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Inheritance of bipyridyl herbicide resistance in *Arctotheca calendula* and *Hordeum leporinum*

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Abstract. The mode of inheritance of resistance to bipyridyl herbicides in bipyridyl-resistant biotypes of *Arctotheca calendula* and of *Hordeum leporinum* was investigated. F_1 plants from reciprocal crosses between diquat-resistant and -susceptible plants of *A. calendula* showed an intermediate response to diquat application that was nuclearly inherited. Treatment of F_2 plants with 100 g ai ha^{-1} of diquat or 800 g ai ha^{-1} of paraquat killed all homozygous-susceptible plants, caused severe injury to heterozygous plants but only slight or no injury to homozygous-resistant plants. Back crosses of F_1 to susceptible plants exhibited intermediate and susceptible phenotypes. The observed segregation ratios in F_2 and test-cross populations fitted predicted segregation ratios, 1:2:1 (R:I:S) and 1:1 (I:S) respectively, showing that bipyridyl resistance is conferred by a single incompletely-dominant gene. Biotypes of paraquat-resistant and -susceptible *H. leporinum* were crossed reciprocally. F_1 plants from reciprocal crosses showed an intermediate response to paraquat application. The F_2 progeny showed segregation ratios that fitted the predicted segregation ratio of 1:2:1(R:I:S) for inheritance of resistance being governed by a single partially-dominant gene.

Key words: Paraquat – Diquat – Herbicide resistance

Introduction

Arctotheca calendula and two annual grass weeds, *Hordeum glaucum* and *H. leporinum*, collectively known as barley grasses, are important weeds in southern Australian agriculture (Powles and Howat 1990). In an alfalfa field in Victoria, these weeds had been successfully controlled by paraquat and diquat over many years (Powles et al. 1989; Tucker and Powles 1991). However, in the early 1980s, resistance appeared in biotypes of *A. calendula*, *H. glaucum* and *H. leporinum* at this site (Powles and Howat 1990). Selection pressure resulting from the repeated use of a similar herbicide for a numbers of years may select for resistant individuals. In the case of paraquat resistance this has only appeared after many applications of herbicide, for example 8–11 years for *Erigeron philadelphicus* (Itoh 1988), 24 years for *H. glaucum* (Powles 1986), 24 years for *A. calendula* (Powles et al. 1989), and 12–24 years for *H. leporinum* (Tucker and Powles 1991; Purba et al., unpublished data).

Studies on the mode of inheritance of herbicide resistance have been reported for a number of herbicide-resistant weed biotypes. Most cases of triazine resistance were found to be maternally inherited (Souza Machado et al. 1978; Scott and Putwain 1981; Darmency and Pernes 1985) and conferred by a mutation in a 32-kDa reaction centre protein (Pfister et al. 1981). In contrast, triazine resistance in *Abutilon theophrasti* was found to be nuclearly inherited and controlled by a single incompletely-dominant gene (Andersen and Gronwald 1987). Inheritance of sulfonylurea resistance in *Lactuca* spp is also controlled by a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990), as is diclofop-methyl resistance in a biotype of Italian ryegrass (*Lolium multiflorum*) (Betts et al. 1992). Paraquat resistance is controlled by a single dominant gene in *Conyza bonariensis* (L.) Cronq. (Shaaltiel et al. 1988). In the case of *H. glaucum* Steud. (Islam and Powles 1988), resistance is

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controlled by a single partially-dominant gene which results in reduced herbicide translocation (Bishop et al. 1987; Preston et al. 1992). Paraquat resistance in *Lolium perenne* is controlled by several genes (Faulkner 1974) and is due to increased levels of protective enzymes (Harper and Harvey 1978).

The objective of the present study was to investigate the mode of inheritance of diquat and paraquat resistance in biotypes of *A. calendula* and *H. leporinum* obtained from a lucerne field in Victoria. These biotypes show high levels of resistance to the bipyridyl herbicides.

Materials and methods

Plant material

Seeds of resistant biotypes of *A. calendula* and *H. leporinum* were originally collected from an alfalfa field in Victoria with a long history of paraquat and diquat use (Powles et al. 1989; Tucker and Powles 1991). When the two biotypes were grown in pots, *A. calendula* survived 200 g ai ha^{-1} of diquat and *H. leporinum* survived 200 g ai ha^{-1} of paraquat. Seeds collected from the surviving plants were used for this study. Susceptible populations of the two species were originally collected from a nearby pasture with no history of paraquat-diquat use. Seeds of both resistant and susceptible biotypes of *A. calendula* were germinated (buried at 2.5–5 mm) in potting soil based on peat and sand in an unheated glasshouse for 16 days. Seeds of *H. leporinum* were germinated on 0.6% (w/v) agar in plastic containers placed in a germination cabinet at 20 °C and 20 $\mu E\,m^{-2}\,s^{-1}$ in a 12 h day/night cycle. Seedlings were transferred separately into 30-cm pots containing potting soil based on peat and sand.

Hybridization

A. calendula. Our investigation has established that *A. calendula* is an obligate outcrossing species. In 1990, crosses between resistant and susceptible biotypes were conducted in an unheated glasshouse. In order to avoid pollination from unwanted pollen the inflorescences of the female parent plants were placed in paper bags before the flowers opened. The flowers were pollinated with a ripened inflorescence from the desired biotype by touching the two. Immediately after pollination the female parents were re-bagged. Reciprocal crosses of resistant and susceptible biotypes were performed to obtain the F_1 generation. Some F_1 plants of the reciprocal crosses, along with the resistant and susceptible parents, were sprayed at the six-leaf stage with diquat. The unsprayed F_1 plants were grown for F_2 seeds which were obtained by hand-pollinating two F_1 plants from the same family.

H. leporinum. H. leporinum is a self-pollinated species and therefore the female parent required emasculation prior to crossing. The anthers of spikes of the female parent were removed at late booting stage to prevent self-pollination. The anthers of *H. leporinum* are minute so emasculation required the aid of a microscope. Immediately after emasculation the inflorescences were bagged. Three to six days after emasculation the plants were hand-pollinated by placing the anthers of the desired male parent onto the stigma with the aid of a microscope. After pollination the inflorescence was bagged again until the seeds were harvested. Each F_1 plant was grown individually in 18-cm pots containing potting soil to produce F_2 seeds by self-pollination.

Response of parents, F_1 and F_2 to herbicide application

F_1, resistant (R) and susceptible (S) seeds of *A. calendula* were germinated in plastic trays (40 × 30 × 12 cm) containing potting soil and were placed outdoors for 18 days. Seedlings at the two-leaf stage were transferred into 18-cm diameter pots containing potting soil at a density of six plants per pot. Plants were maintained outdoors during the normal winter growing season (average 15 °C day and 5 °C night). Plants at the 6–7 leaf stage were sprayed with diquat plus 0.2% (v/v) non-ionic surfactant in a laboratory spray cabinet delivering 113 L ha^{-1}. Plants were sprayed at dusk, kept indoors in the dark overnight and returned outdoors the following morning. Survival and shoot-dry-matter production were recorded 22 days after spraying.

The crosses of *H. leporinum* yielded 13 plants, 12 from the S × R crosses and one from the R × S crosses. F_1 seedlings were germinated on agar and transferred to 18-cm diameter pots containing potting soil and placed outdoors during the normal winter growing season. To increase F_1 plant numbers, F_1 hybrid plants were divided at the three-to-four-tiller stage by separating tillers to produce 3–4 individual clones from one plant. The same procedure was also applied to both parents in order to maintain all plants at the same stage. F_1 plants from the reciprocal crosses along with the parents (resistant and susceptible biotypes) were treated with paraquat 4 weeks after cloning. Six F_1 clones, four S × R and two R × S were sprayed with 100 g ai ha^{-1} of paraquat and four F_1 clones (S × R) were sprayed with 200 g ai ha^{-1} of paraquat. Some unsprayed F_1 clones from all families were maintained to produce F_2 seeds by self pollination. F_2 seeds were collected from all F_1 clones and bagged separately to ensure that segregation in the F_2 population generated from each cross could be detected and to identify whether hybridization between R and S had been conducted successfully. F_2 plants were grown in plastic trays (40 × 30 × 12 cm) containing potting soil with growing conditions as described above. Each tray contained 140–180 F_2 plants and 11 plants each of the resistant and susceptible biotypes as controls. Plants were sprayed at the three-leaf stage with spraying conditions as described above and phenotypic response was scored 6–14 days after spraying.

Chi-square analysis of the segregation of the F_2 and backcross populations was performed as described by Goodenough (1978). Segregation ratios for reciprocal crosses were compared using a chi-square homogeneity test of observed values. The dose of herbicide causing 50% mortality was calculated by logarithmic regression.

Results and discussion

A. calendula

Reciprocal crosses between the resistant and susceptible plants produced large numbers of seed. A dose response to diquat was conducted with resistant, susceptible, and F_1 plants (Fig. 1). The susceptible biotype was killed by rates of diquat as low as 50 g ai ha^{-1} whereas the resistant biotype was only slightly affected at 200 g. The F_1 plants from the reciprocal crosses were intermediate between R and S. Both phenotype and mortality (Fig. 1A) in the F_1 (S × R) and F_1 (R × S) crosses were identical, which demonstrates that bi-

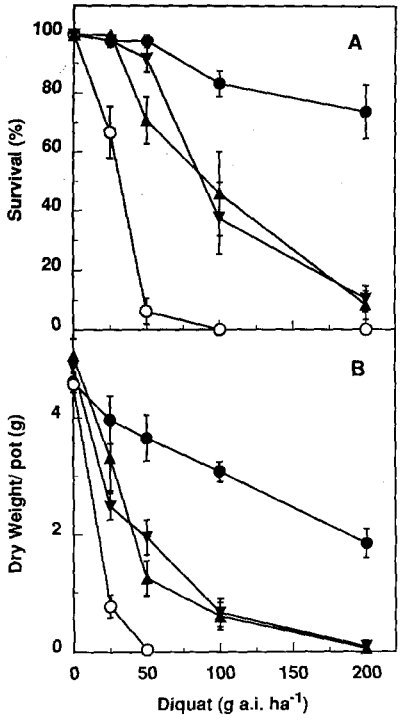

Fig. 1. Effect of diquat on (**A**) survival and (**B**) dry weight of diquat-resistant (●), susceptible (○), and F_1(S × R), ▼, F_1(R × S), ▲, *A. calendula* plants 28 days after application of herbicide. Each point represents 48 plants (six per pot) except for F_1(S × R) with 24 plants. Standard errors are represented by *vertical bars*

pyridyl resistance in *A. calendula* resides in the nuclear genome and is not maternally inherited. Both F_1 populations were intermediate in response and this phenotype could be clearly observed 2–4 days after spraying. The response of reciprocal F_1 progenies was averaged and the LD_{50} was estimated at 80 g ai ha^{-1} whereas the LD_{50} for the resistant biotype was > 200 g ai ha^{-1} and that of the susceptible biotype was 30 g ai ha^{-1}. Dry matter production of the F_1 was intermediate to dry matter production of the parent R and S biotypes (Fig. 1B). The intermediate survival and dry matter production observed in the F_1 plants suggests that bipyridyl resistance may be conferred by an incompletely-dominant allele(s).

F_2 and backcross plants were obtained to further examine the mode of inheritance. F_2 plants generated by crossing F_1 plants were treated with 100 g ai ha^{-1} of diquat or 800 g ai ha^{-1} of paraquat at the 5–6 leaf stage (rates lethal to susceptible, but not resistant, plants). Assessment on the treated F_2 plants was conducted 3 and 4 days after spraying. Plants were scored based on phenotypic responses to herbicide applications which were divided into three groups: resistant plants with no or slight injury, intermediate plants with severe damage to leaves (bleach), and susceptible plants where death had occured. The 742 F_2 plants treated with 100 g ai ha^{-1} of diquat showed a segregation ratio of 1:2:1 (R:I:S) (Table 1) and treatment of the 336 F_2 plants with 800 g ai ha^{-1} of paraquat also had a segregation ratio of 1:2:1 (R:I:S) (Table 1). Chi-square analysis of the observed segregation ratio in F_2 populations was not significantly different from the predicted *P* value whether treated with 100 g of diquat or 800 g of paraquat (Table 1). In all experiments the reciprocal crosses were shown to be homogeneous by the chi-square homogeneity test of observed values (data not shown).

The progeny of nine backcross families (284 plants) treated with 100 g ai ha^{-1} of diquat were separated, based on phenotypic responses, into groups of intermediate and susceptible individuals. The hypothesis predicts a segregation ratio of 1:1 (I:S). The phenotypic response observed showed that the number of intermediate plants was approximately equal to the number of susceptible plants (Table 2). A homogeneity test for the total observed values in backcrosses showed that there is no difference between the two backcross populations (data not shown). Chi-square analysis of the

Table 1. Chi-square analysis of the segregation of the phenotype of F_2 populations of *A. calendula* in response to the application of 100 g ai ha^{-1} of diquat or 800 g ai ha^{-1} of paraquat 3 days after treatment

F_2 population	Segregation by phenotype[a]			Total	χ^2	*P*
	R	I	S			
Diquat						
S × R[b]	135	335	158	628	4.492	0.10–0.20
R × S	29	66	19	114	4.587	0.10–0.20
Total	164	401	177	742	5.306	0.05–0.10
Paraquat						
S × R	11	20	3	34	4.822	0.05–0.10
R × S	76	151	75	302	0.0006	> 0.99
Total	87	171	78	336	0.588	0.70–0.80

[a] R, resistant; I, intermediate; S, susceptible
[b] $S \times R = S_{♀} \times R_{♂}$

Table 2. Chi-square analysis of the segregation of the phenotype of backcross populations of *A. calendula* in response to the application of 100 g ai ha^{-1} of diquat 3 days after treatment

Backcross population	Segregation by phenotype			χ^2	P
	Intermediate	Susceptible	Total		
$S \times F_1(S \times R)$[a]	98	109	207	0.584	0.30–0.50
$S \times F_1(R \times S)$	42	35	77	0.636	0.30–0.50
Total	140	144	284	0.056	0.80–0.90

[a] $S \times F_1(S \times R) = S_♀ \times F_{1♂}$, $S \times R = S_♀ \times R_♂$

goodness of fit of the observed segregation ratio to a 1:1 could not be rejected as the value of P is greater than 0.05. The uniformity of F_1 population phenotypic responses to diquat, and segregation ratios of 1:2:1 (R:I:S) in F_2 populations treated with diquat or paraquat, lead to the conclusion that diquat resistance in *A. calendula* is controlled by a nuclear, partially-dominant gene.

H. leporinum

Reciprocal crosses of *H. leporinum*, between the R and S biotypes produced 20 F_1 hybrid seeds. In the following winter growing season all germinable F_1 hybrid seeds were grown along with parental seed and susceptible, resistant, and F_1 plants at the three-to-four-tiller stage were divided into clones. Four weeks after cloning, some of the F_1 plants and parents (R and S) were treated at 100 and 200 g ai ha^{-1} of paraquat. All resistant plants survived both rates, whereas none of the susceptibles survived at either rate. The six plants of the F_1 treated with 100 g ai ha^{-1} of paraquat were severely damaged and all four plants treated with 200 g ai ha^{-1} died. This intermediate response on the F_1 populations suggest that paraquat resistance may be conferred by a partially-dominant allele(s).

Unsprayed F_1 plants from each family were selfed to obtain F_2 seed and the F_2 seedlings were treated with paraquat at 200 g ai ha^{-1} in the following normal winter growing season. This resulted in a segregation ratio of 1:2:1 (R:I:S) with a P value greater than 0.05 (Table 3). Identical results were obtained from F_2 progeny of the S × R cross and the R × S cross (Table 3) and a homogeneity test showed that the two reciprocal crosses are homogenous which indicates that the resistance gene(s) resides in the nuclear genome. Application of 50 g ai ha^{-1} of paraquat on F_2 plants only affected susceptible, but not intermediate and resistant, plants (Table 3). The segregation ratios of 3:1[(R + I):S] obtained were as expected. The survivors at 50 g ai ha^{-1} of paraquat were allowed to recover for 3 weeks and then re-sprayed with 400 g ai ha^{-1} of paraquat which killed all of the intermediate plants. This second application showed a segregation ratio of 1:3[R:(I + S)] and the value of P observed is not significantly different from the value of P predicted (Table 3). The

Table 3. Chi-square analysis of the segregation of the phenotype of F_2 populations of *H. leporinum* in response to the application of paraquat 1 week after treatment

F_2 population	Paraquat g ai ha^{-1}	Segregation by phenotype[a]				χ^2	P
		R	I	S	Total		
$S \times R$[b]	200	126	214	110	450	2.21	0.30–0.50
R × S	200	46	80	38	164	0.876	0.50–0.70
Total		172	294	148	614	2.97	0.20–0.30
		Segregation by survival					
		R + I[c]	S		Total		
R × S	50	115	34		149	1.228	0.20–0.30
		R	S + I[c]				
R × S	400	39	110		149	0.8495	0.30–0.50

[a] R, resistant; I, intermediate; S, susceptible

[b] $S \times R = S_♀ \times R_♂$

[c] The intermediate biotype survives application of paraquat at 50 g ai ha^{-1} but not at 400 g ai ha^{-1}

two applications, 50 g and 400 g ai ha^{-1} of paraquat, therefore resulted in a segregation ratio of 1:2:1 (R:I:S) which indicates that paraquat resistance in *H. leporinum* is controlled by an incompletely-dominant gene.

Paraquat and diquat resistance in both *H. leporinum* and *A. calendula* are the result of the expression of single incompletely-dominant nuclear genes. The mode of inheritance in these two resistant biotypes is identical to that found in a biotype of paraquat-resistant *H. glaucum* (Islam and Powles 1988). The two paraquat-resistant biotypes, *H. leporinum* and *H. glaucum*, posses a similar mechanism of paraquat resistance (Preston et al. 1992) and, therefore, the genetic factors endowing resistance would be expected to be similar. Paraquat resistance in other biotypes, such as *L. perenne*, is conferred by a polygenic inheritance (Faulkner 1974), or in the case of *C. bonariensis* (Shaaltiel et al. 1988) and *E. philadelphicus* (Itoh and Miyahara 1982) by a single dominant gene and in *E. canadensis* (Yamasue et al. 1992) by a single dominant or partially-dominant gene. In the *H. glaucum* and *H. leporinum* biotypes the mechanism endowing paraquat resistance involves reduced herbicide translocation (Preston et al. 1992); however, in *A. calendula* the mechanism of resistance is not known.

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