RESEARCH REPORTS

Occurrence and distribution in south-eastern Australia of barley grass (*Hordeum glaucum* Steud.) resistant to paraquat

E. S. Tucker and S. B. Powles
Department of Agronomy, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia, 5064.

Summary

Paraquat-resistant barley grass, identified as *Hordeum glaucum* Steud. was first observed and reported in 1982 on a lucerne (*Medicago sativa* L.) field at Willaura, Victoria. A survey aimed at determining the occurrence and distribution of this biotype was undertaken by collections from fields in Victoria and South Australia. Chromosome counts were made to confirm the species collected in each case. Tests for resistance were carried out by observing the effect of low concentrations (10 mM) of paraquat on seedling germination and resistance was confirmed after spraying plants with paraquat at the recommended rate of 200 g a.i. ha^{-1}. The survey reveals that resistance to paraquat was evident only in the Willaura–Ararat region on four independently owned and operated farms that are well separated from each other. Resistant populations were also encountered on other fields within these areas but these were determined as having been introduced through movement of stock, machinery and hay. No resistant biotypes were found in other regions of Victoria or in fields surveyed in South Australia. The reasons underlying the appearance of the resistant biotypes are discussed.

Introduction

A recent review states that populations of 55 weed species world-wide have developed resistance to various herbicides to which they were previously susceptible (LeBaron 1985). Over three-quarters of the reported cases involve specific resistance to the triazine herbicides. Resistance to paraquat has occurred in biotypes of the weed species *Poa annua* L. and *Conyza* sp. (Gressel *et al.* 1982) and *Erigeron* spp. (Saka *et al.* 1982; Watanabe *et al.* 1982; Kato and Okuda 1983).

A paraquat-resistant biotype of an important grass weed, barley grass (*Hordeum glaucum* Steud.), became evident in 1982 in a field at Willaura, Victoria (Warner and Mackie 1982). Studies have confirmed that this biotype is resistant to paraquat; the LD_{50} for the resistant biotype is 3.2 kg a.i ha^{-1}; that for the normal susceptible biotype is 0.025 kg a.i. ha^{-1} (Powles 1986). The resistant biotype was infesting a lucerne (*Medicago sativa* L.) field. The standard practice employed for weed control in such fields is the application of paraquat and/or diquat to control winter-growing weeds. Previously the use each year of these herbicides on this field had been successful in controlling barley grass for more than 15 years.

The appearance of a paraquat-resistant barley grass biotype within the cereal-cropping zone of southern Australia is of both practical and scientific concern. Accordingly, this study was undertaken to identify areas in south-eastern Australia where such populations might occur.

Materials and methods

Species identification

Three species of barley grass commonly occur in mainland Australia, namely *Hordeum glaucum*, *H. leporinum* and *H. marinum*. A fourth species, *H. murinum* is found only in Tasmania (Cocks et al. 1976) *H. murinum* has a sessile central spikelet which readily distinguishes it from *H. glaucum* and *H. leporinum*. *H. marinum* is readily distinguished from either *H. leporinum* and *H. glaucum* by having the lemmas of the lateral spikelet (including the awns) much shorter than the glumes (including the awns). *H. glaucum* retains dark-coloured anthers within its florets at maturity, whereas these are exerted in *H. leporinum*. This characteristic was used to identify *H. glaucum* in the field during the survey. Collected samples were stored in the laboratory for 3 months before growing plants from them. For each population root tips from at least one randomly selected individual were taken and chromosome counts made using the procedure described by Morrison (1959). *H. glaucum* is a diploid ($2n = 14$) whereas *H. leporinum* is a tetraploid ($2n = 28$).

Survey

Surveys were carried out during November and December 1984 and 1985 in lucerne-growing districts in Victoria and South Australia. Samples were collected prior to the first lucerne cuts so that mature spikes could be gathered before they shed or were harvested with the lucerne. The epicentre of the survey was the lucerne field in the Willaura region first found to have paraquat-resistant barley grass. Within a 100 km radius of this field a total of 99 fields were surveyed. Of these, 20% were lucerne fields with a history of paraquat and/or diquat usage. Along the Pyrenees Highway between Ararat and Avoca another 32 fields (22 of them lucerne) were surveyed. Eight lucerne fields were surveyed within the Bendigo area and 10 lucerne fields within the Boort area. Two lucerne fields at Casterton, near the South Australian border with Victoria, were also surveyed. In South Australia, 23 lucerne fields were surveyed within the Keith and Bordertown regions and 10 in the Langhorne Creek area (Table 1). These regions represent the main lucerne-growing regions within these States (Figure 1).

On each field, matured spikes of *H. glaucum* were randomly collected from as many individual plants as possible. Fields in which only *H. leporinum* or *H. marinum* were present were recorded and the seeds of these species were then discarded.

Determination of resistance

Effect of paraquat on germinating seedlings

A seedling bioassay test was used to provide an initial evaluation of the response of the samples to paraquat. At low concentration (1.5 μM) primary shoot elongation is greatly inhibited in susceptible biotypes of *H. glaucum*, while growth of the resistant biotype is unaffected (Powles 1986). Fifty seeds from each sample were incubated in the dark at 20°C in 9 cm Petri dishes given 5 ml of 10 μM paraquat solution. *H. glaucum* seeds collected from the same region and previously tested and found to be susceptible to paraquat, and seeds known to be paraquat resistant, were used as controls. All samples were also incubated in distilled water under the same conditions. Germinated seedlings were examined after 10 days. Samples were then classed as resistant or susceptible on the basis of the inhibition of shoot growth.

Effect of foliar application of paraquat

Paraquat at 200 g a.i ha^{-1} with 0.2% surfactant was sprayed on to intact plants at the 2–3 tiller stage using the sprayer and procedure described by Powles (1986).

Table 1 Occurrence of various species/biotypes of barley grass in fields surveyed. All fields contained *Hordeum leporinum*

Region	No. of fields surveyed	No. of fields with Resistant *H. glaucum*	Susceptible *H. glaucum*	*H. leporinum* only
		Victoria		
Willaura	99	6	11	82
Ararat	32	3	23	6
Bendigo	8	0	2	6
Boort	10	0	1	9
Casterton	2	0	0	2
		South Australia		
Langhorne Creek	10	0	8	2
Bordertown-Keith	23	0	7	16
Total	184	9	52	123

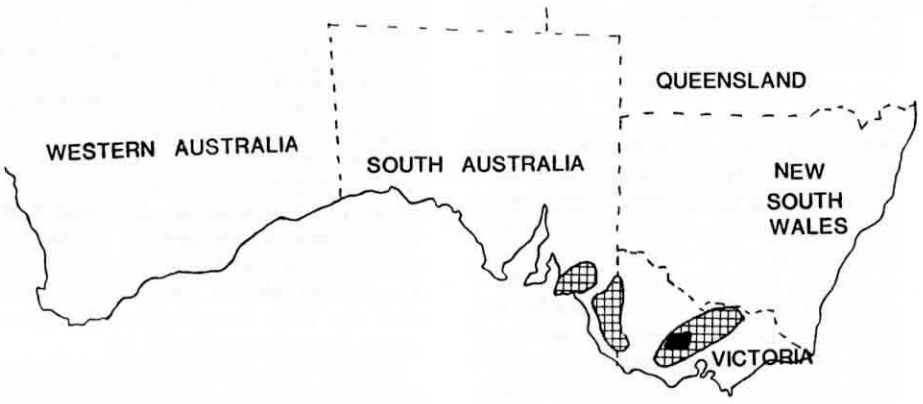

Figure 1 Map of southern Australia showing the survey region (hatched area) and the area in which paraquat-resistant *H. glaucum* was found (solid area).

Control plants were as described in the seedling bioassay test. Twenty plants of each sample were sprayed and resistance or susceptibility was determined on the basis of plant survival 10 days after treatment.

Results

The survey reveals that, with the exception of fields infested by the resistant *H. glaucum* biotype, *H. leporinum* is the dominant barley grass species present in the fields surveyed (Table 1). Normal paraquat-sensitive *H. glaucum* was present in a large number of fields but was not as prevalent as *H. leporinum*. Less than 5% of the lucerne fields surveyed had *H. marinum*. It is emphasized that *H. glaucum* (except for the resistant biotype), *H. leporinum* and *H. marinum* are normally susceptible to paraquat.

The survey documents a paraquat-resistant biotype of *H. glaucum* present in populations of barley grass on nine of the 184 fields surveyed (Table 1). This represents 4.9% of the total number of fields surveyed. All of the resistant samples were found in lucerne fields surveyed in the Willaura–Ararat region of Victoria (Figure 1). There was complete correlation between resistance evident in the seedling bioassay test and resistance of potted plants to treatment with 200 g a.i ha^{-1} paraquat.

Victoria

Willaura region Lucerne fields on three separate farms were found to be infested with paraquat-resistant biotypes of *H. glaucum*. On one farm, 'Edgarley', four lucerne fields (including the field in which the resistant *H. glaucum* was first discovered) were severely infested with the resistant biotype.

Ararat region The resistant *H. glaucum* biotype was found on one lucerne field and two non-lucerne fields in this region.

Bendigo and Boort regions The resistant biotype was not found in any of the fields surveyed in these two regions. Only lucerne fields with a history of paraquat and/or diquat use were surveyed in these areas.

South Australia

The resistant biotype was not found in any of the fields surveyed in South Australia. Only lucerne fields with a history of paraquat and/or diquat use were surveyed in South Australia.

Discussion

The paraquat-resistant biotype of the grass weed *H. glaucum* was found to be present in populations of barley grass on a small number of lucerne fields in the Willaura–Ararat region only. The three farms in the Willaura region where resistant biotypes were found are independently owned and operated enterprises that are well separated from one another. One of the farms, 'Edgarley', had four lucerne fields infested with the paraquat-resistant biotype. This includes the field in which the resistant biotype was first discovered. At the time, the other three fields did not have the problem. The appearance of resistant biotypes in these other lucerne fields at the time of our survey is likely to be due to an introduction from the originally infested field as there has been considerable movement of stock, hay and machinery between the fields. We established that two fields (non-lucerne) in the Ararat region have also become infested with the resistant biotype as a result of the transfer from 'Edgarley' of hay containing the seeds of the resistant biotype. However, there was no reason to suspect that movement of seed of the resistant biotype from 'Edgarley' could explain the infestations on the other three enterprises. Thus, the paraquat-resistant biotype was evident at four independent locations.

Common factors associated with the appearance of paraquat resistance.

The lucerne fields with the original infestation at 'Edgarley', the lucerne fields on the other two farms in the Willaura region and the lucerne field in the Ararat region, had all been sown to lucerne for considerable periods. All had received once-yearly applications of paraquat and/or diquat as the sole method of weed control. The common factors can be summarised as:

1. All fields contained the same perennial crop for a period exceeding 10 years.
2. Paraquat and/or diquat was used once-yearly.
3. No other herbicides, or other forms of weed control, were used.
4. No cultivation had occurred for at least 10 years.

Obviously the long-term combination of the above-mentioned factors has provided positive selective pressure for the paraquat-resistant biotype. Studies with herbicide-resistant weed species in the Northern Hemisphere have also been able to identify common factors associated with the appearance of herbicide resistance (Holliday *et al.* 1976; Putwain 1982; Gressel and Segel 1982; Bachthaler *et al.* 1984).

Despite the positive selection pressure operating (consistent and prolonged use of paraquat as the sole means of weed control on a perennial crop), we found that only a small percentage of lucerne fields surveyed were infested with the resistant

biotype. If the resistant biotype occurs randomly in the barley-grass population we should expect that the resistant biotype would appear on many lucerne fields at several locations, but our survey revealed that the paraquat-resistant biotype occurred only in the Willaura–Ararat area. It is possible that the resistant biotype appeared following a mutation after commencement of paraquat use in this area. However, we found resistant populations at four distinct sites. We have no evidence that the resistant biotype could have been introduced from a single site. It is, therefore, not possible to define whether paraquat resistance was originally present within the barley-grass population or has occurred as the result of a mutation following paraquat use.

Conclusion

Our survey shows that a paraquat-resistant biotype of the weed *H. glaucum* is presently confined to a small number of lucerne fields in the Ararat area where paraquat has been consistently used for a long time. We have identified a number of common factors associated with the occurrence of this resistant biotype. Potential exists for this biotype to be transferred and established in other areas by the movement of stock, machinery and hay.

Acknowledgments

The assistance of Mr Tom Morgan, District Agronomist, Department of Agriculture and Rural Affairs, Ararat, is gratefully acknowledged. The cooperation of Mr Richard Warner of I.C.I. Australia was indispensible. Financial assistance was provided by the Australian Development Assistance Bureau for a large portion of the travel cost of this work.

References

Bachthaler, G., Kees, H., and Dinzenhofer, B. (1984). The development of resistance in weeds after repeated application of herbicides, particularly triazoles. *BASF Agricultural News* **4/84**, 16–24.

Cocks, P. S., Boyce, K. G., and Kloot, P. M. (1976). The *Hordeum murinum* complex in Australia. *Australian Journal of Botany*, **24**, 651–62.

Gressel, J., Ammon, H. U., Fogelfors, H., Gasquez, J., Kay, Q. O. N., and Kees, H. (1982). Discovery and distribution of herbicide-resistant weeds outside North America. *In* 'Herbicide Resistance in Plants'. pp. 31–55, eds. H. M. LeBaron and J. Gressel. (John Wiley & Sons: New York.)

Gressel, J. and Segel, L. A. (1982). Interrelating factors controlling the rate of appearance of resistance: The outlook for the future. *In* 'Herbicide Resistance in Plants', pp. 325–347, (eds. H. M. LeBaron and J. Gressel). (John Wiley & Sons: New York.)

Holliday, R. J., Putwain, P. D., and Dafni, A., (1976). The evolution of herbicide resistance in weeds and its implication to the farmer. *In* 'Proceedings 1976 British Crop Protection Conference – Weeds, pp.937–46.

Kato, A., and Okuda, Y. (1983). Paraquat resistance in *Erigeron canadensis*. *Weed Research (Japan)* **28**, 54–6.

Lebaron, H. M. (1985) Herbicide resistance in plants. Proceedings, 37th California Weed Conference, pp. 38–49.

Morrison, J. W. (1959). Cytogenetic studies in the genus Hordeum; 1. Chromosome morphology. *Canadian Journal of Botany* **37**, 527–38.

Powles, S. B. (1986). Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. *Weed Research* **26**, 167–72.

Putwain, P. D. (1982) Herbicide resistance in weeds – an inevitable consequence of herbicide use? *In* Proceedings 1982 British Crop Protection Conference – Weeds, pp. 719–28.

Saka, H., Chisaka, H., and Uesono, T. (1982). Paraquat resistance in *Erigeron philadelphicus* L. *Weed Research, (Japan)* **28** (Suppl.), 61–2.

Warner, R. B., and Mackie, W. B. C. (1983). A barley grass "*Hordeum leporinum* spp. *glaucum* Steud." population tolerant to paraquat (Gramoxone). *Australian Weed Research Newsletter* **31**, 16.

Watanabe, Y., Honma, T., Ito, K., and Miyrahara, M. (1982) Paraquat resistance in *Erigeron philadelphicus* L. *Weed Research (Japan)* **27**, 49–54.