Can herbicide safeners allow selective control of weedy rice infesting rice crops?

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Abstract

BACKGROUND: Rice is a major field crop of paramount importance for global food security. However, the increased adoption of more profitable and resource-efficient direct-seeded rice (DSR) systems has contributed to greater weed infestations, including weedy rice, which has become a severe problem in several Asian regions. In this study we have developed a conceptually novel method to protect rice plants at high doses of clomazone and triallate.

RESULTS: The insecticide phorate applied to rice seeds provided a substantial level of protection against the herbicides clomazone or triallate. A quantity of 15 kg phorate ha−1 significantly increased the LD50 values, which were more than twofold greater than for rice plants treated only with clomazone. A quantity of 20 kg phorate ha−1 in combination with 2000 g triallate ha−1 safened rice plants (80% survival) with LD50 >3.4-fold greater than in phorate-untreated rice. Weed control efficacy was not lowered by the presence of phorate-treated rice seeds.

CONCLUSION: Weedy rice is one of the most damaging global weeds and a major threat to DSR systems. In this study we have developed a proof-of-concept method to allow selective weedy rice control in rice crops. We call for herbicide discovery programmes and research to identify candidate safener and herbicide combinations to achieve selective herbicide control of weedy rice and alleviate weed infestations in global rice crops.

Keywords: agricultural pesticides; pest management systems; rice; seed physiology; seed treatment; weed management

1 INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food source in Asia, which provides the major part (approximately 90%) of global rice production.[1,2]

In Asia, rice has traditionally involved manual transplanting of seedlings into flooded soil. However, growers are shifting towards direct-seeded rice (DSR) systems, which are considered to be more profitable and sustainable than flooded transplanted rice because they require less water and less labour than transplanted rice. Factors such as limited water resources, expansion of urban and industrial sectors, with subsequent competition for land, and increase in labour costs have led to a major shift and change from flooded transplanted to DSR.[3,4]

Weeds, including weedy rice, often a conspecific or congeneric weed of cultivated rice (*Oryza sativa* L.), remain among the major constraints to productive DSR systems.[5,6] In Asian countries, such as Malaysia, Philippines, Sri Lanka, Thailand and Vietnam, DSR has become the dominant method to establish rice crops, and weedy rice infestations represent a severe problem. The main reasons for the association of weedy rice with DSR systems are the absence of the suppressive effect of standing water on weedy rice growth at crop emergence, the simultaneous emergence of weedy rice and cultivated rice and the physiological and morphological similarities of weedy rice to cultivated rice.[7] In DSR, hand-weeding is also more problematic because weeds are in far greater numbers and there is little discriminative size difference between weed and rice seedlings.[8,9]

Owing to many morphological and physiological similarities between weedy rice and rice, the control of weedy rice is a difficult long-term endeavour.[10] Preplant herbicides can provide effective weed control of a major proportion of the soil weed seed bank. However, selective in-crop control of weedy rice with post-emergence herbicide is generally not possible, as rice and weedy rice respond identically to herbicides. Herbicide selectivity relies on the crop's ability to detoxify the herbicide more rapidly than the weed species. Thus far, post-emergent selective weedy rice control has been achieved with herbicide-tolerant Clearfield rice varieties.[11 – 13]

There are numerous examples of herbicides that are toxic to crop seedlings unless a specific herbicide safener is combined with a specific herbicide.[14] A herbicide safener is a chemical that induces protection in a specific crop to a specific herbicide while retaining efficacy on the target weed species.[15] There are several commercial herbicide safeners for cereal crops.[16] Plants possess detoxification systems, such as cytochrome P450 monooxygenases, glutathione transferases and others, that are expressed both constitutively and can be induced by herbicide safeners, enabling enhanced herbicide metabolism and thus increasing herbicide tolerance of cereal crops at the recommended herbicide dose relative to competing weeds.[17] However, in the case of rice and weedy rice, because of their extreme similarity, it is difficult to discover a safener that, applied at the same time to crop and weedy rice plants, can safen rice but not weedy rice.[18] Here, we present proof-of-concept results showing that a chemical (safener) applied directly to rice crop seeds before crop seeding can safen rice against post-seeding pre-emergence herbicides that would be lethal to rice and weedy rice, and how this practice can enable selective weedy rice control without rice damage.

2 MATERIALS AND METHODS

2.1 Clomazone and triallate dose–response studies

In this study, a medium-grain Australian rice variety (Reziq) and a population of the globally distributed grass weed *Echinochloa colona* (L.) Link, collected in Western Australia,[19] were subjected to herbicide dose – response assays. Plants were grown during the Australian summer season (December–February) in a glasshouse environment at warm temperatures ranging from 16 °C (night) to 32 °C (day), with mean temperatures of 18 °C (night) and 25 °C (day). Rice seeds were imbibed and pregerminated on solidified 0.6% (w/v) agar medium for 3 days and planted at the eruption of the primordial root at 0.5cm depth in 17cm diameter pots in commercial potting soil (50% peat, 25% sand and 25% pine bark). Fifteen rice seeds were placed in each individual pot. The pots were treated immediately after seed transplanting with 0, 125, 250, 500, 750, 1000 and 2000 g ha^{-1} of either clomazone {2-[(2-chlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one} (Command, 48% clomazone; FMC, Brisbane, Qld) or trial- late in pre-emergence [S-(2,3,3-trichloroprop-2-enyl) *N*,*N*-di (propan-2-yl)carbamothioate] (Avadex Xtra, 50% triallate; Nufarm, Melbourne, Vic.). The clomazone recommended label dose for rice ranges between 240 and 290gha^{-1}, whereas the labelled dose of triallate is between 800 and 1500gha^{-1}, depending on the target weeds. The putative safener, the insecticide phor- ate [diethoxy-(ethylsulfanylmethylsulfanyl)-sulfanylidene-l(5)- phosphane] (Thimet; Barmac Industries, Gold Coast, Qld), was applied to the soil surface at 0.1 g pot^{-1} , corresponding to 10 kg phorate AI ha^{-1} just prior to the herbicide treatments (soil 10 treatment versus seed 0 (no phorate) treatment indicating her- bicide treatment only), or added with the germinating rice seeds on agar via a 10 mL aqueous solution at rates of (a) 0.66% wt/wt phorate/rice seed (seed 5 treatment corresponding to 5 kg phor- ate ha^{-1} at a seeding rate of 150 kg rice seed ha^{-1}), (b) 1.3% wt/wt (seed 10 treatment corresponding to 10 kg phorate ha^{-1}), (c) 2.0% (seed 15 treatment corresponding to 15 kg phorate ha^{-1}) or (d) 2.66% (seed 20 treatment corresponding to 20 kg phorate ha^{-1}). Plants were grown in optimal conditions and regularly watered to achieve >90% field capacity. Nitrogen (as NH_4NO_3) was applied (50 mg kg^{-1}) at weekly intervals over the course of the experiment. After 21 days, herbicide efficacy was assessed by counting the number of emerged plants and above-ground plant biomass, and dry weights were determined after drying at 60 °C for 4 days. There were four replicated pots per herbicide dose, and an individual pot represented an experimental unit. Dose – response studies were conducted 4 times in total.

Approximately 33 *E. colona* seeds in admixture with five rice seeds were scattered in 17cm pots prior to herbicide applica- tion to establish whether the rice seeds imbibed with the highest tested phorate dose (20 kg ha^{-1}) could potentially lower the herbi- cide efficacy on weeds (i.e. prevent the bioactivation of the herbi- cide clomazone or triallate). Five untreated versus phorate-treated (20 kg ha^{-1}) rice seeds, corresponding to a crop density of 220 plants m^{-2}, were placed in pots together with *E. colona* seeds. Dose – response studies compared the two main treatments: (1) seed admixture with rice seed not safened with phorate (seed 0 treatment) versus (2) phorate-safened rice seed (seed 20 treat- ment). Subsequently, the pots were treated with 0, 125, 250, 1000 and 2000 g ha^{-1} of either clomazone or triallate. There were three replicated pots per herbicide dose, and an individual pot repre- sented an experimental unit. The herbicide dose – response studies were conducted twice.

2.2 Statistical analysis

Following appropriate statistical investigation, datasets of the final dose–response studies were pooled prior to analysis as veri- fied not to be significantly different. Survival and above-ground biomass data were subjected to non-linear regression analysis as described elsewhere.[20,21] The responses of rice and *E. colona* plants to different herbicide modes of action in the presence (seed 20, 15, 10 and 5 treatments and soil 10 treatment) versus the absence (seed 0) of the insecticide phorate were measured, and LD_{50} and GR_{50} values were estimated, allowing calculation of a crop selec- tivity index (CSI) comparing dose – response curves in the presence and in the absence of phorate (e.g. CSI = LD_{50} for seed 10/LD_{50} for seed 0). The statistical difference was assessed by the SI function in the *drc* package in the software program R v.3.0.2. Graphical data are presented as rice and *E. colona* plant survival (%) or dry weight (% of untreated control).

3 RESULTS

Rice response to a range of clomazone or triallate doses in com- bination with phorate applied to rice seeds or to the soil surface is shown in Figs 1 and 2 respectively. The level of clomazone or triallate control of the major weed *E. colona* in the presence of phorate-treated versus phorate-untreated rice seeds is displayed in Fig. 3. As expected, without herbicide, phorate (up to 20 kg phor- ate ha^{-1}) had no effect on plant growth, when compared with phorate-untreated control plants ($P > 0.2$; data not shown).

3.1 Clomazone

As expected, the rice herbicide clomazone was safe to rice plants at the recommended label dose, with plant survival of >97% (Fig. 1) and no growth reduction (data not shown). Clomazone dose – response analysis showed that complete control of rice plants (<3 ± 1.2% SE survival) could be achieved at doses of 1000 g clomazone ha^{-1} (Fig. 1A). It is well known that the soil-applied insecticide phorate can provide protection against clomazone injury in cotton.[22] Here we confirmed that phorate protected rice plants against clomazone injury. The estimated values of LD_{50} and GR_{50} show that a dose of 10 – 15 kg phorate ha^{-1} significantly increased the level of protection,

with estimated LD_{50} values more than twofold greater than plants treated with clomazone alone (Table 1). At 500 g clomazone ha^{-1} , >80% rice seedling emergence was achieved with phorate-treated seeds versus 31% without phorate (Figs 1C and D; Table 1). A further increase in the phorate dose did not deliver significantly greater protection to rice plants against clomazone (Fig. 1E; Table 1). The highest level of rice protection against clomazone was achieved with 10 kg phorate ha^{-1} applied to the soil surface or directly to rice seeds (Figs 1C and F; Table 1).

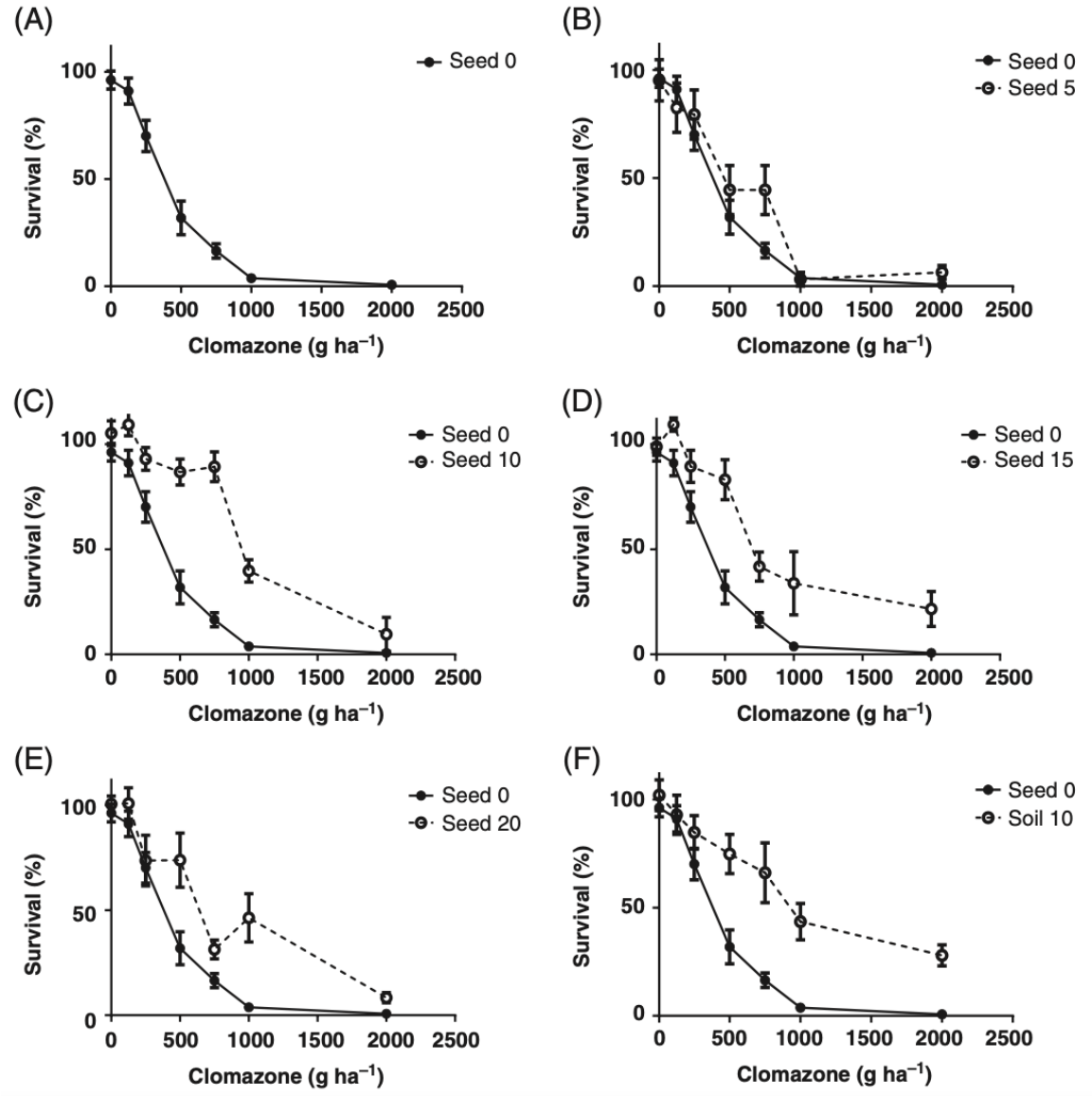

Figure 1. Mean plant survival (emergence as % of untreated control) ± standard errors in clomazone dose – response of rice plants: (A) filled circles and solid line refer to clomazone dose – response alone with seeds not previously exposed to phorate (seed 0); (B) open circles and dashed line refer to clomazone dose – response with seed treated with 5 kg phorate ha^{-1} (seed 5); (C) open circles and dashed line refer to clomazone dose – response with seed treated with 10 kg phorate ha^{-1} (seed 10); open circles and dashed line refer to clomazone dose – response with seed treated with 15 kg phorate ha^{-1} (seed 15); (E) open circles and dashed line refer to clomazone dose – response with seed treated with 20 kg phorate ha^{-1} (seed 20); (F) open circles and dashed line refer to clomazone dose – response with untreated seed and phorate at 10 kg phorate ha^{-1} applied to the soil surface of pots prior to triallate treatments (soil 10); symbols are observed means ± SE (n = 12). Predictive lines connect mean values of survival response at different herbicide doses.

3.2 Triallate

Triallate is lethal to rice, with Fig. 2A showing that 2000 g triallate ha^{-1} caused 98 ± 1.2% SE rice mortality. However, in the presence of phorate at rates above 5 kg ha^{-1}, phorate provided significant protection against rice triallate injury (Figs 2C to E; Table 1). At the highest tested rate of phorate (20 kg ha^{-1}) with 2000 g triallate ha^{-1}, 80% of rice plants survived, with above-ground biomass production 60% that of untreated plants (Fig. 2E). Thus, at this phorate dose a significant level of protection for rice plants against triallate activity was conferred by phorate seed treatments, with estimated values of triallate LD_{50} and GR_{50} > 3.4-fold greater than in phorate-untreated rice (Table 1).

3.3 E. colona response to clomazone or triallate

As expected, clomazone and triallate were confirmed to be lethal to the grass weed species *E. colona*, with >90% mortality achieved at doses near the recommended field rate of either herbicide (Fig. 3). At 125 g clomazone ha^{-1}, *E. colona* control was 94%, whereas 1000 g triallate ha^{-1} caused 97% plant mortality (Fig. 3). Unlike for rice, the presence of phorate did not protect *E. colona* against clomazone or triallate, as the presence of five rice seeds in each pot (equivalent to a density of approximately 220 plants m^{-2}) previously imbibed in water or treated with a rate of 20 kg phorate ha^{-1} did not lower herbicide activity (Figs 3A and B). The estimated LD_{50} and GR_{50} values for *E. colona* treated with clomazone or triallate were much lower than for rice, and values were not significantly different ($P > 0.44$) in the presence or absence of phorate-treated rice seeds (Table 1).

4 DISCUSSION

4.1 Novelty of this study

In this study we have developed a proof-of-concept methodol- ogy by applying a safener to rice seeds to protect rice plants against a herbicide dosage that will be lethal to weedy rice, enabling selective weedy rice control.[18] Weedy rice is one of the most damaging weeds infesting rice globally, especially in DSR systems. In the past two decades, in particular in India and south-east Asian countries, a major shift has occurred in rice establishment methods, with an increasing adoption of DSR.[23] Thus, weedy rice in DSR is a major threat to global rice/food production.

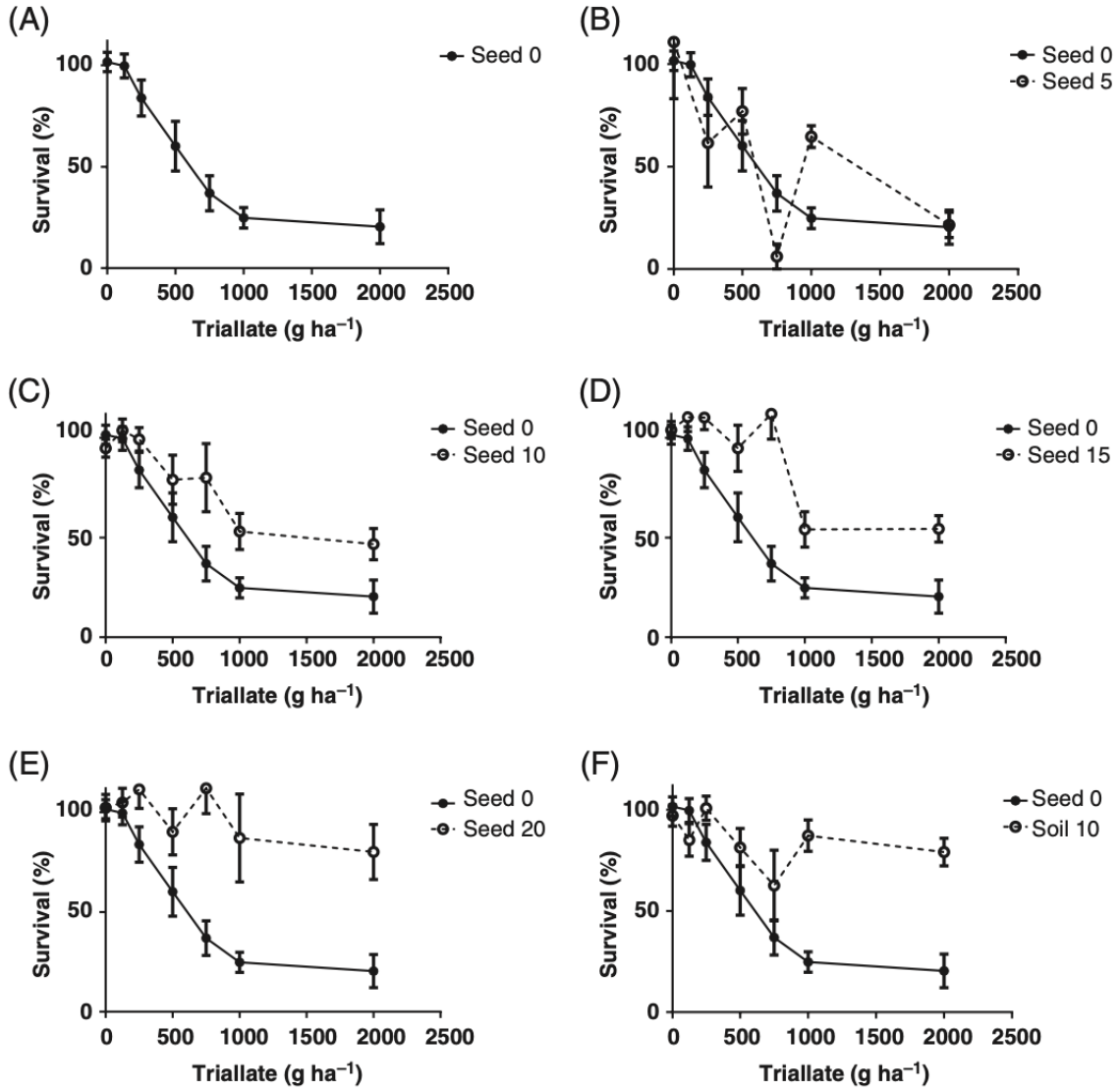

Figure 2. Mean plant survival (emergence as % of untreated control) ± standard errors in triallate dose – response of rice plants: (A) filled circles and solid line refer to triallate dose–response alone with seeds not previously exposed to phorate (seed 0); (B) open circles and dashed line refer to clomazone dose – response with seed treated with 5 kg phorate ha^{-1} (seed 5); (C) open circles and dashed line refer to clomazone dose – response with seed treated with 10 kg phorate ha^{-1} (seed 10); (D) open circles and dashed line refer to clomazone dose – response with seed treated with 15 kg phorate ha^{-1} (seed 15); (E) open circles and dashed line refer to clomazone dose – response with seed treated with 20 kg phorate ha^{-1} (seed 20); (F) open circles and dashed line refer to clomazone dose – response with untreated seed and phorate at 10 kg phorate ha^{-1} applied to the soil surface of pots prior to triallate treatments (soil 10); symbols are observed means ± SE (n = 12). Predictive lines connect mean values of survival response at different herbicide doses.

Clomazone is used as a global rice herbicide for weed control,[24] but it could be effective against weedy rice only at high dosages (Fig. 1A). Here, we report the protection of rice against clomazone by treatment of rice seed with the organophosphate insecticide phorate. Thus, the phorate acts as a safener to the rice seed against injury from the subsequent herbicide treatment. Similarly, substantial weedy rice control was achieved with triallate plus phorate, with minimal rice damage.

To the best of our knowledge, such selective herbicide con- trol of weedy rice in rice crops (herbicide±safener) has very rarely been achieved in commercial rice crops. In one study, Shen *et al*.[25] showed that the safener fenclorim applied to rice seeds, followed by pre-emergence application of the chloroacetamide herbicide pretilachlor, achieves selective control of weedy rice in rice crops. Similarly, a clomazone dose – response study was conducted to assess the efficacy of dietholate and phorate to safen rice against clomazone.[18] However, subsequent commercial and large-scale field validation research has not been reported. Here, we attempted to calibrate an effective dose of phorate to be applied as herbicide safener to rice crop seeds prior to crop seed- ing, and to identify the most discriminative herbicide dose applied in pre-emergence post-seeding to enable rice crop safening and subsequent putative weedy rice control. Therefore, our demon- stration of phorate safening of rice is a 'proof of concept' of a rice safener enabling selective herbicidal control of weedy rice infest- ing rice crops with clomazone or triallate. A chemical discovery programme could identify rice safeners considerably more effec- tively than phorate, and with a more suitable toxicological profile. Research and field trials are warranted to explore the potential for herbicide and rice safeners to provide selective weedy rice control, and to validate the results in rice field crops.

4.2 Potential mechanistic basis

Herbicide safeners can strongly induce herbicide metabolism via overexpression of defence genes such as cytochrome P450 monooxygenases (P450s), glutathione *S*-transferases (GSTs) or other detoxifying enzymes such as glucosyltransferases (GTs) or ABC transporters.[17,26,]

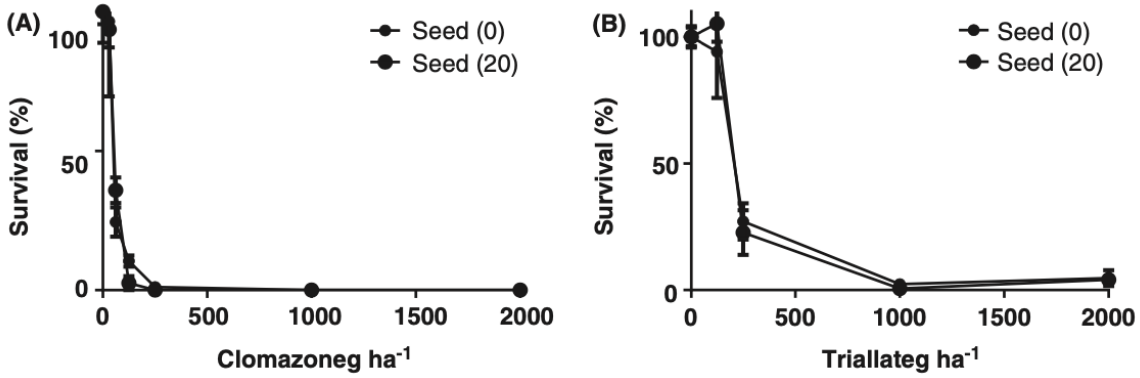

Figure 3. Mean plant survival (emergence as % of untreated control) ± standard errors of the grass weed *E. colona* in clomazone dose – response studies in admixture with the rice crop: (A) filled circles and solid line refer to clomazone dose – response of *E. colona* seeds in admixture with rice seeds (five seeds per pot = 220 rice crop plants m^{-2}) not previously exposed to phorate (seed 0), open circles and solid line refer to clomazone dose – response of *E. colona* seeds in admixture with rice seed previously treated with 20 kg phorate ha^{-1} (seed 20); (B) filled circles and solid line refer to triallate dose – response of *E. colona* seeds in admixture with rice seeds (five seeds per pot = 220 rice crop plants m^{-2}) not previously exposed to phorate (seed 0), open circles and solid line refer to triallate dose – response of *E. colona* seeds in admixture with rice seed previously treated with 20 kg phorate ha^{-1} (seed 20). Predictive lines connect mean values of survival response at different herbicide doses.

Table 1. Pooled data from three herbicide dose–response studies (plant survival and growth) to assess clomazone and triallate efficacy in rice and *E. colona* in the presence or absence of the organophosphate insecticide phorate. Estimated LD_{50} and GR_{50} values are expressed as herbicide g ha^{-1}, with standard errors in parentheses, and the crop–herbicide safety index (CSI) compared with phorate-untreated rice seeds. Probability values (*P*) of difference between rice response to each herbicide with or without phorate were assessed by the SI function in the *drc* package in the software program R v.3.0.2 (2013). Parameters *b*, *d* and *e* of equation (1) are given for each population tested

Herbicide	Species	Phorate (kg ha^{-1})	LD_{50}	*b*	*d*	*e*	CSI	*P*	GR_{50}	*b*	*d*	*e*	CSI	*P*
Clomazone	Rice	Seed 0	392 (44)	2.6	70.1	392	–	–	345 (39)	2.5	102	345	–	–
Clomazone	Rice	Seed 5	548 (159)	12	71.5	548	**1.4**	0.20	543 (141)	41	110	543	**1.6**	0.040
Clomazone	Rice	Seed 10	974 (35)	2.4	62.9	974	**2.5**	<0.001	988 (128)	2.1	128	988	**2.9**	<0.001
Clomazone	Rice	Seed 15	790 (127)	1.7	80.1	790	**2.0**	<0.001	702 (113)	2.0	105	702	**2.0**	<0.001
Clomazone	Rice	Seed 20	662 (127)	1.4	79.3	662	**1.7**	<0.001	628 (135)	1.4	103	628	**1.8**	<0.001
Clomazone	Rice	Soil 10	1087 (176)	1.4	73.0	1087	**2.8**	<0.001	1067 (132)	2.1	95	1067	**3.1**	<0.001
Triallate	Rice	Seed 0	572 (84)	1.7	75	572		–	590 (64)	2.7	101	590		
Triallate	Rice	Seed 5	240 (186)	0.65	75	239	**0.5**	0.46	1274 (452)	2.2	87	1274	**2.2**	0.002
Triallate	Rice	Seed 10	1504 (320)	1.4	100	1503	**2.6**	<0.001	1239 (212)	1.7	115	1239	**2.1**	<0.001
Triallate	Rice	Seed 15	1520 (353)	1.4	78	1520	**2.7**	<0.001	1223 (258)	1.7	108	1223	**2.1**	<0.001
Triallate	Rice	Seed 20	>2000	1.3	73	4024	**>3.5**	<0.001	>2000	1.1	98	2487	**>3.4**	<0.001
Triallate	Rice	Soil 10	>2000	0.4	72	4145	**>3.5**	<0.001	>2000	1.33	89	–	**>3.4**	<0.001
Clomazone	ECHCO	Seed 0	51.0 (5)	5.6	96	51		–	35.4 (6)	6.9	102	35	–	–
Clomazone	ECHCO	Seed 20	54.8 (5)	4.7	86	54	**1.0**	0.60	28.2 (17)	0.7	97	28	**0.8**	0.75
Triallate	ECHCO	Seed 0	208 (24)	5.4	94	208	–	–	172 (22)	3.7	82	171	–	–
Triallate	ECHCO	Seed 20	230 (128)	15	110	230	**1.1**	0.86	151 (12)	4.0	117	150	**0.9**	0.44

It is also well documented that organophosphate insecticides can interact with P450 enzymes, and such an interaction can affect the rate of herbicide efficacy and metabolism.[28 – 30] Simi- larly, in this study we hypothesised that the insecticide phorate (safening agent) would have interacted with P450s involved in herbicide metabolism, and this would have significantly reduced the herbicide phytotoxicity and injury to rice plants via a P450-mediated lower rate of bioactivation of clomazone or trial- late. It is likely that cytochrome P450 mediates *in vivo* metabolic bioactivation processes through hydroxylation (clomazone)[22,31] or sulfoxidation (triallate).[32] Organophosphate insecticides such as phorate can react with cytochrome P450 enzyme, which causes oxidative desulphuration in the organophosphate insecticide molecule.[33] This possible competition between the insecticide and the herbicide as accidental P450 substrate can minimise the herbicidal effect. Similar mechanisms were also hypothesised and observed in soybean (*Glycine max* L.), where lower rates of herbicide bioactivation could explain and confer protection against clomazone.[34] Similarly, in cotton, enhanced protection was conferred by the organophosphate insecticides phorate or disulfoton, which inhibit P450-mediated bioactivation via oxida- tion of the herbicide clomazone, preventing the production of phytotoxic clomazone metabolites.[22] Similarly, reduced herbicide activation has been suggested to confer triallate resistance in the grass weed *Avena fatua* (L.).[35,36]

4.3 Improved herbicide management of evolving populations of weedy rice

In the face of the threat to rice production from weedy rice issues with DSR systems, effective management strategies have been deployed in rice ecosystems in the Americas and Europe.[37 – 41]

Thus far, the Clearfield rice technology and imidazolinone her- bicides (e.g. imazethapyr, imazamox, imazapic, imazapyr) have proved to be highly effective against weedy rice and well suited for DSR systems in Europe and Americas varieties.[11–13] However, bidirectional gene flow between weedy rice and rice crop plants has allowed ALS-resistant weedy rice plants to evolve globally in fields where Clearfield rice is grown.[42] In parallel to the evolution of ALS-resistant weedy rice populations, the repeated use of ALS her- bicides has led to the selection and coevolution of ALS-resistant weeds belonging to the globally distributed weed genera *Cype- rus* and *Echinochloa*.[43] Novel solutions and technologies have been proposed to contain gene flow,[44] and specific research needs have been identified to face the global challenge presented by the evo- lution of herbicide-resistant weeds.[45]

This study shows that weedy rice control could be achieved with high doses of clomazone or triallate, which is not currently registered in rice crops. This methodology could also improve the level of control of other weeds, as we have shown for the major grass weed *E. colona*.

5 CONCLUSION

Within a collective and global effort to control weeds, the results of this study suggest a simple methodology for selective control of weedy rice that could be adopted by smallhold and profit-oriented farmers. Research is needed to identify seed-specific candidate safener and herbicide combinations that could deliver effective weedy rice control without causing agronomic (crop safety), economic

(cost-effective weed control) and environmental (herbicide/insecticide fate) concerns and offer additional tools for integrated management of evolving weed populations in global rice crops.

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