Weed Research, 1986, Volume 26, 167–172

Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat

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Accepted 22 June 1985

Summary: Résumé: Zusammenfassung

A biotype of the grass weed *Hordeum glaucum* Steud. infesting a site at Willaura, Victoria, Australia has resistance to paraquat. Application of the recommended rate of paraquat does not cause death of the resistant biotype at any stage of growth. The LD_{50} for the resistant biotype is 6·4 kg active ingredient ha^{-1} which is 250 times greater than for the normal susceptible biotype (25 g active ingredient ha^{-1}). Growth of the resistant biotype is checked by paraquat with a clear dosage response evident. The paraquat resistant biotype is also resistant to diquat but is normally affected by herbicides with different modes of action. In addition to continued foliage growth of the resistant plants after paraquat application, seeds of these plants can germinate and seedlings elongate in the dark whereas seeds of susceptible plants germinate but there is no further growth. This suggests that studies of the mechanism(s) conferring resistance will have to consider both the effect of paraquat on the chloroplast and a non-photosynthetic effect on cell growth.

Un biotype de la mauvaise herbe Hordeum glaucum *Steud. résistant à l'herbicide paraquat*

Un biotype de la graminée *Hordeum glaucum* Steud. à Willaura, Victoria, Australie, s'est montré résistant au paraquat. L'application de la dose préconisée de paraquat ne provoque pas la mort de ce biotype, quel qu'en soit le stade végétal. La LD_{50} pour le biotype résistant est 6,4 kg matière active ha^{-1}, c'est-à-dire 250 fois plus grande que pour le biotype normal sensible (25 g matière active ha^{-1}). Le paraquat provoque chez le biotype résistant une inhibition de croissance qui se rapporte à la dose. Le biotype résistant au paraquat l'est également au diquat mais réagit normalement envers les herbicides à mode d'action différente. Non seulement la croissance foliaire continue normalement après une application de paraquat chez les plantes résistantes, mais les graines sont capables de germer et les jeunes plants de s'allonger à l'obscurité, tandis que les graines de plantes sensibles germent à l'obscurité mais ne croissent pas. Il semble donc que les études des mécanismes qui produisent la résistance devront examiner l'influence du paraquat sur le chloroplaste ainsi qu'un effet non-photosynthétique sur la croissance cellulaire.

Ueber das Auftreten eines gegen Paraquat resistenten Biotyps von Hordeum glaucum *Steud.*

Bei Willaura, Victoria (Australien) tritt ein gegen Paraquat resistenter Biotyp von *Hordeum glaucum* Steud. auf. Die Application der normalerweise empfohlenen Dosierung Paraquat tötet den resistenten Biotyp in keinem Wachstumsstadium ab. Die Ld_{50} für den resistenten Typ beträgt 6,4 kg ai ha^{-1}; dies ist 250 mal mehr als beim normal sensiblen Typ (25 g ai ha^{-1}). Das Wachstum des resistenten Biotyps wird durch steigende Dosen von Paraquat beeinträchtigt. Der gegen Paraquat resistente Typ ist auch gegen Diquat unempfindlich, weist aber gegenüber Herbiziden mit anderen Wirkungsmechanismen die normale Empfindlichkeit auf. Resistente Pflanzen zeigen nach Paraquatbehandlung ein weitergehendes Blattwachstum. Ihre Samen keimen und die Sämlinge entwickeln sich im Dunkeln weiter, während die Samen sensibler Pflanzen zwar kei-

men, sich aber nicht weiterentwickeln. Diese Beobachtungen weisen darauf hin, dass bei Forschungen zur Aufklärung der Resistenzmechanismen, sowohl die Wirkung von Paraquat auf die Chloroplasten als auch einen nicht photosynthetisch wirksamen Effekt auf das Zellwachstum berücksichtigen müssen.

Introduction

Weed populations with resistance to a particular herbicide are being increasingly reported in the northern hemisphere. There is now widespread resistance among several weed species to the triazine type herbicides (Bandeen, Stephenson & Cowett, 1982; Gressel *et al.*, 1982). However only one studied weed species, *Conyza bonariensis* (L.) Cronq., has been confirmed to have developed resistance to a bipyridyl herbicide (Youngman & Dodge, 1981; Harvey & Harper, 1982).

Bipyridyl herbicides were used successfully on a mixed cereal cropping and sheep grazing enterprise at Willaura (state of Victoria, Australia) over a 15-year period to control winter-growing weeds in fields of lucerne (*Medicago sativa* L.). Throughout this period good control was achieved of the widespread and important grass weeds, known locally as barley grasses (*Hordeum leporinum* Link, *Hordeum glaucum* Steud. and *Hordeum marinum* Huds. var. (ssp.) *gussoneanum* (Part.) Thel. (= *H. hysterix* Roth.)). However in 1981, following application of the recommended rate of 200 g ai ha^{-1} paraquat, poor control of barley grass was evident in one of four fields sown to lucerne. Again in 1982 the same rate of herbicide was ineffectual in controlling barley grass. Applications in 1982 of 230 g ai ha^{-1}, followed 2 weeks later by 280 g ai ha^{-1} were ineffectual. Samples of the barley grass population were identified as *H. glaucum* and were confirmed to survive applications up to 1·6 kg ai ha^{-1} paraquat (Warner & Mackie, 1983). Barley grass populations infesting three other lucerne fields on this farm showed no evidence of resistance and were controlled by the recommended rate of paraquat. No other instances of resistance to paraquat were reported in the area.

The appearance of herbicide resistance is a new phenomenon in Australia. A weed showing herbicide resistance is of economic and scientific significance, and studies were commenced on this resistant population to establish a dose mortality curve and the effect of applications at different growth stages.

Materials and methods

Plant material

Seeds of *H. glaucum* were collected at Willaura from the population showing survival under high rates of application of paraquat. Seedlings were grown at a density of four plants per pot, in pots containing 2 *l* of loamy soil. Seeds of *H. glaucum* collected at Palmer in South Australia (500 km from Willaura), with normal susceptibility to paraquat, were grown as control plants. Following germination and emergence in a glasshouse, the plants were maintained outdoors for the remainder of the experiment. Plants were grown throughout the winter months, corresponding to the normal growth period for this species. (Adelaide has a mediterranean climate with a cool, wet winter growing season.)

Herbicide application

The plants were sprayed using a hydraulic pressure nozzle (Hardi Tee-Jet 8001 1-E) mounted over a variable speed continuous belt circulating at a speed of 1·43 m s^{-1}. The spray mixture (herbicide plus 0·2% surfactant) was pumped via a regulator and filter to the nozzle at a pressure of 200 kPa and directed at the target plants 26 cm below the nozzle. The output of the nozzle at the height of the foliage was evenly dispersed over a 38 cm fan width, as was verified using a patternator. Total spray output with this nozzle, pressure and belt speed was 100 *l* ha^{-1}.

The plants were treated while in deep shade and within 30 min of sunset and were then placed in darkness until the following day. This procedure was adopted to ensure a maximum effect from the paraquat (Brian & Headford, 1968). Following a 12-h dark period the treated and control plants were moved outdoors and kept under the natural prevailing conditions.

Measurements

Dry matter was measured 21 days after herbicide treatment by harvesting the above-ground green tiller and leaf tissue from each pot, drying for 24 h at 80°C and measuring the total dry weight of the four plants. In experiments determining the effect of herbicide treatment on survival the plants were maintained through anthesis. Determination of leaf area (10 days after treatment with paraquat) was made by harvesting the above-ground green leaf tissue (leaf tissue bleached by paraquat was excluded) of four plants in each pot and measuring the total leaf area (Paton electronic leaf area meter).

Results

Plant appearance following paraquat application

Application to the foliage of 200 g ai ha^{-1} (i.e., the recommended rate of a commercial preparation of paraquat) caused death of susceptible *H. glaucum* but not of the resistant biotype from Willaura. The typical paraquat toxicity symptoms of rapid (within 24 h) and massive bleaching of green tissue, followed by plant death, occurred with the susceptible *H. glaucum*. However, with the resistant biotype only localized damage and limited chlorophyll bleaching occurred. The oldest leaves and leaf tips of individual leaves were partially bleached whereas the young leaves and the younger sections of mature leaves remained green. The most striking difference between the susceptible and resistant biotypes was that the young emerging leaves of the resistant biotype remained green, and continued to grow after herbicide treatment. Although the growth rate was checked because of some bleaching and death of older tissue, the emerging leaf tissue led rapidly to new growth.

Survival of H. glaucum *following paraquat application*

Effect of plant age. It was of interest to know if the difference in resistance between resistant and susceptible biotypes changed with leaf age. Both resistant and susceptible plants were treated at the rate of 200 g ai ha^{-1}. The effects of the herbicide were assessed by the survival of treated plants and the production of viable seed. Treatment of the susceptible biotype, at different stages of development (from single leaf through to post-anthesis), always resulted in death. By contrast, the resistant biotype was not killed and set viable seed (data not shown).

Table 1 Effect of paraquat on survival of *H. glaucum*

Application rate kg ai ha^{-1}	No. of plants sprayed	No. survived	% survival
Susceptible biotype			
0·025	29	11	38
0·05	30	0	0
0·1	200	0	0
0·2	200	0	0
Resistant biotype			
0·2	212	212	100
0·4	275	275	100
0·8	243	243	100
1·6	250	250	100
3·2	241	225	93
4·8	233	190	82
6·4	177	72	41

Plants were sprayed at the three tiller stage.

Effect of herbicide rate. The effects of spraying young susceptible and resistant plants at different rates of paraquat is shown in Table 1. The plants were sprayed 36 days after seeding when at the three tiller stage. An application of 50 g ai ha^{-1} paraquat (1/4 the recommended application rate), or higher, resulted in death of the susceptible population. The LD_{50} for this population was approx. 0·025 kg ai ha^{-1}. However the Willaura biotype showed no mortality until the application rate reached 3·2 kg ai ha^{-1}, with the LD_{50} being around 6·4 kg ai ha^{-1}. Thus the lethal dose of paraquat required for *H. glaucum* infesting the field at Willaura is some 250 times higher than for *H. glaucum* collected in South Australia.

Effect of paraquat application on dry matter production. The results presented above indicate the resistant biotype is only killed by paraquat applied at extremely high rates; and it is also important to know if the recommended rate will reduce the vigour of the weed. Plants sprayed at the one, two and three tiller stage showed reduced growth dependent upon the dose of paraquat applied. Figure 1 shows the effect of different rates of paraquat on the dry matter production of the resistant biotype. The plants were sprayed at the three different stages of development and harvested 21 days later, along with unsprayed plants as a control. Increased doses of paraquat caused further reduction in plant weight with a significant response to herbicide dosage evident (Fig. 1).

Table 2 Effect of paraquat (200 g ai ha^{-1}) applied at anthesis on subsequent spike development and seed germination of the resistant *H. glaucum*

	Control (Unsprayed)	% germination	Sprayed	% germination
Mature spikes collected from 25 plants	1144	78	993	78
Bleached, sterile spikes	—	—	200	0

Effect of paraquat application on leaf area. As was found above for the dry matter (Fig. 1) the application of the recommended rate (200 g ai ha^{-1}) resulted in approximately 43% loss of green leaf tissue as a result of bleaching. Increasing doses of paraquat caused little further loss of leaf area. In contrast, treatment of the susceptible *H. glaucum* at 200 g ai ha^{-1} caused total leaf destruction (results not shown).

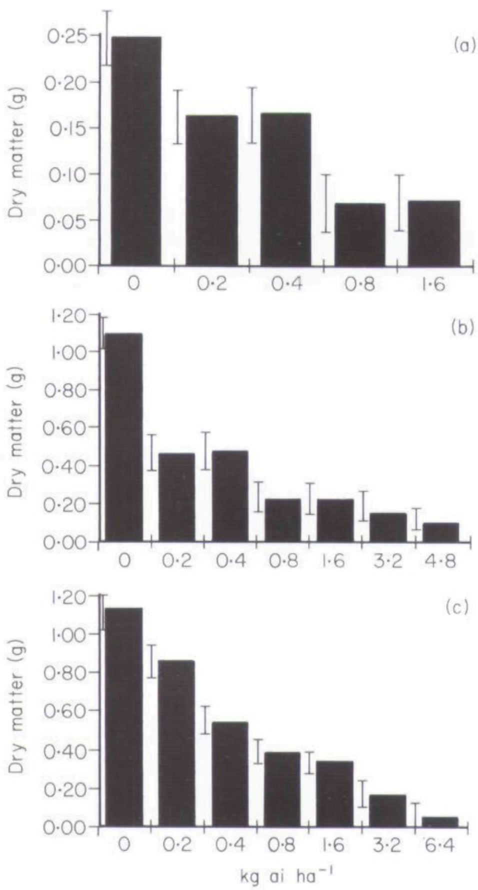

Fig. 1 Effect of increasing dosage of paraquat on the dry matter production (21 days after treatment) of the resistant biotype of *Hordeum glaucum* sprayed at the single (a), two (b) or three (c) tiller stage. Bars are least significant difference at 5% level.

Effect of application at anthesis on subsequent inflorescence development. The effect of spraying resistant plants at anthesis on the subsequent production of spikes is shown in Table 2. In these experiments, uniformly-sized plants were treated at anthesis with 200 g ai ha^{-1} paraquat and compared with unsprayed plants for production of spikes. Spraying did not reduce the number of spikes that developed but did cause approximately 10% of the spikes to bleach and these spikes did not develop seed. The remainder of the spikes developed normally. Except for the bleached spikes the seed from spikes of both unsprayed and treated plants had the same germination rate of around 78%. Application of the same rate of paraquat to susceptible *H. glaucum* caused plant death without the production of any viable seed (results not shown).

Effect of alternative herbicides to paraquat. Table 3 shows that the resistance to paraquat evident in the Willaura biotype of *H. glaucum* is specific to the bipyridyl herbicides. Application of the recommended rate of three herbicides with different modes of action caused death of both the Willaura biotype and *H. glaucum* collected in

Table 3 Effect of application of alternative herbicides to paraquat on survival of *H. glaucum*

Herbicide	Rate kg ai ha^{-1}	% survival Susceptible	Resistant
Diquat (bipyridyl)	0·2	60	100
Diuron	2·5	0	0
Fluazifop-butyl	0·2	0	0
Glyphosate	0·36	0	0

Plants (minimum of 50 plants treated) were sprayed at the three tiller stage.

South Australia. The bipyridyl herbicide diquat (recommended for dicotyledonous species) was ineffective in controlling the resistant biotype and only partially successful in controlling the susceptible biotype.

Effect of paraquat on seed germination. Seeds of the resistant and the susceptible biotypes were incubated in the dark at 19°C on filter paper pre-soaked with water containing low concentrations of herbicide. In these experiments only the active ingredient (methylviologen) was used to eliminate the possibility that effects may be due to additional compounds present in commercial preparations of paraquat herbicide. Percentage germination, shoot and root length were assessed 10 days after incubation. Seed from the resistant biotype and the susceptible biotype showed similar rates of germination (assessed as emergence of the shoot and root tip) of approximately 78% at all levels of methylviologen. Inhibition of the elongation of the seminal root was evident by methylviologen with equal inhibition of root growth in seed from resistant or susceptible plants.

There was a difference between seeds of the resistant and the susceptible biotypes in the ability of the primary shoot to elongate in the presence of herbicide. With the seed from plants resistant to paraquat the emerging shoot was able to elongate at levels of methylviologen from 0 to 155 μM. There was no significant difference in the length of the primary shoot of seeds incubated at low concentrations of methylviologen, although at 155 μM inhibition of shoot length was evident (Fig. 2a). By contrast, with seeds from the susceptible biotype, incubation even at 1·5 μM methylviologen resulted in significant inhibition of shoot elongation and at high concentration very little shoot elongation occurred (Fig. 2b).

In other experiments seeds of resistant and susceptible *H. glaucum* were placed on the soil surface and the pots sprayed with paraquat at 200 g ai ha^{-1}. There was no effect of paraquat on % germination or emergence (data not shown) confirming that paraquat is inactivated by contact with soil.

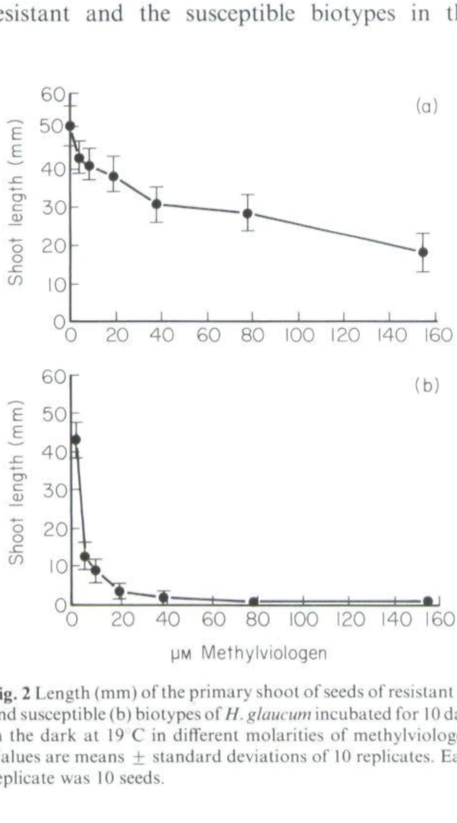

Fig. 2 Length (mm) of the primary shoot of seeds of resistant (a) and susceptible (b) biotypes of *H. glaucum* incubated for 10 days in the dark at 19°C in different molarities of methylviologen. Values are means ± standard deviations of 10 replicates. Each replicate was 10 seeds.

Discussion

It is clear that the biotype of *H. glaucum* at Willaura is resistant to the bipridyl herbicides. When plants grown from seed collected at this site were spayed at the recommended rates of paraquat there was 100% survival regardless of the age of the plants at spraying. The same treatments applied to *H. glaucum* collected from a separate location resulted in death of all plants.

While paraquat does not cause death of the resistant biotype it does cause some damage to the foliage of the plants. Leaf tips and oldest leaves dessicate and bleach. However, the younger leaves are unaffected and lead to new growth with the result that within approximately 4 weeks the effects of paraquat are difficult to detect. The amount of leaf damage to the resistant biotype is greater at higher rates of paraquat with a dose response for leaf damage and growth evident (Fig. 1). Treatment of normal barley grass results in total leaf bleaching and rapid death of the plant.

It is clear that the accepted practice of employing paraquat at the recommended rate of 200 g ai ha^{-1} (applied to young, actively growing weeds) is ineffective in controlling the resistant biotype infesting the farm at Willaura. Similarly, the use of paraquat at anthesis (spray-topping to prevent seed set) is ineffective with the resistant biotype as it can still produce fertile seed after treatment at twice the recommended rate (Table 2).

The resistance evident in the Willaura biotype of *H. glaucum* is specific to the bipyridyl herbicides. Table 3 shows that herbicides with different modes of action are effective in controlling this weed. Therefore, from a practical viewpoint, control of the paraquat resistant biotype can be obtained by the use of a non-bipyridyl herbicide.

The herbicidal effect of paraquat is much greater in the presence of light (Brian & Headford, 1968) consistent with its mode of action as an acceptor of electrons at photosystem one within the chloroplast (Youngman & Dodge, 1981). The observation (Fig. 2a) that the shoot of germinating seeds of the resistant biotype could grow in the dark in the presence of paraquat, whereas the susceptible type could not (Fig. 2b) is of considerable importance. This indicates that paraquat has effects in addition to disrupting photosynthesis and shows that the resistant biotype displays resistance also to this (unknown) site of action.

The appearance of the resistant biotype within the cereal growing region of southern Australia may cause problems to farmers. Studies are in progress to determine the current areas of infestation by this resistant biotype and its possible dispersion into areas where bipyridyl herbicide is widely used. Other studies are underway to determine the genetical basis of the resistance and the physiological mechanism(s) involved.

Acknowledgment

The encouragement of Dr R. Knight and Professor C.J. Driscoll is gratefully acknowledged. This work was initiated while the author was the holder of a Research Fellowship (Agriculture) from the Reserve Bank of Australia and continued by a grant from the Wheat and the Barley Industry Research Councils.

(Received 14 February 1985)

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