Eco-physiology of Primula farinosa Linn,: and some allied species

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Eco-Physiology of *Primula farinosa* Linn.

and some allied species

by

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Primula farinosa Linn. and its allies survived through the winter at Great Dun Fell Radar Station, Cumbria, January 1987.

The results in this thesis are entirely my own work and no part of this thesis has been submitted for any degree in this or any other University.

Dedicated to my parents and to my wife, Suchitra.

ABSTRACT

Ecological and physiological comparisons were made mainly between two populations of *Primula farinosa* Linn. from northern England and some of their closely related arctic-alpine species:- *P. frondosa, P. darialica, P. halleri, P. laurentiana, P. modesta, P. scotica, P. scandinavica,* and *P. stricta,* which have contrasting habitats and natural distribution.

The germination of primulas showed a negative or neutral response to the density of seeds sown. They also showed intrinsic seed-dormancy which can be overcome by chilling treatment. Most of the species tested had significantly higher percentage germination in a diurnally fluctuating temperature regime than at 15°C constant temperature.

The results from analyses of leaf characteristics showed significant variation between species studied. Significant correlations were found between chromosome numbers of the species studied and some leaf characteristics, e.g. cell size, stomatal index.

Plant growth analyses were investigated along an altitudinal gradient in the north of England. Both vegetative and reproductive growth was clearly affected by microclimate. The primulas showed more sensitive responses to drought than frost as regards to their survival. They responded to water stress by accumulating proline as well as increasing their total protein contents.

Photosynthesis measurements showed optimum temperature for O_2 evolution at warm temperatures of 20-25°C. The difference in physiological performances of the primulas is discussed in relation to their leaf characteristics, ploidy levels and habitats of origin.

This study demonstrates clearly that the two populations of *P. farinosa* differed in a number of morphological and physiological characteristics; some of which could make it possible for the different races to occupy different ecological habitats.

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ABBREVIATIONS

A	absorbance
ABA	abscisic acid
ANOVA	analysis of variance
A-S	August-September
BSA	bovine serum albumin
°C	degrees celsius
CAP	number of capsules
Ch	chapter
chl	chlorophyll
chl a	chlorophyll a
chl b	chlorophyll b
chl a/chl b	chlorophyll a per chlorophyll b
cm	centimetre
cm^2	square centimetre
cm^3	cubic centimetre
d	day(s)
DF, df	degrees of freedom
dm	decimetre
dw, DW	dry weight
EDTA	Ethylene Diamine Tetra-acetic Acid
ESH	Esh, County Durham
F	variance ratio
FAB	*Primula farinosa* (b)
FAC	*Primula farinosa* (c)
Fig., Figs.	figure, figures
FR	number of florets per plant
FRO	*Primula frondosa*

fw, FW	fresh weight
g	gram(s)
GDF	Great Dun Fell Radar Station, Cumbria
h	hour(s)
HAL	*Primula halleri*
HAR	Hartside Nursery Garden, Alston, Cumbria
J-A	July-August
J-J	June-July
l	litre
ln	logarithm to base e
LAD	leaf area duration
LAI	leaf area index
LAU	*Primula laurentiana*
LF	number of leaves per plant
LMgraph	light micrograph
LSD	least significant difference
m	metre
m	milli- ($10^{-3}\times$)
M	molar
M-J	May-June
min	minute
mg	milligram
mm	millimetre
mol	mole
n	nano- ($10^{-9}\times$)
N	normal, north
NaOH	sodium hydroxide
P	probability
PAR	photosynthetically active radiation
PFD	photon flux density

PROB.	probability
r	correlation coefficient
RLaGR	relative leaf area growth rate
RuBisCo	Ribulose Bisphosphate Carboxylase/oxygenase
ROS	number of rosettes per plant
s	second
SE	standard error of the mean
SCC	*Primula scotica*
sq	square
STT	*Primula stricta*
SUN	South Bents, Sunderland
TCA	trichloroacetic acid
Tris	Tris (hydroxymethyl)-aminomethane
TW	turgor weight
UV	ultraviolet
V	volt
v/v	volume per volume
W	watt(s)
W	west
WDF	Widdybank Fell, Upper Teesdale
wk	week(s)
wt	weight
μ	micro- ($10^{-6}\times$)

SIGNIFICANCE SYMBOLS

NS	non-significant, $P > 0.05$
*	significant at $P \leq 0.05$
**	significant at $P < 0.01$
***	significant at $P < 0.001$

CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

"Eco-Physiological studies of *Primula farinosa* Linn. and some allied species" was selected as the title for this thesis as a theme likely to improve our knowledge of the ecology of plants and of their physiological responses to their environment. It is generally known that the establishment of plant life on the earth's surface took place about 500 million years ago,and in order to survive and evolve since that time, each individual plant has needed to compete with its neighbours as well as adapt to its environment. The environment of an individual is continually changing throughout its lifetime, some of this change being cumulative and some cyclic (Billings, 1952). If an environmental factor becomes critical simultaneously with a critical stage in the life cycle of the plant, death of the individual or of many individuals in the local population may happen. The adaptation of populations to local climatic conditions ensures that the individual is unlikely to develop at an unfavourable time and so its dispersion then will be limited. Each geographical area, and habitat, poses its own particular problems to plant survival, and the flora of each area consists only of the species suitably adapted to survive these conditions. The successful plant populations are those which have evolved the most appropriate physiological mechanisms.

It is the aim of physiological plant ecology to explain the present day distributions of wild plants by measuring their physiological, morphological, and reproductive adaptations to environmental factors. The potential environmental tolerance ranges of plant taxa is also in need of investigation (Billings, 1985). It has been pointed out by Billings (1985) in his review of the historical development of physio-

logical plant ecology that "... We should continue the use of physiological ecology to help us toward a better understanding of the evolution of taxa at any level. We need more comparative studies of related species within a genus or within subgenera. ..." In the light of these suggestions, *P. farinosa* and its allies seem to serve well, as some of these show very close relationships with respect to both morphological and cytological features. In the past, some species have been determined as subspecies or varieties of *P. farinosa,* but all are currently given the rank of individual species (Wright-Smith & Fletcher, 1943). *P. farinosa* is the most widespread species in the genus (Wright-Smith & Fletcher, 1943); whereas some of its relatives are locally endemic species, e.g. *P. scotica* -growing naturally only in northern Scotland (Ritchie, 1954; 1955); *P. scandinavica* -confined to Scandinavia (Brunn, 1938); *P. laurentiana* -found in SubArctic regions of North America (Fernald, 1928); and *P. frondosa* -growing wild only in the Balkan mountains (Polunin, 1980).

Studies on ecological races have provided some information on morphological, phenological, and physiological variations between populations from different habitats (Heslop-Harrison, 1964; Holmgren, 1968). Species occupying diverse habitats are often genetically differentiated into ecologically-adapted races, or ecotypes (Turesson, 1922). Evidence in favour of this interpretation has been produced by Björkman & Holmgren (1963) who examined differences in the photosynthetic response to light intensity between ecotypes of *Solidago virgaurea* from shaded and exposed habitats. Subsequent studies have shown that similar differences exist amongst both species and races from habitats with contrasting light intensities (Björkman & Holmgren, 1966). Working with arctic and alpine populations of *Oxyria digyna,* Mooney & Billings (1961) concluded that some seeds in a population have a light requirement for germination whilst others do not; light requiring seeds being mostly found in alpine populations rather than in arctic ones. They also noted that the arctic plants had a higher photosynthetic rate and attained their maximum rate at lower temperatures than the alpine plants. The arctic plants also had higher respiration rates at all temperatures, but reached photosynthetic light

saturation at lower light intensities than the alpine plants. This concurs with a previous study (Mooney & Johnson, 1965), which compared the physiology of an alpine population of *Thalictrum alpinum* from California with that of an arctic population from Alaska and found that the temperature peak for the photosynthetic rate of the arctic population was some 5°C below that of the alpine population. *P. farinosa* and its allies provide an excellent opportunity to evaluate and compare interspecific and ecotypic variations in physiological ecological attributes.

Accordingly, the physiological ecology of seed germination was investigated in terms of pre-chilling requirement, duration of chilling and the effect of the cumulative density of the seed on the percentage germination. Leaf characteristics were analysed in conjuction with physiological ecological studies. The response of plants to environmental stress, e.g. water stress, was investigated by measuring the accumulation of the amino acid, proline, and the insoluble and soluble protein contents. The relationships between photosynthetic rate and both temperature and light intensity were investigated using an oxygen electrode in the gas phase. The methods of plant growth analysis were used to evaluate growth responses at different altitudes in the north of England.

1.2 General materials and methods

1.2.1 The biology of the plant material

Primula is one of the largest flowering plant genera, consisting of 538 species which are divided into 32 sections (Wright-Smith & Forrest, 1929). Its prominent character, "heterostyly," was first noted by Clusius in 1583 (van Dijk, 1943) and has interested many biologist since the publication of "The different forms of flowers on plants of the same species" by Darwin in 1877. Since then some species have been studied in relation to the genetics of heterostyly (e.g. Bateson & Gregory, 1905; Crosby, 1940; Bodmer, 1958).

1.2.1.1 First records of native *Primula* in Britain

Primula farinosa was named *Primula veris flora rubro* by Clusius, the plant specimens having been collected from "Harwood neere to Blackburne in Lancashire." This nomenclature was published in "The Herbal, or General Historie of Plantes" by John Gerard in 1597 (see Clarke, 1900; Heslop-Harrison, 1948). *P. scotica* was found by Mr.Gibb, of Inverness, on Holborn Head, near Thurso, in Caithness in 1819 (see Clarke, 1900; Wright-Smith & Fletcher, 1943) and the identity given by Hooker in 1821.

1.2.1.2 Taxonomy

The present research has been carried out mainly on *Primula farinosa* from two populations in the north of England, and the following allied species:- *P. frondosa, P. darialica, P. halleri, P. laurentiana, P. modesta, P. scotica, P. scandinavica,* and *P. stricta.* These members of the genus are all classified into the Section Farinosae, Subsection Eu-farinosae (Wright-Smith & Forrest, 1929). The history of the classification of this group of taxa has been a chequered one; some species have initially been determined as subspecies or varieties of *P. farinosa,* but all are currently given the rank of individual species by Wright-Smith & Fletcher (1943). The taxonomic treatment and nomenclature used in this thesis follows Wright-Smith & Fletcher (1943).

1.2.1.3 General Morphology

All of the species studied are perennial herbs with short rhizomes. The short life span of many of the species under garden conditions has been regarded as a result of poor development of the rhizome (Wright-Smith & Fletcher, 1943). The size of the plants varies from the moderate size of *P. frondosa, P. laurentiana,* and *P. farinosa* to the small size of *P. scotica.* There is much variation in leaf-shape from elliptic to oblanceolate and leaf size also varies (see Table 1.1); the leaf margin is, however, usually entire (except *P. farinosa* from upland sites which has

a denticulate margin).

Farina is found on the lower surface of the leaf and usually throughout the scape. It may be white or yellow and sometimes cannot be observed on older leaves (e.g. in *P. laurentiana, P. stricta*). The inflorescence is a simple umbel, carried upon a scape which varies in length. The number of florets varies from few to many. Corollas are lilac, mauve, or purple to violet. Both the usual heterostyly and also the unusual are found in this group (Wright-Smith & Fletcher, 1943). The capsule is cylindrical, ellipsoid or ovoid, and as long as the calyx or longer. Seeds vary in size, shape and surface pattern, but are, in general, about 0.5 mm in diameter, and ovoid to quadrate-angular.

1.2.1.4 Farina

Farina has been described as wax-like in some botanical literature (Oliver, 1895), but however, what we call farina is powdery and is secreted by glandular hairs (see Fig. 3.1 and 3.2), and its nature differs from the wax-like substances which are found on the surfaces of leaves, stems or fruits of many plants (Blasdale, 1947). Miller (1915) showed that farina is composed of flavone and related substances (see Blasdale, 1947). Flavone is insoluble in water but readily dissolves in most of the common organic solvents. Harborne (1968) found that 71% of species in the genus *Primula* contain flavone and this gives a unique characteristic of the primulas. Flavones are found not only in leaf farina but also in flower pigments and they are grouped into the Flavonoids (Gibbs, 1974).

The advantages or disadvantages of farina production to the primulas themselves are still dubious. Oliver (1895) noticed that the upper side of the *Primula* leaf is quite free from stomata, whereas on the underside the stomata are protected by farina. Some workers have suggested that farina is a toxic or waste product, secreted by the secretory hairs as part of a mechanism for protecting the living tissues (Gibb, 1974). It is possible that flavone functions as a protective agent against UV-light or against infection by phytopathogenic organisms as has been suggested by Hahlbrock

Table 1.1 Some morphological characters of *Primula farinosa* Linn. and its allied species.

Primula spp.	Common English name	Leaves			Flowers	
		shape	size (cmXcm)	farina	colour	form
1.*P.farinosa* Linn.	Bird's eye primrose	oblanceolate or oblong-obovate to elliptic or ovate	2-10X0.3-2	white	lilac or dark purple, deep yellow in the throat	hetero-morphic
2.*P.frondosa* Janka	Pink-flowered primrose	spathulate or oblong or obovate	2-8X1-3	white	rose-lilac to reddish-purple	hetero-morphic or mono morphic
3.*P.daliarica* Ruprecht	—	narrowly obovate to oblong or spathulate	2-10X1-3	white	rose or carmine red	hetero-morphic
4.*P.halleri* J.F.Gmel.	Long-flowered primrose	oblong-obovate or elliptic or oblanceolate	2-8X0.5-3	yellow	violet	mono-morphic
5.*P.laurentiana* Fernald	—	oblanceolate to spathulate or rhombic-ovate	2-8X0.3-3	white	lilac to pinkish -purple with an orange eye	mono-morphic or hetero-morphic
6.*P.modesta* Bisset et Moore	—	spathulate to oblong-elliptic	2-8X1-2	yellow	pinkish-purple	hetero-morphic
7.*P.scotica* Hook.	Scottish primrose	spathulate or elliptic or oblong	1-5X0.4-1.5	white	dark purple with a yellow throat	mono-morphic
8.*P.scandinavica* Brunn	Northern primrose	spathulate to narrowly obovate	2-3X0.5-1	white	purplish violet	mono-morphic
9.*P.stricta* Hornem	—	oblanceolate or narrowly obovate to elliptic	0.5-4X0.5-1	white	violet or lilac	mono-morphic

Sources:-Clapham, Tutin & Warburg, 1962; Tutin et al., 1972; Wilson & Blamey, 1979; Wright-Smith & Fletcher, 1943.

Figure 1.1 *Primula farinosa* Linn. grows at Blackhall Rocks, County Durham.

Figure 1.2 Associated species of *Primula farinosa* at Blackhall Rocks, County Durham.

(1981). It is believed that various species of alpine plants are adapted to a high intensity UV-environment by an increased content of UV-absorbing flavonoids, a low epidermal UV transmission, and/or high leaf reflectance of UV (see Caldwell, 1968). The composition of farina and its possible function either as an insecticide or an allelopathic agent is being investigated[1].

1.2.1.5 Homostyly and heterostyly

Botanists have long shown a special interest in the genus *Primula* because many of the species are heterostylous, individuals differring reciprocally in filament and style length (Ganders, 1979). In his studies of the heterostylous plants Bruun (1938) found a correlation between polyploidy and homostyly in the Section Farinosae, Subsection Eu-farinosae; the diploids being heterostylous and self-incompatible, whereas the polyploids are homostylous and self-compatible. With reference to this previous study, Vogelmann (1960) found heterostyly in the North American diploid species, *P. mistassinica* (2n=18) and homostyly in *P. laurentiana* (2n=72). On the other hand Wright-Smith & Fletcher (1943) suggested the existence of both homostyly and heterostyly in *P. frondosa* (2n=18) and *P. laurentiana* (2n=72).

1.2.1.6 The variation of *Primula farinosa* in Britain

The variation of *Primula farinosa* in northern England was investigated by Heslop-Harrison (1921), the variety *littoralis* being named by him from the population of *P. farinosa* found on the Magnesian Limestone of the Durham coast. The distinctions between coastal and mountain populations were described by him as follows :-

> "...When the flowering period is at its height the seaside plants are distinguishable at a glance by their clear light green leaves which contrast greatly with the darkish greyer green of the others. In shape too the leaves differ, for those of *littoralis* are broader, shorter and even fleshier, and in many cases, entire, whereas those from the hills are longer and denticulated. In fact on the one hand, I have seen plants from

[1] Dr. J.A. Pearson, personal communication

the coast indistinguishable just before their flowers expand from *P. scotica,* and, on the other, mountain examples as strongly toothed as *P. marginata*...As already stated, the majority of flowers may be regarded as, roughly, stellate in shape, with the space between the petals varying enormously in breadth. In general shape, the individual petals are cordate, with the sides of the heart straight or even concave in the alpine flowers, but for the most part convex in the case of *littoralis*. Moreover, in the latter plant, the corolla is slightly flatter, and this, coupled with the convexity, causes the space between the petals to vanish, thereby in many cases obscuring the characteristic flower shape. The reverse tendency is exhibited by the mountain variety, for sometimes the petals become 'carrot' shaped and the whole corolla wheel-like; this shape, as well as the true star form, never appears in *littoralis* or *scotica*. In flower size, the alpine plant has the advantage, its flowers averaging 11.4 mm in diameter, whilst those on the coast only attain an average of 10.2 mm ..."

1.2.1.7 The Genetics of ecological races and species

Primula farinosa and some of its allied species show very close relationships with respect to both morphological and cytological features. Cytological evidence shows that their chromosome numbers relate in such a manner that they are all multiples of the same basic number (Bruun, 1932). Bruun (1932) in his extensive cytological study of *Primula* found a series of chromosome numbers in the Subsection Eufarinosae with the numbers 9, 18, 27, 36, and 63. Bruun (1932; 1938) showed that *P. scotica* var. *scandinavica* (2n=72) was cytologically and geographically distinct from *P. scotica* (2n=54), consequently *P. scotica* var. *scandinavica* was renamed as *P. scandinavica* Bruun. The chromosome numbers of the diploid cells in *P. farinosa* and its allies are shown in Table 1.2. (after Bruun 1932, 1938 and other workers). It can be seen that *P. farinosa, P. frondosa, P. darialica,* and *P. modesta* are all diploids with 2n = 18. Bruun (1932) notes that *P. farinosa* has been the centre of evolution for several allied members of the Farinosae. Accordingly, *P. halleri, P. scotica, P. laurentiana, P. scandinavica,* and *P. stricta,* with diploid chromosome numbers of 36, 54, 72, 72, and 126, can be regarded as tetraploid, hexaploid, octaploid, and tetrakaidekaploid (14-ploid) respectively in relation to *P. farinosa.* The chromosome numbers of 126 in *P. stricta* is the highest known for the genus, and one of the highest amongst the flowering plants (Bruun, 1932).

Table 1.2 Chromosome numbers of the *Primula farinosa* complex (after Bruun, 1932; Davies, 1953; Vogelmann, 1960).

Primula spp.	Chromosome numbers (2n)
1.*P.farinosa*	18, 36
2.*P.frondosa*	18
3.*P.darialica*	18
4.*P.halleri*	36
5.*P.laurentiana*	72
6.*P.modesta*	18
7.*P.scotica*	54
8.*P.scandinavica*	72
9.*P.stricta*	126

In agreement with the results of Bruun (1932), Vogelmann (1960) in his cytological studies of American *Primula,* found that eight populations of *P. laurentiana* from the Gaspes Penninsula, Newfoundland and the north shore of the St.Lawrence River, sampled by both transplants and plants grown from seed, were all octaploid, (2n = 72).

The discovery of a tetraploid form of *P. farinosa* on the Baltic island of Gotland (Davies, 1953) supported the idea that *P. farinosa* has been the centre of evolution for the other member of the Farinosae. Davies (1953) reported both diploid and tetraploid forms of *P. farinosa* on Gotland, which is generally rich in endemic species. He also re-examined the chromosome numbers of *P. farinosa* from both upland and coastal populations, and confirmed that both are the diploid form, 2n = 18. The occurrence of the closely related species *P. farinosa* and *P. scotica* in Britain, led Melderis (1953) to suggest that *P. scotica* (2n=54) has originated by multiplication of the chromosome set from *P. farinosa.* Knaben (1982), studying the evolution of intra and inter-specific polyploidy in Europe during the Quaternary period, has discussed the ancestor of *P. scandinavica.* Using evidence from morphological analyses he deduced that *P. scandinavica* (2n=72) arose from the hybrid between *P. farinosa* (2n=18)×*scotica* (2n=54). However, attempts to hybridize these two species have been abortive (Dovaston, 1955).

1.2.1.8 Distribution

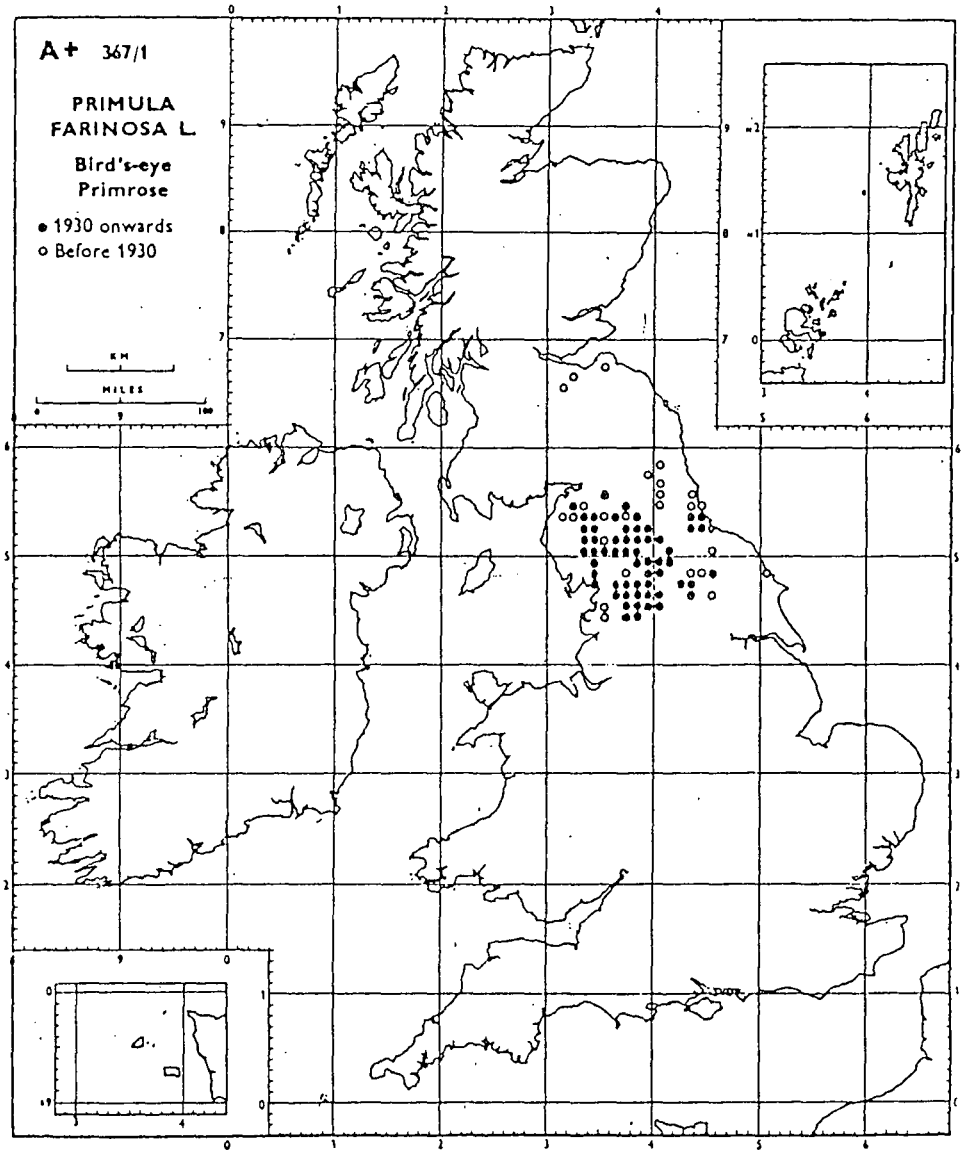
Primula farinosa is the most widespread species (Table 1.3), in the genus (Wright-Smith & Fletcher, 1943). It occurs throughout the European countries, except for Iceland (Ostenfeld & Grontved, 1934), Norway (Brunn, 1938), Ireland, and Greece (Wright-Smith & Fletcher, 1943). Its occurence throughout northern Asia to the shores of the North Pacific and southwards to the Altai and the Tian Shan, led Pax & Knuth to suggest that its centre of distribution is in Asia (see Baker, 1959).

In Britain *P. farinosa* is restricted to a small area (Fig. 1.3) as Watson (1849)

Table 1.3 Distribution of *Primula farinosa* and its allied species.

Primula spp.	Locality or country of distribution	Sources
1.*P.farinosa*	Northern England and Southern Scotland; all the countries of Europe with exception of Iceland, Ireland, Norway and Greece; Northern Asia to the shores of the North Pacific and southwards to the mountain of Altai and Tian Shan.	Farrer, 1919; Hulten, 1950; Ostenfeld & Grontved, 1934; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.
2.*P.frondosa*	Balkans: The Stara Planina, Central Bulgaria.	Polunin, 1980; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.
3.*P.darialica*	N.E.Caucasus.	Pax, 1889; Wright-Smith & Fletcher, 1943.
4.*P.halleri*	Easthern and Westhern Alps, in the Carpathians, Crotia, Bosnia, Albernia, Serbia, Bulgaria, Caucasus and Armenia.	Hermann, 1956; Polunin, 1980; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.
5.*P.laurentiana*	Northern America: Labrador, Newfoundland, Quebec, Nova Scotia, Maine and Magdalen Island.	Britton & Brown, 1897; Fernald, 1928; Wright-Smith & Fletcher, 1943.
6.*P.modesta*	Japan: extending from Yezo as far south as Shikoku, Nyohozan near Nikko, Mount Komagatage.	Wright-Smith & Fletcher, 1943.
7.*P.scotica*	Northern Scotland: Caithness, Sutherland and Orkney.	Clapham, Tutin & Warburg, 1962; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.
8.*P.scandinavica*	Scandinavia: Dalarna, Harjedalen, Jamland, Lycksele lappmark, Torne lappmark, Hedmark, Opland, Buskerud, Telemark, Hordaland, Sogn og fjordane, More, S.Trondelag, N.Trondelag, Saegnes, Oppdal, Nordland, Troms., Finnmark.	Brunn, 1938; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.
9.*P.stricta*	Iceland: Eujafjorour. Northern America: Labrador, Quebec, Ontario, Alberta, Manitoba, Mackenzie, Yukon, Greenland. Scandinavia: Norway: -Dovrefield, Oppdal. Sweden: -Jemtland, Torne, Lappmark. Finland Lapland: -Vardo, Niva	Hermann, 1956; Hulten, 1950; Ostenfeld & Grontved, 1934; Tutin et al., 1972; Wright-Smith & Fletcher, 1943.

Figure 1.3 The distribution in the British Isles of *Primula farinosa* Linn., reproduced with permission from Botanical Society of the British Isles, F.H.Perring and S.M.Walters, Atlas of the British Flora, 1976, Maps Nos. 367/1.


A+ 367/1
PRIMULA FARINOSA L.
Bird's-eye Primrose
• 1930 onwards
o Before 1930
KM
MILES

noted a southern limit in Lancashire and a northern limit near Peebles, Edinburgh. Accordingly he regarded *P. farinosa* as having a Scottish type of distribution (Watson, 1849) or an Intermediate type of distribution (Turrill, 1958). Watson (1849) noted that it lies between latitude 54-56° N. Details of its stations in Britain are shown in Table 1.4. It can be seen that *P. farinosa* occurs from sea level on the Durham coast up to an upper limit of 547 m on Cronkley Fell (Matthews, 1937). According to Wilson (1956) British populations of *P. farinosa* can be divided into upland colonies (300 m above sea level), lowland colonies (50-300 m above sea level), and coastal colonies (0-50 m above sea level).

From Table 1.6 it can be seen that its altitudinal range outside Britain is somewhat more extensive. For example, in the Pyrenees it is found at levels of 1,100-1,800 m (Lofthouse, 1927; Polunin & Smythies, 1973); and in the Vitosa to 1860 m (Polunin, 1980). In Southern Europe it occurs in the Alps at up to 2,900 m (Hoffmann, 1927; Matthews, 1937; Knaben, 1982), whereas in the North West Himalayas it grows at 3,600-5,100 m (Mani, 1978). Even though *P. farinosa* occupies a high altitudinal range outside Britain, Matthews (1937) placed it in the Northern-Montane Element of the British Flora. He indicated that the type of distribution shown by members of this group is not greatly different from that of the Arctic-Alpine Element, but the species are neither characteristically arctic nor alpine and they cannot properly, therefore, be included in the Arctic-Alpine Element.

By contrast, *P. scotica* is a member of the Arctic-SubArctic Element (Matthews, 1937), with its present locations lying between latitude 58-60° N (Watson, 1849). As a closely related species, *P. scotica* occurs about 320 km further north from the nearest British sites for *P. farinosa* and is an endemic species there (Dovaston, 1955). Its southern limit is in coastal pastures and cliff tops south of Wick in Caithness. It extents westwards to Cape Wrath in Sutherland (see Table 1.5 and Fig. 1.4), whilst its northernmost sites are on islands of the Orkney group (Watson, 1849; Dovaston, 1955).

Table 1.4 Distribution of *Primula farinosa* in Britain.

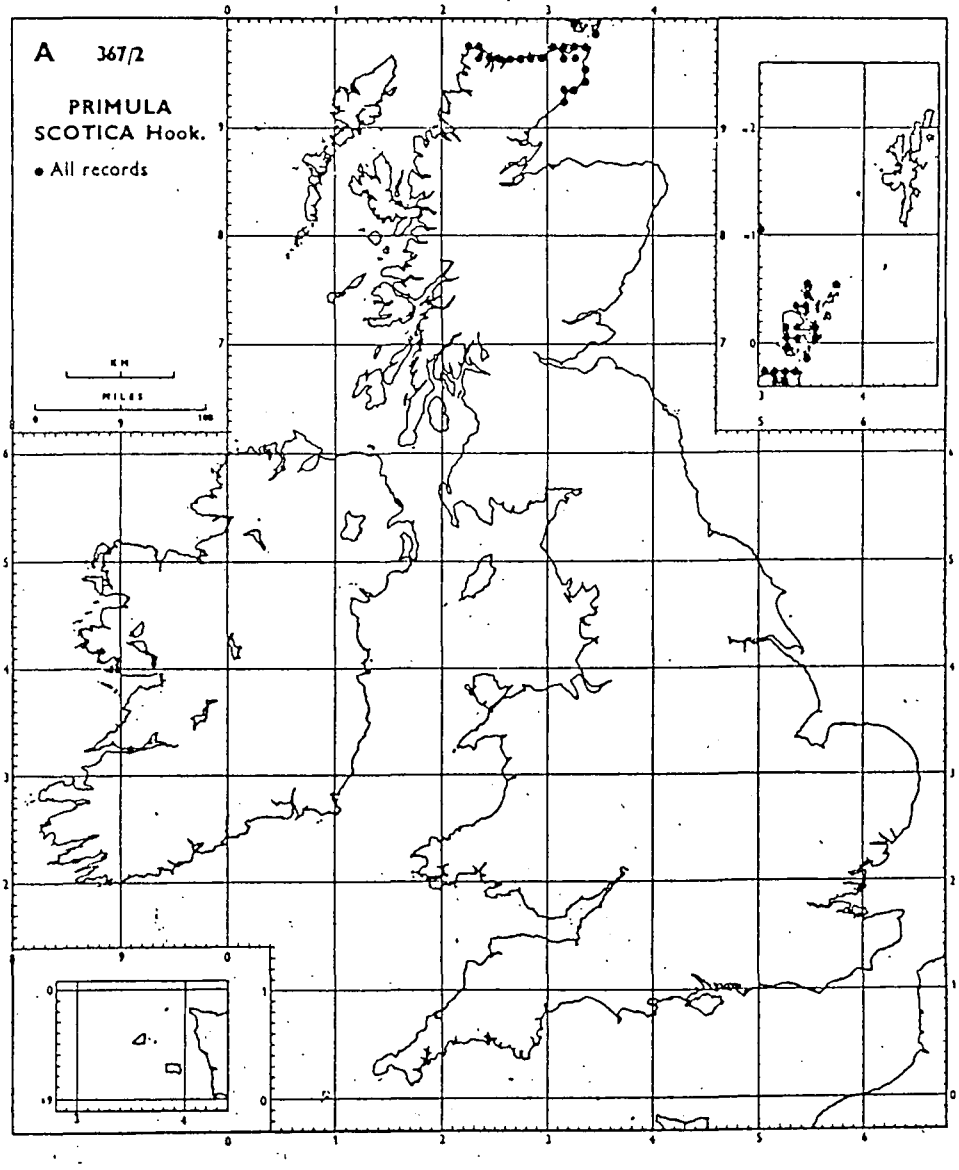
	Stations	Sources
South Lancashire:	Pendleton near Clitheroe, Pendle Hill, near Marsden Hall between Clithroe and Chatburn, Headhill common between Clithroe and Whalley, field between Mitton and Whally	Simpson, 1867; Savidge, Heywood & Gordon, 1963
West Lancashire:	Ease Gill between Carnforth Nether Kellet, Leighton Hall, Brock sills, Fairsnape Fell, Wyresdale Fishey, Balton Roughs and near Lickhurst Leagram	Wheldon & Wilson, 1907
West Yorkshire:	Ingleton, Settle, Copgrove, Knaresborough, Upper part of Wharfedale, Upper part of Airedale	Lees, 1888
North Yorkshire:	Hawkswell Moor near Bellerby, Kirklington, Camphill, Newby Wiske, Dishforth, Woodend, Marderby, Stillington, Cleveland at Ingleby and Seamer, Gurtof Gill near Boltly and Ballast hole near Thirsk Junction, Cronkley Fell	Baker, 1906 Matthews, 1937
Westmorland:	Crosby Ravenswaith, Crag-close	Wilson, 1938
Cumberland:	Windermere, Ullswater, Ireby, Bolton, from Tallentire and Gileruse to Bothel and Ireby, eastward of Oughterside mill, Penrith, Eden hall, Aspatria	Baker, 1885; Hodgson, 1898
Lake District:	Kirkstone Pass, Kendal, Shap, Kirkby Lonsdale, Arnside Knot, Catlands near Wigton, St.John's Vale	Baker, 1885
Durham:	Blackhall Rocks, sea-banks between Ryhope and Seaham, Easington, Penshaw, Hylton, Ferry hill, East Murton, Dalton le-Dale, Castle Eden, Quarrington Hill, Sedgefield, Town Kelloe Bank, Pig Hill, Haswell, Hesledon moor, Elwick, Hulam farm, Widdybank Fell, YHA site, Thrislington Plantation, Cassop Vale, Sprucely	Tate & Baker, 1867; Heslop-Harison, 1957; Winch, 1838; Doody, 1980; Wheeler, 1980;
Northumberland:	Wansbeck, Chollerford, Ovingham	Tate & Baker, 1867
Scotland:	West Linton, Dolphinton, Peebles, East Lothian, Mid Lothian, Pentland	Clapham, Tutin & Warburg, 1962; Stuart, 1881; Wright-Smith & Fletcher, 1943

Table 1.5 Distribution of *Primula scotica* Hook. in Britain.

Stations	Sources
Caithness: Swiney, Keiss Links, Bulno, Reay Links, Thurso, Dunnet Links	Ritchie, 1954; Wright-Smith & Fletcher, 1943
Orkney: Shapinsay, Bay of Moclett, Papa Westray, Stromness, Rousay, Stenness Black, Craig, Westray, Rousay, Walls, North Ronaldshay	Ellison, 1946; Johnston, 1882; Wright-Smith & Fletcher, 1943
Sutherland: Cape Wrath, Durness, Farr Bay, Torrisdale, Bay, Strathy Point, Arnadale Bay, Achininver, Farr Point	Huntley & Huntley, 1985*; Watson, 1849; Wright-Smith & Fletcher, 1943

* personal communication

Figure 1.4 The distribution in the British Isles of *Primula scotica* Hook. reproduced with permission from Botanical Society of the British Isles, F.H.Perring and S.M.Walters, Atlas of the British Flora, 1976, Maps Nos. 367/2.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PRIMULA
SCOTICA Hook.
• All records
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A closely related species, probably phylogenetically close to *P. scotica* and *P. farinosa,* is *P. scandinavica* (Knaben, 1982). In Sweden, *P. farinosa* is a lowland plant of the south-east, reaching up to the calcareous plains of Dalecarlia and Jamtland, whereas *P. scandinavica* is a mountain limestone plant which also grows well along the Norwegian west coast (Bruun, 1938). According to Bruun *P. farinosa* and *P. scandinavica* have no locality in common, and they are ecologically separated. *P. scandinavica* has been regarded as a plant with a bicentric distribution, i.e. (Fig. 1.5), a southern area of distribution from Vaga-Lom-Lesja to Dovre and Trondheim, and a separate northern area from Saltan to West Finnmark and parts of Swedish Lappland (Böcher, 1951). Björkman (see Böcher, 1951) pointed out that such bicentric distribution in Fennoscandia is characteristic of plants confined to base-rich subtrates.

From Table 1.3 it can be seen that *P. laurentiana* and *P. stricta* are both members of the American flora. *P. stricta* occurs between latitude 54-73° N, whilst *P. laurentiana* is found between 48-57° N (Fernald, 1928; Scoggan, 1979; Campbell *et al.,* 1986). Owing to its occurence on both sides of the Atlantic Ocean, *P. stricta* is placed amongst the amphi-atlantic plants by (Hultén, 1958). In Europe, it is found in the Scandinavian countries, Iceland, and Russia, but it is wholly absent from Siberia (Baker, 1959). In North America it occurs in Greenland and is widely distributed in arctic America. Famous & Campbell (1984) studied the distribution of *P. laurentiana* in the eastern United States, finding that it is limited to eastern Maine and is threatened or endangered in New England. They also noted that it is rare in Nova Scotia.

P. halleri occurs in the Alps in the alpine zone, and is reported as a rare species in some areas (Hoffmann, 1927; Thompson, 1911). *P. darialica* is found in the Caucasus in the subalpine region, whilst *P. modesta* occurs in alpine mountains of Japan, and is an endemic species there (Wright-Smith & Fletcher, 1943). *P. frondosa* grows wild only in the Balkan mountain (Polunin, 1980).

1.2.1.9 Habitat

From Table 1.6 it can be seen that *P. farinosa* and its allies are exclusive to soils rich in lime and with a high water content.

They are plants of open habitats, ranging from coastal dune slacks to high mountains. Amongst the *Primula farinosa* complex, *P. farinosa* shows a very wide range of habitats, although this may be due, at least in part, to the fact that this species occupies such a wide distribution. Lousley (1950) in his "Wild Flowers of Chalk & Limestone" described one habitat of *P. farinosa* on Carboniferous Limestone of the Craven District of Yorkshire as follows:-

> "...There are few more exciting places for the botanist in Britain. The sides of these two valleys are fairly steep and there is much bare limestone, but in many places there are also narrow strips of open scrub woodland -so open that much of it we should hardly regard as worthy of the name of wood in the south of England. Below the scrub on the open ground the limestone is often damp -at least locally and here the most characteristic plant is the Bird's-eye Primrose, *P. farinosa*. This little gem has numerous small lilac purple flowers arranged on a common stalk like a Cowslip, and the lower surface of the leaves is mealy. ..."

P. frondosa grows on shady cliffs or rock ledges in limestone mountains (Polunin, 1980). It is a well-known garden primula which was introduced into Britain in 1892 (Wright-Smith & Fletcher, 1943). *P. darialica,* another plant of wet shady rocks in the subalpine region, is very similar to *P. frondosa,* and garden specimens usually, in fact prove to be *P. frondosa* (Wright-Smith & Fletcher, 1943). *P. halleri,* a plant of alpine meadows or pastures, often grows on limestone in the Eastern, Central and Western Alps. *P. laurentiana* occupies meadows, ledges and cliffs in coastal north eastern North America (Fernald, 1928). Famous & Campbell (1984), in their studies of distribution of *Lomatogonium rotatum* and *P. laurentiana,* noted that these two boreal species reach the southern limit of their range along the coastal headlands and islands of the west coast of the Bay of Fundy. This region has a maritime climate of cool summer temperatures, extensive rainfall and fog, and low evapo-transpiration. *P. laurentiana* and *Lomatogonium rotatum* grow side by side

Figure 1.5 The distribution of *Primula scandinavica* Bruun in Scandinavia, after Bruun (1938).

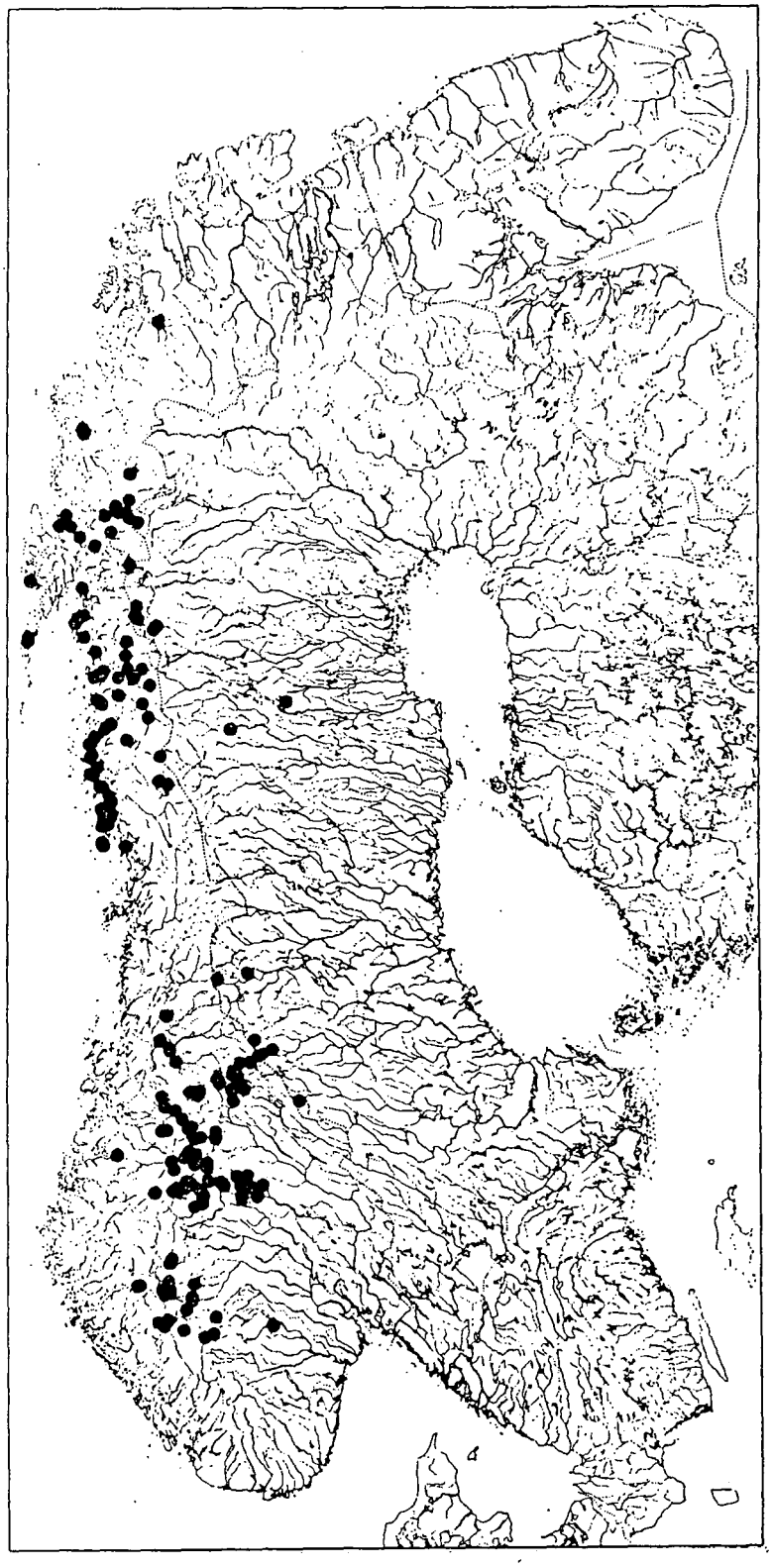


Table 1.6 Habitats of *Primula farinosa* and its allied species.

Primula spp.		Description of habitats(altitude in m)	Sources
P.farinosa	Britain	:Leeds and Halifax district:-on calcareous pasture	Moss,1911
		:Upper Teesdale :-Hay meadow(523),Turfy marshes (calcareous boulder clay)(523)	Pigott,1956
		:Westmorland:-damp mountain pastures and boggy ground mostly on limestone(600)	Wilson,1938
		:Cumberland:-bogs and meadows(450)	Hodgson,1898
		:The Lake district:-swampy fields	Baker,1885
		:North Yorkshire:-frequent in the swamps in the western dales,ascending to the plateau of Cronkley Fell (547)	Baker,1906
		:South Lancashire:-in a boggy field and moist pasture on calcareous soil(300)	Simpson,1867; Savidge et al. ,1963
		:West Lancashire:-damp mountain pastures,and boggy ground,mostly on limestone(7.5-240)	Wheldon & Wilson ,1907
		:Lowland Durham:-in calcareous grassland, restricted to limestone soils;in a small flushed site,in a pasture near Sprucely	Doody,1980 Wheeler,1980
		:Blackhall Rock:-on the Magnesian limestone cliff(10)	Preston,1915
		:Edinburgh:-boggy ground,above Woodhouselee	Stuart,1881
	Spain	:The Pyrenees:-open valley in the boggy ground(1800); marshy valley(1138)	Lofthouse,1927
	The Alps	:Eastern,Central and Western Alps:-Damp,grassy pastures and meadows from the plains to the Alpine region upto 2500 m often in great abundance on limestone	Thompson,1911; Bennett,1897; Hoffmann,1927; Danesch & Danesch,1969
	Bulgaria	: Rila Planina:-damp valley(2000)	Polunin,1980
		:Vitosa:-in wet flushes(1860)	
		:Pirin Planina:-on limestone rocks(1950)	
	Sweden	:Southeastern Sweden:-calcareous mires	Bruun,1938
		:Dalcarlia and Jamtland:-calcareous plains	Malmer,1965
P.frondosa	Bulgaria	:Stara Planina:-on damp sheltered rock ledges in lime-stone mountains(1330);shady cliffs near melting snow	Polunin,1980; Tutin et al., 1972
		:Northern Thrace:-on steep shady cliffs moistened by melting snow.	Wright-Smith & Fletcher,1943; Farrer,1919

Table 1.6 Continued.

Primula spp.	Descriptions of habitat(altitude in m)	Sources
P.darialica	N.E.Caucasus:on wet shady rock in the subalpine region (300–3000)	Wright-Smith& Fletcher,1943
P.halleri	Central Europe:in alpine meadows and in rock crevices often on limestone(1500–2700)	Wright-Smith& Fletcher,1943
	The Alps:Eastern,Central and Western Alps:–High alpine pastures,on primary rocks at about 2000 m; rather rare	Thompson,1911; Bennett,1897; Hoffmann,1927
P.laurentiana	Northern America:Labrador:–banks of Naskaupi River Newfoundland:–turfy limestone barrens,gravelly,limestone shore,wet limestone ledges,calcareous rocks and talus,conglomerate limestone,boggy spots on rocky crests,dry sea–cliffs,bare spots,cliffs near Franchmans'bank Quebec:–limestone and calcareous sandstone terrace, limestone headland,conglomerate(calcareous)sea–cliff ,cliffs and ledges,limestone cliffs,wooded bank of the St.Lawrence Matane Nova Scotia:–dripping cliffs,cliffs and ledges,crest of basalt cliffs,turfy crests and slopes of exposed headlands Maine:–foot of Mt.Kineo,northside of Mt.Kineo	Wright-Smith& Fletcher,1943; Fernald,1928; Britton&Brown, 1897
P.modesta	Northern Japan:in alpine regions	Wright-Smith& Fletcher,1943
P.scotica	Northern Scotland:on maritime cliffs where it is confined to areas with high water–table and stable substratum; on coastal dune slacks and in inland semi–flushed heathy pastures or natural sedgy pasture; on poor bare alluvial soil the grass around them being very short	Ritchie,1954; Ellison,1946
P.scandinavica	Scandinavia:Norway:–along the west coast Sweden:–on mountain limestone	Wright-Smith& Fletcher,1943; Brunn,1938
P.stricta	Scandinavia:on sea–coasts and cliffs, as well as in meadows and by the banks of streams upto 1250 m; occurs sparsely on a small damp spot in the beach zone	Wright-Smith& Fletcher,1943;
	Iceland :on moist clayey soil	Ostenfeld&Grontved,1934
	Northern America:Labrador:–moist banks Ontario:–growing below high–water mark Mackenzie:–arctic seacoast Yukon:–near mouth of Lewis River	Lagerkranz, 1950;Coombe& White,1951; Fernald,1928

on well-drained soils over a granite bedrock. Soil tests from five localities for *P. laurentiana* indicate a pH range from 5.9 to 7.5 with excessive levels of calcium.

P. modesta is found on alpine mountains in various parts of Japan. An endemic species of Scotland, *P. scotica,* occupies coastal cliffs, often with a soil developed on boulder clay, inland habitats (more than 1 km from the coast) with soils of either clay or base-rich flagstone origin, and also dune 'slack' or pasture sites with high, fluctuating water tables (Ritchie, 1954; Gimingham, 1964). Thirty six known populations were reported by Ritchie (1955) and it is not regarded as an endangered species (Perring & Farrell, 1977). *P. scandinavica* occurs along the west coast of Norway and on mountain limestones in Sweden. *P. stricta* is found growing on arctic sea-coasts and cliffs, moist banks of streams, and in meadows (Fernald, 1928; Wright-Smith & Fletcher, 1943; Scoggan, 1979).

1.2.1.10 Associated species

It is my intention here to summarize some of the associated species of *Primula farinosa* and its allies, and to compare their ecological and physiological performance from the available literature. In Table 1.7, all the associated species of *P. farinosa* reported from Britain, Spain, and Bulgaria are included, plus the associated character species of *P. farinosa* from South Germany and South and Southeast Sweden; (limits have been put on the data from these latter areas to avoid an excess of information). Available evidence suggests that *Bartsia alpina, Molinia caerulea, Parnassia palustris, Carex capillaris, Carex pulicaris, Trollius europaeus,* and *Pinguicula vulgaris* are commonly found with *P. farinosa.* According to Matthews (1937) *Bartsia alpina* and *Carex capillaris* are members of the Arctic-Alpine Element; *Carex pulicaris, Parnassia palustris* and *Pinguicula vulgaris* are members of the Continental Northern Element and are also found in arctic Europe. *Trollius europaeus* is in the same element as *P. farinosa,* the Northern-Montane Element. *Pinguicula alpina* an arctic-alpine plant, is found associated with *P. scandinavica* (Table 1.8) in Scandinavia (Böcher, 1951) whilst *Sedum rosea* another plant of the

same group, is associated with *P. laurentiana* in Northeast North America (Campbell *et al.*, 1986).

The species named in Table 1.9 are associated with *P. scotica,* although none of them is placed in the same group with *P. scotica* by Matthews (1937). Amongst the associated species of *P. farinosa* and its allies (Tables 1.7-1.9), *Plantago maritima* is found with *P. farinosa, P. scotica,* and *P. stricta. Parnassia palustris, Pinguicula vulgaris, Selaginella selaginoides,* and *Succisa pratensis* are associated with both *P. farinosa* and *P. scotica.* Only a small numbers of these associated species of *P. farinosa* and its allies have been the subject of ecophysiological studies, for example *Plantago maritima* (Arnold, 1973; 1974), *Sedum rosea* (Woodward & Pigott, 1975), and *Thallictrum alpinum* (Mooney & Johnson, 1965).

1.2.1.11 Flowering periods

The primula is one of the earliest blooming plants (Mani, 1978). In general *P. farinosa* and its allies bloom in May to June and flower once a year, whereas in contrast, *P. scotica* can flower up to 3 times a year (Grant, 1887; Ritchie, 1954; Johnston, 1881), with the first blossoms from May until early July, the second from early July until mid August, and the third from mid August until mid September. Two flowering periods in *P. scotica* are quite common and most plants flower during both periods (Ritchie, 1954). It is interesting to note that in northern England upland populations of *P. farinosa* flower some days earlier than coastal populations (Heslop-Harrison, 1921; Farrer, 1919). A possible explanation of this difference in flowering period is that the growing season for the upland plants is shorter than for the lowland plants (Farrer, 1919; Mani, 1978), hence the upland plants need to rush into flower. This is supported by the observation (Manley, 1945) that in the northern Pennines a slight increase in elevation is accompanied by a remarkably large decrease in the length of the growing season (a shortening of 10 days for 80 m).

In the Alps, *P. farinosa* is amongst the earliest blooming plants, e.g. spring

Table 1.7 Associated species of *Primula farinosa* Linn.

Species	Localities					
	1	2	3	4	5	6
Acrocladium cuspidatum		+			+	
Anacaptis pyramidalis		+				
Antennaria dioica		+		+		
Arenaria biflora				+		
Bartsia alpina	+	+	+		+	
Bryum pseudotriquetrum	+					+
Briza media	+					
Bupleurum ranunloides			+			
Caltha palustris				+		
Campanula patata				+		
Campylium stellatum					+	+
Cardamine raphanifolia				+		
Carex capilaris	+	+			+	
C.dioica		+				
C.flacca	+				+	
C.flava		+			+	
C.glauca		+				
C.hostiana	+	+			+	+
C.lepidocarpa	+	+				
C.panicea	+				+	+
C.pulicaris	+	+			+	
Circium heterophyllum		+				
Crocus veluchensis				+		
Ctenidium molluscum	+	+				+
Drepanocladus revolvens					+	+
Epipactis atrorubens		+				
E.palustris		+				+
Eleocharis quinqueflora		+			+	
Eriophorum alpinum			+			
E.latifolium		+				
Festuca ovina	+					
Filipendula ulmaria				+		
Fissidens cristatus		+				
Gentiana bivarica			+			
G.verna	+		+			
Geranium pyrenaicum				+		
G.sanguineum		+				
Geum montanum				+		
Gymnadenia conopsea	+	+				
Helianthemum vulgare		+				
Homogyne alpina				+		
Kobresia simpliciuscula	+				+	
Linum catharticum	+					+
Leontodon hispidus	+					
Lotus corniculatus	+					
Molinia caerulea	+	+			+	+
Menyanthes trifolia		+				
Myosotis sysvatica				+		

Table 1.7 Continued.

Species	Localities					
	1	2	3	4	5	6
Parnassia palustris		+	+		+	+
Pedicularis orthantha				+		
Pinguicula balcanica				+		
P.vulgaris	+	+			+	+
Plantago maritima	+	+				
P.amara		+				
Polygonum viviparum			+			
Potentilla erecta					+	+
Primula elatior				+		
P.imbricata			+			
Pyrola minor				+		
P.rotundifolia		+				
Rhytidiadelphus squarrorus		+				
Saxifraga aizoides			+		+	
S.stellaris			+			
Scabiosa columbaria	+					
Schoenus ferrugineus					+	+
S.nigricans					+	+
Scilla bifolia				+		
Selaginella selaginoides	+	+				+
Sedum villosum			+			
Senecio panacii				+		
Sesleria coerulea	+					+
Succisa pratensis					+	+
Thallictrum alpinum		+				
Thymus drucei	+					
Tofieldia pusilla	+	+			+	
Tozzia alpina				+		
Trollius europaeus		+	+		+	
Valeriana dioica		+				+
Veronica bellidioides				+		
Viola dacica				+		

Localities:

1. UpperTeesdale(Pigott,1956;Shimwell,1968;Jones,1973).
2. Lowland Northern England:- Westmorland(Lousley,1950; Shimwell,1968); Blackhall Rocks(Heslop-Harrison, 1921;Preston,1915); Lowland Durham(Doody,1980); Thrislington Plantation(Doody,1980;Shimwell,1968) Cassop Vale(Doody,1980); Town Kelloe(Doody,1980); Sprucely(Wheeler,1980).
3. Spain:-Pyrenees(Lofthouse,1927;Polunin&Smythies,1973).
4. Bulgaria:-The Stara Planina(Polunin,1980). Vitosa(Polunin,1980).
5. South Germany(Oberdorfer,1977,1978,1983).
6. South and Southeast Sweden(Tyler,1979).

(+) represented occurrences of the species in the localities.

Table 1.8 Associated species of the primulas.

Primula spp.	Associated species	Sources
P.frondosa	*Cortusa matthioli*	Polunin,1980
	Daphne blagayana	
	D.oleoides	
	Haberlea rhodopensis	
	Micromeria frivalszkyana	
	Rhododendron myrtifolium	
	Rhynchocorys elephas	
P.laurentiana	*Sedum rosea*	Campbell et al.,
	Viola septentrionalis	1986
	Lomatogonium rotatum	Famous &
	Agrostis stolonifera	Campbell, 1986
	Aster novi-belgii	
	A.nemoralis	
	Campanula rotundifolia	
	C.viridula	
	Deschamsia flexuosa	
	Empetrum nigrum	
	Euphrasia canadensis	
	E.randii	
	Festuca rubra	
	Iris hookeri	
	Juncus filiformis	
	Plantago juncoides	
	var.decipiens	
	Prenanthes trifoliata	
	Sagina nodosa	
	Solidago bicolor	
	Triglochin maritima	
	Viola septentrionalis	
P.scandinavica	*Astragagalus frigichus*	Bocher,1951
	Minuartia stricta	
	Pinguicula alpina	
P.stricta	*Carex aquatilis*	Lagerkranz,1950
	C.subspathacea	
	Plantago maritima	
	Puccinella angustata	
	var.vaginata	
	P.phryganodes	

Table 1.9 Associated species of *Primula scotica*.

Species	Localities				
	i	ii	iii	iv	v
Flowering plant					
Primula scotica	+	+	+	+	+
Achillea millefolium			+		
Agrostis tenuis			+		+
A.canira				+	
Aira praecox				+	
Angelica sylvestris	+				
Antennaria dioica			+		
Bellis perennis	+	+	+		
Calluna vulgaris	+		+	+	
Carex arenaria		+			
C.flacca	+	+	+		+
C.nigra		+		+	
C.pauciflora	+				
Coeloglossum viride		+			
Cochleria sp.					+
Cerastium holosteoides				+	+
Cynosurus cristatus	+				
Empetrum nigrum	+				
Erica cineria			+		
E.tetralix	+			+	
Eriophorum angustifolium				+	
Euphrasia brevipila	+	+	+		
E.foulaensis		+			
E.sp.					+
Festuca ovina	+	+	+	+	+
Galium verum		+			
Gnaphalium sylvaticum			+		
Gentianella baltica		+			
G.campestris		+	+		
Hieracium pilosella			+		
Holcus lanatus				+	+
H.mollis	+				
Hypericum pulchrum			+		
Juncus articulatus	+			+	
J.squarrosus	+				
Koeleria gracilis	+				
Leontodon autunnalis				+	+
L.hispidus			+		
Linum catharticum	+	+			
Lotus corniculatus	+	+	+	+	+
Luzula multiflora				+	

Table 1.9 Continued.

Species	Localities				
	i	ii	iii	iv	v
Parnassia palustris	+	+			
Pinguicula vulgaris	+	+			
Plantago caronapus					+
P.lanceolata	+	+	+	+	+
P.maritima	+	+	+	+	+
Potentilla erecta	+		+	+	
Prunella vulgaris	+	+	+	+	+
Ranunculus cf.R.acris					+
Salix repens		+			
Scilla verna	+			+	+
Selaginella selaginoide		+	+		
Sieglingia decumbens	+		+	+	
Succisa pratensis			+		
Thymus drucei		+			
Trifolium pratensis	+		+		
T.repens				+	+
Ulex europaeus			+		
Viola riviniana			+		
Non-flowering plants					
Bryum pallens	+				
Campylium stellatum	+				
Climacium dendroides		+			
Dicranum fuscescens		+			
Hylocomium splendens	+	+			
Hypnum cupressiforme	+	+	+	+	
Mnium affine		+			
Pseudoscleropodium puru			+		
Rhacomitrium canescens	+				
Rhytidiadelphus squarro		+			
Cladonia impexa				+	
C.pyxidata		+	+		
C.rangiferina	+	+	+		
C.sylvatica		+			
C.(basal squannules)				+	
Peltigera canina		+	+		
P.polydactyla	+	+	+		

Localities:Caithness:- i.Swiiney(coastal cliff) near Lybster.
ii.Keiss Links(dune)north of Wick.
iii.Bulno(moor)near Dunbeath.
Sutherland:- iv.Strathy Point by Totegan(Soligenous mire)
v.Strathy Point(cliff tops)north west of Totegan.

Data i-iii from Ritchie(1954).
iv-v from Huntley & Huntley(1985) personal communication.

(+) represents occurrence of the species in each locality.

gentians, spring anemones, -Arber (1910), studying the plant life in the Alps, wrote:-

> "... *Primula farinosa* is one of the earliest spring blossoms in the damper pasturages, where it flowers in countless millions. The leaves are green and smooth above, but are covered below by a white mealy wax or bloom..."

1.2.1.12 History

As has been noted above, significant upland populations of *Primula farinosa* in Britain occur in Upper Teesdale, an area famous for its arctic-alpine rare plants (Wilmott, 1935). These species are survivors of a late-glacial flora which spread to Teesdale after the retreat of the Devensian ice-sheet (Godwin, 1949; Pigott, 1956). This is supported by the pollen analyses of peat deposits from Upper Teesdale which show that a herb-rich flora, including many of the rare plants of Teesdale, has existed there since the late glacial period (Turner *et al.*, 1973). Heslop-Harrison & Richardson, (1953) who studied the origin of the flora on the Magnesian Limestone area of East Durham, pointed out the existence of an important group of plants, e.g. *Trollius europaeus, Antennaria dioica, P. farinosa, Sesleria caerulea* etc. in both Upper Teesdale and on the Magnesian Limestone. During the late-glacial era these species apparently spread from periglacial areas to colonize the newly open areas, so becoming widespread in Britain. Their presence today on the Magnesian Limestone of East Durham reflects a relict status as it does in Teesdale. Heslop-Harrison & Richardson (1953) also pointed out that the coastal colonies of *P. farinosa* are genetically separate from those in Upper Teesdale, and this may be due, at least in part, to the fact that they have been so isolated. If this is so, it is possible to suggest that the ecological races of the relict species could have arisen and evolved to be well adapted to their particular habitats (Turessen, 1922; 1925).

Macrofossils (seeds) collected from deposits in the Cam Valley in Cambridgeshire dated by Godwin (1953) to the last full-glacial period proved to belong to *P. scotica* (Dovaston, 1955). This indicates that *P. scotica* occupied a greater area in the full and late glacial periods than it does today (Ritchie, 1955). Melderis (1953) has suggested that *P. scotica* and other species which today have restricted distributions

in the Inner and Outer Hebrides and Shetland may have survived the Quaternary glaciations near to Scotland in some sheltered ice-free areas near the continental shelf. *P. scotica* is, therefore, probably best considered as a species which has shown a wide, full and late-glacial distribution where there are post glacial stations in northern Scotland (Dovaston, 1955).

1.2.2 Sources of plant materials

A list of the plant species studied and their origins is shown in Table 1.10. Some of these species have been used only for analyses of leaf characteristics or germination experiments. Details of the plant materials used can be seen in the relevant chapter concerned. In all cases, fully expanded mature leaves from adult plants with no sign of senescence were used.

1.2.3 Cultivation

All the primulas were planted in 8 cm plastic pots with J.Arthur Bower's seed and potting compost, unless stated otherwise. They were cultivated in a growth room, Department of Botany, University of Durham. Light was supplied by fluorescent lights (daylight tubes) with a 16-hour photoperiod. Light intensity was approximately 100 μmol m^{-2} s^{-1} (PAR) at plant height. Temperature and humidity were not controlled. The primulas were sprayed with pesticides from time to time to control red spiders and aphids. Every two days the plants were watered with tap water.

1.2.4 Sources of chemicals

All chemicals used were obtained either from BDH chemicals Ltd or Sigma Chemicals Co, Ltd. They were of analytical grade, unless stated otherwise.

1.2.5 Statistical Analysis of Data

Statistical analyses were performed on all data to determine if the treatment means were significantly different at the 0.05 confidence level. An analysis of vari-

Table 1.10 Origin of seeds or plant materials used in this thesis.

Primula spp.	Sites or origin	Habitat	Altitude (m)	Grid reference
1.*P.farinosa(a)*	Lechtaler,Alpen, Vqlluge,Tirol Austria	-	2,000	-
2.*P.farinosa(b)*	Blackhall Rocks, County Durham	on Magnesian Limestone cliff,clay soil	10	NZ/473387
3.*P.farinosa(c)*	Cronkley Fell, UpperTeesdale, North Yorkshire	flat ground, on a thin rendzina soil	547	NY/848283
4.*P.frondosa*	seeds from Jardin Alpin garden	-	-	-
5.*P.darialica*	mature plants from Holden Clough nersery, Lancashire	-	-	-
6.*P.halleri*	seeds from Royal Botanic Garden, Edinburgh	-	-	-
7.*P.laurentiana*	seeds from Royal Botanic Garden, Edinburgh	-	-	-
8.*P.modesta*	mature plants from Holden Clough nersery, Lancashire	-	-	-
9.*P.scotica(a)*	Strathy Point, Sutherland by Totegan	Soligenous mire	150	GR/827689
10.*P.scotica(b)*	Strathy Point, Sutherland north west of Totegan	Cliff top	50	GR/826690
11.*P.scandinavica*	near biological station at Kongsvold,County Oppdal, Norway	-	-	-
12.*P.stricta*	Karasfok, Norway	on gravel bank,open	150	-

ance (ANOVA) followed by either a multiple range test, e.g. Least Significant Difference (LSD), or a Student's t-test, was performed in order to test the significance of the difference between the means (Parker, 1979). Product-moment correlation coefficients were also tested using a Pearson Correlation (Haber & Runyon, 1977). The software package SPSS-X was used for all analyses.

All data were analysed on the mainframe Computer at the Computer Centre, University of Durham, an Ahmdahl 470/V8, under the Michigan Terminal System (MTS).

1.2.6 Text processing and Graphics

This thesis was prepared using the NUMAC Screen Editor on a TVI 910 Terminal to enter text into the mainframe computer. The TEX text-processing program (Knuth, 1986) and GIMMS (Waugh & McCalden, 1983) graphics-processing program were then used to format text and plot graphs respectively. Text and Graphics were printed on a QMS Lasergrafix 800 laserprinter.

1.3 Aims

From the information above, it can be seen that the wide range of latitudes and altitudes occupied by *Primula farinosa* and its allies means that they encounter, and can be expected to have adapted to, a wide range of major environmental parameters. The aim of this thesis is to evaluate the extent and nature of the adaptations shown by populations of *P. farinosa* from different areas, and by related species of more restricted distribution.

CHAPTER 2

SEED GERMINATION

2.1 Introduction

Seed germination is one of many factors which determine whether a particular species is able to maintain itself in a given locality. It has long been known that before a seed can germinate, it must be placed in favourable environmental conditions for this process to occur. Amongst the primary environmental factors which influence germination are an adequate supply of water and oxygen, a suitable temperature, and also light for certain seeds. The availability of these factors changes from time to time and differs from one locality to another (Pollock, 1972). The requirements for these factors vary according to the species and variety and depend on both the conditions during seed development and hereditary factors (Mayer & Poljakoff-Mayber, 1982). The environmental requirements for germination and the ecological conditions in the habitat of the plant and its seeds seem to be correlated.

The only primary environmental factor to be studied here will be temperature. Different seeds have different temperature ranges within which they germinate, and the temperature requirements of seeds vary not only with species, variety, origin and degree of maturity of the seeds but also with their physiological condition (Lovato, 1981). The responses of seeds to temperature are complicated, and are usually concerned with the changes in the dormancy level. In order to overcome dormancy, some seeds require such conditions as stratification, alternating temperature, temperature shifts or, rarely, high temperatures (Bewley & Black, 1982; Wareing & Phillips, 1970; Mayer & Poljakoff-Mayber, 1982). Hydrated seeds of many woody and herbaceous species are freed from dormancy when they are subjected to low temperatures between 1-15°C (Bewley & Black, 1982). Thomas (1972), discussing the works of Frankland and Wareing (1966), Wareing (1965,1969), Amen (1968), Ware-

ing & Saunders (1971) concluded that seeds with a chilling requirement to overcome dormancy often contain both growth inhibitors and growth promoters. Research has shown that this kind of dormancy is controlled by an inhibitor/promoter balance that alters when the seed is chilled. Chilling treatment allows the seeds to germinate when they later experience a more favourable temperature for growth (Black, 1970). This result corresponds with the work of Bradbeer (1968) and Ross and Bradbeer (1968; 1971a; 1971b) who concluded that chilling appears to remove a check to gibberellin biosynthesis, although gibberellins are apparently not subsequently synthesised in quantities sufficient to promote germination until the seed is transferred to a higher temperature (see Thomas, 1972). In nature it appears that seeds with a chilling requirement have to experience winter conditions before germinating in spring or summer (Black, 1970). Chilling or stratification stimulates germination not only in dormant seeds but also in non-dormant seeds. In their studies upon *Eucalyptus pauciflora,* Beardsell & Mullett (1984) concluded that stratification assists the establishment of seedlings from lowland populations (non-dormant seeds) in spring due to an increase in the rate of germination.

Reaction to stratification by seeds of a single species may vary. Baskin & Baskin (1975) studied seed dormancy and ecological aspects in *Isanthus brachiatus* (Labiatae), a summer annual limestone plant. It is interesting to note that in this species freshly matured seeds are dormant and show physiological polymorphism with respect to the conditions necessary to overcome dormancy. 15-35% of the seeds in a seed crop require only one stratification treatment and germinate the first spring. The remainder of the seeds require two, three, or more treatments and thus do not germinate until after two, three, or more overwintering periods in the field. The type of physiological polymorphism exhibited by seeds of *I.brachiatus* may be important to the continued survival of the species in local habitats of a region since it spreads the germination of a particular seed crop over several years and ensures that a reserve of ungerminated seeds is always present in the soil. This versatility of seed germination may be an example of the adaptations displayed by

annual species in order to overcome severe environmental conditions and to survive in an unfavourable habitat.

The duration of chilling required seems to differ in each species; for example *Pinus strobus* needs at least 32 d for a full effect, whereas a few hours of chilling is adequate in lettuce (Bewley & Black, 1982). It seems likely that *Primula farinosa* can germinate at rates of up to 63% if chilling is extended to a duration of 7 wk whereas a rate of only 38% is reached after 4 wk of chilling (Thompson, 1970). Bewley & Black (1982) noted that the required period of chilling is related to the depth of dormancy. Chilling requirements can be shortened by removal of the pericarp and endosperm; for example, an isolated embryo of apple needs less chilling than intact seeds (Black, 1970; Bewley & Black, 1982). It is believed that these effects are due to the enclosing tissue restricting water entry.

Some genera, such as *Cy nodon, Typha* and *Lycopus,* are adapted to respond to alternating temperatures (Bewley & Black, 1982). Lovato (1981) noticed that many kinds of seed germinate better with alternating temperatures than with constant temperatures. He also showed that seeds of *Xanthium pennsylvanicum* fail to germinate at constant temperatures below 25°C, but germinate well with alternating temperatures of 16°C and 23°C. Fluctuating temperatures seem likely to affect species in marshes, bogs, stream sides and other wetland habitats. Several ruderal species have also been studied in which germination was found to depend on fluctuating temperatures, e.g. *Chenopodium rubrum, Polygonum persicaria,* and *Polygonum lapathifolium* (Thompson, Grime & Mason, 1977). Thompson (1974) stressed the importance of fluctuating temperatures as a sensor system capable of responding to the diurnal temperature changes characteristic of the surface layers of the soil, which would act to promote germination of seeds close to the surface and to prevent germination of deeply buried seeds.

It should be noted that the release of dormancy in any one species may require a combination of treatments, such as stratification, fluctuating temperatures and

after-ripening. Interaction of light and temperature has also been found to be necessary in many species in order to break dormancy (Bewley & Black, 1982).

In a survey of the ecological physiology of the seeds of arctic-alpine species Amen (1966) notes that 60-70% of alpine seeds have no intrinsic dormancy. Most dormancy is caused by seed-coat inhibition and can be overcome by scarification, chilling, light, or elapsed time (Billings & Mooney, 1968). Seeds of alpine species are mostly produced rather late in the growing season and do not germinate until the following year. Working with arctic and alpine populations of *Oxyria digyna,* Mooney & Billings (1961), concluded that although the optimum germination temperature is 20°C, germination still occurs at both 10 and 30°C, but not at 3°C. Amen (1966) emphasized that there was no evidence to suggest that any alpine seeds can germinate at temperature below 10°C, but in contrast, some later authors have reported the germination of arctic-alpine seed below 10°C (Mayer & Poliakoff-Mayber, 1982; Holtom & Greene, 1967).

Mooney and Billings (1961) found that seeds of four populations of *Oxyria digyna* germinated poorly at constant temperatures below 10°C, but rather well at higher temperatures. But despite this, if temperatures were alternated every 24 h between 13 and 2°C (mean 7.5°C), germination rates of up to 65% were found in all populations. Corresponding results were presented by Sayers & Ward (1966), working with seeds of six species of alpine plants, who showed that high percentage germination rates were found with alternating temperature ranges of 10-20°C for the low temperatures and 25-30°C for the high temperatures. It has been suggested by Thompson (1968) that the speed of germination at low temperatures and the values of temperature minima for germination, may be correlated with the geographical distribution of different species of Caryophyllaceae. Billings and Mooney (1968) noted that most alpine seeds germinate in early summer after snowmelt and after daytime soil surface temperatures have risen to 10-15°C. They noted also that the optimum germination temperatures are rather high for tundra species (20-30°C).

Before leaving this topic, it is worth noting that in some years the weather during the growing season in arctic or alpine regions is so cold that flowering and fruiting are inhibited and little or no viable seed is produced (Billings & Mooney,1968).

Whilst preparing the plant material grown for this work it was noted that the number of seeds which germinated in each Petri dish (which were not equal in number or density of seeds sown) were somewhat different both within the same, and also between species. A brief review of the literature suggested that the density of seeds sown can effect germination rate (Linhart, 1976). Accounts of the effect of clustering of seeds on the rate of seed germination are, however, rather rare in the literature and the results reported have included both increases and decreases as a consequence of greater density of seeds (Palmblad, 1968; Ballard, 1958). Linhart (1976) conducted a series of experiments designed to test density-dependent germination rates in seeds sown contiguously in clumps of different size. Using the results from his studies and data obtained from the literature, he concluded that positive density-dependent responses appeared to be characteristic of species from closed habitats, whilst neutral or negative responses were found in species of open habitats.

It was, therefore, of interest to examine the effects of seed-clump density and pre-chilling requirements on the percentage germination in species of the *Primula farinosa* complex from contrasted habitats.

2.2 Materials and methods

Seed used in these experiments was partly supplied by growers or Botanic Gardens in Britain and Europe, and partly collected from plants growing in their natural habitats in Britain. Practically all the seed was collected during the summer of 1985 or received between August and December 1985; the experiments being performed within 6 months of reception. The conditions of storage abroad or elsewhere in this country were not known, but upon receipt of seed from, and prior to, these experiments, all seeds were kept in a 4°C room. In an attempt to determine if there were differences in the characteristics of seed germination between the species in the *Primula farinosa* complex, two experiments were performed. Seeds were exposed to light during the course of all the experiments. In each Petri dish 50 seeds were sown; the filter paper was initially moistened with 2 cm^3 of distilled water and further distilled water added as needed from time to time. The presence of a visible radicle was taken as the criterion for germination.

2.2.1 The effect of the cumulative density of the seed

Seeds of *Primula farinosa* (a), *P. farinosa* (b), *P. farinosa* (c), *P. frondosa, P. halleri, P. scotica, P. scandinavica,* and *P. stricta* were used in these experiments. Seeds were imbibed in the dark at 4°C for 4 wk. after which time they were placed singly at least 1 cm part or in clumps of 10, 25, or 50, each clump being at least 1 cm distant from other clumps, in covered Petri dishes. Seeds within clumps were in contact with each other and in monolayers in order to ensure firm contact with the substrate. Petri dishes were placed in a growth chamber with a daily fluctuation in temperature, the daytime temperature being in the range 22-25°C. Counts of seed germination were made 7 and 14 d after sowing. Fifty seeds in each of three replicate dishes were used in calculating the germination percentages.

2.2.2 Cold-Stratification

Seeds of the primulas were the same as 2.2.1 except that *P. farinosa* (a) was not

used. Chilling treatments at 4°C were applied to imbibed seeds stored in the dark for 2, 4, or 6 wk before transfer to an incubator at 15°C. The unchilled control series were sown at the beginning of the experiment in March 1986. Seeds were placed singly at least 1 cm part so as to allow comparison of the percentage germination of 4 wk-chilled seeds in this experiment with that of the singly sown seeds in the previous experiment. Counts of seed germination were made 7, 14, and 21 d after sowing. Fifty seeds in each of four replicate dishes were used in calculating the germination percentages.

2.3 Results

2.3.1 The effect of the cumulative density of the seed

The results presented in Table 2.1 show the cumulative percentage germination of primula seeds sown in sets of 1, 10, 25, and 50 seeds, 7 and 14 d after sowing. *P. farinosa* (a), *P. farinosa* (c), and *P. scandinavica* showed decreased germination at higher densities, whereas *P. farinosa* (b), *P. halleri, P. frondosa, P. scotica,* and *P. stricta* showed no effect of seed-clump density, and germinate more than 65% in *P. frondosa,* and *P. stricta.* The results of an ANOVA for each species 7 d after sowing (Table 2.2) and 14 d after sowing (Table 2.3) show that the decreased seed germination at higher densities is significant only in *P. farinosa* (c) ($P<0.01$) 14 d after sowing. Table 2.4 summarizes the results of the LSD tests on the percentage germination between seed-clump densities in each species. Only some pairs of seed-clump densities in *P. farinosa* (c), e.g. (1):(25), (1):(50) show significant differences.

The difference between species at each seed clump density was also examined; the results from an ANOVA for 7 d after sowing (Table 2.5) and for 14 d after sowing (Table 2.6) show that there were highly significant differences ($P<0.001$) between species at every seed-clump density for both 7 and 14 d after sowing. Detailed results of the differences for each species pair can be seen in Tables 2.7-2.10. For the three populations of *P. farinosa* there were non-significant differences between an alpine population and an upland population in England in all seed clump densities except singly-sown seeds, for both 7 and 14 d after sowing. Similar results were also found between the alpine population and a coastal population from England, except at a clump density of 50 and 14 d after sowing. The two populations of *P. farinosa* from northern England showed a slightly significant difference in percentage germination at clump densities of 25 and 50.

In summary, *P. farinosa* from these three populations exhibited almost the same pattern of germination, but whereas *P. farinosa* (a) and *P. farinosa* (c) showed decreased germination at higher densities, a significant decrease was found only in

Table 2.1 The cumulative percentage germination of seed of *Primula farinosa* and its allied species measured at various densities of sowing.

Primula spp.	Number of seeds used per set	Time (days)	Percentage germination in set of (1)	(10)	(25)	(50)
1.*P.farinosa(a)*	150	7	42.0	35.3	32.7	26.7
		14	55.3	45.3	46.7	42.7
2.*P.farinosa(b)*	150	7	29.3	35.4	38.0	40.0
		14	60.7	52.3	55.3	62.7
3.*P.farinosa(c)*	150	7	24.0	23.3	20.0	12.7
		14	71.3	58.0	44.7	41.3
4.*P.frondosa*	150	7	92.0	78.0	68.7	83.3
		14	96.0	89.3	95.3	98.0
5.*P.halleri*	150	7	58.0	60.0	63.0	67.0
		14	92.0	88.0	85.0	91.0
6.*P.scotica*	150	7	42.7	30.7	29.3	47.3
		14	52.0	42.7	44.7	69.3
7.*P.scandinavica*	150	7	46.7	44.0	14.0	30.0
		14	92.0	88.0	84.0	82.7
8.*P.stricta*	150	7	93.3	92.0	93.3	78.7
		14	96.0	98.7	100.0	86.7

Table 2.2 ANOVA of the cumulative percentage germination of seed of *Primula* spp. measured at various degrees of density, 7 days after sowing.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(a)					
BETWEEN GROUPS	3	363.6667	121.2222	1.6606	.2516
WITHIN GROUPS	8	584.0000	73.0000		
TOTAL	11	947.6667			
P.farinosa(b)					
BETWEEN GROUPS	3	193.2100	64.4033	1.3485	.3258
WITHIN GROUPS	8	382.0867	47.7608		
TOTAL	11	575.2967			
P.farinosa(c)					
BETWEEN GROUPS	3	242.6667	80.8889	2.6377	.1213
WITHIN GROUPS	8	245.3333	30.6667		
TOTAL	11	488.0000			
P.frondosa					
BETWEEN GROUPS	3	859.6667	286.5556	3.8378	.0569
WITHIN GROUPS	8	597.3333	74.6667		
TOTAL	11	1457.0000			
P.halleri					
BETWEEN GROUPS	3	108.4000	36.1333	.0943	.9604
WITHIN GROUPS	6	2300.0000	383.3333		
TOTAL	9	2408.4000			
P.scotica					
BETWEEN GROUPS	3	710.3333	236.7778	2.2694	.1575
WITHIN GROUPS	8	834.6667	104.3333		
TOTAL	11	1545.0000			
P.scandinavica					
BETWEEN GROUPS	3	2028.0000	676.0000	1.2488	.3548
WITHIN GROUPS	8	4330.6667	541.3333		
TOTAL	11	6358.6667			
P.stricta					
BETWEEN GROUPS	3	458.6667	152.8889	.7683	.5432
WITHIN GROUPS	8	1592.0000	199.0000		
TOTAL	11	2050.6667			

Table 2.3 ANOVA of the cumulative percentage germination of seed of *Primula* spp. measured at various degrees of density, 14 days after sowing.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(a)					
BETWEEN GROUPS	3	270.3333	90.1111	1.0560	.4197
WITHIN GROUPS	8	682.6667	85.3333		
TOTAL	11	953.0000			
P.farinosa(b)					
BETWEEN GROUPS	3	203.5833	67.8611	1.4887	.2896
WITHIN GROUPS	8	364.6667	45.5833		
TOTAL	11	568.2500			
P.farinosa(c)					
BETWEEN GROUPS	3	1691.6667	563.8889	9.5574	.0051
WITHIN GROUPS	8	472.0000	59.0000		
TOTAL	11	2163.6667			
P.frondosa					
BETWEEN GROUPS	3	125.3333	41.7778	1.7653	.2313
WITHIN GROUPS	8	189.3333	23.6667		
TOTAL	11	314.6667			
P.halleri					
BETWEEN GROUPS	3	69.6000	23.2000	1.2000	.3869
WITHIN GROUPS	6	116.0000	19.3333		
TOTAL	9	185.6000			
P.scotica					
BETWEEN GROUPS	3	1323.6667	441.2222	2.1843	.1677
WITHIN GROUPS	8	1616.0000	202.0000		
TOTAL	11	2939.6667			
P.scandinavica					
BETWEEN GROUPS	3	160.0000	53.3333	1.3223	.3331
WITHIN GROUPS	8	322.6667	40.3333		
TOTAL	11	482.6667			
P.stricta					
BETWEEN GROUPS	3	325.3333	108.4444	.9799	.4491
WITHIN GROUPS	8	885.3333	110.6667		
TOTAL	11	1210.6667			

Table 2.4 Statistical test of significance (LSD) of the percentage germination of the primula seeds measured at various degrees of seed density.

Primula spp.	Time (days)	Levels of significance between the seed in set of: (1): (10)	(1): (25)	(1): (50)	(10): (25)	(10): (50)	(25): (50)
1.*P.farinosa(a)*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
2.*P.farinosa(b)*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
3.*P.farinosa(c)*	7	NS	NS	*	NS	*	NS
	14	NS	**	**	NS	*	NS
4.*P.frondosa*	7	NS	*	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
5.*P.halleri*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
6.*P.scotica*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
7.*P.scandinavica*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS
8.*P.stricta*	7	NS	NS	NS	NS	NS	NS
	14	NS	NS	NS	NS	NS	NS

Table 2.5 ANOVA of *Primula* spp. on percentage germination of seed, measured at various degrees of seed density, 7 days after sowing.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
SET OF 1					
BETWEEN GROUPS	7	14519.3333	2074.1903	19.8171	.0000
WITHIN GROUPS	16	1674.6667	104.6667		
TOTAL	23	16191.0000			
SET OF 10					
BETWEEN GROUPS	7	12590.8783	1798.6969	8.0582	.0003
WITHIN GROUPS	16	3571.4200	223.2137		
TOTAL	23	16162.2983			
SET OF 25					
BETWEEN GROUPS	7	15415.1594	2202.1656	21.8952	.0000
WITHIN GROUPS	15	1508.6667	100.5778		
TOTAL	22	16923.8261			
SET OF 50					
BETWEEN GROUPS	7	13556.1449	1936.5921	7.0656	.0008
WITHIN GROUPS	15	4111.3333	274.0889		
TOTAL	22	17667.4783			

Table 2.6 ANOVA of *Primula* spp. on percentage germination of seed, measured at various degrees of seed density, 14 days after sowing.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
SET OF 1					
BETWEEN GROUPS	7	7695.8333	1099.4048	25.5676	.0000
WITHIN GROUPS	16	688.0000	43.0000		
TOTAL	23	8383.8333			
SET OF 10					
BETWEEN GROUPS	7	10963.6250	1566.2321	25.6935	.0000
WITHIN GROUPS	16	975.3333	60.9583		
TOTAL	23	11938.9583			
SET OF 25					
BETWEEN GROUPS	7	11758.5797	1679.7971	21.8850	.0000
WITHIN GROUPS	15	1151.3333	76.7556		
TOTAL	22	12909.9130			
SET OF 50					
BETWEEN GROUPS	7	9396.9565	1342.4224	10.9795	.0001
WITHIN GROUPS	15	1834.0000	122.2667		
TOTAL	22	11230.9565			

Table 2.7 Statistical test of significance (LSD) of the percentage germination between *Primula* spp. measured for sets of 1 seed density.

Primula spp.	Time (days)	Levels of significance between species 1.	2.	3.	4.	5.	6.	7.	8.
1.*P.farinosa(a)*	7	–	NS	*	NS	NS	NS	NS	***
	14	–	NS	**	***	***	NS	***	***
2.*P.farinosa(b)*	7		–	NS	***	**	NS	NS	***
	14		–	NS	***	***	NS	***	***
3.*P.farinosa(c)*	7			–	***	***	*	*	***
	14			–	***	**	**	**	***
4.*P.frondosa*	7				–	***	***	***	NS
	14				–	NS	***	NS	NS
5.*P.halleri*	7					–	NS	NS	***
	14					–	***	NS	NS
6.*P.scotica*	7						–	NS	***
	14						–	***	***
7.*P.scandinavica*	7							–	***
	14							–	NS
8.*P.stricta*	7								–
	14								–

Table 2.8 Statistical test of significance (LSD) of the percentage germination between *Primula* spp. measured for sets of 10 seed density.

Primula spp.	Time (days)	Levels of significance between species 1.	2.	3.	4.	5.	6.	7.	8.
1.*P.farinosa(a)*	7	–	NS	NS	**	NS	NS	NS	***
	14	–	NS	NS	***	***	NS	***	***
2.*P.farinosa(b)*	7		–	NS	**	NS	NS	NS	***
	14		–	NS	***	***	NS	***	***
3.*P.farinosa(c)*	7			–	***	**	NS	NS	***
	14			–	***	***	*	***	***
4.*P.frondosa*	7				–	NS	**	*	NS
	14				–	NS	***	NS	NS
5.*P.halleri*	7					–	*	NS	*
	14					–	***	NS	NS
6.*P.scotica*	7						–	NS	***
	14						–	***	***
7.*P.scandinavica*	7							–	**
	14							–	NS
8.*P.stricta*	7								–
	14								–

Table 2.9 Statistical test of significance (LSD) of the percentage germination between *Primula* spp. measured for sets of 25 seed density.

Primula spp.	Time (days)	Levels of significance between species 1.	2.	3.	4.	5.	6.	7.	8.
1.*P.farinosa(a)*	7	–	NS	NS	***	**	NS	*	***
	14	–	NS	NS	***	***	NS	***	***
2.*P.farinosa(b)*	7		–	*	**	*	NS	*	***
	14		–	NS	***	**	NS	**	***
3.*P.farinosa(c)*	7			–	***	***	NS	NS	***
	14			–	***	***	NS	***	***
4.*P.frondosa*	7				–	NS	***	***	**
	14				–	NS	***	NS	NS
5.*P.halleri*	7					–	**	***	**
	14					–	***	NS	NS
6.*P.scotica*	7						–	NS	***
	14						–	***	***
7.*P.scandinavica*	7							–	***
	14							–	*
8.*P.stricta*	7								–
	14								–

Table 2.10 Statistical test of significance (LSD) of the percentage germination between *Primula* spp. measured for sets of 50 seed density.

Primula spp.	Time (days)	Levels of significance between species 1.	2.	3.	4.	5.	6.	7.	8.
1.*P.farinosa(a)*	7	–	NS	NS	***	*	NS	NS	**
	14	–	*	NS	***	***	**	***	***
2.*P.farinosa(b)*	7		–	NS	**	NS	NS	NS	*
	14		–	*	**	*	NS	*	*
3.*P.farinosa(c)*	7			–	***	**	*	NS	***
	14			–	***	***	**	***	***
4.*P.frondosa*	7				–	NS	*	**	NS
	14				–	NS	**	NS	NS
5.*P.halleri*	7					–	NS	*	NS
	14					–	*	NS	NS
6.*P.scotica*	7						–	NS	*
	14						–	NS	NS
7.*P.scandinavica*	7							–	**
	14							–	NS
8.*P.stricta*	7								–
	14								–

P. farinosa (c) at 14 d after sowing.

2.3.2 Cold-Stratification

The effects of the pre-chilling treatment on percentage germination are shown in Table 2.11. It can be seen that no germination had occurred in any unchilled seeds 7 d after sowing. 14 d after sowing, however, one species showed >50% germination, although in the remainder no germination, or only very low percentage germination, was found; *P. frondosa* is the one species that germinated well with or without chilling. In contrast, no germination occurred in unchilled seed of *P. scandinavica* even after 6 wk and only a rather poor percentage germination in unchilled seed of *P. farinosa* (b), *P. farinosa* (c), *P. halleri, P. scotica,* and *P. stricta.* An ANOVA for the effect of chilling duration for 0, 2, 4, 6 wk, respectively, in each species revealed that these effects were significant (Tables 2.13-2.19). It should be noted that seeds of the two populations of *P. farinosa* from Upper Teesdale and Blackhall Rocks showed non-significant differences in the effect of chilling duration at days 7 and 14 after sowing (Tables 2.13 -2.14). However, at day 21 after sowing there was a significant difference in percentage germination for different pre-chilling treatments.

The results for LSD tests between chilling durations (0, 2, 4, 6 wk) for each species can be seen in Tables 2.20-2.26. In *P. farinosa* (b) (Table 2.20 and Fig. 2.1a) it can be seen that both chilling duration and the number of days after sowing have a profound effect on germination rate.

No significant differences in percentage germination were found for either unchilled or chilled seed of this population at day 7 after sowing. A significant difference in percentage germination was found at day 14. 2 wk-chilled seed of *P. farinosa* (b) gave a significantly lower in percentage germination than 4 wk-chilled seed or 6 wk-chilled seed 21 d after sowing. Comparisons between 4 wk-chilled seed and 6 wk-chilled seed showed that there were no significant differences between these two treatments. Briefly, we can say that seed of *P. farinosa* from Blackhall Rocks required a chilling duration of 4 wk and show improved percentage germination 14

Table 2.11 Effect of the length of chilling treatment and number of days after sowing on the germination of *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Percent of germination after chilling 0	2	4	6 (wk)
1.*P.farinosa(b)*	7	0.0	1.5	2.0	4.5
	14	0.0	11.0	20.0	15.5
	21	1.0	17.0	34.0	42.0
2.*P.farinosa(c)*	7	0.0	1.0	1.0	0.0
	14	0.0	11.5	14.0	13.0
	21	0.5	18.5	29.0	36.5
3.*P.frondosa*	7	0.0	15.5	74.5	80.5
	14	56.5	91.5	96.5	96.0
	21	73.0	95.5	97.5	96.5
4.*P.halleri*	7	0.0	0.0	19.5	6.0
	14	0.5	45.0	83.5	85.0
	21	4.5	80.0	91.0	91.5
5.*P.scotica*	7	0.0	0.0	27.5	0.0
	14	0.0	24.5	55.5	27.0
	21	0.5	27.0	58.0	40.5
6.*P.scandinavica*	7	0.0	0.0	3.5	12.5
	14	0.0	66.0	74.5	85.0
	21	0.0	94.5	78.0	93.5
7.*P.stricta*	7	0.0	2.0	55.0	-
	14	3.0	10.0	61.5	-
	21	4.5	13.0	61.5	-

Table 2.12 Effect of different duration of preliminary chilling treatments at 20°C on subsequent germination of *Primula* spp. in the glasshouse (from Thompson, 1970).

Primula spp.	Length of chilling treatment: (wk)			
	0	2	4	8
P.farinosa	4	48	52	64
P.frondosa	80	100	88	72
P.halleri	68	72	76	60
P.stricta	0	16	20	36

Table 2.13 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula farinosa* (b).

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	42.0000	14.0000	1.7872	.2031
WITHIN GROUPS	12	94.0000	7.8333		
TOTAL	15	136.0000			
Day 14 after sowing					
BETWEEN GROUPS	3	882.7500	294.2500	4.7017	.0215
WITHIN GROUPS	12	751.0000	62.5833		
TOTAL	15	1633.7500			
Day 21 after sowing					
BETWEEN GROUPS	3	4004.0000	1334.6667	16.0160	.0002
WITHIN GROUPS	12	1000.0000	83.3333		
TOTAL	15	5004.0000			

Table 2.14 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula farinosa* (c).

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	4.0000	1.3333	.6667	.5885
WITHIN GROUPS	12	24.0000	2.0000		
TOTAL	15	28.0000			
Day 14 after sowing					
BETWEEN GROUPS	3	506.7500	168.9167	2.6991	.0926
WITHIN GROUPS	12	751.0000	62.5833		
TOTAL	15	1257.7500			
Day 21 after sowing					
BETWEEN GROUPS	3	2922.7500	974.2500	7.1768	.0051
WITHIN GROUPS	12	1629.0000	135.7500		
TOTAL	15	4551.7500			

Table 2.15 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) in percentage germination in *Primula frondosa*.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	20012.7500	6670.9167	25.9149	.0000
WITHIN GROUPS	12	3089.0000	257.4167		
TOTAL	15	23101.7500			
Day 14 after sowing					
BETWEEN GROUPS	3	4430.7500	1476.9167	32.0488	.0000
WITHIN GROUPS	12	553.0000	46.0833		
TOTAL	15	4983.7500			
Day 21 after sowing					
BETWEEN GROUPS	3	1664.7500	554.9167	13.9602	.0003
WITHIN GROUPS	12	477.0000	39.7500		
TOTAL	15	2141.7500			

Table 2.16 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula halleri*.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	1014.7500	338.2500	11.4338	.0008
WITHIN GROUPS	12	355.0000	29.5833		
TOTAL	15	1369.7500			
Day 14 after sowing					
BETWEEN GROUPS	3	19220.7500	6406.9167	39.2862	.0000
WITHIN GROUPS	12	1957.0000	163.0833		
TOTAL	15	21177.7500			
Day 21 after sowing					
BETWEEN GROUPS	3	21005.0000	7001.6667	75.4795	.0000
WITHIN GROUPS	12	146.0000	12.1667		
TOTAL	15	21151.0000			

Table 2.17 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula scotica*.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	2268.7500	756.2500	36.1554	.0000
WITHIN GROUPS	12	251.0000	20.9167		
TOTAL	15	2519.7500			
Day 14 after sowing					
BETWEEN GROUPS	3	6189.0000	2063.0000	83.0738	.0000
WITHIN GROUPS	12	298.0000	24.8333		
TOTAL	15	6487.0000			
Day 21 after sowing					
BETWEEN GROUPS	3	7058.0000	2352.6667	51.7849	.0000
WITHIN GROUPS	12	186.0000	15.5000		
TOTAL	15	7244.0000			

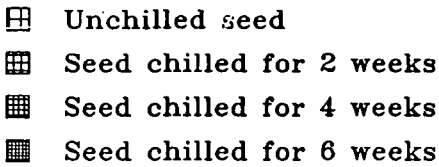
Table 2.18 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula scandinavica*.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	3	418.0000	139.3333	26.9677	.0000
WITHIN GROUPS	12	62.0000	5.1667		
TOTAL	15	480.0000			
Day 14 after sowing					
BETWEEN GROUPS	3	17674.7500	5891.5833	77.6059	.0000
WITHIN GROUPS	12	911.0000	75.9167		
TOTAL	15	18585.7500			
Day 21 after sowing					
BETWEEN GROUPS	3	24270.0000	8090.0000	22.0870	.0000
WITHIN GROUPS	12	230.0000	19.1667		
TOTAL	15	24500.0000			

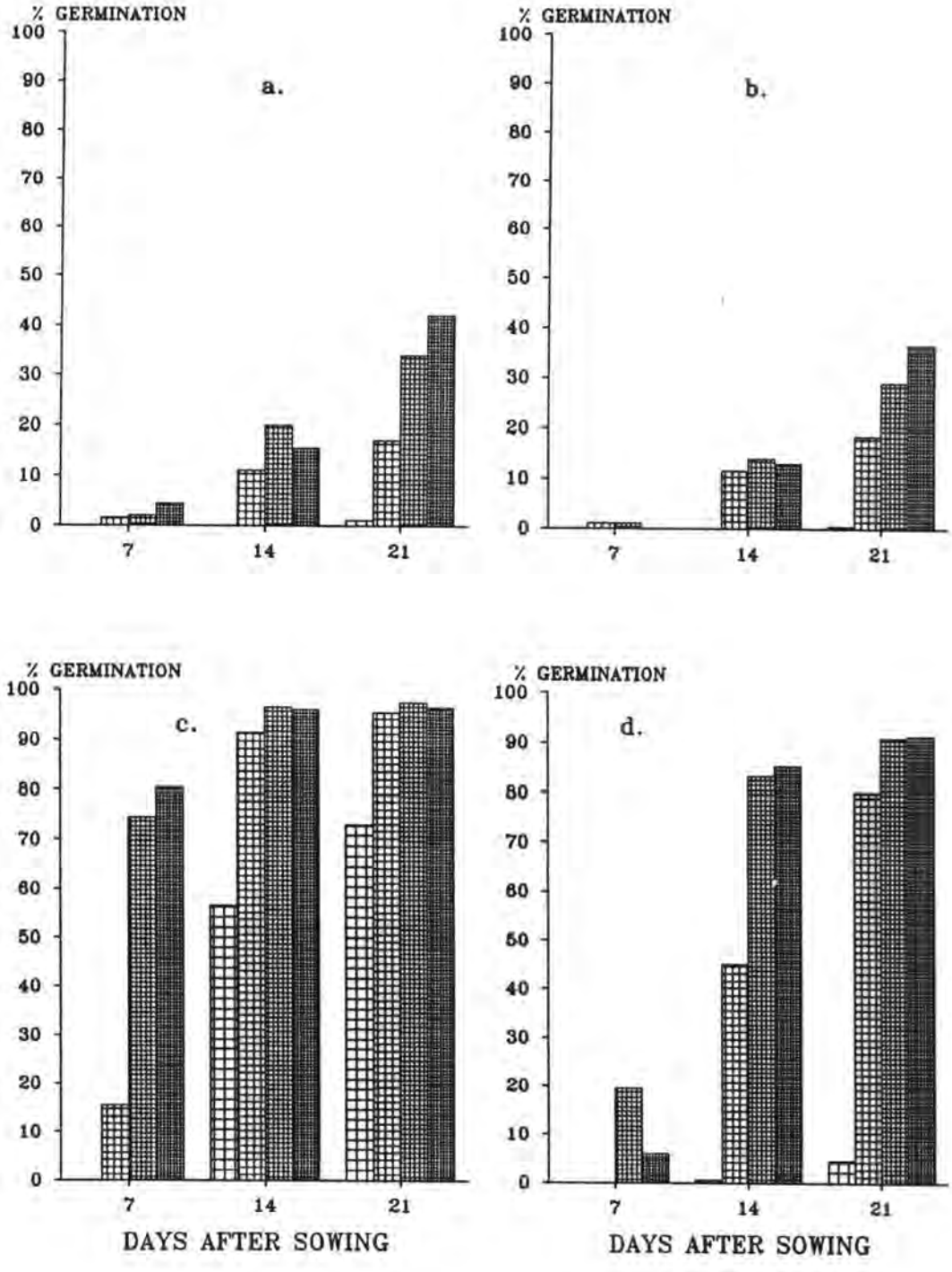
Table 2.19 ANOVA of effect of chilling duration (0, 2, 4, 6 wk) on percentage germination in *Primula stricta*.

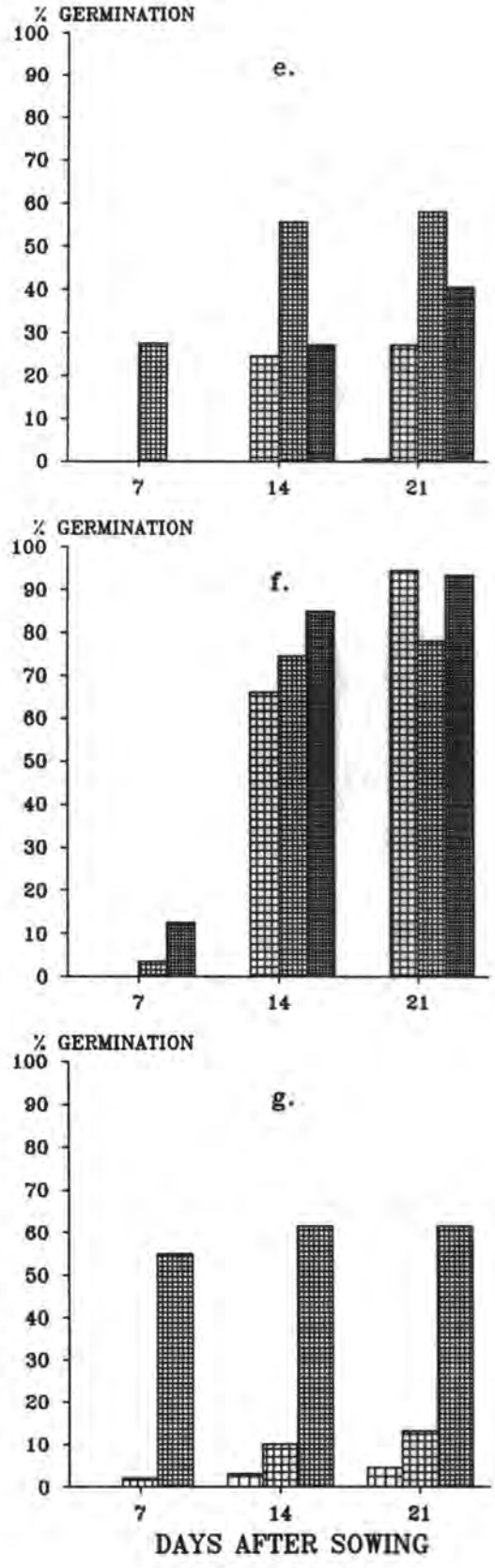
SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Day 7 after sowing					
BETWEEN GROUPS	2	7784.0000	3892.0000	43.4118	.0000
WITHIN GROUPS	9	102.0000	11.3333		
TOTAL	11	7886.0000			
Day 14 after sowing					
BETWEEN GROUPS	2	8164.6667	4082.3333	24.5458	.0000
WITHIN GROUPS	9	295.0000	32.7778		
TOTAL	11	8459.6667			
Day 21 after sowing					
BETWEEN GROUPS	2	7564.6667	3782.3333	40.6653	.0000
WITHIN GROUPS	9	242.0000	26.8889		
TOTAL	11	7806.6667			

Figure 2.1 Effect of chilling treatment on percentage germination in *Primula farinosa* and its allies.



a. *Primula farinosa*(b)
b. *Primula farinosa*(c)
c. *Primula frondosa*
d. *Primula halleri*
e. *Primula scotica*
f. *Primula scandinavica*
g. *Primula stricta*


% GERMINATION
100
90
80
70
60
50
40
30
20
10
0
a.
7
14
21
% GERMINATION
b.
c.
d.
DAYS AFTER SOWING
DAYS AFTER SOWING


% GERMINATION
100
90
80
70
60
50
40
30
20
10
0
e.
7
14
21
% GERMINATION
f.
% GERMINATION
g.
DAYS AFTER SOWING

d or more after sowing. Similar results can be seen from Table 2.21 for the population of *P. farinosa* from Upper Teesdale (Fig. 2.1b). The two populations of *P. farinosa* only differed slightly in chilling duration requirement. These very different environments made it seem likely that the upland population might require a longer chilling period than the coastal population. However, 4 wk-chilled seed and 6 wk-chilled seed of the upland population showed no significant difference in percentage germination.

From Table 2.22 and Fig. 2.1c it can be concluded that although seeds of *P. frondosa* can germinate to some extent without chilling, nevertheless chilling treatment still improved the percentage germination. A chilling duration of 4 wk significantly improved percentage germination and reduced germination time after sowing. A percentage germination of more than 70% could be found in this species within 7 d of sowing. Statistical analyses of percentage germination of *P. halleri* seed are summarized in Table 2.23. Both chilling duration and germination time had a marked effect on seed germination. Again, chilling for 4 wk was sufficient to improve percentage germination and significantly reduce germination time (Fig. 2.1d).

Table 2.24 shows the results of LSD tests on the percentage germination in *P. scotica*. Seed of *P. scotica* required an optimum chilling period of 4 wk. Extending chilling for longer than 4 wk resulted in significant decreases in the germination rate (Fig. 2.1e). Seed of *P. scandinavica* required a chilling treatment of at least 2 wk and 14 d-germination time to improve germination (Table 2.25 and Fig. 2.1f). Its counterpart northern species, *P. stricta* (Table 2.26), required a chilling treatment of 4 wk to give improved percentage germination 14 d after sowing (Fig. 2.1g). Unfortunately, the small number of available seeds limited the experiments which could be performed with this species.

Comparisons of the germination response to chilling treatment between species of the *P. farinosa* complex revealed some interesting information and are summa-

Table 2.20 Statistical tests of significance (LSD) on percentage germination in *Primula farinosa* (b).

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	NS	NS
	14	–	NS	**	*
	21	–	*	***	***
2	7		–	NS	NS
	14		–	NS	NS
	21		–	*	**
4	7			–	NS
	14			–	NS
	21			–	NS
6	7				–
	14				–
	21				–

Table 2.21 Statistical tests of significance (LSD) on percentage germination in *Primula farinosa* (c).

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	NS	NS
	14	–	NS	*	*
	21	–	*	**	***
2	7		–	NS	NS
	14		–	NS	NS
	21		–	NS	*
4	7			–	NS
	14			–	NS
	21			–	NS
6	7				–
	14				–
	21				–

Table 2.22 Statistical tests of significance (LSD) on percentage germination in *Primula frondosa*.

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	***	***
	14	–	***	***	***
	21	–	***	***	***
2	7		–	***	***
	14		–	NS	NS
	21		–	NS	NS
4	7			–	NS
	14			–	NS
	21			–	NS
6	7				–
	14				–
	21				–

Table 2.23 Statistical test of significance (LSD) on percentage germination in *Primula halleri*.

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	***	NS
	14	–	***	***	***
	21	–	***	***	***
2	7		–	***	NS
	14		–	**	***
	21		–	***	***
4	7			–	**
	14			–	NS
	21			–	NS
6	7				–
	14				–
	21				–

Table 2.24 Statistical tests of significance (LSD) on percentage germination in *Primula scotica*.

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	***	NS
	14	–	***	***	***
	21	–	***	***	***
2	7		–	***	NS
	14		–	***	NS
	21		–	***	***
4	7			–	***
	14			–	***
	21			–	***
6	7				–
	14				–
	21				–

Table 2.25 Statistical tests of significance (LSD) on percentage germination in *Primula scandinavica*.

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4	6
0	7	–	NS	NS	***
	14	–	***	***	***
	21	–	***	***	***
2	7		–	NS	***
	14		–	NS	**
	21		–	***	NS
4	7			–	***
	14			–	NS
	21			–	***
6	7				–
	14				–
	21				–

Table 2.26 Statistical tests of significance (LSD) on percentage germination in *Primula stricta*.

Chilling duration(wk)	Days after sowing	Significance levels 0	2	4
0	7	–	NS	***
	14	–	NS	***
	21	–	*	***
2	7		–	***
	14		–	***
	21		–	***
4	7			–
	14			–
	21			–

Table 2.27 ANOVA of percentage germination in *Primula* spp.; effect of chilling treatment.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Unchilled seed, day 14 after sowing					
BETWEEN GROUPS	6	10748.8571	1791.4762	145.8178	.0000
WITHIN GROUPS	21	258.0000	12.2857		
TOTAL	27	11006.8571			
Unchilled seed, day 21 after sowing					
BETWEEN GROUPS	6	17452.0000	2908.6667	148.2573	.0000
WITHIN GROUPS	21	412.0000	19.6190		
TOTAL	27	17864.0000			
Chilled seed 2 wk, day 7 after sowing					
BETWEEN GROUPS	6	761.4286	126.9048	1.28	.3086
WITHIN GROUPS	21	2082.0000	99.1429		
TOTAL	27	2843.4286			
Chilled seed 2 wk, day 14 after sowing					
BETWEEN GROUPS	6	24346.8571	4057.8095	22.0021	.0000
WITHIN GROUPS	21	3873.0000	184.4286		
TOTAL	27	28219.8571			
Chilled seed 2 wk, day 21 after sowing					
BETWEEN GROUPS	6	35707.4286	5951.2381	91.024	.0000
WITHIN GROUPS	21	1373.0000	65.3810		
TOTAL	27	37080.4286			
Chilled seed 4 wk, day 7 after sowing					
BETWEEN GROUPS	6	19779.4286	3296.5714	60.3031	.0000
WITHIN GROUPS	21	1148.0000	54.6667		
TOTAL	27	2 927.4286			
Chilled seed 4 wk, day 14 after sowing					
BETWEEN GROUPS	6	23212.8571	3868.8095	111.7538	.0000
WITHIN GROUPS	21	727.0000	34.6190		
TOTAL	27	23939.8571			

Table 2.27 Continued.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Chilled seed 4 wk, day 21 after sowing					
BETWEEN GROUPS	6	16857.4286	2809.5714	52.7737	.0000
WITHIN GROUPS	21	1118.0000	53.2381		
TOTAL	27	17975.4286			
Chilled seed 6 wk, day 7 after sowing					
BETWEEN GROUPS	5	19629.5000	3925.9000	93.8462	.0000
WITHIN GROUPS	18	753.0000	41.8333		
TOTAL	23	20382.5000			
Chilled seed 6 wk, day 14 after sowing					
BETWEEN GROUPS	5	3 435.3333	6087.0667	166.5155	.0000
WITHIN GROUPS	18	658.0000	36.5556		
TOTAL	23	31 93.3333			
Chilled seed 6 wk, day 14 after sowing					
BETWEEN GROUPS	5	17719.5000	3543.9000	63.3468	.0000
WITHIN GROUPS	18	1007.0000	55.9444		
TOTAL	23	18726.5000			

rized by the results of an ANOVA in Table 2.27. Statistically significant differences ($P<0.0001$) in percentage germination were found between species in all treatments except the 2 wk-chilling treatment at day 7 after sowing. Species varied significantly in magnitude of germination response; detailed results by LSD tests can be seen in Tables 2.28-2.31. Figs. 2.2-2.4 depict the percentage germination of *P. farinosa* and its allies 7, 14, and 21 d after sowing respectively.

In unchilled seed of *Primula* spp., only *P. frondosa* is significantly higher in percentage germination than its allies 14 d after sowing. The other species did not germinate or germinated at very low percentages even up to 21 d after sowing. In 2 wk-chilled seed (Table 2.29), no significant differences between species were found 7 d after sowing. *P. farinosa* from both populations showed the same performance with regard to pre-chilling treatment and time after sowing. They also exhibited a similar response to chilling treatment to some of their allies, e.g. *P. scotica, P. stricta.* Again, *P. frondosa* showed significantly higher percentage germination than its allies 14 d after sowing. However, 21 d after sowing one species, i.e., *P. scandinavica* matched its performance. *P. scandinavica* showed significantly higher percentage germination than the closely related *P. scotica* 14 d after sowing. The European alpine species, *P. halleri,* is also significantly different from all of the other species 14 d after sowing. Arctic *P. stricta* shows significantly lower percentage germination than alpine *P. halleri.* Amongst the northern latitude species, *P. scandinavica* showed significantly higher percentage germination than *P. stricta.*

In 4 wk-chilled seed (Table 2.30), *P. farinosa* from upland and coastal populations still exhibited the same performance. All of their allied species in this experiment showed significantly higher percentage germination, the only exception being *P. scandinavica* 7 d after sowing. 14 d after sowing arctic *P. stricta* and *P. scotica* showed the same response to pre-chilling treatment. In general, in 4 wk-chilled seed significant differences in percentage germination between species can be seen at day 7, a week earlier than in 2 wk-chilled seed. The remainder of the species examined showed a similar performance to that exhibited in the experiment

Figure 2.2 Effect of chilling treatment on percentage germination in *Primula* spp. 7 days after sowing.

a. Unchilled seed
b. Seed chilled for 2 weeks
c. Seed chilled for 4 weeks
d. Seed chilled for 6 weeks

FAB = *Primula farinosa*(b)
FAC = *Primula farinosa*(c)
FRO = *Primula frondosa*
HAL = *Primula halleri*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

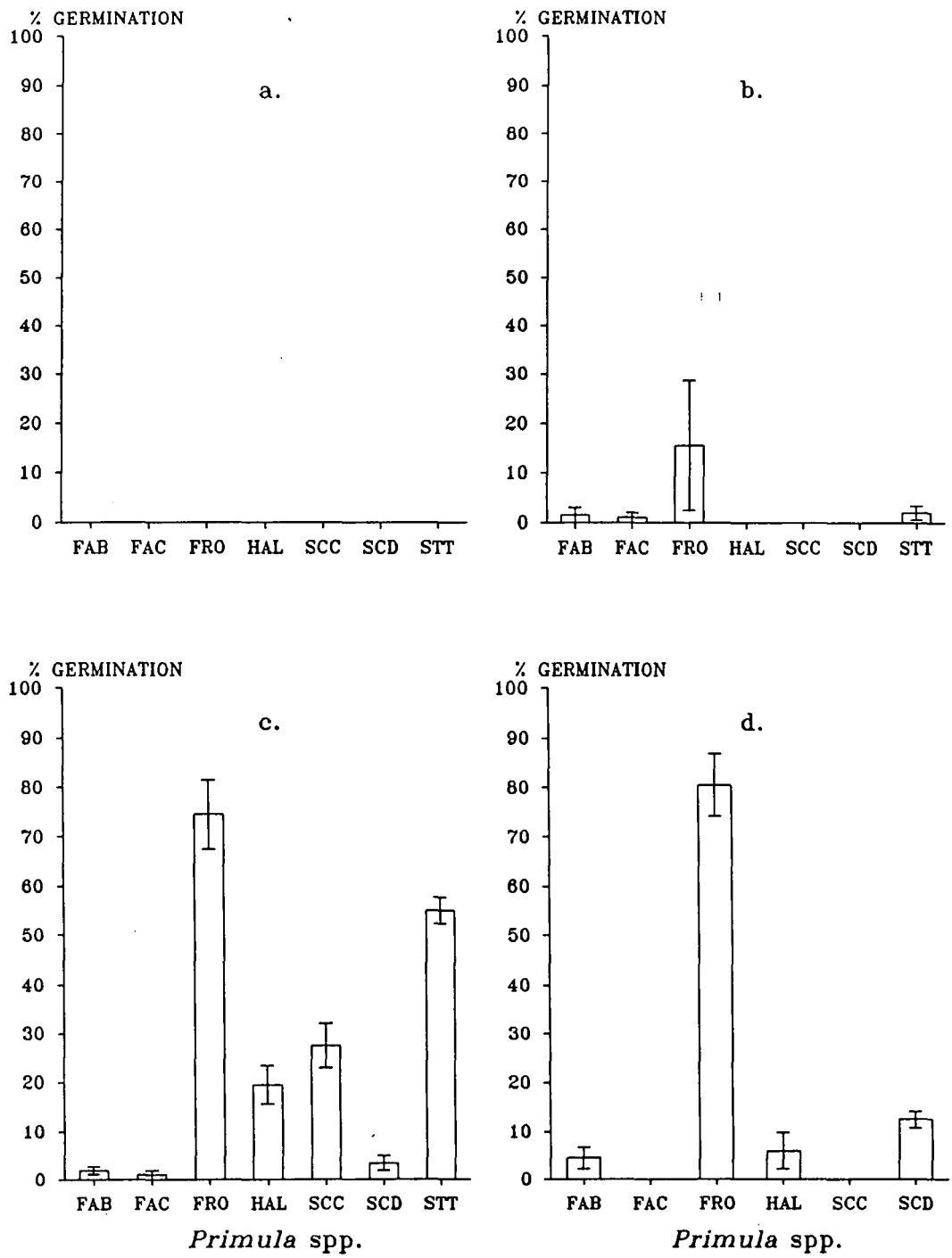

% GERMINATION
a.
b.
c.
d.
100
90
80
70
60
50
40
30
20
10
0
FAB FAC FRO HAL SCC SCD STT
Primula spp.

Figure 2.3 Effect of chilling treatment on percentage germination in *Primula* spp. 14 days after sowing.

a. Unchilled seed
b. Seed chilled for 2 weeks
c. Seed chilled for 4 weeks
d. Seed chilled for 6 weeks

FAB = *Primula farinosa*(b)
FAC = *Primula farinosa*(c)
FRO = *Primula frondosa*
HAL = *Primula halleri*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

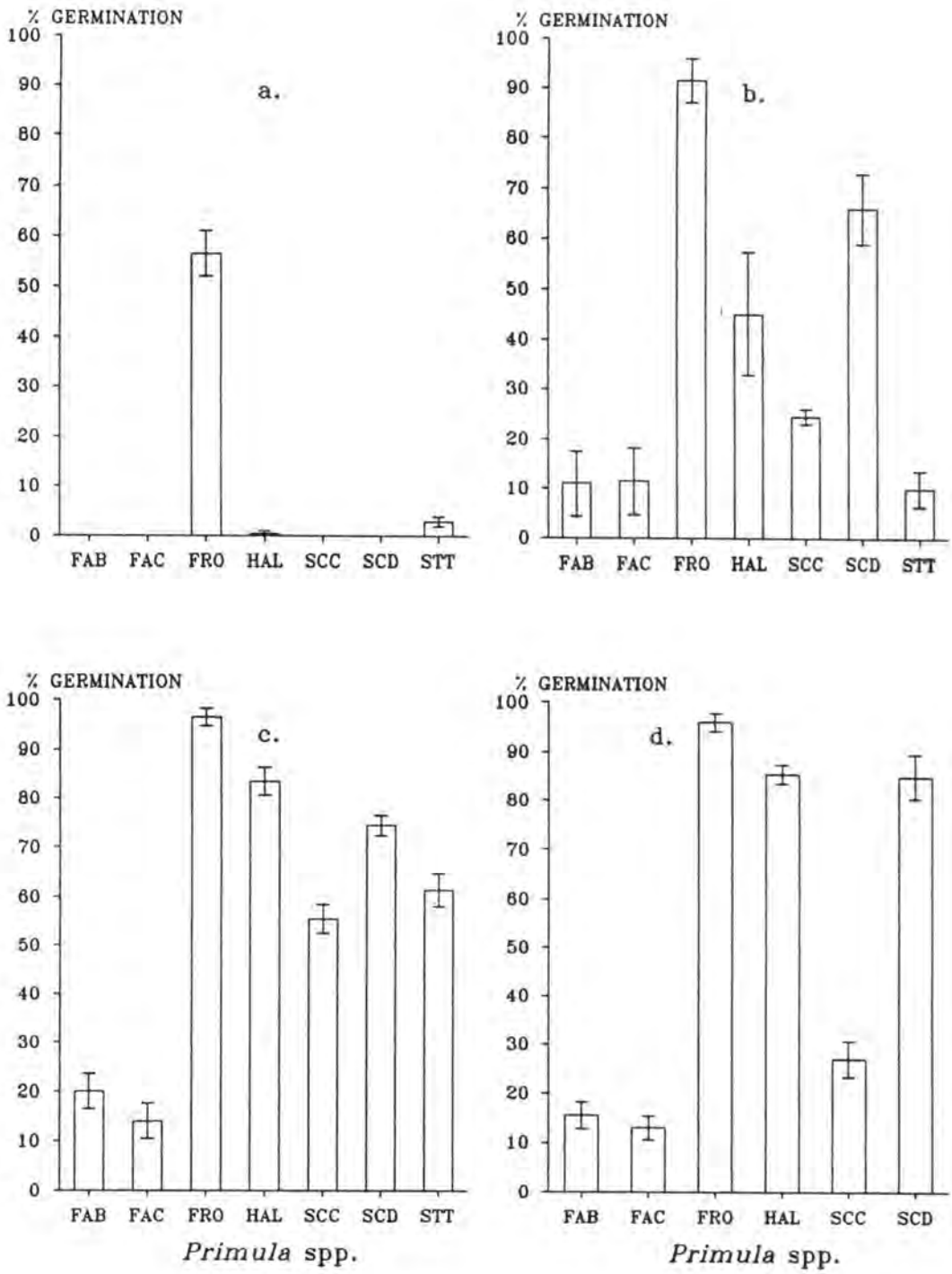

% GERMINATION
a.
b.
c.
d.
100
90
80
70
60
50
40
30
20
10
0
FAB FAC FRO HAL SCC SCD STT
Primula spp.

Figure 2.4 Effect of chilling treatment on percentage germination in *Primula* spp. 21 days after sowing.

a. Unchilled seed
b. Seed chilled for 2 weeks
c. Seed chilled for 4 weeks
d. Seed chilled for 6 weeks

FAB = *Primula farinosa*(b)
FAC = *Primula farinosa*(c)
FRO = *Primula frondosa*
HAL = *Primula halleri*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

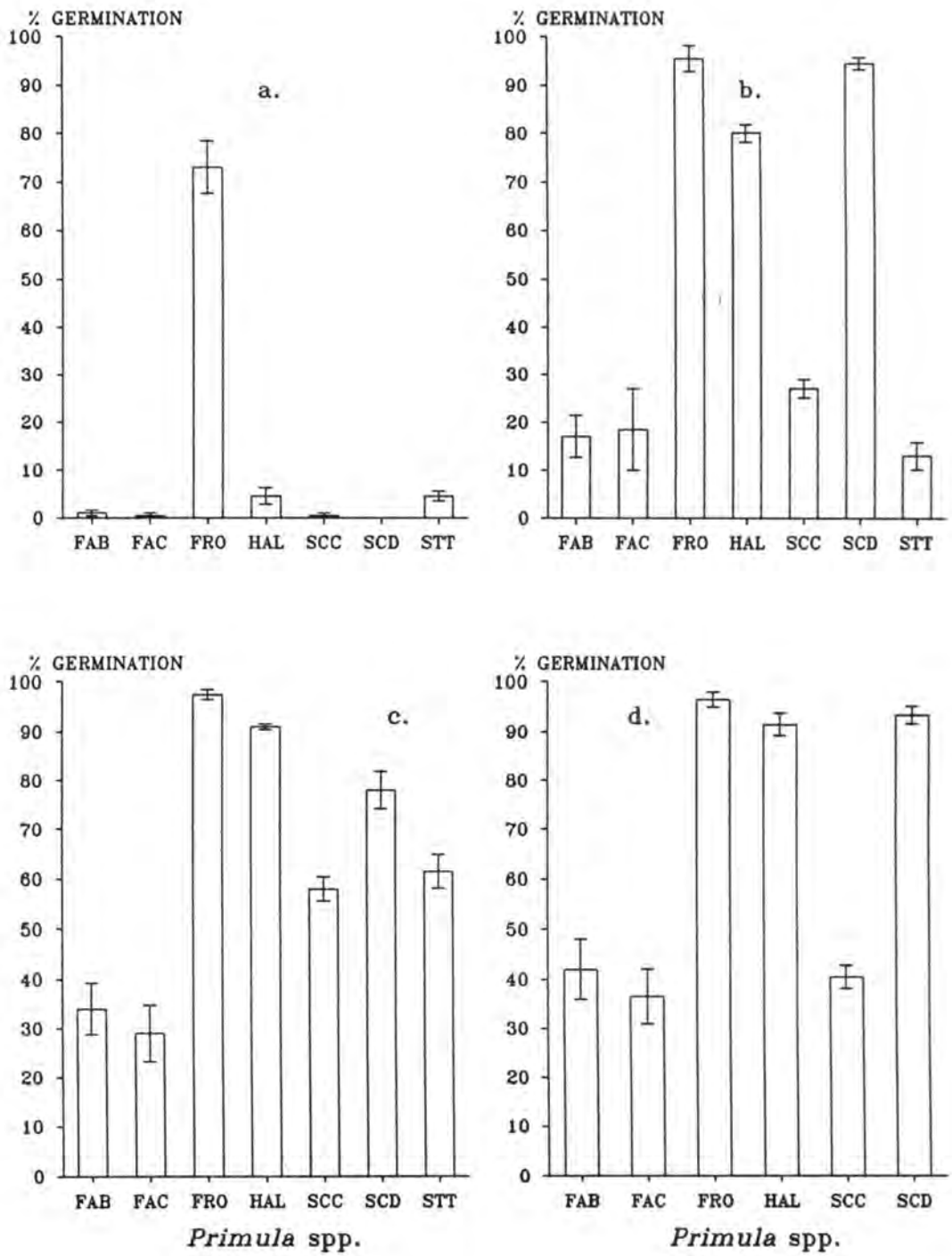

% GERMINATION
a.
b.
c.
d.
100
90
80
70
60
50
40
30
20
10
0
FAB
FAC
FRO
HAL
SCC
SCD
STT
Primula spp.

Table 2.28 Statistical test of significance (LSD) on percentage germination of unchilled seeds in *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Significance levels 1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	7	–	NS	NS	NS	NS	NS	NS
	14	–	NS	***	NS	NS	NS	NS
	21	–	NS	***	NS	NS	NS	NS
2.*P.farinosa(c)*	7		–	NS	NS	NS	NS	NS
	14		–	***	NS	NS	NS	NS
	21		–	***	NS	NS	NS	NS
3.*P.frondosa*	7			–	NS	NS	NS	NS
	14			–	***	***	***	***
	21			–	***	***	***	***
4.*P.halleri*	7				–	NS	NS	NS
	14				–	NS	NS	NS
	21				–	NS	NS	NS
5.*P.scotica*	7					–	NS	NS
	14					–	NS	NS
	21					–	NS	NS
6.*P.scandinavica*	7						–	NS
	14						–	NS
	21						–	NS
7.*P.stricta*	7							–
	14							–
	21							–

Table 2.29 Statistical test of significance (LSD) on percentage germination after chilling for 2 weeks in *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Significance levels						
		1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	7	–	NS	NS	NS	NS	NS	NS
	14	–	NS	***	**	NS	***	NS
	21	–	NS	***	***	NS	***	NS
2.*P.farinosa(c)*	7		–	NS	NS	NS	NS	NS
	14		–	***	**	NS	***	NS
	21		–	***	***	NS	***	NS
3.*P.frondosa*	7			–	NS	NS	NS	NS
	14			–	***	***	*	***
	21			–	*	***	NS	***
4.*P.halleri*	7				–	NS	NS	NS
	14				–	*	*	**
	21				–	***	*	***
5.*P.scotica*	7					–	NS	NS
	14					–	***	NS
	21					–	***	*
6.*P.scandinavica*	7						–	NS
	14						–	***
	21						–	***
7.*P.stricta*	7							–
	14							–
	21							–

Table 2.30 Statistical test of significance (LSD) on percentage germination after chilling for 4 weeks in *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Significance levels 1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	7	–	NS	***	**	***	NS	***
	14	–	NS	***	***	***	***	***
	21	–	NS	***	***	***	***	***
2.*P.farinosa(c)*	7		–	***	**	***	NS	***
	14		–	***	***	***	***	***
	21		–	***	***	***	***	***
3.*P.frondosa*	7			–	***	***	***	**
	14			–	**	***	***	***
	21			–	NS	***	**	***
4.*P.halleri*	7				–	NS	**	***
	14				–	***	*	***
	21				–	***	*	***
5.*P.scotica*	7					–	***	***
	14					–	***	NS
	21					–	***	NS
6.*P.scandinavica*	7						–	***
	14						–	**
	21						–	**
7.*P.stricta*	7							–
	14							–
	21							–

Table 2.31 Statistical test of significance (LSD) on percentage germination after chilling for 6 weeks in *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Significance levels 1.	2.	3.	4.	5.	6.
1.*P.farinosa(b)*	7	–	NS	***	NS	NS	NS
	14	–	NS	***	***	*	***
	21	–	NS	***	***	NS	***
2.*P.farinosa(c)*	7		–	***	NS	NS	*
	14		–	***	***	**	***
	21		–	***	***	NS	***
3.*P.frondosa*	7			–	***	***	***
	14			–	*	***	*
	21			–	NS	***	NS
4.*P.halleri*	7				–	NS	NS
	14				–	***	NS
	21				–	***	NS
5.*P.scotica*	7					–	*
	14					–	***
	21					–	***
6.*P.scandinavica*	7						–
	14						–
	21						–

with 2 wk-chilled seed.

In 6 wk-chilled seed, the two populations of *P. farinosa* confirmed their common performance whilst their allies still exceeded them in percentage germination, except for *P. scotica* at day 7 and 21, and *P. halleri* and *P. scandinavica* at day 7. Extended chilling treatment minimized the differences between species with regard to germination rates; for example no significant difference was found between *P. halleri* and *P. scandinavica* in this experiment.

In order to investigate the interaction between pre-chilling treatment duration and the effect of time after sowing on the percentage germination in each species, a Two-Way ANOVA has been performed and the results are summarized in Table 2.32. The length of chilling treatment, number of days after sowing, and their interactions showed a profound effect on germination ($P<0.0001$), except in one species. In *P. stricta,* the interaction between length of chilling treatment and number of days after sowing was not significant.

The effects of temperature during the germination period were extracted from Tables 2.1 and 2.11 and are summarized in Table 2.33 and Figs. 2.5-2.6. In the experiment on the effect of cumulative density of seed on percentage germination the seeds were maintained at a 22-25°C day temperature alternating with a cooler night temperature, and were kept in the dark at 4°C for 4 wk. Accordingly, seed set in clump of 1 may be compared with 4 wk-chilled seed in this experiment. From Tables 2.33 and 2.34 it can be seen that percentage germination was significantly higher in the diurnally fluctuating temperature regime than at 15°C constant temperature, except in two species, *P. frondosa* and *P. scotica.* The two populations of *P. farinosa* showed a higher percentage germination than their closedly related species, *P. scotica* in the fluctuating temperature regime.

Table 2.32 ANOVA on percentage germination in *Primula* spp.; effects of chilling treatment periods (CT) and numbers of day after sowing.

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F RATIO	F PROB.
Primula farinosa(b)					
MAIN EFFECTS	6829.083	5	1365.817	26.650	0.000
CT	3117.583	3	1039.194	20.277	0.000
DAYS	3711.500	2	1855.750	36.210	0.000
INTERACTIONS	1811.167	6	301.861	5.890	0.000
RESIDUAL	1845.000	36	51.250		
TOTAL	10485.250	47	223.090		
Primula farinosa(c)					
MAIN EFFECTS	5339.833	5	1067.967	15.993	0.000
CT	1921.667	3	640.556	9.592	0.000
DAYS	3418.167	2	1709.083	25.594	0.000
INTERACTIONS	1511.833	6	251.972	3.773	0.005
RESIDUAL	2404.000	36	66.778		
TOTAL	9255.667	47	196.929		
Primula frondosa					
MAIN EFFECTS	40278.917	5	8055.783	70.407	0.000
CT	18196.250	3	6065.417	53.012	0.000
DAYS	22082.667	2	11041.333	96.501	0.000
INTERACTIONS	7912.000	6	1318.667	11.525	0.000
RESIDUAL	4119.000	36	114.417		
TOTAL	52309.917	47	1112.977		
Primula halleri					
MAIN EFFECTS	62283.500	5	12456.700	182.441	0.000
CT	30017.000	3	10005.667	146.544	0.000
DAYS	32266.500	2	16133.250	236.288	0.000
INTERACTIONS	11223.500	6	1870.583	27.397	0.000
RESIDUAL	2458.000	36	68.278		
TOTAL	75965.000	47	1616.277		

Table 2.32 Continued.

SOURCES	SUM OF SQUARES	DF	MEAN SQUARE	F RATIO	F PROB.
Primula scotica					
MAIN EFFECTS	18960.750	5	3792.150	185.738	0.000
CT	13499.583	3	4499.861	220.401	0.000
DAYS	5461.167	2	2730.583	133.743	0.000
INTERACTIONS	2016.167	6	336.028	16.459	0.000
RESIDUAL	735.000	36	20.417		
TOTAL	21711.917	47	461.956		
Primula scandinavica					
MAIN EFFECTS	65594.417	5	13118.883	392.585	0.000
CT	29584.250	3	9861.417	295.105	0.000
DAYS	36010.167	2	18005.083	538.805	0.000
INTERACTIONS	12778.500	6	2129.750	63.733	0.000
RESIDUAL	1203.000	36	33.417		
TOTAL	79575.917	47	1693.105		
Primula stricta					
MAIN EFFECTS	23820.444	4	5955.111	251.624	0.000
CT	23460.222	2	11730.111	495.638	0.000
DAYS	360.222	2	180.111	7.610	0.002
INTERACTIONS	53.111	4	13.278	0.561	0.693
RESIDUAL	639.000	27	23.667		
TOTAL	24512.556	35	700.359		

Table 2.33 Effect of the subsequent temperature after pre-chilling treatment for 4 weeks on the percentage germination of *Primula farinosa* and its allies.

Primula spp.	Days after sowing	Percentage germination 15°C	22-25°C
1.*P.farinosa(b)*	7	2.0	29.3
	14	20.0	60.7
2.*P.farinosa(c)*	7	1.0	24.0
	14	14.0	71.3
3.*P.frondosa*	7	74.5	92.0
	14	96.5	96.0
4.*P.halleri*	7	19.5	58.0
	14	83.5	92.0
5.*P.scotica*	7	27.5	42.7
	14	55.5	52.0
6.*P.scandinavica*	7	3.5	46.7
	14	74.5	92.0
7.*P.stricta*	7	55.0	93.3
	14	61.5	96.0

Table 2.34 Statistical test of significance (t-tests) on percentage germination after chilling for 4 weeks in *Primula farinosa* and its allies, at two different subsequent temperatures.

Primula spp.	Days after sowing	Significance levels
1.*P.farinosa(b)*	7	**
	14	***
2.*P.farinosa(c)*	7	***
	14	***
3.*P.frondosa*	7	NS
	14	NS
4.*P.halleri*	7	*
	14	*
5.*P.scotica*	7	NS
	14	NS
6.*P.scandinavica*	7	***
	14	**
7.*P.stricta*	7	**
	14	**

Table 2.35 Relationship between percentage germination and ploidy levels in *Primula farinosa* complex; effects of chilling treatments and day after sowing.

Treatments of seed	Days after sowing	Correlation Coefficient (r)	Significance levels
Unchilled seed	7	–	–
	14	−0.29998	NS
	21	−0.31117	NS
Chilled seed 2 wk	7	−0.29775	NS
	14	−0.21940	NS
	21	−0.18883	NS
Chilled seed 4 wk	7	+0.26551	NS
	14	+0.22228	NS
	21	+0.11439	NS
Chilled seed 6 wk	7	+0.29448	NS
	14	+0.22339	NS
	21	+0.08287	NS

Table 2.36 Relationship between percentage germination and latitude of origin in *Primula farinosa* complex; effects of chilling treatments and day after sowing.

Treatments of seed	Days after sowing	Correlation Coefficient (r)	Significance levels
Unchilled seed	7	-	-
	14	-0.7598	NS
	21	-0.7861	NS
Chilled seed 2 wk	7	-0.7618	NS
	14	-0.4389	NS
	21	-0.3379	NS
Chilled seed 4 wk	7	-0.6341	NS
	14	-0.4307	NS
	21	-0.4852	NS
Chilled seed 6 wk	7	-0.7162	NS
	14	-0.4245	NS
	21	-0.3997	NS

Figure 2.5 Effect of subsequent temperature on percentage germination in *Primula* spp. after chilling for 4 weeks.

▦ 7 days after sowing, at 15°C
▩ 7 days after sowing, at 22–25°C

FAB = *Primula farinosa*(b)
FAC = *Primula farinosa*(c)
FRO = *Primula frondosa*
HAL = *Primula halleri*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

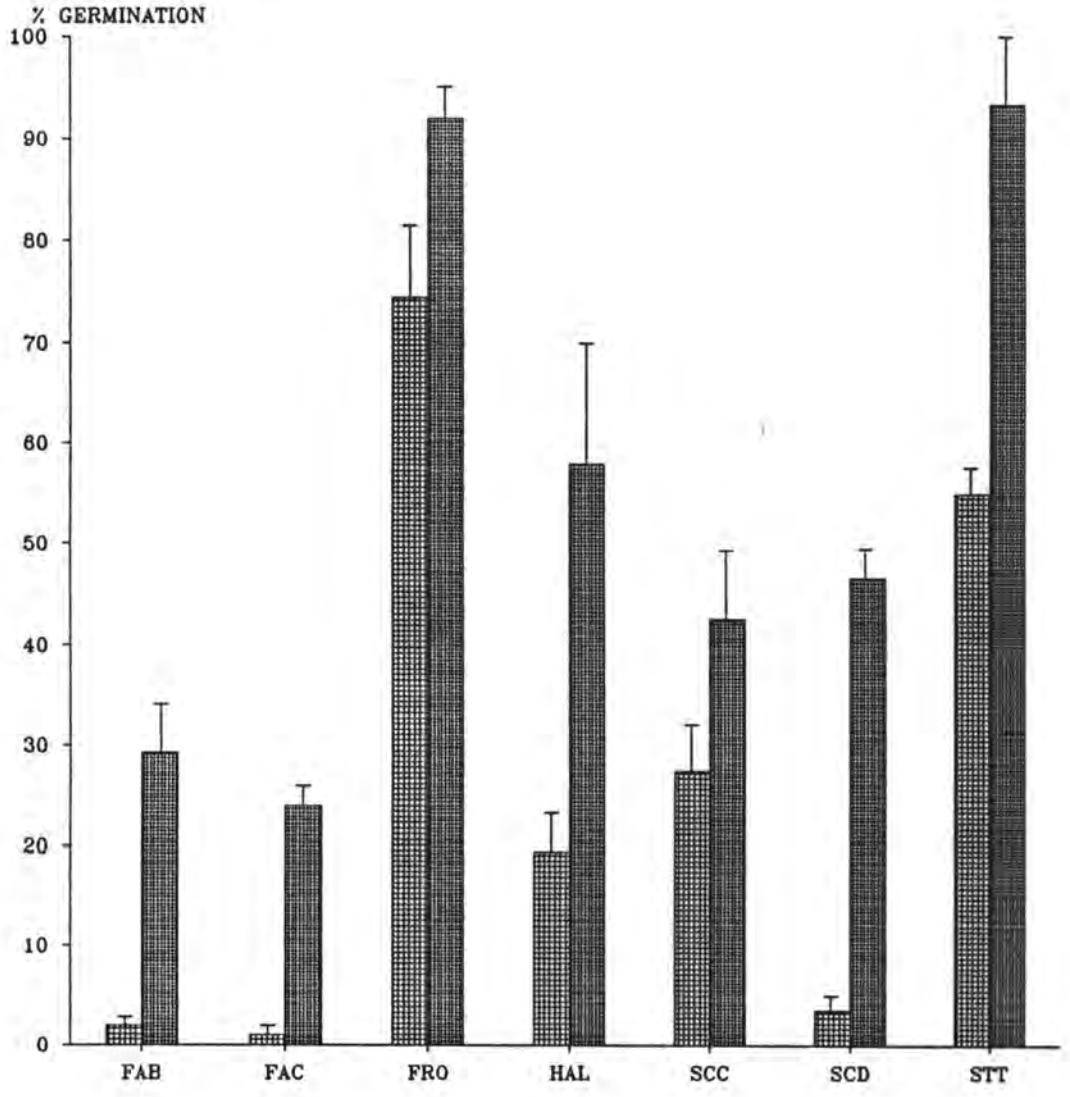
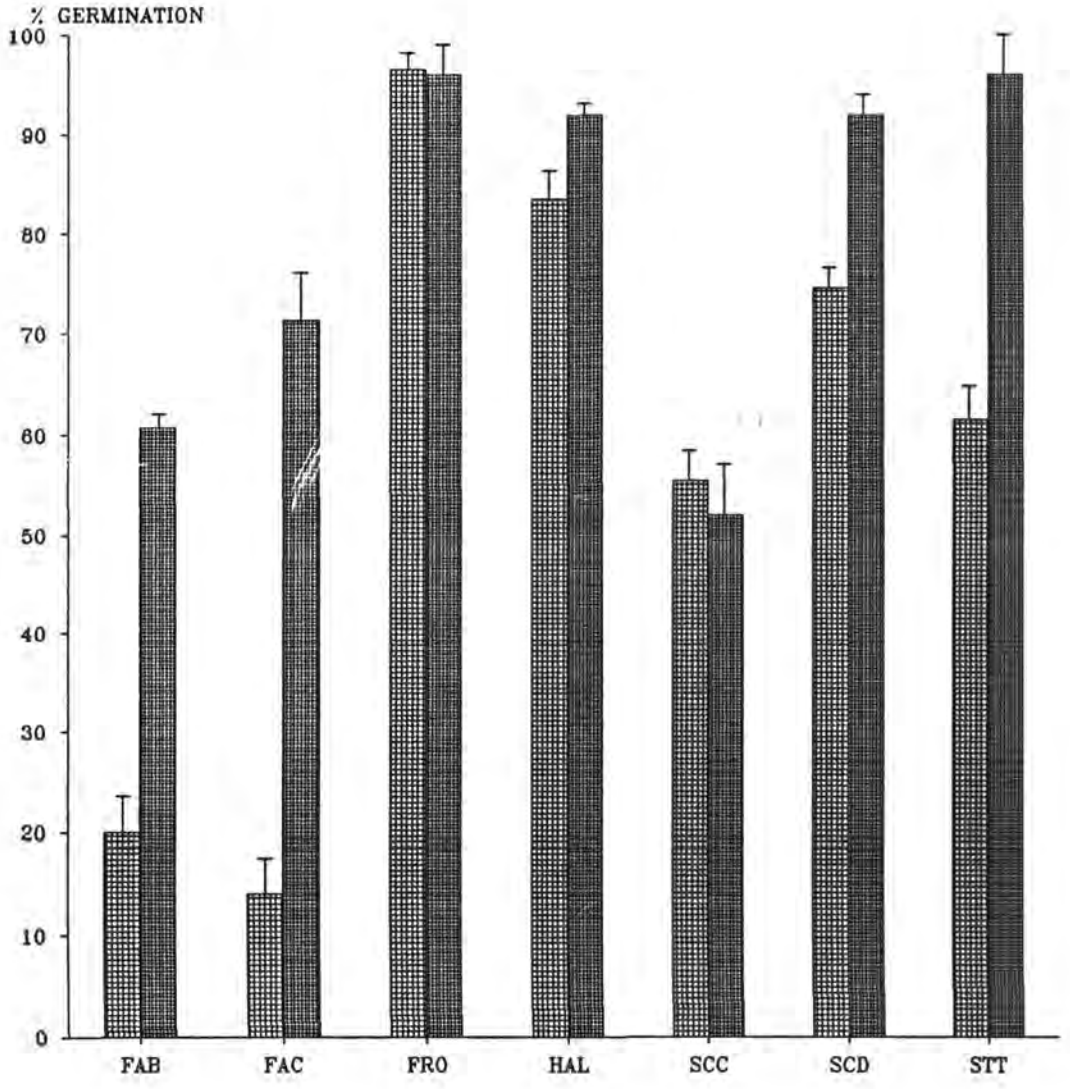

% GERMINATION
100
90
80
70
60
50
40
30
20
10
0
FAB
FAC
FRO
HAL
SCC
SCD
STT

Figure 2.6 Effect of subsequent temperature on percentage germination in *Primula* spp. after chilling for 4 weeks.

▦ 14 days after sowing, at 15°C
▦ 14 days after sowing, at 22–25°C

FAB = *Primula farinosa*(b)
FAC = *Primula farinosa*(c)
FRO = *Primula frondosa*
HAL = *Primula halleri*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.


% GERMINATION
100
90
80
70
60
50
40
30
20
10
0
FAB
FAC
FRO
HAL
SCC
SCD
STT

2.4 Discussion

The results of these experiments showed a negative or neutral effect on percentage germination due to increased densities of seed. These results are in agreement with the conclusions of Linhart (1976). He also suggested that the physiological mechanism responsible for negative responses to density is likely to involve germination inhibitors. A large number of publications demonstrate the occurrence and liberation of germination-inhibiting substances from seeds and fruits (Evenari, 1949). Inhibitors of various chemical classes have been found in seeds of many species, e.g. ABA, Phenolic acids, Coumarin (Boerner, 1960; Bewley & Black, 1985). Evenari (1949) pointed out that germination inhibitors are non-specific, for example tomato juice is effective on tomato seeds, wheat, barley, oat and maize grains. However, the discovery of an inhibitor in a seed does not necessarily mean that it functions in that seed's dormancy mechanism (Bewley & Black, 1985) and an inhibitor may not be liberated under natural conditions in sufficient quantities to inhibit seed germination (Boerner, 1960).

The nature of the habitats of the three populations of *P. farinosa,* have been considered, but unfortunately no details of habitat are available for the seed obtained from the site at 2,000 m above sea level in the mountains of Austria. Of the two sites in northern England, Cronkley Fell, Upper Teesdale is more open than the coastal site at Blackhall Rocks (Heslop-Harrison, 1921). This may account, at least in part, for the difference in response of seed from those two sites with *P. farinosa* from Blackhall Rocks showing a neutral response to increase seed-clump density. Another species, *P. scandinavica,* which showed decreased germination at higher density also showed significant differences from *P. farinosa* (c) at all seed-clump densities. Amongst the species tested *P. frondosa, P. halleri,* and *P. stricta* exhibited high percentage germination at all seed-clump densities but also showed neutral responses to increased seed-clump densities. *P. scotica* seed collected from a sea-cliff site in northern Scotland also showed a neutral response to increased seed-clump densities.

As has been pointed out by Linhart (1976) the effect of seed density upon germination has received scant attention. Clearly, further work is required before the nature of the density-dependent seed germination effect can be fully understood. It should be pointed out that negative density-dependent responses provide a population-regulating mechanism (Palmblad, 1968). Negatively responding species, e.g. many ruderals, produce a large number of seeds under favourable conditions, but only a few germinate at any one time, in order to maintain a seed reservoir over extended periods (Linhart, 1976).

It is widely believed amongst growers that primula seed responds to chilling treatments and various authors have suggested that primula seeds should be exposed to freezing temperatures during the winter in order to improve germination (e.g. Cross, 1940; Amsler, 1945). Germination responses of primula seeds to different pre-chilling durations suggest that germination of these species is enhanced by chilling treatment. However, *P. farinosa* and its allies vary in their germination response to each chilling treatment. They showed statistically significant differences in percentage germination, and on this basis it may be inferred that they differ widely in the depth of dormancy. It has been reported that the depth of dormancy achieved by seeds during maturation depends upon the age of the mother plant and previous climatic adaptation (see Lewak & Rudnicki, 1977). The evidence from this experiment reveals that their dormancy can be overcome by pre-chilling treatments. It should be noted, however, that pre-chilling treatment alone will not usually release seed dormancy. Pre-chilling must usually be followed by subsequent exposure to light or higher temperature or both (Grime *et al.*, 1981; Thompson, 1970).

Thompson (1970) worked on the germination responses of primulas to temperature and found that several species in the Section Farinosae germinated well without chilling and showed little or no response to this treatment. He further concluded that *P. farinosa* and *P. stricta* failed to germinate without chilling, and that all showed improved rates after treatment. This conclusion concurs exactly with the results reported in this experiment. However, from the results in Table

2.11 and Table 2.12 it can be seen that there are some differences in percentage germination in *P. farinosa, P. halleri,* and *P. stricta.* The germination percentages reported by Thompson are somewhat higher than my results for *P. farinosa* and *P. halleri;* but lower for *P. stricta.* In unchilled seed of *P. halleri,* Thompson reports 68% germination, whereas only 4.5% was found in my experiments at day 21 after sowing. The discrepancies between these two experiments may be due to differences in the performance of the different seed lots, differences in the origin of the seeds, differences in the subsequent treatment after chilling and possibly all of these.

From the results of Thompson (1970) and the results reported here, it is reasonable to conclude that cold-requiring seed is an intrinsic characteristic of *P. farinosa* and its allied species. However, no relationship between germination behaviour and ploidy levels was found (Table 2.35). Whittington (1973) in a review of the genetic control of germination concluded that germination characteristics are at least partially under genetic control, and that the pattern of behaviour in cultivated and wild species is likely to have resulted from selection. It is surprising, in view of the fact that some species, e.g. *P. frondosa,* and *P. halleri,* have been in cultivation for a long period (Wright-Smith & Fletcher, 1943), during which time many generations of seedlings have been raised, that these species still show significant dormancy and effects of chilling. It might have been expected that some of the specific requirements, e.g. the chilling requirement, would have been lost as a consequence of artificial selection. In the case of *P. frondosa,* unchilled seeds germinate at rates of up to 50%, but chilling treatment still significantly improves percentage germination. This result shows the existence of the intrinsic characteristic of chilling requirement in even this long-cultivated species.

Results from LSD tests between species indicate that seeds of *P. farinosa* from Upper Teesdale and Blackhall Rocks showed the same performance in all of the treatments. It appears that the long-term isolation of the two populations has not resulted in changes in their specific requirements for germination. In contrast, Heslop-Harrison (1921) reported that seeds of *P. farinosa* from the two populations

germinated at markedly different rates, and about a year later in the upland population. However, the treatment of the seeds and the environment in which the germinating seeds were kept are not recorded in his paper. Comparisons of the germination responses of different populations of a species frequently show the differences between populations, e.g. McNaughton (1966), working with *Typha spp.* and Stearns & Olson (1958) with *Tsuga canadensis,* found differences in germination responses which could be related to variations in the natural origin of the seed. However, there has been evidence reported which supports the results given in this thesis, e.g. Helliwell & Harrison (1978). In studies on the germination, growth and development of *Acer pseudoplatanus* and *Betula verrucosa* they found very few differences in the germination properties of different populations of either species.

The low percentage germination in *P. farinosa* from both populations should not be taken as indication that seeds of this species had lost their viability. Germination behaviour after pre-chilling treatment must also be considered (Thompson, 1973). It can be seen from Table 2.33 that *P. farinosa* from both populations had a germination rate up to 60% within 14 d at 22-25°C instead of only 14-20% at 15°C. These results show the profound effect of subsequent temperature after chilling treatment. The same conclusion can be drawn from the results for the allied species. A further consideration must be that the improvement of germination may be due, at least in part, to the alternation of day and night temperatures. Many authors, e.g. Thompson (1974), working with *Apium graveolens* and *Silene dioica* and Mooney & Billings (1961), with arctic and alpine populations of *Oxyria digyna* reported high percentage germination with alternating temperatures. The results here which report a high percentage germination of primula seeds at higher temperatures (22-25°C) confirm the conclusions of Billings & Mooney (1968) and Sayers & Ward (1966) that the optimum germination temperatures for arctic and alpine plants are rather high (25-30°C).

Non-significant differences in the percentage germination between the two temperature regimes in *P. frondosa* probably indicate that for this species the optimum

temperature is lower than that for its allies. *P. scotica* showed an improved germination rate at 15°C, although it seems unlikely that it reached it optimum germination potential at this temperature. *P. scotica* also showed a significant reduction in percentage germination in the 6 wk- chilling treatment. This same result was obtained by Thompson (1970) for a variety of *Primula* spp.; 19 out of 57 species he examined showed decreased germination after an 8 wk-chilling treatment. However, Thompson (1970) did not show any statistical analysis of his results. It appears likely that prolonged chilling treatment (longer than 4 wk) followed by transfer to a relatively high temperature (15-25°C) results in secondary dormancy (Lewak & Rudnicki, 1977).

The discrepancies in the rate of germination between *P. farinosa* and its allies probably correlate with climatic conditions, or other, characteristics of their natural area of distribution and result from adaptive responses of germination to the environmental conditions (Thompson, 1973; 1981). This is supported by the observation that three closely-related species of *Banksia,* which occur under different conditions along the coasts of eastern Australia differ widely in their temperature responses to germination in ways which could be correlated with particular climatic conditions characteristics of the range or habitat of each species within the area (Sonia & Heslehurst, 1978). Mayer & Poljakoff-Mayber (1982) in a review of the ecology of germination also cited examples of responses in a variety of species which appear to be directly correlated with features of their natural habitats.

It is known that *P. scandinavica* is naturally confined to Scandinavia and that *P. stricta* is a boreal plant (Wright-Smith & Fletcher, 1943; Hultén, 1950; 1958). In this experiment it was found that *P. scandinavica* was very sensitive to chilling treatment and after 2 wk in a cold room (4°C) seed of this species germinated well within 4 d at a warmer temperature. *P. stricta* required a longer period of chilling treatment than *P. scandinavica*; this may be related to the long winter in the Arctic. The long chilling period requirement of *P. stricta* implies that the seed remains viable over the winter. It has been pointed out by Bliss (1962) that the

overwintering of viable seed with germination occurring in moist soil the following spring may be of importance for survival. It is worth noting that Linnington *et al.* (1979) reported that as they increased the period of exposure to low temperature in *Festuca pratensis* var. *apennina* this resulted in increased germination at lower temperatures.

Seed germination under the severe conditions of the Arctic was studied by Sørensen (1941), who found that germination occurred even though the seed remained frozen for half of each 24-h period. Environmental conditions in alpine and arctic habitats seem to promote seed production and germination in particular years; this can have a profound effect on the vegetation pattern of these regions (Bliss, 1962). The two northern species, *P. scandinavica* and *P. stricta* both showed a high rate of germination at warm temperatures after a pre-chilling treatment. Sayers & Ward (1966) pointed out that species with seeds capable of rapid germination could germinate and become established better than species with a slow rate. Ecologically speaking, such an adaptation is important to plants with a northern distribution, since it enables them to make the most of a relatively short growing season.

An attempt to relate percentage germination to latitude of origin in *P. farinosa* and its allies failed, and no relationship was found in any case. (Table 2.36). A lack of correlation between the cold requirement to break dormancy and the altitude from which a population was collected was previously reported by Linnington *et al.* (1979) working with eleven populations of the tetraploid *Festuca pratensis* var. *apennina* from the Italian Alps and Apennines.

From the overall discussion of the ecophysiology of germination in *P. farinosa* and its allies it is clear that much remains to be investigated in this respect. Several possibly important aspects of germination, for example, year to year variation in seed materials, after-ripening, photoperiodic effects, etc. have not been considered here. However, the fact that a good range of germination percentages occurred

in all of the species, and that this related to pre-chilling requirements and the subsequent temperature, increases our understanding of the response of members of the *P. farinosa* complex to these factors and provides an important base for further studies.

CHAPTER 3

ANALYSES OF LEAF CHARACTERISTICS

3.1 Introduction

It has long been known that the characteristic features of leaves are particularly important in determining both the physiological and ecological adaptations of plants, examples being rates of gaseous exchange and heat transfer between plants and their environment. It has become increasingly evident since the work of Raschke (1960) that the morphological and anatomical structure of leaves, leaf size and shape as well as plant habitat have a profound influence on the physiological behaviour of the plant. This is supported by the works of Holmgren (1968), Slatyer (1967) and Gates (1968). From an ecological point of view it is believed that a leaf may be regarded as interacting with its environment in different ways, such as radiation exchange of solar and thermal energy, photosynthesis and respiration heat convection (Raschke, 1960; Slatyer, 1967).

Earlier workers emphasized the significant effects of environmental factors on leaf structure, for example sun and shade leaves are generally known to have marked differences in internal structure (Turrel, 1936). It is generally stated that the leaves of shade plants are thinner and richer in chlorophyll than the leaves of sun plants (Rabinowitch, 1945). Similarly, Wilson & Cooper (1969c), with *Lolium* genotypes, noted that leaves grown in stronger light tended to be thicker than those grown in weak light. Light intensity at which plants were grown also affected the production of palisade tissue -for example, it was found that leaves of ivy responded to strong light by producing palisade tissue, whereas when the same plant was grown in shade (Watson, 1942) there was no similar production. It is only within the last few years that there has been increasing evidence of the physiological consequences

on the internal structure of leaves. It has been reported that with increasing light intensity during leaf development, the mesophyll area per leaf area is positively correlated to mesophyll thickness, and this results in different amounts of internal leaf area being available for the absorption of carbon dioxide (Nobel, 1980). It can be seen that a change in leaf anatomy thus effects not only photosynthesis but also water use efficiency (the ratio of photosynthesis to transpiration) (Nobel, 1980).

The nature of the habitat of a plant also affects leaf size. Raunkiaer (1934) noted that the smallest leaves are associated with the most open communities. This same result was obtained by Böcher (1944), with *Veronica officinalis* (see Lewis, 1972), Lewis(1969), with *Geranium sanguineum* and Valentine (1961), with *Viola riviniana,* who found increasing leaf size with a change from open to shaded habitats. Lewis (1969) also notes that the smaller-leaved types from open habitats are characterized by greater development of palisade mesophyll resulting in greater leaf thickness and higher stomatal frequencies.

In the literature there have been attempts to relate physiological processes with the anatomical structure of leaves of different species. For example, Turrel (1936) found a positive correlation between the internal exposed surface of leaves and the transpiration rate. It has been suggested that thick leaves, which probably have large internal exposed surfaces, transpire more intensively per unit external surface area than thinner leaves, which probably have small internal exposed surfaces. El-Sharkawy & Hesketh (1965) reported that there is a significant negative correlation between the photosynthetic rate and leaf thickness. Again, the data of Dornhoff & Shibles (1976), working with soy beans, may be expressed in the same way and shows the relationship between leaf thickness and the carbon dioxide exchange-rate. Since cell size, cell number and leaf thickness are usually correlated, it is not surprising that mesophyll cell size and cell number of *Lolium perenne* are related to photosynthetic rate (Wilson & Cooper, 1969a,b). It has been pointed out by Wilson & Cooper (1969c), working with *Lolium* genotypes, that the effects of light intensity in which plants are grown on subsequent photosynthesis determination can

be explained in terms of changes in stomatal size. The close relationship between stomatal size and photosynthetic rate has been confirmed by Dornhoff & Shibles (1976), working with soy bean leaves.

Almost all plants experience water stress at some stage of their life cycle. It has been reported that a reduction in cell size is one of the most general anatomical observations which develop in leaves under water stress (Henckel, 1964). It has been suggested that small cell size can be advantageous to plants growing in water deficit conditions, due to the fact that small cells are more elastic resulting in increasing turgor maintenance (Turner, 1979). Accordingly, this may provide an explanation for the different behaviour patterns of hardened and non-hardened plants, small and large cell plants of different species, and small-and large-celled tissues within the same plant.

With respect to the relationships between ploidy levels and leaf characteristics, researches have showed that in *Triticum* species, diploids have smaller cells than hexaploids, which results in them having smaller leaves than the hexaploids (Austin, Morgan, & Ford, 1986). Sax (1938) found a positive correlation between stomatal count and chromosome number. On the other hand, Tan & Dunn (1973), working with *Bromus inermis* found that with increasing ploidy stomatal frequency decreased, but stomatal length increased. Amongst the populations of arctic and alpine *Thalictrum alpinum,* it was found that the arctic triploid plant population had the largest stomata, then the arctic diploid and finally the alpine diploid population the smallest (Mooney & Johnson, 1965).

For some time, attention has been focussed on leaf surface characteristics and their roles in the adaptation of plants. Bliss (1962) noted that a thick cuticle and an abundance of epidermal hairs are believed to be common adaptations of alpine plants. In arid areas, it has also been reported that cuticle thickness increases with increasing aridity (Grieve & Hellmuth, 1970). The upper surface of green leaves normally absorbs 80 to 90% of the Photosynthetically Active Radiation (PAR)

(Björkman, 1973). In contrast, it is found that the pubescence in *Encelia farinosa,* a desert species of the Asteraceae, reduces PAR as much as 56% more than a closedly related but non pubescent species, and accordingly, the photosynthetic rate is reduced (Ehleringer, Björkman & Mooney, 1976). Similarly, Pearman (1965), working with the succulent leaves of *Carpobrotus,* reports that pubescence reduces light absorption by up to 68%. Leaf temperature has also long been recognized to be reduced by the presence of surface features; -this leads to a reduction in transpiration rate and may also prevent inhibition of photosynthesis (Dell & McComb, 1978).

The leaf characteristics of the *P. farinosa* complex have been investigated here as a basis for comparisons of their physiological performances in controlled experiments.

3.2 Materials and methods

3.2.1 Plant materials

Seeds of *Primula farinosa* (b), *P. farinosa* (c), *P. frondosa, P. halleri, P. laurentiana, P. scandinavica, P. scotica,* and *P. stricta* were sown on moist filter paper in Petri dishes at 20°C. Seedlings were transferred to 8 cm plastic pots containing J.Arthur Bower's seed and potting compost. For the other two species, *P. darialica* and *P. modesta,* mature plants were obtained from a garden source (see Table 1.10); the old leaves trimmed and the plants then repotted using the same compost as for the seedlings. The primulas were then placed in a growth chamber, the conditions being as previously described (1.2.3).

3.2.2 Plant microtechnique

The techniques described by Galigher& Kozloff (1964) and Koch (1973) were used throughout.

3.2.2.1 Leaf sampling

Fully expanded leaves were cut and immediately put into water. Each leaf was cut with a sharp razor blade under water into pieces about 3-4 mm long.

3.2.2.2 Fixation

Each piece of leaf, including a section of the mid-rib and a transverse portion from the middle of the blade, was fixed in a mixture of Formalin-Acetic-Alcohol[1] for at least 24 h.

3.2.2.3 Dehydration

After fixing, leaf pieces were washed in 70% ethyl alcohol, then placed in absolute ethyl alcohol for 15 min and transferred to fresh absolute ethyl alcohol for a

[1] prepared from 100 cm^3 of 50% ethyl alcohol, 65 cm^3 of formalin and 2.5 cm^3 of glacial acetic acid

further 15 min. After this time leaf pieces were progressively soaked, for 30 min at each step, in a sequence of mixtures of Histoclear and absolute ethyl alcohol, i.e. (v/v):- 1:3, 1:1, 3:1 respectively, and finally in Histoclear.

3.2.2.4 Wax Infiltration and Embedding

Leaf pieces were placed in a vial, containing a fresh change of Histoclear with melted or shaved Histoplast added to saturate the Histoclear from time to time. The leaf specimens were allowed to stand in the mixture at room temperature overnight.

The vial was kept gently warm for 2-3 h at temperature 30-40°C. Histoplast was added gradually until the mixture was approximately 75% of Histoplast. Leaf specimens were transferred to the vial with pure melted Histoplast and left for at least 2 h, then the Histoplast was replaced by more pure melted Histoplast and left overnight in warm oven at about 56°C (2-3°C above the melting point of the Histoplast). Leaf specimens were embedded in a fresh Histoplast block, which was allowed to harden slowly in a cool water bath.

3.2.2.5 Sectioning

Transverse sections, 7-10 μm thick, were cut using a rotary microtome (Leitz 1512). Leaf sections in wax ribbons were mounted on to clean slides and dried on a warm plate.

3.2.2.6 Staining and Mounting

Staining was performed in Coplin jars containing the following series of mixtures :-

(a) Histoclear I, for 30 min;

(b) Histoclear II, for 15 min;

(c) 1:1 (v/v) of Histoclear/absolute ethyl alcohol, for 15 min;

(d) absolute ethyl alcohol, for 15 min;

(e) 70% ethyl alcohol, for 15 min;

(f) Safranin in 70% ethyl alcohol for at least 2 h.

After these stages the slides were taken back through these steps in reverse order. The leaf sections were counter-stained with light green[2] for 1 min and then mounted with Histomount.

3.2.3 Epidermal peels

Epidermal strips from both upper and lower surfaces of the leaves were obtained by the procedure described by Meidner (1984). An incision was made through the upper epidermis and into the underly ing mesophyll tissue, leaving the lower mesophyll tissue and the lower epidermis intact. The tissue strip was placed on a microscope slide with the upper epidermis facing down. The lower epidermis was lifted with a pair of forceps and bent back sharply. While the epidermis was pulled free of the other tissue the leaf tissue was held on to the slide with another pair of forceps. The lower epidermis came readily off the remainder of the leaf. The angle between the epidermal peel and the remainder of the tissue was kept obtuse to avoid damaging the epidermal cells. Accordingly, the upper epidermis was obtained in the same way. Epidermal strips about 1.0 cm long and 0.5 cm width from the leaf tip, leaf margin and leaf base were mounted together on the same slide, in order to allow average counts to be taken from a single leaf.

3.2.4 Leaf characteristic counts and measurements

Leaf thickness, mesophyll thickness and cuticle thickness were measured from permanent slides, whereas stomatal pore length, width and length of the stomatal apparatus from both surfaces were measured from the freshly mounted epidermal peels using an eyepiece graticule and stage micrometer. The number of mesophyll

[2] prepared from 1 gm light green dye, 75 cm^3 clove oil, and 25 cm^3 absolute ethyl alcohol

cells per sq mm were counted from permanent slides and the number of epidermal cells per sq mm were counted from epidermal strips. Cell size was measured from photographs of epidermal cells using a hand planimeter (Allbrit, England). In this way a high degree of accuracy was attained. The numbers of stomatal apparatus per sq mm (Stomatal frequency) were counted and used as primary data to calculate the Stomatal Index from the formula given below (Salisbury, 1928):-

$$Stomatal\ Index = \frac{number\ of\ stomata\ mm^{-2}}{number\ of\ stomata\ mm^{-2} + number\ of\ epidermal\ cells\ mm^{-2}} \times 100 \quad 3.1$$

All counts and measurements were performed using a light microscope (C. Baker, London) in transmitted light at 10× and 40× magnification and the quoted measurements were the mean values of 9 readings.

3.2.5 Light microscopy

Photomicrographs of leaf sections and epidermal strips were taken using a Leitz Dialux 20 microscope fitted with a Pentax camera body. Images were recorded using Kodak XP1 black and white film, 50 A.S.A.

3.3 Results

Analyses were carried out on 18 leaf characteristics which might be responsible for the differences established in the physiological processes of the tested species. Microscopic observations of leaf anatomy (Fig. 3.1) revealed that the leaves of all species are dorsiventral (Haberlandt, 1914). The mesophyll comprises an adaxial palisade layer, one cell thick, and a spongy mesophyll (Fig. 3.2). The palisade parenchyma consists of cells elongated perpendicular to the surface of the blade, whereas the spongy mesophyll consists of more-or-less isodiametric cells. Both the palisade and the spongy cells contain chloroplasts. It is interesting to note that the unusual features of the primulas' leaves are related to the use of their epidermal layer as chlorenchyma (Fig. 3.3). Occurrences of this characteristic are also found in a number of higher vascular plants, e.g. ferns, flowering aquatic and land plants, especially those of shady habitats (Esau, 1953). Glandular hairs occur on the lower epidermis which secrete a white or yellow powder, commonly known as 'farina' (Blasdale, 1947; Haberlandt,1914). The cuticle is found on both surfaces but its thickness varies between the two surfaces and also differs between species. The surface of the cuticle (Fig. 3.4) is either smooth, e.g. *P. darialica,* or roughened by irregular cracks, e.g. *P. frondosa.* Air-spaces are well marked in the lower mesophyll or beneath the stoma pore. Stomata are present on both surfaces, located at the same level as the ordinary epidermal cells, e.g. *P. farinosa* (b), *P. frondosa, P. darialica* etc., or slightly elevated, e.g. *P. laurentiana, P. scotica* (Fig. 3.5)

3.3.1 Leaf thickness

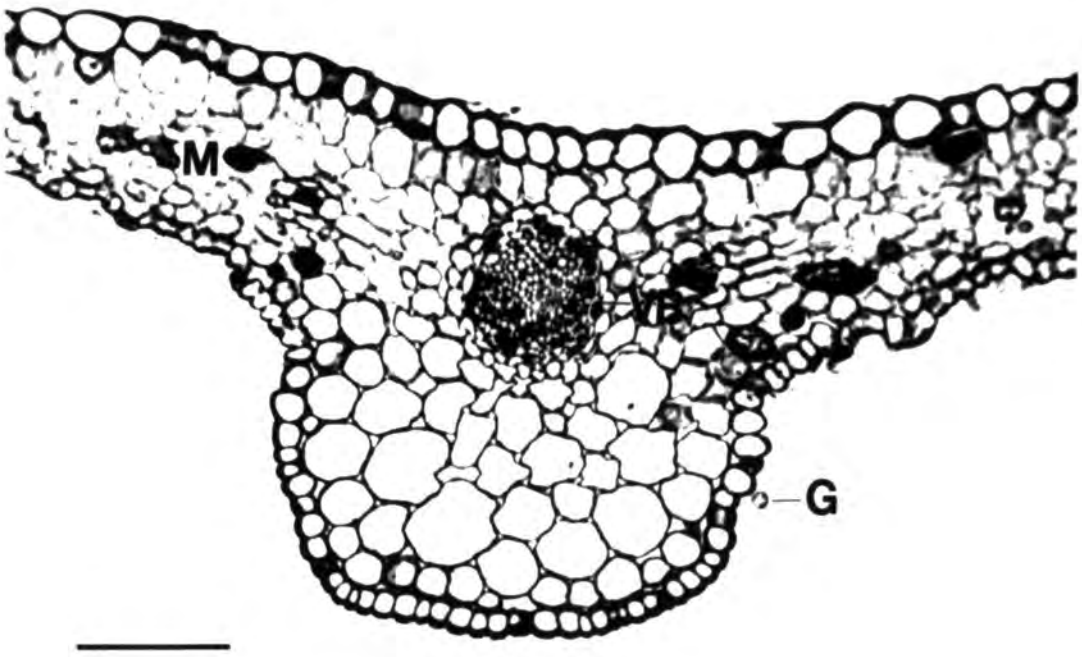
The mean values for the thickness of leaves and their SE are given in Table 3.2. It can be seen that *P. farinosa* from Blackhall Rocks had the thinnest leaf measurement taken and *P. halleri,* and *P. laurentiana* had the thickest leaf measurements recorded. ANOVA from Table 3.1 revealed that there are statistically highly significant differences amongst the mean values of all leaf characteristics in the *P. farinosa* complex ($P<0.001$). The data for leaf thickness was further analyzed

Figure 3.1 LMgraph of cross section through midrib of *Primula farinosa* leaf. Bar represent 15μm.

VB = Vascular bundle
M = Mesophyll
G = Glandular hair

Figure 3.2 LMgraph showing cross section of *Primula farinosa* leaf. Bar represent 5μm.

A = Airspace
G = Glandular hair
P = Palisade parenchyma
S = Spongy parenchyma


M
G

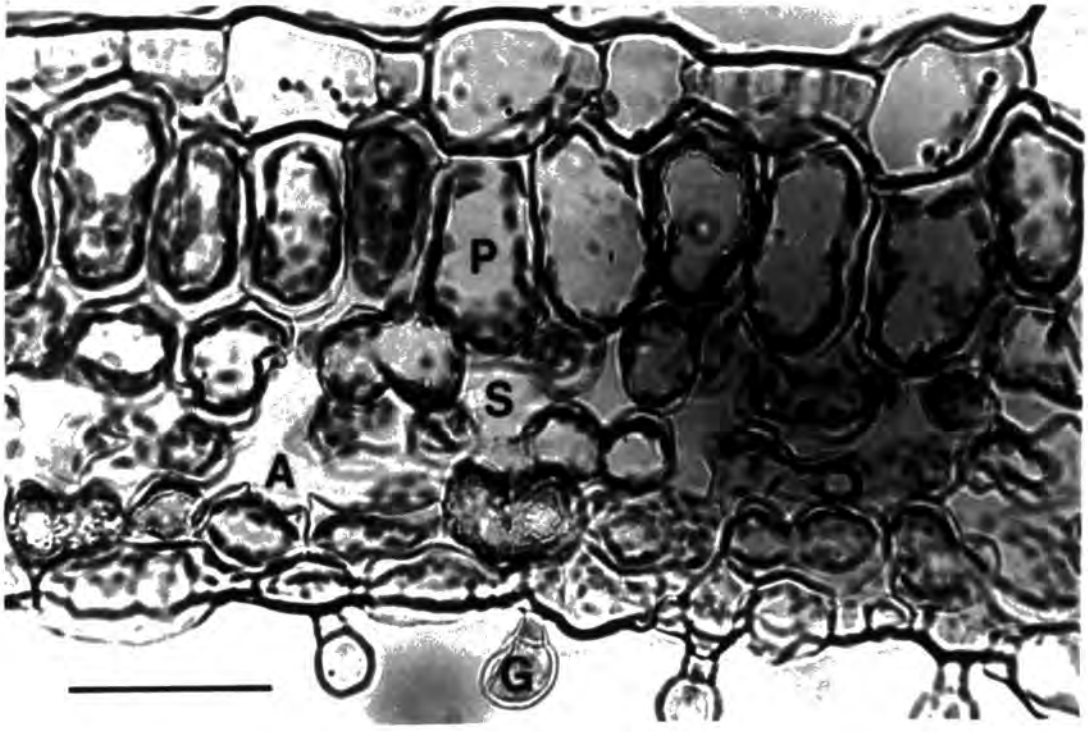
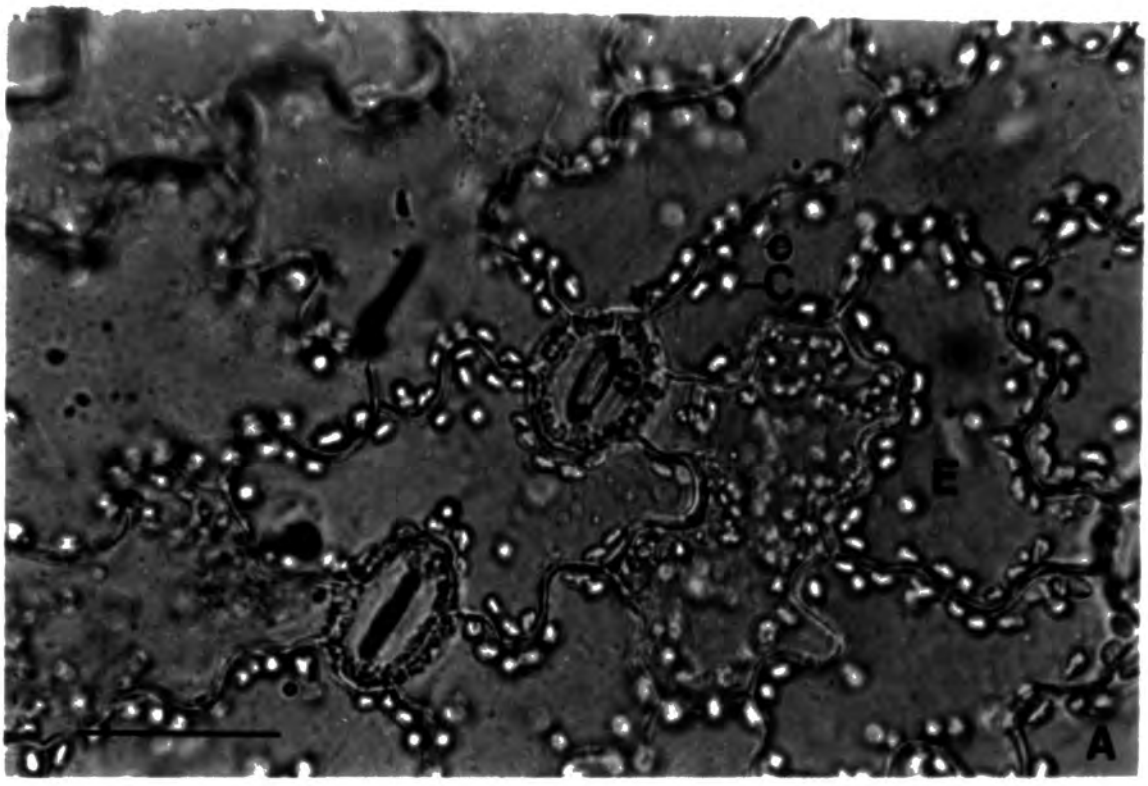

P
S
A
G

Figure 3.3 (A) Adaxial epidermis of *Primula farinosa* showing abundance of chloroplasts in epidermal cells. Bar represent 4μm. Epidermal cell (E); Stomatal apparatus (S); Chloroplast (C).

Figure 3.3 (B) Cross section of *Primula laurentiana*. Notice chloroplasts in epidermal cells and spongy mesophyll cells. Arrows indicate stomatal pores.


C
E
A

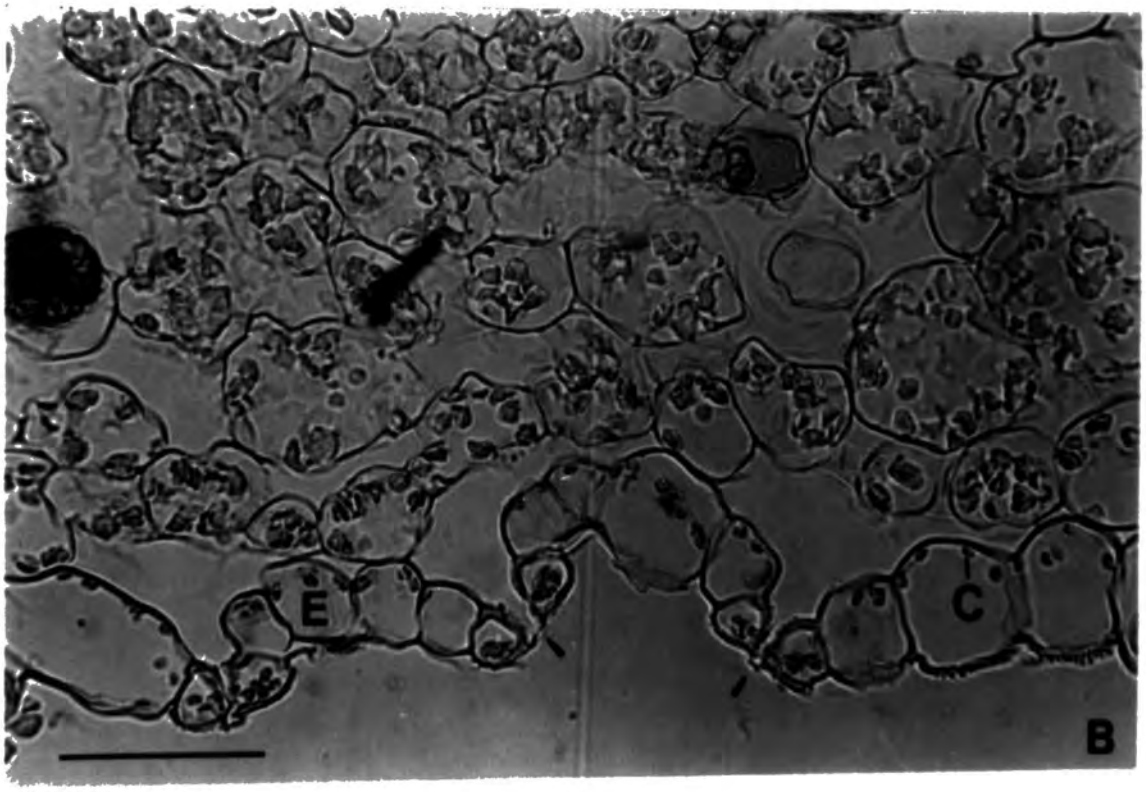

E
C
B

Figure 3.4 Cross sections of primula leaves showing the surface of cuticle.

A *Primula farinosa*(b)
B *Primula farinosa*(c)
C *Primula frondosa*
D *Primula darialica*
E *Primula halleri*
F *Primula laurentiana*
G *Primula modesta*
H *Primula scotica*
I *Primula scandinavica*
J *Primula stricta*

Bar represent 4μm.

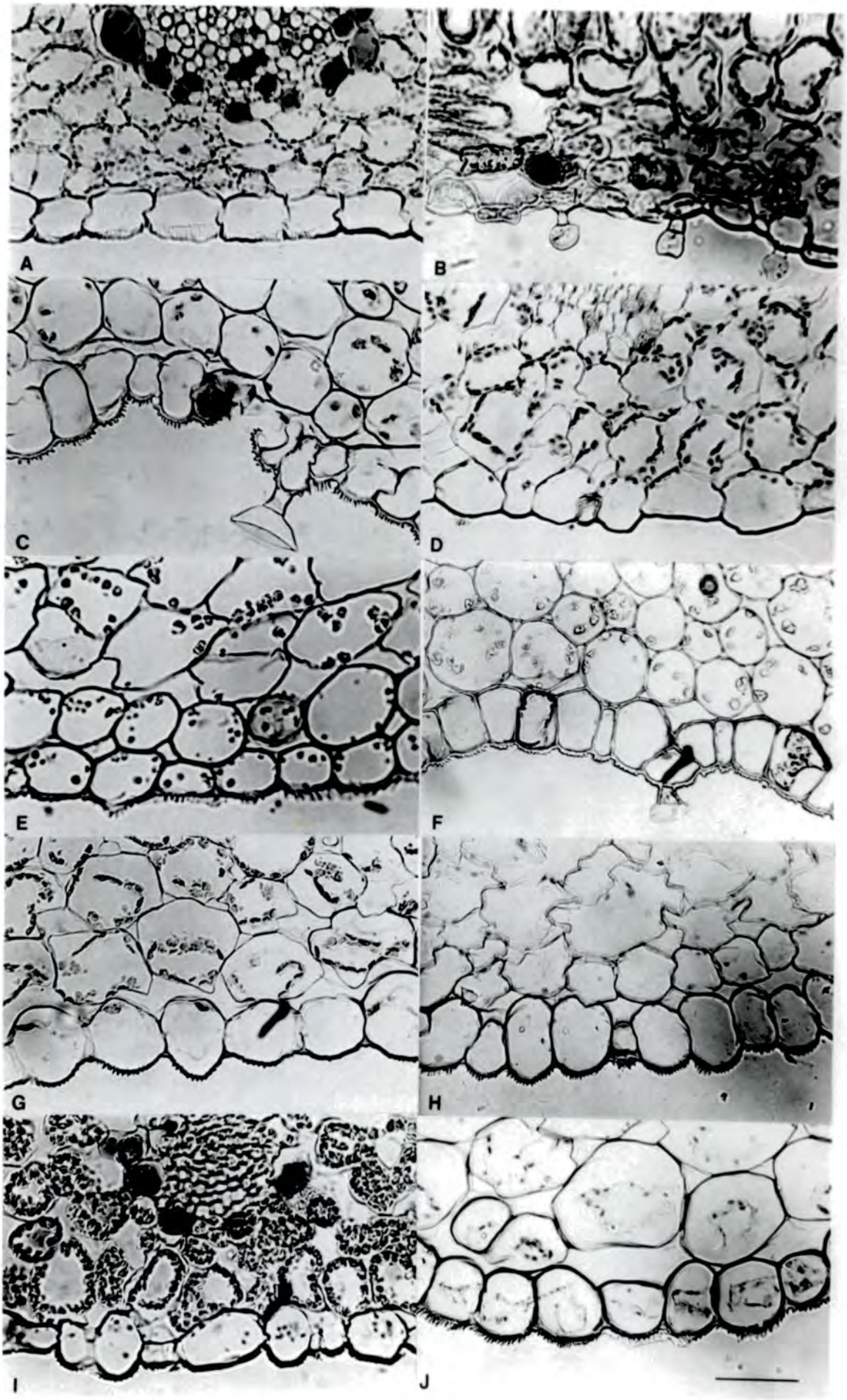

A
B
C
D
E
F
G
H
I
J

Figure 3.5 LMgraph showing cross sections of primula leaves. Notice position of stomata on abaxial surface.

A *Primula farinosa(b)*
B *Primula farinosa(c)*
C *Primula frondosa*
D *Primula darialica*
E *Primula halleri*
F *Primula laurentiana*
G *Primula modesta*
H *Primula scotica*
I *Primula scandinavica*
J *Primula stricta*

Bar represent 4μm.

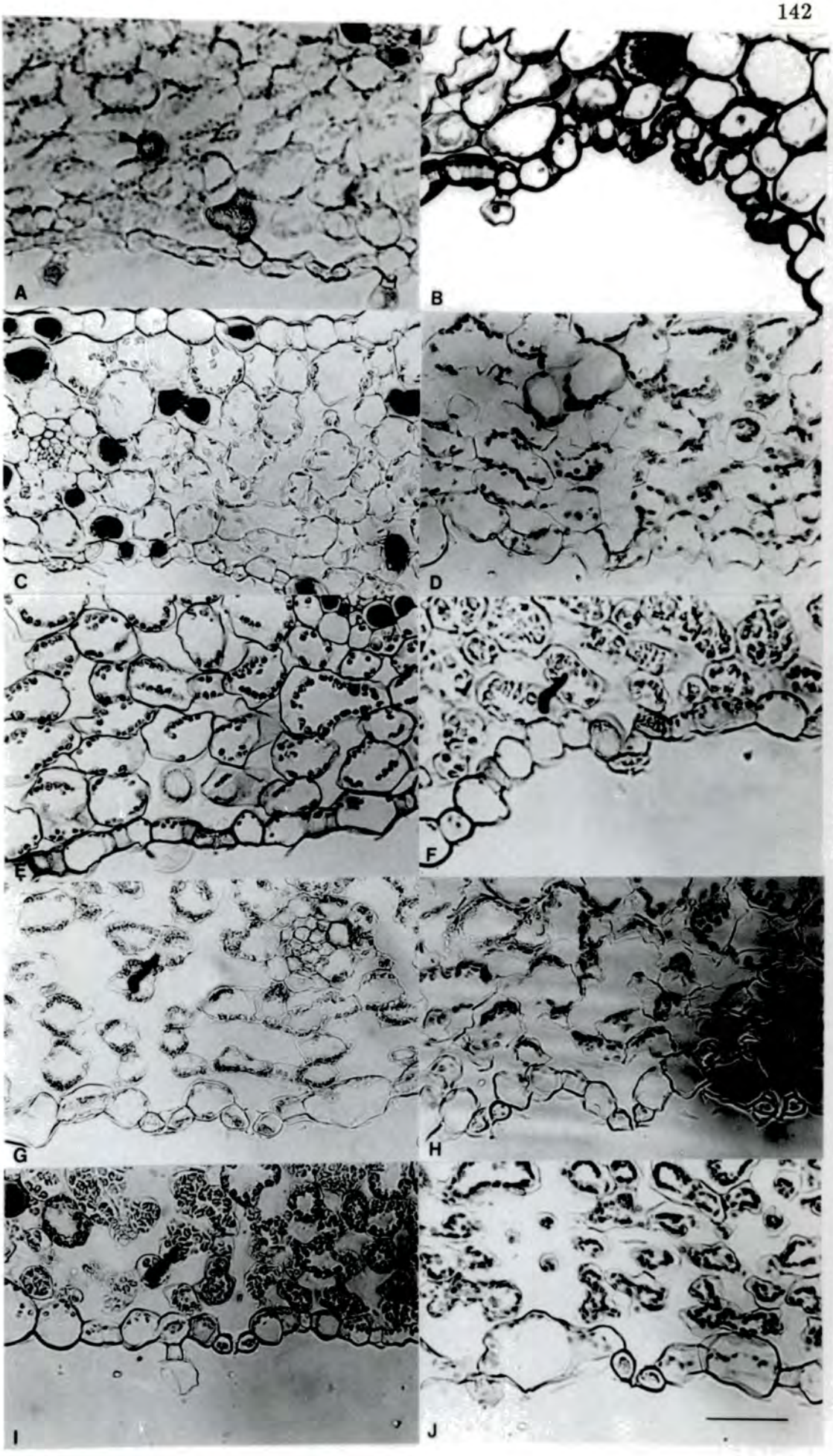

A
B
C
D
E
F
G
H
I
J

Table 3.1 ANOVA of measured leaf characteristics in *Primula farinosa* and its allies.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
THICKNESS OF LEAF (Thickest part of leaf)					
BETWEEN GROUPS	9	.3386	.0376	7.5018	.0000
WITHIN GROUPS	80	.4012	.0050		
TOTAL	89	.7398			
THICKNESS OF LEAF (Thinnest part of leaf)					
BETWEEN GROUPS	9	.0545	.0061	8.0867	.0000
WITHIN GROUPS	80	.0600	.0007		
TOTAL	89	.1145			
THICKNESS OF MESOPHYLL (Thickest part of leaf)					
BETWEEN GROUPS	9	.2035	.0226	9.7983	.0000
WITHIN GROUPS	80	.1846	.0023		
TOTAL	89	.3881			
THICKNESS OF MESOPHYLL (Thinnest part of leaf)					
BETWEEN GROUPS	9	.0391	.0043	9.4997	.0000
WITHIN GROUPS	80	.0366	.0005		
TOTAL	89	.0757			
NUMBER OF MESOPHYLL CELL (per sq mm)					
BETWEEN GROUPS	9	9315430.278	1035047.809	9.9634	.0000
WITHIN GROUPS	80	8310765.778	103884.5722		
TOTAL	89	17626196.06			
THICKNESS OF CUTICLE(upper surface)					
BETWEEN GROUPS	9	86.9694	9.6633	4.0593	.0003
WITHIN GROUPS	80	190.4444	2.3806		
TOTAL	89	277.4139			
THICKNESS OF CUTICLE(lower surface)					
BETWEEN GROUPS	9	211.2250	23.4694	7.5589	.0000
WITHIN GROUPS	80	248.3889	3.1049		
TOTAL	89	459.6139			

Table 3.1 Continued.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
CELL SIZE(sq mm)					
BETWEEN GROUPS	9	98.4362	10.9374	34.2805	.0000
WITHIN GROUPS	80	25.5244	.3191		
TOTAL	89	123.9606			
STOMATAL PORE LENGTH (upper surface)					
BETWEEN GROUPS	8	.0009	.0001	84.6713	.0000
WITHIN GROUPS	72	.0001	.0000		
TOTAL	80	.0010			
STOMATAL PORE LENGTH (lower surface)					
BETWEEN GROUPS	9	.0006	.0001	34.3916	.0000
WITHIN GROUPS	80	.0002	.0000		
TOTAL	89	.0008			
STOMATAL APPARATUS LENGTH (upper surface)					
BETWEEN GROUPS	8	.0043	.0005	178.2867	.0000
WITHIN GROUPS	72	.0002	.0000		
TOTAL	80	.0045			
STOMATAL APPARATUS LENGTH (lower surface)					
BETWEEN GROUPS	9	.0025	.0003	98.2065	.0000
WITHIN GROUPS	80	.0002	.0000		
TOTAL	89	.0027			
STOMATAL APPARATUS WIDTH (upper surface)					
BETWEEN GROUPS	8	.0024	.0003	125.7329	.0000
WITHIN GROUPS	72	.0002	.0000		
TOTAL	80	.0026			
STOMATAL APPARATUS WIDTH (lower surface)					
BETWEEN GROUPS	9	.0011	.0001	62.1849	.0000
WITHIN GROUPS	80	.0002	.0000		
TOTAL	89	.0012			

Table 3.1 Continued.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
STOMATAL FREQUENCY (upper surface)					
BETWEEN GROUPS	9	32.3976	3.5997	7.8159	.0000
WITHIN GROUPS	80	36.8453	.4606		
TOTAL	89	69.2430			
STOMATAL FREQUENCY (lower surface)					
BETWEEN GROUPS	9	2054.1234	228.2359	91.5913	.0000
WITHIN GROUPS	80	199.3515	2.4919		
TOTAL	89	2253.4750			
STOMATAL INDEX (upper surface)					
BETWEEN GROUPS	9	752.4365	83.6041	17.4099	.0000
WITHIN GROUPS	80	384.1669	4.8021		
TOTAL	89	1136.6034			
STOMATAL INDEX (lower surface)					
BETWEEN GROUPS	9	1435.3024	159.4780	37.9911	.0000
WITHIN GROUPS	80	335.8221	4.1978		
TOTAL	89	1771.1245			
NUMBER OF EPIDERMAL CELLS(upper surface)					
BETWEEN GROUPS	9	1192617.822	132513.0914	52.6018	.0000
WITHIN GROUPS	80	201534.0000	2519.1750		
TOTAL	89	1394151.822			
NUMBER OF EPIDERMAL CELLS(lower surface)					
BETWEEN GROUPS	9	4679435.110	519937.2344	151.1606	.0000
WITHIN GROUPS	80	275170.7667	3439.6346		
TOTAL	89	4954605.876			
PRODUCTS OF TOTAL STOMATAL FREQUENCY AND MEAN STOMATAL PORE LENGTH					
BETWEEN GROUPS	8	39.3522	4.9190	52.4927	.0000
WITHIN GROUPS	72	6.7470	.0937		
TOTAL	80	46.0993			

Table 3.2 Leaf thickness in the primulas.

Primula spp.	Leaf thickness (mm)			
	mean	SE	mean	SE
1.*P.farinosa(b)*	0.083	0.004	- 0.200	0.012
2.*P.farinosa(c)*	0.107	0.004	- 0.204	0.006
3.*P.frondosa*	0.135	0.018	- 0.243	0.034
4.*P.darialica*	0.093	0.010	- 0.193	0.015
5.*P.halleri*	0.124	0.008	- 0.376	0.058
6.*P.laurentiana*	0.175	0.009	- 0.357	0.017
7.*P.modesta*	0.137	0.008	- 0.229	0.015
8.*P.scotica*	0.135	0.005	- 0.235	0.007
9.*P.scandinavica*	0.115	0.007	- 0.208	0.011
10.*P.stricta*	0.122	0.008	- 0.257	0.007

to determine whether or not the differences in means of leaf thickness between the species were significant. Detailed results of LSD tests of leaf thickness are summarized in Tables 3.3 and 3.4. *P. farinosa* from both populations in northern England were not statistically different in their leaf thickness. At the thickest part of leaves, *P. halleri* and *P. laurentiana* were not statistically different, but they were highly significantly thicker than the other species. Two populations of *P. farinosa, P. frondosa, P. darialica, P. modesta, P. scotica, P. scandinavica,* and *P. stricta* showed no significant differences in their leaf thickness.

3.3.2 Mesophyll thickness

The mean data with SE for mesophyll thickness measured at the thinnest and the thickest parts of the leaves are shown in Table 3.5. Table 3.1 gives details of ANOVA, showing a highly significant difference in mesophyll thickness within the tested species ($P<0.0001$). Detailed results of the differences between each pair of species can be seen from Tables 3.6-3.7.

3.3.3 Number of mesophyll cells

ANOVA results are summarized in Table 3.1, which shows that there are highly significant differences in the number of mesophyll cells between species of the *P. farinosa* complex ($P<0.0001$). The mean number of mesophyll cells and SE can be seen from Table 3.8, which shows the lowest mean value in *P. stricta* and the highest mean value in *P. farinosa* (b). Table 3.9 shows the results of LSD tests, with a highly significant difference in the number of mesophyll cells between two populations of *P. farinosa.*

3.3.4 Cuticle thickness

The mean values of cuticle thickness from both adaxial and abaxial surfaces and SE of 10 genotypes/species are presented in Table 3.10. Amongst the species, *P. darialica* had the lowest mean cuticle thickness and the highest mean value was found in *P. scandinavica* on the adaxial surface of leaves. On the abaxial surface, *P.*

Table 3.3 Statistical tests of significance (LSD) in leaf thickness (thickest part of leaf) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	NS	***	***	NS	NS	NS	NS
2.*P.farinosa(c)*			NS	NS	***	***	NS	NS	NS	NS
3.*P.frondosa*				NS	***	***	NS	NS	NS	NS
4.*P.darialica*					***	***	NS	NS	NS	NS
5.*P.halleri*						NS	***	***	***	***
6.*P.laurentiana*							***	***	***	**
7.*P.modesta*								NS	NS	NS
8.*P.scotica*									NS	NS
9.*P.scandinavica*										NS
10.*P.stricta*										-

Table 3.4 Statistical tests of significance (LSD) in leaf thickness (thinnest part of leaf) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	***	NS	**	***	***	***	*	**
2.*P.farinosa(c)*			NS	NS	NS	***	*	*	NS	NS
3.*P.frondosa*				**	NS	**	NS	NS	NS	NS
4.*P.darialica*					*	***	**	**	NS	*
5.*P.halleri*						***	NS	NS	NS	NS
6.*P.laurentiana*							**	**	***	***
7.*P.modesta*								NS	NS	NS
8.*P.scotica*									NS	NS
9.*P.scandinavica*										NS
10.*P.stricta*										-

Table 3.5 Leaf mesophyll thickness in the primulas.

Primula spp.	Mesophyll thickness (mm)			
	mean	SE	mean	SE
1.*P.farinosa(b)*	0.058	0.003	- 0.120	0.006
2.*P.farinosa(c)*	0.066	0.003	- 0.137	0.006
3.*P.frondosa*	0.094	0.010	- 0.198	0.033
4.*P.darialica*	0.070	0.009	- 0.128	0.010
5.*P.halleri*	0.097	0.007	- 0.243	0.030
6.*P.laurentiana*	0.133	0.007	- 0.271	0.010
7.*P.modesta*	0.100	0.004	- 0.155	0.009
8.*P.scotica*	0.082	0.005	- 0.156	0.008
9.*P.scandinavica*	0.072	0.009	- 0.146	0.010
10.*P.stricta*	0.074	0.008	- 0.167	0.007

Table 3.6 Statistical tests of significance (LSD) in thickness of mesophyll (thickest part of leaf) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	***	NS	***	***	NS	NS	*	*
2.*P.farinosa(c)*			*	NS	***	***	NS	NS	NS	NS
3.*P.frondosa*				*	NS	NS	NS	NS	*	NS
4.*P.darialica*					***	***	NS	NS	NS	NS
5.*P.halleri*						NS	***	***	***	*
6.*P.laurentiana*							***	***	***	***
7.*P.modesta*								NS	NS	NS
8.*P.scotica*									NS	NS
9.*P.scandinavica*										NS
10.*P.stricta*										-

Table 3.7 Statistical tests of significance (LSD) in thickness of mesophyll (thinnest part of leaf) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	***	NS	***	***	***	*	NS	NS
2.*P.farinosa(c)*			*	NS	**	***	***	NS	NS	NS
3.*P.frondosa*				*	NS	***	NS	NS	*	*
4.*P.darialica*					*	***	**	NS	NS	NS
5.*P.halleri*						***	NS	NS	*	*
6.*P.laurentiana*							**	***	***	***
7.*P.modesta*								NS	*	*
8.*P.scotica*									NS	NS
9.*P.scandinavica*										NS
10.*P.stricta*										-

Table 3.8 Number of mesophyll cells in the primulas.

Primula spp.	No. of mesophyll cells per sq mm	
	mean	SE
1.*P.farinosa(b)*	1879.555	67.834
2.*P.farinosa(c)*	1314.111	58,546
3.*P.frondosa*	1527.000	127.330
4.*P.darialica*	1443.666	92.087
5.*P.halleri*	1295.666	168.404
6.*P.laurentiana*	851.222	58.533
7.*P.modesta*	1541.111	166.936
8.*P.scotica*	1240.000	83.818
9.*P.scandinavica*	1129.000	110.310
10.*P.stricta*	721.444	62.113

Table 3.9 Statistical tests of significance (LSD) in number of mesophyll cells between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		***	*	**	***	***	*	***	***	***
2.*P.farinosa(c)*			NS	NS	NS	**	NS	NS	NS	***
3.*P.frondosa*				NS	NS	***	NS	NS	*	***
4.*P.darialica*					NS	***	NS	NS	*	***
5.*P.halleri*						*	NS	NS	NS	***
6.*P.laurentiana*							***	*	NS	NS
7.*P.modesta*								NS	*	***
8.*P.scotica*									NS	**
9.*P.scandinavica*										**
10.*P.stricta*										-

Table 3.10 Cuticle thickness in the primulas.

Primula spp.	Cuticle thickness(μm) upper surface mean	SE	lower surface mean	SE
1.*P.farinosa(b)*	3.722	0.630	3.055	0.358
2.*P.farinosa(c)*	4.611	0.439	3.944	0.386
3.*P.frondosa*	3.333	0.607	3.777	0.334
4.*P.darialica*	1.722	0.345	1.055	0.176
5.*P.halleri*	1.888	0.217	5.500	0.866
6.*P.laurentiana*	2.777	0.401	4.777	0.596
7.*P.modesta*	3.777	0.703	5.555	0.852
8.*P.scotica*	4.111	0.309	5.116	0.493
9.*P.scandinavica*	4.777	0.813	1.888	0.536
10.*P.stricta*	3.666	0.333	5.666	0.816

stricta had the thickest and *P. darialica* had the thinnest cuticle. ANOVA results of the cuticle thickness are summarized in Table 3.1, showing a highly significant difference between species with respect to the upper surface of leaves ($P<0.001$) as well as for the lower surface ($P<0.0001$). Tables 3.11 and 3.12 are the summaries of LSD tests on both leaf surfaces. When the two populations of *P. farinosa* were compared there was no significant difference in cuticle thickness.

3.3.5 Cell size

The mean values of epidermal cell size and SE are tabulated in Table 3.13. Comparisons between the species showed that there were no significant differences in cell size between two populations of *P. farinosa* (Table 3.14). With all species taken into account, the mean lowest cell size was found in *P. darialica,* whilst the mean highest cell size was found in *P. stricta.* Table 3.1 shows the summaries of ANOVA, with a highly significant difference in cell size between the primulas ($P<0.0001$).

3.3.6 Stomatal pore length

The mean stomatal pore length on both leaf surfaces and SE are summarized in Table 3.15. ANOVA of the stomatal pore length on both surfaces revealed that there were highly significant differences in stomatal pore length between species ($P<0.0001$). From Table 3.15, it can be seen that *P. stricta* had the highest mean value of the stomatal pore length on both surfaces, whilst *P. frondosa* had the smallest mean value on the upper surface and *P. darialica* had the smallest mean value on the lower surface. Detailed results of LSD tests are shown in Table 3.16 and 3.17, from which it can be concluded that the two population of *P. farinosa* were significantly different with regard to their stomatal pore length.

3.3.7 Length and width of stomatal apparatus

The mean values of the length and width of stomatal apparatus revealed the largest size of stomatal apparatus in *P. stricta* for both leaf surfaces, the smallest

Table 3.11 Statistical tests of significance (LSD) in leaf cuticle thickness (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	**	*	NS	**	**	NS	NS
2.*P.farinosa(c)*			NS	***	***	*	NS	NS	NS	NS
3.*P.frondosa*				*	NS	NS	NS	NS	NS	NS
4.*P.darialica*					NS	NS	*	*	***	*
5.*P.halleri*						NS	*	**	***	*
6.*P.laurentiana*							NS	NS	**	NS
7.*P.modesta*								NS	NS	NS
8.*P.scotica*									NS	NS
9.*P.scandinavica*										NS
10.*P.stricta*										–

Table 3.12 Statistical tests of significance (LSD) in leaf cuticle thickness (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	NS	*	*	*	*	NS	**
2.*P.farinosa(c)*			NS	***	NS	NS	NS	NS	*	*
3.*P.frondosa*				**	*	NS	*	NS	*	*
4.*P.darialica*					***	***	***	***	***	***
5.*P.halleri*						NS	NS	NS	***	NS
6.*P.laurentiana*							NS	NS	***	NS
7.*P.modesta*								NS	***	NS
8.*P.scotica*									***	NS
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.13 Epidermal cell size in the primulas.

Primula spp.	Cell size (sq mm)	
	mean	SE
1.*P.farinosa(b)*	1.138	0.082
2.*P.farinosa(c)*	1.347	0.211
3.*P.frondosa*	1.070	0.116
4.*P.darialica*	0.866	0.097
5.*P.halleri*	1.772	0.203
6.*P.laurentiana*	2.885	0.295
7.*P.modesta*	1.065	0.052
8.*P.scotica*	2.219	0.122
9.*P.scandinavica*	1.835	0.229
10.*P.stricta*	4.452	0.286

Table 3.14 Statistical tests of significance (LSD) in leaf epidermal cell size between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	NS	*	***	NS	***	*	***
2.*P.farinosa(c)*			NS	NS	NS	***	NS	**	NS	***
3.*P.frondosa*				NS	**	***	NS	***	**	***
4.*P.darialica*					**	***	NS	***	***	***
5.*P.halleri*						**	**	NS	NS	***
6.*P.laurentiana*							***	NS	***	***
7.*P.modesta*								***	**	***
8.*P.scotica*									NS	***
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.15 Stomatal pore length in the primulas.

Primula spp.	Stomatal pore length(mm)					
	upper surface		lower surface		both surface	
	mean	SE	mean	SE	mean	SE
1.*P.farinosa(b)*	0.019	0.001	0.018	0.000	0.019	0.000
2.*P.farinosa(c)*	0.018	0.000	0.013	0.001	0.015	0.000
3.*P.frondosa*	0.017	0.000	0.014	0.000	0.016	0.000
4.*P.darialica*	0.018	0.000	0.012	0.001	0.015	0.000
5.*P.halleri*	0.018	0.000	0.015	0.000	0.016	0.000
6.*P.laurentiana*	0.025	0.000	0.017	0.000	0.021	0.000
7.*P.modesta*	-	-	0.014	0.000	0.007	0.000
8.*P.scotica*	0.019	0.000	0.019	0.000	0.019	0.000
9.*P.scandinavica*	0.019	0.001	0.017	0.001	0.018	0.000
10.*P.stricta*	0.027	0.001	0.020	0.001	0.023	0.000

Table 3.16 Statistical tests of significance (LSD) in leaf stomatal pore length (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		*	***	*	*	***	–	NS	NS	***
2.*P.farinosa(c)*			NS	NS	NS	***	–	**	*	***
3.*P.frondosa*				NS	NS	***	–	***	***	***
4.*P.darialica*					NS	***	–	**	**	***
5.*P.halleri*						***	–	***	**	***
6.*P.laurentiana*							–	***	***	NS
7.*P.modesta*									–	–
8.*P.scotica*									NS	***
9.*P.scandinavica*										***
10.*P.stricta*										–

Table 3.17 Statistical tests of significance (LSD) in leaf stomatal pore length (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*	***	***	***	***	NS	***	NS	NS	**	
2.*P.farinosa(c)*		NS	NS	**	***	NS	***	***	***	
3.*P.frondosa*			**	NS	***	NS	***	***	***	
4.*P.darialica*				***	***	*	***	***	***	
5.*P.halleri*					**	*	***	***	***	
6.*P.laurentiana*						***	**	NS	***	
7.*P.modesta*							***	***	***	
8.*P.scotica*								*	*	
9.*P.scandinavica*									***	
10.*P.stricta*										-

size in *P. frondosa* with respect to the upper surface and in *P. darialica* for the lower surface (Fig. 3.6 and Table 3.18). Summaries of the results from ANOVA revealed highly significant differences in the length and width on both surface between the primulas ($P<0.0001$). Results from LSD tests showed a significant difference in the size of stomatal apparatus between two populations of *P. farinosa,* except stomatal width on lower surface (Table 3.19 to 3.22).

3.3.8 Stomatal frequencies

The mean stomatal frequencies for both surfaces are shown in Table 3.23. Fig. 3.15 depicts the total stomatal frequency in the primulas. It can be seen that stomata are scattered on both surfaces with a rather higher frequency on the lower surface. Accordingly, the leaves of primulas are said to be amphistomatous (Meidner & Mansfield, 1968). However, there are two species, *P. modesta* and *P. scotica,* which showed relatively low stomatal frequencies on the upper surfaces. In contrast *P. modesta* showed the highest stomatal frequency on the lower surfaces. Comparisons between the species indicated that there were highly significant differences in stomatal frequencies for both surfaces ($P<0.0001$).

3.3.9 Stomatal indices

From Table 3.23 it can be seen that on the upper surfaces the lowest mean value of the stomatal index was in *P. modesta* and the highest mean was *P. stricta.* On the lower surfaces the highest mean value was found to be in *P. scandinavica* and the lowest mean value found in *P. darialica.* Table 3.1 shows the results of ANOVA, and it can be seen that there were highly significant differences ($P<0.0001$) in the stomatal index between species for both surfaces. LSD tests (Tables 3.26 and 3.27) revealed that there were significant differences between two populations of *P. farinosa.*

3.3.10 Number of epidermal cells

Table 3.28 gives the mean values of the number of epidermal cells and SE

Figure 3.6 LMgraph showing adaxial epidermis from epidermal strips of *Primula farinosa* and its allies. Notice size of stomatal apparatus in relation to chromosome numbers.

A *Primula farinosa(b)*, 2n = 18
B *Primula farinosa(c)*, 2n = 18
C *Primula frondosa*, 2n = 18
D *Primula darialica*, 2n = 18
E *Primula halleri*, 2n = 36
F *Primula laurentiana*, 2n = 72
G *Primula scotica*, 2n = 54
H *Primula modesta*, 2n = 18
I *Primula scandinavica*, 2n = 72
J *Primula stricta*, 2n =126

Bar represent 5μm.

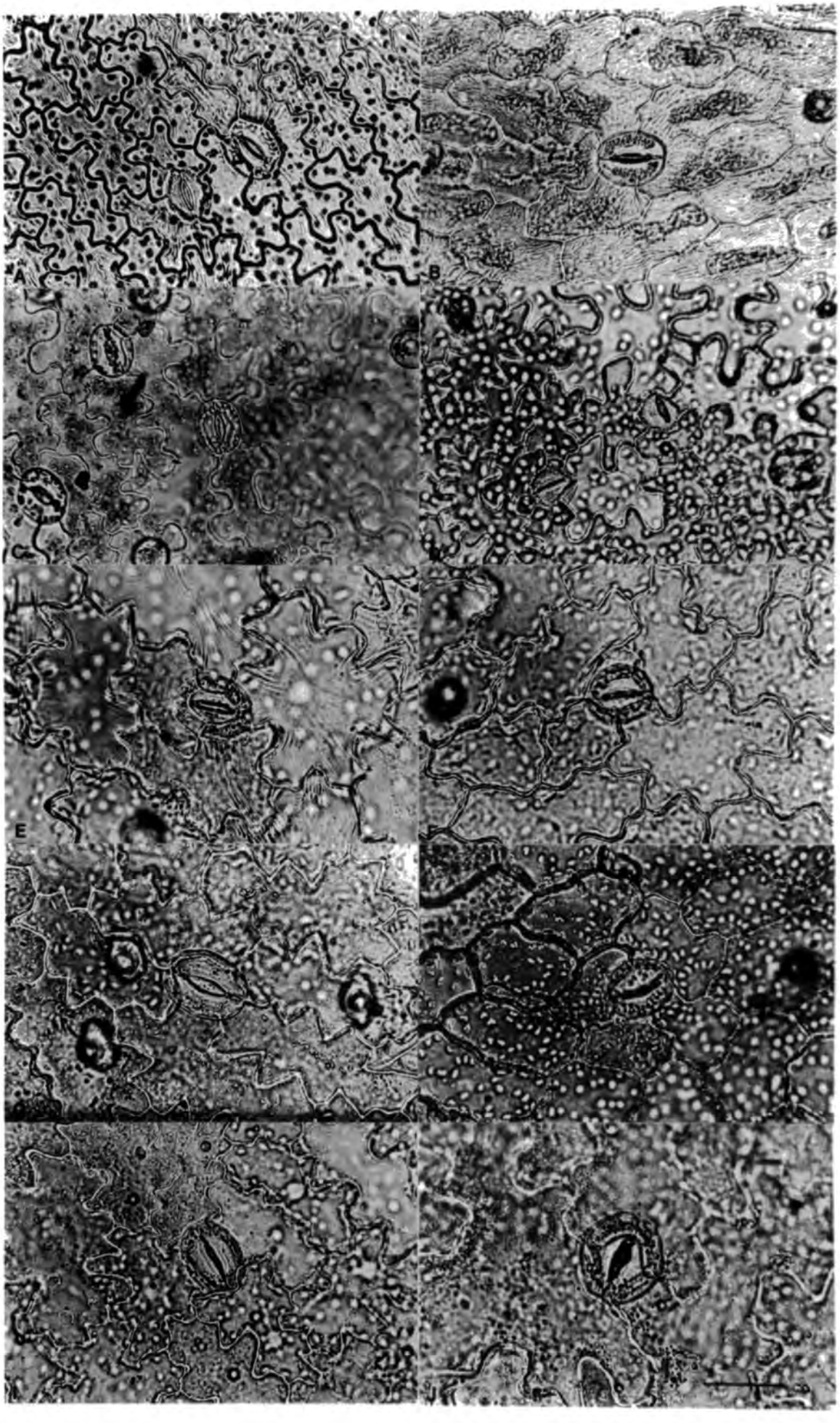

A
B
E

Table 3.18 Length and width of the stomatal apparatus of the primulas.

Primula spp.	Stomatal apparatus upper surface length(mm) mean	SE	width(mm) mean	SE	Stomatal apparatus lower surface length(mm) mean	SE	width(mm) mean	SE
1.*P.farinosa(b)*	0.034	0.001	0.026	0.001	0.035	0.001	0.027	0.000
2.*P.farinosa(c)*	0.036	0.000	0.029	0.001	0.030	0.001	0.028	0.000
3.*P.frondosa*	0.030	0.001	0.024	0.000	0.031	0.001	0.026	0.000
4.*P.darialica*	0.031	0.001	0.024	0.001	0.027	0.000	0.025	0.000
5.*P.halleri*	0.036	0.000	0.030	0.000	0.032	0.000	0.029	0.001
6.*P.laurentiana*	0.047	0.001	0.037	0.000	0.038	0.001	0.032	0.001
7.*P.modesta*	–	–	–	–	0.028	0.000	0.025	0.000
8.*P.scotica*	0.036	0.001	0.033	0.001	0.039	0.001	0.031	0.001
9.*P.scandinavica*	0.039	0.000	0.033	0.000	0.036	0.001	0.029	0.001
10.*P.stricta*	0.054	0.001	0.040	0.001	0.044	0.001	0.037	0.001

Table 3.19 Statistical tests of significance (LSD) in leaf stomatal apparatus length (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		*	***	***	*	***	–	*	***	***
2.*P.farinosa(c)*			***	***	NS	***	–	NS	***	***
3.*P.frondosa*				NS	***	***	–	***	***	***
4.*P.darialica*					***	***	–	***	***	***
5.*P.halleri*						***	–	NS	***	***
6.*P.laurentiana*							–	***	***	***
7.*P.modesta*									–	–
8.*P.scotica*									***	***
9.*P.scandinavica*										***
10.*P.stricta*										–

Table 3.20 Statistical tests of significance (LSD) in leaf stomatal apparatus length (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		***	***	***	*	***	***	***	NS	***
2.*P.farinosa(c)*			NS	***	**	***	NS	***	***	***
3.*P.frondosa*				***	NS	***	***	***	***	***
4.*P.darialica*					***	***	*	***	***	***
5.*P.halleri*						***	***	***	***	***
6.*P.laurentiana*							***	NS	NS	***
7.*P.modesta*								***	***	***
8.*P.scotica*									***	***
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.21 Statistical tests of significance (LSD) in leaf stomatal apparatus width (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		***	**	*	***	***	–	***	***	***
2.*P.farinosa(c)*			***	***	NS	***	–	***	***	***
3.*P.frondosa*				NS	***	***	–	***	***	***
4.*P.darialica*					***	***	–	***	***	***
5.*P.halleri*						***	–	***	***	***
6.*P.laurentiana*							–	***	***	***
7.*P.modesta*								–	–	–
8.*P.scotica*									NS	***
9.*P.scandinavica*										***
10.*P.stricta*										–

Table 3.22 Statistical tests of significance (LSD) in leaf stomatal apparatus width (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	***	**	***	***	***	**	***
2.*P.farinosa(c)*			*	***	*	***	***	***	NS	***
3.*P.frondosa*				**	***	***	**	***	***	***
4.*P.darialica*					***	***	NS	***	***	***
5.*P.halleri*						***	***	**	NS	***
6.*P.laurentiana*							***	NS	***	***
7.*P.modesta*								***	***	***
8.*P.scotica*									**	***
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.23 Stomatal Indices and Stomatal frequencies of the primulas.

Primula spp.	Stomatal Indices				Stomatal frequencies					
	upper surface		lower surface		upper surface		lower surface		total	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
1.*P.farinosa(b)*	3.8	1.7	16.4	1.1	17.7	2.7	102.4	5.5	120.1	6.4
2.*P.farinosa(c)*	1.3	1.3	12.1	0.5	6.7	2.5	118.5	4.8	125.2	4.6
3.*P.frondosa*	2.5	2.1	12.1	0.9	11.1	2.8	58.7	5.4	69.8	6.5
4.*P.darialica*	2.8	1.4	10.9	0.6	13.6	2.0	89.9	5.7	103.5	6.6
5.*P.halleri*	4.1	1.6	17.9	0.5	14.4	2.1	181.8	5.7	196.2	6.4
6.*P.laurentiana*	8.6	0.6	21.9	0.5	19.6	2.2	115.5	5.1	135.1	4.4
7.*P.modesta*	0.1	0.3	19.9	0.5	0.4	0.4	211.5	6.6	211.9	6.5
8.*P.scotica*	0.5	0.9	17.1	0.4	1.5	0.8	91.8	2.8	93.3	2.7
9.*P.scandinavica*	3.9	1.2	23.8	0.8	11.1	3.5	143.6	6.7	154.7	7.0
10.*P.stricta*	9.0	1.4	18.3	0.6	11.1	1.8	53.7	2.6	64.8	3.2

Table 3.24 Statistical tests of significance (LSD) in leaf stomatal frequencies (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		***	*	NS	NS	NS	***	***	*	*
2.*P.farinosa(c)*			NS	*	*	***	NS	NS	NS	NS
3.*P.frondosa*				NS	NS	*	**	**	NS	NS
4.*P.darialica*					NS	NS	***	***	NS	NS
5.*P.halleri*						NS	***	***	NS	NS
6.*P.laurentiana*							***	***	*	**
7.*P.modesta*								NS	**	**
8.*P.scotica*									**	**
9.*P.scandinavica*										NS
10.*P.stricta*										-

Table 3.25 Statistical tests of significance (LSD) in leaf stomatal frequencies (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		*	***	NS	***	NS	***	NS	***	***
2.*P.farinosa(c)*			***	***	***	NS	***	***	**	***
3.*P.frondosa*				***	***	***	***	***	***	NS
4.*P.darialica*					***	***	***	NS	***	***
5.*P.halleri*						***	***	***	***	***
6.*P.laurentiana*							***	**	***	***
7.*P.modesta*								***	***	***
8.*P.scotica*									***	***
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.26 Statistical tests of significance (LSD) in leaf stomatal indices (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		*	NS	NS	NS	***	***	**	NS	***
2.*P.farinosa(c)*			NS	NS	**	***	NS	NS	*	***
3.*P.frondosa*				NS	NS	***	*	NS	NS	***
4.*P.darialica*					NS	***	**	*	NS	***
5.*P.halleri*						***	***	**	NS	***
6.*P.laurentiana*							***	***	***	NS
7.*P.modesta*								NS	***	***
8.*P.scotica*									**	***
9.*P.scandinavica*										***
10.*P.stricta*										–

Table 3.27 Statistical tests of significance (LSD) in leaf stomatal indices (Lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		***	***	***	NS	***	NS	NS	***	NS
2.*P.farinosa(c)*			NS	NS	***	***	***	***	***	***
3.*P.frondosa*				NS	***	***	***	***	***	***
4.*P.darialica*					***	***	***	***	***	***
5.*P.halleri*						***	NS	NS	***	NS
6.*P.laurentiana*							***	***	NS	***
7.*P.modesta*								NS	***	NS
8.*P.scotica*									***	NS
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.28 Number of epidermal cells on upper and lower leaf surface of the primulas.

Primula spp.	Number of epidermal cell/sq mm upper surface mean	SE	lower surface mean	SE
1.*P.farinosa(b)*	441.7	15.4	529.1	22.7
2.*P.farinosa(c)*	428.2	23.6	863.8	21.2
3.*P.frondosa*	415.7	33.4	429.6	27.9
4.*P.darialica*	475.4	14.3	734.2	14.7
5.*P.halleri*	332.7	14.5	831.7	24.7
6.*P.laurentiana*	203.5	8.9	410.4	13.9
7.*P.modesta*	370.8	11.7	967.3	25.2
8.*P.scotica*	237.5	8.5	443.1	12.6
9.*P.scandinavica*	244.0	12.4	458.4	14.1
10.*P.stricta*	109.4	3.9	239.5	7.0

for the upper and lower surfaces. ANOVA (Table 3.1) reveals that there was a highly significant difference in the mean number of epidermal cells for both surfaces ($P<0.0001$). Detailed results from LSD tests showed no significant difference in the number of epidermal cells on the upper surfaces between two populations of *P. farinosa* (Table 3.29), but revealed highly significant differences on the lower surfaces (Table 3.30).

3.3.11 Stomatal frequency and size

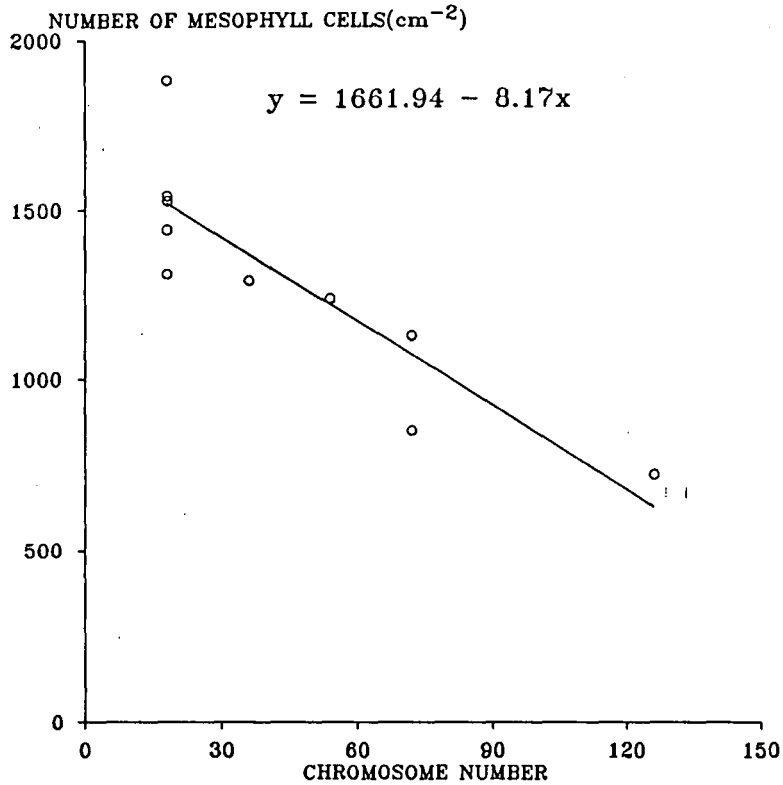
The products of mean stomatal pore length (Table 3.15) and total stomatal frequencies (Table 3.23) are given in Table 3.31 and Fig. 3.16. The values give a rough estimation of the relative surface area covered by stomata in each genotype/species, assuming equal aperture. ANOVA (Table 3.1) reveals highly significant differences between species ($P<0.0001$), and the results of LSD tests are showed in Table 3.32.

3.3.12 Relationship between leaf characteristics and ploidy levels

A summary of the correlation between leaf characteristics and chromosome number in the *P. farinosa* complex is given in Table 3.33. It can be seen that leaf thickness showed a positive weak correlation at the thickest part of leaf ($r = +0.28$) and at the thinnest part of leaf ($r = +0.33$). Similar results were also found with mesophyll thickness, $r = +0.21$, at the thickest part of a leaf and $r = +0.10$ at the thinnest part of a leaf. A strong correlation ($r = -0.87$, $P<0.01$) was demonstrated between the number of mesophyll cells and ploidy levels (Fig. 3.7). In contrast, no correlations were found between cuticle thickness and ploidy levels for the upper surfaces ($r = +0.17$) and lower surfaces ($r = +0.31$). A strong positive correlation ($r = +0.96$, $P<0.001$) was found between cell size and ploidy levels (Fig. 3.8). There was a good correlation found between the number of epidermal cells and ploidy levels (Fig. 3.14) on upper surfaces ($r = -0.94$, $P<0.001$) and on lower surfaces ($r = -0.71$, $P<0.05$). Strong correlations ($r = +0.88$ to $+ 0.92$, $P<0.001$) were found between stomatal size (length and width) and ploidy levels for both surfaces (Fig. 3.9 to 3.12).

Figure 3.7 Relationship between ploidy levels and number of mesophyll cells in *Primula farinosa* and its allies.

Figure 3.8 Relationship between ploidy levels and epidermal cell size in *Primula farinosa* and its allies.


NUMBER OF MESOPHYLL CELLS(cm^{-2})
y = 1661.94 - 8.17x
2000
1500
1000
500
0
0
30
60
90
120
150
CHROMOSOME NUMBER

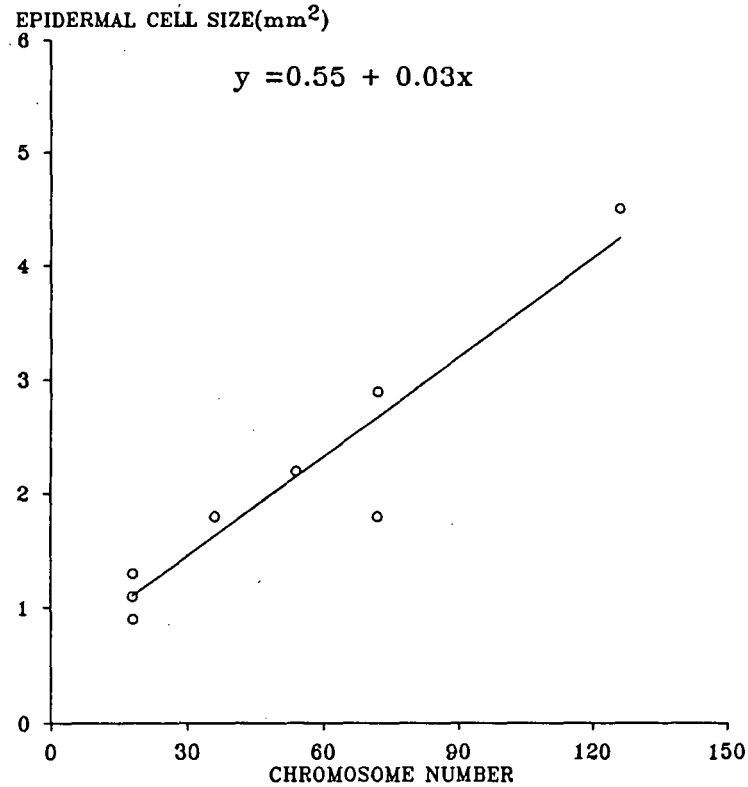
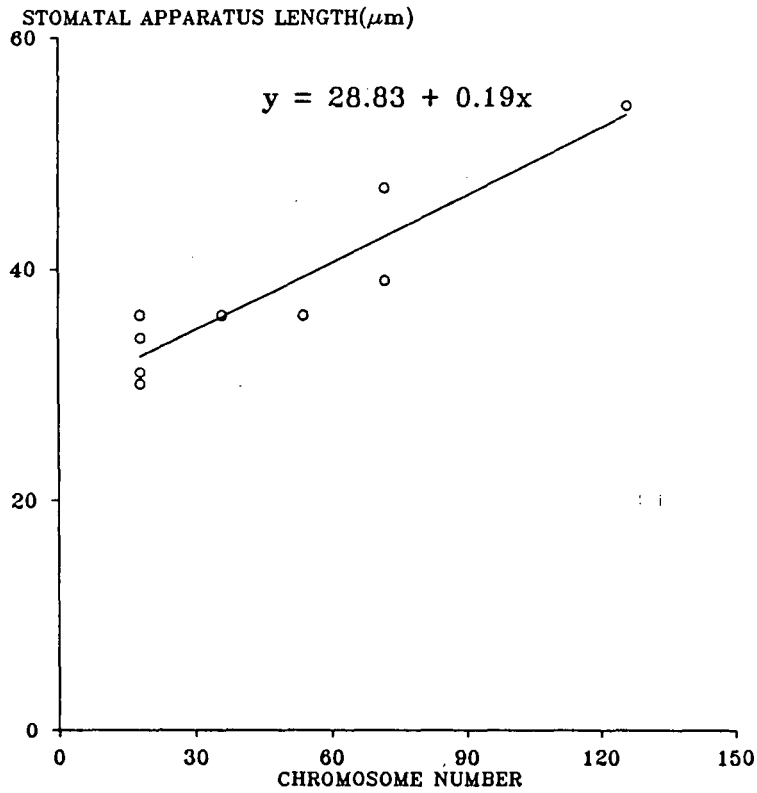

EPIDERMAL CELL SIZE(mm^2)
y =0.55 + 0.03x
6
5
4
3
2
1
0
0
30
60
90
120
150
CHROMOSOME NUMBER

Figure 3.9 Relationship between ploidy levels and stomatal apparatus length on upper epidermis in *Primula farinosa* and it allies.

Figure 3.10 Relationship between ploidy levels and stomatal apparatus length on lower epidermis in *Primula farinosa* and it allies.


STOMATAL APPARATUS LENGTH(μm)
y = 28.83 + 0.19x
60
40
20
0
0
30
60
90
120
150
CHROMOSOME NUMBER

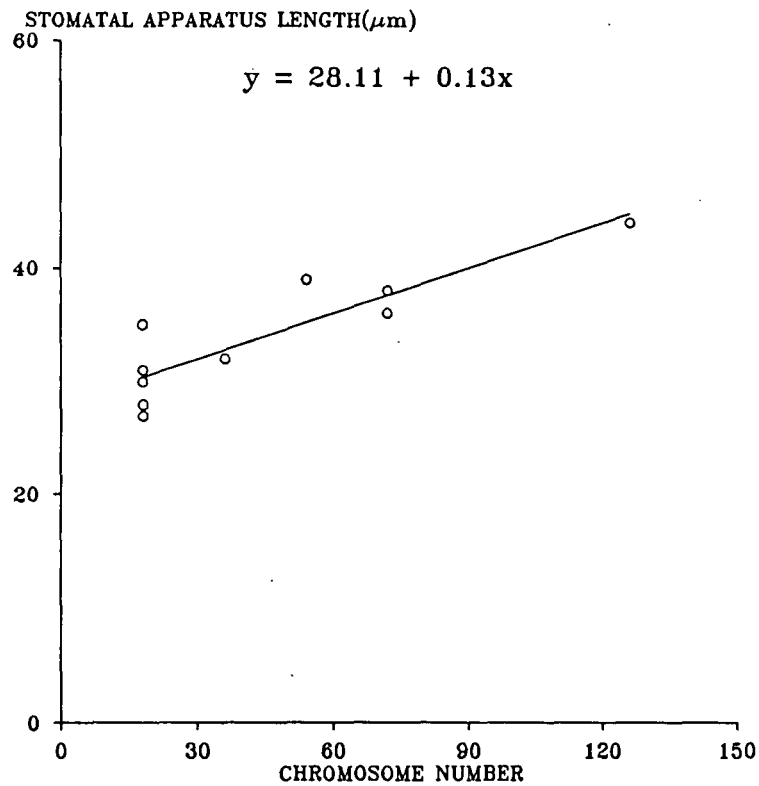
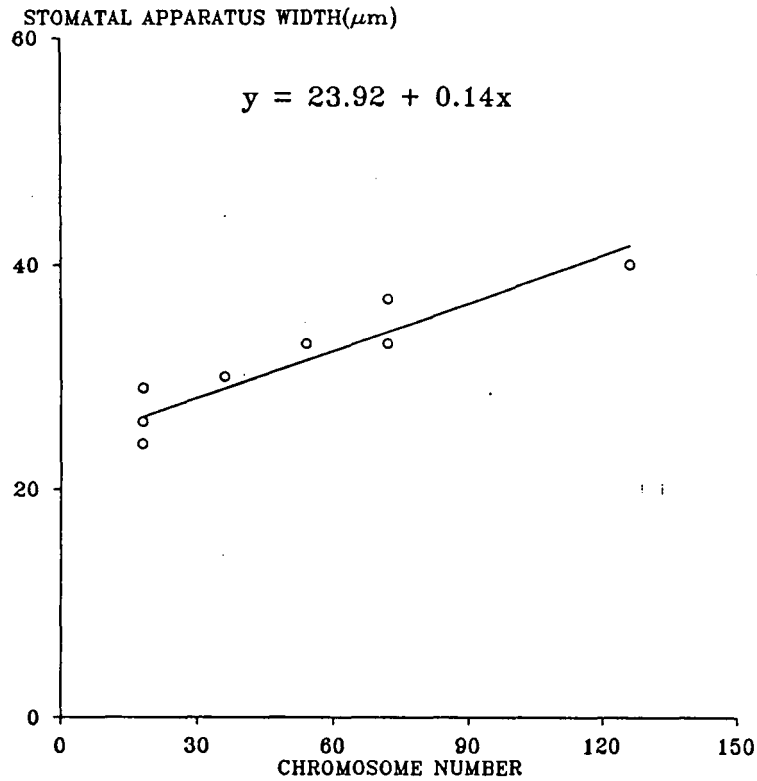

STOMATAL APPARATUS LENGTH(μm)
y = 28.11 + 0.13x
60
40
20
0
0
30
60
90
120
150
CHROMOSOME NUMBER

Figure 3.11 Relationship between ploidy levels and stomatal apparatus width on upper epidermis in *Primula farinosa* and its allies.

Figure 3.12 Relationship between ploidy levels and stomatal apparatus width on lower epidermis in *Primula farinosa* and its allies.


STOMATAL APPARATUS WIDTH(μm)
y = 23.92 + 0.14x
60
40
20
0
0
30
60
90
120
150
CHROMOSOME NUMBER

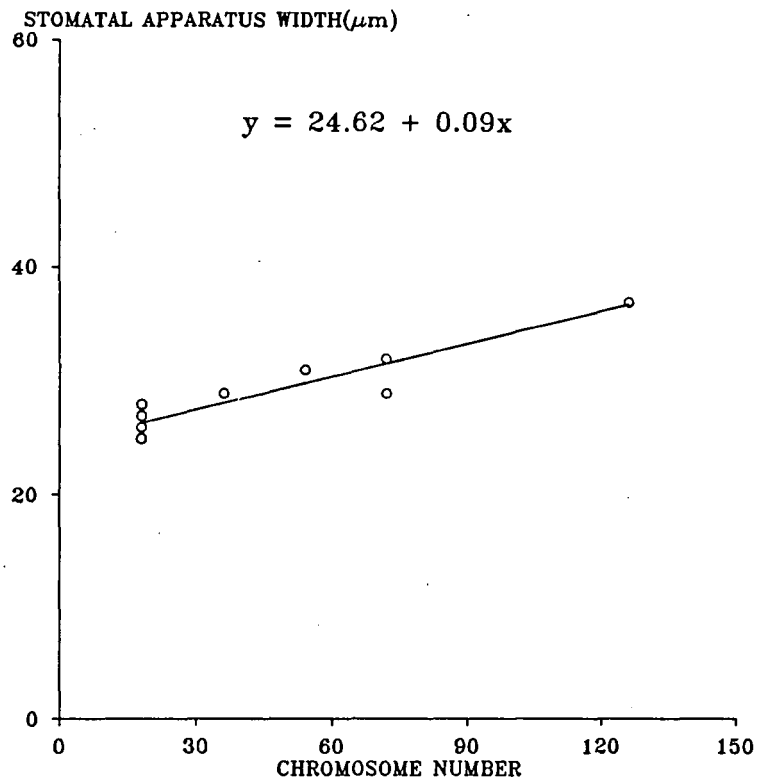
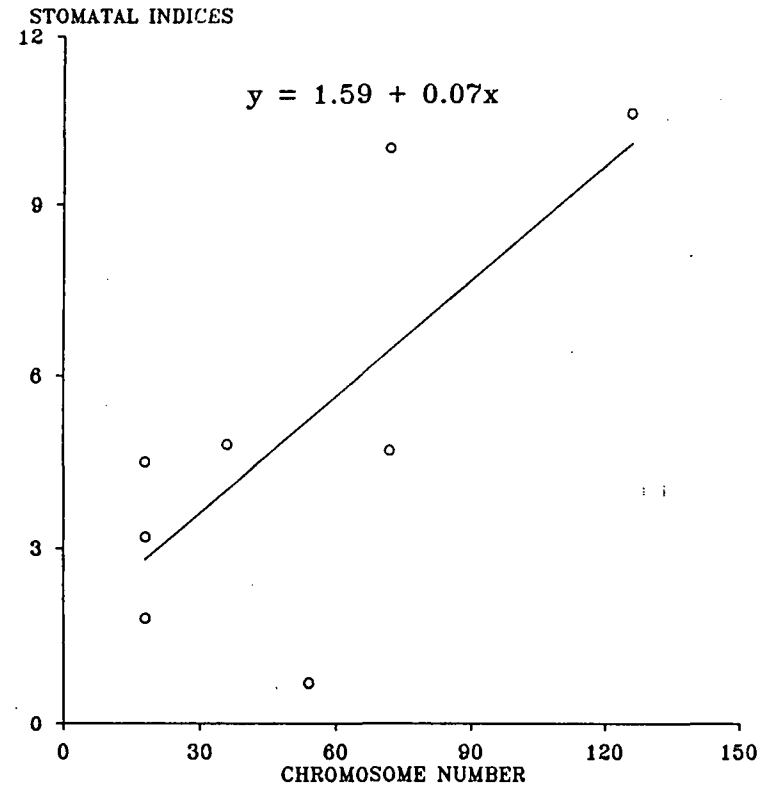

STOMATAL APPARATUS WIDTH(μm)
y = 24.62 + 0.09x
60
40
20
0
0
30
60
90
120
150
CHROMOSOME NUMBER

Figure 3.13 Relationship between ploidy levels and stomatal indices on upper epidermis in *Primula farinosa* and its allies.

Figure 3.14 Relationship between ploidy levels and number of epidermal cells per sq mm on upper epidermis in *Primula farinosa* and its allies.


STOMATAL INDICES
y = 1.59 + 0.07x
12
9
6
3
0
0
30
60
90
120
150
CHROMOSOME NUMBER

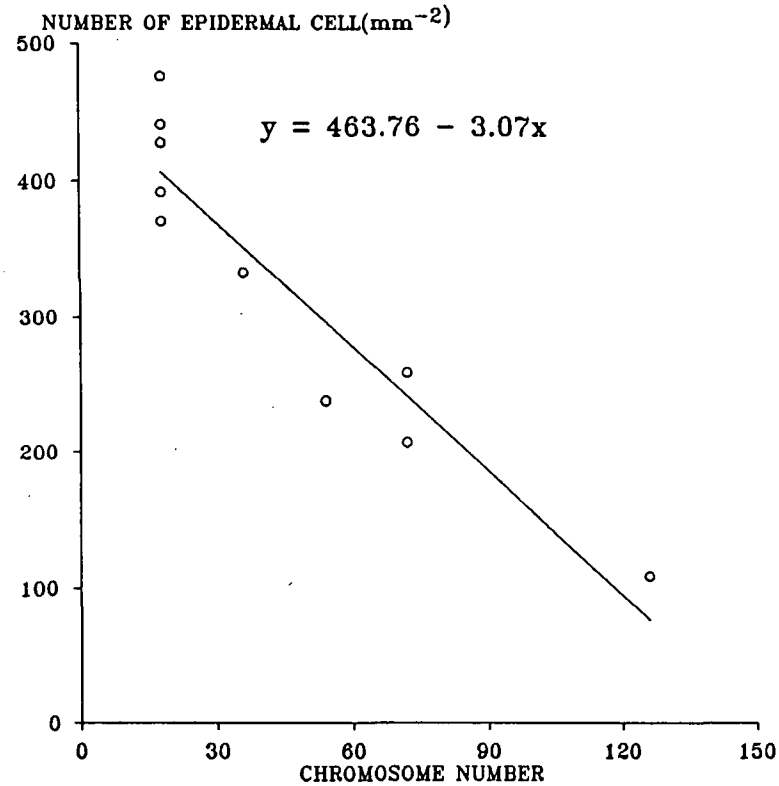

NUMBER OF EPIDERMAL CELL(mm^{-2})
y = 463.76 - 3.07x
500
400
300
200
100
0
0
30
60
90
120
150
CHROMOSOME NUMBER

Figure 3.15 Total stomatal frequency in *Primula farinosa* and its allies.

FAB = *Primula farinosa(b)*
FAC = *Primula farinosa(c)*
FRO = *Primula frondosa*
DAR = *Primula darialica*
HAL = *Primula halleri*
LAU = *Primula laurentiana*
MOD = *Primula modesta*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

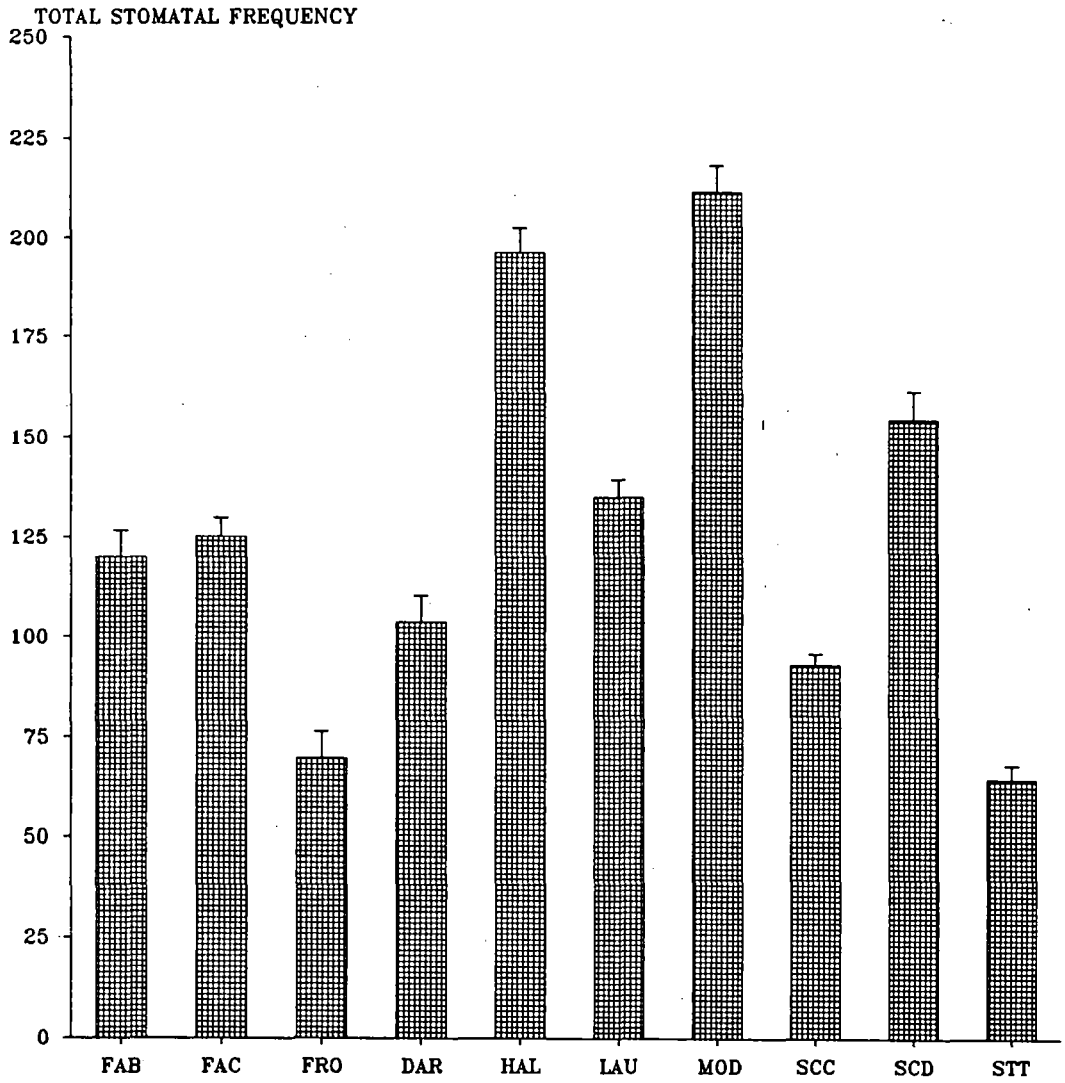

TOTAL STOMATAL FREQUENCY
250
225
200
175
150
125
100
75
50
25
0
FAB
FAC
FRO
DAR
HAL
LAU
MOD
SCC
SCD
STT

Figure 3.16 Products of total stomatal frequency and mean stomatal pore length in *Primula farinosa* and its allies.

FAB = *Primula farinosa(b)*
FAC = *Primula farinosa(c)*
FRO = *Primula frondosa*
DAR = *Primula darialica*
HAL = *Primula halleri*
LAU = *Primula laurentiana*
MOD = *Primula modesta*
SCC = *Primula scotica*
SCD = *Primula scandinavica*
STT = *Primula stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

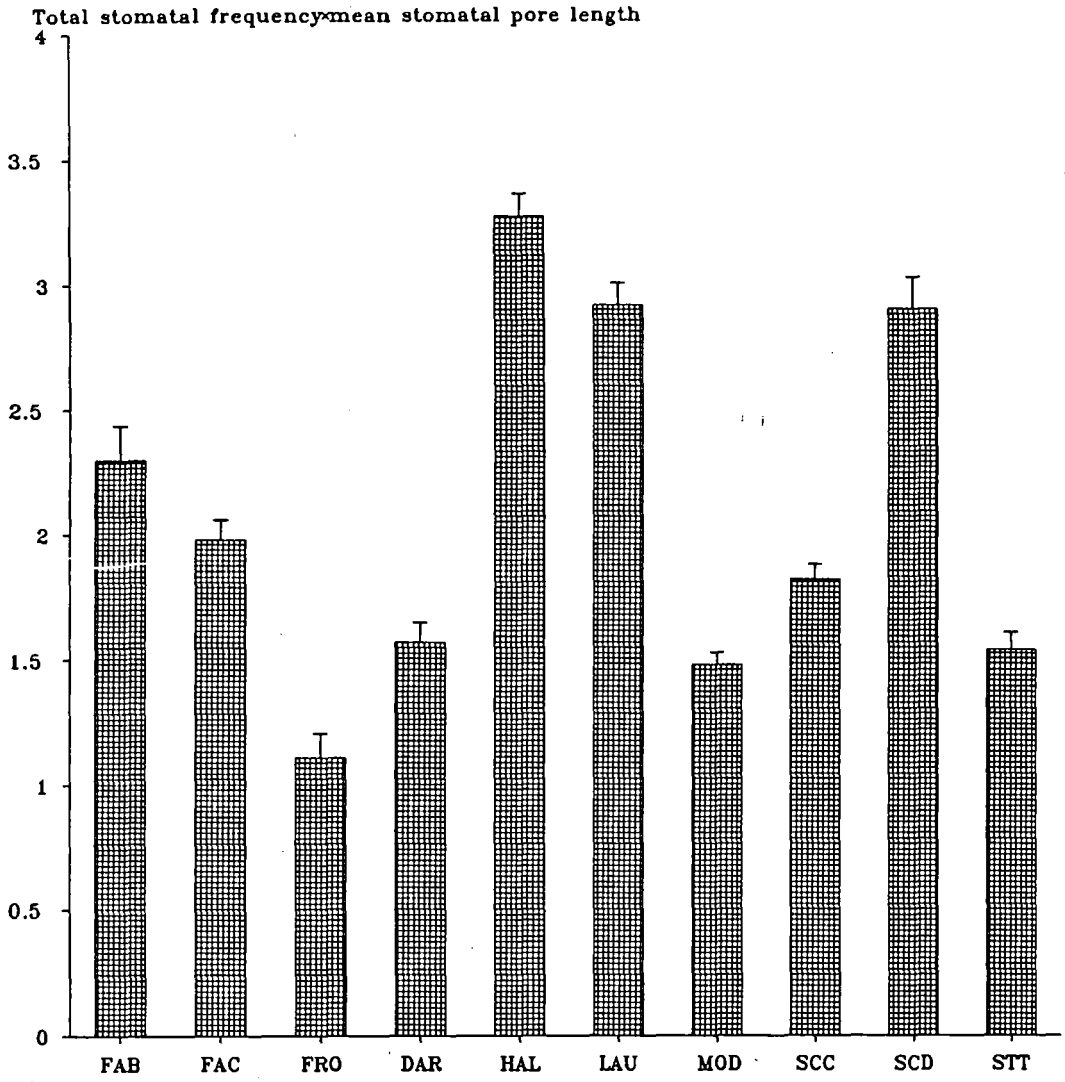

Total stomatal frequency×mean stomatal pore length
4
3.5
3
2.5
2
1.5
1
0.5
0
FAB
FAC
FRO
DAR
HAL
LAU
MOD
SCC
SCD
STT

Table 3.29 Statistical tests of significance (LSD) in number of epidermal cell (upper surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		NS	NS	NS	***	***	**	***	***	***
2.*P.farinosa(c)*			NS	NS	***	***	*	***	***	***
3.*P.frondosa*				NS	***	***	NS	***	***	***
4.*P.darialica*					***	***	***	***	***	***
5.*P.halleri*						***	NS	***	***	***
6.*P.laurentiana*							***	NS	NS	***
7.*P.modesta*								***	***	***
8.*P.scotica*									NS	***
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.30 Statistical tests of significance (LSD) in number of epidermal cell (lower surface) between species in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*	***	***	***	***	***	***	**	*	***	
2.*P.farinosa(c)*		***	***	NS	***	***	***	***	***	
3.*P.frondosa*			***	***	NS	***	NS	NS	***	
4.*P.darialica*				***	***	***	***	***	***	
5.*P.halleri*					***	***	***	***	***	
6.*P.laurentiana*						***	NS	NS	***	
7.*P.modesta*							***	***	***	
8.*P.scotica*								NS	***	
9.*P.scandinavica*									***	
10.*P.stricta*										-

Table 3.31 Products of total stomatal frequency and mean stomatal pore length.

Primula spp.	mean	SE
1.*P.farinosa(b)*	2.30	0.14
2.*P.farinosa(c)*	1.88	0.08
3.*P.frondosa*	1.12	0.10
4.*P.darialica*	1.55	0.09
5.*P.halleri*	3.14	0.10
6.*P.laurentiana*	2.84	0.10
7.*P.modesta*	1.48	0.05
8.*P.scotica*	1.77	0.06
9.*P.scandinavica*	2.78	0.14
10.*P.stricta*	1.49	0.08

Table 3.32 Statistical tests of significance (LSD) in products of total stomatal frequency and mean stomatal pore length in *Primula farinosa* complex.

Primula spp.	Significance levels									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.*P.farinosa(b)*		*	***	***	***	***	***	***	***	***
2.*P.farinosa(c)*			***	**	***	***	***	NS	***	**
3.*P.frondosa*				**	***	***	*	***	***	**
4.*P.darialica*					***	***	NS	NS	***	NS
5.*P.halleri*						**	***	***	**	***
6.*P.laurentiana*							***	***	NS	***
7.*P.modesta*								*	***	NS
8.*P.scotica*									***	*
9.*P.scandinavica*										***
10.*P.stricta*										-

Table 3.33 Relationship between measured leaf characteristics and ploidy levels in *Primula farinosa* complex.

Measured leaf characteristics	r	Significance levels
Leaf thickness		
thickest part of leaf	+0.28125	NS
thinnest part of leaf	+0.33525	NS
Mesophyll thickness		
thickest part of leaf	+0.21300	NS
thinnest part of leaf	+0.10560	NS
Number of mesophyll cells/sq mm	−0.87354	**
Cuticle thickness		
upper surface	+0.16995	NS
lower surface	+0.31598	NS
Epidermal cell size	+0.95674	***
Number of epidermal cells/sq mm		
upper surface	−0.94580	***
lower surface	−0.71143	*
Stomatal apparatus length		
upper surface	+0.92940	***
lower surface	+0.88849	***
Stomatal apparatus width		
upper surface	+0.92746	***
lower surface	+0.92097	***
Stomatal pore length		
upper surface	+0.86273	**
lower surface	+0.76638	**
Stomatal frequencies		
upper surface	+0.13251	NS
lower surface	−0.31353	NS
total	−0.30448	NS
Stomatal indices		
upper surface	+0.75907	*
lower surface	+0.60443	NS
Product of total stomatal frequency and mean stomatal pore length	+0.14688	NS

Also stomatal pore length and ploidy levels showed a strong correlation for both surfaces ($r = +0.76$ and $+0.86$, $P<0.01$). However, stomatal frequency for both surfaces and total stomatal frequency were weakly correlated with ploidy levels. A correlation was found between ploidy level and stomatal index (Fig. 3.13) for upper surface ($r = +0.76$, $P<0.05$), but no relationship was found with ploidy levels on lower surface ($r = +0.59$). Lastly, no relationship was found between ploidy levels and the products of stomatal frequency and mean stomatal pore length.

3.3.13 Relationship between leaf characteristics

Overall correlation of leaf characteristics in *P. farinosa* complex are summarized in Table 3.34.

Table 3.34 Relationship between leaf characteristics in Primula farinosa complex.

---------PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS --------

	DATA1	DATA2	DATA3	DATA4	DATA5	DATA6	DATA7	DATA8	DATA9	DATA10	DATA11
DATA1	1.0000	.4950***	-.0801	.1625	.1669	.2423	.2478**	.2544	.0924	.2106	.1617
DATA2	.4950***	1.0000	.1006	.3375***	.1736	.2650**	.2117	.3613***	.1252	.2962**	.0635
DATA3	-.0801	.1006	1.0000	.1623	.1283	.0862	.0797	.1208	.1894	.0335	-.0318
DATA4	.1625	.3375***	.1623	1.0000	.2204	.3678***	.3466***	.4084***	.1933	.2146	.1064
DATA5	.1669	.1736	.1283	.2204*	1.0000	.7629***	.8418***	.7881***	.8858***	.7352***	-.3854***
DATA6	.2423*	.2650**	.0862	.3678***	.7629***	1.0000	.8498***	.9108***	.5588***	.8813***	-.1357
DATA7	.2478**	.2117*	.0797	.3466***	.8418***	.8498***	1.0000	.8614***	.6505***	.7478***	-.3301***
DATA8	.2544*	.3613***	.1208	.4084***	.7881***	.9108***	.8614***	1.0000	.5680***	.7904***	.0147
DATA9	.0924	.1252	.1894*	.1933*	.8858***	.5588***	.6505***	.5680***	1.0000	.5678***	-.2985**
DATA10	.2106*	.2962**	.0335	.2146*	.7352***	.8813***	.7478***	.7904***	.5678***	1.0000	-.2738**
DATA11	.1617	.0635	-.0318	.1064	-.3854***	-.1357	-.3301***	.0147	-.2985**	-.2738**	1.0000
DATA12	.0973	-.1007	-.0413	-.0794	.1127	.1200	.0753	.0015	.1046	.1730	-.1773*
DATA13	-.5094***	-.4575***	-.1337	-.0763	-.4684***	-.6102***	-.5260***	-.6440***	-.2924**	-.5771***	.1592
DATA14	.8865***	.6806***	-.0200	.1454	.1341	.2177*	.2038*	.2420*	.0624	.2206*	.0972
DATA15	.4800***	.8562***	-.0648	.3535***	.0221	.1698	.0343	.2333*	.0200	.2130*	.1628
DATA16	.2711**	.2058*	.0273	.2602**	.7435***	.8535***	.8308***	.8217***	.5408***	.7597***	-.2914**
DATA17	-.2269*	-.3254***	-.0256	-.2653**	-.7744***	-.7941***	-.8007***	-.8498***	-.6095***	-.7148***	.1051
DATA18	.0188	-.1197	-.0671	.0206	-.7530***	-.5620***	-.6180***	-.4936***	-.6634***	-.6293***	.7000***

	DATA12	DATA13	DATA14	DATA15	DATA16	DATA17	DATA18
DATA1	.0973	-.5094***	.8865***	.4800***	.2711**	-.2269	.0188
DATA2	-.1007	-.4575***	.6806***	.8562***	.2058	-.3254***	-.1197
DATA3	-.0413	-.1337	-.0200	-.0648	.0273	-.0256	-.0671
DATA4	-.0794	-.0763	.1454	.3535***	.2602**	-.2653**	.0206
DATA5	.1127	-.4684***	.1341	.0221	.7435***	-.7744***	-.7530***
DATA6	.1200	-.6102***	.2177	.1698	.8535***	-.7941***	-.5620***
DATA7	.0753	-.5260***	.2038	.0343	.8308***	-.8007***	-.6180***
DATA8	.0015	-.6440***	.2420	.2333	.8217***	-.8498***	-.4936***
DATA9	.1046	-.2924**	.0624	.0200	.5408***	-.6095***	-.6634***
DATA10	.1730	-.5771***	.2206	.2130	.7597***	-.7148***	-.6293***
DATA11	-.1773	.1592	.0972	.1628	-.2914**	.1051	.7000***
DATA12	1.0000	-.0254	.1850	.0014	.0889	.1218	-.2583**
DATA13	-.0254	1.0000	-.5170***	-.2800**	-.5742***	.5621***	.3244***
DATA14	.1850*	-.5170***	1.0000	.6677***	.2301	-.1888	-.0751
DATA15	.0014	-.2800**	.6677***	1.0000	.0527	-.1716	-.0091
DATA16	.0889	-.5742***	.2301*	.0527	1.0000	-.7750***	-.5755***
DATA17	.1218	.5621***	-.1888*	-.1716	-.7750***	1.0000	.5922***
DATA18	-.2583**	.3244***	-.0751	-.0091	-.5755***	.5922***	1.0000

(1-TAILED, " . " PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)

DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest) in mm
DATA15 = thickness of mesophyll (thinnest) in mm
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)

3.4 Discussion

As would be expected from the results of studying leaf anatomy, e.g. leaf dorsiventral structure, having amphistomatous leaves is a characteristic of plants from a mesophytic habitat (Esau, 1953). This may be characteristic of alpine plants as has been reported by Körner & Mayr (1981), who found that 70% of the species in alpine plant communities living between 600 and 2,600 metres above sea level had amphistomatous leaves. Contrary to expectations, the occurrence of chloroplasts in epidermal cells is, however, a characteristic of plants from shady habitats (Esau, 1953), -not a natural habitat for primulas in this study. However, the presence of chloroplasts in the epidermis and the palisade mesophyll as well as the spongy mesophyll can be expected to give a high photosynthetic rate measured on a leaf area basis.

Leaf thickness varied between species, but significant differences between each pair of species were found only with *P. halleri* and *P. laurentiana* which had the thickest leaves. The two populations of *P. farinosa* and the rest of the species did not differ as regards leaf thickness. In an attempt to find a relationship between leaf anatomy and photosynthetic rate, El-Sharkawy & Hesketh (1965) noted that there was a significant negative correlation between leaf thickness and the photosynthetic rate. They also suggested that differences in leaf thickness may account for some of the differences in photosynthesis amongst the species. A relationship between leaf thickness and carbon dioxide exchange rate was also found by Dornhoff & Shibles (1976), working with soy beans. These results could lead to an expectation that *P. halleri* and *P. laurentiana* may show lower rates of photosynthesis than the other species as a consequence of their greater leaf thickness. It might also be suggested that the sensitive response of these two species to drought stress is due to a higher transpiration rates by their thick leaves (Turrel, 1936). The good correlation between leaf thickness and stomatal size indicates that the high transpiration rates of thick leaved species may be due, at least in part, to the increase in stomatal dimension.

A strong positive correlation between leaf thickness and mesophyll thickness suggests that the thickness of the leaf depends largely and on this basis perhaps totally, on mesophyll thickness. Leaf thickness is also highly correlated with cuticle thickness on the lower surface. This finding may be useful in explaining the insensitivity to drought in some thick leaf species. It is, however, surprising that the abaxial cuticle thickness in the primulas is mostly thicker than the adaxial ones. This result contradicts the general finding of many authors (Martin & Juniper, 1970) and the reason for this is not known.

It has been frequently found that hardiness varies inversely with the size characteristics of the plant, e.g. height, leaf length, and especially cell size (Levitt, 1956). Wilson & Cooper (1969a) also found that *Lolium* genotypes with smaller cells show the fastest rates of photosynthesis per unit volume, whilst those with the largest cells had the slowest rates. They also suggested that in conditions of light limitation, cell size might be related to photosynthetic activity through some indirect association, e.g. amount of light-intercepting pigment, or an association with photochemical processes. The results from these analyses showed that the primulas do vary in cell size, e.g. stomatal apparatus size, epidermal cell size. It might be expected therefore that *P. farinosa* and its allies will exhibit different performances with regard to photosynthesis and adaptation to environmental stress.

Due to the fact that all of the species were raised in the same environment, the discrepancies in leaf characteristics between species should be the consequence of their different genotypes. The results presented in Table 3.33 show the relationships between measured leaf characteristics and chromosome number. It can be inferred that the "thickness-of-leaf characteristics", e.g. thickness of leaf, mesophyll, cuticle, etc., are under genetic control, but ploidy could not alone account for these patterns. In contrast on the basis of cell size, chromosome number did affect both the size of guard cells and epidermal cells. These results are in broad agreement with Smith (1946), working with diploid, tetraploid, and hexaploid races of *Sedum puchellum* and Tan & Dunn (1973), working with *Bromus inermis*. Bruun (1938)

in his study on heterostylous plants also noted statistical differences in morphology, e.g. in stomatal pore length, between *P. farinosa* and the polyploid species. He also emphasized the unique large stomata of *P. laurentiana* (2n=72).

The number of cells, e.g. mesophyll cells, or epidermal cells, were highly correlated with ploidy levels, but in the opposite direction. It can be concluded that increased chromosome numbers result in increased cell size but decreased cell number. Negative correlations between the number of mesophyll cells and leaf thickness and mesophyll thickness implies that thickness of leaf or mesophyll depends largely on mesophyll cell size. Unfortunately, mesophyll cell size was not investigated in this study. The present results showed no relationship between overall stomatal frequency on both surfaces and chromosome number, whereas a positive correlation was found between stomatal index on the upper surface alone and chromosome number. In contrast with the results reported here, Tan & Dunn (1973), working with *Bromus inermis* found that stomatal frequency decreased with increasing ploidy. However, Sax (1938) noted that stomatal frequency could not be used as an absolute index to polyploidy.

Comparisons between species show that there was no statistical difference in thickness-of-leaf characteristics, e.g. leaf thickness between two populations of *P. farinosa* from Upper Teesdale and Blackhall Rocks, but they did significantly differ in their cell size, except stomatal apparatus width on lower surface and epidermal cell size. In general, *P. farinosa* from Blackhall Rocks was superior to *P. farinosa* from Upper Teesdale in the length of cell, but inferior in the width. Due to the difficulty in measuring the width of stomatal pore, pore area was not measured. Thus, derived data, such as stomatal resistance could not be investigated. However, differences in stomatal resistance could be simply looked at in terms of stomatal frequency, or size of stomata or the product of the two values (Lloyd, 1974). Taking stomatal frequency and stomatal pore length into account, the present data revealed that the two populations of *P. farinosa* were significantly different, similar results also being found in some pairs of other species. From the total stomatal frequency

(upper surface + lower surface), the highest mean value was found in *P. modesta* (mean = 211.9), the endemic alpine species of Japan, and the lowest mean value was for *P. stricta* (mean = 64.8) the arctic species.

Differences in stomatal pore length and frequency have been found in populations or varieties of other species. For example, Wilson and Cooper (1969a) found statistical differences in length and frequency of stomata in different genotypes of *Lolium perenne*. They also found a good correlation between rate of photosynthesis and pore length, but stomatal resistances were not determined. Salisbury (1928) noted that a mean for total stomatal frequency for alpine plants of 242 (cells mm^{-2}), which compared with 167 for the marginal flora of an English woodland. He also suggested that the high stomatal frequency of alpine plants could be correlated with the much greater exposure in the mountain habitat. Mooney & Johnson (1965) working with an arctic and an alpine populations of *Thalictrum alpinum* found that the alpine diploid population had the highest stomatal frequency (326), the arctic diploid next (264), and the arctic triploid population the lowest (240). A striking feature of the figures for the primulas is that 9 out of the 10 genotypes/species had stomatal frequencies lower than the average from the marginal flora of an English woodland; only 1 species *P. modesta* had stomatal frequencies in the same range as the mean values quoted above for alpine plants.

The differences between the results reported here and the results from the other authors may be due both to the variation between species and to the influence of environmental factors during growth (Meidner & Mansfield, 1968; Losch & Tenhunen, 1981). Amongst species of the *P. farinosa* complex, it seems likely that the variation in stomatal frequency is the consequence of different genotypes and of adaptation to their habitats of origin. For example, the lowest stomatal frequency in *P. stricta* may be the result of its adaptation to the low light intensity in the high Arctic summer. The differences in some leaf characteristics between the two populations of *P. farinosa* may be due to their long-term geographic isolation. Evidence in favour of this interpretation has been produced in the classic work of

Clausen, Keck, & Hiesey (1940), who demonstrated continuous temporal variation in long-term studies of populations of *Potentilla glandulosa* in North America. Also, the data of Lewis (1969) working with *Geranium sanguineum,* may be interpreted in the same way.

CHAPTER 4

PLANT GROWTH ANALYSES

4.1 Introduction

It is generally accepted that the geographic distribution of a plant species is restricted, to some extent at least, by the physiological response of the plants to various aspects of their environment (e.g. Kellman, 1974; Bradshaw, 1959; 1960). The simplest and most direct method by which physiological characteristics may be investigated is by transplant experiments in which samples of a population and/or related species are grown in a series of contrasting natural environments, e.g. along the gradient of altitude (Clausen *et al.*, 1940; 1948; Pigott, 1978). Early workers (e.g. Clausen *et al.*, 1940; 1948) were able to demonstrate considerable physiological differences in various species. For instance, lowland plants that were seriously affected by winter conditions in alpine environments, and alpines that grew with much reduced vigour and flowering in the lowland environments. Bukharin (1961) reported that plants from several ecological habitats were transplanted to various altitudes (340 to 1000 m) in the Murmansk region. All species were reduced in growth and their rate of development slowed with increase in altitude. It has been noted that the reaction patterns observed after transplanting a number of plants indicate the complex interaction between plants and their environment (Clausen *et al.*, 1940).

With increasing elevation there is a strong decrease in the vigour of vegetation; for example, trees usually become more stunted and grasses tend to be shorter (Pigott, 1978). Elevation limits on plant distribution have long been attributed to specific physical factors of the environment (Whittaker, 1975). It is noted that temperatures usually decrease and the amount of precipitation usually increases

with increasing altitude (Pearsall, 1950; Geiger, 1965; Nobel & Hartsock, 1986). Low temperature often sets the upper altitudinal limit of a species, whereas high temperature may set the lower altitudinal limit (Leith & Whittaker, 1975). Since environmental factors like temperature and rainfall vary with elevation, it would be expected that physiological plant response, e.g. growth and development, also varies with elevation. It is suggested that in order to understand how climatic factors affect plants one would have to consider the influence of whole seasons upon the growth of a selected plant (Pearsall, 1950).

Prince (1976) studied the effect of altitude on the growth of barley, and he found a significantly lower final weight of grain from the upland site (460 m) than from the lowland site (46 m). This result is related to the lower grain and day temperatures of the upland site than those of the lowland site. Comparisons between the growth of upland and lowland plants by transplant experiments were made by Woodward & Pigott (1975). They found that *Sedum rosea* (upland species) rarely responds to altitude, whilst *S. telephium* (lowland species) was very sensitive to increasing altitude by a reduction in size when these two species are grown in competition. They suggest that these responses appear to be the result of differences of climate associated with altitude. Parallel work shows that the growth rate of *S. rosea* is rarely affected by temperature within the range it usually experiences in its natural habitat, whilst *S. telephium* grows vigorously at high temperatures and its growth-rate decreases at lower temperatures (Woodward, 1975). Subsequent work on the growth rates of lowland species, *Dactylis glomerata* and *Phleum bertolonii*, showed a greater response to an increase in day temperature from 10-20°C, whilst upland species, *Sesleria albicans* (0-900 m), and *P. alpinum* (600 to 1200 m) showed little response (Woodward, 1979). He noted that these differences are due to a greater leaf-area ratio, and to the increased rates of both leaf-initiation and leaf-expansion of the lowland species at higher temperatures.

In severe environments like arctic and alpine regions, it is found that the growth of plants is rather slow, and indeed many species, especially woody plants require

many years before they flower and set fruit for the first time (Porsild, 1951; Billings & Mooney, 1968). The limitation of growth of arctic plants has been ascribed to either low light intensity (Miller *et al.*, 1976), low air temperature (Bliss, 1962; Warren Wilson, 1966; Billings & Mooney, 1968) or low nutrient availability (Haag, 1974; Russell, 1940; Sørensen, 1941), and it is generally assumed that most members in each community are limited primarily by the same factor (Shaver & Chapin, 1980). The higher intensity of ultraviolet light in the alpine environment has led some authors to speculate that this could be the cause of the low growth form of alpine plants, however, evidence in favour of this assumption is still limited (Bliss, 1962).

Of the various environmental factors, temperature is the most important limiting factor with regard to plant growth and development in the Arctic (Bliss, 1962). However, it is found that differences in growth rate and/or production among communities within the Arctic correlate more closely with variation in soil moisture, aeration, and nutrient availability than with air temperature (Miller, 1982). This explains why arctic plants have been so successful in adapting to low temperatures and that other ecological factors are more important in explaining patterns of community structure and production within the Arctic. In fact, the other factors, e.g. poor soil aeration, low nutrient supply, etc. are indirect consequences of low temperatures (Chapin & Shaver, 1985a).

In general, patterns of growth in arctic and alpine plants are similar to those in temperate species, but growth takes place at lower temperatures and within the limit of a growing season (Chapin & Shaver, 1985a). Scott (1970) noted that arctic and alpine plants have a lower optimum temperature (15-20°C) for shoot growth as compared with temperate species (25-30°C). Not surprisingly, arctic and alpine plants can adjust themselves to grow at low temperatures and also can resist sudden drops in temperature to levels below freezing, with slight or no sign of frost damage (Bliss, 1962). The temperature of a plant is usually determined by the temperature of the air surrounding it (Geiger, 1965). During the day the temperature of a leaf is

usually higher, and at night lower, than the surrounding air temperature. However, the temperature of a plant is also related to the amount of incoming radiation and wind speed (Geiger, 1965). For example, Tikhomirov *et al.* (1960) shows that on a sunny day at Tiksi, Yakutsk, USSR (71° 35′ N), the temperature of plant parts was higher than the air temperature by 2 to 5°C, whilst on a cloudy day the plant temperatures may fall below that of the air. Nevertheless, the fall of leaf temperatures below that of the air rarely exceeds 2°C because the amount of radiation exchange is rather small during night time (Geiger, 1965). The temperature of the leaf is effectively reduced by air movement, particularly at the colder air temperatures.

A wind speed of 3.2 km h^{-1} may reduce leaf temperature as much as 15 to 20°C compared with the temperature without wind if the absorbed radiation is as much as 1.4 cal cm^{-2} min^{-1} (Gates, 1968). Wind is an ever-present environmental factor in both arctic and alpine habitats (Bliss, 1962), thus upsetting the more favourable temperature microclimate of arctic and alpine plants. Warren Wilson (1959; 1960) working with *Oxyria digyna, Salix herbacea* and *Sibbaldia procumbens,* concludes that the vigorous effect on the plant of wind in arctic regions is a result of the sensitivity of plant growth to wind, and that the sensitivity is due less to excessive transpiration and more to temperature conditions as they affect net assimilation and shoot growth rates. When plants were grown in wind tunnels with wind speeds approximating to those commonly found in mountain regions, Whitehead (1962) showed that the whole plant becomes smaller with increasing windspeed and that this is the result of the development of a smaller leaf area and not by a reduction of photosynthetic rates per unit leaf area. He also concluded that the reduction of dry weight yield with increasing wind speed, must be due to the smaller leaf area attained. Russell & Grace (1978), using *Festuca arundinacea* and *Lolium perenne* showed that increasing windspeed reduces the rate of leaf extension and the relative growth rate, but there is no effect of windspeed on the rate of appearance of the leaves or on the leaf water potential. In some experiments, the relative growth rate of plants decreased with increasing wind-speed (Wadsworth, 1959), but in others,

using water culture, there is no effect (Wadsworth, 1960); this suggests that water stress is an important cause of reduced growth in wind. However, Grace (1981) noted that wind does not always increase transpiration rate and in many ordinary conditions an increase in wind causes a decrease in transpiration.

In most low-arctic and alpine regions, there are species that are associated with snowbanks (Bliss, 1962). These plants must be able to complete their vegetative life cycle under snow in some years. A snow cover means both protection and danger (Geiger, 1965). Plants under snow are protected against severe winter cold, and are completely shielded from wind, but on the other hand, icing up of the surface may close the ventilation within the snow. The depth of snow cover determines the length of the growing season, which depends on the area and topography (Kuramoto & Bliss, 1970). Some species begin growth under as much as 50 to 100 cm of snow, whereas other species await the melting of the snow before starting to grow (Billings & Bliss, 1959).

The simplest index of flowering is the appearance of visible flower buds or open flowers; differences in the time of appearance are taken as an index of such time or degree of initiation (Lang, 1965). The development of the primordia to buds and flowers is obviously affected by environmental conditions, e.g. temperature, photoperiod (Billings & Mooney, 1968; Billings, 1974). Temperature is clearly important both as a regulatory factor and as a selective force effecting genetically determined differences in responses to flowering. For instance, ecotypes of the grass *Phalaris tuberosa* from Morocco and Israel show only a quantitative cold requirement, whereas ecotypes from Greece and Turkey require up to 8 wk vernalization for complete flower induction and these cold requirements are related to the severity of the winters at the collection site (Cooper & McWilliam, 1966; McWilliam, 1968). In addition, recent research shows that at anthesis, panicle length of *Pennisetum americanum* is positively correlated with temperature prior to panicle initiation (Coaldrake & Pearson, 1986).

In general, leaf and flower buds of arctic and alpine plants over-winter in an advanced state of development and spring growth starts as soon as bud temperature increases to 0°C for even just a few hours each day (Sørensen, 1941; Bliss, 1962; Billings & Mooney, 1968; Mark, 1970). Flowering of alpine plants is usually coincident, reaching a peak 2-3 wk after snowmelt (Bliss, 1985). The diurnal rhythms of flowering and pollinating agents of arctic plants were studied by Shamurin (1958). He showed that flowers of many species open at minimal air temperatures of 3 to 8°C, with a great number of flowers at 5 to 12°C.

Many arctic plants are "long day" plants, and when transplanted or grown from seed in lower latitudes, they flower poorly or not at all (Porsild, 1951). For example, in *Oxyria digyna,* Mooney & Billings (1961) found that there were no populations which flowered under a 12 h photoperiod, populations from about 40° latitude flowered with a 15 h photoperiod. Billings (1974) adds to this by pointing out that the higher the latitude of origin, the greater the number of hours of continuous light or the longer the photoperiod needed for flowering. He also emphasized that this is true not only for the elongation and flowering of the preformed bud, but also for the production of the preformed flowering primordia themselves. To sum up, alpine plants are better adapted to shorter photoperiods and higher temperatures, while arctic plants are better fitted to long photoperiods and lower temperatures (Porsild, 1951; Chapin & Shaver, 1985a; Bliss, 1985).

The purpose of this study was to compare growth response vs of the primulas to microclimate along an altitudinal gradient in northern England as well as to determine the ability of species, which originate from contrasting habitats to tolerate different extreme conditions, e.g. water stress during summer months and/or cold stress during winter.

4.2 Materials and methods

4.2.1 Plant materials

Five genotypes/species of the primulas were used to study the effects of microclimate on plant growth along the altitude gradient in the north of England. Seeds of *Primula farinosa* were collected from two natural habitats, in lowland and upland sites. Seeds of *P. scotica* were also collected from their natural habitat in northern Scotland. Seeds from the other two species, *P. frondosa* and *P. laurentiana* originated from garden sources (see Table 1.10).

4.2.1.1 Growth conditions

The seeds were germinated on moist filter paper in Petri dishes at 20°C, to provide uniform starting conditions. After 4 d from sowing, healthy seedlings were transferred to plastic bowls (30×20×15 cm^3) with holes in the bottom for drainage. A mixture of J. Arthur Bower's seed and potting compost and gravel was used for planting medium and gravel was also put on the mixture surface to maintain moisture. An equal volume of compost mixture was put into each bowl and a space left of about 3 cm from the top margin. The outer surface of the bowls were painted the same colour (green) to ensure the same amount of heat conduction. On the surface of the planting mixture, 12 holes per bowl were marked and arranged in a 3×4 grid pattern. Three seedlings were put into each hole to ensure survival and the idea was to thin to 1 plant per hole so leaving 12 plants for each bowl.

4.2.1.2 Plant treatments

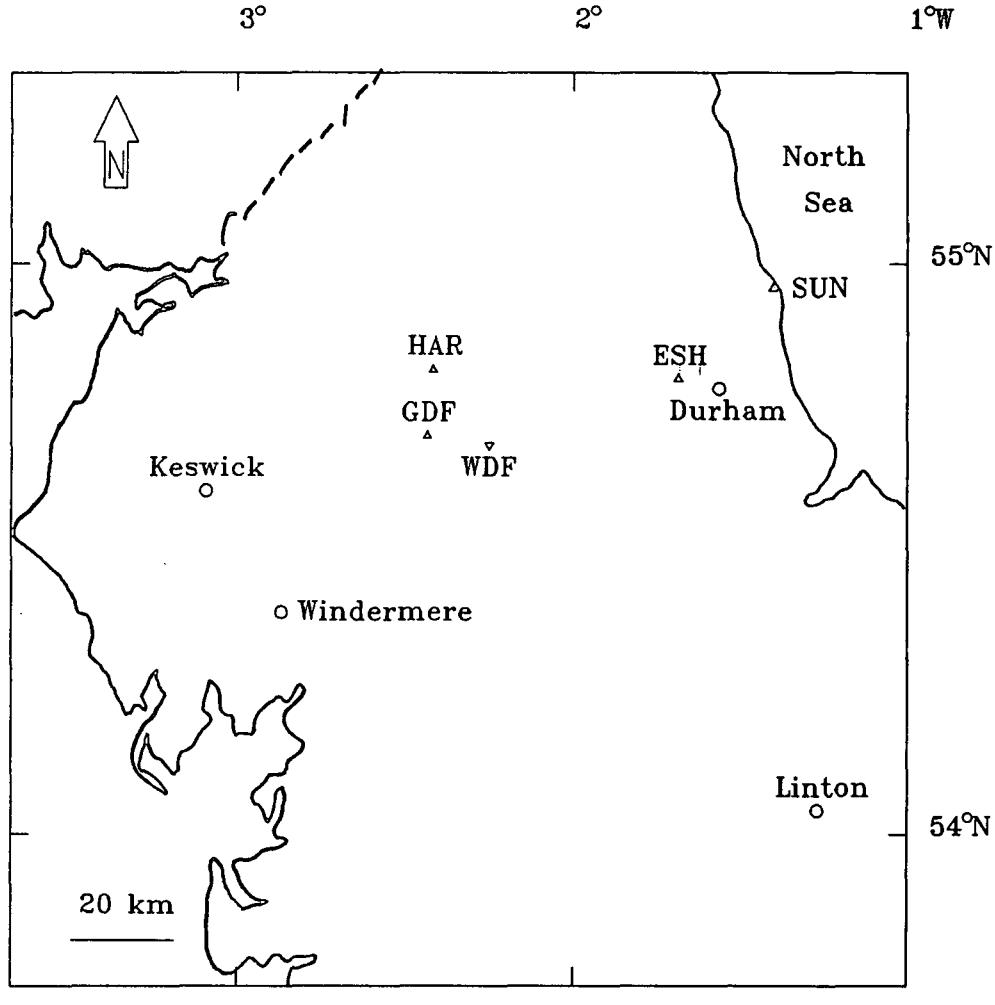
After young plants were established in the bowls, the bowls were moved to the Botanic Gardens, Durham University, in order to keep them in identical conditions (Table 4.1). The bowls were distributed randomly on the floor in a naturally-lit glass house, where temperatures and humidities were not controlled. Before the bowls were placed in the selected sites in the field (Fig. 4.1), they were first moved into a plastic tunnel to let the primulas acclimatize to the natural environment.

Table 4.1 Treatments of *Primula farinosa* and its allies before moving the plants to the selected sites.

Treatments	Days from sowing
1.Chilling the seeds in a cold room (4°C),dark.	---
2.Sowing the seeds in an incubator at 22°C, 24 hours photoperiod.	---
3.Transplanting the seedlings to the bowls(36 seedlings/bowl), 16-24 hours photoperiod.	4 - 14
4.Moving the bowls to the greenhouse at the Botanic Garden, University of Durham, natural daylight, day temperature 17-22°C.	35
5.Moving the bowls to a plastic tunnel, natural day light and temperature; Botanic Garden, University of Durham.	90
6.Trimming the surplus plants, keeping 12 plants per bowl; putting 4 granules of fertilizer around each plant.	115
7.Moving the bowls to the selected sites.	128 - 130

Figure 4.1 Map of northern England showing the approximate positions of the selected sites for plant growth analysis study and environmental stress experiment.

KEY : ▲ Selected site
GDF = Great Dun Fell Radar Station, Cumbria (847 m)
WDF = Widdybank Fell, Upper Teesdale (510 m)
HAR = Hartside Nursery, Alston, Cumbria (330 m)
ESH = Esh, Durham (210 m)
SUN = South Bents, Sunderland (4 m)


3°
2°
1°W
N
North
Sea
55°N
SUN
HAR
ESH
Durham
GDF
WDF
Keswick
Windermere
Linton
54°N
20 km

After 115 d from sowing the surplus plants were thinned out and 3 granules of fertilizer (Gromore) were put around each plant. After 130 d from sowing the bowls were placed in the field, and by that time some species, such as *P. farinosa* (b), *P. frondosa* had started to flower. Due to limitations of space, the difficulties of germination as well as the low percentage survival of the seedlings, only one bowl of each species was put in the field for each site and just three bowls of *P. scotica* were put in 3 sites out of 5. One bowl of each species was kept for the source of leaf area calibration (see 4.2.3.1).

The bowls were sunk together in the ground in existing grass swards so as to present a uniform surface, and they were enclosed by fences of 12 mm mesh netting to exclude small herbivores. Slugs were controlled with metaldehyde pellets.

4.2.2 Study sites

Sites were considered for a number of reasons; e.g. different altitude ranges, nearness to the natural habitat of *P. farinosa,* well exposed places, safety from intrusion, distance from polluted areas, nearness to a Meteorological station, and nearness to Durham. Accordingly, five sites from northern England were chosen. The lowland sites were at South Bents, Sunderland (SUN) and Esh, Durham (ESH). The upland sites in the northern Pennines were at Hartside, Alston, Cumbria (HAR); Widdybank Fell, Upper Teesdale (WDF) and Great Dun Fell Radar Station, Cumbria (GDF). All the study sites lie between latitude 54-55° N, longitude 1-3° W (Fig. 4.1).

4.2.2.1 Great Dun Fell Radar Station (GDF)

This site is situated at latitude 54° 41′ N, longitude 2° 27′ W at altitude 847 m, the second highest of the Pennines, 3.2 km south-south-east of Cross Fell(897 m) (Manley,1942). As a founder of the Meteorological station on Dun Fell, Manley (1942) has described the climate of Great Dun Fell:-

> "...an excessively windy and pervasively wet autumn, a very variable and stormy winter with long spells of snow cover, high humidity and extremely bitter wind, alternating with brief periods of rain and thaw. April has a mean temperature little above freezing-point and sunny days in May are offset by cold polar air; while the short and cloudy summer is not quite warm enough for the growth of trees. Through out the year indeed the summits are frequently covered in clouds..."

From these characteristics, it seems reasonable to regard the climate as sub-Arctic, with a resemblance to the climate at sea-level in South Iceland (Pearsall,1950). The primulas were put into the grounds of the Meteorological station, which is on the hill top and therefore very exposed.

4.2.2.2 Widdybank Fell, Upper Teesdale (WDF)

Widdybank Fell lies on the latitude 54° 40′ N, longitude 2° 16′ W in the middle of Upper Teesdale. The data obtained from the Meteorological station at 510 m on Widddybank show low temperatures for most of the year, high annual precipitation (1523 mm), highest windspeed in winter and lowest in summer (Pigott, 1956; 1978).

As the nearby selected sites in the northern Pennines, Widdybank Fell and Great Dun Fell are somewhat different as regards their climate. Rainfall on Great Dun Fell is higher with an estimated annual average about 2,290 mm, and wind-speed on Great Dun Fell is also about double (10 m s^{-1}) that of Widdybank Fell (Pigott, 1978). The growing season, measured as the number of days with a mean temperature above 6°C is 177 d on Widdybank Fell and 145 d on Great Dun Fell (Jones, 1973). The differences of local climate between these two sites are to be expected as Widdybank Fell is 337 m lower in altitude.

Two ecotypes of *P. farinosa, P. frondosa,* and *P. laurentiana* were put inside the area of the Meteorological station, which is situated on the crest of a slope. One ecotype of *P. farinosa* occurred naturally on this site.

4.2.2.3 Hartside nursery, Alston, Cumbria (HAR)

This site can be regarded as the middle altitude for the selected sites (330 m,

54° 48′ N, 2° 26′ W). The site is open and well exposed, on flat ground. All the selected species were put at this site.

4.2.2.4 Esh, Durham (ESH)

This site is situated on a hill top about 210 m above sea level (54° 47′ N, 1° 42′ W) and about 8 km from Durham City. The site is open and well exposed. The nearest Meteorological station is Durham University Observatory at an altitude of 102 m. It can be concluded from the long-term data recorded from the Meteorological station (1906-1935) that the climate at Durham is much milder than that at Widdybank Fell on average. July and August are the wettest months but still drier than the driest month at Widdybank Fell. Winter months at Durham are rather dry, in contrast to the wet winter at Widdybank Fell. Also the total number of hours of sunshine per month at Durham is much higher than at Widdybank Fell especially during the winter months.

All the selected species, except *P. scotica* were put at this site.

4.2.2.5 South Bents, Sunderland (SUN)

This site has the lowest altitude of the study sites, about 4 m above sea-level (54° 58′ N, 1° 21′ W), and about 800 m from the coast. The site is open and exposed, and the nearest Meteorological station is about 4 km away at Sunderland Polytechnic. This site represents the climate of the coastal habitat of *P. farinosa* at Blackhall Rocks, which is 21 km away. The climate of the area is maritime, with an annual precipitation in an average 592 mm and a mean annual temperature of 9.2°C. All the selected species were put at this site.

It can be seen from the information above that the study sites were well separated over a wide area of northern England. However, the measurement of primary data were made within the same week and took at least 3 d to complete.

4.2.3 Growth measurement

The method of plant growth analysis has been widely used to study the quantitative response of plant growth to various environmental factors, especially light and temperature (Blackman, 1961). This method is well developed in the field of agriculture and botany where the majority of species are annual, biennial, or short-lived perennials (Evans, 1972; Hunt, 1982). In growth analysis, a distinction is usually made between 'measured quantities' or 'primary data', e.g. total leaf area, total plant dry weight, and 'derived valued' or 'growth characteristics' or 'growth attributes', e.g. Relative Growth Rate (RGR) (Květ *et al.*, 1971; Evans, 1972; Hunt, 1982). Sampling of a representative set of plants usually means they are harvested destructively. The limitations of space did not permit sufficient plants for frequenting harvest of the growing plants. Accordingly, non-destructive methods have been adopted. Leaf area is the most important factor determining plant productivity (Watson, 1956) and it is usually assumed to be the size-attribute that best measures its capacity for photosynthesis. Accordingly, leaf area was estimated and growth characteristics values can be calculated. An attempt has been made to estimate plant dry weight or leaf dry weight from the length of the shoot or leaf area, as determined by some workers (Goodal, 1945). There is difficulty in obtaining accurate measurement of the length of the shoot in the primulas and the ratio of leaf area to leaf dry weight has been reported to change during plant growth and with environmental conditions (see Aase, 1978). Consequently, plant growth characteristics related to leaf area [for example, Relative Leaf area Growth Rate (RLaGR), Leaf Area Index (LAI), and Leaf Area Duration (LAD)] were determined.

4.2.3.1 Estimation of leaf area

Leaf area measurements are often necessary as an index of growth and for assimilation and transpiration determination in agronomic and plant physiological studies (Aase, 1978; Donald & Black, 1958). One of the most common non-destructive methods is the formulation of a mathematical relationship between the

linear measurements of the leaf, such as the product of length and breadth, or simply, the leaf length with leaf area (Kemp, 1959; Pereira, 1977; Marshall, 1968; Singh & Kashyap, 1983).

Leaves of the selected species from the reserved bowls were sampled and an outline of each leaf was obtained by photocopying. The area within the outline was measured using a hand planimeter (Paquin & Coulombe, 1959). Planimetering was repeated three times (with the arms of the instrument in different positions) and the mean of the values indicated by the counter was then recorded. The length of the leaf was measured along the line joining the tip to the base. The width of the leaf was measured, perpendicular to the above line and at a point where it was at maximum.

The full form of the standard linear regression equation is :-

$$y = a + bx \qquad 4.1$$

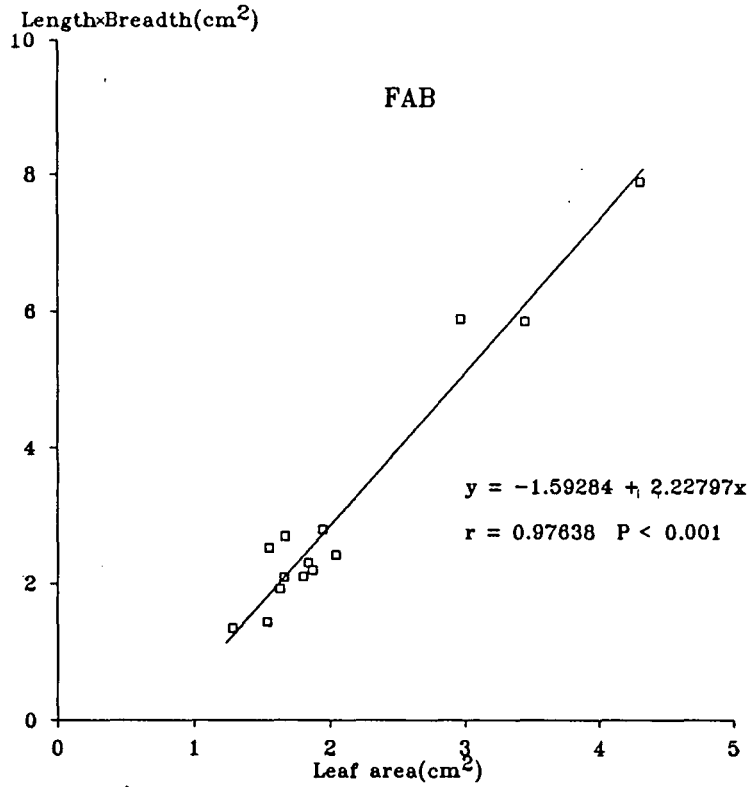
and this has also been frequently used, where y = leaf area, a and b are constants and x is the product of leaf length and leaf breadth. Regression equations were determined for each species and proved to be highly significant ($P<0.001$). The correlation coefficients (r) as well as the regression equations are shown in Fig. 4.2.

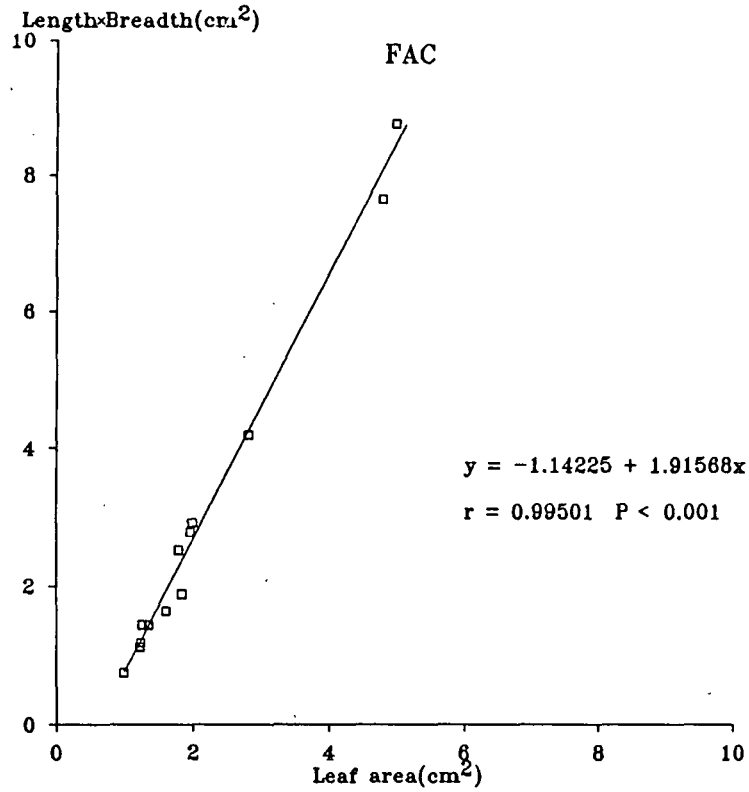
4.2.3.2 Field measurements

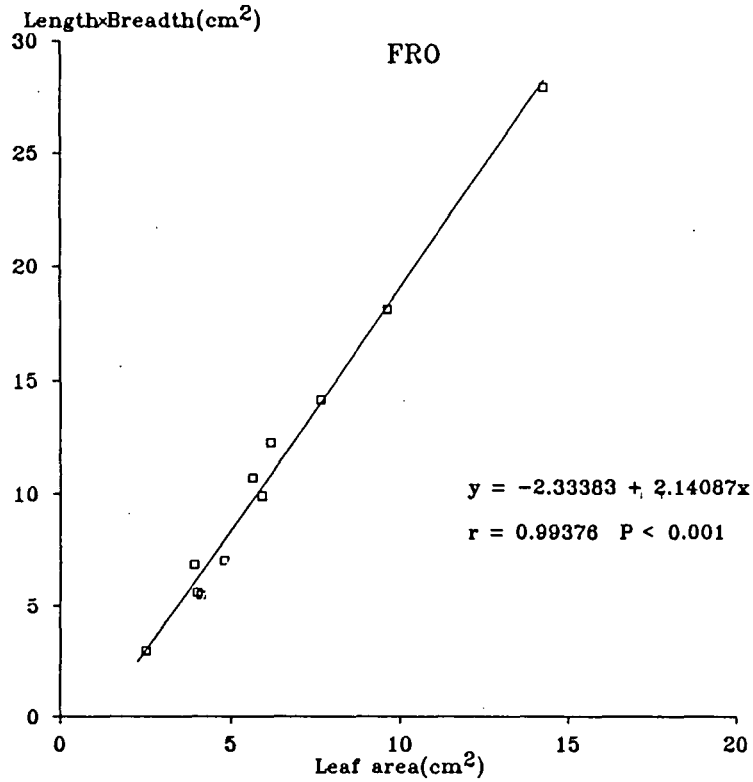
Ten out of twelve plants from each bowl were used for plant growth analyses. The other two were used for studying the effects of the microclimate due to different altitudes on protein/proline levels. In each bowl, individual plants were marked and thus it was possible to follow the growth of the same plant throughout the experimental period. When the weather did not permit outdoor work (e.g. on a

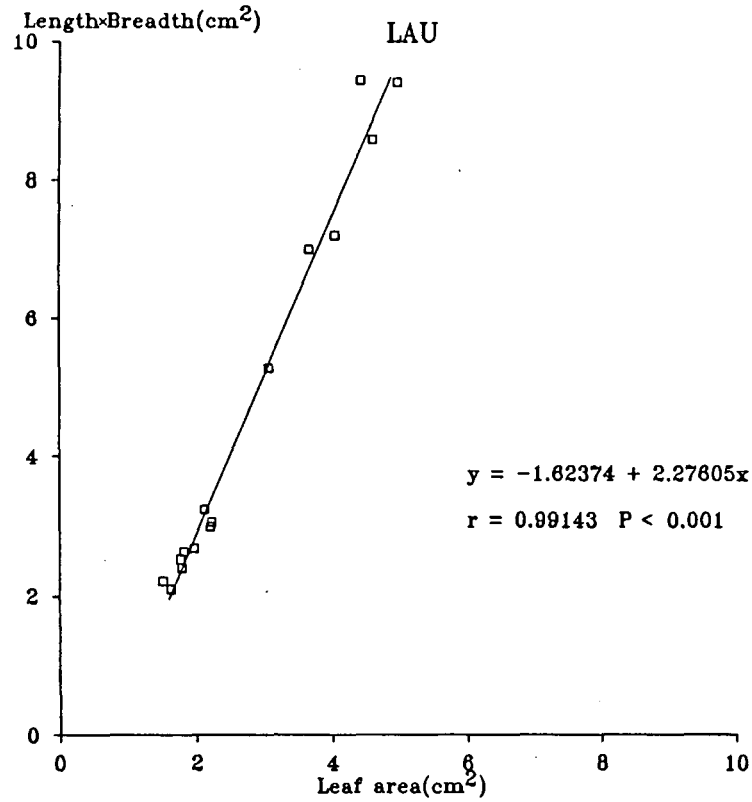
Figure 4.2 Relationships between the product of length×breadth and leaf area of *Primula spp.*

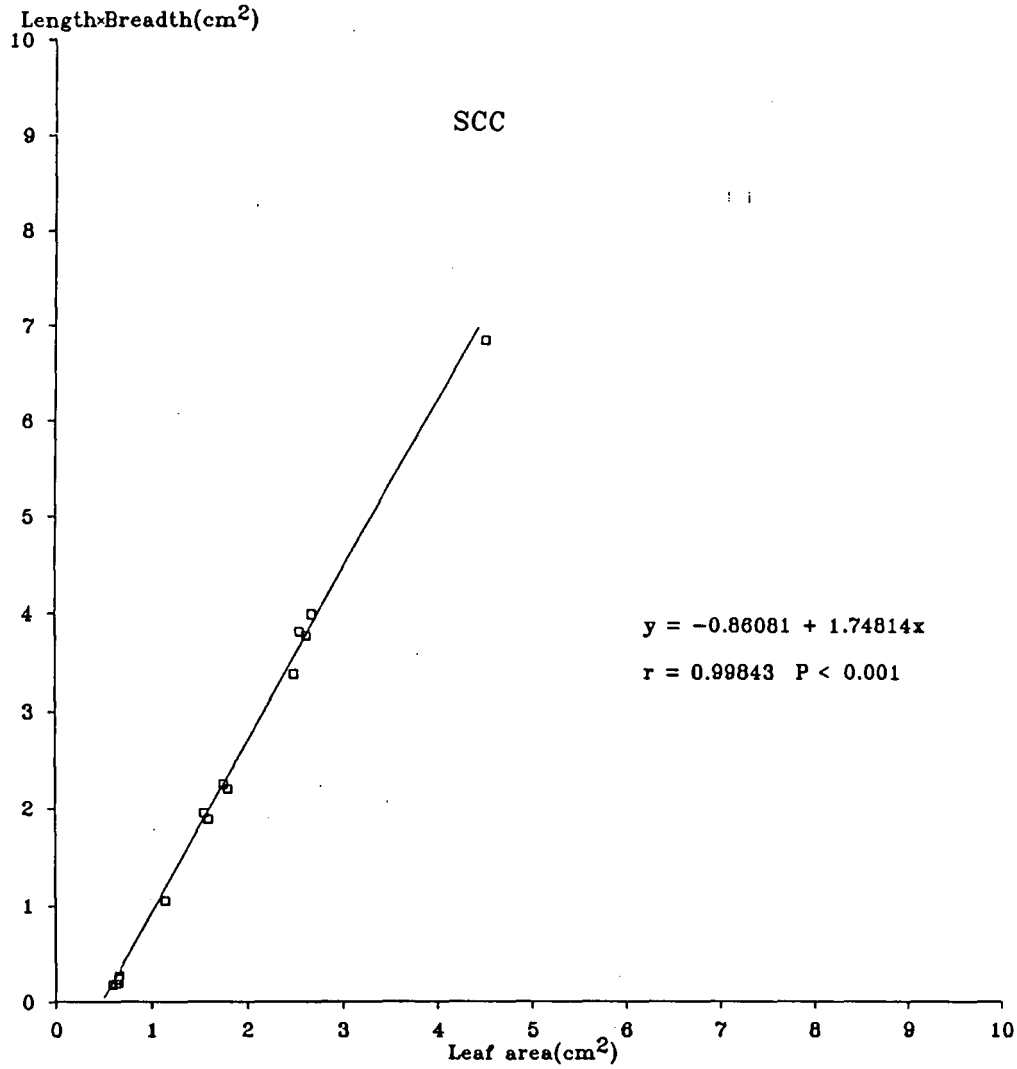
FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
SCC = *P.scotica*


Length×Breadth(cm²)
FAB
y = −1.59284 + 2.22797x
r = 0.97638 P < 0.001
Leaf area(cm²)


Length×Breadth(cm²)
FAC
y = −1.14225 + 1.91568x
r = 0.99501 P < 0.001
Leaf area(cm²)


Length×Breadth(cm²)
FRO
30
25
20
15
10
5
0
0
5
10
15
20
Leaf area(cm²)
y = -2.33383 + 2.14087x
r = 0.99376 P < 0.001


Length×Breadth(cm²)
LAU
10
8
6
4
2
0
0
2
4
6
8
10
Leaf area(cm²)
y = -1.62374 + 2.27605x
r = 0.99143 P < 0.001


Length×Breadth(cm²)
SCC
y = −0.86081 + 1.74814x
r = 0.99843 P < 0.001
Leaf area(cm²)
0
1
2
3
4
5
6
7
8
9
10

windy or wet day), that usually happened for all the three sites in the northern Pennines, then the bowls were taken indoors (temperature 10-15°C) for 20-30 min in order for the measurement to be made; the effect on soil temperature would have very small. For each plant the length and the breadth of a fully expanded green leaf was measured; the number of leaves, number of rosettes, number of scapes, number of florets, and number of survivors were counted each month. When the capsules had nearly ripened each scape was covered with a small paper bag in an attempt to protect the seed from blowing away. At the end of the growing season of 1986, the capsules were counted and collected for seed count. Measurements and counts of the primulas were made from May to October 1986. By the end of the growing season, most of the plants had become senescent preventing any further measurements; this event coinciding with the severe weather in the upland sites. Consequently, the primulas were left over-winter. The number of winter survivors and the number of scapes were counted at the end of April 1987.

4.2.4 Micrometeorological measurements

Many attempts have been made to measure the microclimate of the primulas at each site. However, the local climatic data from the vicinity of the sites during the experimental period as well as long-term weather records were also collected and used in the case of missing data.

4.2.4.1 Temperature

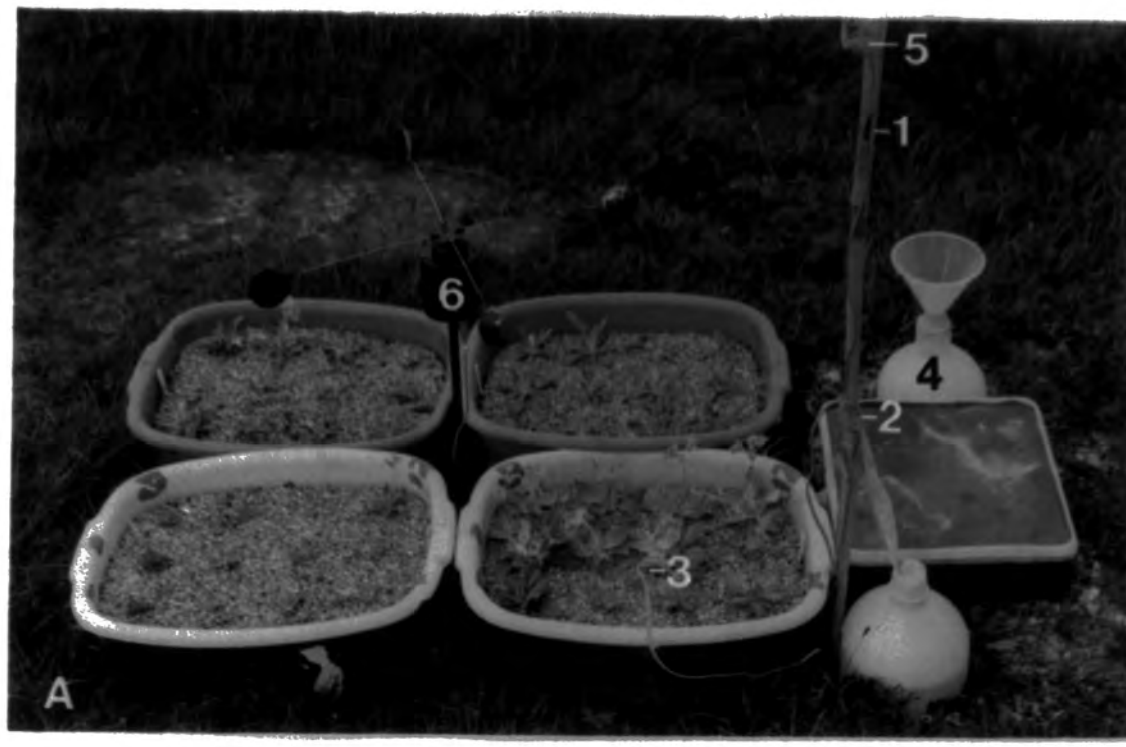
Dry and Wet bulb air temperatures and soil temperatures were recorded at each site from May 1986 to April 1987 with a sensor and integrator for temperature (Woodward & Yaqub, 1979), calibrated at several different temperatures against a standard mercury thermometer. Dry and Wet bulb sensor were put at a height of 40 and 25 cm above the primula canopy surfaces respectively. A soil sensor was sunk to about 5 cm depth in the bowl (Fig. 4.3).

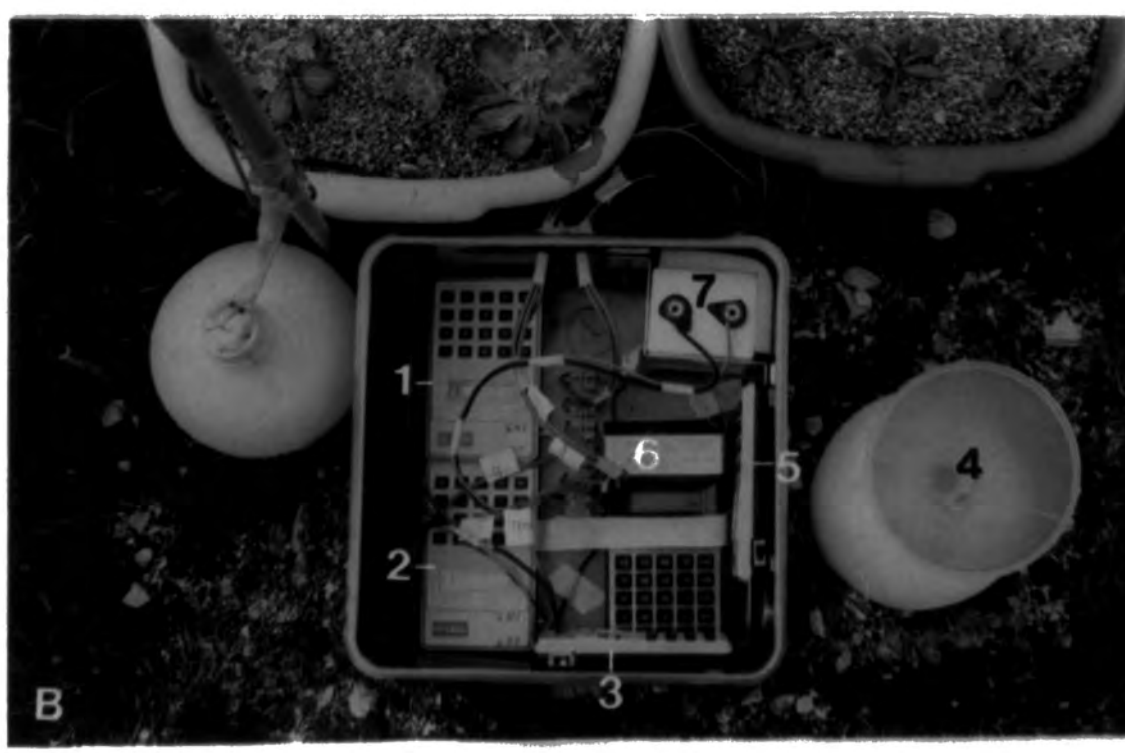
4.2.4.2 Precipitation

Figure 4.3 *Primula farinosa* and its allies grown at Widdybank Fell Meteorological Station (510 m), Upper Teesdale.

A 1 = sensor for Dry-bulb air temperature
2 = sensor for Wet-bulb air temperature
3 = sensor for soil temperature
4 = rain-guage
5 = sensor for irradiance
6 = anemometer

B 1 = integrator for Dry-bulb air temperature
2 = integrator for Wet-bulb air temperature
3 = integrator for soil temperature
4 = rain-guage
5 = integrator for irradiance
6 = integrator for anemometer
7 = battery


5
1
6
4
2
3
A


7
1
6
5
4
2
3
B

Rainfall and other kinds of precipitation were collected in a rain-guage (consisting of a plastic funnel, 10 cm in diameter and 2,500 cm^3 plastic bottle). The unit was sunk into the ground, with the top of the funnel about 20 cm above the margin of the bowl. Precipitation was measured in cm^3 by a measuring cylinder.

4.2.4.3 Radiation

Total photosynthetic photon flux densities (PFD) at 60 cm above the primula canopy surfaces were assessed throughout the whole experiment with a sensor and integrator for irradiance, the unit being designed to record photosynthetically active radiation (PAR) in a 400-700 nm wave band (Woodward & Yaqub, 1979). The sensor was calibrated against a Skye Instruments sensor and meter over a range of PFD under a fluorescent light(daylight) for 1 h at each PFD.

4.2.4.4 Wind speed

Wind speed was recorded at each site by a 4-cup anemometer and integrator originated by Dr. F.I.Woodward, at a height of 45 cm above the primula canopy surfaces.

All the integrators were operated by rechargeble nickel-cadmium batteries (RS Components, Ltd). Data were read and measured monthly and daily mean data were calculated from the regression equation. The integrators and batteries were put together in a sealed plastic box and covered with plastic bag. Moisture inside the box was absorbed by using self indicating Silica Gel.

4.2.5 Calculation of growth characteristics

Three growth characteristics were calculated each month in terms of the changes during the course of development in the leaf area. The area is in units of sq cm and the unit of time course is a day.

4.2.5.1 Relative Leaf area Growth Rate (RLaGR)

Relative Growth Rate (RGR) is one of the most appropriate measurements of growth (Květ *et al.*, 1971). The mean relative growth rate of different plant parts, such as shoot, root, leaf and leaf area can be estimated separately in the same way. The sum of all the relative growth rates of the parts of a plant equals the relative growth rate of the whole (Květ *et al.*, 1971).

For example, the mean relative growth rate of leaf area, RLaGR, over the interval (t2-t1) can be calculated from the following equation (Fisher,1921) :-

$$RLaGR = \frac{ln\,A2 - ln\,A1}{t2 - t1} \qquad (area\ area^{-1}\ time^{-1}) \qquad 4.2$$

where A1 and A2 are the leaf areas at times t1 and t2 respectively.

4.2.5.2 Leaf Area Duration (LAD)

Leaf Area Duration is the relationship between leaf area and time and can be calculated from the formula (Květ *et al.*, 1971) :-

$$LAD = \frac{(A2 - A1)(t2 - t1)}{ln\,A2 - ln\,A1} = \frac{(A2 - A1)}{RLaGR} \qquad (area\ time) \qquad 4.3$$

4.2.5.3 Leaf Area Index (LAI)

Leaf Area Index has been introduced by Watson (1947), as the leaf area per unit of ground area. It is used to characterize crops for interception and penetration of PAR (Anderson, 1971; Montieth, 1965). LAI is given by :-

$$LAI = \frac{A}{P} \qquad (area\ area^{-1}) \qquad 4.4$$

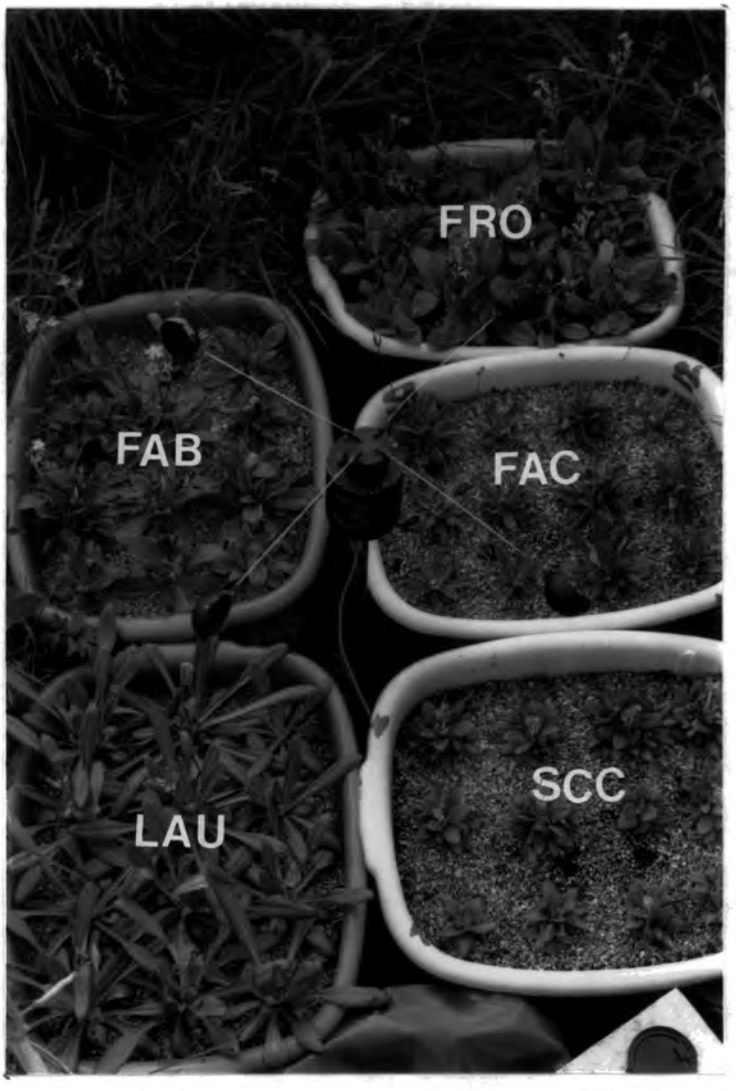
where A is the leaf area and P is the ground area.

In a bowl of primula (surface area = 867 cm^2), containing 12 plants (Fig. 4.4), leaves of two of them were taken for physiological studies and the leaf area from these was omitted in the calculation, thus the LAI values presented are a systematic underestimate of the true value, but consistently so amongst species.

FORTRAN programmes were written to enable all calculations to be made which were run on the mainframe computer, Computer centre, University of Durham.

Figure 4.4 *Primula farinosa* and its allies grown at Hartside nursery (330 m), Alston, Cumbria.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
SCC = *P.scotica*


FRO
FAB
FAC
SCC
LAU

4.3 Results

4.3.1 Analyses of growth

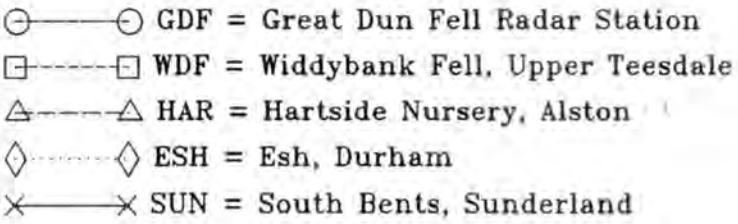
4.3.1.1 Species performance at different sites

(a) *Primula farinosa* (b)

Fig. 4.5 and Fig. 4.10a depict mean monthly values of growth characteristics for *P. farinosa* (b) grown at five sites. ANOVA results of growth characteristics compared between sites are shown in Table 4.2. Comparisons of growth characteristics between each pair of sites using LSD tests are summarized in Tables 4.3 to 4.10. An ANOVA result revealed a highly significant difference in RLaGR between the sites during the growing season except at the beginning of the experiment. The values of RLaGR at all sites were highest at the beginning of the experiment (J-J) and declined slightly later on until the end of growing season where RLaGR was negative at the three upland sites. There was a marked fluctuation of RLaGR at SUN which coincided with the aspects of drought and wetness at this site. During mid-summer (J-A) the highest values of RLaGR were observed at WDF, however, the results of LSD tests showed non-significant differences with HAR.

An ANOVA result for *P. farinosa* (b) showed highly significant differences in LAD between the sites during the growing season. The values of LAD for *P. farinosa* (b) were lowest at the beginning of growing season and then an increase was noted at the end of summer (A-S) and at the beginning of autumn (S-O). By this time the value of LAD at HAR was significantly higher than at the other sites, except WDF. The values of LAD at GDF were rather stable during the growing season. Comparisons of the values of LAI for *P. farinosa* (b) using ANOVA revealed highly significant differences between the sites during the growing season. The values of LAI for *P. farinosa* (b) showed a peak at the end of summer and dropping at the beginning of autumn at all sites except ESH. Similar to RLaGR, the values of LAI at SUN showed a response to the microclimate at the site, as can be seen in Fig.

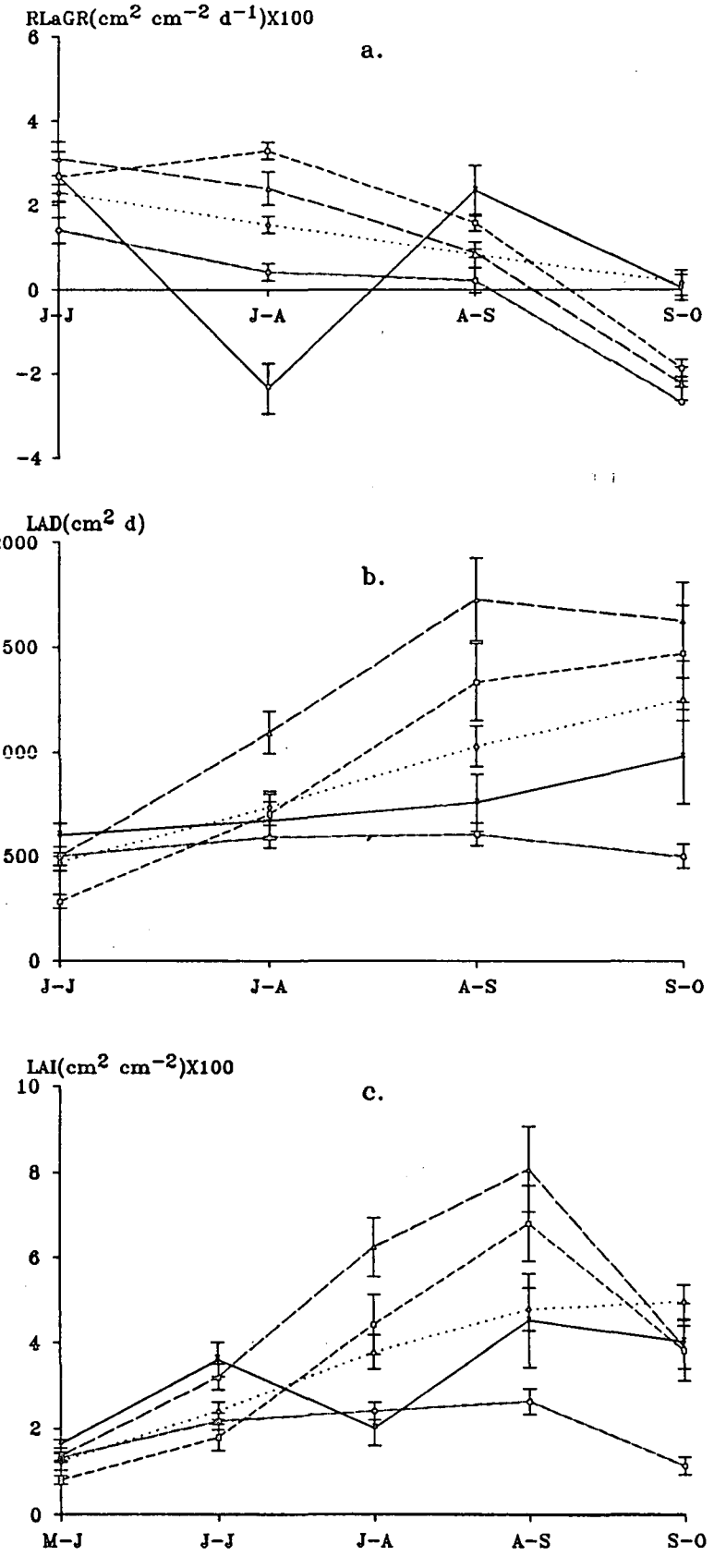
Figure 4.5 Mean monthly values of growth characteristics of *Primula farinosa*(b) grown at five sites in the field during May–October, 1986.

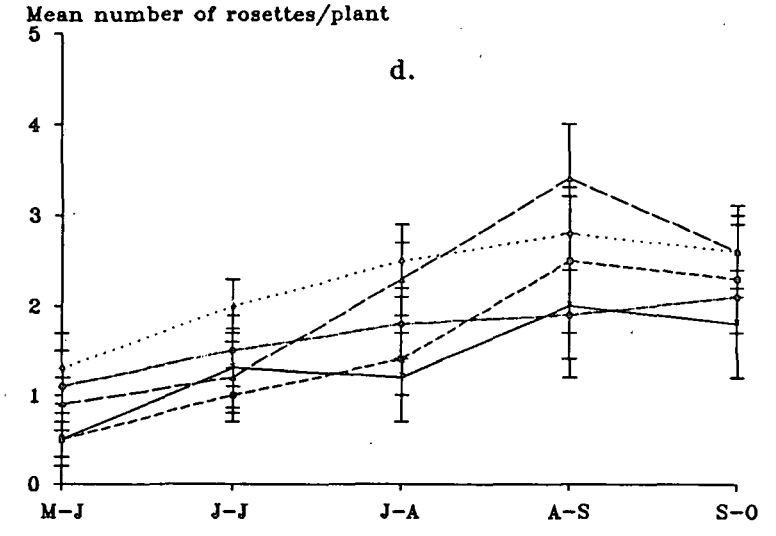


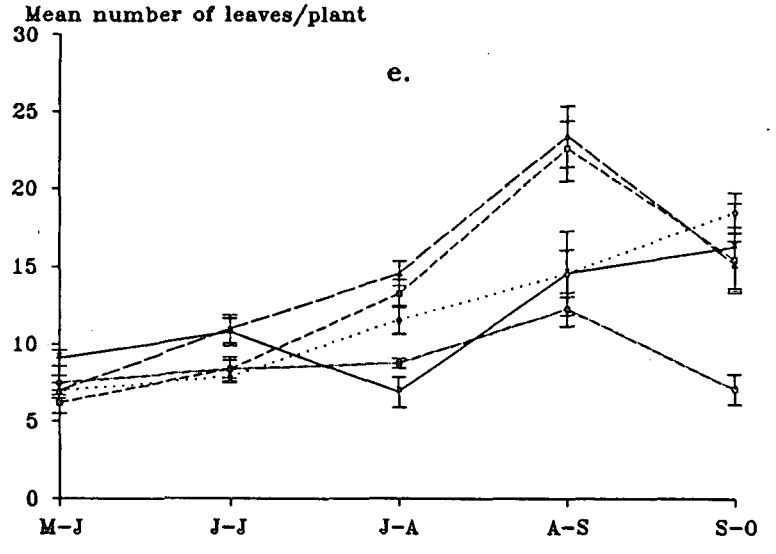
M–J = May–June
J–J = June–July
J–A = July–August
A–S = August–September
S–O = September–October

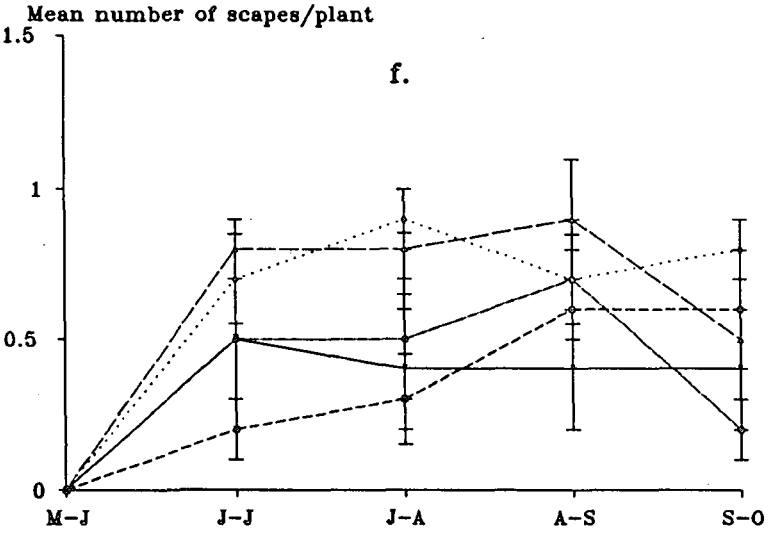
Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

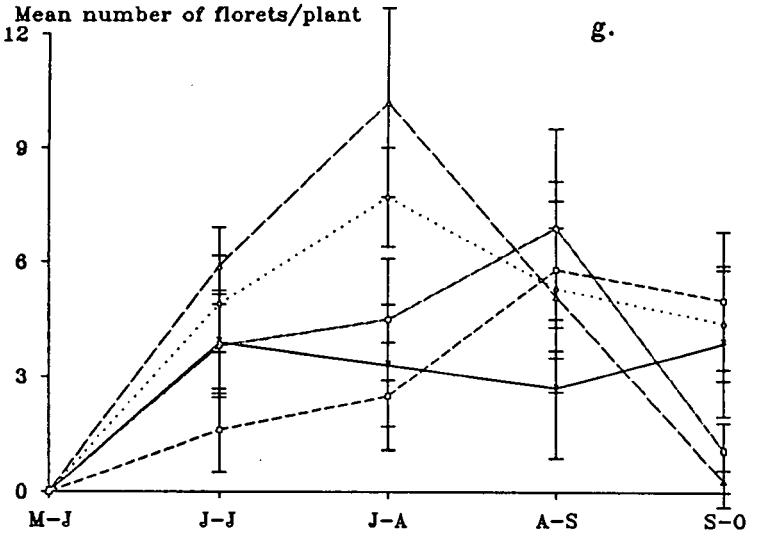
a.– b. data during June–October
c.– f. data during May–October
h.– i. data during September–October

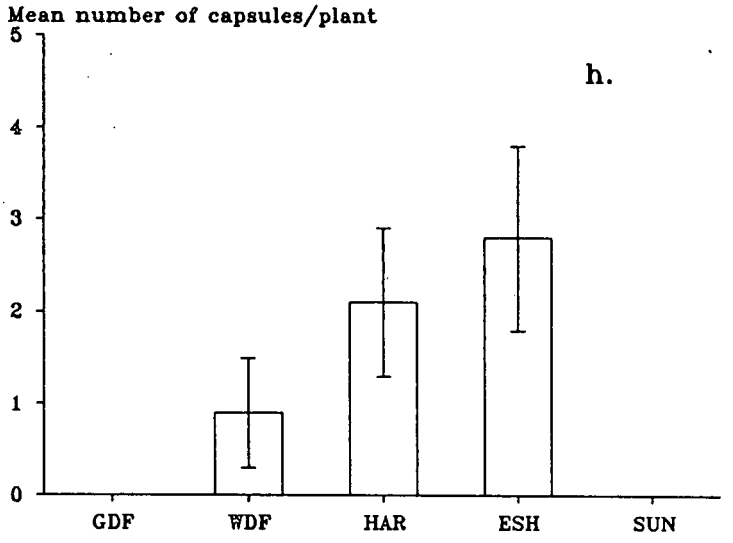

RLaGR(cm^2 cm^{-2} d^{-1})X100
a.
6
4
2
0
-2
-4
J-J
J-A
A-S
S-O
LAD(cm^2 d)
b.
2000
1500
1000
500
0
J-J
J-A
A-S
S-O
LAI(cm^2 cm^{-2})X100
c.
10
8
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of rosettes/plant
d.
5
4
3
2
1
0
M-J
J-J
J-A
A-S
S-0


Mean number of leaves/plant
e.
30
25
20
15
10
5
0
M-J
J-J
J-A
A-S
S-0


Mean number of scapes/plant
f.
1.5
1
0.5
0
M-J
J-J
J-A
A-S
S-0


Mean number of florets/plant
g.
12
9
6
3
0
M-J
J-J
J-A
A-S
S-O


Mean number of capsules/plant
h.
5
4
3
2
1
0
GDF
WDF
HAR
ESH
SUN

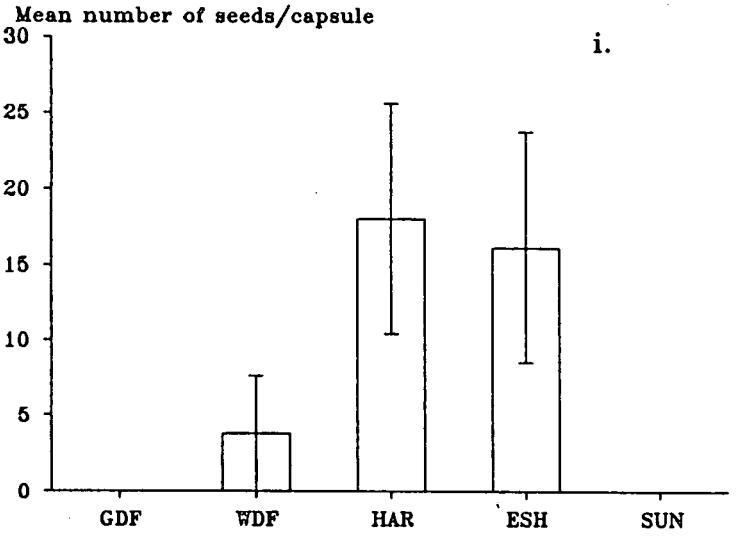

Mean number of seeds/capsule
i.
30
25
20
15
10
5
0
GDF
WDF
HAR
ESH
SUN

Table 4.2 ANOVA of growth characteristics in *Primula* spp. grown at five sites in the field during May – October, 1986.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b)	M-J	–	–	**	NS	**	–	–	–	–
	J-J	NS	***	***	NS	**	NS	NS	–	–
	J-A	***	**	***	NS	***	*	*	–	–
	A-S	***	***	***	NS	***	NS	NS	***	–
	S-O	***	***	***	NS	***	NS	NS	*	*
P.farinosa(c)	M-J	–	–	***	NS	***	**	**	–	–
	J-J	NS	***	***	NS	NS	NS	NS	–	–
	J-A	***	*	***	NS	**	*	NS	–	–
	A-S	***	***	***	NS	***	*	NS	**	–
	S-O	***	***	***	NS	***	NS	***	***	*
P.frondosa	M-J	–	–	NS	NS	NS	***	***	–	–
	J-J	***	*	*	NS	**	**	**	–	–
	J-A	***	***	***	NS	***	NS	**	–	–
	A-S	***	***	*	NS	**	*	NS	***	–
	S-O	***	**	NS	NS	*	***	***	***	**
P.laurentiana	M-J	–	–	**	NS	***	–	–	–	–
	J-J	**	***	***	NS	***	***	***	–	–
	J-A	***	***	***	NS	***	***	***	–	–
	A-S	***	***	***	**	***	***	***	NS	–
	S-O	***	***	***	***	***	NS	*	**	NS
P.scotica	M-J	–	–	***	NS	NS	–	–	–	–
	J-J	**	***	***	**	NS	–	–	–	–
	J-A	*	***	***	***	*	***	***	–	–
	A-S	***	***	***	***	***	***	***	–	–
	S-O	***	***	NS	***	NS	***	***	***	***

Note RLaGR = Relative leaf area growth rate LAD = Leaf area duration

LAI = Leaf area index ROS = Number of rosettes

LF = Number of leaves FR = Number of florets

SC = Number of scapes CAP = Number of capsules

SEED = Number of seeds

Table 4.3 Statistical test of significance (LSD) of RLaGR in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	*	**	NS	*
	J-A	-	***	***	*	***
	A-S	-	**	NS	NS	***
	S-O	-	NS	NS	***	***
2.WDF						
	M-J		-	-	-	-
	J-J		-	NS	NS	NS
	J-A		-	NS	***	***
	A-S		-	NS	NS	NS
	S-O		-	NS	***	***
3.HAR						
	M-J			-	-	-
	J-J			-	NS	NS
	J-A			-	NS	***
	A-S			-	NS	**
	S-O			-	***	***
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	***
	A-S				-	**
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.4 Statistical test of significance (LSD) of LAD in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	**	NS	NS	NS
	J-A	-	NS	***	NS	NS
	A-S	-	***	***	*	NS
	S-O	-	***	***	**	NS
2.WDF						
	M-J		-	-	-	-
	J-J		-	**	**	***
	J-A		-	**	NS	NS
	A-S		-	NS	NS	*
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	-	-
	J-J			-	NS	NS
	J-A			-	*	***
	A-S			-	**	**
	S-O			-	NS	*
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.5 Statistical test of significance (LSD) of LAI in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	**	NS	NS	NS
	J-J	-	NS	*	NS	**
	J-A	-	**	***	NS	NS
	A-S	-	***	***	NS	NS
	S-O	-	**	***	***	***
2.WDF						
	M-J		-	**	*	***
	J-J		-	**	NS	**
	J-A		-	*	NS	**
	A-S		-	NS	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	NS
	J-A			-	***	***
	A-S			-	**	**
	S-O			-	NS	NS
4.ESH						
	M-J				-	*
	J-J				-	**
	J-A				-	*
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.6 Statistical test of significance (LSD) of number of rosettes in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	NS	NS
	J-J	-	NS	NS	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	NS	NS
	S-O	-	NS	NS	NS	NS
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	NS	*	NS
	J-A		-	NS	NS	NS
	A-S		-	NS	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	*
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.7 Statistical test of significance (LSD) of number of Leaves in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	NS	*
	J-J	-	NS	*	NS	*
	J-A	-	***	***	NS	NS
	A-S	-	***	***	NS	NS
	S-O	-	**	**	***	***
2.WDF						
	M-J		-	NS	NS	***
	J-J		-	*	NS	*
	J-A		-	NS	NS	***
	A-S		-	NS	**	**
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	**
	J-J			-	**	NS
	J-A			-	*	***
	A-S			-	***	**
	S-O			-	NS	NS
4.ESH						
	M-J				-	**
	J-J				-	**
	J-A				-	***
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.8 Statistical test of significance (LSD) of number of scapes in *P.farinosa* (b) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	NS	NS	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	NS	NS
	S-O	-	NS	NS	*	NS
2.WDF						
	M-J		-	-	-	-
	J-J		-	**	*	NS
	J-A		-	*	**	NS
	A-S		-	NS	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	-	-
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	*
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.9 Statistical test of significance (LSD) of number of florets in *P.farinosa* (b) grown at five sites in the field during May–October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M–J	–	–	–	–	–
	J–J	–	NS	NS	NS	NS
	J–A	–	NS	*	NS	NS
	A–S	–	NS	*	NS	NS
	S–O	–	*	NS	NS	NS
2.WDF						
	M–J		–	–	–	–
	J–J		–	*	NS	NS
	J–A		–	**	*	NS
	A–S		–	NS	NS	NS
	S–O		–	*	NS	NS
3.HAR						
	M–J			–	–	–
	J–J			–	NS	NS
	J–A			–	NS	**
	A–S			–	NS	NS
	S–O			–	*	NS
4.ESH						
	M–J				–	–
	J–J				–	NS
	J–A				–	*
	A–S				–	NS
	S–O				–	NS
5.SUN						
	M–J					–
	J–J					–
	J–A					–
	A–S					–
	S–O					–

Table 4.10 Statistical test of significance (LSD) of number of capsules and seeds in *P.farinosa* (b) grown at five sites, at the end of growing season, 1986.

Sites		Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	capsule/plant	–	NS	*	**	NS
	seed/capsule	–	NS	*	*	NS
	seed/plant	–	NS	NS	NS	NS
2.WDF						
	capsule/plant		–	NS	*	NS
	seed/capsule		–	NS	NS	NS
	seed/plant		–	NS	NS	NS
3.HAR						
	capsule/plant			–	NS	*
	seed/capsule			–	NS	*
	seed/plant			–	NS	NS
4.ESH						
	capsule/plant				–	**
	seed/capsule				–	*
	seed/plant				–	NS
5.SUN						
	capsule/plant					–
	seed/capsule					–
	seed/plant					–

4.5c. During A-S, the highest values of LAI were found at HAR, significantly higher than all the other sites except WDF.

ANOVA performed on the number of rosette for *P. farinosa* (b) showed non-significant differences between the sites for all months during the growing season. A marked increase in the number of rosettes was found at HAR during A-S and the lowest number of rosettes was at GDF. However, there was no significant difference between each pair of sites with only two exceptions (see Table 4.6). An ANOVA result showed highly significant differences in the number of leaves for *P. farinosa* (b) between the sites. At the upland sites, the maximum numbers of leaves were found during A-S, whilst at the lowland site the number of leaves were still increasing. However, the two upland sites, WDF, HAR showed significantly higher leaf numbers than the other sites during A-S (Table 4.7). The number of leaves for *P. farinosa* (b) at SUN showed the lowest numbers during J-A due to drought conditions at this site. Comparisons of the number of scapes for *P. farinosa* (b) showed significant differences between the sites only during mid-summer (J-A). By this time the highest number of scapes was found at ESH, however, during J-A it was only significantly higher than the other site was found only with WDF which had the lowest numbers of scapes (Table 4.8). The number of scapes at GDF was intermediate amongst the 5 sites and did not show any significant differences with the other sites. The number of scapes at WDF showed a slight increase and the maximum number was found during A-S through S-O.

An ANOVA result of the number of florets was similar to that of the number of scapes, with significant differences between the sites found only during J-A. The number of scapes at HAR and ESH were significantly higher than at the other sites during J-A. The maximum numbers of florets at GDF and WDF were found during A-S which was later than at the other sites. No capsule formation was found at two sites, GDF and SUN and this resulted in highly significant differences between the sites during A-S. The highest number of capsules per plant was at ESH and it was significantly higher than at WDF (Table 4.10). In contrast the number of

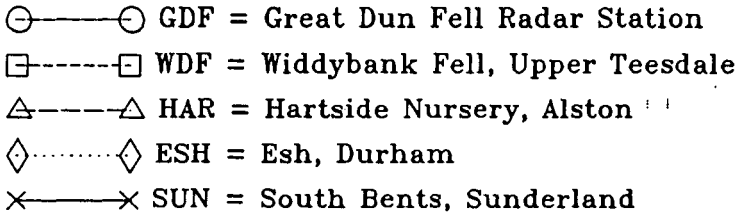
seeds per capsule was highest at HAR, but no significant differences were observed. The number of seeds per plant (calculated values) also did not show significant differences between each pair of sites.

(b) *Primula farinosa* (c)

Detailed comparisons of growth characteristics between the sites for *P. farinosa* (c) can be seen in Fig. 4.6 and Fig. 4.10b. Comparisons of growth characteristics between each pair of sites are summarized in Tables 4.11-4.18. At the beginning of the experiment, the highest values of RLaGR for *P. farinosa* (c) were found at ESH, whilst the lowest values were at WDF. During J-A, the two upland sites (WDF and HAR) showed an increase in RLaGR. However, after mid-summer (J-A) the values of RLaGR declined and the minimum values were observed at the end of growing season (S-O). *P. farinosa* (c) at the lowland sites showed significantly higher values of RLaGR than at all the upland sites by the end of the growing season (Table 4.11). In contrast to the values of RLaGR, LAD showed the highest values at the end of growing season at all sites except GDF. The values of LAD at GDF were significantly lower than at the other sites (Table 4.12). The lowest and the highest altitudinal sites had the lowest values of LAD. Comparisons of LAD between the sites showed significant differences for all months (Table 4.3).

An ANOVA of LAI showed highly significant differences between the sites during the growing season. The maximum values of LAI were found during A-S at all sites and declined markedly at the end of the growing season. At the peak of LAI, the highest values were at HAR and the lowest at GDF. However, the values at WDF, HAR and ESH were not significantly different from each other (Table 4.13). ANOVA performed on the number of rosettes, comparing between the sites showed no significant differences. The highest number of rosettes for *P. farinosa* (c) was found at its natural habitat (WDF) and the lowest number was found at GDF. However, there were no significant differences between each pair of sites (Table 4.14). An ANOVA of the number of leaves showed significant differences between

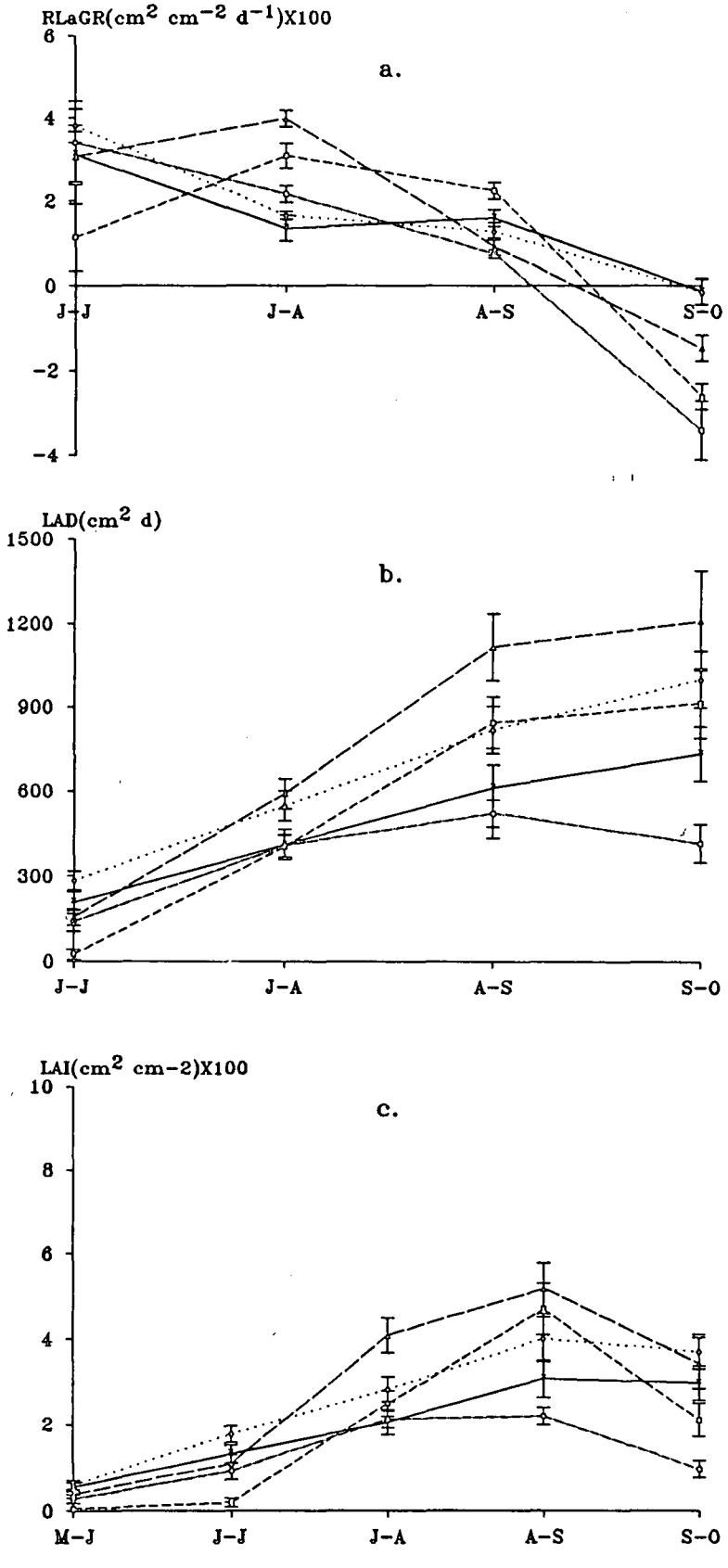
Figure 4.6 Mean monthly values of growth characteristics of *Primula farinosa*(c) grown at five sites in the field during May–October, 1986.

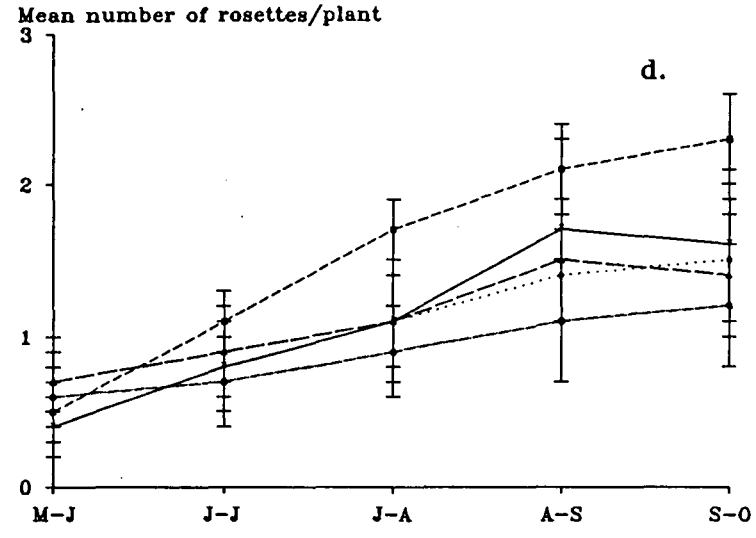


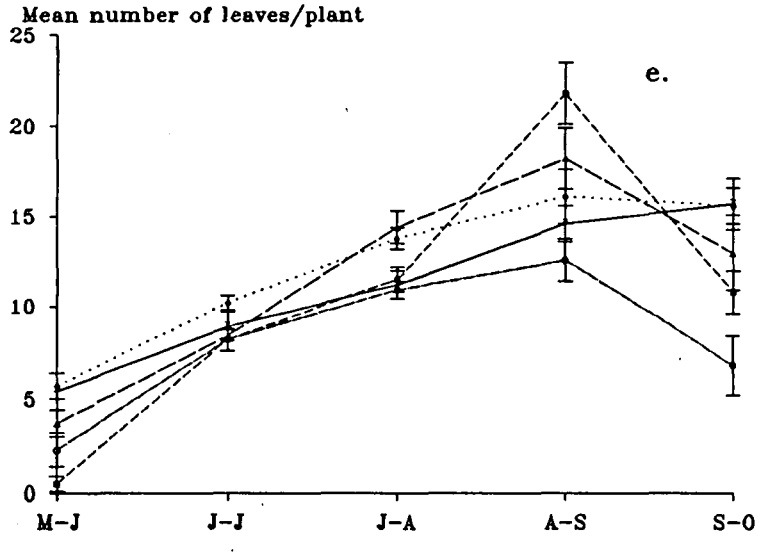
M–J = May–June
J–J = June–July
J–A = July–August
A–S = August–September
S–O = September–October

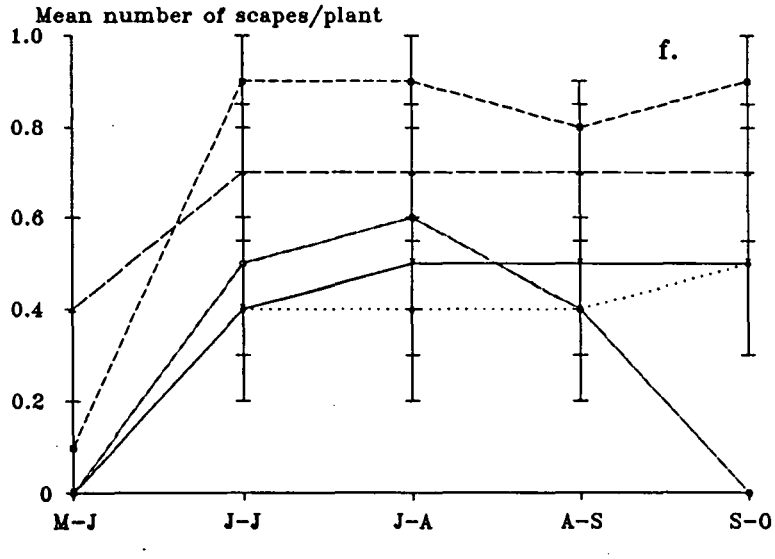
Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

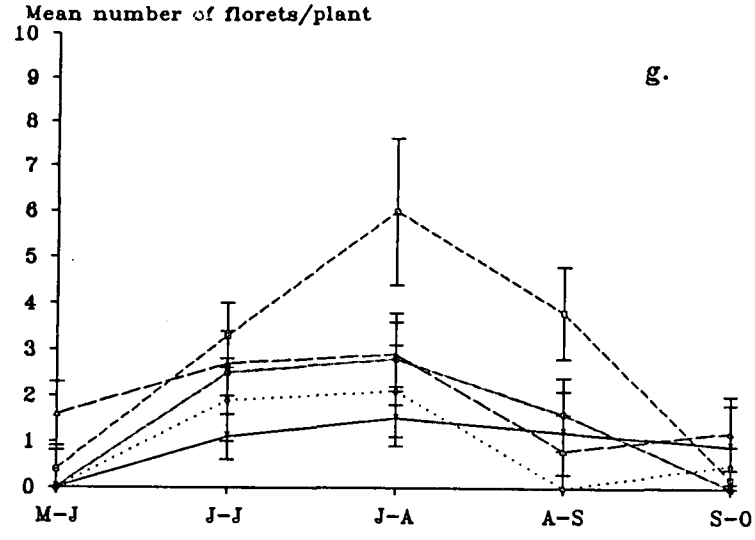
a.– b. data during June–October
c.– f. data during May–October
h.– i. data during September–October

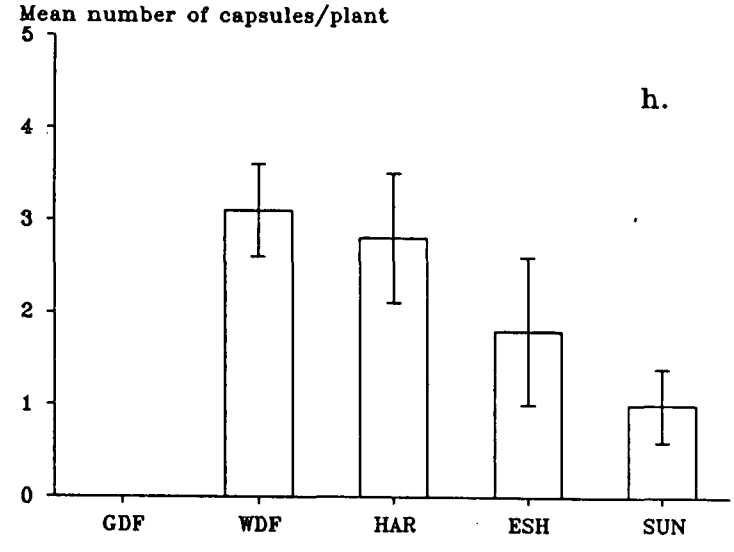

RLaGR(cm2 cm-2 d-1)X100
a.
6
4
2
0
-2
-4
J-J
J-A
A-S
S-O
LAD(cm2 d)
b.
1500
1200
900
600
300
0
J-J
J-A
A-S
S-O
LAI(cm2 cm-2)X100
c.
10
8
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of rosettes/plant
3
2
1
0
d.
M-J
J-J
J-A
A-S
S-0


Mean number of leaves/plant
25
20
15
10
5
0
e.
M-J
J-J
J-A
A-S
S-0


Mean number of scapes/plant
1.0
0.8
0.6
0.4
0.2
0
f.
M-J
J-J
J-A
A-S
S-0


Mean number of florets/plant
g.
10
9
8
7
6
5
4
3
2
1
0
M-J
J-J
J-A
A-S
S-O


Mean number of capsules/plant
h.
5
4
3
2
1
0
GDF
WDF
HAR
ESH
SUN

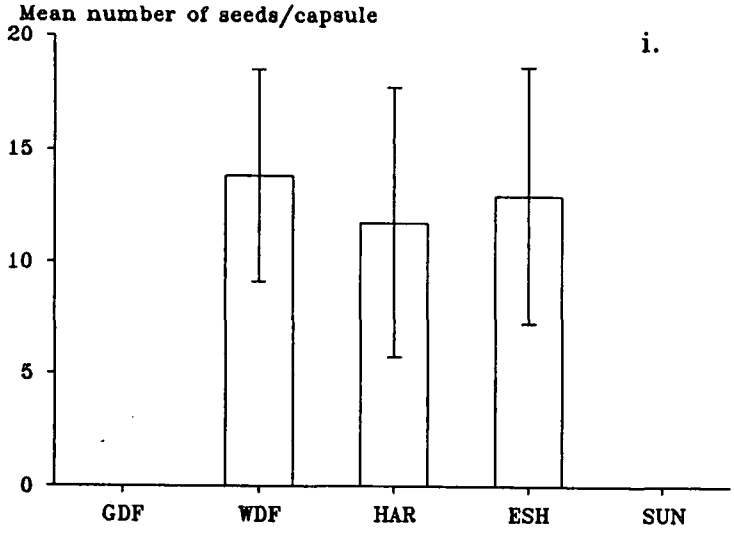

Mean number of seeds/capsule
i.
20
15
10
5
0
GDF
WDF
HAR
ESH
SUN

Table 4.11 Statistical test of significance (LSD) of RLaGR in *P.farinosa* (c) grown at five sites in the field during May-October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	*	NS	NS	NS
	J-A	-	*	***	NS	*
	A-S	-	***	NS	NS	**
	S-O	-	NS	*	***	***
2.WDF						
	M-J		-	-	-	-
	J-J		-	NS	*	NS
	J-A		-	*	***	***
	A-S		-	***	**	*
	S-O		-	*	***	***
3.HAR						
	M-J			-	-	-
	J-J			-	NS	NS
	J-A			-	***	***
	A-S			-	NS	*
	S-O			-	**	*
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.12 Statistical test of significance (LSD) of LAD in *P.farinosa* (c) grown at five sites in the field during May-October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	–	–	–	–	–
	J-J	–	*	NS	**	NS
	J-A	–	NS	*	*	NS
	A-S	–	*	***	*	NS
	S-O	–	**	***	**	*
2.WDF						
	M-J		–	–	–	–
	J-J		–	**	***	***
	J-A		–	**	*	NS
	A-S		–	*	NS	NS
	S-O		–	NS	NS	NS
3.HAR						
	M-J			–	–	–
	J-J			–	**	NS
	J-A			–	NS	*
	A-S			–	*	***
	S-O			–	NS	**
4.ESH						
	M-J				–	–
	J-J				–	NS
	J-A				–	NS
	A-S				–	NS
	S-O				–	NS
5.SUN						
	M-J					–
	J-J					–
	J-A					–
	A-S					–
	S-O					–

Table 4.13 Statistical test of significance (LSD) of LAI in *P.farinosa* (c) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	*	*
	J-J	-	*	NS	**	NS
	J-A	-	NS	***	NS	NS
	A-S	-	***	***	*	NS
	S-O	-	NS	***	***	**
2.WDF						
	M-J		-	*	***	***
	J-J		-	**	***	***
	J-A		-	***	NS	NS
	A-S		-	NS	NS	*
	S-O		-	*	*	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	*	NS
	J-A			-	**	***
	A-S			-	NS	**
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.14 Statistical test of significance (LSD) of number of rosettes in *P.farinosa* (c) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	NS	NS
	J-J	-	NS	NS	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	NS	NS
	S-O	-	NS	NS	NS	NS
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	NS	NS	NS
	J-A		-	NS	NS	NS
	A-S		-	NS	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.15 Statistical test of significance (LSD) of number of leaves in *P.farinosa* (c) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	**	**
	J-J	-	NS	NS	*	NS
	J-A	-	NS	**	**	NS
	A-S	-	***	**	NS	NS
	S-O	-	NS	**	***	***
2.WDF						
	M-J		-	**	***	***
	J-J		-	NS	*	NS
	J-A		-	**	*	NS
	A-S		-	NS	**	***
	S-O		-	NS	*	*
3.HAR						
	M-J			-	NS	NS
	J-J			-	*	NS
	J-A			-	NS	**
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	*
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.16 Statistical test of significance (LSD) of number of scapes in *P.farinosa* (c) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	**	NS	NS
	J-J	-	*	NS	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	NS	NS
	S-O	-	***	***	*	*
2.WDF						
	M-J		-	*	NS	NS
	J-J		-	*	*	*
	J-A		-	NS	*	NS
	A-S		-	NS	NS	NS
	S-O		-	NS	*	*
3.HAR						
	M-J			-	**	**
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.17 Statistical test of significance (LSD) of number of florets in *P.farinosa* (c) grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	**	NS	NS
	J-J	-	NS	NS	NS	NS
	J-A	-	*	NS	NS	NS
	A-S	-	NS	NS	NS	NS
	S-O	-	NS	NS	NS	NS
2.WDF						
	M-J		-	*	NS	NS
	J-J		-	NS	NS	*
	J-A		-	*	*	*
	A-S		-	**	**	*
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	**	**
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.18 Statistical test of significance (LSD) of number of capsules and seeds in *P.farinosa* (c) grown at five sites, at the end of growing season, 1986.

Sites		Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	capsule/plant	–	***	***	*	NS
	seed/capsule	–	*	NS	*	NS
	seed/plant	–	*	NS	NS	NS
2.WDF						
	capsule/plant		–	NS	NS	**
	seed/capsule		–	NS	NS	*
	seed/plant		–	NS	NS	*
3.HAR						
	capsule/plant			–	NS	*
	seed/capsule			–	NS	NS
	seed/plant			–	NS	NS
4.ESH						
	capsule/plant				–	NS
	seed/capsule				–	*
	seed/plant				–	NS
5.SUN						
	capsule/plant					–
	seed/capsule					–
	seed/plant					–

the sites during the growing season except during J-J. The maximum numbers of leaves were found during A-S at all sites except SUN where the number of leaves was still increasing through to the end of the growing season (S-O). During A-S, the highest and the lowest number of leaves were observed at WDF and GDF respectively. Nevertheless, there were no significant differences in the number of leaves between WDF and HAR (Table 4.15).

The result of ANOVA revealed significant differences in the number of scapes per plant between the sites only at the beginning and the end of the growing season. The number of scapes was generally found to be higher at WDF than the other sites, but only some pairs of sites showed significant differences (Table 4.16). The number of scapes at GDF was highest during J-A and dropped sharply at the end of the growing season. An ANOVA of the number of florets for *P. farinosa* (c) was exactly the same as that of the number of scapes. However, the shapes of the curves (Fig. 4.6g) were somewhat different. The peak numbers of florets were found during J-A at all sites and the significantly highest number of florets was observed at WDF, whilst the lowest number of florets was at SUN which did not show significant differences from GDF, HAR and ESH (Table 4.17).

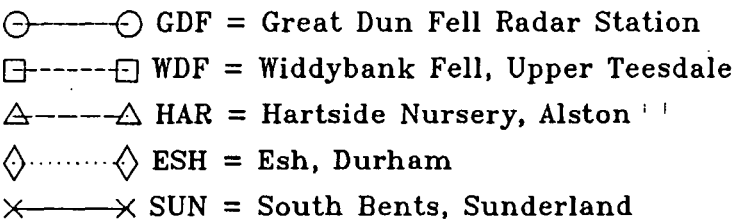
Comparisons of the number of capsules per plant for *P. farinosa* (c) showed highly significant differences between the sites. The highest number of capsules for *P. farinosa* (c) was found at its natural habitat, WDF; whilst there was no capsule formation at GDF. Nevertheless, a significantly higher number of capsules at WDF than the other sites were found only at GDF and SUN (Table 4.18). The number of seed per capsule for *P. farinosa* (c) showed significant differences between the sites. There was no seed formation for *P. farinosa* (c) at GDF and SUN. Amongst the three sites where seed formation was observed, there were no significant differences from each other. This is also true for numbers of seeds per plant (Table 4.18).

(c) *Primula frondosa*

Fig. 4.7 and Fig 4.10c illustrate the responses of growth characteristics to microclimates for *P. frondosa* during the growing season. Comparisons of growth characteristics between the sites are summarized in Table 4.2. The values of RLaGR demonstrated highly significant differences between the sites. The highest values of RLaGR were found at the beginning of the experiment for the three upland sites, whilst the lowland sites showed a peak of RLaGR during A-S. During J-A, RLaGR for *P. frondosa* showed a sensitive response to the microclimate of the sites at GDF, ESH and SUN showing negative values (see Fig. 4.7a). However, during A-S, RLaGR at these three sites showed a marked increase, whilst RLaGR at WDF and HAR was still declining. During this time the values of RLaGR at the lowland sites were significantly higher than at the upland sites (Table 4.19). At the end of growing season (S-O), all the three upland sites showed a sharp decrease of RLaGR to negative values, whilst the decreased values of RLaGR at the lowland sites were still positive. and significantly higher RLaGR values than those at the upland sites were observed. An ANOVA result showed significant differences in the values of LAD for *P. frondosa* between the sites. The maximum values of LAD were found at the end of growing season at all sites except GDF which showed only a slight decrease of LAD from the beginning of the experiment. The two upland sites, WDF and HAR, showed higher values of LAD than the other sites, but significant differences were found in some pairs of sites (Table 4.20). The values of LAD at GDF, ESH and SUN did not show significant differences from each other.

A summary of ANOVA results of LAI for *P. frondosa* showed significant differences between the sites, but not at the beginning and the end of the growing season. The maximum values of LAI at the two upland sites, WDF and HAR were found during A-S, whereas at the lowland sites the maximum values were found at the end of growing season (S-O). The values of LAI for *P. frondosa* at GDF showed the highest values during J-J and then declined slightly to values lower than at the other sites during A-S, but significant differences from ESH, or SUN were not

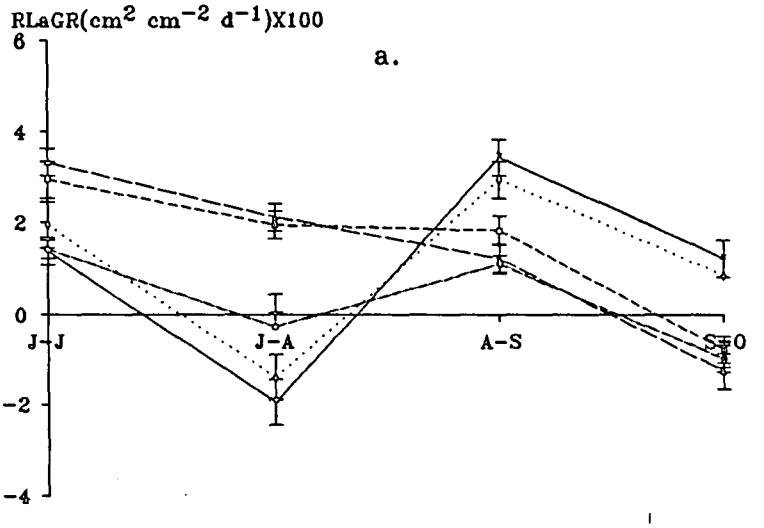
Figure 4.7 Mean monthly values of growth characteristics of *Primula frondosa* grown at five sites in the field during May–October, 1986.

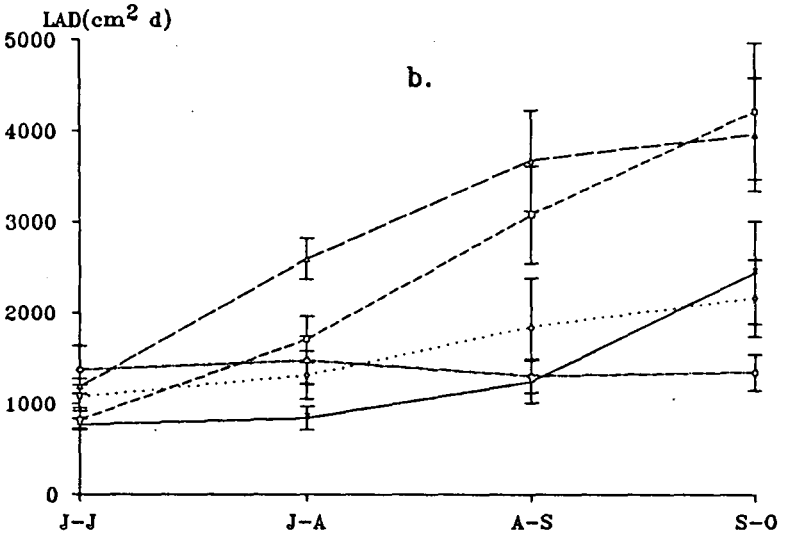


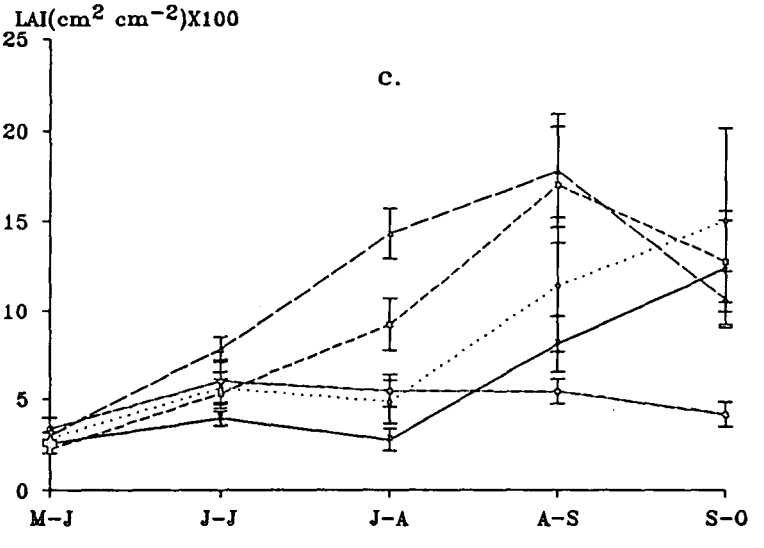
M–J = May–June
J–J = June–July
J–A = July–August
A–S = August–September
S–O = September–October

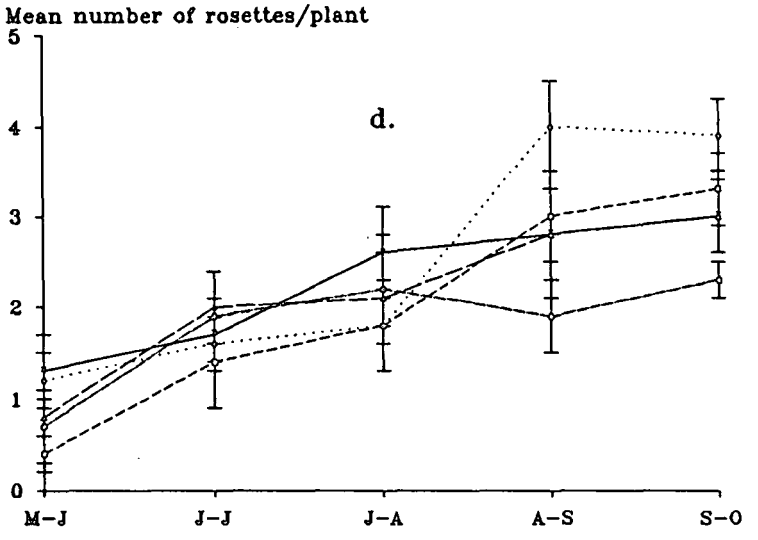
Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

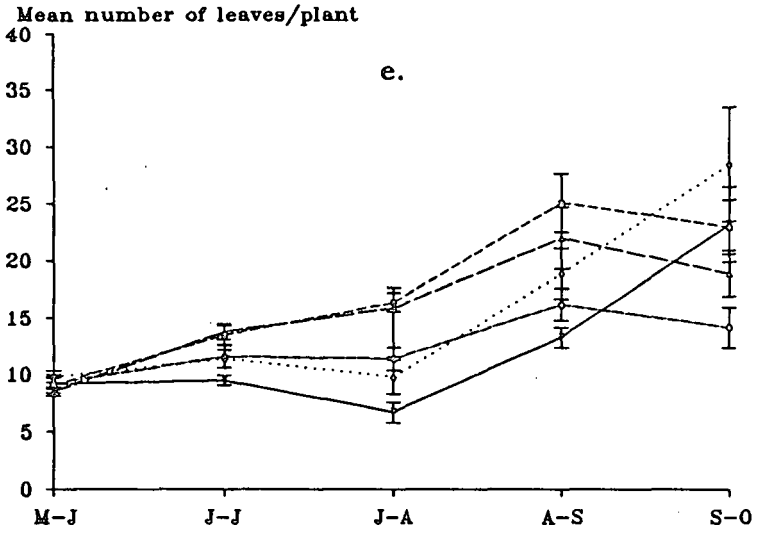
a.– b. data during June–October
c.– f. data during May–October
h.– i. data during September–October

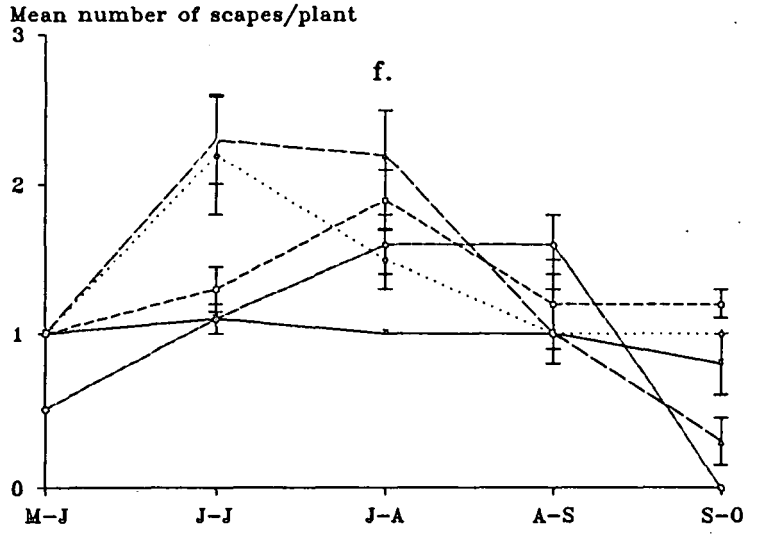

RLaGR(cm2 cm-2 d-1)X100
a.
6
4
2
0
-2
-4
J-J
J-A
A-S
S-O

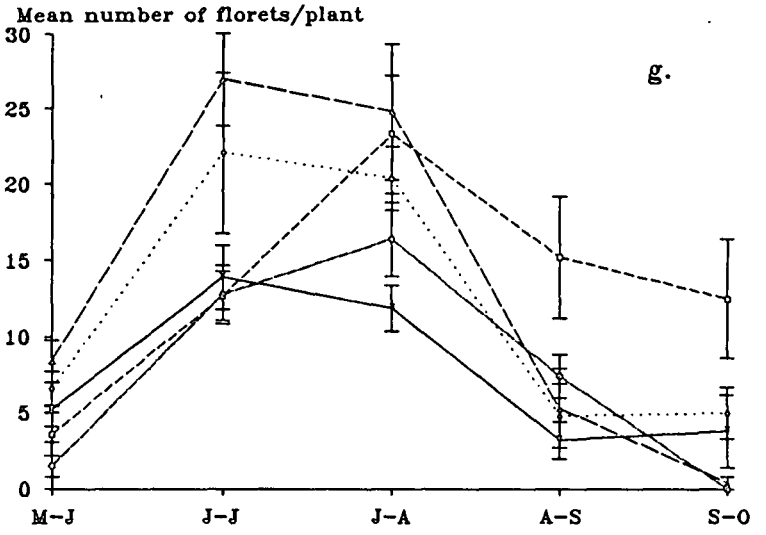

LAD(cm2 d)
b.
5000
4000
3000
2000
1000
0
J-J
J-A
A-S
S-O

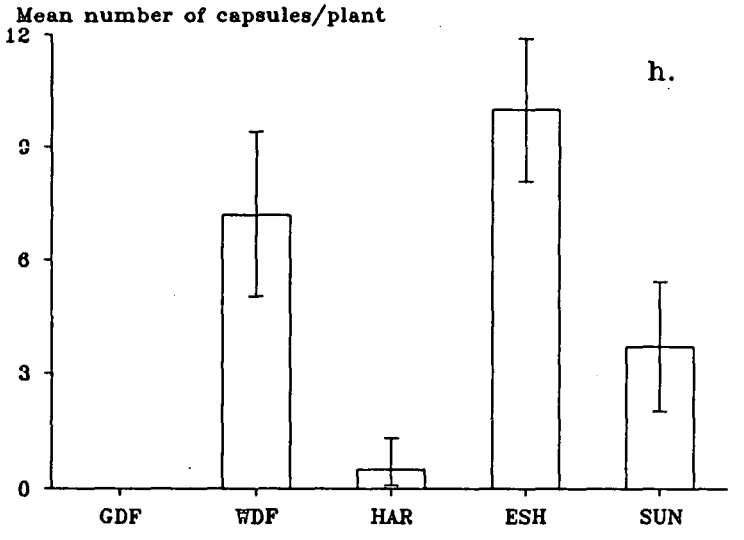

LAI(cm2 cm-2)X100
c.
25
20
15
10
5
0
M-J
J-J
J-A
A-S
S-O


Mean number of rosettes/plant
d.
5
4
3
2
1
0
M-J
J-J
J-A
A-S
S-O


Mean number of leaves/plant
e.
40
35
30
25
20
15
10
5
0
M-J
J-J
J-A
A-S
S-O


Mean number of scapes/plant
f.
3
2
1
0
M-J
J-J
J-A
A-S
S-O


Mean number of florets/plant
30
25
20
15
10
5
0
g.
M-J
J-J
J-A
A-S
S-O


Mean number of capsules/plant
12
6
3
0
h.
GDF
WDF
HAR
ESH
SUN

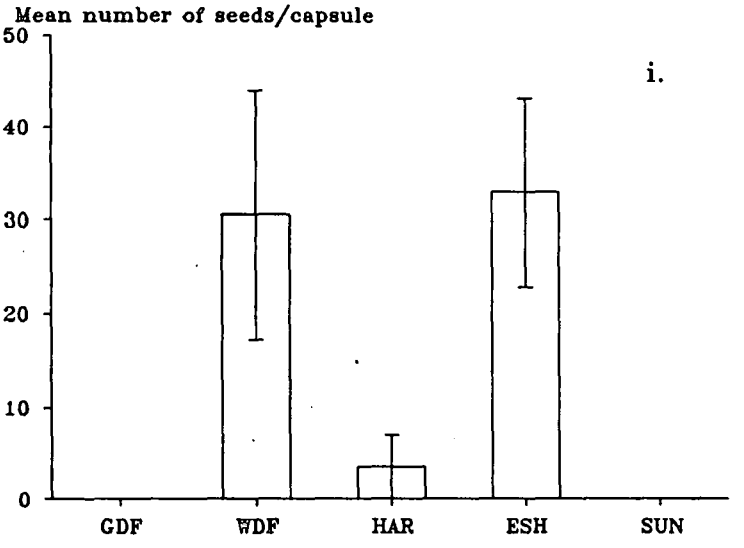

Mean number of seeds/capsule
50
40
30
20
10
0
i.
GDF
WDF
HAR
ESH
SUN

Table 4.19 Statistical test of significance (LSD) of RLaGR in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	**	**	NS	NS
	J-A	-	***	***	*	**
	A-S	-	NS	NS	***	***
	S-O	-	NS	NS	***	***
2.WDF						
	M-J		-	-	-	-
	J-J		-	NS	NS	**
	J-A		-	NS	***	***
	A-S		-	NS	*	**
	S-O		-	NS	***	***
3.HAR						
	M-J			-	-	-
	J-J			-	*	***
	J-A			-	***	***
	A-S			-	***	***
	S-O			-	***	***
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.20 Statistical test of significance (LSD) of LAD in *P.frondosa* grown at five sites in the field during during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	**	NS	NS	**
	J-A	-	NS	**	NS	NS
	A-S	-	**	***	NS	NS
	S-O	-	**	**	NS	NS
2.WDF						
	M-J		-	-	-	-
	J-J		-	NS	NS	NS
	J-A		-	*	NS	*
	A-S		-	NS	NS	*
	S-O		-	NS	*	NS
3.HAR						
	M-J			-	-	-
	J-J			-	NS	*
	J-A			-	***	***
	A-S			-	**	**
	S-O			-	*	NS
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.21 Statistical test of significance (LSD) of LAI in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	*	NS	NS	NS
	J-J	-	NS	NS	NS	NS
	J-A	-	**	***	NS	*
	A-S	-	**	**	NS	NS
	S-O	-	*	NS	**	NS
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	NS	NS	NS
	J-A		-	**	*	***
	A-S		-	NS	NS	*
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	**
	J-A			-	***	***
	A-S			-	NS	*
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.22 Statistical test of significance (LSD) of number of rosettes in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	NS	NS
	J-J	-	NS	NS	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	*	NS
	S-O	-	NS	NS	*	NS
2.WDF						
	M-J		-	NS	NS	*
	J-J		-	NS	NS	NS
	J-A		-	NS	NS	NS
	A-S		-	NS	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.23 Statistical test of significance (LSD) of number of leaves in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	NS	NS	NS
	J-J	-	NS	NS	NS	*
	J-A	-	**	*	NS	*
	A-S	-	**	NS	NS	NS
	S-O	-	*	NS	*	NS
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	NS	NS	***
	J-A		-	NS	***	***
	A-S		-	NS	NS	**
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	*	***
	J-A			-	**	***
	A-S			-	NS	*
	S-O			-	*	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.24 Statistical test of significance (LSD) of number of scapes in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	***	***	***	***
	J-J	-	NS	**	**	NS
	J-A	-	NS	*	NS	*
	A-S	-	NS	NS	*	NS
	S-O	-	***	NS	***	***
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	**	NS	NS
	J-A		-	NS	NS	**
	A-S		-	NS	NS	NS
	S-O		-	***	NS	*
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	**
	J-A			-	*	***
	A-S			-	NS	NS
	S-O			-	***	**
4.ESH						
	M-J				-	NS
	J-J				-	**
	J-A				-	*
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.25 Statistical test of significance (LSD) of number of florets in *P.frondosa* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	NS	***	***	**
	J-J	-	NS	**	NS	NS
	J-A	-	NS	NS	NS	NS
	A-S	-	NS	NS	NS	*
	S-O	-	***	NS	NS	NS
2.WDF						
	M-J		-	***	*	NS
	J-J		-	**	*	NS
	J-A		-	NS	NS	*
	A-S		-	*	*	**
	S-O		-	***	*	*
3.HAR						
	M-J			-	NS	*
	J-J			-	NS	**
	J-A			-	NS	*
	A-S			-	NS	NS
	S-O			-	NS	NS
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.26 Statistical test of significance (LSD) of number of capsules and seeds in *P.frondosa* grown at five sites, at the end of growing season, 1986.

Sites		Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	capsule/plant	–	***	NS	***	NS
	seed/capsule	–	*	NS	**	NS
	seed/plant	–	NS	NS	**	NS
2.WDF						
	capsule/plant		–	**	NS	NS
	seed/capsule		–	*	NS	*
	seed/plant		–	NS	NS	NS
3.HAR						
	capsule/plant			–	***	NS
	seed/capsule			–	*	NS
	seed/plant			–	**	NS
4.ESH						
	capsule/plant				–	*
	seed/capsule				–	*
	seed/plant				–	*
5.SUN						
	capsule/plant					–
	seed/capsule					–
	seed/plant					–

observed (Table 4.21). At the end of the growing season (S-O), the highest values of LAI were found at ESH, but only some pairs of sites showed significant differences. An ANOVA result of the number of rosettes per plant for *P. frondosa* revealed no significant differences between the sites for all months. At the end of the growing season (A-S and S-O), the highest number of rosettes was found at ESH, but significantly higher than the other sites was observed only with GDF (Table 4.22).

Comparisons of the number of leaves per plant for *P. frondosa* demonstrated significant differences between the sites during the growing season except at the beginning of the experiment. The three upland sites showed the maximum numbers of leaves during A-S, whilst the two lowland sites showed a maximum during S-O. However, significant differences between pairs of sites were observed only in some pairs (Table 4.23). The number of scapes per plant for *P. frondosa* showed significantly differences between the sites during the growing season except during A-S. This species produced scapes early in the experiment. The number of scapes at HAR and ESH showed a marked decrease during J-J and then dropped when the plants become mature. The number of scapes at GDF showed maximum numbers during J-A, A-S and then dropped to zero at the end of growing season. At SUN, the number of scapes was rather small, but the number of scapes declined slightly as compared with the other sites.

An ANOVA result of the number of florets per plant for *P. frondosa* showed significant differences between the sites during the growing season, but not during J-A. Flowers were observed for *P. frondosa* at the beginning of experiment at all sites. The numbers of florets increased at all sites during J-J and declined later at HAR, ESH and SUN. The number of florets at GDF and WDF were still increasing during J-A and then dropped at the end of the growing season. By this time the number of florets at WDF was significantly higher (Table 4.25). The number of capsules per plant for *P. frondosa* showed significant differences between the sites. No capsule formation was observed at GDF, while the highest number of capsules was found at ESH. However, the number of capsules at ESH and WDF did not

show significant differences. The number of capsules at HAR, SUN also did not show significant differences from GDF which had no capsule formation at all (Table 4.26).

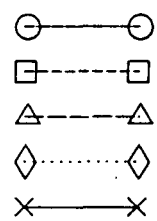
The numbers of seeds per capsule for *P. frondosa* showed significant differences between the sites. Seed production at GDF and SUN was not observed. Seed production at HAR was rather low and significantly lower than at WDF and ESH were observed (Table 4.26). The numbers of seeds per capsule at WDF and ESH did not show significant differences. The number of seeds per plant was higher at ESH than at WDF, but no significant difference was observed.

(d) *Primula laurentiana*

Fig. 4.8 and Fig. 4.10d depict the response of growth characteristics to microclimate at five sites during the growing season. ANOVA results of growth characteristics as compared between the sites are shown in Table 4.2. The values of RLaGR of *P. laurentiana* showed significant differences between the sites. The highest values of RLaGR were found at the beginning of the experiment and then declined to negative values at the end of the growing season (S-O). An exception was observed at lowland sites which showed re-growth during A-S after recovery from drought stress and thus showing significantly higher RLaGR than at the three upland sites (Table 4.27). An ANOVA of LAD for *P. laurentiana* showed significant differences between the sites. The two lowland sites, ESH and SUN showed maximum values at the end of the growing season (S-O). The values of LAD at GDF were rather low and only slight changes were observed during the growing season. These resulted in showing significant differences when compared with the other sites (Table 4.28).

Comparisons of LAI for *P. laurentiana* using ANOVA showed highly significant differences between the sites at all months. The maximum values of LAI at the three upland sites occurred during J-A, whilst at the lowland sites they occurred during A-S. The values of LAI were similar to LAD at GDF which showed low values and small changes. At the end of the growing season, the values of LAI declined at all

Figure 4.8 Mean monthly values of growth characteristics of *Primula laurentiana* grown at five sites in the field during May–October, 1986.

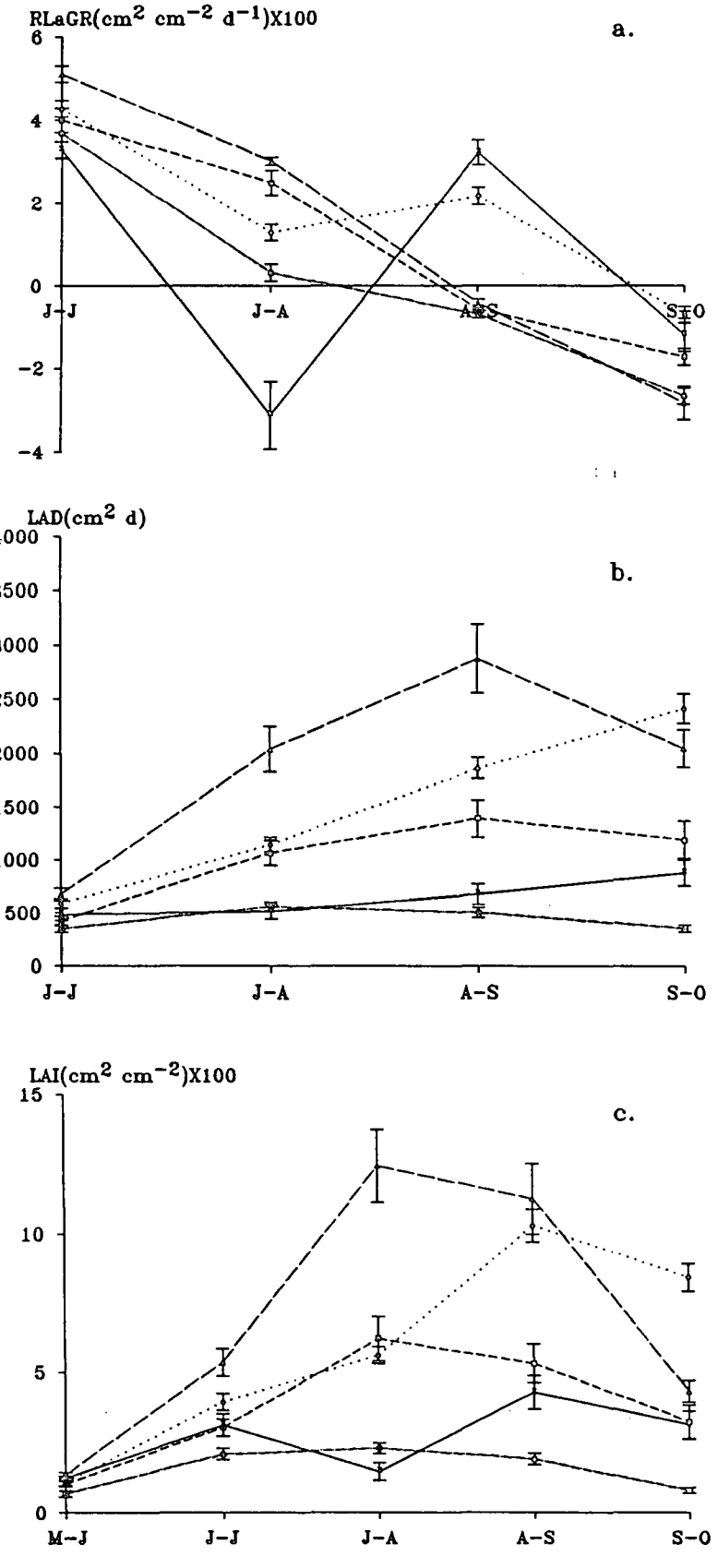


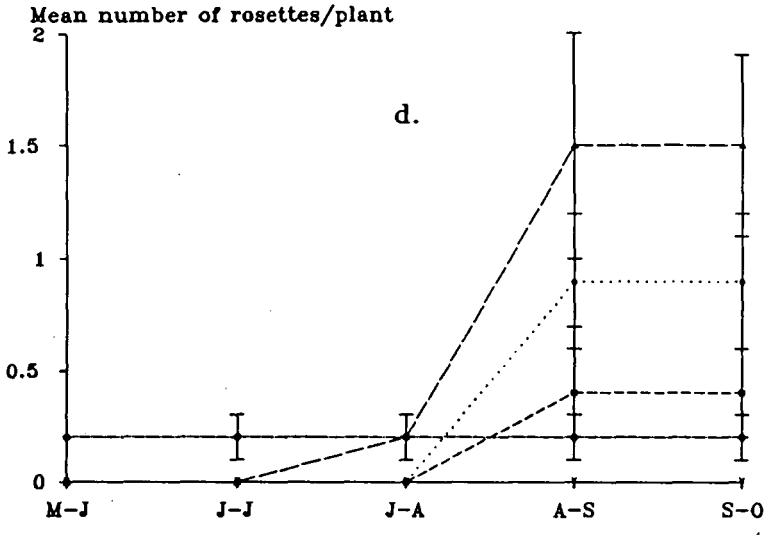
GDF = Great Dun Fell Radar Station
WDF = Widdybank Fell, Upper Teesdale
HAR = Hartside Nursery, Alston
ESH = Esh, Durham
SUN = South Bents, Sunderland

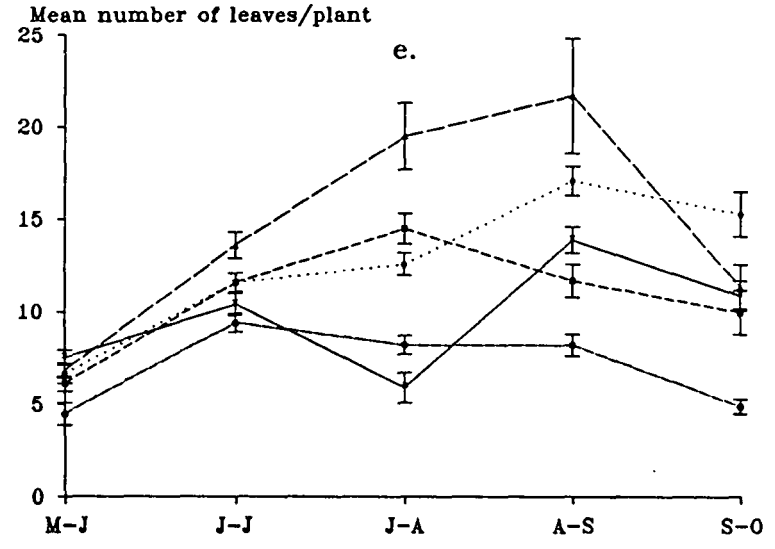
M–J = May–June
J–J = June–July
J–A = July–August
A–S = August–September
S–O = September–October

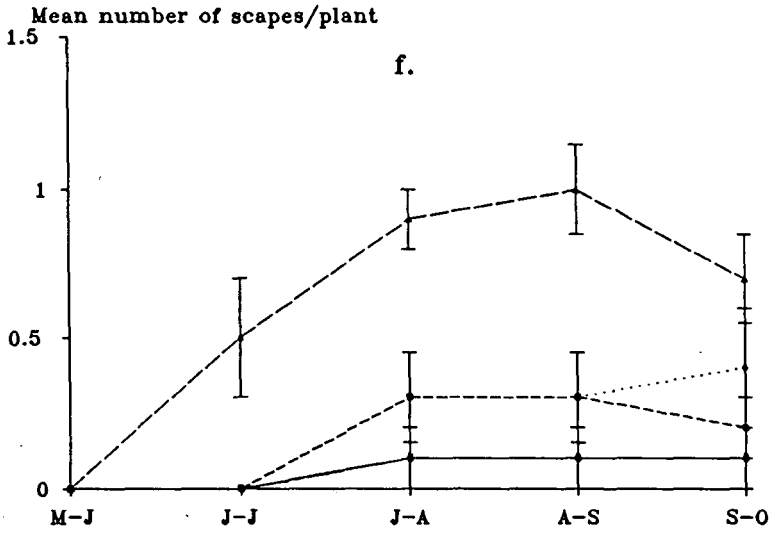
Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

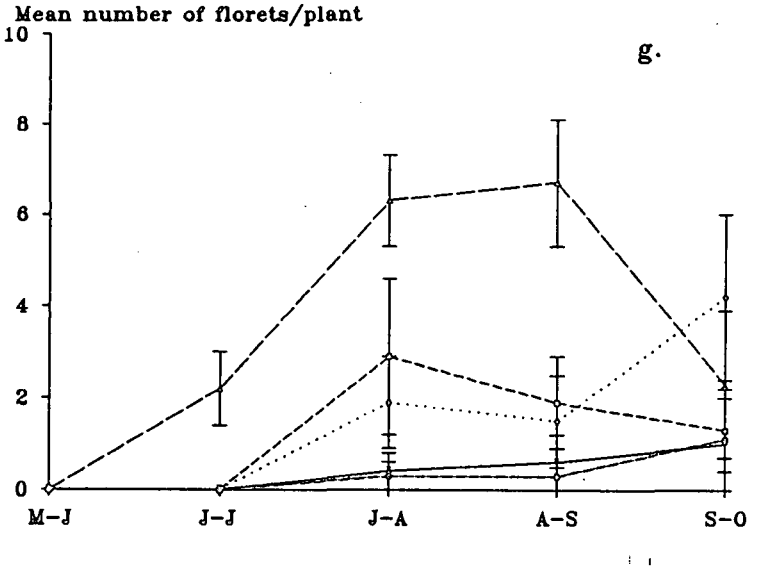
a.– b. data during June–October
c.– f. data during May–October
h.– i. data during September–October

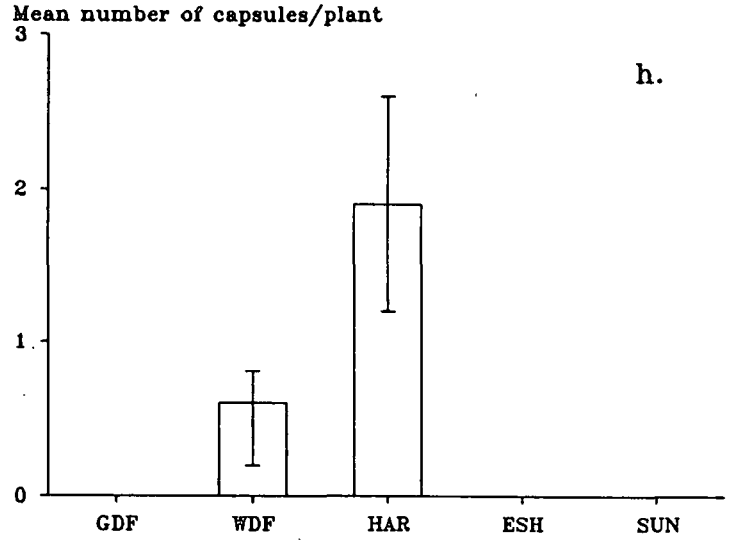

RLaGR(cm2 cm-2 d-1)X100
a.
6
4
2
0
-2
-4
J-J
J-A
A-S
S-O
LAD(cm2 d)
b.
4000
3500
3000
2500
2000
1500
1000
500
0
J-J
J-A
A-S
S-O
LAI(cm2 cm-2)X100
c.
15
10
5
0
M-J
J-J
J-A
A-S
S-O


Mean number of rosettes/plant
d.
2
1.5
1
0.5
0
M-J
J-J
J-A
A-S
S-O


Mean number of leaves/plant
e.
25
20
15
10
5
0
M-J
J-J
J-A
A-S
S-O


Mean number of scapes/plant
f.
1.5
1
0.5
0
M-J
J-J
J-A
A-S
S-O


Mean number of florets/plant
g.
10
8
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of capsules/plant
h.
3
2
1
0
GDF
WDF
HAR
ESH
SUN

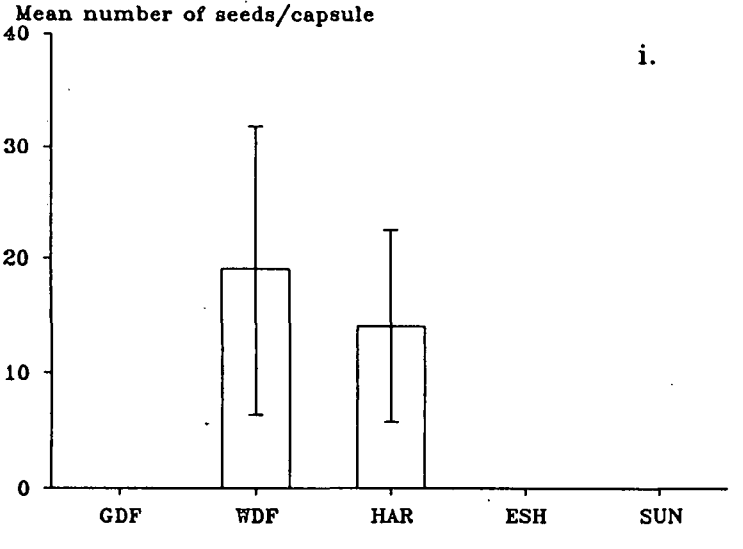

Mean number of seeds/capsule
i.
40
30
20
10
0
GDF
WDF
HAR
ESH
SUN

Table 4.27 Statistical test of significance (LSD) of RLaGR in *P. laurentiana* grown at five sites in the field during May – October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	–	–	–	–	–
	J-J	–	NS	*	NS	NS
	J-A	–	***	***	NS	***
	A-S	–	NS	NS	***	***
	S-O	–	*	NS	***	***
2.WDF						
	M-J		–	–	–	–
	J-J		–	*	NS	NS
	J-A		–	NS	*	***
	A-S		–	NS	***	***
	S-O		–	**	*	NS
3.HAR						
	M-J			–	–	–
	J-J			–	NS	***
	J-A			–	**	***
	A-S			–	***	***
	S-O			–	***	***
4.ESH						
	M-J				–	–
	J-J				–	*
	J-A				–	***
	A-S				–	***
	S-O				–	NS
5.SUN						
	M-J					–
	J-J					–
	J-A					–
	A-S					–
	S-O					–

Table 4.28 Statistical test of significance (LSD) of LAD in *P. laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1. GDF						
	M-J	-	-	-	-	-
	J-J	-	NS	***	**	NS
	J-A	-	**	***	**	NS
	A-S	-	***	***	***	NS
	S-O	-	***	***	***	*
2. WDF						
	M-J		-	-	-	-
	J-J		-	***	*	NS
	J-A		-	***	NS	**
	A-S		-	***	NS	**
	S-O		-	***	***	NS
3. HAR						
	M-J			-	-	-
	J-J			-	NS	**
	J-A			-	***	***
	A-S			-	***	***
	S-O			-	NS	***
4. ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	***
	A-S				-	***
	S-O				-	***
5. SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.29 Statistical test of significance (LSD) of LAI in *P. laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1. GDF						
	M-J	-	*	***	*	***
	J-J	-	NS	***	***	NS
	J-A	-	***	***	**	NS
	A-S	-	**	***	***	*
	S-O	-	***	***	***	***
2. WDF						
	M-J		-	NS	NS	NS
	J-J		-	***	NS	NS
	J-A		-	***	NS	***
	A-S		-	***	***	NS
	S-O		-	NS	***	NS
3. HAR						
	M-J			-	NS	NS
	J-J			-	**	***
	J-A			-	***	***
	A-S			-	NS	***
	S-O			-	***	NS
4. ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	***
	A-S				-	***
	S-O				-	***
5. SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.30 Statistical test of significance (LSD) of number of rosettes in *P.laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	*	*	*	*
	J-J	-	*	*	*	*
	J-A	-	*	*	*	*
	A-S	-	NS	**	NS	NS
	S-O	-	NS	**	NS	NS
2.WDF						
	M-J		-	NS	NS	NS
	J-J		-	NS	NS	NS
	J-A		-	NS	NS	NS
	A-S		-	*	NS	NS
	S-O		-	**	NS	NS
3.HAR						
	M-J			-	NS	NS
	J-J			-	NS	NS
	J-A			-	NS	NS
	A-S			-	NS	**
	S-O			-	NS	***
4.ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	NS
	A-S				-	*
	S-O				-	*
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.31 Statistical test of significance (LSD) of number of leaves in *P. laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1. GDF						
	M-J	-	*	**	**	***
	J-J	-	*	***	**	NS
	J-A	-	***	***	**	NS
	A-S	-	NS	***	***	*
	S-O	-	***	***	***	***
2. WDF						
	M-J		-	NS	NS	*
	J-J		-	**	NS	NS
	J-A		-	***	NS	**
	A-S		-	**	*	NS
	S-O		-	NS	***	NS
3. HAR						
	M-J			-	NS	NS
	J-J			-	*	***
	J-A			-	***	***
	A-S			-	*	**
	S-O			-	**	NS
4. ESH						
	M-J				-	NS
	J-J				-	NS
	J-A				-	***
	A-S				-	NS
	S-O				-	**
5. SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.32 Statistical test of significance (LSD) of number of scapes in *P. laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1. GDF						
	M-J	-	-	-	-	-
	J-J	-	NS	***	NS	NS
	J-A	-	NS	***	NS	NS
	A-S	-	NS	***	NS	NS
	S-O	-	NS	**	NS	NS
2. WDF						
	M-J		-	-	-	-
	J-J		-	**	NS	NS
	J-A		-	**	NS	NS
	A-S		-	***	NS	NS
	S-O		-	*	NS	NS
3. HAR						
	M-J			-	-	-
	J-J			-	***	***
	J-A			-	**	***
	A-S			-	***	***
	S-O			-	NS	**
4. ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5. SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.33 Statistical test of significance (LSD) of number of florets in *P. laurentiana* grown at five sites in the field during May - October, 1986.

Sites	Months	Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	M-J	-	-	-	-	-
	J-J	-	NS	***	NS	NS
	J-A	-	NS	***	NS	NS
	A-S	-	NS	***	NS	NS
	S-O	-	NS	NS	NS	NS
2.WDF						
	M-J		-	-	-	-
	J-J		-	***	NS	NS
	J-A		-	*	NS	NS
	A-S		-	***	NS	NS
	S-O		-	NS	NS	NS
3.HAR						
	M-J			-	-	-
	J-J			-	***	***
	J-A			-	**	***
	A-S			-	***	***
	S-O			-	NS	NS
4.ESH						
	M-J				-	-
	J-J				-	NS
	J-A				-	NS
	A-S				-	NS
	S-O				-	NS
5.SUN						
	M-J					-
	J-J					-
	J-A					-
	A-S					-
	S-O					-

Table 4.34 Statistical test of significance (LSD) of number of capsules and seeds in *P. laurentiana* grown at five sites, at the end of growing season, 1986.

Sites		Significance levels				
		GDF	WDF	HAR	ESH	SUN
1.GDF						
	capsule/plant	–	NS	***	NS	NS
	seed/capsule	–	NS	NS	NS	NS
	seed/plant	–	NS	NS	NS	NS
2.WDF						
	capsule/plant		–	*	NS	NS
	seed/capsule		–	NS	NS	NS
	seed/plant		–	NS	NS	NS
3.HAR						
	capsule/plant			–	***	***
	seed/capsule			–	NS	NS
	seed/plant			–	NS	NS
4.ESH						
	capsule/plant				–	NS
	seed/capsule				–	NS
	seed/plant				–	NS
5.SUN						
	capsule/plant					–
	seed/capsule					–
	seed/plant					–

sites. During J-A, the value of LAI was significantly higher at HAR than at the other sites (Table 4.29). An ANOVA result of the number of rosettes showed significant differences between the sites during the last two months of the growing season. No rosette was observed at SUN during the growing season, whereas the number of rosettes at GDF was low and stable throughout. At the end of the growing season, the number of rosettes at HAR was the highest, but significant differences were found only between GDF and SUN (Table 4.30). ANOVA performed on number of leaves per plant for *P. laurentiana* revealed highly significant differences between the sites throughout the growing season. The number of leaves at GDF was rather low and showed only a slight change, whereas the number of leaves at SUN fluctuated as a response to microclimate. During A-S, the number of leaves was significantly higher at HAR than at the other sites (Table 4.31).

Comparisons of the number of scapes of *P. laurentiana* showed significant differences between the sites throughout the growing season. During A-S, the number of scapes at HAR was significantly highest (Table 4.32), whilst there were no significant differences between each pair of the sites GDF, WDF, ESH and SUN. An ANOVA result of the number of florets for *P. laurentiana* showed significant differences between the sites during the growing season except during S-O. *P. laurentiana* at HAR flowered earlier than at the other two sites and the number of florets was significantly higher throughout the growing season except during S-O (Table 4.33). There were highly significant differences in the number of capsules for *P. laurentiana* when compared between the sites. Capsule formation was observed only at WDF and HAR and the number of capsules at HAR were significantly higher than WDF (Table 4.34). Comparisons of the number of seeds per capsule for *P. laurentiana* revealed no significant differences between the sites. The number of seeds per capsule and the number of seeds per plant did not show any significant differences between the two sites.

(e) *Primula scotica*

The responses of growth characteristics of *P. scotica* to microclimate are shown in Fig. 4.9 and Fig. 4.10e and comparisons between the three sites using ANOVA are summarized in Table 4.2. There were significant differences of RLaGR for *P. scotica* as comparisons were made between the sites. The maximum values of RLaGR were found at the beginning of the experiment and then declined to negative values at the end of the growing season at all sites. During J-A, the values of RLaGR at SUN decreased markedly and showed significantly lower values than at the other two sites (Table 4.35). An ANOVA result of LAD for *P. scotica* showed significant differences between the sites. The values of LAD at HAR were significantly higher throughout the growing season, whereas the values of LAD at GDF and SUN were not significantly different except during J-J (Table 4.36). Comparisons of LAI for *P. scotica* using ANOVA showed significant differences between the sites throughout the growing season except during S-O. The values of LAI at HAR were significantly higher than the other two sites with a peak occurring during A-S (Table 4.37).

The numbers of rosettes per plant for *P. scotica* were significantly different for the sites throughout the growing season except only during the first month of the experiment. The number of rosettes at HAR was significantly higher throughout the growing season except during M-J (Table 4.38). The numbers of rosettes at GDF and SUN did not show significant differences throughout the growing season except during J-A. An ANOVA result of the number of leaves for *P. scotica* showed significant differences between the sites during J-A and A-S. During A-S, the number of leaves at HAR and GDF were significantly higher and lower respectively (Table 4.39). At the end of the growing season, the number of leaves at HAR dropped markedly and did not show any significant differences from the other sites. Comparisons of the number of scapes for *P. scotica* between the sites showed significant differences. During A-S, the number of scapes at HAR was significantly higher, whilst the number of scapes at SUN was significantly lower (Table 4.40). An ANOVA result of the number of florets for *P. scotica* showed significant differences

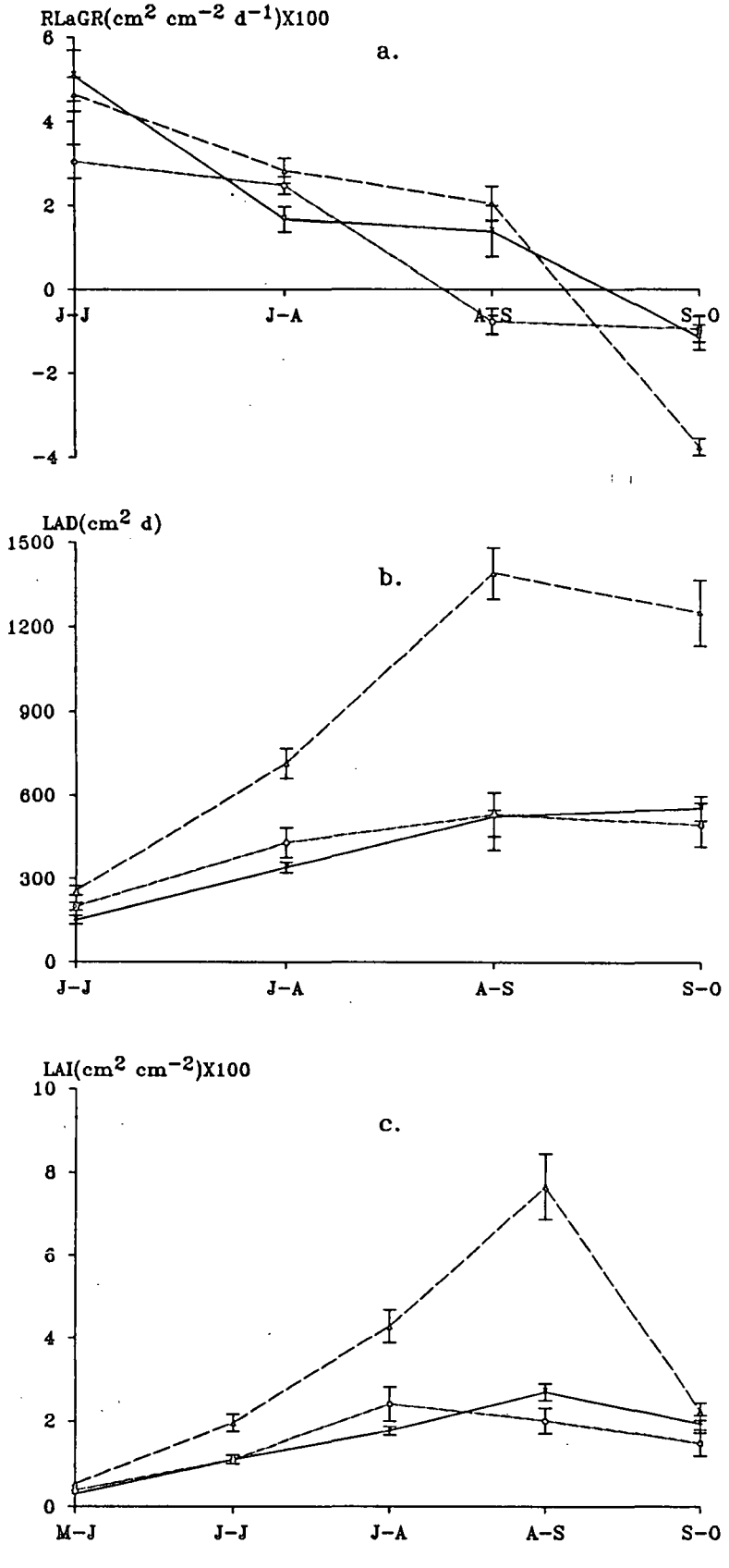
Figure 4.9 Mean monthly values of growth characteristics of *Primula scotica* grown at three sites in the field during May–October, 1986.

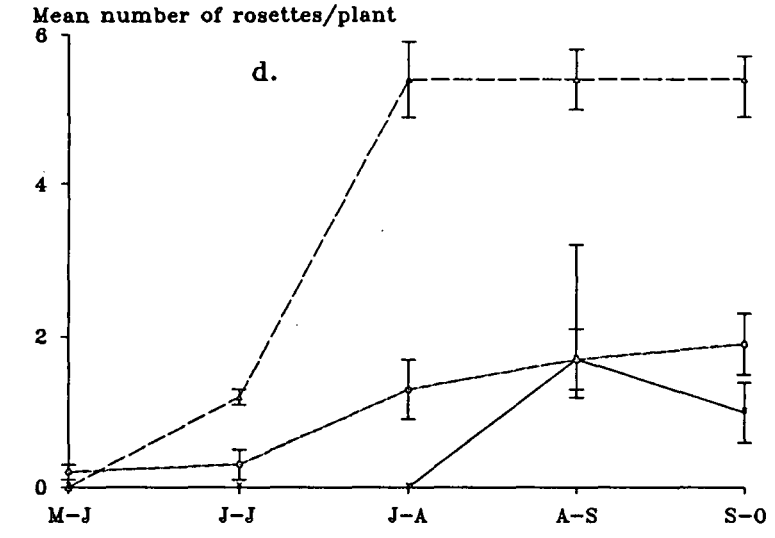
⊖———⊖ GDF = Great Dun Fell Radar Station
△———△ HAR = Hartside Nursery, Alston
×———× SUN = South Bents, Sunderland

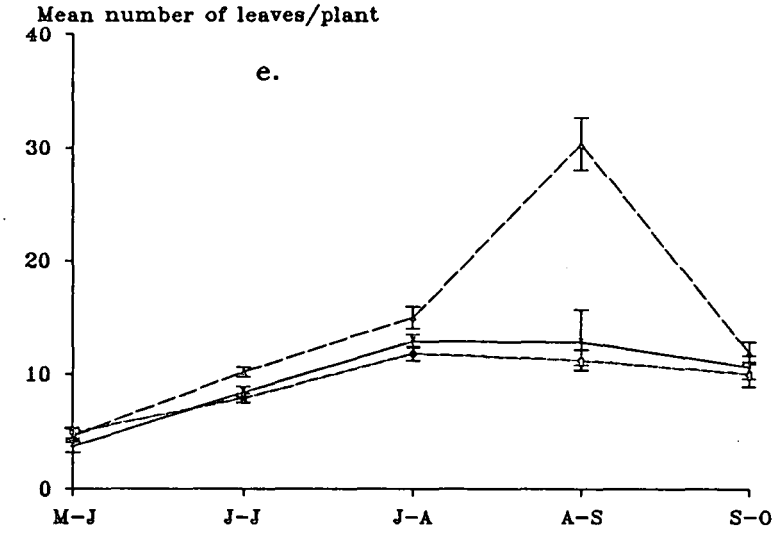
M–J = May–June
J–J = June–July
J–A = July–August
A–S = August–September
S–O = September–October

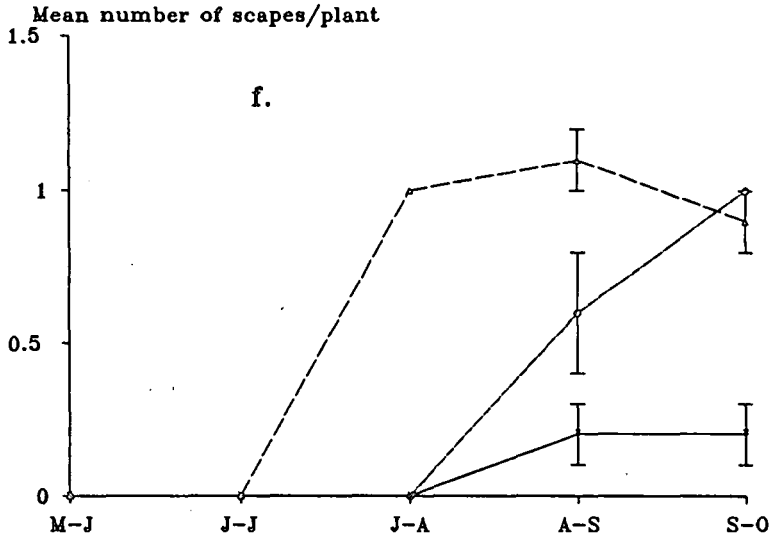
a.– b. data during June–October
c.– f. data during May–October
h.– i. data during September–October

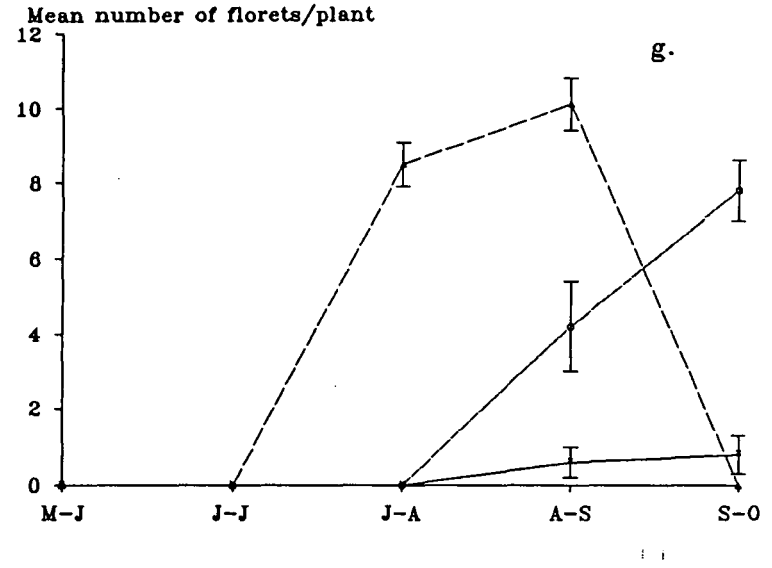
Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

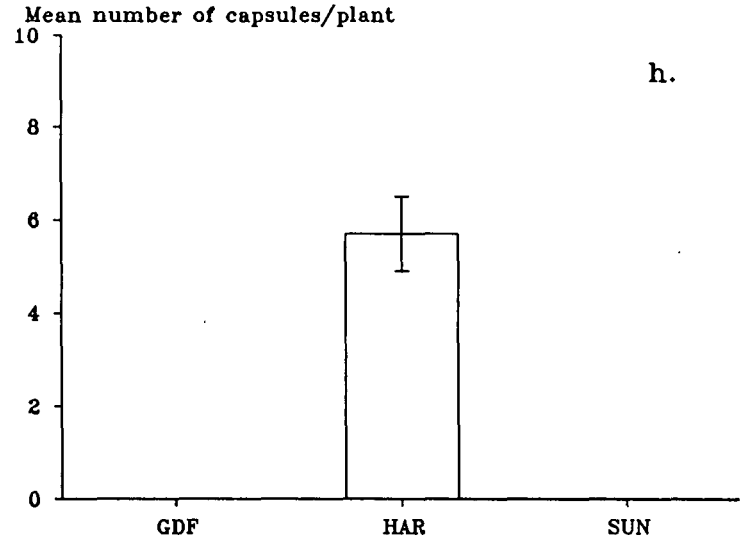

RLaGR(cm^2 cm^{-2} d^{-1})X100
a.
6
4
2
0
-2
-4
J-J
J-A
A-S
S-O
LAD(cm^2 d)
b.
1500
1200
900
600
300
0
J-J
J-A
A-S
S-O
LAI(cm^2 cm^{-2})X100
c.
10
8
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of rosettes/plant
d.
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of leaves/plant
e.
40
30
20
10
0
M-J
J-J
J-A
A-S
S-O


Mean number of scapes/plant
f.
1.5
1
0.5
0
M-J
J-J
J-A
A-S
S-O


Mean number of florets/plant
g.
12
10
8
6
4
2
0
M-J
J-J
J-A
A-S
S-O


Mean number of capsules/plant
h.
10
8
6
4
2
0
GDF
HAR
SUN

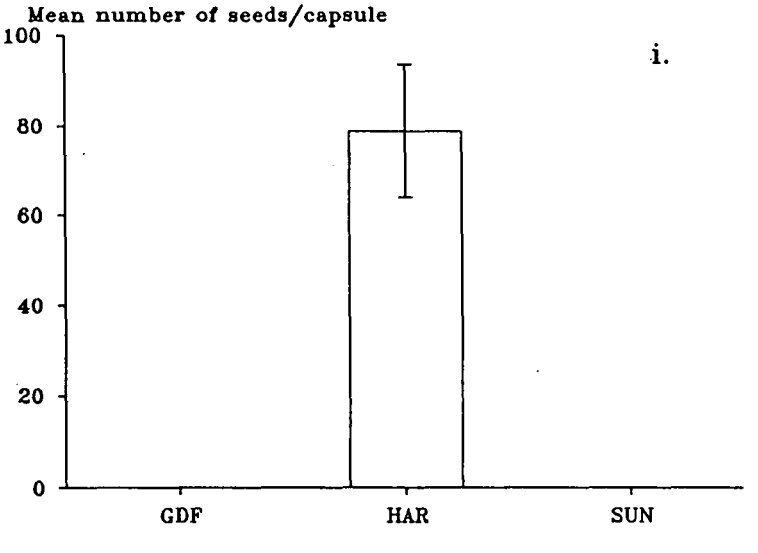

Mean number of seeds/capsule
i.
100
80
60
40
20
0
GDF
HAR
SUN

Figure 4.10 Mean number of seeds per plant in *Primula* spp. grown at five sites in the field at the end of growing season, 1986.

GDF = Great Dun Fell Radar Station
WDF = Widdybank Fell, Upper Teesdale
HAR = Hartside nursery, Alston
ESH = Esh, Durham
SUN = South Bents, Sunderland

a.*P.farinosa*(b)
b.*P.farinosa*(c)
c.*P.frondosa*
d.*P.laurentiana*
e.*P.scotica*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

Note *P.scotica* grown at three site:– GDF,HAR, and SUN.

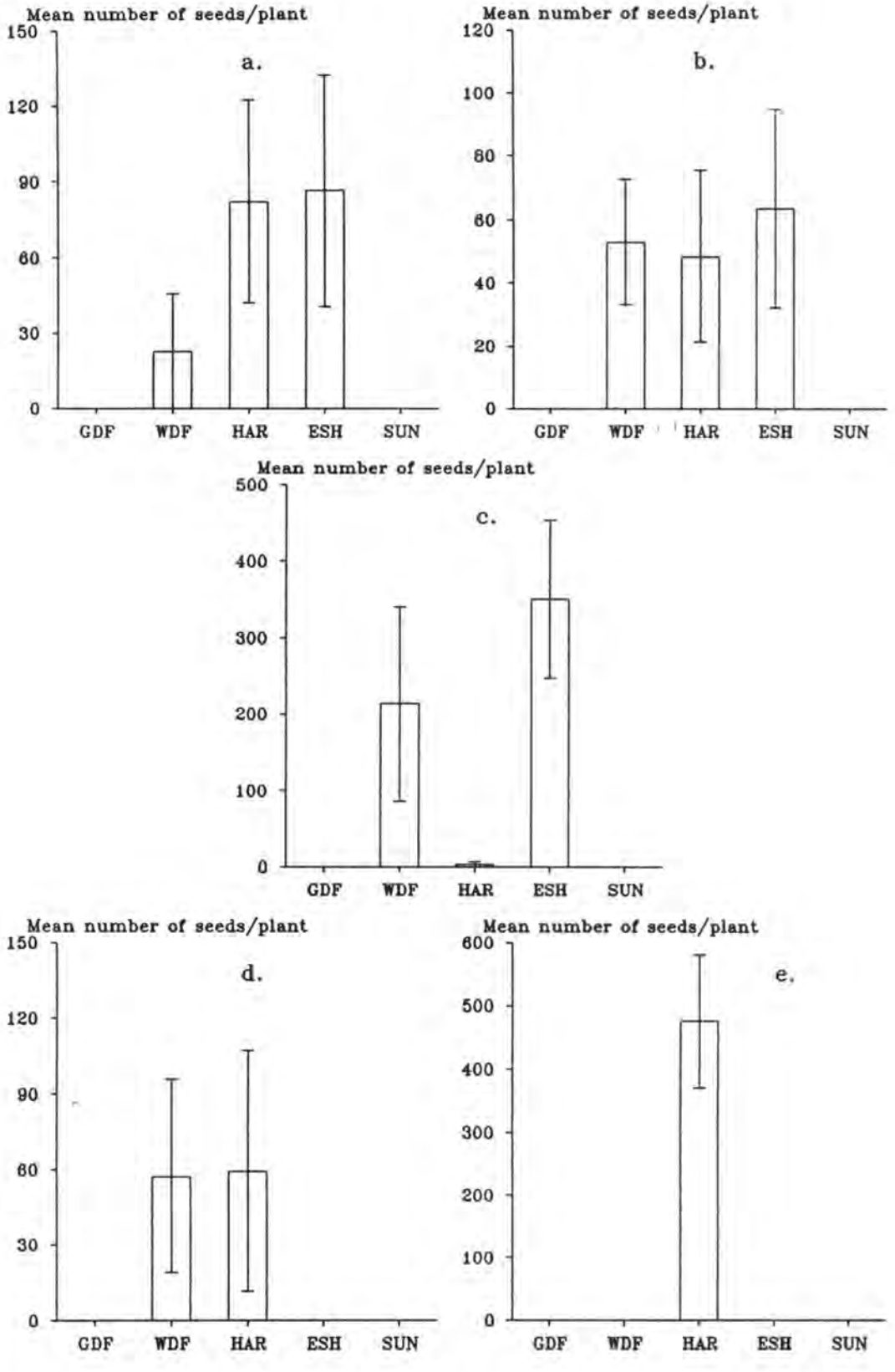

Mean number of seeds/plant
a.
150
120
90
60
30
0
GDF
WDF
HAR
ESH
SUN
Mean number of seeds/plant
b.
120
100
80
60
40
20
0
GDF
WDF
HAR
ESH
SUN
Mean number of seeds/plant
c.
500
400
300
200
100
0
GDF
WDF
HAR
ESH
SUN
Mean number of seeds/plant
d.
150
120
90
60
30
0
GDF
WDF
HAR
ESH
SUN
Mean number of seeds/plant
e.
600
500
400
300
200
100
0
GDF
WDF
HAR
ESH
SUN

Table 4.35 Statistical test of significance (LSD) of RLaGR in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	-	-
	J-J	-	*	**
	J-A	-	NS	*
	A-S	-	***	**
	S-O	-	***	NS
3.HAR				
	M-J		-	-
	J-J		-	NS
	J-A		-	**
	A-S		-	NS
	S-O		-	***
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.36 Statistical test of significance (LSD) of LAD in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	-	-
	J-J	-	*	*
	J-A	-	***	NS
	A-S	-	***	NS
	S-O	-	***	NS
3.HAR				
	M-J		-	-
	J-J		-	***
	J-A		-	***
	A-S		-	***
	S-O		-	***
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.37 Statistical test of significance (LSD) of LAI in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	**	NS
	J-J	-	***	NS
	J-A	-	***	NS
	A-S	-	***	NS
	S-O	-	*	NS
3.HAR				
	M-J		-	***
	J-J		-	***
	J-A		-	***
	A-S		-	***
	S-O		-	NS
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.38 Statistical test of significance (LSD) of number of rosettes in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	NS	NS
	J-J	-	*	NS
	J-A	-	***	*
	A-S	-	***	NS
	S-O	-	***	NS
3.HAR				
	M-J		-	NS
	J-J		-	**
	J-A		-	***
	A-S		-	***
	S-O		-	***
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.39 Statistical test of significance (LSD) of number of leaves in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	NS	NS
	J-J	-	**	NS
	J-A	-	**	NS
	A-S	-	***	*
	S-O	-	NS	NS
3.HAR				
	M-J		-	NS
	J-J		-	**
	J-A		-	NS
	A-S		-	***
	S-O		-	NS
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.40 Statistical test of significance (LSD) of number of scapes in *P.scotica* grown at three sites in the field during May - October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	-	-	-
	J-J	-	-	-
	J-A	-	NS	NS
	A-S	-	*	*
	S-O	-	NS	***
3.HAR				
	M-J		-	-
	J-J		-	-
	J-A		-	NS
	A-S		-	***
	S-O		-	***
5.SUN				
	M-J			-
	J-J			-
	J-A			-
	A-S			-
	S-O			-

Table 4.41 Statistical test of significance (LSD) of number of florets in *P.scotica* grown at three sites in the field during May – October, 1986.

Sites	Months	Significance levels		
		GDF	HAR	SUN
1.GDF				
	M-J	–	–	–
	J-J	–	–	–
	J-A	–	***	NS
	A-S	–	***	**
	S-O	–	***	***
3.HAR				
	M-J		–	–
	J-J		–	–
	J-A		–	***
	A-S		–	***
	S-O		–	NS
5.SUN				
	M-J			–
	J-J			–
	J-A			–
	A-S			–
	S-O			–

Table 4.42 Statistical test of significance (LSD) of number of capsules and seeds in *P.scotica* grown at three sites, at the end of growing season, 1986.

Sites		Significance levels		
		GDF	HAR	SUN
1.GDF				
	capsule/plant	–	***	NS
	seed/capsule	–	***	NS
	seed/plant	–	***	NS
3.HAR				
	capsule/plant		–	***
	seed/capsule		–	***
	seed/plant		–	***
5.SUN				
	capsule/plant			–
	seed/capsule			–
	seed/plant			–

between the sites throughout the flowering period. *P. scotica* at HAR flowered earlier than at any other sites, and showed significantly higher number of florets than the other sites during J-A and A-S (Table 4.41). Capsules and seeds for *P. scotica* were observed only at HAR.

4.3.1.2 Comparisons between species at individual sites

Detailed comparisons of the values of RLaGR at five sites have been made between the species and are shown in Figs. 4.5a-4.9a. ANOVA results (Table 4.43) revealed that there were significant differences in the values of RLaGR between the species at all sites throughout the growing season. At the beginning of the experiment (J-J), *P. laurentiana, P. farinosa* (c) and *P. scotica* showed significantly higher value of RLaGR than *P. farinosa* (b) and *P. frondosa* (Table 4.44). During J-A, the values of RLaGR decreased in all species. The values of RLaGR for *P. farinosa* (c) and *P. scotica* were significantly higher than *P. laurentiana, P. farinosa* (b) and *P. frondosa.* In contrast, during A-S the highest value of RLaGR was found in *P. frondosa* and the lowest value was in *P. farinosa* (c) during A-S and S-O. At the end of the growing season (S-O) the values of RLaGR became negative in all species. By this time, *P. frondosa* and *P. scotica* showed significantly higher values of RLaGR than *P. farinosa* (b), *P. laurentiana* and *P. farinosa* (c).

At WDF, there were no significant differences in RLaGR between *P. farinosa* (b) and *P. farinosa* (c) throughout the the growing season (Table 4.45). During J-A, *P. farinosa* (b) and *P. farinosa* (c) showed significantly higher RLaGR than *P. frondosa. P. laurentiana* showed significantly lower values of RLaGR during A-S. At the end of the growing season, *P. frondosa* exhibited significantly higher RLaGR. At HAR, *P. farinosa* (b) and *P. farinosa* (c) did not show any significant differences in the values of RLaGR throughout the growing season except during J-A when *P. farinosa* (c) showed significantly higher values of RLaGR than the other species. During A-S, *P. scotica* exhibited significantly higher values of RLaGR than the other species. In contrast, at the end of the growing season *P. scotica* showed a lower value

Table 4.43 ANOVA of growth characteristics between *Primula* spp. grown at selected sites in the field during May - October, 1986.

Sites	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
Great Dun Fell Radar Station	M-J	–	–	***	NS	***	**	***	–	–
	J-J	*	***	***	***	***	***	***	–	–
	J-A	***	***	***	**	***	***	***	–	–
	A-S	***	***	***	**	***	**	***	–	–
	S-O	***	***	***	***	***	***	***	–	–
Widdybank Fell, Upper Teesdale	M-J	–	–	***	NS	***	***	***	–	–
	J-J	*	***	***	*	***	***	*	–	–
	J-A	**	***	***	***	***	***	***	–	–
	A-S	***	***	***	**	***	**	*	–	–
	S-O	***	***	***	***	***	***	***	***	NS
Hartside nursery, Alston	M-J	–	–	***	**	***	***	***	–	–
	J-J	***	***	***	**	***	***	***	–	–
	J-A	***	***	***	***	NS	***	***	–	–
	A-S	***	***	***	***	*	*	NS	*	–
	S-O	***	***	***	***	*	NS	NS	***	***
Esh, Durham	M-J	–	–	***	**	***	***	–	–	–
	J-J	***	***	***	***	***	***	***	–	–
	J-A	***	***	**	***	*	***	***	–	–
	A-S	***	***	**	***	NS	*	**	**	–
	S-O	**	**	**	***	**	NS	*	***	*
South Bents, Sunderland	M-J	–	–	***	**	***	–	–	–	–
	J-J	***	***	***	***	NS	***	***	–	–
	J-A	***	***	NS	***	***	***	***	–	–
	A-S	**	**	***	*	NS	NS	**	***	–
	S-O	***	***	***	**	***	NS	*	***	–

Table 4.44 Statistical test of significance (LSD) of growth characteristics in *Primula* spp. grown at Great Dun Fell Radar Station during May - October, 1986.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b):	M-J	–	–	*	NS	***	NS	NS	–	–
P.farinosa(c)	J-J	*	*	NS	NS	NS	NS	NS	–	–
	J-A	***	NS	NS	NS	*	NS	NS	–	–
	A-S	NS	NS	NS	NS	NS	*	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	***	NS	NS	**	***	–	–
P.frondosa	J-J	NS	***	***	NS	**	***	***	–	–
	J-A	*	***	***	NS	**	***	***	–	–
	A-S	**	***	***	NS	*	NS	**	–	–
	S-O	*	***	***	NS	***	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	NS	*	**	NS	NS	–	–
P.laurentiana	J-J	**	NS	NS	**	NS	*	**	–	–
	J-A	NS	NS	NS	**	NS	*	NS	–	–
	A-S	**	NS	NS	**	**	**	*	–	–
	S-O	NS	NS	NS	***	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	*	*	**	NS	NS	–	–
P.scotica	J-J	NS	NS	NS	**	NS	*	**	–	–
	J-A	***	NS	NS	NS	**	*	*	–	–
	A-S	**	NS	NS	NS	NS	NS	NS	–	–
	S-O	**	NS	NS	NS	NS	***	***	NS	NS
P.farinosa(c):	M-J	–	–	***	NS	***	**	***	–	–
P.frondosa	J-J	*	***	***	**	***	***	***	–	–
	J-A	***	***	***	*	NS	***	***	–	–
	A-S	NS	***	***	NS	*	*	***	–	–
	S-O	***	***	***	*	***	NS	NS	NS	NS
P.farinosa(c):	M-J	–	–	NS	NS	*	NS	NS	–	–
P.laurentiana	J-J	NS	NS	NS	NS	NS	NS	**	–	–
	J-A	***	*	NS	NS	**	NS	*	–	–
	A-S	***	NS	NS	NS	**	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(c):	M-J	–	–	NS	NS	**	NS	NS	–	–
P.scotica	J-J	NS	NS	NS	NS	NS	NS	**	–	–
	J-A	NS	NS	NS	NS	NS	**	**	–	–
	A-S	***	NS	NS	NS	NS	NS	NS	–	–
	S-O	***	NS	NS	NS	NS	***	***	NS	NS

Table 4.44 Continued.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.frondosa: *P.laurentiana*	M-J	–	–	***	NS	***	**	***	–	–
	J-J	**	***	***	**	*	***	***	–	–
	J-A	NS	***	***	***	***	***	***	–	–
	A-S	***	***	***	**	***	**	***	–	–
	S-O	*	***	***	***	***	NS	NS	NS	NS
P.frondosa: *P.scotica*	M-J	–	–	***	NS	***	*	***	–	–
	J-J	*	***	***	***	***	***	***	–	–
	J-A	***	***	***	NS	NS	***	***	–	–
	A-S	***	***	***	NS	**	NS	***	–	–
	S-O	NS	***	***	NS	*	***	***	NS	NS
P.laurentiana: *P.scotica*	M-J	–	–	NS	NS	NS	NS	NS	–	–
	J-J	NS	NS	NS	NS	NS	NS	NS	–	–
	J-A	***	NS	NS	*	***	NS	NS	–	–
	A-S	NS	NS	NS	**	*	NS	NS	–	–
	S-O	**	NS	NS	**	***	***	***	NS	NS

Table 4.45 Statistical test of significance (LSD) of growth characteristics in *Primula spp.* grown at Widdybank Fell, Upper Teesdale during May - October, 1986.

Primula spp.	Months	Significance levels								
		RLGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b):	M-J	–	–	***	NS	***	NS	NS	–	–
P.farinosa(c)	J-J	NS	**	*	NS	NS	NS	*	–	–
	J-A	NS	NS	NS	NS	NS	NS	*	–	–
	A-S	NS	NS	NS	NS	NS	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	***	NS	***	***	***	–	–
P.frondosa	J-J	NS	***	***	NS	***	***	NS	–	–
	J-A	**	***	***	NS	**	***	***	–	–
	A-S	NS	***	***	NS	NS	**	*	–	–
	S-O	**	***	***	NS	**	*	**	***	NS
P.farinosa(b):	M-J	–	–	NS	NS	NS	NS	NS	–	–
P.laurentiana	J-J	NS	NS	NS	*	**	NS	NS	–	–
	J-A	NS	NS	NS	**	NS	NS	NS	–	–
	A-S	***	NS	NS	**	**	NS	NS	–	–
	S-O	NS	NS	NS	**	*	NS	NS	NS	NS
P.farinosa(c):	M-J	–	–	***	NS	***	***	***	–	–
P.frondosa	J-J	*	***	***	NS	***	***	NS	–	–
	J-A	**	***	***	NS	***	***	***	–	–
	A-S	NS	***	***	NS	NS	**	NS	–	–
	S-O	***	***	***	NS	***	***	NS	NS	NS
P.farinosa(c):	M-J	–	–	***	NS	***	NS	NS	–	–
P.laurentiana	J-J	**	***	***	*	**	*	**	–	–
	J-A	NS	**	**	***	*	NS	*	–	–
	A-S	***	NS	NS	*	***	NS	NS	–	–
	S-O	*	NS	NS	**	NS	NS	**	NS	NS
P.frondosa:	M-J	–	–	***	NS	***	***	***	–	–
P.laurentiana	J-J	NS	***	**	**	*	***	*	–	–
	J-A	NS	**	*	***	NS	***	***	–	–
	A-S	***	***	***	***	***	***	**	–	–
	S-O	*	***	***	***	***	***	***	***	NS

of RLaGR than that of the other species, but no significant difference was observed when compared with *P. laurentiana* (Table 4.46). At ESH, there were no significant differences in RLaGR between *P. farinosa* (b) and *P. farinosa* (c) except during J-J. All species showed a marked decrease of RLaGR during J-A and significantly lowest RLaGR was observed in *P. frondosa* (Table 4.47). In contrast, after recovering from drought stress, *P. frondosa* showed the highest values of RLaGR during A-S. At the end of the growing season (S-O) the value of RLaGR declined in all species, and negative values were observed in *P. farinosa* (c) and *P. laurentiana.* At SUN, *P. farinosa* (b) and *P. farinosa* (c) showed highly significant differences in RLaGR during J-A (Table 4.48). By that time, the values of RLaGR dropped markedly in all species and fell to negative values in *P. farinosa* (b), *P. frondosa,* and *P. laurentiana.* However, during A-S the values of RLaGR increased in *P. farinosa* (b), *P. farinosa* (c), *P. frondosa* and *P. laurentiana,* whilst the RLaGR of *P. scotica* was still falling. At the end of the growing season, the values of RLaGR declined in all species with the observed negative values in *P. farinosa* (c), *P. laurentiana* and *P. scotica.*

Comparisons of LAD between species of the primulas are shown in Figs. 4.5b-4.9b. ANOVA results of LAD showed that there were highly significant differences between species at all sites throughout the growing season (Table 4.43). At GDF and WDF the values of LAD were significantly higher in *P. frondosa* throughout the growing season. *P. farinosa* from both populations did not show significant differences in LAD except during J-J. LAD of all species were rather stable throughout the growing season at GDF. In contrast, LAD at WDF were increased markedly, especially in *P. frondosa.* At HAR, LAD of all species increased with time. *P. frondosa* showed significantly higher LAD than the other species throughout the growing season with only the exception of *P. laurentiana* during A-S. *P. farinosa* from Blackhall Rocks showed significantly higher LAD than *P. farinosa* from Upper Teesdale during J-J, and J-A (Table 4.46). At ESH, there were no significant differences in LAD between *P. farinosa* (b) and *P. farinosa* (c) throughout the

Table 4.46 Statistical test of significance (LSD) of growth characteristics in *Primula* spp. grown at Hartside nursery, Alston during May - October, 1986.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b):	M-J	–	–	***	NS	***	NS	***	–	–
P.farinosa(c)	J-J	NS	***	**	NS	**	NS	NS	–	–
	J-A	***	*	NS	*	NS	*	NS	–	–
	A-S	NS	NS	NS	**	NS	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	***	NS	*	***	***	–	–
P.frondosa	J-J	NS	***	***	NS	**	***	***	–	–
	J-A	NS	***	***	NS	NS	***	***	–	–
	A-S	NS	***	***	NS	NS	NS	NS	–	–
	S-O	*	***	***	NS	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	NS	**	NS	NS	NS	–	–
P.laurentiana	J-J	**	*	***	*	**	NS	NS	–	–
	J-A	NS	***	***	***	*	NS	NS	–	–
	A-S	**	*	NS	**	NS	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	***	**	**	NS	NS	–	–
P.scotica	J-J	*	**	*	NS	NS	**	**	–	–
	J-A	NS	**	NS	***	NS	NS	NS	–	–
	A-S	**	NS	NS	**	NS	NS	NS	–	–
	S-O	**	NS	NS	***	NS	NS	NS	***	***
P.farinosa(c):	M-J	–	–	***	NS	***	***	***	–	–
P.frondosa	J-J	NS	***	***	*	***	***	***	–	–
	J-A	***	***	***	NS	NS	***	***	–	–
	A-S	NS	***	***	NS	NS	NS	NS	–	–
	S-O	NS	***	***	*	*	NS	NS	*	NS
P.farinosa(c):	M-J	–	–	***	*	***	NS	***	–	–
P.laurentiana	J-J	***	***	***	NS	***	NS	NS	–	–
	J-A	*	***	***	*	**	NS	NS	–	–
	A-S	**	***	*	NS	NS	*	NS	–	–
	S-O	**	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(c):	M-J	–	–	NS	*	NS	NS	***	–	–
P.scotica	J-J	**	NS	NS	NS	*	NS	**	–	–
	J-A	**	NS	NS	***	NS	NS	NS	–	–
	A-S	**	NS	NS	***	**	NS	NS	–	–
	S-O	***	NS	NS	***	NS	NS	NS	**	***

Table 4.46 Continued.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.frondosa:	M-J	–	–	***	**	*	***	***	–	–
P.laurentiana	J-J	**	***	***	***	NS	***	***	–	–
	J-A	*	*	NS	***	NS	***	***	–	–
	A-S	***	NS	**	NS	NS	NS	NS	–	–
	S-O	**	***	***	*	**	NS	NS	NS	NS
P.frondosa:	M-J	–	–	***	**	***	***	***	–	–
P.scotica	J-J	*	***	***	NS	***	***	***	–	–
	J-A	NS	***	***	***	NS	***	***	–	–
	A-S	*	***	***	***	*	NS	NS	–	–
	S-O	***	***	***	***	**	NS	**	***	***
P.laurentiana:	M-J	–	–	***	NS	**	NS	NS	–	–
P.scotica	J-J	NS	***	***	*	***	NS	*	–	–
	J-A	NS	***	***	***	*	NS	NS	–	–
	A-S	***	**	NS	***	*	NS	NS	–	–
	S-O	NS	NS	NS	***	NS	NS	NS	***	***

Table 4.47 Statistical test of significance (LSD) of growth characteristics in *Primula* spp. grown at Esh, Durham during May – October, 1986.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b):	M-J	–	–	**	NS	NS	NS	NS	–	–
P.farinosa(c)	J-J	**	NS	NS	**	**	NS	NS	–	–
	J-A	NS	NS	NS	**	NS	**	*	–	–
	A-S	NS	NS	NS	**	NS	**	NS	–	–
	S-O	NS	NS	NS	*	NS	NS	NS	NS	NS
P.farinosa(b):	M-J	–	–	***	NS	***	***	NS	–	–
P.frondosa	J-J	NS	***	***	NS	***	***	***	–	–
	J-A	***	**	NS	NS	NS	***	*	–	–
	A-S	***	*	**	*	NS	NS	NS	–	–
	S-O	NS	**	**	*	**	NS	NS	***	NS
P.farinosa(b):	M-J	–	–	NS	**	NS	NS	NS	–	–
P.laurentiana	J-J	***	NS	*	***	***	NS	NS	–	–
	J-A	NS	*	*	***	NS	**	**	–	–
	A-S	***	*	*	***	NS	*	NS	–	–
	S-O	*	***	NS	**	NS	NS	*	*	NS
P.farinosa(c):	M-J	–	–	***	NS	***	***	NS	–	–
P.frondosa	J-J	***	***	***	*	NS	***	***	–	–
	J-A	***	***	*	NS	**	***	***	–	–
	A-S	***	**	**	***	NS	*	**	–	–
	S-O	*	***	***	***	***	*	*	***	*
P.farinosa(c):	M-J	–	–	NS	NS	NS	NS	NS	–	–
P.laurentiana	J-J	NS	**	**	*	NS	NS	NS	–	–
	J-A	NS	**	**	*	NS	NS	NS	–	–
	A-S	*	**	**	NS	NS	NS	NS	–	–
	S-O	NS	***	NS	NS	NS	NS	NS	NS	NS
P.frondosa:	M-J	–	–	***	**	***	***	NS	–	–
P.laurentiana	J-J	***	***	*	***	NS	***	***	–	–
	J-A	***	NS	NS	**	*	***	***	–	–
	A-S	NS	NS	NS	***	NS	NS	**	–	–
	S-O	***	NS	*	***	***	NS	**	***	**

Table 4.48 Statistical test of significance (LSD) of growth characteristics in *Primula* spp. grown at South Bents, Sunderland during May - October, 1986.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.farinosa(b): *P.farinosa(c)*	M-J	–	–	***	NS	***	NS	NS	–	–
	J-J	NS	***	***	NS	NS	NS	NS	–	–
	J-A	***	*	NS	NS	***	NS	NS	–	–
	A-S	NS	NS	NS	NS	NS	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.farinosa(b): *P.frondosa*	M-J	–	–	***	*	NS	***	NS	–	–
	J-J	NS	*	NS	NS	NS	***	***	–	–
	J-A	NS	NS	NS	*	NS	***	**	–	–
	A-S	NS	*	**	NS	NS	NS	*	–	–
	S-O	*	***	***	NS	*	NS	NS	***	NS
P.farinosa(b): *P.laurentiana*	M-J	–	–	*	NS	NS	NS	NS	–	–
	J-J	NS	NS	NS	**	NS	**	**	–	–
	J-A	NS	NS	NS	*	NS	*	NS	–	–
	A-S	NS	NS	NS	*	NS	NS	NS	–	–
	S-O	**	NS	NS	**	NS	NS	NS	NS	NS
P.farinosa(b): *P.scotica*	M-J	–	–	***	NS	***	NS	NS	–	–
	J-J	***	***	***	**	*	**	**	–	–
	J-A	***	**	NS	*	***	*	*	–	–
	A-S	NS	NS	NS	NS	NS	NS	NS	–	–
	S-O	**	NS	NS	NS	*	NS	NS	NS	NS
P.farinosa(c): *P.frondosa*	M-J	–	–	***	**	***	***	NS	–	–
	J-J	*	***	***	NS	*	***	***	–	–
	J-A	***	***	NS	**	***	***	**	–	–
	A-S	**	***	***	NS	NS	NS	*	–	–
	S-O	**	***	***	*	*	NS	NS	**	NS
P.farinosa(c): *P.laurentiana*	M-J	–	–	***	NS	*	NS	NS	–	–
	J-J	NS	***	***	NS	NS	NS	*	–	–
	J-A	***	NS	NS	*	***	NS	*	–	–
	A-S	*	NS	NS	*	NS	NS	NS	–	–
	S-O	*	NS	NS	*	NS	NS	NS	NS	NS
P.farinosa(c): *P.scotica*	M-J	–	–	NS	NS	NS	NS	NS	–	–
	J-J	**	NS	NS	NS	NS	NS	*	–	–
	J-A	NS	NS	NS	*	NS	*	**	–	–
	A-S	NS	NS	NS	NS	NS	NS	NS	–	–
	S-O	*	NS	NS	NS	*	NS	NS	*	NS

Table 4.48 Continued.

Primula spp.	Months	Significance levels								
		RLaGR	LAD	LAI	ROS	LF	FR	SC	CAP	SEED
P.frondosa:	M-J	–	–	***	***	NS	***	NS	–	–
P.laurentiana	J-J	**	***	NS	***	NS	***	***	–	–
	J-A	NS	**	**	***	NS	***	***	–	–
	A-S	NS	**	**	**	NS	NS	***	–	–
	S-O	***	***	***	***	***	NS	**	***	NS
P.frondosa:	M-J	–	–	***	***	***	***	NS	–	–
P.scotica	J-J	***	***	***	***	NS	***	***	–	–
	J-A	***	***	*	***	***	***	***	–	–
	A-S	**	***	***	NS	NS	NS	***	–	–
	S-O	***	***	***	**	***	NS	*	***	NS
P.laurentiana:	M-J	–	–	***	NS	***	NS	NS	–	–
P.scotica	J-J	**	***	***	NS	NS	NS	NS	–	–
	J-A	***	*	NS	NS	***	NS	NS	–	–
	A-S	**	NS	NS	*	NS	NS	NS	–	–
	S-O	NS	NS	NS	NS	NS	NS	NS	NS	NS

growing season (Table 4.47). The values of LAD in *P. frondosa* were significantly higher than *P. farinosa* (b) and *P. farinosa* (c) at all months, whilst *P. frondosa* showed significantly higher values of LAD than *P. laurentiana* only during J-J. At SUN, *P. farinosa* (b) showed significantly higher LAD than *P. farinosa* (c) during J-J and J-A. *P. frondosa* exhibited significantly higher LAD than the other species throughout the growing season except with *P. farinosa* (b) during J-A.

Figs. 4.5c-4.9c compares the values of LAI between *Primula* spp. at five sites. ANOVA results (Table 4.43) revealed that there were highly significant differences between species in LAI at all sites except during J-A at SUN. *P. frondosa* showed significantly higher LAI than the other species at all three sites throughout the growing season except with *P. laurentiana* during J-A. At GDF, *P. farinosa* (b) showed significantly higher values in LAI than *P. farinosa* (c) only during M-J and there were no significant differences in LAI between *P. farinosa* (b), *P. farinosa* (c), *P. laurentiana,* and *P. scotica,* except during M-J when there was significant differences between *P. farinosa* (b) and *P. scotica* (Table 4.44). At WDF and HAR, most species showed the maximum values of LAI during A-S, then LAI dropped at the end of the growing season. At lowland sites, *P. frondosa* usually showed significantly higher LAI than the other species, especially during the last two months of the growing season (Table 4.47 and Table 4.48). *P. farinosa* (b) showed significantly higher values of LAI than *P. farinosa* (c) only during the first two months of the growing season. There was a fall of LAI at ESH during J-A in *P. frondosa* as a response to drought (see also Fig. 4.25) whilst at SUN *P. frondosa, P. farinosa* (b) and *P. laurentiana* showed a simultaneous decrease of LAI during J-A.

Figs. 4.5d-4.9d illustrates the comparisons of the numbers of rosettes between *Primula* spp. ANOVA results showed significant differences between species in the number of rosettes during the growing season at all sites except during M-J at GDF and WDF (Table 4.43). At GDF, there were no significant differences in the number of rosettes between the two populations of *P. farinosa* throughout the growing season. *P. laurentiana* had a significantly lower number of rosettes than

the other species, especially during the last three months of the growing season. At WDF, the number of rosettes were not significantly different amongst *P. farinosa* (b), *P. farinosa* (c) and *P. frondosa*. *P. laurentiana* had a significantly lower number of rosettes than the other species except during M-J. At HAR, *P. scotica* showed a significantly higher number of rosettes than the other species during the last three months of the growing season. *P. farinosa* (b) had a significantly higher number of rosettes than *P. farinosa* (c) only during J-A and A-S. *P. farinosa* (b) and *P. frondosa* also showed no significant differences in the number of rosettes (Table 4.46). At ESH, *P. farinosa* (b) had a significantly higher number of rosettes than *P. farinosa* (c) throughout the growing season except during M-J. *P. farinosa* (b) and *P. frondosa* usually showed a significantly higher number of rosettes than the other species, especially when compared with *P. laurentiana* (Table 4.47). At SUN, *P. frondosa* usually showed a higher number of rosettes than the other species. The number of rosettes in *P. farinosa* (b) and *P. farinosa* (c) were not significantly different throughout the growing season. *P. scotica* produced the rosettes later than the other species, whereas *P. laurentiana* had no rosettes at all.

Figs. 4.5e-4.9e shows the number of leaves compared between the species of primulas at five sites. ANOVA results revealed significant differences in the number of leaves between species in most of the studied months (Table 4.43). At GDF, *P. frondosa* usually showed significantly higher number of leaves than the other species (Table 4.44). The number of leaves of all the species increased slowly and tended to be rather stable with a slight decrease at the end of the growing season. At WDF, *P. frondosa* had most significantly high numbers of leaves than the other species. *P. farinosa* (b) and *P. farinosa* (c) had no significant differences in the number of leaves during the growing season except during M-J. The maximum number of leaves were observed during A-S in all species except *P. laurentiana*. At HAR, all the species showed the maximum number of leaves during A-S. By that time *P. scotica* exhibited a significantly higher number of leaves than the other species except *P. farinosa* (b) (Table 4.46). However, *P. farinosa* (b) showed a significantly

higher number of leaves than *P. farinosa* (c) but only in the first two months of the experiment. At ESH, all species showed a steadily increasing number of leaves with time, except *P. frondosa,* which showed a fall in the number of leaves during J-A, then another increase and reaching a peak at the end of the growing season. During M-S, all species showed a similar number of leaves, However, significant differences between pairs of species were observed mostly with *P. frondosa* (Table 4.47). At SUN, *P. farinosa* (b), *P. frondosa* and *P. laurentiana* showed a fall in the number of leaves during J-A, while the other two species, *P. farinosa* (c) and *P. scotica,* were still increasing the number of leaves and they showed a significantly higher number of leaves than *P. farinosa* (b), *P. frondosa* and *P. laurentiana* (Table 4.48).

Figs. 4.5f-4.9f depicts the number of scapes per plant, comparing between the species at five sites. ANOVA results of the number of scapes showed significant differences between the species at all sites with only two exceptions at HAR during A-S and S-O. At GDF, *P. farinosa* (b) and *P. farinosa* (c) showed no significant differences in the number of scapes throughout the growing season (Table 4.44). *P. frondosa* exhibited a significantly greater number of scapes than the other species throughout the growing season, except at the end. *P. scotica* produced scapes later than the other species and reached the maximum number at the end of the growing season. At WDF, *P. farinosa* (c) exhibited significantly higher number of scapes than *P. farinosa* (b) during J-J and J-A. *P. frondosa* showed a significantly higher number of scapes than *P. farinosa* (b) and *P. laurentiana* throughout the growing season, whilst *P. farinosa* (b) and *P. laurentiana* did not show any significant differences in the number of scapes. At HAR, *P. frondosa* produced scapes early and the maximum number of scapes was observed earlier than in the other species. As a result, *P. frondosa* showed a significantly higher number of scapes than the other species during the first three months of the experiment. *P. farinosa* (b) and *P. farinosa* (c) did not show significant differences in the number of scapes during M-J (Table 4.46). All species except *P. frondosa,* showed the maximum number of scapes during A-S and then declined at the end of the growing

season. At the two lowland sites, *P. frondosa* usually showed a significantly higher number of scapes than the other species, whilst the lowest number was found in *P. laurentiana*. *P. farinosa* (b) and *P. farinosa* (c) showed no significant differences in the number of scapes throughout the growing season, except during J-A at ESH (Table 4.48).

Figs. 4.5g-4.9g shows how the number of florets in the primulas changed with time at five sites. ANOVA results mostly revealed significant differences between species in the number of florets except at the end of the growing season at HAR, ESH and SUN (Table 4.43). At GDF, *P. frondosa* showed a significantly greater number of florets than the other species during the first three months of the experiment. *P. farinosa* (b) showed a significantly higher number of florets than *P. farinosa* (c) only during its maximum number of florets (A-S). *P. scotica* flowered later than the other species and it had the maximum number of florets at the end of the growing season while the number of florets of the other species showed a marked fall. At WDF, most species showed the maximum number of florets during J-A. *P. frondosa* had the significantly greatest number of florets throughout the growing season (Table 4.45). There was no significant difference in the number of florets between *P. farinosa* (b) and *P. farinosa* (c) during the growing season. At HAR, *P. frondosa* showed the maximum number of florets during J-J, whilst the other species peaked during J-A or A-S. This resulted in a significantly greater number of florets than the other species during the first three months of the experiment (Table 4.46). *P. farinosa* (b) showed a significantly greater number of florets than *P. farinosa* (c) only during its peak number of florets (J-A). There was no significant difference in the number of florets between *P. farinosa* (b) and *P. laurentiana* throughout the growing season. At ESH and SUN, *P. frondosa* showed a similar pattern of flower development to *P. frondosa* at HAR. *P. farinosa* (b) at ESH showed a significantly greater number of florets than *P. farinosa* (c) during J-A, A-S, whilst no significant difference was observed between these two populations at SUN throughout the growing season. *P. farinosa* (c) and *P. laurentiana* showed no significant difference in the number of

florets at both sites.

Figs. 4.5h-4.9h depicts the comparison of the number of capsules per plant of the primulas at five sites at the end of growing season. ANOVA results show highly significant differences between species in the number of capsules per plant (Table 4.43). None of the species grown at GDF formed capsules. At WDF, *P. frondosa* showed a significantly higher number of capsules than *P. farinosa* (b) and *P. laurentiana,* but no significant difference was found with *P. farinosa* (c). *P. farinosa* (c) produced a higher number of capsules than *P. farinosa* (b), but no significant difference was observed (Table 4.45). *P. laurentiana* produced the lowest number of scapes but no significant difference was found when compared with *P. farinosa* (b) and *P. farinosa* (c). At HAR, *P. scotica* showed a significantly higher number of capsules than the other species. There was no significant difference in the numbers of capsules between *P. farinosa* (b), *P. farinosa* (c) and *P. laurentiana. P. frondosa* had the lowest number of capsules, but no significant difference was observed when compared with *P. farinosa* (b) and *P. laurentiana* (Table 4.46). At ESH, the significantly greatest number of capsules was found in *P. frondosa,* whereas there was no capsule formation in *P. laurentiana.* There was no significant difference in the number of capsules between *P. farinosa* (b) and *P. farinosa* (c). Only two species, *P. farinosa* (c) and *P. frondosa,* produced capsules at SUN and *P. frondosa* showed a significantly higher number of capsules than *P. farinosa* (c).

The number of seeds per capsule and number of seeds per plant are presented graphically in Figs. 4.5i-4.9i and Fig. 4.10. Seed formation was observed in 3 out of 5 sites. There were highly significant differences between species in the number of seeds per capsule at HAR and ESH (Table 4.43). At WDF, the number of seeds per capsule did not show significant differences between the species (Table 4.45). *P. scotica* showed the significantly highest number of seeds per capsule at HAR, whilst the rest of species showed no significant differences. At ESH, there was no significant difference in the number of seeds per capsule between *P. farinosa* (b) and *P. farinosa* (c). *P. frondosa* had the highest number of seeds per capsule, but

a significant difference was found only with *P. farinosa* (c). Seed production was not observed in *P. laurentiana* at this site.

4.3.2 Summer and winter survival

Fig. 4.11 depicts the percentage of dead plants counted at the end of the growing season of 1986. It can be seen that no plants died at WDF and HAR. For *P. frondosa* the percentages of dead plants at GDF and ESH were 10% and 20% respectively, whereas at SUN the number reached 40%. At this site there were also two more species, *P. farinosa* (b) and *P. laurentiana* for which 20% dead plants were observed.

At the end of April 1987 the numbers of winter survivors were counted and it was found that at all sites all of the summer survivors survived through the winter 1986-1987.

4.3.3 Second year flowering

At the beginning of spring 1987 the number of scapes was counted for each species. ANOVA performed on the mean number of scapes showed highly significant differences between species ($P<0.0001$) at all sites (Table 4.49). Comparisons between species of the number of scapes at each site are summarized in Table 4.50. Scapes were observed in *P. farinosa* (c), *P. frondosa* and *P. scotica* at GDF and all of the florets were found to be still in bud. *P. frondosa* had the significantly highest number of scapes. Though scapes were observed in *P. farinosa* (c) no significant difference in the number of scapes between *P. farinosa* (b) and *P. farinosa* (c) was found. At WDF, scapes with most of the flowers still in bud were found in *P. farinosa* (c), whereas scapes with most of the flowers opened were observed in *P. frondosa*. No scapes were observed in the other two species: *P. farinosa* (b) and *P. laurentiana*. *P. frondosa* exhibited a significantly highest number of scapes. All species growing at HAR flowered with the significantly highest number of scapes being found in *P. scotica*. *P. farinosa* (c) had a significantly higher number of

Figure 4.11 Percentage of dead plants in *Primula* spp. at the end of the growing season, 1986.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
SCC = *P.scotica*

GDF = Great Dun Fell Radar Station
WDF = Widdybank Fell, Upper Teesdale
HAR = Hartside nursery, Alston
ESH = Esh, Durham
SUN = South Bents, Sunderland

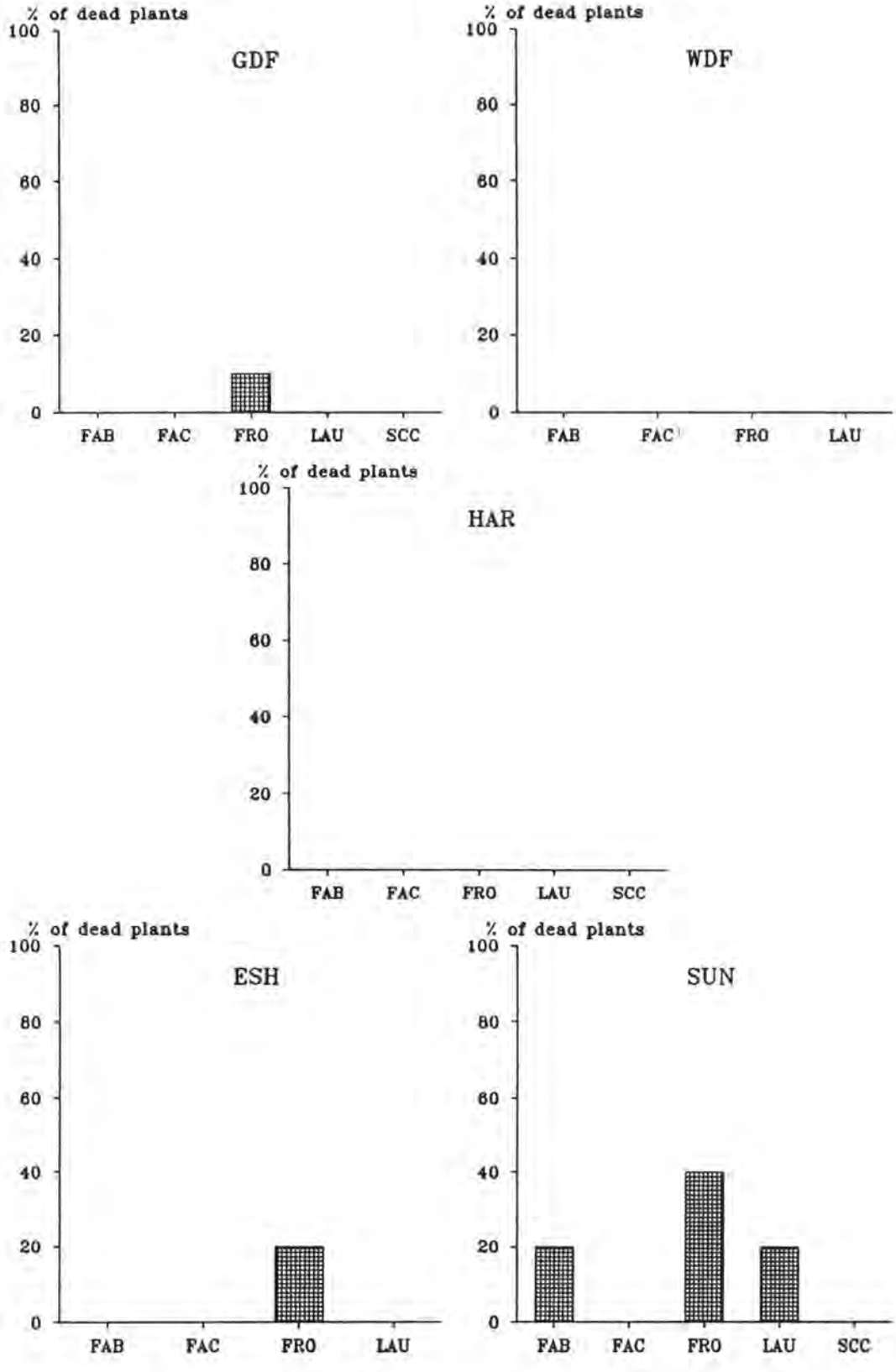

% of dead plants
100
80
60
40
20
0
GDF
FAB
FAC
FRO
LAU
SCC
% of dead plants
100
80
60
40
20
0
WDF
FAB
FAC
FRO
LAU
% of dead plants
100
80
60
40
20
0
HAR
FAB
FAC
FRO
LAU
SCC
% of dead plants
100
80
60
40
20
0
ESH
FAB
FAC
FRO
LAU
% of dead plants
100
80
60
40
20
0
SUN
FAB
FAC
FRO
LAU
SCC

Table 4.49 ANOVA of mean number of scapes, comparisons between species at each site, at the beginning of spring, 1987.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
GDF					
BETWEEN GROUPS	4	23.8222	5.9556	11.5471	.0000
WITHIN GROUPS	43	22.1778	.5158		
TOTAL	47	46.0000			
WDF					
BETWEEN GROUPS	3	181.1000	60.3667	25.2698	.0000
WITHIN GROUPS	36	86.0000	2.3889		
TOTAL	39	267.1000			
HAR					
BETWEEN GROUPS	4	415.8800	103.9700	15.7318	.0000
WITHIN GROUPS	45	297.4000	6.6089		
TOTAL	49	713.2800			
ESH					
BETWEEN GROUPS	3	166.9408	55.6469	23.9143	.0000
WITHIN GROUPS	33	76.7889	2.3269		
TOTAL	36	243.7297			
SUN					
BETWEEN GROUPS	4	125.7043	31.4261	21.6186	.0000
WITHIN GROUPS	41	59.6000	1.4537		
TOTAL	45	185.3043			

Table 4.50 Statistical test of significance (LSD) of mean number of scapes in *Primula* spp. at each site in the field, at the beginning of spring, 1987.

Sites	Primula spp.	Significance levels 1.	2.	3.	4.	5.
GDF	1.*P.farinosa(b)*	–	NS	***	NS	NS
	2.*P.farinosa(c)*		–	***	NS	NS
	3.*P.frondosa*			–	***	***
	4.*P.laurentiana*				–	NS
	5.*P.scotica*					–
WDF	1.*P.farinosa(b)*	–	NS	***	NS	–
	2.*P.farinosa(c)*		–	***	NS	–
	3.*P.frondosa*			–	***	–
	4.*P.laurentiana*				–	–
	5.*P.scotica*					–
HAR	1.*P.farinosa(b)*	–	**	***	NS	***
	2.*P.farinosa(c)*		–	NS	*	***
	3.*P.frondosa*			–	**	**
	4.*P.laurentiana*				–	***
	5.*P.scotica*					–
ESH	1.*P.farinosa(b)*	–	***	***	NS	–
	2.*P.farinosa(c)*		–	*	***	–
	3.*P.frondosa*			–	***	–
	4.*P.laurentiana*				–	–
	5.*P.scotica*					–
SUN	1.*P.farinosa(b)*	–	***	***	NS	***
	2.*P.farinosa(c)*		–	***	***	NS
	3.*P.frondosa*			–	***	***
	4.*P.laurentiana*				–	***
	5.*P.scotica*					–

scapes than *P. farinosa* (b). At ESH, *P. frondosa* showed the significantly highest number of scapes. *P. farinosa* (c) had a significantly higher number of scapes than *P. farinosa* (b) with most flowers still in bud. At SUN, no scapes were observed in *P. farinosa* (b) or *P. laurentiana*, and *P. frondosa* showed the significantly highest number of scapes at this site.

ANOVA results of the mean number of scapes revealed significant differences between the sites in all species except *P. farinosa* (b) (Table 4.51). LSD tests of the comparison of number of scapes between the sites for each species are summarized in Table 4.52. The mean numbers of scapes, compared between the sites for each species are presented in Fig. 4.12. Scapes of *P. farinosa* (b) were observed at HAR and ESH, but there were no significant differences between the sites in the number of scapes. *P. farinosa* (c) flowered at all sites and the highest number of scapes was found at HAR, but no significant differences were found when compared with ESH. Scapes were observed at all sites for *P. frondosa* and the number of scapes at GDF was significantly the lowest. Scapes of *P. laurentiana* were found at HAR and ESH and the number of scapes at HAR was significantly higher than at ESH. Scapes of *P. scotica* were observed at all of the three sites. The significantly highest and lowest number of scapes were found at HAR and GDF respectively.

4.3.4 Microclimates

4.3.4.1 Temperatures

Studies of temperature over a period of time suffered from incomplete records. Missing data were found to be either due to a dead battery at the beginning of the experiment at all sites (except GDF) or failure of the integrator for recording temperatures in the following months. The recorded temperatures as calculated from calibration curves also proved to be markedly higher or lower than the mean monthly air temperatures as collected from the Meteorological stations in some months and some sites (Table 4.53). Accordingly, all the temperature records were abandoned and were replaced by mean monthly air temperatures from the Meteo-

Figure 4.12 Comparisons of mean number of scapes in *Primula* spp. grown at five sites, at the beginning of spring, 1987.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
SCC = *P.scotica*

GDF = Great Dun Fell Radar Station, Cumbria
WDF = Widdybank Fell, UpperTeesdale
HAR = Hartside nursery, Alston
ESH = Esh, Durham
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

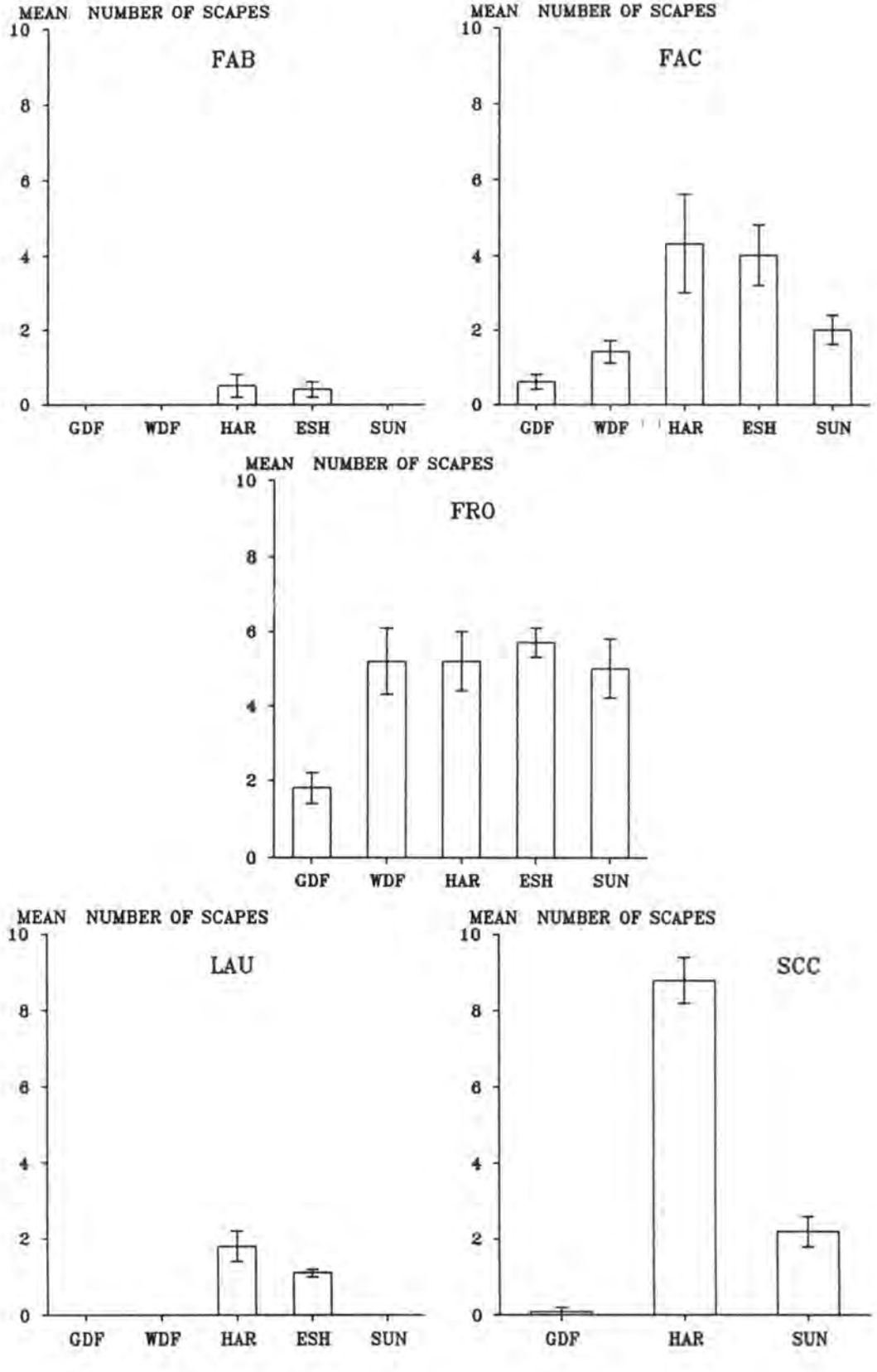

MEAN NUMBER OF SCAPES
FAB
FAC
FRO
LAU
SCC
10
8
6
4
2
0
GDF
WDF
HAR
ESH
SUN

Table 4.51 ANOVA of mean number of scapes in the primulas, comparisons between the sites, at the beginning of spring, 1987.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	4	2.4800	.6200	1.8725	.1318
WITHIN GROUPS	45	14.9000	.3311		
TOTAL	49	17.3800			
P.farinosa(c)					
BETWEEN GROUPS	4	105.5200	26.3800	4.4813	.0039
WITHIN GROUPS	45	264.9000	5.8867		
TOTAL	49	370.4200			
P.frondosa					
BETWEEN GROUPS	4	84.8762	21.2191	4.2984	.0058
WITHIN GROUPS	38	187.5889	4.9365		
TOTAL	42	272.4651			
P.laurentiana					
BETWEEN GROUPS	4	27.5111	6.8778	6.3678	.0000
WITHIN GROUPS	44	18.4889	.4202		
TOTAL	48	46.0000			
P.scotica					
BETWEEN GROUPS	2	398.0490	199.0245	2.2578	.0000
WITHIN GROUPS	26	56.0889	2.1573		
TOTAL	28	454.1379			

Table 4.52 Statistical test of significance (LSD) of means number of scapes in *Primula* spp. grown at five sites in the field, at the beginning of spring, 1987.

Primula spp.	Sites	Significance levels				
		GDF	WDF	HAR	ESH	SUN
P.farinosa(b)	GDF	–	NS	NS	NS	NS
	WDF		–	NS	NS	NS
	HAR			–	NS	NS
	ESH				–	NS
	SUN					–
P.farinosa(C)	GDF	–	NS	**	**	NS
	WDF		–	*	*	NS
	HAR			–	NS	*
	ESH				–	NS
	SUN					–
P.frondosa	GDF	–	**	**	***	*
	WDF		–	NS	NS	NS
	HAR			–	NS	NS
	ESH				–	NS
	SUN					–
P.laurentiana	GDF	–	NS	***	***	NS
	WDF		–	***	***	NS
	HAR			–	*	***
	ESH				–	***
	SUN					–
P.scotica	GDF	–	–	***	–	**
	WDF		–	–	–	–
	HAR			–	–	***
	ESH				–	–
	SUN					–

Table 4.53 Mean monthly air temperatures (data from Meteorological Station); Dry-and wet bulb air temperatures and soil temperatures recorded at study sites during the growing season 1986.

Months	Sites	Mean monthly air temperatures(°C)	Temperatures(°C)		
			Dry-bulb	Wet-bulb	Soil
J-J	GDF	8.6	8.2	7.3	8.6
	WDF	10.7	-	-	-
	HAR	13.2	-	-	-
	ESH	12.8	-	-	-
	SUN	12.8	-	-	-
J-A	GDF	9.1	-	6.6	-
	WDF	11.8	7.0	7.9	-
	HAR	14.0	-	-	14.5
	ESH	14.3	-	-	-
	SUN	15.4	22.9	12.0	73.8
A-S	GDF	7.3	4.6	4.5	-
	WDF	9.4	-	3.7	-
	HAR	10.7	-	-	-
	ESH	11.6	9.9	9.0	10.6
	SUN	12.8	31.5	9.9	50.8
S-O	GDF	6.2	3.8	3.7	-
	WDF	8.1	-	1.9	-
	HAR	8.0	8.8	-	13.3
	ESH	10.6	3.8	-10.8	7.3
	SUN	12.1	30.8	15.7	-

rological station at each site or nearby stations. Mean monthly air temperatures at ESH were calculated from the data obtained from Durham University Observatory, using a lapse rate of 0.67°C with an increasing altitude of 100 m (Savidge *et al.*, 1963; Pigott, 1978). Mean monthly air temperatures at HAR were obtained from private records by Mr. A.R. Huntley. Mean monthly air temperatures at HAR during the experimental period and long term mean monthly air temperatures of the other 4 sites are presented in Fig. 4.13. From these data, the lowest and the highest mean monthly air temperatures were at GDF and SUN respectively. It also can be seen that the mean monthly air temperatures of the three upland sites during winter months were much lower than those of the lowland sites.

4.3.4.2 Precipitation

Table 4.54 shows the mean daily rainfall at each site and higher rainfall was found with increasing altitude during the first two months. During August, there were widespread heavy rain conditions throughout the British Isles, and this resulted in high rainfall at two lowland sites and HAR. July was the driest month at the lowland sites, whilst September was rather dry at WDF and HAR. Detailed July daily rainfall records at ESH and SUN are presented in Fig. 4.14 which shows that there was a dry period over the first 3 wk.

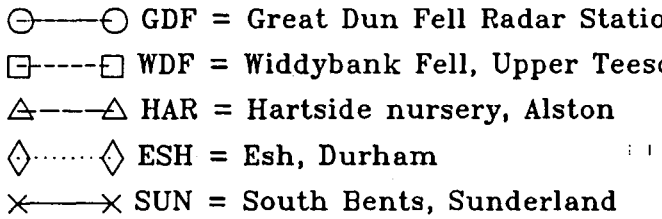
4.3.4.3 Radiation

Missing data during J-J at all sites were due to a dead battery. The pattern of PFD received at each site was quite different during the growing season (Table 4.54). The highest recorded value was 55.4 mol m^{-2} d^{-1} at HAR during S-O, whilst the lowest value was 4.9 mol m^{-2} d^{-1} at GDF during J-A. HAR was the site that had the greatest PFD throughout the growing season.

4.3.4.4 Wind speed

The anemometers located at GDF, WDF, and ESH were unfortunately blown down in strong wind and there were missing data from HAR and SUN due to failure

Figure 4.13 Mean monthly air temperatures (data from Meteorological station) at the selected sites.



GDF data during 1938–1940, after Manley (1942)
WDF data during 1968–1975, after Pigott (1978)
HAR data during 1986–1987, personal communication
ESH data during 1906–1935, from Durham Observatory
SUN data during 1980–1985, from Sunderland Polytechnic Meteorological station

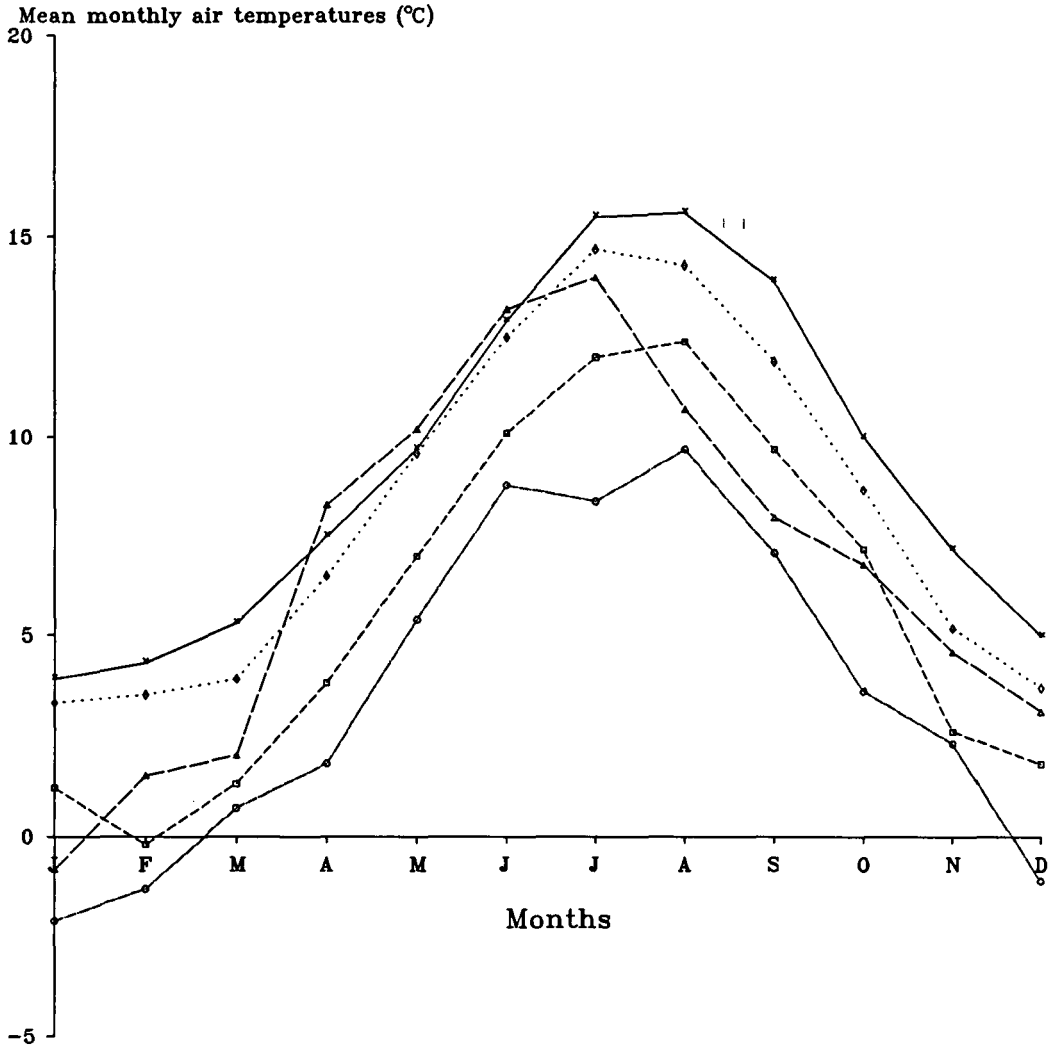

Mean monthly air temperatures (°C)
20
15
10
5
0
-5
J
F
M
A
M
J
J
A
S
O
N
D
Months

Figure 4.14 Daily rainfall at ESH and SUN in July (the driest month) during the experiment.

 denotes date of leaf sampling for determination of protein/ proline levels.

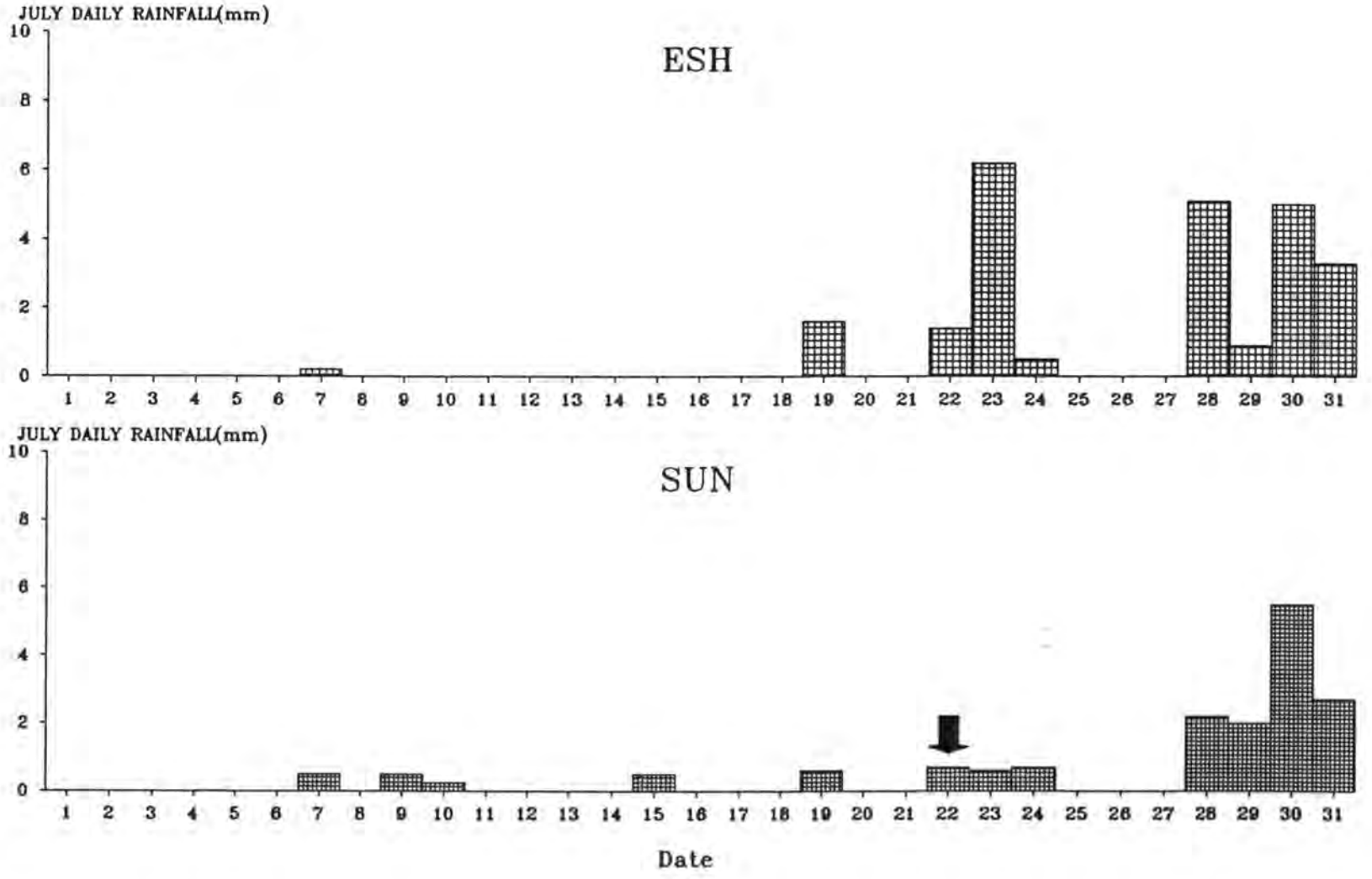

JULY DAILY RAINFALL(mm)
ESH
JULY DAILY RAINFALL(mm)
SUN
Date

Table 4.54 Mean daily rainfall, PFD measured at each site and mean monthly wind speed (data from Meteorological station) during growing season 1986.

Months	Sites	Mean daily		Mean monthly
		Rainfall cm^3	PFD mol /sq m/d	Wind speed m/s
J-J	GDF	21.4	-	8.5
	WDF	11.2	-	5.6
	HAR	8.9	-	-
	ESH	7.7	-	2.1
	SUN	6.4	-	3.6
J-A	GDF	47.0	4.9	8.6
	WDF	25.5	22.5	6.5
	HAR	28.7	30.7	-
	ESH	11.2	25.1	2.8
	SUN	6.2	27.7	4.0
A-S	GDF	57.9	12.1	7.8
	WDF	43.7	17.7	5.7
	HAR	57.5	33.6	-
	ESH	62.4	10.6	2.7
	SUN	27.2	15.5	3.3
S-O	GDF	14.2	11.3	7.5
	WDF	2.6	18.9	5.1
	HAR	4.2		-
	ESH	7.0	7.0	2.9
	SUN	9.6		4.2

Table 4.55 Climatic data obtained from Meteological Station (air temperature and wind speed) and measured data at each site, during January-April 1987.

Months	Sites	Temperature	Rainfall	PFD	Wind speed
		°C	cm^3	mol/sq m /d	m/s
JAN	GDF	-	-	11.8	-
	WDF	-2.1	-	-	5.6
	HAR	-0.9	-	21.7	-
	ESH	-	4.8	2.1	-
	SUN	2.2	12.9	1.3	4.3
FEB	GDF	-1.9	-	11.8	7.5
	WDF	0.2	-	-	5.2
	HAR	1.5	-	21.7	-
	ESH	2.7	16.1	-	3.1
	SUN	4.4	11.8	7.8	3.2
MAR	GDF	-2.4	-	11.8	9.2
	WDF	-0.1	-	-	6.9
	HAR	2.0	-	21.7	-
	ESH	3.9	25.3	7.3	3.4
	SUN	4.4	-	-	4.7
APR	GDF	-	-	11.8	-
	WDF	6.2	-	-	5.8
	HAR	8.3	-	21.7	-
	ESH	9.3	12.5	16.3	2.5
	SUN	9.4	16.2	13.4	4.5

of the integrators. As a result, the mean monthly wind speeds at the Meteorological station at, or nearby each site were collected and are summarized in Table 4.54. It can be seen that the higher wind speeds were found at two upland sites (GDF and WDF) throughout the growing season.

Climatic data during January to April of 1987 are presented in Table 4.55 which shows a relative warmer temperature in April than that of the average long-term air temperature (Fig. 4.13).

4.3.5 Relationship between growth and climates

Table 4.56 summarizes the relationships between microclimates and growth characteristics in *Primula* spp. A very strong significant correlation between RLaGR and mean monthly air temperatures was observed in all species. Rainfall was found to be significantly correlated with RLaGR in *P. farinosa* (c) and *P. frondosa.* A significant negative correlation between PFD and RLaGR was found in all species except *P. farinosa* (c). In contrast no significant linear relationship between RLaGR and wind speed was found in any species. A significantly negative correlation between LAD and mean air temperatures was found in 3 out of 5 species:- *P. farinosa* (c), *P. frondosa* and *P. scotica.* Rainfall was significantly correlated with LAD in all species except *P. frondosa.* A very good positive relationship was observed between PFD and LAD in all species, whilst negative correlations between LAD and wind speed were observed in *P. farinosa* (b), *P. farinosa* (c), and *P. laurentiana.* Air temperatures were found to be significantly correlated with LAI only in *P. farinosa* (b) and *P. laurentiana.* However, a very high relationship between rainfall and LAI was found in all species. PFD was significantly correlated with LAI in *P. farinosa* (b), *P. farinosa* (c) and *P. scotica,* whilst wind speed correlated significantly with LAI in *P. farinosa* (b), *P. farinosa* (c) and *P. laurentiana.*

The number of rosettes correlated significantly with rainfall in *P. farinosa* (b), *P. laurentiana* and *P. scotica,* but PFD correlated significantly with only two species, *P. laurentiana* and *P. scotica.* Wind speed was negatively correlated with

Table 4.56 Relationships between microclimates and growth characteristics in *Primula farinosa* and its allies, during the growing season 1986.

Growth Characteristics	Microclimates			
	TEMP	RAIN	PFD	WIND
P.farinosa(b)				
RLGR	.4402***	.1173	-.1506*	-.1294
LAD	-.1288	.1306*	.3866***	-.2214*
LAI	.1454*	.3041***	.1992*	-.2747**
ROS	-.0806	.1659*	.0591	-.1310
LF	-.0545	.2786***	.1767*	-.1886*
FR	.1506*	.1436*	-.0921	-.0904
SC	.1045	.1040	.0011	-.1818*
CAP	-.0838	.1720**	.0429	-.2937**
SEED	-.1503*	-.1919**	.1950*	-.1327
P.farinosa(c)				
RLGR	.4925***	.1542*	-.0517	-.0937
LAD	-.1889**	.1779**	.3266***	-.2843**
LAI	.0829	.3746***	.2007**	-.3290**
ROS	-.0767	.0288	.0172	-.0520
LF	.0828	.3651***	.0856	-.2816**
FR	.0974	.0398	.0266	.2070*
SC	.0132	-.0309	.1630*	.0883
CAP	-.2040**	.0068	.2237**	-.2131*
SEED	-.2073**	-.2318***	.1075	-.0982
P.frondosa				
RLGR	.2355***	.2427***	-.2149**	-.0807
LAD	-.2575***	.0747	.3714***	.0056
LAI	-.0548	.2023**	.1467	-.1020
ROS	-.1049	.0937	-.0213	-.1641*
LF	.2632***	.1455*	.0227	-.0436
FR	.3780***	-.0865	-.0857	-.0285
SC	.3617***	.0480	-.1442	-.0806
CAP	-.0467	-.0294	-.2647**	-.3135**
SEED	-.1625*	-.2141**	-.1265	-.1068

Table 4.56 Continued.

Growth Characteristics	Microclimates			
	TEMP	RAIN	PFD	WIND
P. laurentiana				
RLGR	.3895***	.0025	-.2325**	-.1269
LAD	.0286	.2423***	.3321***	-.4247**
LAI	.2948***	.3331***	.1412	-.4882**
ROS	-.2092**	.1795**	.2635***	-.1217
LF	.2740***	.2741***	.1745*	-.4397**
FR	.0835	.1265*	.1822*	-.0996
SC	.0704	.1465*	.3686***	-.1059
CAP	-.1410*	.0186	.2973***	-.0805
SEED	-.1704**	-.1486*	.1785*	.0069
P. scotica				
RLGR	.4885***	.0303	-.4007***	-.1413
LAD	-.1965*	.2974***	.6200***	.0319
LAI	.1146	.5192***	.2283*	-.0661
ROS	-.1214	.2626**	.5288***	.2080*
LF	.1129	.4928***	.2150*	-.2101*
FR	-.1718*	.4611***	-.0238	.3275*
SC	-.3310***	.2696**	.3273***	.2609*
CAP	-.2749**	-.2878***	.6749***	.
SEED	-.2613**	-.2736**	.6407***	.

(1-tailed, "." printed if a coefficient cannot be computed)

Note TEMP = mean monthly air temperature

RAIN = mean daily rainfall

PFD = photon flux density

WIND = mean monthly wind speed

the number of rosettes in *P. frondosa,* whilst a positive relationship was found with *P. scotica.* A significant association between air temperatures and the number of leaves was observed only in *P. frondosa* and *P. laurentiana.* Nevertheless, a very strong relationship between rainfall and the number of leaves was found in all species. Leaf numbers for *P. farinosa* (b), *P. laurentiana* and *P. scotica* were significantly correlated with PFD. Wind speed showed a significant negative correlation with leaf numbers in all species except *P. frondosa.* A significant positive relationship between air temperatures and the number of florets was found only in *P. farinosa* (b) and *P. frondosa,* whereas a good correlation was found between rainfall and the number of florets in *P. farinosa* (b), *P. laurentiana* and *P. scotica.* A poor relationship between the number of florets with PFD and wind speed was usually observed in most of species. The number of scapes was found correlate very well with rainfall and PFD in arctic and subarctic species, i.e. *P. laurentiana, P. scotica* respectively; whilst a poor relationship was observed in the other species. A significant negative correlation between wind speed and the number of scapes was found in *P. farinosa* (b), whereas a significant positive correlation was observed in *P. scotica.*

A significant negative correlation was generally found between the number of capsules and the number of seeds with air temperatures and rainfall. In contrast a significant positive correlation was often observed between the number of capsules and the number of seeds with PFD. Wind speed was significantly correlated with the number of capsules in *P. farinosa* (b), *P. farinosa* (c) and *P. frondosa,* whilst very poor correlation was observed between wind speed and number of seeds in all species.

4.4 Discussion

Within the considered range of altitudes in the north of England, there were well defined differences between the selected sites as regards plant growth and development. For instance, during mid-summer of 1986, there was a period of drought at the lowland sites (ESH and SUN), whilst rainfall at the three upland sites was plentiful. This resulted in a marked decrease in the values of RLaGR at the lowland sites to a level below zero in some species. It is known that soil water stress has a profound effect on reducing leaf area development as has been reported by many workers (e.g. Martin, 1940; Asana & Saini, 1958; Shaw & Laing, 1966). Stocker (1960) noted that for sugar beet, the first effect at the onset of water stress is the decrease of photosynthesis coinciding with the increase of respiration. He also noted that a retardation of plant growth due to dry conditions is much more pronounced in leaves than in stems. Denmead & Shaw (1960) working with corn, found that stress imposed while the plant is actively expanding retarded further development. Most of the reduction in leaf area appears to be the consequence of slowed cell expansion (Hsiao *et al.*, 1976a). However, explanations of these results are rather complicated since water stress not only affects cell division and cell expansion but also affects indirectly photosynthesis and translocation which will depress the amount of metabolites available for growth of the expanding leaves (Dale, 1982). When the stress was removed the growth rate did not immediately return to normal, but after several days was almost normal (Denmead & Shaw, 1960). This also appeared to be the case in the primulas, where, a period of 4 wk was long enough to enable them to recover from stress and they showed a marked increase in RLaGR in the following month after the end of the drought.

Another effect of drought on the primulas can be seen from the results of the number of capsules per plant as well as the number of seeds per capsule at SUN. Only 2 out of the 5 species produced capsules, but none of them was fertile. There are numerous reports in the literature showing that a water deficit limits yield. For example, Asana & Saini (1958) working with two varieties of wheat, found that

drought reduced the grain number of both varieties to the same extent. Phillis (1956) noted that water stress may influence both the initiation phase and subsequent development of floral structures. Hsiao *et al.* (1976a) noted that in sorghum varieties water stress imposed during flowering and fruit setting usually resulted in abortion of fertilized ovaries as well as the death of young fruits. Nevertheless, the results of Product-moment correlation coefficients indicated a significantly negative correlation between the number of seeds per capsule and rainfall in all species studied because no seeds were produced at GDF (the wettest site) and optimum seed production was at ESH (the second driest).

Amongst the 5 sites studied GDF is both the highest and the most exposed. A striking feature of climatic factors is that this site was the coldest, highest in rainfall and wind speed, and the lowest in radiation. As would be expected the amount of incoming radiation decreases with increasing altitude. This is due to increased cloud cover (Harding, 1979). It is evident that during the first two months of this experiment rainfall increased about 3-4 fold from SUN (4m) to GDF (847m). Records of the annual climate of 1986 showed that there were 168 d of gale at GDF and the mean high gust was 40 m $s.^{-1}$ This led to problem for an anemometer that was installed at this site, a top part collapsed within 1 wk and had to be replaced twice. It became obvious that none of these instruments could withstand the strong winds at this site. Though the mean monthly air temperatures during June-September of 1986 were well above 5°C, the primulas grown at this site also often experienced temperatures near freezing point at night.

Based upon the plant responses and growth parameters studied all species usually showed the lowest values of growth characteristics at GDF. Plant sizes were the smallest and very little vegetative reproduction was observed. However, all species studied produced flowers with a considerable size of scapes, but without fertile capsules or no capsule formation. These results are in agreement with the findings of Pearsall (1950) who found that the moor-rush (*Juncus squarrorus*) rarely produces viable seeds when they grow above about 750 m to 810 m although vigorous plants

of this species can be found at the higher ranges of altitude. He noted that this effect is evidently due mainly to the retardation of flower and fruit development.

There was some overlaping of the values of growth characteristics of the primulas grown at WDF, HAR and ESH and also between ESH and SUN. However, HAR as the mid-altitude (330 m) site seems to be the most suitable of the 5 sites for the primulas. All species showed a considerable growth in both vegetative and reproductive phases. Flowering of *P. laurentiana* and *P. scotica* at this site was also sooner than at the other sites. The outstanding features of this site may be explained in terms of a better climatic regime than the other sites. During June-August, mean air temperatures at HAR were above 10°C, with no period of drought, with the highest PFD of the 5 sites and a mild wind. Due to the failure of an integrator of the anemometer at this site and there being no available climatic data from the nearby station, there was no information on wind speed from here. Nevertheless, wind speed at this site seems to be lower than that at WDF and ESH, since the top of anemometer was still working throughout the growing season, whilst those at WDF and ESH had both collapsed. However, the only climatic characteristic which differed from the other sites was the amount of radiation. The strong relationship between growth characteristics with the PFD also indicated the importance of light to the primula growth.

In this context, Hodgson (1967), working with *Helianthus annuus* and *Vi cia faba* and Hegarty (1973) with *Helianthus annuus* and *Phaseolus vulgaris* found a positive dependence of RGR on light and temperature. This is exactly in agreement with the results reported herein. However, a negative rather than positive relationship between RLaGR and PFD was observed in this study. Blackman *et al.* (1955), with *Helianthus annuus* found that the relative growth rate of the whole plant, as well as that of the plant parts was positively dependent on light alone. Rorison & Sutton (1975) noted that the major effect of solar radiation on plant growth in Lathkill Dale, a valley running E-W through the limestone plateau, is through evaporation infuencing soil moisture. It is found that temperature influences the rate

that leaves are produced but the photoperiod may also be concerned and the two effects have not always been clearly distinguished (Humphries & Wheeler, 1963). Rosini (1984) also pointed out that air temperatures and net radiation are closely interconected, and it is rather difficult to distinguish between the effects on plant growth of these two parameters. Recently, Chapin & Shaver (1985b), studying the growth of vascular species in undisturbed arctic tussocks and wet meadow tundras in Alaska, found a different pattern of growth response to alternation of light, air temperatures, and nutrient regimes. They concluded that no single factor limits growth of all species in these communities.

A marked contrast between upland and lowland sites in this study was seen in the length of the growing season. It can be seen that all species studied showed a significant higher value of RLaGR and LAI at lowland sites than at upland sites during S-O. The primulas grown at lowland sites were still increasing in leaf numbers at this time whereas leaf numbers at the upland sites were showing a marked decrease in all species. The results of this experiment, based on the primula responses, suggested that the length of the growing season at the upland sites is shorter than that at the lowland sites by approximately 1 month. Manley (1945) noted that at 100 m, at Durham the growing season is April 2-November 30 (223 d) whereas at 821 m at GDF, the growing season is May 23-September 28 (128 d). The data given by Manley for Durham give a rather longer season than my observations at ESH and SUN would suggest as I found that at the end of October 1986 the leaves of all the species became senescent, although the mean monthly air temperatures during October-November 1986 were still above 6.0°C at both sites. Woodward & Pigott (1975) also noted a marked decrease of the growing season with increasing altitude. In addition, Pigott (1978) concluded that there is a fall in mean temperature of 0.67°C with an increasing altitude of 100 m, and the growing season is shortened by over 2 wk.

Upland populations of *P. farinosa* in northern England reach their known highest range of altitude at 547 m at the summit of Cronkley Fell, whilst the coastal

populations occupy the area from sea-level upto 50 m. It is interesting to note that when these two populations of *P. farinosa* were grown side by side at WDF (510 m) there were no significant differences in their values of RLaGR throughout the growing season. Although this site is about 500 m above the natural habitat of *P. farinosa* at Blackhall Rocks. However, when these two populations were grown at GDF, which was much above their known altitudinal ranges, both populations grew with reduced vigour. Nevertheless, *P. farinosa* (c) had a significantly higher value of RLaGR than *P. farinosa* (b) at the beginning and middle of the growing season. This result indicated that *P. farinosa* from Upper Teesdale had more successfully adapted to grow at the lower temperatures than *P. farinosa* from Blackhall Rocks.

At HAR which was the most suitable site for the primula growth, it was found that the RLaGR of the two populations were almost the same except during mid-summer when *P. farinosa* (c) had a significantly higher value of RLaGR than *P. farinosa* (b). *P. farinosa* (b) was expected to show higher performance in RLaGR at the lowland sites. However, the results from this experiment showed that the two populations of *P. farinosa* generally exhibited the same growth rate with only a few exceptions i.e., *P. farinosa* (c) had significantly greater values of RLaGR than *P. farinosa* (b) at the beginning of the growing season at ESH and during the drought stress period at SUN. This is in agreement with results reported previously by Woodward & Pigott (1975), when they found the growth rate of *Sedum rosea* (upland species) was rarely affected by altitudes or by varied temperatures within the range it usually experiences in its natural habitat. In contrast, *S. telephium* (lowland species) showed a sensitive response to increasing altitude by a reduction in size, but *P. farinosa* populations from Blackhall Rocks showed less response to altitudes in comparison with *P. farinosa* from Upper Teesdale. In addition, Pigott (1978) noted that the presence of some arctic-alpine plants e.g. *Gentiana verna, S.rosea,* at sea-level in Scotland or the west of Ireland, is associated with the decrease in summer temperatures at sea-level, due to both the higher latitudes and the influence of the Atlantic.

Amongst the species studied, it is worth noting that the values of RLaGR in *P. frondosa* were rather sensitive to both high and low altitudes and this species appears to adapt itself to the different climates, e.g. at GDF its value of RLaGR was the lowest during J-A, then became higher than that of some species in the following months. At the lowland sites *P. frondosa* also showed a decrease in RLaGR during drought followed by an increase after recovery from drought. At the end of the growing season, (S-O), *P. frondosa* generally showed a significantly higher value of RLaGR than the other species at all sites and the values of RLaGR were still positive at lowland sites, whilst the RLaGR of some species went down below zero. *P. laurentiana* and *P. scotica* had the highest values of RLaGR at all sites (significantly higher than the other species in some pairs) at the beginning of the growing season this appears to be a characteristic of arctic plants which have rapid growth as soon as bud temperature is above freezing point (Chapin & Shaver, 1985a). However, the value of RLaGR of *P. laurentiana* decreased faster and sooner than the other species at all of the upland sites. This may be due to the intrinsic growth characteristics of arctic plants to grow within a short growing season. It may be relevant to this characteristic of *P. laurentiana* to point out that Pigott (1978) noted that the ability of a plant to become dormant varies genetically between populations of cocksfoot.

At all study sites, *P. farinosa* (b) grew to a larger size than *P. farinosa* (c) and also showed higher values of LAD and LAI than *P. farinosa* (c) in some months generally at the beginning of the growing season. *P. frondosa* mostly exhibited the highest values of LAD at all sites throughout the growing season. This result is usually associated with the size of the plant, as demonstrated by Duncan & Hesketh (1968). They worked with 22 races of maize and showed statistically that plants with larger leaf area at the initial measurement usually had larger leaf area at the final harvest. The greater size of *P. frondosa* than the other species at all sites, as well as its variable size at different sites indicated that the size of a plant depends on both genotype and environmental factors.

The ability to reproduce vegetatively by production of rosettes was found to be nearly the same for *P. farinosa* (b) and *P. farinosa* (c) at GDF, WDF and SUN. However, at HAR and ESH, *P. farinosa* (b) showed higher performance in vegetative reproduction than *P. farinosa* (c). This result suggested that the vegetative reproduction of *P. farinosa* (b) is favoured by a more lowland climate. It might be argued that there were no significant differences in the number of rosettes between *P. farinosa* (b) and *P. farinosa* (c) at the lowest-altitude site (SUN). This may be explained by the fact that *P. farinosa* (b) tends to be more sensitive to drought than *P. farinosa* (c) as can be seen from the results of RLaGR during J-A. This result might in part be reflected in the ability of *P. farinosa* (b) to produce rosettes. *P. frondosa,* in general, had a higher number of rosettes than the other species at all sites except HAR, at which *P. scotica* showed marked vegetative reproduction by producing rosettes. In contrast, the ability of *P. laurentiana* to reproduce vegetatively by producing rosettes was very poor. The rosettes of *P. laurentiana* were produced both later and smaller in number than those of the other species at all sites, and no rosettes were produced at all at SUN. The latter result may be in part due to the effect of water stress at this site during which period most plants showed severe wilt. In addition, it is pertinent to note that many arctic plants have little or no power of vegetative reproduction (Savile, 1972).

As has been mentioned earlier (Ch 1), upland populations of *P. farinosa* flower sometimes earlier than lowland populations. It is obvious from the results reported herein that when the two populations of *P. farinosa* were grown side by side, there were highly significant differences in their times of flowering especially at HAR and the two lowland sites. Of the 5 species studied, *P. frondosa* flowered significantly earlier than the other species with the only exception at HAR. *P. scotica* grown at HAR showed significantly higher numbers of scapes than the other species. HAR is suitable not only for vegetative growth of the primulas but also for their reproductive growth. It can be seen that all species were flowering by the end of April 1987, and this was rather sooner than for their time of flowering, and indeed was about 1

month earlier than in spring 1986. A striking difference between climate of both spring may be responsible (Table 4.55), as April and May 1986 were rather cold, in contrast to the warm April of 1987. This resulted in large number of scapes being observed at the two lowland sites and HAR.

The influence of temperatures on flower development can be seen both from the mean number of scapes per plant and the opening of the florets. At GDF 3 out of the 5 species produced scapes but no florets opened. In contrast, at HAR, all species produced scapes and opening florets were observed in two species, i.e., *P. frondosa* and *P. scotica*. Amongst the 5 species studied, the flowering of *P. laurentiana* was the poorest and this species also rarely produced fertile seeds. This results may be due to the nature of arctic plants which require a long day photoperiod combined with low light intensity for flowering and seeds development (Porsild, 1951).

P. frondosa produced scapes and flowers sooner than the other species in both 1986 and 1987. This species also produced greater number of capsules and seeds than the other species, but there was one exception. Looking at HAR during the growing season of 1986, we can see that *P. frondosa* had significantly greater values of LAD, LAI and number of scapes as well as number of florets than the other species. In contrast, *P. frondosa* had the smallest number of capsules per plant and number of seeds per capsule. This result may be explained by the work of Kruger (1977) who studied the effect of plant density on LAI and yields of *Pisum sativum*. He found that when plant density increased there was a reduction in the number of pods per plant and the number of peas per pod.

All species which produced flower buds over-winter in an advanced state, which is a characteristic of arctic and alpine plants (e.g. Sørensen, 1941; Billings & Mooney, 1968) and this can be inferred from the earlier flowering during the milder spring. The differences between the time of scapes development and the opening of the flowers are largely under genotypic control and also depend on the interaction of the genotype and environmental conditions (Lang, 1965).

In terms of the parameters studied, it is quite clear that rainfall had a profound effect on the life of the primulas. Results from the numbers of survivors after the drought period of mid-summer 1986 indicated that *P. farinosa* and its relatives were markedly different in their response to water stress. Amongst the 5 species studied, *P. frondosa, P. farinosa* (b) and *P. laurentiana* were more sensitive to water stress than *P. farinosa* (c) and *P. scotica,* as can be seen from Fig. 4.11. *P. frondosa* had the highest number of dead plants whilst *P. farinosa* (b) and *P. laurentiana* had the same percentage of dead plants. As would be expected from the nature of plants which are usually confined to a damp or wet soil , they showed a sensitive response to drought.

The results of this experiment suggested that there might be some association between plant size and the response to water stress in primulas. *P. scotica* and *P. farinosa* (c) were small-size plants and they showed the same slight sensitivity to water stress, but in contrast, *P. frondosa, P. laurentiana* and *P. farinosa* (b) were bigger plants and they exhibited a severe response to drought. From this standpoint, Levitt (1956) noted that smaller leaf area is one of the primary factors that are associated with frost and drought hardiness and this factor is also related to the physiological properties of the plants. In view of this, Kramer (1983) noted that plants with large leaf area usually transpire more than those with smaller leaf areas. The smaller leaf areas of *P. farinosa* (c) and *P. scotica* compared with those of the other species may be related to their ability to withstand longer periods of drought than some of their allies. In addition, a laboratory experiment was performed to investigate the effect of water stress on nitrogen metabolism in the primulas (Ch 6).

Finally, as all of the summer survivors at all sites survived through the winter of 1986-1987, this indicated that *P. farinosa* and its allies are more sensitive to drought than frost. They also showed rapid growth by producing a number of leaves and scapes in the early spring of 1987. There is no doubt that frost-tolerance and the early production of flowers in the primulas are the characteristics of arctic

and alpine plants (Bliss, 1985; Chapin & Shaver, 1985a).

CHAPTER 5

PHOTOSYNTHESIS MEASUREMENTS

5.1 Introduction

Photosynthesis, the most important fundamental process of plants is very dependent on environmental conditions as well as plant characteristics. Previous studies have demonstrated diversity among species, as well as ecotypes of a species, in their photosynthetic rates which relate to their natural origins. It was found that in the climatic races of *Solidago virgaurea* from Scandinavia, the temperature optimum for net photosynthesis was related to climatic origin (see Cooper, 1963). A maritime population from Skane showed an optimum rate at 20°C, one from the inland climate of Uppsala had a peak rate at 24°C, and another one from Lapland at 16°C. It was noted that natural selection had markedly influenced photosynthetic capacities of closedly related plants. Milner *et al.* (1959) found that high and low altitude specimens of *Mimulus* differed in the optimum temperatures for assimilation, light saturation intensities, respiration rate and photosynthetic rate over diverse conditions.

Billings *et al.* (1961) reported that *Oxyria digyna* plants grown from seed collected at 2,027 m in Montana had higher photosynthetic rates than seedlings from sea-level populations in Alaska. They proposed that the higher photosynthetic capacity discovered in the alpine populations was a response to the lower CO_2 concentrations occurring at high elevations. Chabot & Billings (1972) noted similar differences in photosynthetic rates between high and low elevation populations of several species which they sampled along an altitudinal gradient from desert to alpine communities on the eastern slope of Sierra Nevada in California. They suggested that higher photosynthetic rates may give alpine populations an ability

to gather an annual energy supply during a rather short growing season, as well as an ability to photosynthesize efficiently under low CO_2 concentrations.

In addition, Björkman & Holmgren (1963, 1966) found assimilation differences in *Solidago vigaurea* ecotypes from sunny and shaded habitats. Subsequent works indicated how these adaptive differences arise out of evolutionary modifications of photochemical, biochemical and morphological properties (Björkman, 1968; Holmgren, 1968; Gauhl, 1969). McNaughton (1973) also noted ecologically significant differences with regard to temperature between the ecotypes of *Typha latifolia* for such factors as net photosynthesis and photosynthetic rate.

The photosynthetic periods for arctic and alpine communities are rather short and growth is confined to a 2-4 month period after snowmelt (Billings & Mooney, 1968). This period varies from year to year; in years of high snow accumulation the plant may not be released from snow at all, or for only a few days of activity, before the low temperatures of autumn, and the new snows return (Billings & Mooney, 1968). At low temperatures the photosynthetic activity is restricted in all plants by a reduced velocity of enzymatically catalyzed photosynthetic reactions (Larcher & Bauer, 1981). The rate of net photosynthesis in arctic and alpine plants may be nil or even negative during winter months (Hadley & Bliss, 1964; Bourdeau, 1959). In contrast, it has been reported that positive net photosynthesis can be observed at temperatures as low as -6°C to -8°C in certain conifers and evergreen broad-leaf species (Pisek *et al.*, 1967). It is to be expected that any plants that grow and survive winter in arctic and alpine communities must be metabolically adapted (Bliss, 1985).

In general, arctic and alpine plants photosynthesize by the C_3 pathway (Chapin & Shaver, 1985a; Bliss, 1985). Their patterns of net assimilation are similar to those of temperate-zone species, but are reached at lower temperatures (Wager, 1941; Tieszen & Wieland, 1975; Lechowicz, 1982). However, photosynthetic rate is not uniform throughout the season (Billings & Mooney, 1968); early in the season net

assimilation in alpine plants may be quite low or even negative due to high respiration rates associated with rapid plant growth (Hadley & Bliss, 1964). Maximum photosynthesis is reached during the flowering state (Glagolev & Filipov, 1965). Billings *et al.* (1961) found greater assimilation rate over a range of CO_2 concentrations in alpine populations of *Oxyria digyna* compared with arctic populations.

Photosynthetic activities of different species may vary with regard to their optimum temperatures or tolerance at extremes of high or low temperatures (Berry & Raison, 1981). In general, species from warm habitats usually reach their optimum rates at somewhat higher temperatures than species from cool habitats. For example, alpine populations of *Oxyria digyna* (Mooney & Billings, 1961), *Thalictrum alpinum* (Mooney & Johnson, 1965) have photosynthetic optima at higher temperatures than do arctic ecotypes whilst the photosynthetic rates of arctic plants are generally highest at 10-15°C (Billings *et al.*, 1971; Johnson & Tieszen, 1976). These ranges of temperatures are 10-30°C lower than those for temperate plants, but they are still higher than the average summer-leaf temperatures (6-10°C). Berry & Björkman (1980) suggested that the low temperature optima for photosynthesis in arctic plants result from high levels of RuBisCo, enabling reasonable photosynthetic rates at low temperatures. It seems that alpine plants are pre-adapted to the alternating temperature regime in contrast to arctic plants which grow in a more temperature stable environment (Bliss, 1985). Accordingly, arctic species acclimatize photosynthetically to changes in temperatures, but not as rapidly or as completely as do alpine species (Billings *et al.*, 1971; Tieszen & Helgager, 1968). In contrast, it has been noted that species adapted to the severe temperatures of arctic and alpine communities rarely have temperature optima below 20°C, leading some authors to suggest that plant may not be successfully adapted to such cold environments (Berry & Raison, 1981).

Increasing light intensities cause an upward displacement of both temperatures optimum and temperature maxima until light saturation is reached (Pisek *et al.*, 1973). It was found that light intensity was highly correlated with the net as-

similation rates in five subalpine species in the Medicine Bow Mountains (Scott *et al.*, 1970). Alpine populations of *Oxyria digyna* achieved light saturation at higher intensities than did arctic populations (Mooney & Billings, 1961) and this could be related to the greater photon flux in the former environment. Scott & Billings (1964) observed that for nine alpine species the light compensation point increased with a rise in temperature and that the maximum net assimilation rates were generally 38-50 μmol CO_2 g^{-1} s^{-1}. Hadley & Bliss (1964) also reported that high light saturation resulted in increasing photosynthetic efficiency of alpine plants e.g. *Geum pechii,* at higher temperatures (20-25°C).

Photosynthetic rates in arctic vascular plants are rarely light-saturated and more closely parallel daily and seasonal patterns of light than temperatures (Tieszen, 1975; Miller *et al.*, 1978). Positive net assimilation rate in arctic plants may be sustained over a whole day (24 h) during much of the growing season in the cloudless night (Tieszen, 1978). Light reaction of photosynthesis in arctic plants has many characteristics in common with shaded plants of the temperate regions (Boardman, 1977), e.g. low Hill reaction rate (Billings *et al.*, 1971; Tieszen & Helgager, 1968); whilst the dark reaction is characterized by a high RuBisCo level, as in sun plants (Chapin & Shaver, 1985a).

The photosynthetic efficiency, at low light intensities, of arctic plants seems due in part to high chl concentrations and low chl a to chl b ratios (Mooney & Billings, 1961; Tieszen, 1978) which is in contradiction to the high chl a to chl b ratios of alpine plants (Tieszen & Wieland, 1975). Alpine species require high light levels for both photosynthetic compensation and saturation (Bliss, 1985), according to Mooney & Billings (1961), photosynthesis of *Oxyria digyna* continued to increase at 56,000 lux for plants collected at 3,740 m, whilst photosynthesis of plants collected at 1,740 m was already saturated at 22,000 lux.

Respiration rates tend to be higher at various temperatures in arctic than in alpine populations of *Oxyria digyna* (Mooney & Billings, 1961) and higher in alpine

than in coastal populations of *Polygonum bistortoides* (Mooney, 1963). Arctic plants have high mitochondrial oxidative rates and therefore high respiration rates for both growth and maintenance processes at low temperatures (Wager, 1941; Tieszen & Wieland, 1975; Billings *et al.*, 1971). This high respiratory capacity is probably necessary to support the observed rapid growth rates and can be achieved through high concentrations of enzymes. These adaptations suggest a high nitrogen requirement per unit of leaf (Chapin & Shaver, 1985a). The high respiratory capacity of arctic species leads to a depletion of stored carbohydrates which results in increasing mortality when they are grown in a warmer environment (Chapin & Chapin, 1981). Billings & Godfrey (1968) and Chabot & Billings (1972) have shown that plants taken from high altitudes or latitudes and grown in warm temperature regimes have lower rates of respiration over the normal range of temperatures than plants from the same sites grown in low temperature regimes. Higher dark respiration rates have often been found in plants from high altitudes or latitudes than in plants from low altitudes and latitudes (Wager, 1941; Money & Billings, 1961) and are thought to be an adaptation that allows these plants to compensate for low temperatures and short seasons.

The following experiments were conducted to investigate if *Primula farinosa* and its allies from arctic and alpine communities show any differences or similarities in their rates of O_2 evolution and dark respiration along a series of photon flux densities and temperatures.

5.2 Materials and methods

5.2.1 Plant material

Plant species used were as in Ch 4 and were grown as described in Ch 1 (see 1.2.3). Leaf discs, 0.7 cm in diameter, were cut from the lamina of fully expanded non-senescing leaves, using a sharp borer. The discs were immediately placed into distilled water in a Petri dish and kept under fluorescent light with a photon flux density of 20 μmol m^{-2} s^{-1} until used.

5.2.2 Photosynthetic O_2 evolution

The simple gas-phase polarographic O_2 electrode system which was developed by Hansatech Ltd., England from the original design of Delieu & Walker (1981), was used in this study.

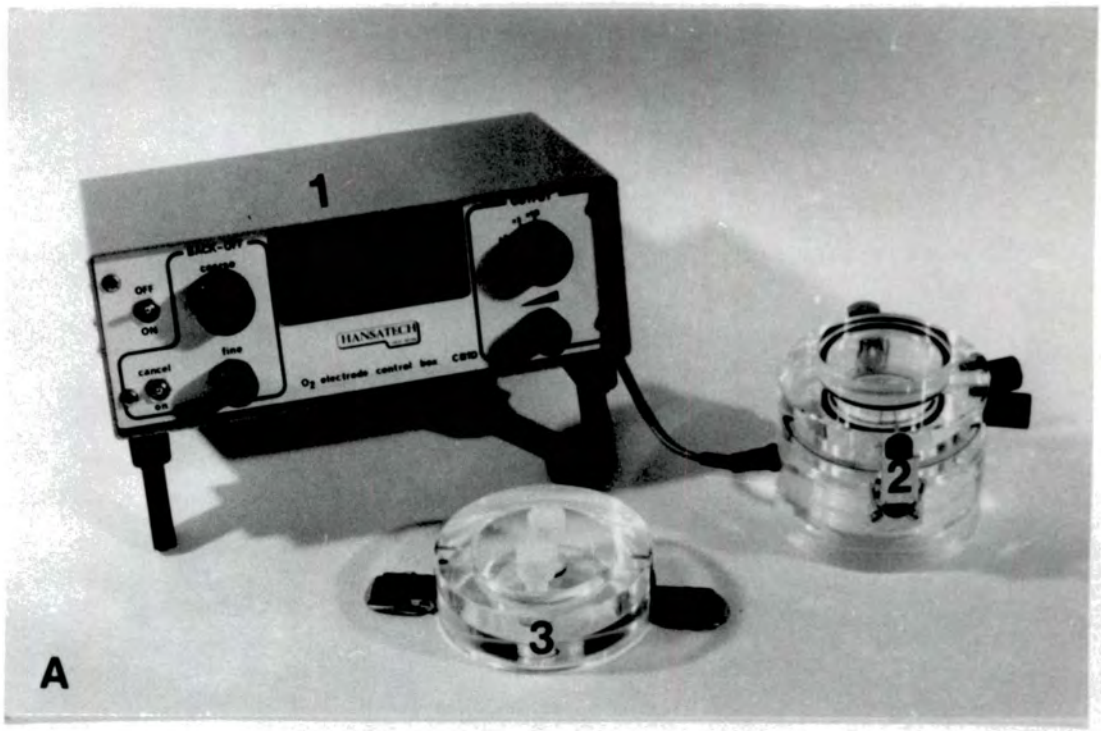
The leaf discs were supported in the cylindrical perspex chamber with a volume of about 4 cm^3. The floor of the chamber had an aperture into which the dome of the electrode protruded (Fig. 5.1). The capillary matting, which was positioned on the floor of the chamber, carried a solution of bicarbonate/carbonate buffer which served as a source of CO_2. The leaf disc was placed on a disc of damp fibre matting and to avoid contact with the high pH buffer this was placed over a perforated stainless plate. The water-jacketed roof of the chamber was flat with a compartment on the top which allowed water to be circulated for controlling temperatures.

The Clark-type O_2 electrode was used for the measurement of O_2 exchange. The amount of O_2 in the leaf disc chamber could be determined by measuring the changes of electric current generated by the electrolysis of O_2 molecules at the electrode. The O_2 electrode system was operated by supplying a negative charge on the platinum electrode and a positive charge on the silver electrode from an O_2 electrode control box (Fig. 5.1). The electrical contact between the two electrode was maintained by a solution of potassium chloride (i.e., 0.1 M KCl in 95% ethylene glycol). The electrodes were separated from the reaction medium, in which O_2 was

Figure 5.1 Instruments for measuring the rate of oxygen evolution.

A 1 O_2 electrode control box
2 Leaf disc electrode unit with a water-jacketed roof in position
3 calibration head

B Leaf disc electrode unit without water-jacketed roof (view from the top)
1 leaf disc chamber with an aperture at the middle
2 a dome of O_2 electrode disc
3 O ring
4 a plug connects with O_2 electrode control box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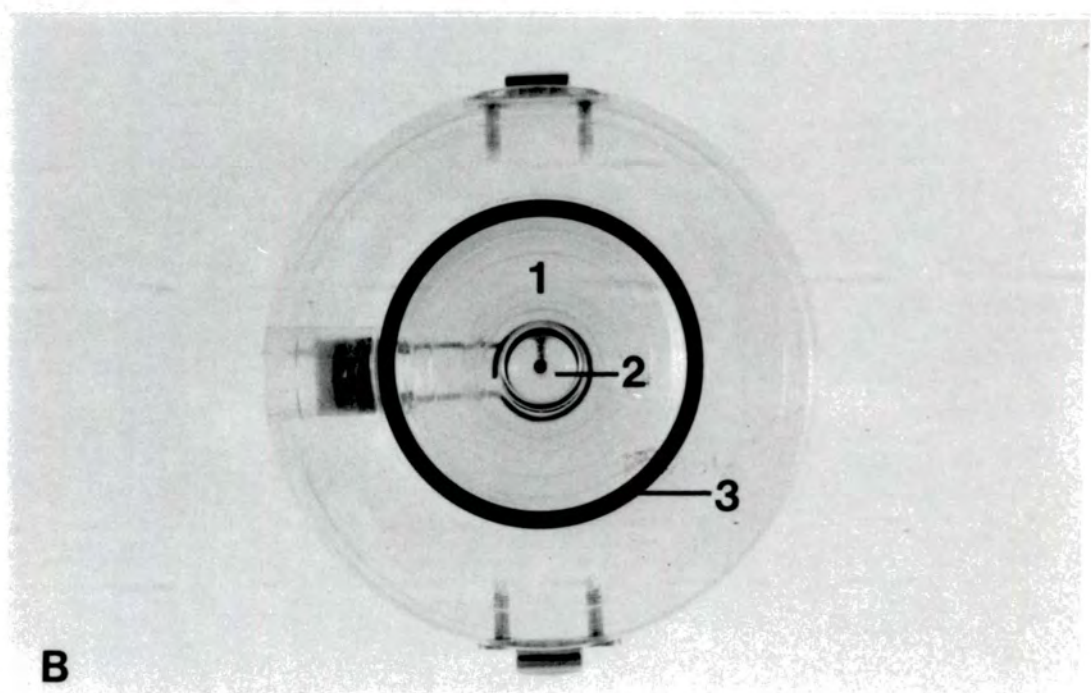
Off
On
cancel
coarse
fine
HANSATECH
O_2 electrode control box
2
3
A


1
2
3
B

to be measured, by a thin Teflon membrance placed over the dome of the electrode. The O_2 electrode control box also contained an amplifier to convert the electrode current into a voltage signal suitable for connection to a pen recorder.

5.2.3 Environmental factors maintenance

5.2.3.1 Light intensity

Light from a fan-cooled slide projector was directed at right angles on to the roof of the leaf disc chamber (Fig. 5.2). When fitted with a 500 W/240 V lamp, a maximum Photon Flux Density (PFD) of approximately 1,800 μmol m^{-2} s^{-1} could be obtained. The PFD was checked frequently between experiments. A series of PFD was obtained by using a regulated switching power supply and neutral density filters. The light output was measured with a Skye Instruments sensor and meter which was designed to measure photosynthetically active radiation (PAR).

5.2.3.2 Temperature

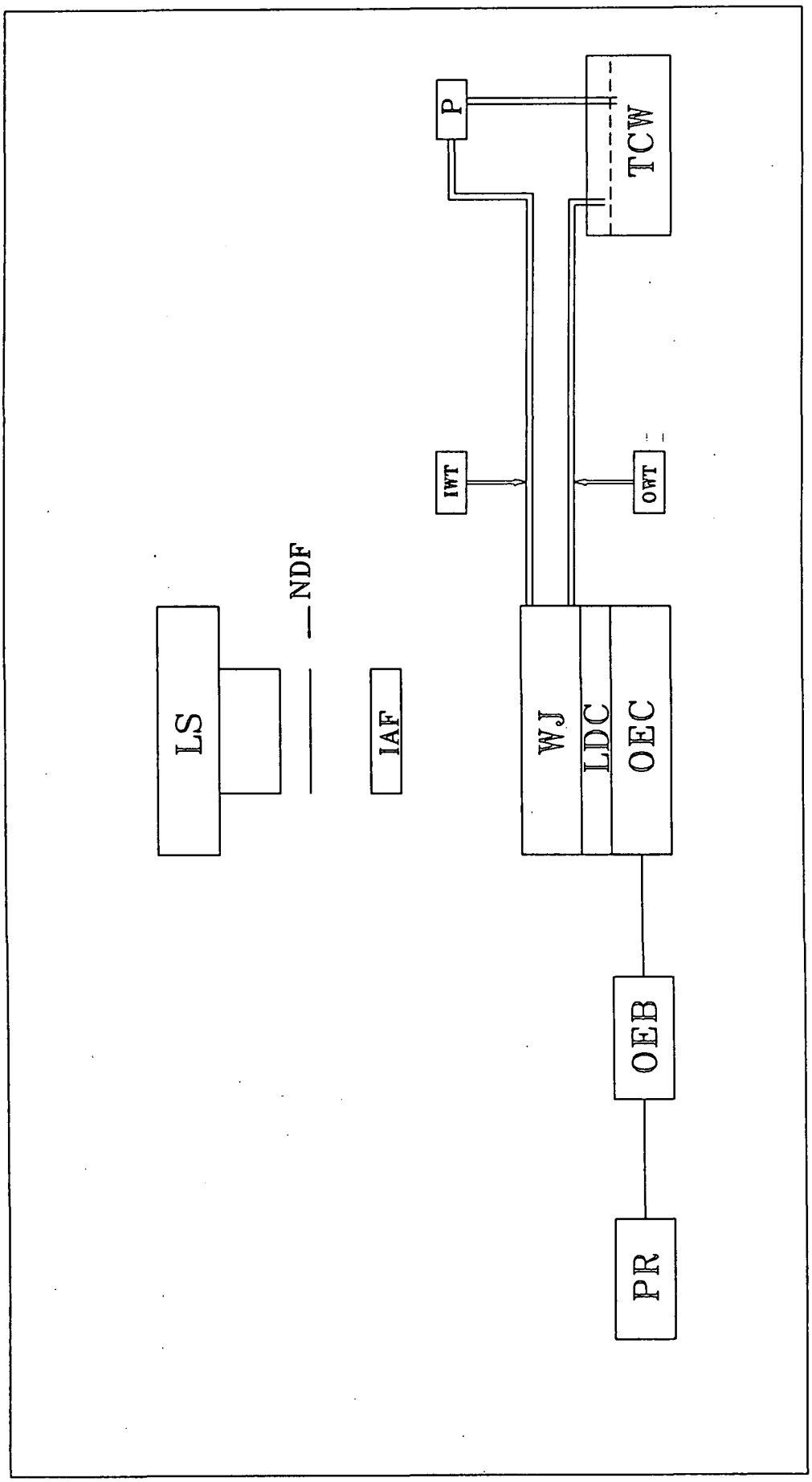
The upper surfaces of the leaf discs were pressed lightly against the surface of a water jacket and their temperatures were controlled by circulating water in and out of the water jacket from an ice or water bath at a series of temperature (5, 15, 20, 25, and 30°C). The temperature of the inlet and outlet water were measured continuously by the use of Copper-constantan thermocouples.

5.2.3.3 Carbon dioxide concentration

The rate of O_2 evolution of the leaf discs was measured at CO_2 saturation. Saturated CO_2 (5%) was provided by damping the capillary matting on the floor of the chamber with a bicarbonate/carbonate buffer (e.g. 2.5 volume 0.1M sodium carbonate, 7.5 volumes 0.1M sodium hydrogencarbonate pH 9.32) which was prepared at working temperatures immediately prior to use (Umbreit, Burris, & Stauffer, 1972; Semikhatova, Chulanovskaya, & Metzver, 1971).

Figure 5.2 Block diagram of instruments for determining the rates of O_2 evolution, and dark respiration at saturated CO_2.

LS = light source(slide projector, 500W)
NDF= neutral density filter
IAF= infrared absorbing filter
WJ = water-jacketed roof
LDC= leaf disc chamber
OEC= O_2 electrode disc chamber
PR = pen recorder
OEB= O_2 electrode control box
IWT= input water thermocouple
OWT= output water thermocouple
P = pump
TCW= temperature control water bath


P
TCW
IWT
OWT
NDF
LS
IAF
WJ
LDC
OEC
OEB
PR

5.2.4 Routine experimental procedures

Measurements of photosynthesis and dark respiration were performed at 5, 15, 20, 25, and 30°C under various PFDs ranging from 25 to 1,800 μmol m^{-2} s^{-1}. O_2 evolution was measured over a 5 min period of illumination started from low to high PFD. For any given determination the chamber was positioned so that the leaf disc received full illumination. The leaf discs were darkened for 1 min after each 5 min period of illumination. This cycle has been shown to establish a steady rate of O_2 evolution (Walker & Osmond, 1986).

After completion of a photosynthetic determination at a series of PFDs the chamber was darkened and measurements were made of the stable dark respiration rate. All results are based on determinations made on five replicates of each species and are presented as mean values.

Rates of O_2 evolution and dark respiration were expressed in term of V m^{-2} s^{-1}, rather than in absolute units of μmol O_2 m^{-2} s^{-1}, due to difficulties in the accurate calibration of the Clark type electrode when using leaf discs in a gas phase, especially over a range of temperatures (Miranda, *et al.*, 1981).

5.2.5 Chlorophyll determination

Leaf discs that had been used for O_2 evolution measurements were used for determinations of chlorophyll (chl) concentration. Leaf discs were placed into a sealed bottle containing 5 cm^3 of 80% acetone and allowed to stand in the dark for 16 h at room temperature. Absorbance(A) of chl was measured using spectrophotometer at 645 and 663 nm. Chl a, Chl b, and Chl (a+b) concentration were calculated from the formula compiled by Arnon (1949) and checked by Bruinsma (1961): –

$$Chl\ a = 12.72A663 - 2.58A645 \quad 5.1$$

$$Chl\ b = 22.87A645 - 4.67A663 \quad 5.2$$

$$Chl\ (a+b) = 8.05A663 + 20.29A645 \quad 5.3$$

5.3 Results

5.3.1 Chlorophyll concentration

Table 5.1 summarizes the averaged chl (a+b) contents on a leaf area basis for the primulas. The highest value was in *P. farinosa* (b) and the lowest was in *P. scotica*. It can be seen that there were highly significant differences ($P<0.001$) in chl contents between species (Table 5.2). Results from LSD tests showed significantly higher chl content in *P. farinosa* (b) than *P. farinosa* (c) and *P. scotica*, whilst *P. farinosa* (b), *P. frondosa*, and *P. laurentiana* did not differ significantly. There was no linear correlation ($r=-0.0$) between chl (a+b) contents and chromosome numbers of the tested species. Table 5.1 also shows the ratios of chl a/chl b with the highest value in *P. farinosa* (b) and the lowest value in *P. scotica*.

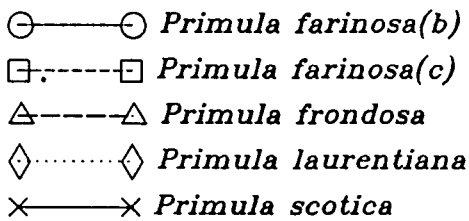
5.3.2 Rates of O_2 evolution

5.3.2.1 Effects of photon flux densities

The rates of O_2 evolution as measured in leaf discs of the primulas at CO_2 saturation and 5°C both on a leaf area and chl basis are summarized in Table 5.4 and Fig. 5.3. ANOVA performed on the data on a leaf area basis (Table 5.5) showed significant variations between species at the PFDs of 200, 600, 1,400, 1,600, and 1,800 μmol m^{-2} s^{-1}. The results from LSD tests (Table 5.6) showed significantly highest value for *P. laurentiana* at the PFDs of 200 and 600 μmol m^{-2} s^{-1}, and for *P. farinosa* (b) at the PFDs of 1,600 and 1,800. No significant difference was found between the two populations of *P. farinosa* at the PFD of 1,400 μmol m^{-2} s^{-1}. On the chl basis, the high rates of O_2 evolution were usually found in species with low chl concentrations, e.g. *P. farinosa* (c), *P. scotica*.

Fig. 5.4 and Table 5.7 summarizes the rates of O_2 evolution at 15°C. The highest rates were found in *P. laurentiana* at the PFDs of 200, 400, and 600 μmol m^{-2} s^{-1}, whilst the lowest rate was in *P. scotica*. At higher PFDs (800-1,800), the highest rates were found in *P. farinosa* (b) and the lowest rates were found

Figure 5.3 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of photon flux densities.



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

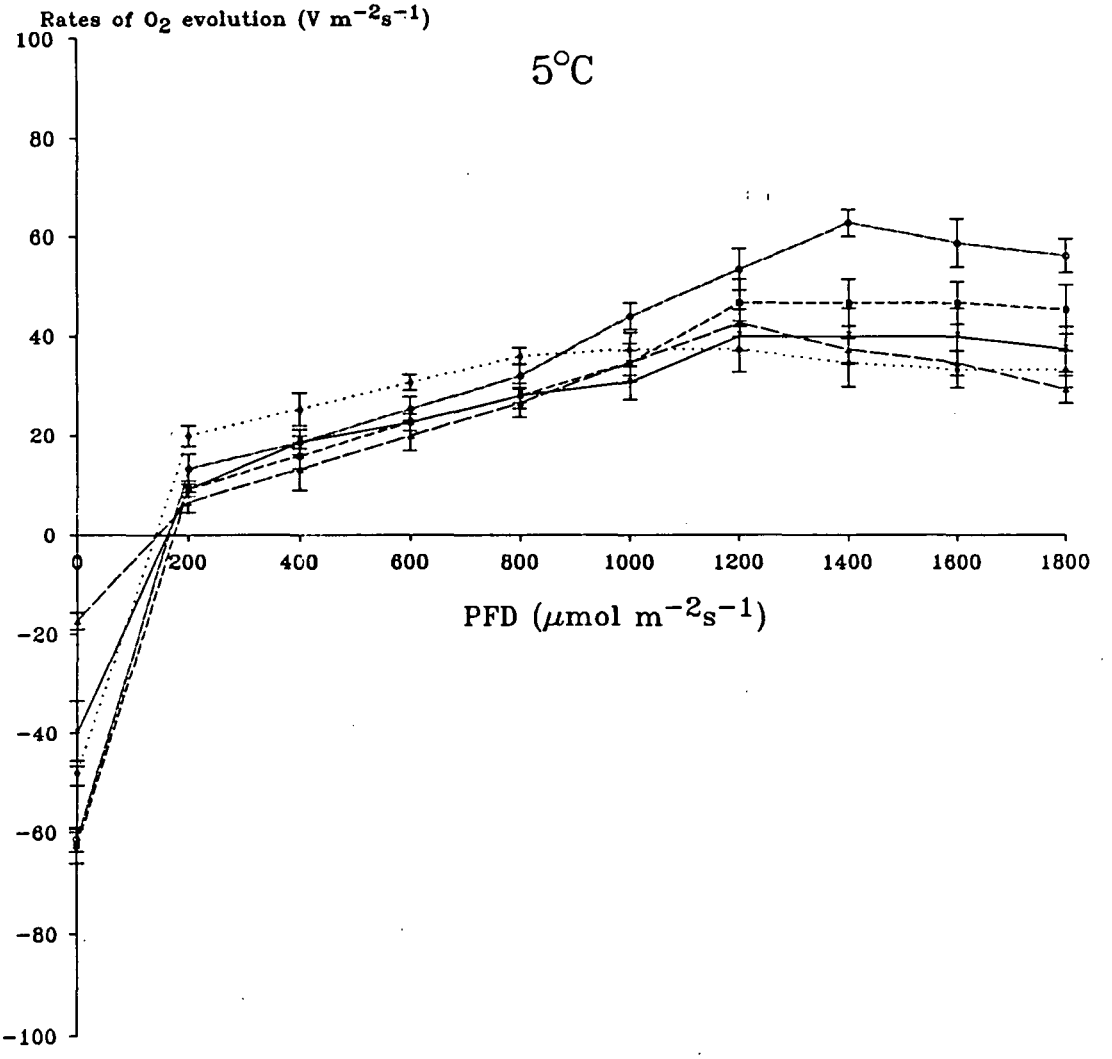

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
5°C
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
200
400
600
800
1000
1200
1400
1600
1800
PFD (μmol $m^{-2}s^{-1}$)

Table 5.1 Chl (a+b) content and chl a/chl b in *Primula* spp.

Primula spp.	Chl (a+b) (mg/sq dm) mean	SE	Chl a/Chl b mean	SE
1.*P.farinosa (b)*	2.42	0.06	2.57	0.03
2.*P.farinosa (c)*	1.64	0.09	2.45	0.03
3.*P.frondosa*	2.20	0.09	2.24	0.10
4.*P.laurentiana*	2.41	0.16	2.37	0.03
5.*P.scotica*	1.42	0.17	2.23	0.09

Table 5.2 ANOVA of chl (a+b) content in *Primula* spp.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	4	3.3835	.8459	12.8720	.0001
WITHIN GROUPS	15	.9857	.0657		
TOTAL	19	4.3693			

Table 5.3 Statistical tests of significance (LSD) of chl (a+b) in *Primula* spp.

Primula spp.	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	–	***	NS	NS	***
2.*P.farinosa (c)*		–	**	***	NS
3.*P.frondosa*			–	NS	***
4.*P.laurentiana*				–	***
5.*P.scotica*					–

Table 5.4 Rates of oxygen evolution of leaf discs of the primulas at carbon dioxide saturation, at 5°C, PFD of 200-1800 µmol/sq m /s.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	200	13.34	2.98	21.34	4.76
P.farinosa(c)		9.33	1.63	22.01	3.84
P.frondosa		6.67	2.10	11.87	3.73
P.laurentiana		20.01	2.10	32.01	3.36
P.scotica		9.33	1.63	25.37	4.43
P.farinosa(b)	400	18.67	2.49	29.87	3.98
P.farinosa(c)		16.00	2.66	37.76	6.27
P.frondosa		13.34	4.21	23.74	7.49
P.laurentiana		25.34	3.26	40.54	5.21
P.scotica		18.67	1.33	50.78	3.61
P.farinosa(b)	600	25.34	2.49	40.54	3.98
P.farinosa(c)		22.67	1.63	53.50	3.84
P.frondosa		20.01	2.98	35.61	5.30
P.laurentiana		30.68	1.63	49.08	2.60
P.scotica		22.67	1.63	61.66	4.43
P.farinosa(b)	800	32.01	2.49	51.21	3.98
P.farinosa(c)		28.01	2.49	66.10	5.87
P.frondosa		26.68	2.98	47.49	5.30
P.laurentiana		36.01	1.63	57.61	2.60
P.scotica		28.01	1.33	76.18	3.61
P.farinosa(b)	1,000	44.02	2.66	70.43	4.25
P.farinosa(c)		34.68	3.88	81.84	9.15
P.frondosa		34.68	2.49	61.73	4.43
P.laurentiana		37.35	3.40	59.76	5.44
P.scotica		30.68	3.40	83.44	9.24
P.farinosa(b)	1,200	53.36	4.21	83.37	6.73
P.farinosa(c)		46.69	4.71	110.18	11.11
P.frondosa		42.68	2.66	75.97	4.73
P.laurentiana		37.35	4.52	59.76	7.23
P.scotica		40.02	2.98	110.85	8.10

Table 5.4 Continued.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	1,400	62.69	2.66	100.30	4.25
P.farinosa(c)		46.69	4.71	110.18	11.11
P.frondosa		37.35	2.66	66.48	4.73
P.laurentiana		34.68	4.90	55.48	7.84
P.scotica		40.02	5.58	108.85	15.17
P.farinosa(b)	1,600	58.69	4.90	93.90	7.84
P.farinosa(c)		46.69	4.21	110.18	9.93
P.frondosa		34.68	2.49	61.73	4.43
P.laurentiana		33.35	3.65	53.36	5.84
P.scotica		40.02	5.58	108.85	15.17
P.farinosa(b)	1,800	56.02	3.40	89.63	5.44
P.farinosa(c)		45.35	4.90	107.02	11.56
P.frondosa		29.34	2.66	52.22	4.73
P.laurentiana		33.35	3.65	53.36	5.84
P.scotica		37.35	4.52	101.59	12.29

Table 5.5 ANOVA of rates of oxygen evolution in *Primula* spp. at 5 °C, PFD of 200–1800 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PFD 200					
BETWEEN GROUPS	4	540.9850	135.2463	5.8462	.0028
WITHIN GROUPS	20	462.6846	23.1342		
TOTAL	24	1003.6696			
PFD 400					
BETWEEN GROUPS	4	398.6205	99.6551	2.2857	.0959
WITHIN GROUPS	20	871.9824	43.5991		
TOTAL	24	1270.6030			
PFD 600					
BETWEEN GROUPS	4	327.4383	81.8596	3.5385	.0244
WITHIN GROUPS	20	462.6846	23.1342		
TOTAL	24	790.1229			
PFD 800					
BETWEEN GROUPS	4	295.4063	73.8516	2.8621	.0502
WITHIN GROUPS	20	516.0712	25.8036		
TOTAL	24	811.4775			
PFD 1000					
BETWEEN GROUPS	4	487.5983	121.8996	2.3621	.0879
WITHIN GROUPS	20	1032.1425	51.6071		
TOTAL	24	1519.7408			
PFD 1200					
BETWEEN GROUPS	4	783.0046	195.7512	2.5581	.0704
WITHIN GROUPS	20	1530.4182	76.5209		
TOTAL	24	2313.4228			
PFD 1400					
BETWEEN GROUPS	4	2516.2922	629.0730	6.8641	.0012
WITHIN GROUPS	20	1832.9427	91.6471		
TOTAL	24	4349.2349			
PFD 1600					
BETWEEN GROUPS	4	2153.2628	538.3157	5.8173	.0028
WITHIN GROUPS	20	1850.7382	92.5369		
TOTAL	24	4004.0010			
PFD 1800					
BETWEEN GROUPS	4	2249.3588	562.3397	7.3488	.0008
WITHIN GROUPS	20	1530.4182	76.5209		
TOTAL	24	3779.7769			

Table 5.6 Statistical tests of significance (LSD) of rate of oxygen evolution between species of the primulas; effects of PFD at 5 °C.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	200	–	NS	*	*	NS
2.*P.farinosa (c)*			–	NS	**	NS
3.*P.frondosa*				–	***	NS
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa (b)*	600	–	NS	NS	NS	NS
2.*P.farinosa (c)*			–	NS	*	NS
3.*P.frondosa*				–	**	NS
4.*P.laurentiana*					–	*
5.*P.scotica*						–
1.*P.farinosa (b)*	1400	–	*	***	***	**
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1600	–	NS	***	***	**
2.*P.farinosa (c)*			–	NS	*	NS
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1800	–	NS	***	***	**
2.*P.farinosa (c)*			–	**	*	NS
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	NS
5.*P.scotica*						–

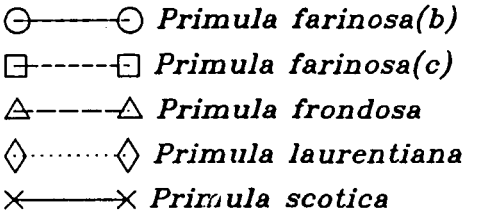
in *P. scotica.* ANOVA results (Table 5.8) revealed highly significant differences in the rates of O_2 evolution in *P. farinosa* (b) than *P. farinosa* (c) at most of PFDs, except at the PFDs of 1,600 and 1,800. The rates of O_2 evolution, calculating on the chl basis showed the highest values in *P. farinosa* (c), whilst the lowest values were found in *P. frondosa* at low PFD (200-600) but at high PFDs (1,000-1,800) the lowest values were found in *P. laurentiana* (Table 5.9).

The rates of O_2 evolution of the primulas at 20°C are presented in Fig. 5.5 and Table 5.10. The lowest rates were found in *P. scotica* at all ranges of PFD, whereas the highest rates were found in different species at different PFDs. However, ANOVA results revealed significant differences between species at all PFDs (Table 5.11). In Table 5.12, it can be seen that rates of O_2 evolution of *P. scotica* were mostly significantly lower than the other species. The two *P. farinosa* populations did not differ significantly from each other. Based on the chl contents, the highest rates were found in *P. farinosa* (c), while the lowest rates were found in different species at different PFDs.

At 25°C, the highest rates of O_2 evolution were mostly found in *P. scotica* calculated both on leaf area and chl content bases, whilst the lowest rates were found in different species at different PFDs (Fig. 5.6 and Table 5.13). ANOVA results also revealed non-significant differences between species at the PFDs of 25 to 600 μmol m^{-2} s^{-1}, but at PFDs of 800-1,200 μmol m^{-2} s^{-1} significant differences in the rates were found (Table 5.14). In Table 5.15, it can be seen that significant differences between each pair of species were found mostly with *P. scotica.* There were no significant differences between the two populations of *P. farinosa* at all PFDs.

The rates of O_2 evolution measured at 30°C (Fig. 5.7 and Table 5.16) were similar to the rates at 25°C in many respects. For example, the highest rates of O_2 evolution were mostly found in *P. scotica* on both area and chl content bases. ANOVA results (Table 5.17) also revealed non-significant differences between species

Figure 5.4 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of photon flux densities.



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

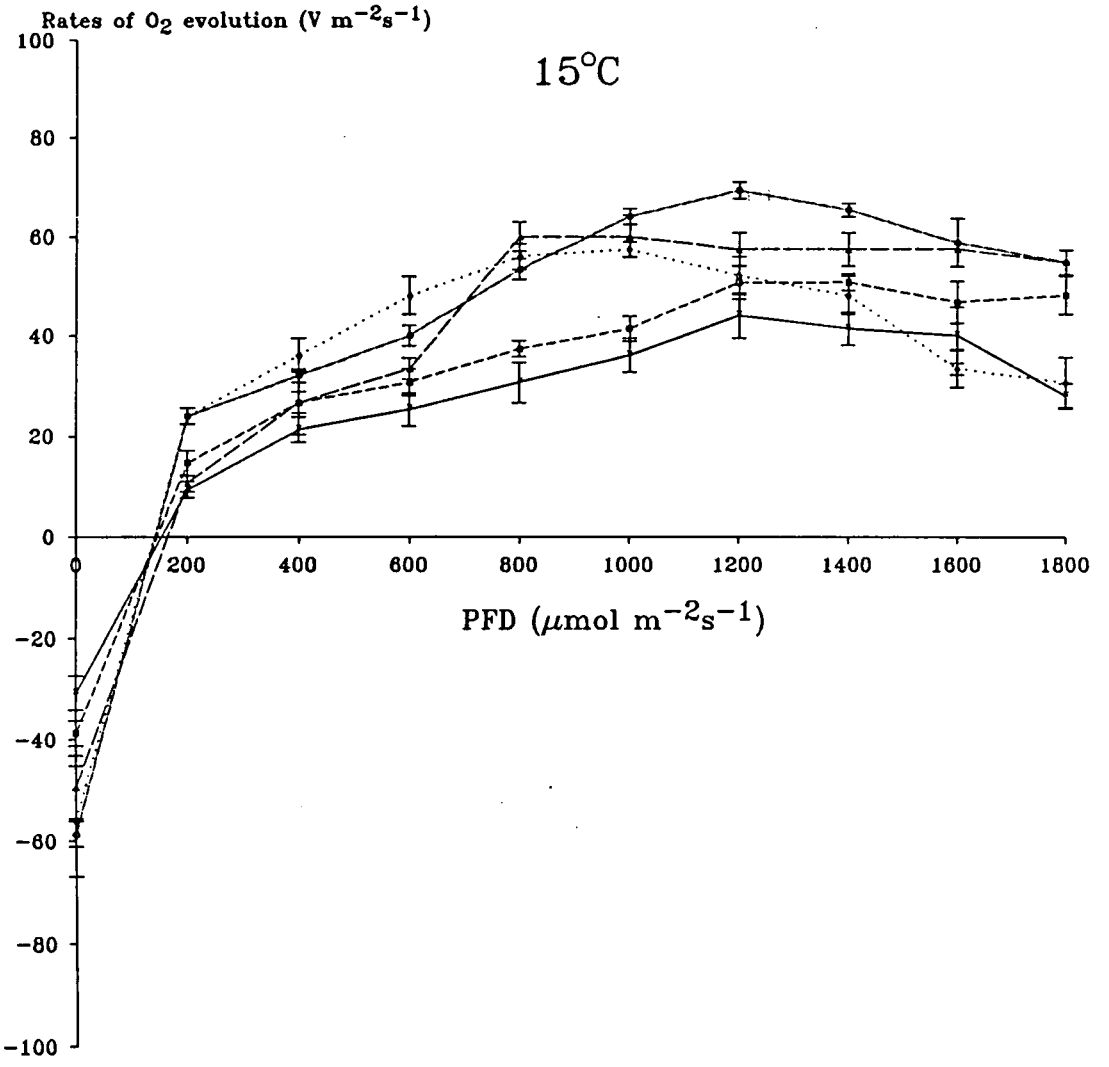
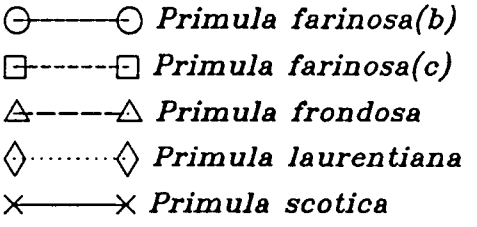

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
15°C
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
200
400
600
800
1000
1200
1400
1600
1800
PFD (μmol $m^{-2}s^{-1}$)

Figure 5.5 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of photon flux densities.



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

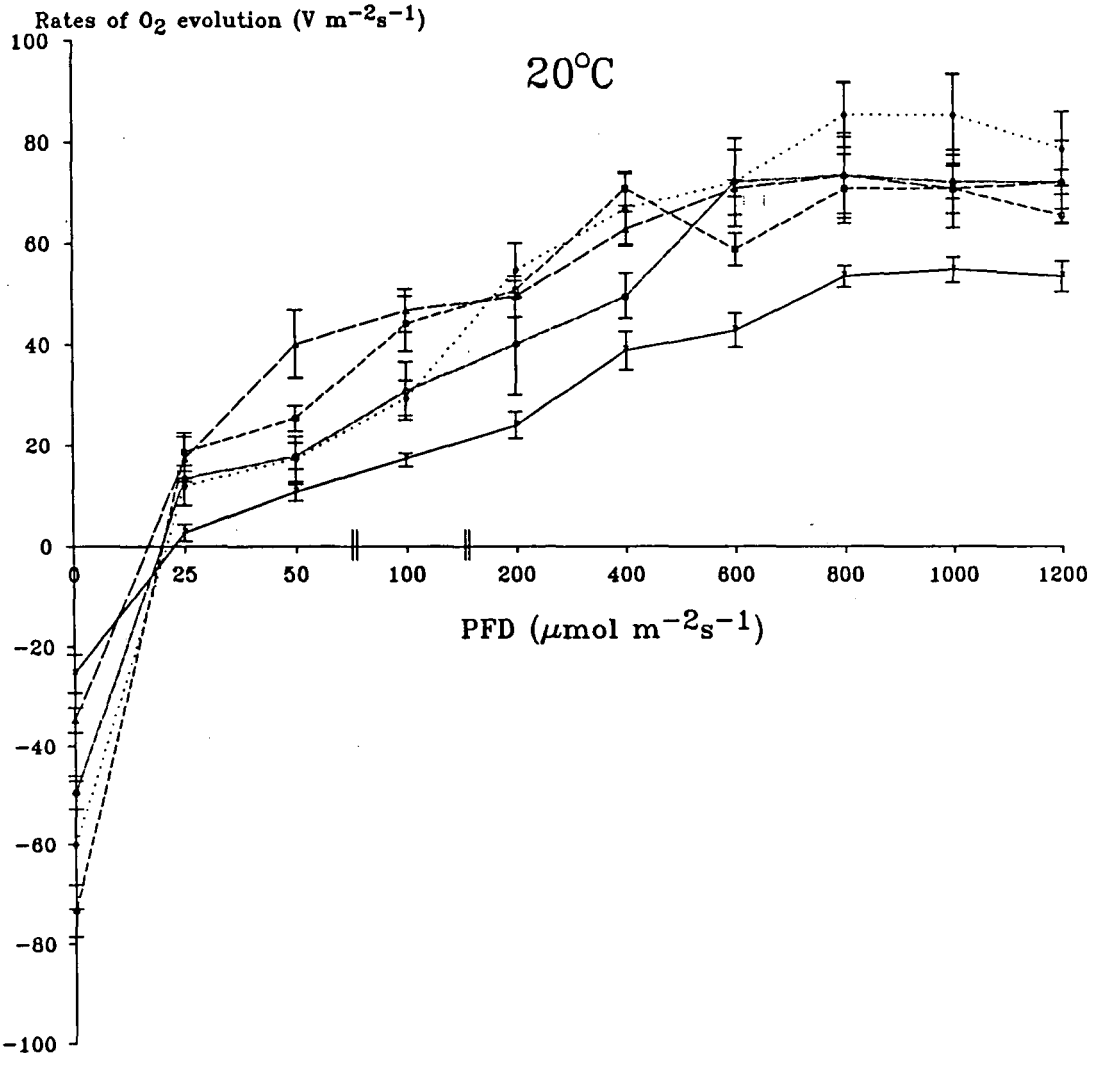

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
20°C
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
25
50
100
200
400
600
800
1000
1200
PFD (μmol $m^{-2}s^{-1}$)

Table 5.7 Rates of oxygen evolution of leaf discs of the primulas at carbon dioxide saturation, at 15 °C, PFD of 200–1800 µmol /sq m/s.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	200	24.01	1.63	38.41	2.60
P.farinosa(c)		14.67	2.49	34.62	5.87
P.frondosa		10.67	1.63	18.99	2.90
P.laurentiana		24.01	1.63	38.41	2.60
P.scotica		9.33	1.63	25.37	4.43
P.farinosa(b)	400	32.01	1.33	51.21	2.20
P.farinosa(c)		26.68	6.32	62.96	14.91
P.frondosa		26.68	2.10	47.49	3.73
P.laurentiana		36.01	3.40	57.61	5.44
P.scotica		21.34	2.49	58.04	6.79
P.farinosa(b)	600	40.02	2.10	64.03	3.36
P.farinosa(c)		30.68	2.66	72.40	6.27
P.frondosa		33.35	2.10	59.36	3.73
P.laurentiana		48.02	3.88	76.83	6.20
P.scotica		25.34	3.26	68.92	8.86
P.farinosa(b)	800	53.36	2.10	85.37	3.36
P.farinosa(c)		37.35	1.63	88.14	3.84
P.frondosa		60.03	2.98	106.85	5.30
P.laurentiana		56.02	2.66	89.63	4.25
P.scotica		30.68	4.00	83.44	10.88
P.farinosa(b)	1,000	64.03	1.63	102.44	2.60
P.farinosa(c)		41.35	2.49	97.58	5.87
P.frondosa		60.03	4.21	106.85	7.49
P.laurentiana		57.36	1.63	91.77	2.60
P.scotica		36.01	3.40	97.94	9.24
P.farinosa(b)	1,200	69.36	1.63	110.97	2.60
P.farinosa(c)		50.69	3.40	119.62	8.02
P.frondosa		57.36	3.40	102.10	6.05
P.laurentiana		52.02	3.88	83.23	6.20
P.scotica		44.02	4.52	119.73	12.29

Table 5.7 Continued.

Primula spp.	PFD (μmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	1,400	65.36	1.33	104.57	2.12
P.farinosa(c)		50.69	1.63	119.62	3.84
P.frondosa		57.36	3.40	102.10	6.05
P.laurentiana		48.02	3.88	76.83	6.20
P.scotica		41.35	3.26	112.47	8.86
P.farinosa(b)	1,600	58.69	4.90	93.90	7.84
P.farinosa(c)		46.69	4.21	110.18	9.93
P.frondosa		57.36	3.40	102.10	6.05
P.laurentiana		33.35	3.65	53.36	5.84
P.scotica		40.02	5.58	108.85	15.17
P.farinosa(b)	1,800	54.69	2.49	87.50	3.98
P.farinosa(c)		48.02	3.88	113.32	9.15
P.frondosa		54.69	2.49	97.34	4.43
P.laurentiana		30.68	4.99	49.08	7.98
P.scotica		28.01	2.49	76.18	6.77

Table 5.8 ANOVA of rates of oxygen evolution in *Primula* spp. at 15 °C, PFD of 200–1800 µmol/sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PFD 200					
BETWEEN GROUPS	4	1007.2287	251.8072	14.8947	.0000
WITHIN GROUPS	20	338.1156	16.9058		
TOTAL	24	1345.3443			
PFD 400					
BETWEEN GROUPS	4	633.5219	158.3805	2.4722	.0775
WITHIN GROUPS	20	1281.2803	64.0640		
TOTAL	24	1914.8023			
PFD 600					
BETWEEN GROUPS	4	1541.0955	385.2739	9.2128	.0002
WITHIN GROUPS	20	836.3913	41.8196		
TOTAL	24	2377.4868			
PFD 800					
BETWEEN GROUPS	4	3249.4693	812.3673	20.7500	.0000
WITHIN GROUPS	20	783.0046	39.1502		
TOTAL	24	4032.4739			
PFD 1000					
BETWEEN GROUPS	4	3032.3634	758.0909	18.5217	.0000
WITHIN GROUPS	20	818.5958	40.9298		
TOTAL	24	3850.9592			
PFD 1200					
BETWEEN GROUPS	4	1797.3516	449.3379	7.3188	.0008
WITHIN GROUPS	20	1227.8936	61.3947		
TOTAL	24	3025.2452			
PFD 1400					
BETWEEN GROUPS	4	1683.4600	420.8650	10.0638	.0001
WITHIN GROUPS	20	836.3913	41.8196		
TOTAL	24	2519.8513			
PFD 1600					
BETWEEN GROUPS	4	3014.5679	753.6420	8.2233	.0004
WITHIN GROUPS	20	1832.9427	91.6471		
TOTAL	24	4847.5105			
PFD 1800					
BETWEEN GROUPS	4	3374.0382	843.5095	14.3636	.0000
WITHIN GROUPS	20	1174.5070	58.7253		
TOTAL	24	4548.5451			

Table 5.9 Statistical tests of significance (LSD) of rates of oxygen evolution between species of the primulas; effect of PFD at 15°C.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	200	–	**	***	NS	***
2.*P.farinosa (c)*			–	NS	**	NS
3.*P.frondosa*				–	***	NS
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	600	–	*	NS	NS	**
2.*P.farinosa (c)*			–	NS	***	NS
3.*P.frondosa*				–	**	NS
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	800	–	***	NS	NS	***
2.*P.farinosa (c)*			–	***	***	NS
3.*P.frondosa*				–	NS	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	1000	–	***	NS	NS	***
2.*P.farinosa (c)*			–	***	***	NS
3.*P.frondosa*				–	NS	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	1200	–	**	*	**	***
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	*
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1400	–	**	NS	***	***
2.*P.farinosa (c)*			–	NS	NS	*
3.*P.frondosa*				–	*	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–

Table 5.9 Continued.

Primula spp.	PFD	Significance levels				
		1.	2.	3.	4.	5.
1.*P.farinosa (b)*	1600	–	NS	NS	***	**
2.*P.farinosa (c)*			–	NS	**	*
3.*P.frondosa*				–	***	**
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1800	–	NS	NS	***	***
2.*P.farinosa (c)*			–	NS	**	***
3.*P.frondosa*				–	***	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–

Table 5.10 Rates of oxygen evolution of leaf discs of the primulas at carbon dioxide saturation, at 20°C, PFD of 25-1200 µmol /sq m/s.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	25	13.34	0.00	21.34	0.00
P.farinosa(c)		18.67	3.88	44.06	9.15
P.frondosa		17.34	4.52	30.86	8.04
P.laurentiana		12.00	3.98	19.20	3.98
P.scotica		2.66	1.63	7.23	4.43
P.farinosa(b)	50	17.84	2.66	27.74	4.25
P.farinosa(c)		25.34	2.49	59.80	5.87
P.frondosa		40.02	6.67	71.23	11.87
P.laurentiana		17.34	4.52	27.74	7.23
P.scotica		10.67	1.63	29.02	4.43
P.farinosa(b)	100	30.68	5.81	49.08	9.29
P.farinosa(c)		44.02	5.41	103.88	12.76
P.frondosa		46.69	4.21	83.10	7.49
P.laurentiana		29.34	3.40	46.94	5.44
P.scotica		17.34	1.63	47.16	4.43
P.farinosa(b)	200	40.02	10.11	64.03	16.17
P.farinosa(c)		50.69	1.63	119.62	3.84
P.frondosa		49.35	4.00	87.84	7.12
P.laurentiana		54.69	5.33	87.50	8.52
P.scotica		24.01	2.66	65.30	7.23
P.farinosa(b)	400	49.35	4.52	78.96	7.23
P.farinosa(c)		70.70	3.40	166.85	8.02
P.frondosa		62.69	3.40	111.58	6.05
P.laurentiana		66.70	6.99	106.72	11.18
P.scotica		38.68	3.88	105.20	10.55
P.farinosa(b)	600	72.03	8.79	115.24	14.06
P.farinosa(c)		58.69	3.26	138.50	7.69
P.frondosa		70.70	1.63	125.84	2.90
P.laurentiana		72.03	6.46	115.54	10.33
P.scotica		42.68	3.40	116.08	9.24

Table 5.10 Continued.

Primula spp.	PFD (μmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	800	73.37	7.60	117.39	12.16
P.farinosa(c)		70.70	6.86	166.85	16.18
P.frondosa		73.37	8.43	130.59	15.00
P.laurentiana		85.37	6.46	136.59	10.33
P.scotica		53.36	2.10	145.13	5.71
P.farinosa(b)	1,000	72.03	3.26	115.24	5.21
P.farinosa(c)		70.70	7.77	116.85	18.33
P.frondosa		70.70	4.99	125.64	8.88
P.laurentiana		85.37	8.00	136.59	12.80
P.scotica		54.69	2.49	148.75	6.77
P.farinosa(b)	1,200	72.03	2.49	115.24	3.98
P.farinosa(c)		65.36	1.33	154.24	3.13
P.frondosa		72.03	8.27	128.21	14.72
P.laurentiana		78.70	7.42	125.92	11.87
P.scotica		53.36	2.98	145.13	8.10

Table 5.11 ANOVA of rates of oxygen evolution in *Primula* spp. at 20°C, PFD of 25–1200 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PFD 25					
BETWEEN GROUPS	4	793.6820	198.4205	4.4600	.0097
WITHIN GROUPS	20	889.7780	44.4889		
TOTAL	24	1683.4600			
PFD 50					
BETWEEN GROUPS	4	2537.6469	634.4117	7.8352	.0006
WITHIN GROUPS	20	1619.3960	80.9698		
TOTAL	24	4157.0428			
PFD 100					
BETWEEN GROUPS	4	2854.4078	713.6020	7.4953	.0007
WITHIN GROUPS	20	1904.1249	95.2062		
TOTAL	24	4758.5327			
PFD 200					
BETWEEN GROUPS	4	3014.5679	753.6420	4.8125	.0070
WITHIN GROUPS	20	3132.0186	156.6009		
TOTAL	24	6146.5864			
PFD 400					
BETWEEN GROUPS	4	3530.6391	882.6598	8.1983	.0004
WITHIN GROUPS	20	2153.2628	107.6631		
TOTAL	24	5683.9019			
PFD 600					
BETWEEN GROUPS	4	3267.2648	816.8162	5.6667	.0032
WITHIN GROUPS	20	2882.8807	144.1440		
TOTAL	24	6150.1455			
PFD 800					
BETWEEN GROUPS	4	2644.4202	661.1051	2.9720	.0445
WITHIN GROUPS	20	4448.8900	222.4445		
TOTAL	24	7093.3102			
PFD 1000					
BETWEEN GROUPS	4	2366.8095	591.7024	3.5561	.0239
WITHIN GROUPS	20	3327.7697	166.3885		
TOTAL	24	5694.5792			
PFD 1200					
BETWEEN GROUPS	4	1840.0609	460.0152	3.2722	.0323
WITHIN GROUPS	20	2811.6985	140.5849		
TOTAL	24	4651.7594			

Table 5.12 Statistical tests of significance (LSD) of rates of oxygen evolution between species of the primulas; effect of PFD at 20°C.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	25	–	NS	NS	NS	*
2.*P.farinosa (c)*			–	NS	NS	**
3.*P.frondosa*				–	NS	**
4.*P.laurentiana*					–	*
5.*P.scotica*						–
1.*P.farinosa (b)*	50	–	NS	***	NS	NS
2.*P.farinosa (c)*			–	*	NS	*
3.*P.frondosa*				–	***	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	100	–	*	*	NS	*
2.*P.farinosa (c)*			–	NS	*	***
3.*P.frondosa*				–	*	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	200	–	NS	NS	NS	NS
2.*P.farinosa (c)*			–	NS	NS	**
3.*P.frondosa*				–	NS	**
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	400	–	**	NS	*	NS
2.*P.farinosa (c)*			–	NS	NS	***
3.*P.frondosa*				–	NS	**
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	600	–	NS	NS	NS	***
2.*P.farinosa (c)*			–	NS	NS	*
3.*P.frondosa*				–	NS	**
4.*P.laurentiana*					–	***
5.*P.scotica*						–

Table 5.12 Continued.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	800	–	NS	NS	NS	*
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	*
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa (b)*	1000	–	NS	NS	NS	*
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa (b)*	1200	–	NS	NS	NS	*
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	*
4.*P.laurentiana*					–	**
5.*P.scotica*						–

at all PFDs except at the PFDs of 800 and 1,200 μmol m^{-2} s^{-1}. The rates of O_2 evolution in *P. farinosa* (c) were lower than for *P. farinosa* (b) but significant differences were found only at the PFD of 1,200 μmol m^{-2} s^{-1} (Table 5.18).

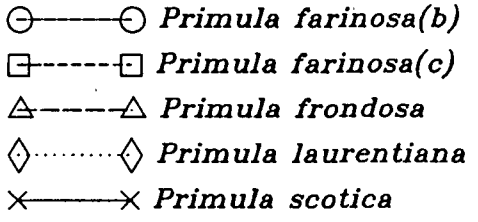
The light compensation points of the primulas were read approximately from Fig. 5.3 to 5.7. At 5 and 15°C, the light compensation points of each species were close together and occurred at the PFDs between 100 and 200 μmol m^{-2} s^{-1}. At 20°C, the highest value was in *P. scotica,* The lowest was in *P. frondosa,* whilst the values for *P. laurentiana, P. farinosa* (b), and *P. farinosa* (c) were nearly the same. At 25°C, the highest value was in *P. laurentiana* which was also close to *P. farinosa* (c); *P. farinosa* (b), and *P. frondosa* were intermediate; whilst the lowest value was found in *P. scotica.* At 30°C, the highest value was in *P. laurentiana, P. scotica* was the second which was also close to *P. farinosa* (b), and the lowest was in *P. frondosa.*

ANOVA was used to compare the rates of O_2 evolution as a function of the PFD at 20°C (Table 5.19). It can be concluded that there were highly significant differences ($P<0.0001$) in the rates of O_2 evolution between working PFDs for all species. On the basis of LSD tests (Table 5.20), light saturation points for photosynthesis of *P. farinosa* (b), *P. farinosa* (c), *P. frondosa, P. laurentiana,* and *P. scotica* were at the PFDs of 600, 400, 400, 600, and 800 μmol m^{-2} s^{-1} respectively.

5.3.2.2 Rates of dark respiration

Table 5.21 and Fig. 5.3 to Fig. 5.7 present the results of the rates of dark respiration of the primulas at 5-30°C. It was found that there were significant differences in the rates of dark respiration between the tested species at all working temperatures (Table 5.22). Comparisons of the rates of dark respiration between species, using LSD tests, are presented in Table 5.23. At 5°C, *P. farinosa* (c) had the highest rates of dark respiration, whilst the lowest rates was found in *P. frondosa.* At this temperature, rate of dark respiration in *P. farinosa* from the two populations were not significantly different. In contrast, at 15°C there were significant differences

Figure 5.6 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of photon flux densities.



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

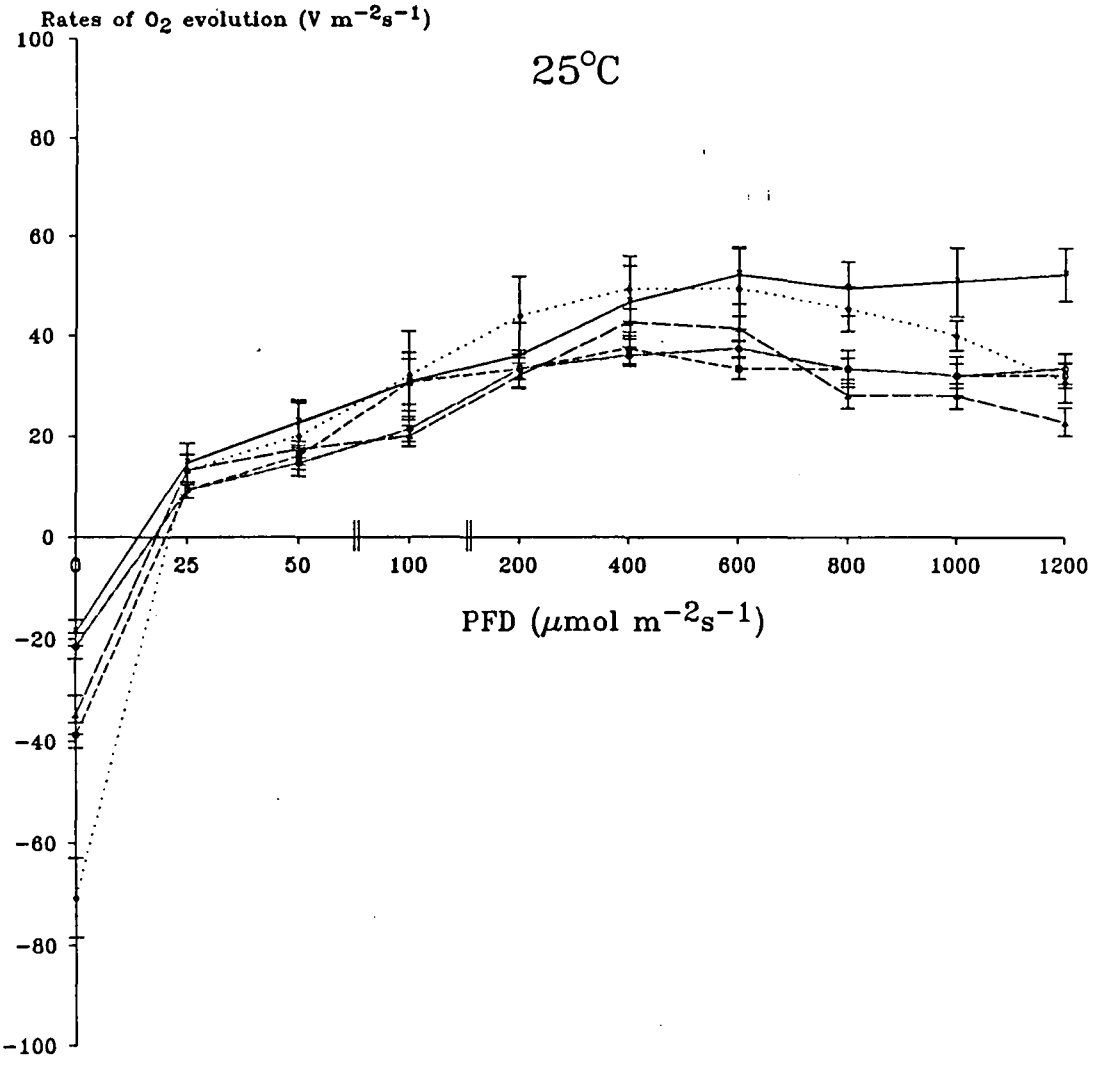
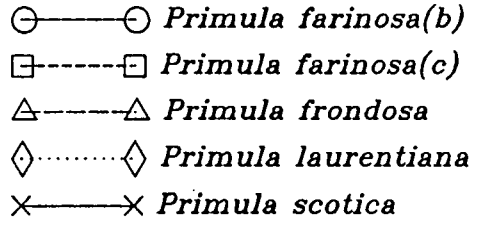

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
25°C
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
25
50
100
200
400
600
800
1000
1200
PFD (μmol $m^{-2}s^{-1}$)

Figure 5.7 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of photon flux densities.



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

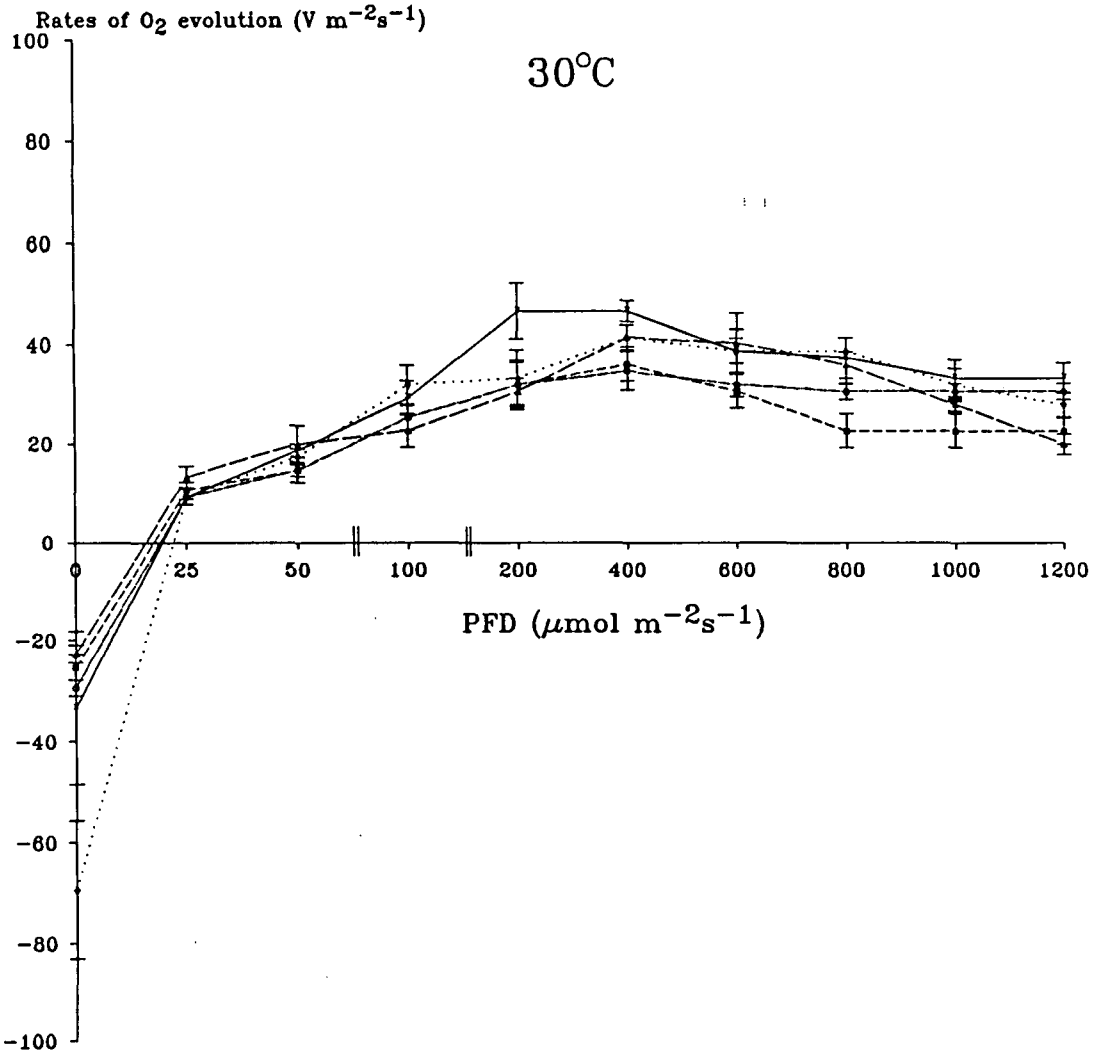

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
30°C
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
25
50
100
200
400
600
800
1000
1200
PFD (μmol $m^{-2}s^{-1}$)

Table 5.13 Rates of oxygen evolution of leaf discs of the primulas at carbon dioxide saturation, at 25 °C, PAR of 25–1200 µmol /sq m/s.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	25	9.33	1.63	14.92	2.60
P.farinosa(c)		9.33	1.63	22.01	3.84
P.frondosa		13.34	2.98	23.74	5.30
P.laurentiana		13.34	2.98	21.34	4.76
P.scotica		14.67	3.88	39.90	10.55
P.farinosa(b)	50	14.67	2.49	23.47	3.98
P.farinosa(c)		16.00	1.63	37.76	3.84
P.frondosa		17.34	1.63	30.86	2.90
P.laurentiana		20.01	6.67	32.01	10.67
P.scotica		22.67	4.52	61.66	12.29
P.farinosa(b)	100	21.34	2.49	34.14	3.98
P.farinosa(c)		30.68	4.52	72.40	10.66
P.frondosa		20.01	2.10	35.61	3.73
P.laurentiana		32.01	8.79	51.21	14.06
P.scotica		30.68	5.81	83.44	15.80
P.farinosa(b)	200	33.35	2.10	53.36	3.36
P.farinosa(c)		33.35	3.65	78.70	8.61
P.frondosa		32.01	2.49	56.97	4.43
P.laurentiana		44.02	7.77	70.43	12.43
P.scotica		36.01	6.53	97.94	17.76
P.farinosa(b)	400	36.01	1.63	57.61	2.60
P.farinosa(c)		37.35	3.40	88.14	8.02
P.frondosa		42.68	2.66	75.97	4.73
P.laurentiana		49.35	6.53	78.96	10.44
P.scotica		46.69	7.30	126.99	19.85
P.farinosa(b)	600	37.35	1.63	59.76	2.60
P.farinosa(c)		33.35	2.10	78.70	4.95
P.frondosa		41.35	2.49	73.60	4.43
P.laurentiana		49.35	8.05	78.96	12.88
P.scotica		52.02	5.73	141.49	15.58

Table 5.13 Continued.

Primula spp.	PFD (μmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	800	33.35	2.10	53.36	3.36
P.farinosa(c)		33.35	3.65	78.70	8.61
P.frondosa		28.01	2.49	49.85	4.43
P.laurentiana		45.35	4.42	72.56	7.07
P.scotica		49.35	5.41	134.23	14.71
P.farinosa(b)	1,000	32.01	2.49	51.21	3.98
P.farinosa(c)		32.01	3.88	75.54	9.15
P.frondosa		28.01	2.49	49.85	4.43
P.laurentiana		40.02	2.98	64.03	4.76
P.scotica		50.69	6.86	137.87	18.65
P.farinosa(b)	1,200	33.35	2.98	53.36	4.76
P.farinosa(c)		32.01	2.49	75.54	5.87
P.frondosa		22.67	2.66	40.35	4.73
P.laurentiana		30.68	4.00	49.08	6.40
P.scotica		52.02	5.33	141.49	14.49

Table 5.14 ANOVA of rates of oxygen evolution in *Primula* spp. at 25°C, PFD of 25–1200 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PFD 25					
BETWEEN GROUPS	4	124.5689	31.1422	.8140	.5311
WITHIN GROUPS	20	765.2091	38.2605		
TOTAL	24	889.7780			
PFD 50					
BETWEEN GROUPS	4	206.4285	51.6071	.6744	.6175
WITHIN GROUPS	20	1530.4182	76.5209		
TOTAL	24	1736.8467			
PFD 100					
BETWEEN GROUPS	4	665.5539	166.3885	1.1688	.3543
WITHIN GROUPS	20	2847.2896	142.3645		
TOTAL	24	3512.8435			
PFD 200					
BETWEEN GROUPS	4	469.8028	117.4507	.9231	.4701
WITHIN GROUPS	20	2544.7651	127.2383		
TOTAL	24	3014.5679			
PFD 400					
BETWEEN GROUPS	4	665.5539	166.3885	1.4167	.2647
WITHIN GROUPS	20	2349.0139	117.4507		
TOTAL	24	3014.5679			
PFD 600					
BETWEEN GROUPS	4	1245.6892	311.4223	2.8000	.0538
WITHIN GROUPS	20	2224.4450	111.2222		
TOTAL	24	3470.1342			
PFD 800					
BETWEEN GROUPS	4	1630.0733	407.5183	5.5854	.0035
WITHIN GROUPS	20	1459.2359	72.9618		
TOTAL	24	3089.3092			
PFD 1000					
BETWEEN GROUPS	4	1630.0733	407.5183	4.8723	.0066
WITHIN GROUPS	20	1672.7826	83.6391		
TOTAL	24	3302.8559			
PFD 1200					
BETWEEN GROUPS	4	2341.8957	585.4739	8.7733	.0003
WITHIN GROUPS	20	1334.6670	66.7333		
TOTAL	24	3676.5627			

Table 5.15 Statistical tests of significance (LSD) of rates of oxygen evolution between species of the primulas; effect of PFD at 25°C.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	800	–	NS	NS	*	**
2.*P.farinosa (c)*			–	NS	*	**
3.*P.frondosa*				–	**	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1000	–	NS	NS	NS	**
2.*P.farinosa (c)*			–	NS	NS	**
3.*P.frondosa*				–	NS	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1200	–	NS	NS	NS	**
2.*P.farinosa (c)*			–	NS	NS	***
3.*P.frondosa*				–	NS	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–

Table 5.16 Rates of oxygen evolution of leaf discs of the primulas at carbon dioxide saturation, at 30 °C, PFD of 25-1200 µmol /sq m/s.

Primula spp.	PFD (µmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	25	9.33	1.63	14.92	2.60
P.farinosa(c)		10.67	1.63	25.18	3.84
P.frondosa		13.34	2.10	23.74	3.73
P.laurentiana		9.33	1.63	14.92	2.60
P.scotica		9.33	1.63	25.37	4.43
P.farinosa(b)	50	14.67	1.33	23.47	2.12
P.farinosa(c)		14.67	2.49	34.62	5.87
P.frondosa		20.01	3.65	35.61	6.49
P.laurentiana		17.34	1.63	27.74	2.60
P.scotica		18.67	1.33	50.78	3.61
P.farinosa(b)	100	25.34	2.49	40.54	3.98
P.farinosa(c)		25.34	2.49	59.80	5.87
P.frondosa		22.67	3.40	40.35	6.05
P.laurentiana		32.01	3.88	51.21	6.20
P.scotica		29.34	3.40	79.80	9.24
P.farinosa(b)	200	32.01	4.42	51.21	7.07
P.farinosa(c)		32.01	4.90	75.54	11.56
P.frondosa		30.68	2.66	54.61	4.73
P.laurentiana		33.35	5.58	53.36	8.92
P.scotica		46.69	5.58	126.99	15.17
P.farinosa(b)	400	34.68	3.88	55.48	6.20
P.farinosa(c)		36.01	3.40	84.98	8.02
P.frondosa		41.35	2.49	73.60	4.43
P.laurentiana		41.35	2.49	66.16	3.98
P.scotica		46.69	2.10	126.99	5.71
P.farinosa(b)	600	32.01	2.49	51.21	3.98
P.farinosa(c)		30.68	3.40	72.40	8.02
P.frondosa		40.32	5.96	71.76	10.60
P.laurentiana		38.68	2.49	61.88	3.98
P.scotica		38.68	4.42	105.20	12.02

Table 5.16 Continued.

Primula spp.	PFD (μmol photon /sq m/s)	V/sq m/s		V/mgChl/s	
		mean	SE	mean	SE
P.farinosa(b)	800	30.68	1.63	49.08	2.60
P.farinosa(c)		22.67	3.40	53.50	8.02
P.frondosa		36.01	2.66	64.09	4.73
P.laurentiana		34.68	2.49	55.48	3.98
P.scotica		37.35	4.00	101.59	10.88
P.farinosa(b)	1,000	30.68	1.63	49.08	2.60
P.farinosa(c)		22.67	3.40	53.50	8.02
P.frondosa		28.01	1.33	49.85	2.36
P.laurentiana		32.01	3.26	51.21	5.21
P.scotica		33.35	3.65	90.71	9.92
P.farinosa(b)	1,200	30.68	1.63	49.08	2.60
P.farinosa(c)		22.67	2.66	53.50	6.27
P.frondosa		20.01	2.10	35.61	3.73
P.laurentiana		28.01	2.49	44.81	3.98
P.scotica		33.35	2.98	90.71	8.10

Table 5.17 ANOVA of rates of oxygen evolution in *Primula* spp. at 30 °C, PFD of 25-1200 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PFD 25					
BETWEEN GROUPS	4	60.5049	15.1262	1.0000	.4307
WITHIN GROUPS	20	302.5245	15.1262		
TOTAL	24	363.0294			
PFD 50					
BETWEEN GROUPS	4	113.8916	28.4729	1.1034	.3822
WITHIN GROUPS	20	516.0712	25.8036		
TOTAL	24	629.9628			
PFD 100					
BETWEEN GROUPS	4	274.0516	68.5129	1.3509	.2861
WITHIN GROUPS	20	1014.3469	50.7173		
TOTAL	24	1288.3985			
PFD 200					
BETWEEN GROUPS	4	879.1007	219.7752	1.9449	.1422
WITHIN GROUPS	20	2260.0361	113.0018		
TOTAL	24	3139.1368			
PFD 400					
BETWEEN GROUPS	4	462.6846	115.6711	2.6531	.0633
WITHIN GROUPS	20	871.9824	43.5991		
TOTAL	24	1334.6670			
PFD 600					
BETWEEN GROUPS	4	373.7068	93.4267	1.1798	.3497
WITHIN GROUPS	20	1583.8048	79.1902		
TOTAL	24	1957.5116			
PFD 800					
BETWEEN GROUPS	4	701.1451	175.2863	4.0204	.0149
WITHIN GROUPS	20	871.9824	43.5991		
TOTAL	24	1573.1275			
PFD 1000					
BETWEEN GROUPS	4	355.9112	88.9778	2.2222	.1031
WITHIN GROUPS	20	800.8002	40.0400		
TOTAL	24	1156.7114			
PFD 1200					
BETWEEN GROUPS	4	612.1673	153.0418	5.2121	.0048
WITHIN GROUPS	20	587.2535	29.3627		
TOTAL	24	1199.4207			

Table 5.18 Statistical tests of significance (LSD) of rates of oxygen evolution between species of the primulas; effect of PFD at 30 °C.

Primula spp.	PFD	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	800	–	NS	NS	NS	NS
2.*P.farinosa (c)*			–	**	**	**
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	1200	–	*	**	NS	NS
2.*P.farinosa (c)*			–	NS	NS	**
3.*P.frondosa*				–	*	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–

Table 5.19 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of a PFD at 20 °C.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	8	24379.9172	3047.4896	17.3662	.0000
WITHIN GROUPS	36	6317.4238	175.4840		
TOTAL	44	30697.3410			
P.farinosa (c)					
BETWEEN GROUPS	8	15768.8434	1971.1054	19.1707	.0000
WITHIN GROUPS	36	3701.4765	102.8188		
TOTAL	44	19470.3199			
P.frondosa					
BETWEEN GROUPS	8	14582.4728	1822.8091	11.8189	.0000
WITHIN GROUPS	36	5552.2147	154.2282		
TOTAL	44	20134.6875			
P.laurentiana					
BETWEEN GROUPS	8	33772.0183	4221.5023	23.8547	.0000
WITHIN GROUPS	36	6370.8105	176.9670		
TOTAL	44	40142.8288			
P.scotica					
BETWEEN GROUPS	8	15851.8894	1981.4862	58.0942	.0000
WITHIN GROUPS	36	1227.8936	34.1082		
TOTAL	44	17079.7830			

Table 5.20 Statistical tests of significance of rates of oxygen evolution in *Primula* spp.; effect of PFD at 20°C.

Primula spp.	PFD	Significance levels								
		1.	2.	3.	4.	5.	6.	7.	8.	9.
P.farinosa (b)										
	1. 25	–	NS	*	**	***	***	***	***	***
	2. 50		–	NS	*	***	***	***	***	***
	3. 100			–	NS	*	***	***	***	***
	4. 200				–	NS	***	***	***	***
	5. 400					–	*	**	*	*
	6. 600						–	NS	NS	NS
	7. 800							–	NS	NS
	8.1000								–	NS
	9.1200									–
P.farinosa (c)										
	1. 25	–	NS	***	***	***	***	***	***	***
	2. 50		–	**	***	***	***	***	***	***
	3. 100			–	NS	***	*	***	***	**
	4. 200				–	**	NS	**	**	*
	5. 400					–	NS	NS	NS	NS
	6. 600						–	NS	NS	NS
	7. 800							–	NS	NS
	8.1000								–	NS
	9.1200									–
P.frondosa										
	1. 25	–	**	***	***	***	***	***	***	***
	2. 50		–	NS	NS	**	***	***	***	***
	3. 100			–	NS	*	**	**	**	**
	4. 200				–	NS	*	**	*	**
	5. 400					–	NS	NS	NS	NS
	6. 600						–	NS	NS	NS
	7. 800							–	NS	NS
	8.1000								–	NS
	9.1200									–

Table 5.20 Continued.

Primula spp.	PFD	Significance levels								
		1.	2.	3.	4.	5.	6.	7.	8.	9.
P.laurentiana										
	1. 25	–	NS	*	***	***	***	***	***	***
	2. 50		–	NS	***	***	***	***	***	***
	3. 100			–	**	***	***	***	***	***
	4. 200				–	NS	*	***	***	**
	5. 400					–	NS	*	*	NS
	6. 600						–	NS	NS	NS
	7. 800							–	NS	NS
	8.1000								–	NS
	9.1200									–
P.scotica										
	1. 25	–	*	***	***	***	***	***	***	***
	2. 50		–	NS	***	***	***	***	***	***
	3. 100			–	NS	***	***	***	***	***
	4. 200				–	***	***	***	***	***
	5. 400					–	NS	***	***	***
	6. 600						–	**	**	**
	7. 800							–	NS	NS
	8.1000								–	NS
	9.1200									–

in the rates between species. The highest rate was found in *P. farinosa* (b), and the lowest in *P. scotica*. However, the rates were not significantly different between *P. farinosa* (b), *P. frondosa,* and *P. laurentiana*. At 20°C, the two populations of *P. farinosa* differed significantly. At 25 and 30°C, the significantly highest rates of dark respiration were found in *P. laurentiana*; *P. scotica* had the lowest rates at 25°C and *P. frondosa* had the lowest rate at 30°C. However, at 30°C the rates of dark respiration were not significantly different between *P. farinosa* (b), *P. farinosa* (c), *P. frondosa* and *P. scotica*.

5.3.2.3 Effects of temperatures

Oxygen evolution was measured over a range of temperature from 5-30°C at PFDs of 200, 400, 600, 800, 1,000, and 1,200 μmol m^{-2} s^{-1} (Fig. 5.8-5.13). In all species, except *P. scotica,* the rate of O_2 evolution at 20°C was significantly higher than at other temperatures at the PFD levels of 200, 400, 600 and 800 μmol m^{-2} s^{-1} (Tables 5.24-5.31). At PFDs of 1,000 and 1,200 μmol m^{-2} s^{-1} some departure from this was seen where the rates at 5 and 15°C were measured (Tables 5.32-5.35). *P. scotica,* however, did not show the same pattern of responses as the other species. This species appeared to have a higher optimum temperature requirement but this was reduced with increasing PFD.

5.3.3 Relationship between photosynthesis and leaf characteristics

Table 5.36 shows 'Pearson product-moment correlations coefficients,' comparing between rates of O_2 evolution, rates of dark respiration, and leaf characteristics in *P. farinosa* (b). It can be seen that positive significant correlations were found between rates of O_2 evolution and the following leaf characteristics:- thickness of leaves (thickest parts), thickness of leaves (thinnest parts), stomatal apparatus length (lower surface), stomatal apparatus length (upper surface), frequency of stomata/area (lower surface), number of mesophyll cells/sq mm, thickness of mesophyll cells (thinnest parts), chlorophyll contents (mg/sq dm), number of epidermal cells (lower surface), and products of stomatal frequency and pore length. In con-

Table 5.21 Rates of dark respiration of leaf discs of the primulas at 5–30 °C.

Primula spp.	Temperatures (°C)	V/sq m/s	
		mean	SE
P.farinosa(b)	5	61.36	2.49
P.farinosa(c)		62.69	3.40
P.frondosa		17.34	1.63
P.laurentiana		48.02	2.49
P.scotica		40.02	6.67
P.farinosa(b)	15	58.69	2.49
P.farinosa(c)		38.68	2.49
P.frondosa		49.35	6.18
P.laurentiana		56.02	10.87
P.scotica		30.68	3.40
P.farinosa(b)	20	49.35	3.40
P.farinosa(c)		73.37	5.16
P.frondosa		34.68	2.49
P.laurentiana		60.03	13.00
P.scotica		25.34	3.88
P.farinosa(b)	25	21.34	2.49
P.farinosa(c)		38.68	2.49
P.frondosa		34.68	3.88
P.laurentiana		70.70	7.77
P.scotica		18.67	2.49
P.farinosa(b)	30	29.34	1.63
P.farinosa(c)		25.34	2.49
P.frondosa		22.67	1.63
P.laurentiana		69.36	13.60
P.scotica		33.35	15.20

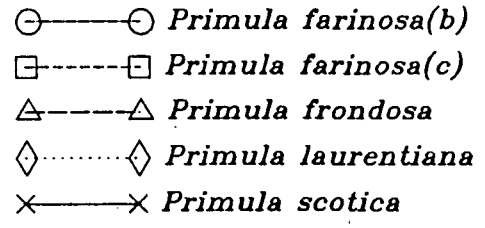
Table 5.22 ANOVA of rates of dark respiration between species of the primulas, at 5-30°C.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
5°C					
BETWEEN GROUPS	4	6879.7635	1719.9409	24.1625	.0000
WITHIN GROUPS	20	1423.6448	71.1822		
TOTAL	24	8303.4083			
15°C					
BETWEEN GROUPS	4	2793.9029	698.4757	3.8670	.0174
WITHIN GROUPS	20	3612.4987	180.6249		
TOTAL	24	6406.4016			
20°C					
BETWEEN GROUPS	4	7395.8347	1848.9587	8.0856	.0005
WITHIN GROUPS	20	4573.4589	228.6729		
TOTAL	24	11969.2937			
25°C					
BETWEEN GROUPS	4	8623.7284	2155.9321	22.8585	.0000
WITHIN GROUPS	20	1886.3294	94.3165		
TOTAL	24	10510.0577			
30°C					
BETWEEN GROUPS	4	7278.3840	1819.5960	4.2516	.0119
WITHIN GROUPS	20	8559.6644	427.9832		
TOTAL	24	15838.0484			

Table 5.23 Statistical tests of significance (LSD) of rates of dark respiration between species of the primulas; effect of temperature.

Primula spp.	Temp. (°C)	Significance levels 1.	2.	3.	4.	5.
1.*P.farinosa (b)*	5	–	NS	***	*	***
2.*P.farinosa (c)*			–	***	*	***
3.*P.frondosa*				–	***	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa (b)*	15	–	*	NS	NS	**
2.*P.farinosa (c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	*
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa (b)*	20	–	*	NS	NS	*
2.*P.farinosa (c)*			–	***	NS	***
3.*P.frondosa*				–	*	NS
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa (b)*	25	–	*	*	***	NS
2.*P.farinosa (c)*			–	NS	***	**
3.*P.frondosa*				–	***	*
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa (b)*	30	–	NS	NS	**	NS
2.*P.farinosa (c)*			–	NS	**	NS
3.*P.frondosa*				–	**	NS
4.*P.laurentiana*					–	*
5.*P.scotica*						–

Figure 5.8 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures at a PFD of 200 μmol $m^{-2}s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

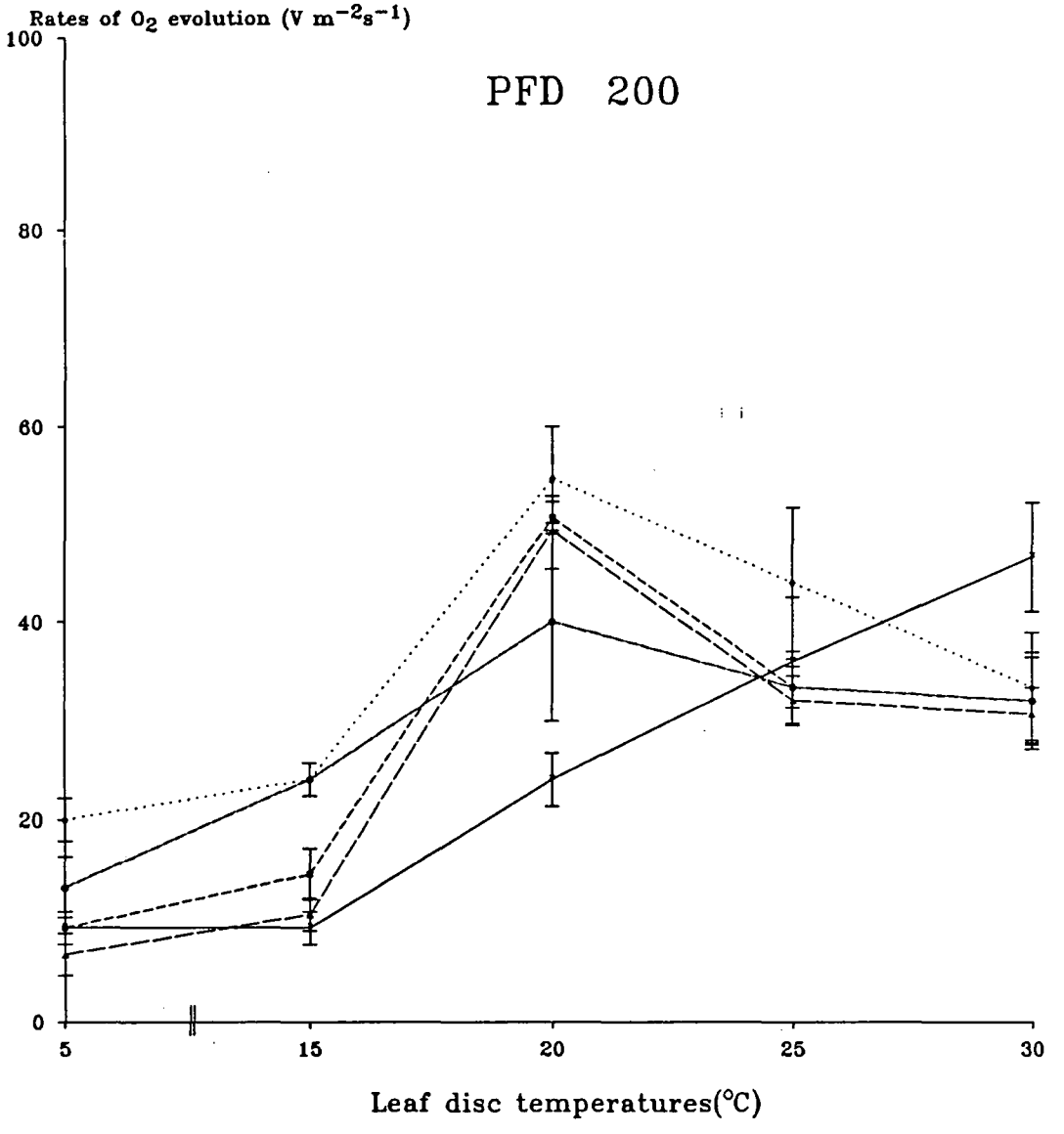
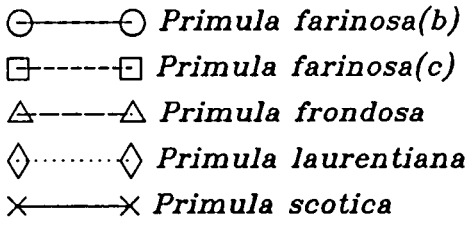

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 200
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

Figure 5.9 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures, at a PFD of 400 μmol $m^{-2}s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

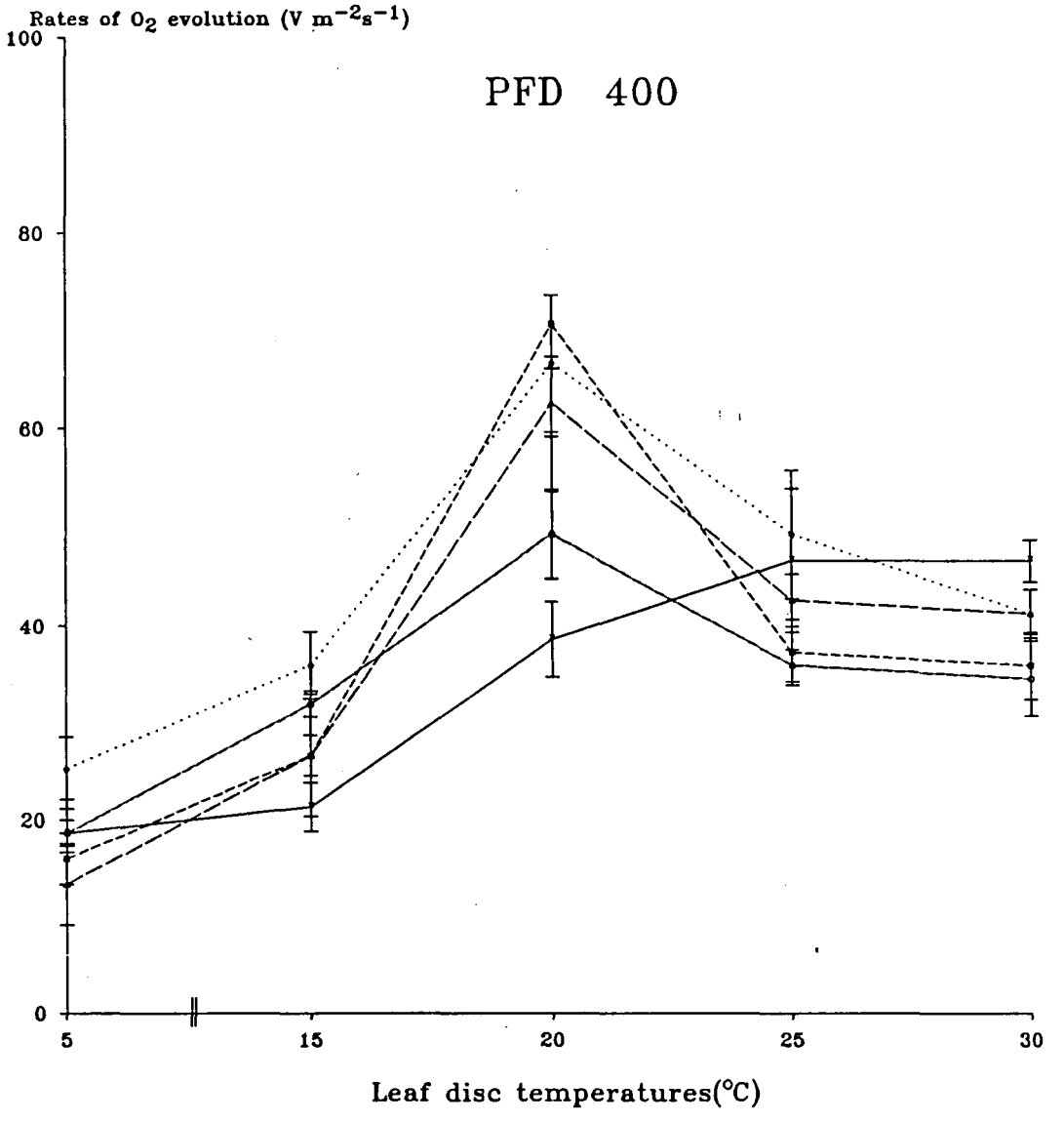
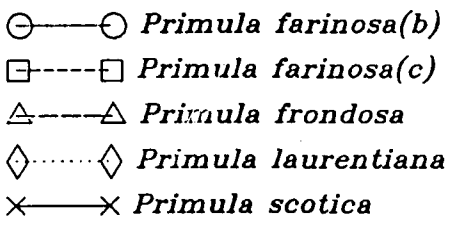

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 400
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

Figure 5.10 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures, at a PFD of 600 μmol $m^{-2}s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

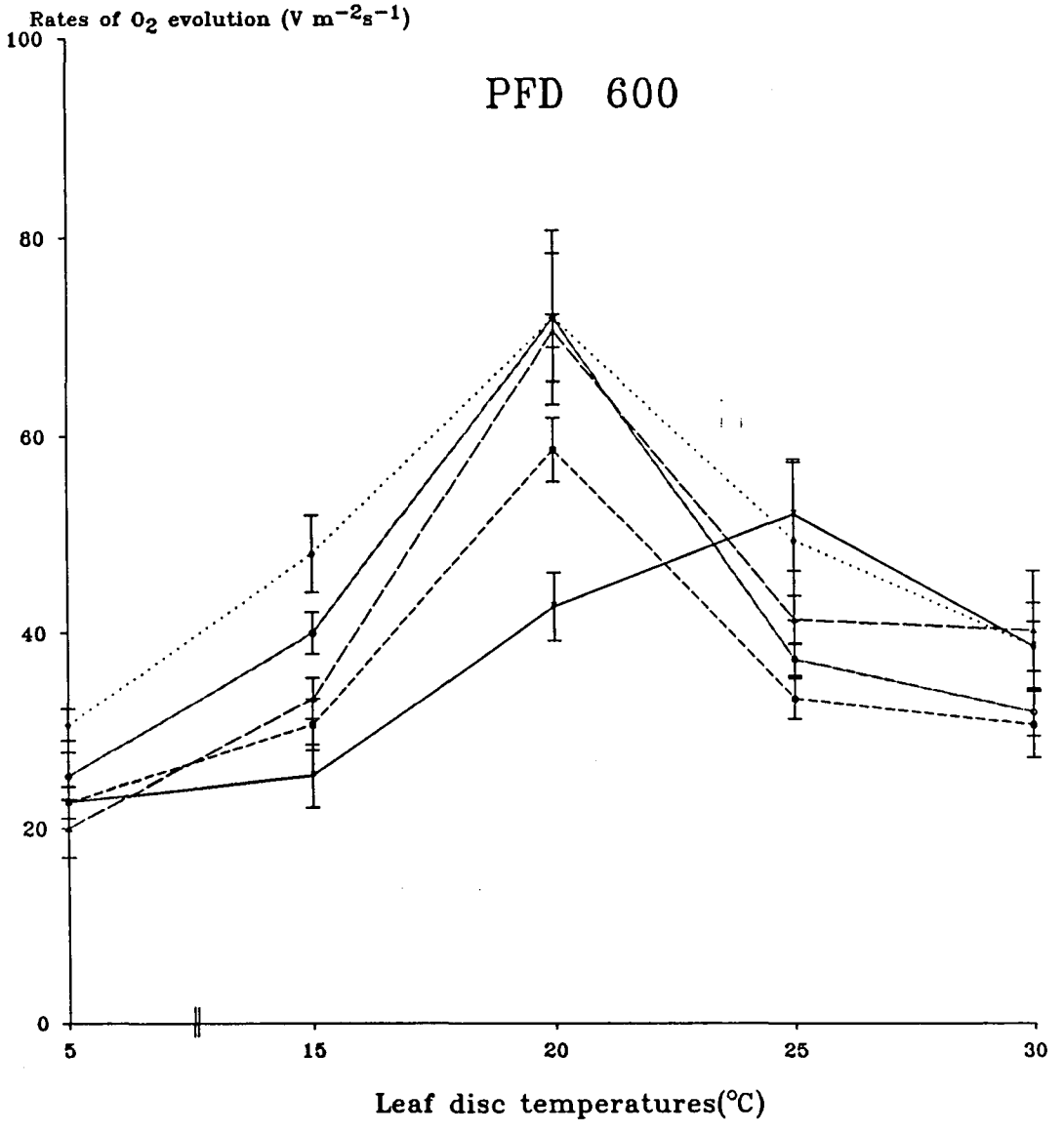
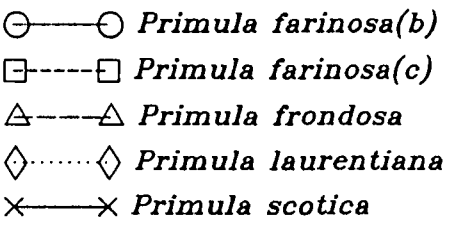

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 600
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

Figure 5.11 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures, at a PFD of 800 μmol m^{-2} $s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

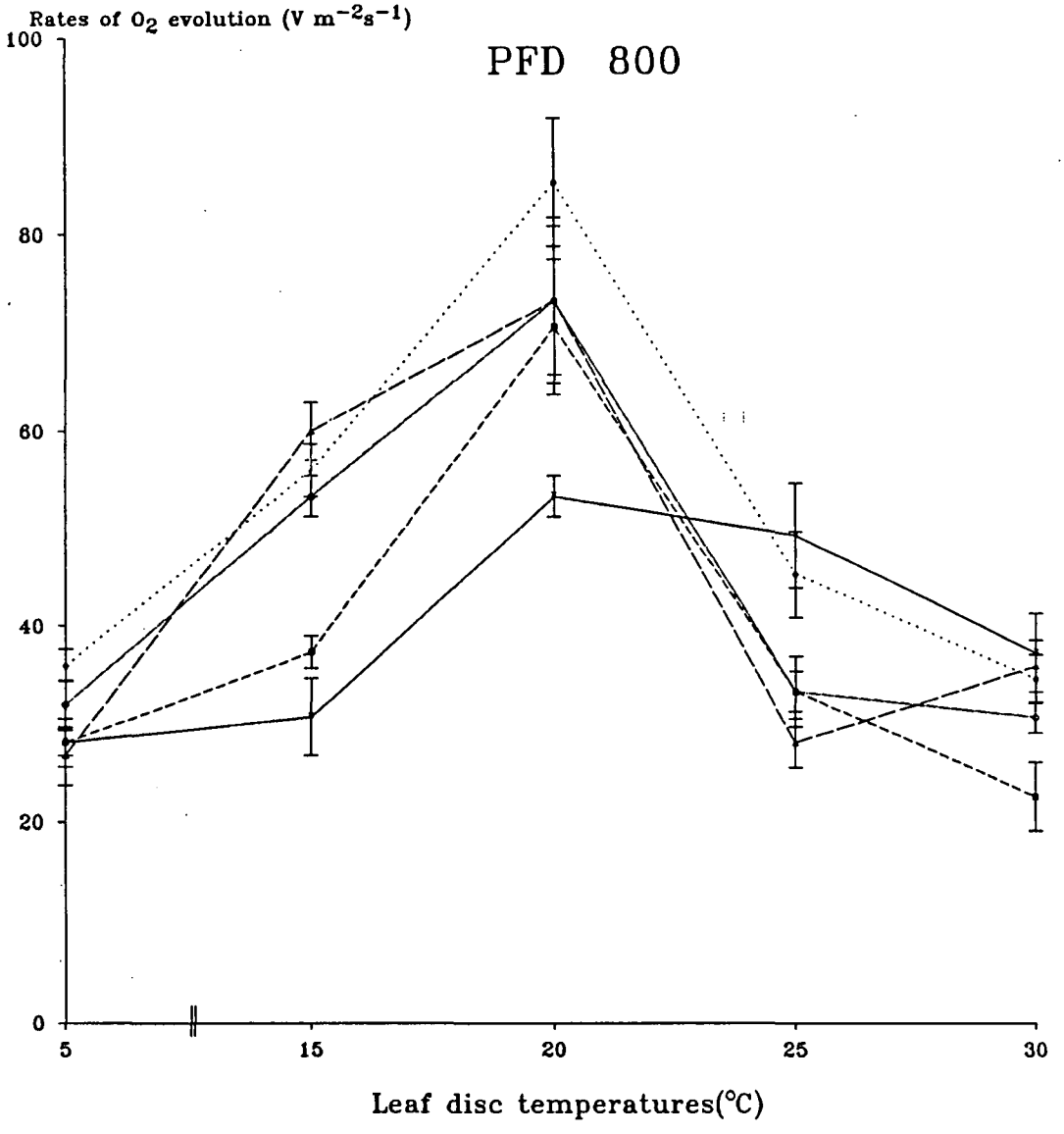
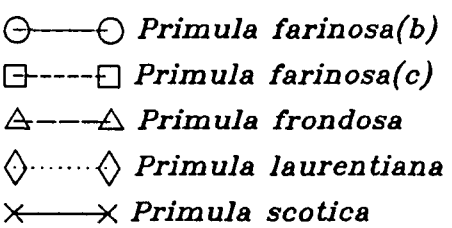

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 800
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

Figure 5.12 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures, at a PFD of 1000 μmol m^{-2} $s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.

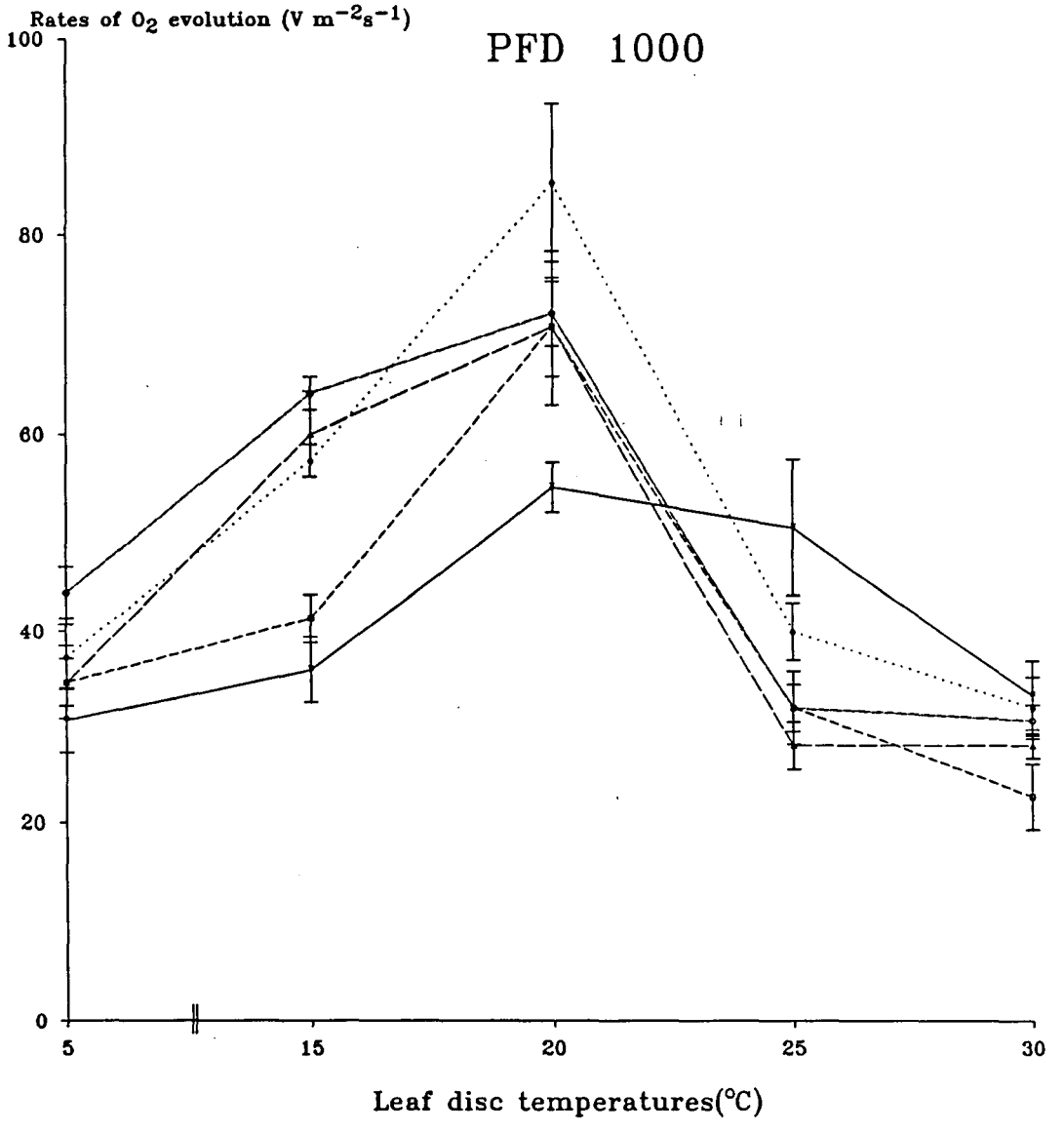
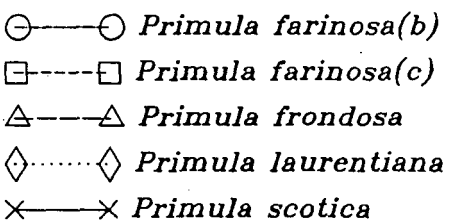
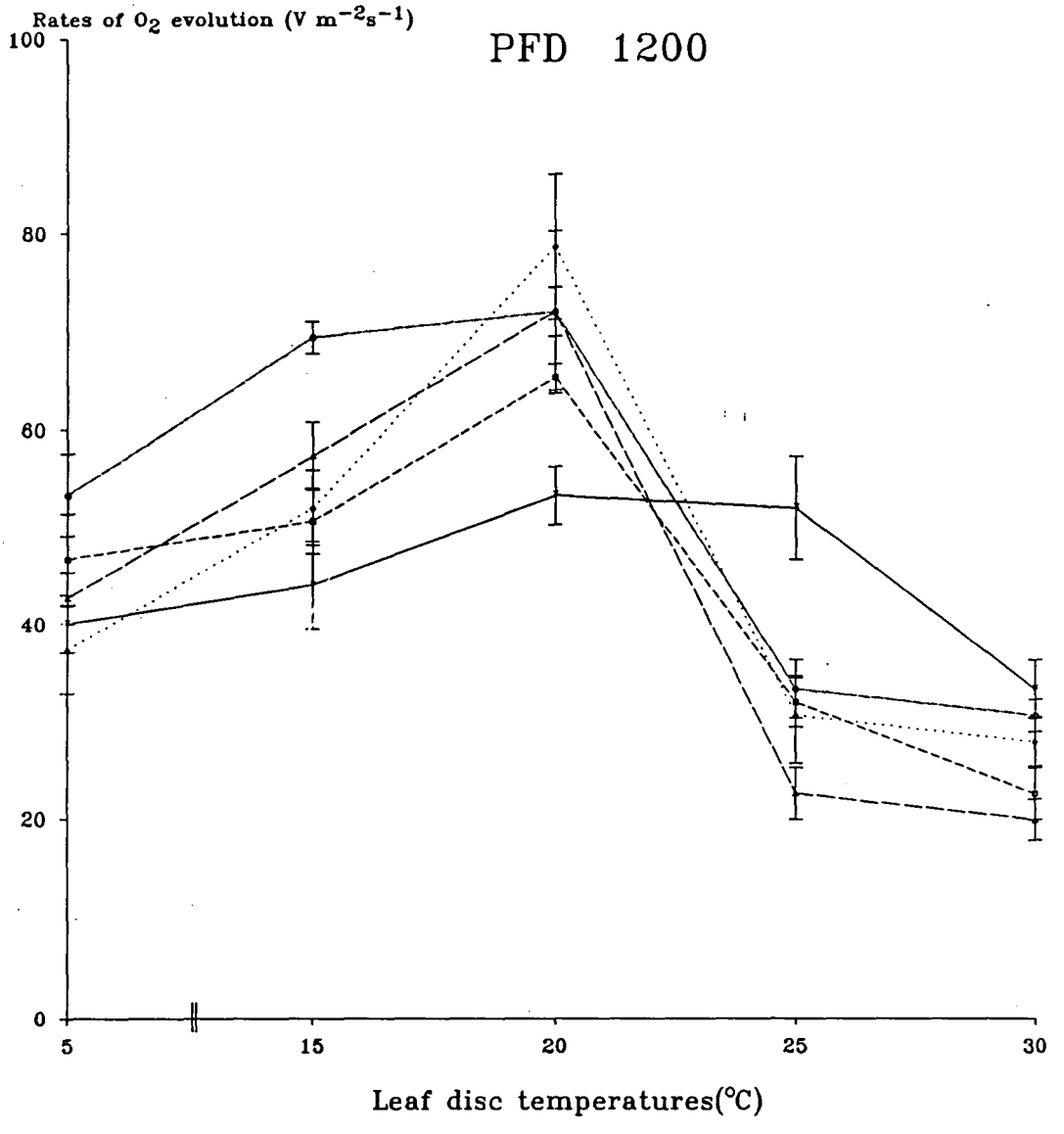

Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 1000
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

Figure 5.13 Rates of oxygen evolution of leaf discs of the primulas at saturated CO_2, effects of temperatures, at a PFD of 1200 μmol m^{-2} $s.^{-1}$



Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols.


Rates of O_2 evolution (V $m^{-2}s^{-1}$)
PFD 1200
100
80
60
40
20
0
5
15
20
25
30
Leaf disc temperatures(°C)

trast, negative significant correlations were found between rates of O_2 evolution and the following leaf characteristics:- thickness of cuticle (upper surface), stomatal pore length (lower surface) stomatal pore length (upper surface), frequency of stomata/area (upper surface). There were negative significant correlations between rates of dark respiration and stomatal apparatus width (upper surface) and thickness of mesophyll cells (thinnest parts).

In *P. farinosa* (c), (Table 5.37) the positive correlations were found between rates of O_2 evolution and the following leaf characteristics:- thickness of leaves (thinnest parts), stomatal apparatus length (lower surface), stomatal apparatus length (upper surface), stomatal apparatus width (lower surface), stomatal apparatus width (upper surface), stomatal pore length (lower surface), thickness of mesophyll cells (thinnest parts), cell size. Negative significant correlations were found with thickness of leaves (thickest parts), thickness of leaves (thinnest parts), stomatal apparatus width (upper surface), number of mesophyll cells/sq mm, thickness of mesophyll cells (thinnest parts), and number of epidermal cell (upper surface). There were negative significant correlations between rates of dark respiration and number of mesophyll cells/sq mm and number of epidermal cell (upper surface).

Results of correlations tests of *P. frondosa* are presented in Table 5.38. It was found that good positive correlations were found between rates of O_2 evolution and the following leaf characteristics:- stomatal apparatus width (upper surface), frequency of stomata/area (upper surface); whereas negative correlations were found with thickness of leaves (thickest parts), thickness of cuticle (upper surface), thickness of cuticle (lower surface), stomatal apparatus length (lower surface), stomatal pore length (lower surface), frequency of stomata/area (upper surface), thickness of mesophyll cells (thickest parts), chlorophyll contents (mg/sq dm), cell size, number of epidermal cell (lower surface). There were no significant correlations between rates of dark respiration and leaf characteristics in *P. frondosa.*

Table 5.39 shows 10 out of 20 leaf characteristics of *P. laurentiana* were signif-

Table 5.24 ANOVA of rates of oxygen evolution in *Primula* spp. effect of temperatures at a PFD of 200 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	4	2092.7579	523.1895	3.7935	.0188
WITHIN GROUPS	20	2758.3118	137.9156		
TOTAL	24	4851.0697			
P.farinosa (c)					
BETWEEN GROUPS	4	5427.6458	1356.9115	27.7273	.0000
WITHIN GROUPS	20	978.7558	48.9378		
TOTAL	24	6406.4016			
P.frondosa					
BETWEEN GROUPS	4	6061.1677	1515.2919	41.5366	.0000
WITHIN GROUPS	20	729.6180	36.4809		
TOTAL	24	6790.7857			
P.laurentiana					
BETWEEN GROUPS	4	4085.8606	1021.4651	8.0280	.0005
WITHIN GROUPS	20	2544.7651	127.2383		
TOTAL	24	6630.6257			
P.scotica					
BETWEEN GROUPS	4	5416.9685	1354.2421	15.6907	.0000
WITHIN GROUPS	20	1726.1693	86.3085		
TOTAL	24	7143.1378			

Table 5.25 Statistical tests of significance (LSD) of rates of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 200 µmol /sq m/s.

Primula spp.	Temperatures (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	NS	**	*	*
	2. 15		–	*	NS	NS
	3. 20			–	NS	NS
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	NS	***	***	***
	2. 15		–	***	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.frondosa						
	1. 5	–	NS	***	***	***
	2. 15		–	***	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	NS	***	**	NS
	2. 15		–	***	*	NS
	3. 20			–	NS	**
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	*	***	***
	2. 15		–	*	***	***
	3. 20			–	NS	***
	4. 25				–	NS
	5. 30					–

Table 5.26 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of temperatures at a PFD of 400 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	4	2395.2824	598.8206	12.9423	.0000
WITHIN GROUPS	20	925.3691	46.2685		
TOTAL	24	3320.6515			
P.farinosa (c)					
BETWEEN GROUPS	4	8417.2999	2104.3250	25.7065	.0000
WITHIN GROUPS	20	1637.1915	81.8596		
TOTAL	24	10054.4914			
P.frondosa					
BETWEEN GROUPS	4	6886.8817	1721.7204	36.5094	.0000
WITHIN GROUPS	20	943.1647	47.1582		
TOTAL	24	7830.0464			
P.laurentiana					
BETWEEN GROUPS	4	4811.9194	1202.9799	10.0148	.0001
WITHIN GROUPS	20	2402.4006	120.1200		
TOTAL	24	7214.3200			
P.scotica					
BETWEEN GROUPS	4	3690.7991	922.6998	11.3956	.0001
WITHIN GROUPS	20	1619.3960	80.9698		
TOTAL	24	5310.1951			

Table 5.27 Statistical tests of significance (LSD) of rates of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 400 µmol /sq m/s.

Primula spp.	Temperatures (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	**	***	***	**
	2. 15		–	***	NS	NS
	3. 20			–	**	**
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	NS	***	**	**
	2. 15		–	***	NS	NS
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.frondosa						
	1. 5	–	**	***	***	***
	2. 15		–	***	**	**
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	NS	***	**	*
	2. 15		–	***	NS	NS
	3. 20			–	*	**
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	**	***	***
	2. 15		–	**	***	***
	3. 20			–	NS	NS
	4. 25				–	NS
	5. 30					–

Table 5.28 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of temperatures at a PFD of 600 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	4	6513.1750	1628.2937	16.7890	.0000
WITHIN GROUPS	20	1939.7160	96.9858		
TOTAL	24	8452.8910			
P.farinosa (c)					
BETWEEN GROUPS	4	3765.5405	941.3851	25.8049	.0000
WITHIN GROUPS	20	729.6180	36.4809		
TOTAL	24	4495.1585			
P.frondosa					
BETWEEN GROUPS	4	6911.7955	1727.9489	29.8769	.0000
WITHIN GROUPS	20	1156.7114	57.8356		
TOTAL	24	8068.5069			
P.laurentiana					
BETWEEN GROUPS	4	4829.7150	1207.4287	9.2313	.0002
WITHIN GROUPS	20	2615.9473	130.7974		
TOTAL	24	7445.6623			
P.scotica					
BETWEEN GROUPS	4	2996.7723	749.1931	9.6782	.0002
WITHIN GROUPS	20	1548.2137	77.4107		
TOTAL	24	4544.9860			

Table 5.29 Statistical tests of significance (LSD) of rate of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 600 µmol /sq m/s.

Primula spp.	Temperature (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	*	***	NS	NS
	2. 15		–	***	NS	NS
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	*	***	*	*
	2. 15		–	***	NS	NS
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.frondosa						
	1. 5	–	*	***	***	***
	2. 15		–	***	NS	NS
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	*	***	*	NS
	2. 15		–	**	NS	NS
	3. 20			–	NS	***
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	**	***	**
	2. 15		–	**	***	*
	3. 20			–	NS	NS
	4. 25				–	*
	5. 30					–

Table 5.30 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of temperatures at a PFD of 800 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	4	6915.3546	1728.8387	22.8588	.0000
WITHIN GROUPS	20	1512.6226	75.6311		
TOTAL	24	8427.9772			
P.farinosa (c)					
BETWEEN GROUPS	4	7125.3422	1781.3356	22.0000	.0000
WITHIN GROUPS	20	1619.3960	80.9698		
TOTAL	24	8744.7382			
P.frondosa					
BETWEEN GROUPS	4	8677.1151	2169.2788	21.2000	.0000
WITHIN GROUPS	20	2046.4894	102.3245		
TOTAL	24	10723.6045			
P.laurentiana					
BETWEEN GROUPS	4	8641.5239	2160.3810	27.9080	.0000
WITHIN GROUPS	20	1548.2137	77.4107		
TOTAL	24	10189.7377			
P.scotica					
BETWEEN GROUPS	4	2516.2922	629.0730	9.3026	.0002
WITHIN GROUPS	20	1352.4626	67.6231		
TOTAL	24	3868.7547			

Table 5.31 Statistical tests of significance (LSD) of rates of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 800 µmol /sq m/s.

Primula spp.	Temperature (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	***	***	NS	NS
	2. 15		–	**	**	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	NS	***	NS	NS
	2. 15		–	***	NS	*
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.frondosa						
	1. 5	–	***	***	NS	NS
	2. 15		–	NS	***	**
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	**	***	NS	NS
	2. 15		–	***	NS	**
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	***	***	NS
	2. 15		–	***	**	NS
	3. 20			–	NS	**
	4. 25				–	*
	5. 30					–

Table 5.32 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of temperatures at a PFD of 1000 μmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa (b)					
BETWEEN GROUPS	4	7022.1280	1755.5320	59.7879	.0000
WITHIN GROUPS	20	587.2535	29.3627		
TOTAL	24	7609.3815			
P.farinosa (c)					
BETWEEN GROUPS	4	6680.4532	1670.1133	15.3852	.0000
WITHIN GROUPS	20	2171.0583	108.5529		
TOTAL	24	8851.5115			
P.frondosa					
BETWEEN GROUPS	4	7837.1646	1959.2912	34.4063	.0000
WITHIN GROUPS	20	1138.9158	56.9458		
TOTAL	24	8976.0805			
P.laurentiana					
BETWEEN GROUPS	4	9438.7650	2359.6913	24.1091	.0000
WITHIN GROUPS	20	1957.5116	97.8756		
TOTAL	24	11396.2766			
P.scotica					
BETWEEN GROUPS	4	2356.1321	589.0330	6.5545	.0015
WITHIN GROUPS	20	1797.3516	89.8676		
TOTAL	24	4153.4837			

Table 5.33 Statistical tests of significance (LSD) of rates of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 1000 μmol /sq m/s.

Primula spp.	Temperature (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	***	***	**	***
	2. 15		–	*	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	NS	***	NS	NS
	2. 15		–	***	NS	*
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.frondosa						
	1. 5	–	***	***	NS	NS
	2. 15		–	*	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	**	***	NS	NS
	2. 15		–	***	*	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	***	**	NS
	2. 15		–	**	*	NS
	3. 20			–	NS	**
	4. 25				–	**
	5. 30					–

Table 5.34 ANOVA of rates of oxygen evolution in *Primula* spp.; effect of temperatures at a PFD of 1200 µmol /sq m/s.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	4	7534.6401	1883.6600	49.2326	.0000
WITHIN GROUPS	20	765.2091	38.2605		
TOTAL	24	8299.8492			
P.farinosa(c)					
BETWEEN GROUPS	4	5527.3009	1381.8252	28.2364	.0000
WITHIN GROUPS	20	978.7558	48.9378		
TOTAL	24	6506.0567			
P.frondosa					
BETWEEN GROUPS	4	9954.8363	2488.7091	25.1982	.0000
WITHIN GROUPS	20	1975.3072	98.7654		
TOTAL	24	11930.1434			
P.laurentiana					
BETWEEN GROUPS	4	8684.2333	2171.0583	19.2126	.0000
WITHIN GROUPS	20	2260.0361	113.0018		
TOTAL	24	10944.2694			
P.scotica					
BETWEEN GROUPS	4	1398.7310	349.6828	4.6235	.0083
WITHIN GROUPS	20	1512.6226	75.6311		
TOTAL	24	2911.3536			

Table 5.35 Statistical tests of significance (LSD) of rates of oxygen evolution in *Primula* spp.; effects of temperature at a PFD of 1200 µmol /sq m/s.

Primula spp.	Temperature (°C)	Significance levels 1.	2.	3.	4.	5.
P.farinosa (b)						
	1. 5	–	***	***	***	***
	2. 15		–	NS	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.farinosa (c)						
	1. 5	–	NS	***	**	***
	2. 15		–	**	***	***
	3. 20			–	***	***
	4. 25				–	*
	5. 30					–
P.frondosa						
	1. 5	–	*	***	**	**
	2. 15		–	*	***	***
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.laurentiana						
	1. 5	–	*	***	NS	NS
	2. 15		–	***	**	**
	3. 20			–	***	***
	4. 25				–	NS
	5. 30					–
P.scotica						
	1. 5	–	NS	*	*	NS
	2. 15		–	NS	NS	NS
	3. 20			–	NS	**
	4. 25				–	**
	5. 30					–

icantly correlated with rates of O_2 evolution. Of these, thickness of leaves (thinnest parts), thickness of cuticle (upper surface), stomatal apparatus length (lower surface), stomatal apparatus width (upper surface), number of mesophyll cells/sq mm, thickness of mesophyll cells (thinnest parts), cell size were positively correlated, whilst thickness of cuticle (upper surface), thickness of cuticle (lower surface), stomatal apparatus length (lower surface), stomatal apparatus width (upper surface), frequency of stomata/area (upper surface), number of mesophyll cells/sq mm, thickness of mesophyll cells (thinnest parts), number of epidermal cell (upper surface) were negatively correlated. There were the positive correlations between rates of dark respiration and stomatal apparatus length (upper surface) and stomatal apparatus width (lower surface).

The results of correlations tests of *P. scotica* (Table 5.40) show that stomatal apparatus length (lower surface), stomatal apparatus width (lower surface), stomatal apparatus width (upper surface), stomatal pore length (lower surface), stomatal pore length (upper surface), frequency of stomata/area (lower surface), frequency of stomata/area (upper surface), thickness of mesophyll cells (thickest parts), thickness of mesophyll cells (thinnest parts), were positively correlated with rates of O_2 evolution. Negative correlations were found with thickness of cuticle (lower surface), stomatal apparatus length (lower surface), stomatal pore length (lower surface), frequency of stomata/area (upper surface), thickness of mesophyll cells (thickest parts), chlorophyll contents (mg/sq dm), number of epidermal cell (upper surface). Only two leaf characteristics were found indirectly correlated with rates of dark respiration:- thickness of cuticle (upper surface) and number of epidermal cell (upper surface).

Table 5.36 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in P.farinosa(b) at 20°C.

PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS

	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	.	−.1462	.7648	.5645	.8996*	−.1042	−.0104	.4184	−.4499	−.6920
DATA2	.	−.6470	.8391*	.0784	.7125	−.2683	−.1737	−.0216	−.2400	−.3988
DATA3	.	−.8394*	−.1164	−.5406	−.3109	−.4973	−.4109	−.7810	.5426	.7351
DATA4	.	−.7187	.5305	−.7006	−.1290	−.5117	−.5482	−.7400	−.1336	.1961
DATA5	.	−.2671	.9359**	.2242	.7304	−.2430	−.2130	.1388	−.5971	−.6476
DATA6	.	−.1572	.3399	.0888	.6223	.8442*	.8664	.7149	.0960	−.6692
DATA7	.	.8750	−.1721	−.1648	−.5160	.2274	.0000	.1021	−.5345	−.1961
DATA8	.	−.1231	.6495	.2029	.7985	.6345	.6478	.7035	−.2303	−.8690*
DATA9	.	−.3430	.0393	.2827	.0759	−.8971*	−.7521	−.4901	.0458	.4372
DATA10	.	.3675	−.8277*	−.2643	−.9063*	−.2229	−.2930	−.4637	.2857	.7600
DATA11	.	.9698**	−.4338	.4579	−.2188	.2106	.1260	.4204	−.2817	−.1504
DATA12	.	−.8385*	.5130	−.5898	.0000	−.5085	−.4903	−.6847	.0000	.2193
DATA13	.	−.5424	−.3883	.2527	.3182	.3114	.5291	.2453	.9029*	.2847
DATA14	.	−.0382	.7355	.2984	.4548	−.6001	−.5870	−.0937	−.7018	−.3950
DATA15	.	−.0483	.9559**	.0849	.6689	.1268	.0705	.3415	−.7137	−.8771*
DATA16	.	.3876	−.0219	.4140	.6460	.9495*	.9710*	.9241*	.2374	−.6005
DATA17	.	.7176	−.3449	−.2350	−.7713	−.2566	−.4453	−.3365	−.4100	.2509
DATA18	.	−.4653	.7057	.1875	.5085	−.6640	−.5732	−.2502	−.3671	−.1782
DATA19	.	−.3392	.8475*	.3413	.7757	−.3220	−.2402	.1284	−.4646	−.5412
DATA20	.	.8545*	−.5648	.3461	−.4523	−.1341	−.2036	.0687	−.1951	.1909

Notes (1-TAILED, " . " PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)

DATA1 = thickness of leaves (thickest part)
DATA2 = thickness of leaves (thinnest part)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = chlorophyll concentrations/sq dm
DATA17 = cell size
DATA18 = number of epidermal cell(upper surface)
DATA19 = number of epidermal cell(lower surface)
DATA20 = products of stomatal frequency and pore length

Table 5.37 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in P.farinosa(c) at 20°C.

	PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS									
	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	-.6827	-.7628	-.4253	.3526	.6113	-.2568	-.4450	-.7632*	-.8638	-.0606
DATA2	-.7312	-.8498*	-.5159	.4278	.9318*	-.8518*	-.5124	-.5066	-.2800	-.1283
DATA3	.5423	.2113	-.3892	.3227	-.6202	.4841	-.0768	-.2034	-.3953	-.7144
DATA4	-.5601	-.3273	.2010	-.1667	.3203	.1667	-.0397	-.3851	-.6124	.5270
DATA5	.4434	.6910	.7203	-.3889	-.1868	-.0278	.6344	.9102*	.9526**	.4392
DATA6	.3430	.5345	.4924	-.4082	-.6864	.9186*	.3885	.1286	-.2500	.3227
DATA7	.8135	.8452	.5839	.0000	-.3101	.0000	.7679	.9491**	.7906	.0000
DATA8	.3800	.5922	.3939	-.7035	-.9415**	.9548**	.1913	.2217	.1846	.3178
DATA9	.0000	.1597	.2942	-.2440	.2344	-.4880	.1741	.5637	.8964*	.3086
DATA10	.5601	.3273	-.2010	.1667	-.3203	-.1667	.0397	.3851	.6124	-.5270
DATA11	.0995	.2655	.6010	.1352	.5518	-.5575	.6350	.6672	.4967	.3738
DATA12	-.1403	-.4911	-.7033	.5832	.4807	-.7919	-.4560	-.2274	.1023	-.6585
DATA13	-.1833	-.6429	-.9211*	.7638	.3669	-.4910	-.5971	-.6417	-.5345	-.8626*
DATA14	.0873	.1360	.4574	.4469	.6532	-.5256	.6230	.4244	.0231	.1692
DATA15	-.6227	-.5796	-.0990	.2686	.9654**	-.8655*	-.1609	-.1316	.0122	.1888
DATA16	.0378	.5235	.8195	-.7867	-.4760	.7750	.4145	.3488	.0000	.8115
DATA17	.7807	.8040	.6852	.2396	-.2131	.2244	.9468**	.7221	.1205	.0224
DATA18	-.0097	-.4676	-.8127*	.7603	.3652	-.6451	-.4495	-.3484	-.1693	-.8743*
DATA19	-.2429	-.2437	.0998	.4150	.5182	-.1366	.1877	-.2509	-.7083	.0844
DATA20	.0794	.1237	.2496	.1305	.4788	-.7211	.3040	.5827	.7412	.1103

Notes (1-TAILED, " . " PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)
DATA1 = thickness of leaves (thickest part)
DATA2 = thickness of leaves (thinnest part)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = chlorophyll concentrations/sq dm
DATA17 = cell size
DATA18 = number of epidermal cell(upper surface)
DATA19 = number of epidermal cell(lower surface)
DATA20 = products of stomatal frequency and pore length

Table 5.38 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in P.frondosa at 20°C.

	PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS									
	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	.2314	.1499	.0491	−.1921	.0470	.2824	−.8939*	−.6151	−.6160	−.3057
DATA2	.4956	.1089	−.1076	−.3448	−.0420	.3722	−.7558	−.5510	−.6062	−.5447
DATA3	.2228	.1453	.0460	−.2034	.0456	.2610	−.8960*	−.6136	−.6183	−.2796
DATA4	.5384	−.6390	−.8660*	−.6086	−.7161	−.3727	.4330	.0000	−.2207	−.4880
DATA5	.0000	−.8944*	−.8839*	−.5590	−.8771*	−.9129*	−.0884	−.4482	−.6307	.0000
DATA6	−.7078	−.0615	.2919	.6975	.0966	−.2010	.5352	.3454	.5257	.3948
DATA7	.1957	.1474	.1166	.1474	.0289	.5417	−.7577	−.6108	−.5109	−.6699
DATA8	.5898	.7500	.3953	−.2500	.6864	.6124	.5929	.8686*	.7655	.1336
DATA9	.6072	−.6864	−.9303*	−.6864	−.8077*	−.3203	.0000	−.3669	−.5847	−.6814
DATA10	−.2107	.3062	.4841	.7485	.2802	.6667	−.2421	−.1909	.0658	−.4910
DATA11	.0984	.2691	.1191	−.4882	.2914	−.2111	−.1617	.1755	.0312	.6735
DATA12	−.8353*	−.3334	.1316	.8055*	−.1963	−.2721	.0658	−.2006	.0401	.0887
DATA13	−.1321	−.0223	−.0352	−.1864	.0878	−.4566	.7597	.6871	.5764	.7175
DATA14	.1383	.1500	.0992	−.0943	.0601	.2831	−.9257*	−.6478	−.6165	−.2888
DATA15	.5101	−.0086	−.2181	−.3563	−.1657	.3214	−.7406	−.6129	−.6734	−.6467
DATA16	.8075	−.0495	−.5038	−.9910**	−.1459	−.0148	.8394	.6125	.3556	−.1739
DATA17	−.9522**	−.1158	.3492	.7512	.0723	−.3607	.1339	.0395	.2581	.5708
DATA18	−.5432	.1443	.3723	.2216	.2235	−.1706	−.7146	−.4060	−.2969	.5278
DATA19	.3684	−.4199	−.5991	−.7066	−.5101	−.3595	−.6396	−.6377	−.8235*	−.2244
DATA20	−.0803	.1525	.0981	−.3900	.2010	−.3382	−.2714	.0301	−.0793	.7167

Notes (1-TAILED, " . " PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)

DATA1 = thickness of leaves (thickest part)
DATA2 = thickness of leaves (thinnest part)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = chlorophyll concentrations/sq dm
DATA17 = cell size
DATA18 = number of epidermal cell(upper surface)
DATA19 = number of epidermal cell(lower surface)
DATA20 = products of stomatal frequency and pore length

Table 5.39 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in P.laurentiana at 20°C.

PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS

	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	−.7546	−.1334	.2997	.1395	.1576	.0777	.4019	.2186	.5689	−.2474
DATA2	−.1935	−.6925	.0928	.7055	.8762*	.7535	.8061*	.8756*	.6519	.3333
DATA3	.8686*	.7741	.1961	−.2500	−.4767	−.5673	−.9541**	−.8750*	−.9429**	.0641
DATA4	.5976	.6594	.2193	−.2795	−.5330	−.4613	−.9225*	−.9317*	−.7028	.1721
DATA5	−.4226	−.9325*	−.6202	.1318	.4020	.7067	.8154*	.8784*	.7100	.2433
DATA6	.6417	.0759	.7399	.7717	.5723	.4245	−.2830	−.2287	−.0924	.8358*
DATA7	.2758	−.4565	.3181	.7003	.6326	.7908	.1065	.1474	.3575	.9833**
DATA8	.8452*	.6994	.3101	.0000	−.1508	−.4892	−.6523	−.5270	−.8519*	−.0811
DATA9	.0546	−.6922	−.0801	.6124	.7785	.7579	.6527	.7825	.4766	.4712
DATA10	−.6290	−.5494	−.6154	−.1961	.0935	.1618	.7687	.7845	.4931	−.4527
DATA11	.1025	−.5090	−.6769	−.0958	.1097	.3165	.3559	.5113	.1034	.1182
DATA12	.5591	.7985	−.0968	−.6464	−.8217*	−.7999	−.9267*	−.9026*	−.9174	−.2843
DATA13	.7638	.8427*	.4804	−.1021	−.3892	−.5474	−.9685**	−.9526**	−.8432*	.1047
DATA14	−.3959	−.1257	.6602	.5610	.4879	.3287	.2824	.1421	.5240	.2049
DATA15	−.5850	−.9084*	−.1626	.4966	.7263	.8135*	.9776**	.9787**	.9248*	.2547
DATA16	.6085	.1646	.6349	.7206	.7165	.1527	.1734	.2488	−.1646	.1032
DATA17	.3339	.8113*	.8262*	.1609	−.0910	−.5183	−.5913	−.6608	−.5060	−.1767
DATA18	.3643	.3945	−.5445	−.7825	−.8375*	−.5571	−.6613	−.6142	−.6619	−.1942
DATA19	−.3900	−.2254	−.1658	.0521	.2540	.0108	.6141	.6409	.2746	−.5110
DATA20	.1858	−.3066	−.7008	−.2618	−.0824	.0885	.1822	.3399	−.1048	−.0402

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DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
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DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = chlorophyll concentrations/sq dm
DATA17 = cell size
DATA18 = number of epidermal cell(upper surface)
DATA19 = number of epidermal cell(lower surface)
DATA20 = products of stomatal frequency and pore length

Table 5.40 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in P.scotica at 20°C.

	PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS									
	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	−.3985	.0762	.7500	−.7966	.2191	−.2055	.7943	.5409	.1926	.6622
DATA2	.7578	.3817	−.2735	.5478	−.4763	−.1834	−.5075	−.3394	−.0780	−.4763
DATA3	.3273	−.3273	−.3273	.8018	−.1833	.4193	−.4226	−.4286	.0000	−.8709*
DATA4	.0000	.2282	−.2282	.6988	.3835	.2193	−.8839*	−.8964*	−.8750*	−.4794
DATA5	.7095	.9798**	.1351	−.5379	−.6103	−.9576**	.1309	.3981	.1851	.6671
DATA6	−.5406	−.1351	.7095	.2276	.7522	.4707	.0000	−.5087	−.6477	−.3123
DATA7	−.2887	.2887	.8660*	−.1768	.4851	.0000	.2236	−.1890	−.4743	.1213
DATA8	−.1667	.1667	1.0000***	−.4082	.2100	−.0801	.6455	.2182	.0000	.2100
DATA9	.9548**	.8040	−.2010	−.2770	−.8866*	−.8690*	.0000	.3948	.4129	.3800
DATA10	−.4082	−.6124	−.6124	.8750*	.5145	7845	−.7906	−.8018	−.5590	−.7717
DATA11	.3058	.0762	.6497	−.5385	−.4659	−.3121	.8886*	.7260	.7332	.2569
DATA12	−.8729*	−.2182	.3273	−.1336	.8709*	.3669	.0000	−.2857	−.5976	.1833
DATA13	.3603	.5413	−.6620	.1478	−.2013	−.4041	−.6998	−.2766	−.3306	.1767
DATA14	.7070	.9464**	.3953	−.4911	−.5636	−.8746*	.2503	.3683	.1770	.5381
DATA15	.8978*	.5343	−.2644	.3272	−.6687	−.4154	−.3626	−.1118	.1056	−.2522
DATA16	−.1946	.0321	−.7692	.7018	.6243	.3823	−.9648*	−.9648*	−.9438*	−.4307
DATA17	−.5322	.0949	.7427	−.6569	.4396	−.0998	.5891	.2917	−.0994	.5818
DATA18	−.4436	−.3912	.1574	.7975	.7194	.7723	−.5173	−.8869*	−.7708	−.8136*
DATA19	.7865	.3482	.0995	.3084	−.5799	−.2419	−.0722	−.0427	.2043	−.3840
DATA20	.5301	.3086	.5879	−.5426	−.6300	−.5076	.7958	.7250	.7279	.3128

Notes (1-TAILED, " . " PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)
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DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = chlorophyll concentrations/sq dm
DATA17 = cell size
DATA18 = number of epidermal cell(upper surface)
DATA19 = number of epidermal cell(lower surface)
DATA20 = products of stomatal frequency and pore length

Table 5.41 Relationship between rates of oxygen evolution, dark respiration and leaf characteristics in *Primula farinosa* complex at 20°C.

	PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS									
	PFD 25	PFD 50	PFD 100	PFD 200	PFD 400	PFD 600	PFD 800	PFD 1000	PFD 1200	DARK
DATA1	.0191	.0569	.0714	.3486*	.3501*	.2487	.1153	.2761	.1510	.0409
DATA2	.0486	.0319	−.0508	.2247	.2828	.1550	.0343	.1917	.0590	−.0609
DATA3	.2418	.1478	.0023	−.3015	−.2965	−.2122	−.5990***	−.5565**	−.4577*	−.2260
DATA4	−.2838	−.3343	−.3388*	−.2074	−.1493	−.2543	−.1065	−.2145	−.2337	−.1168
DATA5	−.5414**	−.6450***	−.6009***	−.3751*	−.4398*	−.3352	−.1001	.0218	−.1510	−.2559
DATA6	−.1877	−.4018*	−.2253	.2617	.2762	.1834	.3661*	.3749*	.2237	.3519*
DATA7	−.3567*	−.4862**	−.4410*	−.1044	−.0749	−.1807	−.0930	.0229	−.1673	.0864
DATA8	−.2663	−.4307*	−.3693*	−.0261	.0091	−.1402	.1183	.1707	−.0288	.1288
DATA9	−.4328*	−.5995***	−.6560***	−.3623*	−.5426**	−.2571	−.1305	.0013	−.0715	−.2897
DATA10	−.1918	−.3338	−.3792*	.2156	.1201	.2069	.3089	.4130*	.3264	.1614
DATA11	.1300	−.2140	−.0372	.1794	.2765	−.0654	.1578	.2953	.0280	.6108**
DATA12	.0917	.1869	.1152	.3446*	.2171	.4015*	.3205	.2940	.3616*	.0298
DATA13	.0748	.0808	−.0061	−.1880	−.2848	.0557	−.0471	−.1923	.0273	−.1548
DATA14	.1396	.2869	.2116	.3694*	.3564*	.2785	.1209	.2719	.1843	.0172
DATA15	.0926	.0313	.0102	.3425*	.3573*	.3268	.2633	.3990*	.2764	.0404
DATA16	.2820	.1230	.1671	.4473*	.3600	.7041***	.5322**	.4878	.5635**	.1518
DATA17	−.1727	−.1959	−.1839	−.0094	−.0410	−.2708	.0342	.0358	−.1521	.0655
DATA18	.4183*	.5368**	.5495**	.1350	.1249	.2393	−.0604	−.0982	.0440	−.0284
DATA19	.3863*	.1207	.4461*	.1920	.3656*	−.1105	−.0527	−.0541	−.1377	.4673**
DATA20	−.0565	−.3780	−.3053	.2225	.1677	.0956	.3075	.4778**	.2413	.4333*

Notes

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5.4 Discussion

Leaf chl content, on an area basis, of the primulas grown in a growth room varied greatly amongst species and was lower than the average for shade and sun plants (Boardman, 1977) in all species. However, these values were higher than the values for arctic and alpine populations of *Oxyria digyna* (Mooney & Billings, 1961) and were in the same range of some cultivated species, e.g. maize (Hesketh, 1963). It was noted that arctic plants are characterized by high chl contents and a low chl a/chl b ratios, whilst alpine plants are lower in chl contents but with a higher chl a/chl b ratios (Mooney & Billings, 1961; Tieszen & Wieland, 1975; Tieszen, 1978). Abdulrahman & Winstead (1977) also reported a general pattern of greater chl levels with increasing latitude of origin from the six different populations of *Xanthium strumarium* which were grown under controlled laboratory conditions. In this study similar results were not observed for all the primulas examined.

P. laurentiana, as an arctic species, had significantly higher chl (a+b) than only 2 species; *P. farinosa* (c) and *P. scotica.* In fact *P. scotica* itself is regarded as a subarctic species, but this species had the lowest chl (a+b) content. Ratios of chl a to chl b were approximately in the same range for the group (2.2 to 2.6). These ratios fit into to the pattern for arctic plants which lie between 1.5 and 2.5 (Tieszen & Johnson, 1968), but the results can not differentiate between species.

It is well established that there is a proportional increase in the rate of photosynthesis with increasing light intensity (e.g. Rabinowith & Godvindjee, 1969; Bannister, 1976). In general, after light saturation the rate remains constant over a certain range of light intensities, but in still stronger light, the rate begins to decline, particularly if the illumination is prolonged. As expected, a similar result was attained for the primulas when photosynthesis was measured as the rate of O_2 evolution. At low temperature (5°C) and low PFD (200 μmol m^{-2} s^{-1}) the arctic species, *P. laurentiana,* had the highest rate whilst *P. farinosa* (b) had the highest rate at high PFD. These findings are in keeping with the general characteristics of

arctic plants (Chapin & Shaver, 1985a). It has been suggested that high photosynthetic rates at low illumination are probably due to high chl concentrations and a low chl a/chl b ratios (Mooney & Billings, 1961). This suggestion can also explain the photosynthetic characteristics of *P. laurentiana* which had the highest chl levels. In addition, it is noted that the low temperature optima of photosynthesis in arctic plants may relate to the high concentrations of RuBisCo (Berry & Björkman, 1980). *P. farinosa* (b) and *P. laurentiana* had nearly the same level of chl content, but chl a/chl b ratios were higher in *P. farinosa* (b) than *P. laurentiana.* It seems that the ratio of chl a to chl b plays an important role in controlling the light response of photosynthesis. At 5°C, the two populations of *P. farinosa* did not differ in their rates of O_2 evolution and they exhibited higher rates than the other species at high PFD (1,200-1,800 μmol m^{-2} s^{-1}). These results confirm the characteristics of alpine plants which exhibit a high rate of photosynthesis at low temperatures and high light levels (Bliss, 1985). At higher temperatures, the two populations of *P. farinosa* showed the same photosynthetic efficiencies at most PFDs. However, as a subalpine species, *P. frondosa* did not show the same performance even though its chl levels were in the same range as *P. farinosa* (b) and significantly higher than *P. farinosa* (c). The discrepancy may be explained by the fact that *P. frondosa* has a lower chl a/chl b ratio than *P. farinosa* (b) and *P. farinosa* (c).

At 15°C the rates of O_2 evolution were similar to the rates at 5°C suggesting unsaturated temperature responses for photosynthesis of the group. It should be noted that in contradiction to the characteristics of arctic plants, *P. scotica* showed the lowest rates at all ranges of PFD. This result may be attributed to its lowest chl level as well as a lower ratio of chl a/chl b than the other species. Nevertheless, at high temperatures, 25-30°C, and high PFD, *P. scotica* mostly show higher rates than the other species.

Shade leaves generally show lower saturation light intensities than do sun leaves (Bannister, 1976; Boardman, 1977; Rabinovith & Godvindjee, 1969). Mooney & Billings (1961) also reported a higher saturation light intensity in alpine populations

of *Oxyria digyna* than the arctic populations. At optimum temperatures for photosynthesis (20°C) light saturation points of the primulas were clearly not different and did not fit the pattern of sun and shade plants or arctic and alpine plants. Similar results were found with various plants from sun and shade habitats grown in South Finland (Aro *et al.*, 1986). This result may be due to their adaptation to the PFD of the growth chamber (Bannister, 1976; Saka & Chisaka, 1985). However, as it has been pointed out by Bannister (1976), it is difficult to define the exact point of saturation even from plant to plant in the same species.

The ecological importance of light compensation points is quite clear in that plants or plant parts with low compensation points are able to make the maximum use of low light intensities (Bannister, 1976). Light compensation points of the primulas were distinctly variable at different temperatures in each species. This result may be due to the rough estimations from the light response curve of O_2 evolution as it was rather difficult to investigate light saturation points by use of the O_2 electrode even though the PFD can be precisely controlled.

Diversities among species in their temperature responses may be characterized by their temperature optima, according to their photosynthetic activities over specific temperature ranges, or according to their tolerance to extremes of high or low temperatures (Berry & Raison, 1981). In general, species from warm habitats usually reach their optimum at somewhat higher temperatures than species from cool habitats (Berry & Björkman, 1980). The present data show that the optimum temperatures for the rates of O_2 evolution of the primulas were found to be at 20°C; the one exception being *P. scotica* where the optimum tends to be between 20 and 25°C. These optima were higher than for some arctic and alpine species, e.g. the temperature optimum of alpine herb, *Selinum vaginatum* was about 10°C (Krishna & Purohit, 1984). Chapin & Shaver (1985a) also noted the maximum rate of photosynthesis of arctic plants to be at 10-15°C. However, photosynthetic activities of arctic and alpine populations of *Thalictrum alpinum* were highest at 20°C and the variation in rates between populations was rather small (Mooney & Johnson, 1965).

This evidence is the most directly comparable to the results reported herein.

It is pertinent to note that the temperature dependence of photosynthesis is strongly influenced by the previous growth temperatures of the plant (Berry & Björkman, 1980), especially when working with leaf discs (Saka & Chisaka, 1985). Temperature optima may shift if the species is capable of acclimatizing to changing temperatures (Berry & Raison, 1981). It was found in an experiment that for many species the optimum appeared to change by about 1°C for each 3°C change in the growth temperatures (Berry & Björkman, 1980). In the case of the primulas, it seems likely that all the species tested could adapt to the day temperatures of the growth room (20-40°C) to a greater or lesser extent. Larcher(1969) also noted that the temperatures of photosynthetic optimum is not a specific characteristic of the species, but is a type of adaptation to the temperature conditions where the plant grows. In possible contradiction to the above discussion, Krishna & Purohit (1984) showed that *Selinum vaginatum*, an alpine perrenial herb which grows under high temperatures (21-34°C) during the growing season at low altitude, had a temperature optimum for photosynthesis at about 10°C. They concluded that photosynthetic characteristics of this species were not modified, but the low temperature optimum and high saturating light intensities were maintained which indicated the intrinsic nature of the alpine plants.

In general, increases in intensity of illumination bring about an upward displacement of both temperature optimum and temperature maximum until light saturation is achieved (Pisek *et al.*, 1973; Tranquillini, 1964). In primulas their optimum temperature did not shift clearly upward with increasing PFD because light saturation points had already been reached at the PFD of 400 μmol m^{-2} s^{-1}. At a PFD of 800 μmol m^{-2} s^{-1} onward, increasing temperatures to 30°C resulted in decreased rates of O_2 evolution, as can be seen from the lowest rates of O_2 evolution at a PFD of 1,200 μmol m^{-2} s^{-1}. This result may be useful to explain the intrinsic sensitivity of arctic and alpine plants to high temperature. Such responses, therefore, indicate that the plant lacks the ability to acclimatize to high temperature

and probably suffers damage to the photosynthetic apparatus (Björkman & Badger, 1977; Bjorkman *et al.*, 1976). Responses similar to those of the primulas have been reported in *Atriplex lentiformis* (Pearcy, 1977), *Agropyron smithii* (Williams, 1974) where natural distribution is limited to cool-temperature environments, and in arctic and alpine populations of *Thalictrum alpinum* (Mooney & Johnson, 1965). As an associated species of *P. farinosa, Thalictrum alpinum* showed a similar temperature response curve for photosynthesis, however, an attempt to compare the rates of photosynthesis failed due to the lack of a valid conversion factor between the two different units used. Berry & Björkman (1980) also emphasized that comparisons between the results from different authors on different plant species and in different environments usually encounter the same problems as the work on temperature dependence of photosynthesis, because even a single leaf is markedly influenced by many environmental factors and also the previous history of the plants can have a profound effect.

Higher dark respiration rates of plants from high altitudes or latitudes compared with plants from low altitudes and latitudes have been suggested to be an adaptation to compensate for low temperatures and short growing seasons (Wager, 1941). Mooney & Billings (1961) found that the arctic plants of *Oxyria digyna* have higher respiration rates at all temperatures than do the alpine plants of the same species. In contrast, Mooney & Johnson (1965), working with arctic and alpine populations of *Thalictrum alpinum,* found that dark respiration rates were similar for plants of all populations at 20°C. Results from experiments reported here have shown significantly higher dark respiration rates at low temperatures in *P. farinosa* from both populations than the other species and again this does not match the pattern of arctic and alpine plants as has been reported by Mooney & Billings (1961). However, at higher temperatures rates of dark respiration were similar in all species, except *P. scotica* which usually showed the lowest rates of dark respiration in the group. This latter result was in agreement with the work of Mooney & Johnson (1965).

There were both positive and negative correlations between leaf thickness and the rates of O_2 evolution in different species over the wide ranges of the PFD measured (Tables 5.36-5.40) and no relationship at all for *P. scotica.* Contrary to the expectation in Ch 3 that *P. laurentiana* might show the lowest rate of photosynthesis due to it having the thickest leaves, this was not so. It was found that leaf thickness of *P. laurentiana* showed positive correlations with the rates of O_2 evolution and this species also showed the highest rates of O_2 evolution at the optimum temperature (20°C) in 5 out of 6 PFD studied. The data from this study (Table 5.41) indicated that there was a tendency to a positive correlation between leaf thickness and the rates of O_2 evolution rather than a negative correlation (El-Sharkawy & Hesketh, 1965). This different result could be due to the occurrence of chloroplasts in the cells of the epidermis as well as mesophyll cells of the tested species. Thus, increased leaf thickness resulted in increased chl content per unit area, and also increased photosynthetic activities.

Wilson & Cooper (1969a) found good correlations between stomatal parameters on the one hand and mesophyll cell size on the other with the rates of O_2 evolution in genotypes of *Lolium perenne,* when there was light limitation for photosynthesis. The results given here showed both positive and negative correlations between stomatal pore length and the rates of O_2 evolution. However, no relationship was found in *P. laurentiana* which had the highest values of stomatal pore length for the species studied in these experiments. The results of correlations between stomatal frequency and the rates of O_2 evolution also showed both positive and negative correlations. It is worth noting that negative correlations between stomatal parameters (e.g. stomatal pore length) and the rates of O_2 evolution, are usually found at low PFDs (Table 5.41). This result is partly in agreement with the work of Wilson & Cooper (1969a). Nevertheless, significant correlations between the products of stomatal frequency and pore length were found only in *P. farinosa* (b) and this corresponds with the work of Hesketh (1963) who found no general relationship between photosynthesis and the products of stomatal frequency and

pore length in 9 cultivated species.

Taking cell size into account, significant negative correlations were found only in *P. frondosa* at a PFD of 25 μmol m^{-2} s^{-1}. This result may be due in part to the fact that epidermal cell size had less importance for photosynthesis even though the primulas had chloroplasts in epidermal cells as well as mesophyll cells. However, the positive correlation between the number of epidermal cells and the rates of O_2 evolution (Table 5.41) indicated an importance of the epidermal cells for photosynthesis. The tendency of positive correlations between thickness of mesophyll and the rates of O_2 evolution in 4 out of 5 species studied may stress the importance of the mesophyll as a photosynthetic tissue, especially in *P. laurentiana* in which good correlations were found at many PFDs. The number of mesophyll cells per unit area correlated with the rates of O_2 evolution in *P. farinosa* (b), *P. farinosa* (c), and *P. laurentiana*. However, the expected positive correlations were found only in *P. farinosa* (b) and *P. laurentiana*. The positive correlation between the rate of photosynthesis and number of mesophyll cells has also been noted by Wilson & Cooper (1969a).

It is generally recognized that chl levels have a profound effects on the rate of photosynthesis, and the maximum photosynthesis may be determined by chl content (Rabinowitch, 1951). In this study the results showed positive significant correlations between chl content and the rates of O_2 evolution on leaf area basis only in *P. farinosa* (b) and these correlations were found at high PFD (600-1,000 μmol m^{-2} s^{-1}). However, when the data were pooled, positive correlations were found at many PFDs (Table 5.41). Working with 9 species from sun and shade plants, Aro *et al.* (1986) likewise did not find any correlation between the rates of O_2 evolution and the chl content.

Significant relationships between leaf characteristics and the rates of dark respiration were found in both direct and indirect ways. Of these, the interesting ones were the positive correlations with stomatal size in *P. laurentiana* as well as the

positive correlations with the products of stomatal frequency and pore length of the group. The large stomatal size may be responsible for the high rates of dark respiration in this species, especially at warm temperatures However, there were no previous results which could support these conclusions.

It should be noted that the measurements of respiration and photosynthesis of small leaf discs can not be generally applied to the whole organ, because the rates for discs differ from the rates for the whole leaves (Zurzycki & Starzecki, 1971). It is likely that the short term exposure of a plant to different environmental conditions is not enough to determine its degree of plasticity since plants in natural habitats are subjected to transient and more persistent variations of environmental factors. An understanding of the effect of environmental factors on photosynthesis in a field situation requires considerably more information than is obtained from just a single factor (Berry & Björkman, 1980).

CHAPTER 6

RESPONSES OF PLANTS TO WATER STRESS

6.1 Introduction

It is generally recognized that terrestrial plants encounter drought stress at some stagesof their life cycle and the ability to cope with this problem is an important factor in determining the natural distribution of plants throughout the world (Fischer & Turner,1978). It seems that some plant species can survive and grow under drier condition than others. Plant adaptations to such environments can be put at four levels: phenological or developmental, morphological, physiological, and biochemical (Hanson, 1980; Turner & Begg, 1981). Of these levels, biochemical adaptation to drought stress is the least known and understood (Hanson & Hitz, 1982).

The development of a water deficit leads to a wide range of responses by plants (Hsiao, 1973). According to Tang (1983), plant water deficit affects every aspect of plant life including photosynthesis, respiration, absorption of water and mineral elements, growth, development, reproduction etc. Hsiao *et al.* (1976b) also summarized the direct effects of a loss of water from tissue as:-

(1) reduction in the chemical potential or activity of waters,

(2) concentration of macromolecules and of solutes of low molecular weight,

(3) changes in spatial relations in membranes and organelles through the reduction in volume

(4) reduction of hydrostatic pressure inside the cells.

Since protein synthesis is closely related to the production of new tissue so

then when water stress inhibits growth, nitrogen metabolism is eventually upset (Kramer, 1983). Early workers, such as Borodin (1876) and Schulze (1879) proposed that plant proteins are not completely stable, but simultaneously experience both synthesis and degradation (see Cooke, 1981). Cooke (1981) also noted the work of Mothes that leaf protein levels are controlled by two systems, one for producing and one for removing proteins. Direct evidence for protein turnover in plants is given by Steward, Bidwell & Yemm (1956), working with carrot root explants, who found that some of the amino acids which arise by protein breakdown might be re-utilized for protein synthesis. They also showed that protein synthesis and breakdown must take place in separate phases of the cell, these results being supported by the work of Ryan & Walker-Simmons (1981).

Wildman & Bonner (1947), working with spinach leaves, found that the cell free protoplasm of leaves comprises of 3 types of nitrogenous materials: the soluble, low molecular weight, nitrogenous material, the particulate matter, and the soluble protein of the leaf cytoplasm (see also Wildman, Cheo, & Bonner, 1949). The soluble cytoplasmic protein has been found to represent between 23-50% of the total leaf protein (Wildman & Cohen, 1955).

Available evidence suggests that protein levels in the leaf vary, depending on many factors, e.g. leaf age and environmental conditions (Huffaker, 1982). It is recognized that growing tissues and organs synthesize protein at a markedly high rate; whereas in ageing tissue and organs, such as old leaves and parts of the flower, protein degradation appears to dominate the whole process (Steward *et al.*, 1965; Larcher, 1980). Mae, Makino & Ohira (1983), working with rice found that in a fully expanded leaf, RuBisCo accounts for half or more of the total soluble protein content and that even in the same leaf the protein content of each region is different. Similar results were reported by Dungey & Davies (1982), working with barley leaves, who found that the rate of protein synthesis is greatest in the cells of the youngest region and that this declines as each region ages. Total soluble protein content changes during leaf ontogeny as reported by Viana & Metivier (1980) work-

ing with sweetgrass (*Stevia rebaudiana*). In contrast, leaf age does not markedly affect the composition of protein in daffodil leaves (Bryant & Fowden, 1958).

Recent biochemical investigations have shown that both chromosomal and genome duplication have marked effects on protein content as well as physiological activities (Resende *et al.*, 1964; Bjurman, 1959; Tal, 1977). Studies by Tal (1977) in tomatoes produced evidence that autotetraploid plants have a lower protein content than diploid plants. In contrast, Leech *et al.* (1985) working with *Triticum* genotypes found that the amount of RuBisCo per cell is highest in hexaploids, then in tetraploids and the lowest in diploids. However, Timko *et al.* (1980) found no differences in protein content per unit fresh weight in leaves of haploid, diploid and tetraploid plants of *Ricinus communis*.

Protein metabolism has been related to the adaptation of plants to environmental change and stress, such as influences of temperature, light, nutrient and water supply (Steward, 1963; Larcher, 1980). Shah & Loomis (1965) found that both soluble and total protein contents of sugar beet leaves decline progressively during water stress. Similar results have been obtained by Stutte & Todd (1969) with wheat leaves, Barnett & Naylor (1966) with bermuda grass, and Shiralipour & West (1984a) with maize seedlings. Similarly, Maranvill & Paulsen (1972) reported that seedlings of corn show a reduction in protein synthesis, instead of increased protease activity causing leaf protein concentration to decrease during severe water stress condition, but significant decreases in protein are not found during mild stress. They also indicated that protein synthesis is inhibited, or proteolysis is increased, or a combination of both occurs as also explained by Hsiao (1973), Cooke (1981), Bewley (1981), and Dungey & Davies (1982). Furthermore, it can be noted that recovery from water stress results in an increase in both insoluble and soluble protein (Shah & Loomis, 1965). In addition it was reported that the more resistant wheat varieties show a greater amount of protein than non-resistant varieties (Stutte & Todd, 1967).

One of the major adaptations of plants to water stress is the ability to synthesize nucleic acids (Kessler & Tishel, 1962) and therefore, to renew proteins during drought and to effect repair rapidly after drought (Henckel, 1970). Studies in excised and attached leaves of many species led Subbotina (1962) to conclude that wilting leads to an increase of the amount of protein nitrogen, which is evidently due to the increased amine form of nitrogen.

There is ample evidence that similar biochemical changes occur when plants are undergoing cold hardening or are subjected to water stress (Hsiao, 1973; Levitt, 1980). For instance, the increase in protoplasmic viscosity correlated with water stress (Startseva & Ishmukhametova, 1973) corresponds to the seasonal increase in total protoplasm during cold acclimation (Siminovitch *et al.*, 1967). Briggs & Siminovitch (1949) and Siminovitch & Briggs (1949) studied the soluble protein content of the bark of the black locust tree (*Robinia pseudo-acacia*) throughout the seasons and found that water-soluble protein increased in concentration in the autumn, coinciding with the development of frost hardiness. The concentration decreased in the spring with the disappearance of hardiness. They suggested that the constituents of the bark bear some casual relationship with the mechanism of development of frost hardiness. Later, Siminovitch (1963), using black locust bark cells and Pomeroy & Siminovitch (1970) using the living bark and needles of red pine (*Pinus resinosa*) found a similar relationship between the seasonal change in soluble protein and in the rate of incorporation of radioactive amino acids into proteins.

In agreement with the previous studies ivy leaves (*Hedera helix*) showed a gradual increase in water-soluble protein from summer to winter (Parker,1962). However, in spring, water-soluble proteins remained at a high level into April and this was true for two following years; whereas the leaves declined markedly in hardiness. Similar results have been obtained by Gerloff, Stahman & Smith (1967) with alfalfa root, Gusta & Weiser (1972) with box wood leaves (*Buxus microphylla* var. *Koreana*) and Morton (1969) with cabbage leaves. Not only soluble protein, but

also total protein was found to increase during developing hardiness (Chen & Li, 1977; Li, Weiser & Van Huystee, 1966). Likewise, Kacperska-Palacz (1978) noted that accumulation of water-soluble proteins is commonly found in herbaceous plants during hardening. The cause of increase has been suggested by Morton (1969) and Kacperska-Palacz *et al.* (1977) to be due to an increase in the concentration of the same kinds of protein which are found in non-hardy plant and/or a synthesis of entirely new proteins. In contrast, Pellett & White (1969) using *Juniperus chinensis* and Young (1969) with grapefruit found no change in total nitrogen or water-soluble protein during hardening.

The response of plants to chilling also results in protein breakdown as was reported by Razmaev (1965), working with wheat and corn. He found that protein synthesis in chill-sensitive plants is replaced by a proteolytic process within one day after low temperature treatment; hence, the protein content decreased, whilst the soluble-N content increased. In contrast, no change occurred in cold resistant plants. Smith & Powell (1976), showed that in cotton, there was a rapid increase in protein breakdown simultaneously with the commencement of membrane changes. Recently, Rosinger *et al.* (1984), working with chill-sensitive and chill-resistant plants, found that four days chilling at 5°C and 85% relative humidity caused a decrease in the leaf soluble protein content in all tested species, the decrease was greatest and most rapid in mung beans (chill-sensitive species). On the other hand, if plants of each species were chilled to 5°C and 100% relative humidity, water loss was greatly reduced and there was no significant decrease in leaf soluble protein. They concluded that the protein decrease which occurred at 5°C and 85% relative humidity was a response to water loss and not a direct response to low temperature. Levitt (1980) suggested that chilling induced respiratory changes and that these could result in a shift from protein synthesis to proteolysis.

There is no doubt, however, that the ability of plants to undergo the cold-hardening process is genetically controlled (see Coleman *et al.*, 1966). Differences in response to low temperatures are found not only in plants of different species but

also in varieties of a single species. This was shown by Coleman *et al.* (1966), who found that 6 genotypes of alfalfa (*Medicago sativa*) reacted differently when exposed to sub-freezing temperatures. Corresponding results were presented by Sugiyama & Simura (1967) for tea plants, where the soluble proteins increased during the attainment of freezing tolerance, under natural and artificial hardening conditions, with the more resistant varieties showing the highest concentrations. In contrast, Marutyan *et al.* (1972) noted that total soluble and water soluble proteins of the non frost-hardy variety of vine were higher than in the frost-hardy variety.

Concomitant with protein synthesis, amino acids play an essential role in plant nitrogen metabolism and exhibit a close relationship with protein metabolism (Fukutoku & Yamada, 1984; Tan & Halloran, 1982). Free proline, which is low in non-stressed plants, is known to accumulate in plant tissue in response to environmental stress and since the first report of its accumulation in wilted tissue of perennial rye-grass (Kemble & Macpherson, 1954), several investigations have been conducted on the influence of environmental stress on its metabolism. Available evidence shows that proline accumulation seems to be a general response to stress since it happens with drought stress (Barnett & Naylor, 1966; Singh *et al.*, 1973; Hanson *et al.*, 1979), salt stress (Stewart & Lee, 1974; Goas *et al.*, 1982; Weimberg *et al.*, 1982), temperature stress (Chu *et al.*, 1974; 1978; Paquin & Pelletier, 1981; Vezina & Paquin, 1982), mineral deficiency (Thompson *et al.*, 1960; Goring & Thien, 1979), waterlog stress (Aloni & Rosenshtein, 1982), heavy metal pollution (Farago & Mullen, 1981) and disease (Seitz & Hochster, 1974).

It has been pointed out by Aspinal & Paleg (1981) that proline accumulation is a primitive response of living organisms due to water deficit. However, the fact that no proline accumulation occurs in some drought-tolerant species of bryophytes, lichens, pteridophytes and flowering plants when subjected to severe water stress led Stewart & Larcher (1980) to conclude that proline accumulation cannot be regarded as an universal response in water-stressed plants. The amount of proline that is accumulated varies with the degree of stress and species (Voetberg & Stewart, 1984).

According to Palfi *et al.* (1974) plants can be grouped into species which either do, or do not accumulate free proline during water deficit. They concluded that mesophytic cultivated plants, for example the entire Solanaceae family and most species of the Fabaceae, Caesalpiniaceae, Mimosaceae, Brassicaceae, Umbelliferae, Asteraceae, and Poaceae accumulate proline under water stress (the proline type). Previously Palfi (1969) had considered all plants to be of the proline type and subdivided them into two subgroups:-

(1) proline increasing to 5-10 fold of the normal level, e.g. maize, wheat, rice

(2) proline increasing 10-100 fold of the amount present in the control plant, e.g. sunflower, peas, tobacco.

Although a very marked increase in free proline content is seen in leaf tissues of many mesophytic plants (Barnett & Naylor, 1966; Waldren & Teare, 1974; McMichael & Elmore, 1977), the levels do not usually exceed 200 μmol g^{-1} dw (Hanson & Hitz, 1982).

Proline accumulation under water stress decreases when normal water relations are restored (Singh *et al.*, 1973; McMichael & Elmore, 1977; Thakur & Rai, 1981; Chang & Lieu, 1984). However, in some species, e.g. sorghum, recovery from water stress is not as complete, and tissue proline concentration may remain above that of non-stressed plants for several days (Aspinal & Paleg, 1981). It was also found in water-stressed barley leaves that free proline only declines after relief of water stress in viable tissue, but not in tissue killed by drought (Hanson *et al.*, 1977). In addition, it was found that proline accumulation is favoured by high leaf carbohydrate (Routley, 1966; Stewart *et al.*, 1966) and also by illumination (Goas *et al.*, 1982; Chu *et al.*, 1974; Stefl *et al.*, 1978).

It is known that proline accumulates in many parts of the intact plant under stress, (Purvis & Yelenosky, 1982; Vezina & Paquin, 1982), but that accumulation is most rapid and extensive in the leaves (Singh *et al.*, 1973; Routley, 1966; Barnett

& Naylor, 1966). In contrast, Ozturk & Szaniawski (1981) reported that the proline content in the root tissue of maize was, on an average, twice that in the leaves when root temperatures were decreased. However, they were unable to show that proline accumulated in the roots of the intact plants was translocated from the shoot system or locally synthesized in the roots. It has been postulated that proline accumulates firstly in leaf and is then transported to the roots during stress periods (Oaks, 1966; Chang & Lieu, 1984). It is reported that the site of cellular proline accumulated is in the cytoplasm (Goring & Thien, 1979; Aspinal & Paleg, 1981).

Intensive studies on proline biosynthesis have revealed that drought stress possibly stimulates its synthesis from glutamate combined with a lowered rate of oxidation and/or reduced incorporation into protein (Barnett & Naylor, 1966; Boggess & Stewart, 1980; Stewart, 1981; Hanson & Hitz, 1982). Accumulation of proline due to other environmental stresses, e.g. temperature, salinity, nutrient deficiencies, may be expressed in the same way, i.e. due to an immediate dehydration of the cytoplasm -commonly called 'physiological drought' (Rosinger *et al.*, 1984; Goring & Thien, 1979; Palfi & Juhasz, 1970). However, it was found that proline accumulates in barley at temperatures below 12°C without any parallel decrease in water potential of the leaf tissue (Chu *et al.*, 1974; 1978), thus casting doubts on the above suggestion.

At present, the role of proline accumulation in adaptation to water stress is not clearly understood, and is still the topic of controversy (Dashek & Erickson, 1981; Fukutoku & Yamada, 1984; Shiralipour & West, 1984b). Possible roles have been proposed by many workers, example being:-

(1) as a cytoplasmic osmoticum (Stewart & Lee, 1974; Stewart & Larcher, 1980),

(2) as an agent protecting against enzyme inactivation (Paleg *et al.*, 1984),

(3) as a stimulant for normal respiration of the cells after stress (Palfi *et al.*, 1974),

(4) as a desiccation protectant (Schobert, 1977; Schobert & Tschesche, 1978),

(5) as an inducer of the biosynthesis of nucleic acid and protein (Stefl *et al.*, 1978),

(6) as a source of energy during recovery from stress (Barnett & Naylor, 1966; Stewart *et al.*, 1966; Blum & Ebercon, 1976; Aloni & Rosenshtein, 1982).

This last idea is the one which has received the most recent attention (Fukutoku & Yamada, 1984). In complete contrast, Hanson *et al.* (1977) suggested that proline accumulation is an incidental consequence of stress.

The consistency of proline accumulation during stress has led many workers to investigate the possibility of its use as an indicator of stress, in particular drought stress. However, Waldren & Teare (1974) working with intact leaves of sorghum and soybean, found that free proline did not accumulate markedly in either species until each was severely stressed, indicating that proline is not a very sensitive indicator of drought stress. McMichael & Elmore (1977), working with cotton leaves, found that proline concentrations remained fairly constant until the stress became severe during the third day and then increased markedly, so they also concluded that concentration of free proline is not a good indicator of the onset of plant drought stress.

It is very obvious that certain plant species show a significantly higher resistance to stress than others and it is usually found that resistant species and susceptible species accumulate proline in different amounts. Accordingly, it has been suggested that proline can be used as a single parameter to measure the magnitude of water stress (Palfi & Juhasz, 1971; Palfi *et al.*, 1973; Singh *et al.*, 1973; Blum & Ebercon, 1976), frost hardiness (Draper, 1975; Paquin & Pelletier, 1981), and flooding stress (Aloni & Rosenshtein, 1982). However, comparisons between drought-resistant and drought-susceptible cultivars do not permit any firm conclusion about the adaptive role of this plant function. According to Palfi & Juhasz (1970), amongst the cultivated varieties of a species, drought-resistant varieties synthesize more proline than less resistant ones, when the water stress is equal. This same result has been reported by Singh *et al.* (1972) with barley varieties, Rao &

Shivraj (1985) with foxtail millet cultivars, Mali & Mehta (1977) with rice cultivars, under water stress, and Benko (1968) with apple varieties under frost stress. In contrast, the drought-tolerant species *Artemesia herba-alba* has a low content of free proline in non-stressed plants and shows no accumulation during period of stress (Pourrat & Hubac, 1974). Rao & Nainawatee (1980) found a higher proline accumulation in less drought-resistant cultivars of wheat under water stress. In agreement with this result, Levy (1983), working with potato cultivars, found a small increase in proline content in drought-tolerant cultivar as compared with a marked increase in proline of the susceptible cultivar during water stress. In addition, the results of Waldren & Teare (1974) working with the drought susceptible species 'sorghum' and drought resistant species 'soybean' and also Patel & Vora (1985) working with wheat, *Plantago,* papaver, and mustard, can be expressed in the same way.

This investigation was carried out to explore possible associations between protein/proline levels and environmental factors, e.g. drought, low temperatures, etc.The initial interest in studies of the response of primulas to water stress and frost stress comes from the characteristic habitats and geographical distribution of this group. Most of them usually occur naturally in damp places and experience various levels of frost stress and water stress during winter. It seems likely that they may be sensitive to drought and frost to different degrees.

6.2 Materials and methods

6.2.1 Plant material

6.2.1.1 Laboratory experiment

Seeds of *P. farinosa* (b), *P. farinosa* (c), *P. frondosa, P. halleri, P. laurentiana, P. scotica,* and *P. stricta* were germinated on moist filter paper in Petri dishes at 20° C. 2 wk after sowing, healthy seedlings were transferred to 8 cm plastic pots filled with J.Arthur Bower's seed and potting compost. The pots were placed on a bench in the green house, Botanic Garden, University of Durham, with a light intensity of 160 μmol m^{-1} s^{-1} (PAR) provided by sodium lamps with a 16 h photoperiod. Temperature and humidity were not controlled. One month after sowing the pots were placed on the floor under natural light. After 20 wk, the plants were moved to a growth chamber with conditions as previously described (see 1.2.3). At this stage, the primulas had 8-12 fully expanded leaves, and roots were observed at the bottom of the pots. The plants were watered regularly with the same amount of water to ensure homogeneus humidity conditions. Water stress was imposed by withholding water when the primulas were 22 wk old. Leaf water saturation deficit (WSD) was used to estimate the internal severity of drought stress.

Samples of fully expanded green leaves were used in the experiments. Three to eight leaves were used for one sample (from 4 plants) to measure WSD and protein/proline levels.

Leaves were severed along the midrib into two and after weighing the samples were put into liquid nitrogen, and stored in a -20°C room prior to determinations being carried out. One set of leaf halves was used for protein determination and the other for proline measurements.

6.2.1.2 Field experiments

Two plants of the five genotypes/species of primulas which had been used for

plant growth analysis (Ch 4):- *P. farinosa* (b), *P. farinosa* (c), *P. frondosa, P. laurentiana,* and *P. scotica,* were preserved for protein/proline determination. Leaf samples were taken in midsummer, after some period of drought, from the three sites:- South Bents, Sunderland (SUN); Hartside nursery, Alston, Cumbria (HAR); Great Dun Fell Radar Station, Cumbria (GDF) (see Fig. 4.1). At the sea level site, SUN, four species; *P. farinosa* (b), *P. farinosa* (c), *P. frondosa* and *P. laurentiana* had wilted but no sign of wilting was found in *P. scotica* (see also Fig. 4.14). At the other two sites no wilted plants were noticed. Leaves were cut, wrapped in muslin sheet, then plunged into liquid nitrogen for return to the laboratory and were stored in the -20°C room before chemical analysis was made.

For all determination of WSD and protein/proline levels, three replicates were taken and mean values were presented in the results.

6.2.2 Measurement of leaf water saturation deficit

Six leaf discs, 1 cm in diameter, were cut using a sharp cork borer from the laminas of a set of leaves and were placed into preweighed vials. After the fresh weight (FW) had been obtained the leaf discs were floated on distilled water in covered Petri dishes which were kept in a high air humidity chamber for six hours at room temperature (about 20-23°C), under fluorescent light (daylight tubes) of 20 μmol m^{-1} s^{-1} (PAR). The discs were surface dried with filter paper and returned to the same vial for turgid weight (TW) determination. Following drying at 70°C for 2 d and dry weight (DW) was determined. Calculation of WSD was carried out by the method reported by Weatherley & Slatyer (1957) and Slatyer (1967). WSD is given by:-

$$WSD = \frac{TW - FW}{TW - DW} \times 100 \qquad 6.1$$

The relative water content can be calculated by subtracting WSD from 100.

6.2.3 Proline determination

Free proline was determined using an acid-ninhydrin method as described by Bates *et al.*, (1973). 0.2 gm of plant sample was ground by mortar and pestle with acid-washed sand and 25 cm^3 of 3% aqueous sulfosalicylic acid. The homogenized sample was filtered through Whatman No. 1 filter paper. 2 cm^3 of filtrate was reacted with 0.15 gm "Amberite" resin in a test tube to remove some amino acids, for example ornithine, lysine and hydroxylysine, which can interfere with proline determination. 2 cm^3 of glacial acetic acid and 2 cm^3 of acid-ninhydrin[1] were then added to 2 cm^3 of the filtrate.

The mixture was heated at 100°C for 1 h, and the reaction was terminated by cooling the tube in an ice bath. Under the conditions specified above, a red, water-insoluble reaction product was formed between proline and ninhydrin at approximately pH 1.0 (Chinard, 1952).

The reaction mixture was extracted with an equal volume of toluene, and following vigorous mixing for 30 sec, was allowed to warm to room temperature. On standing the pigment partitioned into the upper toluene layer. Absorbance was measured at 520 nm using a spectrophotometer. Toluene was used as a blank and the proline concentration was determined from a standard curve, prepared using L-proline. Calculation of the proline concentration was carried out on a fresh weight basis using the equation (Bates *et al.*, 1973):-

$$\mu mol\ proline\ g^{-1}\ fw = \frac{(\mu g\ proline\ cm^{-3} \times cm^3\ toluene)}{115.5\ \mu g\ \mu mol^{-1}} \times \frac{2}{(g\ fw\ sample)} \qquad 6.2$$

The proline concentration on a dry weight basis was also calculated, using the relationship between fresh weight and dry weight of each species.

[1] Acid-ninhydrin was prepared by warming 1.25 gm ninhydrin in 30 cm^3 glacial acetic acid and 30 cm^3 6M phosphoric acid, stirred, until completely dissolved.

6.2.4 Protein determination

0.2 gm of plant sample was ground using a pestle with 4 cm^3 of Tris buffer. [2] The homogenate was transferred into a centrifuge tube and centrifuged for 10 min in a top bench centrifuge (Griffin Christ) at full speed.

6.2.4.1 Extraction of soluble protein

The water soluble protein in the supernatant fraction was precipitated by adding an equal volume of 10% Trichloroacetic acid (TCA) followed by standing for 30 min in an ice bath. The protein was pelleted by centrifugation at full speed for 10 min, and was washed by re-suspending in 2 cm^3 of 5% TCA followed by re-centrifugation. The protein precipitate was dissolved in 1 cm^3 of 1 N NaOH by warming to 80°C in a water bath.

6.2.4.2 Extraction of insoluble protein

The protein pellet (from 6.2.4) was decolourized by washing twice in 2 cm^3 of 1:1 (v/v) mixture of chloroform and methanol followed by one wash in 2 cm^3 of absolute methanol. Protein was dissolved as in section 6.2.4.1.

6.2.4.3 Protein measurement

Protein was measured by the method of Lowry *et al.* (1951). 5 cm^3 of copper sulphate reagent [3] was added to each dissolved protein mixture and allowed to stand for 10 min. After this time, 0.5 cm^3 of 1N Folin & Ciocalteu's phenol reagent was added and the mixture allowed to stand for a further 30 min. The absorbance of the blue-coloured solution was measured at 520 nm, using the spectrophotometer with distilled water as a blank.

The protein concentration were determined from a standard curve, using known

---

[2] 0.04 M Tris (hydroxymethyl) methylomine, pH 7.5; 0.1 M magnesium sulphate, and 0.025 M EDTA

[3] 0.5 cm^3 1% copper sulphate, 0.5 cm^3 2% of sodium/potassium tartrate, and 50 cm^3 of 2% sodium carbonate

concentrations of Bovine serum albumin fraction V (BSA V).

6.3 Results

6.3.1 Laboratory experiment

Fig. 6.1 shows the relative water contents of the leaves measured at 1 wk before the onset of water stress. ANOVA results are summarized in Table 6.1, indicated there was a highly significant difference ($P<0.0001$) between species, with *P. halleri* having the lowest relative water content (Table 6.2). There was no significant difference in relative water content between the two populations of *P. farinosa*. No relationship was found ($r = -0.49$) between relative water content and chromosome numbers. Comparisons of total protein contents between species (Fig.6.2) in non-stressed plants, measured on the same day as relative water content, showed the lowest values in *P. stricta,* whilst *P. scotica* was the highest. Table 6.3 shows that there were highly significant differences between species in total protein content. Significant differences were seen between many pairs of species, particularly between *P. farinosa* (b) and *P. farinosa* (c) (Table 6.4). Total protein content of the tested species was rather variable and only a poor correlation was found ($r = -0.33$) with the chromosome number.

6.3.1.1 Leaf water saturation deficit

It can be seen from Fig. 6.3a to Fig.6.9a that WSD was lowest in all the tested species at day 0. Increasing water deficit resulted in increased WSD which dropped suddenly after re-watering in all the species examined and termination of water stress in 3 species. However, the results of ANOVA (Table 6.5) for each species showed significant differences in WSD during water stress for *P. farinosa* (b), *P. frondosa, P. laurentiana,* and *P. stricta,* but not in *P. farinosa* (c), *P. halleri,* and *P. scotica.* On the other hand, comparisons between controls and treated plants (Table 6.6) revealed no significant differences for all species at day 0. From day 1 onward, there were significant differences in all species except *P. scotica* and *P. stricta.* However, *P. stricta* showed significant differences to the controls on day 4, whilst on day 6 *P. scotica* still showed no significant difference. After re-watering,

Figure 6.1 Relative leaf water content in *Primula farinosa* and its allies.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
HAL = *P.halleri*
SCC = *P.scotica*
STT = *P.stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

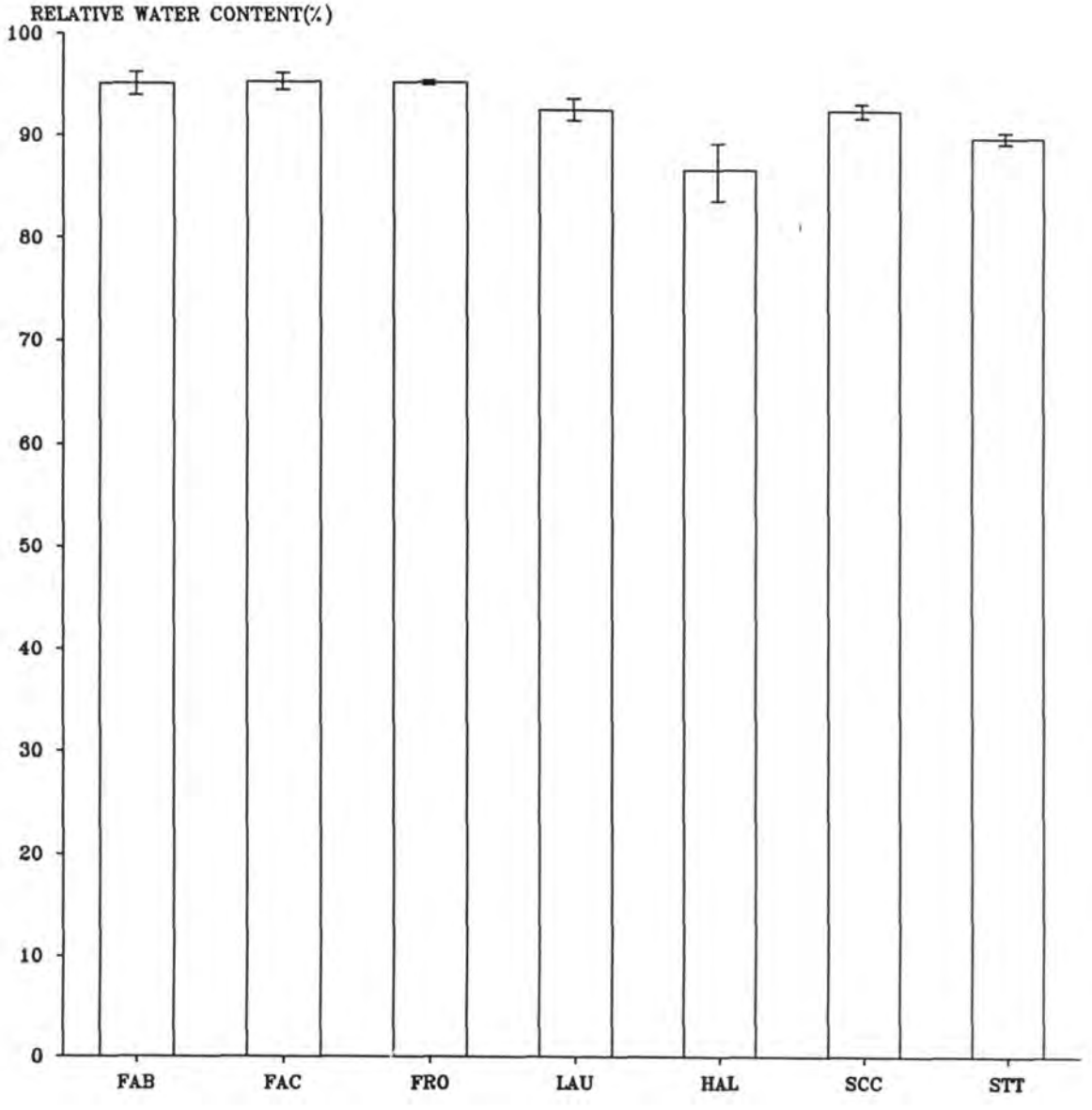

RELATIVE WATER CONTENT(%)
100
90
80
70
60
50
40
30
20
10
0
FAB
FAC
FRO
LAU
HAL
SCC
STT

Figure 6.2 Total protein content in *Primula farinosa* and its allies.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
LAU = *P.laurentiana*
HAL = *P.halleri*
SCC = *P.scotica*
STT = *P.stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

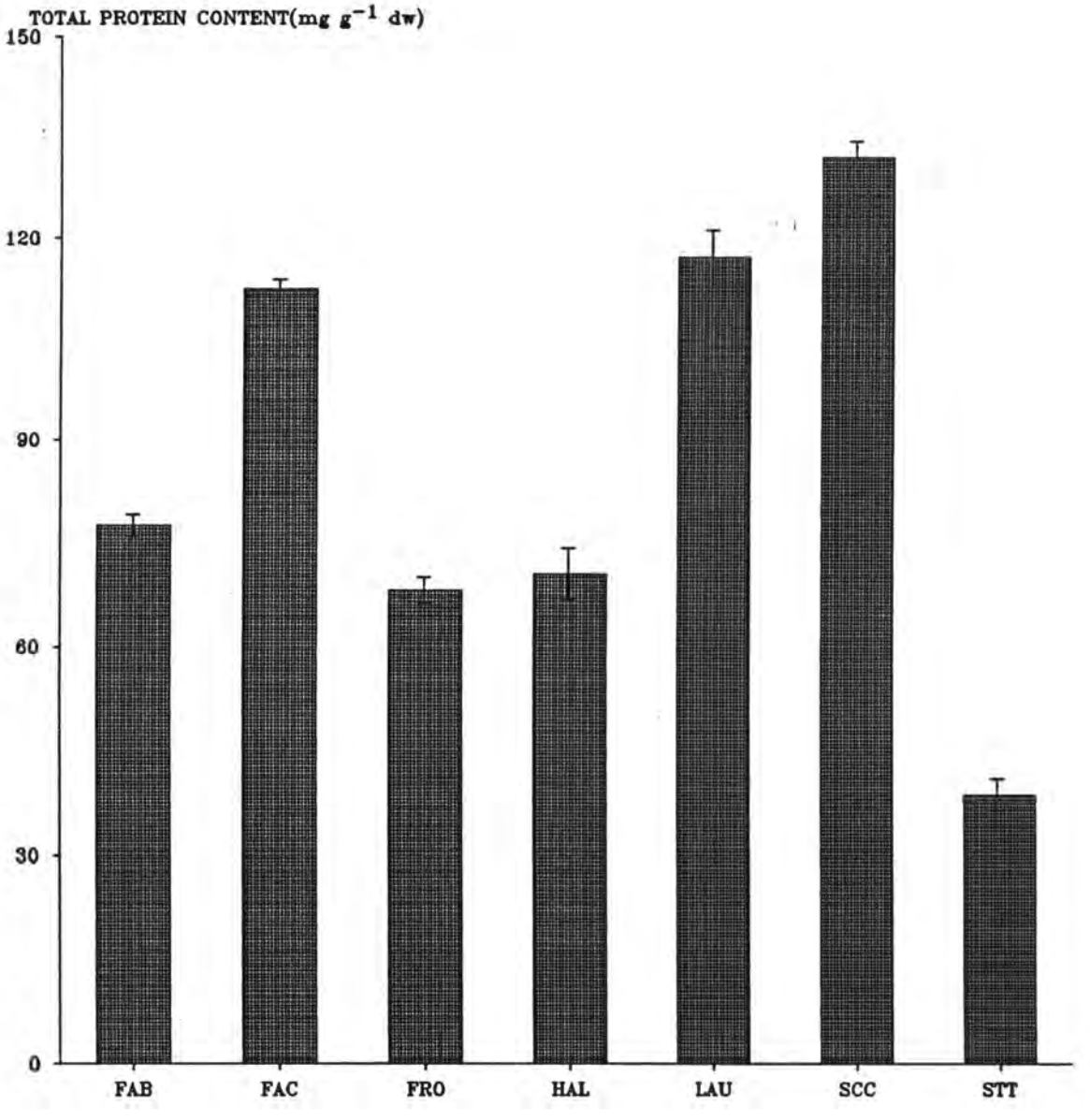

TOTAL PROTEIN CONTENT(mg g^{-1} dw)
150
120
90
60
30
0
FAB
FAC
FRO
HAL
LAU
SCC
STT

Figure 6.3 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula farinosa(b)*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟——⊟ Treated plants

Denotes date of rewatering
(water withheld before and after the date).
Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

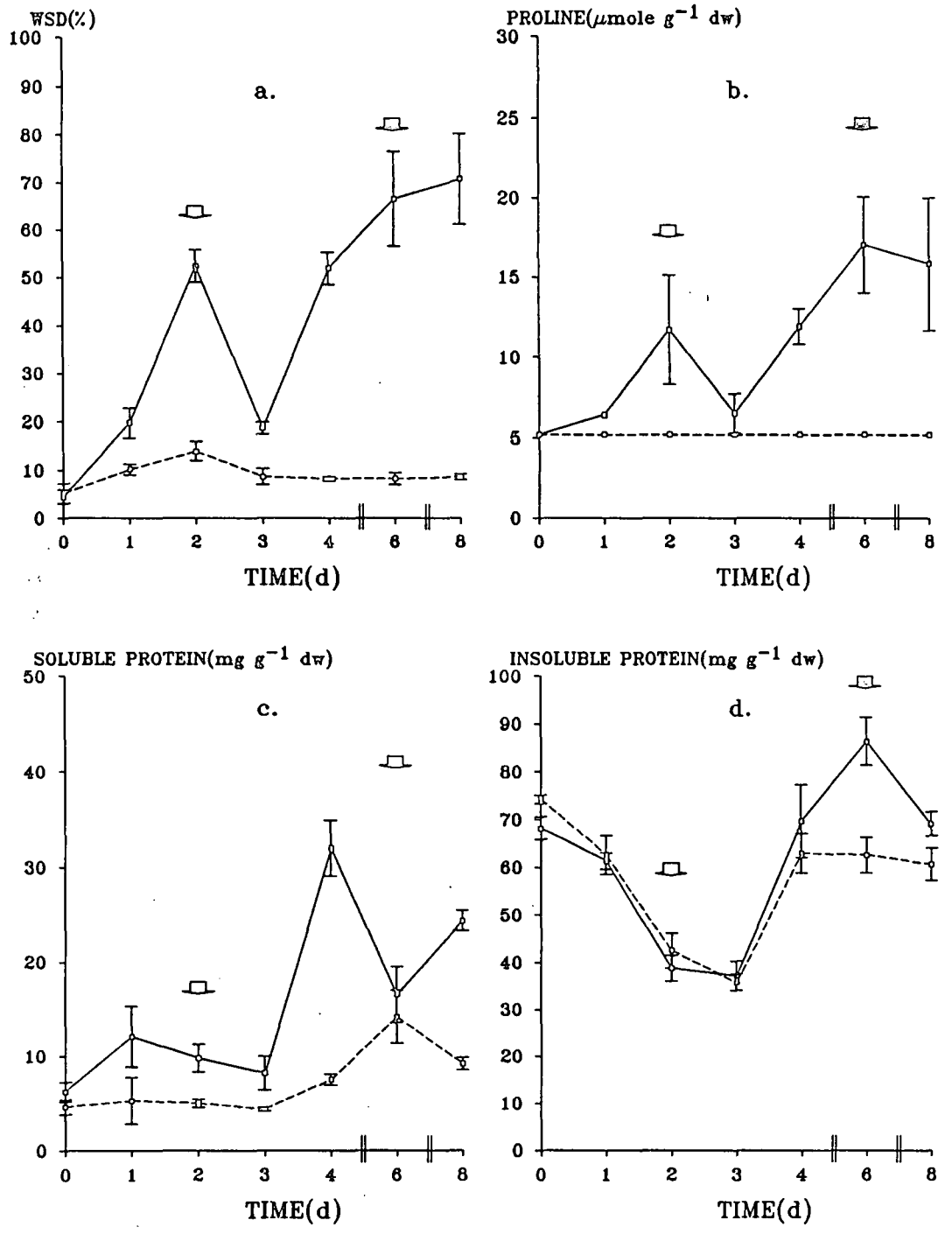

WSD(%)
a.
TIME(d)
PROLINE(μmole g⁻¹ dw)
b.
TIME(d)
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
TIME(d)
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)

Figure 6.4 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula farinosa(c)*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊡------⊡ Controls
⊡——⊡ Treated plants

⇩ Denotes date of rewatering
(water withheld before and after the date).
⇩ Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

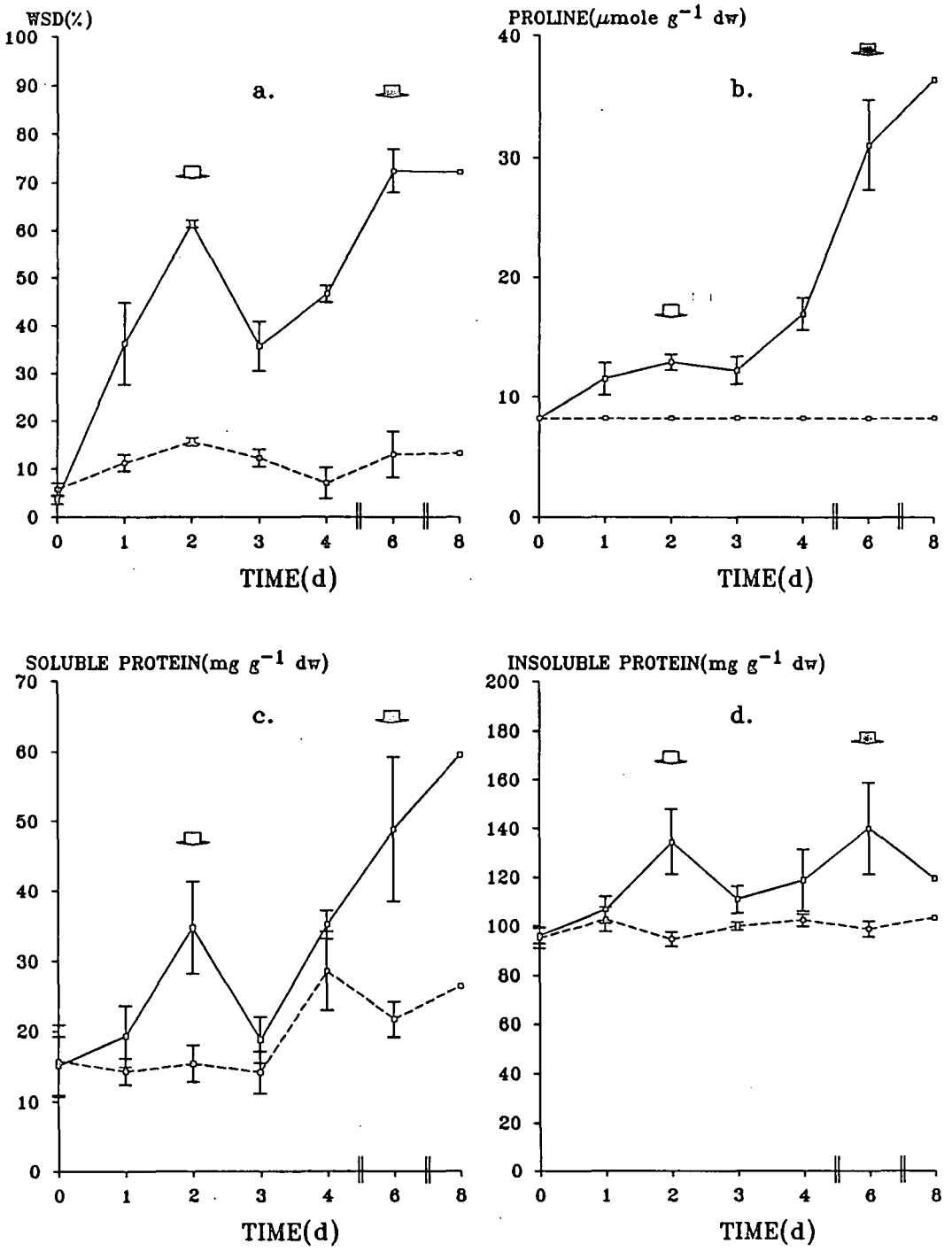

WSD(%)
a.
TIME(d)
PROLINE(μmole g⁻¹ dw)
b.
TIME(d)
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
TIME(d)
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)

Figure 6.5 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula frondosa*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟——⊟ Treated plants

⇩ Denotes date of rewatering
(water witheld before and after the date)
⇩ Denotes date of the termination of water stress treatment

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

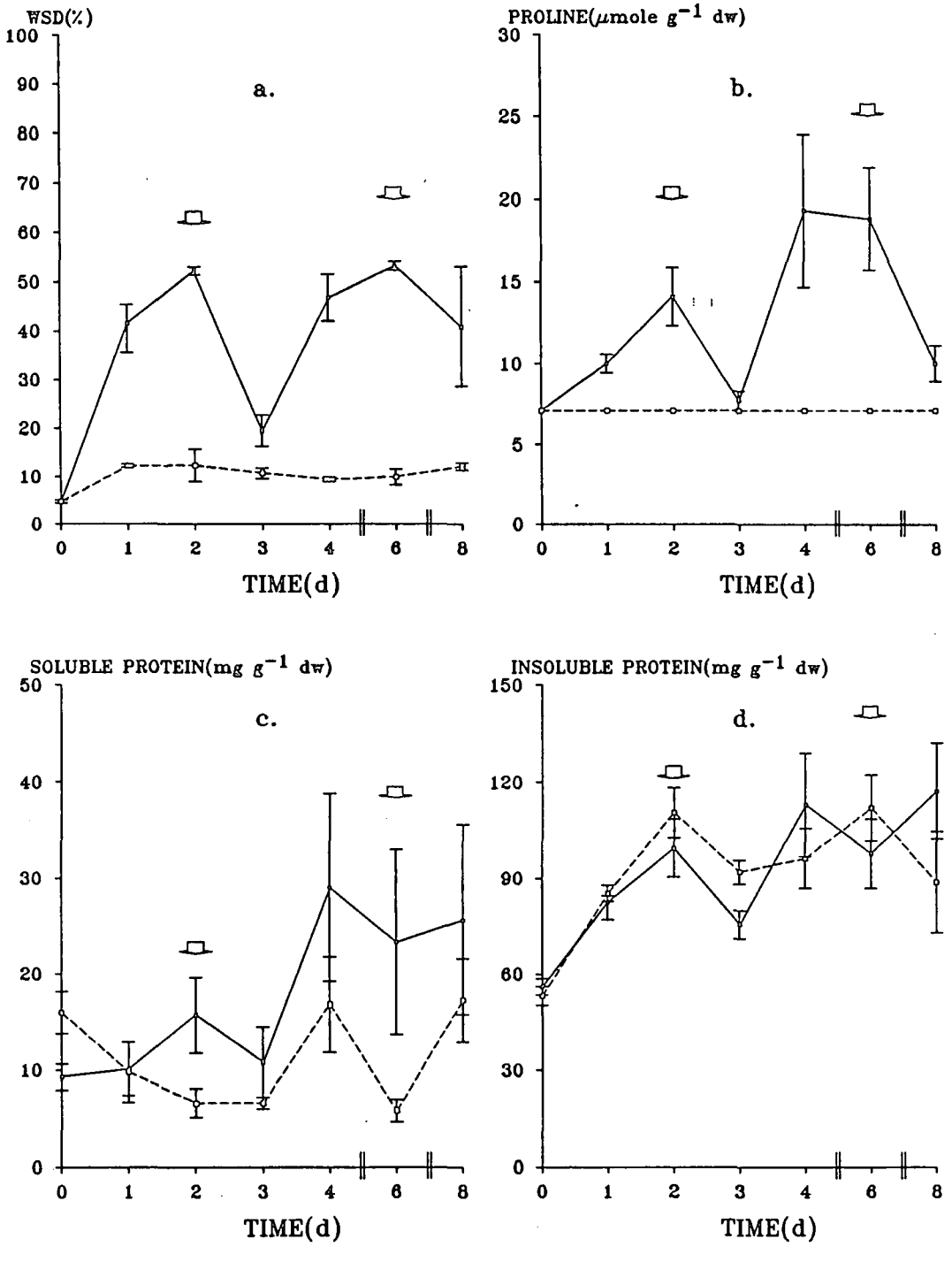

WSD(%)
a.
PROLINE(μmole g⁻¹ dw)
b.
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)
TIME(d)
TIME(d)
TIME(d)

Figure 6.6 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula halleri.*

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟——⊟ Treated plants

⇩ Denotes date of rewatering
(water withheld before and after the date).
⇩ Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

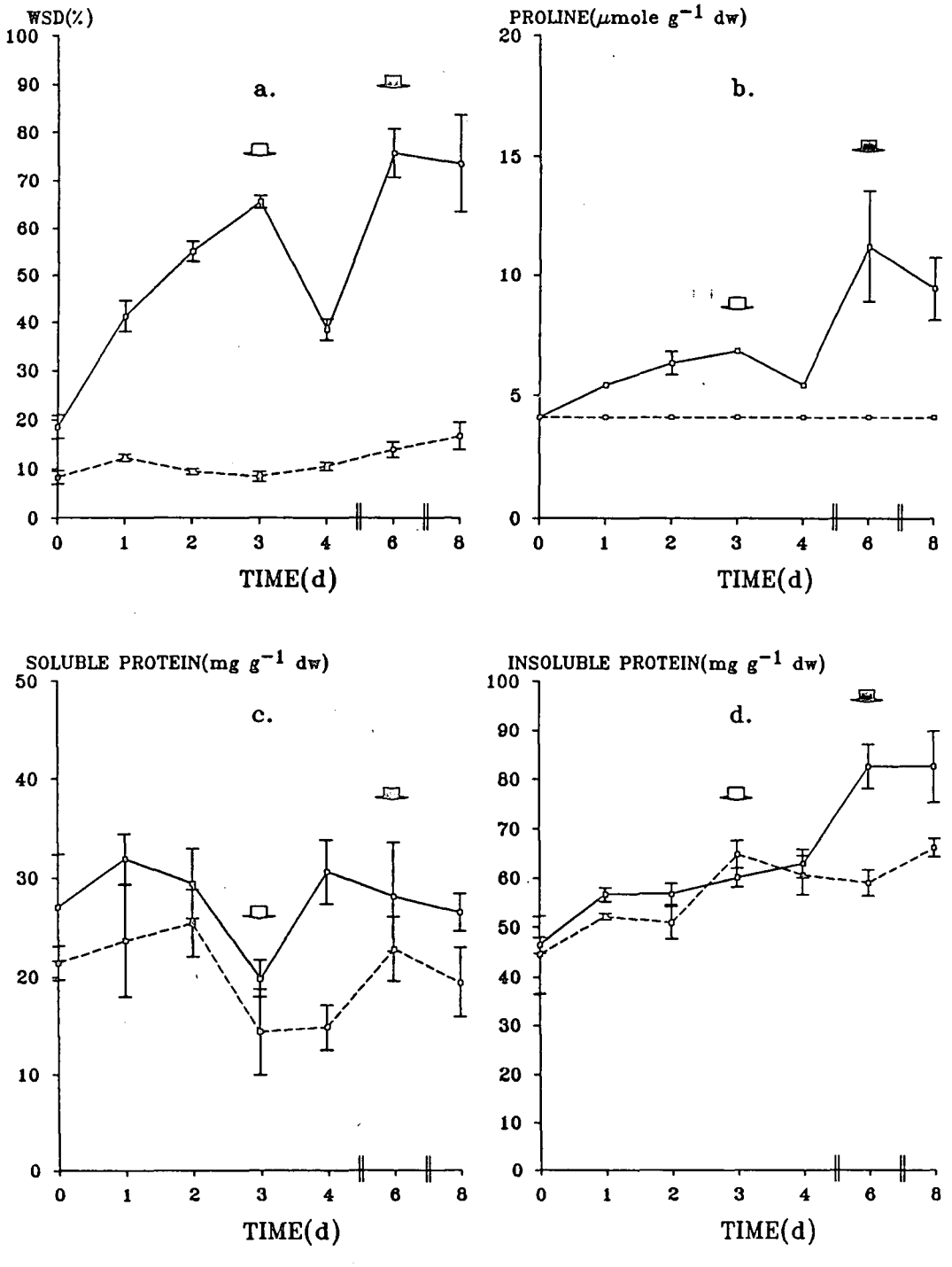

WSD(%)
a.
TIME(d)
PROLINE(μmole g⁻¹ dw)
b.
TIME(d)
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
TIME(d)
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)

Figure 6.7 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula laurentiana*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟———⊟ Treated plants

⇩ Denotes date of rewatering
(water withheld before and after the date).
⇩ Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

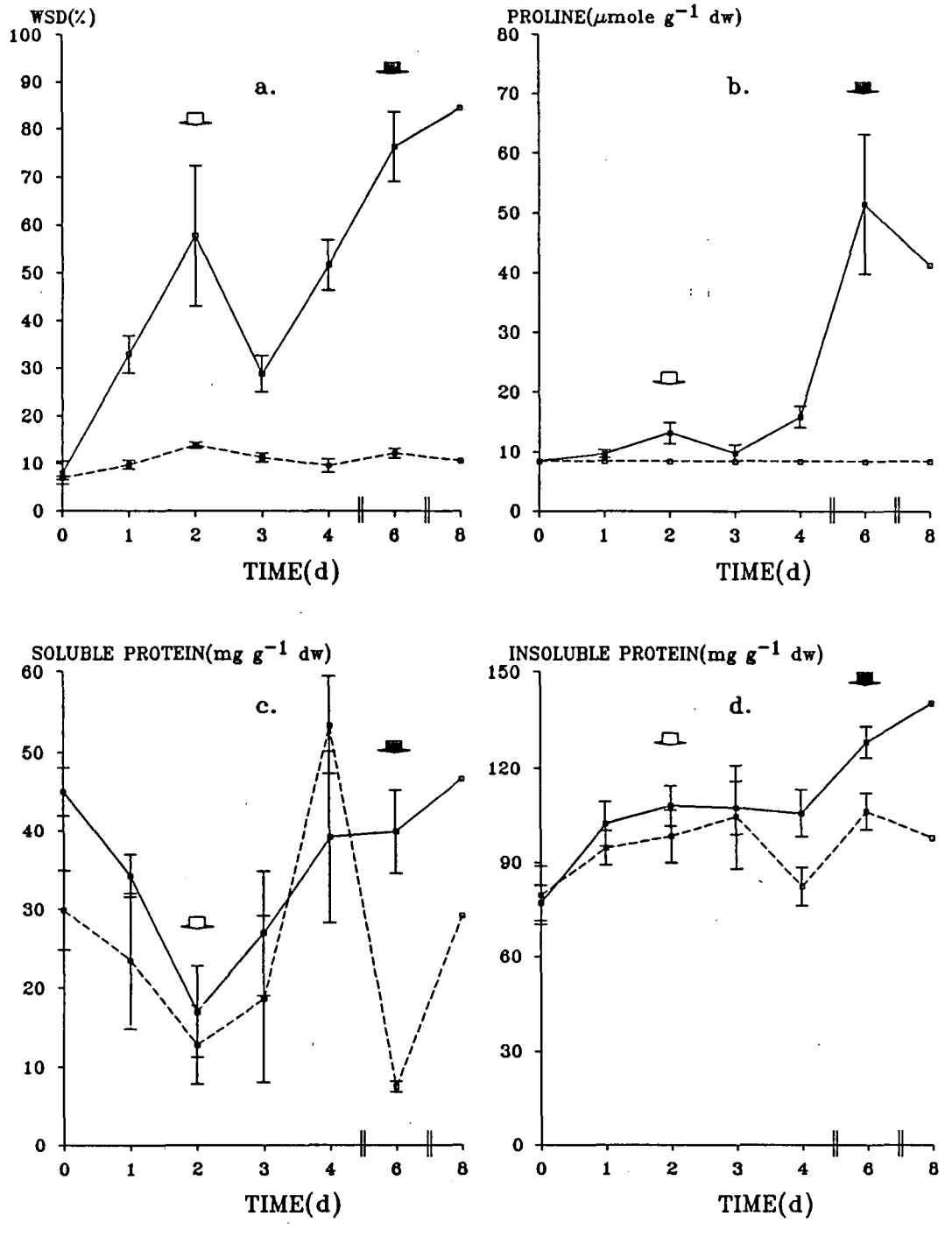

WSD(%)
a.
PROLINE(μmole g⁻¹ dw)
b.
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)
TIME(d)
TIME(d)
TIME(d)

Figure 6.8 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula scotica*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟——⊟ Treated plant

⬇ Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

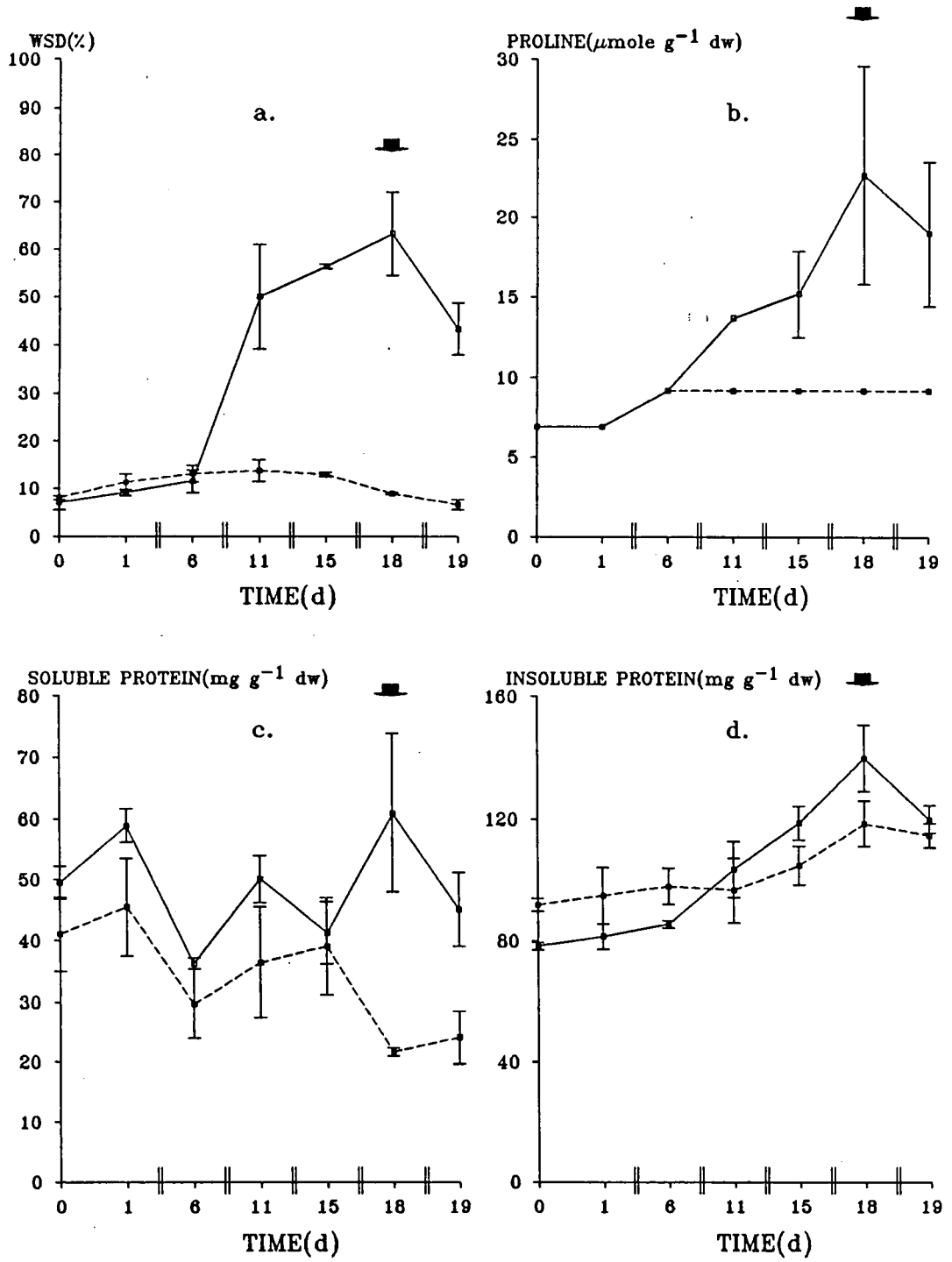

WSD(%)
a.
TIME(d)
PROLINE(μmole g⁻¹ dw)
b.
TIME(d)
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
TIME(d)
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)

Figure 6.9 Effect of duration of water stress and rewatering on protein/proline levels in leaf of *Primula stricta*.

a. WSD
b. Proline
c. Soluble protein
d. Insoluble protein

⊟------⊟ Controls
⊟——⊟ Treated plants

⬇ Denotes date of the termination of water stress treatment.

Vertical bars indicate SE; where bars are not shown, the limits were within the area of the symbols or at the top of the bargraph.

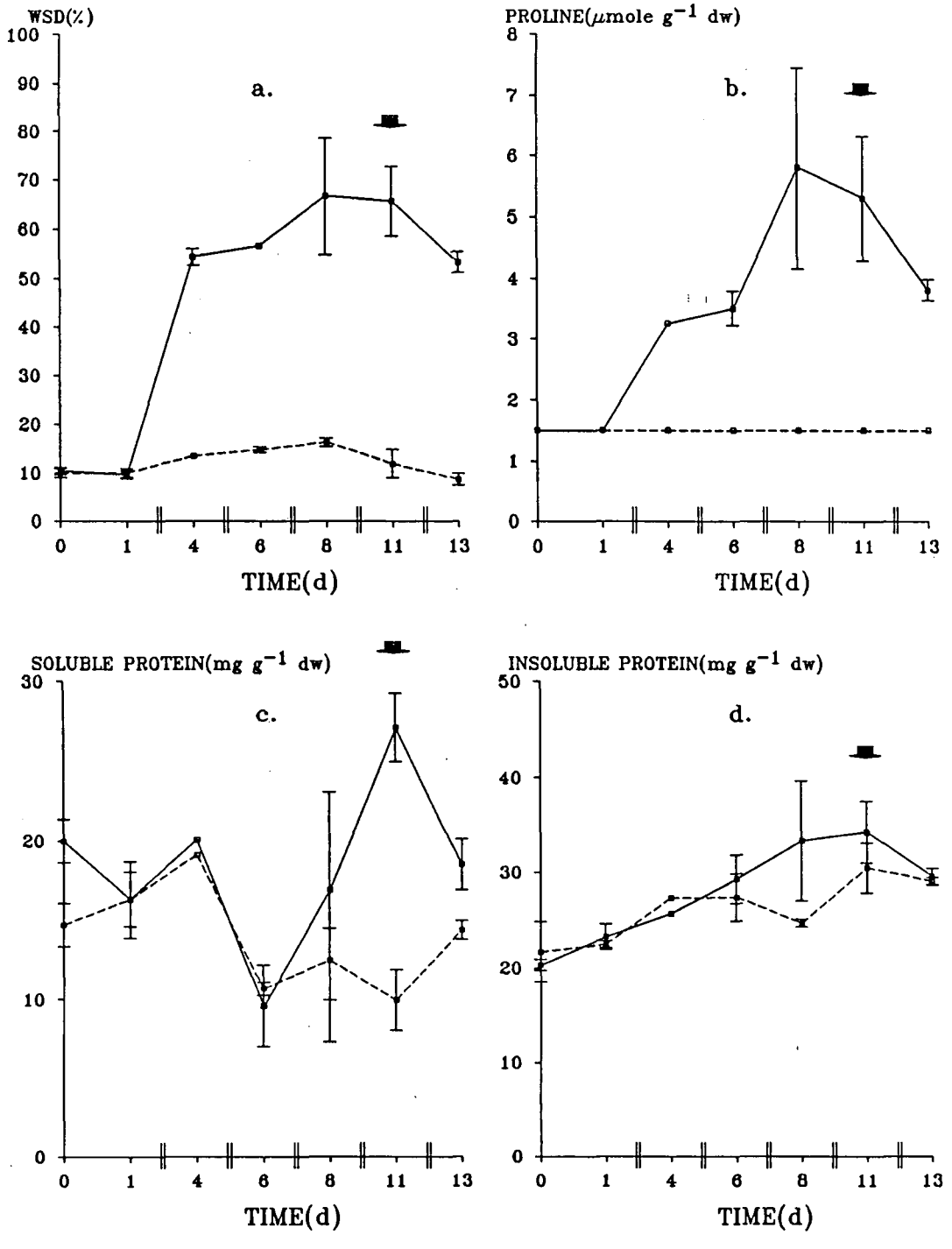

WSD(%)
a.
TIME(d)
PROLINE(μmole g⁻¹ dw)
b.
TIME(d)
SOLUBLE PROTEIN(mg g⁻¹ dw)
c.
TIME(d)
INSOLUBLE PROTEIN(mg g⁻¹ dw)
d.
TIME(d)

Table 6.1 ANOVA of relative leaf water content between species of *Primula farinosa* complex.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	383.6633	63.9439	6.8208	.0001
WITHIN GROUPS	35	328.1217	9.3749		
TOTAL	41	711.7850			

Table 6.2 Statistical test of significance (LSD) on relative leaf water content between species in *Primula farinosa* complex.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	NS	NS	***	NS	NS	**
2.*P.farinosa(c)*		–	NS	***	NS	NS	**
3.*P.frondosa*			–	***	NS	NS	**
4.*P.halleri*				–	**	**	NS
5.*P.laurentiana*					–	NS	NS
6.*P.scotica*						–	NS
7.*P.stricta*							–

Table 6.3 ANOVA of total leaf protein content between species of *Primula farinosa* complex.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	36638.1107	6106.3518	48.1204	.0000
WITHIN GROUPS	35	4441.4105	126.8974		
TOTAL	41	41079.5212			

Table 6.4 Statistical test of significance (LSD) on total leaf protein content between *Primula spp.*

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	***	***	NS	***	***	***
2.*P.farinosa(c)*		–	***	***	NS	**	***
3.*P.frondosa*			–	NS	***	***	***
4.*P.halleri*				–	***	***	***
5.*P.laurentiana*					–	*	***
6.*P.scotica*						–	***
7.*P.stricta*							–

Table 6.5 ANOVA of WSD in *Primula farinosa* and its allies during water stress experiments at various states of water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	6	12143.0448	2023.8408	20.9119	.0000
WITHIN GROUPS	14	1354.9133	96.7795		
TOTAL	20	13497.9581			
P.farinosa(c)					
BETWEEN GROUPS	6	66.5235	11.0873	1.7591	.1907
WITHIN GROUPS	12	75.6333	6.3028		
TOTAL	18	142.1568			
P.frondosa					
BETWEEN GROUPS	6	83.9724	13.9954	4.3006	.0115
WITHIN GROUPS	14	45.5600	3.2543		
TOTAL	20	129.5324			
P.halleri					
BETWEEN GROUPS	6	65.2924	10.8821	1.3709	.2922
WITHIN GROUPS	14	111.1333	7.9381		
TOTAL	20	176.4257			
P.laurentiana					
BETWEEN GROUPS	6	103.8277	17.3046	7.2879	.0019
WITHIN GROUPS	12	28.4933	2.3744		
TOTAL	18	132.3211			
P.scotica					
BETWEEN GROUPS	6	46.1390	7.6898	.9684	.4808
WITHIN GROUPS	14	111.1733	7.9410		
TOTAL	20	157.3124			
P.stricta					
BETWEEN GROUPS	6	10662.2611	1777.0435	17.8206	.0000
WITHIN GROUPS	12	1196.6200	99.7183		
TOTAL	18	11858.8811			

Table 6.6 Statistical test of significance (t-tests) of WSD in leaf of *Primula* spp. between controls and treated plants.

Primula spp.	Day of treatment		Significance levels
P.farinosa(b)	0		NS
	1		*
	2	+	***
	3		**
	4		***
	6	++	**
	8		**
P.farinosa(c)	0		NS
	1		*
	2	+	***
	3		*
	4		***
	6	++	***
	8		***
P.frondosa	0		NS
	1		**
	2	+	***
	3		NS
	4		***
	6	++	***
	8		NS
P.halleri	0		*
	1		**
	2		***
	3	+	***
	4		***
	6	++	***
	8		**
P.laurentiana	0		NS
	1		**
	2	+	*
	3		**
	4		***
	6	++	***
	8		NS

Table 6.6 Continued.

Primula spp.	Day of treatment	Significance levels
P.scotica	0	NS
	1	NS
	6	NS
	11	*
	15	***
	18 ++	**
	19	**
P.stricta	0	NS
	1	NS
	4	***
	6	*
	8	**
	11 ++	***
	13	NS

Note + = Date of rewatering(water withheld before and after the date).

++ = Date of termination of water stress.

WSD dropped in all species but remained significantly higher in the treated plants of all species except *P. frondosa.*

At day 6, when the drought treatment was terminated for *P. farinosa* (b), *P. farinosa* (c), *P. frondosa, P. halleri, P. laurentiana,* significant differences in WSD were found between the species with *P. laurentiana* giving the highest value. However, drought stress was continued up until day 18 for *P. scotica* and 11 for *P. stricta* before any wilting was seen. At the 5% level of significance the primulas can be divided into 3 subgroups:- (1) *P. scotica* (2) *P. frondosa, P. stricta, P. farinosa* (b), (3) *P. farinosa* (c), *P. halleri, P. laurentiana.* Overall, *P. laurentiana* was the most sensitive to water deficit, whilst *P. scotica* was the least sensitive. However, *P. farinosa* (b) could be included in subgroup 2 or 3 since it showed similarities with members of both of these categories.

6.3.1.2 Proline accumulation

It can be seen from Figs.6.3b-6.9b that proline levels in non-drought stressed plants were stable over the experimental period. In contrast, from the onset of water stress the treated plants accumulated proline to a significantly higher level than the controls. In general, the curves of WSD and proline accumulation were very similar for an individual species. Proline levels dropped on recovery from water stress (re-watering or termination of water stress). However, the results in Table 6.7 shows that there were significant differences between day of treatment only in four species, *P. farinosa* (b), *P. farinosa* (c), *P. scotica,* and *P. stricta.* Table 6.8 summarizes the results of t-tests for all the tested species. It can be seen that no significant difference was found between controls and treated plants on day 0, but on the following days there were some. By day 1, significant differences between controls and treated plants were found in only *P. farinosa* (b), *P. frondosa,* and *P. halleri.* After rewatering, proline accumulation dropped to the same level as controls only in *P. farinosa* (b), *P. frondosa,* and *P. laurentiana.* In fact, proline accumulation in controls and treated plants of *P. laurentiana* did not significantly

Table 6.7 ANOVA of proline accumulation in *Primula farinosa* and its allies during water stress experiments at various states of water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	6	403.5095	67.2516	3.8152	.0183
WITHIN GROUPS	14	246.7800	17.6271		
TOTAL	20	650.2895			
P.farinosa(c)					
BETWEEN GROUPS	6	136.8596	22.8099	7.8957	.0013
WITHIN GROUPS	12	34.6667	2.8889		
TOTAL	18	171.5263			
P.frondosa					
BETWEEN GROUPS	6	69.5114	11.5852	1.3192	.3118
WITHIN GROUPS	14	122.9467	8.7819		
TOTAL	20	192.4581			
P.halleri					
BETWEEN GROUPS	6	16.7695	2.7949	1.0740	.4229
WITHIN GROUPS	14	36.4333	2.6024		
TOTAL	20	53.2029			
P.laurentiana					
BETWEEN GROUPS	6	101.0000	16.8333	1.9042	.1612
WITHIN GROUPS	12	106.0800	8.8400		
TOTAL	18	207.0800			
P.scotica					
BETWEEN GROUPS	6	101.0362	16.8394	5.6113	.0038
WITHIN GROUPS	14	42.0133	3.0010		
TOTAL	20	143.0495			
P.stricta					
BETWEEN GROUPS	6	50.7895	8.4649	4.4165	.0138
WITHIN GROUPS	12	23.0000	1.9167		
TOTAL	18	73.7895			

Table 6.8 Statistical test of significance (t-tests) of proline accumulation in leaf of *Primula* spp. between controls and treated plants.

Primula spp.	Day of treatment		Significance levels
P.farinosa(b)	0		NS
	1		***
	2	+	NS
	3		NS
	4		**
	6	++	*
	8		NS
P.farinosa(c)	0		NS
	1		NS
	2	+	**
	3		*
	4		**
	6	++	**
	8		**
P.frondosa	0		NS
	1		**
	2	+	*
	3		NS
	4		NS
	6	++	*
	8		NS
P.halleri	0		NS
	1		***
	2		**
	3	+	***
	4		***
	6	++	*
	8		*
P.laurentiana	0		NS
	1		NS
	2	+	NS
	3		NS
	4		*
	6	++	*
	8		NS

Table 6.8 Continued.

Primula spp.	Day of treatment	Significance levels
P.scotica	0	NS
	1	NS
	6	NS
	11	NS
	15	**
	18 ++	NS
	19	NS
P.stricta	0	NS
	1	NS
	4	*
	6	NS
	8	*
	11 ++	***
	13	NS

Note + = Date of rewatering(water witheld before and after the date).

++ = Date of termination of water stress.

Table 6.9 ANOVA of soluble protein contents in *Primula farinosa* and its allies during water stress experiments at various states of water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	6	1611.1048	268.5175	17.9051	.0000
WITHIN GROUPS	14	209.9533	14.9967		
TOTAL	20	1821.0581			
P.farinosa(c)					
BETWEEN GROUPS	6	32.7828	5.4638	.4932	.8017
WITHIN GROUPS	12	132.9267	11.0772		
TOTAL	18	165.7095			
P.frondosa					
BETWEEN GROUPS	6	61.0762	10.1794	1.6104	.2164
WITHIN GROUPS	14	88.4933	6.3210		
TOTAL	20	149.5695			
P.halleri					
BETWEEN GROUPS	6	72.1362	12.0227	2.4968	.0743
WITHIN GROUPS	14	67.4133	4.8152		
TOTAL	20	139.5495			
P.laurentiana					
BETWEEN GROUPS	6	66.1712	11.0285	1.5183	.2531
WITHIN GROUPS	12	87.1667	7.2639		
TOTAL	18	153.3379			
P.scotica					
BETWEEN GROUPS	6	44.0048	7.3341	1.3704	.2924
WITHIN GROUPS	14	74.9267	5.3519		
TOTAL	20	118.9314			
P.stricta					
BETWEEN GROUPS	6	535.8853	89.3142	2.1203	.1262
WITHIN GROUPS	12	505.4800	42.1233		
TOTAL	18	1041.3653			

Table 6.10 Statistical test of significance (t-tests) of soluble protein content in leaf of *Primula* spp. between controls and treated plants.

Primula spp.	Day of treatment		Significance levels
P.farinosa(b)	0		NS
	1		NS
	2	+	*
	3		NS
	4		***
	6	++	NS
	8		***
P.farinosa(c)	0		NS
	1		NS
	2	+	NS
	3		NS
	4		NS
	6	++	NS
	8		NS
P.frondosa	0		NS
	1		NS
	2	+	NS
	3		NS
	4		NS
	6	++	NS
	8		NS
P.halleri	0		NS
	1		NS
	2		NS
	3	+	NS
	4		*
	6	++	NS
	8		NS
P.laurentiana	0		NS
	1		NS
	2	+	NS
	3		NS
	4		NS
	6	++	**
	8		NS

Table 6.10 Continued.

Primula spp.	Day of treatment		Significance levels
P.scotica	0		NS
	1		NS
	6		NS
	11		NS
	15		NS
	18	++	*
	19		*
P.stricta	0		NS
	1		NS
	4		NS
	6		NS
	8		**
	11	++	NS
	13		NS

Note + = Date of rewatering(water witheld before and after the date).

++ = Date of termination of water stress.

Table 6.11 ANOVA of insoluble protein contents in *Primula farinosa* and its allies during water stress experiments at various states of water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	6	5687.8248	947.9708	19.3702	.0000
WITHIN GROUPS	14	685.1533	48.9395		
TOTAL	20	6372.9781			
P.farinosa(c)					
BETWEEN GROUPS	6	5031.3161	838.5527	.5885	.7340
WITHIN GROUPS	12	17099.5133	1424.9594		
TOTAL	18	22130.8295			
P.frondosa					
BETWEEN GROUPS	6	3070.2324	511.7054	.5708	.7473
WITHIN GROUPS	14	12550.2400	896.4457		
TOTAL	20	15620.4724			
P.halleri					
BETWEEN GROUPS	6	22.6362	3.7727	.4168	.8558
WITHIN GROUPS	14	126.7133	9.0510		
TOTAL	20	149.3495			
P.laurentiana					
BETWEEN GROUPS	6	7558.5130	1259.7522	1.1623	.3867
WITHIN GROUPS	12	13005.9333	1083.8278		
TOTAL	18	20564.4463			
P.scotica					
BETWEEN GROUPS	6	15533.2257	2588.8710	6.7796	.0016
WITHIN GROUPS	14	5346.0800	381.8629		
TOTAL	20	20879.3057			
P.stricta					
BETWEEN GROUPS	6	459.3709	76.5618	2.5462	.0794
WITHIN GROUPS	12	360.8333	30.0694		
TOTAL	18	820.2042			

Table 6.12 Statistical test of significance (t-tests) of insoluble protein content in leaf of *Primula* spp. between controls and treated plants.

Primula spp.	Day of treatment	Significance levels
P.farinosa(b)	0	NS
	1	NS
	2 +	NS
	3	NS
	4	NS
	6 ++	*
	8	NS
P.farinosa(c)	0	NS
	1	NS
	2 +	*
	3	NS
	4	NS
	6 ++	NS
	8	NS
P.frondosa	0	NS
	1	NS
	2 +	NS
	3	*
	4	NS
	6 ++	NS
	8	NS
P.halleri	0	NS
	1	*
	2	NS
	3 +	NS
	4	NS
	6 ++	*
	8	NS
P.laurentiana	0	NS
	1	NS
	2 +	NS
	3	NS
	4	NS
	6 ++	*
	8	NS

Table 6.12 Continued.

Primula spp.	Day of treatment	Significance levels
P.scotica	0	**
	1	NS
	6	NS
	11	NS
	15	NS
	18 ++	NS
	19	NS
P.stricta	0	NS
	1	NS
	4	NS
	6	NS
	8	NS
	11 ++	NS
	13	NS

Note + = Date of rewatering(water witheld before and after the date).

++ = Date of termination of water stress.

Table 6.13 ANOVA of total protein contents in *Primula farinosa* and its allies during water stress experiments at various states of water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	6	10172.3962	1695.3994	19.8985	.0000
WITHIN GROUPS	14	1192.8333	85.2024		
TOTAL	20	11365.2295			
P.farinosa(c)					
BETWEEN GROUPS	6	14014.3386	2335.7231	3.5169	.0303
WITHIN GROUPS	12	7969.7867	664.1489		
TOTAL	18	21984.1253			
P.frondosa					
BETWEEN GROUPS	6	15366.0324	2561.0054	3.3601	.0289
WITHIN GROUPS	14	10670.6133	762.1867		
TOTAL	20	26036.6457			
P.laurentiana					
BETWEEN GROUPS	6	6454.6340	1075.7723	2.9466	.0525
WITHIN GROUPS	12	4381.0733	365.0894		
TOTAL	18	10835.7074			
P.halleri					
BETWEEN GROUPS	6	16500.1790	2750.0298	7.3453	.0011
WITHIN GROUPS	14	5241.4733	374.3910		
TOTAL	20	21741.6524			
P.scotica					
BETWEEN GROUPS	6	12728.8829	2121.4805	5.9255	.0029
WITHIN GROUPS	14	5012.3267	358.0233		
TOTAL	20	17741.2095			
P.stricta					
BETWEEN GROUPS	6	1134.9386	189.1564	1.6941	.2057
WITHIN GROUPS	12	1339.8867	111.6572		
TOTAL	18	2474.8253			

Table 6.14 Statistical test of significance (t-tests) of total protein content in leaf of *Primula* spp. between controls and treated plants.

Primula spp.	Day of treatment	Significance levels
P.farinosa(b)	0	NS
	1	NS
	2 +	NS
	3	NS
	4	*
	6 ++	NS
	8	**
P.farinosa(c)	0	NS
	1	NS
	2 +	*
	3	NS
	4	NS
	6 ++	NS
	8	NS
P.frondosa	0	NS
	1	NS
	2 +	NS
	3	NS
	4	NS
	6 ++	NS
	8	NS
P.halleri	0	NS
	1	NS
	2	NS
	3 +	NS
	4	**
	6 ++	*
	8	NS
P.laurentiana	0	NS
	1	NS
	2 +	NS
	3	NS
	4	NS
	6 ++	*
	8	NS

Table 6.14 Continued.

Primula spp.	Day of treatment		Significance levels
P.scotica	0		NS
	1		NS
	6		NS
	11		NS
	15		NS
	18	++	NS
	19		**
P.stricta	0		NS
	1		NS
	4		NS
	6		NS
	8		**
	11	++	NS
	13		NS

Note + = Date of rewatering(water witheld before and after the date).

++ = Date of termination of water stress.

Table 6.15 ANOVA of WSD and proline/protein levels in *Primula* spp. on day 6 during water stress.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
WATER SATURATION DEFICIT					
BETWEEN GROUPS	6	9340.5562	1556.7594	17.4294	.0000
WITHIN GROUPS	14	1250.4533	89.3181		
TOTAL	20	10591.0095			
PROLINE ACCUMULATION					
BETWEEN GROUPS	6	4751.8733	791.9789	10.6362	.0002
WITHIN GROUPS	14	1042.4467	74.4605		
TOTAL	20	5794.3200			
SOLUBLE PROTEIN CONTENT					
BETWEEN GROUPS	6	3404.2257	567.3710	4.7748	.0075
WITHIN GROUPS	14	1663.5667	118.8262		
TOTAL	20	5067.7924			
INSOLUBLE PROTEIN CONTENT					
BETWEEN GROUPS	6	23316.6495	3886.1083	16.4958	.0000
WITHIN GROUPS	14	3298.1400	235.5814		
TOTAL	20	26614.7895			
TOTAL PROTEIN CONTENT					
BETWEEN GROUPS	6	42727.0981	7121.1830	12.2247	.0001
WITHIN GROUPS	14	8155.3133	582.5224		
TOTAL	20	50882.4114			

Table 6.16 Statistical test of significance (LSD) of WSD in leaves of *Primula* spp.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	NS	NS	NS	NS	***	NS
2.*P.farinosa(c)*		–	*	NS	NS	***	*
3.*P.frondosa*			–	**	*	***	NS
4.*P.halleri*				–	NS	***	*
5.*P.laurentiana*					–	***	*
6.*P.scotica*						–	***
7.*P.stricta*							–

Table 6.17 Statistical test of significance (LSD) of proline accumulation in leaf of *Primula* spp.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	NS	NS	NS	***	NS	NS
2.*P.farinosa(c)*		–	NS	*	***	**	**
3.*P.frondosa*			–	NS	***	NS	*
4.*P.halleri*				–	***	NS	NS
5.*P.laurentiana*					–	***	***
6.*P.scotica*						–	NS
7.*P.stricta*							–

differ from day 0 to day 3. At the termination of water stress, proline accumulation in treated plants decreased but did not differ significantly from the controls in *P. farinosa* (b), *P. frondosa, P. laurentiana, P. scotica,* and *P. stricta.*

Comparisons between species, showed that there were highly significant differences between species ($P<0.001$) on day 6 during water stress (Table 6.15). The lowest and the highest values of proline levels were found in *P. stricta* and *P. laurentiana* respectively and *P. scotica* was the second lowest. LSD tests (Table 6.17) showed significant difference in each pair of species with *P. stricta, P. scotica,* and *P. laurentiana.* The primulas showed the lowest to highest proline levels in the order: *P. stricta, P. scotica, P. halleri, P. farinosa* (b), *P. frondosa, P. farinosa* (c), *P. laurentiana.* As has been noted above, the primulas did not show equal water deficient conditions for the same duration of water stress treatment, e.g. day 6. By day 6, it was found that there were a 3.3 fold increase in proline levels in *P. farinosa* (b), 3.9 fold in *P. farinosa* (c), 2.7 fold in *P. frondosa,* 2.7 fold in *P. halleri,* 6.1 fold in *P. laurentiana,* and 2.3 fold in *P. stricta,* but no proline accumulation in *P. scotica.* There were strong positive correlations between WSD and proline accumulation in all the tested species (Table 6.21). Significant proline accumulation for each species also differed for the day of withholding of water at which it occurred. It was recorded that *P. farinosa* (b) showed significant proline accumulation on day 1 with WSD 19.7%, *P. farinosa* (c) on day 2 with WSD 61.3%, *P. frondosa* on day 1 with WSD 41.5%, *P. halleri* on day 1 with WSD 41.2%, *P. laurentiana* on day 4 with WSD 51.6%, *P. scotica* on day 15 with WSD 56.3% and *P. stricta* on day 4 with WSD 56.8%. Significant proline accumulation also coincided with the visible wilting of the tested species. It can be seen that only *P. farinosa* (b) accumulated proline significantly at low WSD (approximately WSD 20%) while significant accumulations were found in the other species after more severe water stress (over WSD 40%). Except for *P. laurentiana, P. scotica* and *P. stricta* such accumulations were found after 1 or 2 d following the onset of water stress, *P. laurentiana* and *P. stricta* showed significant proline accumulation on day 4, whereas it took 15 d for

Table 6.18 Statistical test of significance (LSD) of soluble protein content in leaf of *Primula* spp.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	**	NS	NS	*	*	NS
2.*P.farinosa(c)*		–	*	*	NS	NS	***
3.*P.frondosa*			–	NS	NS	NS	NS
4.*P.halleri*				–	NS	NS	NS
5.*P.laurentiana*					–	NS	**
6.*P.scotica*						–	**
7.*P.stricta*							–

Table 6.19 Statistical test of significance (LSD) of insoluble protein content in leaf of *Primula* spp.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	***	NS	NS	**	NS	***
2.*P.farinosa(c)*		–	**	***	NS	***	***
3.*P.frondosa*			–	NS	*	NS	***
4.*P.halleri*				–	**	NS	***
5.*P.laurentiana*					–	**	***
6.*P.scotica*						–	***
7.*P.stricta*							–

Table 6.20 Statistical test of significance (LSD) of total protein content in leaf of *Primula* spp.

Primula spp.	Significance levels						
	1.	2.	3.	4.	5.	6.	7.
1.*P.farinosa(b)*	–	***	NS	NS	*	NS	**
2.*P.farinosa(c)*		–	**	**	NS	**	***
3.*P.frondosa*			–	NS	*	NS	***
4.*P.halleri*				–	*	NS	**
5.*P.laurentiana*					–	*	***
6.*P.scotica*						–	***
7.*P.stricta*							–

Table 6.21 Relationship between proline accumulation and WSD in *Primula farinosa* and its allies.

Primula spp.	Correlation coefficient (r)	Significance levels
P.farinosa(b)	+0.92249	***
P.farinosa(c)	+0.82722	***
P.frondosa	+0.79899	***
P.halleri	+0.84585	***
P.laurentiana	+0.79003	***
P.scotica	+0.84232	***
P.stricta	+0.92750	***

Table 6.22 Relationship between WSD and protein content in *Primula farinosa* and its allies.

Primula spp.	Types of protein	Correlation coefficient (r)	Significance levels
P.farinosa(b)	Soluble protein	+0.7138	***
	Insoluble protein	+0.2852	NS
	Total protein	+0.5060	***
P.farinosa(c)	Soluble protein	+0.7648	***
	Insoluble protein	+0.7482	***
	Total protein	+0.8265	***
P.frondosa	Soluble protein	+0.5457	***
	Insoluble protein	+0.4139	**
	Total protein	+0.5371	***
P.halleri	Soluble protein	+0.3798	*
	Insoluble protein	+0.6000	***
	Total protein	+0.7364	***
P.laurentiana	Soluble protein	+0.2362	NS
	Insoluble protein	+0.6125	***
	Total protein	+0.6886	***
P.scotica	Soluble protein	+0.4122	**
	Insoluble protein	+0.6055	***
	Total protein	+0.7519	***
P.stricta	Soluble protein	+0.3578	*
	Insoluble protein	+0.5947	***
	Total protein	+0.6005	***

Table 6.23 Relationship between proline accumulation and protein content in *Primula farinosa* and its allies.

Primula spp.	Types of protein	Correlation coefficient (r)	Significance levels
P.farinosa(b)	Soluble protein	+0.6074	***
	Insoluble protein	+0.3738	*
	Total protein	+0.5304	***
P.farinosa(c)	Soluble protein	+0.7797	***
	Insoluble protein	+0.6492	***
	Total protein	+0.7712	***
P.frondosa	Soluble protein	+0.5639	***
	Insoluble protein	+0.3566	*
	Total protein	+0.4966	***
P.halleri	Soluble protein	+0.3477	***
	Insoluble protein	+0.6384	***
	Total protein	+0.7519	***
P.laurentiana	Soluble protein	+0.2838	NS
	Insoluble protein	+0.5286	***
	Total protein	+0.6524	***
P.scotica	Soluble protein	+0.4575	**
	Insoluble protein	+0.6666	***
	Total protein	+0.8308	***
P.stricta	Soluble protein	+0.4833	**
	Insoluble protein	+0.7093	***
	Total protein	+0.7539	***

P. scotica. P. laurentiana, P. stricta, and *P. scotica* also showed significant proline accumulation at approximately the same levels of water deficient conditions.

6.3.1.3 Protein content

Determinations of protein contents were made on soluble and insoluble protein and total protein content was derived from the sum of these values. It can be seen from Figs. 6.3c-6.9c and Figs. 6.3d-6.9d that both soluble and insoluble protein contents fluctuated throughout the stress period in both controls and treated plants and there appeared to be no overall pattern. However, an increase in total protein content was found in all species when WSD increased (Table 6.22). Table 6.9 shows there were significant differences in soluble protein content during the experiment only in *P. farinosa* (b). The results from t-tests (Table 6.10) showed mostly non-significant differences between controls and treated plants. In particular no significant differences were found on the date of re-watering or termination of water stress except in *P. farinosa* (b), *P. laurentiana,* and *P. scotica.*

However, on day 6 after the commencement of water stress, the results of ANOVA (Table 6.15) showed there were significant differences between species in soluble protein content. The highest soluble protein levels were found in *P. farinosa* (c) and the lowest levels in *P. stricta.* Results from LSD tests (Table 6.18) showed highly significant difference between the two population of *P. farinosa.* The primulas show an apparent ranking from lowest to highest soluble protein of *P. stricta, P. farinosa* (b), *P. frondosa, P. halleri P. scotica, P. laurentiana, P. farinosa* (c).

Similarly, Fig. 6.3d to Fig. 6.9d and Table 6.12 illustrates that the insoluble protein content during stress showed largely non-significant variation in controls for each species. The results of ANOVA presented in Table 6.8 show that there were significant differences in insoluble protein content only in *P. farinosa* (b) and *P. scotica.* Significant differences in insoluble protein content between controls and treated plants on the date of rewatering and/or termination of water stress were found only in *P. farinosa* (b), *P. farinosa* (c), *P. halleri,* and *P. laurentiana.*

By day 6, there were significant differences between species ($P<0.0001$) in insoluble protein content (Table 6.15). The highest insoluble protein content was found in *P. farinosa* (c), whilst the lowest level was in *P. stricta.* Comparisons between each pair of species showed highly significant differences between the two populations of *P. farinosa* and most species showed significant differences from *P. farinosa* (c) and *P. stricta.* Ranking at the 5% level of significance, the primulas gave *P. stricta, P. laurentiana, P. scotica, P. farinosa* (b), *P. frondosa P. halleri, P. farinosa* (c). Table 6.22 showed a strong correlation between WSD and insoluble protein content during the stress period in all species except *P. farinosa* (b).

There were significant differences in total protein content during stress experiments in all the tested species, except *P. laurentiana* and *P. stricta* (Table 6.13). Table 6.14 shows that there were no significant difference between controls and treated plants in all species. Except for two species, *P. halleri* and *P. laurentiana* showed significant differences between controls and treated plants on the day of termination of water stress. Comparisons between species on day 6 (Table 6.15) showed highly significant differences in total protein content between the tested species. The highest total protein content was found in *P. farinosa* (c) and the lowest one was in *P. stricta* (Fig. 6.10). LSD tests (Table 6.20) showed significant differences between each pair of species from *P. farinosa* (c) and *P. stricta.* The primulas showed lowest to highest total protein content in the order *P. stricta, P. farinosa* (b), *P. laurentiana, P. frondosa, P. scotica P. halleri, P. farinosa* (c).

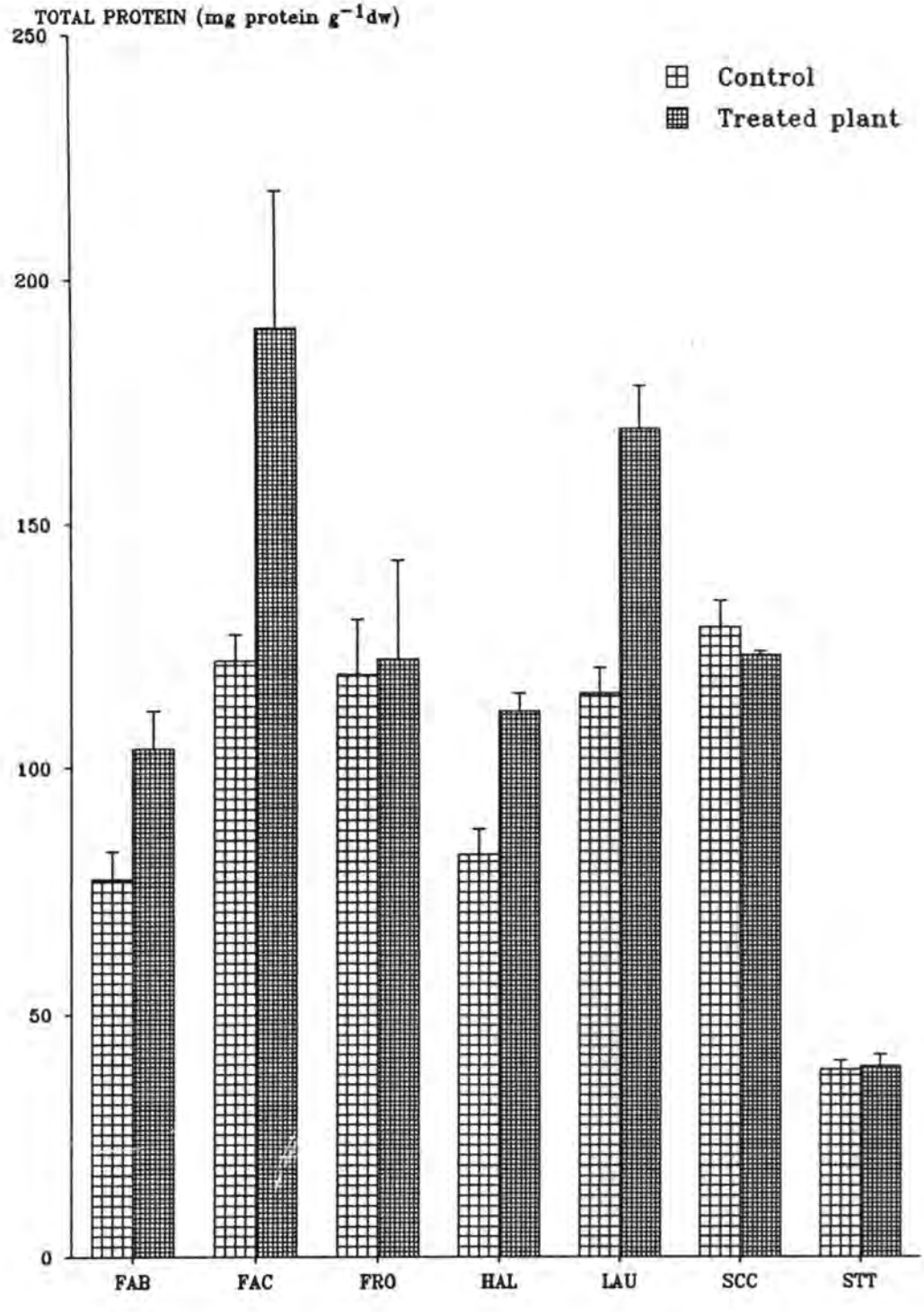
There was a very high correlation between WSD and total protein content in all species (Table 6.22).

Table 6.23 summarizes the relationship between proline accumulation and protein content. It was found that there was a strong positive correlation between proline accumulation and soluble protein content in all species except one, *P. laurentiana,* where only a poor correlation ($r = 0.28$) was found. In contrast, proline accumulation correlated significantly with insoluble protein content and total pro-

Figure 6.10 Levels of total protein in *Primula spp.* on day 6, compare with controls.

FAB = *P.farinosa*(b)
FAC = *P.farinosa*(c)
FRO = *P.frondosa*
HAL = *P.halleri*
LAU = *P.laurentiana*
SCC = *P.scotica*
STT = *P.stricta*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.


TOTAL PROTEIN (mg protein g⁻¹dw)
250
200
150
100
50
0
Control
Treated plant
FAB
FAC
FRO
HAL
LAU
SCC
STT

tein content in all species.

6.3.2 Field experiments

6.3.2.1 Proline accumulation

Fig. 6.11a to Fig. 6.14a depict the proline levels for *P. farinosa* (b), *P. farinosa* (c), *P. frondosa,* and *P. laurentiana* respectively at three study sites. ANOVA results (Table 6.24) revealed highly significant differences in proline levels between the sites. LSD tests (Table 6.28) show that proline levels at SUN were significantly higher than at GDF and HAR and this coincided with the visible wilting of the plants. In contrast, Fig. 6.15a shows that the same level of proline was present in *P. scotica* at all sites. Wilting also could not be observed in this species.

Fig. 6.16 compares the effect of elevation and microclimate on protein/proline levels between the tested species grown at GDF. It can be seen that proline levels (Fig. 6.16a) in *P. scotica* were the highest and the lowest levels were found in *P. farinosa* (b). At this site, no visible wilting was observed in any of the tested species. Comparisons between Fig. 16a and Fig. 17a reveal that proline levels at GDF and HAR were exactly identical for each species. In contrast, at SUN all species except *P. scotica* had higher levels of proline than the plants from GDF and HAR and this coincided with wilting in all species except *P. scotica.* The results from ANOVA (Table 6.34) showed that there were significant differences in proline levels between species. Amongst the species that had high levels of proline, *P. laurentiana* was the highest and *P. farinosa* (b) was the lowest. Table 6.35 shows the results of LSD tests; there were significantly higher proline levels in *P. farinosa* (c), *P. frondosa* and *P. laurentiana* than *P. farinosa* (b). There were no significant differences between *P. farinosa* (b) and *P. scotica.*

6.3.2.2 Protein content

Fig. 6.11b to Fig. 6.15b illustrate soluble protein content in the primulas from three study sites. ANOVA results (Table 6.25) showed significant differences in

Figure 6.11 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula farinosa*(b).

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

GDF = Great Dun Fell Radar Station, Cumbria
HAR = Hartside nursery, Alston
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

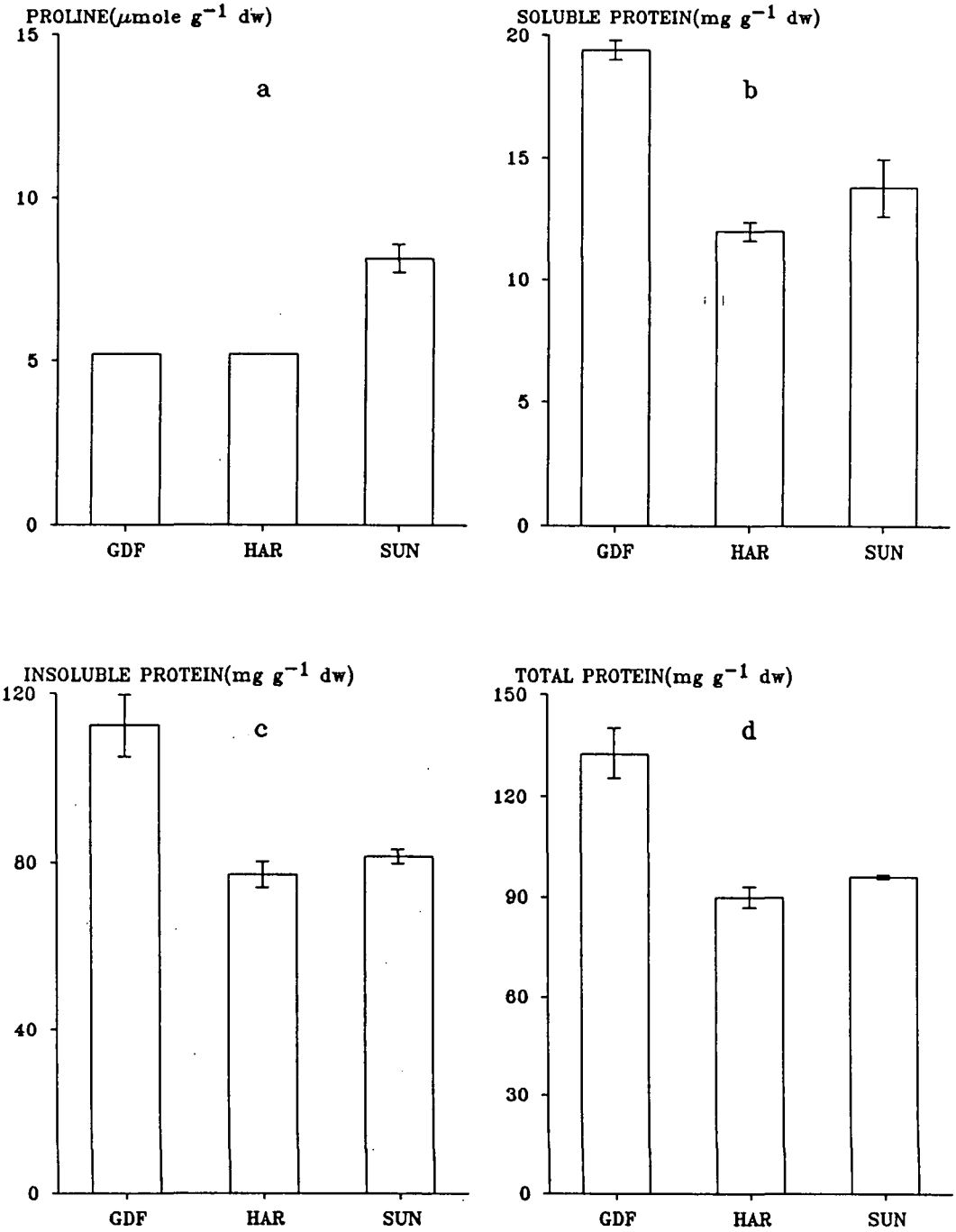

PROLINE(μmole g⁻¹ dw)
15
10
5
0
a
GDF
HAR
SUN
SOLUBLE PROTEIN(mg g⁻¹ dw)
20
15
10
5
0
b
GDF
HAR
SUN
INSOLUBLE PROTEIN(mg g⁻¹ dw)
120
80
40
0
c
GDF
HAR
SUN
TOTAL PROTEIN(mg g⁻¹ dw)
150
120
90
60
30
0
d
GDF
HAR
SUN

Figure 6.12 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula farinosa*(c).

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

GDF = Great Dun Fell Radar Station, Cumbria
HAR = Hartside nursery, Alston
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

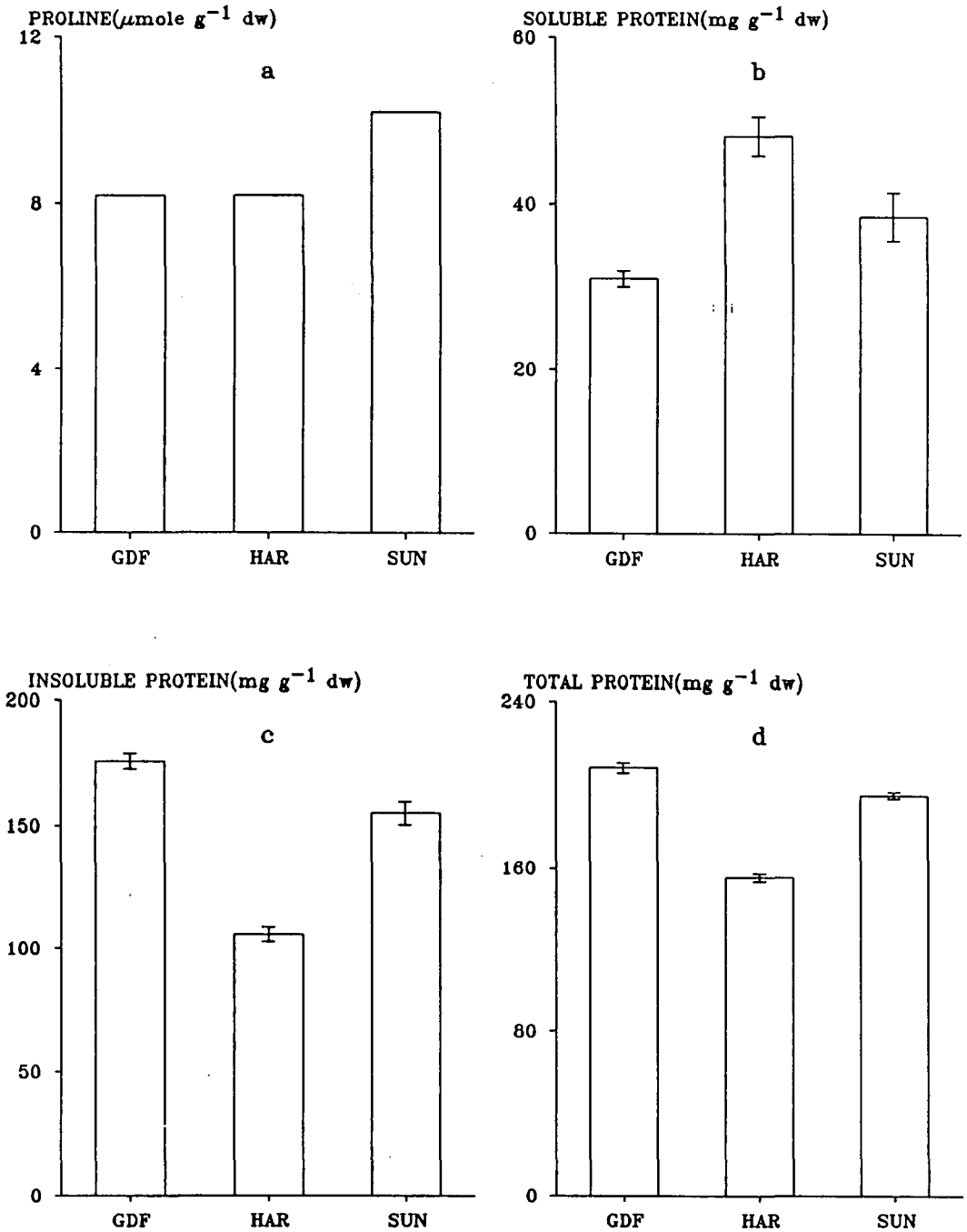

PROLINE(μmole g⁻¹ dw)
12
8
4
0
a
GDF
HAR
SUN
SOLUBLE PROTEIN(mg g⁻¹ dw)
60
40
20
0
b
GDF
HAR
SUN
INSOLUBLE PROTEIN(mg g⁻¹ dw)
200
150
100
50
0
c
GDF
HAR
SUN
TOTAL PROTEIN(mg g⁻¹ dw)
240
160
80
0
d
GDF
HAR
SUN

Figure 6.13 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula frondosa*.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

GDF = Great Dun Fell Radar Station, Cumbria
HAR = Hartside nursery, Alston
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

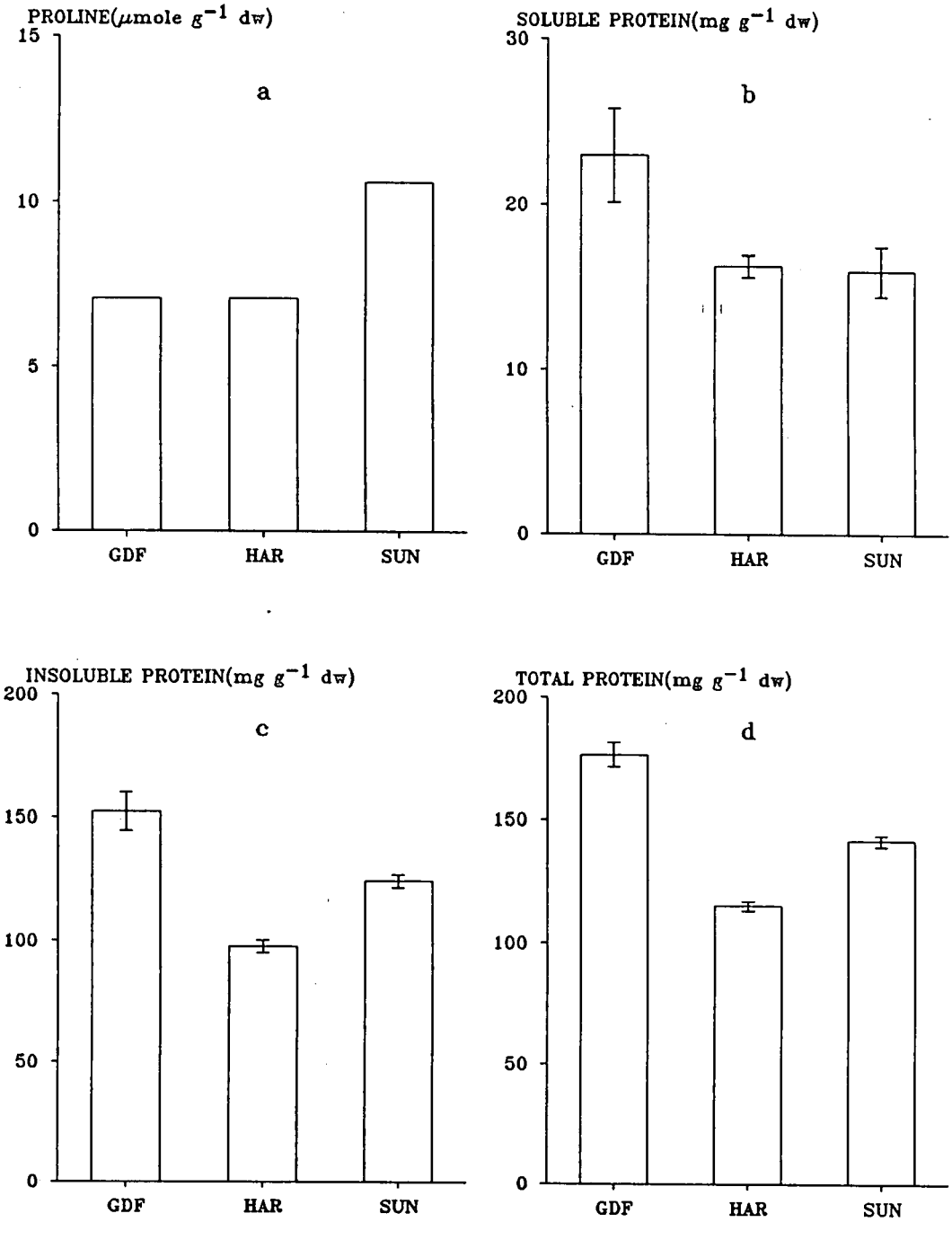

PROLINE(μmole g⁻¹ dw)
15
10
5
0
a
GDF
HAR
SUN
SOLUBLE PROTEIN(mg g⁻¹ dw)
30
20
10
0
b
GDF
HAR
SUN
INSOLUBLE PROTEIN(mg g⁻¹ dw)
200
150
100
50
0
c
GDF
HAR
SUN
TOTAL PROTEIN(mg g⁻¹ dw)
200
150
100
50
0
d
GDF
HAR
SUN

Figure 6.14 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula laurentiana*.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

GDF = Great Dun Fell Radar Station, Cumbria
HAR = Hartside nursery, Alston
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

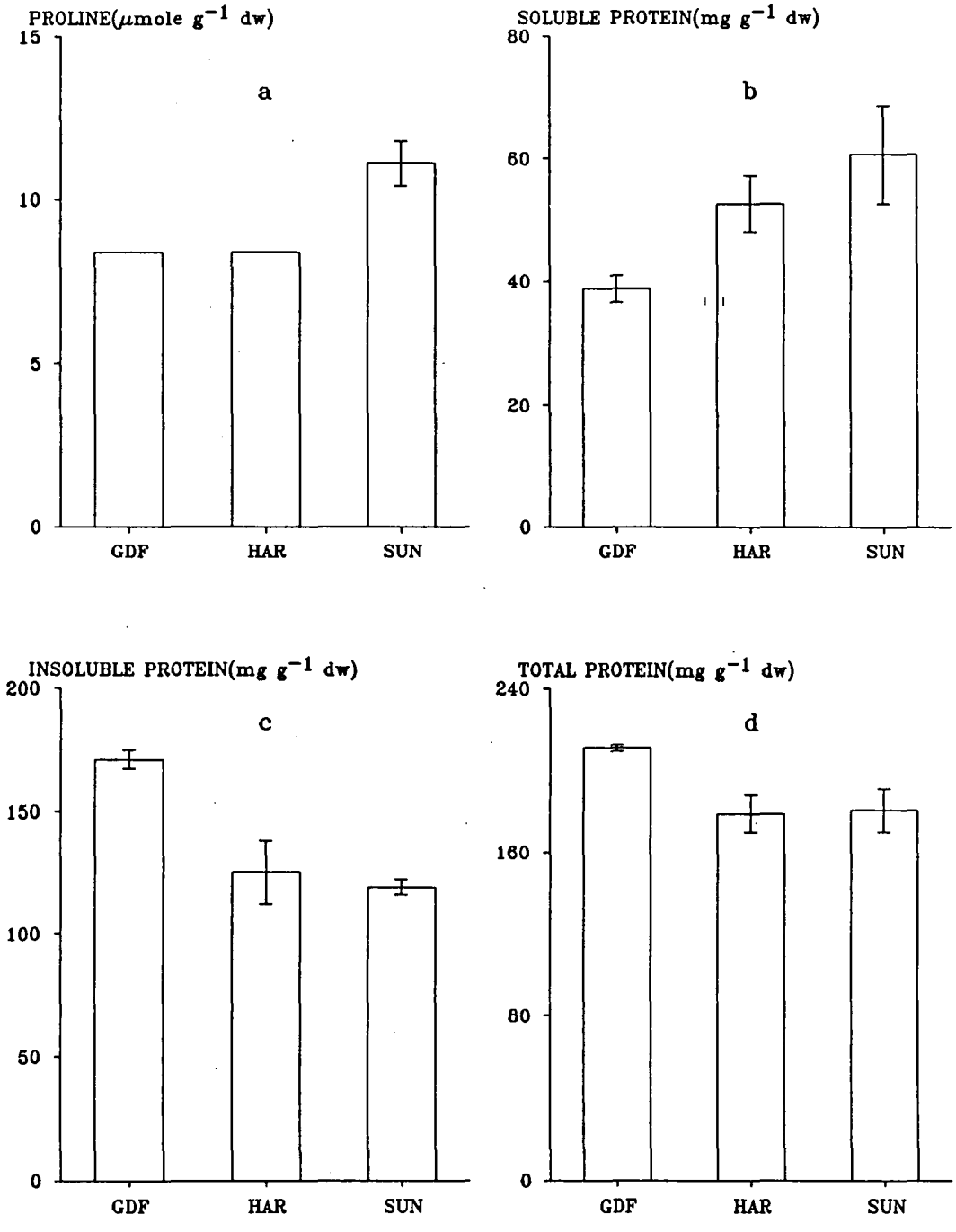

PROLINE(μmole g⁻¹ dw)
15
10
5
0
a
GDF
HAR
SUN
SOLUBLE PROTEIN(mg g⁻¹ dw)
80
60
40
20
0
b
GDF
HAR
SUN
INSOLUBLE PROTEIN(mg g⁻¹ dw)
200
150
100
50
0
c
GDF
HAR
SUN
TOTAL PROTEIN(mg g⁻¹ dw)
240
160
80
0
d
GDF
HAR
SUN

Figure 6.15 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula scotica*.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

GDF = Great Dun Fell Radar Station, Cumbria
HAR = Hartside nursery, Alston
SUN = South Bents, Sunderland

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

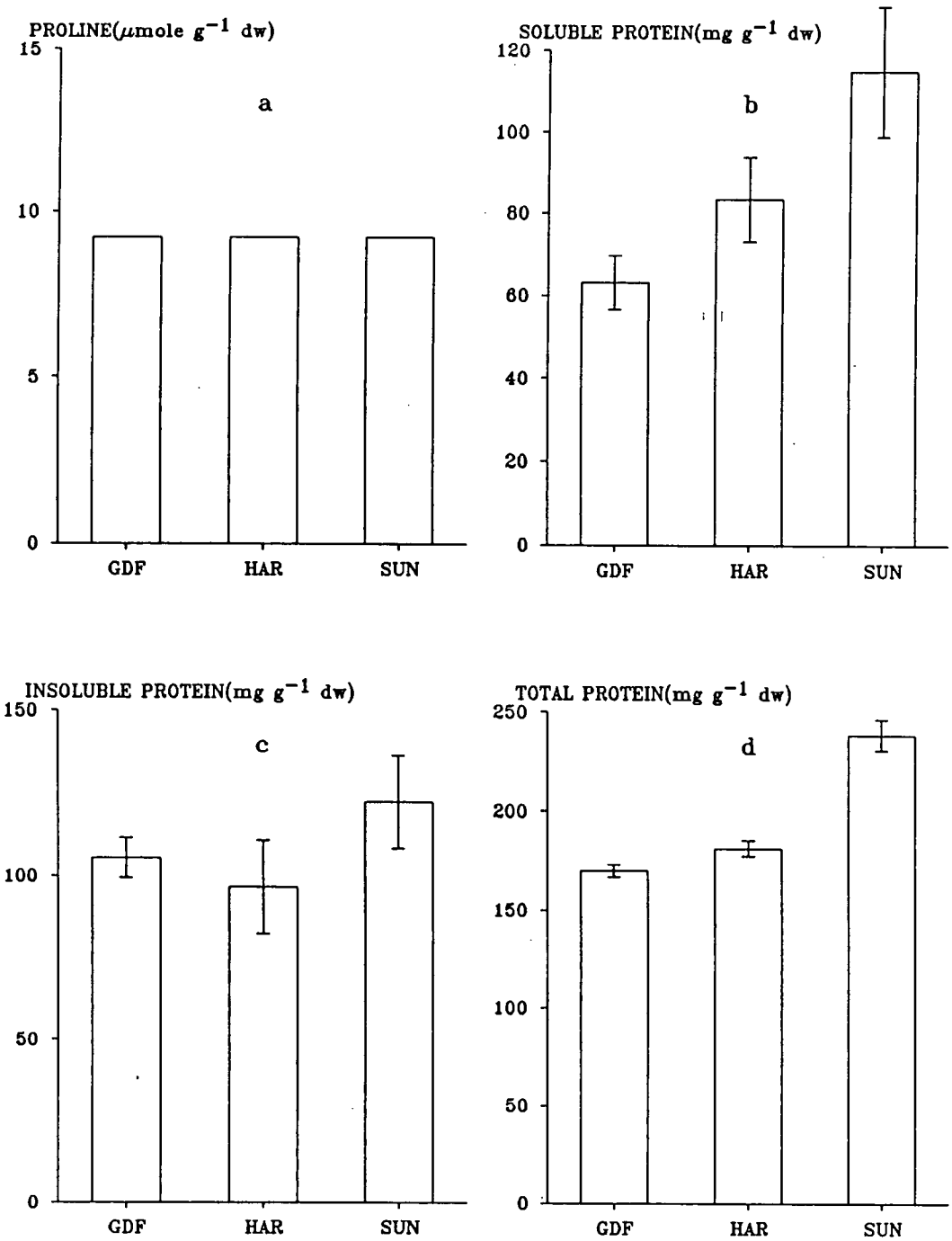

PROLINE(μmole g⁻¹ dw)
15
10
5
0
a
GDF
HAR
SUN
SOLUBLE PROTEIN(mg g⁻¹ dw)
120
100
80
60
40
20
0
b
GDF
HAR
SUN
INSOLUBLE PROTEIN(mg g⁻¹ dw)
150
100
50
0
c
GDF
HAR
SUN
TOTAL PROTEIN(mg g⁻¹ dw)
250
200
150
100
50
0
d
GDF
HAR
SUN

Figure 6.16 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula farinosa* and its allies, at Great Dun Fell Radar Station, Cumbria.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

FAB = *Primula farinosa(b)*
FAC = *Primula farinosa(c)*
FRO = *Primula frondosa*
FAB = *Primula laurentiana*
SCC = *Primula scotica*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

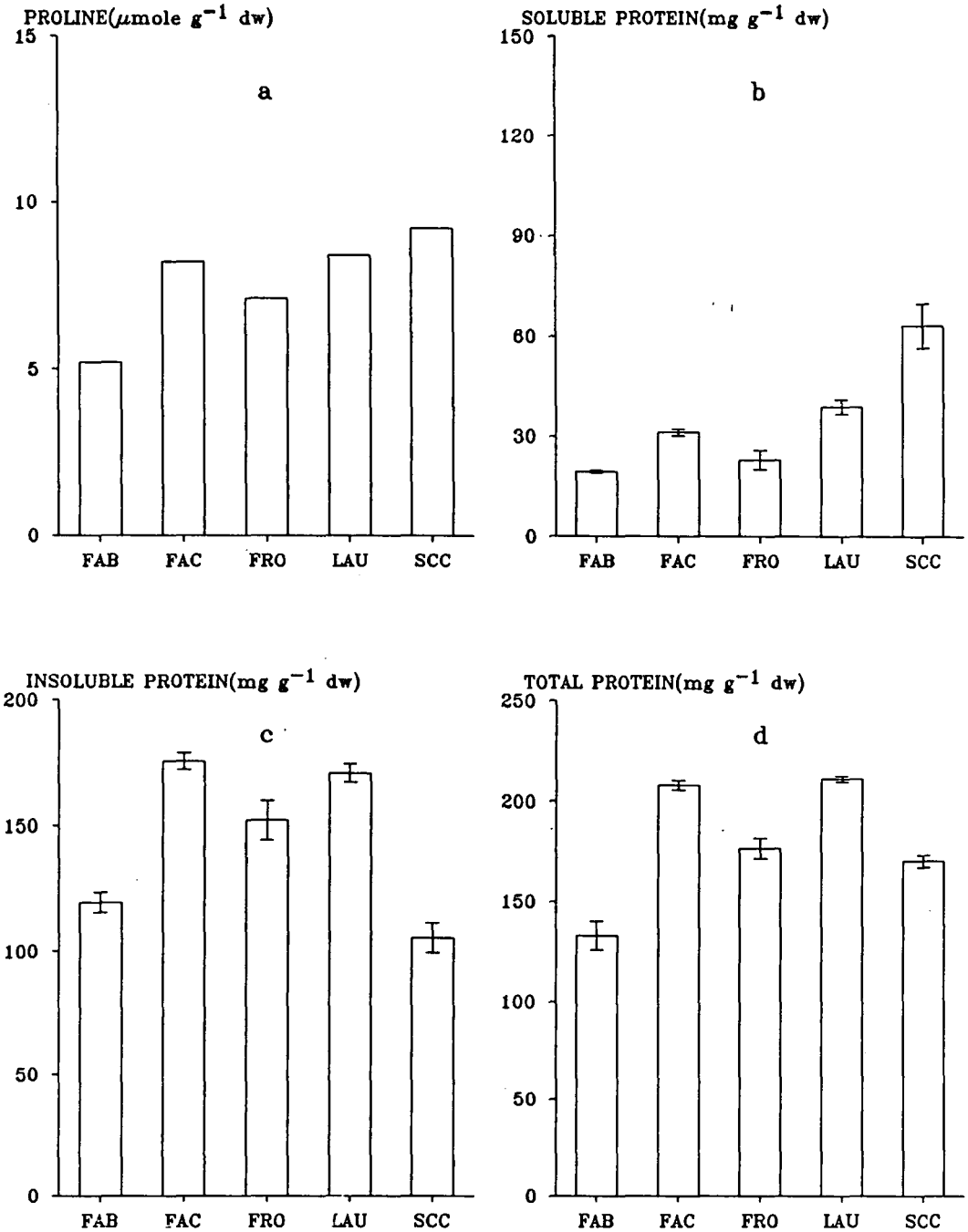

PROLINE(μmole g⁻¹ dw)
a
15
10
5
0
FAB
FAC
FRO
LAU
SCC
SOLUBLE PROTEIN(mg g⁻¹ dw)
b
150
120
90
60
30
0
FAB
FAC
FRO
LAU
SCC
INSOLUBLE PROTEIN(mg g⁻¹ dw)
c
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC
TOTAL PROTEIN(mg g⁻¹ dw)
d
250
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC

Figure 6.17 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula farinosa* and its allies, at Hartside nursery, Alston, Cumbria.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

FAB = *Primula farinosa(b)*
FAC = *Primula farinosa(c)*
FRO = *Primula frondosa*
FAB = *Primula laurentiana*
SCC = *Primula scotica*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

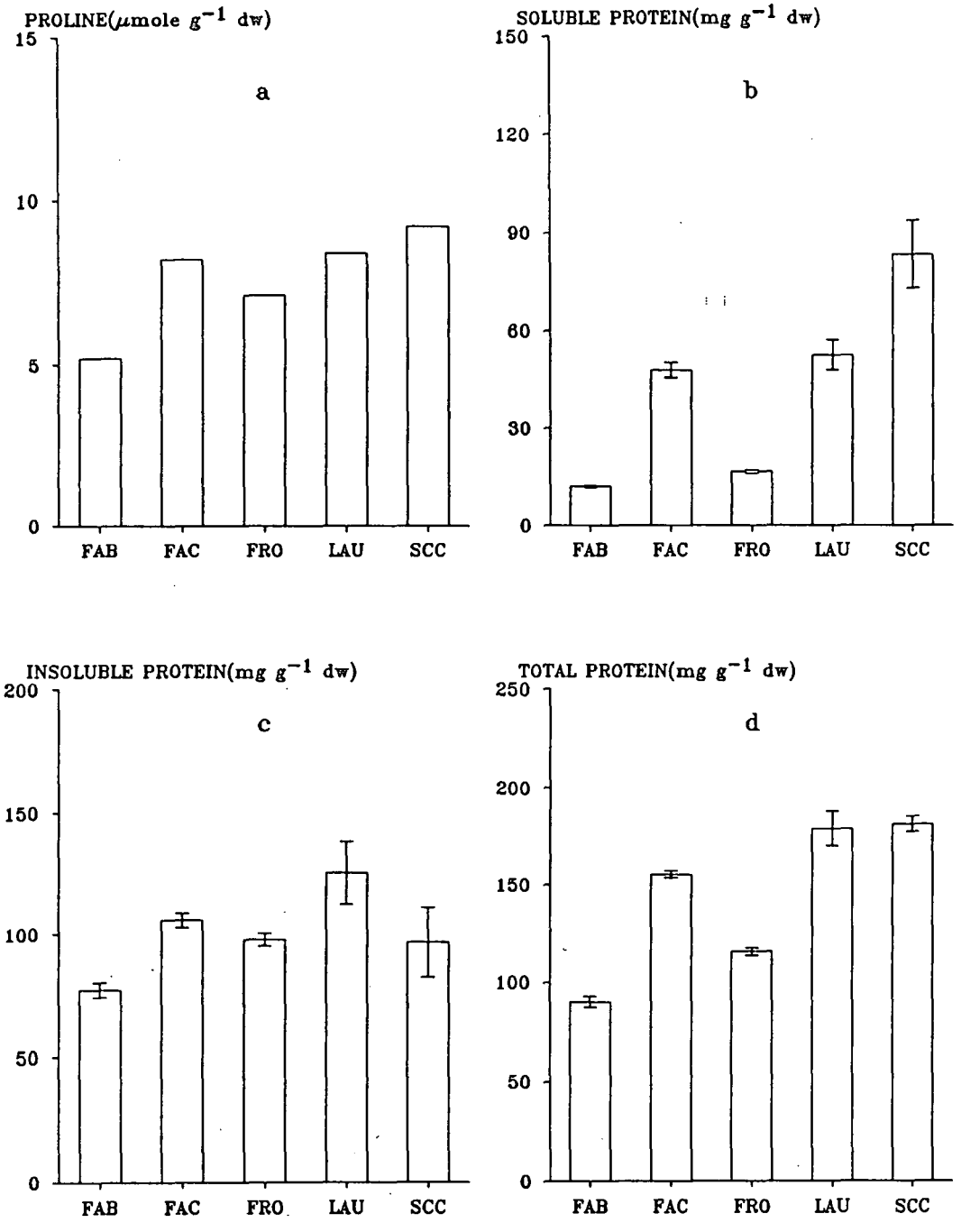

PROLINE(μmole g⁻¹ dw)
a
15
10
5
0
FAB
FAC
FRO
LAU
SCC
SOLUBLE PROTEIN(mg g⁻¹ dw)
b
150
120
90
60
30
0
FAB
FAC
FRO
LAU
SCC
INSOLUBLE PROTEIN(mg g⁻¹ dw)
c
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC
TOTAL PROTEIN(mg g⁻¹ dw)
d
250
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC

Figure 6.18 Effect of elevation and microclimate on protein/proline levels in leaf of *Primula farinosa* and its allies, at South Bents, Sunderland.

a. Proline
b. Soluble protein
c. Insoluble protein
d. Total protein

FAB = *Primula farinosa(b)*
FAC = *Primula farinosa(c)*
FRO = *Primula frondosa*
FAB = *Primula laurentiana*
SCC = *Primula scotica*

Vertical bars indicate SE; where bars are not shown, the limits were at the top of the bargraph.

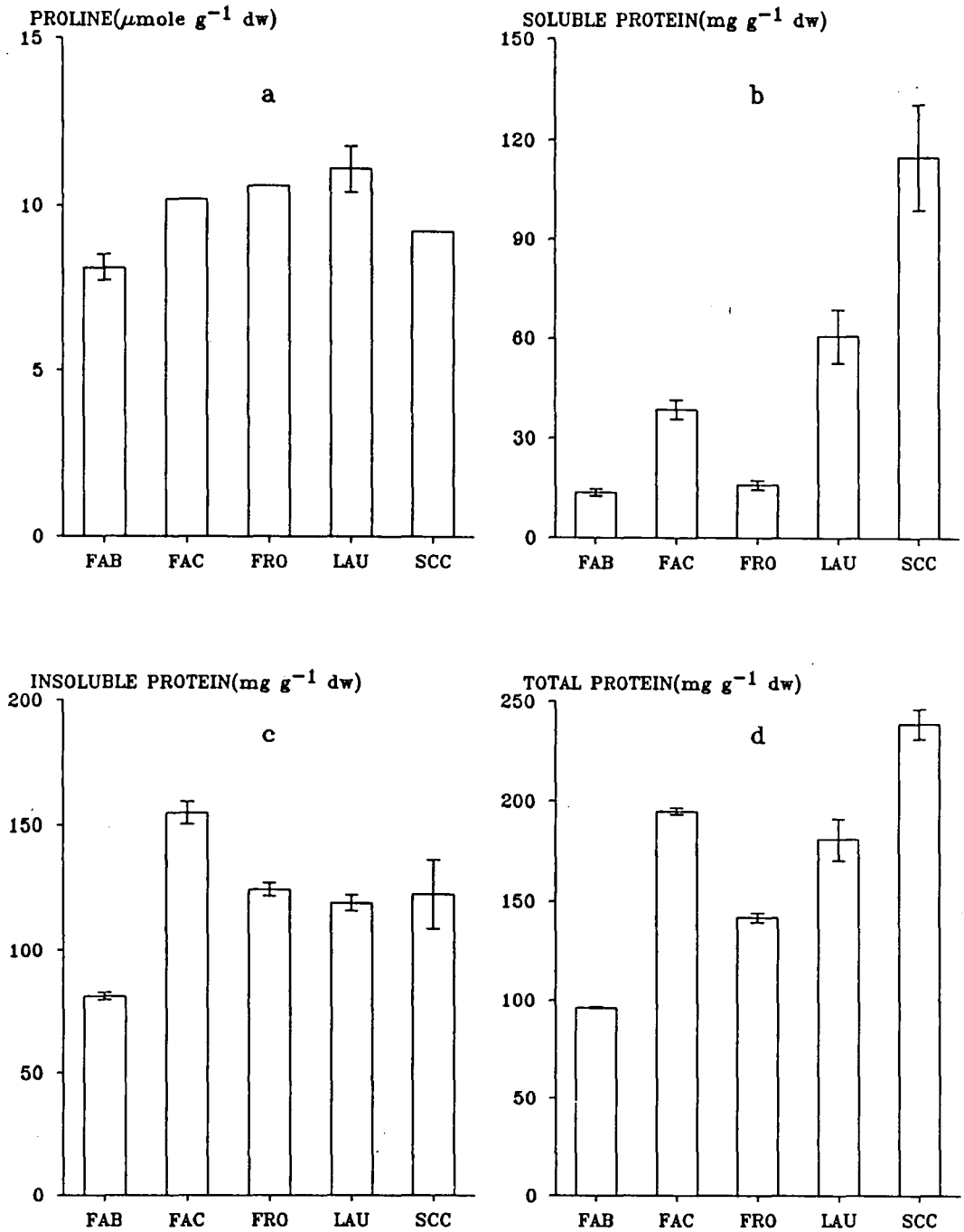

PROLINE(μmole g⁻¹ dw)
a
15
10
5
0
FAB
FAC
FRO
LAU
SCC
SOLUBLE PROTEIN(mg g⁻¹ dw)
b
150
120
90
60
30
0
FAB
FAC
FRO
LAU
SCC
INSOLUBLE PROTEIN(mg g⁻¹ dw)
c
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC
TOTAL PROTEIN(mg g⁻¹ dw)
d
250
200
150
100
50
0
FAB
FAC
FRO
LAU
SCC

Table 6.24 ANOVA of proline accumulations in *Primula* spp. grown at Great Dun Fell Radar station, Cumbria; Hartside Nursery, Alston; and South Bents, Sunderland during mid-summer, 1986.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	2	17.2089	8.6044	45.8225	.0002
WITHIN GROUPS	6	1.1267	.1878		
TOTAL	8	18.3356			
P.farinosa(c)					
BETWEEN GROUPS	2	6.0000	3.0000	+	+
WITHIN GROUPS	5	.0000	.0000		
TOTAL	7	6.0000			
P.frondosa					
BETWEEN GROUPS	2	24.5000	12.2500	+	+
WITHIN GROUPS	6	.0000	.0000		
TOTAL	8	24.5000			
P.laurentiana					
BETWEEN GROUPS	2	14.5800	7.2900	14.8776	.0047
WITHIN GROUPS	6	2.9400	.4900		
TOTAL	8	17.5200			
P.scotica					
BETWEEN GROUPS	2	.0000	.0000	+	+
WITHIN GROUPS	6	.0000	.0000		
TOTAL	8	.0000			

Note + = value cannot be computed.

Table 6.25 ANOVA of soluble protein contents in *Primula* spp. grown at Great Dun Fell Radar station, Cumbria; Hartside nursery, Alston; and South Bents, Sunderland during midsummer, 1986.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	2	90.2289	45.1144	26.6599	.0010
WITHIN GROUPS	6	10.1533	1.6922		
TOTAL	8	100.3822			
P.farinosa(c)					
BETWEEN GROUPS	2	442.3683	221.1842	20.1125	.0041
WITHIN GROUPS	5	54.9867	10.9973		
TOTAL	7	497.3550			
P.frondosa					
BETWEEN GROUPS	2	92.6467	46.3233	4.3692	.0675
WITHIN GROUPS	6	63.6133	10.6022		
TOTAL	8	156.2600			
P.laurentiana					
BETWEEN GROUPS	2	730.0689	365.0344	4.0399	.0774
WITHIN GROUPS	6	542.1400	90.3567		
TOTAL	8	1272.2089			
P.scotica					
BETWEEN GROUPS	2	4071.6822	2035.8411	5.0230	.0523
WITHIN GROUPS	6	2431.8467	405.3078		
TOTAL	8	6503.5289			

Table 6.26 ANOVA of insoluble protein content in *Primula* spp. grown at Great Dun Fell Radar station, Cumbria; Hartside nursery, Alston; and South Bents, Sunderland during midsummer, 1986.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	2	2437.7454	18.8727	57.3245	.0004
WITHIN GROUPS	5	106.3133	21.2627		
TOTAL	7	2544.0587			
P.farinosa(c)					
BETWEEN GROUPS	2	7637.1817	3818.5908	118.6647	.0001
WITHIN GROUPS	5	160.8983	32.1797		
TOTAL	7	7798.0800			
P.frondosa					
BETWEEN GROUPS	2	4445.1622	2222.5811	29.7123	.0008
WITHIN GROUPS	6	448.8200	74.8033		
TOTAL	8	4893.9822			
P.laurentiana					
BETWEEN GROUPS	2	4846.6022	2423.3011	12.3425	.0075
WITHIN GROUPS	6	1178.0267	196.3378		
TOTAL	8	6024.6289			
P.scotica					
BETWEEN GROUPS	2	1034.3889	517.1944	1.1883	.3675
WITHIN GROUPS	6	2611.3667	435.2278		
TOTAL	8	3645.7556			

Table 6.27 ANOVA of total protein contents in *Primula* spp. grown at Great Dun Fell Radar station, Cumbria; Hartside nursery, Alston; and South Bents, Sunderland during mid-summer, 1986.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
P.farinosa(b)					
BETWEEN GROUPS	2	3226.5489	13.2744	25.9949	.0011
WITHIN GROUPS	6	372.3667	62.0611		
TOTAL	8	3598.9156			
P.farinosa(c)					
BETWEEN GROUPS	2	4437.1821	2218.5910	180.4711	.0000
WITHIN GROUPS	5	61.4667	12.2933		
TOTAL	7	4498.6487			
P.frondosa					
BETWEEN GROUPS	2	5635.9400	2817.9700	81.0771	.0000
WITHIN GROUPS	6	208.5400	34.7567		
TOTAL	8	5844.4800			
P.laurentiana					
BETWEEN GROUPS	2	1941.5356	970.7678	4.9033	.0547
WITHIN GROUPS	6	1187.8867	197.9811		
TOTAL	8	3129.4222			
P.scotica					
BETWEEN GROUPS	2	8124.5422	4062.2711	47.2241	.0002
WITHIN GROUPS	6	516.1267	86.0211		
TOTAL	8	8640.6689			

Table 6.28 Statistical test of significance (LSD) of proline accumulation in leaf of *Primula* spp. grown in the selected sites in the field.

Primula spp.	Sites	Significance levels
P.farinosa(b)	GDF - HAR	NS
	GDF - SUN	***
	HAR - SUN	***
P.farinosa(c)	GDF - HAR	+
	GDF - SUN	+
	HAR - SUN	+
P.frondosa	GDF - HAR	+
	GDF - SUN	+
	HAR - SUN	+
P.laurentiana	GDF - HAR	NS
	GDF - SUN	*
	HAR - SUN	*
P.scotica	GDF - HAR	NS
	GDF - SUN	NS
	HAR - SUN	NS

Note GDF = Great Dun Fell Radar station, Cumbria

HAR = Hartside nursery, Alston

SUN = South Bents, Sunderland

\+ = value cannot be computed.

Table 6.29 Statistical test of significance (LSD) of soluble protein content in leaf of *Primula* spp. grown in the selected sites in the field.

Primula spp.	Sites	Significance levels
P.farinosa(b)	GDF – HAR	***
	GDF – SUN	**
	HAR – SUN	NS
P.farinosa(c)	GDF – HAR	**
	GDF – SUN	NS
	HAR – SUN	*
P.frondosa	GDF – HAR	*
	GDF – SUN	*
	HAR – SUN	NS
P.laurentiana	GDF – HAR	NS
	GDF – SUN	*
	HAR – SUN	NS
P.scotica	GDF – HAR	NS
	GDF – SUN	*
	HAR – SUN	NS

Note GDF = Great Dun Fell Radar Station, Cumbria

HAR = Hartside nursery, Alston

SUN = South Bents, Sunderland

Table 6.30 Statistical test of significance (LSD) of insoluble protein content in leaf of *Primula* spp. grown in the selected sites in the field.

Primula spp.	Sites	Significance levels
P.farinosa(b)	GDF - HAR	***
	GDF - SUN	***
	HAR - SUN	NS
P.farinosa(c)	GDF - HAR	***
	GDF - SUN	*
	HAR - SUN	***
P.frondosa	GDF - HAR	***
	GDF - SUN	**
	HAR - SUN	**
P.laurentiana	GDF - HAR	**
	GDF - SUN	**
	HAR - SUN	NS
P.scotica	GDF - HAR	NS
	GDF - SUN	NS
	HAR - SUN	NS

Note

GDF = Great Dun Fell Radar Station, Cumbria

HAR = Hartside nursery, Alston

SUN = South Bents, Sunderland

Table 6.31 Statistical test of significance (LSD) of total protein content in leaf of *Primula* spp. grown in the selected sites in the field.

Primula spp.	Sites	Significance levels
P.farinosa(b)	GDF - HAR	***
	GDF - SUN	**
	HAR - SUN	NS
P.farinosa(c)	GDF - HAR	***
	GDF - SUN	**
	HAR - SUN	***
P.frondosa	GDF - HAR	***
	GDF - SUN	***
	HAR - SUN	**
P.laurentiana	GDF - HAR	*
	GDF - SUN	*
	HAR - SUN	NS
P.scotica	GDF - HAR	NS
	GDF - SUN	***
	HAR - SUN	***

Note

GDF = Great Dun Fell Radar Station, Cumbria

HAR = Hartside nursery, Alston

SUN = South Bents, Sunderland

soluble protein content between sites only in *P. farinosa* (b) and *P. farinosa* (c). It can be seen that soluble protein content of *P. farinosa* (b) at GDF was significantly higher than at the other two sites (Table 6.29). In contrast, the values for *P. farinosa* (c) at HAR were significantly higher than at GDF and SUN. ANOVA also showed significant differences in insoluble protein content of *P. farinosa* (c) from the three sites. In *P. frondosa* no overall significant differences were found between the sites, however, LSD tests revealed significantly higher soluble protein content at GDF than at HAR and SUN. Table 6.29 shows significantly higher values for *P. laurentiana* at SUN than at GDF. Soluble protein content at SUN was also higher than at HAR, but was not significantly different. Soluble protein content of *P. scotica* at SUN was significantly higher than at GDF but not at HAR.

Fig. 6.16b shows the soluble protein content of the primulas grown at GDF. There was a highly significant difference between species (Table 6.32). The highest soluble protein content was found in *P. scotica* while *P. farinosa* (b) was the lowest, the values for *P. farinosa* (c) were significantly higher than *P. farinosa* (b). Significant difference between species were also found at HAR (Table 6.33) and SUN (Table 6.34). Soluble protein content of *P. scotica* was significantly higher than any other species at both sites. Again, soluble protein content of *P. farinosa* (b) was the lowest at both sites. *P. farinosa* from upland populations were significantly higher in soluble protein content at HAR but not at SUN (Table 6.36).

Levels of insoluble protein content for the three study sites are presented in Fig. 6.11c to Fig. 6.15c. It can be seen that the insoluble protein content was significantly different between the sites in all the species, except *P. scotica* (Table 6.26). From Table 6.30, it can be seen that insoluble protein content of *P. farinosa* (b) at GDF was significantly higher than at HAR and SUN. However, the values at HAR and SUN were not significantly different. Insoluble protein content of *P. farinosa* (c) and *P. frondosa* was significantly highest at GDF and lowest at HAR. However, the insoluble protein content of *P. laurentiana* at GDF was significantly higher than at HAR and SUN but HAR and SUN were not significantly different

Table 6.32 ANOVA of protein/proline levels in leaf of *Primula farinosa* and its allies; effect of elevations and microclimate, at Great Dun Fell Radar Station, Cumbria.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PROLINE LEVELS					
BETWEEN GROUPS	4	28.7040	7.1760	+	+
WITHIN GROUPS	10	.0000	.0000		
TOTAL	14	28.7040			
SOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	3622.9307	905.7327	26.7926	.0000
WITHIN GROUPS	10	338.0533	33.8053		
TOTAL	14	3960.9840			
INSOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	10993.8921	2748.4730	32.2683	.0000
WITHIN GROUPS	9	766.5800	85.1756		
TOTAL	13	11760.4721			
TOTAL PROTEIN CONTENTS					
BETWEEN GROUPS	4	12290.7907	3072.6977	53.6760	.0000
WITHIN GROUPS	10	572.4533	57.2453		
TOTAL	14	12863.2440			

Note + = value cannot be computed.

Table 6.33 ANOVA of protein/proline levels in leaf of *Primula farinosa* and its allies; effect of elevations and microclimate, at Hartside nursery, Alston, Cumbria.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PROLINE LEVELS					
BETWEEN GROUPS	4	28.7040	7.1760	+	+
WITHIN GROUPS	10	.0000	.0000		
TOTAL	14	28.7040			
SOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	10269.8840	2567.4710	31.6318	.0000
WITHIN GROUPS	10	811.6733	81.1673		
TOTAL	14	11081.5573			
INSOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	3614.5627	903.6407	3.7469	.0411
WITHIN GROUPS	10	2411.7267	241.1727		
TOTAL	14	6026.2893			
TOTAL PROTEIN CONTENTS					
BETWEEN GROUPS	4	19504.1173	4876.0293	69.9574	.0000
WITHIN GROUPS	10	697.0000	69.7000		
TOTAL	14	20201.1173			

Note + = value cannot be computed.

Table 6.34 ANOVA of protein/proline levels in leaf of *Primula farinosa* and its allies; effect of elevations and microclimate, at South Bents, Sunderland.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
PROLINE LEVELS					
BETWEEN GROUPS	4	16.7169	4.1792	9.2491	.0030
WITHIN GROUPS	9	4.0667	.4519		
TOTAL	13	20.7836			
SOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	20603.4752	5150.8688	23.7366	.0001
WITHIN GROUPS	9	1953.0133	217.0015		
TOTAL	13	22556.4886			
INSOLUBLE PROTEIN CONTENTS					
BETWEEN GROUPS	4	6935.9910	1733.9977	11.7593	.0013
WITHIN GROUPS	9	1327.1183	147.4576		
TOTAL	13	8263.1093			
TOTAL PROTEIN CONTENTS					
BETWEEN GROUPS	4	34522.8438	8630.7110	72.1274	.0000
WITHIN GROUPS	9	1076.9333	119.6593		
TOTAL	13	35599.7771			

Table 6.35 Statistical test of significance (LSD) of proline levels in *P.farinosa* and its allies; effects of elevations and microclimates at three selected sites in the field, during mid-summer, 1986.

Primula spp.	Sites	Significance levels				
		1.	2.	3.	4.	5.
1.*P.farinosa(b)*	GDF	–	+	+	+	+
2.*P.farinosa(c)*			–	+	+	+
3.*P.frondosa*				–	+	+
4.*P.laurentiana*					–	+
5.*P.scotica*						–
1.*P.farinosa(b)*	HAR	–	+	+	+	+
2.*P.farinosa(c)*			–	+	+	+
3.*P.frondosa*				–	+	+
4.*P.laurentiana*					–	+
5.*P.scotica*						–
1.*P.farinosa(b)*	SUN	–	**	**	***	NS
2.*P.farinosa(c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	*
4.*P.laurentiana*					–	**
5.*P.scotica*						–

Note + = value cannot be computed.

Table 6.36 Statistical test of significance (LSD) of soluble protein levels in *P.farinosa* and its allies; effects of elevations and microclimates at three selected sites in the field, during mid-summer, 1986.

Primula spp.	Sites	Significance levels				
		1.	2.	3.	4.	5.
1.*P.farinosa(b)*	GDF	–	*	NS	**	***
2.*P.farinosa(c)*			–	NS	NS	***
3.*P.frondosa*				–	**	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa(b)*	HAR	–	***	NS	***	***
2.*P.farinosa(c)*			–	**	NS	***
3.*P.frondosa*				–	***	***
4.*P.laurentiana*					–	**
5.*P.scotica*						–
1.*P.farinosa(b)*	SUN	–	NS	NS	**	***
2.*P.farinosa(c)*			–	NS	NS	***
3.*P.frondosa*				–	**	***
4.*P.laurentiana*					–	**
5.*P.scotica*						–

Table 6.37 Statistical test of significance (LSD) of insoluble protein levels in *P.farinosa* and its allies; effects of elevations and microclimates at three selected sites in the field, during mid-summer, 1986.

Primula spp.	Sites	Significance levels				
		1.	2.	3.	4.	5.
1.*P.farinosa(b)*	GDF	–	***	**	***	NS
2.*P.farinosa(c)*			–	*	NS	***
3.*P.frondosa*				–	*	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa(b)*	HAR	–	*	NS	**	NS
2.*P.farinosa(c)*			–	NS	NS	NS
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	*
5.*P.scotica*						–
1.*P.farinosa(b)*	SUN	–	***	**	**	**
2.*P.farinosa(c)*			–	*	**	*
3.*P.frondosa*				–	NS	NS
4.*P.laurentiana*					–	NS
5.*P.scotica*						–

Table 6.38 Statistical test of significance (LSD) of total protein levels in *P.farinosa* and its allies; effects of elevations and microclimates at three selected sites in the field, during mid-summer, 1986.

Primula spp.	Sites	Significance levels				
		1.	2.	3.	4.	5.
1.*P.farinosa(b)*	GDF	–	***	***	***	***
2.*P.farinosa(c)*			–	***	NS	***
3.*P.frondosa*				–	***	NS
4.*P.laurentiana*					–	***
5.*P.scotica*						–
1.*P.farinosa(b)*	HAR	–	***	**	***	***
2.*P.farinosa(c)*			–	***	**	**
3.*P.frondosa*				–	***	***
4.*P.laurentiana*					–	NS
5.*P.scotica*						–
1.*P.farinosa(b)*	SUN	–	***	***	***	***
2.*P.farinosa(c)*			–	***	NS	**
3.*P.frondosa*				–	**	***
4.*P.laurentiana*					–	***
5.*P.scotica*						–

from each other. In *P. scotica,* it was found that no significant differences were found between the sites.

Significant differences between species in insoluble protein content were found at all sites (Figs. 6.16c-6.18c; Tables 6.32-6.34). From Table 6.37 it can be seen that at GDF, insoluble protein content of *P. scotica* was significant lower than the other species except *P. farinosa* (b). At HAR, *P. farinosa* (b) had the lowest values, but significant differences were found only with *P. farinosa* (c) and *P. laurentiana.* The insoluble protein content of *P. laurentiana* was also significantly higher than *P. farinosa* (b) and *P. scotica,* but no significant difference was found with *P. frondosa* and *P. farinosa* (c). Significantly higher values were found between *P. farinosa* (c) and *P. farinosa* (b) for all 3 sites. Details comparisons of total protein content between the sites showed significantly higher values for upland populations compared with lowland populations.

Table 6.39 summarises the relationship between protein/proline levels and climatic data. It can be seen that there were good correlations between proline levels and the amount of mean monthly rainfall (July, 1986; Fig. 4.14) at the study sites in all species except in *P. scotica.* In this species the proline levels were the same at all sites. Mean monthly air temperature correlated significantly with proline levels in *P. farinosa* (b) and *P. frondosa.* Soluble protein content correlated significantly with temperatures in *P. farinosa* (b), *P. laurentiana,* and *P. scotica.* In contrast, soluble protein content correlated significantly with the amount of rainfall only with *P. scotica.* Correlation between temperatures and insoluble protein content was found in *P. farinosa* (b) and *P. laurentiana.* Rainfall was correlated with insoluble protein content only in *P. laurentiana.* Total protein content was correlated with temperature in *P. farinosa* (b) and *P. scotica* and a good relationship with rainfall was found only in *P. scotica.*

6.3.3 Relationships between species and their leaf characteristics

The relationship of leaf characteristics (Ch 3) and protein/proline levels were

Table 6.39 Relationship between microclimate and protein/proline levels in *Primula* spp. grown at Great Dun Fell Radar Station, Cumbria; Hartside nursery, Alston; and South Bents, Sunderland at midsummer 1986.

Variation	Proline	Soluble protein	Insoluble protein	Total protein
P.farinosa(b)				
rainfall	-.8663*	.6488	.7111	.7150
temperature	.7563*	-.7791*	-.8444*	-.8291*
P.farinosa(c)				
rainfall	-.8657*	-.4155	.3132	.2739
temperature	.7343	.5916	-.5121	-.4773
P.frondosa				
rainfall	-.8942**	.6602	.4372	.5086
temperature	.7807*	-.7285	-.6048	-.6731
P.laurentiana				
rainfall	-.8157*	-.7403	.7982*	.6346
temperature	.7122	.7575*	-.8661*	-.7182
P.scotica				
rainfall	.0000	-.7896*	-.3683	-.9238**
temperature	.0000	.7899*	.4130	.9532**

Table 6.40 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula farinosa* (b).

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	−.4256	.0832	−.6403	−.3075	−.4409
DATA2	−.9599	−.6915	−.9996**	−.9166	−.9645
DATA3	−.0940	−.5787	.1604	−.2194	−.0771
DATA4	−.7228	−.9714	−.5249	−.8046	−.7110
DATA5	−.8644	−.4977	−.9633	−.7937	−.8728
DATA6	−.5420	−.0498	−.7366	−.4311	−.5561
DATA7	.	.	.	.	.
DATA8	−.6036	−.1246	−.7853	−.4977	−.6170
DATA9	.6910	.2376	.8512	.5939	.7032
DATA10	.6910	.2376	.8512	.5939	.7032
DATA11	.8675	1.0000***	.7139	.9236	.8590
DATA12	−.7228	−.9714	−.5249	−.8046	−.7110
DATA13	.8415	.9989*	.6778	.9031	.8322
DATA14	−.9599	−.6915	−.9996**	−.9166	−.9645
DATA15	−.8395	−.4559	−.9495	−.7639	−.8486
DATA16	.4822	−.0198	.6878	.3673	.4970
DATA17	−.9715	−.9600	−.8802	−.9937*	−.9673
DATA18	−.7853	−.3710	−.9162	−.7005	−.7956
DATA19	.9986*	.8385	.9797	.9838	.9993*

Notes
DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = productss of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.41 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula farinosa* (c).

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	−.5870	−.9878*	−.4611	−.8969	−.7770
DATA2	−.0046	−.8931	.1431	−.4705	−.2639
DATA3	.8197	.8825	.7261	.9922*	.9402
DATA4	−.9946*	−.3592	−.9990*	−.8315	−.9337
DATA5	−.4076	.6286	−.5379	.0654	−.1567
DATA6	−.4076	.6286	−.5379	.0654	−.1567
DATA7	.5870	.9878*	.4611	.8969	.7770
DATA8	−.4076	.6286	−.5379	.0654	−.1567
DATA9	−.1036	−.9333	.0444	−.5556	−.3581
DATA10	.9946*	.3592	.9990*	.8315	.9337
DATA11	−.9946*	−.3592	−.9990*	−.8315	−.9337
DATA12	.6838	−.3398	.7840	.2645	.4710
DATA13	.8096	−.1555	.8874	.4423	.6295
DATA14	−.4076	.6286	−.5379	.0654	−.1567
DATA15	−.2897	−.9843	−.1452	−.7027	−.5281
DATA16	.1698	.9552	.0225	.6099	.4197
DATA17	.8392	−.1036	.9103	.4887	.6694
DATA18	−.8096	.1555	−.8874	−.4423	−.6295
DATA19	.4076	−.6286	.5379	−.0654	.1567

Notes

DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.42 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula frondosa*.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	-.2744	.2643	.5285	.3878	.4556
DATA2	.4011	.8182	-.1335	-.2883	-.2159
DATA3	-.3480	.1890	.5925	.4579	.5231
DATA4	.9864	.9286	-.9030	-.9595	-.9358
DATA5	.7701	.3273	-.9159	-.8411	-.8791
DATA6	-.3507	-.7857	.0794	.2358	.1625
DATA7	.6379	.9449	-.4013	-.5409	-.4766
DATA8	-.3480	.1890	.5925	.4579	.5231
DATA9	.9864	.9286	-.9030	-.9595	-.9358
DATA10	.	.	.	.	.
DATA11	-.9602	-.6759	1.0000***	.9868	.9961*
DATA12	.0223	-.4997	-.2966	-.1421	-.2156
DATA13	-.9375	-.9820	.8055	.8890	.8523
DATA14	-.5676	-.0581	.7723	.6623	.7164
DATA15	.6522	.9508	-.4183	-.5565	-.4929
DATA16	-.4474	-.8465	.1840	.3369	.2656
DATA17	-.8390	-.9996**	.6569	.7677	.7177
DATA18	.9918*	.9143	-.9182	-.9692	-.9481
DATA19	-.9978*	-.8872	.9412	.9827	.9661

Notes

DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.43 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula halleri*.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	−.9605	−.0862	−.8226	.2335	−.9463
DATA2	−.9338	−.6700	−.9991*	.7730	−.5594
DATA3	.7608	−.3317	.5155	.1876	.9955*
DATA4	−.9834	−.1844	−.8749	.3287	−.9096
DATA5	.9834	.1844	.8749	−.3287	.9096
DATA6	−.9109	.0571	−.7330	.0922	−.9828
DATA7	−.3642	−1.0000***	−.6409	.9895*	.2360
DATA8	−.9834	−.1844	−.8749	.3287	−.9096
DATA9	.8623	.7828	.9784	−.8667	.4156
DATA10	.3343	−.7590	.0180	.6536	.8146
DATA11	−.9060	−.7210	−.9935*	.8161	−.4992
DATA12	−.6490	−.9434	−.8569	.9822	−.0950
DATA13	−.9834	−.1844	−.8749	.3287	−.9096
DATA14	−.6490	−.9434	−.8569	.9822	−.0950
DATA15	−.6327	.4948	−.3543	−.3599	−.9625
DATA16	.6403	−.4862	.3635	.3507	.9651
DATA17	.2297	.9907*	.5267	−.9594	−.3703
DATA18	−.9319	−.6739	−.9988*	.7764	−.5550
DATA19	−.8091	−.8395	−.9537	.9111	−.3254

Notes
DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.44 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula laurentiana*.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	.9172	.9828	.0223	-.8570	-.4643
DATA2	-.9611	-.8757	-.6532	.3333	-.2053
DATA3	.	.	.	.	.
DATA4	.4725	.2648	.9982*	.4431	.8445
DATA5	-.5270	-.7027	.5511	.9979*	.8860
DATA6	-.9547	-.9970*	-.1297	.7966	.3662
DATA7	-.7157	-.8530	.3345	.9842	.7487
DATA8	-.4725	-.2648	-.9982*	-.4431	-.8445
DATA9	-.9995*	-.9675	-.4471	.5548	.0415
DATA10	.5270	.7027	-.5511	-.9979*	-.8860
DATA11	-.8809	-.9641	.0609	.8968	.5362
DATA12	.9995*	.9675	.4471	-.5548	-.0415
DATA13	.	.	.	.	.
DATA14	-.9995*	-.9675	-.4471	.5548	.0415
DATA15	-.9778	-.9999**	-.2192	.7384	.2802
DATA16	-.0321	.1909	-.9211	-.7951	-.9945*
DATA17	.8420	.7010	.8425	-.0497	.4767
DATA18	-.0549	.1684	-.9297	-.7810	-.9918*
DATA19	-.9930*	-.9944*	-.3087	.6727	.1901

Notes
DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.45 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula scotica*.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	.9953*	.	−.9820	.5000	−.3273
DATA2	−.9537	.	.9998**	−.6682	.1250
DATA3	−.9105	.0000	.7559	.0000	.7559
DATA4	−.5781	.0000	.7857	−.9820	−.5000
DATA5	.7250	.	−.5000	−.3273	−.9286
DATA6	.5817	.	−.3273	−.5000	−.9820
DATA7	.8159	.	−.6186	−.1890	−.8660
DATA8	.9953*	.	−.9820	.5000	−.3273
DATA9	.5817	.	−.3273	−.5000	−.9820
DATA10	−.9953*	.	.9820	−.5000	.3273
DATA11	.6885	.0000	−.8658	.9450	.3715
DATA12	.5817	.0000	−.3273	−.5000	−.9820
DATA13	−.4135	.0000	.6547	−1.0000***	−.6547
DATA14	.8387	.	−.6500	−.1489	−.8450
DATA15	−.9259	.	.9950*	−.7269	.0433
DATA16	.9845	.0000	−.8944	.2473	−.5705
DATA17	−.9293	.0000	.9959*	−.7206	.0524
DATA18	−.7941	.0000	.5892	.2250	.8839
DATA19	.7673	.	−.9176	.9012	.2624

Notes

DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyllcell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.46 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula stricta*.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	.7628	.6466	.8360	.0885	.9126
DATA2	1.0000***	.0000	.9925*	-.5766	.4318
DATA3	.	.	.	.	.
DATA4	-1.0000***	.0000	-.9925	.5766	-.4318
DATA5	-.5000	-.8660	-.6019	-.4193	-.9970*
DATA6	-.1147	.9934*	.0073	.8778	.8465
DATA7	.5000	.8660	.6019	.4193	.9970*
DATA8	1.0000***	.0000	.9925*	-.5766	.4318
DATA9	-.1890	-.9820	-.3074	-.6934	-.9673
DATA10	.	.	.	.	.
DATA11	-.7557	-.6549	-.8299	-.0994	-.9171
DATA12	-.6932	.7207	-.6001	.9886*	.3507
DATA13	-1.0000***	.0000	-.9925*	.5766	-.4318
DATA14	.5000	.8660	.6019	.4193	.9970*
DATA15	-.5000	-.8660	-.6019	-.4193	-.9970*
DATA16	.7220	.6919	.8010	.1490	.9358
DATA17	-.9766	-.2153	-.9955*	.3871	-.6159
DATA18	.8030	-.5960	.7242	-.9499	-.1909
DATA19	-.9999**	-.0112	-.9938*	.5673	-.4419

Notes

DATA1 = thickness of leaves (thickest parts)
DATA2 = thickness of leaves (thinnest parts)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll cell/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = products of stomatal frequency and pore length
PROL = proline levels
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

Table 6.47 Relationship between leaf characteristics and protein/ proline levels on day 6 during water stress, in *Primula farinosa* complex.

PEARSON CORRELATION COEFFICIENTS					
	WSD	PROL	SOL	INS	TOTAL
DATA1	.2797	.5179**	.0744	.2673	.2182
DATA2	.1140	.4145*	.1452	.3000	.2653
DATA3	-.3148	-.2495	-.0518	.0506	.0209
DATA4	-.3103	.1048	-.0998	-.0767	-.0847
DATA5	-.2950	-.1753	-.3419	-.5637**	-.5181**
DATA6	.0177	.0281	-.1994	-.3797*	-.3404
DATA7	-.2610	-.2522	-.2346	-.5012**	-.4392*
DATA8	-.1399	.0363	-.0703	-.3247	-.2584
DATA9	-.1876	-.2346	-.4697*	-.6366***	-.6118**
DATA10	.0675	.1772	-.1683	-.3110	-.2803
DATA11	.4392*	.2175	.4475	.4804*	.4903*
DATA12	.4729*	.2165	-.2169	-.0458	-.1037
DATA13	-.0566	-.2348	-.1163	.0755	.0179
DATA14	.3187	.3869*	.0849	.3038	.2476
DATA15	.3444	.5430**	.1706	.3800*	.3308
DATA16	-.1604	-.2045	-.2347	-.4867*	-.4292*
DATA17	.1771	.0305	.1110	.3916*	.3198
DATA18	.3943*	.1348	.3609	.5226**	.4935*
DATA19	.5878**	.4035*	.3658	.3935*	.4010*

Notes

DATA1 = thickness of leaves (thickest)
DATA2 = thickness of leaves (thinnest)
DATA3 = thickness of cuticle (upper surface)
DATA4 = thickness of cuticle (lower surface)
DATA5 = stomatal apparatus length (lower surface)
DATA6 = stomatal apparatus length (upper surface)
DATA7 = stomatal apparatus width (lower surface)
DATA8 = stomatal apparatus width (upper surface)
DATA9 = stomatal pore length (lower surface)
DATA10 = stomatal pore length (upper surface)
DATA11 = frequency of stomata/area(lower surface)
DATA12 = frequency of stomata/area(upper surface)
DATA13 = number of mesophyll/sq mm
DATA14 = thickness of mesophyll (thickest parts)
DATA15 = thickness of mesophyll (thinnest parts)
DATA16 = cell size
DATA17 = number of epidermal cell(upper surface)
DATA18 = number of epidermal cell(lower surface)
DATA19 = product of stomatal frequency and pore length
PROL = proline level
SOL = soluble protein content
INS = insoluble protein content
TOTAL = total protein content

analysed on day 6 during water stress treatment. Table 6.40 summarizes the results of the correlation of leaf characteristics and WSD, protein/proline levels in *P. farinosa* (b). There was a significant correlation between WSD and the product of stomatal frequency and pore length. The positive correlations were found between proline levels and frequency of stomata per area (lower surface) and number of mesophyll cell per sq mm. Strong relationships were found between soluble protein content and the product of stomatal frequency and pore length, and thickness of mesophyll (thickest parts). Insoluble protein content was significantly correlated only with the number of epidermal cells (upper surface) but a good correlation was also found between total protein content and the product of stomatal frequency and pore length.

Pearson correlation coefficients presented in Table 6.41, indicate the good correlation of WSD in *P. farinosa* (c) with thickness of cuticle (lower surface), stomatal pore length (upper surface), and frequency of stomata per area (lower surface). Proline accumulation in this species was also statistically correlated with thickness of leaves (thickest parts) and stomatal apparatus width (lower surface). The negative relationships were found between thickness of cuticle (lower surface) and frequency of stomata per area (lower surface) whilst a positive correlation was found with stomatal pore length (upper surface). Only 1 out of 19 comparisons between insoluble protein content and leaf characteristics [thickness of cuticle (upper surface)] correlated significantly. In the other hand no significant correlation was found between total protein content and 19 leaf characteristics of *P. farinosa* (c).

As shown in Table 6.42 only a few pairs of leaf characteristics of *P. frondosa* were correlated with WSD and protein/proline levels. However, good correlations between WSD and number of epidermal cells (lower surface) and product of stomatal frequency and pore length were found. Only one leaf characteristics, number of epidermal cell (upper surface) was significantly correlated with proline levels. In contrast, a strong positive correlation was found between soluble protein content and frequency of stomata per area (lower surface). No relationship was found between

leaf characteristics of *P. frondosa* and insoluble protein content. However, a good correlation was found between total protein content and frequency of stomata per area (lower surface).

In *P. halleri*, no significant relationship was found between WSD and leaf characteristics (Table 6.43). However, proline levels were significantly correlated with stomatal apparatus width (lower surface) and number of epidermal cell (upper surface). Good relationships between soluble protein content and thickness of leaves (thinnest parts), and soluble protein content were observed. There was a significant correlation between insoluble protein content and stomatal apparatus width (lower surface) while the total protein content was significantly correlated with thickness of cuticle (upper surface).

It is evident from Table 6.44 that there were significant correlations between soluble protein content and thickness of cuticle (lower surface) and stomatal apparatus width (upper surface). Negative correlations were demonstrated between total protein content and cell size and number of epidermal cell (lower surface). There were 3 out of 19 leaf characteristics that correlated significantly with WSD of *P. scotica*:- thickness of leaves (thickest parts), stomatal apparatus width (upper surface), stomatal pore length (upper surface) (Table 6.45). In contrast, no leaf characteristic was significantly correlated with proline levels and total protein content. Three leaf characteristics, namely product of stomatal frequency and pore length, thickness of mesophyll (thinnest parts), and number of epidermal cells (upper surface) were found to have a good positive relationship with soluble protein content. A strong negative correlation was found between insoluble protein content and number of mesophyll cells per sq mm.

Table 6.46 shows the very strong relationships between WSD and five leaf characteristics in *P. stricta*:- product of stomatal frequency and pore length, thickness of cuticle (lower surface), stomatal apparatus width (upper surface), number of mesophyll per sq mm, and product of stomatal frequency and pore length. In contrast,

there was a direct relationship between proline levels and stomatal apparatus length (upper surface). There were significant correlations between soluble protein content and product of stomatal frequency and pore length, stomatal apparatus width (upper surface), number of mesophyll per sq mm, number of epidermal cell (upper surface), and product of stomatal frequency and pore length. Insoluble protein content correlated significantly with frequency of stomata per area (upper surface) whilst total protein content was correlated with 4 leaf characteristics, namely stomatal apparatus length (lower surface), stomatal apparatus width (lower surface), thickness of mesophyll (thickest parts), and thickness of mesophyll (thinnest parts).

6.4 Discussion

In agreement with the other authors, the results of these investigations indicated that water stress produced an increase of WSD of leaves of all the tested species (Jäger & Meyer, 1977). Leaf WSD dropped markedly in response to rehydration. However, significant differences in WSD between controls and treated plants still existed after the date of rewatering or termination of water stress in some species, e.g. *P. farinosa* from both populations, *P. halleri, P. scotica,* and this can be attributed to the difference in severity and recovery from stress of the tested species. As can be seen in the case of *P. laurentiana* which had the highest WSD on day 6, at termination of water stress, WSD still increased. This event coincided with the visible severe wilting of this species as compared with the other species. In contrast, WSD of *P. scotica* was the lowest on day 6 and no differences between controls and treated plants were found before day 11. Working with the cultivars of *Cicer arietinum,* Singh & Rai (1981) found that a sensitive cultivar had a higher WSD than a resistant cultivar. On this basis, the results obtained in the present study indicated that *P. laurentiana* was the most sensitive species, whilst *P. scotica* was the most resistant species. The differences in response to water stress were probably due to their differences in leaf characteristics. 12 out of 19 leaf characteristics were correlated with WSD. Amongst these leaf characteristics, products of stomatal frequency and pore length seems to be the most important as it governs the resistance to water loss in the leaf (Jarvis & Morison, 1981; Kramer, 1983). Likewise these parameters correlated well with WSD for most species in this study (Table 6.47). The highest value was found in *P. halleri,* with a ranking of other species in the order *P. laurentiana, P. farinosa* (b), *P. farinosa* (c), *P. scotica, P. stricta,* and *P. frondosa.* The comparable ranking for WSD was *P. laurentiana, P. halleri, P. farinosa* (c), *P. farinosa* (b), *P. stricta, P. frondosa* and *P. scotica.* However, there were significant negative correlations rather than positive correlations in some species which would be expected if differences in relative surface area covered by stomata were the sole cause of difference in stomatal resistance. The results pre-

sented here for *Primula* spp. are in agreement with the results of Holmgren (1968) for ecotypes of *Solidago virgaurea.*

As indicated in Ch 3, *P. halleri* and *P. laurentiana* may show a sensitive response to water deficit due to their greater leaf thickness than the other species studied. However, no significant correlation was found between leaf thickness and WSD of these two species and the expectant positive correlations were found only with *P. laurentiana, P. scotica* and *P. stricta.* In addition, *P. scotica* was the only species where the leaf thickness correlated significantly with WSD. In relation to stomatal frequency, *P. laurentiana* was the only species that showed a good positive significant relationship with WSD. On the whole, many of the leaf characteristics of *P. laurentiana* appeared to play an important role in its sensitivity to water stress.

Plant hardiness has been associated with cell size as reported by many workers (Levitt, 1980; Cutler *et al.,* 1977; Turner, 1979) and it has been found that cell size showed a strong relationship with chromosome numbers of the treated plants (see 3.3.12). The data presented here, however, did not fit this postulation as it can be seen that only poor correlations were found in relation to WSD. Lea *et al.* (1977) compared stomatal diffusion resistances of 4n, 6n, and 8n races of *Bromus inermis* and found that the octoploid strain had the lowest mean stomatal resistance. Based upon the responses of *P. laurentiana,* which is n=8 in relation to *P. farinosa,* the results on WSD can be expressed in the same way. However, Tal & Gardi (1976) reported that autotetraploid tomatoes have a lower transpiration rate than the diploids, suggesting higher leaf resistance. Smith (1946) working with diploid, tetraploid and hexaploid races of *Sedum pulchellum* reported that hexaploids were best able to withstand both an excess and a shortage of water, whilst the diploids are the least able to withstand these extreme conditions. In contrast, no differences in leaf resistance of diploids and polyploids of *Viola adunca* were reported by Mauer *et al.* (1978).

Plant hardiness, and especially drought hardiness, seems to be related to the

relative amount of bound water in the tissue; the more bound water in the tissue, the hardier the plant (Vaadia *et al.*, 1961). Noggle (1946) reviewed the literature on the physiology of polyploidy in plants concluded that polyploid plants have a higher water content than diploids. In contrast, Faberge (1936) found that diploids and tetraploids do not differ significantly in their water content. The results reported here indicated that no significant correlation was found between chromosome numbers and relative water content of the primulas. The use of WSD as an indicator of drought in the leaf tissue seems to give recognizable values and has successfullly given results when compared with proline levels during water stress experiments (Jäger & Mayer, 1977; Singh & Rai, 1981; Patel & Vora, 1985).

In the course of the experiments reported here, it was found that in the case of water deficiency in intact plants, the total free proline of leaves increased considerably. This increase in proline levels as indicated by many authors (Barnett & Naylor, 1966; Boggess & Stewart, 1980; Hanson & Hitz, 1982; Shiralipour & West (1984b) is a result of stagnant protein synthesis, lowered rate of proline oxidation, as well as a stimulation of the rate of proline synthesis from glutamate. In fact proline is not the only free amino acid that accumulate during stress, but it is found to increase to a higher extent than any other amino acids. Studying the free amino acids content of the arctic and alpine populations of *Oxyria digyna,* Mooney & Billings (1961) found that free proline was rarely found in non-stressed plants, whilst the other amino acids, e.g. serine, glutamine etc. were found in all populations.

The proline content of leaves under drought conditions may reach a level a hundred times more than the concentration in well-watered controls as the stress is increased (Barnett & Naylor, 1966; Palfi, 1968; Waldren & Teare, 1974; McMichael & Elmore, 1977). However, the concentrations which accumulate in some species are rather low (Stewart & Larcher, 1980) or none at all (Pourrat & Hubac, 1974). According to Palfi (1969) and Palfi *et al.*, (1974), the results reported here indicate that the primulas are low proline accumulators since they accumulate less than a 10-fold increase in the levels of proline when compared with controls.

Earlier workers noted that accumulation of proline could serve as an index of magnitude of stress (e.g. Barnett & Naylor, 1966; Palfi & Juhasz, 1970; Singh *et al.*, 1973). On the basis of WSD and association with leaf characteristics *P. laurentiana* and *P. scotica* were assumed to be relatively more sensitive and resistant respectively than any other species tested. Results obtained in the present study indicate that the primulas accumulated proline as a result of water stress and direct correlations were observed between WSD of the leaf and the proline levels during the course of water stress experiments. Comparisons between species indicated that *P. laurentiana* was more susceptible to drought stress, as measured by proline accumulation, than *P. scotica.*

According to the results of many workers (e.g. Palfi & Juhasz, 1970; 1971; Singh *et al.*, 1972; Rao & Shivraj, 1985; Mali & Mehta, 1977) when the conditions of water deficit are equal, drought-resistant varieties produced significantly more proline than drought-susceptible varieties. In contrast, the reverse can be true as has been reported by many workers (e.g Pourrat & Hubac, 1974; Waldren & Teare, 1974; Patel & Vora, 1985) suggesting no common conclusion about the adaptive role of proline. In the case of the primulas the sensitive species seem to synthesize more proline than the resistant ones. However, the conditions of water deficit were not equal on the same date of sampling even though the primulas were treated precisely the same, their differences were probable due to the variations in leaf characteristics as well as the discrepancy in adaptation to stress. Both positive and negative correlations were found with the same leaf characteristics, but with different species [e.g. stomatal apparatus length (upper surface), number of epidermal cell (upper surface)], suggesting the different response of the species. However, when all the data of each species were pooled, good positive relationships were found with proline levels and some leaf characteristics, e.g. leaf thickness, products of stomatal frequency and pore length.

Patel & Vora (1985) working with wheat, *Plantago,* papaver, and mustard reported that wheat began to accumulate proline when WSD was about 28% and

increased at higher water deficit, while papaver, *Plantago* and mustard accumulated proline at 13, 12, 4 percent respectively. They concluded that wheat, *Plantago* and papaver are drought-tolerant species, whereas mustard is a drought-sensitive plant, and they suggested that proline accumulation may be an indicator of drought resistance or susceptibility. In the case of the primulas, if viewed from the relationship of WSD and significant proline accumulation, the assumption that *P. laurentiana* is the most sensitive species to drought may be changed. It has already been noted above (see 6.3.1.2) that *P. farinosa* (b) accumulated proline significantly at the lowest WSD (19.7%) on day 1, whilst WSD of *P. frondosa* and *P. halleri* were 41.3 and 41.2 % respectively, on the same day. *P. farinosa* (c) started significant proline accumulation on day 2 at WSD 61.3%, while *P. laurentiana* and *P. stricta* responded on day 4, at WSD 51.8 and 56.8% respectively. In contrast, *P. scotica* accumulated proline significantly at WSD 56.3% on day 15. Judging from the scale of WSD, *P. farinosa* (b) would be sorted as the most sensitive species and *P. scotica* would be still regarded as the most resistant species to drought. Comparisons between the two populations of *P. farinosa,* base on the date of sampling, e.g. day 6, *P. farinosa* (c) was significantly higher in proline levels than *P. farinosa* (b) and assumed to be more sensitive to drought than *P. farinosa* (b). On the other hand, if viewed at significant accumulation, WSD and the date, *P. farinosa* (c) appeared to be more resistant than *P. farinosa* (b). In the case of *P. laurentiana* significant proline accumulation was found three days after *P. farinosa* (b) and at the higher percentage WSD, this means that *P. laurentiana* was more resistant to drought than *P. farinosa* (b). However, these results still need more explanation, as during mild water stress (day 1 to day 4) *P. laurentiana* did not accumulate proline, but after having wilted over a period of time this species did accumulate proline much more than the other species.

These results suggest that the duration of water stress must be taken into account when considering the relationship between drought resistance and proline accumulation. Corresponding results in favour of this suggestion were presented by

Singh & Rai (1981) working with cultivars of *Cicer arietinum* and was re-enforced by Stewart (1981). It should be stressed that the primulas accumulated proline significantly at rather high WSD as compared with some plant species (e.g. Patel & Vora, 1985) which was concomitant with severe wilting in some species, e.g. *P. laurentiana, P. scotica.* Thus it can be concluded from results obtained by Waldren & Teare (1974) and McMichael & Elmore (1977) that proline concentration is not a sensitive indicator of the onset of plant drought stress in the primulas studied.

In keeping with the results from the laboratory, it was found that the results from field experiments can be expressed in the similar way. Of the three study sites, SUN was the driest site during midsummer, 1986 (see also Ch 4), whereas GDF was both the highest and the most exposed sites and HAR seemed to be the optimum site for the primulas. Based upon the plant responses studied, there were no significant differences in the proline levels of *P. scotica* at all sites, whilst *P. farinosa* (b), *P. farinosa* (c), *P. frondosa* and *P. laurentiana* showed significantly higher proline levels at SUN than the other two sites which coincided with the severe wilting of those species. Furthermore, the close relationship between the amount of rainfall and proline levels in each species emphasised the effect of water stress on biochemical responses of the primulas. These findings indicated that under natural conditions during the long drought period the primulas did respond to water stress by accumulating proline. These results also show that *P. laurentiana* was the most sensitive species to drought, whilst *P. scotica* was the most resistant species. There were considerably lower proline levels in *P. farinosa* (b) than *P. farinosa* (c) suggesting that *P. farinosa* (b) was the population more resistant to drought. The probable explanation for the discrepancy in response to drought of the two populations may be that *P. farinosa* populations from Upper Teesdale were adapted to the high rainfall condition of the upland climate all the year round, whilst the population from Blackhall Rocks would have experienced drought stress, especially during the summer months and would have been able to adapt themselves to these severe conditions. Again, the differences in some of their leaf characteristics

may be the result of adaptation to drought stress. However, this is not a clear-cut explanation and is worth studying in more detail. In addition, it has been pointed out by Aspinal & Paleg (1981) that differences between cultivars of a certain species are most commonly associated with differences in leaf-water status due to the relationship between water potential, tissue and proline accumulation and genetic differences in the ability of tissue to accumulate proline in response to a specific water deficit also exist.

As the species most resistant to drought, (according to the evidence presented in these studies), *P. scotica* did not show any leaf characteristics which would indicate its superiority or inferiority to other species. However, from consideration of the general morphology of the primulas (Table 1.1), it can be seen that the leaf areas of *P. scotica* and *P. stricta* are smaller than in the other species, e.g. *P. frondosa, P. laurentiana, P. farinosa* (b). As has already been discussed in Chapter 4, the smaller leaf area of *P. scotica* thus may explain its ability to withstand longer period of drought than some of its allies.

As there were the equal levels of proline in all species at GDF and HAR this suggested that the temperatures of the summer months at GDF were not severe enough to initiate the change in the proline levels. The higher levels of proline in *P. scotica* at GDF and HAR than the other species and the higher levels of proline in *P. scotica* than in *P. farinosa* (b) at SUN suggested that proline may play a role as a desiccation protectant and/or as a source of energy during recovery from stress, as indicated by Schobert (1977) and by Barnett & Naylor (1966) respectively. However, the results reported here did not prove the above proposal. In addition, it was pointed out by Jäger & Mayer (1977) that proline accumulation is possibly involved in an adaptation mechanism of plants to drought stress, thus, being of ecological importance.

Although drought stress usually decreases plant protein content, Chen *et al.* (1964) found a circular pattern of decrease increase and restitution in citrus leaves.

By contrast, the present results support the conclusion of Subbotina (1962) that wilting leads to an increase of the amount of protein nitrogen. However, significant increases in soluble, insoluble, as well as total protein were found only in some species and at certain states of water deficit suggesting that species respond differently during drought stress. Strong direct correlations between protein content and WSD on the one hand and proline levels on the other suggested a close connection between protein metabolism and proline accumulation during drought stress. In fact there are many reports e.g. Barnett & Naylor (1966), Fukutoku & Yamada (1984) suggesting that the association of these two processes is an inverse pattern. Water stress usually results in a reduction of leaf protein with a concomitant accumulation of free amino acids, especially free proline (Barnett & Naylor, 1966; Cooke, 1981; Dungey & Davies, 1982). In contrast, the response of plants during low temperature stress result in both increased and decreased protein contents in different species (Razmaev, 1965; Kacperska-Palacz, 1978; Rosinger *et al.*, 1984). The physiological response of plants to drought or frost stress is usually the same due to the dehydration of the cells (Hsiao, 1973; Levitt, 1956; 1980). In addition, decreased protein content during water stress, due to decreased synthesis and energy requirements, has been suggested as a biochemical adaptation which increases the tolerance of plants to stress (Huffaker *et al.*, 1970).

In the case of increased protein content during low temperature stress, Sugiyama & Simura (1967) noted that the more resistant varieties of tea plant showed a higher content of soluble protein. Similar results have been obtained by Stutte & Todd (1967), who reported that wheat varieties which are more resistant to water stress maintain a higher protein content under water stress conditions than the non-resistant varieties. In contrast, non-frost hardy varieties of vine contained greater amounts of soluble protein than frost-hardy varieties (Marutyan *et al.*, 1972). Duisberg (1952) found that in the extremely drought-resistant plant, *Larrea*, the protein content changed very little with the water status of the leaves. However, total protein levels showed some correlation but not consistently. Some species did increase

protein, e.g. *P. farinosa* (c) and *P. laurentiana* whilst other showed a decrease. Of interest here is *P. laurentiana,* for which work on WSD and proline appeared to demonstrate more drought sensitivity, but actually showed an increase in protein. In contrast, *P. scotica* which is drought resistant, did not show any change in protein level. This could be related to overall metabolic changes under stress and may not bear any relationship to the relative abilities of the plants to resist drought. It can be seen from the results of this study that association of protein content with drought resistance does not show the same pattern as WSD and proline levels. Indeed, protein levels of the primulas fluctuated throughout the course of the water-stress experiments in both controls and treated plants and therefore can not be used as a valuable index for changes in water-deficit conditions or as an indicator of drought stress.

Finally, it is important to note that plants respond with many protective adaptations toward frost or drought stress. There appears to be no universal mechanisms of drought or frost tolerance; however, it has been suggested that there are certain types of adaptation against drought within each ecological-physiological group (Henckel, 1964). Stress resistance or susceptibility is unlikely to depend on a single factor. Studies of the responses of plants to environmental stress suggest rather that resistance results from the possession of a number of characteristics (Levitt, 1980; Kramer, 1983). Attempts to explain susceptibility or resistance of plants to environmental stress in terms of single factors are therefore likely to result in specious theories. Changes in nitrogenous compounds can only be regarded as a factor of resistance or tolerance mechanisms (Stewart & Larcher, 1980).

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

The preceeding chapters have given the results of investigations into the ecological physiology of *Primula farinosa* and some closely related species. Discussions in previous chapters have already emphasized the effect of environmental factors on physiological performance of the primulas. In order to link the results of each chapter together the following points are discussed here.

7.2 Polyploidy and plant distribution

As has been mentioned earlier (Ch 1) *Primula farinosa* and its related species form a polyploid complex (n=9) composed of diploids, tetraploids, hexaploids, octaploids, and tetrakaidekaploid. It is believed that in a polyploid series like this, the lower numbers are primitive and the higher ones are derived (Stebbins, 1971). The *Primula farinosa* complex is cosmopolitan in the northern hemisphere, with the widest distribution found in *P. farinosa,* whilst most of its allies are endemic species. It is recognized that species with the widest distribution appear to have the most variations and are often composed of ecological races or ecotypes, each of these being genotypically adapted to its particular habitat (Berry & Björkman, 1980). In contrast, species with a narrow distribution have fewer races and are less variable (Clausen *et al,* 1940). *P. farinosa* and its allies exactly match these patterns as can be seen from the works of Wright-Smith & Fletcher (1943), who reported 7 varieties of *P. farinosa* from Eurasia.

The distribution of arctic-alpine plants seems to be mainly a response to climate, with edaphic factors playing a secondary role (Larsen, 1980). The boundaries

of most plant distributions are controlled by a complex series of climatic factors, and any changes in these factors will cause a species to spread or reduce its areas, depending on the nature of the change (Stott, 1981). It has been suggested that the polyploids of a group are more widely distributed because of a greater tolerance of severe environments (Cain, 1944; Löve & Löve, 1949; Johnson & Packer, 1965). Löve & Löve (1943) studying the flora of Europe, found that ploidy, within a species, generally increases with latitude, and concluded that the polyploids were hardier than their diploid ancestors. The finding of a tetraploid form of *P. farinosa* from Gotland (Davies, 1953) seems to support their work. Unfortunately, I was unable to obtain seeds of *P. farinosa* from that area in order to ascertain the validity of above interpretation.

With regard to the distribution of species studied here, with the highest chromosome number of the group, *P. stricta* (2n=126), has a circumpolar distribution and probably encounters the most severe climate in the Arctic, whereas the other northern-latitude species, e.g. *P. laurentiana, P. scandinavica,* are octaploids in relation to *P. farinosa.* The closely related species, *P. scotica* is probably derived from *P. farinosa,* and is confined to the far north of Scotland, where the climate appears to be milder than the natural habitat of the three species mention above. It can be seen that the distribution of the primulas studied favoured the assumption of Löve & Löve (1949). However, the results from this study (6.3) did not give any clear cut evidence that the polyploids are hardier than their diploid-related species. From the results of the water stress experiment, it is evident that *P. laurentiana* (2n=72) is the species most sensitive to drought, and *P. scotica* (2n=54) is the most resistant, whereas *P. stricta, P. halleri* (2n=36), *P. frondosa* (2n=18) and *P. farinosa* (2n=18) are intermediate. The sensitivity to drought of *P. laurentiana* seems to be best explained by its particular leaf characteristics.

In addition, the result of summer survivals (4.3.2) lead to similar conclusions. Based upon the result of winter survivals (4.3.2), all summer survivals can survive through the winter at all sites. This also shows that ploidy alone can not account

for the hardiness of the primulas.

Clausen *et al.* (1940) noted that polyploidy does not show the same effect in all plants due to the interaction of many genes and also on the type of genes concerned. Smith (1946) working with diploid and polyploid races of *Sedum pulchellum,* concluded that doubling of chromosome numbers alone does not enable a plant to withstand more adverse conditions in every taxa. Tal (1980) added to this by pointing out that the effect of genome multiplication *per se* can be studied independently of other effects only in autopolyploids newly derived from ancestral strains. Studying polyploidy and distribution, Ehrendorfer (1980) concluded that there are no direct and general fundamental associations between polyploidy on the one hand and ecology, habitat, or distribution of angiosperms on the other.

7.3 Species distribution and climate

Since physiological processes which are modified by differences in temperature, moisture supply, and other external variables, are profoundly influenced by environment, successful plants must be fairly accurately adjusted to their surroundings. Of the environmental variables studied, there is no doubt that temperature has the most profound effect upon the physiology of the primulas. For example, innate seed-dormancy of the primulas required chilling treatment, followed by warm temperatures, in order to improve percentage germination. Seeds with a small chilling requirement are expected to be confined to warm areas at low altitude (or near the sea), whilst seeds from plants grown in high altitudes may have a large chilling requirement that would not be met at lower altitudes. However, based on the response of the two populations of *P. farinosa* from northern England, there were no significant differences in percentage germination for all chilling treatment times. In contrast, northern latitude species *P. scandinavica,* showed a very sensitive response to chilling treatment.

Percentage germination of most species also improved significantly when seeds were sown in a diurnally fluctuating temperature regime rather than at 15°C con-

stant temperature. The primulas also had the highest photosynthetic rate at the somewhat warm temperatures of 20-25°C, which is higher than the average of arctic-alpine plants (Bliss, 1985; Chapin & Shaver, 1985a). However, some alpine plants, e.g. *Caltha leptosepala,* which is the plant of the wet meadow had highest photosynthetic rates at 25-30°C, and a rapid decline in the rate was observed at 35°C (Kuramoto & Bliss, 1970). A reduction in the growth of the primulas grown at GDF may be partly due to the upset of photosynthesis because of the low summer temperatures at this site.

One of the principle aims of this study was to determine the mechanism by which climate may control the altitudinal distribution limits of the primulas. The results from growth analyses indicated the profound effects of altitude at GDF. At this site we could see the combination of environmental factors, e.g. low air temperatures of the summer months, low irradiation and high wind speed, which resulted in reduced vigour of all the species. Vegetative growth, such as the production of rosettes, seemed to be inhibited in all species. However, all species produced scapes and florets to a considerable size, whereas in contrast, capsules and seed production were inhibited. The general effect of altitude within specific area has been described by Pearsall (1950), working with *Juncus squarrosus*, who wrote thus:-

> "...the effects of altitude are differential, affecting the seed-production most, flower-production less and vegetative growth least. The analysis of these effects shows that they vary little as between district receiving great differences in rainfall and they can thus be attributed mainly to the diminution of mean temperature with increasing altitude..."

Billings & Mooney (1968) also noted that the weather during some growing seasons in the Arctic or in the high mountains is so cold that flowering and fruiting are seriously upset and little or no viable seed is produced.

It appears likely that the upper altitudinal limit for the distribution of the primulas is restricted by low summer temperatures due to the failure of capsule and seed setting. Pigott (1974) also showed that the northern distribution of *Cirsium acaule* is limited by failure to set seed. Pigott & Huntley (1981) working with

Tilia cordata found that individuals at the northern limit of distribution do not set seed unless there is a particular warm summer. The abortive capsule and seed production at low temperatures may be due to the reduction of pollen germination and function as has been reported in wild and cultivated tomatoes (Zamir *et al.*, 1981). It has been suggested that low summer temperatures affect plants differently, i.e. by inhibiting a complete life cycle of the species rather than damaging vegetative organs (Grace, 1987). In addition, in the case of the primulas which are insect-pollinated species the failure of capsule and seed setting may be partly due to scarcity of the pollinator at high altitude as has been pointed out by Mani (1978) and Bierzychudek (1981).

As they are restricted to damp or moist places, it might be expected that the primulas would show a sensitive response to drought. It is obvious that this was true, as the results from laboratory and field experiment (6.3.1 and 6.3.2) both showed that they accumulated proline significantly during drought. Furthermore, water stress is most likely to limit primula growth to a varying extent. Based on the results from this study it is reasonable to conclude that low summer temperature is the limiting factor at the upper limit of high altitudes, whilst drought is the limiting factor at low altitudes.

As has been discussed earlier, the effect of temperature and other environmental factors are rather difficult to separate, especially when working in an uncontrolled environment. For example, analyses of the primula's growth along an altitudinal gradient in this study appeared to emphasize the interaction of the environmental factors since many correlations between climatic factors and growth characteristics were found. Cain (1944) has emphasized that physiological processes are multi-conditioned and it is impossible to speak of a single condition or factor as being the cause of a particular observed effect in an organism.

7.4 Physiological performance in the primulas

One of the main aims of this study was to ascertain whether there was any evidence for the existence of ecological races in the populations of *P. farinosa* from Teesdale and a coastal habitat which is considered to have been isolated since the late-glacial period. Different performances from comparative studies done under uniform conditions can be attributed to different genotypes. The results from the chilling treatments showed that the two populations of *P. farinosa* had a similar percentage germination in all the treatments. The results of plant growth analyses and photosynthesis also showed a similar physiological performance of these two populations, although the population of *P. farinosa* from Blackhall Rocks showed many greater values of leaf characteristics e.g. number of cell, stomatal size, etc. than the population from Upper Teesdale. There are somewhat different results in the response to water stress as seen in the determination of protein/proline levels and growth analyses. *P. farinosa* from Upper Teesdale had significantly higher protein/proline levels than *P. farinosa* from Blackhall Rocks and this has been ascribed to the more sensitivity to drought of Teesdale population. In contrast, *P. farinosa* from Upper Teesdale showed less sensitivity to drought than *P. farinosa* from Blackhall Rocks on the basis of RLaGR determinations. The different results from these two experiments may be in part due to the variable response to drought of individual plants within a bowl.

A more striking difference between the two populations of *P. farinosa* from northern England is in the time of flowering. It was observed that *P. farinosa* from upland populations produced flowers earlier than a lowland or coastal populations, and this characteristic was still retained when the plants were grown side by side at all sites along the altitudinal gradient studied, thus indicating genotypic differences. Turessen (1922) emphasized the physiological characteristics at time of flowering, e.g. earliness, as significant indicators of the ecotypes. He found, in general, that southern European alpine and high nordic ecotypes were the earliest to flower, whilst the lowland forms from northern and central Europe were the last. The performance

of the two populations of *P. farinosa* exactly matched the above findings. In evolutionary terms, it might be expected that the two populations which experience different environmental conditions will gradually diverge from one another. This divergence will be most pronounced if the populations are geographically isolated, so that gene flow does not act to keep the genetic composition of the populations identical (Stebbins, 1974).

In short, this study demonstrates clearly that the two populations of *P. farinosa* differ in a number of morphological and physiological characteristics; some of which could make it possible for the different races to occupy different ecological habitats.

Amongst the closely related species of *P. farinosa, P. scotica* raises some interesting points. Although it is a member of the Arctic-SubArctic Element (Matthews, 1937), this species showed somewhat different characteristics and physiological performances to those expected of arctic plants (see Chapin & Shaver, 1985a,) e.g. low total chl content (compared with the other species studied), and having the highest rate of photosynthesis at rather warm temperatures (25-30°C), etc. *P. scotica* produced scapes and florets earlier than the other species and at the optimum site (HAR) this species had vigorous vegetative (producing rosettes) and reproductive growth (highest number of seeds per capsule). Its 2-3 flowering periods and free setting of seed may partly compensate for low percentage germination and slow seedling establishment, which may result in their death if the climate is not favourable. Ritchie (1954) also noted that *P. scotica* is tolerant of a particular type of edaphic conditions. It also exhibited both frost and drought tolerance. Based on performances and features mention above, it is reasonable to postulate that *P. scotica* is very far from endangered species, even though its natural distribution is restricted to a small area of the far north of Scotland.

It is, however, surprising that as an endemic species, *P. scotica* has relatively broader tolerance ranges and more advantageous characteristics than its allies, especially *P. farinosa* which is supposed to show the most variable physiological per-

formance of the group. The restricted distribution of *P. scotica* at present has been suggested by Ritchie (1955) to be due to two natural barriers:-

(1) the prevalence of moorland and peat-bog vegetation over much of the land which is contiguous with the present area of the plant, and

(2) the insularity of the localities in Orkney.

He also showed experimentally that the dispersal of seeds by the wind is not sufficient to overcome these barriers.

Finally, it should be noted that the response of a species in its natural habitat may not be the same as its response to the conditions that prevail when it is grown in a laboratory or green house. There are many eco-physiological processes which have not been considered in the present study. However, as one of the people who value the flora for both its hereditary beauty and scientific interest, I have high hopes that others may have the possibility of future study and hope that the information from this study will provide a platform on which future work, either in the laboratory or the field, can be based.

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