for the greatest withdrawal from the soil seedbank and provides an opportunity for chemical or mechanical control. ROXY RPS® rice technology and associated chemistry, oxyfluorfen, was tested as a viable program for general weed management as well as early-season weedy rice control. Field studies demonstrated that novel ROXY RPS® rice systems will be a highly efficacious opportunity for rice-weed management. The half-life of oxyfluorfen is not well-understood in flooded soils and is only 2-3 days in puddled soils. ROXY RPS®

controlled all early-emerging rice-field weeds, but there was uncertainty around whether or not this system would control weedy rice due to the observed differences in timing of emergence of accessions buried deeper (2.5 cm compared with 1.3 cm) in the soil. Additional studies with pre-plant applied oxyfluorfen were conducted in a greenhouse setting with California weedy rice accessions buried at 1.3 and 2.5 cm depths to determine if the timing of emergence based on burial depth would fall outside of the window of oxyfluorfen efficacy. There was no difference in timing of emergence of weedy rice seeds buried at different depths, and oxyfluorfen did not suppress seedling emergence the way it appeared to suppress other rice-weeds in field studies. Oxyfluorfen did cause lasting stunting and necrotic injury despite the high rate of emergence of all weedy rice accessions. These results provide information on how California weedy rice accessions are likely to behave under various environmental conditions, and what to expect when implementing various weed management methodologies.

Acknowledgements

I would like to my express utmost gratitude to Kassim Al-Khatib and all of the lab mates I have had the pleasure of working with during the time I have been at UC Davis. Kassim's decision to take me on as a graduate student has undoubtedly changed my life and consequently the lives of my family, friends and community. I will never be able to repay this debt to him and will always remember to pay this act forward to future colleagues, students, and mentees.

I also want to acknowledge my peers and mentors in the greater UC weed science program and community, specifically Brad Hanson and Whitney Brim-DeForest, for their support, encouragement, and feedback. It has been the highest honor and privilege to work alongside all of these individuals. I am forever grateful to have access to such an intellectual, collaborative, hardworking and successful community of scientists.

I would also like the thank my friends from all sectors of life, whom are too numerous to name, that have believed in me from the start. The women in my life have kept me grounded, reminded me that work is not all there is to life, and acted as outstanding role models of success, strong-will, perseverance, and strength.

Lastly, I would like to thank my entire extended family, especially my parents, Mark and Jennifer Galvin, for their unyielding love and support. My ability to obtain this degree is a reflection of their own struggles in life and the effort they put into building a comfortable life for their children. Words cannot express what an absolute privilege it is to be their daughter and I will always be grateful to them for never doubting my ability to achieve my goals.

Table of Contents

List of Tables

Chapter 3

List of Figures

Chapter 1

Chapter 2

Introduction

Weedy rice is a problematic weed species of interest in California due to its phenotypic conspecificity with cultivated rice (*Oryza sativa*). These similarities cause difficulty in identification during vegetative stages, resulting in proliferation of this pest across cultivated rice fields (Singh et al., 2013). Most rice-weeds are chemically controlled early in the season to reduce competition with cultivated rice. There are currently no herbicides that will control California weedy rice without simultaneously causing harm to cultivated rice, thus, non-chemical methods are needed for managing this pest (Espino et al., 2018). Weed control techniques that do not solely rely on chemical applications often require greater knowledge of the pest, e.g., ideal temperatures for growth and development. The goal of this dissertation is to provide biological knowledge of California weedy rice accessions paired with management opportunities associated with those research outcomes.

California rice cropping systems are unique compared with other rice growing regions of the world due to the continuous, year-round flooding practices in combination with direct water-seeding methods (Kanapeckas et al., 2018). Deep flooding has traditionally been used to suppress rice-weed species, however, years of continuous flooding in California has likely had significant influence and selected for flooding-tolerant species (Nadir et al., 2017). **In Chapter one**, California weedy rice accessions 1, 2, 3, 4, and 5 were subjected to various flooding and burial depth combinations to determine the limitations of flooding and whether or not deep burial would have any influence over weedy rice emergence. Weedy rice did not emerge when buried at or below 5 cm in rice field soil, but easily emerged through 15 cm of flood water. The combination of deep flooding (15 cm) and deep burial (2.5 cm) had greater control compared with weedy rice seeds buried at more shallow depths (1.3 cm). A 15 cm flooding depth is standard for conventional rice growers in California. Deep burial of seeds is not often considered as a management option for weed seeds due to dormancy concerns. Research suggests that accession 2 and 5 may have a short seed-dormancy period (< 5 years) compared accessions 1, 3, and 4 (>5 years) (Espino et al., 2018). In the absence of seed dormancy information, burying weedy rice seeds to depths of 5 cm or more will result in no seedling emergence for a given season and could be used as an option for suppression.

The Southern United States has utilized herbicide-resistant rice cultivars to combat weedy rice with varying degrees of success (Burgos et al., 2014). As of 2022 California did not have any herbicide-tolerant cultivars on the market. Beginning in 2019, Albaugh LLC in partnership with the University of California Rice Experiment Station in Biggs, CA, began to develop an oxyfluorfen-tolerant, ROXY RPS® rice program. Oxyfluorfen is a protox inhibitor; carfentrazone is the only other PPO inhibitor available to California rice producers. There are currently no known weeds that possess herbicide-resistant traits for this mode of action (Linquist et al., 2018). **Chapter two** details the outcomes of ROXY RPS® program demonstration in the field from 2019-2022. These fields purposefully do not have weedy rice infestations, so additional studies were conducted in a greenhouse environment to determine efficacy of oxyfluorfen on California weedy rice.

Field studies demonstrated ROXY RPS® rice incurred minimal stunting injury in the first 28 days after soil-applied oxyfluorfen applications. This injury did not negatively affect yields while providing greater than 90% control of all problematic sedge and grass-weed species, *Echinochloa* spp., *Leptochloa fascicularis*, and *Cyperus difformis*. Weedy rice accessions 1, 2, 3, and 5 seeds were buried at 1.3 and 2.5 cm in the soil and exposed to soil-applied oxyfluorfen to determine efficacy of this herbicide. Previous studies indicated an increase in the soil burial depth of weedy rice seed reduced seedling emergence and required more time for seedlings to emerge through the soil and water surfaces compared with seeds buried at shallower depths. The half-life of oxyfluorfen in puddled soils is 2-3 days while the half-life in flooded soils is not well documented (Weed Science Society of America, 2014). There were concerns around late-emerging weedy rice escaping the window of oxyfluorfen efficacy, however, burial depth did not significantly influence weedy rice stunting or necrosis injury. All weedy rice was completely necrotic by 28 days after soil-applied oxyfluorfen applications and was severely stunted compared to untreated accessions that had begun to tiller by this time. ROXY RPS® rice and oxyfluorfen represent a significant technological advancement in rice-weed management in California and will provide efficacious weedy control over weedy rice accessions as well as other problematic weed species.

The stale seedbed technique is a pre-season management strategy that hinges its success on maximizing germination of the weed seedbank (Travlos et al., 2020). This methodology is implemented by flushing a rice field to induce germination of weed seeds, then those seedlings are controlled with mechanical and/or chemical control methods.

The stale seedbed technique requires extended periods of time for fields to dry in order to utilize spraying or tillage equipment, and is often constrained by early planting dates or late winter rains (Linquist et al., 2008). These temporal requirements are a major limiting factor in successful implementation of this methodology. **Chapter three** describes how temperature and moisture are the dominant factors influencing weedy rice seed germination (Finch-Savage & Leubner-Metzger, 2006) and thus were used to determine the limitations and ideal conditions for implementing this methodology. Results of this research indicated that California weedy rice accessions have varying degrees of moisture-stress and temperature-stress tolerances. Cooler temperatures at or below 15°C as well as moisture stress conditions (-0.4 and -0.8MPa) required more time for germination to begin and reach maximum. In years when moisture is limited, either due to drought or regulatory limitations, a stale seedbed methodology will not result in maximum germination. Similarly, if a grower wants to implement the stale seedbed methodology in April, when average temperatures are often below 20°C, it may be too cold to maximize germination.

All weedy rice accessions require complete soil saturation (0 MPa) and temperatures between 25-35°C for maximum germination. If these conditions are present, then the stale seedbed methodology is a viable option for optimizing withdrawals from the soil seedbank. Weedy rice accessions have similar temperature and moisture requirements as *Echinochloa* species. Targeting the germination requirements of weedy rice in the stale seedbed application will encompass other problematic grass-weed species for maximum rice-weed control.

References

Burgos, N. R., Singh, V., Tseng, T. M., Black, H., Young, N. D., Huang, Z., Hyma, K.D., Gealy, D. R., Caicedo, A. L. (2014). The Impact of Herbicide-Resistant Rice Technology on Phenotypic Diversity and Population Structure of United States Weedy Rice. *Plant Physiology*, *166*(3), 1208–1220.

Espino, L., Brim-DeForest, W., & Johnson, T. (2018). Weedy Rice in California: Addressing an Emerging Pest Through Research and Outreach. *CAPCA Adviser*, *11*(4), 44.

Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, *171*(3), 501–523.

Kanapeckas, K. L., Tseng, T.M., Vigueira, C. C., Ortiz, A., Bridges, W. C., Burgos, N. R., Fischer, A.J., Lawton-Rauh, A. (2018). Contrasting patterns of variation in weedy traits and unique crop features in divergent populations of US weedy rice (*Oryza sativa* sp) in Arkansas and California. *Pest Management Science*, *74*(6), 1404–1415.

Linquist, B., Al-Khatib, K., Swett, C., Espino, L., Leinfelder-Miles, M., Brim-DeForest, W., & McKenzie, K. (2018). *Rice Production Manual*. Davis, CA.

Linquist, B., Fischer, A., Godfrey, L., Greer, C., Hill, J., Koffler, K., Moeching, M.,

Mutters, R., Van Kessel, C. (2008). Minimum tillage could benefit California rice farmers. *California Agriculture*, *62*(1), 24–29.

Nadir, S., Xiong, H.-B., Zhu, Q., Zhang, X.-L., Xu, H.-Y., Li, J., Dongchen, W., Henry, D., Guo, X-Q., Khan, S., Suh, H-S., Lee, D. S., Chen, L.-J. (2017). Weedy rice in sustainable rice production. A review. *Agronomy and Sustainable Development*, *37*(46), 1–14.

Singh, K., Kumar, V., Saharawat, Y., Gathala, M., Ladha, J. K., & Chauhan, B. S. (2013). Weedy Rice: An Emerging Threat for Direct-seeded Rice Production Systems in India. *Journal of Rice Research*, *1*(106).

Travlos, I., Gazoulis, I., Kanatas, P., Tsekoura, A., Zannopoulos, S., & Papastylianou, P. (2020). Key Factors Affecting Weed Seeds' Germination, Weed Emergence, and Their Possible Role for the Efficacy of False Seedbed Technique as Weed Management Practice. *Frontiers in Agronomy*, *2*, 1.

Weed Science Society of America. (2014). *Herbicide Handbook*. (D. L. Shraner, Ed.) (10th ed.). Weed Science Society of America.

Chapter 1

Flooding depth and burial effects on seedling emergence of five California weedy rice (*Oryza sativa spontanea*) accessions

Liberty B. Galvin, Deniz Inci, Mohsen Mesgaran, Whitney Brim-DeForest, Kassim Al-Khatib

Formatted for and published in Weed Science, March 2022, 70(2):213-219

Abstract

Weedy rice or weedy red rice (*Oryza sativa* f. *spontanea* Roshev.) has recently become a significant pest in California rice production systems. The conspecificity of this pest with cultivated rice, *Oryza sativa* (L.), negates the use of selective herbicides, rendering the development of non-chemical methods a necessary component of management strategies for this weed. Experiments were therefore conducted to determine the emergence and early growth responses of *O. sativa spontanea* to flooding and burial. Treatment combinations of four flooding depths (0, 5, 10, and 15 cm) and four burial depths (1.3, 2.5, 5, and 10 cm) were applied to test the emergence of five *O. sativa spontanea* accessions as well as 'M-206' (medium grain, median maturity rice cultivar) for comparison. Results revealed that burial depth had a significant effect ($P < 0.0001$) on seedling emergence. There was a 43-91% decrease in emergence between 1.3 and 2.5 cm burial depending on the flooding depth and accession, and an absence of emergence from soil burial depths at or below 5 cm. Flooding depth did not affect emergence ($P = 0.0723$), but there was a significant interaction between burial and flooding treatments (P < 0.0001). There was no significant difference between total *O. sativa spontanea* emergence from the soil and water surfaces regardless of burial or flooding depths, implying that once the various accessions have emerged from the soil, they will also emerge from floodwaters. Overall, most accessions had similar emergence rates compared to M-206 cultivated rice but produced more dry weight than M-206 when planted at 1.3 cm. The results of this experiment can be used to inform stakeholders of the flooding conditions necessary to promote or inhibit the emergence of California *O. sativa spontanea* accessions from the weed seedbank.

Keywords: Conspecific, *O. sativa spontanea*, weedy (red) rice

Introduction

Most rice producers in California practice aerial, wet-direct seeding of their cultivars, incorporate continuous annual flooding, and typically do not practice crop rotation. Minimal crop rotation and fallowing as well as a lack of adherence to purchasing certified seed has been credited to the most recent appearance of weedy rice, also known as "red rice" (*Oryza sativa f. spontanea Roshev)*, in California (De Leon et al., 2019). Roughly 5,600 hectares spread across all 9 rice-growing counties in California had been infested with one or more of five *O. sativa spontanea* accessions as of 2019 (Karn et al. 2020). At the time of this study, only five accessions had been documented and were simply referred to as accession 1, 2, 3, 4, and 5, respectively (De Leon et al. 2019). These weeds are thought to be the result of hybridization between various wild, weedy, and cultivated rice types from across the United States (US) and are genetically different from one another (De Leon et al. 2019).

O. sativa spontanea has several traits that allow for long-term persistence including conspecific features with cultivated rice, early maturity (Zhao et al. 2015), high seed production and grain shattering (Kanapeckas et al. 2016), prolonged seed dormancy, and subsequent seedbank persistence (Delouche et al. 2007). Additionally, there is evidence indicating a competitive advantage of *O. sativa spontanea* in an elevated CO_2 environment (Ziska et al. 2010) as well as the ability for higher uptake of nitrogen compared with cultivated relatives (Chauhan & Johnson 2011).

There are multiple cultural factors that contribute to the difficulty in controlling *O. sativa spontanea* in California in addition to the biological mechanisms that limit control of *O. sativa spontanea*. These include absence of herbicide-tolerant rice varieties and a reduced number of herbicides available for weed control compared with the Southern US rice-growing region (Al-Khatib 2017). Flooding has been suggested as an effective method of controlling rice weeds in the absence of chemical control options (Chauhan 2012a; Dorji et al. 2013; Ziska et al. 2015). The efficacy of flooding as a control strategy for *O. sativa spontanea*, however, has not been well studied in California rice systems.

Several studies have indicated that soil burial depth of seeds has an effect on seedling emergence patterns and is important for understanding seed longevity and persistence in the seed bank (Benvenuti et al. 2001; Ghosh et al. 2017). Several *O. sativa spontanea* accessions from Arkansas, Louisiana, and Mississippi (Southern US) were able to emerge from soil burial depths as deep as 7.5 cm in clay and silt loam soils in dry direct-seeded conditions in the absence of standing water (Gealy et al. 2000). Drill-seeded, or dry direct-seeded rice, is less common in California compared with wet direct seeding methods. It is important to acknowledge that seed burial depth is an important factor in weed suppression, but anaerobic conditions induced by floodwaters also act as a suppressive force (Ismail et al. 2012). This illustrates how the rice-growing culture and production practices are different between the two US regions, equating to the likelihood that California *O. sativa spontanea* may exhibit differential seedling emergence responses compared to those studied by Gealy et al. (2000).

The objectives of this study were to determine the effects of flooding and seed burial depth on seedling emergence of the five California *O. sativa spontanea* accessions and determine if there are differences in seedling emergence between these accessions compared with M-206, a medium grain, median maturity rice cultivar. The overall goal of this research was to provide information to support future tillage and flooding depth management strategies aimed at reducing *O. sativa spontanea* in California rice production systems.

Materials and methods

Seed material and pre-treatment protocols

Localized seed collections were organized by University of California Cooperative Extension staff during a field survey conducted in 2016 (Espino et al. 2018). Seed multiplication of all *O. sativa spontanea* accessions was done in controlled greenhouse environments on University of California Davis campus from 2016-2019 with plants physically separated to reduce the likelihood of cross-pollination. All plants were grown in

3.79-liter pots that were submerged in trays with 5 cm of standing water. Table 1 describes each *O. sativa spontanea* accession as well as M-206, a common cultivar in California. The accessions described in Table 1 were the only well-documented *O. sativa spontanea* in California at the time of study. Seeds of each *O. sativa spontanea* accession were arranged into separately sealed plastic bags which contained a dry paper towel, and were placed in a dark incubation chamber for five days at 50°C to break dormancy (Waheed et al. 2012, Shiratsuchi et al. 2017).

Table 1. Descriptions of California *Oryza sativa spontanea* accessions and cultivated rice, M-206 (De Leon et al., 2019, Karn et al., 2020). Average observed 1000-seed weight includes standard error.

Accession	Hull color	Awns	Grain type	Shattering	1000-seed weight (g)
M206	Straw	Absent	Medium	None	26.36 ± 0.3
Type 1	Straw	Absent	Short	high	19.88 ± 0.2
Type 2	Bronze	Absent	Medium	high	27.04 ± 0.3
Type 3	Straw	Long	Medium	high	25.87 ± 0.3
Type 4	Black	Long	Short	high	26.02 ± 0.2
Type 5	Straw	Partial	Medium or long	high	26.68 ± 0.3

The germination process mimicked common practice for pre-germinating cultivated varieties for wet-direct seeded practices (Linquist et al. 2018). Following the dry-hot incubation period, all seeds, including M-206, were wetted by saturating the paper towel within each plastic bag with deionized water. The plastic bags were subsequently placed back into the dark chamber for three days at 30°C. Deionized water was added as needed to ensure the paper towel remained saturated and all seeds received constant moisture.

Plant growth conditions

This experiment was conducted outdoors on University of California Davis campus due to limited greenhouse space. Field soil from a site historically planted in rice (>5 years prior to experimentation) at the Rice Experiment Station in Biggs, California, was used to mimic field-soil conditions. Soil from this site is described by the Web Soil Survey (2019) as Esquon-Neerdobe clay soils and contains 20% sand, 30% silt, 50% clay, 2.65% soil organic matter, with a pH of 5.88, 0.06% total N, 13 ppm Olsen-P, and 250 ppm K. The soil was placed directly into 46 x 66 x 38 cm clear, polycarbonate tubs, wetted, and leveled before planting to ensure all seeds were buried and subsequently flooded to similar treatment depths.

Once seeds had germinated (within incubated plastic bags), those with radicles protruding at 1-3 mm were planted into tubs using a grid to help demarcate different *O. sativa spontanea* accessions from one another. Seeds were placed on the soil surface and then covered with soil to the designated burial depth level, as described by Chauhan (2012a). Irrigation water from a municipal tap was applied immediately after seed burial to achieve the desired flooding depth.

Experimental design

The experiment was repeated in time in order to expose *O. sativa spontanea* accessions to various environments with one replicate beginning in July and the second in August. Four flooding depths of 0 (fully saturated soil absent of standing water), 5, 10, and 15 cm, in combination with four burial depths of 1.3, 2.5, 5, and 10 cm were used. Treatments were arranged as a factorial-split plot in a randomized complete block design with four replicates. Each tub contained a single flood by burial depth combination, representing the whole plot, with the six plant types (five *O. sativa spontanea* accessions and M-206) constituting the sub-plot; 15 seeds of each plant type were planted in rows within each tub.

Data collection

Water temperature was recorded hourly from a single treatment combination replicate with HOBO® Pendant™ MX2201 (Onset Computer Corporation, 470 MacArthur Blvd. Bourne, MA 02532, USA) water temperature data loggers. Ambient air temperature was also recorded hourly with a single HOBO® MX100 Temperature Data Logger. Average minimum and maximum temperatures were 15°C and 39°C, respectively, for run 1 and 13°C and 34°C, respectively, for run 2 accompanied by an absence of rainfall typical to this region during July and August.

Seedling emergence from both the soil and water surfaces was recorded daily when applicable. Seedling emergence from the soil was defined by a visible cotyledon of 3 mm or greater, and water emergence was noted when a leaf visibly permeated the water surface. Any non-rice weeds were immediately removed by hand whilst copper sulfate (CuSO4; Millipore Sigma, PO Box 14508 St. Louis, MO 63178 USA) was applied in liquid formulation at a rate of 13.45 kg/ha to each tub at two, nine and sixteen days after planting to prevent algae growth. Plants were harvested at the soil surface 21 days after planting then placed in paper bags which were dried at 70°C for 3 weeks after which dry biomass was weighed.

Data analysis

O. sativa spontanea seedling emergence from the soil, and water, as well as seedling dry weight data were analyzed using PROC MIXED in SAS version 9.4 (100 SAS Campus Drive Cary, NC 27513, USA). Soil-burial depth of seeds, flooding depth, accession, and their associated interactions were included as fixed effects. Experimental run was incorporated as a random intercept (Yang 2010). Residuals were visually inspected to ensure assumptions of ANOVA in regards to homogeneity, and normality of residuals were met. Initial analysis included all treatments, however, to create a less skewed distribution of the data, seedlings buried at 5 and 10 cm in the soil were excluded from the analysis due to the lack of seedling emergence from those treatment groups. Treatment effects and interactions were subjected to a type III ANOVA followed by a Tukey HSD post-hoc test at a significant level of α = 0.05. A one-tailed, Welch two-sample t-test was conducted using R (2021) programming software to determine differences between average seedling emergence from the soil and water surfaces.

Results and discussion

Burial depth alone had significant influence ($P < 0.0001$) over seedling emergence from the soil and water surfaces as well as dry weight. However, once the 5 and 10 cm soil burial depth treatments were removed from the analysis, burial alone was no longer significant. Soil burial by flooding depth interactions had significant effect on emergence from the soil (P < 0.0001) as well as emergence from the water (P < 0.0001), but not on seedling dry weight (P = 0.0975). Soil burial by accession interactions also had significant effect on seedling emergence from the soil (P = 0.0203) as well seedling emergence from the water (P = 0.0116) and dry weight (P = 0.0091). There were no significant three-way interactions for seedling emergence from the soil (P = 0.9815), emergence from the water (P = 0.9162), or dry weight (P = 0.9814).

There results of the t-test comparing total emergence from the soil and water surfaces showed no significant differences between them regardless of seed burial depth, flooding depths, or accession including M-206.

Seedling emergence from soil and water surfaces

Seed burial depth had significant effects on seedling emergence (P < 0.0001) of M-206 and all *O. sativa spontanea* accessions where no pre-germinated seeds buried at or below 5 cm of soil emerged regardless of flooding depth or rice accession (Figure 1.1). Decreasing weed seeding emergence with increasing soil burial depth may be due to several factors such as increased soil physical impediment or decreased oxygen concentrations in deeper soil depths leading to hypoxia (Ismail et al. 2012, Setter et al. 1988). Accessions 2 and 5 have a short dormancy period (Table 1), so burial at 5 cm or deeper may be a control option for these California *O. sativa spontanea* seeds present in the soil seedbank (Marambe 2009).

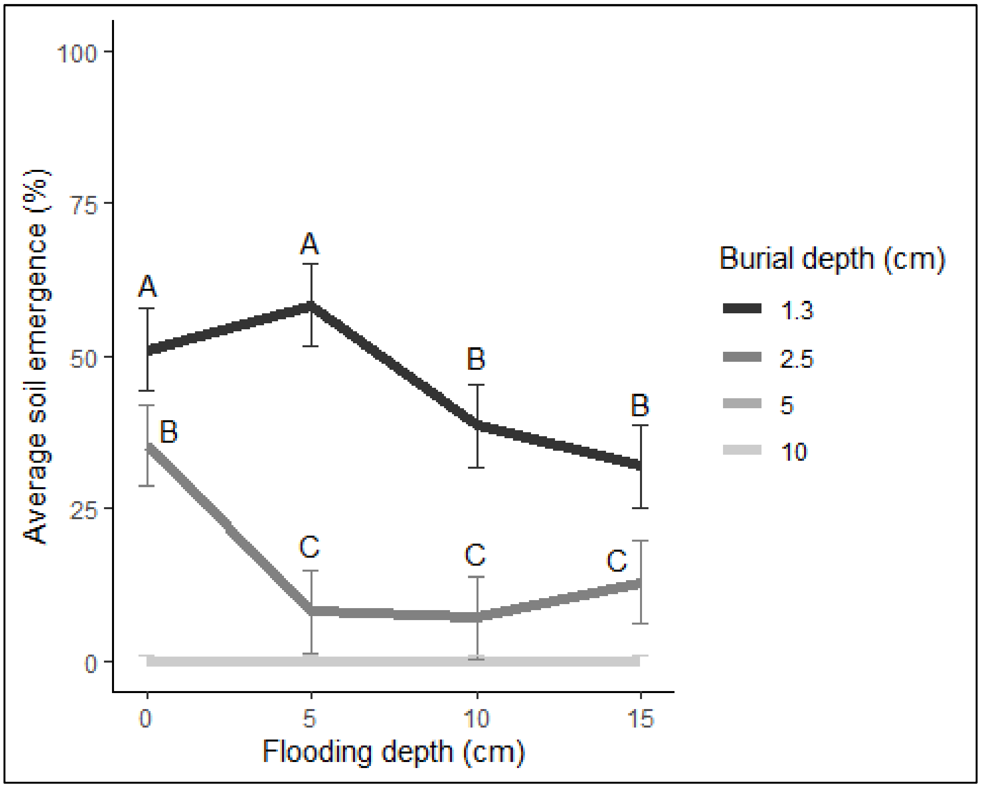

Figure 1.1 Burial and flooding depth interactive effects on soil surface emergence for all accessions of weedy rice combined. Different letters indicate significant differences between treatment means.

There was significant burial depth by flooding depth interactions ($P < 0.0001$) on soil surface emergence. There was no statistical difference ($P = 0.2439$) in seedling emergence from 1.3 cm of soil when no flood as well as 5 cm flood were applied (Figure 1.1). Similarly, there was no difference in seedling emergence from the 1.3 cm in the soil ($P = 0.2884$) when 10 and 15 cm flooding treatments were applied (Figure 1.1). There was a 20% decrease in seedling emergence from 1.3 cm of soil between shallow flooding (0 cm and 5 cm) and deeper flooding (10 cm and 15 cm; $P = 0.0017$) (Figure 1.1). The decrease in soil emergence with increasing flooding depth supports the current practice of using deep flooding as a weed suppression method in flooded rice systems (Ismail et al. 2012). Our research suggests that the flood must be at least 10 cm to have an impact on California *O. sativa spontanea* seeds located at the soil surface (1.3 cm).

Seeds buried at 2.5 cm in combination with no flooding exhibited significantly higher emergence from the soil ($P < 0.0001$) than other flooding levels. Average seedling emergence from 2.5 cm in the soil decreased from 35% to 8% when exposed to 0 cm, and 5 cm of flooding, respectively (Figure 1.1). There was no significant difference ($P > 0.05$) in average seedling emergence from 2.5 cm of soil when 5, 10, and 15 cm floodings were applied. It appears that *O. sativa spontanea* seeds become more sensitive to flooding when buried deeper in the soil. That is, seed burial at or below 2.5 cm in combination with flooding at or greater than 5 cm are likely to suppress the emergence of California *O. sativa spontanea*.

There was significant burial by flooding depth interactions ($P < 0.0001$) that had influence over seedling emergence from the water surface (Figure 1.2). There was no significant difference ($P = 0.3860$) in overall seedling emergence from the water between the 10 and 15 cm flooding depths when seeds were buried at 1.3 cm in the soil (Figure 1.2). There was a significant difference ($P < 0.050$) between these two flooding depths and the 5 cm flooding depth for overall water emergence when accessions were buried at 1.3 cm. Seedling emergence from the water decreased from 55% at 5 cm flood to 36% at the 10 cm flooding depth. Seeds buried at 1.3 cm in the soil exhibited a 6% decrease in emergence from the water when transitioning from 10 cm to 15 cm flooding depths (Figure 1.2).

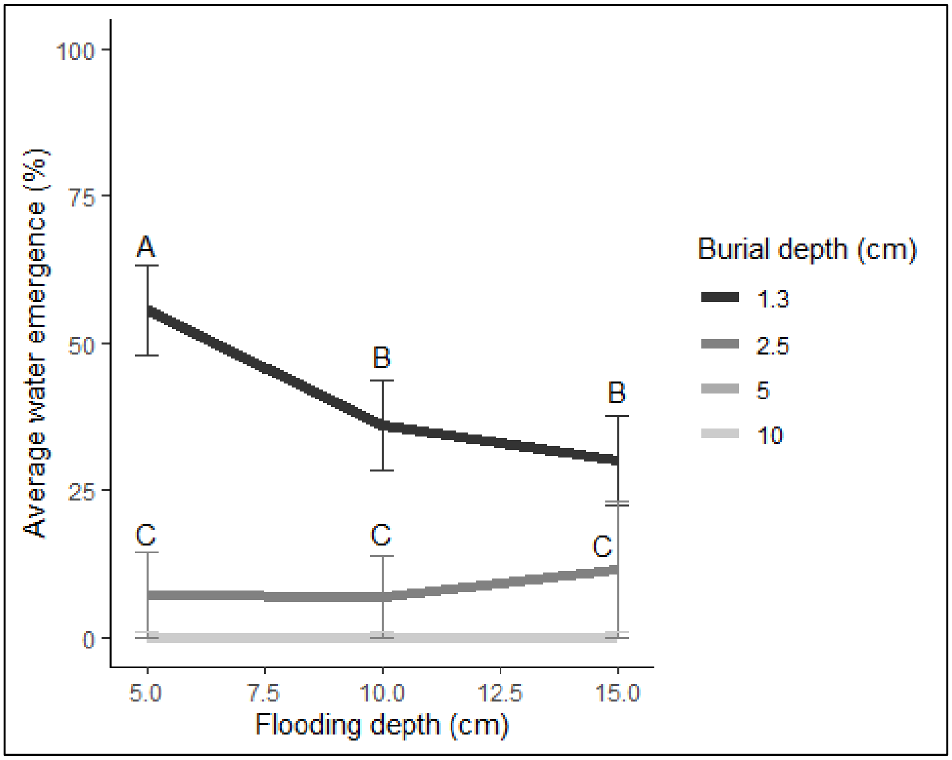

Figure 1.2 Burial and flooding depth interactive effects on water surface emergence for all accessions of weedy rice combined. Different letters indicate significant differences between treatment means.

Accessions buried at 2.5 cm in the soil expressed no significant difference (P > 0.05) in emergence from the water between the 5, 10, and 15 cm flooding depths (Figure 1.2). These results mirror seeding emergence from 2.5 cm burial depths that occurred with increasing flooding depth. Results from the t-test showed no significant differences (P = 0.2786, n =576) in emergence between soil and water surfaces. These results imply that once *O. sativa spontanea* seedlings emerge from the soil, there is a high likelihood that seedling will also emerge through floodwaters. Some rice cultivars have been found to increase coleoptile elongation in the presence of floodwater containing low oxygen concentrations (Turner et al. 1981). This response could also be an explanation for the similarities seen between weedy rice seedling emergence from the soil and water surfaces. The results from this experiment provide evidence that flooding depth does play a role in the emergence of *O. sativa spontanea*, but its effect depends more on the distribution of seeds in the soil, i.e., burial depth, than flooding depth.

Differences between *O. sativa spontanea* accessions

There were significant interactions (P = 0.0203) between burial depth and accession which influenced soil emergence. There was no significant difference (P > 0.05) between average seedling emergence from 1.3 cm of soil for accessions 1, 2, 3, 5, and M-206 (Figure 1.3). Accession 4 had significantly lower seedling emergence than other accessions with 26% less emergence from the soil (P = 0.006) compared with M-206 (Figure 1.3). There was no significant difference (P > 0.05) between emergence from 2.5 cm of soil for all types including M-206 (Figure 1.3). Accession 4 is phenotypically very different from the other accessions despite its close genetic relationship with accession 3 (De Leon et al. 2019). Based on greenhouse growth observations, accession 4 has a dwarf-like growth habit, long awns with a higher shattering rate, a high tiller and panicle number, and low shoot and root dry weight compared with the other *O. sativa spontanea* accessions. Karn et al. (2020) found that accession 4 growth is greatly reduced in the presence of competition from other rice plants. The planting density of this experiment could be a partial factor in the reduced seedling emergence of accession 4 in addition to the biological differences with other accessions.

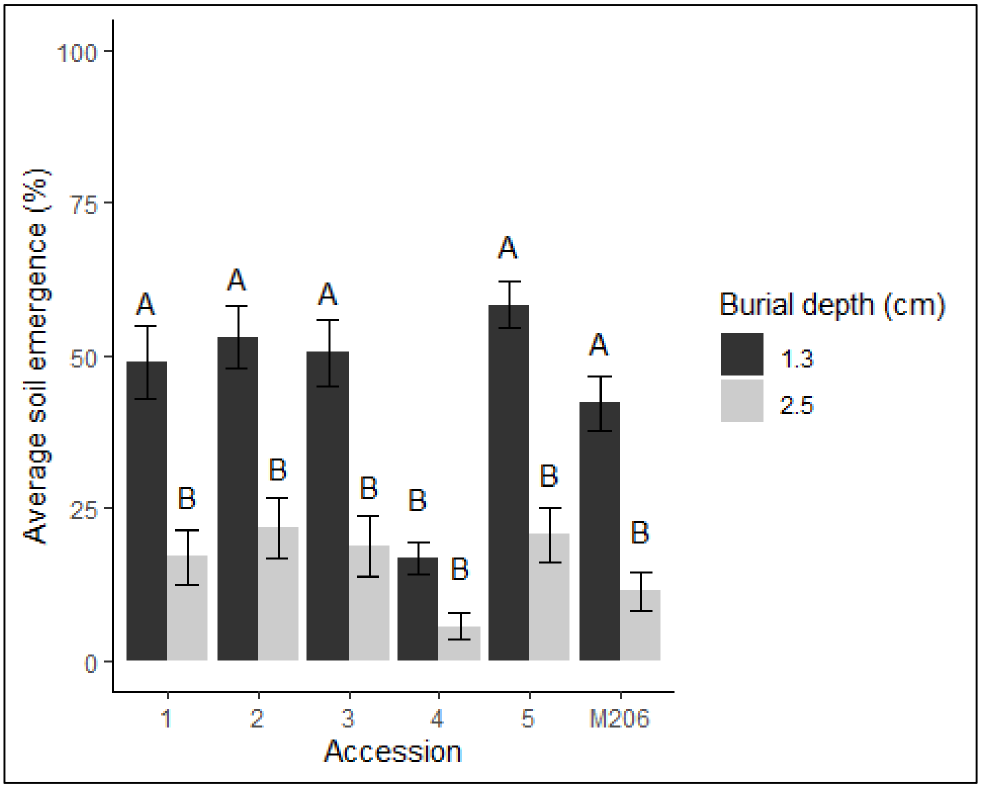

Figure 1.3 Burial effects on average emergence from the soil surface of each *Oryza sativa spontanea* accession and cultivated rice M-206 when planted at 1.3 or 2.5 cm burial depths. Different letters indicate significant differences between treatment means.

Accessions 1, 2, 3, and 5 had 42%, 47%, 50%, and 54% emergence from the water, respectively, and were not significantly different from each other, however, only accessions 1, 2, 3 were not significantly different from M-206 (Figure 1.4). Accessions 4 was again significantly different from all other types with only 16% emergence from the water surface when buried at 1.3 cm regardless of flooding depth. There was no significant difference ($P > 0.05$) between emergence from the water for all types including M-206 when buried at 2.5 cm (Figure 1.4). Similar emergence patterns have been demonstrated through the burial by flooding interactions as well as the burial and accession interactions. Several factors may be contributing to rapid growth after germination in the presence of a flood including early maturation (Zhao et al. 2015) and morphological features such as rapid shoot elongation, and vertical leaf position (Voesenek et al. 2006).

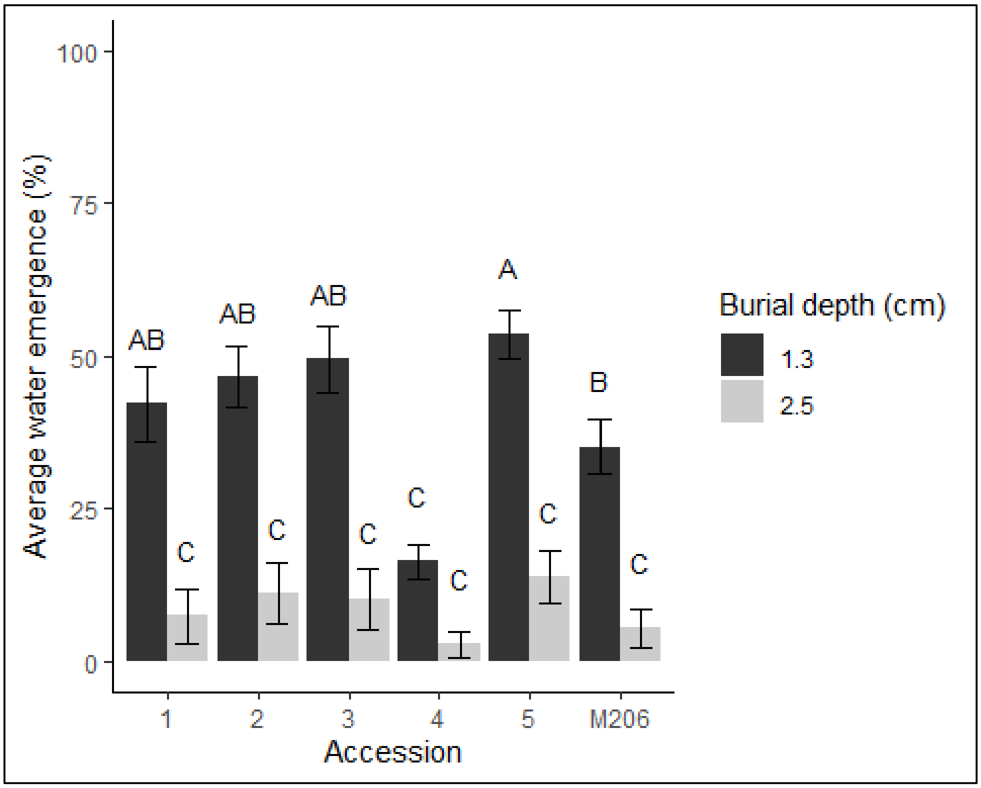

Figure 1.4 Burial effects on average emergence from the water surface of each *Oryza sativa spontanea* accession and cultivated rice M-206 when planted at 1.3 or 2.5 cm burial depths. Different letters indicate significant differences between treatment means.

The general ability to emerge from the soil and floodwaters at equivalent or greater rates compared with M-206 highlights the *O. sativa spontanea* accessions' probable adaptation to the continuous annual flooding (Delouche et al. 2007) common in the majority of California rice cropping systems.

Biomass production

Average dry weight was significantly affected by burial and accession interactions (P = 0.0091). Weedy accessions 2 (106.2 mg; P = 0.0371), 3 (146.4 mg; P = 0.0003), 4 (107.5

mg; P = 0.0427), and 5 (110.0 mg; P = 0.0255) yielded significantly greater dry weight compared with M-206 (53.72 mg) buried at 1.3 cm in the soil. Accession 1 (83.44 mg) did not have significantly different dry weight (P = 0.2400) compared with M-206 seeds buried at 1.3 cm in the soil (Figure 1.5). Accession 1 has a short-grain seed size (Karn et al. 2020) and a 1000-seed weight that is less than the other weedy accessions including M-206 (Table 1). The 1000-seed weight may account for the reduced dry weight accumulated during early stages of development (Roy et al. 1996). This research highlights the importance of early-season control of these accessions to prevent *O. sativa spontanea* from gaining a competitive advantage over cultivated varieties (Kanapeckas et al. 2018).

Accession 3 (169.5 mg) accumulated significantly more dry weight compared to M-206 (70.42 mg; P = 0.0028), as well as accession 1 (50.44 mg; P < 0.0001), 2 (91.81 mg; P = 0.0070), and 5 (76.48 mg; P = 0.0013) when seeds were buried at 2.5 cm. There was not enough plant material from accession 4 to statistically analyze the data (Figure 1.5). Currently, it is assumed that accession 3 has existed in the rice growing region of California for considerably longer than the other *O. sativa spontanea* accessions included in this experiment (Kanapeckas et al. 2016). A genetic divergent from California cultivated rice (De Leon et al. 2019), accession 3 may have had a longer period of time to adapt to California production systems, providing evidence for the ability to produce more dry weight in early growth stages compared with M-206 and other *O. sativa spontanea* accessions.

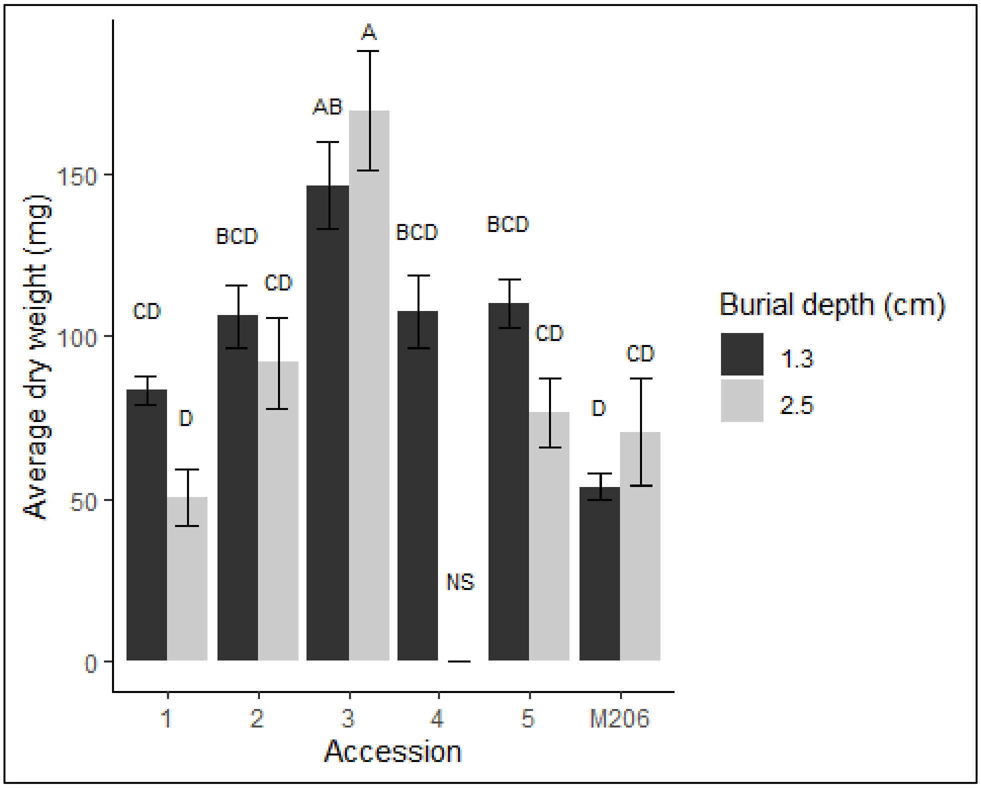

Figure 1.5 Average dry weight of the five *Oryza sativa spontanea* accessions and cultivated rice (M-206) when planted at either 1.3 or 2.5 cm burial depths. Accession 4 seedlings did not emerge often enough from 2.5 cm burial depths to produce significant dry weight results. Different letters indicate significant differences between treatment means.

In this experiment the greatest total emergence of any *O. sativa spontanea* occurred at shallow burial (1.3 cm) and shallow flooding (0-5 cm) depths; emergence and dry weight varied among the different California *O. sativa spontanea* accessions depending on flooding depth, burial depth and accession. Burial depth played the most important role in emergence patterns, demonstrated by the lack of emergence from depths deeper than 5 cm in the soil. Flooding depth alone did not significantly influence emergence, nor did an increase in flooding depth at 1.3 cm burial depth caused a consistent decline in emergence patterns of weedy or M-206. Deep flooding in general is an important weed control tool for California rice producers to control grass-type weeds, but flooding alone will not greatly reduce *O. sativa spontanea* emergence. This information could be used by California rice growers to promote emergence when implementing a stale seedbed methodology, currently one of the only conventional practices available for controlling this weed in California rice cropping systems (Karn et al. 2020). Organic growers can use the outcomes of this research to ensure that their field is properly flooded to depths of 15 cm or greater in order to suppress as many *O. sativa spontanea* plants as possible.

Acknowledgements

This research was supported by the California Rice Research Board and the Melvin D. Androus Endowment. The authors acknowledge Dr. John Madsen for technical support. No conflicts of interest are declared.

References

Al-Khatib K (2017) Spread and management of herbicide resistant weeds in California rice. Page 50 *in* Proceedings of the 2017 California Weed Science Society. Monterey, CA: California Weed Science Society.

Benvenuti S, Macchia M, Miele S (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. Weed Sci 49:528–535

Chauhan BS (2012a) Weedy rice (*Oryza sativa*) II. response of weedy rice to seed burial and flooding depth. Weed Sci 60:385–388

Chauhan BS (2012b) Weed Ecology and Weed Management Strategies for Dry-Seeded Rice in Asia. Weed Technol, 26(1), 1–13

Chauhan BS, Johnson DE (2011) Competitive interactions between weedy rice and cultivated rice as a function of added nitrogen and the level of competition. Weed Biol Manage 11:202–209

De Leon TB, Karn E, Al-Khatib K, Espino L, Blank T, Andaya CB, Andaya VC, Brim-DeForest W (2019) Genetic variation and possible origins of weedy rice found in California. Ecol Evol 1–14

Delouche, JC, Burgos NR, Gealy DR, Zorrilla de San Martin G., Labrada R, Larinde M, Rosell C (2007) Weedy rices: Origin, biology, ecology and control. Rome, Italy: Food and Agricultural Organization of the United Nations Paper 188. 103 p

Dorji, S, Chauhan BS, Baltazar AM, Johnson D (2013) Effect of flooding depth and pretilachlor rate on emergence and growth of three rice weeds: Junglerice (*Echinochloa colona*), smallflower umbrella sedge (*Cyperus difformis*), and ludwigia (*Ludwigia hyssopifolia*). Can J Plant Prot 1:43–48

Espino L, Brim-DeForest W, Johnson T (2018) Weedy rice in California: Addressing an emerging pest through research and outreach. CAPCA Adviser 8:44-48

Gealy DR, Saldain NE, Talbert RE (2000) Emergence of red rice (*Oryza sativa*) ecotypes under dry-seeded rice (*Oryza sativa*) culture. Weed Technol 14:406–412

Ghosh D, Rathore M, Brahmachari K, Singh R, Kumar B (2017) Impact of burial and flooding depths on Indian weedy rice. Crop Prot 100:106–110

Ismail AM, Johnson DE, Ella ES, Vergara GV, Baltazar AM (2012) Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. AoB Plants 2012

Kanapeckas, KL, Tseng TM, Vigueira CC, Ortiz A, Bridges WC, Burgos NR, Fischer AJ, Lawton-Rauh AL (2018) Contrasting patterns of variation in weedy traits and unique crop features in divergent populations of US weedy rice (*Oryza sativa* sp) in Arkansas and California. Pest Manage Sci 74:1404–1415

Kanapeckas, KL, Vigueira CC, Ortiz A, Gettler KA, Burgos NR, Fischer AJ, Lawton-Rauh AL (2016) Escape to ferality: The endoferal origin of weedy rice from crop rice through de-domestication. PLoS One 11:9

Karn E, De Leon T, Espino L, Al-Khatib K, Brim-DeForest W (2020) Effects of competition from California weedy rice (*Oryza sativa* f. *spontanea*) biotypes on a cultivated rice variety. Weed Technol 1-9

Linquist, B, Al-Khatib K, Swett C, Espino L, Leinfelder-Miles M, Brim-DeForest W, McKenzie K (2018) Rice Production Manual. Davis, CA: University of California Agriculture and Natural Resources. Pp 4.5

Marambe B (2009) Weedy rice: Evoluation, threats, and management. Trop Agric 157:43–64

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Roy SKS, Hamid A, Giashuddin MM, Hashem A (1996) Seed size variation and its effects on germination and seedling vigour in rice. J Agron Crop Sci 176:79–82

Setter TL, Kupkanchanakul T, Kupkanchanakul K, Bhekasut P, Wiengweera A, Greenway H (1988) Environmental factors in deepwater rice areas in Thailand: O_2, CO_2, ethylene. Page 55-66 *in* Proceedings of the 1987 International Deepwater Rice Workshop. Los Baños, Philippines: International Rice Research Institute.

Scherner A, Schreiber F, Andres A, Concenco G, Martins MB, Pitol A (2018) Rice crop rotation: A solution for weed management. Pages 83-98 *in* Shah F, Kha Z, Iqbal A, eds. Rice Crop Current Developments. 1st ed. IntechOpen

Shiratsuchi H, Ohdaira Y, Yamaguchi H, Fukuda A (2017) Breaking the dormancy of rice seeds with various dormancy levels using steam and high temperature treatments in a steam nursery cabinet. Plant Prod Sci, 20:183–192

Turner T, Chen CC, McCauley GN (1981) Morphological development of rice seedlings in water at controlled oxygen levels. Agron J 73:566–570

Voesenek LACJ, Colmer TD, Pierik R, Millenaar FF, Peeters AJM (2006) How plants cope with complete submergence. New Phytol 170:213–226

Waheed A, Ahmad H, Abbasi FM (2012) Different treatment of rice seed dormancy breaking, germination of both wild species and cultivated varieties (*Oryza sativa* L.). J Mater Environ Sci 3:551–560

Yang RC (2010) Towards understanding and use of mixed-model analysis of agricultural experiments. Can J Plant Sci 90:605–627

Zhao C, Xu W, Song S, Dai W, Dai L, Zhang Z, Qiang S (2015) Early flowering and rapid grain filling determine early maturity and escape from harvesting in weedy rice. Pest Manag Sci 74:465–476

Ziska LH, Gealy DR, Burgos NR, Caicedo AL, Gressel J, Lawton-Rauh AL, Avila L, Theisen G, Norsworthy J, Ferrero A, Vidotto F, Johnson DE, Ferreira FG, Marchesan E, Menezes V, Cohn MA, Linscombe S, Carmona L, Tang R, Merotto A (2015) Weedy (red) rice: An emerging constraint to global rice production. Adv Agron 129:181-228

Ziska LH, Tomecek MB, Gealy DR (2010) Competitive interactions between cultivated and red rice as a function of recent and projected increases in atmospheric carbon dioxide. Agron J 102:118-123

Chapter 2

Assessment of oxyfluorfen-tolerant rice systems and implications for rice-weed management in California

Liberty B. Galvin, Aaron Becerra-Alvarez, Kassim Al-Khatib

Formatted and accepted for publication in Pest Management Science, August 2022

ABSTRACT

BACKGROUND

Weeds are a significant barrier to rice production in California, exacerbated by lack of chemical control options and herbicide-resistance in persistent aquaphilic species. Oxyfluorfen-tolerant rice, created at the Rice Experiment Station (RES) in Biggs, California, was developed to provide an agronomic program for managing problematic grass and sedge rice-weeds including *Oryza sativa* f. *spontanea* Roshev. (weedy "red" rice). Hand-pulling is the most common removal method for *O. sativa spontanea* because there are no herbicides registered for this pest in California. Oxyfluorfen was used in combination with oxyfluorfen-tolerant rice in 2019 and 2021 field studies to evaluate rice injury and weed control efficacy on prevalent rice-weed species. Additional studies were conducted in 2021 on University of California Davis campus to determine pre-emergent oxyfluorfen efficacy on four California *O. sativa spontanea* accessions.

RESULTS

Fields studies indicated minimal crop injury in the first 28 days after seeding (DAS), but no observable injury at 60 DAS in both years. Weed control with oxyfluorfen alone was

87% or greater for all weeds rated with the exception of *Schoenoplectus mucronatus* (L.) Palla (ricefield bulrush), and *Leptochloa fascicularis* (Lam.) A. Gray (bearded sprangletop) in both years. All *O. sativa spontanea* exposed to soil-applied oxyfluorfen successfully emerged through the soil surface, but became completely necrotic 28 days after flooding.

CONCLUSION

Oxyfluorfen-tolerant rice system was demonstrated to be a viable management strategy for California rice growers who struggle with grass- and sedge-weed control as well as provide a novel herbicide option for California *O. sativa spontanea* management.

Keywords: water-seeded rice, weedy (red) rice

INTRODUCTION

The US is the fifth largest exporter of rice in the world, with approximately 1.23 million ha of rice planted in 2020 including 0.21 million ha in California alone.(Childs, 2021) California rice production is unique in part due to access to aerial equipment, and lack of labor availability compared to other rice-growing regions of the world.(Rao, Brainard, Kumar, Ladha, & Johnson, 2017) Most growers implement early flooding for weed control and utilize aerial seeding with pre-germinated seed.(Kumar & Ladha, 2011) Heavy clay soil in this region make it difficult to rotate crops, so many growers practice continuous rice (Linquist et al., 2008), sometimes for decades.

Persistent aquaphilic rice-weed species have been selected for in California due to long-term monoculture rice production in combination with a lack of available herbicides and year-round flooding. *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass), *Echinochloa oryzicola* (Vasinger) Vasinger (late watergrass), and *L. fasicularis*, major grass-weed species common in California rice, can cause up to 79%, 59%, and 36% in yield losses, respectively, if left uncontrolled for a growing season.(Brim-Deforest, Al-Khatib, & Fischer, 2017)

There are currently seven modes of action available for use in California rice, all of which have only a single active ingredient available with the exception of ALS inhibitors which have six available active ingredients.(Linquist et al., 2018) The major populations of grass weeds, *E. crus-galli*, *Echinochloa oryzoides* (Ard.) Fritsch (early watergrass), and *E. oryzicola*, collectively referred to as *Echinochloa* spp, and *L. fasicularis* have exhibited some resistance to all of the available modes of action with selectivity for grasses. Benzobicyclon (an HPPD inhibitor) which was recently registered in 2017, is the only exception, but this active ingredient is only effective on *L. fasicularis* and *E. crus-galli*. The California rice growing culture has led to nine herbicide-resistant weed species, some of which exhibit multiple-resistance.(Becerra-Alvarez & Al-Khatib, 2022)

O. sativa spontanea, a “weedy” rice that competes with its conspecific relative, cultivated rice (*Oryza sativa*), is a notably problematic rice-weed in California as well.(Delouche et al., 2007) California *O. sativa spontanea* accessions are likely the result of wild, weedy and cultivated rice parentage from within and outside of California.(De Leon et al., 2019) *O. sativa spontanea* has caused up to \$274 ha^{-1} in losses in Arkansas, the largest rice producing state in the US.(Burgos, Norsworthy, Scott, & Smith, 2008) Rice varieties grown in California have higher yields and market values compared to Arkansas varieties,(Childs, 2021) thus an uncontrolled *O. sativa spontanea* outbreak in California could cause considerable losses.

Oryza sativa spontanea was found in California in 2003, and by 2016 there were 4,050 ha reportedly infested with one or more accessions.(De Leon et al., 2019) Cultural control methods are the only options for managing *O. sativa spontanea*. Control strategies include purchasing certified seed,(California Department of Pesticide Regulation, 2017) sanitation of field equipment, hand-pulling, and permitted field burning.(Espino, Brim-DeForest, & Johnson, 2018) However, these practices seldom provide adequate *O. sativa spontanea* control. The Southern US rice growing regions have quizalofop- and imidazolinone-resistant cultivars available for aiding in the control of this pest. California did not have any herbicide-tolerant rice varieties available to growers at the time of this study. However, increased weed pressures have encouraged research on adapted herbicide-resistant varieties for the temperate California environment.

A single recessive gene responsible for oxyfluorfen resistance, “ROXY”, was developed through non-transgenic methods by breeders at the RES.(20180070548, 2018) Albaugh LLC (Ankeny, Iowa, USA) partnered with the RES to develop proprietary formulations of oxyfluorfen and oxyfluorfen-tolerant rice utilizing the “ROXY” gene. Oxyfluorfen is a Herbicide Resistance Action Committee, Weed Science Society of America group 14 herbicide, (PubChem, 2017) that inhibits the protoporphyrinogen oxidase (protox) enzyme in the tetrapyrrole biosynthesis pathway before leading to heme and chlorophyll synthesis.(Matringe et al., 1992) Inhibition of this enzyme results in accumulation of protoporphyrinogen IX, which leaks from the plastid into the cytoplasm and is rapidly oxidized by protoporphyrin IX.(Lee, Duke, & Duke, 1993) This enzyme reacts with light to generate singlet-oxygen in the cytoplasm, causing rapid peroxidation of the cell membrane and consequently cell damage.(Becerril & Duke, 1989; Jacobs, Jacobs, Shreman, & Duke, 1991) Injury symptoms associated with pre-emergent oxyfluorfen application include rapid leaf bleaching and wilting upon emergence, necrosis and desiccation.(Weed Science Society of America, 2014) There is one other herbicide, carfentrazone, with the same mode of action available to California rice growers, which has no known rice-weed resistance issues.

Oxyfluorfen-tolerant rice and associated chemistry represent a significant technological advancement in California rice-weed management. Despite this, no research has been published to date on the efficacy of this technology in water-seeded rice. Therefore, the objectives of these studies were to evaluate the level of weed control and crop injury in oxyfluorfen-tolerant rice systems.

MATERIALS AND METHODS

Weed control and crop injury assessment in oxyfluorfen-tolerant rice

Field site characteristics and preparation

Field studies to assess crop injury, weed control, and grain yield in ROXY RPS® (oxyfluorfen-tolerant) rice systems were conducted at the RES (39.46°N, 121.74°W) in

2019 and 2021. Field soil at this location is Esquon-Neerdobe clay soil, containing 28% sand, 41% silt, and 31% clay with 2.54% organic matter and a pH of 4.91.

All oxyfluorfen was applied in liquid formulation as “ALB2023”, a novel and proprietary formulation created by Albaugh LLC. Applications were soil-applied, i.e., pre-flood and pre-seeding of rice, using a 6-m boom sprayer with six, 8003XR flat-fan nozzles (TeeJet, Camarillo, CA, USA), calibrated to deliver 187 L ha^{-1} application volume at 275 kPa pressure. ALB2023 was applied at five rates, 560, 840, 980, 1123, 1262 g ai ha^{-1} to determine the best rate for weed control. A sixth treatment, 1123 g ai ha^{-1} of ALB2023 followed by 35 g ai ha^{-1} penoxsulam (Granite® GR, Corteva Agrisciences, Wilmington, DE, USA) at the five leaf-stage of rice, was included to represent an additional sequential herbicide treatment option. One to three days after ALB2023 was applied, all plots were flooded and maintained at a 10 cm average depth for the entire growing season. ROXY RPS® rice seeds were soaked in tap water for 24-hours prior to planting and spread by hand into standing flood waters at a rate of 160 kg ha^{-1} on June 15th in 2019 and June 1st in 2021.

Weed Control Assessment

Weed control was visually estimated on a 0 to 100 scale where 0 represented no control and 100 represented complete control. Common rice-weeds in this field were the *Echinochloa* spp. group, *L. fasicularis*, *S. mucronatus*, *Cyperus difformis* L. (smallflower umbrella sedge), *Heteranthera limosa* (Sw.) Willd (ducksalad), *Monochoria vaginalis* (Burm. f.) C. Presl ex Kunth (monochoria), *Bacopa monnieri* (L.) Pennell (water hyssop), and *Ammannia auriculata* Willd (redstem). Weed control was rated at 7, 14, 28, and 60 DAS.

Crop injury evaluation

ROXY RPS® rice was visually evaluated for shoot and leaf bleaching, chlorosis, plant stunting and overall stand reduction at 3, 7, 14, 28, and 60 DAS in both years. Injury metrics were rated on a similar scale as the weed control ratings, where 0 represented no symptoms, and 100 represented plant death. Rice grain was harvested on October 28th in 2019 and October 16th in 2021, with a small-plot combine (Almaco, Nevada, IA, USA); grain weights were adjusted to 14% moisture.

Experimental design and data analysis

Fields were arranged into 3 x 6-m treatment plots, separated by levees to prevent flood water and, consequently, herbicide movement among treatments. Treatments were organized in a randomized complete block design with 3 replicates in 2019 and 4 replicates in 2021. Weed control and crop injury ratings were collected on a time-course and separated by collection date during data analysis. Weed control, crop injury, and yield data were subjected to an ANOVA test using the lm function in *stats* package in R version 4.1.2.(R Core Team, 2022) Treatment means were separated with Fisher's least significant difference test (α=0.05).

Oryza sativa spontanea response to oxyfluorfen

Seed material

The *O. sativa spontanea* accessions chosen for this study, simply referred to as accessions 1, 2, 3, and 5, comprised 95% of the field infestations reported in 2020 (Brim-Deforest, unpublished). All California *O. sativa spontanea* accessions have characteristics that differentiate them from one another as well as cultivated rice, including some amount of seed dormancy and high seed shattering (Table 2.1). *Oryza sativa spontanea* plants were first collected from field surveys conducted in 2016 and used for seed multiplication efforts.(Espino et al., 2018) All accessions used in this experiment were grown in controlled greenhouse environments at Orchard Park Greenhouse complex on the University of California Davis campus from 2016-2019 with plants separated in space to prevent cross-pollination. Once harvested, seeds were stored at 0°C until needed for experimentation. “M-206” rice, a medium-maturity, medium-grain cultivar was compared to weedy rice in this study (Table 2.1).

Table 2.1 Descriptions of California M-206 rice as well as the four *Oryza sativa spontanea* accessions used in this study. “High” dormancy is considered more than 5 years, “low” dormancy is less than 5 years. Average observed 1000-seed weight includes standard error.(De Leon et al., 2019; Espino et al., 2018; Galvin, Inci, Mesgaran, Brim-Deforest, & Al-Khatib, 2022)

Accession	Hull color	Grain type	Dormancy	Shattering	1000-seed weight (g)
M-206	Straw	Medium	None	None	26.36 ± 0.3
Accession 1	Straw	Short	High	High	19.88 ± 0.2
Accession 2	Bronze	Medium	Low	High	27.04 ± 0.3
Accession 3	Straw	Medium	High	High	25.87 ± 0.3
Accession 5	Straw	Long	Low	High	26.68 ± 0.3

Growing conditions

Studies were replicated in time, once in March and again in June 2021, at the Orchard Park Greenhouse complex. Grow lights emitting an average 8,297 lumens $m^{2\ -1}$ were utilized for 12/12 hours day/night for both experiments. Ambient air temperature was recorded using a HOBO® TidbiT MX2203 data logger (Onset Computer, Bourne, MA, USA). Greenhouse air temperature in experimental replicate 1 averaged 26.9°C, ranging from 21-36°C; temperature in experimental replicate 2 averaged 27.9°C, ranging from 19-35°C.

All *O. sativa spontanea* accessions have some level of seed dormancy and underwent priming before planting; M-206 seed do not have a dormancy trait. Seeds of each *O. sativa spontanea* accession were placed into paper coin envelopes in a dry, dark incubation chamber for 5 days at 50°C. Seeds were planted on day six after the priming process was completed.(Galvin et al., 2022)

Soil was taken from historical rice fields at the RES and has similar properties as field soil previous described. Square, plastic pots measuring 8.9 x 8.9 x 8.9 cm with drainage holes were partially filled with soil, then 10 primed weedy rice seeds of a single accession were placed on the soil surface. Pots were then filled with a remaining 1.3 or 2.5 cm soil to ensure differentiation between planting depths. Once the 8.9 cm pots were filled with soil, they were nested within 46 x 66 x 38 cm clear, polycarbonate tubs to maintain a continuously flooded environment.

Oxyfluorfen application

Oxyfluorfen (Goal® 2XL, Nufarm, Melbourne, Australia) was applied in liquid formulation the day after planting using a closed spray chamber (Technical Machining Inc., Folsom, CA, USA) equipped with an 8002E flat-fan nozzle (TeeJet), calibrated to deliver 187 L ha^{-1} at 275 kPa pressure. Oxyfluorfen was soil-applied at 0, 560, 1120, 2240, or 4480 g ai ha^{-1}. Tubs were filled with water on the day of spraying to an 8-cm depth that did not cover the soil surface. 24-hours after spraying, all tubs were flooded to and maintained at a 10 cm depth above the soil surface. Copper sulfate (Millipore Sigma, St. Louis, MO, USA) was mixed with deionized water and applied in liquid formulation at a rate of 500 g ai ha^{-1} at 5, 12, 19, and 26 days after flooding (DAF) to discourage algae interference with rice emergence.(Ohadi, Laguerre, Madsen, & Al-Khatib, 2021)

***Oryza sativa spontanea* injury assessment**

Accessions were not removed from the water for the duration of the experiments to mimic the field environment to the best extent possible. Cumulative seedling emergence was recorded daily beginning one DAF, and was determined by a visible cotyledon of 5 mm or greater emerging from the soil surface.

Stunting and necrosis injury were visually assessed at 7, 14, 21, and 28 DAF and compared to the equivalent untreated control (UTC) accession. Stunting injury was observed in oxyfluorfen-tolerant rice and was determined to be an important indicator of *O. sativa spontanea* stress, or lack thereof. Necrosis was determined by the presence of leaf browning and/or crinkling, and a lack of visible turgor which became apparent in plants that would sway significantly in the water compared with UTC accessions. These metrics were rated on a 0 to 100 scale where 0 represented no injury, and 100 represented plant death. Plants were removed from the water at 28 DAF, harvested at the soil surface, and dried at 50°C for 3 weeks to collect dry weight measurements.

Experimental design and data analysis

This experiment was designed as a 3-factor, split-split plot with repeated measures in time (Altman & Krzywinski, 2015). Herbicide rate represented the whole plot factor, burial depth represented the sub-plot factor, and timing of data collection represented the sub-subplot factor. The entire experiment was repeated in time. Herbicide treatments (5 including the UTC) were contained within tubs and distributed in the greenhouse environment in a complete randomized design with 4 replicates of each herbicide rate for each experimental run. Randomly distributed within each block were 2 replicates of each soil burial depth by accession combination.

Emergence data were reported as a total, and thus was analyzed as a split-plot design. Herbicide rate represented the whole plot factor, and burial depth represented the sub-plot factor. Stunting and necrosis data were collected on a time-course, and thus analyzed as a split-split plot design with repeated measures. Herbicide rate and interactions with burial depth were incorporated into the emergence, stunting, and necrosis models as sources of error using the aov function in the *stats* package in R.(R Core Team, 2022) Relevant treatment means were separated with Fisher's least significant difference test (α=0.05).

RESULTS

Field assessment of oxyfluorfen-tolerant rice system

Weed control

There was a three-way interaction between weedy species, herbicide treatment, and year ($P < .001$), thus results are reported across all combinations of these variables for each data collection point. All herbicide treatments including the sequential treatment had greatest control over broadleaf weeds, *H. limosa*, *M. vaginalis*, *B. monnieri*, and *A. auriculata*, compared to grasses and sedges in both 2019 and 2021. Oxyfluorfen applied at 840 g ai ha^{-1} or greater had at least 96% control of *H. limosa* for the duration of the 2019 field season (Table 2.2). Plots treated with the lowest rate of oxyfluorfen, 560 g ai ha^{-1}, in 2019 had less control, 87%, over *H. limosa* at 28 DAS compared with all other treatments. Control of *H. limosa* at this same rate increased to 97% at 60 DAS. Oxyfluorfen applied at 980 g ai ha^{-1} or more had at least 98% control of *H. limosa* for the duration of the 2021 field season. Oxyfluorfen applied at 560 and 840 g ai ha^{-1} had 100% control of *H. limosa* until 28 DAS at which time control was slightly reduced, but returned to 100% at 60 DAS.

Table 2.2 Broadleaf weed control as affected by oxyfluorfen applied at different rates in water-seeded rice in 2019 and 2021 field studies. Data are separated by collection date, either 28 or 60 days after seeding. Letters designate significant differences (α=0.05) between treatments in a given year at a specific collection date.

		Heteranthera limosa				*Monochoria vaginalis*			
		2019		2021		2019		2021	
		28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)
Herbicide	Rate (g ai ha $^{-1}$)	% Control							
Oxyfluorfen	560	87 b	97 a	90 c	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen	840	96 a	98 a	95 b	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen	980	97 a	98 a	100 a	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen	1123	100 a	99 a	98 ab	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen	1262	99 a	100 a	98 ab	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen fb penoxsulam	1123 fb 35	97 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Herbicide	Rate (g ai ha $^{-1}$)	*Bacopa monnieri*				*Ammannia auriculata*			
Oxyfluorfen	560	90 b	100 a	97 b	100 a	100 a	92 b	100 a	100 a
Oxyfluorfen	840	100 a	100 a	100 a	100 a	100 a	98 a	100 a	100 a
Oxyfluorfen	980	100 a	100 a	100 a	100 a	100 a	98 a	99 a	100 a
Oxyfluorfen	1123	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen	1262	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Oxyfluorfen fb penoxsulam	1123 fb 35	100 a	100 a	100 a	100 a	100 a	98 a	100 a	100 a

DAS – days after seeding, fb – followed by

M. vaginalis was not observed in any herbicide treated plot in 2019, and was controlled at 98% or greater in 2021 regardless of oxyfluorfen application rate (Table 2.2). Plots treated with 840 g ai ha $^{-1}$ oxyfluorfen or more had 100% control of *B. monnieri* for the duration of the 2019 field season (Table 2.2). Plots treated with 560 g ai ha $^{-1}$ oxyfluorfen maintained 100% control of *B. monnieri* until 28 DAS, at which time control decreased to 90%. Control at this same rate increase back to 100% at 60 DAS for the remainder of the

2019 field season. In 2021, Oxyfluorfen and the sequential treatment had 98% control or greater of *B. monnieri*, regardless of application rate, for the duration of the season.

Plots treated with 840 g ai ha^{-1} oxyfluorfen or more had 98% or greater control of *A. auriculata* for the duration of the 2019 season (Table 2.2). Plots treated with 560 g ai ha^{-1} oxyfluorfen maintained 100% control of *A. auriculata* until 60 DAS, at which time control decreased to 92% for the remainder of the 2019 field season. In 2021, Oxyfluorfen and the sequential treatment had 99% control or greater of *A. auriculata*, regardless of application rate, for the duration of the season.

Oxyfluorfen had greater, more consistent control of *Echinochloa* spp. compared with *L. fasicularis* in both years. *Echinochloa* spp. control was 93% or greater for all herbicide treatments for the duration of the 2019 field season (Table 2.3). Plots treated with the lowest rate of oxyfluorfen, 560 g ai ha^{-1}, in 2021 had less control, 94%, of *Echinochloa* spp. at 28 DAS compared to all other herbicide treatments. Outside of this specific time point, all herbicide treatments, including the lowest rate, maintained 98% or greater control of *Echinochloa* spp. for the duration of the 2021 field season.

L. fasicularis control was 93% or greater for all herbicide treatments for the first 28 DAS in 2019 (Table 2.3). Plots treated with 840 g ai ha^{-1} oxyfluorfen had 72% control of *L. fasicularis* at 60 DAS, significantly less than plots treated with 980 g ai ha^{-1}, 95%, in 2019. In 2021 *L. fasicularis* was 100% controlled by all herbicide treatments from 7-28 DAS. At 60 DAS The lowest rate of oxyfluorfen had 74% control, less than all other treatments.

Table 2.3 Grass-weed control as affected by oxyfluorfen applied at different rates in water-seeded rice in 2019 and 2021 field studies. *Echinochloa crus-galli*, *Echinochloa oryzoides*, and *Echinochloa oryzicola* are included in "*Echinochloa* spp." Data are separated by collection date, either 28 or 60 days after seeding. Letters designate significant differences (α=0.05) between treatments in a given year at a specific collection date.

		Echinochloa spp.				*Leptochloa fasicularis*			
		2019		2021		2019		2021	
		28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)
Herbicide	Rate (g ai ha $^{-1}$)	% of control							
Oxyfluorfen	560	93 a	95 a	99 a	94 b	93 a	82 ab	100 a	74 b
Oxyfluorfen	840	96 a	95 a	100 a	100 a	96 a	72 b	100 a	98 a
Oxyfluorfen	980	100 a	98 a	100 a	98 a	100 a	95 a	100 a	95 a
Oxyfluorfen	1123	99 a	98 a	100 a	100 a	99 a	87 ab	100 a	99 a
Oxyfluorfen	1262	99 a	99 a	100 a	100 a	99 a	91 ab	100 a	97 a
Oxyfluorfen fb penoxsulam	1123 fb 35	100 a	99 a	100 a	100 a	100 a	82 ab	100 a	97 a

DAS – days after seeding, fb – followed by

We observed a similar difference between sedge-weeds, *S. mucronatus* and *C. difformis,* as was observed between grass-weeds, *Echinochloa* spp. and *L. fasicularis*. *C. difformis* control with all herbicide application rates was greater than 95% for the duration of the 2019 and 2021 field seasons (Table 2.4).

Control of *S. mucronatus* was 100% in all herbicide treated plots for the first 28 DAS in 2019 (Table 2.4). Control of *S. mucronatus* in plots treated with 560 g ai ha^{-1} of oxyfluorfen was 77% at 60 DAS in 2019, less than plots treated with 1262 g ai ha^{-1} of oxyfluorfen and the sequential treatment. Plots treated with 840 g ai ha^{-1} oxyfluorfen had 80% control at 60 DAS, less than the sequential treatment, but similar control to all other oxyfluorfen application rates at this time in 2019. Control of *S. mucronatus* was 100% in all herbicide treated plots for the first 14 DAS in 2021. At 28 DAS in 2021, control of *S. mucronatus* was less, 96%, when 560 g ai ha⁻¹ oxyfluorfen was applied compared to 100% control when 980, 1,123 g ai ha⁻¹ oxyfluorfen, and the sequential treatment were applied (Table 2.4). Differences in *S. mucronatus* control were more pronounced between treatments at 60 DAS in 2021. Plots treated with 560 g ai ha⁻¹ oxyfluorfen had only 44% control, less than all other application rates. Plots treated with 840 g ai ha⁻¹ oxyfluorfen had 68% control, similar to plots treated with 980 g ai ha⁻¹, but less control than plots treated with 1123, 1262 g ai ha⁻¹ oxyfluorfen, and the sequential treatment.

Table 2.4 Sedge-weed control as affected by oxyfluorfen applied at different rates in water-seeded rice in 2019 and 2021 field studies. Data are separated by collection date, either 28 or 60 days after seeding. Letters designate significant differences (α=0.05) between treatments in a given year at a specific collection date.

		Schoenoplectus mucronatus				*Cyperus difformis*			
		2019		2021		2019		2021	
		28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)	28 (DAS)	60 (DAS)
Herbicide	Rate (g ai ha ⁻¹)	% of control							
Oxyfluorfen	560	100 a	77 c	96 b	44 d	100 a	100 a	95 b	100 a
Oxyfluorfen	840	100 a	80 bc	99 ab	68 c	100 a	100 a	100 a	100 a
Oxyfluorfen	980	100 a	88 abc	100 a	76 bc	100 a	99 a	98 ab	100 a
Oxyfluorfen	1123	100 a	86 abc	100 a	91 ab	100 a	100 a	98 ab	100 a
Oxyfluorfen	1262	100 a	91 ab	98 ab	93 ab	100 a	99 a	98 ab	100 a
Oxyfluorfen fb penoxsulam	1123 fb 35	100 a	98 a	100 a	100 a	100 a	100 a	100 a	100 a

DAS – days after seeding, fb – followed by

Oxyfluorfen-tolerant rice injury and yield

Stunting and stand reduction were the only injury metrics observed in either year. There was a 2-way interaction between year and treatment (P=0.012) that influenced stunting injury, therefore results for each combination were reported for each data collection point.

Oxyfluorfen-tolerant rice exhibited some stunting at 7 DAS in 2019 (Table 2.5). The lowest rate of oxyfluorfen, 560 g ai ha⁻¹, and plots treated with 840 g ai ha⁻¹ had similar rates of stunting, less than rates at or above 980 g ai ha⁻¹ which ranged from 45-72% stunted. There was no stunting observed at 14 DAS or later in 2019. At 7 DAS in 2021 oxyfluorfen-tolerant rice was observed to have similar amounts of stunting injury for all herbicide application rates. There was a negligible amount of stunting in 2021 at 14 DAS. There was a complete lack of observable stunting at 28 DAS in both years.

Table 2.5 Oxyfluorfen-tolerant rice stunting injury and yield as affected by oxyfluorfen applied at different rates in water-seeded rice in 2019 and 2021 field studies. Letters designate significant differences (α=0.05) among treatments within rating dates in a given year. Any treatment without a letter indicates insignificance.

		Stunting				Yield	
		2019		2021		2019	2021
		7 (DAS)	28 (DAS)	7 (DAS)	28 (DAS)		
Herbicide	Rate (g ai ha $^{-1}$)	Injury (% of control)				kg ha $^{-1}$	
Oxyfluorfen	560	7 b	0 -	25 a	0 -	8445 a	7776 c
Oxyfluorfen	840	40 ab	0 -	31 a	0 -	8348 a	9281 abc
Oxyfluorfen	980	45 a	0 -	31 a	0 -	8793 a	8847 bc
Oxyfluorfen	1123	72 a	0 -	31 a	0 -	9155 a	9638 ab
Oxyfluorfen	1262	53 a	0 -	35 a	0 -	9197 a	8669 bc
Oxyfluorfen fb penoxsulam	1123 fb 35	55 a	0 -	38 a	0 -	9794 a	10 868 a
Untreated control		NA	NA	NA	NA	9015 a	5591 c

DAS – days after seeding, fb – followed by; NA – not applicable

There was some stand reduction at 14 DAS in 2019, but rice recovered by 28 DAS and was not observed at 60 DAS (data not shown). There was no stand reduction observed in 2021 for any herbicide treated plot. There were no differences in yield for any treatment including the UTC in 2019 (Table 2.5). UTC yields were similar to plots treated with 560, 840, 980, and 1262 g ai ha^{-1} oxyfluorfen, but less than plots treated with 1123 and the sequential treatment in 2021.

***Oryza sativa spontanea* response to oxyfluorfen**

Total emergence of *O. sativa spontanea* in the greenhouse was not influenced by oxyfluorfen rate or experimental run. Additionally, there were no interactions between rate and burial depth. There were significant interactions between burial depth and accession (P< .001), therefore data were pooled across experimental run and application rate for all accession by burial depth combinations (N =64). Differences in timing of emergence based on seed burial depth were not observed, but there was less total emergence from 2.5 cm soil burial depths compared with 1.3 cm for all weedy accessions (Fig. 2.1). Accession 3 had the greatest emergence, 62%, at 1.3 cm burial depth, followed by accession 5, 48%, accession 2, 35%, and accession 1, 21%. Accession 3 had 30% emergence at 2.5 cm burial depth, similar to accession 5, 27%, but greater than accession 2, 20%, and accession 1, 7%. M-206 did not emerge more than 5% at either burial depth.

Differences in *O. sativa spontanea* stunting and necrosis data were found between accessions for each data collection day as well as between experimental run 1 and 2. Neither stunting or necrosis data were influenced by herbicide rate or burial depth, so data were pooled across these variables (N = 64).

There was no observed stunting injury for any accession at 7 DAF in experimental run 1 (Fig 2.2). The greatest differences between accessions were at 14 DAF in experimental run 1, with M-206 having the least stunting injury, 44%, compared with accessions 1, and 2, 72 and 78%, respectively, and accessions 3 and 5, 89 and 91%, respectively.

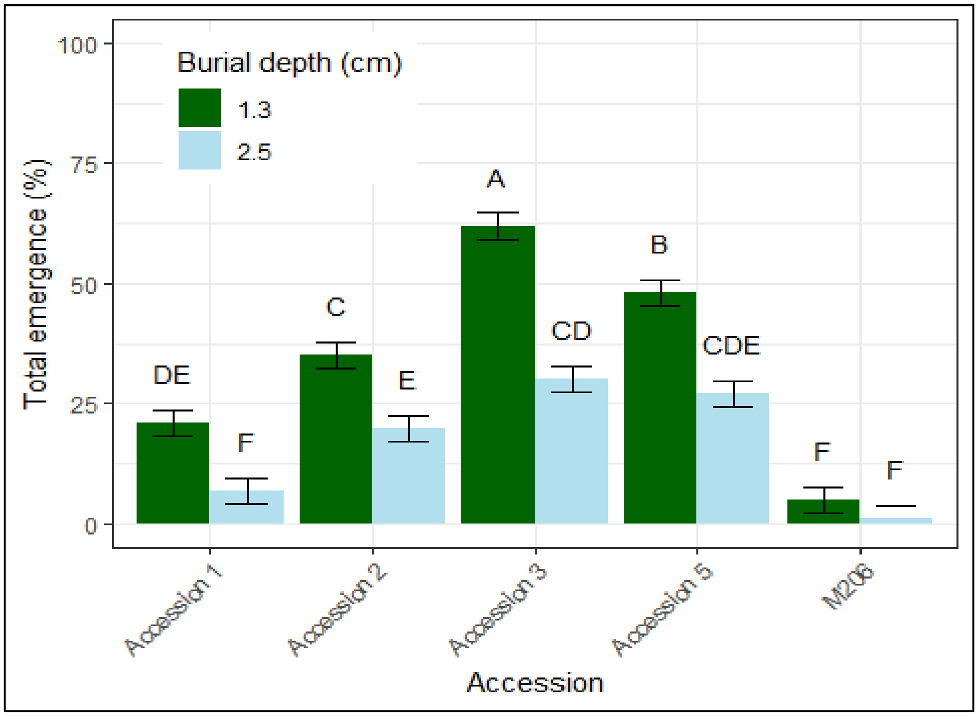

Figure 2.1 The interaction between weedy accession and burial depth influenced total *Oryza sativa spontanea* emergence ($p < 0.001$). There were no differences in emergence between oxyfluorfen application rates in the greenhouse study. Data were pooled for each rice accession at each burial depth across all oxyfluorfen rates (N=64). Error bars illustrate standard error and letters indicate significant differences (α=0.05) between accession and burial depth combinations.

There was no difference in stunting injury between accessions, 86-92%, at 21 DAF in experimental run 1. Differences between *O. sativa spontanea* accessions was more pronounced at 7 DAF than any other time point in experimental run 2. M-206 was stunted 7% at 7 DAF, followed by accession 1, 23%, accession 5, 41%, and accessions 2 and 3, 58%. M-206 had less stunting at 14 DAF in experimental run 2, 69%, compared with all other accessions which ranged from 90-92%.

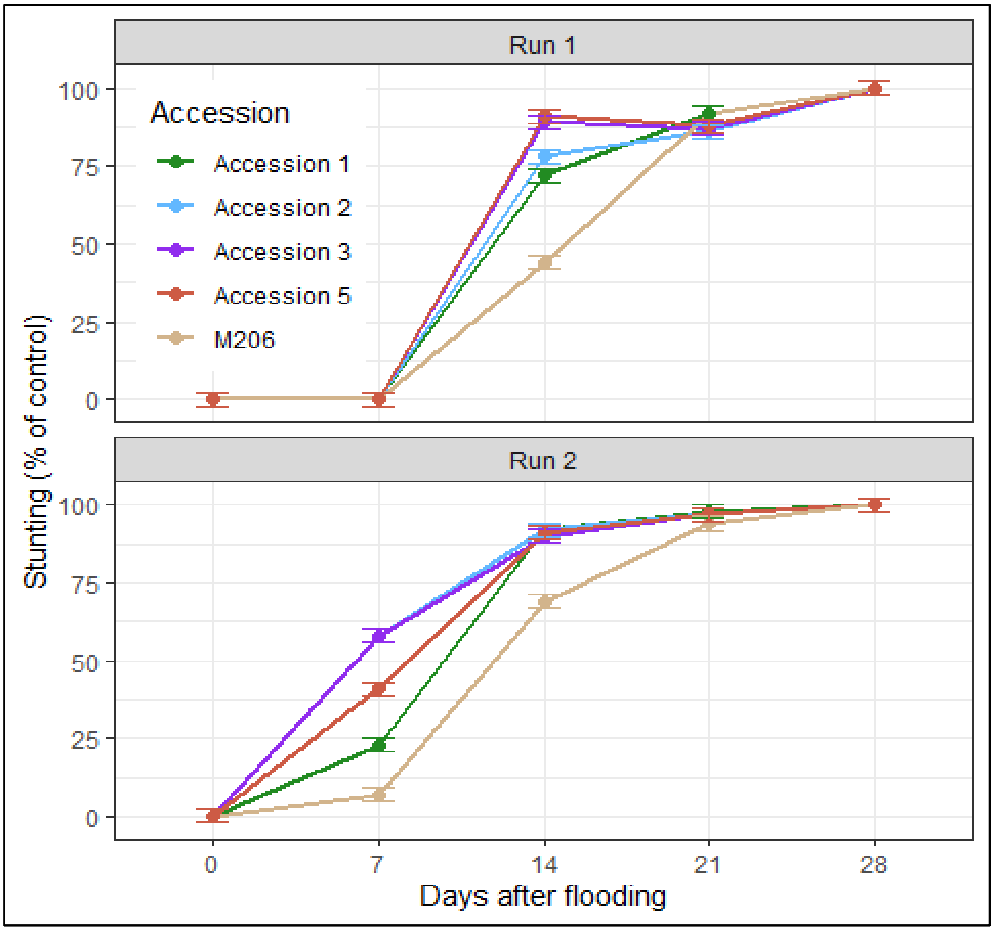

Figure 2.2 Stunting ratings from greenhouse studies for all rice accessions over time for both experimental run 1 and 2. Accession by collection date interactions as well as run influenced stunting ratings, so data were combined for all oxyfluorfen rates and burial depths. Error bars represent standard error between accessions at a single data collection point.

There was no difference in stunting injury between accessions, 94-98%, at 21 DAF in experimental run 2. At 28 DAF in both experimental run 1 and 2, all accessions were greatly stunted with less than three leaves per plant compared to the UTC. It was noted that almost all accessions in UTC blocks had begun to tiller and were on average twice the height of the tubs (76 cm) by 28 DAF.

There was no necrotic injury observed at 7 DAF in experimental run 1 (Fig 2.3). Accession 1 and 2 had similar injury, 20 and 15%, respectively at 14 DAF in experimental run 1, less than all other accessions which ranged from 33-39%. Accession 2 had less necrotic injury, 51%, compared with all other accessions at 21 DAF in experimental run 1. All other accessions had similar injury at this time point which ranged from 60-70%. Necrotic injury was observed earlier in experimental run 2 compared with run 1. Accession 2 had less necrotic injury, 6% at 7 DAF in experimental run 2 compared with accession 1, 13%. Accession 2 at 14 DAF had 40% necrotic injury, less than all other accession which ranged from 49-53%. Outside these specific instances, necrotic injury was similar for all accessions in experimental run 2 at 7, 14, and 21 DAF, respectively. All accessions were completely necrotic by 28 DAF in both experimental run 1 and 2.

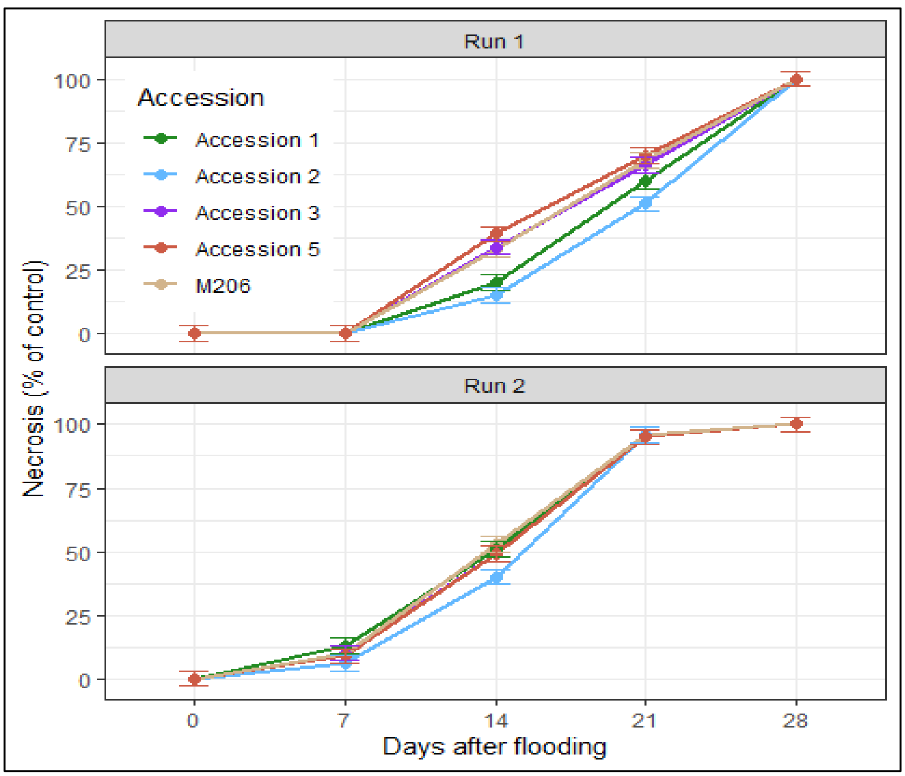

Figure 2.3 Necrosis ratings from greenhouse studies for all rice accessions over time for both experimental run 1 and 2. Accession by collection date interactions as well as run influenced stunting ratings, so data were combined for all oxyfluorfen rates and burial depths. Error bars represent standard error between accessions at a single data collection point.

DISCUSSION

Oxyfluorfen efficacy in combination with oxyfluorfen-tolerant rice

Weed Control

Oxyfluorfen demonstrated excellent weed control of most weeds observed, notably *Echinochloa* spp. which are resistant to many rice-weed herbicides available in California. Oxyfluorfen had reduced control of *Leptochloa fasicularis* and *Schoenoplectus mucronatus* compared with all other broadleaf, sedge, and grass rice-weeds. Species-specific seed germination requirements and emergence timing is likely why there was less control of certain weeds at specific times in the season. *H. limosa* and *B. monnieri* rapidly emerge in large flushes and likely contributed to reduced control at 28 DAF, specifically.(Oraze & Grigarick, 1992) *Leptochloa fasicularis* seed priming requirements are relatively long(Driver, Al-Khatib, & Godar, 2020), thus this species germinates and emerges later in the season compared to *Echinochloa* spp. (Song, Shi, & Song, 2015). *Schoenoplectus mucronatus* has also been observed to emerge later than other weeds in this field as well (Brim-Deforest et al., 2017).

A suggested sequential herbicide treatment, 1123 g ai ha^{-1} of oxyfluorfen followed by 35 g ai ha^{-1} of penoxsulam applied at the 5-leaf stage of rice, was incorporated into field studies to demonstrate the benefit of including herbicides with a later application timing. This sequential herbicide treatment accomplished 95% or greater control of *S. mucronatus* in both years with the later application of penoxsulam. Reduced control of these specific weeds also highlights the need to incorporate species-specific biological knowledge, e.g., timing of emergence, when making weed management plans and not sole reliance on chemical control.

Crop injury and yield

Water-seeded, oxyfluorfen-tolerant rice demonstrated some short-term (7-28 DAS) crop injury from oxyfluorfen, but was not observable at 60 DAS in either year. Stunting injury from residual oxyfluorfen applications (150-600 g ai ha^{-1}) was observed on conventional transplanted rice cultivars in India.(Sathya Priya, Chinnusamy, Murali Arthanari, & Janaki, 2017) Stunting observed in both California and India was likely a response to protox enzyme inhibition, which rapidly effects chlorophyll synthesis and causes cell membrane destruction in susceptible populations.(Weed Science Society of America, 2014) Oxyfluorfen-tolerant rice and associated chemistry have been developed together to reduce crop injury to the greatest extent possible.

This research demonstrates that oxyfluorfen-tolerant rice systems have the potential to provide yields as high as current averages, 11200-13500 kg ha^{-1}.(Linquist et al., 2018) Any rice injury observed in plots treated with oxyfluorfen at 980 g ai ha^{-1} or more did not affect final yield outcomes. The sequential treatment also had one of the highest yields in 2021, significantly greater than the UTC plots. This was expected due to the increase in control of *S. mucronatus* with penoxsulam. Yield outcomes reinforce the practice of mixing of modes of action with different application timings for obtaining the best rice-weed control.

Using oxyfluorfen as a weed control option for *Oryza sativa spontanea*

It was hypothesized that *O. sativa spontanea* may not emerge through the soil surface upon exposure to soil-applied oxyfluorfen. This hypothesis was incorrect; total emergence was not different between application rates in either run. Protox enzyme inhibition can occur very quickly in the presence of light. [17] Seed germination and initial emergence, however, are enabled by carbohydrate reserves in the seeds, and is an independent process from the protox enzyme activity.(Bewley, Bradford, Hilhorst, & Nonogaki, 2013)

Oxyfluorfen has a half-life of 2-3 days in water,(PubChem, 2017; Weed Science Society of America, 2014)and 12 days in puddled soils found in transplanted rice systems,(Das, Debnath, & Mukherjee, 2003) and is highly susceptible to photolysis degradation. [19]Despite this knowledge, there is a lack of published information on the half-life of oxyfluorfen in water-seeded rice systems found in California.

Separate planting depths were selected because *O. sativa spontanea* seeds buried deeper in the soil have been shown to emerge later.(Galvin, Brim-DeForest, & Al-Khatib, 2021) Grounded in current oxyfluorfen half-life information, *O. sativa spontanea* emerging later was hypothesized to experience fewer injury symptoms. There was a difference in total emergence between *O. sativa spontanea* seeds buried at 1.3 and 2.5 cm, but no difference in timing of emergence initiation or injury symptoms. Additional information is needed on the longevity of oxyfluorfen in water-seeded rice in California to determine if the window of efficacy encompasses *O. sativa spontanea* accessions emerging later, e.g., 12 days after flooding.

Stunting injury was not consistent across *O. sativa spontanea* accessions in the greenhouse study, with greater initial injury observed in accession 2, 3, and 5. These accessions had greater total soil emergence, produced more dry weight at the end of 28 DAF, and have a greater seed weight and size compared to accession 1 (Table 2.1). Large seed size and weight have been found to result in more vigorous seedlings, providing more plant material to express injury symptoms.(Roy et al., 1996) M-206 emerged later and is slower to mature compared with weedy accessions. [33] This is likely why reduced stunting injury was observed in this cultivar compared with rapidly growing weedy accessions under the same conditions.

Many *O. sativa spontanea* accessions may have become completely necrotic earlier than 21 DAF. Accessions were completely submerged under water for the duration of the experiment and remained upright, giving the appearance of viability. Once removed from the water at 28 DAF, however, it became clear that plants would not be able to out-grow injury symptoms and the assumption of complete plant death was accepted.

CONCLUSION

Oxyfluorfen caused lasting necrotic injury to all weeds tested regardless of species, application rate, testing location, year, or burial depth. Rice growers are encouraged to consider incorporating herbicide treatments with later application timings when using the oxyfluorfen-tolerant rice system to control weeds emerging later in the season and ensure long-term efficacy against major weed pressures. Oxyfluorfen and associated rice technology is likely to provide a reliable, efficacious weed control option for rice growers who struggle with management of sedge- and grass-weed species including California *O. sativa spontanea*.

ACKNOWLEDGEMENTS

This research was financially supported by the Melvin Androus Endowment. The authors would like to acknowledge the California Rice Research Board, Rice Experiment Station staff, as well as Albaugh LLC for their physical and material support in conducting field research.

REFERENCES

1. Childs N, Rice Sector at a Glance 2021. https://www.ers.usda.gov/topics/crops/rice/rice-sector-at-a-glance/#production [accessed 17 January 2022]

2. Rao AN, Brainard DC, Kumar V, Ladha JK, Johnson DE, Preventive Weed Management in Direct-Seeded Rice: Targeting the Weed Seedbank, ed. Sparks DL, Advances in Agronomy 144th ed, Elsevier Science Publishers, London, pp. 114 (2017).

3. Kumar V, Ladha JK, Direct Seeding of Rice. Recent Developments and Future Research Needs, ed. Sparks DL, Advances in Agronomy 3rd ed. Elsevier Science Publishers, London, pp. 297–414 (2011).

4. Linquist B, Fischer A, Godfrey L, Greer C, Hill J, Koffler K, Moeching M, Mutters R, Van Kessel C, Minimum tillage could benefit California rice farmers. *Calif Agric* 62:24–9 (2008).

5. Brim-Deforest WB, Al-Khatib K, Fischer AJ, Predicting yield losses in rice mixed-weed species infestations in California. *Weed Sci* 65:61–72 (2017).

6. Linquist B, Al-Khatib K, Swett C, Espino L, Leinfelder-Miles M, Brim-DeForest W, McKenzie K, Weeds and Rice growth and development, in Rice Production Manual. University of California Agriculture and Natural Resources, Davis, CA, USA, pp. 9.6, 1.1 (2018).

7. Becerra-Alvarez A, Al-Khatib K, editors. Tracking herbicide-resistant weeds in California through a community-driven approach: Proceedings of 75th Western Society of Weed Science annual meeting; 2022 March 7-10; Newport Beach, CA, USA. Western Society of Weed Science, http://www.wsweedscience.org/publications/proceedings/ (2022).

8. Delouche JC, Burgos NR, Gealy DR, Zorrilla de San Martin G, Labrada R, Larinde M, Rosell C, Introduction in Weedy Rices: Origin, Biology, Ecology and Control, FAO, Plant Production and Protection, Rome, pp. 1 (2007).

9. De Leon TB, Karn E, Al-Khatib K, Espino L, Blank T, Andaya CB, Andaya VC, Brim-DeForest W, Genetic variation and possible origins of weedy rice found in California. *Ecol Evol* 1–14 (2019).

10. Burgos NR, Norsworthy JK, Scott RC, Smith KL, Red rice (*Oryza sativa*) status after 5 years of imidazolinone-resistant rice technology in Arkansas. *Weed Technol* 22:200–8 (2008).

11. California Department of Pesticide Regulation, A Guide to Pesticide Regulation in California. https://www.cdpr.ca.gov/docs/pressrls/dprguide.htm [accessed 10 January 2022]

12. Espino L, Brim-DeForest W, Johnson T. Weedy Rice in California: Addressing an Emerging Pest Through Research and Outreach. *CAPCA Advis* 11:44-48 (2018).

13. McKenzie K, Oxyfluorfen resistant rice lines. US Patent 2 018 007 0548 (2018).

14. National Library of Medicine. Hazardous Substances Data Bank. https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7507 [accessed 12 January 2022]

15. Matringe M, Camadro J, Block M, Joyard J, Scalla R, Labbe P, Douce R, Localization within the chloroplast of protoporphyrinogen oxidase the target enzyme for diphenyl ether-like herbicides. *J Biol Chem* 267:4646–4651 (1992).

16. Lee H, Duke M, Duke S, Cellular localization of protoporphyrinogen-oxidizing activities of etiolated barley (Hordeum vulgare) leaves. *Plant Physiol* 102:881–889 (1993).

17. Becerril J, Duke S, Protoporphyrin IX content correlates with activity of photobleaching herbicides. *Plant Physiol* 90:1175–1181 (1989).

18. Jacobs J, Jacobs N, Shreman T, Duke S, Effect of diphenyl ether herbicides on oxidation of protoporphyrinogen to protoporphyrin in organellar and plasma membrane enriched fractions of barley. *Plant Physiol* 97:197–203 (1991).

19. Weed Science Society of America. Oxyfluorfen in Herbicide Handbook ed. Shraner DL, Weed Science Society of America, pp. 14, 335-336 (2014).

20. R Core Team, R Foundation for Statistical Computing, Version 4.1.2. Vienna, Austria (2022).

21. Galvin L, Inci D, Mesgaran M, Brim-Deforest W, Al-Khatib K, Flooding depths and burial effects on seedling emergence of five California weedy rice (*Oryza sativa spontanea*) accessions. *Weed Sci* 2:213-219 (2022).

22. Ohadi S, Laguerre G, Madsen J, Al-Khatib K, Toward understanding the impact of nuisance algae bloom on the reduction of rice seedling emergence and establishment. *Weed Sci* 1:95–102 (2021).

23. Altman N, Krzywinski M, Points of significance: Split plot design. *Nat Methods* 3:165–166 (2015).

24. Brim-DeForest WB, Al-Khatib K, Linquist BA, Fischer AJ, Weed community dynamics and system productivity in alternative irrigation systems in California rice. *Weed Sci* 1:177–188 (2017).

25. Oraze MJ, Grigarick AA, Biological control of ducksalad (*Heteranthera limosa*) by the waterlily aphid (*Rhopalosiphum nymphaeae*) in rice (*Oryza sativa*). *Weed Sc*i 2:333–336 (1992).

26. Driver KE, Al-Khatib K, Godar A, Bearded sprangletop (*Diplachne fusca* ssp. *fascicularis*) flooding tolerance in California rice. *Weed Technol* 34:193–196 (2020).

27. Song B, Shi J, Song S, Dormancy release and germination of *Echinochloa crus-galli* grains in relation to galactomannan-hydrolysing enzyme activity. *J Integr Agric* 8:1627–1636 (2015).

28. Sathya Priya R, Chinnusamy C, Murali Arthanari P, Janaki P, Carryover effect and plant injury from oxyfluorfen herbicide applied in transplanted rice. *Int J Chem Stud* 3:535–539 (2017).

29. Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H, Structure and composition, in Seeds: Physiology of Development, Germination, and Dormancy. Springer, New York, pp. 7 (2013).

30. Das AC, Debnath A, Mukherjee D, Effect of the herbicides oxadiazon and oxyfluorfen on phosphates solubilizing microorganisms and their persistence in rice fields. *Chemosphere* 3:217–21 (2003).

31. Galvin L, Brim-DeForest W, Al-Khatib K. editors. Thermal Time to Emergence of California Weedy Rice (*Oryza sativa* f. *spontanea*) Under Flooded Field Conditions: A 2-year Study: Proceedings of the 61st Weed Science Society of America Annual Meeting; 2021 February 15-19; Virtual. Weed Science Society of America (2021).

32. Roy SKS, Hamid A, Giashuddin Miah M, Hashem A, Seed size variation and its effects on germination and seedling vigour in rice. *J Agron Crop Sci* 2:79–82 (1996).

33. Karn E, De Leon T, Espino L, Al-Khatib K, Brim-DeForest W, Effects of competition from California weedy rice (*Oryza sativa* f. *spontanea*) biotypes on a cultivated rice variety. *Weed Technol* 34:666-674 (2020).

Chapter 3

Utilizing germinability thresholds for optimizing stale seedbed applications to control weedy rice (*Oryza sativa spontanea*) in California rice cropping systems

Liberty B. Galvin, Kassim Al-Khatib

Formatted for dissertation submission

Abstract

Weedy rice (*Oryza sativa spontanea*) is a problematic weed due to its phenotypic similarities with cultivated rice (*Oryza sativa*). Limited herbicide availability has driven a need for non-chemical control options for managing this pest. One pre-planting strategy that is being explored is the stale seedbed methodology which aims to maximize soil seedbank withdrawals via germination. This technique is adapted in rice by flooding a field, waiting for germination and emergence of weed seedlings, and completed with a mechanical or chemical control application. Optimization of this process is dependent on maximizing weed seed germination which is primarily influenced by temperature and moisture. Germinability across a range of these factors is not well understood in California weedy rice. Thus, this study aimed to determine germinability of California weedy rice accessions under various temperature and water potential treatments. Previously described weedy rice accessions 1, 2, 3, and 5 along with M-206, a common California rice cultivar, were exposed to temperatures from 10-40°C in combination with water potentials of 0, -0.2, -0.4, or -0.8 MPa until either germination or weed seed decay occurred. Statistical analysis indicated a 3-way interaction between accession, temperature, and water potential. Germination reached 95% when seeds were exposed to temperatures between 20-35°C in combination with 0 or -0.2 MPa. Germination was lowest when seeds were under water stress (-0.8 MPa), temperatures were colder than 20°C, or warmer than 35°C. These results could be utilized in the decision-making process for successfully implementing the stale seedbed methodology targeting weedy rice via maximizing germination.

Keywords: Accession, “red” rice, temperature, water potential, water-seeded rice

Introduction

Weedy (“red”) rice, *Oryza sativa spontanea*, is a problematic weed species due to its conspecificity with cultivated rice (*Oryza sativa*). Weedy rice has several traits that make this pest undesirable for cultivation, e.g., high shattering, seed dormancy (Kanapeckas et al., 2018), and poor cooking quality (David R. Gealy & Bryant, 2009). It is more competitive than its cultivated relatives (Karn et al., 2020), and increases milling costs if found in harvested grain (Ottis et al., 2005). Durand-Morat et al. (2018) estimated economic losses in the United States of 38 million USD per year under a moderate weedy rice infestation scenario. The most common method of weedy rice removal in California is hand-pulling due to the limited number of herbicides available for selectively managing weedy rice in cultivated rice fields (Espino et al., 2018). Due to these constraints, non-chemical control methods are needed to manage weedy rice in California rice cropping systems.

Early season control of all weed species is critical to reduce competition with cultivated rice. One opportunity for early-season management of weedy rice is with the stale ("false") seedbed methodology (Espino et al., 2016). The foundational goal of this technique is to germinate as many weed seeds as possible from the seedbank and eliminate them with nonselective pre-plant control strategies. This method is implemented in rice by flooding the field to some degree for some amount of time (Ceseski et al., 2022; Dilipkumar et al., 2022; Singh et al., 2018). Flooding ceases after weed seeds germinate and seedlings emerge. Once the field is accessible by machinery, either mechanical (organic) or chemical (conventional) control strategies can be implemented to destroy weed seedlings (Singh et al., 2018). However, there is uncertainty about whether current practices maximize weed seed germination, because the amount of water and time needed for the flood waters to remain on the field is not well-documented for water-seeded rice systems.

Weed seed germination is a response to ideal environmental conditions including temperature, moisture, oxygen, light or solar radiation, and nitrogen availability (Travlos et al., 2020). California weedy rice can germinate in the dark without the addition of nitrogen (Galvin et al., 2022) while preliminary studies suggest accessions can germinate anaerobically. Thus, the most influential factors over weedy rice germination are temperature and moisture availability. In addition, some weedy rice accessions are believed to have been present in the California environment for some time (Kanapeckas et al., 2016), and have likely adapted to water-seeded conditions. Regardless of these assumptions, the range of temperatures necessary as well as the amount of soil moisture for maximizing the germinability of California weedy rice is currently unknown, which may be limiting the effectiveness of the stale seedbed methodology. In particular, understanding how different accessions respond to a range of soil temperature and moisture levels is necessary to develop site-specific management approaches for different fields, as growers often know which weedy rice accession they are dealing with.

Temperature and water potential were chosen for this study to determine germinability because they are grower-accessible metrics and can be controlled to some degree. Flood waters used by rice producers in California are usually sourced from deep reservoirs and are often too cold for rice production (Roel et al., 2005). Most growers utilize warming basins to increase water temperatures, and consequently soil temperatures (Al-Kayssi et al., 1990). Knowing how much water to apply for how long at a given temperature would allow growers to have greater influence on weedy rice seed germination. This study tested the hypothesis that germination would decrease under temperature and moisture stress, but specific thresholds would be different for different weedy rice accessions. The objective of this study was to determine total germination of California weedy rice under a range of temperatures and water potentials.

Materials and Methods

Seed multiplication protocols

California weedy rice accessions were first identified during a 2016 survey of infested rice fields (Espino et al., 2018). Accessions were first differentiated by phenotypic traits and later confirmed by phylogenetic heredity (De Leon et al., 2019). Accessions were simply referred to as 1, 2, 3, and 5. Seed multiplication was conducted from 2016-2019 in a controlled greenhouse on University of California Davis campus with accessions identified during the 2016 survey. Plants were grown in 3.8-L pots that were submerged in trays holding 5 cm of standing water through the plant's lifecycle. Plants were kept separate once flowering commenced to reduce cross-pollination between distinct accessions. Upon maturity, seeds were harvested and stored at 0°C until experimentation began. Table 3.1 describes each accession as well as M-206, a medium-maturity, medium-grain variety utilized frequently in California rice production.

Table 3.1 Description of M-206 and weedy rice accessions used for experimentation. "High" dormancy is defined as 5 years or more, and "low" dormancy is less than 5 years. Standard error is presented with average observed 1000-seed weight (Galvin et al. 2022).

Accession	Grain type	Hull color	Shattering	Dormancy	1000-seed weight (g)
Accession 1	Short	Straw	High	High	19.88 ± 0.2
Accession 2	Medium	Bronze	High	Low	27.04 ± 0.3
Accession 3	Medium	Straw	High	High	25.87 ± 0.3
Accession 5	Long-	Straw	High	Low	26.68 ± 0.3
M-206	Medium	Straw	None	None	26.36 ± 0.3

Germination experiments

Weedy rice seeds were primed with heat treatment, 50°C for 120 hours in a dry, dark growth chamber to break dormancy prior to experimentation (Galvin et al. 2022). M-206 is nondormant and was not subjected to the priming treatment. Water potential solutions of -0.2, -0.4, and -0.8 MPa were made using polyethylene glycol (PEG 8000) and deionized water (Boddy et al., 2012; Hardegree & Emmerich, 1990). Solutions containing -0.4 and -0.8 MPa were mixed for a minimum of 24-hours, and -0.2 MPa was mixed for a minimum of 12-hours using a stir plate and magnetic rod. Solutions were measured for accuracy with a frequently calibrated WP4-T Dewpoint PotentiaMeter (Meter Group, Pullman, Washington, USA) to ensure proper mixture of the solutions. A control treatment of 0 MPa, i.e., deionized water, was also incorporated into the experiment. Once solutions were mixed and measured, 25 sheets of 90 mm diameter filter paper (GE, Waukesha, Wisconsin, USA) were submerged in beakers of each water potential solution for 24-hours prior to experimentation to ensure full saturation of the filter paper (Hardegree & Emmerich, 1990b). Beakers containing solutions were sealed with 3 layers of parafilm (Fisher Scientific, Waltham, Massachusetts, USA) at all times to prevent evaporation of deionized water from PEG solutions.

A single saturated filter paper was placed into each 100 x 15 mm petri dish (Fisher Scientific, Waltham, Massachusetts, USA), then 25 primed seeds of a given accession were placed on top of the filter paper and wetted with an additional 5 mL of the respective water potential solution. Petri dishes were wrapped with parafilm and checked frequently for cracks. Petri dishes exposed to temperatures at and above 30°C, received more parafilm compared with cooler temperatures and were rewrapped when necessary. There were four petri dishes of each accession by water potential combination for each temperature treatment.

Petri dishes were placed into dark growth chambers (CMP6050 or CMP6010 Conviron, Pembina, North Dakota, USA) and kept at one of the following constant temperatures for the duration of the experiment: 10, 15, 20, 24, 30, 35, or 40°C. Each temperature treatment containing all accession by water potential treatment combinations was repeated 3 times. A seed was considered germinated when the radicle reached 1 mm or longer. Seed germination was evaluated daily, and petri dishes were not opened for the duration of the experiment in order to prevent contamination. Decomposition of the seeds, defined by the presence of mold, determined the length of each study, defined in 7-day increments, either 7, 14, 21, or 28 days.

Data analysis

This study was arranged in a split-split plot design with temperature representing the whole plot, and water potential representing the sub-plot factor, both applied in combination to each accession (Altman & Krzywinski, 2015). A Welch's t-test was used to compare experimental replicates to one another and ensure homogeneity of variance between sample means, e.g., accession 1 exposed to 10C and 0MPa in run 1 was compared to the same treatment combination in run 2. Any whole experimental replicate that did not meet homogeneity of variance was not included in the analysis. Data that did meet homogeneity of variance were pooled for the analysis of variance. Temperature, water potential, accession, and their interactions were incorporated into a statistical model using the lm function in the *stats* package in R, and subjected to an ANOVA (R Core team). Treatment means were separated with Fisher's least significant difference test ($\alpha=0.05$).

Results

Water potential, temperature, and accession (each $p< 0.0001$), as well as a 3-way interaction between these variables ($p< 0.0001$) significantly influenced germination. To illustrate this interaction, a quadratic regression and confidence intervals were fit to the water potential by temperature data to best represent the response of each accession. Table 3.2 lists equations for each regression to depict similarities between responses to water potential for each accession across temperature. R^2 values are also listed to elucidate the quadratic fit to the data. Results of the 3-way interaction are described by their means to give the most accurate depiction of seed germination responses.

Seeds exposed to 0 or -0.2 MPa in combination with temperatures between 20-35°C had an average of 95% or greater total germination regardless of accession (Figure 3.1). All seeds began to germinate after 3-4 days and reached maximum germination within 10 days at these temperatures. Total germination decreased when seeds of any accession were exposed to temperatures colder than 20°C and warmer than 35°C. All seeds exposed to -0.8 MPa typically had less total germination compared with all other water potentials, regardless of temperature (Figure 3.2).

Table 3.2 Quadratic equations as well as R^2 values for water data distributed across water potential and temperature combinations for each accession tested for germination.

Water potential (MPa)	quadratic equation	R^2
	Accession M206	
0	$y = 105.63 + 0.38(x) - 0.42(x-24.69)^2$	0.79
-0.2	$y = 107.87 + 0.36(x) - 0.44(x-24.83)^2$	0.83
-0.4	$y = 109.38 + 0.23(x) - 0.46(x-25.06)^2$	0.86
-0.8	$y = 60.28 + 0.87(x) - 0.35(x-26)^2$	0.63
	Accession 1	
0	$y = 64.41 + 1.81(x) - 0.30(x-24.88)^2$	0.81
-0.2	$y = 64.07 + 1.82(x) - 0.32(x-24.5)^2$	0.78
-0.4	$y = 84.13 + 1.23(x) - .44(x-24.17)^2$	0.85
-0.8	$y = 58.57 + 1.76(x) - 0.47(x-24.57)^2$	0.79
	Accession 2	
0	$y = 56.70 + 2.16(x) - 0.29(x-24.05)^2$	0.76
-0.2	$y = 71.22 + 1.62(x) - 0.32(x-24.63)^2$	0.77
-0.4	$y = 86.16 + 1.20(x) - 0.45(x-23.92)^2$	0.83
-0.8	$y = 64.41 + 1.29(x) - 0.47(x-24.62)^2$	0.82
	Accession 3	
0	$y = 59.36 + 1.87(x) - 0.29(x-25.13)^2$	0.88
-0.2	$y = 59.56 + 1.79(x) - 0.38(x-24.55)^2$	0.91
-0.4	$y = 79.87 + 0.89(x) - 0.43(x-24.75)^2$	0.85
-0.8	$y = 25.41 + 1.52(x) - 0.30(x-23.98)^2$	0.71
	Accession 5	
0	$y = 83.68 + 1.04(x) - 0.29(x-25)^2$	0.73
-0.2	$y = 92.94 + 0.69(x) - 0.42(x-24.74)^2$	0.86
-0.4	$y = 92.86 + 0.47(x) - 0.42(x-24.85)^2$	0.88
-0.8	$y = 51.18 + 1.03(x) - 0.36(x-24.72)^2$	0.80

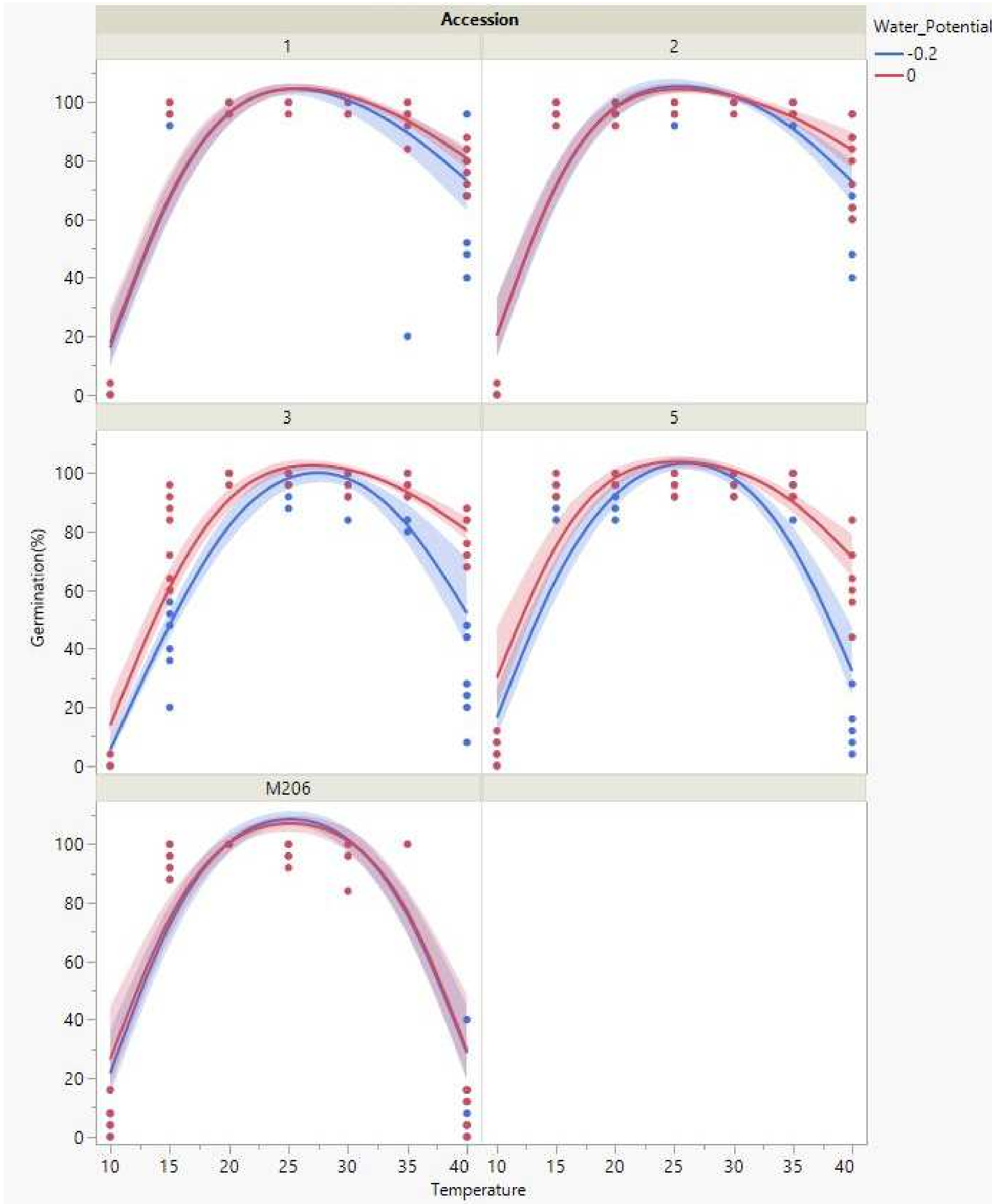

Figure 3.1 Total germination response for each accession across temperature when exposed to adequate moisture (0, -0.2 MPa). Scatter points represent raw data from each replicate, lines represent a quadratic fit for the data, and shaded areas are confidence of fit.

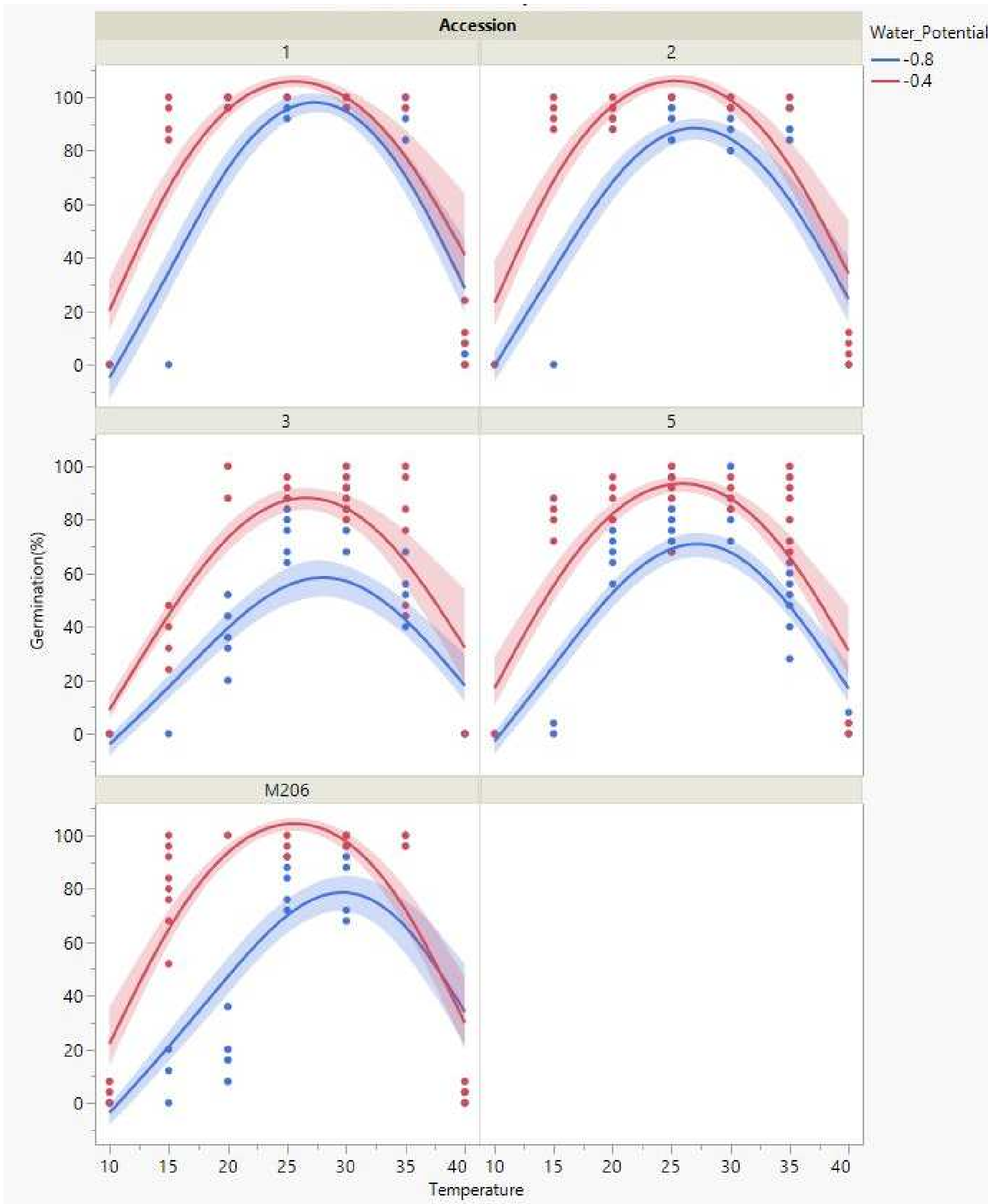

Figure 3.2 Total germination response for each accession across temperature when exposed to water stressed (-0.8, -0.4 MPa) conditions. Scatter points represent raw data from each replicate, lines represent a quadratic fit for the data, and shaded areas are confidence of fit.

Germination responses to temperature stress

Accession 3, followed by accession 1, 2, M206 and accession 5 were least to most cold-tolerant. M206 and accession 5 seeds exposed to 10°C had an average of 6% or less total germination regardless of water potential (Figure 3.1, 3.2). Accessions 1, 2, and 3 did not germinate at this temperature. Accession 3 seeds had a total average germination of 80% at 15°C and 0 MPa, all other accessions had a total average seed germination of 91% or more when water potential was -0.2 or 0 MPa (Figure 3.1). M206 followed by accession 5, 3, 1, and 2 were least to most heat tolerant. M206 had the lowest total seed germination at 40°C, 5%, when exposed to 0 MPa compared with an average 63 to 79% total germination for weedy accessions under the same treatment combinations.

Germination responses to moisture stress

Accession 3 followed by M-206, accession 5, 2 and 1 seeds were greatest to least sensitive to moisture stress. M-206, accession 2, 3, and 5 seeds exposed to 15 and 20°C had less germination at -0.8 MPa compared with all other water potential treatments at these temperatures (Figure 3.2). M-206, Accession 3 and 5 exposed to 25°C had less total germination at -0.8 MPa compared with all other water potential treatments at the same temperature. M-206 and accession 3 seeds had less total germination at -0.8 MPa and 30°C. Accession 3 and 5 seeds had less total germination at 35°C and -0.8 MPa compared with less negative water potentials at the same temperature. Accessions 3 and 5 seeds exposed to 40°C had less total germination with more negative water potentials. Accession 1 and 2 seeds had less total germination at -0.4 MPa in combination with 40°C compared with less negative water potentials (Figure 3.2). Accession 1 was least moisture sensitive and had similar total germination regardless of water potential at 20, 25, 30 and 35°C temperature treatments, respectively.

Discussion

Time to decomposition of seeds

Greater than 95% of seeds within a petri dish would develop mold spores within 3 days of initial observation of mold. It was noted that mold decreased visibility of radicle protrusion and induced decomposition of the seed. It was assumed once mold was observed, seeds were no longer viable and would not germinate successfully. Seeds decayed faster at warmer temperatures compared with cooler temperatures. Seeds exposed to 10 and 15°C developed mold by 28 days after the start of experimentation. Seeds exposed to either 20 or 25°C required 21 days to develop mold, 14 days for seeds exposed to 30°C, and 7 days for seeds exposed to either 35 or 40°C.

Ideal conditions for stale seedbed efficacy on California weedy rice

A field is considered "saturated" with water, i.e., at or near 0 MPa, once all soil pores have been filled and water begins to accumulate on the soil surface (O'Geen, 2013). Time to saturation and amount of water needed to reach saturation is dependent on soil properties. California rice fields are mostly dense clay and have a hard-pan layer allowing for continuous flooding (Sass et al., 1994). A water potential at or near 0 MPa can be assumed without measurement once consistent standing water is observed in a field, regardless of soil texture and properties.

Pre-season weed management strategies, such as the stale seedbed technique, work best when germination of the target weed species is maximized. This allows for destruction of the greatest number of weed seedlings before the crop is planted and withdrawal from the soil weed-seedbank for long-term management (Rao et al., 2007). All California weedy rice accessions in this study had greatest total germination at or near 0 MPa, indicating that fields must be flooded to complete saturated conditions with visible standing water on the soil surface to induce maximum germination. Weedy seed germination is connected to its habitat of origin (Evans & Etherington, 1990). Year-round flooding strategies that are foundational to the California rice growing culture (Brim-DeForest et al., 2017) likely have had significant selection pressure on California weedy rice.

Total weedy rice seed germination for all accessions occurred rapidly at temperatures between 20-35°C. Historical average air temperatures from 2018-2020 in this area during the rice planting period, April, May, and early June, were 16, 21, and 24°C, respectively (UCIPM, 2022). Temperatures in May and June were within ideal ranges for California weedy rice germination, however, temperatures in April were less than optimal for maximizing germination of accession 3.

Previous research suggests that weedy rice seedlings within the top 2.5 cm of the soil surface will emerge through both the soil and 15 cm of flood waters (Galvin et al. 2022, chapter 1). Considering historical temperature ranges and standard flooding practices, weedy rice seeds within the top 2.5 cm of the soil would be expected to germinate and emerge in May and June with full field saturation maintained for a minimum of 7 days.

Disadvantageous conditions for stale seedbed efficacy on weedy rice

Results from this study suggest that cooler temperatures in April, e.g., at or less than 15°C, would require complete soil-saturated conditions for achieving maximum germination. Germination decreased significantly for -0.4 and 0.8 MPa treatments, but was relatively high for -0.2 and 0 MPa water potential, except for accession 3. California has been experiencing severe drought on and off since 2000 which may pose challenges for maintaining saturated field conditions during stale seedbed implementation. Moreover, weedy rice seeds exposed to cooler temperatures required longer amounts of time to germinate, regardless of accession. The amount of time a field is maintained at fully saturated conditions would need to be more than 10 days in order to achieve maximum germination from the soil seedbank, especially for accessions 1, 2, and 3 which were more sensitive to cold compared with M206 and accession 5. If pre-season temperatures are low and water availability is scarce, the stale seedbed methodology may be ineffective for controlling California weedy rice.

The stale seedbed methodology can also be time consuming which could delay rice planting and increase the risk of yield losses. Heavy clay soil in the rice growing regions of California are used for rice production due to their water holding capabilities (Rosenberg et al., 2022). There is some time between removal of flood waters and when it is appropriate to operate heavy machinery, e.g., cultivation or spraying equipment, in the field. This drying period is different depending on the soil texture (O'Geen, 2013) and water availability, which is regulated for the given year by irrigation districts; growers may not have control of when they receive water. Winter rains have also proceeded into April in recent years, making it temporally difficult to implement a stale seedbed methodology before the time comes to prepare the soil for planting cultivated rice. The time required for soil drying, and minimal access to flood water in combination with cool temperatures are not conducive for stale seedbed application. Under these conditions, the stale seedbed methodology would not be financially or temporally efficacious in controlling all California weedy rice accessions.

Post-harvest management of weedy rice

Current best management practices of weedy rice suggest growers reduce fall tillage in order to minimize incorporation of seeds into the soil seedbank (Espino et al., 2016). Results from this study support these practices in a post-harvest setting. If temperatures are high, and moisture is being applied to the field for winter flooding, reduced fall tillage could be a potential strategy for causing either germination or decay of seeds that have shattered onto the soil surface (Linquist et al., 2008). This opportunity would be most efficacious for accessions 1 and 2 which had highest total germination of all accessions at 35°C under water stressed conditions (-0.8 MPa) which could occur between the harvest and winter flooding periods.

Holistic stale seedbed applications for water-seeded rice

A stale seedbed application should ideally control all problematic weeds present. Grass-weed species, Echinochloa spp. (*Echinochloa crus-galli*, i.e., barnyardgrass, *Echinochloa oryzoides*, early watergrass, and *Echinochloa oryzicola*, late watergrass) and bearded sprangletop (*Leptochloa fascicularis*), as well as sedges, ricefield bulrush

(*Schoenoplectus mucronatus*) and smallflower umbrella sedge (*Cyperus difformis*), are highly problematic due to resistance to most available rice-herbicides in California (Becerra-Alvarez & Al-Khatib, 2022; Hill et al., 2006). These species require alternative control methods, e.g., stale seedbed application incorporating herbicides, to maximize control. Ceseski et al. (2022) reported >50% control of all *Echinochloa* spp. and bearded sprangletop species when applying a stale seedbed in advance of drill-seeded rice. The rice field was flooded and herbicide applied 7 days before cultivated rice emergence. Average air temperatures in Ceseski et al. (2022) were within optimal range for weedy rice germination. However, weedy rice cotyledon elongation and shoot emergence through the soil and flood waters may have occurred at the same time as drill-seeded rice emergence based on field observations of weedy rice emergence (Galvin et al., 2021). The conspecificity between weedy- and cultivated rice is a core issue in managing weedy rice; similar emergence timing between these rice-relatives is not ideal for management efficacy.

Boddy et al., (2012) found *Echinochloa phyllopogon* (late watergrass), specifically, had greatest germination under high moisture availability and when temperatures were 35°C with some germination occurred between 15 and 31°C. Barnyardgrass has similar moisture and temperature requirements as late watergrass (Song et al., 2015). Conversely, sprangletop requires 2 weeks of wet-chilling to germinate (Driver et al., 2020). Both of the aforementioned *Echinochloa* species as well as weedy rice would likely germinate and emerge in a stale seedbed application that was made during May or June of a given year. A stale seedbed that takes into consideration the 2-week, wet-chilling requirements for sprangletop seed germination would likely encompass the moisture requirements for other grass-weed species. However, 2 weeks of flooding may not be practical due to time restraints between the drying period and preparation for planting.

Smallflower umbrella sedge has similar temperature requirements for germination compared to weedy rice. Pedroso (2011) found that maximum germination occurred after 14 days or less depending on accession when seeds were exposed to temperatures between 22.3 and 33.7°C under fully saturated conditions. This study took into account various resistant and susceptible features which differentiated accessions. Unlike Southern accessions of weedy rice, California weedy rice has no known herbicide resistance issues at this time. The development of herbicide resistance in one accession could change the thermal and moisture requirements for germination. Field observations of smallflower umbrella sedge demonstrated a required 18 days to reach 100% germination (Lundy et al., 2014). Time constraints are the major factors in whether or not a field manager will choose this methodology for controlling these species.

Ricefield bulrush has been observed to emerge later in the season compared with other rice, grass-weeds (Becerra-Alvarez et al., 2022; Brim-DeForest et al., 2017). Similarly, to sprangletop, ricefield bulrush is not likely to reach maximum germination in a timeframe that is conducive for efficacious stale seedbed and subsequent field preparation, pre-planting. The stale seedbed is only a component of a greater weed management plan. Managers should consider the germination requirements of their most problematic species before implementation of this strategy to optimize the time and effort required for maximum germination and consequently efficacy.

Conclusions

Flooding a field to full soil capacity for a minimum of 7 days when average water temperatures are between 20-35°C will allow for the maximum number of weedy rice seeds to germinate, regardless of accessions present in the field. Historical average temperatures during April-June, the time when stale seedbed applications are made, will likely fall between these temperature ranges. Results from this study suggest that cooler temperatures (<20°C) will begin to affect accessions differently and may result in less-than ideal germination, especially if water availability is scarce. Growers can use the biological information detailed in this research to determine if a stale seedbed is the best option given the accessions present, precipitation, temperature, and water availability in a given year, as well as the other problematic weed species present in their fields.

References

Al-Kayssi, A. W., Al-Karaghouli, A. A., Hasson, A. M., & Beker, S. A. (1990). Influence of soil moisture content on soil temperature and heat storage under greenhouse conditions. *Journal of Agricultural Engineering Research*, *45*(C), 241–252.

Al-khatib, K. (2017). Spread and Management of Herbicide Resistant Weeds in California Rice. In *California Weed Science Society* (p. 1). California Weed Science Society.

Altman, N., & Krzywinski, M. (2015). Points of significance: Split plot design. *Nature Methods*, *12*(3), 165–166.

Becerra-Alvarez, A., & Al-Khatib, K. (2022). Tracking herbicide-resistant weeds in California through a community-driven approach. In *WSWS annual meeting*. Newport Beach, CA.

Becerra-Alvarez, A., Ceseski, A. R., & Al-Khatib, K. (2022). Rice Response and Weed Control from Clomazone Applied at Different Timings in a Continuous Flood System. *Weed Technology*.

Becerril, J., & Duke, S. (1989). Protoporphyrin IX content correlates with activity of photobleaching herbicides. *Plant Physiology*, *90*, 1175–1181.

Benvenuti, S., Macchia, M., & Miele, S. (2001). Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science*, *49*(4), 528–535.

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds: Physiology of Development, Germination, and Dormancy* (third). New York: Springer.

Boddy, L. G., Bradford, K. J., & Fischer, A. J. (2012). Population-based threshold models describe weed germination and emergence patterns across varying temperature, moisture and oxygen conditions. *Journal of Applied Ecology*, *49*(6), 1225–1236.

Brim-Deforest, W. B., Al-Khatib, K., & Fischer, A. J. (2017). Predicting Yield Losses in Rice Mixed-Weed Species Infestations in California. *Weed Science*, *65*, 61–72.

Brim-DeForest, W. B., Al-Khatib, K., Linquist, B. A., & Fischer, A. J. (2017). Weed Community Dynamics and System Productivity in Alternative Irrigation Systems in California Rice. *Weed Science*, *65*(1), 177–188.

Burgos, N. R., Norman, R. J., Gealy, D. R., & Black, H. (2006). Competitive N uptake between rice and weedy rice. *Field Crops Research*, *99*(2–3), 96–105.

Burgos, N. R., Norsworthy, J. K., Scott, R. C., & Smith, K. L. (2008). Red Rice (Oryza sativa) Status after 5 Years of Imidazolinone-Resistant Rice Technology in Arkansas. *Weed Technology*, *22*(1), 200–208.\

California Department of Pesticide Regulation. (2017). *A Guide to Pesticide Regulation in California*. Sacramento, California.

Ceseski, A. R., Godar, A. S., & Al-Khatib, K. (2022). Combining stale seedbed with deep rice planting: a novel approach to herbicide resistance management? *Weed Technology*, *36*(2), 261–269.

Chauhan, Bhagirath S. (2012). Weed Ecology and Weed Management Strategies for Dry-Seeded Rice in Asia. *Weed Technology*, *26*(1), 1–13.

Chauhan, Bhagirath S., & Johnson, D. E. (2011). Competitive interactions between weedy rice and cultivated rice as a function of added nitrogen and the level of competition. *Weed Biology and Management*, *11*(4), 202–209.

Chauhan, Bhagirath Singh. (2012). Weedy rice (Oryza sativa) II. Response of Weedy Rice to Seed Burial and Flooding Depth. *Weed Science*, *60*(03), 385–388.

Childs, N. (2021). *Rice Sector at a Glance*. Retrieved from https://www.ers.usda.gov/topics/crops/rice/rice-sector-at-a-glance/#production

Das, A. C., Debnath, A., & Mukherjee, D. (2003). Effect of the herbicides oxadiazon and oxyfluorfen on phosphates solubilizing microorganisms and their persistence in rice fields. *Chemosphere*, *53*(3), 217–221.

De Leon, T. B., Karn, E., Al-Khatib, K., Espino, L., Blank, T., Andaya, C. B., Andaya, V. C., Brim-DeForest, W. (2019). Genetic variation and possible origins of weedy rice found in California. *Ecology and Evolution*, 1–14.

Delouche, J. C., Burgos, N. R., Gealy, D. R., Zorrilla de San Martin, G., Labrada, R., Larinde, M., & Rosell, C. (2007). *Weedy Rices: Origin, Biology, Ecology and Control* (Plant Production and Protection No. 188). Rome.

Dilipkumar, M., Mohamad-Ghazali, M. S. S., Shari, E. S., Chuen, N. L., Chauhan, B. S., & Chuah, T. S. (2022). Integrated use of the stale seedbed technique with preemergence herbicides to control weedy rice in wet seeded rice. *Weed Technology*, *36*(3), 373–378.

Dorji, S., Chauhan, B. S., Baltazar, A. M., & Johnson, D. (2013). Effect of flooding depth and pretilachlor rate on emergence and growth of three rice weeds: junglerice (Echinochloa colona), smallflower umbrella sedge (Cyperus difformis), and ludwigia (Ludwigia hyssopifolia). *Canadian Journal of Plant Protection*, *1*(2), 43–48.

Driver, K. E., Al-Khatib, K., & Godar, A. (2020). Bearded sprangletop (*Diplachne fusca* ssp. *fascicularis*) flooding tolerance in California rice. *Weed Technology*, *34*(2), 193–196.

Durand-Morat, A., Nalley, L. L., & Thoma, G. (2018). The implications of red rice on food security. *Global Food Security*, *18*(September), 62–75.

Espino, L., Brim-DeForest, W., & Johnson, T. (2018). Weedy Rice in California: Addressing an Emerging Pest Through Research and Outreach. *CAPCA Adviser*, *11*(4), 44.

Espino, L., Leinfelder-Miles, M., Brim-DeForest, W., Al-Khatib, K., & Linquist, B. (2016). *Best Management Practices for Weedy Rice*. Davis.

Evans, C. E., & Etherington, J. R. (1990). The effect of soil water potential on seed germination of some British plants. *New Phytologist*, *115*(3), 539–548.

Galvin, L., Brim-DeForest, W., & Al-Khatib, K. (2021). Thermal Time to Emergence of California Weedy Rice (Oryza sativa f. spontanea) Under Flooded Field Conditions: A 2-year Study. In *WSSA Virtual Annual Meeting* (p. 244). Maui, HI: Weed Science Society of America.

Galvin, L., Inci, D., Mesgaran, M., Brim-Deforest, W., & Al-Khatib, K. (2022). Flooding depths and burial effects on seedling emergence of five California weedy rice (Oryza sativa spontanea) accessions. *Weed Science*, 1–7.

Gealy, D.R., Saldain, N. E., & Talbert, R. E. (2000). Emergence of Red Rice (Oryza sativa) Ecotypes Under Dry-Seeded Rice (Oryza sativa) Culture. *Weed Technology*, *14*(2), 406–412.

Gealy, David R., & Bryant, R. J. (2009). Seed physicochemical characteristics of field-grown US weedy red rice (Oryza sativa) biotypes: Contrasts with commercial cultivars. *Journal of Cereal Science*, *49*(2), 239–245.

Ghosh, D., Rathore, M., Brahmachari, K., Singh, R., & Kumar, B. (2017). Impact of burial and flooding depths on Indian weedy rice. *Crop Protection*, *100*, 106–110.

Hardegree, S. P., & Emmerich, W. E. (1990a). Effect of Polyethylene Glycol Exclusion on the Water Potential of Solution-Saturated Filter Paper. *Plant Physiology*, *92*(2), 462–466.

Hardegree, S. P., & Emmerich, W. E. (1990b). Effect of Polyethylene Glycol Exclusion on the Water Potential of Solution-Saturated Filter Paper. *PLANT PHYSIOLOGY*, *92*(2), 462–466.

Hill, J. E., Williams, J. F., Mutters, R. G., & Greer, C. A. (2006). The California rice cropping system: Agronomic and natural resource issues for long-term sustainability. *Paddy and Water Environment*, *4*(1), 13–19.

Ismail, A. M., Johnson, D. E., Ella, E. S., Vergara, G. V., & Baltazar, A. M. (2012a). Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. *AoB PLANTS*, *2012*.

Ismail, A. M., Johnson, D. E., Ella, E. S., Vergara, G. V, & Baltazar, A. M. (2012b). *Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment* (pls No. 019). *AoB Plants*. Philippines.

Jacobs, J., Jacobs, N., Shreman, T., & Duke, S. (1991). Effect of diphenyl ether herbicideson oxidation of protoporphyrinogen to protoporphyrin in organella and plasma membrane enriched fractions of barley. *Plant Physiology*, *97*, 197–203.

Kanapeckas, K. L., Tseng, T.-M., Vigueira, C. C., Ortiz, A., Bridges, W. C., Burgos, N. R., … Lawton-Rauh, A. (2018). Contrasting patterns of variation in weedy traits and unique crop features in divergent populations of US weedy rice (Oryza sativa sp) in Arkansas and California. *Pest Management Science*, *74*(6), 1404–1415.

Kanapeckas, K. L., Vigueira, C. C., Ortiz, A., Gettler, K. A., Burgos, N. R., Fischer, A. J., & Lawton-Rauh, A. L. (2016). Escape to ferality: The endoferal origin of weedy rice from crop rice through de-domestication. *PLoS ONE*, *11*(9).

Karn, E., De Leon, T., Espino, L., Al-Khatib, K., & Brim-DeForest, W. (2020). Effects of competition from California weedy rice (Oryza sativa f. spontanea) biotypes on a cultivated rice variety. *Weed Technology*, *34*(5), 666–674.

Kumar, V., & Ladha, J. K. (2011). Direct Seeding of Rice. Recent Developments and Future Research Needs. In D. L. Sparks (Ed.), *Advances in Agronomy* (3rd ed., Vol. 111, pp. 297–414). Burlington: Academic Press.

Lee, H., Duke, M., & Duke, S. (1993). Cellular localization of protoporphyrinogen-oxidizing activities of etiolated barley (Hordeum vulgare) leaves. *Plant PHysiology*, *102*, 881–889.

Linquist, B., Al-Khatib, K., Swett, C., Espino, L., Leinfelder-Miles, M., Brim-DeForest, W., & McKenzie, K. (2018). *Rice Production Manual*. Davis, CA.

Linquist, B., Fischer, A. J., Godfrey, L., Greer, C., Hill, J., Koffler, K., Moeching, M., Mutters, R., Van Kessel, C. (2008). Minimum tillage could benefit California rice farmers. *California Agriculture*, *62*(1), 24–29.

Lundy, M. E., Hill, J. E., van Kessel, C., Owen, D. A., Pedroso, R. M., Boddy, L. G., Fischer, A. J., Linquist, B. A. (2014). Site-specific, real-time temperatures improve the accuracy of weed emergence predictions in direct-seeded rice systems. *Agricultural Systems*, *123*, 12–21.

Marambe, B. (2009). Weedy Rice: Evoluation, Threats, and Management. *Tropical Agriculturist*, *157*, 43–64.

Matringe, M., Camadro, J., Block, M., Joyard, J., Scalla, R., Labbe, P., & Douce, R. (1992). Localization within the chloroplast of protoporphyrinogen oxidase the target enzyme for diphenyl ether-like herbicides. *Journal of Biological Chemistry*, *267*, 4646–4651.

McKenzie, K. (2018). *20180070548*. United States: U.S. Provisional Patent Application Office. Retrieved from https://patents.justia.com/patent/20180070548

O'Geen, A. T. (2013). Soil Water Dynamics. *Nature Education Knowledge*, *4*(5), 9.

Ohadi, S., Laguerre, G., Madsen, J., & Al-Khatib, K. (2021). Toward understanding the impact of nuisance algae bloom on the reduction of rice seedling emergence and establishment. *Weed Science*, *70*(1), 95–102.

Oraze, M. J., & Grigarick, A. A. (1992). Biological Control of Ducksalad (Heteranthera limosa) by the Waterlily Aphid (Rhopalosiphum nymphaeae) in Rice (Oryza sativa) Author (s): Michael J . Oraze and Albert A . Grigarick Published by : Cambridge University Press on behalf of the Weed Sc. *Weed S1*, *40*(2), 333–336.

Ottis, B. V., Smith, K. L., Scott, R. C., & Talbert, R. E. (2005). Rice yield and quality as affected by cultivar and red rice (*Oryza sativa*) density. *Weed Science*, *53*(4), 499–504.

Pedroso, R. M. (2011). *Modeling Germination and Emergence of Smallflower Umbrella Sedge (Cyperus difformis L.) Resistant and susceptible to ALS-inhibiting Herbicides*. University of California Davis. Retrieved from

PubChem. (2017). Hazardous Substances Data Bank. Retrieved from https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7507

R Core Team. (2022). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rao, A. N., Brainard, D. C., Kumar, V., Ladha, J. K., & Johnson, D. E. (2017). Preventive Weed Management in Direct-Seeded Rice: Targeting the Weed Seedbank. In D. L. Sparks (Ed.), *Advances in Agronomy* (144th ed.). Cambridge: Elsevier.

Rao, A. N., Johnson, D. E., Sivaprasad, B., Ladha, J. K., & Mortimer, A. M. (2007). Weed Management in Direct-Seeded Rice. *Advances in Agronomy*, *93*, 153–255.

Roel, A., Mutters, R. G., Eckert, J. W., & Plant, R. E. (2005). Effect of Low Water Temperature on Rice Yield in California. *Agronomy Journal*, *97*(3), 943.

Rosenberg, S., Crump, A., Brim-DeForest, W., Linquist, B., Espino, L., Al-Khatib, K., … Pittelkow, C. M. (2022). Crop Rotations in California Rice Systems: Assessment of Barriers and Opportunities. *Frontiers in Agronomy*, *0*, 37.

Roy, S. K. S., Hamid, A., Giashuddin Miah, M., & Hashem, A. (1996). Seed size variation and its effects on germination and seedling vigour in rice. *Journal of Agronomy and Crop Science*, *176*(2), 79–82.

Sass, R. L., Fisher, F. M., Lewis, S. T., Jund, M. F., & Turner, F. T. (1994). Methane emission from rice fields: Effect of soil properties. *Global Biogeochemical Cycles*, *8*(2), 135–140.

Sathya Priya, R., Chinnusamy, C., Murali Arthanari, P., & Janaki, P. (2017). Carryover effect and plant injury from oxyfluorfen herbicide applied in transplanted rice. *International Journal of Chemical Studies*, *5*(3), 535–539.

Singh, K., Kumar, V., Saharawat, Y., Gathala, M., Ladha, J. K., & Chauhan, B. S. (2013). Weedy Rice: An Emerging Threat for Direct-seeded Rice Production Systems in India. *Journal of Rice Research*, *1*(106).

Singh, M., Bhullar, M. S., & Gill, G. (2018). Integrated weed management in dry-seeded rice using stale seedbeds and post sowing herbicides. *Field Crops Research*, *224*, 182–191.

Song, B., Shi, J., & Song, S. (2015). Dormancy release and germination of Echinochloa crus-galli grains in relation to galactomannan-hydrolysing enzyme activity | Elsevier Enhanced Reader. *Journal of Integrative Agriculture*, *14*(8), 1627–1636.

Travlos, I., Gazoulis, I., Kanatas, P., Tsekoura, A., Zannopoulos, S., & Papastylianou, P. (2020). Key Factors Affecting Weed Seeds' Germination, Weed Emergence, and Their Possible Role for the Efficacy of False Seedbed Technique as Weed Management Practice. *Frontiers in Agronomy*, *2*, 1.

Turner, F. T., Chen, C.-C., & McCauley, G. N. (1981). Morphological Development of Rice Seedlings in Water at Controlled Oxygen Levels 1. *Agronomy Journal*, *73*(3), 566–570.

UCIPM. (2022). California Weather Data: Set Dates and Variables. Colusa.C. Retrieved August 17, 2022, from http://ipm.ucanr.edu/calludt.cgi/WXSTATIONDATA?STN=COLUSA.C

Voesenek, L. A. C. J., Colmer, T. D., Pierik, R., Millenaar, F. F., & Peeters, A. J. M. (2006). How plants cope with complete submergence. *New Phytologist*, *170*, 213–226.

Weed Science Society of America. (2014). *Herbicide Handbook*. (D. L. Shraner, Ed.)

(10th ed.). Weed Science Society of America.

Yang, R. C. (2010). Towards understanding and use of mixed-model analysis of agricultural experiments. *Canadian Journal of Plant Science*, *90*(5), 605–627.

Zhao, C., Xu, W., Song, X., Dai, W., Dai, L., Zhang, Z., & Qiang, S. (2015). Early flowering and rapid grain filling determine early maturity and escape from harvesting in weedy rice. *Pest Management Science*, *74*, 465–476.

Ziska, L. H., Gealy, D. R., Burgos, N., Caicedo, A. L., Gressel, J., Lawton-Rauh, A. L., Avila, L. A., Theisen, G., Norsworthy, J., Ferrero, A., Vidotto, F., Johnson, D. E., Ferreira, F. G., Marchesan, E., Menezes, V., Cohn, M. A., Linscombe, S., Carmona, L., Tang, R., Merotto, A. (2015). *Weedy (Red) Rice. An Emerging Constraint to Global Rice Production. Advances in Agronomy* (Vol. 129). Elsevier.

Ziska, L. H., Tomecek, M. B., & Gealy, D. R. (2010). Competitive Interactions between Cultivated and Red Rice as a Function of Recent and Projected Increases in Atmospheric Carbon Dioxide. *Agronomy Journal*, *102*(1), 118.