REVIEWS

Weeds resistant to herbicides in Australia and contributing factors leading to their appearance

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Summary

Two species of plants have been confirmed as being resistant to herbicides in Australia. A population of *Hordeum leporinum* ssp. *glaucum* Steud., at Willaura, Victoria, was found to be resistant to paraquat. The mechanism of this resistance was identified as the binding of paraquat within the cell walls of the resistant plants, thus excluding it from symplasm. The second resistant species is *Lolium rigidum* Gaudin which had developed in various areas of Australia. The initial populations were identified as resistant to diclofop-methyl but further work showed them to be resistant to a wide range of grass herbicides. The mechanism of this resistance has not been identified.

The factors that may contribute to the appearance of resistance: generation time, initial mutation frequency, selection pressure, fitness and seed carryover are discussed with reference to these two resistant species.

Introduction

Resistance of plants to herbicides in Australia has been confirmed in two species, *Hordeum leporinum* ssp. *glaucum* Steud. (barley grass) and *Lolium rigidum* Gaudin (annual ryegrass), while investigations are being undertaken for resistance in populations of *Artcotheca calendula* (capeweed) and *Avena* sp. (wild oats/black oats).

Resistant barley grass

A resistant population of *H. leporinum* ssp. *glaucum*, isolated from a lucerne paddock near Willaura in the Western District of Victoria, was first reported by Warner and Mackie 1983. Subsequent articles have also reported the level of this resistance (Powles 1986; Warner 1984) and findings of studies of its mechanism (Powles 1986).

The original resistant population of *H. leporinum* ssp. *glaucum* was derived from a continuous stand of lucerne (*Medicago sativa* L.) which had been sprayed annually for 15 years for the control of winter-growing weeds. Throughout this period good control was achieved of the local barley grass species: *Hordeum leporinum* Link, *Hordeum glaucum* Steud. and *Hordeum marinum* Huds. However, in 1981, following application of the recommended rate of 200 g a.i. ha^{-1} paraquat, poor control of barley grass was evident in one of the four paddocks sprayed.

The barley grass survived an application of 325 g a.i. ha^{-1} paraquat plus 195 g a.i. ha^{-1} diquat (2.6 L ha^{-1} Sprayseed) in early July 1982 and a test application of 560 g a.i. ha^{-1} paraquat (2.8 L ha^{-1} Gramoxone plus wetter) made in mid-July 1982. Plants were taken from this remaining population and a previously unsprayed barley grass population growing nearby. Individual plants from each source were separated out, transplanted into plastic pots and grown outside. The plants were sprayed with 100, 200, 400, 800 g a.i. ha^{-1} paraquat in 200 L ha^{-1}. The resistant barley grass, although scorched initially, survived at the 800 g a.i. ha^{-1} rate whilst the susceptible barley grass was killed at the 200 g a.i. ha^{-1} rate.

The resistant barley grass was identified as *Hordeum leporinum* ssp. *glaucum* Steud. and the susceptible form as *Hordeum leporinum* Link (Warner and Mackie 1983).

It was established in experiments with isolated chloroplasts and protoplasts from the resistant population that the paraquat-resistant biotype has no difference in the active site of paraquat interaction with photosystem 1 nor any changes in permeability of paraquat through the plasmalemma or chloroplast envelope membranes. Also, there was no difference between biotypes in the enzymic capacity to detoxify excited oxygen states (Powles and Cornic, personal communication). Further studies (Bishop *et al.* 1986) showed that the mechanism of resistance was the binding of paraquat within the cell walls thus excluding it from the symplasm.

The resistant *H. leporinum* ssp. *glaucum* has now been reported to infest five paddocks within the vicinity of the original paddock. Two of these are believed to have arisen from hay cut in the original resistant paddocks (Powles, personal communication). The remaining three paddocks are believed to have populations that have established separately. Further studies of *H. leporinum* ssp. *glaucum* populations in Victoria and South Australia have not shown any resistant populations (S. B. Powles, personal communication).

Resistant annual ryegrass

In 1980, a farmer reported that he believed a population of annual ryegrass (*Lolium rigidum* Gaudin) had developed on his property near Bordertown, South Australia, that was no longer controlled by diclofop-methyl. Over several years the farmer had noted a diminution in the ability of diclofop-methyl to control the annual ryegrass population.

Heap and Knight (1982) compared the suspected ryegrass population with a population growing on part of the property that had never been treated with diclofop-methyl. For an application of diclofop-methyl at 375 g a.i. ha^{-1}, the commercial recommended rate, there was only 14% mortality of the resistant population and 74% mortality of the susceptible population. At 1500 g a.i. ha^{-1} diclofop-methyl the mortalities were 8% and 98% respectively.

Heap and Knight (1986) showed that the resistant population in Bordertown was both cross-resistant (within chemically similar groups) and wide-resistant (between chemically dissimilar

Table 2 Herbicide resistant paddock histories of annual ryegrass populations

Year	Bordertown S.A.	Esperance W.A. (Koecher)	Esperance W.A. (T. Piper)	Dowerin W.A.	Glenrowan Vic.
1968	B (1968–1976 bracketed)				
1969					
1970					
1971					
1972	T (possibly yearly)				
1973					
1974		no herbicide			
1975		S 2.5			pasture
1976		S 1.2	trifluralin?		–
1977	H 2.0	S 2.0	trifluralin?		trifluralin
1978	H 2.0	S 2.0	H 1.0		–
1979	H 1.5 + H 0.75	S 2.0 + H 1.5L	H 1.5L	H 0.75	H 2.0 + S 2.0
1980	H 3.0 + H 2.0	H 1.5	H 1.0	H 1.0	H 1.5
1981		S 2.0 + H 1.0L	H 1.0	H 0.75	H 1.5
1982		H 1.0*	H 2.0	H 0.75	H 1.5 + S 2.0
1983		S 2.0	H 1.5 + G 20	S 1.75	H 1.5
1984				H 1.0#	H 1.5
1985				S 1.75 + F 0.5	F 0.5 + H 1.0 ~ H 1.5

* ryegrass recovered and choked crop; # poor control; ~ very poor control

Herbicides

G = Glean (g) 750 g a.i. kg^{-1} chlorsulfuron
H = Hoegrass (L) 375 g a.i. L^{-1} diclofop-methyl
S = Simazine (L) 500 g a.i. L^{-1} simazine
F = Fusilade (L) 212g a.i. L^{-1} fluazifop-butyl
T = Treflan (L) 400 g a.i. L^{-1} trifluralin
B = Balan (L) 200 g a.i. L^{-1} benfluralin

groups). The three diphenyl-ether-type herbicides tested were fluazifop-butyl, oxyfluorfen and CGA82725, and the two sulfonyl urea herbicides were chlorsulfuron and metsulfuron-methyl. The results show that the diclofop-methyl-resistant biotype was resistant also to fluazifop-butyl, CGA82725, chlorsulfuron and metsulfuron-methyl but not to oxyfluorfen. Further work has shown this population to have varying levels of resistance to the wide range of herbicides that is shown in Table 1.

Heap (1984) indicated that the Bordertown resistant population was less competitive than the susceptible population, but this relative competitive ability is reversed when 94 g a.i. ha^{-1} diclofop-methyl is applied.

Recent research on a population of annual ryegrass from Bordertown that had received applications of trifluralin or benfluralin for 10 years, but no diclofop-methyl, showed that it was susceptible to diclofop-methyl. However, the use of sethoxydin for 2 years after the application of diclofop-methyl increased the level of resistance to diclofop-methyl (J. Heap, personal communication).

In 1982, at the Esperance Research Institute in Western Australia, two populations of *L. rigidum* were reported to be resistant to diclofop-methyl (T. Piper, personal communication; H. Koecher, personal communication). The herbicide paddock histories of these two populations, the Bordertown population and two populations where resistant ryegrass is expected but not confirmed, are shown in Table 2.

A population from the long-term continuous rotation trial at Esperance was tested by Koecher (personal communication) for resistance. The population was shown to be resistant to isoproturon, chlorsulfuron, alloxydim, fluazifop-butyl but not to sethoxydim.

Table 1 Annual ryegrass cross-resistance (Bordertown biotype)

Resistant (> 5-fold)
Hoegrass – diclofop-methyl
Fusilade – fluazifop-butyl
Topik – CGA-82725
Glean – chlorsulfuron
Ally – DPX-T6376
Logram/Amber – CGA-131036

Moderate resistance (2 < Res < 5-fold)
Treflan – trifluralin
Fervin – alloxydim
Lexone – metribuzin

Slight resistance (< 2-fold)
Kerb – propyzamine
isoproturon
Gesatop – simazine
Sertin – sethoxydim

No resistance
Goal 2E – oxyfluorfen
Gramoxone – paraquat

The confirmed resistant populations of annual ryegrass at Bordertown and Esperance have remained restricted to discrete paddocks. This was confirmed by Heap and Knight (personal communication) who sampled along a transect commencing in the Bordertown paddock and continuing into adjoining paddocks. The resistance stopped dramatically at the uncultivated fence line and only increased slightly in the adjoining paddock, which had received some herbicide usage. Thus, it is concluded that the risk through pollen dispersal is minimal.

Since the confirmation of the original populations being resistant to diclofop-methyl, resistance has been confirmed on one property at Dowerin, Western Australia, and a number of other properties around Bordertown, South Australia. It is expected that resistant populations may be confirmed at Clare (South Australia), Howlong (New South Wales), and Glenrowan (Victoria) in tests to be carried out in early 1987 on samples collected in November 1986.

Results from experiments conducted by Dr H. Koecher, of Hoechst A.G. and Dr S. Powles of the Waite Agricultural Research Institute in Adelaide (personal communication) have indicated that there appears to be no difference between the Bordertown resistant population and susceptible strains of *L. rigidum* as far as the following parameters are concerned. These parameters are: uptake and translocation of diclofop-methyl, metabolism of diclofop-methyl in the plant, inhibition of lipid bio-synthesis, respiratory rates, and genetic composition.

Discussion

Thus, in Australia there are two grass species with populations that have confirmed resistance to herbicides. For each species, the extent of its distribution, the possible cross-resistance, the mechanisms of resistance and the selection criteria appear to be different, so it may be possible to use these variations when looking at the factors leading to the appearance of resistance.

Gressel and Segel (1978) and Gressel (1979*a*; 1979*b*) listed the following factors that they believed would affect the rate of build-up of resistance within a population: generation time, initial mutation frequency, selection pressure, fitness, and seed carryover.

Generation time

One reason put forward for the low occurrence of herbicide resistance, when compared with both insecticide- and fungicide-resistance, has been that usually only one generation is contacted by the herbicide during an agricultural year. We believe this to be true for both resistant populations discussed in this paper.

Initial mutation frequency

In neither of the resistant populations has the initial level of resistant plants within the population been established. This initial frequency depends on the number of genes involved, the dominance and the ploidy. Work is being undertaken to establish if the gene for resistance in *H. leporinum* ssp. *glaucum* can be isolated and transferred to other species (Cornic, personal communication).

Selection pressure

The greater the kill rate of the herbicide, the more rapidly a population will become enriched with resistant strains, unless 100% kill can be achieved. Most herbicides are applied at rates that give 90–95% kill. At 90% kill, without intervening factors, there would be a ten-fold yearly enrichment of resistance in the population. This rate of enrichment is reduced in the field situation with the capacity of the surviving weeds to increase seed production and the ability to produce plants that effectively miss application by late germination. Le Baron and Segel (1982) estimated the variation in effective kill among herbicides to be between 40–70%.

In both the *H. leporinum* ssp. *glaucum* (Willaura) and *L. rigidum* (Bordertown) resistant populations, we would expect that this level of effective kill to be quite high. This is especially so in the former case where spraying is undertaken when the plants are well established and the infested crop is cut to produce hay, thus reducing the seeding of missed plants or late germinating plants.

We may also expect that the effective kill achieved with diclofop-methyl is high due to its normally good level of effectiveness (90–95%) (Anderson, personal communication).

Fitness

Haldane (1960) described the loss of fitness of a resistant population compared to the wild-type population as the 'cost' of selection. This reduction of fitness was shown for the Bordertown *L. rigidum* biotype (Heap 1984). The relative fitness was 0.81 when grown in pure stands and 0.64–0.67 when grown in mixed stands. This level of fitness variation has not been determined for the resistant *H. leporinum* ssp. *glaucum* (Willaura) population.

Seed carryover (seasonality/seed bank)

Spaced-out germination of weeds within the one season and the carryover of viable seeds to form a seed bank are important in the selection pressure exerted on the weed population.

Smith (1966) measured the germination of *L. rigidum* and *H. leporinum* under controlled temperature conditions with a diurnal range of 20–30°C. He recorded germination percentages of 96% for both species.

McGowan (1970) found that most *L. rigidum* seed, about 80%, had germinated by the end of May, although 5% of germination could occur in the field as late as July or August. *H. leporinum* germination was above 90% before the end of May and only 1% or less germinated after July. He concluded that differences between *L. rigidum* and *H. leporinum* in their proportion of late-germinating seed would be quite important in the infestation of winter cereals and possible carryover.

Gramshaw and Stern (1977), Cocks and Donald (1973) and Smith (1968) investigated the factors affecting the level of germination in the field of *L. rigidum*. They found that the depth of seeding, effect of light, diurnal temperatures and moisture all affected the percentage germination measured in the field. These three factors will affect the actual level of germination in any one year.

Cheam (personal communication) showed in Western Australia that 15–25% of seed production remained ungerminated after the first season if seeds had been incorporated shallowly into the soil. The proportion of ungerminated seeds remaining alive two years after burial also varied according to depth of burial and district conditions. Deep burial resulted in a greater loss of seed viability. For example, at 1 cm depth 9.8% of the seeds remained alive, whereas at 15 cm only 3.3% remained alive (based on results averaged over three burial sites: Mt Barker, Northam and Chapman). The seed longevity data therefore questions the previously held belief that ryegrass seed does not persist for more than one season.

Popay (1981) concluded after his experiments on the germination of five annual barley grass species, including *H. leporinum* ssp. *glaucum*, that most seeds of all the species germinated readily in response to autumn rains and relatively few weeds survived to germinate in the winter and spring. He also suggested that there is little, if any, long-term dormancy in barley grass seeds and that very few seeds are likely to be present after a year (<1%).

The early germination and very low level of seed carryover of *H. leporinum* ssp. *glaucum* would combine to make a large proportion of the population subject to selection from paraquat. For *L. rigidum* its ability to continue germinating later in the season and build up a seed bank with viable seeds present 2 years after burial would reduce the percentage of the population exposed to any one herbicide application. Thus we may expect that the effect of enrichment of resistance in a population would be lower in *L. rigidum* than in *H. leporinum* ssp. *glaucum*.

Interrelationships between these factors

Gressel and Segel (1978) developed a series of mathematical considerations to evaluate the relative effect of each of the above factors. From this model the greatest effect on reducing the rate of appearance of herbicide resistance is the level of selection pressure, which can be considered as the effective kill. The fitness differential is a less important modifier, while the seed bank would be a major modifier. It was concluded that only at high selection pressure should resistance appear within 10 years, although the examples studied in Australia have shown this enrichment to occur—in the case of *L. rigidum*, after only four applications of diclofop-methyl.

The resistant *H. leporinum* ssp. *glaucum* biotype enrichment occurred within a perennial crop that has received the same herbicide every year for over 12 years. It has been subject neither to cultivation nor to strong crop competition, as the paraquat application is carried out during late winter when the lucerne is semi-dormant. These factors would fit well into the model suggested by Gressel and Segel (1978) to lead to the appearance of a resistant biotype.

With the appearance of the resistant ryegrass we have a greater variation in conditions under which the resistant populations have arisen. It may be considered that this enrichment is due to:

(i) the proportion of resistant individuals within the original population being

higher than experienced in other resistant species;
(ii) little seed carryover; and
(iii) a high level of effective kill.

These factors are experienced for a large proportion of the areas of herbicide usage in Australia. To determine the present situation in respect of resistant populations and what can be expected in the future will require the combined resources of the agricultural chemical industry, government institutions and, most importantly, the herbicide users.

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Erratum

There was an error in the references of the paper by P. D. Howat 'Weeds resistant to herbicides in Australia and contributing factors leading to their appearance' in Volume 2, Number 2 of *Plant Protection Quarterly*.

The references should have been attributed to Heap, I. (1984) not Heap, J. (1984) and to Heap, I., and Knight R. (1986) not Heap, J. and Knight, R. (1986).