Evolution of paraquat resistance in barley grass (*Hordeum leporinum* Link. and *H. glaucum* Steud.)

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Summary The usefulness of herbicides can be eliminated by herbicide resistance in weed species. In Australia 25 weed species have been documented with resistance to one or more of nine herbicide groups. Two weedy barley grass species *Hordeum glaucum* Steud. and *H. leporinum* Link., infest crops and pastures in southern Australia. The intensive use of paraquat on these two species, principally in lucerne cropping and in grain crops, has resulted in the evolution of paraquat resistance at a number of sites in southern Australia. The evolution of paraquat resistance occurs after a prolonged period of use, often up to 20 years, suggesting that resistance genes are rare. A recent random survey of 50 *Hordeum* spp. populations from cropping fields in South Australia found a single population of *H. glaucum* resistant to paraquat. This population is highly resistant (~200 fold) during winter conditions; however, resistance decreases under spring and summer conditions, suggesting that the resistance mechanism is strongly influenced by temperature. Knowledge of the variation both within and between populations enables genetic relationship to be established. Preliminary results using RAPD markers for both *Hordeum* spp. indicate that within population variation is low and that RAPDs can be used to distinguish between these two species. This work will lead to a better understanding of the how resistance is spread as well as the evolution of paraquat resistance in field populations.

Keywords *Hordeum glaucum*, *Hordeum leporinum*, paraquat, resistance, RAPD markers.

INTRODUCTION

The non-selective and non-residual herbicide paraquat is widely used for weed control worldwide. This herbicide disturbs the photosynthetic electron transport system within thylakoid membranes (Harris and Dodge 1972) by acting as an electron acceptor (Fuerst and Norman 1991). Plant death is caused by membrane damage due to the accumulation of toxic oxygen radicals (Kunert and Dodge 1989).

The barley grasses (*Hordeum* spp.) are annual winter growing plants that are important weeds in both pastures and crops in southern Australia (Smith 1972, Dashost and Jessop 1998) and are considered difficult weeds to control (Jones *et al*. 2000). These species are highly competitive in crop and can act as alternative hosts for cereal diseases (Code 1986). The awns of the *Hordeum* spp. can aggravate the mouth, eyes, and nose of cattle or sheep as well as contaminate wool and hides (Smith 1968). Paraquat resistance in barley grass was first reported in 1983 from a lucerne field in western Victoria (Warner and Mackie 1983). In the succeeding two decades, only a few other populations resistant to paraquat have been reported in Australia (Tucker and Powles 1991, Purba *et al*. 1995, Alizadeh *et al.* 1998), despite the continued widespread use of paraquat.

In 1999, a random survey of barley grass populations from cropping fields in South Australia indicated that resistance might be more widespread than previously anticipated. This survey of 50 populations suggested up to ten populations might contain resistant individuals. It was suspected that one possible reason for this high frequency could be the movement of resistant seed between fields, for example in hay (see Tucker and Powles 1988). The aim of this study was to investigate the evolution and spread of resistant populations and to determine whether resistant populations have evolved from local susceptible populations or been imported in from elsewhere. This report contains some preliminary information on this topic.

MATERIALS AND METHODS

Plant material Seed of a resistant population of *H. glaucum* (SHG7) was collected from a lucerne field near Jamestown, South Australia during a random survey in 1999. Seed of other populations used in this study came from stocks held at the Waite Campus. The resistant populations THL1, THL2, THL3, and VHG1 were originally from three lucerne fields near Ouse, Tasmania and a lucerne field near Ararat, Victoria, respectively. The resistant population SHG4 came from a no-till grain cropping field near Avon, South Australia. The two susceptible populations, THL4 and VHG2, were originally from pastures adjacent to lucerne fields near Ouse and Ararat respectively. Seeds of all populations were germinated on 0.6% agar in

1 L plastic containers for six days in an incubator set at 19°C with a 12 h 30 µmol m^{-2} s^{-1} light period and a 12 h dark period. At the one leaf stage seedling were transplanted to pots containing potting soil, with ten individuals in each pot, and grown outdoors.

Herbicide application At the 4–5 leaf stage, plants were sprayed with paraquat at rates ranging from 0 to 1600 g ha^{-1} using a moving-boom laboratory herbicide sprayer delivering 117 L ha^{-1} of water through nozzles positioned 40 cm above the foliage. Plants were kept inside without light overnight and transferred outdoors the following day. Survival was assessed three weeks after herbicide application. Plants were considered alive if they had new green leaves emerging.

PCR-RAPD Genomic DNA was extracted from leaf material using the CTAB method (Rogers and Bendich, 1985) with minimal modifications. The set of OPT primers (purchased from Operon Technology Inc., WA) were used for the RAPD shown here. PCR amplifications were carried out in 30 µl total volume containing 25 ng DNA, 3 µl of 10 x buffer, 2mM $MgCl_2$, 0.25nM primer, 200µM dNTP, 1U Taq DNA Polymerase (Geneworks, Australia). The PCR reaction was run in a MJ Research Programmable Thermal Cycler with the following program: 15 s at 94°C, 15 s at 36°C 30 s at 72°C, repeated for 30 cycles 72°C with a final extension step at 72°C for two minutes (Muralidharan and Wakeland 1993). The amplification products were separated by electrophoresis using 1.5% agarose gels and detected by staining with ethidium bromide.

RESULTS

A random field survey collected barley grass samples (both *H. glaucum* and *H. leporinum*) from grain cropping and lucerne fields in South Australia in 1999. Preliminary studies suggested ten of these populations might contain individuals resistant to paraquat (Preston, unpublished). Further studies established that only one of these ten populations, identified as *H. glaucum*, was resistant to paraquat.

This newly discovered resistant population of *H. glaucum* (SHG7) showed significant resistance to paraquat during winter (Figure 1) and was as resistant as VHG1, a previously well-studied resistant population (Powles 1986). The application of paraquat at 1600 g ha^{-1} was unable to control either SHG7 or VHG1. In contrast, paraquat completely controlled the susceptible populations of *H. glaucum* and *H. leporinum* (VHG2 and THL4, respectively) at the field rate of 200 g ha^{-1} (Figure 1). In winter, plant death (an absence of any green tissue) in susceptible plants was observed about one week after herbicide application. However, a faster response to paraquat was apparent for spring and summer applications in which susceptible plants were killed within three days following paraquat application.

Resistance in SHG7 and another resistant *H. glaucum* population from South Australia, SHG4, declined if plants were treated in spring or summer (Figure 1). This finding conform with previous studies that have shown paraquat resistance in *Hordeum* spp. is greatly reduced under higher temperatures (Purba *et al.* 1995, Alizadeh 1999). Therefore, this temperature sensitivity of paraquat resistance seems to be a characteristic feature of resistant *Hordeum* spp.

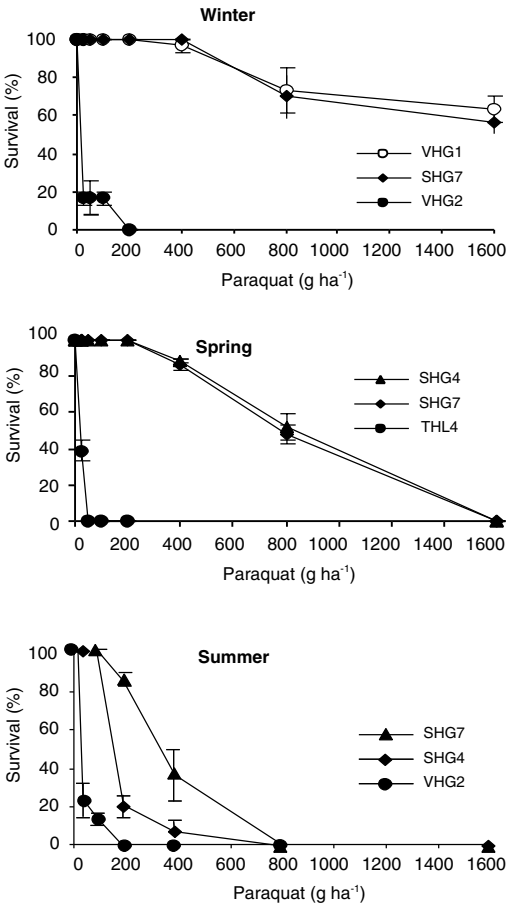

Figure 1. Dose response of resistant (VHG1, SHG4, SHG7) and susceptible (THL4, VHG2) populations of *Hordeum* spp. treated with paraquat in winter, spring or summer. Data are means of 30 plants ± SE.

populations and suggests that a single, temperature-dependent, mechanism of resistance occurs in all these populations.

The RAPD profiles of resistant and susceptible *Hordeum* spp. were examined with two primers (OPT7 and OPT15). The size of the DNA fragments ranged from 300–2000 bp (Figure 2). The number of bands amplified differed between species. The arrow in Figure 2A indicates the most obvious band, around 1200 bp, that occurs only in the two *H. glaucum* populations but not *H. leporinum* populations. Using this primer it is possible to distinguish between these two species. Variation was also observed between the two resistant *H. glaucum* populations from South Australia, SHG4 and SHG7, shown in lanes 5 and 6 (Figure 2A). Likewise, the populations of *H. leporinum* (lanes 1–4) also display some differences in banding patterns. Interestingly, there is a single unique band that occurs in the susceptible THL4 population that is not present in the other *H. leporinum* populations.

Some polymorphisms are evident between individuals within THL4 and an arrow indicates one of these. In contrast, no polymorphisms have been observed within the resistant populations using this primer (not shown). Overall the level of within population variation in these self-pollinating *Hordeum* spp. populations is very low.

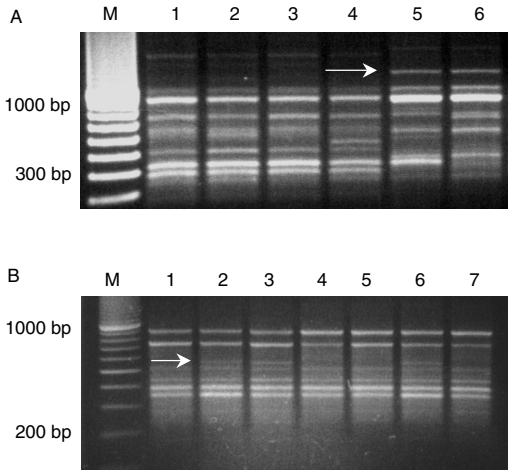

Figure 2. Randomly Amplified Polymorphic DNA profiles for *Hordeum* spp. with primer OPT7. (A) Variations between *H. leporinum* and *H. glaucum* populations. 1–4 *H. leporinum* (THL1, THL2, THL3, THL4), 5,6 *H. glaucum*. (SHG7, SHG4). (B) Variation within susceptible population THL4. 1–7 individual plants within THL4. Lane M designates the molecular weight markers.

DISCUSSION

Paraquat resistant populations of *Hordeum* spp. are known from both long-term lucerne fields and from no-till grain cropping (Powles 1986, Tucker and Powles 1991, Alizadeh *et al*. 1998). Resistance occurs in both situations following a significant history of paraquat application, usually over more than 15 years (Preston 1994, Alizadeh *et al.* 1998). A random survey of barley grass populations from fields in South Australia identified ten populations with paraquat survivors. However, only one of these populations, SHG7, proved to be resistant to paraquat. This population was highly resistant to paraquat during winter; however, resistance was reduced during spring and summer (Figure 1). This temperature dependence of paraquat resistance appears to be a common property of paraquat resistant *Hordeum* spp. populations (Purba *et al.* 1995, Alizadeh 1999). An obvious conclusion is that these paraquat resistant *Hordeum* spp. populations have the same mechanism of resistance and carry the same resistance gene. Resistance to paraquat in *Hordeum* spp. appears to result from a reduction in the translocation of paraquat, particularly to the shoot meristem and leaves that have not yet emerged (Preston *et al.* 1992, Purba *et al.* 1995).

The similarity of paraquat resistance mechanisms in *Hordeum* spp. may indicate resistance has spread from a single or a few sites. Barley grass seeds have sharp awns that easily catch in fleece, fur and machinery. This means barley grass seed can be easily transported from one property to another. Indeed, Tucker and Powles (1988) documented the possible spread in hay of paraquat-resistant *H. glaucum* seed between farms in western Victoria. Therefore, seed spread may be an important reason for the wide incidence of paraquat resistance. Here, RAPD is being used to determine genetic relationships between resistant and susceptible populations of *Hordeum* spp. Preliminary investigations indicate that the two *H. glaucum* paraquat-resistant populations identified in South Australia are not identical (Figure 2). Equally, there is genetic variation between populations of *H. leporinum* collected from Tasmania. These latter populations were collected from fields close together. From these results, it would seem that evolution of paraquat-resistant populations *in situ* is an important factor in the current distribution of paraquat resistant *Hordeum* spp. populations.

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