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Short Communication

Sensitivity of weedy rice populations to Quizalofop

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The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Highlights

- Weedy rice accessions were controlled 100% with quizalofop at 50 g i.a. ha^{-1}.
- Quizalofop can be used to manage volunteer CL rice and IMI-resistant weedy rice.
- Rice lines SC 964 and SC 965 are resistant to quizalofop.

Abstract

Background: Rice lines displaying resistance to quizalofop-p-ethyl (quizalofop), an acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicide, were developed to facilitate control of imidazolinone-resistant weedy rice (red rice), as well as other susceptible grasses.
Objective: To assess the sensitivity of weedy rice accessions from Brazil to quizalofop.
Methods: Two bioassays were conducted on the greenhouse from March to June 2016, in a completely random design with a two-factor factorial combination of treatments and four replications. The factor A was weedy rice accession and factor B was quizalofop rates (0, 25 and 50 g a.i. ha^{-1}). It was assessed a total of 84 genotypes in the two bioassays, composed of 78 weedy rice accessions, 4 rice cultivars, and 2 lines resistant to aryloxyphenoxypropionate (AOPP) herbicides. Quizalofop was applied the 2-leaf stage. Weedy rice control was evaluated at 28 and 22 days after treatment (DAT), respectively for bioassay I and II, using a scale of 0% (no control) to 100% (dead).
Results: All weedy rice accessions were susceptible to quizalofop, being controlled 100% with 50 g a.i. ha^{-1}. Rice lines SC 964 and SC 965, resistant to AOPP herbicides, were not injured by quizalofop.
Conclusions: quizalofop is an effective tool for weedy rice control in ACCase-resistant rice.

Weedy rice (red rice) is one of the most important weeds in paddy rice fields, occurring in all world regions where rice is grown (Delouche et al., 2007; Sudianto et al., 2013). In Southern Brazil, weedy rice is frequently found in different rice production systems (Ulguim et al., 2018), causing losses in yield and grain quality (Menezes et al., 1997). Weedy rice is the most competitive weed in paddy rice because it is a close relative of cultivated rice (*Oryza sativa* L.) and, therefore, has the same morphology and the same environmental and resource requirements as the crop (Burgos et al., 2006; Delouche et al., 2007). The main characteristics of most weedy rice populations are being taller than commercial cultivars, susceptibility to lodging, early to intermediate maturity, high seed shattering index, and high dormancy (Schwanke et al., 2008). However, more recently, it is not unusual to find weedy rice plants in the field that are very similar to modern rice cultivars: short-stature, erect leaves, long-grains, but with reddish pericarp. These are outcrossed plants that have introgressed enough crop genes after years of recurrent interbreeding. Although the crop and weed are of the same species, their outcrossing percentage is low (generally less than 1%) (Shivrain et al., 2009) because this specie is primarily selfing. It used to be that outcrosses barely noticeable in the field, if at all. However, with the high level and sustained adoption of imidazolinone-resistant (Clearfield™) rice to control weedy rice, the outcrosses (which now carry the resistance gene) are being selected with imidazolinone herbicides in the Clearfield™ rice system (Sudianto et al., 2013). Thus, we are seeing higher frequency of crop-like weedy rice in fields with a history of Clearfield™ rice production (Burgos et al., 2014).

The Clearfield™ rice production system (CL) is a unique technology, which is available to the Brazilian rice growers, as it is in North and Central America, a few countries in Europe, and in Malaysia. This technology, if used well in conjunction with other rice herbicides, provides excellent control of weedy rice. The CL rice technology enables selective control of weedy rice using imidazolinone (IMI) herbicides any sequential or premix combination of imazethapyr, imazapyr, imazapic, and imazamox. The resistance to IMI herbicides was obtained by chemically-induced mutation of the acetolactate synthase (ALS) enzyme (Webster and Masson, 2001). However, due to the inability of rice growers to consistently adhere to the stewardship guidelines for CL rice adoption, the outcrosses ensuing from gene flow between CL cultivars and weedy rice resulted in the evolution of IMI-resistant weedy rice populations in Brazil and other world regions (Roso et al., 2010; Goulart et al., 2012; Sudianto et al., 2013). There are many reasons why rice growers could not follow the technology stewardship guidelines; some by choice, others are due to factors beyond their control (Burgos et al., 2008).

A survey conducted to assess the occurrence and distribution of imidazolinone-resistant (IMI-R) weedy rice populations in Rio Grande do Sul State showed that out of 228 sites sampled, 56% of the populations were IMI-R (Menezes et al., 2009). Furthermore, fields practices used in Southern Brazil have contributed to IMI-R spread. Kalsing et al. (2019) reported that crop rotation systems and certified seed were adopted by only 30% of the surveyed farmers and the adoption rates were lower in the regions where IMI-R weedy rice first evolved. Due the continuing evolution of herbicide-resistant weeds, new traits are needed to sustain an effective weed control. New rice genotypes resistant to ACCase-inhibiting herbicides (Provisia™), have been developed. The Provisia™ technology (BASF) will provide rice cultivars resistant to quizalofop (Lancaster et al., 2018a). This technology was developed also by chemically-induced mutation of the ACCase enzyme, producing rice plants resistant to AOPP herbicides (Mankin et al., 2014). A similar technology (Max-Ace™ by RiceTec) also confers resistance to AOPP herbicides by a point mutation at G-2096-S in ACCase enzyme (Hinga et al., 2013).

The Institution for Agricultural Research and Extension for Santa Catarina State - Epagri, Brazil) developed rice lines resistant to AOPP herbicides through induced mutation with gamma rays. A point mutation, G-2027-T, was identified to have caused resistance to AOPP herbicides. This target-site mutation confers more than 70-fold resistance to quizalofop and haloxyfop in these rice lines (Andrade et al., 2018). Research carried out by Lancaster et al. (2018b) demonstrated that quizalofop is selective to Provisia™ rice cultivar with less than 5% injury. Moreover, their research showed that quizalofop is effective in controlling *Oryza sativa*, *Echinochloa crus-galli, Urochloa platyphylla, Panicum dichotomiflorum* and *Leptochloa panicoides* when sprayed at the 2-3 leaf stage. Hence, this research was conducted to assess the sensitivity of 78 weedy rice accessions collected in 11 counties in Brazil to quizalofop, before the commercialization of ACCase resistant rice in Brazil.

Two bioassays were conducted in the greenhouse at Epagri-Itajaí Experiment Station from March to June, 2016. The experimental design was completely randomized with treatments organized in a two-factor factorial scheme, with four replicates. The first factor (A) consisted of 78 weedy rice accessions ,4 rice cultivars (SCS121 CL, SCS117 CL, SCS118 Marques and SCS116 Satoru), and 2 lines resistant to AOPP herbicides (SC 964 and SC 965). The weedy rice seeds were obtained from Epagri´s weedy rice germplasm bank (identified as BAD) located at Itajaí Experiment Station. The accessions were collected in rice fields from the season 1995/96 until 2014/15 (Table 1). The second factor (B) was quizalofop rate (0, 25 and 50 g a.i. ha^{-1}). The commercial formulation Targa 50 EC (quizalofop, 50 g a.i. L^{-1}, EC) was used. The rates were based on previous research with the rice resistant lines (Andrade et al., 2018).

The experimental units consisted of 500-cm^3 pots, filled with soil from a paddy rice field. The soil was sieved prior to use. The soil series was classified as Haplic Entisols. Seeds of all genotypes were pre-germinated in BOD chamber at 30 °C, in the dark. After three days, two seedlings were transplanted to each pot. Permanent flood was established

Table 1 - Counties of sampling (CS) and pericarp color of weedy rice accessions

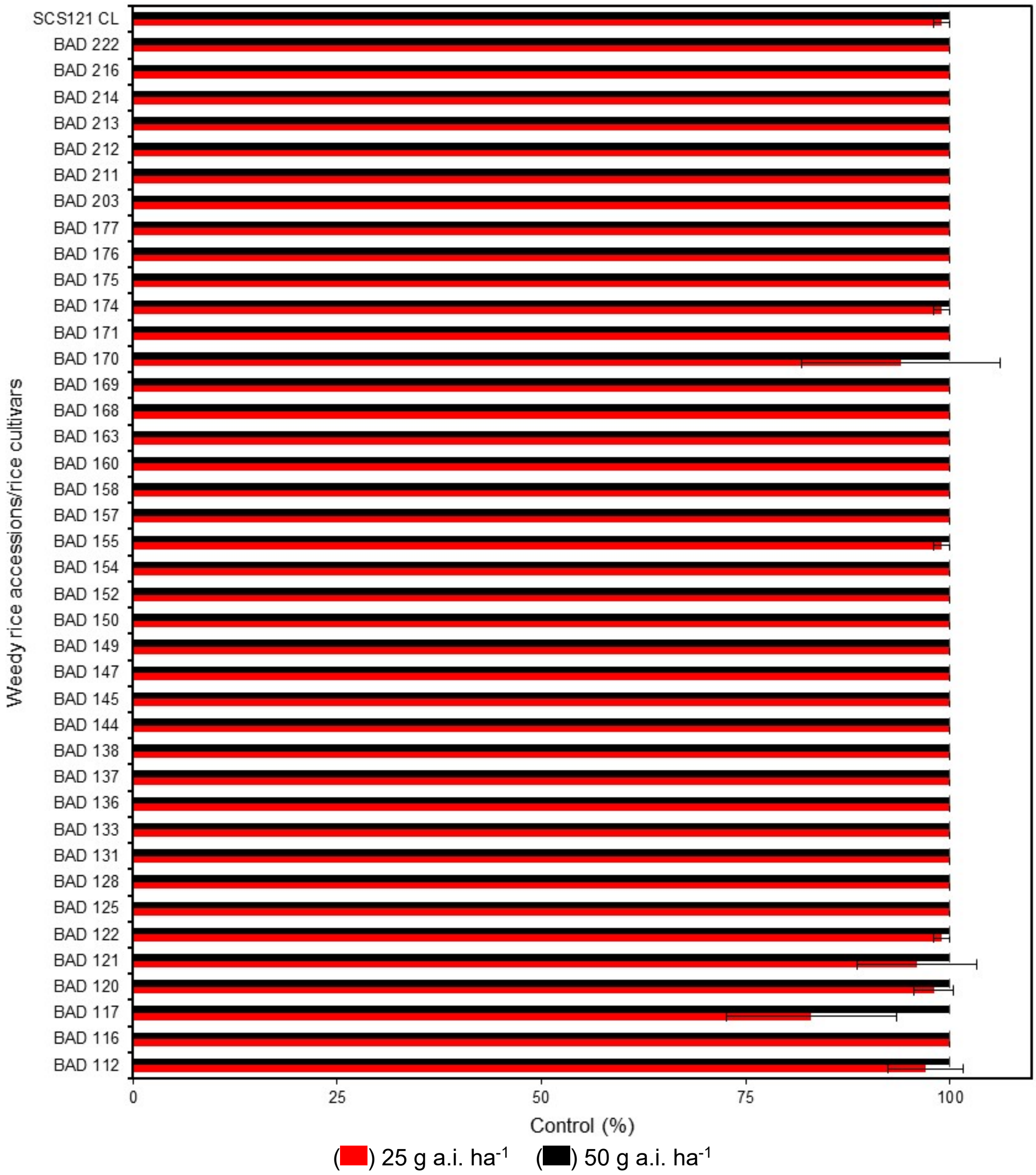
Accession	CS[(1)]	Pericarp	Bioassay	Accessions	CS	Pericarp	Bioassay
BAD 40	ITJ	Red	II	BAD 154	POS	Red	I
BAD 43	ITJ	Red	II	BAD 155	ITJ	Red	I
BAD 46	ITJ	Red	II	BAD 156	ITJ	Red	II
BAD 81	ITJ	Red	II	BAD 157	PEL	Red	I
BAD 82	ITJ	Red	II	BAD 158	SVP	Red	I
BAD 91	ITJ	Red	II	BAD 160	ITJ	Red	I
BAD 93	ITJ	Red	II	BAD 162	ITJ	Red	II
BAD 94	ITJ	Red	II	BAD 163	ITJ	Red	I e II
BAD 96	ITJ	Red	II	BAD 168	GAS	Red	I
BAD 99	ITJ	White	II	BAD 169	GAS	Red	I
BAD 101	ITJ	Red	II	BAD 170	NAV	Red	I e II
BAD 104	ITJ	Red	II	BAD 171	GAS	Red	I
BAD 109	ITJ	Red	II	BAD 172	GAS	Red	II
BAD 112	ITJ	Red	I	BAD 173	MAS	Red	II
BAD 113	ITJ	Red	II	BAD 174	MAS	Red	I
BAD 115	ITJ	Red	II	BAD 175	RDC	Red	I
BAD 116	ITJ	Red	I	BAD 176	GAS	Red	I
BAD 117	ITJ	Red	I e II	BAD 177	GAS	Red	I
BAD 118	ITJ	Red	II	BAD 178	GAS	Red	II
BAD 119	ITJ	Red	II	BAD 179	GAS	Red	II
BAD 120	ITJ	Red	I e II	BAD 180	GAS	Red	II
BAD 121	ITJ	Red	I e II	BAD 182	GAS	Red	II
BAD 122	ITJ	Red	I e II	BAD 184	GAS	Red	II
BAD 124	ITJ	Red	II	BAD 186	GAS	Red	II
BAD 125	ITJ	Red	I	BAD 188	GAS	Red	II
BAD 128	ITJ	Red	I	BAD 190	GAS	Red	II
BAD 131	ITJ	Red	I	BAD 203	GAS	Red	I
BAD 133	ITJ	Red	I	BAD 206	GAS	Red	II
BAD 134	ITJ	Red	II	BAD 208	GAS	Red	II
BAD 135	ITJ	Red	II	BAD 211	GAS	Red	I
BAD 136	ITJ	Red	I	BAD 212	MOG	Red	I
BAD 137	ITJ	Red	I	BAD 213	GUA	Red	I
BAD 138	ITJ	Red	I	BAD 214	BOV	Red	I
BAD 139	ITJ	Red	II	BAD 216	BOV	Red	I
BAD 140	ITJ	Red	II	BAD 222	TAI	Black	I
BAD 141	ITJ	Red	II	BAD 223	TAI	Black	II
BAD 144	ITJ	Red	I	SCS116 Satoru	Cultivar		II
BAD 145	ITJ	Red	I	SCS118 Marques	Cultivar		II
BAD 147	ITJ	Red	I	SCS117 CL	CL cultivar		II
BAD 149	ITJ	Red	I	SCS121 CL	CL cultivar		I e II
BAD 150	ITJ	Red	I	SC 964	Line AOPP-R		II
BAD 152	ITJ	Red	I	SC 965	Line AOPP-R		II

[(1)] ITJ=Itajaí/SC, POS = Pedro Osório/RS, PEL = Pelotas/RS, SVP = Santa Vitória do Palmar/RS, GAS = Gaspar/SC, NAV = Navegantes/SC, MAS = Massaranduba/SC, MOG = Morro Grande/SC, GUA = Guaramirim/SC, BOV = Boa Vista/RR, TAI = Taió/SC.

immediately after transplanting and maintained until the plants were sprayed with herbicide. Quizalofop was sprayed early post-emergence, when weedy rice had two visible leaf collars – V_2 stage (Counce et al., 2000). The herbicide treatments were applied with a CO_2-pressured backpack sprayer, attached to a handheld boom with four flat-fan nozzles (110.015), calibrated to deliver 150 L ha^{-1} at 207 kPa and a traveling speed of 1.0 m s^{-1}. The boom height was set at 0.5 m from the plant canopy. Herbicide application was done in the morning with adequate weather conditions (air temperature ≥ 26 °C, relative humidity ≥ 80% and wind speed of 0.5 to 1.7 km h^{-1}).

Weedy rice control and rice injury were evaluated visually at 28 and 22 DAT, respectively, for bioassays I and II. The level of control was assessed on a scale of 0 – 100 where 0% = no control and 100% = dead (SBCPD, 1995; Kuva at al., 2016). The data were analyzed by confidence interval (p<0,05) and treatments were considered distinct when the confidence intervals did not overlap (Cumming et al., 2004; Concenço et al., 2018).

Weedy rice and commercial rice cultivars treated with 25 g a.i. ha^{-1} showed different sensitivity to quizalofop in bioassay I (Figure 1). In other words, this rate was a sublethal dose to some genotypes. BAD 117 was the most tolerant to quizalofop at 25 g ha^{-1} with 83% control. Eight weedy rice accessions (BAD 112, BAD 120, BAD 121, BAD 122, BAD 155, BAD 170, BAD 174) and the cv. SCS121



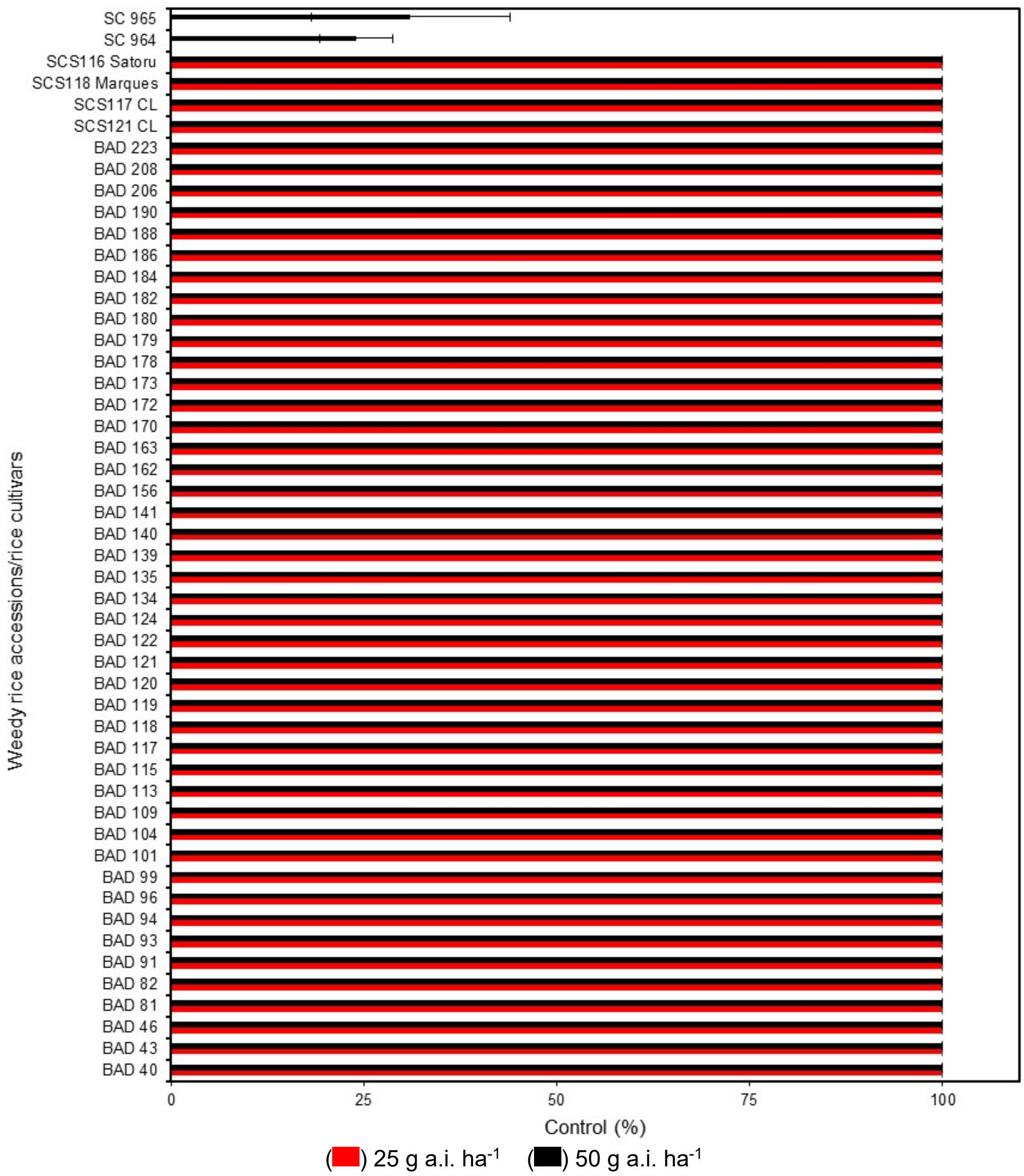
Bars indicate the confidence intervals (p<0.05).

Figure 1 - Weedy rice accessions control (%) treated with quizalofop at 25 and 50 g a.i. ha^{-1}, 28 DAT. Results from bioassay I. Epagri, Itajaí/SC, 2016.

CL were highly susceptible to quizalofop, with control ranging from 94% to 99%. Other accessions (32) in bioassay I were extremely susceptible to quizalofop with 100% control at the lowest rate (25 g a.i. ha^{-1}). All weedy rice genotypes and the cv. SCS121 CL were controlled 100% at the rate of 50 g a.i. of quizalofop (Figure 1). In bioassay II (Figure 2), all weedy rice accessions and the four commercial rice cultivars were susceptible to quizalofop, even at the low rate, including BAD 117, BAD 120, BAD 121, BAD 122 and BAD 170 that were not 100% controlled by quizalofop 25 g a.i. ha^{-1} in the bioassay I. The differential sensibility of some weedy rice accessions between the bioassays can be assigned to no-controlled factor, since the quizalofop rate, stage of application and environmental conditions were similar in both bioassays. The mutant lines SC 964 and SC 965 were resistant to quizalofop, with only 25% injury at the highest rate. The observed injury symptoms were chlorotic spotting, but under field conditions the injury level is expected to be lower, compared to the greenhouse condition. Lancaster et al. (2018b) related that under field condition the injury of quizalofop reached 5% at 21 DAT.

In summary, all weedy rice accessions and cultivars were highly susceptible to quizalofop at 50 g a.i. ha^{-1} (Figure 1 and 2).

Differential sensibility to quizalofop was also reported of *Echinochloa crus-galli* (Lancaster et al., 2018b). Lancaster and colleagues evaluated the response of 126 *E. crus-galli* accessions to



Bars indicate the confidence intervals ($p<0.05$).

Figure 2 - Weedy rice accessions control (%) treated with quizalofop at 25 and 50 g a.i. ha^{-1}, 22 DAT. Results from bioassay II. Epagri, Itajaí/SC, 2016.

80 g a.i. ha^{-1} quizalofop. Of these, 89.7% were completely controlled by quizalofop; the remaining accessions were controlled at least 92%. It turned out that weedy rice is equally sensitive, if not more sensitive, to quizalofop as *E. crus-galli*. All weedy rice genotypes assessed in bioassay I and II were completely controlled by 50 g a.i. ha^{-1} quizalofop (Figures 1 and 2). Similarly, Camacho et al. (2019) also demonstrated that cultivars Catahoula and Mermentau were extremely susceptible to quizalofop and the rate of 2 mg L^{-1} resulted in complete mortality of 2-3 leaf seedlings. Lancaster et al. (2018b) reported 97 and 99% control of red rice with quizalofop (80, 120, or 160 g ha^{-1}) applied at 6- and 2-leaf stage, respectively.

The results from the assay II agreed with those reported by Camacho et al. (2019) pertaining to the response of five quizalofop-resistant rice lines to quizalofop. Camacho and colleagues concluded that the rice lines they tested were indeed resistant, showing no phytotoxicity effects 14 days after treatment. In general, quizalofop appeared to be consistently effective in controlling a broad array of weedy rice genotypes, showing high efficacy even at rates as low as 25 g a.i. ha^{-1}, when applied at 2-leaf stage. Quizalofop also controlled the Clearfield rice cultivars SCS117 CL and SCS121 CL, which indicates that this herbicide is effective in controlling volunteer CL rice and IMI-resistant weedy outcrosses. In the United State of America, the label rate of quizalofop in ACCase-resistant rice ranges from 100 to 138 g a.i. ha^{-1} for a single application with a maximum allowable rate of 240 g a.i. ha^{-1} in one year (Lancaster et al., 2018b). The herbicide rate used by Lancaster et al. (2018b) is 2 to 5 times the rate that provided 100% control of weedy rice and commercial cultivars evaluated at Epagri.

Thus, the development and availability of quizalofop-resistant cultivars to the rice growers will contribute to a better weedy rice management (Andrade et al., 2018; Camacho et al., 2019), as long as the stewardship program is respected. We conclude that weedy rice accessions from rice fields in Brazil are susceptible to quizalofop at the rate of 50 g a.i. ha^{-1}, applied at V_2-leaf stage. Rice lines SC 964 and SC 965 are resistant to quizalofop. Quizalofop can be used to manage volunteer CL rice and IMI-resistant weedy rice.

CONTRIBUTIONS

All authors conceived and designed the research. AMON, MC, AA, and JAN: conducted the experiments. AMON, and NG: analyzed the data, prepared figures and wrote the manuscript. NRB: revised the manuscript. All authors reviewed and approved the manuscript.

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