The Dynamics Of Natural Selection For Herbicide Resistance In Grass Weeds

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by

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ABSTRACT

Peter Flemming Ulf-Hansen: **The Dynamics Of Natural Selection For Herbicide Resistance In Grass Weeds**

The presence and rate of evolution for herbicide resistance in natural populations of weeds depends on genetic variation for resistance, fitness differences associated with this variation and inheritance. This thesis examines natural selection in a species having evolved resistance to the herbicide chlorotoluron, *Alopecurus myosuroides* Huds., and a species which has been identified as a candidate for evolution of resistance to glyphosate herbicide, *Elymus repens* (L.) Gould.

In *E. repens*, populations were sampled from environments contrasted in past herbicide use. Population and clonal-level variation in glyphosate herbicide response was studied in nutrient solution experiments. Population differentiation was weak and clones accounted for about 14% of total variation. Populations with a history of herbicide use did not show clonal differentiation, whereas in herbicide-unexposed populations about 23% of variation was attributable to clones. Both population and clonal variation were more significant for growth and life-history characters, when measured on the same populations in a common-garden experiment. No evidence of past selection was found for herbicide tolerance or life-history characters.

Population variation in herbicide response was also demonstrated in *A. myosuroides*. Furthermore, a half-sib analysis showed that heritability of chlorotoluron response, in nutrient solution, was present (0.21-0.34). Resistant and susceptible populations did not differ in this genetic response.

Ecological factors important in spread of resistance were considered by comparisons of resistant and susceptible biotypes of *A. myosuroides* in different experiments. In a growth analysis of isolated plants, resistant plants produced relatively more tillers but there were no other major biotypic differences. Competition was examined in an experiment with independently varying densities of both biotypes and three herbicide treatments. In the control, the susceptible biotype was more fecund at low densities. Competitive ability was dependent on density: the resistant biotype was more competitive at high densities. As modelled by response surface analysis, this decided the outcome of competition over a number of generations: the resistant biotype caused extinction of the susceptible biotype. In herbicide treatments, positive density-dependent effects were shown on survival of the susceptible biotype.

Population dynamics of the *A. myosuroides* biotypes were studied, and selection intensity measured, in the field with herbicide treatments. Positive density-dependent survival of resistant plants was detected in control plots, whilst with herbicide the susceptible biotype showed negative density-dependent survival. Population growth patterns resulting from such processes showed a novel shape of reproduction curve in some herbicide treatments and contained both unstable and stable equilibrium points. Population trajectories will differ crucially depending on starting density and herbicide exposure.

The intensity of selection for resistance in the field was considerable in full-rate herbicide treatments, even considering contributions of all cohorts. Fecundity selection could be more acute than mortality selection. In control treatments, selection against the resistant biotype was negligible.

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TABLE OF CONTENTS

CHAPTER 1
GENERAL INTRODUCTION

1.1 Occurrence and Nature of Herbicide Resistance in Weeds

Predictions of the evolution of herbicide resistance were made 10-15 years before the event by Abel (in Harper, 1956) and Harper (1956) based on an understanding of (1) the presence of genetic variation in weed species, (2) the selection pressures of recurrent herbicide treatment and (3) the example of the evolution of resistance to fungicides and insecticides (e.g. Georghiou, 1972). Subsequent to the first report of herbicide resistance in *Senecio vulgaris* L. by Ryan (1970), the phenomenon has been reported widely. Rates of evolution of herbicide resistance have been rapid: in many cases resistance has evolved after less than 10 years of regular herbicide use (Bandeen & McLaren, 1976; Holliday & Putwain, 1977). The evolution of herbicide resistance provides a useful model of the evolution in general, because of the speed of evolutionary change (in relatively few plant generations). Additionally, the relationship between the trait and fitness is unambiguous, unlike so many biochemical traits (Endler, 1986; Venable, 1984), but in the same way as metal-tolerance (Bradshaw, 1984a). Herbicide resistance is also of interest because of its widespread occurrence and economic significance (1.3).

Evolution by natural selection requires genetic variation, fitness differences related to that variation and their inheritance. The demonstration of minor differences in sensitivity to herbicides amongst collections of weeds (Albrecht, 1947; Bucholtz, 1958; Santelmann & Meade, 1961) initially suggested that genetic variation for herbicide response existed in natural populations of weeds. The presence of genetic variation between individuals, within populations previously unexposed to herbicides

(Price, Hill & Allard, 1983; Thai, Jana & Naylor, 1985), provided more conclusive evidence that variation was available for selection. The degree of mortality caused by herbicide treatments is often over 90-95% (e.g. Clarke, 1987), which indicates that the fitness of a genotype persisting in such an environment will be high. Phenotypic selection is relatively easy to demonstrate in studies of herbicide resistance, although few studies (e.g. Putwain, Scott & Holliday, 1982) have measured more than percentage mortality in field conditions.

The pattern of appearance of resistance (see 1.2 for definition of resistance) to the triazine herbicides over time is shown in Fig. 1.1. The majority of cases of resistance was recorded 10-20 years ago after the widespread introduction and use of herbicides. By 1987 resistance to triazines had been reported in 13 monocotyledonous species and 37 dicotyledonous weed species (LeBaron, pers. comm.). Resistance to other herbicides varying widely in modes of action also had been recorded in 12 species by this date. A widespread distribution of cases occurs, with resistance currently recorded in 10 European countries, Canada, the U.S.A, Israel, Egypt, Japan, Australia and New Zealand.

Most reports of herbicide-resistance are to the triazine group of herbicides (81% of cases, defined as species-herbicide-U.S. State/Canadian Province/country combinations), which inhibit photosynthesis by interference with photosystem II (PSII) (LeBaron, 1984). The plastids of resistant plants fail to bind the triazine herbicide. This is controlled by a change in the chloroplast DNA coding for one protein found in the PSII complex (Hirschberg & McIntosh, 1983). Detoxification may also reduce herbicide concentrations at the binding site (Gressel, *et al.*, 1983; Andersen & Gronwald, 1987).

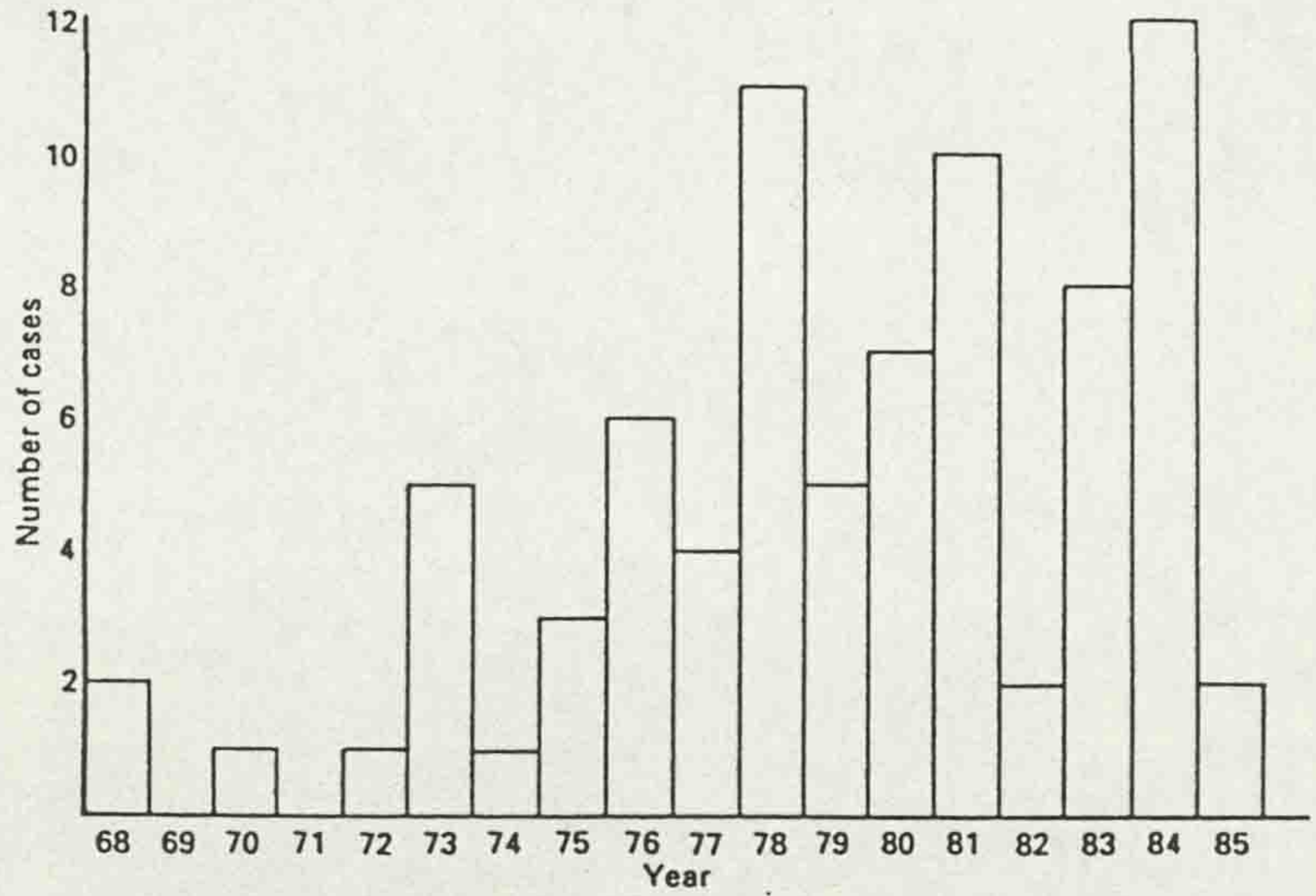

Figure 1.1 The number of new cases of herbicide-resistant biotypes occurring each year. A case represents a species/herbicide/country combination not previously recorded. (Source: LeBaron, pers. comm.).

More recently, resistance has been reported to other chemicals. Resistance to the oxidant-generating herbicide, paraquat, in *Hordeum glaucum* Steud. (Powles, 1986) and *Conyza bonarensis* (Gressel *et al.*, 1982) have been attributed to enhanced levels of oxygen-detoxifying enzymes (Schaaltiel & Gressel, 1986; Gressel, 1987). Fuerst *et al.* (1985) suggest that resistance may also be due to exclusion of paraquat from its site of action.

Resistance has been found in two grass species to the herbicides diclofop-methyl in *Lolium rigidum* Gaud. (Heap & Knight, 1986) and chlorotoluron in *A. myosuroides* Huds. (Moss & Cussans, 1985). Although the two herbicides have different modes of action, resistance may be due to greater monoxygenase activity enabling the herbicide to be rapidly degraded and detoxified (Gressel, 1987; Kemp & Casely, 1987).

Initially, most reports of resistance were to triazine herbicides in broad-leaved species. In the last five years the incidence of triazine-resistant grass species has increased and has been reported in e.g. *Brachypodium distachyon* Beauv. (Gressel *et al.*, 1983), *Phalaris paradoxa* L. (Rubin *et al.*, 1985) and *A. myosuroides* (Rubin *et al.*, 1985). Resistance in grasses has also been found to herbicides other than triazines. The paraquat-resistant *Hordeum glaucum* (Powles, 1986) and chlorotoluron-resistant *A. myosuroides* (Moss & Cussans, 1985) are two examples.

Cross-resistance often occurs between groups of chemicals that share the same mode of action, as reported for different herbicides in the triazine group (Radosevich, Steinbeck & Arntzen, 1979; Fuerst *et al.*, 1986). Recently, however, cross-resistance has been found for chemicals which have different modes of action. Pölös *et al.* (1987) have found a *Conyza canadensis* L. population resistant to both atrazine and paraquat, herbicides with distinctively different sites of action (see above). *L.*

rigidum resistant to diclofop-methyl was found to possess resistance to chlorsulfuron and metsulfuron (Heap & Knight, 1986; Heap, 1987).

1.2 Resistance Defined

The degree of resistance to triazines is usually absolute: resistant plants tolerate doses of over 50 times those that affect susceptible plants, when measured as whole plant injury or inhibition of photosynthetic electron transport (Fuerst *et al.*, 1986). Yet there is a continuum of degrees of resistance (Putwain & Collin, 1988). Resistance is usually defined as cases where plants do not respond to agricultural dose-rates of herbicide. However, in some cases plants show no or slight (< 10%) mortality, but biomass is affected by variable amounts. Compared to controls, about 20-30% reductions in shoot weight occur in *A. myosuroides* resistant to chlorotoluron (Moss & Cussans, 1987; Chapter 4) and *L. rigidum* resistant to diclofop-methyl (Heap & Knight, 1986), when exposed to agricultural rates of the herbicide. Such biotypes are variously defined as showing 'enhanced tolerance' or 'resistance'. The latter usage is adopted for this thesis.

1.3 Agronomic Significance of Herbicide Resistance

The presence of weeds in crops results in yield decreases, seed contamination and consequent control costs. The presence of resistant weeds necessitates a change in the practice of these controls and represents an opportunity cost. Large areas of agricultural land have infestations of herbicide-resistant weeds: in France over 200,000 ha are affected (Gressel *et al.*, 1982); 75% of monoculture maize in Hungary contained resistant *Amaranthus retroflexus* L. (Hartman, 1979); in western Washington, U.S.A., resistant *Senecio vulgaris* L. occurs in over 250,000 ha (LeBaron & Gressel, 1982).

Recently, therefore, reviews have attempted to identify agronomic procedures which will minimise or control such losses and costs (Gressel & Segel, 1982; Putwain, 1982). One such solution is the use of crop rotations. Indeed in areas of the mid-west U.S.A corn-belt, this has slowed the rate of appearance of triazine-resistance, together with the employment of herbicides with different modes of action.

Cross-resistance can have significant agronomic implications. For instance, herbicide-resistant *L. rigidum* is not controlled by the only two herbicides registered for post-emergence control in Australian wheat and barley crops (Powles, 1987).

1.4 Ecology of Herbicide-resistant Plants

1.4.1 Relative fitness of biotypes

Current knowledge of the ecological factors which lead to the evolution or subsequent spread of resistance rests firstly on the relative fitness of resistant and susceptible biotypes. Comparisons of biotypes are usually made from field collected material. Plants of the same species may be sampled from within the same field (Holt, 1988), from adjacent fields (Weaver & Warwick, 1982) or from different geographic locations (Weaver *et al.*, 1982). Secondly, subsequent experiments comparing the biotypes are usually conducted in glasshouse environments and measure biomass of plants either grown as spaced plants or in simple mixtures. Table 1.1 summarises such collective evidence from the literature and uses relative fitness to describe the outcome, measured as a simple coefficient: the performance of the resistant biotype divided by that of the susceptible biotype.

Where significant differences between biotypes were demonstrated, the resistant biotype tended to be less fit (e.g. *Chenopodium album* L.: Warwick & Black, 1981). Fitness is here defined as the chance of leaving

Table 1.1 Literature survey of differential fitness of herbicide-resistant (R) versus susceptible (S) biotypes. Indicated are: species; number of populations compared; days to harvest (M= to maturity); variable measured (SDW= shoot biomass, LDW= leaf biomass, RDW= reproductive biomass, RW%= reproductive biomass as % of total, RWP= reproductive biomass unit area^{-1}; RPP= reproductive biomass plant^{-1}) and treatments, where results are partitioned; fitness differential, measured as performance of S biotype/R biotype (* significant difference at $P < 0.05$); and the published reference. For mixture experiments, only data from replacement series designs are presented and where absolute data (i.e. not proportions) could be extracted. Fitness differential is then calculated at equal mixture frequency, i.e. 50% R, 50% S plants.

Species	No. pops.	Growth (days)	Measure		Fitness differential	Reference
MONOCULTURE EXPERIMENTS						
Senecio vulgaris	2	86	SDW		1.4*	Conard & Radosevich (1979)
			RDW	low density	1.1	
				high density	1.3	
	8	60	SDW		1.1	Varwick (1980)
			RDW		1.1	
	2	M	SDW	high light	2.0*	Holt & Radosevich (1983)
				low light	2.4*	
			RW%	high light	1.4*	
				low light	0.8*	
	2	147	RPP		1.1*	Watson (1987)
	2	49	SDW		1.6*	Holt (1988)
			RDW		2.1*	
Chenopodium album	2	M	SDW		1.6*	Varwick & Black (1981)
			RDW		1.4*	
	8	M	SDW		1.3	Varwick & Marriage (1982)
			RDW		1.5	
	2	56	LDW		0.8*	Jansen *et al.* (1986)
			RDW		1.7*	
			RPP		0.9*	
	2	60	SDW		1.0	Elliot & Peirson (1983)
	2	63	SDW	25°C	0.8*	Bhomik (1982)
				30°C	0.7*	
			RDW	25°C	1.3*	
				30°C	0.9	
	2	75	SDW		1.4	Gasquez *et al.* (1981)
			RDW		1.6*	
C. strictum	2	M	SDW		1.2*	Varwick & Black (1981)
			RDW		1.0	
Amaranthus retroflexus	5	M	LDW		0.72*	Weaver *et al.* (1982)
			RPP		1.0	
	2	90	SDW		1.2	Weaver & Varwick (1982)
			RSA		1.1	
A. powellii	4	M	LDW		1.6*	Weaver *et al.* (1982)
			RPP		1.0	
	2	97	SDW		1.6*	Conard & Radosevich (1979)
			RDW	low density	1.0	
				high density	1.1	
Eleusine indica	10	169	SDW	1983	0.9	Murphy *et al.* (1986)
				1984	0.8	
			RDW		1.3*	
			RPP		1.1	
Phalaris paradoxa	2	150	SDW		0.9	Rubin *et al.* (1985)
			RDW		0.8*	
Solanum nigrum	2	43	SDW		1.5	Gasquez *et al.* (1981)
			RDW		1.4*	
Polygonum lapathifolium (4 separate collections)	2	75	SDW	col. 1	1.2	Gasquez *et al.* (1981)
				col. 2	2.0*	
				col. 3	1.4*	
				col. 4	2.1*	
			RDW	col. 1	0.9	
				col. 2	3.3*	
				col. 3	1.1	
				col. 4	1.9*	
MIXTURE EXPERIMENTS						
Senecio vulgaris	2	68	SDW		3.5*	Conard & Radosevich (1979)
			RW%		3.0*	
	2	68	SDW		5.1*	Holt (1988)
			RDW		2.8*	
Chenopodium album	2	56	SDW		0.53*	Jansen *et al.* (1986)
			RDW		0.67	
A. powellii	2	86	SDW		1.6*	Conard & Radosevich (1979)
A. hybridus	2	84	SDW		3.6*	Ahrens & Stoller (1983)
			RDW		5.7*	
Phalaris paradoxa	2	150	SDW		1.2	Rubin *et al.* (1985)

descendents and is probably best measured as fecundity, but can be estimated by other biomass-based measure assumed to be correlated to fecundity. In fewer cases the susceptible biotype was significantly less fit (e.g. *C. album*: Bhomik, 1982; *A. myosuroides*: Rubin *et al.*, 1985). A number of other points are suggested by the data. Firstly, outcomes may vary within a study: relative fitness depended on the character measured (Jansen *et al.*, 1986; Murphy *et al.*, 1986) or when harvest was taken (Weaver *et al.*, 1982; Murphy *et al.*, 1986). Secondly, a number of studies showed no significant biotypic differences (e.g. Weaver & Warwick, 1982; Elliott & Peirson, 1983), particularly where a number of resistant and susceptible populations were compared (e.g. Warwick, 1980; Warwick & Marriage, 1982). Thirdly, where biotypes were grown in competition, the relative fitness of susceptible biotypes was sometimes greater in magnitude than under spaced plant conditions (Warwick & Black, 1981; Ahrens & Stoller, 1983). In a few studies the resistant biotype was found to be more competitive than the susceptible biotype (Warwick & Black, 1981; Jansen *et al.*, 1981).

1.4.2 Field studies

Field studies of the population dynamics of biotypes in similar environments can provide comparative estimates of potential rates of increase and identify environmental factors which determine relative success. Crucial to an understanding of population growth is a knowledge of density-dependent processes which commonly act to stabilise population numbers.

However, there have been very few detailed comparative field studies of the population ecology of resistant (R) and susceptible (S) biotypes within a species. One species has received some attention, *S. vulgaris*, which shows differences in seed bank dynamics (Watson, 1987), seedling

emergence patterns (Putwain, Scott & Holliday, 1982; Watson, 1987) and adult plant fecundity (Watson, 1987).

1.5 Species Descriptions

This thesis examines aspects of the evolution of herbicide-resistance by focusing on two species, *A. myosuroides* and *Elymus repens* (L.) Gould. These species differ in two important respects. *A. mysuroides* is an annual grass that has already evolved resistant populations to two related herbicides (Moss & Cussans, 1985). Moreover it is convenient for population studies because of its annual life cycle and lack of seed dormancy (see 1.4.2). The other species considered here, *Elymus repens*, contrasts with *A. myosuroides* in that it has not yet developed herbicide-resistance. However, it was identified as a potential candidate for evolution of resistance by Putwain (1982); since then enhanced tolerance has apparently been detected (Sampson, pers. comm.) in a Canadian collection. Furthermore, it is a perennial species and will probably respond differently to selection than an annual species (Harper, 1977).

1.5.1 *Elymus repens* (L.) Gould

A rhizomatous perennial grass, it is considered the worst perennial weed of the U.K. (Ingram, 1975) and Northern Europe (Håkansson, 1975). It has been found in 30-40% of arable crops surveyed (Ingram, 1975; Chancellor & Froud-Williams, 1984) and as serious infestations (large patches or unsprayed strips) in 45% of infested fields (Chancellor & Froud-Williams, 1984).

It is a significant weed of agriculture because of its competitive effects on crops and persistence. Although an extravagant consumer of nutrients (Werner & Rioux, 1977), which may exert considerable competitive pressure on the crop through both below-ground and shoot

interactions (Welbank, 1961), it is itself sensitive to crop presence (Mortimer, 1984).

Spread of the species is primarily vegetative: buds on underground rhizomes will give rise to new plants if fragmented and have a high probability of establishment (McMahon, 1982). following cultivation in August, new shoots develop at the growing point of the existing plant or from dormant buds on rhizome fragments. These remain at 2-3 leaves over winter. This vernalisation is followed by active growth in March (Palmer & Sagar, 1963). After about five aerial leaves are formed, a large proportion of apices initiate inflorescences which emerge in June-July. Plants will have tillered at the 4-6 leaf stage and rhizomes usually produced at the 6-8 leaf stage (Palmer & Sagar, 1963).

Seed matures during late summer and can germinate immediately after flowering, although fluctuating temperatures are required (Sagar, 1961). Seed production is very variable, but can amount to 15-400 seeds per flowering culm (Williams & Atwood, 1971).

Elymus repens is wind-pollinated and, although considered to be self-sterile, Beddows (1931) has found some seed set in enclosed spikes in a few clones. The species is very genetically variable when grown from seed (Williams, 1973a) but it has also been shown to possess considerable genetic variation at the between-population or -clone level in morphological and growth characters (Holly & Parker in Palmer & Sagar, 1963; Haddad & Sagar, 1968; Williams, 1973b; Neuteboom, 1975).

1.5.2 *Alopecurus myosuroides* Huds.

A strict annual weed of arable cultivation, occurring over a range of soil types (Brenchley, 1913; Naylor, 1972a), it is most commonly found as infestations of winter cereal crops. It now probably affects more than the 650,000 ha of arable land (Elliot *et al.*, 1979). Early germinating

plants are competitive with the crop and yield losses of 50% have been recorded in high density infestations (e.g. Moss, 1985). Naylor (1972b) found buried viable seed populations of 23-26 x 10^3 seed m^{-2}.

Seeds (= spikelets, containing a caryopsis with lemma and glume) germinate and emerge from the top 2-5 cm of soil (Naylor, 1970; Moss, 1981) in two emergence pulses: most (80%) emerge in autumn with a later and smaller peak in spring (Wellington & Hutchings, 1966). Plants are shallow rooting, overwinter with 2-5 tillers (Naylor, 1972a) and mature plants may have up to 80 tillers and can produce up to 7600 seeds (Naylor, 1972a). Innate dormancy is low (Wellington & Hitchings, 1966), only a small proportion of seeds surviving for longer than a year (Naylor, 1972b). Viability is low and variable, usually less than 50% (Naylor, 1972a).

The species is wind pollinated and is usually allogamous. Beddows (1931) has reported small amounts of self-fertilisation. Genetic variability is present for many characters (Menck, 1968; Bulke *et al.*, 1973) and has been commonly described at the between-population level (Exley, 1985; see Table 8.1).

The increasing acreage of continuous wheat and reduced cultivation regimes, characteristic of East Anglia and southern Britain, have facilitated the spread of the species (Moss, 1979). The release of seeds prior to crop harvest and their persistence on the soil surface combined with a lack of significant soil disturbance, such as ploughing, and the species' lack of seed dormancy enable rapid and abundant germination following harvest.

1.6 Thesis Content

1.6.1 Objectives

The objectives of the work presented here are:

(1) to examine the presence of genetic variability for herbicide response in populations of two contrasted grass weed species.

(2) to assess the influence of herbicides on the natural regulatory processes of *A. myosuroides* populations.

(3) to compare the relative fitness of herbicide-resistant and susceptible biotypes of *A. myosuroides*.

(4) to accurately assess the magnitude of selection for resistance in field populations of *A. myosuroides*.

1.6.2 Structure

The presence of genetic variation amongst populations can indicate the action of past selection (Bradshaw, 1984a). In **Chapter 2**, populations of *E. repens* were sampled from apparently contrasting environments to investigate the occurrence of population differentiation in respect of herbicide resistance. The potential for evolution was examined by comparison of clones within populations and by parent-progeny relationships.

There is substantial genetic variability in morphology of *E. repens* (Williams, 1973b; Neuteboom, 1975), but the relative magnitude of population and clonal variation is not known. A detailed examination of this is made in **Chapter 3** together with an evaluation of the influence of selection on life history and morphological characters.

In **Chapter 4**, genetic variation at the population level in herbicide response is examined using populations of *A. myosuroides* known to differ in herbicide resistance. The presence of appropriate genetic variation within both unselected and selected populations was investigated in an

experiment comparing the variation in root growth in herbicide solution among half-sibs in the presence of the herbicide.

Ecological factors which may determine the rate of spread of resistance are addressed by comparisons of R and S biotypes under different conditions. **Chapter 5** quantifies growth patterns of *A. myosuroides* biotypes over time grown as spaced plants in a glasshouse environment. A functional growth analysis was used to enable statistical comparison of their relative growth.

Interference between biotypes will determine relative success in mixed populations. Therefore, mixtures of *A. myosuroides* biotypes were grown in a competition experiment (**Chapter 6**) which maintained realism by independently varying densities of both species and investigated the effect of different herbicide treatments on the competitive interaction .

Valid predictions concerning the potential rates of increase of R biotypes cannot be made in the absence of field data. A field experiment which estimates selection pressures imposed by herbicides on *A. myosuroides* is reported in **Chapter 7**. The influence of these herbicides on processes of population regulation of both biotypes are considered here.

A collective discussion of the ecological implications and evolutionary significance of the results of the experimental work is presented in **Chapter 8**.

CHAPTER 2
INTER AND INTRA POPULATION-LEVEL VARIATION FOR HERBICIDE RESPONSE IN *ELYMUS REPENS*

2.1 Introduction

The perennial grass *Elymus repens* (L.) Gould is a serious weed of arable agriculture in Britain (Ingram, 1975) as well as in other parts of the world (Holm *et al.*, 1977; Westra, 1980). In a survey of central southern England, it was the most frequent grass weed of winter cereals and was present as large patches or greater in over 40% of infested fields (Chancellor & Froud-Williams, 1984). *E. repens* is genetically variable for several morphological and life history characters (Palmer & Sagar, 1963; Williams, 1973b; Neuteboom, 1975). The usual method of control is the herbicide glyphosate, to which differences in response between collections have been reported (Westra, 1980). Variation in response to other herbicides has also been noted (Bucholtz, 1958; Haddad & Sagar, 1968).

This Chapter describes the assessment of genetic variation for herbicide response in natural populations of *E. repens*, using three approaches. Firstly, the response of a limited number of populations was compared, when grown in nutrient culture and when sprayed as whole plants grown in soil. Secondly, all populations were compared for herbicide response in nutrient culture. Thirdly, replicated clonal material was used to examine the extent of within-population variation and finally a comparison of parental genotypes and their offspring was made using seed produced from parents grown as a polycross in a greenhouse. At the majority of sampled sites, populations were separately collected from areas sprayed or untreated with glyphosate in order to detect the possible effects of past selection by herbicide treatment.

2.2 Materials and Methods

2.2.1 Plant material

During 1984-5, plants were collected from farms at the locations shown in Table 2.1. At 12 of these farms, rhizome fragments were collected from populations either 1) in a crop which had been treated with glyphosate in the past ('exposed' populations) or 2) at the nearest area not treated with glyphosate, usually at the crop margin, in hedgebanks or road verges ('unexposed' populations). Up to 25 rhizome samples were taken per population, fragments being collected at least 2 m apart to minimise repetitive sampling of genotypes. Samples from a further two sites (M2, N2) were derived from rhizomes collected in treated crops on farms reporting poor glyphosate control (courtesy of Monsanto plc).

For most populations, it was known that samples were from collections of separate plants. These samples were kept as separate 'clones' by propagation from the initial rhizome fragment. No assessment of clonal identity could be made for the two additional populations (M2, N2), except that connected rhizome lengths in the sample obtained were treated as individual 'clones'. Plants were propagated in loam-based compost over a one year period to reduce carry-over effects. They were periodically repotted and fertilised to maintain vigour and encourage rhizome production.

2.2.2 Experimental procedure

<u>Experiments 1 and 2: Dose response</u>

In Experiment 1, rhizome bud fragments from a single node were sprouted on paper towels in seed trays in a mist unit at 27°C. Sprouted rhizome segments were placed on a 100 cm^3 raft of polypropylene beads in plastic pots containing 200 cm^3 of 1.0 g l^{-1} $Ca(NO_3)_2$ nutrient solution

Table 2.1 Populations studied, number of clones sampled, environment from which populations were collected and recent history of glyphosate use for collections of *Elymus repens*. Populations M2 and N2 were kindly provided by Monsanto plc. Glyphosate use is indicated thus: [1] frequency of herbicide use, where 0= not used, 1= used yearly, 2= used every two years, etc. [2] rate of glyphosate applied in usual treatment, where 1= full-rate (4 l ha^{-1}), ¾= three-quarter rate, etc.

Site Code	Source of Material (NGR)	No. Clones	Cultural Regime	Glyphosate Use: frequency (rate)
A1	Penketh, Merseyside	11	Hedgebank	0[1]
A2	(SJ 548877)	19	Winter wheat	3 (½)[2]
B1	Croft, Greater Manchester	22	Field margin	0
B2	(SJ 620959)	20	Winter wheat	3 (1)
C1	Nr. Croft, Cheshire	18	Hedgebank	0
C2	(SJ 624934)	26	Winter wheat	
D1	Halebank, Merseyside	18	Hedgebank	0
D2	(SJ 470840)	21	Winter wheat	3 (¼)
E1	Tadcaster, West Yorkshire	25	Field margin	0
E2	(SE 455471)	25	Spring barley	5 (1)
F1	Harewood, West Yorkshire	22	Field margin	0
F2	(SE 320460)	25	Winter wheat	2 (¼)
G1	Hagley, Hereford & Worcs.	16	Hedgebank	0
G2	(SO 907812)	21	Spring barley	2 (¾)
H1	Blakedown, Hereford & Worcs.	18	Field margin	2 (½)
H2	(SO 899774)	22	Sugar beet	
I1	Mamble, Hereford & Worcs.	15	Hedgebank	0
I2	(SO 690710)	20	Winter barley	2 (¾)
J1	Shifnal, Shropshire	20	Field margin	0
J2	(SJ 772077)	21	Winter wheat	2 (½)
K1	Wellington, Shropshire	15	Field margin	0
K2	(SJ 606081)	18	Spring barley	3 (1)
L1	Hordley, Shropshire	20	Hedgebank	0
L2	(SJ 385310)	22	Sugar beet	3 (1)
M2	Alderford, Norfolk (TG 125186)	?	Cereals	1 (1)
N2	Colney, Norfolk (TG 180085)	?	Cereals	1 (1)

and varying amounts of glyphosate. Glyphosate (formulated as 'Roundup') was added to the nutrient solution to give six concentrations: 0.0000036, 0.000018, 0.000036, 0.00018, 0.00036 and 0.0036 g a.e. l^{-1}, together with a control. The experimental design was a randomised complete block with 10 replicates and two populations, B1 and D2, chosen randomly from all 26 populations (Table 2.1). Each replicate pot contained two sprouted rhizome segments, randomly chosen from a bulked collection of rhizome segments from the two populations (B1, D2).

Pots were placed in a growth chamber with a 12 hour daylength of fluorescent lighting at an intensity of 100 μe cm^{-2} s^{-1}, a temperature cycle of 20°C (day) and 15°C (night), and relative humidity at 85/55%. To minimise evaporation losses during the experiment, pots were stood in a 10 cm depth of water and each block was surrounded by a guard row of water filled pots.

In Experiment 2, populations B2, D2 and K1 were used. Rhizome segments were taken from all clones of each population, randomised within each population and sprouted under the same conditions as for Experiment 1. Four uniform-sized, sprouted rhizome segments from a population were then planted into each 9.0 cm diameter plastic pot filled with J.I No. 1 loam-based compost. Plants were grown for four weeks in a glasshouse until each plant had four expanded leaves and 2-3 tillers.

Pots were randomly allocated to a herbicide treatment and were sprayed with an Oxford Precision Sprayer at seven rates: 0.0, 0.054 (not D2), 0.108, 0.216, 0.432, 0.864, 1.728 (not D2) kg a.e. glyphosate ha^{-1}, in 180 l ha^{-1} water at 2.3 bar. Pots were laid out in a completely randomised design with four replicates of herbicide treatments for two populations (B1, K1) and three replicates of five doses for population D2. After a further 25 days, shoots were cut at soil level and rhizomes

excavated. Green shoot and healthy rhizome material (not dark-coloured or black; both buds and rhizomes firm) were oven dried at 60°C for 5 days and weighed.

Experiment 3: Population variation

Treatment of rhizome segments and experimental conditions were the same as in Experiment 1. The design was a randomised complete block with three replicate pots of the 26 populations (Table 2.1). Each pot contained five rhizome segments, each segment representing a randomly chosen clone from a population. Thus, 15 different clones were sampled for each population, except A1 where repetitive sampling of four clones was needed. The performance of untreated rhizomes was compared with that of rhizomes treated with glyphosate applied at a single dose of 0.00036 g a.e. l^{-1}, being chosen from the results of Experiment 1 as likely to differentiate the response of separate populations, in which most plants were not dead at harvest.

Experiment 4: Clonal variation

This experiment was set up concurrently to and as an extension of Experiment 3. The five randomly chosen clones in block 1 of Experiment 3 were replicated in two further blocks. One population (G2) was excluded because insufficient replicate rhizome segments were available. Treatments and experimental conditions were the same as for Experiment 3.

Experiment 5: Parent-offspring regression

Two randomly chosen populations (A2, G2) were used. Twenty mature plants of both populations were grown together in two separate glasshouses and allowed to set seed as a polycross. Seed collected from these parents was germinated in soil, seedlings individually transplanted into 7.5 cm diam. pots containing a loam-based compost and allowed to

grow in a heated glasshouse under the same conditions (see Experiment 2) as the parents, until rhizomes were produced (usually by 10 weeks).

Rhizome bud segments were taken from both progeny and parents. Between three and eight progeny for nine (A1) or 11 (G2) parents were each replicated four times. Each pot in the completely randomised design contained one replicate of a randomly chosen parent and one replicate of four randomly chosen progeny. The plants were grown in nutrient solution containing 0.00036 g a.e. glyphosate l^{-1} in the same conditions as described for Experiment 1.

Elymus repens, although generally regarded as self-sterile (Palmer & Sagar, 1968), has been recorded as setting seed in some enclosed spikes (Beddows, 1931). Spikes of each population were therefore bagged during this experiment, but showed no seed set.

2.2.3 Analysis

Analysis of variance (ANOVA) for experiments 1-4 was carried out using PROC GLM of SAS (SAS Inst. Inc., 1985) with Type III sums of squares to correct for non-orthogonality and missing values. Initial shoot length was a significant covariate of percentage increment growth. Least squares means, adjusted for the covariate, are therefore presented where appropriate and the covariate included in the ANOVA table. In Experiments 1-4 a log transformation was used to minimise heterogeneity of variances.

In Experiment 3, populations were treated as a random ANOVA factor, whilst in Experiment 4 both populations and clones nested within populations were considered random model effects. In the latter experiment, variance components were calculated from the sets of linear equations for an overall model and for each individual population. Broad

sense heritability (h^2_B) was calculated as $h^2_B = V_g / V_g + V_e$, where $V_e = \sigma^2_w$ and $V_g = \sigma^2_b$ (Burton & DeVane, 1953; Falconer, 1981).

In Experiment 5, the mean response of individual progeny and maternal parents over replicates was treated as the regression data. Estimates of narrow sense heritability (h^2_N) are obtained by a maternal parent-progeny regression from the slope of the regression (b), which is equal to ½ h^2_N. This analysis assumes that random mating occurs within a population and that maternal effects are absent .

2.3 Results

2.3.1 Dose response

When grown in nutrient solution (Experiment 1), over 80% of shoot segments were dead at harvest in the highest herbicide dose and the two populations had equal mortality (G_{adj}= 0.63, 1 d.f., N.S.). Increasing herbicide dose significantly affected increment growth ($P \leq 0.001$), but the two populations did not differ in response (Table 2.2). Doses up to 0.00018 g a.e. l^{-1} had little effect on shoot increment (Fig. 2.1) but at 0.00036 g a.e. l^{-1} growth was reduced by approx. 50% in both populations..

For whole plant response measured as shoot growth, in Experiment 2, populations differed significantly ($P \leq 0.05$) in response to herbicide dose (Table 2.3). Examination of means showed that population B1 was most responsive at low dose rates (Fig. 2.2). Populations were generally similar in response at the highest dose rates. For rhizome growth, there was no significant population main effect or population interaction. Dose rate had strong effects ($P \leq 0.001$), as expected.

2.3.2 Population variability

A significant ($P \leq 0.05$) block effect was found in Experiment 3 (Table 2.4) and probably reflected conditions in the growth chamber, where the middle block grew best. Populations differed significantly ($P \leq 0.05$) in

Table 2.2 Analysis of variance of shoot increment growth for populations of *Elymus repens* grown in nutrient solution and exposed to increasing glyphosate herbicide doses. Shoot increment is expressed as a percentage and transformed to $\log_{10}(x + 101)$. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1982). The covariate is initial shoot length. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Source	d.f.	M.S.	*F*
Block	9	0.061	0.54
Herbicide	6	1.068	9.5***
Population	1	0.133	1.01
Herbicide x Population	6	0.123	1.09
Covariate	1	4.854	43.19***
Error	168	0.112	
Total	191		

Table 2.3 Analysis of variance of shoot growth (dry weight) and rhizome growth (dry weight) for three populations of *Elymus repens* treated with glyphosate as whole plants. Both variables are transformed to $\log_{10}(x + 1)$. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). Significance levels as in Table 2.2.

Source	d.f.	M.S.	*F*
Shoot growth			
Herbicide	6	2.651	106.71***
Population	2	0.125	5.02*
Herbicide x Population	10	0.066	2.65*
Error	50	0.025	
Total	68		
Rhizome growth			
Herbicide	6	1.990	51.15***
Population	2	0.049	1.25
Herbicide x Population	10	0.037	0.96
Error	36	0.039	
Total	54		

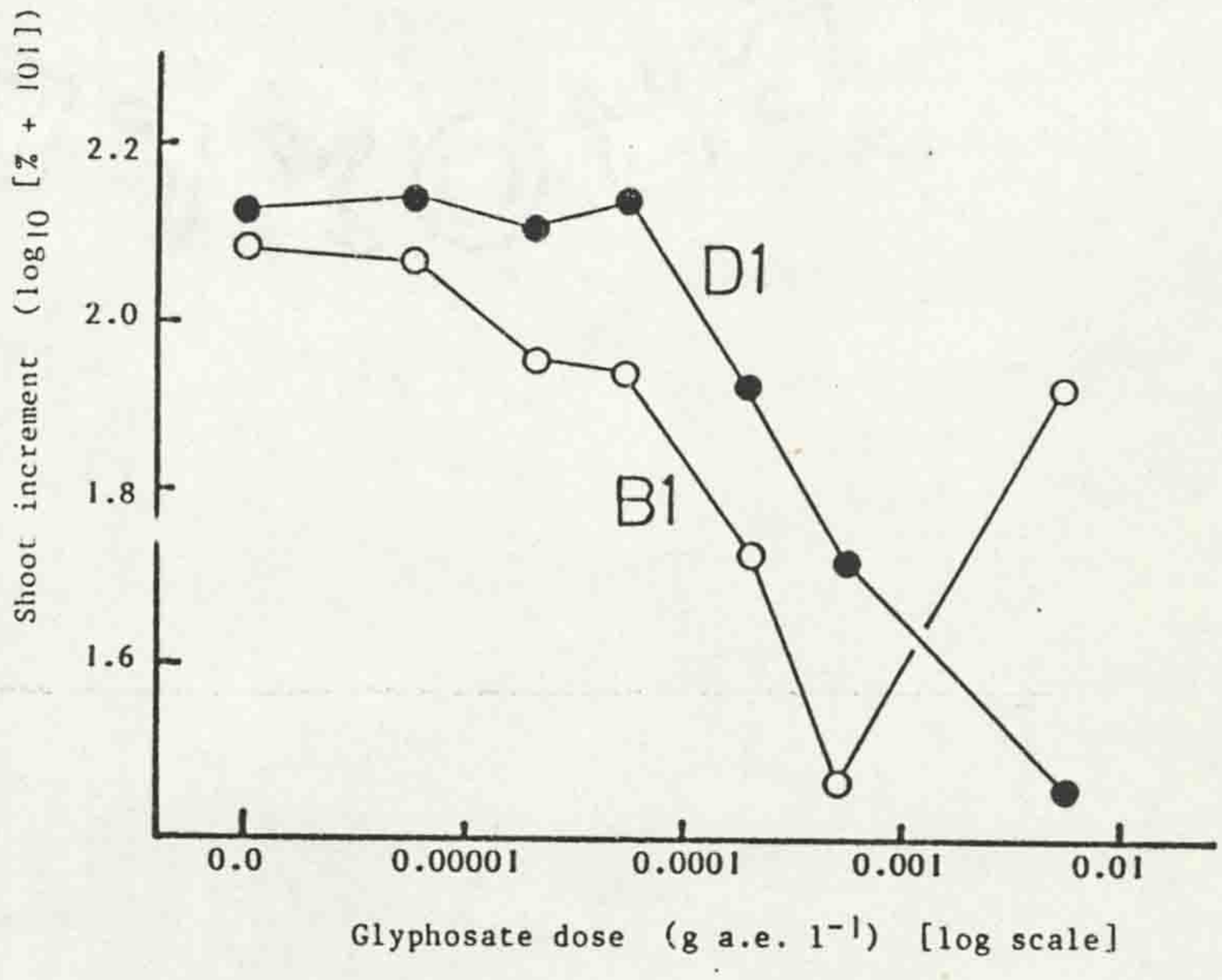

Figure 2.1 The relationship between shoot increment growth and glyphosate herbicide dose for two populations of Elymus repens (B1 and D1), grown in nutrient solution. Response represented is least squares mean shoot increment growth, adjusted for initial shoot length.

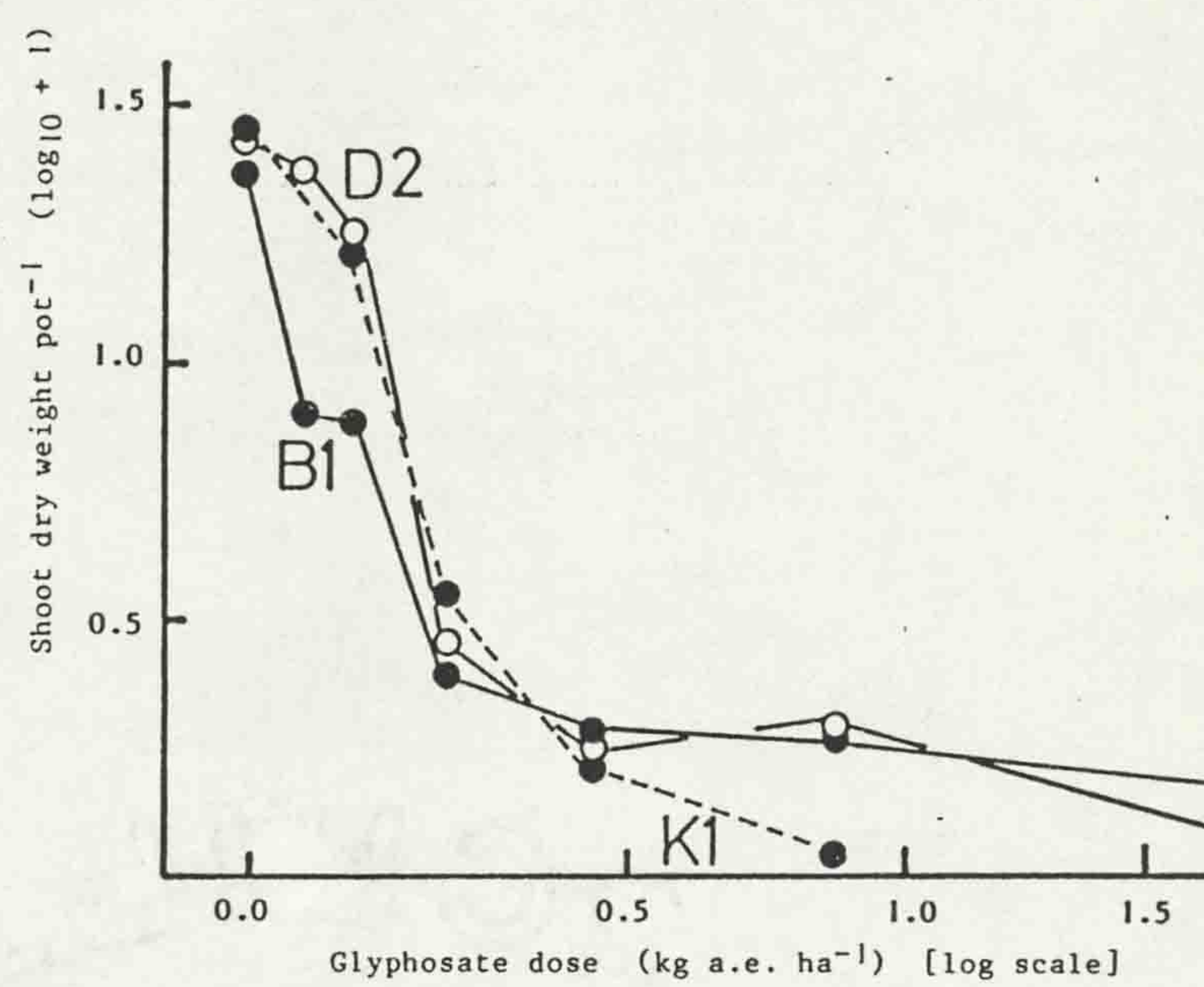

Figure 2.2 The relationship between shoot growth per pot and glyphosate dose for three populations of Elymus repens (B1, D2 and K1), grown in soil.

herbicide response and means for each population are shown in Fig. 2.3. Variability amongst populations is clearly greater under herbicide treatment. The majority of populations responded in a similar fashion, with a few exceptions. Two herbicide-exposed populations (N2, L2) showed a greater than average growth in nutrient solution in the absence of nutrient solution; another population sampled from the same environment (B2) showed the least response to herbicide application (i.e. the flattest slope).

There was no simple association between past herbicide regime experienced by a population and its performance in response to the herbicide (Fig. 2.3).

2.3.3 Within population variation

In Experiment 4, populations did not differ in response to herbicide (*contra* the results of Experiment 3), although the main effect of populations was significant ($P \leq 0.01$) (Table 2.5) .Significant clonal differentiation in herbicide response was evident ($P \leq 0.01$). Variance component analysis showed that clonal differences in response to herbicide was the largest source of assumed genetically determined variation, amounting to about 14% (Table 2.6). Population level variation in total only amounted to about 6%. Most variation (80%), however, was attributable to differences between replicates of clones and thus represented error variation.

Unexposed populations were analysed separately from those exposed to herbicides and different results were obtained (Table 2.7). The separate analyses showed that unexposed populations did not differ in response to herbicide, but that clonal response to herbicide was significantly different ($P \leq 0.001$). For exposed populations, the only significant effect was the main effect of herbicide ($P \leq 0.001$). The amount of population-

Table 2.4 Analysis of variance of shoot increment growth for 26 populations of *Elymus repens* treated with glyphosate in nutrient solution. Shoot increment is expressed as a percentage and transformed to $\log_{10}(x + 101)$. F-ratios are calculated according to a mixed model for a randomised block design with population as random and block and herbicide as fixed effects. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). The covariate is initial shoot length. Significance levels as in Table 2.2.

Source	d.f.	M.S.	*F*
Block	2	1.33	3.43*
Herbicide	1	101.71	262.51***
Population	25	0.89	2.29***
Herbicide x Population	25	0.59	1.52*
Covariate	1	19.38	50.01***
Error	660	0.39	
Total	714		

Table 2.5 Analysis of variance of shoot increment growth for clones in 25 populations of *Elymus repens*. Shoot increment is expressed as a percentage and transformed to $\log_{10}(x + 101)$. F-ratios are calculated according to a mixed model for a randomised block design with population and clone as random effects and block and herbicide as fixed effects. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). The covariate is initial shoot length. Populations mean square is tested against Clones (within Populations) and Herbicide x Population against Herbicide x Clones (within Populations). Other effects are tested against Error Mean Square. One population (N2) was excluded because of insufficient sample size. Significance levels as in Table 2.2.

Source	d.f.	M.S.	*F*
Block	2	0.55	1.73
Herbicide	1	95.84	301.63***
Population	24	0.97	2.06**
Clones within Population	97	0.47	1.49*
Herbicide x Population	24	0.75	1.54
Herbicide x Clones (Pop)	97	0.49	1.53**
Covariate	1	9.78	30.77***
Error	483	0.32	
Total	729		

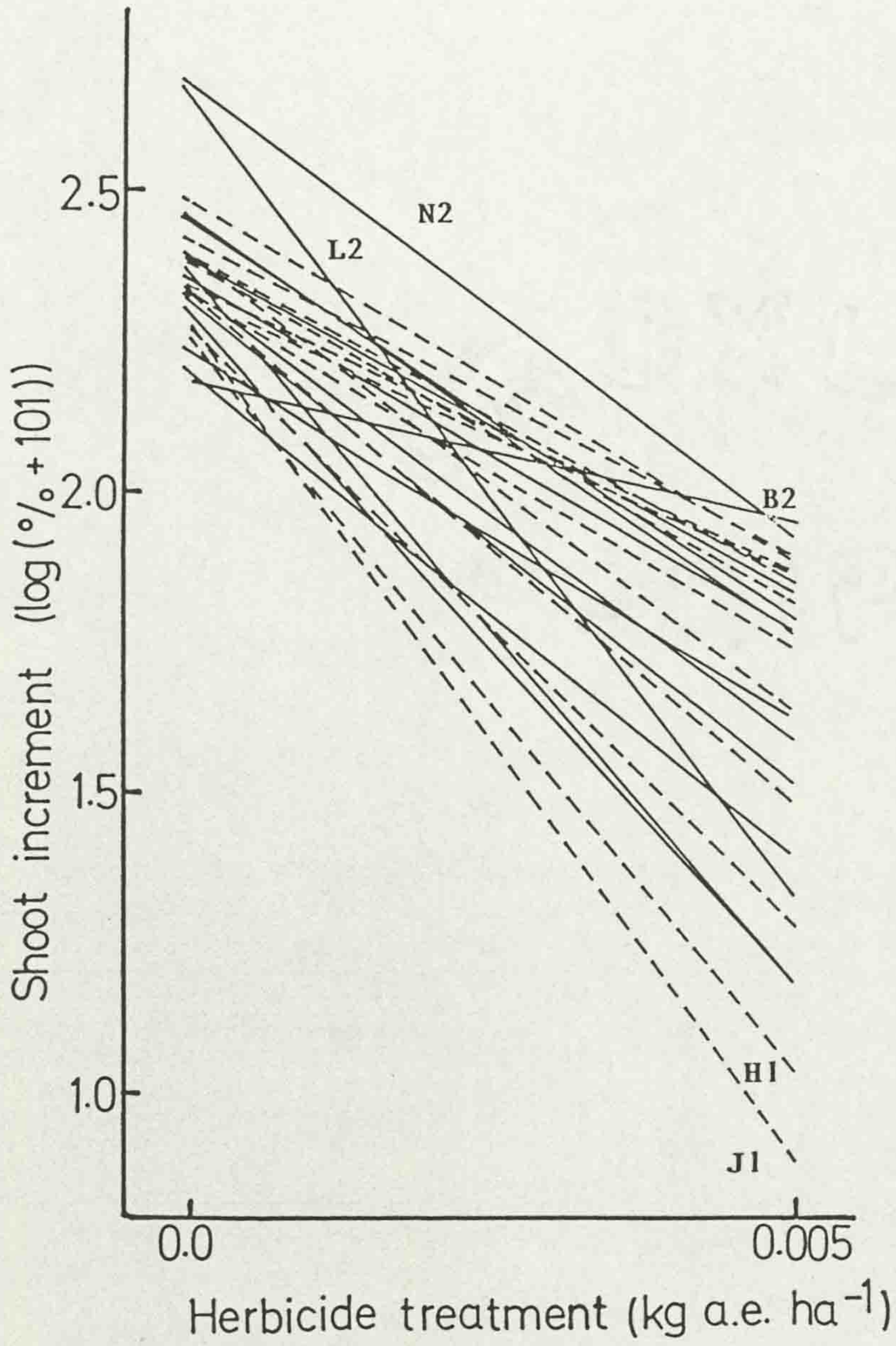

Figure 2.3 Herbicide response of 26 populations of Elymus repens, measured as least squares mean percentage increment growth, adjusted for initial shoot length. Populations were originally collected in areas unexposed (– – – -) or exposed (————) to glyphosate herbicide. Some populations are indicated.

Table 2.6 Variance components calculated from sets of linear equations of the analysis of variance model. The proportion of total variance components is given in parentheses. Herb.= herbicide effect. Significance levels, taken from Tables 2.5 and 2.7, as in Table 2.2.

Analysis	Source of Variance				
	Population (Pop.)	Clones	Herb.x Pop.	Herb.x Clone	Error
All populations	0.0084** (2.1)	-0.0023* (-0.6)	0.0181 (4.5)	0.0567** (14.2)	0.3177 (79.7)
Unselected populations	0.0067* (1.9)	-0.0029** (-0.8)	0.0238 (6.8)	0.0806*** (22.9)	0.2439 (69.3)
Exposed populations	0.0039 (0.9)	-0.0017 (-0.4)	0.0150 (3.4)	0.0367 (8.3)	0.3862 (87.9)

Table 2.7 Separate analyses of variance for *Elymus repens* shoot increment growth for clones in either 12 populations not previously exposed to glyphosate (Unexposed), or 13 populations exposed to glyphosate (Exposed). Shoot increment, expressed as a percentage, is transformed to $\log_{10}(x + 101)$. F-ratios are calculated according to a mixed model for a randomised block design with population and clone as random effects and block and herbicide as fixed effects. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). One population (N2) was excluded from analysis for Unexposed populations because of insufficient sample size.The covariate is initial shoot length. Tests of mean squares as in Table 2.5. Significance levels as in Table 2.2.

Source	d.f.	M.S.	*F*
Unexposed Populations			
Block	2	0.26	1.08
Herbicide	1	39.52	162.02***
Population	11	0.98	2.13*
Clone within Population	47	0.46	1.90**
Herbicide x Population	11	0.82	1.71
Herbicide x Clone (Pop)	47	0.48	1.97***
Covariate	1	2.91	11.94***
Error	226	0.24	
Total	346		
Exposed Populations			
Block	2	0.20	0.51
Herbicide	1	57.00	147.59***
Population	12	0.86	1.77
Clone within Population	50	0.49	1.26
Herbicide x Population	12	0.72	1.44
Herbicide x Clone (Pop)	50	0.50	1.29
Covariate	1	6.80	17.60***
Error	254	0.39	
Total	382		

level variation (about 9%) was small in unexposed populations compared to clonal variation in response (23%) (Table 2.6).

Analyses conducted on each population separately showed that only five populations contained significant, presumed genetic variation among clones as indicated by significant herbicide x clones interactions, namely populations A2, B2, I2 and J2 ($P \leq 0.05$), and G1 ($P \leq 0.01$). The main effect of clones was significant in populations I2 and J2 ($P \leq 0.05$), and in G1 ($P \leq 0.01$).

Regression statistics calculated for Experiment 5 showed that the relationship between parents and progeny was not significant for both populations (Fig 2.4). Heritabilities cannot be derived from the slope of the regression if there is no significant relationship (Falconer, 1981). This indicates a lack of genetic determination of glyphosate response in these two populations.

2.4 Discussion

2.4.1 Nutrient culture

The use of sprouted rhizome segments growing in herbicide nutrient solution in the present study is analogous to the technique recommended by Coupland and Wyatt (1982). It was efficient and negated the potential influence of the leaf system morphology on the measure of tolerance. Since glyphosate is translocated, leaf morphology can influence absorbtion. *Elymus repens* is known to vary in colour, attributable to a leaf wax layer, in hairyness of the leaf surface (Neuteboom, 1975) and in various shoot characters (Palmer & Sagar, 1968; Williams, 1973b; Neuteboom, 1975). Herbicide in the experimental system used here could be absorbed by diffusion through the rhizome segment, through any root developed (Penn & Lynch, 1982) and bud surfaces in contact with the solution. The disadvantage to this approach is that tolerance in the field

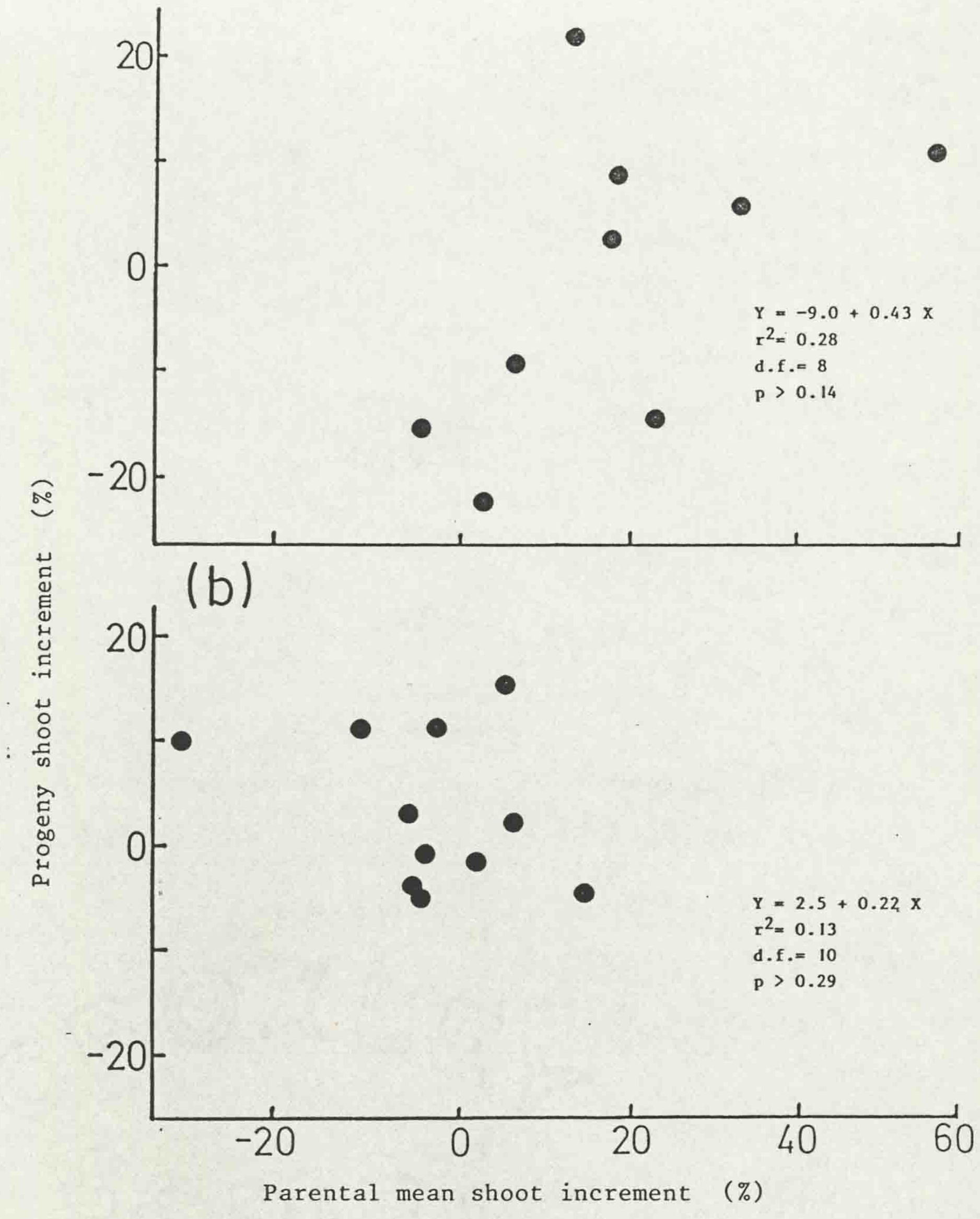

Figure 2.4 Regression of mean glyphosate herbicide tolerance of progeny on tolerance of female parents of two populations of Elymus repens. Tolerance is measured as percentage shoot increment growth, untransformed. (a) population A1 (b) population G2.

may be expressed in other characters, e.g. leaf structure. The dose-response results of Experiments 1 and 2 on two and three populations, respectively, showed that populations responded differently to herbicide as whole plants, but did not differ when grown in nutrient solution. This appears to confirm that other, morphological or anatomical, characters may be important. Yet with a greater number of populations, significant population variation was detected in nutrient culture (Experiment 3).

2.4.2 Genetic variability

Significant between-population variation in response to glyphosate was demonstrated in an experiment where herbicide was sprayed on mature plants (Experiment 2). Other studies have demonstrated population variability in response to herbicides using a similar experimental system: Westra (1980) found differences between seven collections of *E. repens* in performance as mature plants when sprayed with glyphosate. Intraspecific variability was also found in response to dalapon (Bucholtz, 1958; Haddad & Sagar, 1968) and aminotriazole (Haddad & Sagar, 1968). However, the experiments examining variation amongst many populations in response to glyphosate in nutrient culture showed conflicting results: a significant ($P \leq 0.05$) herbicide x population interaction was found in Experiment 3, but not in Experiment 4 where clonal differences were partitioned out of the total variation. Furthermore, when only two populations were compared (Experiment 1), no population x herbicide effect was present. This suggests that population differences in response were weak and that most populations appeared to have a similar degree of response to the herbicide (Fig. 2.3), certainly when exposed to the herbicide directly through the rhizome system. The incremental growth of the population which responded least to the herbicide was still reduced by 70% by herbicide treatment (Fig. 2.3).

There has been little experimental work examining both clonal and population-level variation in *E. repens*. Most other comparable studies of response to herbicide compare single clones drawn from different localities: such samples should be regarded as single-clone population samples. The present study used a hierarchical approach, sampling a number of clones from within each of many populations. Significant clonal variation was demonstrated here (Experiment 4), although not in populations exposed to herbicide when these were analysed separately (Table 2.7). The degree of presumed genetic determination was revealed by the clonal variance components. Most of the clonal variation was due to the herbicide interaction effect: clones responded differently to herbicide solution. However, the small magnitude of the variance component in relation to within clone (error) variation showed (Table 2.6) that the degree of genetic determination was low. This may help to explain the failure to detect a significant parent-offspring relationship in Experiment 5. The degree of apparent genetic control of the trait was too low or the precision of the experiment was not high enough to expose any possible genetic determination due to high error variation. Indeed, only about 10 parental plants were included in the experiment; this is probably insufficient for regression purposes.

2.4.3 Selection

Given the relatively large number of populations sampled (12 from each environment), the action of past selection pressures could be inferred if there were consistent associations between the degree of tolerance of a population and the environment from which it was drawn. However, no consistent pattern of increased tolerance in herbicide-exposed populations was found, although in very few comparisons did they grew better than unexposed populations from the same site.

These results suggest that evolution of enhanced tolerance has not occurred, at least as a consistent genetic response across populations. Explanation for this may involve the lack of sufficient selection pressure, absence of genetic determination of herbicide response or factors masking the operation of selection. The intensity of selection may have been insufficient in the cultural conditions experienced by the populations. Glyphosate is relatively expensive to the farmer and is not employed regularly as are the triazines, for example. Glyphosate is often used only every few years, applied at reduced rates or applied to localised infestations selectively. Even where glyphosate is sprayed each year (as in pre-harvest treatment in cereal crops), there will only be one application and it is unlikely that the entire rhizome population will be exposed to herbicide.

Selection may also be reduced because of the perennial nature of *E. repens*. Rhizomatous spread is a major means of propagation and rhizome buds remain in various states of dormancy (Dekker & Chandler, 1985). Selection operating on particular genotypes in one season may not be reflected in the genotype composition of the next season, because of the presence of a bud 'bank'. Also, some of the bud population may escape selection, depending on the timing of the spray treatment. A further hypothesis to account for lack enhanced tolerance in 'selected' populations is that phenotypic plasticity for response to herbicide could buffer the genotype against the effects of selection (Sultan, 1987).

If selection had in fact operated sufficiently in the exposed populations to cause genetic change, the experiment may still not have detected any resultant, enhanced tolerance. Other, unmeasured variables such as leaf morphology or rate of translocation may determine tolerance in these populations of *E. repens*. Alternatively, heritable variation may

be absent. In an analysis of all populations clonal differences were detected (Experiment 4), but the amount of presumed genetic control was low and transmission of genetically-determined sensitivity to glyphosate from parent to offspring was not shown (Experiment 5). It is very interesting that exposed populations, when analysed separately, showed no evidence of clonal differentiation, whereas populations from unexposed environments possessed significant clonal differences. Furthermore, the two populations included (randomly) in the parent-offspring trial were both from environments exposed to glyphosate. One explanation is that fixation of any genetic variation for herbicide response had already occurred from the selection already experienced. On the other hand, the exposed populations may have been depleted of genotypes and thus not show sufficient, detectable clonal differences. This could be tested by comparisons of genotype composition using electrophoretic techniques.

CHAPTER 3
LIFE HISTORY AND MORPHOLOGICAL VARIATION IN *ELYMUS REPENS* POPULATIONS FROM CONTRASTING ENVIRONMENTS GROWN UNDER UNIFORM CONDITIONS

3.1 INTRODUCTION

Many weed species contain high levels of genetic variation for a range of characters (Barrett, 1981). Selection, acting on such levels of variation, can produce considerable population differences in morphological (Clegg & Allard, 1972), life history (Law, Bradshaw & Putwain, 1977; Warwick & McNeill, 1982) and electrophoretic characters (Hamrick & Allard, 1972). Where such selection pressures are strong (and directional) this represents a powerful agent of evolutionary change, as exemplified by the evolution of metal tolerance (Jain & Bradshaw, 1966; Bradshaw, 1984a) and herbicide resistance (LeBaron & Gressel, 1982). Even when the link between the selective agent and fitness is less clear, selection can be strong. For instance Davies and Snaydon (1976) have shown that selective coefficients of up to 0.8 are measurable against an ecotype in a pasture environment.

Elymus repens (L.) Gould. is a primary colonist of uncultivated waste areas and is a noxious weed of both arable, horticultural and lightly-grazed grassland communities (Palmer & Sagar, 1963; Boyall, Ingram & Kynot, 1981). Genetic variation is present in *E. repens* for many morphological and growth characters, both between populations (Palmer & Sagar, 1968; Williams, 1973; Neuteboom, 1975) and between clones (Williams, 1973; Westra, 1980). However, there are no quantitative estimates of the relative importance of the between- and within-population variation. Furthermore, Williams (1973) has indicated that considerable phenotypic or within-clone variation occurs in the species. The magnitude of this component is not known.

Many species have been shown to possess life history variation (Venable, 1984). The wide variety of habitats occupied by *E. repens* suggests that it possesses sufficient flexibility to respond to changed environments and cultural factors. If additive genetic variation for life history and growth characters has been present in the past, *E. repens* populations from contrasting environments may show adaptations to the properties of different cultural regimes.

Using *E. repens* clones from populations growing in cropped and uncropped sites, three objectives of this study were: (1) to determine the relative magnitude of between-population and between-clone variation; (2) to estimate the genetic component of variation for life history and morphological characters; (3) to identify any effects of past selection on current features of the populations.

3.2 Materials and Methods

3.2.1 Plant material

Plants were collected from the locations shown in Table 2.1, as described in Chapter 2. Rhizomes were sampled as described above (2.2.1) from 26 populations. At each of 12 sites, two contrasting populations were sampled: one from the standing crop and the other from the unsprayed crop margin or hedgebank. Plants were grown in a polythene tunnel house for two years before the experiment commenced, they were regularly rerandomised and repotted twice from new rhizome material.

3.2.2 Experimental procedure

Rhizome bud fragments were sprouted on paper towels in seed trays placed in a mist unit. Sprouted rhizome segments were planted singly in 7.5 cm diameter pots containing a soil-based compost (J.I. No. 2). The experimental layout was a randomised, complete block design with three replicates. Each block contained six randomly chosen clones from each of

the 26 populations. The experiment was laid out on one bench in a glasshouse with background heating above a minimum of 15°C and watered as necessary. After 72 days, the following characters were measured or counted on the primary shoot complex: maiximum shoot length (cm), number of tillers, number of leaves, maximum third leaf width (mm), number of leaf hairs unit area^{-1} (33.2 mm^2), erectness score (scaled from prostrate (1) to erect (5)) and score of hairyness of leaf sheaths (scaled from glabrous (1) to hairy (5)): the number of secondary shoot complexes initiated from rhizomes was also counted.

The rhizome complex of each plant was excavated and the following characters were recorded: total rhizome length (mm); number of primary rhizomes; total number of rhizome buds; rhizome width (mm) at the fifth internode from the apex of the longest primary rhizome or, if less than 5 internodes, at the internode closest to the shoot complex.

3.2.3 Analysis

A range of derived variables were calculated: mean number of leaves tiller^{-1}, mean primary rhizome length (mm), mean number of buds primary rhizome^{-1} and mean internode length (mm). To minimise the heterogeneity of error variance (Sokal & Rohlf, 1981), transformation either by square root or logarithms to base 10 (see Table 3.1) was performed where necessary. Choice of variables and type of transformation was made after examination of scatter plots of residuals and univariate histograms. The univariate normality of transformed variables was improved in all cases, when assessed by examination of normal probability plots (Ryan, Joiner & Ryan, 1981).

Analysis of variance was carried out on all 16 variables separately using a mixed model. Replicate blocking was treated as a fixed effect: populations and clones nested within populations as random effects.

Variance components were calculated by PROC VARCOMP (SAS Inst. Inc., 1982) both for the whole design and for each population separately. Multivariate analysis of variance (PROC GLM) was used to compare the mean population and clone responses over all variables and canonical discriminant analysis (PROC CANDISC) to display population centroids on canonical variates.

3.3 Results

3.3.1 Univariate analysis

Block effects were significant ($P < 0.05$) for three variables (Table 3.1), but block means showed no trend with spatial or other arrangement of blocks. Significant population effects were apparent for all characters (Table 3.1). In addition to population differences, significant clone effects were detected for 10 variables indicating that there was a genetic component of the within-population phenotypic variation. Clonal differences were not present for four characters: leaves $tiller^{-1}$, mean rhzome length, rhizome internode length and rhizome width (Table 3.1).

The proportions of between-population, between-clone and within-clone (error) variation of total variation were calculated by variance components analysis (Table 3.2). The proportion of the total variation attributable to clones is equivalent to the intraclass correlation coefficient and, for clonal material, provides an estimate of 'broad-sense' heritability (Burton & DeVane, 1953), i.e. that proportion of phenotypic variation attributable to genetic sources (Falconer, 1981; Bradshaw, 1984b). Morphological variables had both large population and clonal components. For leaf hair density, only 23% of variation was environmental (error) variation. For growth variables, evidence of population differentiation was strong: variation in leaf and tiller number was greater than 30% of total variation.

Table 3.1 Probability levels of block, population and clone main effects from univariate analyses of variance on populations of *Elymus repens* grown in a common environment. Population mean squares were tested against clone (nested within populations) mean squares. Transformations of variables are indicated by: † $\log_{10}$ and †† square root.

	Analysis of Variance Effect		
Variable	Block	Population	Clone
Morphological			
Erectness	0.373	0.001	0.001
Leaf sheath hairs	0.432	0.001	0.001
Leaf width	0.025	0.001	0.001
Leaf hairs†	0.003	0.001	0.001
Shoot			
No. shoot complexes	0.055	0.001	0.356
Stem height	0.051	0.001	0.001
Tiller number†	0.104	0.001	0.001
No. leaves†	0.002	0.001	0.001
Leaves tiller^{-1}	0.105	0.001	0.265
Rhizome			
No. primary rhizomes	0.275	0.040	0.001
Total rhizome length††	0.143	0.001	0.001
Mean rhizome length††	0.612	0.001	0.350
Total no. buds††	0.163	0.004	0.001
Mean buds per rhizome††	0.656	0.001	0.037
Internode length†	0.269	0.001	0.484
Rhizome width	0.191	0.001	0.699

Table 3.2 Variance components estimated from analysis of variance model for random effects for three types of characters, morphological, shoot and rhizome, measured on populations of *Elymus repens* grown in a common environment. Each variance component is expressed as a fraction of total variance components (in parentheses) and represents, for populations and clones respectively, the intraclass correlation coefficients t_{pop} and t_{clone}. Variance components for significant factors in the analysis of variance (Table 3.1) are emboldened.

	Variance Component		
Variable	Population	Clone	Error
Morphological			
Erectness	**0.115**	**0.215**	0.726
	(0.11)	(0.20)	(0.69)
Leaf sheath hairs	**0.752**	**0.383**	1.293
	(0.31)	(0.16)	(0.53)
Leaf width	**0.612**	**0.660**	1.583
	(0.21)	(0.23)	(0.56)
Leaf hairs	**0.145**	**0.160**	0.091
	(0.37)	(0.40)	(0.23)
Shoot			
No. shoot complexes	**0.073**	0.016	0.893
	(0.08)	(0.02)	(0.91)
Stem height	**13.00**	**15.88**	**44.35**
	(0.19)	(0.16)	(0.65)
Tiller number	**0.008**	**0.004**	0.014
	(0.31)	(0.16)	(0.53)
No. leaves	**0.010**	**0.005**	0.016
	(0.32)	(0.18)	(0.50)
Leaves tiller^{-1}	**0.037**	0.010	0.308
	(0.10)	(0.03)	(0.87)
Rhizome			
No. primary rhizomes	**0.094**	**0.403**	1.397
	(0.05)	(0.21)	(0.83)
Total rhizome length	**4.31**	**6.37**	**28.46**
	(0.11)	(0.16)	(0.73)
Mean rhizome length	**1.275**	0.176	8.814
	(0.12)	(0.02)	(0.86)
Total no. buds	**0.159**	**0.363**	1.153
	(0.10)	(0.22)	(0.69)
Mean buds per rhizome	**0.040**	**0.024**	0.228
	(0.14)	(0.08)	(0.78)
Internode length	**0.009**	0.000	0.012
	(0.07)	(0.00)	(0.93)
Rhizome width	**0.032**	-0.004	0.159
	(0.17)	(-0.24)	(0.85)

Low population components (< 10%) were found for characters with little clonal variation but high error variation (no. of shoot complexes, internode length) and for characters with relatively large clonal components (rhizome no., rhizome bud no.). Genetic variation at the clone level was important for both shoot and rhizome characters. Over 20% of total variation was clonal in origin for number of rhizomes and rhizome buds and greater than 15% for shoot height, tiller and leaf number and rhizome length.

3.3.2 Within population variation

Analysis of clonal differences within individual populations revealed that some contained more genetically determined variation than others (Table 3.3). For instance, population D2 possessed significant ($P \leq 0.05$) clonal differences for eight of 15 characters, whilst two populations (G2, N1) showed no significant clone effects for any characters. Fewer populations (n=11) had significant clonal variation in rhizome characters compared to shoot (n=18) and morphological variables (n=20) (Table 3.3). For all population-rhizome character combinations, 15% of clone effects were significant at $P \leq 0.05$. Shoot characters had 18% significant combinations, but morphological characters had a greater proportion (32%) of significant clone effects. Overall, 20% of possible population-character combinations were significant at the 95% level.

3.3.3 Multivariate analysis

When all characters were considered jointly, both the population and clone effects were highly significant (Table 3.4a). Although the first seven derived canonical variates were significant (Likelihood ratio test, $P \leq 0.05$), interpretation of the multivariate data structure is limited here to the first three variates which together explained 53% of total variation (Table 3.4b). Correlations between the original variables and

Table 3.3 Broad sense heritability values representing the proportion of total variance components attributable to clone differences from analyses of variance on individual populations of *Elymus repens* grown in a common environment. Only values for populations with significant ($P < 0.05$) clone effect are shown.

Morphological

Population	A1	B1	B2	C1	C2	D1	D2	E2	F1	F2	H1	H2	I1	I2	J1	J2	K1	K2	L1	L2
Erectness	-	-	-	0,57	0,61	-	0,68	-	-	0,68	-	-	-	0,54	-	0,78	-	-	-	-
Leaf width	-	0,65	-	-	0,40	0,59	0,66	0,68	-	0,73	-	0,52	-	-	0,35	-	-	-	0,54	-
Leaf hairs	0,46	0,75	0,86	-	0,68	0,85	0,86	-	0,49	-	0,66	0,69	0,60	-	-	-	0,87	0,40	0,93	0,93
Stem hairs	-	-	0,82	-	-	0,91	0,60	-	-	-	0,44	-	-	-	-	-	0,64	-	-	-

Shoot

Population	A1	A2	B1	B2	C1	C2	D1	D2	E1	F2	G1	I1	I2	J1	K2	L1	L2	M2
Shoot height	-	-	-	-	-	-	-	0,47	-	0,52	-	0,64	0,52	-	0,52	-	0,66	-
No. shoots	-	-	0,56	-	-	-	-	-	-	-	0,64	-	-	-	-	-	-	-
No. tillers	-	-	-	-	-	0,59	0,54	-	-	-	-	-	-	-	-	0,56	-	0,63
No. leaves	-	0,47	0,61	-	0,57	-	0,56	-	-	-	-	-	-	-	-	0,52	-	-
Lvs tiller^{-1}	0,50	-	-	0,47	-	0,49	-	-	0,71	-	-	0,51	-	0,42	-	-	-	-

Rhizome

Population	A1	B1	B2	C2	D2	F1	H2	I2	J1	K1	L1
No. rhizomes	0,55	0,67	0,63	0,67	0,57	-	0,71	0,61	-	-	-
Total length	0,46	-	-	-	0,71	-	0,51	-	-	0,57	0,65
Mean length	-	-	-	-	-	0,45	-	-	0,36	-	-
No. buds	0,83	-	0,71	-	0,83	-	0,64	0,58	0,35	-	0,66
Buds rhizome^{-1}	-	-	-	-	-	-	0,51	-	0,39	-	-
Internode length	-	0,74	-	-	-	-	-	-	-	-	-

Table 3.4 Significance of factors in multivariate analysis of variance (MANOVA) (a), and correlations between original variables and canonical variates from canonical discriminants analysis (b), for clone means of morphological, shoot and rhizome characters measured on 26 populations of *Elymus repens* grown in a common environment. MANOVA was carried out on data assuming blocks were replicates (i.e. no block effects). The variance extracted by each canonical variate is given in (b).

(a) Multivariate Analysis of Variance

Factor	Wilks'	F approximation	d.f.	*P*
Population	0.00144	2.43	400, 1558	0.0001
Clone	0.00014	1.59	2016, 4287	0.0001

(b) Canonical Discriminant Analysis

Variable	Canonical Variate U_1	U_2	U_3
Erectness	-0.286	0.001	-0.363
Leaf sheath hairiness	0.145	0.559	-0.421
Leaf width	-0.690	-0.058	-0.024
Leaf hairs	0.117	0.755	0.281
Shoot complexes	-0.234	-0.131	0.363
Stem height	0.075	-0.192	0.476
Tiller number	0.690	0.256	-0.262
Leaf number	0.724	0.169	-0.234
Leaves tiller^{-1}	0.044	-0.357	0.152
Rhizome number	-0.321	0.014	0.119
Total rhizome length	-0.328	-0.084	0.418
Mean rhizome length	-0.130	-0.048	0.474
Number rhizome buds	-0.400	-0.030	0.298
Mean buds rhizome^{-1}	-0.240	0.017	0.357
Internode length	0.081	-0.092	0.558
Rhizome width	-0.681	0.294	0.052
Variance extracted	0.204	0.180	0.143

canonical variates showed that the first canonical variate, U_1, differentiated clones with narrow leaves and poor rhizome development from vigorously tillering plants which produced many leaves. The amount of leaf and leaf sheath hairyness defined the second canonical variate, U_2 (18% of variance extracted). On the third variate, which accounted for 14% of total variance, clones with longer rhizomes contrasted with erect and sparsely tillering plants.

Population centroids, plotted in the space of U_1 and U_2, and U_1 and U_3, showed that few distinctive groupings of populations emerged (Figs. 3.1). The plots showed that: (a) in some cases contrasted populations (hedgerow vs crop) from the same site resembled each other strongly, e.g. populations from sites D and K and from J (Fig. 3.1); (b) groupings of populations from similar cultural environments (e.g. all hedgerow) could be more alike than to populations from the same site but different environments, i.e. the comparison between hedgerow populations from sites L, I at U_1: 2.0, U_3: 0.5, and populations from the crop environment at sites L, I (Fig 3.1). However, there was no consistent overall pattern in relation to population source.

3.4 Discussion

Experiments involving clonal material allow greater precision in estimating environmental variance (between replicate clones) than those with progeny arrays. However, inheritance over a generation from parent to progeny is not observed and it is less relevant to the ecogenetics of natural populations because seed and seedling stages of the life cycle are not considered (although *E. repens* seedling recruitment is thought to be rare in the field). Furthermore, common garden experiments under artificial conditions, as here, will overestimate additive variation because environmental variation is less in controlled environments

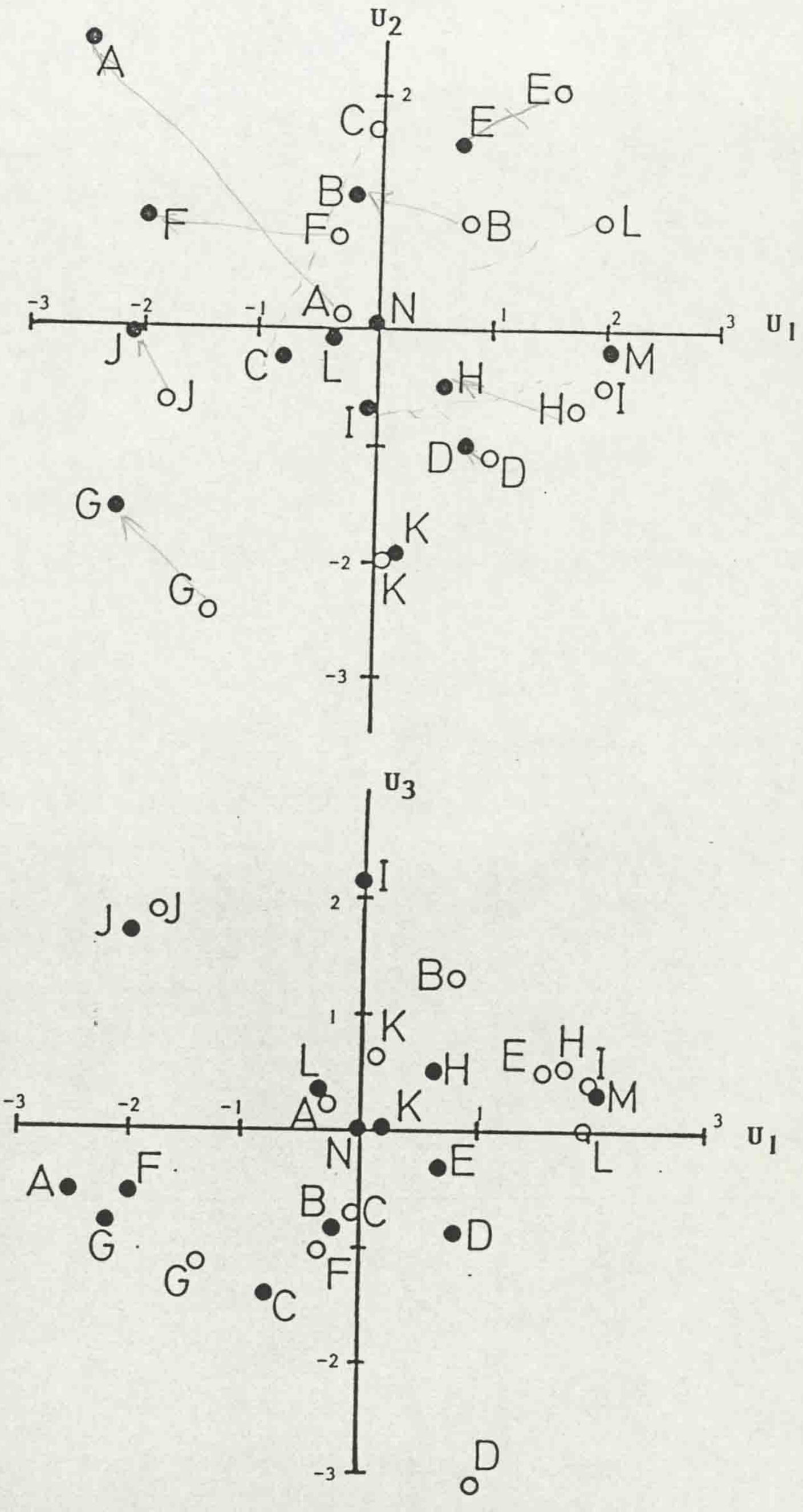

Figure 3.1 Disposition of population means on three canonical variates describing the growth and morphology of 26 Elymus repens populations, from a canonical discriminant analysis. Canonical variates, U_1–U_3, summarise population response on 16 original variables. Site affiliations are indicated by letter. Symbols: (○) population previously unexposed to herbicide; (●) population with history of herbicide use.

(Venable, 1984). This method therefore does not completely separate phenotypic from genetically based differences, but reveals differences between genotype-specific norms of reaction under controlled conditions. The use of clonal material has the further drawback of carry-over effects (Falconer, 1981; Evans, 1986). Such effects were unlikely in this study because plants were grown in a common environment for two years prior to the experiment.

3.4.1 Population and clonal variation

Evidence of strong population differentiation was found for all characters. This confirms the observations on *E. repens* morphology of Holly and Parker (in Palmer & Sagar, 1963) and Neuteboom (1973), who found population level variants in growth habit, hairyness of leaves and sheath bases and in leaf width. Within-clone variation was relatively high for morphological variables (> 50%), except for leaf hair density. For the latter character, population and clonal variation were about equal in magnitude and greater than within-clone variation (23%). Two shoot characters, the number of leaves $tiller^{-1}$ and number of shoot complexes initiated, were not genetically controlled in this experiment. Both characters had high levels of error variation which may be attributable to growth stage differences at transplanting. For the latter character, Williams (1973) found conflicting evidence of genetic determination: no differences were evident between seedlings from different areas in the number of secondary shoots but in another experiment he found differences between clones, established from rhizomes, within areas.

Variation amongst populations, representing about 18% of total variation, was generally less than for other sources of variation. Clonal differentiation was important in all characters which measured the extent or size of rhizome matter, except for mean rhizome length. The two

variables expressing rhizome form, internode distance and rhizome width, showed no significant clone effects. Neuteboom (1975), however, did find evidence of clones with significantly different rhizome thicknesses. Where significant clonal variation was recorded in the present study, it accounted for 8-22% of total variation, irrespective of character type.

Populations varied considerably in the presence of clonal differentiation, ranging from populations with no genetic component for any characters to those with genetic determination of both shoot, rhizome and morphological characters. In this study the explanation for fewer populations showing clonal variation in rhizome characters, compared to other types of character, may be the duration of the experiment. There was less opportunity for clone effects to be expressed in rhizome variables because rhizomes were either initiated or not: this meant that more data were absent for all rhizome variables (except for the numbers of rhizomes initiated).

3.4.2 Selection on life-history characters

Fitness of a genotype is difficult to define in perennial species, such as *E. repens*, which spread vegetatively as well as by seed and therefore characters expressing both aspects require consideration. In this study, fecundity was not measured directly. It has been shown, however, that tiller number is often a good measure of fitness (Turkington, 1983; Silander, 1985) through association with survival, dry matter production or fecundity. Total rhizome length and, in particular, the number of rhizome buds will also estimate fitness. In a purely vegetatively spreading population of *E. repens*, e.g. maintained by grazing, mowing or recurrent disturbance, such measures may have more ecological relevance for persistence in a habitat than measures of sexual fecundity. The presence of considerable variation under genetic control for such

characters, if closely linked to fitness, runs counter to Fisher's (1958) prediction that additive genetic variation will be driven to zero by periods of directional selection for fitness. Yet, Venable (1984) reviewed many studies and described heritable variation in many life history traits which were considered to be correlated to fitness.

The degree of variation between clones can reflect the potential response to selection. The product of selection, however, is evident in variation between populations (Bradshaw, 1984a). Population differentiation has been demonstrated for all characters measured. It is reported to be very common in many plant species (Heslop-Harrison, 1964; Bradshaw, 1984a). Rather than only demonstrating such patterns, it is more interesting to interpret them in terms of past selection pressures. Caution should be exercised in such investigation not to invoke adaptation, where simpler hypotheses would do (Gould & Lewontin, 1979). Here, a simple association between population differentiation and past cultivation regime is hypothesised and tested on a large number of populations. Although populations were differentiated in multivariate space, the pattern of variation was essentially continuous. It is therefore necessary to reject the possibility of selection by cultivation practices having influenced the complex of characters describing growth and morphology of these *E. repens* populations. Neuteboom (1975) also found no apparent correlation between type of field (arable, ley or mown grasslands) and shoot characters or secondary shoot complex initiation.

Although cultivation regime can be a strong selective agent for growth form (Warwick & Briggs, 1978a; 1978b) and life history characters (Law *et al.*, 1977), this study suggests that it has not acted in *E. repens*, at least within the populations sampled. This is despite the demonstration that additive genetic variation was present. The presence

of rhizome buds in the soil, not eliminated in herbicide-treated cultivated areas, may buffer selection. Secondly, phenotypic plasticity may be considerable and will not allow expression of the genotype (Schlichting, 1986, Sultan, 1987). Of course, the intensity and direction of selective pressures may be insufficiently different in the two apparently contrasted cultivation regimes.

CHAPTER 4
GENETIC VARIATION FOR HERBICIDE RESPONSE IN *ALOPECURUS MYOSUROIDES*

4.1 Introduction

The extent and nature of genetic variability among weed populations in herbicide response is of theoretical and practical interest because of the widespread, long-term use of herbicides and the evolution of resistant populations in over 50 weed species (LeBaron, pers. comm.),. Evidence for the evolution of herbicide resistance has primarily come from demonstrations of significant variation between populations in herbicide response where a single population fails to respond to herbicide doses which kill susceptible populations (Lutman & Lovegrove, 1985; Moss & Cussans, 1985). Such genetic changes probably result from the strong selection pressures imposed by persistent herbicide application (LeBaron & Gressel, 1982; Bradshaw, 1984a). Stronger evidence comes from selection experiments which demonstrate increased tolerance to herbicides over generations (Grignac, 1978; Jana & Naylor, 1982) and, to a lesser degree, studies which have compared herbicide response of populations either unexposed or exposed to herbicides (Thai, Jana & Naylor, 1985).

The potential for evolution of a character in natural populations depends on the presence of genetic variability *within* populations. Weed species are known to contain high levels of genetic variation (Barratt, 1981) and within population variability in herbicide response has been demonstrated in such species as *Senecio vulgaris* L. (Holliday, 1978), *Brassica olearacea* L. (Sykes, 1980), and *Avena barbata* Brott. and *A. fatua* L. (Price, Hill & Allard, 1983). Selection acting on such levels of variation is thought to have led to the evolution of herbicide resistance in a number of weed species (Putwain, Holliday & Sykes, 1982).

Intraspecific variation in urea herbicide response has been detected in the grass *A. myosuroides* (Niemann and Pestemer, 1984; Moss & Cussans, 1985, 1987). Niemann and Pestemer (1984) showed enhanced tolerance in two of 13 populations. Moss and Cussans (1985; 1987) compared about 80 populations and documented greatly enhanced tolerance to chlorotoluron in populations from one farm, together with a similar degree of tolerance to that found by Niemann and Pestemer (1984) in two other populations. In these reports a herbicide dose in the range 1-2 times the recommended agricultural rate was required to reduce shoot fresh weight of the most resistant population by about 50%. At such rates, mortality was only about 10% (Moss & Cussans, 1987). Dose-response trials based on biomass are affected by time of harvest and therefore can overestimate the degree of resistance, calculated by mortality response, if the effect of the herbicide is only temporary.

To further quantify the variation between and within populations of *A. myosuroides* three approaches were taken. Firstly, mortality and shoot biomass reduction were examined in relation to herbicide dose for 10 populations in a soil-based experiment. Populations of known degree of resistance (Moss & Cussans, 1985; 1987) were included for comparison with new collections of populations. Secondly, for two populations known to differ in herbicide response, dose-response in nutrient solution was studied to eliminate any possible population differences in leaf morphology, leaf uptake or rooting depth. Thirdly, within population variation in resistance was examined for three populations in nutrient culture in a half-sib trial. The objectives of these investigations were to determine: (1) the pattern of response among families and individuals; (2) the magnitude of genetic effects; and (3) population differences in genetic response.

4.2 Materials and Methods

4.2.1 Experimental procedure

Experiment 1: Soil dose-response

Seed (= spikelets) was collected from populations in fields regularly treated with urea herbicides where poor control had been reported and from populations with a known degree of resistance to chlorotoluron (Moss and Cussans, 1985, 1987) (Table 4.1). The latter ranged from populations designated as 'susceptible' (Rothampsted), 'intermediate resistant' (Brickhouse) and 'resistant' (Peldon) (Table 4.1). Seed stocks were bulked up by propagation over one to two generations, each collection being raised as an isolated interbreeding population in a glasshouse or polythene tunnel.

Between 120-200 seeds (120: Faringdon; 150: Gosberton, Denby, Strainfield; 200: other populations) were sown at a depth of 0.5 cm in 10 cm diam. pots containing J.I. No. 1 loam-based compost. Pots were laid out in a randomised, complete block design with five replicates, on two benches in a heated glasshouse. Pots were watered and covered for 10 days with clear polythene to encourage germination. After 19 days the number of established seedlings was counted. Establishment varied significantly between populations (analysis of variance [ANOVA] on arcsin square root emergence proportion, $P \leq 0.05$) and ranged (± standard error) from 17 (±1) to 108 (±1) plants pot^{-1}. Plants were not thinned, to ensure sufficient numbers to accurately determine mortality after spraying and to prevent disturbance to young seedlings.

When plants were at the 1-2 leaf stage, pots were sprayed with the following doses: 0.0, 0.2 (Faringdon excepted), 0.4, 0.8, 1.6, 3.2, and 6.4 kg a.i. chlorotoluron (formulated as 'Dicurane 500 FW') ha^{-1}. Peldon and Brickhouse populations were additionally sprayed with: 12.8 and 25.6 kg

Table 4.1. Location, year of seed collection and history of herbicide application for *Alopecurus myosuroides* populations used in experiments examining genetic variation for herbicide response. Some collections of seeds were kindly provided by others and the source of these is indicated by: + Weed Research Organisation, ++ May and Baker Ltd.

Population	Location (O.S. Grid Ref.)	Year originally collected	Years urea herbicides used (to 1984)
Faringdon	Oxfordshire (SU 290950)	1984+	10
Rothampsted	Hertfordshire (TL 140130)	1984+	0
Vare	Hertfordshire (TL 356145)	1983++	2
Tillingham	Essex (TL 995036)	1983++	2+
Whitton	Humberside (SE 900240)	1985	6
Denby	Lincolnshire (TF 430820)	1983++	2+
Strainfield	Lincolnshire (TF 110730)	1983++	2+
Gosberton	Lincolnshire (TF 240310)	1985	>10
Peldon	Essex (TL 985169)	1984+	9
Brickhouse	Essex (TQ 990160)	1984+	9

a.i. ha^{-1}. Herbicide was applied with an Oxford Precision Sprayer in 400 l ha^{-1} water at 2.3 bar with Teejet nozzles. Pots were kept in a glasshouse for a further 45 days and watered with a fine rose from above as necessary. At harvest, 64 days after sowing, the number of survivors was counted. Shoot material was clipped at soil level and dry weight determined after drying for 4 days at 80°C.

Experiment 2: Nutrient solution dose-response

Seeds were collected as in Experiment 1. The experiment was laid out as a completely randomised, block design in a growth chamber with 10 replicates of two populations, Peldon and Rothampsted. Each block contained eight pots of each population. Lots of 50 seeds of each population were floated on a 80 cm^3 raft of polypropylene beads in 250 cm^3 of 0.5 g l^{-1} $Ca(NO_3)_2$ nutrient solution in each pot. After 16 days pots were removed and seedlings thinned to 10 per pot. Nutrient solution was replaced with a fresh solution containing: 0.0, 0.00001, 0.00005, 0.0001, 0.0005, 0.001, 0.01, and 0.1 ml l^{-1} a.i. chlorotoluron. Pots were re-randomised when replaced and after a further 16 days, the length of the longest root (mm) of each seedling was measured. The status (dead/alive) of the shoot system was also noted.

The growth chamber had a 12 hour daylength, with light intensity at 100 $\mu e\ cm^{-2}s^{-1}$, a temperature cycle of 20/15°C and relative humidity of 85/55%.

Experiment 3: Within population variation

Seed was collected from three of the sites in Table 4.1: (1) plots never previously sprayed with herbicides in Broadbalk Field, Rothampsted Experimental Station, Harpenden, Herts (Rothampsted); (2) a continuous wheat field recurrently sprayed with urea herbicide (see Table 1.1 for herbicide use) (Peldon) ; (3) a similar field at Brickhouse. At the Peldon

and Brickhouse sites, 50 plants with more than four seed heads were sampled from areas of approximately 20 x 10 m in the standing crop. Plants roughly 1 m apart were selected systematically, starting with a randomly-sited plant. Sixty plants were sampled from the Rothampsted site in the same manner, but only from the edge of pre-existing experimental plots. Mature seeds from each individual plant (= family) were collected and kept separate. Twenty-five families were randomly chosen from plants at each site and the seed from each family divided into three approximately equal lots. Three populations were used: Peldon, Brickhouse and Rothampsted.

The three seed populations were sown in a randomised, complete block design with three replicates. Each block contained all 25 families of each population. Seed lots were sown in the same manner as described for Experiment 2 and were grown under the same growth chamber conditions. After 18 days pots were removed, seedlings thinned to five per pot and the longest root length (mm) measured. Nutrient solution was replaced with fresh solution containing 0.001 ml l^{-1} a.i. chlorotoluron. Similar harvest procedures were carried out as for Experiment 2 except that plants were finally harvested after 14 days in herbicide solution.

4.2.2 Analysis

Experiment 1

Mortality was initially analysed by probit analysis (Finney, 1971). However, evidence of systematic departures from the probit model were found. At low doses fewer plants died than expected and at intermediate doses, more plants died than was expected by the probit model. Heterogeneity chi-squared (χ^2) values, indicative of lack of fit, were highly significant for nine of the 10 populations.

An alternative dose-response model, based on a logistic curve (Finney, 1978) was therefore used. This technique is applicable to both quantal and quantitative data. When the minimum and maximum responses are 0.0 and 1.0 respectively, the model is:

$$Y = \frac{1}{1 + (X / C)^b}$$

where Y= response, X= dose (untransformed), and **C** and **b** are experimentally determined parameters. The parameter **C** is the dose at which the expected response is 0.5 and is termed the ID_{50} (Finney, 1978). It is equivalent to the ED_{50} for continuous data and the LD_{50} for quantal responses. Quantitative data were expressed as a proportion of the mean control (unsprayed) response. The model was fitted using SAS NLIN procedure using an iterative least squares algorithm (SAS Inst. Inc., 1985). To reduce heterogeneity of error variances, a Box-Cox transformation was used with λ= 0.25 (Draper & Smith, 1981; Streibig, 1987).

Experiment 2

Mean root growth per pot was expressed as a proportion of root growth in control solution and analysed by the logistic model (as in Experiment 1). Seedling mortality was pooled over blocks. At each dose, proportionate mortality of the two populations was compared by a G-test (Sokal & Rohlf, 1981).

Experiment 3

Individual seedling root length increment was transformed to square root (x + 0.5) to eliminate dependence of variance on mean (Sokal & Rohlf, 1981) of family data. Block effects were not significant (Table 4.3) and were ignored in subsequent calculation of genetic parameters. Estimates of variance components, intraclass correlation coefficients and

heritabilities were obtained from an analysis of variance of half-sibs (Falconer, 1981; Faulkner, 1981). The population mean square was tested over the family mean square. The family mean square, if significant, shows that the character is heritable. The intraclass correlation coefficient, t_r, expresses the degree of resemblance between relatives without taking into account the breeding system.

Mating was assumed to be at random because *A. myosuroides* is an outbreeder (Beddows, 1931). A number of spikes from the three populations were individually bagged to assess outbreeding, but no selfing was recorded. Genotype-environment interactions were assumed to be absent because of randomisation procedures during the experiment. The model for estimation of heritability assumes that no maternal or dominance effects were present.

4.3 Results

4.3.1 Soil dose-response

Survival

The effect of herbicide dose rate on plant survival (Experiment 1) is presented in Fig. 4.1. Comparison of survival at 6.4 kg ha^{-1} illustrates variation among populations. In most populations 30-40% of plants survived, whereas survival in the two resistant populations (Peldon, Brickhouse) was about 70% . Indeed, at two to four times that dose, survival was still 30-40% in the latter populations. Two other populations had above average survival. In the Faringdon population, survival was similar to the resistant populations over the dose range up to 6.4 kg ha^{-1}. Also, at 6.4 kg ha^{-1} about 60% of Whitton plants survived. Calculated values of the estimated dose required to kill 50% of individuals in each population are given in Table 4.2. For six populations, ID_{50} values were in the range 4.7-7.3 kg ha^{-1}. One

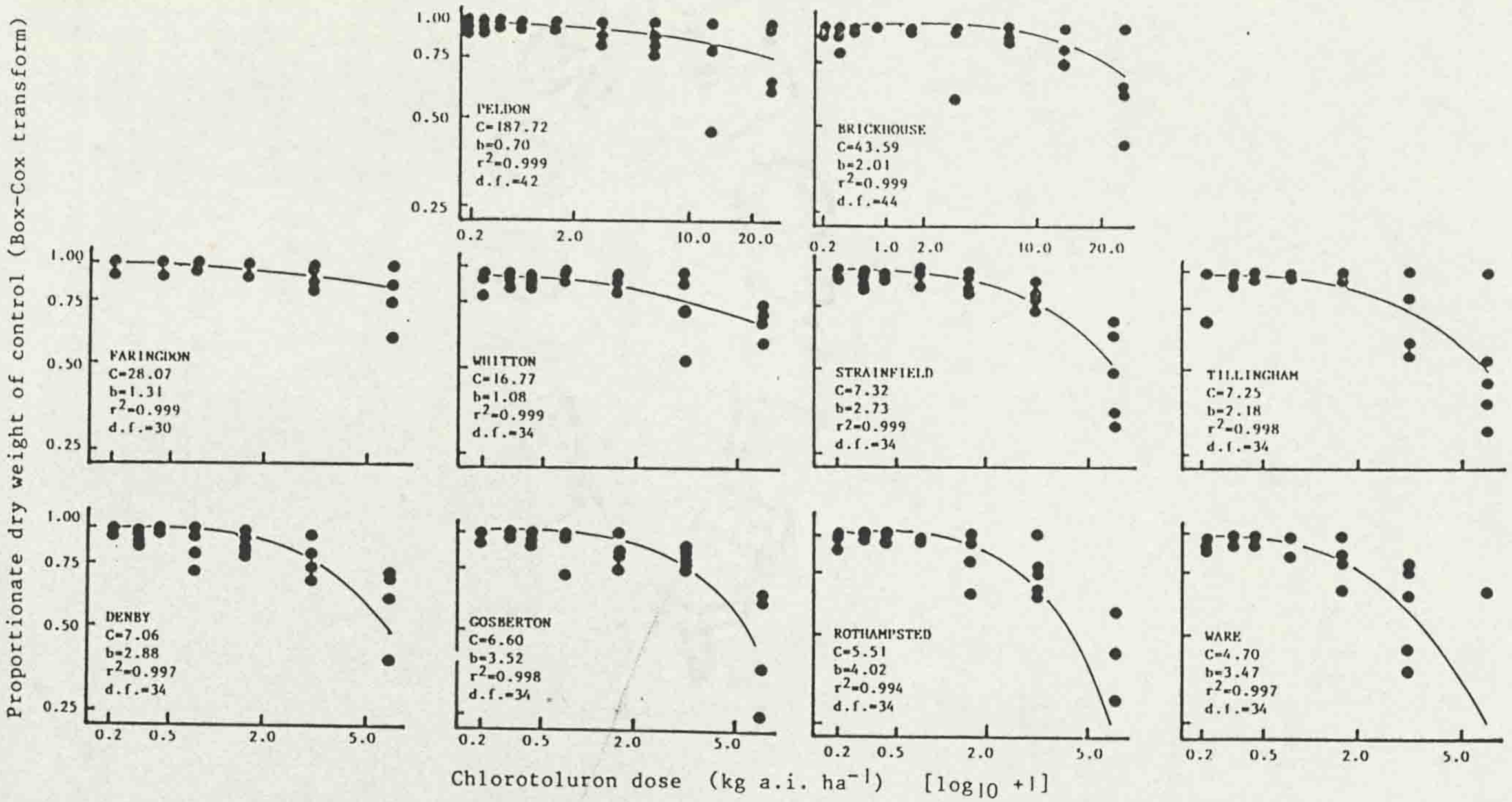

Figure 4.1 Relationship between proportionate survival (Y) and dose (X) for Alopecurus myosuroides populations. Fitted lines are non-linear regressions using the model: $Y^{0.25} = [1/(X/C)^b]^{0.25}$, where C= an estimate of ID_{50} and b= a slope parameter. Coefficient of determination (r^2) and d.f. are given for each population. Note the different horizontal scale for the Peldon and Brickhouse populations.

Table 4.2 Response of *Alopecrus myosuroides* populations to chlorotoluron sprayed post-emergence, as measured by the estimated dose required to cause 50% mortality or a 50% reduction in dry weight plant^{-1} [ID_{50} values (± S.E.)]. Populations are ranked by mortality ID_{50}.

	ID_{50}, kg ha^{-1} (± S.E.)			
Population	Mortality		Dry weight plant^{-1}	
Ware	4.70	(0.32)	2.75	(2.75)
Rothampsted	5.51	(0.41)	2.41	(0.14)
Gosberton	6.60	(0.27)	3.23	(0.17)
Denby	7.06	(0.53)	3.44	(0.12)
Tillingham	7.25	(0.60)	2.81	(0.20)
Strainfield	7.30	(0.33)	4.20	(0.22)
Whitton	16.77	(4.43)	2.81	(0.18)
Faringdon	28.07	(16.93)	3.86	(0.31)
Brickhouse	43.59	(11.60)	10.19	(0.83)
Peldon	187.72	(182.01)	9.44	(1.13)

Table 4.3 Hierarchical analysis of variance of root length increment, measured in herbicide solution, for half-sibs of three populations of *Alopecurus myosuroides*. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1982).

Source	d.f.	M.S.	*F*	*P*
Block	2	1.05	0.45	> 0.63
Populations	2	363.62	78.99	< 0.0001
Families	72	4.60	1.98	< 0.0001
Error	1000	2.32		
Total	1076			

population, Ware, was significantly ($P<0.05$) more sensitive than all of these except Rothampsted. Whitton had an ID_{50} value double that of this group, yet was not significantly different from three of the populations in the group.

For other populations showing various degrees of resistance, ID_{50} values were more poorly estimated. Particularly for Peldon and Brickhouse, a number of pots showed little or no response to the highest doses (Fig. 4.1). The magnitude of ID_{50} values, however, showed that Faringdon was four times as resistant as other sensitive populations and Brickhouse was twice as resistant as Faringdon. By contrast, Peldon was four times as resistant as the Brickhouse population.

Dry matter production

Dry weight, expressed per surviving plant to compensate for variation in initial seedling density, showed a greater response to herbicide dose rate than survival (Fig. 4.2). All populations, except Brickhouse and Peldon, exhibited reductions in dry weight $plant^{-1}$ at 1.6 kg ha^{-1} and some at 0.8 kg ha^{-1}. Depression of dry weight of the same order for the two resistant populations required a dose of 6.4 kg ha^{-1}. Variation in quantitative response was more successfully modelled than survival: standard errors of ID_{50} values were relatively lower (Table 4.2). In terms of biomass reduction, the Whitton population was not differentiated from other populations sensitive to chlorotoluron (ID_{50}: 2.7-4.2 kg ha^{-1}). In these populations, the Strainfield population had significantly ($P<0.05$) greater biomass than all except Faringdon. Resistant populations (Brickhouse, Peldon) had similar ID_{50}'s, two to three times larger than all other populations.

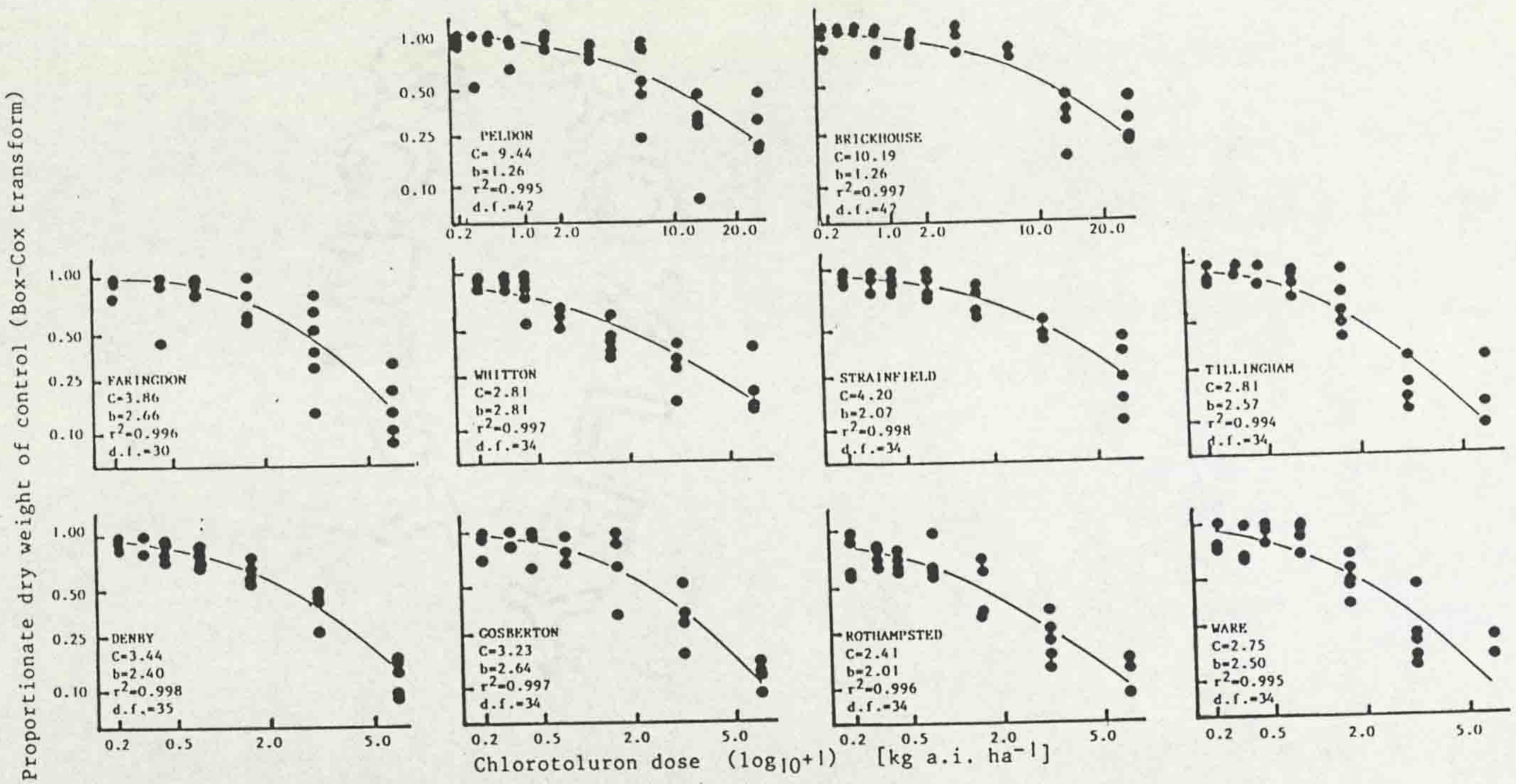

Figure 4.2 Relationship between dry weight plant^{-1} expressed as a proportion of control (X) and dose (X) for Alopecurus myosuroides populations. Details as for Figure 4.1.

4.3.2 Nutrient solution dose-response

The difference between populations described in Experiment 1 was much reduced in nutrient culture at the highest doses (Fig. 4.3), as shown by the greater effect on the Peldon population. The fitted curve for the Rothampsted population was considerably flatter than for Peldon, but growth was affected more at low doses. Comparison of 95% confidence limits of ID_{50} values showed a significant difference: growth of the Peldon population (± S.E.) was reduced by 50% at 0.0026 (± 0.0006) ml l^{-1} chlorotoluron while the Rothampsted population required 0.00015 (± 0.00008) ml l^{-1}.

Few plants were recorded as dead in either population up to 0.0005 ml l^{-1}. Thereafter, higher doses killed more Rothampsted than Peldon plants. At 0.001 ml l^{-1} only Rothampsted plants died (G_{adj}=13.0, $P \leq 0.05$) and at 0.01 ml l^{-1} 43% died, compared to only 6% of Peldon plants (G=39.0, $P \leq 0.001$). Ninety-three percent of Rothampsted plants had died at the highest dose, but only 54% of Peldon plants (G=41.8, $P \leq 0.001$).

4.3.3 Within population variation

Analysis of variance on root increment (Table 4.3) showed that the families effect was highly significant ($P \leq 0.0001$), indicating that variation in this character has a heritable component. A significant population mean squares indicated that there were systematic differences in root growth response to chlorotoluron between populations, over and above that between families within the populations. Estimates of genetic parameters were calculated by separate analyses for each population (Table 4.4) and show that all family effects were significant ($P \leq 0.05$). The degree of resemblance between relatives, t_r, was similar for the three populations and heritability values, though higher for Rothampsted and Brickhouse populations, were not significantly different.

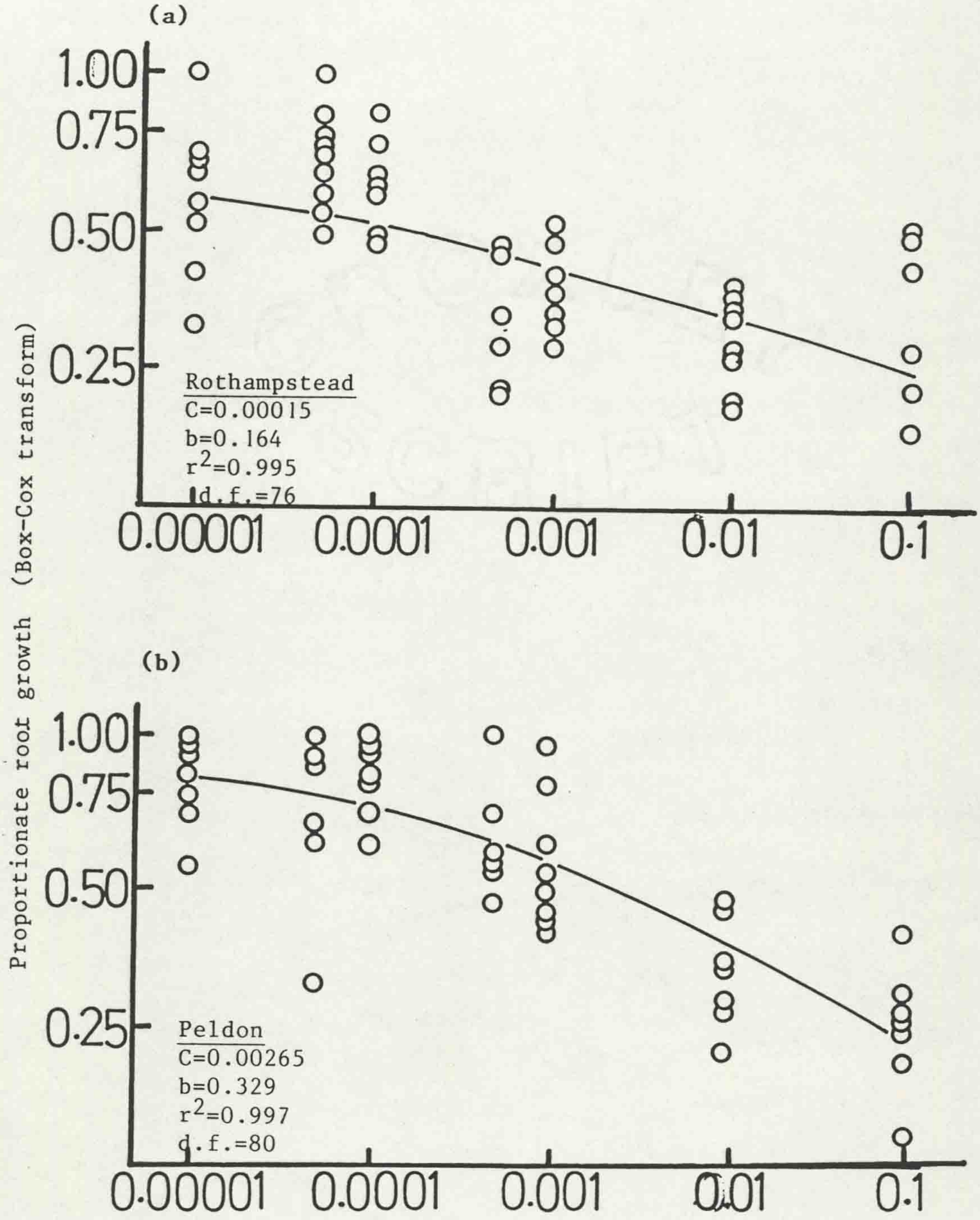

Figure 4.3 The relationship between root growth increment, expressed as a proportion of growth in control, and chlorotoluron herbicide dose for two populations (biotypes) of Alopecurus myosuroides, grown in nutrient solution. (a) Rothampstead and (b) Peldon population. Fitted lines are non-linear regressions. See Figure 4.1 for details.

Table 4.4 Significance of the family effect in separate analyses of variance and genetic parameters for three *Alopecurus myosuroides* populations. σ^2_T and σ^2_W are the between and within families variance components, respectively, t_r is the intraclass correlation coefficient and h^2_N is the narrow-sense heritability (± Standard Error).

Population	Probability of family effect	σ^2_T	σ^2_W	t_r	h^2_N	±S.E.
Peldon	0.0123	0.193	3.480	0.053	0.210	±0.131
Brickhouse	0.0014	0.176	2.121	0.076	0.306	±0.152
Rothampsted	0.0006	0.121	1.303	0.085	0.340	±0.160

Table 4.5 Population means, sample size (n) and standard error (S.E.) of untransformed, and square root (+ 0.5) transformed, root increment (mm) for three *Alopecurus myosuroides* populations, grown in herbicide solution. Data is pooled over 25 families. Coefficient of variation (C.V.) is given for transformed data.

Population	n	Untransformed root increment ± S.E.	Transformed root increment ± S.E.	C.V.
Peldon	366	25.78 ± 0.99	4.757 ± 0.100	40
Brickhouse	355	12.97 ± 0.57	3.344 ± 0.080	45
Rothampsted	356	8.68 ± 0.37	2.784 ± 0.064	43

Frequency distributions of root increment pooled over families showed that there was overlap between distributions, particularly between Brickhouse and Rothampsted populations (Fig. 4.4). About 14% of plants in these two populations showed no root growth in the herbicide solution. Roots of these plants had ceased active growth and were senescing at harvest, though shoots were still green. Family mean distributions showed that only two means overlap between between Peldon and Rothampsted populations (Fig. 4.4). Mean untransformed root increment was 200% and 50% greater in Peldon and Brickhouse than in the Rothampsted population (Table 4.5). The Peldon population produced almost 100% more root growth than the Brickhouse population.

4.4 Discussion

4.4.1 Measurement of response

Seedling numbers at spraying in Experiment 1 varied by a factor of six and were high: equivalent to densities of 1000-6000 seedlings m^{-2}. The population with the highest seedling density, Whitton, had an elevated ID_{50} as assessed by mortality. However, when measured by biomass (dry weight $plant^{-1}$), it was as sensitive as other populations including the known susceptible population, Rothampsted. This may suggest that survival was enhanced by high seedling density but that the biomass response corrected for density. Positive (Mortimer, 1985; Chapter 7) and negative (Firbank, Mortimer & Putwain, 1985; Chapters 6, 7) density-dependent as well as density-independent (Manlove, 1985) seedling survival have been detected in the few studies of the interaction of density and herbicide on plant survival.

Estimation of ID_{50} values was adequate, as judged by standard errors, for most sensitive populations. When survival was high, either for resistant populations or because of density, values were more poorly

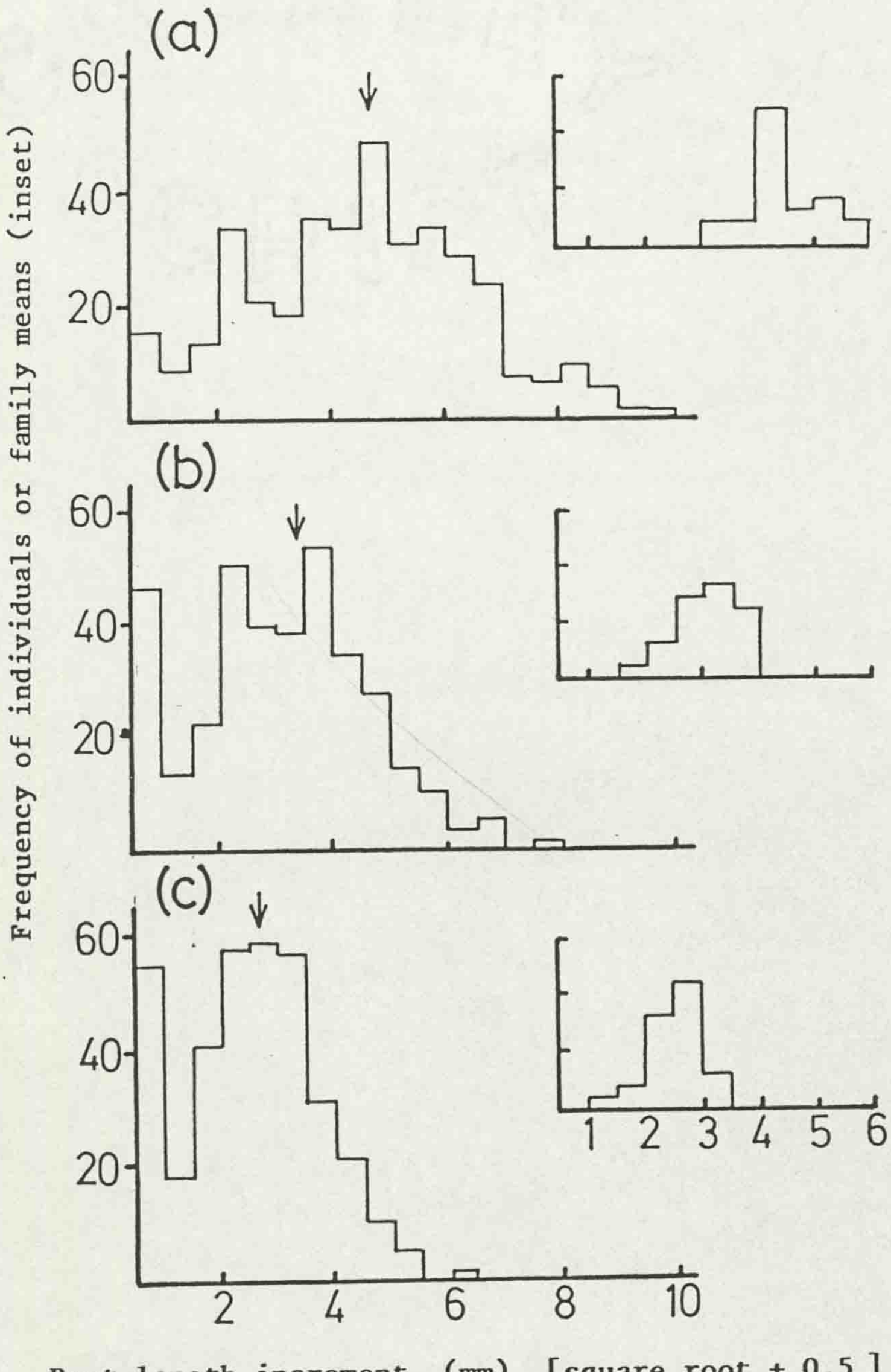

Figure 4.4 Frequency distributions of root length increment growth for three populations of Alopecurus myosuroides grown in nutrient solution with herbicide. (a) Peldon, (b) Brickhouse and (c) Rothampsted. Arrows represent the mean individual root length increment. Insets are distributions of family means.

estimated. This can be attributed to the presence of zero response at the lower end of the dose range and a lack of sufficient response at high doses. Higher doses are therefore needed for the resistant populations. Much improved accuracy was shown in quantitative response, which suggests that the dose range was satisfactory for this character. In Experiment 2, the higher doses in nutrient culture elicited sufficient response in the resistant population.

Dose ranges of up to three or four times recommended agricultural doses (Moss and Cussans, 1985, 1987) and greater ranges (Moss, 1987) of chlorotoluron have been used in previous studies of the Peldon population in soil culture. The response variable usually measured was reduction in shoot fresh weight. Up to two times recommended rates were reported to cause fresh weight reductions of about 50% in the Peldon population (recommended rate: about 2.5-2.8 kg a.i. ha^{-1}). In the present study, three times the recommended rate was needed. Estimates of ED_{50} are known to vary between experiments (Nyffeler *et al.*, 1982). For two populations studied by Moss and Cussans (1985), ED_{50} values varied by a factor of two in separate experiments. At dose rates of this magnitude, mortality of resistant populations has been reported as only about 10% (Moss & Cussans, 1987; Chapter 7).

If plants show growth reductions to herbicide treatment but can recover, dose-response experiments based on biomass may be inaccurate. Herbicide response will then be overestimated and the degree of resistance underestimated. Ratios of ID_{50} values for chlorotoluron of the Peldon (resistant) to the Rothampsted (susceptible) population provide a measure of degree of resistance. For the biomass data presented here, the ID_{50} ratio was 3.9 but equalled 34.0 when assessed by mortality. These values indicate that measures based on biomass can underestimate the

level of resistance. They span the range of fresh weight values found in other comparisons of the same two populations. In post-emergence experiments the ED_{50} ratio was 16.0 (Moss & Cussans, 1985) and 7.7 (Moss, 1987), while in a pre-emergence comparison the ED_{50} ratio was 6.6 (Moss & Cussans, 1987). The most accurate measure of herbicide response is a measure of fecundity reduction, which is also relevant to future infestations, but this is rarely estimated (but see Chapters 6 and 7).

In nutrient solution (Experiment 2), the Peldon population had an ID_{50} value of at least one order of magnitude greater than Rothampsted. The resistance ratio here was 18.0. Although the logistic model fitted these data well, at low dose rates many points fell above the predicted curve for the Rothampsted population (Fig. 4.3). This suggests that some stimulation of root growth may occur, though it was not observed in the other population. Kemp and Casely (1987) report a similar effect in nutrient culture in *A. myosuroides* and it appears to be an occasional phenomenon (Streibig, 1987).

4.4.2 Genetic potential

The expression of resistance to chlorotoluron may include some natural variation in root growth. Analysis of root length before herbicide was introduced into nutrient solution showed significant family effects. However, the order of population means was the reverse of that recorded after exposure to herbicide. Resistance variation is thus probably expressed at the population level and also within populations, but against a background of innate root growth variation.

The results of Experiment 3 confirm that heritable variation for chlorotoluron response was present in the *A. myosuroides* populations examined and that they differed in response. Populations were similar in the magnitude of heritability estimates. However, such values represent

the upper limits of the potential for response to selection (Mitchell-Olds & Rutledge, 1986). Because some families may contain full-sibs, true estimates of heritability may range to half the values reported here (Lawrence, 1984). The failure to detect any selfing in limited bagging of spikes (4.2.2 Experiment 3) of the three populations suggests that this possibility of error is reduced here.Secondly, if present, dominance and maternal effects can inflate heritability (Falconer, 1981). In this study, only three populations were investigated in one environment, therefore generalisations are necessarily cautious, although the similarity of heritabilities amongst populations is encouraging.

Substantial between-population variability in response to chlorotoluron was found here in Experiment 1 and also in the results of Moss and Cussans (1985, 1987). Examination of the magnitude of variation attributable to populations in these experiments (Table 4.4) indicates that a greater portion of total phenotypic variation is due to population differences than to family effects.

This study is the first demonstration of genetic variation in chlorotoluron response within natural populations of *A. myosuroides*. Heritable variation existed in both a population unexposed to any herbicide and in those exposed to recurrent chlorotoluron use (and which have already evolved resistance). The values of heritability (narrow sense) of 0.21-0.34 found here are low to moderate in terms of potential response to selection (Falconer, 1981). If selection is intense in a crop community recurrently treated with persistent herbicides (Chapter 7), the rate of evolutionary advance may be rapid.

Other studies have shown similar amounts of heritable variation. Price *et al.* (1985) found broad sense heritabilities of 0.11-0.63 for barban response in populations of three weed species not previously

exposed to the herbicide. Sykes (1980) showed narrow sense values of response to simazine of 0.32-0.37 in wild *Brassica olearacea* L. An experimental population of *Lolium perenne* L. had narrow sense heritabilities of paraquat response of 0.51-0.72 (Faulkner, 1974). There is therefore little evidence from this study that the herbicide-exposed populations lack or have less additive variation, compared to the susceptible population, as would be expected ultimately under severe directional selection (e.g. Fisher, 1958). Possible explanations are that too few generations of selection have elapsed, and/or that selection is weaker than anticipated (Gressel & Segel, 1978).

The agricultural consequences of the results presented here are that there is a likelihood of further spread of the resistant biotype, because of the possibility of resistance being transmitted by pollen flow. Indeed, Moss (1987) has shown by intensive sampling that collections show various degrees of resistance up to 11 km from the site of the original resistant *A. myosuroides* population. Furthermore, the presence of heritable variation in a previously unexposed population suggests that, given sufficient selection, evolution of resistance may reoccur elsewhere.

CHAPTER 5
PATTERNS OF GROWTH IN HERBICIDE-RESISTANT AND SUSCEPTIBLE *ALOPECURUS MYOSUROIDES*

5.1 Introduction

Changes in cultivation practices over the last 20-30 years and the intrinsic biology of *A. myosuroides* have resulted in this species becoming a noxious weed of winter cereals in Great Britain (Baldwin, 1979). *A. myosuroides* occurs widely in central, southern England, often as serious infestations (as large patches, unsprayed strips or worse) (Chancellor & Froud-Williams, 1984). Consequently, over the last 10-15 years urea herbicides have been increasingly used as a means of economically feasible control (Baldwin, 1979; Barnes, 1981). However, one population has been found showing resistance to agriculturally recommended dose-rates (2-2.5 kg a.i. l ha^{-1}) of the widely used urea herbicide, chlorotoluron (Moss & Cussans, 1985; Moss, 1987). This population also exhibited a variable degree of cross-resistance to both chemically similar and unrelated herbicides (Moss & Cussans, 1985; Moss, 1987). The population originated from a continuous wheat field with minimum cultivation, where urea herbicides have been used recurrently (Moss & Cussans, 1985).

The evolution of herbicide-resistant populations has often been attributed to the high selection pressures imposed by the regular application of persistent herbicides (LeBaron & Gressel, 1982). Further lateral spread of the resistant biotype and other occurences will be influenced by, among other factors, their ecological fitness in a specified environment (Gressel & Segel, 1982). A suitable measure of this is population growth rate (λ) (Nur, 1984). Hence fitness can apply in a herbicide treated environment (where the resistant biotype is obviously more fit) or in unsprayed conditions. Furthermore, fitness may be defined

for spaced-plant experiments or in competition with susceptible biotypes, i.e. 'relative fitness'.

Rarely are population rates of increase, λ, measured. Rather, related traits such as vegetative biomass or reproductive dry matter are recorded. In this manner, most spaced-plant experiments have shown that resistant plants are less 'fit' than susceptible plants (e.g. Conard & Radosevich, 1979; Warwick & Black, 1981). However, some studies have shown either no biotypic differences (Warwick, 1980; Weaver & Warwick, 1982) or indeed that resistant plants outyielded susceptible plants (Rubin, Yaacoby, & Schonfeld, 1985; Jansen *et al*, 1986). These findings are confined to triazine-resistant broadleaf weeds; only a few studies have examined other herbicides or grass species (McWhorter & Jordan, 1976; Murphy, Gossett & Toler, 1986).

Experiments on spaced plants have usually been limited to one or a few harvests. However, the relative performance of biotypes can change with time (Bhomik, 1982; Holt & Radosevich, 1983). Detailed studies over sequential harvests are therefore important to clarify any differences in growth patterns (Elliot & Peirson, 1983) and to shed light on interpretations of competitive ability (Roush & Radosevich, 1985).

This study examines the growth of chlorotoluron-resistant and sensitive biotypes of the grass *A. myosuroides* grown as isolated plants. The objective was to determine any differences between biotypes in temporal growth pattern by formal growth analysis.

5.2 Materials and Methods

5.2.1 Plant material

Seeds (i.e. spikelets) of the resistant (R) biotype were derived from an original collection at a farm near Peldon in Essex (NGR: TL 985169) and of the susceptible (S) biotype from plots at Broadbalk Field,

Rothampsted, Hertfordshire (NGR: TL 140130) which have remained unsprayed for 140 years. Seed from each collection was bulked up over one seed generation in separate polythene tunnel houses.

5.2.2 Experimental design and procedure

The experiment was designed to compare the performance of biotypes over a series of destructive harvests over a period of almost 7 months. Eight plants of each biotype were laid out in a randomised block design, with two plants of each biotype randomised within four blocks arranged on benches in a heated glasshouse at the University of Liverpool Botanic Gardens, Ness, The Wirral.

Pots (18 cm diameter) were filled with J.I. No. 2 loam-based compost and arranged on two benches. Seed was germinated on soil in seed trays on soil under a mist unit. Uniform seedlings at the one tiller, 1-2 leaf stage were transplanted into pots on 14-15th September 1985, 40 days after sowing. Additional pots containing plants of each biotype were established at the same time; they were used to replace pots with transplant failures that had occurred by 2nd October 1985. Pots were watered from below through capillary matting throughout the experiment. Supplemental lighting was given by mercury fluorescent incandescent bulbs (400W) to extend daylength to a uniform 12 hours after transplanting. Plants were sprayed prophylactically with bupirimate ('Nimrod') and pirimicarb ('Pirimor') for mildew and aphid control, respectively, throughout the experiment.

The first four harvests were made weekly (11, 18, 26, and 31 days after transplanting), the next three at two-week intervals (47, 61, 82 days) and subsequently at monthly intervals (110, 141, 169, 201 days). The final harvest was made on 4 April 1986. At each harvest, tiller number was recorded and plants were cut at soil level. Shoot material was

separated into leaves, stems, and reproductive material (spikes) and fresh weight determined. Plant parts were oven dried separately at 80°C for 4 days and weighed. Area of green leaf tissue was measured until the fifth harvest to determine a leaf area-leaf water relationship following Evans (1972).

5.2.3 Analysis

Leaf area was predicted for all plants using the following linear regression:

$$\ln(\text{leaf area}) = 1.586 + 0.479 \ln(\text{leaf water content})$$

$$r^2=0.34,\ \text{d.f.}=63,\ P \leq 0.0001,$$

where leaf water content=leaf fresh wt - leaf dry weight. The primary data were described by stepwise polynomial models fitted using the computer programme of Hunt & Parsons (1974). Plant growth analytical quantities and 95% confidence limits were derived using natural logarithm transformations of the primary data. Fitted curves were generated of whole plant dry weight (W), leaf area (LA), leaf dry weight (LW) and tiller number (TL), against time (t) in days. Derived quantities were calculated as: $RGR_w = (1/W)(dW/dt)$, $RGR_{lw} = (1/LW)(dLW/dt)$, $RGR_{tl} = (1/TL)(dTL/dt)$, $LAR = LA/W$; $NAR = (1/LA)(dW/dt)$, where RGR= relative growth rate, LAR= leaf area ratio and NAR= net assimilation rate.

5.3 Results

5.3.1 Germination

Although seedlings were selected for uniformity, germination was slightly quicker in the S biotype (22% and 9% of final S and R numbers had germinated 17 days after sowing, respectively). Final germination proportion (± S.E.) of S plants was lower (S: 0.40 ± 0.02; R: 0.47 ± 0.01). Although all plants were at the one tiller stage, initial seedling size measured as height was 15% greater in S plants ($t_{174} = 3.54$, $P \leq 0.01$) as a

result of these germination patterns. Moreover, unequal variance in seedling size ($F_{77,77}$= 2.80, $P \leq 0.01$) was found and the relative magnitude of variances indicated that germination was more uniform in the R biotype.

5.3.2 Growth

All data sets were fitted by cubic polynomial functions which accounted for 90-97% of variation (Table 5.1). Biotypic differences were found for the intercept term ($P \leq 0.05$), but all other coefficients were similar, by comparison of 95% confidence intervals.

Leaf dry weight

Leaf dry weight (LW) increased rapidly with time over the first 7-8 harvests in both biotypes and subsequently declined until the final harvest (Fig. 5.1a). The S biotype produced more leaf material over the first 31 days from transplanting ($P \leq 0.05$). Thereafter, biotypes were similar until the penultimate harvest: at the final harvest the R biotype had greater LW ($P \leq 0.05$). RGR_{LW} did not differ between biotypes until 31 days (Fig. 5.1b). At this time R biotype growth was increasing faster than in S plants ($P \leq 0.05$) until about 30 days later.

Tiller number

Tiller number increased rapidly until 110 days from transplanting, after which it declined with a slight increase at final harvest (Fig. 5.2a). Susceptible plants possessed the most tillers during the first 18 d, but after 72 days R plants displayed significantly ($P \leq 0.05$) more tillers. Mature plants of the R biotype had 40-80% more tillers than the S biotype during this period. Analysis of RGR_{TL} showed that R plants added new tillers at a faster rate ($P \leq 0.05$) than S plants for 61 days after transplanting (Fig. 5.2b).

Table 5.1 Best-fit growth analysis functions of leaf dry weight, tiller number and plant dry weight (transformed to natural logarithms) with time from transplanting for herbicide-resistant (R) and susceptible (S) biotypes of *Alopecurus myosuroides* grown as isolated plants under greenhouse conditions.

Response variable	Biotype	Response Function	Coefficient of determination (r^2)
Leaf dry weight	R	$\ln Y = -5.789 + 0.195t - 0.0014t^2 + 0.3x10^{-4}t^3$	0.947
	S	$\ln Y = -4.494 + 0.160t - 0.0010t^2 + 0.2x10^{-4}t^3$	0.925
Tiller number	R	$\ln Y = 0.177 + 0.123t - 0.0011t^2 + 0.3x10^{-4}t^3$	0.941
	S	$\ln Y = 0.691 + 0.100t - 0.0009t^2 + 0.2x10^{-4}t^3$	0.904
Plant dry weight	R	$\ln Y = -5.821 + 0.200t - 0.0015t^2 + 0.4x10^{-4}t^3$	0.966
	S	$\ln Y = -4.571 + 0.170t - 0.0013t^2 + 0.3x10^{-4}t^3$	0.955

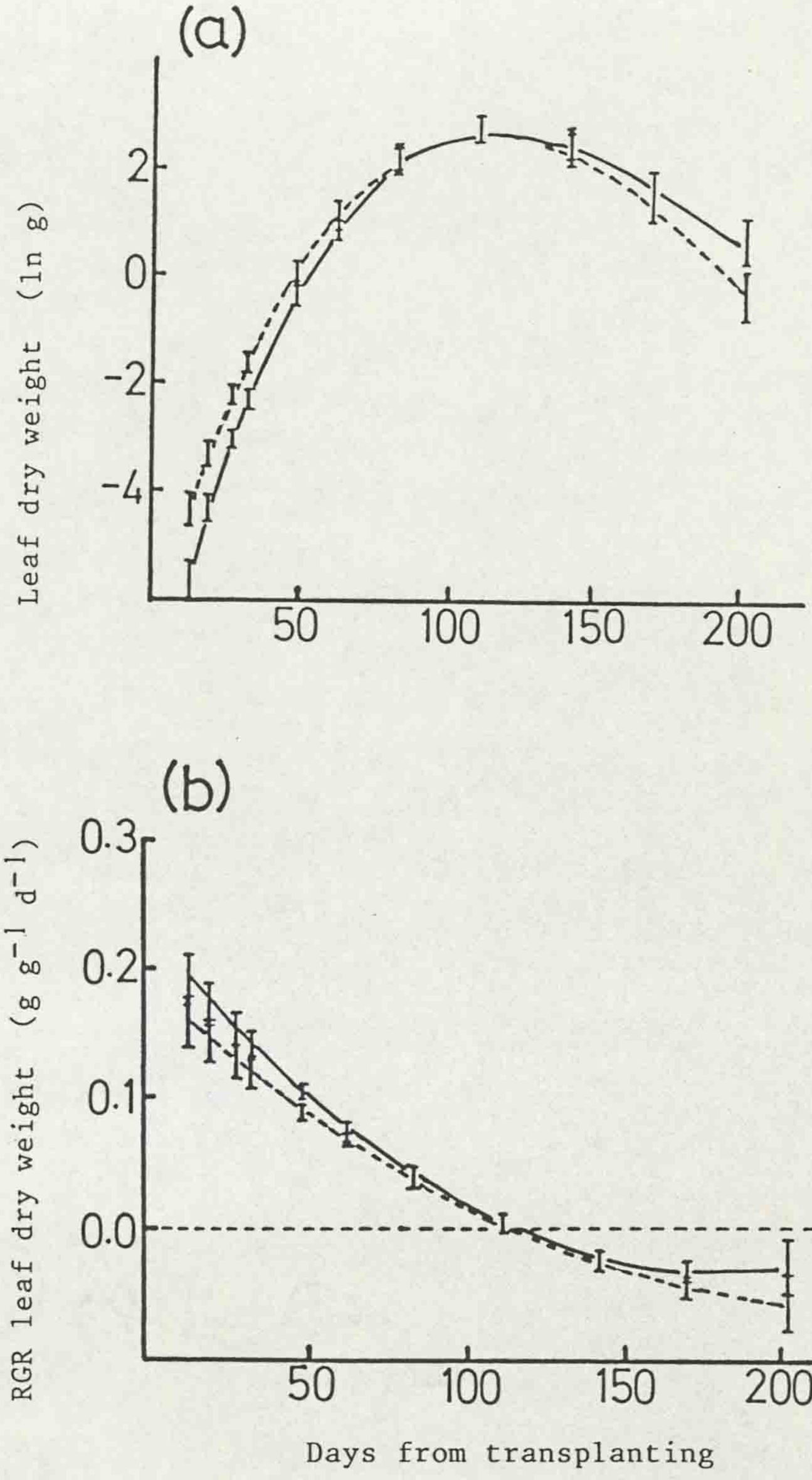

Figure 5.1 Fitted curves of leaf dry weight (a), and progressions of relative growth rate (RGR) (b), for herbicide-resistant (————) and susceptible (- - - - -) Alopecurus myosuroides biotypes. Curves in (a) represent best fit functions, vertical bars are 95% confidence limits for fitted values and in (b) for derived values, at harvest times.

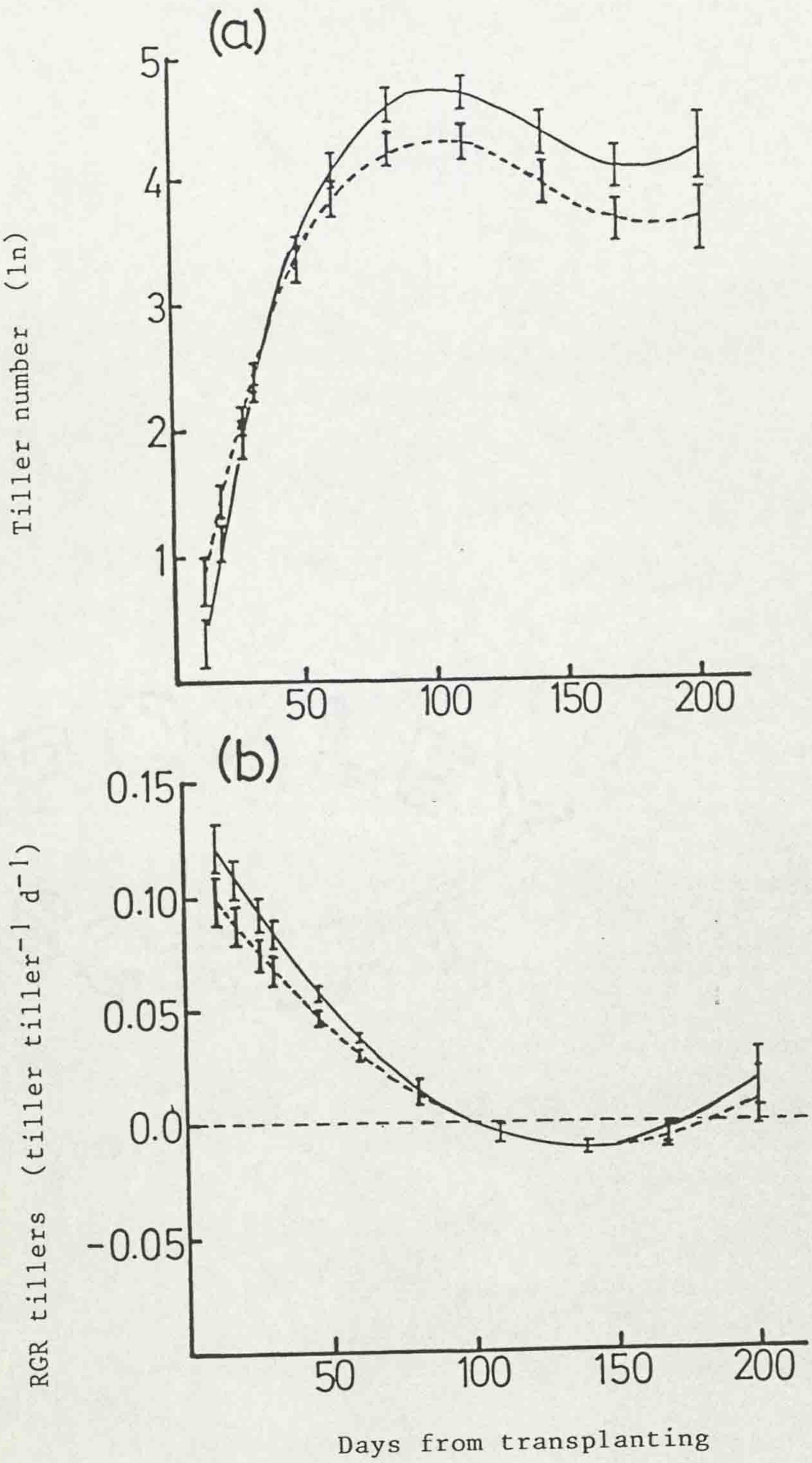

Figure 5.2 Fitted curves of (a) tiller number and (b) progressions of relative growth rate (RGR) for a herbicide-resistant (————) and a susceptible (------) Alopecurus myosuroides biotype. Details as in Figure 5.1.

Plant dry weight

In both biotypes rapid gains in dry weight were followed by a period of stasis from about 110 days after transplanting (Fig. 5.3a). Susceptible plants had greater W during early growth, but subsequently there were no significant differences between biotypes. Resistant plants grew at a faster rate than S plants in the period from 31-61 days after transplanting (Fig. 5.3b). During the entire experiment there was no period when RGR_ω fell significantly below zero.

Derived growth indices

Calculated values of ULR (Fig. 5.4) gave no differences between biotypes. Unit leaf rate showed an initial rise until 82 days after transplanting, followed by decline to an almost negative ULR at 141 d. Leaf area ratio declined steeply from the outset; the R biotype had a LAR 30-50% greater than the S biotype ($P \leq 0.05$) until 21 days later (Fig. 5.5). Biotypic differences were not present after then until the penultimate harvest.

5.3.3 Reproduction

Flowering spikes were present in a few S plants as early as 82-110 days after transplanting but only represented above 10% of total dry weight by the penultimate harvest. No significant differences were detected in reproductive allocation or dry weight, until the final harvest (Fig. 5.6). Resistant plants produced about 76% more ($t_{14} = 6.76$, $P \leq 0.001$) weight of reproductive material than S plants. Resistant plants were slightly later to reach maturity (no plants reproduced until 140 days after transplanting).

5.4 Discussion

Trends in the data were well described by cubic polynomials, as confirmed by examination of observed means and lack of fit, possible with

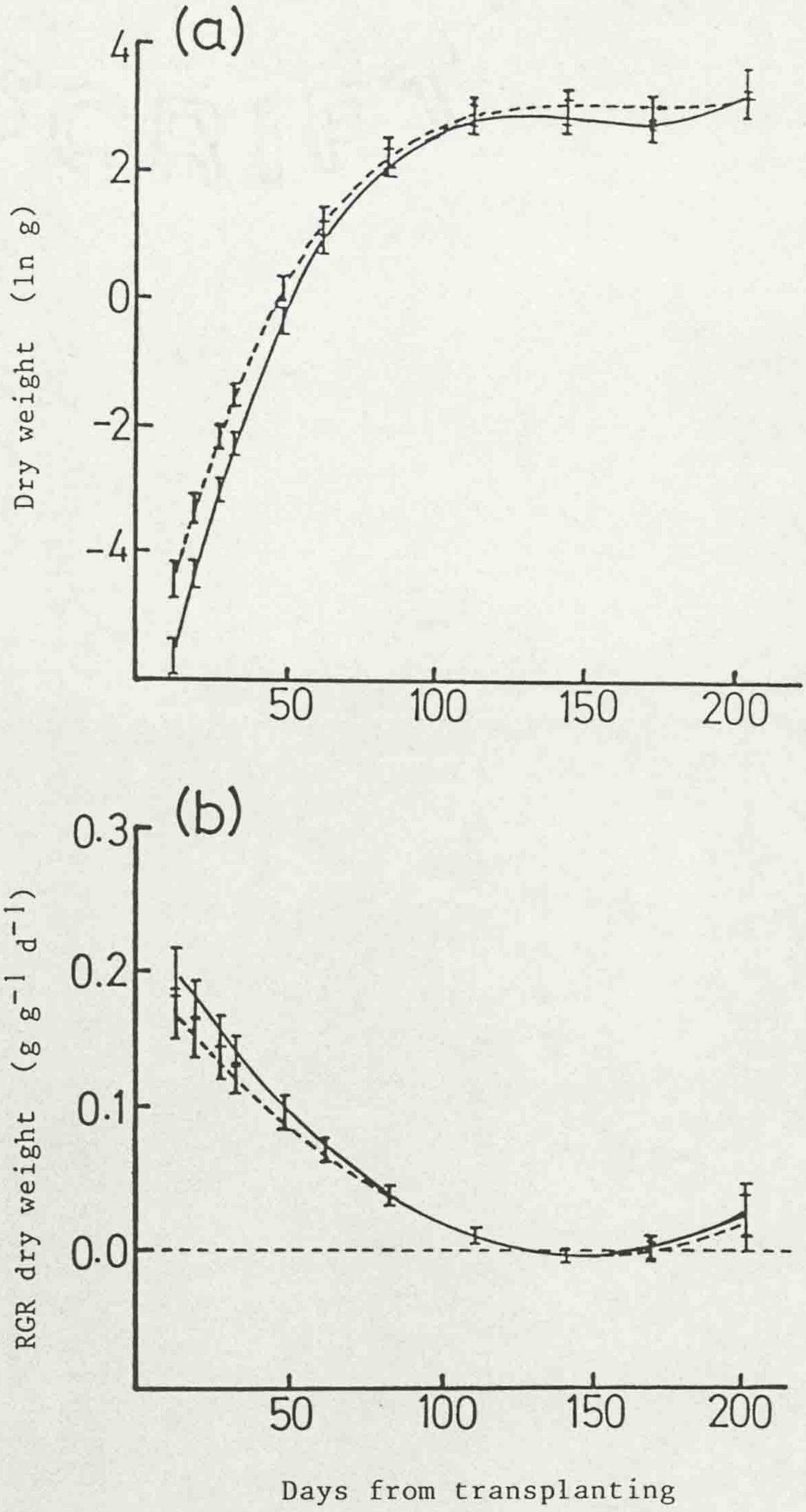

Figure 5.3 Fitted curves of (a) plant dry weight and (b) progressions of relative growth rate (RGR) for herbicide-resistant (————) and susceptible (------) Alopecurus myosuroides biotypes. Details as in Figure 5.1.

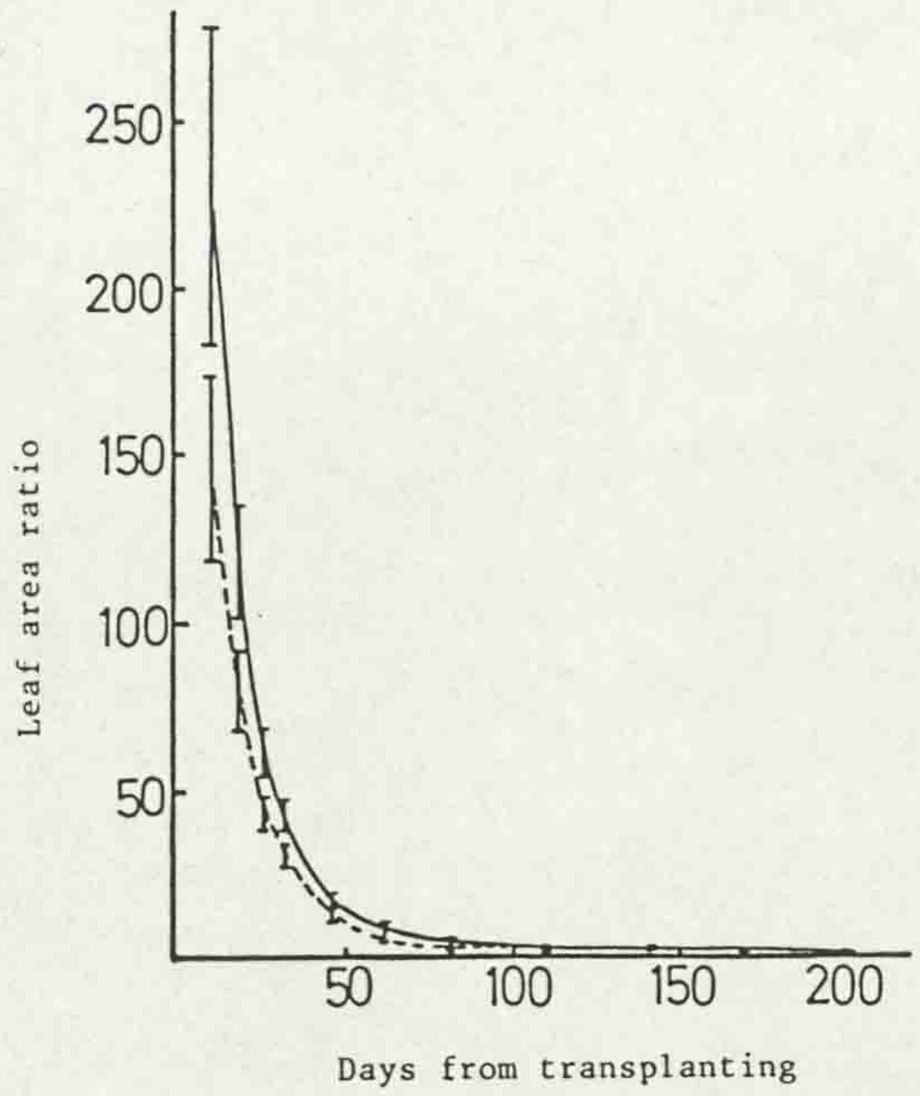

Figure 5.5 Progressions of leaf area ratio for herbicide-resistant (————) and susceptible (------) biotypes of Alopecurus myosuroides. Vertical bars represent 95% confidence limits for the derived values. Curves were fitted by eye through mean values.

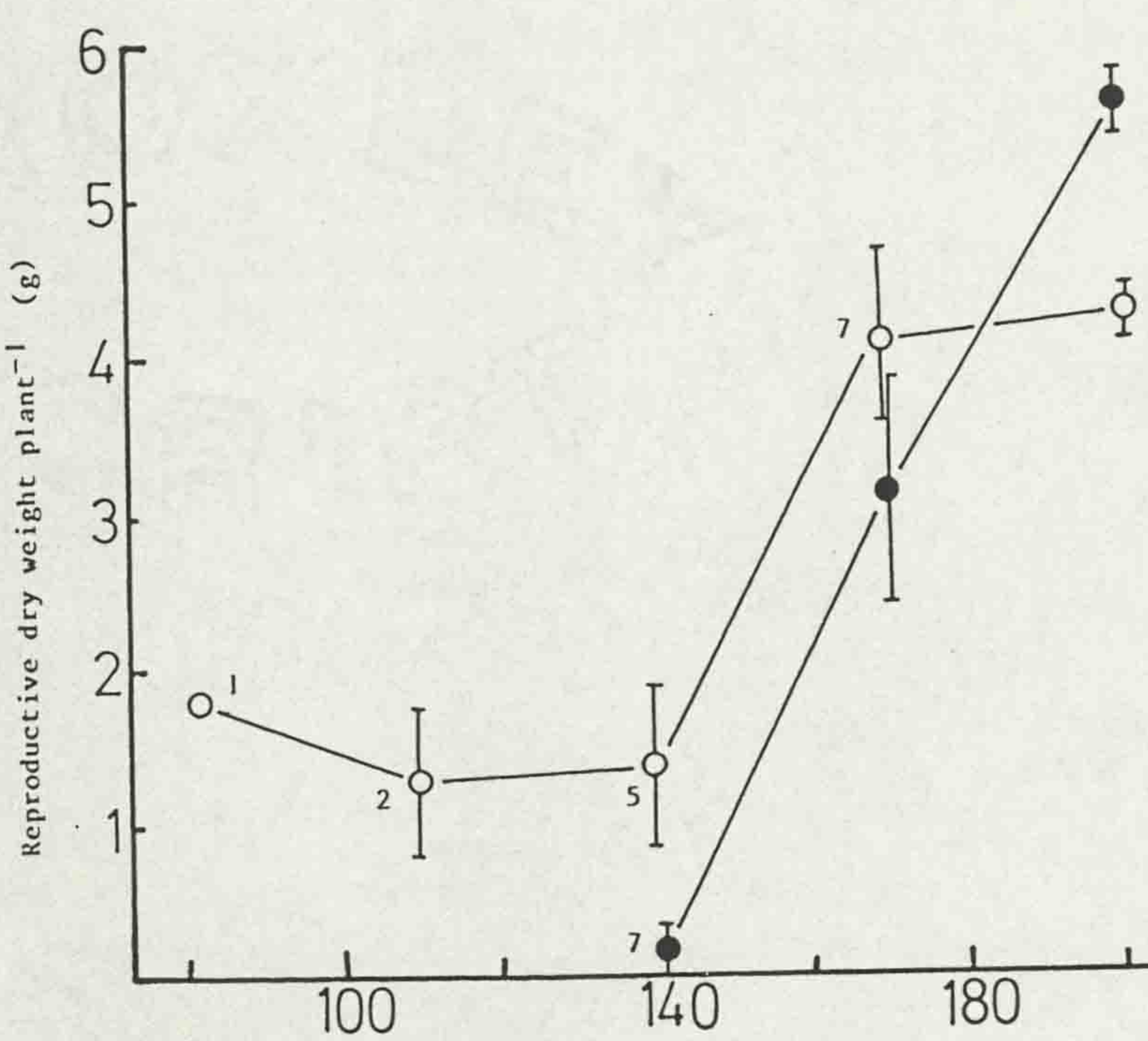

Fig. 5.6 Reproductive dry weight plant^{-1} for Alopecurus myosuroides biotypes over the last five harvests. Vertical bars represent ± 1 S.E. Where number of plants included was <8, sample sizes are given in parentheses. Symbols: (●) resistant biotype, (○) susceptible biotype.

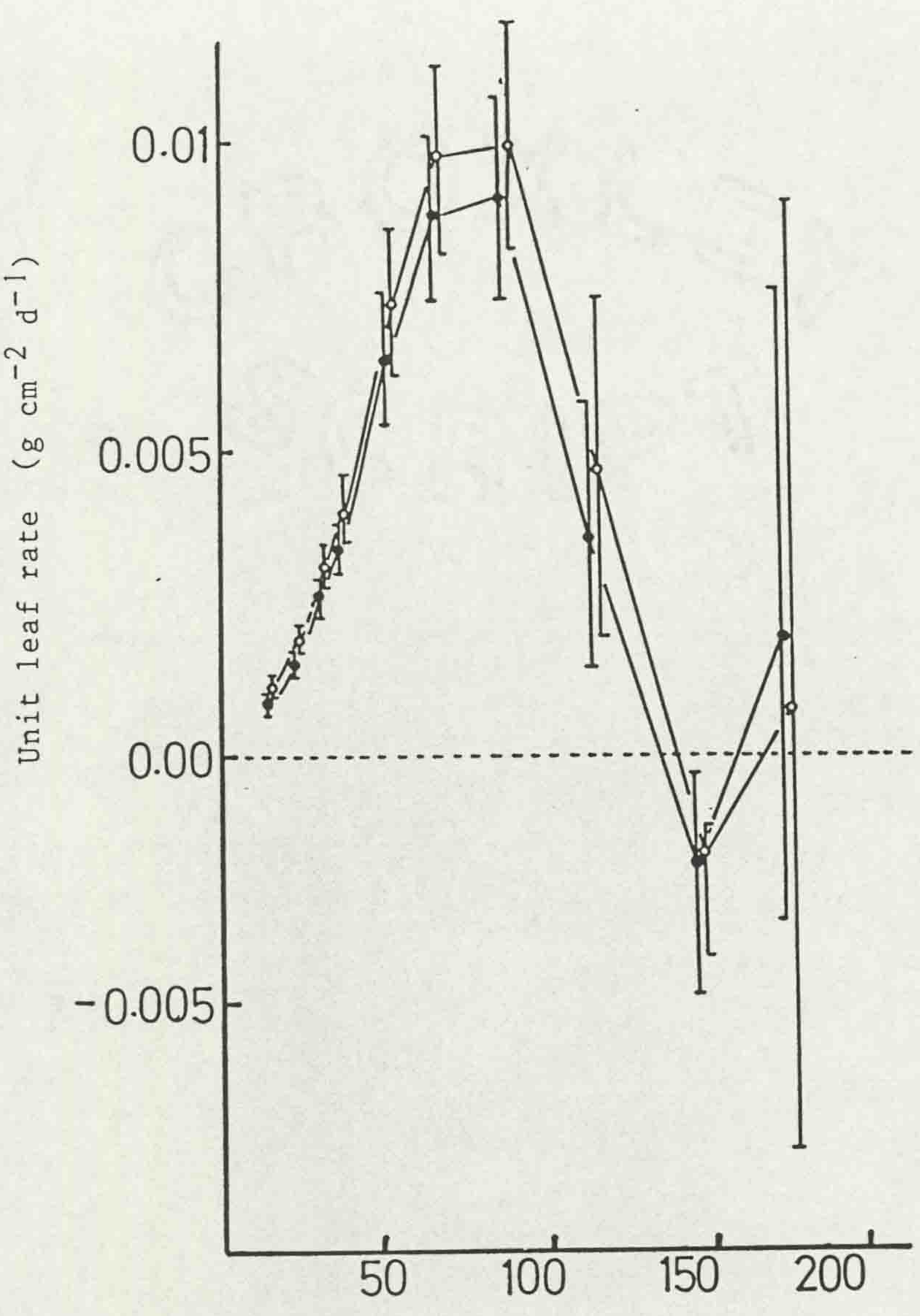

Figure 5.4 Progressions of unit leaf rate for herbicide-resistant (————) and susceptible (------) biotypes of Alopecurus myosuroides. Vertical bars represent 95% confidence limits of derived values. Points at the last harvest (201 days) have been omitted because of large error.

replicated plants at each harvest (not shown). Cubic functions of dry weight or tiller number accomodated either downturns in LW (Fig. 5.1a) or more complicated patterns (Fig. 5.2a). Caution is needed in interpreting the upturn in tiller number at the final harvest, which may be an artifact of the cubic curve. Progressions of derived RGRs are quadratic in shape and suggest declines in RGR followed in some cases by slight, non-significant, upturns caused by features of the primary data (see below). If functional analysis was required over a longer period than considered here, undoubtably the data would have to be segmented or more complicated spline curves used (Hunt, 1982). Over the first two harvests RGR_W, as calculated using the conventional approach, would increase for the R biotype. Resistant plants were, apparently, sufficiently young to show increasing growth rate. Such data cannot be modelled by quadratic RGR curves but could be more precisely modelled with a cubic RGR fit derived from a quartic function of W with time. This approach, together with the alternative, fitting splines, does not facilitate statistical comparison of biotypes. Such comparison was a primary objective of the analysis here and thus only cubic polynomials were fitted to the primary data

Small differentials in seedling age or size are known to strongly affect later growth stages (Cook, 1980; Roach, 1986) and were observed in the present study. The initial selection of plants varied slightly between biotypes as a consequence of germination patterns: the R biotype emerged more uniformly and later. The resultant initial size advantage of S plants may explain the observed patterns in leaf dry weight growth. Susceptible plants were larger at the start but were subsequently overtaken in leaf growth rate for a short period by R plants. At the last three harvests, allocation of resources had apparently switched to reproduction and RGR_{LW}

became negative. More leaf material had been lost by S plants, probably because of their slightly advanced growth stage.

Pre-transplanting size differences may also explain the higher tiller numbers of S plants during the first three weeks and the faster tiller number increase of R plants. Tiller number decline after the eighth harvest is thought to reflect death of vegetative tillers as resources were concentrated into reproduction. The upturn in tiller numbers at final harvest was probably an artifact of the fitting process in that observed means were declining. There was no period when the RGR_ω fell significantly below zero, so any leaf or tiller death must have been compensated for by reproductive gain.

In general, the impact of seedling size had disappeared 61 days after transplanting into pots. Biotypic differences not attributable to early size were detected. Resistant plants produced consistantly about 50% more tillers in the second half of the experiment. Since total plant dry weight was similar over this period, tiller size was probably smaller given that allocation of resources to unmeasured (e.g. roots) components was not changed. Leaf area was estimated with limited precision (r^2=0.3) and so trends in derived quantities incorporating LA are probably only broadly accurate. Although ULR showed that the biotypes had a similar carbon assimilatory capacity, with resistant plants showing signs of greater leafiness in a higher early LAR, this was not consistent.

Frequent harvests and empirical model fitting characteristic of the functional approach to growth analysis have been employed by Elliot and Peirson (1983) to compare R and S biotypes of *Chenopodium album* L. They found differences in leaf architecture, growth rate and onset of leaf senescence. If germination effects are removed from this study of *A. myosuroides*, biotypic divergence is not as great as found by Elliott and

Peirson (1983). Many less intensive studies (e.g. Warwick & Black, 1981; Gasquez, Darmency & Compoint, 1981; Watson, 1987) have shown that S biotypes have a yield advantage when grown as spaced plants. Such presumptive fitness advantages are often attributed to photosynthetic efficiency/capability (Warwick, 1980), which have followed demonstrations of reduced rates of electron transfer in Photosystem II in R plants (Bowes, Crofts and Arntzen, 1980). However, other studies have shown no such reductions (Jansen *et al*, 1985) and that yield advantages to S plants may be environment-dependent (Bhomik, 1982; Ahrens & Stoller, 1983). Together with the results of this study on *A. myosuroides*, such findings must place doubt on hypotheses of a general fitness advantage to susceptible plants.

CHAPTER 6
INTER-BIOTYPE COMPETITION BETWEEN HERBICIDE-RESISTANT AND SUSCEPTIBLE *ALOPECURUS MYOSUROIDES*

6.1 Introduction

Weed management practices have caused shifts in species composition of arable flora (Haas & Streiberg, 1982) and evolutionary changes within a species (LeBaron & Gressel, 1982). The widespread evolution of herbicide-resistant populations of many species is one example of the capacity of weed control practices to influence weed communities and populations. The phenomenon of herbicide resistance has important implications for weed control strategies. *A priori*, knowledge is needed on the impact of the effects of herbicide treatment on weed populations and on the relative performance of resistant (R) and susceptible (S) biotypes in comparison to the effects of natural regulatory processes.

Many previous studies on relative fitness of R and S biotypes have concentrated on comparisons of spaced plants in glasshouse environments (e.g. Warwick, 1980). However, fitness differences between populations or genotypes are often more noticeable when plants are in competition in mixtures (Snaydon, 1962). The majority of such studies, whether on spaced plants or on plants in mixture (e.g. Conard & Radosevich, 1979; Warwick & Black, 1981), appear to document an advantage to the S biotype of about 10-40% in terms of total plant or plant part weight (Table 1.1). Three further points emerge from such studies (Chapter 1): (1) S advantage was often magnified in competition, (2) the R biotype either equals or outyields the S biotype in a sizeable minority of cases and (3) that numbers of seeds produced (a true fitness measure, in ecological terms) are rarely counted.

All published experiments examining performance in mixture of such biotypes (Table 1.1) have been substitutive designs, with total density

held constant, but the frequency of biotypes varied (De Wit, 1960). This approach ignores situations where the two components vary independently in density, as is the case under natural conditions (Connolly, 1986; Law & Watkinson, 1987). Predictions over more than one generation are impossible because succeeding generations inevitably start at densities different from those at the start of the experiment (Inouye & Schaffer, 1981; Law & Watkinson, 1987). Response surface analysis provides a possible solution to such difficulties (Firbank & Watkinson, 1985).

The experiment described in this Chapter compares the performance of R and S biotypes of *Alopecurus myosuroides* Huds. in competition. Biotypes were grown in large pots at a range of densities and frequencies in the presence of a crop species to mimic the field situation and were subjected to herbicide treatment. Plants were grown through to seed production, allowing analysis over a whole generation by response surface analysis as well as by standard statistical means. The following questions were addressed: (1) are biotypic differences apparent in performance, density response or competitiveness? (2) is the effect of herbicide density-related? (3) does the herbicide alter the competitive balance in a predictable way?

6.2 Materials and Methods

6.2.1 Plant material

Seeds (spikelets) of R and S biotypes were derived from original collections at Peldon, Essex (NGR: TL 990160), and from un-sprayed plots at Rothampsted Experimental Station, Hertfordshire (NGR: TL 140130). Seed from each collection was bulked up for two generations, raising plants of each biotype in separate greenhouses. The resistance of the seed stocks to chlorotoluron, a urea-based herbicide, has been verified in Chapter 4

and by Moss and Cussans (1985). Winter wheat (*Triticum aestivum* L.) cv. Avalon was the companion crop species.

6.2.2 Experimental design and treatment

Sowing densities were chosen to achieve final emerged densities of 0, 1, 3, 30, 100 plants pot^{-1} in 30 x 30 cm square pots (= 0, 11, 33, 333, 1111 plants m^{-2}). All mixture combinations over this density range were included as well as monocultures. There were three herbicide treatments, control and chlorotoluron at 1.38 kg a.i. ha^{-1} (half-rate) and 2.75 kg a.i. ha^{-1} (full-rate), formulated as 'Dicurane 500 FW' in 400 l ha^{-1} of water and applied with an Oxford precision sprayer.

The experiment was laid out in two completely randomised blocks, with one extra replicate in both blocks for mixtures 0R-1S, 1R-0S and 1R-1S, and one extra replicate in only one block (randomly chosen) for 3R-1S, 1R-3S and 3R-3S. Wheat was sown at a constant density of 28 seed pot^{-1} (= 311 seed m^{-2}). The experiment therefore comprised 186 experimental units (pots) in total.

6.2.3 Execution of experiment

The experiment was conducted in an unheated, polythene tunnel-house at the University of Liverpool Botanic Gardens, The Wirral, in the first half of 1986. During 13-19 January 1986 pots were filled with J.I. No. 1 loam-based compost, wheat was sown at 7.5 cm depth, *A. myosuroides* seed sown on the surface and finally covered with a 0.5 cm layer of soil. *A. myosuroides* seed was sown in alternate rows at the two higher mixture densities, but was randomly scattered over the soil in monocultures and in other mixtures with paper cups covering the minority biotype. Seedling emergence was counted in March and transplanting/thinning carried out at the two lowest densities. In mixtures, seedlings of one biotype were marked with coloured wire rings.

On 10 April, replicate pots of herbicide treatments were sprayed: control pots were left unsprayed. After spraying the pots were re-randomised before replacing into blocks. On 25 April wheat was thinned to 20 plants pot^{-1} (= 222 plants m^{-2}) and loops of string supported by split canes placed around each pot to minimise shading between pots. Thereafter, overhead watering by mist lines ceased and pots were individually watered until 27 June, after which no further watering occurred. Plants were harvested after seed set, after a period of 25 weeks growth, over the period 2-30 July. Wheat was clipped at soil level and *A. myosuroides* plants were separated out. For each *A. myosuroides* plant, the numbers of tillers and length of flowering spikes were recorded. At this time the wheat had flowered but wheat seed was not fully ripe (Zadoks G.S. 75-77). Wheat yields were not recorded.

6.2.4 Analysis

Estimation of seed numbers

An allometric relationship of seed number with spike length was used. Measurements of spike length (mm) were taken from a stratified sample of experimental plants; after air-drying, seed numbers were counted for these spikes. Linear regressions of logarithmically (base 10) transformed data explained 92% and 88% of the variance for the R and S biotype, respectively. Analysis of covariance (Sokal & Rohlf, 1981) showed that slopes were homogenous ($F_{1,123}=0.83$, $P > 0.36$) and furthermore, that intercepts were not different ($F_{1,124}=0.25$, $P > 0.25$). Data were therefore pooled over biotypes and the following relationship used to predict seed numbers per spike from the length:

$$\log_{10}(\text{seed no.}) = -0.414 + 1.302 \log_{10}(\text{spike length}),$$
$$r^2=0.90,\ n=127,\ P<0.0001.$$

Statistical analysis

Data analysis depended on response variable: survival and flowering were analysed with log-linear models for categorical data. These are the counterpart for attribute data of the linear analysis of variance (ANOVA) model for continuous variables (Sokal & Rohlf, 1981). A four-way analysis of herbicide, R density, S density and flowering or mortality was carried out on data summed over blocks using PROC CATMOD of SAS (SAS Inst. Inc., 1985), with a maximum likelihood algorithm. For continuous responses, seeds plant^{-1} and reproductive tillers plant^{-1}, ANOVA was used (PROC GLM, SAS Inst. Inc, 1985). Tests were based on Type III sums of squares, correcting for unbalanced designs, because of extra replicates at low densities. Both variables were logarithmically transformed before analysis, following inspection of residuals.

Response surface analysis

Response surfaces were fitted to observed data using models of two species competition (following Firbank & Watkinson, 1985; Law and Watkinson, 1987). For mortality, the following was used:

$$N_{B,f} = N_{B,i} \, (1 + m_B \, (N_{B,i} + \gamma_B \, N_{B',i}))^{-1} \qquad (1)$$

where N are densities of B and B', the two biotypes. Subscripts f and i refer to initial sown seed densities (i) and final harvest plant densities (f). γ_B is the equivalence coefficient describing the comparative effect of B' in terms of B. For seed production per plant:

$$S_B = w_B \, (1 + a_B \, (N_{B,i} + \alpha_B \, N_{B',i}))^{-b} \qquad (2)$$

where S is seeds plant^{-1} at harvest, **w** is seeds plant^{-1} in the absence of intra- and inter-biotypic competition, **a** is a density related parameter, α an equivalence coefficient describing the effect of B' on B in terms of seed yield and **b** is a power parameter thought to be related to the efficiency of resource utilisation (Watkinson, 1980).

Law and Watkinson (1987) proposed a new model which incorporates density-dependent competitive ability and takes the form:

$$S_B = w_B \left(1 + N_{B,i}^{\delta_{BB}} + N_{B',i}^{\delta_{BB'}}\right)^{-1} \quad (3)$$

where δ are power parameters for each input density. The competitive ability of a component (B or B') at a particular density combination can be estimated for component B' as:

$$\frac{\delta_{B,B'} N_{B',i}^{(\delta_{B,B'}-1)}}{\delta_{B,B} N_{B,i}^{(\delta_{B,B}-1)}} \quad (4)$$

All models were fitted to logarithmically (base 10) transformed data, to normalise the distribution of error terms, using PROC NLIN of SAS (SAS Inst. Inc., 1985) with an iterative least-squares algorithm.

6.3 Results

6.3.1 Seedling emergence

Previous seedling emergence tests had shown that emergence in the S biotype was higher than in the R biotype (73% vs. 66%, respectively) and seeding densities were chosen to correst for this. Sample sizes were too low to test emergence differences at the two lower densities. At the two high densities, emergence was the same for both biotypes (G=1.14, d.f.=3, N.S.). This resulted in 9% more R seedlings emerging (t=2.95, d.f.=58, $P \leq 0.01$) at the highest density. There was no significant difference (t=0.82, d.f.=58, N.S.) at the second highest density.

6.3.2 Mortality

Survival proportions were calculated summed over the three low densities (1-3 plants pot^{-1}) and over blocks to achieve adequate sample sizes.

<u>Resistant biotype</u>

Resistant biotype

There was a significant four-way interaction of survival with R density, S density and herbicide (G=17.7, d.f.=8, $P \leq 0.025$). Since this overall interaction was present, further tests were made within each herbicide treatment.

In the control treatment, there was no evidence for a three-factor interaction (G=3.7, d.f.=4, N.S.) or any dependence upon biotype density (G=9.1, d.f.=6, N.S.). Although the effect on survival of R density (G=11.0, d.f.=6, N.S.) and S density (G=10.0, d.f.=6, N.S.) are not in themselves significant, the decrease in fit from dropping these two factor effects was significant (G=7.3 and 6.6, d.f.=2, $P \leq 0.05$, respectively). Survival in the R biotype was thus affected independently by R and S density. Survival proportions (Fig. 6.1a-c) show that survival declines over the two highest densities and that the lowest density of R (1-3 plants pot^{-1}), based on less plants, only contributes to the trend at intermediate mixture density.

At half-rate chlorotoluron, the three-way interaction of R density, S density and survival was significant (G=17.8, d.f.=4, $P \leq 0.01$) indicating that the degree of association between R density and mortality differed according to S density. Fig. 6.1b indicates little depression in survival proportion by S density at the two lower R densities, but at high R density, the highest S density reduced survival from over 90% to 84%. Trends in survival were more clear-cut at full rate chlorotoluron (Fig. 6.1c). Three-way and two of the two-way interactions were not significant and although the effect of R density was marginally non-significant, the decrease in fit from dropping the interaction was important (G=11.7, d.f.=2, $P \leq 0.01$). A test for complete independence of S density from R

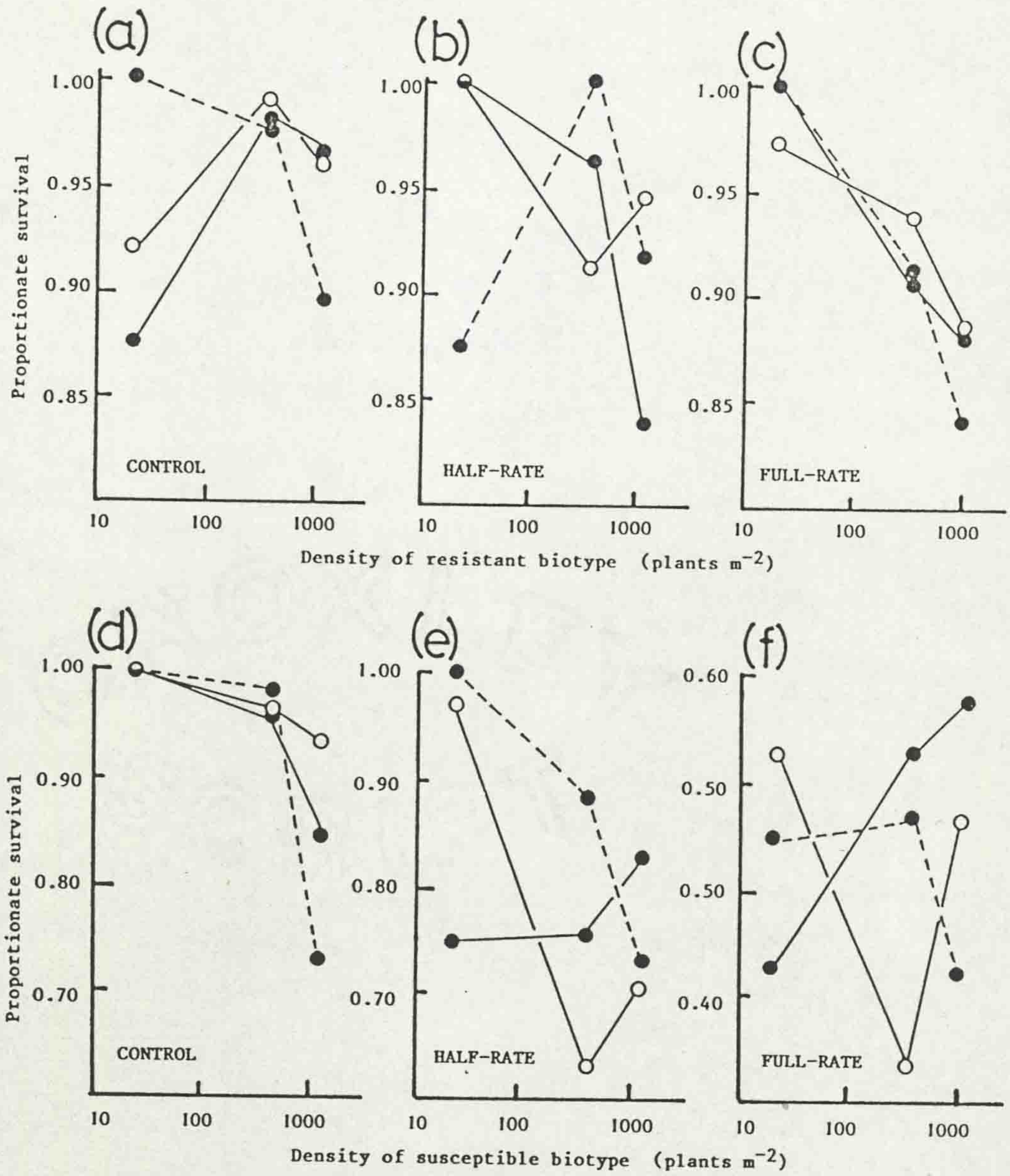

Figure 6.1 Survival proportion as affected by mixture densities for two biotypes of Alopecurus myosuroides grown in competition and treated with three rates of chlorotoluron herbicide. Data for two lowest densities (11, 33 plants m^{-2}) were pooled for the target biotype and the three lowest (0, 11, 33 plants m^{-2}) for the associated mixture biotype. (a)-(c) Resistant biotype, (d)-(f) Susceptible biotype. Note the change in vertical scales. Symbols: mixture biotype at densities of (○——○) 0-33 plants m^{-2}; (●----●) 333 plants m^{-2}; (●——●) 1111 plants m^{-2}.

density and survival showed that S density had no effect (G=6.8, d.f.=10, N.S.) and thus that survival was only affected by R density.

Susceptible biotype

No significant joint interaction between the four factors was found when considered simultaneously (G=5.4, d.f.=8, N.S.). Of the three-factor interactions, the association between R density and S density did not depend on herbicide rate (G=11.1, d.f.=16, N.S.). However, herbicide level significantly conditioned the association between survival and S density (G=47.1, d.f.=12, $P<0.0001$) and R density (G=38.9, d.f.=12, $P<0.01$). Futhermore, the effect of S density on its own survival was dependent on R density (G=30.8, d.f.=12, $P<0.01$).

In the control treatment, increasing S density decreased survival and the two higher R densities reduced survival at the highest S density (Fig. 6.1d) . At half-rate chlorotoluron, increased S density decreased survival at the two lower R densities, but survival actually increased with S density at the highest R density (Fig. 6.1e) . The only clear trend at full-rate chlorotoluron was that survival increased with increasing S density at the highest mixture R density (Fig. 6.1f).

6.3.3 Flowering

Resistant biotype

Flowering proportion was uniformly high, being over 95% in almost all treatments. There was no evidence of any significant interactions (all N.S.) of treatments with flowering. Fig. 6.2a-c confirms that the proportion of R plants flowering was unaffected by sowing density or herbicide treatment.

Susceptible biotype

No high order interactions were present in the S biotype (all N.S.), but herbicide treatment strongly affected flowering proportion (G=160.6,

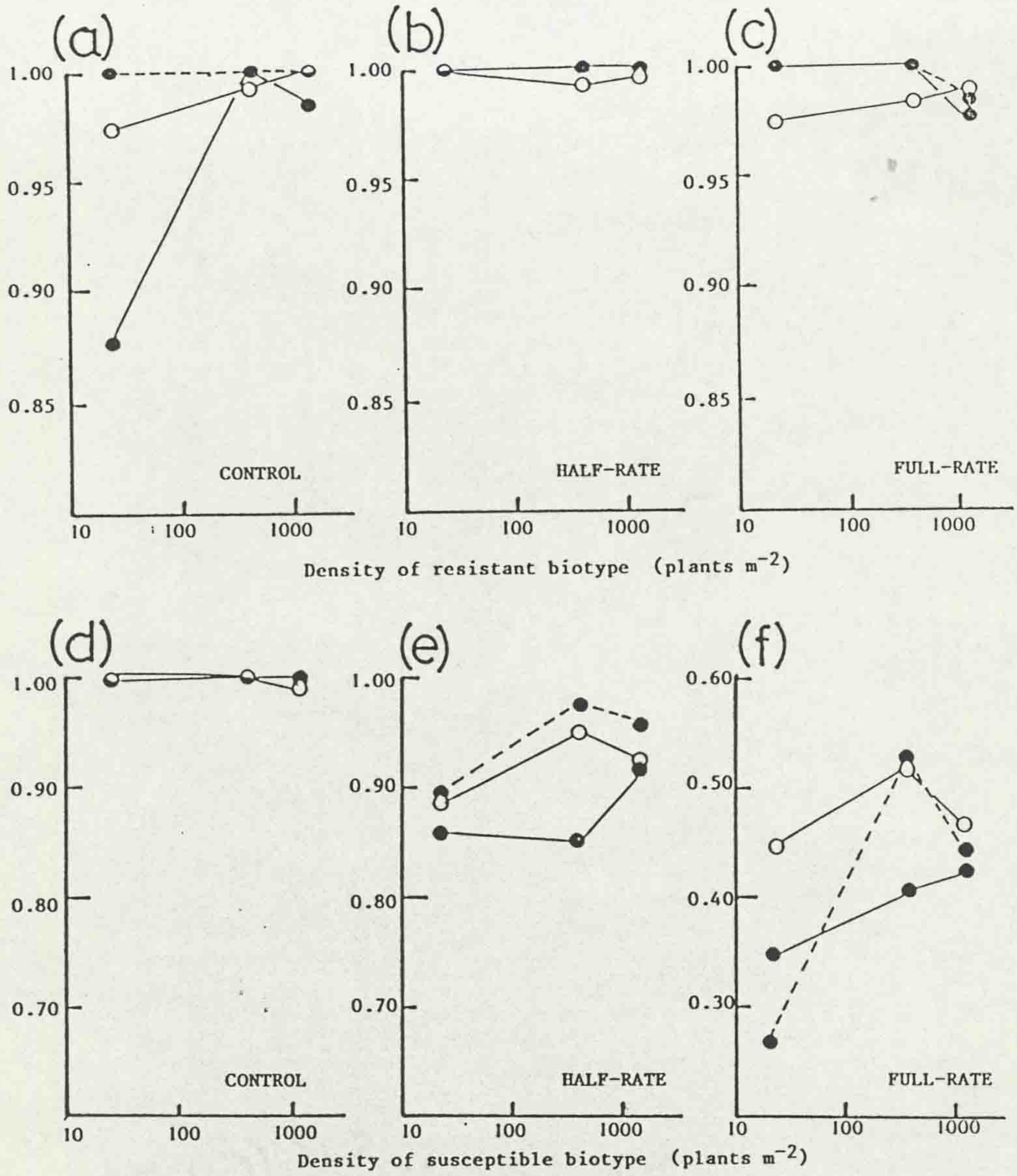

Figure 6.2 Flowering proportion as affected by mixture densities for two biotypes of Alopecurus myosuroides treated with three rates of chlorotoluron herbicide. (a)-(c) Resistant biotype, (d)-(f) Susceptible biotype. Note the difference in vertical scales. Details as in Figure 6.1.

d.f.=30, $P<0.0001$). Mean flowering proportion (Fig. 6.2d-f) revealed this strong herbicide effect, which reduced the frequency of flowering plants from 100% to below 90% at full-rate chlorotoluron. There was some evidence that flowering was enhanced at higher S densities when herbicide was applied, but without statistical significance attached.

6.3.4 Tillering

Resistant biotype

A significant block effect was found (Table 6.1a), with block 2 containing plants with more tillers. This block was harvested about 1-2 weeks later than block 1. Herbicide treatment had no effect on tiller production. Both increasing R density ($P<0.0001$) and S density ($P<0.05$) reduced tiller number, but no interactions between biotypes were present. High density of the other (S) biotype dampened the response in tiller production in relation to its own density (Fig. 6.3a) in control and half-rate chlorotoluron treatments, but not at full-rate herbicide.

Susceptible biotype

Table 6.1b shows that herbicide treatment reduced reproductive tillers plant $^{-1}$ significantly ($P<0.0001$) and conditioned the effect of S density on tiller production ($P<0.05$). The density of R plants had a strong effect ($P<0.0001$) and there were no differences between the three low R densities (Fig. 6.3b). In the control treatment, the effect of R density was greater at low densities. At high R density, tiller numbers were not responsive to S density. At half-rate and full-rate chlorotoluron, tiller production was not affected by S density, beacuse S numbers had been reduced by mortality (S surviving numbers are plotted on ordinate, Fig. 6.3b). R density effects were only apparent at the highest density.

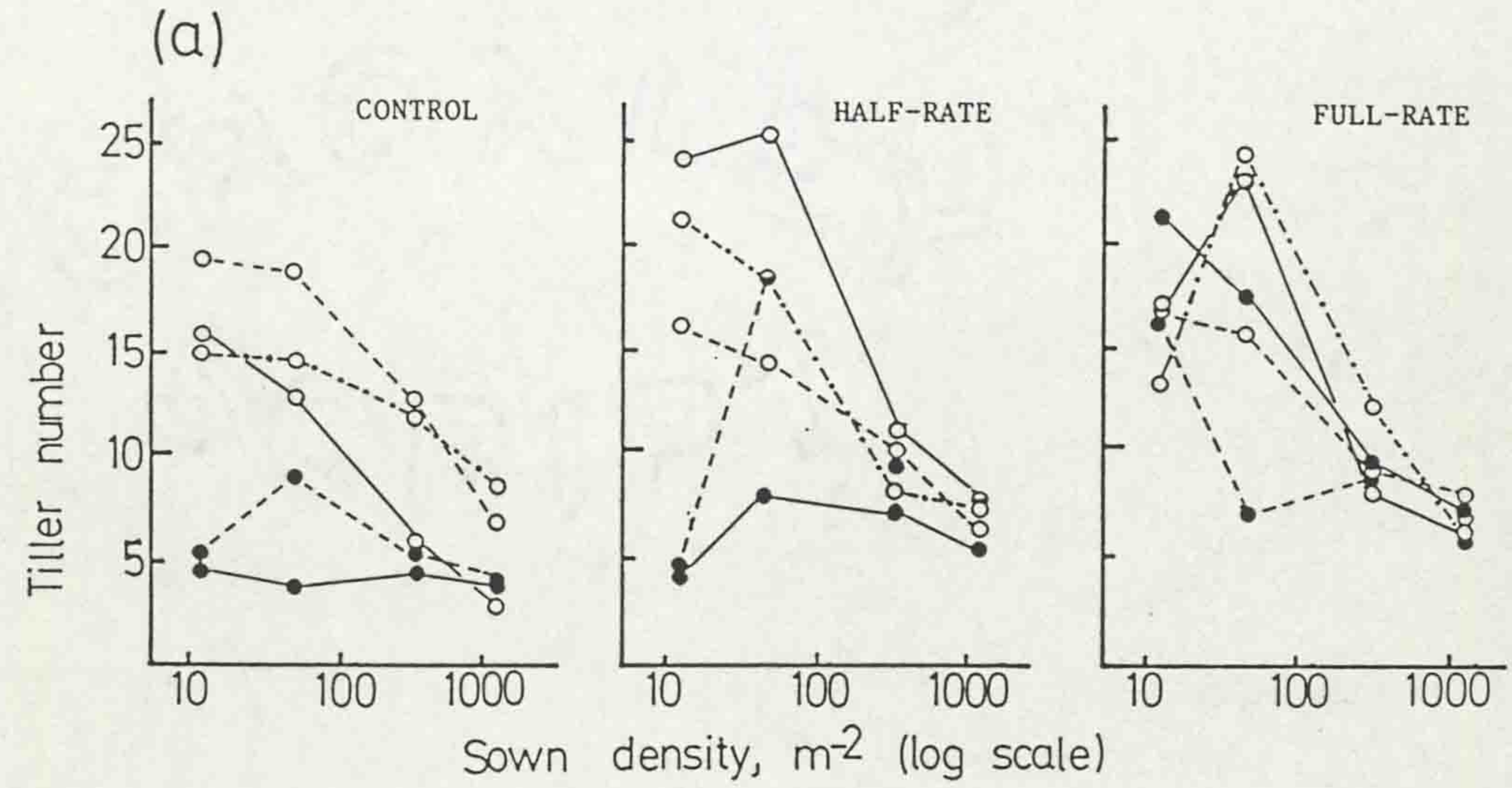

Figure 6.3 Mean tiller number per plant of the (a) Resistant and (b) Susceptible biotypes of Alopecurus myosuroides, when grown in mixture and treated with three rates of chlorotoluron herbicide. Mixture densities of the associated biotype are indicated by symbols: (O-·-·-O) 0 plants m^{-2}; (O---O) 11 plants m^{-2}; (O——O) 33 plants m^{-2}; (●---●) 333 plants m^{-2}; and (●——●) 1111 plants m^{-2}. For the susceptible biotype (b) the mean number of survivors at each sowing density is plotted along the horizontal axis.

Table 6.1 Analysis of variance of numbers of reproductive tillers plant^{-1} for resistant (a) and susceptible (b) biotypes of *Alopecurus myosuroides* grown in competition and exposed to herbicide treatments. Tillers plant^{-1} is transformed to $\log_{10}(x + 1)$. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). Significant effects are indicated by: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. For interactions N.S. indicates that those not specified are not significant ($P > 0.05$). Only significant, or close to significant interaction effects, are indicated separately.

Source	d.f.	M.S.	*F*
(a) Resistant Biotype			
Main Effects			
Block	1	0.876	13.69***
Herbicide	2	0.035	0.76
R Density	3	0.562	8.78***
S Density	4	0.182	2.84*
Interactions			
Others (N.S.)	50	0.040	
Error	83	0.064	
Total	143		
(b) Susceptible Biotype			
Main Effects			
Block	1	0.081	1.76
Herbicide	2	1.540	33.47***
S Density	3	0.036	0.78
R Density	4	0.362	7.87
Interactions			
Herbicide x S Density	6	0.107	2.32***
Others (N.S.)	43	0.040	
Error	74	0.046	
Total	133		

Table 6.2 Analysis of variance of seeds plant^{-1} for (a) herbicide-resistant (R) and (b) susceptible (S) biotypes of *Alopecurus myosuroides*, grown in competition and exposed to herbicide treatments. The variable is transformed to $\log_{10}(x)$. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). Only significant, or close to significant interaction effects, are indicated separately. Details as in Table 6.1.

Source	d.f.	M.S.	*F*
(a) Resistant Biotype			
Main Effects			
Block	1	0.398	3.83
Herbicide	2	0.007	0.07
R Density	3	0.704	6.78***
S Density	4	0.224	2.16
Interactions			
Others (N.S.)	50	0.040	
Error	82	0.104	
Total	142		
(b) Susceptible Biotype			
Main Effects			
Block	1	0.000	0.00
Herbicide	2	6.635	42.26***
S Density	3	0.016	0.10
R Density	4	0.969	6.17***
Interactions			
Herbicide x S Density	6	0.304	1.94
S Density x R Density	12	0.285	1.81
Others (N.S.)	31	0.091	
Error	71	0.157	
Total	130		

6.3.5 Fecundity

Resistant biotype

Fecundity was unaffected by herbicide treatment (Table 6.2a). The major effect was intra-biotype (R) density ($P \leq 0.001$), while S density was not quite significant ($P \leq 0.08$). Compared to the two low R densities, at 333 plants m^{-2} fecundity was reduced by 26% and by 51% at 1111 plants m^{-2} (Fig. 6.4a). The two highest S densities reduced seeds $plant^{-1}$ by 37%, compared to the three low S densities.

Susceptible biotype

Herbicide treatment significantly ($P \leq 0.001$) reduced seeds $plant^{-1}$ (Table 6.2b). At half-rate herbicide, plants produced 63% less seeds than unsprayed ones and at full-rate, 85% less. The density of the R biotype had a significant effect ($P \leq 0.001$) on S fecundity, but its own density did not. Nearly significant interactions of S x R density and Herbicide x S density showed (Fig. 6.4b) that the effect of herbicide was less at high densities than at low ones, e.g. with full-rate herbicide, seeds $plant^{-1}$ were depressed by 84% at low densities but only by 68% at the highest joint density. Furthermore, there were indications (Fig. 6.4b) that in both herbicide treatments at the lowest density of the R biotype, seed production actually increased with increasing S density, though the numbers of surviving plants at low S densities was small.

6.3.6 Rate of population growth

Finite rate of increase, λ, can be calculated as the proportionate change in numbers from one generation to the next, i.e. N_{t+1}/N_t. Here, N_t is sowing density at time t and N_{t+1} is seeds produced at harvest (= post-dispersal) per unit area.

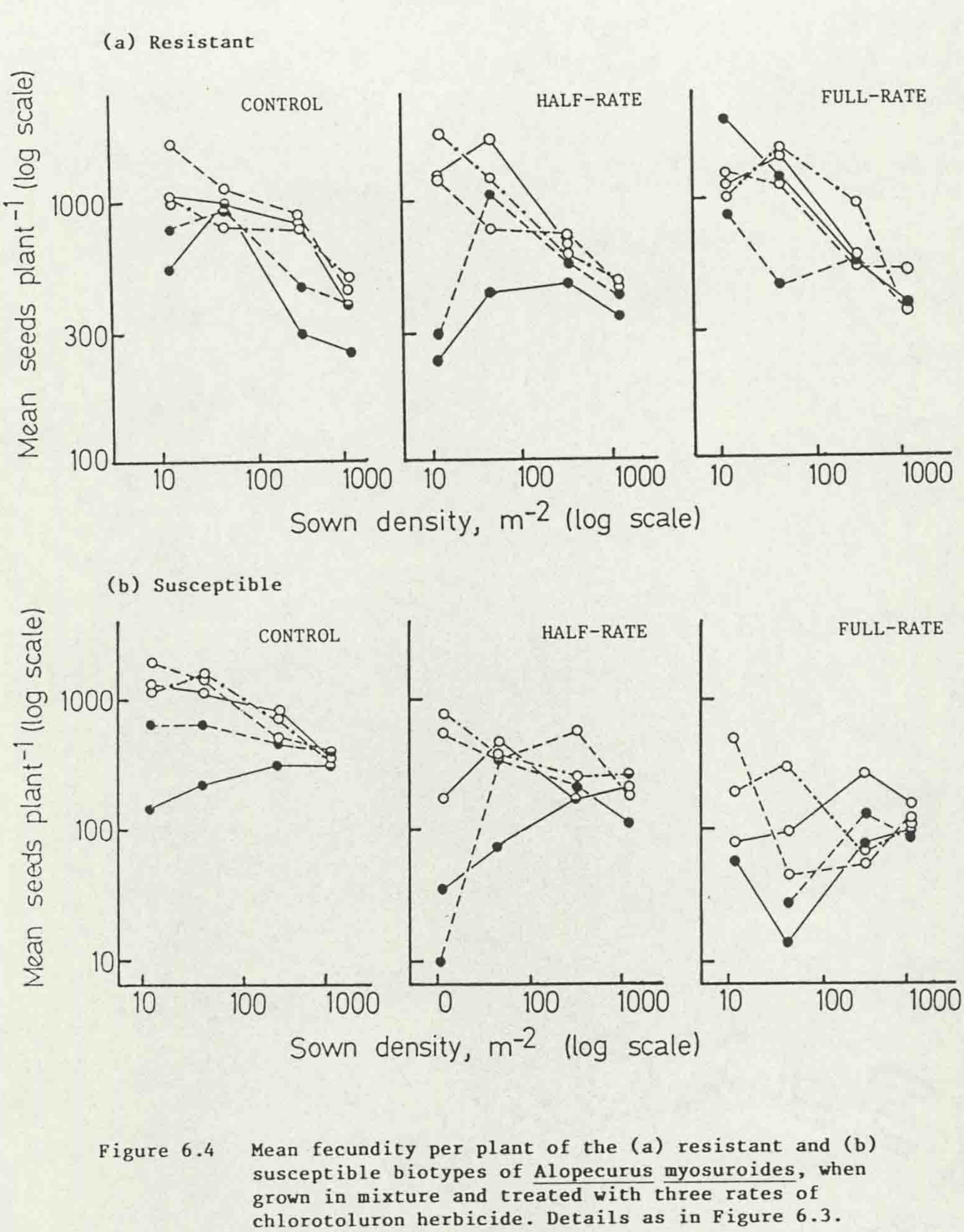

Figure 6.4 Mean fecundity per plant of the (a) resistant and (b) susceptible biotypes of Alopecurus myosuroides, when grown in mixture and treated with three rates of chlorotoluron herbicide. Details as in Figure 6.3.

Resistant biotype

Herbicide treatment had no significant effect and only R density significantly reduced λ values (Table 6.3a). Statistical contrasts showed a significant difference in the low vs. high density comparison ($P<0.001$), but no significant differences between 1 and 3 plants pot^{-1}, or 30 and 100 plants pot^{-1}. A significant low vs. high S density effect was found, despite the non-significant main effect of S density.

Mean values show (Table 6.4a) that reductions in λ with herbicide are only apparent at the full-rate herbicide (compare λ values at low S density across herbicide treatments). Mortality of the S biotype in herbicide treatments has in some cases increased R rate of growth.

Susceptible biotype

Analysis of variance on λ values (Table 6.3b) showed that herbicide strongly ($P<0.0001$) affected the rate of increase, but that S density effects were dependent on herbicide treatment ($P<0.01$). Mean λ values (Table 6.4b) showed that response to S density was minimal at the high herbicide rate and λ mostly below 50.0, while at half-rate herbicide λ was reduced by S density only at the low R densities. In the control and at low densities, λ was greater than 600.0 but was reduced to 100-150 at high densities.

6.3.7 Response surface analysis

Application of models

Mortality.- Negative density-dependence has been detected in control treatments (Fig. 6.1) and therefore the mortality model (equation [1]) of Firbank and Watkinson (1985) is applicable. In herbicide treatments, density effects were also evident (Fig. 6.1), but were variable. The R biotype showed a stronger, negative density response and equation (1) is still suitable. The S biotype, however, responded differently according to

Table 6.3 Analysis of variance of reproductive rate, λ, for (a) herbicide-resistant (R), and (b) susceptible (S) biotypes of *Alopecurus myosuroides* grown in competition and exposed to herbicide treatments. Mean squares (M.S.) are corrected for non-orthogonality because of missing values (Type III sums of squares, SAS Inst. Inc., 1985). Only significant, or close to significant interaction effects, are indicated separately. Details as in Table 6.1.

Source	d.f.	M.S.	*F*
(a) Resistant Biotype			
Main Effects			
Block	1	351289	2.55
Herbicide	2	12807	0.09
R Density	3	1305202	9.47***
S Density	4	176722	1.28
Interactions			
Others (N.S.)	50	47880	
Error	82	137779	
Total	142		
(b) Susceptible Biotype			
Main Effects			
Block	1	16946	0.48
Herbicide	2	1293934	36.53***
S Density	3	299327	8.45***
R Density	4	156060	4.41**
Interactions			
Herbicide x S Density	6	128581	3.63**
Others (N.S.)	43	38199	
Error	71	35417	
Total	130		

Table 6.4 Mean finite rate of increase, λ, for (a) resistant (R), and (b) susceptible (S) biotypes of *Alopecurus myosuroides* grown in competition, for combinations of R and S initial sowing densities, with either no herbicide (control) or with half- and full-rate chlorotoluron.

(a) Resistant Biotype

		S Density				
Herbicide	R Density	0	1	3	30	100
Control	1	543	856	509	384	276
	3	488	468	577	559	439
	30	364	342	351	193	155
	100	209	182	151	155	94
Half-rate	1	905	583	602	151	115
	3	715	444	998	571	257
	30	280	328	288	288	263
	100	180	160	163	168	137
Full-rate	1	490	634	560	421	595
	3	878	661	720	271	694
	30	349	219	215	264	228
	100	134	163	122	129	146

(b) Susceptible Biotype

		R Density				
Herbicide	S Density	0	1	3	30	100
Control	1	634	967	705	342	64
	3	934	881	659	383	134
	30	283	243	361	284	164
	100	130	145	110	120	118
Half-rate	1	379	274	122	10	16
	3	231	214	273	216	44
	30	61	110	43	100	72
	100	80	53	48	77	36
Full-rate	1	94	258	38	-	23
	3	67	16	45	10	5
	30	8	12	62	40	26
	100	20	46	37	17	27

mixture density: both negative and positive effects of density were apparent (Fig. 6.1e,f). The implication for modelling S biotype response was that an additional model parameter was incorporated into equation (1) to account for the very high mortality at low sown S densities, when the R biotype was present at high density. This intercept term was added to give the model:

$$N_{B,f} = \tau_B + N_{B,i} \; (1 + m_B \; (N_{B,i} + \gamma_B \; N_{B',i}))^{-1} \qquad (4)$$

where τ represents the intercept parameter.

Fecundity.- Individual plant fecundity was negatively density-dependent in control treatments (Fig. 6.4) and models of the type represented by equation (2) suit such data. However, the depression in fecundity as a result of competition from the other biotype depended on sown initial density. For this reason model (3) was considered more appropriate. Nevertheless, both models were tested on the data to compare their relative success. The decline in plant yield at high densities was not sufficient to give a constant final yield and therefore the power term, **b**, was set equal to unity. This also facilitated comparison of these models, because both were then specified by only three parameters (if incorporated into the model fitting described below **b** was not significantly different from unity, for both biotypes).

For herbicide treatments, R responses are able to be fitted by models (2) or (3), but the pattern of S response (Fig. 6.4b) means that reciprocal models cannot be fitted.

Control treatment

Mortality response.- Plots of survivors against seedlings on log scales (Fig. 6.5) showed little evidence of density-dependent mortality, even at high mixture densities where mortality amounted to 10%, despite previous statistical analysis having shown significant density effects on survival

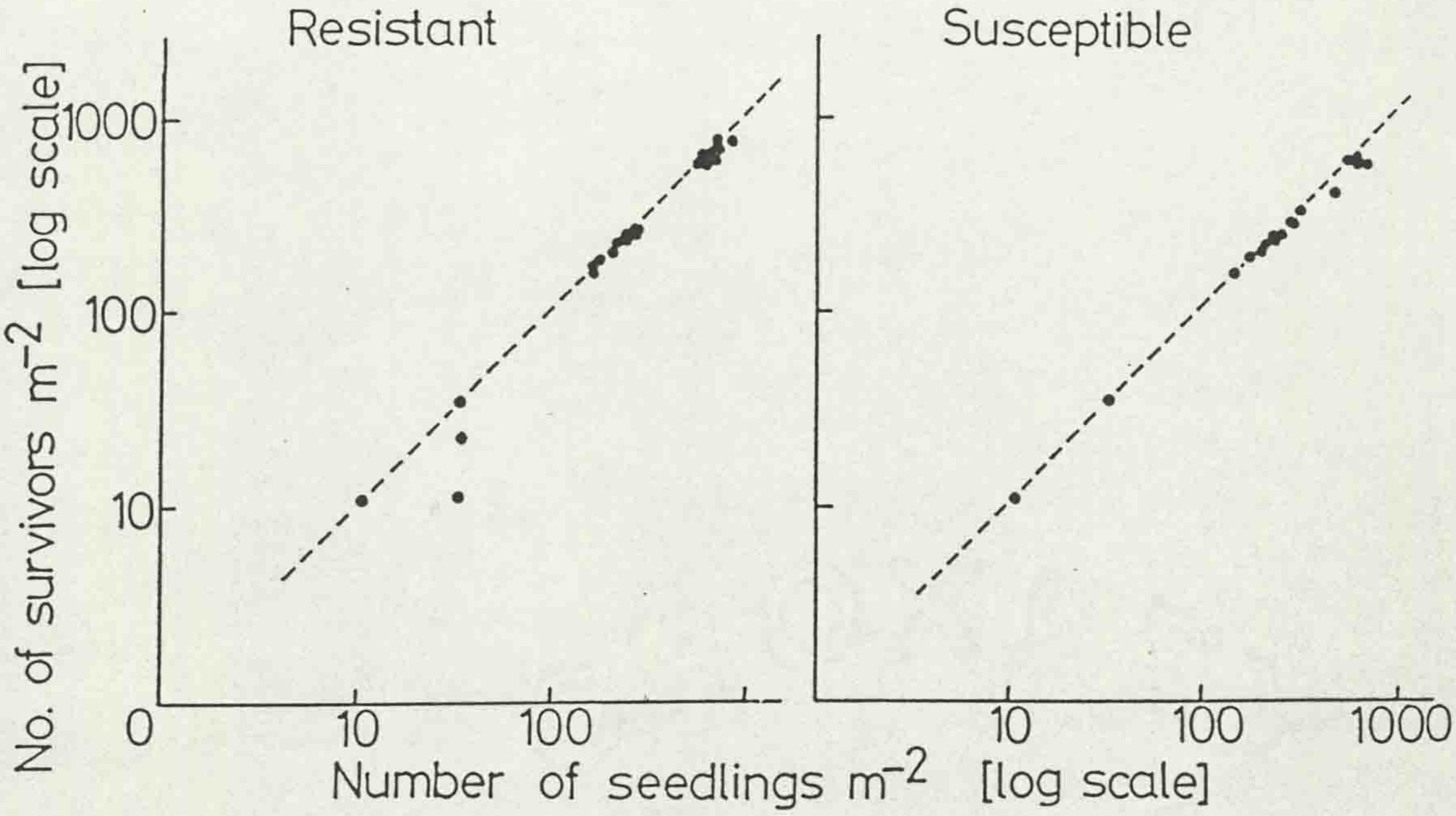

Figure 6.5 The relationship between number of seedlings and number of survivors at harvest of herbicide-resistant and susceptible biotypes of Alopecurus myosuroides in control treatments. Data for all mixture densities are included. Dashed line is of unit slope and represents complete survival.

(6.6.2, Fig. 6.1). There were no evident biotypic differences (Fig. 6.5) and applying model (1) gave the following relationship for the R biotype:

$$N_{R,f} = N_{R,i} \, (1 + 0.00006 \, (N_{R,i} + 1.21 \, N_{S,i}))^{-1},$$
$$r^2=0.9980, \text{ d.f}=47, P<0.001$$

For the S biotype:

$$N_{S,f} = N_{S,i} \, (1 + 0.00021 \, (N_{S,i} + 0.08 \, N_{R,i}))^{-1},$$
$$r^2=0.9998, \text{ d.f}=48, P<0.001$$

Although model-fitting explained much of the variation in the data (r^2 values), all parameters were poorly estimated: coefficients of variation (CV) of m_R, γ_R, γ_S were in fact over 1000%. It is clear that the upper density range was not high enough to cause sufficient mortality for modelling purposes and to detect biotype differences by this means.

Fecundity response.- Individual plant fecundity, when plotted in three dimensions, was visibly affected by density (Fig. 6.6) and two biotype differences were noticeable in this graph. The S biotype produced more seed at low densities and R fecundity was not suppressed greatly at high S-low R densities. Model (2), with **b**= 1, gave results which broadly agreed with this interpretation. Estimates of **w** were significantly different ($P<0.05$): back-transformed values were 935 seeds plant^{-1} for the R biotype and 1678 seeds plant^{-1} for the S biotype. Precision of the competition coefficients and **a** values was much lower (CV: 210-340%) and biotypes were not different. Qualitatively, they indicated that S plants were sensitive to R plants (α_S=1.91), whilst R plants experienced a S plant as equivalent to a R plant (α_R=1.10).

Applying model (3) gave no overall improvement in regression fit ($r^2 \simeq$ 0.99 for both biotypes), but standard errors for parameter values were much reduced (all CV < 100%, except $b_{ij,R}$). The models fitted to the data gave the following relationships:

$$S_R = 5636 \, (1 + N_{R,i}^{0.36} + N_{S,i}^{0.24})^{-1},$$
$$r^2=0.996, \text{ d.f.}=46, P<0.0001$$

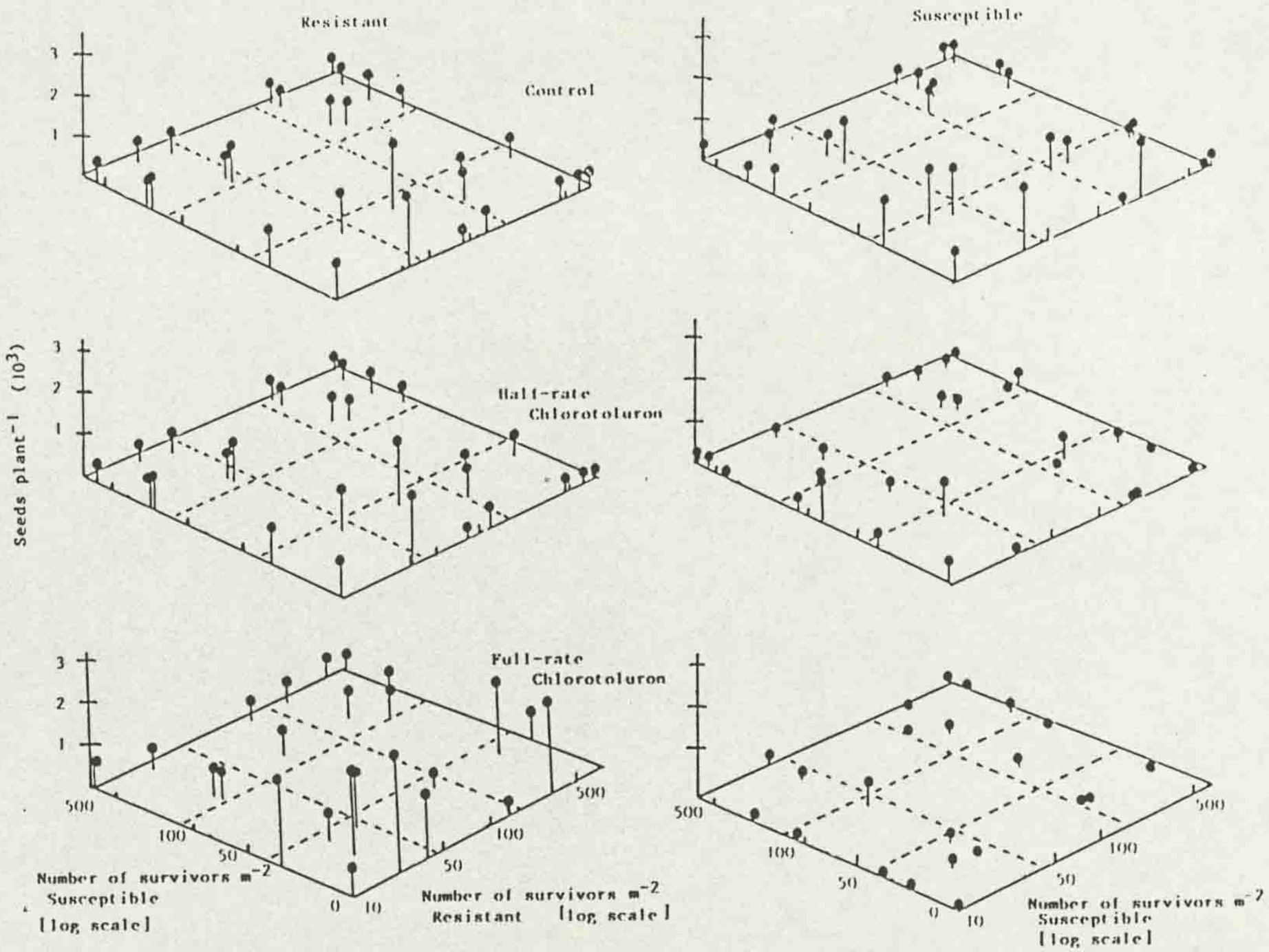

Figure 6.6 The relationship between mean seeds plant^{-1} and number of surviving plants at harvest for mixtures of herbicide-resistant and susceptible biotypes of Alopecurus myosuroides in a pot experiment, treated with three rates of herbicide. Numbers of survivors are represented for both target biotype and associated mixture biotype on the horizontal axes.

$$S_S = 15066\ (1 + N_{S,1}{}^{0.51} + N_{R,1}{}^{0.62})^{-1},$$
$$r^2=0.993,\ d.f.=36,\ P<0.0001$$

Comparison of parameter values (**w**) showed that the S biotype produced about 67% more seed in the absence of competition ($P<0.01$). The power terms, b_{ij}, were significantly different ($P<0.001$), but the b_{ii} terms were not. To describe relative competitive ability, model (4) was used for a range of initial densities and frequencies. The results (Table 6.5) show that only at particular high R densities does the R biotype have greater competitive ability. At low densities, the S biotype is more competitive both in equal mixtures and in minority. At high (1000 seeds pot^{-1}) R density, S competitiveness is greater than R at an intermediate S density.

Herbicide Treatments

Mortality response.- No clear mortality was evident for the R biotype in response to either density or herbicide in monocultures (Fig. 6.7). Attempts to detect density-dependent effects by regression analysis, indicated by a slope not equal to unity, were hampered by little mortality at the lowest densities for the S biotype (Fig. 6.7). Where numbers of plants were greater and sampling error thus diminished, mortality was evident. Regression on such data gave positive non-zero intercept terms, which have no biological significance: they would imply that numbers of survivors was greater than seedling numbers!

Parameter values from fitting model (5) to S data and model (1) to R data are presented in Table 6.6, with regression statistics. Although standard errors for parameters were large except for τ and m_S, parameter values fall in an intuitive pattern. With increasing herbicide dose, S competitiveness (α_{FR}) declined to zero. At the same time the intercept parameter, τ became negative, which would describe mortality at low

Table 6.5 Relative competitive ability calculated from model of Law and Watkinson (1987), based on parameter values estimated from analysis of competition between a herbicide-resistant (R) and a susceptible (S) biotype of *Alopecurus myosuroides*. Competitive coefficients are given for representative initial density combinations.

	Competitive ability of component j	
j:i density	R	S
1:1	0.68	1.22
10:10	0.52	1.57
10:1000	0.02	0.27
1000:1	55.50	35.84
1000:10	9.63	14.96
1000:1000	3.43	0.39

Table 6.6 Parameter values, associated standard errors (in parentheses) and regression statistics for models fitted to density response of the herbicide-resistant (R) and susceptible (S) biotypes of *Alopecurus myosuroides*, when grown in competition and under three chlorotoluron herbicide treatments.

	Regression Parameters and Statistics								
	R Biotype				S Biotype				
Herbicide	m	γ	r^2	d.f.	τ	m	γ	r^2	d.f.
Control	0.00006 (0.00009)	1.21 (3.1)	0.998	48	0.006 (0.005)	0.00023 (0.00004)	0.15 (0.16)	0.999	49
Half-rate	0.00014 (0.00004)	0.29 (0.38)	0.999	50	-0.029 (0.017)	0.00060 (0.00020)	-0.10 (0.20)	0.998	47
Full-rate	0.00022 (0.00004)	0.06 (0.20)	0.999	48	-0.183 (0.060)	0.00123 (0.00060)	-0.37 (0.32)	0.983	43

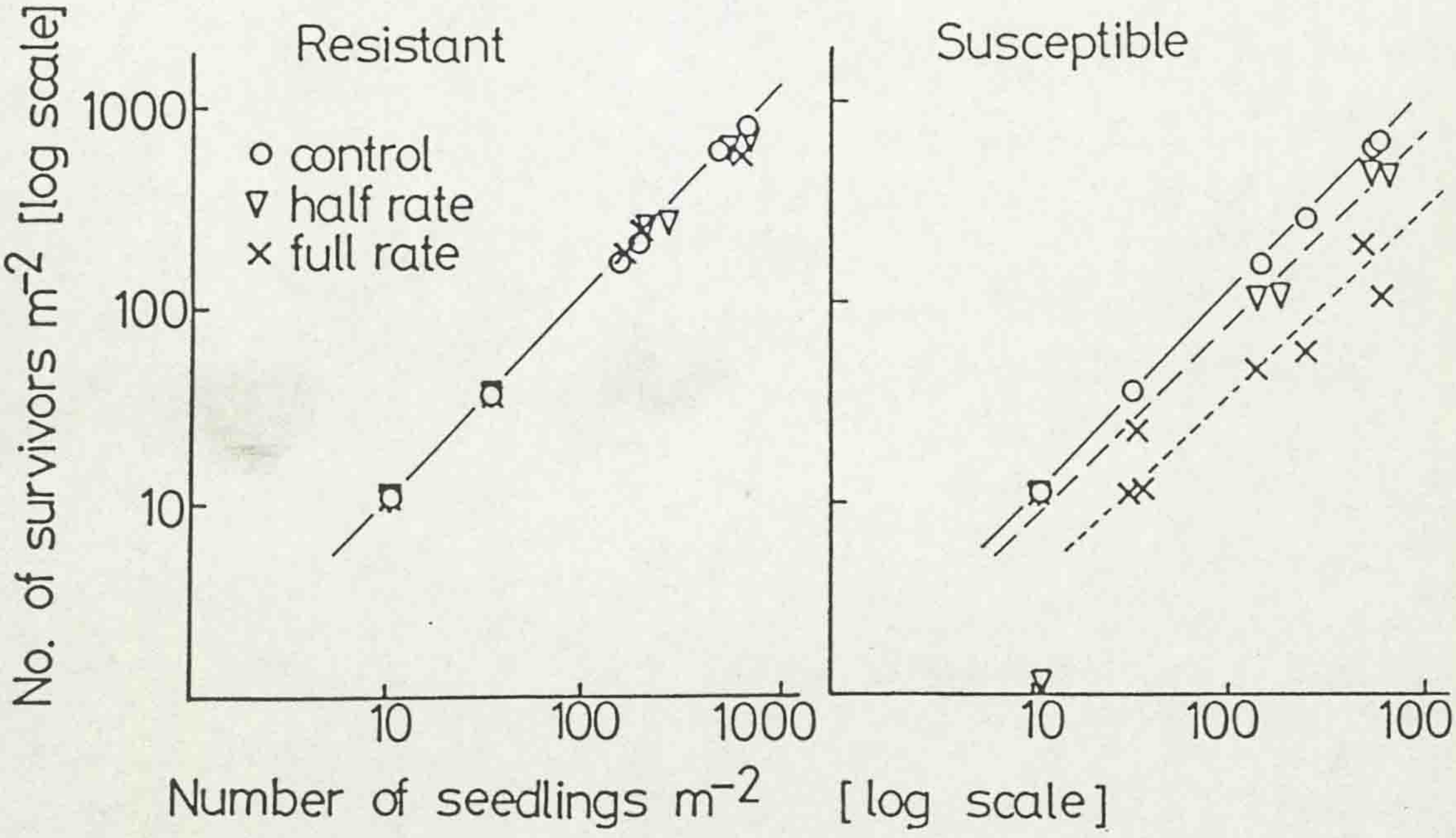

Figure 6.7 The relationship between seedling density and density of survivors at harvest in monocultures of herbicide-resistant and susceptible biotypes of Alopecurus myosuroides in different chlorotoluron herbicide treatments.

densities. Additionally, α_{RS} values become negative, implying that R plants act to reduce mortality of S plants.

Fecundity response.- The relationships between surviving plant density and individual plant density are shown in Fig. 6.6. The variation in seeds plants^{-1} was greater in herbicide-treated pots. For example at low R density, S plants present at high density varied more (half-rate: CV=7%; full-rate CV=18%) compared to control (CV=3%). The raw data show that S fecundity was strongly depressed by herbicide application. Fecundity of the R biotype was reduced slightly by herbicide, but in mixtures seed yield appeared to be unaffected. Compensation in yield for lower S density may have tempered the herbicide effect in such mixtures.

If model (2) is fitted to the R biotype data at each herbicide rate, the competition coefficient representing S effect on R seed yield is 2.2 for half-rate chlorotoluron and 0.3 for full-rate. The high value in the former treatment is unexpected (in control treatment= 1.1) and it may be attributable to very low R yields at the lowest R density with high associated S densities (see Fig. 6.4a).

6.4 Discussion

6.4.1 Biotype differences

The biotypes used here were genetically distinct because they were collected from different sites. An ideal study would use material isogenic except for the resistance trait. The closest to this has been a study (Gressel & Ben Sinai, 1985) on triazine-resistant, cultivated *Brassica napus* L., which used back-crossed plants. For practical reasons almost all studies, including this one, used seed either collected from one area and screened for resistance (e.g. Elliott & Peirson, 1985) or from different areas (e.g. Warwick & Black, 1981). An improved approach, recommended by Warwick (1980), uses a number of R and S collections.

This is feasible for growth comparisons on spaced plants, but is much less practical for competition studies (but see Burdon & Müller [1987] with disease-resistance). Studies based on genetically distinct biotypes must generalise to situations where the two biotypes have been brought together by dispersal or invasion events.

In strict statistical terms, estimates of competitive effect of one mixture component on another are only appropriate for one component, since reciprocal effects are not independent. Law and Watkinson (1987), using small sized pots in a two-species competition experiment, doubled the number of experimental units to eliminate this problem. Such increase in the size of the present experiment was precluded in this study because of the numbers of pots already employed, and by the use of large pots to minimise edge effects and better estimate 'isolated plant' yield. In modelling competition, estimates of parameters representing isolated plant yield were indeed adequate here, but the lack of sufficiently high densities prevented accuracy in estimates of the other two parameters of the reciprocal model. Lack of precision in the competition coefficients may be attributable to the presence of substantial genetic variability in *A. myosuroides* (Chapter 4, Table 8.1) as an outbreeder (Beddows, 1931) and to the use of bulked seed from collections made in natural populations. Taken together with the similarity between *A. myosuroides* biotypes relative to interspecific comparisons (e.g. Law & Watkinson, 1987), greater replication than used here is suggested, even at low densities.

The S biotype was more fecund at low densities than the R biotype and models describing the mixture data confirmed this. Relatively more tillers were produced by the R biotype, however, and it appeared to be less sensitive to increasing densities than the S biotype. Seed yield per

unit area was higher for the S biotype at low densities at equal mixtures, while the R biotype produced relatively more in majority or minority mixtures. Estimates of competitive ability based on seed yield depended on the model, one (Firbank & Watkinson, 1985) predicting R superiority in competition. The more recent model of Law & Watkinson (1987) is more flexible in allowing competitive ability to vary with density. The data supported the use of this model: at low densities the S biotype was more competitive, but at high densities the R biotype outperforms the other biotype. Despite the overall superiority of the R biotype at high densities, at one particular mixture combination, the S biotype would be more competitive (Table 6.5).

Such competitive interactions between biotypes will only influence the rate of spread of a biotype when selection pressure from herbicide is relaxed and mixed populations occur. Cases where the selective agent is not operating can occur through spatial or temporal 'escape'. Spatial escape will occur: when the herbicide is not applied, such as in a crop rotation, when the herbicide fails to perform, e.g. in sub-optimal weather conditions; when the herbicide misses the target, perhaps through faulty spray nozzles or by shielding of target plants by other target plants or crop plants; by dispersal of a biotype to an area not sprayed, such as a field margin or different crop. Temporal escape may result from seedling emergence after a herbicide application, as can happen easily with contact herbicides, or after herbicidal activity has declined, with residual herbicides (Putwain *et al.*, 1982).

6.4.2 Herbicide and density

Few studies have examined individual and population responses to herbicides over density ranges commonly found in the field. The results of this study suggest that there is a need to develop appropriate models

to describe interactions of herbicide with natural regulatory processes (see also Mortimer, Sutton & Gould, in press). For mortality, model (1) was unable to describe S biotype response to density when sprayed and a futher parameter (intercept term) was needed (model (5)). Although variation in the data was increased by herbicide treatment and parameter values were more poorly estimated, mortality competition coefficients became negative with increasing herbicide dose. This suggests that presence of R plants increased S biotype survivorship. More conclusive evidence of postive density-dependence came from statistical analysis of survival proportion.

The action of herbicides is usually taken to be independent of weed density (e.g. Doyle *et al.*, 1986). Manlove (1985) has provided evidence to support this assumption with *Avena fatua* L. and a post-emergence herbicide. Yet as density increases to a point where self-thinning occurs, an intuitive expectation is that herbicide dose received per plant declines. Whether the decline is sufficient to allow survival at high densities depends on the toxicity of the herbicide. A clear demonstration of such positive density-dependence was reported in *Bromus sterilis* population responses to pre-emergence herbicide (Mortimer, 1985). Below a threshold density all seedlings were killed, but above this positive density-dependent seedling survivorship occurred up to densities of 10^4-10^5 seeds m^{-2}, where survival equalled that in control plots.

The mechanism allowing positive density effects in *B. sterilis* and *A. myosuroides* may be similar. Susceptible plants, in this study, may have experienced reduced herbicide dose because of the presence of R individuals and surviving S plants. Shielding of target plants from herbicide spray is possible by high densities of other plants. Additionally, the mean dose received per plant could be reduced by other

plants through uptake of the herbicide in sufficient quantity, making it unavailable for target plants. This may be more likely here, with chlorotoluron, than shielding, because most of chlorotoluron activity is in root rather than foliar uptake (Blair, 1978).

6.4.3 Prediction of competitive outcome

Predicting competitive outcome over generations is easily possible with response surface analysis for two-species mixtures, but is complicated for the two biotypes used here by the breeding system of the species and lack of knowledge of the mode of inheritance of resistance. If resistance is maternally inherited in *A. myosuroides*, as in most triazine-resistant weeds, the composition of successive generations would reflect relative success of biotypes in competition in the previous generation. However, if resistance, competitive ability, or aspects of morphology relating to competitive ability were transmitted by pollen, then genetic change would occur between generations unless incompatibility or assortative mating between biotypes was assumed. The mode of inheritance of resistance is not known for *A. myosuroides* (Chapter 4) and because of the difficulties involved in simultaneously modelling population growth and genetic changes, the assumption is made here that R and S plants produce only R and S offspring, respectively. This then implies either maternal inheritance or total inbreeding of homozygous R and S plants.

In a herbicide-treated environment, predictions are hampered further by the lack of suitable models displaying patterns of response as seen in the data, notably positive density-dependence. Yet given the magnitude of both mortality and fecundity reductions in surviving S plants, the outcomes are likely to be simple with repeated herbicide application: elimination of the S biotype.

In an unsprayed situation, two further assumptions are needed: that emergence proportions are 0.60 and 0.65 for S and R biotypes respectively, and that mortality patterns and competition coefficients for mortality are equal for both biotypes (see 6.3.7). Outcomes of competition are now determined by the reciprocal model (3), describing seed production. Iterating the model over a number of generations results in the R biotype eliminating the S biotype: the S biotype increases for 2-3 generations before declining slowly to extinction in over 20 generations. If mortality models are set equal for both biotypes with their respective competition coefficents at unity, extinction of the S biotype is more rapid. When the S biotype is the majority mixture component initially, elimination of S plants occurs in 16-20 generations: if both biotypes are at equal initial densities, it is eliminated in 15 generations; if S plants are in minority in the starting mixture, extinction takes 10-14 generations. Despite the greater reproductive capacity of S plants at initial low densities, R plants can produce enough seed within one or two generations to allow its superior competitive ability to dominate the mixture.

CHAPTER 7
POPULATION DYNAMICS AND SELECTION IN HERBICIDE-RESISTANT AND SUSCEPTIBLE *ALOPECURUS MYOSUROIDES*

7.1 Introduction

Although the factors regulating population size in weed species are beginning to be understood (Mortimer, 1983; Firbank & Watkinson, 1985; Mortimer, 1987), the effects of natural and artificial perturbations on populations are far from understood (Mortimer, 1985). Several factors may influence the demography and growth of weed populations, e.g. pathogens (Burdon *et al.*, 1984; Paul & Ayres, 1987), herbivory (Pyke, 1987) and cultivation regime (Wilson & Cussans, 1975; Moss, 1981). Furthermore, the potential of herbicides to influence weed populations has long been recognised (Harper, 1956). Herbicides can have considerable effects on species composition (Haas & Streibig, 1982; Mahn, 1984), population size (Manlove, 1985; Mortimer, 1987; Fernandez-Quintanilla *et al.*, 1986), and the evolution of herbicide-resistant populations through selection (LeBaron & Gressel, 1982). In an agronomic context, such effects have far-reaching implications and warrant detailed investigation.

The high rates of control (90-95%) often achieved by agricultural dose rates of herbicides (e.g. Clarke, 1987) suggest that strong selection pressures for herbicide tolerance (Putwain, Scott & Holliday, 1982). This is supported by the existence of numerous resistant populations in many species (LeBaron & Gressel, 1982). However, it has been claimed that the frequency of cases is less than expected because selection is weaker than is commonly assumed (Gressel & Segel, 1978). To this end, intrinsic features of weed biology, e.g. dormancy, may facilitate a dilution of the selection pressures (Putwain, 1982; Gressel & Segel, 1982).

An understanding of the potential rate of evolution of resistance and the development of efficient and rational weed management strategies in the face of resistance will require both knowledge of the factors regulating weed populations and of population response to control measures (Mortimer, 1983). For instance, herbicide treatment has been traditionally viewed as a density-independent control practice (Pollard, 1982; Doyle, Cousens & Moss, 1986). Perplexingly, the very limited experimental evidence both supports (Manlove, 1985) and contradicts (Mortimer, 1985) this assumption. Furthermore, to accurately assess selection pressures, studies should take place in the field (Endler, 1986) and should measure reductions in seed production and increased mortality to quantify selection by herbicide treatment. Finally, phenological escape from herbicide may occur (Putwain *et al.*, 1982), hence seed production should be measured for all cohorts recruited to a population.

This study describes an experimental, field-based examination of the interaction of weed density and herbicide control on the dynamics of two biotypes of *A. myosuroides* were in a wheat crop. One biotype was resistant to the urea herbicide chlorotoluron, the other susceptible to the herbicide (Chapter 4; Moss & Cussans, 1985). The objectives were to: (1) test for a density-dependent basis to herbicide control; (2) describe and compare age-specific and density effects on plant response with and without herbicide treatment; (3) assess the magnitude of selection by the herbicide in a field environment.

7.2 Materials and Methods

7.2.1 Plant material

Seeds (spikelets) of resistant (R) and susceptible (S) biotypes of *A. myosuroides* were derived from original collections made at a farm in Essex (NGR: TL 985169) (R) and from experimental plots which have never received herbicide at Broadbalk Field, Rothampsted Experimental Station, Harpenden, Herts (NGR: TL 140130) (S). Seed from each collection was bulked up over two seed generations in isolated, interbreeding populations in polythene tunnel houses. Resistance and susceptibility was confirmed in pot experiments (Chapter 4).

7.2.2 Experimental procedure

The experiment was laid out in an area of 768 m^2 on a sandy loam soil on a gentle, south-west facing slope at the University of Liverpool Botanic Gardens, Ness. The plot layout was a randomised block, split-plot design with three replicates. Main plot treatments were four herbicide regimes and sub-plots were six sowing densities of each *A. myosuroides* biotype sown as monocultures. There was one extra replicate per block of the two lowest densities of each biotype. Individual plot size was 1.5 x 1.5 m with 1 m guard areas at the edge of the experimental area.

The crop, winter wheat cv. Avalon, was sown on 22 October 1985 at 250 kg ha^{-1} (8 cm depth) with 9:24:24 NPK fertilizer incorporated in the seed bed at 375 kg ha^{-1}. *Alopecurus myosuroides* seed was scattered by hand over each plot on 25 October at six densities (1, 10, 30, 100, 300, 1000 'germinable' seed m^{-2}; where germinability was assessed in a soil test under a mist unit) and raked into the soil surface. Four herbicide treatments were applied in the last week of March 1986 with an Oxford field plot precision sprayer in 400 l ha^{-1} water at 3 bar.

Treatments were: (1) no herbicide applied; (2) half-rate (1.38 kg a.i. ha^{-1}) and (3) full-rate (2.75 kg ha a.i. ha^{-1}) chlorotoluron, formulated as 'Dicurane 500 FW'; (4) full-rate (2.1 kg a.i. ha^{-1}) isopoturon, formulated as 'Tolkan'. Subsequently three spring top dressings of N ('Nitram') were applied on 18 April, 19 May and 9 June 1986 to a total of 200 kg N ha^{-1}.

Emerging *A. myosuroides* seedlings were ringed with coloured wire at 2-3 week intervals in either 1 m^2 or 0.25 m^2 (highest density) plots starting on 23 November 1985. Seedling fate was followed subsequently until harvest, after 300 days growth, which took from 4 August to 5 September 1986. At harvest, inflorescence spike lengths (mm) of individual reproductive tillers were recorded for all plants and a stratified sample of heads (n=52) retained to predict seed number.

7.2.3 Data analysis

Seed number

Seed numbers were estimated by measuring the inflorescence length. An allometric relationship was calculated between $\log_{10}$ inflorescence length and $\log_{10}$ seed number. Inflorescence lengths ranged from 7 mm to 141 mm in the retained sample. Separate regressions accounted for 87% and 68% of the variation for the R and S biotype, respectively. Analysis of covariance showed that the slopes were homogeneous ($F_{1,49}$=0.61, N.S.) and further, that intercepts were not significantly different ($F_{1,49}$=0.98). A joint regression, $\log_{10}$ seed no.= -0.221 + 1.165 $\log_{10}$ spike length (r^2=0.80, df=51, $P<0.0001$), was therefore used.

Germination

Survival analysis (e.g. Lee, 1980) was applied to germination responses to detect any biotype differences in germination rate and its distribution over time (Scott, Jones & Williams, 1984). The method sums germination responses within an experimental treatment and uses life table analysis to estimate the germination rate at defined intervals. The germination rate is measured by the probability density function (PDF), which estimates the probability that a seed will germinate in a given time interval. The probability density can then be interpreted as the distribution of germination rates over time (Scott *et al.*, 1984). Estimates of the PDF were generated using PROC LIFETEST of SAS (SAS Inst. Inc., 1985) and herbicide treatments or biotypes were compared using 95% confidence intervals of the PDF. Likelihood ratio (G) tests were used to compare final percent germination between biotypes.

Survival

Survival is a quantal response and appropriate analysis of survival involves log-linear models. These are the counterpart, for attribute data, of analysis of variance on continuous data (Sokal & Rohlf, 1981). Based on a multinomial distribution, a series of log-linear models are fitted, testing the association between survival and experimental treatments (here, density and herbicide) at a high level (e.g. 4-factor interaction) before proceeding to lower level interactions.

Final proportional survival, as analysed by log-linear models, does not address the pattern of survival over time of a cohort or population, however. Examination of survivorship curves and thus lifespans are therefore needed (Pyke & Thompson, 1986). Lifespans were

estimated as the number of days between emergence (= census date when tagged) and death (= census date when recorded as dead). Plants dead at the final census (harvest) were treated as dead on the first day of harvesting. Plants alive at the final census were treated as dead on the last day of harvesting, 32 days later, on the assumption that these plants would have died on reaching the end of the annual life span, or from cultural operations such as harvest. Analysis of this uncensored data compared frequency distributions of lifespans using the Kruskal-Wallis test (Sokal & Rohlf, 1981).

Fecundity

Because of lack of data at low densities where some or most cohorts were not represented, seeds plant^{-1} (X) was first pooled over certain densities and cohorts. Data were transformed to $\log_{10}$ (X + 1) before analysis to homogenise variances. To correct for non-orthogonality, analyses of variance were based on Type III sums of squares (SAS Inst. Inc., 1985).

Population growth

Population change through one generation was modelled using a difference equation originally due to Hassell (1975):

$$N_{t+1} = N_t \lambda (1 + a N_t)^{-b} \quad (1)$$

where N_t and N_{t+1} are population numbers at intervals t and t+1, λ = net reproductive rate under uncrowded conditions, and **a** and **b** are parameters which measure response to density and are to be estimated. To avoid overfitting (a result of high parameter/sample ratios) and reduce the standard errors of parameters, equation (1) was simplified by assuming exact compensation for density and thus setting **b** = 1. There was *a posteriori* evidence for this: **b** was not significantly ($P > 0.05$) different from unity. Hence, the model becomes:

$$N_{t+1} = N_t \lambda (1 + a N_t)^{-1} \quad (2)$$

To model more than one equilibrium point a second equation was employed:

$$N_{t+1} = \tau N_t^2 (\rho + N_t^2)^{-1} \quad (3)$$

where τ and ρ are shape-parameters to be estimated. All models were fitted using SAS PROC NLIN (SAS Inst. Inc., 1985). The transformation $\log_{10}(X + 1)$ was used to homogenise variances for equation (2).

7.3 Results

7.3.1 Emergence

Seedlings of *A. myosuroides* emerged over a 25 week period starting in mid-November (about 30 days after sowing) and were grouped into eight, roughly equally spaced, 2-3 week cohorts. The first three cohorts recruited between November 1985 and January 1986 formed about 85% of total numbers. Emergence was not density-dependent, seedling density per plot showing a linear relationship to sowing density (Fig. 7.1). Seedling emergence between biotypes was compared with data pooled over all densities. Total emergence proportion was significantly ($G=224.6$, d.f.=1, $P<0.001$) greater in the R (0.34) than the S biotype (0.28).

Comparisons at each census showed that the rate of emergence also differed (by comparison of 95% confidence interval of PD function). When data was pooled over herbicide and density, rate of emergence was greatest for the S biotype until 60-70 days after sowing (Fig. 7.2a). However, the emergence rate of R plants was higher at census points after 150 days (from April to May). Cumulative emergence showed that by 75 days 71% (± S.E. 0.7) of R plants and 77% (± S.E. 0.8) of S plants had emerged; by 100 days, 87% (± S.E. 0.5) and 90% (± S.E. 0.5) of R and S plants, respectively, had emerged.

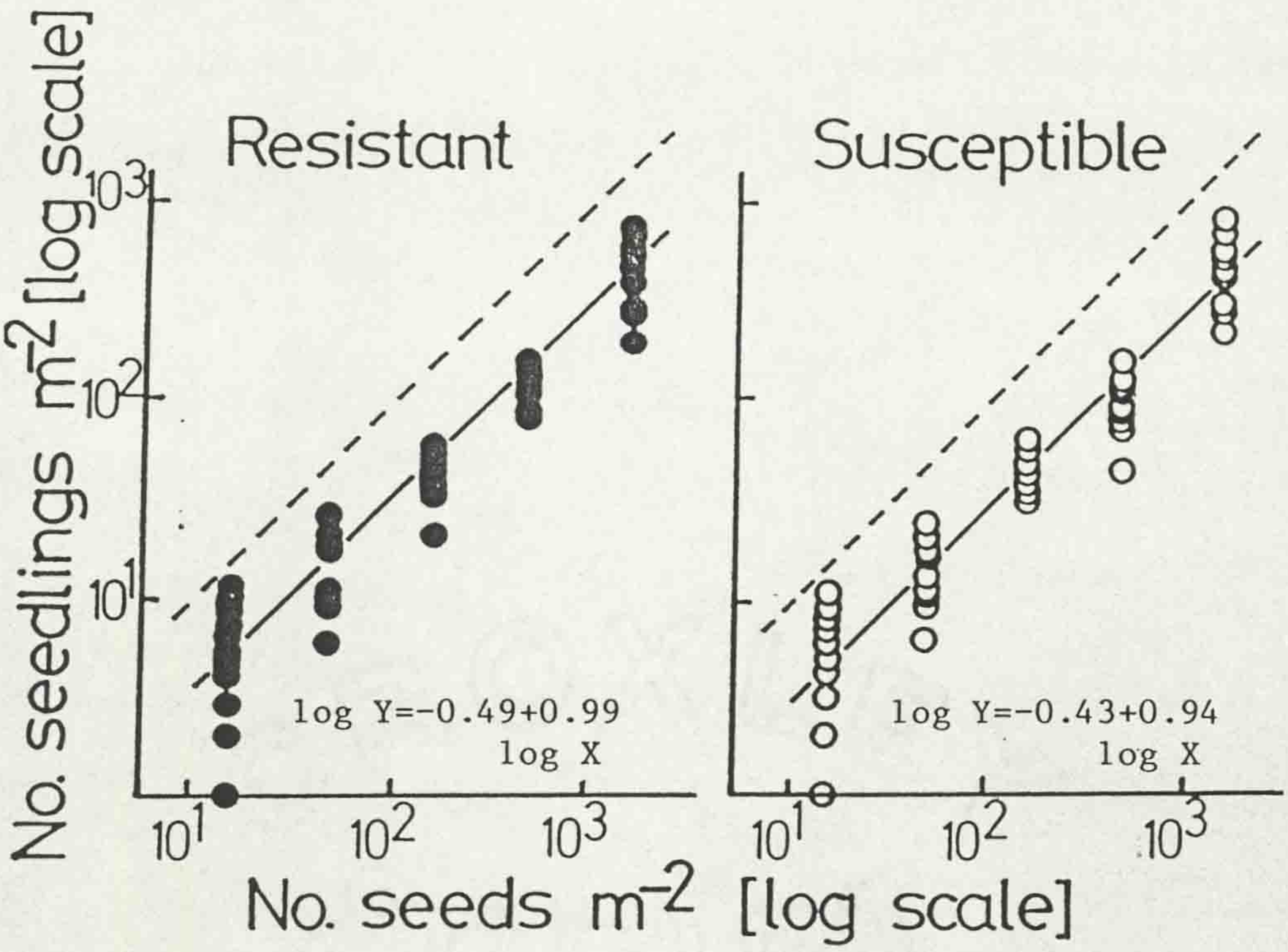

Figure 7.1 The relationship between sowing density (seeds m^{-2}) and number of seedlings emerging (plants m^{-2}) for herbicide-resistant and susceptible biotypes of Alopecurus myosuroides. Data were pooled over herbicide treatments. Linear regressions (solid line) were fitted to $\log_{10}$ transformed data. Regression statistics for both biotypes were: r^2= 0.933, d.f.= 53 and $p < 0.0001$. Dashed line is of unit slope and represents complete emergence of seeds sown.

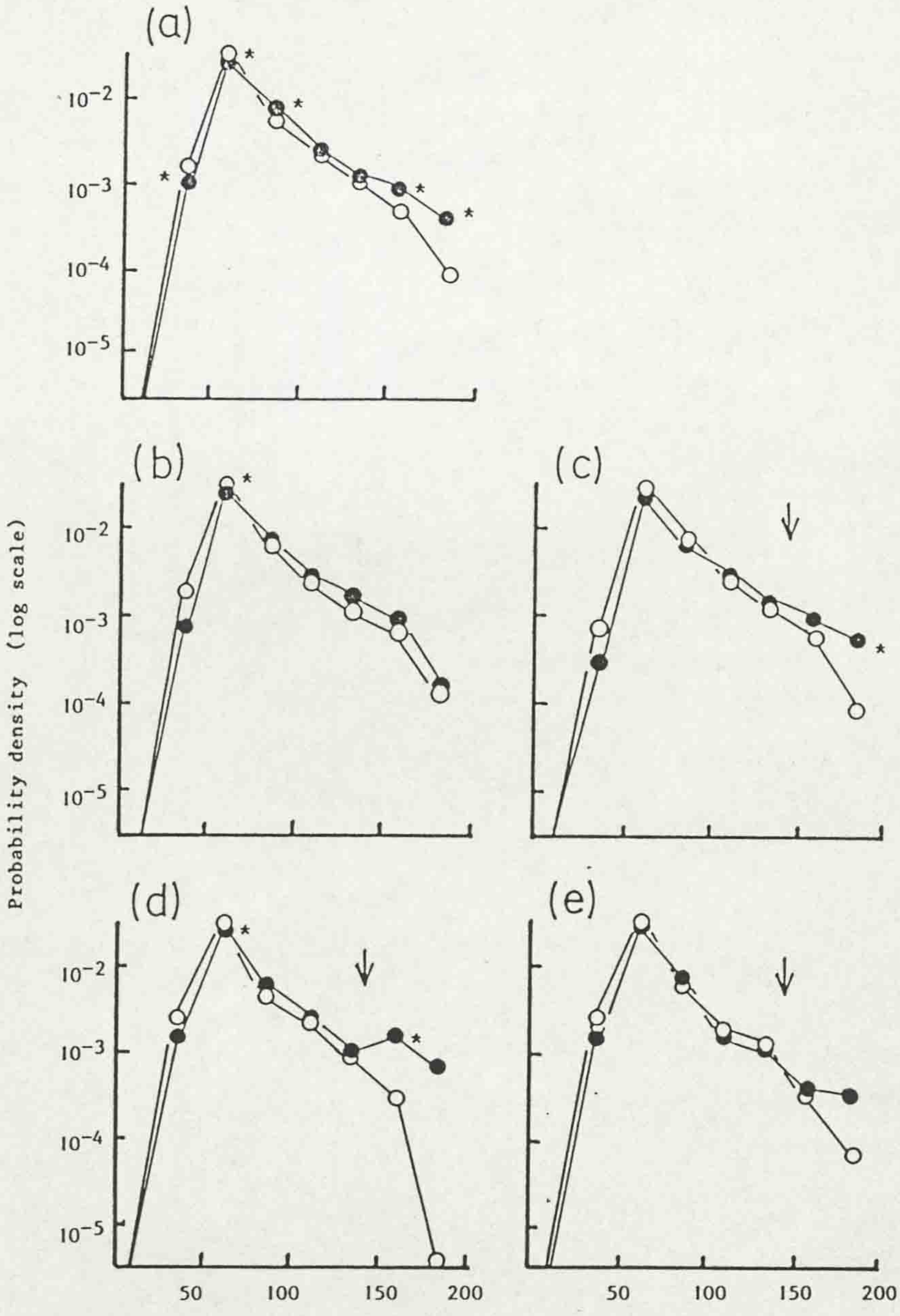

Figure 7.2 Germination rates expressed as probability density functions for herbicide-resistant (●——●) and susceptible (○——○) biotypes of Alopecurus myosuroides grown as field populations and exposed to four herbicide treatments. (a) all data pooled; (b) control; (c) half-rate chlorotoluron; (d) full-rate chlorotoluron; (e) full-rate isoproturon. Asterisks indicate significant differences between biotypes from comparison of 95% confidence limits. Arrows indicate the time of spraying with herbicide.

When examined separately for each herbicide treatment, no differences between biotypes were found in emergence rate in unsprayed plots, after the date of herbicide treatment (Fig. 7.2b). Higher R emergence ($P \leq 0.05$) was found after about 175 days in the half-rate chlorotoluron treatment (Fig. 7.2c) and after about 150 days with full-rate chlorotoluron (Fig. 7.2d). No difference occurred in isoproturon-treated plots (Fig. 7.2e).

7.3.2 Overall survival

Density-dependent effects on survival are suggested if the slope of a seedlings-survivors regression deviates from unity. If the slope equals unity, the presence and magnitude of density-independent mortality is detectable by a significant non-zero intercept. In unsprayed plots, there was no evidence of density-dependent mortality or biotype differences (Fig. 7.3a). Chlorotoluron caused slight, and isoproturon substantial, density-independent mortality in the R biotype. All herbicides acted in a density-dependent manner on S plants (Figs. 7.3b-d). Density-dependence was negative: at higher seedling densities, herbicide killed proportionately more plants. Isoproturon had a stronger effect than chlorotoluron, as judged by the value of the slope.

Log-linear model analysis confirmed that the response of survival to density was dependent on herbicide treatment for both R ($G=33.5$, d.f.=12, $P \leq 0.001$) and S ($G=18.2$, d.f.=12, $P=0.05$) biotypes. Associations between density and survival were therefore examined for each herbicide separately. In unsprayed plots survival of the R biotype increased with density ($G=18.2$, d.f.=4, $P \leq 0.001$) except at the highest density treatment (Fig. 7.4a). Survival increased more clearly (Fig 7.4a) with density at half-rate chlorotoluron ($G=9.6$,

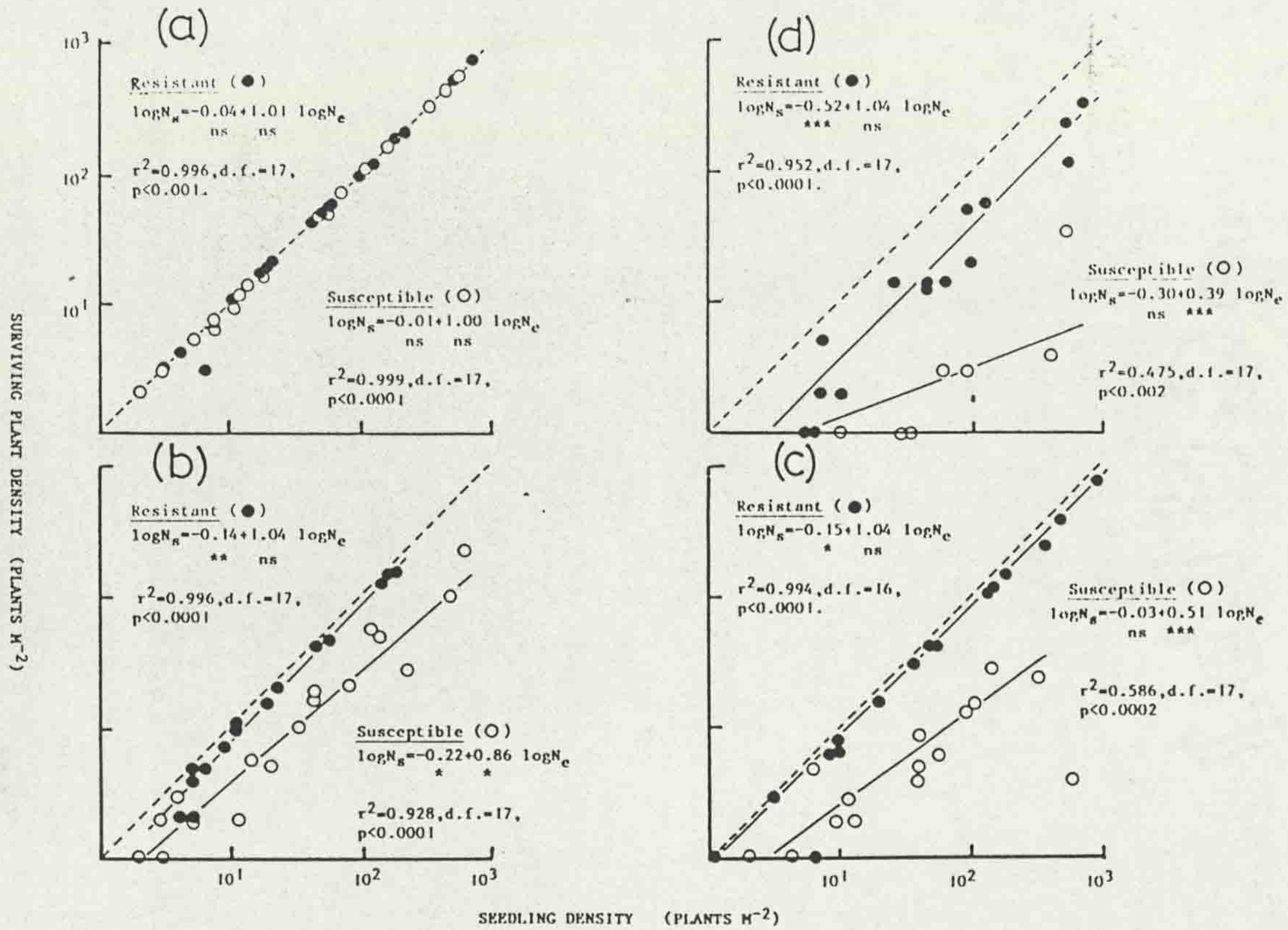

Figure 7.3 The relationship between seedling density and surviving plant density at harvest (plants m^{-2}) for field populations of herbicide-resistant (●) and susceptible (○) biotypes of Alopecurus myosuroides treated with herbicide. (a) control; (b) half-rate and (c) full-rate chlorotoluron; (d) full-rate isoproturon. Regression equations and statistics are given. Also given are significance levels of F-tests for H_0: intercept=0, H_0: slope=1. Significance levels are indicated by: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant. One outlier was excluded from the regression for the susceptible biotype at full-rate chlorotoluron.

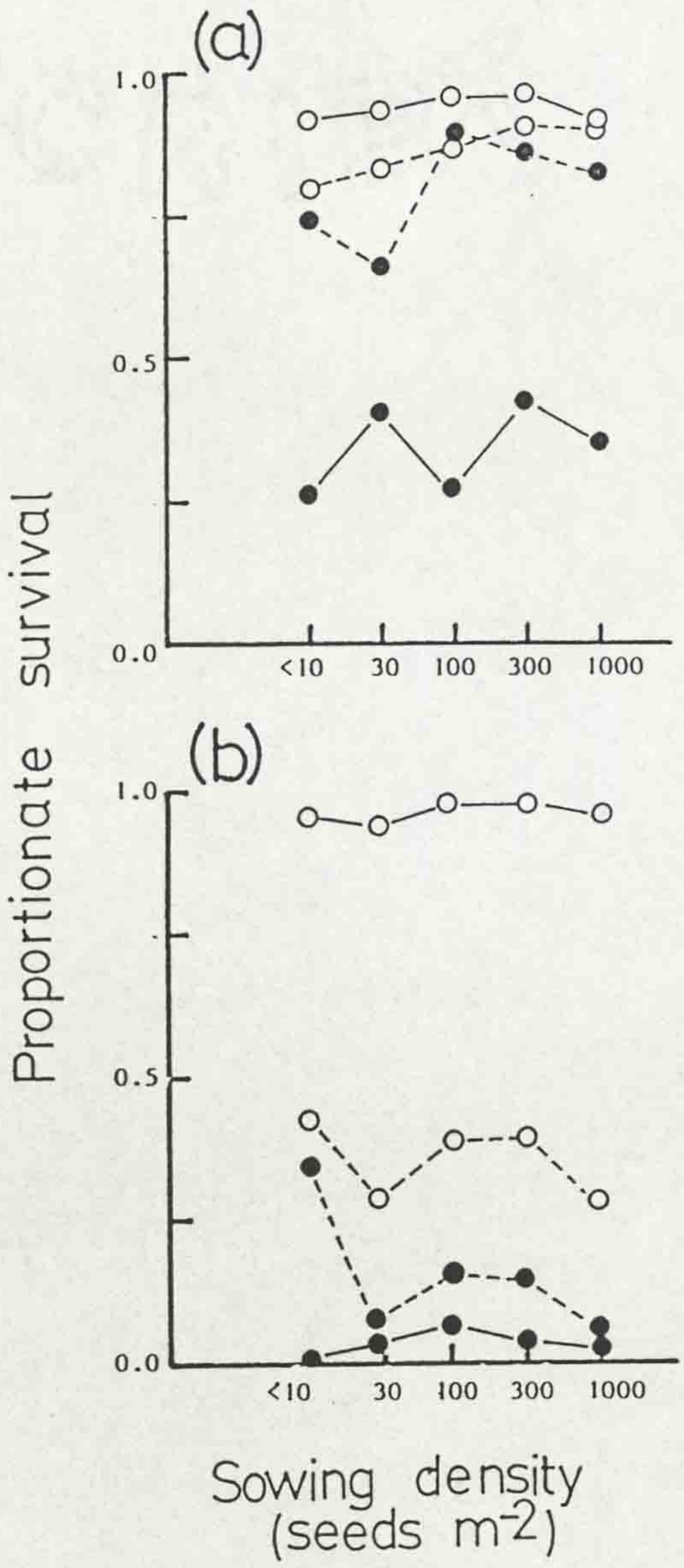

Figure 7.4 Mean proportionate survival in relation to sowing density treatments for (a) herbicide-resistant and (b) susceptible biotypes of Alopecurus myosuroides, exposed to four herbicide treatments as field populations. Survival of the two lowest density treatments has been pooled. Symbols: (O——O) control; (O---O) half-rate chlorotoluron; (●---●) full-rate chlorotoluron; (●——●) full-rate isoproturon.

d.f.=4, $P<0.05$) by 13% from the lowest to the highest density. Survival was also affected by density at full-rate chlorotoluron (G=14.1, d.f.=4, $P<0.01$). The two higher densities had increased survival compared to the two lowest densities (Fig. 7.4a). Survival was more variable for isoproturon, but was statistically dependent on density (G=11.1, d.f.=4, $P<0.025$).

Survival of unsprayed and isoproturon-treated S plants did not alter in relation to density (unsprayed: G=9.0, d.f.=4, N.S.; isoproturon: G=2.1, d.f.=4, N.S.) (Fig. 7.4b). Survival significantly decreased with density for both half-rate (G=20.7, d.f.=4, $P<0.0001$) and full-rate chlorotoluron (G=48.8, d.f.=4, $P<0.0001$).

7.3.3 Lifespan

Because no consistent block effects were found, tests for density effects were carried out on data pooled over blocks. In the R biotype, unsprayed plants from sowings at the three highest densities (100, 300, 1000 seed m^{-2}) had greater ($P < 0.05$) lifespans than the two lowest densities (Table 7.1). Populations of *A. myosuroides* from the two higher density plots had greater mean lifespans than at the three lower densities in plots sprayed with half-rate chlorotoluron ($P<0.01$) and isoproturon ($P<0.01$). Mean lifespans were 11-21 days longer at the higher densities. No density effects were found for the R biotype sprayed with full-rate chlorotoluron or for the S biotype in unsprayed and isoproturon treatments (Table 7.1). At both half-rate ($P<0.001$) and full-rate chlorotoluron ($P<0.01$), density effects were evident: intermediate sowing densities of 100 and 300 seeds m^{-2} had plant populations with mean lifespans 10-20 days longer than at extreme densities (except for the lowest density, full-rate chlorotoluron).

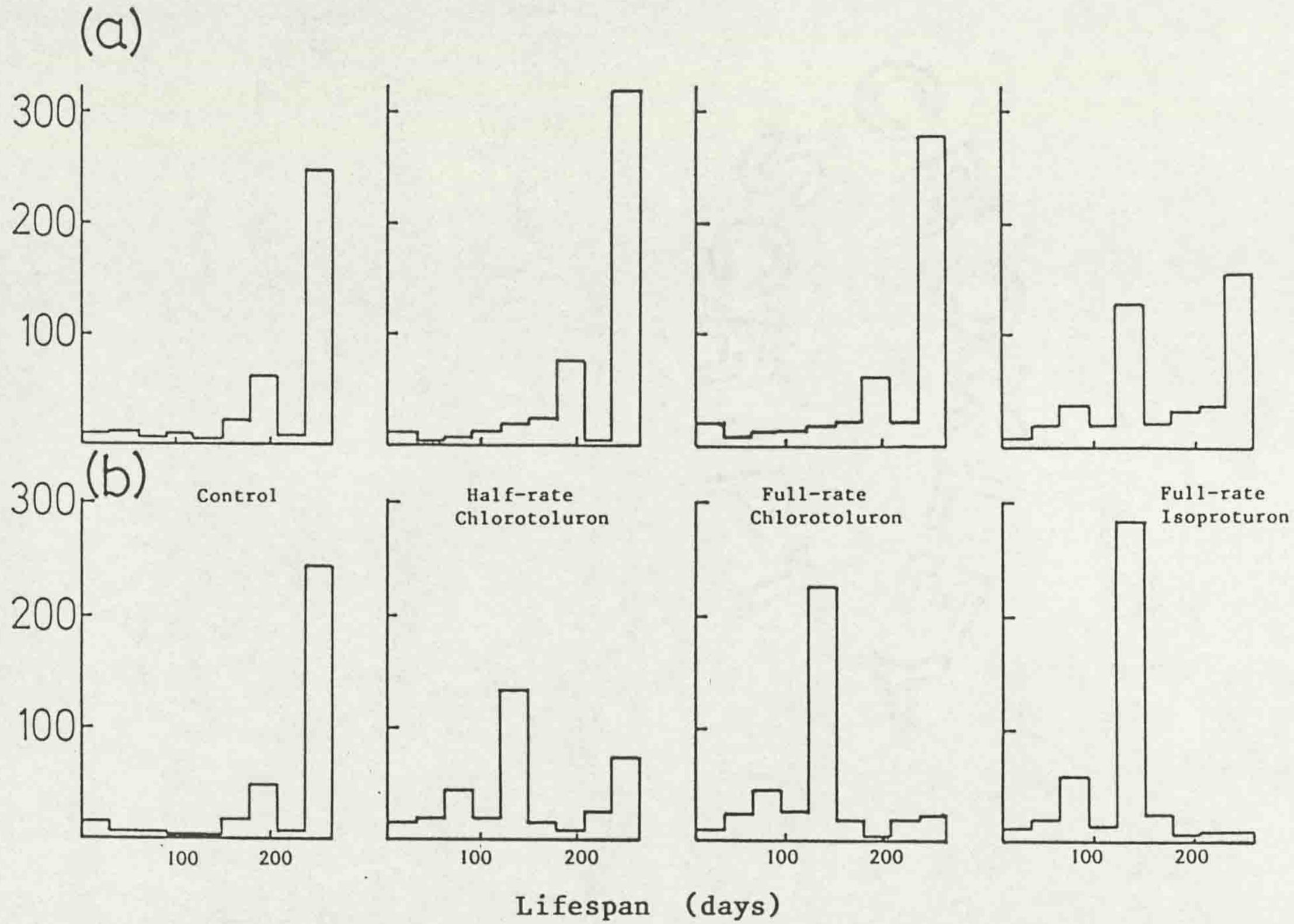

Figure 7.5 Frequency distributions of individual plant lifespans (days) for (a) herbicide-resistant and (b) susceptible biotypes of Alopecurus myosuroides grown in the field, 1985-1986, and exposed to four herbicide treatments. Data are given for high density (1000 seeds sown m^{-2}) plots and are pooled over three replicate blocks.

Table 7.1 Mean lifespan (X) in days and sample size (n) of field populations of herbicide-resistant and susceptible *Alopecurus myosuroides* biotypes, in five density treatments and exposed to four herbicide rates, pooled over blocks. Differences, in frequency distributions of lifespans between densities, within biotype-herbicide combinations are tested by Kruskal-Wallis tests (χ^2 approximation with all d.f.= 4). Significance is indicated by: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ^{NS} non-significant.

		Biotype									
		Density (seeds m^{-2})									
		Resistant					Susceptible				
		≤10	30	100	300	1000	≤10	30	100	300	1000
Control											
	X	192.0	219.8	231.4	235.1	235.4	225.0	233.3	232.9	237.1	236.6
	n	75	63	145	426	350	48	49	177	301	322
				$\chi^2=10.80^{*}$					$\chi^2=2.60^{NS}$		
Half-rate Chlorotoluron											
	X	208.1	219.8	220.6	234.0	235.7	163.3	162.1	185.4	183.4	160.6
	n	46	40	127	471	470	37	46	112	350	328
				$\chi^2=17.73^{**}$					$\chi^2=23.20^{***}$		
Full-rate Chlorotoluron											
	X	214.2	223.3	227.3	223.2	226.2	164.8	139.4	156.8	151.4	139.5
	n	42	48	130	452	428	46	28	110	288	356
				$\chi^2=1.89^{NS}$					$\chi^2=14.12^{**}$		
Full-rate Isoproturon											
	X	164.1	162.9	159.5	180.7	184.4	135.8	132.7	140.0	142.0	135.6
	n	50	51	148	331	458	39	27	96	175	357
				$\chi^2=22.19^{***}$					$\chi^2=4.43^{NS}$		

If biotypes are compared, R plants survived on average about 55 and 78 days longer than S plants at half and full-rate chlorotoluron, respectively. Lifespan was about equal in unsprayed plots and about 34 days longer for R plants in isoproturon plots. Distributions of lifespans for representative high density treatments show right-skewed distributions in unsprayed plots for both biotypes and in chlorotoluron plots for the R biotype (Fig. 7.5). Bimodal distributions were found for half-rate chlorotoluron in the S biotype (Fig. 7.5b) and isoproturon plots in both biotypes (Fig. 7.5a, b).

7.3.4 Cohort survival

Final proportionate survival at harvest is presented for seven cohorts in Figs. 7.6 and 7.7 for the three highest densities, because these treatments ensured sufficient sample sizes (i.e. main cohorts of > 10 individuals) to statistically examine survival. Mortality in unsprayed plots was only appreciable at the highest density: cohorts of plants emerging after March-April experienced higher mortality ($P \leq 0.01$) than ones emerging earlier in both R (Fig. 7.6) and S (Fig. 7.7) biotypes and reached over 50% of S plants emerging after March. In the R biotype, earlier cohorts had better survival at all high densities ($P \leq 0.01$) when treated with herbicide. Although this pattern was observable at other densities (Fig. 7.6), it was only significant ($P \leq 0.05$) with the G-test at 300 seeds m^{-2} in full-rate chlorotoluron plots. Herbicide killed most (> 80%) plants in each cohort for the S biotype, but earlier cohorts appear to have survived slightly better (Fig. 7.7).

7.3.5 Overall fecundity

Over the range of surviving plant densities, seed yield was reduced for both biotypes from about 800 to 100-200 seeds $plant^{-1}$ with

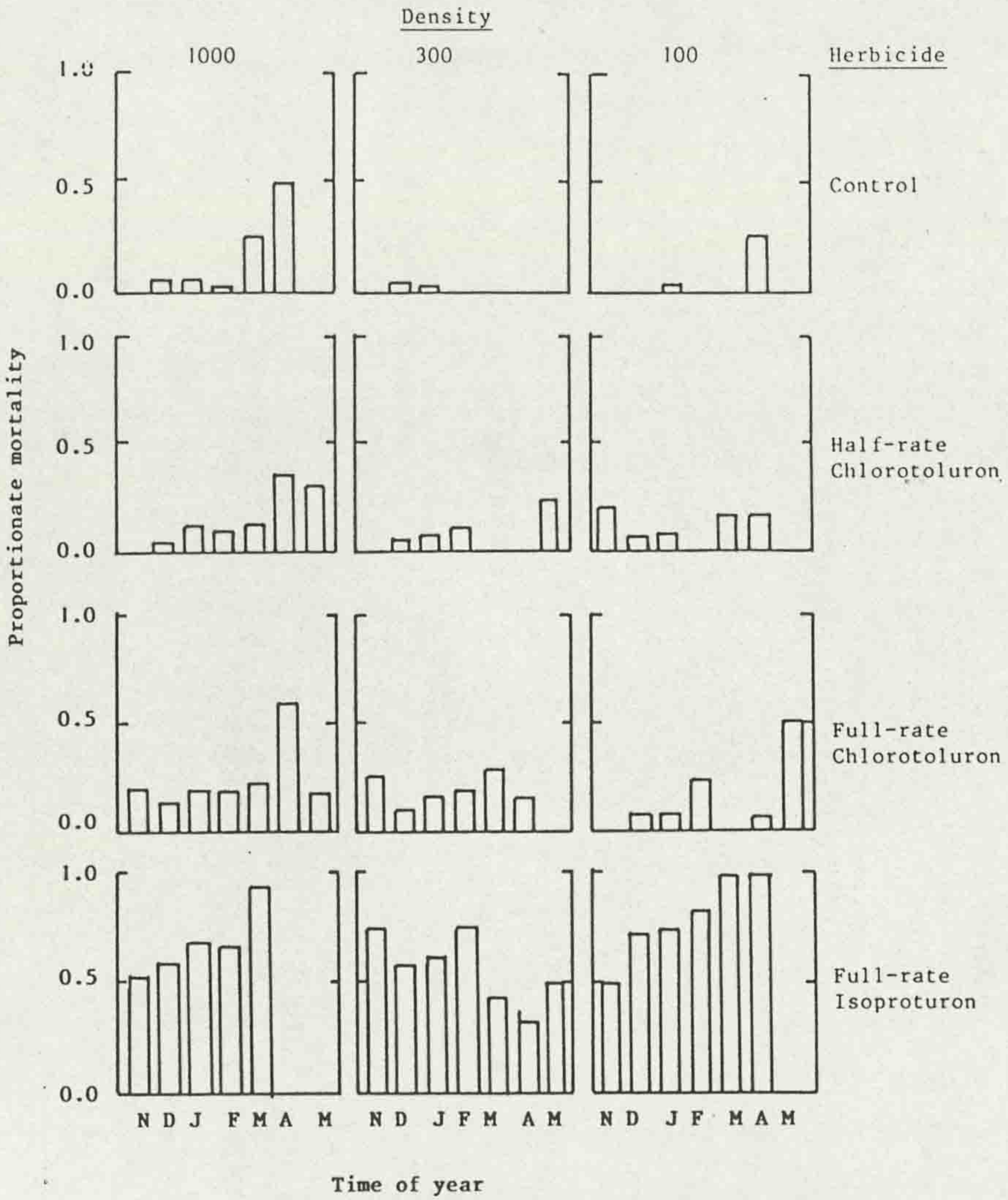

Figure 7.6 Mean cohort mortality of the herbicide-resistant biotype of Alopecurus myosuroides in field populations at three densities and exposed to four herbicide treatments, 1985-1986. Mortality is mean of three replicate plots. On horizontal axis, N= November, D= December etc.

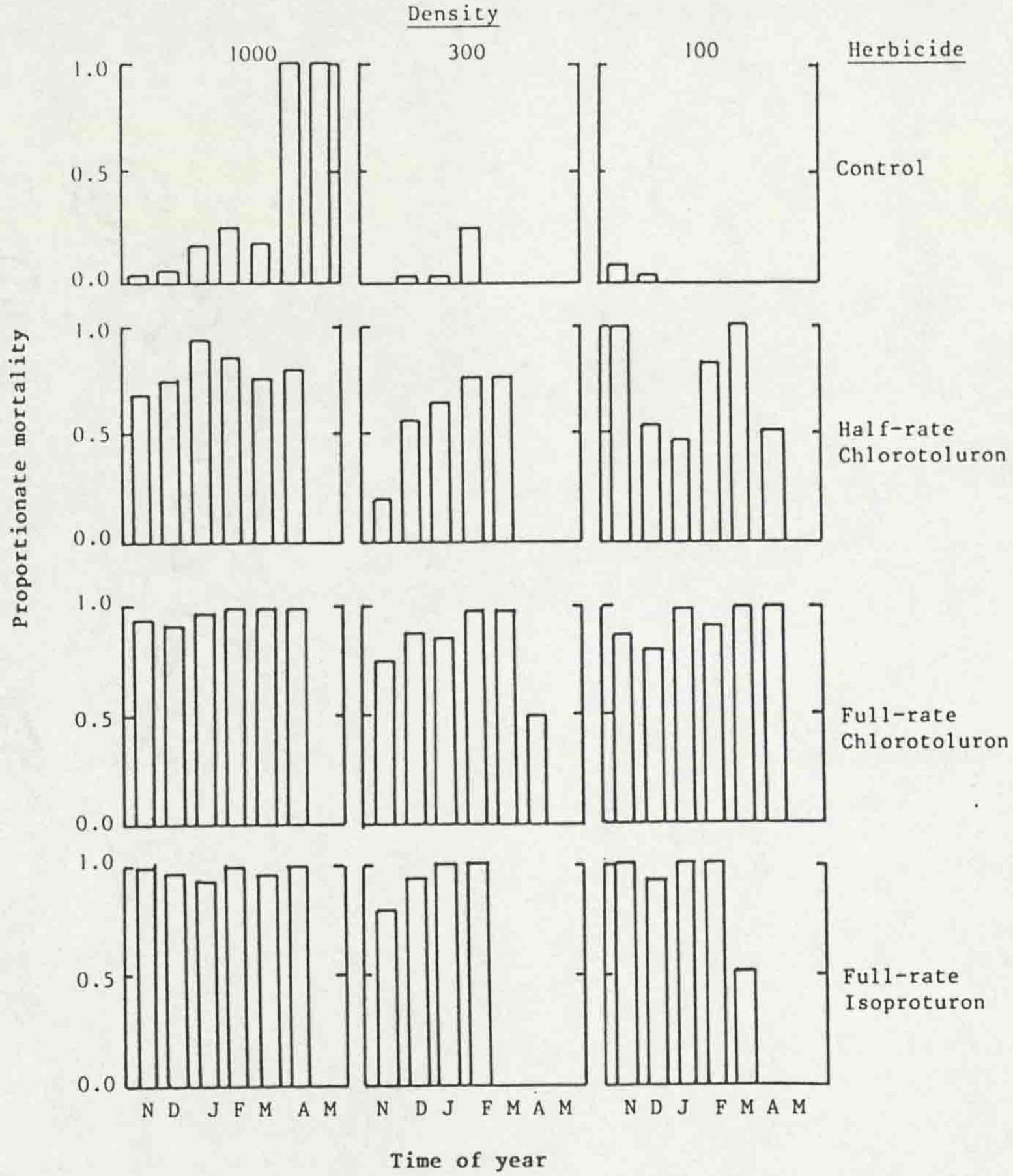

Figure 7.7 Mean cohort mortality of the herbicide-susceptible biotype of Alopecurus myosuroides in field populations at three densities and exposed to four herbicide treatments, 1985-1986. Details as in Figure 7.6.

increasing density in control plots (Fig. 7.8). At half-rate chlorotoluron individual plant fecundity in the R biotype was depressed relative to unsprayed plants and density effects remained evident (Fig. 7.8). The overall reduction in the mean number of seeds plant^{-1} due to herbicide application was about 31%. There was no effect of density on seed yields under treatments of full-rate chlorotoluron and isoproturon and yields were depressed by 52% and 67%, respectively.

For the S biotype all herbicide treatments reduced the number of seeds plant^{-1} and surviving plant numbers so that no seed yield-density relationship was apparent (Fig. 7.8). Relative to yield for unsprayed plots, plant fecundity was reduced by 88% and 92% respectively with half- and full-rate chlorotoluron, and by 91% with isoproturon.

7.3.6 Cohort fecundity

The analysis of cohort fecundity was carried out on the number of seeds plant^{-1}, by pooling data from the three lowest sowing densities and the four latest-emerging cohorts. Analysis of variance showed that for the R biotype there were no significant interaction effects and that the main effects of herbicide, density and cohort were significant ($P \leq 0.001$) . Examination of R biotype untransformed means (Fig. 7.9) showed the major effect of time of emergence: plants in early emerging cohorts produced over 300 seeds plant^{-1}, whereas those emerging after March yielded less than 50 seeds plant^{-1}. Herbicide only reduced plant fecundity noticably in isoproturon plots (Fig. 7.9).

For the S biotype the effect of cohort on plant fecundity depended on the herbicide-density combination ($P \leq 0.05$). The influence of plant

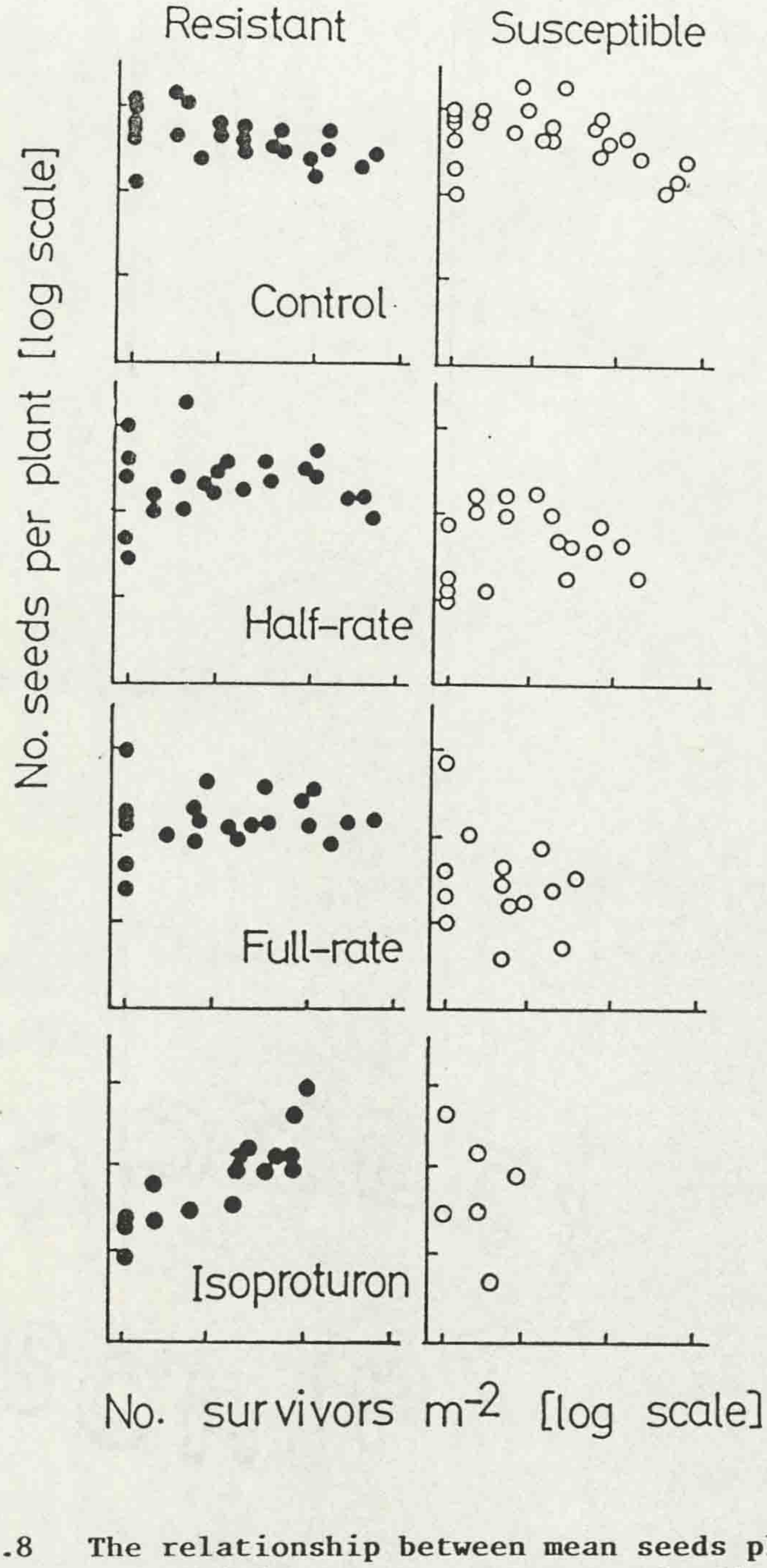

Figure 7.8 The relationship between mean seeds plant^{-1} and number of surviving plants at harvest in field populations of herbicide-resistant and susceptible biotypes of Alopecurus myosuroides, exposed to four herbicide treatments. Half- and full-rate refer to chlorotoluron herbicide, whilst isoproturon was applied at full-rate.

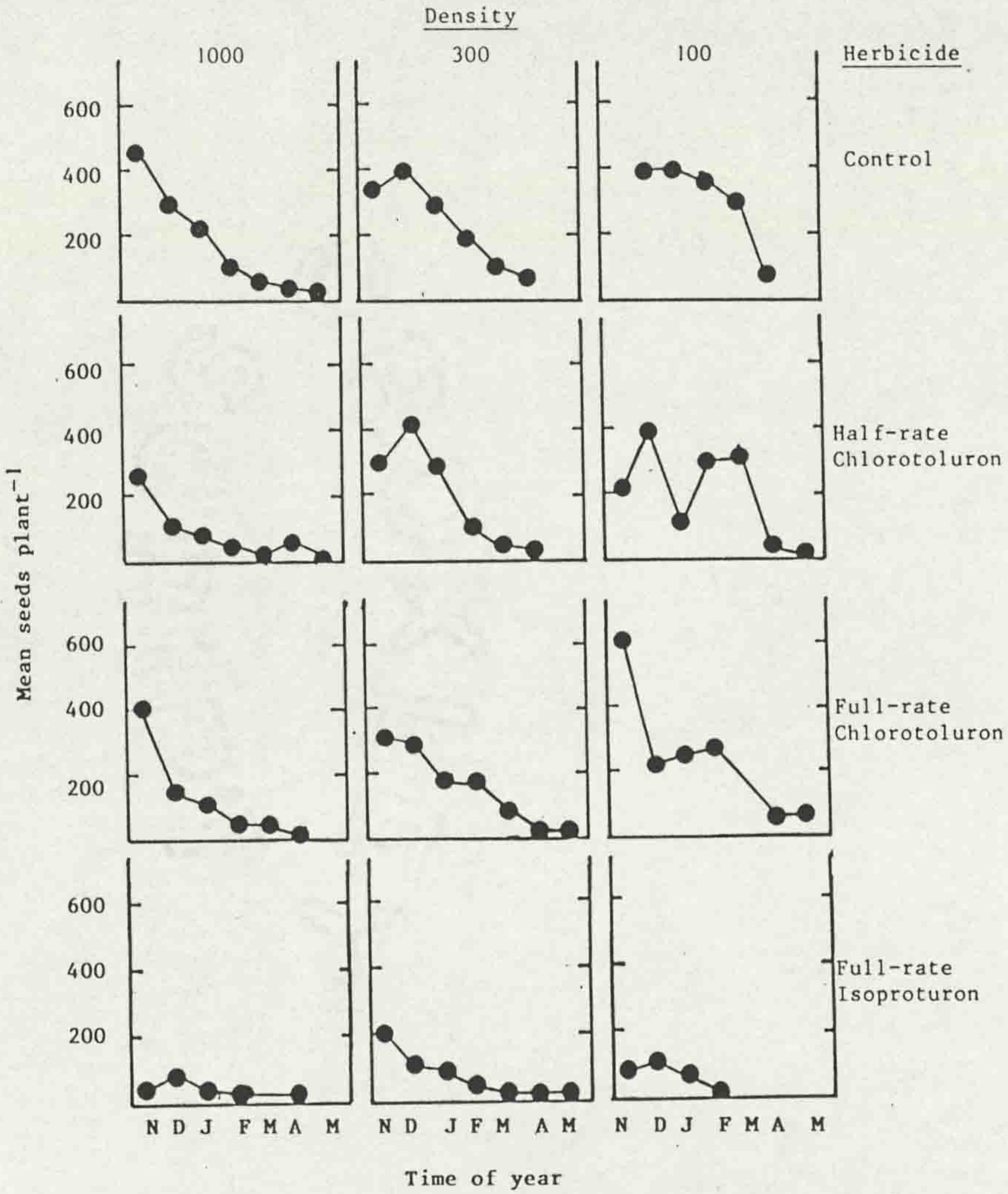

Figure 7.9 Mean cohort fecundity of the herbicide-resistant biotype of Alopecurus myosuroides in field populations at three densities and exposed to four herbicide treatments, 1985-1986. Fecundity is mean seeds plant^{-1} of three replicate blocks. Details as in Figure 7.6.

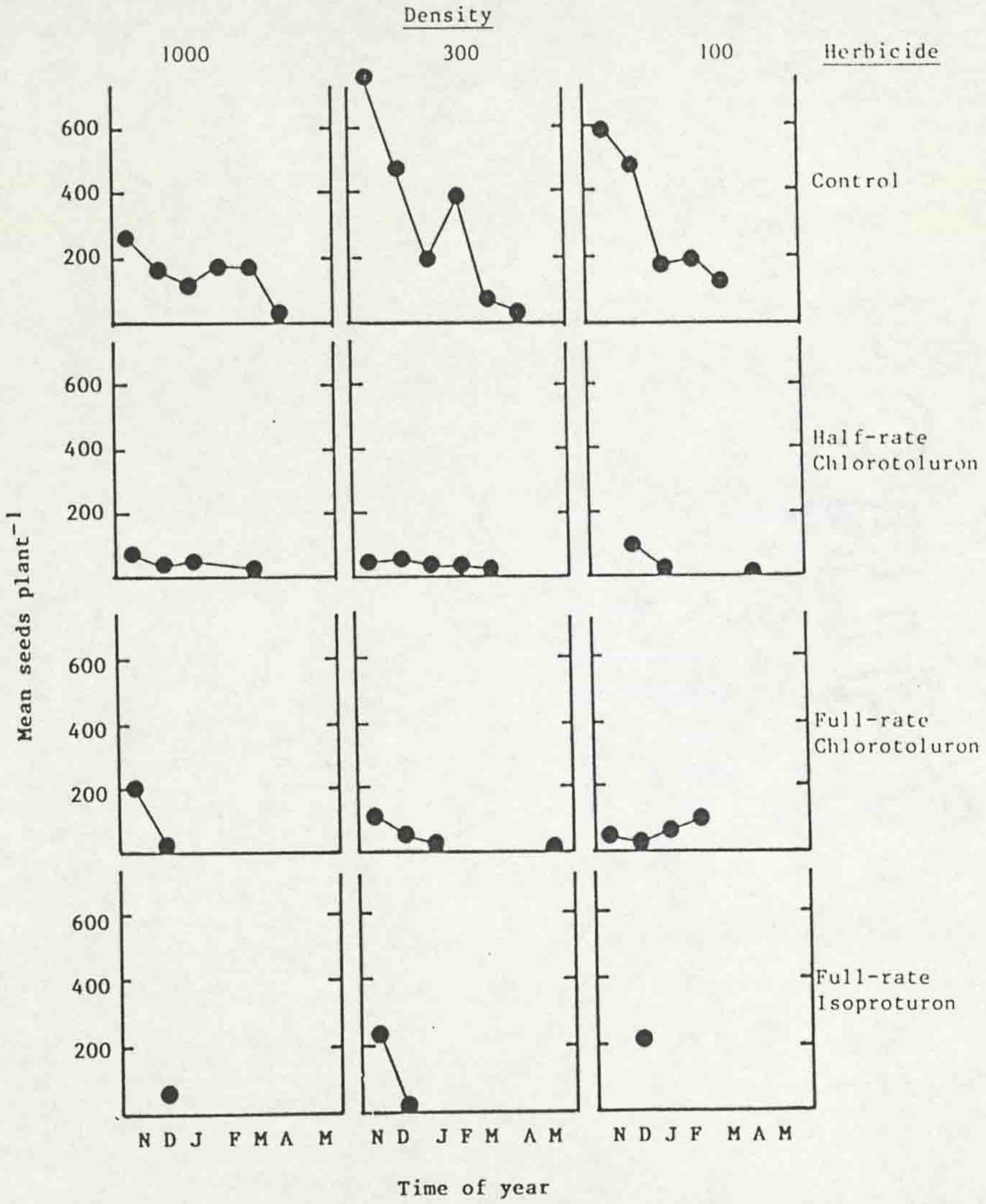

Figure 7.10 Mean cohort fecundity of herbicide-susceptible biotype of Alopecurus myosuroides in field populations at three densities and exposed to four herbicide treatments, 1985-1986. Details as in Figures 7.6 and 7.9.

age was only apparent in unsprayed plots (Fig. 7.10), because of the high mortality induced by herbicide treatment (see Fig. 7.7). Density had more obvious effects in unsprayed plots than in the R biotype, particularly for the first two emerging cohorts. In herbicide-treated plots only plants in early emerging cohorts produced seed and fecundity was much reduced (Fig. 7.10).

7.3.7 Population growth

The effects of density and herbicides described above for life-cycle stages from emergence, seedling survival through to reproduction can be summarised by considering the change in numbers (N) from one generation (t) to the next (t+1). The numbers of seeds sown per unit area (N_t) and the numbers of seeds harvested per unit area (N_{t+1}) represent two censuses which, assuming no loss of seed in the period after dispersal and before germination, span a complete generation. Curves of N_{t+1} against N_t can be plotted to describe the course of population growth. Points on such graphs falling on the line of unit slope indicate a static population ($N_{t+1}=N_t$). Above the line population numbers increase over one generation and below it, decline.

The data showed a range of forms depending on biotype and herbicide treatment (Fig. 7.11). Over all densities numbers of the R biotype increased under all herbicide treatments except at low density and full-rate isoproturon (Fig. 7.11a). Chlorotoluron application reduced the rate of population increase relative to the unsprayed treatment, but by considerably less at higher densities. Sown densities were not sufficiently high to yield equilibrium population densities in the field but some density-dependent regulation was apparent due to reduced rates of increase at the higher density sowings. A different pattern occurred in isoproturon treated plots. At

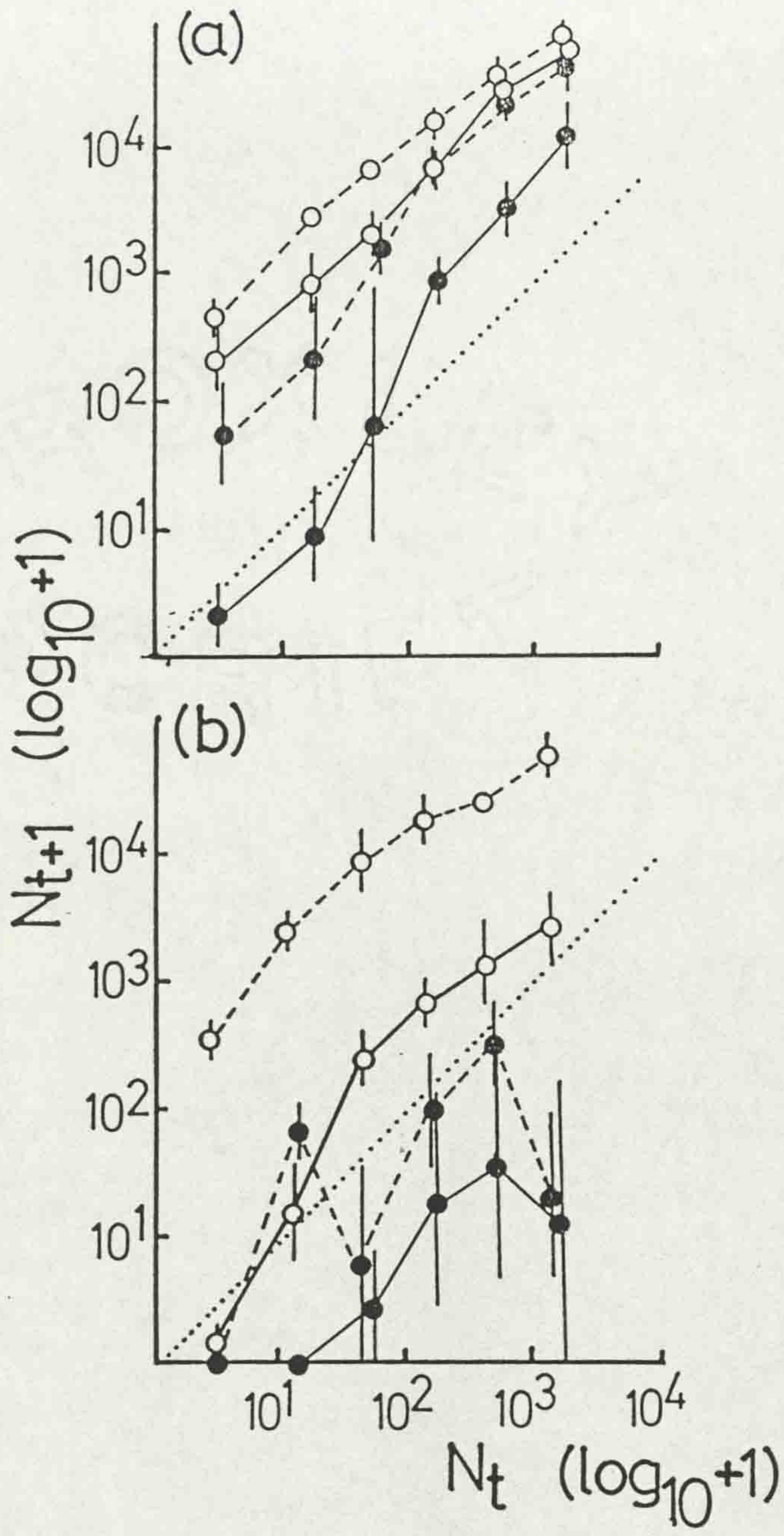

Figure 7.11 Curves of N_{t+1} against N_t (reproduction curves or generation maps) for field populations of resistant and susceptible biotypes of Alopecurus myosuroides exposed to different herbicide treatments. Points are means of three or six (two lowest densities) plots. Vertical bars represent ± 1 S.E. of the mean. Symbols for resistant (a) and susceptible (b) biotypes are: (○----○) control, (○——○) half-rate and (●----●) full-rate chlorotoluron, (●——●) full-rate isoproturon.

low densities final seed populations were less than those originally sown, but at higher densities numbers increased. Variation amongst plots was high at sowing densities less than 10^2 seeds m^{-2}.

There was little difference between R and S biotypes in the unsprayed treatment. At half-rate chlorotoluron, plots of the S biotype sown at the lowest densities returned less seed than at sowing (Fig. 7.11b). At higher sowing densities numbers increased over one generation, but in a diminishing fashion above 10^2 seeds m^{-2}. With full-rate chlorotoluron and isoproturon variability was substantially increased and population decline was apparent at most infestation densities. Assuming no seed dormancy, plants of the S biotype would be eliminated at the two lowest densities by spraying with isoproturon and at the lowest density with full-rate chlorotoluron.

Equation (2) was fitted to unsprayed treatments of both biotypes and to both chlorotoluron treatments of the R biotype. Equation (3) was fitted to half-rate chlorotoluron treated S plants and to isoproturon treated R plants. Estimated values of λ and other parameters are shown in Table 7.2. In unsprayed treatments, values of λ were similar for both biotypes. Reproductive rate (λ) of the R biotype was reduced three-fold by half-rate chlorotoluron and further halved at full-rate chlorotoluron. For the S biotype under herbicide treatment, only model (3) could describe the data for half-rate chlorotoluron. There was no discernable pattern in the data for full-rate chlorotoluron or isoproturon and no models could be fitted.

7.4 Discussion

This investigation has clearly demonstrated interactions between herbicide rate and population density on the life history of two

Table 7.2 Parameter estimates and statistics for models fitted to N_{t+1}/N_t data, shown in Fig. 11, of population growth patterns for herbicide-resistant (R) and susceptible (S) biotypes of *Alopecurus myosuroides* exposed to herbicide treatments. Model (2) was used for both biotypes in control and for the resistant biotype at half-rate and full-rate chlorotoluron. Model (3) was used for the susceptible biotype at half-rate chlorotoluron and for isoproturon in the resistant biotype. Models were fitted using a non-linear maximum likelihood algorithm (PROC NLIN, SAS Inst. Inc, 1985) and the significance of all regressions was $P < 0.0001$. These models could not fit the data for the susceptible biotype at full-rate chlorotoluron and isoproturon.

Herbicide	Biotype	Model	Parameter Estimates (± S.E.)				d.f.	r^2
			R/τ		a/ρ			
Control	R	(2)	R=229	±38	a=0.003	±0.001	24	0.995
	S	(2)	R=234	±50	a=0.003	±0.002	24	0.992
Half-rate Chlorotoluron	R	(2)	R=78	±21	a=0.005	±0.001	24	0.983
	S	(3)	τ=3574	±2582	ρ=1263	±588	24	0.475
Full-rate Chlorotoluron	R	(2)	R=30	±13	a=-0.0002	±0.001	24	0.946
	S	-	-		-		-	-
Full-rate Isoproturon	R	(3)	τ=517827	±326235	ρ=20714	±8015	23	0.588
	S	-	-		-		-	-

biotypes of *A. myosuroides* differing in their herbicide tolerance. However, two caveats limit inferences which may be made. Firstly, only one generation (or cropping season) has been investigated. Previous workers have demonstrated between year variation in demographic parameters of weed populations (Reader, 1985; Fernandez-Quintanilla *et al.*, 1986) and in herbicide effects (Fernandez-Quintanilla *et al.*, 1987). Secondly, seed population densities were not sufficiently high to enable accurate assessment of density-dependent mortality (self-thinning) patterns in unsprayed plots.

There is little experimental evidence of the densities at which *A. myosuroides* populations self-thin, despite several studies on its population ecology (Naylor, 1972b; Moss, 1981). In field trials, the maximum seedling density recorded has been 7200 m^{-2} (Cussans, Moss, & Wilson, 1987), counted in October before drilling with the crop. However, this sheds little light on self-thinning because final numbers of plants at harvest were not recorded. The highest seedling densities achieved in the present study were about 750 m^{-2} and mortality in unsprayed plots at such densities only amounted to about 5% (Fig. 7.4). Doyle *et al.* (1986) assumed, for modelling purposes, that the maximum supportable density was 500 plants m^{-2}, but maximum harvest densities in this study reached 750 m^{-2}. Therefore, asymptotic population densities of *A. myosuroides* at harvest may reach 1000 plants m^{-2}.

7.4.1 Dynamics of unsprayed populations

Although more R plants emerged in total, S plants emerged faster. There was no detectable density-dependence in either biotype at the seedling emergence stage, nor in seedling survivorship for the S biotype. Statistical analyses revealed evidence of positive density-

dependence of total seedling survival in the R biotype (7.3.2), though this was not obvious from graphs of seedling-survivor regression plots (Fig. 7.3). Another source of evidence was the pattern of survival over time, examined by recording lifespans for individual plants. Although the general shapes of frequency distributions of lifespans did not change with density, mean lifespan increased with density in the R biotype (Table 7.1). At the highest densities R plants on average lived 15-40 days longer than at the lowest densities.

Previous studies have shown positive density-dependence in seedling establishment for *Boisduvalia glabella* (Linhart, 1976) and *Floerkia prosepinacoides* Willd. (Smith, 1983b), and in early survival of *Prunus ilicifolia* (Bullock, 1981). Changes in microclimate with high density have been suggested as a mechanism for postive density effects (Bullock, 1981). Since the S biotype showed no sign of a response similar to that of the R biotype, but emerged at comparable densities, such an explanation is unlikely in the present study.

What processes allowed plants at higher densities to live longer? One explanation is that interference between individuals at higher densities suppressed growth and prolonged the annual lifespan because the terminal event, seed set, was delayed. However, this appears to be unusual in comparison with studies of natural populations. Palmbald (1968) and Symonides (1978), for instance, found that high densities accelerated development in certain annual species. In these cases nutrients were probably in short supply. In the present study, nutrients were unlikely to have been limiting because of high fertilizer inputs. Smith (1983a) demonstrated for a forest-floor annual that most life history processes were independent of density, except for death.

Germination or emergence time is a powerful determinant of subsequent individual plant performance (Ross & Harper, 1972; Cook, 1980; Howell, 1981). In the present study, earlier emerging plants survived better and were more fecund. In contrast, some studies have shown that earlier emerging seedlings survive less well, despite survivors accruing an advantage in fecundity or yield (Baskin & Baskin, 1971; Howell, 1981). This is probably a feature of environments where early germination exposes seedlings to drought or high summer temperatures (Kalisz, 1986). The survival or fecundity advantage of *A. myosuroides* possibly accrues from a competitive advantage gained by early emergers with respect to other *A. myosuroides* plants and/or to crop plants. Low densities of *A. myosuroides* also showed marked cohort effects in survival and fecundity, which suggests that crop competition was significant. Similar trends are present in studies on the effect of crop planting date on weed competition (e.g. Moss, 1985). The earlier the crops were shown, the greater the suppression of weed numbers and biomass that occurred.

The action of population regulation through the stabilising effects of negative density-dependence was not found at early growth stages such as seedling emergence or survival over the range of densities used here. Regulation was concentrated at the reproductive stage, by reductions in fecundity with increasing density.

7.4.2 Herbicide-density interactions

Few experimental studies have examined herbicide effects on density responses of weed populations. Consequently, herbicide action has commonly been assumed to be density-independent (e.g. Pollard, 1982; Doyle *et al.*, 1986). Whilst evidence from post-emergence

herbicide treatment (flamprop-isopropyl) in an experiment on *Avena fatua* L. has supported this assumption (Manlove, 1985), another field study has shown a positive density-dependent seedling survivorship response to pre-emergence herbicide (triallate) in *Bromus sterilis* L. (Mortimer, 1985). In the results reported here, positive density-dependence was found in the R biotype. Survival of this biotype was enhanced by increased density when treated with chlorotoluron (Fig. 7.4). Such a phenomenon may be attributable either to shielding of plants by their neighbours or by a dilution of herbicide dose to sub-lethal or non-phytotoxic levels. That it has been previously observed under pre-emergence treatment (Mortimer, 1985) confirms dilution as a feasible mechanism.

However, the results presented here conclusively demonstrate that negative density-dependence also occurred. Mortality of the S biotype was proportionately greater at higher densities under all herbicide treatments. One simple explanation is that interference between seedlings occurred before herbicide treatment and delayed growth. Plants at the higher densities would thus be at an earlier growth stage and more sensitive to herbicide. Growth of these plants is reduced more by the herbicide relative to plants at lower densities (and at later growth stages). Detailed growth stage recording was not carried out during the experiment, but in view of the strong effects of age on survivorship and fecundity (e.g. Figs. 7.7, 7.10) this hypothesis remains tenable. Negative density-dependence has also been reported at the seedling emergence stage of *Bromus sterilis* (Firbank, Mortimer, & Putwain, 1985).

7.4.3 Herbicide effects on individual plants

Frequency distributions of lifespans were used to examine herbicide effects on individual plant survival. A strongly right-skewed distribution was found where the R biotype was not affected by the herbicide, i.e. with chlorotoluron. Here most plants lived a long time and early emerging cohorts contributed most individuals. Survivorship curves based on these data will be Deevey Type I and similar to that found for most other annuals (Watkinson, 1981). In some cases where the populations responded to herbicide the distribution of lifespans was bimodal (Fig. 7.5), the rightmost peak representing individuals from large cohorts that survived herbicide treatment. The middle peak and the enhanced left tail comprised plants which had reduced lifespans resulting from herbicide treatment. The lifespan data (Table 7.1) confirmed the positive density-dependence detected by overall survival rate for the R biotype.

Herbicide treatment in the S biotype reduced mean lifespan by 21-32% and 27-41% at half- and full-rate chlorotoluron, respectively. It also affected mean lifespan differently according to density. Sowings of plants at two intermediate densities had longer mean lifespans than those at the extreme density treatments (Table 7.1). Negative density-dependent effects on overall mortality (Fig. 7.3) have possibly caused low lifespans at the highest density. However, the peak at intermediate densities is perhaps due to positive density-dependence at these densities.

The demonstration of similar emergence rates of the two biotypes when unsprayed suggests that any decrease in emergence rate in treated plots is attributable to herbicide effects. Herbicide treatment proved to strongly influence the size of cohorts emerging after the date of herbicide spray (7.3.1). Herbicide application affected plants before

emergence: either through seed mortality, germination inhibition or reduced seedling development. Post-emergence application of both chlorotoluron and isoproturon therefore exerted a similar degree of control as pre-emergence treatments did on later germinating cohorts; as detected by monitoring emergence of controlled sowing densities.

7.4.4 Population growth patterns

Ricker (1954) first used graphical plots of population sizes (N_{t+1}/N_t) to describe population growth, which were termed 'reproduction curves'. These curves were improved by logarithmic transformations of both axes by Varley, Gradwell & Hassell (1975). Evidence on the shape of reproduction curves for plants was summarised by Mortimer (1987), who termed such curves 'generation maps'. Of the limited studies completed, he found that curves were essentially similar in shape: rates of growth were high at low density, but increased density led to population regulation with curves approaching an asymptotic equilibrium density. In one case (Watson, 1987) the final equilibrium was lower than intermediate ones.

Population growth in unsprayed plots in the present study was only beginning to be restricted by density-dependent processes and any approach to equilibrium was not detected (Fig. 7.11), because of the limited density range used. However, this form of relationship was adequately modelled by equation (2) (Hassell, 1975). Reproductive rate (λ) values of about 230 were found for both biotypes in unsprayed conditions. No other comparable data exist for the species because seed numbers have not been measured in previous field studies. If λ values are weighted by the low seed viability of *A. myosuroides*, they are similar to those for to other weedy annuals in the presence of the

companion crop. In a winter wheat crop $\lambda = 97$ was found for *A. fatua* (Mortimer, 1987) and $\lambda = 127$ for *B. sterilis* (Sutton, 1989).

At half-rate chlorotoluron the analysis indicated a novel shape of generation map for the S biotype (Fig. 7.11; raw data in Appendix). Low sowing densities led to population decline, while higher densities gave increasing populations with clear indication that the population would achieve an equilibrium density within a few generations. Figure 7.12 shows a schematic curve of this type, with arrows indicating the course of a population over a few generations starting from two different densities (X_1, X_2). Point U in Fig. 7.12 indicates a 'threshold' density. Above this point the trajectory of the population increases towards the stable equilibrium (point Q), whilst below it, the trajectory leads to extinction of the population. A difference equation first suggested by Holling (1965) was used to describe this generation map and is given in equation (3). The model represented the overall trend of the data but because of increased variability in the data, perhaps as a result of herbicide application, and the lack of very high densities the fit was less successful than the model fitting to other data, evidenced by the standard errors of parameters and r^2 values in Table 7.2.

The same pattern appears in the R biotype in response to isoproturon treatment (Fig. 7.11). Biological explanation of such relationships may involve spatial factors not examined in this experiment. One possibility is that at low densities plants are sparsely distributed and the herbicide kills almost all plants. At higher densities the chance of individual plants being missed by herbicide spray through increases. Also, less herbicide reaches the ground and so less uptake through roots will occur. Such survivors may

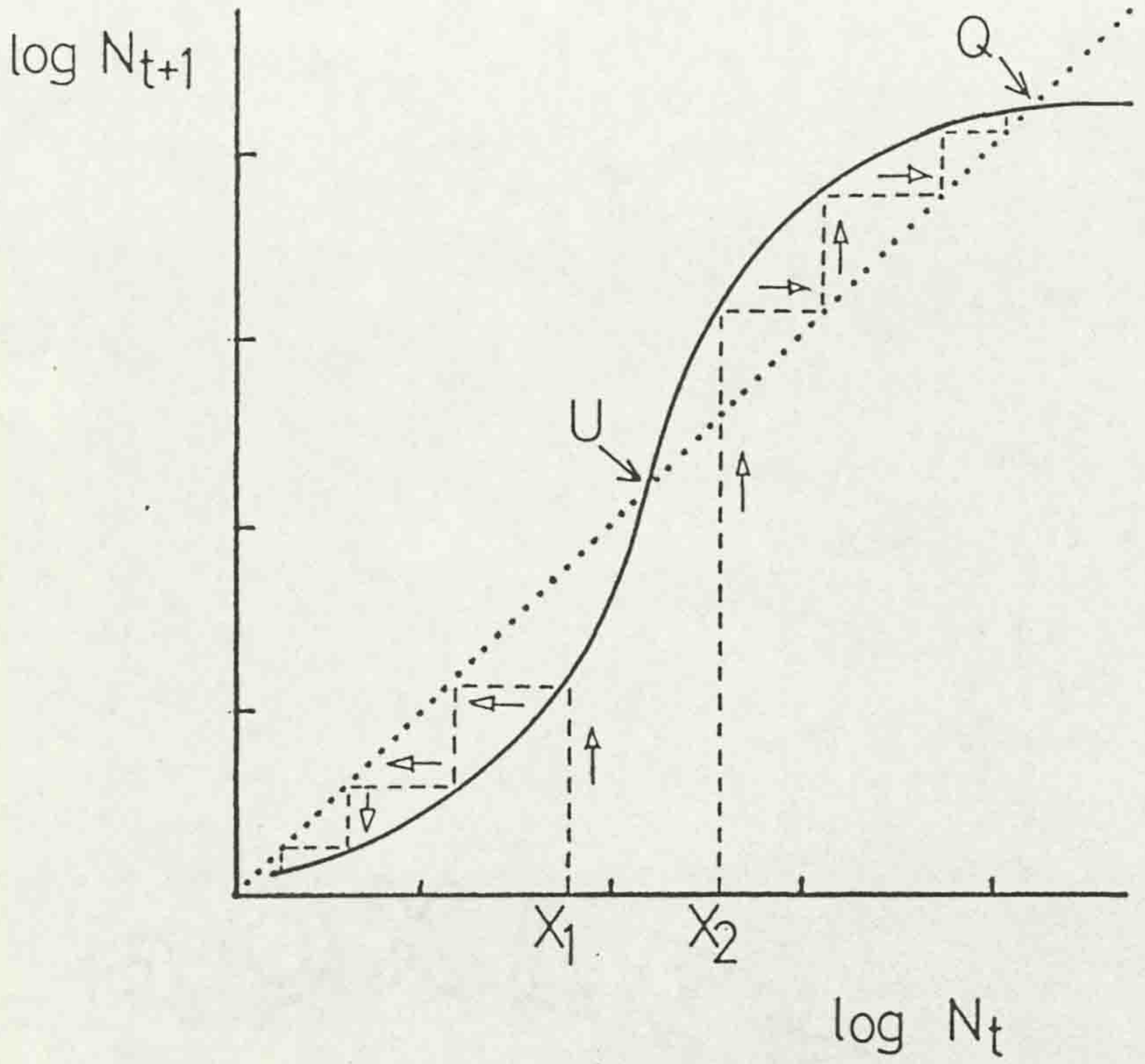

Figure 7.12 Schematic diagram of model (3) (see text), representing a reproduction curve or 'generation map' of N_{t+1} versus N_t on logarithmic axes. Points at X_1 and X_2 represent starting (input) densities. The line of unit slope (·····) is equivalent to $N_{t+1}=N_t$; lines from X_1, X_2 (------) represent the progression of numbers from one generation to the next, as determined by the curve (——————), in the direction indicated by arrows. The locus Q is a stable equilibrium point whilst U is an unstable one.

allow the population to increase. The lack of fecundity reductions with density (Fig. 7.8) under herbicide treatment gives no stabilising properties to the population. However, negative density-dependent survival (Fig. 7.3) may act to stabilise population numbers.

7.4.5 Magnitude of selection

The intensity of selection may be inadequately estimated if only mortality is measured (Putwain, 1982). Endler (1986) has also stressed that natural selection includes fecundity (and fertility) selection as well as mortality selection, although it can proceed with mortality selection alone. Fecundity selection can be measured by differences between genotypes or populations in the reduction in seed numbers over a cropping season by herbicide treatment. Selection measured in this way is overestimated by mortality-based measures if recovery and regrowth of survivors occurs, or plants germinate after herbicide treatment (Putwain *et al.*, 1982). On the other hand, selection may be underestimated if the action of a residual herbicide extends beyond a count of mortality or if crop competition reinforces selection by the herbicide (Putwain, 1982).

Selection intensities, measured as selection coefficients (McGraw & Antonovics, 1983), showed that in unsprayed plots mortality selection was non-existent to weak against the R biotype, though reaching statistical significance at the highest density (Table 7.3). As expected, the intensity of mortality selection against S plants was strong in all herbicide treatments, but did not show obvious trends with density. Fecundity selection intensity was weak in unsprayed plots and in the opposite direction to mortality at higher densities. In all herbicide treatments, selection acting on fecundity was in the expected direction and was related to density. These results confirm

Table 7.3 Selection coefficients comparing the performance of herbicide-resistant and susceptible biotypes of *Alopecurus myosuroides*, based on either survival or seed production, in herbicide and density treatments of field populations. Coefficients of selection were calculated as (1-[performance of given biotype/performance of best-performing biotype]). Performance was measured as survival proportion or seeds m^{-2}. The direction of selection is indicated by sign: negative values indicate selection against the susceptible biotype. Statistical comparison of biotypic performance was based on G-tests of survival proportion or t-tests of log (x +1) transformed seeds m^{-2}. Significance indicated by: NS not significant at $P = 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$.

		Density (seeds m^{-2})				
Herbicide	Performance	≤10	30	100	300	1000
Control	Survival	0.05^{NS}	0.00^{NS}	0.01^{NS}	0.01^{NS}	0.03*
	Seeds m^{-2}	0.15^{NS}	0.52^{NS}	0.38^{NS}	-0.36^{NS}	-0.19^{NS}
Half-rate Chlorotoluron	Survival	-0.47**	-0.66**	-0.56**	-0.58**	-0.70**
	Seeds m^{-2}	-0.97**	-0.86*	-0.89*	-0.94*	-0.95**
Full-rate Chlorotoluron	Survival	-0.52**	-0.89**	-0.82**	-0.83**	-0.93**
	Seeds m^{-2}	-0.82^{NS}	-0.96*	-0.98**	-0.98**	-1.00**
Full-rate Isoproturon	Survival	-1.00**	-0.92**	-0.78**	-0.91**	-0.90**
	Seeds m^{-2}	1.00^{NS}	-0.99^{NS}	-0.87^{NS}	-0.96^{NS}	-0.90^{NS}

the view that selection measured by mortality substantially underestimates the intensity of selection as measured by fecundity.

The intensity of selection against susceptible *A. myosuroides* plants in a herbicide environment amounted to 0.82-1.00 (Table 7.3). This is comparable to intensities measured for metal tolerance (Jain & Bradshaw, 1966) even though Bradshaw (1982) considers the persistance of heavy metal toxicity to result in greater selection pressure than is present in herbicide-contaminated environments. Although selection for resistance is potentially very strong, evolution will ultimately depend on the degree of heritability of resistance to herbicide (Chapter 4) and breeding system of the species concerned. It has been frequently suggested that in a herbicide-free environment, R plants will be selected against due to poor competitive ability (Warwick, 1980; Gressel & Segel, 1982). Most experiments which have confirmed this view (e.g. Warwick & Black, 1981; Holt & Radosevich, 1983) were not undertaken in the field. The study presented here estimated selection in a field environment similar to that experienced by natural infestations of *A. myosuroides* and included a range of sowing densities. Selection against the R biotype in a herbicide-free environment was negligible (0.0-0.03) and of a much lower magnitude than that previously recorded for herbicide-resistant biotypes or most metal tolerant ecotypes (Bradshaw, 1972).

CHAPTER 8
GENERAL DISCUSSION

Selection is used in this thesis to mean phenotypic selection, a within-generation change in distribution of a trait. This phenotypic selection can result from either mortality or fecundity differences between individuals or genotypes. *Natural* selection, however, implies a genetic response, i.e. inheritance of the trait in question (Falconer, 1981; Lande & Arnold, 1983). Evolution is then limited to cases where natural selection results in some change in trait distribution (see Endler, 1986). The evolution of herbicide resistance thus implies a directional change in genetic composition away from 'wild-type' susceptible plants to plants possessing a degree of tolerance or resistance to the selective agent, the herbicide.

Natural selection for herbicide resistance thus delimits the conditions within which evolution can take place and includes: (1) genetic variation for herbicide response, (2) fecundity or survival differences associated with the genetic variation and (3) inheritance of resistance between parents and offspring. This thesis has considered (1) and (3) for *E. repens* and (1) to (3) for *A. myosuroides*, allowing an assessment of factors which will determine the rate of evolution of resistance. The work has also compared the population growth patterns of resistant and susceptible *A. myosuroides* biotypes. Together with competitive interactions (Chapter 6) such patterns will locally limit the spread of resistant plants. These considerations are discussed in more detail in this Chapter.

8.1 Models, Density Phenomena and Herbicide

8.1.1 Density response of weed populations

Results from experiments reported in this thesis (Chapter 6, 7) indicate that positive density-dependence occurred in populations of *A. myosuroides*. Firstly, it was found for seedling survivorship of susceptible plants in mixture, under herbicide treatment (6.3.2). In the second case, field-grown resistant plants showed positive response to density in both survival and lifespan, in all treatments (7.3.1, 7.3.2). Mortimer (1985) also demonstrated such effects in weed response to herbicide. Taken together, these results suggest that the influence of herbicide treatment on populations of arable weeds is considerably more complex than assumed by Pollard (1982) and Doyle *et al.* (1986), when modelling population responses in the presence of herbicidal control agents.

Whether this pattern of density-dependent response is a more general phenomenon or specific to certain species-herbicide combinations awaits further studies with high weed densities and with measurement of individual plant performance in response to herbicide. Positive density-dependence has previously been demonstrated for seed germination (Linhart, 1976), seedling emergence (Smith, 1983b) and survival (Schmitt & Antonovics, 1986). The effect was only present in the latter study in aphid-attacked plants and together with the results presented here, suggests that density and competition experiments should more often include complicating effects such as herbivory (e.g. Windle & Franz, 1979), pathogens (e.g. Burdon *et al.*, 1984) and in weed studies, herbicides.

8.1.2 Modelling change in populations

A further significant finding of this thesis is a novel type of population response which was found in both biotypes (Fig. 7.11). Reproduction curves, or generation maps, characteristically are asymptotic resulting from negative density-dependent regulation, or decline after reaching an peak (Mortimer, 1987a). They indicate a stable equilibrium population size is attainable. It is a property of such models, described by difference equations, that following disturbance populations will show either exponential damping or damped oscillations back to the stable equilibrium point (Hassell *et al.*, 1976; Watkinson, 1980), depending on the precise model formulation.

The course of growth in a population described by the curve in Fig. 7.12 is qualitatively different, however. Disturbance to the population following this trajectory can result in two outcomes. Firstly, the population may track to a stable equilibrium in the same fashion as the models of Hassell *et al.* (1976) and Watkinson (1980). On the other hand, the population may move away from this equilibrium towards low population size and possibly extinction. The fate of a population following a trajectory away from the threshold or 'repelling' point (May, 1977) (point U in Fig. 7.12 will be determined by the shape of the curve at low population sizes. May (1977) gives an example of such a difference equation, for an animal population experiencing predation, where a further stable equilibrium point exists at population sizes nearer to the origin than the repelling point. There is no evidence for this particular pattern of population behaviour in the results presented here and the population reproduction curve used was one described originally by Holling (1965) for animal populations.

The use of this particular model was purely empirical--the model was originally developed for animal predator-prey relationships and attachment of biological meaning to parameters is difficult. However it is clear that the data fit the basic form of the model: values fell below the equilibrium (1:1) line at low population sizes, but with a reversion to a negative density-dependent levelling of the growth curve at high population size (Fig. 7.11). Variance in the data was considerably enhanced near the 'repelling' or 'threshold' point and this may reflect real processes in the data. The underlying, causative mechanisms of this curve are not known but they are probably linked to the positive density-dependence found under herbicide treatment.

8.2 Natural Selection

8.2.1 Genetic variation and inheritance

Population variation in herbicide response

Intraspecific variation for herbicide response in natural populations of weeds was probably first reported by Hanson (1956). Since then it has been described, as population-level variation, for many species, particularly annuals. Frequently such studies have described a degree of variation which represents inherent variation in the species (Santelmann & Meade, 1961; Jacobsohn & Anderson, 1968; Ellis & Kay, 1975). This level of variation is thus unrelated to selection for enhanced tolerance or resistance by herbicide application. Alternatively, populations can be considerably outside this range of variation (e.g. Moss & Cussans, 1985; Lutman & Lovegrove, 1985). Resistance is attributed to the population, implying that evolution has occurred as a result of natural selection.

Perennial species have also been shown to possess genetic variation for herbicide response, e.g. *Cynodon dactylon* (L.) Pers. (Hanson, 1963) and *Cirsium arvense* (L.) Scop. (Hodgson, 1970). Similar demonstrations

have been made for *E. repens*, a species considered in this thesis (see Chapter 2). Buchholtz (1958) found differences in response to dalapon between 14 'clones' of the species. Haddad and Sagar (1968) established significant variation between four samples in response to both dalapon and aminotriazole. The only study of *E. repens* response to glyphosate, its current main agent of control, is that of Westra (1980). He showed differences between seven biotypes, where biotypes comprised single clones collected from a number of separate locations.

These previous studies on *E. repens* herbicide response appear to have used samples consisting of a single clone from a number of locations. Significant differences between these samples have then been interpreted as 'clonal' variation. However, they can equally and more properly be treated as population level variation, where each population is represented by a single clone. In order to distinguish adequately between true population level and clonal variation, replicated samples are needed of a number of clones from a number of populations (significant population variation, compared to that between clones, then represents true population variation).

In the present study, significant population differentiation for glyphosate response was found (Chapter 2). There was no evidence, however, for major genetic shifts away from the modal response: most populations were similar in magnitude of response. Variation in response to herbicide stimulus was thus close to the pattern of innate variability expected in samples from natural populations of an outbreeding species and as demonstrated for other perennial weeds (e.g. Albrecht, 1947; Hodgson, 1970; McWhorter & Jordan, 1975).

In contrast, *A. myosuroides* showed evidence of major variability in response to chlorotoluron (Chapter 4) which confirms previous work; the species also possesses population variability for many other characters (Table 8.1). The magnitude of differences in herbicide response was unlikely to be part of natural population variation. Two populations of the 10 studied were much less responsive to increasing herbicide dose (ID_{50} values were 3+ times greater; 4.3). This confirms the results of Moss and Cussans (1985, 1987) for biomass and furthermore, shows that mortality response provides greater discrimination between populations, despite less statistical precision because of experimental limitations (Chapter 4). Two of these populations are considered to be resistant biotypes, as indeed they were by Moss and Cussans (1985; 1987).

Nevertheless, the demonstration that a population is well outside the 'normal' range of variation is only strong circumstantial evidence of selection. Selection can only be inferred because resistance is only observed in natural populations *post hoc*. More conclusive evidence can be obtained from selection experiments. Artificial selection has shown that both relatively small improvements in tolerance can be achieved (mainly demonstrated for crop species: Karim & Bradshaw, 1968; Warwick, 1973; Sykes, 1980) as well as large responses amounting to resistance as in *Poa annua* L. (Grignac, 1978) and *S. vulgaris* (Holliday & Putwain, 1977).

The degree of resistance, i.e. the difference between resistant and susceptible biotypes, found in *A. myosuroides* both here and in Moss and Cussans' studies, was not as high as that typical of triazine resistant populations. Fuerst *et al.* (1986) found that ratios of ID_{50} (= ED_{50}) values (based on fresh weight reductions) of triazine-resistant to susceptible biotypes were over 1000 for *Senecio vulgaris* L., about 500 for *Amaranthus hybridus* L. and 50 for *Chenopodium album* L. Equivalent values

Table 8.1 Evidence of between population variability in *A. myosuroides* from published sources and this thesis. For most studies, characters with significant ($P < 0.05$) inter-population differences are shown with the number of populations and source of evidence.

Character	No. of Populations	Reference
Seed		
Seed viability	3	Wellington & Hitchings (1966)[a]
Presence of caryopsis	4[b]	Naylor (1972)[a]
Germination proportion	5[c]	Naylor & Abdalla (1982)
Germination proportion	10	Chapter 4
Emergence pattern	2	Chapter 7
Growth		
DW[d] growth in nutrient solution	43	Darmency (1981)
Tiller number[e]	2	Chapter 5
Plant height	20	Exley (1985)
Plant DW	20	Exley (1985)
Competitive ability	2	Chapter 6
Reproduction		
Spike emergence	20	Exley (1985)
Self-fertilisation	3	Beddows (1931)[a]
Seedhead no./length/DW	20	Exley (1985)
Spikelet weight	4[b]	Naylor (1972)
Chlorotoluron Response		
Seedling shoot FW[d] reduction	9	Niemann & Pestemer (1984)
ED_{50} of shoot FW	19	Moss & Cussans (1985)
Shoot FW reduction, 2.5 kg ha^{-1}	22	Moss & Cussans (1987)
Shoot FW reduction, 4.0 kg ha^{-1}	38	Moss (1987)
ID_{50} of shoot DW $plant^{-1}$ and mortality	10	Chapter 4
Seedling root length growth	3	Chapter 4

[a] Studies where statistical comparison of populations was not carried out, or possible.
[b] 9 collections from 4 sites.
[c] 12 collections from 5 sites.
[d] DW = dry weight, FW = fresh weight.
[e] Exley (1985) found no significant difference between 20 populations.

for biomass response of *A. myosuroides* were 4.0 in the present study, 6.6 in Moss and Cussans (1987), 7.7 in Moss (1987) and 16.0 in Moss and Cussans (1985). The resistance ratio is higher (34.0) if mortality is used as the response (Chapter 4). Surprisingly, few other studies have accurately measured weed mortality over a dose range (but see Heap, 1987; Harrington & Popay, 1987).

Within-population variation

Genetic variation between populations reflects past responses to selection (Bradshaw, 1984; Venable, 1984). More significant for examination of the dynamics of natural selection is within-population variability, which can allow prediction of the potential response to selection (Antonovics, 1976; Venable, 1984). Considerable amounts of such variation exists in plant populations in characters ranging from enzyme polymorphisms (Hamrick, Linhart & Mitton, 1986), morphology (Warwick & Briggs, 1978a) and life-history (Law *et al.*, 1977; Mitchell-Olds, 1986). Much of this variation is polygenic in origin (Lawrence, 1984) and can be described by quantitative-genetic parameters, e.g. heritability (Falconer, 1981).

Quantitative genetic variation for herbicide tolerance has been found in a number of weed species and in populations differing in their histories of herbicide selection (see 4.4.2). It was demonstrated in this thesis (Chapter 4) that heritable variation for chlorotoluron response was present in *A. myosuroides*. Narrow-sense heritabilities of 0.21-0.34 were found for populations (two) treated recurrently with herbicides and one never exposed to herbicides. These findings therefore confirm the earlier work of Thai *et al.* (1985) and Price *et al.* (1983). Interestingly, Price *et al.* (1983) found heritable variation in populations previously unexposed to any herbicide. Two of four *A. barbata* Brott. populations

showed significant broad-sense heritabilities, of 0.11-0.39. All except one of six *A. fatua* populations were reported to have heritable variation in the range 0.12-0.63. It follows that wild populations of weeds possess variation for herbicide response, with the attendant potential for evolution of resistance given the appropriate degree and direction of selection.

In *E. repens*, although no parent-offspring relationship was found in two specimen populations (2.3.2), significant clonal differentiation was observed in another experiment. The latter is strongly suggestive of genetic determination of the trait. However, clonal differences in *E. repens* glyphosate response disappeared when populations previously exposed to the herbicide were condidered separately. This lack of a presumed genetic determination in these populations may reflect past selection--slightly more tolerant genotypes have been favoured and genetic variation lost, compared to unselected populations. Thai *et al.* (1985) have shown a similar trend in triallate response of *A. fatua*. An alternative explanation is that genotypic diversity within these populations is low for other reasons, e.g. founder effects (Mayr, 1963; Barrett, 1981). The demonstration that both classes of *E. repens* populations possess considerable intrapopulation variation for many other, morphological and life-history characters (3.3.2) suggests that this is unlikely.

8.2.2 Phenotypic selection

Relative fitness of biotypes in unselected environments

The relative performance of R and S biotypes when not exposed to herbicide is of considerable interest (see Table 1.1) because it will determine the rate of spread or decline of the R biotype in such environments. At low densities, patterns of vegetative growth and

subsequently reproductive output may determine relative success. In mixed populations of R and S plants, competitive ability and biotype-specific density response will decide outcomes of interactions between biotypes.

Comparisons of growth patterns of the two *A. myosuroides* biotypes in spaced plant conditions revealed a similar development over time, except that R plants had more tillers and possibly produced more reproductive material (5.3). When grown in mixture with a crop species at low *A. myosuroides* density, tiller numbers of the R biotype were again higher (6.3.4). However, at these low densities the S biotype proved more fecund, conflicting with the tentative results of Chapter 5. Yet it is difficult to compare the two experiments because the mixtures were grown in the presence of a companion crop. No evidence of fecundity differences could be detected in the field (Chapter 7).

When interactions between the biotypes were taken into account (Chapter 6), the results indicated that the R biotype outyielded the S biotype in high density mixtures. It would thus usually become dominant in competition over time. In the field, without interference, high density responses were similar for both biotypes (Chapter 7). This may reflect the higher environmental variance under field conditions.

A review of published work examining differences between biotypes (1.3, Table 1.1) showed that S biotypes tended to out-yield R biotypes in biomass and reproductive matter, both as spaced plants and when grown in competition. However, a significant number of studies contradicted this view, either by showing no differences or a R advantage. This presumptive fitness advantage attached to the S biotype has been attributed to physiological differences associated with the resistance trait (e.g. Warwick, 1980). Yet this question of enhanced fitness is difficult to answer unequivocally because comparisons are usually made with field

collected material and because few have sampled R and S biotypes from populations differing only in the resistance trait.

One partial solution is to examine many R and S biotypes from different collections. In two such studies, Warwick (1981) and Murphy *et al.* (1987) have shown little evidence of S biotype advantage. Taken together with experiments that have revealed a R advantage (Rubin *et al.*, 1985; Jansen *et al.*, 1985), the hypothesis of reduced fitness in the R biotype is difficult to sustain and raises a number of possibilities. Firstly, in results where the S biotype has a fitness advantage ('S>R' effect) is found, two explanations are feasible: (1) there is a real penalty or cost in possession of the resistance trait, or (2) that other aspects of the genome determine the observed result and (1) is false. If there is no biotypic difference recorded, or indeed a 'R>S' effect, it follows that either: (3) there is no 'S>R' effect, or (4) there is an 'S>R' effect but that other aspects of the genome outweigh it, or this is achieved by experimental error. Studies with material where the genetic background is as similar as possible (ideally isogenic lines) are needed to distinguish between (1) and (2), or between (3) and (4). There is no published work with weed species, but Gressel and Ben-Sinai (1985) have compared a *Brassica napus* L. cultivar to the same cultivar backcrossed to a resistant *B. campestris* L. biotype. They found that the 'S>R' effect was present.

In field environments, plants are selected for many aspects of performance (Antonovics, 1979), e.g. competitive ability with a crop and performance at high densities in the case of *A. myosuroides* and this may be concurrent with selection for resistance. Realistically, hypotheses (2) and (4) above may be more accurate and the 'S>R' effect not expressed under field conditions. It is extremely likely, given the mechanisms of

weed seed spread through grain contamination (Horne, 1953), farm machinery etc., that R biotypes will spread into new geographic locations and compete with S plants evolved in different environments. Further, R biotypes themselves are likely to be genetically distinct. Biotype fitness, as determined by selection for many aspects of performance, is therefore as important, or more so, for the spread of a biotype than the effect of the resistance gene alone.

Herbicide selection in field environments

Estimates of the intensity of phenotypic selection were obtained in a field experiment with biotypes of *A. myosuroides*. Plants were grown in a model cropping system, in winter wheat, and sprayed with three herbicide treatments. These estimates perhaps represent the best we have for herbicide selection because: (1) they were obtained in the field, (2) a range of densities was included, (3) the contribution of all cohorts within a generation was assessed and (4) they represent lifetime seed production.

The intensity of selection against the S biotype in a herbicide-treated environment was obviously great, but that of mortality selection was considerably less than through fecundity differences. This discrepancy widened at the lower selection intensities, e.g. with half-rate herbicide. In cereal growing areas of E. Anglia, where *A. myosuroides* is a serious weed, it is conceivable that actual selection will be less than that demonstrated for full-rate chlorotoluron. The experiment was conducted in a freely drained, sandy soil. Chlorotoluron probably performs best under these conditions and markedly worse in the cultivation systems of Eastern England. In particular, minimum cultivation systems encourage the build-up of absorbtive, organic matter. Straw residues remaining from cultivation in the surface layers decrease the performance

of the herbicide (Nyfeller & Blair, 1978; Moss, 1981; Orson & Livingstone, 1987) because the major activity of chlorotoluron is through root uptake (Blair, 1978).

The close study of some of the population biology of *A. myosuroides* revealed factors which may further act to weaken selection. For example, early germinating seedlings proved less responsive to the herbicide in survival and concomitant seed production (7.3). Phenotypic selection for resistance is therefore underestimated on these seedlings, unless pleiotropic linkages existed with characters expressing germination time. The presence of a seed bank, though relatively short-lived (Moss, 1980), in *A. myosuroides* means that selection on a set of genotypes in one year is not consistently related to genotype composition in the next. The small proportion of such dormant seed (Naylor, 1970) suggests that it introduces only limited error, but does indicate the need for study of the relative seed bank dynamics of the two biotypes, an aspect not covered in this thesis.

In *E. repens*, no selection was detected on either herbicide response (Chapter 2) or life history characters (Chapter 3), despite the finding of genetic differentiation, with a presumed genetic basis, for many characters including herbicide response. This does suggest that selection was not sufficiently strong in the environments experienced by the populations sampled. Characteristics of glyphosate use that may reduce actual selection pressure were discussed in Chapter 2. Other possibilities that have the same effect of weakening selection and apply also to life history variation are: (1) negative genetic correlations or pleiotropy amongst major fitness (represented by life history characters) components limit the response to selection (Antonovics, 1976; Lande & Arnold, 1983) and indeed Williams (1973b) reported a strong negative relationship

between proportion of dry weight allocated and to shoot or rhizome, suggesting that negative genetic correlations are possible in the species; (2) selection may be variable spatially or temporally (e.g. Kalisz, 1986); (3) not sufficiently intense because of recurrent disturbance; (4) sampling was not extensive enough to reveal clonal differentiation, paricularly for herbicide response (only five clones sampled); or (5) that clonal variation is actually passively maintained by phenotypic plasticity in natural habitats (Sultan, 1987).

8.3 Conclusions

(1) Genetic potential for natural selection and evolution of herbicide resistance exists in natural populations of weeds, previously unexposed to herbicides. Genetic variation had not been reduced by selection in the field-collected samples of *A. myosuroides*.

(2) Selection, acting on such variation, was strong for herbicide resistance in *A. myosuroides*, even when assessed against the contribution of all cohorts in a population. Fecundity selection can be more acute than mortality selection.

(3) Models to describe the influence of perturbations on natural populations experiencing density regulation are not well developed. Further study may lead to the detection and accurate description of patterns of population behaviour which are novel in character, at least for plants.

APPENDIX

Raw data, shown in Figure 7.11, representing seed output per plot from herbicide-resistant (R) and susceptible (S) biotypes of *Alopecurus myosuroides* grown in the field under four herbicide treatments and six initial sowing densities (1=1515 seeds m^{-2} R, 1370 S; 2=455 R, 411 S; 3=152 R, 137 S; 4=46 R, 41 S; 5= 15R 14 S; 6=1 R, 1 S).

	Resistant				Susceptible			
Sowing density	Control	Chlorotoluron Half-rate	Chlorotoluron Full-rate	Isoproturon Full-rate	Control	Chlorotoluron Half-rate	Chlorotoluron Full-rate	Isoproturon Full-rate
1	163223	86189	24094	4215	132318	6174	0	0
	79712	66170	95783	28763	63786	4804	31	0
	52314	62915	60624	18156	44671	920	248	3206
2	93212	41274	19824	1798	29789	2365	580	0
	25432	36461	39082	6660	32829	4097	80	453
	28074	27796	17760	4458	30843	352	789	99
3	27114	10923	4156	488	16373	1367	19	20
	12553	8765	7606	1249	10214	911	291	418
	12133	3949	15711	1639	56856	336	216	0
4	8947	5265	2133	0	4511	898	0	0
	5508	2086	2436	2102	5136	130	0	0
	8025	1062	977	117	37334	173	202	28
5	3821	2064	0	0	862	302	21	0
	4242	9277	526	9	5458	0	21	0
	5525	966	145	19	4272	76	692	0
	1724	195	312	138	11202	37	21	0
	4075	473	1134	39	1240	21	115	0
	1362	280	3361	0	1389	0	131	0
6	402	361	0	16	613	10	0	0
	861	236	48	0	197	0	0	0
	1056	44	153	16	412	0	0	0
	477	863	185	0	789	0	0	0
	115	921	911	0	698	0	0	0
	590	27	27	0	87	0	0	0

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