

EXPERIMENTAL INVESTIGATIONS OF COMPETITION
AND ALLELOPATHY IN HERBACEOUS PLANTS

By

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EXPERIMENTAL INVESTIGATIONS OF COMPETITION AND ALLELOPATHY IN
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SUMMARY

This thesis consists of two parts. Part I describes field investigations carried out to examine the characteristics of the major species present in the herb layer in a range of perennial communities established at various sites in the Sheffield region. At each site quantitative measurements of seasonal changes in shoot biomass were conducted in association with analyses of soil mineral nutrient status and bioassays designed to allow seasonal release of phytotoxins to be detected. From the results of these studies it would appear that dominance in relatively productive and undisturbed vegetation is strongly associated with the capacity to develop a large summer peak in shoot biomass. This pattern is evident in the widely successful grass, *Holcus lanatus*, although in this species the size of the summer peak was found to vary considerably from year to year. From soil analyses it was apparent that mineral nutrient status plays an important part in determining the types of phenology represented in the vegetation.

The results of bioassays conducted on various soils suggested the release of toxins from certain plants. At two sites toxicity appeared to be related to the rapid efflux of organic solutes from deciduous tree litter. Toxic effects were also detected in an area of derelict grassland colonized by *Holcus lanatus*. Evidence of autotoxicity in *H. lanatus* was obtained from the field and in a garden experiment.

The experimental studies in Part II involved attempts to measure the effects of toxin production and competition by *H. lanatus* upon the growth of species with which *H. lanatus* is frequently associated in the field. In an attempt to manipulate the vigour and competitive ability of *H. lanatus*, studies were conducted to measure the influence of temperature and mineral nutrient supply upon a mixed sward. The results revealed a strong capacity in *H. lanatus* to suppress the vigour of *Lolium perenne* and this ability was only marginally affected by variation in temperature and clipping regime. The results of the laboratory experiments supported the field evidence of toxic effects originating from *H. lanatus*. A technique was developed which effectively distinguishes between allelopathy and effects resulting from the depletion of mineral nutrients in the soil solution.

الخلاصة :

بحثت هذه الرسالة في جزئين الاول منها يتعلق بدراسة عقلية بجامعة من الاعشاب المعمرة حول مدينة شنفيلد ان تم اخسند عينات من موافقع مختلفة محطة بهذه المدينة واجريت عليها فحوصات مختبرية لقياس التغيريـم الحاصل في اناجينة القسم الغضري خلال فصول السنة .

كذلك اجريت تحاليل للتربة في ذات المواقع المشار اليها لمعرفة القسم فيما ان اكانت هناك مواد مبيطة فيها . ابرزت نتائج البحث في هذا القسم ان امكانية تغلب الجزء الغضري في بعض انواع هذه الاعشاب وخاصة في فصل الصيف كبيرة جدا ومثال على ذلك هو (*Holcus lanatus*) لقد اوضحت النتائج ان له القابلية على الانتعاش الواسع كما لوحظ ان امكانيته في التغلب متفاوتة من سنة الى اخرى نتيجة لاختلاف الظروف المناخيـة ان تحاليل التربة اظهرت ان المواد الغذائية فيها تلعب دورا مهما في تحديد الطواهر الفينولوجية في معيشة النباتات من خلال الفحص المختبري لانواع مختلفة في التربة تبين وجود بعض المواد المبيطة فيها وهذ هـ النتائج فيـم ابرزت بعد سقوط اوراق الاشجار في موقعين وايضا لوحظت هذه النتائج في موقع اخر حيث يكثر فيه (*Holcus lanatus*) . الشواهد التجريبية اشارت الى ان هذا النوع من النبات يفرز مواد مبيطة مودة فترة على نسوه . القسم الثاني منها يبحث في تأثير المواد المبيطة التي تنتج من النباتات سواء من النمو او التفسخ وكذلك تأثيرها على بعض انواع من النباتات التي تنمو معها في الحقل . اجريت تجارب اخرى لمعرفة قابلية التناقص (*Holcus lanatus*) من خلال تأثير درجة الحرارة والمواد الغذائية وقد تبين ان (*Holcus lanatus*) له القابلية كبيرة وتأثيره واسع على نمو الـ *Iolium perenne* وتفاوت قابليته هذه على اختلاف درجات الحرارة .

ولست النتائج على ان *Holcus lanatus* يفرز مواد مبيطة ايضا ختامـا تم تطوير محاولة علمية من خلال البحث تتركز حول امكانية التفریق بين تأثير المواد المبيطة والمواد الغذائية .

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CHAPTER 1

INTRODUCTION

INTRODUCTION

In certain extreme habitats, e.g. deserts, the distribution of plant species and the structure of the vegetation are determined primarily by the direct effects of environmental factors. In the majority of field situations, however, vegetation is strongly affected by interactions between plants. This is especially true for perennial communities of trees, shrubs or herbs in temperate environments.

The problems which have limited progress in previous attempts to investigate the role of interactions as determinants of vegetation are both technical and philosophical and arise from the fact that neighbouring plants interact through a variety of different but inter-related mechanisms. In particular, a distinction must be drawn between two major types of interactions. The first concerns the ability of one plant to modify the environment of another through resource depletion by shading or absorption of water and mineral nutrients, whilst the second relates to the possibility that substances released into the environment from a plant may exert an inhibitory effect upon the germination, establishment, growth or regeneration of its neighbours. Harper (1961) has recognized the need for such a distinction and suggests that effects due to resource depletion and to phytotoxicity (allelopathy) may be brought together under the general term "interference" which he applied to any negative interaction between neighbouring plants.

Competition: Publications dealing with the capture and utilization of resources by neighbouring plants frequently involve use of the word "competition". As pointed out by Milne (1961) this term has been used in very different ways by different biologists. Some authors have used the word as a synonym for the "struggle for existence" (Darwin 1859) and have even applied the term to situations where differences in the vigour or survival of neighbours are determined by differential impacts of environmental factors or predators. A more restricted use of the term is evident in a definition by Harper (1961) in which competition is described as "short and long-term hardships that result to an organism from the direct proximity of neighbours, except that it does not include the direct effects of parasites and predators upon their prey and hosts". Even here, however, competition is being used to describe several related phenomena including not only the attempt by neighbours to utilize the same local supply of resources but also the reactions and relative tolerances of the plants to the associated decline in supplies of light, water, mineral nutrients and space. Scaife (1976) is one of the few biologists who has distinguished the interacting effects of differential rates of resource capture and differences in response to resource depletion in plant interactions.

More recently it has been suggested (Grime 1973, 1979) that analysis of the mechanisms of interactions between plants may be assisted by using the term merely to describe the immediate activity whereby a plant attempts to capture resources in the presence of other plants. In this approach, applied henceforward

in this thesis, competition is defined as "the tendency of neighbouring plants to utilize the same quantum of light, ion of a mineral nutrient, molecule of water or volume of space" and competitive ability is recognized as "a function of the area, the activity and the distribution in space and time of the plant surfaces through which resources are absorbed".

Allelopathy. All plants contain compounds which are potential inhibitors of the germination and growth of the same or of different species. However, many of these substances, e.g., lignin, have essential functions in the plant and there is no evidence to suggest that their phytotoxicity is more than an incidental attribute. However, the presence in many species of high concentrations of potential inhibitors in leaves, stems, roots, flowers, fruits, seeds and litter has prompted some ecologists to suggest that such compounds function as a defence against predation or alternatively have evolved as part of a mechanism limiting the ability of neighbouring organisms to compete for resources. The latter phenomenon has been described as allelopathy which Muller (1969) defines as "the process by which a plant releases into the environment a chemical compound which inhibits the growth of another plant in the same or a neighbouring habitat".

In order for allelopathy to occur it is necessary not only for toxic substances to be produced but for these to be released into the environment and to persist for a period long enough to affect neighbouring plants. Caution must be applied in interpreting the results of the large number of studies in which powder prepared by grinding plant material has been added directly to soil or leached

to provide water soluble extracts and measurements of plant response have been made (Muller *et al.* 1968; Wilson and Rice 1968; McPherson and Muller 1969; del Moral and Muller 1970; Bell and Muller 1973; Parks 1970; Abdul-Wahab and Rice 1967; Glass 1975; Overland 1966; Turner and Quarterman 1975; Stowe 1979).

However, when such unrealistic investigations are discounted there remains a considerable number of reports in which phytotoxic substances such as amino acids, sugars, phenols, terpenes and organic acids have been identified in root exudates, leaf leachates, decomposing shoots and roots and as volatiles released into the atmosphere (e.g., Guenzi, Kehr and MaCalla 1964; Muller *et al.* 1964; Muller 1965,1966; Rice 1964; Tukey 1969; Tukey and Morgan 1964; Persidsky, Loewenstein and Widle 1965; Rovira 1969; del Moral and Muller 1970; Lehman and Rice 1972; Newman and Rovira 1975; June 1976; Firth 1977; Al-Mashhadani, Sydes and Grime 1979).

In addition, measurements have been made of the effect of various factors such as light quality, intensity and photoperiod, mineral nutrient supply, water stress, temperature and plant maturity upon the production of particular toxins; reviews of these studies are included in Rice (1974,1979).

Allelopathic effects have been suggested on the basis of field observations of bare ground, stunted vegetation or a scarcity of seedlings in close proximity to certain species. These reports have been mainly associated with vegetation in relatively dry climates (Bonner 1950; Rice 1964,1972,1974,1979; Muller 1966; Rice and Panchoy 1973).

Even where there is strong evidence either from the laboratory or the field (or from both sources) for the release of phytotoxins two further difficulties are often encountered in attempts to interpret such phenomena.

The first of these problems is to ascertain whether a toxic effect arises from natural selection for allelopathic ability or is merely an incidental consequence of a quite different adaptation such as the production of compounds which provide a defence against predation or microbial attack.

The second difficulty arises from the need to determine whether the release of a toxin secures an advantage for the producer. As Newman (1978) points out, if a plant can, by producing a toxic substance, reduce the growth of other plants in its vicinity, this is likely to increase the amount of available resources and so be an advantage to the plant producing the toxin. However, this hypothesis depends upon the idea that the toxin-producer is resistant to the effects of its own toxins or, in some way escapes their impact. There is a great deal of laboratory data (Benedict 1941; Milton 1943; Curtis and Cottam 1950; Keever 1950; Guyot 1957; Voight 1959; Grant and Sallans 1964; Muller 1966; Abdul-Wahab and Rice 1968; Hanes 1971; Tinnin and Muller 1972; Newman and Rovira 1975; Al-Mashhadani, Sydes and Grime 1979, Stowe 1979) suggesting that many of the plants which are known to produce toxins are themselves susceptible to their effects. This problem has been considered by Grime (1979) and a model has been devised describing a hypothetical situation in which the phenology of a plant may allow it to escape the harmful effect of its own toxins.

The objective in the studies described in this thesis is to investigate allelopathy in a broader context than has been considered in most studies. In the herbaceous species under study and particularly with respect to the perennial grass *Holcus lanatus** an attempt has been made not only to detect the production of toxic substances but also to study their phenology, growth characteristics and competitive ability and to examine the extent to which these features influence their performance in interactions with other species and modify the influence of phytotoxins.

The thesis consists of two parts. In Part I, field investigations are described in which the main objective was to examine the characteristics of the major species present in the herb layer in a range of perennial communities established at various sites in the Sheffield region. At each site quantitative measurements of seasonal changes in shoot biomass were conducted in association with analyses of soil mineral nutrient status and bioassays designed to allow seasonal release of phytotoxins to be detected.

The second part of the thesis contains an account of garden and laboratory experiments involving herbaceous plants known to be capable of releasing toxic substances. The number and identity of the species used varied from experiment to experiment but in all cases *Holcus lanatus* was included. Evidence of the ability of this species to dominate herbaceous vegetation and to inhibit the vigour of neighbouring species was obtained in the field investigations described in Part I and was available from previous studies (Beddows 1961; Riveros 1963; Thurston 1969; Watt 1977; Remison and Snaydon 1978; McGrath 1979; Turkington and Harper 1979) and there

* Nomenclature of higher plants follows Clapham, Tutin and Warburg (1973).

was some published evidence of the production of toxins by this species (Newman and Rovira 1975; Newbery 1976; Firth 1977; Newman and Miller 1977; Watt 1977; Newbery 1979). The experimental studies described in Part II involve attempts to measure effects of toxin production and competition by *Holcus lanatus* upon the growth of species with which *Holcus lanatus* is frequently associated in the field. Studies were also conducted to measure the influence of temperature and mineral nutrient supply upon a mixed sward in an attempt to manipulate the vigour and competitive ability of *Holcus lanatus* (Chapter 6). Further studies were designed to examine the toxicity of residues accumulated in sand previously exploited by *Holcus lanatus* (Chapters 7 and 9). In an attempt to distinguish effects of allelopathy from those due to mineral nutrient depletion, experiments were carried out (Chapters 8 and 9) and in the final chapter, the effect of *Holcus lanatus* toxins upon seedling growth are examined under various levels of mineral nutrient supply.

PART I

CHAPTER 2

PHENOLOGICAL STUDIES

2.1 INTRODUCTION

In ecological research there have been two main types of phenological study. In the first, attempts have been made to record qualitatively seasonal variation in phenomena such as leaf expansion, flowering and seed production. Using this approach several investigators have described the phenologies of all the main component species in a stand of vegetation and have summarised the results in one diagram, the so-called phenospectrum (Salisbury 1916; Ellenberg 1938; Ahshapanek 1962; Holway and Ward 1965; Jankowska 1967; Dierschke 1970; Lieth 1970; Monasterio and Sarmiento 1976).

The second method is quantitative and relies upon harvests and sorting of the standing crop conducted at intervals throughout the year. This approach has been used in conjunction with measurements of other seasonal variables such as chlorophyll content and leaf area index (e.g. Lieth 1970).

Quantitative studies of the shoot phenology of the constituent species in various types of herbaceous vegetation established in open habitats have been carried out by several investigators (Odum 1960; Iwaki *et al.* 1964; Bliss 1967; Golley and Gentry 1966; Traczyk 1968; Egunjobi 1974; Williamson 1976; Haggard 1976; Al-Mufti *et al.* 1977; McGrath 1979; Furness 1980; Sydes 1980). In other studies (Struik 1965; Kubicek and Brechtl 1970; Hughes 1971; Bazzaz and Bliss 1971; Eber 1971; Hughes 1975; Ford and Newbould 1977; Al-Mufti *et al.* 1977; Al-Mufti 1978) measurements of seasonal change in shoot biomass in herbaceous plants have been made in temperate deciduous woodlands.

The purpose in the studies described in this chapter was to examine quantitatively the phenologies of the common herbs present in seven contrasted types of herbaceous vegetation in the Sheffield region. The harvest method was used in order to determine the seasonal change in dry weight of living shoot material of each constituent species. The measurements also allowed estimates to be made of the seasonal change in total biomass of living herbaceous shoots, herb litter and tree leaf litter at each site.

As explained in the introductory chapter, the objective in these phenological studies was to identify the patterns of shoot growth exhibited by dominant species and to attempt to relate these to seasonal changes in soil nutrient status and in the inhibitory effects of soil toxins. At five of the sites described in this chapter, concurrent soil samplings and seedling bioassays were conducted; these are described in Chapters 3 and 4, respectively.

2.2 THE STUDY AREA AND THE SAMPLING SITES

Seven sites were chosen: all were situated within a radius of 25 km from the centre of Sheffield. The sites represented several types of habitat including a woodland herb layer, a lightly-shaded river terrace, derelict pasture, two sown pastures, a stand of bracken, and an area of heathland. The distribution of the sites within the study area is shown in Figure 2.1. The following descriptions provide more details concerning the sites:

Site 1 Totley Wood

The first site was situated on the Coal Measures in an area previously described by Woodhead (1906), Pigott (1956) and Al-Mufti (1978). The study area occurred within the south-west boundary of the city of Sheffield on a river terrace where the vegetation is rather disturbed and the tree canopy is discontinuous and composed of shrubs, e.g. *Salix capraea*, *Crataegus monogyna*, *Sambucus nigra*, and small specimens of *Fraxinus excelsior* and *Acer pseudoplatanus*. The site was located on an alluvial soil (pH 6.2 at the surface) 10 m from the River Sheaf, the summer level of which was approximately 3 m below the soil surface.

The major species in the ground flora were *Urtica dioica*, *Poa trivialis* and *Ranunculus ficaria*. Local patches of *Mercurialis perennis*, *Hedera helix* and *Veronica montana* occurred and *Galium aparine* was conspicuous during the spring. The remaining herbaceous species were present either as scattered individuals or in local patches. The site also contained bryophytes, most of which occurred

Figure 2.1

Map showing the location of the study sites (1-7) used in the phenological studies, in relation to geological boundaries.

S - Sheffield

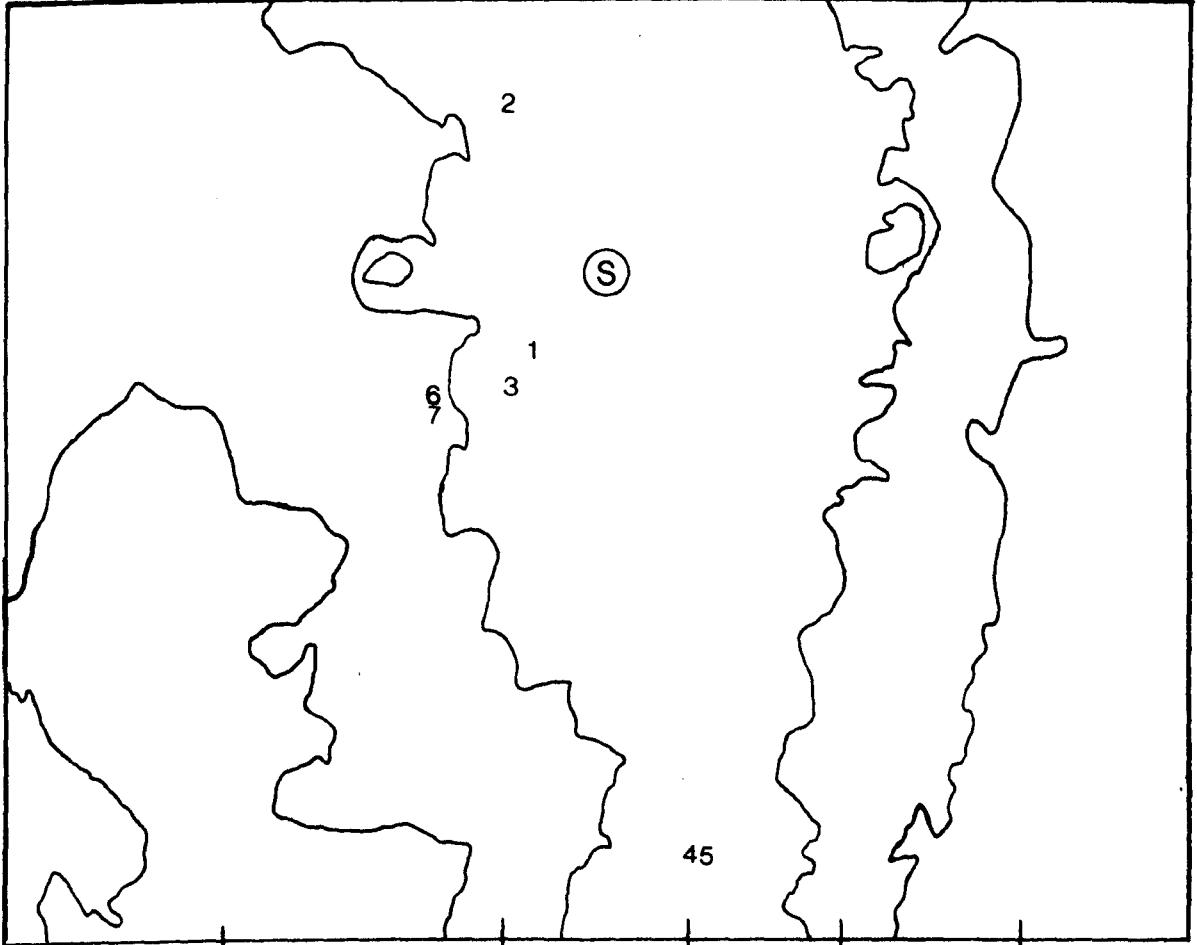
CL - Carboniferous Limestone

MG - Millstone Grit

CM - Coal Measures

ML - Magnesian Limestone

BS - Bunter Sandstone



CL

MG

CM

ML

BS

on the decaying stem litter of *Urtica dioica*. At this site the sampled area was 20 m x 10 m.

Site 2 Beely Wood

This site was situated on a deep alluvial soil (pH 6.7 at the surface) on the edge of the River Don and was shaded from one side by a tree canopy mainly composed of *Acer pseudoplatanus*. The site occupied an area of 13 m x 4 m on the edge of the river and was subject to flooding, silting and scouring during the winter. During the summer the vegetation was composed mainly of a dense stand of *Impatiens glandulifera* with an understorey of *Poa trivialis*. The vegetation also contained a small number of scattered individuals of other species such as *Agrostis stolonifera*, *Cardamine flexuosa*, *Rumex obtusifolius*, *Stellaria alsine* and *Festuca gigantea*. A small amount of bryophyte was also present. Samples were removed from within a fairly homogeneous area of 2 m x 10 m.

Site 3 New Totley

The derelict pasture sampled at New Totley had not been grazed for a period of at least ten years. The site was situated on a Coal Measures brown earth soil with a mean pH value of 5.5 in the surface zone of the profile.

Yorkshire Fog (*Holcus lanatus*) dominated the grassland at this site (see Plate 2.2), although additional species such as *Agrostis tenuis*, *Festuca rubra* and *Poa pratensis* occurred,* along with small amounts of other species which are listed in Appendix 1. The sampling site occupied an area of 4 m x 10 m.

Sites 4 and 5 Littlemoor

Two sites were located on a farm, near Clay Cross in Derbyshire. Site 4 occurred in a field which had been ploughed and sown with barley and various grassland species (listed in Table 2.1) in April 1974. The barley was harvested in August leaving the grass about 4 inches (10.2 cm) in height. The turf was then grazed down to 1-1½ inches (3.9 cm) in the following spring at which time fertilizer was applied and the grass was cut for hay in June, then fertilized again and grazed in August. Fertilizer was applied and grazing was recommenced in October. Management followed the same pattern in each succeeding year. At the time of this study the main species present were *Lolium perenne* and *Lolium multiflorum* together with small amounts of other species such as *Agropyron repens*, *Agrostis stolonifera* and *Poa annua*. The pH at the soil surface at Site 4 was 6.2 and the sampled area was 2 m x 10 m.

Site 5 had been planted in April 1974 with spring barley together with the other species listed in Table 2.2. The barley was harvested in September, leaving the grass at a height of about 2 inches (5.1 cm). In the following spring the vegetation was cut for hay in June, and subjected to fertilizer application and grazing in August and November. During the following year (1976) the field was used for strip grazing by dairy cattle and was treated five times with nitrogenous fertilizer and in 1977 lime was applied during the winter. During 1977, there was an apparent loss of vigour in the sward and only four grazing sessions were carried out. At this site the predominant species during the period of study were *Holcus lanatus* and *Agrostis stolonifera*. The *Lolium* species

Table 2.1 Sown grassland species

Species	Weight of seed per acre (lbs)
<i>Dactylis glomerata</i> (Cocksfoot, English)	1
<i>Festuca pratense</i> (Meadow Fescue)	1
<i>Lolium multiflorum</i> (Italian Ryegrass, Danish EF486)	3
<i>Lolium perenne</i> (Perennial Ryegrass, Danish)	11
<i>Phleum pratense</i> (Timothy, Canadian)	2
<i>Trifolium dubium</i> (Trefoil, English)	1
<i>Trifolium hybridum</i> (Alsike, Canadian)	1
<i>Trifolium pratense</i> (Broad Red Clover, English)	3
	23

Table 2.2 Sown grassland species

Species	Weight of seed per acre (lbs)
<i>Lolium perenne</i> (Perennial Ryegrass, Devon Eaver)	6
<i>Lolium perenne</i> (Ryegrass, H/1 Short Rotation, Manawa)	2
<i>Lolium perenne</i> (Perennial Ryegrass, Danish)	4
<i>Lolium multiflorum</i> (Italian Ryegrass, Danish EF486)	4
<i>Phleum pratense</i> (Timothy, S.48)	2
<i>Phleum pratense</i> (Timothy, Canadian)	4
<i>Trifolium pratense</i> (Broad Red Clover, English)	2
<i>Trifolium pratense</i> (Late flowering Red Clover, Canadian)	2
<i>Trifolium repens</i> (White Clover, N.Z.Cert. Permanent pasture)	1
<i>Trifolium hybridum</i> (Alsike)	1
	28

(*perenne* and *multiflorum*) composed 15% of the biomass in spring and summer while in autumn they contributed 60% of the total living plant material. A list of the minor species present at this site is given in Appendix 1. The site occupied a homogeneous area of 2 m x 10 m. The mean pH at the soil surface (0-5 cm) was 5.8.

Sites 6 and 7 Stoney Ridge

The Stoney Ridge sites were situated on Millstone Grit approximately 8 km from the centre of Sheffield. The first site (Site 6) was located within an extensive stand of bracken (*Pteridium aquilinum*) and contained small amounts of *Deschampsia flexuosa*, while Site 7 was situated in adjacent heathland. Site 7 was dominated by *Calluna vulgaris*, and as in Site 6 was associated with a podzolic soil (mean pH 3.5 at 0-5 cm). Site 6 occupied an area of 5 m x 10 m.

The vegetation at Site 7 was composed of a dense stand of *Calluna vulgaris*, containing a small number of scattered individuals of other species such as *Empetrum nigrum* and *Deschampsia flexuosa*. At the centre of this site a small homogeneous area of 5 m x 10 m was selected. The soil at this site had a pH of 3.6 at the soil surface (0-5 cm).

2.3 PROCEDURE

2.3.1 Sampling

On each sampling occasion the above-ground vegetation was removed along with the soil to a depth of 10 cm in 0.25 m^{-2} quadrats each located at random within a subsection of the sampled area. At Sites 1, 2, 3, 6 and 7, five replicates were taken, but at Sites 4 and 5 seven samples were removed. Sampling commenced in April 1978 at Sites 1, 6 and 7, in March at Sites 2 and 3 and in February 1977 at Sites 4 and 5. The samples were transported to the laboratory and stored in a cold room at 5°C .

2.3.2 Sorting

The vegetation in each quadrat was sorted by hand into the following components:

- a) living shoots of each herbaceous species;
- b) total living bryophytes;
- c) herbaceous litter;
- d) tree leaf litter.

After sorting the material was dried in an oven at 90°C for 48 hours and weighed.

2.3.3 Data analysis

Graphs have been drawn for each site, showing the seasonal change in standing crop and in shoot biomass of individual species and in density of herbaceous litter and tree litter. The data obtained for minor contributors to the vegetation, and for those species showing a scattered but contagious distribution within the sampling area are listed in Appendix 1.

3.4 RESULTS

Site 1

Figure 2.2 illustrates the seasonal variation in the dry weight of herbaceous shoot material, herbaceous litter and tree leaf litter at Site 1. The maximum standing crop (350 g m^{-2}) occurred in July. There was little variation in the density of tree litter during spring and summer with a mean density of about 1.0 g m^{-2} , rising to a maximum of approximately 130 g m^{-2} during the autumn. The density of herbaceous litter remained below 45 g m^{-2} throughout the investigation.

Seasonal patterns of shoot biomass for ten herbaceous species and for the bryophyte component are shown in Figure 2.3. *Poa trivialis*, *Galium aparine*, *Anemone nemorosa* and *Ranunculus ficaria* attained maximum shoot biomass during the spring and early summer. These four species were characterized by rapid shoot expansion and this was associated with early flowering and seed production.

In *Ranunculus ficaria* and *Anemone nemorosa* maximum biomass and flowering occurred before the canopy of the trees was fully expanded. The maximum standing crop attained by *Poa trivialis* was 35 g m^{-2} while that for *Galium aparine* was 8 g m^{-2} . Both of the latter species reached their maximum shoot biomass in July; the leaves persisted rather longer into the shaded phase and the biomass declined only after the onset of seed production. The evergreen *Hedera helix* showed a slight increase in dry weight and reached maximum biomass (85 g m^{-2}) in July and then declined in

Figure 2.2

Seasonal change in the dry weight of three above-ground components at Site 1.

○—○ total living shoot material

●—● herbaceous litter

■---■ tree leaf litter

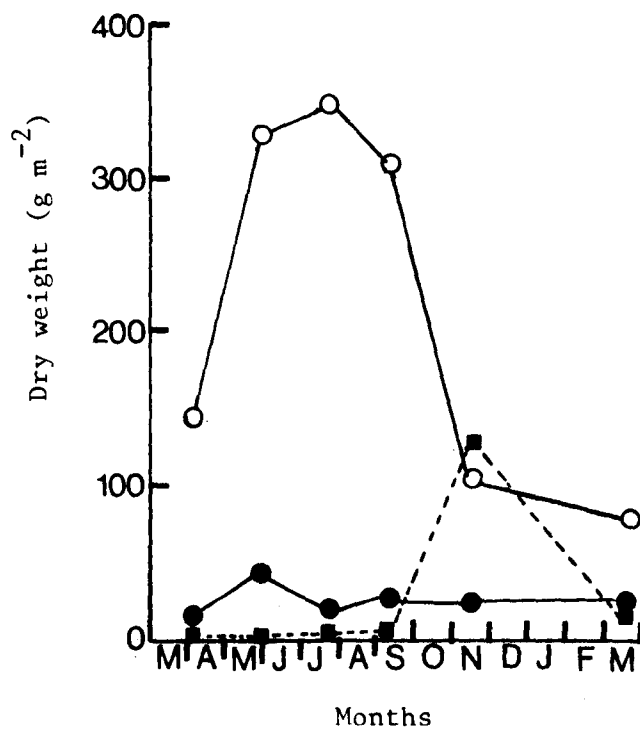
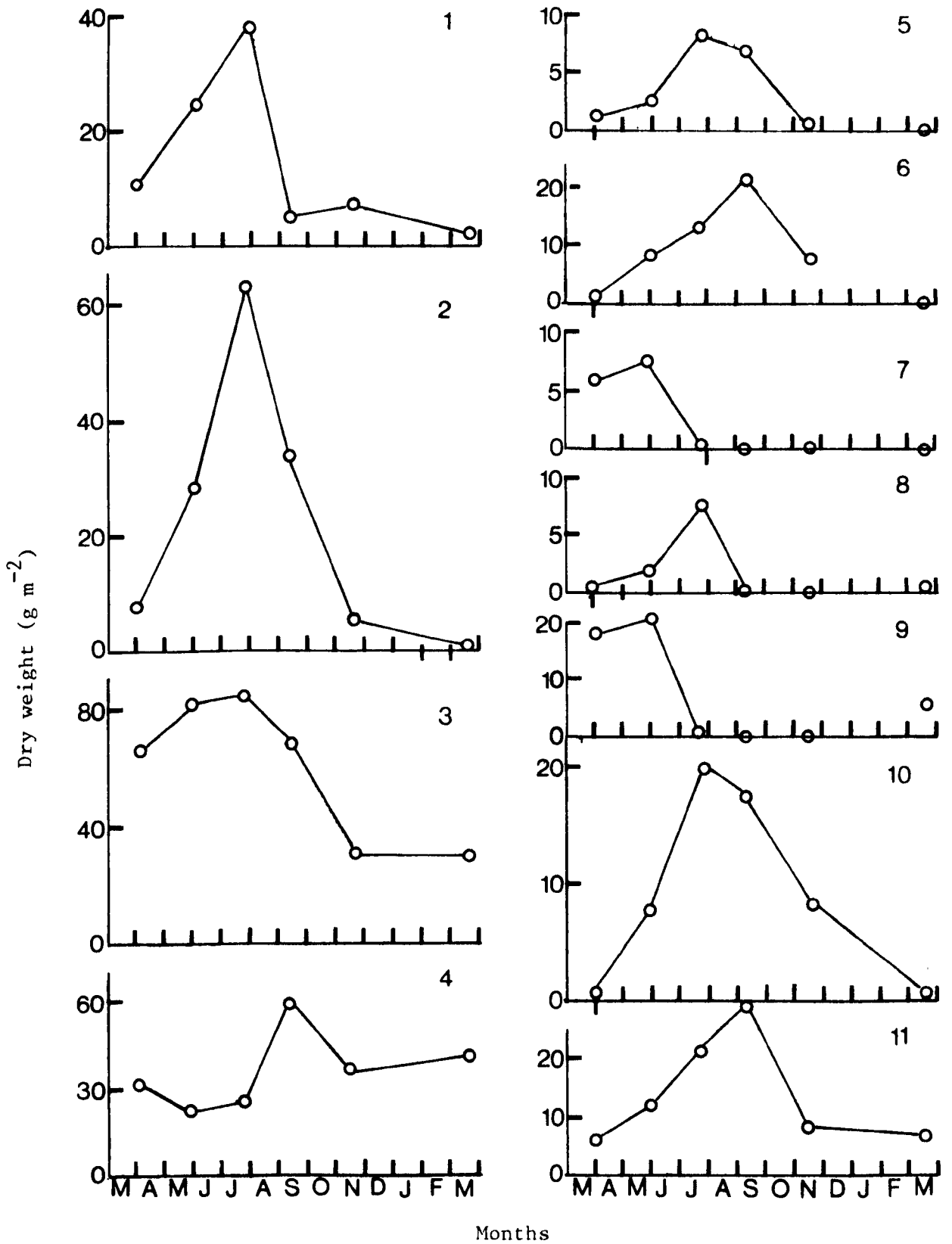


Figure 2.3

Seasonal changes in the shoot biomass of the main constituents of the herb layer at Site 1:

- (1) *Poa trivialis*, (2) *Urtica dioica*, (3) *Hedera helix*,
(4) Bryophytes, (5) *Circaea lutetiana*, (6) *Festuca gigantea*, (7) *Anemone nemorosa*, (8) *Galium aparine*,
(9) *Ranunculus ficaria*, (10) *Mercurialis perennis*,
(11) *Veronica montana*.



winter. *Urtica dioica* produced a peak biomass (64 g m^{-2}) during July; but this was followed by a decline in shoot dry weight during the summer shade phase, at which time many of the nettle plants became etiolated and failed to flower and produce seeds.

Mercurialis perennis and *Veronica montana* exhibited rather different shoot phenologies from the above in that there was a peak in shoot biomass much later in the summer. *M. perennis* attained maximum biomass in July while *V. montana* showed a different pattern with a maximum in late summer. Maximum standing crop for *Circaea lutetiana* occurred in the late spring although some shoots persisted during the shade phase. The bryophyte component showed two peaks in standing crop. The first occurred in spring whilst the second, in September, coincided with the decline of the herbaceous canopy.

The remaining species listed in Appendix 1 were present either in smaller patches or as scattered individuals and made only a minor contribution to the shoot biomass.

Site 2

Seasonal changes in the mean dry weight of living and dead shoot material and tree litter at Site 2 are described in Figure 2.4. The shoot biomass showed a well-defined peak in late summer with a maximum standing crop of 355 g m^{-2} , about two-thirds of which was contributed by *Impatiens glandulifera*. This was followed by a decline to a value of approximately 44 g m^{-2} in November at which time the herbaceous litter reached a maximum of 32 g m^{-2} . The density

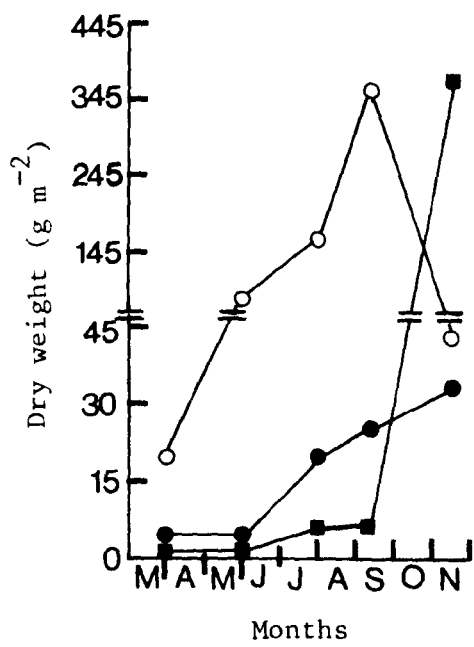
Figure 2.4

Seasonal changes in the dry weight of three above-ground components at Site 2:

○—○ total living shoots

●—● herbaceous litter

■—■ tree leaf litter



of tree litter showed a sharp increase and reached a maximum of 370 g m^{-2} in November.

In Figure 2.5 graphs are presented describing the shoot phenology of the main constituents of the standing crop. The annual species *Impatiens glandulifera* was scarcely detected in April but then expanded its shoots extremely rapidly to achieve a maximum in late summer. At this time the shoots of *I. glandulifera* were about 1.25 m in height (Plate 2.1). After seed production the biomass declined sharply to 10 g m^{-2} in November. It is interesting to observe that *Poa trivialis* reached maximum biomass (70 g m^{-2}) in early summer and persisted, but with declining vigour, through the summer shade phase. This pattern was similar to that recorded for *P. trivialis* at Site 1. *Stellaria alsine* and *Cardamine flexuosa* were both characterized by narrow peaks in biomass occurring in spring and declining in summer. *Agrostis stolonifera* reached maximum standing crop (20 g m^{-2}) in July.

The shoot phenology of *Festuca gigantea* resembled that described for the same species at Site 2. *Rumex obtusifolius*, which was mainly represented by seedlings, reached a peak in standing crop during the summer. The bryophyte component expanded to a peak in April and declined progressively throughout the summer and autumn.

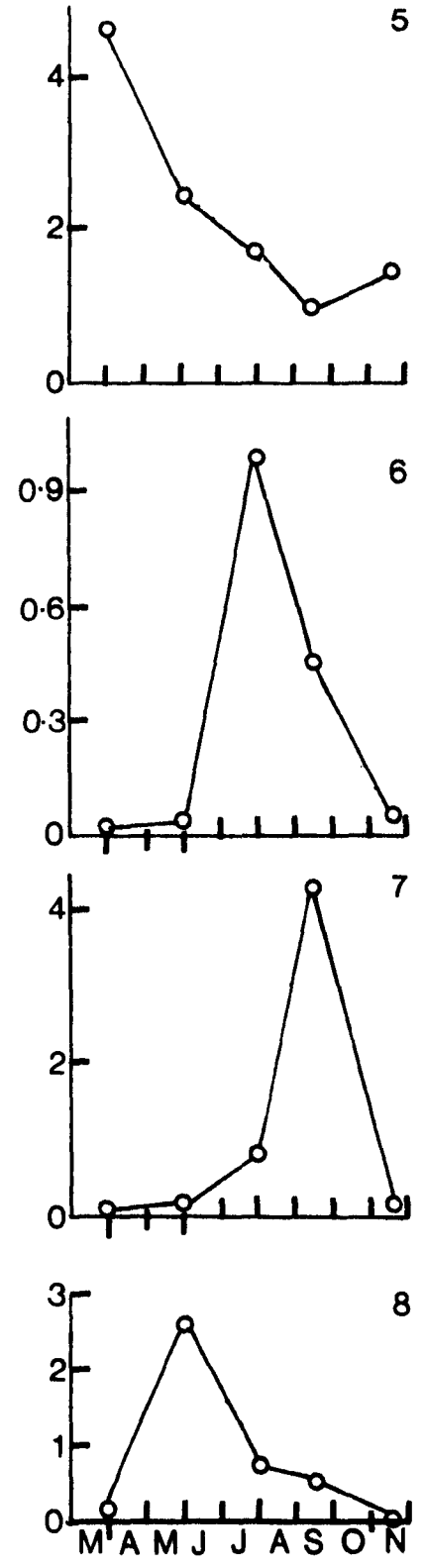
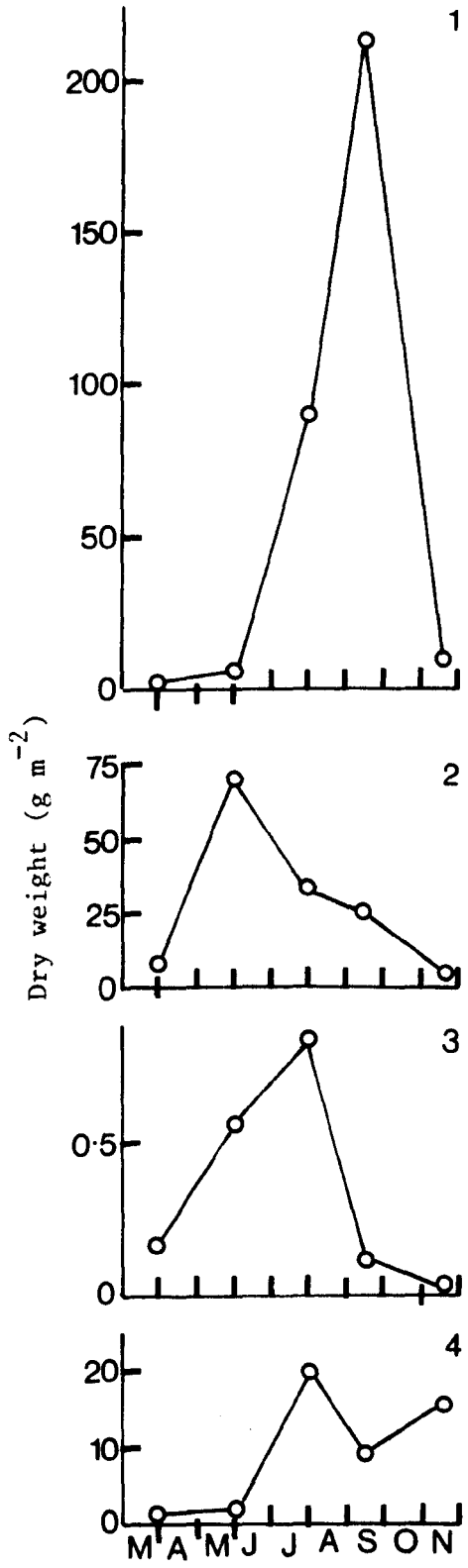
Site 3

Figure 2.6 illustrates the changes in the dry weight of living shoot material and litter measured in the derelict grassland

Figure 2.5

Seasonal changes in shoot biomass of the main constituents of the herb layer at Site 2:

- (1) *Impatiens glandulifera*, (2) *Poa trivialis*,
(3) *Stellaria alsine*, (4) *Agrostis stolonifera*,
(5) Bryophytes, (6) *Rumex obtusifolius*, (7) *Festuca gigantea*, (8) *Cardamine flexuosa*.



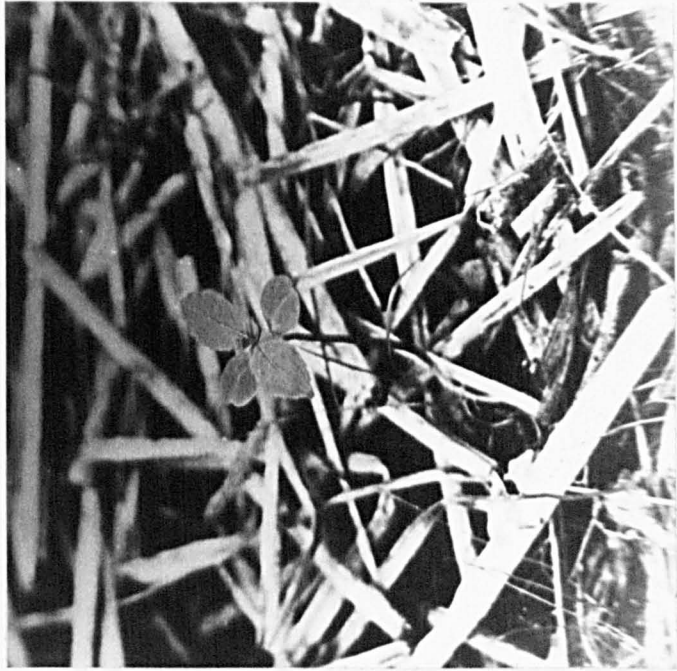
Months

PLATE 2.1

Two phases in the life-cycle of the large summer annual, *Impatiens glandulifera*, on a river bank in North Derbyshire, England. The plate (a) shows the large seedling emerging in the spring through the stem litter remaining from the previous year whilst (b) is a vertical section through the canopy of three neighbouring plants photographed in the early summer.



b



a

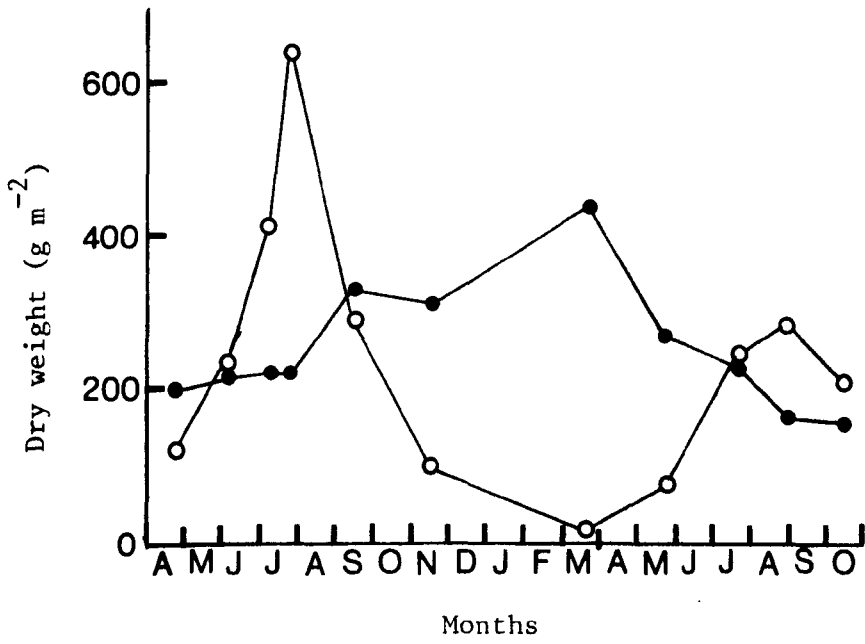
Figure 2.6

Seasonal changes in the dry weight of the shoot

Figure 2.6

Seasonal changes in the dry weight of the shoot material and litter at Site 3.

○—○ total living shoots
●—● herbaceous litter



examined at New Totley. The data show a marked seasonal fluctuation in the standing crop which reached maxima of 643 g m^{-2} in July 1978 and 284 g m^{-2} in August 1979. Herbaceous litter composed almost exclusively of the shoots of *Holcus lanatus* was present in abundance throughout the period of study, remaining about 160 g m^{-2} and rising to 441 g m^{-2} by March 1979.

In the spring and summer of 1978 the shoots of *H. lanatus* occurred in July but with the onset of flowering there was a sharp decline and the quantity of green material fell to a level comparable with that recorded in the spring. The amount of living material of *H. lanatus* was reduced in 1979, as compared with the spring and summer of the previous year. Plate 2.2 shows the great change in *H. lanatus* biomass between July 1978 and July 1979.

Agrostis tenuis developed a peak in shoot biomass of 90 g m^{-2} during July 1978 but, in contrast to *H. lanatus*, the standing crop declined much later in summer, a pattern which was correlated with seed production which occurs comparatively late in this species. In marked contrast to the pattern observed in *H. lanatus*, the summer maximum in shoot biomass in *A. tenuis* was considerably greater in 1979 than that measured in the previous year.

The shoot phenology of *Festuca rubra* showed a peak (25 g m^{-2}) during the spring of 1978 and declined slowly during the summer. The amount of living material of *F. rubra* was reduced in 1979, as compared with the values attained during the spring and summer of the previous year.

PLATE 2.2

Appearance of the sampling site at New Totley in July 1978 (top) and July 1979 (bottom).

Whitish *Holcus* inflorescences were numerous in 1978 but were rare in 1979 following severe winter conditions.



Figure 2.7

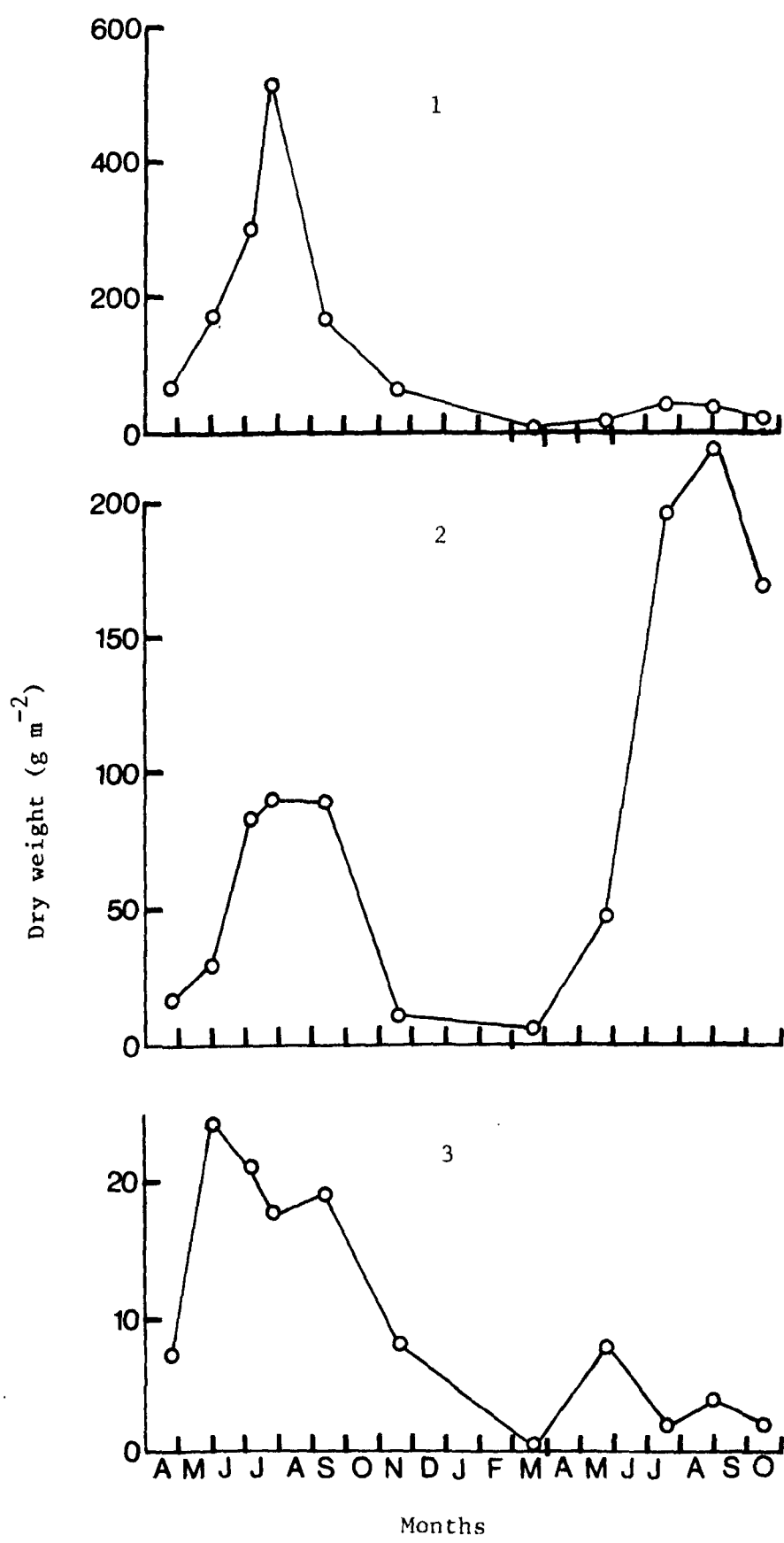
Seasonal changes in the shoot biomass of the main



Figure 2.7

Seasonal changes in the shoot biomass of the main constituents of the herb layer at Site 3:

- (1) *Holcus lanatus*, (2) *Agrostis tenuis*,
- (3) *Festuca rubra*



Site 4

Figure 2.8 illustrates the seasonal variation in dry weight of living shoot material and herbaceous litter at Site 4. Two peaks in standing crop are apparent. Following the first peak in June, the standing crop was drastically reduced when the field was cut for hay. A second peak occurred in September and was largely due to expansion of the two species of *Lolium* (*multiflorum* and *perenne*) which were major components of the sward and formed 93% of the shoot biomass in May. The amount of herbaceous litter at this site reached a maximum of 210 g m^{-2} in July. Figure 2.9 shows the phenology of four species present as minor sward components. *Agropyron repens* attained its maximum standing crop (28 g m^{-2}) in June while the highest percentage contribution to the total shoot biomass was 13% in May. *Poa annua* showed a maximum percentage contribution in October (12%) while the maximum biomass occurred in June (19 g m^{-2}). The maximum standing crop of *Agrostis stolonifera* (15 g m^{-2}) appeared in August while the maximum percentage (12%) was attained in October.

Site 5

As shown in Figure 2.10, the standing crop at Site 5 was lower than that at Site 4. The maximum in living shoots (170 g m^{-2}) occurred in August, while the herbaceous litter declined in the spring, then slightly increased during the summer. The graphs in Figure 2.11 show the seasonal variation in shoot biomass in various components of the standing crop. A range of different patterns are

Figure 2.8

Seasonal changes in the dry weight of shoot material and herbaceous litter at Site 4.

○—○ total living shoot material

●—● herbaceous litter

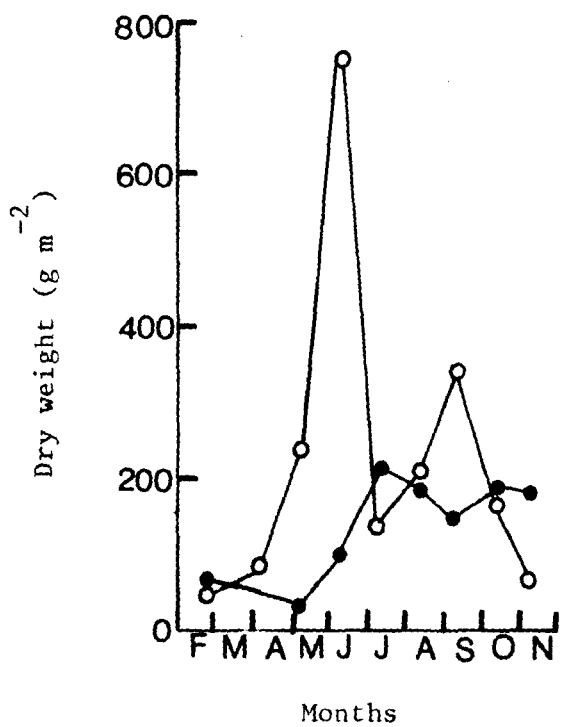
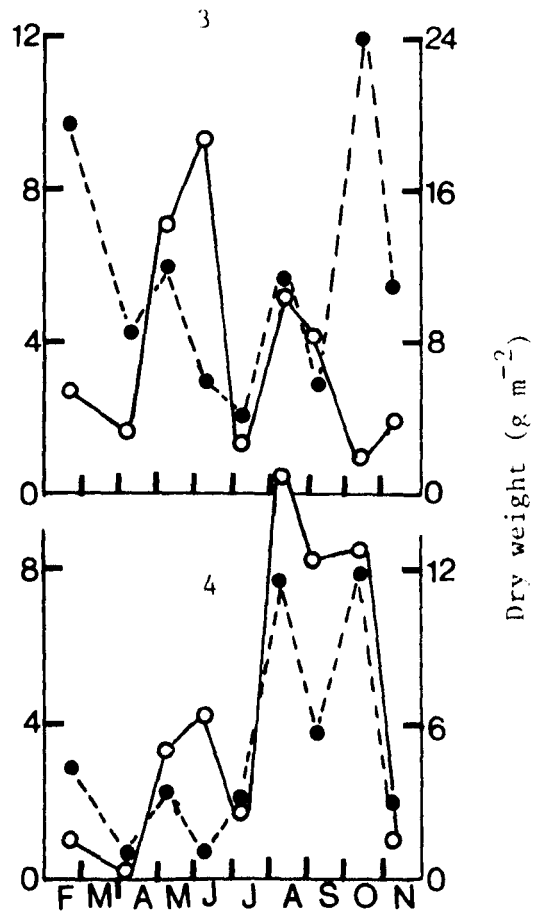
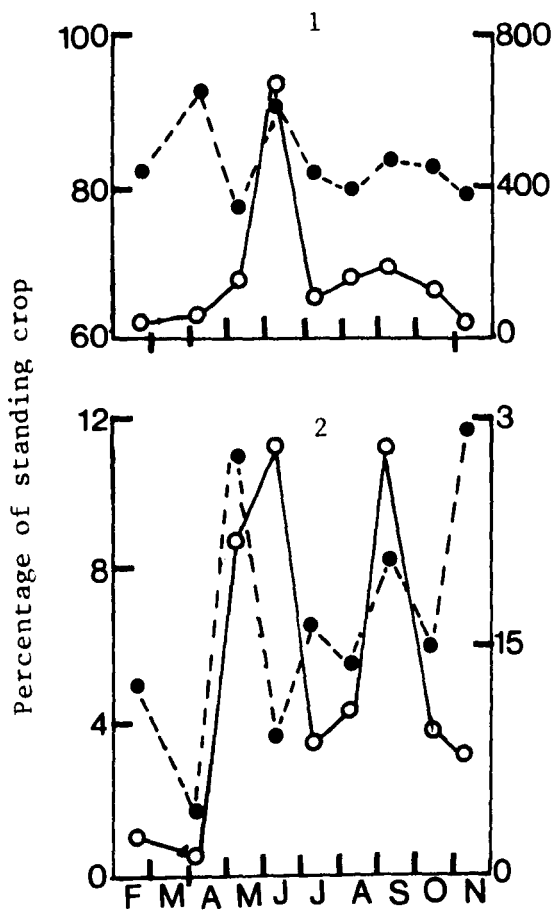


Figure 2.9

Seasonal changes in dry weight (o—o), and percentage contribution (●---●) of component species to the standing crop at Site 4:

- (1) *Lolium* (*perenne* and *multiflorum*), (2) *Agropyron repens*,
(3) *Poa annua*, (4) *Agrostis stolonifera*.



Months

Figure 2.10

Seasonal changes in the dry weight of shoot material (o—o)
and litter (●—●) at Site 5.

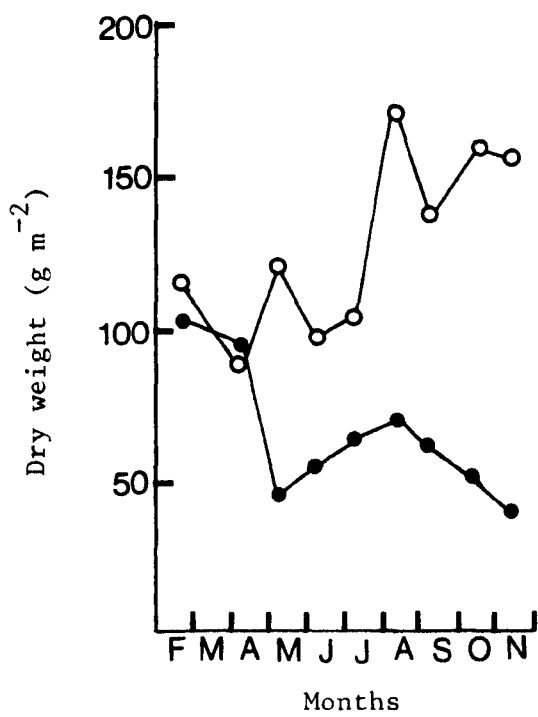
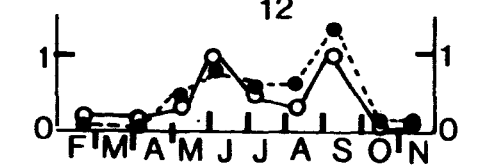
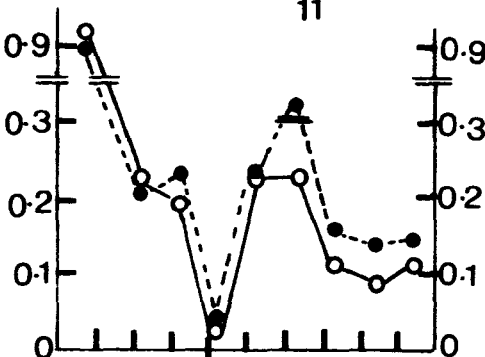
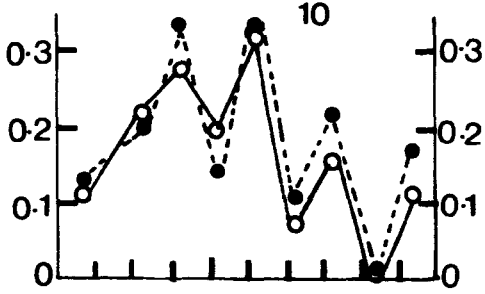
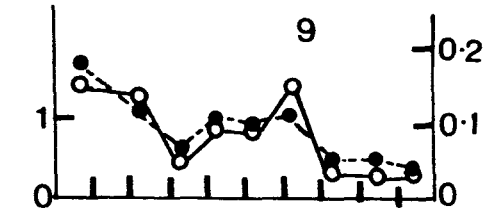
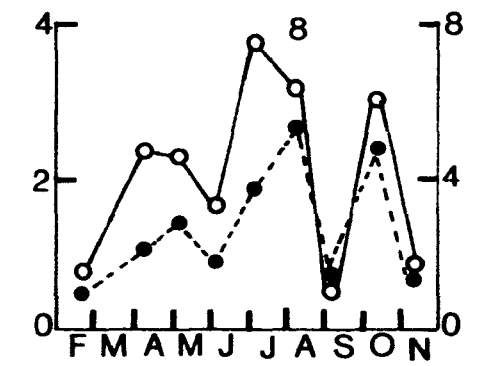
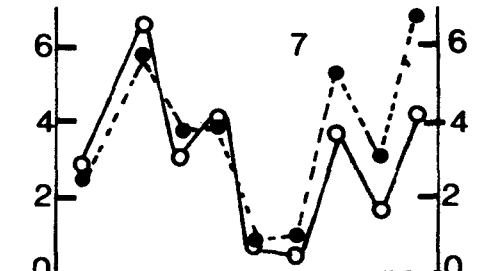
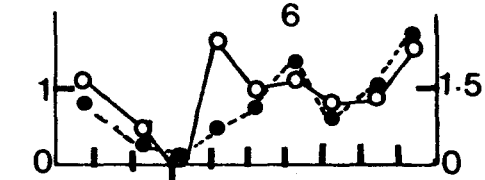
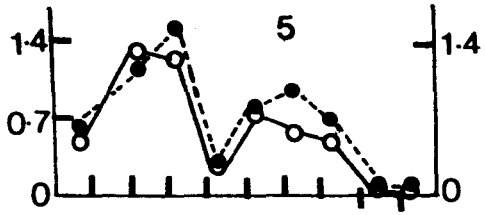
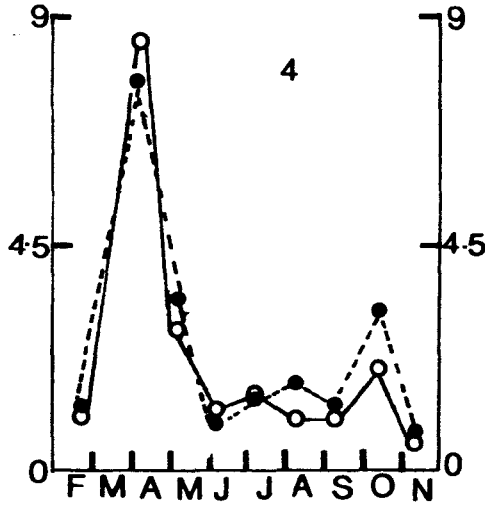
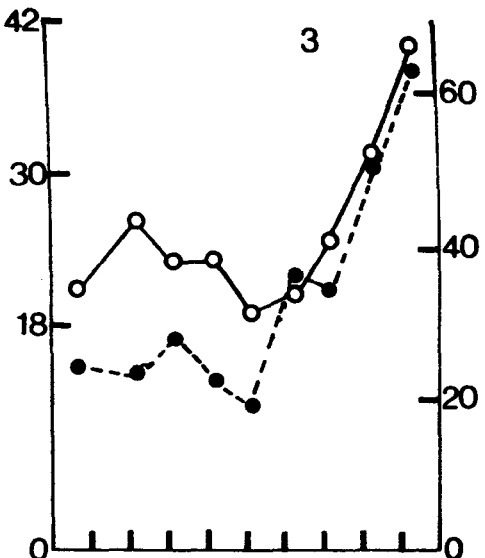
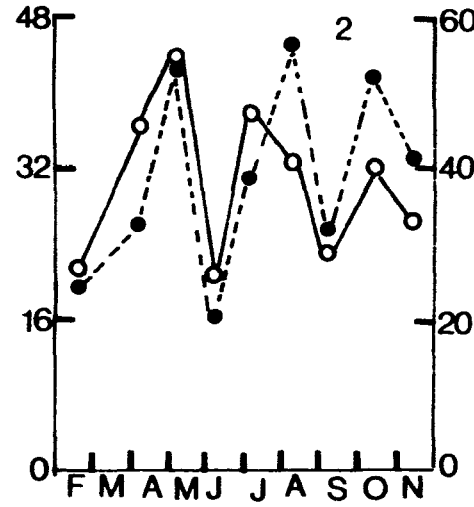
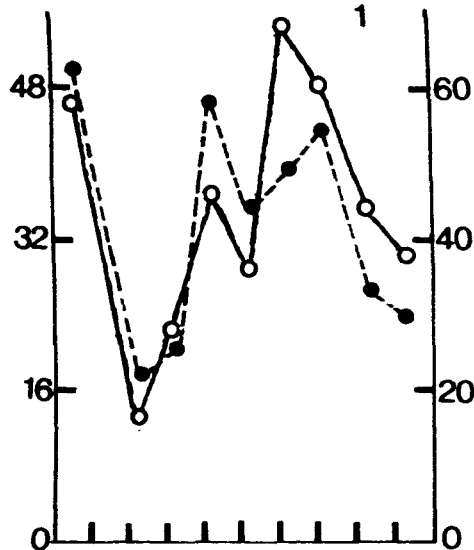
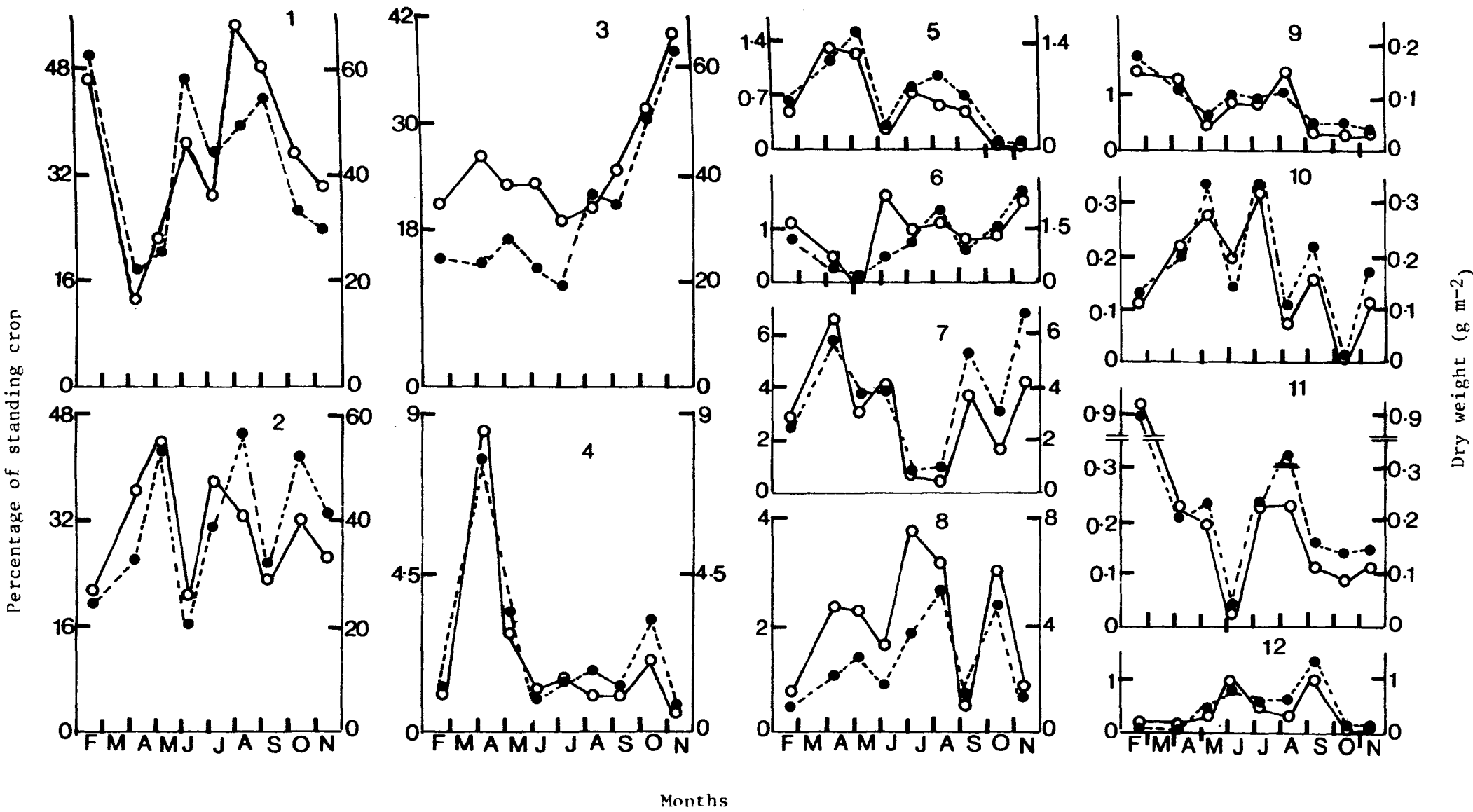


Figure 2.11

Seasonal changes in dry weight (o—o), and percentage contribution (●---●) of component species to the standing crop at Site 5:

- (1) *Agrostis stolonifera*, (2) *Holcus lanatus*, (3) *Lolium* (*perenne* and *multiflorum*), (4) *Anthoxanthum odoratum*,
- (5) *Trifolium pratense*, (6) *Trifolium repens*,
- (7) *Plantago lanceolata*, (8) *Ranunculus repens*,
- (9) Bryophytes, (10) *Taraxacum officinale*,
- (11) *Cerastium holosteoides*, (12) *Hypochoeris radicata*.



apparent among the eleven species for which data were obtained. The two major component species at this site were *Agrostis stolonifera* and *Holcus lanatus*.

The phenology of *Agrostis stolonifera* was characterized by a seasonal maximum of 68 g m^{-2} in August. When expressed as a percentage of the standing crop two peaks were apparent, the first in June and the other in September. *Holcus lanatus* exhibited a shoot phenology in which there was a small peak in June followed by a decline in July associated with cattle grazing.

Following the cessation of grazing a second peak in *H. lanatus* shoot material occurred in August. It is interesting to note that *H. lanatus* recovered quickly after being grazed in July and during the following month this species formed one-third of the total shoot biomass.

The two *Lolium* species (*multiflorum* and *perenne*) and *Anthoxanthum odoratum* showed two peaks, one in spring and the other in autumn.

A small number of additional species of scattered occurrence and low contribution to the total shoot biomass were also present at this site. *Plantago lanceolata* reached a maximum of 7 g m^{-2} in November and two peaks appeared in the graph. The amount of *Ranunculus repens* was extremely low and the shoot biomass did not exceed 2.6 g m^{-2} . *Trifolium pratense*, *Trifolium repens*, *Taraxacum officinale*, *Cerastium fontanum*, *Hypochoeris radicata*, and bryophytes were minor components at this site and none exceeded 1.5% of the total dry weight of living shoots. However, even among the minor species different seasonal patterns of shoot development were apparent.

Trifolium pratense showed two peaks, one in June and the second in August, while in *T. repens* the maximum occurred in August. The bryophytes displayed two peaks, one in spring and the other in late summer.

Only one peak in shoot biomass was observed in *Taraxacum officinale* and *Cerastium holosteoides* both of which expanded to a maximum during the summer.

Site 6

The area of bracken examined at Stony Ridge (Figure 2.12a) attained a very high standing crop; the density of shoot material increased throughout the summer to a peak value of 1700 g m^{-2} in August. This maximum was followed by a steep decline during the autumn. The vegetation at this site consisted almost exclusively of *Pteridium aquilinum* and the pattern shown in Figure 2.13(2) is clearly a function of the phenology of this species. The standing crop of *Pteridium aquilinum* reached a value of 1699 g m^{-2} in August. At this time the shoots of *Pteridium aquilinum* were about 1.5 metres in height. *Deschampsia flexuosa*, the only herbaceous species present at Site 6, attained a maximum shoot biomass of 4 g m^{-2} .

A striking feature of Site 6 was the amount of herbaceous litter, which exceeded that of the living component by a considerable margin except during the summer peak in standing crop.

Figure 2.12

Seasonal changes in the dry weight of living shoot material (o—o) and litter (●—●) at Sites 6 and 7.

(a) Site 6

(b) Site 7

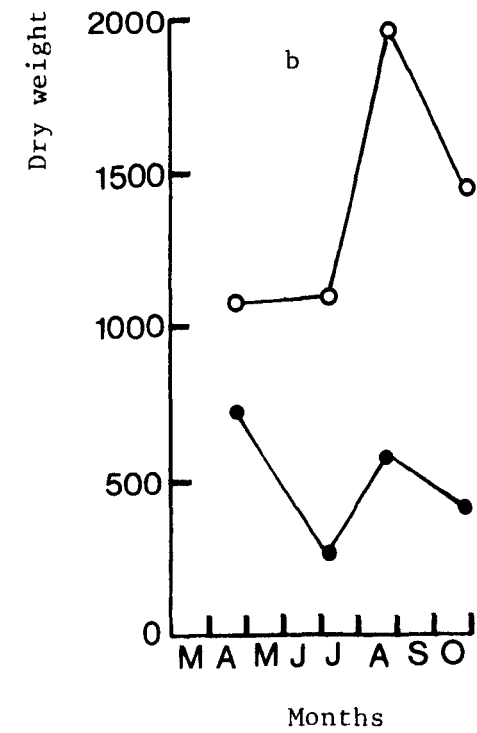
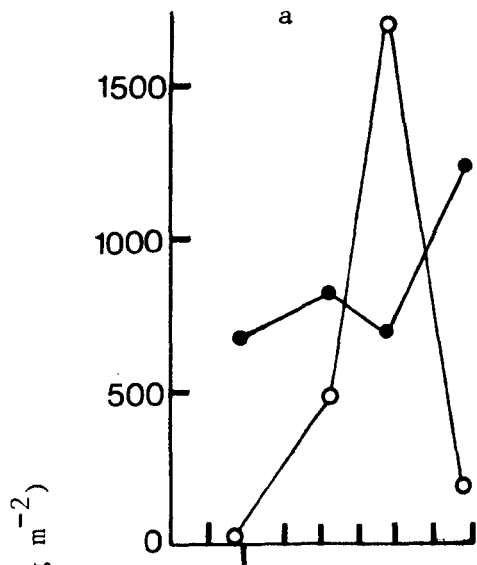


Figure 2.13

**Seasonal changes in shoot biomass of the main
constituents of**

Site 6:

(1) *Deschampsia flexuosa*

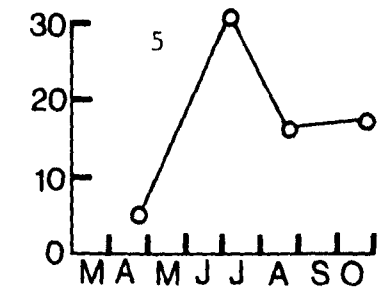
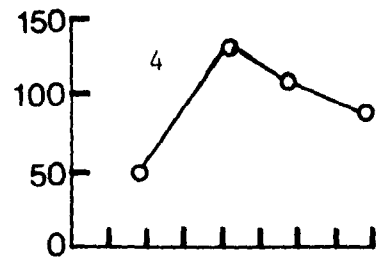
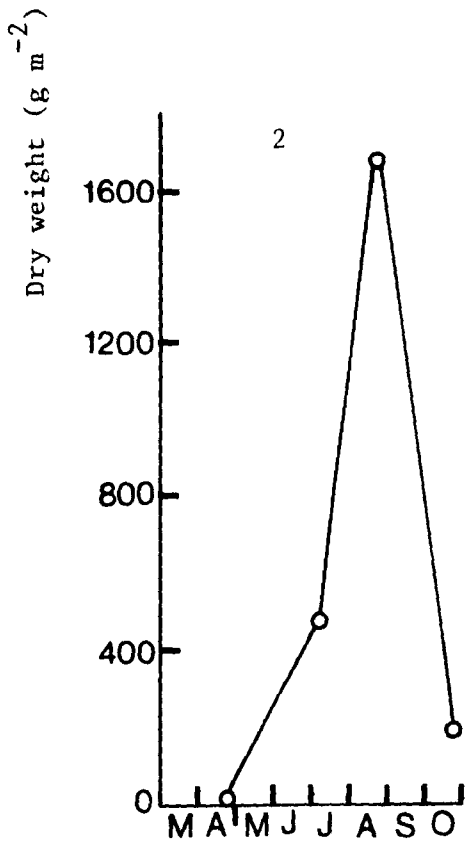
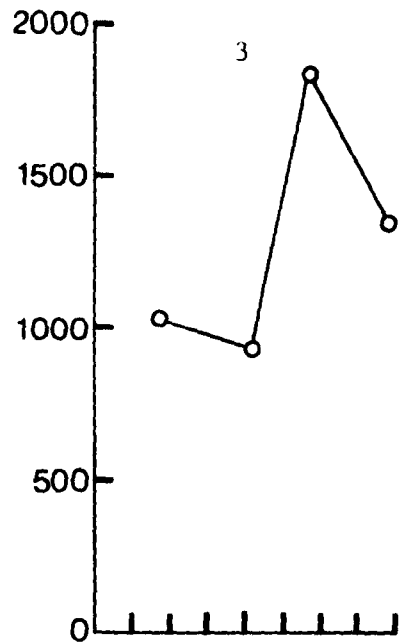
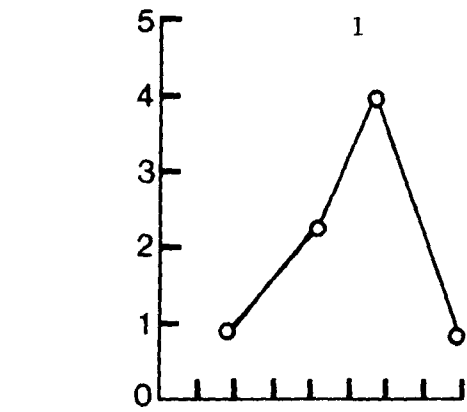
(2) *Pteridium aquilinum*

Site 7:

(3) *Calluna vulgaris*

(4) *Empetrum nigrum*

(5) *Deschampsia flexuosa*



Months

Site 7

Although data for this site were collected on only four occasions it is apparent that at Stoney Ridge (Figure 2.12b) the peak in standing crop occurred in August. The dry weight of litter, most of which was composed of the dead stems of *Calluna vulgaris*, remained above 250 g m^{-2} throughout the period of study. Two further species occurred in the site (Figure 2.13). The shrub *Empetrum nigrum* which was the second most abundant component at the site, reached a maximum standing crop (132 g m^{-2}) in July. *Deschampsia flexuosa* produced a greater yield than in Site 6 and reached a maximum of 30 g m^{-2} in July.

2.5 DISCUSSION

Quantitative studies of the shoot phenology of herbaceous species in woodland herb layers, in grassland, and in other common habitats of the British Isles are few in number and the results obtained in the present study are therefore of interest since they allow an attempt to be made to classify the phenologies into basic types.

Using the data from Site 1, three groups of species may be distinguished. In the first group, the species reached maximum shoot weight during the spring and this was followed by a rapid decline to zero after full expansion of the tree canopy. It would appear that species in this group are unable to tolerate low light intensities (Blackman and Rutter 1946; Goryshina 1972; Koyama and Kawane 1973; Ford and Newbould 1977; and Al-Mufti 1978). In the present study this group was represented by two geophytes, *Anemone nemorosa* and *Ranunculus ficaria* and by certain other species such as *Poa trivialis* and *Galium aparine* which resemble the geophytes in phenology but are rather more persistent after expansion of the tree canopy. All four species are characterized by rapid shoot expansion during the spring light phase. A second phenological type which could be recognized at Site 1 was represented by *Urtica dioica*, a species which commonly occurs in unshaded habitats. In this species the peak in shoot biomass occurred in early summer at a time when the tree canopy was fully expanded. After reaching the peak in shoot biomass there was a gradual decline during the low-light phase. The third phenological group recognized at Site 1 included *Circaea lutetiana*, *Mercurialis perennis* and *Veronica montana*.

The species in this group exhibited peaks in shoot development during the summer but there was only a relatively small decline in shoot biomass during the shaded phase.

The physiology of certain of the species encountered in this study has been investigated previously (Lieth and Ashton 1961). These workers measured the light compensation points and respiration rates of severed leaves from the vernal species *Ranunculus ficaria*, *Anemone nemorosa* and *Allium ursinum*, and on the basis of these measurements they concluded that *Allium ursinum* was more shade-tolerant than *Anemone nemorosa* while *Ranunculus ficaria* was intermediate in tolerance.

In Canada, Sparling (1964,1967) classified a range of woodland herbs according to their phenologies and recognized (1) vernal shade-intolerant plants, (2) intermediate semi-shade-tolerant species and (3) late, shade-tolerant species. Sparling (1967) also showed that plants from these groups differ in their photosynthetic response to light intensity. The vernal species exhibited light-saturation of photosynthesis at intensities higher than those recorded for intermediate and late-expanding species.

Physiological studies on species of contrasted phenology were also conducted by Taylor and Pearcy (1976). These authors employed 22°C as the experimental temperature for all species in an open-system gas analysis apparatus which was used to investigate the seasonal trends in the photosynthetic characteristics of two representative species from each of three phenological types present in the deciduous forests near New York. It was found that these species in which photosynthetic activity was mainly restricted to

the period before tree canopy development were characterized by high maximum light-saturated net photosynthetic rates and high leaf and mesophyll conductances.*

In vernal species retaining some foliage during both the light and shade phases it was found that the potential for moderately rapid light-saturated rates of CO_2 uptake occurred during the light phase but these rates dropped remarkably in response to canopy closure.

In the third group, composed of species with foliage persistent during the summer under strongly reduced light intensities, photosynthesis was associated with a low light saturation point and low leaf conductances. In this group of species low dark respiration rates were recorded and these resulted at low light intensities in rates of net CO_2 uptake higher than those observed in species from the other phenological groups.

The present study has revealed a range of phenological types in woodland herbs similar to that examined by the North American workers and it seems likely that comparative studies on British species will expose comparable differences in photosynthetic capacity and acclimation to light intensity.

Another phenological type recognizable in the present study in a wide range of habitats was exemplified by *Urtica dioica*, *Agrostis stolonifera*, *Holcus lanatus* and *Pteridium aquilinum*. All these species appear to have the potential to produce a large peak in biomass during the summer although under certain conditions (e.g. *Urtica dioica* at Site 2 (Figure 2.3)) shading appeared to limit production well below this potential. A good example of

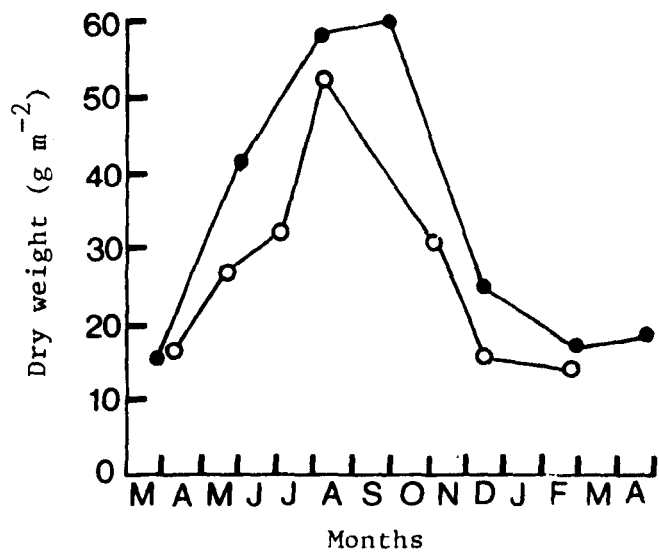
*Leaf conductance to CO_2 exchange was calculated from the equation: $CL = [T / (e_L - e_a)] (D_{\text{CO}_2} / D_{\text{H}_2\text{O}})$, where CL = Leaf (stomatal and boundary layer) conductance in cm sec^{-1} , T = transpiration rate in $\text{g cm}^{-2} \text{sec}^{-2}$, e_L = saturated water vapour density at leaf temperature in g ml^{-1} , e_a = water vapour density of the photosynthesis chamber atmosphere in g ml^{-1} , D_{CO_2} and $D_{\text{H}_2\text{O}}$ = diffusion coefficients of CO_2 and H_2O .

this type of phenology is evident in Figure 2.13.2 which describes the seasonal change in biomass in a vigorous stand of the perennial fern *Pteridium aquilinum* in which the density of living shoot material reached a summer maximum of approximately 1700 g m^{-2} . A rather different species which adheres to this phenological type occurred at Site 2. Here, a large peak in shoot biomass was recorded in the large annual *Impatiens glandulifera* (Plate 2.1) which in Europe colonizes extensive areas at the margins of water courses and produces a large shoot biomass which, as shown in Figure 2.5, is capable of dominating the shoot biomass during the summer.

It is clear that in contrast to the perennial dominants (e.g. *Urtica dioica*, *Pteridium aquilinum*) where individual plants may occupy extensive areas the tendency of *Impatiens glandulifera* to suppress the growth of other species can be related to the combined effect of a dense population of short-lived plants of similar size and maturity. At Site 3, the derelict grassland of New Totley, *Holcus lanatus* was the major component species during 1978 and formed a virtual monoculture during the main growing season (Figure 2.7). It is interesting to examine the phenological patterns of the few species (e.g. *Festuca rubra*, *Agrostis tenuis*) associated with *H. lanatus* at Site 3. *Festuca rubra* showed a phenology completely different from that described for the same species by Williamson (1976) and Al-Mufti *et al.* (1977) (Figure 2.14) in that a decline in shoot biomass of *F. rubra* coincided with peak biomass development in *H. lanatus* which produced many tillers and a very dense canopy at this time. It seems reasonable to conclude that

Figure 2.14

Seasonal change in the shoot biomass of the perennial grass, *Festuca rubra*, measured in derelict calcareous pastures at two sites in Northern (o—o) and Southern (●—●) England (Williamson 1976; Al-Mufti *et al.* 1977).



these phenological data indicate a strong negative effect of *Holcus lanatus* upon *Festuca rubra* during the summer. In a study conducted concurrently at the same site McGrath and Al-Mashhadani (1980) found evidence that intraspecific interactions within the population of *H. lanatus* reached a maximum during the summer at which time high mortalities occurred in seedlings and small plants.

The results from the second year showed that there was a corresponding increase in the amount of litter in May 1979. It seems possible that the marked difference in performance of *H. lanatus* in the two years could be related to the difference in climatic conditions between the two periods; *H. lanatus* is reported to be frost-sensitive (Vries and Hart 1941; Oberdorfer 1949). During 1978, the late winter and spring was severely cold and snow lay on the ground for many weeks. From Figure 2.7 it is apparent that during the second growing season the reduced shoot biomass of *H. lanatus* coincided with an increase in that of *Agrostis tenuis*. Because *A. tenuis* is a rhizomatous species it is likely that this species may be less liable to frosting and may recover more quickly due to the possession of underground storage organs and growing points.

From the data presented in this chapter it would appear that dominance in relatively productive and undisturbed vegetation is strongly associated with the capacity to develop a summer peak in shoot biomass. It is also clear from the data for *H. lanatus* that the size of the summer peak can be affected by short-term climatic effects which may reduce the vigour of a dominant species possibly allowing other potential dominants (e.g. *A. tenuis*) to expand. In attempting to explain the dominant effects of species such as

Holcus lanatus, *Impatiens glandulifera*, *Pteridium aquilinum* and *Calluna vulgaris* the possibility of allelopathic effects cannot be discounted. In the present study it was evident that all of the dominant species released considerable quantities of litter which in some instances was persistent in the habitat. Later in this thesis attention will be drawn to the problem of assessing the role of toxic products from shoot and root residues in dominance.

The association between dominance and the production of a large shoot biomass during the summer is also evident in the data from Site 4 where *Lolium perenne* and *L. multiflorum* (Figure 2.9) produced a large peak in July. Only a small number of native species had successfully invaded this site and several of the sown species had virtually disappeared (Appendix 1). This site was subjected to light grazing and it seems likely that this allowed the canopy to be dominated by the two *Lolium* species. In marked contrast, at Site 5 which was under heavy grazing pressure, no dominant species was recognizable. *Agrostis stolonifera* and *Holcus lanatus* formed a major part of the biomass during the summer while *Lolium* was prominent in spring and autumn. It is also interesting to note (Appendix 1) that the number of native species which invaded Site 5 was higher than at Site 4. This was almost certainly because of the intensive grazing at Site 5 which caused severe disturbance and tended to create large gaps in the vegetation. In a field experiment conducted at this site (Thompson 1977) it was established that natural invasion by seedlings of native species such as *Holcus lanatus* is particularly common in patches of bare soil arising from natural or experimental disturbance of the sward.

The results of the phenological studies at Sites 4 and 5 therefore suggest that the degree of dominance exerted in herbaceous vegetation is strongly reduced by grazing, trampling and mowing which tend to prevent the development of a large biomass by any of the component species and limits the amount of litter production.

CHAPTER 3

PHENOLOGICAL CHANGE AND SEASONAL
VARIATION IN THE AVAILABILITY OF MINERAL NUTRIENTS

3.1 INTRODUCTION

The purpose of the study described in this chapter was to measure the seasonal variation in the levels of various soil constituents (ammonium-nitrogen, nitrate-nitrogen, phosphorus and exchangeable Na, Ca, Mg, Mn, Fe and Al) at five of the sites (Sites 1, 2, 3, 6 and 7) subjected concurrently to phenological analysis (Chapter 2) and seedling bioassays (Chapter 4).

The objective was to examine the relationship between phenological changes and seasonal variation in the availability of mineral nutrients and organic toxic elements.

3.2 MATERIALS AND METHODS

3.2.1 Sampling

On each sampling occasion at each site, soil was removed from beneath the area from which the vegetation had been removed for the phenological study. A sharp knife was used to excavate a block of soil extending to a depth of 10 cm. This procedure was not followed at Site 6; here the soil contained two very distinct horizons which were sampled individually. The first consisted of an organic layer approximately 8 cm in thickness, whilst the second was mainly of mineral soil situated at a depth of about 10 cm. The soil used as a control in the investigation was collected in bulk on one sampling occasion from a garden plot at Tapton Elms, Sheffield 10, and, after thorough mixing, was stored in a cold room at 5°C. The procedure used to prepare each replicate soil sample for

chemical analysis and for use in the seedling bioassay (Chapter 4) was as follows:

The soil block was broken up and sieved to pass 1.0 cm in order to remove stones and coarse root fragments. After sieving the soil was mixed thoroughly and divided into two portions, one of which was used in the seedling bioassay experiment whilst the other was subjected to chemical analysis.

3.2.2 Chemical analysis

Extraction

a) Exchangeable cations (Ca, Mg, K, Na, Mn, Fe and Al)

Two subsamples were used from each replicate. Each was weighed and placed in a 100 ml centrifuge tube, and normal ammonium acetate (pH 7.0) extractant was added. After continuous shaking for four hours on a rotary shaker, the mixture was centrifuged for 15 minutes at 4000 rpm at 20°C. The supernatant was filtered through Whatman No.1 filter paper and the filtrate was collected in 100 ml volumetric flasks. The residue was washed with two 25 ml aliquots of ammonium acetate solution and after repeating the centrifuging and filtration procedure, the total volume of filtrate was made up to 100 ml with distilled water.

b) Exchangeable phosphorus

The procedure used for this extraction* was the same as that for the cations except that the extractant was a 50 ml solution of sodium bicarbonate (pH 8.5) and the shaking period was reduced to 90 minutes.

*The method was modified from Allen *et al.* (1974).

c) Ammonium- and nitrate-nitrogen

The extractant for ammonium-nitrogen* was 50 ml of 6% NaCl solution while that used for nitrate-nitrogen[†] was 50 ml 0.02 N copper sulphate solution. The samples were shaken for 90 minutes in the ammonium-N extraction and for 60 minutes in the case of nitrate-N. The subsequent procedure was the same as in the cation extraction except that the filtrate was centrifuged once only.

Analysis

a) i. Calcium, magnesium, manganese, iron and aluminium

Concentrations of these five elements were estimated by atomic absorption spectrophotometer (Unicam SP190) calibrated using a range of known concentrations prepared from standard solutions of $MnCl_2$, $MgCl_2$, $FeCl_2$, $CaCl_2$ and $Al(NO_3)_3$.

ii. Potassium and sodium

The concentrations of these two elements were measured using an EEL flame photometer calibrated against standard solutions of potassium sulphate and sodium chloride.

b) Phosphorus

Two replicates, each of 3 ml of the extract were used from each sample. Each was placed in a 25 ml volumetric flask to which the following reagents were added:

*The method for ammonium-N was modified from Allen *et al.* (1974).

[†]The method for nitrogen-N was modified from Oien and Selmer-Olsen (1969).

- a. 1-2 drops 2-4 dinitrophenol
- b. 1-2 drops 10% NaOH
- c. 2 drops 2N HCl
- d. 10 ml distilled water
- e. 1 ml sulphomolybdic acid
- f. 1 ml freshly prepared chlorostanous solution

Distilled water was added to make the volume up to 25 ml and the concentration of phosphorus was estimated in an EEL Colorimeter using a red filter and calibrated against solutions of known phosphorus concentration.

c) Ammonium- and nitrate-nitrogen

i. Ammonium-nitrogen

Ammonium-nitrogen was estimated spectrophotometrically using an AC60 batch-analyser which automatically mixed 3 ml of each extract with a reagent consisting of 1 ml phenonitroprusside and 0.4 ml hypochlorite, and measured the intensity of colour-development at 600 nm. Calibration of the spectrophotometer was carried out using standard solutions of NH_4Cl .

ii. Nitrate-nitrogen

Nitrate in the soil extract was measured using a nitrate electrode (Model 92-07 Electronic Instruments Ltd.) connected to a laboratory pH meter. The electrode was calibrated using standard solutions of known concentration of NaNO_3 . Recalibration of the instrument was carried out between successive batches each of which contained five soil extracts.

3.3 RESULTS

The results of analyses carried out between April and September 1978 are included in Table 3.1. From these data it is apparent that between sites there were major differences in soil fertility and in Chapter 4 an attempt will be made to relate the differences in levels of nutrient elements and potentially toxic cations to differences in the growth of *Rumex acetosa* in the bioassay experiment. The analytical results will now be considered for each site in turn.

Control site (garden soil)

No marked changes in soil chemistry were detected during the investigation and this was not surprising since the soil was stored in a dry condition at a temperature of 5°C. Calcium concentrations measured in the extract from this soil were higher than those recorded from the other soil types and the high values for P and NO₃-N and very low concentrations of Fe, Mn, NH₄-N are consistent with the conditions of high soil fertility expected in a garden soil.

Site 1 (Totley Wood)

The amount of calcium was very high, but was less than that recorded for the garden soil. The elements Fe, Na, K and Mn remained of fairly constant concentration during the investigation. It is interesting to note, however, that certain constituents (Al, Ca, K, P, NH₄-N and NO₃-N) declined in concentration during the spring. This pattern was correlated with the expansion of

Table 3.1 Estimates of the extractable levels ($\mu\text{g g}^{-1}$) of nine soluble constituents, and soil pH in soil samples from five sites. All estimations were conducted on samples removed between April and September 1978. 95% confidence limits are included. N.D. - none detected.

Sites	Date	NO ₃	NH ₄	P	Ca	Mg	K	Na	Mn	Fe	Al	pH
Tapton soil	April	48.8± 1.7	1.4±0.10	38.4± 1.7	5435± 9	82± 4	181± 3	92± 2	0.5± 0.13	1.4± 0.3	4.3± 0.7	6.8
	July	53.2± 9.0	1.3±0.12	35.9± 3.0	5817±524	94± 4	208± 66	108±16	0.5± 0.16	1.3± 0.3	4.1± 1.9	
	Aug	59.3±10.8	1.7±0.84	41.9± 8.8	5462±985	90±15	214± 42	81±11	0.6± 0.2	1.4± 0.3	4.1± 0.8	
	Sept	53.0± 7.6	1.3±0.24	38.6± 6.1	5638±593	92±10	201± 36	91±18	0.6± 0.14	1.6± 0.6	4.0± 0.6	
1 Totley Wood	April	33.0±21.0	6.4±0.91	5.8± 1.6	2498± 56	165±14	212± 50	65± 4	0.5± 0.3	0.8± 0.3	2.5± 0.8	6.2
	May	8.5± 1.7	3.9±1.00	4.0± 0.9	2216±854	165±14	114± 27	78±26	0.9± 0.5	1.1± 0.5	1.6± 1.7	
	July	30.0± 6.3	1.0±0.20	5.6± 1.6	2972±256	187±17	194± 68	99±12	0.6± 0.4	1.1± 0.4	3.7± 1.5	
	Sept	49.0±16.0	4.2±0.11	6.3± 3.4	2391±233	175±16	156± 19	80± 6	0.9± 0.4	1.8± 0.6	3.4± 0.8	
2 Beely Wood	March	27.5± 8.1	2.0±0.41	23.1± 8.4	2283± 33	212±33	202± 47	95±31	2.5± 1.8	4.6± 0.9	2.7± 1.1	6.7
	May	7.0± 2.2	1.4±1.20	8.3± 2.7	2862±737	196±70	157± 92	99±23	2.5± 2.2	3.7± 1.8	2.0± 0.7	
	Aug	25.1± 7.3	0.8±0.41	19.0± 8.7	2804±903	224±40	183± 48	126±30	3.6± 2.6	6.4± 3.4	4.9± 3.0	
	Sept	53.0±13.4	1.9±0.52	23.1±14.0	2025±980	220±51	159± 70	96±32	3.0± 2.1	7.5± 4.0	5.8± 2.7	
3 New Totley	April	9.2± 8.8	12.6±4.91	6.9± 1.4	411±152	55±14	210± 24	98±29	33.1±11.3	23.2±11.0	35.6±14.1	5.5
	May	4.7± 2.5	5.8±1.63	6.4± 1.4	411±116	52± 9	192± 47	63±10	29.4±10.5	22.2± 7.1	30.3± 7.3	
	July	14.3± 5.5	3.3±2.01	4.6± 0.9	544±210	58± 7	229± 25	75± 6	25.6± 6.3	15.3± 5.4	14.4± 7.5	
	Sept	16.3± 5.5	10.6±2.54	7.9± 1.9	454±176	50±15	150± 39	40± 6	21.5±11.1	20.5± 8.2	16.9± 8.2	
6 Stony Ridge Organic layer	April	N.D.	55.1±9.20	29.3±12.3	2043±132	167±44	570±179	215±36	19.1± 5.7	18.3± 4.4	16.3± 5.2	3.5
	July	N.D.	82.3±23.1	31.4±24.7	3627±655	254±44	660±164	408±64	29.4± 4.5	52.1±31.5	24.5±14.3	
	Aug	N.D.	59.4±20.5	27.1± 9.6	2206±1.77	200±107	543±165	243±84	19.3±11.3	19.5±11.6	25.6±12.1	
6 Stony Ridge Mineral soil	April	N.D.	9.8±6.23	182± 69	429±162	81±42	544±320	240±61	6.1± 3.7	262.1±233.3	43.2±29.5	3.6
	July	N.D.	27.2±8.24	55± 15	798±213	88±20	537±123	377±42	4.9± 1.9	591.4±158.4	28.5±13.6	
	Aug	N.D.	16.4±10.12	44± 8	525±160	117±42	389±136	269±92	4.9± 2.7	263.5±229.6	22.7±10.5	
7 Stony Ridge	April	N.D.	4.4±1.50	15.6± 3.5	1164±201	94±32	348±119	205±24	14.2± 7.3	55.3±34.5	33.4±14.3	3.6
	July	N.D.	5.6±1.90	20.1± 8.0	1707±279	172±30	483±108	334±35	29.5±18.6	54.5±43.1	27.6±10.4	
	Aug	N.D.	14.5±9.40	12.3± 3.5	1377±547	276±50	514±149	370±177	19.1±14.5	41.7±28.2	18.7± 4.8	

growth in the ground flora and in the trees. At this site these same constituents tended to rise in concentration during the summer.

Site 2 (Beely Wood)

In comparison with the control site, the level of magnesium, iron, manganese and ammonium-N were relatively high and the concentrations of mineral nutrients indicated conditions of high fertility. Phosphorus, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ showed two peaks in concentration, one in the spring and the other in September. The decline in levels during the summer coincided with the maximum biomass of the herbaceous biomass, most of which was composed of *Impatiens glandulifera*. The other constituents analysed at Site 2 showed no major changes with season during the study.

Site 3 (New Totley)

In the moderately-acidic derelict grassland dominated by *Holcus lanatus*, the concentrations of Al, Mn and Fe were relatively high and the general level of concentrations measured in calcium, magnesium, phosphorus and nitrate-nitrogen was consistent with conditions of moderately low fertility. Phosphorus, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ showed two peaks, one in April and the other in September. In the result for Mn, Mg, Fe, K and Ca there was little evidence of seasonal variation in concentration.

Site 6 (Stony Ridge)

For this site results are available for two depths in the soil profile. The concentrations of calcium, phosphorus, magnesium

in the organic horizon were considerably higher than those measured in the mineral layer. For sodium and potassium there were no differences in concentration between the two layers. The levels of iron and $\text{NH}_4\text{-N}$ in the mineral layer were higher than those measured in the organic layer. Nitrate-nitrogen was not detected in either of the horizons.

Site 7 (Stony Ridge)

The levels of nutrient elements were relatively low, and the concentrations of potentially toxic elements (Mn, Al) were high. The concentration of calcium and magnesium was very low in this site. Most of the elements did not show any differences in the concentration during the period of the investigation.

3.4 DISCUSSION

The results for the Tapton soil suggest that during storage in the cold room no significant changes in mineral status occurred; this confirms the reliability of this soil as the control in the studies described in Chapter 4.

In the deciduous woodland site (Site 1), the seasonal decline in concentration of most elements was due to the uptake of mineral nutrients during the spring flush of growth by the herbs and trees. At this site the vernal species declined in biomass during the late spring, and it seems likely that the summer peak in levels of mineral elements was due to their release from the senescent foliage of the vernal herbs. It seems probable also that, at both of the sites with trees (Sites 1 and 2), additional mineral nutrients were derived in the autumn from the deposition and decay of tree litter.

The soils at Sites 3, 6 and 7 were highly acidic and it is not surprising to find that the concentration of sesquioxides (Al, Mn and Fe) was relatively high and coincided with low levels of calcium and nitrate-nitrogen. From the work of other investigators (Pearsall 1938; Boswell and Gover 1946; Scurfield and Boswell 1953; Jarvis 1964) we may also expect that the more acidic soils will exhibit reduced earthworm activity and will contain a microflora which is less conducive to nitrification and mineralization of nutrient elements.

The soil analyses confirm that the sites examined in this study form a series including fertile soils (Sites 1 and 2), moderately fertile soil (Site 3) and highly infertile soils (Sites 6 and 7).

CHAPTER 4

SEASONAL CHANGE IN SOIL FERTILITY

4.1 INTRODUCTION

The development of phytotoxic properties in soils beneath natural plant communities and crops is a well known phenomenon. In agriculture, 'soil sickness' has been recognized in a number of investigations. The main problem in interpreting such phenomena is to distinguish phytotoxic effects originating from plant secretions or decomposition products from those due to mineral nutrient deficiencies or toxicities arising from inorganic soil constituents. Börner (1960) in his review suggested that there is enough evidence to be certain that, in some circumstances, agricultural soils are 'poisoned' by organic residues from preceding crops. In one particular study for example Patrick (1955), investigating the nature of soil sickness in old orchards, concluded that amygdalin originating from dead roots was converted by microorganisms to soluble toxic substances highly detrimental to living peach roots. The amounts of these toxins and, thus, the degree of toxicity produced, appeared to depend upon, first, the amount of old peach roots remaining in the soil after the old trees had been removed and, second, upon their amygdalin content. On this basis we would expect that toxic effects would be greatest for the first year or two after tree removal and should gradually diminish subsequently. As Patrick pointed out this diminishing toxicity would explain, at least in part, the frequent observation that the 'replant problem' associated with peach orchards diminishes two or three years after removal of the old trees.

In a quite different context Guenzi and MacCalla (1966a) showed that organic compounds capable of markedly inhibiting the

respiration, germination and growth of tobacco seedlings were obtained after residues from timothy, corn, rye or tobacco plants had been allowed to decompose under appropriate soil conditions. Wang *et al.* (1967) demonstrated that phenolic acids at concentrations commonly occurring in soils were capable, when applied to plants growing in nutrient culture solution, of suppressing the growth of seedlings of wheat, corn and soya bean. Several workers (Muller 1966; Rice 1964,1965,1976; Wilson and Rice 1968; Floyd and Rice 1967; Abdul-Wahab and Rice 1967; Gant and Clebsch 1975; Hull and Muller 1977) have concluded that harmful effects upon the survival, yield and reproduction of certain native plants arising from the release of inhibitory substances either by direct secretion or as microbial decomposition products is one of the most important factors determining vegetation pattern, succession and dominance in certain grassland and shrub communities.

A small number of reports have been concerned with the allelopathic interaction of tree species with the understorey vegetation in forests. In one study, del Moral and Muller (1970) have suggested that in semi-arid conditions in N.America certain types of annual herbaceous vegetation are severely inhibited by toxins originating from adjacent stands of *Eucalyptus camaldulensis*. In this investigation an attempt was made to measure effects of competition for light and mineral nutrients and the data obtained suggested that competition was not likely to be an important mechanism of inhibition of herb growth in this case. In the studies described by Al-Mousawi and Al-Naib (1975) evidence was obtained of a major reduction in the growth of herbaceous plants growing

under the canopy of the tree *Eucalyptus microtheca*. The same herbaceous species were observed to grow well under adjacent trees of *Casuarina cunninghamiana* at the same site. The effect of *E. microtheca* did not appear to be the result of competition in that the depletion of soil moisture and nutrient elements and the intensity of shading did not exceed the levels associated with *C. cunninghamiana*.

From a number of investigations, therefore, we have circumstantial evidence of the possible role of allelopathy as a determinant of plant yield and vegetation composition. However, most of this evidence is drawn from vegetation types other than those associated with temperate grasslands. The aim of the investigation described in this chapter was to attempt to detect by means of bioassay the presence of toxic substances in the soils associated with a range of vegetation types of common occurrence in Britain, and to measure seasonal change in their production or persistence.

4.2 MATERIALS AND METHODS

Soil samples from five sites were removed at intervals during the year and seedlings of *Rumex acetosa* were grown in pots in a standardized growth-room experiment. In order to recognize effects due to seasonal variation in mineral nutrient availability measurements of yield were made on seedlings grown with and without the addition of Hewitt nutrient solution to the soil samples. A garden loam from Tapton Elms was used as a control and a second control treatment involved seedlings grown on sand treated with a volume of nutrient solution the same as that applied to the test soils. Phenological studies and soil analyses were carried out at each of the sites over same period of time and these are described in Chapters 2 and 3. By this approach an attempt could be made to relate the results of the bioassays to measurements of shoot phenology and seasonal variation in soil nutrients.

At four of the sites the soil samples used in the bioassay were taken from a depth of 0-10 cm. This zone was selected in order to examine the part of the soil profile exploited during seedling establishment and containing the main concentration of roots of the established herbaceous vegetation. Soil from two depths was sampled from one site (Site 6) where, as explained on page 30, the soil consisted of two distinct horizons.

4.2.1 Choice of species used in the bioassays

In order to assay soils for toxic effects, *Rumex acetosa* was chosen, because:



- (1) the species has a rapid growth-rate;
- (2) it is capable of growth on a wide range of natural soils (Figure 4.1);
- (3) the seed populations are rather uniform with respect to germination rate and seedling growth, and
- (4) the species has been used previously for similar purposes (Grime 1963a; Rorison 1967).

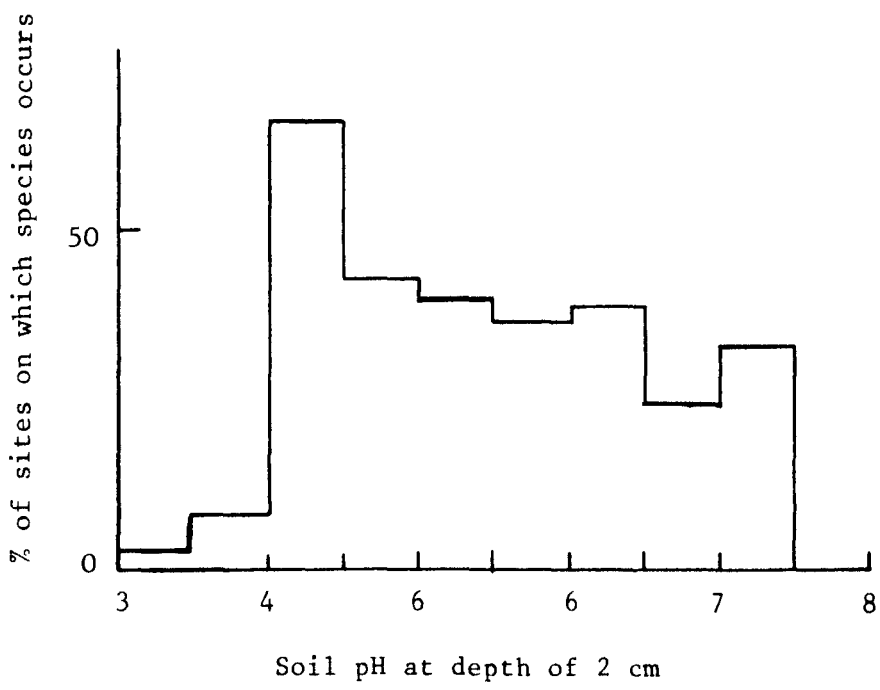
4.2.2 Procedure

As explained in Chapter 3, the soil used in this investigation was derived as subsamples of the soil samples removed for chemical analysis. For each site five subsamples were used to fill sixty plastic pots of 7.5 cm diameter. Twenty-four pots of the garden soil were used and a further eight pots containing washed sand were employed as an additional control. Thirty pots of each soil, and twelve containing the control garden soil received only distilled water throughout the experiment. Each of the remaining containers (30 pots of each soil, 12 garden soil, and 8 filled with sand) received aliquots of 120 ml Long Ashton nutrient solution throughout the experiment. Nutrient solution was added at a rate of 40 ml per pot at intervals of seven days.

At the commencement of each bioassay two eight-day-old seedlings of *R. acetosa* were planted at the centre of each pot. Seven days later one of the two seedlings was removed from each pot. The experiment was carried out in a growth-room in which the pots were arranged in six randomised blocks. Watering of the pots with distilled water was carried out at regular intervals in order

Figure 4.1

Histograms showing the frequency of occurrence of *Rumex acetosa* over a range of soil surface pH. Data provided from 340 random m⁻² quadrats in established grassland from 41 sites in the Sheffield area.



to maintain the soil near to field capacity. A daylength of 18 hours was employed, with a constant temperature of 20°C day and night, and relative humidity maintained above 75%.

The plants were harvested three weeks after the beginning of the experiment, and the roots were carefully separated and cleaned by hand under running tap water. The plants were oven-dried for 48 hours at 80°C, and the dry weights of the roots and shoots were determined.

4.2.3 Data analysis

At the end of the practical work, graphs including mean values and 95% confidence limits were plotted describing the seasonal changes in the yield of *R. acetosa* on the five soil types and in the two control treatments.

4.3 RESULTS

Figures 4.2 and 4.3 illustrate the main results of the investigation. On the garden soil (Figure 4.2.2) which was used as the control, the yield of *Rumex acetosa* both with and without the addition of mineral nutrients showed no significant differences in yield throughout the investigation, and a similar pattern occurred with respect to seedling yield in sand culture (Figure 4.2.2). All three control treatments produced yields higher than those obtained on the natural soils. Clear differences in yield were associated with soil type. Samples from the two acidic soils (Sites 6 and 7) produced much lower yields than those associated with the remaining sites (Sites 1, 2 and 3) and on these soils *R. acetosa* did not respond to the same extent to the addition of mineral nutrients.

At Site 1 there were clear and statistically-significant ($P < 0.05$) reductions in the yield of *R. acetosa* in March in both treatments. Site 2 showed a seasonal pattern rather similar to that obtained for Site 1, the only major difference being the generally lower yields obtained both in the presence and in the absence of additional nutrients. Although Site 3 produced yields of *R. acetosa* which were relatively high in both treatments at the beginning of the experiment in April, yields then showed a continuous decline throughout the remainder of the investigation.

For the samples from Sites 6 and 7 (Figures 4.3.1, 4.3.2 and 4.3.3) no significant seasonal differences in yield were observed in the absence of mineral nutrient treatment. In the treatments involving nutrient additions, however, a clear decline in yield occurred in October. In the samples taken from the

Figure 4.2

The dry weight of *R. acetosa* grown in different soils.
95% confidence limits are included.

(1) Site 3

(2) Garden soil

- nutrient treatment
- distilled water treatment
- sand/solution culture

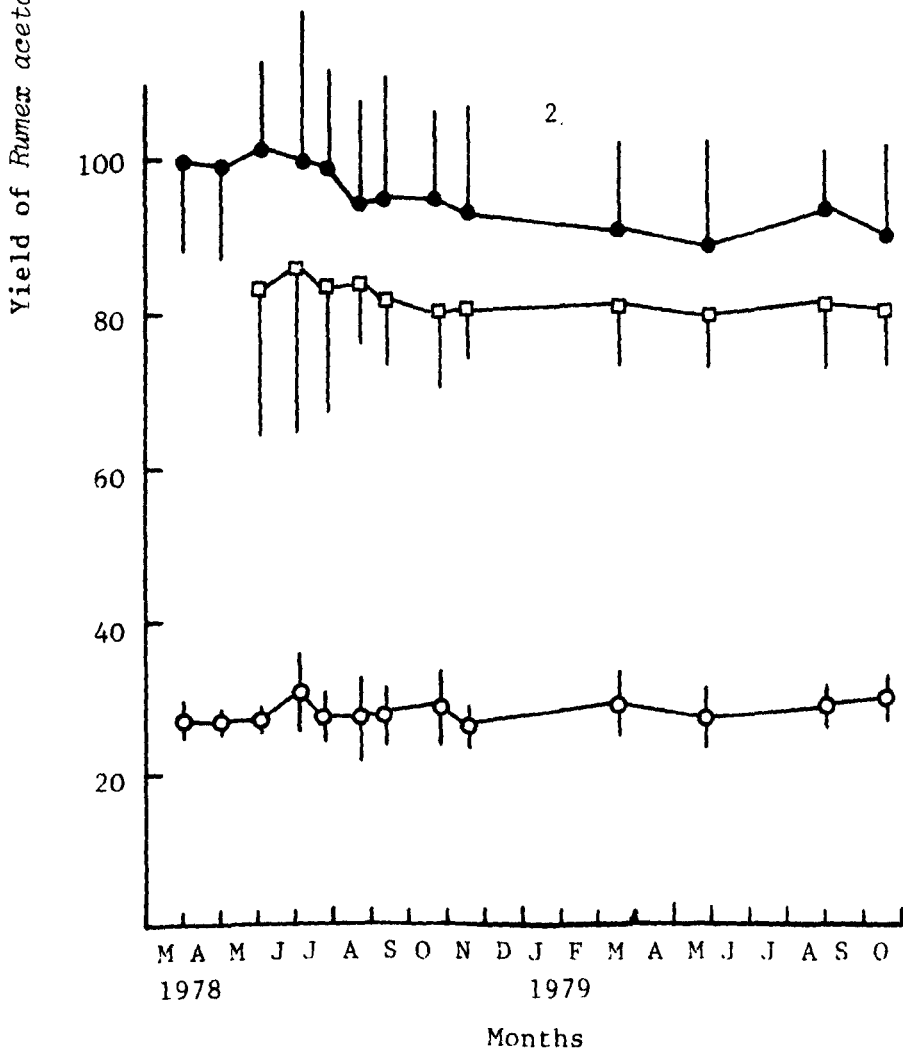
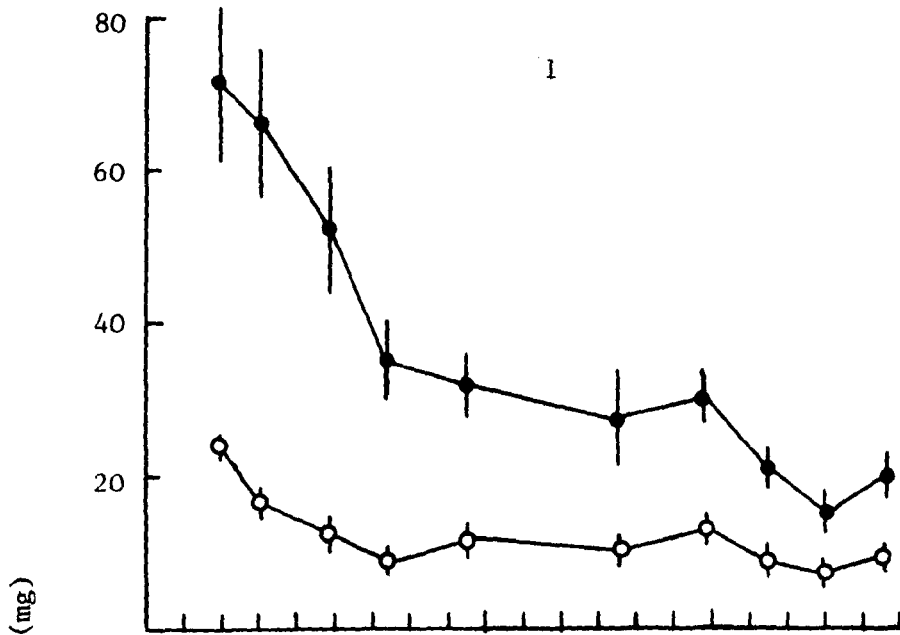


Figure 4.3

The dry weight of *R. acetosa* grown on soil samples removed at intervals from four different sites.

95% confidence limits are included.

(1) Site 7 (0-10 cm)

(2) Site 6 (0-8 cm)

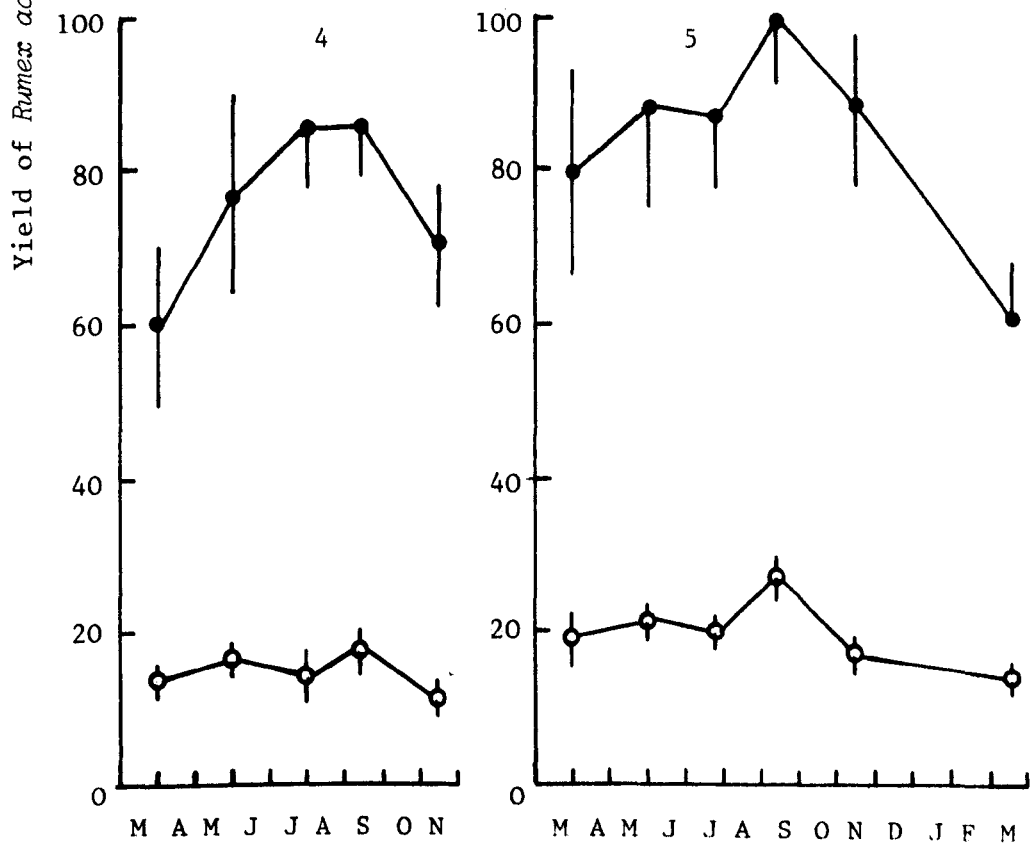
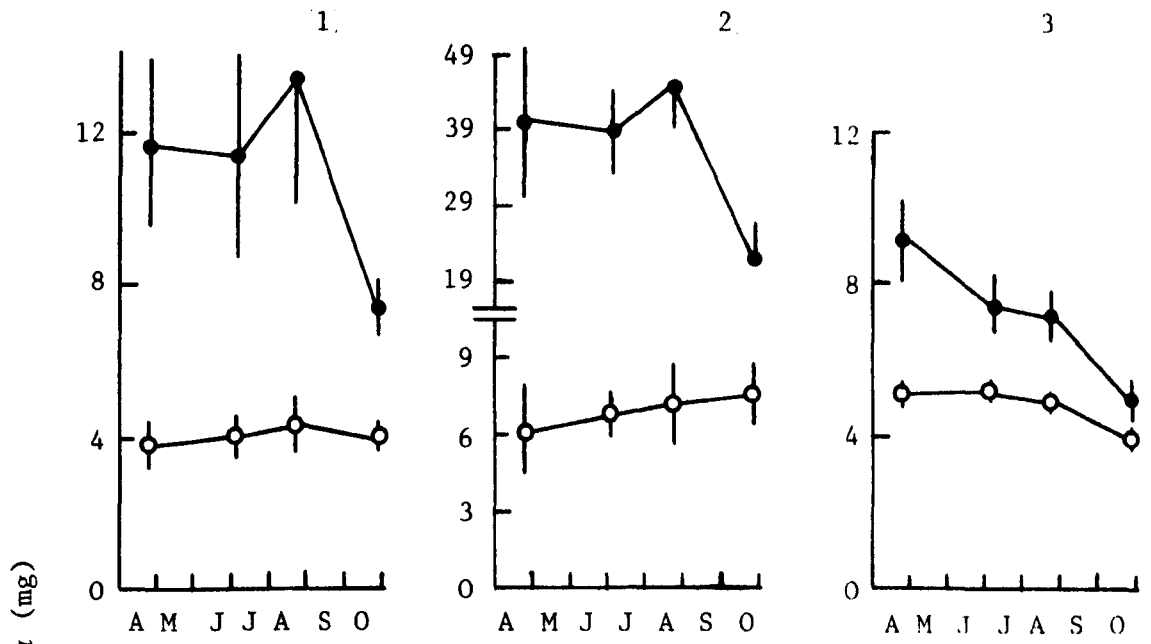
(3) Site 6 (8-18 cm)

(4) Site 2 (0-10 cm)

(5) Site 1 (0-10 cm)

●—● + additional mineral nutrients

○—○ - additional mineral nutrients



Months

organic layer of the soil profile at Site 6, the yield was increased to a major extent by the addition of mineral nutrients but this effect was much less pronounced for the mineral soil removed from the same site.

4.4 DISCUSSION

On the control soils no significant differences in the yield of *Rumex acetosa* were detected, indicating that the growth-room environment and the experimental procedure were maintained relatively constant throughout the investigation.

It may be significant that the yield of *R. acetosa* at Sites 1 and 2 showed a decline at the time when the deposition of tree litter reached a maximum (see Chapter 2). Decomposition of the tree litter was very rapid in both sites. Whilst this may have resulted in an increase in mineral nutrient supply (see Chapter 3) at this time of the year, it is also possible that there would have been a release of toxic substances into the soil during this period. Sydes (1980) provides evidence of this phenomenon in an experiment in which litter from a range of common British trees and shrubs was compared with respect to the rate of decay and the ability of leachates to inhibit seedling growth (Table 4.1). It is interesting to note that in the experiment described in this chapter a decline in yield coinciding with the occurrence of the seasonal maximum in tree litter was detected in the treatments involving additional mineral nutrients. This suggests that the effect was unlikely to be the result of mineral nutrient depletion and could be related to the rapid efflux of organic solutes from the freshly-deposited litter (Al-Naib and Rice 1971; Lodhi and Rice 1971; McPherson and Thompson 1972; Lodhi 1975, 1976).

The progressive decline in the yield of *R. acetosa* on the soil samples removed from Site 3 is a striking phenomenon and in contrast with the results for Site 1 and 2, the effect of mineral nutrients was to produce only a small improvement in yield. This

Table 4.1 Comparison of the litter of eighteen trees and shrubs of common occurrence in Britain with respect to (a) weight loss during initial period of decay and (b) inhibitory effect of litter leachates on plant growth. Measurements of decay and the preparation of leachates both involved litter maintained in a moist aerobic condition at warm temperatures (20°C day, 15°C night) over a three-week period immediately following leaf fall. Growth inhibition was based upon reductions in yield of seedlings of the perennial herb *Rumex acetosa*, grown for 3 weeks in nutrient-sufficient sand culture (Sydes 1980).

	(b)	(b)
	Weight loss by decay and leaching (% dry weight)	Phytotoxic effect of leachate (% reduction in yield of <i>Rumex acetosa</i> seedlings)
<i>Sambucus nigra</i>	57	50
<i>Frangula alnus</i>	52	45
<i>Thelycrania sanguinea</i>	46	45
<i>Ulmus glabra</i>	42	31
<i>Fraxinus excelsior</i>	42	31
<i>Alnus glutinosa</i>	33	19
<i>Acer campestre</i>	31	42
<i>Salix capraea</i>	30	10
<i>Acer pseudoplatanus</i>	28	39
<i>Corylus avellana</i>	25	3
<i>Populus canescens</i>	24	17
<i>Betula pendula</i>	23	42
<i>Tilia cordata</i>	23	7
<i>Quercus robur</i>	23	7
<i>Sorbus aucuparia</i>	23	44
<i>Castanea sativa</i>	17	31
<i>Crataegus monogyna</i>	17	18
<i>Fagus sylvatica</i>	15	16

suggests that at Site 3 phytotoxins originating during the seasonal peak of growth were released into the soil by the dominant species. (*Holcus lanatus*).

The yield of *R. acetosa* on the soil from Site 7 was very low in both treatments, and the growth was suppressed throughout the whole experiment. This result is in close agreement with the finding of Laing (1932) who reported an almost total absence of root development by spruce (*Picea abies*) rooting in the organic layers of heathland soils. The failure of *R. acetosa* to respond to a major extent following the addition of mineral nutrients suggests that organic toxins originating from the dominant species, *Calluna vulgaris*, were a major factor limiting seedling yield on this soil. In a number of investigations (Malcolm 1975; Mantilla, Arines and Vieitez 1975; Jalal and Read, unpublished) it has been shown that various living parts of *C. vulgaris* contain phenolic compounds which when added to soil are capable of inhibiting the growth of flowering plants. However, the results of extraction experiments of this type must be treated with caution since most plant material contains chemical substances capable of inhibiting the germination and growth of plants. Under field conditions, soil microorganisms may exert an important role in modifying the chemical structure and phytotoxicity of decomposing plant remains. It is important, moreover, in explaining the poor yields of *R. acetosa* observed in this investigation, to bear in mind that the levels of inorganic and potentially-toxic elements (Al and Mn) are relatively high at Site 7 (Chapter 3).

At Site 6 which was dominated by *Pteridium aquilinum*, the yield of *R. acetosa* was reduced significantly below that of the control

and, as in the case of Site 7, the effect of additional mineral nutrients was relatively small. Gliessman and Muller (1972) have drawn attention to the occurrence of phenolic acids in the rhizosphere of *P. aquilinum* and in laboratory experiments they have demonstrated that the concentrations occurring in nature are capable of strong phytotoxic effects, especially under nutrient-deficient conditions. This suggests the hypothesis that allelopathy is an important part of the mechanism whereby bracken attains dominance over extensive areas of Upland Britain.

CHAPTER 5

A STUDY OF SPATIAL VARIATION IN SOIL FERTILITY

5.1 INTRODUCTION

The aim of the investigation described in this chapter was to seek evidence of the presence of toxic substances in the rhizosphere of naturally established plants of *Holcus lanatus*. The site chosen for the study consisted of a fairly homogeneous area (60 x 60 m) of derelict grassland situated on the Coal Measure Sandstone at New Totley on the south-west boundary of the city of Sheffield. The grassland was subject to local disturbance by trampling and horse-riding but no grazing animals were present and scrub encroachment was occurring at the margins of the site. Patches of vegetation dominated by *H. lanatus* occurred throughout the central part of the field and these tended to be more frequent in areas marginal to a path which traversed the site.

The method consisted of the removal of turves from the patches dominated by *H. lanatus* and comparison of the growth of seedlings on samples of surface soil from these turves with that of seedlings grown on soil removed from randomly-selected samples of turf taken from the field.

The site used in this investigation was also the subject of phenological investigations (Chapter 2) and studies of seasonal variation in soil fertility (Chapter 4).

5.2 MATERIALS AND METHODS

5.2.1 Sampling and sorting

Samples of turf, 0.25 m^{-2} in area and extending to a soil depth of 10 cm were removed in May 1976 from ten distinct *H. lanatus* patches widely distributed over the central area of the field. Using random co-ordinates, a further ten samples were collected from positions within the same general area. In the laboratory the vegetation in each sample was sorted into its component species and the oven-dried weight of each was determined. The soil from each sample was mixed thoroughly and coarse roots, litter and stones were removed. The soil was divided into two subsamples one of which was used in a bioassay whilst the other was subjected to chemical analysis.

5.2.2 Bioassay

Soil from each of the twenty samples was used in an experiment in which seedlings of *Rumex acetosa* were grown in the presence and absence of additional mineral nutrients.

The soil in each subsample was used to fill fourteen 6.5 cm diameter pots, seven of which received additional nutrients whilst the remainder were supplied with distilled water. At the beginning of the experiment a single two-week-old seedling was sown into each pot. In the nutrient treatment each pot received a 50 ml aliquot of Hewitt nutrient solution before planting and subsequently at intervals of 3 days the soil was watered alternately with 35 ml of Hewitt solution and 35 ml distilled water. Each pot was provided

with a separate saucer. Twelve pots of washed sand were used to grow the control seedlings and these received nutrients and water at rates comparable to the pots in the nutrient treatment. In the treatment involving soil without additional nutrients, distilled water was applied at rates equivalent to the total volumes of nutrient solution and distilled water used in the nutrient treatment.

The plants were harvested three weeks after the beginning of the experiment; the oven-dried weights of the shoots were then determined.

5.2.3 Chemical analysis

Using the methods described in Chapter 3 (pages 31 to 33) each of the soils used in the bioassays was subjected to an extraction procedure followed by analysis of the concentration of Ca, Fe, Mn, K, Mg, Na, phosphorus, ammonium-N and nitrate-N.

5.3 RESULTS

Figure 5.1 describes the composition of the vegetation in the patches of *Holcus lanatus* and in the randomly located samples. The results confirm the high density of living shoot material of *H. lanatus* in the *H. lanatus* patches and reveal only trace quantities of other species. *Holcus lanatus* was only the fifth commonest species present in the random samples; here the dominant species at the time of sampling were *Festuca rubra* and *Poa pratensis* and the total shoot biomass was on average 2.59> that attained in the *H. lanatus* patches. Both sets of samples contained a high density of herbaceous litter, most of which appeared to have originated from *H. lanatus* in the samples from the *H. lanatus* patches.

The pH of the soil from the *H. lanatus* patches was not significantly different from that from random patches (Table 5.1). The results of the chemical analyses (Table 5.1) indicated that levels of eight of the constituents examined were closely similar in the soils from the *Holcus* patches and the random samples. With respect to ammonium-nitrogen, however, significantly ($P < 0.05$) higher levels were associated with the *H. lanatus* patches.

The yield of *Rumex acetosa* on the control pots of washed sand was considerably higher than that of seedlings grown on the soil samples (Figure 5.2). In the presence and in the absence of added mineral nutrients, soil from the random samples produced yields of *R. acetosa* significantly higher than those on the soil samples from the *H. lanatus* patches. Both sets of soil samples showed a major increase in fertility after the addition of mineral nutrients but this stimulus did not bring the yield up to the level attained in the controls.

Figure 5.1

Mean dry weight of herbaceous litter and of the shoot material of each species in patches of *Holcus lanatus* and in random samples of vegetation from within the same area of derelict grassland at New Totley.

Dry weight (g 0.25 m⁻²)

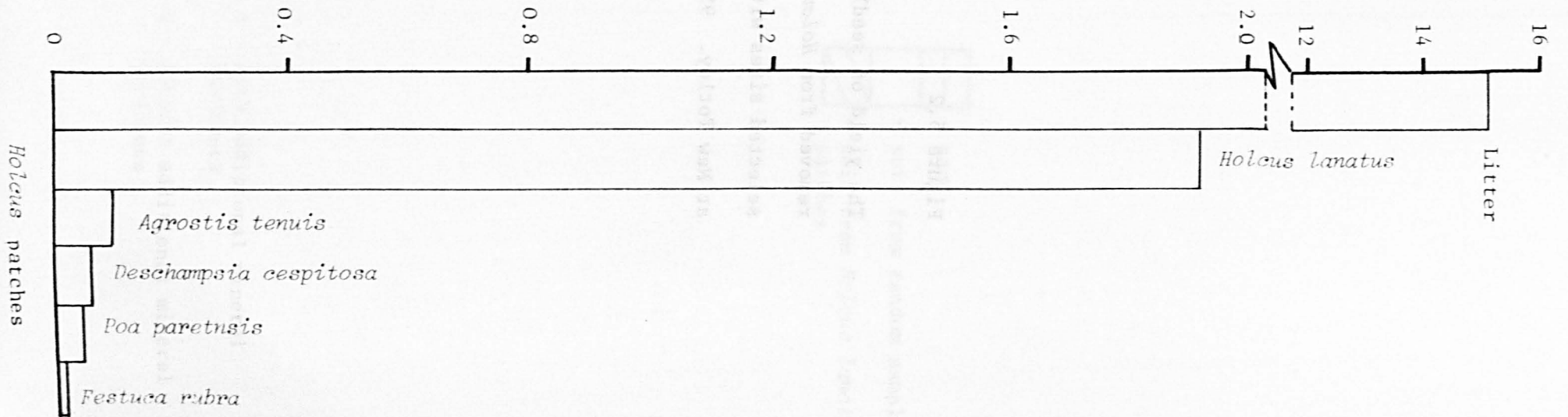
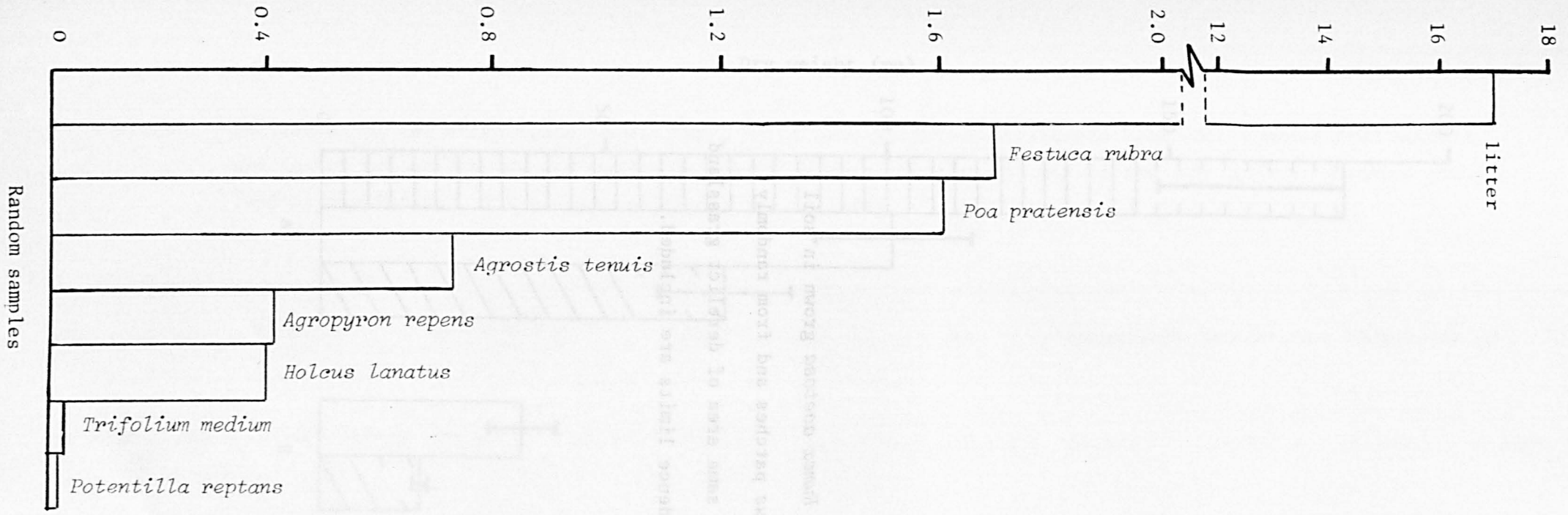
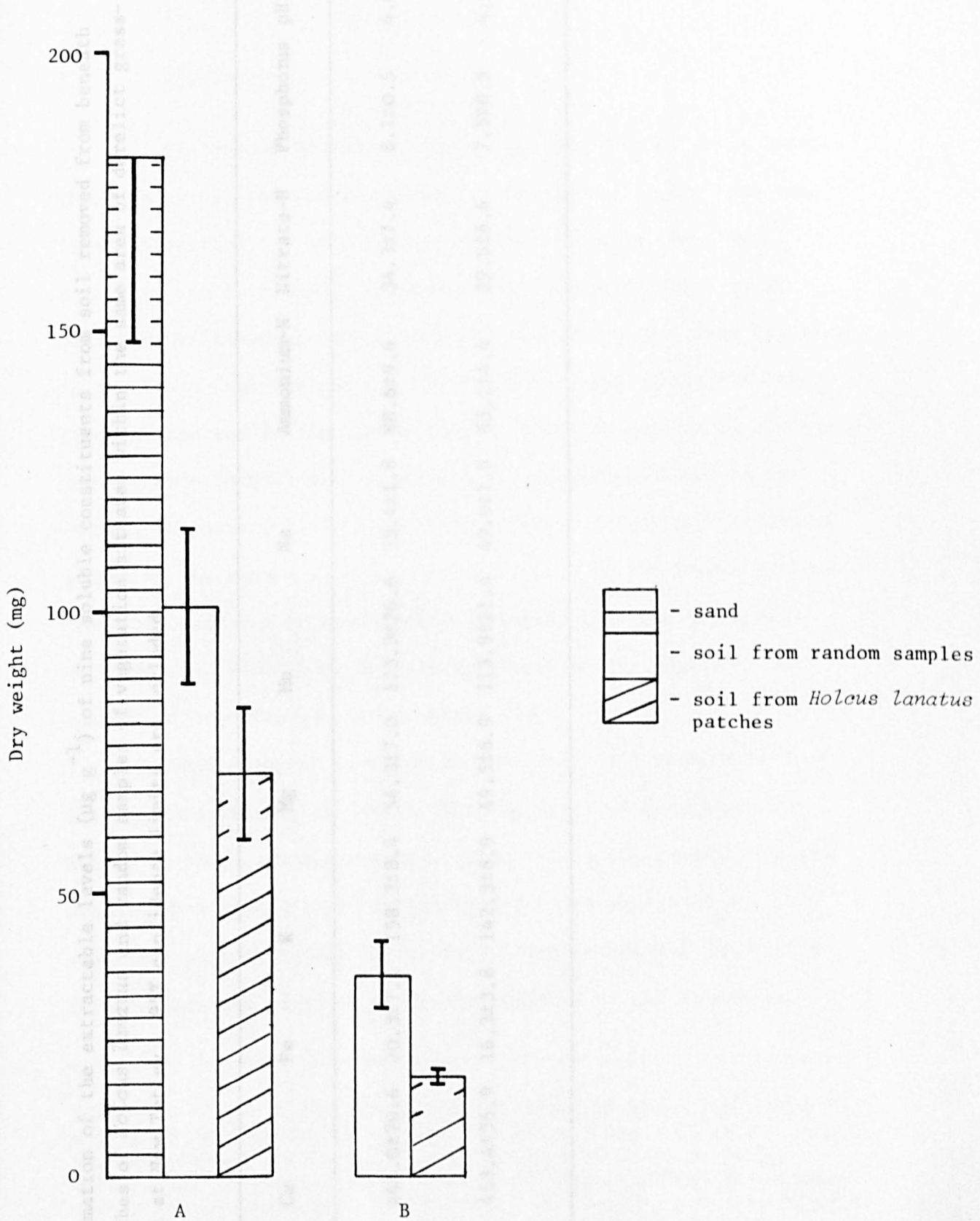


Figure 5.2

The yield of seedlings of *Rumex acetosa* grown in soil removed from *Holcus lanatus* patches and from randomly selected sites within the same area of derelict grassland at New Totley. 95% confidence limits are included.



A = with additional mineral nutrients

B = without additional mineral nutrients

Table 5.1 Estimation of the extractable levels ($\mu\text{g g}^{-1}$) of nine soluble constituents from soil removed from beneath patches of *Holcus lanatus* and random samples of vegetation situated within the same area of derelict grassland at New Totley. 95% confidence limits are included.

	Ca	Fe	K	Mg	Mn	Na	Ammonium-N	Nitrate-N	Phosphorus	pH
<i>Holcus lanatus</i> patches	444.0±70.6	20.3±7.1	158.3±8.4	54.3±7.0	123.3±26.6	55.4±1.8	88.6±9.6	34.3±7.4	8.1±0.5	4.68±0.18
Random samples	453.4±56.9	16.3±3.8	142.3±8.9	49.5±6.9	113.9±21.4	49.9±1.9	63.2±4.4	29.6±6.6	7.5±0.5	4.76±0.17

5.4 DISCUSSION

The *Holcus lanatus* patches contained a lower shoot biomass than the turves sampled at random in the area of study. One possible explanation for this difference is related to the time of sampling. In *H. lanatus*, maximum shoot biomass is not usually obtained until summer (see Chapter 2). However, this does not seem to provide a full explanation for the difference, since the most frequent species in the random samples (*Festuca rubra*, *Poa pratensis* and *Agrostis tenuis*) also produce peaks in biomass later in the summer. Moreover, it is clear from Figure 5.1 that the density of litter in the random samples was higher than in the *Holcus* patches. From this evidence it seems rather unlikely that the dominance exerted by *H. lanatus* can be related simply to the vigour of the species or to excessive litter production.

Soil analyses revealed no consistent differences between the two sets of samples except that the level of ammonium-N was higher in the *H. lanatus* patches. This raises the possibility that ammonium-N toxicity could be involved to some extent in the suppression of productivity in the *H. lanatus* patches and in the effects upon the yield of *R. acetosa* observed in the bioassays.*

* Polizotto *et al.* (1975) found that NH_4 -treated plants had lower Ca and Mg concentrations in exudates collected from cut stems than did NO_3 -fed plants. Barta (1977) suggests that this is an indirect effect, due to reduced shoot growth with NH_4 which has an indirect effect on ion transport. Accumulation of free NH_4 ions may restrict photosynthesis by uncoupling noncyclic photophosphorylation in isolated chloroplasts (Walker and Crofts 1970), although it is not known whether photosynthesis in intact plants is so affected. There is also evidence that NH_4^+ inhibits electron transfer in respiration (Vines and Wedding 1960).⁴

An additional or alternative explanation for the reduced yield of *R. acetosa* grown on soil from the *H. lanatus* patches is the presence of an allelopathic effect. However, from the data examined in this chapter it is not possible to assess the importance (if any) of either NH_4 -toxicity or allelopathic effects in the inhibitory effect associated with the *H. lanatus* patches.

PART II

CHAPTER 6

THE INFLUENCE OF TEMPERATURE UPON THE
CONTRIBUTION OF *HOLCUS LANATUS* TO A MIXED SWARD

6.1 INTRODUCTION

Although *Holcus lanatus* varies in frequency of occurrence according to soil type and grassland management (Grime and Lloyd 1973), the species is capable of dominance not only in systems exposed to defoliation by grazing or cutting but also in derelict grasslands. The species is also commonly found in pastures and meadows containing a mixture of sown and native grasses. In order to understand the phenomena controlling the contribution of *H. lanatus* to the vegetation of pastures and meadows it is necessary to consider the possible impacts of a wide range of factors. Certain of these factors, such as the frequency and height of defoliation, have been the subject of previous studies (Riveros 1963; Remison 1976; Watt 1977). The purpose of the experiments described in this chapter is to examine the influence of one of these factors, namely temperature, upon the vigour of *H. lanatus* in a mixed sward.

From the phenological studies described in Chapter 2, it is evident that *H. lanatus* has a characteristic seasonal pattern of shoot expansion and flowering and contrasts markedly with the phenology of certain other sward components, most notably the sown species *Lolium perenne* and *Lolium multiflorum* which tend to exhibit a bimodal pattern of shoot production with peaks coinciding with the cooler conditions of spring and autumn. This observation suggests that differences in phenology and temperature response may be of major importance in determining the extent of dominance/co-existence between *H. lanatus* and the *Lolium* spp.

Two experiments will be described. In the first, blocks of turf and soil were removed from an area of uniform turf in a

sown pasture and these were then maintained under a variety of conditions in order to examine the response of the constituent species to various temperature regimes. The second experiment involved measurement of the effect of temperature upon monocultures and mixtures of *H. lanatus* and *L. perenne* established from seedlings in pots of soil.

6.2 EXPERIMENT 1

6.2.1 Materials and Methods

On the 6th April 1977, thirty-two blocks of turf and soil were removed from a uniform area within the sown pasture at Littlemoor (Site 5). The blocks were taken in close proximity to the area used for the phenological study (Chapter 2) and each was 0.25 m² in area and extended to a depth of 15 cm. The turf on each block was trimmed to a height of 5 cm above the soil surface. Seven blocks were then selected at random and the total shoot biomass on each was removed and sorted into its constituent species in order to obtain an estimate of the initial composition of the turf. At this stage it became apparent that only three species, *Holcus lanatus*, *Lolium perenne* and *Agrostis stolonifera* were major components and in order to simplify the experiment it was decided to remove by hand shoots of all the other species present in the remaining turves. It seems likely that a small amount of *Lolium multiflorum* occurred in the turf; this was not removed and data for this species have been included with that for *L. perenne*.

The remaining twenty-five blocks were placed individually into rectangular plastic tanks (Plate 6.1) each of which was provided with two large drainage holes, 5 cm above the base. The blocks were then allocated at random to five temperature regimes:

(a) A 'natural' outdoor environment at the Botany Department

Experimental Garden in Sheffield.

(b) A growth-room environment providing 15°C day and 10°C night.

(c) A growth-room environment providing 25°C day and 20°C night.



PLATE 6.1

Photograph showing one of the turves of Treatment (a) in Experiment 1.

- (d) A growth-room environment alternating at intervals of three days between high temperatures (25°C day, 20°C night) and low temperatures (15°C day, 10°C night).
- (e) A growth-room environment alternating at intervals of fifteen days between high temperatures (25°C day, 20°C night) and low temperatures (15°C day, 10°C night).

Daylength in the growth-room treatments was 18 h. The light intensity at plant height was 14500 lux. Relative humidity was maintained at c. 85% in each growth-room. Mean minimum and maximum daily temperatures recorded using a Stevenson screen situated 1 m above a clipped sward at a site close to that used for Treatment (a) are presented in Table 6.1.

Table 6.1 Mean minimum and maximum daily temperatures recorded at Western Bank over the experimental period.

Temperatures °C	<u>Months</u>						
	April	May	June	July	Aug	Sept	Oct
Mean of daily max.temps.	10.6	14.6	16.4	19.6	19.2	16.6	13.9
Mean of daily min.temps.	3.7	6.1	8.3	11.8	11.7	9.7	8.3

The experiment was set on 7th April 1977. Each plastic tank was watered every week with 250 ml of complete Hewitt solution, and tap water was added regularly to keep the soil at field capacity. All the treatments were clipped at 5 cm from the

Differences between the species in vegetative response to treatment are evident when we examine the dry weight of the clippings collected from the turves (Figure 6.1, 6.2; Table 6.3).

Table 6.3 Mean dry weight (g) per container of accumulated clippings of *Holcus lanatus*, *Agrostis stolonifera* and *Lolium perenne**. 95% confidence limits are inserted in brackets.

Species	Garden	Growth-room			
		15°C	25°C	Fluctuation between 15°C and 25°C every 3 days	Fluctuation between 15°C and 25°C every 15 days
<i>Holcus lanatus</i>	20.0 (±3.4)	36.6 (±9.3)	53.1 (±2.4)	41.8 (±11.9)	42.8 (±15.9)
<i>Agrostis stolonifera</i>	6.6 (±2.1)	5.6 (±3.4)	7.6 (±3.2)	8.4 (±3.6)	7.6 (±5.1)
<i>Lolium perenne</i>	11.0 (±5.9)	16.0 (±1.1)	13.5 (±1.3)	13.5 (±3.7)	10.9 (±2.0)
Total	37.6 (±4.4)	58.2 (±8.5)	74.5 (±5.5)	63.8 (±5.9)	61.4 (±9.5)

* A small amount of *Lolium multiflorum* occurred with *L. perenne*.

The data presented in Table 6.3 show that under all treatments the total weight of clippings harvested from *H. lanatus* was higher than that produced by the other two species. *H. lanatus* showed a marked stimulation by warm temperatures and this was the main reason for the large total weight of clippings produced in the

25°C treatment. With respect to the yield of clippings, *Agrostis stolonifera* did not show any significant effect of treatment. *Lolium perenne* produced more clippings at 15°C than in the other three warmer growth-room conditions and in this respect showed a major difference in response when compared with *H. lanatus* and *A. stolonifera*. The mean dry weights of the clippings collected from successive cuts (Figure 6.1) show that in the outdoor treatment, peaks of production occurred during the spring and autumn and the contribution of *L. perenne* to the total weight of clippings remained relatively high throughout the period of the experiment.

In all of the growth-room treatments, the level of production of clippings increased rapidly during the first few weeks and remained at a high level throughout the experiment. In these treatments most of the weight of clippings was due to *H. lanatus* and with the passage of time, the quantity of *L. perenne* and *A. stolonifera* fell to a low level regardless of the temperature regime. It is interesting to compare the two main grass species with respect to their yield of clippings in the 15°C and 25°C treatments. *H. lanatus* attained greater yields of clippings at 25°C while the maximum for *L. perenne* occurred at 15°C.

Three-day fluctuations between high and low temperatures (Treatment (d)) produced a pattern similar to that occurring in the 25°C treatment except that the amount of clippings of *H. lanatus* was lower. In Treatment (e) it was apparent that *H. lanatus* yielded more clippings during the warmer 15-day period. However, this effect was not apparent in either *A. stolonifera* or *L. perenne* except at the beginning of the experiment.

Figure 6.1

Dry weight of *Holcus lanatus* ●—●, *Lolium perenne* ■—■, *Agrostis stolonifera* ○—○, in clippings removed on successive occasions from turf subjected to the following treatments:

- (a) Garden environment
- (b) 15°C
- (c) 25°C
- (d) Three-day fluctuations between 15°C and 25°C
- (e) Fifteen-day fluctuations between 15°C and 25°C

95% confidence limits are included.

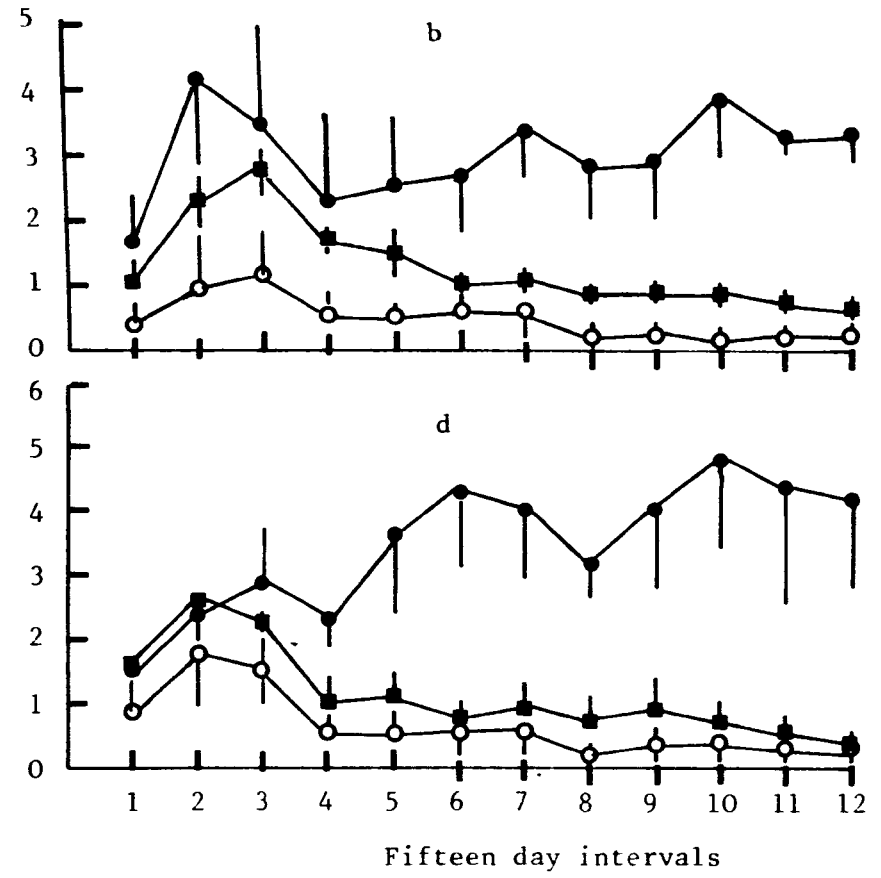
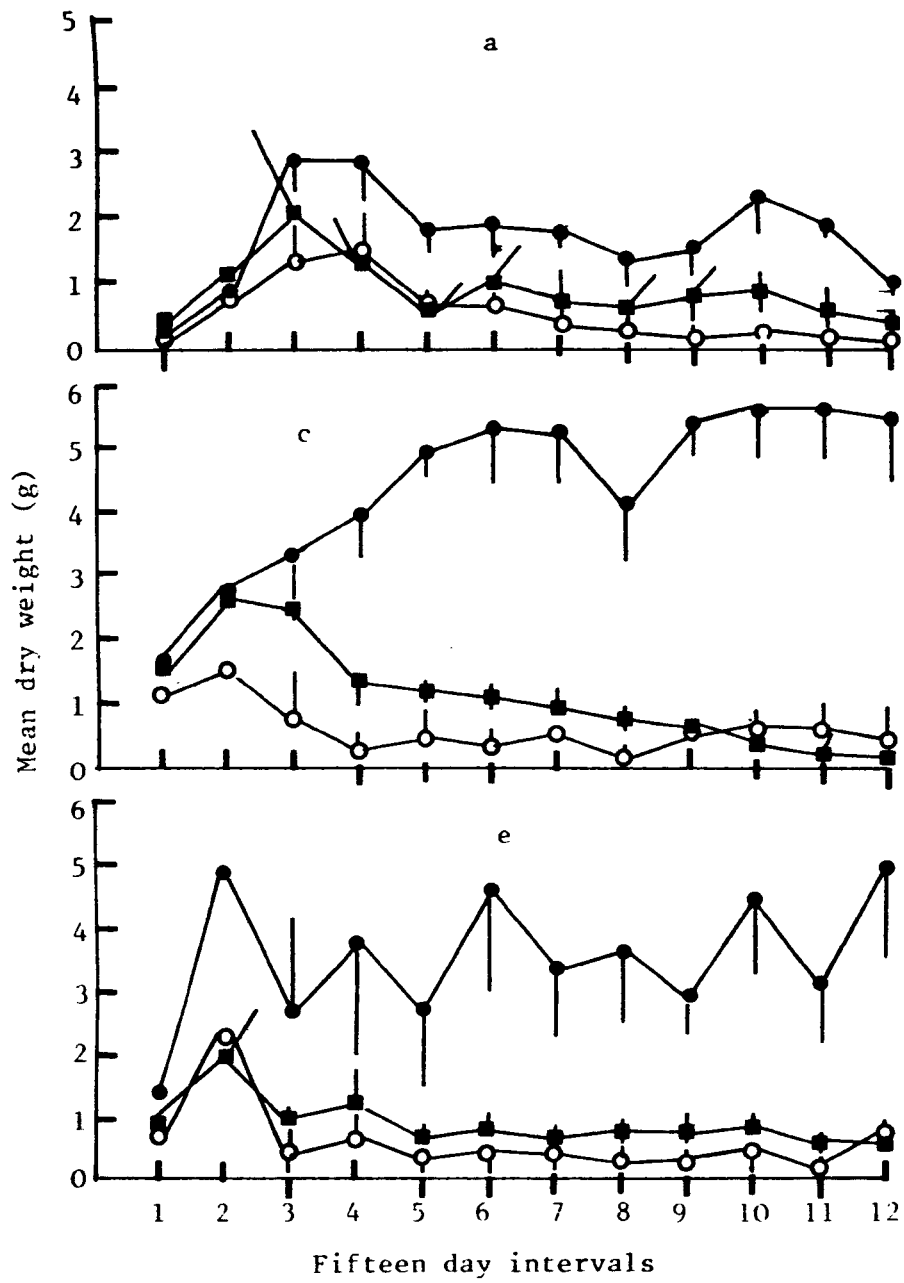
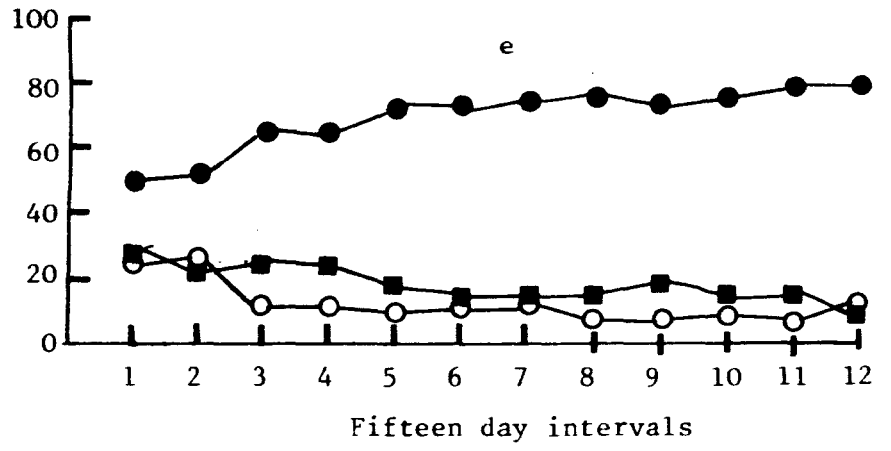
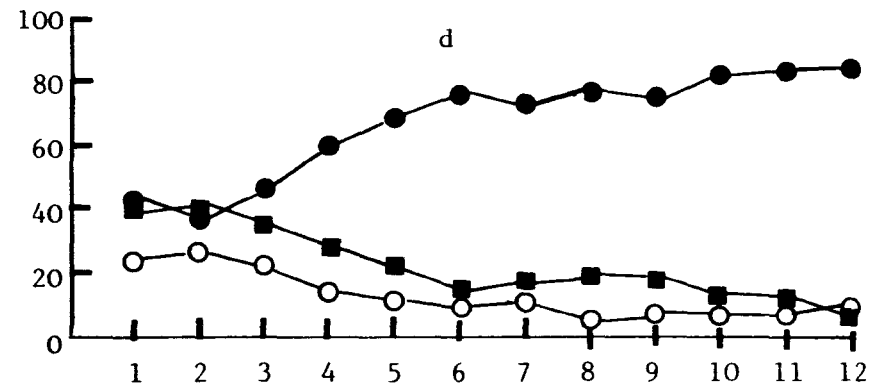
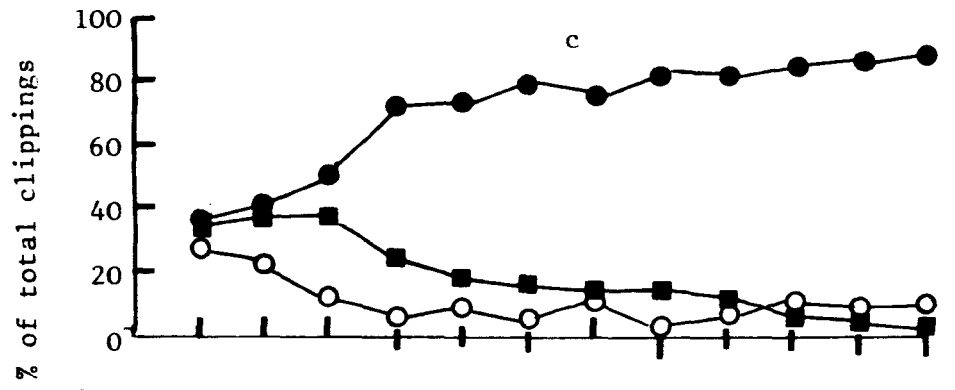
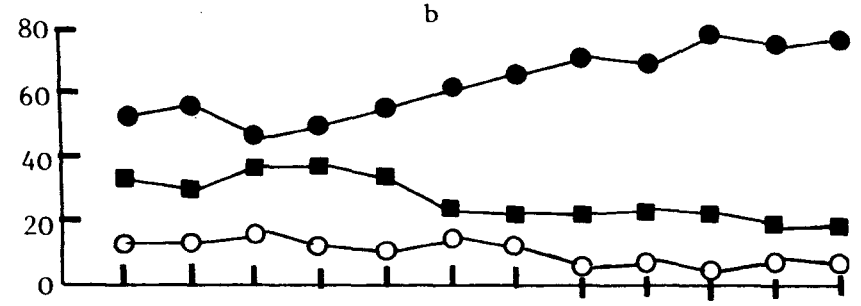
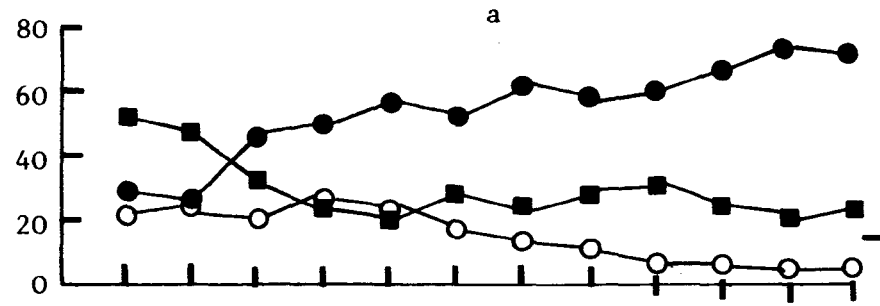


Figure 6.2

Percentage contribution of *Holcus lanatus* (●—●), *Lolium perenne* (■—■) and *Agrostis stolonifera* (○—○) to the clippings removed on successive occasions from turf subjected to the following treatments:

- (a) Garden environment
- (b) 15°C
- (c) 25°C
- (d) Three-day fluctuations between 15°C and 25°C
- (e) Fifteen-day fluctuations between 15°C and 25°C



Fifteen day intervals

Table 6.4 presents the mean dry weight of shoots harvested at the beginning and end of the experiment. The dry weight of shoots of *H. lanatus* in the initial harvest was very much higher than that of the other two species. In the final harvest, *H. lanatus* produced a large quantity of shoot material in all treatments. The yield of *L. perenne* at the final harvest was greatest at low temperatures (Treatments (a) and (b)) and at higher temperatures (Treatments (c), (d), (e)) the dry weight of shoots was less than that recorded at the initial harvest. It is interesting to note that despite its low contribution to the clippings *A. stolonifera* developed a comparatively large amount of shoot material in each experimental treatment. This was due to the fact that *A. stolonifera* produced an abundance of leaves and stolons close to the ground surface below the height of clipping.

Values for total shoot production (estimated by adding the dry weight of the plants at the final harvest to the weight of the accumulated clippings) are presented in Table 6.5 and very clearly depend on the temperature treatment. This Table confirms that *H. lanatus* attained maximum yield at 25°C and outyielded *A. stolonifera* and *L. perenne* in all treatments. *L. perenne* showed maximum yield at 15°C and in this respect was markedly different from both *A. stolonifera* and *H. lanatus*.

Table 6.4 Mean dry weight (g) of shoots and litter at final harvest. 95% confidence limits are included.

<u>Species</u>	<u>Initial harvest</u>	<u>Final harvest</u>				
		Outdoor	15°C	25°C	Fluctuation between 15/10°C and 25/20°C every 3 days	Fluctuation between 15/10°C and 25/20°C every 15 days
<i>Holcus lanatus</i>	2.04(±0.83)	9.34(±0.93)	13.55(±0.97)	11.49(±2.32)	13.40(±2.04)	10.06(±2.16)
<i>Lolium perenne</i>	1.52(±0.92)	1.62(±1.17)	1.97(±0.78)	0.46(±0.11)	1.05(±0.41)	0.69(±0.10)
<i>Agrostis stolonifera</i>	0.98(±0.93)	8.75(±1.77)	8.80(±4.51)	5.23(±1.68)	7.79(±5.42)	5.79(±4.09)
Total	4.54(±0.68)	19.69(±2.68)	24.31(±2.23)	19.31(±2.51)	22.43(±6.47)	18.55(±7.75)
Litter	5.98(±0.84)	4.75(±1.35)	7.20(±1.53)	8.46(±2.49)	7.08(±2.90)	7.71(±3.54)

6.3 EXPERIMENT 2

6.3.1 Materials and Methods

In order to obtain seedlings of *Holcus lanatus* and *Lolium perenne* Var.S.23 to be used in the experiment, seeds of both species were germinated on filter paper in plastic boxes in a growth-room environment with a daylength of 18 h and temperatures maintained at 20°C by day and 15°C by night. Fourteen-day-old seedlings of *H. lanatus* and ten-day-old seedlings of *L. perenne* of uniform size were transplanted into 13 cm pots of garden loam. The level of the soil in each pot was adjusted to exactly 5 cm below the rim. Three sowing arrangements were used as follows:

- (1) A monoculture of *L. perenne* (12 seedlings/pot)
- (2) A monoculture of *H. lanatus* (12 seedlings/pot)
- (3) A mixture of *L. perenne* and *H. lanatus* (6 seedlings/pot of each species)

All pots were then allowed to remain in the growth-room for a period of two weeks.

During this time and subsequently the pots were watered every week with 100 ml Hewitt nutrient solution and tap water was added regularly to keep the soil at field capacity. The vegetation in each pot was then clipped at 5 cm and the pots associated with each sowing arrangement were allocated at random (5 pots/treatment) to five treatments ((a)-(e)) identical to those applied in Experiment 1. In one additional treatment (Treatment (f)) pots experiencing three-day fluctuations in temperature between 15°C and 25°C were subject to clipping at intervals of three days. The pots in

each experimental treatment were arranged in five randomized blocks.

Clipping of the foliage in each pot was conducted at a height of 5 cm every 15 days (3 days in Treatment (f)), sorted by species and weighed. After the final clipping at the end of the experiment the stubble remaining in each pot was cut off at ground level, separated into component species and weighed.

6.3.2 Results

The dry weights of clipping of each species in monocultures and mixture together with the total weight of clippings (*H. lanatus* + *L. perenne*) from the mixture in various treatments are presented in Figure 6.3. Despite the difference in number of seedlings sown at the beginning of the experiment, no statistically significant difference could be established between the dry weights of clipping of *H. lanatus* in monoculture and mixture and although, in all treatments, the total weight of clippings from the mixture usually remained marginally greater than the yield from *H. lanatus* in monoculture, no significant difference was detected.

In *L. perenne* the dry weight of clippings recorded from the mixture fell very far below the levels recorded for the monocultures in all treatments. It is interesting to note, however, that with the exception of treatments (c) and (d), the yield of clippings of *L. perenne* in monoculture did not fall much below that recorded for the total weight of clippings removed from the mixture.

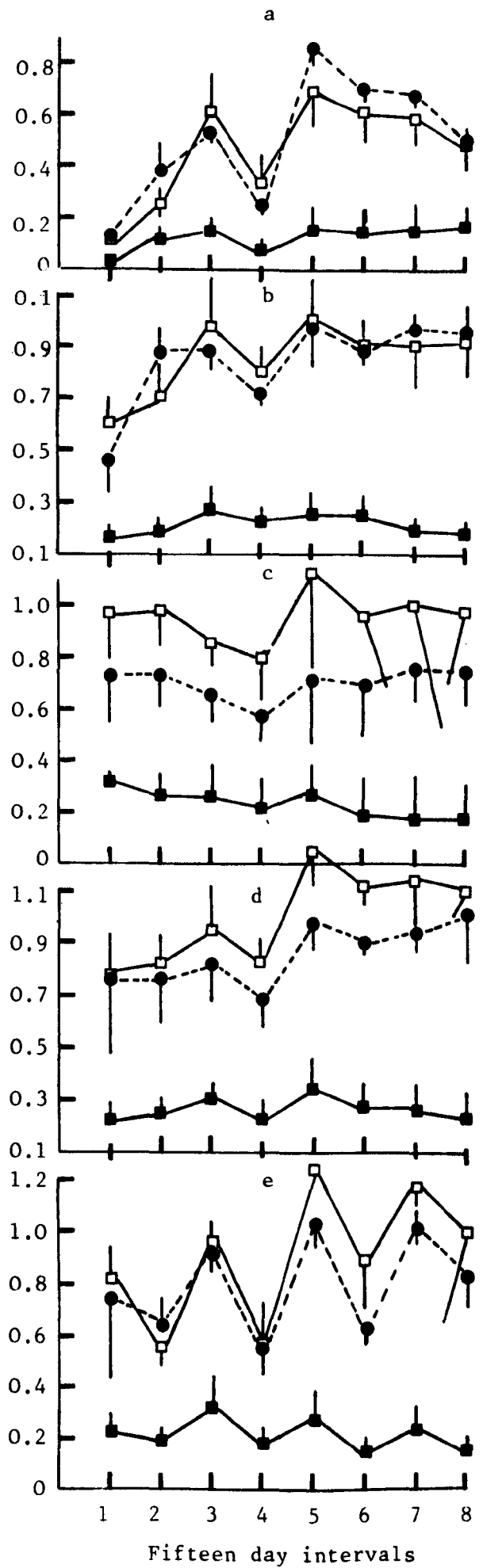
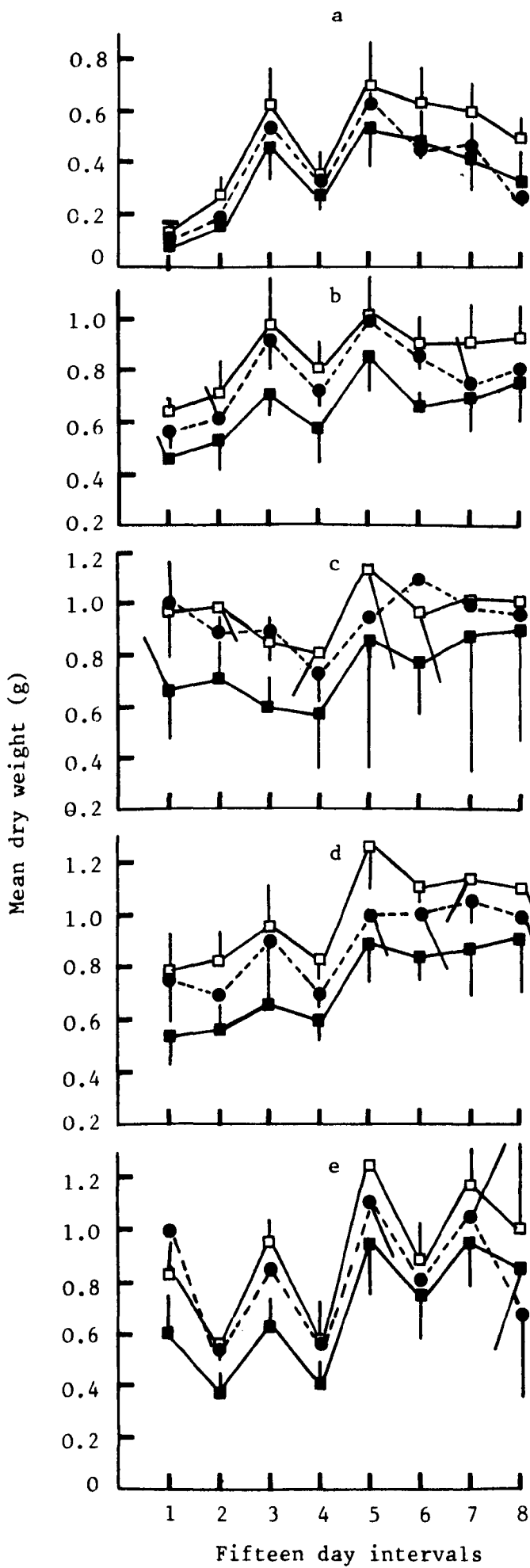
Figure 6.3

Dry weight of clippings of *Holcus lanatus* (left) and *Lolium perenne* (right) removed on successive occasions from pots subjected to the following treatments:

- (a) Garden environment
- (b) 15°C
- (c) 25°C
- (d) Three-day fluctuations between 15°C and 25°C
- (e) Fifteen-day fluctuations between 15°C and 25°C

95% confidence limits are included.

Total dry weight □—□, monoculture ●—●, mixture ■—■
(*H. lanatus* + *L. perenne*)





In *H. lanatus* the highest quantities of clippings were recorded at 25°C, whilst the maximum for *L. perenne* occurred at 15°C. In Treatment (e) *H. lanatus* responded to the warmer periods with a marked increase in weight of clippings and this effect was observed in monoculture and mixture. *L. perenne* exhibited a similar response in monoculture but the stimulating effect of warm temperature was scarcely detectable in the mixture.

Figure 6.4 describes the percentage contribution of *H. lanatus* and *L. perenne* to the total weight of clippings in the mixture in each treatment. A high proportion of *H. lanatus* was evident in all treatments, although the species was most abundant at warmer temperatures. In the 'natural' (i.e. garden) treatment *L. perenne* contributed more clippings during the spring and autumn and in this respect the results are in agreement with those obtained in Experiment 1.

The data for the total weight of clippings presented in Table 6.6 show that with the exception of Treatment (d) no significant differences were established for *H. lanatus* between monoculture and mixture. This was in sharp contrast to the result for *L. perenne* where the accumulated weight of clippings from the mixture was consistently small in comparison with that recorded for the monoculture.

It is particularly interesting to note that the total weight of clippings from *L. perenne* in monoculture in the garden environment (Treatment (a)) exceeded that of the monoculture of *H. lanatus* in the same treatment. The highest yields of clippings from *L. perenne* occurred in two of the treatments ((b) and (d)) involving cooler temperatures.

Figure 6.4

Contribution of *Holcus lanatus*  and *Lolium perenne* 
to the clippings removed on successive occasions from
turf subjected to the following treatments:

- (a) Garden environment
- (b) 15°C
- (c) 25°C
- (d) Three days fluctuation between 15°C and 25°C
- (e) Fifteen days fluctuation between 15°C and 25°C

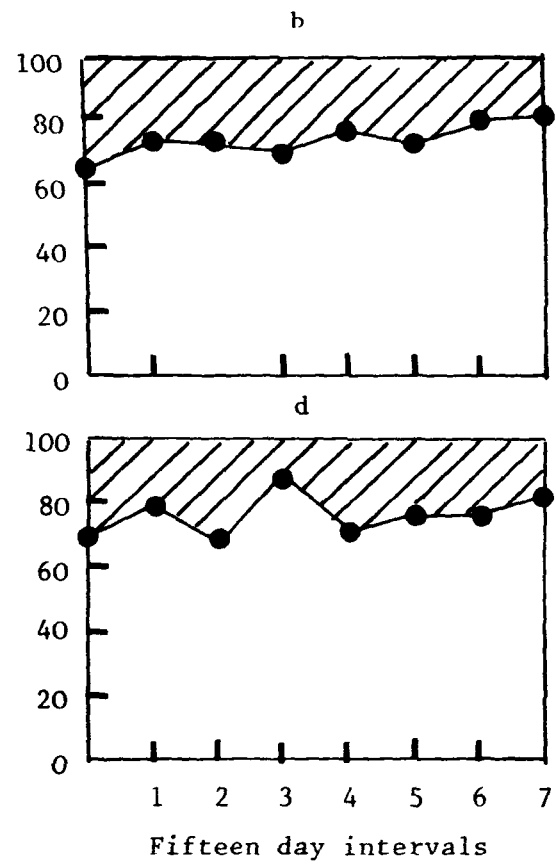
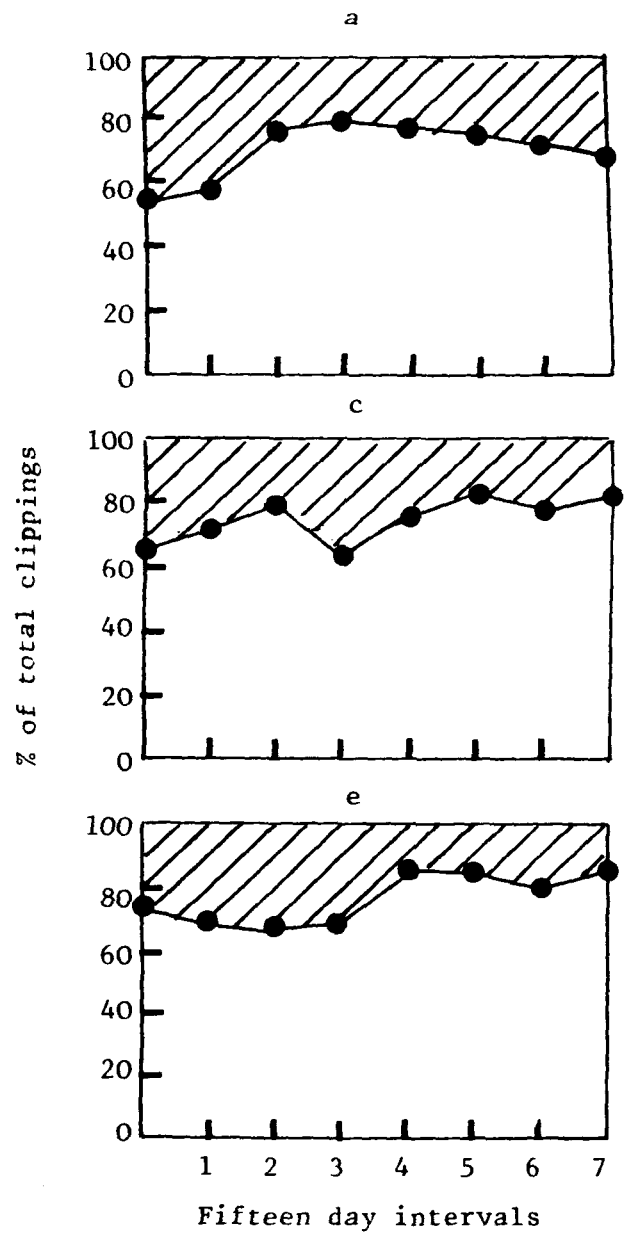


Table 6.6 Mean dry weights (g/pot) of accumulated clippings from successive cuts. 95% confidence limits are inserted in brackets.

<u>Species</u>	(a) Garden	Growth-room			
		(b) 15°C	(c) 25°C	(d) Fluctuation between 15°C and 25°C every 3 days	(e) Fluctuation between 15°C and 25°C every 15 days
<i>Holcus lanatus</i> (monoculture)	2.99(±0.31)	6.15(±0.67)	7.43(±1.46)	7.16(±0.35)	6.59(±0.99)
<i>Holcus lanatus</i> (mixture)	2.66(±0.60)	5.23(±0.66)	5.96(±1.75)	5.84(±0.66)	5.51(±0.78)
<i>Lolium perenne</i> (monoculture)	4.01(±0.14)	6.77(±0.24)	5.54(±1.03)	6.85(±0.78)	6.23(±0.62)
<i>Lolium perenne</i> (mixture)	1.03(±0.22)	1.71(±0.25)	1.70(±0.19)	2.09(±0.51)	1.71(±0.41)
Total mixture (<i>L. perenne</i> + <i>H. lanatus</i>)	3.63(±0.65)	6.89(±0.48)	7.83(±1.50)	7.93(±0.50)	7.26(±0.40)

From Table 6.7 it is apparent that by the time of the final harvest there were no marked or statistically significant effects of treatment upon the amount of shoot material of *H. lanatus* remaining in the unclipped stratum. In contrast, *L. perenne* showed a clear reduction in the yield in the mixture in all treatments. It is also apparent from these data that in both monoculture and mixture the shoot material of *H. lanatus* exceeded that of *L. perenne* in all treatments.

Values for total shoot production (estimated by adding the dry weight of shoots at final harvest to the accumulated clippings) are presented in Table 6.8. In *H. lanatus* no significant differences between monoculture and mixture occurred except in Treatment (d). *L. perenne* showed a clear reduction in yield in the presence of *H. lanatus* and this effect was statistically significant in all treatments.

In Treatment (f) where 3-day temperature fluctuations were associated with a 3-day clipping regime the results (Figure 6.5; Table 6.9) show that *H. lanatus* responded rapidly to the short-term temperature fluctuations producing approximately twice as much clippings during the 25°C periods. In this treatment no significant differences in dry weight of clippings were detected between plants growing in monoculture and in mixture and the weight of clippings in monoculture was only marginally below the total weight of clippings recovered from both species in the mixture. In Figure 6.6 and Table 6.9 there is clear evidence of a major reduction in the weight of clippings of *L. perenne* when this species was grown in mixture.

Table 6.7 Mean dry weight (g) of plant material at final harvest. 95% confidence limits are included.

<u>Species</u>	<u>Garden</u>	<u>Growth-room</u>			
		15°C	25°C	Fluctuation between 15°C and 25°C	Fluctuation between 15°C and 25°C
<i>Holcus lanatus</i> (monoculture)	2.83(±0.83)	3.24(±1.08)	2.24(±0.24)	2.96(±0.42)	2.14(±0.79)
<i>Holcus lanatus</i> (mixture)	2.50(±0.76)	2.56(±0.47)	1.52(±0.46)	2.35(±0.31)	2.05(±0.41)
<i>Lolium perenne</i> (monoculture)	2.80(±0.39)	1.89(±0.14)	1.12(±0.38)	1.63(±0.20)	1.31(±0.11)
<i>Lolium perenne</i> (mixture)	0.63(±0.22)	0.42(±0.03)	0.29(±0.09)	0.42(±0.09)	0.33(±0.14)




Table 6.8 Total weight of shoot material* (g/pot) of *Holcus lanatus* and *Lolium perenne* grown under different temperature regimes. 95% confidence limits in brackets.

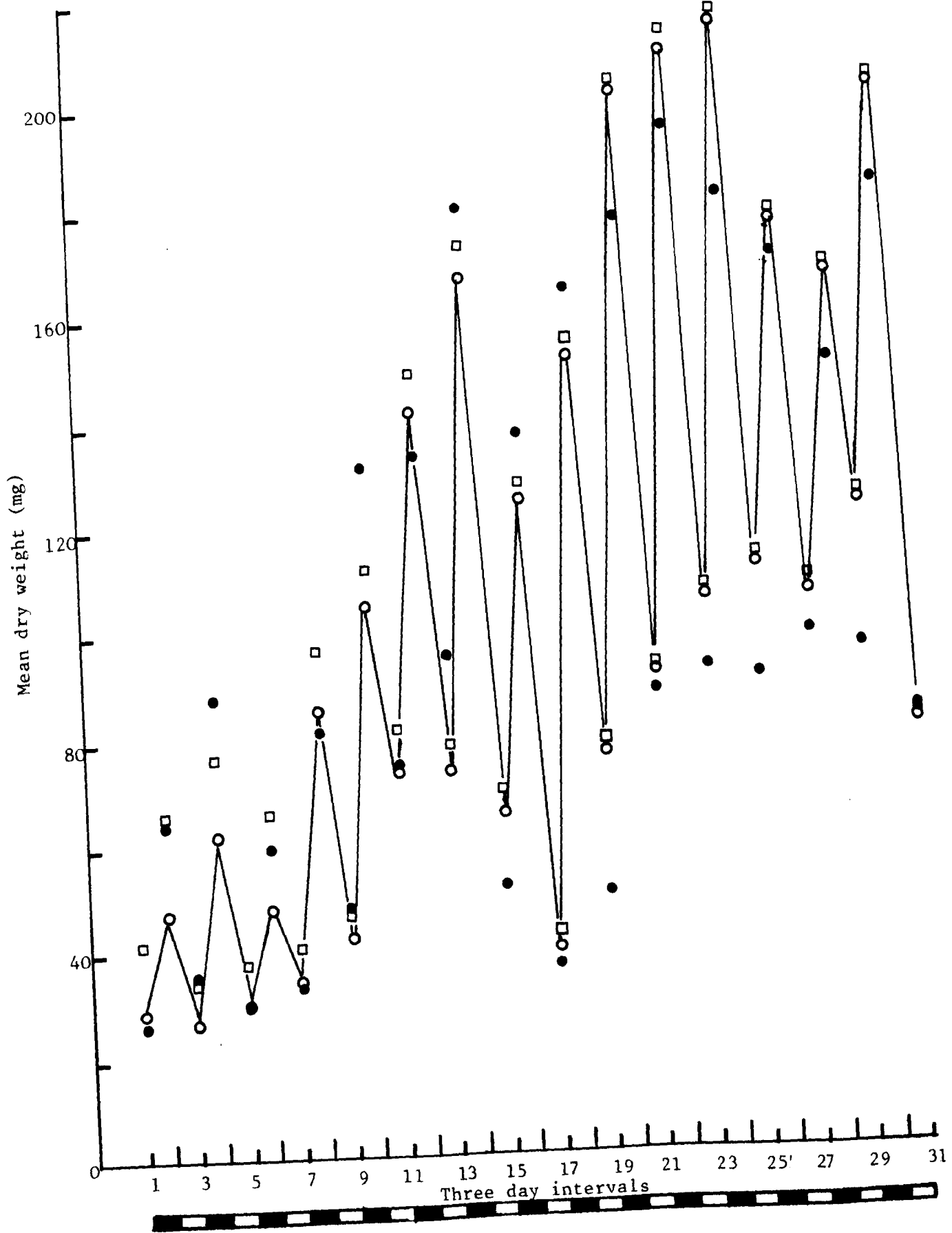
Species	(a) Garden	Growth-room			
		(b) 15°C	(c) 25°C	(d) Fluctuation between 15°C and 25°C every 3 days	(e) Fluctuation between 15°C and 25°C every 15 days
<i>Holcus lanatus</i> (monoculture)	5.82(±0.54)	9.39(±0.74)	9.89(±2.03)	10.12(±0.77)	8.73(±1.66)
<i>Holcus lanatus</i> (mixture)	5.26(±0.87)	7.78(±1.03)	7.48(±2.17)	8.19(±6.78)	7.36(±0.84)
<i>Lolium perenne</i> (monoculture)	6.81(±0.3)	8.65(±0.2)	6.62(±1.29)	8.28(±0.94)	7.54(±0.64)
<i>Lolium perenne</i> (mixture)	1.65(±0.42)	2.11(±0.22)	1.99(±0.24)	2.46(±0.67)	2.04(±0.53)
Total mixture (<i>L. perenne</i> + <i>H. lanatus</i>)	6.75(±0.25)	9.88(±0.84)	9.61(±1.51)	10.48(±0.72)	9.88(±0.84)

* accumulated clippings + final harvest

Figure 6.5

Dry weight of clippings collected from successive cuts from a mixture of *Holcus lanatus* and *Lolium perenne* clipped at 5 cm every 3 days and subjected to fluctuating temperatures.

- (a) Total mixture (*H. lanatus* + *L. perenne*) 
- (b) *H. lanatus* with *L. perenne* 
- (c) Monoculture (*H. lanatus*) 



25°C
 15°C

Table 6.9 Production of dry matter by *Holcus lanatus* and *Lolium perenne* in monocultures and mixtures subjected to 3-day fluctuations in temperature (15°C and 25°C) and clipped at intervals of 3 days. 95% confidence limits are in brackets.

	<u><i>Holcus lanatus</i></u>		<u><i>Lolium perenne</i></u>	
	monoculture	mixture	monoculture	mixture
Mean dry weight (g) of accumulated clippings	3.11(±0.13)	3.23(±0.4)	1.50(±0.25)	0.16(±0.04)
Mean dry weight (g) of shoot at final harvest	1.84(±0.14)	1.78(±0.42)	0.74(±0.23)	0.02(±0.01)
Mean dry weight (g) of root at final harvest	0.83(±0.19)	0.63(±0.09)	0.17(±0.05)	0.007(±0.004)
Total production by adding the dry weight of the plant at final harvest (shoot + root) to the weight of accumulated clippings	5.78(±0.34)	5.64(±0.51)	2.41(±0.48)	0.19(±0.03)




Table 6.9 Production of dry matter by *Holcus lanatus* and *Lolium perenne* in monocultures and mixtures subjected to 3-day fluctuations in temperature (15°C and 25°C) and clipped at intervals of 3 days. 95% confidence limits are in brackets.

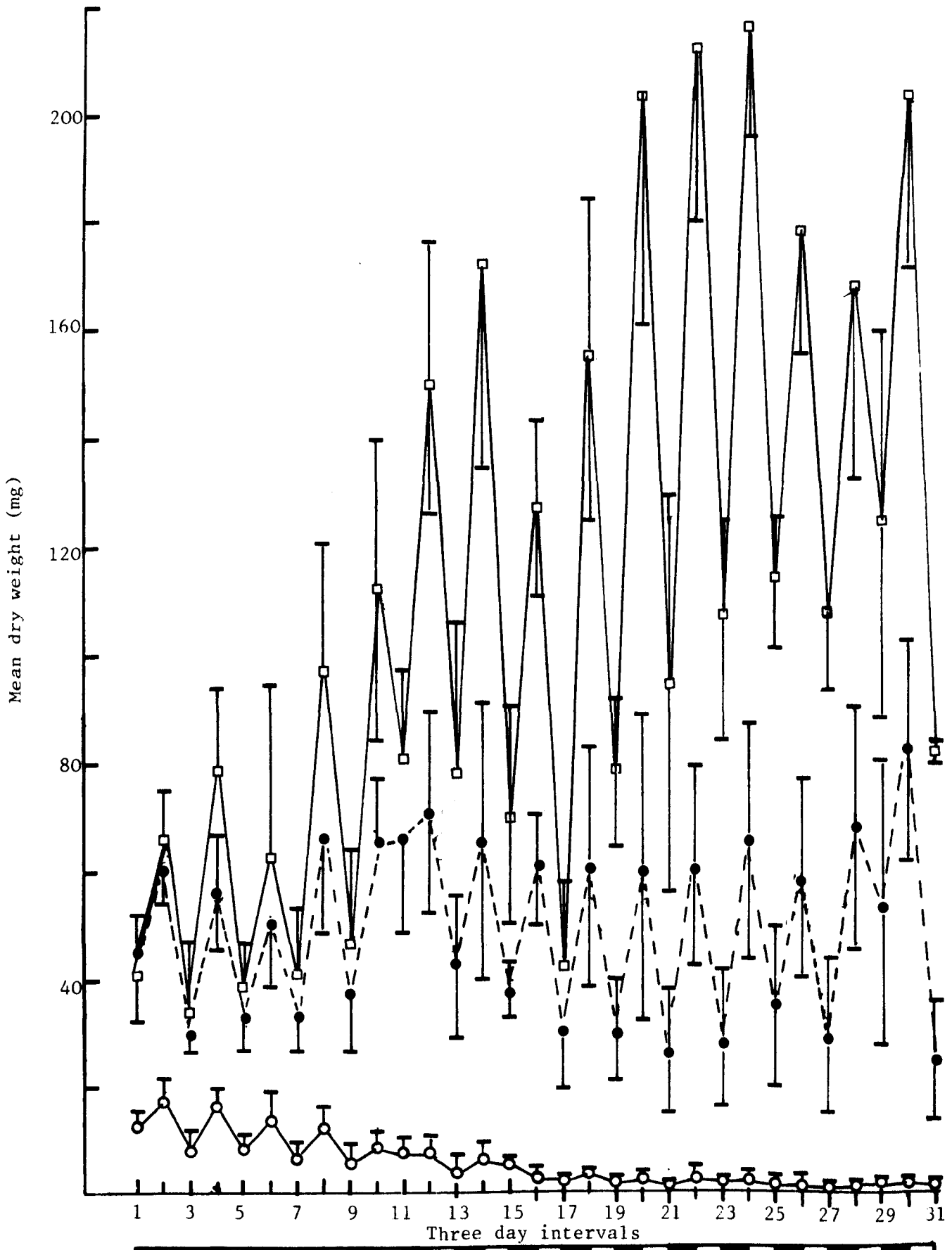
	<u><i>Holcus lanatus</i></u>		<u><i>Lolium perenne</i></u>	
	monoculture	mixture	monoculture	mixture
Mean dry weight (g) of accumulated clippings	3.11(±0.13)	3.23(±0.4)	1.50(±0.25)	0.16(±0.04)
Mean dry weight (g) of shoot at final harvest	1.84(±0.14)	1.78(±0.42)	0.74(±0.23)	0.02(±0.01)
Mean dry weight (g) of root at final harvest	0.83(±0.19)	0.63(±0.09)	0.17(±0.05)	0.007(±0.004)
Total production by adding the dry weight of the plant at final harvest (shoot + root) to the weight of accumulated clippings	5.78(±0.34)	5.64(±0.51)	2.41(±0.48)	0.19(±0.03)

Figure 6.6

Dry weight of clippings collected from successive cuts from a mixture of *Holcus lanatus* and *Lolium perenne* clipped at 5 cm every 3 days and subjected to fluctuating temperatures.

95% confidence limits are included.



- (a) Total mixture (*H. lanatus* + *L. perenne*) 
- (b) *L. perenne* with *H. lanatus* 
- (c) Monoculture (*L. perenne*) 

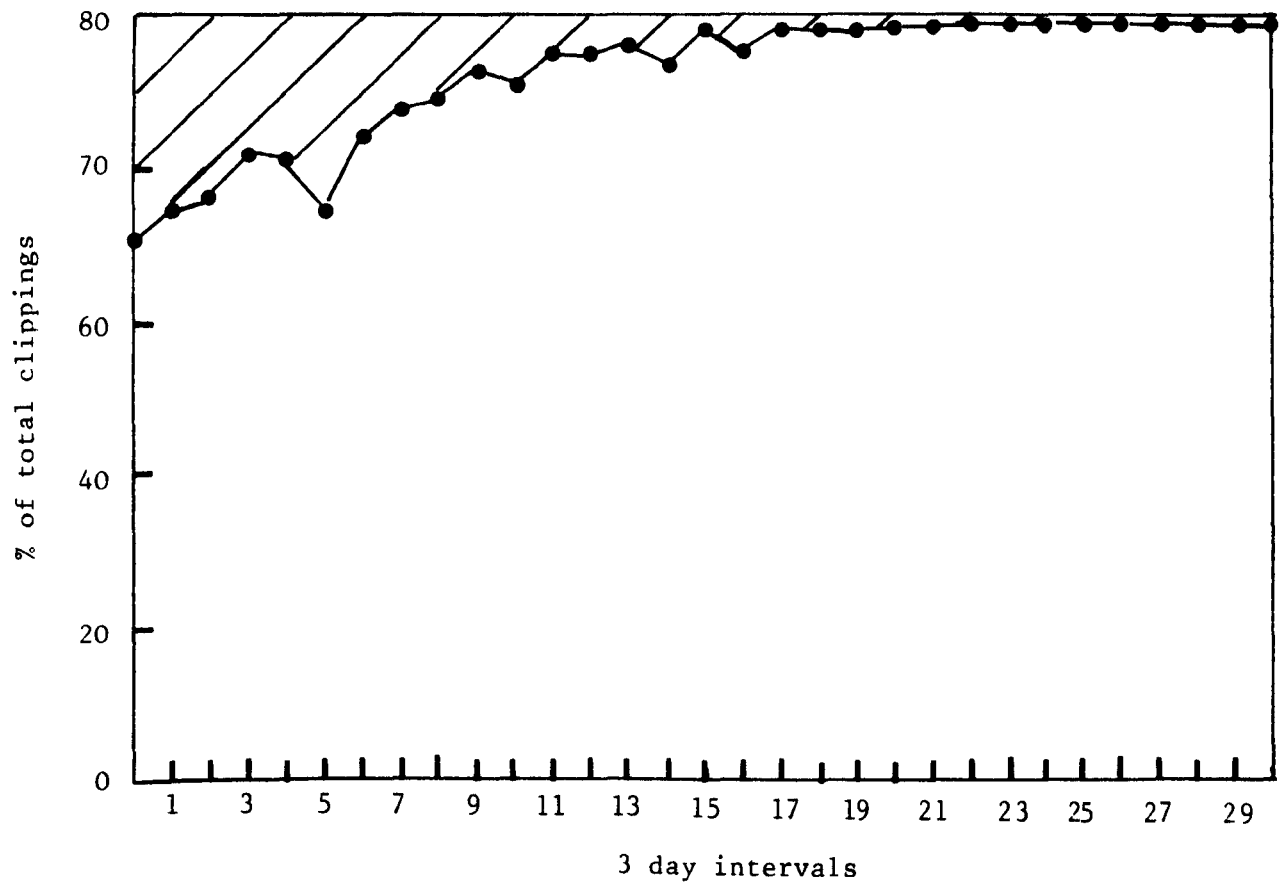


25°C
 15°C

In Figure 6.7 the yield of clippings of the two species from the mixture in Treatment (f) are expressed as percentages. From this figure it is apparent that *L. perenne* declined in its contribution to the clippings throughout the experiment.

Figure 6.7

Contribution of *Holcus lanatus* , and *Lolium perenne* 
to the dry weight removed at 3-day intervals from a
mixture subjected to 3-day fluctuations between 15°C
and 25°C.



6.4 DISCUSSION

The first experiment described in this chapter involved samples of a sown sward removed to controlled environmental conditions and provided an opportunity to observe the response of three constituent grass species to manipulation of the temperature regime. In the second experiment only two species, *Lolium perenne* and *Holcus lanatus*, were included and the design involved planting of the two species in standardized monocultures and mixture. It is clear that although both experiments provide insights into competitive interactions, only the second experiment allows an assessment of the impact of one species upon another and a comparison of the productivity of monocultures and mixtures under constant and fluctuating temperature conditions.

Response to temperature

Optimal temperatures for dry matter production in the range 20–25°C have been recorded (Mitchell 1956; Evans *et al.* 1964; Tainton 1967; Mahmoud 1973; Al-Mufti 1978) for a number of temperate herbaceous species including *Lolium perenne*, *Dactylis glomerata*, *Agrostis tenuis*, *Deschampsia flexuosa*, *Arrhenatherum elatius*, *Holcus lanatus*, *Holcus mollis*, *Urtica dioica* and *Zerna erecta*. The results in this chapter were consistent with their findings although it is evident that the optimum temperature for growth of *Lolium perenne* is appreciably lower than that of *Holcus lanatus* and *Agrostis stolonifera*. The relatively high temperature optimum in *H. lanatus* is consistent with the fact that this species continues to exhibit vegetative growth during the summer (Chapter 2) and attains maximum shoot biomass at this time.

The lower temperature optimum in *L. perenne* is again consistent with the phenology of the species which appears to produce two peaks of shoot production, one in spring and the other in autumn (Haggar 1976, Chapter 2).

In view of the difference between *H. lanatus* and *L. perenne* in temperature optimum it is tempting to suppose that differential responses to seasonal and short-term changes in temperature could be conducive to co-existence between the two species. However, examination of the data from both experiments reveals that despite the difference in temperature optimum, none of the experimental conditions allow the quantities of dry matter produced by *L. perenne* to exceed that of *H. lanatus*. In both experiments and under all temperature regimes, mixtures were dominated by *H. lanatus* and although this species attained its highest rates of growth at warm temperatures, dry matter production in the cooler growth-room environment exceeded that of *L. perenne*. It is noteworthy, however, that in the garden environment the yield of clippings of *L. perenne* in Experiment 1 exceeded that of the other two species during the early spring and it seems possible that conditions advantageous to this species might have been found in experiments involving temperatures lower than those employed in this investigation.

Response to clipping

Observations during the course of the experiments revealed that *L. perenne*, *H. lanatus* and *A. stolonifera* produced rather different growth forms which caused the plants to suffer different intensities of damage during clipping. In particular it was clear

that the erect shoots of *L. perenne* were more severely affected than the creeping tillers of *A. stolonifera*. From previous studies (e.g. Sullivan and Sprague 1943; Mahmoud 1973; Watt 1977) it seems likely also that the species differed in their morphogenetic responses to defoliation. In *L. perenne* and to a lesser extent in *H. lanatus* clipping resulted in the projection of more foliage into the clipped stratum. This was in marked contrast to the response of *A. stolonifera* which produced numerous tillers and stolons beneath the level of clipping.

In Experiment 2 where the turf was established from seedlings, the effect of defoliation and slow rate of tillering upon canopy development by *L. perenne* was quite pronounced. In Plate 6.2 it is evident that even in the absence of competition from *H. lanatus*, the extent of lateral spread and tiller formation in *L. perenne* was insufficient to produce a continuous sward even by the time of the final harvest.

Competitive interactions

From both experiments there was conclusive evidence of the ability of *H. lanatus* to suppress the vigour of *L. perenne* and this ability was only marginally affected by variation in temperature or clipping regime. Previous investigators (e.g. Riveros 1963; Chadokar and Humphreys 1973; Rebson 1973) have drawn attention to the importance, in competitive interactions, of dense leaf canopies and in this respect the ability of *H. lanatus* to produce numerous tillers under a wide range of conditions is of particular importance.



a

b

c

Plate 6.2

Appearance of plants of *Holcus lanatus* and *Lolium perenne* after three months of growth under fluctuating temperature conditions (15°C and 25°C) and subjected to clipping every 3 days.

(a) *Lolium perenne* monoculture

(b) *Holcus lanatus* monoculture

(c) Mixture (6 plants *H. lanatus*, 6 plants *L. perenne*)

Scale units are cm.

It seems likely that progressive submergence of the leaves of other species may be an important part of the mechanism whereby the competitive superiority of *H. lanatus* is exerted. However, it seems unlikely that the advantage of *H. lanatus* over *L. perenne* in the present experiment was merely the result of leaf shading. It has been noted already that the growth form of *L. perenne* causes the species to suffer greater intensities of damage under clipping regimes. The resulting loss of photosynthate is likely to weaken the competitive ability of *L. perenne* both above and below ground. Defoliation not only prevents the development of an effective leaf canopy but also causes the diversion of photosynthesis into leaf growth at the expense of the roots (Troughton 1963).

Hence we may suggest that the impact of *H. lanatus* upon *L. perenne* can be related also to competitive interactions within the rhizosphere. Even in circumstances where root development in *L. perenne* is unaffected by defoliation there is reason to expect that the species would be subject to severe effects of root competition from *H. lanatus*. This may be predicted from the extremely high rates of root extension and root hair development characteristic of *H. lanatus*.

An additional possible factor, that of allelopathy, must be recognized in any attempt to explain the suppression of *L. perenne* by *H. lanatus*. This possibility has been recognized already by Newman and Rovira (1975) and is re-examined in the final discussion (Chapter 11) in relation to the evidence presented in Chapters 4, 5, 7, 8, 9 and 10.

CHAPTER 7

AN INVESTIGATION OF SOME EFFECTS OF ESTABLISHED
PLANTS UPON THE GROWTH OF THE SEEDLINGS

7.1 INTRODUCTION













The two experiments described in this chapter were designed to investigate the effects of established plants upon the growth of seedlings. In both experiments attention was confined to below-ground interactions. The first experiment, conducted in a garden, allowed the possibility of both competition for mineral nutrients and allelopathic interactions. The second experiment was concerned exclusively with the attempt to detect phytotoxic effects originating from established plants.

7.2 EXPERIMENT 1

7.2.1 Materials and Methods

The established plants used in this experiment consisted of various species which had been grown outdoors at the Botany Department experimental garden in sand culture by A. M. Neal and J. P. Grime for a period of four years prior to the beginning of these studies. With the exception of *Mercurialis perennis*, for which transplants were used, each of the species was established from seed collected from sites in the Sheffield area (Table 7.1). The plants were allowed to establish in square plastic tubs each containing 30 l of washed sand; three replicate tubs were provided for each species. Each tub was placed upon an individual plastic tray in which drainage water collected. At monthly intervals during the spring and summer, one litre of Hewitt nutrient solution was added to each container and watering was conducted as required to maintain the sand in a moist condition. During the winter months the volume of nutrient solution

Table 7.1 Source of seeds, seedlings and plants which were used in the established plant. Also explained is the phenology.

<u>Species</u>	<u>Source of plants</u>	<u>Phenology</u>
<i>Acer pseudoplatanus</i>	Tapton Garden	
<i>Agropyron repens</i>	Worksop	
<i>Agrostis tenuis</i>	Lathkilldale	
<i>Arrhenatherum elatius</i>	Lathkilldale	
<i>Chamaenerion angustifolium</i>	Millers Dale	
<i>Festuca ovina</i>	Lathkilldale	
<i>Festuca rubra</i>	Winnats Pass	
<i>Holcus lanatus</i>	Press Reservoir	
<i>Mercurialis perennis</i>	Winnats Pass	
<i>Poa trivialis</i>	Cadeby Common	
<i>Urtica dioica</i>	Coombsdale	  E S

S - start of experiment
E - end of experiment

was reduced to 500 ml/month. Control tubs containing sand but no plants were maintained by exactly the same procedure throughout the four years.

In October 1975 the main part of the experiment was commenced by sowing six 10 day-old seedlings of *Rumex acetosa* into each container. In order to eliminate the possibility of effects due to the above-ground parts of the established plants the seedlings were introduced to the sand through holes in the base of an opaque V-shaped plastic screen (Figure 7.1). For the first two weeks of the experiment each container was examined daily and dead or missing seedlings were replaced. The seedlings were then allowed to grow for a period of seven months during which aliquots of 250 ml Hewitt solution were added weekly to each container through the holes in the plastic screen. In April 1976 the plants of *R. acetosa* were harvested individually (shoots only), oven-dried at 30°C for 48 h and weighed.

7.2.2 Results

The number of plants of *Rumex acetosa* surviving to the end of the experiment in each replicate is recorded in Table 7.2. It is clear that the majority of the seedlings survived although high mortalities occurred in certain replicates of *Chamaenerion angustifolium* and *Urtica dioica*. As we might expect, the most vigorous growth in *R. acetosa* was observed during the autumn and spring. The first few months of the experiment were fairly warm (Appendix 2) and this fact may have contributed to the high rates of establishment and survival. Growth of *R. acetosa* in the controls was rapid (Plate 7.1)

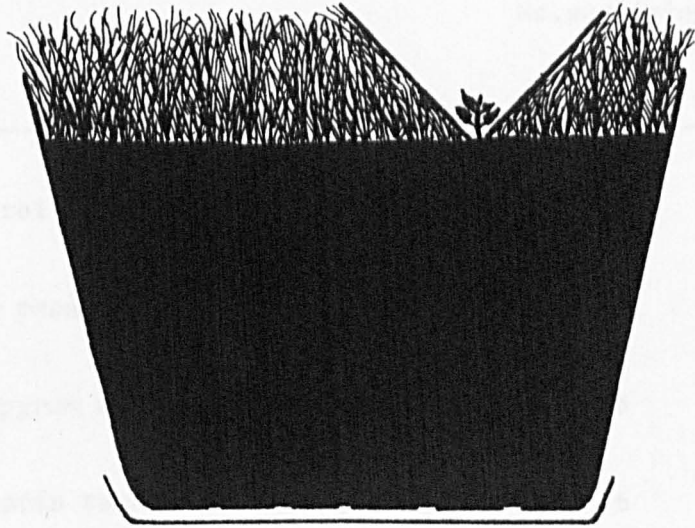


Figure 7.1

Vertical section illustrating the container and the V-shaped plastic screen.

Table 7.2 Number of surviving plants of *Rumex acetosa* at final harvest in each replicate.

Species	No. surviving in 3 replicates*		
	1	2	3
Control	6	6	6
<i>Acer pseudoplatanus</i>	6	6	6
<i>Agropyron repens</i>	6	6	6
<i>Agrostis tenuis</i>	6	6	6
<i>Arrhenatherum elatius</i>	5	6	6
<i>Chamaenerion angustifolium</i>	6	2	2
<i>Festuca ovina</i>	6	4	5
<i>Festuca rubra</i>	4	4	5
<i>Holcus lanatus</i>	5	5	6
<i>Mercurialis perennis</i>	6	6	6
<i>Poa trivialis</i>	6	6	6
<i>Urtica dioica</i>	5	2	3

* maximum possible 6.



PLATE 7.1

Rumex acetosa seedlings grown for seven months in the control treatment.

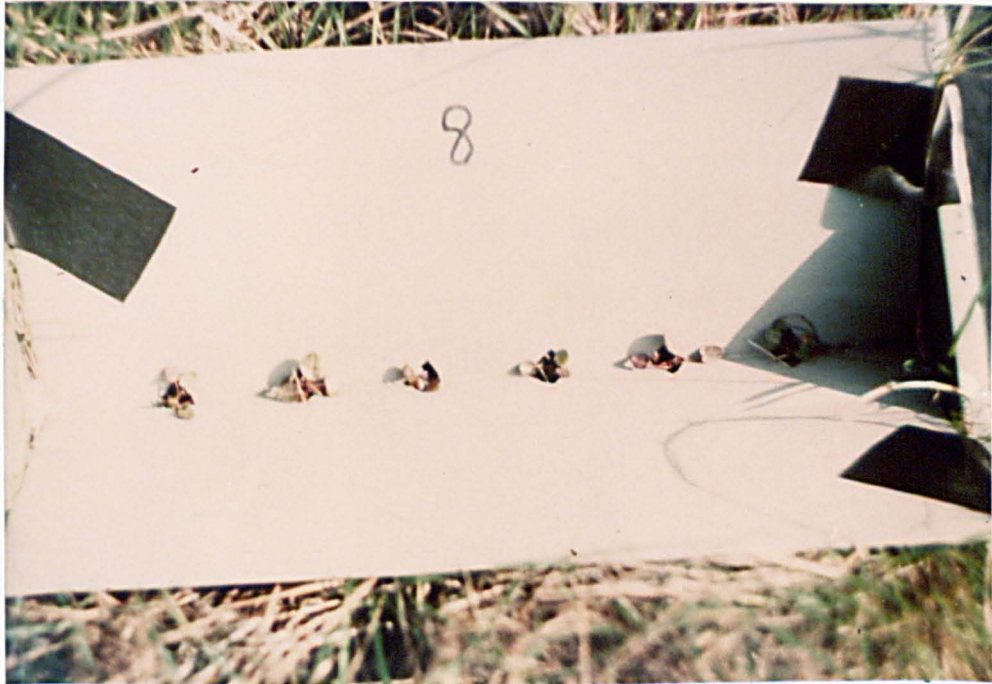


PLATE 7.2

Rumex acetosa seedlings grown for seven months in a monoculture of *Agrostis tenuis*.

and provided a marked contrast with performance in tubs occupied by established plants of species such as *Agrostis tenuis*, *Chamaenerion angustifolium* and *Holcus lanatus* (Plates 7.2, 7.3, 7.4). In the latter, the seedlings remained small to the end of the experiment and the majority of leaves showed red or bronzed discoloration. These symptoms were much less pronounced in seedlings associated with three species, *Mercurialis perennis*, *Poa trivialis* and *Urtica dioica*.

Table 7.3 presents the yields of shoot material of the seedlings of *Rumex acetosa* grown in the control tubs and in combination with established plants of each of eleven species. Marked differences are apparent in the extent to which the various species affected the yield of *R. acetosa*. *Festuca ovina* and *Festuca rubra* caused reductions in the yields of *R. acetosa* greater than those occurring in association with any other species. *Acer pseudoplatanus*, *Agropyron repens*, *Agrostis tenuis*, *Arrhenatherum elatius*, *Chamaenerion angustifolium* and *Holcus lanatus* also produced large and statistically significant differences from the yield of the control seedlings. *Mercurialis perennis*, *Poa trivialis* and *Urtica dioica* brought about reductions in yield but these were not statistically significant.

7.2.3 Discussion

The experimental results provide strong evidence of the inhibitory influence of established plants upon the growth of seedlings of *Rumex acetosa*. The results are particularly interesting in that they were obtained over a time of year in which many species including *R. acetosa* normally pass through the phase of germination

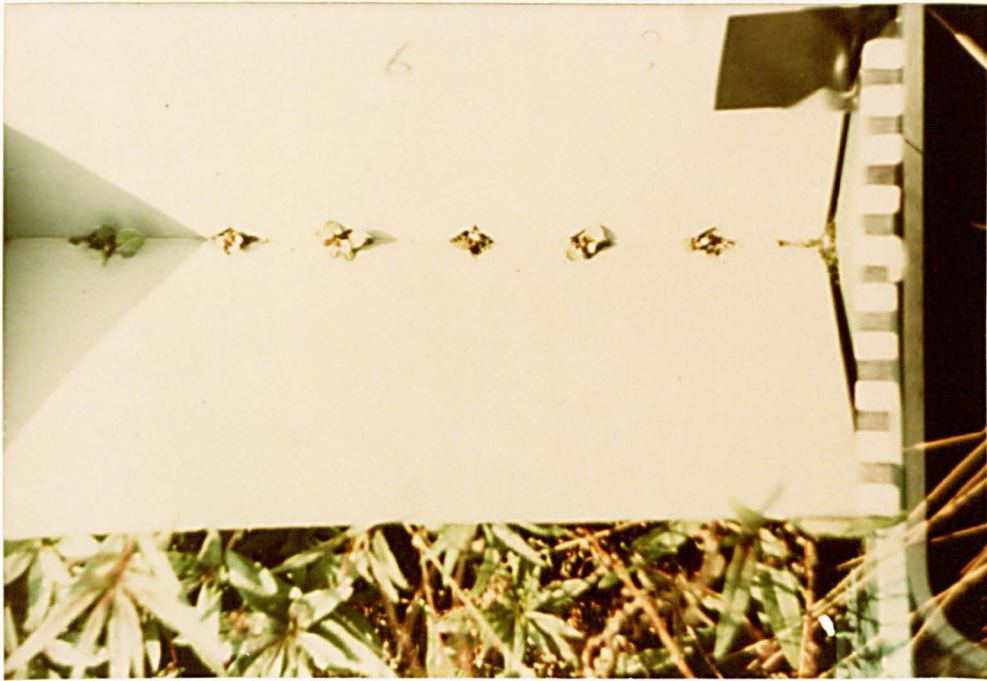


PLATE 7.3

Rumex acetosa seedlings grown for seven months in a monoculture of *Chamaenerion angustifolium*.

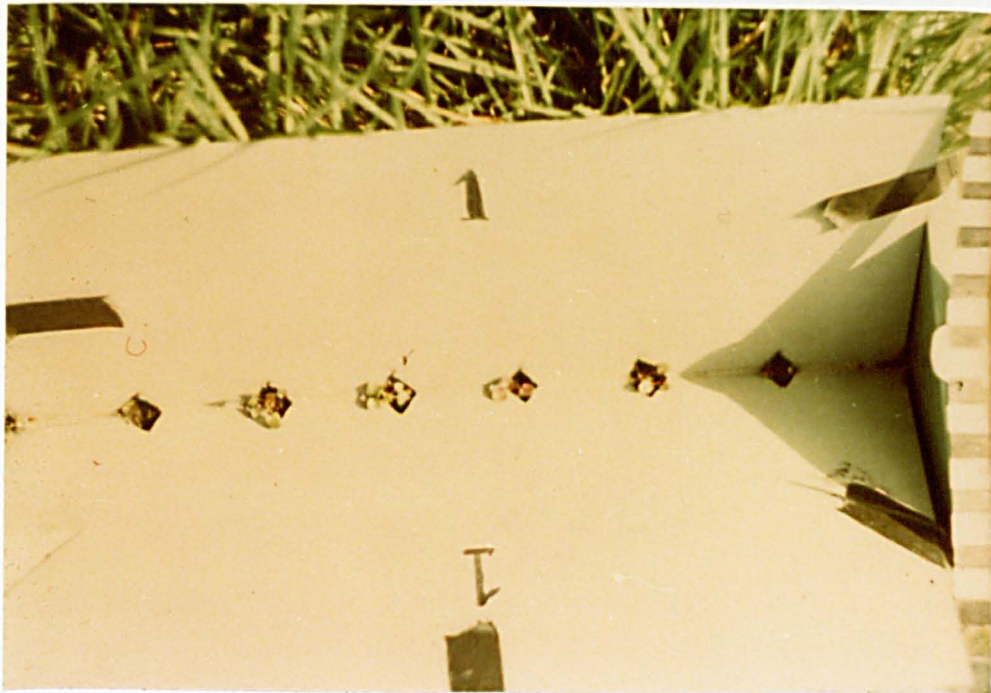


PLATE 7.4

Rumex acetosa seedlings grown for seven months in a monoculture of *Holcus lanatus*.

Table 7.3 Yield of *Rumex acetosa* grown for seven months in monocultures of various species. 95% confidence limits between brackets.

Species	Yield of shoot	
	dry weight (mg)	% of control
Control	95.14(±18.77)	-
<i>Acer pseudoplatanus</i>	23.82(± 7.25)*	25.04
<i>Agropyron repens</i>	16.13(± 4.47)*	16.95
<i>Agrostis tenuis</i>	32.45(±15.87)*	34.12
<i>Arrhenatherum elatius</i>	13.09(± 6.00)*	13.76
<i>Chamaenerion angustifolium</i>	5.14(± 3.33)*	5.40
<i>Festuca ovina</i>	2.84(± 1.12)*	2.99
<i>Festuca rubra</i>	1.82(± 0.64)*	1.91
<i>Holcus lanatus</i>	12.20(± 7.39)*	12.82
<i>Mercurialis perennis</i>	61.14(±19.20)	64.26
<i>Poa trivialis</i>	67.72(±31.74)	71.11
<i>Urtica dioica</i>	45.51(±33.44)	47.83

* yield significantly different from control (P<0.05)

and establishment. A further point to be noted here relates to the fact that the experimental conditions restricted the impact of the established plants to below-ground interactions occurring during the period October-April. In this connection it is interesting to observe that the strongest impact upon the yield of the seedlings occurred in association with *Festuca ovina* and *Festuca rubra*, two species which are evergreens and produce very dense root systems close to the ground surface. It seems possible therefore that despite the weekly additions of nutrient solution the inhibiting effect of these two grasses could have been due at least in part to depletion of the level of mineral nutrient supply to the seedlings. A similar hypothesis could be advanced to explain the suppression of seedling growth associated with other grasses such as *Agrostis tenuis*, *Arrhenatherum elatius* and *Agropyron repens*, all of which had formed dense root systems in the sand. It is interesting to observe, however, that a strong inhibition occurred in association with *Holcus lanatus*, a species which after an initial phase of vigorous growth was in a rather moribund condition by the time that the seedlings were introduced to the tubs. *Holcus lanatus* and several other species (e.g. *Agrostis tenuis*, *Festuca* spp.) causing severely reduced seedling growth had accumulated a considerable amount of litter and it is therefore quite possible that some inhibitory effects may have originated from decomposition products.

7.3 EXPERIMENT 2

In an attempt to detect the inhibitory effect, if any, of toxic substances released into the sand, a second experiment was conducted. In order to exclude the possibility of effects caused by mineral nutrient depletion, the procedure involved removal of samples of sand from the tubs and growth of seedlings of *Rumex acetosa* over a short period during which the supply of mineral nutrients was maintained at a high level.

7.3.1 Materials and Methods

In January 1976 samples of sand were removed from the containers used in Experiment 1. Samples were taken from the surface (0-6 cm) layer of sand in the controls and in the experimental monocultures of each species. The sample of sand from each tub was sieved to remove fragments of litter and roots and was used to fill three 2½ inch plastic pots. Seeds of *Rumex acetosa* were germinated on moist filter paper in plastic petri dishes in a growth-room providing 20°C by day and 15°C by night. Two seedlings were transferred to each pot which was allowed to stand in a separate saucer and received a 50 ml aliquot of Hewitt nutrient solution before planting. Seven days later one of the two seedlings was removed from each pot. Subsequently at intervals of six days each pot received 35 ml of Hewitt nutrient solution. Three days after each addition of mineral nutrients, 35 ml of distilled water was supplied to each pot. In addition to the studies involving *Rumex acetosa* further tests were conducted on sand samples removed from

the monocultures of *Agrostis tenuis*, *Holcus lanatus*, *Festuca rubra* and *Urtica dioica*. For each of these additional tests growth was examined in seedlings of the species which had occupied the sand. Seedlings of the four species were germinated in conditions identical to those used for *R. acetosa* and the controls were again based upon the growth of seedlings in sand from containers which had been unoccupied. The seedling bioassay was carried out in a growth-room experiencing 20°C by day and 15°C by night, relative humidities exceeding 60% and a daylength of 18 hours. After three weeks the plants were harvested, oven-dried and the roots and shoots were then weighed separately.

7.3.2 Results

In Table 7.4 the yields of *R. acetosa* grown on sand removed from beneath experimental monocultures of the eleven species are presented. Sand associated with five of the species induced statistically significant reductions in yield, compared with that of the controls. *Holcus lanatus* caused the greatest inhibition and Plate 7.5 illustrates the magnitude of this effect. *Acer pseudoplatanus*, *Agropyron repens*, *Agrostis tenuis* and *Festuca rubra* also caused major reductions in yield. Smaller depressions in yield were associated with sand from the remaining species but these were not statistically significant effects. Table 7.5 describes the extent of the autotoxic effects of sand removed from the four species, *A. tenuis*, *F. rubra*, *H. lanatus* and *U. dioica* and Plates 7.6 and 7.7 illustrate the appearance of the harvested seedlings of *A. tenuis* and *H. lanatus*. From these data there is strong evidence of autotoxicity in *H. lanatus* and *A. tenuis*.

Table 7.4 Yield of *Rumex acetosa* on sand removed from beneath experimental monocultures of various species. 95% confidence limits are in brackets.

Species	Shoot		Root	
	dry weight (mg)	% of control	dry weight (mg)	% of control
Control	48.92(± 3.59)		16.94(±1.58)	
<i>Acer pseudoplatanus</i>	32.08(± 3.48)*	66	10.45(±2.75)*	62
<i>Agropyron repens</i>	38.50(± 4.26)*	78	13.46(±1.28)*	79
<i>Agrostis tenuis</i>	29.70(± 9.87)*	60	12.10(±3.79)*	71
<i>Arrhenatherum elatius</i>	41.77(± 9.53)	85	13.40(±3.27)	79
<i>Chamaenerion angustifolium</i>	46.83(±10.21)	95	12.78(±4.35)	75
<i>Festuca ovina</i>	41.92(± 8.57)	85	13.95(±2.13)	82
<i>Festuca rubra</i>	33.84(± 6.71)*	69	10.88(±3.92)*	64
<i>Holcus lanatus</i>	15.75(± 9.15)*	32	5.18(±3.44)*	30
<i>Mercurialis perennis</i>	42.08(± 4.82)	86	14.76(±2.87)	87
<i>Poa trivialis</i>	41.62(± 7.51)	85	15.66(±4.01)	92
<i>Urtica dioica</i>	37.51(± 9.96)	76	11.68(±4.06)	68

* yield significantly different from control at (P<0.05)



PLATE 7.5

Seedlings of *Rumex acetosa* grown on sand removed from control (left) and from beneath established plants of *Holcus lanatus* (right).



PLATE 7.6

Seedlings of *Holcus lanatus* grown on sand removed from control (left) and from beneath established plants of *Holcus lanatus* (right).

Table 7.5 The results of autotoxicity tests in four herbaceous species

Species	Shoot dry weight (mg)			Root dry weight (mg)		
	control sand	sand + species	% of control	control sand	sand + species	% of control
<i>Agrostis tenuis</i>	10.67(±5.90)	3.60(±0.92)*	34	3.75(±2.14)	1.10(±0.32)*	29
<i>Festuca rubra</i>	21.90(±2.35)	18.27(±5.19)	83	6.08(±1.04)	4.70(±1.59)	77
<i>Holcus lanatus</i>	49.90(±9.12)	23.02(±8.72)*	46	18.46(±3.82)	8.80(±2.34)*	48
<i>Urtica dioica</i>	29.88(±8.25)	23.07(±9.98)	77	6.95(±2.09)	5.50(±2.87)	79

* yield significantly different ($P < 0.05$) from control.



PLATE 7.7

Seedlings of *Agrostis tenuis* grown on sand removed from control (left) and from beneath established plants of *A. tenuis* (right).

...ing the test and it seems most unlikely that the low yields obtained in certain treatments could be attributed to nutrient deficiency, the plants obtained from the rather similar control pots were rather similar to those from the other pots. The levels of nutrients in the control plants in the present experiments were likely to be superoptimal for the growth of *A. tenuis*.

The most recent toxicity suggested by the data is that associated with Helvetic Limestone and it is interesting to note that there is strong evidence of an autotoxic effect in this species. This result is in agreement with that of Newman and Kovacs (1975) and may be related to some observations (Grime, pers. communication) made during the preliminary stages of the experiment described in this chapter. During the first growing season, the monocultures of *A. tenuis* were observed to grow exceedingly rapidly but in subsequent years there was a progressive decline in both vegetative and reproductive vigour. It seems possible, therefore, that, even prior to

7.3.3 Discussion

The experiment revealed some major differences between the growth of *Rumex acetosa* upon sand removed from beneath established species and that observed in the controls. Since the control pots had received during the previous four years nutrient additions comparable to those supplied to monocultures we may suppose that in the absence of vegetation the control sand would accumulate mineral nutrients. Thus we must consider in particular whether the greater growth of seedlings on the control sand could be due to a higher nutrient level. However, in an attempt to avoid this effect, a considerable volume of full Hewitt nutrient solution was added to each pot during the test and it seems most unlikely that the low yields obtained in certain treatments could be attributed to nutrient deficiencies. Moreover, the results obtained from the rather similar experiments described in Chapter 9 suggest that the levels of nutrient supply to the control plants in the present experiments were likely to be superoptimal for the growth of *R. acetosa*.

The most potent toxicity suggested by the data is that associated with *Holcus lanatus* and it is interesting to note that there is strong evidence of an autotoxic effect in this species. This result is in agreement with that of Newman and Rovira (1975) and may be related to some observations (Grime, pers. communication) made during the preliminary stages of the experiment described in this chapter. During the first growing season, the monocultures of *H. lanatus* were observed to grow exceedingly rapidly but in subsequent years there was a progressive decline in both vegetative and reproductive vigour. It seems possible, therefore, that, even prior to

the seedling bioassay, effects due to autotoxicity were apparent. The possibility may be considered that autotoxicity was involved in the pattern of declining vigour in *H. lanatus* observed under natural conditions (Chapter 2).

In contrast with *H. lanatus*, several of the species which strongly inhibited the growth of *R. acetosa* in Experiment 1 (e.g. *Agropyron repens*, *Festuca ovina*, *Chamaenerion angustifolium*), produced relatively slight effects in Experiment 2. This strongly suggests that the inhibitory effect of such species in the first experiment was mainly due to depletion of mineral nutrients. By the same criteria, the results suggest that toxicity contributed to the inhibitory effects exerted by *Agrostis tenuis*, *Festuca rubra* and *Acer pseudoplatanus*. For each of the latter species there have been previous reports (Welbank 1963; Firth 1977; Sydes 1980) of inhibitory effects due to the release of toxic residues from litter.

CHAPTER 8

EXPERIMENTAL STUDIES OF THE INHIBITORY EFFECTS OF
AGROSTIS TENUIS, *HOLCUS LANATUS* AND
DESCHAMPSIA FLEXUOSA UPON *LOLIUM PERENNE*

8.1 INTRODUCTION

In circumstances where the yield of a plant is suppressed by the presence of neighbours, it is usually extremely difficult to distinguish between reductions in growth due to competition and those which might arise from the release of phytotoxins, i.e. allelopathic effects. A further complication is the possibility, supported by some evidence (Loche and Chouteau 1963; Armstrong *et al.* 1970; del Moral 1972; Lehman and Rice 1972) that the production of some potentially phytotoxic substances may be increased under conditions of mineral nutrient depletion such as might be expected to coincide with intense competition.

Earlier in this thesis (Chapters 4 and 7) attempts were made to detect the presence of residual toxic effects in soil samples removed from natural soils and sand cultures. In the investigations described in this chapter, pot experiments were conducted to study interactions directly and to attempt to distinguish between inhibitory effects due to resource depletion and those arising from the production of toxic substances. The three grasses selected for study were *Holcus lanatus*, *Agrostis tenuis* and *Deschampsia flexuosa*. For each of these species there is circumstantial evidence either from this study or from previous investigations (Welbank 1960, 1963; Jarvis 1964; Grant and Sallans 1964; Newman and Rovira 1975; Newbery 1976; Firth 1977; Newman and Miller 1977) of the capacity to produce toxic substances. The experiments involved the maintenance of clipped turf in split-pots and were carried out at two levels of mineral nutrient supply. Inhibitory effects of the grasses were measured in terms of the reduction in yield of *Lolium perenne*.

8.2 MATERIALS AND METHODS

The experiment involved sand cultures in 9 cm pots, each of which was divided into two equal parts by a vertical partition (Plate 8.1) which separated the shoots on either side and was sealed to the inner wall and floor of the pot with contact cement* to prevent roots penetrating from one side to the other. The base of each pot was perforated on both sides of the partition by several holes. Each pot was provided with a separate plastic saucer in which mixing of water and solutes draining from the sand on the two sides of the partition occurred. Through reabsorption from the drainage water collected in the saucer it was therefore possible for root exudates and soluble decomposition products to pass from one side of the partition to the other, under conditions in which the roots of different species growing on opposite sides were prevented from establishing direct contact with each other. The supply of mineral nutrients was maintained by application of equal volumes of nutrient solution to the sand surface on each side of the partition. In this way, an attempt was made to isolate the effect of root toxins from that due to competition for mineral nutrients. A possible weakness in this experimental design was related to the possibility of indirect competition for nutrients arising from differences in the ability of the species to draw on the nutrients accumulated in the drainage water. Particularly where there are substantial differences in the rate of growth of the grasses on the two sides of the partition, we may anticipate that reabsorption of water and mineral nutrients would also proceed at

* Petroleum mixture

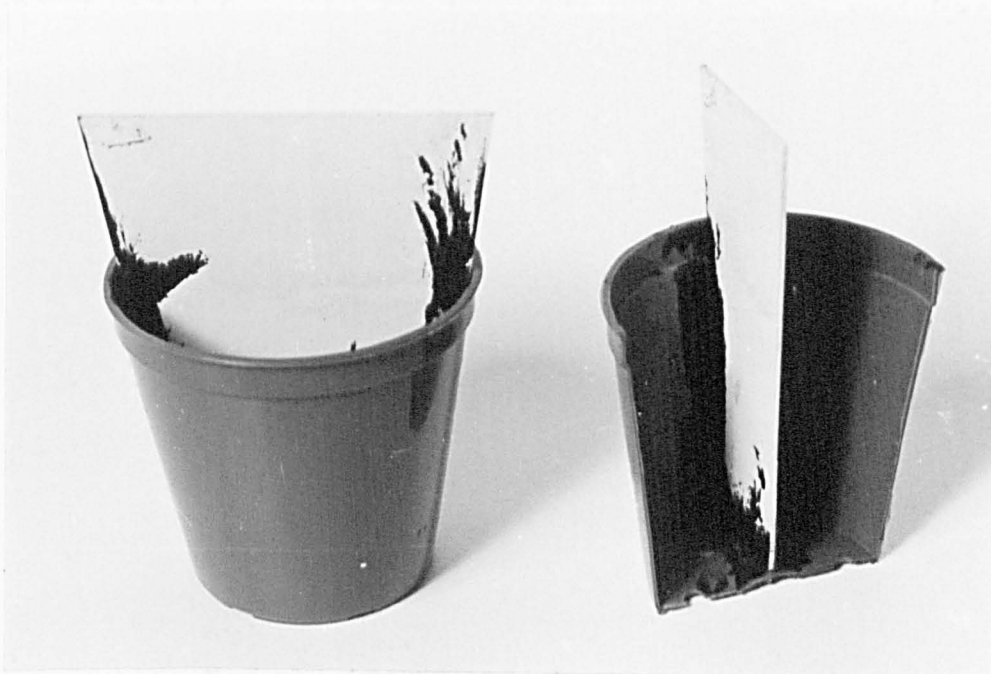


Plate 8.1

The divided pot used in the experiment. Left: side-view. Right: vertical section through the pot at right angles to the partition.

different rates. With this in mind two points are worth noting. Firstly, the level of watering was adjusted to allow drying out of the saucer. This effectively prevented extension of roots into the drainage water. Secondly, all plants were clipped at a standard height at regular intervals throughout the experiment, minimizing differences in canopy size and transpiration on the two sides of the partition. Despite these procedures it seems likely that the experiment allowed some differences in rates of mineral nutrient capture by different species and this possibility has been recognized in attempting to interpret the results of the experiment.

The experiment included three treatments which are illustrated in Figure 8.1. These were

- (a) monocultures each consisting of two plants of the same species situated on one side of the partition; the remaining half of the pot contained sand but no plants;
- (b) cultures in which two pairs of plants, each pair consisting of a different species, were planted on opposite sides of the partition;
- (c) mixtures consisting of two plants of each of the two species all situated on one side of the partition.

The experiment was commenced by planting one-week-old seedlings of *Lolium perenne* and uniform tillers of each of the three species. The plants in mixtures were arranged alternately and were planted 4 cm apart as shown in Figure 8.1(c). In the monocultures, the plants were arranged in the same pattern as that used in the mixture, i.e. the areas corresponding to those occupied by the other species in the mixture were allowed to remain bare. As explained in Mahmoud and Grime (1976) this arrangement permits a

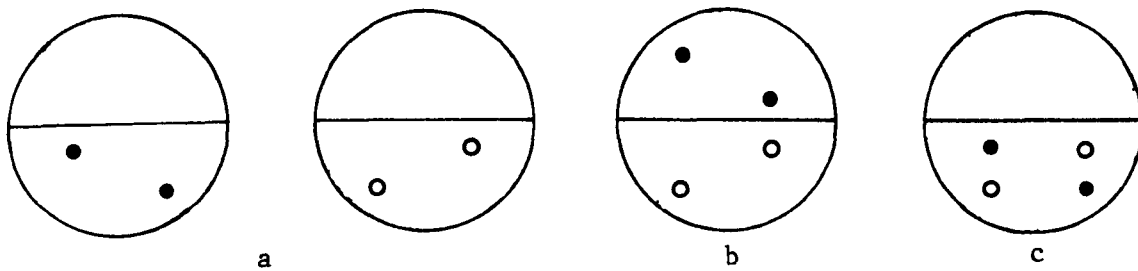


Figure 8.1

Pattern of planting of tillers of either *Agrostis tenuis*,
Deschampsia flexuosa or *Holcus lanatus* (o) and seedlings
of *Lolium perenne* (●).

- (a) monocultures
- (b) 'separate' culture
- (c) mixture

comparison between the yield of each species in the presence and in the absence of another. The experiment was carried out at two levels of mineral nutrient supply. The high nutrient treatment was applied as full Hewitt nutrient solution whilst the second involved 1/20 dilution of Hewitt nutrient solution. Each half of each pot was supplied every week with 50 ml of the appropriate nutrient solution, and distilled water was added as required to maintain the sand at field capacity. All additions were made from the top of the pot and run-off was allowed to collect in individual plastic saucers. The pots were arranged in seven randomized blocks in a growth-room with a temperature of 20°C by day and 15°C by night.

After twenty days, the foliage in each pot was clipped at 5 cm and the plant material removed from each pot was sorted into species, dried and weighed. Clipping was repeated at intervals of two weeks and the experiment was allowed to run for ninety-eight days. The number of tillers of each species in each pot was counted before the final harvest. The plants were then removed from the pots and the shoots and roots were separated, oven-dried and weighed.

8.3 RESULTS

The differences in nutrient level produced two distinct types of turf. The low nutrient level was associated with an 'open' structure whilst, at full nutrient level, a dense closed sward was produced.

Figure 8.2(a) describes the dry weights of clippings of *Holcus lanatus* from plants grown at full nutrient level in the three treatments. It is interesting to note that the yield of clippings reached a peak six weeks after the beginning of the experiment, then decreased continuously towards the end of the experiment.

At the low level of nutrient supply (Figure 8.2(b)) the dry weight fell sharply after the first clipping, then recovered to reach a maximum at the fifth clipping, followed by declining yield in the last two clippings.

At both levels of nutrient supply, the yield of clippings from *H. lanatus* did not show any statistically significant difference between treatments, a result which is in close agreement with the result of the experiment described in Chapters 6 and 9.

The yield of clippings recovered from *A. tenuis* (Figure 8.2(c),(d)) also did not show any significant effect of treatment at either level of nutrient supply. At full nutrient level the dry weight of clippings of *A. tenuis* at the beginning of the experiment was very low, compared with the yield from *H. lanatus* and in both treatments the dry weight of clippings in monoculture and mixtures continued to rise during later stages of the experiment.

Figure 9.2(e) describes the dry weight of clippings of *D. flexuosa* obtained in the full nutrient treatment. In comparison

Figure 8.2

Dry weights of clippings collected on the successive occasions plants grown at two nutrient levels in three different treatments. 95% confidence limits are included.

(a) *Holcus lanatus*

(b) *Holcus lanatus*

(c) *Agrostis tenuis*

(d) *Agrostis tenuis*

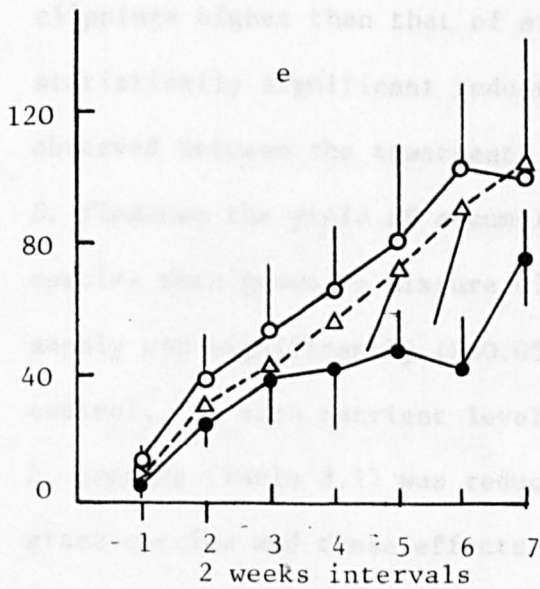
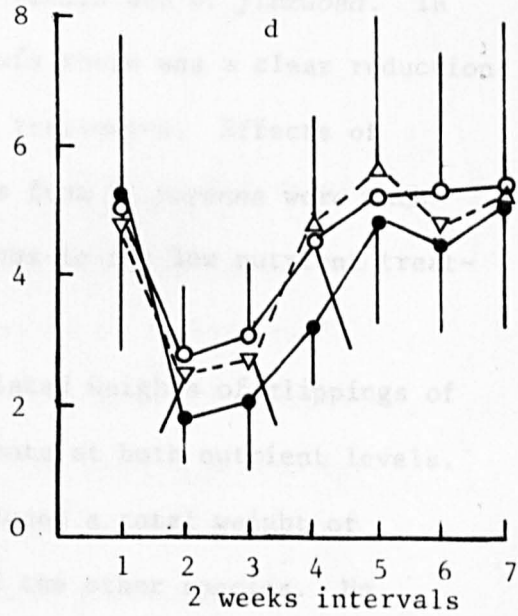
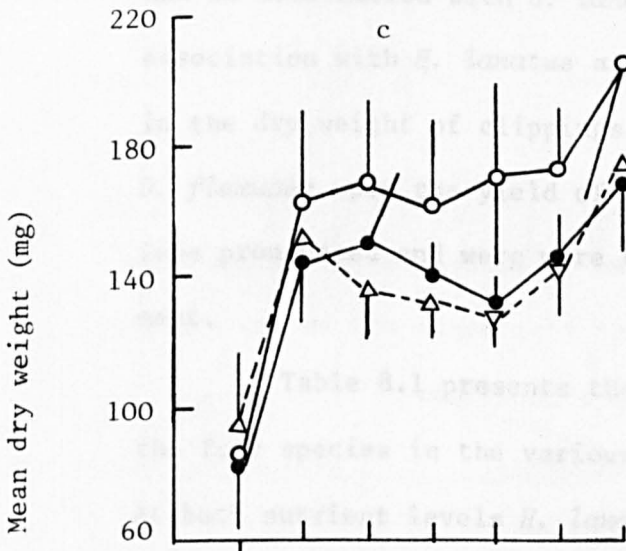
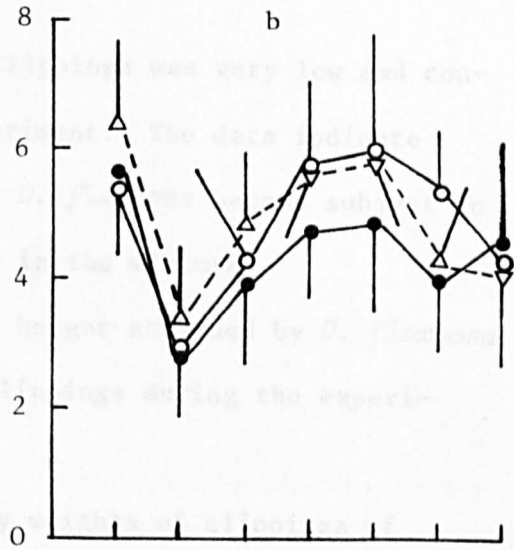
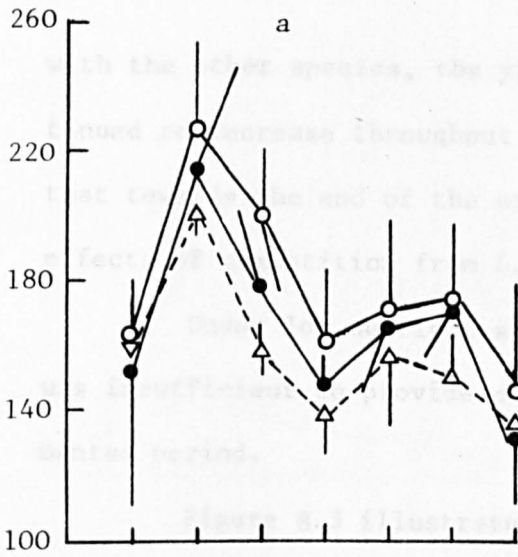
(e) *Deschampsia flexuosa*

(a), (c), (e) - high nutrient level

(b), (d) - low nutrient level

○—○ monoculture Δ---Δ roots and shoots separated

●—● mixture



with the other species, the yield of clippings was very low and continued to increase throughout the experiment. The data indicate that towards the end of the experiment *D. flexuosa* became subject to effects of competition from *L. perenne* in the mixture.

Under low nutrient supply the height attained by *D. flexuosa* was insufficient to provide adequate clippings during the experimental period.

Figure 8.3 illustrates the dry weights of clippings of *L. perenne* removed at high and low nutrient levels in monoculture and in combination with *H. lanatus*, *A. tenuis* and *D. flexuosa*. In association with *H. lanatus* and *A. tenuis* there was a clear reduction in the dry weight of clippings in both treatments. Effects of *D. flexuosa* upon the yield of clippings from *L. perenne* were much less pronounced and were more conspicuous in the low nutrient treatment.

Table 8.1 presents the accumulated weights of clippings of the four species in the various treatments at both nutrient levels. At both nutrient levels *H. lanatus* produced a total weight of clippings higher than that of either of the other species. No statistically significant reductions in yield of clippings were observed between the treatments in *H. lanatus* and *A. tenuis*. In *D. flexuosa* the yield of accumulated clippings produced by the species when grown in mixture with *L. perenne* at high nutrient supply was significantly ($P < 0.05$) lower than that recorded in the control. At both nutrient levels the total yield of clippings from *L. perenne* (Table 8.1) was reduced by the presence of the other grass species and these effects were statistically significant in the majority of treatments.

Figure 8.3

Dry weight of clippings collected on successive occasions from *Lolium perenne* in three different treatments and at two nutrient levels. 95% confidence limits are included.

(a) *Lolium perenne* with *Holcus lanatus*

(b) *Lolium perenne* with *Holcus lanatus*

(c) *Lolium perenne* with *Agrostis tenuis*

(d) *Lolium perenne* with *Agrostis tenuis*

(e) *Lolium perenne* with *Deschampsia flexuosa*

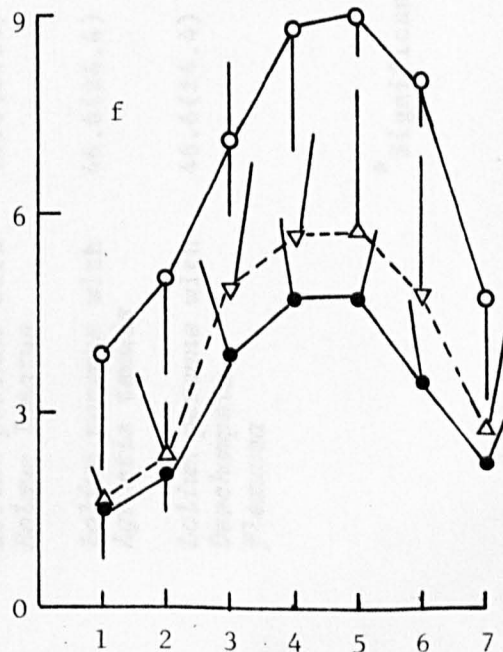
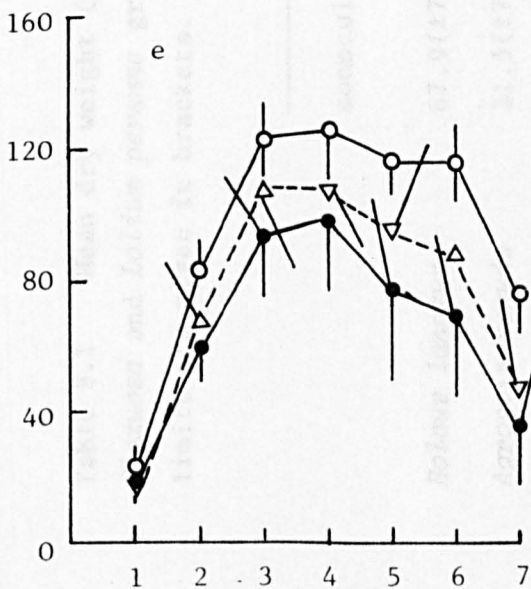
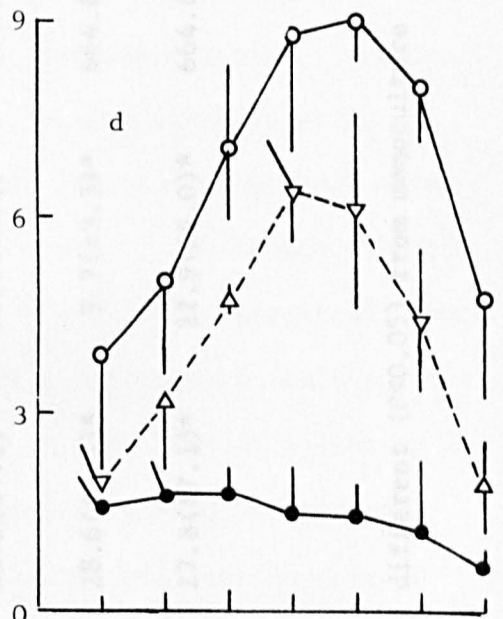
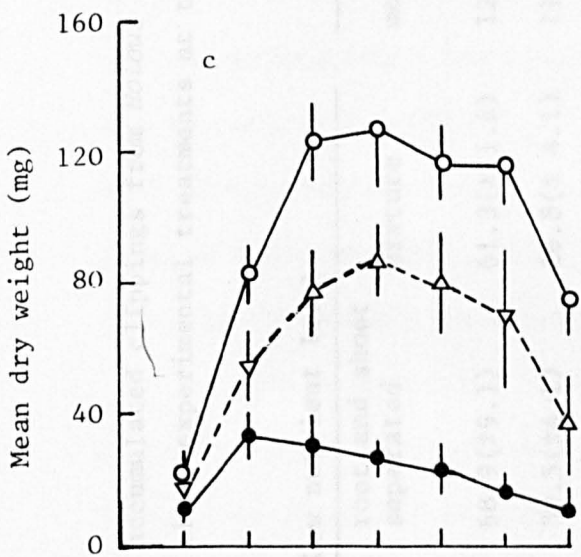
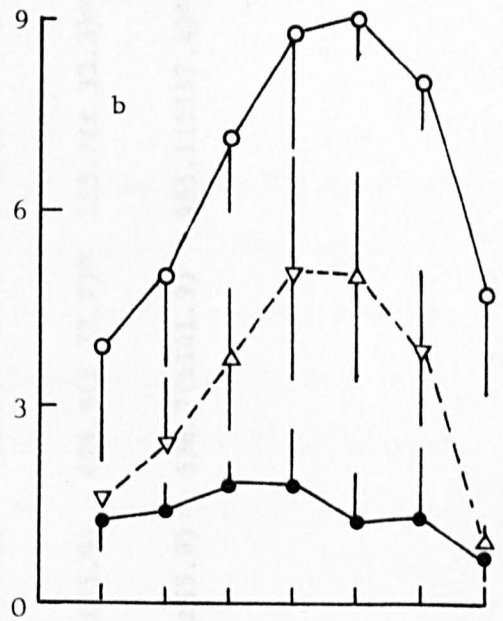
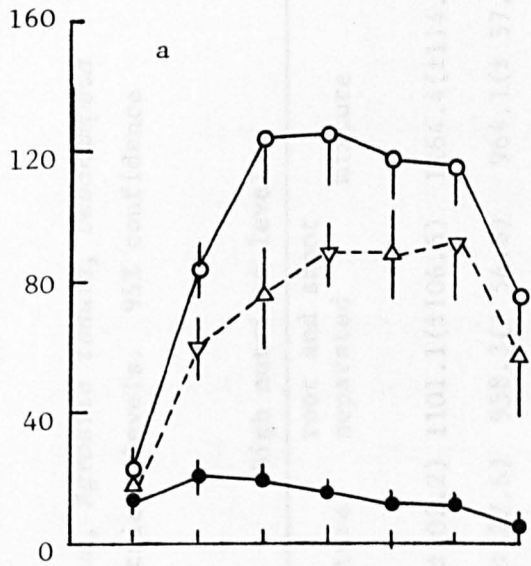
(f) *Lolium perenne* with *Deschampsia flexuosa*

(a), (c), (e) - high nutrient level

(b), (d), (f) - low nutrient level

○—○ monoculture Δ---Δ root and shoot separated

●—● mixture



2 weeks intervals

2 weeks intervals

Table 8.1 Mean dry weight (mg) of accumulated clippings from *Holcus lanatus*, *Agrostis tenuis*, *Deschampsia flexuosa* and *Lolium perenne* grown in three experimental treatments at two nutrient levels. 95% confidence limits are given in brackets.

	Low nutrient level			High nutrient level		
	monoculture	root and shoot separated	mixture	monoculture	root and shoot separated	mixture
<i>Holcus lanatus</i>	67.9(±7.5)	68.9(±9.1)	61.3(±11.6)	1235.5(±102.2)	1101.1(±106.5)	1164.4(±114.7)
<i>Agrostis tenuis</i>	31.5(±7.1)	30.5(±4.1)	26.8(± 4.1)	1128.9(±122.6)	958.3(± 54.8)	964.1(± 57.6)
<i>Deschampsia flexuosa</i>	-	-	-	454.4(±122.0)	402.6(±106.4)	281.8(± 62.2)
<i>Lolium perenne</i> with <i>Holcus lanatus</i>	46.6(±4.4)	22.5(±4.9)*	9.7(±3.1)	664.8(±55.9)	483.9(± 65.4)*	101.8(± 8.7)*
<i>Lolium perenne</i> with <i>Agrostis tenuis</i>	46.6(±4.4)	28.6(±3.5)*	9.7(±3.3)*	664.8(±55.9)	424.8(± 73.2)*	155.7(± 33.3)*
<i>Lolium perenne</i> with <i>Deschampsia flexuosa</i>	46.6(±4.4)	27.8(±7.1)*	22.9(±5.0)*	664.8(±55.9)	536.7(±141.9)	455.1(±112.4)*

* Significantly different (P<0.05) from monoculture

Tables 8.2-8.5 present further data recording the response of the four grasses to the experimental treatments. From these results it is apparent that the susceptibility of *L. perenne* to the presence of the other species, whether in mixture or within a divided pot, is reflected in shoot and root weight at final harvest, in total production (final harvest + total clippings) and in tiller number.

Two incidental features of these experimental results are worthy of note. In the first place it is apparent that *D. flexuosa* showed comparatively low responsiveness to mineral nutrient level and to combination with *L. perenne*. This finding is in close agreement with previous reports (Hackett 1965; Hunt 1970; Mahmoud 1973; Mahmoud and Grime 1974; Grime and Hunt 1975) of slow potential growth rate, low phenotypic plasticity and stress tolerance in this species.

The second point is related to the low rate of tillering in *L. perenne*. In the low nutrient treatment no plants progressed beyond the first tiller stage and even in the high nutrient treatment few tillers were produced by plants growing in mixture with either *H. lanatus* or *A. tenuis*.

Table 8.2 Mean dry weight (mg) of the shoots of *Holcus lanatus*, *Agrostis tenuis*, *Deschampsia flexuosa* and *Lolium perenne* at final harvest. 95% confidence limits are given in brackets.

	Low nutrient level			High nutrient level		
	monoculture	root and shoot separated	mixture	monoculture	root and shoot separated	mixture
<i>Holcus lanatus</i>	152.3(±32.0)	123.3(±12.8)	134.6(±13.9)	602(±171)	648(±120)	558(±116)
<i>Agrostis tenuis</i>	171.3(±20.6)	169.0(±34.0)	172.8(±19.5)	1298(±104)	998(±136)	969(±139)
<i>Deschampsia flexuosa</i>	199.3(±51.7)	204.5(±43.0)	211.8(±21.1)	907(±246)	870(±157)	892(±172)
<i>Lolium perenne</i> with <i>Holcus lanatus</i>	29.2(± 4.0)	17.7(± 3.3)*	12.3(± 3.1)*	260(±110)	138(± 48)	33(± 8)*
<i>Lolium perenne</i> with <i>Agrostis tenuis</i>	29.2(± 4.0)	20.6(± 2.6)*	12.5(± 3.2)*	260(±110)	121(± 43)	37(± 10)*
<i>Lolium perenne</i> with <i>Deschampsia flexuosa</i>	29.2(± 4.0)	19.5(± 5.1)*	19.5(± 4.5)*	260(±110)	186(± 45)	102(± 26)*

*Significantly different (P<0.05) from monoculture.

Table 8.3 Mean dry weight (mg) of the roots of *Holcus lanatus*, *Agrostis tenuis*, *Deschampsia flexuosa* and *Lolium perenne* at final harvest. 95% confidence limits are given in brackets.

	Low nutrient level			High nutrient level		
	monoculture	root and shoot separated	mixture	monoculture	root and shoot separated	mixture
<i>Holcus lanatus</i>	225.5(±58.2)	236.3(± 28.3)	149.3(± 6.1)	373.4(± 92.1)	409.0(± 81.5)	353.3(± 65.9)
<i>Agrostis tenuis</i>	334.0(±62.1)	326.5(± 41.6)	323.1(±96.1)	1089.6(±196.9)	1094.4(±208.4)	902.4(±242.6)
<i>Deschampsia flexuosa</i>	150.3(±84.1)	181.0(±102.3)	122.5(±55.5)	154.0(± 55.8)	198.3(± 46.4)	82.7(± 21.9)
<i>Lolium perenne</i> with <i>Holcus lanatus</i>	44.0(± 6.5)	26.1(± 9.0)*	13.0(± 6.8)*	200.9(± 47.1)	122.9(± 31.7)*	23.3(± 4.5)*
<i>Lolium perenne</i> with <i>Agrostis tenuis</i>	44.0(± 6.5)	24.8(± 4.7)*	13.3(± 8.3)*	200.9(± 47.1)	108.1(± 23.4)*	40.7(± 7.4)*
<i>Lolium perenne</i> with <i>Deschampsia flexuosa</i>	44.0(± 6.5)	23.6(± 6.3)	24.1(±17.0)*	200.9(± 47.1)	161.7(± 46.3)	89.9(± 41.8)*

* Significantly different (P<0.05) from monoculture.

Table 8.4 Mean dry weight (mg) of total plant material (clippings + final harvest) of *Holcus lanatus*, *Agrostis tenuis*, *Deschampsia flexuosa* and *Lolium perenne*. 95% confidence limits are given in brackets.

	Low nutrient level			High nutrient level		
	monoculture	root and shoot separated	mixture	monoculture	root and shoot separated	mixture
<i>Holcus lanatus</i>	445.1(± 50.3)	428.1(±38.1)	428.5(± 74.3)	2212(±232)	2172(±245)	2075(±233)
<i>Agrostis tenuis</i>	537.1(± 72.0)	525.3(±43.3)	496.8(± 62.4)	3516(±286)	3050(±324)	2837(±360)
<i>Deschampsia flexuosa</i>	350.6(±126.4)	328.0(±60.0)	385.5(±140.1)	1515(±355)	1470(±249)	1256(±190)
<i>Lolium perenne</i> with <i>Holcus lanatus</i>	122.6(± 9.6)	64.3(±17.8)*	35.3(± 12.4)*	1595(±281)	744(±121)*	156(± 25)*
<i>Lolium perenne</i> with <i>Agrostis tenuis</i>	122.6(± 9.6)	75.3(± 7.1)*	35.5(± 13.7)*	1595(±281)	667(±124)*	232(± 47)*
<i>Lolium perenne</i> with <i>Deschampsia flexuosa</i>	122.6(± 9.6)	70.7(±17.1)*	65.6(± 25.2)*	1595(±281)	885(±193)*	630(±149)*

* Significantly different (P<0.05) from monoculture.

Table 8.5 Mean number of tillers at final harvest of four species subjected to three treatments at two nutrient levels. 95% confidence limits are given in brackets.

	Low nutrient level			High nutrient level		
	monoculture	root and shoot separated	mixture	monoculture	root and shoot separated	mixture
<i>Holcus lanatus</i>	7.7(±1.6)	6.4(±0.9)	7.6(±0.9)	41.8(± 4.7)	41.7(± 5.4)	40.8(± 3.6)
<i>Agrostis tenuis</i>	11.6(±1.3)	9.6(±2.2)	10.0(±2.1)	85.4(±10.0)	74.4(±11.8)	70.7(±10.6)
<i>Deschampsia flexuosa</i>	15.1(±1.7)	15.9(±1.4)	15.1(±1.9)	76.4(± 1.9)	77.1(±15.3)	76.1(±14.4)
<i>Lolium perenne</i> with <i>Holcus lanatus</i>	1.0	1.0	1.0	14.0(± 1.9)	9.3(± 3.4)*	2.3(± 0.5)*
<i>Lolium perenne</i> with <i>Agrostis tenuis</i>	1.0	1.0	1.0	14.0(± 1.9)	8.3(± 1.9)*	3.7(± 1.1)*
<i>Lolium perenne</i> with <i>Deschampsia flexuosa</i>	1.0	1.0	1.0	14.0(± 1.9)	11.4(± 3.4)	7.1(± 1.6)*

*Significantly different (P<0.05) from monoculture

8.4 DISCUSSION

From the results described in this chapter there is evidence that the four species investigated responded differently to the various experimental treatments. Although the main objective of the experiment was to study interactions between species it is important to take account of the fact that in the experiment the grasses used were subjected to regular clipping. It is necessary, therefore, to consider how far differences in response were conditioned by defoliation. Both Milton (1940) and Mahmoud (1973) have shown, for example, that there are profound differences between species and varieties of grasses with respect to their response to defoliation and we may expect that such differences will strongly influence the competitive abilities of grasses growing in mixture in a grazed or mown sward.

At the high nutrient level (Plate 8.2) the main reaction of *Lolium perenne* to clipping appeared to consist of a rapid and almost vertical regrowth of the damaged leaves, whereas, under the same treatment, *Agrostis tenuis* responded by producing a large number of small tillers and leaves, many of which were not projected into the clipped stratum but produced tillers and stolons capable either of lateral spread over bare sand or of infiltrating areas of the pot which were already occupied by other species. In comparison with *Lolium perenne*, both *Agrostis tenuis* and *Holcus lanatus* formed a denser canopy close to the sand surface and situated to a much greater extent below the level subject to clipping.

In the treatment involving separation of the roots and shoots, *H. lanatus*, *A. tenuis* and *D. flexuosa* suppressed the yield of



PLATE 8.2

Response of four species to clipping every two weeks

at 5 cm. Left to right: *Lolium perenne* var.S23,

Holcus lanatus, *Agrostis tenuis* and *Deschampsia flexuosa*.

Photograph taken 7 days after clipping.

L. perenne and this effect was observed at both nutrient levels. The three species differed in the extent to which they suppressed the yield of *L. perenne* and the strongest effect was recorded in treatments including the two faster-growing species. This effect could be related either to the production of phytotoxins or to the greater capacity of *H. lanatus* and *A. tenuis* to absorb mineral nutrients by direct absorption from the sand or following their reabsorption from the saucer. As mentioned on page 81, the design of the experiment does not appear to allow an adequate means of discrimination between effects of competition for mineral nutrients and effects of toxic substances. The possibility remains, however, that more satisfactory evidence can be obtained from divided-pot experiments by conducting additional measurements to detect the levels of mineral nutrient depletion and/or toxin accumulation on the two sides of the partition. This possibility is examined in the next chapter.

It is interesting to note that despite its comparatively low yield *D. flexuosa* reduced the yield of *L. perenne* to a considerable extent at both high and low levels of nutrient supply. In one respect, however, this inhibition differed from that caused by *A. tenuis* and *H. lanatus* in that reduction in the yield of *L. perenne* in the treatment separating roots and shoots was almost comparable in extent to that occurring in the mixture. The failure of *D. flexuosa* to exert a stronger effect in mixture could be due to the slow rate of leaf canopy development in this species. An alternative explanation is that the effect of *D. flexuosa* was primarily due to the release of toxic substances able to effectively reach both sides of the pot.

CHAPTER 9

INTERACTIONS BETWEEN
HOLCUS LANATUS AND *FESTUCA RUBRA*

9.1 INTRODUCTION

The split-pot experiments described in Chapter 8 did not effectively distinguish between possible effects of mineral nutrient depletion and those due to the production of toxic substances. In the investigation described in this chapter an attempt was made to improve the technique as follows:

- (1) The investigation was carried out in two stages. In the first stage (Experiment 1) measurement was made of the inhibitory effect exerted by *Holcus lanatus* and *Festuca rubra* when the two species were combined in split-pot treatments of the same type as those described in Chapter 8. The second stage (Experiment 2) was designed to attempt to detect the presence of toxic substances in the sand cultures used in the first experiment and involved growing seedlings of *Rumex acetosa* on samples of sand removed from the split-pots.
- (2) The experiment was maintained for a longer period in order to increase the chance of toxin accumulation.
- (3) Two additional control treatments were employed in the tests for toxic effects. The first involved sand from split-pots without plants but maintained with comparable nutrient additions throughout the experimental period. In the second control treatment tests were conducted on fresh sand supplied with nutrient solution at the commencement of Experiment 2.

9.2 EXPERIMENT 1

9.2.1 Materials and Methods

The experiment involved sand cultures in 12.5 cm plastic pots each of which was divided into two equal parts by a plastic partition. The experimental procedure was identical with that used in the experiments described in Chapter 8 except that an additional control treatment was included as described under (3) in the Introduction. The experiment was commenced by planting ten-day-old seedlings of two species using the sowing patterns illustrated in Figure 8.1.

The experiment was conducted in a growth-room with a temperature of 20°C by day and 15°C by night and a daylength of 18 hours. Each pot received 200 ml of Hewitt nutrient solution every week, supplemented by quantities of distilled water sufficient to maintain the sand at field capacity. In each pot the run-off was collected in a plastic saucer and was allowed to become reabsorbed into the sand. Commencing after three weeks, the plants were clipped at 5 cm from the sand surface at intervals of two weeks. The number of tillers per pot was counted before each clipping.

The clippings collected from each pot at each cut were sorted into species, dried, and weighed. The plants were harvested after 27 weeks and the shoots and roots were oven-dried and weighed separately.

Sand analysis

At the end of the experiment the sand was removed separately from the two halves of each pot in each experimental treatment and

analyses for mineral nutrients (Ca, K, Na, Mg, P, ammonium-N and nitrate-N) were carried out using the methods applied in Chapter 3.

9.2.2 Results

Tiller number

Figure 9.1 describes variation with time and treatment in tiller number of *H. lanatus*. There were no significant differences between treatments, and at the end of the experiment the number of tillers of *H. lanatus* was four times that of *F. rubra* (Figure 9.1b). Subsequent to the fifth clipping the tiller number produced by *F. rubra* in monoculture was higher than that measured in the two remaining treatments, although the difference was statistically significant ($P < 0.05$) only with respect to the species mixture. Representative plants of *F. rubra* photographed at the end of the experiment are illustrated in Plate 9.1.

Dry weight of clippings and total yield

Figure 9.2a records the dry weight of clippings recovered from *H. lanatus* and shows that there were no significant differences between the treatments. In comparison with the results obtained in monoculture, the dry weight of clippings in plants of *F. rubra* in mixture with *H. lanatus* or separated from *H. lanatus* by root and shoot partitions showed clear and statistically significant reductions ($P < 0.05$).

From the results presented in Table 9.1 it is apparent that there were no significant effects of treatment for *H. lanatus* in total dry weight of clippings and in dry weight of shoot and root at final harvest.

analyses for mineral nutrients (Ca, K, Na, Mg, P, ammonium-N and nitrate-N) were carried out using the methods applied in Chapter 3.

9.2.3 Results

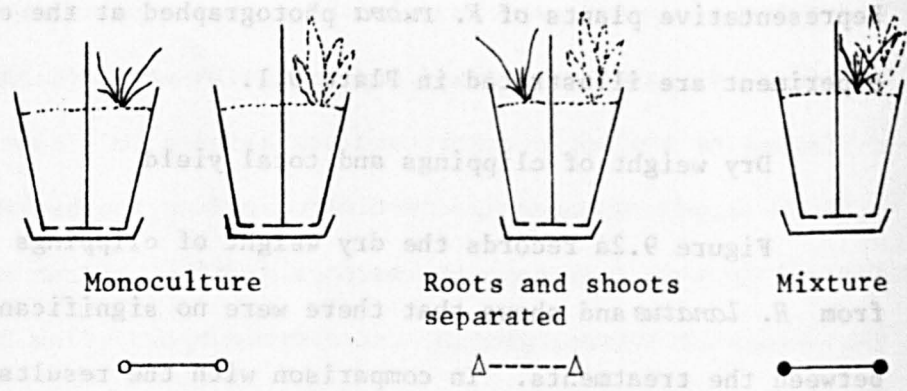
Tiller number

Figure 9.1 describes variations with time and treatment in tiller number of *H. lanatus*. There were no significant differences between treatments, and at the end of the experiment the number of tillers of *H. lanatus* was four times that of *F. rubra* (9.1b).

Figure 9.1

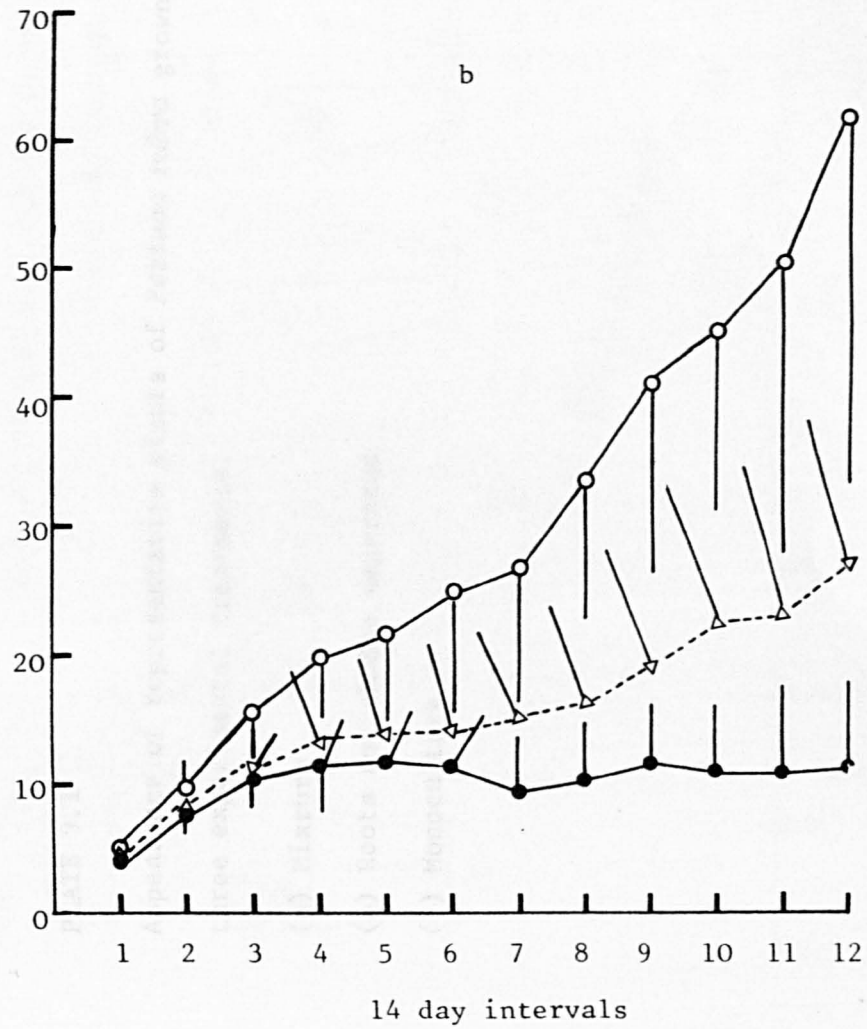
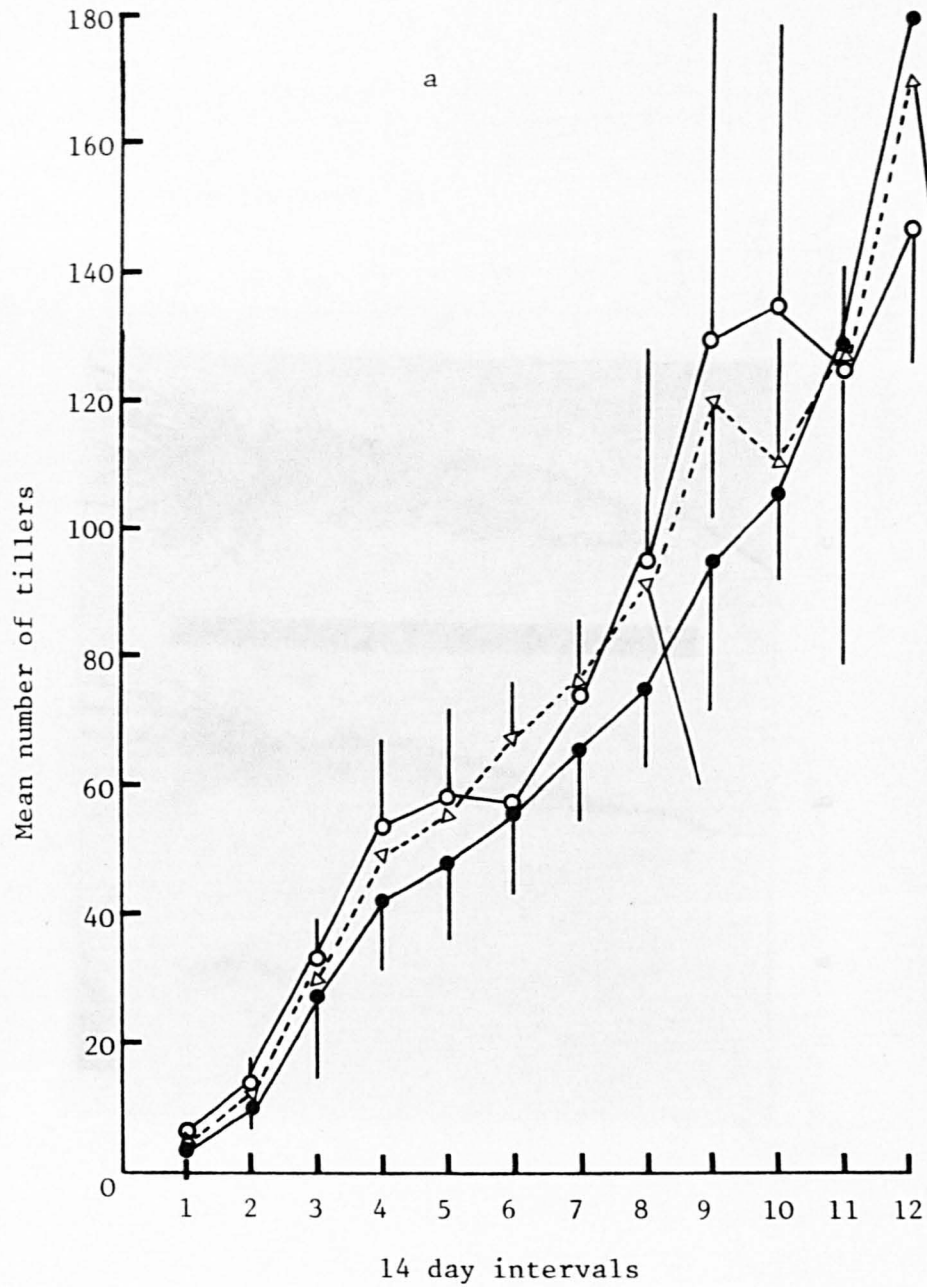
Tiller production in plants of *Holcus lanatus* (a) and *Festuca rubra* (b) in four different treatments.

95% confidence are included.



From the results presented in Table 9.1 it is apparent that there were no significant effects of treatment for *H. lanatus* in monoculture, the dry weight of clippings in plants of *F. rubra* in mixture with *H. lanatus* or separated from *H. lanatus* by root and shoot partitions showed clear and statistically significant reductions ($P < 0.05$).

From the results presented in Table 9.1 it is apparent that there were no significant effects of treatment for *H. lanatus* in monoculture, the dry weight of clippings in plants of *F. rubra* in mixture with *H. lanatus* or separated from *H. lanatus* by root and shoot partitions showed clear and statistically significant reductions ($P < 0.05$).



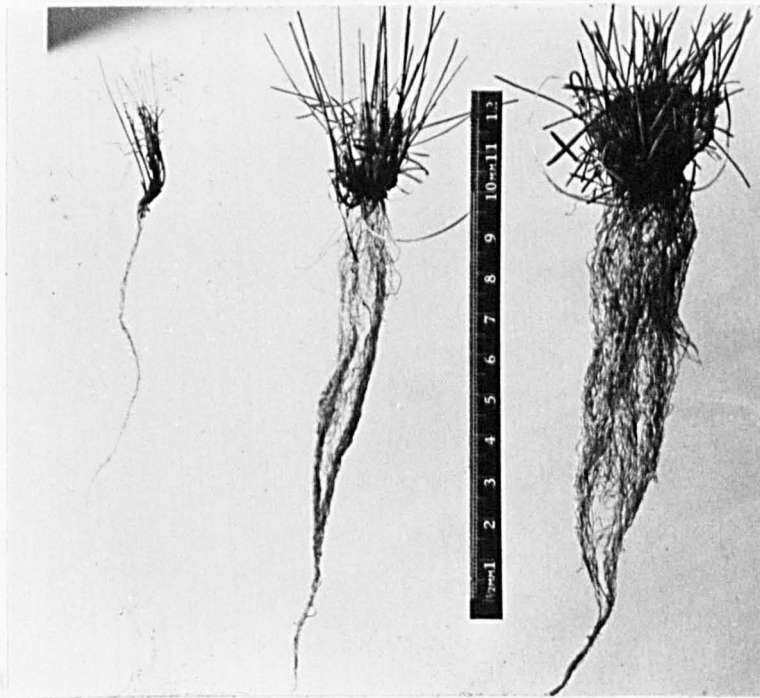


PLATE 9.1

Appearance of representative plants of *Festuca rubra* grown in three experimental treatments:

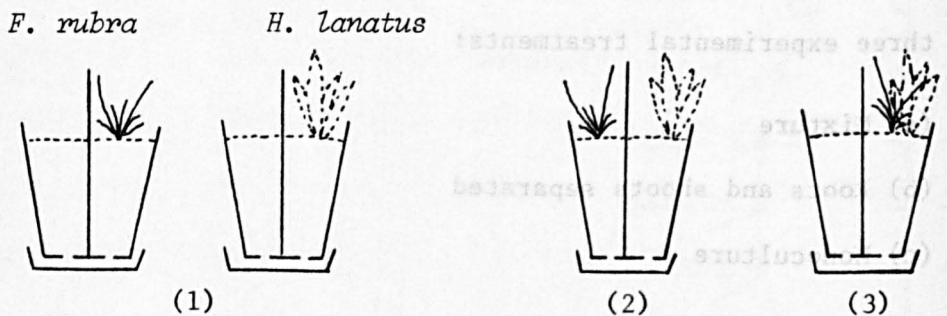
- (a) Mixture
- (b) Roots and shoots separated
- (c) Monoculture

Figure 9.2

Dry weights of clippings collected at successive cuts from plants of *Holcus lanatus* (a) and *Festuca rubra* (b) in four different treatments.

95% confidence limits are included.

- (1) Monoculture (o—o)
- (2) Roots and shoots separated (Δ ----- Δ)
- (3) Mixture (●—●)



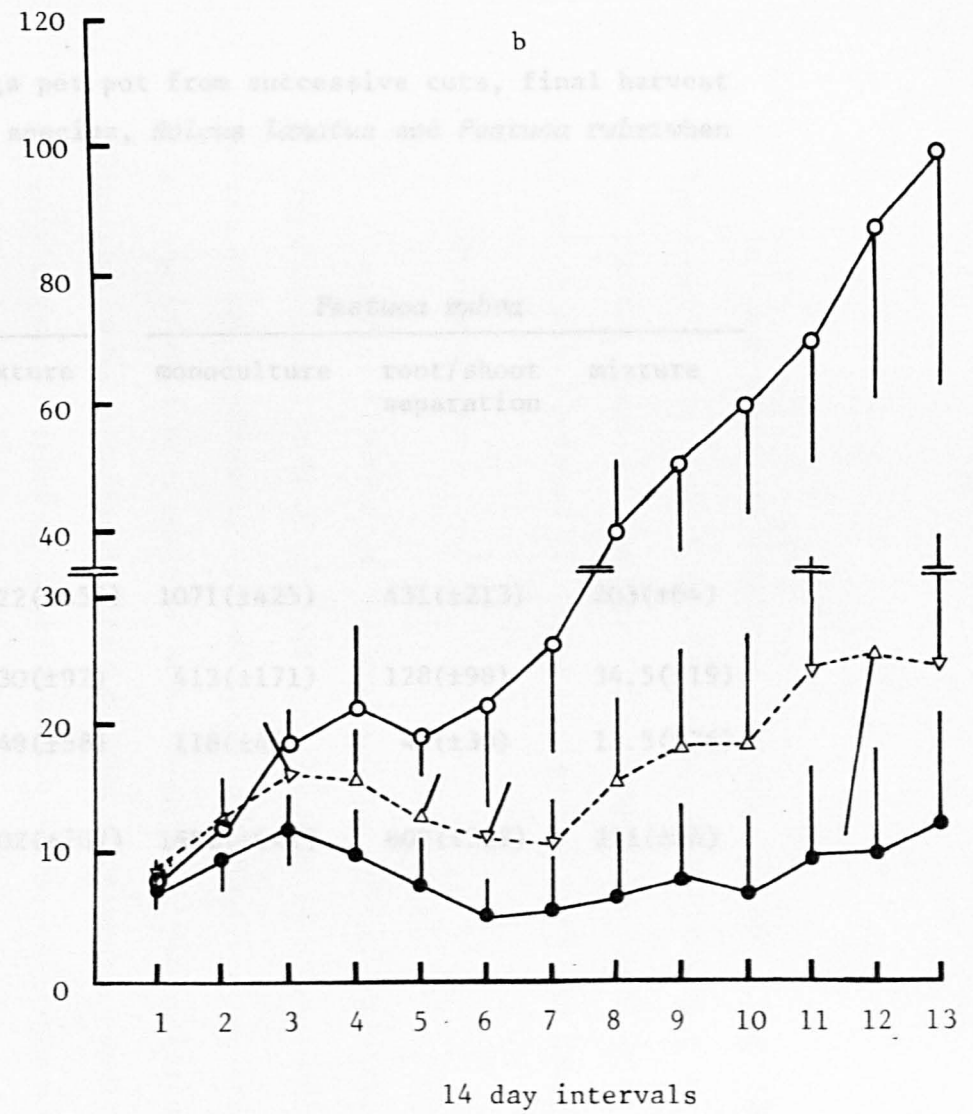
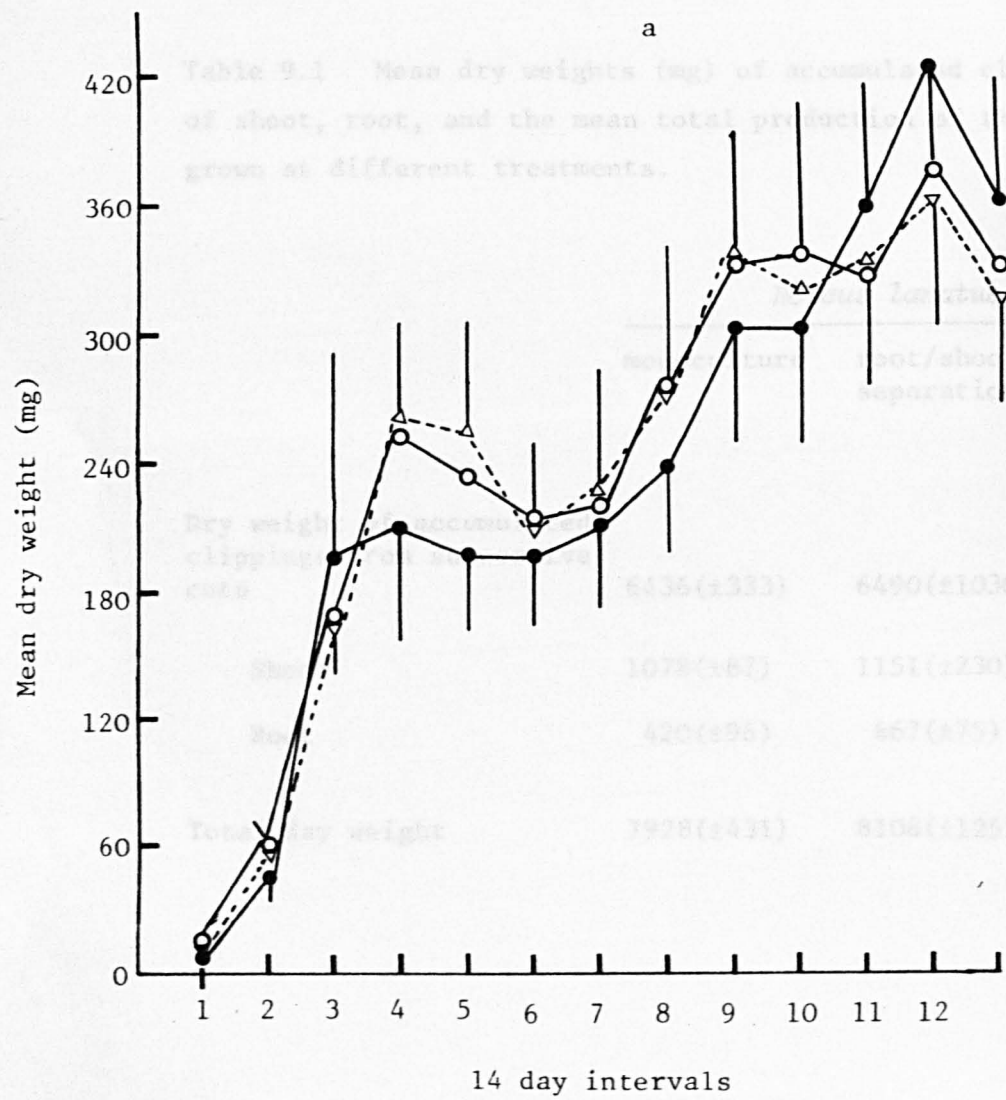


Table 9.1 Mean dry weights (mg) of accumulated clippings per pot from successive cuts, final harvest of shoot, root, and the mean total production of the two species, *Holcus lanatus* and *Festuca rubra* when grown at different treatments.

	<i>Holcus lanatus</i>			<i>Festuca rubra</i>		
	monoculture	root/shoot separation	mixture	monoculture	root/shoot separation	mixture
Dry weight of accumulated clippings from successive cuts	6436(±333)	6490(±1030)	6322(±455)	1071(±425)	431(±213)	203(±64)
Shoot	1078(±87)	1151(±230)	1130(±92)	413(±171)	128(±98)	34.5(±19)
Root	420(±96)	467(±75)	449(±58)	118(±48)	43(±30)	13.5(±76)
Total dry weight	7928(±431)	8108(±1251)	7902(±707)	1602(±641)	602(±328)	251(±86)

In *F. rubra* the dry weight of clippings showed a significant ($P < 0.05$) decline in mixture and in the treatment involving partitioning of the two species. Similar differences in *F. rubra* were detected at final harvest in the dry weight of shoot and roots.

Sand analyses

Table 9.2 illustrated the analyses for mineral nutrients in the sand from each side of the pot partition in each treatment at the end of the experiment. In sand from the control pots (Treatment A) all the constituents analysed were recorded at concentrations higher than those associated with the other treatments. Depletion was more marked in calcium, phosphorus and nitrate-nitrogen and tended to occur to a greater extent in sand exploited by *H. lanatus*.

Table 9.2 Estimation of the extractable levels ($\mu\text{g g}^{-1}$) of seven soluble constituents on each side of the pot at different treatments. 95% confidence limits are included.

Treatments	K	Na	Ca	Mg	P	Ammonium-N	Nitrate-N
Control (A)	70.0±13.2	65.3± 2.8	526± 14	276±14	8.8±1.0	25.8±6.3	30.4± 7.1
<i>Festuca rubra</i> (B ₁)	64.0±45.5	55.3±24.5	356± 52*	222±40*	6.7±2.7	21.8±6.5	27.8± 4.7
<i>Festuca rubra</i> (B ₂)	46.6±28.2	60.6±11.5	376±100*	210±24*	7.1±5.5	20.5±2.0	16.3± 8.7
<i>Holcus lanatus</i> (C ₁)	55.3±41.6	56.3± 7.5	316±114*	220±24*	4.0±2.2*	22.8±7.2	17.7± 7.2*
<i>Holcus lanatus</i> (C ₂)	62.0± 5.0	62.0± 5.0	463± 38*	206±23*	3.8±2.5*	25.6±10.0	28.9±14.0*
(D ₁)	50.0± 8.6*	58.3± 5.7	320± 49*	226±28*	2.5±3.6*	17.9±2.2	13.5± 2.0
(D ₂)	48.0± 8.6	60.0±13.1	483± 37*	236±24*	2.8±2.6*	18.2±6.9	21.9± 5.3
(E ₁)	50.0±25.0	54.0± 8.6	410± 65*	230±24*	3.0±0.1*	14.0±6.9	13.0± 0.9*
(E ₂)	50.0± 8.6	59.3± 2.8	473± 14*	223±14*	3.3±1.0*	18.6±5.3	19.6± 6.9



A
Control



B
F. rubra



C
H. lanatus



D
Roots and shoots
separated



E
Mixture

* significantly different ($P < 0.05$) from Control (A)

9.3 EXPERIMENT 2

9.3.1 Materials and Methods

The sand from each half-pot in each treatment in Experiment 1 was removed and used to fill two 6.5 cm plastic pots and in addition 12 control pots were filled with clean sand. Fifty ml of Hewitt nutrient solution was added to the sand surface in each pot and a one-week-old seedling of *R. acetosa* was planted in each pot. In order to minimize leaching and to prevent desiccation, the run-off from each pot was allowed to collect in separate saucers. At intervals of 6 days, further additions of Hewitt solution, each of 35 ml, were made to each pot and distilled water was added regularly to each pot to keep the sand at field capacity. Each treatment was therefore represented by 14 seedlings (two replicates from each of seven half-pots). The pots were arranged in seven random blocks in a growth-room providing a temperature of 20°C by day and 15°C by night with a day length of 18 hours.

After three weeks the plants were harvested and the roots and shoots were oven-dried and weighed separately.

9.3.2 Results

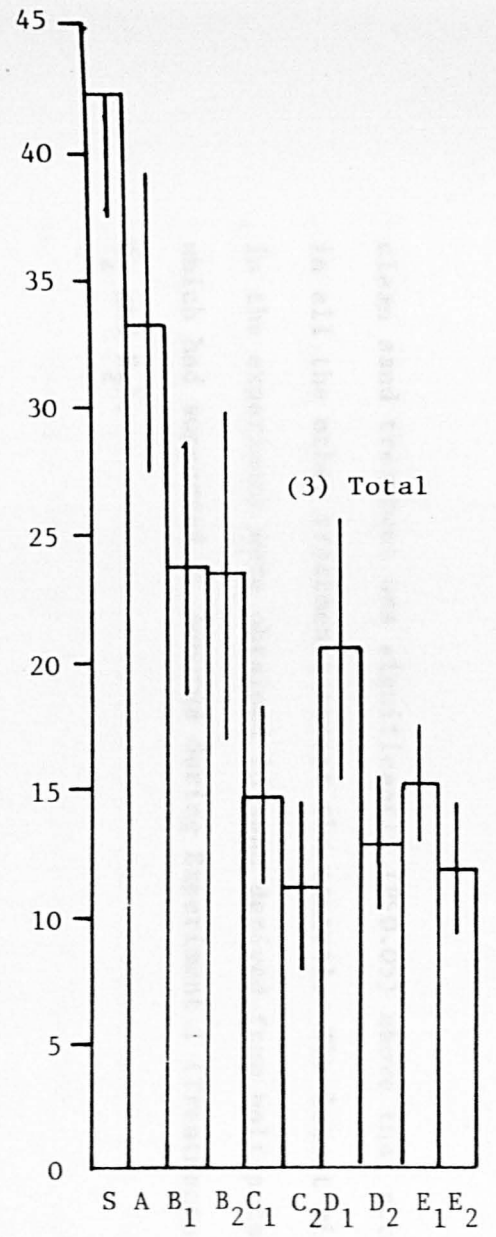
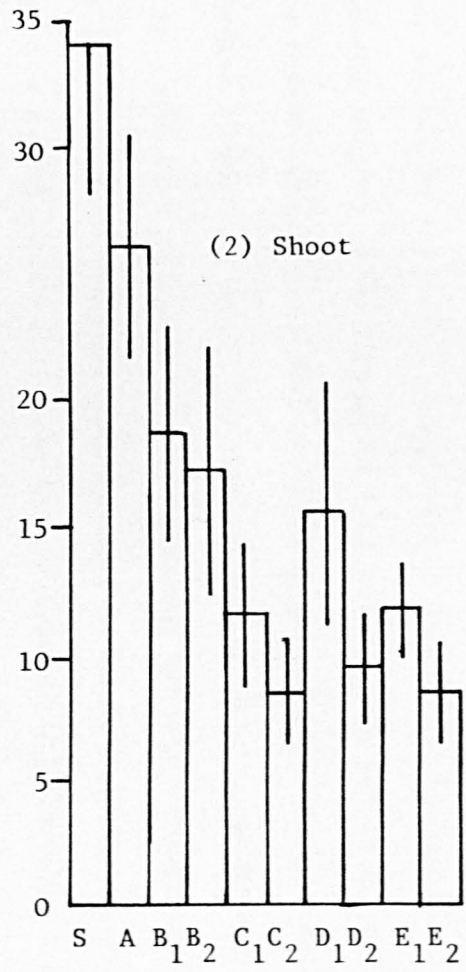
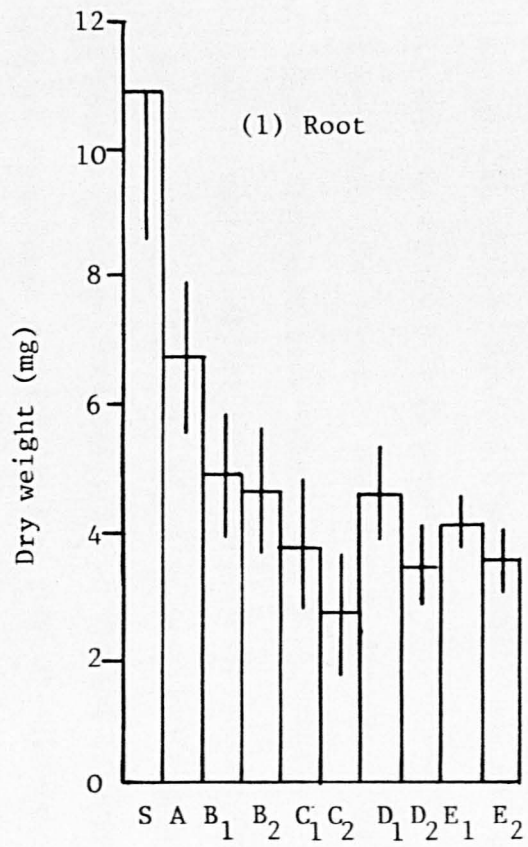
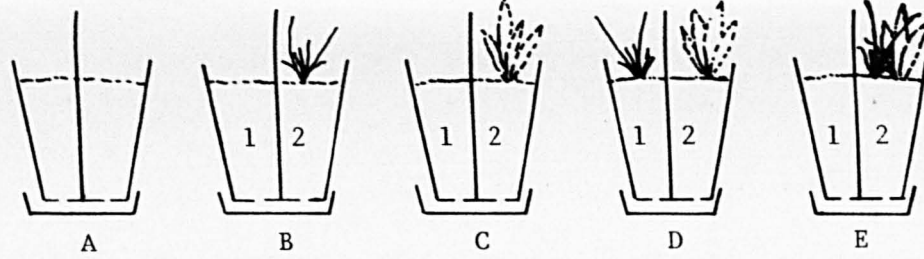
Figure 9.3 records the yield of *R. acetosa* in the two control treatments and in sand removed from the various treatments in Experiment 1. The yields of root, shoot, and the total dry weight of *R. acetosa* in the controls were higher than those from all the other treatments. The yield of *R. acetosa* grown on sand maintained in pots containing no plants (Treatment A) was only marginally below that of plants grown in clean sand. The yield of *R. acetosa* in the

Figure 9.3

The yield of *Rumex acetosa* grown on sand removed from Experiment 1 at the end of the experiment (S = washed sand).

95% confidence limits are included.

- (A) Control
- (B) Monoculture of *Festuca rubra*
- (C) Monoculture of *Holcus lanatus*
- (D) Roots and shoots separated
- (E) Mixture



clean sand treatment was significantly ($P < 0.05$) above that attained in all the other treatments except the control. The lowest yields in the experiment were obtained in sand derived from half-pots which had supported *H. lanatus* during Experiment 1 (Treatments C₂, D₂ and E₂).

9.4 DISCUSSION

From the data in Table 9.2 it is clear that during Experiment 1 differences in nutrient levels developed on opposite sides of the partition in those treatments which involved *H. lanatus*. In general higher nutrient levels occurred at the end of the experiment in the sand which had been exploited by *H. lanatus*. In view of the rapid growth by *H. lanatus* relatively higher rates of transpiration might be expected, allowing this species to obtain a major share of the run-off (including mineral nutrients) by reabsorption from the saucer. From the analytical results, it would appear that this effect occurred and was sufficient to offset the high rates of nutrient absorption by *H. lanatus*. From the results of Experiment 1 it is clear that the yield of *F. rubra* was severely inhibited by the presence of *H. lanatus* and this effect was observed not only when the two species were grown in mixture but also when they were prevented from direct contact by means of the partition. There are two possible explanations for this effect. The first is that *H. lanatus* through its higher rates of transpiration and nutrient absorption was able to obtain a high proportion of the mineral nutrients draining into the saucer. The second relates to the possibility that toxic substances were released by *H. lanatus*.

Evidence in support of the latter hypothesis can be deduced from data in Figure 9.3 recording the yield of *R. acetosa* seedlings grown on sand samples removed from the pots at the end of Experiment 1. These results show large and significant ($P < 0.05$) reductions in all treatments as compared with clean sand (Treatment S) and the control treatment (Treatment A) and these reductions are most

pronounced in treatments involving contact with *H. lanatus*. During the seedling growth experiment, all the pots were supplied with the same volume of nutrient solution and the high yield of the plants grown on clean sand (Treatment S) is proof that this rate of supply was adequate to sustain high rates of growth. Thus it would appear that nutrient depletion cannot explain the low yield of the *R. acetosa* seedlings on sand previously exploited by *H. lanatus*. Moreover the analytical data in Table 9.2 show that the levels of mineral nutrients in sand associated with *H. lanatus* tended to be relatively higher than in sand supporting *F. rubra*. This might be interpreted as evidence that mineral nutrient toxicity occurred in *R. acetosa* seedlings grown on sand exploited by *H. lanatus*. However, this possibility is excluded by the fact that considerably greater yields were obtained on sand from the control treatment (Treatment A) which contained higher levels in all the nutrients analysed. On this basis, therefore, the experiment appears to provide strong circumstantial evidence of the presence of an inhibitory influence of *H. lanatus* additional to that attributable to competition for light, water or mineral nutrients.

CHAPTER 10

THE EFFECT OF LEACHATES FROM THE RHIZOSPHERE OF ESTABLISHED
PLANTS OF *HOLCUS LANATUS* UPON THE GROWTH OF SEEDLINGS
OF *HOLCUS LANATUS* AND *RUMEX ACETOSA*

10.1 INTRODUCTION

The experiment described in this chapter was designed to investigate further the presence of phytotoxic substances in the rhizosphere of *Holcus lanatus*. Earlier in this thesis (Chapters 4, 5, 7 and 9) evidence was obtained, from laboratory and field investigations, of inhibitory effects upon seedling growth resulting from previous occupation of sand or soil by the roots of *H. lanatus*. Although these experiments involved the addition of mineral nutrient solutions to the soils and sand cultures used in the bioassays and were supported by chemical analyses, the possibility remains that the effect of *H. lanatus* could arise, at least in part, from reductions in mineral nutrient supply resulting from high rates of nutrient absorption by *H. lanatus*. The procedure adopted in the experiment to be described here was designed specifically to take account of the possible effect of mineral nutrient depletion by *H. lanatus*.

A further consideration in the experimental design was that of autotoxicity. Many workers have reported the occurrence of phytotoxic effects active against the plants which produce them (Milton 1943; Curtis and Cottam 1950; Voight 1959; Webb, Tracey and Haydock 1967; Hanes 1971; Tinnin and Muller 1972; Newman and Rovira 1975) and already in two parts of this investigation (Chapters 2 and 7) evidence has been found of autotoxicity in *H. lanatus*. This evidence is a complication in any attempt to attach an ecological significance to the phenomenon of toxin production by *H. lanatus* and suggests the need to determine whether *H. lanatus* is more or less susceptible to its own toxins than the species with which it occurs in the field.

With this in mind, therefore, an attempt was made to compare the effect of leachates from the rhizosphere of established plants of *H. lanatus* upon seedlings of *H. lanatus* and its common associate *Rumex acetosa*.

10.2 MATERIALS AND METHODS

The experiment involved 'donor pots' and 'receiver pots'. Leachate from donor pots was applied to the receiver pot, and the yield of seedlings in the receiver pot was used to assess response to the leachates.

The donor pots were 13 cm in diameter and at the beginning of the experiment each was filled with washed sand. In half of the pots ten-day-old seedlings (8/pot) of *Holcus lanatus* were planted whilst in the remaining control pots there were no seedlings. All of the pots were then placed in a growth-room with temperatures of 20°C by day and 15°C by night and an 18 h daylength. Full strength Hewitt nutrient solution in aliquots of 200 ml was added every ten days to each pot. After two months the turf of *H. lanatus* in each pot was clipped at a height of 5 cm. The sand in each pot was then washed with 3 l of distilled water, and the experimental and control pots were divided into four groups each composed of five replicates. For the remainder of the experiment each of the four groups of pots received a different concentration of nutrient solution. The levels applied were 0.5 N, 1 N, 2 N and 4 N Hewitt nutrient solution.

The receiver pots containing the same washed sand as the donor pots were 9 cm in diameter and each was provided with a plastic saucer. Ten-day-old seedlings of *H. lanatus* and *Rumex acetosa* were planted singly in each pot. There were ten receiver pots of each species for each strength of nutrient solution (i.e. 2 receiver pots/1 donor pot). The pots were arranged in randomized blocks within the same growth-room as the donors. The seedlings in the receiver pots were allowed to establish for 18 days during which time two aliquots, each of 100 ml full Hewitt nutrient solution, were supplied to each pot. After 18 days application of leachates commenced. Leachate was collected from each donor pot by adding 200 ml of the appropriate nutrient solution to the surface of the sand in the donor pot and collecting the runoff which was then applied again to the sand surface. After four passages through the sand, the leachate was applied to the receiver pots. Following this procedure 50 ml of leachate was added to each receiver pot every week.

10.3 RESULTS

In Figures 10.1 and 10.2 the yields of *Rumex acetosa* and *Holcus lanatus* seedlings harvested at the end of the experiment are plotted against the concentration of Hewitt nutrient solution supplied to the donor pots and used to elute the leachates. A more detailed presentation of these data is included in Appendix 3. From these results it is apparent that seedlings of both species were reduced in yield when subjected to leachates from established plants of *H. lanatus* and this effect extended across the range of nutrient concentrations. For both species asymptotic responses to nutrient concentration occurred both in the control plants and in those subjected to *H. lanatus* leachates. It is interesting to note that for *R. acetosa* the highest yield in the control plants occurred at 2.0 N Hewitt solution whereas the 1.0 N solution was optimal in the experimental treatment. In *H. lanatus*, maximum yield was associated with a concentration of 2.0 N Hewitt solution in both treatments.

When the yields of seedlings treated with leachates from *H. lanatus* are expressed as a percentage of the yield of the control plants it is clear that the extent of inhibition was rather greater in *R. acetosa* than *H. lanatus*. However, because of the larger initial size of the seedlings of *R. acetosa*, the effect of this inhibition was to cause seedlings of the two species to attain quite similar yields.

Figure 10.1

The yield of seedlings of *Rumex acetosa* after six weeks' growth in sand cultures, supplied with leachates from pots of sand containing no plants (o) and established plants of *Holcus lanatus* (●).

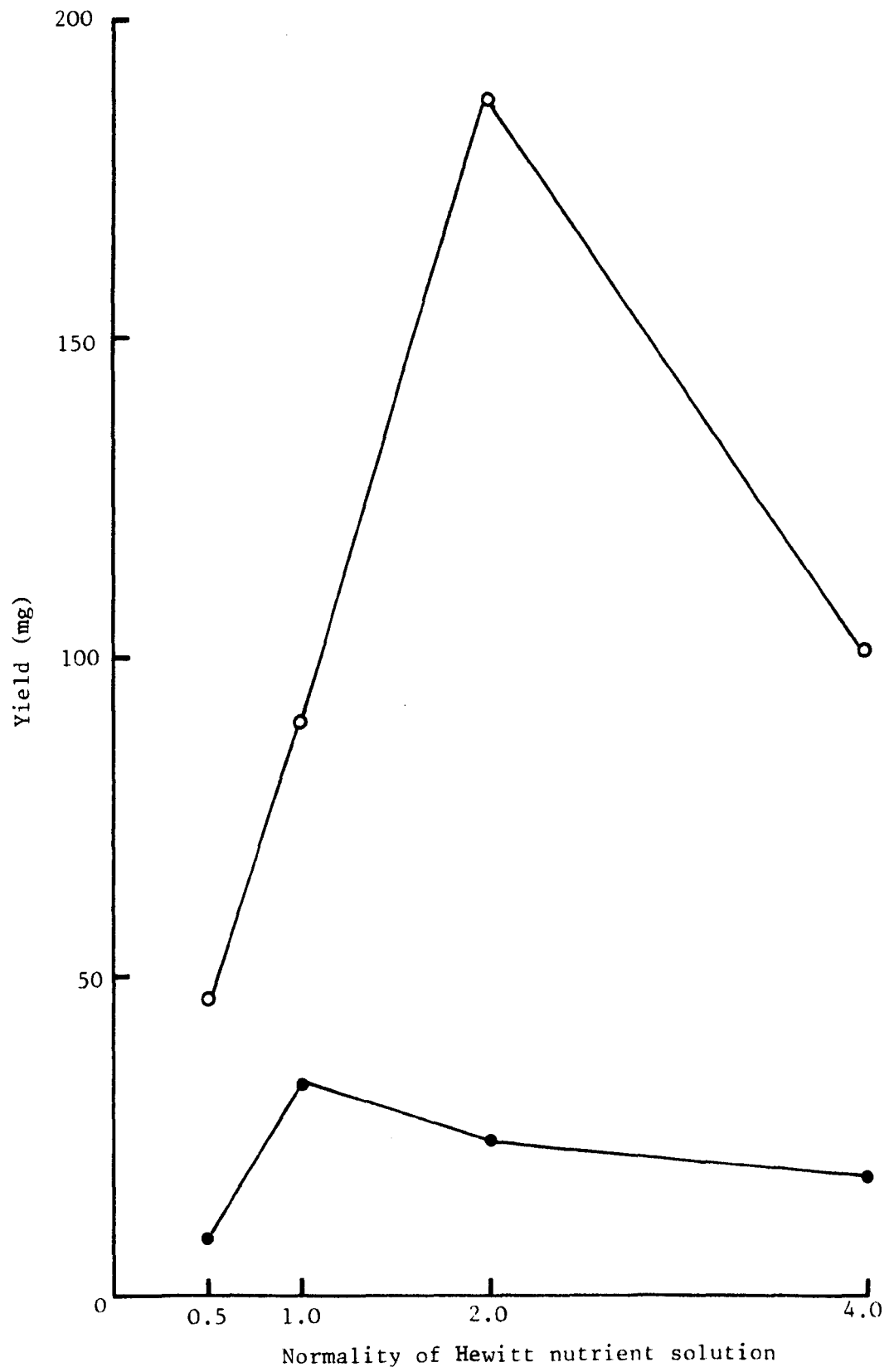
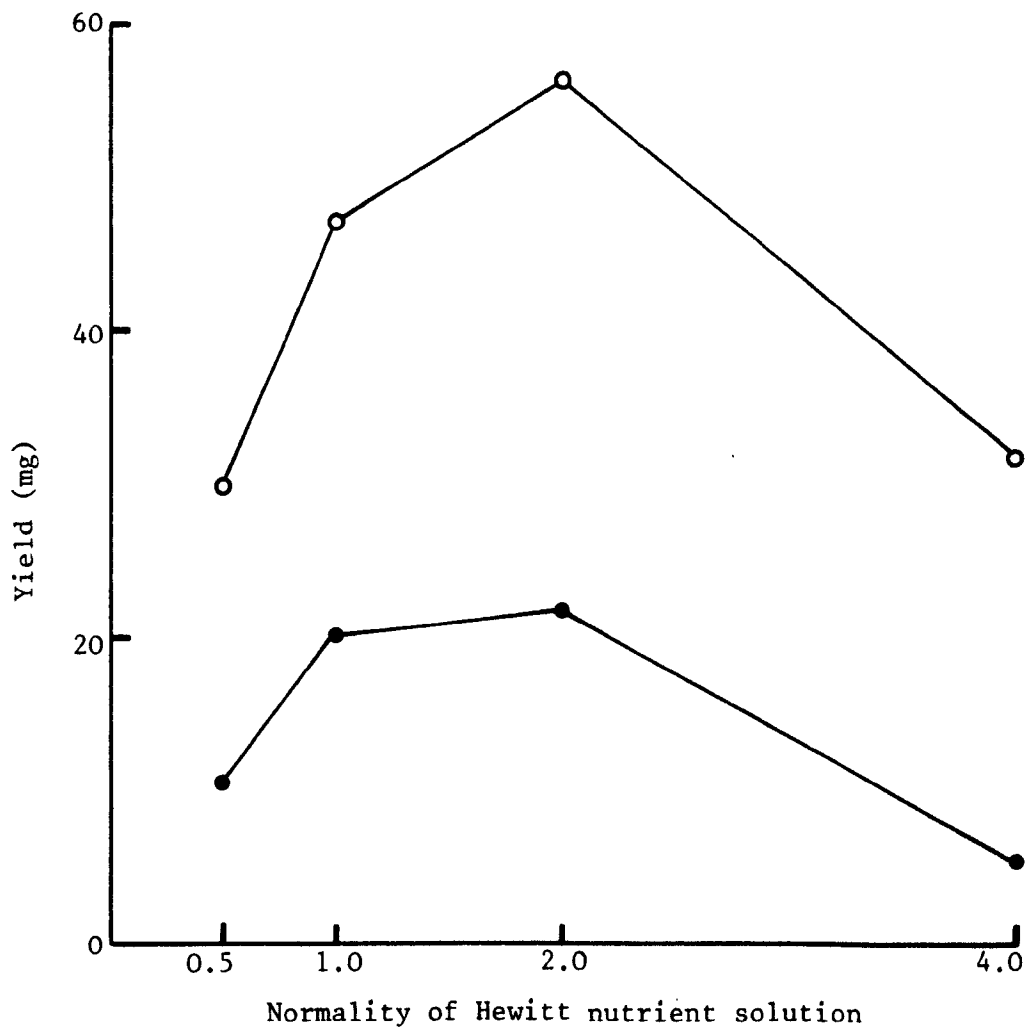


Figure 10.2

The yield of *Holcus lanatus* after six weeks' growth in sand culture, supplied with leachates from pots of sand containing no plants (o) and established plants of *Holcus lanatus* (●).



10.4 DISCUSSION

Seedlings of both *Rumex acetosa* and *Holcus lanatus* showed an asymptotic response in yield to increasing mineral nutrient supply and this pattern obtained in the presence and in the absence of leachates from sand containing the roots of established plants of *H. lanatus*. It seems likely that the reduced yield at 0.5 N Hewitt solution was due to mineral nutrient shortage whilst that observed at a concentration of 4 N was the result of some form of mineral nutrient toxicity. It is of considerable interest, therefore, to observe that in both species the leachate from *H. lanatus* produced a major reduction in seedling yield *across the complete range of nutrient concentrations*. This provides evidence of a toxic effect originating from *H. lanatus* which can be clearly distinguished from effects arising from competition for mineral nutrients. It is also worth mentioning that in common with the results of the studies described in Chapters 2, 4 and 9, this experimental data suggests that toxic effects can be produced relatively early in the life-cycle of *H. lanatus* and do not depend upon the accumulation of large quantities of litter or root debris.

The results also indicate that *R. acetosa* was more susceptible than *H. lanatus* to the inhibitory effect of the *H. lanatus* leachate. This finding is consistent with the findings of Newman and Rovira (1975) and with those described in Chapter 7.

CHAPTER 11

GENERAL DISCUSSION

11.1 INTRODUCTION

In any attempt to consider the involvement of plant phytotoxins in ecological processes there is a need to review evidence from both field and laboratory sources and careful analysis is required in order to relate such evidence to information concerning other aspects of the mechanisms by which interactions occur between neighbouring plants. Moreover, as explained in Chapter 1, extreme caution is justified, where the research objective is to attempt to recognize plants in which the production of phytotoxins is an adaptive feature allowing the species concerned to suppress the vigour of potential competitors.

With these considerations in mind therefore, the procedure in this Discussion will be first to review the evidence obtained from the present studies for the production of phytotoxins by selected herbaceous plants of the Sheffield flora. Secondly, an attempt will be made to consider whether the production of phytotoxins confers an ecological advantage upon the plants which produce them and comments will be made concerning the possible importance of allelopathy, relative to that of other recognized determinants of success in plant interactions.

11.2 EVIDENCE OF THE PRODUCTION AND RELEASE OF PHYTOTOXINS

11.2.1 Field evidence of phytotoxic effects

(1) Woodland sites (Sites 1 and 2)

The results of the seedling bioassays described in Chapter 4 suggest that an inhibitory effect in samples of surface soil occurred in the period following the deposition of deciduous tree litter at Sites 1 and 2. The data indicate that this effect was not due to mineral depletion and it seems reasonable to suspect that inhibition was related to the release of organic toxins during the initial phase of microbial decay of the tree leaves. In attempting to assess the impact of this phenomenon on the growth of ground flora species it is worth noting that the effect appeared only for a short period at a time when most of the herbaceous species were not in an active phase of growth (see Chapter 2). Further studies will be necessary before it is possible to determine whether such temporary effects influence the success of germination and seedling establishment in woodland plants. Evidence suggesting that certain tree species produce inhibitory chemical effects upon adjacent plants has been reported in several earlier studies (Cook 1921; Massey 1925; Mergen 1959; del Moral and Muller 1969; Al-Naib and Rice 1971; Lodhi and Rice 1971; McPherson and Thompson 1972; Lodhi 1975, 1976) and there is now a major requirement for investigations testing the hypothesis that seasonal release of toxins influences phenological events on the woodland floor.

(2) Heathland sites (Sites 6 and 7)

The bioassays conducted on soils from Sites 6 and 7 in Chapter 4 produced consistently low yields throughout the seasonal studies. This could be attributed to several factors. The soil was extremely acidic (Table 3.1) and elements such as Mn, Fe and Al were present in very high concentrations and mineral nutrients such as nitrate-nitrogen were scarcely available. In these studies the yield of *Rumex acetosa* seedlings remained low on soil samples provided with additional mineral nutrients suggesting that toxic substances could have been released by the dominant species (*Calluna vulgaris*, *Pteridium aquilinum*) at the two sites. It is interesting to note that for both of these species there is previous evidence of the occurrence of toxic effects.

Bracken (*P. aquilinum*) grows in environments ranging from tropical to boreal, and there are references in the literature to its dominating influence in many types of vegetation (Gliessman and Muller 1972). Most investigators have attributed dominance to the superior competitive ability of the species, but Gliessman and Muller (1972) reported that water extracts of dead fronds collected near Santa Barbara, California, were inhibitory to seed germination and radical growth of two grass species. Leachates of green fronds were found to be non-toxic but 'artificial rain' leachates from bracken litter were shown to be inhibitory to the radical growth of grass seedlings and residues of bracken fronds in the soil also exerted a toxic effect. In a further study with the same species Stewart (1975) concluded that allelopathic interactions may explain the relative absence of shrubs from sites dominated by bracken.

Whitehead (1964) found that soil associated with bracken contained P-hydroxybenzoic acid, vanillic acid, P-hydroxycinnamic acid, and ferulic acid at concentrations of 3.9×10^{-5} M, 419×10^{-5} M, 4.2×10^{-5} M and 0.4×10^{-5} M respectively. Fronds were found to contain these same compounds in high concentrations throughout the growing season, and rain leaching and decomposition of the fronds apparently introduced the compounds into the soil (Glass and Bohm 1969). Glass (1976) treated six grasses and a species of clover to conditions intended to reproduce exactly the concentrations of major phenolic acids present in soil associated with bracken. The first experiment was carried out using 0.5 mM CaSO_4 solution as the control medium together with an experimental treatment of CaSO_4 solution plus the phenolic acids. Root growth was severely inhibited in comparison with the controls in all species except one (*Agropyron repens*). In a further experiment an attempt was made to examine the influence of mineral nutrients upon the effect of phenolic acids. No inhibitory effects of the phenolic acids upon barley root growth were detected in the presence of a complete inorganic nutrient medium. Recently Gliessman and Muller (1978) have completed a very comprehensive study of bracken toxicity. From the results, these authors suggested that competition cannot account for the lack of herbs in bracken stands. Measurements of soil pH, soil texture, water-holding capacity, and organic matter content in bracken patches and in neighbouring areas of vegetation indicated no consistent correlations with soil factors and it was concluded that phytotoxins leached from the dead standing bracken fronds by the first few rains of the wet season were largely responsible for herb suppression.

These toxins were isolated in rain-drip from bracken foliage in the field and from soil inside the fern stands. Moreover, herbs reinvaded the stands after several seasons when fronds were removed before rains could leach them, and conversely, placing fronds over the herbs in neighbouring grassland resulted in herb inhibition. It would be most interesting to conduct similar experiments upon British populations of *P. aquilinum*.

From studies conducted in Galicia in Spain situations have been described (Ballester and Vieitez 1971; Ballester, Arines and Vieitez 1972; Salas and Vieitez 1972; Vieitez and Ballester 1972) in which attempts to utilize heathlands for agriculture have resulted in strongly inhibitory effects upon crop plants, particularly various grass species. These investigators found that *Calluna vulgaris* and several other Ericaceous species all contained substances which were inhibitory in the *Avena coleoptile* straight growth test. Phenolic compounds have been identified in various parts of these heath plants and the same substances were found to be present in the soil under certain of these species. It has been established that tree species, such as birch (*Betula pendula*) and spruce (*Picea abies*) often fail to develop in association with heather (*Calluna vulgaris*). Handley (1963) reported that in the presence of heather the growth of mycorrhizae on spruce was inhibited and he concluded that this caused the failure of spruce to establish in heather. Robinson (1972) employed experimental techniques which demonstrated conclusively that run-off from roots of living *Calluna vulgaris* and raw humus of this species contained a factor toxic to several mycorrhizal fungi. More investigations are now required in

order to study the failure of many grasses, forbs and tree seedlings to establish in heathland. In particular it is necessary to assess the importance of toxins derived from *Calluna vulgaris* in inhibiting mycorrhizal associations.

(3) The grassland site (Site 3)

In the investigation described in Chapter 5 samples of soil collected from local areas of a derelict grassland dominated by *Holcus lanatus* were found to reduce seedling growth to an extent consistently greater than that observed for samples collected from random positions within the same field. This effect could not be removed by the addition of mineral nutrients to the soil and the results obtained in a seasonal study (Chapter 4) suggested that phytotoxins originating from *H. lanatus*, although persistent, showed some seasonal variation in effect and in this respect appeared to be intermediate between those associated with the heathland and woodland sites.

11.2.2 Experimental evidence of phytotoxic effects

As explained in Chapter 1, evidence of the production of phytotoxins by plants is available from numerous experimental investigations. The evidence obtained in the present study can be considered under two headings which refer to different experimental techniques.

(1) Long-term experiments

In the published studies of the toxic effects of decomposing plant materials reviewed by Rice (1974,1979) attention has been concentrated upon short-term effects upon germination and early seedling growth resulting from the addition of litter or plant extracts to soil or sand cultures. In a recent investigation of this type Newbery (1976,1979) adopted a broad 'screening' approach using many species to investigate allelopathic effects of decomposing roots and shoots of one species upon the growth of the same or another species under a range of experimental conditions. From the results of this study it was apparent that decaying roots and shoots of a range of British herbaceous plants are capable of affecting plant growth. It is of interest to note that in Newbery's investigation inhibitory effects were recorded in experiments involving residues of *Holcus lanatus* since evidence of the inhibition of plant growth by decomposition products or toxic secretions from *H. lanatus* was obtained in the present study in the experiments described in Chapters 7, 8 and 9. In view of this evidence of the production of phytotoxins in short-term experiments it is interesting to consider what is the likely course of events in circumstances where species such as *H. lanatus* occupy the same soil for an extended period. Some evidence on this point is available from the experiment described in Chapter 7. The results of this study show that, after the first year, monocultures of several species, including *H. lanatus*, accumulated toxic substances to an extent which appeared to induce severe autotoxicity. In this respect the results accorded with the field

evidence of autotoxicity in *H. lanatus* patches described in Chapter 5. However, in assessing the significance of the long-term experiments it may be important to remember that the rooting medium was sand and may have contained a microflora more conducive to toxin accumulation than those occurring in natural soils.

(2) Short-term experiments

In relatively short-term experiments there is an opportunity to study mechanisms of allelopathy under more closely-controlled conditions. In particular it is easier to ensure that effects due to depletion of mineral nutrient supply are either eliminated or taken into account. The technique applied in the experiment described in Chapter 10 appears to be very effective in distinguishing between effects of phytotoxins and mineral nutrient supply in leachates from cultures of *H. lanatus* and perhaps could be applied more generally in future screening experiments.

A second advantage of the short-term experiment is related to the possibility that it may provide opportunities to distinguish between toxic secretions and inhibitors originating as decomposition products. However, as several authors (e.g. Shamoot *et al.* 1968; Barber and Gunn 1974) have pointed out, even in experiments involving seedlings and young plants as donors, it seems certain that leachates will contain not only substances which have been actively exuded but also decomposition products either from root hairs and other cells which have been sloughed off the roots or from the root surface microflora.

11.3 THE ECOLOGICAL SIGNIFICANCE OF PHYTOTOXIN PRODUCTION

From the results obtained in this investigation and from many previous studies there is circumstantial evidence that toxins, originating by secretion from plants or as products of the decay of litter, contribute to the mechanisms limiting rates of dry matter production in natural habitats. However, the present state of development of experimental techniques does not provide unequivocal proofs of the impact of allelopathy. Even under controlled conditions in the laboratory there are likely to be formidable technical problems where an attempt is made to investigate the role of plant toxins under conditions which resemble (nutritionally and microbiologically) those occurring in the field. Moreover, before we can judge the role of phytotoxins as determinants of vegetation composition it will be necessary to assess the extent to which their effects are (1) selective between species and (2) important relative to other environmental factors.

(1) Selectivity

From field investigations (e.g. Milton 1943; Curtis and Cottam 1950; Voight 1959; Webb, Tracey and Haydock 1967; Hanes 1971; Tinnin and Muller 1972; Newman and Rovira 1975; Newbery 1976) and evidence such as that in Figure 10.2, it is apparent that many phytotoxins are effective against the plants which produce them. This does not mean, however, that we should assume that under field conditions these plants derive no advantage from the production and release of toxins. There are several possible mechanisms whereby such plants may escape the major impact of their toxins. In the case of *Pteridium aquilinum*, for example, it seems likely that the main concentration of toxic residues from the litter will be present in the surface layers of the soil profile and will exercise a more potent effect upon shallow rooting grasses than upon *P. aquilinum* which tends to exploit deeper horizons of the soil profile.

A second mechanism which could allow a plant to evade the effect of its toxins is related to phenology and has been summarised in the form of the 'Loch-Ness' model (Grime 1979). This model

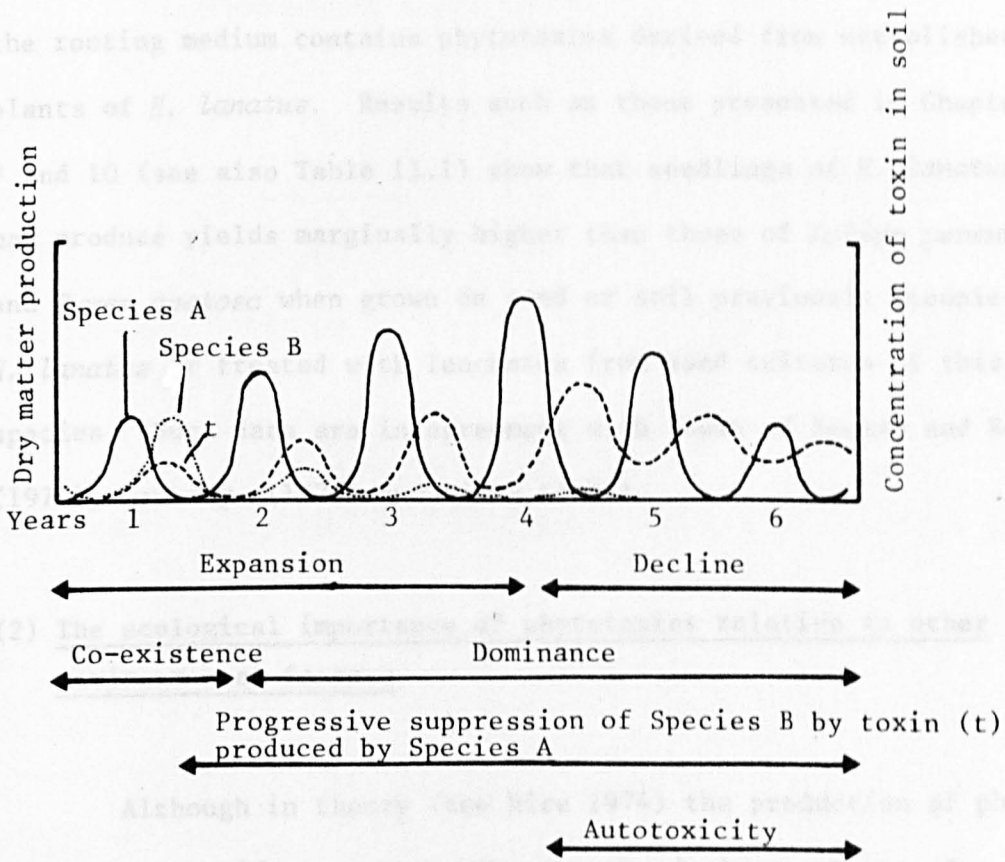
(Figure 11.1) describes the possible sequence of events, in a circumstance in which the growth of the toxin producer is restricted to one season in the year during which toxins accumulate either by secretion or as decomposition products from senescent roots and shoots. Under these hypothetical conditions the highest concentrations of toxin in the environment will occur after the main growth-period of the producer and may be expected therefore to exert a selective effect against species with complementary phenologies. However, a continued advantage can be expected from such a mechanism only provided that the rate of breakdown of the toxin is sufficient to reduce the concentration to a low level before the next growth-period of the toxin-producer and, as shown in Figure 11.1, alteration of the balance between rates of production and decomposition of toxin could result in conditions favourable either to co-existence or to autotoxicity. At the present time, there is only circumstantial evidence (e.g. Robinson 1971) of such 'seasonal' allelopathy. However, as pointed out by Grime (1979), it may be significant that phytotoxic effects have been reported for a considerable number of competitive-ruderals, many of which achieve local or temporary dominance in herbaceous vegetation. Examples include annual forbs, such as *Hordeum vulgare* (Pickering 1919; Overland 1966), *Camelina alyssum* (Grümmer and Beyer 1960), *Helianthus annuus* (Wilson and Rice 1968), and *Avena fatua* (Tinnin and Muller 1972), grasses such as *Lolium multiflorum* (Welbank 1963) and *Holcus lanatus* (Al-Mashhadani 1980), and perennial forbs such as *Trifolium* spp. (Chang *et al.* 1969; Katznelson 1972) and *Andropogon virginicus* (Rice 1972). In this connection it is also interesting to note

Figure 11.1

Model describing the possible role of accumulating plant toxins in mechanisms of co-existence, dominance, and autoxicity involving two herbaceous species.

that cycles of colonization, dominance and reduced productivity have been recognized in acidic heathlands and grasslands of the British Isles (Watt 1947, 1955; Wilson 1963; Connell, Green and Merrifield 1967).

The present study has provided some evidence that *H. lanatus* is able to outlast certain other species in circumstances where the rooting medium contains phytochemicals derived from established plants of *H. lanatus*. Results such as those presented in Figures



Although in these few lines (1974) the production of phytotoxins can provide a partial explanation of the success of particular species in certain vegetation types it is doubtful whether the success of any plant can be attributed exclusively to allelopathy. In the present study, each of the species for which there is evidence of toxin production exhibits additional characteristics facilitating resource capture and the exclusion of taller bearing plants. In *H. lanatus*, for example, these features include extensive lateral spread by rhizomes, the development of a

that cycles of colonization, dominance and reduced productivity have been recognized in acidic heathlands and grasslands of the British Isles (Watt 1947,1955; Grime 1963b;Grubb, Green and Merrifield 1967).

The present study has provided some evidence that *H. lanatus* is able to outyield certain other species in circumstances where the rooting medium contains phytotoxins derived from established plants of *H. lanatus*. Results such as those presented in Chapters 7 and 10 (see also Table 11.1) show that seedlings of *H. lanatus* may produce yields marginally higher than those of *Lolium perenne* and *Rumex acetosa* when grown on sand or soil previously occupied by *H. lanatus* or treated with leachates from sand cultures of this species. Such data are in agreement with those of Newman and Rovira (1975), Newbery (1976) and Firth (1977).

(2) The ecological importance of phytotoxins relative to other environmental factors

Although in theory (see Rice 1974) the production of phytotoxins can provide an explanation for the dominant status of particular species in certain vegetation types it is doubtful whether the success of any plants can be attributed exclusively to allelopathy. In the present study, each of the species for which there is evidence of toxin production exhibits additional characteristics facilitating resource capture and the exclusion of neighbouring plants. In *Pteridium aquilinum*, for example, these features include extensive lateral spread by rhizomes, the development of a

Table 11.1 The yield of seedlings of *Holcus lanatus*, *Lolium perenne* and *Rumex acetosa* after three weeks' growth in soil dominated by *H. lanatus*, supplied with different Hewitt nutrient solution concentrations. 95% confidence limits are included. (Al-Mashhadani unpublished)

	Concentration of Hewitt solution			
	0.5	1.0	2.0	4.0
<i>Holcus lanatus</i>	20.9±6.4	15.7±4.27	14.2±6.9	8.4±3.8
<i>Lolium perenne</i>	10.3±2.8	7.2±1.6	5.7±2.0	4.0±1.3
<i>Rumex acetosa</i>	14.8±4.5	13.0±3.4	11.1±4.5	7.1±2.4

high leaf canopy during the summer, the tendency to accumulate a dense layer of persistent litter on the ground surface and the production of robust fronds capable of penetrating the litter. In *Holcus lanatus*, dominance is associated with the capacity for rapid growth over a wide range of temperatures (Chapter 6), the production of a dense leaf canopy under clipped and unclipped conditions and the formation of an exceedingly large root surface area composed of finely-divided roots and root-hairs.

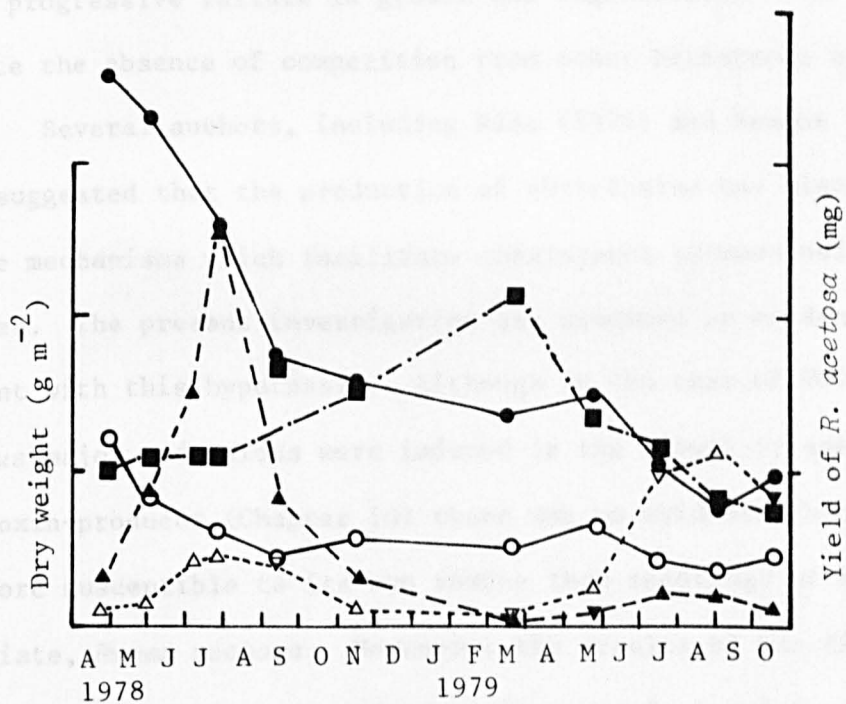
Circumstantial evidence consistent with the 'Loch-Ness' model is evident in Figure 11.2 which summarizes the phenological and bioassay data obtained in the present study from the grassland site at New Totley (Chapters 2 and 4). From the bioassays it is apparent that during the summer of 1978 there was a progressive decline in soil fertility which followed the peak in shoot biomass in *Holcus lanatus*, coincided with the build up of *H. lanatus* litter and was only partially removed by application of mineral nutrients during the bioassays. The data indicate that phytotoxic effects persisted during 1979 and were associated with a very reduced phenological peak in *H. lanatus* and an increase in the shoot biomass of *Agrostis tenuis*. Whilst it is tempting to conclude that the decline in vigour of *H. lanatus* in 1979 was the result of auto-toxicity, other possible explanations (e.g. frost damage during the relatively severe winter) must be considered and further investigation will be required in order to test this hypothesis. It is interesting to note, however, that sequences of vigorous growth followed by marked decline have been recorded for *H. lanatus* in earlier studies. Dunbar (1971,1974) examined *H. lanatus* colonies

Figure 11.2

Estimates of seasonal variation in the shoot biomass in two constituent grasses in an area of derelict grassland (see Chapter 2) combined with the results of seedling bioassays on soil samples removed concurrently from the same site (see Chapter 3).

- *R. acetosa* yield + nutrient addition
- *R. acetosa* yield + distilled water
- - -■ Dry weight of litter (mainly from *H. lanatus*)
- ▲- - -▲ Dry weight of *H. lanatus*
- △- - -△ Dry weight of *A. tenuis*

over a five-year period. At several sites, *R. acetosa* showed vigorous growth and the most complete ground cover in 1978, but occurred in the first season, but thereafter declined rapidly as *R. lanatus* was replaced by other species. While it is possible that the patterns recognized by Dunbar would reflect a relatively short life-history in the species it is not clear if Dunbar's toxicity in the example of declining vigor of *R. lanatus* found in the sand culture experiment described in Dunbar (1974). In 1978 there was a progressive failure to grow and regenerate in 1979 despite the absence of competition from other species.



Several authors, including King (1974) and Dunbar (1974), have suggested that the inhibition of *R. lanatus* is due to the presence of a toxic substance in the soil. This substance is thought to be a secondary metabolite of *R. lanatus* which inhibits the growth of other species. The results of laboratory experiments described in Dunbar (1974) indicate that under conditions of high soil moisture there is a strong tendency for *R. lanatus* to be the dominant component of the vegetation. The present study (Chapter 3) and the results of other recent studies of ground cover (Mortimer 1974; Thompson 1977) also suggest the existence between *R. lanatus* and other ground cover species of a phenomenon unrelated to gibberellin, which is a common

over a five-year period at several sites and found that the most vigorous growth and the most complete ground cover at each site occurred in the first season, but thereafter declined rapidly as *H. lanatus* was replaced by other species. Whilst it is possible that the patterns recognized by Dunbar merely reflect a relatively short life-history in the species it is not easy to discount auto-toxicity in the example of declining vigour of *H. lanatus* found in the sand culture experiment described in Chapter 7. Here there was a progressive failure in growth and regeneration of *H. lanatus* despite the absence of competition from other herbaceous species.

Several authors, including Rice (1974) and Newman (1978), have suggested that the production of phytotoxins may also contribute to the mechanisms which facilitate coexistence between neighbouring species. The present investigation has produced no evidence consistent with this hypothesis. Although in the case of *Holcus lanatus* major reductions were induced in the growth of seedlings of the toxin-producer (Chapter 10) there was no evidence that *H. lanatus* was more susceptible to its own toxins than seedlings of its common associate, *Rumex acetosa*. Moreover, the results of the field and laboratory experiments described in Chapters 2, 5 and 6, 7, 9, indicate that under conditions in which toxin accumulation occurs there is a strong tendency for *H. lanatus* to occur as the dominant component of the vegetation. The phenological studies (Chapter 2) and the results of other recent studies of grassland dynamics (Mortimer 1974; Thompson 1977; Watt 1977) suggest that coexistence between *H. lanatus* and other grassland herbs is dependent upon phenomena unrelated to allelopathy, such as differential responses

to seasonal variation in temperature, grazing and mowing regimes and complementary patterns of vegetative spread and seedling regeneration.

APPENDIX 1

Lists of the species of flowering plants present within each of the samples sites.

Site 1

Allium ursinum
Anemone nemorosa
Arum maculatum
Chrysosplanium oppositifolium
Circaea lutetiana
Festuca gigantea
Galium aparine
Hedera helix
Heracleum sphondylium
Mercurialis perennis
Myosotis sylvatica
Myrrhis odorata
Poa trivialis
Ranunculus ficaria
Ranunculus repens
Silene dioica
Urtica dioica
Veronica montana

Site 2

Agrostis stolonifera
Anthriscus sylvestris
Arrhenatherum elatius
Cardamine flexuosa
Deschampsia cespitosa
Epilobium adenocaulon
Epilobium hirsutum
Festuca gigantea
Heracleum sphondylium
Holcus lanatus
Impatiens glandulifera
Poa trivialis

Ranunculus repens
Rorippa islandica
Rumex obtusifolium
Silene dioica
Stellaria alsine
Trifolium repens
Urtica dioica

Site 3

Agropyron repens
Agrostis tenuis
Anthoxanthum odoratum
Arrhenatherum elatius
Chamaenerion angustifolium
Dactylis glomerata
Deschampsia cespitosa
Festuca rubra
Holcus lanatus
Poa pratense
Rumex acetosa

Site 4

Acer pseudoplatanus
Agropyron repens
Agrostis stolonifera
Bromus mollis
Cerastium fontanum
Dactylis glomerata
Equisetum arvense
Galium aparine
Holcus lanatus

APPENDIX 1 (Contd)

Site 4 (contd)

Lolium multiflorum
Lolium perenne
Poa annua
Poa pratense
Poa trivialis
Ranunculus acris
Stellaria media
Trifolium pratense
Trifolium repens

Site 5

Agrostis stolonifera
Anthoxanthum odoratum
Bellis perennis
Cerastium fontanum
Cerastium holosteoides
Chrysanthemum leucanthemum
Galium aparine
Holcus lanatus
Hypochoeris radicata
Lolium multiflorum
Lolium perenne
Plantago lanceolata
Poa annua
Poa trivialis
Polygonum aviculare
Ranunculus acris
Ranunculus repens
Rumex obtusifolius
Stellaria media
Taraxacum officinale
Trifolium pratense
Trifolium repens

Site 6

Deschampsia flexuosa
Pteridium aquilinum

Site 7

Calluna vulgaris
Deschampsia flexuosa
Empetrum nigrum

APPENDIX 2

Climatic data from Western Park (2 km from experimental site).

Max = mean maximum daily air temperature °C

Min = mean minimum daily air temperature °C

Month	1973		1974		1975		1976		1977		1978	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Jan	6.7	2.8	8.1	2.9	8.9	3.3	7.6	3.2	4.6	0.4	5.3	3.0
Feb	7.4	2.2	7.9	3.1	8.4	1.7	6.7	2.1	6.2	1.5	4.3	-0.4
Mar	10.6	3.6	8.5	2.3	6.8	1.7	7.8	1.4	9.4	3.4	10.1	3.4
Apr	12.1	4.7	11.5	3.6	12.2	5.1	11.8	4.4	10.6	3.7	9.3	3.2
May	15.5	7.1	15.6	6.9	13.8	5.6	15.7	8.1	14.6	6.1	16.4	7.2
June	20.0	10.8	17.7	9.8	20.6	9.7	22.3	12.0	16.4	8.3	17.4	9.7
July	19.7	12.2	18.7	11.7	21.7	14.3	24.0	13.2	19.6	11.8	18.2	10.8
Aug	20.6	12.1	19.5	11.5	24.6	13.9	23.0	12.0	19.2	11.4	18.6	11.7
Sept	17.9	10.5	15.7	8.8	17.3	9.5	16.4	9.6	16.2	9.7	17.2	11.0
Oct	12.8	5.6	10.0	5.2	13.3	6.5	13.0	7.5	13.9	8.3	15.3	9.2
Nov	8.5	3.6	8.7	3.7	8.8	3.3	8.7	3.2	8.6	3.2	11.3	6.1
Dec	7.3	2.3	9.9	5.1	8.2	3.5	3.9	0.3	7.5	3.6	5.0	0.9

APPENDIX 3

The yield of seedlings of *Rumex acetosa* and *Holcus lanatus* after six weeks' growth in sand cultures, supplied with leachates from pots of sand containing no plants (control) and established plants of *Holcus lanatus*. 95% confidence limits are in brackets.

Species	Concentration of Hewitt solution	Dry weight (mg) of the yield received leachate from control pot			Dry weight (mg) of the yield received leachate from <i>H. lanatus</i> pots		
		Root	Shoot	Total	Root	Shoot	Total
<i>Rumex acetosa</i>	0.5	18.9(±14.8)	27.8(±12.7)	46.7(±26.9)	2.9(±1.1)	6.5(± 3.1)	9.4(± 4.1)
	1.0	17.2(± 9.1)	52.9(±24.1)	70.1(±29.9)	9.5(±4.3)	23.9(±10.9)	33.4(±15.1)
	2.0	66.3(±23.8)	126.0(±28.5)	192.3(±33.1)	8.8(±6.5)	15.4(±13.2)	24.2(±14.1)
	4.0	27.9(±20.0)	73.5(±33.1)	101.4(±47.0)	7.0(±3.1)	11.6(± 6.0)	18.6(± 7.9)
<i>Holcus lanatus</i>	0.5	5.7(± 1.5)	25.4(± 3.6)	31.1(± 8.1)	2.9(±0.7)	7.8(± 5.7)	10.7(± 6.1)
	1.0	6.9(± 1.3)	40.3(±21.2)	47.2(±22.2)	5.0(±1.1)	15.1(± 6.8)	20.1(± 7.7)
	2.0	6.7(± 2.7)	49.6(±30.2)	57.3(±32.9)	3.9(±1.9)	17.7(±10.4)	21.6(±12.7)
	4.0	5.1(± 2.0)	26.7(± 7.0)	31.8(± 9.0)	1.7(±0.3)	3.7(± 1.2)	5.5(± 1.5)

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