Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales

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Summary
In 2007 a random survey was conducted across the cereal cropping zone of southern New South Wales to determine the level of herbicide resistance in annual ryegrass populations. In total, 181 paddocks were visited resulting in 137 samples of annual ryegrass seed collected for testing. These samples were then screened to the commonly used herbicide groups (A, B, C, D and M) for annual ryegrass control in Australia. The majority of samples were resistant to Group A 'fop' (81%) and Group B 'SU' (70%) and 'Imi' (65%) herbicides. These represented increases from the 10–14% experienced in the previous survey in 1991. Seventy six percent of the 117 samples tested to five herbicide Groups (A 'fop, A 'dim, B, C or D) were resistant to two or more of the herbicide groups tested and only 9% were susceptible to all herbicides. Of particular interest is the minimal increase in resistance to simazine and trifluralin in the 16 years since the last survey, and only low incidence of glyphosate resistance was identified in this survey. The rapid increase in the incidence of herbicide resistance in annual ryegrass, particularly in Group A and Group B herbicides, highlights the importance of adopting an integrated approach in weed management. This integrated approach is also necessary for maintaining the low level of resistance in Groups C, D and M, and extending the commercial life of these effective chemicals.

Introduction
Herbicide resistant weeds are a major problem in the cropping regions of Australia. In the southern cropping region of New South Wales annual ryegrass (*Lolium rigidum* Gaud.) is considered to be the weed of greatest importance (Lemerle *et al.* 1996). Annual ryegrass is a highly genetically variable cross-pollinating species which is present in high numbers across the southern Australian cereal zone as a result of its suitability to the climate, extensive use as a pasture species and high seed production (Gill 1996). The pollen produced by this species can move large distances via the wind, at least three kilometres, and while this may be of importance in the spread of rare herbicide resistance genes (e.g. glyphosate) (Busi *et al.* 2008) normally the more common forms of herbicide resistance develop *in situ* through repeated exposure to herbicides.

Annual ryegrass is commonly controlled by a number of different herbicides, many of which can be used in both the cropping and pasture phases of a rotation. The increased uptake of minimum tillage and stubble retention in many regions has increased the emphasis on herbicides for annual ryegrass control. Since the release of the first Group A 'fop' herbicide (diclofop-methyl) in 1978 and Group B herbicide (chlorsulfuron) in 1982 these and other herbicides within the same herbicide groups have been extensively used for annual ryegrass control.

As a result of the extensive use of these herbicide groups for annual ryegrass control, resistance to these and other herbicide groups was quick to develop in Australia. These include Group A, both 'fop' (aryloxyphenoxypropionate) and 'dim' (cyclohexandione) (Heap and Knight 1982, Heap and Knight 1986), Group B (sulfonylurea and imidazolinone) (Matthews *et al.* 1990, Powles and Howat 1990, Christopher *et al.* 1991), Group C (triazine) (Burnet *et al.* 1991, Pratley *et al.* 1991), Group D (dinitroaniline) (Pratley *et al.* 1991, McAlister *et al.* 1995) and Group M (glycine) (Pratley *et al.* 1996, Powles *et al.* 1998, Pratley *et al.* 1999). Annual ryegrass populations have also developed complex resistance patterns, both cross and multiple resistance, to several different herbicide groups (Heap and Knight 1986, Christopher *et al.* 1992, Powles *et al.* 1997, Broster and Pratley 2006).

While time consuming and expensive, identifying the underlying levels of resistance across large areas is important in better understanding herbicide resistance and in designing integrated weed management strategies. Information gained from surveys can aid in the planning of herbicide resistance research and extension for specific areas, as growers with high levels of resistance may have different information needs compared to those who do not (Llewellyn and Powles 2001).

A previous survey conducted in 1991 established baseline levels for herbicide resistance in southern new South Wales (Pratley *et al.* 1993). However, as annual ryegrass continues evolving in the presence of herbicides, these surveys need to be conducted regularly. Two surveys of the Western Australian wheat belt, conducted in 1998 and 2003 showed marked changes in the level of herbicide resistance in the five year period (Llewellyn and Powles 2001, Owen *et al.* 2007). As no survey had been conducted in southern New South Wales in the 16 years since the 1991 survey, the changes in resistance levels could be greater than experienced between the two Western Australian surveys.

This paper reports the findings of a random survey conducted across the southern New South Wales cereal belt in 2007 to determine the extent and distribution of resistance in annual ryegrass samples to commonly used herbicides. Changes in the extent of herbicide resistance since the last survey (Pratley *et al.* 1993) was conducted in this region are also quantified.

Materials and methods
Sample collection
Cropping paddocks in southern New South Wales were surveyed over a four week period in November and December 2007 prior to the commencement of harvest (Figure 1). Paddocks were randomly selected at ten kilometre intervals, alternating left and right hand side of the survey transects where possible. The location of all sites was recorded using a GPS unit. Seed was unable to be collected from some areas surveyed as seasonal conditions had resulted in crop failure.

The paddocks were surveyed by two people walking across them for a ten to fifteen minute period. Mature seed heads were collected from plants along the sampling path. After collection the samples obtained by the two people were bulked to obtain a single sample for the paddock. In total 181 paddocks were visited of which 137 contained sufficient annual ryegrass plants to allow for resistance screening. Immediately after collection the seed samples were stored in a glasshouse until February 2008 when they were threshed and cleaned.

Resistance screening
In August 2008 0.2 g of seed for each of the 137 samples were planted in plastic punnet trays (330 mm × 280 mm × 60 mm). Each tray contained 14 different samples sown in rows 25 mm apart and 5 mm deep and then covered. The trays were filled with either a 50:50 peat:sand mix or

a soil mix (50:50 loam:river wash sand) depending upon the herbicide to be applied. For all herbicides except for imazapic/impazapyr the seedlings were sown in the peat:sand mix. Trays were kept in a temperature controlled glasshouse (10°C minimum, 25°C maximum) and were watered and fertilized as required. Two weeks after sowing all samples in the post-emergent herbicide treatments were counted and thinned to a maximum of 20 plants per sample. Three replicates were sown for all samples except where seed numbers were limited.

The ryegrass samples were screened with five post-emergent herbicides across Groups A, B, and M. Diclofop-methyl, clethodim, glyphosate and imazapic/impazapyr were all applied when the plants were at growth stage Z12–13 (Zadoks *et al*. 1974). Seedlings which survived the diclofop-methyl application were sprayed with sethoxydim. Seedlings which survived the glyphosate application were then sprayed at twice the rate of the first screening to confirm resistance.

Three pre-emergent herbicides, chlorsulfuron, simazine and trifluralin (Groups B, C and D) were screened in this survey. For all three herbicides the seeds were sown in the soil mix. For both the chlorsulfuron and simazine treatments, the seeds were sown in the rows, covered with 5 mm of the soil mix and sprayed with the herbicide and the herbicide watered in. For the trifluralin treatment the trays were sprayed then raked to incorporate the herbicide, the seed was then sown in rows on top of the herbicide and covered with 5 mm of soil.

The herbicide resistance testing protocol was adopted from Broster and Pratley (2006) although for this experiment herbicides were only applied at the label recommended rate (Table 1). All herbicides were applied at the recommended stage of growth using an automated laboratory-sized cabinet sprayer with a moving boom applying a water volume of 77 L ha^{-1} from a flat fan nozzle at 300 kPa pressure. Adjuvants were added to herbicides as required by label requirements (Table 1). A standard susceptible biotype and a known resistant biotype, where available, were included with each cohort of samples. Due to limited seed availability for some samples not all of the 137 samples were screened to all herbicides, or for all replicates.

Herbicide evaluation

All samples were assessed between 21 and 28 days after treatment. Seedlings in post-emergent treatments were counted before and after treatment to enable survival percentages to be calculated. Samples sprayed pre-emergent were rated visually from 0 (no germination) to 10 (no visual difference from susceptible control). Results were analysed by ANOVA using GenStat version 11.1 (GenStat 2008) and the standard error for each herbicide determined.

Samples were classified as resistant if the mean survival percentage for all replicates was greater than 20% for post-emergent herbicides or a visual score of greater

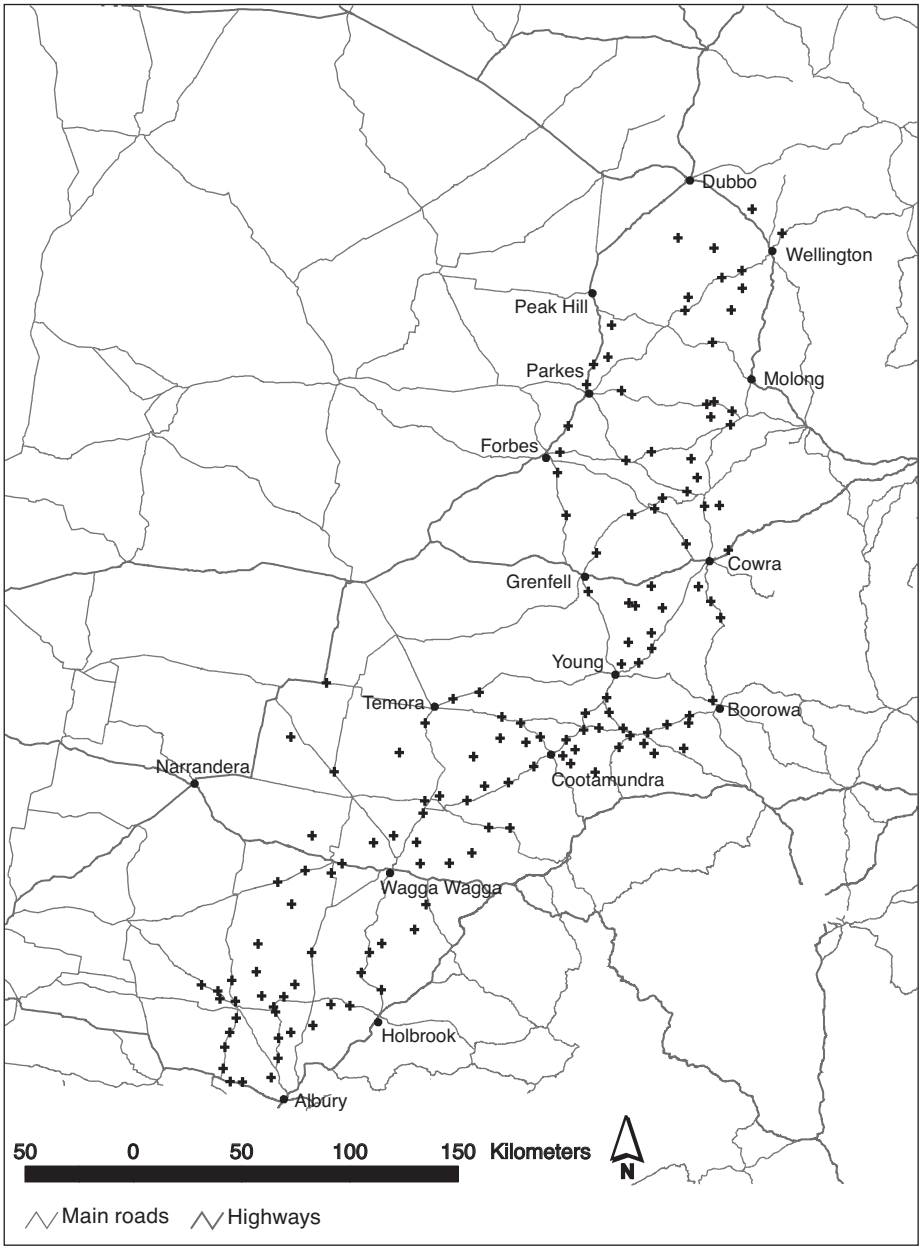

Figure 1. Annual ryegrass sample locations for the 2007 survey.

Table 1. Herbicides and rates used for annual ryegrass herbicide resistance screening.

Herbicide	Group	Rate (g a.i. ha^{-1})	Adjuvant	Adjuvant rate (% v/v)
Diclofop-methyl	A 'fop'	375	BS1000	0.25
Clethodim	A 'dim'	60	Hasten	1
Sethoxydim	A 'dim'	72	DC Trate	1
Chlorsulfuron	B 'SU'	15	-	–
Imazapic/imazpyr	B 'Imi'	28	Hasten	0.5
Simazine	C	1200	–	–
Trifluralin	D	800	–	–
Glyphosate	M	576	BS1000	0.1

than 2.5 for pre-emergent herbicides or a visual score higher than that of the susceptible control by twice the standard error (whichever was the greater) (Broster and Pratley 2006). Samples with survival percentages of between 10 and 19% for post-emergent herbicides or a visual score of between 1.5 and 2.5 for pre-emergent herbicides or a visual score higher than that of the susceptible control by the standard error (whichever was the greater) were classed as developing resistance. Samples were classed as susceptible if survival was less than 10% or a visual score of below 1.5 or a visual score less than the standard error higher than that of the susceptible control (whichever was the greater).

Results

Resistance to diclofop-methyl was common among samples collected for this survey. Of the 134 samples screened to diclofop-methyl, 81% (109 samples) were classed as resistant or developing resistance (Table 2). Upon screening to sethoxydim, 43% of the diclofop-methyl resistant samples were also resistant or developing resistance to sethoxydim, equating to 35% of samples with target site resistance to Group A herbicides. Twenty one percent of the samples were resistant or developing resistance to clethodim.

No samples susceptible or developing resistance to diclofop-methyl were resistant to clethodim. Nineteen samples classified as resistant to diclofop-methyl and developing resistance to sethoxydim were classed as susceptible to clethodim. Two samples classified as resistant to diclofop-methyl and developing resistance to clethodim were classed as susceptible to sethoxydim (Table 3). Of the nine resistance combinations possible among samples resistant to diclofop-methyl, eight were identified in this survey. Among the 94 samples tested resistant to diclofop-methyl, 46 samples (49%) were susceptible to both dim herbicides (clethodim and sethoxydim), 23 samples (24%) were susceptible to either clethodim or sethoxydim, and 25 samples (27%) were cross-resistant to both clethodim and sethoxydim.

In total, 120 samples were screened to two Group B herbicides; one, chlorsulfuron was a sulfonylurea (SU) and the other imazapic/impazapyr, an imidazolinone (imi) herbicide. Similar levels of resistance were found to both herbicides, 70% compared to 65%. A higher proportion of samples were classed as developing resistance when screened to chlorsulfuron, this resulted from increased variability in the level of control in the three replicates leading to a higher threshold for classification as resistant (Table 2).

In the 120 samples screened to both chlorsulfuron and imazapic/impazapyr, 68 (57%) of the samples were resistant or developing resistance to both and 26 (22%) were susceptible to both herbicides. Sixteen samples (13%) were resistant or developing resistance to chlorulfuron but susceptible to imazapic/impazapyr while ten samples (8%) were susceptible to chlorulfuron but resistant or developing resistance to imazapic/impazapyr. Samples resistant to both herbicides were most likely to be found in the central or southern areas of the survey while the susceptible samples were located in the north and around Wagga Wagga (Figure 2).

The vast majority of samples was susceptible to the other three herbicides tested, simazine, trifluralin and glyphosate. Ninety four percent of samples were susceptible to trifluralin while for both simazine and glyphosate only one sample was resistant (1%) to each of these herbicides (Table 2).

Table 3. Relationships between the three Group A herbicides screened on 134 annual ryegrass populations (S – susceptible <10% survival; DR – developing resistance 10–20% survival; R – resistance >20% survival; n.t. – not tested).

Samples	Diclofop-methyl (A 'fop')	Clethodim (A 'dim')	Sethoxydim (A 'dim')	%
24	S	S	n.t	17.9
1	S	n.t	n.t	0.7
12	DR	S	S	9.0
2	DR	S	DR	1.5
1	DR	S	R	0.7
46	R	S	S	34.3
12	R	S	DR	9.0
7	R	S	R	5.2
2	R	DR	S	1.5
2	R	n.t.	S	1.5
0	R	R	S	0.0
2	R	DR	DR	1.5
8	R	DR	R	6.0
1	R	R	DR	0.7
14	R	R	R	10.4

Table 2. Resistance levels for the screened herbicides (R – resistant; DR – developing resistance; S – susceptible, TR – total resistant = resistant and developing resistant combined).

	Diclofop	Sethoxydim[A]	Clethodim	Chlorsulfuron	Imazapic /imazapyr	Simazine	Trifluralin	Glyphosate
R	94	30	15	59	68	1	2	1
DR	15	17	12	25	10	0	5	0
S	25	32	104	36	42	119	113	126
Tested	134	109	131	120	120	120	120	127
% TR	81	43	21	70	65	1	6	1

[A] sethoxydim was only screened to the diclofop resistant populations.

R: diclofop, sethoxydim, clethodim, imazapic-imazapyr, glyphosate survival >20%, simazine, trifluralin score >2.5, chlorsulfuron score >4.67 (2 × S.E. >susceptible control).

DR: diclofop, sethoxydim, clethodim, imazapic-imazapyr, glyphosate survival 10–20%, simazine, trifluralin score 1.5–2.5, chlorsulfuron score 4.0-4.67 (1 × S.E. >susceptible control).

S: diclofop, sethoxydim, clethodim, imazapic-imazapyr, glyphosate survival <10%, simazine, trifluralin score <1.5, chlorsulfuron score <4.0 (<1 × S.E. >susceptible control).

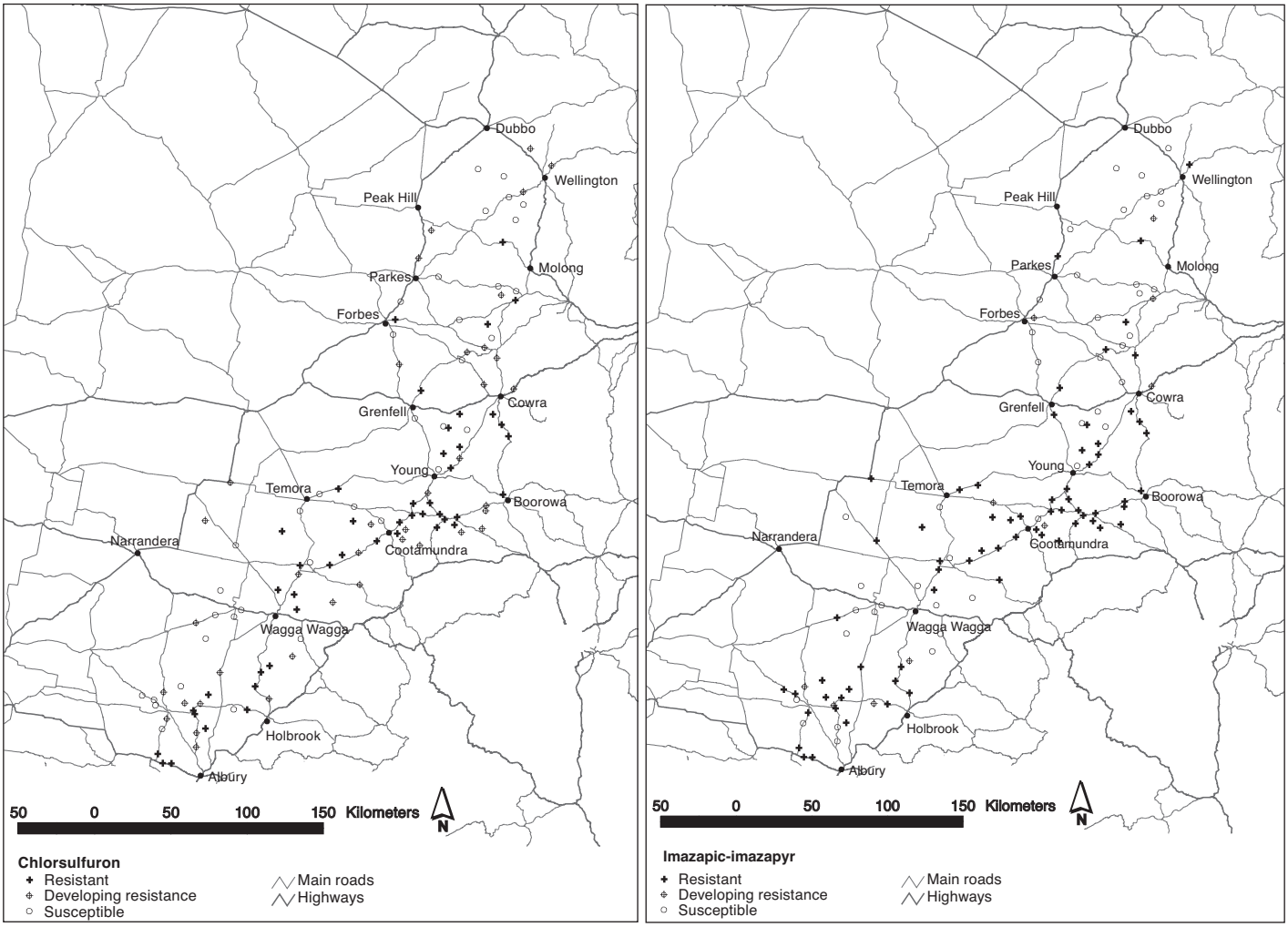

Figure 2. Location of samples resistant, developing resistance or susceptible to two Group B herbicides.

Multiple resistance

Of the 137 samples, 117 were screened to all five groups of selective herbicides screened (Groups A 'fop', A 'dim', B, C and D). If a sample was resistant or developing resistance to either herbicide in two groups (A 'dim' and B) where two herbicides were tested, it was classified as resistant to that group. Of the 117 samples only 9% were susceptible to all five herbicide groups. Although no population was identified to develop multiple resistance to all the five groups, three percent were resistant to four groups, 31% resistant to three and 42% resistant to two. Of the 117 samples 69% were resistant to both Group A 'fop' and B herbicides while 32% were resistant to Group A 'fop', 'dim' and B herbicides. Samples with higher levels of multiple resistance were more likely to have come from the central or southern regions of the surveyed area reflecting the higher level of Group B resistance in these regions (Figure 3).

Comparison to previous survey

The previous survey conducted in 1991 collected samples over a smaller area than the 2007 survey (Figures 1 and 4), collecting no samples north of Cowra. The 1991 survey did however collect some samples from further west than in 2007.

Herbicide resistance levels for Group A 'fop' and 'dim' and Group B increased markedly between the 1991 and 2007 surveys. Resistance to diclofop-methyl was recorded in 14% of samples in 1991 compared to 81% in the 2007 survey. A similar increase, 11% to 70%, was recorded for chlorsulfuron (Table 4). The increase in resistance to sethoxydim between the two surveys while still large was not as great as experienced for either diclofop-methyl or chlorsulfuron

The level of trifluralin resistance recorded decreased between the two surveys. However, many of the trifluralin resistant samples in the 1991 survey came from an area between Temora and Narrandera not surveyed in 2007 due to the dry seasonal conditions (Figures 1 and 4).

Discussion

This survey confirms that increases in resistance have occurred in southern New South Wales since the last survey. The level of resistance for the Groups A (fop) and B herbicides had increased significantly since the last survey, from 14% to 81% and 11% to 70% respectively. This was expected as the last survey was conducted 16 years before. During this period the annual ryegrass populations would have had additional applications of all herbicides tested in the 1991 survey, thereby further increasing the selection pressure for resistance development. The annual rate of increase for both Group A (fop) and B herbicides in the surveyed region was 4%, similar to that experienced by the Western Australian survey (Llewellyn and Powles 2001, Owen *et al.* 2007).

This finding differs from the data from the commercial resistance testing service operated at Charles Sturt University which have shown limited annual variability in resistance levels over this period (Broster and Pratley 2006). Commercial samples are different to samples obtained through random surveys. The commercial samples were highly selective. The seeds were collected from suspected populations which have failed in herbicide applications. The levels of resistance in these commercial samples are therefore expected to be high and less variable. However, in each year during this period (1991–2004) samples

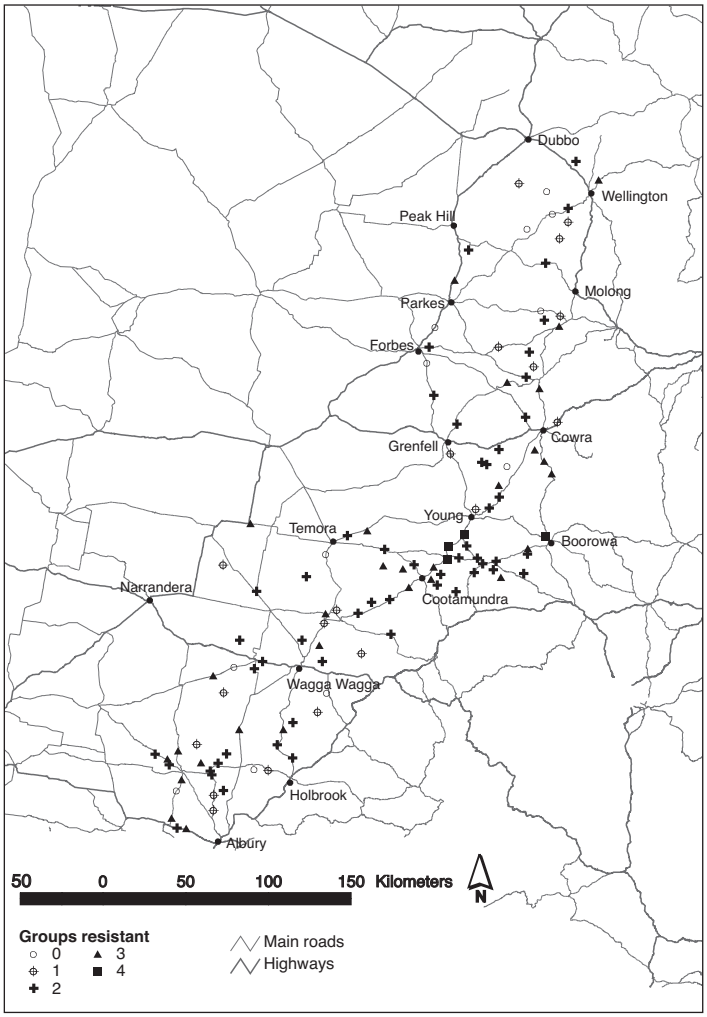

Figure 3. Location of samples with different levels of multiple resistance.

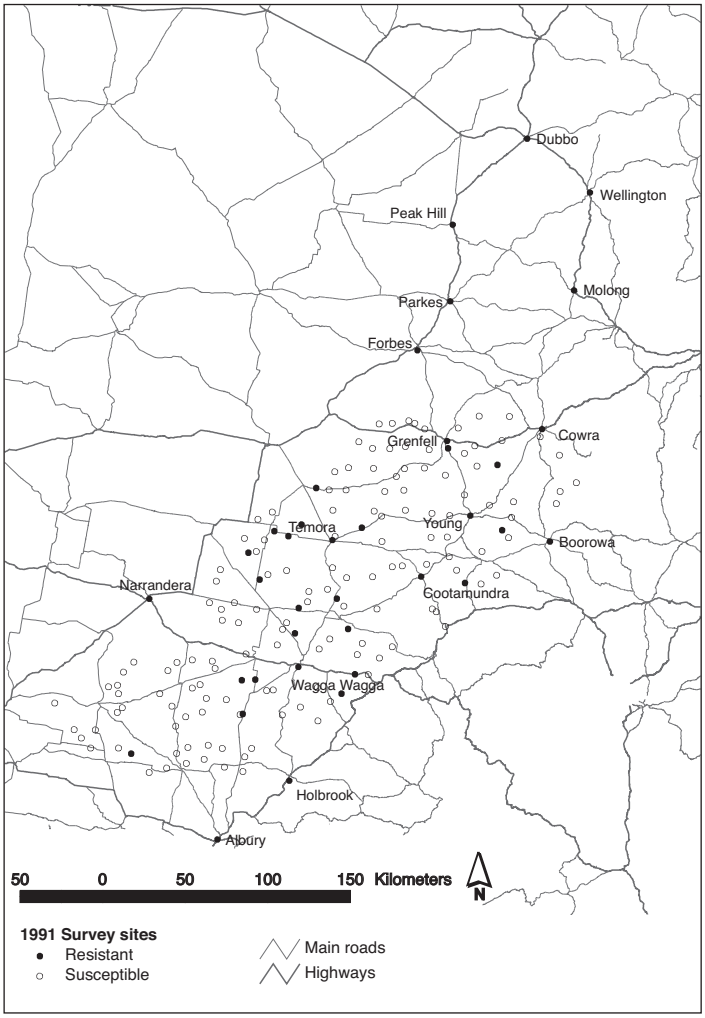

Figure 4. Sample locations for 1991 survey showing locations of trifluralin resistant samples.

were received for resistance testing from postcode areas which had not previously provided samples.

As was the situation in previous surveys, in this survey the level of herbicide resistance in Group A 'fop' and B herbicides were similar and higher than the other herbicide groups (Gill 1993, Pratley *et al.* 1993, Henskens *et al.* 1996, Llewellyn and Powles 2001, Owen *et al.* 2007). In many cases Group A 'fop' herbicides are the last used on a crop and as such there are limited salvage options for their failure compared to Group B, C or D herbicides. While Group B herbicides can be applied earlier in the crop's growth, they control a wide range of weeds, both grass and broadleaf species (Parsons 1995). Therefore, they apply selection pressure for resistance development in annual ryegrass even when it is not the primary weed target.

Despite the level of 'fop' resistance (81% cf. 68%) being higher and B (70% cf. 88%) lower in southern New South Wales compared to Western Australia the proportion of populations resistant to both is similar (62% cf. 64%) (Owen *et al.* 2007). In this survey the areas of more intensive cropping have the higher proportion of samples with high levels of multiple resistance as was the case in the 1991 survey (Pratley *et al.* 1995).

Table 4. Percentage of ryegrass populations resistant to herbicides screened in both the 1991 and 2007 surveys.

	Group	2007	1991
Diclofop-methyl	A 'fop'	81	14
Sethoxydim	A 'dim'	43[A]	12
Chlorsulfuron	B (SU)	70	11
Simazine	C	1	0
Trifluralin	D	6	12

[A] Sethoxydim was only screened to the diclofop resistant populations in 2007.

While the level of resistance to both the Group A 'fops' and B herbicides is high, it will continue to rise. The continued use of these herbicides is expected as they are effective in a wide range of crop and weed combinations. However, it is extremely unlikely that 100% of annual ryegrass populations will become resistant to these herbicides. While the development of herbicide resistance is linked to generations of exposure (Maxwell and Mortimer 1994), the low cropping intensity of some paddocks mean that herbicides are only a minor factor in the evolutionary process of those paddocks. While possibly unaware of the fact, the managers of those paddocks are actually practising a very effective version of integrated weed management with respect to herbicide resistance development.

The proportion of 'fop' resistant samples also resistant to sethoxydim is lower than experienced in the Western Australian survey (43% cf. 61%) (Owen *et al.* 2007). Sethoxydim is unable to be metabolized by annual ryegrass therefore all resistant populations are the result of target site mutations (Tardif and Powles 1994). Therefore, while the incidence of

resistance to 'fop' herbicides was higher in New South Wales, the level of target site resistance was lower.

While the level of sethoxydim resistance is lower (35% cf. 42%) than in the Western Australia survey, the level of clethodim resistance is higher (21% cf. 8.5%). Clethodim has been shown to be different from the other Group A 'dim' herbicides in resistance development (Roy and Jackson 1996, Llewellyn and Powles 2001, Broster and Pratley 2006). This may represent an increased use of the 'dim' herbicides before the release of clethodim in Australia in 1993 due to a greater long-term use of both lupins and canola in Western Australian rotations.

The finding in this survey of two samples susceptible to sethoxydim but developing resistance to clethodim is unusual. However, while classed as developing resistance to clethodim, these samples were at the lower end of the class (10.9 and 13.9% survival respectively) while they did have some plants survive the sethoxydim application (7.7 and 6.1% survival respectively). Whilst populations susceptible to sethoxydim and resistant to clethodim are rare, one has previously been tested by the commercial herbicide resistance testing service at Charles Sturt University (J. Broster unpublished data).

The lower level of trifluralin resistance reflects its reduced use compared to the Group A and B herbicides especially in minimum tillage situations. However, as a result of the high level of Group B resistance experienced in this survey, the use of trifluralin would be expected to increase placing additional selection pressure on this herbicide in the future. This hypothesis is supported by the finding in Western Australia where 24% of the populations were trifluralin resistant in 2003 despite it not being tested in 1998 when 64% of populations were Group B resistant (Llewellyn and Powles 2001, Owen *et al.* 2007).

While this survey showed a decrease in trifluralin resistance compared to the 1991 survey it is understandable. The region where the majority of resistant populations were found in 1991 was not sampled in 2007 due to seasonal conditions. If this region was sampled it could be expected that the incidence of trifluralin resistance would be equal to, or greater than, that detected in the 1991 survey.

The low incidence of simazine resistance is surprising given the high incidence of resistance to the triazine herbicides worldwide (Heap 2009). With the use of simazine and other triazine herbicides limited to the grain legume phase of rotations, this has placed less selection pressure for resistance on this herbicide group. However, the high uptake of triazine tolerant canola varieties in recent years has increased selection pressure for resistance development.

The low incidence of glyphosate resistance in this survey is encouraging. Glyphosate is of major importance to the conservation farming systems used since the 1980s. Nevertheless, resistance to glyphosate has occurred throughout Australia in annual ryegrass populations (Preston 2009), as well as in awnless barnyard grass (*Echinchloa colona* (L.) Link) and liverseed grass (*Urochloa panicoides* Beauv.) (Heap 2009). With Roundup Ready® canola varieties now being grown on a limited scale, and the probability of an increased area being sown in the future, the management of this herbicide to avoid the build-up of resistance is of vital importance in Australian farming systems.

While high levels of resistance were present to diclofop-methyl, chlorsulfuron and imazapic/impazapyr across the surveyed area, a positive finding was the low level of resistance to simazine, trifluralin and glyphosate. Of concern is the moderate but significant level of resistance to both sethoxydim and clethodim. These and other Group A 'dim' herbicides are the major alternatives to Group A 'fop' herbicides for in-crop annual ryegrass control.

An inability to use those herbicides with a high incidence of resistance will place increased pressure on the remaining herbicides which currently experience a low incidence of resistance. These results show the importance of using non-chemical weed control methods, or integrated weed management, to reduce the impact of herbicide resistance. Increased emphasis on reducing the number of individuals in a weed population treated with herbicides will prolong the effective life of the herbicides which currently have a high incidence of resistance, while also reducing the pressure on resistance development in the herbicides with lower incidence of resistance.

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