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Weed Management—Major Crops

Incidence of Herbicide Resistance in Rigid Ryegrass (*Lolium rigidum*) across Southeastern Australia

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Herbicide resistance in rigid ryegrass is an escalating problem in grain-cropping fields of southeastern Australia due to increased reliance on herbicides as the main method for weed control. Weed surveys were conducted between 1998 and 2009 to identify the extent of herbicide-resistant rigid ryegrass across this region to dinitroaniline, and acetolactate synthase- and acetyl coenzyme A (CoA) carboxylase-inhibiting herbicides. Rigid ryegrass was collected from cropped fields chosen at random. Outdoor pot studies were conducted during the normal winter growing season for rigid ryegrass with PRE-applied trifluralin and POST-applied diclofop-methyl, chlorsulfuron, tralkoxydim, pinoxaden, and clethodim. Herbicide resistance to trifluralin in rigid ryegrass was identified in one-third of the fields surveyed from South Australia, whereas less than 5% of fields in Victoria exhibited resistance. In contrast, resistance to chlorsulfuron was detected in at least half of the cropped fields across southeastern Australia. Resistance to the cereal-selective aryloxyphenoxypropionate-inhibiting herbicides diclofop-methyl, tralkoxydim, and pinoxaden ranged between 30 and 60% in most regions, whereas in marginal cropping areas less than 12% of fields exhibited resistance. Resistance to clethodim varied between 0 and 61%. Higher levels of resistance to clethodim were identified in the more intensively cropped, higher-rainfall districts where pulse and canola crops are common. These weed surveys demonstrated that a high incidence of resistance to most tested herbicides was present in rigid ryegrass from cropped fields in southeastern Australia, which presents a major challenge for crop producers.
Nomenclature: Chlorsulfuron; clethodim; diclofop-methyl; pinoxaden; tralkoxydim; trifluralin; rigid ryegrass, *Lolium rigidum* Gaudin; canola, *Brassica napus* L.
Key words: Random weed survey.

La resistencia a herbicidas en *Lolium rigidum* es un problema creciente en los campos de cultivo de granos en el sureste de Australia, debido al incremento en la dependencia a herbicidas como el método principal para el control de malezas. Estudios observacionales de malezas se realizaron entre 1998 y 2009 para identificar el alcance en esta región de la resistencia de *L. rigidum* a los herbicidas dinitroaniline, inhibidores acetolactate synthase y acetyl CoA carboxylase. *L. rigidum* se recolectó en campos de cultivo seleccionados al azar. Se realizaron estudios al aire libre con macetas durante la temporada normal de crecimiento en invierno para *L. rigidum* con trifluralin aplicado PRE y diclofop-methyl, chlorsulfuron, tralkoxydim, pinoxaden y clethodim aplicados POST. La resistencia de *L. rigidum* al herbicida trifluralin fue identificada en un tercio de los campos muestreados en el sur de Australia, mientras que menos del 5% de los campos en Victoria mostraron resistencia. En contraste, la resistencia al chlorsulfuron fue detectada en al menos la mitad de los campos de cultivo en el sureste de Australia. La resistencia a los herbicidas selectivos a cereales, inhibidores aryloxyphenoxy propionate, como son diclofop-methyl, tralkoxydim y pinoxaden, varió entre 30 y 60% en la mayoría de las regiones, mientras que en áreas marginales de cultivo, menos del 12% de los campos mostraron resistencia. La resistencia al clethodim varió entre 0 y 61%. Niveles más altos de resistencia a clethodim se identificaron en los distritos de mayor intensidad de cultivo y mayor precipitación, donde los cultivos de especies leguminosas y *Brassica napus* son comunes. Estos estudios observacionales de malezas demostraron que existe una alta incidencia de resistencia en *L. rigidum* a la mayoría de los herbicidas estudiados en los campos de cultivo en el sureste de Australia, lo cual representa un importante reto para los productores.

The intensive use of herbicides has resulted in the evolution of herbicide-resistant weeds. Currently there are over 30 weed species where populations have evolved herbicide resistance in Australia, with thousands of fields infested with herbicide-resistant rigid ryegrass (Heap 2011). This makes rigid ryegrass the most important weed species of winter grain crops in Australia (Jones et al. 2005). The ability of rigid ryegrass to rapidly evolve resistance to herbicides makes it a difficult weed to control, particularly in cereal crops. In many situations, the level of rigid ryegrass infestation in the previous season dictates what rotational crops farmers can grow. A significant contributor to the rapid appearance of herbicide resistance in rigid ryegrass has been the widespread adoption of minimum tillage, which offers many benefits including reduced erosion and improved moisture conservation (Jones et al. 2005). However, the reduction in soil disturbance in grain-cropping fields across southern Australia has greatly increased the reliance on herbicides for weed control (Chauhan et al. 2006).

Wheat (*Triticum aestivum* L.) is one of the less risky crops for producers in southern Australia to grow, although herbicide options are more limited in wheat because of herbicide-resistant weeds (Anonymous 2008). Farmers are sometimes forced into growing less-profitable crops where more diverse control measures can be used; such crops include pulses (use of butroxydim or clethodim) or triazine-resistant

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Table 1. Distribution of crop type and occurrence of rigid ryegrass that was encountered in each of the major survey regions.

Region	Year	Fields sampled	Ryegrass (%)	Crop Wheat (%)	Crop Barley (%)	Crop Legume crops (%)
South Australia						
Mallee	2007	156	72	66	24	10
Southeast	2007	77	70	53	18	29
Central	1998	196	85	66	10	24
Central	2003	187	87	55	22	23
Central	2008	270	93	59	23	18
Eyre Peninsula	2009	177	93	87	5	8
Kangaroo Island	2009	26	92	50	12	38
Victoria						
Western	2005	125	90	61	18	21
Northern	2006	118	96	67	17	16
Southern	2009	104	88	47	23	30

canola where atrazine can be used (Potter and Salisbury 1993). In marginal cropping districts with low annual rainfall and soil fertility, restricted rotational options, such as pastures, with fewer inputs favor poorer weed control. Additionally, in marginal regions, the use of effective (and often more expensive) herbicides may not be economically viable.

In 1982, the first case of herbicide resistance in rigid ryegrass was identified only four years after the acetyl CoA carboxylase (ACCase)-inhibiting herbicide diclofop-methyl became commercially available (Heap and Knight 1982). Similarly, resistance in rigid ryegrass to the acetolactate synthase (ALS)-inhibiting herbicide chlorsulfuron was confirmed 4 yr after its release in 1982 (Heap and Knight 1986). Additional ACCase- (fluazifop-butyl, haloxyfop-methyl, quizalofop-*P*-ethyl, tralkoxydim, sethoxydim, clethodim, butroxydim, and pinoxaden) and ALS-inhibiting herbicides (triasulfuron, iodosulfuron, imazethapyr, pyroxsulam) have since become available to selectively control rigid ryegrass in many grain crops. Over the past three decades, thousands of rigid ryegrass populations have been confirmed resistant not only to ACCase- and ALS-inhibiting herbicides, but also to other modes of action (MOA) such as the triazine (inhibition of photosynthesis at photosystem II) and dinitroaniline (inhibition of microtubule assembly) herbicides (Boutsalis and Broster 2006; Broster and Pratley 2006; Broster et al. 2011; Llewellyn and Powles 2001; Owen et al. 2007; Pratley et al. 1993). A number of rigid ryegrass populations in Australia have evolved resistance to six different herbicide MOA, although such populations are not common (Preston et al. 1996).

Information from farmers and commercial resistance testing services has indicated that in rigid ryegrass, herbicide resistance remains a significant issue, with 80 to 90% of samples tested from southern Australia resistant to ACCase- and ALS-inhibiting herbicides (Boutsalis and Broster 2006). However, it is difficult to quantify the number of fields with resistance across different grain-growing regions solely on the basis of such information. In addition, knowledge of the magnitude of herbicide resistance in key farming regions can create opportunities for chemical companies to develop new herbicides to control herbicide-resistant rigid ryegrass.

A method commonly used for quantifying the incidence of herbicide resistance is to conduct weed seed collection surveys across large agronomic regions from fields selected at random. To date, random weed surveys have been conducted in two Australian states, Western Australia and southern New South Wales, where significant incidence of herbicide resistance in rigid ryegrass, particularly to ACCase- and ALS-inhibiting herbicides, has been reported (Llewellyn and Powles 2001; Owen et al. 2007; Pratley et al. 1993). On the basis of the frequency that resistance has been detected and the area surveyed, these findings suggest that herbicide-resistant rigid ryegrass occurs across millions of hectares. However, there is a lack of information on the incidence of resistance in rigid ryegrass from two other important cereal-growing states, South Australia (SA) and Victoria (Vic). This paper reports the incidence of resistance in rigid ryegrass populations to dinitroaniline and ACCase- and ALS-inhibiting herbicides using outdoor pot studies. The samples were collected between 1998 and 2009 in 10 separate surveys across key agronomic regions in SA and Vic covering an area greater than a quarter of a million km^2.

Materials and Methods

Survey. Ten random weed surveys totaling 1,436 fields were conducted in 1998, 2003, and annually from 2005 to 2009, with multiple regions surveyed in some years (Table 1, Figure 1). The central SA region was surveyed three times at 5-yr intervals in 1998, 2003, and 2008 (Table 1, Figure 2). Of the total fields surveyed, rigid ryegrass was collected from 1,252 fields. Fields were randomly chosen within key grain-cropping districts in SA and Vic. (Figure 1). The same fields were not surveyed each time. Sampling was performed just before crop harvest once rigid ryegrass seed had matured. The dates the surveys were conducted varied between years on the basis of the season and the location of the region(s) surveyed. Within each district, fields were selected by traveling for a predetermined distance (5 or 10 km) on minor roads where possible. At each stop, a single field was surveyed. Rigid ryegrass sampling of mature spikes commenced 10 m in from the edge of the crop and continued in an inverted W pattern through at least 1 ha of the field. When a large patch of rigid ryegrass plants was encountered, only up to 20 spikes were collected from the patch. Sampling was discontinued once

Figure 1. Map of surveyed regions across southeastern Australia showing the location of surveyed fields where rigid ryegrass populations were collected for herbicide resistance testing. Names in capitals are the main agronomic regions with the subregions in small font.

about 100 spikes had been collected or after 30 min, whichever occurred first. Fields that had been harvested were still sampled since rigid ryegrass plants with intact seed heads were often found lying prostrate on the soil surface. Seed heads were placed in labeled paper bags and the location of each sample noted either by distance from the previous location (in the 1998 and 2003 SA surveys) or in later surveys by a global positioning system (GPS) navigational unit recording longitude and latitude. The GPS positions of the earlier surveys were identified using Google Earth (Anonymous 2011) to identify the fields sampled. The populations were kept in a covered enclosure and subjected to fluctuating summer temperatures for 4 mo to allow complete after-ripening of the seed. The seed was subsequently threshed from the heads and stored at room temperature until tested.

POST Resistance Screening. Rigid ryegrass seed (0.2 g = 50 to 60 seeds) from the survey samples, as well as two standard susceptible populations, SLR4 and VLR1 (McAlister et al. 1995) and two standard herbicide-resistant populations, SLR31 (Holtum et al. 1991; McAlister et al. 1995) and L322 (confirmed in dose–response pot trials to ACCase- and ALS-inhibiting herbicides), was sown directly into 0.55-L square pots, covered with 5 mm of cocoa peat potting mix (cocoa peat potting mix produced by mixing 540 L of coco peat, 220 L of water, and 60 L of sand before steaming for 1 h). The following additives were then mixed to the pasteurized mix: 180 g of Dolomite lime; 600 g of agricultural lime; 240 g of hydrated lime; 180 g of gypsum; 180 g of superphosphate; 450 g of iron sulfate; 30 g of iron chelate; 180 g of micromax trace elements; 450 g of calcium nitrate, and 1,800 g of Osmocote mini 3 to 4 m (16-3-9+te), and watered. Each sample was sown into separate pots, transferred outdoors, and placed on the soil surface. One pot per herbicide treatment including an untreated check was sown. Pots were watered as required. Each trial was conducted in mid-autumn under environmental conditions grain growers would normally be using when applying herbicides in the field. Before treatment, the number of seedlings per pot was counted. Chlorsulfuron (Glean, 750 g kg^{-1} chlorsulfuron, DuPont) at 20 g ai ha^{-1} was applied to rigid ryegrass at the Z11 growth stage, whereas diclofop-methyl (Hoegrass 500, 500 g L^{-1} diclofop-methyl, Bayer CropScience) at 500 g ai ha^{-1}, tralkoxydim (Achieve WG, 400 g kg^{-1} tralkoxydim, Crop Care) at 160 g ai ha^{-1}, pinoxaden (Axial 100EC, 100 g L^{-1} pinoxaden, Syngenta) at 30 g ai ha^{-1}, and clethodim (Select, 240 g L^{-1} clethodim, Sumitomo) at 60 g ai ha^{-1} were applied at growth stage Z12 to Z13 (Zadoks et al. 1974). Herbicide was applied using a laboratory herbicide applicator with a twin-nozzle (Hardi ISO F-110-01 standard flat fan, Hardi, Adelaide) moving boom situated

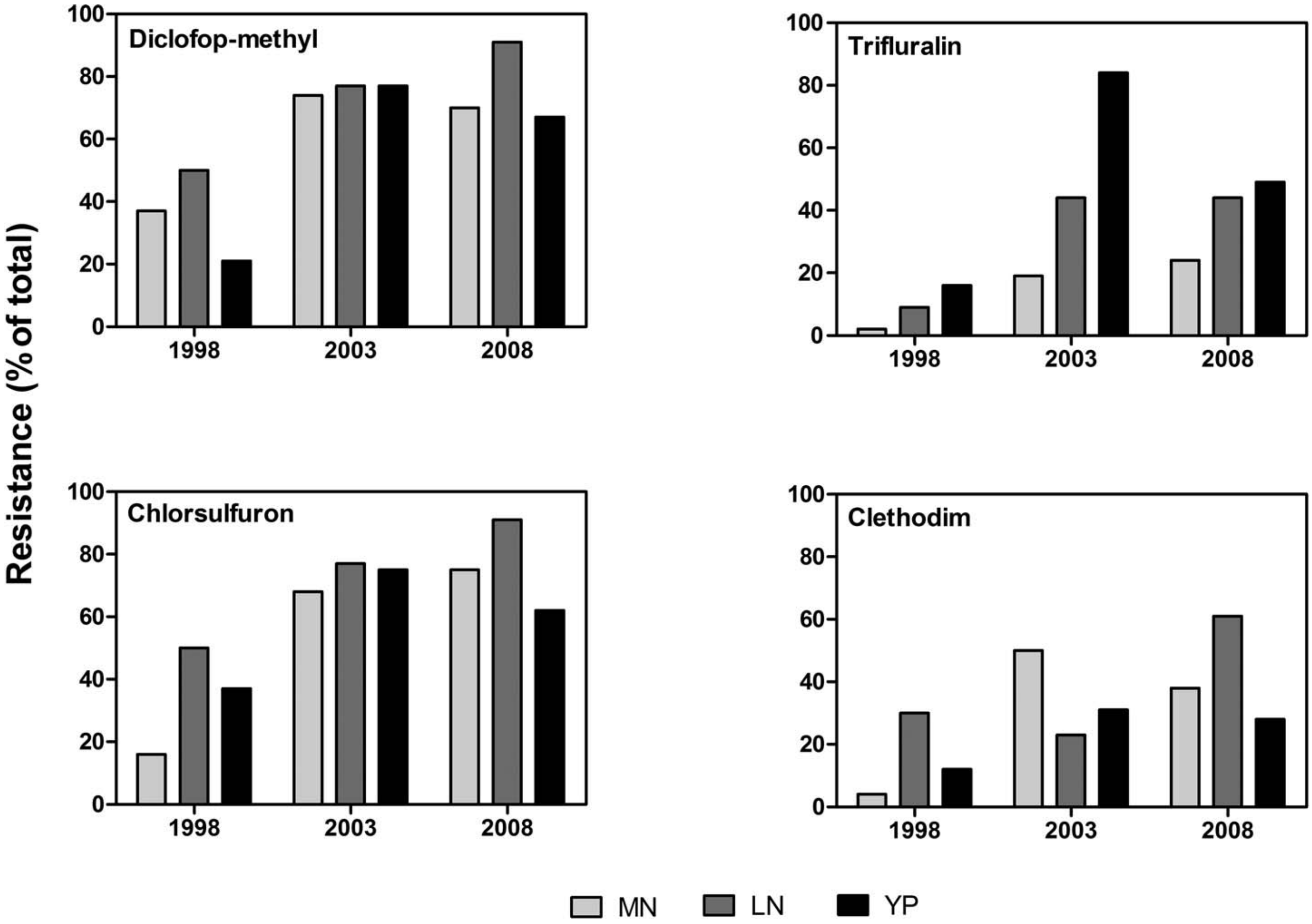

Figure 2. Change in the incidence of resistance in rigid ryegrass to trifluralin, diclofop-methyl, chlorsulfuron, and clethodim between 1998 and 2008 in central SA. The three regions are abbreviated as YP (Yorke Peninsula), MN (mid-north), and LN (lower north). The same subregion (YP, MN, and LN) was surveyed at 5-yr intervals, no effort made to survey the same fields. The fields were chosen at random at 5-km intervals. Resistance is defined as populations with 20% survival or greater to each herbicide.

40 cm above the pots and delivering 103 L ha^{-1} at 1 m s^{-1} and 250 kPa. Nonionic surfactant (alcohol alkoxylate, BS1000, Crop Care) was added to diclofop-methyl and chlorsulfuron at 0.2% (v/v), whereas crop oils were added to the remaining herbicides as recommended by the herbicide manufacturers. Ethyl and methyl esters of vegetable oil (Hasten, Victorian Chemicals) at 1% (v/v) were added to clethodim, 1% (v/v) mineral oil (mineral oil, Supercharge, Crop Care) was added to tralkoxydim, and 0.5% (v/v) methyl esters of canola oil fatty acids (Adigor, Syngenta) were added to pinoxaden. Survival of plants in the pot tests was assessed 21 to 28 d after treatment by counting the surviving plants and dividing by the total number of plants that were present in each pot before herbicide treatment.

PRE Resistance Screening. Every rigid ryegrass seed sample that was tested with POST-applied herbicides was also tested with trifluralin in a PPI trial. Each trial was conducted at the same time as the POST-applied trials. Seeds from each sample were weighed (0.2 g = 50 to 60 seeds) and sprinkled onto the surface of cocoa peat potting soil. Trifluralin (Triflur X, 480 g L^{-1} trifluralin, Nufarm) at 200 g ai ha^{-1} was applied directly onto the seed with the same laboratory herbicide applicator and the same conditions as described above. The seed was then covered with cocoa peat potting mix to a depth of 5 mm and watered. A control pot for each sample was also sown and was not sprayed with trifluralin. In every trial, this application system resulted in the total control of the susceptible population with 200 g ha^{-1} trifluralin. Assessment of seedling emergence was made 28 d after herbicide application, with the number of emerged seedlings in the trifluralin-treated pots compared with the seedlings that emerged in the control pots. Seedlings were considered emerged if they had reached the Z12 to Z13 growth stage 28 d after treatment. Populations were classed as resistant if emergence after trifluralin application was at least 20% of the emergence in the control pots. On each occasion, VLR1 and the resistant biotype SLR31,which has moderate resistance to trifluralin, were included (McAlister et al. 1995).

Statistical Analysis. For each experiment the pots were randomized. Each seed sample (representing a single field) was

Table 2. The incidence of herbicide-resistant rigid ryegrass populations (% of total) detected in the major winter cropping zones in southeastern Australia.[a,b,c]

Region	Year	Trifluralin	Diclofop-methyl	Chlorsulfuron	Tralkoxydim	Pinoxaden	Clethodim
South Australia							
Mallee	2007	19	6	67	2	2	2
Northern mallee		5	2	75	2	2	2
Southern mallee		35	12	59	2	2	2
Southeast	2007	39	60	69	50	53	41
Upper southeast		43	60	71	50	53	43
Lower southeast		12	62	58	52	52	29
Central	2008	40	76	73	64	59	40
Mid-north		24	70	75	63	60	38
Lower north		44	91	81	76	66	61
Yorke Peninsula		49	67	62	53	49	28
Eyre Peninsula	2009	5	30	78	29	30	11
Upper Eyre Peninsula		1	2	71	5	3	0
Lower Eyre Peninsula		10	66	87	60	64	25
Kangaroo Island	2009	0	46	67	55	50	9
Victoria							
Western	2005	5	35	57	28	30	12
Wimmera		2	60	60	55	54	26
Mallee		7	12	54	3	7	0
Northern	2006	2	40	43	nt	34	11
North central		2	18	19	nt	6	4
Northeast		2	63	68	nt	63	18
Southern	2009	0	79	88	84	68	23

[a] Resistant populations were defined as those with 20% survival or greater.

[b] The data represent the percentage of resistant populations of the total fields that contained ryegrass.

[c] Abbreviation: nt, not tested.

represented by a single pot, whereas five pots of the standard resistant and susceptible populations were sown. Populations were classed as resistant if at least 20% of seedlings survived each herbicide treatment. Populations with survival less than 20% were scored as nonresistant.

The independence of resistance to herbicides among populations across the whole data set was analyzed using a *G*-test (Sokal and Rohlf 1981). The null hypothesis tested was that resistance to any one herbicide was independent of resistance to other herbicides. That is, populations resistant to one herbicide would have a distribution of resistance to all other herbicides that was the same as the data set as a whole. The *G*-test is a contingency test comparing observed frequencies with expected frequencies (Sokal and Rohlf 1981). The *G* statistic and probabilities were calculated using the method described in Sokal and Rohlf (1981).

Results and Discussion

Fields in key grain-growing regions were surveyed between 1998 and 2009. The location of each field in surveys that were conducted annually from 2005 to 2009 is shown in Figure 1. Rigid ryegrass was detected in 70 to 96% of fields across both states (Table 1). Cereals dominated the crop types, with 47 to 87% of the fields visited containing wheat, 5 to 24% containing barley, and 8 to 38% containing a pulse or canola crop (Table 1). The lower rainfall zones, such as the SA mallee and Eyre Peninsula, were dominated by cereal crops, whereas regions with more consistent and higher rainfall, such as southern Vic, Kangaroo Island, and southeast SA, contained at least one-third pulse or canola crops (Table 1).

Resistance to Sulfonylurea Herbicides. The greatest incidence of resistance in rigid ryegrass across all regions was to chlorsulfuron (Table 2). All major cropping regions in SA and Vic, irrespective of whether they were marginally or intensively cropped, contained a high level of resistance to chlorsulfuron (43 to 88%; Figure 1, Table 2). The cost effectiveness of sulfonylurea herbicides and their broad-spectrum weed control has contributed to the widespread resistance detected across southeastern Australia. In addition, the high frequency of ALS-inhibiting herbicide-resistant individuals that occurs naturally in weed populations accounts for the accelerated evolution of resistance (Preston and Powles 2002). The presence of widespread resistance to sulfonylurea herbicides as identified here suggests that these herbicides are likely to be ineffective in controlling rigid ryegrass across southerneastern Australia. Information from weed surveys conducted in New South Wales and Western Australia has also confirmed that chlorsulfuron resistance in rigid ryegrass was the most common form of resistance (Broster et al. 2011; Owen et al. 2007). In contrast, higher resistance levels to ACCase-inhibiting herbicides compared with sulfonylureas was reported in a survey of Italian ryegrass in northern Idaho and eastern Washington, as a result of the greater use of ACCase-inhibiting herbicides in those regions (Rauch et al. 2010).

Resistance to Dinitroaniline Herbicides. Two rates were applied for the trifluralin test: 0 and 200 g ha^{-1}. Using the

Table 3. *G* statistic (upper value) and probability (lower value) for *G*-test of whether resistance to the two herbicides was randomly distributed among populations of rigid ryegrass collected across South Australia and Victoria combined for individual pairs of herbicides.

		Second herbicide				
First herbicide		Tralkoxydim	Pinoxaden	Clethodim	Chlorsulfuron	Trifluralin
Diclofop-methyl	*G* P	533, 3×10^{-115}	498, 2×10^{-107}	237, 5×10^{-51}	68, 10×10^{-14}	44, 2×10^{-9}
Tralkoxydim	*G* P		555, 7×10^{-120}	250, 6×10^{-54}	59, 8×10^{-13}	34, 2×10^{-7}
Pinoxaden	*G* P			240, 8×10^{-52}	85, 3×10^{-18}	23, 4×10^{-5}
Clethodim	*G* P				49, 1×10^{-10}	30, 1×10^{-6}
Chlorsulfuron	*G* P					9 3, $\times 10^{-2}$

testing procedure, volatilization losses were minimized by the immediate and even incorporation of trifluralin. This technique closely mimics normal no-till (low disturbance) use of trifluralin in Australian grain crops where the herbicide is applied to weed seeds and soil, and then incorporated by the seeding operation (Chauhan et al. 2006, 2007). However, under the conditions of this pot assay, trifluralin activity was greater than in the field because of the absence of stubble, minimal volatilization losses, and greater herbicide–seed contact than is usually achieved in the field.

The greatest incidence of trifluralin resistance in rigid ryegrass was in SA and the lowest was in Vic, with the percentage resistance ranging from 0% in southern Vic and Kangaroo Island to 49% in the populations from the Yorke Peninsula (Table 2). The incidence of trifluralin resistance in the southern mallee and upper southeast of SA was similar (35% and 43%, respectively; Figure 1, Table 2). Both districts encompass a similar region in eastern SA where broadleaf crops are an important component of the cropping rotation. Restrictions in recropping intervals from sulfonylurea herbicide residues (Brown 1990) and high levels of resistance to ACCase-inhibiting herbicides have resulted in the heavy reliance on trifluralin as an alternative MOA herbicide for rigid ryegrass control in numerous cereal and broadleaf crops. In areas where the percentage of fields with resistance to ACCase-inhibiting herbicides and trifluralin was low, such as in the northern SA mallee (2% and 5%, respectively), upper Eyre Peninsula (2% and 1%, respectively), Vic mallee (12% and 7% respectively), and north-central Vic (18% and 2%, respectively), rotations including these two MOA groups are still predominately effective (Table 2).

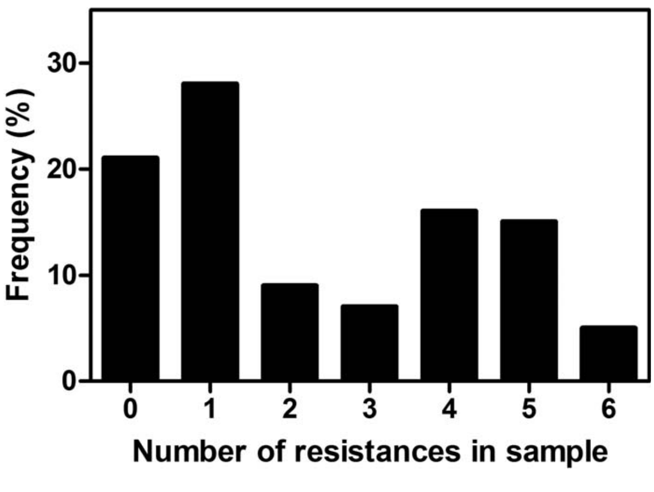

Figure 3. Percentage of rigid ryegrass populations from the 2005–2009 survey collections that were resistant to none, one, or up to six of the following herbicides: diclofop-methyl, chlorsulfuron, tralkoxydim, pinoxaden, clethodim, trifluralin. Resistance is defined as populations with 20% survival or greater to each herbicide.

Weed Surveys from Other Key Cereal-Growing States. Western Australia and New South Wales have identified low levels of trifluralin resistance (0.2 to 6% of populations) (Broster et al. 2011; Owen et al. 2007). A key factor for the high incidence of trifluralin resistance in certain regions of SA can be attributed to the wide distribution of alkaline soils, as detailed in the Atlas of Australian Soils (Anonymous 1968; Hollaway et al. 2006a; Stork 1995). Degradation of sulfonylurea herbicides has been reported as significantly slower in alkaline soils than in neutral or acidic soils, which can lead to damage in sensitive crops that form an integral component of cropping rotations (Brown 1990; Hollaway et al. 2006b). Strict guidelines on minimum recropping intervals have been imposed on sulfonylurea herbicide product labels. As a result, a large proportion of farmers in SA have continued using trifluralin, in addition to sulfonylureas, which has contributed to the high resistance incidence to both herbicide groups.

Resistance to ACCase-Inhibiting Herbicides. In contrast to chlorsulfuron resistance, which was detected at significant levels throughout the entire survey region, considerable variation in resistance to ACCase-inhibiting herbicides was identified between regions. In marginal cropping areas, such the northern SA mallee and upper Eyre Peninsula, less than 5% of populations tested exhibited resistance to diclofop-methyl, whereas in most other regions resistance to diclofop-methyl was greater than 60% (Table 2).

Populations resistant to one herbicide were more likely to be resistant to a second herbicide with the same MOA. Rigid ryegrass that exhibited resistance to one ACCase-inhibiting herbicide, such as diclofop-methyl, tralkoxydim, or pinoxaden, was often resistant to the other two, a finding reported previously (Ruchs et al. 2006; Table 2). This strong relationship is illustrated by the most significant probability values, which ranged between 2×10^{-107} and 7×10^{-120} (Table 3). These findings indicate a common mechanism of resistance, most likely ACCase target-site resistance (Yu et al. 2007), that is contributing to the strong relationship between these herbicide pairs. A high incidence of cross-resistance can lead to reduced efficacy by new herbicides with the same MOA. In Australia, pinoxaden was first registered in cereal crops in 2006; however, 40 and 30% of the fields surveyed before 2006 in the 2003 and 2005 surveys, respectively, contained pinoxaden-resistant rigid ryegrass (Table 2). This highlights that cross-resistance in rigid ryegrass can reduce the effectiveness of herbicides that have not been exposed to rigid ryegrass in commercial situations.

In certain situations, diclofop-methyl-, tralkoxydim- and, pinoxaden-resistant populations were also resistant to clethodim. The relationship between resistance to clethodim and to diclofop-methyl, pinoxaden, and tralkoxydim was, however, not as strong as indicated by lower probability values ranging between 5×10^{-51} and 6×10^{-54} (Table 3). In clethodim-resistant rigid ryegrass, molecular studies have shown that a resistant ACCase target site was responsible and that biotypes resistant to clethodim were also resistant to tralkoxydim and pinoxaden (Yu et al. 2007). For populations resistant to diclofop-methyl, tralkoxydim, and pinoxaden, but not to clethodim, resistance is likely to be endowed by a mutation(s) that does not confer resistance to clethodim. This property has been identified in rigid ryegrass biotypes possessing the Gly-2096-Ala mutation that confers resistance to some cyclohexanedione and phenylpyrazole herbicides, but does not confer field-level resistance to clethodim (Delye 2005). However, the involvement of nontarget-site resistance cannot be discounted (Preston et al. 1996; Preston and Powles 1998; Tardif and Powles 1994).

Multiple Resistance. In only 21% of all fields sampled across all the survey regions was no resistance detected in rigid ryegrass and a further 28% of fields contained rigid ryegrass with resistance to one of the tested herbicides (Figure 3). All other fields contained rigid ryegrass with resistance to more than one herbicide. Approximately 35% of rigid ryegrass populations exhibited resistance to four or more herbicides (Figure 3). The distribution of fields by numbers of herbicides with resistance was bimodal (Figure 3). Fields were likely to have resistance to less than two herbicides or four or more herbicides. This pattern is likely to be a reflection of intensity of herbicide use. In marginal regions such the northern SA mallee and upper Eyre Peninsula fewer herbicides are used, with these regions accounting for many of the fields where resistance to fewer than two herbicides was detected (Table 1). Fields in regions with greater intensity of crop production are more likely to have resistance to multiple herbicides.

Rigid ryegrass biotypes with resistance to different MOA herbicides such as trifluralin, chlorsulfuron, and ACCase-inhibiting herbicides (diclofop-methyl, tralkoxydim, pinoxaden, and clethodim) are likely to possess different mechanisms that confer resistance to each herbicide MOA group. Rigid ryegrass biotypes have been confirmed that possess multiple mechanisms of resistance in the same individual, such as target-site resistance and nontarget-site resistance, in most cases the latter being enhanced metabolism (Preston et al. 1996; Tardif and Powles, 1994). In contrast, rigid ryegrass biotypes have been confirmed to exhibit resistance to herbicides from different MOA herbicides due to a single mechanism, commonly enhanced metabolism (Christopher et al. 1991; Preston et al. 1996). One such biotype is rigid ryegrass biotype SLR31, which has been confirmed as resistant to diclofop-methyl and chlorsulfuron due to enhanced metabolism (Christopher et al. 1991). McAlister et al. (1995) suggested that enhanced metabolism may also be responsible for the trifluralin resistance that biotype SLR31 exhibits. These findings suggest that for rigid ryegrass biotypes with target-site resistance to ACCase- or ALS-inhibiting herbicides, it is also possible that these biotypes possess enhanced metabolism as a secondary mechanism.

Changes in Herbicide Resistance. The central SA region was surveyed in 1998, 2003, and 2008 (Figure 2). Over this decade, the greatest increase in herbicide resistance occurred between 1998 and 2003. Diclofop-methyl was the first cereal-selective POST herbicide registered for rigid ryegrass control in 1978. Subsequently, chlorsulfuron, the first sulfonylurea herbicide to be registered in Australia, became available in 1982. Both of these herbicides were registered for selective control of rigid ryegrass in cereals. In the 1998 survey, 38% of populations were identified as resistant to diclofop-methyl and 22% to chlorsulfuron (Figure 2). The rapid adoption of both herbicides was a key factor in the evolution of herbicide resistance as identified in the 1998 survey. Longer exposure to diclofop-methyl is likely to have contributed to the greater resistance detected than to chlorsulfuron. In addition, 9% of fields exhibited resistance to trifluralin (registered in 1975) and 19% to clethodim (registered in 1992). In 2003, resistance to diclofop-methyl and clethodim doubled, whereas there was a 5-fold increase in resistance to trifluralin and a 3.5-fold increase in chlorsulfuron resistance. These increases suggest a heavy reliance on sulfonylurea herbicides and trifluralin to control rigid ryegrass due to the reduced effectiveness of diclofop-methyl in earlier years. In addition, restrictions in broadleaf crop rotations due to persistent sulfonylurea residues in alkaline soils, which dominate central SA, is likely to have increased reliance on trifluralin as the dominant herbicide for rigid ryegrass control in cereals. Consequently, trifluralin resistance was detected in 49% of fields surveyed in 2003. No increase in trifluralin resistance was, however, detected between 2003 and 2008, suggesting that the adoption of integrated weed management (IWM) involving the use of new MOA herbicides, such as prosulfocarb, was effective in reducing the incidence of resistance. A 12% reduction in the incidence of clethodim resistance in the mid-north region between 2003 and 2008 is probably related to increasing use rates for clethodim, leading to greater weed control. In addition, lower rainfall between 2006 and 2008 in this region is likely to have had an impact on fewer broadleaf crops grown and therefore less use of clethodim, resulting in reduced selection pressure.

The results of these surveys have highlighted that herbicide resistance in rigid ryegrass is likely to significantly affect weed control across southeastern Australia. This is of particular concern in intensively cropped areas where the majority of fields were infested with rigid ryegrass with complex resistance patterns to both ACCase- and ALS-inhibiting herbicides. A significant proportion of rigid ryegrass populations from SA also possessed resistance to trifluralin, further restricting herbicide options. Across southeastern Australia, no-till farming is the dominant method used to sow crops with minimal soil disturbance. Thus, the lack of mechanical weed control imposes greater pressure on herbicides to control rigid ryegrass. For this reason farmers are encouraged to incorporate

IWM strategies, irrespective of whether they have resistance or not. IWM strategies include techniques such as using nonselective herbicides presowing, hay, silage, preventing seed-set by late application of nonselective herbicides in pulse crops, and removing weed seeds during the harvest. In addition, the availability of new MOA herbicides such as prosulfocarb (registered in 2008) and pyroxasulfone (registered in 2012) will provide additional options for managing herbicide-resistant rigid ryegrass (Matthews 1994; Ruchs 2008).

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Literature Cited

Anonymous. 1968. The soil map of South Australia is a simplified version of The Atlas of Australian Soils (Division of Soils of the CSIRO, Ninth International Congress of Soil Science, Adelaide).

Anonymous. 2008. Australian Bureau of Statistics. Year Book Australia. Canberra. Number 90, Pp. 477–508.

Anonymous. 2011. http://www.google.com/earth/index.html. Accessed: March 2, 2011

Boutsalis, P. and J. C. Broster. 2006. Herbicide resistance in *Lolium rigidum* by commercial institutions. Pages 488–490. *in* C. Preston, J. H. Watts and N. D. Crossman, eds. Fifteenth Australian Weeds Conference. Adelaide, South Australia: Weed Management Society of South Australia, Adelaide.

Broster, J. C., E. A. Koetz, and H. Wu. 2011. Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. Aust. J. Plant Prot. Quart. 26:22–28.

Broster, J. C. and J. E. Pratley. 2006. A decade of monitoring herbicide resistance in Lolium rigidum in Australia. Aust. J. Exp. Agric. 46:1151–1160.

Brown, H. M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29:263–281.

Chauhan, B. S., G. S. Gill, and C. Preston. 2006. Tillage system effects on weed ecology, herbicide activity and persistence: a review. Aust. J. Exp. Agric. 46:1557–1570.

Chauhan, B. S., G. S. Gill, and C. Preston. 2007. Effect of seeding systems and dinitroaniline herbicides on emergence and control of rigid ryegrass (*Lolium rigidum*) in wheat. Weed Technol. 21:53–58.

Christopher, J. T., S. B. Powles, D. R. Liljegren, and J.A.M. Holtum. 1991. Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*). Plant Physiol. 95:1036–1043.

Delye, C. 2005. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci. 53:728–746.

Heap, I. M. 2011. International survey of herbicide resistant weeds. http://www.weedscience.org. Accessed: May 24, 2011.

Heap, I. M. and R. Knight. 1982. A population of ryegrass tolerant to the herbicide diclofop-methyl. J. Aust. Inst. Agric. Sci. 48:156–157.

Heap, I. M. and R. Knight. 1986. The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. Aust. J. Agric. Res. 37:149–156.

Hollaway, K. L., R. S. Kookana, D. M. Noy, J. G. Smith, and N. Wilhelm. 2006a. Persistence and leaching of sulfonylurea herbicides over a 4-year period in the highly alkaline soils of southeastern Australia. Aust. J. Exp. Agric. 46:1069–1076.

Hollaway, K. L., R. S. Kookana, D. M. Noy, J. G. Smith, and N. Wilhelm. 2006b. Crop damage caused by residual acetolactate synthase herbicides in the soils of southeastern Australia. Aust. J. Exp. Agric. 46:1323–1331.

Holtum, J.A.M., J. M. Matthews, R. E. Hausler, D. R. Liljegren, and S. B. Powles. 1991. Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*): III. On the mechanism of resistance to diclofop-methyl. Plant Physiol. 97:1026–1034.

Jones, R. E., D. T. Vere, Y. Alemseged, and R. W. Medd. 2005. Estimating the economic cost of weeds in Australian annual winter crops. Agric. Econ. 32:253–265.

Llewellyn, R. and S. B. Powles. 2001. High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across the Western Australian wheatbelt. Weed Technol. 15:242–248.

Matthews, J. M. 1994. Management of herbicide resistant weed populations. Pages 317–335. *in* S. B. Powles and J.A.M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis Publishers.

McAlister, F. M., J.A.M. Holtum, and S. B. Powles. 1995. Dinitroaniline herbicide resistance in rigid ryegrass (*Lolium rigidum*). Weed Sci. 43:55–62.

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

Potter, T. D. and P. A. Salisbury. 1993. Triazine resistant canola in southern Australia. *In* Proceedings of the 7th Australian Agronomy Conference. Adelaide, South Australia: Australian Society of Agronomy.

Pratley, J. E., R. J. Graham, and A. R. Leys. 1993. Determination of the extent of herbicide resistance in Southern NSW. Pages 286–288. *in* Proceedings of the 10th Australian and 14th Asian-Pacific Weeds Conference: Weed Society of Queensland, Brisbane.

Preston, C. and S. B. Powles. 1998. Amitrole inhibits diclofop metabolism and synergises diclofop-methyl in a diclofop-methyl-resistant biotype of *Lolium rigidum*. Pestic. Biochem. Physiol. 62:179–189.

Preston, C. and S. B. Powles. 2002. Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. Heredity 88:8–13.

Preston, C., F. J. Tardif, J. T. Christopher, and S. B. Powles. 1996. Multiple herbicide resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pestic. Biochem. Physiol. 54:123–134.

Rauch, T. A., D. C. Thill, S. A. Gersdorf, and W. J. Price. 2010. Widespread occurrence of herbicide resistant Italian ryegrass (*Lolium multiflorum*) in northern Idaho and eastern Washington. Weed Technol. 24:281–288.

Ruchs, C. 2008. Boxer® Gold, a flexible new pre-emergent herbicide alternative for the control of annual ryegrass (*Lolium rigidum* Gaudin) and toad rush (*Juncus bufonius* L.) in wheat and barley. Pages 291–293. *in* Proceedings of the 16th Australian Weeds Conference. Cairns, Queensland: Weed Society of Queensland, Brisbane.

Ruchs, C. A., P. J. O'Connell, and P. Boutsalis. 2006. Axial®, a cereal selective graminicide for the control of annual ryegrass (*Lolium rigidum* Gaudin) and other major grass weeds. Pages 838–841. *in* Proceedings of the 15th Australian Weeds Conference. Adelaide, South Australia: Weed Management Society of South Australia.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. 2nd ed. San Francisco: Freeman.

Stork, P. R. 1995. Field leaching and Degradation of soil applied herbicides in a gradationally textured alkaline soil: chlorsulfuron and triasulfuron. Aust. J. Agric. Res. 46:1445–1458.

Tardif, F. J. and S. B. Powles. 1994. Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: isolation of a subset with resistant acetyl-CoA carboxylase. Physiol. Plant. 91:488–494.

Yu, Q., A. Collavo, M. Q. Zheng, M. Q. Owen, M. Sattin, and S. B. Powles. 2007. Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: Evaluation using clethodim. Plant Physiol. 145:547–558.

Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415–421.

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