FACTORS INFLUENCING THE DEVELOPMENT OF RESISTANCE TO THE BIPYRIDYL HERBICIDES IN AUSTRALIA

Edison Purba

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ABSTRACT

Paraquat and diquat resistance in the grass weed species *Hordeum leporinum* Link., *H. glaucum* Steud., and *Vulpia bromoides* SF Gray, and in the broadleaf weed *Arctotheca calendula* (L.) Levyns were examined in pot experiments during normal winter growing seasons. Three biotypes of *H. leporinum* and one biotype each of *H. glaucum, V. bromoides* and *A. calendula* were documented as resistant to the bipyridyl herbicides paraquat and diquat. All the resistant biotypes were obtained from lucerne fields where paraquat and diquat had been used as the sole weed control method for a long period of time. None of the paraquat and diquat resistant biotypes displayed any cross-resistance to alternative herbicides.

Bipyridyl resistant biotypes of *H. leporinum* from different lucerne fields with different histories of bipyridyl use were examined for a correlation between the level of bipyridyl resistance and the period of bipyridyl exposure in the field. No relationship between the length of bipyridyl use and the level of resistance in *H. leporinum* was observed in pot experiments. *H. leporinum* biotypes THL1 and THL3 selected for 12 years were more resistant to paraquat and diquat than biotypes THL2 and VHL1 that had been selected for 24 years.

Replacement series experiments were conducted to investigate the fitness of a resistant biotype (THL1) compared to a susceptible biotype (THL4) in the absence of herbicide. The results indicated that the two biotypes were equally fit. No difference was found in the productivity of the resistant and susceptible biotypes when the plants were grown in the field under non-competitive conditions during the winter growing season. Although the number of tillers of the susceptible biotype increased faster than that of the resistant biotype early in ontogeny, later in ontogeny no difference was found in the number of spike inflorescences, number of seed per inflorescence, seed weight or above-ground dry

matter production. The results from these experiments indicate that once a resistant population appears in a field it will persist in the absence of herbicide use.

Seed dormancy of the resistant (THL1) and susceptible (THL4) biotypes of *H. leporinum* was investigated. Germinability of fresh seeds and seeds buried under 2 cm below the soil surface were examined at different times. The results demonstrated that there is no difference in germinability between the two biotypes and both biotypes have no seed dormancy.

A field experiment was conducted to investigate the initial frequency of resistance in a population of *Hordeum* with no herbicide history. More than six million *Hordeum* plants were treated with either 100 or 200 g a.i ha^{-1} paraquat during the winter growing season and none of the plants survived the herbicide applications. This result demonstrates that the initial frequency of bipyridyl resistance in *Hordeum* spp. is low, less than 1 in 10^6. Model estimates of the initial frequency of bipyridyl resistance in *Hordeum* spp were from 10^{-11} to 10^{-23}.

The mode of inheritance of bipyridyl resistance in *A. calendula* and *H. leporinum* were investigated by crossing resistant and susceptible plants. Progenies from crosses along with both parents were grown outdoors during the winter growing season and then sprayed with paraquat or diquat herbicides. Response of F_1 plants from reciprocal crosses of resistant and susceptible biotypes of *A. calendula* to diquat were similar and showed an intermediate response to the resistant and susceptible parents. Segregation ratios in F_2 plants and backcross plants were 1 : 2 : 1 and 1 : 1 respectively. These results indicated that resistance in *A. calendula* resides in nuclear genome and controlled by a single partially dominant gene. Likewise, F_1 plants from reciprocal crosses of resistant and susceptible biotypes of *H. leporinum* showed an intermediate response to the resistant and suceptible parents indicating that resistance resides in the nuclear genome. The segregation ratios of 1 : 2 : 1 in F_2 populations indicated that bipyridyl resistance is controlled by a single partially dominant gene. Thus paraquat resistance in

the two resistant biotypes of *A. calendula* and *H. leporinum* is nuclearly inherited and controlled by a single partially dominant gene.

Seasonal effects on bipyridyl resistance in *A. calendula*, *H. glaucum* and *H. leporinum* were examined in pot experiments in summer and winter. A decrease in resistance was observed in resistant biotypes of *H. glaucum* and *H. leporinum* but not in *A. calendula* when the plants were treated in summer. Field experiments in summer and winter where light intensity was manipulated by use of a shadehouse indicated that the decrease of resistance in the two resistant biotypes of *H. glaucum* and *H. leporinum* is due to high temperature not light intensity. The results from these field experiments were supported by a growth room experiment where the resistant plants grown at 15°C and then placed at 30°C immediately after treatment showed reduced resistance compared to plants kept at 15°C. The physiological and biochemical reasons for this reduction in resistance were investigated in a biotype of *H. leporinum.* No difference was found in the photosynthetic activity of the two biotypes when O_2 evolution was measured at low or high temperature. Similarly, there was no difference in the interaction of paraquat with PS I between the two biotypes at low or high temperature. These results indicate that the decreased resistance in the resistant biotypes in summer is not due to any change in the photosynthetic apparatus or at the active site PS I.

Experiments investigating uptake and distribution of ^{14}C-paraquat at low and high temperature revealed that there was no difference in herbicide uptake between the resistant and susceptible biotypes. Both biotypes absorbed more paraquat at lower temperatures than at higher temperatures. Translocation of the herbicide to young tissue in the resistant biotype at low temperature was found to be less than that at high temperature. In addition, photosynthesis of young tissue is less inhibited at low temperature than at high temperature. Therefore the reduction in the level of bipyridyl resistance in the resistant biotype of *H. leporinum* at high temperature is due to increased translocation of the herbicide to the growing tissue.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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CHAPTER 1

GENERAL INTRODUCTION

Herbicides and other pesticides are used successfully throughout the world to suppress the pests that interfere with crops. Persistent application of these pesticides, however, has led to the evolution of mechanisms that enable pests to detoxify or otherwise to resist the chemical toxicants. This adverse impact of chemical pest control is now a global phenomenon that has occurred with insecticides, bactericides, fungicides, rodenticides and herbicides (Georghiou 1986). Herbicide resistance was first reported in *Senecio vulgaris* in 1970 (Ryan 1970) and twenty years later herbicide resistance has been reported in more than 100 weed species and to many classes of herbicides. Most of these cases involve resistance to triazine herbicides where these herbicides have been used repeatedly for a long time (LeBaron 1991; Holt et al 1993).

Herbicide resistance in Australia has been reported in a number of weed species with the first report being resistance to diclofop-methyl in *Lolium rigidum* in 1982 (Heap and Knight 1982). Paraquat resistance was first reported in *Hordeum glaucum* (Warner and Mackie 1983), followed by *Arctotheca calendula* (Powles et al. 1989) and *H. leporinum* (Tucker and Powles 1991). These three weed species are important and widespread annual weeds of southern Australian agriculture. Poor control of these species with paraquat and diquat occurred in lucerne fields after these fields had been exposed once a year for 15-24 years to these herbicides. Paraquat- and diquat-resistant biotypes of all three species have been observed in one lucerne field (Tucker 1989). Recently there have been further failures of paraquat and diquat to control weeds in lucerne-growing operations across southern Australia.

The increasing occurrence of herbicide-resistant weed biotypes throughout the agricultural districts of southern Australia is becoming a serious problem (Powles and Howat 1990).

This resistance is of major practical significance due to the increasing reliance on chemical methods of weed control in agricultural operations in Australia. In particular, the development of multiple- and cross-resistance to a wide range of herbicides observed in biotypes of *Lolium rigidum* (Burnet et al. 1991; Powles and Howat 1990; Heap and Knight 1990) threatens the viability of some cropping operations. The appearance of paraquat and diquat resistant weed populations has not, so far, been a serious practical problem as they can be controlled by alternative herbicides, nevertheless, the increasing numbers of such populations must be of concern. The studies reported in this thesis were carried out to investigate some of the factors which may influence the development of resistance to the bipyridyl herbicides. In particular the following aspects were addressed:

1. The appearance of new paraquat- and diquat-resistant biotypes.
2. The correlation, if any, between the level of resistance to paraquat and diquat and the length of herbicide use for several *H. leporinum* biotypes.
3. The relative fitness of a paraquat-resistant *H. leporinum* biotype compared to a susceptible biotype in the absence of herbicides.
4. The gene frequency for paraquat resistance in a population of *Hordeum* spp. which had never been exposed to paraquat and diquat herbicides.
5. The mode of inheritance of paraquat resistance in a *H. leporinum* biotype and of diquat resistance in an *A. calendula* biotype.
6. The effects of environmental factors, in particular temperature, on the level of paraquat-resistance observed in one biotype of *H. leporinum*.
7. Mechanisms involved in paraquat resistance.

CHAPTER 2

LITERATURE REVIEW

2. 1. INTRODUCTION

The herbicides paraquat and diquat have been used for a long period of time in Australia and elsewhere in the world and the continued use of these herbicides as the only form of weed control has resulted in the appearance of a number of resistant biotypes of weed species in formerly susceptible populations. In this chapter I will review three areas that are relevant to this study. Firstly, I will consider the phenomenon of herbicide resistance with emphasis on the agro-ecological factors that affect the development of resistance. Secondly, I will review the chemistry and use of the bipyridyl herbicides and resistance to these herbicides, concentrating on possible mechanisms of resistance. Finally, I will consider the biology of those genera in which bipyridyl resistance has occurred in Australia with the view to elucidating any common factors which may predispose these genera to develop resistance to paraquat.

2. 2. RESISTANCE TO HERBICIDES

The idea that weeds can become resistant to herbicides is not new and was predicted in the 1950s by Abel (1954) and Harper (1956) who recognised the need for herbicide rotations. The first reported case of herbicide resistance was triazine-resistant *Senecio vulgaris* found in Washington (Ryan 1970). By 1993, biotypes of at least 121 weed species have been reported to display resistance to herbicides, of which 57 species (40 broadleaf weeds and 17 grasses) have biotypes resistant to triazine herbicides and 64 species with biotypes resistant to another 14 groups of herbicides. These herbicide resistant weeds have been reported from many countries around the world (LeBaron 1991; Holt et al. 1993).

In Australia, herbicide resistant weed biotypes of *Lolium rigidum* were first reported in 1982 (Heap and Knight 1982) and now at least nine weed species have resistance to one or more classes of herbicides (Table 2. 1). Of these species *L. rigidum* is of the greatest practical and scientific concern as resistant biotypes of this species can display cross resistance to a number of chemically dissimilar herbicides (Powles and Howat 1990) through multiple mechanisms of resistance (Powles and Matthews 1992).

Table 2. 1. Herbicide resistant weed biotypes in Australia.

Species	Herbicide(s) to which resistant	References
Lolium rigidum	aryloxyphenoxypropanoates, cyclohexandiones, triazines, sulfonylureas, triazinones, dinitroanalines and others.	Heap and Knight 1982, 1986, 1990; Burnet et al.1991; Christopher et al. 1992
Hordeum glaucum	paraquat and diquat	Warner and Mackie 1983; Powles 1986
Hordeum leporinum	paraquat and diquat	Tucker and Powles 1991
Arctotheca calendula	paraquat and diquat	Powles et al. 1989
Avena fatua	aryloxyphenoxypropionates	Mansooji et al. 1992
Avena sterilis	aryloxyphenoxypropionates	Mansooji et al. 1992
Vulpia bromoides	paraquat and diquat	this thesis
Sisymbrium orientale	ALS-inhibitors	P. Boutsalis, Personal comm.
Sonchus oleraceus	ALS-inhibitors	P. Boutsalis, Personal comm.

2. 2. 1. The appearance of herbicide resistant biotypes

The rate of appearance of resistance to herbicides depends on the interaction between features of the weed and features of the herbicides used. The rapidity of development of resistance is influenced by various factors including initial gene frequency, selection pressure and the relative fitness of the resistant population (Gressel and Segel 1982). These will be dealt with in more detail below.

2. 2. 1. 1. Initial gene frequency

The initial gene frequency of resistant genes in a population is influenced by the mode of inheritance. Gressel and Segel (1982) predicted that the frequency of a single dominant gene, without selection, is about 1×10^{-5} to 1×10^{-6} whereas single recessive genes are present at a much lower frequency of around 1×10^{-9} to 1×10^{-11}. Darmency and Gasquez (1990a) found that the frequency of triazine resistance is relatively high, ranging from 1×10^{-4} to 3×10^{-3}. The frequency of diclofop resistant individuals in *Lolium rigidum* populations collected from non-farm sites was around 2×10^{-3} (Matthews and Powles 1992). Therefore, alleles conferring resistance are likely to be present in any large population and the frequency at which they occur influences the rate at which resistance appears. A higher initial gene frequency will lead to an earlier appearance of resistance. In addition, selection pressure and immigration rates may impose unstable equilibrium gene frequencies, below which resistance alleles decrease in fitness and above which they increase (Gressel 1986).

2. 2. 1. 2. Selection pressure

Selection pressure in the case of herbicide use can be thought of in terms of an effective kill. If there is a high effective kill of weeds after herbicide use, then the vast majority of survivors are likely to be resistant. A lower effective kill will leave more susceptible individuals and the selection pressure will be lower. Enrichment of a population with

herbicide-resistant individuals occurs every time herbicides are used; however, the rate of enrichment will vary with the selection pressure. It can take a long time for the frequency of resistant weeds to become noticeable and so this enrichment of herbicide resistant individuals is often not realised until it has become a practical problem (Gressel 1986).

Resistance does not evolve at the same rate for all organisms that come under selection pressure. In one species resistance may develop rapidly whereas in another species it may develop more slowly or not at all (Georghiou and Taylor 1986). The frequency of resistant individuals in a weed population is, in large part, a result of the selection pressure from herbicide use. Less frequent applications of herbicides reduce the selection pressure over time and therefore, reduce the probability and rate of resistance development. Paraquat-resistant weeds for example, have evolved a number of times following frequent application of this herbicide. All of the paraquat-resistance problems have been found in perennial crop areas where weeds had been exposed to this herbicide for a long time (Fuerst and Vaughn 1990). For example, in Australia, paraquat-resistant weeds were found in lucerne fields with a long history of paraquat and diquat use (Powles and Howat 1990). Resistance to herbicides with other modes of action has also appeared after a number of exposures to the herbicide(s) for example, 3-5 applications for ACC-ase inhibitors (Heap and Knight 1982), 5-7 applications for ALS inhibitors (Christopher et al. 1992; Mallory-Smith et al. 1990) and >10 applications for PS II inhibitors (Bandeen et al. 1982; Gressel et al. 1982). The selection pressure on weeds exerted by herbicide application can also be influenced by the presence of other species in the area. For example, Sharma and Vanden Born (1983) reported that application of diclofop-methyl to wild oat plants in a wheat crop decreased the dry weight of survivors less than for such plants treated in the absence of crop.

2. 2. 1. 3. Fitness

It is generally considered that selection for a particular trait brings a penalty to the organism in terms of fitness or the ability to survive and reproduce. The selected trait is

often ecologically less fit compared to the "normal" trait. Under competitive conditions the normal type will outcompete the selected type and maintain them at a low frequency within the population (Radosevich and Holt 1982). Fitness can be defined as the ability of an individual plant to leave a proportion of genes in the gene pool of the population in the next generation (Holt 1990). The characteristics of fitness of a plant are determined by a number of factors such as seed germination and dormancy, and physiological processes that affect growth rate, seed size and yield per plant. Moreover, fitness is also mediated by interactions of the phenotype of an organism with its environment (Holt 1990).

Many triazine-resistant biotypes, such as *Senecio vulgaris* (Conard and Radosevich 1979; Warwick 1980; Holt 1988), *Amaranthus retroflexus* (Conard and Radosevich 1979; Weaver and Warwick 1982), *A. hybridus* (Ahrens and Stoller 1983), *A. powellii* (Weaver and Warwick 1982), *Brassica campestris* (Mappleback et al. 1982), and *Chenopodium album* (Warwick and Black 1981) were found to be ecologically less fit than triazine-susceptible biotypes of these species. Productivity of a triazine-resistant biotype of *S. vulgaris* was also found to be less than that of a susceptible biotype under non-competitive conditions (Holt 1988). The lower ecological fitness of most triazine-resistant biotypes is considered to be a consequence of an alteration of the D1 protein, the active site, which results in less efficient electron transfer at PS II (Conard and Radosevich 1979; Radosevich and Holt 1982; Ahrens and Stoller 1983; Ort et al. 1983; Stowe and Holt 1988; Holt 1990). However, in some cases, there are other influences on productivity which modify the effect of the D1 mutation. For example, a triazine-resistant biotype of *C. album* was found to be a better competitor compared to a susceptible biotype and was also better in growth under non-competitive conditions (Jansen et al. 1986). A triazine-resistant population of *C. strictum* was equally competitive to the susceptible biotype (Warwick and Black 1981) and a triazine-resistant *Phalaris paradoxa* had equal productivity to a susceptible biotype grown under non competitive conditions (Scönfeld et al. 1987). In these cases continued selection pressure may have selected for genes which have modified the negative effect of the mutation on fitness (Holt 1990).

The situation is less clear when fitness of biotypes which are resistant to non-triazine herbicides is examined. Productivity under non-competitive conditions of dinitroaniline-resistant and -susceptible biotypes of *Eleusina indica* were the same (Murphy et al. 1986) but under competitive conditions the resistant biotype was less fit (Valverde et al. 1988). A sulfonylurea-resistant biotype of *Lactuca serriola* produced less above-ground biomass and had a lower relative growth rate than the susceptible biotype under both competitive and non-competitive conditions (Alcocer-Ruthling et al. 1992a). However, in this case, there was no difference in competitive ability between the resistant and susceptible biotypes of *L. serriola*. A study of the seed biology of the same biotypes found that fecundity, seed viability, and seed longevity were the same for both resistant and susceptible biotypes (Alcocer-Ruthling et al. 1992b), although seed of the resistant biotype germinated faster than seed from the susceptible biotype. Lower ecological fitness has also been reported for paraquat-resistant biotypes of *Hordeum glaucum* (Tucker 1989) and *Erigeron philadelphicus* (Itoh and Matsunaka 1990). Tucker (1989) also found that paraquat-resistant *H. glaucum* grown under non-competitive conditions produced less biomass than the susceptible biotype.

The lower competitiveness of a herbicide-resistant biotype would tend to decrease its proportion in a population in the absence of herbicide selection and, under such conditions, after a suitably long period of time its presence would then depend upon replenishment via natural mutation. In contrast, a biotype which was an equal or better competitor would be able to maintain, or even increase, its proportion in a population in the absence of the herbicide selection pressure.

2. 2. 2. Evolution of herbicide resistance

2. 2. 2. 1. Genetic variation

The evolution of herbicide resistance in weed species occurs when genetic variation for resistance is present (Holliday et al. 1976). Individual plants which can survive a

herbicide application at a normal field rate may do this as a result of genetically controlled characteristics or may have 'escaped' the application (Gasquez and Darmency 1991). Some reasons why plants could survive herbicide application are a thicker cuticle, the presence of hair, narrower leaves, longer roots, differential translocation of herbicide or detoxification of herbicide before reaching its target. The plant may also increase the production of protective enzymes or mutate the target site (Gasquez and Darmency 1991). The presence of genetic variation to herbicides in natural populations has been documented, for example *Poa annua* to metoxuron (Grignac 1978), *Avena fatua* to flamprop, difenzoquat, MSMA, diclofop (Somody et al. 1984), barban, and bromoxynil (Price et al. 1983), *A. barbata* to barban and bromoxynil (Price et al. 1983), and *Convolvulus arvensis* to glyphosate (DeGennaro and Weller 1984).

Selection favouring genes conferring herbicide resistance occurs in a normal population following herbicide treatment. For example, *Senecio vulgaris* populations showed a positive linear relationship between percentage survival of a population and the number of consecutive years of herbicide application (Holliday and Putwain 1980). A single ecotype of *P. annua* was found to be more tolerant of metoxuron after being subjected to strong selection pressure by repeated metoxuron application. The percentage of survival was as high as 46.5% after 18 selections with the herbicide. In contrast only 3.5% of the progeny of an untreated population survived (Grignac 1978). A susceptible population of *L. rigidum* selected with diclofop-methyl over two generations had become resistant to both diclofop-methyl and chlorsulfuron (Matthews and Powles 1992). Grignac (1978) predicted that the evolution of resistance under natural conditions is subjected to both gene flow and the number of resistance genes that exist in a population.

2. 2. 2. 2. Inheritance of resistance

The rate of evolution of herbicide resistance is partly determined by the mode of inheritance of herbicide resistance gene(s) (Holliday and Putwain 1977). The dominance level of a resistance gene is closely related to the rate of evolution of resistance to different

chemical groups. The rate of evolution of herbicide resistance is not only dependent upon selection pressure, although it is an important determinant, but the genetic structure of population, such as the number of genes controlling resistance, the level of dominance, mutation rate and the amount of recombination. These factors are also influenced by the breeding system (Holliday and Putwain 1977) as an efficient outcrossing species can rapidly build up resistance.

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 are maternally inherited (Darmency and Pernes 1985; Scott and Putwain 1981; Souza Machado et al. 1978) and conferred by a mutation in a 32kD reaction centre protein (Pfister et al. 1981). In contrast, triazine resistance in *Abutilon theophrasti* is nuclearly inherited and controlled by a single incompletely dominant gene (Andersen and Gronwald 1987) due to enhanced glutathione S-transferase activity for atrazine (Anderson and Gronwald 1991) which results in an enhanced capacity to detoxify the herbicide via glutathione conjugation (Gronwald et al. 1989). Inheritance of ALS-herbicide resistance in *Lactuca* spp., a target site mutant, is controlled by a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990) as is ACCase-herbicide resistance in biotypes of *Lolium multiflorum* (Betts et al. 1992) and *Avena sterilis* (Barr et al. 1992). Paraquat resistance is controlled by a single dominant gene in *Conyza bonariensis* (L.) Cronq. (Shaaltiel et al. 1988), and a single gene (no clear dominance level) in *Erigeron canadensis* (Yamasue et al. 1992). Paraquat resistance in biotypes of *E. philadelphicus* (Itoh and Miyahara 1984) and *H. glaucum* (Islam and Powles 1988) was found to be controlled by a single, partially dominant gene. In the case of *H. glaucum,* resistance is a result of 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 (Harvey et al. 1978). From this it can be seen that there are a wide variety of modes of inheritance for resistance genes. Also the mode of inheritance is dependent on the mechanism that endows resistance.

2. 2. 3. Spread of resistance

Due to the chance nature of the distribution of resistance genes, some populations of weeds may or may not contain resistant plants (Darmency and Gasquez 1990b). The presence of resistance may occur as a result of mutation, although there is no evidence yet that herbicide resistance in the field has resulted from mutation during the period of selection pressure. Nevertheless, in glasshouse experiments, mutation at the target site for triazine herbicides has been observed on plants which previously had never been treated with herbicides and proved to be susceptible to the herbicide (Darmency and Gasquez 1990a; b). A population with no resistant precursor has little risk of the emergence of resistance, but a population which contains resistant individuals can develop resistance. The spread of this resistance is influenced by fitness of the resistant precursor and mode of inheritance of the resistance gene (Darmency and Gasquez 1990b). For example, herbicide resistance in an outcrossing species with a nuclear encoded resistance gene can be spread through pollen flow. The farming system can also play an important role in spreading resistance by moving resistant individuals from one field to another via equipment, hay, stock or animals. This has been observed in the case of paraquat-resistant *H. glaucum* (Tucker and Powles 1988). Seed growing and distribution enterprises may also 'assist' in spreading resistance by contaminating of seed crops with resistant seeds.

2. 2. 4. Environmental effects on herbicide resistance

Studies of environmental effects on herbicide resistance have been confined to comparisons of triazine-resistant and -susceptible biotypes. These studies have mainly emphasised the influence of light and temperature on growth and photosynthetic performance as will be reviewed in more detail below.

2. 2. 4. 1. Light

A triazine-resistant biotype of *Brassica napus* L. grown under moderate to high photon flux density (PFD) resulted in decreased photon yield, decreased light-saturated O_2 evolution and slower growth than the triazine-susceptible biotype. However, resistant plants grown under low PFD exhibited similar photon yield and light saturated O_2 evolution to the triazine-susceptible biotype (Hart and Stemler 1990a). Lower photon yield and O_2 evolution in the triazine-resistant biotype grown under high PFD is probably due to the increased sensitivity to photoinhibition in this biotype (Hart and Stemler 1990b). This is reflected in studies conducted in a glasshouse and growth chambers which found that the growth of triazine-resistant *B. napus* was not different from that of susceptible plants under low PFD. In contrast, the growth of the triazine-resistant lines was significantly less than that of the susceptible lines under high PFD in both glasshouse or growth chamber studies (Hart et al. 1992). These authors concluded that photoinhibitory damage occurred in the resistant plants at high PFD which led to reduced productivity. Similarly a physiological study on triazine-resistant and -susceptible biotypes of *Senecio vulgaris* found that net carbon fixation, quantum yield and O_2 evolution at all light levels were higher in the resistant biotype than in the susceptible biotype (Holt et al. 1981). This study also indicated that under high PFD the triazine-resistant biotype tends to have photoinhibitory damage and less productivity. As a consequence of triazine resistance, the resistant biotypes will have reduced productivity in the field where light intensities will be high for at least a part of the day.

A study of the effect of the diurnal light period has been conducted on triazine-resistant and -susceptible biotypes of *B. napus* during ontogeny. During the vegetative stage the total assimilation of CO_2 over the whole day was lower in the resistant biotype than in the susceptible biotype, although early and late in the day the resistant biotype had higher photosynthetic rates. In contrast, at the reproductive stage the resistant biotype assimilated more carbon throughout the day. It was concluded that the superior

productivity of the susceptible biotype was due to increased assimilation at higher light intensities and early in development (Dekker and Burmester 1992).

2. 2. 4. 2. Temperature

Temperature effects on herbicide resistance have also been examined mainly on triazine-resistant biotypes and the results obtained vary from one study to another. For example, no effect of temperature was found on the growth of triazine-resistant and -susceptible biotypes of *Solanum nigrum* (Jacobs et al. 1988) and *Chenopodium album* (Vencill et al. 1987). However the growth of a triazine-resistant biotype of *Amaranthus hybridus* was less vigorous than that of a triazine-susceptible biotype at low temperature (Vencill et al. 1987).

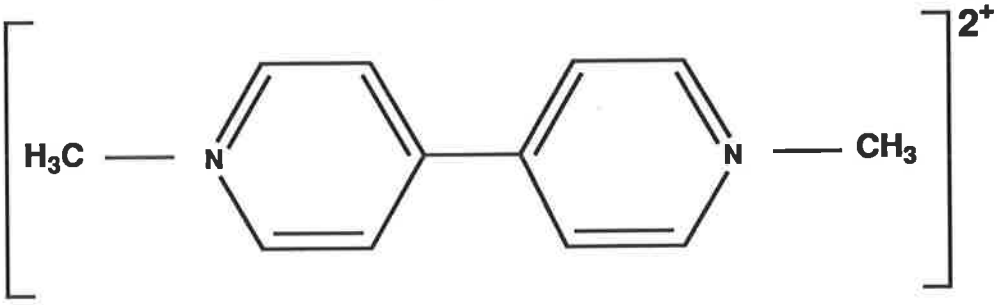
Photosynthetic performance of triazine-resistant biotypes of *S. nigrum, Poa annua* and *C. album* at high temperature was observed and the resistant plants were found to be less tolerant to heat stress (Fuks et al. 1992) compared to susceptible biotypes. These authors concluded that heat tolerance is related to the differences in the chlorophyll antennae organisation between the two biotypes. This conclusion was supported by studies which found that high temperature was shown to inhibit photosynthesis in the resistant plants as a result of inhibition of electron transfer between the primary and secondary quinone acceptors of Photosystem II (Ducruet and Ort 1988; Ducruet and Lemoine 1985; Havaux 1989) due to alteration of the D1 protein of PS II. Photosynthetic electron transport in chloroplasts of a triazine-resistant biotype of *Polygonum lapathifolium* was more sensitive to high temperature than that of the susceptible biotype (Darmency and Gasquez 1982). Thus, triazine-resistant plants probably grow better at cooler temperatures.

2. 3. BIPYRIDYLIUM HERBICIDES

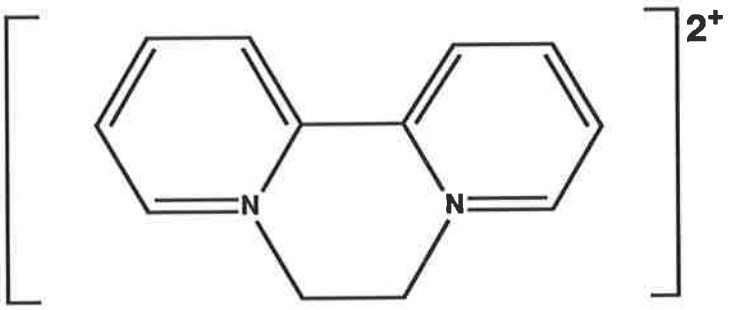
The bipyridylium herbicide group contains two commercially available members, diquat and paraquat (Fig. 2. 1) (Calderbank and Slade 1976). These herbicides are non-selective and act rapidly on plant tissue. They are also non-residual herbicides due to a rapid, irreversible adsorption on contact with soil colloids which renders them inactive (Summers 1980). Their unique combination of properties have made them a discovery of major importance to agriculture. The inactivation of both of these herbicides in soil allows immediate sowing after application, and their use has been instrumental in the adoption of minimum tillage and direct-drill cropping. The implementation of direct-drilling and minimum-tillage operations has been of value in eliminating the negative impact of soil erosion and in providing more flexible systems for farm management (Calderbank and Slade 1976). Both diquat and paraquat can be used for control of both terrestrial and aquatic weeds (Calderbank and Slade 1976).

2. 3. 1. History

The herbicidal properties of the bipyridylium herbicides were discovered at Jealott's Hill Laboratories of Imperial Chemical Industries Ltd. in the 1950s. The first herbicide of this class discovered was diquat (1,1'-ethylene-2,2'-bipyridylium dibromide) followed by paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride) (Dodge 1971). Paraquat, which was originally prepared by Weidel and Russo as the diiodide salt in 1882, had been used as an oxidation-reduction indicator under the name methyl viologen since 1932 (Summers 1980). Diquat was first synthesised by R. J. Fielden and its structure was assigned by R. F. Homer (Brian et al. 1958). Paraquat was first marketed for agricultural purposes in 1962 (Tsunenari 1975).



Paraquat



Diquat

Figure 2. 1. Structures of paraquat and diquat.

2. 3. 2. Herbicidal use

Both paraquat and diquat are recognised as knockdown herbicides with similar actions. Paraquat has a greater effectiveness against grass species (Jeater 1964; Summers 1980) while diquat is superior against some broadleaf weeds (Calderbank and Slade 1976). The reason for this difference in efficacy of the two herbicides against monocotyledonous and dicotyledonous weed species is still not understood. Diquat and paraquat herbicides are used as general contact weed control agents and are applied to the foliage. They have been used selectively for the control of annual species in herbaceous perennial crops where perennial species are either not exposed to, or will recover from contact spray, whereas the annual weed species will die. These herbicides are strongly bound by soil particles (Damanakis et al. 1970) which makes them safe for use shortly prior to sowing the crop or before emergence of the crop (Ashton and Crafts 1981). Paraquat residues remaining on the soil surface can inhibit germination of some weed species such as *Lolium perenne* (Watkin and Sagar 1971); however, this toxicity disappears immediately the soil is disturbed mechanically (Watkin and Sagar 1971).

2. 3. 3. Degradation and metabolism of paraquat and diquat

Herbicides can be removed from the environment by degradation and metabolism. Fungi such as *Penicillium frequentans, Aspergillus niger* (Smith et al. 1976), *Lipomyces starkeyi* (Carr et al. 1985) and *Neocosmospora vasinfecta* (Funderburk and Bozarth 1967) are able to slowly degrade paraquat. *A. niger* also degrades diquat (Smith et al. 1976). Degradation of paraquat and diquat also occur photochemically under ultra violet light (Funderburk and Bozarth 1967; Slade 1966; Smith and Grove 1969). The potential for metabolism of paraquat and diquat in higher plants has been examined by feeding the plants with ^{14}C-labelled paraquat and diquat. These studies found that these two herbicides were not metabolised by plants (Funderburk and Lawrence 1964; Slade 1966).

2. 3. 4. The effect of environmental factors on herbicide efficacy

Environmental factors have long been known to influence the activity of diquat and paraquat. Temperature dependence was found in the herbicidal activity of paraquat on the membranes of plants where at low temperature paraquat was less effective than at higher temperatures in causing membrane leakage (Merkle et al. 1965). In another study, low temperatures (5° to 6°C) delayed paraquat activity in treated plants in the short term, but in the long term the effectiveness of paraquat at low temperatures and higher temperatures was equal. In the field, visible symptoms of paraquat action can be delayed by up to 10 days at lower temperatures compared to at higher temperatures (Bovey and Davis 1967). Soil moisture and relative humidity influence uptake and movement of the paraquat. The downward movement of paraquat within treated plants is increased in a moist atmosphere combined with dry soil conditions. In contrast the movement of paraquat is limited when plants are in wet soil (Brian and Headford 1968).

2. 3. 5. Uptake and translocation of herbicide

Uptake of these herbicides is rapid with more than 30% of the applied herbicide being absorbed in the first hour after treatment (Brian 1967a). Light and dark conditions following application influence the uptake and subsequent translocation of these herbicides. A period of darkness following application of diquat (Brian 1969; Smith and Sagar 1966) and paraquat (Brian 1969; Slade and Bell 1966) is necessary for the herbicides to be widely distributed within the plant. The darkness is required for adequate penetration and local distribution of the herbicides (Mees 1960; Brian 1969; Smith and Sagar 1966). Plants kept in the dark for a period of time following herbicide application were killed when subsequently exposed to light whereas treated plants kept in the light after treatment showed only localised damage (Baldwin 1963; Brian 1967b). This has led to the recommendation that farmers apply these herbicides close to dusk (Anonymous 1992). Exposure of treated plants to strong light causes damage to the tissue which

impedes further movement of the herbicides (Slade and Bell 1966). In contrast light or dark conditions before application had no effect on the uptake of diquat (Brian 1966).

Translocation of paraquat and diquat has been observed in a number of studies (Slade and Bell 1966; Thrower et al. 1965; Smith and Sagar 1966; Brian 1969; Baldwin 1963). All of these studies found that paraquat and diquat herbicides were translocated within treated plants kept in the darkness for a period of time following treatment, except for Thrower et al. (1965) who suggested that translocation of diquat occurred readily in either light or darkness. Translocation of these herbicides takes place predominantly in the xylem and is associated with the transpiration stream (Slade and Bell 1966; Thrower et al. 1965). Uptake and translocation of the herbicides were also influenced by other environmental factors. For example, high humidity of the environment, particularly before application of these herbicides, increased the uptake and translocation of both herbicides (Brian 1966; Brian and Ward 1967).

2. 3. 6. The active site at Photosystem I

The bipyridylium herbicides, following application and uptake into the plants, are reduced at the terminal end of Photosystem I (PS I). The PS I reaction centre is a thylakoid-embedded chlorophyll-protein complex which, in participation with the PS II complex and the cytochrome b_6/f iron sulfur-complex, functions to transfer electrons from water to $NADP^+$. PS I consists of a series of redox components with mid-potentials ranging from around -880 to -320 mV (Fig. 2. 2) (Bowyer and Camilleri 1987). In PS I the absorption of a photon results in a charge separation between the primary donor (P700) and acceptor (A_0) molecules (Golbeck and Bryant 1991). The primary electron acceptor in PS I is called A_0, which is probably a chlorophyll monomer. The electron then passes to A_1, a phylloquinone molecule, which has a mid-potential around -730 mV (Bowyer and Camilleri 1987; Golbeck and Bryant 1991). The electron then traverses three iron-sulphur clusters known as F_X, F_A and F_B with mid-potentials of -705 to -530 mV (Golbeck and Bryant 1991). These iron-sulphur clusters are the source of electrons for

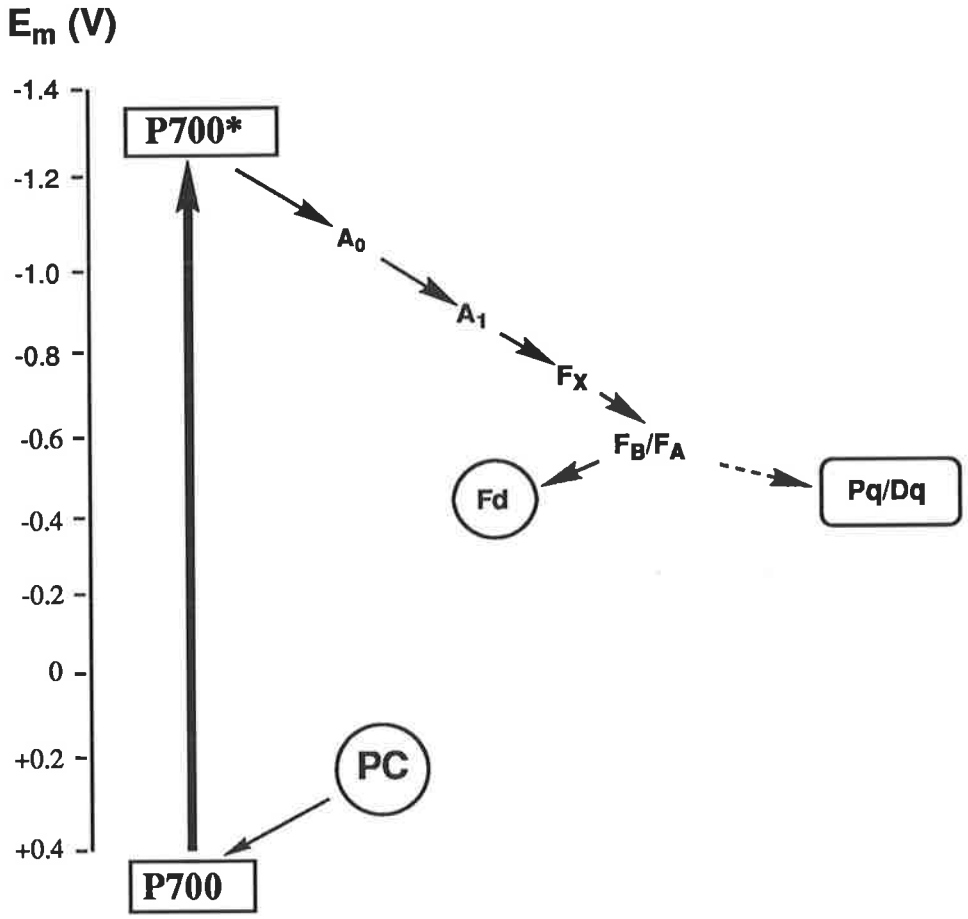


Figure 2. 2. Electron transfer around Photosystem I including paraquat and diquat as electron acceptors (modified after Golbeck and Bryant 1991).

the photoreduction of ferredoxin (Fd) on the outside of the thylakoid membrane which reduces $NADP^+$ to yield NADPH which is used for CO_2 fixation.

2. 3. 7. Mode of action

Bipyridylium herbicides are redox active compounds which interact with Photosystem I. Paraquat and diquat have mid-point redox potentials of -446mV and -349mV respectively (Homer et al. 1960). In the presence of light and oxygen, paraquat and diquat compete with ferredoxin for electrons (Bowyer and Camilleri 1987) from PS I (Summers 1980). Electrons are diverted from one of the iron-sulphur centres, F_B (Golbeck and Cornelius 1986), forming a bipyridyl cation radical (BP^{+*}, equation 1) and this unstable bipyridyl cation radical reacts with O_2 to form superoxide (equation 2). Superoxide can be detoxified by the plant via superoxide dismutase to produce hydrogen peroxide and molecular oxygen (equation 3). Hydrogen peroxide can be further detoxified by the enzymes of the ascorbate-glutathione cycle. The net result of these reactions is the consumption of NADPH (equation 4).

1) $BP^{2+} + e^- \longrightarrow BP^{+*}$

2) $BP^{+*} + O_2 \longrightarrow BP^{2+} + O_2^{-*}$

3) $2O_2^{-*} + 2H^+ \longrightarrow H_2O_2 + O_2$

4) $H_2O_2 + NADPH + H^+ \longrightarrow 2H_2O + NADP^+$

Other reactions also can occur within the chloroplast involving H_2O_2. In the presence of ferrous salts and H_2O_2, hydroxyl radicals (OH*) can be produced in the Fenton reaction (Cobb 1992) (equations 5, 6 and 7). Hydroxyl radicals can also be produced in Winterbourn's reactions (Winterbourn 1981) (equations 8 and 9):

5) $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^*$

6) $Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + O_2^- + H^+$

7) $Fe^{3+} + O2^{-*} \longrightarrow Fe^{2+} + O_2$

8) $BP^{+*} + H_2O_2 \longrightarrow BP^{2+} + OH^- + OH^*$

9) $BP^{+*} + Fe^{3+} \longrightarrow BP^{2+} + Fe^{2+}$

The hydroxy radical, rather than superoxide, is probably the damaging species. Considerable quantities of hydroxyl radicals have been observed to be produced in plants following paraquat application (Babbs et al. 1989). The hydroxyl radicals are highly reactive and will react with biological molecules in the vicinity to produce secondary radicals (Cobb 1992). These radicals attack double bonds in the fatty acid side chains of lipids with the net result of these chains being broken (Kunert and Dodge 1989). The membrane disruption that results causes the death of the plant cell. These herbicides, in particular paraquat, are also able to interact with electron transfer systems in animals which create lung pathology and finally death due to failure of the respiratory system (Fridovich and Hassan 1979), and so are toxic to animals. They can also be toxic to plants kept in the dark, but the herbicidal action does not occur as rapidly as in the light. In addition, the toxic symptoms in the light of blackening followed by wilting are different to those in the dark where the black colour only appears in very wilted plants (Mees 1960). The mechanism leading to death in the dark is not clear but some evidence suggests that these herbicides, as in animals, may interact with other electron transport systems forming bipyridyl radicals (Homer et al. 1960; Calderbank and Slade 1976).

2. 4. BIPYRIDYLIUM HERBICIDE RESISTANCE

2. 4. 1. Occurrence

The first reported case of bipyridyl resistance was paraquat resistance in *Lolium perenne* which was reported by Faulkner in 1976. There are now 16 species of weeds which have biotypes resistant to the herbicides paraquat and diquat. Four of these paraquat-diquat resistant species were found in Australia (Table 2. 2). In general, most of the paraquat and diquat resistant biotypes have appeared in cropping areas after repeated use

of these herbicides for a long period of time as the only form of weed control. Resistance to these herbicides has only occurred following exposure of a population in the field to these herbicides several times each year for 5 to 10 years (Pölös et al. 1987; Clay 1989) or following annual applications for up to 24 years (Powles 1986). Resistance is mainly found in perennial cropping areas, for example, resistant *Poa annua* appeared in hop gardens in the UK (Putwain 1982), *Erigeron philadelphicus* in mulberry fields and *E. canadensis* in vineyards in Japan (Watanabe et al. 1982), *Conyza bonariensis* in vineyards and citrus plantations in Egypt (Gressel et al. 1982), and *Hordeum glaucum*, *H. leporinum* and *A. calendula* in alfalfa fields in Australia (Powles and Howat 1990).

Table 2. 2. Occurrence and Distribution of Weed Biotypes Resistant to Paraquat and Diquat

Species	Origin	References
Amaranthus lividus	Malaysia	Itoh et al. 1992
Arctotheca calendula	Australia	Powles et al. 1989
Conyza bonariensis	Egypt	Gressel et al. 1982
Crassocephalum crepidioides	Malaysia	Itoh et al. 1992
Epilobium ciliatum	Belgium	Bulcke et al. 1987
Erigeron canadensis	Hungary	Lehoczki et al. 1984
	Japan	Hirata and Matsunaka 1985
Erigeron philadelphicus	Japan	Itoh and Miyahara 1985
Erigeron sumatrensis	Japan	Hanoika 1987
	Malaysia	Itoh et al. 1992
Hordeum glaucum	Australia	Powles 1987
Hordeum leporinum	Australia	Tucker and Powles 1991
Parthenium hysterophorus	Kenya	LeBaron 1991
Poa annua	U. Kingdom	Gressel et al. 1982
Solanum americanum	USA	Bewick et al. 1991
Solanum nigrum	Malaysia	Itoh et al. 1992
Vulpia bromoides	Australia	this thesis
Youngia japonica	Japan	Hanoika 1987

2. 4. 2. Mechanisms of resistance to paraquat or diquat

Mechanisms of paraquat and diquat resistance may be due to one or more possibilities as follows: reduced uptake of herbicide, modification of site of action, increased metabolism of the herbicide, detoxification of the toxic products of herbicide action, reduced herbicide translocation, and compartmentation.

Reduced Uptake of Herbicide

Bipyridyl resistant biotypes would survive and grow normally if the herbicides could not penetrate the leaves. However, paraquat uptake in the resistant biotypes of *Hordeum glaucum* (Bishop et al. 1987), *Conyza bonariensis* (Fuerst et al. 1985), *Lolium perenne* (Harvey et al. 1978), and *A. calendula* (C. Preston, personal comm.) was not significantly different to the susceptible biotypes.

Modification of the Site of Action

The possibility that a modification of the site of action in Photosystem I resulting in paraquat resistance has been examined in paraquat-resistant and -susceptible biotypes of *H. glaucum* (Powles and Cornic 1987), *H. leporinum* (this thesis), *C. bonariensis* (Fuerst et al. 1985) *L. perenne* (Harvey and Harper 1982) and *A. calendula* (C. Preston, personal comm.). These studies found that no alterations at the active site have occurred in the resistant biotypes.

Metabolism of Herbicide

The possibility that paraquat and diquat are metabolised is unlikely because these herbicides have not been shown to be metabolised in plants (Funderburk and Lawrence, 1964). Investigations with resistant biotypes of *L. perenne* (Harvey et al. 1978) and *C.*

bonariensis (M. Norman, P. Fuerst, R. Smeda and K. Vaughn, unpublished results) found no metabolism of these herbicides.

Reduced Translocation of Herbicide

Paraquat and diquat are translocated within the plants when kept in the dark following application of the herbicide. Studies of the translocation of paraquat in the leaf of resistant plants have been conducted in biotypes of *H. glaucum* (Bishop et al. 1987), *E. philadelphicus* (Tanaka et al. 1986) and *C. bonariensis* (Fuerst et al. 1985). In these studies ^{14}C-labelled paraquat was fed through the petiole or cut leaf base and reduced translocation of the herbicide within the resistant biotype compared to the susceptible biotype was observed. Preston et al. (1992) measured the amount of paraquat in young tissue of resistant biotypes of *H. glaucum* and *H. leporinum* as the result of basipetal translocation and found reduced translocation of the herbicide occurred in the resistant biotypes. This reduced translocation was claimed to be the mechanism of resistance for these biotypes.

Detoxification of Toxic Products of Herbicide Action

Resistance to paraquat and diquat could result from an increase in the activity of protective enzymes (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase) which are able to detoxify the oxygen radicals and therefore prevent lipid peroxidation. Studies on these protective enzymes in paraquat-resistant and -susceptible biotypes of *H. glaucum* (Powles and Cornic 1987), *Conyza bonariensis* (Vaughn and Fuerst 1985), *Ceratopteris richardii* (Carroll et al. 1988), and *L. perenne* (Harper and Harvey 1978) found no difference of the protective enzyme activity with the exception of *L. perenne*. The paraquat-resistant biotype of *L. perenne* has increased activity of superoxide dismutase, catalase and peroxidase by 1.6, 1.3 and 1.4 fold respectively compared to the susceptible biotype. In the case of paraquat-resistant *C. bonariensis* a more detailed study on the role of these protective

enzymes found that the activity of these enzymes did not differ in whole leaf extracts but differed in chloroplasts where the activity of superoxide dismutase, ascorbate peroxidase and glutathione reductase were 1.6, 2.5 and 2.9 times higher respectively in the resistant biotype than in the susceptible (Shaaltiel and Gressel 1986).

Compartmentation of Paraquat

Compartmentation has been suggested as a mechanism of paraquat resistance in *H. glaucum*, where compartmentation outside the cell has been suggested as a mechanism (Powles and Cornic, 1987; Preston et al. 1992). This mechanism has also been suggested for *C. bonariensis* where resistance has been attributed either to binding to a cellular component (Fuerst et al. 1985), or active sequestration in the vacuole (Fuerst and Vaughn 1990).

In conclusion, in most cases paraquat resistance is often associated with decreased herbicide translocation, which may be due to compartmentation of the herbicide. In some cases increased detoxification of active oxygen species has been suggested to be the mechanism of resistance. This may be the case for *L. perenne* which has a low level of resistance but seems unlikely in *C. bonariensis* as the increased levels of enzymes appear to be too small to account for the >100 fold level of resistance observed (Fuerst and Vaughn 1990).

2. 5. BARLEY GRASSES (*HORDEUM* SPP.)

2. 5. 1. Introduction

The genus *Hordeum* in the family of Graminae is composed of many species (Smith 1972). The members of the genus *Hordeum*, except *Hordeum vulgare* (barley), are not economically important crop species. However, a number of species of *Hordeum* are an

important component of grasslands in some parts of the world. *Hordeum* spp. can be either annual or perennial species. Three species, *H. leporinum, H. glaucum* and *H. murinum*, which belong to the *Hordeum murinum* complex, are the major weed species of this genus present in Australia (Cocks et al. 1976).

2. 5. 2. Seed characteristics and seed production

The seeds of *Hordeum* are carried in florets which break from the rachis in three groups, with one central fertile floret and two sterile lateral florets. The size of the seeds are relatively large compared to other grass weeds. The long, barbed awns of the seeds cause irritation of the mouths, eyes, and noses of cattle and sheep and can become entangled in wool (Smith 1968a). Barley grass seeds cause considerable stock damage and reduced productivity of pastures (Kloot 1981). The sharp awn of the seed also plays an important role in dispersal of the seed which can be carried a long way in the hair or hide of many animals. Seed contamination of forage for domesticated animals is another factor involved in dispersal, particularly in moving species to new continents (Smith 1972). Barley grass is characterised by early seasonal maturity and seeds are produced copiously during the spring (Grant and Rumball, 1971). Seed production as high as 25,000 seeds m^{-2} has been recorded in Australia (Smith 1968c).

2. 5. 3. Dormancy and germination

Summer dormancy is a characteristic of *Hordeum* seed and freshly matured seeds do not germinate readily in the first summer (Rossiter 1966; Powles et al. 1992). This may be largely a result of the high soil surface temperatures in summer which can induce temporary dormancy even in the presence of abundant moisture (Smith 1968a). This characteristic resistance of the seed to germination at high temperatures increases the possibility of survival in southern Australia where germination following summer rains can result in seed wastage of many pasture species (Smith 1968a). Germination appears to be controlled in a complex way through the interaction of temperature and moisture in

summer and by day and night in autumn. In the field, 99% germination of *H. leporinum* in north-east Victoria had occurred by the end of May or June in any one year (McGowan 1970). In a laboratory study, freshly matured seeds of *H. leporinum* had low germination at all temperatures tested; however, after being stored for around one month a large proportion of seeds germinated, suggesting dormancy in this species is short (Popay 1981). Similarly, in a field study *Hordeum* seed showed a high percentage germination after 2 months in the soil over summer (Powles et al. 1992).

Light has no effect on the germination of *H. murinum* (Davison 1971) and *H. leporinum* (Cocks and Donald 1973). Similarly shallow burial does not affect germination of *H. murinum* (Popay and Sanders 1975) or *H. glaucum* (Tucker 1989).

2. 5. 4. Distribution

The distribution of barley grasses is influenced by climatic factors. They are typical mediterranean annual-type plants and are found where the climate is characterised by mild wet winters and hot dry summers (Rossiter 1966). In Australia, *Hordeum glaucum* is prevalent in the drier areas whereas *H. leporinum* is commonly found in the wetter areas and it is considered that the 425 mm isohyet divides the two habitats (Cocks et al. 1976). Along this isohyet the two species, *H. glaucum* and *H. leporinum*, often grow together. *H. murinum* in New Zealand invades pastures in high rainfall areas (1050 mm average annual precipitation) (Smith 1972) and the same may be true in Australia. Davison (1971) found that in Europe *H. murinum* is found in northern and cooler climates while *H. leporinum* is more restricted to mediterranean climates. *H. hystrix* and *H. marinum* are found in saline environments (Popay and Allen, 1988).

In southern Australia the life cycle of barley grasses is extraordinarily well adapted to the climatic and biotic factors prevailing over much of the grazed lands which leads not only to persistence, but also to abundance (Smith 1972). The adaptation of *H. leporinum* to environmental conditions in southern Australia results in high herbage production in the

early part of the winter growing season, frequently a time of acute shortage of forage. This benefit is however, offset by the stock damage caused by the sharp awns later in the growing season.

2. 5. 5. Soil factors

Hordeum plants prefer fertile soils (Smith 1968b; Davison 1971) with the nutrient status (particularly nitrogen and phosphate) in the soil playing an important role in invasion of barley grasses. *Hordeum murinum*, for example, enters annual pastures only when superphosphate has been added at a high rate and legumes have increased the nitrogen status (Davison 1971). The frequencies of barley grass plants are positively correlated with levels of available soil nitrogen (Grant and Ball 1970) and their presence is associated with high soil phosphate status (Rossiter 1964). In addition, barley grass is more abundant on mildly acid, pH 5.0 - 6.8, soil (Tiver and Crocker 1951). Cocks (1974) found that with low soil nitrogen *H. leporinum* was a better competitor than *Lolium rigidum* and suggested this was due to *Hordeum* plants quickly depleting the available soil nitrogen as a result of rapid germination and early growth. Smith (1968a) reported that *Hordeum* spp. are dependent upon soils of high fertility and at low fertility they are very susceptible to frost damage. The success or failure of *Hordeum* as a persistent pasture species in most soils is probably related to its ability to produce copious seed and its long bristly awns which both discourage grazing animals and aid in its dispersal (Smith 1972; Cocks and Donald 1973).

2. 6. CAPEWEED (*ARCTOTHECA CALENDULA* (L.) LEVYNS)

2. 6. 1. Introduction

A. calendula (L.) Levyns is an annual dicot herb, a member of the Asteraceae. Leaves are divided into lobes and their under surface are covered with downy hairs. Outer

florets of the inflorescences are long and spreading and yellow in colour. Disk florets are tubular and bisexual and mature seed is covered with a pinkish, tangled, wooly covering (Lamp and Collet 1979).

The genus of *Arctotheca* is a native of South Africa. Four species of this genera, *Arctotheca prostrata, A. nivea* (= *A. populifolia*), *A. calendula*, and *A. forbesiana* are found in South Africa and two of these species, *A. calendula* and *A. populifolia*, occur in Australia (Hallet 1971). In Australia, *A. calendula* is a weed of cultivation and waste places and can be abundant in pastures (Lamp and Collet 1979).

2. 6. 2. Distribution

The distribution of *Arctotheca* outside South Africa is largely restricted to areas with similar climatic conditions to its original habitat. *A. calendula* was accidentally introduced last century to Australia and is now widespread throughout Southern Australia (Levyns 1950). *A. calendula* is abundant in areas with a Mediterranean climate and has been observed to be the dominant species in some pastures (McIvor 1972).

A. calendula is apt to affect stock with prolonged feeding, causing mild gastro-enteritis. Eating large amounts of this weed has been considered to be the cause of death in cows and lambs in New South Wales. Ingestion of *A. calendula* has also been reported to cause diarrhoea and weight loss in sheep. In addition, pollen of *A. calendula* can cause hay fever in humans (Hurst 1942).

2. 6. 3. Climatic factors

The presence of *A. calendula* in pastures is related to the amount of annual rainfall. *A. calendula* does not appear in areas where the average annual rainfall is less than 254 mm and its incidence increases with increasing annual rainfall (Hallet 1971). *A. calendula*

was observed to be a dominant species over the rainfall range of 305 mm to 635 mm per annum whereas beyond 762 mm *A. calendula* densities decreased (Rossiter 1966).

2. 6. 4. Seed, germination and establishment

A. calendula frequently appears as isolated single plants in pastures. The plant spreads through the large number of seeds produced by this plant. For example McIvor (1972) found that *A. calendula* is able to produce 4,000 seeds per plant. He also found the seed of this plant developed rapidly after flowering. This is, in his opinion, the reason why *A. calendula* may be able to maintain seed production at high stocking rates. Seeds of *A. calendula* are able to germinate at temperatures between 8.5°C and 32°C; however, reduced germination occurs above 20°C (Hallet 1971).

The successful establishment of *A. calendula* in pastures is determined by several factors such as the predominant germination at higher temperatures and the tolerance of seedlings to drought which gives an advantage in competition with other species at the start of the season. *A. calendula* also has the ability to establish on bare ground (Hallet 1971).

2. 6. 5. Soil factors

Soil fertility affects the content of *A. calendula* in pastures. The increase of soil fertility due to sub clover growth and superphosphate applications can result in an increase in *A. calendula* content of pastures (Tiver and Crocker 1951; Rossiter 1952). Rossiter (1964) found that there was a positive correlation between the increase in percentage of *A. calendula* and the rate of superphosphate application in a subclover-capeweed pasture. *A. calendula* is also highly responsive to soil nitrogen in autumn and spring (McIvor 1972).

2. 7. SILVERGRASS (*VULPIA* SPP.)

2. 7. 1. Introduction

The genus *Vulpia* contains diploid (2n=14) (*V.bromoides, V. fotquerana, V. membranacea, V. muralis, V. octoflora)*, tetraploid (2n=28) *(V. ambigua, V. ciliata, V. fasciculata)* and hexaploid (2n=42) *(V. hirtiglumis, V. megalura, V. microstachys, V. myuros, V. persica, V. sciurea)* species (Cotton and Stace 1976). They occur in Europe, North Africa, West and Central Asia, and North and South America (Cotton and Stace 1976). Six species, *V. fasciculata, V. ciliata, V. megalura, V. myuros, V. muralis* and *V. bromoides,* are widely distributed in southern Australia (Jessop and Toelken 1986).

2. 7. 2. Distribution

V. bromoides and *V. myuros* occur extensively in southern Australia but *V. myuros* is the most widespread (Dillon and Forcella 1984). Both species, *V. bromoides* and *V. myuros* are common weeds of pastures in southern Australia. The plants contribute to livestock carcass irritation but they are more widely known as a contaminant of clipped wool (Forcella 1984). Silvergrasses, with the exception of *V. fasciculata*, are not common weeds of cropping (Jessop and Toelken 1986) due to their susceptibility to cultivation (Dillon and Forcella 1984).

2. 7. 3. Inflorescence and flowering

The length of the inflorescence of *Vulpia* sp. at and after anthesis is a valuable character to identify the members of this genus (Cotton and Stace 1977). In particular, it is a discriminant between *V. bromoides* and *V. myuros*, and a partial discriminant between *V. membranea* and *V. fasciculata.* This character is subject to ecophenetic variation such

that in very dry conditions the normally exserted inflorescences of *V. bromoides* may become included in the uppermost leaf sheath and closely resemble those of *V. myuros*.

Flowering in *V. bromoides* occurs over a wide range of conditions but is limited by temperature and photoperiod (Dillon and Forcella 1984) with the number of flowering culms greater at low temperatures. Intermediate temperatures (23°/15°C) promoted flowering under a short or long photoperiod but flowering is inhibited at high temperatures (Dillon and Forcella 1984). *V. bromoides* has the ability to produce a higher number of flowering culms in a wide range of temperatures/photoperiods than *V. myuros* (Dillon and Forcella 1984). In South Australia, *V. bromoides* flowers in September through January and occasionally at other times (Jessop and Toelken 1986).

2. 7. 4. Dormancy and germination

Seeds of *V. bromoides* have innate dormancy and freshly matured seeds do not germinate for 2-3 months after seed shed (McGowan 1970). Seeds in soil banks cultured in a glasshouse did not germinate until 4-5 months after seed shed (Dillon and Forcella 1984). *V. bromoides* and *V. myuros*, germinate well in a wide range of temperatures from 2°C-31°C (Dillon and Forcella 1984). The optimum temperatures for germination were 12°-23°C for both species in the darkness, and 12°-31°C and 9°-28°C for *V. myuros* and *V. bromoides* respectively, in the light. *V. bromoides* seed germinated less well than *V. myuros* at high temperatures in both light and darkness. Light stimulates the two species to germinate and germination is more rapid in the light than if seeds are kept in the dark (Dillon and Forcella 1984). The depth of seed burial influences both the germination of seeds and seedling emergence (Dillon and Forcella 1984). Burial of seeds of *V. bromoides* at a depth of more than 5 cm results in a low germination and emergence. This sensitivity to burial may be a cause of the absence of silvergrass from conventionally cultivated crops (Dillon and Forcella 1984). The emergence of seedlings can occur at any season of the year (McGowan 1970) but are restricted by the requirement of least 50 mm of precipitation after completion of the dormancy period (Dillon and Forcella 1984).

2. 7. 5. Soil factors

Silvergrasses grow well in pastures with light to medium acidic soils (Rossiter 1966). In the Perth region *V. bromoides* is common in sandy soils (Marchant et al. 1987). Silvergrass is not overly sensitive to soil fertility (Dillon and Forcella 1984) and has the ability to grow well in low nutrient soils (Rossiter 1966).

2. 8. CONCLUSION

The incidents of herbicide resistance show a positive correlation between the development of resistance and the period of herbicide selection pressure. The time taken for resistance to appear is influenced by a number of factors such as the initial gene frequency of resistance, mode of inheritance, seedbank density, seed longevity, and seed production. The mechanisms of resistance may also impact on the resistance development time. The development of bipyridyl resistance has required a long period of herbicide exposure. A number of the factors mentioned above have probably had an impact here. In particular, the low probability of active site changes and herbicide metabolism as mechanisms of resistance to bipyridyl herbicides probably means that resistance to these herbicides will occur at lower frequency than resistance to some other herbicides. There are no obvious clues as to why paraquat resistance has developed in only a few weed species in Australia other than the presence of these weeds in large numbers in those areas where perennial lucerne growing operations occur.

CHAPTER 3

APPEARANCE AND CONTROL OF BIPYRIDYL-RESISTANT WEED SPECIES IN AUSTRALIA

3. 1. INTRODUCTION

Paraquat and diquat have been successfully used to control weeds in Southern Australia over a considerable period. In 1982 a population of *H. glaucum* Steud. infesting a lucerne field at Willaura, Victoria could no longer be effectively controlled with paraquat. This was the first reported case of bipyridyl-herbicide resistance in Australia (Warner and Mackie 1983). This population of *H. glaucum* was subsequently shown to be resistant to both paraquat and diquat (Powles 1986). Tucker and Powles (1988) conducted a survey of 184 lucerne fields in Southern Australia and found paraquat-resistant populations of *H. glaucum* in a number of fields in the Willaura and Ararat regions of Victoria. These authors concluded that there had been at least four separate appearances of paraquat resistance in *H. glaucum* and that the other occurrences observed during the survey were due to the introduction of resistant seed from other fields. One field in which paraquat-resistant *H.glaucum* occurred, was also infested with *Arctotheca calendula* (L.) Levyns (Powles et al. 1989) and *H.leporinum* Link. (Tucker and Powles 1991) resistant to paraquat and diquat.

In 1989, *H. leporinum* from three lucerne fields at Ouse, Tasmania (hereafter referred to as THL1, THL2 and THL3) were not controlled satisfactorily by paraquat and diquat. Each of these lucerne fields had been treated with paraquat-diquat over a long period; the lucerne fields where THL1, THL2 and THL3 seed were collected had been treated annually for 12, 24 and 12 years respectively. The paraquat-resistant population of *H. leporinum* from Victoria had a 24-year history of paraquat and diquat use (Tucker 1989; Tucker and Powles 1991). As the history of paraquat and diquat use in each of the

lucerne fields were different, the effects of paraquat and diquat on all the resistant populations was examined. The relationship between the period of paraquat-diquat use in each field and the level of resistance of these populations was also investigated.

In 1990, a *Hordeum glaucum* population from a lucerne field near Spalding, South Australia which had been treated for at least nine years with paraquat and diquat survived herbicide treatment. Prior to 1987, satisfactory control of *H. glaucum* had been achieved with a combination of paraquat and diquat. In 1990 the application of paraquat and diquat at 250 g and 150 g a.i. ha^{-1} respectively (mixed together in a commercial formulation) failed to control *H. glaucum*. The response of this purported resistant *H. glaucum* to bipyridyl herbicides was also investigated.

In the lucerne field at Elmhurst, Victoria, treated with paraquat and diquat for 24 years and infested with three annual weed species, *H. glaucum*, *H. leporinum* and *A. calendula*, resistant to paraquat and diquat there were some other annual weed species. In this field, *Poa annua, Bromus mollis, Avena fatua* and *Vulpia bromoides,* were tested and found to be susceptible to paraquat and diquat (Tucker 1989). However, in 1989, silvergrass, *V. bromoides*, was observed to survive paraquat application on this field and therefore this species was collected for further testing.

In this chapter the response of these weed populations to paraquat and diquat were examined to determine the level of resistance to these herbicides. The response of these populations to other herbicides was also investigated. The weed populations used in this thesis are listed in Table 3. 1 with their resistance status and collection location.

Table 3. 1. Bipyridyl herbicide-resistant weed species examined in this study

Biotype number	Species	Status	Collection location
SHG1	*H. glaucum*	Resistant	Spalding, S. Australia
SHG2	*H. glaucum*	Susceptible	Spalding, S. Australia
VHL1	*H. leporinum*	Resistant	Elmhurst, Victoria
VHL2	*H. leporinum*	Susceptible	Elmhurst, Victoria
THL1	*H. leporinum*	Resistant	Ouse, Tasmania
THL2	*H. leporinum*	Resistant	Ouse, Tasmania
THL3	*H. leporinum*	Resistant	Ouse, Tasmania
THL4	*H. leporinum*	Susceptible	Ouse, Tasmania
VVB1	*V. bromoides*	Resistant	Elmhurst, Victoria
SVB1	*V. bromoides*	Susceptible	Waite Inst., S. Australia
VAC1	*A. calendula*	Resistant	Elmhurst, Victoria
VAC2	*A. calendula*	Susceptible	Elmhurst, Victoria

3. 2. MATERIALS AND METHODS

3. 2. 1. Plant material

3. 2. 1. 1. *H. glaucum* from Spalding, South Australia (biotype SHG1)

Forty randomly-selected barley grass plants which had survived a field application of 250 and 150 g a.i. ha^{-1} of paraquat and diquat respectively were collected from a lucerne field near Spalding, South Australia in 1990. This population has been designated SHG1 (Table 3. 2). At the same time forty plants were collected from a laneway bordering the lucerne field which had no history of bipiridylium use (SHG2). These plants were transplanted into pots and when growing normally all SHG1 and 20 SHG2 plants were

sprayed with 200 g a.i. ha^{-1} paraquat. All SHG1 plants survived and set seed whereas all SHG2 plants died. Seed was collected from the surviving SHG1 plants and from unsprayed SHG2 plants.

Table 3. 2. Herbicide use record in the lucerne field near Spalding, South Australia where SHG1 biotype was collected.

	Bipyridyl herbicide	
Year[a]	diquat	paraquat
	g a.i. ha^{-1}	
1982	150	250
1983	150	250
1984	150	250
1985	150	250
1986	0	140
	0	160
1987	150	250
1988	150	250
1989	150	250
1990[b]	150	250

[a]This field had been sown to lucerne prior to 1982; however, no herbicide history was available.

[b] applied with diuron at 2 L ha^{-1}.

3. 2. 1. 2. *Hordeum leporinum* from Elmhurst, Victoria (biotype VHL1)

Seeds of a population of *H. leporinum* resistant to the herbicides paraquat and diquat (VHL1) originally obtained from a lucerne field at Elmhurst in Victoria (Table 3. 3)

(Tucker and Powles 1991) were collected from plants which had survived 200 g a.i. ha^{-1} paraquat. Seeds of a susceptible population (VHL2) were collected from plants obtained from a nearby pasture which had no history of bipyridylium herbicide use (Tucker and Powles 1991).

3. 2. 1. 3. *H. leporinum* from Ouse, Tasmania (biotypes THL1, THL2 and THL3)

Seeds of *H. leporinum* plants were obtained from three lucerne fields near Ouse, Tasmania infested with barley grass. These three fields (populations designated THL1, THL2 and THL3) had a long history of paraquat and diquat use having been sprayed once annually with these herbicides for 12, 24 and 12 years respectively (Table 3. 4). Seeds of *H. leporinum* from a nearby pasture with no history of bipyridylium herbicide use were also obtained (THL4). Seed was germinated and the resulting plants sprayed with 200 g a.i. ha^{-1} paraquat. All plants of THL4 died whereas those of the other three populations survived. Seed was collected from the surviving plants and from unsprayed THL4 plants.

3. 2. 1. 4. *Arctotheca calendula* from Elmhurst, Victoria (biotype VAC1)

Seeds of a population of *A. calendula* resistant to the herbicides paraquat and diquat (VAC1) originally obtained from a lucerne field at Elmhurst in Victoria (Table 3. 3) (Powles et al. 1989) were collected from plants which had survived 200 g a.i. ha^{-1} diquat. Seeds of a susceptible population (VAC2) were collected from plants obtained from a nearby pasture which had no history of bipyridylium herbicide use (Powles et al. 1989).

3. 2. 1. 5. *Vulpia bromoides* from Elmhurst, Victoria (biotype VVB1)

Seeds of a *V. bromoides* population (VVB1) which had survived a field application of 125 and 75 g a.i. ha^{-1} paraquat and diquat respectively were collected from a lucerne field at

Table 3. 3. Herbicide use history in the lucerne field at Elmhurst, Victoria where VHL1[a],VAC1[a] and VVB1 biotypes were collected.

Year	Bipyridyl herbicide	
	diquat	paraquat
	g a.i. ha^{-1}	
1963	150	0
1964	150	0
1965	0	0
1966	150	0
1967	150	0
1968	150	150
1969	150	150
1970	150	150
1971	150	150
1972	150	150
1973	300	300
1974	300	300
1975	300	300
1976	300	300
1977	300	300
1978	300	300
1979	300	300
1980	300	300
1981	75	125
1982	0	0
1983	75	125
1984	75	125
1985	150	250
1986	75	125
1987[b]	0	0
1988[b]	0	0
1989	75	125

[a] biotypes VHL1 and VAC1 were collected prior to 1987.

[b] fluazifop-butyl applied at 106 g a.i. ha^{-1}.

Table 3. 4. Herbicide use history of lucerne fields at Ouse, Tasmania where THL1 THL2 and THL3 seeds were collected.

Year	THL1, THL3		THL2	
	Bipyridyl herbicide		Bipyridyl herbicide	
	diquat	paraquat	diquat	paraquat
	g a.i. ha^{-1}		g a.i. ha^{-1}	
1965			0	200
1966			0	200
1967			0	200
1968			0	200
1969			0	200
1970			0	200
1971			0	200
1972			0	200
1973			0	200
1974			0	200
1975			0	200
1976			0	200
1977			0	200
1978	0	200	0	200
1979	0	200	0	200
1980	0	200	0	200
1981	0	200	0	200
1982	0	200	0	200
1983	0	200	0	200
1984	0	200	0	200
1985	0	200	0	200
1986	0	200	0	200
1987	75	125[a]	75	125
1988	75	125	75	125
1989	75	125[a]	75	125

[a] diuron was used for control of shepherd's purse, *Capsella bursa-pastoris*, in the area from where THL1 was collected.

Elmhurst in Victoria (Table 3. 3). Seeds of a susceptible population (SVB1) with no history of bipyridylium herbicide use were collected from the Waite Agricultural Research Institute Arboretum. These seeds were germinated and the plants at 4-5-tiller stage sprayed at 50 g a.i. ha^{-1} paraquat. All SVB1 plants died whereas 92% of VVB1 plants survived. Seed was collected from the surviving VVB1 plants and from unsprayed SVB1 plants.

3. 2. 2. Germination

Seeds of *H. glaucum, H. leporinum* and *V. bromoides* were germinated on 0.6% (w/v) agar for five days in a seed germinator with 21°C, 14 h, 20-30 μmol photons m^{-2} s^{-1} light period/ 15°C, 10 h dark period. When seedlings were 2.5 cm high they were transplanted into 18-cm pots containing potting soil. There were 12 seedlings of barley grass and 21 seedlings of *V. bromoides* per pot. Seeds of *A. calendula* were germinated in plastic trays (42 x 30 x 12 cm) containing potting soil and placed outdoors during the winter for 18 days. Seedlings at the 2-leaf stage were transplanted into 18-cm pots containing potting soil (6 plants per pot). The seedlings were grown outdoors during winter (June - August) which is the normal growing season for these species in southern Australia and were kept well watered.

3. 2. 3. Laboratory herbicide application

Herbicide applications were conducted in a laboratory spray cabinet using a hydraulic laboratory sprayer. The nozzles were positioned 40 cm above the leaves. The output of the sprayer was 115 L ha^{-1} at a pressure of 250 kPa and a speed of 1 m s^{-1}. The herbicides were applied as commercial formulations with 0.2% (v/v) Agral 60 added. Plants were sprayed in the evening and kept indoors overnight (in the dark), to allow the herbicides to be translocated within the plants, and then the pots were placed outdoors the following morning. The number of surviving plants was recorded from 21 to 38 days after spraying. The survivors were harvested by removing the above-ground parts of the

plants which were dried in an oven at 80°C for 24 to 48 hours and dry matter per pot was recorded.

3. 2. 4. Field Experiment

In 1990, in a lucerne paddock near Spalding, a field experiment was conducted to examine possible cross resistance of a paraquat-resistant *H. glaucum* biotype to other herbicides. The dose of each herbicide used was the recommended rate, namely: fluazifop-butyl at 318 g a.i. ha^{-1}, haloxyfop-ethoxyethyl at 12.5 g a.i. ha^{-1}, quizalafop-ethyl at 48 g a.i. ha^{-1}, sethoxydim at 187 g a.i. ha^{-1}, glyphosate at 450 g a.i. ha^{-1}, paraquat at 200 g a.i. ha^{-1} and an unsprayed control. Glyphosate and paraquat were applied with 0.2% (v/v) Agral 60 and all the others were applied with 2% (v/v) DC Tron. The herbicides were applied with a hand-held boom sprayer at a pressure of 250 kPa. The output of sprayer was 133 L ha^{-1} at a speed of 1 m s^{-1}. The position of the nozzles was approximately 40 cm above the plants. The plants were sprayed at 18-25 cm high and at pre-booting to booting stage. Spraying conditions were a temperature of 13°C, light wind and light drizzle one hour after spraying was completed. The design of the experiment was a randomized complete block with three replications. Plot size was 5 m x 2 m with 25 cm borders. Five weeks after spraying the number of inflorescence in eight quadrats (1 quadrat = $0.1m^{-2}$) per plot was determined.

3. 2. 5. Leaf area measurement

The effect of paraquat application on the leaf area of VHL1 and THL1 populations was studied in 1990. Plant material and spraying conditions were the same as described above. Five plants were grown in each 18-cm pot. Plants were sprayed at the 2-3-tiller stage at 0, 200, 400 and 800 g a.i. ha^{-1} of paraquat. There were 16 plants sprayed for each rate of herbicide. The green leaf area 21 days after herbicide application was measured using a Paton electric planimeter (Paton Industries Pty, Ltd)

3. 2. 6. Effect of other herbicides

In these experiments possible cross resistance of some populations to other herbicides was investigated. The herbicides were applied at the recommended rate as follows: glyphosate at either 360 g or 720 g a.i. ha^{-1}, fluazifop-butyl at 200 g a.i. ha^{-1}, sethoxydim at 187 g a.i. ha^{-1}, metsulfuron-methyl at 6 g a.i. ha^{-1}, metribuzin at 300 g a.i. ha^{-1}, trifluralin at 600 g a.i. ha^{-1}, 2,4-D at 850 g a.i. ha^{-1}, diuron at 425 g a.i. ha^{-1}, and terbutryn at 425 g a.i. ha^{-1}. All of these herbicides were applied with 0.2% (v/v) Agral 60.

3. 2. 7. Effect of paraquat at different plant age

The effect of plant age on the level of paraquat resistance was examined in *H. leporinum* biotypes THL1 and THL4. In this experiment plants (8 pots at 4 plants pot^{-1}) of each biotype were sprayed with 0 to 6400 g a.i. ha^{-1} paraquat at the 3-leaf, 3-tiller, 15-tiller and 22-tiller stages. The number of surviving plants and dry weight were recorded 21 days after spraying.

3. 2. 8. Effect of paraquat on seed production

The effect of paraquat application at anthesis (known as spraytopping or pasture topping) on the subsequent seed production was examined on *V. bromoides* biotypes VVB1 and SVB2 and *H. leporinum* biotypes THL1 and THL4. Plants were grown outdoors during the winter growing season. The two biotypes of *V. bromoides* were sprayed with paraquat either at 100 or 200 g a.i. ha^{-1} at anthesis using the laboratory sprayer as previously described. Each treatment comprised four pots. Untreated controls were sprayed with 0.2% (v/v) non-ionic surfactant only. Inflorescences were harvested when all of the plants were mature, the number of seeds per plant was recorded and these seeds subsequently germinated on 0.6% (w/v) agar. The number of seeds germinated was recorded 7 days after incubation.

Seeds of paraquat-resistant (THL1) and -susceptible (THL4) biotypes of *H. leporinum* collected from a productivity experiment (see Chapter 4) were used in this study. Seedlings were transplanted in 18-cm pots with 5 plants per pot. At anthesis plants were sprayed with either paraquat at 150 g a.i. ha^{-1} or glyphosate at 360 g a.i. ha^{-1}. Seeds from treated and untreated plants were collected and then stored for six months at room temperature prior to test of germinability. Germination of seeds were tested on agar 0.6% (w/v) as described previously. Number of germinable seeds were recorded 10 days after incubation.

3. 3. RESULTS

3. 3. 1. Response of *H. glaucum* biotypes to diquat and paraquat

The result of paraquat application at different rates on the two *H. glaucum* biotypes is presented in Fig. 3. 1A and shows that there was no mortality of the SHG1 biotype at the recommended rate of 200 g a.i. ha^{-1} paraquat. In contrast, this rate produced 100% mortality of the susceptible *H. glaucum* biotype, SHG2. Higher rates of paraquat resulted in some mortality of the resistant biotype SHG1 with 38% mortality at 1600 g a.i. ha^{-1} paraquat. Paraquat application at 200 g a.i. ha^{-1} resulted in a 55% reduction of dry weight of SHG1 whilst dry weight of SHG2 was reduced to 0 (Fig. 3. 1C). Diquat application at 200 g a.i. ha^{-1} killed 96% of susceptible biotype (SHG2) but only 5% of the resistant biotype (SHG1) when the plants were sprayed at the 3-leaf stage (Fig. 3. 1B). There were no survivors of SHG2 at 400 g a.i. ha^{-1} whereas 87% of the resistant biotype SHG1 survived. Higher rates of diquat resulted in increasing mortality of SHG1. Dry weight of SHG2 was more affected by diquat application than that of SHG1 at all rates (Fig. 3. 1D).

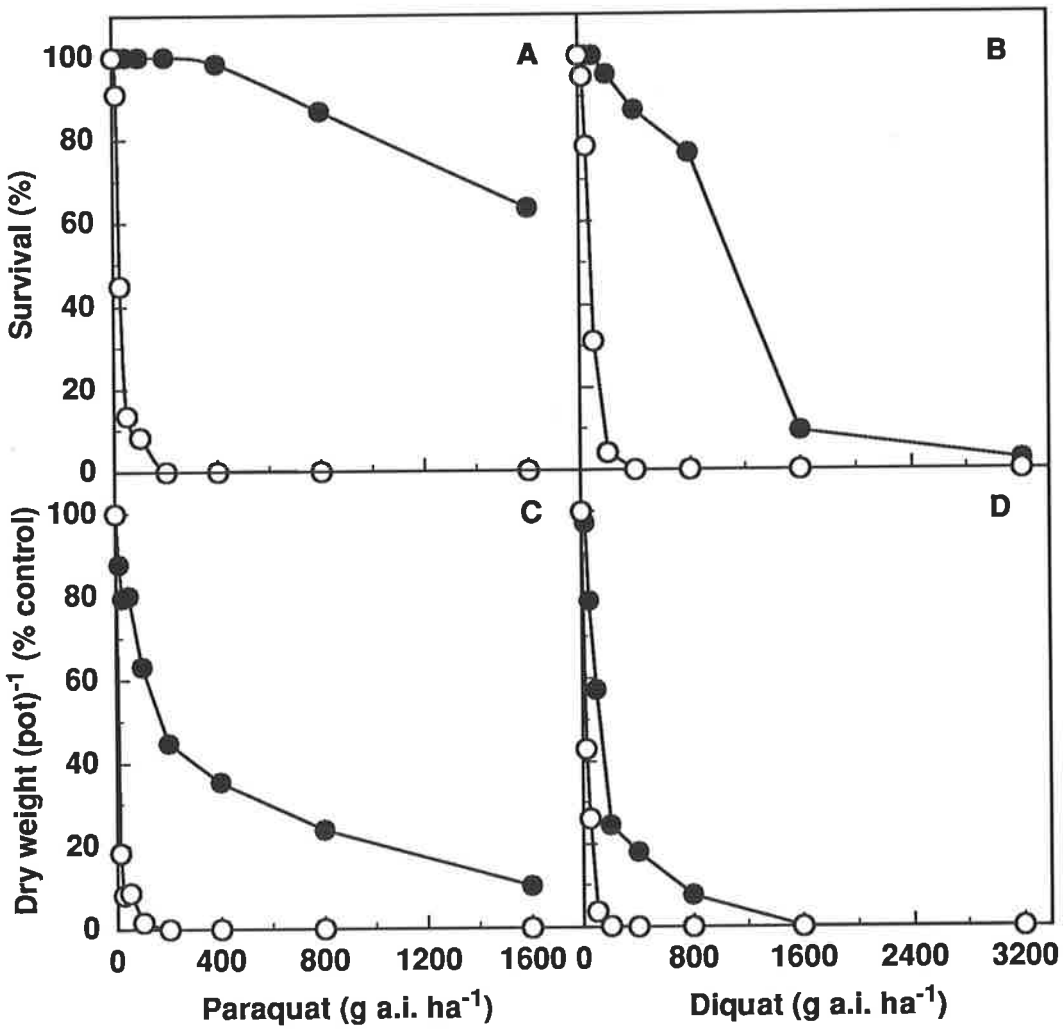


Figure 3. 1. Response of biotypes SHG1 (●) and SHG2 (O) to paraquat (A, C) and diquat (B, D) application. Survival (A, B) and dry weight (C, D) were determined 28 days after spraying. Each point is the mean of 60 plants.

3. 3. 2. Response to alternative herbicides

The results of application of paraquat and alternative herbicides to SHG1 plants in a field experiment is shown in Table 3. 5. All the herbicides used significantly reduced the number of inflorescences of these plants compared to plants sprayed with paraquat or the unsprayed control. Paraquat had no effect on the number of inflorescences produced by these plants.

Table 3. 5. Control of *H. glaucum* biotype SHG1 with alternative herbicides in the field. The number of inflorescence of SHG1 per m^2 was recorded five weeks after spraying.

Herbicide	Dose (g a.i. ha^{-1})	Inflorescence. m^{-2} [a]
Fluazifop-butyl	318	1 b
Haloxyfop-methyl	187.5	0 b
Quizalafop-ethyl	48	33 b
Sethoxydim	187	166 b
Glyphosate	450	0 b
Paraquat	200	820 a
Unsprayed control		880 a

[a] Values in each row followed by the same letter are not significantly different at the 5% level.

3. 3. 3. Response of *H. leporinum* biotypes to diquat and paraquat

The three biotypes of *H. leporinum* from the lucerne fields at Ouse, Tasmania were all resistant to both paraquat and diquat with varying levels of resistance to these herbicides.

Paraquat did not control the *H. leporinum* biotypes (THL1, THL2 and THL3 biotypes) but did control the susceptible biotype (THL4) (Fig. 3. 2A). Biotypes THL1 and THL3 proved very resistant to paraquat application with this herbicide causing little mortality to these biotypes. Biotype THL2 was less resistant to paraquat with 62% survival at the recommended rate, 200 g a.i. ha^{-1} (compared to 5% survival of biotype THL4). When paraquat was applied at a high rate of 1600 g a.i. ha^{-1}, 89% and 88% of THL1 and THL3 biotypes survived respectively, whereas 37% of THL2 survived and none of biotype THL4. The mean dry weights of the four biotypes of *H. leporinum* are shown in Fig. 3. 2C. The mean dry weight for the three resistant biotypes (THL1, THL2, and THL3) was greater than that of the susceptible THL4 biotype at all paraquat rates. The three paraquat-resistant biotypes of *H. leporinum* (THL1, THL2 and THL3) were all more resistant to diquat when compared to the susceptible THL4 biotype (Fig. 3. 2B). The application of 200 g a.i. ha^{-1} (the recommended rate of diquat) resulted in no mortality of biotypes THL1 and THL3. At this rate of herbicide application 59% of THL2 biotype survived; however, only 20% of THL4 biotype survived. At higher rates of herbicide application some mortality was apparent for all biotypes with THL4 clearly the most susceptible biotype, followed by THL2, then THL1 and THL3 which were the most resistant biotypes. A comparison of the responses of these biotypes to paraquat with diquat (Fig. 3. 2 A, B) indicates that THL4 is more tolerant of diquat than paraquat. This is readily explained by the fact that diquat has reduced efficacy against grass species (Calderbank and Slade, 1976). In contrast, the three paraquat-resistant biotypes, THL1, THL2 and THL3, whilst all resistant to diquat, show greater resistance to paraquat. For example, LD_{50} values to diquat for the resistant biotypes were 1.8 to 8 times that of the susceptible THL4, whereas LD_{50} values to paraquat for the resistant biotypes were 19 to >80 times that of the susceptible. The mean dry weight per pot of surviving plants was greater for each of the three resistant biotypes compared to that of the susceptible THL4 biotype after diquat application (Fig. 3. 2D).

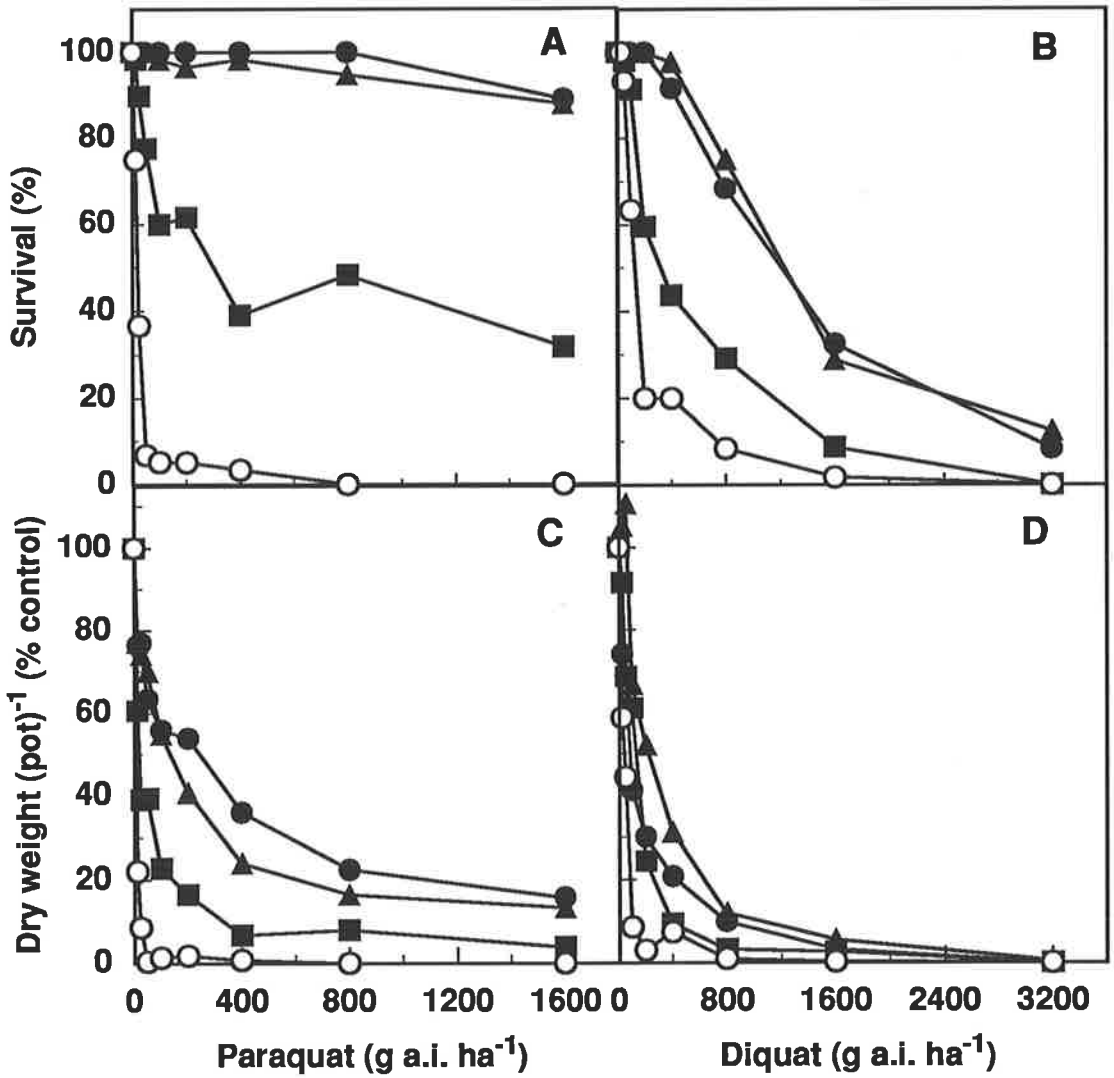


Figure 3. 2. Effect of paraquat on survival (A) and dry weight (C), and of diquat on survival (B) and dry weight (D) of *H. leporinum* biotypes THL1 (●), THL2 (■), THL3 (▲) and THL4 (O) 28 days after spraying. Each point is the mean of 60 plants.

3. 3. 4. Comparison of the level of resistance in resistant *H. leporinum* biotypes

Among the three resistant populations described above, THL1 and THL3 were clearly more resistant to paraquat than THL2. These studies therefore establish that there are now four known *H. leporinum* populations in Australia resistant to the herbicides paraquat and diquat and these have been exposed to different periods of treatment with the herbicides ranging from 12 to 24 years of mostly continuous use (Tables 3. 3 and 3. 4). The level of resistance for each of these resistant biotypes to paraquat and diquat differs markedly (Fig. 3. 3A, B). Biotype THL1 is the most resistant to paraquat followed closely by THL3 with THL2 then VHL1 the least resistant (Fig. 3. 3A). Biotype THL3 is the most resistant to diquat followed closely by THL1. Biotypes THL2 and VHL1 are the least resistant (Fig. 3. 3B). As biotypes THL2 and VHL1 had been exposed to the most herbicide, then it is clear that there is no correlation between the amount of herbicide applied over time and the level of resistance in these four biotypes.

3. 3. 5. Effect of paraquat application on leaf area

The application of paraquat at the recommended rate (200 g a.i. ha^{-1}) on the resistant biotypes THL1 and VHL1 resulted in approximately 41% and 78% loss of green leaf tissue respectively as a result of bleaching (Fig. 3. 4). The loss of green leaf area increased as the dose of paraquat increased with biotype VHL1 more affected than THL1 at all rates of paraquat. In contrast all green leaf tissue of the susceptible biotypes (THL4 and VHL2) was totally bleached at 200 g a.i. ha^{-1} (data not shown). The difference between the two resistant biotypes observed in this experiment correlates well with the level of resistance observed at the whole plant level. This experiment also shows that considerable herbicide damage can occur to resistant plants even though all plants survive treatment.

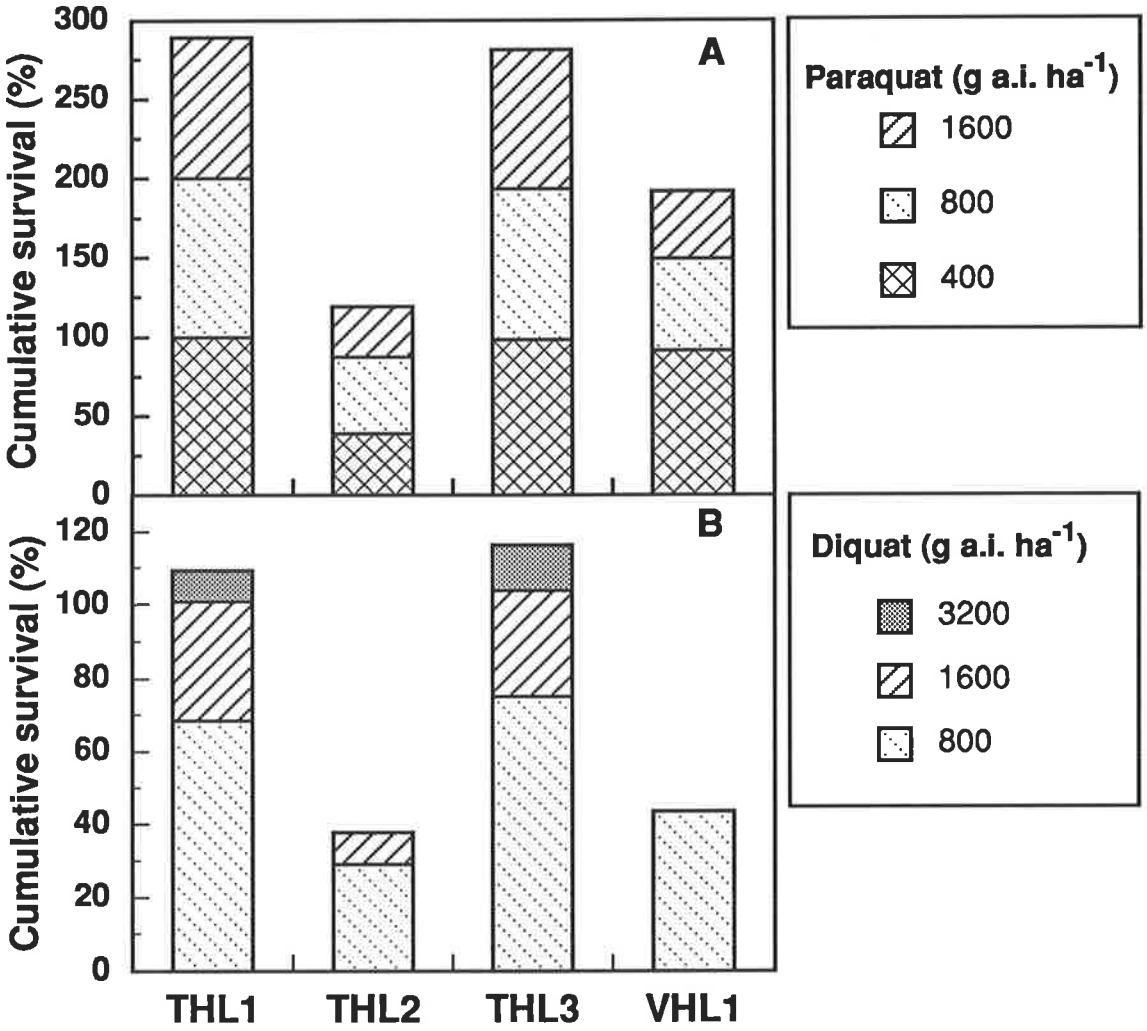


Figure 3. 3. Cumulative survival of THL1, THL2, THL3, and VHL1 biotypes of *H. leporinum* treated with high rates of paraquat (A) and diquat (B). These biotypes were obtained from lucerne fields with different histories of bipyridyl herbicide use. The cumulative survival was determined by adding the percent survival of each population at the three herbicide rates. Different fill patterns indicate different herbicide rates.

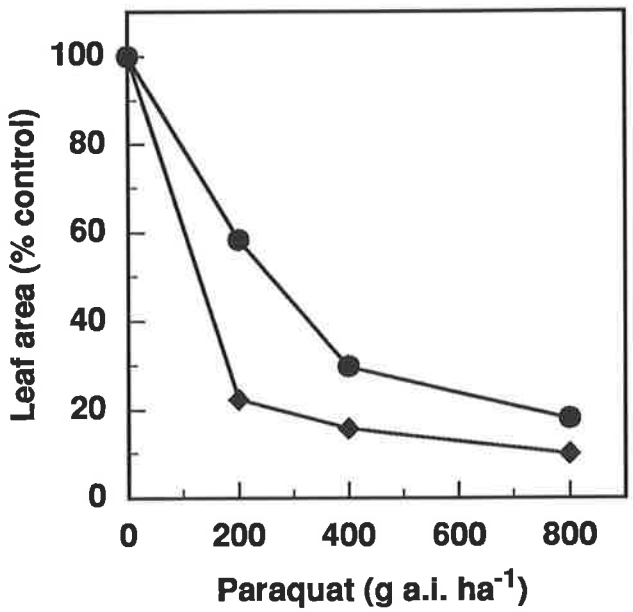


Figure 3. 4. Green leaf area of *H. leporinum* resistant biotypes THL1 (●) and VHL1(◆) after paraquat application. Green leaf area was measured 21 days after spraying. Each point is the mean of 16 plants.

3. 3. 6. Effect of paraquat on survival and dry matter at different plant age

The percentage survival of the resistant biotype of *H. leporinum* THL1 after paraquat application increased with increasing plant age (Fig. 3. 5A). Very young plants could be killed by much lower rates of paraquat than older plants. Plants at the 22-tiller stage showed no mortality even at 6.4 kg a.i. ha^{-1} paraquat. The result of paraquat application on the dry matter of the resistant biotype of *H. leporinum*, THL1, at different plant ages is presented in Fig. 3. 5B. With older plants paraquat tended to have less effect on dry matter production; however, the use of higher rates of paraquat on these resistant plants did reduce dry matter production. Even relatively low rates of paraquat application could dramatically reduce the dry matter production of the resistant plants.

3. 3. 7. Response of *H. leporinum* biotypes to other herbicides

The effectiveness of alternative herbicides for the control of the paraquat-resistant biotype THL1 was examined in pot experiments (Table 3. 6). All plants of the resistant biotype survived application of paraquat and diquat at the recommended rate. All plants of the susceptible biotype were killed by paraquat and 40% by diquat. Paraquat-resistant biotype THL1 and -susceptible biotype THL4 both suffered 100% mortality when treated with fluazifop-butyl, sethoxydim or glyphosate. Thus the resistant biotype of *H. leporinum*, THL1, is only resistant to the bipyridyl herbicides and shows no cross-resistance to other herbicides which can, therefore, be used to control this biotype.

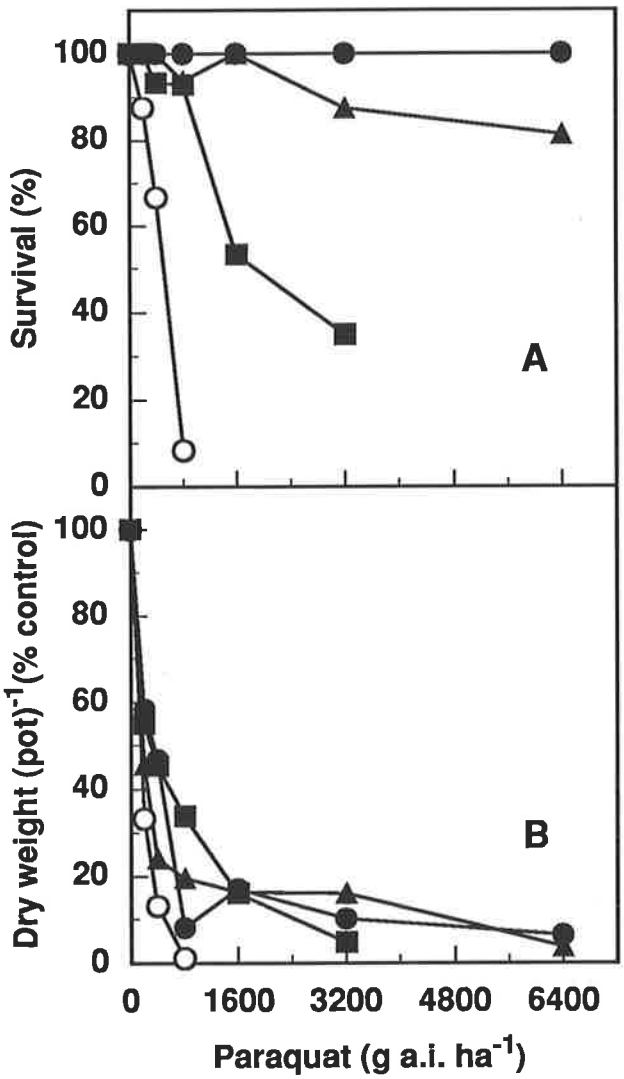


Figure 3. 5. Effect of paraquat on survival (A) and dry matter (B) at different ages of *H. leporinum* biotypes THL1 (21 days after spraying), O: 3-leaf stage, ■: 3-tiller stage, ▲: 15-tiller stage and ●: 22-tiller stage.

Table 3. 6. Effect of alternative herbicides on survival of paraquat-resistant and -susceptible *H. leporinum* populations. Fourty plants in pots were treated with each herbicide for each biotype.

Herbicide	Dose	Survival (%)	
	g a.i. ha^{-1}	Resistant (THL1)	Susceptible (THL4)
Paraquat	200	100	0
Diquat	200	100	60
Fluazifop	200	0	0
Sethoxydim	187	0	0
Glyphosate	360	0	0

3. 3. 8. Response of *A. calendula* biotypes to diquat and paraquat

Two biotypes of *A. calendula* which have previously been determined to be resistant (VAC1) and susceptible (VAC2) to diquat from field data (Powles *et al.*, 1989) were examined in pot experiments (Fig. 3. 6A). VAC1 was clearly resistant in pot experiments whereas VAC2 biotype was found to be susceptible to diquat. With the resistant biotype VAC1, 97% of the plants survived the recommended rate (200 g a.i. ha^{-1}) of diquat whereas all VAC2 plants died within 5-7 days after spraying. Higher rates of diquat application resulted in increased mortality of VAC1 and at 1600 g a.i. ha^{-1} all VAC1 plants were killed. The mean dry weights per pot of surviving plants (Fig. 3. 6C) were greater for VAC1 than for VAC2 at all herbicide rates except 1600 g a.i. ha^{-1}. The growth reduction of VAC1 biotype in terms of dry matter at the recommended rate was 48%, whereas it was 100% for the susceptible biotype.

VAC1 proved to be very resistant to paraquat (Fig 3. 6B) whereas VAC2 was largely susceptible. All of the VAC1 plants survived at all rates of paraquat applied up to 800 g a.i. ha^{-1}. Higher rates of paraquat application resulted in increased mortality of VAC1

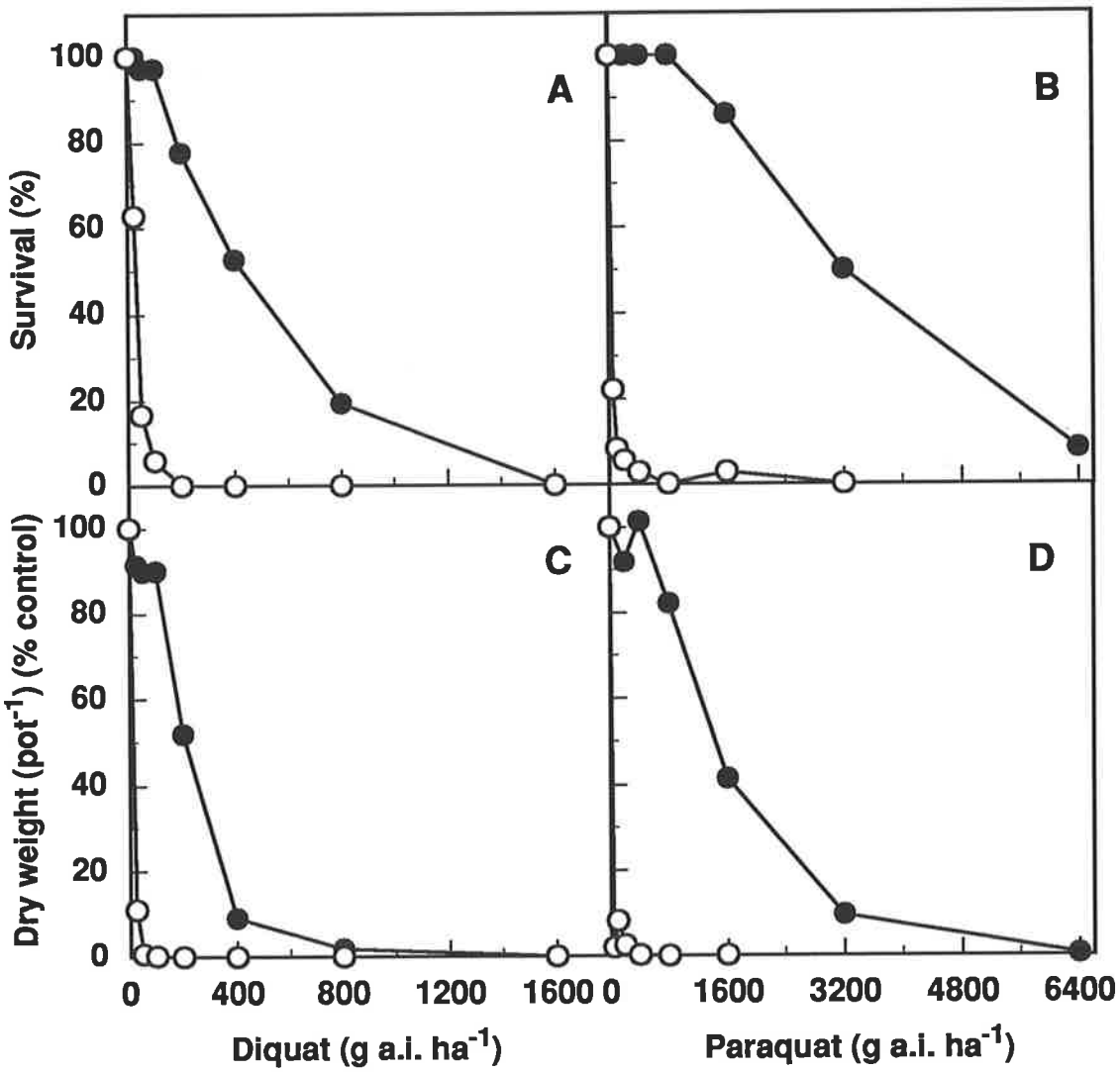


Figure 3. 6. Effect of diquat on survival (A) and dry matter production (C) and paraquat on survival (B) and dry matter production (D) of VAC1 (●) and VAC2 (O) biotypes of *A. calendula* 21 days after spraying. Each point is the mean of 36 plants.

and at 6400 g a.i. ha^{-1} 92% of VAC1 were killed. In contrast, only 5.5% of VAC2 survived at 200 g a.i. ha^{-1} of paraquat and all were killed at 800 g a.i. ha^{-1}. The VAC1 plants had a greater dry weight per pot than those of VAC2 at all rates of paraquat application (Fig. 3. 6D). Hence,the resistant population, VAC1, is clearly resistant to both diquat and paraquat when compared to the susceptible population VAC2.

3. 3. 9. Response of *A. calendula* to other herbicides

The control of diquat-resistant *A. calendula* by other herbicides was examined in pot experiments (Table 3. 7). Diquat application at 200 g a.i. ha^{-1}, the recommended rate, caused 100% mortality of VAC2 plants whereas 75% of VAC1 plants survived. Both VAC1 and VAC2 were effectively controlled by diuron, glyphosate, metribuzin and terbutryn applications, when applied at field recommended rates. Application of 2,4-D at 850 g a.i. ha^{-1} resulted in 25% and 22% survival of VAC1 and VAC2 respectively.

Table 3. 7. Effect of alternative herbicides on survival of diquat-resistant and -susceptible *A. calendula* populations. Sixty plants in pots were treated with each herbicide for each biotype.

Herbicide	Dose	Survival (%)	
	(g a.i. ha^{-1})	Resistant (VAC1)	Susceptible (VAC2)
Diquat	200	75	0
Diuron	425	0	0
2,4-D	850	25	22
Glyphosate	720	0	0
Metribuzin	300	0	0
Terbutryn	425	0	3.3

Therefore, the diquat-resistant biotype VAC1 was not cross resistant to any of the herbicides examined and all herbicides used, except 2,4-D, which would need to be applied at a higher rate, gave satisfactory control of the resistant biotype of *A. calendula.*

3. 3. 10. Response of *V. bromoides* biotypes to diquat and paraquat

After successfully using fluazifop-butyl to control paraquat-resistant *H. glaucum* and *H. leporinum* in a lucerne field in Victoria for two years the farmer noticed an increase in the number of *Vulpia* spp. Fluazifop-butyl does not control *Vulpia* plants and its applications during the two consecutive years led to a heavy infestation of *Vulpia* plants in the lucerne field. The successful control of the *Hordeum* plants also meant that the *Vulpia* could grow free from competition from *Hordeum.* Field application of paraquat and diquat in 1989 failed to control the *Vulpia bromoides.* The resistance status of these plants was examined and they proved to have some resistance to paraquat (Fig. 3. 7A) with 42% surviving 200 g a.i. ha^{-1}. The susceptible population, SVB1, suffered 100% mortality at this application rate. The LD_{50} for VVB1 was approximately 170 g a.i. ha^{-1} whereas that for SVB1 was approximately 30 g a.i. ha^{-1}. Therefore, there is a 5-6 fold difference between the VVB1 biotype and a susceptible biotype. Dry weight of VVB1 was greater than that of SVB1 at all rates of paraquat up to 200 g a.i. ha^{-1} (Fig. 3. 7C). The VVB1 biotype was also resistant to diquat as 82% of VVB1 plants survived diquat application at 800 g a.i. ha^{-1} whereas only 3.2% of SVB1 survived (Fig. 3.7B). Dry weight of VVB1 was greater than that of SVB1 at all rates of diquat. Application of diquat at 200 g a.i. ha^{-1} (recommended rate) reduced the dry weight of VVB1 30% whereas the dry weight of SVB1 was reduced up to 80% (Fig. 3. 7D). Susceptible *Vulpia* plants collected from a nearby pasture proved to be *V. myuros* not *V. bromoides* and these *V. myuros* plants were susceptible to paraquat with a similar response to the SVB1 population from the Waite Institute (data not shown). Thus resistance to paraquat and diquat has occurred in a species which was initially present in the area at a low frequency. This is the fourth paraquat- and diquat-resistant biotype to appear in this lucerne field after biotypes of *H. glaucum, H. leporinum* and *A. calendula* (Tucker and Powles, 1991).

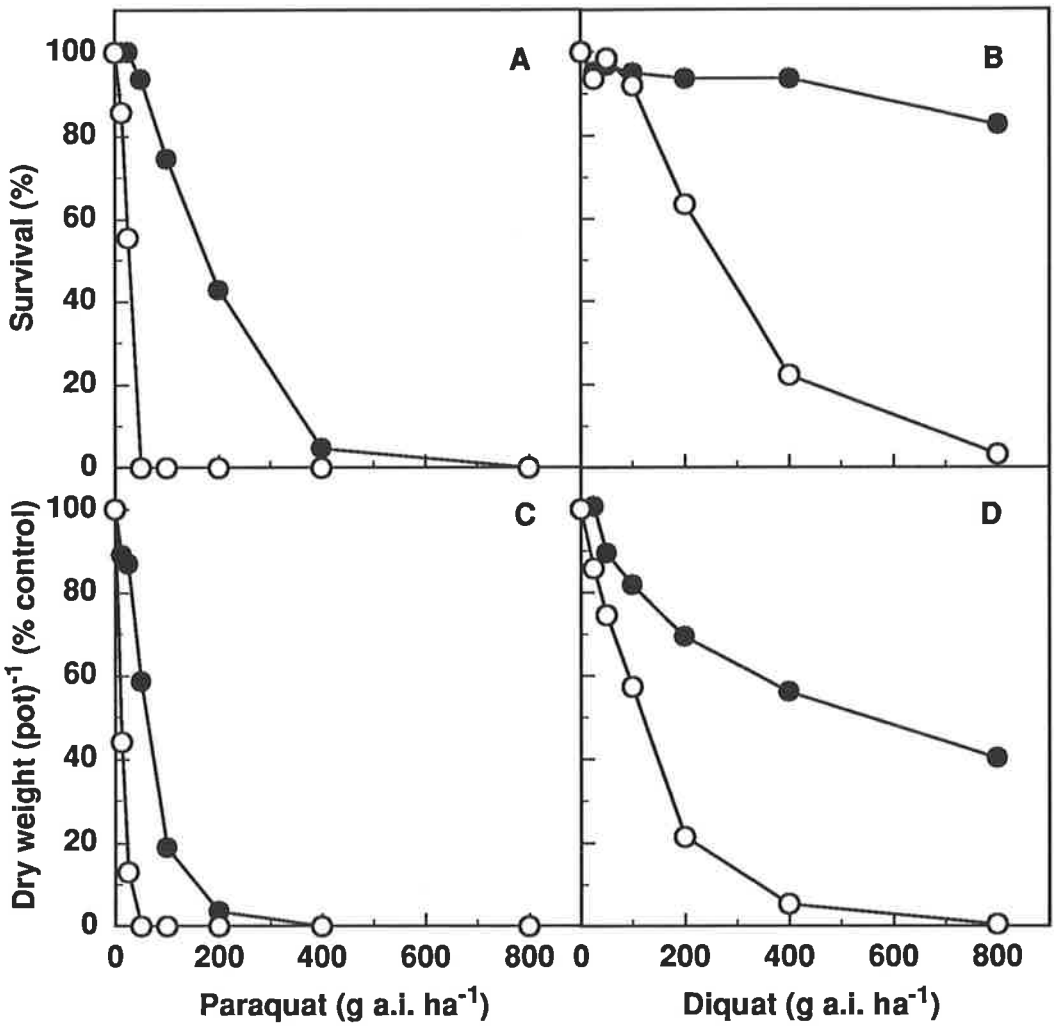


Figure 3. 7. Effect of paraquat on survival (A), dry weight (C) and diquat on survival (B) and dry weight (D) of VVB1 (●) and SVB1 (O) biotypes of *V. bromoides* 38 days after spraying. Each point represents 63 plants.

3. 3. 11. Response of *V. bromoides* biotypes to other herbicides

The effectiveness of alternative herbicides for the control of paraquat-resistant *V. bromoides* was examined in pot experiments (Table 3. 8). Paraquat application at 200 g a.i. ha^{-1}, the recommended rate, controlled the susceptible population, however 55% of the resistant biotype survived. Glyphosate at 720 g a.i. ha^{-1} and metribuzin at 300 g a.i. ha^{-1} both gave good control of both biotypes. Hence, the resistant biotype exhibits resistance only to paraquat and diquat but not to glyphosate and metribuzin at normal field rates.

Table 3. 8. Effectiveness of alternative herbicides on bipyridylium-resistant and -susceptible biotypes of *Vulpia bromoides*. Sixty-three plants grown in pots were treated for each herbicide and each biotype.

Herbicide	Dose	Survival (%)	
	g a.i. ha^{-1}	VVB1	SVB1
Paraquat	200	55	0
Glyphosate	720	0	0
Metribuzin	300	0	0

3. 3. 12. Effect of paraquat on subsequent seed production

In Australian agriculture paraquat is often applied to pasture fields containing annual grass weed species at anthesis (known as spraytopping or pasture topping) (Leys et al. 1991; Jones et al. 1984). Paraquat is normally applied at 100 to 120 g a.i. ha^{-1} for pasture topping. Therefore, experiments were conducted to examine the effect of paraquat application at anthesis on the subsequent seed production of resistant biotypes of *V. bromoides* and *H. leporinum*. Seed production of both resistant and susceptible biotypes

of *V. bromoides* was reduced by this treatment. Application of 100 g a.i. ha^{-1} paraquat at anthesis completely prevented seed production of the susceptible plants and reduced that of the resistant plants by 95% (Table 3. 9). Increasing the paraquat concentration to 200 g a.i. ha^{-1}, further reduced seed set of the resistant plants. The seed that was produced was largely viable as more than 80% germinated. Thus, whilst paraquat application at anthesis would dramatically reduce seed set in this biotype, viability of surviving seed was not diminished.

Table 3. 9. Effect of paraquat application at anthesis on subsequent seed set of paraquat-resistant and -susceptible biotypes of *V. bromoides*. 84 plants (21 plants $(pot)^{-1}$) were sprayed per treatment.

Paraquat	Seeds per plant		% germination	
(g a.i. ha^{-1})	Susceptible	Resistant	Susceptible	Resistant
Control	626	748	98	99
100	0	39	-	83
200	0	9	-	82

Application of 150 g a.i. ha^{-1} paraquat at anthesis on the paraquat-resistant and -susceptible biotypes of *H. leporinum* reduced the production of germinable seeds (Table 3. 10). Paraquat application on the resistant biotype reduced seed viability of the resistant biotype by 38% and the susceptible biotype by 88%. Glyphosate application at 360 g a.i. ha^{-1} reduced seed viability of the both biotypes by 96%. Therefore, the resistant biotype of *H. leporinum* is also resistant at anthesis.

Table 3. 10. Effect of paraquat and glyphosate application at anthesis on subsequent seed viability of paraquat-resistant and -susceptible biotypes of *H. leporinum*. Five hundred seeds (100 seeds replication^{-1}) per treatment were randomly taken out of the seeds collected from 40 plants.

Biotype	Germinable seeds (%)		
	Untreated	Paraquat 150 g a.i. ha^{-1}	Glyphosate 360 g a.i. ha^{-1}
Resistant	100	62	4
Susceptible	100	12	3

3. 3. 13. Comparison of paraquat and diquat efficacy against all biotypes

The LD_{50} and GR_{50} for each biotype against paraquat and diquat were calculated from the dose response curves shown above and are given in Table 3. 11. This table also gives the resistance index for all biotypes, which is calculated as the LD_{50} (or GR_{50}) of the resistant biotypes divided by that of the susceptible biotypes. A resistance index of 1 would indicate no difference between the two biotypes. All of the paraquat-resistant biotypes show increased tolerance to diquat and vice versa; however, the resistance index for the two herbicides can vary greatly. The other point to notice is that the resistance index based on GR_{50} is often considerably lower than that based on LD_{50}.

Table 3. 11. The effect of paraquat and diquat on the bipyridyl-resistant and susceptible biotypes as represented by LD_{50} and GR_{50} [a]. LD_{50} values were calculated from the dose response curves in Figs. 3. 1, 3. 2, 3. 5, 3. 6, and 3. 7.

Biotype	Paraquat				Diquat			
	LD_{50} (g a.i. ha^{-1})	R. I.[b]	GR_{50} (g a.i. ha^{-1})	R. I.	LD_{50} (g a.i. ha^{-1})	R. I.	GR_{50} (g a.i. ha^{-1})	R. I.
SHG1 (R)	>1600	>66	160	23	1400	18	116	6
SHG2 (S)	24		7		75		20	
THL1 (R)	>>1600	>>80	200	22	1200	8	85	2
THL2 (R)	377	19	18	2	275	1.8	130	3
THL3 (R)	>>1600	>>80	130	14	1225	8	220	5.5
THL4 (S)	20		9		150		40	
VHL1 (R)	1040	69	125	21	730	5	215	11
VHL2 (S)	15		6		140		20	
VAC1 (R)	3200	>64	1300	>>26	425	15	225	19
VAC2 (S)	<50		<<50		28		12	
SVB1 (S)	27		11		250		120	
VVB1 (R)	175	6.5	60	5.5	>800	>3.2	540	4.5

a) >: greater than the highest rate applied, >>:much greater than the highest rate applied.

b) R. I. (resistance index) was calculated using the susceptible population collected from the same geographical area except in the case of *V. bromoides*.

3. 4. DISCUSSION

In this chapter the level of resistance of a number of biotypes from four species has been examined. Five suspected new paraquat-resistant biotypes were examined for the first time. These were one biotype of *H. glaucum,* three of *H. leporinum* and one of *V. bromoides*. In all cases the susceptible populations which were collected from adjacent pastures (except for *V. bromoides* which was collected from the Waite Institute) proved susceptible to paraquat. The suspected paraquat-resistant biotype of *H. glaucum* from South Australia (SHG1) proved to have a high level of resistance to paraquat (Fig. 3. 1A). The level of resistance, although high, is not as high as that reported for other resistant biotypes of *H. glaucum* from Victoria (Powles 1986; Tucker 1989). This biotype was also resistant to diquat but did not show the same level of resistance to this herbicide as to paraquat (Fig. 3. 1B). The level of resistance to diquat was also less than has been observed for other resistant *H. glaucum* biotypes (Tucker 1989). This resistant population could be readily controlled in the field with a variety of alternative herbicides (Table 3. 5) and therefore shows no cross-resistance to other herbicides.

H. leporinum populations infesting three lucerne fields in Tasmania also exhibited resistance to the bipyridyl herbicides (Fig. 3. 2). The bipyridyl herbicides, paraquat and diquat, at the recommended rate easily controlled a susceptible population of *H. leporinum* collected from an adjacent area which had no history of bipyridyl herbicide use. The resistant plants exhibited damage to the older leaves only and the plants subsequently recovered such that 3-4 weeks after spraying it was difficult to distinguish between sprayed plants and unsprayed plants. Spraying of older plants resulted in reduced mortality for both resistant (Fig. 3. 5) and susceptible populations (data not shown); however, the effect was much more dramatic for the resistant population and no mortality occurred at 6400 g a.i. ha^{-1} paraquat applied to plants with 22 tillers.

The four paraquat-resistant *H. leporinum* populations now available were compared to see if there is any relationship between the level of resistance to paraquat and the length of

selection pressure. No such correlation could be observed as biotypes THL1 and THL3 were the most resistant to both paraquat and diquat and biotypes THL2 and VHL1 the least resistant (Fig. 3. 3A). The latter two biotypes had been exposed to paraquat and diquat for twice as long as the first two (Tables 3. 2 and 3. 3).

Despite the high level of resistance in the *H. leporinum* populations these plants do suffer from paraquat and diquat application in that dry matter production is almost always reduced by herbicide application even at lower rates when there is no mortality of the plants (Fig. 3. 2C). One factor that may influence the amount of dry matter is that these herbicides are able to partly bleach the leaves of the resistant biotypes. This was quantified by measuring the leaf area of green tissue 21 days after application of paraquat (Fig. 3. 4). At 200 g a.i. ha^{-1}, a rate which gives no mortality of resistant biotypes, green leaf tissue of these biotypes was substantially reduced, more so for VHL1 compared to THL1. This reduction in green tissue may be due to two causes, some bleaching of the existing tissue and inhibition of growth such that new green tissue is not produced as rapidly. This reduction in leaf tissue active in photosynthesis would lead to reduction in rate of growth of the plants.

A biotype of *A. calendula*, VAC1, from the lucerne field in Victoria had been reported as resistant to diquat and paraquat on the basis of field experiments (Powles et al. 1989). In pot experiments this biotype was confirmed as resistant to diquat. The LD_{50} for diquat of VAC1 population is about 425 g a.i. ha^{-1} whereas that of VAC2 is about 28 g a.i. ha^{-1}, and the resistance index is about 15-fold (Table 3. 11). Survivors were only damaged on the tips of the older leaves and new leaves grew unaffected. Moreover, the plants recovered from the damage in 2-3 weeks after spraying with only a small decrease in dry weight. In contrast, VAC2 the susceptible population, suffered 100% mortality at 200 g a.i. ha^{-1} diquat. The VAC1 plants were also very resistant to paraquat, with paraquat up to 800 g a.i. ha^{-1} unable to kill any of these plants. Although paraquat is not recommended for *A. calendula* control, the effect of paraquat application on the two

biotypes showed a large difference. Both biotypes VAC1 and VAC2 could be effectively controlled by other herbicides (Table 3. 7) and hence no cross-resistance was apparent.

V. bromoides (VVB1) was collected from the same lucerne field as VHL1 and VAC1, but after that field had received two years of fluazifop-butyl and a further year of paraquat and diquat use. This population proved to be resistant to paraquat but only at a level 6-7-fold that of a susceptible population. This biotype also showed resistance to diquat even though diquat is not very effective against *V. bromoides*. The low level of resistance to paraquat is more reminiscent of the 4-10 fold resistances which have appeared following laboratory selection rather than the >100 fold resistances more typical of field situations (Fuerst and Vaughn 1990). This suggests that resistance development may be just beginning in this population. Indeed in 1987 *Vulpia* plants taken from this field showed no apparent resistance to paraquat (Tucker 1989). The use of fluazifop-butyl, which does not control *V. bromoides*, for two years to control resistant *H. glaucum* and *H. leporinum* (Powles et al. 1989; Tucker and Powles 1991) has undoubtedly led to the proliferation of the paraquat-resistant *V. bromoides* through the reduction of competition from other weed species. The VVB1 population could be controlled with glyphosate and metribuzin and showed no cross-resistance. This is the fourth species to develop resistance to paraquat and diquat in the same field.

Application of knockdown herbicides such as paraquat or glyphosate at anthesis is a common practice in pasture management in southern Australia. This practice, known as "spraytopping", when applied to grass species with a non-residual seed bank, can significantly reduce the number of viable seeds produced and therefore the number of plants emerging the following year. Leys et al. (1991) reported that paraquat application at 100 g a.i. ha^{-1} at anthesis in a field trial resulted in a 72% reduction in the number of *V. bromoides* plants emerging in the following year. This was attributed to a reduction in seed set as a result of the herbicide treatment. In the present study, paraquat application at 100 g a.i. ha^{-1} at anthesis prevented seed production in the susceptible biotype of *V. bromoides*. Seed production of the resistant biotype (VVB1) was reduced by 95% at 100

g a.i. ha^{-1} and by 99% at 200 g a.i. ha^{-1} paraquat (Table 3. 9). Similarly seed viability in the subsequent seed production of *H. leporinum* as the result of 150 g a.i. ha^{-1} paraquat application at anthesis was reduced by 88% in the susceptible biotype but only by 38% in the resistant biotype (Table 3. 10). In comparison, application of paraquat at anthesis to a paraquat-resistant biotype of *H. glaucum* , with a 250 fold level of resistance, reduced the seed set and viability by only 10% (Powles 1986).

In conclusion, paraquat and diquat resistance has developed in four species after a number of years of herbicide use across three states of Southern Australia. To date resistance has only been observed in lucerne fields in Australia, whilst it has commonly appeared in orchards and vineyards in other parts of the world (Faulkner 1976; Gressel et al. 1982; Harvey and Harper 1982; Watanabe et al. 1982). Neither the number of years of herbicide use nor the amount of herbicide used are correlated with the level of resistance obtained. Hence the level of resistance observed may largely depend on the mechanism for resistance that is selected in each field. All of the biotypes that are resistant to one bipyridyl herbicide show greater tolerance to the other; however, the level of increased tolerance can vary considerably. None of the paraquat or diquat resistant biotypes displays cross resistance to any other herbicides which means that control of these resistant plants may not be a practical problem as alternative herbicides are available. The appearance of four species with resistant biotypes in one field is; however, a practical problem as there are few herbicides available for the control of *V. bromoides* (Sparrow 1991; Leys et al. 1991). This means that other, non-chemical methods may have to be instituted to control weeds in this field.

CHAPTER 4

COMPARATIVE FITNESS OF A PARAQUAT-RESISTANT BIOTYPE OF *H. LEPORINUM* LINK.

4. 1. INTRODUCTION

The fitness of an organism is determined by its ability to compete and leave viable descendants (Holt 1990; Hickney and McNeilly 1975). This is influenced by environmental factors, genetic factors and the interaction of these two. An individual is said to have increased relative fitness if its activities decrease the number of descendants left by its neighbours (Harper 1977). In general there is competition among plants for water, light, nutrients and space (Hickney and McNeilly 1975). Thus, the ability of a plant to compete successfully for resources and thereby grow larger than its neighbours should lead to an increase in seed production. Herbicide-resistant individuals are by their nature more fit than susceptible individuals under herbicide stress. However, the selection of a particular trait within an organism often leads to a penalty in the fitness of that organism when the selective pressure is removed (Holt 1990). In the absence of herbicide a number of studies have shown a decrease in fitness of herbicide-resistant biotypes compared to susceptible biotypes (Gressel and Ben-Sinai 1985; Holt 1988; Ahrens et al. 1983; Weaver and Warwick 1982). These studies have mainly been performed on triazine-resistant biotypes which show reduced vigour, growth and photosynthetic efficiency when compared to the susceptible biotypes (Conard and Radosevich 1979; Holt 1988). Studies on a dinitroaniline-resistant biotype of *Eleusine indica* indicate that it also is less fit than the susceptible biotype (Valverde et al. 1989). A paraquat-resistant *Hordeum glaucum* biotype (VHG1) has been reported to have lower fitness than either paraquat-susceptible *H. glaucum* or paraquat-susceptible *H. leporinum* biotypes (Tucker 1989). No previous study on the fitness of paraquat-resistant *H. leporinum* compared to paraquat-susceptible *H. leporinum* biotypes has been undertaken.

A replacement series experiment (de Wit 1960) was performed to obtain an indication of field performance and fitness of paraquat-resistant and -susceptible biotypes of *H. leporinum* over an extended period under field conditions.

4. 2. MATERIALS AND METHODS

4. 2. 1. Experimental site

The experiment was conducted during the winter of 1990 in a field at the Waite Agricultural Research Institute. The area was ploughed using a mouldboard plough in spring 1989. The soil type is a red-brown earth with fine sandy loam topsoil over a clay subsoil. In the previous two years the experimental site had been sown to wheat and faba bean.

4. 2. 2. Experimental design

In a replacement series experiment (de Wit 1960), the two biotypes of *H. leporinum* were grown in competition at two densities, namely 100 and 400 plants m^{-2}. The resistant plants and susceptible plants were planted at the following seven relative proportions:

Resistant	:	Susceptible
0	:	100
10	:	90
25	:	75
50	:	50
75	:	25
90	:	10
100	:	0

There were three replications each arranged in a randomised block design.

4. 2. 3. Field establishment

To delineate plant position, permanent grid meshes were laid over the soil prior to transplanting. Two different mesh sizes, 5 cm x 5 cm and 10 cm x 10 cm were used to give the densities of 400 and 100 plants m^{-2} respectively. The grid meshes had been painted using two different colours, red and green, to indicate the position of resistant and susceptible plants respectively. Each plot consisted of 100 plants (10 x 10 grid) surrounded by three buffer rows. The ratio of plant biotypes in the buffer rows was consistent with that of the biotype(s) in the plot.

4. 2. 4. Seedling Establishment

Seeds of paraquat-resistant and -susceptible biotypes come from almost identical sites (except for paraquat history) and were collected and maintained under identical conditions. The seeds of the resistant biotype were not contaminated with susceptible seed or vice versa, as determined from dose response experiments (see Chapter 3). The seeds of the resistant biotype, THL1, were collected from a lucerne paddock in Tasmania in 1989 that had been treated with paraquat-diquat herbicides for previous 12 years and were resistant to paraquat (Chapter 3). The seeds of susceptible biotype, THL4, were collected from an immediately adjacent area which had no history of paraquat or diquat use (see Chapter 3). Seeds of both biotypes were separately sown in 42 x 30 x 12 cm plastic trays containing perlite and placed in a growth room for 5 days. The growth room was maintained at 65% relative humidity, 16 h, 300 μmol m^{-2} s^{-1}, 28°C, light period/ 8 h, 21°C dark period. Three days prior to transplanting the trays were transferred from the growth room to outdoors to acclimatise the seedlings to field conditions. Seedlings were transplanted into the field plots on 3rd and 4th July 1990. Throughout the experiment the plots were kept free of other plants by hand weeding.

4. 2. 5. Plant harvest

Plants were harvested 135 days after transplanting at which time the majority of spikes were still green except at the spikelet tips where seeds had matured. Prior to harvest the buffer rows were cut and removed to avoid contamination of experimental plots. Plants were harvested individually by excising at the soil surface and removing the above-ground parts which were placed in individual, identified, paper bags (15 cm x 35 cm). The number of tillers and the number of spike inflorescences produced by the individual plants was determined. Dry weight was measured after plants had been dried in an oven at 80°C for 24 h. Data were subjected to analysis of variance. It was impractical to measure seed number as the spike inflorescences tended to shatter on maturity, scattering seed within the bag.

4. 2. 6. Productivity under non-competitive conditions

Productivity of both biotypes was determined in the absence of competition. Plots of each biotype were placed separately 1 m from the competition experiments. Plant materials used were the same as for the competition experiment. To eliminate neighbour effects, seedlings were transplanted at 50 cm intervals and throughout the experiment the plots were kept free of other plants. The number of spike inflorescences on each plant was determined at 124 and again at 149 days after transplanting when plants were harvested by cutting at the soil surface. The number of tillers and dry weight per plant were recorded at harvest. Seed weight and the number of seeds per inflorescence (2 weeks prior to harvest) were also recorded.

4. 2. 7. Seed dormancy

Seeds of the paraquat-resistant and paraquat-susceptible *H. leporinum* biotypes collected from plants used in the productivity experiment described above were used to determine seed dormancy for these biotypes. The seed of both biotypes was therefore produced

under identical conditions and were collected on the same day. The seeds were immediately placed in polypropylene fibre net bags (20 cm x 30 cm) with each bag containing approximately 3500 seeds mixed with 200 g potting soil to simulate field conditions, with four replicates. Previously no difference had been observed in germination of *H. glaucum* seed which had been stored on the soil surface, under wheat straw or 2 cm below the soil surface (Powles et al. 1992), so a single treatment, burial at 2 cm, was chosen here. The experiment was conducted in the same field as the competition experiment described above. Samples of seeds of each biotype were removed from the bag every 15 days and fifty seeds from each bag were tested for germination by placing on 0.6% (w/v) agar in a germination cabinet with 20°C, 14 h, 50 μmol photons m^{-2} s^{-1} light period/ 20°C, 10 h dark period. Before the seeds were placed on the agar surface the sterile lateral florets were detached from the fertile floret. The seeds were examined every 4 days over a 20-day period and germinated seeds were counted and discarded.

4. 2. 8. Seedling emergence

To determine whether the resistant biotype and susceptible biotype differed in time to emergence a separate experiment was conducted during the winter growing season. Three hundred seeds of each biotype were separately sown in 42 x 30 x 12 cm plastic trays containing potting soil. The seeds were evenly covered with 0.5 cm potting soil. Trays were placed in an unheated glasshouse, watered twice daily and emergence was recorded up to eight days after sowing.

4. 2. 9. Plant Growth and Maturity

In 1991, and 1992, experiments were established to examine the growth of the paraquat-resistant and paraquat-susceptible *H. leporinum* biotypes. Seeds from the original collection and seeds collected from the productivity experiment described previously (where seeds from resistant and susceptible biotypes were grown under identical conditions and harvested at the same time) were germinated as described above. Seedlings

at 2.5 cm high were transplanted into 30 cm diameter pots containing potting soil. There were four plants per pot and each biotype was grown in a different pot to avoid inter-biotype competition. The experiments were conducted outdoors during winter and spring which are the normal growing seasons for this species. The number of tillers and spike inflorescences for each plant were counted at several intervals. The experiments were terminated either 203 or 215 days after transplanting. Dry weight of the above-ground parts of the plants was measured after drying in an oven at 80°C for 24 h.

4. 3. RESULTS

4. 3. 1. Productivity of THL1 and THL4 biotypes grown under competitive conditions

The productivity of the resistant and susceptible biotypes, THL1 and THL4, grown in competition at various proportions with an overall plant density of 100 plants m^{-2} are presented in Table 4. 1. The results show that dry matter and the number of tillers of each biotype at the corresponding proportion are statistically not different. However the resistant (THL1) biotype produced a significantly greater number of spike inflorescences per m^2 than did the susceptible (THL4) biotype. At an equal planting proportion (50:50 resistant : susceptible) the resistant biotype produced 57% of the total number of spike inflorescences. At the higher overall plant density of 400 plants m^{-2} (Table 4. 2) there was no difference in dry matter production between resistant and susceptible biotypes but there were differences in both number of tillers and number of spike inflorescences. The resistant biotype (THL1) produced significantly fewer tillers m^{-2} compared to the susceptible biotype but more spike inflorescences. At an equal proportion (50:50) the resistant biotype produced 44% and 54% of the total number of tillers and spike inflorescences respectively. In this case the number of spike inflorescences produced is not consistent with the dry matter production of the two biotypes.

Table 4. 1. Dry weight, number of tillers and number of spike inflorescences of paraquat-resistant and -susceptible *H. leporinum* biotypes when grown under competitive conditions at various ratios with an overall density of 100 plants. m^{-2}.

Plant Proportion R : S	Dry Weight (g. m^{-2})	Tiller number (m^{-2})	Inflorescence number (m^{-2})
Resistant biotype			
100 : 0	859	3165	1194
90 : 10	782	2863	1097
75 : 25	634	2172	874
50 : 50	387	1382	570
25 : 75	356	1246	486
10 : 90	86	295	113
Susceptible biotype			
100 : 0	831	3023	934
90 : 10	743	2939	751
75 : 25	507	2146	543
50 : 50	366	1609	421
25 : 75	187	808	196
10 : 90	87	390	78
P-value [a]	ns[c]	ns	P<0.01
SED[b]	38.3	150.1	48.6

[a] test of difference between resistant and susceptible biotypes.

[b] standard error of difference between biotypes.

[c] not significantly different

Table 4. 2. Dry weight, number of tillers and number of spike inflorescences of paraquat-resistant and -susceptible *H. leporinum* when grown under competitive conditions at various ratios with an overall density of 400 plants. m^{-2}.

Plant Proportion R : S	Dry Weight (g. m^{-2})	Tiller number (m^{-2})	Inflorescence number (m^{-2})
Resistant biotype			
100 : 0	847	4284	1416
90 : 10	827	3892	1292
75 : 25	701	3420	1148
50 : 50	381	1924	640
25 : 75	255	912	320
10 : 90	59	320	112
Susceptible biotype			
100 : 0	909	4808	1116
90 : 10	662	4064	860
75 : 25	640	3492	804
50 : 50	453	2480	552
25 : 75	204	1180	244
10 : 90	64	364	72
P-value [a]	ns[c]	P<0.01	P<0.01
SED[b]	32.7	110.4	29.84

[a] test of difference between resistant and susceptible biotypes.

[b] standard error of difference between biotypes.

[c] not significantly different.

The results of this competition experiment are further illustrated in replacement series diagrams (Fig. 4. 1 and 4. 2) to illustrate the nature of the competition between the two biotypes. The curves for dry matter, number of tillers and number of spike inflorescences for both biotypes at 100 plants m^{-2} (Fig. 4. 1) are not linear which suggests that the resistant biotype is doing better under competition than is the susceptible biotype, particularly with regard to number of spike inflorescences. With a density of 400 plants m^{-2}, the shapes of curves for dry matter and number of tillers for the two biotypes are close

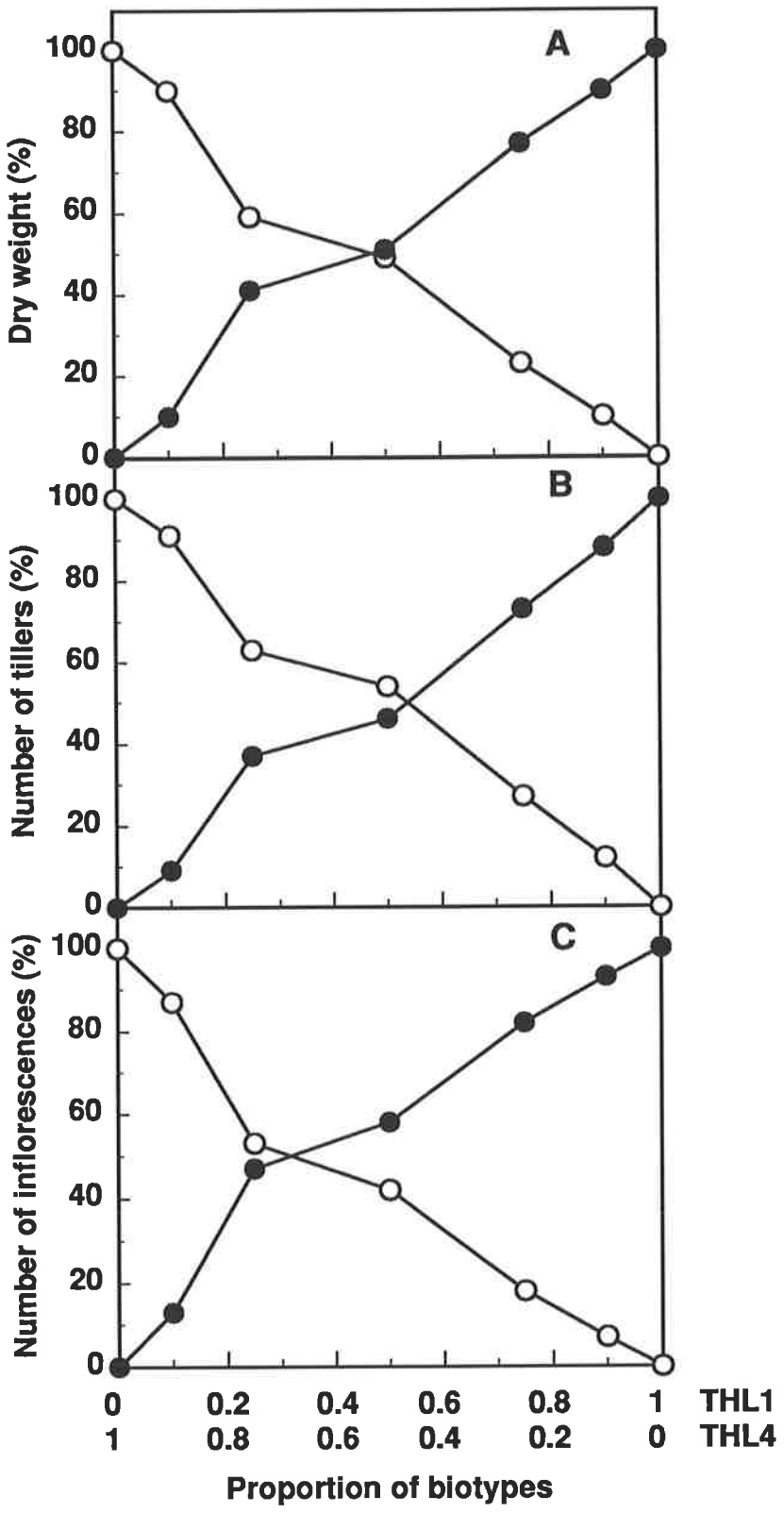


Figure 4. 1. The proportion of total dry weight (A), number of tillers (B) and number of heads (C) of resistant (THL1) (●) and susceptible (THL4) (O) biotypes of *H. leporinum* grown together in various proportions at a constant plant density of 100 plants m^{-2}. Plants were harvested 135 days after transplanting.

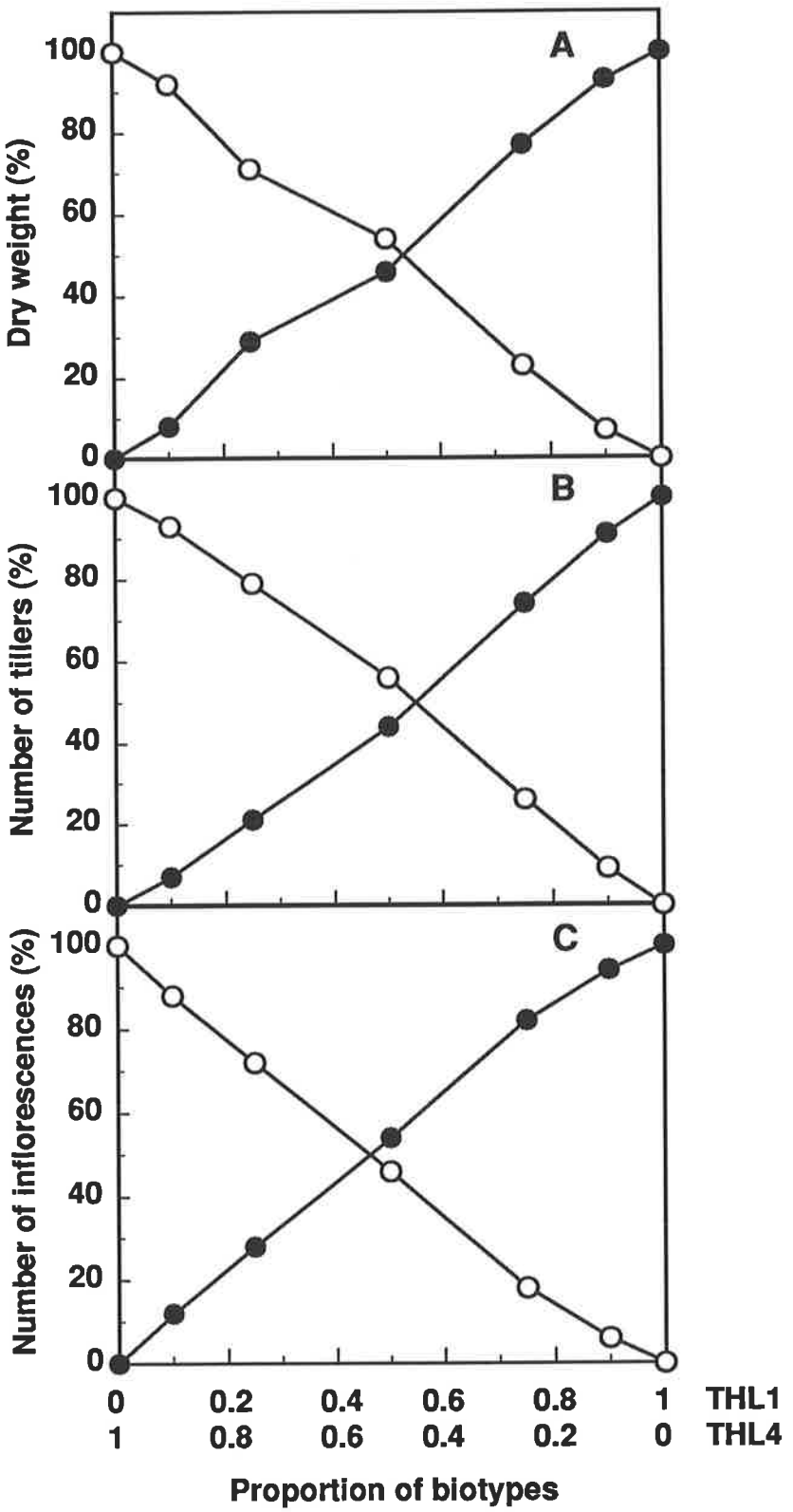


Figure 4. 2. The proportion of total dry weight (A), number of tillers (B) and number of heads (C) of resistant (THL1) (●) and susceptible (THL4) (O) biotypes of *H. leporinum* grown together in various proportions at a constant plant density of 400 plants m^{-2}. Plants were harvested 135 days after transplanting.

to linear suggesting that the two biotypes do equally well at this density. In contrast, the shape of the curve for the number of spike inflorescences is slightly concave for THL4 suggesting the resistant biotype does better in this parameter. Overall it is clear from this experiment that the susceptible biotype does not have a competitive advantage over the resistant biotype in the absence of herbicide.

4. 3. 2. Productivity of THL1 and THL4 biotypes of *H. leporinum* grown without competition

Dry matter production, number of tillers and number of spike inflorescences per plant for resistant (THL1) and susceptible (THL4) biotypes grown under non-competitive conditions are shown in Table 4. 3. In the absence of any plant competition dry matter production and number of tillers of the resistant biotype were less than that of the susceptible biotype. However the number of spike inflorescences of the resistant biotype was not different from that of the susceptible biotype at harvest, 149 days after transplanting. Earlier, at 124 days after transplanting, the resistant biotype had more spike inflorescences per plant than did the susceptible biotype. Hence, this experiment showed that dry matter production of the susceptible biotype is greater in the absence of competition (Table 4. 3) but not under competitive conditions (Tables 4. 1, 4. 2)

Seed weight was measured by weighing 1000 seeds (100 x 10 seeds) of each biotype with seed awns removed prior to weighing. No difference was observed between the seed weight of the resistant and the susceptible biotypes (Table 4. 4). Weight of individual seed averaged 5.77 and 5.86 mg for resistant and susceptible biotypes respectively. Similarly the number of seeds per spike inflorescence of the resistant biotype was not different from the susceptible biotype (Table 4. 4). The number of seeds per inflorescence was 29.8 for the resistant and 30.4 for the susceptible biotype.

Table 4. 3. Dry matter, number of tillers and number of spike inflorescences per plant of resistant (THL1) and susceptible (THL4) biotypes of *H. leporinum* grown in non-competitive conditions [a].

Biotype	Dry matter	Number of tillers	Number of spike inflorescences	
	(g)		124 days	149 days
Resistant	23.17a	91.87a	11.65a	62.61a
Susceptible	35.1b	159.15b	5.1b	64.25a

[a] Means in each column followed by the same letter are not significantly different (P=.01; t-test)

Table 4. 4. Seed weight and number of seeds per spike inflorescence of resistant (THL1) and susceptible (THL4) biotypes.

Biotype	Seed Weight (mg)	Number of seeds per inflorescence
Resistant	5.77 ± 0.68	29.8 ± 0.3
Susceptible	5.86 ± 0.56	30.4 ± 0.3

4. 3. 3. Seed dormancy

Seed of both biotypes of *H. leporinum* was harvested on the same day (149 days after transplanting) and then stored 2 cm below the soil surface the day after harvest. Germination was then determined by exhuming seeds. The fresh seeds of the two biotypes germinated at 90% and 96% respectively (Table 4. 5). The germination percentage for seeds of both biotypes did not significantly change during storage over 45 days under field conditions 2 cm below soil surface. These results show that both

biotypes of *H. leporinum* have no seed dormancy and there is no difference in dormancy of the two biotypes.

Table 4. 5. Germination of exhumed seed of paraquat-resistant and -susceptible biotypes of *H. leporinum* maintained in the field during summer, 2 cm below the soil surface. Seed were harvested on 12 December 1990 and samples exhumed every 15 days. Percentages represent total germination of four replications 20 days after incubation.

Days after harvesting	Germination (%)	
	Resistant	Susceptible
1	90	96
15	92	97
30	95	91
45	91	90

4. 3. 4. Seedling emergence

No seedlings emerged for the first four days after sowing. After that seedlings emerged rapidly until 8 days after sowing when 94% of seeds of both biotypes had emerged (Table 4. 6). There was no difference in emergence between the paraquat-resistant and -susceptible biotypes of *H. leporinum*.

Table 4. 6. Rate of seedling emergence of resistant (THL1) and susceptible (THL4) biotypes of *H. leporinum*. A total of 300 seeds were seeded in each tray.

Days after sowing	Number of seedlings emerged [a]	
	THL1 (R)	THL4 (S)
1	-	-
2	-	-
3	-	-
4	-	-
5	38 (13)	41 (14)
6	211 (70)	224 (75)
7	267 (89)	269 (90)
8	283 (94)	284 (94)

[a] numbers in parentheses are percentages of seeds sown.

4. 3. 5. Plant growth and maturity

The competition experiment indicated that the resistant biotype, THL1, was as fit as the susceptible biotype under competition; however, the dry matter production of the susceptible biotype was greater in the absence of competition. The resistant and susceptible plants grown in the absence of competition were allowed to proceed for 14 days longer than for the competition experiment and so there may be an influence of plant maturity on these fitness experiments. This was further indicated by the fact that the resistant biotype had produced more spike inflorescences 124 days after transplanting (Table 4. 3) than had the susceptible biotype; however, at 149 days the number of inflorescences was the same. The potential difference in maturity between the two biotypes was examined by measuring the number of tillers and spike inflorescences of resistant and susceptible plants during the growing season. The number of tillers of

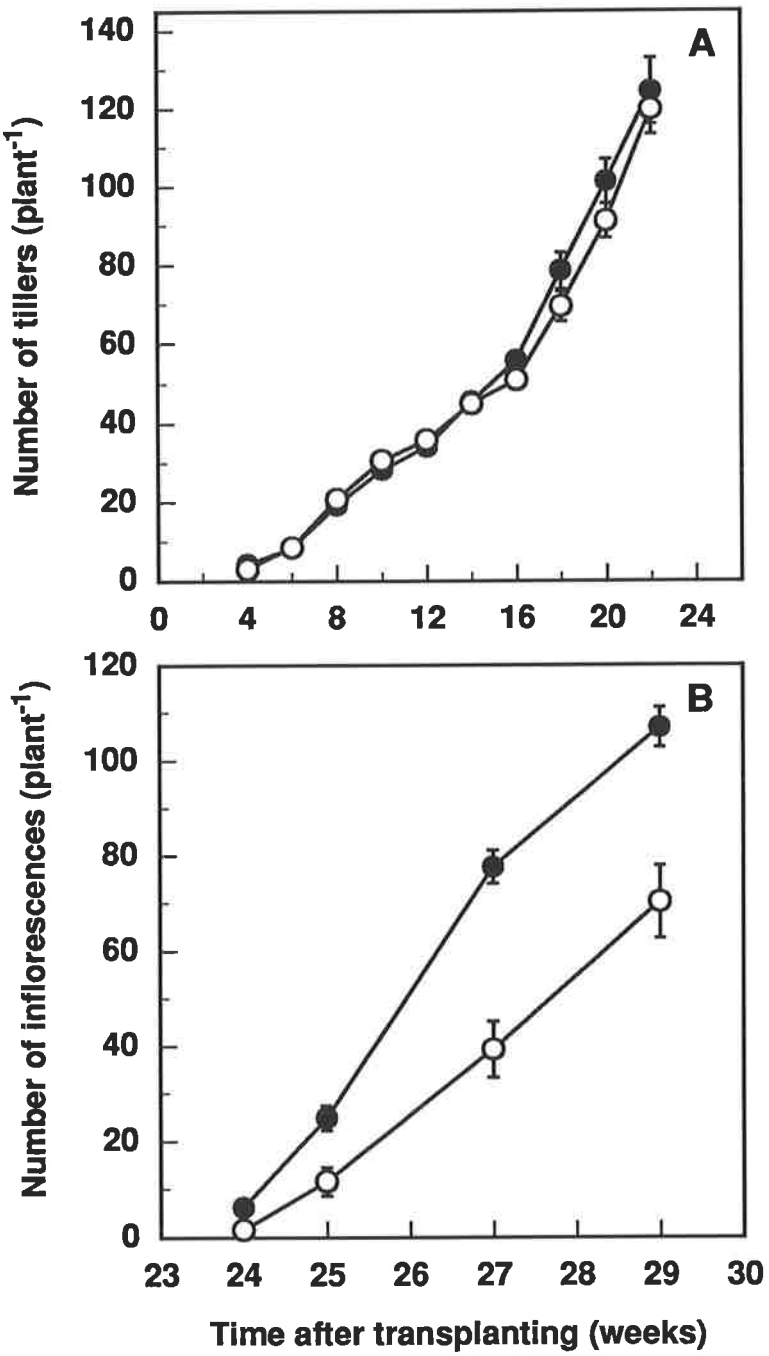


Figure 4. 3. Number of tillers (A) and spike inflorescences (B) per plant of resistant (THL1) (●) and susceptible (THL4) (O) biotypes of *H. leporinum* over time. Plant material was from original seed collected in the field in 1990. Plants were grown in monoculture in 30 cm pots with four plants per pot. Each point is the mean of 24 plants. Vertical bars are the standard errors.

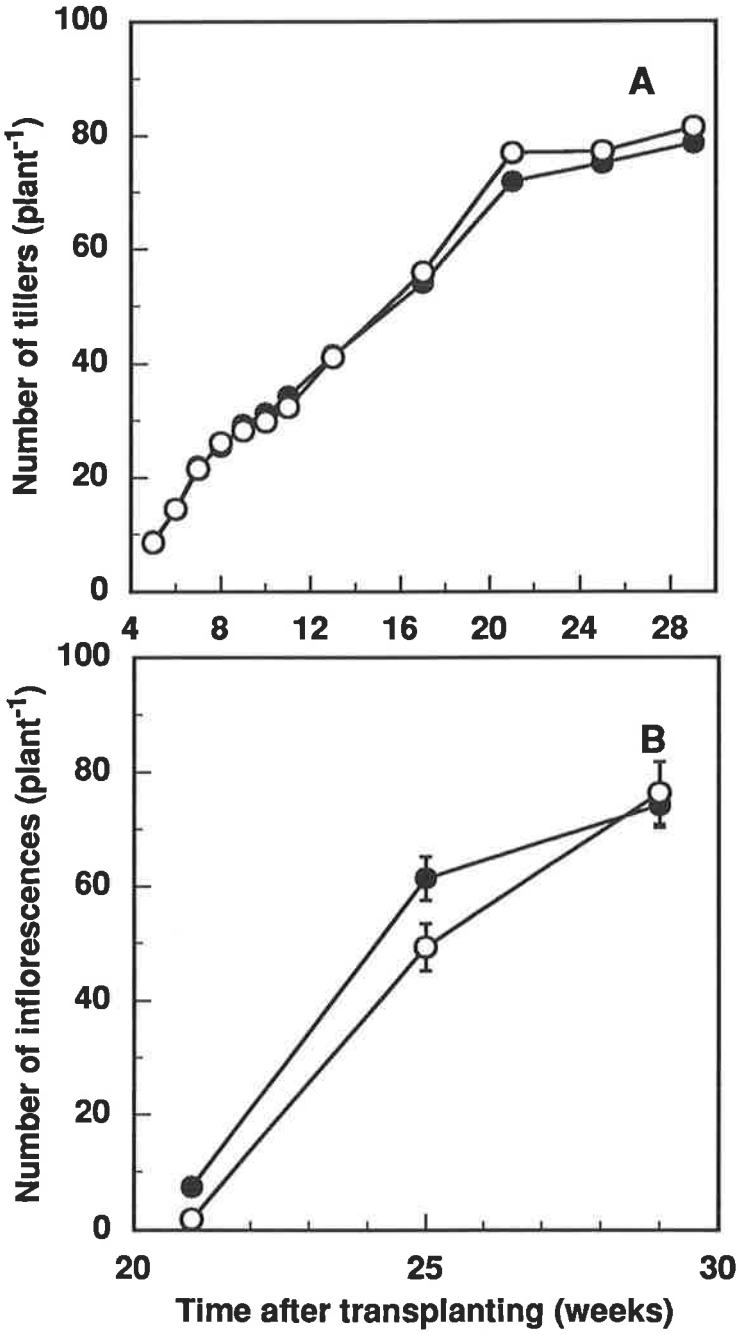


Figure 4. 4. Number of tillers (A) and spike inflorescences (B) of resistant (THL1) (●) and susceptible (THL4) (O) biotypes of *H. leporinum* over a period of time. Plants were grown from seed collected in 1991 from plants grown at the Waite Institute. Plants were grown in monoculture in 30 cm diameter pots with four plants per pot. Each point is the mean of 24 plants. Vertical bars are the standard errors.

resistant and susceptible biotypes was not different at any time during the growing season (Figs. 4. 3A, 4. 4A), for either the original seed stock or for seed grown at the Waite Institute.

Table 4. 7. Number of green (fresh) and dry spike inflorescences and number of days to emergence of first spike inflorescence (DTH) of THL1 and THL4 biotypes of *H. leporinum* grown in monoculture at density of four plants per 30 cm pot. Seeds used were collected from the productivity experiment in 1991.

Biotype	DTH	Number of spike inflorescences per pot at 27 weeks[a]		
	(day)	Green	Dry	Total
THL1	146	254 (17%)a	1243 (83%)a	1497a
THL4	149	925 (61%)b	586 (39%b	1511a

[a] Values in the same column followed by different letters are significantly different (P=.01, t-test)

In contrast to the tiller number, there was a difference between the two biotypes in the ontogeny of inflorescence development. The time to emergence of the first spike inflorescence was 21 weeks for the resistant biotype compared to 22 weeks for the susceptible biotype for plants grown from the original seed collection (Fig. 4. 3B). The resistant biotype produced more inflorescences at each time point up to 29 weeks (Fig. 4. 3B). This earlier maturity of the resistant biotype was an inherent property of this biotype as plants grown from seed obtained from the productivity experiment (grown and harvested under identical conditions) also showed earlier maturity in the resistant biotype (Fig. 4. 4). In this experiment the time to emergence of the first spike was 146 days (21 weeks) for the resistant biotype compared to 149 days (21.5 weeks) for the susceptible biotype (Table 4. 7) The resistant biotype produced more inflorescences at all times up to

27 weeks at which time the total number of spike inflorescences was the same for each biotype. The number of mature inflorescences at this time was significantly greater in the resistant biotype (Table 4. 7) and correspondingly there were more green inflorescences in the susceptible biotypes. Clearly reproductive maturity occurs earlier in the resistant biotype than in the susceptible biotype; however, the total reproductive productivity is the same.

4. 4. DISCUSSION

Studies on the competitiveness of herbicide-resistant weeds have observed that resistance can carry a penalty in terms of fitness and competitive ability compared to the susceptible biotype (Gressel and Ben-Sinai 1985; Holt, 1988; Valverde et al. 1989; Conard and Radosevich 1979; Tucker, 1989). In the absence of herbicides, productivity of triazine-resistant biotypes of *Amaranthus retroflexus*, *A. powelli*, and *Senecio vulgaris* was less than the susceptible ones (Warwick 1980; Weaver and Warwick 1982; Conard and Radosevich 1979; Holt 1988). In contrast, productivity of a triazine-resistant biotype of *Phalaris paradoxa* was shown to be equal or superior to the susceptible biotype when they were grown under non-competitive conditions (Schönfeld et al. 1987).

The reduced fitness observed for triazine-resistant populations is likely to be the result of reduced photosynthetic efficiency in these plants (Holt 1988; LeBaron 1991; Ort et al. 1983); however, other factors are also involved (Holt 1988). This reduced photosynthetic efficiency is due to a change at the active site in Photosystem II which confers resistance, but which also reduces electron flow through Photosystem II (Ireland et al. 1988; Stowe and Holt 1988; Ort et al. 1983). Hart and Stemler (1990a) observed that productivity of a triazine-resistant biotype of *Brassica napus* L. was reduced compared to the susceptible biotype when plants were grown in high light. Under high light intensities, the resistant biotype had a lower photon yield than the susceptible one, but when grown under low light intensity there was no difference in the photon yield of the two biotypes. These authors

concluded that the lower productivity observed in triazine-resistant biotypes was due to increased sensitivity of the resistant plants to photoinhibition as a result of reduced capacity for electron flow from Q_A to Q_B (Hart and Stemler 1990b). Thus, the reduced fitness in these species is mostly a result of a penalty associated with the mechanism of resistance. Reduced fitness has also been observed in a paraquat-resistant biotype of *H. glaucum* (Tucker 1989) and a dinitroaniline-resistant biotype of goosegrass (Valverde et al. 1989); however, in these cases it is not apparent whether the reduced fitness is a result of changes associated with the mechanism of resistance or of associated deleterious genetic traits. However, sulfonylurea-resistant and -susceptible biotypes of *Lactuca serriola* were shown to be equally competitive (Alcocer-Ruthling et al. 1992a) demonstrating that there was not a fitness penalty in this case.

In the present study the paraquat-resistant biotype of *H. leporinum*, THL1, is not less productive than the susceptible biotype, THL4, when grown in monoculture at either 100 plants m^{-2} or 400 plants m^{-2} (Tables 4. 1, 4. 2). This performance of THL1 was evident in a replacement series competition experiment which revealed that the resistant biotype was at no competitive disadvantage compared to the susceptible biotype (Figs. 4. 1, 4. 2). In contrast to the results of the competition experiment, the susceptible biotype produced greater dry matter and number of tillers in the absence of competition than did the resistant biotype; however, the number of spike inflorescences was the same (Table 4. 3). Similarly seed weight and the number of seeds per spike inflorescence were not different between the two biotypes (Table 4. 4), and therefore, total fecundity did not differ.

The growth and maturity of the two biotypes were examined and although the production of tillers was the same, inflorescences were produced and matured earlier in the resistant biotype than the susceptible biotype. This earlier maturity of the resistant biotype comes as some surprise as the two biotypes were collected from adjacent paddocks. This would seem to preclude the earlier maturity being the result of different environmental influences on these biotypes and, therefore, the earlier maturity may have occurred as a result, either active or passive, of selection for herbicide resistance. In addition, this earlier maturity

was maintained for one generation when plants were grown in a different climate (Fig. 4. 4B). The reasons for this earlier maturity need to be investigated; however, the differences observed between the competition experiment and productivity in the absence of competition appear to be largely a result of the earlier maturity of the resistant biotype. The replacement series experiments (de Wit, 1960) have been criticised when used to determine competitive ability between species because such experiments make no allowance for differences in maturity of the different species (Cousens, 1990). The same problem has arisen in this study where differences in maturity between the two biotypes have influenced the results obtained. Taking the difference of maturity into account, it is clear that whilst biomass production may be less in the absence of competition, the resistant biotype is not at a competitive disadvantage compared to the susceptible biotype. In addition, the level of dormancy, the rate of emergence of seedlings of the resistant biotype, and the total fecundity do not differ and therefore, these factors will not reduce competitive ability in the field.

Seed dormancy investigations show that both biotypes of *H. leporinum,* THL1 and THL4, have no innate dormancy. Each biotype has a high percentage of germination (90% and 96% for resistant and susceptible biotypes respectively) with freshly harvested seeds. Germination of the two biotypes did not change over a period of two months storage. This is in contrast to the study of Popay (1981) who reported that freshly harvested seeds of biotypes of *H. leporinum* collected from four different areas had low germination ranging from 19-20%. Similarly Powles et al. (1992) found that seeds of both paraquat-resistant and -susceptible biotypes of *H. glaucum* from Victoria bore innate dormancy for eight weeks. The lack of innate dormancy in biotypes studied here would therefore appear to be a result of selection for the environment where they were collected. These biotypes were collected from an area with high and non-seasonal rainfall distribution and a lack of dormancy may be a successful strategy in this environment.

The equal fitness of this paraquat-resistant biotype of *H. leporinum* has important implications for the control of this biotype. Where a herbicide-resistant biotype is less fit

compared to the susceptible biotype then, over time, the numbers of resistant individuals would decrease through competition for resources provided the selection pressure was removed. This will not occur for the resistant biotype of *H. leporinum* and therefore passive measures will be insufficient to reduce the numbers of resistant individuals. Instead an active program for the control of this biotype must be implemented.

CHAPTER 5

MODE OF INHERITANCE OF BIPYRIDYL HERBICIDE RESISTANCE IN *A. CALENDULA* (L.) LEVYNS AND *H. LEPORINUM* LINK.

5. 1. INTRODUCTION

Genes for herbicide resistance may or may not exist in natural weed populations (Darmency and Gasquez 1990a); however the larger a population the greater the likelihood of a resistance gene being present. The frequency of plants resistant to any given herbicide in a wild population is unpredictable and depends on the number of genes for resistance, dominance and the ploidy of the plant involved (Gressel 1979). Gressel (1979) made a prediction that the frequency of resistant plants in a natural population would be between 1×10^{-10} and 1×10^{-5}. Selection pressure resulting from the repeated use of a similar herbicide for a number of years selects for resistant individuals. Resistant plants in a weed population usually become noticeable after repeated use of herbicide for a period of time. For example, resistance appeared to triazine herbicides after the use of the herbicide for about 5 to 10 years (Gressel et al. 1982), whereas *Kochia scoparia* resistant to sulfonylurea herbicides (Mallory-Smith et al. 1990; Primiani et al. 1990) and *Lolium rigidum* resistant to aryloxyphenoxypropionate herbicides (Heap and Knight 1982) appeared after 3 to 7 years and 3 to 4 years of treatment, respectively. In the case of paraquat resistance, this has only appeared after the herbicide had been used for 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 to 24 years for *H. leporinum* (Chapter 3) which suggests that genes for paraquat resistance may be rarer than resistance to some other herbicides.

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, for example atrazine resistance in *Brassica campestris* (Souza

Machado et al. 1978), triazine resistance in *Senecio vulgaris* (Scott and Putwain 1981) and *Setaria viridis* (Darmency and Pernes 1985). Maternal inheritance of most cases of triazine resistance is due to resistance being caused by a mutation in the D1 protein of the Photosystem II reaction centre, the binding site of triazine herbicides at Photosystem II (Pfister et al. 1981). In contrast triazine resistance in *Abutilon theoprasti* was found to be nuclearly inherited and controlled by a single incompletely dominant gene (Andersen and Gronwald 1987). In this case resistance was due to a detoxification of the herbicide via glutathione conjugation (Gronwald et al. 1989), which resulted from an increased activity of glutathione S-transferase (Anderson and Gronwald 1991). Inheritance of sulfonylurea resistance in *Lactuca* spp. is controlled by a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990) as is diclofop resistance in a biotype of Italian ryegrass (*Lolium multiflorum*) (Betts et al. 1992) and resistance to ACCase-inhibiting herbicides in *Avena sterilis* (Barr et al. 1992). The inheritance of paraquat resistance has been reported in a number of paraquat-resistant weed species and has been shown to be controlled by a single dominant gene in *Conyza bonariensis* (L.) Cronq. (Shaaltiel et al. 1988), and *Erigeron philadelphicus* L. (Itoh and Miyahara 1984). A single dominant or partially dominant gene controls resistance in *E. canadensis* (Yamasue et al. 1992) whereas resistance in *H. glaucum* Steud. is due to a single incompletely dominant gene (Islam and Powles 1988). In contrast paraquat resistance in *Lolium perenne* L. is controlled by several genes (Faulkner 1974) whereas paraquat resistance in *Ceratopteris richardii* is controlled by a single recessive gene (Hickok and Schwarz 1986).

The objectives of this study were (1) to investigate the initial gene frequency of paraquat resistance in a natural population of *Hordeum* spp. and (2) 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 which show high levels of resistance to the bipyridyl herbicides.

5. 2. MATERIALS AND METHODS

5. 2. 1. Frequency of paraquat-resistant plants in a natural population

In July and August 1991 (the winter growing season) a field experiment was conducted at Bordertown, South Australia to attempt to identify the initial gene frequency for paraquat resistance in a *Hordeum* population. The field chosen was infested by a large number of *Hordeum* spp. which had no history of paraquat or diquat use. Paraquat was applied at two rates, 200 g (the recommended rate) and 100 g a.i. ha^{-1}. The herbicide was applied with a hand-held boom sprayer at a pressure of 250 kPa with an output of 133 L ha^{-1} at a speed of 1m s^{-1} with nozzles 40 cm above the plants. 0.2% (v/v) non-ionic surfactant (Agral 600) was added to all treatments. The plants were sprayed at the 2-3 leaf stage, temperature was 14°C with no wind and no rain. The experiment was a randomised block design with six replications and a plot size of 25 m x 4 m with 25 cm borders. Prior to, and three weeks after spraying, the number of surviving plants in five quadrats (1 quadrat = 0.1 m^{-2}) in each plot were counted.

5. 2. 2. Modelling

A prediction of the development of paraquat resistance in *H. leporinum* was simulated using a herbicide resistance model which was developed by Rick Roush. Data used for this model are based on the seedbank study conducted by Powles et al. (1992) and also data from the study in this thesis. Parameters used are :

- initial frequency of the R allele: variable
- inbreeding coefficient (0-1; outcrossing = 0) : 1
- survival of RR homozygotes (0-1; 1 = 100%): 1
- survival of RS homozygotes (0-1; 1 = 100%): 0.05
- survival of SS homozygotes (0-1; 1 = 100%): 0.01
- the proportion of seeds that die between years (0-1; 1 = 100%): 0.5
- the proportion of seeds staying in bank (0-1; 1 = 100%): 0.1

- the non-herbicidal mortality of seedlings (0-1; 1 = 100%): 0.62
- the initial seed density per meter: variable
- the treatment threshold density for barley grass: 0
- the production of seeds per plant: variable

By inserting the data above into the model a prediction of the development of paraquat resistance in barley grass can be made.

5. 2. 3. Crossing experiments

5. 2. 3. 1. Plant material

Seeds of resistant biotypes of *A. calendula* (VAC1) and *H. leporinum* (VHL1) were originally collected from an alfalfa field in Victoria with a long history of paraquat and diquat use (Chapter 3). When the two biotypes were grown in pots, *A. calendula* survived 200 g a.i. ha^{-1} diquat and *H. leporinum* survived 200 g a.i. ha^{-1} 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 or diquat use. Seeds of both resistant and susceptible biotypes of *A. calendula* were germinated (buried at 2.5 to 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 as described in Chapter 3. Seedlings were transferred separately into 30-cm pots containing potting soil based on peat and sand and then maintained in the glasshouse.

5. 2. 3. 2. Hybridisation

A. calendula

Capeweed (*A. calendula*) is an obligate outcrossing species (F. van de Loo and S. B. Powles, unpublished). 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 crossing 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 bagged. Reciprocal crosses of resistant (VAC1) and susceptible (VAC2) 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 6 days after emasculation the plants were hand-pollinated by placing the anthers of the desired male parent on to 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.

5. 2. 3. 3. Response of parents, F_1 and F_2 to herbicide application

F_1, resistant parent (VAC1) and susceptible parent (VAC2) seeds of *A. calendula* were germinated in plastic trays (40 x 30 x 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 (approximately 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 (36 plants at each rate) 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 x R crosses and one from the R x 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, 4 S x R and 2 R x S, were sprayed with 100 g a.i. ha^{-1} paraquat and four F_1 clones (S x R) were sprayed with 200 g a.i. ha^{-1} 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 hybridisation between R and S had been conducted successfully. F_2 plants were grown in plastic trays (40 x 30 x 12 cm) containing potting soil with growing conditions as described above. Each tray contained 140 to 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 to 14 days after spraying.

Chi-square analysis of segregation of the F_2 and backcross populations was performed as described by Goodenough (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.

5. 3. RESULTS

5. 3. 1. Initial gene frequency

A large field population of *Hordeum* spp. with no history of bipyridyl herbicide use was sprayed with either 100 or 200 g a.i. ha^{-1} paraquat. The density of *Hordeum* plants before, and three weeks after spraying, is shown in Table 5. 1. At the time of spraying there was an average of 5180 plants m^{-2} in the plots but no plants in any of the sprayed plots survived paraquat application at either rate. The total number of plants sprayed at the two rates was about 6.2 million yielding no paraquat-resistant individuals. Therefore the frequency of resistant individuals in this population is less than 1 in 10^6. The rate of 100 g a.i. ha^{-1} was used in an attempt to identify any heterozygous individuals (see section 5. 3. 3) in the field; however, none were detected.

Table 5. 1. Density (plants m^{-2}) of *Hordeum* plants in a population with no history of bipyridylium herbicide use before and after spraying with paraquat.

Paraquat	Plants m^{-2}	
(g a.i. ha^{-1})	At the time of spraying	3 weeks after spraying
100	5100	0
200	5260	0

5. 3. 2. Paraquat resistance model

As the field experiment did not yield an initial gene frequency for paraquat resistance, modelling was performed in an attempt to estimate the possible gene frequencies of resistance. In this model three components were varied: soil seedbank density, initial gene frequency for resistance and seed production. The results of computer simulation of

the dynamics of resistance development reveal that initial soil seed-bank density has little effect in the development of paraquat resistance in *Hordeum* spp (Fig. 5. 1A). The development of paraquat resistance is predicted to be only 2 years slower with the initial seed density of 10 seeds m^{-2} than with 1000 seeds m^{-2}. However, the initial gene frequency dramatically influences the development of resistance. With an initial resistance gene frequency of 1×10^{-10} and a seed density of 10 seeds m^{-2}, the model predicts resistance to appear after 11 years (Fig. 5. 1A). Decreasing the initial resistance gene frequency to 1×10^{-20} increases the time for resistance to appear to 19 years. The time taken for resistance to appear was estimated using various initial resistant gene frequencies at initial seed densities of 10, 100 and 1000 seeds m^{-2} (Fig. 5. 1A). The field experience has been that paraquat resistance in *Hordeum* spp. has taken 12 to 24 years of herbicide selection pressure for resistance to appear hence, from the model the initial resistant gene frequency should have been in the order of 1×10^{-11} to 1×10^{-23} (Fig. 5. 1A). The model therefore supports the field observations that the initial resistant gene frequency for paraquat resistance in *Hordeum* spp. is low.

It is important to realise that when left undisturbed a barley grass plant can produce up to 1900 seeds (Chapter 4); however, in lucerne fields plants are rarely allowed to produce the full complement of seeds. Harvesting the lucerne removes many of the *Hordeum* seed heads from the field. The model was used to examine the effects of seed removal on the development of resistance. At the initial resistance gene frequency of 10^{-12} the time for paraquat resistance in *Hordeum* spp. is predicted at various levels of seed production per plant (Fig. 5. 1B). With a seed production of 100 seeds per plant, the model predicts resistance will appear after 11 years whereas with a seed production of 25 seeds per plant resistance takes 20 years to appear. Decreasing seed production further to 15 seeds per plant increases the predicted time for resistance to appear to 49 years, although this amount of seed production per plant hardly occurs in the field as the seed production observed in the field averaged 30 seeds per spike inflorescence (Chapter 4). In contrast, increasing seed production from 100 seeds to 800 seeds per plant decreases the predicted time for resistance to appear by only 3 years (Fig. 5. 1B).

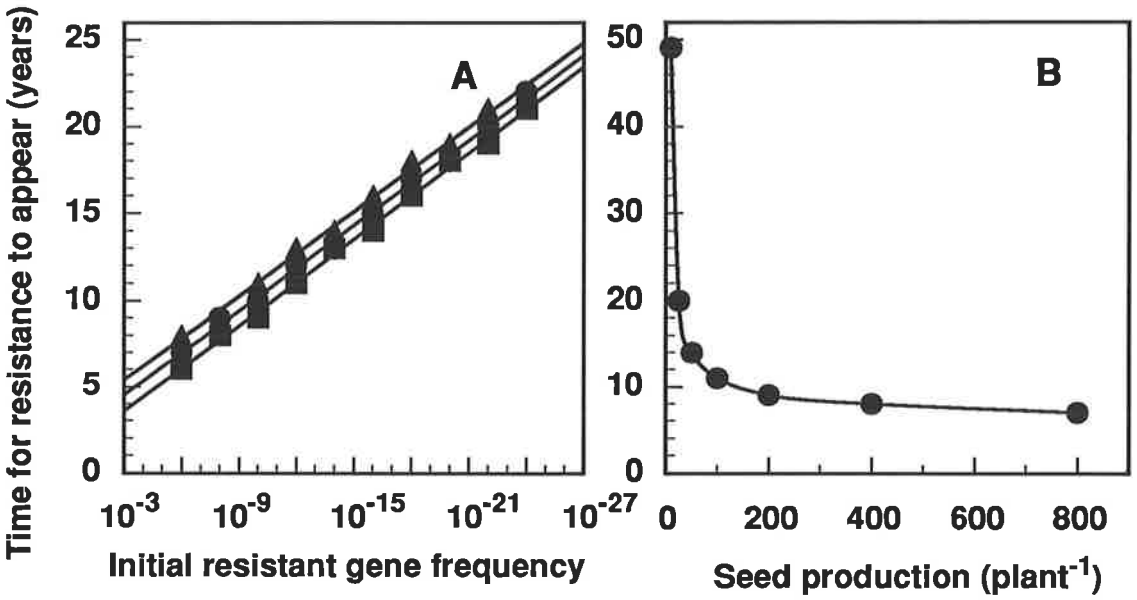


Figure 5. 1. Model of paraquat resistance in *Hordeum* spp. (A) with various initial soil seed-bank densities (▲: 10, ●: 100, ■: 1000 seeds m^{-2}) and seed production of 100 seeds per plant and (B) with various seed productions per plant at an initial resistant gene frequency of 10^{-12} and an initial seed-bank density of 1000 seeds m^{-2}.

5. 3. 3. Crossing experiment

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. 5. 2). The susceptible biotype was killed by rates of diquat as low as 50 g a.i. ha^{-1} whereas the resistant biotype was only slightly affected at 200 g. Both phenotype and mortality (Fig. 5. 2) in the F_1 (VAC2 x VAC1) and F_1 (VAC1 x VAC2) crosses were identical and intermediate between the resistant and susceptible parent populations. A similar result was seen with the production of dry matter by these plants (Fig. 5. 2B).

F_2 and backcross plants were obtained to further examine the mode of inheritance. F_2 plants generated by crossing F_1 plants with each other were treated with 100 g a.i. ha^{-1} diquat or 800 g a.i. ha^{-1} paraquat at the 5- to 6-leaf stage (rates lethal to susceptible plants but not to resistant plants (see Chapter 3). Assessment on the treated F_2 plants was conducted 3 and 4 days after spraying. Plants were scored based on phenotypic responses to herbicide injury 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 occurred. The 742 F_2 plants treated with 100 g a.i. ha^{-1} diquat showed a segregation ratio of 1 : 2 : 1 (R : I : S) and treatment of the 336 F_2 plants with 800 g a.i. ha^{-1} paraquat also displayed a segregation ratio of 1 : 2 : 1 (R : I : S) (Table 5. 2). 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 a.i. ha^{-1} diquat or 800 g a.i. ha^{-1} paraquat (Tables 5. 2). In all experiments the reciprocal F_1 crosses were shown to be homogeneous by the chi-square homogeneity test of observed values (not shown).

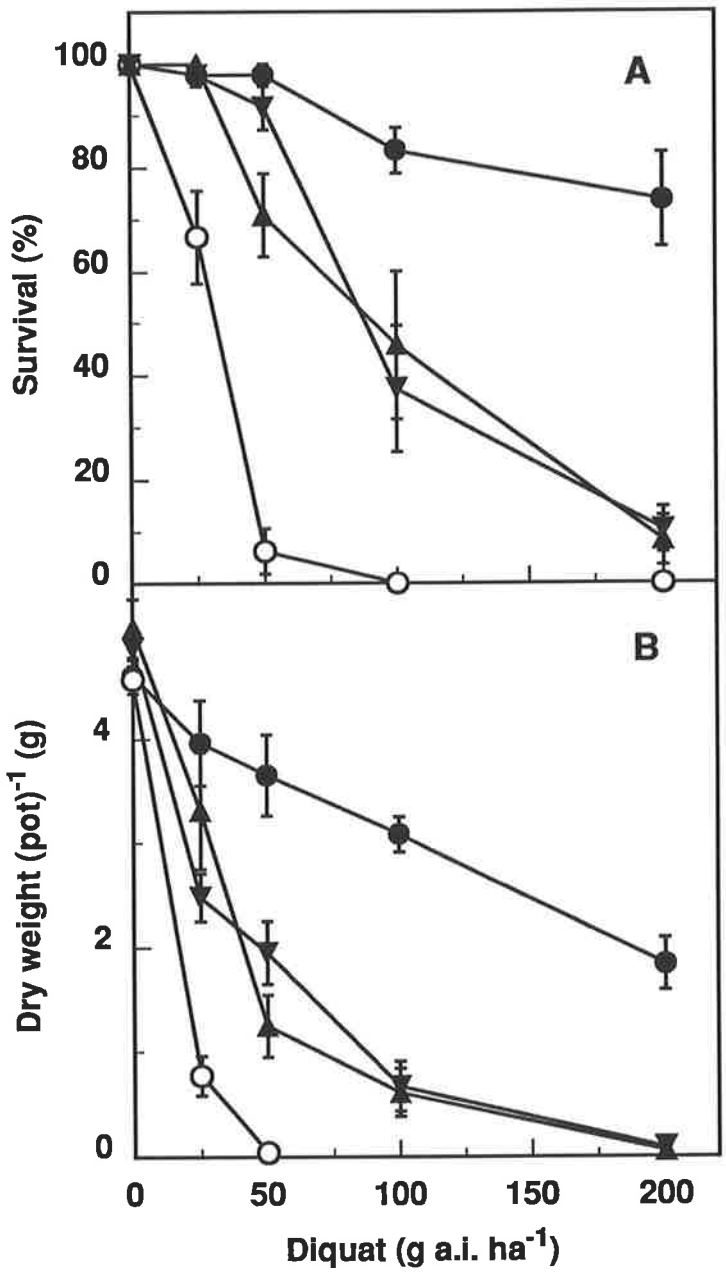


Figure 5. 2. Effect of diquat, 21 days after treatment, on survival (A) and dry weight (B) of susceptible VAC2 (O) and resistant VAC1 (●) biotypes of *A. calendula* and of the reciprocal F1 crosses (VAC2 x VAC1) (▲); and (VAC1 x VAC2) (▼) between the susceptible and resistant biotypes. Each point is the mean of 36 plants and vertical bars are the standard errors.

Table 5. 2. Chi-square analysis of the segregation of phenotype of F_2 populations of *A. calendula* in response to the application of 100 g a.i. ha^{-1} diquat or 800 g a.i. ha^{-1} paraquat 3 d after treatment.

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

[a]R: resistant, I: intermediate, S: susceptible.

[b]R x S = $R_{♀}$ x $S_{♂}$.

The progeny of nine backcross families (284 plants) treated with 100 g a.i. ha^{-1} 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 5. 3). Homogeneity test for the total observed values in backcrosses showed that there is no difference between the two backcross populations (not shown).

Table 5. 3. Chi-square analysis of the segregation of phenotype of backcross populations of *A. calendula* in response to the application of 100 g a.i. ha^{-1} diquat 3 d after treatment.

Backcross	Segregation by phenotype[a]			X^2	P
Population	Intermediate	Susceptible	Total		
S x F_1(SxR)[b]	98	109	207	0.584	0.30-0.50
S x F_1(RxS)	42	35	77	0.636	0.30-0.50
Total	140	144	284	0.056	0.80-0.90

[a]R: resistant, I: intermediate, S: susceptible.

[b]S x F_1(SxR) = $S_♀$ x $F_{1♂}$, S x R = $S_♀$ x $R_♂$.

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 along with parental seed were grown 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 a.i. ha^{-1} paraquat. All resistant plants survived both rates, whereas none of the susceptible plants survived at either rate. The 6 F1 plants treated with 100 g a.i. ha^{-1} paraquat were severely damaged and all 4 plants treated with 200 g a.i. ha^{-1} paraquat died. This intermediate response of the F_1 population suggests that paraquat resistance may be conferred by a partially dominant allele(s).

Unsprayed F_1 plants from each family were allowed to self to obtain F_2 seed and the F_2 seedlings were treated with paraquat at 200 g a.i. 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 5. 4). Identical results were obtained from F_2 progeny of the S x

R cross and the R x S cross (Table 5. 4) and homogeneity test showed that the two reciprocal crosses are homogeneous which indicates that the resistance gene(s) reside in the nuclear genome. Application of 50 g a.i. ha^{-1} paraquat to F_2 plants distinguished susceptible plants from intermediate and resistant plants (Table 5. 4). The segregation ratios obtained of 3 : 1 [(R+I) : S] were as expected. The survivors at 50 g a.i. paraquat were allowed to recover for 3 weeks and then resprayed with 400 g a.i. ha^{-1} 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 5. 4).

Table 5. 4. Chi-square analysis of the segregation of phenotype of F_2 populations of *H. leporinum* in response to the application of paraquat 7 d after treatment.

F_2	Paraquat	Segregation by phenotype[a]				X^2	P
population	g a.i. ha^{-1}	R	I	S	Total		
S x R[b]	200	126	214	110	450	2.21	0.30-0.50
R x 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				
R x S	50	115	34		149	1.228	0.20-0.30
		R	S + I[c]				
R x S	400	39	110		149	0.849	0.3-0.50

[a]R: resistant, I: intermediate, S: susceptible.

[b]S x R = $S_{♀}$ x $R_{♂}$.

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

5. 4. DISCUSSION

5. 4. 1. Initial gene frequency for paraquat resistance in *Hordeum*

Screening of wild populations with no herbicide history for resistant plants have rarely identified resistant individuals; however, considerable variation in responses of populations to herbicides has been observed. For example, examination of a large number of *Brassica* spp. showed no individuals with resistance to simazine (Putwain 1982). When 46 populations of *Senecio vulgaris* that had been exposed to simazine from 0 to 12 years were examined a positive correlation between survival and history of simazine use was observed, but no population was truly resistant (Holliday and Putwain 1980). These authors concluded that the appearance of resistance to triazines in a population is most likely due to new mutation events in the psbA gene encoding the D1 protein. In fact most triazine-resistant biotypes show a mutation in this gene (Darmency and Gasquez 1990b). When a large number of *Chenopodium album* families were treated with atrazine about 3% of individuals from five populations showed some resistance and proved to have a mutated psbA gene (Darmency and Gasquez 1990b). Somody et al. (1984) screened 908 wild oat populations from across North America and observed considerable variation to diclofop-methyl, flamprop, diallate and other herbicides. Similarly, high genetic variability has been found in *Avena barbata, A. fatua* and *Clarkia williamsonii* populations to barban and bromoxynil (Price et al. 1983). A study of genetic variability of *Convolvulus arvensis* which was originally collected from a single population later showed a striking difference in response to glyphosate (DeGennaro and Weller 1984). Application of glyphosate at 2.24 kg ha^{-1} only decreased dry weight of a tolerant biotype by 40% while the susceptible biotype was completely killed. The authors in these two studies, DeGennaro and Weller (1984) and Price et al. (1983) suggested that genes conferring herbicide tolerance occur in natural populations. Matthews and Powles (1992) who studied the frequency of diclofop resistance in *L. rigidum* populations collected from farm sites and non-farm sites which had never been exposed to the herbicide diclofop-methyl found a high frequency of resistance. The frequency of

resistant individuals of *Lolium rigidum* in the farm site populations was 2×10^{-2} whereas in non-farm populations was 2×10^{-3}. As *L. rigidum* is an obligate outcrossing species, there remains the possibility that resistant genes have moved from fields containing resistance to these areas via pollen flow.

However, in the study reported here in which a wild *Hordeum* population with no herbicide history was treated with paraquat at normal field rates revealed that the initial gene frequency of paraquat resistance in this naturally occurring, unsprayed *Hordeum* population was low, less than 1×10^{-6} (Table 5. 1). Modelling estimates of the initial resistant gene frequency for paraquat resistance in *Hordeum* populations ranged from 1×10^{-11} to 1×10^{-23} (Fig. 5. 1B). This is much lower than the initial gene frequency for triazine resistance of 1×10^{-4} to 3×10^{-3} (Darmency and Gasquez 1990a) or for diclofop of 2×10^{-3} resistant individuals in *L. rigidum* (Matthews and Powles 1992). A low initial gene frequency for resistance will delay the appearance of resistance in the field because the resistant population will take longer to build up compared to a population with a higher initial gene frequency of resistance. The low frequency for paraquat resistance may be one reason why it has taken so long, 12 - 24 years of continuous use of the same herbicides, for paraquat resistance to appear in Australia (see Chapter 3). No annual species are naturally resistant to paraquat and therefore there are no pre-existing resistance mechanisms. Hence any resistance genes are likely to be very rare. The low estimates of gene frequencies for paraquat resistance raise the possibility that in most fields there are no resistant genes present. If this was the case then the paraquat-resistant biotypes that have appeared may not have come from pre-existing resistant individuals, but from new mutational events during the course of the selection period.

Selection pressure and the genetic structure of the population are important determinants of the rate of evolution of resistance. For example the number of genes controlling resistance, dominance, the rate of mutation and the amount of recombination which occurs, will all affect the spread of resistance genes. As genetic recombination is determined by the breeding system, a rapid build-up of resistance is more likely to occur in

a species with effective outcrossing (Harper 1956) such as *A. calendula*. Since *H. leporinum* is a self-pollinating species, recombination would be a relatively rare occurrence; however, once a highly resistant genotype appears, then self-pollination would help to maintain its frequency in the population even in the absence of selection by paraquat or diquat. The relative fitness of the resistant biotype will affect the stability of the resistance gene in the population in the absence of selection. For example the paraquat-resistant *H. leporinum* biotype THL1 (see Chapter 4), which is as fit as the susceptible biotype, will remain in the population.

5. 4. 2. Inheritance of resistance in *A. calendula*

Reciprocal crosses between resistant (VAC1) and susceptible (VAC2) plants produced large numbers of F_1 hybrid seeds. The F_1 plants from the reciprocal crosses were intermediate in response between the VAC1 and VAC2 (Figure 5. 2). Both phenotype and mortality (Figure 5. 2A) in the F_1 (VAC2 x VAC1) and F_1 (VAC1 x VAC2) crosses were identical which demonstrates that bipyridyl 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 to 4 days after spraying. Response of reciprocal F_1 progenies was averaged and LD_{50} was estimated to be 80 g a.i. ha^{-1} whereas the LD_{50} for the resistant biotype was >200 g a.i. ha^{-1} and that of the susceptible biotype was 30 g a.i. ha^{-1}. Dry matter production of F_1 was intermediate to the dry matter production of the parent VAC1 and VAC2 biotypes (Figure 5. 2B). The intermediate survival and dry matter production observed with the F_1 plants suggests that bipyridyl resistance is conferred by an incompletely dominant allele(s). F_2 plants treated either with 100 g a.i. ha^{-1} diquat or 800 g a.i. ha^{-1} paraquat had a segregation ratio of 1 : 2 : 1 (R : I : S) (Table 5. 2). These results suggest that resistance in *A. calendula* is probably controlled by a single gene. To test this possibility the progenies of backcrosses were treated with diquat. Of the 284 plants produced from backcrosses treated with 100 g a.i. ha^{-1} diquat, 140 plants behaved as the intermediate phenotype and 144 plants displayed the susceptible phenotype (Table 5. 3). Chi-square analysis of 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 and 1 : 1 (I : S) in backcross populations treated with diquat or paraquat lead to the conclusion that diquat and paraquat resistance in *A. calendula* is controlled by a single nuclear, partially dominant gene.

5. 4. 3. Inheritance of resistance in *H. leporinum*

Reciprocal crosses of paraquat-resistant and susceptible biotypes of *H. leporinum* resulted in F_1 progeny of which most were severely damaged by the application of 100 g a.i. ha^{-1} paraquat and died at 200 g a.i. ha^{-1} paraquat. These rates of paraquat killed all susceptible parent individuals but not the resistant parent plants. Herbicide symptoms on the F_1 plants were clearly intermediate between the two parents. Paraquat resistance in this biotype of *H. leporinum* resides in the nuclear genome. Application of 50 g a.i. ha^{-1} paraquat to the F_2 population resulted in a segregation ratio of 3:1, whereas application of 400 g a.i. ha^{-1} paraquat resulted in a segregation ratio of 1:3. The explanation for the different segregation ratios at the two herbicide rates is that the intermediate phenotype is resitant at 50 g a.i. ha^{-1}, but susceptible at 400 g a.i. ha^{-1}. The intermediate phenotype does not show a high level of resistance to paraquat. The results from paraquat applications on F_1 and F_2 progeny show that paraquat resistance in *H. leporinum* is controlled by a single, incompletely dominant gene.

Therefore, in conclusion paraquat and diquat resistance in both *H. leporinum* and *A. calendula* are the result of expression of single incompletely dominant nuclear genes. The mode of inheritance in these two resistant biotypes is thus identical to that found in a biotype of paraquat-resistant *H. glaucum* obtained from the same field (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 genetics endowing resistance would be expected to be similar. Paraquat resistance in biotypes of *Conyza bonariensis* (Shaaltiel et al. 1988) and *Erigeron philadelphicus* (Itoh and Miyahara

1984) is conferred by a single dominant gene and in *E. canadensis* by a single dominant or semi dominant gene (Yamasue et al. 1992). However, in *Lolium perenne* resistance is polygenic (Faulkner 1974). In the resistant *H. glaucum* and *H. leporinum* biotypes the mechanism endowing paraquat-resistance involves reduced herbicide translocation and / or penetration of paraquat to the active site (Preston et al. 1992). The mechanism of resistance to diquat in *A. calendula* is similar (C. Preston, pers. communication). In these three species the mode of inheritance is correlated with the mechanism involved. It is interesting to speculate as to whether the same mutation to give resistance has occurred in these three different species.

CHAPTER 6

ENVIRONMENTAL FACTORS INFLUENCING THE LEVEL OF BIPYRIDYLIUM RESISTANCE

6. 1. INTRODUCTION

Environmental influences have long been recognised to alter the effectiveness of bipyridyl herbicide action in plants. The most important of these environmental factors so far identified is light, with higher light intensities immediately after treatment known to reduce diquat effectiveness (Mees 1960). The rapid phytotoxic action of paraquat and diquat at high light quickly damages leaf tissue resulting in limited herbicide translocation. In contrast, low light intensities immediately after treatment can increase bipyridyl herbicide activity by increasing translocation throughout the plant (Brian 1967b). Humidity also affects paraquat and diquat effectiveness as these herbicides are more effective at high humidities because increased humidity promotes herbicide uptake (Thrower et al. 1965). In particular, high atmospheric humidity and low soil moisture were shown to dramatically increase the movement of these herbicides into plants (Thrower et al. 1965). Conversely, low humidity and high temperature can prevent movement of herbicide from the treated to untreated parts of the plants due to desiccation of the treated leaves (Thrower et al. 1965). The effects of temperature on paraquat activity have not been extensively studied; however, cooling plants to 0°C increased paraquat effectiveness (Brian and Headford 1968). Another study which examined cell membranes suggested that paraquat tended to be less effective at low temperatures (Merkle et al. 1965).

In 1990, during a screening test for resistance in *H. leporinum* biotype THL1, there was some indication that a large seasonal difference in the level of resistance in this biotype may have been apparent. The objective of this study was to investigate the effects of

seasonal factors on the level of resistance in paraquat- and diquat-resistant biotypes of *H. leporinum*, *H. glaucum* and *A. calendula*.

6. 2. MATERIALS AND METHODS

6. 2. 1. Plant materials

Biotypes used in this study were resistant biotypes of *H. leporinum* (THL1), *H. glaucum* (SHG1), *A. calendula* (VAC1) and susceptible biotypes of *H. leporinum* (THL4), *H. glaucum* (SHG2) and *A. calendula* (VAC2). The histories of these biotypes have been described in Chapter 3. Seedling preparation, transplanting and herbicide application were the same as protocols mentioned previously (see Chapter 3).

6. 2. 2. Pot experiments

Light intensity and temperature were varied by growing plants in full sun or in a closely adjacent shadehouse in summer and winter. The shadehouse consisted of a simple framework covered with shade-cloth to reduce the light intensity. During the experiments both light intensity and temperature in the shadehouse and in the sun were recorded at 2 pm every day. There was no difference in temperature between the sun and shade treatments, but the shade-cloth decreased the light intensity by 75%. During summer peak noon light intensities reached 2200 $\mu E\ m^{-2}\ s^{-1}$ (550 $\mu E\ m^{-2}\ s^{-1}$ in shade treatment) and in winter 180 $\mu E\ m^{-2}\ s^{-1}$ (45 $\mu E\ m^{-2}\ s^{-1}$ in shade treatment).

A. calendula

Seeds of both VAC1 and VAC2 were germinated as described in Chapter 3 in an unheated glasshouse for the summer experiment or outdoors for winter experiment. Seedlings at the 2-leaf stage were transplanted into 18-cm diameter pots (6 plants per pot) containing

potting soil. The two biotypes, VAC1 and VAC2, were grown in both the shade and full sun. Plants at 6- to 8-leaf stage were sprayed with diquat at different rates using the laboratory spray cabinet with conditions as described previously (see section 3. 2. 3). After spraying, plants were kept indoors overnight and transferred back to either the shade or to full sun the following morning. Plant survival and dry matter of above-ground parts were recorded 30 days after spraying.

***Hordeum* spp.**

Plants were germinated as described in section 3. 2. Seedlings were transplanted into 18-cm diameter pots containing potting soil with 12 plants per pot. To protect transplanted seedlings from initial desiccation in summer the pots were kept in the shade for 3 days after transplanting prior to being placed in to the shade or full sun treatment. At the 3-leaf stage both biotypes were sprayed with paraquat at different rates. Plant survival and dry matter production were recorded 30 days after spraying.

6. 2. 3. Effects of temperature on the level of resistance

As temperature could not be manipulated with outdoors grown plants the effect of temperature was evaluated under growth room conditions. Seeds of paraquat-resistant and -susceptible biotypes of *H. leporinum* (THL1 and THL4) were germinated as described previously (see section 3. 2). Seedlings (12 plants per pot) were transplanted into 14-cm square pots containing potting soil. Plants were grown and maintained in two growth rooms with a 12 h/ 12 h, 15°C/10°C or 30°C/25°C light/dark cycle with a light intensity of 500 $\mu E\ m^{-2}\ s^{-1}$ and 75% RH. Pots were repositioned every three days to minimise any effect of variation in light intensity within the growth room. Plants, at 3- to 4-leaf stage, were sprayed with paraquat at rates of 0, 25, 50, 100, and 200 g a.i. ha^{-1} with the susceptible biotype and of 0, 50, 100, 200, and 400 g a.i. ha^{-1} with the resistant biotype. Spraying conditions were the same as described previously. Plants were either

returned to the same growth room or swapped between temperatures. For the first 12 h after treatment plants were kept in the dark. Plant mortality and dry weight were recorded 16 days after spraying.

6. 2. 4. Photosynthetic measurements

Plants (THL1 and THL4) were grown in a growth room with a 14 h/10 h, 20°C/15°C, light/dark cycle and a light intensity of 300 μE m^{-2} s^{-1}. Plants were grown in 15-cm pots containing potting soil with 4 plants pot^{-1}. Plants used in experiments were 6 to 8 weeks old.

6. 2. 4. 1. Measurement of O_2 evolution

O_2 evolution was measured with a Hansatech leaf disc O_2 electrode at 25°C and 750 μE m^{-2} s^{-1}. Leaf segments, about 2 cm long, from the second youngest fully expanded leaf were cut under water and placed in an irrigated brass clip (to prevent desiccation) in the chamber. Leaf segments were incubated for 10 minutes at 750 μE m^{-2} s^{-1} at 25°, 38°, 39,°, 40°, 41°, 42°, 43°, and 44°C and then transferred into another chamber and allowed to equilibrate for 10 minutes at 25°C and 750 μE m^{-2} s^{-1} prior to measuring the photosynthetic activity. Photosynthetic activity was measured in an atmosphere of 20% O_2, 5% CO_2, and 75% N_2 to minimise stomatal contributions.

6. 2. 4. 2. Paraquat interaction with Photosystem I

Thylakoid isolation

Leaves, approximately 10 g, were ground in 50 mL of buffer containing 50 mM Hepes, pH 7.3, 500 mM sorbitol, 20 mM NaCl, 5 mM $MgCl_2$, 0.5 mM PMSF and 0.5% PVP-40 using Sorval Omnimixer for 20 s. The grindate was filtered through four layers of miracloth and centrifuged at 200 x *g* for 2 minutes. The supernatant was collected and

centrifuged at 10,000 x *g* for 10 minutes. The pellet from this spin was resuspended with a soft paint brush in 20 mL of 20 mM Hepes, pH 7.3 and 20 mM NaCl and centrifuged at 10,000 x *g* for 10 minutes. The final pellet was resuspended in 1 mL of 40 mM Hepes, pH 7.0, 400 mM Sucrose and 5 mM $MgCl_2$. Chlorophyll content was determined in 80% acetone as described by Arnon (1949).

Measurement of thylakoid O_2 consumption

Rates of oxygen consumption of isolated thylakoid membranes were measured using a Clark-type oxygen electrode in a continuously stirred reaction vessel at 15°C, 25°C and 35°C. Illumination was provided by a 250 W halogen quartz globe of a slide projector filtered through a 15-cm diameter water filter. The light intensity at the electrode chamber was 2000 $\mu E\ m^{-2}\ s^{-1}$. Photosystem I (PS I) activity of the isolated thylakoid membrane preparations was measured in 3 mL of 25 mM Hepes, pH 7.8, 400 mM sucrose, 5 mM $MgCl_2$, 50 µM DCIP, 1 mM Na ascorbate, 1 µM DCMU, and 1 mM NaN_3. Paraquat was added as the electron acceptor.

6. 2. 5. Effect of temperature on herbicide uptake and translocation

Seedlings were grown as described previously (section 3. 2) and transferred into 5-cm square pots containing sterilised sand at one plant per pot. The pots were placed in plastic trays (42 x 30 x 12 cm) containing 2 cm Hoagland's nutrient solution (Hoagland and Arnon 1938). Plants were maintained in a growth room with 15° ± 3°C, 12h/12h light and dark cycle with light intensity of 300 $\mu E\ m^{-2}\ s^{-1}$ and 70% RH. Plants (at two-leaf stage) were treated with ^{14}C-paraquat. Radiolabelled paraquat with a specific activity of 820 MBq. $mmole^{-1}$ was diluted with Gramoxone® and 0.2% non-ionic surfactant to a concentration of 3 mM paraquat (equivalent to the concentration of paraquat when sprayed at 100 g a.i. ha^{-1} paraquat) with a final radioactivity of 1.67 kBq. Ten µL of this solution was applied to the adaxial midsection (about 2 cm) of the first leaf. Treated plants were kept in the growth room in the dark at a constant temperature of either 15°C or 30°C.

Plants were harvested 24 or 48 h after herbicide application and were sectioned into five parts: a) treated zone of the first leaf b) tip of the treated leaf c) base of the treated leaf d) second leaf and e) roots. The unabsorbed ^{14}C was removed by washing the treated zone with 15 mL (3 x 5 mL) of wash solution consisting of 10% ethanol and 0.1% Triton X-100 in H_2O. The radioactivity in the rinse solution was quantified by Liquid Scintillation Spectrometry (LSS). The plant sections were oven dried at 40°C and radioactivity in each section of the plants was determined by combustion using a Model OX600 biological oxidizer (R. J. Harvey Instrument Corp. Hillsdale, N. J.) where ^{14}C was trapped in a 15 mL scintillation liquid (Carbon 14 cocktail, R. J. Harvey Instrument Corp. Hillsdale, N. J.). Radioactivity was quantified using LSS.

6. 2. 6. Photosynthetic activity of young leaf tissue from within the leaf sheath

Both the resistant and susceptible biotypes of *H. leporinum* (5 plants per pot) were grown in 18-cm diameter pots containing potting soil and placed in a growth room with 12 h at 500 $\mu E\ m^{-2}\ s^{-1}$ light intensity and a constant temperature of 15°C. At the two- to three-tiller stage the plants were sprayed with 200 g a.i. ha^{-1} paraquat in the spray laboratory with conditions as described previously. The treated plants were kept in the dark at either 4°C or 30°C for 24 hours. At this time, emerging leaf tissue still within the leaf sheath (not directly exposed to paraquat) was excised and photosynthetic activity of this young tissue of the resistant and susceptible biotypes was measured with a Hansatech leaf disc O_2 electrode, using the same protocols as described in section 6. 2. 4. 1. The objective of measuring photosynthesis in this leaf sheath tissue not directly exposed to paraquat was to investigate the effect of temperature on the translocation of paraquat to growing tissue.

6. 3. RESULTS

6. 3. 1. Seasonal effect on the level of resistance

The response of paraquat-resistant *H. leporinum* biotype (THL1) to paraquat application in summer and winter is shown in Figure 6. 1. The resistant biotype proved to be highly resistant to paraquat compared to the susceptible biotype when the plants were grown and sprayed during winter (Fig. 6. 1A), which is the normal growing season for this species. In contrast, plants of the THL1 biotype grown and sprayed during summer displayed a dramatically reduced level of resistance (Fig. 6. 1B). Application of paraquat at 800 g a.i. ha^{-1} caused no mortality in winter but was sufficient for 100% mortality of THL1 in summer. A small increase in the level of tolerance of the susceptible biotype to low rates of paraquat was also observed in plants sprayed in summer (Fig. 6. 1B).

A possible explanation for the greatly increased mortality evident in the resistant biotype treated under summer versus winter conditions is the much higher light levels prevailing in summer. However, summer conditions with high light intensity also involve high temperature and it is possible that higher temperature increased the effectiveness of paraquat. To examine whether high light intensity or high temperature was responsible for the dramatic decrease in resistance of THL1 in summer (Fig. 6. 1B) an experiment was set up in the field where these components could be manipulated. Plants were grown either in summer or winter, in full sun or in the shade as described. In winter the resistant biotype (THL1) is highly resistant to paraquat application (Figs. 6.1 A and 6. 2A). The same biotype grown in summer showed a marked reduction in the level of resistance regardless of whether plants were grown in the sun or the shadehouse (Fig. 6. 2C, D). In contrast the susceptible biotype (THL4) showed only a small increase in tolerance at low rates of paraquat in the summer (Fig. 6. 2C, D). Growing plants in the shade increased the effectiveness of paraquat on both biotypes in winter and on the resistant biotype in summer (Fig. 6. 2B, D). A similar response was also observed in the production of dry matter (not shown).

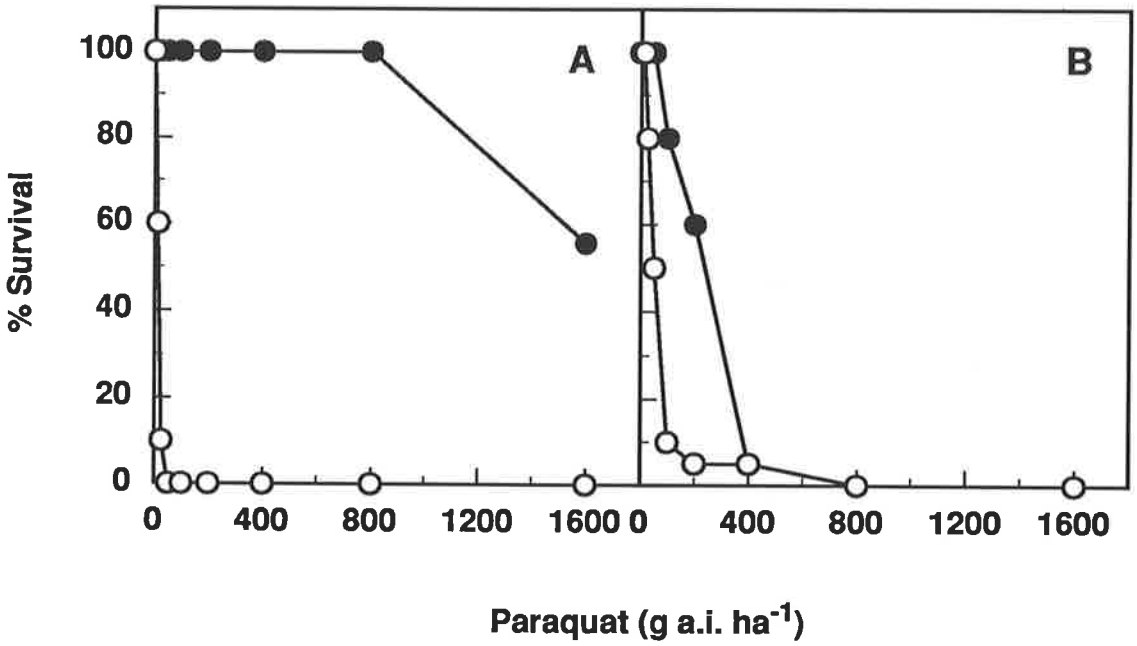


Figure 6. 1. Response of THL1 (●) and THL4 (○) biotypes of *H. leporinum* to paraquat application in winter (A) and summer (B).

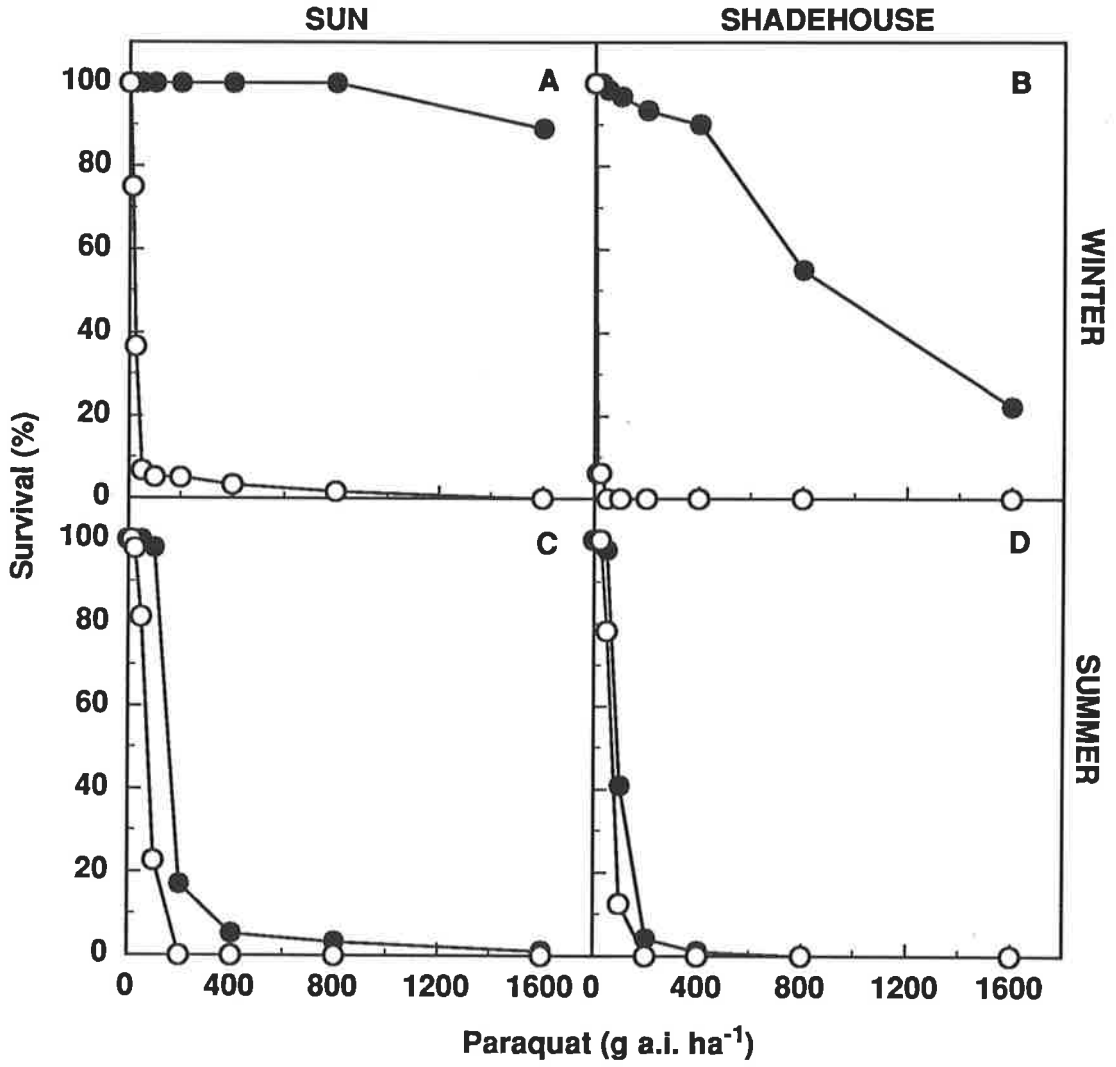


Figure 6. 2. Seasonal and light intensity effects on the survival of THL1 (●) and THL*A* (O) biotypes of *H. leporinum* sprayed with different rates of paraquat in winter (A, B) and summer (C, D). Plants were grown either in the sun (A, C) or shadehouse (B, D).

It is clear from Fig. 6. 2 that light intensity is not responsible for the dramatically reduced level of resistance of biotype THL1 in summer. Rather the comparison of Fig. 6. 2A versus Fig. 6. 2C strongly indicates that temperature is the influencing factor.

The seasonal effects on the level of resistance to diquat in THL1 are shown in Fig. 6. 3. Unlike paraquat, diquat did not show differential effectiveness on the resistant biotype between treatment in winter or summer (Fig. 6. 3A, C). In both seasons THL1 showed a similar response to diquat application. However the susceptible biotype (THL4) showed a small increase of tolerance in summer compared to winter (Fig. 6. 3A, C). Plants grown in the shadehouse versus full sun showed increased effectiveness of diquat in winter but not in summer. These results indicated that application of diquat to THL1 and THL4 is not greatly affected by temperature and light intensity. In addition, it appears that light intensity has little effect on the level of resistance of THL1 to diquat (Fig. 6. 3A, C, D) except at low light intensities (shadehouse in winter) where there is increased effectiveness of diquat (Fig. 6. 3B). A similar response was also observed in the production of dry matter (not shown).

A similar experiment was conducted for paraquat-resistant (SHG1) and susceptible (SHG2) biotypes of *H. glaucum* to determine whether the reduction in paraquat resistance at high temperature evident in *H. leporinum* also occurs in this *H. glaucum* biotype. The resistant biotype (SHG1) showed high resistance to paraquat in winter both in full sun and in the shade (Fig. 6. 4A, B) where no mortality of the resistant biotype was observed even at 1600 g a.i. ha^{-1} paraquat. As for *H. leporinum* there was a dramatic reduction of the level of resistance in the resistant biotype (SHG1) in summer in full sun (Fig. 6. 4C). This dramatic decrease of resistance in summer was also observed in the shade (Fig. 6. 4D) where only 40% of plants survived at 50 g a.i. ha^{-1} paraquat. The susceptible biotype (SHG2) was highly susceptible to paraquat in either season and, unlike THL4, did not show any increased tolerance to paraquat in summer (Fig. 6. 4). Resistant plants treated with paraquat in summer also showed decreased dry matter production compared with

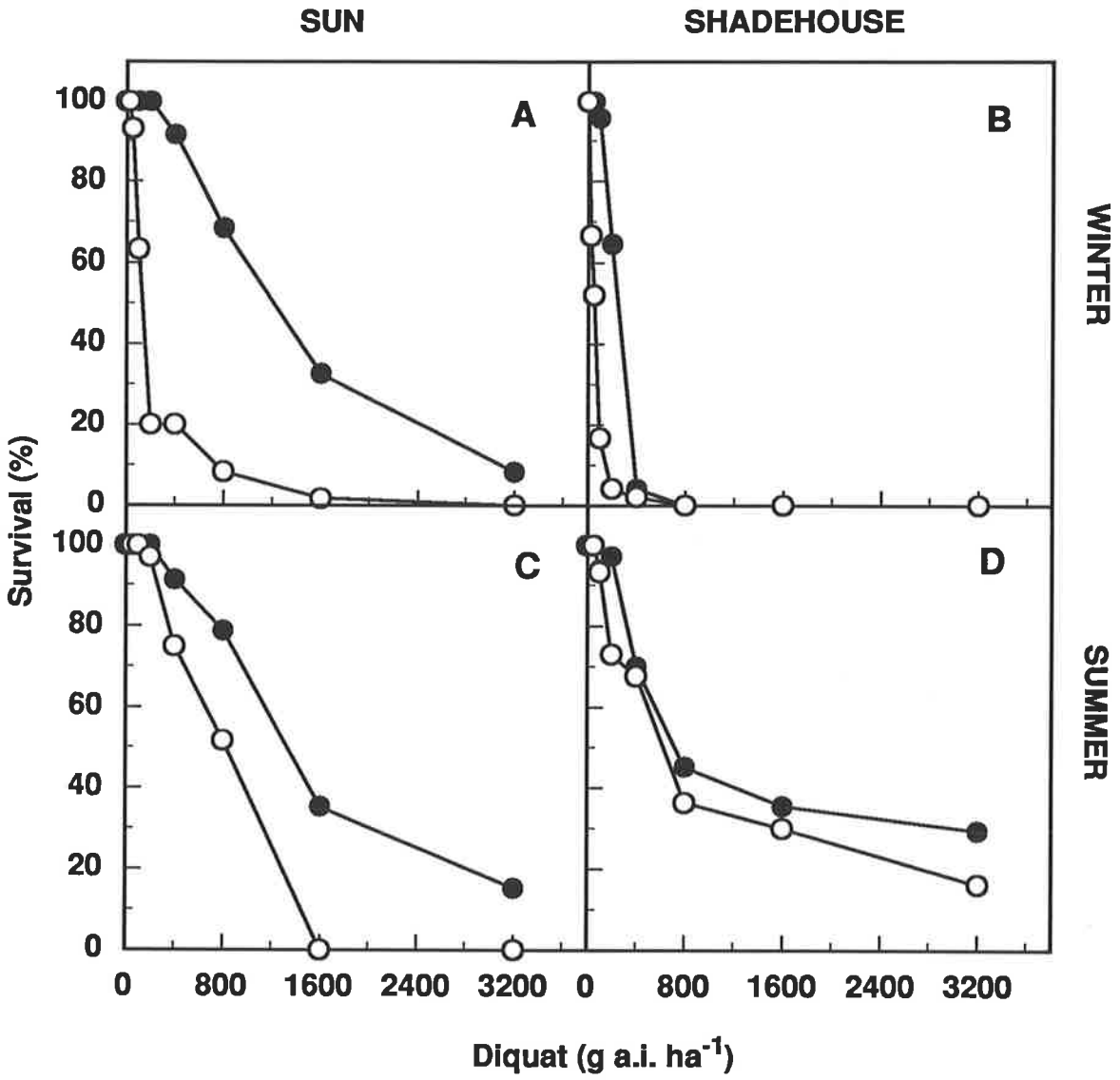


Figure 6. 3. Seasonal and light intensity effects on the survival of THL1(●) and THL4 (O) biotypes of *H. leporinum* sprayed with different rates of diquat in winter (A, B) and summer (C, D). Plants were grown either in the sun (A, C) or shadehouse (B, D).

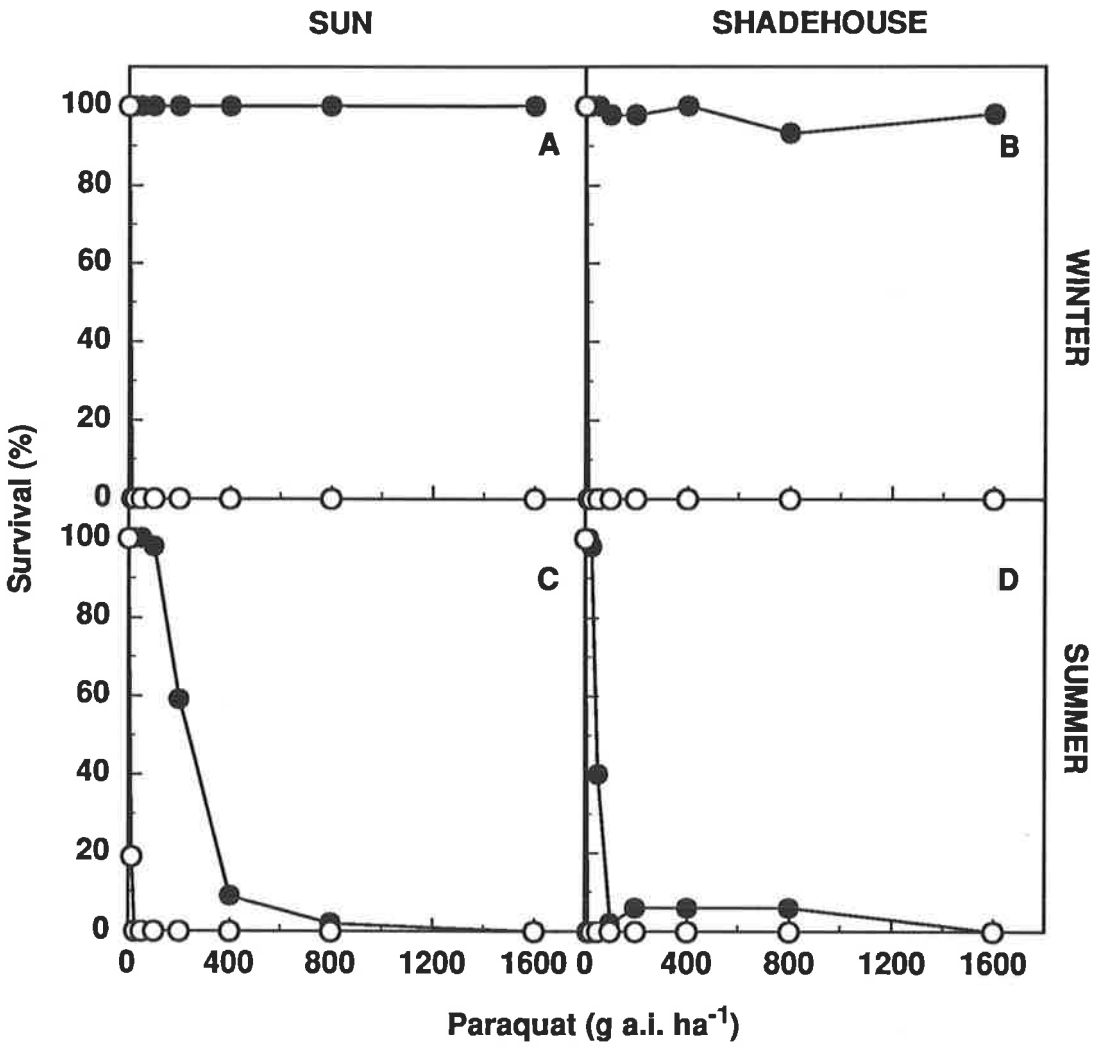


Figure 6. 4. Seasonal and light intensity effects on the survival of SHG1(●) and SHG2 (O) biotypes of *H. glaucum* sprayed with different rates of paraquat in winter (A, B) and summer (C, D). Plants were grown either in the sun (A, C) or shadehouse (B, D).

plants treated in winter (not shown). The decrease in resistance of SHG1 in summer is also probably a result of high temperatures and not increased light intensity.

In order to determine whether the reduction in resistance at high temperatures is specific to the resistant *Hordeum* biotypes or is a more general property of paraquat and diquat resistant weed biotypes a similar experiment was also conducted on a diquat-resistant biotype of *A. calendula* (VAC1). The resistant biotype showed 53% survival at 400 g a.i. ha^{-1} diquat in winter in full sun (Fig. 6. 5A), but showed 100% survival at this herbicide rate in summer in full sun (Fig. 6. 5C). The susceptible *A. calendula* biotype, VAC2, also showed a small increase in the tolerance to diquat in summer (Fig. 6. 5C) compared to winter (Fig. 6. 5A). The biggest effect in this experiment was the increase in effectiveness of diquat on both biotypes in the shade compared to those in full sun. In winter following application 400 g a.i. ha^{-1} of diquat, 53% of VAC1 survived in the sun; however, only 15% of this biotype survived this rate of herbicide in the shadehouse (Fig. 6. 5B). Likewise in summer this rate of herbicide application resulted in no mortality of sun grown plants compared to 74% of shadehouse grown plants (Fig. 6. 5A, B). An increase in effectiveness of diquat in the shadehouse could also be observed with susceptible VAC2 in both summer (Fig. 6. 5D) and winter (Fig. 6. 5B). In conclusion, unlike paraquat resistance in *Hordeum leporinum* and *H. glaucum*, the level of diquat resistance in VAC1 is not substantially affected by temperature. The level of resistance in summer is only slightly increased compared to that in winter.

It was observed that even with *H. leporinum* there was no influence of temperature on the level of resistance to diquat (Fig. 6. 3). An experiment was therefore conducted on VAC1 and VAC2 in summer and winter to determine if there are temperature influences on paraquat resistance in these biotypes as was the case for THL1 and SHG1. No difference of mortality in winter and summer was observed in VAC1 (Fig. 6. 6). Although the susceptible biotype, VAC2, showed slightly increased tolerance to paraquat treatment in summer. This result confirms that resistance to paraquat or diquat in VAC1 is not influenced by temperature.

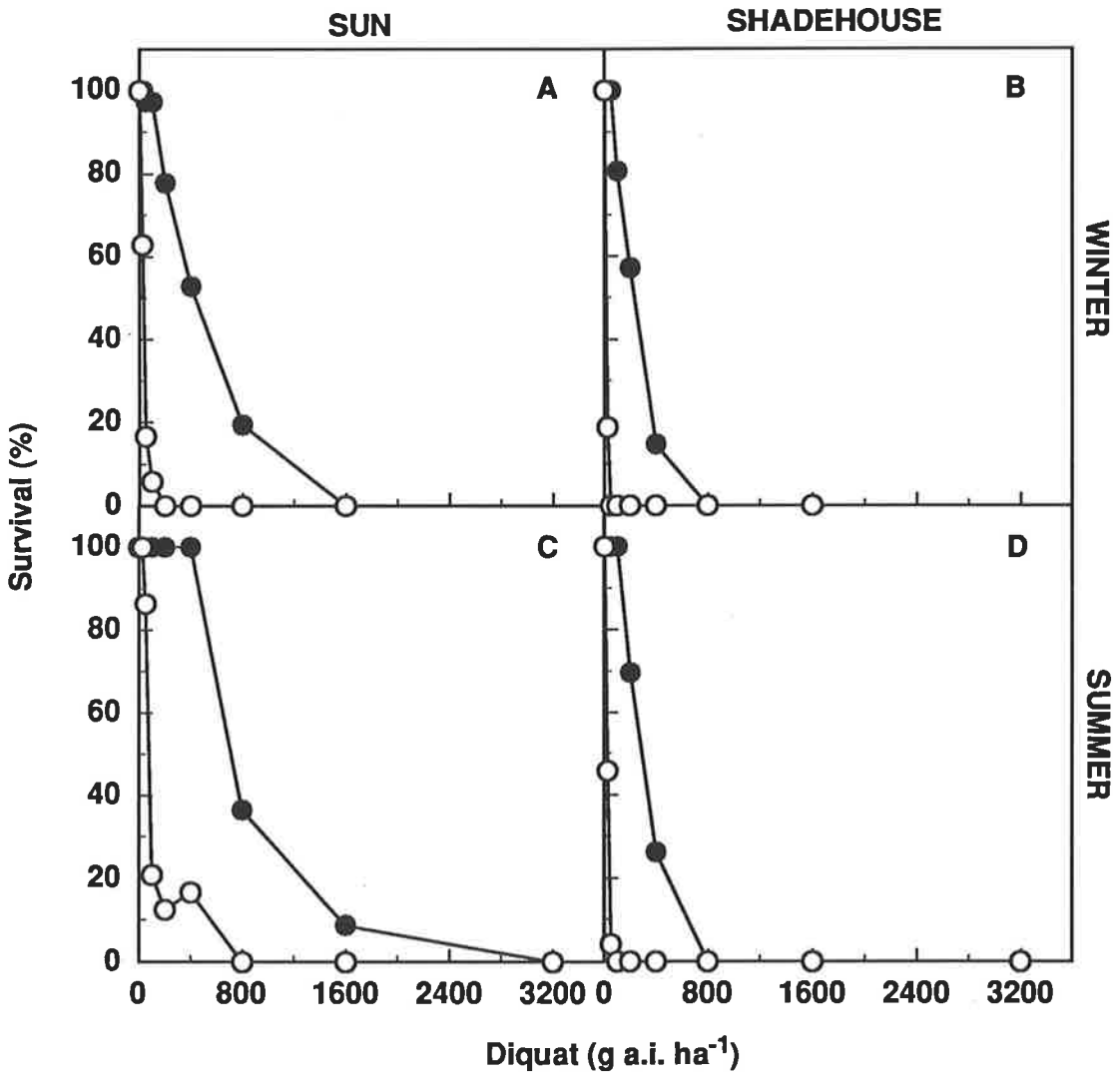


Figure 6. 5. Seasonal effects on the survival of VAC1 (●) and VAC2 (O) biotypes of *A. calendula* following diquat application in winter (A, B) or summer (C, D). Plants were grown either in the sun (A, C) or shadehouse (B, D).

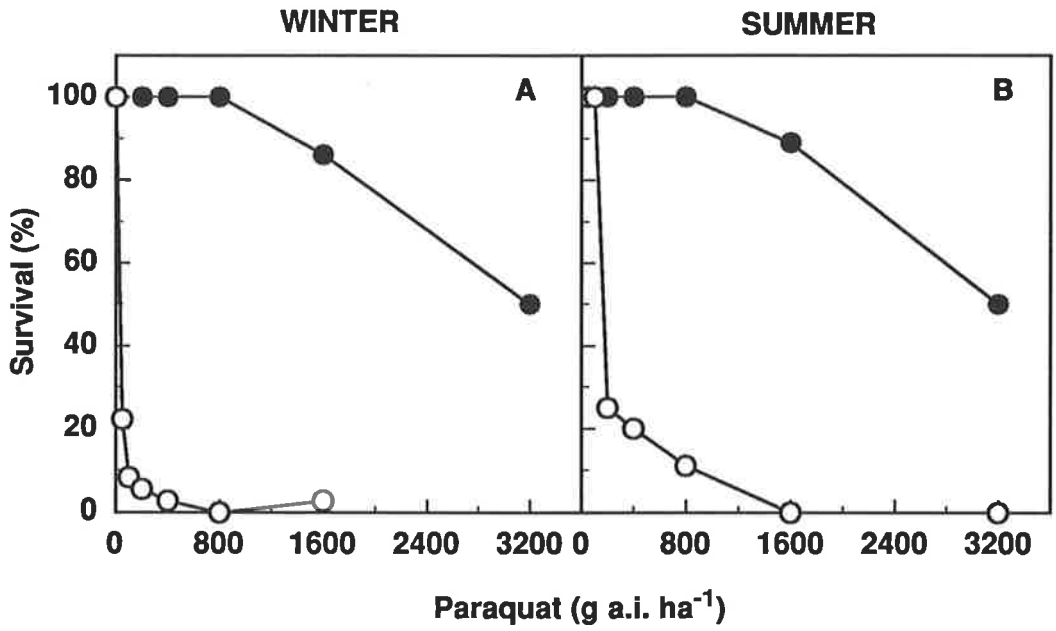


Figure 6. 6. Response of VAC1 (●) and VAC2 (○) biotypes of *A. calendula* to paraquat application in winter (A) and summer (B).

To prove that the decreased level of resistance of biotype THL1 in summer compared to winter is due to temperature, an experiment was conducted in growth rooms with different temperatures but with other environmental factors similar. In these experiments plants were grown at either 15°C or 30°C. Plants that were grown at 15°C, sprayed with paraquat and then transferred to 30°C after treatment were much less resistant than plants kept at 15°C before and after treatment (Fig. 6. 7). Treatment of THL1 plants with 100 g a.i. ha^{-1} paraquat and subsequent transfer to 30°C resulted in 98% mortality compared to only 12% mortality if plants were maintained at 15°C after treatment. This experiment also demonstrated that the degree of resistance is due to the temperature after paraquat application as the THL1 plants grown either at 30°C or 15°C and transferred to 15°C after treatment were much more resistant than those plants transferred to 30°C (Figs. 6. 7 A and 6. 8 A). However, a small increase of resistance was observed in plants grown at 30°C prior to paraquat application (Fig 6. 8) compared to those grown at 15°C (Fig. 6. 7). A small increase in tolerance of the susceptible biotype to paraquat was also seen in plants transferred to 30°C compared to those kept at 15°C.

The results of growth room studies together with the outdoors study provide compelling evidence that high temperature strongly reduces the resistance level of THL1 to paraquat. The level of resistance to paraquat is markedly reduced if high temperatures prevail after paraquat treatment in the resistant biotypes of *H. leporinum* and *H. glaucum*. Little effect of temperature was observed on the response of resistant biotype of *A. calendula* to diquat or paraquat. The effects of high temperature are markedly different between the three resistant biotypes and the two herbicides, therefore, this phenomenon is probably not a general property of paraquat and diquat resistant plants, but may be biotype specific, perhaps based on the specific resistance mechanisms involved.

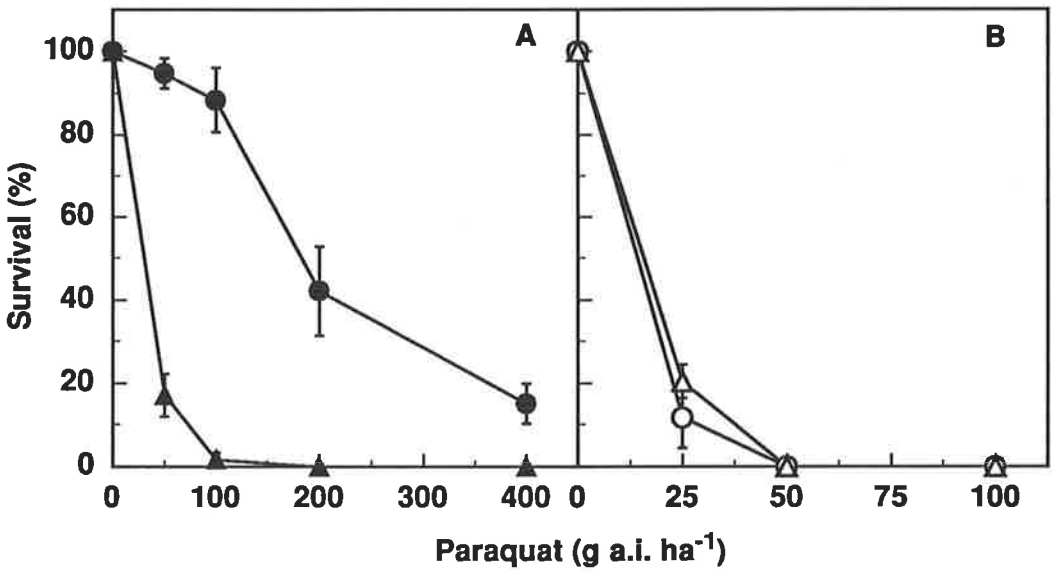


Figure 6. 7. Response of THL1 (A) and THL4 (B) biotypes of *H. leporinum* to paraquat. Plants were grown at 15°C, and then maintained at 15°C (●, O) or transferred to 30°C (▲, Δ) after paraquat treatment. Vertical bars are standard errors of 5 replications of two experiments.

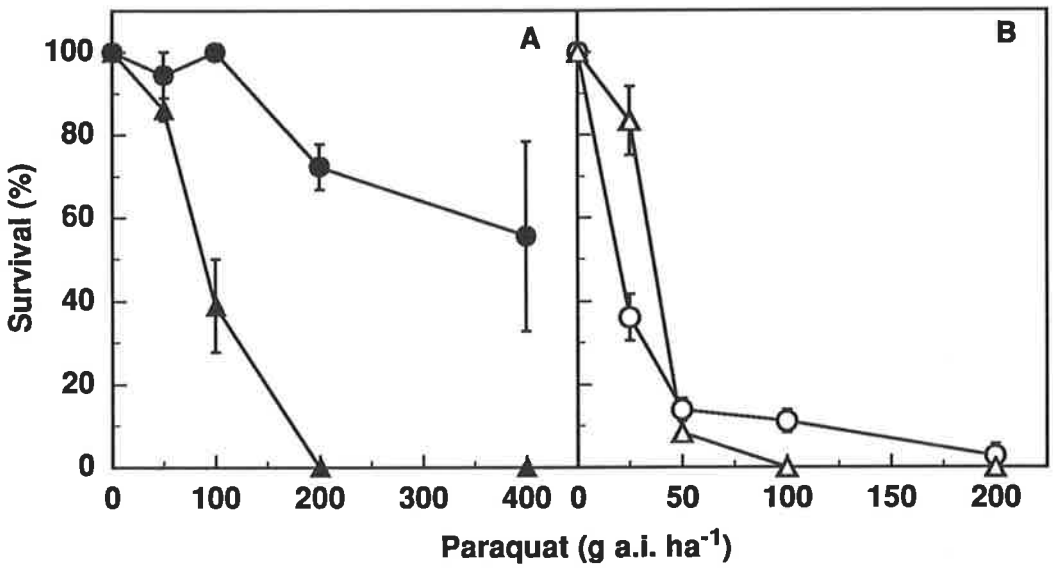


Figure 6. 8. Response of THL1 (A) and THL4 (B) biotypes of *H. leporinum* to paraquat. Plants were grown at 30°C, and then maintained at 30°C (▲, Δ) or transferred to 15°C (●, O) after paraquat treatment. Vertical bars are standard errors of 3 replications of one experiment.

6. 3. 2. Effects of temperature on photosynthesis

The biochemical mechanism for the high-temperature induced decrease in the level of paraquat resistance was investigated in *H. leporinum* biotype THL1. Several studies have shown that triazine-resistant biotypes show increased sensitivity to high temperatures as a result of increased heat-induced disruption of photosynthetic activity (Ducruet and Lemoine, 1985; Donnelly and Hume, 1984). The response of photosynthetic activity of *H. leporinum* biotypes THL1 and THL4 to a short heat stress was examined. Detached leaves were exposed for 10 minutes to high temperature, allowed to re-equilibrate to 25°C and O_2 evolution was measured in a leaf disc O_2 electrode. The rate of O_2 evolution of both biotypes declined rapidly with heat stress at temperatures greater than 38°C but no difference in inhibition was observed between the two biotypes (Fig. 6. 9A). This experiment was repeated using plants grown outdoors in winter, which were subject to lower temperatures and light intensities and reduced photoperiod compared to the growth room plants. Photosynthetic activity of the outdoor-grown plants declined with temperature stress above 39°C and again no difference was observed between the two biotypes (Fig. 6. 9B). These results indicate that the decreased level of paraquat resistance induced by high temperature is not a result of increased heat sensitivity of the photosynthetic apparatus in the paraquat-resistant biotype.

6. 3. 3. Temperature effect on the reaction of paraquat with PS I

The possibility of changes in the action of paraquat at the active site at higher temperatures was also examined. PS I activity in isolated thylakoids of THL1 and THL4 biotypes was measured with paraquat as an electron acceptor. The interaction of paraquat with PS I was determined at 15°C, 25°C and 35°C by the rate of oxygen consumption as a function of paraquat concentration at these three temperatures. The thylakoid preparations had low levels of endogenous Mehler reaction and O_2 consumption increased with increasing paraquat concentration to a maximum at about 20 μM paraquat at all temperatures (Fig. 6. 10). Increasing the paraquat concentration to 100 μM did not further increase activity.

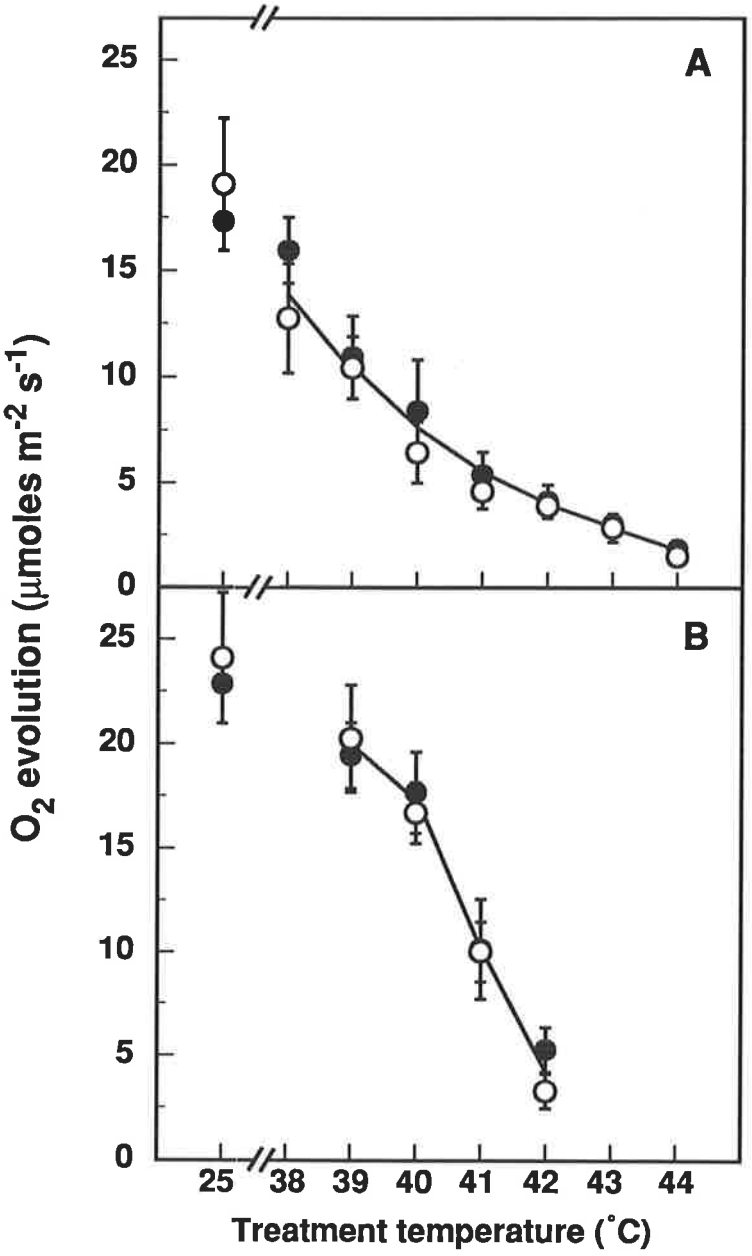


Figure 6. 9. Effect of a 10 minute pre-incubation at high temperature on the photosynthetic activity of leaf segments from THL1 (●) and THL4 (O) biotypes of *H. leporinum*. Plants were grown either in a growth room (A) or outdoors in winter (B). Following incubation of leaf segments at high temperature for 10 minutes the segments were allowed to re-equilibrate to 25°C prior to measuring O_2 evolution at 25°C. Points are means (± SE) of four determinations.

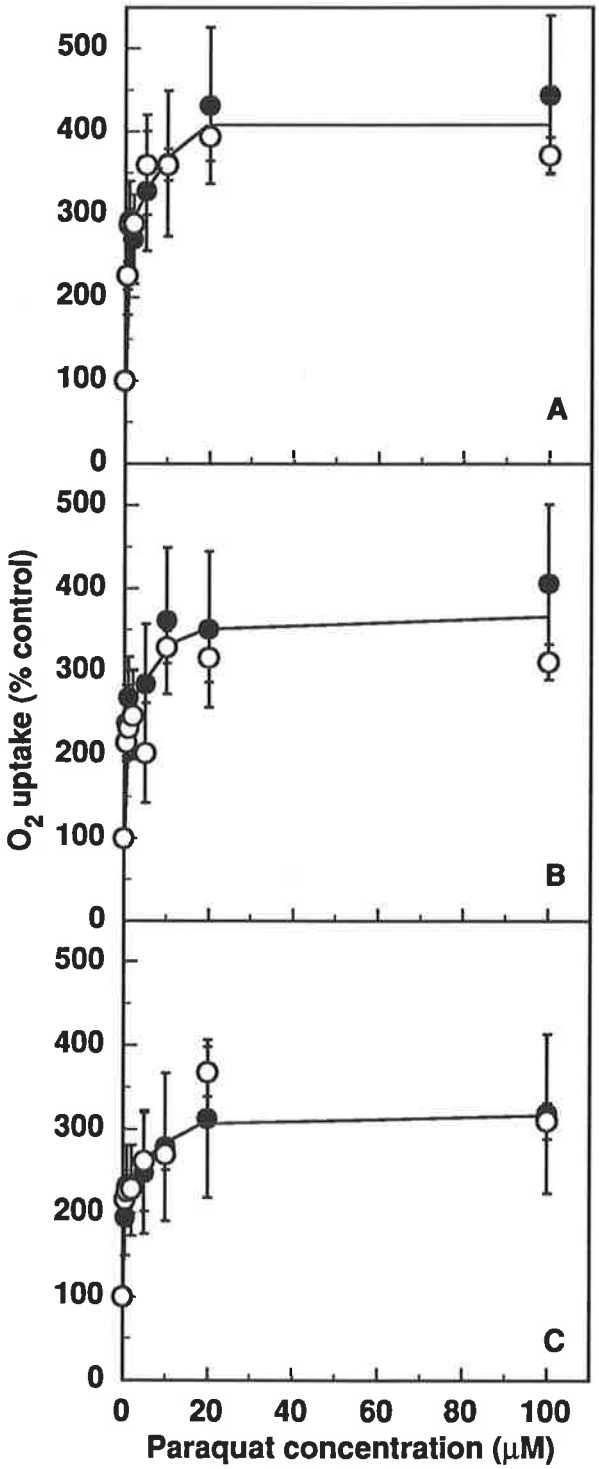


Figure 6. 10. Photosystem I activity of isolated thylakoid membrane preparations with paraquat as the electron acceptor at 15°C (A), 25°C (B) or 35°C (C). Thylakoid membranes were prepared from THL1 (●) or THL4 (O) biotypes of *H. leporinum*. Each point is the mean (± SE) of four experiments. 100% levels of activity, which represent the endogenous Mehler reaction, were THL1: 15°C=156.7; 25°C=157.5; 35°C= 196.2; THL4: 15°C=111.6; 25°C=145.4; 35°C=136.1 μmoles O_2 mg Chl^{-1} h^{-1}.

The maximum stimulation of O_2 consumption by paraquat decreased with increasing temperature from about 400% of control at 15°C (Fig. 6. 10A) to about 350% of control at 35°C (Fig. 6. 10C). No difference in the stimulation of the O_2 consumption by paraquat was observed between the two biotypes at any of the temperatures. These results show that the high temperature-induced decrease in paraquat resistance of THL1 is not due to a change in the interaction of paraquat at the active site. In addition, changes at the active site are not the mechanism of paraquat resistance in this biotype.

6. 3. 4. Effects of temperature on herbicide uptake

The uptake of paraquat by resistant and susceptible biotypes of *H. leporinum* at high and low temperatures was examined. The amount of ^{14}C-paraquat absorbed by the resistant biotype (THL1) and susceptible biotype (THL*A*) is affected by temperature (Table 6. 1). Greater than 95% of the applied herbicide was absorbed at 15°C in both biotypes. At 30°C the uptake of the herbicide in both biotypes was lower than at 15°C both 24 h and 48 h after treatment. This reduction in herbicide uptake may be a result of more rapid drying of the application solution; however, this was not examined. There were no differences in uptake between the resistant and susceptible biotypes at either 15° or 30°C.

Table 6. 1. Uptake of ^{14}C- paraquat in paraquat-resistant and -susceptible biotypes of *H. leporinum* 24 and 48 hours after treatment. Plants were grown at 15°C and placed at either 15°C or 30°C after treatment. Data is the mean of eight samples from two replications ± standard error (SE).

	^{14}C-paraquat uptake (%)			
Time after	15°C		30°C	
treatment	Resistant	Susceptible	Resistant	Susceptible
24 h	96 ± 1	95 ± 2	52 ± 8	65 ± 5
48 h	99 ± 0	98 ± 1	66 ± 6	54 ± 4

6. 3. 5. Effects of temperature on herbicide translocation

The effects of temperature on the translocation of ^{14}C-paraquat is shown in Table 6. 2. Paraquat is normally translocated in xylem tissue (Brian, 1969), and hence considerable amounts of herbicide were translocated to the leaf tips. No difference was observed in the distribution of ^{14}C-paraquat between the resistant and susceptible biotypes in the applied zone, tip and base treated leaf sections measured 24 h after application when the plants were maintained at temperatures of 15° or 30°C. Much smaller amounts of paraquat were translocated in a basipetal direction, suggesting a small component of phloem translocation. There was a small, but obvious, increase in paraquat translocation to the second leaf evident in the susceptible biotype at 15°C compared to the resistant biotype. This increased translocation to the second leaf of the susceptible biotype in plants maintained at 15°C was also evident 48 h after treatment with 1.8% of paraquat uptake translocated to the second leaf while in the resistant biotype only 1% of the herbicide was translocated (Table 6. 2). In contrast, when plants where maintained at 30°C after treatment, increased paraquat translocation to the second leaf was evident in the resistant biotype but not in the susceptible biotype. Similar amounts of paraquat were translocated to the second leaf of both biotypes at 30°C 48 h after treatment (Table 6. 2). The differential paraquat translocation to the second leaf at different temperatures between the two biotypes was more distinct 24 h after treatment than 48 h after treatment. These data suggest that paraquat translocation from the treated leaf is more rapid in the resistant biotype than in the susceptible biotype at 30°C although, 48 h after treatment the amount of translocated paraquat in the second leaf was not different between the two biotypes.

In an attempt to gauge the effect of paraquat translocation at 15°C and 30°C, an experiment was conducted where plants were sprayed with 200 g a.i. ha^{-1} paraquat and placed in the dark at either 4°C or 30°C. Twenty-four hours after treatment the young leaf tissue remaining within the leaf sheath which had not been exposed to herbicide was dissected out. Photosynthetic activity of this tissue was measured as CO_2-dependent O_2 evolution. Photosynthetic activity of the unexposed young leaf tissue of the susceptible biotype was

Table 6. 2. Translocation of ^{14}C-paraquat in the paraquat-resistant (THL1) and -susceptible (THL4) biotypes of *H. leporinum* 24 and 48 hours after treatment. Plants were grown at 15°C and placed at either 15°C or 30°C after treatment (10 µL of 3 mM paraquat on midsection of 2nd leaf). At harvest each plant was divided into applied zone, tip of treated leaf, base of treated leaf, second leaf and roots. The data are the mean of eight samples from two replications ± standard errors (SE).

Plant Section	Temperature following paraquat application			
	15°C		30°C	
	Resistant	Susceptible	Resistant	Susceptible
	^{14}C-Paraquat detected in plant section (% absorbed)			
24 hours after treatment				
Treated Zone	62 ± 4	63 ± 4	29 ± 3	35 ± 4
Tip of Treated Leaf	35 ± 4	32 ± 4	63 ± 4	60 ± 4
Base of Treated Leaf	1.92 ± 4	3.15 ± 1	5.3 ± 1.6	3 ± 1
Second Leaf	0.63 ± 0.1	0.96 ± 0.1	1.5 ± 0.4	0.9 ± 0.2
Root	0.56 ± 0	1.46 ± 0.2	0.8 ± 0.2	0.5 ± 0.1
48 hours after treatment				
Treated Zone	38 ± 1	41 ± 4	34 ± 5	31 ± 6
Tip of Treated Leaf	56 ± 1	51 ± 4	62 ± 5	65 ± 6
Base of Treated Leaf	2.35 ± 0.58	4.15 ± 1.1	2 ± 0.34	1.7 ± 0.3
Second Leaf	1.0 ± 0.2	1.8 ± 0.3	1.17 ± 0.3	1.15 ± 0.24
Root	0.8 ± 0.06	1.45 ± 0.2	0.5 ± 0.08	0.45 ± 0.02

completely inhibited 24 h after treatment where plants were maintained at 4°C; however, O_2 evolution of the young tissue of the resistant biotype was unaffected by paraquat treatment if the plants were kept at 4°C for the 24 h before measurement. In contrast, O_2 evolution of unexposed young leaf tissue of the resistant biotype was inhibited by 70% in

plants kept at 30°C for 24 h after treatment (Table 6. 3). Photosynthetic activity in the susceptible biotype at this temperature was inhibited by 90%. This experiment shows that in plants maintained at 4°C after paraquat application there is little paraquat appearing at the active site in young leaf tissue of the resistant biotype because photosynthesis in this tissue is unaffected, whereas at 30°C there is substantial inhibition of photosynthesis as a result of paraquat at the active site.

Table 6. 3. Oxygen evolution activity of young leaf tissue from within the leaf sheath of paraquat-resistant and susceptible biotypes of *H. leporinum* following paraquat treatment measured at 25°C and 750 μE m^{-2} s^{-1}. Plants were grown at 15°C, sprayed with 200 g a.i. ha^{-1} paraquat and then kept in the dark at 4°C or 30°C for 24 hours. Unexposed young leaf tissue from leaf sheath was dissected out for measurements of photosynthetic activity. The data are the mean of three experiments ± standard errors (SE) except for the susceptible biotype control at 30°C which is one measurement.

	O_2 evolution (μmoles (mg chl)$^{-1}$ h^{-1}			
	4°C		30°C	
Biotype	Untreated Control	Paraquat 200 g a.i.. ha^{-1}	Untreated Control	Paraquat 200 g a.i. ha^{-1}
Resistant	318 ± 35	298± 18	453 ± 127	135 ± 38
Susceptible	188 ± 25	0	277	27 ± 27

6. 4. DISCUSSION

Changes in environmental conditions are well known to influence the activity of bipyridyl herbicides by influencing uptake, movement and activity of diquat and paraquat (Thrower et al. 1965). Light is clearly the most important of these factors and Brian (1967b) showed that plants kept in darkness for a period after application of diquat were rapidly

killed once illuminated, whereas these that were kept in the light showed only localised damaged. Brian and Ward (1967) found that more herbicide was taken up at low light intensities than at high light intensities. It was concluded that increased uptake of these herbicides occurs in the dark or low light whereas strong sun light immediately after treatment results in rapid tissue damage which limits herbicide movement (Slade and Bell, 1966). Humidity also influences herbicide uptake with increased paraquat and diquat uptake under humid conditions (Brian, 1966). Conditions of high humidity and low soil moisture readily promote herbicidal movement (Brian and Headford, 1968; Brian and Ward, 1967). Thrower et al. (1965) showed that high temperature and low humidity decreased diquat transport by desiccating treated leaves.

The effects of temperature on paraquat and diquat activity have not been extensively studied and has never been studied for paraquat-resistant plants. In the studies reported in this chapter a dramatic reduction in the level of resistance in the paraquat-resistant biotype of *H. leporinum* (THL1) was observed in summer compared to winter (Fig. 6. 1). A dramatically decreased level of resistance in summer compared to winter was also observed in the paraquat-resistant biotype of the closely related species *H. glaucum* (SHG1) (Fig. 6. 4) but not in the dicot species *A. calendula.* This differential response to paraquat treatment was shown to be the result of the high temperatures of summer, as reducing light intensity by placing plants in the shade did not increase the level of resistance but decreased it (Figs. 6. 2 and 6. 4). This is consistent with the increased effectiveness of paraquat at low light intensities reported elsewhere. Growth room experiments clearly demonstrated that the temperature following paraquat treatment had the most influence on paraquat efficacy in the resistant biotype as the resistant biotype was much less resistant at 30°C after spraying than at 15°C (Figs. 6. 7 and 6. 8). Growing plants at 30°C before herbicide application slightly increased the level of tolerance of both resistant and susceptible biotypes (Fig. 6. 8) compared to the same biotype grown at 15°C (Fig. 6. 7). This was probably due to morphological modifications that influence paraquat performance. The susceptible biotype of *H. leporinum* (THL4) was also affected by temperature and showed some increased tolerance to paraquat in summer.

No differential response of the resistant (THL1) and susceptible (THL*A*) biotypes to diquat was observed in winter and summer. In both seasons about 70% of the resistant biotypes survived 800 g a.i. ha^{-1} diquat application (Fig 6. 3). These results are different from those observed with paraquat application in winter and summer, and may be due to the different chemical structures of the herbicides, or to differences in uptake or translocation characteristics. Similar experiments conducted on a diquat-resistant biotype of *A. calendula* (Fig. 6. 5) showed a small increase in the level of resistance to diquat in summer. This increase was possibly due to high light intensities and not high temperature. No difference of response in the two seasons was also observed to paraquat treatment (Fig. 6. 6) The variability in the response of the paraquat and diquat resistant biotypes to high temperature suggests that decreased levels of resistance in summer is not likely to be a general property of all paraquat and diquat resistant plants and is probably directly related to the resistant mechanism operating in *H. glaucum* and *H. leporinum.*

All biotypes showed an increased effectiveness of herbicide when maintained in the shade compared to full sun after treatment. Increased light intensities are known to decrease herbicidal activity (Brian and Ward, 1967); however, as the plants were kept in the dark for 16 h after treatment this point might be of minor importance. The shade maintained plants were also exposed to more humid conditions which promote herbicidal activity (Brian and Headford, 1968). However as the plants in each season received similar conditions for the first 16 h after treatment, the increased humidity may also not be relevant. Anatomical and ultrastructural changes, such as decreased cuticle thickness, which occur in shade grown plants (Hathway 1986; Kirkwood 1987) may play a more important role as a thinner cuticle will allow more herbicide to penetrate. Another possibility is physiological changes as PS I is less efficient in the plants grown under high light than shade plants (Boardman, 1977).

Several studies on triazine-resistant weeds have observed that triazine-resistant biotypes are more susceptible to heat stress compared to the susceptible biotypes (Ricroch et al.

1987; Ducruet and Lemoine, 1985; Ducruet and Ort, 1988); however Vencill et al. (1987) were unable to demonstrate this. The increased heat stress in the triazine-resistant biotypes was due to decreased stability of the photosynthetic apparatus at high temperatures as a result of the decreased electron transfer from Q_A to Q_B in these plants caused by the mutation in the Q_B binding site. Heat tolerance of the photosynthetic apparatus of the paraquat-resistant and -susceptible biotypes of *H. leporinum* was examined here and found to be the same in both resistant and susceptible biotypes, both in plants grown in the growth room and plants grown outside in winter (Fig. 6. 9). In addition no temperature related differences in the action of paraquat with the active site, PS I, were observed between the two biotypes (Fig. 6. 10). These experiments also demonstrated that the mechanism of paraquat resistance in this biotype is not due to changes at the active site.

Uptake of paraquat into the treated leaf is not different between the resistant and susceptible biotypes at low or high temperatures. However, increased paraquat uptake occurs in both biotypes at lower temperature. This could explain why the susceptible biotype is slightly more tolerant to paraquat at high temperature compared to lower temperatures. These results agree with Caseley (1970) who found that low rates of paraquat were effective against *Agropyron repens* only at 6°C whereas at 16° and 26°C the plants regrew. This was in contrast to other herbicides that had a high effective kill at higher temperatures. This phenomenon is still not understood as normally uptake increases greatly at higher temperatures (Kirkwood, 1987). However, clearly the lower rates of leaf uptake at higher temperature do not explain the dramatically reduced resistance level at higher temperature compared to low temperature in the resistant biotype. These experiments also show that decreased herbicide uptake is not the mechanism of paraquat resistance in this biotype.

There was more ^{14}C- paraquat translocation to the second leaf of the susceptible biotype when maintained at 15°C for 24 h after treatment than for the resistant biotype. At 30°C translocation to the second leaf of the resistant biotype was slightly higher than for the

susceptible biotype. (Table 6. 2). This increased translocation of paraquat to young tissue at high temperature may be responsible for the marked reduction of resistance level in the resistant biotype. Increased translocation of paraquat to young tissue of the resistant biotype at high temperature was shown to affect the photosynthetic activity of young leaf tissue not directly exposed to herbicide (Table 6. 3). Inhibition of photosynthesis in this tissue is due to presence of the herbicide translocated from other parts of the plant which had been directly exposed to paraquat treatment. There was no difference in photosynthetic activity of the young tissue between the untreated and treated resistant plants maintained at 4°C after treatment. In contrast photosynthetic activity in the treated resistant plant was reduced by 70% compared to the control at 30°C. Photosynthesis in the susceptible plants was markedly reduced at both low and high temperatures although some activity was observed at 30°C (Table 6. 3). The results clearly indicate that not only is more paraquat translocated to young tissue at high temperature but more of the herbicide is reaching the active site and inhibiting photosynthesis. Reduced translocation of paraquat could explain paraquat resistance in this biotype; however decreased penetration of paraquat to the active site may also be involved. Clearly the herbicide that is being translocated to the young tissue at low temperature is not reaching the active site as photosynthesis in the young tissue of the resistant biotype is not inhibited, whereas photosynthesis of young tissue in the susceptible biotype is. At high temperature photosynthesis is inhibited in both biotypes. This suggests that in addition to increased paraquat translocation at 30°C, there may also be increased tissue sensitivity to paraquat, such that more herbicide reaches the active site, in the resistant biotype.

In order to kill a plant, there has to be translocation of paraquat from the exposed leaves to young leaf tissue not directly exposed to herbicide. Reduced translocation of paraquat has been shown to be a mechanism of resistance in biotypes of *H. glaucum* (Bishop et al. 1987), *H. leporinum* (Preston et al. 1992), *A. calendula* (Preston, pers. communication), *E. canadensis* (Tanaka et al. 1986), and *C. bonariensis* (Fuerst et al. 1985). In the case of *H. glaucum* (Preston et al. 1992) and *A. calendula* (Preston, pers. communication) decreased movement of herbicide into the cells of the resistant biotype has been

demonstrated. The differential resistance level in the resistant biotypes in winter and summer documented in this chapter is probably due to differences in the amount of herbicide translocated to the young tissue in the two biotypes in response to temperature, but may also be influenced by differences in tissue sensitivity to paraquat as seen for *H. glaucum* (Preston et al. 1992) and *A. calendula* (Preston, pers. communication). The difference of the amount of herbicide translocated to the young tissue (second leaf) between the two biotypes is small (Table 6. 2); however, it is sufficient to kill the susceptible biotype but not the resistant biotype. Clearly, small increases in herbicide translocation in the resistant biotype can have dramatic effects on the level of resistance observed.

CHAPTER 7

GENERAL DISCUSSION

Herbicide-resistant weed populations normally appear following the repeated use of herbicides with the same mode of action. These herbicides select for resistant individuals in the population and these individuals go on to produce the most progeny for later generations. The length of herbicide use until herbicide resistance becomes noticeable varies with herbicide and weed species. Paraquat and diquat resistance have generally appeared after repeated uses of these herbicides several times a year for 5 to 10 years or with annual use for 12 to 24 years (Chapter 3). Resistance to other herbicides is, with a few exceptions such as in the cases of cross-resistance, also a result of repeated use of the same chemicals. For example, resistance to the triazine herbicides was reported after 5 to 10 years of use (Gressel et al. 1982), to diclofop-methyl after 3 to 4 years of use (Heap and Knight, 1982) and to sulfonylureas after 3 to 7 years of use (Mallory-Smith et al. 1990); Primiani et al. 1990). Therefore, although there is considerable variation in the time taken for herbicide resistance to develop, it is obvious that resistance to paraquat and diquat takes considerably longer to appear than does resistance to some other chemical groups. The rate at which resistance appears is not only related to the chemical group but will also vary between species. For example resistance to diclofop-methyl in *L. rigidum* occurs much more readily than in *A. fatua* and *A. sterilis* (Mansooji et al. 1992). The nature of the breeding system (selfing versus outcrossing) and of the seed bank (short versus long) will influence the speed at which resistance develops. Here I would like to address the point of why it takes so long for resistance to paraquat and diquat to develop.

There are many factors which can influence the rate of development of resistance, some of which have already been alluded to. In this thesis I have examined some of these factors, such as: length of herbicide use, breeding system and inheritance of resistance, resistance

frequency in a natural population, dormancy, growth and maturity and environmental influences on resistance.

In Australia, paraquat- and diquat-resistant weed species have appeared in a number of lucerne fields which have been treated once annually with these herbicides for a long period of time (12 to 24 years). In total there are 11 biotypes of four weed species with confirmed resistance to paraquat and diquat. In these fields paraquat and diquat have been the major form of weed control used. It was evident in this study that the history of paraquat and diquat application is not correlated to the level of resistance in barley grass (Chapter 3). For example, a biotype of resistant *H. leporinum* that had been treated with the herbicides for 12 years was more resistant than a biotype that had been treated for 24 years. Thus length of time of paraquat and diquat application does not affect the level of resistance but will affect the appearance of resistance. Resistance to these herbicides in Australia has not appeared unless the population had been treated annually with paraquat and diquat for more than 10 years.

The appearance of paraquat resistance is not only a result of a long history of use of the herbicides but other factors such as weed management, frequency of the resistance gene in a population, fitness of the resistant individuals, selection pressure and management of lucerne growing operations also contribute. For example, weed management in a lucerne field in Victoria had allowed a paraquat-resistant biotype of *V. bromoides* to become abundant where it had been undetected 3 years earlier. Continuous use of paraquat and diquat to control weeds in this field led to resistance in three species, *H. glaucum, H. leporinum* and *A. calendula* (Tucker and Powles 1988; Tucker and Powles 1991; Tucker et al. 1989). At that time *Vulpia* plants collected from this field did not exhibit resistance to these herbicides. The farmer switched to fluazifop-butyl and diuron to control paraquat-resistant barleygrasses and diquat-resistant capeweed for two years, during which time the number of *Vulpia* plants increased as they are not controlled by fluazifop-butyl and diuron. The absence of competition from *Hordeum* and *Arctotheca calendula* (controlled by fluazifop and diuron) led to an increase in the *Vulpia* population. When

paraquat was re-applied to control the *Vulpia*, some plants which were resistant to paraquat and diquat were able to survive. The resistant plants were probably present originally at a low frequency; however, the increase in *Vulpia* numbers allowed a concomitant increase in the numbers of the resistant biotype. Therefore the management of weed control in this field inadvertently allowed *Vulpia* to be noticeable as resistant to paraquat and diquat. This is the fourth species in this one lucerne field to develop resistance to paraquat and diquat.

In comparison to resistance to some other herbicides, resistance to paraquat and diquat has taken a considerable length of time to develop. In part this may be due to the nature of the herbicide itself. Paraquat reacts with PS I as a redox agent and probably does not bind to the active site. A change in the active site would be difficult to accomplish whilst still allowing photosynthetic electron transport to continue. Secondly, unlike many other herbicides, there are no known mechanisms for metabolism of paraquat in higher plants and therefore a mechanism of resistance involving metabolism of paraquat would require the development of a new catabolic enzyme or pathway. Changes at the active site and metabolism of the herbicide are, by far, the most common mechanisms of herbicide resistance (Holt et al. 1993). For this reason it might be expected that the number of resistant individuals in any population would be low. In an experiment to test this point no resistant individuals were detected in more than 6 million *Hordeum* plants sprayed (Table 5. 1), thus the resistance frequency in this population is less than 1×10^{-6}. In addition, modelling of the development of herbicide resistance using data from *Hordeum* spp. suggested that the initial resistant gene frequency lies between 1×10^{-11} to 1×10^{-23} and is therefore quite rare (Fig. 5. 1).

Another factor which affects the appearance of the resistant biotypes is seed longevity in the soil seedbank. The soil seedbank is a source of susceptible individuals which can dilute the resistant individuals. In lucerne fields, maturing seeds of weed species are regularly removed from the field with the first seasonal lucerne harvest and consequently the injection of seed into the seedbank is reduced. In general, a species with a persistent

seedbank that survives for many years will develop resistance more slowly than one with a smaller, readily-depleted seedbank. The seedbank of *H. glaucum* was shown to be depleted within three years if seed set could be totally stopped (Powles et al. 1992). In contrast *A. calendula* has a larger seedbank and the seeds survive longer in the soil (S. Powles, pers. comm.). This may, in part, contribute to the fact that the majority of paraquat-resistant biotypes in Australia are *H. glaucum* and *H. leporinum.*

Seed dormancy might also influence the development of resistance. Increased seed dormancy, by delaying emergence of weeds until after herbicide application, could potentially delay resistance by keeping susceptible individuals in the population. Conversely, early germination and emergence, resulting in large plants at the time of herbicide application, might also affect the development of resistance. As has been shown here (Fig. 3. 5) larger plants are less susceptible to paraquat. Seed dormancy of four weeks has been observed in paraquat-resistant and -susceptible biotypes of *H. glaucum* (Powles et al. 1992) with no difference between the biotypes. In paraquat-resistant (THL1) and -susceptible (THL4) biotypes of *H. leporinum* there is no innate dormancy (Table 4. 6). In these two biotypes, at least, seed dormancy does not appear to have been affected by the development of resistance.

The mode of inheritance of paraquat and diquat resistance will also affect the appearance of resistance. Paraquat resistance and diquat resistance in the resistant biotypes of *H. leporinum* and *A. calendula* have been shown to be controlled by single partially dominant genes (Chapter 5). Heterozygous individuals in these biotypes are fairly susceptible to bipyridyl herbicides and therefore the application of these herbicides will kill many of the heterozygous plants (Chapter 5). This will delay the appearance of herbicide resistance. Homozygous plants, on the other hand, are highly resistant to the herbicides which means that once a homozygous resistant individual has appeared in the population then the resistance will remain in the population. *H. leporinum* individuals homozygous for herbicide resistance rapidly build up as this species is self-pollinating. On the other hand, with *A. calendula*, an obligate outcrossing species, diquat-resistance genes might spread

rapidly, but the homozygous individuals will build up more slowly than for paraquat-resistant *H. leporinum* biotypes.

Temperature may also affect the appearance of paraquat resistance in barley grass. The level of resistance in the resistant biotype of *H. leporinum* and *H. glaucum* is much lower when treated with paraquat during warm conditions (Chapter 6). In the field, seeds of barleygrasses, *H. leporinum* and *H. glaucum*, have no or possess a very short innate dormancy which enables them to germinate soon after rain in autumn at which time the temperature may still be warm. If spraying of these plants occur when the temperature is still warm, many of the resistant individuals may be killed. In particular, paraquat application to a population with low level of resistance to paraquat may result in some control of this population and thereby delay the development of resistance.

The fitness of an individual in the different phases of a management system will affect the continued presence of a herbicide resistant population. By definition herbicide-resistant individuals are fitter under herbicide application and therefore paraquat-resistant individuals will proliferate so long as the selection pressure is maintained. It has generally been observed that herbicide resistance carries a fitness penalty; however, in the case of *H. leporinum* biotype (THL1) this is not so (Chapter 4). Therefore, when the herbicide selection pressure is lifted these plants will remain in the population. An active program of weed control needs to be implemented for control of such populations.

In conclusion the factors that are most responsible for the limited number of paraquat-diquat resistant weeds and for the long lag period for their appearance in response to herbicide application are the low frequency of resistant individuals, or a slow rate of mutation of the gene to the resistant form, in natural populations, the susceptibility of heterozygote resistant individuals to herbicide application, seed removal and the effects of environmental conditions at the time of spraying. The lack of correlation between years of paraquat and diquat use and the level of resistance to these herbicides in four populations

of *H. leporinum* (Chapter 3) suggests that a single resistance occurrence has taken place in each population.

All of the paraquat- and diquat-resistant weed biotypes studied here have appeared after a long period of use of these herbicides as the only form of weed control in lucerne. As there is no cross resistance to other herbicides, resistance to paraquat and diquat would not appear to be a major practical problem; however, the appearance of resistant biotypes of four annual weed species in the same field presents a serious problem as control methods using alternative herbicides are severely limited.

This study has highlighted the necessity for good weed management to avoid herbicide resistance. The weed management of agricultural operations should aim to delay the onset of resistance. At present paraquat and diquat herbicides are commonly used as a sole weed control method in lucerne. This should be changed by including the use of alternative herbicides with dissimilar modes of action and other methods of weed control. The choice of alternative herbicides needs to be carefully considered such that the alternative herbicides do not allow some weed species an advantage as has happened for *V. bromoides* (Chapter 3). Rotation of crops to allow non-chemical methods of weed control should also be considered. Improving farm hygiene to avoid transferring seeds of resistant plants to other fields needs to be implemented. Once a herbicide resistant biotype has appeared in a lucerne field new management options need to be implemented. The use of alternative herbicides as discussed above, of crop rotation and grazing animals should be implemented to control the resistant weeds.

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