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STUDIES ON PLANT-ANIMAL INTERACTIONS:  
TERRESTRIAL MOLLUSCS AND THEIR FOOD PLANTS

A thesis presented for the degree of

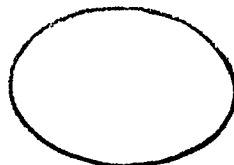
Philosophiae Doctor

of the University of Wales

by

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1980

"....., if we wish in imagination to give the plant the power of increasing in number, we should have to give it some advantage over its competitors, or over the animals which prey on it".

Charles Darwin

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## ABSTRACT

Several aspects of plant-animal interaction were studied using terrestrial molluscs and their food plants.

1. Seedling monocultures of 7 species of plants with different acceptability (measured previously using leaf discs) were grazed by slugs (Agriolimax caruanae) for controlled periods.

(i) The risks of death due to slug grazing varied with plant size and growth form. Acceptability was a poor predictor of the risks of death due to grazing.

(ii) Seedlings were killed by slugs, remained intact or were damaged but not killed; the acceptability to slugs of mature leaves was a poor predictor of the damage done to seedlings.

(iii) Plants of at least some of the species had an increased risk of death after grazing. Those grazed plants that survived were usually smaller.

(iv) It is suggested that for most species there is a 'critical size of escape' when the plants are able to tolerate grazing without being killed.

2. Monocultures of Capsella bursa-pastoris and Poa annua were grown, at densities high enough for plants to interfere with each other's growth and survival, in the presence and absence of slugs (A. caruanae).

(i) Slugs behaved quite differently towards the two plant species. They ate whole, or parts of, leaves of C. bursa-pastoris, but rarely killed the plants. They ate P. annua (only after a 'starving threshold') by chewing through shoots at ground level, 'felling' but not consuming the shoots; many of the damaged plants of Poa died. Grazed and ungrazed populations

of both species conformed to the  $-3/2$  thinning law.

(ii) The compensatory effects of competition and predation are discussed.

3. Populations of Capsella bursa-pastoris were grown at two densities in a background of plant diversity given by the seedlings emerging naturally from soil collected from areas where the slugs Agriolimax caruanae and A. reticulatus are abundant. The populations were grazed by both slugs and the plants of Capsella were monitored for most of their life cycle.

(i) C. bursa-pastoris was clearly the species most preferred by slugs.

(ii) Grazing of Capsella by slugs affected plant survival, rosette diameter, number of flowering and fruiting plants and the number of capsules per plant. The effects were almost exclusive to the populations of low density.

The reproductive potential of Capsella was also affected by defloration.

(iii) It was suggested that the diversity of the vegetation that is relevant to a given herbivore depends on its search and range and that studies on the role of herbivores on a population must take into account the diversity of the whole community (inter- and intraspecific).

4. The intraspecific variation in plant acceptability was studied by examining the response of slugs to the cyanogenesis polymorphism in Trifolium repens.

(i) Molluscs failed to distinguish between cyanogenic and acyanogenic leaf discs or detached clover leaves but experiments with intact growing plants, as well as field surveys, showed clearly that molluscs preferred the acyanogenic morphs. The pattern of grazing was characterised by an excess of nibbling on the cyanogenic plants and an excess of heavy damage on the acyanogenic forms.

(ii) Animals fed on monotonous diets of lettuce grew considerably faster than slugs feeding on diets of cyanogenic and acyanogenic clover leaves but there was a marginally lower rate of growth of the slugs fed on cyanogenic leaves.

(iii) There was a significant association between the spatial distribution of mollusc activity and clover morphs in the field: areas of intense mollusc activity had an excess of cyanogenic morphs while in areas of low mollusc activity the acyanogenic morphs were represented in excess.

(iv) Homogenates made from the digestive tract of slugs produced cyanogenic reactions with clover leaves that had cyanoglucosides but lacked hydrolising enzymes.

(v) A tentative coevolutionary model of the clover-mollusc interaction is suggested.

5. The performance of transplanted cuttings of cyanogenic and acyanogenic morphs was observed in the field for 1 year.

(i) There was some superiority of the acyanogenic morphs for several growth parameters and plant survival during the growing season of the sward.

(ii) There was much heterogeneity between clones of both morphs with respect to their susceptibility to grazing by several herbivores; nevertheless, there was a consistent preference for the acyanogenic forms by molluscs. Weevils and sheep showed preference for one morph at one time of the year, and for the other morph at another time.

(iii) Damage by the rust Uromyces trifolii was almost entirely confined to the cyanogenic morph.

(iv) It is concluded that studies on the interaction between clover and mollusc must also take into account the intraspecific (or even intraclonal) variation in reaction of clover to grazing, pathogenic infection and response to competing neighbours.



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GENERAL INTRODUCTION

A successful species (plant or animal) is one whose ancestors have displayed the necessary genetic flexibility to change in response to the selective forces operating through a long evolutionary history of life (up to 200,000 million years). The fact that organisms leave more progeny than can possibly survive has the direct implication that there is usually a surplus of individuals (and genotypes) upon which natural selection can act. Ultimately, the crucial issue is that some individuals leave more descendants than others and that the attributes that determine these differences are heritable. The particular set of climatic and geological conditions of a given area simply provide the scenario in which organisms live or die, and leave more or less descendants, but these events occur in the context of a complex biological matrix of neighbours (of the same and of other species), hosts and pathogens, and predators and prey.

This contrast of emphases on the nature of the selective forces - physical or biotic - reflects a difference in the approach of Wallace (physical forces) and Darwin (biotic forces) (Harper, 1977, p.749). There is one crucial difference between natural selection by the physical elements of the environment and by biotic pressure; against an unchanging physical environment there is generally an ultimate evolutionary solution, e.g. an ecotype specialised to life at low temperature; such an 'ultimate solution' may never be reached in response to biotic selection because living organisms form part of each other's environment and a change in any component leads to a change in the environment of the others: this in time feeds back in reciprocating selection of each on the other (Pimentel, 1964).

The present work focuses on biotic interactions, specifically the interface between plants and herbivores that prey upon them.

Traditionally, studies of plant/animal interactions have had a clear zoocentric bias, with emphasis on trophic chains (plants as the trophic level that forms the base of energy transfer through food chains) (cf. Lindeman, 1942; Odum, 1960; Slobodkin, 1960), and behavioural aspects of search and choice by the animal (e.g. Huffaker, 1958; Owen, 1971). There are some exceptional botanical contributions but these come mainly from agronomic studies, particularly the experimental approach and stimulating repercussions on ecological thinking in the classic works of Martin Jones (Jones, 1933 a,b,c,d,e) and Milton (1940, 1947). A vast source of information is also provided by the literature of forestry and crop production (Bennett, 1955; Bruce, 1956; Churchill et al., 1964; Horton, 1964), in which the emphasis has been largely on the effects of predators on some product of economic interest such as timber, grain and herbage yield, etc. (see Jameson, 1963 and refs.). The common denominator of this wide range of studies has been that the animal is studied as a predator. In contrast, mutualistic relationships between plants and animals have received a disproportionately large share of attention from biologists, particularly studies of insect pollination (e.g. Faegri and van der Pijl, 1971; Frankie, 1975 and refs.; Free, 1970; Proctor and Yeo, 1973).

Ehrlich and Raven's fundamental essay on the coevolution of butterflies and plants (Ehrlich and Raven, 1965) stimulated a number of biologists, particularly in U.S.A., to study the coevolution of plants and animals (mostly insects); the result of this has been a plethora of reports suggesting one way, or reciprocal interactions between plants and animals. Many of these studies have

emphasised biochemical coevolution as a common reaction to coevolutionary pressures (see e.g. Dollinger et al., 1973; Gilbert and Raven, 1975; Harborne, 1978; Wallace and Mansell, 1976).

A formal theoretical treatment for the interaction of predators and prey when both are animals was developed by Lotka (1925) and Volterra (1926). A comparable theoretical treatment of situations in which plants are the prey was not developed until 50 years later (Caughley, 1976).

This long delay in development of models of plant/herbivore interactions is due, in part, to the lack of basic knowledge of the mechanisms by which predation occurs and on the most immediate repercussions of predation on a plant population. The relevance of this interaction was emphasised over 120 years ago:

".....if we wish in imagination to give the plant the power of increasing in number, we should have to give it some advantage over its competitors, or over the animals which prey on it".

Darwin (1859)

There is an important distinction between predation on animals and on plants. When an animal eats another animal, the prey is generally killed, and the demographic consequences of the interaction are generally immediate for the populations of the prey and delayed for the predator; moreover, the density of the prey population can be readjusted through mobility of the animal. When an animal eats a plant, the consequences to the prey can be at two levels: (i) immediate, if the plant is killed, and (ii) long-term, if the plant remains alive (e.g. if only parts are eaten). Even when the plant prey is killed by the predator, the parallel with the animal-animal interaction is only partial because plants are sessile: if a slug preferentially kills most of the seedlings of say,

Capsella bursa-pastoris from a mixed patch of Capsella and Poa annua, the remaining seedlings of Poa have no other option but to face intraspecific competition! Quite clearly, the role of a plant predator, from the plant point of view, is not simply to reduce the density of the prey population.

Direct death of the prey in the animal-plant interaction is rather uncommon (usually restricted to the seedling stage); more frequently, only plant parts are taken and the plant is left to regenerate the animal's meal. Although regeneration can occur, two common consequences of this are: (i) the position of the plant in the hierarchical organisation of the population may be damaged (Harper, 1977 and refs.), and (ii) the shape or morphological development of the plant can be altered (see e.g. Grant and Hunter, 1966). It follows that the role of a grazing animal on a plant population will generally have to be seen in the context of these post-predation effects.

The present work attempts to explore some elementary questions about the interactions of plants and animals, from a 'phytogenic' point of view. For the most part, the work has been carried out in simple systems under controlled conditions. The interactions between terrestrial molluscs and some of the plants they eat was chosen because it appeared to be simple enough for fundamental analysis. As an experimental model it was also cheap. It was hoped that the technical and logistic difficulties of, for example, keeping a group of herbivorous tortoises within enclosures of different densities of Opuntia (or other acceptable plants) in one of the Galápagos Islands, or the expense of holding cattle or sheep on experimental fields, could be avoided, yet as readily generalisable results obtained by grazing 'flocks' of slugs on seed trays of plants.

The first stage of this study was concerned with a broad comparison of the acceptability of 30 species of higher plants to the slug Agriolimax caruanae (Dirzo, 1980); some other details of the feeding behaviour of this herbivore have been reported elsewhere (Dirzo, 1977). This preliminary work exposed some of the technical problems in manipulating the slug/plant system, raised some topics for further study and identified plant species of contrasting acceptability to this slug. It was clearly shown in this work that plants vary in their acceptability to slugs; some of the causes and consequences of these differences in plant acceptability are examined in this thesis. "Palatability (acceptability\*) is perhaps most usefully regarded not as a plant characteristic but as a function of plant and animal attributes under particular environmental conditions" (Grime et al., 1968). This point is kept in mind throughout the development of this work and although the emphasis is placed more heavily on plant responses, some sections of the thesis contain doses of zoocentric analysis; a rather comparable study of slug-plant interactions from the zoological point of view is at present being carried out in this School by R. Whelan (in prep.).

Much of the relevant literature concerning the interaction of terrestrial molluscs with their food plants (with a major concern with the acceptability of different species) has been reviewed in Dirzo (1977, 1980) with an emphasis on slugs, and in Miller (1979) with a major emphasis on snails. This introductory literature review is not repeated in this thesis.

The thesis contains (i) a brief description of the effects of grazing on monocultures of seedlings of 6 species that previously were found to be of

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\* The word acceptability is used to describe the position of a plant (or plant part) in the hierarchy of choice made by slugs rather than the word palatability which, although more commonly used, carries the implication that taste is the determinant of choice (see Dirzo, 1980).

contrasting acceptability in laboratory tests (Dirzo, 1980); (ii) an account of the effects of slug grazing on plant populations of two species of contrasting acceptability and biology grown for most of their life-cycle in high density monocultures; (iii) a study of some of the demographic consequences of grazing on a plant species grown under semi-natural conditions; (iv) a field and laboratory study of the interaction of molluscs and the intra-specific variation in plant acceptability using the system of cyanogenesis polymorphism in Trifolium repens; (v) a report of the performance of two acceptability-contrasting phenotypes of T. repens in the field. This research programme explores aspects of the interaction of molluscs and plants at two levels of plant diversity: interspecific (aspects (i) to (iii)), and intraspecific (aspects (iv) and (v)).



## CHAPTER 1

# THE EFFECTS OF GRAZING. BY SLUGS ON POPULATIONS OF SEEDLINGS OF A VARIETY OF PLANTS

".....on a piece of ground three feet long and two wide, dug and cleared, and where there could be no choking from other plants, I marked all the seedlings of our native weeds as they came up, and out of 357 no less than 295 were destroyed, chiefly by slugs and insects".

Charles Darwin

## INTRODUCTION

Given a range of potential food plants, most herbivores are likely to make choices among them; this is probably true even for highly polyphagous herbivores (Harper, 1977; p.418). This being so, it should be possible to order a group of potential food plants along an acceptability gradient for a given herbivore. Such an ordering has been shown for terrestrial molluscs (Dirzo, 1980) - a group of animals that have a reputation for being highly polyphagous. Table 1.1 lists Acceptability Indices (AI's) and rankings of 30 species of higher plants determined by the choices made by the slug Agriolimax caruanae. The frequency distribution of the AI's is shown in Fig. 1.1. The strong skewness of the histogram reflects the large proportion of unacceptable species. Most species can be allocated to three broad categories, Unacceptable, Moderately Acceptable, Highly Acceptable. The present short investigation explores the acceptability of some species whose potential acceptability as leaves of mature plants is towards the extremes of the range shown in Fig. 1.1. Since seedlings are more likely than mature plants to be damaged by slugs, this study was designed to determine the effects of slug grazing on monocultures of seedlings of species with contrasting acceptabilities. The aim of this experiment was to establish how much of the effect of slug grazing on populations of seedlings can be predicted on the basis of the relative acceptabilities of the species (determined on mature plants).

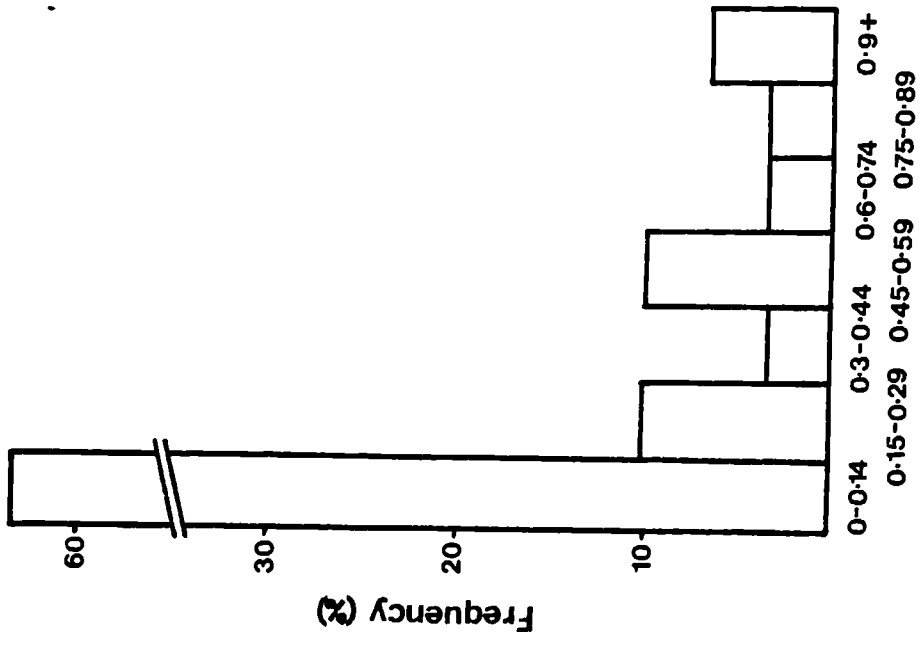
Table 1.1. Acceptability indices (and ranking) of 30 plant species offered as leaf discs to the slug *Agriolimax caruanae*. (From Dirzo, 1980).

Species tested	Species code	Acceptability Index $\pm$ S.E.		Species rank <sup>a</sup>
		1977	1978 mean of 2yrs	
<u>Anagalistenella</u>	At	0.57 $\pm$ 0.10	-	-
<sup>s</sup> <u>Bellis perennis</u>	Bp	0	0	=17
<u>Brassica</u> (cultivated)	B(c)	0.26(0.33) <sup>b</sup> $\pm$ 0.13(0.07)	0.36 $\pm$ 0.05	7
<u>Brassica napus</u>	Bn	0.66 $\pm$ 0.23	0.26 $\pm$ 0.07	6
<u>Brassica nigra</u>	Bni	0.65(0.81) <sup>b</sup> $\pm$ 0.26(0.16)	0.27 $\pm$ 0.07	5
<u>Brassica oleracea</u>	Bo	0.14 $\pm$ 0.08	0	=11
<u>Brassica rapa</u>	Br	0.03 $\pm$ 0.02	0	=15
<u>Capsella bursa-pastoris</u>	Cbp	1.05 $\pm$ 0.01	1.0 $\pm$ 0.003	1
<u>Chenopodium album</u>	Cha	0.20 $\pm$ 0.05	-	-
<u>Epilobium hirsutum</u>	Eh	0	0	=17
<u>Epilobium parviflorum</u>	Ep	0	0	=17
<u>Lamium purpureum</u>	Lp	0.07 $\pm$ 0.05	0	=13

<u>Lotus corniculatus</u>	Lc	0	0	0	0	=17
<u>Papaver dubium</u>	Pd	$0.53(0.89) \pm 0.30(0.10)$	$0.75(0.83) \pm 0.09(0.07)$	0.86	3	
<u>Plantago lanceolata</u>	Pl	0	0	0	=17	
<u>Plantago major</u>	Pm	0	0	0	=17	
<u>Plantago media</u>	Pmd	0	0	0	=17	
<u>Poa annua</u>	Pan	0	0	0	=17	
<u>Polygonum aviculare</u>	Pa	$0.28(0.11) \pm 0.10(0.02)$	$0.33 \pm 0.11$	0.22	8	
<u>Ranunculus acris</u>	Ra	$0.01 \pm 0.002$	$0.34 \pm 0.7$	0.17**	9	
<u>Ranunculus bulbosus</u>	Rb	$0.04 \pm 0.03$	0	0.02	=15	
<u>Ranunculus repens</u>	Rr	$0.10 \pm 0.04$	$0.03 \pm 0.01$	0.07	=11	
<u>Senecio jacobaea</u>	Sj	0	$0.01 \pm 0.01$	0.01	16	
<u>Senecio squalidus</u>	Ss	0	0	0	=17	
<u>Senecio vulgaris</u>	Sv	$0.18 \pm 0.04$	$0.07 \pm 0.03$	0.13	10	
<u>Stellaria media</u>	Sm	$0.91 \pm 0.07$	$0.42 \pm 0.09$	0.67***	4	
<u>Taraxacum officinale</u>	T	$0.98 \pm 0.11$	$0.83 \pm 0.14$	0.91	2	
<u>Trifolium pratense</u>	Tp	$0.14(0.08) \pm 0.08(0.02)$	$0.04 \pm 0.03$	0.06	12	
<u>Trifolium repens</u>	Tr	0	$0.08 \pm 0.03$	0.04	=13	
<u>Veronica persica</u>	Vp	0	$0.05 \pm 0.03$	0.03	14	

- a Ranks given to the species are for mean of 2 years and exclude values from 'anomalous' slugs.  
(i.e. slugs that consistently made choices different from the rest of the replicates).
- b Numbers in parenthesis show the values obtained when results from 'anomalous' slugs are omitted.
- l Complete leaflets were used instead of discs.
- r Leaf rectangles were used instead of discs.
- s Small leaf discs ( $0.57 \text{ cm}^2$ ) were used because of the small size of the leaves.
- \* ,  $P < 0.05$ ; \*\* ,  $P < 0.01$ ; \*\*\*;  $P < 0.001$  for the comparison of  $|A|$  between the 2 years; values with no asterisk are not significantly different between years.

Fig. 1.1. Distribution of Acceptability Indices of 30 plant species to the slug Agriolimax caruanae. (From Dirzo, 1980).



AI Classes

## MATERIALS AND METHODS

### The plant species

Six plant species were chosen for this study. Two of them, Capsella bursa-pastoris and Papaver dubium, had been classed as of high acceptability (see Table 1.1); the other four, Poa annua, Trifolium repens, Lotus corniculatus and Plantago lanceolata, had low Acceptability Indices (Table 1.1). The control plant used for the acceptability trials of Table 1, lettuce, was also included (this was also highly acceptable). Within each of these 2 groups of contrasting acceptability, the species vary in seedling characteristics; these are summarised in Table 1.2. Seed of the cyanogenic form of T. repens was obtained from the Welsh Plant Breeding Station, Aberystwyth. The seed of L. corniculatus and the control (Lactuca sativa) were obtained from commercial suppliers. Seed of the remaining four species was collected from plant growing in the surroundings of the Pen-y-Ffridd (PyF) Field Station, U.C.N.W., Bangor (Nat.Grid.Ref. SH 564705).

### The herbivores

Mature individuals, ca 2.5 cm long, of the slug Agriolimax caruanae were collected during early January 1979 from trapping sites established at PyF Field Station, Bangor (see Dirzo, 1980). Individual slugs were then kept in separate Petri dishes under laboratory conditions (15°C) temporarily, and fed with a mixture of bran cereal and lettuce until one day before the experiment was made.



Table 1.2. Characteristics of the seedlings of the six species (and the control) used for this experiment.

Acceptability category	Species	Growth form	Leaf area exposed to grazing	Presence of chemical substances
	Control ( <u>Lactuca sativa</u> )	Rosette-like; leaves + erect, annual	L	Latex
Acceptable	<u>Capsella bursa-pastoris</u>	Prostrate, rosette, annual	L	Mustard oils (glucosinolates)
	<u>Papaver dubium</u>	Erect shoots	S	Possibly alkaloids (latex)
	<u>Lotus corniculatus</u>	Erect shoots	S	Cyanogenic glucosides <sup>2</sup>
Unacceptable	<u>Poa annua</u>	Erect shoots	S	Silica granules
	<u>Plantago lanceolata</u>	Prostrate, rosette perennial	L	?
	<u>Trifolium repens</u>	+ erect, perennial	M	Cyanogenic glucosides <sup>3</sup>

<sup>1</sup> Arbitrary scorings: L, large; M, medium; S, small.

<sup>2</sup> This species is polymorphic for the presence of glucosides.

<sup>3</sup> The cyanogenic form was used.

### The experiments

Populations of seedlings of the seven species were obtained by scattering seed randomly on John Innes compost (No.1) in plastic pots (12 cm diameter). The density of seed sown had been adjusted, after germination tests, to give equal populations of seedlings and after emergence these were hand-thinned to a final density of 50 seedlings per pot of Capsella, Papaver and Poa (small seedlings), and 30 per pot of lettuce, Lotus, Plantago and Trifolium (larger seedlings); each population was replicated 5 times. On the day that the experiment was started, slugs were weighed and identified in their Petri dishes with a replicate number, 1 to 5, and a code for the species of plant to which they were assigned. A slug was introduced into each of the 5 replicates of each seedling population on the night of 8 January 1979, when seedlings were approximately 13 days old. To prevent the animals escaping from the experimental pots, an empty pot of the same size was prepared by sealing its base with perforated mesh; this sealed pot was placed on top of the experimental one. Next morning, this cover was removed and the animals were returned to their respective petri dishes. Every morning, recordings were made of the number of seedlings that had been damaged or killed. At night 1, the spatial pattern of damage was assessed by superimposing on the pots a grid of 16 small quadrats (2 x 2 cm) outlined on a sheet of transparent material (Melanex). The number of damaged seedlings per quadrat was counted and the frequency distribution was compared against a Poisson distribution by the variance/mean ratio method (Greig-Smith, 1957). Notes were also made describing any aspect of the grazing activity. This procedure (except the pattern analysis) was repeated for 5 days until January 13 1979. Two control pots (populations) of each plant species were treated in exactly the

same way, but without slugs.

In all the seedling populations some grazed and ungrazed plants remained after 5 nights of slug-grazing. These 2 groups of plants were labelled with plastic rings of different colour and allowed to grow for a further 10 days in the absence of slugs. On January 23, recordings were made of the number of surviving plants (grazed and ungrazed); the surviving plants were clipped at soil level, oven dried for 10 days and individual dry weights were determined.

The live weight of the slugs was measured again after the 5 night grazing period so that for each animal there was a recording of initial and final live weight.

## RESULTS

### General description of the effects of grazing

At the end of the grazing period, all the seedlings in the control populations of all the species were still alive; it is assumed therefore that the following description relates to the effects of slug-grazing alone.\*

### Types of damage

Seedlings of lettuce, Capsella and Papaver were readily eaten by slugs. The animals tended to concentrate their grazing in patches as indicated by the variance/mean ratio (Table 1.3). The seedlings of these three species differed markedly in the size of cotyledons and the extent to which they constituted a meal for a slug. The tiny individual seedlings of Papaver were almost totally stripped of green material, the larger seedlings of Capsella and lettuce satiated the slugs before grazing was too drastic. There were also differences in the effects of slugs on the 'unacceptable' species. Seedlings of Poa were often 'felled' by slugs chewing at the base of the plants; the felled leaves usually were left intact; a few leaves were eaten but only small areas of leaf were removed. There was no evidence of clumped grazing (Table 1.3), presumably because, faced with an 'unacceptable' plant, the slugs did not settle and feed locally. Lotus corniculatus was occasionally taken by slugs, sometimes felled by the slug by chewing through the hypocotyl. The spatial pattern of grazing was significantly clumped; Lotus was the only 'unacceptable' species to be grazed in a patchy manner (Table 1.3). Seedlings of Plantago appeared to be relatively unacceptable to slugs: many plants were 'nibbled' (producing small holes in the cotyledons) and only occasionally

\* N.B. In the tables and figures of this Chapter, "Control" refers to lettuce.

Table 1.3. Assessment of the spatial pattern of damage by slugs  
at night 1.

Species	$\frac{s^2}{\bar{x}}$ ratio	t	P	Pattern
Control	1.939	5.900	<0.01	Clumped
<u>Capsella bursa-pastoris</u>	1.476	2.991	<0.01	Clumped
<u>Papaver dubium</u>	2.158	7.288	<0.01	Clumped
<u>Lotus corniculatus</u>	1.633	3.979	<0.01	Clumped
<u>Poa annua</u>	1.151	0.949	n.s.	Random
<u>Plantago lanceolata</u>	1.190	1.194	n.s.	Random
<u>Trifolium repens</u>	0.917	0.542	n.s.	Random

were complete leaves or large pieces felled and left uneaten; the random pattern of grazing during the first night (Table 1.3) may be correlated with the low acceptability of this species. In populations of Trifolium repens (cyanogenic form), many leaves were 'nibbled', or complete leaves were felled but not eaten, after the petioles had been chewed. Damaged plants were distributed at random (Table 1.3)

### Survival

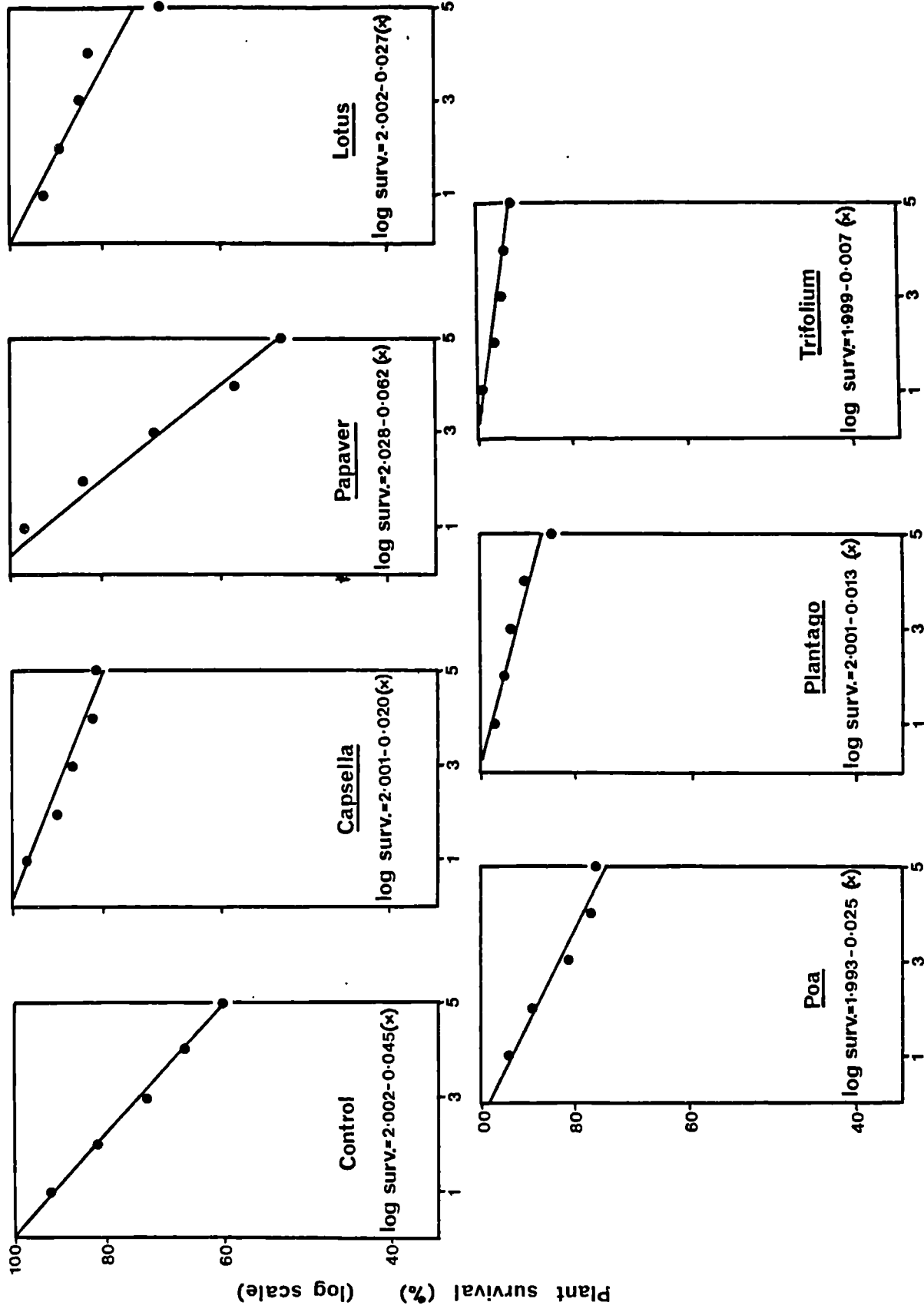
In all the grazed plant populations the slugs removed a more or less constant proportion of individuals from the populations per unit time. Table 1.4 shows the results of analyses of variance of the regressions of the log percentage of surviving plants against time. For all the species (except Lotus corniculatus) over 90% of the variance in survivorship was accounted for by the corresponding regressions on time. The mortality risk, although constant over time, varied from species to species; this is shown graphically, and numerically, in Fig. 1.2. The second terms in the equation describing the relationship between survival and time for each species gives a numerical measure of the rate of mortality; values varied more than 8-fold from -0.062 for Papaver to -0.007 for Trifolium. Three families of mortality regressions can be recognised from the steepness of the slopes: (i) intense risk - lettuce and Papaver; (ii) intermediate risk - Capsella, Lotus and Poa, and (iii) slight risk - Trifolium and Plantago. The mortality risks to seedlings, calculated in this way, do not correspond with the order of Acceptability Indices determined on leaf discs (Table 1.2). Capsella and Poa, for example, occupy extreme positions on a scale of AI but their mortality risks as seedlings are of the same order.

Table 1.4. Analyses of variance for the regressions log percentage of surviving individuals - time for the six species and the control.

Species	Source of variation	df	MS	F	P	Proportion of variance accounted for by regression (%)																																																								
Control	Regression	1	$0.3615 \cdot 10^{-1}$	2244.0	~0	99.82																																																								
	Residuals	4	$0.1611 \cdot 10^{-4}$				<u>Capsella bursa-pastoris</u>	Regression	1	$0.6875 \cdot 10^{-2}$	161.2	0.002	97.58	Residuals	4	$0.1705 \cdot 10^{-4}$	<u>Papaver dubium</u>	Regression	1	$0.6804 \cdot 10^{-1}$	135.0	0.0003	97.12	Residuals	4	$0.5039 \cdot 10^{-3}$	<u>Lotus corniculatus</u>	Regression	1	$0.1288 \cdot 10^{-1}$	34.02	0.0043	89.48	Residuals	4	$0.3786 \cdot 10^{-3}$	<u>Plantago lanceolata</u>	Regression	1	$0.2919 \cdot 10^{-2}$	60.13	0.0015	93.76	Residuals	4	$0.4854 \cdot 10^{-4}$	<u>Poa annua</u>	Regression	1	$0.1081 \cdot 10^{-1}$	99.10	0.006	96.12	Residuals	4	$0.1091 \cdot 10^{-3}$	<u>Trifolium repens</u>	Regression	1	$0.8957 \cdot 10^{-3}$	175.2	0.0002
<u>Capsella bursa-pastoris</u>	Regression	1	$0.6875 \cdot 10^{-2}$	161.2	0.002	97.58																																																								
	Residuals	4	$0.1705 \cdot 10^{-4}$				<u>Papaver dubium</u>	Regression	1	$0.6804 \cdot 10^{-1}$	135.0	0.0003	97.12	Residuals	4	$0.5039 \cdot 10^{-3}$	<u>Lotus corniculatus</u>	Regression	1	$0.1288 \cdot 10^{-1}$	34.02	0.0043	89.48	Residuals	4	$0.3786 \cdot 10^{-3}$	<u>Plantago lanceolata</u>	Regression	1	$0.2919 \cdot 10^{-2}$	60.13	0.0015	93.76	Residuals	4	$0.4854 \cdot 10^{-4}$	<u>Poa annua</u>	Regression	1	$0.1081 \cdot 10^{-1}$	99.10	0.006	96.12	Residuals	4	$0.1091 \cdot 10^{-3}$	<u>Trifolium repens</u>	Regression	1	$0.8957 \cdot 10^{-3}$	175.2	0.0002	97.77	Residuals	4	$0.5113 \cdot 10^{-5}$						
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	Residuals	4	$0.5113 \cdot 10^{-5}$																																																											

**Fig. 1.2.** Mortality rates for the six species and the control.  
(Means of five replicates).





### The fates of grazed seedlings

In a population of plants grazed by slugs, an individual plant may either (i) escape damage and remain intact, (ii) be grazed and killed at the same time, or (iii) suffer the loss of some of its parts but remain alive. The number of plants suffering these different fates was determined in each population; the results are shown in Fig. 1.3. There were considerable differences between the fates of the different species, which again failed to parallel the known AI's determined on mature leaves. The greatest contrast was found between Capsella and Papaver, both classified as 'highly acceptable'. Of those seedlings attacked by slugs (damaged and killed), 82% of Papaver seedlings were killed and only 27% of the attacked seedlings of Capsella were killed. Lettuce suffers damage similar to, but not as dramatic as, Papaver.

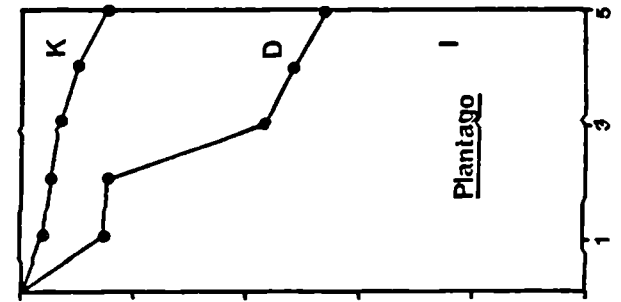
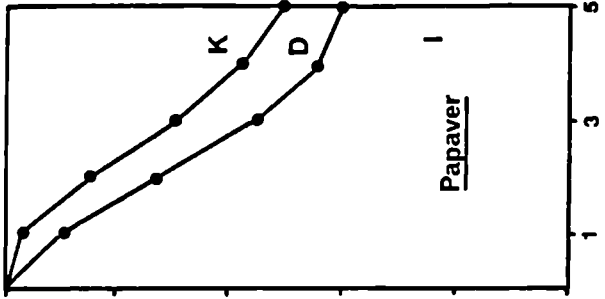
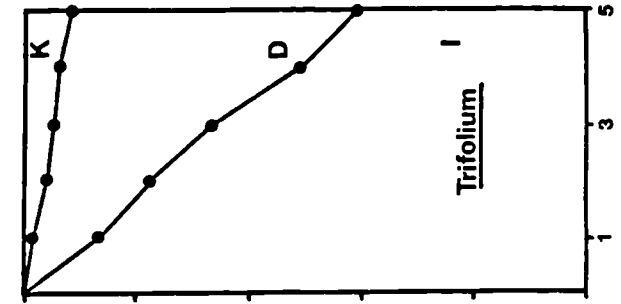
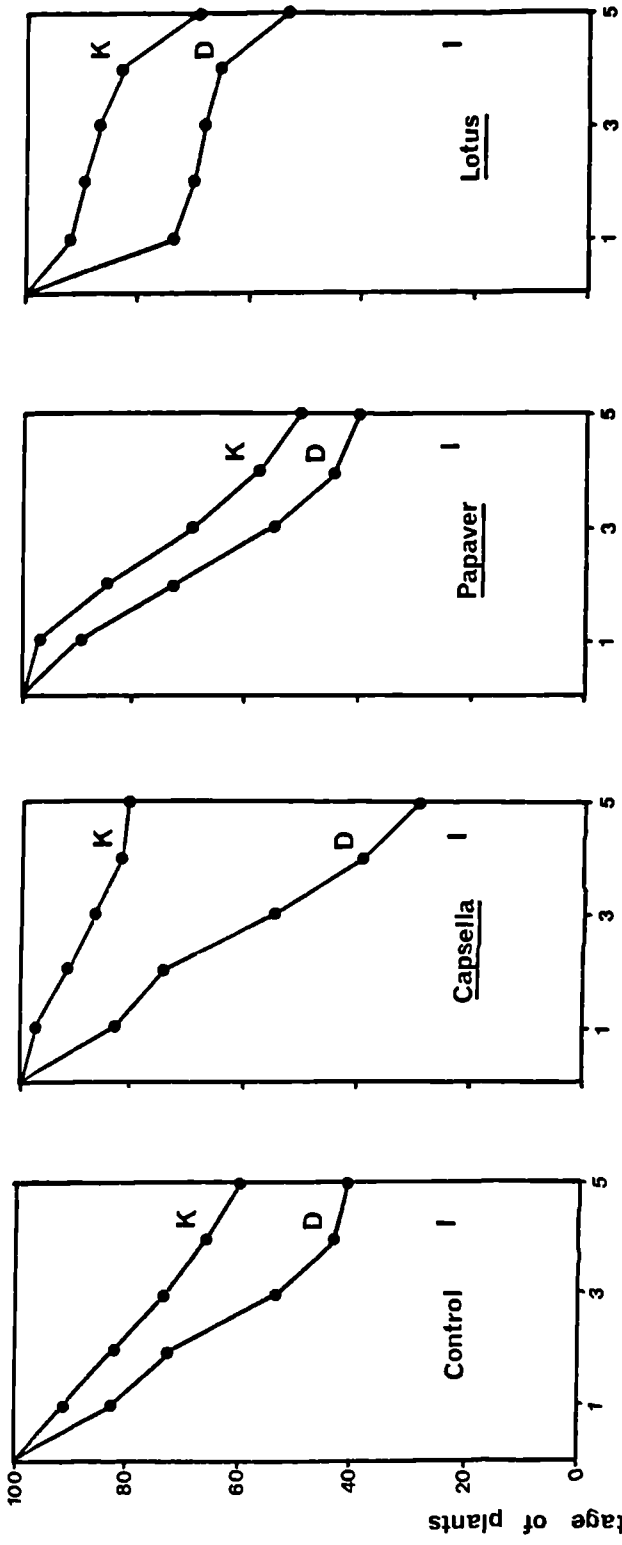
Among the 'unacceptable' species, many seedlings of Plantago and Trifolium were damaged (slightly) but few were killed. In Lotus and Poa some of the attack by slugs were lethal.

### The after-effects of grazing

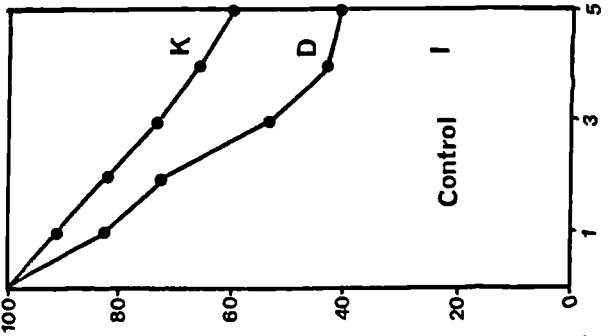
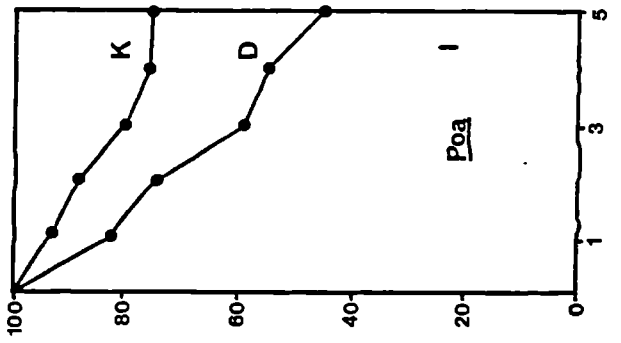
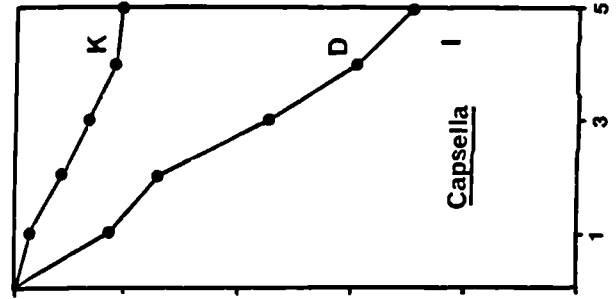
After a 10 day recovery period following grazing, measurements were made of the residual survivorship of the plants and the dry weight of survivors.

The frequency distribution of the weights of individual plants normally becomes skewed as a hierarchy of size develops. The effects of treatments are therefore most validly compared as medians rather than means. The results of such comparisons are shown in Table 1.5, which also shows the ratio of the mean dry weight per plant grazed/ungrazed as a guide to the direction of the median tests. Grazed plants of most species tended to have lower dry weights than

Fig. 1.3. The three alternative fates of the seedlings during the grazing period. I, Intact; D, Damaged; K, Killed.



Nights of grazing



Percentage of plants

Table 1.5. Comparison (median tests) of the after-effects of grazing on individual dry-weight production.

	No. of plants		Median test		Relative dry-weight per plant Grazed/Ungrazed
	Grazed	Ungrazed	$\chi^2$	P	
Control	9 > median < median	38 24	3.63	0.1 > P > 0.05	0.84
<u>Capsella bursa-pastoris</u>	48 > median < median	53 32	6.0	< 0.025	0.71
<u>Papaver dubium</u>	5 > median < median	62 34	11.13	< 0.001	0.50
<u>Lotus corniculatus</u>	6 > median < median	30 32	0.12	> 0.50	0.86
<u>Poa annua</u>	15 > median < median	79 35	37.04	< 0.001	0.56
<u>Plantago lanceolata</u>	14 > median < median	34 27	1.94	0.25 > P > 0.10	0.76
<u>Trifolium repens</u>	14 > median < median	31 21	3.28	0.10 > P > 0.05	0.70

controls, but the extent of this trend varied considerably and was significant ( $P < 0.05$ ) for only 3 of the species (Capsella, Papaver and Poa), the comparison nearly reached significance for lettuce and Trifolium ( $0.10 > P > 0.05$ ). It should be borne in mind that the median test has only 60% of the power of the t-test (Zar, 1974) and real effects may not be detected.

Some plants died during the 10 day period following slug grazing. Information from all replicates was pooled and the calculated death risks for each species are shown in Table 1.6. Plants that had been damaged by slugs suffered a higher risk of death during the 'recovery' period than those that had escaped damage (Table 1.6, last column). There again, the effect varied across the acceptability categories.

#### The effects of the different food diets on the weight of the animals.

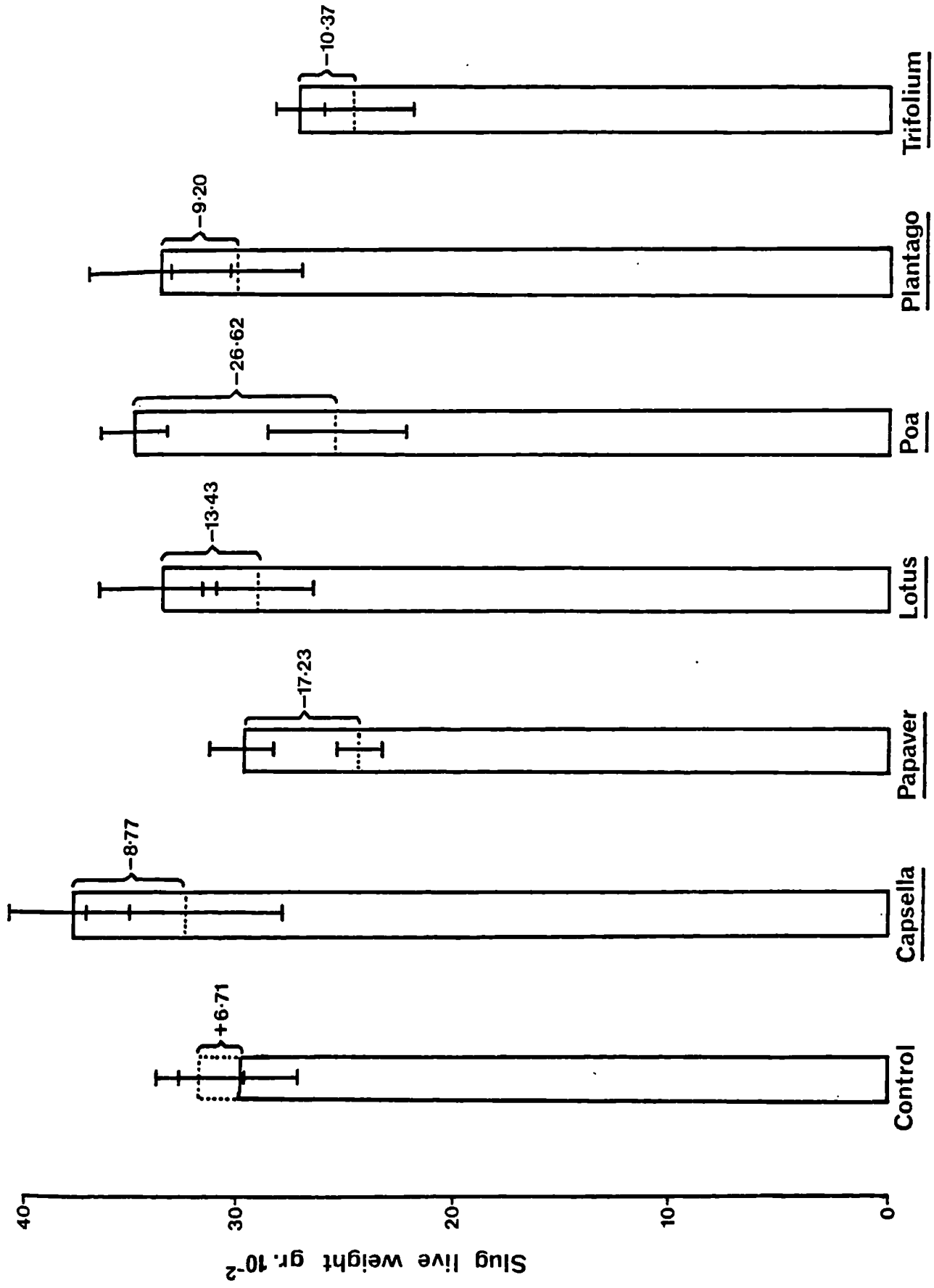
The live weights of the animals before and after the grazing period are shown in Fig. 1.4. All slugs (except those fed on the control, lettuce) lost weight during the experiment. *The animals that lost least weight were those feeding test species with high AI values (e.g. Capsella).* In contrast, animals grazing on Poa ( $AI = 0$ ) lost 26.62% of their original live weight. The slugs that grazed Papaver ( $AI = 0.86$ ) also lost much weight (17.23%). There was a minor increase in weight (statistically non-significant) of the animals feeding on lettuce. A multi-sample comparison of the mean live weight of slugs at the end of the grazing period was carried out by means of an analysis of covariance (Table 1.7) to control for the differences in the original weights. Neither in this analysis nor in the analysis of variance of the original and final weights (separately) were the changes in slug live weight significant - though the data are

Table 1.6. The mortality of damaged and undamaged plants during a 10 day recovery period after slug grazing.

Species	No. of plants surviving after 5 nights of grazing		No. of plant surviving after 10 days of further growth		Prob. survival after the 10 further days		Relative prob. of survival Grazed/Ungrazed
	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	
Control	28	53	25	53	0.89	1.0	0.89
<u>Capsella bursa-pastoris</u>	126	77	121	77	0.96	1.0	0.96
<u>Papaver dubium</u>	28	101	19	101	0.67	1.0	0.67
<u>Lotus corniculatus</u>	24	82	10	78	0.42	0.95	0.44
<u>Poa annua</u>	76	116	65	116	0.86	1.0	0.86
<u>Plantago lanceolata</u>	57	70	36	65	0.63	0.93	0.68
<u>Trifolium repens</u>	76	62	43	51	0.57	0.82	0.70

Fig. 1.4. Slug live-weights before (—), and after (·····) five nights of grazing on each of the seven populations of seedlings. Means of five replicates  $\pm$  S.E. (The values in each bar show the proportional changes in live weight after the grazing period).





Seedling population

Table 1.7. Analysis of covariance of the initial and final live weights of slugs feeding on the different food plants..

Source	df	SSx	SP	SSy	df	SSŷ	MSŷ	F
Among means	6	0.0325	0.0212	0.0476	6	0.0339	0.0057	1.6960
Within groups	33	0.1221	0.0866	0.1681	32	0.1066	0.0033	n.s.
TOTAL		0.1546	0.1078	0.2157		0.1405		

highly suggestive. The effects of plants diet on the growth of slugs warrants more extended and detailed study.

## DISCUSSION

Surprisingly there is very little information from natural systems with which to confront the realism of the experimental results described here.

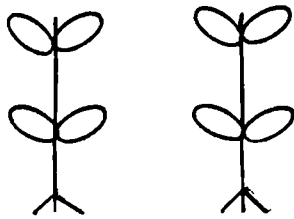
In the field, Agriolimax caruanae can reach fairly high densities in communities of plants on waste sites. In spring and early summer when seedlings of many of the plants of waste land communities are abundant (e.g. Capsella, Poa, etc.), I have counted up to 200 surface-feeding animals of this species in a search period of 25 minutes when weather conditions are suitable (see Crawford-Sidebotham, 1972a). Under these conditions the effect of slugs on populations of seedlings can be quite catastrophic on a local scale; indeed, slugs of this, and other species, have been seen devastating newly-germinated patches of seedlings. One of the few measurements of this effect is Darwin's field observations (Darwin, 1859) in which 83% of the seedlings of weeds were destroyed mainly by slugs and insects.

The relevant parameters to be measured in order to describe the effects of predators on plant populations will depend on the nature and conditions of the system to be studied. In the case of populations of seedlings probably 'lethal predation' and the short-term after-effects of grazing are good parameters to be measured. On the one hand, it is of direct demographic interest to define the extent to which this concept of predation (in the sense of direct killing) can be applied to plant populations; in this study, all the species showed a constant mortality risk over time during the grazing period ( $P < 0.005$ ), but the extent of this varied with the species. Although the populations produced for this study were extremely homogeneous (e.g. the populations were even-aged to within 1

day, etc.), probably these experimental results are not too unrealistic since it is quite common to find in the field, patches of even-aged seedling populations and commonly on a scale similar to these experimental populations. However, any degree of heterogeneity even within this fine-scale pattern is likely to be of great importance. For example, some seedling monocultures (patches) observed in the field show a very heterogeneous grazing due to factors apparently as trivial as a few days - difference in germination data, with younger (and therefore smaller) seedlings being killed first. Probably for most species there is a 'critical size of escape' from lethal predation. On the other hand, non-lethal predation is of interest from the point of view of the subsequent plant-to-plant interactions; depending on the degree of grazing, a defoliated plant can be placed at a competitive disadvantage in the proximity of a non-defoliated neighbour. The assessments in terms of dry-weight production in the post-grazing period would appear to indicate this effect in the populations of lettuce, Capsella and Poa ( $P < 0.05$ ). In a population in which grazing is non-selective, the effects of grazing will be simply to reduce the photosynthetic area and to bring the plants to an earlier stage of growth (Harper, 1977, p.392); by contrast, in a situation in which grazing is selective the effect is more subtle: not only is the growth of the grazed plant retarded but plants that escape grazing are left interacting with less aggressive neighbours; under these circumstances, the growth of the defoliated plant is likely to be worse than it would be if it was growing in a population in which all its neighbours were defoliated at the same degree; Harper (1977) has summarised this situation in the diagram shown in Fig. 1.5.

Some evidence pointing out in this direction comes from the analysis of individual dry weights collected at the end of the experiment (Fig. 1.6). With

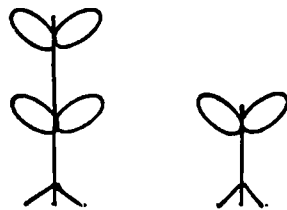
Fig. 1.5. Diagrammatic description of the effects of selective and non-selective defoliation within a density-stressed population of plants. (From Harper, 1977).



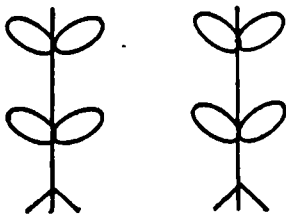
Before defoliation .



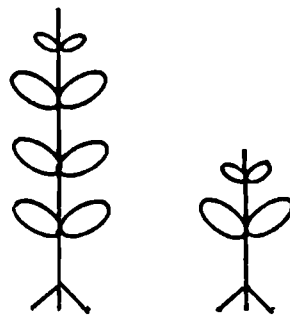
After non-selective  
defoliation



After selective  
defoliation



After recovery



After recovery

the exception of Trifolium, populations of all the species show a frequency distribution of dry-weights with a clear tendency to positive skewing; Table 1.8 shows a numerical measurement (the statistic  $g_1$ ; Zar, 1974) of the degree of departure from normality. Skewed distributions are good indicators of density stress within a population due to the development of a hierarchial organisation of sizes (see Bazzaz and Harper, 1976; Obeid et al., 1967; Ogden, 1970), with many suppressed, fewer intermediate and a few dominant plants; probably minor individual differences between plants can determine whether a plant will grow to become one of the 'elite' of dominant or to the 'mass' of suppressed plants. The role of herbivores can be of paramount importance on this respect since they can generate these differences. If the activity of the predator puts some plants in a subordinate position, the effect of grazing is much greater than the simple removal of photosynthetic area. The lower bars of the histograms in Fig. 1.6 show the proportion of grazed plants falling into any of the size classes after the recovery period. With the exception of the populations of Lotus and to a lesser extent of Plantago, the grazed individuals tended to be over-represented in the smaller or smallest size categories of the population. The statistical significance of these different distributions was assessed with the log likelihood ratio test\*; the obtained  $\chi^2$ 's (shown with the histograms) were significant ( $P < 0.01$ ) for all the species except Lotus and Plantago; when significant, these tests are indicative of a greater probability for the grazed plants to belong to the sub-ordinate size class in a density-stressed population.

In this study some observations were made on the survival component of the fitness of the seedlings. In slug grazed plants all fitness is lost if a slug

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\* N.B. The tests were made with actual numbers, not the percentages.



kills a plant either as a direct consequence of grazing or by increasing the subsequent risk of death.

Most of the data obtained in this study show that it is unsafe to use the Acceptability Index of a species (determined in experiments with mature leaves) as a means of predicting the effects of slug grazing on populations of seedlings. It was surprising to find that species that were 'unacceptable' to slugs nevertheless suffered from grazing because slugs tended to attack a different plant part in place of the unacceptable leaves; e.g. the tender tissue at the base of the shoots of Poa, or the base of the stems or petioles of Lotus; felling (without eating) of complete leaves or even whole plants was a common hazard to 'unacceptable' species.

One aspect that appears to correlate reasonably well with the acceptability ranges of the different species was the spatial pattern of damage at the onset of the experiment (Table 1.3). Unacceptable species tended to be attacked at random while populations of acceptable species were grazed in patches or clumps.

Neither the mortality rates (Fig. 1.2) nor the after-effects of damage in the different species showed any clear relationship with AI.

Very reliable, accurate and repeatable measurements of a plant's acceptability to slugs can be obtained from laboratory tests. These have little predictive value for the fate of seedlings in the field where a host of other variables - accessibility, regenerative ability, seedling size and form, interact to determine the ecological consequences of grazing by slugs.

CHAPTER 2

THE EFFECT OF GRAZING  
BY SLUGS ON HIGH DENSITY MONO-  
CULTURES OF CAPSELLA BURSA-PASTORIS  
AND POA ANNUA

## INTRODUCTION

The presence and persistence of a plant population in an area implies that it and its ancestors have survived the recurrent physical hazards typical of that area and these hazards may no longer be the dominant forces directing selection. However, the role of biotic forces in natural selection is such that no equilibrium may ever be reached - each evolutionary change in one organism affects the selective forces operating on its competitors, predators or prey, with the consequence that ever-continuing evolutionary processes are maintained and an equilibrial solution may be quite unlikely (Levins, 1968; Harper, 1977).

Two biotic hazards of prime importance for a plant are (i) the presence of neighbours making demands on the same, or similar resources and (ii) at a different trophic level, the activities of herbivores (or pathogens or both); presumably in many situations neighbours and herbivores can both act and interact.

The effects of herbivores on their plant prey can vary depending on the kind and amount of plant material removed by the predator, as well as the age or physiological status (or both) of the plant that is damaged (Harper, 1977, p.386). The harm done to the plant will rarely, if ever, be simply measured by the amount of tissue removed. A unit weight removed may, if the plant is a seedling, mean inevitable death, whereas the same unit weight removed from an adult plant may be an almost insignificant fraction of a senile leaf. Moreover, the after-effects of the removal of some plant parts by a predator will depend on the extent to which neighbouring plants are already interfering with each other's activities, because partial defoliation may increase the disadvantage that a plant experiences in the presence of a non-defoliated and competing neighbour.

High density in a plant population causes mortality or plasticity or both amongst its members (Harper and Gajic, 1961); the response follows a formal mathematical relationship, the  $-3/2$  power law (Yoda et al., 1963) which is described by  $W = Cp^{-3/2}$  (where  $W$  is individual plant weight,  $p$  is the density of surviving individuals and  $C$  a constant). The wide generality of this rule is established by White (1979) and Gorham (1979). Plant populations in which competition is occurring develop a hierarchical structure of suppressed, intermediate and dominant individuals (Obeid et al., 1967; Ogden, 1970; White and Harper, 1970; Bazzaz and Harper, 1976). The effect of predators on vegetation may therefore need to be seen in the context of the position of the grazed plants in an established hierarchy of a naturally self-thinning population.

The experiments described in this paper were designed to study the influence of a herbivore on plant populations which were dense enough for individual plants to interfere with each other's growth and survival.

## MATERIALS AND METHODS

### The species

A previous study of the acceptability of a range of plant species to the slug Agriolimax caruanae Pollonera (Dirzo, 1977) showed that of the two common weedy annuals Capsella bursa-pastoris and Poa annua, Capsella was consistently preferred, whether compared as leaf discs or as intact seedlings. Leaves of Capsella were preferred over roots and stems. In the absence of any alternative food, slugs would feed on Poa only after a "starving threshold" of 66 hours.

In nature, Agriolimax caruanae has an annual life cycle with overlapping generations. It is abundant in open and semi-open habitats, commonly in early phases of colonisation, e.g. arable lands and gardens but particularly in waste areas. It is absent or very rare in plant communities dominated by grasses (further details are given in Dirzo, 1980). A. caruanae is commonly found in areas where C. bursa-pastoris and P. annua are abundant. Plants of these two species can be found vegetative and often flowering in all seasons and are a potential food resource for the slugs throughout the year.

### Experiments

Seeds of the two plant species were obtained from commercial suppliers. Seed of Capsella requires chilling for a month at 4°C to give satisfactory (55%) germination (Popay and Roberts, 1970). Seed of Poa germinated readily (85%) without pre-treatment. One month chilled seeds of Capsella and non-chilled seeds of Poa were used to produce the experimental populations.

Plastic trays (21 x 16 x 5 cm) were filled to within 1.5 cm of the top with John Innes Compost No.1. A further layer of finely sifted compost was applied to give a smooth soil surface and seeds were scattered at random. The number of seeds was calculated to give 400 seedlings per tray ( $12\ 085\ m^{-2}$ ). After sowing, the trays were placed in growth cabinets set at 24°C during the day, 18°C during the night with 9 hours light per day. Germination was complete after 7 days and the trays were then transferred to a cool glasshouse for three days for acclimatisation. Seeds of Capsella gave a slightly higher germination than expected and the seedling populations were thinned by hand to 420 per tray. Seed of Poa gave populations of seedlings close to the expected density but these were contaminated with other grass species which were removed leaving a realised density of 376 seedlings of Poa per tray.

The experimental design involved two treatments (slugs and no-slugs) x 2 species of plant x 5 harvests x 2 replicates in a completely randomised design. The five harvests (H1-H5) were taken 9, 17, 25, 39 and 53 days after seedling emergence (10 July 1977). At each harvest the plants were cut at soil level and the above-ground parts were oven-dried and weighed.

Slugs were introduced 4 days after seedling emergence (14 July 1977). A density of two slugs per tray was chosen, based on evidence from previous studies (Hatto and Harper, 1969; Harper, 1977). Slugs are nocturnal feeders and they were introduced to the plant populations every evening (around 6 pm) and removed the following morning (around 9 am). To prevent their escape, a removable, completely sealed, fence of plastic mesh was fitted to the trays every evening when the slugs were introduced and removed when the slugs were removed in the morning. The slugs were not fed during the day, and an individual slug was always put in the same tray throughout the study.

## RESULTS

### Ungrazed populations

#### Biomass production

In the ungrazed populations (Fig. 2.1), Capsella achieved a higher dry weight per plant and per unit area than Poa until harvest 3, but after H3 the yield of Poa (per plant and per unit area) became much higher than that of Capsella, and continued to increase until the end of the study. Capsella is a plant of determinate growth; the first flowering stages were visible after H3 and at that time the rate of dry matter production declined. Poa on the other hand, is a plant of indeterminate growth, and no flowering tillers appeared until the end of the study (H5) at which time vegetative growth and tillering were still active.

Measures of the dry weight of plants per unit area give an incomplete picture of the growth of the population since yield is a function of (i) survivorship and (ii) the distribution of plant weight amongst the survivors.

#### Survivorship

The trends of survivorship with time (expressed on a logarithmic scale in Fig. 2.2) show that populations of both species were declining continuously during the course of the experiment. The shapes of the survivorship curves differ in interesting ways. The ungrazed populations of Capsella suffered an essentially exponential rate of decay (i.e. individuals were lost from the population at a constant rate) and the risk of death was much greater than in Poa in which it remained low and constant until H4 and then increased rapidly. The death risks

Fig. 2.1.

The growth and survival of plants of control (a) and grazed (b) populations of Capsella bursa pastoris and of Poa annua (means of 2 replicates and range). ●—●, plant survival (%), ○—○, dry weight per plot (g), ▲—▲, dry weight per plant (mg).



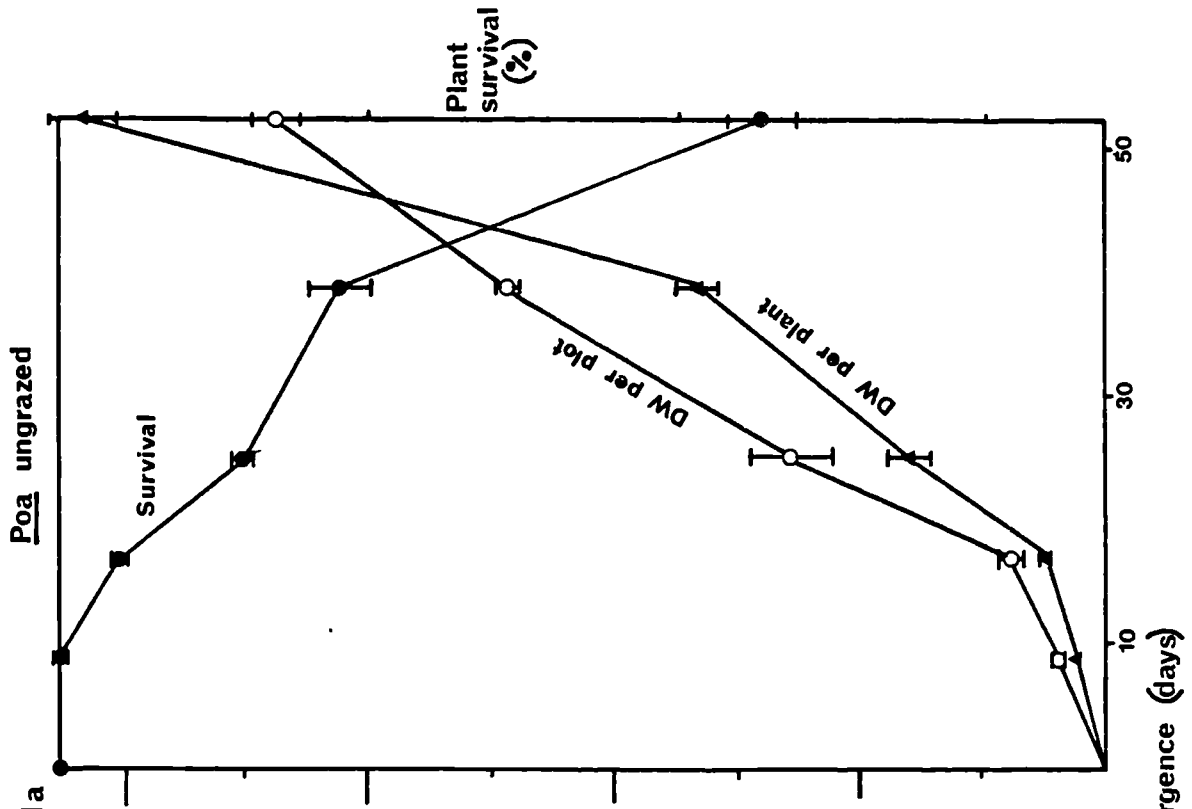
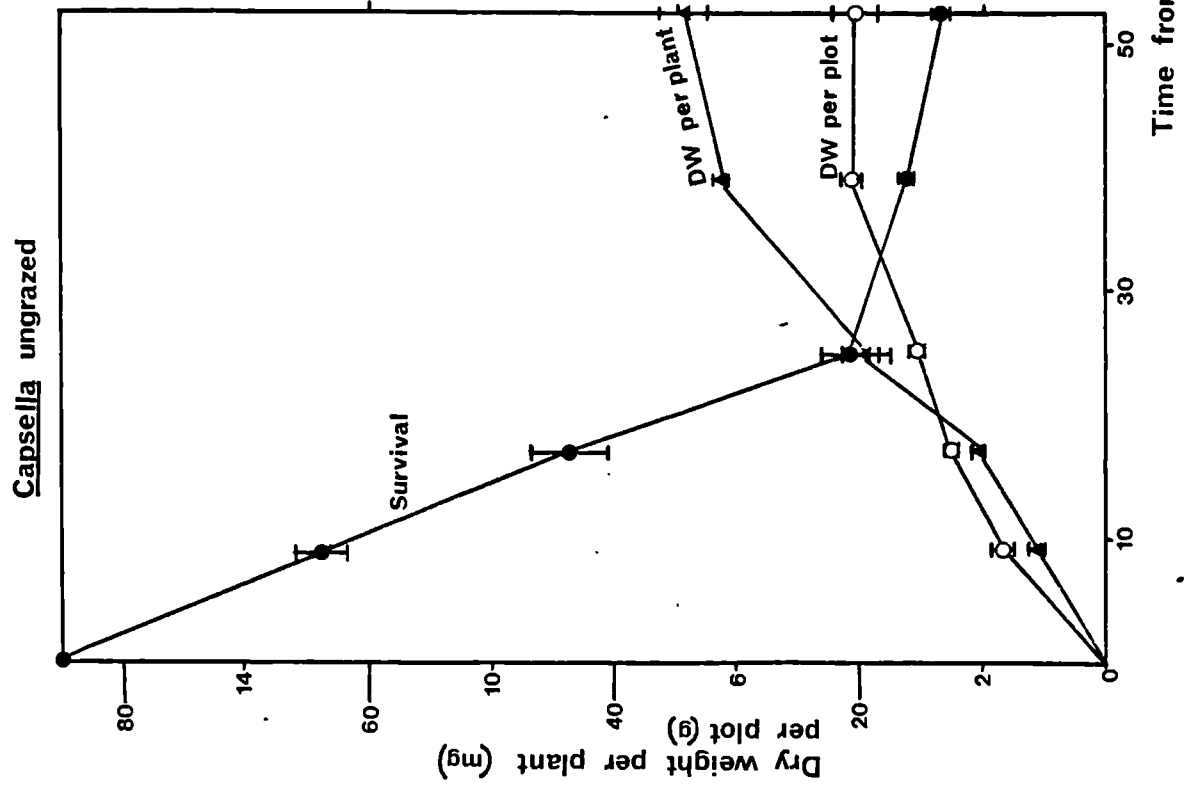


Fig. 2.1a



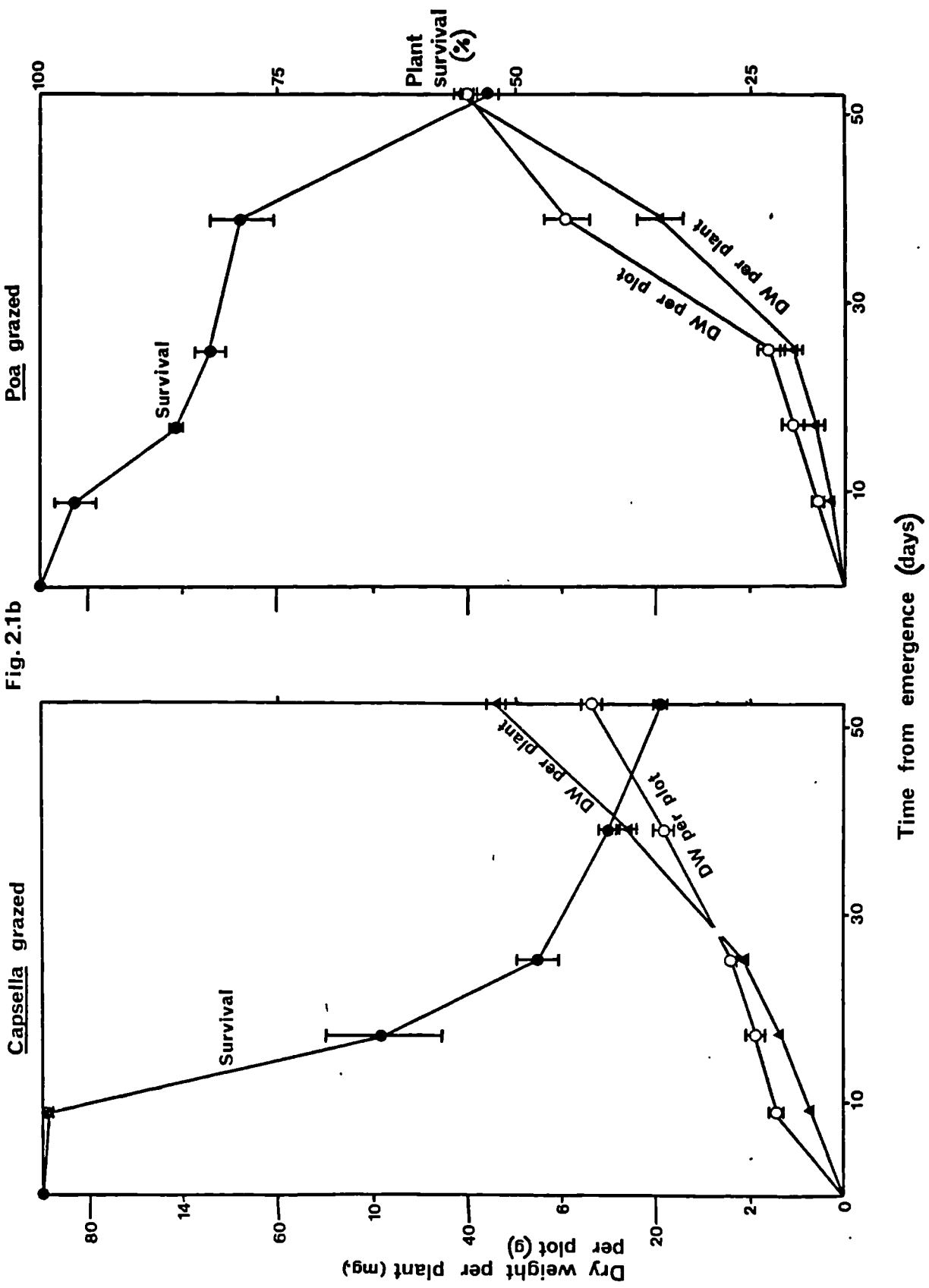
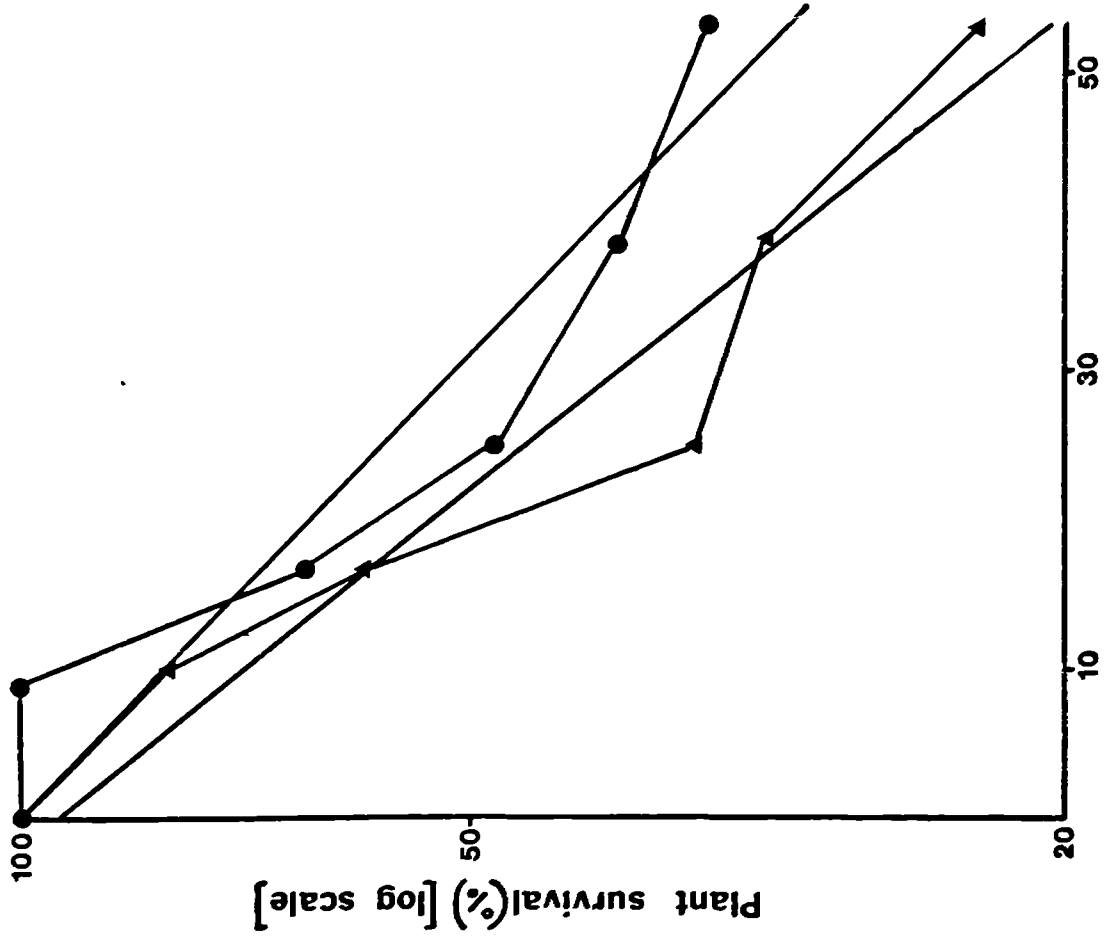


Fig. 2.1b

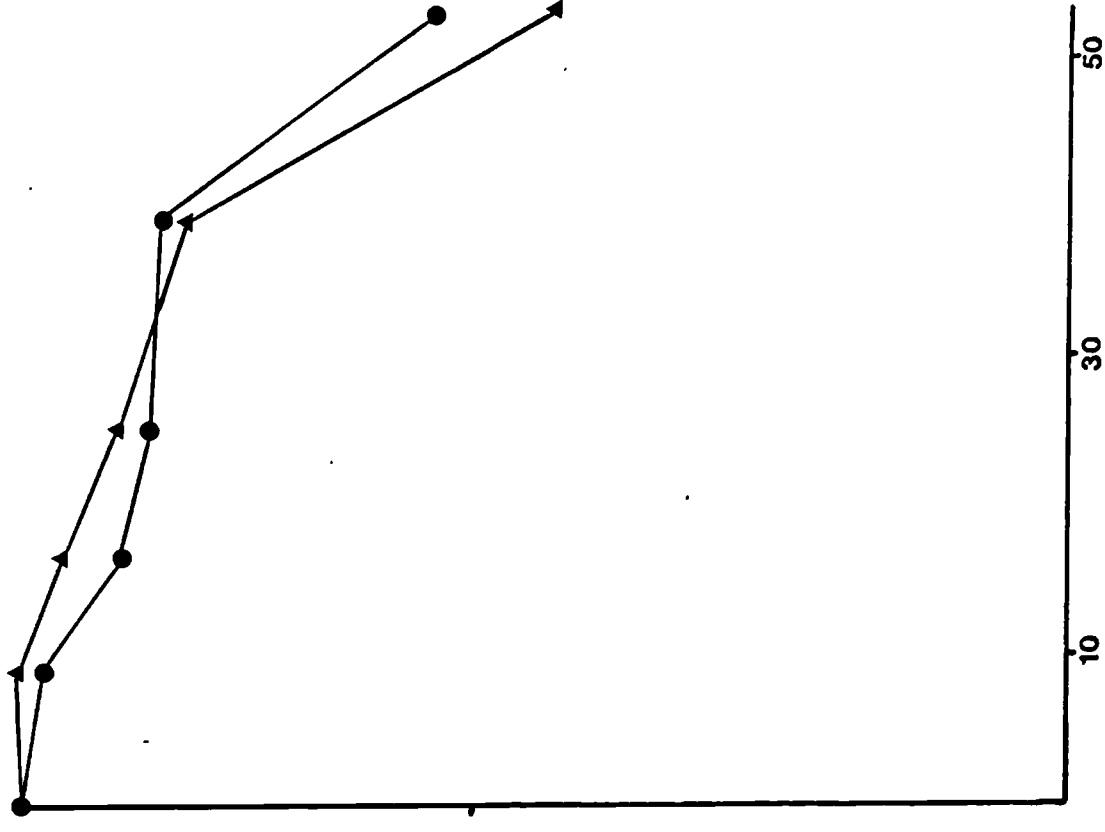
Fig. 2.2.

The time course of survival of plants of Capsella bursa-pastoris and Poa annua in grazed and control populations. The fitted linear regressions for Capsella are shown as straight lines.  
▲—▲ , ungrazed, ●—●, grazed.

Capsella



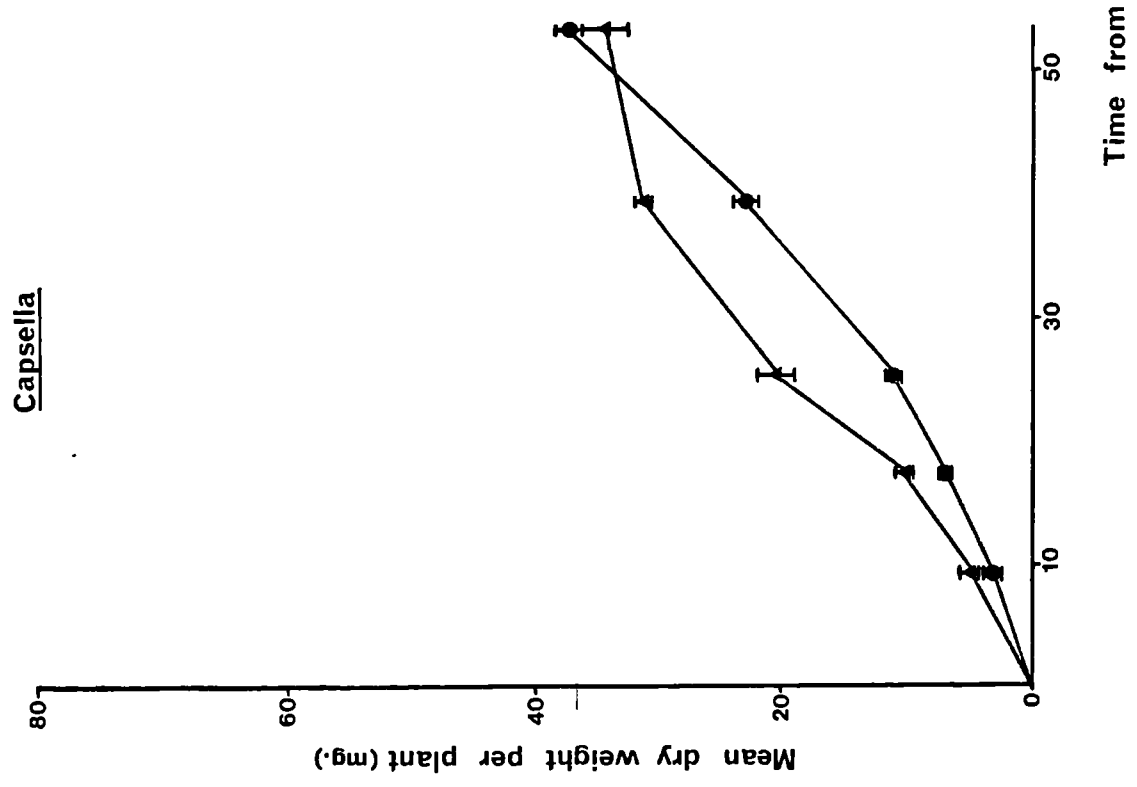
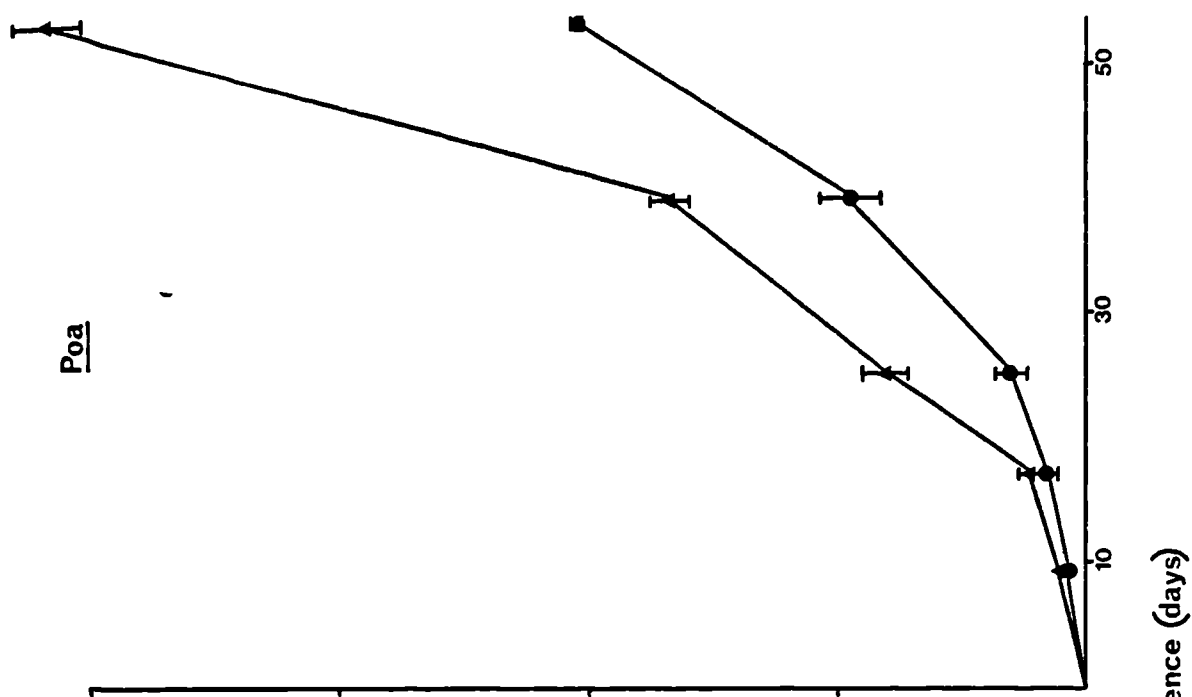
Poa



Time from emergence (days)

Fig. 2.3.

Changes with time in the mean dry weight per plant in populations of Capsella bursa-pastoris, and Poa annua (means of 2 replicates and range). ▲—▲ , ungrazed, ●—●, grazed.



in the populations of the two species are readily interpretable in relation to the yield of dry matter per plot (Fig. 2.1). In Capsella dry weight developed quickly and ceiling yield was reached by H4 - interference between individual plants is expected to be intense within such populations and the early onset of self-thinning is not surprising. In contrast, the yield of populations of Poa fell below those of Capsella until H3 but then surpassed it. A later start to intense self-thinning could therefore be expected in the populations of Poa.

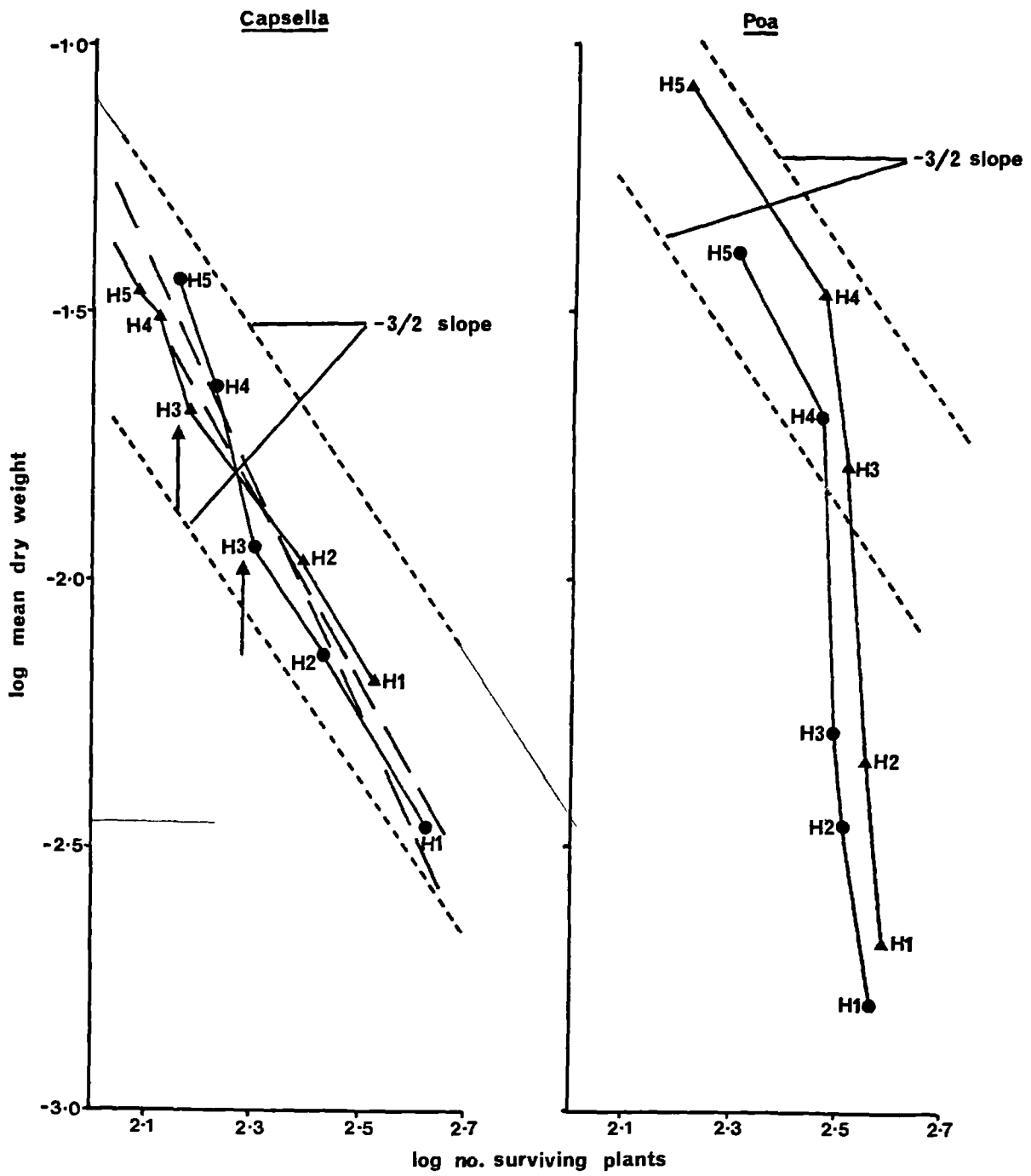
#### Correlated changes of survivorship and the weight of surviving plants

The interpretation of mortality patterns as a response to the growth made by the survivors appeared even more clearly when log mean plant weight was plotted against log density of survivors (Fig. 2.4) in order to test for the fit of the data to the  $-3/2$  power law (Yoda et al., 1963). Populations of Capsella displayed a reasonably close fit to this law (Fig. 2.4) with a slope of  $-1.8$ , not significantly different ( $P < 0.05$ ) from  $-1.5$ . The regression accounted for up to 96% of the variation. The arrows in Fig. 4 indicate the onset of flowering in Capsella. At that time both the growth rate and the risk of death are reduced so that the points on the graph representing different harvest dates come to lie close together. In populations of Poa the  $-3/2$  power relationship did not become apparent until H4 and H5 (Fig. 2.4). The individual plants were not large enough to interfere with each other significantly and to cause mortality amongst neighbours until after H3.

Fig. 2.4.

Correlated changes in plant dry weight and the number of survivors in populations of Capsella bursa-pastoris (a), and Poa annua (b). Arrows in Capsella show the onset of flowering. The straight lines in Capsella are the fitted linear regressions. ▲—▲ , ungrazed, ●—●, grazed.





### The frequency distribution of individual weight

The frequency distribution of plant weight becomes positively skewed in dense populations as a hierarchical structure develops through time with a few large plants and many small (Obeid et al., 1967; Bazzaz and Harper, 1976). This is shown in Fig. 2.5 for Poa and Capsella at harvests 1, 3 and 5. The degree of departure from normality (i.e. skewness) can be quantified by  $g_1$ , relative skewness (Zar, 1974, p.72), which is an estimator of  $\gamma_1$ , the third moment about the mean of a distribution. A value of  $g_1 < 0$  indicates that the sample comes from a population whose distribution is skewed to the left, while  $g_1 > 0$  indicates a distribution skewed to the right. A harvest H0 was made of Capsella populations 4 days after seedling emergence and at that stage there was no significant departure from a normal distribution ( $P > 0.05$ ). Skewness then developed very rapidly (Fig. 2.6) and departure from normality was already significant ( $P < 0.01$ ) at H1. This is further confirmation of the very early stage at which interference develops between individuals in this species. Individual dry weight of Poa (Fig. 2.5) by contrast is normally distributed at H1 ( $g_1 = 0.21$ ,  $P > 0.05$ ) and significant skewness develops more slowly through H3 and H5 (Fig. 2.6). This again confirms that interference between individuals is slower to develop in populations of Poa than in Capsella.

Trends of relative skewness ( $g_1$ ) with time (Fig. 2.6) summarise the differences between the species. Skewness increases as if towards an asymptote in populations of Capsella - this is to be expected as the determinate growth habit involves a transition of flowering and an end of growth towards the end of the experiment. In populations of Poa, skewness is still increasing rapidly at H5, the individuals have indeterminate growth and yield per plot was still increasing

Fig. 2.5.

The frequency distribution of dry weight in control (a) and grazed (b) populations of Capsella bursa pastoris and of Poa annua at four harvest times, H0, H1, H3, H5.

Fig. 2.5 a

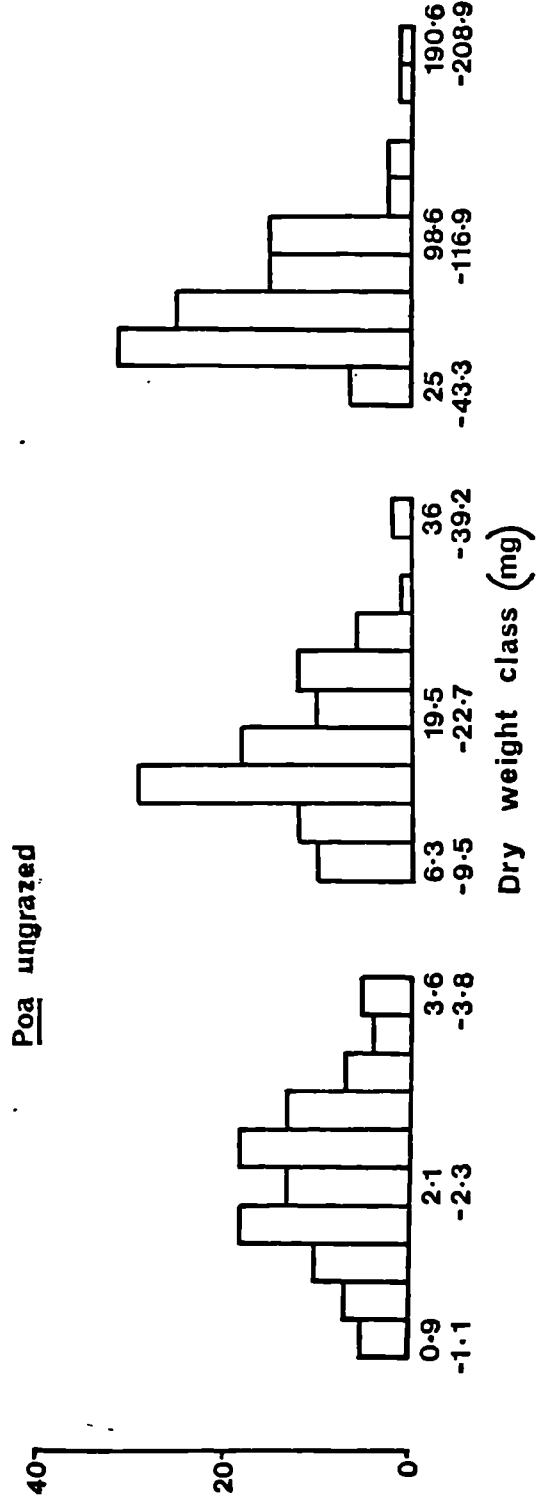
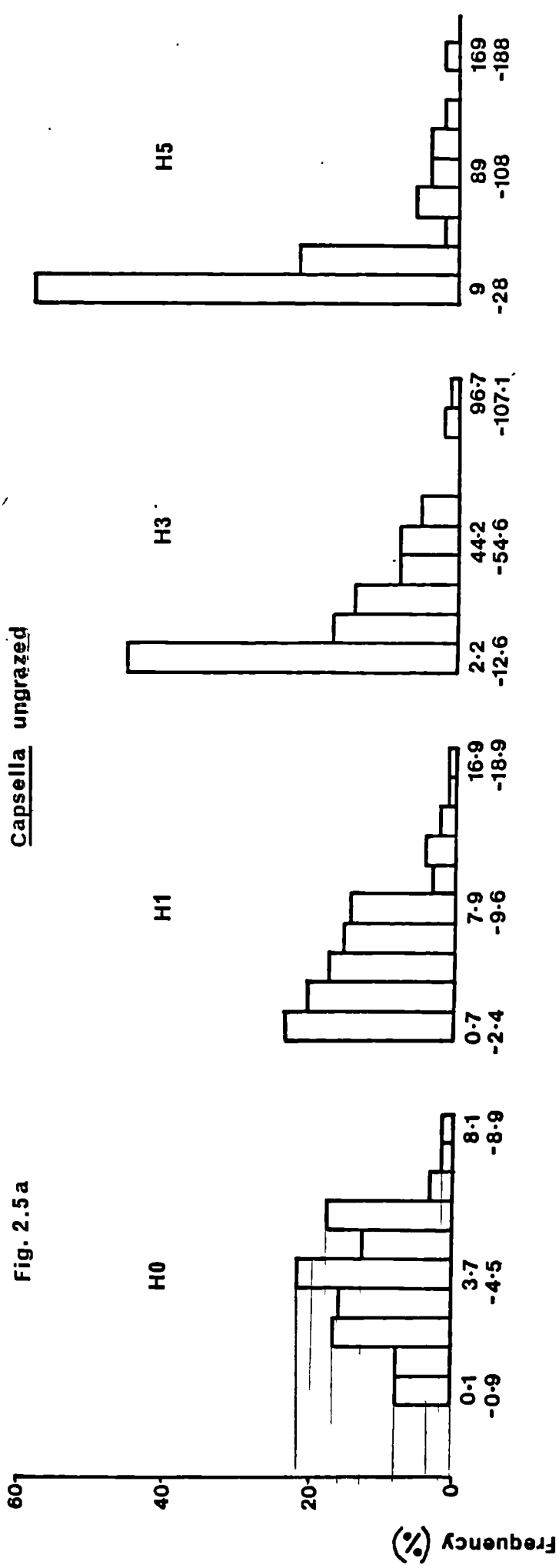
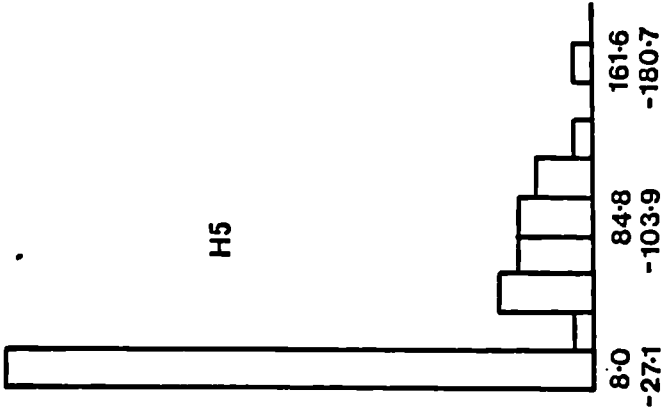
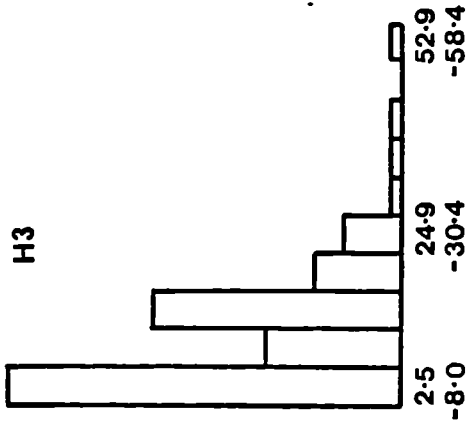
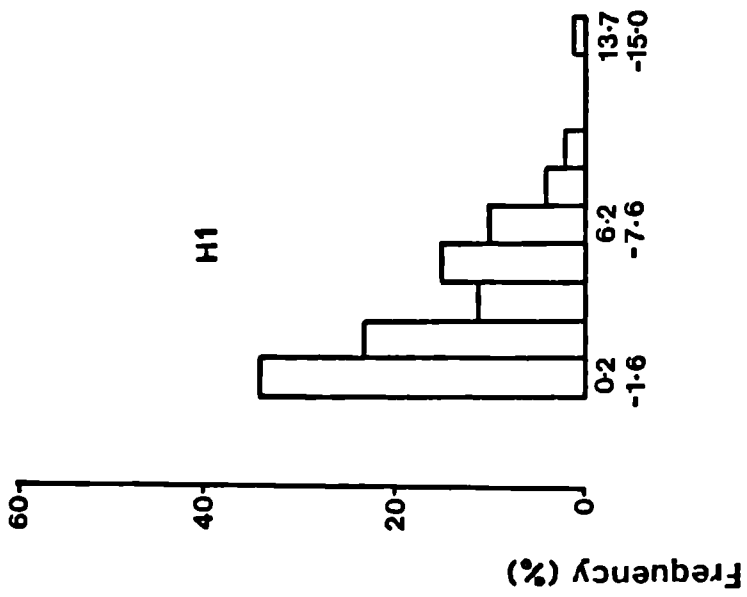
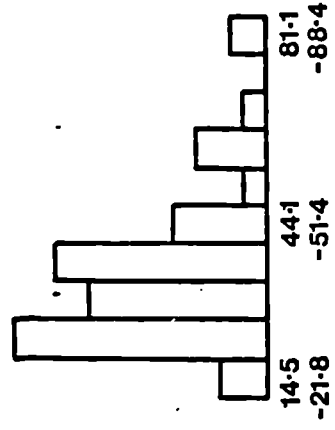
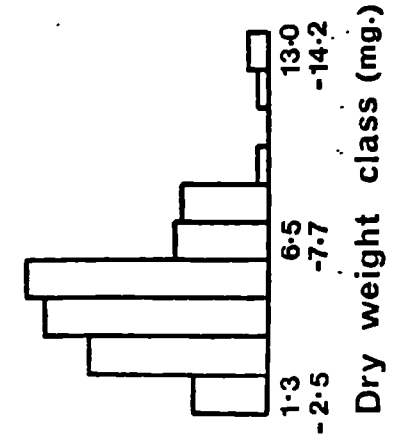
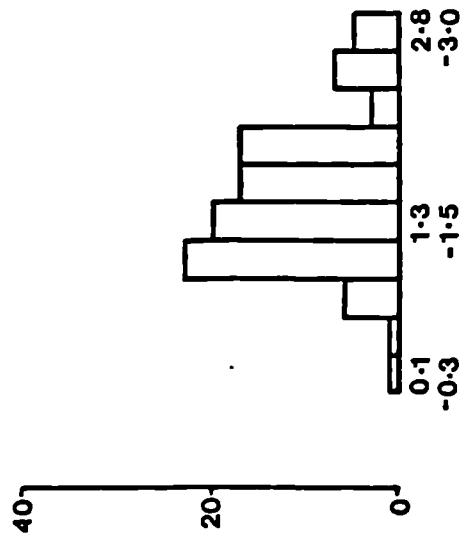


Fig. 2.5 b

Capsella grazed



Poa grazed

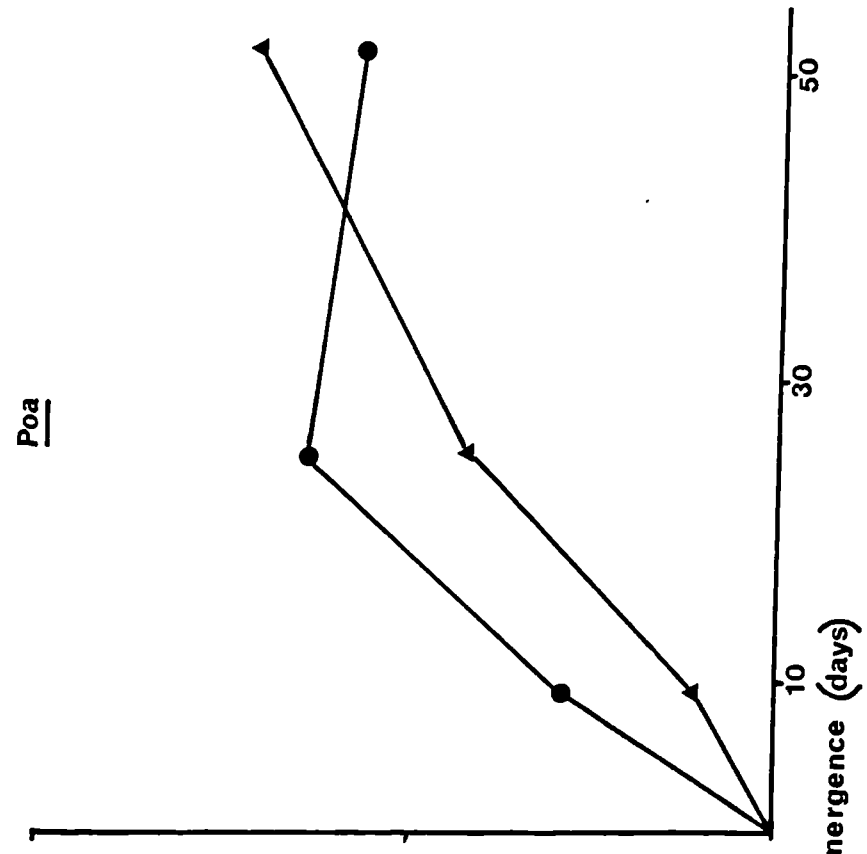
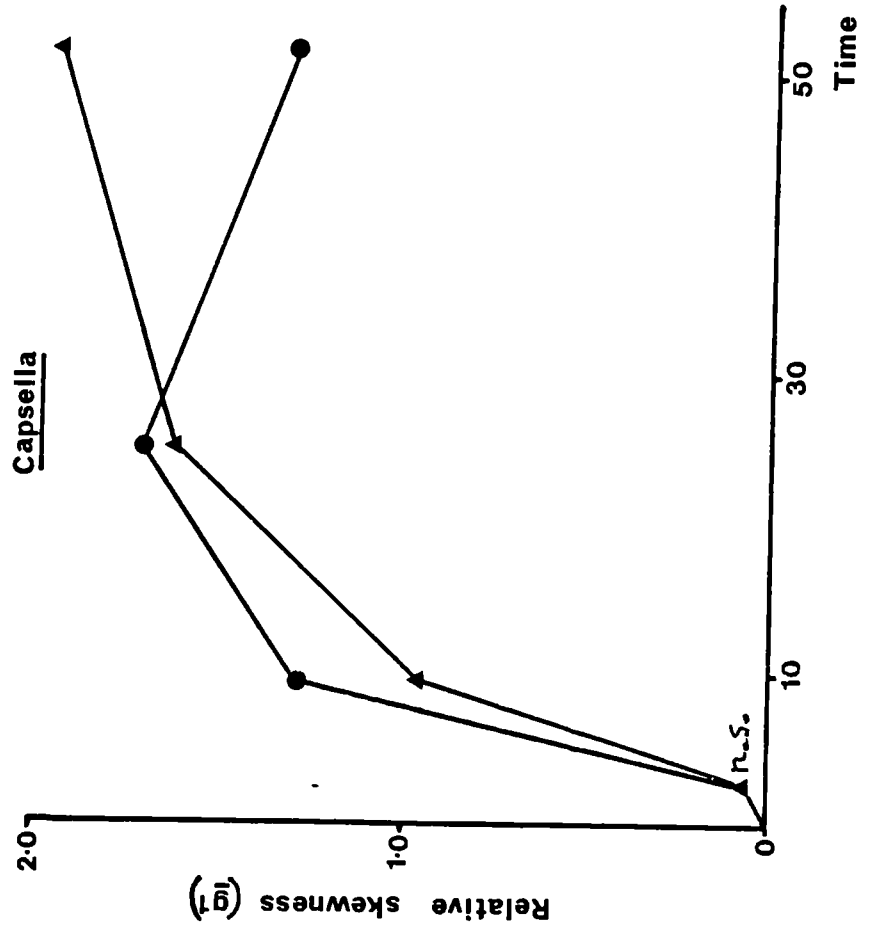


Dry weight class (mg.)

Fig. 2.6.

The trends of relative skewness ( $g_1$ ) with time in populations of Capsella bursa-pastoris and Poa annua. All points except those indicated as n.s. are statistically significant ( $P < 0.01$ ).

▲—▲ , ungrazed, ●—●, grazed.



rapidly at the end of the experiment.

The behaviour of the slug grazed populations has to be seen against this background of different growth rates, thinning regimes and frequency distribution that develop in the control populations of Capsella and Poa.

### Grazed populations

#### General features of slug damage

Capsella was readily accepted by the slugs and defoliation started on the first night of their introduction. Poa, in contrast, was not eaten during the first two nights but grazing started after a "starving threshold" of ca 66-68 hours.

The slugs attacked Capsella and Poa in quite different ways. Parts of the leaves of Capsella were eaten and sometimes whole leaves were separated from the plant as the slugs chewed through the petioles. The slugs attacked Poa by chewing through the young shoots at ground level. Some of the grass plants damaged in this way were marked with plastic rings to follow the consequences of this type of damage. Most often the damaged plants died quickly; in a few cases the seedlings remained alive and regenerated but died later, perhaps as a consequence of the combination of defoliation and suppression by undamaged neighbours.

The spatial pattern of damage was determined by dividing the whole of each tray into twenty small quadrats, counting the number of damaged plants per quadrat and comparing the frequency of damage against a Poisson distribution. After 1 week of grazing the variance/mean quotient was significantly greater than 1 (Table 2.1) for both species indicating a contagious probability of



Table 2.1. The spatial pattern of damaged plants in populations of Capsella bursa-pastoris and Poa annua after one week of grazing by the slug Agriolimax caruanae

	<u>Capsella</u>	<u>Poa</u>
Total no. of damaged plants per plot	45	22
Mean per quadrat 4 x 4 cm ( $\bar{x}$ )	2.3	1.1
Variance ( $S^2$ )	14.0	4.8
Ratio Variance/Mean	6.2	4.4
Significance ( $H: S^2/\bar{X} = 1$ )	$P < 0.001$	$P < 0.001$

damage, i.e. slugs tended to graze neighbouring plants. After a further period of grazing, damage became widespread through the populations, most of the quadrats contained a considerable number of damaged plants and there was no longer evidence of clumped grazing.

### Biomass production

The yield of the grazed populations is shown in Fig. 2.1b. Dry matter production followed the same general trends as the controls (Fig. 2.1) but comparison of grazed and control populations showed the following differences. The effect of slug grazing on the yield of Capsella was most marked early in the development of the populations; grazed plots yielded 87% and 74% of the controls at H1 and H2 respectively. The effect gradually decreased despite the continued presence of the slugs and by H5 the yield of the grazed populations was not significantly different from that of the controls ( $P > 0.10$ ). Although Poa was much less acceptable than Capsella to the slugs and grazing started later, the slugs caused more damage to Poa. Grazed populations of Poa yielded 30-60% of the controls, with the exception of the value 74% at H1. Despite this considerable yield reduction relative to the controls the grazed populations of Poa eventually achieved a much greater biomass than grazed populations of Capsella.

### Survivorship

As in the controls, some individuals in grazed populations of both Capsella and Poa died (Fig. 2.2) but the risk of death in grazed populations of Capsella was conspicuously smaller than in the control plots. This suggests that the activity of the slugs reduced the intensity of natural density-dependent thinning -

presumably by defoliating the plants and making them smaller so that the damaged plant interfered with each other less than the vigorously growing controls. The risk of death in Poa was less affected by the presence of slugs. In the period H1-3, when there was little if any density-dependent thinning, survivorship was marginally higher in the ungrazed populations. In the period H4-5 when competitive interaction had developed strongly in the control populations and self-thinning was occurring, the presence of slugs slightly decreased the death risk of the surviving grass plants.

Values for changes in the dry weight per plant (Fig. 2.3) complement the results for yield per plot and survivorship. The mean weight per plant in grazed populations of Capsella was consistently lower than that of ungrazed plants except at the last harvest. The weight per plant of Poa in the grazed populations was only marginally less than that of ungrazed plants at H1 and H2 but was dramatically reduced from H3 to H5.

#### Correlated changes of survivorship and individual plant weight

Patterns of self-thinning in the grazed populations (Fig. 2.4) were again analysed and tested for conformity with the  $-3/2$  power law. The results were essentially similar to those obtained in the control populations. Self-thinning in Capsella (Fig. 2.4) gave an exponent of  $-2.2$  which does not differ significantly from  $-1.5$  ( $P > 0.05$ ). The regression accounted for up to 92% of the variation. Anomalous values occurred after the onset of reproduction (see arrow in Fig. 2.4) when the growth rate and risk of death both declined. If analysis is carried out eliminating the data for the flowering period H4-5 a slope of  $-1.6$  is obtained which does not depart significantly from  $-1.5$  ( $P > 0.05$ ). The interpretation of

self-thinning in grazed populations of Poa (Fig. 2.4) is the same as for the controls: density-dependent thinning is delayed until H4 when the plants are large enough for individuals to interfere with each other. The values of H4 and H5 reasonably conform to the expected slope of  $-3/2$ .

#### The frequency distribution of individual plant weight

Analysis of the frequency distribution of dry weight was made at harvests 1, 3 and 5 (Fig. 2.5). Populations of Capsella showed a significant departure from normality at all harvests (except H0). At H1 the populations of Poa showed a weak though significant tendency to depart from normality ( $P < 0.01$ ), ( $g_1 = 0.58$ ) which perhaps reflects an alteration of the population structure by slug damage rather than by density or sampling bias. At H3 and H5 departures from normality remained significant ( $P < 0.01$ ).

Trends of the statistic  $g_1$  (relative skewness) with time (Fig. 2.6) show a general tendency for initial increase and later approach to an asymptote although the populations of Capsella show a rather low value of  $g_1$  at H5.

#### Effects of slug grazing on reproduction and tillering

Observations on the number of tillers in Poa were made at harvests 4 and 5 (Table 2.2). In the grazed plots, the number of tillers increased both per plant and per plot, at both harvests, though the effects failed to reach significance at  $P = 0.05$ . Some plants of Capsella had started to flower at H3 (Table 2.3), but no plants of Poa flowered until the end of the study (H5). Flowering in Capsella was slightly delayed by grazing, but the effects on fruiting were more marked (Table 2.3). At H5, 59% of the control plants bore developing

Table 2.2. The number of tillers produced by grazed and ungrazed populations of Poa annua; mean values (range).

	Grazed		Ungrazed	
	Total no. per plot	Mean per plant	Total no. per plot	Mean per plant
H4	805.5 (866-745)	2.7 (2.8-2.6)	687 (694-680)	2.4 (2.4-2.3)
H5	706 (741-671)	3.6 (3.6-3.5)	562.5 (568-557)	3.5 (3.7-3.2)

Table 2.3. Flowering and fruiting in grazed and control populations of Capsella bursa-pastoris and Poa annua;

values given are for the average number of plants flowering or fruiting and the same values expressed as a percentage of the number of surviving plants at each harvest.

	Grazed populations						Control (ungrazed) populations					
	(a)		(b)		(c)		(a)		(b)		(c)	
	Flowering N (range)	%	N (range)	%	Total N	%	Flowering N (range)	%	Fruiting N (range)	%	Total N	%
H3 Capsella	0.5(1-0)	0.3	0	0	0.5	0.3	7(8-6)	4.8	0	0	7	4.8
H4 Capsella	6.5(9-4)	3.8	8(10-6)	4.8	14.5	8.6	15(16-14)	11.3	20 (22-18)	15.0	35	26.3
H5 Capsella	18.0(24-12)	12.4	30(31-29)	20.7	48.0	33.0	15(20-10)	12.8	54.5(67-42)	46.2	69.5	59.0
Poa	2.0(3-1)	1.0	0	0	2.0	1.0	11(13-9)	7.1	0	0	11.0	7.1

capsules compared with 21% of the grazed plants. Few plants of Poa had flowered at H5, but grazing appeared to delay flowering in this species also.

## DISCUSSION

In laboratory tests in which leaf discs of various plant species had been offered in Petri dishes to the slug Agriolimax caruanae there was a strong dietary preference for Capsella and Poa was eaten only after a "starving threshold" of 66 hrs. In the present study in which free ranging slugs were offered actively growing populations of Capsella or Poa, Capsella was eaten readily and there was a comparable "starving threshold" before the animals would eat Poa. The comparability of slug behaviour in the two situations is important as it implies that the results of leaf disc experiments have validity beyond the Petri dish in which they were made. However, the effects of slugs grazing on the two plant species were quite different. They ate whole, or parts of, leaves of Capsella, but rarely, if ever, killed the plants. In the case of Poa, they chewed through the young shoots at a ground level, "felling" but not consuming the leaves or shoots. Some damaged plants died, some showed slight regeneration but died later, and a few of them remained alive but stunted. A similar sort of damage was caused by the slug Agriolimax reticulatus grazing on Lolium perenne (Hatto and Harper, 1969). The effect of slugs was therefore generally, in the case of Capsella, to change the size of plants but not their density and in the case of Poa, to change the density of plants rather than their size, at least in the early phases of the study. Despite the different effect that slugs had on the two food plants, populations of both species responded to plant density by reasonable conformity to the  $-3/2$  power law. In control populations of both Capsella and Poa, self-thinning occurred within the populations - there was both density-dependent mortality and density-dependent plasticity. Grazing by slugs substituted for death or plastic change



that would have been produced by density alone (Fig. 2.2). It appears that slug damage simply removed or reduced the size of individuals that would have been affected by the more intense interference between individuals in the absence of grazing. The substitution of one cause of plant death or plastic change by another in this study has much relevance to interpreting field experiments in which the activity of herbivores is measured by using exclosures (see for example, Harper, 1977, p.433). Changes that occur in exclosure plots will represent not only the results of preventing grazing but also the consequent increased level of interference that occurs between the ungrazed plants. This will almost inevitably mean that exclosure experiments underestimate the direct effects of herbivores on both biomass and species composition.

The wide generality of the  $-3/2$  power law has been demonstrated for a wide variety of plant species and monospecific populations. It has also been shown to hold true for a mixture of two species in experiments by Bazzaz and Harper (1976). The present study extends this generalisation to include monospecific plant populations in the presence of predators. An obvious next stage in development of this type of study would involve introducing predators to naturally thinning mixed populations of two or more plant species.

The effects of a predator on plant survivorship and dry weight represent only elements in its effects on plant fitness which must take account of whole life histories. The present study was not specifically designed to look at this effect but differences in the reproductive behaviour of grazed and ungrazed populations emerged quite clearly in the delayed reproduction of grazed plants of Capsella (Table 2.3). A similar delay in the onset of reproduction consequent on defoliation has been observed by Collins and Aitken (1970) in Trifolium subterraneum

where defoliation delayed flowering by as much as 30 days.

Most notably, the experiments show that slugs attack different species of plant in quite different ways and with quite different consequences. If we are to ascribe changes in vegetation to the activity of herbivores it is insufficient to measure simply the amount of plant material taken or its relative acceptability compared to other available species. The precise form that the damage takes may override these other considerations in determining how much harm is done to the plants.

Further studies are needed to examine the response of plant populations at different density to the presence of a herbivore where both search range and the level of interference between neighbouring plants is varied. Studies are also needed of the behaviour of herbivores such as slugs in mixed plant populations where the interspersions of acceptable and unacceptable plants may modify the amount of damage that is done and in which conditioning or habituation of slugs to available food plants may perhaps influence decisions about what is eaten and what is not.

CHAPTER 3

THE EFFECT OF FREE-RANGING SLUGS  
ON POPULATIONS OF CAPSELLA BURSA-  
PASTORIS GROWN IN DIVERSE  
PLANT COMMUNITIES

## INTRODUCTION

Perhaps the most fundamental way of defining the ecology of plant species is by demographic analysis directed to observe and measure the numerical changes occurring among individuals within populations, focussing attention on the life-span and reproductive capacity of individuals as well as on the time and causes of individual death and differential reproduction. This area of plant ecology has remained largely 'dormant' and it is not until relatively recently that some notable 'germination flushes' have occurred (cf. Harper, 1967; Sarukhán and Harper, 1973; Harper and White, 1974; Sarukhán, 1974). However, very little information (either descriptive or experimental) exists on the role that biotic agents (e.g. herbivores) play in shaping the life-tables of plant populations, despite the fact that agronomic literature provides plenty of examples of the potentially catastrophic effect of herbivores on plants of economic interest and despite some very spectacular instances of biological control of weeds (see De Bach, 1964; Huffaker and Messenger, 1976).

A crucial component of the ecological stage on which the drama of plant/animal interaction is played, is the biological matrix of plant neighbours of the same or of other species occurring at different relative abundances - the diversity of the plant community. Neighbours will usually make demands on the same, or similar resources as the plant under study. Any exploration of the role of herbivores on the demography of a population of plants has to be seen in the context of the interactions of neighbouring plants that may or may not share the same herbivore.

The aim of this study is to examine the demographic response of a plant species, Capsella bursa-pastoris, grown in populations of different densities in the presence of free-ranging slugs. The study attempts also to incorporate the

effects of a background of acceptable and unacceptable plants in semi-natural communities of weeds typical of areas where the slugs Agriolimax caruanae and A. reticulatus are very abundant and constitute the major component of the surface-feeding mollusc fauna.

## MATERIALS AND METHODS

### The plant communities and the species

The cultivated areas around the Pen-y-Ffridd Field Station, Bangor, have been closely observed for over two years, especially during the periods of spring-summer when there is a considerable recruitment of seedlings of the weeds and ruderals typical of disturbed areas, and when the number of active molluscs is also high. A number of nocturnal observations were made during the springs and early summers of 1977 and 1978 to define whether this ecosystem of waste and cultivated land and its associated molluscan fauna could be suitable for study.

A number of species of plant were observed to be heavily grazed by slugs and the number of active slugs on some nights appeared to be high enough severely to affect the growth of plants, especially seedlings. By far, the most abundant species of molluscs were the slugs Agriolimax caruanae and A. reticulatus; the mean  $\pm$  SE number of active animals per 30 x 30 cm quadrats was  $3.25 \pm 0.79$  (N = 20). The two species were present at about the same densities (A. caruanae =  $1.55 \pm 0.47$ ; A. reticulatus =  $1.70 \pm 0.45$  per quadrat). Capsella bursa-pastoris was one of the most abundant plant species present and was also one of the most commonly grazed at almost all stages of its life cycle by both species of slug. It was decided to use this trio for the present work. Capsella is an extremely variable species and two leaf-shape morphs were included in the study to test the hypothesis that slugs might discriminate between leaves with entire and divided leaf margins (see Dirzo, 1977).

### Collection of slugs and seeds of *Capsella bursa-pastoris*

Mature slugs of both species were collected from the weeded areas around Pen-y-Ffridd Field Station, Bangor, as described in Dirzo (1980).

Seeds of both leaf shape morphs of *Capsella* were provided by K. Garbutt and were collected from progenies of 2 genotypes: (i) an inbred line designated as P-G 61 (entire leaf margin) and (ii) an F<sub>2</sub> generation designated as P-G 39 (dissected leaf margin). Some aspects of the genetics of a population of *Capsella bursa-pastoris* including these 2 genotypes are given in Garbutt (1980).

### The experiment

Populations of the native weed flora were grown for the experiment by using the natural buried seed bank as the source of seed. In late spring (May 26 and 27, 1979) soil was sampled from all the fields of the Station by collecting a superficial layer of 0-10 cm depth. The samples of all fields were bulked and air dried in the laboratory for 2 days. The soils were then thoroughly mixed and sifted and the mixture was separated into the 2 fractions which passed or were retained by a 7 mm sieve. The 2 soil fractions were stored in a cold room (4°C) and then used to fill plastic trays (35.5 x 21.5 x 6 cm). Three cm of John Innes sterile compost No.1 was placed in each tray followed by a layer of 2 cm of the coarse fraction of the mixed soil sample. A 1 cm layer of the finely sifted fraction was then added. Seed of *Capsella* was sown on the soil surface. It is extremely difficult to obtain random distribution of seeds by scattering seed and so the following procedure was adopted. The area of each tray was divided into a grid of 1 x 1 cm squares; then 2 random numbers were called to give the co-ordinates of each sowing point. These positions were marked with a small triangular peg

(3 x 8 mm) of white plastic inserted into the soil so that it was barely visible; each peg was numbered to identify each of the 750 plants of Capsella observed in this study. A reference map was also drawn for each of the experimental trays. Two densities of Capsella were sown: 10 and 50 seeds per tray. A final layer of finely sifted John Innes compost was then applied to cover the sown seed. Seedlings of Capsella emerging from the natural seed bank were removed to avoid confusion with those that were deliberately sown and to ensure that each tray contained an even-aged population of Capsella of the desired genotype and density.

A cool glasshouse was especially prepared for this experiment. The roof and upper sides of the glasshouse were lightly covered with white paint to prevent excessive temperatures during the day; there were 2 benches in the house; thick matting was placed on the benches and soaked to give a continuously damp surface under the plastic trays. A colony of 36 slugs (1:1 mixture of the 2 species) was established on one of the benches. Slug-shelters were provided by placing rectangular pieces of hardboard in between the trays. The legs of the benches stood in plastic containers filled with water to prevent escape of, and/or invasion by slugs. The trays of seedlings were distributed at random on each bench and re-randomised twice a week. The experimental design consisted therefore of 2 grazing treatments: grazed and ungrazed x 2 densities of Capsella (50 and 10 plants per tray) x 2 leaf morphs of Capsella (entire (E) and dissected (D)) x 3 replicates (= 24 plots and 720 plants of Capsella).

Every plant in the population was examined each week (July 31, August 8, 13-14, September 1, 1979) to record presence or absence, intensity of grazing, rosette diameter, and phenological states (vegetative or reproductive). The number of flowers and/or capsules was counted on reproductive plants. By



September 1 most of the plants had become reproductive and many had started to senesce; the plants were then cut at soil level and oven-dried for dry weight analysis.

An additional set of 10 trays in which no Capsella seed was sown was prepared in the same way as, and randomly arranged among, the experimental plots. A floristic analysis was made of the seedlings present after 2 weeks.

## RESULTS

### Floristic analysis of the plots

Table 3.1 summarises the floristics of the weedy communities that developed in the trays. Within a very short period (1-1.5 weeks) the seed bank produced species-rich (ca 14 spp.) communities of seedlings\* (average of 89.4 seedlings per tray =  $1171 \text{ m}^{-2}$ ). Five taxa were strongly represented, (Ranunculus spp., Grasses, Capsella bursa-pastoris, Epilobium sp. and Stellaria media) and accounted for ca 71% of the total number of seedlings present. These 5 taxa and Matricaria sp. occurred in every tray; other species (e.g. Plantago lanceolata) appeared only occasionally, while other species like Senecio vulgaris appeared in nearly every tray but in very low numbers. The 5 most abundant and frequent taxa could be arranged in the following relative order of acceptability to slugs: Capsella > Stellaria > Ranunculus > Epilobium = Grasses based on acceptability trials in which these species had been offered to the slug A. caruanae (Dirzo, 1980). (Grasses are, in general, unacceptable to slugs but Holcus lanatus (one of the 4 most common grasses in these trays) has been reported to be a major component of the diet of A. reticulatus (Pallant, 1972)).

The densities of Capsella deliberately sown in this experiment were (10 sown Capsella) at, or slightly below, the mean density (12.2) of Capsella emerging when it had not been sown and, (50 sown Capsella) ca 4 times the mean density of the seedlings of Capsella that emerged 'naturally' in the trays.

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\* N.B. The different species of grasses could not be distinguished at this stage and are therefore grouped together. Also, Ranunculus spp. may have included more than 1 sp. of buttercups.

Table 3.1. Floristic composition of the communities developed from the seed bank of soils collected from waste and cultivated land at Pen-y-Ffridd Field Station, Bangor, N. Wales. (Data per tray of ca 763.25 cm<sup>2</sup>).

Species	Number of plants per tray*		$\bar{X} \pm$ S.E.
	Maximum	Minimum	
<u>Ranunculus</u> spp.	28	8	17.90 $\pm$ 1.96
Grasses ( <u>Agrostis stolonifera</u> , <u>Poa annua</u> , <u>Lolium perenne</u> , <u>Holcus lanatus</u> )	21	9	14.10 $\pm$ 1.33
<u>Capsella bursa-pastoris</u>	20	6	12.20 $\pm$ 1.30
<u>Epilobium</u> sp.	15	7	10.30 $\pm$ 0.90
<u>Stellaria media</u>	18	2	9.10 $\pm$ 1.31
<u>Matricaria</u> sp.	10	3	6.70 $\pm$ 0.68
<u>Rumex</u> sp.	6	0	3.60 $\pm$ 0.64
<u>Chenopodium album</u>	6	0	2.70 $\pm$ 0.54
<u>Veronica persica</u>	5	0	2.30 $\pm$ 0.63
<u>Lamium purpureum</u>	5	0	2.00 $\pm$ 0.60
<u>Polygonum aviculare</u>	7	0	1.80 $\pm$ 0.65
<u>Urtica dioica</u>	4	0	1.40 $\pm$ 0.43
<u>Cyperaceae</u> (unidentified)	3	0	1.30 $\pm$ 0.42
<u>Senecio vulgaris</u>	3	0	1.30 $\pm$ 0.40
<u>Trifolium repens</u>	2	0	1.00 $\pm$ 0.26
<u>Fumaria</u> sp.	4	0	0.80 $\pm$ 0.42
<u>Oxalis</u>	1	0	0.40 $\pm$ 0.32
<u>Cardamine</u> sp.	2	0	0.40 $\pm$ 0.22
<u>Plantago lanceolata</u>	2	0	0.30 $\pm$ 0.21
No. plants per tray (of all species)	107	66	89.40 $\pm$ 3.89
No. species per tray	18	10	13.90 $\pm$ 0.92

\* 10 trays analysed.

The number of grazed plants of all the species

The number of grazed plants of all the species was determined at the end of the study, i.e. 5 weeks after the emergence of Capsella. Table 3.2 lists the species in decreasing order of abundance (cf. Table 3.1); it also shows the mean number of grazed but still living plants of each species per plot. This procedure underestimates slug damage since, at least in Capsella, some individuals had been grazed and killed by slugs before the end of the study. The various species were ranked according to the number of plants that had been grazed (Table 3.2). At both sown densities, Capsella was clearly the most grazed species, although at low density it ranked only 3 in terms of abundance; when sown at high density, when it was the most abundant species present, the preference was even more marked. It appears that the intensity of grazing suffered by many of the other species (particularly the four most abundant) was reduced by increased density of Capsella; the sum of damage to all species (excluding Capsella) at the bottom of Table 10 reflects this point clearly.

The performance of the populations of Capsella bursa-pastoris

A summary of the behaviour of the populations of Capsella towards the end of the study is shown in Table 3.3. For the eight variables measured, comparisons were made between the leaf shape morphs and the grazed and control populations within each density treatment. None of the comparisons between leaf shape morphs were significant but the effects of grazing were very clear in low density populations. In dense populations of Capsella the effects of grazing were much less obvious and with the exception of two comparisons (no. of plants flowering at week 4 and no. of plants fruiting at week 5), differences

**Table 3.2.** The number of grazed plants of all species per tray 5 weeks after emergence of Capsella bursa-pastoris. Data are given both for plots in which Capsella was sown at low and high density (see text). Species are listed in decreasing order of abundance.

Species	Low density of <u>Capsella</u>		High density of <u>Capsella</u>	
	No. grazed	Rank of grazing	No. grazed	Rank of grazing
<u>Ranunculus</u> spp.	2.17 ± 0.60	4=	1.17 ± 0.40	5
Grasses	4.0 ± 0.82	3	3.50 ± 0.81	3
<u>Capsella bursa-pastoris</u>	7.5 ± 0.85	1	44.67 ± 1.65	1
<u>Epilobium</u> sp.	4.33 ± 0.71	2	2.17 ± 0.75	2
<u>Stellaria media</u>	2.17 ± 0.31	4=	1.67 ± 0.49	4
<u>Matricaria</u> sp.	0.17 ± 0.17	10=	0.50 ± 0.34	7=
<u>Rumex</u> sp.	0.17 ± 0.17	10=	0.50 ± 0.34	7=
<u>Chenopodium album</u>	0.33 ± 0.21	9=	0.83 ± 0.40	6=
<u>Veronica persica</u>	1.33 ± 0.33	5	0.17 ± 0.17	9=
<u>Lamium purpureum</u>	0.67 ± 0.33	7	0.83 ± 0.31	6=
<u>Polygonum aviculare</u>	0.50 ± 0.22	8=	0.33 ± 0.21	8=
<u>Urtica dioica</u>	0.33 ± 0.21	9=	0.17 ± 0.17	9=
Cyperaceae	0.83 ± 0.40	6	0.83 ± 0.40	6=
<u>Senecio</u> sp.	0.33 ± 0.21	9=	0	10=
<u>Trifolium repens</u>	0.17 ± 0.17	10=	0.17 ± 0.17	9=
<u>Fumaria</u> sp.	0.33 ± 0.21	9=	0.33 ± 0.21	8=
<u>Oxalis</u>	0.50 ± 0.22	8=	0	10=
<u>Cardamine</u> sp.	0	11=	0	10=
<u>Plantago lanceolata</u>	0	11=	0	10=
Unidentified	0.17 ± 0.17	10=	0.17 ± 0.17	9=
Σ of all species	18.50 ± 2.29		13.50 ± 1.31	

Table 3.3. A summary of the performance of the populations of *Capsella bursa-pastoris* towards the end of the study (weeks 4 and 5 after seedling emergence). Means of 3 replicates (range).

Parameter measured	Low density populations		High density populations	
	Dissected	Entire	Dissected	Entire
1) No. surviving plants at week 5				
Control	10	9.67(10-9)	49.33(50-48)	47.67(49-45)
Grazed	8.33(10-6)	6.67(9-5)	45.67(49-39)	45.67(47-44)
2) No. of grazed plants at week 5				
Control	-	-	-	-
Grazed	8.33(10-6)	6.67(9-5)	45.67(49-39)	43.67(46-42)
3) Mean rosette diameter at week 4				
Control	17.40(19.20-15.95)	13.63(15.20-11.60)	10.87(11.42-10.34)	9.96(10.85-7.55)
Grazed	11.37(14.05-6.33)	6.77(8.67-4.92)	10.91(12.06-9.18)	8.86(11.48-5.90)
4) No. of plants flowering at week 4				
Control	** 9.33(10-9)	8.67(10-6)	38.33(44-26)	41.67(44-38)
Grazed	3.33(4-2)	2.00(3-1)	22.33(27-17)	24.67(33-15)
5) No. of plants fruiting at week 4				
Control	* 8.00(10-6)	8.33(10-6)	33.33(46-21)	40.00(44-38)
Grazed	3.33(4-2)	2.00(3-1)	19.67(26-14)	27.00(33-15)
6) No. of plants fruiting at week 5				
Control	** 9.67(10-9)	9.67(10-9)	44.67(50-37)	42.67(46-41)
Grazed	4.67(6-3)	3.33(4-3)	28.33(33-20)	33.67(39-24)
7) No. of capsules per plant at week 5				
Control	101.35(188-110)	80.83(96-53)	51.27(68-34)	58.82(73-43)
Grazed	50.76(88-26)	23.61(37-14)	49.58(62-31)	41.60(78-23)
8) Final dry weight per plant (g)				
Control	0.807(1.111-0.633)	0.495(0.655-0.339)	0.279(0.332-0.235)	0.290(0.368-0.225)
Grazed	0.313(0.531-0.112)	0.084(0.099-0.066)	0.256(0.266-0.150)	0.198(0.348-0.188)

\* P < 0.05; \*\* P < 0.01

were statistically significant in only the low density populations. Clearly the damage done by slugs to Capsella (and the other species) is diluted when this favoured food plant is abundant.

Since there were no significant differences between leaf shape morphs, their values are pooled and the subsequent description of the effects of grazing is based on this increased sample size.

### Plant survival

Observations of plant survival in the present study underestimate early mortality since the experimental design limited the observations to a restricted part of the life-cycle of the plants. Figures 3.1 and 3.2 show the number of surviving individuals through the period of study at both densities of Capsella. There was very little mortality of plants during the experiment though the grazed populations suffered more than the ungrazed controls and there were some deaths in high density controls but more at low density (cf. Figs. 3.1 and 3.2). Plants of Capsella die soon after reproduction and with the exception of some plants that remained vegetative, most individuals were showing signs of senescence by week 5. All plants would probably have been dead soon after week 5.

### Defoliation by slugs

An estimate of the extent of grazing on the populations of Capsella is given by the number of plants that had been damaged (Figs. 3.1 and 3.2). At both densities there was a rapid increase in the number of grazed plants; at low density, after week 1, almost every plant had been grazed to some degree and indeed the number of grazed plants quickly became the same as the number of

Fig. 3.1.

The number of surviving and grazed plants of Capsella bursa-pastoris at low density in grazed and control populations. Means of 6 replicates  $\pm$  S.E.  $\blacktriangle$ — $\blacktriangle$ , surviving plants in ungrazed populations;  $\bullet$ — $\bullet$ , surviving plants in grazed populations;  $\blacksquare$ — $\blacksquare$ , grazed plants.



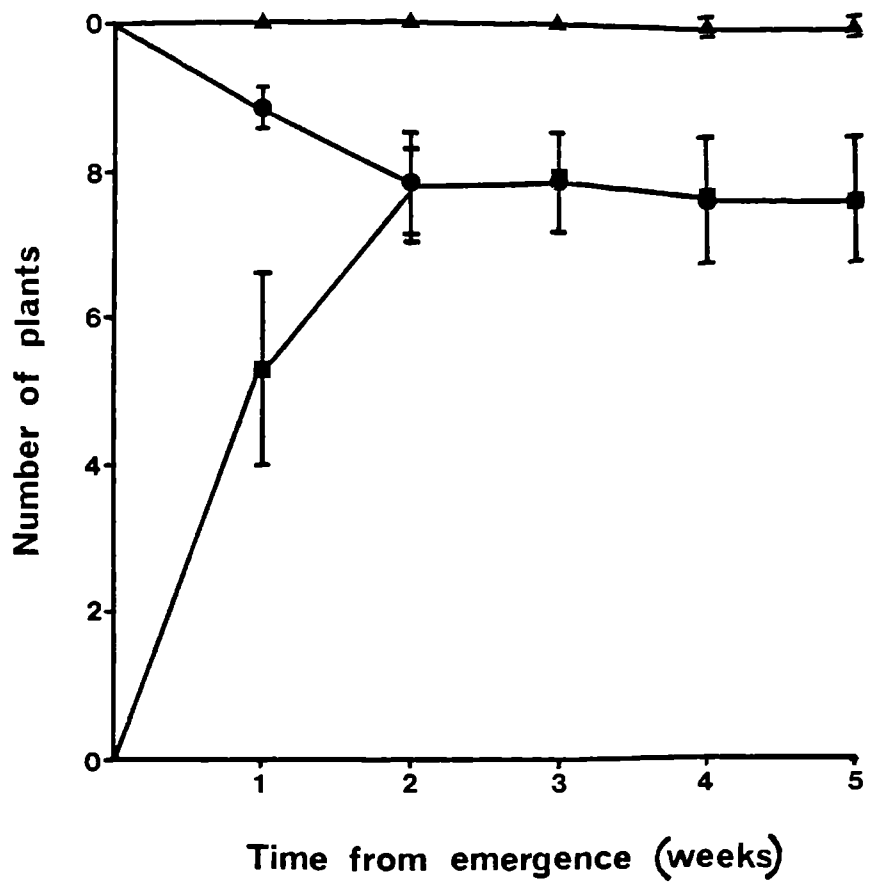
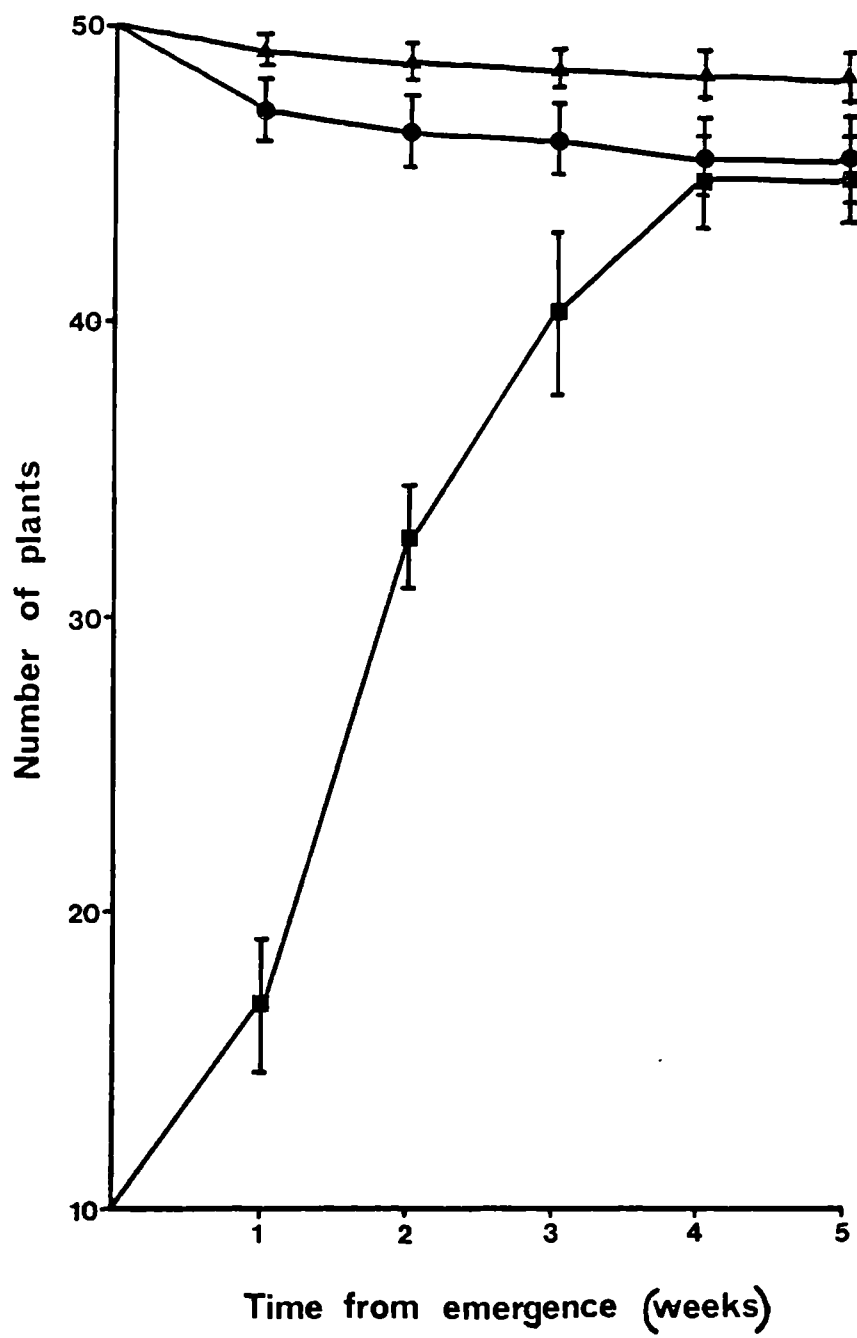


Fig. 3.2.

The number of surviving and grazed plants of Capsella bursa-pastoris at high density in grazed and control populations. Means of 6 replicates  $\pm$  S.E.  $\blacktriangle$ — $\blacktriangle$ , surviving plants in ungrazed populations;  $\bullet$ — $\bullet$ , surviving plants in grazed populations;  $\blacksquare$ — $\blacksquare$ , grazed plants.



surviving plants (Fig. 3.1). In the high density populations, the number of damaged plants continued to increase until after week 3, and by week 4 almost every surviving plant had been damaged (Fig. 3.2).

When plants are heavily grazed by slugs this is reflected in the diameters of the individual rosettes. Figure 3.3 shows the change in rosette diameters with time. Data are given only to week 4 after which rosette diameters declined rapidly during flowering. In the low density populations, grazing considerably reduced rosette diameter; up to week 3, the average rosette diameter of ungrazed plants was ca twice that of the grazed plants; the effect was much less marked at week 4 due to the decline in rosette size of the actively reproductive plants in the control plots and was not significant at high density.

### The effects of grazing on the reproduction of Capsella

#### (i) The frequency distribution of rosette diameter and reproduction

Mean values for plant performance are often misleading, particularly in populations at high density where frequency distributions are often severely skewed. The frequency distribution of rosette diameters in the grazed populations is shown in Fig. 3.4; data from all replicates were included to give a large sample size. The range of sizes is extremely large - from ca 1.0 to 33.0 cm - and with a tendency for positive skewness. Reproductive and non-reproductive plants are distinguished in the Figure. There is a clear tendency for non-reproductive individuals to be over-represented in the smaller diameter classes, while plants in the largest rosette diameter categories are mainly reproductive. Since, in this histogram, the actual values (rather than percentages) were used, the representation of reproductive and non-reproductive plants in each diameter

Fig. 3.3.

The mean rosette diameter (cm) per plant in populations of low (a) and high (b) density. Means of 6 replicates  $\pm$  S.E.  $\blacktriangle$ — $\blacktriangle$ , ungrazed;  $\bullet$ — $\bullet$ , grazed.

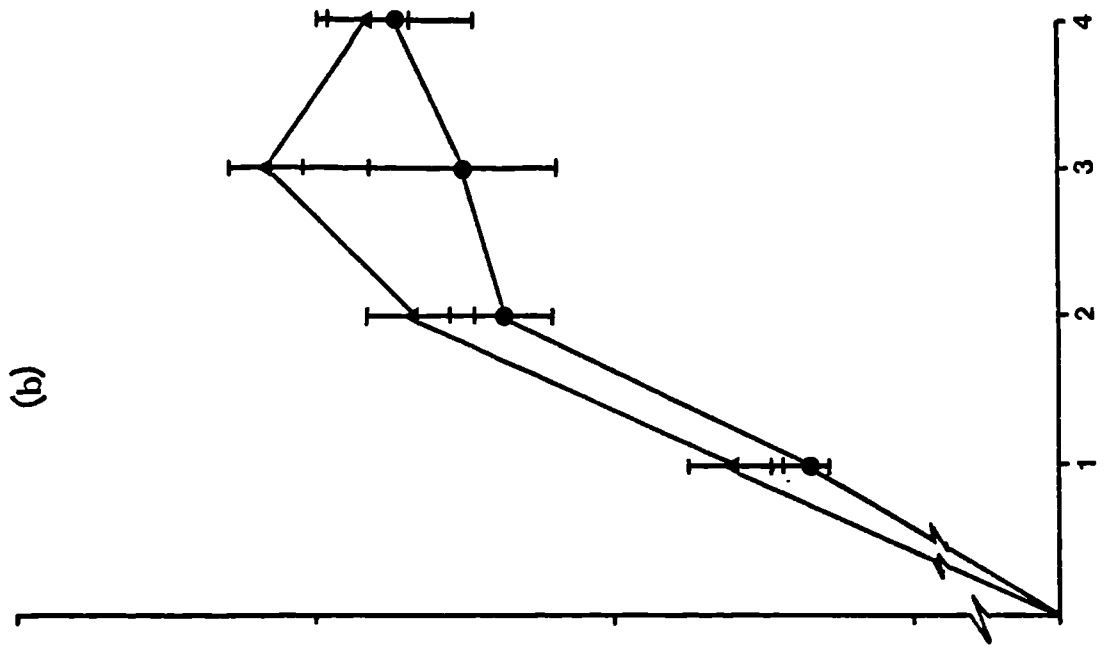
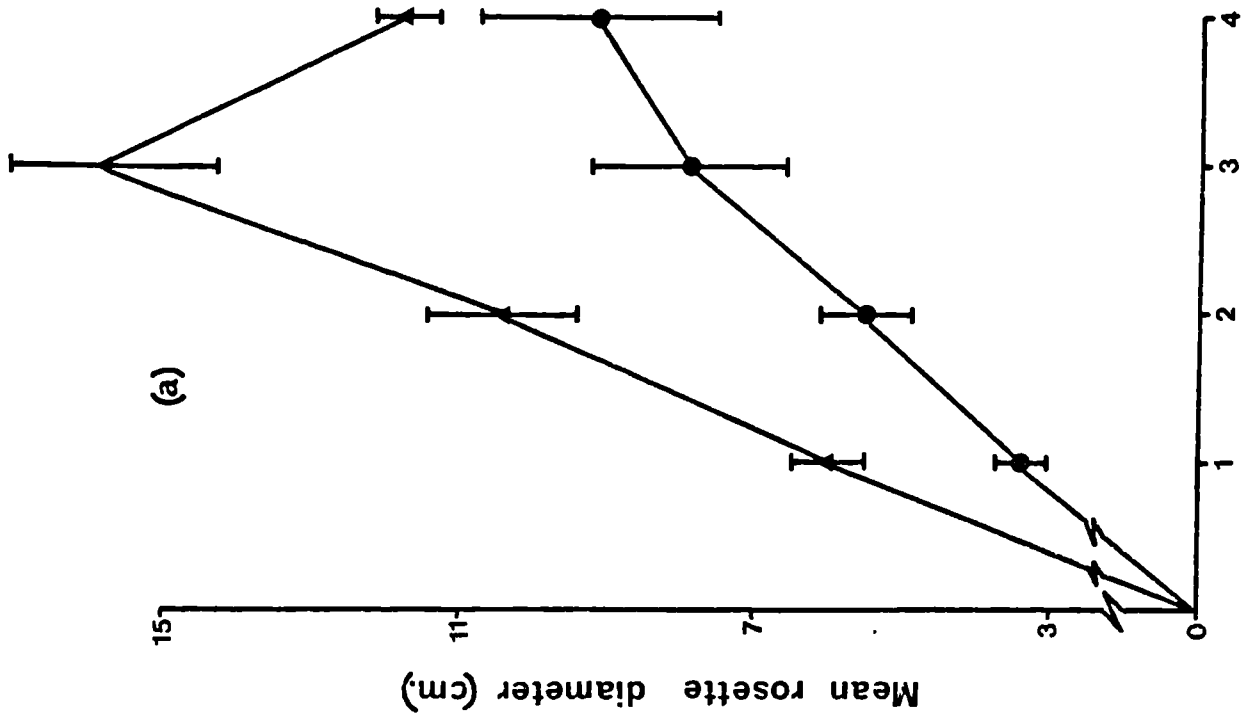


Fig. 3.4.

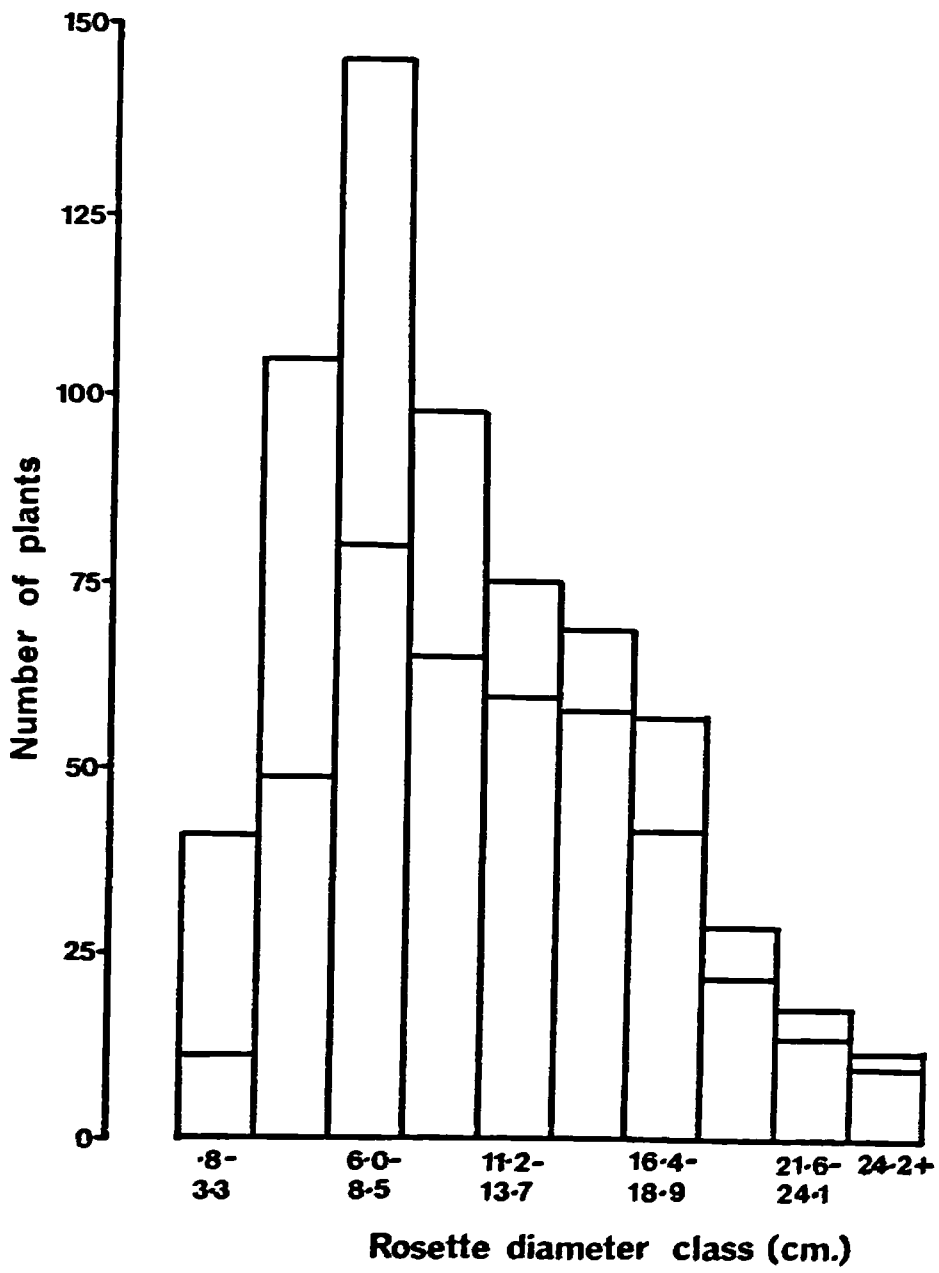
The frequency distribution of rosette diameters of plants of Capsetla bursa-pastoris in the grazed plots at week 4. Bottom bars show the frequencies of reproductive individuals and upper bars those of non-reproductive plants.

Sample size = 649

Reproductive = 411

Non-reproductive = 238

$\chi^2 = 66.52; P < 0.01$





category can be legitimately compared with a  $X^2$  test; the applied contingency test gave a highly significant  $X^2$  ( $P < 0.001$ ) - grazing by slugs reduced plant size and therefore the likelihood of flowering.

(ii) Defloration by slugs

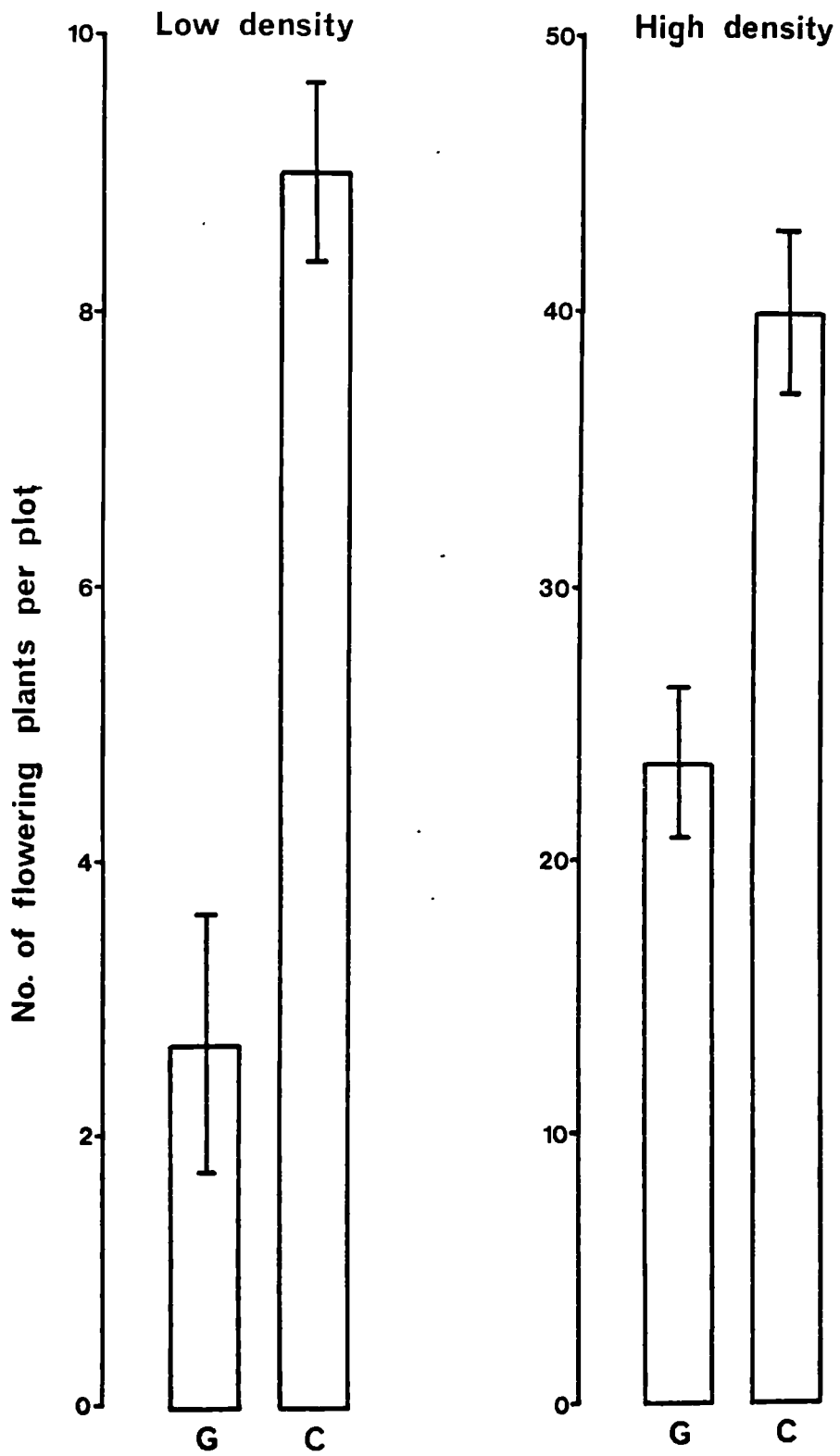
Slugs grazed some flowers as well as leaves of Capsella in weeks 3-5; defloration occurred in the following ways: (i) slugs climbed the inflorescence axis and ate flowers in situ; (ii) most commonly, slugs felled the whole inflorescence by chewing through the main axis, and (iii) in the case of plants with branched inflorescences slugs occasionally felled only some of the branches. At the onset of reproduction (week 3) only a few (i.e. 10) plants had suffered defloration (3 and 7 plants in the low and high density plots respectively). At the peak of flowering (week 4) the total number of deflorated plants increased to 29 (4 and 25 plants of the low and high density plots respectively); this number, although relatively low, constituted 18.5% of the total of plants flowering at this date (i.e. 157). The partially deflorated plants (i.e. those whose flowers were eaten in situ and those in which slugs removed some branches only) set some seed but those whose inflorescence was wholly removed remained sterile at the end of the study.

(iii) The combined effects of defoliation and defloration on plant reproduction

Flowering starts very early in Capsella (some plants bore visible floral buds as early as week 2!) and the combined effects of defoliation and defloration may not only prevent flowering but they may also delay and/or reduce fecundity. Figure 3.5 shows the effect of grazing in reducing the number of plants flowering

Fig. 3.5.

The number of plants of Capsella bursa-pastoris flowering at week 4 after emergence in populations of low and high density. Means of 6 replicates  $\pm$  S.E. G, grazed; C, control.



in the peak flowering period (week 4). The effect was less marked at high density where the abundance of Capsella diluted the extent of damage.

Although some plants bore capsules at week 4, the peak of capsule production did not occur until week 5. The effects of defoliation and defloration on this second measure of reproductive activity (Fig. 3.6) were essentially the same as on flowering (cf. Fig. 3.5).

The effects of slug grazing may be measured on populations or on individuals. A measurement of the effects of slug grazing on plant fitness was made by comparing the mean number of capsules per plant at the end of the study (Fig. 3.7). Slugs dramatically reduced the number of capsules per plant in the plots of low density: grazed plants that bore capsules produced on average only 37% of the number of capsules produced by the ungrazed plants. The number of capsules per plant was not significantly reduced by slugs in the high density populations. Figure 3.7 also permits a comparison of the effects of density; while there was no significant effect of density in the plants of the grazed plots, in the controls the plants growing at low density produced on average more than twice the number of capsules produced by the plants growing at high density.

Fig. 3.6.

The number of plants of Capsella bursa-pastoris fruiting at week 5 after emergence in populations of low and high density. Means of 6 replicates  $\pm$  S.E. G, grazed; C, control.

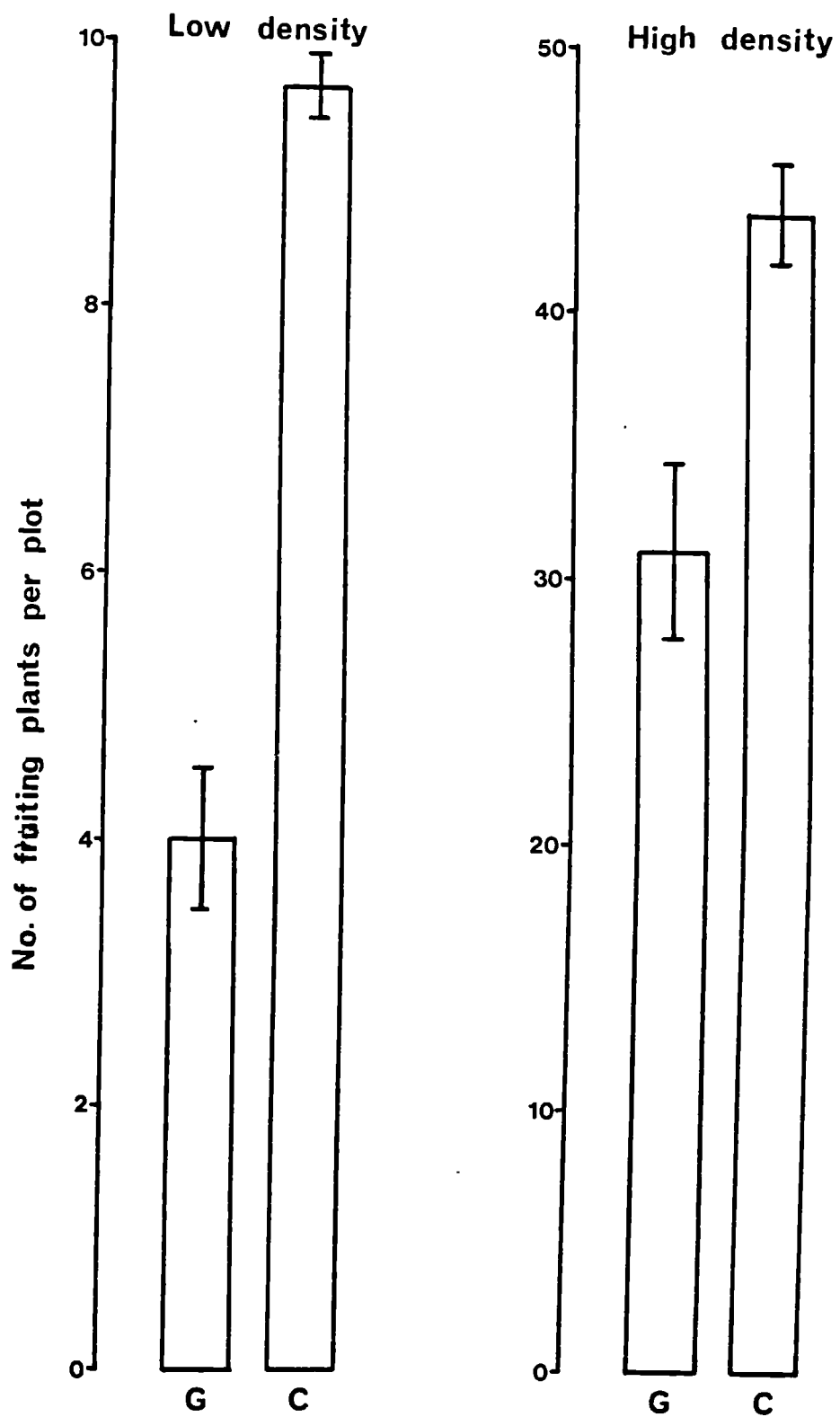
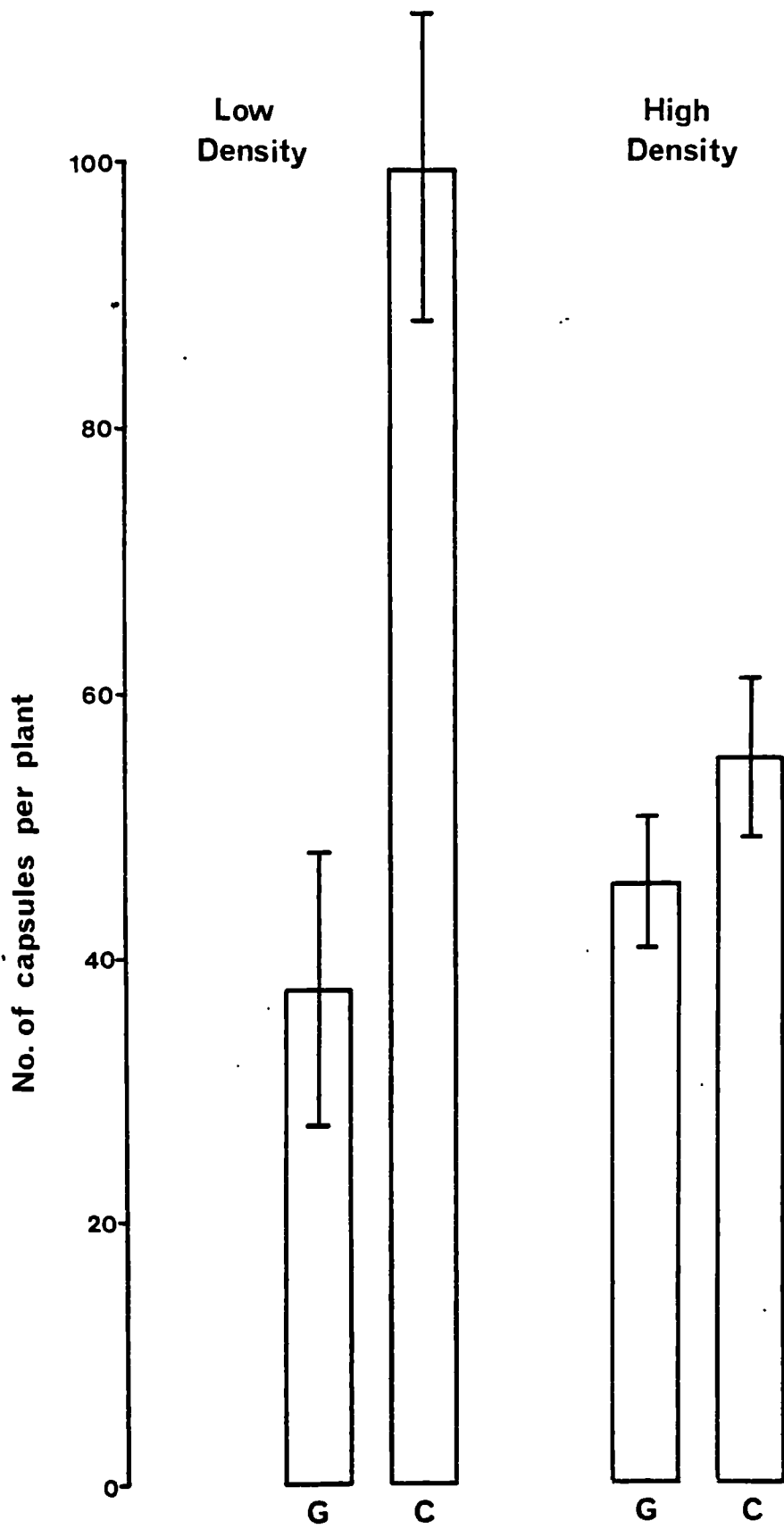


Fig. 3.7.

The number of capsules per plant of Capsella bursa-  
pastoris at week 5 after emergence in populations of  
low and high density. Means of 6 replicates  $\pm$  S.E.  
G, grazed; C, control.





## DISCUSSION

### Grazing and plant diversity

The diversity of plant communities is described in many ways but is usually expressed as the number of species present corrected to take account of the relative representation of each species. To a herbivore a relevant aspect of diversity of vegetation is determined by its feeding range - the diversity of plants encountered in a day, a year or a life-time of grazing. The diversity of a pasture that is relevant to a slug will be very different from that relevant to a sheep. The relevant grain of the environment is determined by the activity of the animal - its search and range. Within the sampled grain, diversity may be represented not only at the classical level of species diversity but also at the level of intraspecific variation. There may indeed be greater differences in acceptability between genetic variants of a species (or even of part of a plant) than between species.

In the semi-natural communities developed for this study there was a clear numerical dominance of (i) potentially unacceptable food plants (i.e. Ranunculus spp. and several species of grasses), (ii) moderately acceptable species (Stellaria media) and (iii) Capsella bursa-pastoris, probably the most acceptable of all plants present in these communities (see Table 3.1). The experimental design of this study (with two contrasting densities of Capsella) gave two contrasting situations: (i) at low density of Capsella (i.e. 10 plants per plot) the herbivore faces a community in which there is a majority of unacceptable plants; (ii) at high densities of Capsella (50 plants per plot), the most acceptable food plant is also the most common. At the end of the study the order of acceptability expected from AI values obtained in the laboratory (Capsella >

Stellaria > buttercups + grasses) was partly confirmed in the overwhelming preference of slugs for Capsella (see Table 3.2) whether it was the majority component, or ranked only 3rd in order of abundance of species present. When Capsella was the majority species in the community other taxa suffered less damage from slugs. It follows that any attempt to evaluate the grazing activities of an animal on a given plant species must take the rest of the vegetation into account. This was hinted as early as 1933 in Jones' classic experiments on sheep grazing (Jones, 1933 a,b,c,d,e). In a mixed plant community, the damage suffered by one species from a predator depends not only on its abundance and its acceptability but also on the acceptability and abundance of its associates.

Besides the interspecific diversity of plant communities, diversity can also be manifested at the intraspecific level; all plant populations are likely to be diverse in one way or another; (i) somatic diversity (e.g. presence of individuals or plant parts of different ages, sizes, phenologies, etc.) and (iii) genetic diversity (e.g. presence or absence of protective substances like cyanogenic glucosides or the foliar white marks of Trifolium repens (Cahn and Harper, 1976). Any attempt to look at the grazing activities of a herbivore on a given plant species will have to take into account whether there are 'herbivore-relevant' differences between the different individuals or parts of such a species. The present study attempted to explore the effects of slug grazing on Capsella, growing in its typical weedy communities using two leaf shape morphs of Capsella (entire and dissected leaf margins). There is anecdotal evidence that the slug Agriolimax caruanae prefers to graze entire leaved plants (Dirzo, 1977), but the present study did not support this contention. Although some parameters (e.g. rosette diameter) showed a tendency for entire leaved morphs to be more affected by slugs,

none of the comparisons reached statistical significance at  $P = 0.05$  (see Table 3.3).

Where there was an over-representation of Capsella in the weedy populations, the effects of grazing were diluted (see Figs. 3.3 and 3.5-3.7). The most likely explanation is that when acceptable plants exceed what the slugs will eat, less tissue is removed per plant. The most extreme example of the dilution effect is seen in the mean rosette diameter which was significantly reduced only in the plots of low density (Fig. 3.3). However, the leaves of the closely packed rosettes were more erect than those at low densities and measurements of rosette diameters made on more or less erect rosettes cannot be made with much precision.

#### The effects of grazing on some demographic parameters of Capsella bursa-pastoris

Two demographic parameters are the essential determinants of the fitness of an organism - differential survival and differential reproduction - and the grazing herbivore can influence either, or both. Slugs can clearly determine whether a plant lives or dies, particularly by grazing seedlings; previous sections of this thesis have explored this aspect of the slug-plant interface. The idea that there is some 'critical size' at which young plants 'escape' lethal damage was introduced in Chapter I (see page 17); in the present study, slugs were introduced after most seedlings had apparently reached this critical size of escape - very little slug-induced mortality occurred. Grazing did, however, have a great effect on the reproduction of Capsella (especially in the low density plots). The probability of a plant becoming reproductive at a given date was found to be positively correlated with rosette-size (Fig. 3.4) and since grazing reduced the rosette diameter (Fig. 3.3) slugs reduced the probability of reproduction; evidence of the existence of a critical

rosette size for flowering was obtained from the teasel, Dipsacus fullonum (Werner, 1975); in this species it appears that the probability that a plant will flower in its second or subsequent years is very low for plants with a rosette diameter of less than 20 cm. Also, the risk of death was very low in the plants with large rosette diameters.

Some evidence arose of a delay in the reproductive activities of plants in the grazed populations. Slug grazing notably reduced both the number of flowering and fruiting plants at the peaks of flowering and capsule production (Figs. 3.5 and 3.6). A delay in the onset of reproduction of up to 30 days has been observed by Collins and Aitken (1970) after defoliation of subterranean clover (Trifolium subterraneum).

This experiment showed that slugs dramatically reduced the mean number of capsules produced per (capsule-bearing) plant in the low density populations (although the effect was diluted in the high density plots) (Fig. 3.7). These data give a crude indication of the effect of slug grazing on the seed yield of plants. Clearly this is the type of information that is required to evaluate the effect of predators on the population biology of plants; outside the agronomic literature (see Jameson, 1963 and refs.) there is little information available from the ecological literature on the effect of defoliation on the reproductive potential of plants (see however, Maun and Cavers (1971), Rockwood (1973)).

A remarkable effect of slug grazing on the reproductive potential of plants detected in this study was that of plant defloration. This special type of grazing damaged not only tissue of the sporophytic (petals, stalks, etc.) and gametophytic (pollen and ovules) phases of a plant growth but also the next sporophytic progeny (fertilised ovules)!. The extent of the demographic effects of

this kind of damage depends on the degree of defloration; the effect will be maximal for those plants from which slugs remove the whole inflorescence, particularly if there is no regeneration. In the present study, the plants of Capsella from which whole inflorescences were removed, remained sterile.

Just as there is a measurable acceptability of leaves (e.g. Dirzo, 1980), there is probably an acceptability of flowers (or petals) with a host of associated consequences. In a survey of the nocturnal activity of slugs in the lawns of the Treborth Botanic Gardens, Bangor, (Dirzo, unpublished), the slug Agriolimax reticulatus was observed in large numbers eating the flower heads (both the periphery and the central florets) of the dandelion, Taraxacum officinale (a very abundant plant in these lawns). On nights of high slug activity, up to 3 slugs per flower head were observed. In contrast, slugs were only occasionally seen eating the flower heads of the daisy, Bellis perennis, another abundant species in these lawns. Acceptability tests of leaf discs with the slug Agriolimax caruanae revealed a high acceptability of the leaves of the dandelion and a total rejection of the leaves of daisy. The possible interactions between leaf and flower acceptability is an aspect that warrants much deeper study.

Each stage in the development of this work on the interaction slugs-plants reveals a new host of variables to be taken into consideration. Extension of this experimental work to field studies where the variables discovered (and probably many more) both act and interact, requires that very sensitive methodologies are developed to test the validity of the conclusions from these simple experimental conditions. Nevertheless, the experiments described here go far towards mimicking the complexity of natural ecosystems in the use of diverse plant communities, 2 species of slug, soil taken directly from the field, and in allowing the slugs free-range.

CHAPTER 4

MOLLUSC GRAZING AND INTRASPECIFIC  
VARIATION IN THE ACCEPTABILITY OF  
PLANTS OF TRIFOLIUM REPENS

## INTRODUCTION

The great variety of monomorphic 'mechanisms of defense' displayed by different species of plants (e.g. spines, silica granules, alkaloids, etc.) against the predatory activities of herbivores are of interest when one is exploring for example, the relative acceptabilities of, or the effects of herbivores on, a range of plant species (Whittaker and Feeny, 1971). However, superimposed on this interspecific diversity is the extent of the diversity displayed within the species. Polymorphic plant populations (sensu Ford, 1971) provide one of the best models (i) for studying the interactions of plants and herbivores and (ii) for assessing the role of a particular 'defense mechanism'. This is because the real significance of a defense mechanism may best be seen in its presence and absence against a background of the highest common factor of similarity in other respects - the polymorphic system. Thus intraspecific comparison should be especially rewarding (see Jones, 1971). On occasions, it may also be possible to alter the expression of a putative defense mechanism by changing the environment of the plant. We can, for example, postulate that the presence of silica granules in the leaves of Poa annua confers the typical unacceptability of this plant to the slug Agriolimax caruanae. We can then grow P. annua in a silica-free medium and feed it to slugs to see whether the acceptability still operates. This experiment has been done and it was found that the silica-free plants became more acceptable to A. caruanae but the silica-free plants were also smaller and thinner than normal plants (Dirzo and Harper, unpublished) and we cannot be sure whether the increased acceptability was due to the absence of silica, or to other changes produced in the plants by the experimental treatment; also, pleiotropic effects could obscure the interpretation

of such experiment. Presumably then, the polymorphic system remains as the best experimental set up.

Defense in some cases may be expressed as the presence or absence of a chemical, or exhibited as a continuous variation of concentration; in the latter case, we may be justified in interpreting the chemical as defensive or protective, if the degree of herbivore attack is greater the lower the concentration of the chemical (see Jones, 1973 and refs.).

One much studied chemical polymorphism in plants is that of cyanogenesis (see e.g. Corkill, 1940, 1942; Daday, 1954 a,b, 1965; Jones, 1972, etc.); it consists of the ability of some individuals to release hydrogen cyanide (HCN), generally as a result of physical damage to the tissue (e.g. leaves). Cyanogenesis is widely distributed in the plant kingdom, e.g. flowering plants, ferns, fungi and bacteria (Jones, 1972 and refs.) but it also extends to animals, e.g. the crushed tissue of all life stages of the moths Zygaena filipendulae and Zygaena lonicerae release detectable amounts of HCN (Jones et al., 1962). A brief historic account of cyanogenesis is given in Jones (1972) and it dates back as early as 1803 with the discovery of Prussic acid (a cyanogenic substance) in bitter almonds.

The biochemistry of cyanogenesis has been studied in detail in some plants of agronomic interest, e.g. Trifolium repens (white clover) (Melville et al., 1940); for a plant to be cyanogenic, cyanogenic glucosides must be present (Linamarin and Lotoaustralin in T. repens) as well as the appropriate  $\beta$  glucosidase enzymes (Linamara in T. repens) capable of hydrolysing the glucosides and releasing HCN. This system is genetically controlled in T. repens by two unlinked loci (Corkill, 1942). One allele pair (generally designated as Ac/ac) controls the production of the cyanogenic glucosides, and the other pair (designated as Li/li) controls production



of the enzymes. There is complete dominance of Ac and Li over ac and li respectively, so populations polymorphic for both loci will include the four gross phenotypes shown in Table 4.1. These four phenotypes can be readily distinguished by simple biochemical tests.

This chapter reports a study of cyanogenesis polymorphism in Trifolium repens and its relevance as a defense against grazing by terrestrial molluscs (slugs and snails). I report a series of laboratory and field studies made to measure the relative acceptability of cyanogenic and acyanogenic morphs of white clover to several species of terrestrial molluscs, and some of the consequences of cyanogenesis to both the plants and the herbivores. The study is concerned only with phenotypes (see Table 4.1) and no attempt is made to identify the heterozygotes which can be distinguished only by appropriate test crosses.

In Lotus corniculatus (another cyanogenesis-polymorphic legume with essentially the same biochemic system as T. repens), observations in natural populations (Jones, 1962) suggested that the acyanogenic forms are preferred by predators and subsequent formal experiments (Jones, 1966), elegantly confirmed this with several species of slugs and snails. The work of Prof. D.A. Jones has pioneered studies of cyanogenesis polymorphism in plants, especially L. corniculatus. After his initial reports, several attempts have been made to test the hypothesis that cyanogenesis in T. repens is also relevant to defense against herbivores, however the literature is somewhat confusing and conflicting (see Table 4.13). Surprisingly, also, most of the studies on white clover appear to have the goal of demonstrating only the defensive role of cyanogenesis (see however, Daday, 1954 a,b, 1965; Foulds and Grime 1972 a,b); some more detailed observations are reported in this chapter.

Table 4.1. The phenotypes of the cyanogenesis polymorphism in T. repens.

Alleles	Plant contents	Notation used here	Reaction
Ac ? Li ?	Cyanogenic glucosides and enzyme	Ac Li	cyanogenic
Ac ? li li	Cyanogenic glucosides but no enzyme	Ac li	acyanogenic
ac ac Li ?	Enzyme but no cyanogenic glucosides	ac Li	acyanogenic
ac ac li li	Neither cyanogenic glucosides nor enzyme	ac li	acyanogenic

## MATERIALS AND METHODS

Only materials and methods are given here that are common to the subsequent experiments and field surveys; more detail is given in the relevant sections.

### The herbivores

In the winter and spring of 1978 I made a series of diurnal and nocturnal observations in order to determine which herbivores were potentially important to white clover. Observations were made in wasteland sites at Pen-y-Ffridd Field Station, Bangor (Nat. Grid. Ref. SH 564705) and in grasslands at Treborth Botanic Gardens, Bangor (Nat. Grid. Ref. SH 553712) and at Henfaes Field, College Farm, Aber (Nat. Grid. Ref. SH 733656). Four molluscs (and sheep at College Farm) were the herbivores most commonly seen eating T. repens: in wastelands the slugs Agriolimax caruanae, A. reticulatus and Arion ater, were the most frequent predators, while in grasslands, the slugs A. reticulatus, Arion ater and the snail Helix aspersa were the most common clover-eaters. These three species of slug and the snail were chosen for subsequent study. Other molluscs were also seen eating clover (e.g. Arion hortensis) but they were less frequent. The activity of some other herbivores (e.g. small mammals and birds) might have been overlooked. The sward at Henfaes Field, is permanently grazed by sheep and these obviously eat large quantities of white clover; a report of some observations made on the sheep/clover interaction is given in the next chapter but a detailed study of sheep grazing on several plant species in this field, including T. repens is given in Peters (1980). Wood pigeons are reported to be important predators of clover

(Murton et al., 1966) but the sites I observed gave little evidence of this; the work reported here therefore deals only with the four species of mollusc.

Molluscs were collected at Pen-y-Ffridd Field Station by trapping technique as described in Dirzo (1980). Molluscs of major abundance in grasslands (e.g. Arion ater) were gathered by direct collection of animals active at night.

#### The experimental plant material

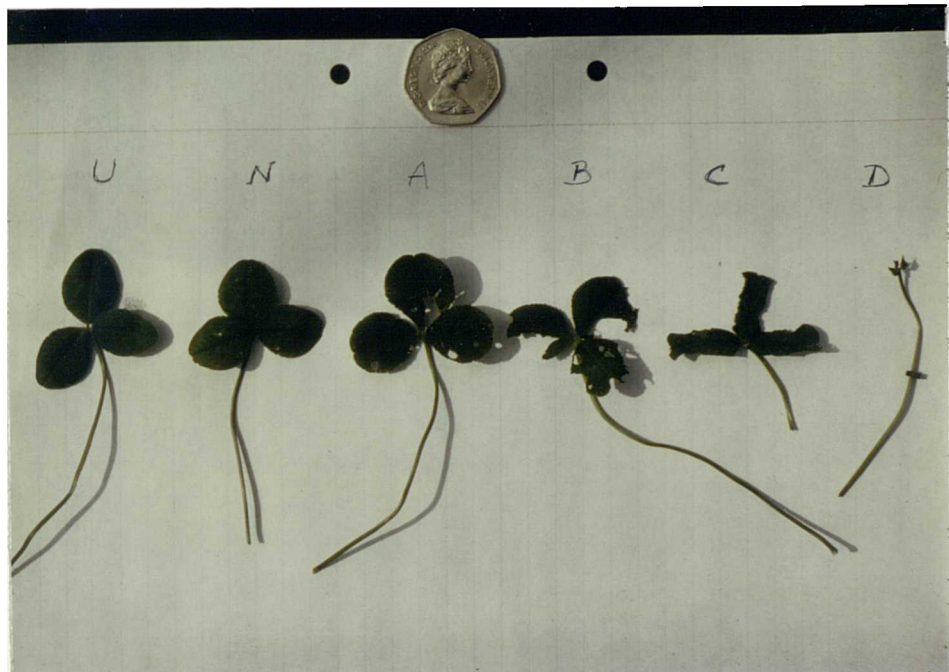
Unless otherwise stated, the plant material used for the experiments here described was obtained from soil cores collected at Henfaes Field, College Farm, in January 1978 and propagated under glasshouse conditions at the Pen-y-Ffridd Research Station.

#### Identification and scoring of mollusc damage on clover leaves

Plots of T. repens were established outdoors at Pen-y-Ffridd Field Station and left to grow for several weeks; initially molluscs were excluded by constant application of molluscicide (Draza) and frequent records were made of the types and intensity of damage that herbivores other than molluscs could do. Afterwards, application of molluscicide was suspended and molluscs of the four chosen species were introduced in large numbers into the plots; this procedure made it possible (i) to recognise the type of damage made by molluscs on clover leaves and (ii) to establish a system of scoring mollusc damage based on the area removed from leaflets. Six categories of damage were defined: untouched (U), nibbled (N), up to 25% of the leaflet taken (A), up to 50% taken (B), up to 75% taken (C) and up to 100% of the leaflet removed (D). Plate 1 shows both the type of damage and the scoring system.

Plate I.

Identification of mollusc damage on the leaves of Trifolium repens, and the system of scoring the damage on the basis of the leaflet area removed: U = untouched, N = nibbled, A = up to 25% removed, B = 25% to 50% removed, C = 50% to 75% removed, D = 75% to 100% removed.



### Cyanogenesis tests in *Trifolium repens*

Cyanogenesis in white clover is detected by the sodium picrate test described by Corkill (1940) and Jones (1966). For the tests carried out in this study, the three terminal leaves of a stolon, including the youngest (unfolded) leaf, were collected in test tubes (5 cm long x 0.9 cm) and two drops of analar toluene were added; the material was then crushed with a glass rod. Strips of Whatman No.1 filter paper (3.7 x 0.7 cm) were soaked in a solution of sodium picrate, the excess removed with filter paper and, while still wet, the paper strips were suspended in the corked test-tube and incubated at 37°C for at least 24 hrs. Plants were scored as cyanogenic (i.e. AcLi) if after the incubation period the sodium picrate paper had changed from its original yellow-lemon colour to different tones of red or brownish (the intensity of the red colour is correlated with the concentration of HCN in the tissue, i.e. it is not an 'all or nothing' response). Samples that produce no colour change can be further tested to distinguish the three possible acyanogenic morphs (cf. Table 4.1); by repeating the whole process but adding two drops of a solution (i) of the glucoside, tests that change colour are then scored as enzymatic (ac Li), or (ii) of the enzyme, and if the reaction then occurs, the plant is scored as glucosidic (Ac li). Material that does not react with either of the two solutions is scored as double recessive (ac li). The glucoside used for these tests (i.e. Linamarin) was obtained from commercial suppliers (Calbiochem); the enzyme (Linamarase) was prepared from linseed according to a recipe provided by Prof. D.A. Jones.

## RESULTS

### Acceptability trials with cyanogenic and acyanogenic plant material

A series of experiments in decreasing order of artificiality was made to determine whether the 4 chosen mollusc species were selective in their grazing on the cyanogenic (Ac Li) and the acyanogenic (ac li) morphs.

#### 1. Experiments with leaf discs in petri dishes

(i) Choice experiments: Leaf discs of cyanogenic and acyanogenic material were obtained by punching leaves with a core borer. For Agriolimax caruanae and A. reticulatus, four  $0.71 \text{ cm}^2$  discs of each morph were offered and three  $1.45 \text{ cm}^2$  discs of each morph were offered to Arion ater and Helix aspersa which have larger appetites. Individual animals of all four species were placed in plastic petri dishes (8.5 cm diameter for the two species of Agriolimax and 16 cm diameter for Arion ater and Helix aspersa); leaf discs of each clover morph were placed alternatively in a ring around the petri dish; a pellet of cotton wool soaked in distilled water was placed in the centre of the dish and the test animal was placed on the pellet. After this, the petri dish was closed and the animals were left for 12 hours (overnight) to feed. During the feeding trials the dishes were kept in a dark refrigerator at a constant temperature ( $15^{\circ}\text{C}$ ). After the feeding period the area of the leaf discs that remained was measured with a leaf area scanner and, subtracted from the original area of disc, gave a measure of the amount of material eaten. This procedure was repeated for three consecutive nights for A. caruanae and A. reticulatus and for two nights for Arion ater and Helix aspersa. Prior to the experiment, the animals had been starved for 24 hours. Animals that ate none



of the available material were eliminated from the analysis. Even so, there was considerable variation between replicates and the results had to be analysed with non-parametric procedures (Table 4.2). It was expected that leaf discs would provide the most suitable experimental material since the physical damage made by punching the leaves would make the cyanogenic plants release HCN immediately. In fact, not only was there no selective eating of the acyanogenic material but individuals of A. caruanae and of Arion ater at night 2 ate significantly more cyanogenic than acyanogenic material! Angseesing (1972) found that in experiments on selective eating, the slug Arion ater was relatively unselective at the beginning of the experiment and selectivity increased with time. There is no evidence of such a behavioural pattern here. There was very high variation in the response of replicated animals of all the species; when anomalous feeding behaviour occurred, it was not consistent, i.e. when deviant animals were re-tested subsequently they did not maintain deviant behaviour (cf. Dirzo (1980) who found consistently anomalous behaviour of some slugs in acceptability trials of several plant species).

The lack of selectivity found in these experiments might have occurred if cyanogenic discs release HCN in gaseous form and this accumulates inside the petri dishes; under these conditions, the animals might be unable to select between morphs. To avoid this possibility the experiments were repeated in a sequential, rather than a simultaneous, design.

(ii) Sequential feeding experiments. Cyanogenic and acyanogenic leaf discs were offered as described before except that on any night discs of only one morph were provided in the petri dishes. After 24 hours of starvation, all animals were given cyanogenic material at night 1, and non-cyanogenic discs on night 2. After

Table 4.2. The mean leaf area eaten from leaf discs of cyanogenic and acyanogenic clover in choice experiments carried out in petri dishes, together with the results of rank comparisons.

Species	Night	Leaf Area Eaten ( $\bar{X} \pm$ S.E.)		Wilcoxon's matched pairs rank test	
		Ac Li	ac li	n	T
<u>Agriolimax caruanae</u>	1	0.70 $\pm$ 0.16	0.67 $\pm$ 0.11	10	27.58
	2	1.07 $\pm$ 0.24	0.37 $\pm$ 0.11	9	5.00
	3	0.59 $\pm$ 0.08	0.63 $\pm$ 0.24	6	10.00
<u>Agriolimax reticulatus</u>	1	1.36 $\pm$ 0.23	1.16 $\pm$ 0.20	10	14.00
	2	1.58 $\pm$ 0.22	1.09 $\pm$ 0.26	10	12.00
	3	1.09 $\pm$ 0.13	0.87 $\pm$ 0.11	10	15.00
<u>Arion ater</u>	1	1.71 $\pm$ 0.44	1.95 $\pm$ 0.48	6	8.00
	2	3.09 $\pm$ 0.45	2.42 $\pm$ 0.31	6	2.00
<u>Helix aspersa</u>	1	3.03 $\pm$ 0.46	2.88 $\pm$ 0.52	6	10.00
	2	2.93 $\pm$ 0.37	2.71 $\pm$ 0.25	6	9.00

(2) = two-tailed test

(1) = one-tailed test

this 2-night period, animals were fed for 12 hours with lettuce, then starved again for another 24 hours after which the cycle was repeated but this time initiated with acyanogenic material (at night 4). This cycle was repeated three times for Agriolimax caruanae and A. reticulatus and twice for Arion ater and Helix aspersa. Here again, there was considerable variation between replicates and the result per cycle were analysed non-parametrically by Mann-Whitney's (non-paired) U test (Table 4.3). In this experiment, there was a tendency for slugs of Agriolimax caruanae and A. reticulatus to eat more acyanogenic material and in 2 of the experiments with A. reticulatus and 1 with A. caruanae the difference was statistically significant. None of the experiments involving Arion ater and Helix aspersa, were statistically significant in any direction. The experimental design does not wholly rule out the possibility that the feeding behaviour of the animals might have been altered in a closed chamber of HCN gas. Crawford-Sidebotham (1972b) carried out sequential experiments with detached leaves of T. repens and L. corniculatus offered to 13 species of mollusc, and some species did discriminate between cyanogenic and acyanogenic morphs but his results were also highly variable.

It can be concluded from the present experiments that selective grazing of the clover morphs does not occur in the petri dish-leaf disc test system. An attempt to reduce artificiality was made in the following experiments.

## 2. Choice experiments in turves

(i) Experiments with leaf discs. Sections of turf were collected from the lawns at Treborth Botanic Gardens, Bangor, where white clover and 3 of the molluscs (not A. caruanae) are abundant. Turves (16 x 16 x 10 cm) were put into plastic

Table 4.3. The mean leaf area eaten from leaf discs of cyanogenic and acyanogenic clover in sequential feeding experiments carried out in petri dishes, together with the results of U-test comparisons.

Species	Night	Ac Li	ac li	n	Mann-Whitney U-test		P
					U <sub>1</sub>	U <sub>2</sub>	
<u>Agriolimax caruanae</u>	1	0.66 ± 0.14	1.60 ± 0.27	9	70.0	11.0	P < 0.005
	2						
	5	0.71 ± 0.61	0.73 ± 0.20	9	42.5	38.5	n.s.
	4						
	7	0.53 ± 0.13	0.91 ± 0.20	9	56.5	24.5	n.s.
	8						
	1	2.00 ± 0.16	2.81 ± 0.04	9	78.0	3.0	P < 0.002
	2						
<u>Agriolimax reticulatus</u>	5	2.04 ± 0.24	2.46 ± 0.50	9	55.0	26.0	n.s.
	4						
	7	2.46 ± 0.18	3.66 ± 0.19	9	75.0	6.0	P < 0.005
	8						
	1	3.68 ± 1.16	3.21 ± 0.86	6	15.0	21.0	n.s.
	2						
	5	1.71 ± 0.51	3.27 ± 1.32	6	21.0	15.0	n.s.
	4						
<u>Helix aspersa</u>	1	5.24 ± 0.88	4.70 ± 5.83	6	16.5	19.5	n.s.
	2						
	4	4.79 ± 0.92	4.68 ± 1.16	6	18.5	17.5	n.s.
	5						

**Table 4.3.** The mean leaf area eaten from leaf discs of cyanogenic and acyanogenic clover in sequential feeding experiments carried out in petri dishes, together with the results of U-test comparisons.

Species	Night	Ac Li	ac li	n	Mann-Whitney U-test		P
					U <sub>1</sub>	U <sub>2</sub>	
<u>Agriolimax caruanae</u>	1	0.66 ± 0.14	1.60 ± 0.27	9	70.0	11.0	P < 0.005
	2						
	5	0.71 ± 0.61		9	42.5	38.5	n.s.
	4						
	7	0.53 ± 0.13		9	56.5	24.5	n.s.
	8						
	1	2.00 ± 0.16		9	78.0	3.0	P < 0.002
	2						
<u>Agriolimax reticulatus</u>	5	2.04 ± 0.24	2.46 ± 0.50	9	55.0	26.0	n.s.
	4						
	7	2.46 ± 0.18		9	75.0	6.0	P < 0.005
	8						
	1	3.68 ± 1.16		6	15.0	21.0	n.s.
	2						
	5	1.71 ± 0.51		6	21.0	15.0	n.s.
	4						
<u>Arion ater</u>	1	5.24 ± 0.88	4.70 ± 5.83	6	16.5	19.5	n.s.
	2						
	4	4.79 ± 0.92		6	18.5	17.5	n.s.
	5						
	1						

Table 4.4. The mean leaf area eaten from leaf discs of cyanogenic and acyanogenic clover in choice experiments carried out in turves, together with the results of rank comparisons.

Species	Night	Leaf Area Eaten ( $\bar{X} \pm S.E.$ )		Wilcoxon's matched pairs range test	
		Ac Li	ac li	n	T
<u>Agriolimax caruanae</u>	1	0.34 + 0.30	0.56 + 0.16	5	3.00
	2	0.57 + 0.43	0.56 + 0.26	5	5.00
<u>Agriolimax reticulatus</u>	1	1.60 + 0.68	3.01 + 0.67	5	3.00
	2	3.24 + 0.52	3.54 + 0.79	5	8.00
	3	1.82 + 0.62	4.30 + 1.08	5	0.00
<u>Arion ater</u>	1	4.75 + 1.53	5.34 + 1.06	5	3.00
	2	6.95 + 0.67	7.78 + 0.32	5	3.00
	3	7.70 + 1.42	8.47 + 1.73	5	4.50
<u>Helix aspersa</u>	1	5.56 + 1.55	5.43 + 1.33	5	5.00
	2	6.31 + 0.70	7.29 + 0.55	5	4.00
	3	4.63 + 1.29	7.42 + 1.76	5	2.00

n.s. (1-tail)  
n.s.

n.s.  
n.s.  
P = 0.05

n.s.  
n.s.  
n.s.

n.s.  
n.s.  
P = 0.10

the animals of each species. Since there was no control of the amount of leaf area (or weight) offered to the animals, records were made of the proportion of the leaflet lamina removed (see Materials and Methods). Only four categories of damage were used by pooling together categories A and B and C and D. It was also necessary to pool the values of all the replicates in order statistically to compare the numbers of leaflets of both morphs falling into each of the categories of damage; sample sizes were still small and the contingency tables were analysed with the log.likelihood ratio test (instead of the typical  $X^2$  test) in order to deal with small sample sizes. Results are given in Table 4.5. Here again, Agriolimax caruanae largely refused to eat the leaves of clover although some other dycots in the sward were eaten; the few clover leaflets damaged did not indicate selection between morphs. The other three species ate more acyanogenic than cyanogenic leaves but only the data for A. reticulatus reached statistical significance ( $P = 0.02$ ); Helix aspersa nearly reached significance ( $P = 0.11$ ) while in the case of Arion ater, there was a probability as large as 25% of obtaining the observed values by chance alone.

(iii) Experiments with naturally occurring clover in turves. From the turves collected at Treborth Botanic Gardens, a number were chosen in which T. repens was particularly abundant. These turves were treated with molluscicide (Draza) for 3 weeks and any leaves visibly damaged by molluscs were recorded and removed at the junction with the stolon node. These turves were not clipped but otherwise were treated as described for the two previous experiments. Each clover-rich turf was allocated to individual replicates of each species of mollusc and 5 animals were allowed to graze freely for 5 nights; they were then removed and damaged leaflets were counted and scored for damage. All the plants were scored for cyanogenesis

**Table 4.5.** The scores of damage to leaflets of cyanogenic and acyanogenic clover in choice experiments carried out in turves together with the results of log. likelihood ratio tests.

Agriolimax caruanae

	Score of damage*					
	U	N	S	H		
Ac Li	50 (48) <sup>1</sup>	8 (8.5)	7 (7.5)	7 (8)	72	$X^2_{(3)} = 0.54$
ac li	46 (48)	9 (8.5)	8 (7.5)	9 (8)	72	$P > 0.90$
					144	

Agriolimax reticulatus

	U	N	S	H		
Ac Li	24 (24)	8 (5.5)	9 (5.5)	16 (22)	57	$X^2_{(3)} = 10.0$
ac li	24 (24)	3 (5.5)	2 (5.5)	28 (22)	57	$P = 0.019$
					114	

Arion ater

	U	N	S	H		
Ac Li	4 (2.5)	3 (2.5)	4 (3)	52 (55)	63	$X^2_{(3)} = 3.14$
ac li	1 (2.5)	2 (2.5)	2 (3)	58 (55)	63	$P > 0.25$
					126	

Helix aspersa

	U	N	S	H		
Ac Li	11 (11.5)	4 (2.5)	6 (3.5)	33 (36.5)	54	$X^2_{(3)} = 6.07$
ac li	12 (11.5)	1 (2.5)	1 (3.5)	40 (36.5)	54	$P = 0.11$
					108	

\* U = Untouched, N = Nibbled, S = Slight, H = Heavy.

<sup>1</sup> Expected numbers are shown in parenthesis



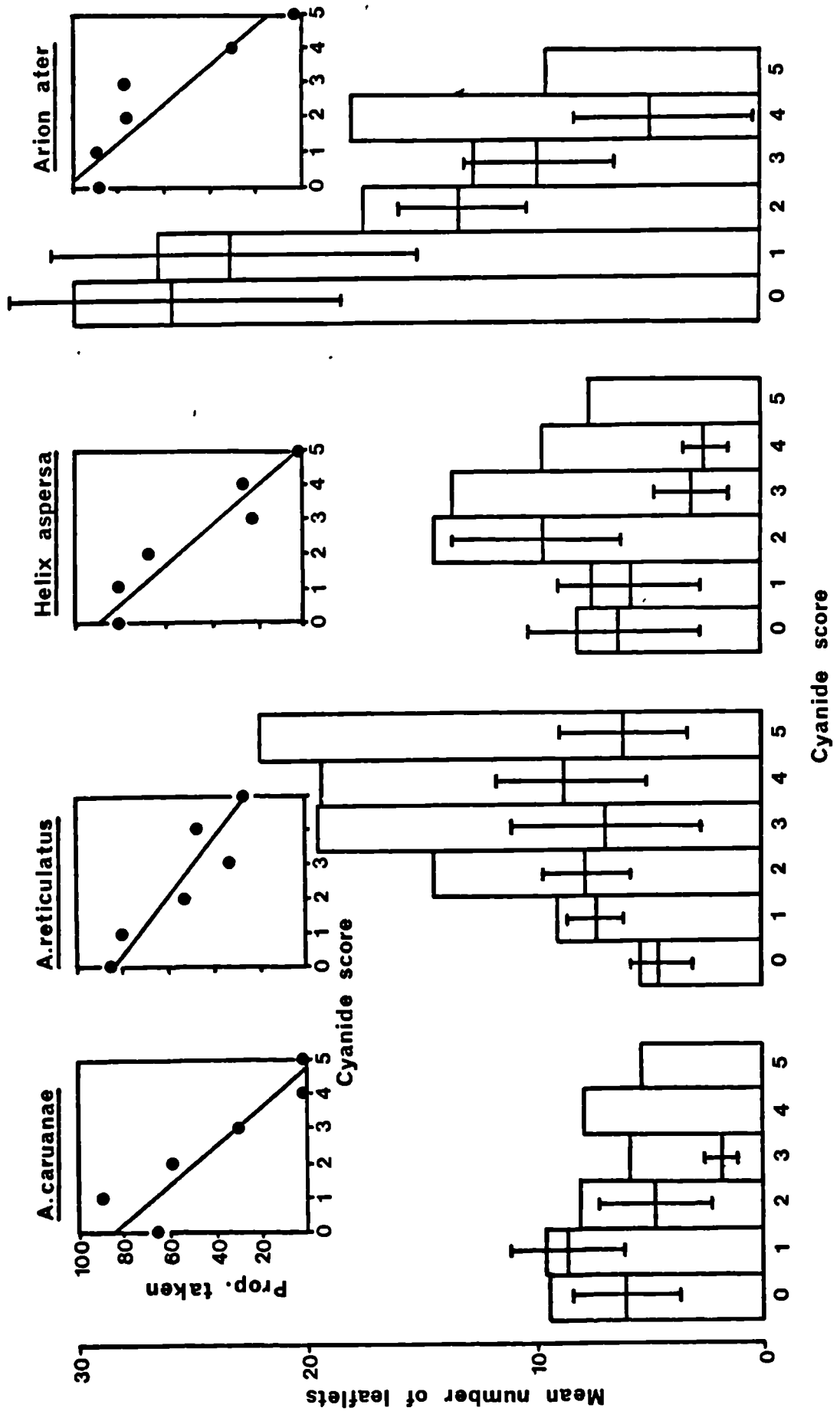
but since there was a marked variation in the response, the degree of cyanogenesis was also scored by comparing the colour of the picrate paper against a standard scale of colours (Munsell) ranging from zero (no change in colour) to 5 (the most intense colour). Histograms in Fig. 4.1 show the number of leaflets damaged by molluscs (regardless of the degree of damage) and the total number available for each category of cyanide score; the inserts show the same data but expressed as proportion of leaflets taken; there is a clear tendency for slugs to take a progressively smaller proportion of leaflets as the cyanide score increased. A. caruanae again ate very little clover in comparison to any of the other species of mollusc. It is odd that for A. caruanae, Helix aspersa and Arion ater the leaflets with the highest cyanide score were not even nibbled; it is difficult to imagine how the molluscs could 'know' without 'tasting' which were the leaflets with the highest cyanide score. It is likely that different leaves of the same plant will show a different cyanogenic reaction (for example, associated with age (cf. Hughes, 1968)) and it may be that after a mollusc has 'tasted' a leaf of high cyanide score, say 4, it moves away from the plant, leaving other leaves of higher score (5) untouched.

### 3. Acceptability trials with intact, growing plants

Plants of a very strongly cyanogenic strain and a completely acyanogenic one were grown from seed provided by the Welsh Plant Breeding Station (WPBS), Aberystwyth. Individual seedlings were grown in plastic pots (10 cm diameter) for a month. Plastic containers (16.5 x 16.5 x 20 cm) were filled to 4 cm from the top with John Innes Compost No.1; two seedlings, one of each morph, (i.e. Ac Li and ac li), were transplanted into opposite corners of the containers and left to establish for 10 days after which individual animals of the 4 mollusc species were

Fig. 4.1.

The mean number of leaflets of Trifolium repens in each cyanide score. Top bars are the total number of leaflets per container and the lower bars are the number of leaflets that were damaged by molluscs of each of the 4 species. Means of 5 replicates  $\pm$  S.E. The inserts show the same data expressed as proportion of leaflets taken in each cyanide score.

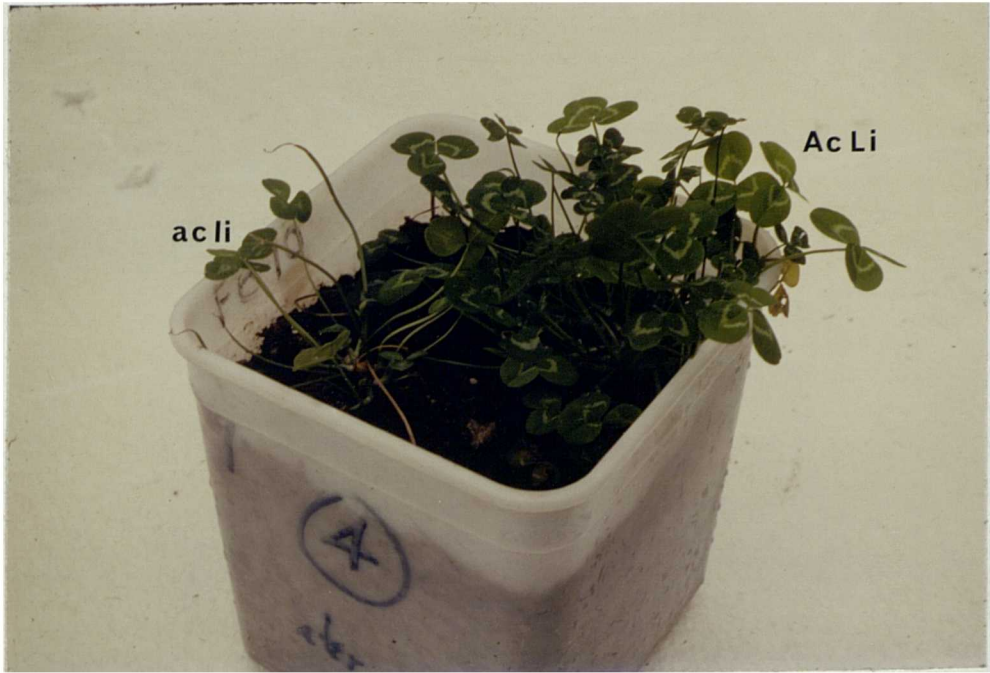


introduced and allowed to graze the plants for 7 nights; escape of the animals was prevented as in the experiments with turves. Recordings of the categories of damage in each leaflet were made daily after night 2, but sufficient data were accumulated only towards the end of the grazing period. Plates 2(a) and 2(b) show the type of results obtained. Tables 4.6 a-d show contingency tables for grazing by the 4 species separately for each replicated animal. The individual sets of data were analysed with the log-likelihood ratio test (Zar, 1974) to deal with small sample sizes. Since the individual sets of data are being tested against the same null hypothesis and in order to decide whether it is valid to combine all the sets in one overall contingency analysis, the data for replicated animals were tested for heterogeneity. In A. caruanae, Arion ater and Helix aspersa, the heterogeneity between replicates was not statistically significant. In A. reticulatus (Table 4.6 b) there was a significant heterogeneity brought about by replicate 3; if this replicate is omitted from the analysis the values of the remaining 4 replicates can legitimately be pooled. The overall (pooled) contingency tables for each species allow a much more robust test to be made and this is highly significant for all 4 species; the source of these high  $X^2$  values can easily be seen by comparing the expected and observed values in the contingencies: there is a clear under-representation of cyanogenic leaflets in heavily grazed categories (C and D) and over-representation of acyanogenic leaflets; conversely, there is an over-representation of cyanogenic leaflets in the U (undamaged) and/or low damage categories, and again, the opposite holds for the acyanogenic leaflets.

Although slug damage was clearly less on cyanogenic clover, there was some degree of grazing or at least nibbling recorded on cyanogenic forms. Clearly, cyanogenesis reduces but in no sense prevents slug grazing on clover.

Plate 2.

The grazing trials with intact, growing plants of cyanogenic (Ac Li) and acyanogenic (ac li) morphs of Trifolium repens. The plates show the results of replicate 4 of Arion ater seen from aside (a) and from above (b).



(a)



(b)

**Table 4.6.** Contingency analysis for the number of leaflets of cyanogenic and acyanogenic morphs in each category of damage.  $\chi^2$  analyses are shown for the individual replicates, the sum of replicates, the pooled values and the heterogeneity between replicates. (a) Agriolimax caruanae, (b) Agriolimax reticulatus, (c) Arion ater, (d) Helix aspersa.

(a) Agriolimax caruanae

Replicate	Morph	No. of leaflets in categories of damage <sup>1</sup>				$\Sigma$	$\chi^2$	df	P
		U	N	A+B	C+D				
1	Ac Li	25	1	2	1	29	6.17	3	>0.10
	ac li	26	1	2	9	38			
		51	2	4	10	67			
2	Ac Li	22	3	8	3	36	5.00	3	>0.10
	ac li	20	1	8	10	39			
		42	4	16	13	75			
3	Ac Li	38	1	3	1	43	2.18	3	>0.50
	ac li	18	1	3	2	24			
		56	2	6	3	67			
4	Ac Li	25	1	2	2	30	2.03	3	>0.50
	ac li	21	2	4	4	31			
		46	3	6	6	61			
5	Ac Li	40	1	2	6	49	4.96	3	>0.10
	ac li	18	2	4	6	30			
		58	3	6	12	79			
$\Sigma$							20.34	15	
Pooled	Ac Li	150 (139.15) <sup>2</sup>	7 (7.7)	28 (26.95)	13 (24.2)	198	13.76	3	<0.005
	ac li	103 (113.85)	7 (6.3)	21 (22.05)	31 (19.8)	162			
		253	14	49	44	360			
Heterogeneity							6.58	12	>0.75

<sup>1</sup> Categories: U = Untouched, N = Nibbled, A = up to 25% removed, B = 25-50% removed, C = 50-75% removed, D = 75-100% removed

<sup>2</sup> Expected numbers are shown in parenthesis

(b) *Agriolimax reticulatus*

Replicate	Morph	No. of leaflets in categories of damage				Σ	X <sup>2</sup>	df	P
		U	N	A+B	C+D				
1	Ac Li	35	9	7	1	52	22.14	3	<0.001
	ac li	21	3	19	11	54			
		56	12	26	12	106			
2	Ac Li	29	4	8	6	47	12.10	3	<0.01
	ac li	24	1	10	23	58			
		53	5	18	29	105			
3	Ac Li	46	2	11	12	71	1.01	3	>0.75
	ac li	31	1	10	12	54			
		77	3	21	24	125			
4	Ac Li	50	3	10	1	64	16.20	3	<0.005
	ac li	22	2	10	10	44			
		72	5	20	11	108			
5	Ac Li	46	6	13	1	66	35.59	3	<0.001
	ac li	20	1	11	21	53			
		66	7	24	22	119			
Σ						87.04	15		
Pooled	Ac Li	206 (172.65)	24 (17.05)	49 (58.08)	21 (52.22)	300	65.27	3	<0.001
	ac li	118 (151.35)	8 (14.95)	60 (50.92)	77 (45.78)	263			
		324	32	109	98	563			
Heterogeneity						21.17	12	≤0.05	
Excluding replicate 3									
	Ac Li	160 (129.14)	22 (15.5)	38 (46.01)	9 (38.69)	229	78.58	3	<0.001
	ac li	87 (117.86)	7 (13.84)	50 (41.99)	65 (35.31)	209			
		247	29	88	74	438			
Σ						86.03	12		
Heterogeneity of 4 replicates						7.45	9	>0.50	



(c) Arion ater

Replicate	Morph	No. of leaflets in category of damage				$\Sigma$	$X^2$	df	P
		U	N	A+B	C+D				
1	Ac Li	55	2	4	28	89	107.41	3	<0.001
	ac li	1	1	2	96	100			
		56	3	6	124	189			
2	Ac Li	61	6	4	29	100	30.06	3	<0.001
	ac li	9	1	2	36	48			
		70	7	6	65	148			
3	Ac Li	50	2	6	19	77	14.33	3	<0.005
	ac li	12	1	1	22	36			
		62	3	7	41	113			
4	Ac Li	41	2	9	20	72	28.82	3	<0.001
	ac li	4	1	2	28	35			
		45	3	11	48	107			
5	Ac Li	60	1	2	29	92	91.55	3	<0.001
	ac li	2	2	2	78	84			
		62	3	4	107	176			
$\Sigma$						272.17	15		
Pooled	Ac Li	267 (130.06)	13 (11.15)	25 (19.95)	125 (225.85)	430	260.55	3	<0.001
	ac li	28 (121.94)	6 (7.85)	9 (14.05)	260 (159.15)	303			
		295	19	34	385	733			
Heterogeneity						11.62	12	>0.25	

(d) Helix aspersa

Replicate	Morph	No. of leaflets in category of damage				$\Sigma$	$\chi^2$	df	P
		U	N	A+B	C+D				
1	Ac Li	63	4	9	13	89	42.88	3	<0.001
	ac li	36	1	2	56	95			
		99	5	11	69	184			
2	Ac Li	60	2	1	10	73	11.15	3	<0.01
	ac li	21	1	3	14	39			
		81	3	4	24	112			
3	Ac Li	65	3	9	6	83	29.13	3	<0.001
	ac li	39	1	1	28	69			
		104	4	10	34	152			
4	Ac Li	70	2	4	3	79	48.76	3	<0.001
	ac li	45	1	3	45	94			
		115	3	7	48	173			
5	Ac Li	74	1	2	23	100	14.47	3	<0.005
	ac li	24	2	5	23	54			
		98	3	7	46	154			
$\Sigma$						146.39	15		
Pooled	Ac Li	332 (271.91)	12 (9.85)	25 (21.34)	55 (120.91)	424	113.89	3	<0.001
	ac li	165 (225.09)	6 (8.15)	14 (17.66)	166 (100.09)	351			
		497	18	39	221	775			
Heterogeneity						32.50	12	<0.025	

In the previous experiments, evidence of selective grazing by slugs was unconvincing - or marginally so. The essential differences of procedure in the present tests were: (i) complete, intact plants were used; (ii) specially selected acyanogenic and highly cyanogenic morphs were tested; and (iii) experiments were allowed to run for a longer time.

#### A field survey of mollusc-grazing on *T. repens* morphs

An attempt was made to determine the relevance of cyanogenesis to grazing by molluscs in a field situation. A survey was made in spring (mid-May) of 1978 in a 1 ha. field of permanent grassland (Henfæes Field, College Farm, Aber). In this field, all four species of mollusc are present; *A. caruanae* is rare though some individuals have been observed in the peripheral (weeded) areas of the field. For this survey, 72 quadrats (1 dm<sup>2</sup>) were randomly positioned in the field by choosing random numbers that defined coordinates. In each quadrat all the clover leaflets were inspected for mollusc grazing and scored with the usual system (U to D); samples (1300 leaflets in all) were collected and tested for cyanogenesis to test for correlation between the cyanogenic reaction and the grazing scores. In the 72 sampled quadrats, 15 (or 20.83%) had no clover, 9 (or 12.5%) had acyanogenic leaves only, and 13 (or 18%) had only cyanogenic leaves; in 35 (or 48.61%) both cyanogenic and acyanogenic leaves were intermingled. The results are shown as a contingency analysis in Table 4.7. Plants were defined as cyanogenic and acyanogenic without distinguishing any of the 3 possible acyanogenic phenotypes (as neither enzyme nor glucoside was available at this time).  $\chi^2$  tests gave very strong evidence of selective grazing of the acyanogenic phenotypes; there was an excess of untouched and slightly damaged leaflets among the Ac Li plants and an

Table 4.7. A contingency table of the numbers of cyanogenic and acyanogenic leaflets of T. repens falling into different scores of mollusc damage. Data is from a field survey; the values for the 'acyanogenic' plants are given without distinction between the 3 possible acyanogenic phenotypes.

Phenotype	Score of mollusc damage					Σ	
	U	N	A	B	C		D
Cyanogenic	267 (257.19)	81 (72.29)	87 (67.05)	34 (37.19)	13 (12.05)	189 (225.24)	671
Acyanogenic	224 (233.81)	57 (65.71)	41 (60.95)	37 (33.81)	10 (10.95)	241 (204.76)	610
Σ	491	138	128	71	23	430	1281

$\chi^2 = 28.41; P < 0.001$   
 $df = 5$

under-representation of those with the highest predation scores; these results are reversed for the acyanogenic phenotypes.

It was noted that the intensity of grazing was patchy with more evidence of grazing at the margins than in the centre of the field.

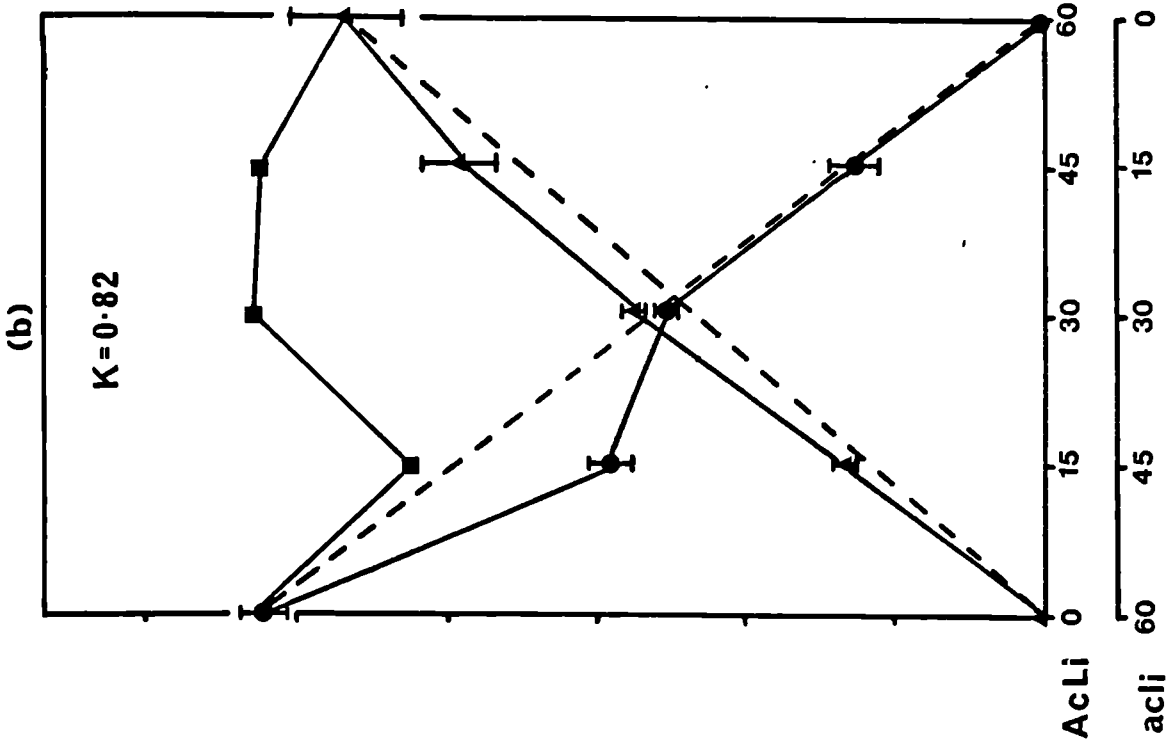
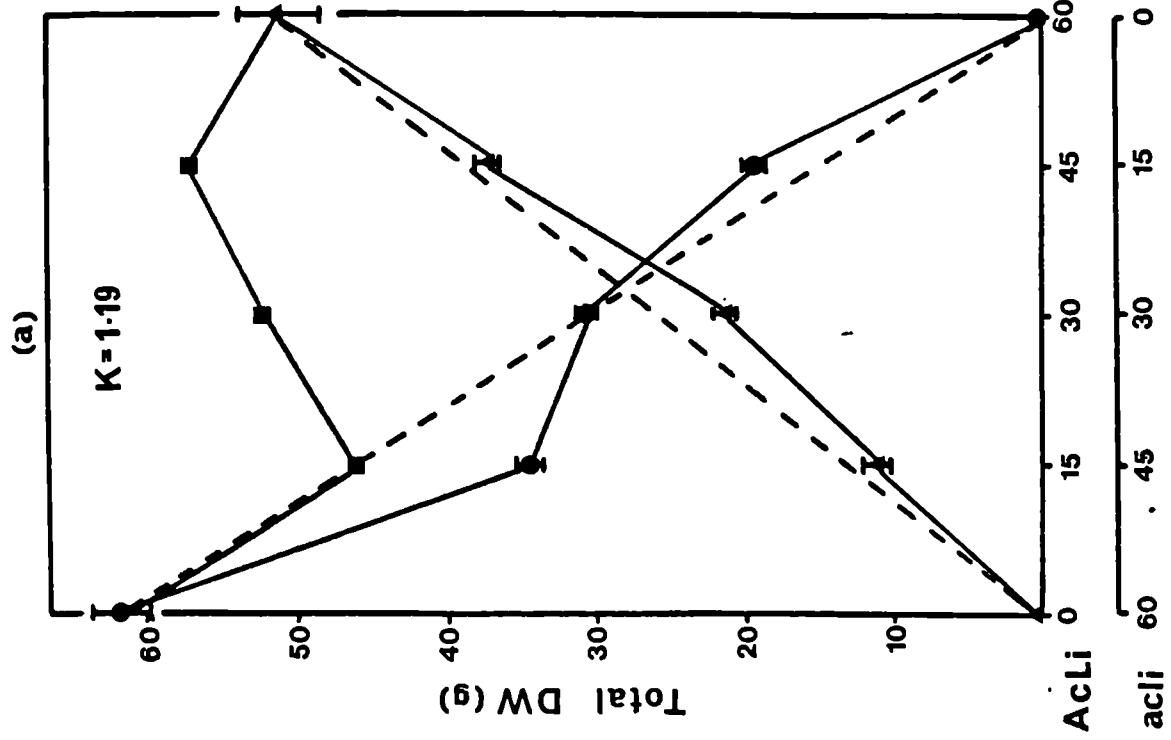
#### Experiments involving grazing and plant to plant interactions

The lower acceptability to slugs of the cyanogenic morphs of white clover might be expected to put them at an advantage in the presence of molluscs. This was tested in experiments in which slugs grazed on mixtures of cyanogenic and acyanogenic morphs grown at a density at which they could be expected to interfere with each other's growth.

A typical replacement series experiment (de Wit, 1960) was made in which cyanogenic and acyanogenic plants were planted at constant overall density but varied proportions 1:0, 3:1, 1:1, 1:3, 0:1. Plastic trays (35 x 21 x 5 cm) were filled with J1 Compost to 1 cm from the top and 60 white clover cuttings, ca 2.5 cm long with a terminal bud, were planted in each tray in a square arrangement with inter-plant distances of 3.5 cm; the positioning of the 2 morphs was made at random. Morphs were labelled with plastic rings of different colours. The experimental design was completely randomised with 2 treatments, ± slugs and replicated 5 times. Agriolimax reticulatus was chosen for this study. To prevent slugs from escaping, each tray was placed in a box of larger dimensions; a sheet of polythene was fixed between the walls of the tray and the inner surface of the box and this 'channel' was filled with water. The plants were allowed to grow for 1 month and were harvested by cutting at soil level on June 24, 1979, oven-dried for at least 2 weeks and dry weights recorded. Figure 4.2 shows the results in the manner conventional

Fig. 4.2.

The yield of cyanogenic ( $\blacktriangle$ ), and acyanogenic ( $\bullet$ ) plants of Trifolium repens in a Replacement Series experiment with (a) and without slugs (b).  $K$  = Relative Crowding Coefficient of ac li towards Ac Li.  $\blacksquare$ — $\blacksquare$ , total yield.



for replacement series. Clearly, the yield of the mixtures is not accurately predictable from the mean monoculture yields but the departures from the expectation vary with the treatments; for example, at the ratio 15 Ac Li/45 ac li, the acyanogenic form makes a poorer contribution to mixture yield than expected, both in the presence and absence of slugs. The relative crowding coefficients (K) of acyanogenic with respect to cyanogenic morphs changed from 1.19 in the ungrazed to 0.89 in the grazed plots, suggesting that a marginal competitive superiority of the ac li morph in the controls is reversed in the presence of slugs.

#### The effects of clover diets on the growth of slugs

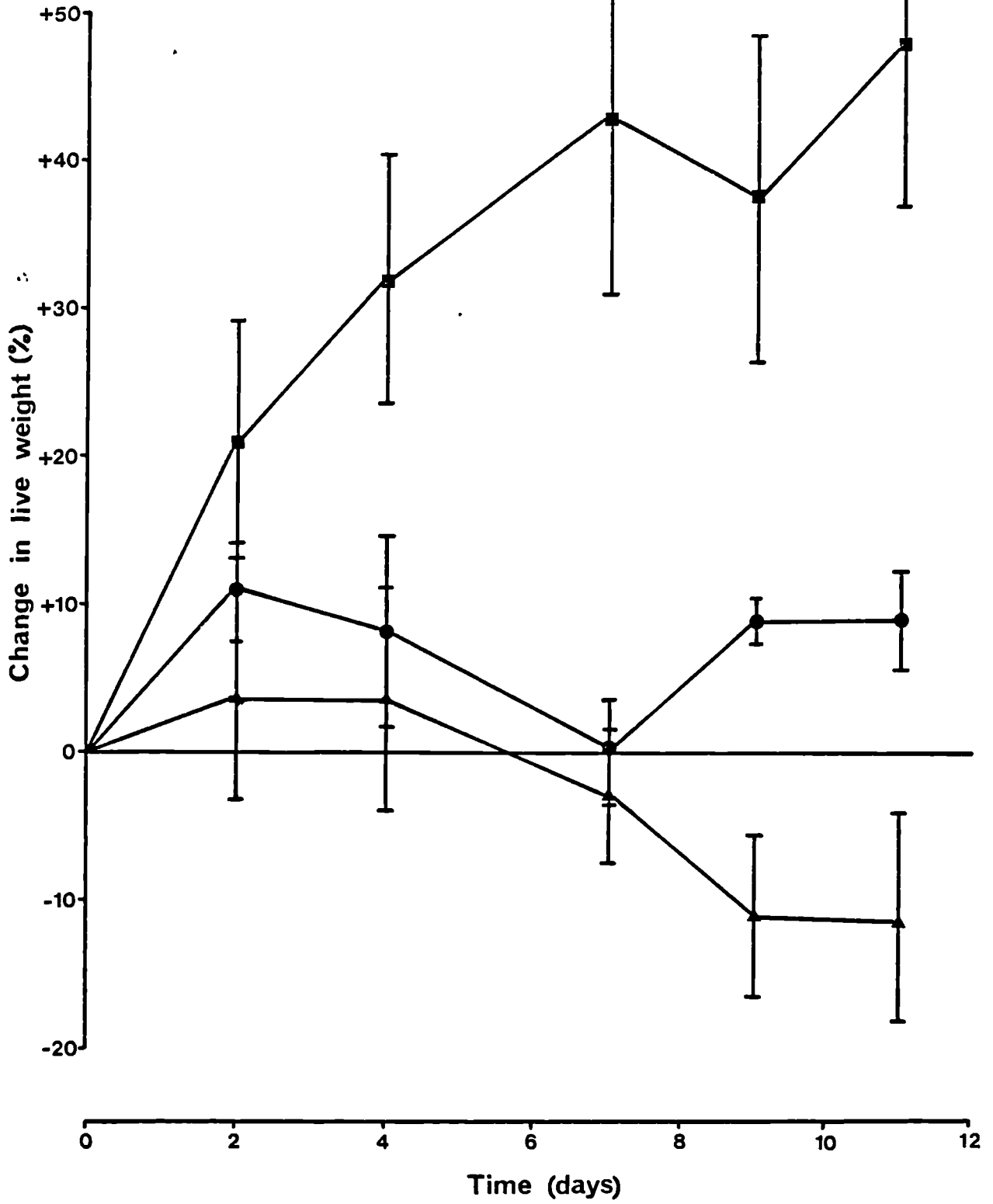
Most of the work described so far has been made with a phytocentric emphasis. However, observations were made that attempt to assess some of the possible consequences of the cyanogenesis-polymorphism on the slugs; experiments were made to determine the effects of a diet of cyanogenic or acyanogenic leaves on the growth of juvenile individuals of Agriolimax caruanae and A. reticulatus and on middle-size individuals of Arion ater; it was necessary to use slugs of pre-reproductive age to avoid the changes in weight that occur when animals become reproductive (Runham, personal communication). Slugs were placed in petri dishes (8.5 cm diameter) in which a cotton wool pellet was kept continuously moist with distilled water; leaves of cyanogenic (Ac Li) and acyanogenic (ac li) plants were offered as monotonous diets for 10 days to A. caruanae and A. reticulatus and for 5 weeks to Arion ater. As a control, an additional set of animals was fed with only lettuce. The food was provided in excess and replaced daily. The live weight of the slugs was recorded at the start of the feeding regimes; individuals of A. caruanae and A. reticulatus were weighed every other day and Arion ater

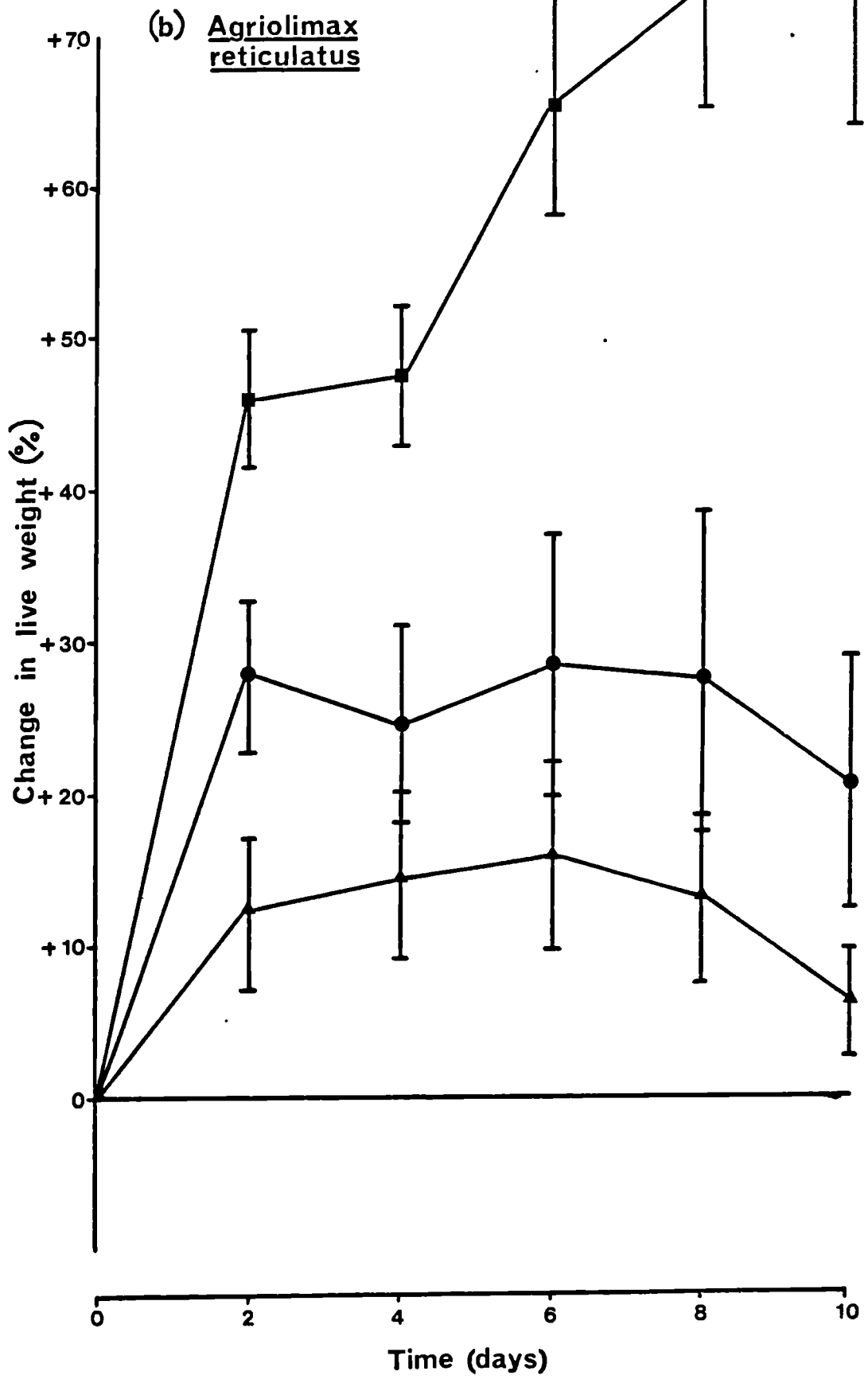


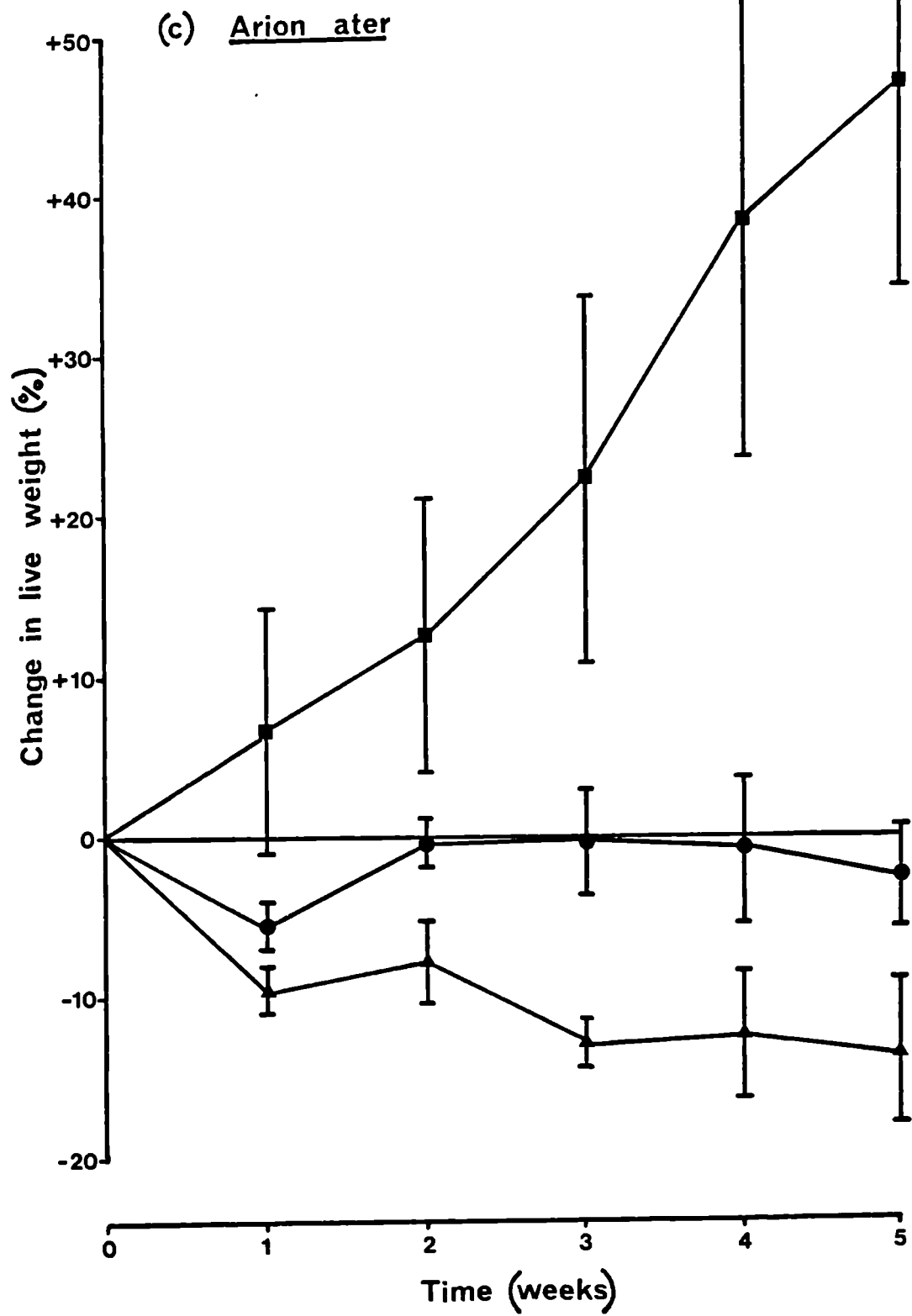
Fig. 4.3.

The effects of diets of lettuce (■), and of cyanogenic (▲), and acyanogenic (●) leaves of Trifolium repens on the live-weight of slugs of Agriolimax caruanae (a), Agriolimax reticulatus (b), and Arion ater (c). Means of 6 replicates  $\pm$  S.E.

(a) Agriolimax caruanae







weekly. Figures 4.3 a,b and c show the changes in live weights expressed as a percentage of the original weight. The results for the three species are very similar: animals feeding on lettuce gained weight rapidly, nearly doubling their original weight, by the end of the experiment some growth was made by A. reticulatus on both clover diets, with a tendency for slugs eating non-cyanogenic leaves to grow faster - but growth in either clover morphs was very much slower than on lettuce. Individuals of both A. caruanae and Arion ater lost ca 10% of their original weight when fed on cyanogenic clover; those eating acyanogenic leaves maintained their weight. Clover (even acyanogenic morphs) was a very poor diet compared with lettuce.

#### Interactions between plant and animal biochemistry

In view of the very definite evidence that slugs select between cyanogenic and acyanogenic morphs of clover, yet very inconsistent results obtained from the early laboratory trials, it seemed worth testing whether there were biochemical features of the slug that might interact with the cyanogenesis polymorphism.

Runham and Hunter (1970, page 48-49) list a number of enzymes present in the crop juice of slugs; the list includes  $\beta$ -glucosidase, an enzyme active against a number of substrates including  $\beta$ -glucosides. The enzyme is reported in Agriolimax reticulatus and Arion ater, which are both important grazers of clover. Thus, a slug that eats an acyanogenic plant containing the glucoside, but not the enzyme (Ac li) probably produces the cyanogenic reaction inside its digestive tract! To test the hypothesis that the clover enzyme is necessary for slugs to distinguish between the clover morphs, two grazing trials were carried out in which the slugs Agriolimax caruanae and A. reticulatus were offered: (i) cyanogenic plants (Ac Li)

together with acyanogenic but glucosidic (Ac li) plants, and (ii) acyanogenic but glucosidic (Ac li) together with acyanogenic, double recessive (ac li) plants. In these experiments, the lack of selectivity between the two morphs in trial (i) and the preference of ac li over Ac li in experiment (i) would suggest that the clover enzyme must not necessarily be present for slugs to be deterred. Trials were carried out with transplanted clones of the appropriate genotypes in the same way as described for experiment 3. After 10 days of grazing, counts were made of the leaflets and their scores of damage. Tables 4.8 (a and b) and 4.9 (a and b) show the contingency analysis of the results. The trial Ac Li/Ac li for A. reticulatus (Table 4.8 a) showed no significant heterogeneity between replicates ( $P > 0.25$ ) and from the pooled contingency it can be seen that slugs clearly preferred the Ac li morph indicating that the enzyme must be present in the plant if the slugs are to discriminate. For the same species of slug in the trial Ac li/ac li (Table 4.8 b) there was a marked heterogeneity between replicates ( $P < 0.001$ ) and 4 of the animals showed either erratic behaviour or a tendency to eat more Ac li than expected, suggesting again that the enzyme must be present in the plant if slugs are to discriminate; however, replicate 4 showed a significant preference ( $P < 0.025$ ) for ac li over Ac li. A tentative interpretation is that the slugs could themselves be enzymatically-polymorphic! The other species, Agriolimax caruanae, gave significant heterogeneity ( $P < 0.001$ ) in the trial Ac Li/Ac li (Table 4.9 a) with animals tending to prefer Ac li over Ac Li but slug (replicate) 1, and 5 to some extent, showed no differential grazing. In the Ac li/ac li (Table 4.9 b) there was also significantly heterogeneous response ( $P < 0.001$ ) between replicates but slugs either made no clear choice (e.g. replicate 1) or preferred Ac li over ac li!

**Table 4.8.** Results of the choice experiments designed to investigate selective grazing in the absence of clover enzyme (B glucosidase) with the slug *Agriolimax reticulatus*. (a) Pair Ac Li/Ac li, (b) Pair Ac li/ac li.

(a) Pair Ac Li /Ac li

Replicate	Morph	No. of leaflets in categories of damage				$\Sigma$	$\chi^2$	df	P
		U	N	A+B	C+D				
1	Ac Li	491 (500.62)	69 (48.35)	28 (31.26)	44 (51.77)	632	20.54	3	<0.001
	Ac li	534 (524.38)	30 (50.65)	36 (32.74)	62 (54.33)	662			
		1025	99	64	106	1294			
2	Ac Li	435 (415.6)	11 (6.47)	11 (14.65)	8 (28.27)	465	29.14	3	<0.001
	Ac li	785 (804.4)	8 (12.53)	32 (28.35)	75 (54.73)	900			
		1220	19	43	83	1365			
3	Ac Li	577 (554.86)	24 (20.55)	27 (30.14)	36 (58.45)	664	19.16	3	<0.001
	Ac li	638 (660.14)	21 (24.45)	39 (35.86)	92 (69.55)	790			
		1215	45	66	128	1454			
4	Ac Li	514 (519.49)	31 (20.10)	15 (14.62)	23 (28.78)	583	13.15	3	<0.005
	Ac li	623 (617.57)	13 (23.9)	17 (17.38)	40 (34.22)	693			
		1137	44	32	63	1276			
5	Ac Li	759 (752.3)	21 (19.47)	16 (23.47)	25 (25.76)	821	6.03	3	n.s.
	Ac li	555 (561.7)	13 (14.53)	25 (17.53)	20 (19.24)	613			
		1314	34	41	45	1434			
$\Sigma$							88.02	15	
Pooled	Ac Li	2776 (2741.95)	156 (111.79)	97 (114.11)	136 (197.15)	3165	73.60	3	<0.001
	Ac li	3135 (3169.05)	85 (129.21)	149 (131.89)	289 (227.85)	3658			
		5911	241	246	425	6823			
Heterogeneity							14.42	12	>0.25

## (b) Pair Ac li/ac li

Replicate	Morph	No. of leaflets in categories of damage				$\Sigma$	$\chi^2$	df	P
		U	N	A+B	C+D				
1	Ac li	591 (599.62)	24 (29.35)	57 (44.33)	60 (59.3)	732	11.72	3	<0.01
	ac li	409 (400.98)	25 (19.65)	17 (29.67)	39 (39.7)	490			
		1000	49	74	99	1222			
2	Ac li	708 (715.86)	32 (30.51)	37 (46.38)	78 (62.25)	855	15.10	3	<0.005
	ac li	465 (457.14)	18 (19.49)	39 (29.62)	24 (39.75)	546			
		1173	50	76	102	1401			
3	Ac li	890 (921.41)	37 (33.86)	51 (45.14)	118 (95.59)	1096	21.93	3	<0.001
	ac li	498 (466.59)	14 (17.14)	17 (22.86)	26 (48.41)	555			
		1388	51	68	144	1651			
4	Ac li	990 (973.38)	32 (31.29)	38 (51.35)	58 (61.98)	1118	9.83	3	<0.025
	ac li	659 (675.62)	21 (21.71)	49 (35.65)	47 (43.02)	776			
		1649	53	87	105	1894			
5	Ac li	570 (601.83)	32 (20.5)	29 (23.91)	83 (67.76)	714	29.36	3	<0.001
	ac li	487 (455.17)	4 (15.5)	13 (18.09)	36 (51.24)	540			
		1057	36	42	119	1254			
$\Sigma$						87.94	15		
Pooled	Ac li	3749	157	212	397	4515	24.15	3	<0.001
	ac li	2518	82	135	172	2907			
Heterogeneity						63.79	12	<0.001	



**Table 4.9.** Results of the choice experiments designed to investigate selective grazing in the absence of clover enzyme (B glucosidase) with the slug *Agriolimax caruanae*. (a) Pair Ac Li/Ac li, (b) Pair Ac li/ac li.

(a) Pair Ac Li/Ac li

Replicate	Morph	No. of leaflets in categories of damage				$\Sigma$	$\chi^2$	df	P
		U	N	A+B	C+D				
1	Ac Li	528 (532.8)	14 (10.3)	8 (5.77)	17 (18.13)	567	3.91	3	< 0.25
	Ac li	765 (760.2)	11 (14.7)	6 (8.23)	27 (25.87)	809			
		1293	25	14	44	1376			
2	Ac Li	733 (715.43)	16 (21.3)	15 (14.5)	28 (40.78)	792	10.56	3	< 0.025
	Ac li	846 (863.57)	31 (25.7)	17 (17.5)	62 (49.22)	956			
		1579	47	32	90	1748			
3	Ac Li	444 (411.63)	18 (20.55)	23 (37.58)	20 (35.23)	505	36.59	3	< 0.001
	Ac li	257 (289.37)	17 (14.45)	41 (26.42)	40 (24.77)	355			
		701	35	64	60	860			
4	Ac Li	1008 (1006.4)	34 (27.01)	20 (24.5)	38 (42.09)	1100	8.17	3	< 0.05
	Ac li	594 (595.6)	9 (15.99)	19 (14.5)	29 (24.91)	651			
		1602	43	39	67	1751			
5	Ac Li	523 (518.36)	13 (11.3)	19 (21.34)	28 (32.01)	583	1.55	3	> 0.50
	Ac li	1129 (1133.64)	23 (24.7)	49 (46.66)	74 (69.99)	1275			
		1652	36	68	102	1858			
$\Sigma$						60.78	15		
Pooled	Ac Li	3236 (3189.17)	95 (86.89)	85 (101.37)	131 (169.57)	3547	24.13	3	< 0.001
	Ac li	3591 (3637.83)	91 (99.11)	132 (115.63)	232 (193.43)	4046			
		6827	186	217	363	7593			
Heterogeneity						36.65	12	< 0.001	

(b) Pair Ac li/ac li

Replicate	Morph	No. of leaflets in categories of damage					$\Sigma$	$X^2$	df	P
		U	N	A+B	C+D					
1	Ac li ac li	577 (579.33)	31 (34.65)	31 (33.85)	42 (43.17)	681	5.24	3	>0.10	
		443 (440.27)	30 (26.35)	11 (18.15)	34 (32.83)	518				
		1020	61	42	76	1199				
2	Ac li ac li	647 (664.58)	40 (33.51)	55 (42.95)	25 (25.96)	767	9.67	3	<0.025	
		761 (743.42)	31 (37.49)	36 (48.05)	30 (29.04)	858				
		1408	71	91	55	1625				
3	Ac li ac li	987 (992.62)	26 (26.5)	26 (33.37)	65 (51.52)	1104	10.21	3	<0.025	
		1036 (1030.38)	28 (27.5)	42 (34.63)	40 (53.48)	1146				
			54	68	105	2250				
4	Ac li ac li	505 (512.09)	12 (18.02)	23 (33.74)	77 (53.15)	617	30.18	3	<0.001	
		603 (595.91)	27 (20.98)	50 (39.26)	38 (61.85)	718				
		1108	39	73	115	1335				
5	Ac li ac li	1052 (1060.39)	17 (16.97)	34 (35.58)	51 (91.06)	1154	5.62	3	>0.10	
		885 (876.61)	14 (14.03)	31 (29.42)	24 (33.94)	954				
		1937	31	65	75	2108				
$\Sigma$						60.92	15			
Pooled	Ac li ac li	3768	126	169	260	4323	19.07	3	<0.001	
		3728	130	170	166	4194				
		7496	256	339	426	8517				
Heterogeneity						41.85	12	<0.001		

Since these results are far from being unequivocal, the hypothesis was tested in a more direct way: the glycoside (linamarin) was added to homogenates of the slug guts. On September 9, 1979, full-sized slugs (mean live weight =  $0.268 \pm 0.008$ ) of A. reticulatus were collected from the lawns at Treborth Botanic Gardens and starved for 48 hrs. The digestive system including buccal organs was then extracted by Dr. N.W. Runham, Department of Zoology, U.C.N.W., and homogenates prepared. Drops of a solution of the clover glucoside linamarin (substrate) were added to the homogenate; sodium-picrate paper tests were carried out with the homogenates and after 48 hrs of incubation (at  $38^{\circ}\text{C}$ ) recordings were made of the picrate reaction: 9 out of the 10 samples gave very clear positive reactions indicating the presence of enzyme in the gut. The remaining sample gave an unclear reaction and was scored as negative. In order to exclude the possibility that  $\beta$  glucosidase in the slugs' gut was derived from residual clover enzyme, another set of 10 animals collected on the same date (September 9, 1979) were starved for a week and gut homogenates were tested as before; 8 of the 10 samples gave positive reaction and 2 were scored as negative. The evidence is therefore fairly conclusive that slugs of A. reticulatus, from this population at least, produce cyanogenic enzymes in the gut - but there is a suggestion again that slugs differ among themselves.

Some field observations on the interaction of molluscs and the cyanogenesis polymorphism in T. repens

Observations had been made on some aspects of the interaction between molluscs and clover in the Henfaes Field, College Farm. This section reports some observations on the spatial distribution of mollusc activity and the spatial distribution

of white clover phenotypes. It was frequently observed that mollusc activity was greater near the edges than in the centre of the field. In the Spring and early Summer of 1979, nocturnal samplings of mollusc activity were made by placing 128 quadrats (35 x 35 cm) at 8 m intervals in a regular pattern throughout the field. A count was made of the number of active molluscs per quadrat. The most abundant species were A. reticulatus, Arion ater and Helix aspersa, but A. caruanae was less common. The mean number of molluscs at each position was calculated and its position in the field defined by a system of X-Y co-ordinates (on the basis of the 8 m grid). Also, in May, small quadrats (10 x 10 cm) were laid in the same positions as those used to sample active molluscs; the number of leaflets of T. repens falling in each of the usual categories of mollusc damage (i.e. U to D) was recorded; from these values a Mollusc Grazing Index (MGI) was calculated (per quadrat) as:

$$\text{MGI} = \frac{A \times 1 + B \times 2 + C \times 3 + D \times 4}{U}$$

and the values of MGI and of active molluscs per quadrat and their co-ordinates were stored in a computer file.

Contour maps of the distribution of (i) mollusc activity and (ii) MGI's in the field were produced by subjecting the data to a Trend Surface Analysis\* (Gittins, 1968). Linear trends (1 to 8 degrees of polynomial fitting) were obtained and maps chosen on the basis of the analyses of variance obtained for each trend. Summaries of the analyses of variance for both features are shown in Table 4.10 a and b. Although F ratios are significant ( $P < 0.05$ ) the proportions

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\* I thank Mr. John Conway (Dept. Soil Science, U.C.N.W.) for making his computer programme available.

**Table 4.10.** Analysis of variance of the regression in the trend surface analysis for the data of number of active molluscs (a), and the Mollusc-grazing Indices (b).

(a)

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F-test
Regression	134.59	14	9.61	2.8075 ; P<0.05
Deviation	95.88	28	3.42	
Total variation	230.47	42		
Goodness of fit	= 0.5840			
Correlation coefficient	= 0.7642			
Trend surface map of degree 4				

(b)

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F-test
Regression	75293.43	35	2151.24	1.7594 ; P<0.05
Deviation	112489.52	92	1222.71	
Total variation	187782.95	127		
Goodness of fit	= 0.4010			
Correlation coefficient	= 0.6332			
Trend surface map of degree 7				

of explained variances are rather low and the maps (Figs. 4.4 a and b) should be regarded with caution; however, the contour map for mollusc activity gave a very simple trend that was easy to compare with a map containing the original field data and a very reasonable fit was obtained which was further improved by correction or addition of information from the original data; therefore, the map for mollusc activity (Fig. 4.4a) is accurate while that for MGI's (Fig. 4.4b) is more complex and less precise. Both maps show a background of symbols which correspond to the location of phenotypes of 100 plants sampled and tested for cyanogenesis in early July 1979.

A tentative correlation between the spatial distributions of the grazing regimes and clover phenotypes was made by counting the numbers of clover samples of each morph that fall into the different grazing areas. The data were assessed by contingency analysis (Tables 4.11 and 4.12). The data for MGI showed no significant associations (Table 4.12), while the contingency of the number of active molluscs shows a tendency for cyanogenic morphs to be over-represented in the areas of heavy grazing and under-represented in the areas of low, or very low grazing activity; the opposite appears to be true for the acyanogenic morphs (Table 4.11 a). Pooling the values for the three acyanogenic phenotypes gives a larger sample size (Table 4.11 b) and shows the associations more clearly ( $P < 0.001$ ). One complication of this analysis is that some clover samples cannot be accurately assigned to a given grazing area. Some of the ambiguous samples were omitted from the analysis. Thus, there appears to be a clear association between mollusc grazing and the distribution of clover morphs in the field.

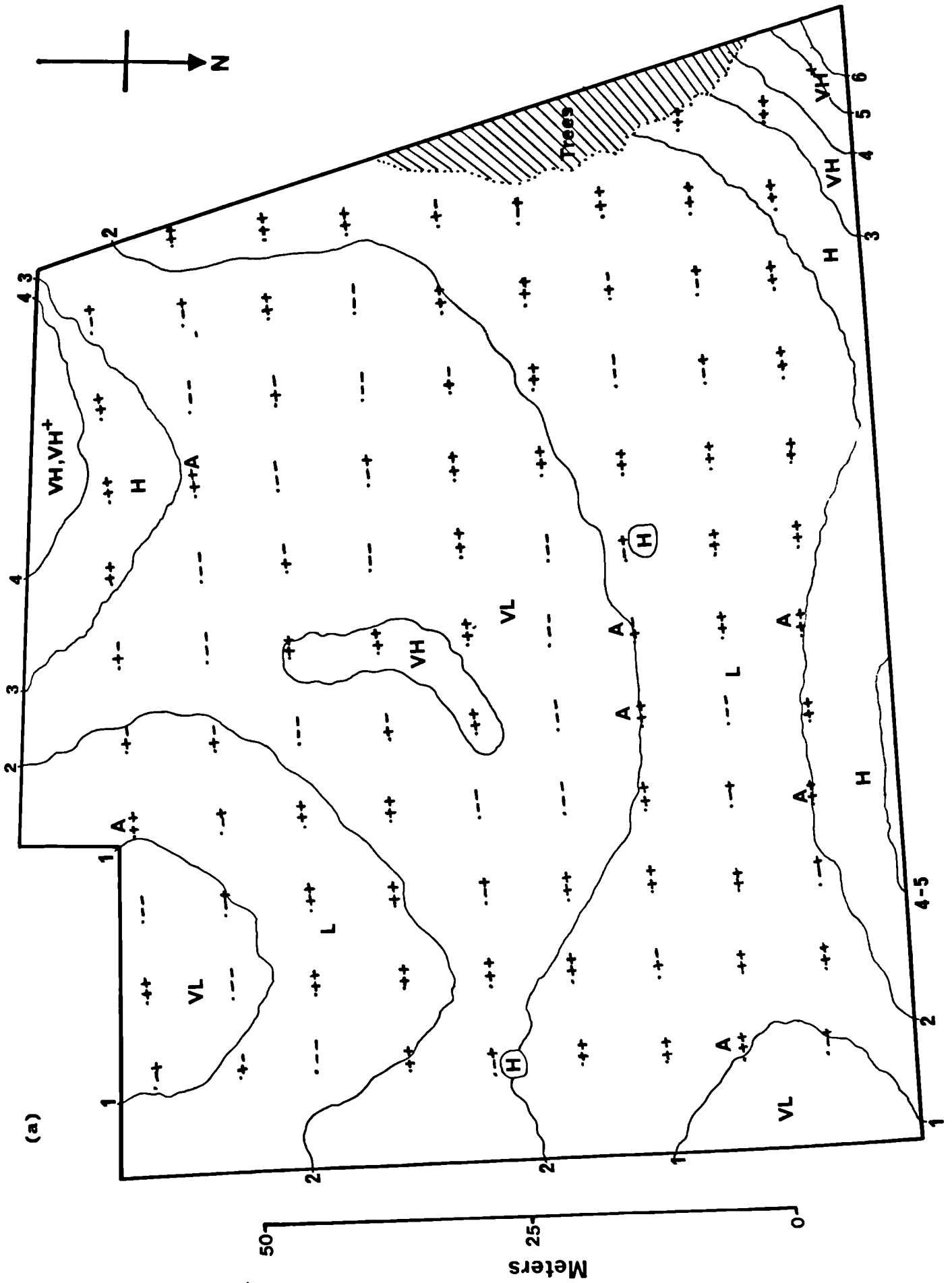
Fig. 4.4.

Contour maps made by a trend-surface analysis of the data of the number of the active molluscs (a) and the Mollusc-grazing Indices (b) at Henfaes Field, College Farm, U.C.N.W., Bangor.

The numbers shown at the edges of the field are the values (the mean number of molluscs or MGI's) that correspond to each contour line.

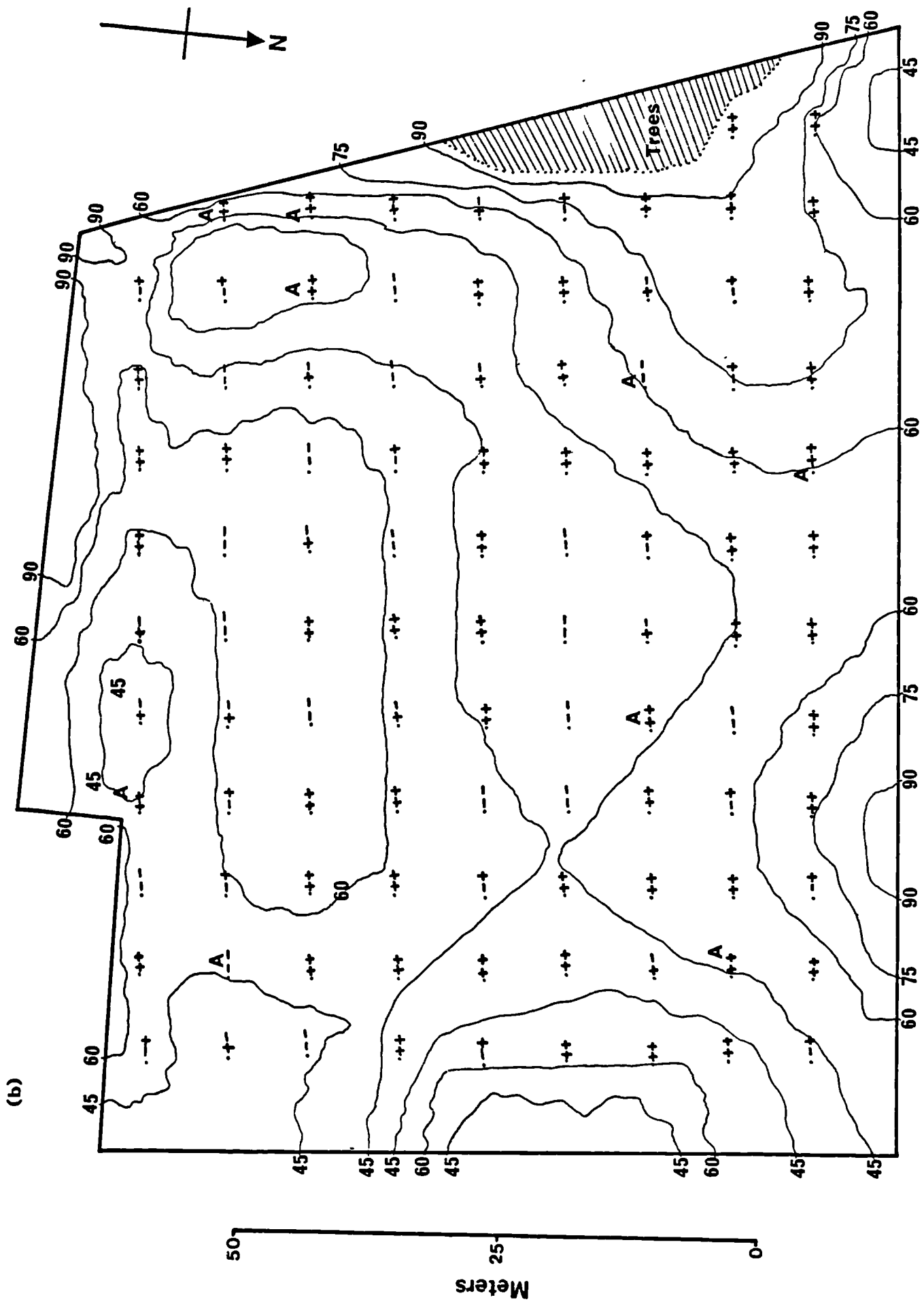
In Fig. 4.4 a: VL = very low (mollusc numbers), L = low, H = high, VH and VH+ = very high.

In both figures the symbols shown indicate the locations of the 4 clover morphs: ++, cyanogenic, +- , glucosidic, -+, enzymatic, --, double recessive. A indicates ambiguous samples not included in the contingency analysis (see text).



(a)





**Table 4.11.** The number of samples of the different morphs and their distribution into areas of different mollusc activity.

(a) Numbers for each of the 4 morphs; (b) Values of the 3 acyanogenic phenotypes pooled into a single group.

(a)

Mollusc Activity Area	Plant Morph				
	++	+-	-+	--	
VH + H	11 (6.45)*	0 (1.42)	1 (1.81)	0 (2.32)	12
L	29 (24.19)	7 (5.32)	6 (6.77)	3 (8.71)	45
VL	10 (19.35)	4 (4.26)	7 (5.42)	15 (6.97)	36
					93

(b)

Mollusc Activity Area	Plant 'phenotype'		
	Cyanogenic	Acyanogenic	
VH + H	11 (6.45)	1 (5.55)	12
L	29 (24.19)	16 (20.81)	45
VL	10 (19.35)	26 (16.65)	36
			93

Log. likelihood test:  $X^2 = 18.78$ ;  $P < 0.001$   
df = 2

\* Expected numbers in parenthesis

Table 4.12. The number of samples of the different morphs and their distribution into areas of different mollusc grazing index (MGI).

(a) Numbers for each of the 4 morphs; (b) Values of the 3 acyanogenic phenotypes pooled into a single group.

(a)

MGI-Area	Plant Morph				
	++	+	-+	--	
75+	5 (3.16)*	0 (0.79)	1 (0.99)	0 (1.05)	6
60-75	10 (11.08)	3 (2.77)	4 (3.46)	4 (3.96)	21
45-60	23 (21.10)	4 (5.27)	7 (6.59)	6 (7.03)	40
45-	10 (12.66)	5 (3.16)	3 (3.96)	6 (4.22)	24
					91

(b)

MGI-Area	Plant 'phenotype'		
	Cyanogenic	Acyanogenic	
75+	5 (3.16)	1 (2.84)	6
60-75	10 (11.08)	11 (9.92)	21
45-60	23 (21.10)	17 (18.90)	40
45-	10 (12.66)	14 (11.34)	24
			91

Log. likelihood ratio test:  $X^2 = 4.02$ ;  $P = 0.26$   
 $df = 3$

## DISCUSSION

The experimentalist usually assumes that if he simplifies a biological system, the major determinant will emerge more clearly than against the full background of natural 'noise'. In the present study this was not the case. Simple experiments involving leaf discs and molluscs in petri dishes gave largely inconsistent and inconclusive results. Similar simple experiments reported in the literature have given similar inconclusive results. For example, Crawford-Sidebotham (1972b) tested the acceptability of cyanogenic and acyanogenic leaves of Trifolium repens and Lotus corniculatus to a large number of species of molluscs in petri dishes and obtained highly variable results. Some mollusc species appeared to select acyanogenic material in both species of plants; some species did not differentiate and others differentiated between phenotypes of only L. corniculatus (e.g. A. reticulatus), while others differentiated between the phenotypes of only T. repens (e.g. Arion hortensis); he suggested that with the molluscs he studied, selection against cyanogenic forms was more apparent on L. corniculatus than T. repens but he was aware that his interpretations were subject to large standard errors, and he commented (page 409): "These conclusions, therefore, should not be treated as exact for any one mollusc/plant combination, but rather all the experiments should be considered together to demonstrate patterns".

The ecological relevance of acceptability trials in petri dishes (e.g. Dirzo, 1980) is thrown into doubt in the light of the experiments described here. In theory, they provide the most direct test of acceptability - in practice, they fail to reveal real differences that are apparent in field studies. The failure of the acceptability

tests with leaf discs in petri dishes may be because: (i) HCN is released in gaseous form from the discs and accumulates on the dishes and hinders choice-making by the slugs, (ii) T. repens (even acyanogenic forms), is a relatively unacceptable diet and it may be that under these circumstances selectivity will not operate until a 'starving threshold' has been reached (see Dirzo, 1977 and Chapter 2), (iii) it is probably over simplistic simply to compare cyanogenic vs. acyanogenic material, because there is great variation in the degree to which the character is expressed.

There is much inconsistency in the literature of cyanogenic and grazing animals; this is shown in Table 4.13. Several points are apparent from this table: (i) of the 22 different reports included, 13 are suggestive of selection between morphs while 9 are not, (ii) some species (e.g. Agriolimax reticulatus, Helix aspersa) are reported to be selective by one author, and non-selective by another, (iii) other species appear to be consistently selective (e.g. Arion ater), (iv) most of the variation of results comes from the differences between the 10 species of molluscs reported; note that some of the herbivores were not identified, (v) only two reports for vertebrate mammals are included here; Corkill's (1952) observations suggest that rabbit-grazing was more marked in plots of his acyanogenic morphs but there was no evidence of selection by sheep.

Although some of the reports cited in Table 4.13 represent casual observations, many come from formal experimental analysis or apparently accurate field observations, and these similarly give conflicting results. The present study makes it clear that quite small variations in the detail of the experimental procedures can lead to quite different results.

It is clear from the present investigation that (both in laboratory and field) cyanogenesis is a relative and not an absolute defense against mollusc grazing: the

Table 4.13. A compilation of reports of experiments or field observations giving evidence of selective or non-selective feeding between cyanogenic and acyanogenic phenotypes of Trifolium repens.

Herbivore	Result	Type of observation and source of information
<u>Agriolimax reticulatus</u>	Selective Selective Non-selective Non-selective	Intact leaves in boxes (Angseesing, 1974) A field survey (Angseesing & Angseesing 1973) Potted plants (Bishop & Korn, 1959) Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Agriolimax sp.</u>	Selective	Observation from experimental field plots (Corkill, 1952)
<u>Arianta arbustorum</u>	Non-selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Arion ater</u>	Selective Selective Selective	Intact leaves in boxes (Angseesing, 1974) A field survey (Angseesing & Angseesing, 1973) Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Arion hortensis</u>	Selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Arion subfuscus</u>	Non-selective	Intact leaves in boxes (Angseesing, 1974)
<u>Cepaea hortensis</u>	Selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Cepaea nemoralis</u>	Non-selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Helicella virgata</u>	Selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Helix aspersa</u>	Selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
<u>Theba pisana</u>	Selective	Detached leaves in petri dishes (Crawford-Sidebotham, 1972b)
'grasshoppers'	Non-selective	Field observations (Daday, 1965)
rabbits	Selective	Observations from experimental field plots (Corkill, 1952)
sheep	Non-selective	Observations from experimental field plots (Corkill, 1952)
'slugs'	Selective	Observations from experimental field plots (Daday, 1955)
unidentified	Non-selective	Seedlings experimentally introduced into the field (Miller <u>et al.</u> , 1975)
unidentified	Non-selective (but tendency)	A field survey

defensive role of cyanogenesis was expressed as (i) an under-representation of heavily damaged cyanogenic leaflets and (ii) an excess of undamaged and nibbled cyanogenic leaflets; the opposite was true for the acyanogenic leaves. A general, ideal model of this interaction derived from the results of contingency analysis is shown in Fig. 4.5, using in this case the data from Agriolimax reticulatus in Experiment 3.

The excess of nibbled leaflets of the cyanogenic phenotype in many of the grazing trials is, presumably, indicative of 'sampling trials' made by the molluscs. Since no aposematic features appear to be associated with the cyanogenesis polymorphism, 'sampling' may be the only mechanism by which animals can determine acceptability or unacceptability. Clearly this is an inefficient mechanism of protection - the best protected plants suffer some damage. Molluscs have poorly developed visual senses and there may be few cues that could deter such predators.

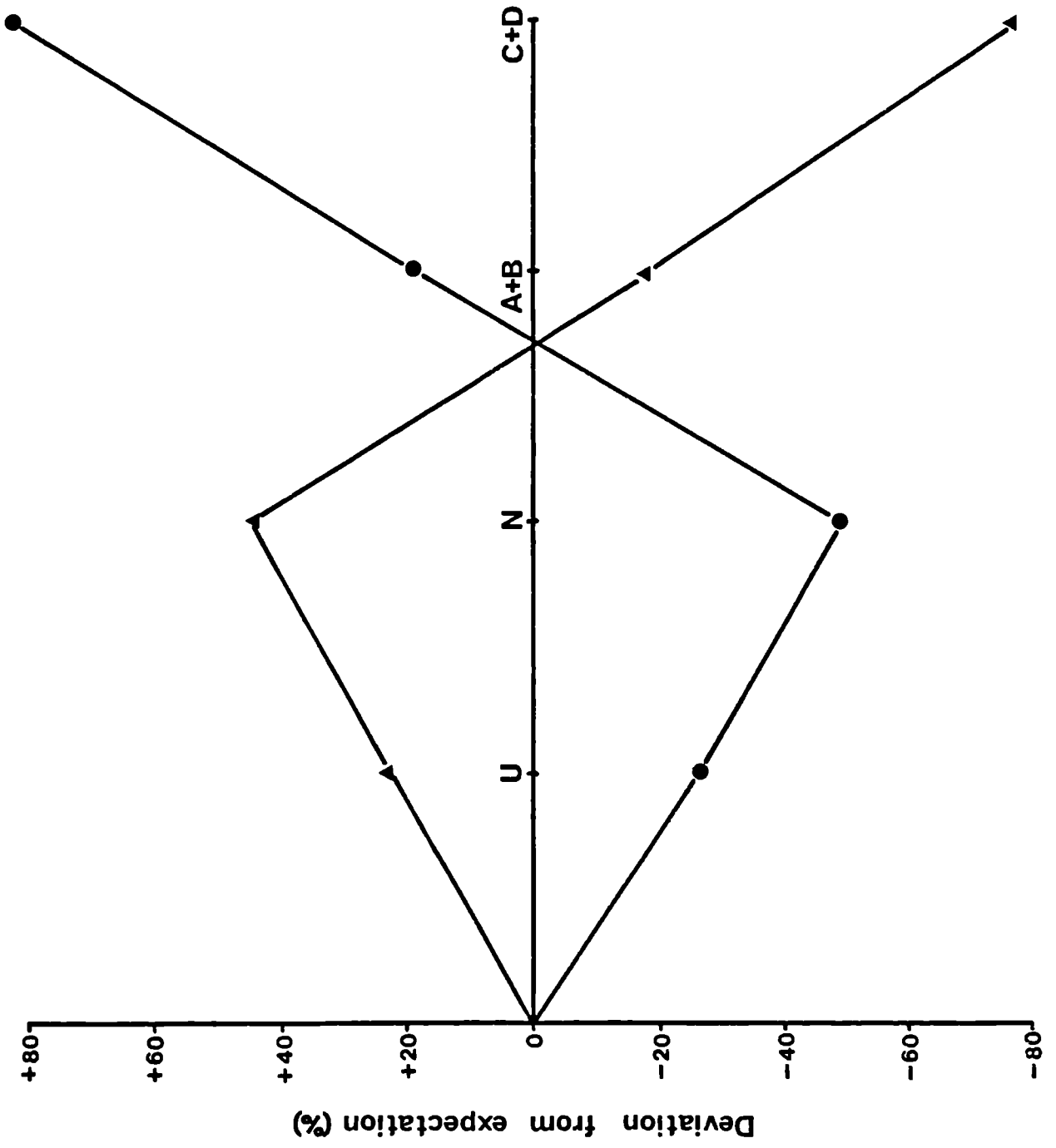
The differences in the growth rate of animals eating cyanogenic and acyanogenic diets were not very conclusive (see Fig. 4.3). Excess food was always provided and the animals ate different amounts. The differences in growth made with the three monotonous diets appeared to be related to the amount of food that was taken. When slugs were offered cyanogenic clover it was left largely uneaten; the fact that cyanogenic plants are nibbled suggests that recognition of distastefulness occurs in the buccal organs.

In grazing trials involving a choice between cyanogenic and glucosidic plants (Tables 4.8 and 4.9), slugs largely preferred glucosidic plants; therefore, it has to be assumed that there are some crucial differences between the response of the slug facing cyanide evolved by the plant and 'tasted' and cyanide formed when the glucoside meets enzymes in the slugs' gut. Detoxifying mechanisms may exist

Fig. 4.5.

Graphical representation of the contingency analysis for Agriolimax reticulatus (pooled values of 4 replicates).- ▲—▲ , cyanogenic, ●—●, acyanogenic. U = untouched, N = nibbled, A = up to 25% of the leaflet taken, B = 25-50% taken, C = 50-75% removed, D = 75-100% taken.





in the digestive tract of the slug. Cyanide-detoxifying mechanisms are not uncommon in other animals; for example, Apanteles zygaenarum which is a parasite of the cyanogenic Zygaena spp. larvae has the enzyme Rhodanase which can detoxify cyanide (Jones, 1962); the diapausing pupae of Hyalophora cecropia and other silk worms are supposed to be cyanide-resistant because of the high ratio of cytochrome oxidase to cytochrome C (Kurland and Schneiderman, 1959).

Cates (1975) has described a situation very similar to that of slugs and white clover. The plant Asarum caudatum is eaten by the slug Ariolimax colombianus. The plants were polymorphic (dimorphic) for acceptability to slugs and also for growth rate and seed production. Where slugs were scarce, populations of A. caudatum were composed mainly of the acceptable morph with rapid growth rates, and high seed outputs; he hypothesised that in the absence of slugs, this morph was at a competitive advantage over the unacceptable morph, the unacceptable morph being favoured under grazing pressure. He tested this hypothesis by artificial grazing of monocultures and 50:50 mixtures, and his results supported the view that the acceptable morphs were the more vigorous competitors. My results from the experiment with free-ranging slugs (Fig. 4.2) show that the competitive superiority of acyanogenic forms is only marginal; clearly the possibility of differences between the clover morphs, other than their relative acceptability (e.g. competitive potential) is an aspect that deserves further attention.

Demonstration of coevolutionary interactions between animals and plants relies on retrospective interpretation of presently existing features in both interactants. Jones (1973) has written a detailed essay on coevolution and cyanogenesis; the arguments he used were the defensive role of cyanogenesis in T. repens and L. corniculatus and physiological or biochemical processes present in animals which

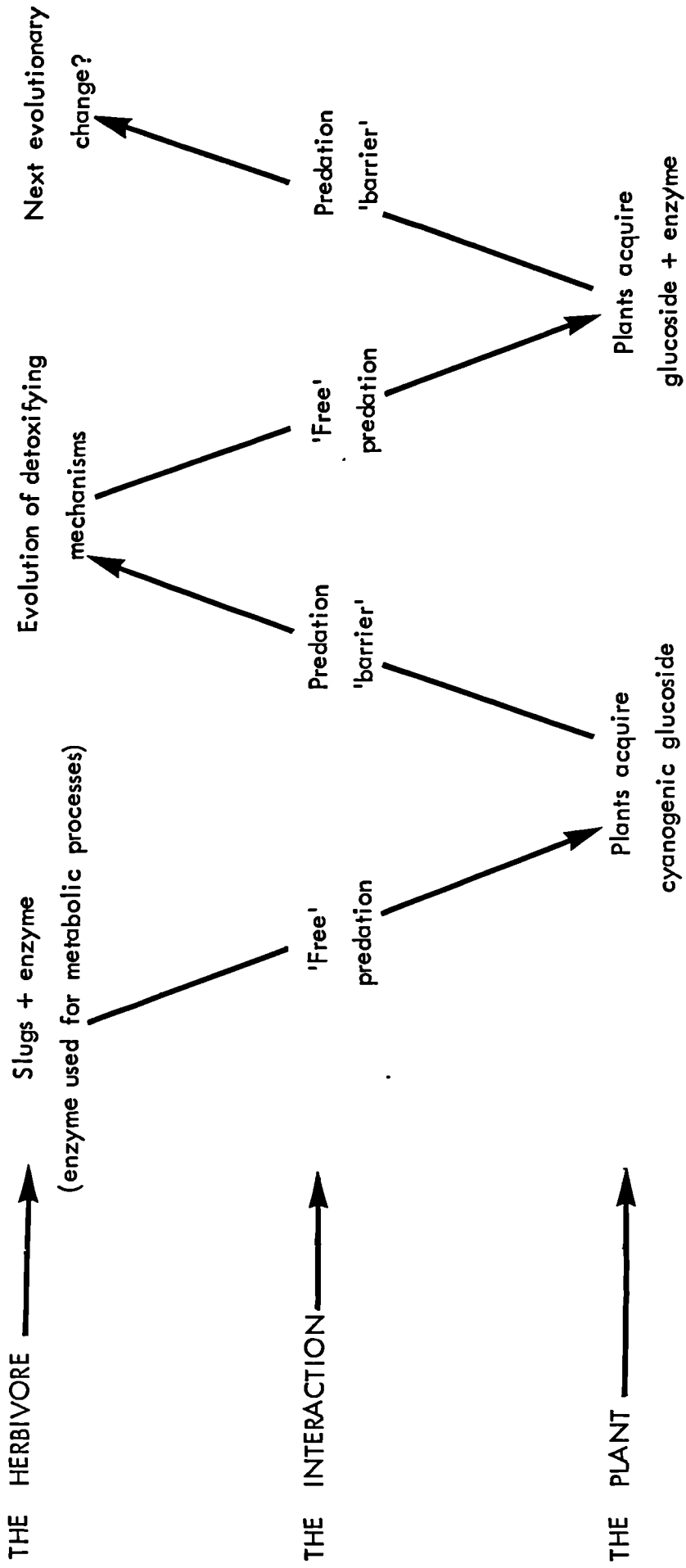
unable them to graze cyanogenic plants. But coevolution in the original conception of Ehrlich and Raven (1965) implies a series of changes of both the plant and the animal in a step-wise fashion and only a few studies exist in which different plant and animal features are (retrospectively) interpreted as the result of step-wise coevolutionary changes. One such case is that of Lupinus spp. (leguminosae) and Glaucopsyche lygdamus (lepidoptera) (Dollinger et al., 1973). The lupins contain a variety of alkaloids which are variable in relation to the distribution of the Glaucopsyche larvae; in localities where the larvae are abundant, even the lupins which contain many alkaloids are heavily predated, but these alkaloids are usually combined in a given predictable mixture; in contrast, plants that contain three or four compounds in individually different mixtures are only lightly predated. The coevolutionary interpretation given is that individual variability has evolved in response to previous especialisation by the predator. Information of this sort appears to be lacking in the cyanogenesis/herbivore system although some of the findings reported in this chapter might be interpreted as compatible with a coevolutionary interpretation. A tentative coevolutionary pathway for the mollusc/clover interaction could be hypothesised as follows. In an initial situation, the molluscs carry  $\beta$  glucosidases (related to food digestion). These animals are common predators of initially acyanogenic plant phenotypes. Mutation and/or recombination are sources capable of including cyanogenic glucosides in the chemical repertoire of the plant. Production of such compounds is likely to be enhanced by natural selection if it confers some protection against herbivory. The animals now face an unacceptability barrier that selects for the evolution of some sort of detoxifying mechanism. In slugs such a mechanism probably does exist already, judging from the readiness with which they accept non-cyanogenic but

glucosidic plants. The next step would be selection for cyanide release as soon as a leaf is bitten - favouring any mutant clover capable of hydrolysing the glucoside. On this interpretation, the herbivore is due to make the next co-evolutionary move. This hypothesis is summarised in Fig. 4.6.

One puzzle in this retrospective interpretation is that it might be expected that natural selection would favour the linkage of the glucosidic and enzymatic loci: in fact, segregation is independent (Corkill, 1942).

A number of studies on different topics of the interaction are needed, for example, (i) further biochemical and physiological study of the feeding activities of molluscs and (ii) a search for differences between the three acyanogenic morphs. There may be aspects of ecological specialisation (e.g. competitive vigour) among these three forms that explain the balancing of the polymorphism in nature. The field data which allowed analysis of the spatial distribution of areas of mollusc activity and cyanogenesis morphs in T. repens should be extended. Ultimately, the interpretation of a polymorphism in nature must depend on observations of the fates of morphs in nature - studies on individuals in the real environment. The interaction between slugs and clover cyanogenesis morphs is only a tiny aspect of the biological interactions affecting clover in the field. Turkington and Harper (1979a) found that there is a very dynamic process of local invasions and extinctions of T. repens in the field, indeed different parts of the field are probably at different stages of the cycle at any given time. If this is true, spatial and temporal variations in the structure of the vegetation may bring with them changes in the temporal and spatial distribution of the cyanogenetic morphs. The distribution of areas of mollusc activity is also likely to vary in details with time, though some general trends, e.g. molluscs being abundant nearby the sheltered areas of the field

Fig. 4.6. A hypothetical coevolutionary interpretation of the mollusc/cyanogenesis interaction in T. repens.



(the north and south edges; see Fig. 4.4) and generally scarce towards the centre of the field, probably persist over periods larger than the clover regeneration cycle. A similar association between the distribution of cyanogenesis morphs in Lotus corniculatus in areas of different mollusc-activity is reported by Ellis et al. (1977a). Some maritime populations of L. corniculatus in Holy Island, Anglesey, were looked at in detail and they showed that the distribution of the cyanogenic form is directly related to the nature of the sites. Areas that were less exposed to wind and wind-borne salt, where terrestrial molluscs occur in greater abundance, had the greatest frequency of cyanogenic plants; in sites on the cliffs close to the sea where wind exposure and salt sprays were more common, molluscs were scarce and non-cyanogenic plants were the most frequent. Neither in this study or in my own is there any indication of how this distribution of phenotypes comes about. Presumably, in areas heavily grazed by molluscs selective grazing puts the acyanogenic morphs at a competitive disadvantage and leads to their exclusion. It may be that this process operates most strongly at the seedling stage, although establishment of T. repens from seed appears to be rare in pastures. In a field survey carried out in October 1978, out of 100 sampling points observed, only 4 seedlings were found. An intra-specific demographic study dealing with cyanogenesis polymorphic plants would be of the greatest value for interpreting several points on the population biology of cyanogenesis.

The various aspects of the interaction mollusc/cyanogenesis polymorphism in white clover described in this chapter, indicate that this is a useful system to combine laboratory experimentation and field observation; moreover, some of the results obtained from laboratory work, were confirmed by field experiments;

the logical extension of this work is to study in more detail the response of cyanogenic and acyanogenic morphs to mollusc grazing as well as to the rest of biological interactions occurring in the field.



CHAPTER 5

THE PERFORMANCE OF CYANOGENIC  
AND ACYANOGENIC MORPHS OF  
TRIFOLIUM REPENS IN THE FIELD

## INTRODUCTION

Perhaps the fundamental test of the performance of a plant population can be made only by following in detail the fate of individuals in the field where the whole spectrum of selective forces (climatic factors, neighbours, predators and pathogens) interact to shape the survivorship and fertility schedules of the population.

Such study - the demography of plants - has been seriously developed only recently (see Harper and White, 1974), but virtually no information exists on the demographic behaviour in the field, of variants or forms of a given species - the intraspecific demography of plants.

No attempts seem to have been made to evaluate at a local scale the performance of cyanogenic and acyanogenic morphs in white clover in the field; instead, studies have been made involving rather large scale patterns. Daday (1954a) studied the distribution of frequencies of the different morphs in Europe. Samples collected from localities of different latitude showed a clear decrease in the frequency of cyanogenic morphs with the increase of latitude; he found a direct relationship between the frequency of both the glucosidic (Ac) and enzymatic (Li) alleles and the mean winter temperature. A similar relationship was found by sampling clover populations from different altitudes in the Alps (Daday, 1954b). Transplants of 6 week old seedlings into coastal (warm) and alpine (cold) habitats in New Zealand showed a superiority (in terms of the proportion of flowering plants and dry matter production) of the cyanogenic (Ac Li), glucosidic (Ac li) and enzymatic (ac Li) phenotypes over the double-recessive (ac li) morphs (Daday, 1965). This set of results clearly suggested a selective advantage of the dominant alleles under warm

conditions and a selective disadvantage at low temperatures. He claimed that winter temperature is a major factor responsible for the maintenance of the polymorphism and proposed that the physiological basis for the selective differences was that cold temperature caused HCN liberation and consequently tissue death. Foulds and Grime (1972 a,b) took this physiological explanation and suggested that, not just cold temperatures, but any agents that caused physical damage to the leaves, could cause cyanide release and tissue death and therefore, should select against cyanogenic morphs. They hypothesised that drought should act in this way and found (i) a reduction in the frequency of the glucosidic plants (regardless of the presence or absence of the Li locus) from populations sampled in Derbyshire and (ii) that under experimental conditions of severe drought there was a greater mortality of plants possessing the Ac allele; sub-lethal drought reduced the reproductive vigour of the cyanogenic (Ac Li) plants in comparison to the other three morphs.

Paim and Dean (1976) studied morphological and physiological characteristics of white clover in an attempt to find differences in fitness between cyanogenic and acyanogenic plants but their study was criticised by Jones (1978) on the grounds of inadequacy of sampling and analysis.

More recent work (Foulds, 1977 and Foulds and Young, 1976) has failed to give definite support to the conclusions of the work of Daday (1954 a,b, 1965) and Foulds and Grime (1972 a,b). Jones (1973) has criticised some of this earlier work and commented on the dangers of judging effects on alleles rather than on phenotypes. Moreover, although this early work is indicative of some of the potential selective forces operating on the cyanogenesis polymorphism it does not reveal the nature of the mechanisms that might maintain the different morphs in a

balanced polymorphic system within a given population. Cahn and Harper (1976), studying another polymorphic system (leaf mark polymorphism) in white clover, suggested that "more detailed analysis (within a localised area), with or without perturbation may be needed to isolate the real events that determine the life and death of individuals".

This chapter describes a study of the behaviour of acceptability.- contrasting plants of Trifolium repens transplanted as cuttings into the field and observed for one year. The aims of the study were: (i) to follow the fates of cyanogenic and acyanogenic individuals in the field, (ii) to evaluate the defensive character of cyanogenesis when the plants are exposed to a range of factors besides the herbivores and (iii) to detect any other selective factors that might interact with the cyanogenesis polymorphism in white clover.

## MATERIALS AND METHODS

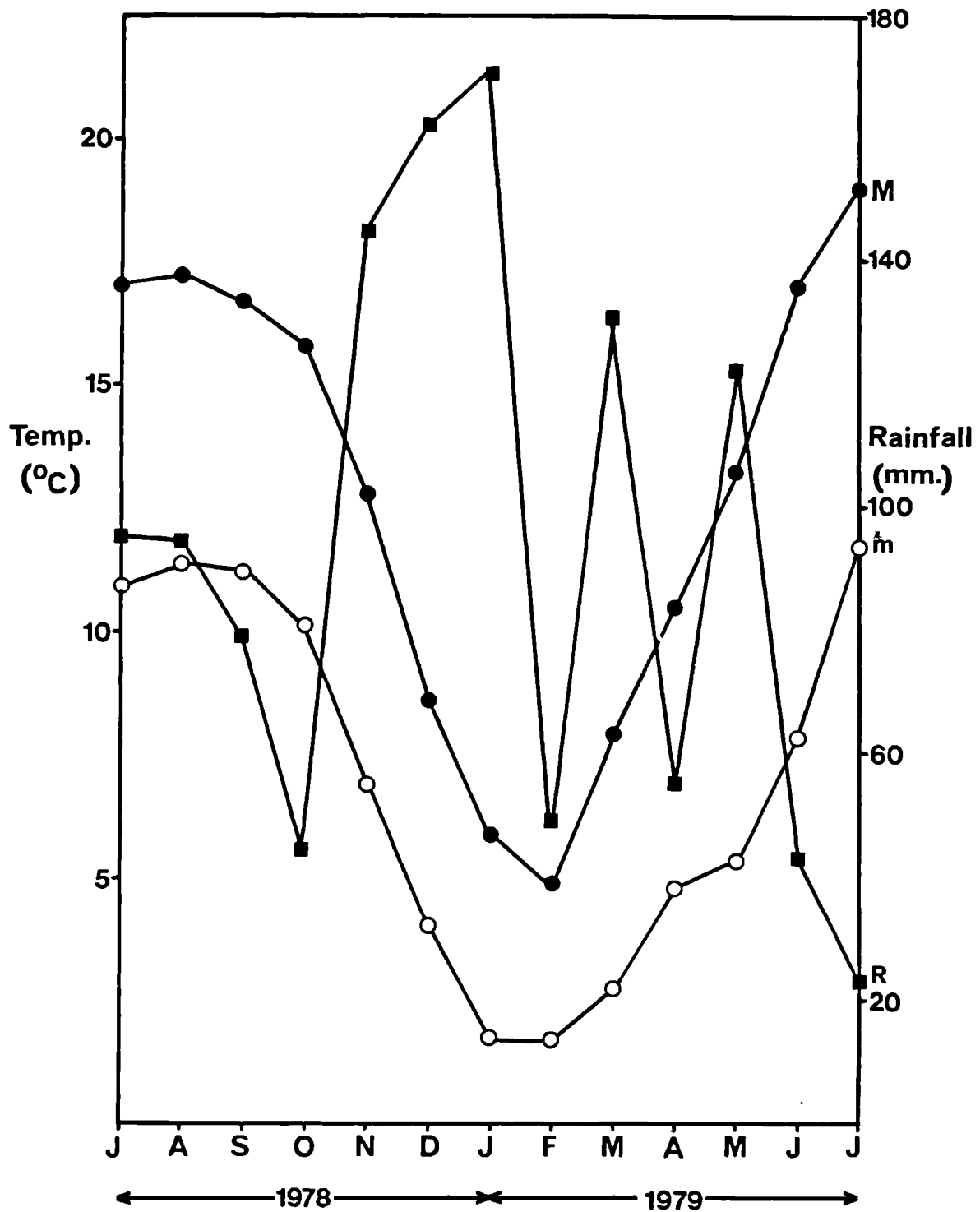
### The site of study

The study was made in a 1 ha. field of permanent grassland at Henfaes, Aber, Gwynedd in North Wales (Nat. Grid. Ref. SH 733656). Details of the history, management as well as descriptions of the vegetation and climate are given in Sarukhán and Harper (1973) and in Turkington and Harper (1979a); the field has not been ploughed nor received any chemical treatment for over 50 years. The field is bounded on two sides by hedges; the other two sides are bounded by a stone wall with a row of ash trees and by wire fencing respectively. Climatic data for most of the period of study was obtained from the College Farm Meteorological Station at Aber; data for the last three months was not available from this station and was obtained from the Meteorological Station at Bangor. Maximum and minimum temperatures and rainfall per month are shown in Fig. 5.1. The winter of 1978-79 was particularly cold with a minimum of only 1.71°C in February; the period November 1978-May 1979, except February and April was also particularly wet. Frost covered the ground for a very long period from November 1978 till May 1979. The sward has a marked season of growth of approximately 6 months (April-September).

Trifolium repens is abundant throughout most of the field except near the areas shaded by the ash trees. During the time of this study (July 1978-July 1979), only sheep have grazed the field but previously it has also been grazed by cattle and horses. Other herbivores occurring in the field include molluscs (Agriolimax reticulatus, Arion ater, Helix aspersa, and less abundantly, Arion hortensis and Agriolimax caruanae) and weevils (unidentified). Rabbits and other small mammals

Fig. 5.1.

Maximum (M) and minimum (m) temperatures and rainfall (R) during the period covered by the study.



were not seen; wood pigeons were seen only occasionally.

### The experiment

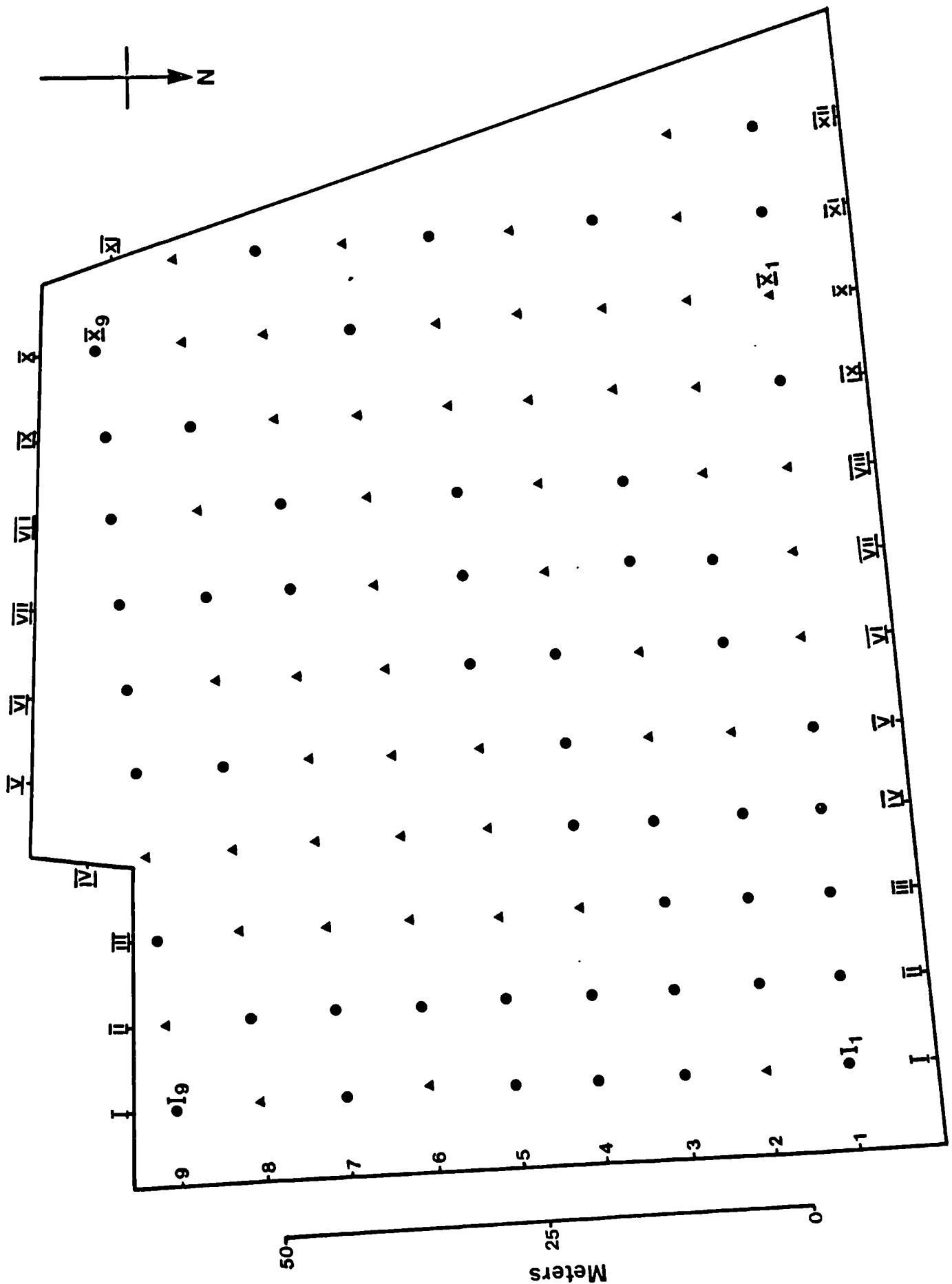
Soil cores were collected from the field in January 1978; from these, stolons of T. repens were sorted and separated and then propagated in wooden boxes in a glasshouse. Sodium-picrate tests (see Materials and Methods, Chapter 4) were made to distinguish the four possible cyanogenesis morphs. In June 1978, cuttings were obtained from 5 strongly cyanogenic (Ac Li) and 5 acyanogenic (ac li) plants. The cuttings were prepared by removing sections of 3 cm of stolon with one leaf and axillary bud; these were then planted into small jiffy pots (3 x 3 x 5 cm) filled with J.I. Compost No.1 and left for 1 week in a mist-spray unit after which 10 cuttings of each of the 5 cyanogenic and acyanogenic clones were chosen (on the basis of uniformity of size) for field transplanting.

For the introduction of transplants, a grid was established in the field with equidistant points 8 m apart (see Fig. 5.2); the cuttings were then randomly allocated to each of the 100 points of the grid. The fifty cuttings of each morph were transplanted into the field on July 3, 1978. For subsequent relocation of the transplants, wooden pegs were sunken into the soil on two parallel sides of the field; the pegs were labelled with numbers I-XII (indicated in Fig. 5.2); a string with labelled distances at 8 m could be fixed between two parallel pegs to indicate with good approximation, the position of the transplants along each line. Also, aluminium pegs were buried in the soil at exactly 25 cm away from the transplants and in line with the string; the location of the aluminium peg with a metal detector accurately showed the position of each cutting. To facilitate location, all introduced cuttings were tagged with a red plastic ring.



Fig. 5.2.

The location of the cyanogenic (▲) and acyanogenic (●) transplants of Trifolium repens in the field at Henfaes, Aber. I-XII indicate the position of parallel pegs used for the relocation of transplants. Numbers 1-9 were used to designate individual transplants, e.g. 1<sub>1</sub>, 1<sub>9</sub>, etc.



Recordings of plant survival were made monthly and observations on plant growth and grazing were made at intervals of ca six weeks. Notes were made of any other events (e.g. pathogen damage) observed at any recording date.

## RESULTS

### Plant survival

A possible way of attaching standard errors to the data on plant survival is by calculating the mean ( $\pm$  SE) number of surviving plants per clone of each morph. Table 5.1 shows this treatment of the data; there is a tendency for better survival of the acyanogenic morph but the standard errors overlap in all but four months (October 1978, March, April and May 1979). An alternative treatment of the data is to pool for each date the values from the five clones of each morph and compare the rates of survival. The validity of this procedure was assessed as follows: where applicable,  $X^2$  tests were made to investigate heterogeneity of plant survival between the clones; in no case was the difference between clones significant (at  $P = 0.05$ ), so the data have been pooled for each morph and shown as a percentage of plant survival (log scale) throughout the period of study (Fig. 5.3). There was a remarkable similarity in the trends of survivorship of both morphs, but the cyanogenic plants showed a consistently lower survival and at the end of the study 22% of the acyanogenic cuttings but only 14% of the cyanogenic remained alive; the difference between morphs appears to be more marked towards the end of winter. Deaths were rare during the resting period of the sward (October-February); in contrast, the risks of death increased in the parts of the growing seasons (of both years) included in this study - the greater hazards in the life of a cutting appeared to be when its neighbours were growing rapidly.

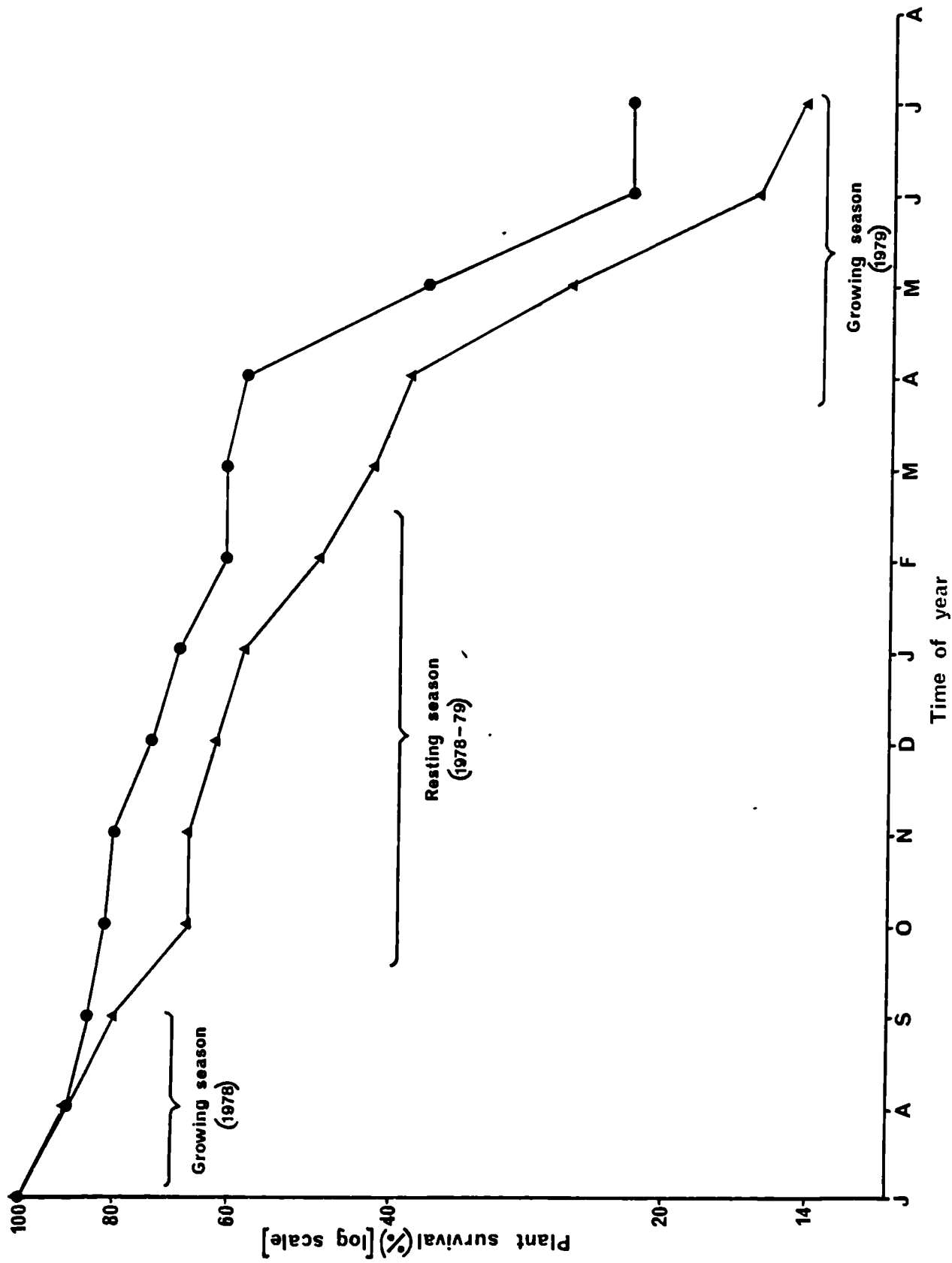
Statistical comparison of the survivorship between morphs was made (i) with the Kolmogorov-Smirnov two sample test (Campbell, 1974); this test failed

Table 5.1. The mean number of surviving plants per cyanogenic (Ac Li) and acyanogenic (ac li) clone of Trifolium repens. Means of 5 clones (+ S.E.); initial number of plants per clone = 10.

Month	Morph	No. of surviving plants per clone $\pm$ S.E.
July 1978	Ac Li	10 $\pm$ 0
	ac li	10 $\pm$ 0
August	Ac Li	9.00 $\pm$ 0.55
	ac li	9.00 $\pm$ 0.45
September	Ac Li	8.00 $\pm$ 0.84
	ac li	8.60 $\pm$ 0.60
October	Ac Li	6.60 $\pm$ 0.75
	ac li	8.20 $\pm$ 0.80
November	Ac Li	6.60 $\pm$ 0.75
	ac li	8.00 $\pm$ 0.95
December	Ac Li	6.20 $\pm$ 0.66
	ac li	7.20 $\pm$ 0.67
January 1979	Ac Li	5.80 $\pm$ 0.58
	ac li	6.80 $\pm$ 0.80
February	Ac Li	4.80 $\pm$ 0.73
	ac li	6.20 $\pm$ 0.66
March	Ac Li	4.20 $\pm$ 0.58
	ac li	6.00 $\pm$ 0.84
April	Ac Li	3.80 $\pm$ 0.66
	ac li	5.80 $\pm$ 1.02
May	Ac Li	2.60 $\pm$ 0.40
	ac li	3.60 $\pm$ 0.40
June	Ac Li	1.60 $\pm$ 0.51
	ac li	2.20 $\pm$ 0.37
July	Ac Li	1.40 $\pm$ 0.51
	ac li	2.20 $\pm$ 0.37

Fig. 5.3.

The time course of survival of transplants of the two morphs of Trifolium repens. ▲—▲, cyanogenic; ●—●, acyanogenic. (Initial sample size = 50).



to detect significant difference ( $P > 0.05$ ), and (ii) with a contingency test of the number of surviving cuttings at each recording date with the following model:

	No. of surviving plants	No. of dead plants	Σ
Ac Li morph			50
ac li morph			50
	<hr/>		
Σ			100

Only one comparison (April 1979) yielded a significant difference ( $P < 0.05$ ), suggesting again, a greater risk of death for the cyanogenic morph towards the end of winter-early spring.

#### The growth of cyanogenic and acyanogenic plants

Plant growth can be measured in a variety of ways ranging from the traditional weight measurements to the recently developed approaches based on a demographic analysis of plant units (see Bazzaz and Harper, 1976). In the present study, estimates of the growth of both morphs were made simply on the basis of counts and measurements of several plant units. A detailed study of the demography of leaves of T. repens and other species in this field was carried out at the same time by Peters (1980).

#### Leaf production

No significant difference in the number of leaves per plant was found between clones of the two morphs; therefore comparisons between morphs were made on the pooled values.



All the transplanted cuttings started with a photosynthetic capital of 2 open leaves, and 1 week after introduction of the transplants they had increased their leaf number per plant (cyanogenic =  $3.94 \pm 0.22$ ; acyanogenic =  $4.53 \pm 0.15$ ). Figure 5.4 shows the number of expanded leaves (including those damaged by herbivores or senescent) per plant. There was a clear seasonality of leaf production with a peak of 23 leaves per plant in September and a minimum of 1 leaf per plant in February. For most of the growing season the rate of leaf production was faster in the acyanogenic morphs, but the difference was un-significant during the 'resting' period (November-April). The large standard errors arising towards the end of the study are largely due to the small number of plants surviving to this time.

#### Stolons per plant

Figure 5.5 shows the mean number of stolons produced by each transplant at each recording date. Stolon branching occurred soon after transplanting. By late July, plants of the acyanogenic morph had on average two stolons per plant and the number continued to increase throughout the growing season. Cyanogenic plants behaved similarly but regularly produced fewer stolons. No new stolons were formed from October onwards and the number present declined between November and April. From December onwards, there was no significant difference in the number of stolons per plant between the two morphs; a tendency for superiority of the acyanogenic morphs is evident again in the growing season of 1979 but the large standard errors (associated with the small sample sizes) overlap throughout.

Fig. 5.4.

The number of open leaves per plant of the two morphs of Trifolium repens. ▲—▲, cyanogenic; ●—●, acyanogenic. (Means of the number of surviving plants at each recording date  $\pm$  S.E.).

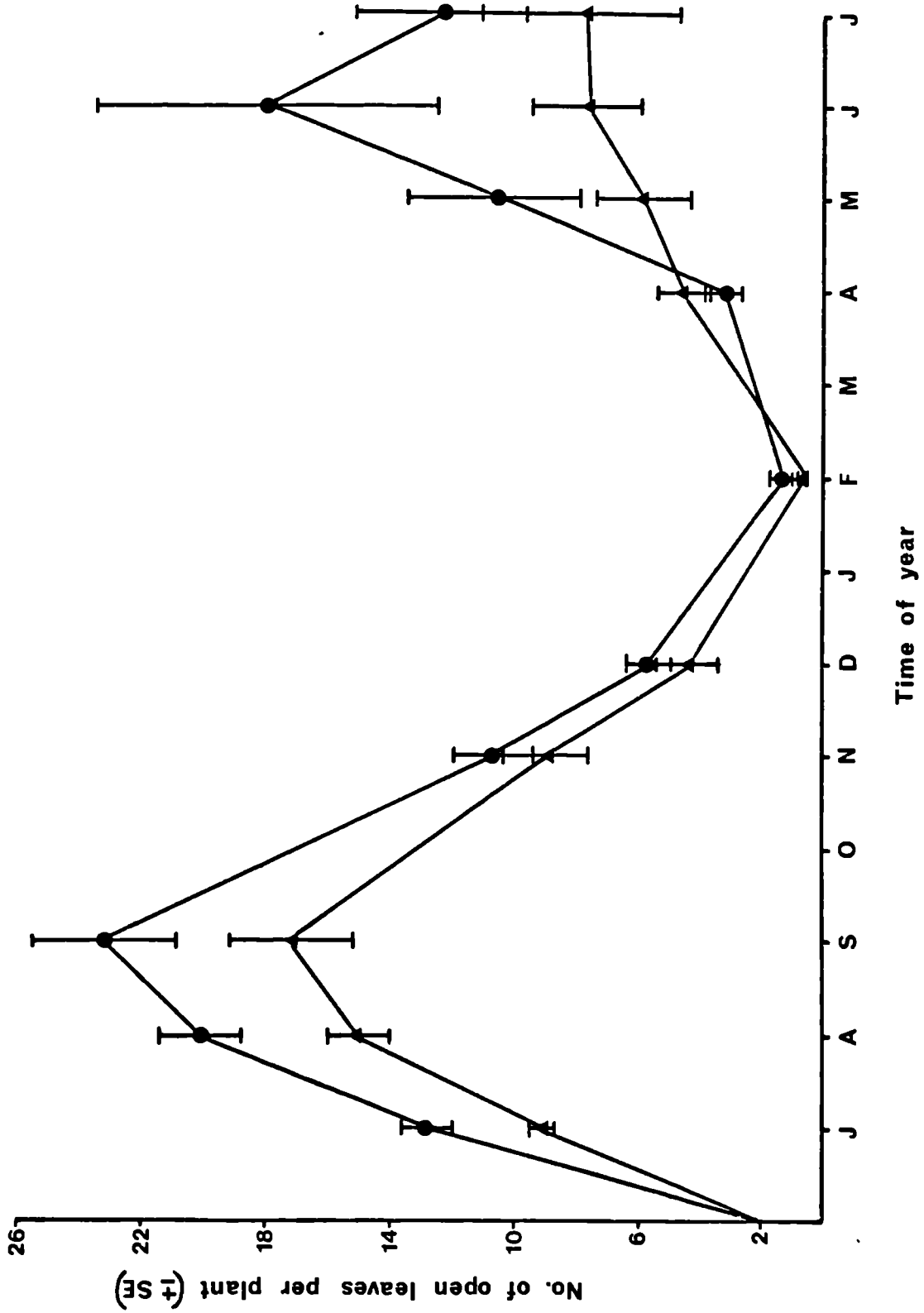
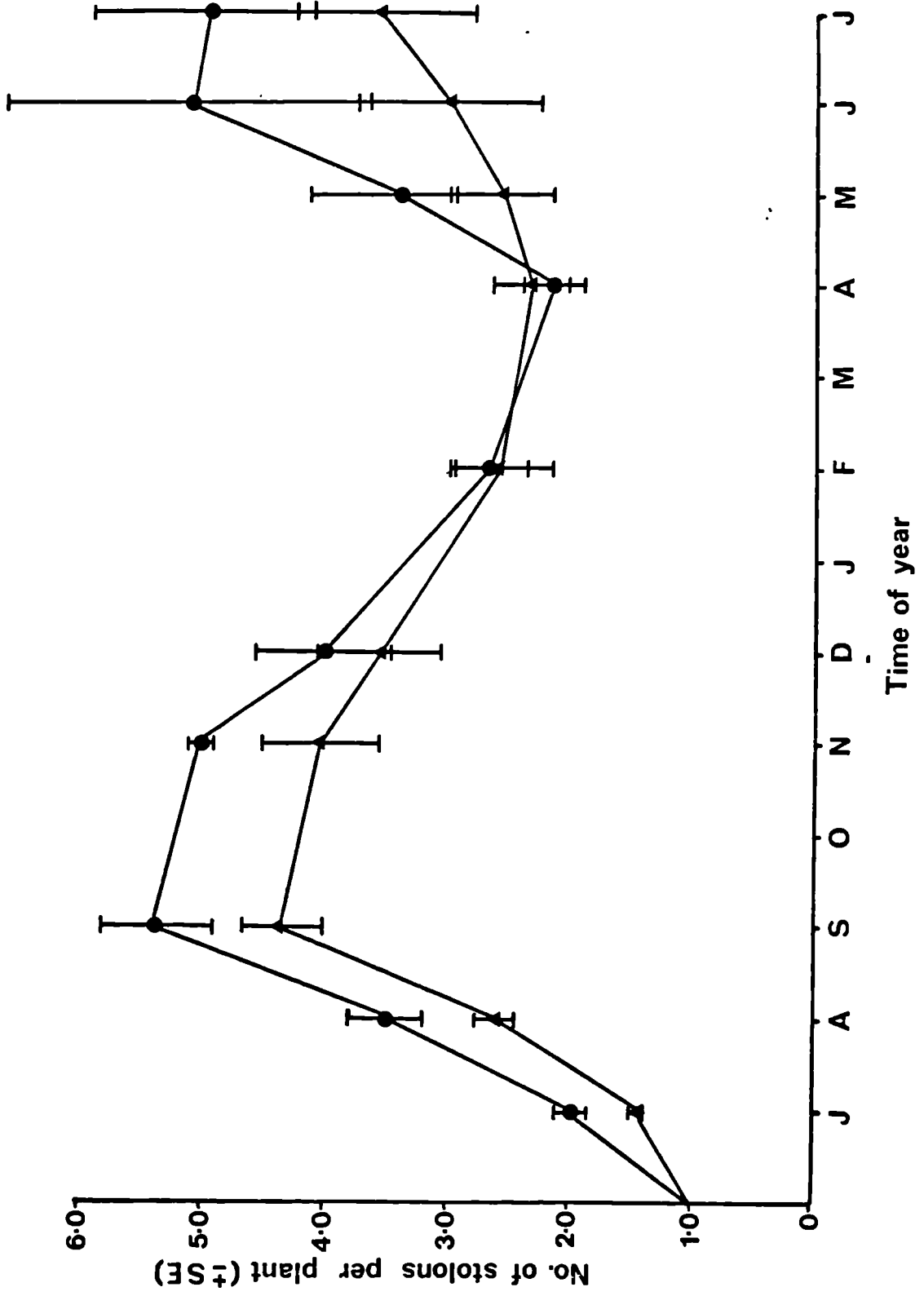


Fig. 5.5.

The number of stolons per plant of the two morphs of Trifolium repens. ▲—▲, cyanogenic; ●—●, acyanogenic. (Means of the number of surviving plants  $\pm$  S.E.).



One of the reasons for the drop in the number of stolons during winter and early spring was stolon decay. A recording was made of the number of stolons decaying in late November-early December; the data for both morphs, together with a  $2 \times 2$  contingency analysis are shown in Table 5.2. There was a significant ( $P < 0.025$ ) excess of decaying stolons of the cyanogenic morph. The consequences for the plant of this tissue decay were of three kinds: (i) loss of whole stolons, (ii) loss of segments of stolons and (iii) a combination of (i) and (ii). Only two plants were observed in which tissue decay separated ramets that both remained alive after winter, usually the separated sections died soon afterwards.

Measurements were made of the length of stolons. Figure 5.6 shows the total length of stolon per plant of the two morphs. On four recording dates (July 1978 and May, June, July 1979) the acyanogenic morphs bore marginally longer stolons but at other times the stolon lengths of the two morphs were virtually the same.

#### Herbivore grazing

Three major types of herbivore damage were detected throughout the study: (i) mollusc damage, (ii) weevil damage and (iii) sheep damage. While damage of types (i) and (ii) was quite unmistakable, damage by sheep might have been confused with the damage made by other large animals (e.g. rodents). 'Sheep' damage occurred continuously throughout the 12 months of study, while damage by molluscs and (especially) weevil damage was very seasonal. Details of the extent of grazing activities of the three herbivore groups in the pasture are reported in Peters (1980); the present study emphasises only the interaction

Table 5.2. The number of decaying stolons of cyanogenic and acyanogenic plants of Trifolium repens in late November - early December and contingency analysis.

	No. of decaying stolons	No. of non-decaying stolons	Σ
Ac Li	31 (22.81)*	103 (111.19)	134
ac li	25 (33.19)	170 (161.81)	195
Σ	56	273	329

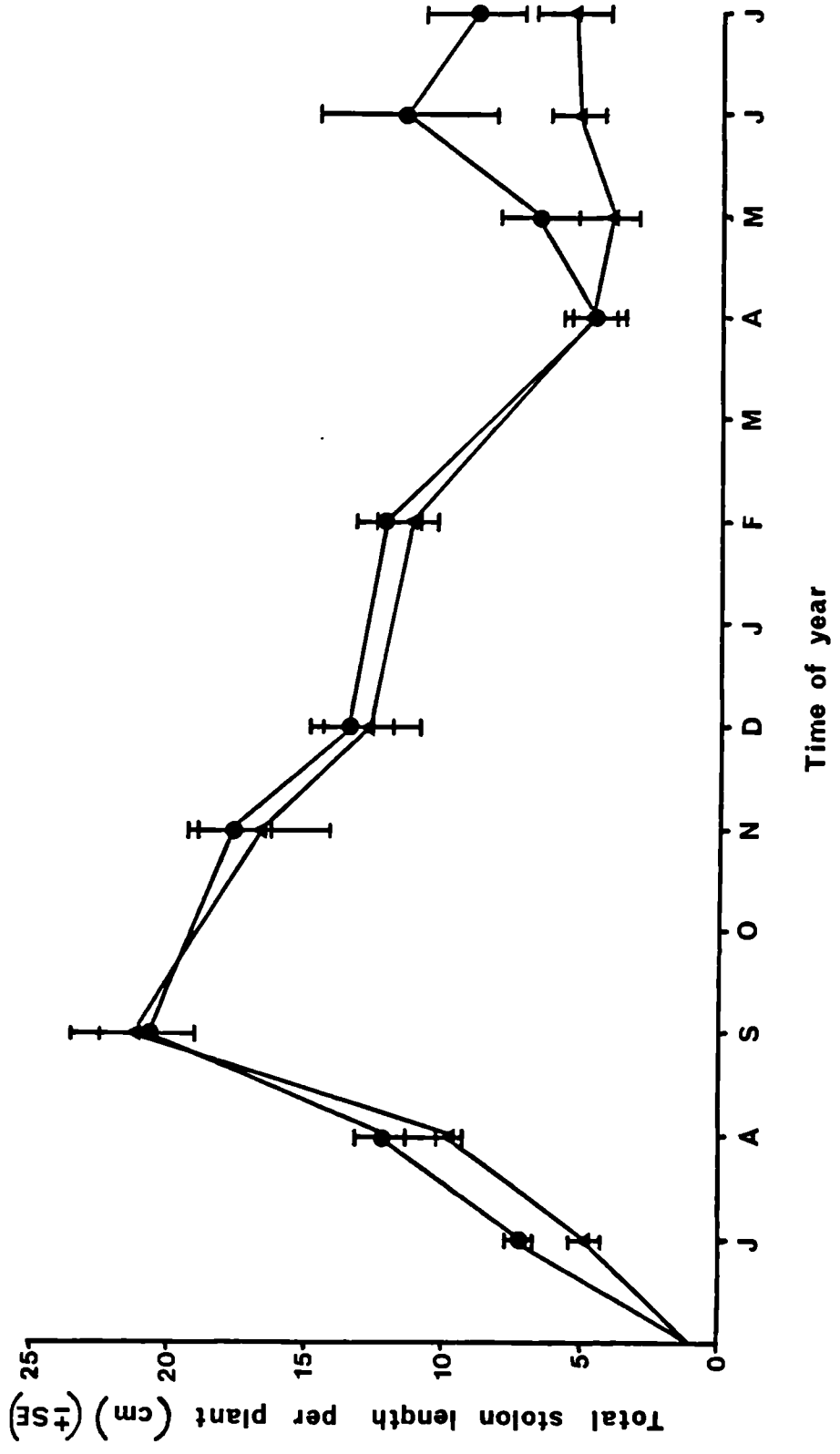
$$X^2 = 5.97; \quad df = 1; \quad P < 0.025$$

\* Numbers in parenthesis are the expected values

Fig. 5.6.

The total stolon length per plant (cm) of the two morphs of Trifolium repens. ▲—▲, cyanogenic; ●—●, acyanogenic. (Means of the number of surviving plants  $\pm$  S.E.).





between grazing and the cyanogenesis polymorphism.

Mollusc grazing

At each recording date, counts were made of the number of leaflets of the cyanogenic and acyanogenic transplants falling into the categories of mollusc damage described in the previous chapter (i.e. U = untouched, N = nibbled, A = up to 25% of the leaflet removed, B = 25% to 50% removed, C = 50% to 75% removed, D = 75% to 100% removed; see Plate 1).  $\chi^2$  heterogeneity tests were made (i) to investigate the existence of inter-clonal differences in susceptibility to mollusc grazing, and (ii) to detect which clones were responsible for heterogeneity. Comparisons (by means of  $\chi^2$  contingency analysis) were then made of the damage suffered by cyanogenic and acyanogenic morphs as described in the previous chapter (see e.g. Table 4.5). There was a considerable heterogeneity between the clones of both morphs and this had the following consequences: (i) comparisons were made separately for each of the clones that caused heterogeneity and (ii) inter-clonal heterogeneity reduced the sample sizes for the comparisons and in the contingency analysis table the damage categories A and B and C and D were pooled; in all cases the contingency table was as follows:

Morph	No. of leaflets in category of damage				Σ
	U	N	(A+B)	(C+D)	
Ac Li					
ac li					
Σ					N

with  $(4-1) \times (2-1) = 3$  degrees of freedom. A summary of the resulting  $X^2$  values is given in Table 5.3. In all, 36 comparisons were made of which 29 gave a significant  $X^2$  indicating preferential grazing of the acyanogenic forms; only 7 comparisons failed to reach significance at  $P = 0.05$ . Most of the significant comparisons showed a pattern similar to the graphic model proposed for the contingency analysis (see Fig. 4.5). Only the data for November and December 1978 could be treated as homogeneous so that comparisons could be made with the pooled data. Results for both months are displayed graphically in Fig. 5.7; again, the picture obtained closely resembles the proposed contingency analysis model (cf. Fig. 4.5).

Quite convincingly, this whole set of results confirm the defensive role of cyanogenesis against mollusc grazing; here again, as concluded in the previous chapter, mollusc damage is markedly reduced on cyanogenic clover but slight damage or at least nibbling occurred in the cyanogenic forms - clearly cyanogenesis greatly reduces but does not wholly prevent molluscs from grazing on clover.

#### Weevil grazing

Weevils leave quite characteristic evidence of their damage on clover leaves; they chew the leaflets from the abaxial side making small circular holes (ca 1 mm diameter), but usually leave a thin layer of upper epidermis intact. Weevil grazing occurs chiefly during summer; in this study, recordings were made which gave sample sizes large enough for analysis only in July, August and September. Three categories of damage were distinguished: U = untouched, N = nibbled (up to 3 holes) and H = more than 3 holes. No distinction could be made between the degree of grazing suffered by the different clones of each

Table 5.3. The resulting  $X^2$  values from the contingency tests comparing the mollusc grazing scores (see text) on cyanogenic and acyanogenic morphs of Trifolium repens. The data are tabulated separately for each recording date. The clones of each morph were arbitrarily designated by the letters A to E (cyanogenic), and a to e (acyanogenic); as a convention, [ ] enclose the non-significantly heterogeneous clones whose values have been pooled for the analysis.

(i) July 1978

[abcd] e

[ABCDE] 30.05\*\*\* 58.31\*\*\*

Result ac li preferred ac li preferred

(ii) August 1978

a b c d e

[ABCDE] 36.50\*\*\*

72.63\*\*\*

101.38\*\*\*

29.50\*\*\*

12.33\*\*\*

Result ac li preferred

ac li preferred

ac li preferred

ac li preferred

ac li preferred

(iii) September 1978

	a	b	c	d	e
A	14.85*** ac li preferred	0.43 n.s. no selection	8.21* ac li preferred	6.40 n.s. no selection	9.36 ac li preferred
B	31.57*** ac li preferred	37.41*** ac li preferred	17.07*** ac li preferred	42.66*** ac li preferred	47.0*** ac li preferred
C	8.65* ac li preferred	9.89* ac li preferred	1.99 n.s. no selection	15.82** ac li preferred	13.51** ac li preferred
D	14.14** ac li preferred	16.58*** ac li preferred	2.69 n.s. no selection	19.56*** ac li preferred	26.25*** ac li preferred
E	13.23** ac li preferred	7.44 n.s. no selection	3.45 n.s. no selection	11.17* ac li preferred	19.24*** ac li preferred

(iv) November 1978

[abcde]

[ABCDE] 18.51\*\*\*  
ac li preferred

(v) December 1978

[abcde]

[ABCDE] 16.67\*\*\*  
Result ac li preferred

(vi) May 1979<sup>1</sup>

---

[abcde]

---

[BCDE]	1.07 n.s.
Result	no selection

---

(vii) June 1979<sup>1</sup>

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[abce]

---

[BCDE]	13.91***
Result	ac li preferred

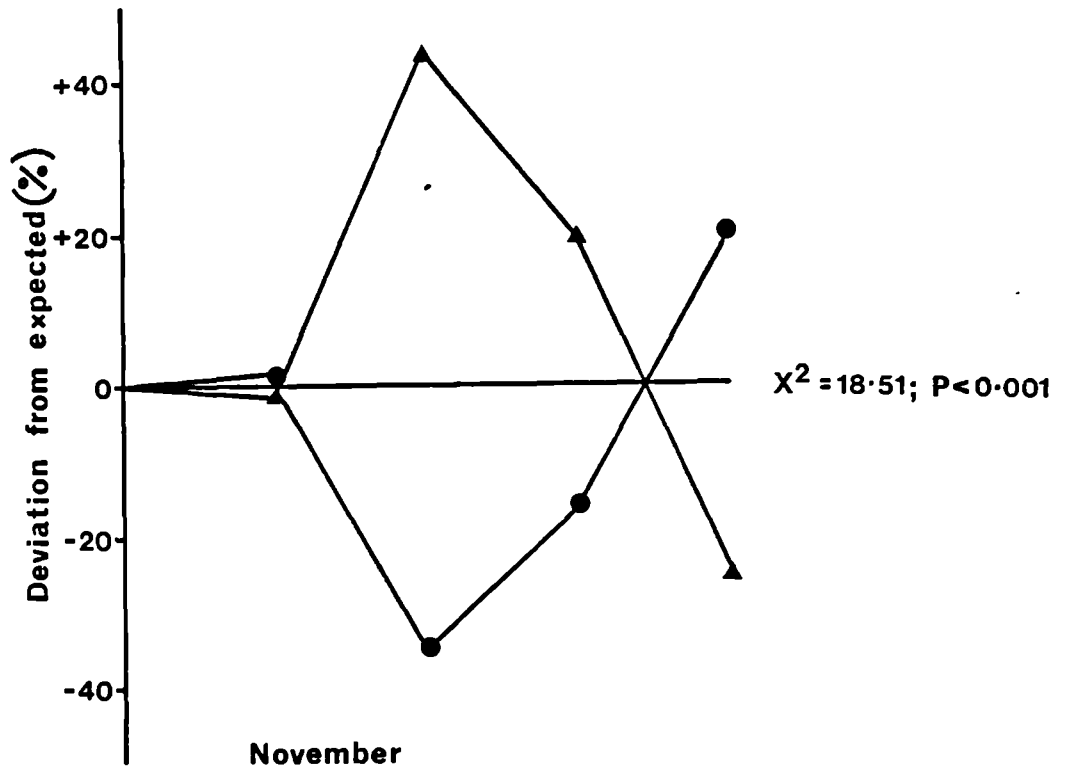
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<sup>1</sup> Small sample sizes; only 2 damage categories included: U = untouched, D = damage; therefore the tests are made with 1 degree of freedom.

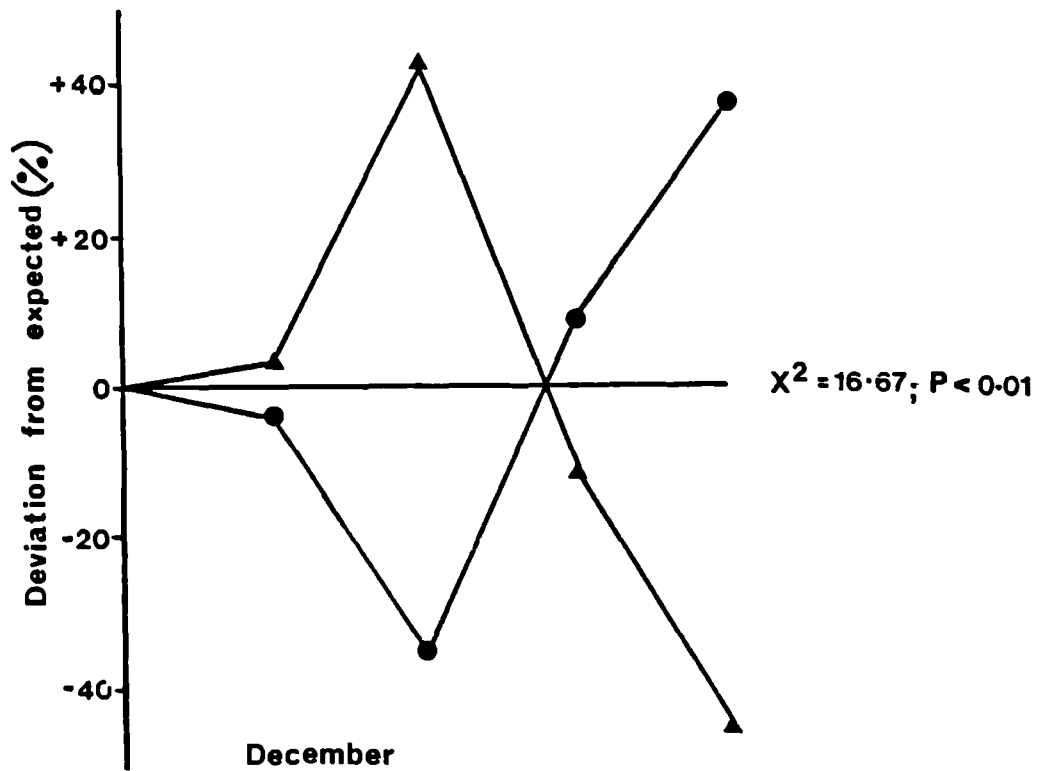
\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001

Fig. 5.7.

Deviations from expected (%) of the numbers of cyanogenic (▲) and acyanogenic (●) leaflets of Trifolium repens in the following categories of damage by molluscs: U = untouched, N = nibbled, AB = up to 50% of the leaflet taken, CD = 50-100% taken.



Damage score U N AB CD





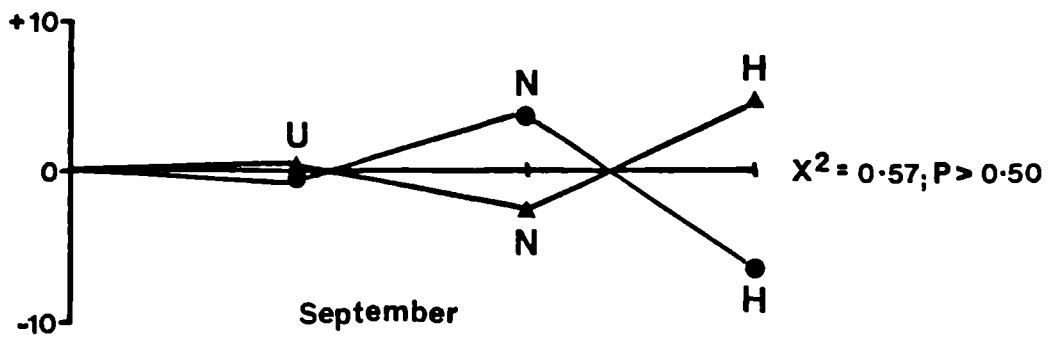
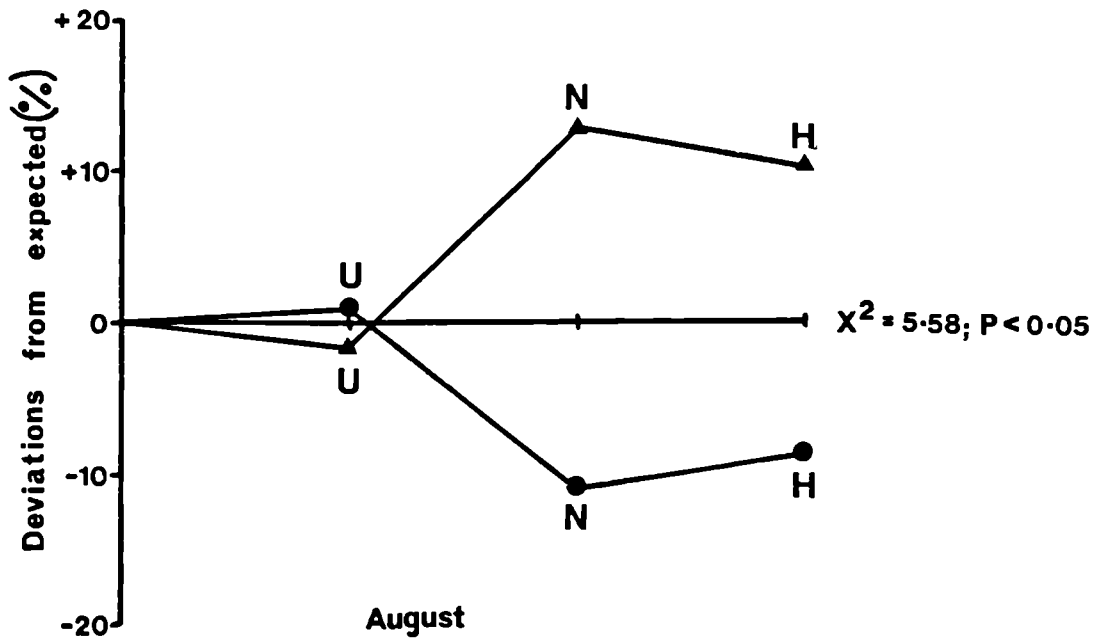
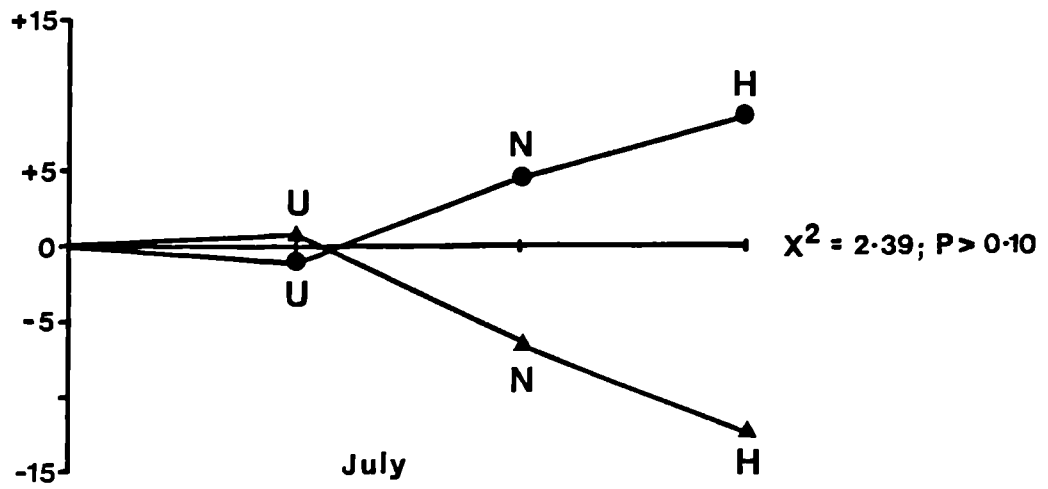
morph and the data have been pooled. Figure 5.8 shows the deviations from expectation of the numbers of cyanogenic and acyanogenic leaflets falling in each category damage and the corresponding  $X^2$  values. In July, acyanogenic leaflets suffered an excess of nibbling and heavy damage. Cyanogenic leaflets were damaged less often than expected by chance but the differences do not reach statistical significance ( $P > 0.10$ ). In August, by contrast, an excess of cyanogenic leaflets had been eaten and acyanogenic leaflets were under-represented in the nibbled and heavily damaged categories; the results significantly departed from expectation ( $P < 0.05$ ); in September, the observed values did not depart from expectation ( $P > 0.50$ ). This whole set of results is quite unexpected, but suggest that the interaction weevil/cyanogenesis may be quite complex. It is possible that the different results obtained in the different months reflect the interaction of clover with different weevil populations including cyanide-tolerant ones, e.g. Hypera plantaginis (Parsons and Rothschild, 1964).

### Sheep grazing

Sheep were the herbivore that removed by far the largest number of clover leaves of both morphs. In general, the size of a sheep 'bite' removed complete leaves and, although at each recording date counts were made of the number of leaflets damaged, analysis of data was made on damage to leaves. Only 2 categories of damage were distinguished: U = undamaged and D = damaged = whole leaf removed. As in the case of mollusc grazing, heterogeneity tests were made to investigate inter-clonal differences in the ratio of U/D leaflets. Enough data for statistical analysis were obtained only for the period July-December 1978. 2 x 2 contingency tests (i.e. 2 damage categories and 2 clover morphs) were made

Fig. 5.8.

Deviations from expected (%) of the numbers of cyanogenic (▲) and acyanogenic (●) leaflets of Trifolium repens in the following categories of damage by weevils: U = untouched, N = nibbled, H = heavy grazing (see text).



to test for preferential grazing between morphs. Table 5.4 shows the inter-clonal heterogeneities and the resulting  $X^2$ 's for the appropriate comparisons. As in the case of molluscs, since all the comparisons have 1 degree of freedom, the  $X^2$  values (when significant) give an indirect measure of the degree of preferential grazing. Of the 25 comparisons made, nearly half (i.e. 12) gave no evidence of preferential grazing ( $P > 0.05$ ). Of the remaining 13, 7 showed significant preference for the acyanogenic and 6 for the cyanogenic morphs. There is evidence that the sheep discriminates some clones between morphs but the direction of discrimination is not consistent, or groups of clones were chosen at one time, but avoided at another. All the instances of preferential grazing on the acyanogenic morphs occurred in July, August and September and most preferential grazing of cyanogenic morphs occurred in November and December. A graphic representation of these results is shown in Fig. 5.9; this marked shift in clover morph preference is quite puzzling and is an aspect that warrants further study.

#### Damage by pathogens

Pathogenic infection of the leaves of white clover was observed soon after the introduction of transplants. Two main pathogens were observed: Cymadothea trifolii and Uromyces trifolii\*. Infection by C. trifolii occurred only during a limited period (August-September) and only a few leaves were damaged. At the peak of infestation by this pathogen (September) a  $X^2$  test revealed no difference ( $P > 0.10$ ) in the number of infected leaflets of the two morphs. The rust, U. trifolii, persisted for a much longer period (July-November) and affected a larger number of leaves. Damage was almost exclusively to the cyanogenic

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\* I thank Dr. J. Burdon for identifying the pathogens.

Table 5.4.

The resulting  $X^2$  values from the 2 x 2 contingency tests (see text) comparing sheep grazing on cyanogenic and acyanogenic morphs of Trifolium repens. The 5 clones of each morph were arbitrarily designated by the letters A to E (cyanogenic) and a to e (acyanogenic); clones enclosed in [ ] were non-significantly heterogeneous and their values have been pooled for the analysis.

(i) July 1978

[abcde]

[ABCDE]

7.47\*\*

Result ac li preferred

(ii) August 1978

a

b

c

d

e

[ABCDE]

0.14 n.s.

6.57\*

0.05 n.s.

7.46\*\*

29.34\*\*\*

Result no selection

ac li preferred<sup>1</sup>

no selection

ac li preferred

ac li preferred

(iii) September 1978

	a	[bcd]	e
A Result	2.68 n.s. no selection	9.74** Ac Li preferred	0.04 n.s. no selection
B Result	4.93* ac li preferred <sup>1</sup>	1.80 n.s. no selection	12.40*** ac li preferred
ECDEJ Result	0.40 n.s. no selection	0.84 n.s. no selection	9.12** ac li preferred

(iv) November 1978

	a	b	c	d	e
[ABCDEJ] Result	1.16 n.s. no selection	9.54** Ac Li preferred	4.05* Ac Li preferred <sup>1</sup>	1.71 n.s. no selection	0.28 n.s. no selection

(v) December 1978

	a	b	c	d	e
[ABCDE]	4.98*	~0 n.s.	0.06 n.s.	3.91*	8.01**
Result	Ac Li preferred <sup>1</sup>	no selection	no selection	Ac Li preferred <sup>1</sup>	Ac Li preferred

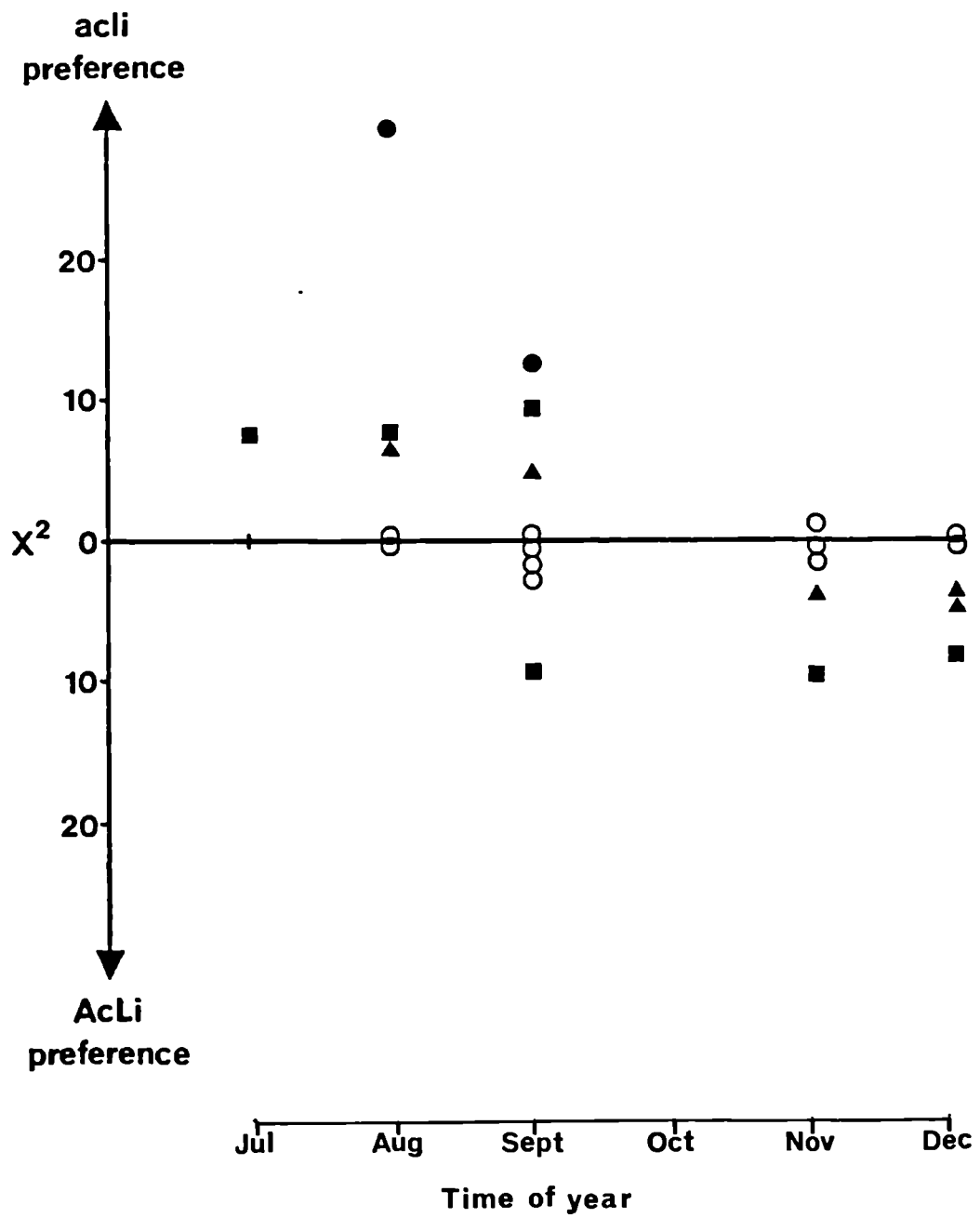
<sup>1</sup> Results near the region of  $P = 0.05$ ; Yate's correction for  $X^2$  continuity was applied.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

Fig. 5.9.

The resulting  $X^2$  values from the contingency tests made to compare sheep grazing on cyanogenic (Ac Li) and acyanogenic (ac li) leaves of Trifolium repens (see Table 5.3). o, n.s.; ▲,  $P < 0.05$ ; ■,  $P < 0.01$ ; ●,  $P < 0.001$ .





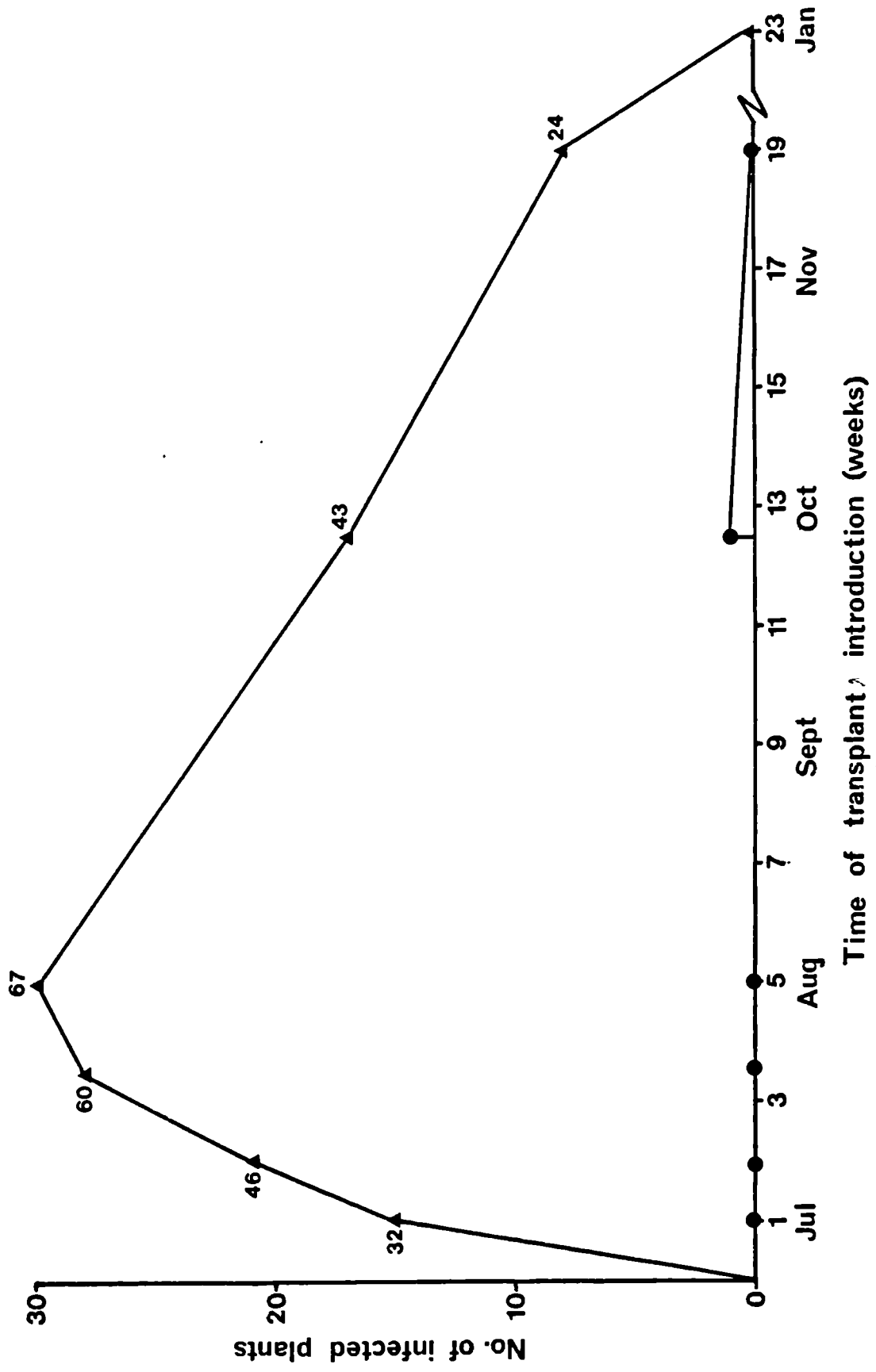
cuttings (Fig. 5.10) and only one acyanogenic plant was infected by this rust (in October). By late September-early October, the cumulative proportion of cyanogenic plants showing infection reached 80%. No fungicide had been applied to the clones while they were propagated in the glasshouse, prior to the introduction to the field, and it is possible that the disease might have been acquired then though this was not apparent at the time of choosing the cuttings for experimental transplanting. An attempt to confirm the differential susceptibility to infection of the two morphs was made by sampling another field near Llanfairpwll, Anglesey, N. Wales, which was heavily damaged by this rust. On September 9 and 10, 1978, leaves of 25 infected and 25 non-infected plants of Trifolium repens were collected at random from this field. Care was taken to collect samples of plants at least 5 m apart. The leaves were immediately tested for cyanogenesis with the sodium picrate paper technique (see Materials and Methods, Chapter 4). The following results were obtained:

	Plant morph		
	Cyanogenic	Acyanogenic	
Infected	18	7	25
Non-infected	19	6	25
Total	37	13	50
Ratio Inf./Non-inf.	0.95	1.17	

The cyanogenic morph was 2.85 times as common as the acyanogenic form and therefore a very small sample size is left to make the comparison. However, the ratio of infected to non-infected leaves was very close to unity in both morphs and this simple observation does not give support to the findings with

Fig. 5.10.

The number of cyanogenic (▲) and acyanogenic (●) cuttings of Trifolium repens infected with the rust Uromyces trifolii. The values shown near the line are the numbers of infected plants as a proportion of the number of surviving plants.



the transplants. More detailed work is required to clarify this point. The data from Henfaes field has proven very striking and it may be that Uromyces resistance and cyanogenesis are linked in this clover population.

#### The fates of the cyanogenic and acyanogenic leaflets

The clover plant can be thought of as a population of leaves that experience different environmental risks, acting and interacting with different degrees of intensity through time. Figure 5.11 summarises the variety of fates of leaflets surveyed in this study; each column has been constructed with the total number of leaflets (shown as N in the diagrams) of all the cyanogenic and acyanogenic plants alive at each sampling date; each complete column can be taken as an idealised picture of a plant (by substituting the N values by 1.0) with its leaflets having different fates. In the diagrams both the shaded areas and the sections marked as Sh (sheep grazing) imply death\* of the leaflets.

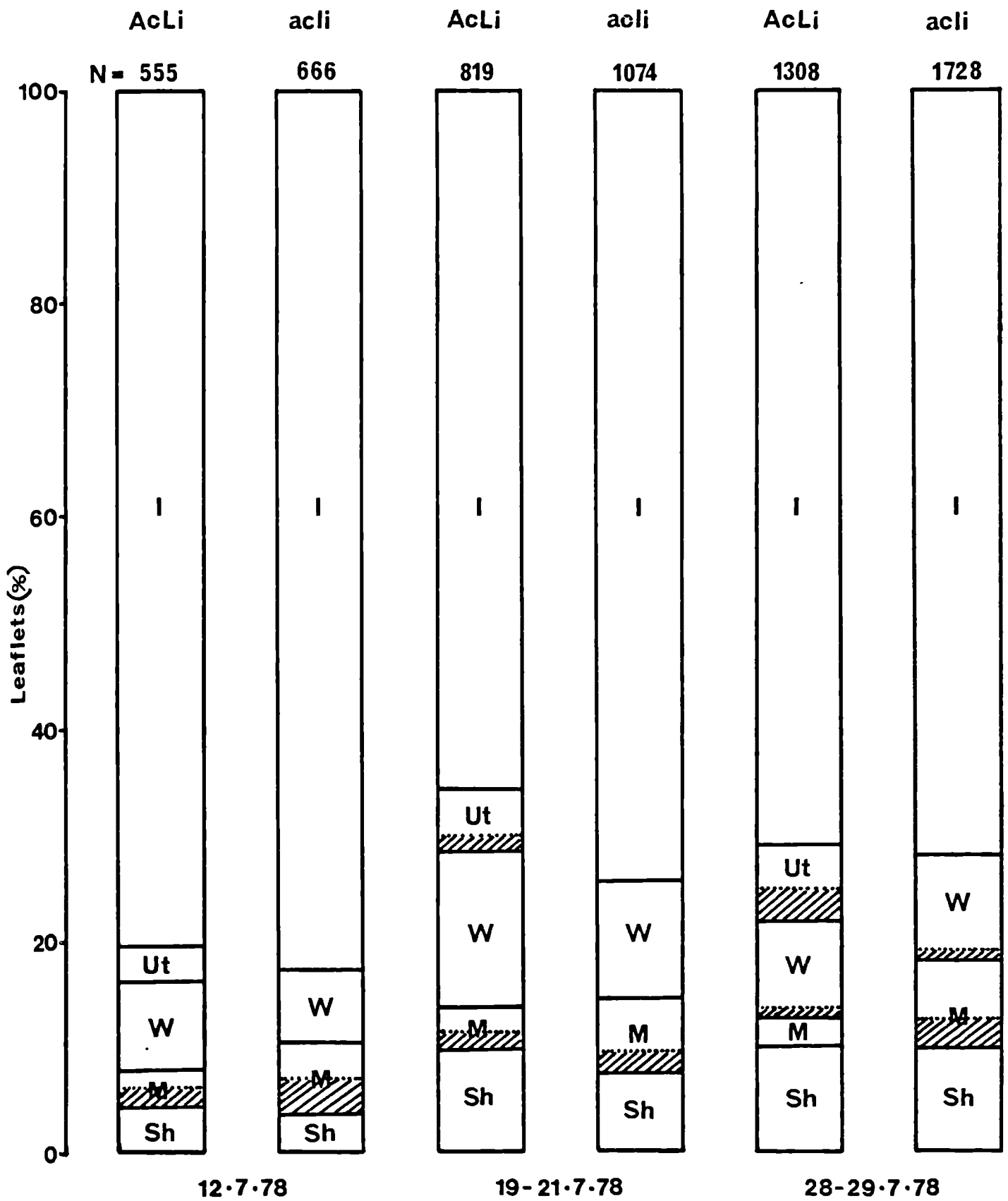
The first months after initiation of the study, detailed samplings were made at short intervals (ca 1 week) and therefore, four sampling dates are given for the summer season; in autumn and winter, details are given for observations early and late in the season; only one sampling date is given for spring. Soon after the introduction of the cuttings (12.7.78), the proportion of intact leaflets of both morphs was high (ca 80%) but this proportion decreased and in early winter it was near 50% and then increased again in spring. In summer (Fig. 5.11 a) the main cause of leaflet death was sheep grazing and a minor proportion of leaflets was lost to mollusc grazing (particularly in the acyanogenic morphs) and to infection by Uromyces trifolii (in the cyanogenic morphs exclusively). A

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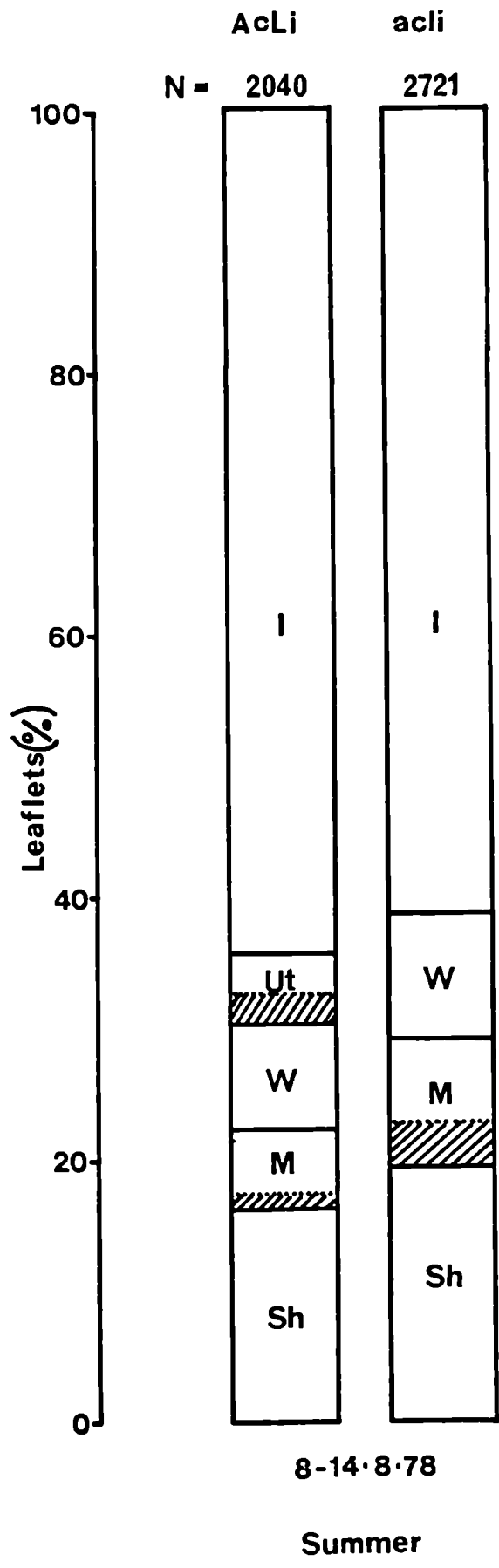
\* Leaflet death is defined as (i) total removal from the plant or (ii) 50% or more of the lamina losing its green colour.

Fig. 5.11.

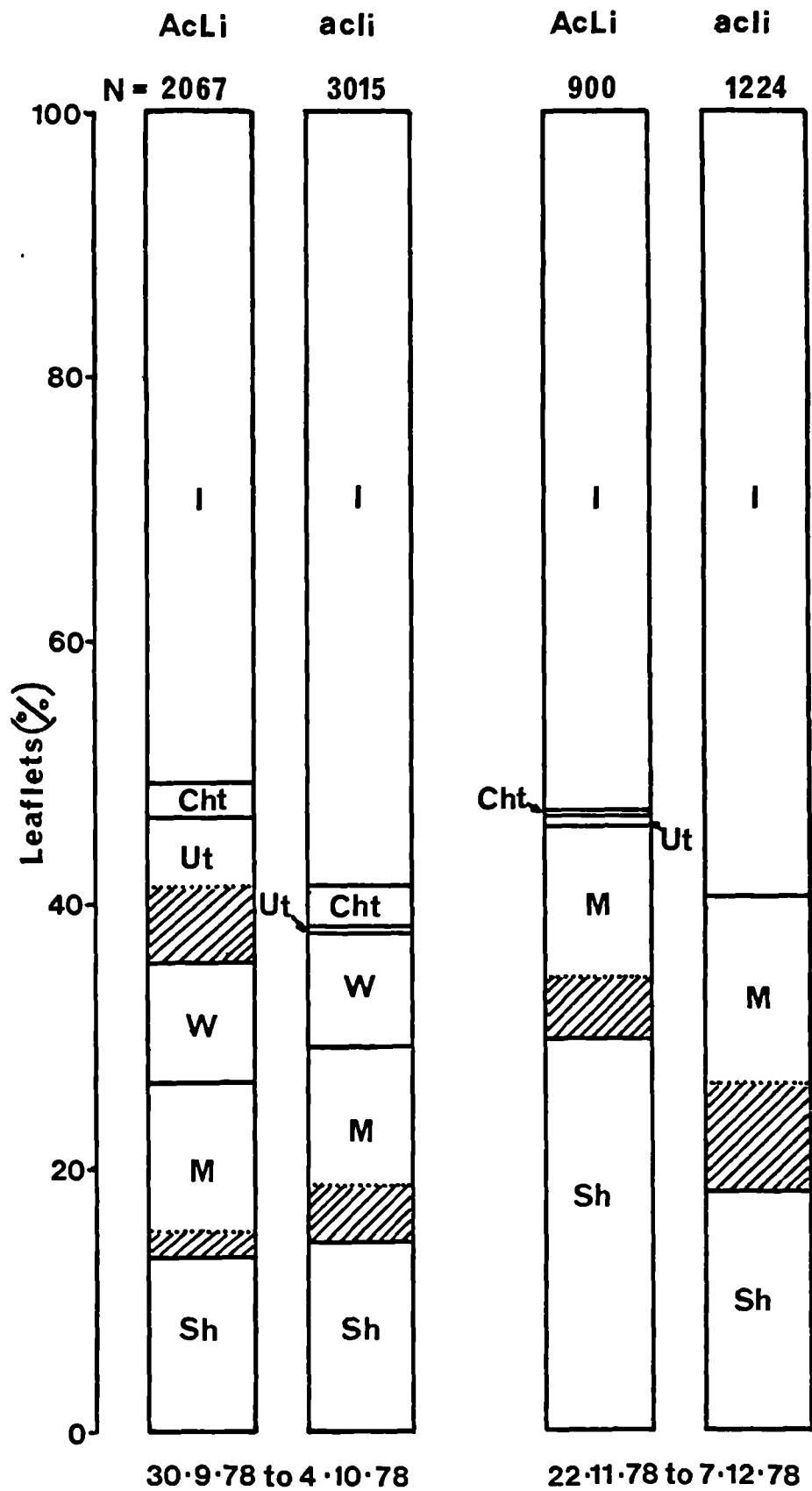
The proportion of leaflets of cyanogenic (Ac Li) and acyanogenic (ac li) morphs of Trifolium repens having different fates through the four seasons of the year (a) summer, (b) autumn, (c) winter, (d) spring. Fates: I = intact, Ut = Uromyces trifolii infection, Cht = Chymadothea trifolii infection, W = weevil damage, M = mollusc damage, Sh = sheep damage, F = frost damage; N = total number of leaflets. (Shaded areas and Sh indicate dead leaflets).



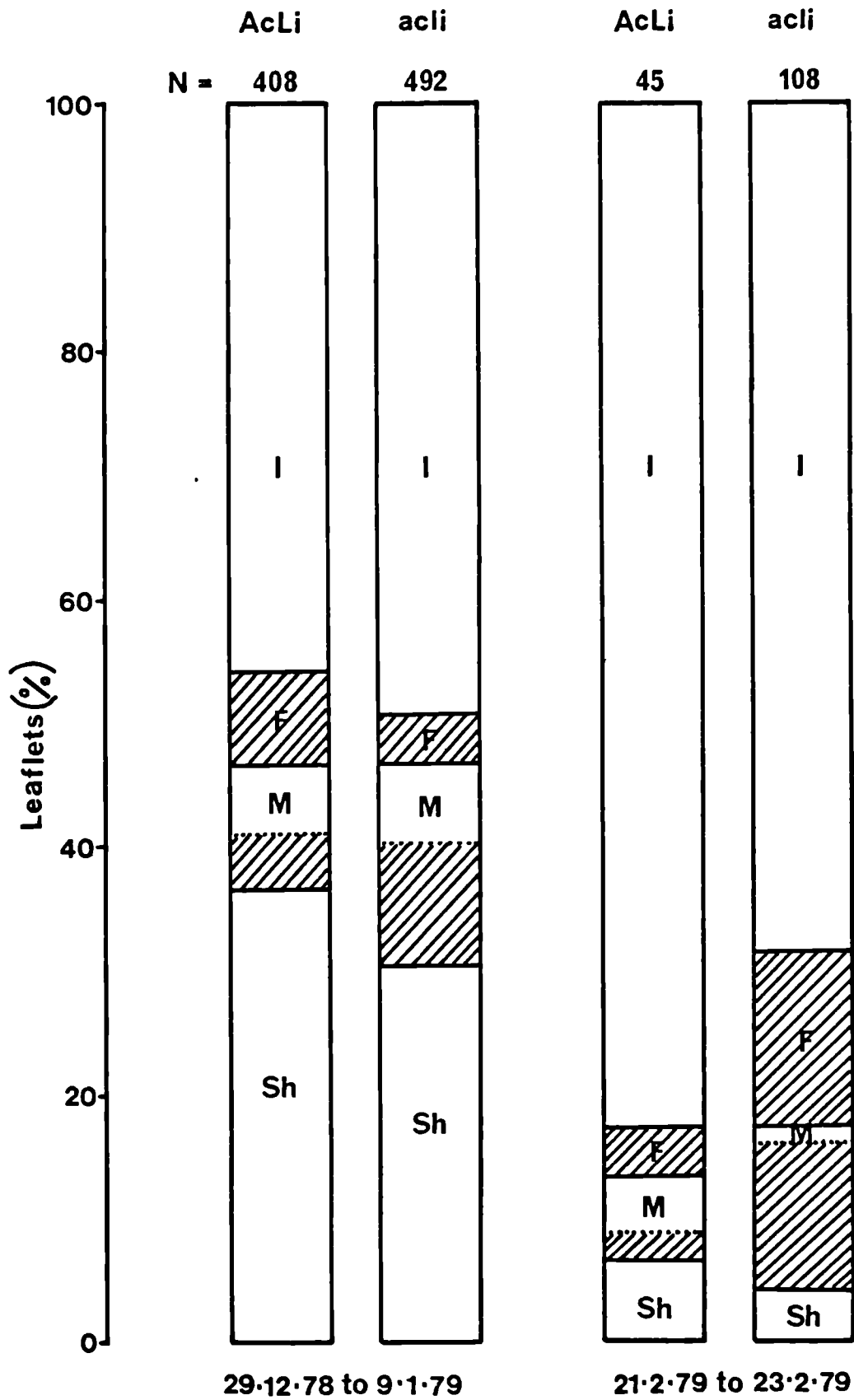
(a) Summer



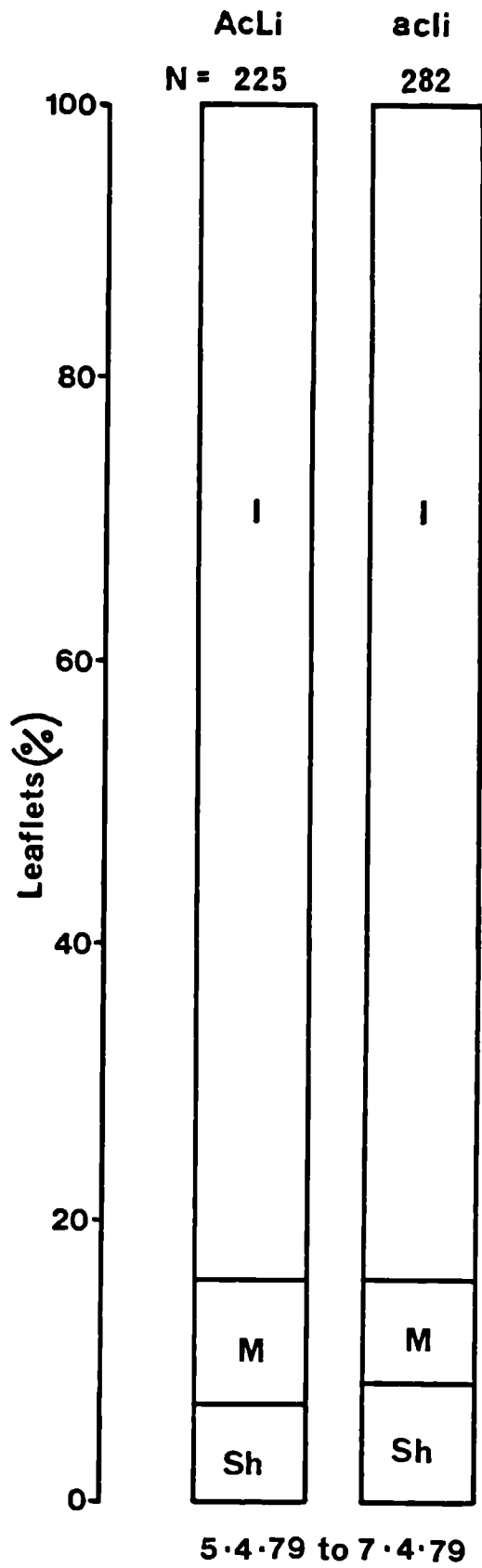




(b) Autumn



(c) Winter



(d) Spring

considerable proportion of leaflets was damaged by weevils but only a tiny fraction of this appeared to have caused leaflet death. At the last sampling of the season (8-14.8.78) there was a marginally lower proportion of intact leaflets in the acyanogenic morph due to the more intense damage by sheep, molluscs and weevils. In early autumn (Fig. 5.11 b) the peak of leaf infection by Uromyces trifolii reduced the proportion of intact cyanogenic leaflets even though there was a greater loss of leaflets of the acyanogenic plants due to grazing by sheep and molluscs; leaflets with pathogen damage had disappeared (died) late in the season but cyanogenic plants still bore fewer intact leaflets than acyanogenic plants because of the unusually larger proportion of cyanogenic leaflets taken by sheep at that time. Early in the winter (Fig. 5.11 c), sheep grazing still concentrated on the cyanogenic plants but this was counterbalanced by increased grazing of acyanogenic plants by molluscs. Frost damage appeared at this time and caused tissue necrosis and death of leaflets. Towards the end of winter the leaflet population decreased substantially (especially in the cyanogenic forms) and the proportions here are subject to bias; in the acyanogenic morph, 30% of the leaflets were lost to the combined effects of grazing by sheep and molluscs and frost damage. In the final survey (spring of 1979; Fig. 5.11 a), the leaflet numbers built up again and the leaflet populations of the two morphs started the new season of growth in the same conditions - 84% of them intact and 16% damaged by sheep and molluscs.

#### Observations on reproductive activity

Only a few of the transplanted cuttings flowered during the season of 1978, but observations on flowering were made on the plants naturally growing in the field during 1979. The grid system established for the transplants (see Fig. 5.1)

was again used to define equidistant transects 8 m apart; along these transects samples were taken at every eight paces. Sampling consisted of finding the flowering plant of clover nearest to a mark on the shoe (the toe-point method (Cahn and Harper, 1976)). Leaves of this plant were collected in test tubes and then taken to the laboratory where sodium-picrate paper tests were made to define the cyanogenic morph; 100 plants were sampled and tested this way. The 4 possible morphs (Ac Li, Ac li, ac Li, ac li; see Table 4.1) were distinguished and this whole procedure (sampling and testing) was repeated on July 11, August 11 and September 12, 1979. On July 13, 1979, a systematic sampling of 100 plants (regardless of their reproductive status) was made to establish the frequency of each morph and serve for comparison with the survey of flowering plants. Table 5.5 shows the frequency of each of the 4 morphs and the number of plants of each morph flowering in the three surveys. The most salient aspect of these results is the consistent under-representation of the cyanogenic morph (Ac Li) in the flowering sample; the enzymatic morph (ac Li) was also under-represented in the July and September surveys. By contrast, both the glucosidic (Ac li), and the double recessive morphs (ac li) were represented in excess in the flowering samples, particularly the Ac li morph. Tests of goodness of fit with the respective frequencies of each morph show a significant departure in July and September but not in August; the departures in this month, however, are, with the exception of ac Li, in the same general direction: under-representation of Ac Li and excess of Ac li and ac li. Detailed work on the intra-specific population biology of white clover is needed to assess the extent of disadvantage associated with the poor flowering of the cyanogenic morph found in this study.

Table 5.5. The phenotypes of 100 reproductive plants (i.e. flowering) of Trifolium repens sampled at 3 dates during the flowering season of 1979.

Survey	Deviation		Ac li	Deviation		ac li	Deviation		Goodness of fit
	Ac Li	from F		from F	ac Li		from F	X <sup>2</sup>	
Frequency (F)	55		12		15		18		
11.7.79	35	(-20)	30	(+18)	10	(-5)	25	(+7)	36.13 <0.001
11.8.79	45	(-10)	17	(+5)	16	(+1)	22	(+4)	4.03 >0.25
12.9.79	39	(-16)	26	(+14)	10	(-5)	25	(+7)	23.26 <0.001

## DISCUSSION

In the original definition of polymorphism (Ford, 1971), a clear emphasis is laid on the occurrence and co-existence of the different morphs in the same habitat. However, in most classical studies on the biology of the cyanogenesis polymorphism in Trifolium repens (e.g. Daday, 1954 a,b, 1965) the emphasis has been placed on the occurrence and variations of the frequency of alleles along extensive geographical ranges and some quite marked clines have been established; this approach has been criticised by Cahn and Harper (1976) who suggested that the existence of clines does not explain the existence of the polymorphism and commented that it is doubtful that large scale surveys can reveal the nature of the selective forces balancing the survivorship of the morphs in a stable polymorphic system. The present study deliberately focuses attention on the behaviour of individuals (leaves and plants) of two morphs growing in the same habitat.

Among the several elements of plant fitness investigated in this study, plant survivorship showed a slight superiority of the acyanogenic form. The major contrasts in survivorship occurred after winter and at the commencement of the growing season when plant interference between plants becomes intense. The superior survivorship of the acyanogenic form was consistent (though not always statistically significant) throughout the period of study. Further analysis of this feature is needed with even larger sample sizes which would allow sensitive Time-Series analyses to be made.

Measurements of several growth parameters observed in this study (e.g. leaf and stolon production) showed some superiority of the acyanogenic morph

during the growing season and during the period when interference between plants is highest; the paramount effect of actively growing neighbours on the survival of plants has been shown in other studies carried out in this field (e.g. Sarukhán and Harper, 1973). It is possible that the observed differences in growth parameters between morphs during the season determines the ability of the morphs to withstand interference.

The defensive role of cyanogenesis against mollusc grazing was clearly shown in this study. There was, however, much heterogeneity both in the degree of susceptibility to grazing of the acyanogenic clones and in the degree of protection against grazing of the cyanogenic clones. The heterogeneity remains even when clones with a closely similar cyanogenic response were chosen to produce the experimental transplants. Ellis et al. (1977b) have shown a considerable degree of plasticity in the intensity of the cyanogenic reaction in clones of Lotus corniculatus and also that some clones are phenotypically unstable (i.e. a given plant may express a different phenotype at different times). A repetition of cyanogenesis tests (with sodium-picrate paper) was made on the transplanted cuttings still surviving in November 1978 and this confirmed that all of them were expressing their original phenotypes although there was some variation in the intensity of the cyanogenic response. De Waal (1942)\* collected samples of Trifolium repens at fixed times during the day for several days, and he found variation, both diurnal and between days, in the cyanogenic response. It is possible that this sort of variation accounts for the observed heterogeneity of grazing on the cyanogenic morph. Spatial heterogeneity is another factor that might have affected the degree of grazing by molluscs; there is not only a gross

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\* I thank H. Prins, J. Groenendael and C. van Gool for their help in translating de Waal's thesis from the Dutch into English.



pattern of distribution of intensity of mollusc grazing in the field (see Chapter 4, Fig. 4.4) but this gross pattern may change in its details due, for example, to minor changes in the neighbouring vegetation - a slug might eat much more clover if it is surrounded by unacceptable rather than acceptable neighbours.

It was somewhat surprising that weevils did not graze selectively between the clover morphs; it could be expected that cyanide would act as an effective deterrent against such small beetles; it may be that they possess some mechanism of cyanide resistance. Several weevils have been found associated with Lotus corniculatus (Jones, 1973) and of these, Hypera plantaginis is known to be able to detoxify cyanide (Parsons and Rothschild, 1964). The dangers of extrapolating the results found for a given group of herbivores (slugs) to another, can hardly be over-emphasised.

A puzzling phenomenon that emerged from the present study was the preferential grazing by sheep on the acyanogenic cuttings in summer, and a shift to the cyanogenic morphs in late autumn. These findings contrast with those reported by other authors, e.g. Corkill (1952), who carried out trials with sheep and found no evidence of preferential eating of the acyanogenic as opposed to the cyanogenic plants of Trifolium repens. The results reported here suggest that the interaction cyanogenesis/sheep may be quite complex and this is an aspect that warrants much deeper study.

Several authors have investigated the expectation that cyanogenesis affords protection against fungal attack. For example, Angseeing and Angseeing (1973) compared the degree of infection by the black blotch fungus (Cymadothea trifolii) on cyanogenic and acyanogenic leaves of T. repens, and found no evidence of preferential attack on either morph; similar results were found for the same

pathogen in the present study. Trione (1960) found no correlation between HCN content of the flax plant and resistance to flax rust (Fusarium oxysporum lini). On the other hand, in the present study, the rust Uromyces trifolii almost exclusively infected the leaves of cyanogenic white clover, although this was not the case in another field. The present results are puzzling, for it was the cyanogenic and not the acyanogenic morph that was preferentially attacked.

In this study, I report some observations on the decay of stolons and the consequent loss of complete stolons or fragments; the number of decaying stolons was significantly greater in the cyanogenic transplants. This phenomenon need not necessarily be interpreted as of selective disadvantage, for the fragmentation of stolon may prevent viruses or systemic pathogens (such as Uromyces trifolii) from spreading through a whole genet.

In the light of this study the suggestion of Daday (1965) that low temperature is the major factor responsible for the cyanogenesis polymorphism in Trifolium repens is somewhat venturesome. On the one hand, the present results (and those reported by other authors, e.g. Jones (1973)) suggest that more than one factor may be important in the maintenance of the polymorphism (e.g. herbivore grazing, differential competitive ability, fungal attack, frost damage, drought, etc.). On the other hand, Daday's physiological explanation for the disadvantage of the cyanogenic form at low temperatures, namely physical damage leading to cyanide release and self-poisoning, strongly challenges the defensive role of cyanogenesis against grazing for it is the physical damage made by the grazing herbivore that causes cyanide release by which the animal is consequently deterred. Also, Solomos (1977) has commented on the widespread resistance to poisonous cyanide found in the plant kingdom.

An inescapable conclusion from the present study is that analysis of the population biology of white clover (even if made at a very local scale) is of only restricted relevance if it does not include intra-specific or even intra-clonal differences to herbivore grazing, differences in reaction to competing neighbours (see Turkington and Harper, 1979b), differential susceptibility to pathogens, etc. Quite likely, the ecological literature of the near future will have to deal with the population biology of morphs in a given area rather than with the population biology of unrealistic entities labelled with a binomial.

GENERAL DISCUSSION

### Studies on plant-animal interactions

One branch of plant ecology that has received very little attention from biologists is that concerning the study of the behaviour of individuals (within populations) at a local scale and their interaction with the biotic elements of the environment (neighbours, predators and pathogens). This markedly contrasts with some other aspects of plant ecology that have been extensively studied, e.g. the correlations between vegetation and the physical aspects of the environment (climate, geology and soils) - the 'vegetationalist' approach (see e.g. Mueller-Dombois and Ellenberg, 1974). This contrast may be due partly to the fact that the methodologies required by the vegetation analyst are somewhat simpler and also because the plasticity of plants and vegetative spread make it difficult to look in detail at individuals in populations (Harper, 1967). Studies of biotic interaction between trophic levels have undoubtedly been hindered by the traditional training of biologists as 'botanists' or 'zoologists' and perhaps also by a holist conviction that natural systems are too complex to be broken down into simpler elements for study. The present thesis represents a deliberate attempt to search for a relatively simple ecological system and to study its elements in simplified models. This involves the assumption that the whole (of an ecological system) is no more than the sum of its parts plus their interactions.

### The slug-plant interaction

An attempt to define patterns or general tendencies in the interface animal-plant can be made on the basis of the position of the herbivore along a gradient of specificity with respect to its food plant. The theoretical range of

this gradient would be from a tight reliance of the herbivore on a specific plant morph or even a plant part of a given morph (e.g. the Heliconine butterflies that specialise on a given Passiflora plant (Gilbert, 1975)), to a herbivore that approaches such an indiscriminate behaviour as that of a grass mower, to which perhaps the closest approximation would be that of a whale grazing on marine phytoplankton. The ecological literature provides abundant examples of specific interaction of herbivores, mostly insects, with a given food plant, but it is quite difficult to find examples of 'true generalists'. Slugs can be assigned to the category of 'acceptability moderated generalists' in that, faced with a variety of food plants, they are not specialised to the extent of monophagous dependence on a particular food plant, yet they clearly define a hierarchy of plant acceptability (see Dirzo, 1980). These distinctions in the degree of specialisation of the herbivore are useful in that they attempt to classify plant-animal interactions and should make it possible to predict the outcome of a given interaction. Subdivisions of these broad classifications can be imposed by behavioural aspects typical of a given herbivore. For example, it was found (Chapters 1 and 2) that the measured acceptability of the leaves of different plant species to slugs was a poor predictor of the extent of damage made on seedlings of the same plant species. Plant attributes such as growth form or size have a major effect on the grazing pattern of slugs. The consequences of damage to seedling populations by wood pigeons (another acceptability moderated generalist (Murton et al., 1964)) would probably be quite different due to the different grazing patterns and mobility of the pigeons.

A relatively recent attempt to establish general patterns in the strategies of chemical defense of plants against herbivores was made by Feeny (1976) on the basis of the concept of "Plant Apparency". He distinguished two groups of plant

apparency to the herbivore: (i) Apparent - 'bound to be found' plants, e.g. persistent and abundant, long-lived plants and (ii) Unapparent - 'hard to find' plants, e.g. ephemeral, sparse, short-lived plants. He suggested the following correlations of apparency with chemical defense: apparent species produce compounds not readily susceptible to counter-adaptation, that affect the fitness of the herbivores and act in a dosage-dependent fashion (e.g. tannins). Unapparent plants produce compounds that are susceptible to counter-adaptation, have little effect on the herbivore's fitness and act in a qualitative (not dosage-dependent) fashion (e.g. glucosinolates). These strategies of chemical defense are respectively equivalent to those defined by Rhoades and Cates (1976) as Digestibility Reducing Systems and Toxic Systems. Unapparent plants (or tissues) are expected to escape predation due to their unpredictability in space and time and under these conditions natural selection would favour two herbivore strategies: (i) the ability to adjust the life-cycle in relation to the availability of resource - the 'tracking' strategy (see Harper, 1977), or (ii) a more or less generalist feeding behaviour. Specialist herbivores would, presumably, be at a disadvantage in communities of unapparent plants; conversely, greater apparency should select for specialism. On the basis of the degree of specialisation of slugs it can be predicted that their most suitable habitat would be that in which plants are located more towards the extreme of unapparency. The tremendous success of slugs in disturbed (commonly man-induced) habitats seems to support this prediction.

Another element that must be super-imposed on these correlations of defense strategy - herbivore specialisation - plant apparency is that apparency is very much dependent on the range and search behaviour of the herbivore. The form and behaviour of the animals determine the way they sample the diversity of

the vegetation. The diversity of vegetation that is relevant for a slug making dietary choices is perhaps contained in a 20 x 20 cm area of sward; the diversity in such area may include different species of plants, different morphs of a given species, plants of different age, etc. - the apparency of plants would be operative at such scale. The equivalent environmental grain that is relevant to a more mobile folivorous animal like the howler monkey Alouatta spp. (Milton, 1979), would be perhaps of the order of several hectares of tropical rain forest. If a slug was to move through the foliage of a tropical rain forest, it would sample the environment in such a coarse-grained fashion that it would spend a large proportion of its lifetime on a single plant and it would have little opportunity of choice. If a howler monkey was to choose its food plant from 20 x 20 cm patches it would probably soon starve to death! (In both cases the concept of plant apparency would be inoperative). In a more realistic comparison, it could be shown that in a day of grazing in a given sward, a sheep will sample several clones of clover, a slug may sample the leaves of a few neighbouring ramets of clover and a weevil will sample only a few clover leaflets.

More detailed studies - both in the field and in the laboratory are needed to establish the complex interdependences between plant defense - herbivore specialisation - plant apparency - feeding range and search that characterise the animal-plant interface. Simple experimental models like the ones used in the present study could be a valuable tool for the ecologist.

#### The effects of grazing on the individual plant

The effects of predation by slugs are greater at the seedling stage and this is probably true for most plant-herbivore systems. It follows that greater



protection against grazing at the seedling stage should be specially advantageous for the plant. In this context, Bernays and Chapman (1976) report that young seedlings of several species of grass are less acceptable to several acridid insects than older seedlings; they found that meals of the locust, Locusta migratoria, are up to nine times larger on the mature than on the seedling leaves of 20 different grasses. Woodhead and Bernays (1978) have shown that the maximum inhibition of feeding by locusts on seedlings of Sorghum bicolor (due to the presence of HCN and phenolic acids) is inversely correlated with the age of the plant. It can be speculated also that in an interacting system, the selective pressure exerted by the chemistry of the plant on the herbivore should be greater for an animal that specialises on seedlings.

For a seedling with poor chemical (or physical) defenses, there may be selective pressure for it quickly to pass the 'critical size of escape' beyond which the predator will remove only plant parts and the plant may then proceed to regenerate the predator's meal. There will, however, for most plant-animal systems, be two major after-effects of a grazing episode: (i) some additional mortality if the grazed plant is not able to withstand the interference exerted by ungrazed neighbours, or (ii) the grazed plant can be placed in a subordinate position in the hierarchy of sizes in the population. Whittaker (1979) has provided several examples of grazing by invertebrates that elegantly illustrate the importance of apparently trivial levels of herbivore grazing on the competitive interactions between plants. It follows that the role of the herbivore will have to be seen in the context of interacting plant neighbours; presumably this generalisation can be applied to most plant-herbivore systems.

### The defensive role of cyanogenesis

Cyanogenesis is a very widespread character in the plant kingdom - over a thousand species are cyanogenic (Gibbs, 1974), yet in only a few species has its defensive role been convincingly demonstrated. Selective grazing both by slugs and snails occurs in the legumes Lotus corniculatus (Jones, 1962, 1966) and Trifolium repens (Angseesing, 1974 and Chapters 4 and 5). Evidence of selective grazing by deer and sheep on the acyanogenic form of bracken (Pteridium aquilinum) in South England, has been reported by Cooper-Driver and Swaine (1976). The same authors showed that the locust Schistocerca gregaria clearly rejected the cyanogenic forms of the fern. Bernays (1977) has shown that HCN release is responsible for the protection of cassava (Manihot esculenta) against grazing by insects (Zonocerus sp.). Some circumstantial evidence of the defensive role of cyanoglucosides present in the seeds of jojoba (Simmondsia chinensis) against several heteromid rodents has been given by Sherbrooke (1976). An exquisite interplay between protection against predation and facilitation of dispersal has been reported for the shrub Heteromeles arbutifolia (Dement and Mooney, 1974). The immature fruits contain high levels of cyanogenic glucosides only in the pulp while the mature fruits have high levels in the seed but not in the pulp. At the same time, maturation of the fruits coincides with anthocyanin production (hence the attractive red colour), and a decrease in the content of tannins (facilitation of dispersal by birds!). Dement and Mooney also report a seasonality of cyanide release by the leaves, which is well correlated with the intensity of insect damage. In a very detailed study of the acceptability of Sorghum bicolor to Locusta migratoria, Woodhead and Bernays (1978) have shown that HCN and phenolic acid had an additive effect that accounted for the unacceptability of the plant at different

stages of development.

Several lines of evidence suggest that protection against grazing of the cyanogenic plants is due to the immediate release of HCN upon damage of the tissue; for example, (i) the pattern of mollusc damage - excess of nibbling on the cyanogenic plants and excess of heavy damage on the acyanogenic plants (Chapters 4 and 5); (ii) the locust Schistocerca gregaria feeds with the same degree of impunity on the glucosidic forms of bracken as on the forms that lack both the enzyme and the glucoside (Cooper-Driver and Swaine, 1976); the same result was found for the slug Agriolimax reticulatus (Chapter 4). These observations suggest that the glucosides themselves have no deterrent effect; (iii) the most elegant demonstration of the mode of action of cyanogenesis comes from the study of Woodhead and Bernays (1978). A cannula was inserted in the pre-oral cavity of individuals of Locusta migratoria; after 5 hrs. of starvation, the insects were allowed to feed on an acceptable diet and while the insect was still feeding, HCN was injected through the cannula; concentrations of HCN that are equivalent to the HCN initially released by seedlings of Sorghum bicolor (the host plant) clearly deterred the insects. An important conclusion of these authors is that the amount of cyanogenic glucosides may not, alone, be a good measure of the deterrent properties of the plant; the availability of the enzyme is also relevant.

The results reported in this thesis (Chapter 4), as well as those of Woodhead and Bernays (op.cit.) and the examples quoted in Bernays (1977) suggested that cyanogenesis may be assigned to the category of Toxic Systems (terminology of Cates and Rhoades (1976)) or to the strategy of Unapparent plants (terminology of Feeny (1976)). The same conclusion was reached by Jones et al. (1978).

### Polymorphism and Coevolution

Coevolutionary interpretations in systems of polymorphic plants and their herbivores can be inadequate (e.g. Cates, 1975) in that the existence of acceptable and unacceptable plant morphs is not sufficient evidence of coevolution if it does not include descriptions of animal features that can be interpreted as having resulted from selective pressure by the plant. In the case of the polymorphic legume Lotus corniculatus, Jones (1973) has proposed that the existence of cyanide-detoxifying mechanisms in the common blue butterfly, Polymatus icarus and the weevil Hypera plantaginis whose larvae feed on the leaves and flowers of L. corniculatus is evidence of coevolutionary interactions. Sherbrooke (1976) extended this interpretation to his findings that the rodent Perognatus baileyi was able to feed on the seeds of Simonsia chinensis, which contain cyanogenic glucosides. No evidence was found in the literature of animal characteristics that justify postulating coevolutionary interactions of slugs with Trifolium repens. Perhaps the closest approximation to this is the finding of enzymatic reactions of homogenates from the slugs' guts with the clover glucoside (linamarin) and the fact that slugs eat glucosidic plants with apparent impunity (see Fig. 4.6). The presence of enzymes capable of hydrolysing cyanogenic glucosides is not unique to slugs. Bernays (1977) has reported that the digestive juices of Locusta contain enzymes capable of releasing HCN from amygdalin and linamarin; however, no coevolutionary interpretations of this finding appear to have been made before. The investigation of enzymatic reactions in the slugs' guts gave tentative evidence of the existence of enzymatic polymorphism in the herbivore and if this is true, it is one of the rare instances in which a plant polymorphism can be coupled with polymorphism in the predator.

The performance of cyanogenic and acyanogenic plants in the field and the  
balance of the polymorphism

A possible interpretation of the observed poor growth and reproduction of the cyanogenic morph of T. repens in the field can be made in terms of partitioning of resources (Cody, 1966). He suggested that the allocation of resources by an organism involves compromises between three elements of fitness: (i) reproduction, (ii) competitive ability and (iii) protection against predators; a disproportionate expenditure in any of the three is made at the expense of the others. Cody proposed this idea originally to assess the fitness of birds, but botanists have adopted it to assess the fitness of plants (e.g. Gadgil and Solbrig, 1972; Abrahamson and Gadgil, 1973) (see, however, Harper (1977) who has indicated the possible limitations of this approach). In the specific case of T. repens, the argument would be that the sacrifices made to competitive ability and reproduction of the cyanogenic plants are compensated for by the benefit of cyanide production and hence protection against predation. This study is too premature to support the argument and confirmation of the poor performance of the cyanogenic form as well as accurate measurement of the selective advantage conferred by protection against grazing awaits further study. Moreover, the results emerging from the field study (Chapter 5) suggest that mollusc grazing is only a minor element affecting the polymorphism and that many other factors (pathogenic damage, differential competitive ability, other herbivores, low temperatures, etc.) probably act and interact in the balance of the polymorphic system. The interplay of all of these elements most likely will vary from area to area and therefore any conclusions must be confined to the particular set of conditions where observations are made. "Different plants in different habitats may respond to selection in entirely different ways and therefore

contrary explanations of the role of cyanogenesis within species, let alone between species, are only to be expected" (Jones et al., 1978).

Although quite illustrative and valuable for their potential to erect hypothesis and point out areas of further study, some of the simplified experimental models described in this thesis lack the realism of a field study; ultimately it is in the field, in the context of individuals in populations, that differential death and reproduction become the essence of the study of the animal-plant interaction.

For the plant ecologist, the incorporation of animals to his study of plant systems offers a fascinating picture of the complexity of interactions that occur in a community: the herbivore's activity affects plant to plant interactions in ways that are dependent on the degree of specialisation of the herbivore, its search and range as well as on the mechanisms of defence of the plants and the environmental grain. In turn, the chemistry of plants affects animal to animal interactions (at one or more trophic levels) in ways that have only recently started to be explored (see Lawton and McNeill, 1979). It is the task of the biologist interested in the study of biotic interactions, to develop more ingenious methods of study adequately to explore the animal-plant interface.

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