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THE AUTECOLOGY OF THE MEADOW THISTLE (Cirsium dissectum L. Hill) ON DEVON RHoS PASTURES, WITH PARTICULAR REFERENCE TO THE EFFECT OF MAJOR ENVIRONMENTAL VARIABLES ON THE POPULATION DYNAMICS

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by

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Abstract

The autecology of the meadow thistle (*Cirsium dissectum* L. Hill) on Devon Rhôs pastures, with particular reference to the effect of major environmental variables on the population dynamics

John Ross

Semi-natural wet grassland communities are rapidly disappearing from the farmed landscape. Protection of remaining areas and restoration of degraded areas has become a priority with organisations concerned with the conservation of biodiversity in the U.K. The research described in this thesis provides an insight into the community dynamics of one particularly rare plant community, the *Cirsio-Molinietum* fen meadow (NVC M24). This was achieved by examining the autecology of *Cirsium dissectum,* one of the key indicator species of this community.

The habitat environment where *C. dissectum* is present was characterised at eight Devon M24 locations and ten non-M24 locations. This revealed a particularly unique suite of environmental conditions. Soil water content was found to be relatively high with little seasonal fluctuation. Mineral nutrient status was characterised by particularly low phosphorus, below optimal nitrogen, ample calcium and potassium and a soil pH which was only mildly acid. The physiological characteristics of *C. dissectum* were determined by a series of controlled experiments. The species exhibited a high water requirement but was relatively tolerant of drought stress and prolonged dehydration. It was demonstrated that *C. dissectum* is well adapted to a low phosphorus environment, is tolerant of nitrogen levels considered below optimum for many plants and is relatively tolerant of shade. It can also recover from partial or total defoliation in a relatively short period of time without any loss in plant mass or carbohydrate reserves. From the physiological characteristics, it was concluded that *C. dissectum* conforms to the "stress-tolerant competitor" functional type. It was also concluded that the decline of the species is directly related to loss of habitat as a result of its degree of specialism and specific niche requirement. The implications for management of existing sites and restoration of degraded sites is discussed and an outline model of appropriate management actions is presented.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

This study was financed with the aid of a studentship from the University of Plymouth and was carried out in collaboration with the Institute of Grassland and Environmental Research who also provided additional financial support.

Relevant scientific seminars, conferences, symposiums and workshops were attended at which work was presented as follows:

Dec 1995	Attended annual British Ecological Society conference and presented a poster on germination characteristics of three fen meadow plant species
Jan 1996	Attended a post-graduate seminar course at Imperial College, Silwood Park, on Population Dynamics
Feb 1996	Attended a Royal Society Seminar on Plant Life Histories (Ecological Correlates and Phylogenic Constraints)
Dec 1996	Attended annual British Ecological Society conference
Sep 1997	Presented a paper at the British Grassland Society 5th Research conference. "Culm measure grasslands: Management implications of rare species research"
Sep 1997	Attended 5th International Workshop on Clonal Plants: Clonal Plants and Environmental Heterogeneity at University of Wales, Bangor
Dec 1997	Attended annual British Ecological Society conference and presented a poster on the response of *C. dissectum* to phosphorus availability.
Jan 1998	Attended post-graduate workshop at Imperial College, Silwood Park on Applying Ecological Theory. Presented a poster on phosphorus response in *C. dissectum.*
Sep 1998	Attended annual British Ecological Society Symposium: Advances in Plant Physiological Ecology.
Jan 1999	Attended and presented a paper at the annual British Ecological Society conference. "The response of *C. dissectum* to phosphorus availability, both continuous and pulsed"

Published paper not relevant to this particular project:

J.D. Goss-Custard, J. Ross, S. McGrorty, S.E.A. Le V. dit Durell, R.W.G. Caldow and A.D. West (1998) Locally stable wintering numbers in the Oystercatcher *Haematopus ostralegus* where carrying capacity has not been reached, *Ibis,* **140**, 104-112.

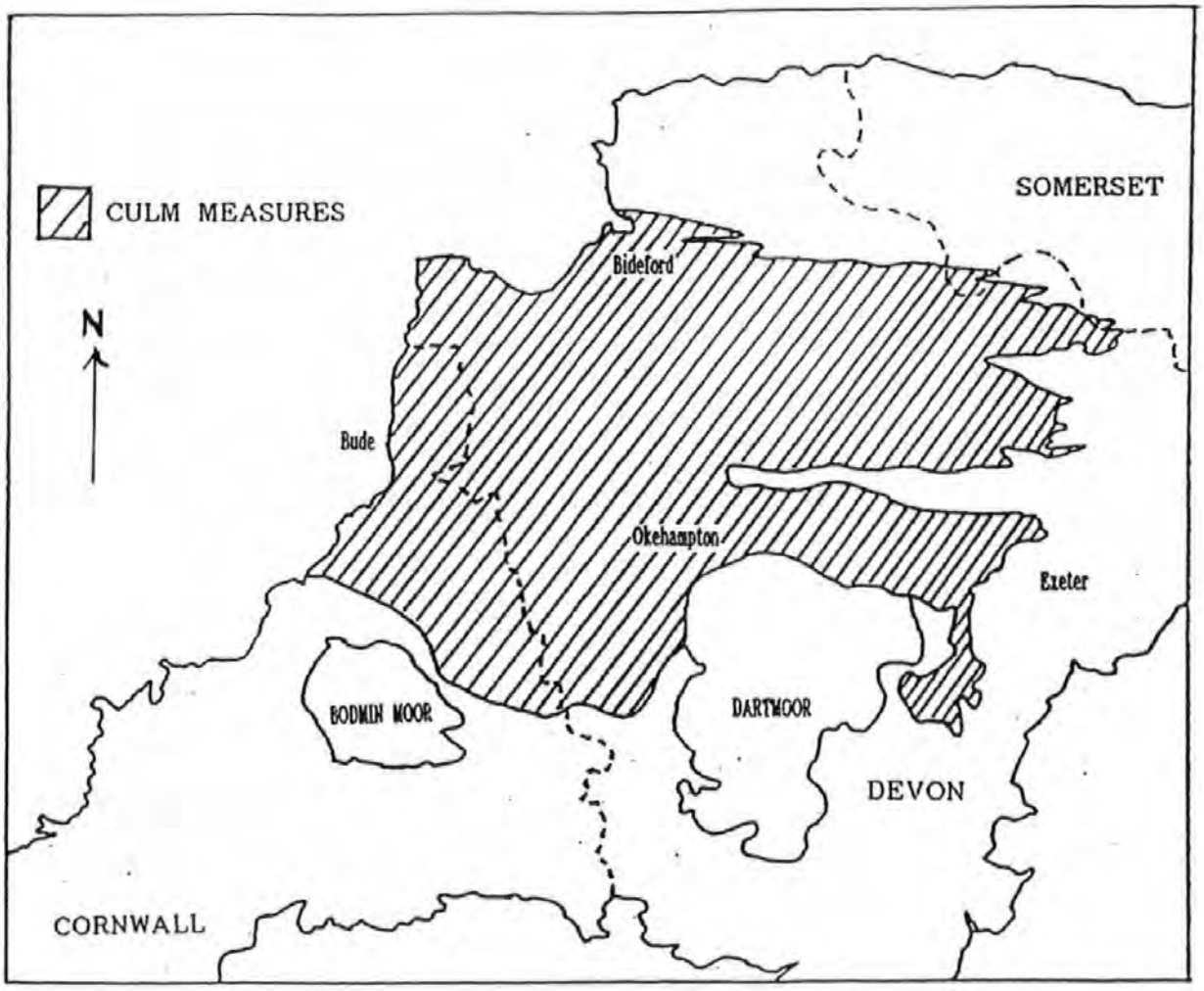
1.0 INTRODUCTION AND RESEARCH RATIONALE

1.1 BACKGROUND

Semi-natural habitats and communities that were once a common feature of the farmed landscape have been subject to ongoing decline (Fuller, 1987; Wells, 1989). Improved drainage and mechanical cultivation techniques have made even the least tractable sites with heavy clay soils and impeded drainage amenable to agricultural intensification. As a result semi-natural wet grassland communities are rapidly disappearing from the farmed landscape. The wildlife species (plants, birds and invertebrates) characteristic of these grasslands have become increasingly rare and threatened, to the point where protection of remaining areas has become important and mechanisms for restoration of degraded areas may be necessary to prevent irrevocable loss (Manchester, *et al.*, 1998). Within the classification of lowland wet grassland, there is a category collectively referred to as unimproved Rhôs pasture. This is defined as wet grasslands occurring on acidic to neutral soils with impeded drainage, more common in areas of high rainfall. The vegetation of these marshy grasslands comprises a distinctive mixture of fen meadow, rush pasture and wet heath, often occurring in mosaic. However this habitat often merges into other drier grassland types, or into heath and bogs, and sites generally contain an element of wet, usually scrub, woodland.

North Devon is of national importance for the extent and types of species-rich (unimproved) wet grasslands present on the Culm Measures which underlie much of the area. A recent report (Cordrey, 1996) has identified that the total UK resource of Rhôs pasture covers approximately 56,000 ha with 7% of this area located on the Devon Culm Measures. In Central Europe the area of these pastures has also been declining and now they are only to be found on the plains north of the Alps (Ellenberg, 1988). The Culm Measures cover an area from north Devon through to north east Cornwall (Figure 1.1) underlain by sandstones and shales of the late Carboniferous period (Durrance and Lamming, 1982).

Figure 1.1 The distribution of the Culm Measures in South-West England (Source: Wolton, 1992)



The soils mainly consist of Brown/Gleyed Brown (52%), Gleys (29%), Podzols (12%) and Pelosols (7%) with impeded drainage and poor structure (Goodwin, 1995). The high annual mean rainfall, exceeding 1000 mm (Ivimey-Cook, 1984), and soil conditions result in soils which are nutrient-poor and difficult to manage agriculturally (Goodwin, 1995). Rhôs pasture grasslands now cover only 2% of the Culm Measures (N.C.C. and D.W.T., 1989), and are known locally as 'Culm Grasslands'. However, they are important in terms of nature conservation as the habitat supports a rich diversity in wildlife, including rare and specialised plants, butterflies and birds. A survey carried out jointly by the Nature Conservancy Council and Devon Wildlife Trust (1989) concluded that, within the sample area, 65% of Culm Grassland had been lost between 1984 and 1989 and only some 8% of that present in 1900 now remains. This decline was directly attributed to agricultural improvement, with the most damaging operations being drainage, ploughing and reseeding. Although agricultural improvement of these habitats has largely ceased, the present threat is currently from neglect and lack of management resulting in natural succession to scrub and ultimately woodland. An action plan which includes Rhôs pasture and marshy grassland was included in the UK Steering Group Report on Biodiversity (H.M.S.O., 1995). Also, the adoption of Environmentally Sensitive Areas (ESAs), Countryside Stewardship Schemes and Wildlife Enhancement Schemes all provide opportunities to encourage the sympathetic management of these grasslands (Wolton, 1992).

1.2 ECOLOGICAL RATIONALE

The main vegetation communities present on the Devon Culm Grasslands are *Ericetum tetralicis* wet heath (National Vegetation Classification M16), *Juncus-Galium* rush pasture (M23), *Molinia-Potentilla* mire (M25) and the very rare *Cirsio-Molinietum* fen meadow (M24). In two surveys carried out by Wolton and Trowbridge (1990; 1992), European plant communities resembling the British M24 were either rare or absent from Galicia (Spain), and in Brittany (France) only small remnants of M24 type communities remain. They concluded that the M24 community type is probably the most threatened Rhôs pasture in Europe and without formal protection at any site. The rarity of the M24 community, totalling only 9% of

the remaining Culm grassland, has resulted in existing M24 sites being targeted as a priority by U.K. regional conservation bodies for management. On agriculturally improved sites, where M24 communities have been lost, the various conservation incentive schemes could enable reconstruction of these types of community. The UK Steering Group Report (H.M.S.O., 1995) has set a target of recreating 500 ha of Rhôs pasture on land adjacent to, or nearby, existing sites by the year 2005. However, it is suggested that successful management or reconstruction will require an understanding of the autecology of the various key species and their interactive behaviour at the both the population and community levels.

An analysis of communities can follow two different lines of research by concentrating its efforts on either the functions or on the structures of the system (Zwolfer, 1987). In the first case, the analysis deals with the interaction between the components of the system and their environment and will attempt to predict the behaviour of the system under defined conditions. The second approach gives emphasis to an explanation of community structure and to predictions concerning structural parameters of the system. This study uses the first, system function, approach. The main community controlling functions are the acquisition and use of resources by the constituent plants. The temporal and spatial variations in resources, especially water and nutrients, seem to be the major environmental factors that determine the distribution of plants along environmental gradients (Schulze and Chapin, 1987). Within the constraints set by overriding physical forces within each community, a knowledge of the physiological behaviour of individual plants can contribute to the explanation of major components of community function (Lange, *et al.*, 1983). Theoretical and experimental work suggests that species must have different resource requirements in order for them to coexist in a community (Berg and Braakhekke, 1978). Plant 'niche' has been defined by Whittaker (1972) as "the way a species is specialised within a community, its position in space and time, and its functional relationship to other species in the community". Using the phytocentric view of plant niches provides a way to express the nature of response of individual populations and species to major factors in the environment (Bazzaz, 1996). Therefore, the general research strategy adopted for this study was to study in detail *Cirsium dissectum*, a key species representative of the M24 community, as a basis

to explain community function. A knowledge of species function and the general trends in critical environmental factors make it possible to make predictions about the future of communities (Bazzaz, 1996). The physiological ecology of resource processing by the selected species was studied by conducting controlled laboratory experiments and combining these with field observations of natural patterns of microenvironments, with an emphasis on physical and chemical fluxes.

1.3 M24 HABITAT DESCRIPTION

C. dissectum occurs in a number of mire and fen meadow community associations and non-NVC associations, detailed in the next section. However, the M24 community is the only community where *C. dissectum* appears as one of the key indicator species. Therefore this community will be the basis for describing the habitat of *C. dissectum*. The M24 community is particularly valued for its rich floristic diversity, characterised by an abundance of sedges such as *Carex panicea, C. pulicaris, C. hostiana* and key species include hemicrytophytes such as *Cirsium dissectum, Succisa pratensis,* and *Potentilla erecta* (Rodwell, 1991b). Also, Culm grasslands in general support a wide range of invertebrates, birds and mammals, notably marsh fritillary *Eurodryas aurinia*, narrow-bordered bee-hawkmoth *Hemaris tityus,* soldier fly *Odontomyia argentat,* curlew *Numenius arquata,* snipe *Gallinago gallinago,* barn owl *Tyto alba,* dormouse *Muscadrinus avellanarius* and the globally threatened southern damselfly *Coenagrion mercuriale* (Cordrey, 1996). In addition, the M24 is unique as it is the only community where *C. dissectum* is present as a key species (Rodwell, 1991b). It is a rare and declining species with losses of 20% in south-west England since 1949. Details of the regional distribution for the present day compared with post 1949 are illustrated in Figure 1.2.

The species has a limited southerly distribution, as does the M24 community, and just under one third of the mainland UK distribution is found in the south-west of England (Rodwell, 1991b). Maps showing the geographical distribution of *C. dissectum* and the M24 community are in Figures 1.3a and 1.3b.

Figure 1.2 Distribution of *Cirsium dissectum* in the British Isles, number of 10 kilometre squares in which the plant has been recorded. Source: Ecoflora Database (Bath Information and Data Services, 1995)

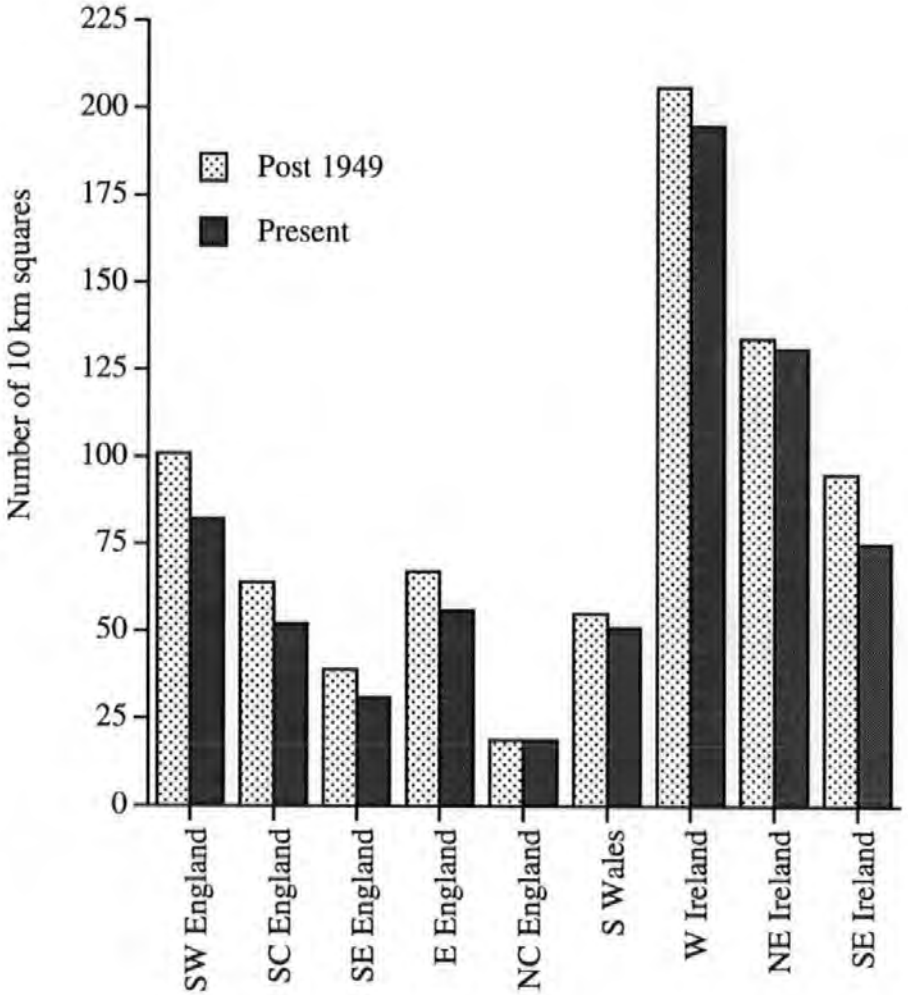
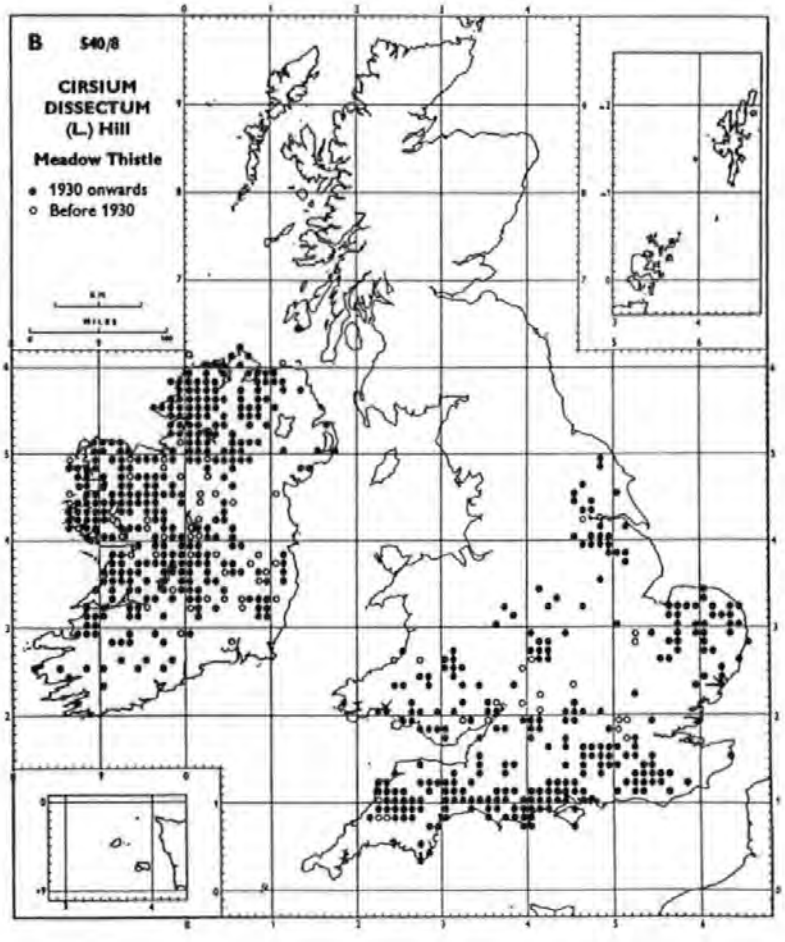


Figure 1.3a U.K. distribution map of *Cirsio-Molinietum* M24 fen meadow communities (Source: Rodwell, 1991b)

Figure 1.3b British Isles distribution map of *Cirsium dissectum*. (Source: Perring and Walters, 1982)



Rodwell (1991b) describes the M24 habitat in some detail and this is summarised as follows:

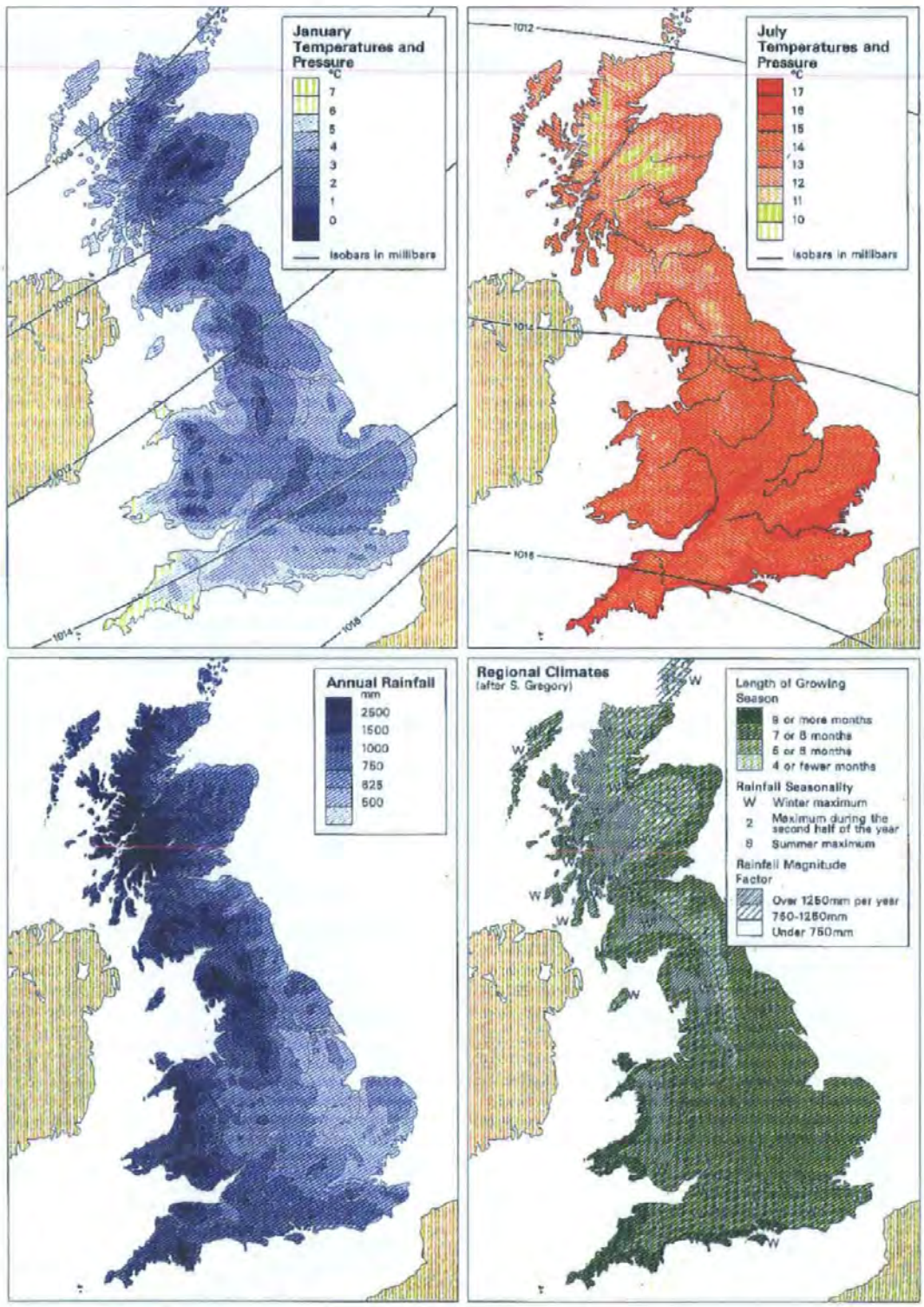
a) **Temperature:** this is a vegetation type of the warmer parts of Britain, almost all the known stands falling within the area where annual accumulated temperature exceeds 1200 day-degrees. Maps detailing temperature, annual rainfall and growing season are illustrated in Figure 1.4.

b) **Soil:** found over organic or strongly humic profiles that are of a generally intermediate character in terms of their moisture regime, base status and nutrient content. This is a community of circumneutral soils, with superficial pH generally within the range 5.0 - 6.5. These conditions set some broad but important limits on the composition of the community and generally confine its occurrence to situations that are transitional between mires on the one hand and grasslands and dry heaths on the other.

c) **Soil moisture:** the profiles range from fairly moist to quite dry and there is normally no marked seasonal fluctuation in water level or throughput.
The soils are seldom flooded to the surface, even in the wettest parts of winter, and they can dry out appreciably above in the summer months. The profiles are thus consistently better aerated than the permanently waterlogged or winter flooded peats.

d) **Floristic composition:** this can be very variable depending on the local edaphic conditions and management regime. However, *Molinia caerulea* is a constant component of the community and it can be abundant, forming the basis of a rough sward or occurring as a more strongly tussocky cover, depending on management.

Figure 1.4 Climate maps for mainland Britain detailing summer and winter temperatures, annual rainfall and length of growing season (Source: Ordnance Survey, 1982).



A comparative survey of habitat conditions of herbaceous rich fen vegetation types by Wheeler and Shaw (1987) included fifteen sites described as *Cirsio-Molinietum* which are equivalent to the NVC M24 classification. They described the habitat conditions as follows:

a) **Base richness:** pH 6.0 - 7.1; Calcium 1200 - 3000 mg l^{-1} peat;

Bicarbonate 251 - 460 mg l^{-1} with 71% of surveyed sites found over calcareous bedrocks.

b) **Water level:** By normal fen standards water level is low i.e. 10 - 25 cm below the soil surface and the community is generally associated with drier conditions found in fen margins but can also be found on flushed slopes where water level is in the range 9 cm below to 1 cm above the soil surface.

c) **Substratum fertility** (phytometrically estimated capacity to support plant growth): Generally classified as very low with a range from < 3 to 9 mg per plant (dry weight). Also, phosphorus concentration is very low between 0.25 to 0.40 mg P l^{-1} peat.

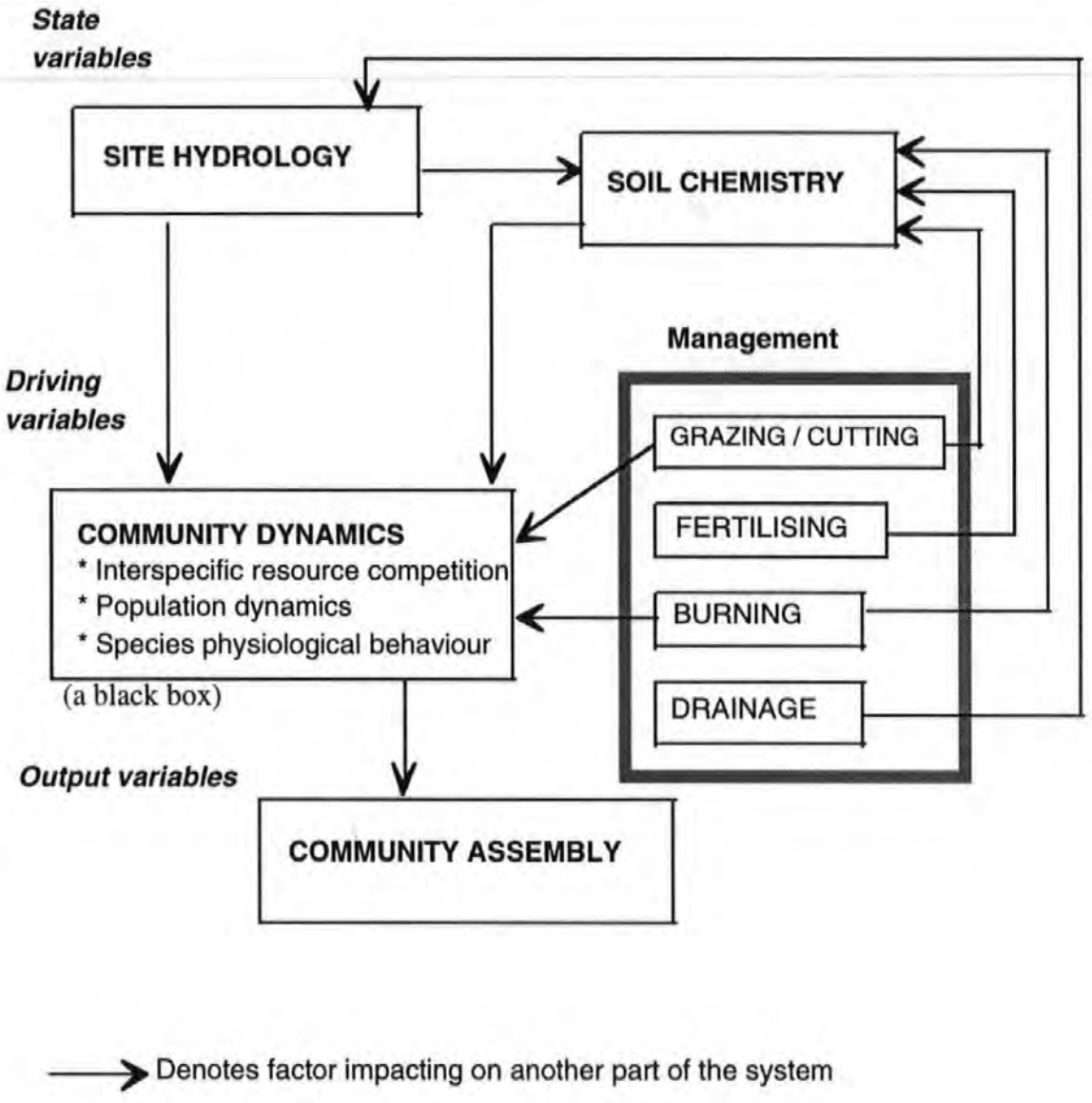
Compared with Rodwell's habitat description, Wheeler and Shaw recorded a slightly higher pH range and observed that soils can be flooded to the surface. Also, Wheeler and Shaw recorded low soil phosphorus content which was not specified by Rodwell.

In total some 90 species are associated with the M24 community at various levels of frequency and abundance. The associations may be categorised as plagioclimax maintained by summer grazing. A summary of the constant species of the community is detailed in Table 1.1.

Table 1.1 Constant species of the *Cirsio-Molinietum* M24 community, range of frequency and abundance (Source: Rodwell, 1991b)

Species	Common name	Frequency (%)	Abundance (% cover)
Molinia caerulea	Purple moor grass	81 - 100	1 - 75
Potentilla erecta	Tormentil	81 - 100	1 - 10
Succisa pratensis	Devil's bit scabious	81 - 100	1 - 33
Cirsium dissectum	**Meadow thistle**	61 - 80	1 - 33
Lotus uliginosus	Marsh bird's foot trefoil	61 - 80	1 - 10
Carex panicea	Carnation sedge	61 - 80	1 - 25

Figure 1.5 A generalised plant/environment model of a *Cirsio-Molinietum* fen meadow (N.V.C. M24) ecosystem



The community exists as a result of interactions between environmental/edaphic variables and the plant community dynamic variables which can both be modified, directly and indirectly, by specific management intervention. This is illustrated by a simple ecosystem model in Fig. 1.5 showing how management can affect the community dynamics directly or indirectly by altering the state variables. There is also an interaction between the state variables, with hydrology affecting soil chemistry. The final system output is the visible plant community which is normally classified by the component species and their individual frequency and abundance. Hydrology and soil chemistry represent sub-models within the system and whilst soil hydrology can be modelled (Gowing, Spoor, and Mountford, 1998), soil ecology models are more complex with certain aspects of the system not fully understood.

The least understood part of the system is the community dynamics with a complex interactions between physiological, intraspecific and interspecific plant processes. With such a complex system (sometimes referred to as a 'black box'), the ability to distinguish between causal and correlative factors becomes extremely difficult. Therefore a complete understanding of the whole system will involve understanding the simultaneous action of several factors.

The term fen is used in the context of this study to refer to the specific fen meadow M24 community. However, the term fen can be a rather vague habitat description, often connected with terms such as mire, bog, wet meadow and wet heath, since a fen is often regarded as a transition between waterlogged and dry ground conditions. Therefore site hydrology is often very variable within any of the above habitat descriptions, often confounding comparisons of community studies in the available literature. Most fen community studies have examined the community end state changes as a result of changes in hydrology, soil chemistry and management, either individually or in various combinations. Grootjans *et al.* (1986) examined the vegetation changes in *Cirsio-Molinietum* stands and the influence of drainage on N - mineralisation. After drainage, N - mineralisation was 2-3 times higher. Vegetation did not respond with increased yields but P availability was

depressed, pointing to P being an important limiting factor in this system. Hayati and Proctor (1990) investigated plant distribution on wet heaths, in relation to mineral nutrient availability. They found the pattern of uptake for every species and every element was different, suggesting this to be a factor in their stable coexistence. Fojt and Harding (1995) surveyed changes over 30 years in three valley mires and detail changes in community types as a result of changes in drainage, nutrients and management regimes. They concluded that a combination of drainage and dereliction resulted in the greatest changes at the community level. Drained mires showed the greatest alteration at the species level whereas dereliction showed the least change in associated flora. Wheeler and Shaw (1991) measured crop mass and species richness of several fen communities. They found that generally, crop mass was negatively correlated with species density and occurrence of rare species, concluding that low growing swards are particularly important for the conservation of many rare species.

As detailed above, the M24 community coincides closely with the mainland British distribution of *C. dissectum* not only in this country, but also in those parts of Ireland (White and Doyle, 1982) and of the Continent (Westoff and den Held, 1969) where similar conditions prevail. Since this is the only community containing *C. dissectum* as a key indicator species, along with it being the only constant phytogeographic indicator of the community, it was chosen as the main subject of this study.

1.4 A REVIEW OF *C. DISSECTUM*

In phytogeographic terms *C. dissectum* is an Oceanic West European plant (Matthews, 1955). Presently, very little published information is available on this species with no detailed Biological Flora and no entry in Comparative Plant Ecology (Grime, Hodgson, and Hunt, 1988). Ellenberg (1988) has produced the only ecological account of *C. dissectum*, where he has classified the plant according to his environmental indicator values for European plants which are detailed in the box below. Interestingly, Ellenberg does not included soil phosphorus within his environmental indicator values.

Light Value (7)

generally in well lit places, but also occurs in partial shade.

Temperature Value (7)

warmth indicator, only on lowland sites in the northern part of Central Europe.

Continentality Value (1)

extreme oceanic, only in a few outposts of Central Europe.

Water Value (8)

between (7) damp site indicator, mainly on constantly damp, but not wet, soils. and (9) wet site indicator, often in water saturated badly aerated soils.

Reaction Value (3)

acid indicator, mainly on acid soils but can also be found where there is a neutral reaction.

Nitrogen Value (2)

between (1) indicator of site poor in available N and (3) more often found on N deficient soils than on richer ones.

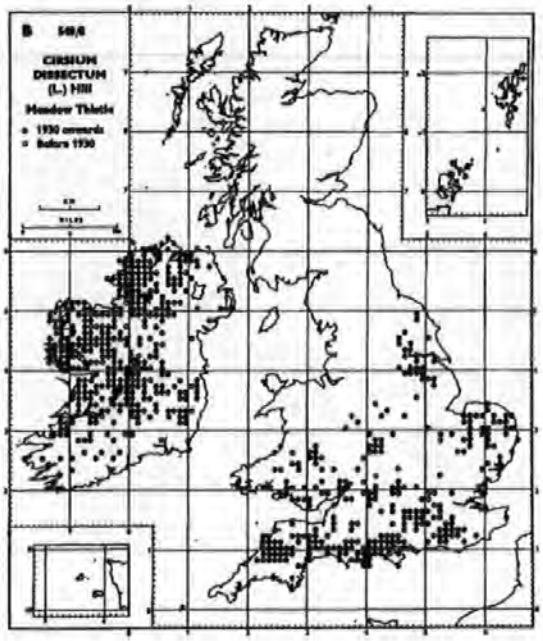
More detailed physiological research is confined mainly to five published papers. Hayati and Proctor (1990) included the species in comparative studies of species distribution in relation to the distribution of major nutrients in the soil. An analysis of the chemical composition of leaf material in June identified relatively high percentages of Ca, Mg, K, Na and P. They also found that plant Ca and Mn were positively correlated with soil Ca and Mn status and plant Mg and Na were negatively correlated with soil Ca. In a subsequent experiment, Hayati and Proctor (1991) investigated plant responses to nutrients (Ca, Mg, N, P, and K) added to pots of wet heath peat. This demonstrated that *C. dissectum* showed a strong positive response to lime but not $CaCl_2$, and to added P but not to N or K. In two reports to the Countryside Council for Wales (Kay and John, 1994; Kay and John, 1995), there were detailed results of seed production and viability of two South Wales populations. They also examined levels of genetic diversity between 33 populations in the UK and Eire and found the species to have a low apparent genetic diversity in mainland Britain. Grootjans, Schipper

and van der Windt (1986) investigated vegetation response to drainage on an M24 community and, although *C. dissectum* was recorded as present in one of their sample plots, the species was not detailed in their table of vegetation changes over the study period. Pegtel (1983) included *C. dissectum* in a glasshouse pot experiment, investigating response to nutrients and reported a moderate response to increased soil fertility and an unexpected phenotypic plasticity.

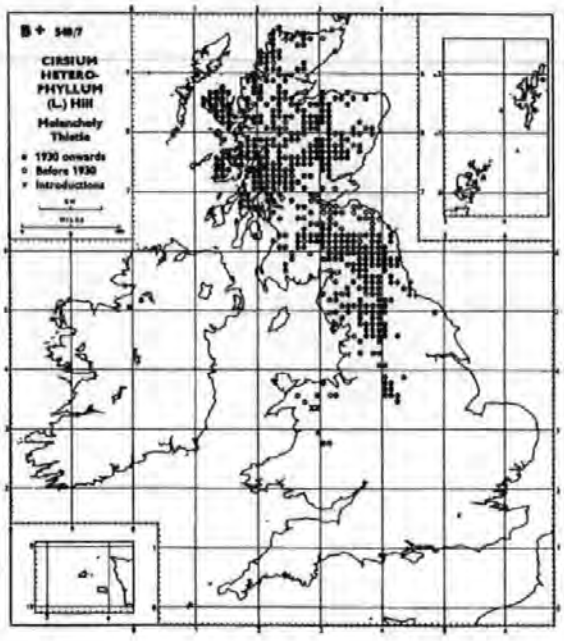
Apart from these publications, available information on *C. dissectum* mainly consists of morphological description, distribution and some floristic ecology (Smith, 1822). *C. dissectum* is described by most morphological texts as a long lived herbaceous perennial with a hemicryptophyte basal rosette growth form (Butcher, 1961; Clapham, Tutin, and Moore., 1987; Rose, 1981; Stace, 1997). It has been observed that the species behaves more like a biennial, flowering in its second year at the earliest, with the individual plant dying after seed set. However, unlike a biennial each plant will have reproduced vegetatively prior to flowering. From earlier experimental work, prior to this project, an unusual overwintering strategy was observed. The species would appear to be semi-deciduous, with the normal photosynthetic leaves senescing with the onset of winter and being replaced with about 4 -5 small narrow fleshy leaves. The plant then overwinters in this form, presumably storing reserves in these leaves which will have a very low photosynthetic rate. Vegetative spread is by underground stolons so that the individual plants form extensive leafy patches. These often occur as single isolated patches, perhaps formed by single clones. Today existing populations may be isolated by several kilometres from the next known site. Population recruitment from seed is low due to low numbers of viable seeds produced per plant and heavy predation (Kay and John, 1994), although viable seeds will germinate readily under a variety of environmental conditions (Ross and Williams, unpublished).

Figure 1.6 U.K. distribution maps for four species of the *Cirsium* genus
(a) *C. dissectum* (b) *C. heterophyllum* (c) *C. acaulon* (d) *C. eriophorum*
(Source: Perring and Walters, 1982)

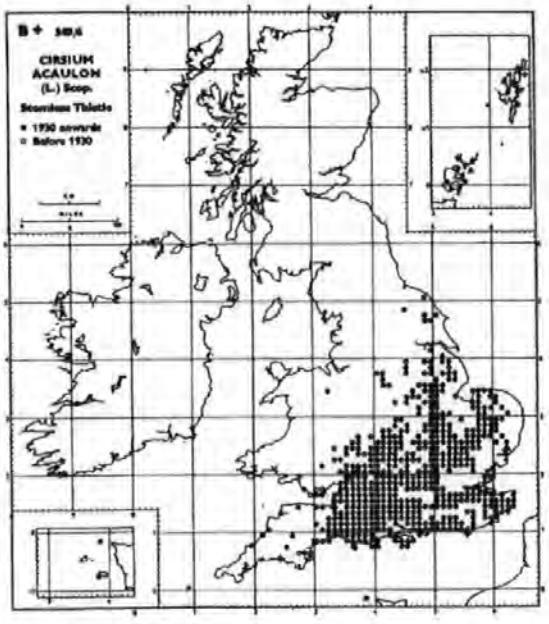
(a)



(b)



(c)



(d)

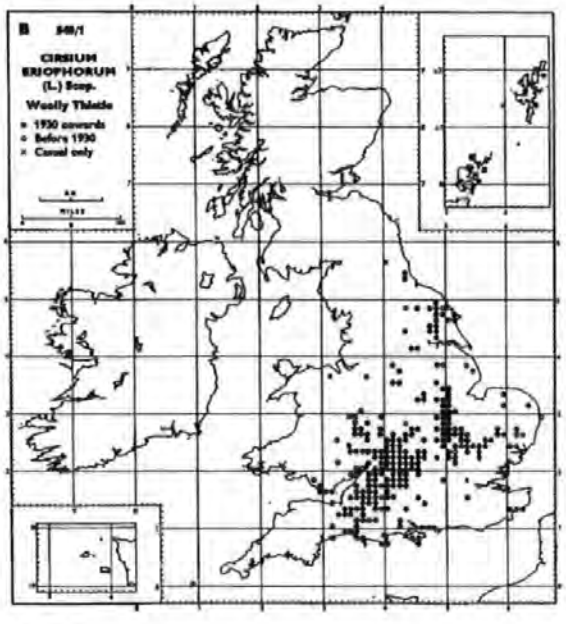


Figure 1.7 A matrtix of hybridisation between the eight British species of the *Cirsium* genus. Source: Stace (1997)
(X denotes two species capable of hybridising)

	C. t	*C. e*	*C. ac*	*C. ar*	*C. v*	*C. p*	*C. h*	*C. d*
C. d			X			X		
C. h						X		
C. p	X		X	X	X			
C. v		X	X					
C. ar			X					
C. ac								
C. e								
C. t								

Key:
C. d - C. dissectum; C. h - C. heterophyllum; C. p - C. palustre;
C. v - C. vulgaris; C. ar - C. arvense; C. ac - C. acaulon;
C. e - C. eriophorum; C. t - C. tuberosum.

There are eight species of the *Cirsium* genus found in the British Isles. Three of the thistles, *C. arvense, C. palustre* and *C. vulgare* are very common, often reaching pest proportions, and are found throughout Britain and Ireland. The other five species, *C. dissectum, C. heterophyllum, C. eriophorum, C. acaulon* and *C. tuberosum* all have a particular patterns of distribution. The closest species to *C. dissectum* would seem to be *C. heterophyllum* which has almost identical botanical characteristics. The main difference between these two species appears to be one of temperature preference which is illustrated by their respective north/south distributions in Britain (Fig 1.6a and 1.6b). *C. heterophyllum* is only found in upland meadows and grasslands in the northern stations of Britain and is virtually absent from Ireland. By comparison, *C. acaulon* and *C. eriophorum* have a southern British distribution but are both absent from Ireland (Fig 1.6d and 1.6e). Their particular preference is for relatively well drained dry soils, usually calcareous (Pigott, 1968; Toffs, 1999). Other differences are that *C. acaulon* is a stemless perennial with a rhizomatous root stock and requires high summer temperatures in excess of 20 °C, whereas *C. eriophorum* is a biennial, reproducing only from seed and is nitrophilous. The rarest of the species is *C. tuberosum* which is only found in a few locations in Wiltshire and South Wales. It is also confined to dry calcareous grasslands and is a long lived perennial with a tuberous root stock and only reproduces from seed. Links appear to exist between the species as all the British *Cirsium* species hybridise to a greater or lesser extent and this is illustrated by a matrix in Fig 1.7. In particular, there appears to be a close link between *C. dissectum, C. heterophyllum* and *C. palustre*. Apart from sharing similar habitats, both *C. dissectum* and *C. heterophyllum* hybridise with *C. palustre* and *C. dissectum* x *C. palustre* =*C. xforesteri* is one of the commonest hybrid thistle (Stace, 1997).

The phytosociological associations of *C. dissectum* in all published NVC communities in the UK are detailed in Table 1.2. This highlights the relatively small number of communities containing *C. dissectum* and the very limited range of habitat. To put this into context, Table 1.3 details all six key M24 species and compares their distribution by the number of different communities within each habitat type where they can be found.

Table 1.2 Summary and comparison of all plant communities containing *Cirsium dissectum* Source: Rodwell (1991a; 1991b; 1992; 1995)

Community	*Habitat*	*Occurrence of C. dissectum in main community and sub-communities*	*% Frequency*	*% Cover*
M13 *Schoenetum nigricantis* mire †(M24 - 4)	peat/mineral soils, valley mires pH 6.5-8.0, low P Ca 20-200 mg l^{-1}	Main	1-20	< 4
		a. *Festuca rubra-Juncus acutifloris*	1-20	< 4
		b. *Briza media-Pinguicula vulgaris*	1-20	< 4
		c. *Caltha palustris - Galium uliginosis*	21-40	< 4
M16 *Ericetum tetralicis* wet heath †(M24 - 1)	mineral soils/shallow peats, seasonally waterlogged pH 3.5-4.5, high Fe	Main	1-20	< 4-20
		a. Typical sub-community	1-20	< 4
		b. *Sucissa pratensis-Carex panicea*	21-40	< 4-20
M21 *Narthecio - Sphagnetum* valley mire †(M24 - 1)	permanently waterlogged acid peats pH 3.5-4.5	Main	1-20	< 4
		a. *Rynchospora alba - Sphagnum auriculatum*	21-40	< 4
M22 *Juncus subnodulosis - Cirsium palustre* fen meadow	moist base-rich peats and mineral soils pH 6.5-7.5	Main	1-20	< 4
		b. *Briza media-Trifolium* spp.	1-20	< 4*
		d. *Iris pseudacorus*	1-20	< 4
M24 *Cirsio-Molinietum* fen meadow	Moist to fairly dry peats and peaty mineral soils, pH 5.0-6.5	Main	61-80	< 4-33
		a. *Eupatorium cannabinum*	61-80	< 4
		b. Typical	61-80	< 4-10
		c. *Juncus acutifloris-Erica tetralix*	61-80	< 4-33
M29 *Hyperico-Potametum polygonifolii* soakaway	shallow soakaway and pools in peats and peaty mineral soils pH 4.0-5.5 low Ca and P	Main (only)	1-20	< 4-10
S24 *Peucedano - Phragmitetum australis* tall-herb fen	fen peats, moderate/high summer water table + some winter flooding pH 6.5-7.5 Ca 60-120 mg l^{-1}	Main	1-20	< 4
		f. *Schoenus nigricans*	1-20	< 4

† Number of key M24 spp. in the key spp. of this community
* With few individuals

Table 1.3 Occurrence of main M24 species in all recorded NVC communities, by habitat group. Numbers indicate the number of different NVC communities the species appears in, within each habitat group.
Sources: Rodwell (1991a; 1991b; 1992; 1995) and NCC (1988)

Habitat group (NVC)	*Molinia caerulea*	*Potentilla erecta*	*Succisa pratensis*	*Cirsium dissectum*	*Lotus uliginosus*	*Carex panicea*
Woodlands (W)	8	10	7	-	3	-
Mires (M)	26	21	17	6	11	23
Heaths (H)	14	21	9	-	-	11
Calcicolous grassland (CG)	4	7	12	-	-	5
Mesotrophic grassland (MG)	-	4	6	-	5	7
Montane & Calcifugous grassland (U)	6	16	6	-	-	7
Swamps & Tall-herb fens (S)	3	1	1	1	9	3
Sand-Dune (SD)	1	1	3	-	2	3
TOTAL	**62**	**81**	**61**	**7**	**30**	**59**

Although the NVC communities are useful as a general classification tool they are based on data sampled from a limited number of sites and do not necessarily reflect specific regional or local site variations. The omission of a species from a particular published community does not mean that it will not be found in the community. Apart from the key constant species, other non-listed species may appear occasionally at a low frequency as a result of a localised colonisation event. For example, *C. dissectum* has been personally observed in dune slack communities (Braunton Burrows, North Devon and Kenfig N.N.R., Glamorgan) but it is not listed in any of the NVC sand dune (SD) community floristic tables. Similarly, J.R.B. Tallowin, Institute of Grassland and Environmental Research (IGER) and R.J. Wolton, English Nature (EN) (personal communications) have both observed the species in mesotrophic grassland communities (MG5 and MG8) but again the species is not listed in those NVC tables. It is therefore possible that, due to the often very localised nature of *C. dissectum* populations, the species has been under-recorded by the limited site sampling method used to compile the NVC community data. All the NVC published communities containing *C. dissectum* have in common impeded drainage, peaty soils and low nutrient availability. However, there is a wide range of pH conditions between 3.5 to 8.0 suggesting the plant does not have any evolved calcicole/calcifuge preference.

Using a single species study to provide a basic understanding of the processes of an entire community has obvious limitations in extrapolating the results from the precision at the low hierarchic level (species ecophysiology) to the generality at the high hierarchic level (community dynamics). However, it has been argued (Bazzaz and Sipe, 1987) that an equally productive method of exploring interactions within the community is to describe the patterns of resource use by participating species, namely, using physiological behaviour of the individual to explain the community function, as mentioned earlier. The rationale for choosing this species to represent the community is twofold. It is confined to the narrowest range of habitat types and is found in fewer communities than any of the other five key M24 species. Also, the M24 is the only community which has *C. dissectum* as a key species, suggesting that this is its optimum ecological niche but not necessarily its physiological optimum. This suggests the species has a narrow niche but not whether this is a result of

narrow tolerances to environmental factors or whether it is a 'realized' niche as a result of competition with other species (Crawley, 1989). However, since *C. dissectum* has the narrowest niche of any of the M24 species, it makes it a logical choice as a diagnostic indicator of the major environmental and plant interaction factors present in the community. Secondly, this study will provide previously unpublished physiological and ecological data on a relatively rare and declining species which, at present, have been largely overlooked. All the other key M24 species, by comparison, have relatively detailed physiological and ecological accounts, e.g. Comparative Plant Ecology (Grime, *et al.*, 1988).

1.5 THEORETICAL PLANT : ENVIRONMENT RELATIONSHIPS

In seeking to understand how vegetation functions, Grime (1977; 1979) has proposed recognising the major adaptive strategies which have evolved in plants and related these to the processes which determine the structure and species composition of vegetation. He defines these strategies as groupings of similar or analogous genetic characteristics which recur widely among species or populations and cause them to exhibit similarities in ecology. The external factors which limit vegetation in any habitat may be classified into two categories. The first is described as *stress*, consisting of phenomena which restrict photosynthetic production, namely shortages of light, water and mineral nutrients. The second category is classified as *disturbance*, associated with partial or total destruction of the plant biomass such as the action of herbivores, pathogens and humans (trampling, mowing and ploughing). Within plant habitats permutations of stress and disturbance occur which influence plant function and these are associated with the evolution of three distinct plant strategies which are detailed in Table 1.4.

Table 1.4 A matrix of stress/disturbance and associated plant strategies

Intensity of **disturbance**	Intensity of **stress**	
	Low	High
Low	Competitors	Stress-tolerators
High	Ruderals	No viable strategy

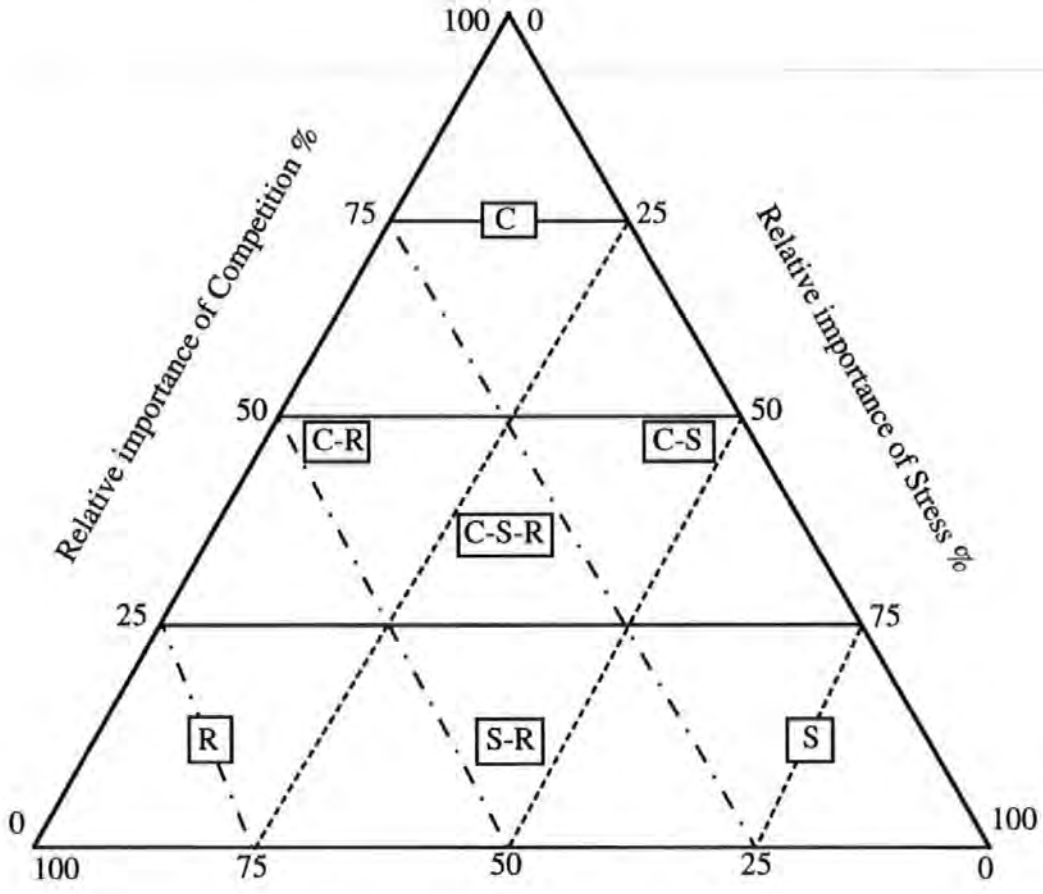
Grime (1979) describes this as the C-S-R theory and classifies species into *competitors, stress-tolerators* and *ruderals*. In addition to the three extremes of evolutionary specialisation, there will be various secondary strategies which will have evolved in habitats with intermediate intensities of competition, stress and disturbance. The intermediate categories are described as *stress-tolerant competitor, competitive ruderal, stress-tolerant ruderal* and *C-S-R strategist*. These functional strategies and various equilibria between stress, competition and disturbance are illustrated by an ordination triangle model (Figure 1.8). The above theory suggests that plants have evolved along three fundamentally different functional pathways:

i) Competitor (C) - high competitive ability which depends on plant characteristics which maximise the capture of resources in productive, relatively undisturbed conditions.

ii) Stress-tolerator (S) - reduced vegetative and reproductive vigour, adaptations which allow endurance of continuously unproductive, relatively undisturbed environments.

iii) Ruderal (R) - short life span with high seed production in severely disturbed but potentially productive environments. With C and S strategists, both adapted to low disturbance, it follows that competitiveness is inversely related to stress tolerance. Therefore stress excludes competitive species and reduces the importance of competition as a community structuring force. It is also suggested (Callaghan and Emmanuelsson, 1985) that at high levels of stress (e.g. arctic tundra habitats) plants can become mutualistic in their interactions. Each of these individual functional types generally have in common a range of physiological characteristics and some examples are detailed in Table 1.5. Therefore the individual physiological characteristics would not only classify a species within a functional type but also give a strong indication of the habitat conditions which should constitute its ecological niche and vice versa. This study used this theory to quantify the physiological characteristics *C. dissectum*, determine its functional type according to the C-S-R ordination model and used this information to make predictions about specific environmental parameters controlling the community dynamics.

Figure 1.8 C-S-R triangular ordination model showing the primary and secondary strategies and the various equilibria between stress, competition and disturbance. (After Grime, 1979)



Key: C *competitor*, S *stress-tolerator*, R *ruderal*,
C-S *stress-tolerant competitor*, C-R *competitve ruderal*,
S-R *stress-tolerant ruderal* and C-S-R *intermediate C-S-R strategist*

——— Relative importance of competition
. . . . . Relative importance of stress
· — · — · — · · Relative importance of disturbance

Table 1.5 Some physiological characteristics of the three plant functional types. Source: Grime (1979)

Physiology	**Competitive**	**Stress-tolerant**	**Ruderal**
Maximum potential relative growth-rate	Rapid	Slow	Rapid
Response to stress	Rapid morphogenetic responses (root:shoot ratio, leaf area, root surface area) maximising vegetative growth	Morphogenetic responses slow and small in magnitude	Rapid curtailment of vegetative growth, diversion of resources into flowering
Photosynthesis and uptake of mineral nutrients	Strongly seasonal, coinciding with long continuous period of vegetative growth	Opportunistic, often uncoupled from vegetative growth	Opportunistic, coinciding with vegetative growth
Acclimation of photo-synthesis, mineral nutrition and tissue hardiness to seasonal change in temperature, light and moisture supply	Weakly developed	Strongly developed	Weakly developed
Storage of photosynthate mineral nutrients	Most photosynthate and mineral nutrients are rapidly incorporated into vegetative structure but a proportion is stored and forms the capital for expansion of growth in the following growing season	Storage systems in leaves, stems and/or roots	Confined to seeds

The above review has focused on general ecological aspects of *C. dissectum*, its habitat and the theoretical relationships between plants and their environment. Specific aspects of plant physiology, namely competition, stress and disturbance, are however areas of scientific specialisation. In addition, stress physiology has many specialised subdivisions, for example water, nutrient, temperature and light stresses. Therefore, literature reviews on specific areas of relevant plant physiology are included in the introduction to those particular chapters.

1.6 OVERALL PROJECT AIMS AND OBJECTIVES

There were three main aims of this study:

1) Characterisation of the main environmental conditions on a range of sites where *C. dissectum* populations are established, in particular the *Cirsio-Molinietum* M24 fen meadow sites in Devon.

2) Examination of the autecology and physiology of *C. dissectum*, establish its functional type (according to the C-S-R theory), describe its optimum ecological niche and identify the key adaptations that enable it to survive in fen meadow habitats.

3) Identification of the main community controlling variables which will provide a basis for predicting responses to certain aspects of site management.

The specific objectives were therefore to:

1) Collect data on abiotic environmental conditions both physiographic and edaphic from a range of *C. dissectum* sites. Measure selected botanical and physiological characteristics of *C. dissectum* field specimens and collect seeds to provide experimental plant specimens.

2) Determine the relative water requirement and water metabolism of *C. dissectum* and the effects of shade and nitrogen availability on water metabolism. At the same time, quantify

the general growth and morphological responses to both shade and nitrogen availability.

3) Quantify the response of *C. dissectum* to two major stresses which are known to exist or could potentially occur on these sites.

i) *Water stress*: Although water does not appear to be limiting in most communities containing *C. dissectum* (Table 1.2), an unusually dry year or changes in site drainage could result in the development of water stress. Therefore it was proposed to examine water uptake and metabolism in *C. dissectum* along with the ability to withstand drought stress and tissue dehydration under controlled conditions.

ii) *Nutrient stress*: this is known to be a major stress factor on fen meadows, particularly the low availability of phosphorus (P). Various aspects of P requirement and metabolism were measured under controlled conditions.

4) Quantify the response of *C. dissectum* to defoliation which is a major disturbance on many M24 sites due to the presence of domestic grazing animals. The effects of defoliation severity and interactions with nutrient stress will be measured under controlled conditions.

To enable accurate determination of the various elements in objectives 2, 3 and 4 an underlying requirement, when designing the various experiments, was the control of a specific set of variables. Controlling variables such as light, temperature, nutrients etc. is virtually impossible under field conditions, therefore all of the subsequent experiments in this study were carried out under controlled conditions in growth chambers.

Achieving the above objectives has provided some indication of plant response to the interaction between the main environmental and management variables namely stress and disturbance. This enabled a better understanding of the community dynamics 'black box' within the ecosystem model (Fig 1.5). It is now possible to make testable predictions of the system output for a given set of environmental variables.

2.0 FIELD OBSERVATIONS OF HABITAT ENVIRONMENT AND GENERAL BOTANICAL CHARACTERISTICS OF *C. DISSECTUM*

2.1 INTRODUCTION

Autecology can be defined as "the study of a single species and its relationship with the environment" (Toothill, 1984). The environment consists of both biotic and abiotic components but this chapter is primarily concerned with the latter, focusing on climatic, edaphic and physiographical factors. Temporal and spatial variations in resources, especially water and nutrients, seem to be the main factors that determine the distribution of plants along environmental gradients (Schulze and Chapin, 1987). The apparent narrow niche and limited distribution of *C. dissectum* suggests that the abiotic environment plays a large part in determining population survival. The environmental conditions in habitats where *C. dissectum* is likely to be present have been documented by several authors (Ellenberg, 1988; Rodwell, 1991b; Wheeler and Shaw, 1987) and these are summarised in section 1.3. However, on any given site there is always a spatial element to the individual species distribution within a plant community. This can often be a result of fine scale differences in environmental heterogeneity. In the case of *C. dissectum,* a species which has a very patchy distribution, the environment and in particular the edaphic conditions, need to be characterised at the very localised scale where the species is found.

Chapter 1 identified Rhôs pasture and in particular the *Cirsio-Molinietum* fen meadow (NVC M24) as the main habitat for *C. dissectum.* The Rhôs pasture is arguably Devon's most important habitat as it represents a particularly large proportion of the UK resource, estimated at 80% of the habitat in England. Within the Rhôs pasture category, the M24 habitat is considered most valuable in terms of biodiversity and has been deemed a priority for conservation (Devon County Council, 1998). As a result this study has a strong regional bias which focuses on *C. dissectum* habitats in Devon and most of the field work in this study concentrates primarily on M24 sites in this county.

The overall aim of the fieldwork component within this study was to describe and quantify the abiotic environmental conditions from a range of sites where there are established *C. dissectum* populations. The specific objectives were to:

* Provide a physiographic description of the sites
* Measure seasonal soil water variations in relation to climate in Devon
* Measure seasonal phosphorus availability, a nutrient known to be limiting on fen meadows (Chapter 5, Section 5.3)
* Measure other edaphic factors, namely pH, calcium, potassium, organic content and soil type
* Measure spring leaf mineral nutrient content and identify whether the species has a mycorrhizal association
* Measure other plant botanical/physiological characteristics to test against the CSR model prediction.

This will provide essential baseline environmental data. Also, this data will be used to compare with a series of controlled environment experiments designed to measure plant response to a range of environmental variables. Those subsequent experiments will follow the 'comparative' approach described by Grime (1979) which will enable recognition of the main avenues of adaptive specialisation and identify characteristics of life-history and physiology which determine fitness (or lack of it) in a particular environment.

2.2 METHODS

Soil water content

Soil water content was recorded during April and July 1997 and April and July 1998 on all the main sites and some of the supplementary sites to compare early spring and mid-summer levels. These sampling times were chosen to represent the potential maximum and minimum soil water content during the main growing season for *C. dissectum* to estimate the degree of soil water fluctuation.

On each occasion recordings were taken at a time when there had been no rain for at least seven consecutive days. All the sites were visited within a 48 hour period. Soil water content was measured using a ML1 Theta Probe (Delta-T Devices, Cambridge). This device measures volumetric soil water content by applying a 100 MHz signal via a specially designed transmission line whose impedance is changed as the impedance of the soil changes. The probe measures the soil water content over a depth of 6 cm. Recordings were taken within an area where there was a well established *C. dissectum* population. The mean soil water content was calculated from a minimum of five readings, taken at random within the area occupied by the population. The readings were taken vertically from the soil surface, which was cleared of any surface vegetation, and therefore represented the soil water content in the surface top 6 cm rooting zone.

Soil sampling

Samples were collected in both April and July of 1997 and 1998, at the same times as the soil water content was recorded, as detailed in the preceding section. Also, two methods of sampling were used. An initial sampling was carried out on the main M24 sites by collecting several small samples (approximately 250 g) from the top 20 cm of soil, at least 3 m apart and in close proximity to a *C. dissectum* population, to measure within-site and between-site variation at each location. Subsequent sampling on the main M24 and supplementary sites involved collecting a single large sample (> 1 kg) from the side walls and core material of a 30 cm wide x 30 cm high x 30 cm depth pit. This bulk material was air-dried then thoroughly mixed to produce a homogenised soil sample to measure between-site variation of the supplementary sites and spring/summer variation of soil P over two years on the main M24 sites. All soil analyses were carried out on air-dried soil samples.

Soil analysis - Phosphorus

Phosphorus is a complex mineral present in soils both in inorganic (mainly soluble) and organic (insoluble) forms. Also the amount of extractable P in soils from Culm grassland sites is known to be low (Goodwin, *et al.*, 1998). As a result of the above and the fact that P may be an important limiting factor in these habitats, particular emphasis has be given to

the analysis and accurate determination of soil P. An estimation of available P is usually measured by replacement with an extractant, which in general measures the intensity factor or extent to which a soil can release phosphate (Allen, 1989). In the case of calcareous soils, a widely used method is extraction with Olsen's reagent (Olsen, *et al.*, 1964). A major problem associated with this method is an overreaction with the reagent, resulting in some normally less available forms of P (i.e. aluminium and calcium phosphates) being replaced by the extractant (Moore and Chapman, 1986). Also, Olsen's reagent has a pH of 8.5, higher than most of the soils in this study, which range from pH 4.5 - 7.0. Goodwin (1998) found that the Olsen's method extracted concentrations of P three orders of magnitude greater than P concentrations of soil solution extracted by centrifuge. The inherently low levels of P in soil solution extracted by centrifuge or suction cup methods, from these types of soils, make detection by colorimetry difficult and prone to errors as the concentrations can be close to (or below) the limits of detection (Goodwin, *et al.*, 1998). The Olsen's method, described in Allen (1989), was used in this study as it has been widely used, enabling comparisons with other published results. In view of the anticipated low amounts of P in the soils, overestimation and lower errors would be preferable from an analytical point of view.

An additional unresolved problem encountered by Goodwin (1995) was the variable coloration of the extracts, probably resulting from high levels of dissolved organic matter associated with peaty soils. This problem has been reported by other researchers (J.R.B. Tallowin (I.G.E.R), *pers comm*). Attempts by Goodwin (1995) and other workers to remove the colouring with activated charcoal were reported to be unsuccessful, resulting in inconsistent P enhancement. Variable degrees of coloration, ranging from pale yellow to dark brown, were found in preliminary extracts of the soil samples in this study. However, using a particular form of activated charcoal the solutions were successfully decoloured. Discussions with various suppliers revealed that many charcoal discolouring products are acid washed using phosphoric acid, resulting in P absorption by the charcoal. One particular form of activated charcoal available from Sigma-Aldrich (Poole, Dorset), product code C4386, is washed using hydrochloric acid, effectively leaching most of the P present in the charcoal and this was used to decolour the extracts.

From a process of trial and error, it was found that the quantity of charcoal required to produce completely clear samples following extraction was 0.5 g. The degree and uniformity of P gain or loss from this treatment was tested by comparing sets of charcoal-treated and untreated samples of the same P solution calibration standards. A comparison of the regression equations of the two calibration lines revealed almost identical slopes which only differed in their intercept. The mean difference between each calibration point on the slope was +0.04 mg kg^{-1} (± SD 0.005) for the treated samples, which suggests that the charcoal is not fixing any P but the presence of the bicarbonate reagent removes a very small amount P still present in the charcoal. This level of contamination (approximately 0.04 mg kg^{-1}) is well below the expected P levels of the soil extracts and would appear to be consistent. Therefore, the following method was devised to decolour the samples:

> Following the sodium bicarbonate extraction, 25 ml of the filtrate was shaken for 30 minutes with 0.5 g charcoal and re-filtered. The set of standard P solutions used to produce the calibration graph are also subjected to the same process, to take into account the additional P extracted from the charcoal. The charcoal-treated solutions are then taken through the rest of the process as normal.

Soil analysis - Calcium

Exchangeable Ca was determined by shaking 5 g of air-dried soil with 100 ml of 1.0 M ammonium acetate at pH 7.0 for two hours. Lanthanum chloride was added to remove interference from elements such as phosphate, aluminium and silicon (Allen, 1989). The Ca content was then determined by air-acetylene flame absorption using an atomic absorption spectrophotometer.

Soil analysis - Potassium

Extractable K was determined by shaking 10 g of air-dried soil with 50 ml of ammonium nitrate (1M) for thirty minutes. After filtering, the extract was then aspirated into an air-acetylene flame and the atomic emission determined using an atomic absorption spectrophotometer.

Soil analysis - pH

Soil pH was determined electrometrically by mixing 10 g of air-dried soil with 25 ml of distilled water, stirred for one minute, left to stand for 15 minutes then a reading taken. Due to the variability in readings encountered with distilled water suspensions of these soils (Goodwin, 1995), a replicate set of samples was also measured using 0.01 M $CaCl_2$, which has been shown to produce less variable, but lower, readings (Skinner, Church, and Kershaw, 1992). The readings from the distilled water samples, in this instance, did not exhibit any particularly high variability. A comparison between the two sets of results revealed that the mean $CaCl_2$ readings were consistently lower by 0.4 pH units (± SD 0.2). Therefore only the distilled water readings are presented in the results.

Plant mineral content analyses

All chemical analyses were carried out using oven-dried (70 °C) finely ground plant material. Due to the very low dry weight of leaves on an individual plant, it was necessary to bulk several plants to provide sufficient analysable material from which three replicates for each site were used. Total N, P, K and Ca were determined from a single wet-ash Kjeldahl digestion of samples using concentrated sulphuric acid and Kjeltabs NACT catalyst tablets (Thompson and Capper Ltd, Runcorn). A 100 mg plant sample was mixed with 10 ml acid plus one catalyst tablet and then digested for approximately one hour on a thermal block at 350 °C, the resulting digest was then made up to 100 ml with distilled water. This is a slightly modified version of a method by Gupta (1987) found to be reliable for small amounts of plant material. The method was substantiated by running a series of test digests with a reference plant material which confirmed that the level of digestion was consistent. The reference material was a large sample of oven-dried beech leaves (2.5 kg), all taken from a single tree, finely ground and stored in an air-tight container. The coefficient of variation between sample digests was 5% for P and Ca and 1% for K and N, which is an acceptable degree of error for the method. The beech powder was routinely included as a reference in all subsequent sample digests. The inorganic P fraction (P_i) was extracted by shaking 100 g

of sample material in distilled water, filtered and made up to 100 ml. This method was similarly calibrated with the beech powder as before and produced a coefficient of variation of 5%. After the digestion process, the following methods were employed to determine the specific mineral elements:

P - Colorimetric, stannous chloride reduction method (Allen, 1989) using a Tecator 5012 Flow Injection Analysis (FIA) system (Perstorp Analytical, Maidenhead). Acid concentration of digests was diluted to 1% to prevent interference with the ammonium molybdate-sulphuric acid reagents

N - Steam distillation with boric acid indicator followed by titration with standard hydrochloric acid (Allen, 1989).

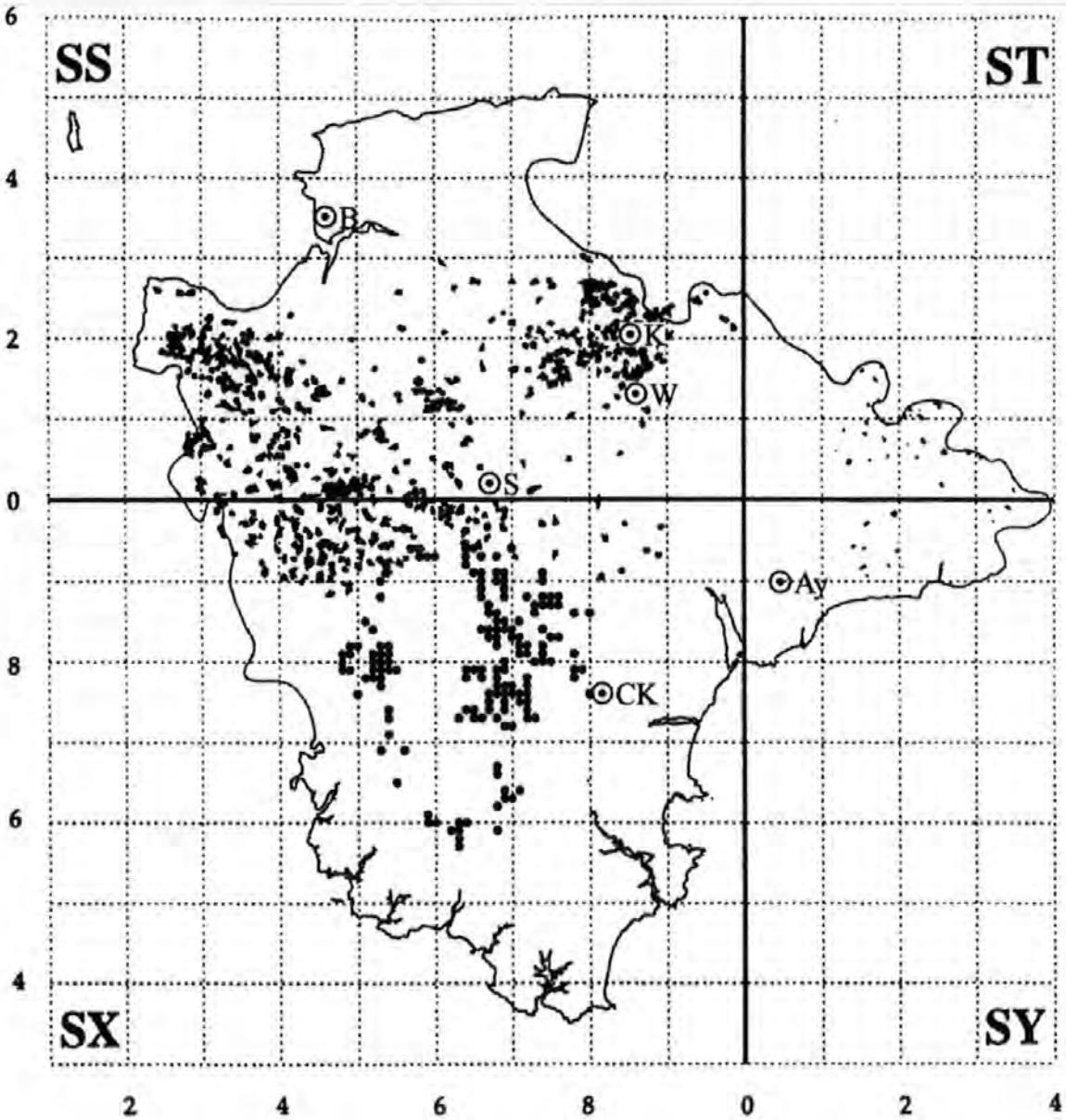
Ca - Air-acetylene flame absorption using an atomic absorption spectrophotometer. Lanthanum chloride was added to remove interference from elements such as phosphate, aluminium and silicon (Allen, 1989).

K - Air-acetylene flame emission using an atomic absorption spectrophotometer.

2.3 FIELD SITE DESCRIPTIONS

Five fen meadow sites throughout Devon were selected for detailed study containing M24 communities and these are referred to as the main field sites. The sites represent locations in North, South and Mid-Devon and vary in size, aspect, surrounding vegetation and degree of management. All the sites contain well established *C. dissectum* populations and information on past/current management was available. Other selection criteria included easy access by car to enable soil moisture data to be collected from all main sites within a two-day period and permission had been granted to carry out the various sampling procedures. A map detailing the location of the Devon field sites is shown in Figure 2.1. Three non-fen meadow sites, one in Devon and two outside (South Wales and Brittany), were also included in the study. These sites were known to contain *C. dissectum* populations and were included to provide a comparison of the edaphic conditions between the two types of site. These are referred to as the supplementary sites.

Figure 2.1 Distribution of Rhôs pasture (dark areas) and location of main and supplementary field sites (open circles) in the county of Devon. Grid squares (10 km) correspond with Ordnance Survey map letters and reference numbers. (Source: Devon County Council, 1998)
Key: Ay - Aylesbere, B - Braunton Burrows, CK - Chudleigh Knighton, K - Knowstone Moor, S - Staddon Moor.



The plant communities detailed in the various site descriptions are identified by the NVC classifications published by Rodwell (1991a; 1991b; 1992; 1995) and English Nature (N.C.C., 1988). The communities are referred to in the text only by their NVC classification and the full community descriptions are as follows:

M16 - *Erica tetralix-Sphagnum compactum* wet heath

M16b - *Succisa pratensis-Carex panicea* sub-community

M16d - *Juncus squarrosus-Dicranum scoparium* sub-community

M21 - *Narthecium ossifragum-Sphagnum papillosum* valley mire

M23 - *Juncus effusus/acutiflorus-Galium palustre* rush-pasture

M24 - *Molinia caerulea-Cirsium dissectum* fen-meadow

M24a - *Eupatorium cannabinum* sub-community

M24b - Typical sub-community

M24c - *Juncus acutiflorus-Erica tetralix* sub-community

M25 - *Molinia caerulea-Potentilla erecta* mire

M29 - *Hypericum elodes-Potamogeton polygonifolius* soakway

H4 - *Ulex gallii-Agrostis curtisii* heath

H4c - *Erica tetralix* sub-community

SD13 - *Salix repens-Bryum pseudotriquetrum* dune slack

SD14 - *Salix repens-Campylium stellatum* dune slack

SD15 - *Salix repens-Calliergon cuspidatum* dune slack

MG8 - *Cynosurus cristatus-Caltha palustris* grassland

W25 - *Pteridium aquilinum-Rubus fruticosus* underscrub

Also, all the geology and soil classifications detailed in the field site descriptions were derived from Findlay *et al* , (1984).

Description of the main field sites

Chudleigh Knighton Heath - (abbreviation code **CK**)

❑ *Location:* (Grid ref. SX 838 776) East of the Bovey basin on the south side of Dartmoor. Comprising a total of 45.6 ha of open lowland humid and wet heath, divided by

roads into three main compartments. The study site is the central largest compartment (20 ha) comprising of *Molinia caerulea* dominated M16 vegetation with birch and willow scrub, bracken and fringed with mature oak and birch to the west. The site grades gently from 45 to 25 m a.s.l., north to south, with wet heath in the lowest parts. Owned by Watts Blake Bearne and Co., Newton Abbot and was re-notified as a S.S.S.I. in 1988

❑ *Geology:* Drift over Jurassic and Cretaceous clay or mudstone.

❑ *Soil Classification:* Surface-water gley, typical stagnogley soil. Slowly permeable seasonally waterlogged fine loamy over clayey, fine silty over clayey and clayey soils over clay-enriched subsoil, prominently mottled above 40 cm depth.

❑ *Management:* Managed by the Devon Wildlife Trust and the site is covered by a Countryside Stewardship agreement. Management consists of grazing by either cattle, ponies or sheep and some ad-hoc scrub clearance.

Aylesbere Common Nature Reserve - (abbreviation codes **Aw** and **Ac**)

❑ *Location:* (Grid ref. SY 054 905) Lying on the dip-slope of the Budleigh Salterton Pebble-beds between 110 and 140 a.s.l., covering an area of 216 ha. The higher parts comprise acid dry heath mainly H4, W25, M16 and M25 communities. Two shallow valleys across the heath cut down into the Pebble-beds and underlying Permian sandstone. The lower slopes of these valleys contain wet heath, fed with water in part by runoff and seepage from the spring-line near the base of the Pebble-beds, and in part by localised springs (sometimes highly calcareous) or more diffuse seepage from the sandstone (Hayati and Proctor, 1990). The wet heath area contains a patchwork of plant associations which include M24a, b and c communities as well as M21 and M16. Two locations were chosen for this study. The first location, referred to as Aylesbeare Central (code: Ac), is a M24c community in a relatively flat dry area at the bottom of a north facing slope. The second location, referred to as Aylesbeare West (code: Aw), is approximately 400 metres west of the Ac location and is a M24 community in a wet tussocky area which follows the line of a spring.

❑ *Geology:* Permo-Triassic reddish mudstone and till.

❑ *Soil Classification:* Surface-water gley (typical stagnogley) soil. Slowly permeable seasonally waterlogged reddish fine loam over clay-enriched subsoil, prominently mottled above 40 cm depth.

❑ *Management:* Managed by the R.S.P.B. as a nature reserve. Light spring and summer grazing by beef suckler cattle at a stocking rate of one animal per hectare, plus occasional cutting and/or burning of selected compartments to remove excess *M. caerulea* litter.

Witheridge Moor - (abbreviation codes **Wm** and **Wt**)

❑ *Location:* (Grid ref. SS 865 155) One of the largest remaining continuous expanses of Culm grassland in Devon comprising 102 ha of moorland occupying an exposed plateau between 190 and 230 m a.s.l. The bulk of the vegetation is *M. caerulea* dominated M16 (45 ha), plus M23, M24, M25, M29, mesotrophic grassland communities and 18 ha of scrub and woodland. The whole site is privately owned by the Knightshayes Estate, Tiverton and for the most part is covered by a S.S.S.I. designation. Two locations on the site were chosen for this study. The first location is referred to as the Main moor (code: Wm), a compartment of 30 ha containing mostly *M. caerulea* dominated M16 with small patches of M23, M24 and M25 at the north east corner and bordered by birch and willow scrub/woodland to the north. The second location is a gently south sloping rectangular 5 ha field sheltered by a hedge to the east and birch woodland on the other three sides. This location is referred to as Tractor field (code: Wt), a species rich mesotrophic grassland with *Agrostis tenuis, Anthoxanthum odoratum, Holcus lanatus, Juncus articulatus* and sedges *Carex panicea, C. nigra* and *C. demissa.* In the south west corner of the field there is a wetter area with a *Molinia* dominated M24 community and large dense patches of *C. dissectum*, particularly where the sward has been kept short.

❑ *Geology:* Drift derived from Carboniferous sandstone and shale.

❑ *Soil Classification:* Surface-water gley (Pelo-stagnogley) soil. Slowly permeable seasonally waterlogged clayey, fine loamy and fine silty soils over clayey subsoil, prominently mottled above 40 cm depth.

❑ *Management:* The site is covered by Countryside Stewardship agreements (1992 and 1995) and is managed by the Devon Wildlife Trust. The Main moor is managed by summer

grazing with cattle at a stocking rate of one suckler with calf per 0.8 ha to maintain the sward at less than 15 cm. Rotational burning of areas in selected compartments is carried out. The Tractor field location is managed as a traditional hay meadow, cut in summer and occasionally grazed by sheep.

Knowstone Moor - (abbreviation codes **Ki** and **Ko**)

❑ *Location:* (Grid ref. SS 850 215) Rackenford and Knowstone Moors Nature Reserve is the largest reserve owned by the Devon Wildlife Trust, comprising over 121 ha wet heathland, rough pasture, scrub and woodland, designated a S.S.S.I in 1983. The reserve is just south of South Molton and Knowstone Moor (71 ha) and is split in two by the A361 North Devon link road, with the Inner Moor to the north and the Outer Moor to the south. Locations on both Inner and Outer Moors were used in this study. The Inner Moor (36 ha, code: Ki) has a gentle south facing slope, grading down from 252 m to 230 m a.s.l. and is dominated by M16b wet heath with some M25 to the north west and a long narrow (north/south strip) of M24 in the southwest corner which appears to follow a spring seepage gulley. There are substantial stands of gorse and scattered bracken stands with scrub bordering the east boundary. The Outer Moor (35 ha, code: Ko) has a complex topography but is essentially a north/west facing bowl grading down a 30% slope from 260 m to 210 m a.s.l. to a shallow valley bottom. The majority of the vegetation is M16b and M16d wet heath with a substantial M24 and M24c strip running east to west along the northern boundary valley bottom and at the highest parts of the slope it grades into H4c dry heath. There are also substantial stands of gorse and bracken.

❑ *Geology:* Drift derived from Carboniferous sandstone and shale.

❑ *Soil Classification:* Surface-water gley (Pelo-stagnogley) soil. Slowly permeable seasonally waterlogged clayey, fine loamy and fine silty soils over clayey subsoil, prominently mottled above 40 cm depth.

❑ *Management:* The reserve is managed for conservation by Devon Wildlife Trust with light summer cattle grazing at a stocking rate of approximately one animal per hectare and rotational winter burning. Gorse and scrub management is practised and extensive bracken control has been carried out.

Staddon Moor - (abbreviation codes **S** and **SI**)

❑ *Location:* (Grid ref. SS 680 025) Two adjacent fields previously studied by Goodwin (1995) one an unimproved species-rich fen meadow (code: S) with M16, M23 and M24 communities and the other an agriculturally improved permanent pasture (code: SI), dominated by *Holcus lanatus.* Situated on a north facing slope (< 5%) 190 m a.s.l., designated a S.S.S.I. in 1991.

❑ *Geology:* Drift derived from Carboniferous sandstone and shale.

❑ *Soil Classification:* Surface-water gley (Pelo-stagnogley) soil. Slowly permeable seasonally waterlogged clayey, fine loamy and fine silty soils over clayey subsoil, prominently mottled above 40 cm depth.

❑ *Management:* Unimproved 4.2 ha field - managed by the same owner for 40 years where store cattle were grazed in the summer and the sward topped in the autumn.
Improved 3.5 ha field - ploughed, reseeded and limed in the late 1940s. Subsequently reseeded in 1980 and received an annual application of 50 kg N, 11 kg P and 21 kg K in the form of a compound fertiliser in early spring. No *C. dissectum* present on this field.

Description of the supplementary field sites

Braunton Burrows - (abbreviation codes **Be** and **Bw**)

❑ *Location:* (Grid ref. SS 450 350) Sand dune and dune slack complex covering an area of approximately 1,500 ha, with *C. dissectum* present in a few dune slack communities, mainly SD14 and SD15. The site is privately owned by Christie Devon Estates, designated as a S.S.S.I., Biosphere Reserve, Area of Outstanding Natural Beauty and Heritage Coast site, but was de-classified as a National Nature Reserve in 1996. Part of the site is used as an M.O.D. training area. The two sampling sites, referred to as Braunton East (code: Be) and Braunton West (code: Bw) are approximately 400 m apart in an area known as Rowan Plain, which is 1,600 metres inland from the beach in the third row of slacks. The area is 8 m a.s.l., subject to seasonal flooding with an average annual rainfall of 879 mm and the prevailing onshore wind is from the west.

❑ *Geology:* Dune sand and marine shingle.

❑ *Soil Classification:* Typical sand-pararendzinas mainly deep well drained calcareous and non-calcareous sandy soils, often seasonally waterlogged in dune slack hollows.

❑ *Management:* Managed as a National Nature Reserve by English Nature until 1996 using light sheep grazing in small selected fenced paddocks and occasional scrub control but not on the areas containing *C. dissectum.*

Kenfig National Nature Reserve - (abbreviation codes **K1, K2** and **K3**)

❑ *Location:* (Grid ref. SS 860 760) Sand dune and dune slack complex covering an area of approximately 10 sq. km, with *C. dissectum* present in a few dune slack communities, mainly SD13 and SD14. Designated a S.S.S.I. and National Nature Reserve. Three dune slack locations all containing *C. dissectum.*were used for this study. The three locations are approximately one kilometre apart and are referred to as K1, K2 and K3.

❑ *Geology:* Dune sand and marine shingle.

❑ *Soil Classification:* Typical sand-pararendzinas mainly deep well drained calcareous and non-calcareous sandy soils, often seasonally waterlogged in dune slack hollows.

❑ *Management:* Managed as a National Nature Reserve by Bridgend County Borough Council using light grazing by sheep and occasional mowing.

Brittany, France - (abbreviation codes **B1a, B1b, B2** and **B3**)

Three sites containing *C. dissectum* were sampled by Dr. E.N.D. Williams (University of Plymouth) in September 1997. They are all within a very flat area of countryside approximately 200 m a.s.l..

Site 1: Guéméné. Located approximately 1.5 km north of the town on the east side of the D3 road. Sites consists of two 1 acre fields (codes: B1a and B1b) of permanent pasture on the east bank of the Scorff river, overgrown and dominated by *Juncus effusus* and *Angelica sylvestris,* with no signs of any recent management.

Site 2: Le Coscro (code: B2). Located approximately 5 km south west of Guéméné on the west side of the D18 road . The site is approximately 3-4 ha of permanent pasture, 500 m

west of the Scorff river with evidence of recent hay making. There was a high diversity of species, including large populations of *Scorzonera humilis*, and many large patches of *C.dissectum.*

Site 3: Kerosen (code: B3). Located 8 km east of La Faouet on the north side of the D782 road. The site is an isolated, unfenced patch 60 m by 40 m surrounded by improved permanent pasture typical of MG6 grassland dominated by *Lolium perenne,* approximately 200 m west of a Scorff river tributary. The patch is dominated by *M. caerulea* and, although there were cattle grazing on the surrounding grassland, there were no signs of incursion or grazing in this patch.

For ease of reference a summary of all the abbreviation field site codes used in subsequent Tables and Figures is contained Table 2.1.

Table 2.1 Summary of all field site abbreviation codes used in Tables and Figures.

Code	Main M24 sites	Code	Supplementary sites
Ki	Knowstone Inner Moor	K1	Kenfig location 1
Ko	Knowstone Outer Moor	K2	Kenfig location 2
Wm	Witheridge Main Moor	K3	Kenfig location 3
Wt	Witheridge Tractor Field	BRe	Braunton Burrows East
Aw	Aylesbere West	BRw	Braunton Burrows West
Ac	Aylesbere Central	B1a	Guéméné, Brittany, field 1
CK	Chudleigh Knighton Heath	B1b	Guéméné, Brittany, field 2
S	Staddon	B2	Le Coscro, Brittany
SI	Staddon Improved	B3	Kerosen, Brittany

2.4 SOIL WATER CONTENT

Soil water content has to be viewed in the context of the general weather conditions around the time of recording. A summary of monthly rainfall and temperature for both years are detailed in Figs 2.2a and 2.2b, which includes a comparison with the 30-year long-term mean (LTM). The data presented are from the Seale-Hayne Faculty weather station (Grid ref. SX 730 829) which is representative of the Devon climate south of the Dartmoor plateau. However, most of the Devon sites, apart from Chudleigh Knighton and Aylesbeare, are north of the Dartmoor plateau where weather conditions can vary from the south. A comparison between Seale-Hayne and North Devon data was made using records from IGER, North Wyke (Grid ref. SX 974 663) There were consistent differences, with the North Devon monthly LTM rainfall 7 mm higher and the temperature 1 °C cooler on average. A comparison between the two fieldwork years revealed that in 1997 the March and April rainfall was about half the LTM, June rainfall was twice the LTM but July was similar to the LTM. The subsequent year, 1998, was wetter, with spring and June rainfall at least twice the LTM and July approximately 1.5 times the LTM. There were no unusually high temperatures in either year, compared to the LTM, which could result in particularly high evaporation rates during the sampling periods. All the sites sampled in both April and July 1998 were at field capacity, sometimes with standing water, and as a result no comparative data are presented. Site comparative data for 1997 are illustrated in Fig 2.3 and each value is the mean of at least five samples. This, for the most part, highlights the similarity in water content between the M24 sites and the very small seasonal differences, with water content mostly above 0.4 $m^3 m^{-3}$. The lowest recorded water content was at Braunton Burrows, one of the two dune slack sites. Kenfig, the other dune slack site, was only recorded in July but had water contents similar to the M24 sites. Also, the Staddon improved site had relatively lower soil water content compared to the Staddon M24 site which was also demonstrated by Goodwin *et al* (1998).

Figure 2.2a Monthly **rainfall** in South Devon for 1997, 1998 and 30 year long-term mean recorded at University of Plymouth, Seale-Hayne Faculty weather station

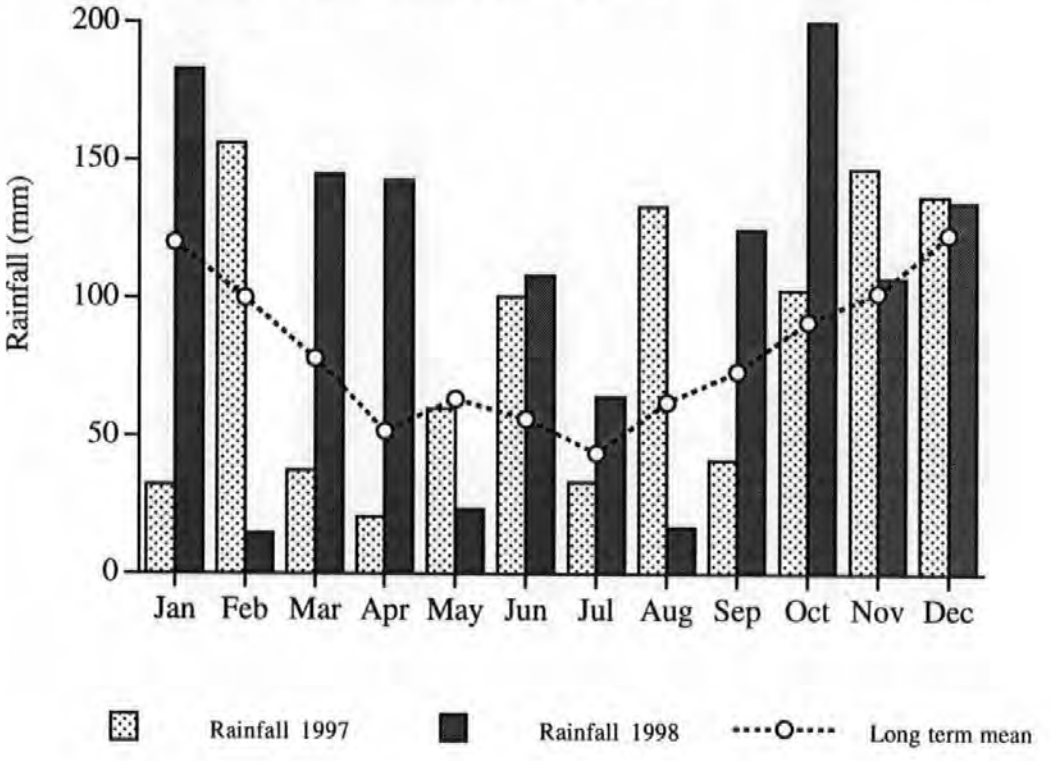


Figure 2.2b Monthly mean **air temperature** in South Devon for 1997, 1998 and 30 year long-term mean recorded at University of Plymouth, Seale-Hayne Faculty weather station

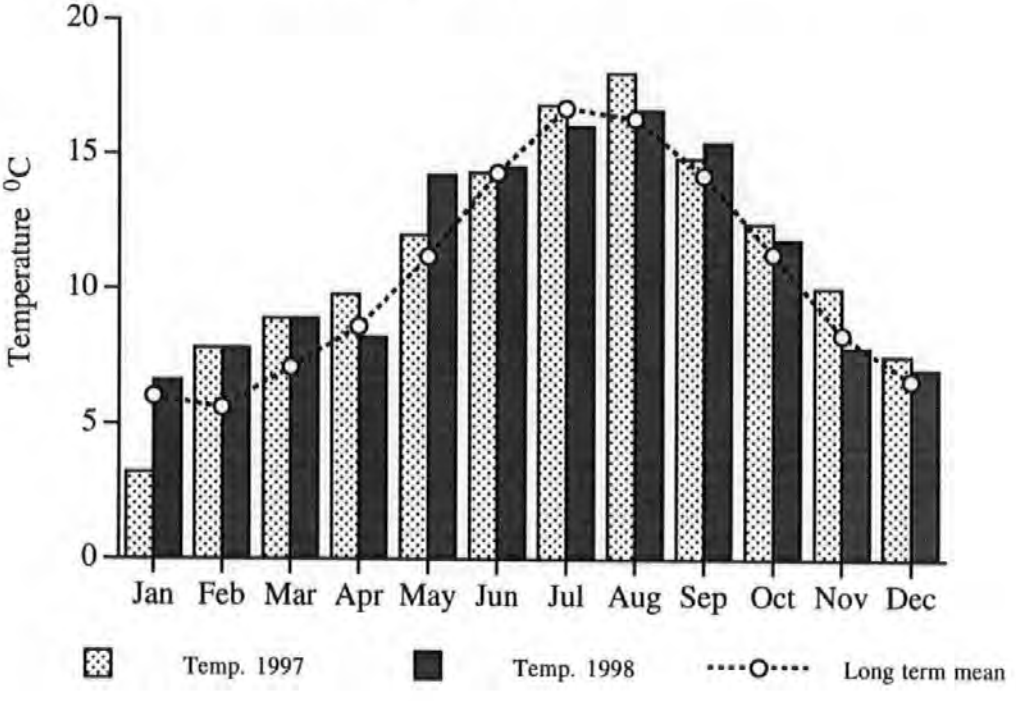
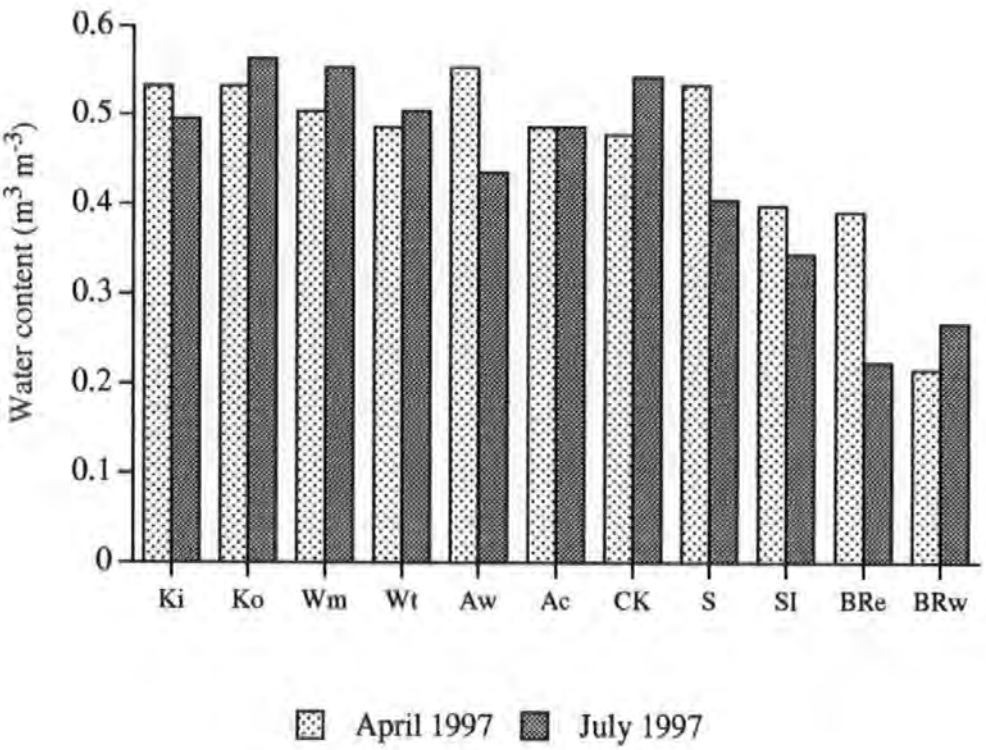


Figure 2.3 Comparison of spring and summer soil water content (volumetric) of field sites during April and July 1997
(See Table 2.1 for key to sites)



It is unfortunate that during the course of this study, it was not possible to monitor edaphic conditions during a particularly dry summer to observe the extreme limits of soil water deficit. There have been only five summers in the last thirty years where the combined June/July rainfall has been less than half the LTM (99 mm), with the lowest in 1976 which had a total rainfall of only 20 mm in June and July. The driest period, relative to LTM, recorded during this study was the combined March/April period in 1997, which was less than half the LTM (129 mm) and was also less than half the June/July period of 1997 (133 mm). The site soil water contents in 1997 (Fig 2.3) do not appear to reflect the fact that summer rainfall was higher than spring rainfall in 1997 (Fig 2.2a). This suggests that soil water may be more a function of site hydrology and water table rather than incident rainfall. Goodwin (1995) reported that the water table on a Culm grassland site (containing *C. dissectum*) did not fall below 5 mm below the soil surface throughout the summer of 1992, a year with a relatively dry spring and summer. It can therefore be assumed that it would require a substantial period of drought to reduce the water table or deplete the ground water recharge, and result in M24 sites drying out.

2.5 SOIL AND PLANT ANALYSES

Various studies have shown that P is a prime limiting nutrient in fen meadows (Egloff, 1983; Grootjans, *et al.*, 1986; Pegtel, 1983). In non-agricultural soils there is generally a predictable spring nutrient flush, particularly of P, associated with a spring increase in microbial activity and freeze-thaw or wetting-drying cycles that lyse microbial cells (Chapin, 1980). A more detailed review of the role and importance of P in plant physiology is contained in Chapter 5. Therefore, a major component of the soil sampling was to determine plant available soil P on the various field sites and whether there were any significant spring/summer fluctuations. The secondary component of the soil sampling was to provide comparative site data on calcium (Ca) and potassium (K) levels, along with soil pH and organic content. Nitrogen has been omitted from this study as values of nitrate and ammonium nitrogen are very variable, subject to environmental conditions and nitrification/denitrification processes. Experiments by Hayati and Proctor (1991)

demonstrated that nitrogen was not a limiting nutrient to *C. dissectum* in these soils.

2.5.1 *Soil Phosphorus*

The relative variability of extractable P was calculated from the coefficient of variation (Zar, 1996) and a comparison of the within-site variability of the main field sites detailed in Fig 2.4. This highlights that P on the Knowstone Inner site is highly variable, closely followed by Knowstone Outer and Witheridge Main, which are both over 40%, and the lowest variability was Staddon Improved field. The three highest variability sites have in common, relatively dense vegetation cover with a predominance of *Molinia caerulea* tussocks and surface standing water quite common. A comparison between the main sites of mean extractable P was analysed with a one-way ANOVA, which revealed significant differences ($P < 0.001$). A 'Tukey' test identified that the Knowstone Inner, Aylesbeare West and Aylesbere Central sites had significantly lower P than the Knowstone Outer and Staddon Improved sites, while all other sites were similar (Fig 2.5). A comparison of extractable P on the supplementary sites (Fig 2.6) illustrates that, with one exception (Brittany 1a), all the sites had relatively similar P levels, within the range 6 - 10 mg kg^{-1}, which closely matches the general range of the main M24 sites. The data for seasonal variation in extractable P on the main sites, over the two sampling years (Figs 2.7a and 2.7b), do not show any consistent patterns that would indicate a spring pulse of increased P. However, the four sampling periods do clearly confirm that extractable P is consistently very low, equivalent to an ADAS index of 0 or 1. This is considered likely to produce a failure in arable and glasshouse crops and is below the level required to produce an economic grazing or conserved grass crop (M.A.F.F., 1994). The maximum extractable P from any of the sites sampled rarely exceeds 10 mg kg^{-1}, which confirms that P is a severely limited resource on any habitats where *C. dissectum* populations are present.

Figure 2.4 Within-site variation of extractable soil Phosphorus on main field sites. Data are coefficient of variation (CV) expressed as a percentage. (See Table 2.1 for site key)

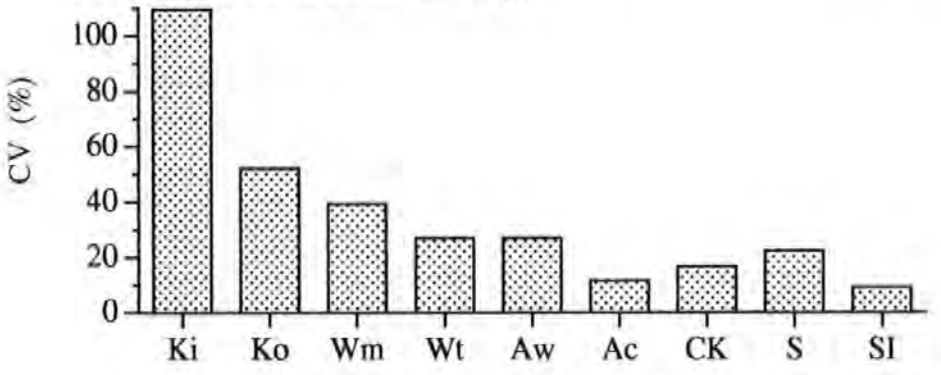


Figure 2.5 Between-site variation in extractable soil Phosphorus on main field sites. Data are means of 3-5 samples. Vertical bars are twice the SE of the mean, letters denote significant differences between means. (See Table 2.1 for site key)

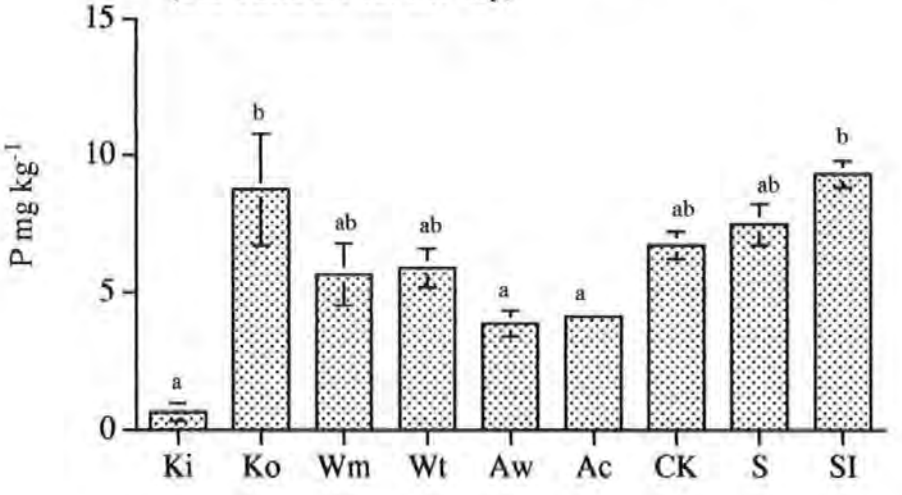
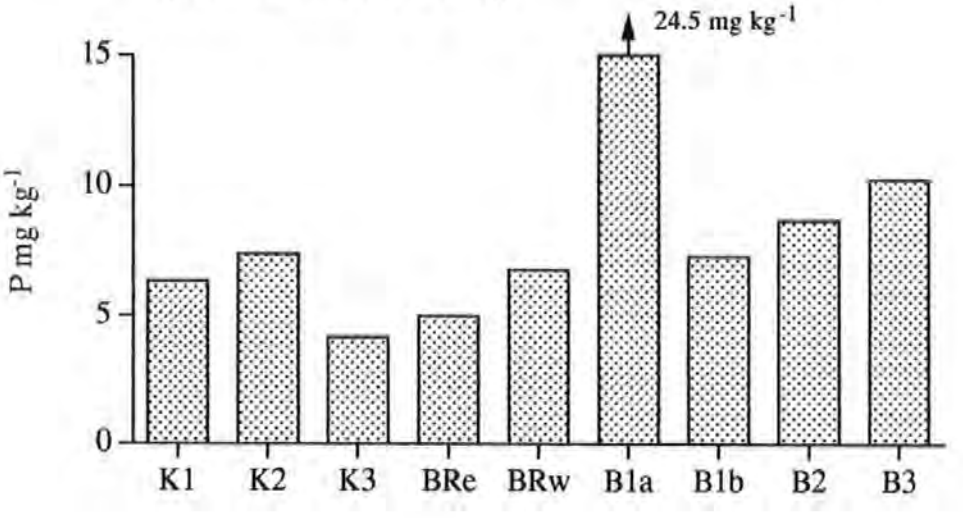


Figure 2.6 Extractable soil Phosphorus on supplementary field sites. Data are means of 3 replicates from a bulk sample. (See Table 2.1 for site key)



2.5.2 *Soil Calcium*

Calcium (Ca) is an essential macro-nutrient required for plant growth and metabolism. Its major plant functions are for cell wall strength, cell division and extension, membrane stability, cation/anion balance and osmoregulation (Marschner, 1995). It also plays an important role in stem elongation, inhibits leaf abscission and delays leaf senescence as well as providing a pH buffering effect in soils (Mengel and Kirkby, 1979). Although quite variable, several authors have reported that Ca is relatively abundant in M24 habitats (Hayati and Proctor, 1991; Rodwell, 1991b; Wheeler and Shaw, 1987), often as a result of calcareous spring-fed ground water. Hayati and Proctor (1990) also found that leaves of C. *dissectum* had relatively high Ca content which was positively correlated with soil Ca.

A comparison of the within site variability of exchangeable Ca on the main field sites detailed in Fig 2.8 which shows a smaller range of coefficient variation than soil P. An ANOVA of the between site variation in exchangeable Ca (Fig 2.9) revealed significant differences ($P< 0.001$) and a 'Tukey' test identified that Witheridge Tractor field and Staddon sites had Ca levels higher than that of Chudleigh Knighton (approximately 2 fold greater), which in turn was higher than that of the four other sites (approximately two fold greater). Therefore the variation in Ca on M24 sites can be about four fold, within the range of 350 to 1400 mg kg^{-1}. A comparison of the extractable Ca on the supplementary sites (Fig 2.10) reflects the high levels of Ca which would be expected on the two sand dune sites (Kenfig and Braunton). However, the Brittany sites all fall well within the range of the main M24 sites.

2.5.3 *Soil Potassium*

After nitrogen, potassium (K) is the mineral nutrient required in the largest amount by plants (Marschner, 1995). The K requirement for optimal plant growth is in the range of 2-5% of the plant dry weight for vegetative parts. It is a highly mobile univalent cation and plays a major role in the osmotic potential of cells and tissues, maintenance of turgor and control of stomatal movement by controlling turgor in guard cells.

Figure 2.7 Extractable soil phosphorus on main M24 field sites in April and July of (a) 1997 and (b) 1998. Data are means of 3 replicates from a bulk sample. (See Table 2.1 for site key)

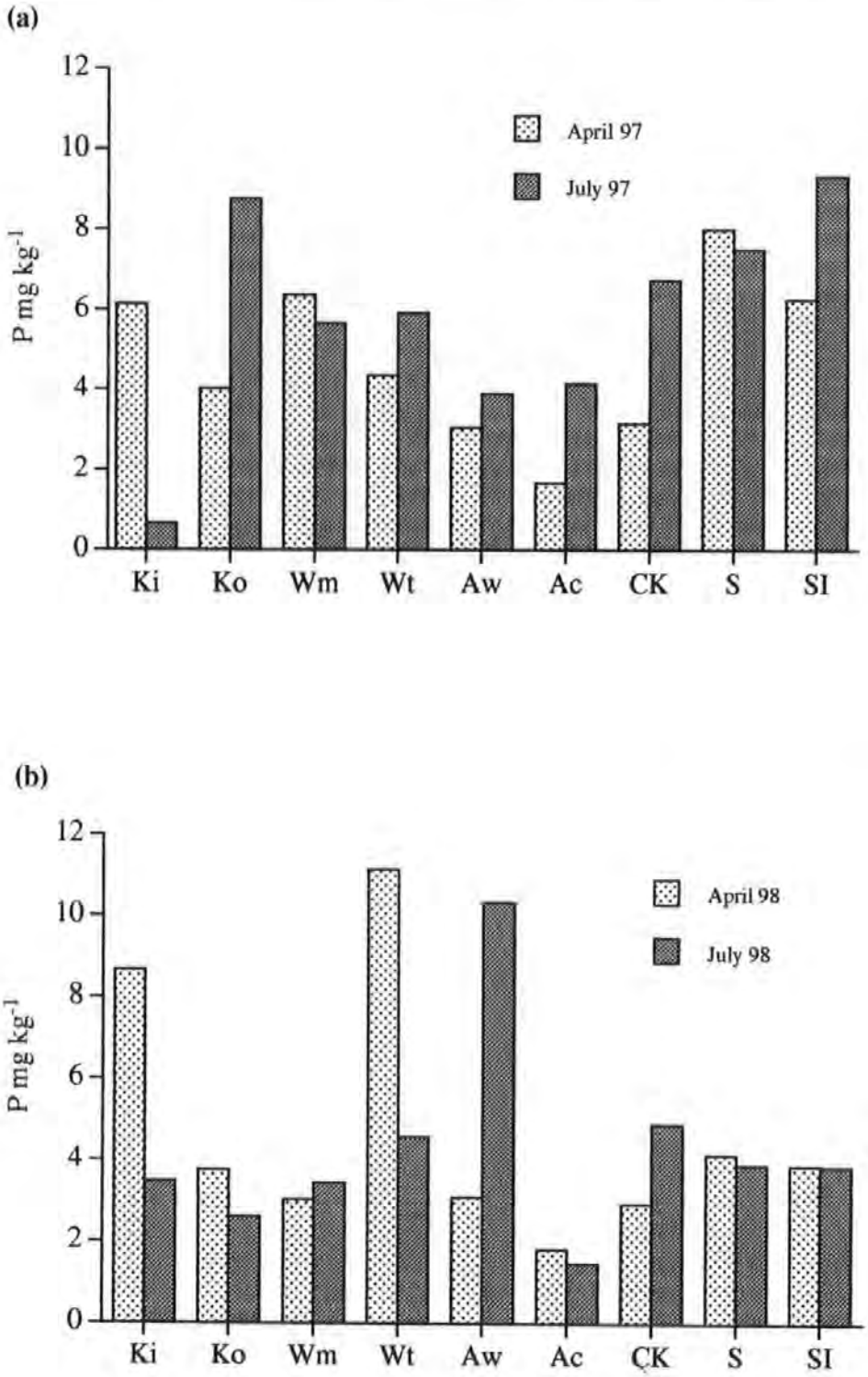


Figure 2.8 Within-site variation of exchangeable soil calcium on main field sites. Data are coefficient of variation (CV) expressed as a percentage. (See Table 2.1 for site key)

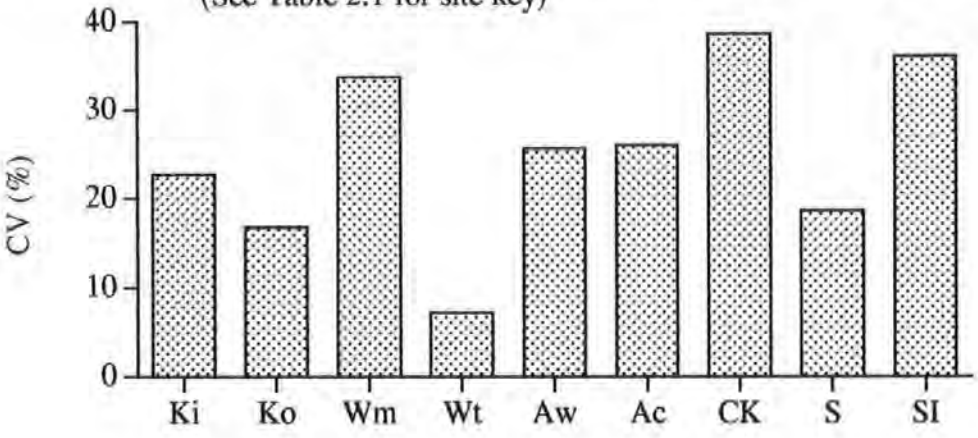
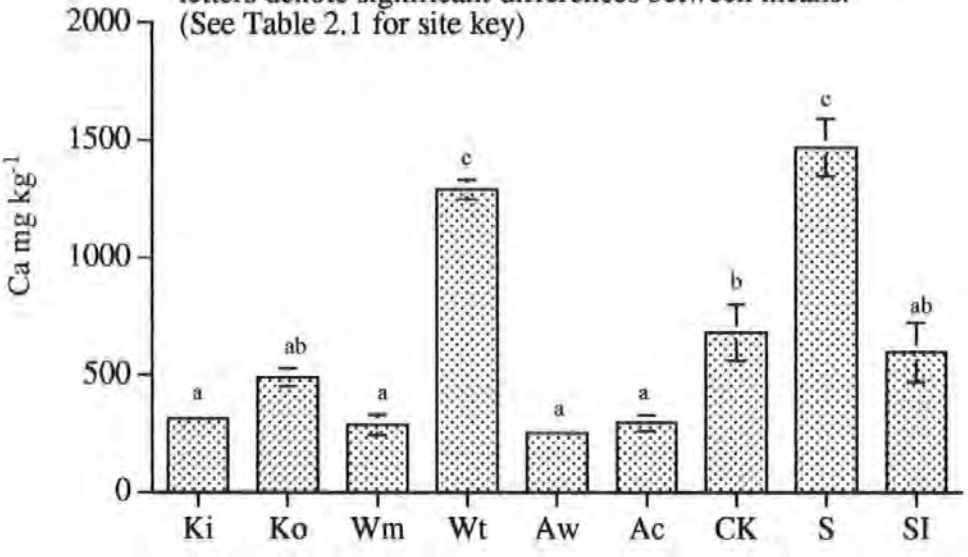
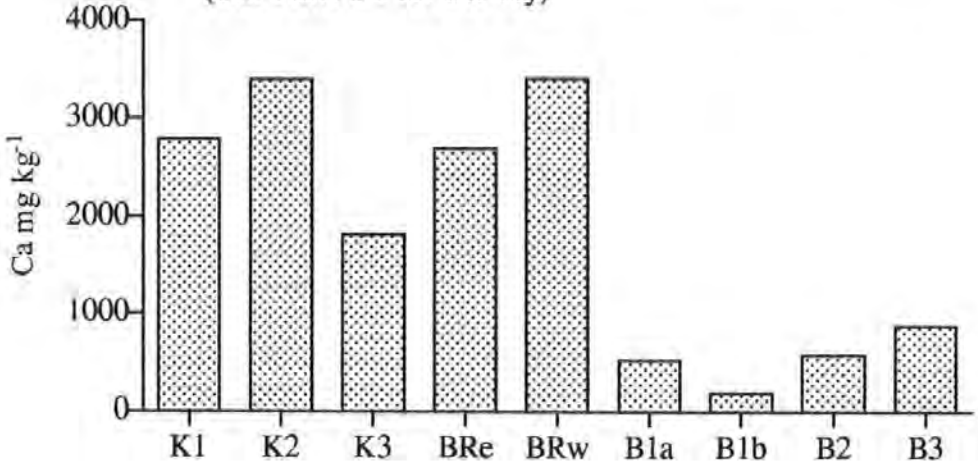


Figure 2.9 Between-site variation in exchangeable soil calcium on main field sites. Data are means of 3-5 samples. Vertical bars are twice the SE of the mean, letters denote significant differences between means. (See Table 2.1 for site key)



[Note: Graph scales differ by a factor of two]

Figure 2.10 Exchangeable soil calcium on supplementary field sites. Data are means of 3 replicates from a bulk sample. (See Table 2.1 for site key)



Other major functions include enzyme activation and carbohydrate metabolism, protein synthesis (e.g. chloroplast RuBP carboxylase), photosynthesis and CO_2 fixation, phloem transport (loading of sucrose) and cation-anion balance (Marschner, 1995; Mengel and Kirkby, 1979). Goodwin (1995) found that K was not particularly limiting on Devon Culm grassland soils, being typically much higher than in acid grasslands and wet heathlands, as a result of the clay-rich Culm soils weathering rapidly and releasing K-bearing minerals such as feldspars and micas. As with the leaf content of Ca, Hayati and Proctor (1990) also found that *C. dissectum* had a high leaf content of K but, in this case, it was not positively correlated with soil K.

The within-site variability of K on the main M24 sites (Fig 2.11) was lower than Ca, generally less than 25%, with five of the sites having a coefficient of 10% or less. An ANOVA of the between-site variation in K (Fig 2.12) revealed significant differences ($P< 0.001$) and a 'Tukey' test identified that Knowstone Outer had the highest K and Staddon the lowest. The range of variation on M24 sites of extractable K is approximately two fold, within the range 100 - 225 mg kg^{-1}, a much lower degree of variation compared to that of exchangeable Ca. A comparison of extractable K on the supplementary sites (Fig 2.13) highlights the low K levels on Kenfig and Braunton, the two sand dune sites, whereas K levels on the Brittany sites were similar to those on the main M24 sites. The lower K levels on the sand dune sites will be mainly due to the much lower clay content of these soils as K is mainly present in the secondary clay minerals which largely make up the clay fraction of the soil (Mengel and Kirkby, 1979). Therefore, with the exception of the sand dune communities, sites containing *C. dissectum* have K levels which are non-limiting and equivalent to an ADAS index 2, considered adequate for both agricultural and horticultural crops (M.A.F.F., 1994).

Figure 2.11 Within-site variation of extractable soil potassium on main field sites. Data are coefficient of variation (CV) expressed as a percentage. (see Table 2.1 for site key)

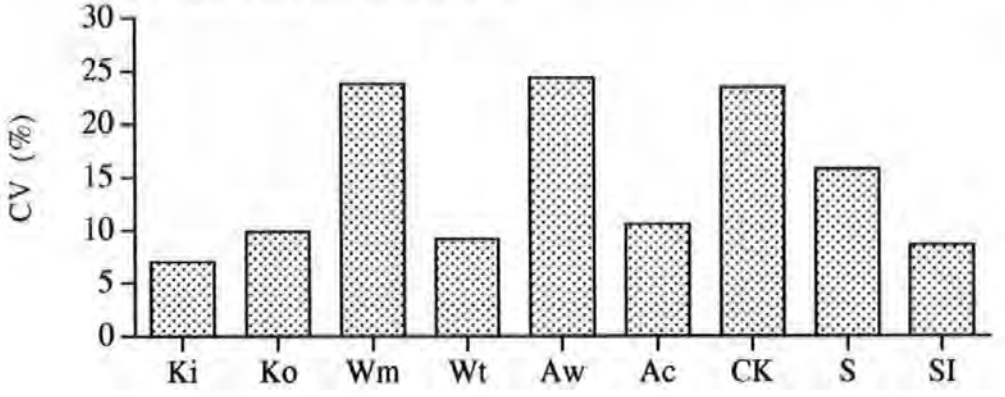


Figure 2.12 Between-site variation in extractable soil potassium on main field sites. Data are means of 3-5 samples. Vertical bars are twice the SE of the mean, letters denote significant differences between means. (See Table 2.1 for site key)

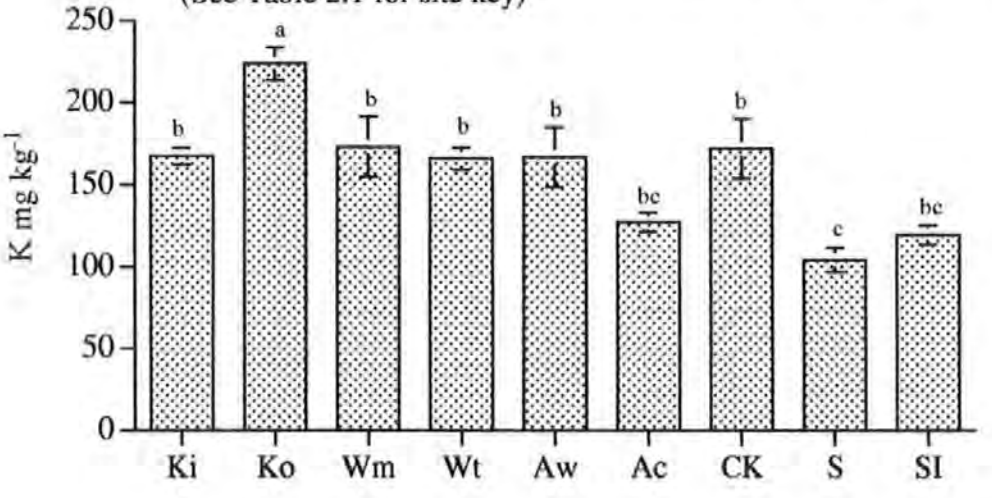


Figure 2.13 Extractable soil potassium on supplementary field sites Data are means of 3 replicates from a bulk sample. (See Table 2.1 for site key)

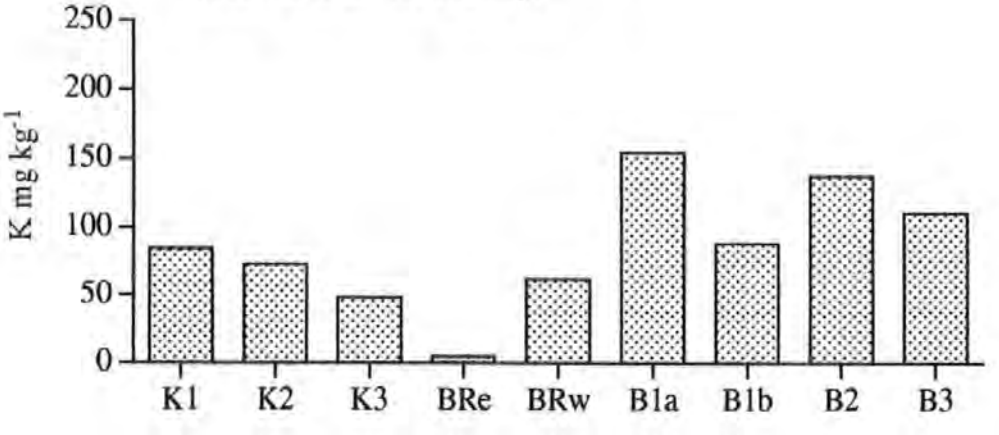
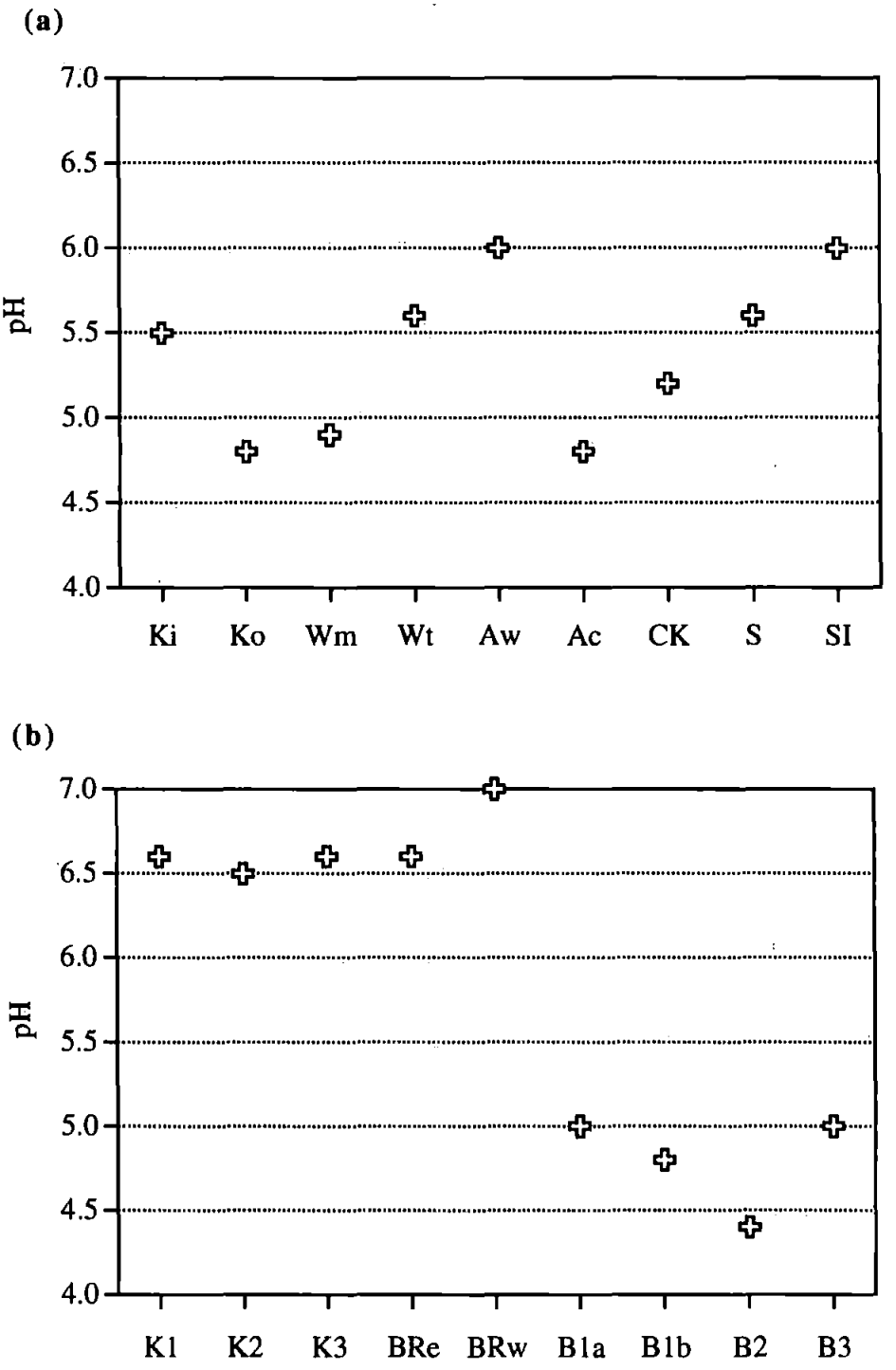


Figure 2.14 Soil pH using distilled water suspensions of air dried soil for (a) main field sites and (b) supplementary sites. (See Table 2.1 for site key)



2.5.4 *Soil pH*

Soil pH is a useful value when considering plant nutrition and chemical properties of soils (Wild, 1988). The optimum pH for crop growth is related to soil texture, being low in organic soils, e.g. peats with organic content greater than 20%; pH 4.0, and rising in mineral soils with increasing clay content, e.g. silty loams and clay with clay content greater than 15%; pH 7.0 (Mengel and Kirkby, 1979). Aluminium salts reduce growth and can be toxic to plants and, at low pH levels, aluminium becomes more soluble and comes into soil solution. Also, as pH levels progressively decline below 5.0, there is a corresponding decline in the availability of major nutrients (e.g. N, P, K and Ca), particularly in organic soils (Mengel and Kirkby, 1979). Conversely, at higher pH levels (greater than pH 6.5), the availability of P is considerably reduced and this aspect is discussed in more detail in Chapter 5.

The main M24 sites had a pH range between 4.8 and 6.0 (Fig 2.14a), with a relatively low between-site coefficient of variation (9%). The supplementary sites (Fig 2.14b), exhibited a higher range (pH 6.3 - 7.0) in the sand dune sites (Kenfig and Braunton) which was to be expected from the much higher Ca present in these soils. The Brittany sites were within the lower range of the main M24 sites.

2.5.5 *Soil organic content*

Arable topsoils commonly contain 1 to 3% of organic carbon, with grassland and forest soils often containing somewhat more, particularly if poorly drained (Wild, 1988). Also, the carbon:nitrogen ratio (C:N) of topsoils is relatively constant, within the range 10 to 14, for most soils, with the exception of strongly acid (pH < 5.0) and poorly drained soils (Wild, 1988). A C:N ratio greater than 14 is a strong indication that the soil contains much partially decomposed plant material.

Three replicates of a bulked soil sample from each site (collected July 1998) were analysed for carbon and nitrogen using a LECO® FP-2000 infrared gas analyser (Leco Corporation, Michigan, USA).

Table 2.2 Soil organic carbon (C) content and C:N ratios for all field sites. Data are means of three replicates from a bulk sample collected in July 1998.

Main M24 sites			Supplementary sites		
Site*	Organic C content (%)	C:N Ratio	Site*	Organic C content (%)	C:N Ratio
Ki	5.2	13.4	BRe	2.1	11.8
Ko	4.3	15.4	BRw	6.8	14.4
Wm	4.8	12.6	K1	6.6	15.8
Wt	4.6	11.6	K2	6.8	15.0
Ac	4.9	14.1	K3	2.8	16.3
Aw	7.8	15.7	B1a	11.7	12.2
CK	6.3	12.5	B1b	4.6	12.1
S	4.6	10.7	B2	9.2	12.2
SI	3.0	10.5	B3	10.6	12.6
Mean (SD)	5.1 (1.34)	12.9 (1.88)	Mean (SD)	6.8 (3.29)	13.6 (1.79)
Coefficient of variation (%)	26.5	14.5	Coefficient of variation (%)	48.0	13.2

*See Table 2.1 for key.

The results are detailed in Table 2.2 which illustrates the range of organic carbon on the main M24 sites between 3 and 7.8%. Organic carbon in the supplementary sites had a wider range, where the Brittany samples generally had higher carbon than the main M24 sites and two of the sand dune sites (Kenfig and Braunton) had carbon contents lower than the main sites. However, there was very little difference in C:N ratio between the main and supplementary sites. Also, the level of variation in C:N ratio between the main M24 sites was relatively low with a coefficient of variation of only 14.5% and only two sites (Knowstone Outer and Aylesbeare West) had ratios greater than 15. Interestingly, all three Kenfig sand dune locations had ratios of 15 or more, suggesting slow decomposition on this site.

2.5.6 *Leaf mineral nutrient content*

The main factors which control the mineral content of plant material is primarily the specific, genetically fixed nutrient uptake potential for the different mineral nutrients and secondly, the availability of plant nutrients in the soil medium (Mengel and Kirkby, 1979). Soil nutrient analysis indicates the overall level of potentially available nutrients under conditions favourable for root growth and root activity, whereas plant mineral nutrient analysis reflects the actual nutritional status of a plant (Marschner, 1995). Therefore, plants were analysed from the main M24 field sites in April 1998 to provide an indication of nutritional status which can be compared against soil nutrient potential. Spring sampling was chosen as this is the period when soil P is likely to be at its highest availability. In general the nutritional status of a plant is better reflected in the mineral element content of the leaves than in any other plant organs (Marschner, 1995). Thus leaf samples were used in the following analyses.

The results of the analyses are summarised in Table 2.3, which illustrates that there is some degree of variation between sites. However, the overall between-site variation is relatively low and within a narrow band, with coefficients of variation for the individual elements ranging from 19.5% (K) to 28% (P). Table 2.3 also includes data from Hayati and Proctor (1990) for *C. dissectum* which indicates that the results of this study have produced similar

results for Aylesbere heath with the exception of K, which this study shows to be higher. To provide a general comparison of these results with other species, two other published sets of data are examined. Table 2.4 details an extract from a study of 83 species by Thompson *et al.* (1997) from sites in the Sheffield area. These particular species were selected as they represent a range of species which have all been recorded in M24 main and sub-communities. This shows *C. dissectum* to be in the middle of the species range for P and Ca content, at the lower end of the range for N content and at the highest end of the range for K content. Table 2.5 details the full results of Hayati and Proctor (1990) for a range of species (including *C. dissectum*) from an M24 site also used in this study. In this case, the results of this study show *C. dissectum* to have a relatively high content of all four minerals, particularly Ca and K, compared to most of the other species. A comparison between the leaf mineral content and the potentially available soil nutrient in the six sites sampled was carried out using regression analyses. This revealed no significant relationship between plant mineral content and soil mineral content for either P, K or Ca. Hayati and Proctor (1990) found leaf Ca in *C. dissectum* to be positively correlated with soil Ca, which was not the case in this study. However they collected a much larger number of both soil and plant samples within a single site compared to this study.

Marschner (1995) identified that for each macro-nutrient mineral element there is a well defined plant nutrient content range (specified as a percentage of plant dry weight) which is required for optimal growth. The results of this experiment indicate that for *C. dissectum* Ca and K content are within the optimal ranges but both P and N are below the optimal ranges (0.3 - 0.5% P; 2 - 5% N). However, when compared with the other species detailed in Table 2.5, similarly low contents of P and N would seem to be common in species from this particular habitat.

Table 2.3 Mineral nutrient content of *Cirsium dissectum* leaf samples collected from main M24 field sites during the second week of April 1998. Calculations are based on dry weight. Data in brackets in second row of Aylesbere Heath are comparative results from work by Hayati and Proctor (1990).

Site*	No. of plants	% P total	Proportion inorganic P	% Ca	% K	% N
Witheridge Tractor field	11	0.33	0.45	1.31	2.30	1.81
Knowstone Outer Moor	7	0.18	0.36	1.67	4.10	1.55
Knowstone Inner Moor	6	0.20	0.26	1.32	4.03	1.42
Aylesbere Heath (Hayati and Proctor, 1990)	8 (42)	0.20 (0.15)	0.33 -	2.09 (1.85)	3.71 (2.27)	1.81 (1.33)
Staddon Moor	1	0.13	*	1.91	3.22	1.32
Chudleigh Knighton Heath	10	0.19	0.34	1.16	3.22	2.30
All sites mean (± SD)		0.21 (0.06)	0.35 (0.07)	1.58 (0.37)	3.43 (0.67)	1.70 (0.36)

*See Table 2.1 for Key

Note: With the exception of Staddon Moor, all data are means of 3 sub-sample replicates of bulked sample material.

Table 2.4 Leaf mineral nutrient content (% dry weight) for a range of species which have all been recorded on *Molinia caerulea-Cirsium dissectum* fen meadow (M24) sites. Source: Thompson *et al.* (1997).

Species	P (total)	Ca	K	N
Angelica sylvestris	0.36	2.06	2.96	3.33
Anthoxanthum odoratum	0.15	0.38	1.99	2.04
Briza media	0.11	0.42	2.05	1.66
Calluna vulgaris	0.11	0.53	0.77	1.46
Cerastium fontanum	0.45	1.11	3.51	2.41
Deschampsia caespitosa	0.19	0.37	2.00	1.82
Eriophorum angustifolium	0.21	0.16	1.28	2.42
Festuca rubra	0.22	0.47	2.00	1.61
Filipendula ulmaria	0.28	0.94	1.94	3.40
Holcus lanatus	0.33	0.57	3.45	2.71
Juncus effusus	0.13	0.15	1.47	1.31
Plantago lanceolata	0.22	1.87	2.09	2.05
Potentilla erecta	0.18	1.47	1.97	2.17
Rumex acetosa	0.32	0.70	3.11	3.37
Mean	**0.34**	**1.38**	**3.03**	**3.35**
(±SD)	(0.03)	(0.96)	(0.11)	(0.03)

Table 2.5 Leaf mineral nutrient content (% dry weight) of a selection of species from Aylesbere Heath, collected in spring 1983 by Hayati and Proctor (1990).

Species	P (total)	Ca	K	N
Molinia caerulea	0.09	0.21	1.84	1.72
Ulex gallii	0.17	0.34	1.54	2.47
Trichophorum cespitosum	0.10	0.21	1.37	0.55
Eriophorum angustifolium	0.09	0.10	0.74	0.57
Nathecium ossifragum	0.10	0.46	2.05	1.07
Cirsium dissectum	**0.15**	**1.85**	**2.27**	**1.33**
Succisa pratensis	0.18	0.85	2.24	0.72
Seratula tinctoria	0.18	0.68	3.19	0.77
Mean	**0.14**	**0.45**	**2.52**	**1.25**
(±SD)	(0.06)	(0.33)	(0.95)	(0.67)

2.5.7 *Mycorrhizal association*

Mycorrhizae are the most widespread associations between microorganisms and higher plants and on a global basis occur in 83% of dicotyledonous plants (Marschner, 1995). Their role has been detailed by Marschner (1995) where, in nutrient poor soils, external mycelia add surface area to roots giving greater access to limiting nutrients, particularly P and N. Vesicular-arbuscular mycorrhizae (VAM) are by far the most abundant of mycorrhizal groups, characterised by the formation of branched haustorial structures (arbuscules) within the cortex cells and by a mycelium which extends well into the surrounding soil. The most distinct growth enhancement effect by VAM occurs by improved supply of mineral nutrients of low mobility in the soil solution, predominantly P. Such an association would therefore have obvious benefits to a species such as *C. dissectum*, growing as it does in a low P environment. However, non-mycorrhizal species can occur in habitats that are waterlogged or where soil fertility is extremely low (Brundrett, 1991). It was therefore deemed useful to establish whether *C. dissectum* does have a mycorrhizal association.

The study of mycorrhiza is a particularly specialised area of soil/plant ecology and it was considered that the detection and identification of such organisms was generally beyond the scope of this study. Therefore, outside assistance was sought to provide the necessary analysis. Dr. A.C. Gange of Royal Holloway, University of London, Egham analysed field samples of root material from the main field sites (five plants from each site). Using an autofluorescence method of detecting arbuscules, it was established that *C. dissectum* was infected with VAM. It was also found, from the percent root length colonised, that the youngest fine roots were the most heavily colonised with very little detectable arbuscule development in the older roots.

2.6 GENERAL BOTANICAL OBSERVATIONS

Subsequent Chapters in this study will detail controlled experiments which measure specific responses of *C. dissectum* to various levels of stress and disturbance. However, over the course of the study some interesting field and glasshouse observations have been made

which have not been empirically tested. In Chapter 1 it was suggested that the limited distribution of *C. dissectum* is a result of 'realised' ecological niche rather than its physiological optimum environment. Specimen glasshouse plants allowed to grow without restriction in relatively nutrient rich composts (e.g. B & Q Multipurpose peat based compost and John Innes loam based potting compost) produced some examples of plants considerably larger and more vigorous than any field specimens. Plates 2.1a, b and c illustrate three specimens grown on compost with leaves in some cases longer than 300 mm, which can be compared with a typical field specimen (Plate 2.1d). This demonstrates the genetic potential of *C. dissectum* which has not been observed in specimens growing under field conditions on British sites during the course of this study. However, the populations observed on the Brittany sites had much larger leaves compared with British specimens.

An unusual change in the leaf structure takes place with the onset of winter. All the normal lanceolate leaves senesce and are replaced by 3 to 5 very small narrow leaves (less than 5 mm wide), often flushed red with anthocyanin. Some examples of glasshouse specimens in this overwintering form are illustrated in Plates 2.2a and b. This change in leaf form would seem to be a response to some temperature threshold rather than day length, as this phenomenon was observed in a growth room under a long day length light regime when the heating failed during a particularly cold spell. The plants remain in this overwintering form until spring when new 'normal' leaves are initiated, replacing the overwintering leaves. Also, flower bud formation is well developed by the time these new leaves are initiated (Plate 2.2c). This suggests that the initiation and development of flower buds takes place over winter using stored carbohydrate reserves. Although *C. dissectum* appears to be dormant over the winter, the maintenance of some green leaf structure suggests that the plant's metabolism is still active with some photosynthesis taking place whilst in this overwintering form.

Plate 2.1 Examples of *Cirsium dissectum* (a), (b), (c) grown in nutrient-rich compost and (d) a typical field specimen (ruler length 300 mm)

(a)

(b)

(c)

(d)

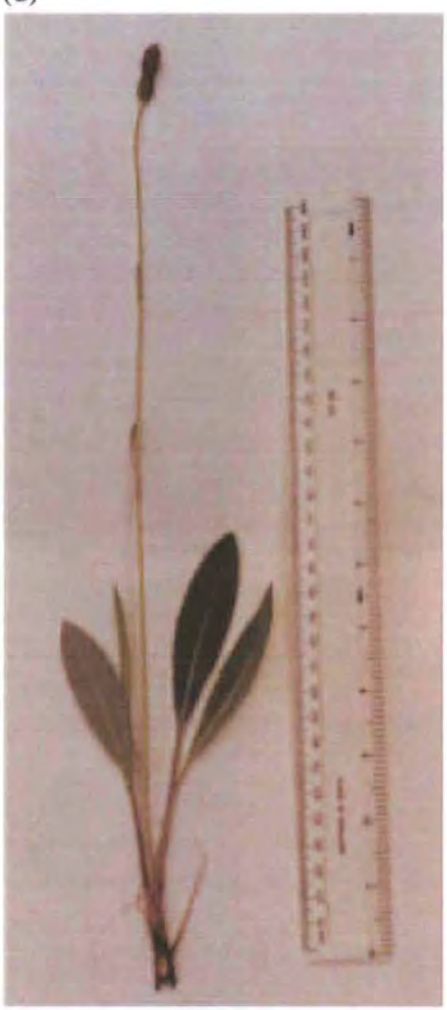


Plate 2.2 Examples of *Cirsium dissectum* (a), (b) in overwintering narrow leaf form and (c) emerging new spring leaves with flower initiation established (circled).

(a)

(b)

(c)

2.7 PREDICTING C-S-R FUNCTIONAL TYPE

The C-S-R theory (Grime, 1979), discussed in Chapter 1, suggests that plants have evolved along three fundamentally different pathways i.e. *competitor, stress-tolerator* and *ruderal.* Each of the functional types have in common a particular suite physiological characteristics. Conversely, this suite of characteristics can be used to provide a general classification of any given species within a functional type category. A recent prediction model has been developed (Hodgson, *et al.*, 1999) which compares a set of simple predictor variables against a set of 'gold standard' indices for the C, S and R dimensions in the ordination triangle (Figure 2.5, Chapter 1). The predictor variables used are Canopy Height, Dry Matter Content, Flowering Period, Flowering Start, Lateral Spread, Leaf Dry Weight and Specific Leaf Area. Raw data on the seven variables is then processed through a series of five stages using an Excel (V 5.0) spreadsheet (obtained from the Unit of Comparative Plant Ecology, University of Sheffield) which calculates the coordinates for each of the three dimensions in the ordination triangle and produces a predicted functional type. Using the botanical observations, this model was employed for *C. dissectum* which predicted an SC/CSR functional type. A detailed print out of the model is contained in Appendix 1. Hodgson *et al.* (1999) estimate, from their validation of the model, the overall sensitivity (i.e. how good is the test at picking up the correct type) to be 74%. The model therefore predicts that *C. dissectum* does not appear to have a particular ordination towards any extremes of the three dimensions but falls between a stress-tolerant competitor and C-S-R strategist. The stress-tolerant competitor functional type is characteristic of species found in derelict grassland, heath and marshland. Such plants are usually robust perennials with a capacity for lateral vegetative spread but with low maximum relative growth rates. The C-S-R strategist is commonly found in unproductive pastures and grazed marshes where mineral nutrient stress and moderate intensities of defoliation by grazing animals are more or less constant features of the habitat (Grime, 1979). Characteristics of the C-S-R strategist are small stature perennials with a moderate maximum potential relative growth rate and many of the dicotyledons are rosette forming species. Two examples of such species, with an established C-S-R strategy, are *Succisa pratensis* and *Potentilla erecta* (Grime, *et al.*, 1988),

both of which are also key M24 community species. Therefore the model does appear to have provided a reasonable indication of the most likely functional type classification for *C. dissectum,* which in turn provides a reasonably accurate prediction of its likely habitat.

2.8 SUMMARY

The results of the fieldwork have provided detailed information relating to the abiotic environmental conditions associated with the distribution of *C. dissectum* populations. The main M24 field sites all have in common a particular combination of physical and edaphic conditions, which all fall within a well defined, relatively narrow range. The soil types are all surface-water gleys, which are slowly permeable, seasonally waterlogged and contain a high proportion of clay-sized particles. Many of the sites are located at valley bottoms or the lower end of a slope. Soil water content is relatively high with very little apparent seasonal fluctuation, which is mainly a function of site hydrology rather than incident rainfall. Soil mineral nutrient conditions on all field sites are uniformly low in phosphorus (between 6 - 10 mg P kg^{-1}) but not limited by calcium or potassium. This study was not able detect any increase in extractable soil P normally attributed to a spring flush of P mineralisation. Soil pH is generally slightly acid to neutral, but generally within a range which does not limit the availability of other major mineral nutrients such as N, P, K and Ca. Although soil organic content is higher than that of arable soils, the C:N ratio is relatively low on the main sites which indicates that decomposition is not particularly inhibited by the high soil water contents. Plant mineral content of field samples indicate that Ca and K content are within the range considered optimal for growth but both N and P content are below optimal but similar to other species from this type of habitat. It has been established that *C. dissectum* does have a vesicular-arbuscular mycorrhiza association which appears to be mainly confined to the youngest fine roots. Finally, from a set of physiological characteristics, a model which predicts C-S-R functional type has classified *C. dissectum* within the categories of stress-tolerant competitor and C-S-R strategist with no particular extreme ordination towards any of the three main functional types.

3.0 WATER UPTAKE

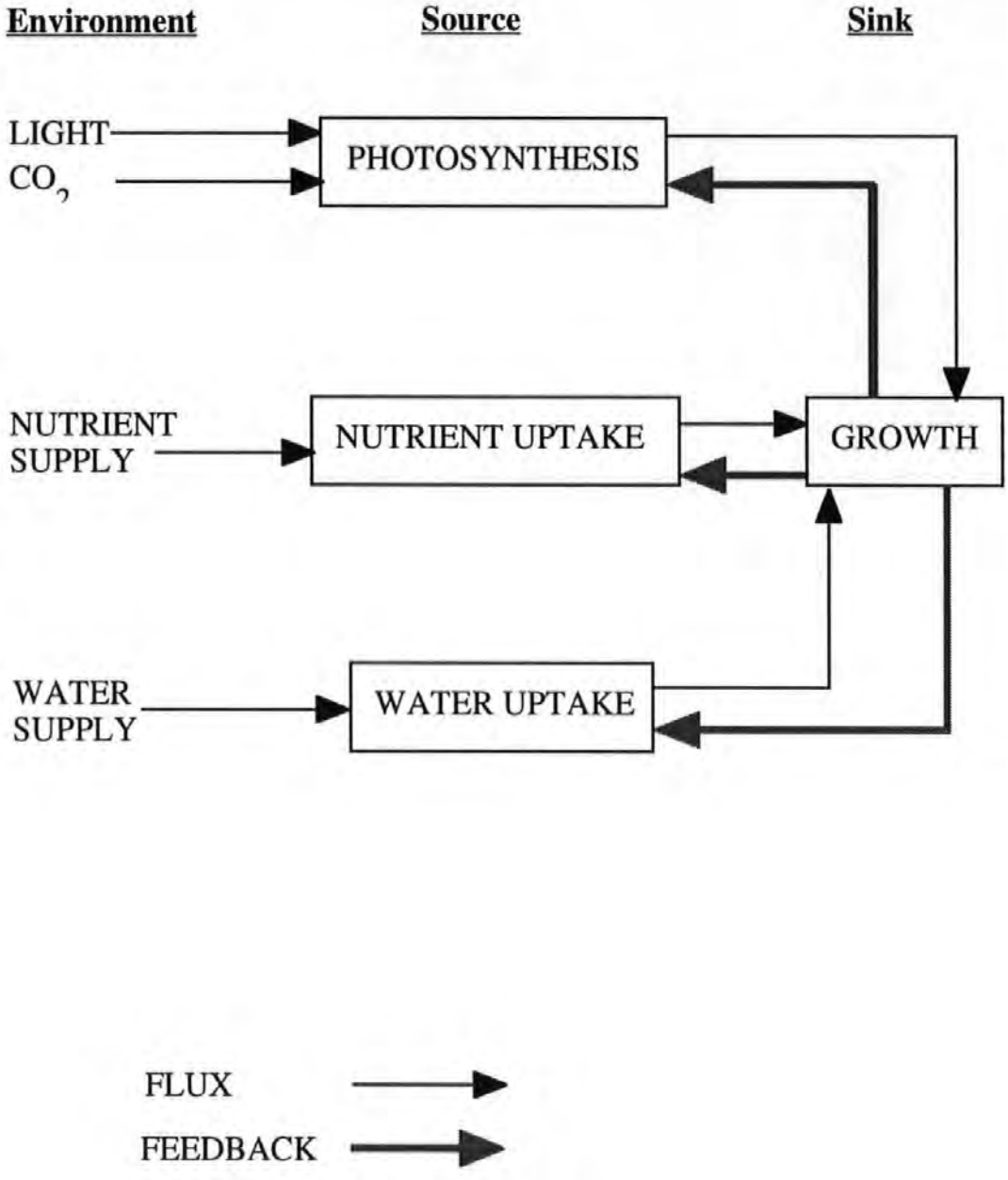
3.1 INTRODUCTION

The review of the M24 habitat descriptions in Chapter 1 (section 1.3) and the results of the soil water field data in Chapter 2 (section 2.4) have highlighted a unique environmental characteristic of this habitat, namely a relatively high soil water content which does not fluctuate to any great degree. This suggests that soil water availability may be an important determinant in the maintenance of this community and, in particular, the distribution of *C. dissectum.*

Resource acquisition and growth by plants is directly controlled by four main environmental variables and a series of source-sink feedbacks (Chapin, 1991) and these are detailed in Figure 3.1. Plants obviously require water for growth because water is essential for cell volume increase by vacuolation which is responsible for growth (Milburn, 1979). Sustained expansive growth is the result of interactions among numerous processes in the plant, both biophysical and biochemical, and these processes are affected by factors other than water (Hsiao, Silk, and Jing, 1985). Firstly, light directly affects photosynthesis which in part determines growth resulting in feedback affecting water uptake (see Fig 3.1). Secondly, Radin and Boyer (1982) suggested that nitrogen limitation causes a reduction in growth as a result of changes in plant water relations. Even in hydroponic systems, nitrogen stress quickly causes a decline in hydraulic conductance of roots (and therefore water uptake) and an associated decline in stomatal conductance and therefore transpirational water loss (Chapin, 1991).

It is hypothesised that one of the possible reasons why *C. dissectum* is confined to poorly drained sites is that it may have a relatively high water requirement due to a specific water metabolism or it may not be particularly efficient in its use of water. Also, *C. dissectum* is a hemi-cryptophytic species and, on fen meadows, will have to compete for light with more dominant and erect species, specifically *Molinia caerulea.*

Figure 3.1 Direct environmental controls and source-sink feedbacks affecting resource acquisition and growth by plants. (Source: Chapin, 1991)



Therefore, it may be shade-tolerant and able to maintain a higher level of growth under shade than a non-tolerant species. If this were the case the water uptake of *C. dissectum* would not be affected by shade. It has also been demonstrated that the North Devon fen meadow communities are low in nitrogen (Goodwin, 1995). Therefore if the water uptake of *C. dissectum* is not reduced under conditions of low nitrogen availability, this would indicate an adaptation to a low nitrogen environment. Since the water usage of a plant depends on a dynamic interaction between environmental conditions and plant factors, the purpose of this chapter is to quantify the dynamics of the water uptake of *C. dissectum* in relation to two environmental variables namely light and nitrogen and compare it with a mesophytic species.

3.2 SOIL-PLANT-ATMOSPHERE CONTINUUM

Water uptake and water flow through a plant are generally described by the widespread concept of the Soil-Plant-Atmosphere Continuum (SPAC) developed by van den Honert (1948) and applied by Cowan (1965) and others. This describes water movement through a plant driven by a water potential gradient that results from a water deficit in the leaves generated by a difference between the extent of water uptake and transpiration.

In its simplest form: $$\text{Water flux} = \frac{\text{difference in water potential}}{\text{resistance}}$$

Water transport through whole plants is complicated by several types of flow occurring simultaneously, namely water uptake, water lost by transpiration, water stored for growth and water stored or released by hydration/dehydration. Whole plant water balance has been characterised by Boyer (1985) in Equation 1. The conservation of mass requires that each of these flows be additive with due regard to whether water is entering, leaving, or being stored by the plant. Accordingly:

Equation 1. $$A + T = G + H$$

where A represents the total water gained by the plant and T is the total water lost by the plant. The sum of A (positive) and T (negative) is the flux available for growth and changes in hydration. G is always positive or zero whereas H can be positive or negative depending on whether the plant is hydrating or dehydrating. Under steady state conditions, such as

those in the following experiment (i.e. unlimited water supply, constant light, temperature and humidity), the water potentials do not change with time and H is zero. The equation can therefore be simplified and rewritten to represent the fate of total water taken up by the plant as follows:

Equation 2. $A = G + T$

How a plant uses the water taken up is largely determined by the its metabolic processes which result in water retention for cell expansion (i.e. growth) and water loss by transpiration according to Equation 2. Therefore by measuring T and G, it was possible to calculate A. In practical terms this involved measuring regular changes (every two days) of total water lost from the system (T) and adding the water content of plant fresh weight gain (G) to calculate the water uptake (A).

3.3 AIMS AND OBJECTIVES

Measuring water uptake will provide comparative baseline data on water uptake, water use efficiency and growth under conditions of unlimited water supply plus an indication of shade tolerance and low nitrogen adaptation. In addition, this information is required to enable the design of further experiments to study plant water relations under conditions of water stress. Such experiments will require combining the rate of plant water uptake with the soil (or growing medium) drying rate to estimate rate of water stress and likely wilting point resulting in loss of turgor. Therefore, the main aims of the following experiment are firstly to compare the water metabolism of *C. dissectum* with a mesophytic species to determine whether it has a relatively high water requirement. Secondly to examine the effect of two environmental factors, namely light and nitrogen availability, on water metabolism, growth and morphology to determine whether *C. dissectum* is relatively tolerant of shade and/or nitrogen stress compared with a mesophytic species. The specific objectives are therefore to:

a) measure water uptake and water use efficiency in relation to growth and the effects of shade and nitrogen availability

b) measure the effects of shade and nitrogen availability on growth, biomass partitioning, plant form and leaf expansion

Plate 3.1 Experimental plant flask system (a) *Cirsium dissectum* plant sealed into a flask (b) foil-lined cylinders with and without shade cover.

(a)

(b)

3.4 MATERIALS AND METHODS

It was originally intended to use potometers for this experiment (Bannister, 1986; Slavik, 1974). This was abandoned at an early stage as experimental attempts to seal plants into the potometers were unsuccessful. It was therefore decided to use the gravimetric method of water loss (Bannister, 1986) by measuring changes in weight of plants growing in 100 ml conical flasks. A weighing lysimeter system has been described by Van Leperen and Madery (1994) which involves two communicating vessels filled with nutrient solution; each placed on an electronic balance. This technique is based on the water balance characterization detailed above and provides high resolution measurement of water uptake and transpiration simultaneously, over a short time span (minutes). However for practical reasons (i.e. 72 balances would not fit in the growth cabinet) a simplified method has been devised which will provide suitable replication over a longer time scale (two weeks) at a lower resolution but was found to be sufficient to identify significant treatment differences.

Helianthus annuus was used as a control species in this experiment, as it is a well established mesophyte, which does not have a particularly high water or nutrient requirement but is not shade tolerant (Andria, *et al.*, 1995; Purseglove, 1969). A dwarf variety of *H. annuus* (cv. Allegro, supplied by CPB, Twyford) was used due to the space restrictions imposed by the growth chamber used for the experiment. *C. dissectum* and *H. annuus* were grown in 100 ml conical flasks filled with Rorison's nutrient solution (Hendry and Grime, 1993). Plants were supported in the flasks by non-absorbent cotton wool and the flasks were wrapped in aluminium foil to prevent light reaching roots and to prevent algal growth (see Plate 3.1). Plants were grown from seed and germinated in an incubator on moist Perlite. Once germinated they were moved to the growth chamber and left to grow in the Perlite, watered with nutrient solution, until they were required for the experiment. This minimised damage to the seedlings as a result of handling.

Due to the vigorous growth of *H. annuus*, one week-old plants were used in the experiment to compare with six week old *C. dissectum* plants. Plants of each species were selected for

the experiment on the basis of size uniformity. A random sample of spare plants was oven dried at 70 °C and dry weight biomass recorded. Similarly, at the end of the experiment all plants were dried as before and dry weight biomass recorded. In addition, leaf area was measured using a Delta-T Area Meter MK II (Delta-T Devices, Cambridge). Environmental conditions were controlled by carrying out the experiment in a SGC 660 series phytotron (Sanyo Gallenkamp PLC, Leicester). The regime was a 16 hour day at 22 °C and 15 °C night, with daytime light supplied at 350 (± 10) μ mol m^{-2} s^{-1} photon flux density, following the Integrated Screening Programme (ISP) standard regime (Hendry and Grime, 1993). Relative humidity was maintained at 85% (± 5%) throughout to minimise water loss by evaporation. To ensure that all plants received equal amounts of light, the flasks were enclosed in 130 mm diameter x 210 mm high cylinders lined with reflective foil. A plastic plant pot holder was used as a base for the cylinder and had holes drilled to allow air circulation. Shade conditions were simulated by placing three layers of nylon gauze over the top, reducing light by approximately 57% to 150 (± 10) μ mol m^{-2} s^{-1}. The composition of the nutrient solution was designed to measure response to reduced nitrogen (N). Three concentrations of N were supplied, namely the full Rorison solution (100%: 4000 μM N), 50% (2000 μM N) and 25% (1000 μM N). All other nutrients were maintained uniform and non-limiting. The composition of these solutions is detailed in Table 3.1.

Although the plant/flask growing system is essentially sealed, some water will be lost by evaporation through the cotton wool at the neck of the flask. This was measured using blank controls with flasks containing distilled water, sealed with cotton wool, placed in cylinders and replicated four times for both light and shade. Total water loss and plant fresh weight was measured every two days and nutrient solutions changed. The whole plant/flask system weight was recorded. Plants were then removed from the flasks, excess moisture was removed from the roots by briefly wrapping in paper towel and then fresh weight recorded. Plants were then replaced in the flasks with fresh solution and the whole system reweighed. Measurements were taken at the same time of day on every occasion, i.e. two hours after the start of the day cycle in the growth chamber. The experiment ran for a total of sixteen days, by which time the growth of *H. annuus* had reached the top of the cylinder.

Table 3.1 Rorison nutrient solution, preparation of 1 litre with variations of N levels. (Adapted from Hendry and Grime, 1993).

Stock solutions		$g\ l^{-1}$	Full basic (ml)	50% N (ml)	25% N (ml)
1	$Ca(NO_3)_2 - 4H_2O$	476.10	1	0.50	0.25
2	$MgSO_4 - 7H_2O$	248.00	1	1	1
3	Fe (Na) EDTA	25.00	1	1	1
4	*Traces* $MnSO_4 - 4H_2O$ H_3BO_3 $(NH_4)_6Mo_7O_{24} - 4H_2O$ $ZnSO_4 - 7H_2O$ $CuSO_4 - 5H_2O$	 2.028 2.863 0.184 0.440 0.393	1	1	1
5	1 Normal H_2SO_4 (pH adj.)	28ml conc./l	1	1	1
6	K_2HPO_4 (anhydrous)	176.03	1	1	1
7	$CaCl_2 - 4H_2O$	294.73	-	0.50	0.75

3.5 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A randomised block design layout was used which contained two species x two light levels x three nutrient levels replicated in three blocks, see Fig 3.2. The block design was used to identify any differences due to the block position as there appeared to be a light gradient in the growth chamber with a reduction in light at the side ends. A preliminary examination of the data was made to check for homogeneity of variances using a F_{max} test. Where data were found to have heterogeneous variances they were stabilised with a log transformation and re-examined. Statistical analyses were carried out using a factorial analysis of variance (ANOVA) or where variances could not be stabilised a 'Kruskal-Wallis' test was employed. All proportional data were arcsine transformed prior to analysis to provide an underlying distribution that is nearly normal (Zar, 1996). Where significant differences within factor levels or interactions between factors were identified, a 'Tukey' test was used as a first analysis. Where there were 'overlapping similarities' in pairwise comparisons, the less conservative "Newman-Keuls' test was used. The trends in incremental fresh weight over the experiment were analysed using regression analysis. Each regression was examined for autocorrelation using the 'Durban-Watson' test statistic and residuals were examined for homogeneity of variances. All statistical tests were derived from texts by Fowler, Cohen and Jarvis(1998), Kanji (1999) and Zar (1996).

Figure 3.2 Layout of randomised block design in the growth chamber.
(Key: **C** = *C. dissectum* , **S** = *H. annuus* ; **L** = light, **D** = shade;
25, 50 or **100** = % N of nutrient solution).

(Back)

BLOCK 1			BLOCK 2			BLOCK3		
CL50	CD50	CD100	SL50	SL100	SD50	CD50	CD100	CL100
SD100	SL100	SD25	CD50	CD100	CL100	SD25	SL50	CL25
CL100	SD50	SL25	CD25	SL25	CL25	CD25	CL50	SL25
CD25	SL50	CL25	CL50	SD25	SD100	SD100	SD50	SL100

(Front)

3.6 RESULTS

The block factor was found to be not significant ($P > 0.05$) in any of the analyses, therefore only species x light/shade x nitrogen results are presented.

Plant water content and evaporation

From the dry weights measured at the beginning and the end of the experiment the proportion of water in the plant weight was calculated for each species. A 't' test analysis revealed that in *C. dissectum* the total plant water proportion was not significantly different with a mean of 0.89. In *H. annuus* there was a significant difference ($P = 0.002$); mean at start 0.93 and finish 0.94, a difference of 0.01. In practical terms the water proportion over the experimental period did not differ by any great order of magnitude. Therefore it was assumed that it was constant in both species. This being the case, it was then possible to correct the water uptake measurements to take account of dry mass gain. Similarly, over the experimental period there were no significant differences in water lost by evaporation, in the blank flasks, between the light and shade replicates. Therefore the total mean evaporation on each measurement day was used to correct the water uptake data. The amount of evaporation varied from 0.15 to 0.77 g per two-day period. The variation in evaporation was the result of two instances where there was a slight technical problem with the humidity control in the growth chamber.

Fresh weight gain

Total fresh weigh gain (FWG) over the experiment of *H. annuus* was seven times higher ($P< 0.001$) than *C. dissectum* (Fig 3.3). Neither shade nor N resulted in any significant difference in FWG for either species. However, *H. annuus* FWG did appear to be lower under shade (Fig 3.3) but a "Kruskal-Wallis' analysis was not able to detect any difference. There was also a difference between the two species in the pattern of fresh weight increment over the experiment (Fig 3.4). The trend in fresh weight increment of *C. dissectum* was a third order polynomial curve, whereas *H. annuus* was a quadratic curve. Also, the shade treatment appeared to produce a lower slope in *H. annuus*, suggesting a lower growth rate.

Figure 3.3 Mean fresh weight gain for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the SE of the mean; letters denote significant differences between means

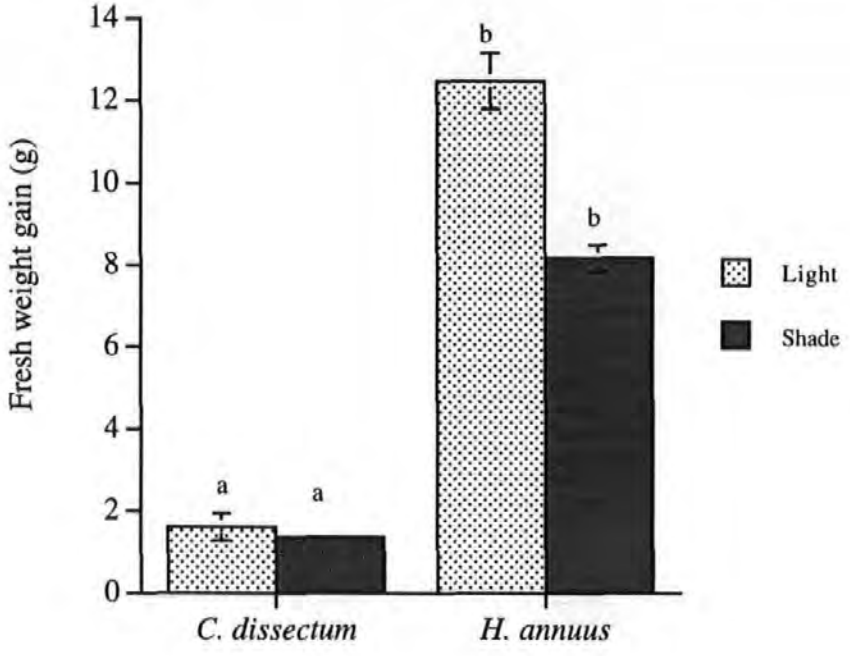
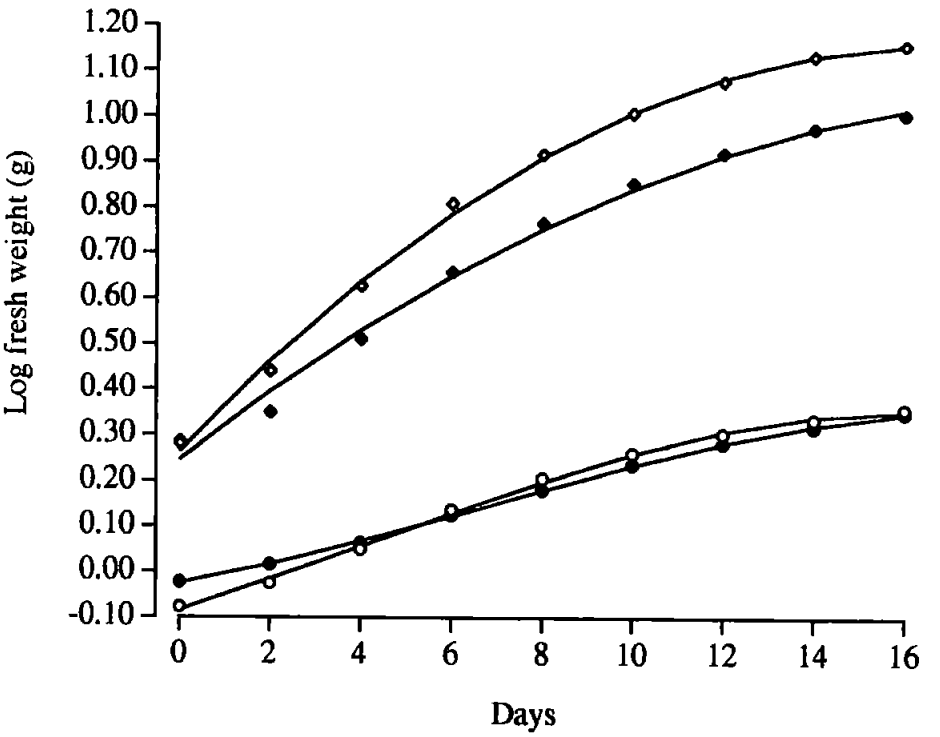


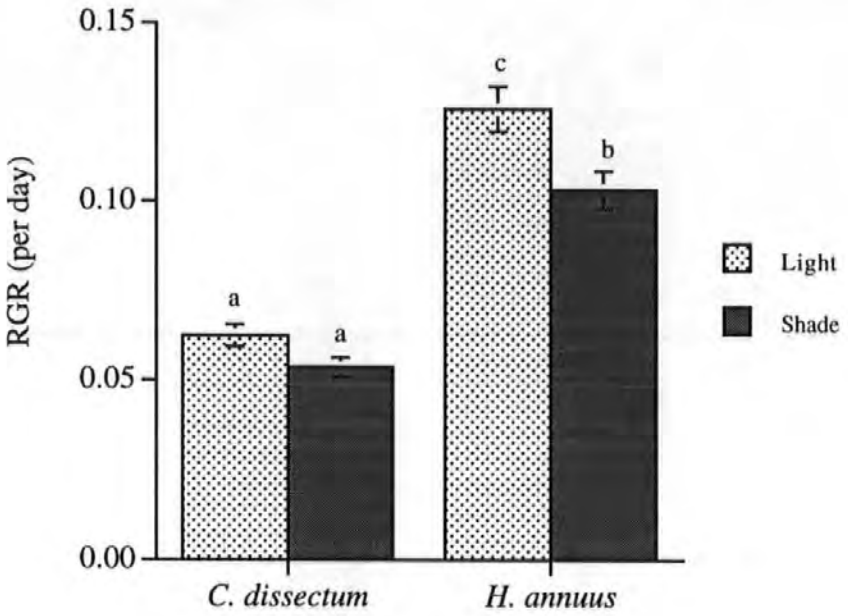
Figure 3.4 Trend in mean incremental fresh weight (log-transformed) under light and shade for *Cirsium dissectum* (Light o; Shade ●) and *Helianthus annuus* (Light ◇; Shade ◆)



Line equations for *H. annuus*
Light: $y=0.264+0.11x+0.003x^2$; R^2 99.8%
Shade: $y=0.246+0.08x+0.002x^2$; R^2 99.2%

Line equations for *C. dissectum*
Light: $y=-0.084+0.03x+0.001x^2-0.00009x^3$; R^2 99.8%
Shade: $y=-0.025+0.02x+0.002x^2-0.00007x^3$; R^2 100%

Figure 3.5 Mean relative growth rate (RGR) per day for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the SE of the mean; letters denote significant differences between means.



Growth rate

To enable direct comparison between the two species and any interactions between species and other factors, it was necessary to allow for differences in species growth vigour. To enable the data to be corrected for different growth rates, it was necessary to calculate relative growth rate (RGR). Computation of mean RGR, over the experimental period was calculated using the formula detailed by Evans (1972) as follows:

Equation 3. $$RGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_2 and W_1 are the total plant dry weights at the beginning and end of the period and $T_2 - T_1$ represents the time period between harvests, in this case 16 days. There was a significant species x RGR interaction (P= 0.037). The RGR of *H. annuus* was reduced by shade but was still twice the RGR of *C. dissectum* which was not affected by shade (Fig 3.5). This confirms the pattern observed in the fresh weight analyses, namely that shade reduces growth in *H. annuus* but has no effect on the growth of *C. dissectum*. There was no effect on RGR of either species as a result of the various N treatments.

Water uptake

Plant water metabolism is largely determined by growth and transpiration (Section 3.2). The growth results detailed above, highlight clear differences between the two species in their growth rates which will result in differences in water metabolism. Therefore, the water uptake data was adjusted to allow for the differential water use by dividing the water uptake of each species by their respective final leaf areas. The adjusted data is referred to as relative water uptake (RWU).

There was significant species x light/shade interaction (P= 0.012). RWU of *C. dissectum* was 150% that of *H. annuus* under full light but shade reduced RWU in *C. dissectum* by 25%, whereas shade reduced RWU in *H. annuus* by 39% (Fig 3.6). Also, at the 25% N level RWU was twice that of the 100% N level (P= 0.002) in both species.

Figure 3.6 Mean relative water uptake (RWU) log-transformed for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means.

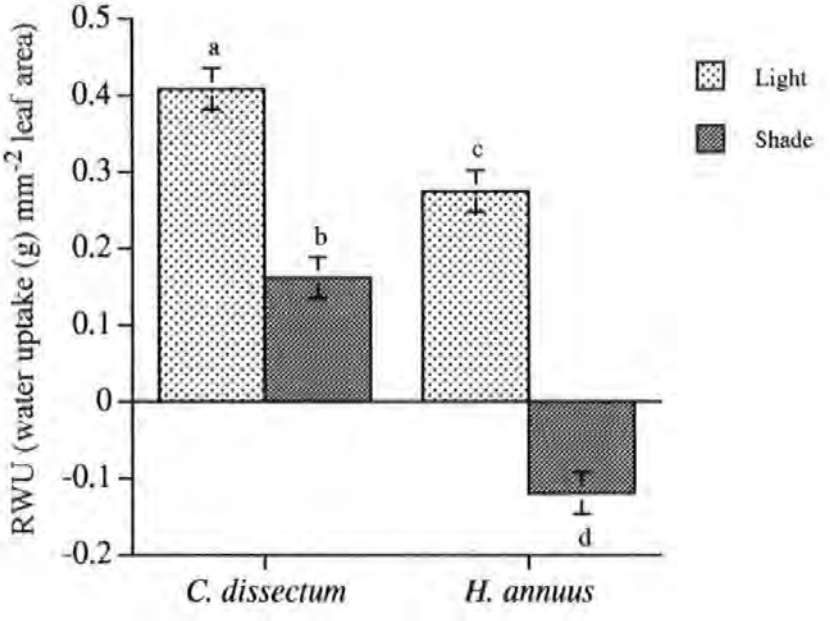
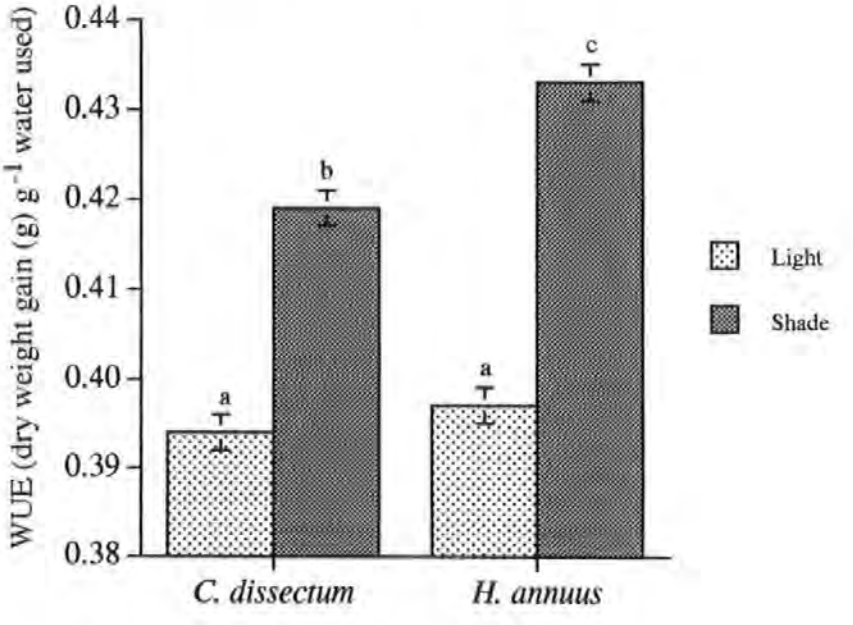


Figure 3.7 Mean water use efficiency (WUE) log-transformed for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the pooled SE of the means; letters denote significant differences between means



Therefore, it would appear that *C. dissectum* has relatively high water requirement which is increased at low N availability. In addition, *C. dissectum* exhibits a lower degree of response to shade, in its water uptake, compared with a mesophytic species.

Water use efficiency

Differences between transpiration and carbon assimilation can be analysed from the water use efficiency (WUE) of a plant. In its simplest form it has been described by Kramer (1983), Teare *et al.* (1973) and Boyer (1996) as follows:

Equation 4. $$WUE = \frac{\text{Dry matter}}{\text{Water used in transpiration}}$$

and is normally expressed in grams of dry weight gain per gram of water used. In this experiment *C. dissectum* had a lower WUE ($P = 0.028$) compared with *H. annuus* but the difference was only 2% (Fig 3.7). Shade increased water efficiency in both species ($P < 0.001$) but less so in *C. dissectum*. N availability had no effect on WUE in either species. Therefore, although *C. dissectum* has a relatively high water requirement, it appears to be similar or only slightly less efficient in its water use compared with a mesophytic species.

Morphological responses to shade and nitrogen

There were no significant differences between the two species in their root:shoot ratio (RS) as a result of the shade treatment. Overall, shade resulted in a slight reduction ($P = 0.031$) in RS (Light 0.54; Shade 0.46) in both species. However, there was a clear difference between the two species in RS as a result of N availability. There was a significant species x N interaction ($P < 0.001$) where *H. annuus* responded with an increase in RS at each level of reduced N, whereas the RS of *C. dissectum* was not affected (Fig 3.8). This suggests that *C. dissectum* has either a low inherent morphological plasticity or has a relatively slow morphological response rate.

Figure 3.8 Mean root:shoot ratio (R:S, log-transformed) for *Cirsium dissectum* and *Helianthus annuus* at three nitrogen levels. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means

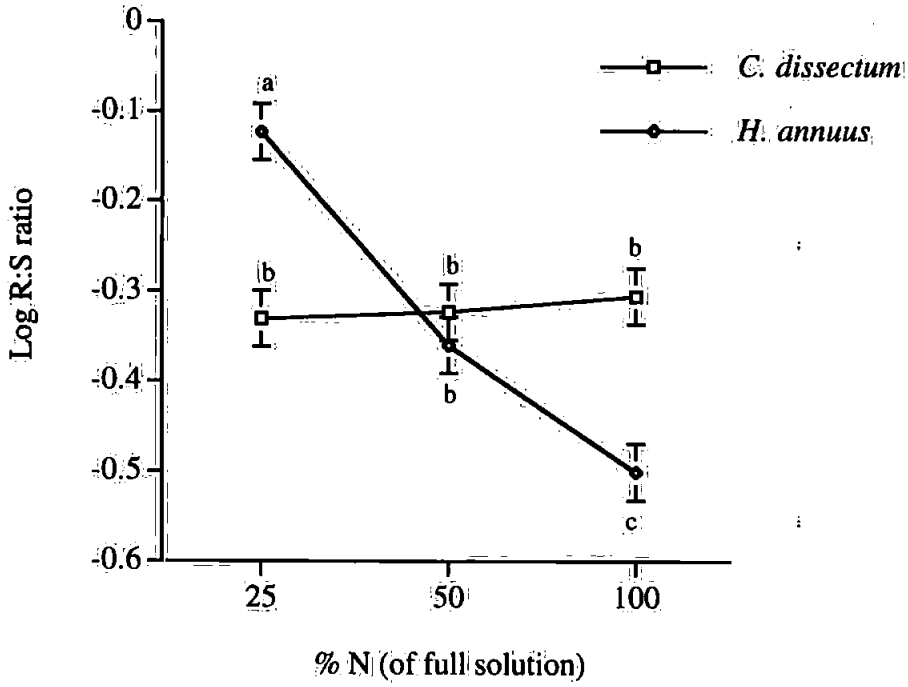


Figure 3.9 Mean leaf area ratio (LAR) for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means

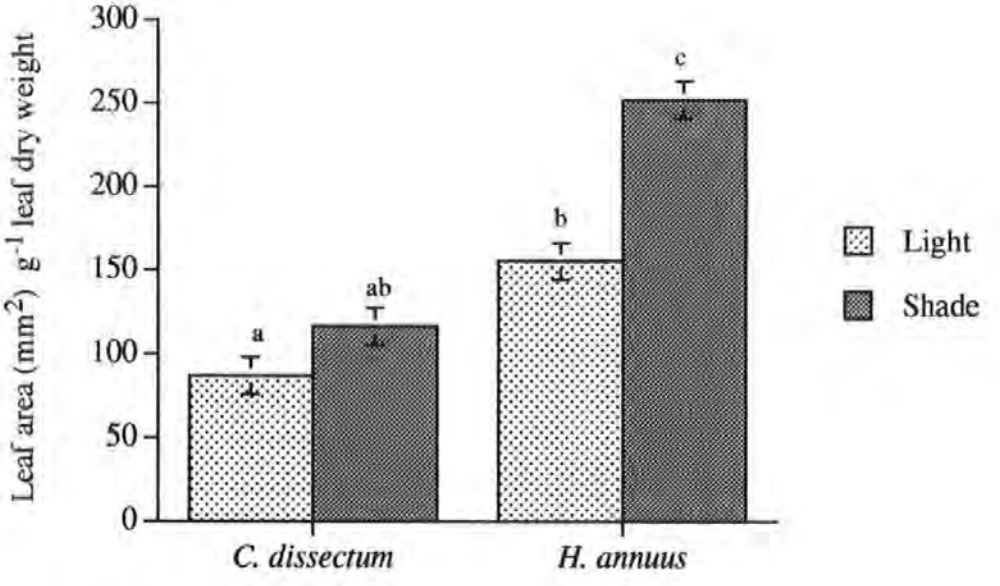
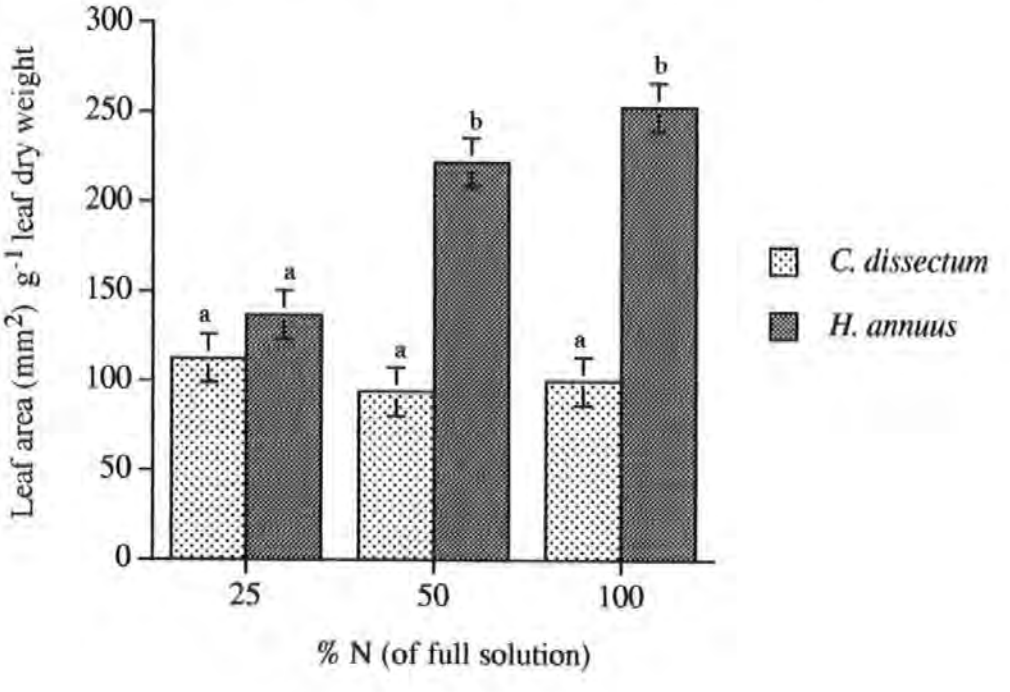


Figure 3.10 Mean leaf area ratio (LAR) for *Cirsium dissectum* and *Helianthus annuus* at three nitrogen levels. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means



A comparison of the differences in plant form between the two species can be made using the morphological index leaf area ratio (LAR), which is the leaf area per unit dry weight of the whole plant (Evans, 1972). This gives a measure of the amount of carbon gain allocated to photosynthetic structure. There was a significant species x light/shade interaction (P= 0.006) in the LAR response. Under shade conditions, *H. annuus* LAR increased whereas LAR in *C. dissectum* was not affected, and LAR was lower in *C. dissectum* compared with *H. annuus* under both light and shade respectively (Fig 3.9). There was also a species x N interaction (P< 0.001) where the lowest N treatment (25%) reduced LAR in *H. annuus* to a similar level as that of *C. dissectum* which was not affected by N availability (Fig 3.10). This suggests that *C. dissectum* allocates a relatively small proportion of carbon gain to photosynthetic structure and does not appear to compensate for low photosynthetic active radiation (PAR) by an increased allocation to photosynthetic area, even when nutrient supply is ample.

A further comparison of morphological differences between the two species is specific leaf area (SLA) which is the average leaf expansion in area per unit dry weight (Evans, 1972). There was a significant species x light/shade interaction (P= 0.009) in the SLA response. *H. annuus* SLA was higher under shade conditions and had a higher SLA compared with *C. dissectum* SLA which was unaffected by shade (Fig 3.11). There was also a species x N interaction (P= 0.008) where the lowest N treatment (25%) reduced SLA in *H. annuus* but was in turn higher than *C. dissectum* SLA which was not affected by N availability (Fig 3.12). Overall, *C. dissectum* has relatively smaller, thicker leaves and leaf expansion is not affected by reduced light or N availability.

Figure 3.11 Mean specific leaf area (SLA) for *Cirsium dissectum* and *Helianthus annuus* under light and shade. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means

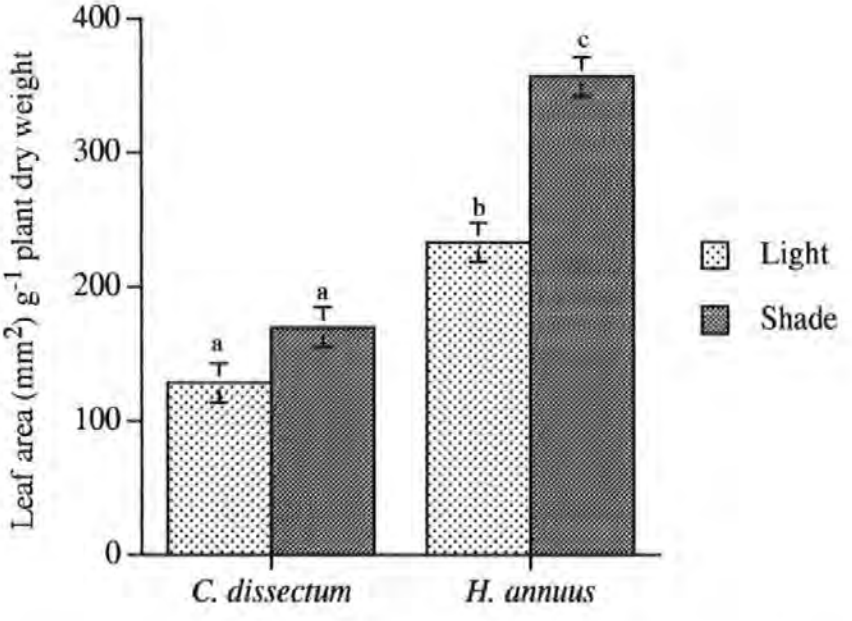
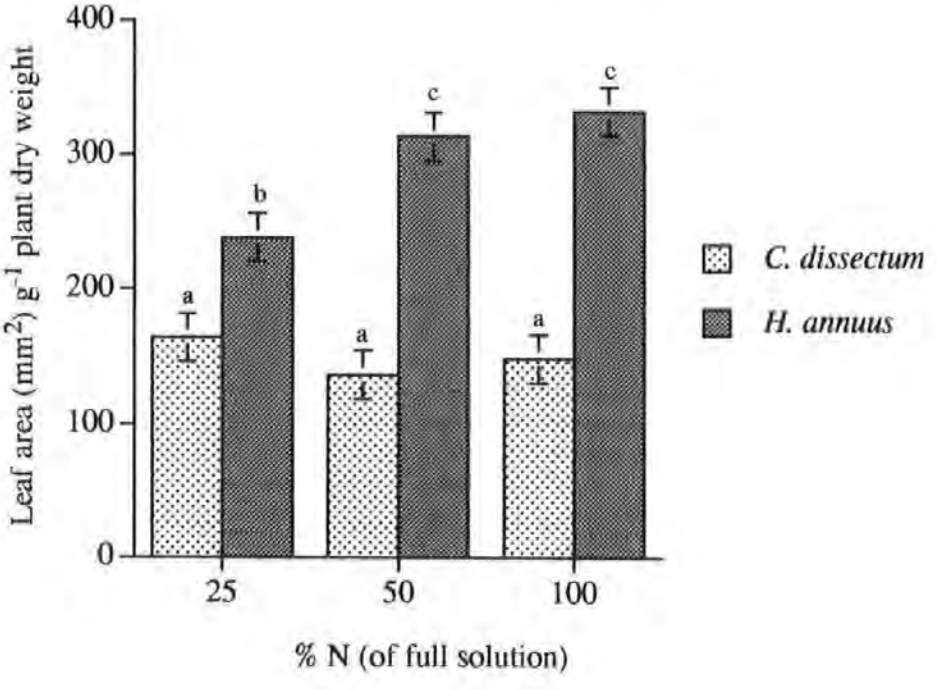


Figure 3.12 Mean specific leaf area (SLA) for *Cirsium dissectum* and *Helianthus annuus* at three nitrogen levels. Vertical bars represent twice the pooled SE of the mean; letters denote significant differences between means



3.7 DISCUSSION

The results have highlighted clear differences between the two species in growth, water use and morphology. The pattern of growth over the experimental period was non-linear for both species, with *C. dissectum* exhibiting a third order polynomial pattern and *H. annuus* a quadratic trend. This makes any comparisons in growth pattern between the species difficult. However, it did appear that incremental fresh weight in *H. annuus* slowed down towards the end of the experiment, whereas *C. dissectum* fresh weight appeared to be increasing. There are two possible explanations for the reduced growth in *H. annuus*. Firstly, the rapid growth of the *H. annuus* plants resulted in the horizontal leaf extension to be restricted by the walls of the tube and the leaf area soon occupied a much greater proportion of the tube diameter than *C. dissectum*. The tube diameter would therefore create finite area of available PAR which, once covered by leaves, would limit photosynthesis of any further leaf material produced. Overcrowding of leaves in the tube could also alter the microclimate in the tube, increasing humidity and possibly reducing the CO_2 concentration. Growth can be reduced under conditions of low evaporative demand and high humidity with reductions up to half normal growth recorded in *H. annuus* (Winneberger, 1958). Secondly, several of the *H. annuus* plants had started flower initiation which would slow vegetative growth as resources were diverted to the development of reproductive structures. However, overall growth rate of *C. dissectum* was considerably lower than *H. annuus*, not reduced by shade and not affected by the N availability in this experiment..

There is a considerable difference in water metabolism between the two species. *C. dissectum* exhibits a relatively higher water throughput but is only slightly less efficient in its water use compared with a mesophytic species. Smith (1992) demonstrated that wetland species have an inherently higher negative water potential compared with mesophytic species and concluded that plants from wetland habitats require high soil water availability in order to obtain sufficient water for their metabolism. As *C. dissectum* is relatively efficient in its use of water, the high water metabolism suggests and adaptation to conditions of high soil water content. This could be linked to the low nutrient availability on fen meadows, as the

concentration of major nutrients, mainly N and P, in the soil solution would be very dilute and a high water throughput may be necessary to acquire an adequate supply of nutrients. Although shade reduced water uptake and improved water use efficiency in both species, there was a significantly lower response of these functions by *C. dissectum*. Therefore transpiration and photosynthesis in *C. dissectum* is less plastic compared with a mesophytic species, which suggests some degree of shade tolerance.

A comparison of morphological differences, under optimal conditions, revealed that *C. dissectum* had a lower RGR, LAR and SLA compared with a mesophytic species. However, under shade conditions the mesophyte increased LAR and SLA but reduced RGR, whereas these responses were absent in *C. dissectum*. The lack of response from *C. dissectum* in these morphological characteristics clearly indicate shade tolerance. Similarly, at the lowest N supply (25%) LAR and SLA were reduced and there was a negative correlation between RS and N supply in the mesophyte, whereas there was no response in *C. dissectum*. This suggests that *C. dissectum* is tolerant of low N availability compared with a mesophytic species.

3.8 CONCLUSIONS

This experiment has demonstrated that *C. dissectum* has a relatively high water requirement but not due to any inefficiency in its use of water. High water uptake would appear to be an adaptive mechanism to enable the acquisition of adequate mineral resources in a nutrient poor, but high soil water content, environment. Conversely, such an adaptation would mean that in a low nutrient environment with dry soil conditions it would not be able to obtain sufficient mineral nutrients. This would explain why *C. dissectum* does not spread beyond the M24 community into the similarly low nutrient status H4 dry heath vegetation which commonly borders M24 communities. Also if *C. dissectum* has an inherent high water metabolism it may be sensitive to tissue dehydration and therefore susceptible to drought. This aspect of *C. dissectum* physiology is investigated further in Chapter 5.

The general physiological and morphological characteristics of *C. dissectum* are those of a relatively slow-growing species which allocates a relatively small proportion of carbon gain to photosynthetic structure and leaf area expansion. It is relatively tolerant of shade and low nitrogen availability and exhibits no morphological plasticity, in terms of root:shoot allocation, in response to reduced nitrogen. These are all characteristics which suggest that *C. dissectum* has evolved a primary strategy of 'stress-tolerator' as described by Grime *et al.* (1988).

It can be concluded that soil water availability is an important environmental factor in controlling the distribution of *C. dissectum*. This experiment has shown that a high water requirement may be an evolved physiological mechanism which is closely linked to an overall adaptation to a low nutrient environment. A more detailed study of low nutrient tolerance (specifically phosphorus) is dealt with in Chapter 6. The relative competitive ability of *C. dissectum* may be such that the realised ecological niche is an environment which has a high water availability combined with low nutrient availability. Exactly the sort of habitat conditions which Chapter 2 has shown to exist where *C. dissectum* populations are found. This relatively narrow range of optimal habitat would in part explain why *C. dissectum* has only been observed in about ten plant communities and is only a key indicator species in one community, the M24 fen meadow, all of which are characteristically wet, low nutrient habitats.

4.0 MEASUREMENT OF PLANT WATER STATUS

4.1 INTRODUCTION

4.1.1 *Rationale*

Chapter 3 identified that, although *C. dissectum* was relatively efficient in its use of water, it has a high water requirement and therefore may not be tolerant of water stress. Therefore soil water availability may be a major environmental variable determining the distribution of this species. To measure water stress in plants requires the determination of plant-water status in relation to soil water availability. The original aim of this part of the study was to evaluate the various methods of measuring plant-water status and develop a suitable technique to quantify the relationship between water availability and plant-water status in *C. dissectum*. Using this knowledge, it was then proposed to measure the response *C. dissectum* to various levels of water stress to determine tolerance limits and whether the species has evolved any particular adaptations to ameliorate the effects of water stress (e.g. osmotic adjustment). An exhaustive study of the current techniques and available equipment to determine water status in plants was carried out and this chapter consists of a technical review of plant water relations measurement and its practical limitations.

4.1.2 *The importance of water to plants*

Almost every plant process is affected directly or indirectly by the supply of water. The importance of water to a plant has been detailed by Kramer (1983) and can be summarised under four general categories of key plant functions:

1) *Constituent:* water constitutes 80-90% of the fresh weight of most herbaceous plants and is as an important a part of the protoplasm as the protein and lipid molecules which constitute the protoplasmic framework.

2) *Solvent:* water functions as a solvent in which gases, minerals and other solutes enter plant cells and move from cell to cell and organ to organ.

3) *Reactant:* water is a reactant or substrate in many important processes, including photosynthesis and hydrolytic processes.

4) *Maintenance of turgidity:* water maintains turgidity which is essential for cell enlargement and growth and for maintaining the form of herbaceous plants. Turgor is also important in the control of stomatal opening and the movements of various plant structures.
Any deficit in plant water therefore affects every aspect of plant growth resulting in stress which impacts on physiology, leaf morphology and biomass production.

4.2 THE CONCEPT OF WATER POTENTIAL

The water status of plants and soil is generally described using thermodynamic terminology and the term 'water potential' proposed by Owen (1952) and Slayter and Taylor (1960) is now widely accepted. Water in plants and soils contains solutes that modify its colligative properties. The chemical potential, vapour pressure, osmotic potential and freezing point are lowered in proportion to the concentration of the solute present. The concept of water potential has been adopted as the best measure of the energy status of water in plants and soil (Kramer, 1983). Plant water potential is the amount by which its chemical potential is reduced below that of pure water. Plant water potential (Ψ) in its simplest form comprises the sum of osmotic potential (π) (the effect of solutes in the cell solution) and turgor pressure (P) (the hydrostatic pressure). Ψ is measured in megapascals (MPa) following the International System (SI) units and is usually negative. For example:

Plant condition	Ψ	=	π	+	P
Fully turgid	0.0	=	-2.0	+	+2.0
Partly turgid	-1.0	=	-2.0	+	+1.0
Flaccid	-2.0	=	-2.0	+	+0.0

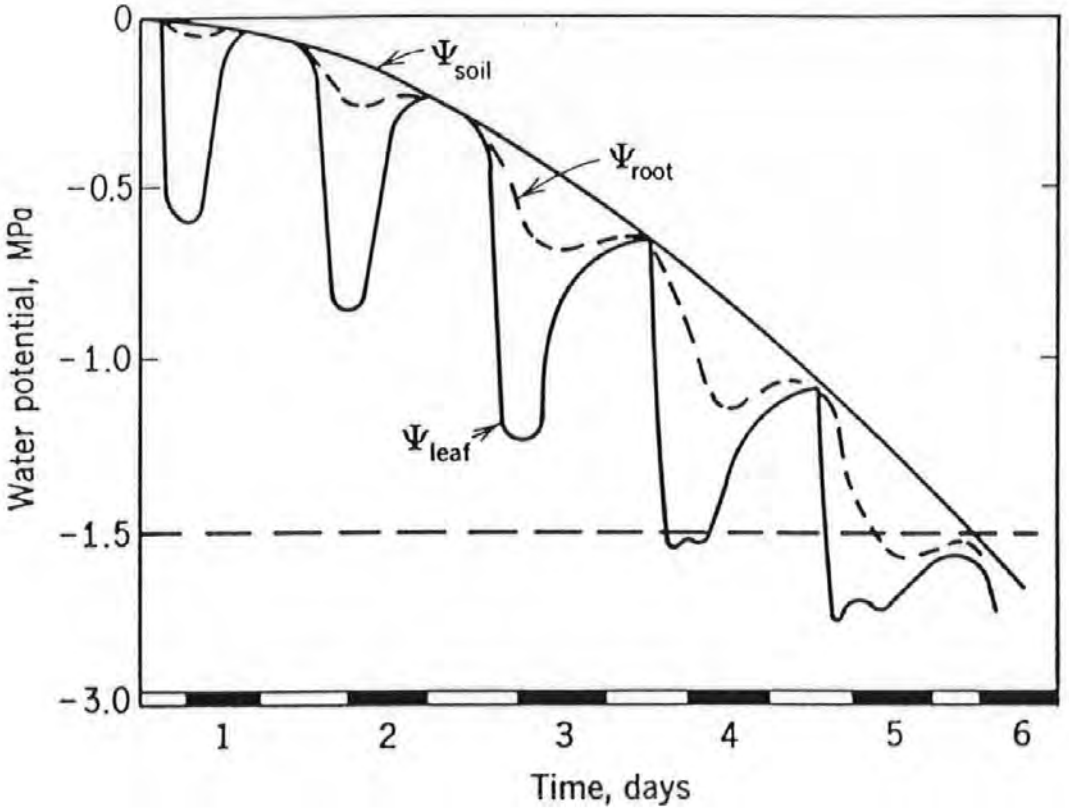
Since Ψ is usually negative, it becomes lower as water stress increases and can therefore be used to measure total plant water status at any given point in time. Water stress tolerance (i.e. before permanent damage occurs) varies widely among species and is characterised by their ability to tolerate the degree of water deficit ranging from -2.5 MPa in sunflower (*H. annuus)* to -10.0 MPa in various xerophytes, and to the air dry condition in a few

exceptional plants (Kramer, 1983). Also, some plants have methods of adaptation to changing water availability. Short-term fluctuations in water stress (eg. seasonal and diurnal fluctuations) are compensated by metabolic processes such as osmotic adjustment to maintain P by lowering of π arising from a net increase in solutes (Kramer, 1983; Morgan, 1984; Turner and Jones, 1980). Water deficits occur routinely during the daily diurnal cycle as a result of transpiration rate exceeding water uptake. However, the development of long-term water deficits, as a result of a progressive reduction in the supply of soil water, results in the eventual inability to recover at night. This cycle was analysed in detail by Slayter (1967) and shows how, as the soil dries, less and less recovery is possible. A reprint of this cycle is detailed in Figure 4.1. This analysis emphasises three factors in the development of water deficits: rate of transpiration, rate of water movement through soil to the roots and relationship of soil water potential to leaf water potential. The ability to measure Ψ and its components π and P would be an essential prerequisite in any study of plant water relations, particularly where specific levels of water stress need to be determined.

4.3 A REVIEW OF MEASUREMENT TECHNIQUES

General experimental techniques currently used in plant water relations study have been detailed and reviewed by several authors (Shackel, 1984; Slavik, 1974; Turner, 1981; Turner and Kramer, 1980). The literature suggests three main methods which have been widely adopted. Two of those, the pressure chamber and thermocouple psychrometry/hygrometry, measure total Ψ and the third is a relative measure of leaf water content. The three methods and their suitability for use in this particular study are evaluated below.

Figure 4.1 A model showing probable changes in leaf water potential (Ψ_{leaf}) and root water potential (Ψ_{root}) of a transpiring plant rooted in soil allowed to dry from a soil water potential (Ψ_{soil}) near zero to a point at which wilting occurs. The dark bars on the x axis indicate darkness and the broken horizontal line represents the typical wilting point (Slayter, 1967)



4.3.1 *Pressure Chamber Technique*

This method was made popular by Scholander *et al.* (1965) and has achieved widespread adoption due to its ease of use, speed, reliability and the fact that it does not require fine control of temperature. In this technique a leaf or branch is cut and placed in a pressure chamber with the cut end of the petiole or stem just protruding from the chamber through a rubber gland which is used to seal the chamber. The pressure in the chamber is gradually increased by compressed air until the sap just returns to the severed ends of the xylem vessels. The recorded pressure at this point is equivalent to total plant Ψ. This method relies entirely on being able to effect an air-tight seal around the protruding excised leaf material. Unfortunately *C. dissectum* is a rosette growing plant with no discernable petiole or stem. An examination of the sealing mechanism on the available unit from Plymouth (Biological Science dept.) clearly highlighted the impossible nature of attempting to seal the end of a cut leaf into the pressure chamber. This method was therefore deemed unsuitable for studying *C. dissectum.*

4.3.2 *Relative Water Content*

Relative water content (RWC) is a gravimetric method for measuring water status in plants based on the water content, as a percentage, in a leaf sample at a given time relative to the water content at full turgor (Slavik, 1974). Excised leaf disc samples are immediately weighed then allowed to absorb water to full turgor (constant weight) under very low light (approx. 10 μmol m^{-2} s^{-1} PAR), where the leaf sample is neither photosynthesising nor respiring They are then re-weighed and RWC calculated. Although relatively simple, this method is not an empirical measurement and a given RWC does not represent the same level of Ψ in leaves of different species or ages, or from different environments (Kramer, 1983). At best RWC can only be used as an indication of relative differences in plant water content over a given period. There is often a correlation between RWC and Ψ but this can only be determined by producing a material specific calibration curve which would require the ability to measure Ψ in the first instance (Slavik, 1974). RWC is only a measure of water content,

unlike the first method which measures the energy status of the water in plant tissue.

4.3.3 *Psychrometric Technique*

The thermocouple psychrometer is based on the principle that the relative vapour pressure (e/e_o) of a solution or piece of plant material is related to its Ψ according to the following equation:

Equation 1. $$\Psi = \frac{RT}{\overline{V}} \ln \frac{e}{e_o}$$

where R is the gas constant, T is the Kelvin temperature, $\overline{V}$ is the partial molar volume of water, e is the vapour pressure of water in the solution or tissue and e_o is the vapour pressure of pure water at atmospheric pressure.

A sample of plant material placed into a hermetically sealed chamber and allowed to equilibrate will generate a relative vapour pressure or relative humidity (RH) in the chamber equivalent to the total water potential of the tissue at the time of sealing into the chamber. The air sample in the chamber is then measured using a thermocouple sealed into the chamber. The use of thermocouple psychrometry/hygrometry was first proposed by Spanner (1951) and has since been developed in great detail by several authors, e.g. (Brown and Collins, 1980; Brown and van Haveren, 1972; Campbell, Campbell, and Barlow, 1973; Wiebe, *et al.*, 1971), and over 1000 papers have been published which use this method of water potential measurement.

There are two basic thermocouple methods:

1) *Psychrometric (Wet Bulb)* - which determines the wet bulb depression temperature. A thermocouple is cooled below the dew point by means of the Peltier Effect, thereby collecting micro-droplets of condensed water on the junction surface. The water is then allowed to evaporate, causing the temperature of the junction to be depressed below the ambient temperature, due to evaporative cooling. The wet bulb temperature depression persists until all the water has evaporated; then the thermocouple returns to the ambient

temperature. The differential temperature of the junction is an explicit function of the relative humidity and hence of the water potential in the media being measured. Thermocouple psychrometers have a typical responsivity near 4.7 $\mu V\ MPa^{-1}$ at 25° C.

2) *Hygrometric (Dew Point)* - which determines the dew point depression temperature. A thermocouple is cooled below the dew point as in the psychrometric method, but the temperature of the thermocouple is then controlled by the heat of condensation from the water condensing on its surface. This causes the thermocouple temperature to converge to the dew point, where it remains with a static amount of water. The dew point measurement is therefore continuous in nature, rather than transitory, as in the wet bulb depression measurement. The electromagnetic field produced by the temperature difference between the junction at the dew point temperature and the ambient temperature is a linear function of the water potential. The proportionality constant is approximately 7.5 $\mu V\ MPa^{-1}$.

The psychrometric/hygrometric method has the added advantage that, in addition to being able to measure total Ψ, it is also able to measure π. The determination of π is needs to be carried out on fixed (killed) plant tissue in order to destroy the semipermeability of the living cytoplasmic membranes, which would prevent release of many osmotically active compounds from the vacuoles of living tissues subjected to pressure. This is normally done by freezing samples in liquid nitrogen and then thawing. The sap is then expressed from the killed tissue, using a specially designed press (Slavik, 1974), absorbed onto a filter paper disc and then sealed into a thermocouple chamber for measurement.

4.4 TESTING THE PSYCHROMETRIC METHOD

As this method has the potential to measure total Ψ, π and, by calculation, P it would appear to be the obvious choice for this study. It was therefore decided that, if suitable equipment was available, this method should be evaluated further and tested for practicality, reliability and accuracy within the range of plant materials and experimental conditions likely to be

used for the study. A Wescor HP-115 Water Potential Data System (Wescor, Logan, Utah) was made available for our unlimited use by the Institute of Grassland and Environmental Research (Aberystwyth research station) and compatible Wescor C-30 thermocouple sample chambers were borrowed from the University of Exeter (Dept. of Biological Sciences). The HP-115 system is a battery powered automatic water potential data acquisition system enabling up to 15 sample chambers to be recorded sequentially over programmed intervals with memory storage which can be down-loaded onto a computer. The C-30 sample chamber is constructed from modified stainless steel tube fittings (12mm diameter x 23mm deep) fitted with a thermocouple psychrometer/hygrometer.

The first step in the evaluation process was to calibrate each chamber in conjunction with an allocated channel on the HP-115. There is an inherent variation in individual chamber response and there can also be variability as a result of chamber/channel interaction. Chambers are calibrated using filter paper discs saturated with NaCl solutions of known molality which correspond to water (or osmotic) potentials within the expected experimental range. The relationship between water/osmotic potential and μV output is linear down to -5 MPa (Slavik, 1974) and some examples of the relationship between molality, osmotic potential and μV is detailed in Table 4.1. The temperature of the solution affects the osmotic potential and at temperatures other than 25 °C a correction needs to be made:
Correct reading = actual reading / (0.325 + 0.027 T), where T is the temperature in °C. As the HP-115 only records a single μV reading, it seemed more appropriate to use in the hygrometric mode rather than the psychrometric mode. Determining the μV which represents the water potential in the psychrometric mode requires identifying a plateau signal before it returns to zero. Therefore the time delay allowed before taking the single reading, after cessation of the cooling current, would be largely guesswork and less accurate than the stable μV output provided by the hygrometric method. Later equipment developed by Wescor (HR-33T model) has an output for a chart recorder enabling more precise determination of the plateau when in the psychrometric mode.

Table 4.1 Relationship between molality and osmotic potential of NaCl solutions.

Molality	Osmotic potential of NaCl solutions @ 25°C (MPa)	Hygrometer equivalent μV output (@ 7.5 μV MPa^{-1})
0.10	-0.46	3.45
0.20	-0.92	6.90
0.30	-1.37	10.28
0.40	-1.82	13.65
0.50	-2.28	17.10

Source: Slavik (1974)

4.4.1 *Equipment calibration*

A series of calibration tests were carried out using the HP-114 and eight C-30 chambers using NaCl molal solutions of 0.10 to 0.35 at 0.05 increments. Theoretically it should be possible to calibrate using a single molal solution as the relationship within the expected range is linear. However, it was felt that the entire range should be tested for any possible deviation from the linear. Numerous replicate runs had to be conducted to determine optimum programme timings for several variable factors which affect the repeatability and therefore reliability of the output data:

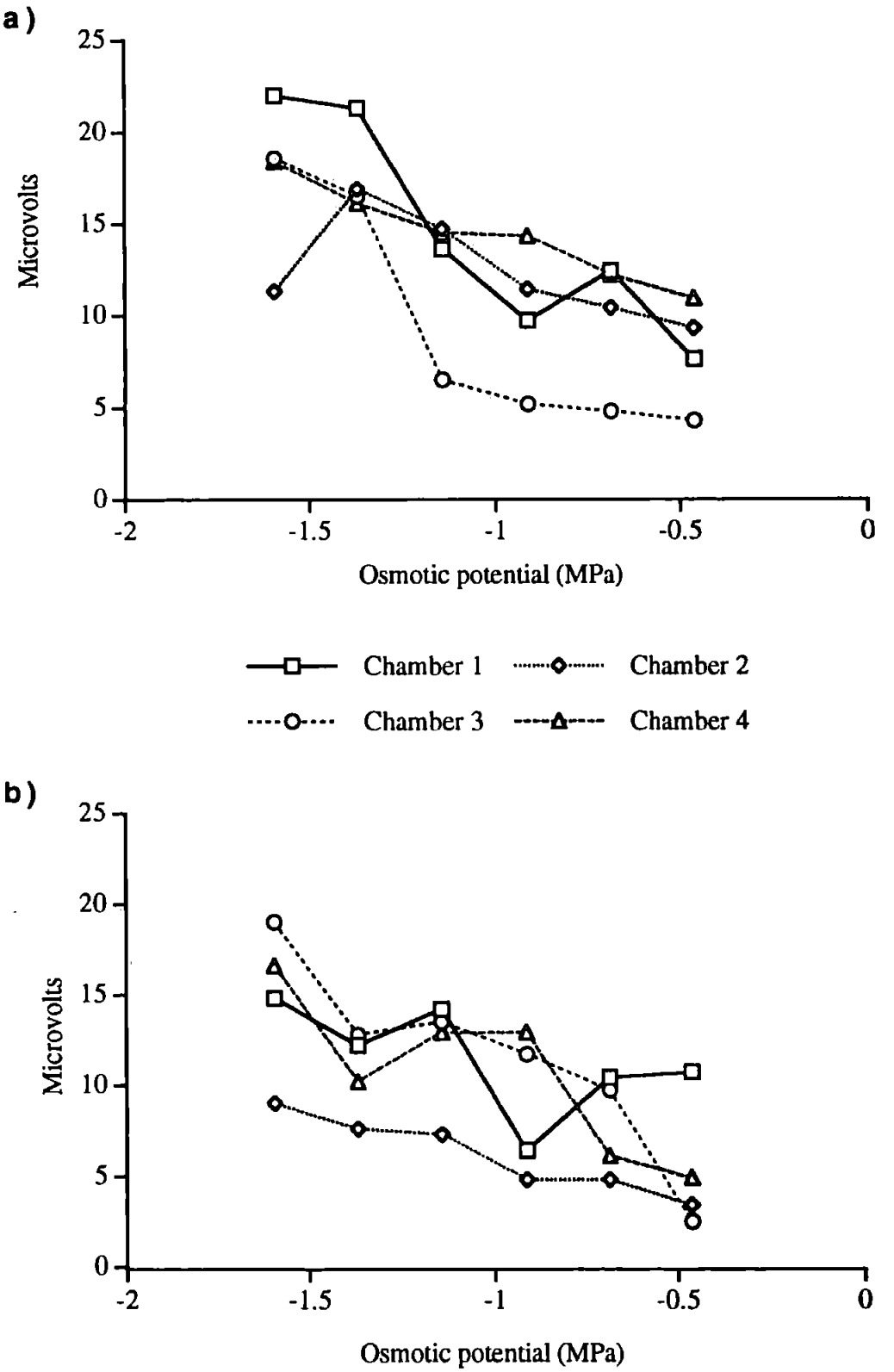
a) Duration of applied cooling current; sufficient length of time to condense a water droplet on the thermocouple - range: 10 to 30 seconds.

b) Delay time; the interval between cessation of cooling current and μV reading to allow the thermocouple temperature to converge to the dew point - range: 10 - 30 seconds.

c) Temperature and vapour pressure equilibration time; to allow stable isothermal conditions and vapour pressure equilibrium to develop in the sample chamber is most critical and can take anything between one and six hours.

It was found that cooling time of 15 seconds, delay time 10 seconds and equilibration time of between 3 - 4 hours were optimal to produce consistent readings. However producing a calibration curve over the range of osmotic potentials proved more difficult.

Figure 4.2 Initial calibration of C-30 sample chambers.
a) chambers 1-4; b) chambers 5-8



An example of an attempted calibration is illustrated in Figure 4.2 and clearly the data were extremely variable, non-linear and unusable. Repeated runs failed to produce anything that resembled a linear (or other) relationship between recorded μV and osmotic potential of the various solutions. No consistent μV output was observed for any of the chambers at any of the osmotic potential levels. The HP-115 machine was checked for reliability by comparing readings with Exeter University's HR-33T system and found to be functioning perfectly, suggesting the fault lay in the C-30 chambers. Subsequent communications with Wescor and their UK agents suggested thermocouple contamination as a possible cause of poor readings.

The thermocouples were cleaned, following the manufacturers recommendations, and one of the chambers was found to be faulty. A further seven chambers were borrowed from Exeter University making a total of 14, and the calibration process was repeated. Five of the 14 chambers were found to have an intermittent fault, where a spurious temperature reading was being recorded resulting in a low μV reading. Of the remaining nine chambers, four were giving highly variable readings with no acceptable means and standard deviations, particularly at low osmotic potentials below -1.0 MPa. The final five remaining 'best' chambers did produce a reasonable linear calibration but even these displayed high standard deviations around the mean readings at low potentials below -1.0 MPa. This whole process was extremely time consuming and the reliability and accuracy of the C-30 sample chamber was felt to be very questionable.

Correspondence with Wescor did nothing to provide any reassurance of the suitability of the C-30 chamber. The chamber was originally designed for large samples (entire leaves or a one inch square portion of larger leaves) and the use of 10mm diameter saturated filter paper discs (or leaf discs) may not provide sufficient water to provide uniform vapour equilibrium within the chamber, therefore measurement of small samples may be outside the chambers original design specification. If it is not possible to calibrate reliably the C-30 chamber using saturated filter paper discs, then obviously the chamber would not be suitable for measuring osmotic potential from expressed leaf sap. Wescor and other sources (Lancaster University

research staff) suggest that the Wescor C-52 sample chamber is more accurate with small leaf disc samples and expressed sap on filter paper discs, however C-52 chambers currently cost £850 each. Also, discussions with Lancaster University research staff highlighted another major source of potential error, namely temperature gradients. Any temperature differences or fluctuations during the measurement process along the length of wire from the meter to the thermocouple can cause inaccurate readings and it has been recommended that the equipment and all measurements are carried out in a temperature controlled room (Turner, 1981). This equally applies to both the C-30 and C-52 chambers. One final drawback associated with this method is the need to remove part of the plant leaf, usually a disc cut with a cork borer. In small plants with only a few leaves, such as *C. dissectum,* repeated water or osmotic potential measurements would result in permanent damage to the leaves of experimental plants, similar to the effect of an invertebrate herbivore. This would confound any experimental results as sampled plants would progressively suffer from defoliation stress in addition to water stress and there could be an interaction effect between the two stresses.

4.4.2 *Logistical problems*

Finally, the calibration process has highlighted some logistical problems. Each sample requires a minimum equilibration time of three hours, possibly more for leaf samples with highly negative Ψ, which would limit the number of possible runs in any single day to four at the most. Normally Ψ is measured at precise times, normally one hour pre-dawn and one hour after mid-day to measure overnight stress recovery and maximum stress level during transpiration respectively, as illustrated by Figure 4.1. Comparative measurement of plant water status needs to be carried out simultaneously on all plants under experiment. Excised leaf disc samples need to be placed into sample chambers immediately, as any delay would result in a change in their water status, therefore samples cannot be stored pending measurement. The inherent variability of the C-30 chambers would require that each sampling episode would need at least three samples from each of three plants for any single treatment measurement to provide a reliable statistical mean. This would necessitate running a minimum of nine chambers for every factor level being measured in an experiment. Even a

simple treatment versus control would need 18 chambers (the HP-115 water potential system only has 15 channels) and a multifactorial randomised block design experiment could not even be contemplated.

4.5 CONCLUSIONS

The use of thermocouple psychrometry/hygrometry, whilst ideal in theory for measuring Ψ and π, is fraught with many technical and logistical problems, not least that the measuring processes itself is intrusive and damaging to the plant. Also, this technique appears to severely restrict experimental design, particularly replication, thereby reducing accuracy and potential for credible statistical analysis. The available equipment and resources are unable to provide the accuracy and reliability of measurement and the necessary replication which would be essential to provide a meaningful study of plant water relations. The cost of purchasing suitable equipment would be prohibitive and well outside the budget of this project. From the review of the above methods for measuring plant water relations, it is clear that reliable and accurate measurements of Ψ, π and P are not possible due to a combination of technical difficulties, resource limitations and logistical problems. The inability to quantify total Ψ and its components π and P therefore precludes any detailed study of metabolic processes and physiological adaptations which could determine tolerance to water stress of *C. dissectum*. Therefore it was decided to limit any subsequent experiments involving water relations to those which only measure basic growth and morphological responses under conditions of slowly induced drought, along with survival and recovery from wilting episodes.

The conclusions of this chapter bring into question the many published experiments which have used these techniques for measuring Ψ, particularly thermocouple psychrometry. Without exhaustively surveying such work in detail, it can only be assumed that other workers have been able to overcome the logistical problems by substantial investment in thermocouple equipment and have appropriate controlled temperature facilities within which to carry out their experiments.

Water relations in plants is a fundamentally important area of plant physiology, as water plays a key functional role in a plant's metabolism and physical structure. An understanding of water relations is also of paramount importance in other areas of stress physiology, such as salt tolerance and frost hardiness. It would appear from the results of this study that the currently available techniques for measuring plant water status, although theoretically sound, all have some drawbacks and limitations in their practical application. The ability to record plant water status rapidly and reliably under a variety of conditions would enable a major advancement of knowledge in this key area of plant physiology. This will not be possible until a technical solution can be found to overcome the current problems and provide a non-invasive method of reliable and affordable measurement.

5.0 DROUGHT TOLERANCE

5.1 INTRODUCTION

The results of the field data in Chapter 2 (section 2.4) identified that the habitat of *C. dissectum* has a relatively high soil water content with very little seasonal fluctuation. Chapter 3 demonstrated that the species has a relatively high water requirement and concluded that soil water availability is an important environmental factor which is closely linked to the distribution of *C. dissectum*. It was also suggested that if *C. dissectum* has an inherent high water metabolism it may be sensitive to tissue dehydration and therefore susceptible to drought. Although drought can result from an extreme meteorological event, it can also result from man-induced activities such as drainage and eutrophication. North devon M24 sites are integrated within an agricultural environment and could therefore be affected by drainage or nutrient run-off from nearby agricultural operations. This could result in a reduction in the soil water status of M24 sites. Therefore the following chapter investigates the effect of soil water deficit on the of physiology of *C. dissectum*.

Dry matter production in vegetation is subject to a variety of environmental constraints, the most frequent of which are related to shortages and excesses in the supply of solar energy, water and mineral nutrients. Earlier chapters examined the effects of stress on *C. dissectum* resulting from shade and low nutrient availability. The latter is an environmental constraint which is known to exist in fen meadows and is relatively constant from year to year. In contrast, water availability in temperate climates is an environmental variable which is very unpredictable and is only a constraining factor when drought conditions develop. Linsley *et al.* (1959) defined drought as a sustained period without significant rainfall. As a meteorological event, drought can be defined as a time period when the amount of precipitation is less than some designated percentage of the long-term mean. However, drought as an environmental event can be described as the absence of rainfall for a period of time long enough to cause depletion of soil moisture and damage to plants (Kramer, 1980).

For a period of dry weather to affect a plant community, the rainfall deficit must lead to a soil water deficit and ultimately to a plant water deficit.

The environmental conditions on fen meadows detailed in Chapters 1 and 2 indicate that water availability is rarely a limiting factor. Data collected during this study in 1997 and 1998 reflect two relatively wet summers and may not be representative of any extremes of soil water deficit during a particularly hot dry summer such as 1976. Long term weather data (Chapter 2) has recorded five very dry summers in the last 30 years. Also, the likelihood of soil water deficit could be greater for *C. dissectum* populations on dune slack habitats such as Kenfig and Braunton Burrows. Data collected on Braunton Burrows (Chapter 2) indicate lower soil water content in summer compared with fen meadow sites. Although drought on fen meadows may be considered to be a relatively rare occurrence, agricultural or other operations (e.g. road building) on or near fen meadows could alter the site hydrology resulting in increased drainage and a lower water table. Also, increased eutrophication caused by nutrient run-off from surrounding agriculturally improved sites can result in more vigorous growth of some grass species (e.g. *Holcus lanatus*) leading to increased transpirational loss on fen meadows. These factors, individually or in combination, could lead to an increased risk of mean summer soil water deficit in a particularly hot dry year and increase the risk of plant water deficit.

The ecological implications for *C. dissectum* under such infrequent conditions are worth considering. Extreme events in nature such as drought, floods, fire etc. are unpredictable but their importance can be enormous and measured in terms of the impacts they could have on a particular ecosystem or species. If drought resulted in the extinction of *C. dissectum* then the probability of a drought episode at a particular location would represent the lowest risk of extinction. If other factors were included such as changes in site hydrology, eutrophication etc., then the probability of extinction would increase.

Earlier chapters have highlighted the fact that *C. dissectum* is generally only present in habitats where soil moisture content remains relatively high throughout the year.

The results of Chapter 3 led to the conclusion that, although the species is relatively efficient in its use of water, it does have a particularly high water uptake rate. This poses the question as to whether *C. dissectum* is confined to relatively wet habitats because it has a particularly high water requirement or whether the high water uptake just an adaptation to a high soil-water environment ?. If the species has a particularly high water requirement or metabolism it would be particularly sensitive to drought or more precisely tissue dehydration. This chapter examines the response of *C. dissectum* to dehydration to assess the affect of a drought episode on plant performance in the medium term, the risk of mortality and whether this could lead to population extinction.

5.2 PLANT STRATEGIES AND DEHYDRATION

Water stress as a result of tissue dehydration induces many morphological, phenological, anatomical and physiological responses (Hsiao, 1973; Paleg and Aspinal, 1981; Turner and Kramer, 1980). Drought tolerance is a generic term used to cover a range of mechanisms, often referred to as strategies, which plants have evolved to enable them to withstand dehydration (Jones, Turner, and Osmond, 1981; Kramer, 1980; Levitt, 1972; Ludlow, 1989). These strategies fall into three distinct categories which are summarised below following the nomenclature of Kramer (1980)

Drought Escape

Plants which are particularly sensitive to dehydration and avoid it completely by completing their life-cycle rapidly (or at least their reproductive cycle) during periods of water availability. This is characteristic of only a few species such as desert ephemerals or species growing in areas with well-defined wet and dry seasons.

Dehydration Postponement

Tissues sensitive to dehydration (lethal leaf water potential -1.5 to -2.5 MPa; lethal relative water content greater than 50%). Plants with this strategy attempt to minimise water loss and maximise water uptake by morphological or physiological

modifications that reduce transpiration or increase absorption. Characteristics include large/deep root systems, stomatal control and shedding of older leaves. However, there is a cost to the plant associated with this strategy. Both photosynthesis and carbon gain are reduced and recovery is slow after stress is relieved. Also, this strategy only provides a short-term survival of dehydration.

Dehydration Tolerance

Tissues able to withstand severe dehydration (lethal leaf water potential less than -13.0 MPa; lethal relative water content less than 25%). Typical responses to dehydration include leaf rolling and high osmotic adjustment (common in tropical C_4 grasses). No particular costs of this strategy have been identified. However plants with this strategy often have a lower overall carbon fixation than plants with the postponement strategy. This provides a good long-term survival during periods of drought.

There is an abundance of literature relating to drought tolerance in plants which mainly concentrates on the effect of drought on yields of economically important crop species in tropical or arid climates. From an ecological viewpoint, drought tolerance in natural plant communities is generally concerned with species survival during conditions of drought rather than phytomass yield. Similarly most ecological studies of drought tolerance have been concerned with plants from arid environments or regions with extreme seasonal variations in water availability. In species of temperate climates, studies generally focus more on response to degrees of water stress rather than drought survival (Pickett and Bazzaz, 1976; Zimmermann and Lectowicks, 1982). Few comparative studies on the influences of drought stress on species of contrasting ecology have been made due to the difficulty in applying drought stress at constant levels below field capacity (Boot, Raynal, and Grime, 1986). It is most likely that *C. dissectum* will fall into the dehydration postponement category. However, species which have a dehydration postponement strategy will vary in their ability to postpone tissue dehydration.

It is possible that species which have evolved or adapted to a high soil moisture environment are likely to be more sensitive to water stress and have a poor ability to postpone dehydration compared with plants from drier habitats.

5.3 PHYSIOLOGICAL RESPONSE TO DEHYDRATION

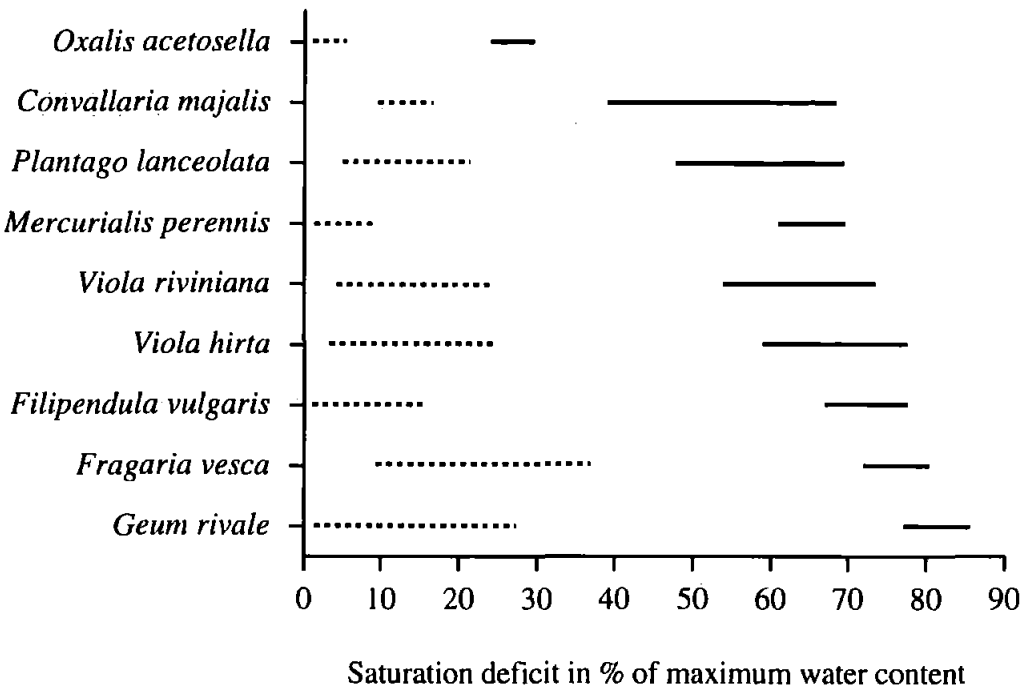
Although it is clear that differences in resistance to dehydration damage do exist amongst plant species, it is difficult to quantify such differences because of uncertainties in establishing suitable indices of water stress (Fitter and Hay, 1987). The primary effect of drought stress is cell dehydration, which is elastic and completely reversible up to a point, beyond which it is plastic, irreversible and therefore injurious. All plants are subject to moderate elastic cell dehydration as shown by diurnal expansion and contraction of leaves (Chaney and Kozlowski, 1969; Slayter, 1967). The effect of such elastic dehydration on growth varies with the species, however, when the dehydration is sufficient to eliminate turgor, growth ceases (Lawlor, 1969). Under natural conditions most plants in temperate climates experience moderate water deficits but these are usually small and fall within a relatively narrow range that is well below the critical saturation deficit (Figure 5.1). The water saturation deficit (WSD) of a plant at any particular moment (at which its fresh weight is referred to as its natural weight) is calculated as follows:

$$\text{WSD} = \frac{\text{saturation weight} - \text{natural weight}}{\text{saturation weight} - \text{dry weight}} \times 100$$

and the critical water deficit is the deficit resulting in mortality (Levitt, 1972).

The species differences in critical water deficit detailed in Fig 5.1 highlight that the wilting point of a species is determined by its inherent water deficit sensitivity rather than a specific leaf or soil water potential. In addition to being dependent on turgor pressure, wilting is also a function of the mechanical properties of cell wall and tissue (Hsiao, 1973). However, as water stress develops, many physiological and metabolic processes are affected before wilting occurs. These responses have been comprehensively detailed in a review by Hsiao (1973) which also highlights the differential sensitivity of twelve major responses to levels of water stress and that the sensitivity of those responses differs among species.

Figure 5.1 Comparison between the highest average natural water deficit (broken lines) and the critical water deficit (solid lines) for the leaves of different species (Adapted from Levitt, 1972)



The most sensitive are cell growth, wall synthesis and protein synthesis which are all affected by a very small reduction in tissue water potential of less than -0.25 MPa. Other general morphological responses to water stress include an increase in root:shoot ratio as a result of the greater sensitivity of leaves to water stress (Sharp and Davies, 1985) and a reduction in leaf area by acceleration of the senescence rate in older leaves (Begg, 1980)

5.4 AIMS AND OBJECTIVES

The main aim of the experiment described in this chapter is to study the effects resulting from a period of drought on *C. dissectum* and the possible ecological implications of such an event. Specific objectives were:

1) To measure the ability of *C. dissectum* to survive and recover from varying degrees of dehydration
2) Determine whether it is particularly sensitive to tissue dehydration by measuring substrate water content at wilting point.
3) Measure the medium term effects of varying degrees of dehydration on the physiology and morphology of *C. dissectum* and whether those effects suggest mechanisms to postpone or tolerate dehydration.

The experiment was designed to measure plant response at two stages of dehydration. The first stage is the dehydration period up to the point where turgor pressure is lost and the plant wilts, which is referred to as 'water stress'. The second stage is the post-wilting period where growth will have ceased and progressive dehydration will eventually lead to irreversible tissue damage. This stage is referred to as 'prolonged dehydration stress'.

5.5 MATERIALS AND METHODS

C. dissectum plants were raised from seed and grown for five months on sand watered with Rorison's solution (10% of full strength) prior to commencing the experiment. The experiment was carried out under controlled environment conditions in a phytotron growth cabinet using the same ISP standard regime detailed in Chapter 3. Plants were transferred to

102 mm diameter plastic plant pots filled with washed 'silver' sand which had a particle size distribution of 88% between the range 0.25 mm to 0.5 mm and 11% between the range 0.125 mm to 0.25 mm. Pots were initially watered with Rorison's nutrient solution (10% of full strength) to maximum water holding capacity (after all free drainage had ceased), approximately 18% water content. Sand was chosen as a growing media because of its moisture characteristic curve, releasing most of its water over a small range of matric potential based on grain size distribution, where it is estimated that water content is approximately 2% (by volume) at permanent wilting point (Marshall and Holmes, 1979). Also sand media allows maximum recovery of root material for weighing at the end of the experiment.

Preliminary trials of growing plants in sand, using a growth chamber, had shown that water evaporates relatively rapidly and it was estimated the plants would reach wilting point after eight days. The soil water release characteristics of sand mean that plants would not experience a substantial increase in matric potential until low levels of sand water content were reached. It was estimated, from the sand drying rate and the plant water uptake rate (from Chapter 3), that the level of sand water content where plants would experience stress from increased matric potential would be approximately day six. Therefore plants would only experience water stress over a period of two days before wilting point was reached. As this degree of exposure to water stress is relatively short, it was decided to recharge the water to half the maximum holding capacity (9%), by adding 50 ml of nutrient solution on day seven, thereby increasing the duration of water stress for the experiment to four days. There was no way of determining at what sand water content plants would begin to experience water stress. However, by doubling the period of time plants were exposed to low sand water content should allow sufficient time for the less sensitive physiological/metabolic processes to be affected.

All plants were allowed to dry until visibly wilted and leaves were all flaccid. Wilting was deemed to have occurred when all leaves on a plant were visibly flaccid at the beginning of a daytime cycle. This specific time was chosen to ensure that any plants wilted at the end of a

daytime cycle did not recover turgor during the night cycle. Six levels of dehydration were then imposed subsequent to wilting, where re-watering was withheld for increasing time intervals post-wilting: 1 = immediate re-watering, 2 = after 4 hours, 3 = after 8 hours, 4 = after 16 hours, 5 = after 32 hours, 6 = after 64 hours. In addition, a comparison treatment was included, which was maintained fully watered throughout the experiment. The mean plant weight within each treatment group was checked to ensure that there were no significant differences at the start of the experiment. Once all plants had wilted and re-watered, they were maintained fully watered for six weeks to allow recovery from dehydration. At that point the experiment was stopped and all plants were harvested and weights etc. recorded. At the start of the experiment all plant fresh weights were recorded and a random sample of plants (n=6) were weighed and dried to record initial water content and root:shoot ratio. During the initial drying period three replicate pot and sand blanks, watered to maximum water capacity, were included to measure water loss by evaporation.

The experimental design was a fully randomised layout with six replicates of each treatment. The raw data were examined for homogeneity of variances using Hartley's F_{max} test. The water stress stage was analysed using a Student's 't' test or where there were heterogeneous variances a Mann-Whitney 'U' test was employed. The prolonged dehydration stress stage was analysed by regression analysis. The each regression analysis was examined for autocorrelation using the Durbin-Watson test statistic and residuals were examined for homogeneity of variances. Where any heterogenous variances were identified, the data were log transformed and re-examined. All proportional data were subjected to arcsine transformation before analysis. Statistical tests were derived from texts by Fowler, Cohen and Jarvis (1998), Kanji (1999) and Zar (1996).

5.6 RESULTS

Drying cycle

The decline in water content of the sand media was estimated from the three replicate pot and sand blanks. Mean maximum water content at the start of the experiment was approximately 16.5% and water loss by evaporation had reduced the content of the blanks to about 2% within seven days. The complete drying cycle is detailed in Fig 5.2 which includes an estimated sand water content of plant replicates based on a plant water uptake of 5 ml per day which was derived from the experiment in Chapter 3. Based on that extrapolation, it can be seen from Fig 5.2 that sand water content should have reached zero by day 15 (water content of sand blanks was < 1%) and in fact the first plants wilted on day 16. Six plants wilted on day 16 followed by 15 plants on day 17 and the remainder wilted on day 18. The drying regime used in this experiment is somewhat artificial, being much faster than would occur in a natural soil other than possibly a sand dune soil. However, the specific objectives of the experiment required the application of dehydration stress which this method achieved in a relatively uniform way.

5.6.1 Water stress

The effect of water stress (defined as the period of dehydration up to plant wilting) on *C. dissectum* was measured by a range of physiological and morphological responses, measured after a recovery period of six weeks, and these are summarised in Table 5.1.

Figure 5.2 Decline in water content of sand media measured by weight loss in control blanks and estimated water content of plant replicates based on evaporation plus plant uptake of 5 ml per day

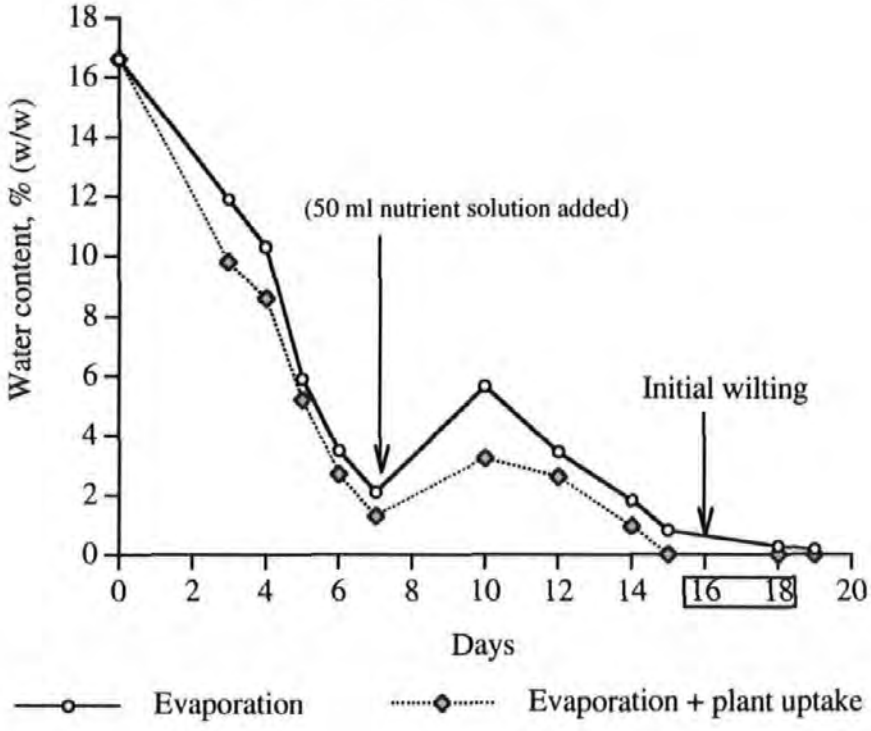


Table 5.1 Comparison of *C. dissectum* responses under conditions of water stress compared with fully watered conditions (n= 6).

Measured parameter (on a dry weight basis)	Unstressed (mean ±SD)	Stressed (mean ±SD)	Significance level
Root % water content	0.714 (0.016)	0.741 (0.014)	P= 0.012
Dry weight gain (g) including senesced leaves	1.55 (0.31)	0.90 (0.25)	P< 0.003
Total relative growth rate (per week)	0.134 (0.009)	0.064 (0.021)	P< 0.001
Root relative growth rate (per week)	0.173 (0.12)	0.102 (0.014)	P< 0.0001
Shoot relative growth rate (per week)	0.068 (0.027)	0.001 (0.044)	P= 0.013
Root:shoot ratio	2.63 (0.80)	2.57 (0.88)	n.s.
Senesced leaf weight as a proportion dry weight gain	0.047 (0.038)	0.282 (0.235)	P= 0.0065
Senesced leaf number as a proportion of total leaf number	0.132 (0.078)	0.284 (0.164)	n.s.

Water content

The water content of roots was higher (P= 0.012) in the water stress treatment compared with the unstressed treatment but leaf water content was not significantly different.

Growth

Total phytomass production, measured as total dry weight gain (DWG) including senesced leaves, was reduced (P< 0.003) 42% by water stress. However, the proportion of senesced leaf material within the DWG was six times greater (P= 0.0065) in the stressed plants compared with the unstressed plants. Relative growth rates (RGR) were calculated without senesced leaf material to give a true measure of live phytomass increment, which is then compatible with root:shoot ratios. Whole plant RGR in the stressed plants was reduced (P< 0.001) to half that of the unstressed plants. There was also a marked difference between root and shoot RGRs of the stressed plants. Shoot RGR was reduced (P= 0.013) by over 98% compared to the unstressed plants, whereas root RGR was reduced (P< 0.0001) to

41% of the unstressed plants. However, there was no significant difference in root:shoot ratio between stressed and unstressed plants.

Leaf turnover

There was no significant difference in the mean total leaf number per plant (including senesced leaves) between stressed and unstressed treatments (mean 10.4; ±SD 1.3) at the end of the recovery period. Similarly, the mean proportion of leaves senesced to total leaves per plant was not affected by the stress treatment.

5.6.2 Prolonged dehydration stress

Increasing degrees of dehydration stress were imposed by delaying post-wilting re-watering by a series of doubling time intervals, namely: 0 (immediate re-watering), 4, 8, 16, 32 and 64 hours. All the plants at the 64 hour treatment died but there was 100% survival at all other treatment levels. The effect of prolonged dehydration stress on *C. dissectum* was measured (after a six week recovery period) by the same range of physiological and morphological responses used in the water stress analysis.

Water content

Although root water content increased under water stress, there was no significant trend with prolonged dehydration. However, there was a positive linear relationship ($P< 0.001$; R^2 38.6%) between shoot water content and dehydration duration (Fig 5.3)

Growth

There was a negative linear relationship ($P= 0.006$; R^2 24%) between total DWG (including senesced leaves) and dehydration duration (Fig 5.4a). Also, there was a positive linear relationship ($P< 0.001$; R^2 42%) between the senesced leaf proportion of total DWG and dehydration duration (Fig 5.4b). Total plant RGR (without senesced leaves) was linear and negative ($P< 0.001$; R^2 41%) in relation to dehydration duration (Fig 5.5a).

Figure 5.3 Relationship between shoot % water (arcsine transformed) and dehydration duration

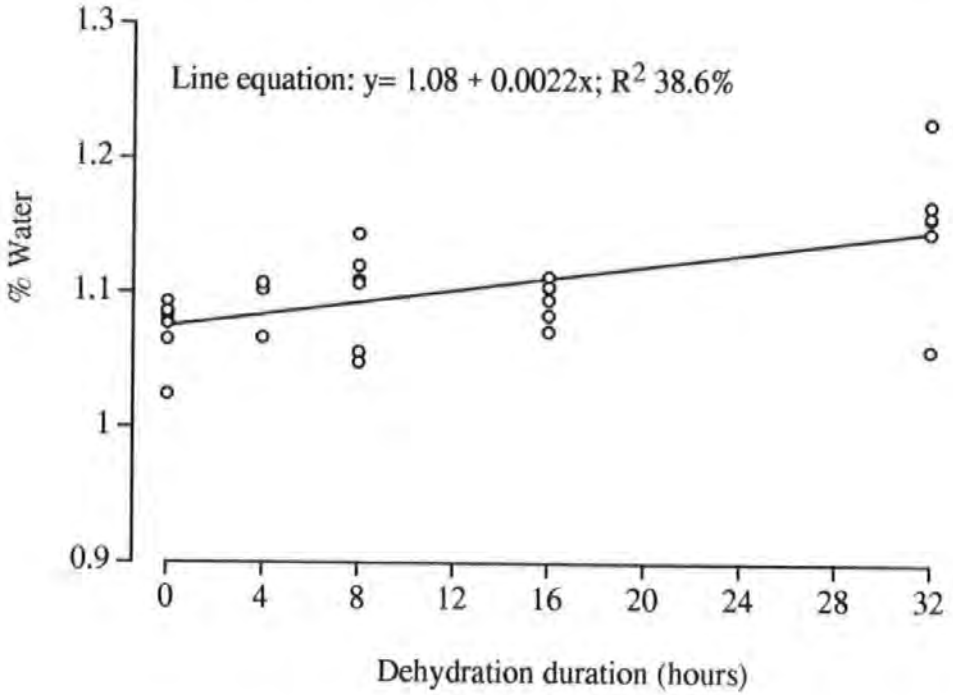


Figure 5.4a Relationship between total dry weight gain (including senesced leaves) and dehydration duration

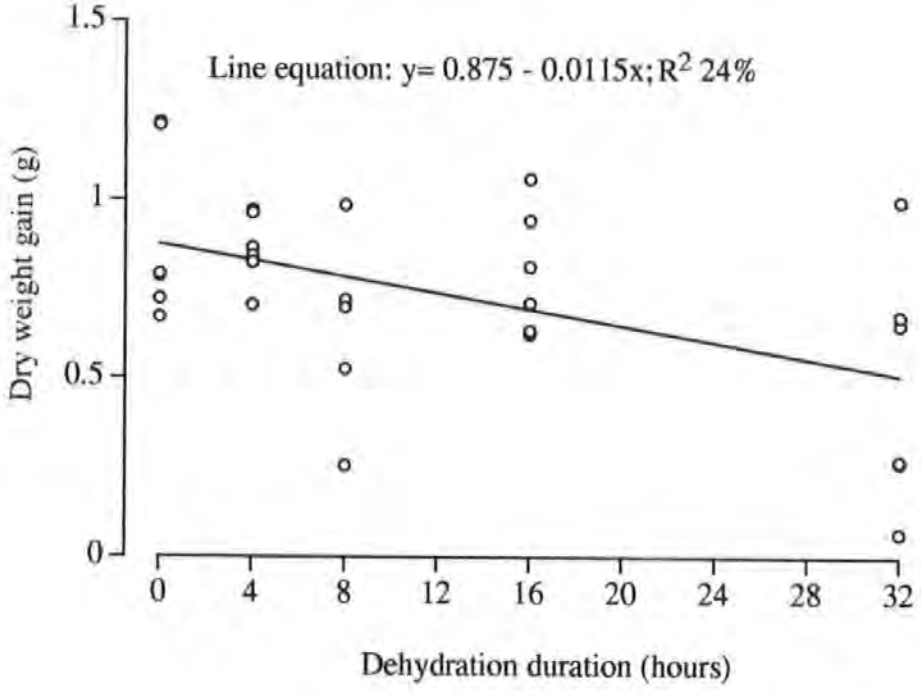


Figure 5.4b Relationship between proportion of senesced leaf dry weight to total dry weight gain (arcsine transformed) and dehydration duration

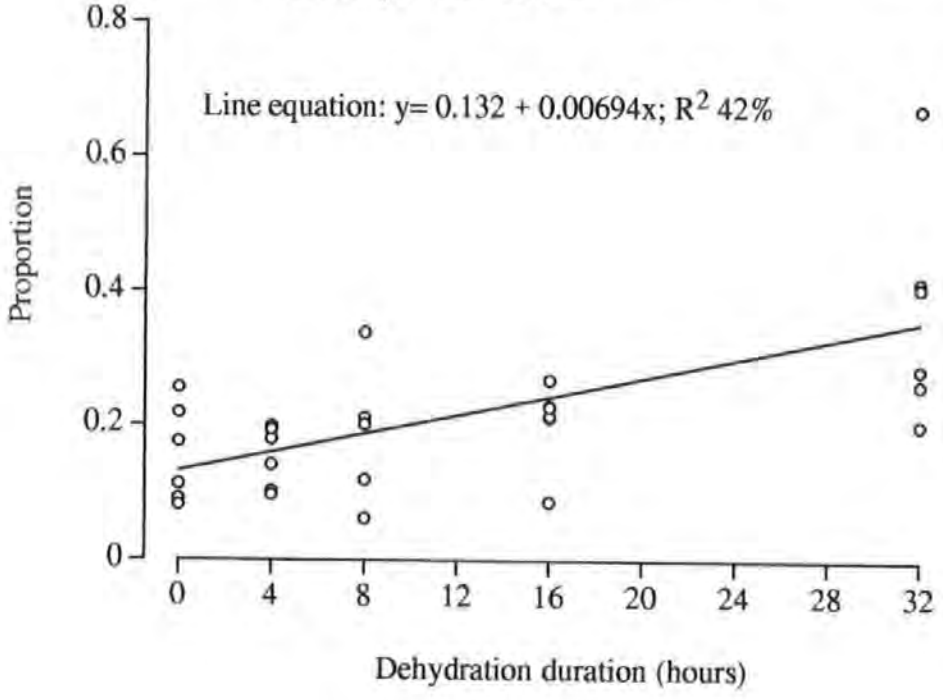


Figure 5.5a Relationship between total dry weight relative growth rate (per week) and dehydration duration

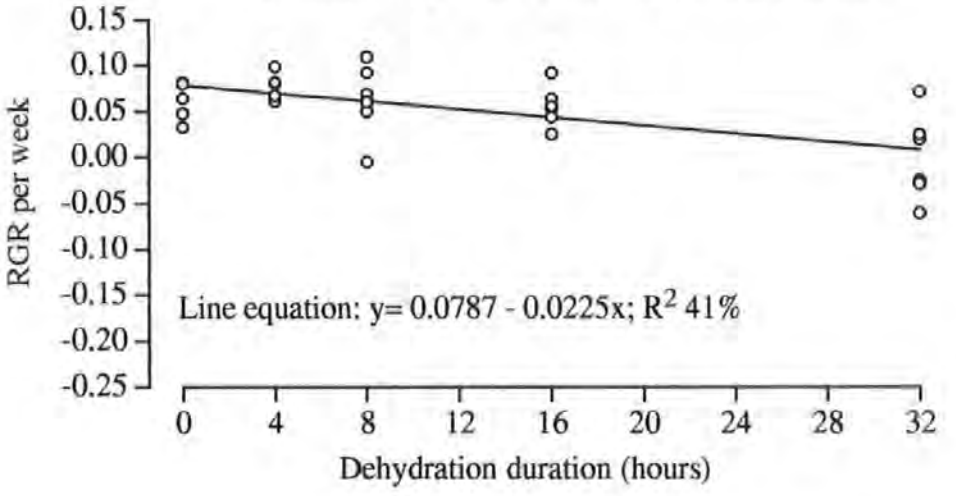


Figure 5.5b Relationship between root dry weight relative growth rate (per week) and dehydration duration

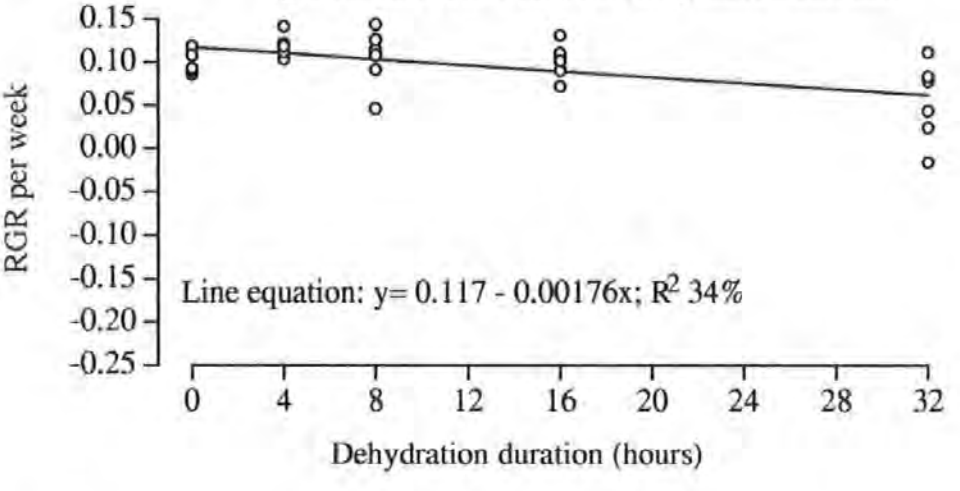
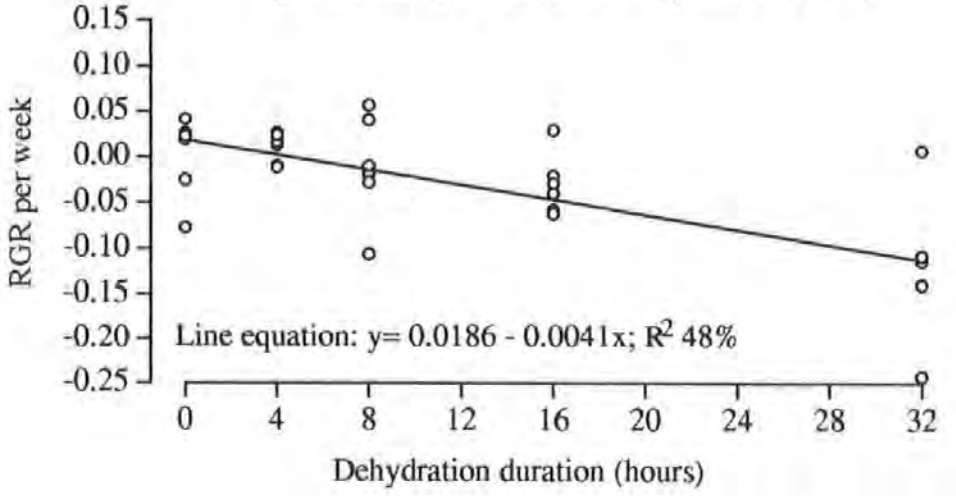


Figure 5.5c Relationship between shoot dry weight relative growth rate (per week) and dehydration duration



The same pattern of negative linear relationship with dehydration duration was observed in both root RGR ($P= 0.001$; R^2 34%) and shoot RGR ($P< 0.001$; R^2 48%)(Figs 5.5b and c). The slope of the root and shoot RGR lines were not significantly different. However, the mean root RGR remained positive, whereas mean shoot RGR became negative at 8 hours and over dehydration. There was a positive linear relationship ($P< 0.001$; R^2 39%) between root:shoot ratio and dehydration duration (Fig 5.6).

Leaf turnover

There was no significant relationship between the final total leaf number per plant (including senesced leaves) and dehydration duration. Which indicates that overall leaf production was not affected by prolonged dehydration. However, there was a positive linear relationship ($P< 0.001$; R^2 39%) between the senesced leaf/total leaf proportion and dehydration duration (Fig 5.7). This shows that leaf senescence rate increased with increasing prolonged dehydration. Observations during the course of the experiment revealed that leaves were senesced in a systematic way, with the oldest leaves senescing first.

5.7 DISCUSSION

The sand water content of the droughted treatments was estimated to be virtually zero by day 15, as the mean water content of the sand blanks was only 0.81% (± 0.19 SE) at this point. When converted to volumetric water content, this represents 1.25% (± 0.31 SE) which is below the 2% generalised permanent wilting point of sand quoted by Marshall (1979). This was at least 24 hours before wilting occurred, which suggests that *C. dissectum* is able to maintain turgor in a very dry soil with a negative soil water potential possibly in excess of -1.5 Mpa. The main responses to water stress during the period up to loss of turgor pressure and wilting demonstrate some lasting negative effects on the plant over the medium term. The results of this experiment clearly illustrate that during the period six weeks after a water stress episode, there was a general reduction in overall plant growth. Total phytomass production was almost halved and there was an increase in the proportion of senesced leaf material. Similarly, total plant relative growth rate was halved.

Figure 5.6 Relationship between dry weight root:shoot ratio (log-transformed) and dehydration duration

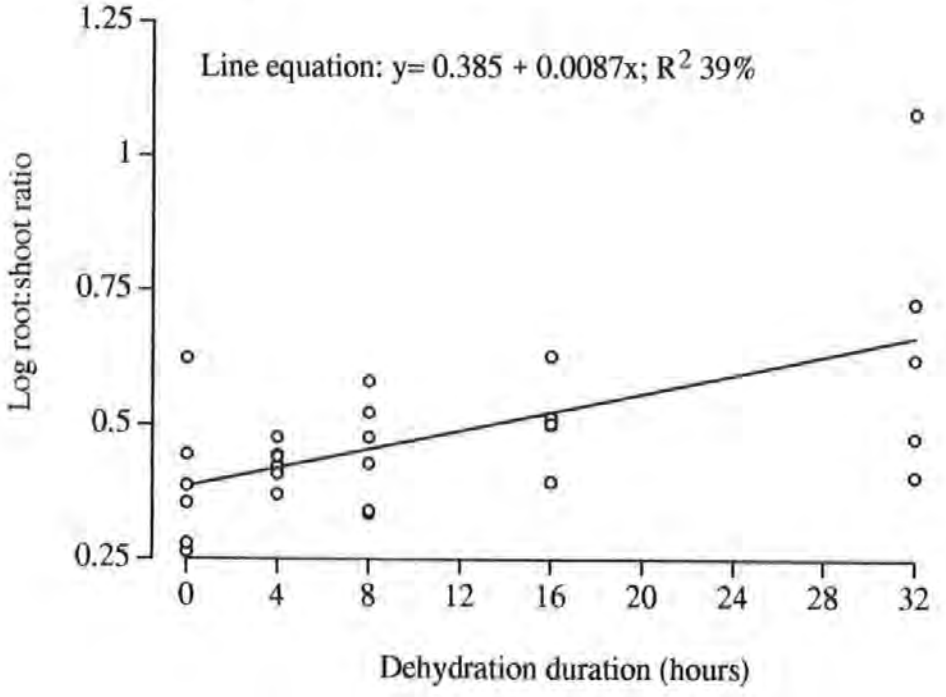
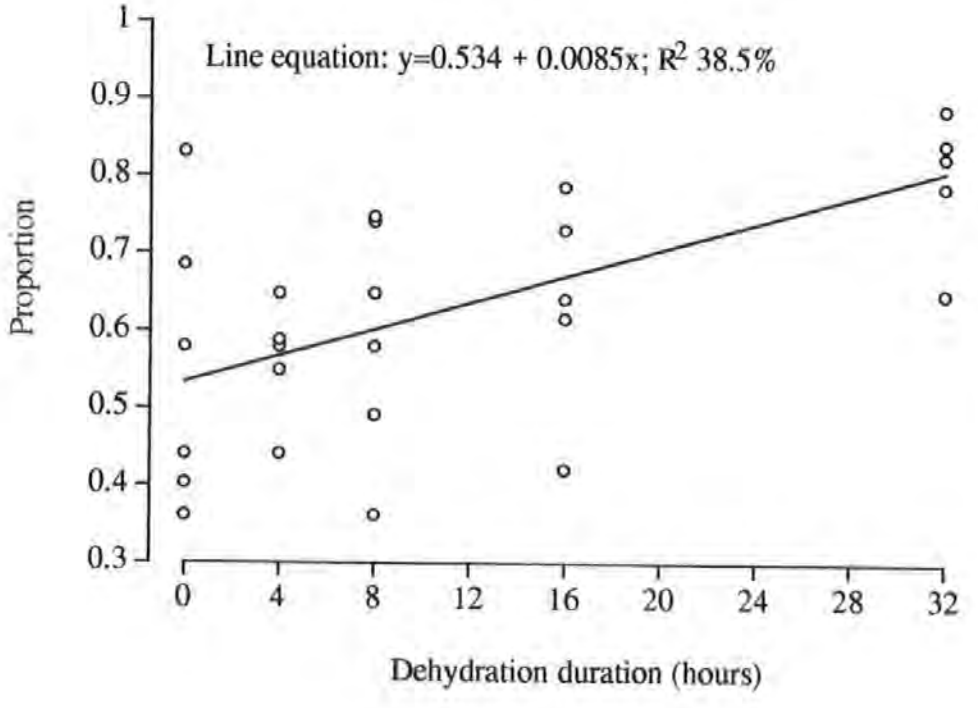


Figure 5.7 Relationship between proportion of senesced leaf number to total leaf number (arcsine transformed) and dehydration duration



Shoot RGR was particularly affected, declining to only 1% of the root RGR. Therefore, although *C. dissectum* was able to maintain turgor until sand water was almost depleted, this caused a substantial reduction in plant performance which would have a lasting effect. Water stress also resulted in increased root water content. This may be an acclimation response to drought, as increased plant water status has been documented as one of the acclimation mechanisms important for survival in natural plant communities (Turner, 1986).

The length of time a plant remained in a wilted state was directly related to plant performance and ultimately survival. The results demonstrate that *C. dissectum* was able to survive in a wilted state for a period of at least 32 hours but a period of 64 hours would be fatal. It is unfortunate that neither of these time periods produced partial mortality giving an indication of the 50% mortality level, which would more accurately determine maximum survival time. As no direct comparisons with other species of survival time are possible, it is difficult to interpret these results in comparative terms. However, the ability of *C. dissectum* to survive in a wilted state for periods of over 32 hours suggests that the species is not particularly sensitive to dehydration but does result in lasting negative effects on plant performance. The effect of increased tissue dehydration post-wilting was evident even after a six week recovery period. Plant growth, both dry weight gain and relative growth rate, declined linearly with increasing dehydration. Shoot relative growth rate showed the greatest response, becoming negative after 8 hours in a wilted state. This was directly related to an increase in leaf senescence rate with increasing dehydration, which was reflected in a corresponding increase in root:shoot ratio. Also, leaf water content increased with prolonged dehydration. This was most likely due to the shedding of older leaves, resulting in a greater proportion of young leaves which would have a higher water content.

The results of water stress and prolonged dehydration both highlight the differential sensitivity between roots and shoots, with shoots being more sensitive. This resulted in a greater reduction leaf RGR (to the extent of being negative) compared with root RGR. The greater sensitivity of leaves to dehydration was reflected by the increase in senescence rate of older leaves, a typical response (Begg, 1980). An interesting characteristic of *C. dissectum*,

highlighted by the unstressed replicates, is the higher root RGR, some 2.5 times the shoot RGR. This reinforces the inherent preferential allocation of phytomass to root production observed in the phosphorus experiments. The least sensitive morphological response appeared to be root:shoot ratio which only increased with prolonged dehydration.

5.8 CONCLUSION

It has been established that *C. dissectum* can maintain turgor at relatively low soil water levels and can survive in a wilted state for a period of longer than 32 hours but less than 64 hours. However, in the field, that period may be shorter as a result of the lower relative humidity (R.H.) under droughted field conditions compared to the higher R.H. (85%) maintained in the growth chamber. Field survival time in the wilted state will be directly related to leaf water evaporation rate which will depend on R.H. and air flow at the leaf surface. However, this may be ameliorated to some extent due the leaf pubescence on the abaxial surface.

It would appear that *C. dissectum* can survive short-term dehydration and exhibits some characteristics of the 'dehydration postponement' strategy (Section 5.2.), namely a large root system and shedding of older leaves, which suggest the species is relatively tolerant of drought. Although the species can survive and recover from an episode of water stress and prolonged dehydration, its growth (particularly leaf growth) would be substantially reduced. Based on historical weather records, the possible timing of a drought episode is likely to be somewhere between mid July to early August. The six-week recovery period used in this experiment suggests that the effect of a summer drought would result in much smaller *C. dissectum* plants by the autumn and the onset of winter dormancy. The effects of this in the subsequent spring growing season could be reduced clonal reproduction and possibly insufficient reserves which would delay flowering until the following season. The reproductive life-cycles, both clonal and sexual reproduction, in this species are not fully understood and are outside the remit of this study. However, it appears that *C. dissectum* is a monocarpic perennial and in such species size is the major determinant of the onset of

flowering (Wesselingh, 1995). The results of this experiment indicate that a reduction of soil water or a short period of drought on Devon M24 sites is unlikely to result in the direct extinction of *C. dissectum* populations. However, the ecological implications are that if summer drought stress became a regular occurrence, due to altered hydrology for example, flowering and vegetative reproduction would be reduced. This would lead to reduced fitness and low genetic diversity, ultimately resulting in population decline and an increased risk of extinction.

As *C. dissectum* is not particularly sensitive to drought and dehydration this suggests that the high water uptake is not a specific metabolic requirement but a functional adaptation to a high soil-water environment. Therefore the distribution of *C. dissectum* is not primarily determined by water availability. Habitats with high summer soil water inhibit soil microbial activity thus maintaining low fertility which would exclude more competitive species. Therefore water availability may only be a secondary environmental factor interacting with soil fertility and only indirectly related to the distribution of the species. This leads once again to the conclusion that the distribution of *C. dissectum* is determined by relative competitive ability in a low nutrient environment, resulting in the realised ecological niche.

6.0 PHOSPHORUS UPTAKE

6.1 INTRODUCTION

The results of the field data (Chapter 2) highlighted the fact that phosphorus availability is particularly low on both the M24 and supplementary field sites containing *C. dissectum*. This agrees with earlier studies on North Devon Culm grassland sites carried out by Goodwin *et al.* (1998). It has been demonstrated that species adapted to nutrient-poor sites adjust their growth rates to the most limiting nutrient (Chapin, 1983). As phosphorus has been identified as a key limiting nutrient within *C. dissectum* habitats, it has been chosen as the main subject for investigation into the nutrient requirement of this species.

6.1.1 *The role of phosphorus in plants*

Phosphorus (P) is a major plant nutrient, essential for most of a green plant's metabolic processes. P is found in plants as a constituent of nucleic acids, phospholipids, nicotinamide adenine dinucleotide (and other cofactors) and, as a constituent of adenosine triphosphate and other high-energy compounds. High concentrations of P are found in the meristematic regions of actively growing plants, where P is involved in the synthesis of nucleoproteins. In addition, the various P compounds are important in oxidation-reduction reactions and essential plant processes such as photosynthesis, respiration, nitrogen metabolism, carbohydrate metabolism and fatty acid synthesis (Devlin and Witham, 1983). The P requirement for optimal growth is in the range of 0.3 - 0.5% of the plant dry matter during the vegetative stage of growth, with the probability of P toxicity increasing at contents higher than 1% in the dry matter (Marschner, 1995). In plants suffering from P deficiency, reduction in leaf expansion, leaf surface area and number of leaves are the most striking effects (Marschner, 1995). In contrast to shoot growth, root growth is much less inhibited under P deficiency. This typically leads to an increase in root-shoot dry weight ratio.

6.1.2 *Phosphorus fractions in plants*

Plant P occurs in inorganic form (P_i) as orthophosphate and to a minor extent as pyrophosphate. The organic forms of P (P_o) are compounds in which the orthophosphate is esterified with hydroxyl groups of sugars and alcohols or bound by a pyrophosphate bond to another phosphate group (Mengel and Kirkby, 1979). The proportion of P_i in the total P content of leaves is highest in older leaves, whereas younger leaves contain relatively high quantities of P_o, predominately in the form of nucleic acids (Mengel and Kirkby, 1979). In vegetative plant organs P_i functions as the reserve form. This can be seen in P-deficient plant tissues where the contents of P_i are particularly depressed, whilst P_o levels are little affected (Hart, 1972). In vacuolated cells of higher plants the vacuole acts as storage pool, or 'non-metabolic pool', of P_i, and at adequate P supply 85-90% of the total P_i is located in the vacuoles (Bieleski and Ferguson, 1983). In contrast, in leaves of P-deficient plants virtually all P_i is found in the cytoplasm and chloroplasts, i.e. in the 'metabolic pool' (Foyer and Spencer, 1986).

6.1.3 *Phosphorus uptake by plants*

The most important P-containing ions in soil solution are pyrophosphate and orthophosphate, with the ratio between the two ion species in soil solution being pH dependent (Mengel and Kirkby, 1979). High H^+ concentrations shift the equilibrium to the more protonated form according to the equation:

$$HPO_4^{2-} + H^+ \rightleftarrows H_2PO_4^-$$

For example at pH 5, pyrophosphate is almost absent, whereas at pH 7 both phosphate species are present in equal proportions (Mengel and Kirkby, 1979). The rate of phosphate uptake by plants is pH dependent (Hendrix, 1967), and declines at both high and low pH solutions. Phosphorus is taken up by plants roots largely as P_i in the soil solution, absorbed by plant cells and rapidly becomes involved in metabolic processes, but thereafter is released again as P_i into the xylem (Marschner, 1995). Jackson and Hagan (1960) reported that after 10 minutes following uptake by roots, 80% of the phosphate absorbed was incorporated into organic compounds, especially sugar phosphates.

6.2 AVAILABILITY OF SOIL PHOSPHORUS

Soil P occurs almost exclusively in the form of orthophosphate with a total content in the range of 200 to 1,500 mg kg^{-1} P (Mengel and Kirkby, 1979). From the viewpoint of plant nutrition, there are three main soil P fractions which are important:

a) P in soil solution

b) P in the labile pool, i.e. solid P which is held on surfaces so that it is in rapid equilibrium with the soil solution phosphate

c) phosphate of the non-labile fraction, i.e. insoluble phosphate.

It is beyond the scope of this study to examine the various soil P forms and the interrelationships between these forms. The experiments in this chapter will only investigate uptake and utilisation by plants of P from the soil solution.

The phosphate concentration of the soil solution is very dilute and in fertile arable soils, usually ranges between 10^{-5} to 10^{-4} M, equivalent to 0.3 to 3 mg l^{-1} P (Bieleski, 1973; Mengel and Kirkby, 1979). Nutrient concentrations in soil solution and therefore nutrient absorption by plants fluctuate considerably during the year. In non-agricultural soils there is generally a predictable spring nutrient flush associated with a spring increase in microbial activity and freezing-thawing or wetting-drying cycles that lyse microbial cells (Chapin, 1980; Turner and Haygarth, 1998). In infertile habitats, it is likely that a large proportion of annual nutrient absorption occurs during nutrient flushes (Chapin, Barsdate, and Barel, 1978), particularly during late winter or early spring (Chapin and Bloom, 1976; Mooney and Rundel, 1979), rather than by steady-state absorption under average conditions. The frequency and duration of these pulses of nutrient release, on infertile soils, are suspected to be a critical determinant of plant success under these conditions (Crick and Grime, 1987; Grime, Crick, and Rincon, 1986).

Table 6.1. Soil phosphorus analyses from a range of sites all containing *C. dissectum*

Source	NVC community or habitat type	Extractable P (mg kg^{-1})	Total soil P (mg kg^{-1})
Goodwin (1998)	M16	4.7	546
Tallowin (unpublished)	M24 M23, 24, 16 M16	9.8 2.0 8.0	425 439 348
Loach (1966)	Valley bog	4.5	n.a.
Hayati & Proctor (1991)	M16	4.7	180
Pegtel (1983)	*Cirsio-Molinietum*	5.0	442
Wheeler & Shaw (1987)	*Cirsio-Molinietum*	-	0.25 - 0.40 mg l^{-1} peat
Dinsdale (1996)	M25	3.0	-
Medcalf (1993)	M24c	5.0	-
This study (Chapter 2)	M24	0.7 - 9.3	-

Table 6.2. Phosphorus availability from a range of sites under agricultural management

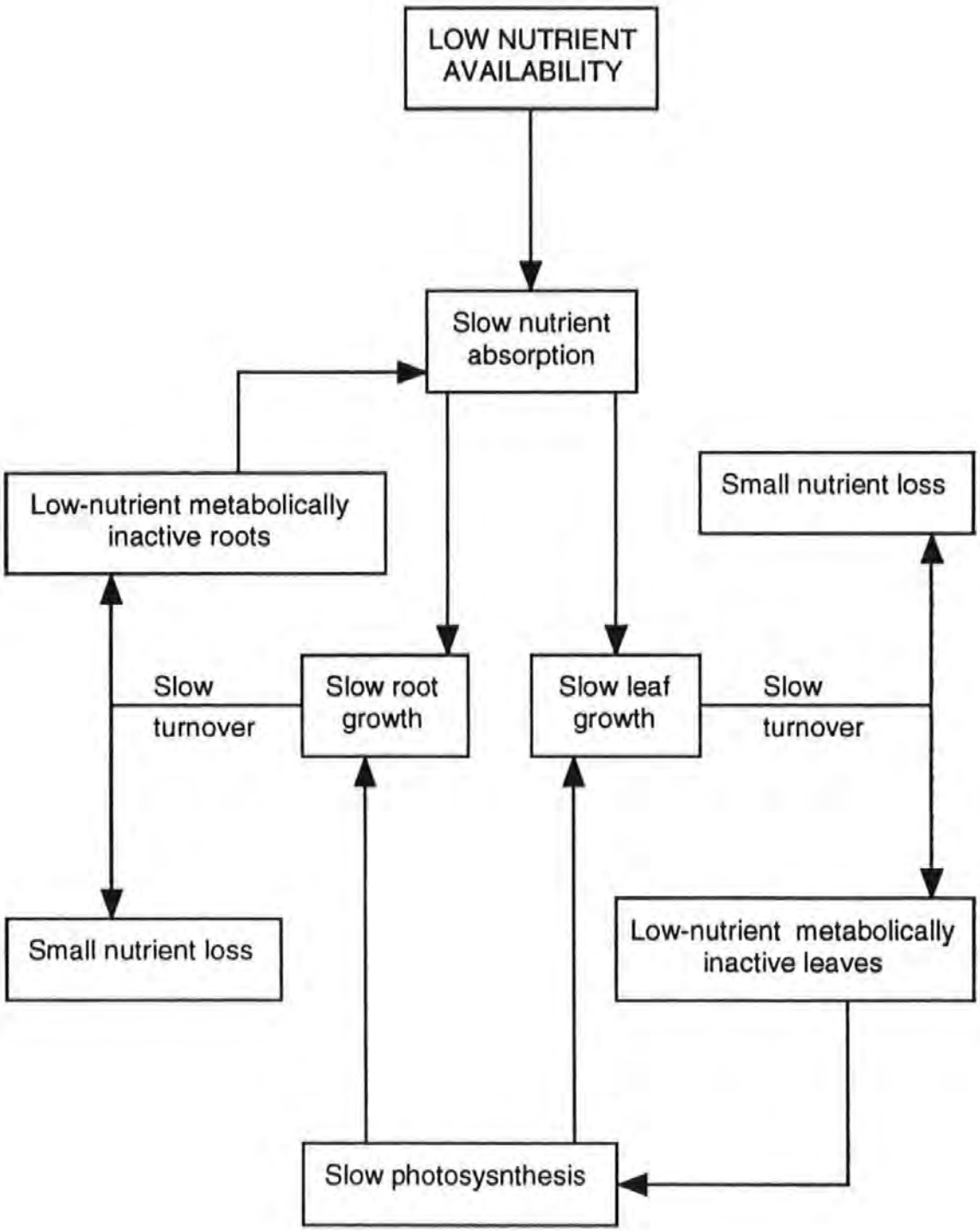
Source	Type of site	Extractable P (mg kg^{-1})
Goodwin (1995)	Improved Culm grassland MG6/MG10	27.0
Gough & Marrs (1990b)	Arable field (abandoned for 4 years) Arable field Intensively managed grassland	89.0 19.6 68.0
Marrs, Gough & Griffiths (1991)	Arable field	62.0
Pywell, Webb & Putwain (1994)	Farmed pasture	42.0

6.3 ECOLOGICAL IMPLICATIONS OF PHOSPHORUS AVAILABILITY

Various studies have shown that P is a prime limiting nutrient in fen meadows (Egloff, 1983; Grootjans, *et al.*, 1986; Pegtel, 1983). The soil P status of sites where *C. dissectum* has been recorded, from previously published work, is detailed in Table 6.1. By comparison with the P status of agriculturally improved or managed sites quoted in Table 6.2, it can be seen that most *C. dissectum* habitats have relatively low P availability (based on extractable soil P). Low soil fertility is a major factor in maintaining floristically rich communities (Grime, 1973; Grubb, 1977), valued for their species diversity and nature conservation interest. The *Cirsio-Molinietum* M24 fen meadow community (Rodwell, 1991b) is an example of an agriculturally unimproved grassland of high conservation value (H.M.S.O., 1995). Also, in low input grassland systems it has been shown that there is an inverse relationship between floristic diversity and P availability (Gough and Marrs, 1990a; Gough and Marrs, 1990b; Rorison, 1971). It was also suggested by Gough and Marrs (1990b) that a low extractable P within the range 5 - 10 mg kg^{-1} was optimum for high floristic diversity. It has been clearly demonstrated that plant species differ in their response to P availability (Chapin, Follet, and O'Connor, 1982; Hayati and Proctor, 1991; Rorison, 1968; Veerkamp, *et al.*, 1980) and that nutrient availability, mainly nitrogen (N) and P, is a major factor in determining the distribution of species in the field (Grime, *et al.*, 1988; Hayati and Proctor, 1990; Loach, 1966).

The correlation between species diversity and low nutrient availability conforms to the C-S-R model (Grime, 1979), which predicts that without the stress imposed by low nutrients, plants of high competitive ability would occupy the site and, over time, a drift towards a monoculture would occur. Conversely, low nutrient sites will generally only contain those species which have evolved a primary strategy of stress tolerance. Although there are several types of stress i.e. nutrient, pH, water (drought/inundation) and shade, the balance of evidence (Chapin, 1980; Grime, 1979) points to all stress tolerators sharing the common underlying stress of low nutrient supply, especially P and N.

Figure 6.1 A model illustrating the characteristics of the nutrient-stress tolerant strategy. (adapted from Chapin, 1980)



6.4 PHYSIOLOGICAL ADAPTATION TO PHOSPHORUS STRESS

Plants of low nutrient habitats with a primary strategy of stress tolerance have evolved a certain combination of physiological traits to enable them to compete and survive in a nutrient-stressed environment. A model of the interacting characteristics of such plants is detailed in Figure 6.1. The general characteristics of stress-tolerators have been discussed in detail by Grime (1979) and adaptations to nutrient stress reviewed by Chapin (1980). A summary of the main characteristics relevant to low P tolerance are detailed as follows.

6.4.1 *Nutrient absorption*

* Low absorption rate: compared with species from high-P environments, species from infertile soils absorb considerably less P under high-P conditions but similar quantities, and in some cases even more P, at extremely low P availability.
* High root to shoot ratio coupled with a low phenotypic plasticity and an uncoupling of growth from nutrient uptake.
* Seasonal patterns: a large percentage of annual P uptake occurs during flushes, particularly during late winter/early spring.
* Rhizosphere interactions: an additional trait critical to plant nutrition at low-P status is root mycorrhizal associations (Bieleski, 1973). Briefly discussed in Chapter 2 (section 2.5.7)

6.4.2 *Longevity*

* Increased leaf longevity associated with a lower rate of leaf production.
* Lower leaf senescence rate, therefore lower P loss as a result of inherent inefficiencies of P translocation from senesced leaves.
* Perennials which are long lived with a large root biomass and slow root turnover.

6.4.3 *Growth rate and luxury consumption*

* Because only slow growth can be supported on infertile soils, a species with an inherently low rate of growth is functioning closer to its optimal growth and metabolic rate and may therefore be better adapted to a low-P environment than a rapidly growing species that experiences a substantial reduction in yield under the same conditions.
* Luxury consumption: slowly growing species that absorb P in excess of immediate growth requirements during P flushes may use these reserves to support growth after soil reserves are exhausted. This is characterised by a high proportion of P_i and a low proportion of structurally bound P_o in the plant tissues.

Chapin (1982) concluded that a low P requirement associated with slow growth is more important than between species differences in P metabolism, phosphate absorption, or efficiency in P utilisation in explaining the success of low-P-adapted species on infertile soils. Also, Chapin (1983) has shown that species adapted to nutrient poor sites adjust their growth rates to the most limiting nutrient, in this case P.

6.5 AIMS AND OBJECTIVES

The restricted P availability on *Cirsio-Molinietum* fen meadows will be a major stress factor and therefore *C. dissectum* should be a stress-tolerator. If this is the case, *C. dissectum* should exhibit many of the characteristics of a species conforming to the stress-tolerator functional type. The following experiments seek to determine whether *C. dissectum* is a low-P adapted species.

The main objectives were therefore to test the key predicted attributes which will confer a selective advantage of this species within the environmental conditions of fen meadows. The following hypotheses are put forward:

a) *C. dissectum* has a low P requirement as a result of a low growth rate (and is therefore functioning closer to its optimal growth and metabolic rate than a faster growing, P-demanding species).

b) *C. dissectum* has low morphological plasticity, where growth and root : shoot allocation is uncoupled from P availability

d) *C. dissectum* does not show a growth response to flushes of high P but absorbs P in excess of immediate growth requirement and stores as P_i

e) *C. dissectum* has a high leaf longevity and low leaf senescence rate

Experiments were designed to measure the response of *C. dissectum* to P availability under conditions of i) continuous supply and ii) pulses of varying duration. In both experiments, the response of *C. dissectum* was compared with *Urtica dioica* (common stinging nettle), chosen for its known classification (by functional type) as a 'competitor' with a high relative growth rate and a high P requirement (Grime, *et al.*, 1988; Rorison, 1968). The experiments carried out by Rorison (1968) and Nassery (1970) demonstrated that *U. dioica* responds markedly to changes in external concentrations of P, shows a sharp cessation of growth at concentrations below 10 μM P and exhibits a short-lived positive response to pulses of high P.

6.6 MATERIALS AND METHODS

All the plant material used in the following experiments was grown from seed. Seeds of *C. dissectum* were from glasshouse-grown stock originating from North Devon M24 sites and *U. dioica* seeds were supplied by Suffolk Herbs, Kelvedon, Essex. Plants were grown until they were large enough to be safely handled, which was five months for the *C. dissectum* and two months for the *U. dioica*. The fresh weights (FW) of all plants were recorded at the start and at various intervals throughout the experiments. Also, leaf number for each plant was recorded at the beginning and end of the experiments and any senesced leaves collected and recorded. Plant material at the end of the experiment, both live and senesced, was analysed for mineral nutrient content of total P, N, K, Ca and P_i (proportion)

using the same methods as the field sample analysis detailed in Chapter 2 (Section 2.3.6). This provided a comparison with the nutrient contents of samples of leaves of *C. dissectum* in the field and was used to indicate whether the nutrient solutions were limiting the availability of nutrients, other than the manipulated P. Prior to the start of the experiment random samples of plants of the two species were measured for water content, root:shoot ratio and mineral content (as above).

The nutrient environment was controlled by growing the plants hydroponically using Rorison's solution (Hendry and Grime, 1993), modified to provide the required experimental levels of P concentration. All other nutrients were supplied at 1/10th Rorison's full solution and full details of the solution formula are contained in Table 6.3.

Table 6.3 Rorison nutrient solution, preparation of 1 litre of basic solution (from standard stock solutions) with four levels of P and all other nutrients at 1/10th of full strength. Adapted from Hendry and Grime (1993).

Stock solutions		gms l^{-1} to make stock solution	ml l^{-1} of diluted stock solution	100 µM P	10 µM P	1.0 µM P	0.1 µM P
				Dilution of stock solution (%)			
1	$Ca(NO_3)_2 - 4H_2O$	476.10	**1**	10			
2	$MgSO_4 - 7H_2O$	248.00	**1**	10			
3	Fe (Na) EDTA	25.00	**1**	10			
4	*Traces* $MnSO_4 - 4H_2O$ H_3BO_3 $(NH_4)_6Mo_7O_{24} - 4H_2O$ $ZnSO_4 - 7H_2O$ $CuSO_4 - 5H_2O$	 2.028 2.863 0.184 0.440 0.393	**1**	10			
5	H_2SO_4 (to adjust pH to 5.8)	28ml conc. l^{-1}	**0.5**	10			
6a	K_2HPO_4 (anhydrous)	176.30	**1**	10	1	0.1	0.01
6b	*K_2SO_4 (0.5 M)	87.50	**(2)**	-	9.9	9.9	9.9

*Used to maintain K balance at concentrations of 10 µM P or lower

A pH of 5.8 was maintained in all solutions by varying additions of dilute H_2SO_4. This equates to the pH generally found on *C. dissectum* field sites (see Chapter 2) and within the range which is not limiting to P uptake (Mengel and Kirkby, 1979). Prior to the experiments

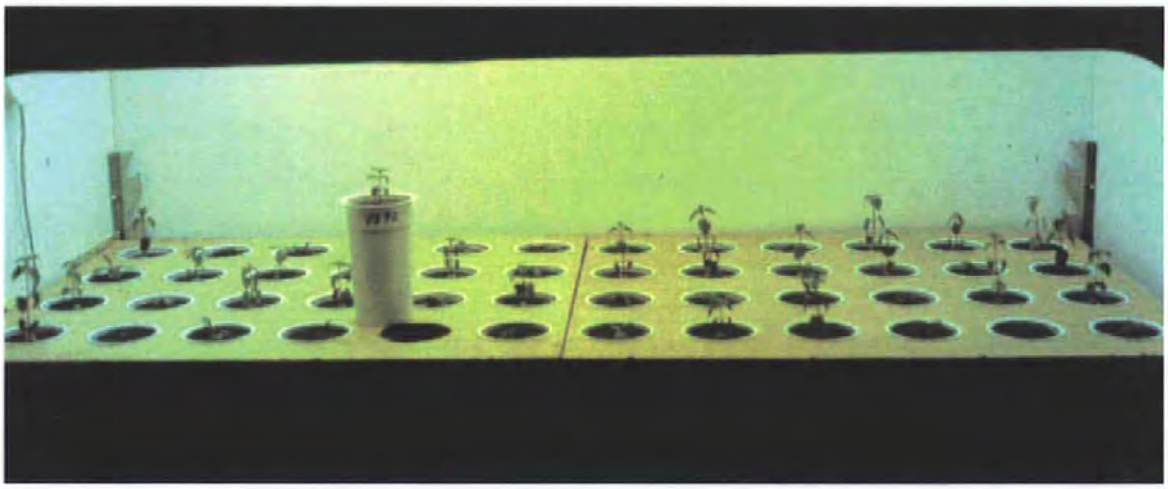
all plant material had been germinated/cultivated in sand supplied with Rorison's solution, as detailed above, with P supplied at 10 μM. This concentration of P was chosen as it is the lowest level considered non-limiting to growth (Rorison, 1968), designed to be sufficiently low to prevent *C. dissectum* from accumulating reserves of P prior to the experiments.

Each plant was grown in a 65 mm diameter, 250 ml capacity "food-grade" plastic container supplied by Insulpak Ltd, Huntingdon. The plant was supported in the nutrient solution by a 62 mm diameter polystyrene float painted dark brown with Dulux water-based emulsion (Dulux reference 18YR 05/072) to prevent light reflectance to the leaf underside and to minimise algal growth. A hole was cut in the float using a cork borer and the plant was secured with non-absorbent cotton wool. To facilitate easy access to the plant at intervals for weighing, a narrow 'V' shaped wedge was also cut from the float which could be removed, allowing the plant to slide out, and replaced once the plant was re-secured with cotton wool. The pots were then suspended in a specially constructed box to exclude light from the roots and to enable a uniform 50 mm spacing between each pot. An illustration of the design is given in Plate 6.1.

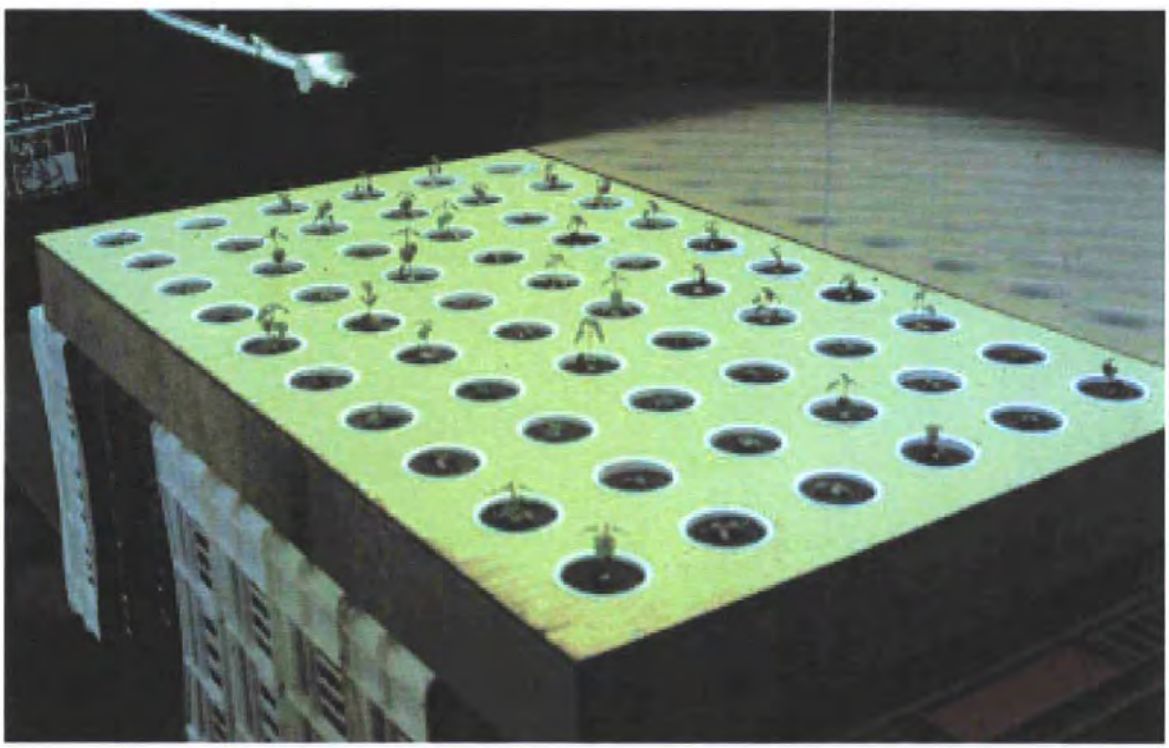
A preliminary experiment was carried out to estimate P depletion rate from the various solution concentrations. Three-month old *U. dioica* plants were used as they were representative of the highest likely P uptake. Three replicates were grown for five days at each of the four P levels under the environment conditions detailed in Experiment 1 (below). The P content of the nutrient solutions was recorded at the start and each day for five days by taking a 5 ml sample of each solution which was then analysed using a Tecator 5012 Flow Injection Analysis (FIA) system (Perstorp Analytical, Maidenhead).

Plate 6.1 Hydroponic growth system and arrangement for (a) Experiment 1 in growth cabinet and (b) Experiment 2 in growth room.

(a)



(b)



Two different aspects of P response were measured in the following experiments:

Experiment 1. Growth response to four P levels under conditions of continuous supply. The design was fully randomised: two species x four P levels, replicated six times laid out in a 12 x 4 matrix. The four treatment levels were 100 and 10 μM P, considered non-limiting to growth, and 1.0 and 0.1 μM P, considered deficient for any soil solution (Rorison, 1968). The experiment was carried out under controlled environment conditions in a SGC 660 series phytotron (Sanyo Gallenkamp, Leicester). The regime was a 16 hour day at 22 °C and 15 °C night, with light supplied at 250 (± 10) μ mol m^{-2} s^{-1} photon flux density, following the ISP standard regime (Hendry and Grime, 1993) and relative humidity was maintained at 80% (± 5%).

Experiment 2. Growth response to pulses of high P (100 μM P) supplied for seven durations, namely 0 (no pulse control), 6, 12, 24, 48, 96 and 192 hours respectively, applied to plants otherwise maintained in a low P solution of 1.0 μM P. Previous work by Naylor (1996) has shown that fast-growing species do not respond to pulses of less than five hours. Design was fully randomised: two species x seven pulse durations, replicated four times laid out in a 10 x 6 matrix (excluding the four corners). The experiment was carried out under controlled conditions using a custom-built growth cabinet. The regime was the same as Experiment 1 except there was no humidity control available. The nutrient solutions were changed every five days in both experiments and between changes any solution lost due to uptake/evaporation was replaced with distilled water to maintain solution volume constant.

The following plant physical characters were measured and between species comparisons were made.

a) Species response to P availability was measured by relative growth rate (RGR), as detailed in Section 3.4.1, and total biomass production.

b) Morphological plasticity was measured by comparing biomass partitioning between roots and shoots.

c) P storage was measured by a chemical analysis of both total P and P_i present in the individual plants along with macro nutrients N, K and Ca.

d) Leaf longevity was measured by recording the number of leaves at the beginning and end of the experiment (including any leaves which senesced during the experiment) for each plant.

Both experiments were planned to run for a minimum period of twelve weeks to allow sufficient time for the expression of any interspecific differences in plant characteristics, as a result of the different treatments.

Data analysis

The data from both experiments were analysed using a factorial analysis of variance (ANOVA). Linear or polynomial models were also examined using linear or polynomial regressions of individual species response against treatment level (i.e. P concentration - Experiment 1; pulse duration - Experiment 2). Residuals, from a preliminary ANOVA of the raw data, were examined for homogeneity of variances. In some instances variances were not homogenous, therefore, in order to stabilise variances, the data were $\log_{10}$ transformed and the residuals re-examined for homogeneity of variances. All proportional data were arcsine transformed prior to analysis to provide an underlying distribution that is nearly normal (Zar, 1996). Data in Experiment 1 were analysed with a two factor ANOVA namely: species (2), P concentration (4) and the interaction term species x concentration (8). A significant interaction term was interpreted as a differential in response between the two species at different concentration levels. Experiment 2 was analysed in a similar manner, namely: species (2), pulse duration (7) and the interaction term species x pulse (14). An additional factor in experiment 2 was the measurement of plant fresh weight gain (FWG) and fresh weight relative growth rate (RGR_{fw}) at three time periods over the experiment namely: 30 days pre-pulse, 10 days post-pulse and the final 20 days. Therefore FWG and RGR_{fw} were also analysed using a three factor ANOVA namely: species (2), pulse (7), period (3) and the interaction terms species x pulse (14), species x period (6) and species x pulse x period (42). Where significant differences within factor levels or interaction between factors were identified from an ANOVA, multiple comparison tests were used to identify differences

between the various means. The 'Tukey' wholly significant test was used as a first analysis. However, where there were overlapping similarities in pairwise comparisons of treatment means, the less conservative 'Newman-Keuls' or 'Dunnet's' test (only valid in Experiment 2) was used. All the above statistical methods have been derived from Zar (1996).

6.7 RESULTS AND DISCUSSION

A major difference was observed between the two species in the way they senesced their leaves in both experiments. *U. dioica* leaves detached from the stem and fell, often before senescence was complete, whereas *C. dissectum* senesced leaves stayed firmly attached to the rosette, and were not removed until the end of the experiment. It was therefore necessary to correct the FW data to allow for the fact that *C. dissectum* FW measurements included a proportion of senesced leaf dry weight (DW). The senesced leaf DW was converted to FW for both species and the total plant FW was corrected. Therefore, all FW results presented include senesced leaf material on a fresh weight basis. Also, adding back the FW of the senesced leaves provides a measure of the total FW accumulated over the experiment.

Nutrient depletion

The preliminary experiment revealed, that over a five-day period, the *U. dioica* plants depleted the 100 and 10 μM P solutions at a rate of approximately 2.4 μM P per day but in the 1.0 and 0.1 μM P solutions there was no detectable change in concentration. The latter could have occurred because *U. dioica* had a poor ability to take up P at such low concentrations (Rorison, 1968) or because such low concentrations are close to the lower limit (0.3 μM P) of accurate detection by the Tecator 5012 FIA . The final mean FW of the plants used in the preliminary experiment was 2.8 g. Therefore, in the main experiment, nutrient solution P may have been limiting in the 10 μM P solution treatment in the final 20 days of the Experiment 1, where the final mean plant FW exceeded 3 g and may have exhausted the available solution P prior to the solutions being changed.

Pre-experiment mineral content and root:shoot ratios

Randomly selected plants not used in the two experiments were analysed for mineral nutrient content and root:shoot ratios to provide baseline comparison data and the results are detailed in Table 6.4. Compared with the mean values for mineral contents of samples of *C. dissectum* leaves collected in the field, the contents of P, N and K of the plants at the start of the experiment were low.

Table 6.4 Summary of mineral content for both shoot (S) and root (R) material of plants prior to the start of Experiments 1 and 2 plus the mean value of leaves collected from the field of *Cirsium dissectum*. Total plant content 'Plant (e)' is an estimated figure calculated from the R:S ratios. Data are means of sub-samples (n=3) of bulked material.

Species (root:shoot ratio)	Root/Shoot	% P (Total)	P_i (prop. of total P)	% Ca	% K	% N
C. dissectum (R:S 5.1)	Shoot (field leaf)	0.13 (0.21)	0.44 (0.35)	1.74 (1.58)	0.98 (3.43)	1.22 (1.70)
	Root	0.07	0.43	0.14	1.00	0.75
	Plant (e)	0.08	0.43	0.40	0.98	0.83
U. dioica (R:S 0.71)	Shoot	0.51	0.36	1.82	2.03	0.97
	Root	0.39	0.54	0.12	1.87	0.55
	Plant (e)	0.46	0.44	1.11	1.96	0.80

6.7.1 Experiment 1 - *Response to four P levels under conditions of continuous supply.*

Fresh Weight

Incremental changes in plant fresh weight are illustrated in Fig. 6.2 and indicate that both species showed a marked increase in fresh weight when 100 or 10 μM of P were supplied but very little change when only 1.0 and 0.1 μM of P were available. Unlike dry weight which was only recorded at the end of the experiment, fresh weight was recorded at intervals over the experiment, enabling a regression analysis of growth. Linear regressions of the responses over time are summarised in Table 6.5 and confirm that both species showed a positive linear response at the higher P concentrations. This would indicate that the potential nutrient depletion problem at 10 μM, mentioned earlier, did not have a noticeable effect.

Figure 6.2 Incremental changes in mean plant fresh weight over the course of the experiment, including senesced leaves, of (a) *Cirsium dissectum* and (b) *Urtica dioica*

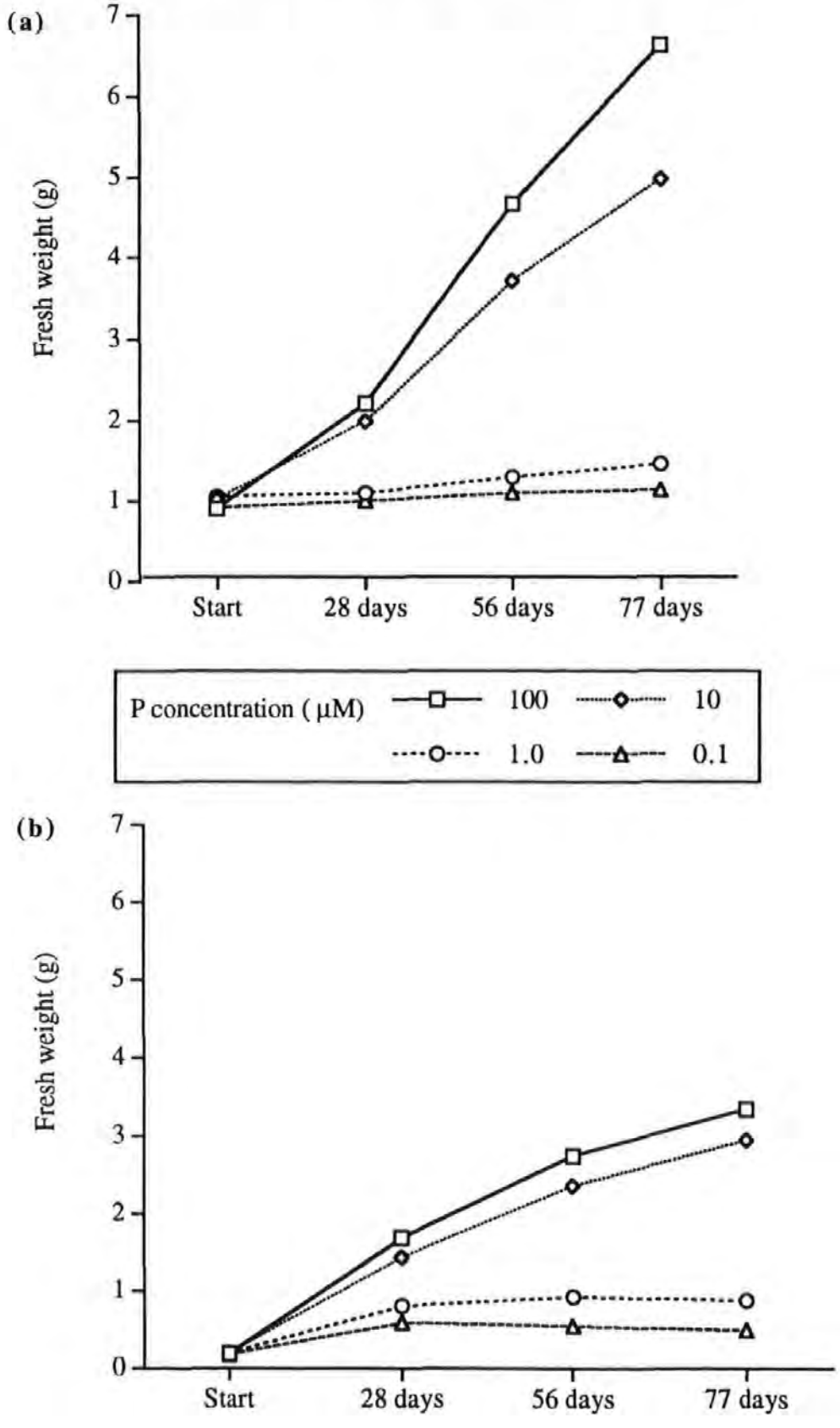


Table 6.5 Summary of incremental fresh weight regression trends of *C. dissectum* and *U. dioica* over the experiment at four P concentrations

Species	P Level	Slope	Slope Coefficient(s)	Significance Level	R^2 (adj.) %
C. dissectum	100 µM	Linear	0.0777	< 0.001	87.1
	10 µM	Linear	0.0569	< 0.001	79.7
	1.0 µM	Linear	0.0085	0.006	26.7
	0.1 µM	-	-	n.s.	-
U. dioica	100 µM	Linear	0.0583	< 0.001	96.1
	10 µM	Linear	0.0491	< 0.001	97.3
	1.0 µM	Quadratic	0.0324 -0.0002 (sq)	0.007	75.5
	0.1 µM	Quadratic	0.0223 -0.0002 (sq)	0.005	70.5

At the two lowest concentration levels, *C. dissectum* showed only a slight positive linear trend at 1.0 µM with a low R^2 and had no significant trend at 0.1 µM. Although *U. dioica* did show a significant positive linear response at the lower P concentrations, the R^2 levels were improved by 8.8% (1.0 µM) and 12.1% (0.1 µM) with a quadratic expression, suggesting that growth rate was declining in the latter stages of the experiment. At the two highest P concentrations the slopes tended to be steeper at 100 µM than 10 µM for both species and overall *C. dissectum* tended to have steeper slopes than *U. dioica.* This suggests that *C. dissectum* was exhibiting a higher rate of weight gain at these P levels compared with *U. dioica.*

Dry Weight

Final total dry weight gain (DWG) at the end of the experiment confirms the response to high and low concentrations of P supply (Fig. 6.3) and an ANOVA revealed significant differences in DWG between P concentrations ($P < 0.001$). *C. dissectum* had a higher DWG at 100 than 10 µM P which in turn was higher than at 1.0 and 0.1 µM P, which were not significantly different. *U. dioica* DWG was the same at 100 and 10 µM P which was greater than both 1.0 and 0.1 µM P, which were not significantly different.

Figure 6.3 Mean total dry weight gain of *Cirsium dissectum* and *Urtica dioica* (over 77 days) at four P concentrations. Vertical bars represent twice the SE of the mean; letters denote significant differences between means

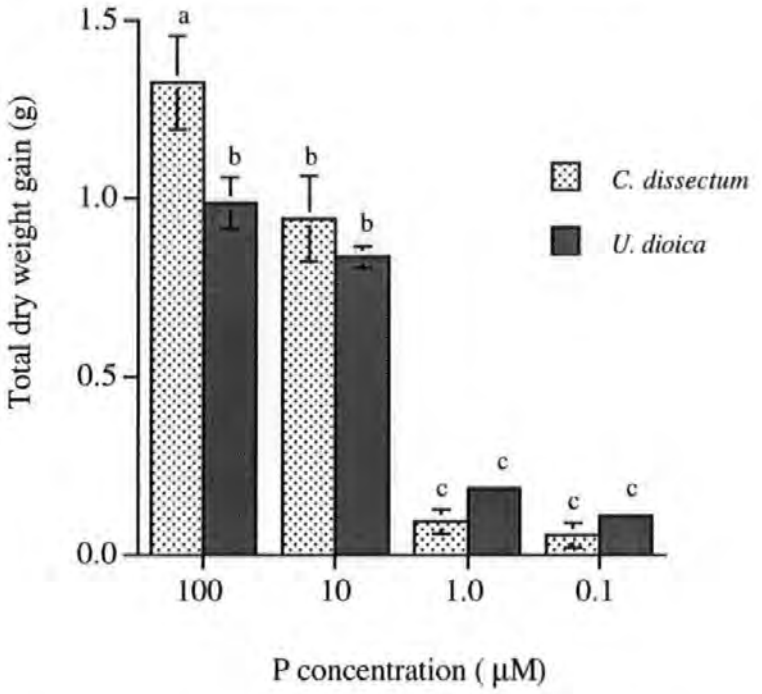
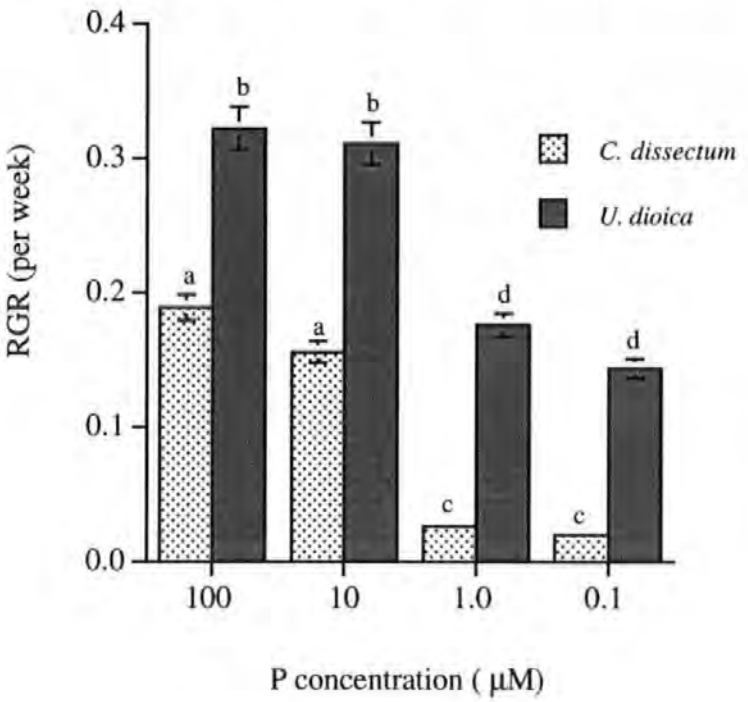


Figure 6.4 Mean dry-weight RGRs for *Cirsium dissectum* and *Urtica dioica* at four P concentrations. Vertical bars represent twice the SE of the mean; letters denote significant differences between means



However, *C. dissectum* DWG was greater than *U. dioica* at 100 μM P but there were no significant differences between the species at 10, 1.0 and 0.1 μM.P respectively.

Relative Growth Rate

Both species clearly responded to differences in P level, but between-species comparison is better illustrated by relative growth rate (RGR) based on dry weights (Fig. 6.4) which takes into account between species differences in starting weights. There was a significant difference in RGR between species ($P < 0.001$) and between P levels ($P < 0.001$) but there was no significant interaction between the factors. *U. dioica* had a higher RGR than *C. dissectum* and both species had higher RGRs at the two highest levels of P compared to the two lowest levels of P which, respectively, were not significantly different. The mean difference in RGR between *C. dissectum* and *U. dioica* was 0.14 (± SD 0.047). This difference between the two species was consistent at all P concentrations, from which it can be concluded that *U. dioica* has an inherently higher RGR than *C. dissectum.*

The recording of fresh weight at intervals during the experiment also enabled a more detailed breakdown of fresh weight RGR (RGR_{fw}) at three stages of the experiment, i.e. first four weeks, second four weeks and the last three weeks. The results are detailed in Fig. 6.5 and would seem to indicate that the pattern of RGR_{fw} is similar to the overall dry weight RGRs (RGR_{dw}) (Fig. 6.4) in the first four weeks, but thereafter there is a distinctly different pattern. There was a significant interaction of the mean of all P levels between species and period ($P < 0.001$) (Fig. 6.6). It can be concluded that the RGR_{fw} of *U. dioica* declined ($P< 0.001$) between the first and second period (five-fold) and there was a further two-fold decline ($P< 0.001$) between the second and final period. Also the RGR_{fw} of *U. dioica* was almost four times greater than *C. dissectum* in the first period but subsequently declined to a similar RGR_{fw} to that of *C. dissectum* by the final period. In contrast, there was only a small decline ($P< 0.001$) in the RGR_{fw} of *C. dissectum* (1.7 fold difference) between the first and last period.

Figure 6.5 Mean fresh weight RGRs for *Cirsium dissectum* and *Urtica dioica* (including senesced leaves), (a) first four weeks, (b) second four weeks and (c) final three weeks. Vertical bars represent twice the SE of the mean.

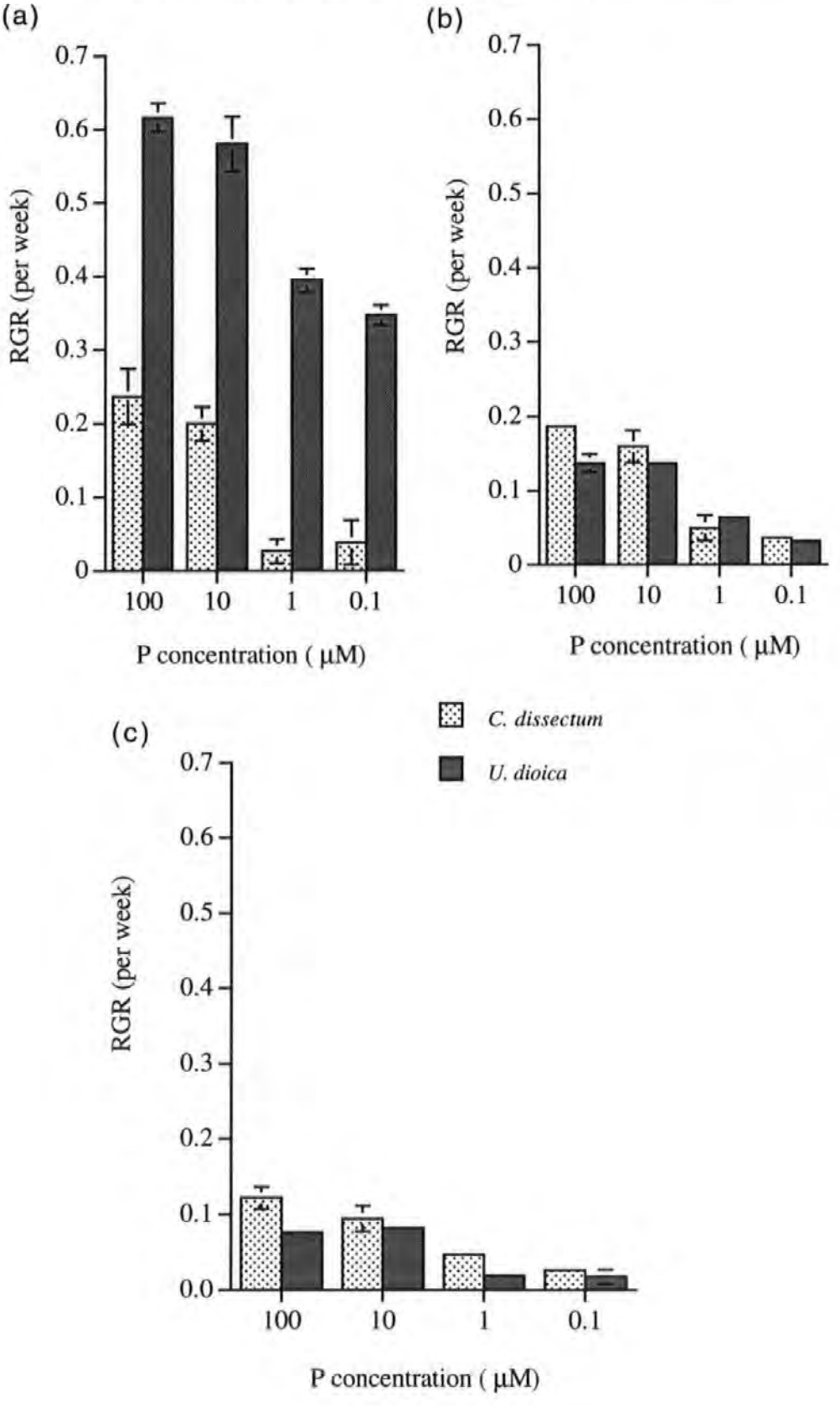


Figure 6.6 Interaction between species and period of fresh weight RGRs including senesced leaves. Vertical bars represent twice the SE of the mean, letters denote significant differences between means.

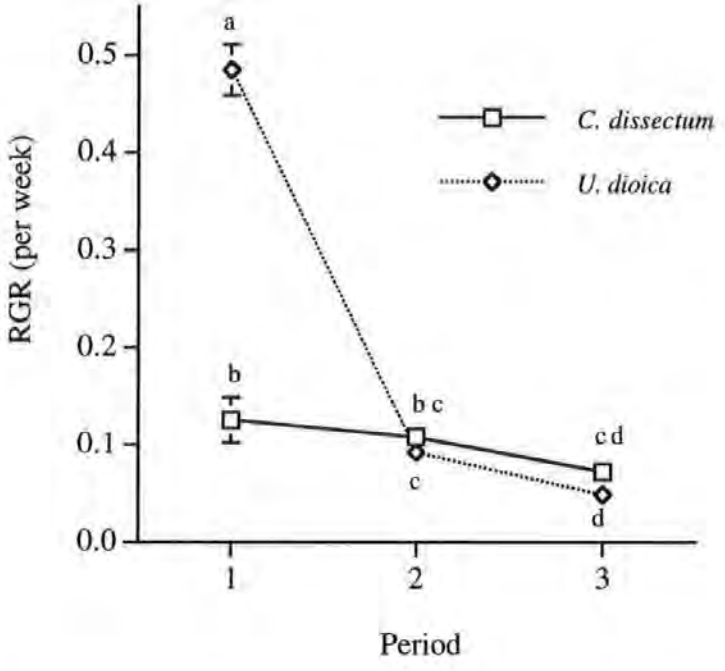
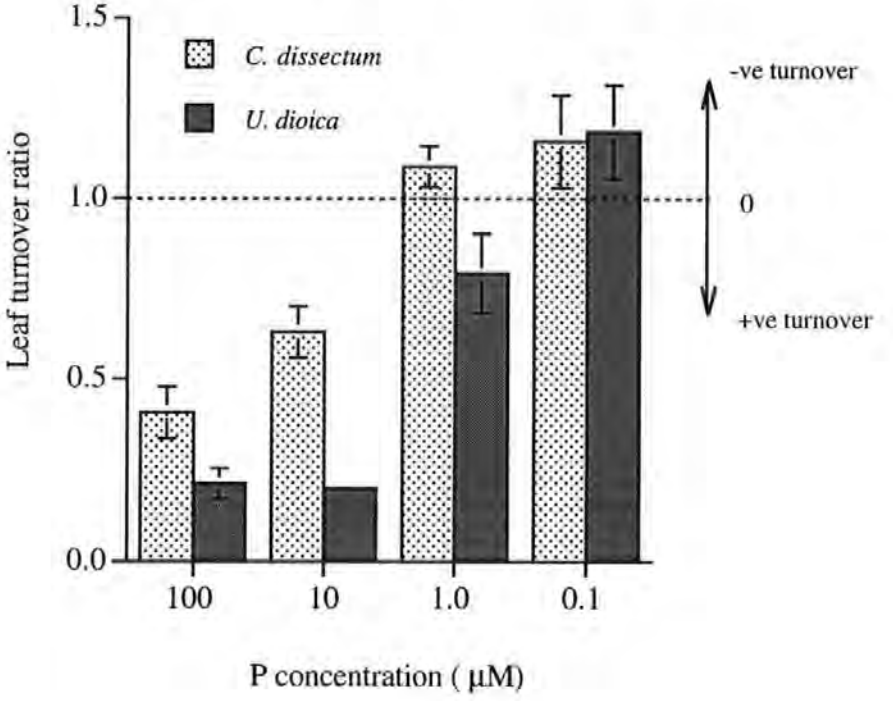


Figure 6.7 Mean leaf turnover of *Cirsium dissectum* and *Urtica dioica*, expressed as the number of leaves senesced per new leaf produced over the experiment. Vertical bars represent twice the SE of the mean.
(Dotted line indicates equilibrium leaf turnover)



The rate of growth in *C. dissectum* was relatively constant over the experiment. In contrast, growth in *U. dioica* declined dramatically after four weeks suggesting it had exhausted its internal reserves of P and that the P concentrations in this experiment were well below the optimum for continued expansive growth.

Leaf Senescence

Leaf senescence over the experiment was recorded both in terms of number of leaves and dry weight of leaves senesced. Mean leaf turnover was calculated on the number of leaves senesced per new leaf produced. Changes in leaf turnover for a given P concentration are shown in Fig. 6.7. There were significant differences between species ($P = 0.001$) and between P levels ($P < 0.001$) but no significant interaction between the two factors. *C. dissectum* showed a significantly higher leaf turnover at the two lower P levels, to the extent of showing a negative leaf turnover (i.e. decline in live leaf number), compared to the two higher P levels which were both positive (i.e. a gain in live leaf number). A similar pattern was observed in *U. dioica,* except that the turnover at 1.0 μM P was significantly lower, and positive, compared with 0.1 μM P. Overall, *C. dissectum* had a higher leaf turnover than *U. dioica*. Senesced leaf dry weight was analysed using the mean ratio of total dry weight gain (DWG) to senesced leaf dry weight (SL)(i.e. the higher the ratio, the lower the proportion of senesced leaf material) providing a measure of leaf longevity. There was a significant interaction ($P < 0.001$) between species and P level and this is illustrated in Fig. 6.8. The DWG:SL ratio of *C. dissectum* was significantly greater ($P< 0.001$) at 100 than 10 μM P which in turn was greater than at either 1.0 or 0.1 μM P which were not significantly different. There were no significant differences in the ratio as a result of P level in *U. dioica*. However, at 100 μM P there was a significant difference ($P< 0.001$) in the ratio between the two species with *C. dissectum* twice that of *U. dioica*. These results suggest that *C. dissectum* leaf longevity is positively correlated with P solution concentration.

Figure 6.8 Interaction between species and P concentration of mean ratio of total dry weight gain to senesced leaf dry weight. Vertical bars represent twice the pooled SE of the mean.

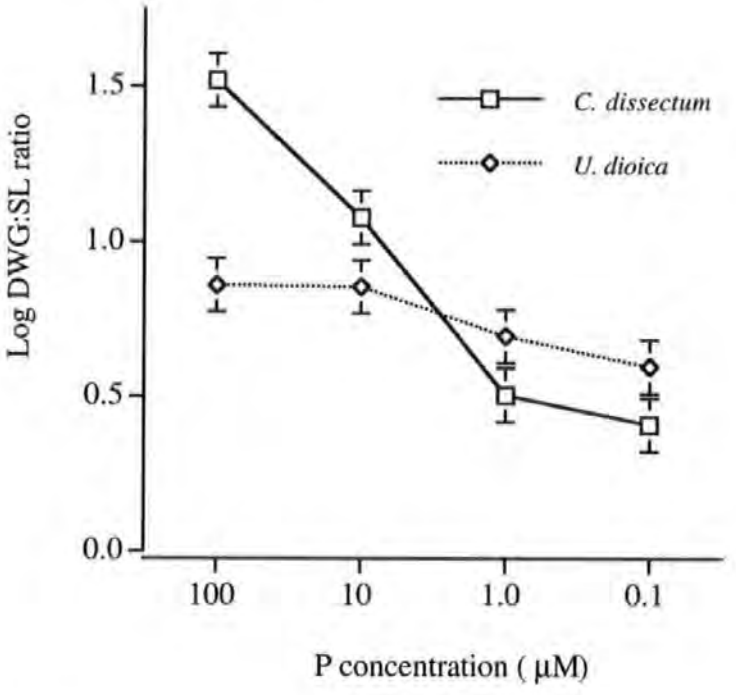


Figure 6.9 Mean root:shoot ratio (log-transformed) of *Cirsium dissectum* and *Urtica dioica* at four P concentrations. Vertical bars represent twice the SE of the mean; letters denote significant differences between means.

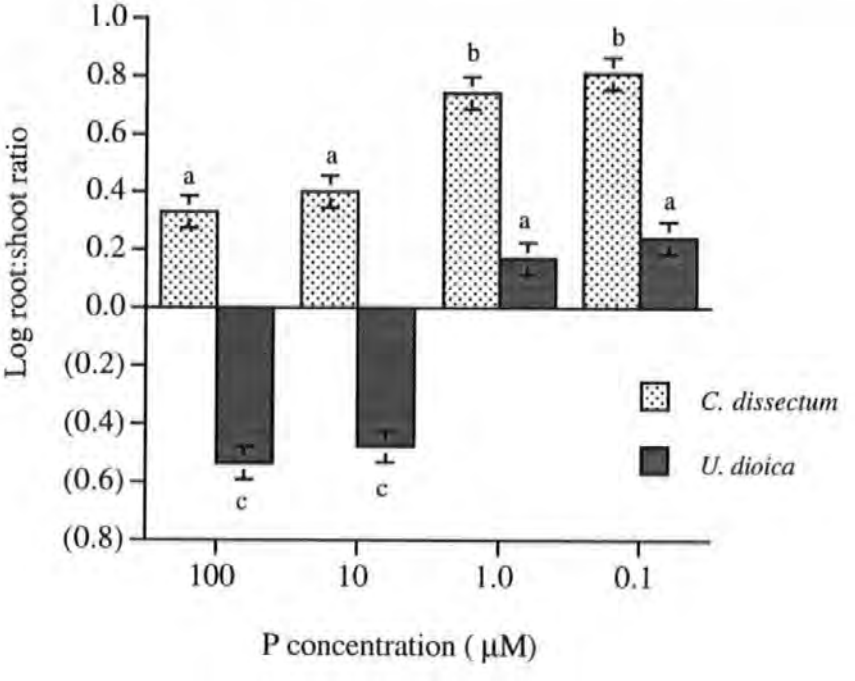
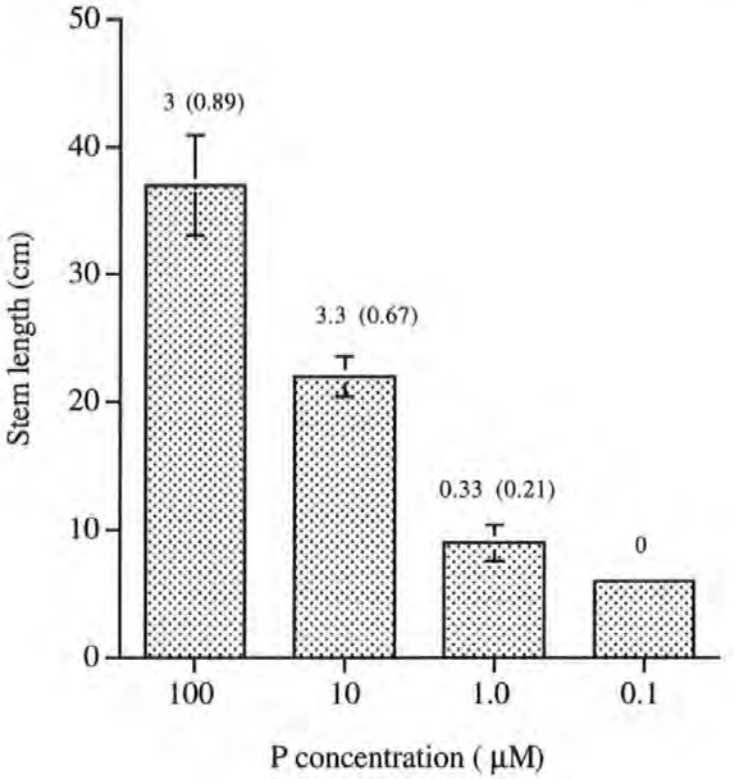


Figure 6.10 Mean stem length of *Urtica dioica* at four P concentrations. Vertical bars represent twice the SE of the mean. (Numbers are mean no. of lateral branches and SE)



Root:Shoot Ratio

An analysis of the dry weight root:shoot (R:S) ratios at the end of the experiment revealed differences in shoot and root phytomass allocation which is illustrated by Fig. 6.9. There was a significant species x P concentration interaction ($P = 0.005$), where *C. dissectum* had a higher R:S ratio than *U. dioica* at both high and low levels of P. However, the difference in R:S ratio between high and low levels in *C. dissectum* was 2.5 fold whereas in *U. dioica* the difference was 5 fold. At the higher P levels there was a 7.5 fold difference in R:S ratio between the two species, which was similar to the difference in ratio at the beginning of the experiment. In *C. dissectum*, the lowest mean R:S ratio was 2, whereas the highest R:S ratio in *U. dioica* was 1.7. This would suggest that *C. dissectum* has an inherently higher R:S ratio compared to *U. dioica.*

Stem Elongation

The response of stem elongation to P concentration in *U. dioica* is illustrated in Fig. 6.10. There were significant ($P < 0.001$) differences in stem length at the various P concentrations. Stem length was greater at 100 μM P compared to 10 μM P, which in turn was greater than both 1.0 and 0.1 μM P which were not significantly different. This confirms that the normal rapidly-ascending tall erect growth habit of this species is positively correlated with P availability.

Mineral Nutrient Content

Analysis of mineral nutrient content had to be carried out on total plant material (roots and shoots combined) to provide sufficient material for full sample replication at 100 and 10 μM P treatments and three sub-sample replicates of bulked sample material at 1.0 and 0.1 μM P treatments and the results are detailed in Table 6.6. Total P content was significantly higher at 100 μM P than 10 μM P ($P < 0.001$) for both species, with no difference between the species at either level. However, the inorganic fraction of total P (Pi proportion) was significantly higher at 100 μM P than 10 μM P for both species and at 100 μM P, the Pi proportion was 1.5 fold higher in *C. dissectum* ($P< 0.001$) compared to *U. dioica*.

Table 6.6 Experiment 1 plant mineral content, expressed as a percentage of dry-weight of total plant material. Means at 100 and 10 μM P are replicates (n=6), means at 1.0 and 0.1 μM P are sub-samples (n=3) of bulked material.

P concentration (μM P)	Species	% P	Pi Proportion	% K	% Ca	% N
100	*C. dissectum*	0.42[a]	0.82[a]	1.53	0.99	0.81
	U. dioica	0.38[a]	0.55[b]	1.69	1.19	0.81
10	*C. dissectum*	0.10[b]	0.38[c]	1.55	0.80	1.03
	U. dioica	0.11[b]	0.42[c]	1.62	1.04	1.13
1.0	*C. dissectum*	0.07	0.50	1.39	0.56	1.43
	U. dioica	0.10	0.40	1.97	0.76	2.01
0.1	*C. dissectum*	0.05	0.37	1.32	0.51	1.37
	U. dioica	0.09	0.35	1.86	0.58	2.07

Table 6.7 Experiment 1 senesced leaf mineral nutrient content. Data are single samples of bulked material, expressed as a percentage of dry weight.

P concentration (μM P)	Species	% P	% Ca	% N
100	*C. dissectum*	0.42	2.80	0.30
	U. dioica	0.54	1.88	0.72
10	*C. dissectum*	0.21	2.53	0.13
	U. dioica	0.29	2.01	0.63
1.0	*C. dissectum*	0.23	1.81	0.67
	U. dioica	0.27	1.86	1.19
0.1	*C. dissectum*	0.24	1.78	0.73
	U. dioica	0.30	1.65	1.95

Regression analyses of mean plant mineral content against log P concentration of nutrient solution highlighted three significant trends:

1) There was a significant positive correlation between plant P_i proportion and P solution concentration in *U. dioica* (P= 0.046; R^2 (adj) 86%; slope +0.063).

2) There was a significant positive correlation between plant Ca content and P solution concentration in both species (*C. dissectum* P= 0.024; R^2 (adj) 93%; slope +0.17 and *U. dioica* P= 0.006; R^2 (adj) 98%; slope +0.21).

3) There was a significant negative correlation between plant N content and P solution concentration in *U. dioica* (P= 0.039; R^2 (adj) 88%; slope -0.446).

The mineral content results for *C. dissectum* are not directly comparable with the field sample mineral content data (Table 2.2, Chapter 2) as the experimental results are for total plant material whereas the field samples are for leaf material which has a higher mineral content than root material (Table 6.4). Therefore whole plant mineral content would be generally relatively lower than leaf content but would depend on the root:shoot ratio, which in this experiment ranged between 2 and 6.5 for *C. dissectum*. However, in the case where whole plant mineral content was equal to or greater than leaf mineral content, it would be reasonable to assume that the comparison was valid. Based on this assumption, both the whole plant total P content and P_i proportion of *C. dissectum* at the 100 µM P treatment in this experiment were higher than the field leaf samples while the P_i proportion at the three other treatment levels were similar to the field samples.

Senesced leaf mineral content

No results are presented for the K analysis as it was found to be contaminated. The remaining results are detailed in Table 6.7 which indicates that P and Ca contents are similar in both species but N content appears to be higher in *U. dioica*. The total P content of *C. dissectum* senesced leaves is similar to the content found in live-leaf field samples which suggests that P is not resorbed for recycling. Conversely, the N content of *C. dissectum* senesced leaves is lower than live-leaf field samples suggesting that N may be resorbed for recycling.

Discussion (Experiment 1)

Growth of *C. dissectum*, in terms of phytomass production, was shown to be greater than, or equivalent to, that of *U. dioica* from the incremental fresh weight and dry weight gain data. Both species appeared to be equally retarded at the lowest P concentrations (1.0 and 0.1 μM), which was to be expected as these concentrations are considered limiting to growth for most species. At 10 μM P both species had similar dry weight gain but at 100 μM P *C. dissectum* had a significantly higher dry weight gain. However, the phytomass production by *C. dissectum* was achieved with an overall 2.4 fold lower RGR than *U. dioica.* The RGR_{fw} results highlighted differences between the two species in their growth pattern over the experimental period. *U. dioica* exhibited a rapid decline in RGR_{fw} after only four weeks whereas that of *C. dissectum* remained almost constant. This is consistent with previous work (Chapin, 1983; Rorison, 1968) which concluded that species that have a high P requirement for optimum growth are unable to absorb P at low levels of availability, resulting in their rapid growth rate exhausting internal P reserves. Rorison (1968) concluded that the optimum P level for growth in *U. dioica* was upwards of 1000 μM. Also, Grime and Hunt (1975) demonstrated a maximum potential RGR for *U. dioica* of 2.2 per week given ample nutrient, which is substantially higher than the 0.6 maximum RGR recorded in this experiment. These findings support the first hypothesis (a) that at low P availability, *C. dissectum,* when compared with *U. dioica*, is functioning closer to its optimal growth at the P levels in this experiment.

The mineral content results determined that at the highest P availability in this experiment (100 μM P) both species had similar total P contents but *C. dissectum* had a 1.5 fold higher proportion of inorganic P (82%), which suggests that P was in excess of immediate requirement and the surplus was being stored. Also, this high (whole plant) inorganic P proportion in *C. dissectum* is more than twice that of field leaf samples. The negative correlation between N content in *U. dioica* and P availability suggests that at higher P availability growth is more vigorous, reducing N content by dilution (Mengel and Kirkby, 1979).

The response of N content and uniform K content in relation to P supply both suggest that the results of this experiment are directly attributable to the experimental manipulation of P availability and not any other macro-nutrient limitation.

Leaf turnover rate was shown to be higher in *C. dissectum*. However comparing leaf numbers may not be a particularly appropriate measure due to the very different growth forms of the two species. *U. dioica* produced many more leaves at the higher P concentrations, some of which were very small, sprouting from the various internodes up the stem and from lateral branches. However, using the DWG:SL ratio as a measure of leaf longevity, *C. dissectum* leaf longevity was twice that of *U. dioica* at the highest P concentration which in part supports hypothesis (e) that *C. dissectum* has a high leaf longevity. Leaf longevity in *C. dissectum* was also seen to be correlated with P availability. By retaining the senesced leaves firmly attached to the plant, it is possible that nutrient reserves are translocated from the senesced leaves of *C. dissectum* and recycled within the plant. However, P content of the senesced leaves was similar to live-leaf field samples within the time scale of this experiment, therefore it would seem that this is not the case. In the case of N, senesced leaf content was lower than field samples suggesting possible N recycling. It could be speculated that, in *C. dissectum*, N is more mobile compared with P which may be a result of a more highly developed adaptation to low P availability rather than low N availability.

The root:shoot ratio analysis clearly identified that the pattern of growth in *C. dissectum*, in terms of phytomass production, was predominantly root growth, even at high levels of P availability. By contrast, phytomass production of *U. dioica* at high P availability was largely in the form of shoot growth. Although both species exhibited a negatively correlated relationship between P concentration and root:shoot ratio, the response of *C. dissectum* was half that of *U. dioica*. Hypothesis (b), that *C. dissectum* has a low morphological plasticity, where root:shoot allocation is uncoupled from P availability is therefore supported by the experimental results. Unfortunately the data analysis in this experiment was not able to show a clear linear trend between root:shoot ratio and P concentration, possibly due to the

range of P concentrations being too narrow. Previous work on *U. dioica* (Nassery, 1970) showed a clear linear response between P concentration and root:shoot ratio using 100 fold steps in solution concentration i.e. 0.1, 10 and 1000 μM P. This suggests that *C. dissectum* response would need to be tested over a greater range of P concentrations to establish its degree of morphological plasticity.

6.7.2 Experiment 2 - *Response to high P applied as pulses of varying duration.*

Fresh and Dry Weight Gain

Incremental changes in fresh weight were recorded at various stages in the experiment and these are detailed in Fig. 6.11. *U. dioica* displayed a significant positive linear increase ($P = 0.038$, R^2(adj) 88.7%) with a slope coefficient of 0.034. In contrast, *C. dissectum* did not exhibit any significant positive trend. This difference in phytomass production is clearly reflected by the significant differences ($P< 0.001$) in mean total dry weight gain (DWG) over the experiment, where *U. dioica* produced 225 times the phytomass of *C. dissectum.* When these data are analysed by the various pulse treatments, it can be seen (Fig. 6.12) that *U. dioica* total DWG was a positively correlated with pulse duration (regression significant at $P< 0.001$; R^2(adj) 72%; slope 0.21) whereas there was no significant difference in total DWG as a result of pulse duration in *C. dissectum.* There was a significant ($P< 0.001$) difference in *U. dioica* total DWG due to pulse duration, where DWG was greater at 192 hours than 24 hours, which was in turn was greater than the 'no-pulse' control.

Mean fresh weight gain (FWG) was first analysed at two stages in the experiment, namely 30 days prior to the application of the pulse and 30 days post-pulse application. The 30 days post-pulse FWG was then further analysed at 10 days post-pulse and the remaining 20 days to the end of the experiment. There was a significant species x pre-/post-pulse interaction ($P< 0.001$) for total FWG. In *U. dioica* total FWG was higher post-pulse than pre-pulse. *U. dioica* was also higher than *C. dissectum* pre-pulse (7.5 fold) and post-pulse (13 fold). The pre- and post-pulse period responses were not significantly different in *C. dissectum.* The response of FWG to the pre- and post pulse periods is illustrated in Fig. 6.13.

Figure 6.11 Incremental change of mean total fresh weight of *Cirsium dissectum* and *Urtica dioica* over Experiment 2 (including senesced leaves).

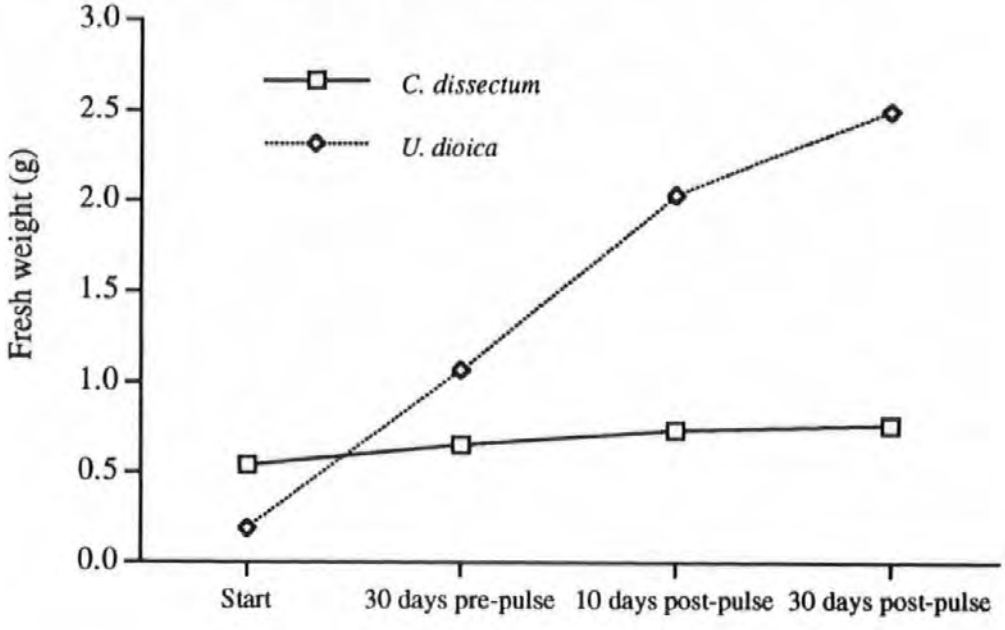
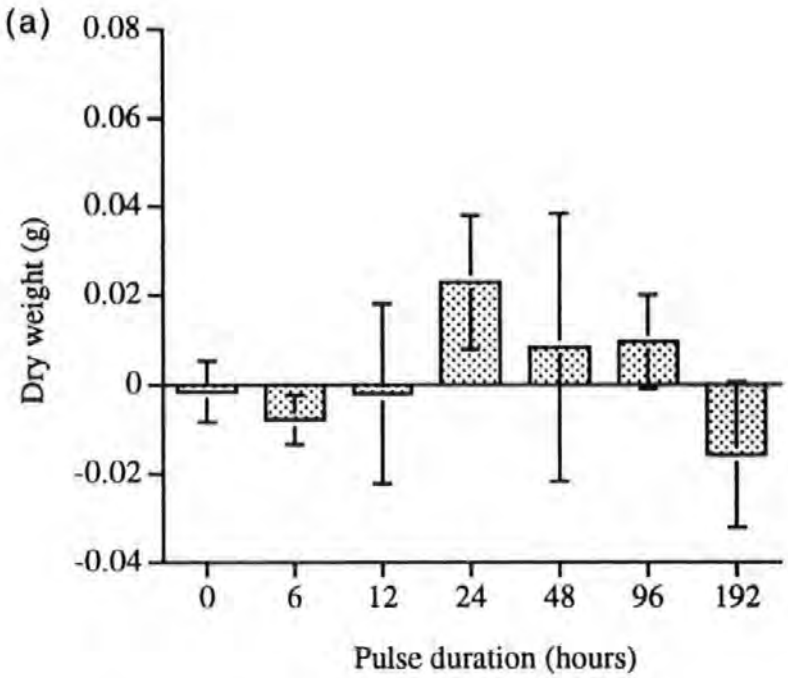


Figure 6.12 Mean total dry weight gain over the experiment of (a) *Cirsium dissectum* and (b) *Urtica dioica*. Vertical bars represent twice the SE of the mean.



[N.B. GRAPH SCALES DIFFER BY A FACTOR OF 10]

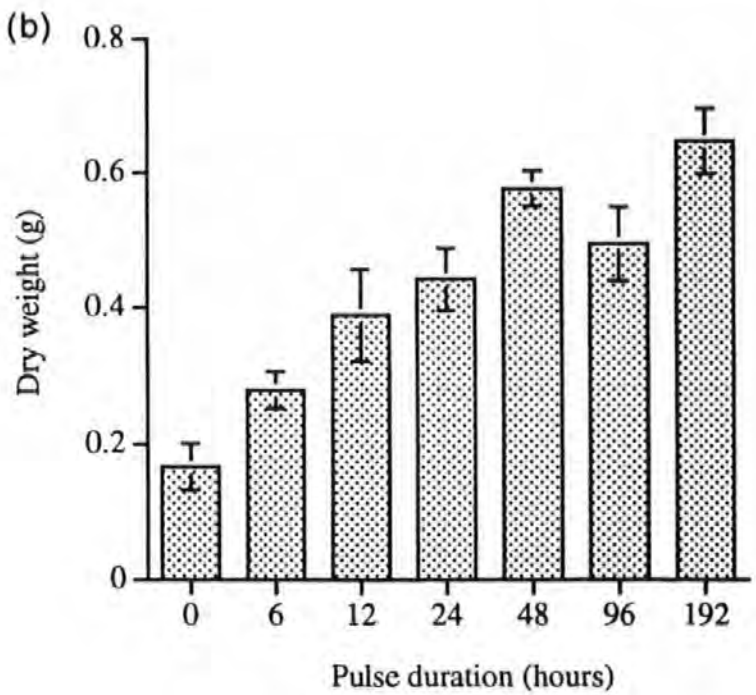


Figure 6.13 Mean fresh weight gain of pulse treatments before and after pulses (including senesced leaves) of (a) *Cirsium dissectum* and (b) *Urtica dioica*. Vertical bars represent twice the SE of the mean. Letters denote significant differences between means and arrowed lines indicate groups of means not significantly different .

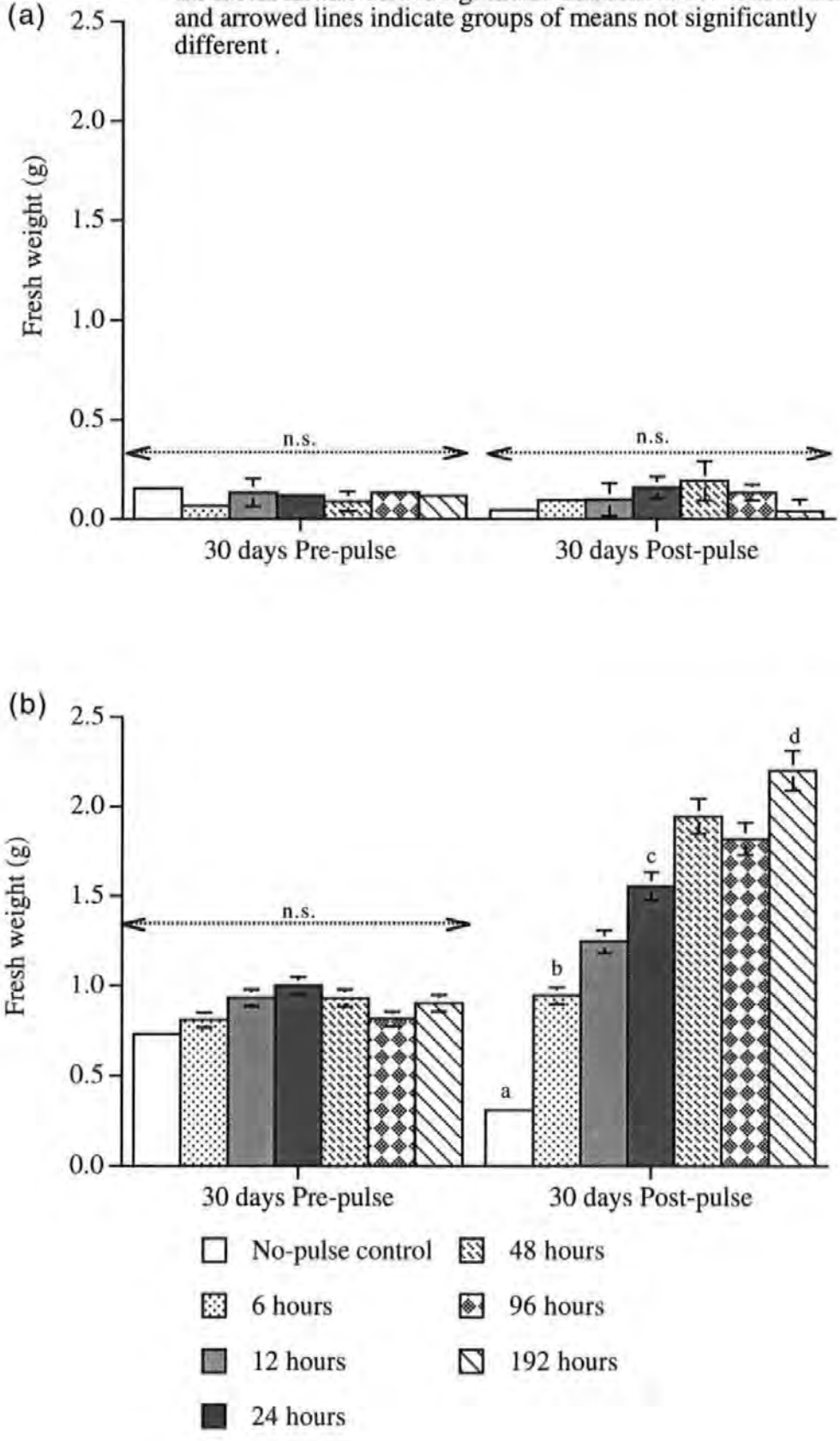
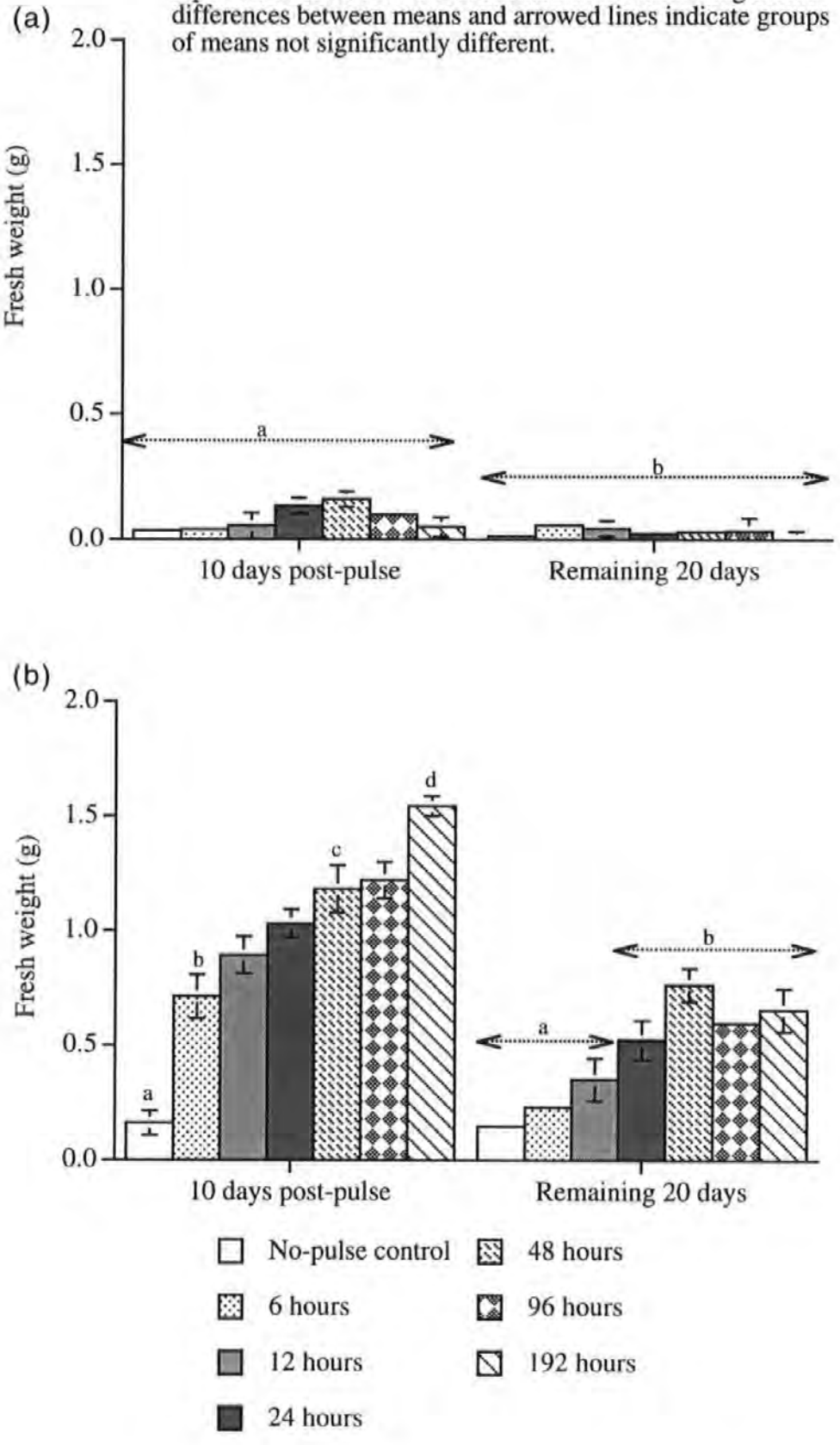


Figure 6.14 Mean fresh weight gain at seven pulse durations over two post-pulse periods (including senesced leaves) for (a) *Cirsium dissectum* and (b) *Urtica dioica*. Vertical bars represent twice the SE of the mean. Letters denote significant differences between means and arrowed lines indicate groups of means not significantly different.



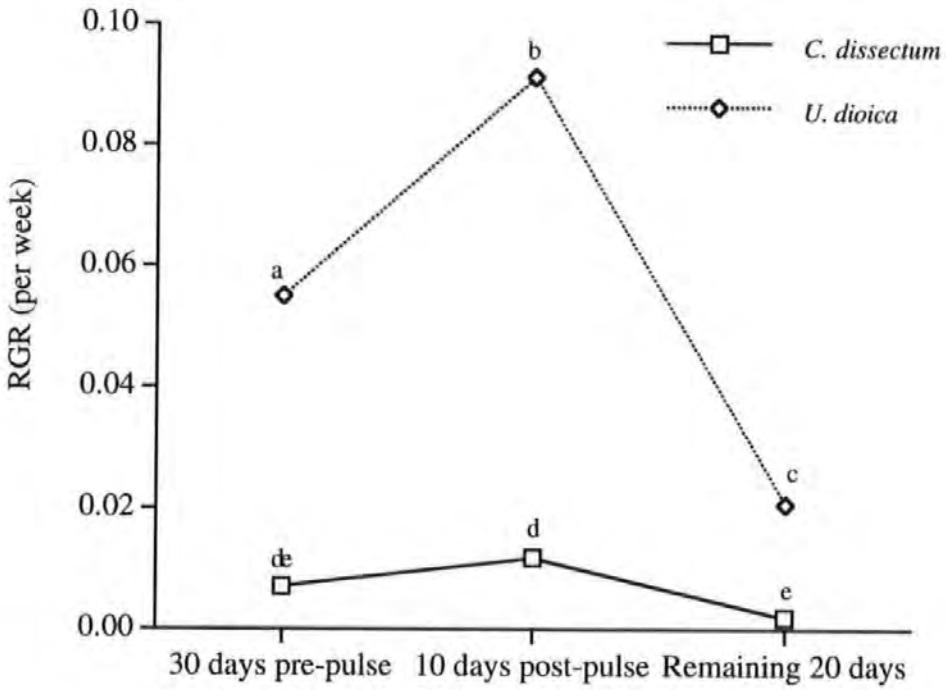
The groups of replicates which received the various pulses were analysed for any differences in FWG in the pre-pulse period to check whether there was any variation due to experimental or specific plant group anomalies. There were no significant differences between the groups for either species. In the post-pulse period, there were no significant differences in FWG as a result of pulses in *C. dissectum* (Fig 6.13a). In *U. dioica* there was a significant ($P < 0.001$) difference in FWG as a result of pulse duration, with FWG at 192 hours greater than 24 hours, which was greater than 6 hours, which were all in turn greater than the control (Fig 6.13b). A more detailed breakdown of post-pulse FWG is illustrated by Fig. 6.14. In the case of *C. dissectum* (Fig. 6.14a), there was a significant three fold difference in overall FWG ($P = 0.007$) between the 10 day post-pulse period and remaining 20 days. There was no significant difference in FWG as a result of pulses and the interaction term was not significant. In *U. dioica* (Fig. 6.14b) there were significant differences in FWG as a result of both pulse and period ($P < 0.001$ both factors), with a significant interaction ($P < 0.001$). The interaction was attributed to the 'no-pulse' control which was not significantly different between the two periods. This was confirmed when the control group data were removed from the analysis which resulted in a loss of significance for the interaction.

U. dioica overall FWG (minus control) in the 10-day post-pulse was twice that of the remaining 20-day period and the effect of the pulse duration was more pronounced in the 10-day post-pulse period compared to the final period. In the 10-day post-pulse period *U. dioica* FWG at 192 hours was greater than 48 hours, which was greater than 6 hours, which in turn was greater than control. This effect declined in the remaining 20 day period, where only the 24 hour and longer pulses were significantly different from the control.

Relative Growth Rate

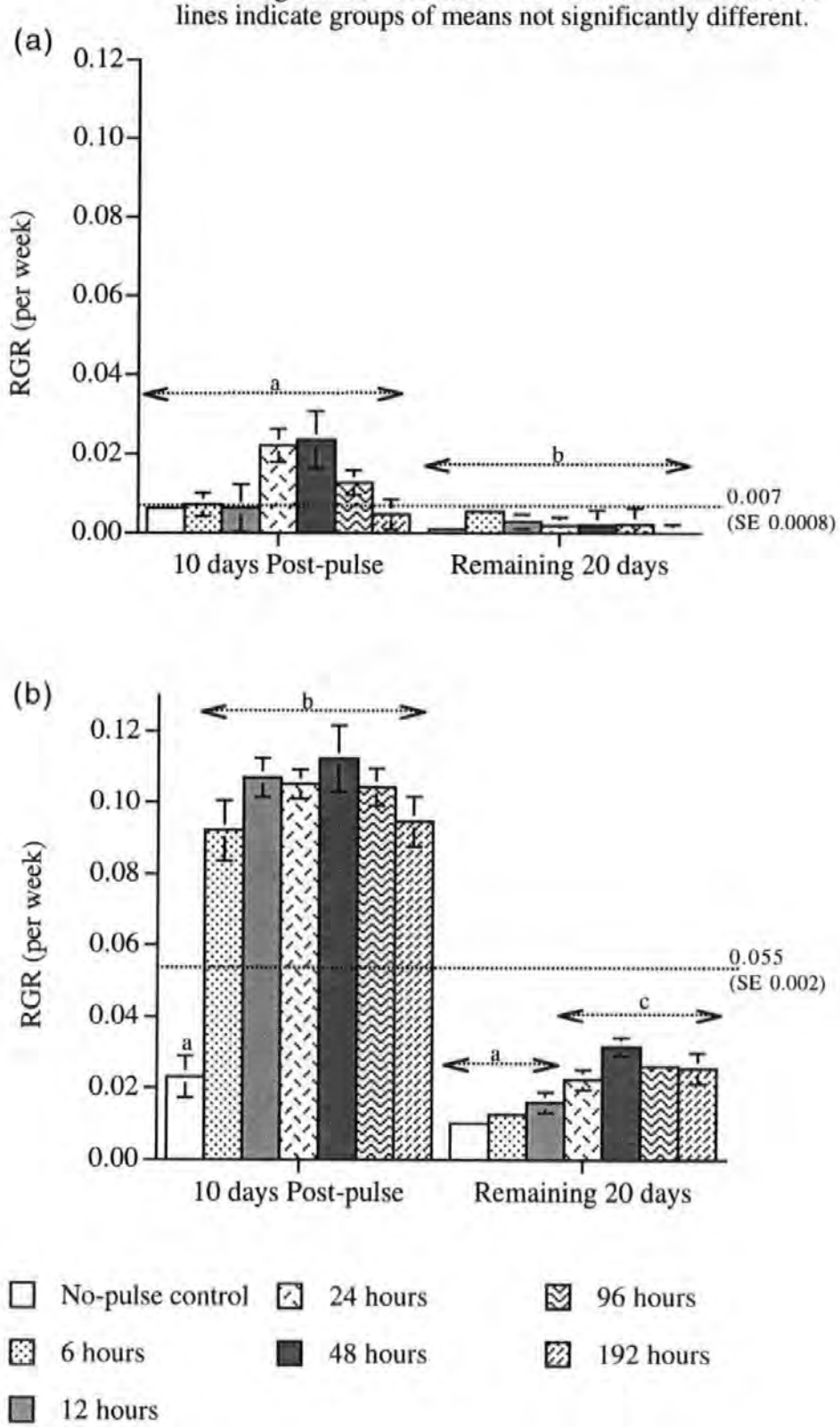
The comparison between species and between the three periods of the experiment was also analysed using the fresh weight RGRs. There were significant differences in RGR between both species and periods ($P < 0.001$) and a significant species x period interaction ($P < 0.001$). The differences in response between the two species is shown in Fig. 6.15.

Figure 6.15 Species x period interaction of mean fresh weight RGR (pooled SE 0.003). Letters denote significant differences between means.



Urtica dioica had a higher RGR than *C. dissectum* in all three periods and the difference was over seven-fold. *U. dioica* also had the highest RGR in the 10-day post-pulse period and the lowest RGR in the final period. In contrast, the only significant difference in *C. dissectum* RGR was between the 10-day post-pulse period and the final 20-day period and this was very small. As in the FWG analysis, variation in mean RGR in the 30-day pre-pulse period, between the replicates predetermined to receive the various pulses, was checked and found to be not significant. Response of both species to the various pulse treatments is illustrated in Fig. 6.16 and clearly illustrates the greater response to pulses by *U. dioica.* Analysis of *C. dissectum* RGR (Fig 6.16a) identified significant differences between periods ($P < 0.001$) and between pulse treatments ($P = 0.037$) but no significant interaction. However, multiple comparison tests did not reveal any differences between the *C. dissectum* pulse treatment means and in neither period were any of the pulsed treatments significantly different to the respective control treatment. In *U. dioica* (Fig. 6.16b), there were significant differences in RGR as a result of both pulse and period ($P < 0.001$ both factors) and a significant pulse x period interaction ($P < 0.001$). However, similar to the FWG analysis, the interaction was largely due to the influence of the control data and when the control data was removed from the analysis, no significant interaction was found. *U. dioica* total RGR was 4.5 times greater in the 10-day post-pulse period compared to the final 20 days (Fig 6.16b). Also, in the 10-day post-pulse period, all the *U. dioica* pulsed treatments resulted in significantly higher RGRs compared to the control, but there were no differences in RGR as a result of pulse duration. However in the final 20 days, only pulses of 24 hours and longer produced a RGR significantly higher than the control.

Figure 6.16 Mean fresh weight RGRs at 7 pulse durations for (a) *Cirsium dissectum* and (b) *Urtica dioica.* Vertical bars represent twice the SE of the mean. Dotted line indicates the 30 days pre-pulse mean total RGR. Letters denote significant differences between means and arrowed lines indicate groups of means not significantly different.



Root:Shoot Ratios

The root:shoot ratios (R:S) for both species were calculated from the final dry weights and these are shown in Fig. 6.17. These clearly illustrate that there was a much higher phytomass allocation to the roots in *C. dissectum* compared with *U. dioica*. By the end of the experiment, *C. dissectum* had a significantly higher R:S ratio ($P < 0.0001$), 4.5 times that of *U. dioica*. Also, there were no significant differences in R:S ratios in *C. dissectum* as a result of the various pulse treatments. However, in *U. dioica* there were significant ($P < 0.001$) differences in R:S ratio as a result of the pulse treatments. The *U. dioica* control R:S ratio was greater than the 6 hour pulse, which was greater than the 48, 96 and 192 hour pulses, which were not significantly different. A regression of *U. dioica* log R:S ratio against log pulse duration was significant ($P < 0.001$; R^2 (adj) 78%) and this is illustrated in Fig. 6.18, which clearly shows that R:S ratio declined as the pulse duration increased.

Senesced Leaves

As in Experiment 1, leaf senescence was recorded both in terms of number of leaves and dry weight of leaves senesced. Mean leaf turnover (number of leaves senesced per leaf produced) was significantly different between the two species ('t' test; $P < 0.0001$) and *C. dissectum* had a turnover 2.5 times that of *U. dioica*. However, neither species had any significant differences in leaf turnover as a result of the pulse treatments. The ratio of dry-weight gain to senesced leaf dry weight (DWG:SL) was, once again, significantly different between the two species ('t' test; $p < 0.0001$), with *U. dioica* 65 times higher than *C. dissectum*. In fact the mean DWG:SL ratio in *C. dissectum* was less than one, indicating that leaf senescence was greater than overall dry weight gain. Also, there was no significant difference in DWG:SL ratio in either species as a result of the pulse treatments.

Figure 6.17 Mean root:shoot ratios (log-transformed) for *Cirsium dissectum* and *Urtica dioica* at 7 pulse durations. Vertical bars represent twice the SE of the mean. Letters denote significant differences between *U. dioica* means (*C. dissectum* means not significantly different).

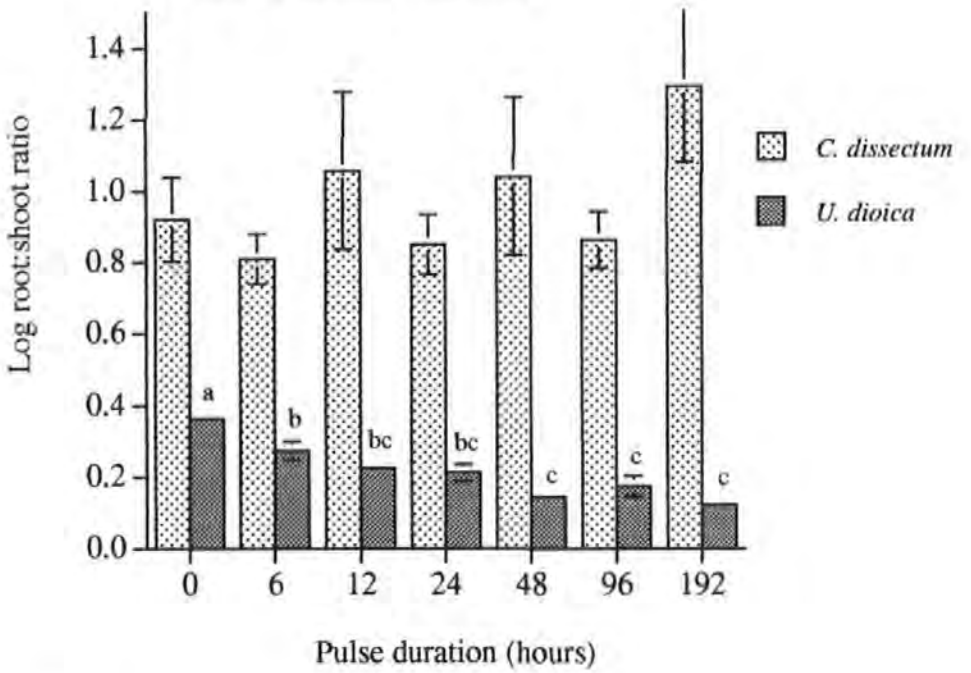
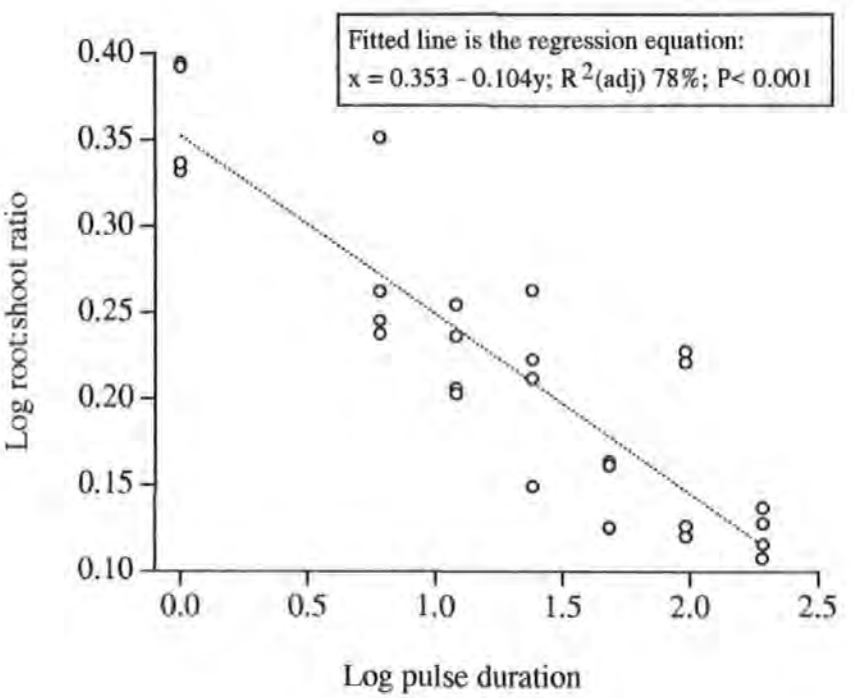


Figure 6.18 *Urtica dioica* log root:shoot ratio against log pulse duration.



Mineral Nutrient Content

Analysis of mineral content was carried out on combined root and shoot material. There was only sufficient *C. dissectum* material for three sub-sample replicates of bulked material within each treatment level. In the case of *U. dioica*, the 24 to 192 hour pulse treatments produced enough analysable material for full replication but the remaining three treatment levels (0 to 12 hours) had to be analysed as three sub-sample replicates of bulked material within each treatment level. The results for both species are detailed in Table 6.8. From the limited replicate data, an ANOVA of *U. dioica* mineral content from the 24 to 192 hour pulse treatments revealed that the only significant difference was in N content, which was significantly lower at the 192 hour pulse compared to the 24 hour pulse (P= 0.005).

A regression of the *C. dissectum* data points and *U. dioica* means against log pulse duration highlighted the following significant trends:

1) There was a significant positive correlation between total P content and log pulse duration in both species (*C. dissectum* P= 0.022; R^2 (adj) 62%; slope +0.041 and *U. dioica* P= 0.009; R^2 (adj) 72%; slope +0.019). There was no correlation between pulse duration and P_i proportion in either species. However, there was a clear difference between the species in overall mean P_i proportion ('t' test significant at $P < 0.0001$), where *C. dissectum* has a 1.5 fold higher P_i proportion than *U. dioica*, which is similar to the results of the first experiment.

2) There was a significant negative correlation between N content and log pulse duration in *U. dioica* (P= 0.002; R^2 (adj) 90%; slope -0.75), which is similar to the relationship between solution P concentration and plant N content seen in the first experiment.

Analysis of mineral content of senesced leaves was limited to a single sample of bulked material at each treatment level for both species and the results are detailed in Table 6.9. A regression of mineral content against log pulse duration produced only one significant trend, where N content of senesced leaves in *U. dioica* was negatively correlated with pulse duration (P= 0.023; R^2 (adj) 61.5%; slope -0.188).

Table 6.8 Experiment 2: plant mineral nutrient content, expressed as a percentage of dry weight of total plant material. In *U. dioica*, data for pulses 0 - 12 are means of sub- samples of bulked material (n=3) but pulses 24 - 192 are means of replicates (n=4). All *C. dissectum* data are single samples of bulked material at all pulses.

Pulse duration (hours)	Species	% P	Pi Proportion	% K	% Ca	% N
no pulse control	*C. dissectum*	0.08	0.61	1.29	0.61	1.89
	U. dioica	0.15	0.31	2.00	0.83	1.83
6	*C. dissectum*	0.11	0.52	1.43	0.67	1.72
	U. dioica	0.14	0.35	2.14	0.97	2.24
12	*C. dissectum*	0.14	0.50	1.35	0.59	1.68
	U. dioica	0.14	0.34	2.05	0.88	1.81
24	*C. dissectum*	0.11	0.52	1.73	0.70	1.33
	U. dioica	0.16	0.37	2.38	0.90	1.69^{a}
48	*C. dissectum*	0.15	0.48	1.51	0.68	1.39
	U. dioica	0.16	0.40	1.94	0.86	1.29
96	*C. dissectum*	0.13	0.64	1.49	0.73	1.66
	U. dioica	0.18	0.39	2.14	0.87	1.40
192	*C. dissectum*	0.20	0.53	1.37	0.60	1.91
	U. dioica	0.18	0.36	1.73	0.78	1.00^{b}

Superscript letters denote significant differences ($P < 0.05$)

Table 6.9 Experiment 2: senesced leaf mineral nutrient content. Data are single samples of bulked material, expressed as a percentage of dry weight.

Pulse duration (hours)	Species	% P	% Ca	% N
no pulse control	*C. dissectum*	0.30	1.97	1.07
	U. dioica	0.40	2.04	1.87
6	*C. dissectum*	0.06	2.20	1.00
	U. dioica	0.31	2.12	1.48
12	*C. dissectum*	0.29	1.77	1.38
	U. dioica	0.34	2.01	1.24
24	*C. dissectum*	0.35	2.09	1.08
	U. dioica	0.42	2.06	1.33
48	*C. dissectum*	0.32	2.13	1.25
	U. dioica	0.51	2.23	0.80
96	*C. dissectum*	0.29	1.81	2.21
	U. dioica	0.37	2.49	1.34
192	*C. dissectum*	0.23	2.10	1.34
	U. dioica	0.50	1.94	0.76

Although there was no correlation between pulse duration and P content in either species, the overall mean senesced leaf P content of all treatments was significantly lower in *C. dissectum* compared to *U. dioica* ('t' test: P= 0.01).

Discussion (Experiment 2)

The results of Experiment 1 demonstrated that when grown at a constant 1.0 μM P concentration (the background level of P supplied in this experiment), both species had similar growth patterns and were equally limited. However, in this experiment, the two species behaved quite differently when the 1.0 μM P was supplemented with a pulse of 100 μM P. The total DWG of *C. dissectum* over the experiment was substantially lower than *U. dioica* and unaffected by any of the pulsed P treatments, unlike *U. dioica,* where DWG was positively correlated with pulse duration. The FWG results showed similar interspecific differences, but also established no difference in *C. dissectum* FWG between the 30-day pre-pulse and 30-day post-pulse periods, whereas *U. dioica* had a higher FWG in the 30-day post-pulse period which was positively associated with pulse duration. However, within the post-pulse period, *C. dissectum* total FWG was three times higher 10 days after the pulses, compared to the final 20 days, but there was no difference between control and pulsed treatments within either of these periods. Similarly, *U. dioica* total FWG in the 10-day post-pulse period was twice that of the final 20-day period, but in addition, FWG was associated with pulses. However, by the final period only the longer pulses of 24 hours and over had a higher FWG than the control.

The fresh weight RGR analysis provides the clearest evidence of how both species responded to pulses of P. In *C. dissectum,* the various pulse treatments did not produce any significant change in RGR when compared with the control replicates. By comparison, in *U. dioica,* all levels of pulse treatments produced a similarly higher RGR, 4.5 times the control replicates in the 10-day post-pulse period. This effect was still present in the final 20-day period, where the RGRs of the 24 hour and over pulses were still twice as high as the control. Therefore, the positive effect on RGR of a pulse as short as 24 hours would still be present for at least 30-days. Also, the fact that the positive effects on RGR from the

shorter pulses had been lost after 30 days, suggests that the span of influence of a pulse is related to the pulse duration. These results, in part, support the hypothesis, that *C. dissectum* does not show a growth response to flushes of high P.

The differences in phytomass allocation are very clear from the R:S ratio results. In *C. dissectum,* none of the pulsed treatments resulted in R:S ratios which were significantly different from the control. However, there was a clear negative correlation between R:S ratio and pulse duration in *U. dioica* and all the pulse treatments had a lower R:S ratio than the control. As would be expected, the R:S ratios of the control replicates of both species were similar to those grown at 1.0 μM P in the first experiment. However, the R:S ratio of *C. dissectum* was lower when supplied with a continuous supply of high P in the first experiment, whereas in this experiment, there was no detectable change in R:S ratio when high P was supplied as a pulse. This reinforces the results from the first experiment, suggesting that *C. dissectum* has a low morphological plasticity, and shows clearly that the expression of any inherent plasticity is very slow, which lends further support to hypothesis (b) that *C. dissectum* has a low morphological plasticity. There was no correlation between leaf senescence/longevity as a result of supplying P in pulses in either species. However, in contrast with the first experiment, *C. dissectum* exhibited a lower degree of mean leaf longevity compared to *U. dioica* in this experiment.

Plant P content was positively correlated with P pulse duration in both species but without the corresponding increase in P_i proportion observed in Experiment 1. This suggests that, in the case of *C. dissectum,* the pulse duration was too short to provide P in excess of immediate requirement. However, in common with the first experiment, *C. dissectum* exhibited an overall P_i content 1.5 times higher than *U. dioica.* This could suggest that *C. dissectum* was experiencing a lower degree of P stress as, in P deficient plants, tissue contents of P_i are depressed, whereas organic P levels are little affected (Mengel and Kirkby, 1979). Although the P content of senesced leaves was lower in *C. dissectum* compared to *U. dioica*, it was still similar to the live-leaf field samples and would confirm that *C. dissectum* does not appear to resorb P for recycling.

6.9 CONCLUSIONS

The results of both experiments demonstrate that the functional response of *C. dissectum* is clearly different to that of a highly competitive species with a high P requirement such as *U. dioica.* Four hypotheses were originally proposed to test predicted plant functional attributes which would suggest an evolved physiological adaptation to a low P environment. In the main, all four hypotheses have been supported by the results of these experiments. One of the major adaptations to a low P environment is a low RGR (Chapin, 1980) and this has been demonstrated in *C. dissectum.* However, DWG was higher in *C. dissectum* compared to *U. dioica,* which illustrates that the yield in *U. dioica* was reduced to a greater degree and that *C. dissectum* was functioning closer to its optimal growth and metabolic rate. In addition, Experiment 2 demonstrated that growth and phytomass allocation in *C. dissectum* is uncoupled from short-term fluctuations in P availability, unlike *U. dioica* which responds immediately with increased growth and altered allocation patterns to very short-term increases (i.e. 6 hours) in nutrient availability. The lack of response by *C. dissectum* to temporary fluctuations in P availability is a fundamental characteristic of species adapted to a low P environment (Chapin, 1980; Grime, 1979). In addition, *C. dissectum* does have a capacity to store P when availability exceeds current requirements, coupled with higher P_i reserves, both characteristics of low P-adapted species (Nassery, 1970). The inherent high root:shoot ratio in *C. dissectum* is a further clear physiological trait associated with plants of low P environments. Although it has been shown that *C. dissectum* exhibits a relatively low morphological plasticity, it has not been possible to determine the full range of plasticity expression from the relatively narrow range of P concentrations used in this experiment. Finally, *C. dissectum* exhibited a relatively higher leaf longevity compared to *U. dioica* which is consistent with the observation that species adapted to infertile sites retain green leaves longer under conditions of lower nutrient availability than do species from fertile environments (Grime and Curtis, 1976).

It can be concluded from the above findings, that *C. dissectum* possesses many of the characteristics of a species conforming to the stress-tolerator functional type and has evolved physiological adaptations to a low P environment. The ecological implications are that the low P availability in *Cirsio-Molinietum* fen meadows plays a significant role in determining the ecological niche of *C. dissectum.* Although the species may not be able to realise its full genetic potential, it can function much closer to its optimal growth and metabolic rate under these conditions compared to a high P-demanding competitive species. This confers a relative competitive advantage, where *C. dissectum* will be better adapted to a low P environment than a rapidly growing competitive species.

The above conclusions are based on experimental results under controlled environment conditions using a hydroponic growth medium. Under field conditions, root infection by mycorrhizae and the possible capacity of *C. dissectum* to modify soil chemistry with root exudates (Helal and Sauerbeck, 1991; Tarafdar and Claassen, 1988) may play an important role in P metabolism under conditions of low P availability. The ability of root exudates to mobilise the substantial reserves of organic P in Culm soils could alter all the assumptions of low P availability in these soils. Therefore some caution should be exercised before extrapolating these results directly to the field situation without further detailed study.

7.0 THE RESPONSE OF *CIRSIUM DISSECTUM* TO DEFOLIATION

7.1 INTRODUCTION

The preceding chapters have been mainly concerned with the response of *C. dissectum* to various forms of stress, either existing or potential. The stresses are mainly as a direct result of edaphic or environmental conditions. These are large-scale processes which vary seasonally and annually and are extremely difficult and costly to manipulate by intervention management. However, the plant:environment model (Fig 1.4, Chapter 1) identifies grazing as one of the plant community driving variables and this is relatively easily controlled by management. It is generally accepted that the botanical characteristics of a grassland community reflect its history of management, including grazing pressures (Archer and Smeins, 1991; Ausden and Treweek, 1995; Crawley, 1997; Duffey, *et al.*, 1974). Historically, the main agricultural use of Rhôs pastures was summer grazing by cattle and in many areas this is still current practice. In the absence of grazing there would be natural succession to scrub and carr (Harper, 1977; Rodwell, 1991b), resulting in a decline of the species diversity for which these sites are particularly valued. The *Molinia caerulea-Cirsium dissectum* (M24) fen meadow community, in particular, owes its maintenance and essential aspects of floristics and structure to particular patterns of mowing and/or grazing (Godwin, 1941). Constant removal of herbage and the effects of trampling by cattle favour the abundance of hemicryptophytes, particulary rosette species such as *C. dissectum*, which are generally limited to short or moderately tall herbage typical of the M24 community (Rodwell, 1991b). Therefore, although grazing is apparently a positive driving force in maintaining *C. dissectum* populations in these particular communities, the precise mechanisms operating at the particular plant species level and interactions with other species are not fully understood.

Unlike experiments in earlier chapters which examined response to stress, removal of shoot material during the growing season represents a major form of disturbance. Grime (1979) defines disturbance as "the mechanisms which limit the plant mass to accumulate by causing its partial or total destruction" and proposes this to be one of two external factors responsible

for limiting vegetation in any habitat, the other factor being stress which restricts photosynthetic production. The preceding stress experiments have established that *C. dissectum* falls into the stress-tolerant functional type category as described by Grime (1979). However, many of the physiological characteristics of this functional type are not particularly compatible with disturbance, namely low RGR, slow leaf turnover rate and low morphological plasticity. Therefore, loss of shoot material during the growing season would appear to be particularly damaging to *C. dissectum,* unless it is able to compensate for lost photosynthetic tissue.

7.2 PLANT RESPONSE TO DEFOLIATION

Much of the ecological literature concerning defoliation relates to herbivory and predator-prey population biology, where plant predators are invertebrates and wild mammals (Crawley, 1983; Harper, 1977; Louda, Keeler, and Holt, 1990). In contrast, studies on the effects of grazing on plants by domestic animals are largely concerned with highly productive grass species and their value for animal production (Spedding, 1971). Although defoliation responses have been studied extensively in turf grasses and species of pastoral importance, the literature on native herbaceous plant species is sparse. There are also important differences between the way cattle defoliate plants compared to invertebrates. Cattle generally clip or tear part or all of a leaf whereas invertebrates damage leaves in a variety of ways, for example skeletonizing, holing, rolling and mining (Crawley, 1983). A study by Morrison and Reekie (1995) demonstrated that the way in which leaf tissue is removed can have a dramatic effect upon the photosynthetic capacity of the remaining tissue. They concluded that the effect of defoliation on photosynthesis appeared to be related to the amount of leaf wounding which they quantified as the length of the cut edge. An increase in the length of the cut edge resulted in reduced photosynthesis of the remaining tissue.

Maschinski and Whitham (1989) reviewed a considerable number of apparently contradictory studies on the impacts herbivores have on plants, the results of which ranged from: herbivory being detrimental, of no consequence or even being beneficial. They went on to show that a plant's response to herbivory is plastic and varies according to the conditions it experiences. Because the effects of herbivory are governed by interactions between the environment and the affected plant (McNaughton, 1986), plant responses vary according to the prevalent biotic and abiotic conditions. Plant responses to herbivore damage vary enormously, but much of the literature agrees that most plants exhibit compensatory growth to some degree (Crawley, 1983; Crawley, 1997; Louda, *et al.*, 1990; Maschinski and Whitham, 1989; Oesterheld, 1992). Factors known to influence the degree of compensatory growth include timing and intensity of grazing (Crawley, 1983; Harper, 1977), water availability (Cox and McEvoy, 1983), nutrient availability (Chapin, Schulze, and Mooney, 1990), type and age of tissue eaten (Crawley, 1997). Individually these factors can influence a plant's physiological state and its ability to compensate for defoliation. This may be further complicated by interactive effects between two or more factors.

The ecological implications of grazing are that differential response to it may be an important determinant of relative abundance in species-rich plant communities and may also help to explain the absence of certain species from these kinds of vegetation (Hendry and Grime, 1993). There are many examples in the literature which clearly demonstrate the ability of grazing or mowing to alter the composition of a plant community (Harper, 1977) and effect a complete change from one community type to another (Godwin, 1941). Grazing impacts at both the population and community level, altering intra- and interspecific relative competitive ability, changing identity of the key community species and these effects are likely to be more pronounced in low productivity open systems (Crawley, 1997).

7.3 MECHANISMS OF PLANT COMPENSATION

Crawley (1983) reviewed the work of many authors and proposed five main processes through which compensation for herbivory can occur and these are briefly summarised below.

Increased unit leaf rate (ULR)

The ULR (net photosynthesis per unit leaf area) of heavily clipped plants increases probably due to stomatal opening of remaining leaves. This has been hypothesised to be caused by a reduction of leaf competition for cytokinins produced in the roots (Wareing, Khalifa, and Treharne, 1968). Also partial leaf removal alters the balance of carbohydrate sources and sinks; leaf removal, by reducing the source, often causes an increase in the ULR of the surviving leaves.

Carbohydrate reserves

These reserves consist of saccharides of various kinds which may be stored during periods when carbohydrate production exceeds the demands of respiration, growth and production. The major storage compounds are starch, fructans or sucrose and can be crucial to plant survival where grazing is so severe that residual green leaf area is negligible.

Distribution of photosynthate

Root:shoot ratios are generally uniform during vegetative growth and are largely determined by water and nutrient availability. Defoliation alters the pattern of distribution of photosynthate within the plant. A defoliated plant has more root than is necessary to supply the transpiration or nutrient requirements of its small residual leaf area. It therefore builds new leaves to redress the balance and allows respiring roots, surplus to requirements, to die without replacement.

Reduced death rate

When plants are source-limited there is greater scope for compensation for the removal of sinks by herbivores. Damage to fruits or flowers can often be compensated for by an increase in proportion of seeds maturing, reduced abortion rate of late flowers and continuation of flowering over a longer period. However, these compensation mechanisms are dependent on the timing of defoliation in the season and adequate supplies of water and nutrients.

Reduced competition

Selective herbivory reduces competition with other plants resulting in relaxed density dependence in either plant death rate or in the growth rate of individual plants. This is only likely to be important in dense monocultures or in sparse populations of high species density. However, compensation for herbivory is unlikely to be important after density-dependent processes have determined plant size, fecundity and survivorship.

Oesterheld (1992) demonstrated that the main mechanism responsible for compensatory growth followed a simple growth model. Grazed and ungrazed plants may have equal absolute growth rates (i.e. full compensation), so long as the shoot relative growth rate (RGR) of grazed plants increases exponentially with grazing intensity (i.e. to the proportion of biomass removed). His experiments demonstrated that plants (in his case two grass species) showed the same above ground growth regardless of defoliation intensity as a result of an exponential stimulation of above ground RGR by defoliation. As a consequence, below-ground RGR was depressed by defoliation. After a 42-day recovery period, basic allometric relationships such as root:shoot and leaf-area:weight ratios were not affected by defoliation intensity. He concluded that exponential above ground compensatory responses represent a key feedback process resulting in constant above ground growth regardless of defoliation intensity and appear to be a simple consequence of strong commitments to certain allometric relationships.

Similar findings were reported by Prins, Verkaar and van den Herick (1989), working on *Senecio jacobaea* and *Cynoglossum officinale.* Also, Thornton (1991) demonstrated that in *Molinia caerulea,* compensatory response mechanisms interact with nutrient availability. At low nitrogen availability, defoliation increased leaf extension rate but reduced root dry weights and resulted in higher root:shoot ratios.

7.4 CARBOHYDRATE RESERVES

Storage of carbohydrates is a characteristic feature of most plants, particularly perennials, and is a major plant process, along with acquisition, transport, growth, defence and reproduction. This subject has been comprehensively reviewed by Chapin (1990). He broadly defines storage as "resources that build up in the plant and can be mobilised in the future to support biosynthesis for growth or other plant functions". His review also highlighted the following points which, in the context of this study, would be relevant to defoliation, particularly if it reduced stored reserves.

1) In both biennials and perennials there is clear evidence of the importance of reserves in supporting reproduction.

2) In biennials, rosette size is a good predictor of the quantity of stored reserves.

3) The dependence of storage reserves for reproduction is more pronounced in habitats with low environmental resources (i.e. nutrients, water, light) .

4) Seasonal and lifetime storage of carbohydrates has a lower opportunity cost and is therefore better developed in plants of low- (compared to high-) environmental resource habitats.

Brocklebank and Hendry (1989) investigated differences in species-specific storage patterns of reserve carbohydrates and associated plant characteristics and these are briefly reviewed as follows. They found that most plant species accumulate more than one of the three principal reserve carbohydrates (starch, fructan and sucrose) at any one time. However, there were species differences as to which carbohydrate was the major reserve. Where fructan is the principal reserve, it is stored largely as an alternative to, and not in addition to, starch.

Appreciable concentrations of sucrose were present in all the 20 species examined at some stage in the year and in seven species it was the principal reserve. Both starch and fructan are stored principally in underground systems, the former in amyloplasts, the latter in vacuoles (Matile, 1987) and accumulate the highest concentrations in the summer (starch 4 - 88 mg g^{-1}; fructan 11 - 295 mg g^{-1} fresh weight) (Hendry, 1987). In contrast, sucrose mainly accumulates in the shoot tissue with the period of maximum accumulation during winter between January and March (7 - 18 mg g^{-1} fresh weight) (Hendry, 1987). Both starch and fructan reserves, being below ground, are inaccessible to leaf herbivores and sucrose only reaches maximum concentrations in winter. Therefore storage reserves are unavailable to most leaf herbivores in either space or time. Further, species storing fructan have attributes which differ from those storing starch or sucrose. Fructan is generally accumulated to considerable concentrations and these are not subject to depletion during tissue growth and development. Environmental stresses such as low temperature, low N and drought promote fructan accumulation (Hendry, 1987) but defoliation by grazing and cutting reduces accumulation (Pollock, 1984). Although phenologies of fructan-storing species differ, all appear to have one common feature, namely to grow, or remain green throughout winter or to undergo growth in the late winter or in early spring in advance of most non-fructan species (Brocklebank and Hendry, 1989). Hendry (1987) also highlighted that the dominant family of fructan-storing species is the Compositae, with an estimated 24,000 species. Many of the features characteristic of fructan-storing species detailed above would appear to closely match many of the known characteristics of *C. dissectum,* suggesting that fructan is the major reserve carbohydrate in this species. It is a member of the Compositae family, retains a very small green leaf area compared to summer (see Chapter 2) and starts growth in early spring.

7.5 AIMS AND OBJECTIVES

Plants which exist under a grazing regime are likely to experience damage from defoliation to some degree, which will impact on the integrated growth of the whole plant in some form over a long or short time period. The aim of the experiment described in this chapter is therefore to examine the impact of a single partial or total defoliation event on *C. dissectum* in terms of its physiological and morphological responses and the possible ecological implications. The specific objectives were to determine the following:

a) Whether *C. dissectum* exhibits compensatory regrowth in response to defoliation

b) The main mechanisms of compensatory regrowth, if present

c) Whether any compensatory response is affected by nutrient availability

d) The principal reserve carbohydrate and whether defoliation depletes reserves

e) Whether defoliation affects vegetative reproduction (if any) within the experimental period.

7.6 MATERIALS AND METHODS

C. dissectum plants were raised from seed and grown for five months on sand watered with Rorison's solution (10% of full strength) prior to commencing the experiment. The experiment was carried out under controlled environment conditions in a phytotron growth cabinet, using the same ISP standard regime detailed in Chapter 3. Prior to commencing the experiment, plants were transferred to individual 120 mm diameter plastic plant pots filled with washed fine grade 'silver' sand and plant fresh weight was recorded. The mean plant weight within each allocated treatment group was checked to ensure that there were no significant differences at the start of the experiment. Plants were then allowed to acclimatise for 24 hours prior to defoliation. Proportional biomass removal was adopted as a means of subjecting all plants to a standardised degree of damage (Hendry and Grime, 1993). Leaf material was removed using scissors. Three levels of defoliation were applied 1) zero defoliation (control), 2) 50% of all leaf material removed and 3) all leaf material removed (100%) by cutting the leaf at the base where it joins the rosette.

In addition, three levels of nutrient were applied to each of the defoliation treatments: 1) balanced (10% of Rorison's solution) , 2) low nitrogen (N) - 10% of balanced and 3) low phosphorus (P) - 5% of balanced. The nutrient regimes were based on Rorison's solution at 10% of full strength with reductions of N and P. Details of concentrations for each of the nutrient treatments are shown in Table 7.1. All other mineral nutrients were maintained at a uniform concentration. The choice of P concentration was a compromise based on results of Chapter 5, which show that 10 μM P (10% of balanced) does not substantially reduce growth (compared to 100 μM P) in *C. dissectum* whereas 1 μM P (1% of balanced) is particularly limiting.

Table 7.1 Nitrogen and phosphorus concentrations in experimental nutrient treatments

Treatment	N concentration (μM)	P concentration (μM)
Balanced (10% Rorison's)	400	100
Low - N	40	100
Low - P	400	5

Following defoliation, plants were allowed to recover for 53 days, at which point the experiment was stopped and root/shoot fresh weights were recorded along with leaf number, senesced leaf number, senesced leaf dry weight and any clonal growth. The plants were then immediately immersed in liquid nitrogen and stored in a freezer to prevent enzymatic changes in the carbohydrates (Allen, 1989), then subsequently freeze dried in a Pirani 10 model EF2 freeze drier (Edwards, Crawley) and finely ground in preparation for carbohydrate analysis. Determination of carbohydrate fractions was carried out using Bioquant® enzyme analysis kits (Merck, Lutterworth) reference number 436862J - Starch kit and 436882N - Glucose/Fructose/Sucrose kit. Fresh weight relative growth rates (RGR) were calculated (Evans, 1972) for both root and shoot as it has been demonstrated (Oesterheld, 1992) that defoliation can stimulate leaf RGR and depress root RGR. It was necessary to use fresh weight RGRs as handling of the roots and shoots during and after the freeze drying process may result in the accidental loss of some material.

The experimental design was a fully randomised layout with five replicates of each treatment (3 defoliation levels x 3 nutrient levels x 5 replicates). Statistical analysis was carried out using a balanced two factor analysis of variance (ANOVA). Prior to conducting the ANOVA, raw data were examined for homogeneity of variances using a F_{max} test (Fowler, *et al.*, 1998). In order to stabilise variances, data were log-transformed where appropriate. Where significant differences within factor levels or interaction between factors were identified, a 'Tukey' test was used as a first analysis. Where there were overlapping similarities in pairwise comparisons, the less conservative 'Newman-Keuls' test was used (Zar, 1996).

7.7 RESULTS

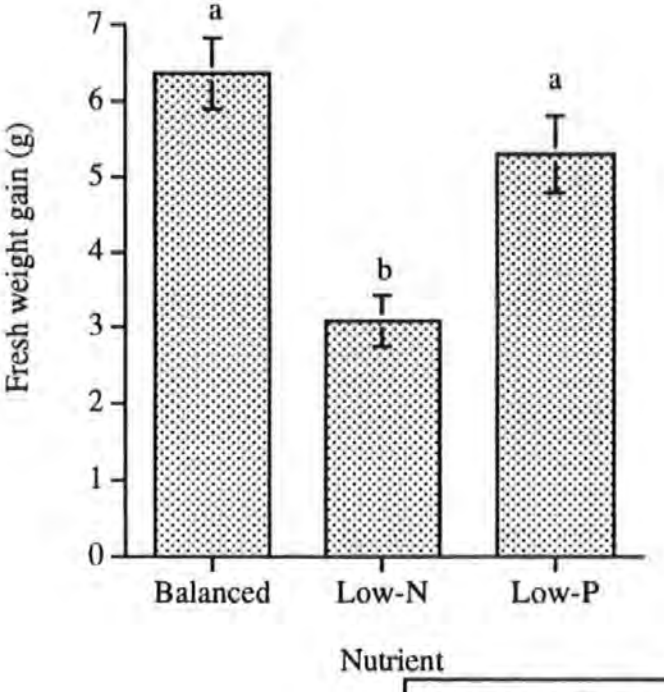
Overall fresh weight gain (FWG) was unaffected by defoliation (P= 0.17). A summary of mean FWG data at each treatment is contained in Table 7.2. The low-N treatment reduced FWG (P< 0.001) to just under half that of the balanced and low-P treatments. There was no significant difference in FWG between the balanced and low-P treatments (Fig 7.1).

Table 7.2 Mean total plant fresh weight gain (g) of *C. dissectum* at three defoliation levels and over three nutrient regimes. Cell contents are means (± SD).

Defoliation	Balanced nutrient	Low-N	Low-P	All
None	6.85 (1.46)	2.82 (0.81)	6.40 (2.10)	5.36 (2.35)
50%	5.19 (1.82)	3.31 (1.63)	4.25 (2.25)	4.25 (1.95)
100%	7.04 (1.69)	3.10 (1.59)	5.24 (1.56)	5.13 (2.17)
All	6.36 (1.76)	3.08 (1.31)	5.30 (1.98)	4.91 (2.17)

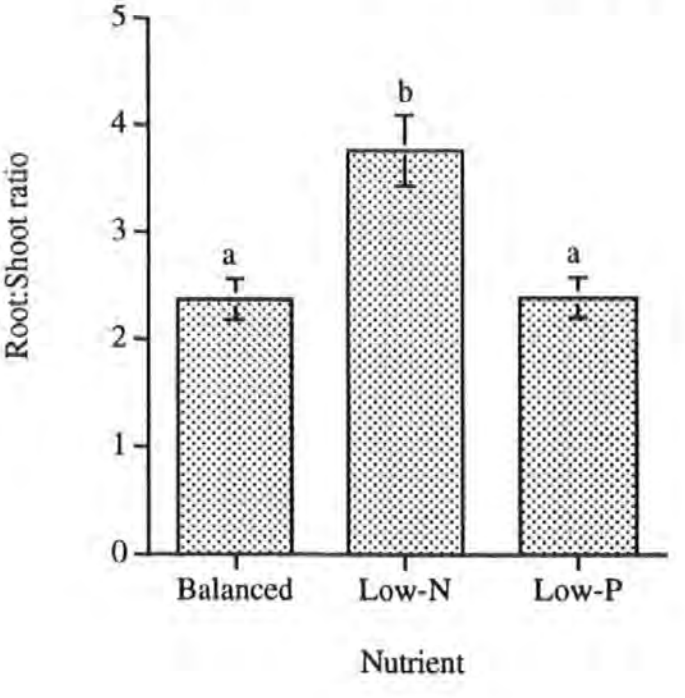
The root:shoot ratios (RS) were unaffected by defoliation (P= 0.63). The low-N treatment increased RS (P< 0.001) by 1.6 times compared with either balanced or low-P treatments. The latter treatments were not significantly different in RS (Fig 7.2).

Figure 7.1 Mean fresh weight gain (g) under three nutrient regimes. Vertical bars represent twice the SE of the mean.



Note: Histograms with different letters are significantly ($P < 0.05$) different. Relevant for all figures where letters are used

Figure 7.2 Mean root:shoot ratio under three nutrient regimes. Vertical bars represent twice the SE of the mean.



Root and shoot RGRs were affected by both defoliation and nutrient supply but there was no significant interaction between the effects of these two factors on RGR. Root RGR was significantly reduced ($P = 0.001$) by about 35% compared with the undefoliated control in both the 50% and 100% defoliation treatments (Fig. 7.3a). The opposite effect was observed in shoot RGR, which was increased ($P = 0.004$) compared with the undefoliated control by 63% in the 50% and 100% defoliation treatments (Fig. 7.3b). Both low-N and low-P reduced root RGR ($P < 0.001$) by about 38% compared with the balanced nutrient regime (Fig. 7.4a). However, the shoot RGR was only reduced ($P < 0.001$) by low-N, resulting in an RGR 56% lower than the balanced and low-P regimes (Fig. 7.4b). The combined effect of defoliation and nutrient concentration for both root and shoot RGRs are detailed in Fig. 7.5, which illustrates that the degree of response to defoliation in shoots is more pronounced than in roots and that N-deficiency (as opposed to P-deficiency) is much more limiting to shoot growth than root growth.

Senesced leaf material was virtually zero in the 100% defoliation treatment, with only one replicate producing any senesced leaves. There was no significant difference in senesced leaf dry weight or senesced leaf number between the undefoliated and 50% defoliation treatments. Also, there were no differences in senesced leaf dry weight or senesced leaf number as a result of the different nutrient treatments. The number of new leaves produced over the experiment was affected by both defoliation and nutrient treatments but there was no significant interaction between the effects of these two factors on new leaf production. The 100% defoliation treatment produced a greater ($P = 0.002$) number of new leaves compared with the 50% treatment (Fig. 7.6a). There were significant differences ($P < 0.001$) in new leaf production between all three nutrient treatments. The balanced nutrient treatment produced the greatest number of new leaves and the low-N treatment produced the least (Fig 7.6b). It is worth noting that in the 50% defoliation treatment, a number of the half leaves from the original defoliation (mean 27%; ± 19% SD) survived to the end of the experiment and were presumably still photosynthetically active.

Figure 7.3 Mean fresh weight RGRs per week at three levels of defoliation for (a) root and (b) shoot. Vertical bars represent twice the SE of the mean

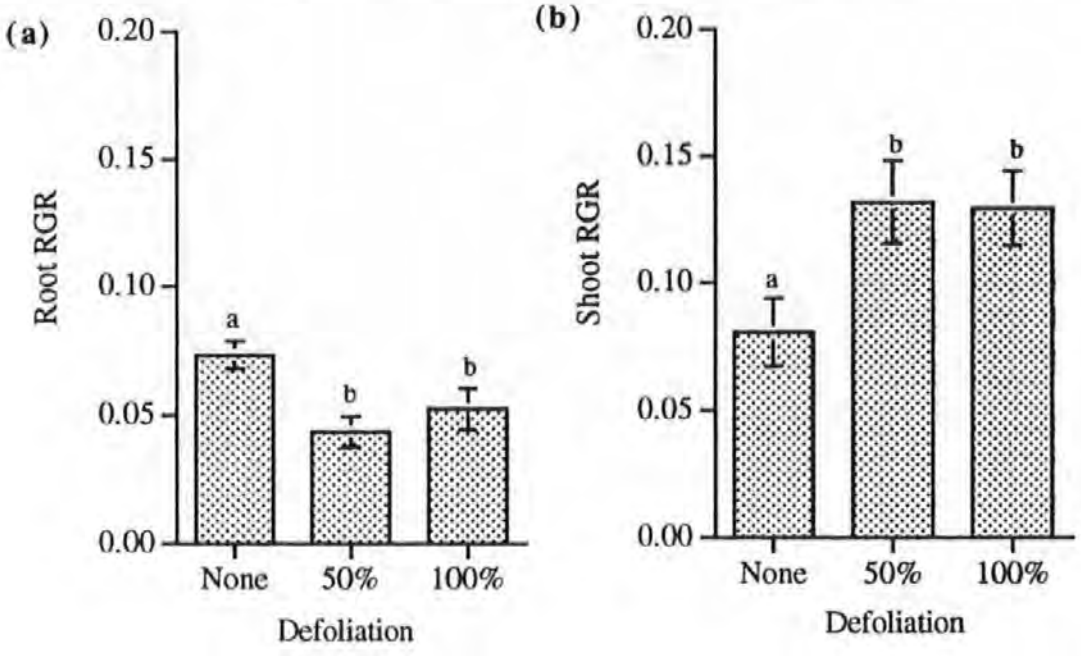


Figure 7.4 Mean fresh weight RGRs per week at three levels of nutrient supply for (a) root and (b) shoot. Vertical bars represent twice the SE of the mean

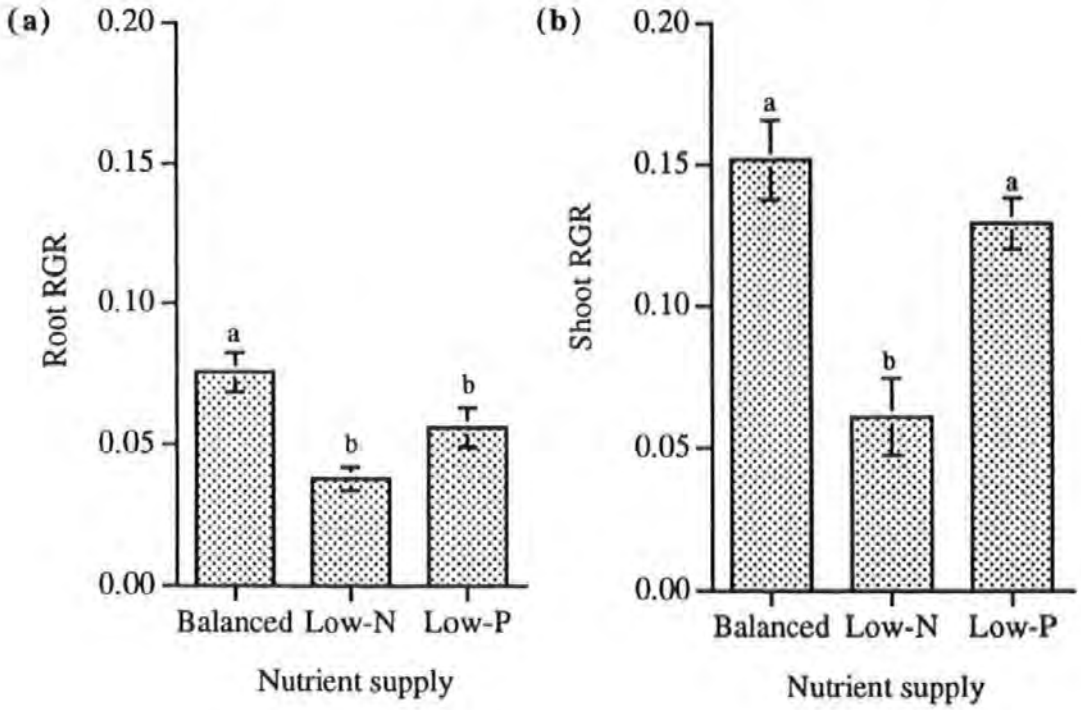


Figure 7.5 The combined effect of defoliation and nutrient on both root and shoot mean RGRs per week

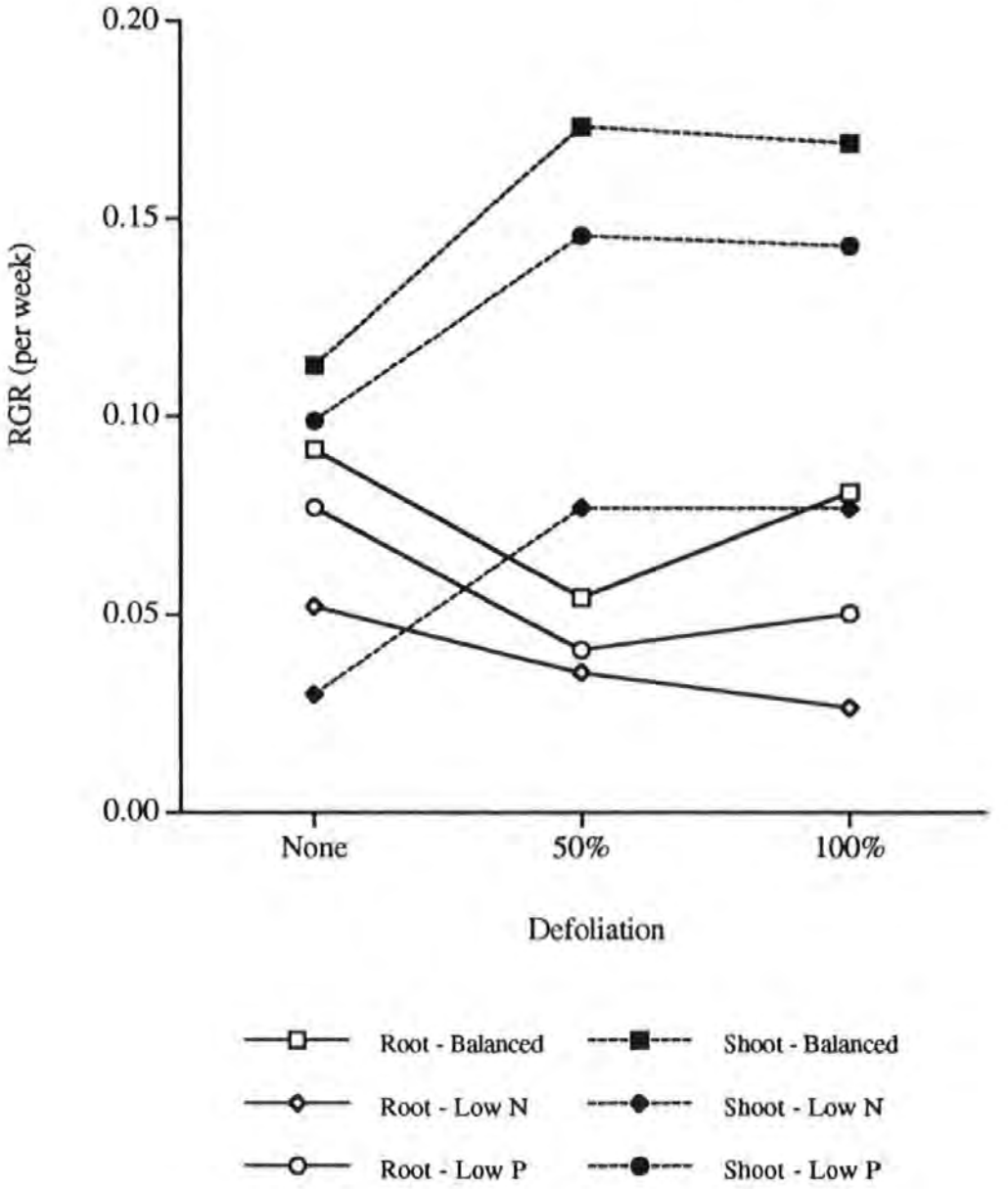
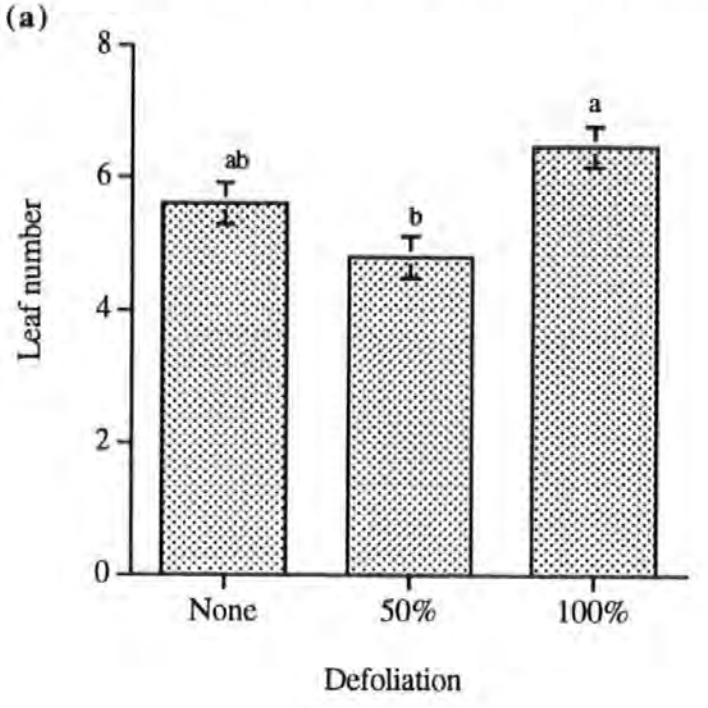
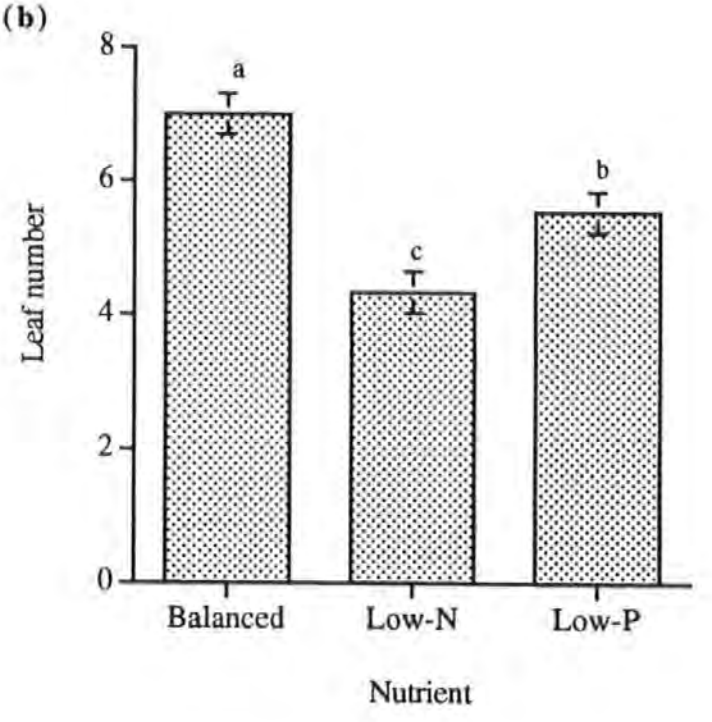


Figure 7.6 Mean number of new leaves produced over the experiment at (a) three levels of defoliation and (b) three nutrient regimes. Vertical bars represent twice the SE of the mean





A comparison between the number of leaves senesced and the number of new leaves produced in the combined undefoliated and 50% defoliation data revealed that mean senesced leaf number was higher (P= 0.042) than mean new leaf number, but the difference was only one leaf. This suggests that leaf senescence and new leaf production are basically in equilibrium.

As a general measure of plant vigour at the end of the recovery period, absolute plant mass, was compared using final plant fresh-weight. There were no significant differences in final root fresh-weight as a result of either defoliation or nutrient treatments. Similarly, there was no significant difference in shoot fresh-weight as a result of defoliation but the low-N regime reduced (P< 0.001) shoot fresh-weight by 41% (Fig 7.7). Defoliation did not affect final shoot fresh-weight but it did affect leaf structure. The 100% defoliation treatment reduced (P< 0.001) final leaf number compared to the 0 and 50% treatments (Fig 7.8). Therefore, regrowth leaves after the 100% defoliation treatment were larger and/or heavier (i.e. thicker), suggesting greater cell division and/or greater cell extension. Also, a comparison between the mean leaf number at the start of the experiment with the final leaf number of the 0 and 50% defoliation treatments revealed no significant differences.

Clonal growth was very limited with only 13 out of 45 replicates having initiated clones and a summary of the number of plants with clonal growth is presented in Table 7.3. Unfortunately the level of replication precludes the use of chi-square frequency analysis, therefore it was not possible to identify any significant differences in clonal growth as a result of any of the treatments. However, it can be seen (Table 7.3) that only the 100% defoliation/balanced nutrient treatment produced clonal growth in all replicates.

Table 7.3 Number of plants with clonal growth at three levels of defoliation and three nutrient regimes.

Defoliation	Balanced	Low-N	Low-P	Total
None	0	2	2	4
50%	2	0	0	2
100%	5	1	1	7
Total	7	3	3	**13**

Figure 7.7 Mean final shoot fresh weight (g) under three nutrient regimes. Vertical bars represent twice the SE of the mean

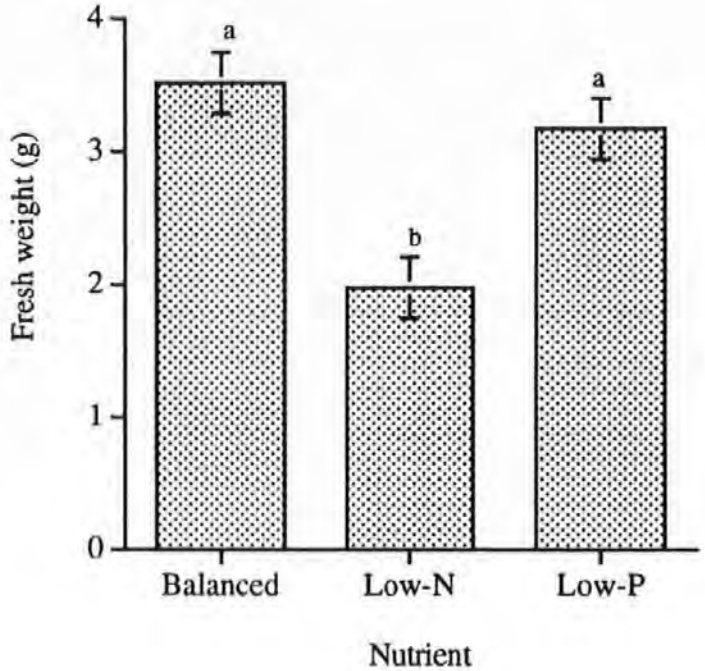


Figure 7.8 Mean final leaf number (log transformed) at three defoliation levels. Vertical bars represent twice the SE of the mean

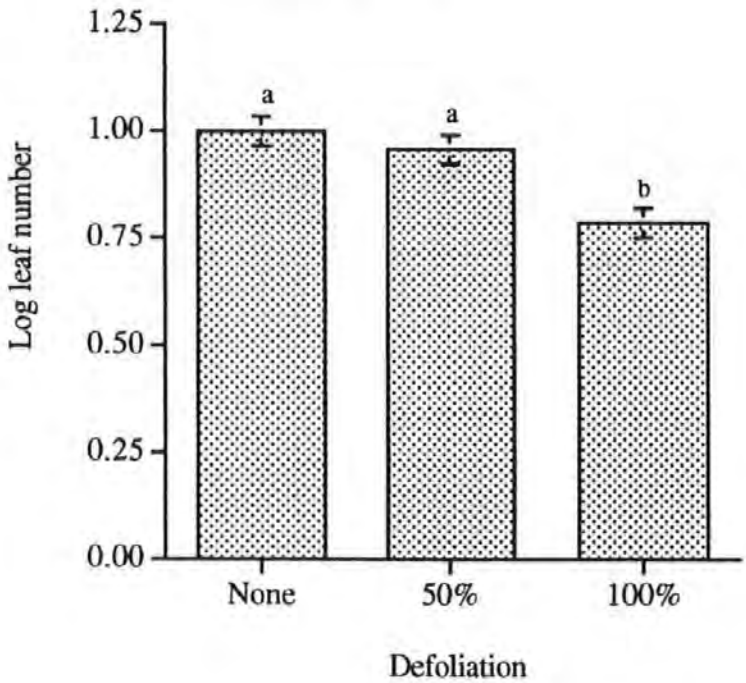


Figure 7.9 Comparative content of sucrose, fructan and starch in roots and shoots. Vertical bars represent twice the SE of the nean

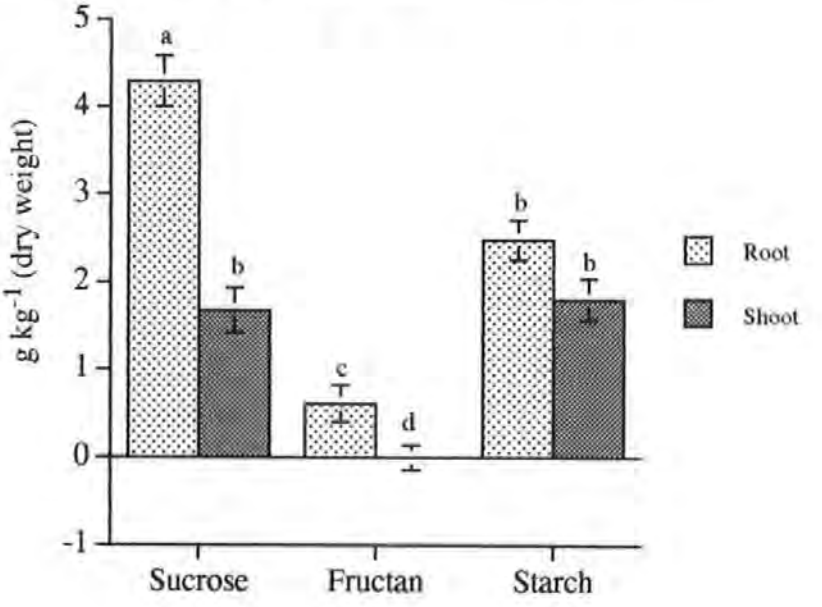
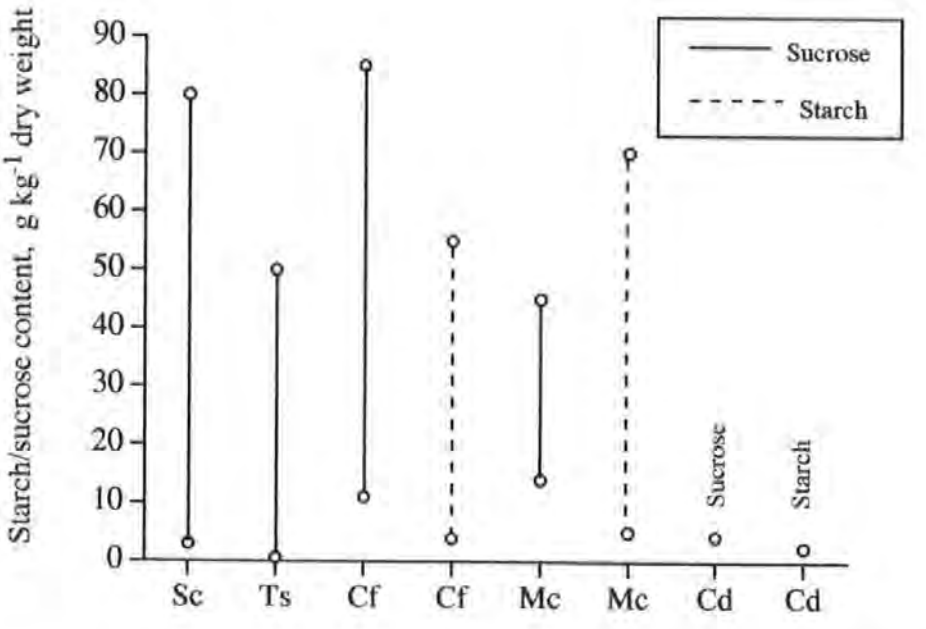


Figure 7.10 Range of sucrose and starch content from field samples of some grassland species which have sucrose as principal or second reserve carbohydrate (Brocklebank & Hendry, 1989), together with *Cirsium dissectum* data from this experiment



Key to species: Sc - *Scabiosa columbaria* Ts - *Teucrium scorodonia* Cf - *Carex flacca* Mc - *Molinia caerulea* Cd - *Cirsium dissectum*

An analysis of the starch, sucrose and fructan content of both roots and shoots revealed that root-sucrose represented the major carbohydrate fraction and fructose the smallest fraction (Fig 7.9). The amount of root sucrose was significantly ($P< 0.001$) greater than the amount of shoot sucrose. The amount of shoot sucrose was not significantly different to the amount of either root or shoot starch. Fructan comprised the smallest carbohydrate reserve fraction in the roots and was absent from the shoot. It would appear, from the results of this experiment, that sucrose is the principal carbohydrate reserve with starch as a second reserve. Neither defoliation nor nutrient had any significant effect on any of the carbohydrate component levels in either root or shoot. However, the sucrose and starch levels, detected in this experiment, seem to be at the low end of the amounts found by Brocklebank and Hendry (1989) in field samples for other grassland species (Fig 7.10).

7.8 DISCUSSION

The results demonstrate that *C. dissectum* is able to compensate fully for a single defoliation event, exemplified by total fresh-weight gain over the experiment and final root to shoot ratios, neither of which were significantly affected by defoliation. However, both of these parameters are more sensitive to low-N rather than low-P. Therefore *C. dissectum* conforms to the pattern of commitment to certain allometric relationships demonstrated by Oesterheld (1992), where R:S ratios of defoliated plants had recovered to the same ratio as control plants by the end of the 53-day recovery period (Oesterheld allowed 42 days recovery). The main mechanism for compensation was differential root and shoot RGRs. Defoliated plants exhibited an increase in shoot RGR with a corresponding decrease in root RGR compared to control plants which maintained equal root and shoot RGRs. However, the degree of compensatory regrowth of shoots is largely dependent on the availability of an adequate supply of nitrogen, but unlike the findings of Thornton (1991), this experiment did not demonstrate any interaction between the effects of defoliation and nutrient supply.

There are also clear differences in the patterns of leaf emergence and senescence as a result of partial defoliation and total defoliation. With partial defoliation, the remaining damaged leaves continue to function with no apparent increase in senescence (compared with undefoliated plants) but new leaf production is reduced compared with total defoliation. This suggests that resources for compensatory regrowth originate from current photosynthate rather than reserves, an observation supported by Chapin (1990). Also, there was no reduction in new leaf production in partly defoliated plants compared with undefoliated plants. This may be due to an increase in the photosynthetic rate of the remaining damaged leaves, a response which has been well documented (Crawley, 1983; Crawley, 1997; Trumble, Kolodny-Hirsch, and Ting, 1993).

After 53 days there were no differences in plant mass, as a result of defoliation. However, the 100% defoliation treatment had altered leaf structure resulting in fewer but larger and/or thicker leaves. It has been demonstrated that severe defoliation results in an increase in leaf area ratio (leaf area per unit weight) (Li, Kemp, and Hodgson, 1997; Prins, *et al.*, 1989), which suggests that the regrowth leaves in this experiment were more likely to be larger rather than thicker. Also, Thornton (1991) proposed that thin leaves offer advantages to the plant in being able to reduce resource expenditure when replacing lost leaf area for carbon assimilation. (It had not been possible to measure leaf area due to the requirement for immediate freezing of excised leaves to prevent enzymatic changes in the composition of carbohydrates).

The final leaf number of both the undefoliated and 50% defoliated plants was the same as the start of the experiment. This suggests that, in the vegetative phase, *C. dissectum* maintains a fixed number of leaves providing optimal photosynthetic leaf area and this would largely govern maximum rosette size. In prostrate rosette growth form, light foraging is essentially on a single horizontal plane and is therefore a function of circular geometry, where the maximum number of leaves in a rosette (without overlapping) is a direct function of the leaf width to length ratio described by equation 1.

Equation 1: $$LN_{max} = \frac{360}{2(\tan^{-1}\frac{w}{l})}$$

where LN_{max} is the maximum leaf number, w is the width and l is the length. Botanical texts (Butcher, 1961) and general observations in the field during this study indicate that the width to length ratio of *C. dissectum* is approximately 0.3. When this value is used in equation 1 this results in a LN_{max} of approximately 11, which corresponds with the starting and final leaf numbers per plant in this experiment, with the exception of the 100% defoliation treatment. Once a rosette has 11 leaves, subsequent emerging leaves will eventually shade existing leaves. At this point the orientation of an emerging leaf would be important in terms of the age of the leaf it shades, as maximum photosynthetic efficiency would be gained by progressively shading the oldest, least photosynthetically efficient, leaf (which may have already started to senesce). In this experiment, plants which had been subjected to complete defoliation did not reach the optimum eleven leaf equilibrium stage after 53 days, with only six leaves per plant.

C. dissectum was able to recover from defoliation without any loss in plant mass in less than eight weeks, which is sufficient time for to recover from early summer grazing before the onset of winter. This may be an important mechanism in maintaining fecundity by maximising potential flowering and seed production in the following season. It has been suggested that plant size is considered to be positively correlated with seed production (Crawley, 1997) and in biennials, rosette size is a good predictor of seed output (Chapin, *et al.*, 1990). Also, in perennials and facultative biennials, there is often a size threshold to flowering (Weaver and Cavers, 1980; Wesselingh, 1995).

From the phenological profile of *C. dissectum,* it was originally suggested that fructan might be the major storage carbohydrate. The results of the carbohydrate analysis clearly indicate that this is not the case. The high proportion of sucrose, relative to starch and fructan, detected in this analysis is not particularly unusual in itself as sucrose is regarded as the principal sugar of vascular plants and found throughout plants, often in high concentrations

(Lewis, 1984). However, what is unusual is the high proportion detected in the roots, as sucrose normally accumulates in shoot tissue (Brocklebank and Hendry, 1989). Some exceptions to this are the bulbils of *Ranunculus ficaria,* the large tap root of *Scabiosa columbaria* (a species with little or no starch or fructan) (Brocklebank and Hendry, 1989) and species with developed storage organs such as carrot, red beet and sugar beet (ap Rees, 1984). In most plants sucrose is the form in which carbohydrate is transported from the sites of synthesis to the sites of utilisation and storage (Duffus and Duffus, 1984). By virtue of its non-reducing nature, sucrose can be moved around plants and stored without being readily metabolised until required (Lewis, 1984). Starch appeared to be the secondary storage compound. Roots and shoots contained similar concentrations which is also unusual, as the principal site of starch storage is in root tissue (Brocklebank and Hendry, 1989). However, overall starch levels were low. Sucrose is considered to be the primary substrate for starch synthesis, but the mechanisms controlling the synthesis of starch are poorly understood (Duffus and Duffus, 1984). In the field, the period of maximum accumulation of starch is summer or autumn, whereas with sucrose the period of minimum accumulation is summer. The conditions of this experiment are somewhat artificial, with plants germinating from seed and growing in the equivalent of seven months of summer. The fact that sucrose content still exceeded starch content after what could be considered a 'long summer' reinforces the conclusion that sucrose is the major storage carbohydrate. However, a more detailed study of *C. dissectum* in the field is required to confirm this conclusion. Neither defoliation nor nutrient availability had any effect on the content or proportions of sucrose, fructan and starch. Although plants at the 50% defoliation treatment may have replaced part or all of the lost leaf area from current photosynthate, the 100% defoliated plants would have certainly had to utilise stored reserves. Therefore, it can be assumed that either the 53-day recovery period is more than adequate to replace any depleted reserves, or the reserves utilised were small in relation to the available reserves. It has been demonstrated that the reserves of *Lolium perenne* decline rapidly after severe defoliation and are restored after three weeks of summer regrowth (Grant, Barthram, and Torvell, 1981). Also, the inherently high root:shoot ratio in *C. dissectum* means that, typically, more than 70% of the total plant mass is root tissue. Therefore, even in the event of total defoliation,

this only represents the loss of a small fraction of the plant biomass and would conversely only require a small proportion of the root carbohydrate reserves to initiate new leaves which would quickly become self sufficient in carbohydrates from photosynthates.

7.9 CONCLUSIONS

This experiment has shown that *C. dissectum* is reasonably robust in its ability to withstand a major disturbance in the form of defoliation. The species has the potential for full compensatory regrowth under suitable conditions in a time period of less than eight weeks. This is achieved by an alteration in the differential root and shoot RGRs. Within this recovery time span, only severely defoliated plants show any structural differences, where the optimum rosette leaf number is reduced as a result of fewer but larger leaves. Overall plant mass is quickly restored, which may be important in terms of potential fecundity. However, the above mechanisms rely on an adequate supply of nitrogen but are not particularly sensitive to low levels of phosphorus. The latter is known to be limited in the M24 community, particularly in summer. This may be, in part, as a consequence of the species being low-P adapted as detailed in Chapter 5. The recovery from defoliation does not deplete the plant's carbohydrate reserves. This will be important in terms of its ability to produce early season growth and to accumulate stores to enable flowering and seed production in the following season (Schulze, 1982).

All of the above may influence relative competitive ability. This however also depends on the differential responses of other species in the community to defoliation, along with their responses to nutrient availability (Berendse, 1985) and any interactions between defoliation and nutrient availability. The only other M24 key species which has been studied in its response to defoliation is *Molinia caerulea.* Work by Thornton (1991) and Torvell, Common and Grant (1988) has shown that *M. caerulea* is highly sensitive to defoliation, because new leaf production is at the expense of overwintering reserves in the basal internodes and the priority for carbon partitioning may also be at the expense of overwintering root dry matter. In addition, Thornton (1991) found that defoliation adversely

affected root dry weight and root:shoot ratios, particularly at low N. This suggests that if both species were grazed (or cut) equally on a regular annual cycle it would suppress *M. caerulea* but favour *C. dissectum.* This effect has been observed at Witheridge Moor Tractor Field site, where areas regularly cut for hay have large dense cover patches of *C. dissectum* and *M. caerulea* is vitually absent. In contrast, an adjacent uncut area in a corner of the field is dominated by *M. caerulea* and *C. dissectum* is present as a few scattered individual plants.

The partitioning and proportions of the major storage carbohydrates require more detailed analysis and investigation in the field, as both content and fractions fluctuate at different times of the year. If sucrose is the major storage carbohydrate, greater concentrations than those detected in this experiment should be present during winter (i.e. January to March). The ecological significance or particular advantage gained from storing one type of carbohydrate rather than another has not yet been demonstrated (Brocklebank and Hendry, 1989; Hendry, 1987). However, carbohydrate reserves are particularly important in herbaceous perennials for early season growth and initiation of flowering, and may also influence the degree of clonal growth.

The above conclusions invite speculation that the accumulation of sucrose in the roots of *C. dissectum* could have several advantages. It could be an adaptation evolved to tolerate defoliation and protect reserves. During the vegetative phase it would provide a highly mobile carbohydrate reserve capable of supporting clonal growth when some size or storage threshold was reached. Most of the M24 field sites in this study have some summer grazing or cutting regimes. Therefore carbohydrate reserves of *C. dissectum* defoliated plants will be depleted during summer to some extent. The lost reserves would have to be replaced during autumn and winter to provide sufficient reserves to support early growth and flowering in the following season. If overwintering accumulated reserves were below some threshold to initiate flowering in the subsequent spring, they would instead be used to support vegetative and clonal growth for the rest of that season.

These hypotheses will require a much more detailed study of carbohydrate metabolism and the reproductive life cycle under field conditions. Specific experiments would need to establish whether reserves increase during winter and that there is a positive correlation between spring carbohydrate reserves and summer flowering. Also, the relationship between defoliation, carbohydate reserves and clonal propagation needs to be explored to establish the specific physiological mechanisms that initiate or stimulate clonal growth and whether grazing/cutting (and the timing of such operations) affect a switch from sexual to vegetative reproduction.

8.0 CONCLUSIONS, IMPLICATIONS AND FUTURE WORK

The original rationale for selecting *C. dissectum* for detailed study was its apparently narrow ecological niche. The species is only known to occur in a total of ten plant community associations and is a key indicator species in only one plant community, the *Cirsio-Molinietum* M24. This made the species a useful indicator of the plant/environment factors present in the M24 habitat. A the beginning of this study very little was known about the physiology or ecology of *C. dissectum*. Field observations and experiments during this study have established its physiological responses to various stresses and disturbance, along with a positive classification of functional type. Understanding its autecology has provided some insight into the community dynamics of the M24 ecosystem and has also provided previously unpublished comparative ecological data on a relatively scarce and declining species.

8.1 THE MICRO-SITE ENVIRONMENT OF *C. DISSECTUM* HABITAT

The first aim was a characterisation of the main environmental conditions at the very localised scale where the species is present. This revealed that M24 communities were confined to a well defined narrow range of environmental parameters. Soil water content is relatively high, often seasonally waterlogged and does not appear to fluctuate to any great degree throughout the year. Low phosphorus (P) availability is a particularly growth-limiting environmental variable but nitrogen availability is also below optimal levels for growth. Soil pH is slightly acid to neutral and not limiting to mineral nutrient availability. However, the Devon sites appear to have lower pH levels compared with sites surveyed by Wheeler and Shaw (1987). It can be concluded that soil water content is the main factor controlling the availability of two macro mineral nutrients. The absence of soil moisture fluctuation and regular wetting/drying cycles inhibits microbial activity. This results in low rates of P mineralisation and low levels of aerobic digestion of organic material reducing nitrogen mineralisation. Also, periods of waterlogging promote anaerobic activity and denitrification, reducing the amount of nitrate available to plants. The inhibiting effect of

ground water level on nitrogen and phosphorus availability has been demonstrated on fen meadows in the Netherlands (Van Der Hoek and Braakhekke, 1998). The timing and duration of waterlogging will also be an important environmental variable. If waterlogging were to extend beyond winter and early spring into the main spring/summer growth season, the ability of *C. dissectum* to tolerate waterlogging and periods of submergence during the active growth phase would be an important attribute which needs to be quantified. The current climatic trends seem to suggest higher spring and summer rainfall which may increase the degree and duration of waterlogging on M24 sites. This may lead to a change in the community assembly favouring an increase in wetland species and altering the M24 to a *Juncus subnodulosus-Cirsium palustre* fen meadow (M22) where the frequency and abundance of *C. dissectum* would be considerably reduced.

The role of mycorrhizae has only been briefly mentioned in this study. In soils of low nutrient status mycorrhizae play an important part in plant nutrient acquisition, particularly in the absorption of P. The enhancing effect of mycorrhizae can increase the uptake rate of P (per unit root length) 2 - 3 times that of non mycorrhizal plants (Tinker, Jones, and Durall, 1992). It has been shown that field specimens of *C. dissectum* are infected with mycorrhizae. Therefore the role of mycorrhizae in relation to mineral nutrition in *C. dissectum* and how this effects relative competitive ability compared with other M24 species, is an area of study which warrants detailed investigation. Although it has been established that extractable soil P is low on M24 sites, there is a large reserve of soil P. Total soil P is in the order of 545 mg kg^{-1} and the largest proportion of this is organic forms of P, almost twice the inorganic fraction (Goodwin, *et al.*, 1998). It has been demonstrated that some plants can modify their soil environment and mobilise organically bound P by root exudates (Helal and Sauerbeck, 1991; Tarafdar and Claassen, 1988). If *C. dissectum* has the capacity to mobilise organic soil P, then many of the assumptions relating to growth in a low P environment may no longer be valid. Therefore the ability of *C. dissectum* to access these forms of P, relative to both M24 and non-M24 species, would be a major determinant of relative competitive ability as P availability would not be as limiting to *C. dissectum* in what is normally deemed a low P environment to many other plants. A complete understanding of

P uptake and metabolism in *C. dissectum* would therefore require further research into the importance of organic soil P in the mineral nutrition of this species. It is suggested that areas of further research should include more detailed investigation of the mineralisation of nutrients, microbial activity and rates of organic decomposition on these sites. Specifically, nitrogen cycling and denitrification are poorly understood in this particular environment.

The narrow range of environmental and edaphic parameters is clearly a major factor in determining the distribution of *C. dissectum* and the M24 community, representing a narrow transition habitat between the drier grassland/heathland communities and valley bog wetland vegetation. The unique combination of geology, hydrology and soil type controls the soil water status of these sites, which in turn directly influences the soil pH and nutrient availability. A common feature of fen meadows is a sloping aspect, creating a gradient of increasing soil water content down the slope. This results in a zonation of plant communities associated with the soil water gradient. The very nature of the M24 habitat, often a relatively narrow intermediate zone between relatively dry and permanently waterlogged soils, makes it particularly vulnerable to any increase or decrease in soil water status. Therefore the existence of this community, or the potential for it to exist, is dependent on this unique combination of environmental variables and a very specific soil water status. Similarly, within any particular fen meadow site, where all other variables are relatively uniform, the distribution and maximum potential size of a M24 community will largely be determined by the microsite soil water status.

8.2 EXPERIMENTALLY DERIVED PHYSIOLOGICAL CHARACTERISATION OF *C. DISSECTUM*

The second and main aim of this study was to examine various physiological characteristics of *C. dissectum* to establish functional type, describe its ecological niche and identify any particular adaptations to the habitat environmental conditions. This was achieved by a series of controlled experiments measuring the requirement for water, light and nitrogen, tolerance of stress (phosphorus and dehydration) and tolerance of disturbance (defoliation). The aims

and objectives of the four experimental chapters (3, 5, 6 and 7) have largely been satisfied and provide a clear characterisation of the major resource requirements (i.e. water, light and nutrients) along with its ability to tolerate varying degrees of stress and disturbance. Key characteristics observed in all the experiments were its inherent low growth rate and low morphological plasticity which are uncoupled from resource availability. *C. dissectum* consistently maintains a high root to shoot ratio, where the priority of phytomass allocation is primarily to root production. This pattern appears constant throughout the vegetative phase, where root:shoot ratio increases with increasing plant maturity. However, this pattern may alter with the onset of vegetative reproduction and/or flower initiation. The reproductive life cycle of *C. dissectum* was outside the remit this study and it is suggested that this should be the main priority of any further investigation into the ecology of this species.

Water requirement of *C. dissectum* is relatively high but this is not due to any particular inefficiency in its use of water or any high degree of sensitivity to water stress. It has the capacity to survive a drought event and can recover from a period of prolonged dehydration. However, such an event would substantially reduce the plant's growth performance within that particular growth season and could reduce clonal growth and/or inhibit flowering in the subsequent growth season. Soil water availability does not appear to be major environmental factor controlling plant metabolism *per se* but high soil water content is important as a secondary factor in the uptake of mineral nutrients, which are present in very low concentrations in the soil water. It can be concluded that the relative high water uptake in *C. dissectum* appears to be a mechanistic adaptation to a low nutrient/high soil water environment.

Stress tolerance in *C. dissectum* focused in detail on phosphorus availability, a macro nutrient which has been demonstrated to be particularly low in M24 habitats. The two experiments in Chapter 5 revealed that *C. dissectum* possesses a suite of functional attributes which are consistent with an evolved physiological adaptation to a low phosphorus environment. It was concluded that *C. dissectum* has a relative competitive advantage as it is

better adapted to a low phosphorus environment than rapidly growing competitive species. Two other areas of stress tolerance were examined at a more superficial level. Shade tolerance was briefly examined over a relatively short experimental period and results indicated that *C. dissectum* was tolerant of shade. However, the longer term effects of shade need to be investigated in more detail, using a more sophisticated experimental regime, which would take account of not only the effects of reduced PAR but also replicating natural shade conditions and the changes in spectral light quality (Stuefer and Huber, 1998). Similarly, a cursory examination of nitrogen availability, included in the water metabolism experiment, indicated that *C. dissectum* was tolerant of reduced levels of nitrogen but extreme limits of low nitrogen tolerance were not quantified. Although nitrogen is generally below optimum growth levels in M24 habitats, it is not considered particularly limiting in comparison with the low phosphorus availability. Of all the stresses examined by the experiments in this study, low phosphorus was the main stress to which *C. dissectum* exhibited the greatest degree of tolerance and the species is well adapted to a low phosphorus environment. This is consistent with the findings of Chapin (1983), that plants of low nutrient environments are adapted the most limiting nutrient element.

A final experiment examined the ability of *C. dissectum* to tolerate disturbance in the form of defoliation. This demonstrated that the species can fully recover from partial or total defoliation in a relatively short time period without any loss in plant mass or depletion of carbohydrate reserves. Recovery was dependent on adequate nitrogen availability but was not inhibited by low phosphorus availability, a further confirmation of low phosphorus adaptation. Direct comparisons of recovery ability with other similar herbaceous species was not possible. However, a comparison with published experiments of defoliation on *M. caerulea,* the dominant species in the M24 community, suggested that *C. dissectum* was less sensitive to defoliation and regular grazing of both species would favour *C. dissectum.*

Growing plants under artificial controlled environment hydroponic conditions can be criticised as the response of plants under natural field conditions may be different. However, it is argued that the underlying requirement to control experimental variables

would make many of the treatments (and treatment interactions) applied in these experiments impossible to recreate in the field. Also, accurately measuring plant responses (e.g. total fresh weight gain, root mass, water uptake etc.) in the field would be extremely difficult and experimentally impractical. Therefore, although these experiments may not reflect exact field responses, they do provide baseline measurements of absolute comparative physiological responses to specific environmental parameters. It is envisaged that these results would form the basis for developing further hypotheses to test predictions of likely outcomes from a series of experiments in which field conditions were manipulated.

The experimental part of this study has been able to place *C. dissectum* generally in the 'stress-tolerator' functional category within the ordination triangle of Grime's C-S-R theoretical model (Grime, 1979). No direct measure of relative competitive ability for *C. dissectum* has been attempted in this study as the ability to predict competitive outcomes between different species is quite limited for higher plants and requires complex simulation models (Grace, 1990). The ability of *C. dissectum* to function closer to its optimum growth under various stresses (particularly low phosphorus) and its ability to recover rapidly from defoliation indirectly implies a comparatively high relative competitive ability under the unfavourable conditions present on the M24 habitat. Thus *C. dissectum* may be more likely to fall within the intermediate functional category of 'stress-tolerant competitor'. The majority of the physiological, morphological and botanical characteristics of *C. dissectum* conform to the predicted attributes of the 'stress-tolerator' or 'stress-tolerant competitor' functional type as proposed by Grime's C-S-R theory and generally confirms the results of the prediction model used in Chapter 2. The conclusions from the experimental work in this study appear to provide a reasonable validation of the C-S-R theory of evolved functional adaptive strategies in plants.

8.3 THE ECOLOGICAL NICHE OF *C. DISSECTUM*

The ecological niche of *C. dissectum* is relatively narrow and its physiological and botanical characteristics clearly reflect several adaptations to its particular habitat environment. Its

degree of specialism can be compared with the other seven species of the *Cirsium* genus found in the British Isles (see Chapter 1, section 1.4). The functional types of the three most common species, *C. arvense, C. palustre* and *C. vulgare* , are 'competitor', 'C-S-R generalist' and 'competitive-ruderal' respectively (Grime, *et al.*, 1988). In common with *C. dissectum,* the other four species, *C. heterophyllum, C. eriophorum, C. acaulon* and *C. tuberosum* all appear to fall into the 'stress-tolerant' functional type category.

C. heterophyllum, botanically very similar to *C. dissectum,* is only found in upland meadows and grasslands in northern parts of Britain and is virtually absent from Ireland. *C. acaulon* and *C. eriophorum* have a southern British distribution (both absent from Ireland) with a particular preference for relatively well drained dry soils, usually calcareous (Pigott, 1968; Toffs, 1999). They differ in their particular environmental requirements in that *C. acaulon* requires high summer temperatures in excess of 20 °C, whereas *C. eriophorum* is nitrophilous. The rarest of the species is *C. tuberosum* which is only found in a few locations in Wiltshire and South Wales. It is also confined to dry calcareous grasslands and is a long lived perennial but only reproduces from seed. It would seem that each of the five 'stress-tolerant' *Cirsium* species have evolved unique characteristics, following distinct pathways of specialism into very specific ecological niches. It has been demonstrated that, in British herbaceous seed plants, distribution range is positively correlated with niche breadth (expressed as diversity of habitats exploited) and habitat availability (Thompson, Gaston, and Band, 1999; Thompson, Hodgson, and Gaston, 1998). In these studies, distribution range was measured at the national level (the number of 10 x 10 km grid squares occupied by a species) and local level (the number of 1 km^2 grid squares occupied). The comparative rarity of *C. dissectum* , along with the other four stress-tolerant species, is largely as a result of the degree of relative specialisation and narrow niche breadth, where distribution will primarily be limited by the availability of suitable habitat which constitutes the ecological niche. Therefore the decline in *C. dissectum* (Chapter 1) can be directly linked to the overall national decline in Rhôs pasture habitat and, at a local level, decline in area of Culm grasslands in Devon, a view promoted by Thompson and Hodgson (1996).

An interesting area of speculation and possible future study is the phylogeny of the *Cirsium* genus. Did the five stress-tolerant species evolve through niche specialisation from one or more of the three competitive species or vice versa ? How genetically different or distant are the individual British species ? The ability of *C. palustre* to form hybrids with all but one of the species suggests it could the common ancestor. Also the high degree of interspecific hybridisation raises the possibility of a viable hybrid, which if self-compatible, could result in a new species. This could be examined by cross-breeding trials. The mutually exclusive north/south distribution of *C. heterophyllum* and *C. dissectum* would also provide an interesting study. As there are only very minor botanical differences between the two species, are the interspecific differences a result of genotype or just an expression of phenotype extremes or as a result of evolved ecotypes ? At an intraspecific level, an preliminary study of selected *C. dissectum* populations in mainland Britain generally indicated low genetic diversity (Kay and John, 1994; Kay and John, 1995). A detailed examination of the within and between population genetic diversity of *C. dissectum* would provide an indication of the degree of regeneration from seed, dispersal/colonisation ability and existence of regional ecotypes. This information would have important implications for management. In particular, the existence of ecotypes would support the argument for using seeds of local provenance in any habitat reconstruction schemes. Transplant experiments, cross-breeding and modern molecular genetic techniques should be able to answer most of these questions.

8.4 THE IMPLICATIONS FOR MANAGEMENT

The final aim of this study was to identify the main community variables to provide a basis for predicting responses to management. Based on the assumption that the desired plant community is the *Cirsio-Molinietum* M24, management of fen meadows will fall into three general categories of plant community manipulation. The first category is maintaining existing communities, secondly restoration of an M24 community where the plant community has altered due to neglect, natural succession or altered site conditions and finally reconstruction of an M24 community from an agriculturally 'improved' site.

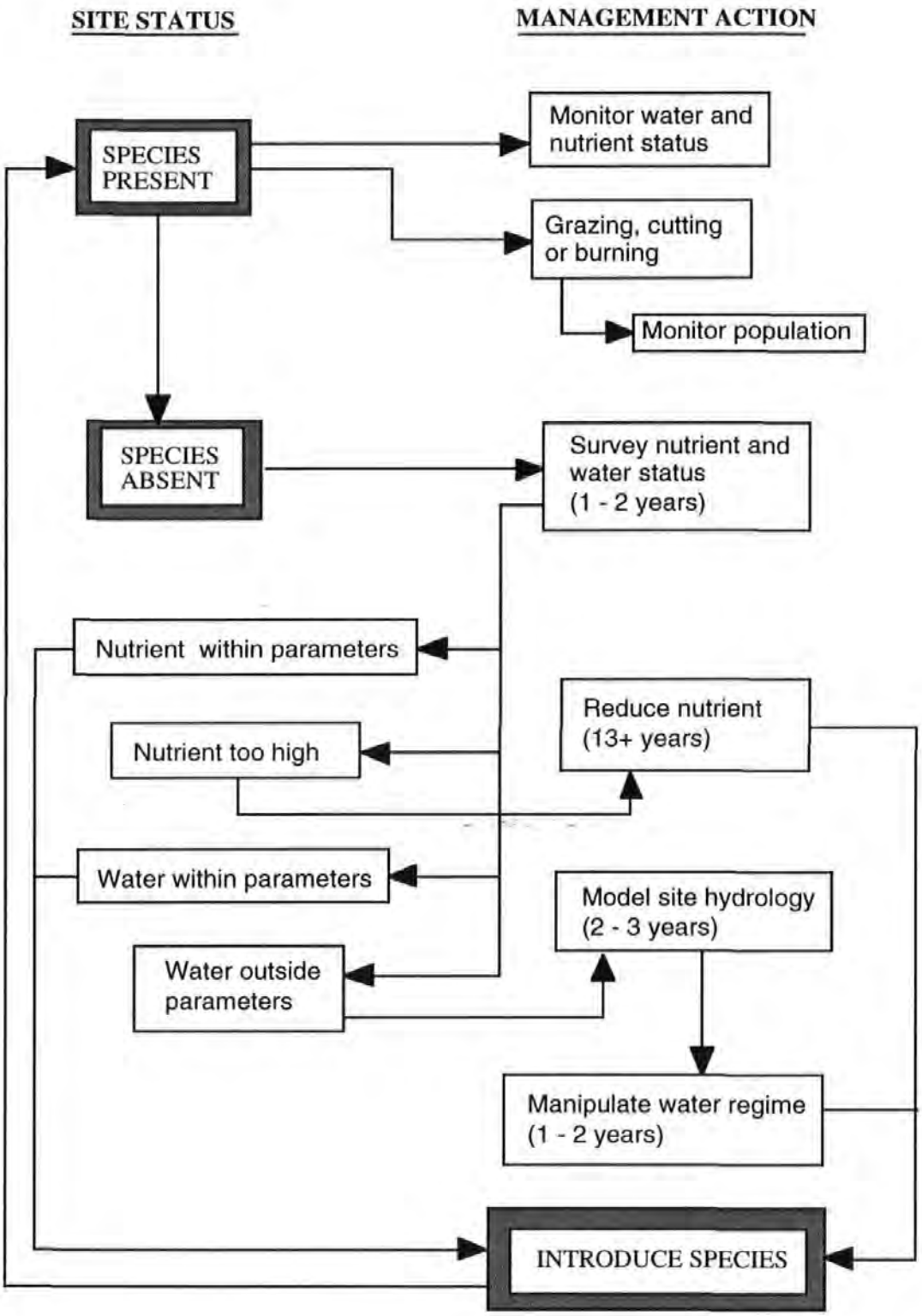
The results of this study have clearly identified that the potential existence of the M24 community depends primarily on a very specific soil water and phosphorus status. Changes in the vegetation composition can be driven by disturbance (i.e. grazing, cutting and burning) but the edaphic conditions are the key determining state variable in the community model (Fig 1.5, Chapt. 1).

In the case of maintaining an existing community, it is recommended that the main focus of management should be on regular monitoring of soil water and nutrient status to detect any changes in status which could be potentially threatening. Similarly, adjoining land should be monitored for any changes in use or management which could alter hydrology or cause increased nutrient run-off and impact on the target site. Restoring a neglected site would require an detailed evaluation of the soil water and nutrient status to determine whether the decline or loss of an M24 community was the result of a change in either or both of the key edaphic parameters. Reconstructing an M24 community from an agriculturally improved site will present the greatest challenge. The 'improvements' carried out in the past are likely to have included at least one or more operations involving drainage, ploughing, re-seeding and fertiliser application. Elevated phosphorus levels will present the greatest long term problem as it has been predicted that it would take a minimum of 13 years to eliminate enhanced phosphorus availability (Tallowin *et al.*, 1998). Also, the seeds of many M24 species would no longer exist in the seed bank as most of the grassland species have a short persistence in the soil (Thompson, Bakker, and Bekker, 1997). Therefore, community reconstruction from improved sites will require the re-introduction of many of the M24 species and will mean collecting suitable seeds, ideally of local provenance, and sowing. This creates a further level of complexity, as knowledge of the germination requirements of the individual species (e.g. light, temperature, humidity, etc.) would be a prerequisite to ensure establishment. Unpublished work carried out prior to this study (Ross and Williams), examining the germination requirements of three key M24 species, found that *C. dissectum* germinated rapidly under a wide range of conditions, *Molinia caerulea* had very specific and narrow requirements and *Carex panicea* seeds could not be made to germinate in the laboratory. This suggests that sowing may not be an option for some of the M24 species

and alternative methods of species introduction, such as turf transplants, may have to be developed.

In both neglected and improved sites, it may be necessary to manipulate the water regime and this will require modelling the specific site hydrology and the necessary infrastructure to control the water regime effectively (Gowing *et al.*, 1998). It is not proposed to discuss the various techniques involved in restoration ecology as this is outside the remit of this project. There are many specialist texts on restoration techniques and wet grassland management and restoration is comprehensively dealt with by several authors in Joyce and Wade (1998). However, a key management component of any conservation or reconstruction initiative must include a clear definition of the target plant community and long-term monitoring of the edaphic conditions and community composition. In the case of the M24 community, *C. dissectum* can be used as a key indicator of the effectiveness of any particular management initiative. This study has shown that it has an extremely specific and narrow range of edaphic requirements and the narrowest niche of all the M24 key species. Therefore, it is suggested that conservation or reconstruction projects should concentrate on the success and vigour of the *C. dissectum* population component within the community. If *C. dissectum* is thriving, then the main state parameters which control this community are being met. A simple schematic model illustrating the range of suggested management actions is detailed in Fig 8.1. In the case where *C. dissectum* is present, management consists of 1) routinely monitoring the edaphic conditions to detect any environmental change and 2) monitoring the *C. dissectum* population to gauge the effectiveness of management operations (i.e. grazing cutting etc.). Reconstruction or re-instatement of an M24 community on a neglected or agriculturally improved site or is a more complex process. If the nutrient status is too high, it will be necessary to reduce nutrient levels. Similarly, if the water status is outside optimum parameters, an understanding of the site hydrology will be a prerequisite to drainage manipulation. Both of these operations are long-term processes and, in the worst case scenario, could take a decade or longer to create the optimum edaphic parameters to support an M24 community.

Figure 8.1 A simple model of management actions required to maintain or introduce a *Cirsium dissectum* population.



Grazing, cutting and burning are well established methods of manipulating plant community composition, with the treatment variables being timing, severity and frequency. This study has only briefly touched on this subject area by demonstrating that *C. dissectum* is relatively tolerant of partial or total defoliation. Therefore, it can be assumed that, in general, the use of grazing or cutting as a management practice on M24 communities should not be detrimental to *C. dissectum* populations and may even be beneficial. Anecdotal observations from several sources suggest that cutting vegetation short in areas containing *C. dissectum* results in increased vigour and vegetative spread. Grazing and/or cutting will be important management operations for both maintaining existing M24 communities and improving neglected sites. Based on the limited information available on other key M24 species, this study has only been able to hypothesise that defoliation would alter the balance between *M. caerulea* and *C. dissectum* in favour of the latter. Differential response to defoliation of the various species in the M24 community needs to be fully quantified and can only be measured by large-scale field experiments. It is recommended that further research should be conducted to determine optimum grazing/cutting regimes for producing a clearly defined target of plant species community composition. It may also be worth including experimental comparisons between the traditional cattle grazing and the use of other domestic grazing animals such as sheep, goats and horses.

8.5 CONCLUSION

In common with many autecological studies, the results and conclusions of this study have provided an understanding of certain aspects of the ecophysiology of *C. dissectum* but have also highlighted other areas where knowledge of this species is still incomplete. The fundamental problem when trying to understand ecosystem function is that the totality of factors can never be assessed (Gigon, 1987). Determining causal factors of ecosystem or plant function is therefore focused on differentiating relatively more important factors from less important ones. The review of the previous authors work and the field data collected during this study provided a characterisation of the environment. As a result, specific key ecophysiological factors were identified and tested experimentally to determine their relative

importance to *C. dissectum*. The results identified soil water and nutrient (particularly P) as important factors which interact to create particular edaphic conditions and appear instrumental in determining the distribution of *C. dissectum*. The role of defoliation, in the form of cutting of grazing, would appear to be important in determining the balance between *C. dissectum* and potentially dominating species such as *M. caerulea*. Remaining gaps in the understanding of this species, which will be linked to the key edaphic factors, include reproductive life-cycle, root/soil interface dynamics, flooding tolerance and relative competitive ability. Other factors which may also play a role in ecosystem/plant function to greater or lesser degree include phylogeny, dispersal ability, invertebrate predation, pathogens, defence chemistry and frost tolerance.

This study has provided important baseline data on the absolute responses of *C. dissectum* to specific key environmental variables and a classification of its functional type. It is hoped that future research will be able to build on this information and the results of this study will be used to design large-scale field experiments to test predicted responses. In addition, some of the speculations derived from the results of the experimental work in this study will provide the basis to formulate further hypotheses for testing in the laboratory and field.

Appendix one

Allocating C-S-R plant functional types: a soft approach to a hard problem

For a full explanation of the method see .....

For NON-GRASSES, etc | ***Cirsium dissectum***

Fill in the red boxes: identifier (optional, above) and predictor values (required, below)

CanopyHeight	49	*(millimetres maximum)*
DryMatterContent	20	*(percent in fully-expanded leaves)*
FloweringPeriod	2	*(months in duration)*
LateralSpread	5	*(special six-point classification, see text)*
LeafDryWeight	847	*(mg per fully-expanded leaf)*
SpecificLeafArea	18	*(square mm per mg dry weight in fully-expanded leaves)*
FloweringStart	4	*(special six-point classification, see text)*

Predicted type is: SC/CSR *based upon the above information*

	C	S	R
with coordinates:	0	0	-1

The second and third pages display the intermediate calculations that led to this prediction

Processed input data

CanopyHeight	1	(now classified as 1-6)
DryMatterContent	4.5	(now square root of the original value)
FloweringPeriod	2	(as original value)
LateralSpread	5	(as original classification)
LeafDryWeight	9.74	(now natural log of original value, plus 3)
SpecificLeafArea	4.24	(now square root of the original value)
FloweringStart	4	(as original classification)

Regression predictions of raw C-S-R dimensions using processed input data

Raw C-dimension	3.014	('dominance index' units)
Raw S-dimension	-27.751	(PCA axis units)
Raw R-dimension	14.529	('ruderality index' units)

Raw C-S-R dimensions converted to raw decimal C-S-R coordinates

C	0.029	(decimal coordinate)
S	-0.225	(decimal coordinate)
R	-0.771	(decimal coordinate)

Correction of raw decimal C-S-R coordinates

(a) Adjusted for high outliers

C	0.029	(decimal coordinate)
S	-0.225	(decimal coordinate)
R	-0.771	(decimal coordinate)

(b) Adjusted for low outliers

C	0.029	(decimal coordinate)
S	-0.225	(decimal coordinate)
R	-0.771	(decimal coordinate)

(c) Coordinates rounded towards zero, with one decimal place

C	0.0	(decimal coordinate)
S	-0.2	(decimal coordinate)
R	-0.7	(decimal coordinate)

Appendix one (contd.)

Identification of closest valid combination of coordinates

Type	*C*	*S*	*R*	*Variance*
C	2	-2	-2	8.93
C/CR	1	-2	-1	4.33
C/SC	1	-1	-2	3.33
CR	0	-2	0	3.73
C/CSR	1	-1	-1	1.73
SC	0	0	-2	1.73
CR/CSR	0	-1	0	1.13
SC/CSR	0	0	-1	0.13
R/CR	-1	-2	1	7.13
CSR	0	0	0	0.53
S/SC	-1	1	-2	4.13
R/CSR	-1	-1	1	4.53
S/CSR	-1	1	-1	2.53
R	-2	-2	2	14.53
SR/CSR	-1	0	0	1.53
S	-2	2	-2	10.53
R/SR	-2	-1	1	7.53
S/SR	-2	1	-1	5.53
SR	-2	0	0	4.53

Minimum variance = 0.13
at position in list = 8

Mean departure 0.204

Predicted functional type

SC/CSR

coordinates

C	S	R
0	0	-1

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Awarding Body : Plymouth

Thesis By : ROSS John

Thesis Title : THE AUTECOLOGY OF THE MEADOW THISTLE (CIRSIUM DISSECTUM L. HILL) ON DEVON RHoS PASTURES, WITH PARTICULAR REFERENCE TO THE EFFECT OF MAJOR ENVIRONMENTAL VARIABLES ON THE POPULATION DYNAMICS