Regression of herbicide resistance in some populations of annual ryegrass (*Lolium rigidum* Gaud.)

John M. Matthews
CRC for Weed Management Systems, Roseworthy Campus, University of Adelaide, Roseworthy, South Australia 5371, Australia

Summary Aspects of the life cycle that contribute to the fitness and persistence of herbicide-resistant annual ryegrass were investigated in three untreated and segregating biotypes of annual ryegrass. Susceptible genotypes drawn from the segregating resistant populations were identified and found to have a greater contribution to the successive generation than the resistant fraction.

An increasing susceptible genetic influence is also identified. Resistant plants decreased in frequency in two field populations and in three isolated control plots.
Keywords Herbicide resistance, annual ryegrass, regression.

INTRODUCTION

The evolution of resistance to many graminicides is an established phenomenon in many thousands of populations of annual ryegrass in southern Australia. During the course of a field experiment, a reduction in the level of resistance was observed in a population of resistant ryegrass, which was not treated with any selective herbicides for three years.

In the resistant population in which resistance regressed, it is surmised that the outcome of random crossing of the incompletely dominant genetic resistance and increasing frequency of the susceptible component with successive generations may have accounted for the observation. This implied that the susceptible component may be more fit than the resistant component of the population and may also additionally have contributed to the susceptible proportion. Fitness is the outcome of many elements of the life cycle and previously unstudied aspects of the life cycle may lead to a better understanding of the factors contributing to the persistence of herbicide resistance.

The prospect of managing the genetic component conferring resistance may have potential for utilisation in many resistant populations.

MATERIALS AND METHODS

Ryegrass populations Three populations of herbicide resistant (R) annual ryegrass were obtained for this study. Population SR31 is a well characterised resistant population (Matthews *et al*. 1990). Population TS was obtained for this study from Bordertown, 300 km south of Adelaide. Population TD was obtained from a field collection prior to the trial initiation from Tarlee 65 km north of Adelaide. All three populations had some resistance to herbicides which inhibit the enzymes ACCase (Group A herbicides) and acetolactate synthase (ALS) (Group B herbicides).

Genetics A classical crossing experiment was undertaken with each population in which five representative resistant plants of each population were crossed individually with susceptible (S) plants. The seeds from each maternal plant were kept separate and further backcrossed to each of the resistant and susceptible parent and crossed on to form an F2 population. Crossing was done by planting the two subjects within a 1.5 m high plastic tubes in the field.

Continuing populations without competition in field plots Subsequent generations of each population were generated each year without herbicide application and in a non-competitive environment shielded from pollen ingress by a 2 m high barrier. Four hundred seeds per square metre were sown and the population grown to maturity without any herbicide treatment. Three generations were called SR31, TD and TS and P0, P1 and P2, respectively, referred to as 'Isolation' plots.

Competition within wheat crop of regressing populations Seeds of SR31 P1, TD P1, TS P1 were sown in a marked grid within a wheat crop. A tiller was cut from each plant and planted in a glasshouse and treated with 540 g ha^{-1} of the selective graminicide diclofop methyl (Hoegrass®). The results of the herbicide application allowed plants to be classified as susceptible or resistant. Cohort performance, dry weights and seed production was measured on plants proven to be R and S from the in-crop screening.

Observation of pollen output and potential fecundity within a wheat crop Several gravid florets were taken from plants and were allowed to mature in a cotton wool stoppered eppendorf tubes in a 25°C incubator. Pollen grains were extracted by crushing florets in 75% methanol, centrifuging and counting in a haemocytometer.

Populations planted at 1:1 ratio of susceptible ryegrass to speed up the onset of susceptibility One plot each of SR31 P0, TD P0 and TS P0 was established at 100 plants m^{-2} within a wheat crop of 400 plants m^{-2}. An equal number of susceptible plants were also established in each plot. Plants were marked and seeds retrieved and final dry weights measured, at least 12 plants per biotype per plot were harvested for weighing.

Trials of herbicide resistance within field plots Three populations were established in plots at two sites. Treatments included Hoegrass® treatments with and without two levels of crop density. One site was free of ryegrass and SR31 and TS were established in wheat plots (site 1). The other site had a residual seedbank of one of the biotypes (TD) and the SR31 biotype was planted in some plots (site 2). The experiment was continued for three years with ryegrass and wheat plots and a 6 m border also planted to wheat. Broadleaf weeds were controlled by herbicides as required. Seed was harvested each year from each plot and resown in the same plot after burning to reduce ryegrass seedbank. Samples from the final year were planted in pots and treated with herbicides Hoegrass® and triasulfuron (Logran®) to evaluate the susceptibility to herbicides.

RESULTS

Genetic experiments Ryegrass is an obligate outcrossing species and in a population every anthesis event can influence the population structure. There was a difference in the mean of all the LD_{50} of the F1s due to maternal influence, the mean of the susceptible material was 400 mL ha^{-1} and the resistant 1.1 L ha^{-1}. The untreated F2's susceptible maternal crosses between several representatives of the SR31, TS and TD are shown in Figure 1.

There is substantial variability around the mean (represented by the heavy line) for both susceptible and resistant biotypes. The R^2 value for the susceptible group is 0.66 and for the resistant group is 0.49, the variability in the results is due largely to the genetic variability of the material. In Figure 1 and 2 the plants grown from resistant and susceptible maternal material has been crossed to either the susceptible or resistant parent. In Figure 1 the LD_{50} of the resistant maternally derived material is about three times that of the susceptible material when treated with Hoegrass® when crossed to a susceptible pollen source. When crossed to a resistant pollen source the LD_{50} increased by about 50 fold. Figure 2 compares the survival of maternally derived susceptible and resistant F2 when mated to a resistant pollen source.

Competition within a wheat crop of susceptible and resistant components of segregating populations There were significant differences between resistant and susceptible components of each population in the P1 and P2 generations. The differences expressed in the means of each of tiller number, (susceptible 2.69 and resistant 1.89 tillers per plant), whole plant dry weight (0.97 g and 0.74 g) and whole plant seed number (Table 1), indicate that there is potential for the susceptible component to increase and contribute

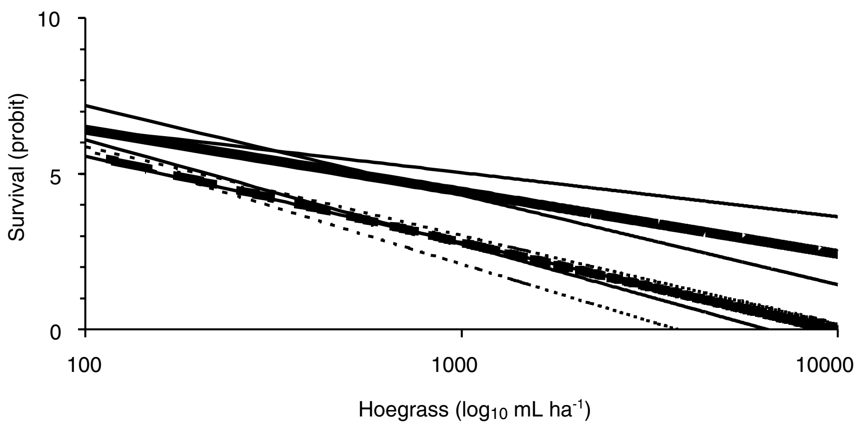

Figure 1. Survival (probits) of susceptible and resistant F2 maternally derived plants backcrossed to susceptible parents. Susceptible plants shown in dashed lines, resistant in solid lines, heavy lines are the mean of three biotypes.

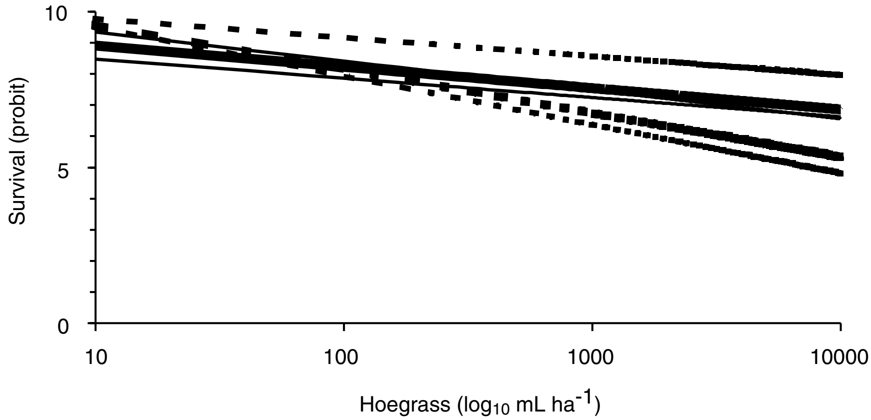

Figure 2. Survival (probits) of susceptible and resistant F2 maternally derived plants backcrossed to resistant parents. Susceptible plants shown in dashed lines, resistant in solid lines, heavy lines are the mean of three biotypes.

both in number and pollen availability to further generations.

Pollen production by resistant and susceptible plants drawn from segregating populations Gravid florets were picked from various plants within segregating populations, pollen from these maturing florets was counted and the relationship to floret fresh weight established (Table 2).

The relationship between pollen grain number and floret weight is not high with R^2 for the susceptible plants 0.42 and for resistant plants 0.32. Some variability due to position on the tiller and the tiller age can be expected. The trend indicates that susceptible plants may have more pollen and it increases with floret weight more than the resistant plants. The average floret and pollen grain count is shown in Table 2.

Populations planted at 1:1 ratio of susceptible ryegrass to speed up the onset of susceptibility Ryegrass seeds of each resistant parental biotype and one susceptible biotype were established in separate plots. In each case the susceptible had a greater dry weight than the resistant biotype. The mean dry weight and the relative proportion of dry weights of the resistant biotype are shown in Table 3.

Although the differences in dry weight were not statistically significant due to variability they showed the potential of susceptible ryegrass to accumulate more biomass than the biotypes of resistant ryegrass and to contribute proportionally more to the subsequent generations. A mixture or better adapted susceptible strains may provide more competition than the susceptible strain used.

Table 1. Seed output for R and S components of the P1 and P2 generations.

Populations	Seed number plant^{-1}	
	Susceptible	Resistant
SR31 P1	19.71	18.36
TS P1	37.10	19.35
TD P1	21.43	23.85
SR31 P2	38.25	18.77
TS P2	43.11	20.32
TD P2	42.83*	25.36[A]

[A] All biotype comparisons are sig. diff. $P < 0.05$ except where indicated.

Table 2. Floret fresh weight (mg) and mean number of pollen gains extracted from at least 35 florets from resistant and susceptible types.

Biotype	Mean floret weight (mg)	Mean pollen grain (number mg^{-1})
Susceptible	16.0	15,034
Resistant	14.8	12,722

Table 3. Mean dry weight (g) of S and R P0 ryegrass plants.

Biotype	Resistant	Susceptible	R/S[A]
SR31 P0	0.577	1.0856	0.532
TS P0	1.245	1.532	0.818
TD P0	2.012	2.101	0.958

[A] The differences in dry weight were not sig. diff.

Results of field trials At one field site resistance to the field rate of Hoegrass® and Logran® declined (Tables 5 and 6). In all plots without herbicide treatment there were no significant differences between treatments. The data from the control plots (isolation plots) is also presented in Tables 5 and 6. All three populations originally displayed high levels of resistance to these herbicides. SR31 P0 was 72% and 48% resistant to the recommended rate of Hoegrass® and Logran respectively, TS P0 87% and 19% and TD P0 79% and 25% resistant. At the second field site with an existing seedbank resistance did not decline. It is assumed that resistant ryegrass at the site and the level of ryegrass pollen from adjoining areas influenced the experimental outcome.

Table 4. Survival of previously resistant ryegrass plants to the field rate of Logran®.

	Survival %[A]		
	SR31	TS	TD
Isolation plots	1	1	1
Field plots	7.5	5.1	–

[A] No sig. differences (P <0.05) within sites.

Table 5. Survival of previously resistant ryegrass plants to the field rate of Hoegrass®.

	Survival %[A]		
	SR31	TS	TD
Isolation plots	8.5	35	20
Field plots	30.4	26	–

[A] No sig. differences (P <0.05) within sites.

DISCUSSION

Several elements of fitness in herbicide resistant annual ryegrass biotypes were investigated. Under competition, susceptible plants generated by the segregating populations tended to be more productive than the resistant component. Pollen and seed production were greater in the susceptible components than in the resistant plants. Thus the susceptible biotypes generated by re-assortment can be fitter than the resistant fraction of the population from which they were drawn and may generate further introgression due to relatively higher output of pollen. There is likely to be a cumulative aspect of an increasing proportion of susceptible plants in a population due to the genetic influence also. For populations that have gone almost to fixation, the introduction of susceptible ryegrass can provide a source of susceptible genes to accelerate the process.

The frequency of resistant plants declined in field plots in one of two sites. After three generations resistance had declined by about 70%. The difference in the isolation plots compared to the field plots is not great and indicates that field based attempts to reverse herbicide resistance may have success. The genetic aspects of resistance can be managed to provide a decrease in resistance such that a return to a high level of susceptibility can be achieved.

ACKNOWLEDGMENTS

The project was supported by the Grains Research and Development Corporation. Technical assistants were Peter Blackburn, Shahajahan Miyan and Darryl Miegel.

REFERENCES

Matthews, J.M., Holtum, J.A.M., Liljegren, D.R., Furness, B. and Powles, S.B. (1990). Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*). *Plant Physiology* 94, 1180-1186.