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Sensitivity of herbicide-resistant rigid ryegrass (*Lolium rigidum*) populations to cinmethylin, a new herbicide site of action

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Abstract

Rigid ryegrass (*Lolium rigidum* Gaudin) is the most problematic weed in Australia, with evolved resistance to multiple herbicide sites of action. Selection pressure by cinmethylin (Group 30, a fatty-acid thioesterase inhibitor) has been limited, because few populations have been exposed to the herbicide since its introduction in 2019. In this study, we examined the sensitivity of *L. rigidum* populations to this new herbicide. From a screening of almost 500 field populations in 2020, 28 potentially resistant populations were further investigated in a dose–response experiment. Seedlings from five populations surviving treatments of 250 or 375 g ai ha^{-1} cinmethylin were grown to maturity and seeds were harvested. The level of resistance found among the five putative-resistant parental populations of *L. rigidum* was negligible. In one population, one round of selection with cinmethylin resulted in a 2-fold increase in the lethal dose causing 50% mortality in the progeny population, although this dose was still only one-sixth of the recommended field rate of cinmethylin. Having a unique site of action, cinmethylin is a viable preemergence herbicide option to control existing multiple-resistance populations of *L. rigidum*. Comprehensive field monitoring and recurrent selection studies under controlled environmental conditions are needed to better ascertain the risk of *L. rigidum* evolving a high level of resistance to cinmethylin, although current data suggest that this risk is relatively low.

Introduction

Rigid ryegrass (*Lolium rigidum* Gaudin) is one of the most troublesome weeds in Australia, and is also found in the Americas, Africa, Europe, and the Middle East (Busi et al. 2020a). In the late 1800s, *L. rigidum* was introduced into southern Australia as a pasture forage. Beginning in the 1970s, many Australian farmers shifted to more profitable annual cropping systems (Henzell 2007). Consequently, *L. rigidum* became an abundant, widespread, and competitive weed species. At high plant densities, this weed can reduce wheat (*Triticum aestivum* L.) yields by 21% to 29% (Palta and Peltzer 2001; Peltzer and Douglas 2022). Therefore, farmers must use all available tools to control *L. rigidum*, but its adaptivity, high fecundity, seed and pollen dispersal capability, and high genetic variability make this a challenging task (Owen et al. 2015).

Herbicides have been the dominant tool to control *L. rigidum* across southern Australia during the past 50 yr. With intensive, recurrent usage of herbicides across millions of hectares annually, *L. rigidum* populations quickly evolved resistance to one or more herbicide sites of action (Owen et al. 2007, 2014, 2015). It is now ranked as the most resistance-prone species globally, with confirmed resistance to up to 14 different herbicide sites of action (Heap 2022). With widespread and high-level resistance of *L. rigidum* to postemergence herbicides such as the acetyl-CoA carboxylase (Group 1) and acetolactate synthase (Group 2) inhibitors, growers now frequently apply soil-residual preemergence herbicides such as trifluralin (Group 3) and prosulfocarb and pyroxasulfone (both Group 15). In Australia over the past 30 yr, there has been extensive outreach to growers on the importance of rotating and mixing different herbicide sites of action within the context of an integrated weed management cropping system. The "mix-and-rotate" strategy is now becoming widely adopted across the country (Busi et al. 2020b).

There is continual pressure on agrichemical companies to discover and develop new molecules. This situation is driven by the increasing frequency and complexity of herbicide resistance in many key economically damaging weeds, including *L. rigidum*. The last major herbicide site of action was introduced in the 1980s (Beckie and Harker 2017). In 2019, BASF introduced cinmethylin (Luximax®, 750 g ai L^{-1}, BASF Australia) as a new chemical for preemergence control of *L. rigidum* in wheat. It belongs to Group 30, the fatty-acid thioesterase inhibitors (Crop Life Australia 2021) and is of moderate volatility (vapor pressure: 10.2 MPa), similar to trifluralin (9.5 mPa) and triallate (12 mPa) (Grayson et al. 1987; Lewis et al. 2016). Volatilization is one of the key factors affecting the longevity of trifluralin and triallate in the soil (Chauhan et al. 2006; Curran 2016) and is more rapid at high temperatures (Bor et al. 1995; Glotfelty et al. 1984). Because cinmethylin is a new herbicide site of action recently introduced into Australia, selection pressure for resistance evolution in *L. rigidum* has been limited, and the inadvertent application of below-label rates due to volatility loss could contribute to selection of less-sensitive weed populations. The potential for rapid evolution of cinmethylin resistance in *L. rigidum* populations is presently unknown, but *L. rigidum* is capable of low levels of metabolic detoxification of cinmethylin, and this capacity is greater in populations with reduced cinmethylin sensitivity (Goggin et al. 2022).

To assess the variability of cinmethylin response in *L. rigidum* populations, baseline monitoring of nearly 500 field populations from across southern Australia was conducted in 2020. Results indicated that several populations exhibited survival in response to the full label rate of the herbicide (Busi et al. 2021). Therefore, the aim of the current study was to ascertain and quantify the level of sensitivity of suspected resistant populations and their cinmethylin-selected progeny to cinmethylin using a pot-based dose–response assay.

Materials and Methods

Plant Material

Populations submitted by growers to the University of Western Australia (UWA) resistance testing service were used in this study. Seed heads of *L. rigidum* plants were collected in individual fields throughout the cropping region of southern Australia in 2019 by growers and agronomists and sent to UWA. Of the nearly 500 populations screened at cinmethylin rates of 375, 500, and 750 g ha^{-1}, 28 populations (24 from Western Australia, 2 from South Australia, and 2 from Victoria) displayed variable levels of resistance to multiple sites of herbicide action (including Groups 1, 2, 3, and 15) and also exhibited a putative reduced sensitivity to cinmethylin (survival ranging from 6% to 30% at the recommended label rate of 375 g ha^{-1}) (Busi et al. 2020a, 2021). These populations, which exhibited healthy shoot and root growth after treatment with cinmethylin, were used for further study. The initial screening described in the following section was performed on the original field-collected populations, while subsequent pot studies were also performed with the progeny of individuals surviving treatment with 250 or 375 g cinmethylin ha^{-1}.

Initial Screening of Putative Cinmethylin-Resistant Populations

A dose–response experiment was conducted with the 28 putative-resistant (P) populations (designated P1 to P28) alongside cinmethylin-tolerant wheat ('Mace') and a well-characterized herbicide-susceptible *L. rigidum* population (VLR1; hereafter referred to as "S"). Wheat was used in lieu of a known cinmethylin-resistant *L. rigidum* population, as none have yet been identified. The experiment was performed in May 2020 and repeated in June 2020. Plastic trays with 20 cells measuring 6.5 cm by 6.5 cm by 6.5 cm were filled with potting soil (50% composted pine bark, 25% peat, and 25% river sand). The potting mix contained 4% organic carbon, which is at the high end of the range of organic carbon concentrations found in Western Australian agricultural soils (0.7% to 4%; Griffin et al. 2013). To ensure 25 individuals in each cell, seeds were pre-germinated on 0.6% (6 g L^{-1}) agar, and those with emerging radicles >0.5 mm were transferred to the surface of the soil in the cell trays after 3 d. Cinmethylin (Luximax®, BASF Australia, Melbourne, Australia) was applied directly to the seeds immediately after sowing, and the seeds were then covered with 1 cm fresh potting mix. Trays were watered and placed in a naturally lit glasshouse at UWA. The herbicide was applied using a dual-nozzle cabinet sprayer calibrated to deliver 110 L of spray solution ha^{-1} at 210 kPa and mounted with flat spray tips (TeeJet® XR 11001 nozzles, Spraying Systems, Wheaton, IL, USA) (Owen et al. 2014). The cinmethylin doses used were 0 (untreated), 50, 125, 250, 375 (label dose), and 500 g ha^{-1}. The soil was kept moist with daily watering, and seedlings were fertilized weekly with commercial soluble fertilizer. These experiments had four replicates of each treatment.

The number of healthy, germinated seedlings was counted at 28 d after herbicide application, and survival rate was calculated as a percentage of the untreated controls (Owen et al. 2015).

Assessing Cinmethylin Sensitivity under Controlled Conditions

Given that weed populations could potentially receive below-label rates of cinmethylin due to evaporative losses at higher temperatures and/or soil moisture contents (Spencer and Cliath 1974), resulting in a perceived resistance problem in susceptible populations, an experiment was conducted to compare the cinmethylin response of four *L. rigidum* populations (S, P11, P22, and P28) in a "closed" versus "open" system under controlled conditions. Wheat (Mace) was also included as a tolerant control. In the closed system, volatility losses of cinmethylin were minimized by germinating seeds on cinmethylin-containing agar in dishes sealed with Parafilm® (Sigma-Aldrich, Sydney, NSW, Australia) and incubating them on a layer of moist paper towel in sealed plastic bags to maintain humidity close to 100%. Cinmethylin concentrations of 0, 1, 5, 10, 20, 40, 60, or 120 nM were used by incorporating the appropriate amount of formulated cinmethylin into the agar. In the open system, pre-germinated seeds were sprayed with formulated cinmethylin at rates of 0, 2.3, 5.0, 23.4, 46.9, 93.8, 188, 375, or 562 g ha^{-1} while situated on the surface of moist potting mix, as described earlier. Seeds were then covered with 1 cm of fresh potting mix and kept moist for the duration of the experiment. Both the closed and open experiments were performed at day/night temperature regimes of 20/10 C and 30/20 C with a 12-h photoperiod of cool white LED light at 300 µmol m^{-2} s^{-1}. In the closed experiment, seedling coleoptile lengths were measured at 7 d after the start of imbibition; in the open experiment, the number of healthy green seedlings with at least two leaves was recorded at 28 d after cinmethylin treatment. There were 10 to 12 seedlings per treatment with three replicates, and each experiment was performed twice.

Parental versus Progeny Response to Cinmethylin

A third experiment compared the parental and progeny response to increasing doses of cinmethylin. Five populations (P11, P17, P18, P22, P28), encompassing a range of LD_{50} values that were 1- to 7-fold higher than that of the S population and thus representative of the variability present in the field, were selected for

Table 1. Survival of field-collected *Lolium rigidum* populations at three rates of cinmethylin.[a]

Dose	No. populations tested	Mean plant survival	No. populations with 0% survival
g ha^{-1}		%	
0	469	100 ± 0	0
375	492	0.18 ± 0.04	457
500	486	0.24 ± 0.08	453
750	487	0.15 ± 0.04	460

[a] Each population was treated once with 375 (recommended field rate), 500, or 750 g cinmethylin ha^{-1}, and survival was assessed after 21 d. There were 200 individuals per population per herbicide treatment.

Table 2. Response of 28 *Lolium rigidum* populations to increasing doses of cinmethylin.[a]

Population	Estimated LD_{50} ± SE	RI	P-value
	g cinmethylin ha^{-1}		
P1	55.0 ± 5.7	5.4	0.010
P2	53.6 ± 4.8	5.3	0.010
P3	50.3 ± 4.7	5.0	0.035
P4	41.8 ± 6.9	4.1	0.029
P5	61.1 ± 6.8	6.0	0.008
P6	64.0 ± 6.0	6.3	0.006
P7	40.3 ± 6.4	4.0	0.032
P8	31.5 ± 8.3	3.1	0.064
P9	37.4 ± 10.1	3.7	0.040
P10	37.8 ± 7.5	3.7	0.043
P11	**67.2 ± 8.8**	**6.6**	**0.003**
P12	59.1 ± 6.5	5.8	0.008
P13	47.2 ± 6.0	4.7	0.018
P14	38.8 ± 7.1	3.8	0.038
P15	42.3 ± 5.9	4.2	0.026
P16	36.3 ± 7.2	3.6	0.048
P17	**10.7 ± 10.1**	**1.1**	**0.956**
P18	**28.6 ± 8.8**	**2.8**	**0.098**
P19	50.3 ± 3.3	5.0	0.031
P20	47.6 ± 4.2	4.7	0.037
P21	18.0 ± 10.1	1.8	0.437
P22	**46.4 ± 12.8**	**4.6**	**0.023**
P23	19.1 ± 12.2	1.9	0.349
P24	44.8 ± 8.3	4.4	0.015
P25	25.9 ± 9.3	2.6	0.147
P26	26.2 ± 9.8	2.6	0.148
P27	37.5 ± 7.2	3.7	0.043
P28	**65.0 ± 6.8**	**6.4**	**0.006**
S	**10.2 ± 12.1**	**1.0**	**1.000**
Wheat ('Mace')	**>500**[b]	**>50**	**<0.001**

[a] LD_{50} is the dose required to kill 50% of the population; resistance index (RI) is calculated by dividing the LD_{50} of the putative-resistant (P) populations by that of the susceptible (S) population (populations highlighted in bold were investigated in dose–response experiments).

[b] Wheat showed >50% survival at the highest cinmethylin dose applied (500 g ha^{-1}), so the LD_{50} could not be calculated accurately.

study. Pre-germinated seeds were treated with 250 or 375 g ha^{-1} cinmethylin as described earlier, and survivors were transplanted into 8-L pots filled with potting mix, covered with pollen-proof nets, and plants of each separate population were cross-pollinated among themselves. At maturity, plants were harvested and threshed, and seeds were cleaned using a forced-air separator. Following harvest, the seeds were dry afterripened for at least 2 mo to relieve dormancy before being used in experiments (Owen et al. 2015). Dose–response experiments were performed in April 2021 and repeated in June 2021 on the parental and progeny populations according to the procedures described earlier for the initial screening (i.e., rates of 0, 50, 125, 250, 375, or 500 g cinmethylin ha^{-1} were applied). In each dose–response experiment, there were four replicated pots for each herbicide dose, and each pot was the experimental unit.

Statistical Analysis

The dose–response studies were repeated at least once and data were pooled before nonlinear regression analysis. Plant survival was expressed as a percentage of total plants (based on untreated controls) treated with herbicide. The DRC package of the statistical software R v. 3.4.2 (R Core Team 2022) was used to calculate the herbicide dose causing 50% plant mortality (LD_{50}) and to estimate the regression coefficients of a three-parameter log-logistic model (Equation 1):

$$Y = \frac{d}{1 + \exp[b(\log x - \log e)]} \qquad [1]$$

where d is the upper limit (100%), b is the slope of the curve, x is the herbicide dose, and e is the dose producing a 50% reduction in response. Nonlinear regression assumptions were met by assuming a continuous Gaussian distribution of errors. Statistically significant differences in estimated LD_{50} values between S and putative-resistant *L. rigidum* populations were assessed using the *EDcomp* function in the DRC package. Estimated LD_{80} and LD_{95} were also calculated. For the agar experiment, coleoptile length was expressed as a percentage of the coleoptile length of the untreated controls, and the cinmethylin concentration causing a 50%, 80%, or 95% reduction in length (ED_{50}, ED_{80}, or ED_{95}) was calculated as described for the survival data. The resistance index (RI) was expressed as the ratio of LD or ED values in the putative-resistant P versus S populations.

Results and Discussion

Initial Screening of Putative Cinmethylin-Resistant Populations

Of the ~500 populations screened at 375, 500, and 750 g cinmethylin ha^{-1}, >450 showed 0% survival at each rate, indicating that most field populations of *L. rigidum* can be classified as susceptible (Table 1; Supplementary Figure S1). The dose–response experiment on 28 putative-resistant populations indicated that none of these had an LD_{50} value approaching the label rate of 375 g ha^{-1} cinmethylin. Of the 28 populations, 21 had LD_{50} values significantly higher ($P < 0.05$) than that of the S population (Table 2). The RI threshold to classify populations as having moderate or high-level resistance is 10 or above, because this represents a resistance level that is visible and problematic in the field (Baucom and Busi 2019). Low-level resistance is more difficult to define (HRAC 2022); for the current study, the 16 populations with an RI value >4 were considered to have reduced sensitivity to cinmethylin compared with the S population. These populations could indicate an early stage of shifting sensitivity to cinmethylin, but it should be noted that the LD_{50} of even the least-sensitive population was less than one-fifth of the recommended rate of this herbicide. Nevertheless, continued intensive selection by cinmethylin could potentially result in agronomically relevant reduced control of *L. rigidum* in the field. Further studies were therefore conducted to better quantify the practical impact of such a low-level resistance (Heap 2022).

Table 3. Comparison of response of susceptible (S) vs. putative-resistant parental *Lolium rigidum* populations (P11, P22, and P28) to cinmethylin under controlled conditions.[a]

Agar 20/10 C	ED_{50}	RI	P-value	ED_{80}	RI	P-value	ED_{95}	RI	P-value
	nM			nM			nM		
S	8.6 ± 1.2			26.3 ± 5.1			92.8 ± 31.8		
P11	11.8 ± 2.1	1.4 ± 0.3	0.24	39.3 ± 8.1	1.5 ± 0.4	0.24	152 ± 58	1.6 ± 0.8	0.45
P22	12.3 ± 2.4	1.4 ± 0.4	0.22	54.4 ± 12.8	2.1 ± 0.6	0.09	290 ± 121	3.1 ± 1.7	0.21
P28	11.5 ± 2.9	1.4 ± 0.4	0.37	59.4 ± 15.4	2.3 ± 0.7	0.09	374 ± 184	4.0 ± 2.4	0.21
Wheat ('Mace')	241 ± 47	28 ± 0	<0.001	1,233 ± 376	47 ± 17	<0.001	7,718 ± 4,352	83 ± 55	<0.001
Agar 30/20 C									
S	14.4 ± 1.7			41.8 ± 6.4			138 ± 39		
P11	20.7 ± 2.9	1.4 ± 0.3	0.09	80.5 ± 13.3	1.9 ± 0.4	0.03	370 ± 111	2.7 ± 1.1	0.13
P22	28.7 ± 3.9	2.0 ± 0.4	0.01	111 ± 18	2.7 ± 0.6	0.01	508 ± 154	3.7 ± 1.5	0.08
P28	18.5 ± 2.8	1.3 ± 0.2	0.24	81.3 ± 14.9	1.9 ± 0.5	0.04	429 ± 144	3.1 ± 1.4	0.12
Wheat (Mace)	460 ± 71	32 ± 0	<0.001	2,171 ± 633	52 ± 17	<0.001	12,415 ± 6,503	90 ± 53	<0.001
Soil 20/10 C	LD_{50} g ha^{-1}	RI	P-value	LD_{80} g ha^{-1}	RI	P-value	LD_{95} g ha^{-1}	RI	P-value
S	5.2 ± 0.4			6.4 ± 3.8			8.0 ± 9.5		
P11	8.3 ± 2.1	1.6 ± 0.4	0.17	34.4 ± 8.8	5.4 ± 3.5	0.21	171 ± 74	21 ± 27	0.06
P22	16.6 ± 3.3	3.2 ± 0.7	0.002	39.9 ± 7.1	6.3 ± 3.9	0.18	107 ± 36	13 ± 16	0.05
P28	9.8 ± 2.6	1.9 ± 0.5	0.09	46.0 ± 11.8	7.2 ± 4.7	0.19	261 ± 109	33 ± 41	0.03
Wheat (Mace)	571 ± 50	111 ± 0	<0.001	893 ± 171	141 ± 88	<0.001	1,478 ± 496	184 ± 227	<0.001
Soil 30/20 C									
S	31.7 ± 3.2			45.6 ± 6.6			68.7 ± 16.9		
P11	60.7 ± 11.8	1.9 ± 0.4	0.03	162 ± 35	3.5 ± 0.9	0.01	486 ± 184	7.1 ± 3.2	0.06
P22	84.9 ± 17.3	2.7 ± 0.6	0.01	180 ± 31	3.9 ± 0.9	0.001	419 ± 143	6.1 ± 2.6	0.05
P28	80.1 ± 14.3	2.5 ± 0.5	0.004	175 ± 30	3.8 ± 0.9	0.001	419 ± 124	6.1 ± 2.3	0.03
Wheat (Mace)	784 ± 382	25 ± 0	<0.001	1,214 ± 1,271	27 ± 28	<0.001	1,986 ± 3,345	29 ± 49	<0.001
Temperature comparison	Agar experiment		Soil experiment						
	High:low	P-value	High:low	P-value					
S	1.7 ± 0.4	0.002	7.8 ± 1.6	<0.001					
P11	1.8 ± 0.3	<0.001	6.1 ± 3.6	<0.001					
P22	2.3 ± 0.8	<0.001	5.7 ± 2.3	<0.001					
P28	1.6 ± 0.5	0.06	4.8 ± 1.6	<0.001					
Wheat (Mace)	1.9 ± 0.4	<0.001	1.4 ± 0.4	0.20					

[a] Dose–response experiments were performed at constant day/night temperatures of 20/10 C or 30/20 C on agar or in pots (soil). The cinmethylin doses (±SE) causing 50%, 80%, or 95% reduction in coleoptile length (agar; ED_{50}, ED_{80}, or ED_{95}) or seedling survival (soil; LD_{50}, LD_{80}, or LD_{95}) were calculated in nM and in g ha^{-1}, respectively. The resistance index (RI; ratio of effective doses for putative-resistant P to susceptible population S) was also calculated, as was the ratio of effective doses (ED_{50} or LD_{50} only) at high (30 C/20 C) and low (20/10 C) temperatures for each population. Data were pooled from two independent experiments ($n = 3$) for both the agar and soil studies.

Assessing Cinmethylin Sensitivity under Controlled Conditions

In the agar experiment performed at low temperature (20/10 C), the ED_{50}, ED_{80}, and ED_{95} values of the putative-resistant P *L. rigidum* populations were not significantly higher than those of the S population (Table 3). At high temperatures (30/20 C), there were no resistance indices above 4-fold, and few instances where the ED_{50} or ED_{95} values of the putative-resistant populations were significantly different from those of the S population (although it should be noted that the ED_{80} values of the putative-resistant populations were significantly higher) (Table 3). In contrast, wheat was ≥30-fold more tolerant than the S population under both temperature regimes.

In the low-temperature pot (soil) experiment, population P22 had significantly higher LD_{50} and LD_{95} values than the S population (P28 also had a higher LD_{95}), while at high temperatures, all three putative-resistant P populations were significantly less sensitive to cinmethylin than the S population, with low to moderate RI values (≤7). Wheat exhibited RI values of ≥100 at low temperature and ≥25 at high temperature (Table 3). A comparison of effective doses for individual populations at high versus low temperatures revealed that both *L. rigidum* and wheat were about 2-fold less sensitive to cinmethylin at high temperature on agar. In soil, *L. rigidum* was >5-fold less sensitive at high temperature, but wheat did not show a significant temperature response (Table 3). The minimal effect of temperature on the response of *L. rigidum* to cinmethylin in the closed agar system, where evaporation of the herbicide was minimized, compared with the more significant effect in the open soil system, indicates that volatility losses of cinmethylin have more effect on cinmethylin response in these populations than any nascent resistance mechanism. This is supported by the results of a previous study in which population P22 was shown to have no greater ability to detoxify cinmethylin than did population S (Goggin et al. [2022], in which population P22 was designated "R1").

With predominantly no-tillage systems and the trend of early sowing in southern Australia (Zaicou-Kunesch et al. 2018), there may be a greater risk of environmental loss of volatile preemergence herbicides because of minimal soil incorporation and higher ambient temperatures. In addition, early sowing is often performed under relatively dry soil conditions, which can reduce the phytotoxicity of preemergence herbicides such as cinmethylin (Russell et al. 1991). In Australia, substantial volatilization losses of cinmethylin due to high temperatures near the time of application may translate into exposure of *L. rigidum* populations to sublethal doses that could accelerate quantitative resistance to this herbicide.

Table 4. Cinmethylin dose–response results for the five investigated *Lolium rigidum* putative-resistant populations (P) (parental and progeny populations), a susceptible population (S), and wheat (‘Mace’).[a]

			P-value				P-value				P-value	
Population	LD_{50}	RI	P vs. S	progeny vs. parental	LD_{80} (g ha^{-1})	RI	P vs. S	progeny vs. parental	LD_{95}	RI	P vs. S	progeny vs. parental
	g ha^{-1}								g ha^{-1}			
P11 parental	9.1 ± 13.9	0.5	0.529	—	23.5 ± 18.1	0.6	0.497	—	67.8 ± 23.4	0.9	0.746	—
P11 progeny	52.6 ± 2.8	2.8	0.284	0.588	88.0 ± 8.1	2.4	0.019	0.346	156.8 ± 27.9	2.0	0.103	0.144
P17 parental	23.5 ± 13.4	1.3	0.801	—	42.4 ± 7.4	1.2	0.621	—	82.4 ± 29.6	1.1	0.887	—
P17 progeny	47.9 ± 2.0	2.0	0.310	0.372	66.4 ± 10.0	1.8	0.103	0.118	95.7 ± 32.7	1.2	0.651	0.780
P18 parental	22.6 ± 16.9	1.2	0.850	—	39.8 ± 10.1	1.1	0.796	—	75.1 ± 28.1	1.0	0.951	—
P18 progeny	36.5 ± 4.3	2.0	0.422	0.614	87.8 ± 8.4	2.4	0.020	0.044	235.3 ± 50.1	3.0	0.043	0.114
P22 parental	31.5 ± 5.6	1.7	0.512	—	60.3 ± 5.2	1.7	0.108	—	124.9 ± 34.4	1.6	0.309	—
P22 progeny	59.2 ± 5.2	3.2	0.259	0.019	152.6 ± 14.6	4.2	0.002	0.000	442.2 ± 81.9	5.7	0.009	0.031
P28 parental	42.0 ± 3.4	2.3	0.359	—	76.8 ± 7.3	2.1	0.036	—	151.4 ± 34.2	2.0	0.151	—
P28 progeny	33.7 ± 4.9	1.8	0.468	0.146	95.3 ± 10.0	2.6	0.015	0.171	306.6 ± 74.3	4.0	0.034	0.127
S	18.5 ± 11.2	1.0	NA	NA	36.3 ± 8.4	1.0	NA	NA	77.2 ± 19.7	1.0	NA	NA
Wheat (Mace)[b]	619.6 ± 50.2	50.2	0.000	—	911.4 ± 163.8	25.1	0.000	—	1,406.5 ± 416.6	18.2	0.000	—
Wheat (Mace)[c]	834.7 ± 160.1	33.5	0.000	0.216	1,605.9 ± 615	44.2	0.000	0.352	3,350.5 ± 2,027.4	43.4	0.000	0.389

[a] Data were pooled from two independent experiments conducted in April and June of 2021. LD_{50}, LD_{80}, and LD_{95} are the lethal doses of cinmethylin in g ha^{-1} required to kill 50%, 80%, and 95% of individuals in a population, respectively; RI is the resistance index, calculated as the ratio of LD values for the putative-resistant (P) populations and susceptible population (S). Differences in LD values between P vs. S or progeny vs. parental populations were considered significant if the P-value was ≤ 0.05.

[b] Wheat present in parental dose–response experiment.

[c] Wheat present in progeny dose–response experiment.

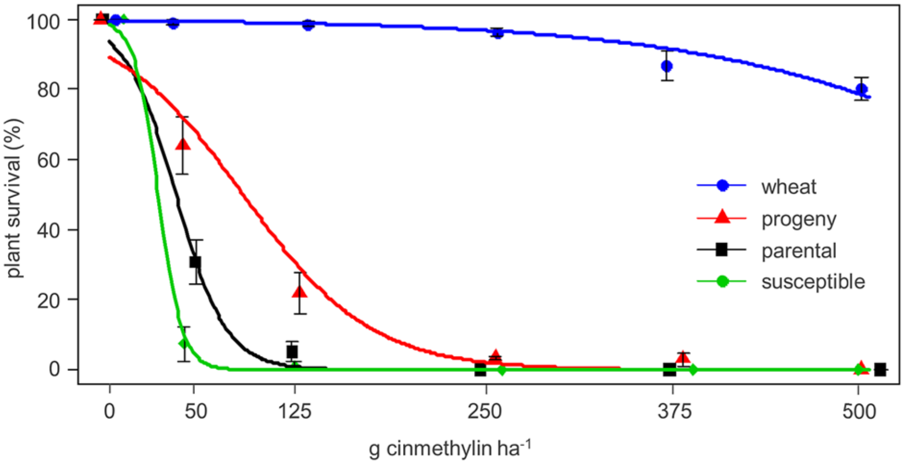

Figure 1. Survival response (% of untreated controls) of P22 progeny and parental *Lolium rigidum* populations, a susceptible (S) control *L. rigidum* population, and wheat (‘Mace’) to increasing doses of cinmethylin (g ai ha^{-1}).

Parental versus Progeny Response to Cinmethylin

None of the five parental or progeny populations had a significantly higher LD_{50} than the S control population, but LD_{80} values were higher than S in progeny P11, P18, P22, and P28 (and parental P28), and LD_{95} values were higher in progeny P18, P22, and P28 (Table 4). A comparison between parental and progeny populations revealed that only the P22 progeny had significantly ($P < 0.05$) higher LD_{50}, LD_{80}, and LD_{95} values than its parent (Table 4; Figure 1), while the LD_{80} for P18 was also higher in the progeny than the parent (Table 4).

Lolium rigidum is a diploid, outcrossing species, which means that there is the potential for accumulation of complex patterns of herbicide-resistance and cross-resistance mechanisms, as well as multiple mechanisms of resistance to a single site of action (e.g., Han et al. 2016; Yu and Powles 2014). The mechanism for reduced cinmethylin sensitivity in the populations used in the current study has not been investigated. However, in two populations with even lower sensitivity, the capacity for metabolic detoxification of cinmethylin was inversely correlated with the level of sensitivity (Goggin et al. 2022). As mentioned earlier, parental population P22 did not display significantly higher levels of cinmethylin metabolism than population S in the previous study, but the higher LD_{50} of its cinmethylin-selected progeny could potentially be linked to an incremental selection for enhanced cinmethylin metabolism. This needs to be demonstrated experimentally.

The observation that parental populations P11, P22, and P28 did not generally exhibit reduced sensitivity relative to the S population in the 2021 experiment comparing parental and progeny populations was in contrast to the results of the initial screening experiment conducted in 2020. The reason for this discrepancy is unclear, but may be due to environmental conditions affecting cinmethylin efficacy. Such variability in calculated LD values in S populations was also observed in a previous study (Busi et al. 2020a). Population P22, which exhibited less sensitivity to cinmethylin in the first experiment, also showed consistent differences in sensitivity between parental and progeny populations. The decrease in cinmethylin sensitivity between P22 parental and progeny populations after only one cycle of selection is consistent with results of previous studies of recurrent selection of herbicide resistance in *L. rigidum*. Populations of *L. rigidum* that were exposed to recurrent selection with pyroxasulfone (Busi et al. 2014), diclofop-methyl (Neve and Powles 2005), or bixlozone (Brunton et al. 2021) evolved high levels of resistance (likely via enhanced herbicide metabolism, at least in the cases of pyroxasulfone and diclofop-methyl) after only two to four generations, thus highlighting the risk of evolution of resistance in *L. rigidum* through repetitive and frequent herbicide use. In the case of cinmethylin, however, any appearance of agronomically relevant levels of resistance is likely to require many more rounds of selection, based on the very low LD_{50} values (relative to the recommended field rate) of both the parental and once-selected progeny populations.

In summary, the collective results of this study highlight the variable sensitivity of field-collected *L. rigidum* populations to cinmethylin. Such a sensitivity analysis is a valuable proactive approach for early monitoring for herbicide resistance to a new herbicide or herbicide site of action (Panozzo et al. 2020). The reduction in sensitivity of progeny versus parental population P22 of *L. rigidum* to cinmethylin after only one cycle of selection indicates the potential for evolution of resistance to cinmethylin. Further rounds of selection are warranted to fully assess the risk of resistance evolution in *L. rigidum* to this herbicide site of action and to determine whether, as suspected, metabolic detoxification of cinmethylin will be the predominant mechanism of reduced sensitivity. Risk assessment of cinmethylin resistance also requires regular monitoring through field surveys and testing of submitted samples at qualified laboratories. Additional practices to mitigate the selection and evolution of resistance to cinmethylin include rotating and mixing different herbicide sites of action within the context of a vigorous, weed-competitive crop and consistently employing harvest weed seed control.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2023.2

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References

Baucom RS, Busi R (2019) Evolutionary epidemiology in the field: a proactive approach for identifying herbicide resistance in problematic crop weeds. New Phytol 223:1056–1058

Beckie HJ, Harker KN (2017) Our top 10 herbicide-resistant weed management practices. Pest Manag Sci 73:1045–1052

Bor G, Berg F van den, Smelt JH, Smidt RA, Peppel-Groen AE van den, Leistra M (1995) Volatilization of Tri-allate, Ethoprophos and Parathion Measured with Four Methods after Spraying on a Sandy Soil. Report 104. Wageningen University, Wageningen, Netherlands. https://edepot.wur.nl/362145. Accessed: June 6, 2022

Brunton DJ, Gill G, Preston C (2021) Resistance to bixlozone and clomazone in cross-resistant rigid ryegrass (*Lolium rigidum*) populations from southern Australia. Weed Sci 69:284–289

Busi R, Beckie HJ, Bates A, Boyes T, Davey C, Haskins B, Mock S, Newman P, Porri A, Onofri A (2021) Herbicide resistance across the Australian continent. Pest Manag Sci 77:5139–5148

Busi R, Dayan FE, Francis I, Goggin D, Lerchl J, Porri A, Powles SB, Sun C, Beckie HJ (2020a) Cinmethylin controls multiple herbicide-resistant *Lolium rigidum* and its wheat selectivity is P450-based. Pest Manag Sci 76:2601–2608

Busi R, Gaines TA, Vila-Aiub MM, Powles SB (2014) Inheritance of evolved resistance to a novel herbicide (pyroxasulfone). Plant Sci 217–218:127–134

Busi R, Powles SB, Beckie HJ, Renton M (2020b) Rotations and mixtures of soil-applied herbicides delay resistance. Pest Manag Sci 76:487–496

Chauhan BS, Gill G, Preston C (2006) Tillage systems affect trifluralin bioavailability in soil. Weed Sci 54:941–947

Crop Life Australia (2021) Herbicide Mode of Action Table. https://www.croplife.org.au/wp-content/uploads/2021/07/2021-Herbicide-MOA-Table-ADJUSTED.pdf. Accessed: May 1, 2022

Curran WS (2016) Persistence of herbicides in soil. Crops Soils 49:16–21

Glotfelty DE, Taylor AW, Turner BC, Zoller WH (1984) Volatilization of surface-applied pesticides. J. Agric Food Chem 32:638–543

Goggin DE, Cawthray GR, Busi R, Porri A, Beckie HJ (2022) Enhanced production of water-soluble cinmethylin metabolites by *Lolium rigidum* populations with lower cinmethylin sensitivity. Pest Manag Sci 78:3173–3182

Grayson BT, Williams KS, Freehauf PA, Pease RR, Ziesel WT, Sereno RL, Reinsfelder RE (1987) The physical and chemical properties of the herbicide cinmethylin (SD 95481). Pestic Sci 21:143–153

Griffin E, Hoyle F, Murphy D (2013) Soil organic carbon. In: Report Card on Sustainable Natural Resource Use in Agriculture. Perth: Department of Agriculture and Food, Western Australia. www.agric.wa.gov.au/sites/gateway/files/2.4%20Soil%20organic%20carbon.pdf. Accessed: October 25, 2021

Han H, Yu Q, Owen MJ, Cawthray GR, Powles SB (2016) Widespread occurrence of both metabolic and target-site herbicide resistance mechanisms in *Lolium rigidum* populations. Pest Manag Sci 72:255–263

Heap I (2022) The International Herbicide-Resistant Weed Database. http://www.weedscience.org. Accessed: May 1, 2022

Henzell T (2007) Grain crops. Pages 1–47 *in* Australian Agriculture: Its History and Challenges. Collingwood, Australia: CSIRO Publishing

[HRAC] Herbicide Resistance Action Committee (2022) Criteria for Confirmation of Herbicide-Resistant Weeds with Specific Emphasis on Confirming Low Level Resistance. https://hracglobal.com/herbicide-resistance/confirming-resistance. Accessed: September 9, 2022

Lewis KA, Tzilivakis J, Warner D, Green A (2016) An international database for pesticide risk assessments and management. Hum Ecol Risk Assess 22:1050–1064

Neve P, Powles S (2005) Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. Theor Appl Genet 110:1154–1166

Owen MJ, Goggin DE, Powles SB (2015) Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). Pest Manag Sci 71:966–971

Owen MJ, Martinez NJ, Powles SB (2014) Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. Weed Res 54:314–324

Owen MJ, Walsh MJ, Llewellyn RS, Powles SB (2007) Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. Aust J Agric Res 58:711–718

Palta JA, Peltzer S (2001) Annual ryegrass (*Lolium rigidum*) reduces the uptake and utilisation of fertiliser-nitrogen by wheat. Aust J Agric Res 52:573–581

Panozzo S, Collavo A, Sattin M (2020) Sensitivity analysis of Italian *Lolium* spp. to glyphosate in agricultural environments. Plants 9:165

Peltzer S, Douglas A (2022) Annual Ryegrass. Perth, Australia: Department of Primary Industries and Regional Development. https://www.agric.wa.gov.au/grains-research-development/annual-ryegrass. Accessed: May 1, 2022

R Core Team (2022) R: A Language and Environment for Statistical Computing. Vienna, Austria: Foundation for Statistical Computing. http://www.R-project.org. Accessed: May 1, 2022

Russell SG, Monaco TJ, Weber JB (1991) Influence of soil moisture on phytotoxicity of cinmethylin to various crops. Weed Sci 39:402–407

Spencer WF, Cliath MM (1974) Factors affecting vapor loss of trifluralin from soil. J Agric Food Chem 22:987–991

Yu Q, Powles S (2014) Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. Plant Physiol 166:1106–1118

Zaicou-Kunesch C, Shackley B, Biddulph B (2018) Frost Risk—Manage Wheat Variety and Sowing Time. https://www.agric.wa.gov.au/frost/frost-risk-%E2%80%93-manage-wheat-variety-and-sowing-time?page=0%2C0. Accessed: June 12, 2022