Summary  The barley grasses (Hordeum glaucum Steud. and Hordeum leporinum Link.) are important weeds of crops and pastures in South Australia. Populations of both species have evolved resistance to paraquat, primarily following intensive use of paraquat for winter weed control in lucerne (Medicago sativa L.) crops. In the past few years, agricultural consultants have been reporting an increase in problems with Lolium spp. in lucerne crops in South Australia. This research was conducted to determine the relative importance of seed movement compared with independent evolution for paraquat resistance in Hordeum spp. Hordeum glaucum and H. leporinum seed was collected from lucerne fields and fields immediately adjacent to the lucerne fields at several sites in South Australia. The seed was tested to determine which fields contained resistant populations. As H. glaucum and H. leporinum are self pollinated, RAPD technology was used to examine diversity within and between populations. Individual resistant populations contained much less genetic diversity than did adjacent susceptible populations indicating the existence of a selection bottleneck caused by herbicide use. Most resistant populations were different genotypes; however, two resistant populations separated by 7 km appeared to be the same genotype. These results suggest that both independent evolution and seed movement are important in the distribution of paraquat-resistant Hordeum spp. in South Australia.

Keywords  Paraquat, herbicide resistance, Hordeum glaucum, gene flow.

INTRODUCTION

Hordeum glaucum Steud. and H. leporinum Link. are two important weedy grasses with populations that have evolved resistance to paraquat in Australia (Warner and Mackie 1983, Tucker and Powles 1991). At present there are more than 20 sites in south-eastern Australia known to harbour paraquat-resistant Hordeum spp. The vast majority of sites are lucerne (Medicago sativa L.) fields; however, paraquat resistance is also known from two wheat (Triticum aestivum L.) fields in South Australia (Alizadeh et al. 1998). Paraquat is still the herbicide of choice for the control of Hordeum spp. in lucerne fields and is being used increasingly widely in other crops for control of weeds prior to crop seeding or at anthesis. Therefore, the spread of resistance to paraquat in cropping fields may threaten the usefulness of this herbicide.

Hordeum spp. seed contain sharp awns that can aggravate the mouths, nose and eyes of grazing animals and also contaminate wool or hides (Smith 1968). These sharp awns provide an effective means for movement of seed on stock or in equipment. Seed may also be moved as a contaminant of hay or grain. Tucker and Powles (1988) concluded from a survey of lucerne-growing properties in Victoria, that there was a high likelihood of movement of paraquat-resistant H. glaucum between properties in hay, on stock or in machinery.

There are increasing reports from agricultural consultants of paraquat-resistant Hordeum spp. in lucerne fields in South Australia. At the same time, paraquat is being widely promoted as a herbicide to be used in the management of glyphosate resistance in Lolium rigidum Gaudin (Neve et al. 2003). If paraquat-resistant Hordeum spp. were to be moving from lucerne fields to cereal fields, then the usefulness of paraquat as an alternative to glyphosate may be compromised. The objective of this study was to determine whether the increasing incidence of paraquat resistance in H. glaucum in South Australia is the result of independent evolution or of seed movement.

MATERIALS AND METHODS

Plant material and assay for resistance  Two physical surveys were conducted in the Mid North and Lower North of South Australia. The first survey was conducted in November 1999 and consisted of sampling Hordeum spp. seed from randomly selected fields in a triangle between Adelaide, Port Wakefield and Jamestown. Fields were selected at random along major and minor roads. Fields were walked in a ‘W’ pattern and any Hordeum spp. seed encountered was collected. The second survey was conducted in November 2001 and specifically surveyed lucerne fields and adjacent crop or pasture fields located between Jamestown and Spalding or between Kapunda and Waterloo. In 1999, 44 samples were collected, whereas in 2001, 23 samples were collected. The seed collected was stored until the following autumn, when it was planted out in pots containing potting mix. For each seed sample collected, between 50 and 100 seeds were sown. Emerged seedlings
were treated at the three leaf stage with 200 g a.i. ha⁻¹ paraquat as a commercial formulation (Gramoxone, 250 g a.i. L⁻¹, CropCare Australasia), with the addition of 0.2% non-ionic surfactant. Typically, susceptible plants show strong symptoms of paraquat damage within one or two days and are bleached and desiccated within seven days. Resistant plants show strong bleaching to existing leaves, but the base of the leaves remains green. Within seven days of paraquat application, new green leaves begin to emerge. Plants were scored for survival 21 days after paraquat application.

**DNA extraction and RAPD analysis** *Hordeum glaucum* is a self-pollinated species, which makes molecular markers a useful technique for determining the origins of genotypes. All progeny of a particular parent will be identical to that parent, saving new mutations, as there is no mixing of genes between individuals.

Fresh young leaves were harvested separately from eight plants for each population. Approximately 0.5 g of leaf material was cut using clean scissors, then wrapped in aluminium foil and frozen in liquid nitrogen. The leaf tissue was ground to a fine powder in liquid nitrogen with a mortar and pestle. Genomic DNA was extracted from the ground leaf tissue using the CTAB (cetyltrimethyl-ammonium bromide) method of Doyle and Doyle (1990). RNA was removed from the DNA with ribonuclease. The final DNA pellet was resuspended in 400 µL of 10mM Tris-HCl (pH 8.0), 1mM EDTA and stored at 4°C.

PCR amplification was performed with a single primer OPT-07 (GGCAGGCTGT). Amplification reactions contained 20–40 ng DNA, 0.25µM primer, 200µM dNTPs, 2mM MgCl₂, 10mM Tris-HCl (pH 8.8), 50mM KCl, 0.1% Triton X-100 and 1 unit of Taq polymerase. A negative control without a DNA template was included in each reaction. Polymerase chain reactions were conducted in a thermal controller programmed for 30 cycles with a thermal profile of 15 s denaturation at 94°C, 15 s for annealing at 36°C, and 30 s extension at 72°C. At the end of 30 cycles, a final extension was applied at 72°C for 2 min.

The PCR products were separated on polyacrylamide gels (Pharmacia Biotech) run at 10°C with running conditions of 200 V/20 mA for 20 min, 380 V/30 mA for 50 min and 450 V/30 mA for 30 min. PCR products were visualised following silver staining using a silver staining kit (Pharmacia Biotech).

**RESULTS AND DISCUSSION**

Of the 44 seed samples collected in 1999, only one proved to contain paraquat-resistant individuals. Of the 23 more targeted samples collected in 2001, seven contained resistant individuals. The site where resistance was detected in 1999 was re-sampled in 2001. Of the seven resistant populations detected, six were determined to be *H. glaucum* (designated SHG7, SHG8, SHG9, SHG10, SHG11 and SHG12) and one *H. leporinum* (designated SHL2). Over the two years of surveys, 13 samples from lucerne fields were tested and five proved to contain paraquat-resistant individuals. The other three resistant populations came from pastures and wheat crops. The locations of paraquat-resistant populations are shown in Figure 1.

In addition to these samples, we had previously obtained four other paraquat-resistant *H. glaucum* populations from South Australia. One was obtained from a lucerne field near Spalding (SHG1), a second from a lucerne field near Mt. Bryan (SHG5) and two others from no-till cereal fields near Avon (SHG3 and SHG4) (Figure 1).

RAPD analysis was performed on five paraquat-resistant *H. glaucum* populations. In all cases, we were unable to detect polymorphisms within resistant populations; however, we could detect polymorphisms within and between susceptible populations (data not shown). This is suggestive of a genetic bottleneck occurring in populations evolving paraquat resistance.

When herbicide resistant populations were compared (Figure 2), the banding patterns for most populations were different. Individual bands varied in their presence between populations. Some populations differed in only a small number of bands, whereas others

**Figure 1.** Map showing the location of known paraquat-resistant *Hordeum* spp. populations in South Australia.
were widely different. The exception was populations SHG8 and SHG9, which contained identical banding patterns, despite being collected from lucerne fields 7 km apart.

The results of this study show that paraquat resistance has evolved independently on a number of occasions in South Australia. In addition to four separate examples of evolution in *H. glaucum*, a paraquat-resistant population of *H. leporinum* was also detected. During our survey we were unable to find paraquat-resistant *Hordeum* spp. in fields adjacent to the lucerne fields containing resistant populations, suggesting that spread of resistance between adjacent fields is not a common phenomenon. However, two resistant populations of *H. glaucum* collected from lucerne fields 7 km apart appear to contain the same genotype. This suggests that paraquat-resistant *H. glaucum* could move between lucerne fields, and possibly also between lucerne fields and grain fields, most likely in hay or equipment. Consequently management strategies for paraquat-resistant *Hordeum* spp. need to not only reduce the selection pressure for resistance evolution, but may also need to consider ways of limiting seed movement.

In this study, local evolution was determined as the main reason for the appearance of paraquat-resistant *Hordeum* spp. However, with other weeds and cropping systems, seed or pollen movement may play an important role. For example, Andrews *et al.* (1998) concluded that seed movement played an important role in the spread of herbicide resistant *Avena fatua* L. Equally, Stankiewicz *et al.* (2001) argued that seed movement was important in the spread of herbicide resistant *Solanum nigrum* L. across Europe. In contrast, Cavan *et al.* (1998) concluded local evolution was most important in the evolution of herbicide resistant *Alopecurus myosuroides* Huds. on farms in the United Kingdom.

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