An evaluation of the resistance of annual and perennial ryegrass to herbicides

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Abstract Ryegrass, both perennial (*Lolium perenne*) and annual (*L. multiflorum*), can be weeds in other arable crops such as wheat (*Triticum aestivum*). They are generally removed using selective herbicides, but herbicide-resistant perennial and annual ryegrasses have been reported anecdotally. Perennial ryegrass seeds that were suspected to be herbicide resistant were sourced from near Methven, Canterbury. They were evaluated for tolerance to five herbicides and were found to be resistant to all of them. In a separate experiment, annual ryegrass seeds that were suspected to be herbicide resistant were also sourced from near Methven, Canterbury. They were evaluated for tolerance to six different herbicides and were found to be resistant to haloxyfop applied at up to four times label rate but susceptible to clethodim. These results have important implications for growers and advisers throughout New Zealand.

Keywords herbicide resistance, annual ryegrass, perennial ryegrass, grass herbicides, sulfonylureas.

INTRODUCTION

In New Zealand, herbicides are classified into 23 groups based on their mode of action (Harrington 2017). The herbicides glyphosate and glufosinate, Groups G and H respectively, kill a wide range of plants including grasses and are not suitable for controlling grass weeds in cereal crops. The Group G herbicide works through the inhibition of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and the Group H herbicide works through the inhibition of glutamine synthase (Harrington 2017). Recently, both annual and perennial ryegrass have been found to be resistant to these two herbicides in vineyards (Ghanizadeh et al. 2013). In contrast, Group A herbicides (also known as FOPs, DIMs and DENs) are selective post-emergence herbicides that are used for the

control of grass weeds in broadleaved crops such as brassicas, clover and peas (Novachem 2016). These herbicides inhibit the enzyme acetyl-CoA carboxylase, the enzyme catalysing the first committed step in *de novo* fatty acid synthesis. Inhibition of fatty acid synthesis blocks the production of phospholipids, which are used in building new membranes required for cell growth (Focke & Lichtenthaler 1987).

The introduction of the selective Group A herbicides in the 1980s enabled control of *Lolium multiflorum* (annual ryegrass) in wheat fields (Stanger & Appleby 1989). Recently, a wheat grower reported *Lolium multiflorum* plants were surviving Group A herbicide treatments applied to wheat in Canterbury. Most farms growing

wheat sow ryegrass seed crops as part of the crop rotation, and *Lolium multiflorum* seed or surviving plants are potential contaminants in subsequent wheat crops. *Lolium multiflorum* is indigenous to Europe, Asia and northern Africa (Edgar & Connor 2010). However, it has spread worldwide and has become a major weed in many different cropping systems (Stanger & Appleby 1989) as it can significantly reduce winter wheat yields (Appleby et al. 1975). Internationally, *L. multiflorum* has developed multiple resistance to herbicides through repetitive exposure to herbicides with the same mode of action (Heap 2017).

Group B herbicides are commonly used to control grass weeds in cereal crops and include imidazolinones, sulfonylureas, and triazolopyrimidines that inhibit acetolactate synthase (ALS; also called acetoxyacid synthase AHAS). This enzyme plays a key role in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (Anon n.d.). The earliest Group B herbicides developed, e.g. chlorsulfuron, thifensulfuron and tribenuron, are recommended only for control of broadleaf weeds in cereal crops as they have reduced efficacy on grass weeds (Rolston et al. 2003). However, their use for broadleaf weed control may result in an increased tolerance of surviving ryegrasses and other grass weeds to more recently developed Group B herbicides that are more active on grass weeds including *L. perenne* (perennial ryegrass). Recently, a wheat grower reported *L. perenne* plants were surviving Group B herbicide treatments applied to control grass weeds in wheat in Canterbury.

This aim of this study was to test the hypothesis that *L. multiflorum* and *L. perenne* found in two separate wheat fields in Methven, Canterbury, New Zealand have developed resistance to herbicides from Groups A and B respectively.

MATERIALS AND METHODS

Experiment 1

This experiment was carried out in the AgResearch Plant Protection glasshouse complex at Ruakura Research Centre in the North Island of New

Zealand. Seed of *L. perenne* that was suspected to be herbicide resistant (based on reports from a wheat grower) was sourced from a field near Methven, Canterbury, in 2014. Non-resistant *L. perenne* (cv. 'Grasslands Samson') seed and non-resistant *L. multiflorum* (cv. 'Progrow') seed provided by the Foundation for Arable Research were used as controls. The seed was sown in 15-cm diameter plastic pots that were filled with commercial potting mix (Daltons) and, after emergence, thinned to one plant per pot. All the ryegrass seed used in this experiment germinated readily and no dormancy breaking methods were required.

Five Group B herbicides (chlorsulfuron, iodosulfuron, pyroxsulam, thifensulfuron and tribenuron) were included in Experiment 1. Herbicides were applied at the standard (1×) label rate, double (2×) and quadruple (4×) the label rate. The herbicide treatments were applied on 18 September 2014 when plants were considered mature (8–12 tillers), on an overcast day with a glasshouse temperature of 21°C. Treatments were applied in the equivalent of 200 litres/ha water at 200 kPa with a moving belt precision sprayer fitted with a single TeeJet® 8001-E even-spray fan nozzle. There were 10 replicate pots of each treatment. The herbicides and their normal application (1×) rates were: chlorsulfuron (Glean® 750 g/kg in the form of a water dispersible granule) at 20 g ai/ha, iodosulfuron-methyl (Hussar® 50 g/kg iodosulfuron-methyl-sodium in the form of a water dispersible granule) at 150 g ai/ha; pyroxsulam (Simplicity™ 30 g/litre pyroxsulam in the form of an oil dispersible liquid) at 500 g ai/ha; thifensulfuron-methyl (Chord™ 750 g/kg thifensulfuron methyl in the form of a water dispersible granule) at 20 g ai/ha and tribenuron-methyl (Sero® WG 750 g/kg tribenuron-methyl in the form of a water dispersible granule) at 25 g ai/ha (Novachem 2013). Treatments were applied with the label recommended adjuvant Actiwett® (950 g/litre linear alcohol ethoxylate) at 0.025% except for iodosulfuron where Partner® (vegetable oil polymer) at 0.5% was used. The herbicide treatments were compared with a water-only

control. After treatment, the pots were arranged in a randomised complete block design in the glasshouse. A visual assessment of percentage damage was carried out at 7, 14 and 21 days after treatment (DAT) (0=nil; 100=dead).

Experiment 2

Seeds of *L. multiflorum* that are suspected to be herbicide resistant were sourced from a field near Methven, Canterbury in 2015. This collection site was different from the one used in Experiment 1. Three Group A herbicides (clethodim, haloxyfop and pinoxaden), a mixture of Group B herbicides (mesosulfuron + iodosulfuron + diflufenican) plus glufosinate and glyphosate were included in Experiment 2. For this trial, seedlings were raised in pots in Plant and Food Research glasshouses at Lincoln and the treatments applied at New Zealand Arable facilities at Templeton. Seeds of both suspected resistant and control lines were initially germinated in plastic trays on seed germination paper soaked in gibberellic acid to break any seed dormancy. On 12 August 2016, two plants per pot were transplanted and grown to an average size of five tillers. Control plants were *L. multiflorum* cv. 'Winterstar' (tetraploid ryegrass, PGG Wrightson seeds). The seedlings were treated with herbicides on 31 August 2016 with a four-nozzle backpack plot sprayer applying the equivalent of 200 litres/ha of water. The herbicides were applied at standard (1×), double (2×) and quadruple (4×) the standard label rates and compared with a water-only control. There were six replicate pots per treatment. The herbicides evaluated and their 1× application label rate were: Group A herbicides, clethodim (Arrow® 240 g/litre clethodim in the form of an emulsifiable concentrate) at 120 g ai/ha with 2 litre/ha crop mineral oil; haloxyfop-P (Gallant™ Ultra 520 g/litre haloxyfop-P present as the haloxyfop-P-methyl in the form of an emulsifiable concentrate at 62 g ai/ha with 1 litre/ha Uptake™ (582 g/litre paraffinic oils and 240 g/litre alkoxyated alcohol non-ionic surfactants); pinoxaden (Twinax® 100 g/litre pinoxaden in the form of an emulsifiable concentrate) at 30 g ai/ha with 0.5% v/v Adigor® (440 g/litre methyl esters of canola fatty acids);

Group G herbicide, glyphosate (Roundup® 360 360 g/litre glyphosate as the isopropylamine salt in the form of a soluble concentrate) at 1440 g ai/ha with 0.1% v/v non-ionic surfactant; Group H herbicide, glufosinate (Buster® 200 g/litre glufosinate-ammonium in the form of water soluble concentrate) at 1500 g ai/ha; and a Group B herbicide mixture mesosulfuron + iodosulfuron + diflufenican (Othello® OD 50 g/litre diflufenican, 7.5 g/litre mesosulfuron methyl, 2.5 g/litre iodosulfuron methyl sodium in the form of an oil dispersion) at 50 + 7.5 + 2.5 g ai/ha respectively. Two days after spray application, the plants were transferred to a greenhouse maintained at 20°C. At 7, 14, 21 and 28 days after treatment (DAT), the pots were visually evaluated for percent damage (0=nil; 100=dead).

Statistical analysis was conducted using a two-way ANOVA, herbicide by rate, test on untransformed data (Genstat® v17).

RESULTS

Experiment 1

The suspected herbicide-resistant *L. perenne* was not damaged at 1×, 2× or 4× label rates of the five Group B herbicides, whereas the control cultivars of *L. multiflorum* and *L. perenne* were severely damaged (>90% damage) by three of the Group B herbicides (chlorsulfuron, iodosulfuron, pyroxsulam) and partially damaged by the other two (thifensulfuron and tribenuron) (Table 1).

Experiment 2

The suspected herbicide-resistant *L. multiflorum* population sprayed with haloxyfop was not damaged by up to four times the standard label rate, whereas the susceptible control *L. multiflorum* was 100% damaged at the standard rate (Table 2). This resistant population also had partial tolerance to both pinoxaden and the mesosulfuron + iodosulfuron + diflufenican mix (Table 2). The resistant *L. multiflorum* population was susceptible to clethodim, although not completely controlled at either the 1×, 2× or 4× rates. The suspected herbicide-resistant *L. multiflorum* was also susceptible to glufosinate and glyphosate at 1× standard label rates.

Table 1 Mean damage score for three *Lolium* lines when scored 21 days after treatment with five 'Group B' herbicides applied at three application rates.

Herbicide	Percentage damage (%)			
	Herbicide rate (times above label)			
	Water only	1×	2×	4×
Susceptible <i>L. multiflorum</i>				
chlorsulfuron	0	95	60	80
iodosulfuron	0	100	100	100
pyroxsulam	0	100	98	100
thifensulfuron	0	20	0	93
tribenuron	0	5	30	45
Susceptible <i>L. perenne</i>				
chlorsulfuron	0	95	85	93
iodosulfuron	0	100	100	100
pyroxsulam	0	100	100	100
thifensulfuron	0	28	75	95
tribenuron	0	45	35	78
Suspected resistant <i>L. perenne</i>				
chlorsulfuron	0	0	0	0
iodosulfuron	0	25	0	0
pyroxsulam	0	0	10	0
thifensulfuron	0	0	0	0
tribenuron	0	0	0	0
Overall LSD _{0.05}			27.9	

Table 2 Mean damage score for two *Lolium multiflorum* lines when scored 21 days after treatment with five herbicides applied at three application rates.

Herbicide	Percentage damage (%)			
	Herbicide rate (times above label)			
	Water only	1×	2×	4×
Susceptible <i>L. multiflorum</i>				
clethodim	0	100	100	100
haloxyfop	0	100	100	100
glufosinate	0	100	100	100
glyphosate	0	100	100	100
meso+iodo+diflu*	0	53	80	100
pinoxaden	0	100	100	100
Suspected resistant <i>L. multiflorum</i>				
clethodim	0	84	91	97
haloxyfop	0	9	3	4
glufosinate	0	100	100	100
glyphosate	0	91	100	100
meso+iodo+diflu*	0	43	64	75
pinoxaden	0	25	33	60
Overall LSD _{0.05}			20	

* = mesosulfuron + iodosulfuron + diflufenican applied as Othello® OD.

DISCUSSION

None of the Group B herbicides effectively controlled suspected herbicide-resistant *L. perenne* in Experiment 1. Chlorsulfuron, iodosulfuron and pyroxsulam resulted in total control of a commercial standard (susceptible) *L. perenne* cultivar and a commercial standard (susceptible) *L. multiflorum* cultivar. Thifensulfuron and tribenuron did not effectively control any of the tested plants. Chlorsulfuron, thifensulfuron and tribenuron are used in cereals for broadleaf weed control and, although they do not kill grass weeds, they do affect their growth (Rolston et al. 2003). It is possible that their long use for broadleaf weed control may have contributed to the evolution of resistance to the newer Group B herbicides iodosulfuron and pyroxsulam.

The suspected herbicide-resistant *L. multiflorum* population tested in Experiment 2 was resistant to the Group A herbicide haloxyfop at up to four times the standard rate and showed increased tolerance towards the Group A herbicide pinoxaden. It was susceptible to the Group A herbicide clethodim at all rates, although the trend of increasing damage with increasing rate suggests that increased resistance to clethodim may be developing in this population.

The suspected herbicide-resistant *L. multiflorum* also had some tolerance to a mixture of Group B herbicides (mesosulfuron + iodosulfuron + diflufenican). However, this was probably due to plant size as the manufacturer recommends this mixture for controlling both annual and perennial ryegrass with up to two tillers, whereas the trial plants had five tillers. The results reported in this paper are the first confirmed cases of Group B herbicide resistance of *L. perenne* and Group A herbicide resistance to *L. multiflorum* within New Zealand. Herbicide resistance in both of these species is not uncommon overseas, with *L. multiflorum* known to be resistant to eight different modes of action in 12 different countries including 20 cases of Group A and 13 cases of Group B resistance reported from seven countries (Heap 2017).

Denmark, another major grass seed production country, with approximately 35000 hectares grown per year reported herbicide resistance in *L. multiflorum* in 2010 for the first time (Anon. 2015; Neve et al. 2014). In contrast, Group B resistance in *L. perenne* has only twice been reported, with sulfometuron-methyl resistance in roadsides and railways in California USA in 1989 and multiple resistance to iodosulfuron-methyl, pyroxsulam and to the Group A herbicide pinoxaden, in Germany in 2008 (Heap 2017). Additionally there is only one other case of Group A resistance, that of diclofop-methyl and clodinafop-propagyl in cereals in Chile in 2001. Farmers who grow continuous wheat crops without rotation, and/or who do not vary the group of herbicide they use are at risk of increased populations of herbicide-resistant weeds. Farmers need to remain vigilant and regularly observe plant species surviving herbicide application. Surviving weeds need to be removed before these plants can set viable seed. Glyphosate is considered an effective herbicide for grasses but there are known cases of glyphosate resistance in populations of both *L. perenne* and *L. multiflorum* in Marlborough vineyards (Ghanizadeh et al. 2013).

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