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Gene flow of quizalofop-p-ethyl resistance from crop to weedy rice

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Abstract: Background: Provisia™ rice was developed as an alternative to Clearfield® rice technology. However, the Provisia™ system's long-term efficacy may be quickly reduced by gene flow from crop to weedy rice.

Objective: This study aimed to assess the pollen-mediated gene flow from Provisia™ to weedy rice.

Methods: Two field experiments were conducted in rectangular design. The pollen donor (Provisia™) was sown upwind of the predominant wind direction and the pollen acceptor (quizalofop-p-ethyl (QPE)-susceptible weedy rice) was placed downwind at 0, 0.5 and 1m from donor. Weedy rice seeds from putative hybrids were sown on Germitest® paper containing QPE solution (1.6 mg ai L^{-1}). The surviving seedlings were transplanted into pots and at V3, seedlings were treated with QPE (120 g ai ha^{-1}). The survivors (putative F1 crosses) were cultured. Seeds (F2) were harvested and used in QPE dose-response assay. Genomic DNA was extracted from Provisia™, QPE-susceptible weedy rice and F1 for analysis of the presence or absence of the $Ile_{1781}Leu$ mutation in ACCase.

Results: A few (0.07%) survived the QPE treatment and no difference between the distances was observed. All F1 survivors carried the same ACCase mutation ($Ile_{1781}Leu$) present in Provisia™. Dose-response assay showed that all F2 were resistant to QPE.

Conclusions: QPE-resistant weedy rice arose from crop-to-weed pollen-mediated gene flow. It is recommended to avoid weedy rice escapes from Provisia™ rice fields.

Keywords: Herbicide-resistant; Provisia™ Rice; Quizalofop-p-ethyl

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1. Introduction

Weedy rice (*Oryza* spp.) is a global problem in rice fields (Burgos et al., 2021) threatening food security. The most troublesome weed species are those that are like the crops they infest. A perfect example of this is weedy rice, belonging to the same taxon as cultivated rice (a conspecific plant), but has weedy traits such as seed shattering, seed dormancy, various pericarp color (red, black, straw), among others (Nadir et al., 2017; Burgos et al., 2021). Conspecific weeds, due to the close evolutionary relationship, are typically compatible with related crops, resulting in gene flow between conspecific crops and weeds (Jia et al., 2014).

Gene flow can occur via dispersal of pollen, seeds, and/or vegetative propagules (Beckie et al., 2019). Pollen-mediated gene flow (PMGF) is primarily facilitated by wind and pollinators (Ganie and Jhala, 2017). Although cultivated, wild, and weedy rice are predominantly autogamous (self-pollinated species), spontaneous PMGF occurs between these species (He et al., 2014; Jia et al., 2014). Under field conditions, the frequency of gene flow from cultivated rice is between approximately 0.011 and 0.046% to weedy rice and between approximately 1.21 and 2.19% to wild rice (Chen et al., 2004).

Intraspecific hybridization between cultivated and weedy rice is modified by biological/ physical factors and generally is less than 1% (Jia et al., 2014). PMGF exists in nature as one of the most important evolutionary sources of plants and can provide useful genes/alleles to weed populations (survival advantages) from their domesticated or their nearby wild relatives (Nadir et al., 2017). Through allelic introgression of sympatric cultivars, intraspecific hybridization allows weedy rice to acquire some morphological and physiological traits of rice crop (He et al., 2014), including the introgression of herbicide resistance (HR) genes (Nadir et al., 2017). As a result, PMGF can contribute to the rapid spread of HR within and among weed populations (Beckie et al., 2019).

In direct-seeded rice, weedy rice post-emergence selective control only became possible with the release of imidazolinone (IMI)-resistant rice – Clearfield® (CL) production system for rice (Sudianto et al., 2013). IMIs are one of the chemical groups of acetolactate synthase (ALS)-inhibiting herbicides, a key enzyme in the biosynthesis of branched-chain amino acids (valine, leucine and isoleucine) (Duggleby et al., 2008). Some point mutations that result in amino acid changes

maintain the ALS functionality but alter the enzyme's structure and compromises the binding of herbicides, conferring resistance to ALS-inhibiting herbicides (Sudianto et al., 2013). Non-transgenic rice, with ALS gene mutated at positions $Gly_{654}Glu$, $Ser_{653}Asp$ and $Ala_{122}Thr$, was commercially released as an important tool to manage weedy rice (Roso et al., 2010; Sudianto et al., 2013).

The main concern in CL rice was the quick evolution of IMI-resistant weedy rice, attributed mainly to PMGF (Goulart et al., 2012). In the early 2000s, studies performed in the United States demonstrated that PMGF transfers resistance traits from CL rice to weedy rice, meaning that resistance evolution can start in the first growing season if weedy rice is in close proximity and flowers simultaneously as the crop (Rajguru et al., 2005; Shivrain et al., 2007). In Brazil, it was found that only three years after the adoption of CL rice, all producing regions had IMI-resistant weedy rice (Roso et al., 2010). In this case, subsequent studies with different populations, using simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers, demonstrated that 98.9% of IMI-resistant weedy rice was due to PMGF and only 1.1% by independent selection (Goulart et al., 2012). Changes in ALS gene are monogenic, dominant or partially dominant and inheritance is related to the nuclear gene (Tranel and Wright, 2002). Thus, all of the weedy rice-rice hybrids and a majority of the succeeding generations are herbicide-resistant (Shivrain et al., 2007).

Crop to weed PMGF is a concern because it can spread HR alleles (Jhala et al., 2021). Even in self-pollinated species, PMGF rates are higher than spontaneous mutation rates (estimated as 10^{-5} or 10^{-6} gametes per locus each generation) (Beckie et al., 2019). Thus, when PMGF introduces alleles from an herbicide-resistant population the main management strategy is to prevent any escape of the pollen acceptor weed during the crop's growing season (Goulart et al., 2012).

Recently, using SNP markers and traditional crossing methods, a QPE-resistant rice was developed through a mutation in the gene coding for enzyme acetyl-CoA carboxylase (ACCase) (Famoso et al., 2019; Camacho et al., 2019). Provisia™ rice is a new alternative for the CL rice technology (Dauer et al., 2018). QPE is a herbicide inhibitor of ACCase effective in controlling IMI-resistant and -susceptible weedy rice and *Echinochloa* spp. However, under the Provisia™ system some plants, especially weedy rice, may escape control (Rustom et al., 2019).

The ACCase gene is encoded in the cell nucleus and a single dominant or co-dominant gene is responsible for resistance (Tal and Rubin, 2004), making the Provisia™ weedy rice hybrids QPE-resistant. In a study evaluating the artificial hybridization of Provisia™ and susceptible rice, it was found that 100% of F1 expressed resistance (Camacho et al., 2019). This raises the same concern about the rapid evolution of QPE-resistant weedy rice, as was observed with CL rice. Thus, this study aimed to assess the PMGF from Provisia™ to weedy rice.

2. Material and Methods

To assess the gene flow from Provisia™ rice to weedy rice the following procedure was adopted: 1) putative hybridization, with possible transfer of the herbicide-resistant trait from crop-to-weed; 2) seed collection from pollen acceptor weedy rice; 3) seed germination in aqueous solution containing QPE to pre-select QPE-resistant weedy rice from pollen acceptor; 4) transplanting of seedlings and spraying QPE at 120 g ai ha^{-1} (plants that survived the germination test and post-emergence treatment were classified as F1 and cultured to produce F2 seeds); 5) Genomic DNA from Provisia™, pollen acceptor weedy rice (before crossing) and from putative weedy rice hybrids (F1) for analysis of the presence or absence of the $Ile_{1781}Leu$ mutation in the ACCase enzyme; and 6) Dose-response assay, using seeds of Provisia™, pollen acceptor weedy rice and weedy rice from F2. Figure 1 illustrates a summary of the entire methodology used.

2.1 Putative hybridization from Provisia™ rice-to-weedy rice and seed collection

Two field experiments (2019/2020 and 2020/2021) were conducted at Centro Agropecuário da Palma (Capão do Leão, RS, 31°48'28.0"S, 52°28'53.6"W), in a rectangular scheme (Jia et al., 2014) and two repetitions. The soil in the area is classified as Haplic Planosol (silty-loam type by the texture), with the following chemical characteristics: pH= 5.6, CEC= 7.1 cmolc dm^{-3}, organic matter = 1.52%, clay content = 18%, phosphorus (P) = 14.2 mg dm^{-3}, potassium (K) = 62 mg dm^{-3}. In the region, rice sowing is recommended during the spring so the reproductive stage coincides with summer – season wherein the predominant wind direction is towards the East (Silva et al., 1997).

In both years, rice crop was sown at the recommended season, using 90 kg seeds ha^{-1}, with a nine-row direct seeder spaced at 0.17 m. At the same day, weedy rice was sown in 10-L pots (keeping 1 plant per pot). The pollen donor (Provisia™ – non-commercial inbred line) was sown upwind of the predominant wind direction and the pollen acceptor (QPE-susceptible weedy rice) was placed downwind at 0, 0.5 and 1 m from pollen donor. In each year, 6 acceptor plants were placed at for each distance along two strips (replicates) of 35 x 1.53 m (Figure 2). The pollen acceptor weedy rice used in this study came from the seedbank bulk of the Weed Science Research Group of Federal University of Pelotas (CEHERB – UFPel). The seeds were collected from commercial paddy rice fields during the 2012 to 2013 season in Rio Grande do Sul state (Brazil) (Piveta et al., 2021).

On the day of rice sowing, 250 kg ha^{-1} of N-P-K (formulated as 05-20-20) was applied. Subsequently, 110 Kg ha^{-1} of Nitrogen (N) was applied as urea; the first application (50%) was at 3–4 leaf stage and the second (50%) was at panicle differentiation (R1). Herbicides for general weed management were applied with a backpack

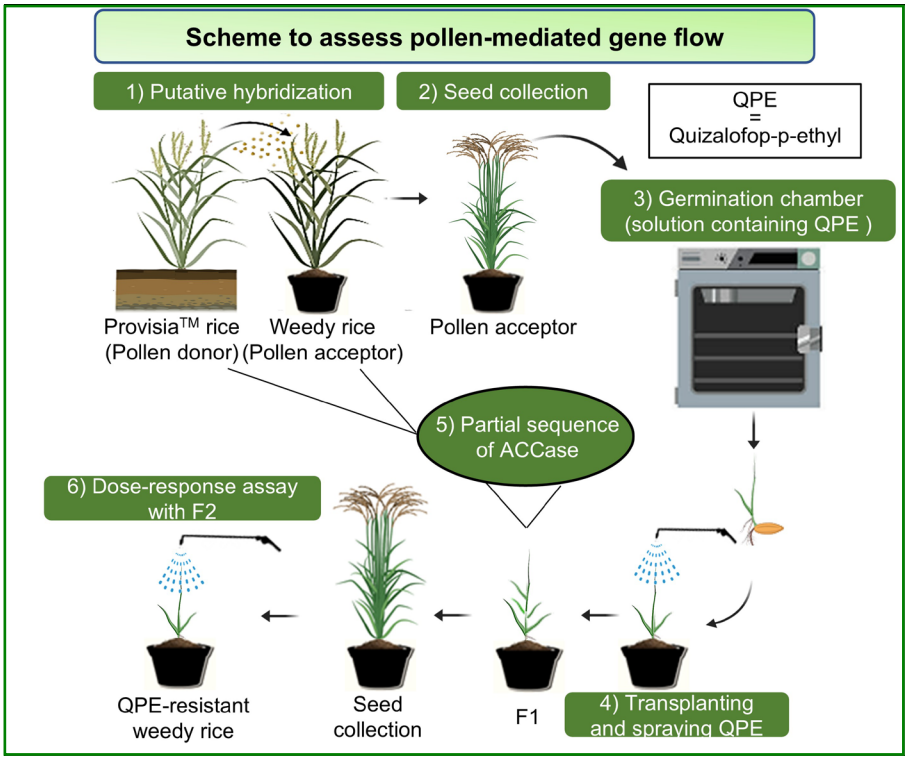

Figure 1 - Scheme to assess pollen-mediated gene flow from Provisia™-to-weedy rice

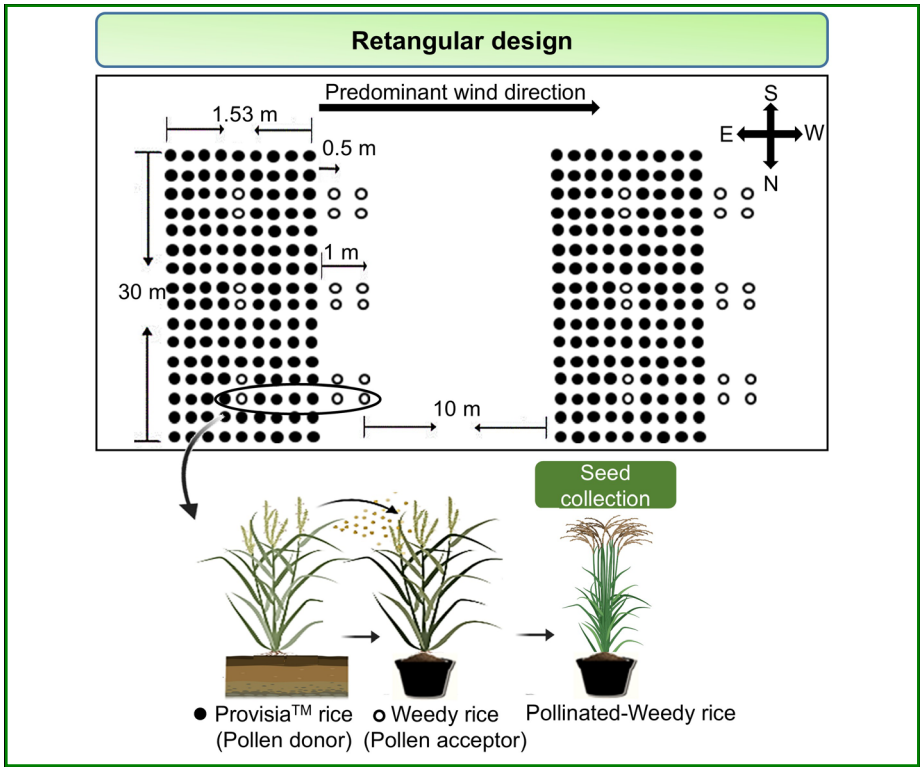

Figure 2 - Rectangular design for putative hybridization of quizalofop-resistant rice (● Provisia™ – pollen donor) and quizalofop-susceptible weedy rice (○ pollen acceptor) Adapted from Jia et al., 2014

sprayer pressurized with CO_2, equipped with 110.015 fan-type nozzles and a spray volume of 150 L ha^{-1}. The treatment used was: glyphosate at 1,440 g ae ha^{-1} (Roundup®, 360 g ae L^{-1}, SL) + clomazone at 252 g ai ha^{-1} (Gamit® 360 CS, 360 g ai L^{-1}, CS) at S3 stage; 100 g ai ha^{-1} of quizalofop-p-ethyl + adjuvant 1% (v/v) (Assist, 756 g L^{-1}, EC) + bentazon 960 g ai ha^{-1} (Basagran®, 600 g ai L^{-1}, EC) at V3 stage; and 100 g ai ha^{-1} of quizalofop-p-ethyl + adjuvant 1% (v/v) (Assist, 756 g L^{-1}, EC) at 5-6 leaf stage. Two days after the V3 treatment, rice was continuously flooded (3-5 cm) to the grain filling stage (mowed to avoid seed dispersal).

After chemical treatment, weedy rice was arranged along the strips as mentioned above. In the grain filling stage, the panicles were wrapped with tulle fabric bags to avoid losses by seed shattering. After physiological maturation, seeds from pollen acceptor weedy rice were collected. Awns were removed and the seeds were counted. Subsequently, the seeds were placed in an oven with forced air circulation (45 °C) for seven days to overcome seed dormancy and were stored in paper bags at room temperature until tested for germination.

2.2 Determining the discriminating dose for QPE resistance in weedy rice

Before starting the main experiments, it was necessary to determine the discriminating dose to select for resistant hybrid seeds in the first generation. Thus, a seed germination assay was performed in June 2020 and repeated in September of the same year for selection of putative QPE-resistant weedy rice. The experimental design was completely randomized, with four repetitions (100 seeds per replicate). The factorial arrangement comprised: A) QPE-susceptible (IRGA 424 RI and IRGA 426 – used as simulators of weedy rice) and -resistant rice (Provisia™); B) QPE rates (Targa®, 50 g ai L^{-1}, EC) selected after three preliminary tests: 0, 0.0128, 0.064, 0.32, 1.6, 8, 40, 200, 1,000, and 5,000 mg ai L^{-1}.

The seeds were sown on Germitest® paper soaked in the herbicide solutions (prepared with distilled water, equivalent to 3x the weight of the paper), folded into rolls and packed in closed plastic bags. The rolls were randomly placed inside a BOD-type (Biological Oxygen Demand) germination chamber, set at 25 °C, 70% relative humidity, and 12-hour daylength. Fourteen days after sowing, normal germinated seeds (compared to control) were counted.

In October 2020 and 2021 seeds from pollen acceptor weedy rice were sown in a QPE solution at 1.6 mg ai L^{-1} as previously selected in this assay. Fourteen days after sowing, the remaining seedlings were transplanted into 10 L pots containing commercial substrate, acclimatized for three days, and then taken to the greenhouse. At 3-4 leaves, the plants were treated with QPE (120 g ai ha^{-1}). Plants that survived the germination test and post-emergence treatment were cultured to produce F2 seeds.

2.3 ACCase partial sequencing

Resistance to QPE is associated with an A (wild type/susceptible)/T (mutant/resistant) SNP that causes an isoleucine (Ile) to leucine (Leu) substitution at amino acid residue 1781 of the chloroplastic ACCase (1781 mutation) (Camacho et al., 2019). Leaf samples were collected from Provisia™ rice plants, pollen acceptor weedy rice (before crossing), and putative weedy rice hybrids (F1) at V6 stage. The samples were sent to a specialized service company (FOODCHAIN ID®) for extraction of genomic DNA (gDNA) and *ACCase* partial sequencing to detect the presence of the Ile_{1781}Leu mutation.

2.4 Dose-response to QPE

A greenhouse experiment was performed in October 2022. The experimental design was completely randomized, with three repetitions. The factorial arrangement comprised: A) QPE-resistant rice (Provisia™), QPE-susceptible weedy rice (SUS) and F2 weedy rice; B) QPE rates (Targa®, 50 g ai L^{-1}, EC) + adjuvant 1% (v/v) (Assist®, 756 g L^{-1}, EC): 0, 3.75, 7.5, 15, 30, 60, 120, and 240 g ai ha^{-1} for SUS; and 0, 60, 120, 240, 480, 960, 1920, and 3840 g ai ha^{-1} for Provisia™ and F2 (120 g ai ha^{-1} is the recommended field rate for weedy rice control).

The seeds were sown in 0.5 L pots containing a commercial substrate, keeping one plant per pot. When the plants reached 3-4 leaves, herbicide was applied with a CO_2 pressurized sprayer equipped with flat fan nozzles (XR 110.015, 0.5 m apart), delivering 150 L ha^{-1} of carrier volume. During application the average temperature (26-28 °C), relative humidity (68%), and wind speed (4-5,4 ms^{-1}) were monitored with a digital Kestrel® 4,500 Weather Meter. Two days after treatment, the pots were kept in continuous flood (3–5 cm).

Herbicide effect was evaluated visually 28 days after chemical treatment, based on a 0–100 percentage scale, where 0% indicated no effect and 100% was dead (data not shown). The surviving plants were cut close to the ground and placed in a forced-air oven at 60 °C until they reached a constant weight to determine the shoot dry weight (SDW).

2.5 Statistical Analysis

The data were analyzed using the statistical program R (R Core Team, 2019). The homogeneity and normal distribution of residuals of all data were graphically tested. Seed germination data (% relative to control) were subjected to analyses of variance (ANOVA, $p < 0.05$) to determine interactions between doses x rice x years. Data were combined across years if the year x treatment interaction effect was not significant. For both germination and dose-response data (% relative to control), the *drc* package was used to fit the curves by a nonlinear model, choosing the three-parameter log-logistic equation (Equation 1).

$$Y = \frac{d}{1 + \exp\{b(\log(x) - \log(e))\}} \quad \text{(Equation 1)}$$

where Y is the response variable (germination or SDW expressed as % of control); *e* is the GR_{50} value (the dose that causes 50% reduction in germination or SDW); *d* is the upper limit; and *b* indicates the slope around *e* (Ritz et al., 2019).

The regression model was subjected to the lack-of-fit test, using the *modelFit* function. When not significant, regression analyses describe the variation in the data similar to ANOVA. The *drm* and *summary* functions estimated the parameters, their standard errors and significance ($p<0.05$). The GR_{50} and the resistance factor (GR_{50} Provisia™/GR_{50} rice; GR_{50} Provisia™/GR_{50} susceptible weedy rice; or GR_{50} F2 weedy rice/GR_{50} susceptible weedy rice) were estimated by the *ED* and *EDComp* functions (95% confidence interval).

Weedy rice seeds that germinated in QPE solution (% relative to control) and seedlings that survived post-emergence treatment were recorded and the data were analyzed using multifactorial mixed-effects ANOVA (assuming years and blocks as random effects), using *lme4* package. The *lmer* and ANOVA functions were used to test significant factors effects ($p<0.05$). The plot function and ggplot were used to create the figures.

3. Results and Discussion

3.1 Putative gene flow hybridization from Provisia™ rice-to-weedy rice

A total of 28,078 seeds were evaluated. In the first year, 8 plants survived the germination test and post-emergence application, out of 13,065 viable seeds (numbered from F1-P1 to F1-P8). In the second year, 11 plants survived, out of 15,013 viable seeds (numbered from F1-P9 to F1-P19). Based on these, the putative gene flow was estimated to be 0.07% regardless of distance from pollen source, up to 1 m away (Figure 3).

Weedy rice outcrosses from PMGF with HR rice can survive the pertinent herbicide (Jia et al., 2014). The current PMGF estimate of 0.07% from Provisia™ rice to weedy rice in Brazil is within the values reported in other studies (0.002% to 0.342%) (Jia et al., 2014). In a hybrid progeny from *Avena fatua* resistant (pollen donor) and susceptible (pollen acceptor) to ACCase inhibitors, the gene flow rate was 0.05 to 0.16% (Murray et al., 2002). However, the hybridization between cultivated rice and weedy rice just can be confirmed inequivocably using SSR markers to show that the weedy rice hybrids indeed have alleles from both parents (Rajguru et al., 2005). This confirmation is necessary because point mutations endowing resistance to herbicides can be present in weed populations as part of their standing genetic variation. In *Alopecurus myosuroides* the observed frequency of $Ile_{1781}Leu$ mutation was 7.3×10^{-4} (Délye et al., 2013) – a value significantly lower than what was found in the present study.

ACCase is encoded in the cell nucleus and a single dominant or partially dominant gene is responsible for resistance in Provisia™ rice (Camacho et al., 2019). Thus, the transfer of resistance genes by PMGF results in the majority of the weedy rice-rice hybrids to be herbicide-resistant (Shivrain et al., 2007), corroborating the results of the present study. Rice has hermaphrodite flowers (spikelets) (Yoshida and Nakato, 2011), but the release of pollen grains occurs at anthesis (Zhu et al., 2004). Therefore, pollen grains can be carried by the wind (Jia et al., 2014) and reach the stigma of adjacent plants, resulting in low-level outcrossing (Nadir et al., 2017).

The synchrony of flowering and anthesis (Zhao et al., 2017), the distance from the pollen donor, wind speed and direction are determining factors for the rate of PMGF (Jia et al., 2014). Generally, there is synchrony in the flowering and anthesis time of cultivated rice and weedy rice (Zhao et al., 2017). The opening of the lemma and palea characterizes anthesis and occurs after the panicle is emitted, even before its complete exertion from the flagleaf (Yoshida and Nakato, 2011). In Provisia™ rice, 50% of the panicles fully emerge and flowering begins 87 days after crop emergence (Famoso et al., 2019). In the region where the study was performed, the variability in the flowering time of weedy rice is 77 to 110 days after emergence, indicating potential for synchronized flowering and spontaneous PMGF (Piveta et al., 2021).

The pollen grain (42–43 µm) settles to the ground relatively quickly after release from the anthers and dispersion (Jia et al., 2014), affecting pollen germination and cross pollination with adjacent plants. There is an exponential reduction in the frequency of gene flow based

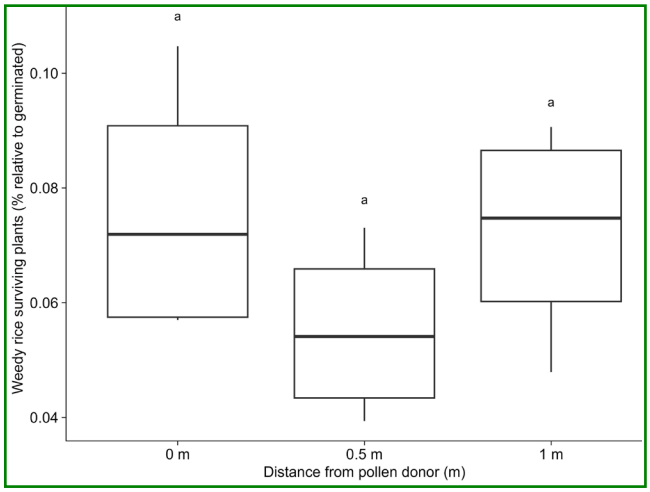

Figure 3 - Weedy rice, originating from putative hybridization with Provisia™ rice, surviving the germination test (% in relation to the total number of seeds able to germinate) and application of quizalofop-p-ethyl in post-emergence, at different distances from the pollen donor.

on distance from the pollen donor source. However, the gene flow distance depends on wind speed and can be 1 m (winds of 1.5 m s^{-1}); 2 m (winds of 2.3 m s^{-1}) or 5-10 m (winds of 4.8 m s^{-1}) (Jia et al., 2014). During the reproductive phase (summer in the region) the average wind speed is greater than 3 m s^{-1} (Silva et al., 1997). Therefore, gene flow from Provisia™ rice to weedy rice can occur at progressively reduced frequencies beyond 1 m, which was the maximum distance assessed in the current study.

3.2 Determining the discriminating dose for QPE-resistant weedy rice

The seed germination assay detected differences between QPE-resistant (Provisia™) and QPE-susceptible rice (IRGA 424 RI and IRGA 426). For Provisia™, the dose to reduce germination by 50% was 195 g ai ha^{-1}; while for IRGA 424 RI and IRGA 426 was 0.18 and 0.17 g ai ha^{-1}. Thus, the dose necessary to reduce Provisia™ germination is 1000 times higher compared to susceptible rice (Table 1). QPE at 1.6 mg ai L^{-1} did not affect QPE-resistant rice germination. This dose was then used for the seed germination assay. However, it did not completely inhibit the germination of susceptible rice (Figure 4), requiring post-emergence application of QPE to eliminate false positives.

For large-scale testing, it is desired to select a concentration that kills or completely inhibits the growth of all susceptible plants, but not resistant ones (Burgos, 2015). In the present study, the germination of resistant rice remained unchanged at 1.6 mg ai L^{-1} of QPE, the same as the nontreated control. A previous study indicated that the QPE dose up to 1 mg ai L^{-1} (close to the dose used in the present study) did not affect the development of resistant rice and can be used to properly separate QPE-resistant and –susceptible rice seeds (Camacho et al., 2019).

Table 1 - Estimated parameters *b*, *d* and GR_{50} (with standard error) by the nonlinear equation[1], based on germination (% relative to control) of quizalofop-resistant (Provisia™) and quizalofop-susceptible rice (IRGA 424 RI and IRGA 426), at 14 days after sowing as a function of quizalofop-p-ethyl rates

Rice	Parameters (*Lack of fit*, p value = 0.18)				
	b	*d*	GR_{50} (g ai L^{-1})	CI[3] (g ai L^{-1})	RF[2]
Provisia™	0.76*** ± 0.04	100.15*** ± 1.02	195.40*** ± 17.04	162 – 229	-
IRGA 424 RI	1.69*** ± 0.14	97.14*** ± 1.65	0.18*** ± 0.01	0.15 – 0.21	1085.55
IRGA 426	1.21*** ± 0.08	99.46*** ± 1.80	0.17*** ± 0.01	0.12 – 0.20	1149.41

[1] Y = d/ (1+ exp[b(log(x) – log (e))]; *** (p<0.001). [2] Resistance factor = GR_{50} Provisia™/ GR_{50} quizalofop-susceptible rice. [3] 95% confidence interval.

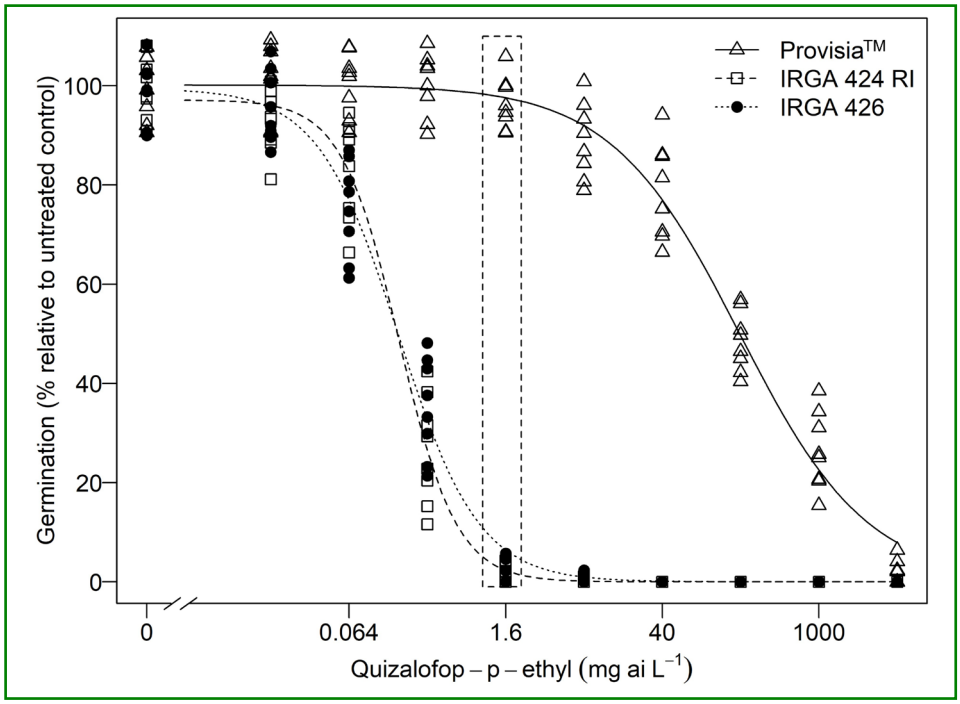

Figure 4 - Seed germination of quizalofop-resistant (Provisia™) and quizalofop-susceptible rice (IRGA 424 RI and IRGA 426), 14 days after sowing as a function of QPE doses. Dotted line = discriminating dose

The seed germination methodology with herbicide solution has proven to be efficient for the rapid detection of herbicide-resistant plants. Putative resistant and susceptible populations are first tested over a wide range of doses to determine the discriminatory dose before carrying out large-scale resistance testing (Burgos, 2015). The seed germination test is fast, economical and useful for evaluating many putative resistant populations and has been used to identify ACCase-resistant populations (Huan et al., 2011).

3.3 ACCase partial sequencing

Partial sequencing of chloroplastic ACCase revealed the presence of $Ile_{1781}Leu$ mutation in all samples of weedy rice plants from F1 (F1-P1 to F1-P19) and in Provisia™ rice; while pollen-acceptor weedy rice harbored the wild type ACCase (data not permitted for publication). This mutation is a SNP in the first nucleotide base at position 1781 that result in the change from isoleucine (Ile) to leucine (Leu) (Tal and Rubin, 2004; Camacho et al., 2019). SNP can be used as molecular markers to infer about PMGF when using a resistant and a susceptible population, as they allow checking the introgression of the HR gene (Roso et al., 2010; Jia et al., 2014). Thus, our detection of the same ACCase mutant in weedy rice from F1 and in Provisia™ rice suggests the occurrence of PMGF. In a previous study, evaluating the controlled hybridization of Provisia™ and susceptible rice, 100% of F1 were resistant. The hybrids were heterozygous, with one mutant allele ($Ile_{1781}Leu$) and one susceptible allele (Camacho et al., 2019). In Arkansas, target-site mutation ($Ile_{1781}Leu$) was the main resistance mechanism to quizalofop in two weedy rice samples (González-Torralva, Norsworthy, 2023).

One concern is that weeds can accumulate mutations in different genes resulting in multiple resistance to ALS- and -ACCase-inhibiting herbicides (Dauer et al., 2018). This is possible, as weedy rice can acquire resistance genes from CL rice and Provisia™ rice through PMGF (He et al., 2014; Nadir et al., 2017). The use of QPE in the chemical management of IMI-resistant weedy rice is effective, but can leave some plant escape (Rustom et al., 2019). Therefore, it is recommended to remove weedy rice escapes from paddy rice fields that use Provisia™.

3.4 Dose-response to QPE

The assay detected differences between QPE-resistant rice (Provisia™), QPE-susceptible weedy rice and plants originating from putative hybridization (F2-P1 to F2-P19). For all weedy rice plants originating from F2, the doses necessary to reduce the SDW by 50% (GR_{50}) were above the recommended rate (120 g ai ha^{-1}). The GR_{50} values of all these plants were 10 times higher than pollen acceptor weedy rice (Table 2; Figure 5).

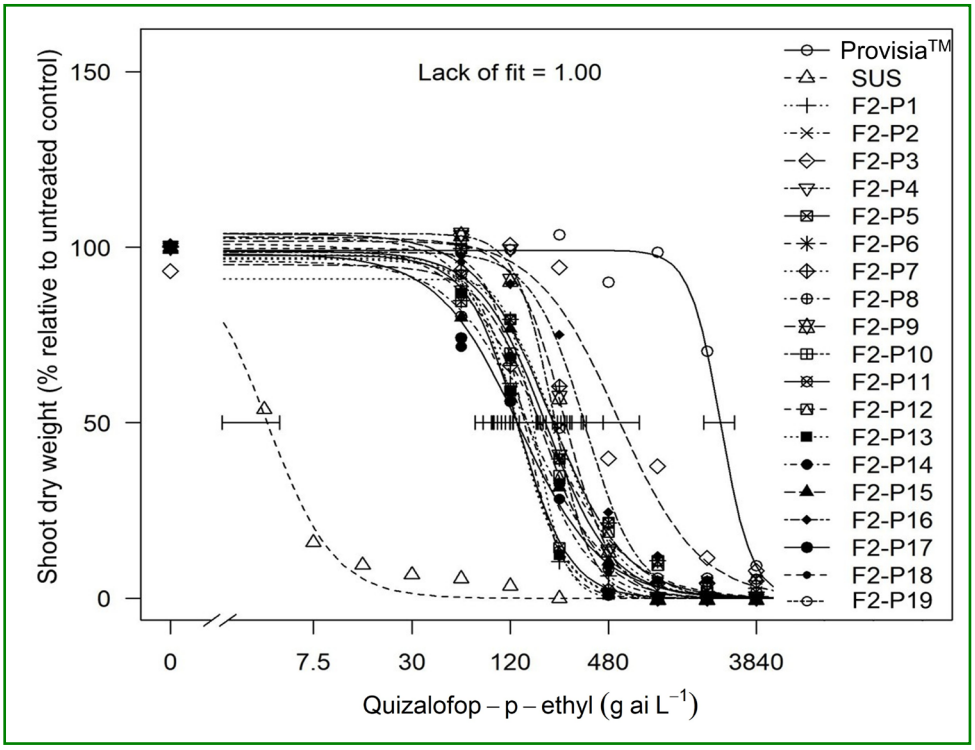

Figure 5 - Shoot dry weight (% relative to untreated control) of quizalofop-resistant rice (Provisia™), quizalofop-susceptible weedy rice (SUS) and weedy rice from putative hybridization (F2-P1 to F2-P19), 28 days after quizalofop-p-ethyl treatment. The horizontal bar represents the 95% confidence interval to obtain a 50% reduction in shoot dry weight (GR_{50}). Label rate = 120 g ai ha^{-1}

Table 2 - Shoot dry weight (% relative to untreated control) parameters (*b*, *d*, GR_{50} – with standard error), by nonlinear equation[1], for quizalofop-resistant rice (Provisia™), quizalofop-susceptible weedy rice and weedy rice from putative hybridization (F2-P1 to F2-P19), 28 days after treatment with different doses of quizalofop-p-ethyl

Rice	Parameters				
	b	*d*	GR_{50} (g ai ha^{-1})	RF[2]	RF[3]
Provisia™	7.42 ± 11.27	98.55*** ± 4.34	1686.41*** ± 331.75	433.52	-
Susceptible	2.09* ± 1.07	100.28*** ± 9.59	3.89*** ± 0.78		-
F2-P1	3.04* ± 1.41	96.94*** ± 9.40	136.65*** ± 20.29	-	35.12
F2-P2	2.32*** ± 0.58	102.50*** ± 8.61	149.03*** ± 24.20	-	38.31
F2-P3	1.74*** ± 0.41	102.85*** ± 6.32	545.26*** ± 95.75	-	140.17
F2-P4	3.80* ± 1.74	103.88*** ± 6.69	221.79*** ± 22.52	-	57.01
F2-P5	2.79** ± 1.03	97.66*** ± 9.23	134.58*** ± 21.09	-	34.60
F2-P6	1.90*** ± 0.51	103.79*** ± 8.17	211.70*** ± 36.87	-	54.42
F2-P7	2.21* ± 0.89	96.62*** ± 10.01	230.12*** ± 52.10	-	59.16
F2-P8	1.90*** ± 0.51	103.79*** ± 8.17	211.70*** ± 36.87	-	54.42
F2-P9	3.01** ± 1.01	101.67*** ± 6.94	258.52*** ± 33.28	-	66.46
F2-P10	1.91*** ± 0.51	103.76*** ± 8.16	211.69*** ± 36.82	-	54.42
F2-P11	2.14** ± 0.66	98.77*** ± 9.06	199.24*** ± 38.57	-	51.22
F2-P12	1.66*** ± 0.45	100.85*** ± 8.93	183.01*** ± 37.40	-	47.05
F2-P13	3.06* ± 1.18	97.83*** ± 8.96	133.92*** ± 19.22	-	34.43
F2-P14	1.82** ± 0.62	95.98*** ± 10.21	158.33*** ± 37.17	-	40.70
F2-P15	2.39* ± 0.97	94.97*** ± 9.16	188.94*** ± 34.30	-	48.57
F2-P16	2.69** ± 1.00	98.60*** ± 6.78	344.46*** ± 48.64	-	88.55
F2-P17	1.73*** ± 0.47	98.08*** ± 9.81	135.69*** ± 30.67	-	34.88
F2-P18	3.85* ± 1.51	99.61*** ± 7.73	147.48*** ± 17.28	-	37.91
F2-P19	4.97* ± 2.15	90.91*** ± 7.25	172.16*** ± 22.77	-	44.26

[1] $Y = d/ (1+ \exp[b(\log(x) - \log(e))]$; [2] Resistance factor = GR_{50} Provisia™/ GR_{50} susceptible; [3] Resistance factor = GR_{50} F2 (P1 to P19)/ GR_{50} susceptible; * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$).

These results demonstrate high levels of resistance (>10-fold), indicative of target-site resistance (Heap and Duke, 2017). Higher resistance level was documented with Provisia™, in which the GR_{50} was 400 times higher, compared to susceptible weedy rice.

ACCase inhibitor herbicides are divided into three chemical groups (aryloxyphenoxypropionates [FOPs], cyclohexanediones [DIMs] and phenylpyrazoline [DEN]). These herbicides act on the carboxyl transferase (CT) domain of the plastid homomeric form of the enzyme (Kaundun, 2014). The occurrence of some SNPs, which result in amino acid substitution and alter the herbicide binding site in the CT domain, results in high resistance to ACCase-inhibiting herbicides (Jhang et al., 2013). Thus, results of the dose-response assays indicate a change in the conformation of the ACCase catalytic site of Provisia™ rice and weedy rice plants resulting from putative hybridization (F2).

The main positions (numbered according to *Alopecurus myosuroides*) where mutations associated with ACCase resistance occur are: Ile_{1781}, Trp_{1999}, Trp_{2027}, Ile_{2041}, Gly_{2066}, Asn_{2078}, Cys_{2088} (Kukorelli et al., 2013). Provisia™ rice was developed through the selection of spontaneous mutations in tissue culture, using molecular markers (SNP) and traditional crossing methods (Pedigree breeding). This rice has an Ile_{1781}Leu mutation in the ACCase gene that confers resistance to QPE (Camacho et al., 2019). Occurrence of the same mutation in QPE-resistant, F2 weedy rice indicates gene flow from Provisia™ rice to weedy is supported by the segregation of the F1 outcrosses.

Resistance levels depend on specific amino acid changes and the number of resistant alleles. In *Lolium rigidum*, it was found that heterozygous plants for the $Ile_{1781}Leu$ mutation were more susceptible to clethodim than homozygous plants (co-dominant inheritance) (Tal and Rubin, 2004). Considering that plants originating from F2 are still segregating, heterozygous plants are likely still predominant. On the other hand, the non-commercial Provisia™ rice line is most likely homozygous. It is important to emphasize adherence to the company's chemical management recommendations, which highlight the sequential application of quizalofop (applied 15 days after the first application, at the V2–V4 stage of rice) to control re-infestations from new germination flushes. In addition, if necessary, remaining weedy rice plants that survive chemical control should be removed during the rice crop season before the reproductive stage to prevent gene flow. The commercial release of Provisia™ rice hybrids (heterozygous plants to quizalofop resistance) can also mitigate the introgression of HR genes.

4. Conclusions

The offspring of susceptible weedy rice outcrossed with Provisia™ rice harbor the expected ACCase mutation carried by Provisia™ rice. The F1 plants are heterozygous for resistant allele and are resistant to the label rate of QPE. Therefore, QPE-resistant weedy rice arose from crop-to-weed gene flow. It is recommended to avoid weedy rice escapes in paddy rice fields managed with Provisia™ to avoid the risk of quick evolution of QPE-resistant weedy rice.

Authors' contributions

All authors read and agreed to the submitted version of the manuscript. All authors: conceptualization of the manuscript and development of the methodology. DMC: data collection and curation. All authors: data analysis and interpretation. LAA, ERC: funding acquisition and resources. LAA, ERC: project administration: all authors; supervision. DMC: writing the original draft of the manuscript. All authors: writing, review and editing.

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Data Availability

The data are available upon reasonable request: https://doi.org/10.48331/SCIELODATA.FHCIWN

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