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Gene flow from single and stacked herbicide-resistant rice (*Oryza sativa*): Modeling occurrence of multiple herbicide-resistant weedy rice

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Abstract

Background: Provisia™ rice (PV), a non-genetically engineered (GE) quizalofop-resistant rice, will provide growers with an additional option for weed management to use in conjunction with Clearfield® rice (CL) production. Modeling compared the impact of stacking resistance traits versus single traits in rice on introgression of the resistance trait to weedy rice (also called red rice). Common weed management practices were applied to 2-, 3- and 4-year crop rotations, and resistant and multiple-resistant weedy rice seeds, seedlings and mature plants were tracked for 15 years.

Results: Two-year crop rotations resulted in resistant weedy rice after 2 years with abundant populations (exceeding 0.4 weedy rice plants m^{-2}) occurring after 7 years. When stacked trait rice was rotated with soybeans in a 3-year rotation and with soybeans and CL in a 4-year rotation, multiple-resistance occurred after 2–5 years with abundant populations present in 4–9 years. When CL rice, PV rice, and soybeans were used in 3- and 4-year rotations, the median time of first appearance of multiple-resistance was 7–11 years and reached abundant levels in 10–15 years.

Conclusion: Maintaining separate CL and PV rice systems, in rotation with other crops and herbicides, minimized the evolution of multiple herbicide-resistant weedy rice through gene flow compared to stacking herbicide resistance traits.

Keywords: quizalofop, imidazolinone, red rice, stacked-trait, Provisia, Clearfield, herbicide-resistant weeds

1 Introduction

Cultivated rice (*Oryza sativa* L.) production in the USA is concentrated in the mid-south (Arkansas, Louisiana, Missouri, Mississippi, and Texas) and California. Throughout the production area, weedy rice (*O. sativa* L., also called red rice) remains a problematic weed because it is the same species as cultivated rice, but with weedy attributes like greater seed dormancy, variation in emergence depth, greater seed shattering, and earlier maturity.[1–4] Importantly, weedy (red) rice has a non-white pericarp which affects the sale price,[5] affects cultivated rice growth and yield through competition,[6] and has impacted cultural practices.[7] Weedy rice reduced rice grain weight by 20–25%[8] and reduced soybean (*Glycine max* L. Merr) yield by 8–10%.[9] Additionally, as atmospheric CO^2 levels continue to rise, the competitive advantage of weedy rice will increase.[10]

Given that cultivated and weedy rice are the same species with genetic and phenotypic similarity between the weedy and the cultivated rice types, weed management options, particularly chemical weed management options, for weedy rice in rice have been limited. In 2001, BASF Corporation commercialized imidazolinone (IMI)-resistant Clearfield (CL) rice to provide chemical control of weedy rice and other grass species.[11] This non-genetically engineered (GE) herbicide-resistant rice allowed in-season application of IMI herbicides. Approximately 60% of US hectares are planted with CL rice, and adoption was equally rapid in Central and South America.[12] A survey of 80 certified crop advisors and consultants in Arkansas, USA, found that 85% of advisors and consultants had observed excellent control of weedy rice with IMI herbicides in rice.[1]

Evolution of IMI-resistant weedy rice has been documented repeatedly 1–4 years after CL was released[12] and has increased in abundance since first documentation.[13] The IMI-resistant allele is dominant[14,15] and can be transferred between cultivated and weedy rice through outcrossing events during flowering.[14,16,17] Successful outcrossing events are largely predicated on overlapping flowering time[18,19] with outcrossing generally being limited to a distance of< 1mfromthe cultivated plant.[14] Outcrossing rates from CL to weedy rice are low for this autogamous species, generally ranging from 0.003% to 0.25% with outcrossing rates dependent on crop variety and weedy rice biotype.[14,17–19]

Management recommendations associated with CL rice production are meant to reduce the evolution of IMI-resistant weed species, including weedy rice. These recommendations include not planting CL rice in successive years, rotating herbicide modes of action in the same field, using the full rates of the herbicides, and actively managing weeds that escape or survive herbicide treatment.[20] Producers reported that crop rotation and use of certified seed were used to reduce the occurrence of weedy rice infestations.[1]

Additionally, most producers (92%) in that survey rotated to other crops between CL rice plantings, often rotating to soybeans. Changing timing of rice planting also was used to reduce the likelihood of flowering overlap between cultivated and weedy rice.

Because IMI-resistant weedy rice may become a significant barrier to continued use of CL rice, BASF Corporation initiated a rice trait development program. The goal of this program was to develop a rice cultivar that would be susceptible to IMI herbicides and resistant to an herbicide able to control weedy rice and volunteer CL rice plants. The Provisia (PV) rice cultivar is resistant to the Acetyl CoA Carboxylase (ACC-ase)-inhibiting herbicide, quizalofop.[21,22] Quizalofop controls weedy rice[8,23–27] and the PV rice plants are susceptible to IMI herbicides.

During development, questions were raised about how to effectively deploy the quizalofop-resistance allele in combination with the IMI-resistance allele to reduce the evolution of multiple herbicide-resistant weedy rice via gene flow. A spatially implicit weed population model was created to assist in the decision-making as to whether to stack the resistance traits in a single cultivar or to maintain separate CL and PV cultivars, each with only a single resistance trait. Specifically, the model was developed to determine whether:

1) multiple-resistant weedy rice produced via gene flow would appear more quickly in cropping rotations with stacked trait rice compared to rotations with rice with a single resistance trait, and
2) one or more crop rotations maximize the time until multiple-resistant weedy rice would occur and reach a high density.

2 Materials And Methods

2.1 Model overview

The model is specific to gene flow as related to single or stacked resistant traits and does not include all management strategies that producers might use to prevent the selection of resistant rice; for example, the application of additional pre-emergence herbicides that could be used in conjunction with the stacked traits. In addition, this model represents commonly used crop and herbicide programs for the USA.

The weed population model tracks weedy rice seeds, seedlings, and mature plants for 15 years while common weed management practices are applied. Factors affecting weed growth, outcrossing, and herbicide survival can vary markedly among sites. This model attempts to capture this variability by selecting variables from a range of possible values for model parameters and repeating this model iteration many times with different variables. The model tracks four weedy rice biotypes during the 15-year simulation: plants susceptible to both IMIs and quizalofop, plants resistant to IMIs, plants resistant to quizalofop, and plants resistant to both IMIs and quizalofop.

2.2 Life cycle parameters

Biological parameters specific to weedy rice were used to track the growth and development through plant life stages and were assumed to be similar among biotypes (**Table 1**). Where data were reported by ecotype, parameters associated with the strawhull ecotype were used because this has been most prevalent historically[28] although other ecotypes have recently increased in prevalence.[29] In a study involving outcrossing from glufosinate-resistant rice to weedy rice, shattering, seed dormancy, and fecundity were not affected by the presence of the resistance allele.[15,30] Seeds produced in fall had a high likelihood of germination the following spring (low dormancy) and the mean and standard deviation for germination from the most recent publication that had a wide variation in sampling locations and ecotypes were used.[29] In the absence of herbicide use, 87–97% of weedy rice germinants survive to seedling stage,[6] whereas survival from seedling to flood and survival during flooding was assumed to be 100% because flooding has been shown to affect weedy rice biomass but not survival.[31]

Reproduction of weedy rice varies based on factors not controlled for in this population model; for example, timing of weedy rice emergence relative to cultivated rice, competition effects, hybrid vigor, and weather conditions, all of which can vary from year to year. The mean and standard deviation per-plant seed production values were from strawhull ecotypes of populations sampled throughout the rice growing region,[32] and seed production was allowed to vary each year by drawing a single per plant fecundity value from

Table 1. Biological parameters for weedy rice growth and reproduction used in the simulation model. During a simulated year, a single value was drawn from a distribution of values (Uniform and Normal) using an estimate of the mean and standard deviation based on data available in the source. Where values of parameters were not published, the authors reached a consensus estimate.

Parameter	Rice crop or weedy rice (WR)	Units	Value (Distribution used)	Source
Outcrossing	Crop ≥ WR	% plants	0–0.21% (Uniform)	17
Dormancy	WR	% seed	2.2%	33
Germination	WR	% seed	97%, SD=4% (Trunc. Normal)	29
Survival to seedling	WR	% seed	97%	6
Seed production	WR	Seeds per plant	1237, SD=574 (Uniform)	32
Survival to pre-flood	WR	% seedlings	100%	Assumption
Summer (flood) survival	WR	% plants	100%	31
Shattering (remain in seedbank)	WR	% seed	65%	29
Collected in harvester	WR	% seed	35%	Assumption

a normal distribution of fecundity values. After seed production, weedy rice seed was either collected during harvest (35%) and removed from the simulation or shattered (65%) and added to the soil seed bank.[30] A small percentage of seed (2.2%) was considered dormant and remained in the seed bank from fall of one year to fall of the following year.[33]

2.3 Herbicide parameters

Herbicides have the greatest effect on weedy rice population survivorship and were considered the main technique for weed management in these simulations (**Table 2**). Where multiple studies have been conducted on herbicide efficacy on weedy rice plants, published survivorship values for the strawhull ecotype (where available) were used to generate a normal distribution of potential weedy rice survivorship values (all truncated at 0% survival). During the simulation, a single survivorship value was randomly selected from this distribution each year.

Herbicide product selection corresponded to standard BASF Corporation recommendations and expected use patterns within the crops (**Table 3**).[20] In rice production systems, herbicides were applied at a combination of timings: 3–5 days after planting (Pre), spike stage through 2-leaf rice (EPOST), and 3-leaf rice stage through tillering (POST). Stacked trait rice herbicide recommendation for timing and products were based on projected use. When weedy rice densities exceed 0.4 plants m^{-2} in the CL and PV rice systems after

Table 2. Herbicide survivorship values used in the simulation model were obtained from published and unpublished sources of data. During a simulated year, a single herbicide survivorship value was drawn from a distribution of values using an estimate of the mean and standard deviation based on data available in the sources. Normal distributions were truncated at 0% survivorship (Trunc. Normal).

Parameter	Rice crop or weedy rice (WR)	Units	Value (Distribution used)	Source
Survival to quinclorac and/or clomazone	WR	% plants	100%	Assumption
Survival to imazethapyr	WR	% plants	8%, SD=2% (Trunc. Normal)	9, 43–46
Survival to imazamox	WR	% plants	3%, SD=1% (Trunc. Normal)	46
Survival to quizalofop	WR	% plants	5%, SD=2% (Trunc. Normal)	8, 24, 25
Survival to metolachlor	WR	% plants	10%, SD=6% (Trunc. Normal)	8, 24, 25, 47
Survival to glyphosate	WR	% plants	4%, SD=2% (Trunc. Normal)	8, 26
Survival to clethodim	WR	% plants	17%, SD=2% (Trunc. Normal)	23
Survival to clethodim	Quizalofop-resistant WR	% plants	10%	BASF unpublished data
Survival to quizalofop (rescue timing)	WR	% plants	0%–1% (Uniform)	BASF unpublished data
Survival to imazamox, imazethapyr, quizalofop	Resistant WR	% plants	100%	Assumption
Threshold for rescue herbicide application	WR	Number of plants	0.4 plants m^{-2}	BASF recommendation

Table 3. Each year of a simulation involved a crop† and the recommended herbicide applications for the crop. Herbicide application timings were: 3–5 days after planting (Pre), spike stage through 2-leaf rice (EPOST), 3-leaf rice stage through tillering (POST). When weedy rice (of any biotype) density reached 0.4 plants m^{-2}, a rescue treatment herbicide was applied.

CONV rice	*Crop*	*Conventional rice*			
	Timing	Pre	EPOST	POST	Rescue
	Herbicide	Quinclorac + Clomazone	Quinclorac	None	None
CL rice	*Crop*	*Clearfield rice*			
	Timing	Pre	EPOST	POST	Rescue
	Herbicide	None	Imazethapyr	Imazethapyr	Imazamox
PV rice	*Crop*	*Provisia rice*			
	Timing	Pre	EPOST	POST	Rescue
	Herbicide	None	Quizalofop	Quizalofop	Quizalofop
ST rice	*Crop*	*Stacked trait rice*			
	Timing	Pre	EPOST	POST	Rescue
	Herbicide	None	Imazethapyr	Quizalofop	Quizalofop
Soy	*Crop*	*Roundup Ready soybean*			
	Timing	Pre	EPOST	POST	Rescue
	Herbicide	Metolachlor	Glyphosate	Glyphosate	Clethodim

Soy, soybeans; CONV, conventional rice; CL, Clearfield rice; PV, Provisia rice; ST, stacked trait rice.

flooding and prior to seed set, producers frequently use an additional herbicide application meant to ‘blank’ weedy rice seeds. The threshold value of 0.4 plants m^{-2} was based on the experience of one of the authors (JH) with rice production for 40 years and the term, rescue application, refers to this specific herbicide application. The rescue application may not kill the weedy rice plants, but it prevents or reduces seed production.[27] Herbicide-resistant biotypes were assumed to have 100% survivorship when treated with herbicides corresponding to their specific resistance trait.

Reproduction was the only time when herbicide-resistant weedy rice biotypes evolved through gene flow from rice to weedy rice. Rice and weedy rice have the potential to outcross and the frequency of these events varies considerably among research trials,[14,16,34–36] ranging from 0 to 0.21%. Given the importance of this parameter and variability reported, a unique value for outcrossing was drawn from a uniform distribution across this range each year of the simulation to approximate the variation in flowering overlap and temperature differences that have been shown to affect outcrossing (Table 1).[17] A successful outcross would be the result of cultivated rice pollinating weedy rice and transferring a herbicide-resistance allele. These seeds were reapportioned to the appropriate biotype prior to the next model iteration. The model reflects the assumption that the resistance allele was transferred to the weedy rice and had no impact on the fitness of the weedy rice.[30]

2.4 Cropping scenarios

Crop rotations allow for a diversity of herbicide use and may change the time to occurrence of herbicide-resistant weedy rice. The simulation model compared 2-, 3-, and 4-year crop rotations including conventional rice (CONV), CL rice (IMI-resistant), PV rice (quizalofop-resistant), stacked trait rice (ST, IMI- and quizalofop-resistant), and soybean (SOY) (**Table 4**). Crop rotations were based on likely rotations and BASF recommendations for CL rice stewardship and meant to represent common US cropping rotations.[20] When rotations included more than one herbicide-resistant rice variety (CL, PV, ST), year 1 was assigned to CL rice system.

Simulations were conducted using a 10 ha field with an initial weedy rice seed bank population of 10 seeds m^{-2} (100 000 seeds ha^{-1}). Unless otherwise indicated, results are median values from 1000 simulated 15-year rotations. The model was created using R v. 3.2 and the package ggplot2.[37,38]

2.5 Sensitivity analysis

There are many factors within any given agronomic production scenario that could impact the occurrence and population growth of a weed species, for example, environmental conditions or flowering overlap. A sensitivity analysis of the model described how the initial conditions for these factors impacted the occurrence and growth of the weedy rice populations. Two factors considered were the initial seed density and the presence of

Table 4. Crop rotations were varied to match potential agronomic practices involving rice production. Rotations are grouped according to their duration before repeating the same pattern. Simulations utilized a static 15-year rotation.

	Year			
Rotation name	1	2	3	4
2 year rotations				
CONV rice, Soy	CONV	Soy	CONV	Soy
CL rice, Soy	CL	Soy	CL	Soy
PV rice, Soy	PV	Soy	PV	Soy
ST rice, Soy	ST	Soy	ST	Soy
3 year rotations				
CL rice, Soy, CONV rice	CL	Soy	CONV	CL
CL rice, Soy, PV rice	CL	Soy	PV	CL
ST rice, ST rice, Soy	ST	ST	Soy	ST
4 year rotations				
CL rice, Soy, PV rice, Soy	CL	Soy	PV	Soy
CL rice, Soy, ST rice, Soy	CL	Soy	ST	Soy

Soy, soybeans; CONV, conventional rice; CL, Clearfield rice; PV, Provisia rice; ST, stacked trait rice.

IMI-resistant weedy rice at the outset of the crop rotations. The same nine crop rotation scenarios were initiated using 0.01 of the initial seed bank, equal to 0.1 seed m^{-2}, resulted in different times to emergence and high density for multiple-resistant weedy rice. The same nine crop rotation scenarios were initiated with half of the initial weedy rice seeds (5 seeds m^{-2}) classified as IMI-resistant weedy rice seeds while looking at the difference in time until emergence and high density for multiple resistant weedy rice.

3 Results and Discussion

There are many considerations during the deployment of an herbicide-resistant crop and the focus of this model was on the occurrence of multiple-resistant weedy rice within cultivated rice because the abundance of weedy rice plants may influence adoption and best practices for the continued cultivation of herbicide-resistant rice. First, consider a baseline or control scenario – rotating CONV rice with soybeans. This scenario allows producers few weedy rice management options because of the similarity between cultivated and weedy rice. Weedy rice in CONV rice will not be controlled by herbicides used in CONV rice; therefore, weedy rice flourishes each time CONV rice is grown. The competition is generally untenable for production given the impacts on crop yield.[8,23] Years with soybeans provide 83–96% (on average) weedy rice control through metolachlor, glyphosate, and clethodim use; however, these measures cannot counter weedy rice seed production during CONV rice plantings, and weedy rice population size increases rapidly.

3.1 Clearfield and Provisia rice rotations

In current rice systems, CL is a major component of the crop rotation.[12] The availability of a second herbicide-resistance trait within cultivated rice renews questions about stewardship of the technology that enhances weed management options for rice producers. In 2-year rotations including CL and PV rice with soybeans, herbicide-resistant weedy rice occurs rapidly and reaches a high density in a short time (**Figures 1 and 2**). The median time for multiple-resistant weedy rice evolution is 3 years during CL rice rotations and PV rice rotations (Figure 2). Resistant weedy rice reaches a high density (> 0.4 plants m^{-2}) in 7 years for CL and PV rotations (Figure 1). The difference in weedy rice population size between these rotations is minimal and due to the variation in herbicide efficacy – slightly fewer weedy rice plants survive the PV rice herbicide program than the CL rice herbicide program. However, plants did survive in both scenarios, outcrossed, and produced herbicide-resistant weedy rice seeds in year 1 in each rotation. These seeds emerged and matured into herbicide-resistant plants in year 3.

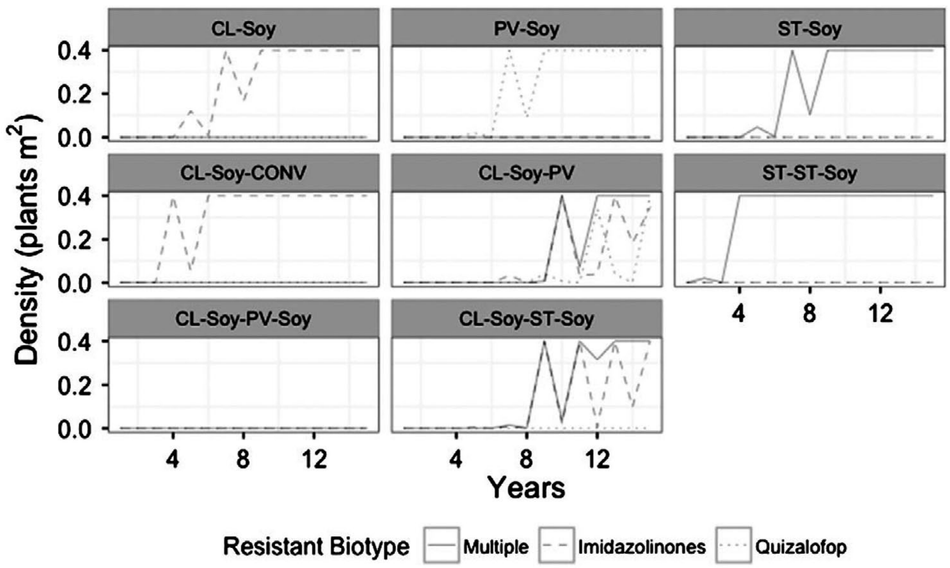

Figure 1. Mature plant density. Median mature weedy rice plant density (1000 simulations) for three herbicide-resistant rice biotypes for each year in 15-year simulations. Susceptible weedy rice plants, the CONV–Soy rotation, and plant densities >0.4 plants m^{-2} are not shown for clarity. Soy, soybeans, CONV, conventional rice; CL, Clearfield rice; PV, Provisia rice; ST, stacked trait rice.

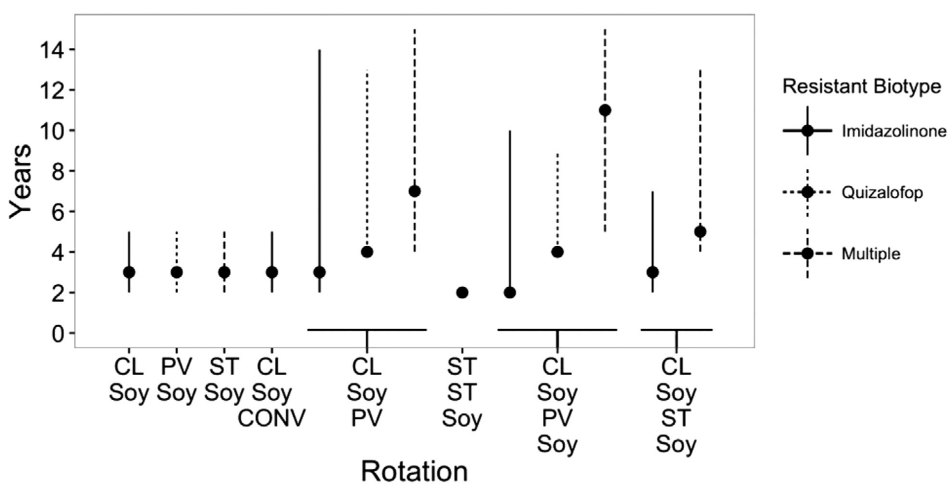

Figure 2. First appearance of weedy rice. Median (solid dot) and minimum and maximum time until herbicide-resistant weedy rice appeared in 1000 simulations of each crop rotation. In the ST–ST–Soy rotation, the minimum, maximum, and median time were identical. Soy, soybeans, CONV, conventional rice; CL, Clearfield rice; PV, Provisia rice; ST, stacked trait rice.

In the model, multiple-resistant weedy rice did not occur in 2-year rice rotations with CONV, CL or PV rice. Multiple-resistant weedy rice in CONV rice would require natural occurrence of mutations leading to resistance to IMI and quizalofop herbicides, and a selective advantage for the population to increase, an unlikely proposition. It is possible for weedy rice plants to outcross with CL or PV rice (Figure 2) followed by a mutation to the other mode of action. However, there would not be a selective advantage for these plants and therefore they are unlikely to reach high densities over time.

Three-year rotations included soybeans, CL rice and either CONV rice or PV rice, and the median time to appearance of IMI or quizalofop-resistant weedy rice was similar to 2-year rotations, occurring in 3–4 years (Figures 1 & 2). Comparing a CL–Soy–CONV rotation with a CL–Soy rotation, inclusion of CONV rice reduced (shortened) the median time to reach high density of IMI-resistant weedy rice to 4 years (Figure 1). As discussed earlier, CONV rice provides producers with no herbicide options for controlling weedy rice and therefore does not reduce the rapid expansion of IMI-resistant weedy rice. Conversely, adding PV rice (CL–Soy–PV) did not affect or extend the median time to high densities to 7 and 10 years for IMI- and quizalofop-resistant weedy rice, respectively, compared with 7 years for CL–Soy and PV–Soy rotations (Figure 1).

Multiple-resistant weedy rice had a median first occurrence time of 10 years in the CL–Soy–PV system although, in some iterations, multiple-resistant weedy rice occurred in as few as 4 years (Figure 2). The multiple-resistant seeds occurred from outcrossing of PV rice with surviving IMI-resistant plants in year 3 and these seedlings survived the herbicide program in the CL rice system. It did take years for multiple-resistant weedy rice population to reach high density, not occurring until year 10 (Figure 1).

One option to slow resistance is to avoid a rotation of PV rice followed by CL rice by adding a second soybean crop, represented in the 4-year CL–Soy–PV–Soy rotation. In this scenario, multiple-resistant weedy rice seeds produced in the PV year would emerge during year 4 and be subjected to the low weedy rice survival soybean herbicide program. The median density for all three biotypes was zero, although IMI-resistant, quizalofop-resistant, and multiple-resistant weedy rice did appear in 41%, 14%, and 22% of the 1000 simulations (Figure 1). When resistant rice did occur, the first occurrence of IMI-resistant and quizalofop-resistant weedy rice in the 4-year rotation was similar to 2- and 3-year rotations because of outcrossing with herbicide-resistant rice (Figure 2). However, the multiple-resistant weedy rice populations had a median appearance in year 11, although some iterations resulted in multiple resistant plants occurring in year 5. High density for resistant weedy rice occurred after 13–15 years, 3–6 years later than the 3-year rotation, showing the positive impact of the additional soybean year between the PV rice and CL rice. Field studies examining soybean, PV rice, and CL rice rotations found similar patterns,[39] with 2-year Soy–CL rice rotations having greater weedy rice densities than a 4-year Soy–PV–Soy–CL rotation suggesting the differences arose from a greater diversity of herbicide use in the rotations.

3.2 Stacked trait rice rotations

Stacked trait (ST) rice allows for more weed management options but brings the additional risk of gene flow between cultivated rice and weedy red rice populations that would give rise to multiple-resistant weedy rice populations. In 2-year rotation of ST rice and soybeans, multiple-resistant weedy rice plants occurred very rapidly, a median of 3 years, similar to the CL and PV 2-year rotations (Figure 2). In some iterations, multiple-resistant seeds were produced during year 1 and appeared in year 2 (soybeans) and escaped the herbicides used in soybeans. Survival to those herbicides is low (< 3%), but not zero and, over a 10 ha field there was sufficient reproduction to maintain the population. Additionally, seed dormancy allowed multiple-resistant weedy rice seeds to remain in the seed bank during soybean years and emerge during ST rice years. In 2-year rotations, glyphosate control of weedy rice plants would need to be >99.9% to delay the occurrence and growth in abundance of multiple-resistant weedy rice populations. Adding an additional year of ST rice (ST–ST–Soy) does not ameliorate the rapid onset of multiple-resistant weedy rice that occurred by year 2, and high density of multiple-resistant weedy rice occurred after only 4 years (Figure 1). Rotating CL rice with ST rice (CL–Soy–ST–Soy) increased the time to first occurrence of multiple-resistant weedy rice to 5 years and time to high density to 9 years.

There were four cultivated rice rotations involving the quizalofop-resistant trait: CL–Soy–PV, ST–ST–Soy, CL–Soy– PV–Soy, and CL–Soy–ST–Soy that each had a longer time until occurrence of a high density of multiple-resistant weedy rice plants. For these potential rotations, ST rice was not helpful because of the rapid occurrence of multiple-resistant plants due to outcrossing in years with ST rice. In fact, in the ST–ST–Soy rotation, multiple-resistant weedy rice occurred as quickly as any 2-year rotation with a single herbicide-resistant trait. Outcrossing had a greater effect than selection of herbicide-resistant plants as seen when comparing rotations with PV and CL rice, and rotations with ST rice. Even though PV rice is followed directly by CL rice in one of the rotations, high density of multiple-resistant weedy rice was not reached for at least 4 years (median of 7 years; Figure 2).

3.3 Sensitivity analysis

For the first sensitivity analysis, the initial weedy rice seed density was changed to a very low density – 10 000 susceptible weedy rice seeds in a

10 ha field (1% of the original simulated density), to represent weedy rice seeds present during planting. In the CL–Soy–PV rotation, the time to first occurrence of multiple-resistant weedy rice increased to 12 years (from 5 years during initial simulations; Figure 2) and high density occurred in year 14. Multiple-resistant weedy rice never occurred in the CL–Soy–PV–Soy rotation. However, in rice rotations containing stacked trait rice (ST–ST–Soy and CL–Soy–ST–Soy), the low initial seed density had no effect, only increasing the time until high density from 4 to 5 years for ST–ST–Soy and from 9 to 11 years for CL–Soy–ST–Soy (Figure 2). Therefore, initial susceptible weedy rice seed density can impact the time until high density of multiple-resistant weedy rice, but only in rotations without ST rice.

For the second sensitivity analysis, the initial seed density was apportioned half to susceptible weedy rice seed and half to IMI-resistant seed. This scenario was meant to approximate the conditions of production fields that already contained IMI-resistant weedy rice populations.[12] With IMI-resistant weedy rice present in the field, time of first occurrence of multiple-resistant weedy rice is reduced from 7 to 4 years in CL–Soy–PV and from 11 to 4 years in CL–Soy–PV–Soy. Similarly, high density of multiple-resistant weedy rice was reached in 6–9 years instead of 10–15 years. The occurrence of some IMI-resistant weedy rice initially had no effect on time until multiple-resistant weedy rice emerged in ST rice rotations. A field that already contains herbicide-resistant plants will almost certainly result in multiple-resistant weedy rice plants given the outcrossing rates.[40]

4 Conclusions

This simulation model weighs the relative risks for multiple-resistant weedy rice to occur and increase in abundance in rice production fields considering two herbicide-resistant traits (one new and one established), either stacked in a single cultivar or in separate cultivars. Regardless of crop rotation, there was a high likelihood that multiple-resistant weedy rice would occur within 15 years, although there was a greater likelihood when using stacked trait rice. Outcrossing between cultivated rice and weedy rice, even though a rare event (< 0.21%), still occurs quite readily and it can be assumed that dominant traits like herbicide-resistant traits will move to the weedy crop. Crop rotations, especially those incorporating soybeans, and the concomitant use of different herbicides or cultural weed control techniques (i.e., rouging weedy rice plants, winter flooding[41]), could prolong the time until the multiple-resistant weedy rice becomes widespread within rice fields. Conversely, in 2-year rotations and continuous rice (data not shown), herbicide-resistant and multiple-resistant weedy rice occurs quickly and continues increasing as the herbicides used in those rotations become ineffective.

Based on the outcomes of this model, BASF Corporation chose to not stack the two herbicide-resistant traits and instead chose to release cultivars with a single resistance trait. Maintaining separate cultivars may delay the occurrence of multiple resistant weedy rice. Although the predictions of this model are specific to rice and weedy rice, the outcomes of this model should be considered for other crops that have sexually compatible relatives where gene flow and introgression are of concern.[40] For example, the herbicide-resistant crops canola (*Brassica napus* L.), sorghum (*Sorghum bicolor ssp. bicolor* (L.) Moench), and sunflower (*Helianthus annuus* L.) among others have weedy sexually compatible relatives.[42] In these cases, stacking traits may lead to multiple resistant weeds more quickly, as with weedy rice, than if the traits were released in separate cultivars.

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