

Rising atmospheric CO₂ concentration affect weedy rice growth, seed shattering and seedbank longevity

Andrisa Balbinot¹  | Anderson da Rosa Feijó²  | Marcus Vinicius Fipke¹  |
Vinicios Rafael Gehrke¹  | Dirceu Agostinetto¹  | Nelson Diehl Kruse³  |
Lewis Hans Ziska⁴  | Edinalvo Rabaioli Camargo¹  | Luis Antonio de Avila¹ 

¹Crop Protection Graduate Program, Federal University of Pelotas (UFPEL), Pelotas, Brazil

²Plant Physiology Graduate Program, Federal University of Pelotas (UFPEL), Pelotas, Brazil

³Crop Protection Department, Federal University of Santa Maria (UFSM), Santa Maria, Brazil

⁴Mailman School of Public Health, Columbia University, New York, New York, USA

Correspondence

Luis Antonio de Avila, Crop Protection Graduate Program, Federal University of Pelotas (UFPEL), Pelotas, 96.010-900, Brazil.
Email: laavilabr@gmail.com

Funding information

Conselho Nacional de Desenvolvimento Científico e Tecnológico; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Subject Editor: Diego Batlla Universidad de Buenos Aires, Buenos Aires, Argentina

Abstract

Weedy rice (*Oryza* sp.) is one of the most troublesome global weeds in cultivated rice. Its troublesome status is associated with characteristics such as seed shattering and dormancy, allowing for long-term reinfestation and persistence in rice fields. However, the role of rising carbon dioxide levels (CO₂) and other climate variables on these characteristics has not, to date, been assessed. The current research objectives were to evaluate two aspects related to climatic change, increased CO₂ concentration (400 ± 50 μmol mol⁻¹ and 700 ± 50 μmol mol⁻¹) and water management (continuous and alternate-wetting drying), to assess plant development, seed shattering and seedbank longevity of weedy rice. Our results indicated that elevated CO₂ (700 ± 50 μmol mol⁻¹) increased weedy rice growth and biomass, seed shattering and lengthened viability within the seedbank. Water management did not affect weedy rice growth, seed shattering and seed dormancy. These results suggest that in areas where weedy rice is dominant, its seed bank persistence and potential competition may be exacerbated with rising CO₂ levels, with negative consequences for rice production.

KEYWORDS

climate change, dormancy, free-air CO₂ enrichment, seed viability

1 | INTRODUCTION

Weedy rice (*Oryza sativa* f. *spontanea*) is a problematic weed widely disseminated in rice production systems worldwide (Avila et al., 2021; Chauhan, 2013; Ziska et al., 2015). Weedy rice is difficult to differentiate from cultivated rice, and is optimised to grow in similar environmental conditions. Contamination in cultivated rice, its ability to shatter and its longevity in the seedbank add to its persistence. It is highly competitive and associated with more significant yield losses than any other weed in rice systems (Noldin et al., 2004; Smith, 1988). In addition, weedy rice has several populations that have evolved resistance to imidazolinone herbicides, limiting chemical management (Piveta et al., 2021; Shivrain et al., 2010).

For weedy rice, seed shattering is an adaptive characteristic that contributes to temporal persistence within the soil seedbank (Ji

et al., 2010; Li et al., 2006; Yoon et al., 2017; Zhao et al., 2019b; Zhou et al., 2012). The degree of shattering is regulated, in part, by environmental conditions during plant development through anthesis (Cheng et al., 2016; Konishi et al., 2006).

Seed shattering and prolonged seed dormancy period can maintain viability and perpetuate weedy rice occurrence in cultivated rice (Agostinetto et al., 2001). Seed dormancy is characterised by the blocking of germination of intact viable seed, even under favourable environmental conditions (Goggin et al., 2010). As with seed shattering, environmental conditions can alter dormancy.

Rising levels of carbon dioxide, [CO₂], in addition to contributing to rising surface temperatures and climatic change, can directly affect plant physiology. In addition to stimulating plant growth, increasing [CO₂] can also reduce stomatal conductance, reduce transpiration and

increase water use efficiency (Kumar et al., 2017; Leakey et al., 2009; Terashima et al., 2014). Water management is a critical agronomic practice in paddy rice that affects rice yield (Massey et al., 2014) and weed–crop competition. Rice production is mainly performed through continuous flooding, which effectively controls a broad spectrum of terrestrial weeds, but it also requires pumping large volumes of water (Avila et al., 2015; Chamara et al., 2018). However, as the climate changes and water costs and availability become issues, intermittent watering of rice-growing systems has been considered a management option to reduce water use (Avila et al., 2015).

Overall, the increase in [CO₂] levels and associated changes in climate, including water availability, are likely to alter rice/weedy rice interactions with consequences for global production (Refatti et al., 2019; Varanasi et al., 2016; Ziska et al., 2019). Although several studies have examined cultivated rice responses to [CO₂] and climate (Kim et al., 2013; Sakai et al., 2019), there is a need for additional information regarding weedy rice's growth, development and reproductive characteristics with projected increases in [CO₂] and changes in water availability. Consequently, the primary aim of the current research was to determine whether increased [CO₂] levels (400 ± 50 μmol mol⁻¹ to 700 ± 50 μmol mol⁻¹) and different water regimes could significantly alter weedy rice growth, development, seed shattering and seedbank longevity.

2 | MATERIALS AND METHODS

The experiment was conducted in 2017/2018 and repeated in 2018/2019. It was performed in an open-top chamber (OTCs) using a method developed by Leadley and Drake (1993), and installed at the Federal University of Pelotas, Capão do Leão, Brazil. The wood-frame chambers measured 1.9 m width (W) × 1.9 m depth (D) × 2.0 m height (H) and were covered with 150-μm plastic film. The [CO₂] level was maintained by a central control system programmed to measure and replace CO₂ in 30-second intervals (Leadley & Drake, 1993). The experimental units consisted of plastic containers measuring 0.36 m (W) × 0.63 m (L) × 0.33 m (H), filled with rice paddy soil (Albaqualf) collected from 0 to 20 cm soil profile from an adjoining rice field. The containers were filled with soil, allowing a space to flood the soil with a 10 cm water layer after crop establishment. Temperature was measured daily throughout the rice growing seasons 2017/2018 and 2018/2019. The average temperatures were 19.8, 25.1 and 30.3°C (minimum, mean and maximum) and 18.9, 27.0 and 35.1°C (minimum, average and maximum) in growing seasons 2017/2018 and 2018/2019, respectively.

2.1 | Experimental design growth conditions

The experimental design was completely randomised with a factorial arrangement of treatments with four replications. Factor A included two levels of [CO₂]: ambient (*a*[CO₂]) at 400 ± 50 μmol mol⁻¹ and elevated (*e*[CO₂]) at 700 ± 50 μmol mol⁻¹. Factor B consisted of two water regimes: continuous flooding and alternate-wetting drying (AWD). Each

regime's flood was initiated at V₄ (four-leaf stage, Counce et al., 2000) as recommended for rice paddy in Southern Brazil (SOSBAI, 2018). A maximum flood depth of 10 cm was used for both irrigation treatments, but the continuous flood was maintained at 10 cm through grain maturity, whereas the AWD was allowed to subside until the soil surface was exposed to air when the flood was re-established.

Weedy rice seeds, characterised by a red pericarp and awnless, were collected from paddy rice fields in the Centro Agropecuário da Palma belonging to Universidade Federal de Pelotas, RS, Brazil. The seeds were hand-planted (90% germination rate) at a seeding rate equivalent to 110 kg rice seeds ha⁻¹ and 17 cm row spacing. Fertilisation followed the recommendation of SOSBAI (2018) for cultivated rice; at sowing NPK (formulation: 5–20–20) was distributed at a rate of 300 kg ha⁻¹. Two applications of urea (45% N) at 150 kg ha⁻¹, immediately before initial flooding and the second at panicle initiation. Two rulers were installed in each experimental unity to determine the daily water consumption and calculate the water use efficiency (WUE).

2.2 | Evaluations during the vegetative stage

At the vegetative stage, the total number of tillers (per container) was evaluated at the V₇ plant stage and plant height in stage R₃ (heading stage).

2.3 | Seed shattering evaluation

During anthesis, 10 panicles were randomly isolated, with transparent polyamide bags, for each treatment. Seed counting was carried out daily from anthesis to harvest. Seeds per panicle (total and shattered) were quantified at the end of the experiment.

When rice grain moisture content reached 21% (w/w), all plants from all treatments were harvested, and the total number of panicles and grain yield (g) per experimental unit was assessed. A subset of 10 panicles was randomly selected from each treatment, and the number of grains per panicle, spikelet sterility (%) and weight per thousand grains was determined. Spikelet sterility was determined by counting the number of spikelets separated from the grain sample. The total aboveground dry weight (ADW) was obtained after the straw samples were dried to a constant weight at 60°C.

2.4 | Weedy rice seedbank longevity

The experiment was conducted over 225 days for each year of the study. The second year of the study was devoted to dormancy experiment.

For the dormancy evaluation, 100 seeds from each treatment and replication were placed in nylon bags (10 cm × 15 cm) with 200 g of soil (rice paddy soil—Albaqualf). Following vegetation removal in the centre experimental area, bags were placed in the middle of each experimental unit at a depth of 10 cm.

The experiment was arranged in a three factor (2x2x6), according to the experiment in OTC (section 2.1; Factor A: two levels of [CO₂], Factor B: consisted of two water regimes) and Factor C: six sampling times (0, 45, 90, 135, 180 and 225 days following burial). After each sampled time, seeds were extracted from the bag by washing the sample under running water using a set of sieves. Sodium hypochlorite solution (2%) was used to disinfect the seeds for 10 minutes to avoid contamination by fungi before the germination test. After cleaning, seeds were weighed, germinated on Germitest® paper (blotter paper) and placed in gerbox boxes, and moistened with distilled water. The germination chamber was maintained a continuous temperature of 25°C and a 12 h day/night photoperiod for 14 days. After the germination test, dormancy and seed viability were evaluated through a tetrazolium test using 2, 3, 5-triphenyl tetrazolium chloride 1.0%; the seeds were split in half and placed in cups with tetrazolium solution for 2 hours at a temperature of 35°C (BRASIL, 2009).

For the remaining seeds (unpredated seeds, i.e. non-dead, predated or spoiled seeds), germination, dead seed percentage and dormancy were evaluated at 14 DAS. The percentage of remaining seeds was calculated based on the initial number of 100 seeds per treatment and replicate. Dead and dormant seed calculations were performed using germination percentages based on the number of remaining seeds. The percentage of viable and non-viable seeds was calculated based on the dormant seeds determined during the germination test. This was used to determine the changes in seed status in the soil.

2.5 | Statistical analysis

The statistical analysis was performed using statistical program R Core Team (2018) and the ExpDes.pt package. Before the analysis of variance, the data were tested for normality. For ANOVA, the growing season effect was considered a random variable. When there was no significant difference between growing seasons, the data were combined. The ANOVA was performed for the variables number of tillers, plant height, aboveground dry weight, number of panicles, number of grains, grains yield and spikelet sterility and the means compared using Tukey's HSD test ($p \leq 0.05$).

For seed shattering, data were analysed as for normality and, subsequently, submitted to ANOVA. Data on dead seeds were submitted to square root type transformation. In case of statistical significance, a regression analysis was performed using SigmaPlot 12.0 program (SIGMAPLOT, 2012). Time variation (days) of the soil's seed status was calculated from the means for germination and seed viability (dead, viable and predated, or deteriorated). Regression analysis was performed for the sampling times.

Seed shattering, remaining seeds, germination, dormancy and mortality data were fitted with a nonlinear sigmoidal, quadratic and hyperbola regression model, according to Equations [1] and [2], respectively:

$$y = a / (1 + \exp(-[x - x_0]/b)) \quad (1)$$

$$y = y_0 + a * x + bx^2 \quad (2)$$

$$y = (a * x) / (b + x) \quad (3)$$

where, for Equation [1], y is the seed shattering (grains) or germination (%), and 'a' the upper asymptotic values of Y ; parameter 'b' denotes the relative slope, 'x₀' corresponding to 50% seed shattering or germination in days and the parameter 'x' is days after anthesis. For Equation [2], y is remaining seeds as a percentage, 'y₀' initial point in y , 'a' is the relative slope and 'b' is the quadratic coefficient. For Equation [3], y is seed dormancy as a percentage, and 'a' the upper asymptotic values of Y ; parameter 'b' denotes the relative slope, and the parameter 'x' is days.

3 | RESULTS

3.1 | Evaluation of growing and production parameters

Except for tiller number, 1000 grains weight, and WUE, all the variables demonstrated the growing season effect (Table 1). For the variables without effect, season growing was combined.

The number of tillers in both growing seasons was not affected by atmospheric [CO₂] or irrigation treatment (data not shown). Weedy rice plants were 5% taller at flowering and harvest in 2017/2018 than in the 2018/2019 growing season (Figure 1). In was both growing seasons, plants growing under e[CO₂] were taller at harvest than those grown under a[CO₂]. No effect of irrigation management observed for either growing season.

Plants grown at e[CO₂] had greater above-ground dry weight (ADW) relative to ambient (Figure 2), and no effect of [CO₂] and irrigation management was observed for panicle number, grains per panicle, grain yield or spikelet sterility for either year of the study (Table 1).

3.2 | Seed shattering and longevity of weedy rice seedbank

Although seed production and yield parameters were not affected by additional CO₂, seed shattering increased during both growing seasons (Figure 3). Anthesis of the panicles at e[CO₂] occurred 3 days earlier, but the harvest was carried out on the same day, and seed shattering started simultaneously. This suggests that increased seed shattering was not associated with a shorter or longer period of plant exposure to [CO₂]. When analysing the period that causes 50% of seed shattering, it can be seen that in 2017/2018 growing season there is no difference between a[CO₂] and e[CO₂] (23.85 and 24.27 days, respectively). However, in 2018/2019, the 50% seed shattering period (Figure 3) was longer in e[CO₂] of 29 days compared to 26 days in a[CO₂].

Longevity study showed that seed numbers declined over time (Figure 4). Dormancy decreased, and germination increased over time (Figure 4B). However, a higher value of dormancy was observed at e

TABLE 1 Effects of CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$) and water regimes (alternate-wetting drying and continuous flooding) on number of panicles, number of grains per 10 panicles, grains yield and spikelet sterility for two growing seasons (2017/2018 and 2018/2019)

[CO ₂]	Panicles (per experimental unit)		Number of grains (10 panicles)		Grain yield (g)		Spikelet sterility (%)	
Season 2017/2018								
	AWD ^e	Continuous	AWD	Continuous	AWD	Continuous	AWD	Continuous
$a[\text{CO}_2]^a$	78 ± 13 ^{ns}	76 ± 17	1002 ± 32 ^{ns}	921 ± 66	76 ± 15 ^{ns}	81 ± 9	14 ± 1 ^{ns}	14 ± 1
$e[\text{CO}_2]^b$	80 ± 8 ^{ns}	82 ± 5	931 ± 58 ^{ns}	1011 ± 56	72 ± 18 ^{ns}	76 ± 11	14 ± 3 ^{ns}	13 ± 3
CV(%) ^d	11.73		15.65		18.13		8.75	
Season 2018/2019								
$a[\text{CO}_2]$	69 ± 4 ^{ns}	70 ± 6	785 ± 32 ^{ns}	732 ± 10	77 ± 14 ^{ns}	73 ± 17	21 ± 2 ^{ns}	26 ± 5
$e[\text{CO}_2]$	72 ± 2 ^{ns}	72 ± 7	686 ± 122 ^{ns}	739 ± 22	68 ± 10 ^{ns}	71 ± 17	22 ± 2 ^{ns}	27 ± 2
CV(%)	16.92		8.73		20.65		17.72	

^a $a[\text{CO}_2] = 400 \mu\text{mol mol}^{-1}$; ^b $e[\text{CO}_2] = 700 \mu\text{mol mol}^{-1}$; ^cMean ± standard deviation ($n = 4$); ^dCoefficient of variation (CV); ^eAlternate-wetting drying (AWD) and continuous (continuous flooding); ^{ns}Indicated there was no significant difference by Tukey's test.

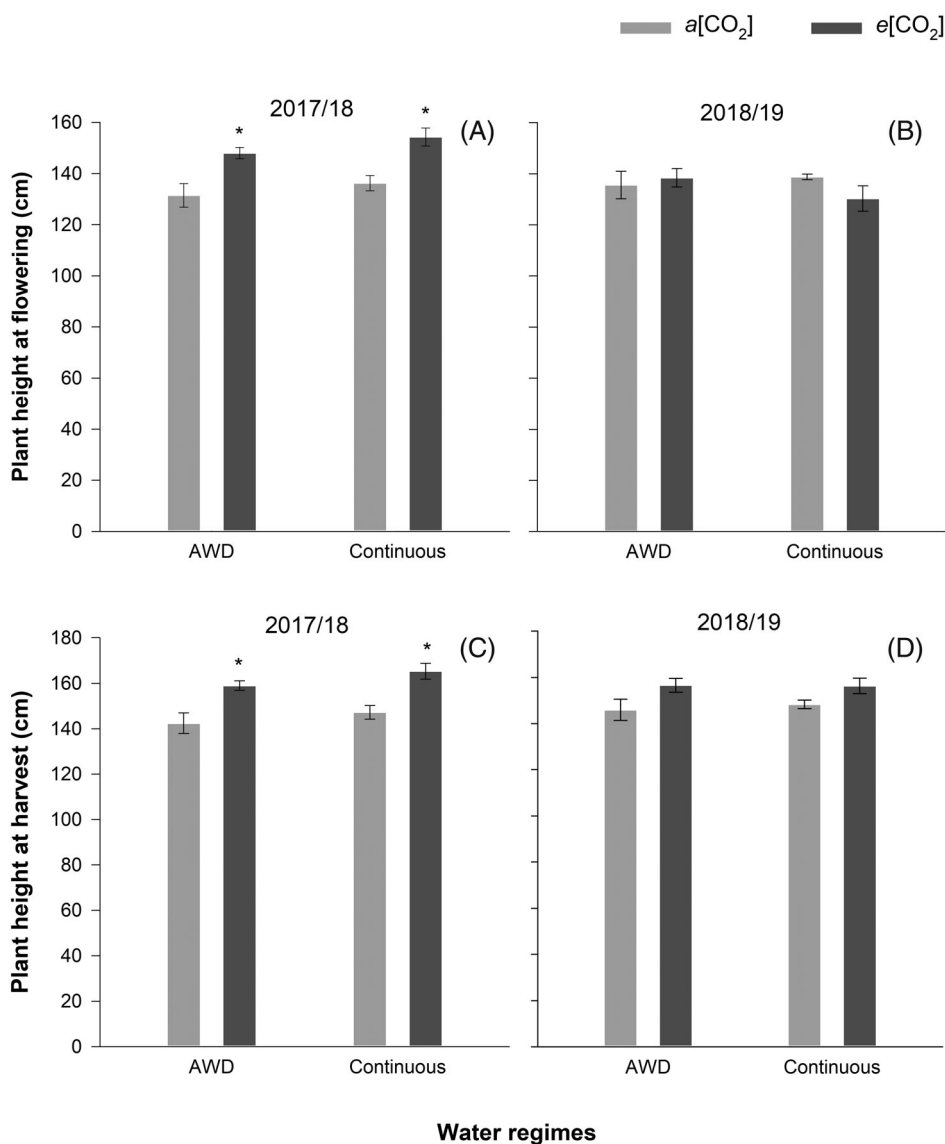


FIGURE 1 Effects of CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$) and water regimes (alternate-wetting drying and continuous flooding) on plant height at flowering (A, B) and plant height at harvest (C, D) two growing seasons (2017/2018 and 2018/2019). Error bars correspond to 95% confidence intervals. * indicates a significant difference between mean values based on Tukey's test ($p < 0.05$)

FIGURE 2 Effects of CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$) and water regimes (alternate-wetting drying and continuous flooding) on the above-ground dry weight in 2017/2018 (A) and 2018/2019 (B) growing seasons. Error bars correspond to 95% confidence intervals. *indicates a significant difference between mean values based on Tukey's test ($p < 0.05$)

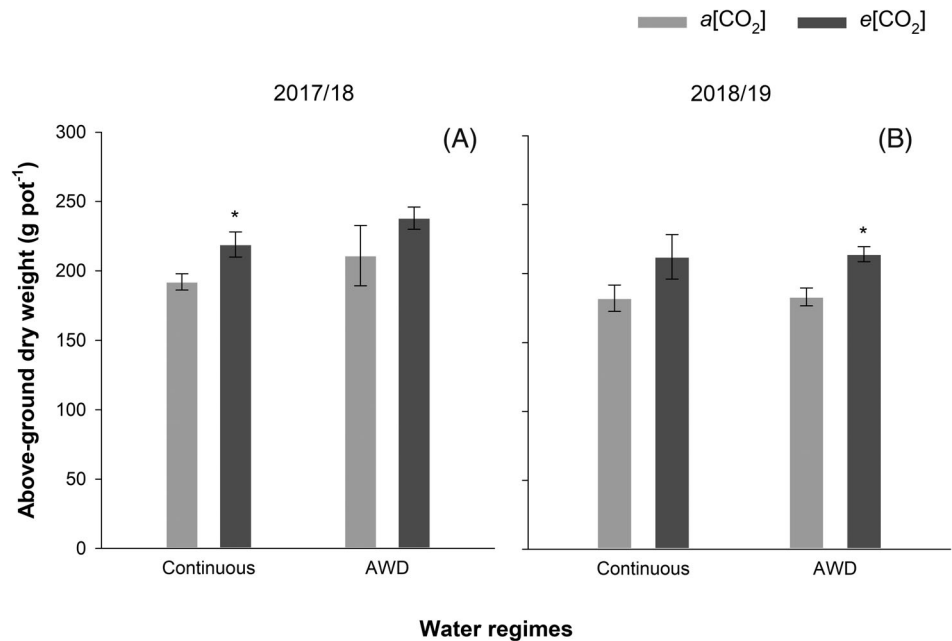
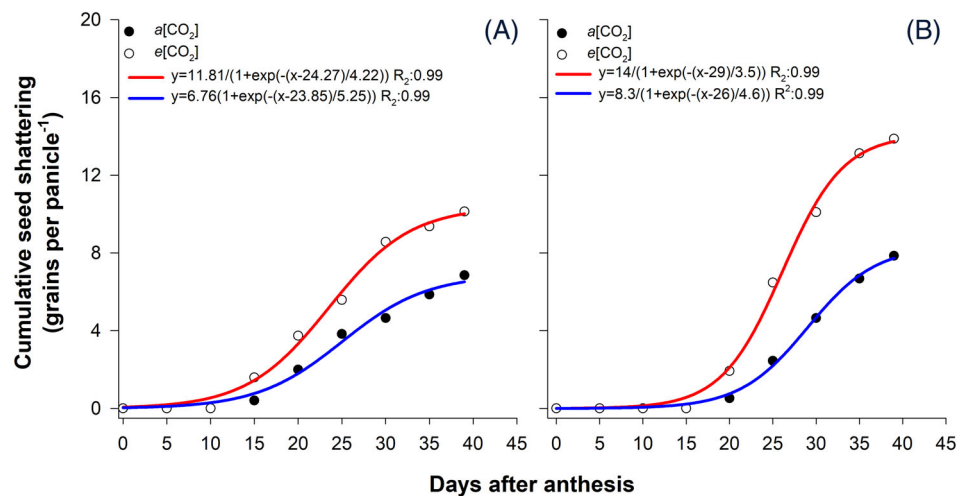


FIGURE 3 Cumulative weedy rice seed shattering as affected by CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$) in 2017/2018 (A) and 2018/2019 (B) growing seasons



[CO₂] relative to $a[\text{CO}_2]$ (Figure 4C). No treatment effects on seed mortality were observed (Figure 4D). For sampling at 45 and 180 days, the remaining seeds (unpredated) were higher at $e[\text{CO}_2]$.

In this study, viable dormant seeds (~20%) were observed even at approximately 200 days after burial (Figure 5). Approximately 25% of the seeds had deteriorated by 225 days. Seed dormancy was affected by [CO₂], with more dormant seed in $e[\text{CO}_2]$.

4 | DISCUSSION

4.1 | Evaluation of growing and production parameters

Weedy rice is noted for its wide phenotypic and genotypic diversity (Piveta et al., 2021; Singh et al., 2013). It is a recognised competitor with cultivated rice at all stages of development with

consequences for crop yield and grain quality (Chauhan, 2013; Munda et al., 2019).

The CO₂-induced increases in ADW have been observed previously for cultivated and weedy rice (Ziska & Mcclung, 2008). In addition to direct stimulation of photosynthesis due to being a C₃ plant, additional CO₂ could improve other aspects related to ADW, as the development of the root system and consequently the better nutrient uptake. It has already been described that weedy rice can absorb and use nutrients more efficiently than cultivated rice (Burgos et al., 2006). In this research, it was observed that ADW increase at $e[\text{CO}_2]$, but no effect was observed for seed production. This may be associated with low and high temperatures during the reproductive phase of rice (Figure 6). The most critical phases determining final grain yield are the reproductive phases (e.g., panicle initiation, booting and flowering) (Ye et al., 2009). Ghadirnezhad and Fallah (2014) indicated that low temperature ranging from 15 to 19°C during the reproductive stage impairs microspore development, increasing sterility

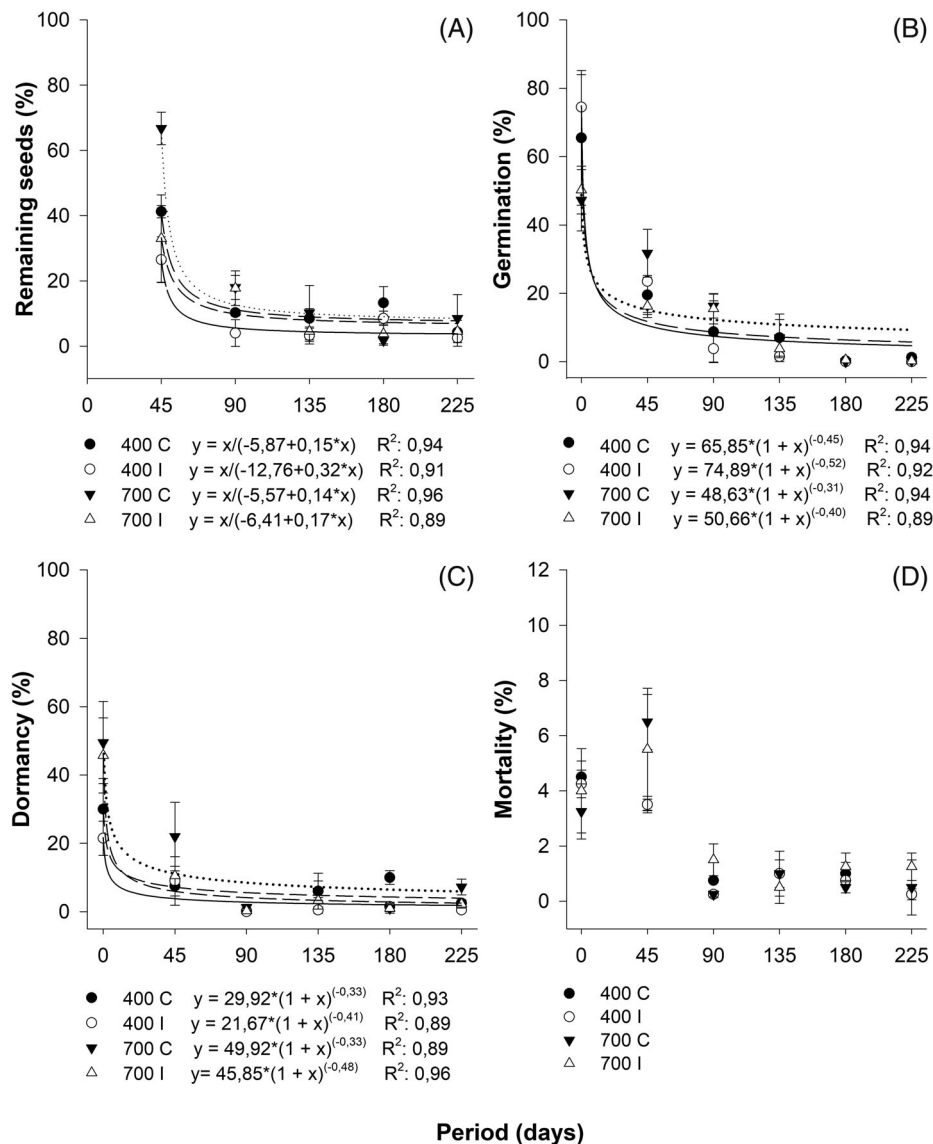


FIGURE 4 Remaining seeds (A), germination (B), dormancy (C) and mortality (D) weedy rice seed after the germination test sowing, according to CO_2 concentrations ($a [CO_2] = 400 \mu mol Mol^{-1}$ and $e[CO_2] = 700 \mu mol Mol^{-1}$) and water regimes (I: Alternate-wetting drying and C: Continuous flooding) for seeds collected period in 2018/2019 growing season. Error bars correspond to 95% confidence intervals

spikelets, thus reducing grain production. Furthermore, Martínez-Eixarch and Ellis (2015) indicated that the most critical phase for cold stress in rice culture would be at the booting stage, particularly at the pollen microspore stage, which occurs approximately 10–12 days before to heading. Shah et al. (2011) reported that spikelet sterility was significantly increased at temperatures higher than 35°C. In the present study, we have both effects at the reproductive stage. Low and high temperatures can be the cause of higher spikelet sterility in 2018/2019 (Figure 6B), which probably reduced production in this growing season.

4.2 | Seed shattering and longevity of weedy rice seedbank

Shattering refers to the release of mature seeds from the parent plant, which occurs through developing a specialised layer of cells, the abscission zone (AZ), located at the junction of the base of grain with

the pedicle (McKim et al., 2008). Abscission is triggered by ethylene production, which inhibits auxin production (Ogawa et al., 2009). Furthermore, through hydrolytic enzymes such as polygalacturonase, endo- β -glucanase and expansin, the middle lamella degradation process of the cell wall occurs, causing seed shattering (Roberts et al., 2002). The formation of the AZ zone and shattering can also be influenced by environmental conditions (Zhao et al., 2019a). Since CO_2 participates in several physiological processes, being indispensable for development, high $[CO_2]$ can affect secondary metabolic processes, acting in different routes and may interfere with cellular metabolism (Kimball, 2016; Noctor & Mhamdi, 2017; Xu et al., 2015). For example, Pan et al. (2019) reported that the transcription levels of multiple genes related to the synthesis of ethylene and heat proteins (HSPs) were induced by high $[CO_2]$ ($800 \mu mol mol^{-1}$) compared to environmental $[CO_2]$ ($400 \mu mol mol^{-1}$) in tomato leaves, grown under controlled temperature conditions (25°C). Previous studies had already indicated that, in addition to being the substrate for carbon assimilation by plants, CO_2 plays a role in redox homeostasis and

hormonal signalling (Shi et al., 2015). Overall, several potential mechanisms may act on the AZ, increasing the weedy rice shattering trait. The potential influence of CO₂-induced changes in genetic differentiation of cells in the abscission zone (Li et al., 2006; Zhao et al., 2019b) or other specialised proteins also deserves study (Konishi et al., 2006) (Thurber et al., 2010). To fully determine any role of CO₂ on

abscission and shattering, it will be necessary to study the effect of increased [CO₂] level on the seed shattering genes' expression with contrasting weedy rice and rice genotypes.

In weedy rice, increased dormancy can allow germination under AWD over time, increasing weed infestation and difficult in management (Li et al., 2011; Tseng et al., 2018). From an evolutionary perspective, dormancy can directly affect seed longevity under field conditions, increasing chances for favourable environmental outcomes and increased fecundity (Gardarin & Colbach, 2015).

Variability in seed dormancy period is quite common in weedy rice; some biotypes germinate shortly after harvest and others can take weeks to months to germinate (Delatorre, 1999). Noldin et al. (2006) found 21% of viable red/weedy rice seeds at a 25 cm depth after 5 months of burial. After 24 months of burial, 10% of the seeds were still viable. However, some other environmental factors can affect viability, including depth (oxygen availability), water, temperature, predation, etc. (Sartori et al., 2014; Zhao et al., 2018).

Seedbank dynamics has a strong temporal influence on weed persistence in agronomic systems and can be influenced by additions from seed production, withdrawals related to deterioration, predation, loss of viability, etc. (Chauhan & Johnson, 2010). The dynamics of the seedbank in the soil involve several interactions depending on the type of weed species (dormancy), environmental conditions (light, temperature and humidity) and soil management practices that can affect persistence (Menalled, 2008; Scherner et al., 2016).

The weedy rice seedbank longevity observed here is consistent with other weed species, including *Lolium multiflorum* (Cechin et al., 2021), *Conyza* sp. (Vargas et al., 2018), *Euphorbia heterophylla* (Asgarpour et al., 2020) and *Amaranthus* sp. (Korres et al., 2018).

The basis for greater dormancy with additional CO₂ remains to be elucidated. Zhu et al. (2018) demonstrated declines in protein and

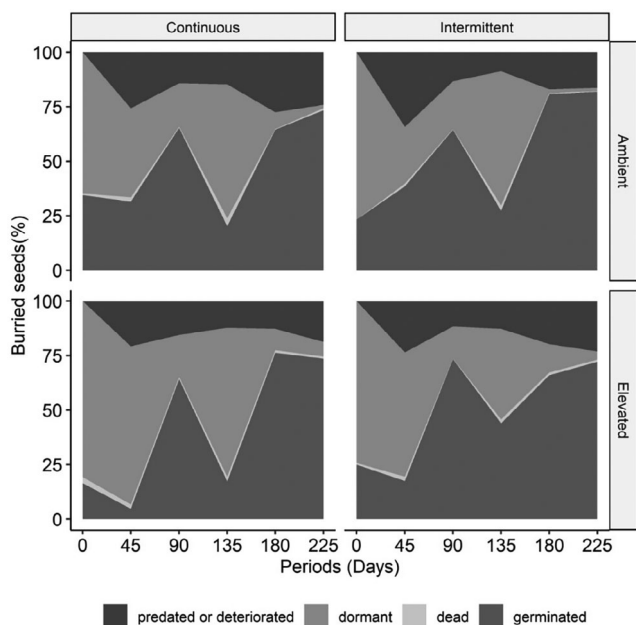


FIGURE 5 Variation in time (days) of the status in the soil of weedy rice seeds obtained of plants grown under CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$) and water regimes (intermittent: Alternate-wetting drying and continuous: Continuous flooding) in 2018/2019 growing season

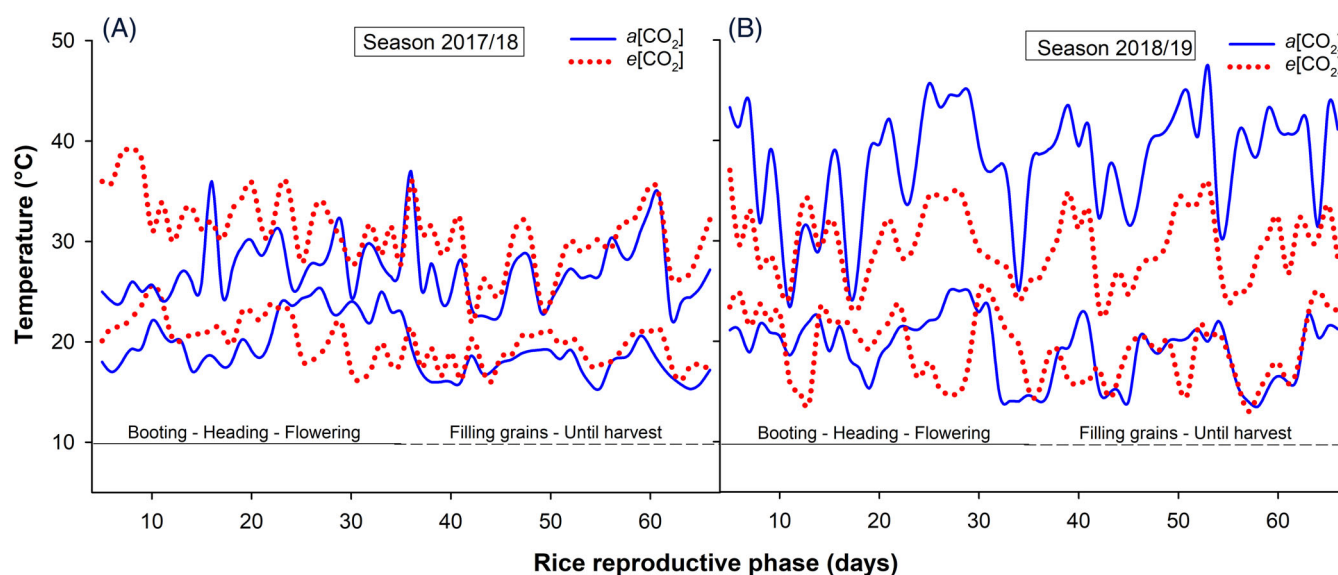


FIGURE 6 Daily average temperature data observed during 2017/2018 (A) and 2018/2019 (B) seasons growing during the reproductive stages of rice (booting–heading–flowering and filling grains–harvest) the OTCs at CO₂ concentrations ($a[\text{CO}_2] = 400 \mu\text{mol Mol}^{-1}$ and $e[\text{CO}_2] = 700 \mu\text{mol Mol}^{-1}$)

mineral contents in cultivated rice seed from plants grown in $e[CO_2]$. Studies have also indicated that soluble sugars, the primary substrate for the germination and seedling process, were reduced in $e[CO_2]$ (Lamichaney et al., 2019). However, similar analyses of weedy rice in the context of higher CO_2 levels are not, to our knowledge, available.

Additional factors can affect dormancy and seed longevity, including genetic and environmental factors (Marty & Bassirirad, 2014). However, to our knowledge, this is the first evidence of elevated CO_2 affecting seed shattering and seed dormancy in a weed species.

This study provides the first information for understanding the longevity and persistence of weedy rice seed and seedbank dynamics associated with $e[CO_2]$ under field conditions. While additional information is needed, these data suggest a role of rising CO_2 in increasing weedy rice infestation and occurrence, with subsequent challenges regarding weed management.

5 | CONCLUSIONS

Under $e[CO_2]$, weedy rice presents greater growth and biomass, which may increase its advantage on the competition with rice crops.

Weedy rice seed shattering was higher in $e[CO_2]$, relative to $a[CO_2]$. The basis for the increase in shattering is unclear and requires further investigation.

Weedy rice seeds from plants grown at $e[CO_2]$ maintained higher viability than those in $a[CO_2]$. Further studies on the topic should be explored since several environmental factors control this trait.

These results suggest that weedy rice traits associated with seed dispersal, persistence and germination may become a greater constraint on rice production in response to rising CO_2 levels.

DATA AVAILABILITY STATEMENT

The datasets used in the current study are available from the corresponding author on reasonable request.

ORCID

Andrisa Balbinot  <https://orcid.org/0000-0002-2380-7523>

Anderson da Rosa Feijó  <https://orcid.org/0000-0003-4606-4563>


Marcus Vinicius Fipke  <https://orcid.org/0000-0002-3057-9522>

Vinicius Rafael Gehrke  <https://orcid.org/0000-0002-8459-9318>

Dirceu Agostinetto  <https://orcid.org/0000-0001-6069-0355>

Nelson Diehl Kruse  <https://orcid.org/0000-0002-5208-0159>

Lewis Hans Ziska  <https://orcid.org/0000-0002-6220-1620>

Edinaldo Rabaioli Camargo  <https://orcid.org/0000-0001-8025-1042>

Luis Antonio de Avila  <https://orcid.org/0000-0002-2532-7152>

REFERENCES

- Agostinetto, D., Fleck, N.G., Rizzardi, M.A., Merotto Junior, A. & Vidal, R.A. (2001) Arroz vermelho: ecofisiologia e estratégias de controle. *Ciência Rural*, 31, 341–349.
- Asgarpour, R., Ghorbani, R., Khajeh-Hosseini, M., Golzari, F. & Ilkhaee, M.N. (2020) Seed longevity of spotted spurge and wild poinsettia under diverse environmental conditions. *Planta Daninha*, 38, 1–11. <https://doi.org/10.1590/S0100-83582020380100002>
- Avila, L.A., Martini, L.F.D., Mezzomo, R.F., Refatti, J.P., Campos, R., Cezimbra, D.M. et al. (2015) Rice water use efficiency and yield under continuous and intermittent irrigation. *Agronomy Journal*, 107, 442–448.
- Avila, L.A., Noldin, J.A., Mariot, C.H.P., Massoni, p.F.S., Fipke, M.V., Gehrke, V.R. et al. (2021) Status of weedy rice (*Oryza sativa* L.) infestation and management practices in southern Brazil. *Weed Science*, 69, 536–546. <https://doi.org/10.1017/wsc.2021.24>
- BRASIL. (2009) *Ministério da Agricultura, Pecuária e Abastecimento. Regras para Análise de Sementes*. Brasília: Mapa/AC.
- Burgos, N.R., Norman, R.J., Gealy, D.R. & Black, H. (2006) Competitive N uptake between rice and weedy rice. *Field Crops Research*, 99, 96–105.
- Cechin, J., Schmitz, M.F., Hencks, J.R., Vargas, A.A.M., Agostinetto, D. & Vargas, L. (2021) Burial depths favor Italian ryegrass persistence in the soil seed bank. *Science in Agriculture*, 78, 1–6. <https://doi.org/10.1590/1678-992X-2019-0078>
- Chamara, B.S., Marambe, B., Kumar, V., Ismail, A.M., Septiningsih, E.M. & Chauhan, B.S. (2018) Optimizing sowing and flooding depth for anaerobic germination-tolerant genotypes to enhance crop establishment, early growth, and weed management in dry-seeded rice (*Oryza sativa* L.). *Frontiers in Plant Science*, 871, 1–15.
- Chauhan, B.S. (2013) Strategies to manage weedy rice in Asia. *Crop Protection*, 48, 51–56.
- Chauhan, B.S. & Johnson, D.E. (2010) The role of seed ecology in improving weed management strategies in the tropics, 1st ed. *Advances in Agronomy*, 105, 221–262. [https://doi.org/10.1016/S0065-2113\(10\)05006-6](https://doi.org/10.1016/S0065-2113(10)05006-6)
- Cheng, J., He, Y., Zhan, C., Yang, B., Xu, E., Zhang, H. et al. (2016) Identification and characterization of quantitative trait loci for shattering in japonica rice landrace Jiuciqing from Taihu Lake Valley, China. *Plant Genome*, 9, plantgenome2016.03.0034. <https://doi.org/10.3835/plantgenome2016.03.0034>
- Counce, p.A., Keisling, T.C. & Mitchell, A.J. (2000) A uniform, objectives, and adaptive system for expressing rice development. *Crop Science*, 40, 436–443.
- Delatorre, C.A. (1999) Dormência em sementes de arroz vermelho. *Ciência Rural*, 29, 565–571.
- Gardarin, A. & Colbach, N. (2015) How much of seed dormancy in weeds can be related to seed traits? *Weed Research*, 55, 14–25.
- Ghadirnezhad, R. & Fallah, A. (2014) Temperature effect on yield and yield components of different rice cultivars in flowering stage. *Int. J. Agron.*, 2014, 1–4.
- Goggin, D.E., Emery, R.J.N., Powles, S.B. & Steadman, K.J. (2010) Initial characterisation of low and high seed dormancy populations of *Lolium rigidum* produced by repeated selection. *Journal of Plant Physiology*, 167, 1282–1288.
- Ji, H., Kim, S.R., Kim, Y.H., Kim, H., Eun, M.Y., Jin, I.D. et al. (2010) Inactivation of the CTD phosphatase-like gene OsCPL1 enhances the development of the abscission layer and seed shattering in rice. *The Plant Journal*, 61, 96–106.
- Kim, H.Y., Ko, J., Kang, S. & Tenhunen, J. (2013) Impacts of climate change on paddy rice yield in a temperate climate. *Global Change Biology*, 19, 548–562.
- Kimball, B.A., 2016. Crop responses to elevated CO_2 and interactions with H_2O , N, and temperature. *Current Opinion in Plant Biology* 31, 36–43. <https://doi.org/10.1016/j.pbi.2016.03.006>
- Konishi, S., Izawa, T., Lin, S.Y., Ebana, K., Fukuta, Y., Sasaki, T., Yano, M., 2006. An SNP caused loss of seed shattering during rice domestication. *Science* 312, 1392–1396. <https://doi.org/10.1126/science.1126410>
- Korres, N.E., Norsworthy, J.K., Young, B.G., Reynolds, D.B., Johnson, W.G., Conley, S.P. et al. (2018) Seedbank persistence of palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) across diverse geographical regions in the United States. *Weed Science*, 66, 446–456.

- Kumar, U., Quick, W.P., Barrios, M., Cruz, p.C.S. & Dingkuhn, M. (2017) Atmospheric CO₂ concentration effects on rice water use and biomass production. *PLoS One*, 12, 1–17.
- Lamichaney, A., Kumar Swain, D., Biswal, P., Kumar, V., Pratap Singh, N. & Krishna Hazra, K. (2019) Elevated atmospheric carbon-dioxide affects seed vigour of rice (*Oryza sativa* L.). *Environmental and Experimental Botany*, 157, 171–176.
- Leadley, p.W. & Drake, B.G. (1993) Open top chambers for exposing plant canopies to elevated CO₂ concentration and for measuring net gas exchange. *Vegetatio*, 104–105, 3–15.
- Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. (2009) Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany*, 60, 2859–2876.
- Li, C., Zhou, A. & Sang, T. (2006) Rice domestication by reducing shattering. *Science*, 311, 1936–1939.
- Li, W., Xu, L., Bai, X., Xing, Y., 2011. Quantitative trait loci for seed dormancy in rice. 427–435. <https://doi.org/10.1007/s10681-010-0327-4>
- Martínez-Eixarch, M. & Ellis, R.H. (2015) Temporal sensitivities of rice seed development from spikelet fertility to viable mature seed to extreme-temperature. *Crop Science*, 55, 354–364.
- Marty, C., Bassirirad, H., 2014. Seed germination and rising atmospheric CO₂ concentration: a meta-analysis of parental and direct effects 401–414
- Massey, J.H., Walker, T.W., Anders, M.M., Smith, M.C. & Avila, L.A. (2014) Farmer adaptation of intermittent flooding using multiple-inlet rice irrigation in Mississippi. *Agricultural Water Management*, 146, 297–304. <https://doi.org/10.1016/j.agwat.2014.08.023>
- McKim, S.M., Stenvik, G.E., Butenko, M.A., Kristiansen, W., Cho, S.K., Hepworth, S.R. et al. (2008) The BLADE-ON-PETIOLE genes are essential for abscission zone formation in Arabidopsis. *Development*, 135, 1537–1546. <https://doi.org/10.1242/dev.012807>
- Menalled, F. (2008) Weed seed bank dynamics & integrated management of agricultural weeds. *Agriculture and Natural Resources*, 1, 1–4.
- Munda, S., Saha, S., Adak, T., Jambhulkar, N., Sanghamitra, P. & Patra, B.C. (2019) Performance of cultivated indica rice (*Oryza sativa* L.) as affected by weedy rice. *Experimental Agriculture*, 55, 875–884. <https://doi.org/10.1017/S0014479718000455>
- Noctor, G. & Mhamdi, A. (2017) Climate change, CO₂, and defense: the metabolic, redox, and signaling perspectives. *Trends in Plant Science*, 22, 857–870. <https://doi.org/10.1016/j.tplants.2017.07.007>
- Noldin, J.A., Chandler, J.M. & McCauley, G.N. (2006) Seed longevity of red rice ecotypes buried in soil. *Planta Daninha*, 24, 611–620. <https://doi.org/10.1590/S0100-83582006000400001>
- Noldin, J.A., Yokoyama, S., Stuker, H., Rampelotti, F.T., Gonçalves, M.I.F., Eberhardt, D.S. et al. (2004) Desempenho de populações híbridas F2 de arroz-vermelho (*Oryza sativa*) com arroz transgênico (*O. sativa*) resistente ao herbicida amônio-glufosinate. *Planta Daninha*, 22, 381–395.
- Ogawa, M., Kay, P., Wilson, S. & Swain, S.M. (2009) Arabidopsis dehiscence zone Polygalacturonase1 (ADPG1), ADPG2, and Quartet2 are polygalacturonases required for cell separation during reproductive development in Arabidopsis. *Plant Cell*, 21, 216–233. <https://doi.org/10.1105/tpc.108.063768>
- Pan, C., Zhang, H., Ma, Q., Fan, F., Fu, R., Ahammed, G.J. et al. (2019) Role of ethylene biosynthesis and signaling in elevated CO₂-induced heat stress response in tomato. *Planta*, 250, 563–572. <https://doi.org/10.1007/s00425-019-03192-5>
- Piveta, L.B., Noldin, J.A., Roma-Burgos, N., Viana, V.E., Benedetti, L., Pinto, J.J.O., Lamego, F.P., Avila, L.A. de, 2021. Weedy rice diversity in southern Brazil. *Weed Science* <https://doi.org/10.1017/wsc.2021.23>, 69, 547, 557
- Refatti, J.P., de Avila, L.A., Camargo, E.R., Ziska, L.H., Oliveira, C., Salas-Perez, R. et al. (2019) High [CO₂] and temperature increase resistance to cyhalofop-butyl in multiple-resistant *Echinochloa colona*. *Frontiers in Plant Science*, 10, 1–11. <https://doi.org/10.3389/fpls.2019.00529>
- Roberts, J.A., Elliott, K.A. & Gonzalez-Carranza, Z.H. (2002) Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology*, 53, 131–158. <https://doi.org/10.1146/annurev.arplant.53.092701.180236>
- Sakai, H., Tokida, T., Usui, Y., Nakamura, H. & Hasegawa, T. (2019) Yield responses to elevated CO₂ concentration among Japanese rice cultivars released since 1882. *Plant Production Science*, 22, 352–366. <https://doi.org/10.1080/1343943X.2019.1626255>
- Sartori, G.M.S., Marchesan, E., Azevedo, C.F., Coelho, L.L. & De Oliveira, M.L. (2014) Germinação de arroz irrigado e de biótipos de arroz-vermelho submetidas a diferentes temperaturas. *Revista Ciencia Agronomica*, 45, 319–326. <https://doi.org/10.1590/1808-1657001242012>
- Schnerer, A., Melander, B. & Kudsk, P. (2016) Vertical distribution and composition of weed seeds within the plough layer after eleven years of contrasting crop rotation and tillage schemes. *Soil and Tillage Research*, 161, 135–142. <https://doi.org/10.1016/j.still.2016.04.005>
- Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Chen, C. et al. (2011) Impact of high-temperature stress on rice plant and its traits related to tolerance. *The Journal of Agricultural Science*, 149, 545–556. <https://doi.org/10.1017/S0021859611000360>
- Shi, K., Li, X., Zhang, H., Zhang, G., Liu, Y., Zhou, Y. et al. (2015) Guard cell hydrogen peroxide and nitric oxide mediate elevated CO₂-induced stomatal movement in tomato. *The New Phytologist*, 208, 342–353. <https://doi.org/10.1111/nph.13621>
- Shivrain, V.K., Burgos, N.R., Sales, M.A. & Kuk, Y.I. (2010) Polymorphisms in the ALS gene of weedy rice (*Oryza sativa* L.) accessions with differential tolerance to imazethapyr. *Crop Protection*, 29, 336–341. <https://doi.org/10.1016/j.cropro.2009.10.002>
- SIGMAPLOT, 2012. Scientific graphing software.
- Singh, K. et al. (2013) Weedy rice: an emerging threat for direct-seeded rice production systems in India. *Rice Research Open Access*, 4, 1–6. <https://doi.org/10.4172/jrr.1000106>
- Smith, R.J. (1988) Weed thresholds in southern U.S. Rice, *Oryza sativa*. *Weed Technology*, 2, 232–241. <https://doi.org/10.1017/s0890037x00030505>
- SOSBAI, S.S.-B. de arroz irrigado, 2018. Recomendações técnicas da pesquisa para o Sul do Brasil, XXIX Reunião Técnica da Cultura do Arroz Irrigado. Cachoerinha.
- Terashima, I., Yanagisawa, S. & Sakakibara, H. (2014) Plant responses to CO₂: background and perspectives. *Plant & Cell Physiology*, 55, 237–240. <https://doi.org/10.1093/pcp/pcu022>
- Thurber, C.S., Reagon, M., Gross, B.L., Olsen, K.M., Jia, Y. & Caicedo, A.L. (2010) Molecular evolution of shattering loci in U.S. weedy rice. *Molecular Ecology*, 19, 3271–3284. <https://doi.org/10.1111/j.1365-294X.2010.04708.x>
- Tseng, T.M., Shivrain, V.K., Lawton-Rauh, A. & Burgos, N.R. (2018) Dormancy-linked population structure of weedy rice (*Oryza* sp.). *Weed Science*, 66, 331–339. <https://doi.org/10.1017/wsc.2017.86>
- Varanasi, A., Prasad, p.V.V. & Jugulam, M. (2016) Impact of climate change factors on weeds and herbicide efficacy. In: *Advances in agronomy*. Amsterdam: Elsevier Inc., pp. 107–146. <https://doi.org/10.1016/bs.agron.2015.09.002>
- Vargas, A.A.M., Agostinetto, D., Zandoná, R.R., Fraga, D.S., Avila Neto, R. C., 2018. Longevity of horseweed seed bank depending on the burial depth. *Planta Daninha* 36, 1–9. <https://doi.org/10.1590/S0100-83582018360100050>
- Xu, Z., Jiang, Y. & Zhou, G. (2015) Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO₂ with environmental stress in plants. *Frontiers in Plant Science*, 6, 1–17. <https://doi.org/10.3389/fpls.2015.00701>

- Ye, C., Fukai, S., Godwin, I., Reinke, R., Snell, P., Schiller, J. et al. (2009) Cold tolerance in rice varieties at different growth stages. *Crop & Pasture Science*, 60, 328–338. <https://doi.org/10.1071/CP09006>
- Yoon, J., Cho, L.H., Antt, H.W., Koh, H.J. & An, G. (2017) KNOX protein OSH15 induces grain shattering by repressing lignin biosynthesis genes. *Plant Physiology*, 174, 312–325. <https://doi.org/10.1104/pp.17.00298>
- Zhao, X., Zhou, N., Lai, S., Frei, M., Wang, Y. & Yang, L. (2019a) Elevated CO₂ improves lodging resistance of rice by changing physico-chemical properties of the basal internodes. *Science of the Total Environment*, 647, 223–231. <https://doi.org/10.1016/j.scitotenv.2018.07.431>
- Zhao, Y., Zhang, J., Zhang, Z. & Xie, W. (2019b) *Elymus nutans* genes for seed shattering and candidate gene-derived EST-SSR markers for germplasm evaluation. *BMC Plant Biology*, 19, 1–15. <https://doi.org/10.1186/s12870-019-1691-4>
- Zhao, Y.F., Sun, H.Z., Wen, H.L., Du, Y.X., Zhang, J., Li, J.Z. et al. (2018) Germination vigour difference of superior and inferior rice grains revealed by physiological and gene expression studies. *The Journal of Agricultural Science*, 156, 350–357. <https://doi.org/10.1017/S0021859618000461>
- Zhou, Y., Lu, D., Li, C., Luo, J., Zhu, B.F., Zhu, J. et al. (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor shattering Abortin1. *Plant Cell*, 24, 1034–1048. <https://doi.org/10.1105/tpc.111.094383>
- Zhu, C., Kobayashi, K., Loladze, I., Zhu, J., Jiang, Q., Xu, X. et al. (2018) Carbon dioxide (CO₂) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. *Science Advances*, 4, 1–9. <https://doi.org/10.1126/sciadv.aag1012>
- Ziska, L.H., Blumenthal, D.M. & Franks, S.J. (2019) Understanding the nexus of rising CO₂, climate change, and evolution in weed biology. *Invasive Plant Science and Management*, 12, 79–88. <https://doi.org/10.1017/inp.2019.12>
- Ziska, L.H., Gealy, D.R., Burgos, N., Caicedo, A.L., Gressel, J., Lawton-Rauh, A.L. et al. (2015) Weedy (red) rice: an emerging constraint to global rice production. *Advances in Agronomy*, 129, 181–228. <https://doi.org/10.1016/bs.agron.2014.09.003>
- Ziska, L.H., McClung, A. (2008) Differential response of cultivated and weedy (Red) rice to recent and projected increases in atmospheric carbon dioxide. *Agronomy Journal*, 100, 1259–1263. <https://doi.org/10.2134/agronj2007.0324>

How to cite this article: Balbinot, A., da Rosa Feijó, A., Fipke, M.V., Gehrke, V.R., Agostinetto, D., Kruse, N.D. et al. (2022) Rising atmospheric CO₂ concentration affect weedy rice growth, seed shattering and seedbank longevity. *Weed Research*, 62(4), 277–286. Available from: <https://doi.org/10.1111/wre.12536>