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Cyperus difformis evolves resistance to propanil

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ABSTRACT

Cyperus difformis L. is one of the worst weeds of rice world-wide and has evolved resistance to acetolactate synthase (ALS)-inhibiting herbicides in rice fields of California. Propanil use was intensified to control the widespread resistant biotypes. Rice growers have recently experienced poor control, suggesting resistance to this photosystem II-inhibiting herbicide may have evolved in *C. difformis* populations. The objectives of this study were to detect the presence of propanil resistance, to establish resistance levels, and to investigate involvement of enhanced herbicide detoxification as mechanism of resistance through the use of metabolic inhibitors. Four *C. difformis* populations collected in rice fields from the Sacramento Valley of California were confirmed resistant to propanil. This is the first case of such resistance outside the *Poaceae* and the first time *C. difformis* exhibits resistance to an herbicide mechanism of action other than ALS inhibition. Carbaryl and malathion applied individually in mixture with propanil had minor effects on herbicide toxicity suggesting metabolic detoxification was not a resistance mechanism. A resistant biotype produced more than 80% biomass after a propanil (6.7 kg a.i. ha^{-1}) and carbaryl (1.9 kg a.i. ha^{-1}) or propanil and malathion (1.0 kg a.i. ha^{-1}) treatment compared to <20% by a susceptible biotype, suggesting substantial resistance still persisted in spite of insecticide addition. Propanil-resistant plants were cross-resistant to bensulfuron-methyl, imazosulfuron, halosulfuron-methyl and penoxsulam, but susceptible to carfentrazone. The loss of propanil to control this important weed of rice underscores the fragility of herbicide-based weed control in monoculture rice. Integrated weed management approaches to decrease herbicide selection pressure are needed to mitigate the evolution of multiple-herbicide resistance in *C. difformis* of California rice.

1. Introduction

Produced on about 200,000 ha, rice is the major crop in California's Sacramento Valley where it has been grown since the early 1900s (Linquist et al., 2008). California rice is typically grown without rotation as a water-seeded monoculture, perpetuating over many decades an aquatic environment to which a number of serious weeds have adapted (Fischer et al., 2000). Prominent among these is *Cyperus difformis* L., a cosmopolitan highly selfing (Merotto et al., 2009a), annual C3-type emergent aquatic sedge (Barrett and Seaman, 1980). *C. difformis* is a weed of rice in 46 countries and infests both dry direct- and wet-seeded systems (Chauhan and Johnson, 2009).

As *C. difformis* is well-adapted to flooding (Chauhan and Johnson, 2009), its control in rice has been largely based on post-emergent herbicides. Consequently, *C. difformis* has evolved resistance to five ALS (acetolactate synthase) -inhibiting herbicide groups in California (Merotto et al., 2009b). This renders twelve different cross-resistance patterns among populations that evolved resistance independently on numerous occasions (Merotto et al., 2010). Worldwide, herbicide-resistance in *C. difformis* is widespread but has thus far only been recorded for ALS inhibitors (Heap, 2013). Faced with the ensuing reduced control options, growers in California have come to rely heavily on propanil (3,4-dichloropropionanilide) for control of sedges and other weeds. Propanil is a Photosystem II (PSII) inhibitor selectively used in rice to control both dicotyledonous and monocotyledonous weeds. Propanil has been available to California rice growers since the 1960s but its use was initially restricted due to drift hazards to susceptible crops. Since 1997, propanil became widely used after

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formulation changes and availability of equipment to apply herbicides by ground in paddies (Seaman, 1983; Hill et al., 1994; Hill et al., 2002). However, growers have recently experienced poor *C. difformis* control with any of the available propanil formulations, suggesting resistance to this PSII inhibiting herbicide may have evolved in some populations.

Resistance to propanil has thus far only been reported in grasses (Heap, 2013), particularly in *Echinochloa* species, in which resistance is associated with an increased propanil hydrolysis into propionic acid and 3,4-dichloroaniline (DCA) by enhanced aryl acylamidase (aryl acylamine amidohydrase EC 3.5.1.a, AAA) activity (Yih et al., 1968a; Leah et al., 1994; Hoagland et al., 2004). Inhibition of propanil hydrolysis by carbamate and organophosphate insecticides provided evidence of the metabolic nature of propanil resistance in *Echinochloa* spp. (Caseley et al., 1996). Similarly, insecticides of these groups at low doses restored propanil efficacy in resistant *Echinochloa colona* and established the basis to commercially develop the rice-selective organophosphorus herbicides piperophos and anilofos as synergists to overcome propanil resistance in Central and South America (Valverde et al., 2001; Valverde, 2007).

The objectives of this study were 1) to establish resistance to propanil in *C. difformis* populations; 2) evaluate cross-resistance to herbicides of varying mechanisms of action; and 3) determine whether resistance involves increased propanil metabolism.

2. Materials and methods

2.1. Plant material and general experimental conditions

C. difformis seeds were collected in 2011 from nine grower rice fields in California within the area located between 39.35° and 38.49° N and 122.11° and 121.29° Where propanil resistance was suspected, and accessions were designated as CYP1 through CYP9. Additionally, a known propanil-susceptible (CYP-S) population collected at the Rice Experiment Station (RES) near Biggs, CA, was included in the study as control. Seeds in each field were gathered from at least 20 fully mature plants and are hereafter referred to as populations. Seeds were allowed to dry in the greenhouse for several weeks prior to testing. To prevent unwanted *C. difformis* emergence from the soil seedbank, seeds were germinated in steam sterilized Yolo clay loam soil (fine-silty, mixed, nonacid, thermic Typic Xerorthents, 36% clay, 1.8% OM) that was placed into 58 cm^2 pots previously filled to approximately 80% capacity with unsterilized soil. Pots were placed in a greenhouse at the RES, where temperatures averaged 24–30 °C. Plants were exposed to 16 h daylight, with 900 μmol m^{-2} s^{-1} photosynthetic photon-flux density from high pressure sodium lamps used to supplement natural light. Water was provided by sub-irrigation, such that soil remained moist but not flooded. Seeds were sprinkled on the moist soil surface and after establishment plants were thinned to five per pot. When plants were approximately at the 3–4 leaf stage, herbicides were applied with a cabinet track sprayer using an 8001-EVS nozzle delivering 375 L ha^{-1} at 250 kPa pressure. Plants were harvested 14–17 days after herbicide application; the number of surviving plants was tabulated for each pot and fresh aboveground plant material was weighed. All experiments were conducted in spring 2012.

2.2. Initial screening for resistance to propanil

Propanil (Ultra Stam 80 EDF, RiceCo, Memphis, Tennessee, USA) was first applied to all ten *C. difformis* populations at 3.4, 6.7, and 13.5 kg a.i. ha^{-1}, equivalent to 0.5, 1 and 2× the field dose, respectively; 0.125% crop oil concentrate was added to all treatments. An untreated control for each population was sprayed with de-ionized water. The average number of surviving plants and the total fresh weight of a treated pot expressed as percent of the average untreated control and subtracted from 100 were used to quantify the percent control by the individual treatment. Each treatment was replicated four times and the experiment repeated.

2.3. Dose response curves of selected populations

Populations CYP-S (propanil susceptible and also known susceptible to ALS inhibitors) and CYP2 (propanil resistant in the initial screening) were subjected to a whole-plant herbicide dose response bioassay, including treatments with carbaryl and malathion to explore the likelihood of AAA or P450 driven metabolism as the mechanism of resistance. Plants were treated with propanil at dosages of 0, 0.8, 1.7, 3.4, 6.7, 13.5, 26.9 and 53.8 kg a.i. ha^{-1} covering a range from 0 to 8× the field dose. Also, across these dosages, plants were treated with either malathion (1.0 kg a.i. ha^{-1}) or carbaryl (1.9 kg a.i. ha^{-1}) applied 3 h before spraying propanil; a 0.125% crop oil concentrate was added to all treatments. Each treatment was replicated three times and the experiments conducted twice.

2.4. Cross-resistance to other herbicides

Two of the propanil resistant populations detected in the initial screening (CYP2 and CYP3), as well as the susceptible control (CYP-S), were further tested against a number of other herbicides currently available to rice farmers for control of this weed, in order to identify field control options. All herbicide treatments were applied to the weed's foliage as previously described. The herbicides and doses (in parentheses, followed by name of commercial formulation) were the following: Propanil (6726 g ha^{-1}, Ultra Stam 80 EDF), bensulfuron-methyl (70 g ha^{-1}, Londax, RiceCo, Memphis, Tennessee, USA), penoxsulam (35 g ha^{-1}, Granite SC, Dow AgroSciences, Indianapolis, Indiana, USA), carfentrazone (224 g ha^{-1}, Shark H_2O, FMC Corpo., Philadelphia, Pennsylvania, USA), halosulfuron-methyl (52.5 g ha^{-1}, Sandea, Gowan Co., Yuma, Arizona, USA) and imazosulfuron (336 g ha^{-1}, V-10142, Valent U.S.A. Corp. Walnut Creek, California, USA). Bensulfuron-methyl, halosulfuron-methyl, imazosulfuron and penoxsulam are ALS-inhibiting herbicides; carfentrazone is a protoporphyrinogen oxidase (protox) inhibitor. The dose of carfentrazone is the one normally used for into-water treatments but in this experiment the herbicide was sprayed to the foliage. Crop oil concentrate (0.125%) was added to all treatments except to carfentrazone. Each treatment was replicated four times and the experiments conducted twice.

2.5. Statistical analyses

All data were subjected to analysis of variance. And treatment means, when appropriate, were compared using Fisher's protected LSD ($\alpha = 0.05$). Dose-response of fresh weight for propanil alone or in mixture with synergists within an experiment were fit with a three-parameter logistic model that assumes a lower limit equal to 0, which is useful when the highest dose is insufficient to reduce growth (fresh weight) to attain a lower limit (Ritz and Streibig, 2005):

$$Y = D/(1 + \exp[b(\log x - \log ED_{50})]) \quad (1)$$

where Y denotes the fresh weight response at dose x of the herbicide; D represents the upper asymptote of fresh weight at dose zero (control treatment). ED_{50} is the dose of herbicide required to reduce

fresh weight half-way between *D* and 0 (50% reduction of fresh weight from that of untreated plants), and *b* is the proportional slope of the curve around ED_{50}. Variance was stabilized as in Seiden et al. (1998). Resistance indices (RI) were computed as resistant to susceptible ED_{50} ratios. Mortality data were subjected to a probit analysis in order to determine LD_{50} or LD_{20} values when 50% reductions were not observed within the range of dosages tested. These computations were made using the *proc probit log10* in SAS software (SAS Institute Inc., Cary, NC). Values of the LD_{50} were not considered the same when their 95% confidence intervals (CI) as calculated by the probit analysis did not overlap. The effects of potentially synergistic combinations using carbaryl or malathion was evaluated by relative potency (RP) departures from one. Thus:

$$RP = ED_{50}(\text{propanil})/ED_{50}(\text{propanil} + \text{synergist})$$

Ratios of ED_{50} values and their 95% confidence intervals (CI) were calculated using the *comped* function in the *drc* package (Ritz and Streibig, 2005) of the statistical freeware program R2.15.2 (R Development Core Team 2012). A synergistic effect (increased potency) would have an RP > 1 considering the amplitude of the corresponding 95% CI. Similarly, when ratios of LD_{50} values with non-overlapping 95% CI indicated RP > 1, the effect was considered synergistic.

3. Results

3.1. Resistance to propanil: initial screening

Fresh weight and survival rates were generally higher for run 1 than run 2 across populations but at 13.5 kg propanil ha^{-1} (or twice the field dose), populations CYP1, CYP2, CYP3 and CYP4 were consistently less affected in their growth (fresh weight) and survived more than populations CYP6, CYP7, CYP8 and CYP-S (Fig. 1). The former group was accordingly categorized as propanil-resistant and the latter as -susceptible. Populations CYP5 and CYP9 displayed an intermediary response.

3.2. Propanil dose response assays

From the initial screening, putative propanil-resistant population CYP2 and -susceptible population CYP-S were selected for full dose response bioassays using propanil alone or in combination with malathion or carbaryl. To facilitate visual comparisons, the response curves are graphically presented on a proportional basis (Fig. 2) although there were no differences in the upper limits (average weight of untreated plants of both biotypes). The two populations had substantially different responses to propanil alone. Based on biomass data, the calculated ED_{50} values for the reference susceptible CYP-S population was 2560 and 1840 g propanil ha^{-1} in the first and second experimental runs, respectively (Table 1). The ED_{50} for the CYP2 population was outside the dose range used resulting in very wide confidence intervals, and was expressed as >>53,800 g propanil ha^{-1}; RI ratios could not adequately be calculated. Similarly, mortality responses of the resistant CYP2 biotype were very low compared to the susceptible CYP-S biotype (Fig. 3). Values of the LD_{50} could only be estimated for CYP-S plants and ranged from 10.5 to 6.9 kg ha^{-1}; values of the LD_{50} and LD_{20} were beyond the maximum dose used (53. 8 kg ha^{-1}) and could not be estimated (Table 2). Therefore, population CYP2 was confirmed as propanil resistant.

Addition of insecticide in the absence of propanil did not change ($P > 0.05$) fresh weight of either biotype compared to untreated plants (data not shown) but shifted dose response curves (Figs. 2 and 3). However, ED_{50}-based RP increases with carbaryl could not be statistically substantiated, because CYP2 responses were so low in spite of the high rate used that ED_{50} values, or their upper 95% CI, were beyond the treatment range (Table 1). Carbaryl synergism of CYP-S plant growth suppression with propanil (Fig. 2) was statistically marginal. The effects of malathion on plant fresh weight

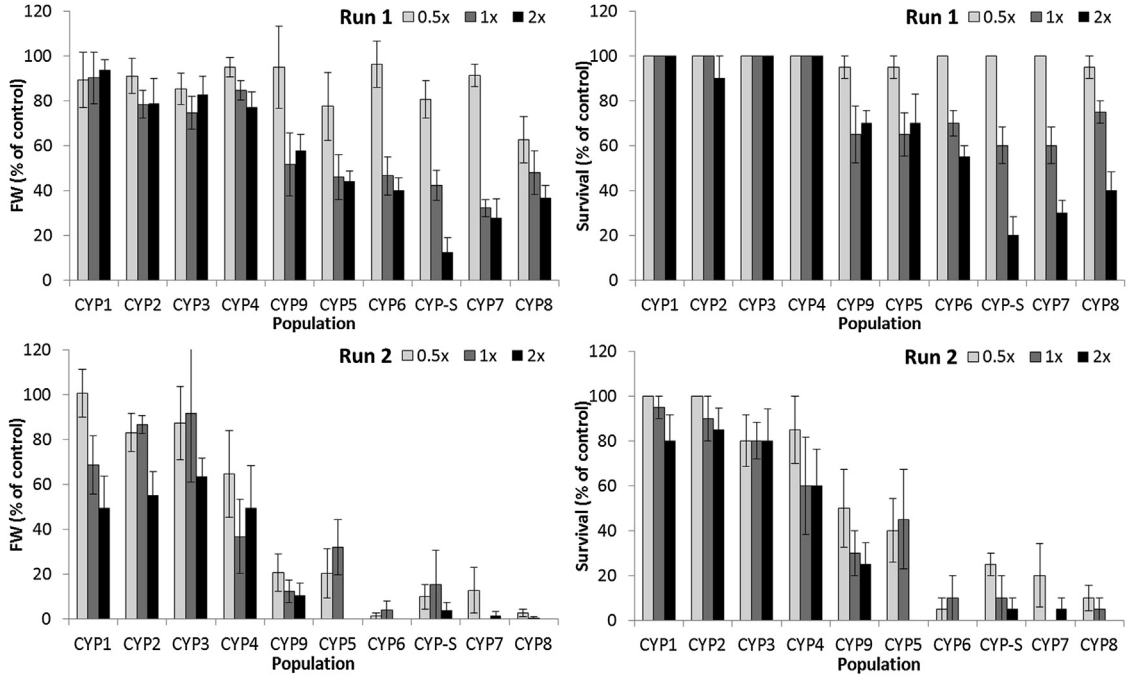

Fig. 1. Fresh weight (FW) and survival of ten *C. difformis* populations subjected to 3.4, 6.7, and 13.5 kg propanil ha^{-1}, equivalent to 0.5, 1 and 2x the field dose, respectively. Columns represent averages of 4 replicates expressed as a fraction of the untreated control, and bars are standard errors. Plant fresh weight $LSD_{0.05}$ = 21 and 26% for runs 1 and 2, respectively; plant survival $LSD_{0.05}$ = 20 and 25% for runs 1 and 2, respectively.

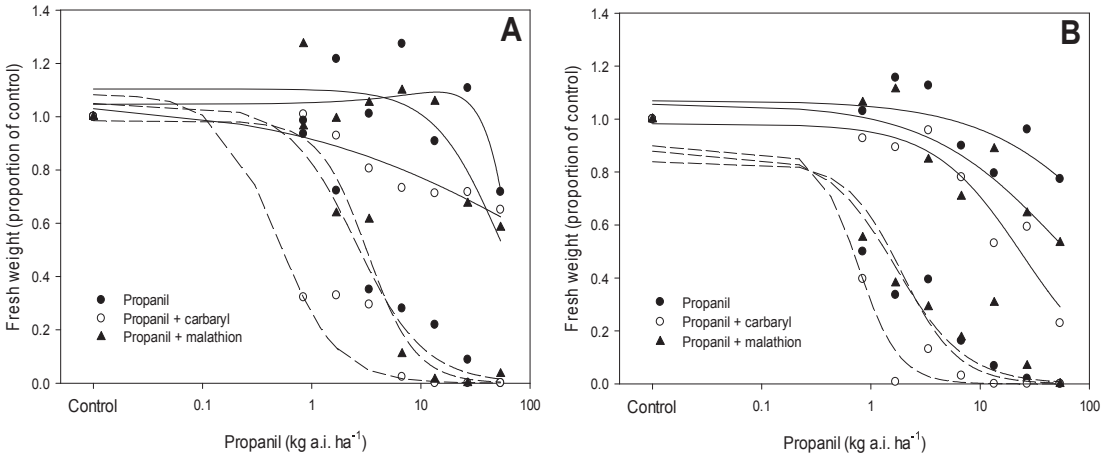

Fig. 2. Dose response of *C. difformis* populations CYP2 (solid lines) and CYP-S (dashed lines) to propanil, with and without malathion or carbaryl; graphs (A and B) correspond to two experimental runs. Symbols are averages of three replicates fitted with log-logistic models for growth responses (fresh weight), model parameters are shown in Table 1. The field dose is 6.7 kg a.i. ha^{-1}.

were minimal to none in both biotypes (Fig. 2, Table 1). Carbaryl enhanced propanil-caused plant mortality of susceptible plants but not of resistant plants (Fig. 3 and Table 2).

3.3. Cross-resistance

Both propanil-resistant populations studied (CYP 2 and CYP3) were also resistant to bensulfuron and imazosulfuron, and resistant or partially resistant to halosulfuron, the third sulfonylurea herbicide tested (Table 3). Penoxsulam controlled CYP3 but was only partially effective on CYP 2, and carfentrazone applied as a foliar spray at this dose was effective across all populations.

4. Discussion

The initial screening provided preliminary confirmation of a differential response to propanil among *C. difformis* populations and a strong indication of the evolution of resistance to a herbicide having a mode of action different to those that inhibit the ALS enzyme, the only class of herbicides to which this species had previously evolved resistance (Heap, 2013). The higher fresh weight and survival rates observed in the first bioassay run may result from spraying the herbicide to somewhat larger (older) plants (4 leaf stage) than in the second run (3 leaf stage). Larger plants usually require higher herbicide doses for the same level of control than

Table 1
Summary of parameters ±95% confidence intervals (CI) describing the response of *Cyperus difformis* fresh weight to increasing doses of propanil alone and in mixture with carbaryl or malathion.[a]

Treatment biotype	*D* (g)	*b*	ED_{50} (g propanil ha^{-1})	RI[b]	RP[c]
Propanil alone					
First run					
CYP2	Model did not fit		>>53,800[d]	NC[d]	
CYP-S	3.71 ± 1.52	1.39 ± 0.76	2560 ± 2340		
Second run					
CYP2	Model did not fit		>>53800[d]	NC[d]	
CYP-S	3.51 ± 1.40	1.50 ± 0.60	1840 ± 1393		
Propani + carbaryl					
First run					
CYP2	4.83 ± 0.67	1.23 ± 0.98	37,900 ± 19,700		NC[d]
CYP-S	4.62 ± 3.09	1.60 ± 0.79	489 ± 521		5.23 ± 6.93
Second run					
CYP2	6.01 ± 1.13	0.50 ± 0.36	36,000 ± 31,000		NC
CYP-S	3.72 ± 2.07	2.44 ± 1.45	794 ± 528		2.31 ± 2.19
Propanil + malathion					
First run					
CYP2	5.07 ± 1.04	1.00 ± 1.30	>>53,800[d]		NC
CYP-S	2.98 ± 0.05	3.44 ± 1.61	3690 ± 1580		0.69 ± 0.66
Second run					
CYP2	4.54 ± 0.74	1.23 ± 1.33	46,400 ± 31,100		NC
CYP-S	2.76 ± 1.56	1.90 ± 0.98	2080 ± 1830		0.89 ± 0.97

[a] Data are described according to the equation $Y = D/(1 + \exp[b(\log x - \log ED_{50})])$ where Y denotes the growth response of *C. difformis* at the x dose of propanil and D represents the upper asymptote of seedling growth (fresh weight) at dose zero (untreated control). ED_{50} denotes the propanil dose required to reduce fresh weight by 50%, and b is the proportional slope of the curve around ED_{50}.

[b] RI is the resistance index = ED_{50} resistant plants = ED_{50} susceptible plants.

[c] RP is the relative potency of propanil as affected by the addition of insecticides and expressed as the ratio of ED_{50} for plants treated with propanil only to ED_{50} for plants treated with propanil + insecticide.

[d] Actual ED_{50}, RI and RP values could not be calculated (NC) because the ED_{50} lies beyond the maximum propanil dose tested (53,800 g propanil ha^{-1}).

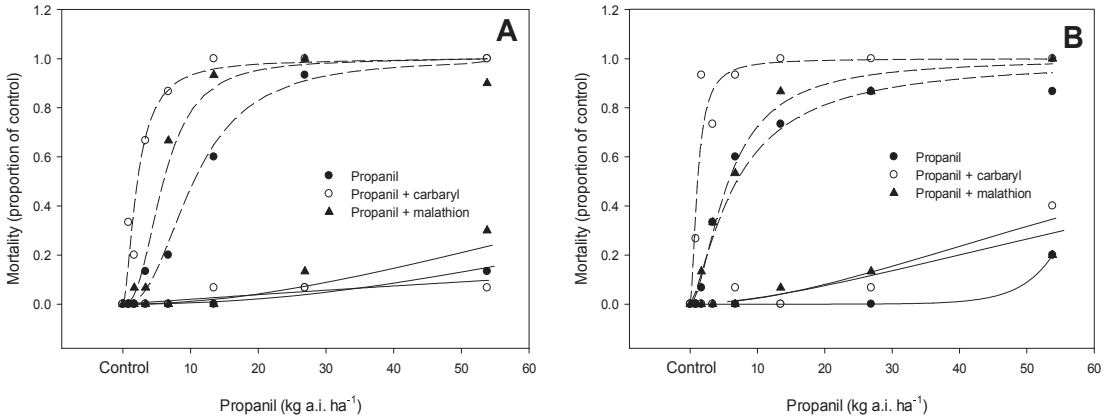

Fig. 3. Dose response of *C. difformis* populations CYP2 (solid lines) and CYP-S (dashed lines) to propanil, with and without malathion or carbaryl. Graphs (A and B) correspond to two experimental runs. Symbols are averages of three replicates fitted with a probit function for plant survival; model parameters are shown in Table 2. The field dose is 6.7 kg a.i. ha^{-1}.

Table 2
Effect of propanil alone and in mixture with carbaryl or malathion on the mortality of the propanil-susceptible (CYP-S) and -resistant (CYP2) populations of *Cyperus difformis*.[a]

Treatment	Susceptible population LD_{50} (g propanil ha^{-1})[a]	RP[b]	Susceptible population LD_{20} (g propanil ha^{-1})	RP	Resistant population LD_{20} (g propanil ha^{-1})	RP
First run						
Propanil alone	10,460 (7830–14,040)	–	5950 (3700–7940)	–	>>53,800[c]	–
Propanil + carbaryl	2140 (1410–3030)	4.88	950 (427–1440)	6.27	>>53,800[c]	NC[c]
Propanil + malathion	5990 (4490–7990)	1.75	3443 (2140–4580)	1.72	48,500 (26,500–>>53,800)	NC
Second run						
Propanil alone	6900 (4640–10,300)	–	2520 (1230–3850)	–	>>53,800[c]	–
Propanil + carbaryl	1100 (566–1600)	6.25	491 (127–827)	5.12	35,651 (19,330–>>53,800)	NC
Propanil + malathion	5700 (4,000–8100)	1.21	2530 (1390–3650)	0.99	39,870 (21,500–>>53,800)	NC

[a] LD_{50} and LD_{20} (propanil doses required to cause 50% and 20% plant mortality, respectively) values and their 95% confidence intervals (in parenthesis) estimated based on probit analysis.
[b] RP is the relative potency of propanil as affected by the addition of insecticides and expressed as the ratio of LD_{50} for plants treated with propanil only to LD_{50} for plants treated with propanil + insecticide; underlined values correspond to ratios of LD_{50} values with non-overlapping CI.
[c] NC, not calculated, actual LD_{50}, RI and RP values could not be adequately estimated because the LD_{50} lied beyond the maximum propanil dose tested (53,800 g propanil ha^{-1}).

smaller plants; also, the level of resistance to propanil was found to decrease at older growth stages in *E. colona* (Leah et al., 1995). However, the consistency of responses across both experimental runs underscores applicability of these results to the usual timing of *C. difformis* control with propanil under field conditions. Resistance to propanil was unequivocally confirmed by the dose response bioassays. Resistance based on plant growth inhibition was so large (at least 25-fold) that ED_{50} and LD_{50} value for the CYP2 population could not be estimated within a dose range that spanned up to eight times the field dose.

Rice can rapidly metabolize propanil to DCA via AAA activity, whereas susceptible *E. cruss-galli* accumulates lethal concentrations of propanil (Yih et al., 1968a). Propanil resistant *Echinochloa* spp. exhibit substantially greater AAA activity than susceptible plants allowing for more rapid propanil detoxification (Leah et al., 1994; Hirase and Hoagland, 2006). Further metabolism involves conjugation by glucosylation followed by complexation with polymeric cell constituents (Still and Kuzirian, 1967, Yih et al., 1968b; Still, 1968; Winkler and Sandermann, 1989; Pogány et al., 1990). Conjugation is also a contributing factor in the resistance of *E. colona* to propanil (Leah et al., 1997). Simultaneous application of some organophosphate and carbamate insecticides overcomes both propanil tolerance in rice and resistance in *Echinochloa* spp. (Bowling and Hudgins, 1966; Matsunaka, 1968; Leah et al., 1994; Daou and Talbert, 1999). Carbaryl is an AAA inhibitor in rice and *Echinochloa* spp. Malathion is a less effective inhibitor of rice AAA than carbaryl (Frear and Still, 1968), but has been shown to reduce propanil resistance by presumably inhibiting the peroxidases involved in the incorporation of DCA into bound residues (Leah et al., 1997). Therefore, a synergistic effect of these insecticides on propanil, conferring susceptibility to propanil resistant plants, would suggest differential propanil metabolism is the basis for the

Table 3
Percent control of *Cyperus difformis* populations with herbicides of contrasting modes of action applied in postemergence.

Population	Weed control (%)[a]					
	Bensulfuron[b]	Carfentrazone	Halosulfuron	Imazosulfuron	Propanil	Penoxsulam
CYP-S	98.00 a	100.00 a	100.00 a	100.00 a	93.34 a	100.00 a
CYP2	4.29 c	100.00 a	24.07 c	7.31 b	21.45 b	73.37 b
CYP3	28.31 b	100.00 a	78.01 b	24.84 b	28.36 b	97.06 a

[a] Herbicide doses in active ingredient are: Propanil (6726 g ha^{-1}), bensulfuron (70 g ha^{-1}), penoxsulam (35 g ha^{-1}), carfentrazone (224 g ha^{-1}), halosulfuron (52.5 g ha^{-1}) and imazosulfuron (336 g ha^{-1}). Crop oil concentrate (0.125%) was added to all treatments except to carfentrazone.
[b] Weed control values are percent reduction of fresh weight compared to untreated plants; values followed by the same letter within columns are not statistically different according to Fisher's Protected LSD with $\alpha = 0.05$.

contrasting response of the two *C. difformis* populations to the herbicide. However, in our experiments the propanil-resistant CYP2 biotype produced more than 80% biomass after a propanil (6.7 kg a.i. ha^{-1}) and carbaryl (1.9 kg a.i. ha^{-1}) or propanil and malathion (1.0 kg a.i. ha^{-1}) treatment compared to <20% by a susceptible biotype (Fig. 3); there also lacked significant increases in relative potency of propanil. All of which suggests substantial resistance to propanil still persisted in spite of the effects by the AAA inhibitor carbaryl or by malathion. Malathion was substantially less effective than carbaryl in synergizing propanil toxicity upon both *C. difformis* populations. Therefore, we can conclude that differential propanil metabolism (susceptible to inhibition by carbaryl and malathion) is not directly responsible for propanil resistance in the CYP2 population, suggesting the absence of a relevant AAA role in this resistance. Other factors that could contribute to resistance, such as target site alterations or differential herbicide uptake and short distance movement should be addressed in future research to properly elucidate the resistance mechanism. Although implicated as herbicide resistance mechanisms in several weed species (Yu et al., 2010; Ge et al., 2011), these processes have neither contributed to propanil resistance in *Echinochloa* spp. (Carey et al., 1995; Leah et al., 1995) nor have explained selectivity between rice and these weeds (Yih et al., 1968a).

Clarifying the relative contributions of independent mutations or gene flow among populations towards the evolution and spread of herbicide resistance is paramount for developing rational mitigation strategies (Tsuji et al., 2003; Merotto et al., 2010). *C. difformis* resistance to ALS-inhibitors evolved since 1992 in California rice fields after only four seasons of bensulfuron-methyl use (Pappas-Fader et al., 1993). Propanil resistance in this species should represent a subsequent evolutionary process, because propanil use was intensified precisely to control bensulfuron-methyl-resistant biotypes (Hill et al., 2002). A study of genetic diversity conducted on 29 *C. difformis* populations collected in rice fields of the Sacramento Valley of California, including the same areas where the accessions in our study originated, revealed little genetic diversity within populations (Merotto et al., 2010), The majority of the genetic variation was partitioned among populations, rather than within populations, and there was limited gene flow between populations. The study further suggested that ALS-inhibitor-resistance in *C. difformis* from California rice fields has spread through multiple independent evolutionary events in different locations rather than through dispersal from a single source (Merotto et al., 2010). With such genetic structure and limited gene flow, it would be logical to infer that propanil resistance may have also resulted from multiple independent evolutionary events within these *C. difformis* populations. Consequently, successful resistance mitigation should emphasize reducing herbicide selection pressure through in-farm integrated weed management practices rather than the control of seed movement among populations (Merotto et al., 2010).

Resistance to propanil in several grass species is rampant in other rice areas (Valverde and Itoh, 2001). To our knowledge, the loss of propanil to control this important weed of rice has not yet been reported and underscores the fragile sustainability of herbicide-based weed control in rice monoculture systems. Our results suggest that the use of carfentrazone within an integrated weed management approach can help mitigate the evolution of propanil resistance in *C. difformis* of California rice.

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