EVALUATION OF THE PETRI DISH ASSAY FOR SCREENING DICLOFOP-METHYL RESISTANCE IN PARTIALLY RESISTANT POPULATIONS OF ANNUAL RYEGRASS, *LOLIUM RIGIDUM*

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Summary. Diclofop-methyl resistance in annual ryegrass, *Lolium rigidum*, has become a common occurrence in wheat growing areas of southern Australia. Farmers are currently being offered a population testing service for herbicide resistance based on the petri dish assay of Heap and Knight (2). Our evaluation of the petri dish assay showed that it underestimated the level of herbicide resistance in annual ryegrass as compared to the foliar application of diclofop-methyl in a pot experiment. In the petri dish assay even resistant ryegrass suffered complete mortality at high concentrations of diclofop. Such a result warrants that caution should be exercised not only while selecting screening concentrations of diclofop for the petri dish assay but also in interpretation of the results obtained.

INTRODUCTION

Annual ryegrass, *Lolium rigidum*, is one of the most common grass weeds of wheat in southern Australia (3). It is a prolific seed producer (4), and if uncontrolled, the seed reserves in the soil can increase rapidly and pose serious infestation problems.

Release of diclofop-methyl on the Australian market in 1978 was a major advance in weed control technology and it appeared to largely overcome ryegrass problem in wheat crops. Early experience with diclofop-methyl showed it to be an extremely effective herbicide, with ryegrass control often in excess of 90%. It soon achieved a high level of adoption by farmers. Some farmers also used diclofop-methyl in lupins and achieved excellent control of ryegrass. In hindsight, it is not really surprising that in response to such high selection pressure, ryegrass populations have evolved resistance to diclofop-methyl (1).

Research is in progress at the W.A. Department of Agriculture to develop strategies for ryegrass control that will delay or even avoid evolution of herbicide resistance in this grass. To monitor genetic shifts in herbicide resistance in weed populations there is a need to develop suitable screening techniques. The studies reported here were designed to evaluate the suitability of the petri dish assay (2) for ryegrass populations with low frequency of diclofop-methyl resistant individuals.

METHODS

Seed source. Seeds of diclofop-methyl resistant ryegrass were collected in 1984 from a farmer paddock under wheat-lupin rotation at Dowerin. The ryegrass population had received 5 successive annual applications of diclofop-methyl before the farmer observed deterioration in the level of ryegrass control. Seeds of the susceptible population were sampled from a pasture paddock at Lake Grace which had never been sprayed with any herbicide. The seeds of both the populations were stored in a refrigerator at approximately 4^{o}C to maintain seed viability.

Petri dish assay. Diclofop-methyl (technical grade) was hydrolysed with sodium hydroxide and then recrystallised from water to obtain diclofop-Na. The solubility and stability of diclofop-Na in phosphate buffer makes it easier to use in petri dish assays. Diclofop-methyl on the other hand is not only relatively insoluble in water but also contains xylene which is known to damage plant cells.

The ryegrass seeds were germinated in large petri dishes (20 cm dia.) on a double layer of filter paper at 16/10°C (12h day/12h night). Seeds were enclosed in aluminium foil to keep them in the dark. Seeds started to germinate within a week. Petri dishes were examined daily for seeds with 2-3 mm radicle length which were transferred to petri dishes (9 cm dia.) containing a range of concentrations of diclofop-Na. Each petri dish had a double layer of filter paper and contained 5 ml of herbicide solution. The petri dishes were sealed with a polyethylene film to prevent drying. The seeds were incubated at 20°C for 7 days following which observations were made on the number of seeds still alive (white root tips) and their coleoptile lengths were recorded.

Foliar application. Ryegrass accessions used in the petri dish assay were also used in a pot experiment to determine their response to diclofop-methyl. Seeds were sprinkled on the surface of a sandy loam soil to give approximately 30-40 seedlings/pot (approx. 1.0 kg air dry soil/pot). The pots were placed in a growth cabinet set to give a temperature regime of 16°/10°C (12h day/12h night) and light intensity at the soil surface of 350 $\mu E/m^2/s$. The pots were fertilised weekly with a dilute nutrient solution (N,P,K and trace elements).

A hand-pushed small plot sprayer was used to spray ryegrass plants with different rates of diclofop-methyl (see results section) at Z12 and Z14-15 stages of growth. The sprayer used water volume equivalent to 200 L/ha at a pressure of 200 kPa. A non-ionic wetting agent (BS 1000) at 0.25% (vol./vol.) was added to all herbicide treatments. Ryegrass seedlings in each pot were counted before the herbicide treatment. Average seedling density was 32 (s.e.m.=1.5) and 26 (s.e.m.=1.5) plants/pot for the susceptible and resistant accessions, respectively. The experiment was terminated at 8 weeks after sowing, at which time all the susceptible plants had died. Pots were watered 3 hours prior to harvesting, to restore complete turgor. At harvest, the number of surviving healthy plants and their fresh weights were recorded.

RESULTS AND DISCUSSION

Petri dish assay. There were clear differences between the two populations in the seedling mortality and coleoptile length of the surviving seedlings (Table 1). The concentration of diclofop-Na required to cause 75% seedling mortality (LD_{75}) or equivalent reduction in coleoptile length were good indicators of the relative responsiveness of the two ryegrass populations (Table 1). Heap and Knight (2) also obtained a similar distinction in the LD_{75} for seedling mortality of a resistant and susceptible population of annual ryegrass. Plotting the proportion of different size classes of coleoptiles at different herbicide rates suggested that there were 1-2% resistant individuals in the Dowerin population of ryegrass (data not presented).

Table 1. The effect of diclofop-Na on the seedling mortality and coleoptile length of a putative resistant (Res.) and susceptible(Sus.) accession of annual ryegrass (petri dish assay).

Diclofop-Na (uM)	Seedling mortality (%)			Coleoptile length (mm)	
	Res.	Sus.		Res.	Sus.
0	0	0		32.7	39.9
11	15	25		25.7	15.9
24	20	35		18.7	14.4
51	41	56		25.7	14.4
110	43	73		13.9	8.3
236	65	80		18.2	6.9
510	85	93		24.2	3.3
1100	96	99		11.7	1.0
5107	100	100		0.0	0.0
LD75 (μM)	375	110	GR75 (μM)	1100	75

Foliar application. The environmental conditions in the growth cabinet were ideal for diclofop-methyl activity and the susceptible population was completely killed at the lowest herbicide rate (93 g/ha). The growth stage of ryegrass at the time of herbicide application did not appear to have a significant impact on the survival of the susceptible population.

Table 2. The effect of diclofop-methyl on the seedling survival and shoot fresh weight of a putative resistant (Res.) and susceptible (Sus.) accession of annual ryegrass (pot experiment).

Diclofop-methyl (kg/ha)		Seedling survival (%)		Shoot fresh weight (g/pot)	
Res.	Sus.	Res.	Sus.	Res.	Sus.
Z12-13					
0.000	0.000	100	100	18.96	21.55
0.187	0.093	24	0	6.42	0.00
0.375	0.187	16	0	12.64	0.00
0.750	0.281	11	0	4.04	0.00
1.500	0.375	7	0	4.29	0.00
3.000	0.750	16	0	6.29	0.00
Z14-15					
0.000	0.000	100	100	18.96	21.55
0.187	0.093	20	0	7.64	0.00
0.375	0.187	15	0	4.55	0.00
0.750	0.281	26	0	10.51	0.00
1.500	0.375	18	0	6.66	0.00
3.000	0.750	7	0	2.15	0.00

The proportion of healthy ryegrass plants decreased sharply with increasing rates of diclofop-methyl but appeared to reach an asymptote at 10-20% survival (Table 2). The shoot biomass of ryegrass showed a similar trend in response to diclofop-methyl with an asymptote of 2.5-5 g/pot as compared to the shoot biomass of 20 g/pot in the untreated controls (Table 2).

The two techniques for screening diclofop-methyl resistance in ryegrass were able to discriminate between the resistant and susceptible populations. However, the petri dish assay did not appear to reach an asymptote, and underestimated the level of resistance in ryegrass (1-2% vs 10-20%).

It is imperative therefore, that the selection of screening rate for the petri dish assay should be done with great caution because at high rates of the herbicide even the truly resistant individuals can be killed. It also appears necessary to validate the results of petri dish assay against a dose response from the pot or field experiments.

ACKNOWLEDGMENTS

The financial assistance of Wheat Research Committee of Western Australia is gratefully acknowledged. Competent technical assistance was provided by Ms M. Allan.

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