Resource allocation in the pseudoviviparous alpine meadow grass (Poa alpina l.)

Simon Pierce

Resource Allocation in the Pseudoviviparous Alpine Meadow Grass (*Poa alpina* L.).

Submitted to the University of Durham
for the degree of Doctor of Philosophy

by

Simon Pierce B.Sc. (Wales)
Department of Biological Sciences

1998

Acknowledgements

The author wishes to thank the following people. In particular Dr. Bob Baxter (Uni. Durham) for his imaginative ideas and Dr. Clare Stirling (Institute of Terrestrial Ecology, Bangor) for her practical suggestions, and both for regular supervision. Dr. Alan Pearson also provided supervision and lab space. This work was funded primarily by a University of Durham scholarship, with an additional studentship provided by the I.T.E.

At the University of Durham, Department of Biological Sciences.

Prof. Nick Harris and Dr. Phil Gates for the use of their fluorescence microscopes and for good humour. Dr. Brian Whitton and lab members for the use of their facilities. Kate Lewthwaite for general help and advice, and more specifically for coaching in the fine art of Skalar. The following technical staff for help on numerous occasions; Eric Henderson, Michael Bone, Gordon Bainbridge, Paul Sidney and Margaret Snow. Also, the following people all made life in Lab. 7 entertaining; Michelle Amor, Dr. Dorothy Catling, Jon Lane, Dr. Michelle Metcalfe, Dr. Jackie Spence (& son), and the inimitable Dr. Laszlo Tecsi.

At the University of Durham, Botanic Gardens.

Mike Hughes and Steve Ansdell for help and plantsmanship, and the grounds staff for general help.

At the Institute of Terrestrial Ecology, Bangor Research Unit.

Dr. Trevor Ashenden for his administrative and organisational skills. Dr. Harry Harmens for the loan of his prized Ciras-1. Laurence Jones, Alison Bostock and Heather Dale for good company during lunch breaks at the solardomes.

At the University of Wales, Bangor, Department of Biological Sciences.

I would like to thank Prof. John Farrar for both putting up with me, and on several occasions putting me up. Also, I would like to thank everyone in the lab, and acknowledge the help of several people in particular. Gareth Glyn Williams for invaluable technical support during ^{14}C experiments, Dr. Sheila Gunn and Lynne Gilbert for the loan of several pieces of equipment, Dr. Bryan Collis and all of the above for help in finding and moving equipment during a busy period.

Finally I would like to thank friends, Graham, Jenny and Phillip Pierce, and especially Jennifer Smith for support, love and pep talks throughout the three years.

Pierce S (1998) Resource allocation in the Pseudoviviparous Alpine Meadow Grass (*Poa alpina* L.). Ph.D. Thesis. University of Durham, UK.

Abstract

Many biotypes of the northern-hemisphere Arctic-Alpine grass *Poa alpina* L. reproduce asexually via prolification of the spikelet axis to produce dehiscing shoots. Although such pseudoviviparous plantlets are capable of photosynthesis, the source-sink characteristics of these synflorescence systems are unknown, including the degree to which plantlets are capable of providing for their own carbon requirements, or contributing to parental sinks.

An initial anatomical investigation of the culm revealed that transpiration flow, as delimited by Lucifer Yellow tracer dye, was maintained despite advanced senescence (as evidenced by loss of chlorophyll and chloroplasts), with plantlet leaves driving transpiration flow. Transpiration flow was not hindered by cavitation or tylosis in older culms, the low frequencies of these processes being bypassed via nodal plexi. Despite this, water content of plantlets declined over time and visual indications of water stress became apparent, suggesting that water supply via the determinate culm was not sufficient for the increasing transpirational demand of indeterminate plantlets.

Photosynthetic rates within the paracladial zone, as determined by infrared gas analysis (IRGA), exceeded respiratory rates by 3 – 4 fold, indicating that plantlets were sources of carbon. ^{14}C tracer studies determined that the paracladial zone was not only as efficient at fixing carbon as the youngest fully expanded leaf, but that both organs exported carbon basipetally (*c.f.* acropetal export from this leaf in seminiferous grasses). Distal plantlets fixed approx. 20 % more ^{14}C than proximal plantlets, by virtue of greater dry weight.

Manipulative growth analysis of the paracladial zone suggests the operation of a system of apical dominance, with distal plantlets becoming dominant over proximal plantlets. At dehiscence, distal plantlets were more likely to become established, and possessed relative growth rates more than ten times those of proximal plantlets. Paracladial heterogeneity was also apparent as an increased proportion of aborted spikelets on proximal paracladia. Data indicate that this abortion was, at least in part, a result of constraint imposed by the pseudostem on the developing synflorescence.

When grown in conditions of differing resource availability (altered nutrient supply and atmospheric CO_2 concentration), low nutrient availability in concert with elevated CO_2 concentration induced particularly low photosynthetic nitrogen and phosphorus use efficiencies in both parent and plantlet tissues. This occurred in concert with acclimatory loss of photosynthetic capacity leading to a decreased reproductive response of the plant; a product of the number of tillers in flower and the subsequent growth of attached plantlets. It is predicted that in future climatic conditions *Poa alpina* will decline in habitats that include species which exhibit less acclimatory loss, no change, or an increase in photosynthetic capacity. These experiments also rule out resource availability as a cause of heterogeneity within the paracladial zone. A direct study of the phytohormonal characteristics of the pseudoviviparous system is therefore proposed in order to elucidate the mechanism of control within the paracladial zone.

Contents

List of plates

List of figures

List of tables

Declaration

I hereby declare that this thesis and material presented herein has not previously been submitted to this or any other institution and is solely the product of the present author.

Signature S. Pierce

Date 5/12/98

Abbreviations

A	Net photosynthetic rate
ABA	Abscisic acid
Abs.	Absorbance
ADC	Analytical Development Company
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
Bq	Bequerel
BV	Blue-violet excitation
Chl_a	Chlorophyll *a*
Chl_b	Chlorophyll *b*
C_i	Sub-stomatal carbon dioxide concentration
CO_2	Carbon dioxide
DW	Dry weight
FW	Fresh weight
H_2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
IRGA	Infrared Gas Analysis
kBq	Kilo bequerel
LA	Long Ashton nutrient solution
LYCH	Lucifer yellow dithionite
m.a.s.l.	Metres above sea level
µCi	Micro curie
Mpa	Megapascal
N	Nitrogen
NAR	Net assimilation rate
NUE	Nutrient use efficiency
O_2	Molecular oxygen
$O_2^{\cdot}$	Superoxide radical
P	Phosphorus
PAR	Photosynthetically active radiation (400 - 700 nm)
PCR	Photosynthetic carbon reduction
PcZ	Paracladial zone
P_i	Inorganic phosphate
PNUE	Photosynthetic nitrogen use efficiency
PPFD	Photosynthetic photon flux density
PPUE	Photosynthetic phosphorus use efficiency
r.p.m.	Revolutions per minute
Ru1,5bisP	Ribulose-1,5-bisphosphate
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
sDW	Structural dry weight
SOD	Superoxide dismutase
SLA	Specific leaf area
tDW	Total dry weight
TNC	Total non-structural carbohydrate
VB	Vascular bundle
WUE	Water use efficiency

1 Introduction

The grass family or Poaceae (formerly Gramineae) is a monocotyledonous family that is of great economic and ecological importance worldwide. Grasses have been extensively studied and reliable summaries include Arber (1934), Clayton & Renvoize (1986) and Chapman (1996). The taxonomy and classification of British species in this text follow that of Hubbard (1992), although in the case of *Festuca vivipara* (L.) Sm., the classification of Frederiksen (1981) is used. Sexual reproduction in grasses is best described by Arber (1934) and the floral biology of the genus *Poa* by Anton & Connor (1995).

This general introduction serves to define some of the asexual processes involved in grass reproduction, which is essential for an understanding of this study and the subject as a whole. Also, the particular process investigated by this study – namely prolification – shall be placed in context with a description of the habitats and cyto-genetics of plants exhibiting this characteristic.

1.1 Apomixis and the mop habit

Strictly, apomicts are plants in which the mechanism for sexual fertilisation has been lost (Gustafsson 1946a). However, the term apomixis is also used to describe any form of asexual reproduction, and is thus sub-divided into two types; agamospermy[1] (the development of an unfertilised ovule into an asexual seed), and vegetative reproduction in general (Gustafsson 1946a). Many forms of vegetative reproduction, such as stoloniferous runners or tillering, do not rely on the production of specialised reproductive structures and/or do not necessarily represent a loss of sexual fertilisation. They are alternative and independent modes of propagation and it is perhaps best to consider each vegetative process separately, and to avoid the use of the term apomixis in this context.

Prolification - the continued growth of the spikelets as leafy plantlets (see Section 1.1.4) - is a vegetative reproductive process which is often confused with

[1] Agamospermy has been recorded in grasses such as *Poa alpina* L. (Müntzing 1940, 1980), *Poa pratensis* L. (Myers 1943), *Poa ampla* Merr. x *Poa pratensis*, *Poa scabrella* (Thurb.) Benth. x *Poa ampla*, *Poa scabrella* x *Poa pratensis* (Hiesey 1953), and *Dichanthium aristatum* Willemet. (Knox 1967). In these cases the apomictic 'seeds' did not germinate while still attached to the parent plant. A grass inflorescence may produce some seed sexually, and some via agamospermy (Müntzing 1980). See Gustafsson (1946a) for a more exhaustive list of grass species exhibiting agamospermy.

vivipary (a sexual process), and proliferation (which has little known reproductive value). These processes occur after elongation of the culm, or true stem, and may result in individuals with a tufted 'mop' or 'pompon' of leaves at the apex of the culm (Arber 1934). Confusion between these processes is compounded by the fact that the term vivipary is frequently used to describe the process of prolification, due to the original misuse of the term vivipary by Carl Linnaeus (Linnaeus 1737, 1759; cited by Gustafsson 1946a and Wycherley 1953a). The ubiquitous and long-term misuse of this terminology forces its further misuse for the sake of consistency. Most authors are aware of this, including a caveat to that effect in their work. However, this study shall not use a caveat, but the correct terminology, which should be borne in mind when consulting the literature. These terms and the processes they describe shall be presented in the following sections.

1.1.1 True vivipary

True vivipary, or 'premature germination' (Mangelsdorf 1930) refers to the germination of seed that is still attached to a parent plant (i.e. seed with no period of dormancy; Pope 1949). Elmqvist & Cox (1996) define true vivipary as a purely sexual event, in which the fruit pericarp is breached by the growing embryo. Vivipary of grasses can therefore be distinguished by the presence of coleoptiles in spikelets (Wycherley 1953a), and is known in *Festuca ovina* L., (Wycherley 1953a), *Spartina* x *townsendii* H. + J. Groves (Merchand & McLean 1965), *Avena fatua* L. (Hsiao & McIntyre 1988), and the genera *Melocalamus*, *Melocanna* (Arber 1934; McClure 1966) and *Dinochloa* (van der Pijl 1972); the latter three genera belonging to the tribe Bambuseae and native to East Asia. In some cereal crops vivipary has been artificially induced by excessive humidity (Pope 1949; Hsiao & McIntyre 1988). A general account of vivipary is also given in van der Pijl (1972).

1.1.2 Abnormal development of the inflorescence axis

Deviation from typical development of the inflorescence axis can result in abnormal inflorescences that superficially resemble the mop habit, and can be misinterpreted as arising from vivipary, proliferation or prolification. For example, Hubbard (1992) states that *Lolium perenne* L. is:

> "very variable in structure, especially so far as its spikes are concerned, these sometimes being loosely branched or shortened and much congested."

Indeed, Arber (1934) displays a variety of abnormal *Lolium* inflorescences. Examples of suppressed and bifurcated inflorescences of *Lolium perenne* can be seen in Fig. 1.1. A recent general account of teratology - the study of 'monstrous' deviations from typical morphological development - is given in Bell (1991), with many striking general examples of deviations in Masters (1869) and Worsdell (1915, 1916).

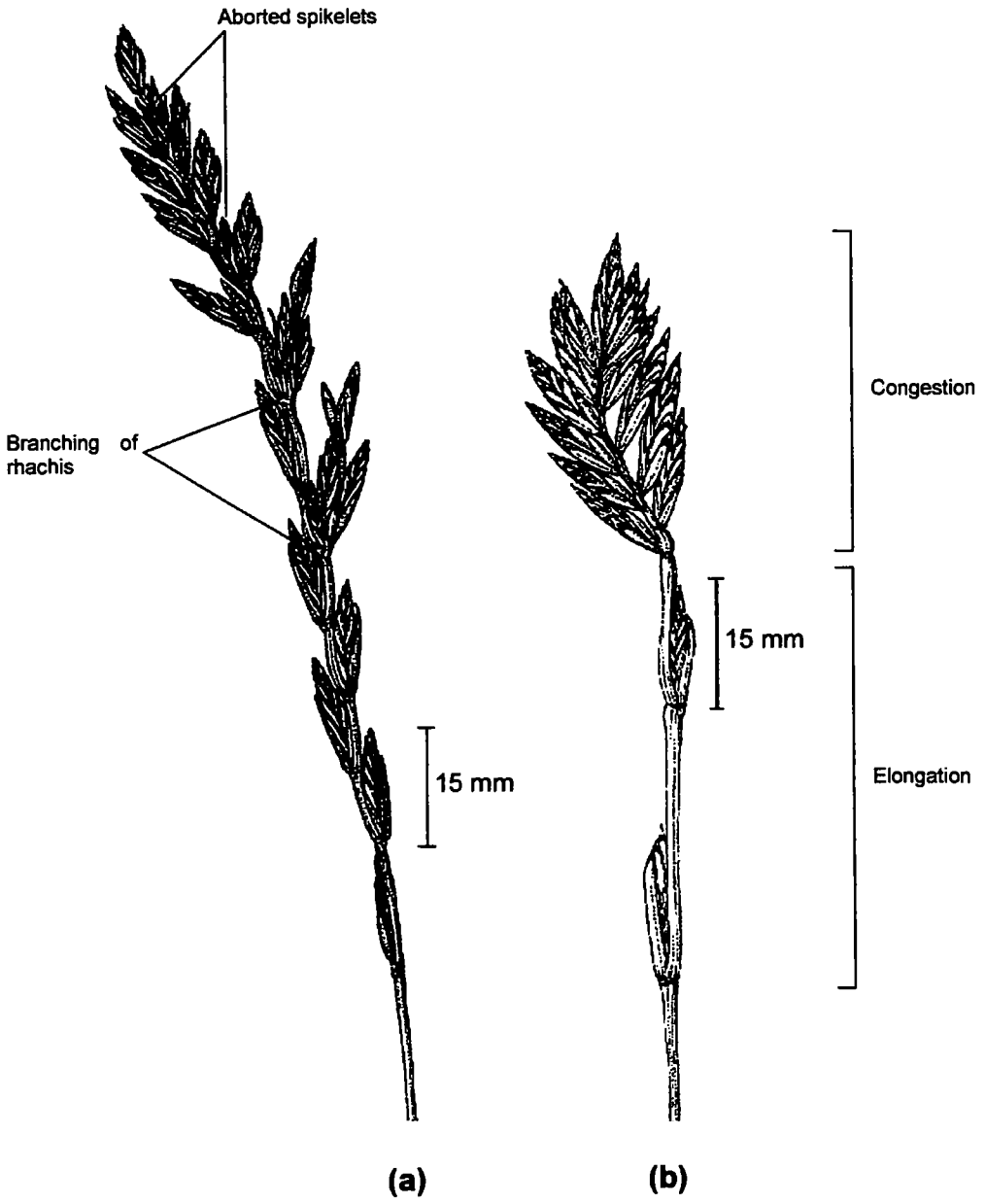


Fig. 1.1. Deviational inflorescence development in *Lolium perenne* L., superficially resembling the 'mop' habit.
(a). Abnormal bifurcation of the rachis and abortion of spikelets. Ten such inflorescences were found in a sward growing in a grass verge on Hollingside Road, Durham, U.K. (outside the University Botanic Gardens) on 1/Aug/1996.
(b). Variation in rachis internode length - elongation (proximally) and suppression ('congestion'; distally) of the axis. This specimen was found on 10/June/1996 in a meadow at Durham University Botanic Gardens. Two other such spikes were found nearby. Five similar spikes were found on 1/Aug/1996 in a sward growing in a verge on Southwark Road, Durham, U.K.

1.1.3 Proliferation

The process of proliferation or false multiplication (Bell 1991) is considered by teratologists to be a specific form of deviation (Worsdell 1915). In grasses proliferation is axillary in nature; i.e. representing the growth of usually suppressed axillary buds. Thus proliferation occurs at the nodes of the stem, and these proliferated side-shoots are tillers. Where the stem is vertical, such as a flowering culm or the extending culm of an intra-vaginal tiller, proliferations are borne above the plant and are known as 'aerial tillers'.

Proliferation producing aerial tillers is more apparent after unusually long growing seasons, with aerial tillers usually being found from September to December in Britain (Arber 1934), after the elongation of the culm. Thus proliferation is possibly the result of a change in the balance of phytohormones with changing daylength and temperature conditions, promoting the growth of axillary buds. Aerial tillers have been recorded in grasses such as those shown in Table 1 of Appendix 1, and examples of aerial tillering observed by the author in *Holcus lanatus* L., and *Agrostis canina* L. are shown in Plates 1.1 and 1.2.

Proliferated aerial tillers do not possess any specialised store of nutrients or water and remain attached to the main tiller axis (Arber 1934). These tillers are dependent on the main shoot for water and nutrients and die along with the main shoot at the end of the growing season. In nature they do not detach, but if provided with a substrate, aerial tillers can root and grow independently (Arber 1934). Root initials have been observed at the base of *Agrostis stolonifera* proliferations (which then grew when placed on sand; the aerial tillers developing into healthy plants), and adventitious roots have been observed growing from the base of proliferated tillers of *Agrostis tenuis* while still attached to the main shoot (personal observations of the author).

Plate. 1.1. Proliferation of *Holcus lanatus* L., resulting in aerial tillers and a 'mop' habit. This specimen was collected by the author on 23/Oct/1995 in a graveyard on Southwark Road, Durham, U.K. Two other such proliferated culms were found on the same individual, and two similar culms were found on a nearby plant, with proliferated shoots arising from all the nodes of the up-right culm. The image was scanned from an herbarium specimen three years after collection.

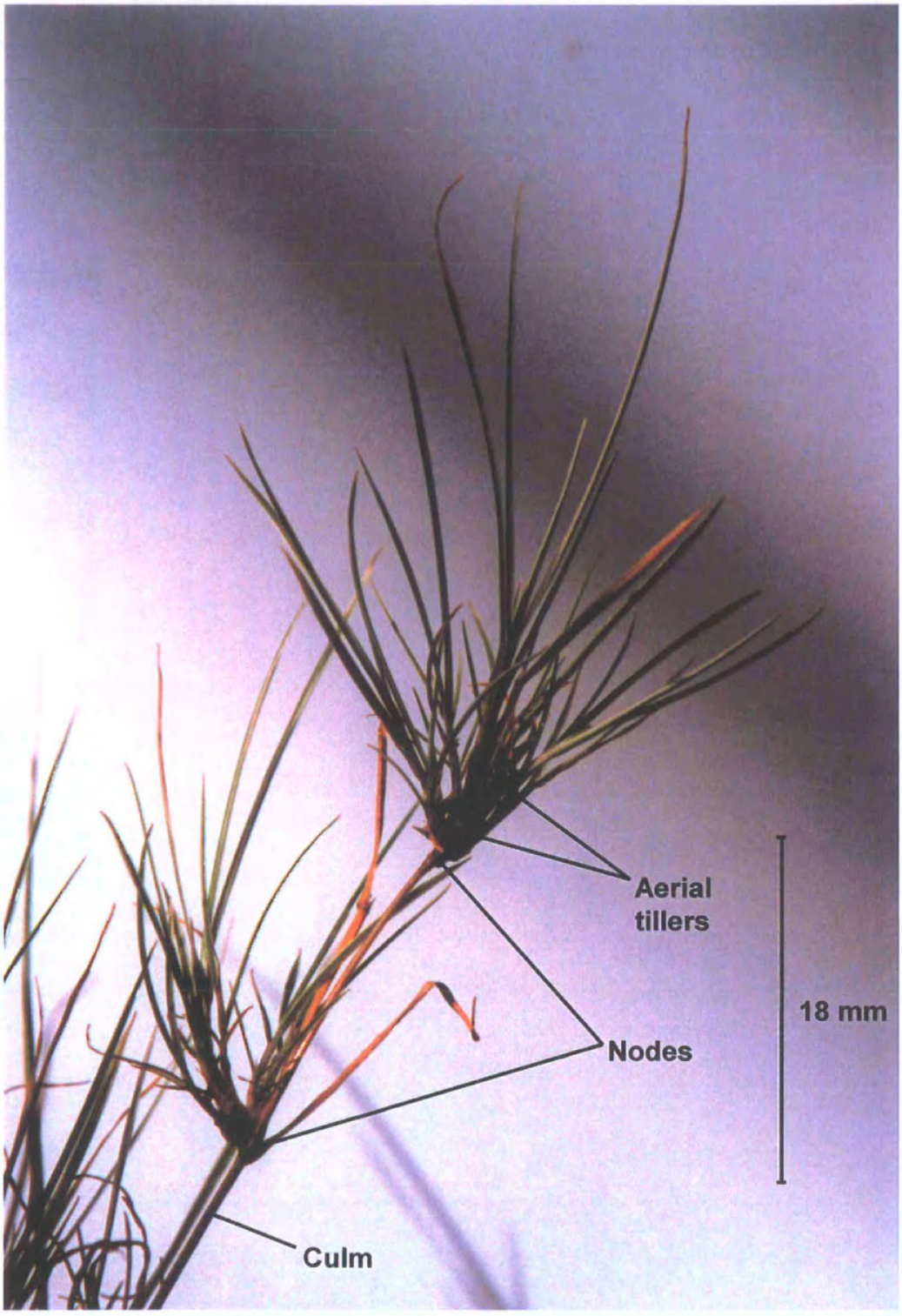


Plate 1.2. Proliferation of *Agrostis canina* L. 'Silver Needles', resulting in aerial tillers. This individual was found amongst plants for sale at Durham Botanic Gardens, 3/Oct/1997. Hubbard (1992) confirms that *Agrostis canina* regularly undergoes proliferation.

1.1.4 Prolification

Prolification (false or pseudovivipary, Bell 1991; see also Elmqvist & Cox 1996) refers to the production of vegetative shoots directly by meristems associated with the spikelets, and from here on the term prolification shall be used to describe the process resulting in the condition of pseudovivipary. The processes of proliferation and prolification in grasses differ in one main aspect – the history of the meristems involved. Meristems involved in proliferation are derived from the apical meristem during its vegetative phase of growth, and meristems involved in prolification are ultimately derived from the apical meristem after its initiation to inflorescence differentiation. Thus prolification marks a reversion of growth back to a vegetative phase and is a process truly distinct from axillary proliferation.

1.2 Ephemeral and habitual prolification

Grasses exhibiting prolification can be sub-divided into two groups. Firstly, species which are usually seminiferous and do not prolificate unless prompted by unusual changes in environmental conditions (these are known as 'ephemerally viviparous' species (Arber 1934) and from here on these shall be referred to as ephemerally pseudoviviparous grasses). Secondly, species in which prolification is the usual state of the inflorescence (these are the true 'viviparous grasses', Hubbard 1992; from here on these shall be referred to as habitually pseudoviviparous grasses).

1.2.1 Ephemeral prolification

The potential for ephemeral prolification possibly occurs in all grasses (Harmer 1978), but has only been recorded in a certain number of species (listed in Table 2 of Appendix 1). Floral induction in grasses usually has a dual environmental requirement consisting of primary induction (i.e. the conversion of the apical meristem into an inflorescence primordium) in short photoperiods and/or low temperature, followed by secondary induction. Coldrake & Pearson (1986) state that in the case of pearl millet (*Pennisetum americanum* (L.) Leeke) secondary induction consisting of short daylength (10 h) and higher temperatures (30 °C *c.f.* 21 °C) corresponded with an increase in the rate of growth of the inflorescence primordium and initiation of differentiation of the inflorescence. Continuation of growth in these conditions resulted in typical development, whereas revertion to lower temperatures - before the completion of inflorescence differentiation (after 10 d in this case) - resulted in abnormal inflorescences. This included the production of more than one inflorescence on the axis, mis-shapen inflorescences (such as those of *Lolium*

perenne detailed in section 1.1.2) and also inflorescences which were composed mainly of leaf material. That this deviation is the mechanism underlying ephemeral prolification is supported by other authors. Heide (1986, 1987) discovered that if secondary induction of *Dactylis glomerata* L. (see Plate 1.3), *Poa nemoralis* L., *Poa alpigena* (Fries) Lindman and a biotype of *Poa alpina* L. occurred in lower temperatures (3 to 6 °C) and short photoperiods (8 h) then prolification was the result. Kjellqvist (1961) supports this by inducing production of plantlets by *Festuca rubra* L., by exposing plants to low temperature and short photoperiods.

Kjellqvist (1961) concluded that *Festuca rubra* possesses a genetic potential for prolification which is only expressed if the environment exhibits radical changes from conditions usually found at a particular time of the growing season. Nielson (1941) suggested that it is the rapidity of environmental change that prompts ephemeral prolification in grasses. These suggestions together may be closer to the truth; a rapid drop in temperature during inflorescence differentiation resulting in abnormal growth forms and vegetative structures within the inflorescence, as seen by Coldrake & Pearson (1986) in pearl millet. Many plantlets of ephemerally pseudoviviparous grasses may themselves be in flower whilst still attached to the parent inflorescence (Plate 1.3 and Fig. 1.3 – see also Foreman (1971) with the example of *Trisetum spicatum* (L.) Richt., and also Hubbard (1992)), which may be an indication of temperature conditions subsequently returning to those favourable for floral differentiation after the production of prolificated spikelets.

Aside from the usual environmental prompts, disease and physical damage can also trigger ephemeral prolification. This is the 'induced vivipary' of Clay (1986); terminology corrected to 'induced pseudovivipary' by Elmqvist & Cox (1996). For example head smut (*Sphacelotheca reilana* (Kuehn) Clint.) or loose kernel smut (*Sphacelotheca cruenta* (Kuehn) Potter) infections can cause prolification in *Sorghum bicolor* (L.) Moench. (Casady 1969). Beetle (1980) also reports prolification, probably initiated via invertebrate damage, in *Bromus unioloides* (Willd.) HBK, *Festuca hieronymi* Hack., *Panicum demissum* Trin., *Poa cusickii* Vasey and *Scleropogon brevifolius* Phil. Inflorescences of *Agrostis* spp., infested with the nematode *Anguillina agrostis* (Steinbuch) Goodey, often produce galls which play no part in the reproduction of the grass (Philipson 1935; Beetle 1980). Akai & Fukutomi (1966) also describe induced pseudovivipary in rice (*Oryza sativa* L. var. *Aya-Nishiki*), *Eleusine indica*, and *Setaria viridis* resulting from infection by the downy mildew *Sclerophthora*

macrospora - which also caused abnormal inflorescences in *Arrhenatherum elatius*, *Dactylis glomerata*, *Imperata cylindrica* var. *koenigii*, and *Miscanthus sinensis*. The physiological mechanisms involved in these incidences currently remain without investigation, but the character of these abnormalities again suggests a disturbance in the development of the inflorescence primordium before the completion of differentiation. Van Beusekom (1907) induced the production of adventitious buds at the leaf tips of *Gnetum gnemon* L. (Gymnospermophyta), both by insect damage and by inflicting stab-wounds with a fine needle; this being viewed as a case of induced deviation of growth form (Worsdell 1915). Thus the literature indicates that ephemeral prolification - involving indiscriminate disruption of meristematic development - represents reversion in the teratological sense, with components of the infloresence reverting to vegetative development. This is borne out by the inconsistent nature of the structures and shoots produced (i.e. the growth response to disruption of the inflorescence primordium is unpredictable), ranging from abnormal and multiple inflorescences to plantlets reminiscent of the habitually pseudoviviparous grasses. The ultimate growth form exhibited is probably dependent on the precise stage of development at which the inflorescence primordium is disrupted, as a number of different meristems are involved (Irish 1997) – the production of spikelet meristems depends on the development of the meristems producing the branches of the inflorescence, and these in tum depend on the development of the main axis apical meristem.

That ephemeral prolification can produce plantlets that are of a similar, but not identical, nature to the plantlets of habitually prolific grasses is illustrated by the following examples. The author observed plantlets of *Festuca rubra* L. ssp. *commutata* Gaud. which consisted of well developed shoots borne on spikelets (Plate 1.4). These possessed root initials (Plate 1.5) which on wetting developed into healthy roots in a matter of days (Plate 1.6) with plantlets becoming established and growing in potting compost, without care or attention other than regular watering. However, these well developed plantlets, and also those of *Dactylis glomerata* observed by the author (Fig. 1.2 & 1.3), did not dehisce in the manner associated with 'viviparous' plantlets, being resistant to all efforts to remove them exempting the blade of a scalpel (note the retention of the glumes and pedicel tissue in Plates 1.5 & 1.6). The morphological inconsistency exhibited, and the inability of the plantlets of many species to dehisce, distinguishes the shoots produced by ephemeral prolification from propagules produced by habitual prolification.

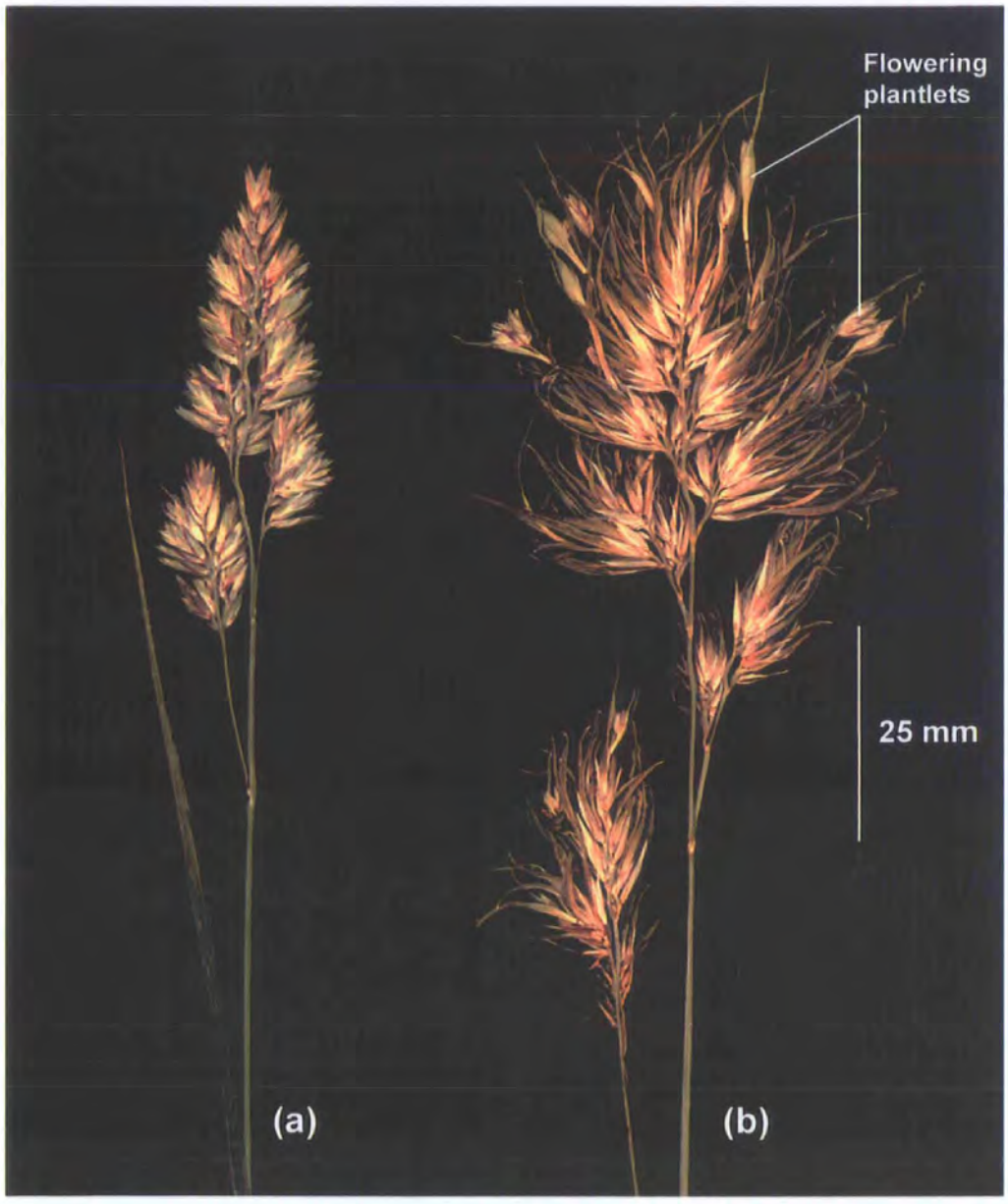


Plate 1.3. **(a)** Seminiferous and **(b)** pseudoviviparous inflorescences of *Dactylis glomerata* L. from neighbouring plants. Specimens were collected by the author at Heath-End Fields, Alsager, South Cheshire on 26/Aug/1993, next to the borough landfill. Note the presence of flowering plantlets. The image was scanned from herbarium specimens five years after collection.

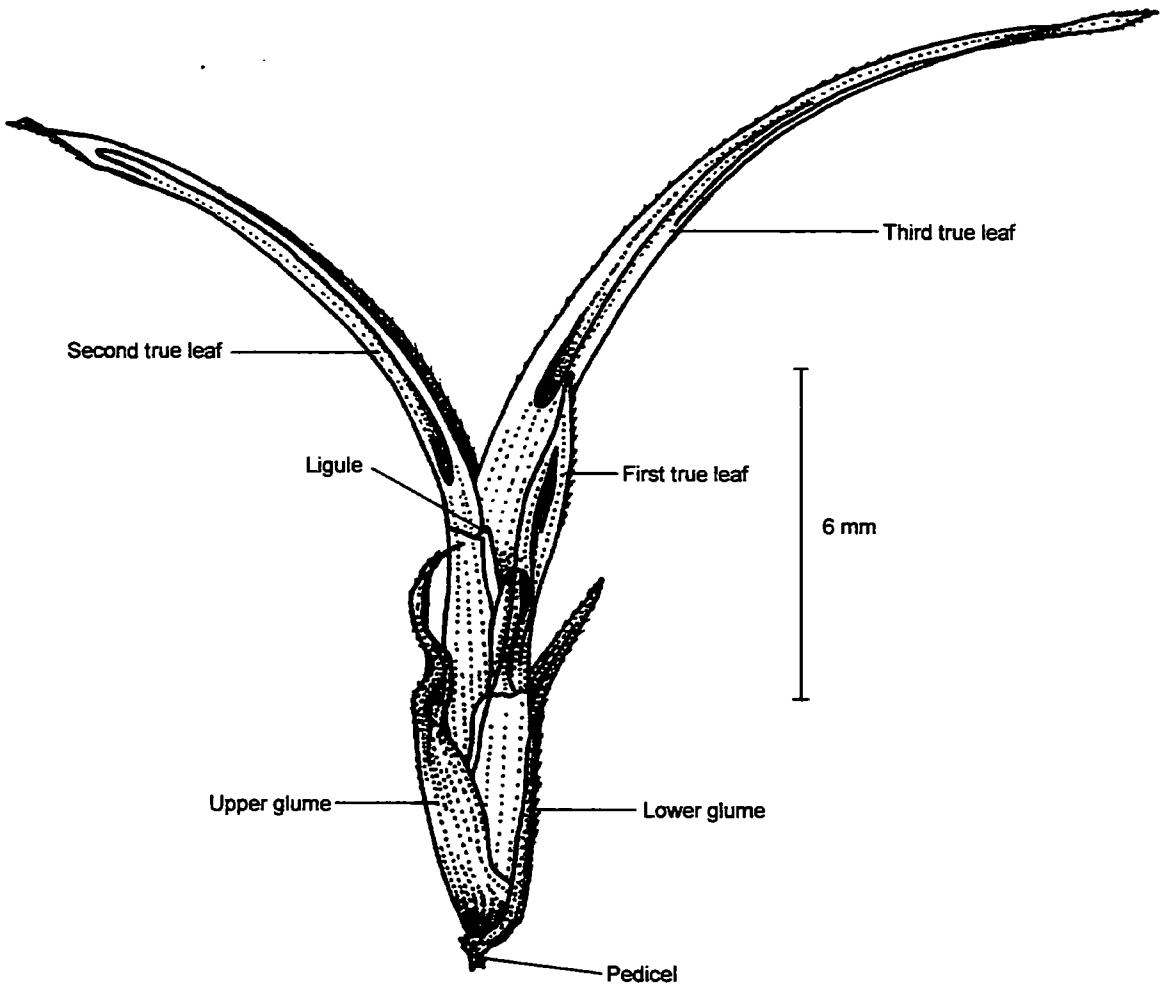


Fig. 1.2. A prolificated plantlet of *Dactylis glomerata* L., one of 26 plantlets found in an inflorescence of a *Dactylis glomerata* plant that was found growing on waste ground near the Department of Computer Science, University of Durham on 4/Sept/1996. On dissection, the upper glume surrounded both the leaves (borne on the rachilla) and aborted floral parts. The lemma and palea of the single floret were undeveloped membranes surrounding the immature anthers and carpel. Lodicules were present. Plantlets did not dehiscence, but had to be removed using a scalpel.

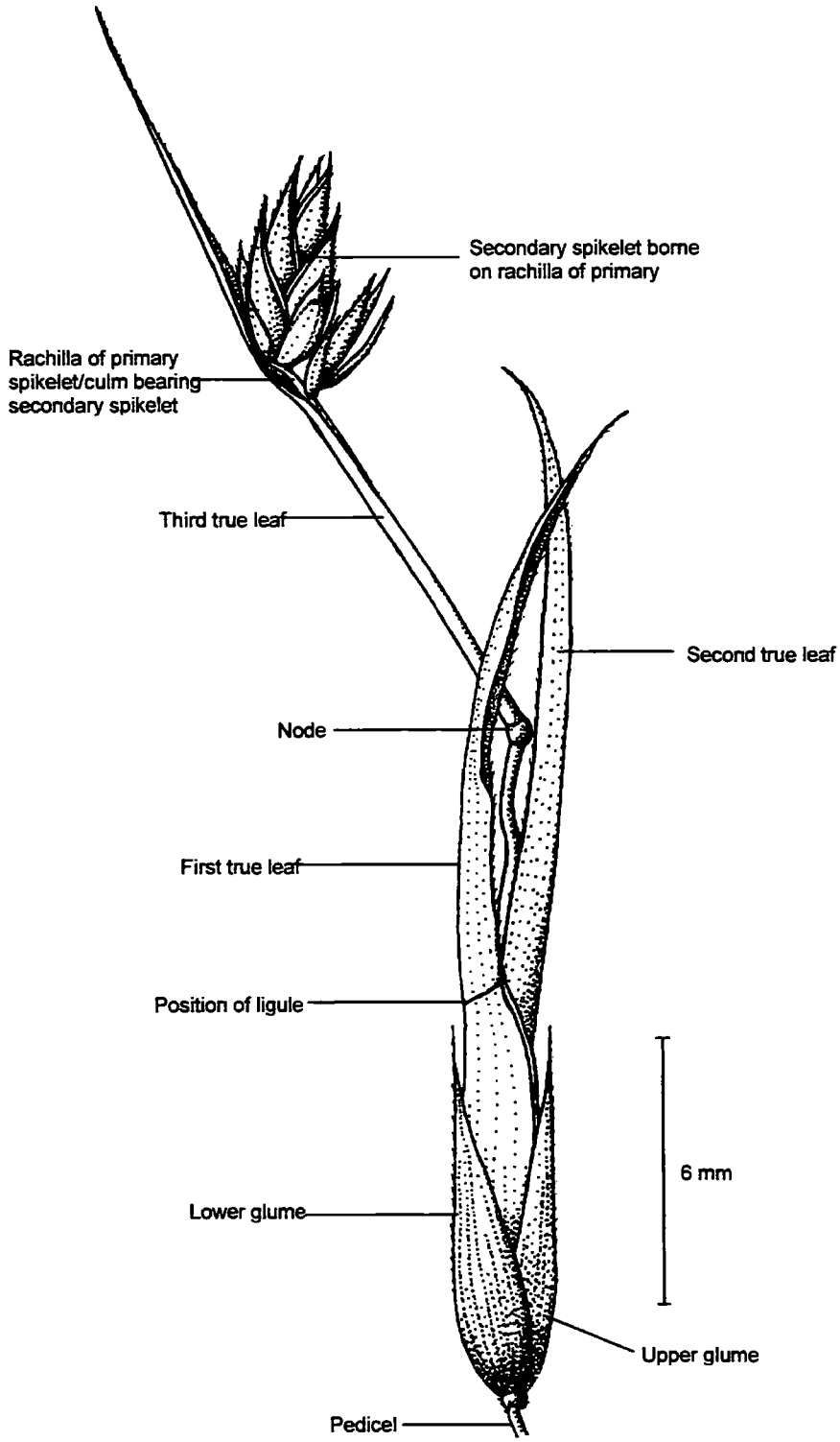


Fig. 1.3. A plantlet of *Dactylis glomerata* L. which was itself in flower while still attached to the parent plant. Thirteen of the twenty six plantlets detailed in Fig. 1.2 were flowering in this manner.

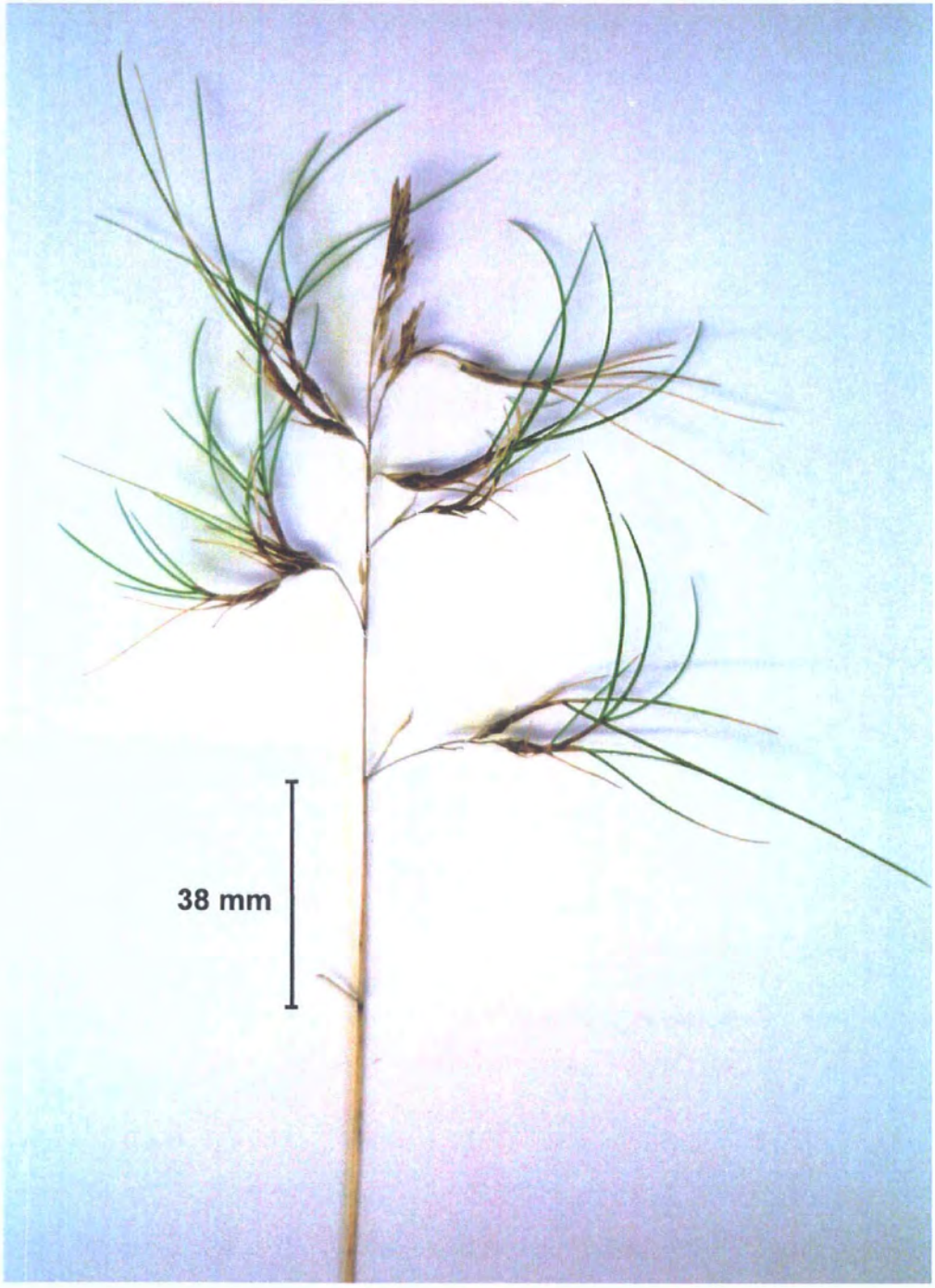


Plate 1.4. Prolification of the usually seminiferous *Festuca rubra* ssp. *commutata*. This inflorescence was found in a verge near to the entrance to the Department of Chemistry at the University of Durham on 4/Oct/1997. Plantlets did not dehisce despite necrosis of the culm and entire main axis.

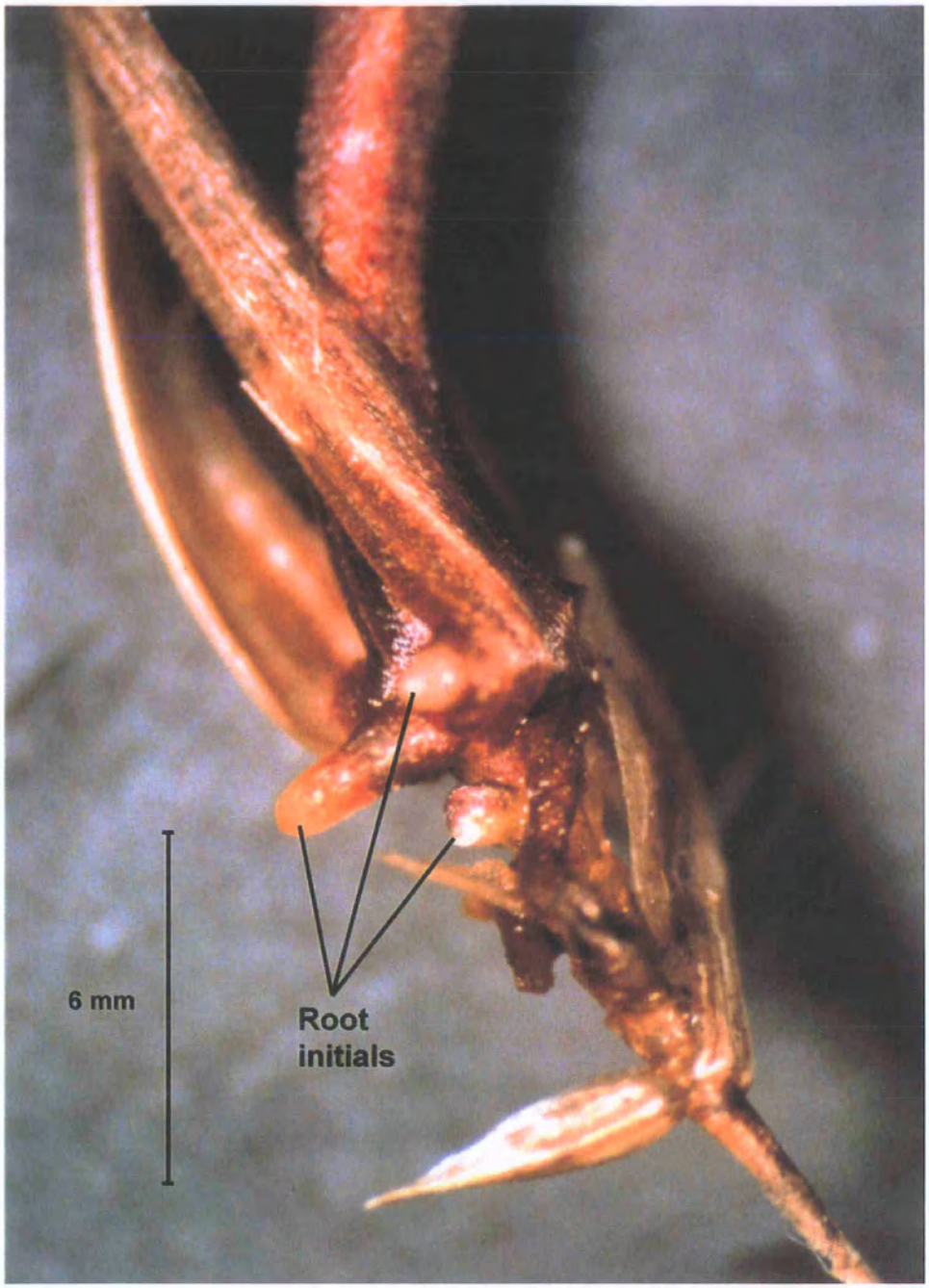


Plate 1.5. Prolification of *Festuca rubra* ssp. *commutata* - root initials present at the base of a plantlet.

Plate 1.6. Prolification of *Festuca rubra* ssp. *commutata* – root growth. The bases of plantlets were dipped into water for 5 days, resulting in the rapid growth of healthy roots. All nine plantlets established and grew when provided with a pot of compost and regular watering. This plantlet was excised proximal to the glumes using a scalpel.

1.2.2 Habitual prolification

Habitually pseudoviviparous grasses stand out both by the consistency with which they produce plantlets, and the consistent nature of the plantlets themselves. Apparently all are capable, under certain environmental conditions, of producing sexual spikelets (e.g. Youngner 1960; Heide 1988) as there is no recorded case of a habitually pseudoviviparous grass that cannot do so. This indicates that habitually pseudoviviparous grasses are more sensitive than other grasses to environmental circumstances which prompt prolification; circumstances resulting in entirely sexual reproduction rarely being met in their particular distributional range, but with the potential for sexual reproduction still inherent in their genome.

This may explain the vast number of ecotypes of *Poa alpina* from habitats all over the northern hemisphere, which possess different tendencies towards prolification (Müntzing 1933, 1936, 1940, 1946, 1954, 1980). Until Müntzing's life-long cytological comparison of these biotypes began, views of *Poa alpina* as either seminiferous or viviparous were blinkered by a comparison of a small number of biotypes in one particular locality (e.g. var. *seminifera* or var. *vivipara*; Hegi 1930, and the original classification as *Poa alpina* L. and *Poa alpina* L. var. *vivipara* L.; Linnaeus 1759). Müntzing's elucidation of *Poa alpina* as a phenotypically diverse aggregate of polyploid ecotypes has often been ignored in favour of the simple assumption that it is one species with a viviparous form. More importantly, these key studies provide evidence that ecotypes from different latitudinal ranges respond to differing degrees to the environmental cues resulting in prolification (see also Heide 1988). Grass species presently recorded as exhibiting habitual prolification are listed in Table 3 of Appendix 1.

As the plantlets of habitually prolific grasses are relatively consistent in form, their morphology can be reliably described. The main axis of the prolificated shoot is a continuation of the rachilla of the spikelet (Philipson 1934; Gustafsson 1946a; Youngner 1960). The spikelet apical meristem is determinate in seminiferous grasses, usually dying after the production of a number of florets. In grasses exhibiting prolification the spikelet apical meristem is not only indeterminate, but also reverts from floral development to a vegetative phase, producing what is essentially a tiller arising from the tip of the spikelet. Thus florets may be present at the base of the prolificated spikelet (the condition of semiviviparae; Turesson 1926, 1930, 1931), or completely absent (viviparae). When present, florets usually possess a typical palea,

but the lemma and sometimes palea may be elongated. As the abnormal lemma may partially sheathe the plantlet axis, casual observations frequently result in descriptions of plantlets as being derived from the lemma, which is not the case. The rudiments of floral structures are often present in florets; as a general rule the larger the lemma the smaller the flower in its axil. *Deschampsia* spp. and *Poa* spp., including *Poa alpina*, that possess well developed plantlets may also possess mature floral organs (Philipson 1934; Wycherley 1953a; Müntzing 1980) although anthers may be indehiscent, as recorded in *Poa* x *jemtlandica* (Wycherley 1953a). Alternatively, floral parts of florets may be completely absent. The rachilla, lemma and floral organs may develop to various extents and the spikelets of habitually pseudoviviparous grasses are morphologically plastic, often grading from a floral to a vegetative state along the axis. Thus the vegetative plantlet and sexual zone of the spikelet should be regarded as poles of a continuous axis, with a physiologically older floral end, and a younger vegetative end. For ease of use the term 'plantlet' can be defined as an indeterminate vegetative spikelet, but the morphological plasticity regarding florets should be borne in mind.

Establishment and/or dispersal of plantlets either occurs when the collapse of the parent plant's culm brings the plantlet into contact with the substrate (Harmer & Lee 1978b), or when the plantlet dehisces. Dehiscence occurs along an abscission zone situated distally to the glumes and proximal to the florets, which becomes lignified towards the end of the growing season (Exo 1916; cited by Gustafsson 1946a, who also reproduces figures). Abscission zones are common to spikelets in the majority of pooid grass species, being the mechanism of fruit shedding. The fruit of grasses is a single-seeded, indehiscent (i.e. the seed is retained in the fruit), dry caryopsis. Being indehiscent the unit of dispersal is usually comprised of the caryopsis surrounded by the lemma and palea – the rachilla of the spikelet breaking up to allow the dispersal of individual fruits, as is the case with *Poa alpina* (Exo 1916; Armstrong 1917; Hubbard 1992), although in some genera, including *Holcus*, *Alopecurus*, and *Panicum*, the unit of dispersal is the entire spikelet. At the floral end of the prolificated spikelet the dehiscence zone is retained, but with the reversion to vegetative growth the rachilla does not break up (Exo 1916; cited by Gustafsson 1946a). Thus the abscission zone is a characteristic of the sexual development of the spikelet; development often continuing as floret production, but ultimately deferring to vegetative development after this point.

The position of plantlets distal to this dehiscence zone is the main characteristic that allows habitually prolificated plantlets to act as propagules, allowing them to shed unlike aerial tillers and ephemerally produced plantlets. This is not an attribute specific to plantlets but one of spikelets in general. Plantlets do not appear to have any other specific advantages over tillers, and appear to be as robust and as likely to establish as detached tillers and ephemerally produced plantlets. To quote Harmer & Lee (1978a):

> "The structural features and the form of carbohydrate and nitrogen storage compounds indicate that plantlets [of *Festuca vivipara*] are more akin to grass stem bases than to seeds".

Even the swollen leaf-sheaths of *Poa bulbosa* var. *vivipara* plantlets mirror the swollen sheaths of the tillers (Kennedy 1929). The ability of plantlets to act as dispersive propagules is therefore due to a combination of three general characteristics of grasses:

1. the dehiscence zone of spikelets,
2. the propensity for vegetative growth,
3. the ability of vegetative organs to produce adventitious roots.

It is clear that several types of primordium are of prime importance in both flowering and prolification – the apical meristem of the main axis (from which the entire shoot and inflorescence are derived), the apical meristem of the spikelet (from which the spikelet is derived), and the apical meristem of the floret (producing individual florets). These have been identified in maize by Irish (1997) who states that the relative development of these meristems provides a framework for the development of the varied inflorescence architectures found in the Poaceae. Two criteria must be met for prolification to occur; the spikelet apical meristem must be indeterminate, and this meristem must also revert to vegetative growth. Therefore it is possible that the secondary induction event resulting in habitual prolification is specifically an effect of environmental cues on these two aspects of spikelet apical meristem development, rather than on the development of an unspecific number and type of inflorescence meristems often apparent during ephemeral prolification.

This suggests that the origin of habitual prolification may have been a deviational reversion event distal to the dehiscence zone of the spikelet. Presumably the advantageous nature of this particular deviational form (i.e. the inherent ability of a structure of this nature to act as a propagule), and the susceptibility of the genotype to environmental cues prompting reversion, resulted in it's retention as the typical mode of development in the pseudoviviparous grasses. Thus, as habitual prolification in grasses comprises the usual morphology of the plant it is not considered to represent a teratological event (Bell 1991), but the evolutionary origins of prolification may well have been teratological (Heide 1989).

1.3 Habitats of habitually pseudoviviparous Poaceae

Ephemerally pseudoviviparous species are usually temperate lowland inhabitants, and habitually pseudoviviparous grasses are native to more extreme habitats, where vegetative reproduction is thought confer advantage (Lee & Harmer 1980; see also Section 1.4). On a global scale habitually pseudoviviparous grasses are usually native to Arctic or Alpine habitats (Arctic being defined simply as regions at latitudes beyond the tree line, and Alpine regions being those at altitudes above the tree line where other plants can grow), with the exception of *Poa bulbosa* var. *vivipara* (usually a sand-dune dweller; Wycherley 1953b). *Poa alpina* has been recorded on every continent in the northern hemisphere, and in the European Alps is one of the most important fodder grasses (Hegi 1930; Steiner *et al.* 1997) consisting of 10 % protein and approximately 3 % 'crude oil' (Hegi 1935; in German, cited by Steiner *et al.* 1997). Indeed:

> "Romeye, Muttern und Adelgrass,
> Das Beste ist, was Chüeli frass."
>
> "Alpine meadow-grass, Cowslip and Plantain,
> The best is what the cow eats."
>
> - saying of Austrian herdsmen,
> from Schröter ([n.d.])

Steiner *et al.* (1997) also report the commercial use of *Poa alpina* for 'greening ski runs and damming of avalanches and torrents', and improvement of this species for these purposes by breeding.

1.3.1 Arctic habitats

In the Arctic strong winds assist dispersal but can incur much mechanical damage (especially abrasion when ice crystals are present), and mechanical damage to roots occurs due to soil movements associated with freeze-thaw processes (Russell 1940). Many environmental factors such as temperature have relatively large ranges. These relatively harsh conditions produce habitats with low density populations and select for tussock growth-forms in monocotyledonous plants (Savile 1972). Relatively small edaphic and climatic changes can have disastrous effects on plant populations, so the Arctic is regarded as a highly unstable environment (Savile 1972).

The low incidence of the sun is apparent as low light intensities (Savile 1972), leading to little re-emission of short-wave radiation from the ground and thus low local atmospheric temperatures (Emiliani 1992). This in turn favours snow formation, the high albedo of snow resulting in still lower absorption of shortwave light; a positive feedback effect exaggerating cooling. Plants in the Arctic must therefore be able to survive prolonged freezing events, and as low temperatures decrease the velocity of enzymatically catalysed photosynthetic reactions (Larcher & Bauer 1961), possess a metabolism geared towards growth in a relatively cold environment. Indeed, Chapin and Shaver (1985) state that Arctic plants may possess a temperature optimum for photosynthesis 10 - 30 °C lower than temperate plants, due to higher concentrations of Rubisco. Arctic (and Alpine) plants also possess relatively high mature tissue respiration rates to ensure adequate energy supply in metabolically-limiting conditions (Chapin & Shaver 1989; Farrar & Williams 1991). These increased respiration rates, being the result of increased concentrations of enzymatic machinery, are thus limited by nitrogen availability. Chapin & Shaver (1989) recorded an increase in shoot growth in a number of tundra species when extra nutrients were provided, concluding that nitrogen was the major limiting nutrient for the particular site that they examined. Bradshaw *et al.* (1964) state that, of a number of species tested, grasses showed the largest increase in dry weight when supplied with additional nitrogen, although some reacted more than others; different species therefore have different nitrogen requirements.

Although rain can provide a small source of nitrogen (Wager 1938), rainfall in the Arctic tundra can be as low as 50 - 250 mm per annum, less than half of which falls in the growing season (Chapin & Shaver 1985). Many plants rely on seasonal snow-

melt as a source of water. Snow melt may only occur over a 3 - 10 day period in some areas, but water is retained in the upper few centimetres of soil as permafrost below prevents drainage, and as a result 90 % of biomass occurs in the upper 5 cm of soil (Chapin & Shaver 1985). Legumes are usually absent and low temperatures lead to slow chemical weathering of soil parent material, slow rates of nitrogen fixation (e.g. nitrogen fixation by *Azotobacter* is negligible below 7 °C; Russell *et al.* 1940) and slow rates of organic matter decomposition, leading to a general scarcity of inorganic nitrogen and other minerals. Plant population densities are closely correlated with the activity of nitrifying bacteria and the availability of nitrogen. For example *Poa alpina* and *Festuca vivipara* are more common on and beneath bird cliffs where they benefit from nitrogen derived from bird excreta (Russell & Wellington 1940). Grasses are scarce in most regions, but dominate nitrophilous communities, such as those found proximal to bird cliffs or human rubbish dumps, where the vegetation in general is relatively lush (Summerhayes & Elton 1928).

Chapin & Bloom (1976) state that three species of tundra graminoids have relatively high rates of phosphate absorption and are relatively insensitive to short term temperature changes in this respect (*c.f.* temperate plants). Active phosphate absorption by tundra graminoids allows uptake from soils of only a few degrees Celsius in temperature (but not frozen soils). This ability is not shared by temperate plants. Chapin & Shaver (1985) report that low root temperature and low nutrient availability generally result in high root:shoot ratios in Arctic plants.

Availability of mineral ions, although undoubtedly of prime importance, is not the sole influence on growth and reproduction in Arctic grasses. Seasonal and diurnal climatic rhythms in the Arctic are different to those of more temperate or tropical latitudes (Barry 1981). Due to its polar situation the Arctic daylength is long in the summer, and at the highest latitudes can last 24 h, and 0 h during the winter (Chapin & Shaver 1989; Solhaug 1991), although long photoperiod is partially offset by the low light intensity. Tiller production and net assimilation rate (NAR) in certain biotypes of *Poa alpina* are dependent on photoperiod (Foreman 1971, Solhaug 1991). Foreman (1971) also states that Arctic ecotypes of *Poa alpina* require low light intensities for the initiation of flowering, whereas Alpine ecotypes require high light intensities.

Between 40 and 60 % of the dry weight of storage tissues of Arctic plants is composed of non-structural carbohydrates (Chapin & Shaver 1985). This may provide

some osmotic protection against chilling or freezing and reserves for re-growth after grazing (Chapin & Shaver 1985), but also Arctic graminoids rely heavily on these stored reserves for growth in the next growth season. Therefore the effects of limiting factors on growth may not be apparent until the following summer. Tundra graminoids remain physiologically active below ground long after shoot senescence (i.e. the shoot growing season is distinct from the root/rhizome growing season).

1.3.2 Alpine habitats

The Alpine environment resembles the Arctic in many respects. In the troposphere[2] temperature drops with distance from the Earth's surface (re-emission being the main source of the troposphere's thermal energy). Hence with increasing altitude there is a decrease in air temperature. Barry (1981) states that on Alpine peaks wind speeds may be extremely high as a result of limited friction between high altitude air currents and the ground. Alpine environments also experience relatively low partial pressures of O_2 and CO_2, and relatively high levels of radiation (Körner & Diemer 1987). In order to cope with low partial pressures of CO_2, herbaceous plants growing at high altitudes have a net rate of photosynthetic uptake approximately 50 % higher than plants growing at lower altitudes, mainly due to larger amounts of chlorenchyma, assimilating more CO_2 per unit leaf area than low altitude grown plants. Nitrogen content of leaf tissues in high altitude plants equals that of low altitude plants (Körner & Diemer 1987).

1.3.3 British upland habitats

In Britain the habitually pseudoviviparous grasses are usually restricted to upland regions, but in areas of very high rainfall their range may extend to sea-level (Wycherley 1953b). Four native species are found; *Deschampsia alpina*, *Deschampsia cespitosa* ssp. *alpina*, *Festuca vivipara* ssp. *vivipara* (Plate 1.5), and *Poa alpina*, along with the native hybrid *Poa* x *jemtlandica* (Wycherley 1953a, 1953b; Frederiksen 1981). In addition, one alien species is also present; *Poa bulbosa* var. *vivipara*, a native of the European continent. There are no endemic species as such, although specific ecotypes could perhaps be considered unique to Britain.

British upland regions, although mainly devoid of trees, may not necessarily be Alpine environments as such, as in many situations trees are excluded by grazing or burning

[2] The troposphere is the lowest thermal layer of the atmosphere, 10 km thick at the poles and 16 km thick at the equator (Emiliani 1992)

rather than climatic or edaphic stresses. Heather or grass communities are therefore not the natural climax vegetation (Batey 1982). In general British uplands possess acidic soils (due to low base status parent material and heavy leaching), forming either thin, freely draining podzols, or relatively thick (up to 2 m) poorly drained gleys on flatter land in wet areas. The average growth season is between 150 to 200 days, and the mean annual rainfall range is 1000 - 3000 mm (Batey 1982).

Dry deposition of air-borne nitrogenous compounds in British uplands has increased with increasing inputs from industry. Present inputs via dry deposition are consequently higher than at any other time during the postglacial period (Batey 1982) and the nitrogen balance is constantly changing. The annual input of total nitrogen may be as high as 14 kg ha^{-1} and total soil nitrogen may range from 6 t ha^{-1} on slopes, to 25 t ha^{-1} on flat or basin sites, but this is mainly organic in form, and low turnover or mineralization rates may lead to low nitrogen availability (Batey 1982).

Hill sheep farming also affects the nitrogen cycle of upland areas; 0.4 kg of nitrogen per hectare per year is removed from the ecosystem via the production of wool or young sheep. Each ewe can contain up to 0.8 kg of nitrogen, and although nitrogen may be returned to the ecosystem via excreta, dead ewes may be removed, taking their nitrogen with them. Relatively small amounts of nitrogen may also be removed in the form of other grazing animals (e.g. deer removed by hunters). Small, but not insignificant, amounts of inorganic nitrogen are lost from streams, and considerable but variable losses result from burning of vegetation.

Plate 1.7. *Festuca vivipara* ssp. *vivipara* growing at an altitude of 700 m.a.s.l. on Tryfan, Snowdonia National park, Gwynedd, Cymru. July 1997.

1.4 The efficiency of habitual prolification as a reproductive strategy in extreme habitats

1.4.1 Production of diaspores

High wind speeds in Arctic-Alpine environments may not permit efficient wind pollination of grasses resulting in varying amounts of seed from year to year in seminiferous species (sometimes none may be produced; Lee & Harmer 1980). Prolification is therefore a more consistent method of producing diaspores in Arctic-Alpine habitats.

1.4.2 Viability and dormancy

Grass seeds must attain a specific stable dry weight, have a low water content and contain specific storage materials in order to achieve viability. The ability of *Festuca vivipara* plantlets to 'germinate' (produce roots) is not determined by the fulfilment of specific criteria (Harmer & Lee 1978a). However, functional propagules must also dehisce, and so plantlets are probably only truly viable as dispersive agents during the later stages of the growth season (Harmer & Lee 1978a). In the case of *Festuca vivipara* the plantlets produced commence establishment immediately prior to leaving the parent plant (Lee & Harmer 1980) without the dormancy period of seeds exhibited by *Festuca ovina* and also by plantlets of *Poa bulbosa* var. *vivipara*, which may undergo a 3 - 6 month period of dormancy (Youngner 1960). *Festuca vivipara* plantlets can survive the winter without establishment (Harmer 1978; Lee & Harmer 1980), but a general lack of dormancy and the vegetative nature of the plantlets presumably results in a relatively transient germ bank compared to the soil seed bank of grasses.

1.4.3 Dispersal

The caryopses of grasses are usually dispersed by anemochory (travelling via the atmosphere; van der Pijl 1972), endozoochory (travelling inside the gut of an animal), epizoochory (travelling attached to an animal; see Arber 1934), hydrochory (travelling via water), and also via synzoochory (actively carried by an animal, i.e. myrmecochory by ants). Many of these dispersal mechanisms are not consistently available in extreme habitats due to the relative lack of animals as vectors (Savile 1972). Both Savile (1972) and van der Pijl (1972) state that plantlets may be scattered like seed, a process aided by strong winds. Plantlets have a relatively large mass and anemochory is not thought to be as efficient as seed anemochory

(Frederiksen 1981). Plantlets may also be dispersed via hydrochory (Wycherley 1953b). Therefore the dispersal of caryopses and plantlets in Arctic-Alpine habitats is similar, both relying on wind and water dispersal, and each dispersal method (i.e. caryopsis or plantlet) may be better suited to a particular local situation.

1.4.4 Nutrient and carbohydrate storage and use

Typical grass seeds show a decline in the proportion of inorganic nutrients over time whereas in plantlets of *Festuca vivipara* most inorganic nutrients are present in the same proportions over time (Harmer & Lee 1978a). Also the proportion of soluble sugars in seeds decreases with a concurrent increase in starch (Stoddart 1964a, 1964b; Escalada & Moss 1976), with a decline in amino acids coupled with a concurrent increase in proportion of protein. Seed production clearly has a disadvantage in extreme habitats, with storage molecules being converted into transportable and usable forms; processes involving metabolic energy. Mobilisation of this kind is not necessary and Harmer & Lee (1978a) reported that it does not occur in plantlets of *Festuca vivipara* (there is little if no starch accumulation, and little change in the proportion of protein over time in *Festuca vivipara* plantlets).

In artificial competitive situations the growth rate, total dry weight and tiller number of *Festuca vivipara* plants derived from plantlets are higher than in plants derived from seed (Lee & Harmer 1980). *Festuca vivipara* plantlets have nutrient reserves 'three or four times' that of seeds (Harmer & Lee 1978a), due to higher total dry weights of plantlets. Also, in low temperatures plantlets more readily root and establish than do seeds (Harmer 1978).

1.4.5 Variation and sex

Too much variation in an extreme environment could easily lead to the expression of characteristics that prove to be a selective disadvantage. The repression of variation via asexual modes of reproduction therefore retains the expression of advantageous genes in a population and prevents the expression of disadvantageous genes, while retaining the potential variability inherent in the total genome. Thus, asexual reproduction may be a disadvantage during episodes of rapid environmental change, during which plants may be reliant on an ability to reproduce sexually in unusual environmental conditions (e.g. in the case of *Festuca vivipara*, when day-length is long and temperatures relatively high; Heide 1988), or via semiviviparae. Thus the

susceptibility of a population to climate change may well be dependent on its tendency to prolificate.

1.4.6 Summary

Plantlet production is potentially more consistent than seed production, with dispersal then usually effected in a similar manner. Vegetative reproduction favours quick establishment in colder habitats with a short growing season; plantlets not having a period of dormancy, not being reliant on mobilisation of storage compounds, and possessing a metabolism geared for vegetative growth in place even before dispersal. Asexual reproduction also conserves advantageous characteristics, but plantlets may be less able to cope with rapid environmental change. As stated by Lee & Harmer (1980), the advantages of pseudovivipary in harsh environments are many, but whether prolification represents an adaptive response to harsh environmental conditions is not entirely clear.

1.5 The cyto-genetics of habitually pseudoviviparous Poaceae in relation to distribution, ecological range and prolification

1.5.1 Polyploidy in habitually pseudoviviparous grasses

The habitually pseudoviviparous grasses are mainly polyploids and are considered to be allopolyploid in origin - that is they contain chromosome sets from two or more different species. Normal diploid hybrids are sterile as respective chromosomes are not homologous and cannot pair, inhibiting meiosis. If hybrids undergo autopolyploidy (i.e. multiplication of their chromosome set) the result is an allopolyploid in which every chromosome has a homologue, permitting meiosis and producing a fertile daughter plant (Lindsey & Jones 1992). This is in effect a new species. Flovik (1938) proposes *Festuca ovina* var. *brevifolia* (R.Br.) Hart. and *Festuca rubra* var. *arenaria* (R.Br.) E.Fries as parent species of *Festuca vivipara*. The parents of *Poa* x *jemtlandica* are considered to be *Poa alpina* and *Poa flexuosa* Sm. (Wycherley 1953a), and *Poa herjedalica* is probably a daughter species of *Poa alpina* and *Poa pratensis*.

1.5.2 General effects of polyploidy

General effects of chromosome multiplication include a slower rate of cell division and an increase in cell size. The subsequent increased ratio of cell volume to surface area results in slower inter- and intra-cellular transport leading to a slower growth rate

(Müntzing 1936). Such species with low growth rates and low yields possess a selective advantage in extreme habitats as they are less likely to exhaust the available nutrient supply and will show sufficient metabolism under low nutrient conditions (Bradshaw *et al.* 1964). Thus, low growth rates extend the time over which existing nutrient reserves are used, increasing the likelihood of survival until the next nutrient flush. Despite low growth rates, polyploid plants are relatively vigorous (i.e. physiologically fit), and are thus ideally suited to stress-tolerant lifestyles (see Grime, 1974). Warner *et al.* (1987) attribute the vigour of polyploids to increased concentrations of enzymes and molecular diversity resulting from increased genetic diversity. Joseph *et al.* (1981) also report increased photosynthetic rates with increasing levels of polyploidy in seminiferous *Festuca arundinacea* Schreb., due to increased concentrations of chlorophyll and Rubisco. There is a definite correlation between the level of polyploidy and morphology, Müntzing (1936) providing the following general list of differences of polyploids from diploids; thicker and stouter stems, taller habit, thicker leaves, darker green leaves, larger leaves, proportionally shorter and broader leaves, larger flowers and floral parts, and larger seeds.

1.5.3 Polyploidy and variation

Allopolyploids, containing the genomes of two or more species, produce a larger proportion of heterozygotes than homozygotes in a population (more allelic combinations are possible) and their capacity for variation is therefore far greater than a diploid counterpart (Lindsey & Jones 1992). Also the expression of specific genes is occasionally altered. This greater variation results in greater potential ecological adaptability. Polyploids in general are more often found in habitats in which climatic fluctuations are frequent (Johnson & Packer 1965). Müntzing (1933) states that chromosome races are ecologically and geographically distinct and Skalinska (1950 & 1951; cited by Müntzing 1980) states that in the Polish mountains the biotypes of *Poa alpina* with the highest chromosome numbers reach the highest altitudes.

1.5.4 Polyploidy and chill resistance

Chill resistance permits quicker establishment in colder regions, such as Arctic-Alpine habitats (Johnson & Packer 1965). In general polyploids are more chill resistant than diploids, for example Nishiyama (1934; cited by Müntzing 1936) noted an increase in the chill resistance of *Avena* species with increasing chromosome number. MacGillivray & Grime (1995) also determined a correlation between higher

chromosome number and increased chill resistance in 51 temperate herbaceous species, but also noted a small number of exceptions. Gustafsson (1946b) asserts that there are a large number of Alpine plants with a low chromosome number, and a large number of lowland plants with a high chromosome number. Thus it is perhaps the case that certain species possess unrelated characteristics which give them an advantage in a particular habitat and lend them a range inconsistent with their level of ploidy.

1.5.5 Polyploidy and water economy

The proportion of polyploids to diploids in a population is higher in wet habitats, and this is particularly true of monocots (Haskel 1951, 1952). Haskel (1952) therefore suggests that polyploidy is inherent in many monocots, being a relict trait from an evolutionary phase in aquatic habitats. Cain (1944) reports a decreased frequency of stomata per unit leaf area in polyploids in general compared to diploids, and that stomatal pores and guard cells are larger in polyploids. However, Byrne *et al.* (1981) state that, in *Festuca arundinacea*, the level of polyploidy merely denotes a different stomatal density, not necessarily a lower density. Byrne *et al.* (1981) also report that with increasing levels of polyploidy in *Festuca arundinacea* there is an increase in the carbon dioxide exchange rate and a decrease in diffusive resistance, and also an increase in the estimated volume of leaf mesophyll cells. Due to the decreased diffusive resistance, the water economy of polyploid forms of *Festuca arundinacea* differs from that of the diploid form. This may possibly be true of other polyploid species, and may restrict their range to habitats in which water is more readily available. Thus a higher frequency of habitually pseudoviviparous grasses in damp habitats could possibly be a consequence of polyploidy; restriction to these habitats resulting from higher water requirements, and restriction of dispersal by water stressed plantlets (Lee & Harmer 1980).

1.5.6 Polyploidy and prolification

Biotypes of *Poa alpina* with a higher chromosome number tend towards prolification (Müntzing 1980). Flovik (1938) determined three genotypes for *Deschampsia alpina*; 2n = 49 (highly pseudoviviparous, robust and with well developed plantlets), 2n = 41 (smaller and less pseudoviviparous than 2n = 49) and 2n = 39 (dwarf with narrow leaves and slight prolification). These biotypes, along with a seminiferous 2n = 26 form were also observed by Nygren (1949). Hence these workers inferred a correlation between chromosome number and prolification. However,

pseudoviviparous diploid biotypes of *Deschampsia setacea* (Nygren 1949), *Deschampsia cespitosa* ssp. *alpina* (Rothera & Davy 1986), and *Poa alpina* (Skalinska 1951; cited by Müntzing 1980) have been recorded. Also, Flovik (1938) states that pseudoviviparous biotypes of *Poa alpigena* exhibit less prolification with increasing levels of polyploidy. Thus, Lee & Harmer (1980) point out that the relationship between polyploidy and pseudovivipary is far from clear.

What is clear is that chromosome number *per se* does not denote the tendency to prolificate. This appears to be a characteristic of each particular ecotype; each ecotype having evolved with different daylength and temperature requirements for the reversion event and concomitant prolification (see Section 1.2.1) depending on the habitat of origin. However, there is a trend for polyploids to be chill resistant, and also prolification may confer reproductive advantage in harsh environments. The relationship between prolification and high ploidy level is therefore clouded by the fact that both characteristics are independently advantageous in colder habitats, and any association between the two could be coincidental.

1.6 Notes on the synflorescence concept

The traditional view of the grass 'inflorescence' is elaborate and was not followed in this study. In this view, flowers (grossly 'florets') are grouped together within spikelets, these spikelets are grouped together on branches, and branches are further grouped together to form the panicle. There are clearly different levels of organisation within the reproductive zone, and the spikelet, branch and the entire panicle can all reasonably be regarded as types of inflorescence.

Despite not having the synflorescence concept in mind, Gram's (1961) description of the grass inflorescence system recognised that the reproductive architecture was organised from repeated units. That the 'inflorescence' of grasses possesses a paracladial structure (i.e. composed of branches of similar or identical construction that are repeated; Bell 1991) has subsequently been asserted by Cámara-Hernández & Rua (1991) and Vegetti & Anton (1996). Paracladial branches may themselves be composed of lower order paracladia, and in the case of grasses these are ultimately composed of the co-florescences which form the spikelets. In grasses, the entire supporting axis - including vegetative organs - is viewed as part of this synflorescence. These concepts (summarised in Fig. 1.5) are useful for the physiologist; rather than focusing on individual organs, they acknowledge the fact that all organs are integral parts of a whole individual. As this study is concerned with

physiology at the whole-plant scale the synflorescence concept is adopted. However, in this study the main florescence was included as a functional part of the paracladial zone, although its different developmental origins should be borne in mind.

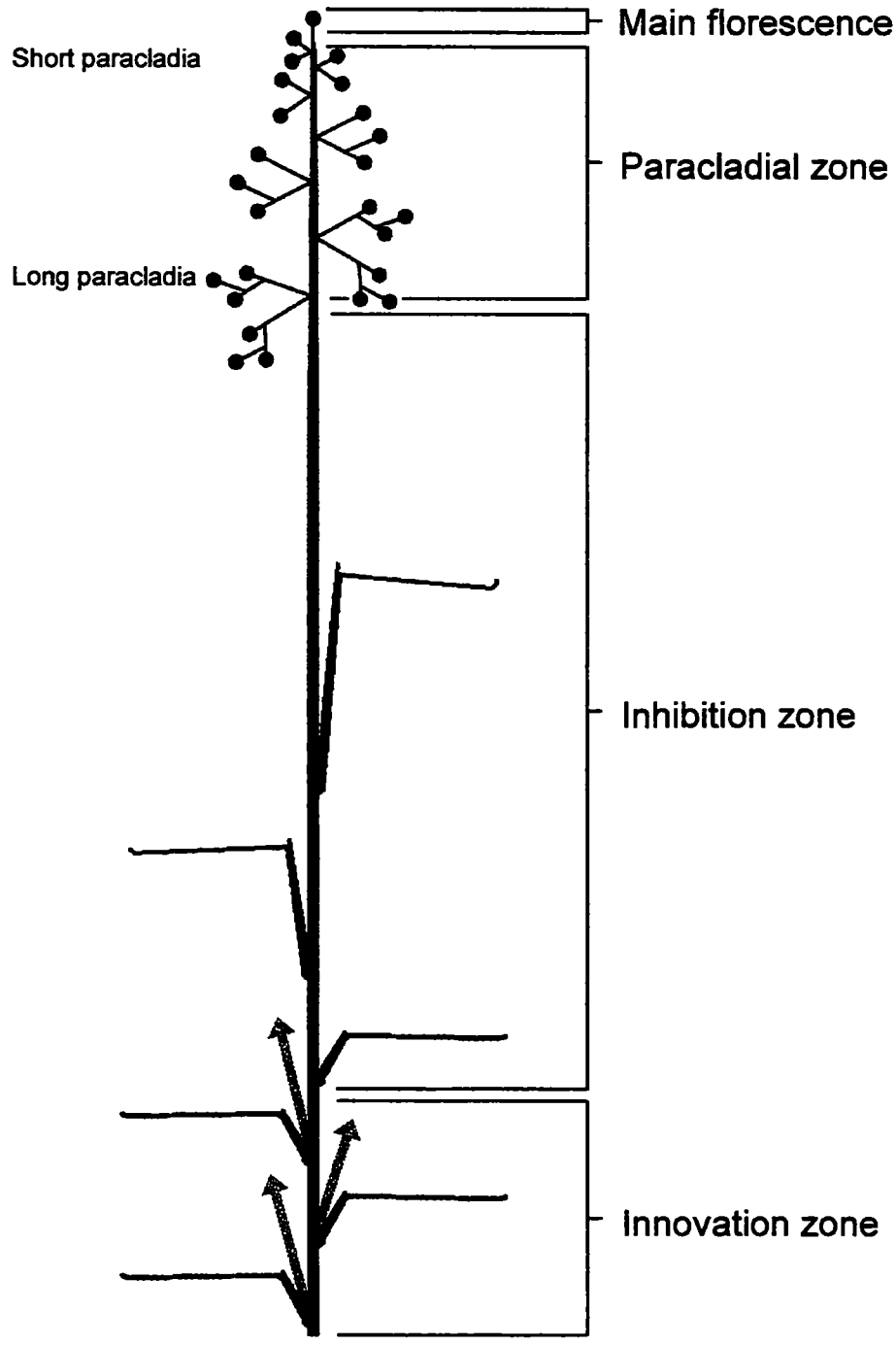


Fig. 1.4. The synflorescence concept as applied to the Poaceae (modified after Vegetti & Anton 1996).

1.7 Aims of the present study

The habitually pseudoviviparous grasses possess characteristics, such as polyploidy and asexual reproduction, which give them an advantage in the habitats in which they live. The teratological origin of plantlets suggests that plantlet leaves are developmentally identical to the leaves of tillers, and are likely to have an identical physiological role. Thus, plantlet leaves not only form the body of an asexual propagule, but are also likely to provide photoassimilate.

Although the green and leafy plantlets of *Festuca vivipara* have been shown to photosynthesise and fix carbon (Lee & Harmer 1980), the worth of this occurrence in pseudoviviparous grasses has not been determined with respect to the carbon requirements of the developing plantlets. Also the occurrence of an excess in carbon fixation by these propagules would define their role as that of carbon sources rather than seed-like sinks, and so an aim of this investigation was to investigate the carbon economy of plantlets and of the inflorescence/parent system, and also to determine the extent of inter-plantlet carbon relations and heterogeneity within the inflorescence. As plantlets are supported both physically and physiologically by the culm of the plant, including a connection to the remaining sources and sinks via this transport system, the functional development of this structure was investigated in terms of both xylem and phloem transport.

Photosynthetic capacity of organs in this reproductive system may be modified by growth in future climatic conditions. Due to the burning of fossil fuels, the concentration of carbon dioxide (CO_2) in the atmosphere has risen by approximately 80 μmol CO_2 mol^{-1} since industrialisation (Gammon *et al.* 1985; DOE 1988), and is predicted to rise by a further 180 μmol CO_2 mol^{-1} by the year 2050 (Watson *et al.* 1990). Increases in this substrate for carboxylation may actually result in decreased photosynthetic rates and dry matter increment in the long term (relative to control plants grown in present CO_2 conditions), in situations in which nutrient availability is low. Baxter *et al.* (1997) concluded that a low supply of nitrogen and phosphorus in conjunction with an elevated atmospheric CO_2 concentration resulted in induced nutrient deficiency within leaf tissues of *Poa alpina*, mainly caused by down-regulation of photosynthesis and remobilization of nitrogen from photosynthetic organs. As plantlets are composed of leaf material the hypothesis tested is that plantlets possess an identical response to nutrient and carbon availability as that of parent vegetative tissues, and that the reproductive phase of the life cycle is limited in an identical manner to the vegetative phase. The specific hypotheses tested are stated in the introductions to the relevant chapters.

2 The culm and long-distance water transport

2.1 Introduction

The organs of the synflorescence are connected both physically and physiologically via the true stem or culm (formerly termed 'haulm'; Arber 1934), which also provides mechanical support. Initial development of the culm and synflorescence has been described in the literature in some detail, starting with a switch from vegetative to reproductive growth by the shoot apical meristem (the environmental cues prompting this change are discussed in Section 1.2). This results in gross elongation of the stem internodes to form the culm, and the elongation of leaf sheaths to form the surrounding pseudostem (Bell 1991). With the elongation of the culm the paracladial zone eventually emerges from the pseudostem, with anthesis usually taking place within each spikelet as it emerges (Arber 1934). Paracladia are initially borne in an upright attitude, but at anthesis pulvini (operating via growth rather than changes in turgor *per se*) reorient each paracladium and also individual spikelets (Arber 1934; Gram 1961), paracladia subsequently being borne more or less horizontally. This spreads the paracladia and spikelets apart, presumably facilitating air movement and therefore wind pollination (Hawley 1989). In many species paracladia revert to their original orientation after anthesis, but in species such as *Agrostis tenuis* and *Festuca gigantea* (L.) Vill. the paracladia remain spread and may even become permanently reflexed as pulvini become lignified with age (Gram 1961).

Although these events are well known, the events within the culm during these later stages of synflorescence development have received little attention, as interest then becomes focussed on the propagules. Harmer and Lee (1978a) suggest that the duration over which the plantlets of pseudoviviparous grasses remain physiologically attached to the parent plant may determine the extent of resource partitioning to them. Indeed, the extent of monocarpic senescence[1] of rice (*Oryza sativa* L.), was found to be inversely proportional to partitioning of dry matter to paracladia (Debata & Murty 1981), with delayed senescence facilitating greater mobilisation of metabolites. Studies of culm development and the cessation of physiological links between the parent and propagules during monocarpic senescence are therefore of great importance to the understanding of assimilate partitioning to propagules.

---

[1] The shoot axes of grass plants - being monopodial and terminating in reproductive growth - senesce in a monocarpic fashion (Wareing & Phillips 1990). In grasses this is preceded by the sequential senescence of individual leaves along the axis. For a general review of senescence see Noodén (1988), and for molecular aspects of senescence see Smart (1994).

Aside from commonly observed senescence processes, Chaffey & Pearson (1985) noted the presence of tyloses[2] in the xylem vessels at the ligule of senescent leaves of *Lolium temulentum*. Tyloses appeared to hinder, but not entirely block, the movement of radiolabelled amino acids in the xylem sap. At first reading, tylosis in functional xylem vessels may seem to be an unlikely occurrence, as the vast majority of investigations into tylosis either state or assume that tylosis is the result of cavitation[3] of the vessel; creating an embolism or air space into which tyloses can grow and seal off vessels (Chattaway 1949, Hutson & Smith 1980, Elmahjoub *et al.* 1984, Pearce & Holloway 1984, Obst *et al.* 1988, Cochard & Tyree 1990, Sano & Fukazawa 1991, Saitoh *et al.* 1992, 1993, Babos 1993, Schmitt & Liese 1994, Rioux *et al.* 1995, Rioux *et al.* 1998). However, Canny (1997) states that in petioles of Sunflower (*Helianthus annuus* L.) tylosis may result from low pressure in the lumen and not specifically to embolism, and that as tyloses block or effectively reduce the diameter of vessels this may act to maintain pressure within the remaining vessels and ensure continuation of transpiration flow. Whatever it's proposed function, it is clear that tylosis is a response to a net loss of water from xylem tissues, and that as living cells may protect against embolism by excluding air (Kramer & Boyer 1995) cavitation events may occur with age, and these events may also effectively block xylem vessels.

Senescence of the grass culm and inflorescence has long been assumed to be associated with water loss and drying - the culm becoming a straw (Hawley 1989), and pseudoviviparous plantlets (including those of *Poa alpina*) becoming water-stressed (Lee & Harmer 1980). The extent of drying, embolism and tylosis during the senescence of the grass culm have not previously been determined, and aside from providing an initial descriptive account of culm phenology this chapter will aim to resolve the hypotheses detailed below.

---

[2] Xylem vessel blockages may take two forms: true tylosis, in which parenchymatous companion cells grow into the vessel via the pit membranes (Chattaway 1949), and also the secretion of pectic polysaccharide gels (formerly known as 'gums') by companion cells also via the pit membranes (Chattaway 1949; Rioux *et al.* 1998). The two forms of blockage may both be present and associated.

[3] See Kramer & Boyer (1995) for general information concerning cavitation.

That:

1. the water content of synflorescence components is dependent on the extent of monocarpic senescence,
2. cavitation events in the xylem of the culm increase in frequency during senescence and ultimately result in extensive dysfunction of xylem tissue,
3. tylosis occurs as a function of embolism in culm xylem vessels, and can be used as an anatomical marker of decreased xylem sap transport.

2.2 Methods

2.2.1 Plant material

Plantlets of a biotype of *Poa alpina* were obtained from an altitude of 2641 m.a.s.l., originating from the Hohe Mut ridge, Oetztal, Austria (46° 50' 12" N, 11° 2' 50" E). A more specific identification of biotype was not possible. Root-tip squash techniques are notoriously difficult to apply to grasses, and to *Poa alpina* in particular (Müntzing 1980, failing on occasion). Müntzing (1980) determined, from a single root tip of *Poa alpina*, cells with chromosome numbers ranging from 37 to 52 (and similar results for other individuals). This was due to the presence of 'minute chromosomes' which were indistinguishable from true chromosomes at metaphase. Thus individual plants should be regarded as having a mean chromosome number, rather than a distinct and specific chromosome number. Also, Müntzing determined that this mean chromosome number was inconsistent; sibling progeny from seminiferous plants all possessing different mean chromosome numbers. Thus it was deemed futile to attempt a definition of the biotype used in this study using this criterion. Steiner *et al.* (1997) also reached this conclusion:

> "for verification of ecotype or variety [of *Poa alpina*]...chromosome counting and nuclear DNA content determination are not useful."

Steiner *et al.* (1997) also showed that storage protein electrophoresis can be used to distinguish between biotypes, but biotypes still had to be referred to using an arbitrary number or label. As the phenotype of the biotype used in this study was constant between asexually produced generations it is assumed that the genetic complement of each generation is also consistent, offspring having the same mean chromosome number as the parent. The biotype used will therefore be distinguished by phenotypic characteristics and location of origin.

2.2.2 Growth medium and nutrition

Plants were grown in 1 litre capacity (11x11x12 cm) injection-moulded square black plastic pots. In each pot a square of net curtain was used to cover drainage holes and retain the growth medium, while still allowing free drainage of liquid. Washed silver sand (William Sinclair Horticulture Ltd., Firth Road, Lincoln, UK) was used as an inert growth medium to which nutrient solution was added. The nutrient solution used was the Long Ashton (LA) nutrient solution described by Hewitt (1966), modified with a 0.2 mM solution of *di*sodium hydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) and 1 mM ammonium nitrate (NH_4NO_3) to provide both nitrate and ammonium at 1 milligram equivalent per litre (obtained when 1 ml stock is diluted to 1 l), and phosphate at 0.6 milligram equivalent per litre. Four other macronutrients and six micronutrients were also provided by this nutrient solution (see Appendix 2 for details). This nutrient solution was then diluted to one-fifth strength before application. Nutrients were given every second day and were applied until the nutrient solution fully saturated the pot. Pots were washed through with tap water on alternate days in order to avoid the concentration of available nutrients by evaporation.

2.2.3 Pest and disease control

Metaldehyde slug pellets, pesticide (permethrin and bioallethrin) and fungicide (copper sulphate and ammonium hydroxide) were applied every two weeks. Earwigs were removed using the plant-pot method of Culpan (1995).

2.2.4 Synflorescence induction

As discussed in Section 1.2.1, a period of vernalisation was required for initiation of synflorescence production. An alpine greenhouse was used to house young plants over winter months in chilling temperatures (e.g. 0.9 ± 0.6 °C minimum and 15.0 ± 0.9 °C maximum averaged over January – April 1998; $n = 20$) and natural daylight (short daylength). When required, batches of plants were moved to a heated greenhouse (11.7 ± 0.7 °C minimum and 28.5 ± 1.5 °C maximum averaged over January – April 1998; $n = 20$), with natural daylight supplemented with 400 W high pressure sodium lighting (Thermoforce Ltd., Heybridge, Maldon, Essex, UK) to provide 16 h daylength. This prompted exertion of the paracladial zone in the majority of plants, with this event occurring simultaneously within the population - although the longer-term timing of this event was unpredictable.

Exertion of the paracladial zone was defined as the date at which the distal spikelet (main florescence) became visible at the ligule of the youngest leaf (i.e. when the

spikelet was no longer surrounded by the leaf sheath). On exertion the pseudostem was tagged with the date.

2.2.5 Non-destructive growth analysis

Using a group of six replicate plants, the distance between the ligules (interligular distance) of the five youngest leaves on the main axis were measured with a plastic rule twice a week (measurements taken three days apart followed by four days apart). At exertion of the main florescence the distance between the base of the main florescence and the ligule of the youngest leaf was measured until the entire paracladial zone had emerged. From this point onwards the length of the rachis, and also the distance from the base of the paracladial zone to the ligule of the youngest leaf (i.e. the length of the visible distal internode or peduncle) were measured. Culms were supported during later stages of growth by a cane in order to prevent folding and collapse.

2.2.6 Gas exchange

During the same period net photosynthetic and respiratory rates of the five youngest leaves on the main axis were determined by infrared gas analysis (IRGA) using an LCA4 (ADC Bioscientific, Hoddesdon, Hertfordshire, UK) with a broad leaf cuvette (PLC 4). Measurements were taken at saturating photosynthetic photon flux density (PPFD) of 1700 µmol m^{-2} s^{-1} PAR, a leaf surface temperature of 20 °C, at an air flow-rate of 300 ml min^{-1}. Dark respiratory rates were made by occluding the cuvette window with a purpose built screen (an aluminium foil and paper sandwich cut to shape and applied using masking tape), and temperature maintained using a peltier device built into the leaf cuvette.

2.2.7 Water content

Throughout reproductive development, plants were harvested one hour after watering (i.e. at approximately 11 am) and the paracladial zone, the distal internode of the culm, and also the youngest fully expanded leaves of two non-flowering tillers were removed. The paracladial zone was divided into distal and proximal halves by spikelet number, and all green material separated from yellowing or brown material, with only green material being used. All excised plant material was placed inside a labelled sealed envelope and placed on ice immediately after removal. The distal internode of the culm was divided longitudinally (one half being used immediately in the photosynthetic pigment analysis detailed in Section 2.2.8). The fresh weight of all excised plant material was taken. Plant material was then oven dried at 70 °C for 48 h

and re-weighed. Absolute water content as a percentage of fresh weight was calculated using equation [1].

$$\text{Absolute water content} = \frac{(FW - DW)}{FW} \times 100\ \% \qquad [1].$$

A cubic polynomial curve ($y = y_0 + ax + bx^2 + cx^3$) was fitted to the data using SigmaPlot graphing software (SPSS Inc., Chicago, Illinois).

2.2.8 Photosynthetic pigment content

The following protocol was carried out in a darkened room, with all plant material and extracts kept on ice and, where possible, covered with aluminium foil to exclude light. Material of the distal internode of the culm reciprocal to that used in Section 2.2.6 was ground in 3 ml of ice-cold 95 % ethanol using a pestle and mortar, aided by a spatula-tip of acid-washed sand. The extract and ground material were then decanted into a centrifuge tube and the pestle washed with two successive 3 ml aliquots of 95 % ethanol. Washings were pooled with the extract and ground material, and then centrifuged at 500 r.p.m. for 10 minutes. Supernatant was then decanted into a 10 ml volumetric flask and made up to volume using ice-cold 95 % ethanol.

A spectrophotometer (Unicam Ltd., Cambridge, UK) was then used to measure the absorbance by the supernatant of light at wavelengths of 470 nm, 649 nm and 665 nm using 95 % ethanol as a blank. The following calculations of Lichtenthaler & Wellburn (1983) were then used to determine the chlorophyll *a* & *b* and carotenoid content, and from this the total photosynthetic pigment content of the distal internode of the culm:

$$\text{Chlorophyll } a \text{ concentration} = 13.95 \times (Abs_{665}) - 6.88 \times (Abs_{649}) \qquad [2].$$

$$\text{Chlorophyll } b \text{ concentration} = 24.96 \times (Abs_{649}) - 7.32 \times (Abs_{665}) \qquad [3].$$

$$\text{Carotenoid concentration} = \frac{1000 \times (Abs_{470}) - 2.05\ ([Chl_a]) - 114.8\ ([Chl_b])}{245} \qquad [4].$$

Where units of concentration are $\mu g\ ml^{-1}$. The pigment content of plant material was then calculated on a fresh weight basis. Using the absolute water content of the reciprocal half of the internode (determined in Section 2.2.7) the dry weight of the material used in pigment analysis was calculated, and the total photosynthetic

pigment content presented on a calculated dry weight basis in order to avoid the complications of possible changing water content of tissues. A single, 2 parameter exponential decay curve ($y = ac^{-bx}$) was fitted to the data using SigmaPlot graphing software (SPSS Inc., Chicago, Illinois).

2.2.9 Cavitation

A modified version of the anatomical technique described by Lo Gullo & Salleo (1991) was used to quantify the degree of cavitation in the xylem of the culm. Reproductive shoots of two ages were used, these ages being 20 d from exertion of the paracladial zone and 40 d from exertion. Shoots were excised from the roots at the root/shoot interface; this being carried out under water to avoid breakage of the transpiration stream. Tillers were excised, leaving the entire main synflorescence. The synflorescence was then 'fed' with fluorescent stain (fluorochrome) by placing the cut end into 2 ml of 1 % (w/v) lucifer yellow dithionite (LYCH) (Oparka & Read 1994) with controls in 2 ml of distilled water. Plants were left for one hour in a controlled environment room, with an air-conditioning unit stirring the air, at 15 °C and PPFD of 250 µmol m^{-2} s^{-1} (PAR), after which the synflorescence was rapidly dissected into component parts, these being:

1. the basal node of the culm,
2. a 1 cm length of the basal internode,
3. the distal-most node of the culm,
4. a 1 cm length of the distal-most internode taken from immediately proximal to the paracladial zone,
5. plantlets and their supporting pedicels.

Throughout the remainder of the procedure light was excluded using aluminium foil to prevent photo-oxidation of the fluorochrome. Material was immediately fixed in immunofix (3 % (w/v) paraformaldehyde, 1.25 % (v/v) gluteraldehyde and 0.05 M phosphate buffer) overnight in a rotator at room temperature. Material was then dehydrated through a series of buffered alcohol solutions (12 %, 25 %, 50 %, 75 %, 95 %, 100% ethanol – one hour in each), and was then washed in histoclear (two washes, each of four hours duration). Following this, material was gradually infiltrated with paraplast embedding wax (25 % paraplast : 75 % histoclear, 50 % paraplast : 50 % histoclear, 75 % paraplast : 25 % histoclear, 100 % paraplast – two hours minimum in each) at 65 °C. Material was then placed in embedding tubes (TAAB, Aldermaston, Berks., UK) in 100 % paraplast and positioned before being left to cool.

Embedding tubes were then cut away from specimens using a hacksaw, and specimens mounted onto wooden blocks using melted wax, prior to sectioning.

Transverse sections of 10 µm thickness were cut on a microtome (Ernst Leitz GmbH, Wetzlar, Germany), dried onto glass slides, and then mounted using Canada balsam and coverslipped. Slides were viewed with a Diaphot TMD-EF inverted microscope (Nikon UK Ltd., Telford, Shropshire, UK) using an HBO 100 W/2 mercury short arc lamp (Osram Ltd., Wembley, Middlesex, UK) as a near ultra-violet light source with which to excite the fluorochrome. The blue-violet (BV) excitation method (Nikon Corp. [n.d.]) was used. The BV filter block incorporated an excitation filter allowing transmission of excitation light from the lamp at wavelengths of 400-440 nm, and a suppression (barrier) filter transmitting only wavelengths greater than 470 nm to the eyepieces and camera port. The extent of autofluorescence within tissues was determined by visual comparison with unstained control material (see Koch 1972).

The number of vascular bundles in which fluorochrome was present was compared with the number in which fluorochrome was completely absent in order to calculate a crude index of cavitation. The mean indices of younger and older material and of different types of vascular bundle (see Section 2.3.5) were then compared using two-way Analysis of Variance (ANOVA).

2.2.10 Tylosis.

These methods diverged from those used in the previous section (2.2.8) in that the 'feed' pre-treatment of fluorochrome was not administered and serial sections (again of 10 µm) were made. Serial sections were stained with toluidine blue (0.05 % (w/v) in benzoate buffer – 0.125 g benzoic acid, 0.145 g sodium benzoate in 100 ml distilled water – at pH 4.4; Bolhàr-Nordenkampf & Draxler (1993)) and dried prior to mounting with Canada balsam. Slides were viewed in bright field on a Diaphot TMD-EF inverted microscope and the presence and position of tyloses noted. The length of occlusions in xylem vessels was calculated by multiplying section thickness by the number of sections in which the occlusion occurred. An index of occluded to unblocked vessels was calculated, with an occluded vascular bundle being defined as a vascular bundle in which one or more elements were either fully or partially blocked.

2.3 Results

2.3.1 Non-destructive growth analysis

A photographic record of the synflorescence at differing stages of development is presented in Plate 2.1. Fig. 2.1 shows that the pseudostem of the synflorescence elongated by virtue of the successive lengthening of the distal two interligular portions (i.e. including stem internodes and leaf sheaths), interligular distances proximal to this remaining short - interligule 5 (proximal) ceasing extension at a mean of 2.17 ± 0.44 mm, and interligule 4 at 3.17 ± 0.5 mm (n = 6). The distal internode of the culm (i.e. the peduncle) was visible 9 d after exertion of the main florescence, at which time the paracladial zone was fully exerted and the rachis fully extended. Extension growth ceased sequentially in each interligule, followed by cessation of culm extension growth by 30 d. At this point the entire synflorescence had achieved a length of 396 ± 40 mm and the culm was observed to be yellow rather than green, with plantlets readily shedding. In unsupported plants grown alongside experimental replicates the culm did not readily collapse before this point, but was prone to collapse after this point in development. Plantlets retained green leaves throughout development, but 'blasting' or 'whitehead' was apparent in older plantlets, as glumes and older plantlet leaves senesced.

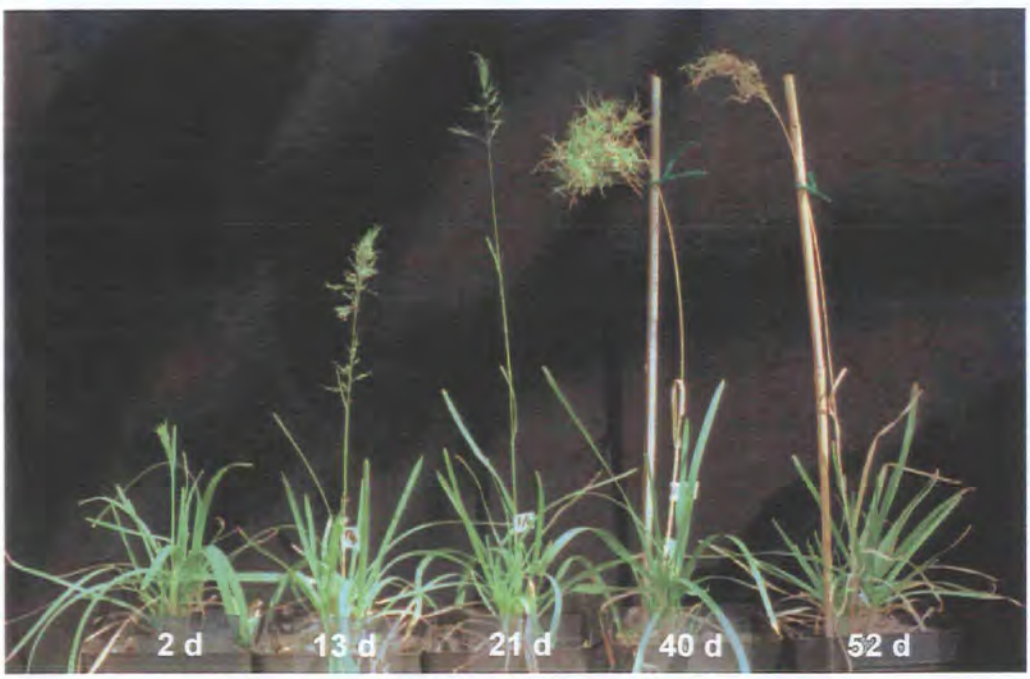


Plate 2.1. The synflorescence of a *Poa alpina* biotype during reproductive development. Canes were used to support the culm during later stages of development.

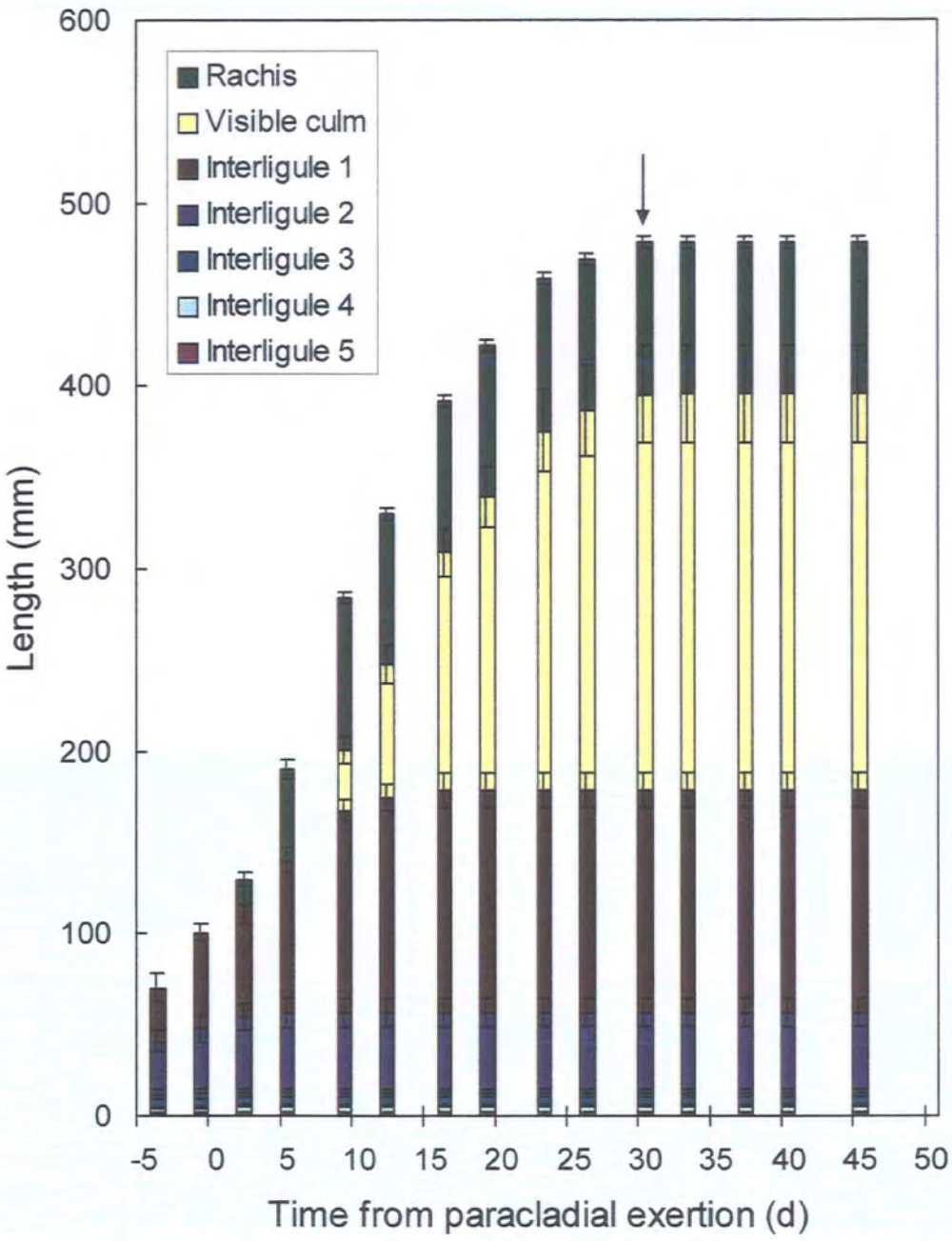


Fig. 2.1. The length of components comprising the synflorescence axis during reproductive development. Data represent the mean ± one S.E. of six replicates. Arrow represents the time from which the culm appeared yellow (previously green), and at which plantlets were observed to readily dehisce.

2.3.2 Gas exchange

Net photosynthetic rates of leaves borne on the culm at 2 d after paracladial exertion decreased with the age of each leaf (Fig. 2.2a), the youngest leaf (leaf 1) having a rate of 16.0 ± 0.2 μmol m^{-2} s^{-1} and the oldest leaf (leaf 5) a rate of 5.1 ± 0.3 μmol m^{-2} s^{-1}. Net photosynthetic rate of the youngest leaf was shown to decline steadily over a 55 d period after exertion, from 16.0 ± 0.2 μmol m^{-2} s^{-1} to zero (Fig. 2.2b). Respiratory rates of all five green leaves on the synflorescence axis 2 d after exertion ranged between 1.4 ± 0.3 μmol m^{-2} s^{-1} and 1.6 ± 0.3 μmol m^{-2} s^{-1} (Fig. 2.2a). Throughout the development of the youngest, distal-most leaf respiratory rates remained between 1.1 ± 0.3 μmol m^{-2} s^{-1} and 1.6 ± 0.2 μmol m^{-2} s^{-1} until immediately prior to necrosis at 55 d (Fig. 2.2b).

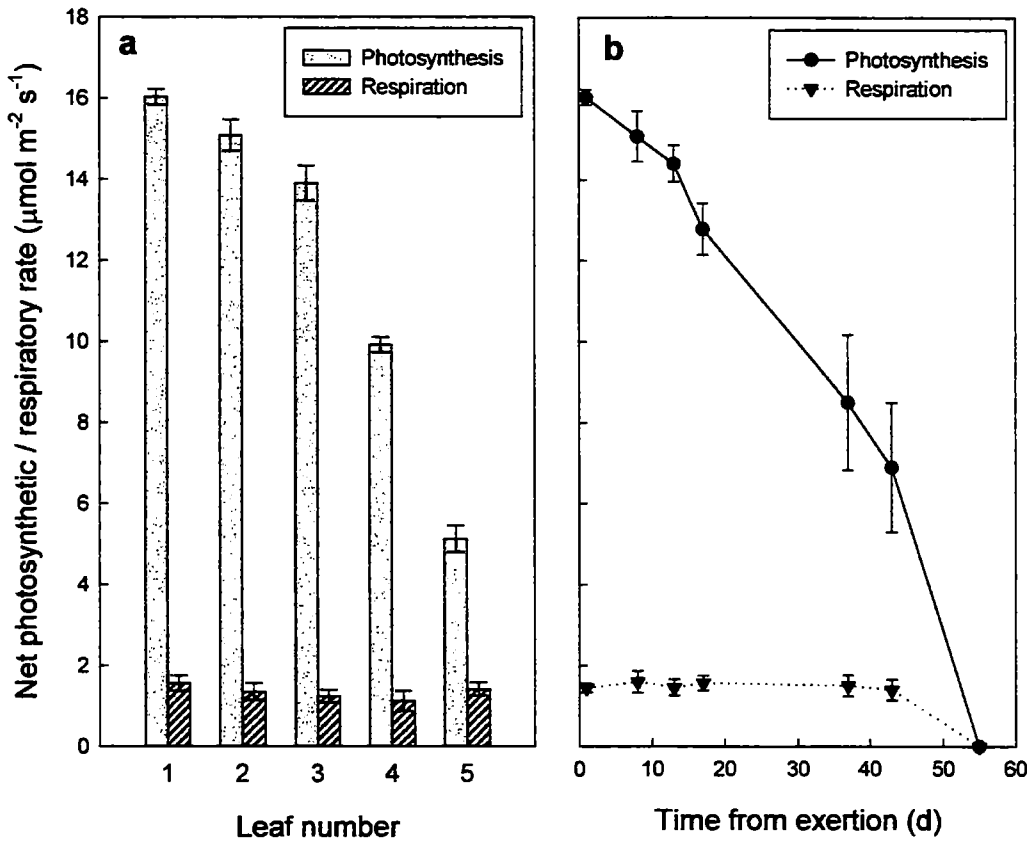


Fig. 2.2. Net photosynthetic and respiratory rates of **(a)** leaves one to five on the main synflorescence axis two days after paracladial exertion and **(b)** leaf 1 during development after paracladial exertion. Leaf 1 = distal and youngest. Data represent the mean ± one S.E. of six replicates.

2.3.3 Water content

The youngest fully expanded leaves of non-flowering tillers showed no change in absolute water content during this period, remaining at approximately 68 % (Fig. 2.3c). Absolute water content of the distal culm internode remained at 70 % until 35 d from exertion, whereupon it decreased to 15 % by 50 d (Fig. 2.3a). Absolute water content of green material in the distal and proximal halves of the paracladial zone were not significantly different ($P = 0.73$ as determined by Student's t-test; see Appendix 5 for full details of statistical tests), and declined from approximately 75 % to 45 % over the 50 d of development studied (Fig. 2.3b).

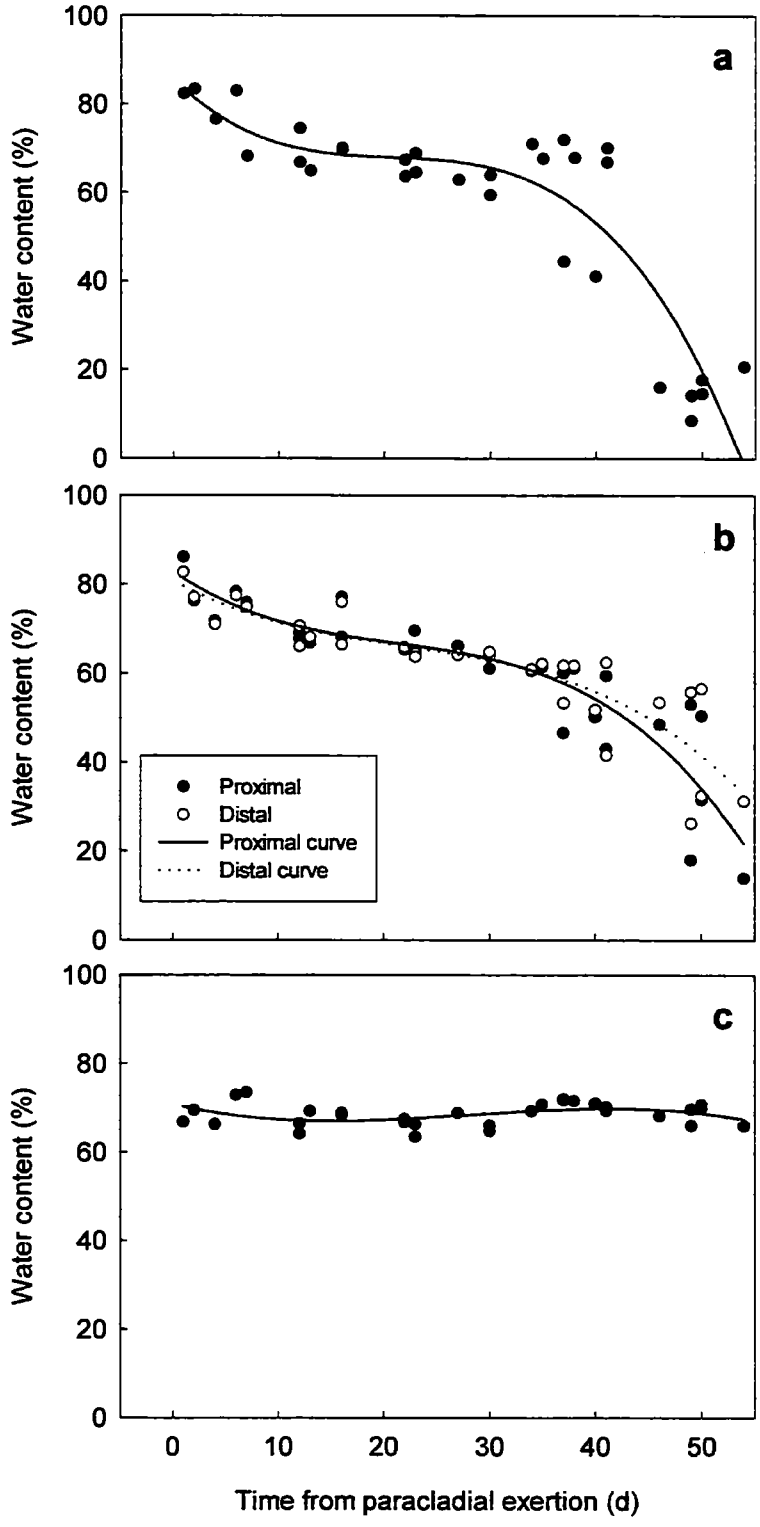


Fig. 2.3. Absolute water content of:

(a) the distal internode of the culm during reproductive development ($n = 30$, $r^2 = 0.81$, $P \leq 0.001$)

(b) green material in both halves of the paracladial zone during reproductive development ($n = 30$, proximal, $r^2 = 0.81$, $P \leq 0.001$; distal, $r^2 = 0.77$, $P \leq 0.001$).

(c) the youngest fully expanded leaf of non-flowering tillers during reproductive development of the main axis ($n = 30$, $r^2 = 0.67$, $P \leq 0.001$).

2.3.4 Photosynthetic pigment content

Culm distal internode total photosynthetic pigment content declined from 0.2 mg g^{-1} $DW_{(calc.)}$ at paracladial exertion to 0.02 mg g^{-1} $DW_{(calc.)}$ at 50 d from exertion, following an exponential decay (Fig. 2.4).

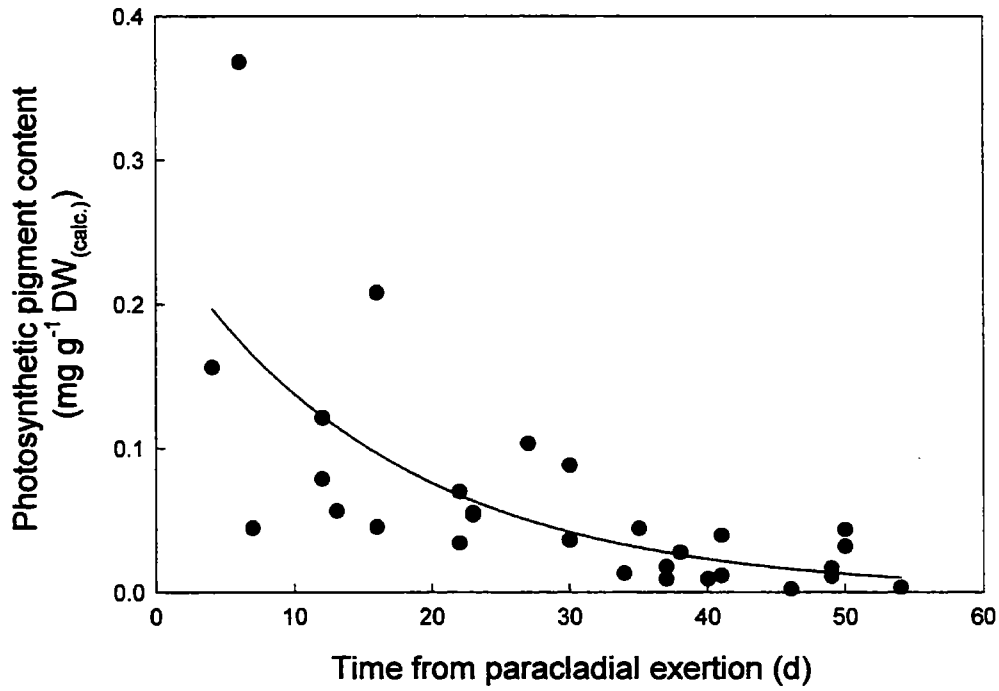


Fig. 2.4. Total photosynthetic pigment content of the distal internode of the culm during reproductive development ($n = 30$, $r^2 = 0.49$, $P \leq 0.001$).

2.3.5 Cavitation

Two types of vascular bundle were present, labelled as major and minor (Plate 2.2), with minor vascular bundles being found on the outer edge of the stele and with xylem elements of smaller dimensions than those of the major vascular bundles. An example of the control material used to gauge the presence or absence of fluorochrome is given in Plate 2.3. Fluorochrome was visible in all vascular bundles of all replicates at 20 d after exertion. At 40 d after exertion fluorochrome was present in 92.3 ± 7.7 % of major vascular bundles and 62.3 ± 19.9 % of minor vascular bundles (Table 2.1), a balanced two-way ANOVA revealing a significant difference between means over time ($P \leq 0.05$; Table 2.2), but not between the two types of vascular bundle. Plate 2.4 shows examples of vascular bundles in which fluorochrome was either absent or present, and photomicrographs of examples of transverse stem sections from these two developmental times can be seen in Plate 2.5. Chlorenchyma and also stomata were observed in culm internodes 20 d after exertion (Plate 2.6a), although at 40 d after exertion no chloroplasts were present (Plate 2.6b). Fluorochrome was present in plantlet tissues (pedicel, glumes, leaf sheaths and blades) at both stages of development (see Plates 2.7 and 2.8 for examples at 20 d from exertion). However, due to the brittle and intricate nature of plantlet material from 40 d, sections were fragmented and not presentable, although fluorochrome could easily be discerned. The presence of fluorochrome in older plantlets can be inferred from the operation of a transpiration stream via culm tissues and a lack of fluorochrome in the proximity of culm stomata, as is also the case at 20 d. Fluorochrome was also detected within plantlet leaf tissues in the veins and stomatal guard cells of plantlet leaves (Plate 2.9), with plantlet leaves being amphistomatic (with stomata on both adaxial and abaxial surfaces). Fluorochrome was present throughout the nodal plexi at both stages of development (e.g. Plate 2.10).

Table 2.1. The proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the paracladial zone in which fluorochrome was present. Data represent the mean ± one S.E. of four replicates.

Time from exertion (d)	Proportion of vascular bundles containing fluorochrome (%)	
	Major	Minor
20	100 ± 0.0	100 ± 0.0
40	92.3 ± 7.7	62.3 ± 19.9

Table 2.2. Balanced two-way ANOVA on the proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the paracladial zone in which fluorochrome was present. Data were arcsin transformed prior to statistical analysis. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Vascular bundle type	n.s.
Time	*
VB type x Time	n.s.

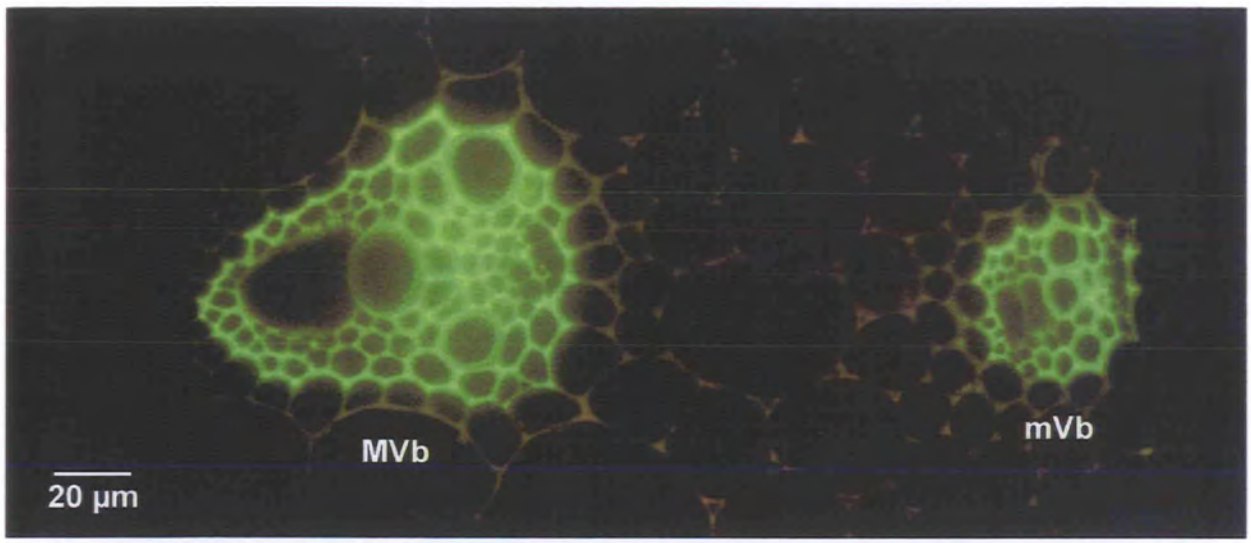


Plate 2.2. Detail of the two forms of vascular bundle present in the culm. The culm pictured was treated with lucifer yellow 20 d after paracladial exertion. MVb: Major vascular bundle. mVb: minor vascular bundle.

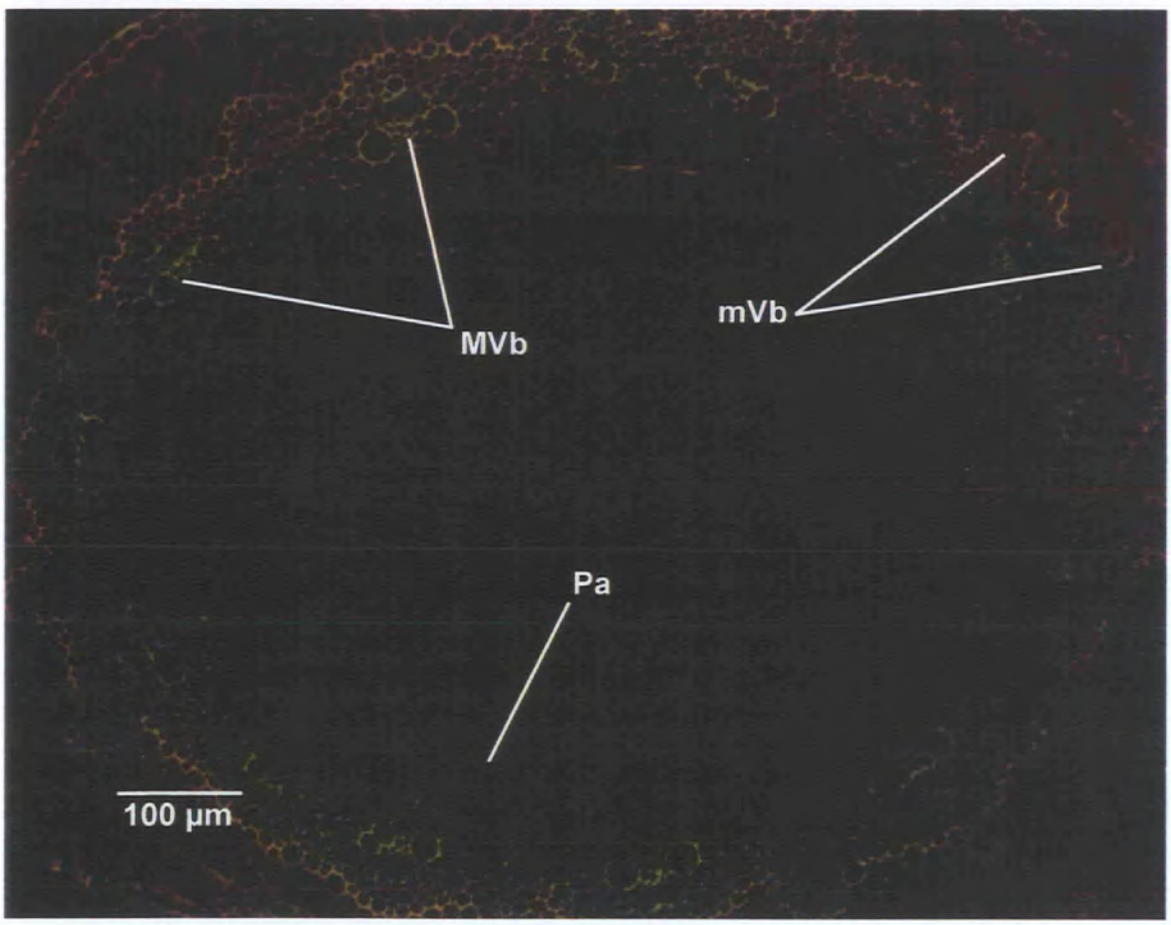


Plate 2.3. Control material from the distal internode of the culm. The culm pictured was not treated with lucifer yellow and was fixed for sectioning at 40 d after paracladial exertion. MVb: Major vascular bundle. mVb: Minor vascular bundle. Pa: parenchyma.

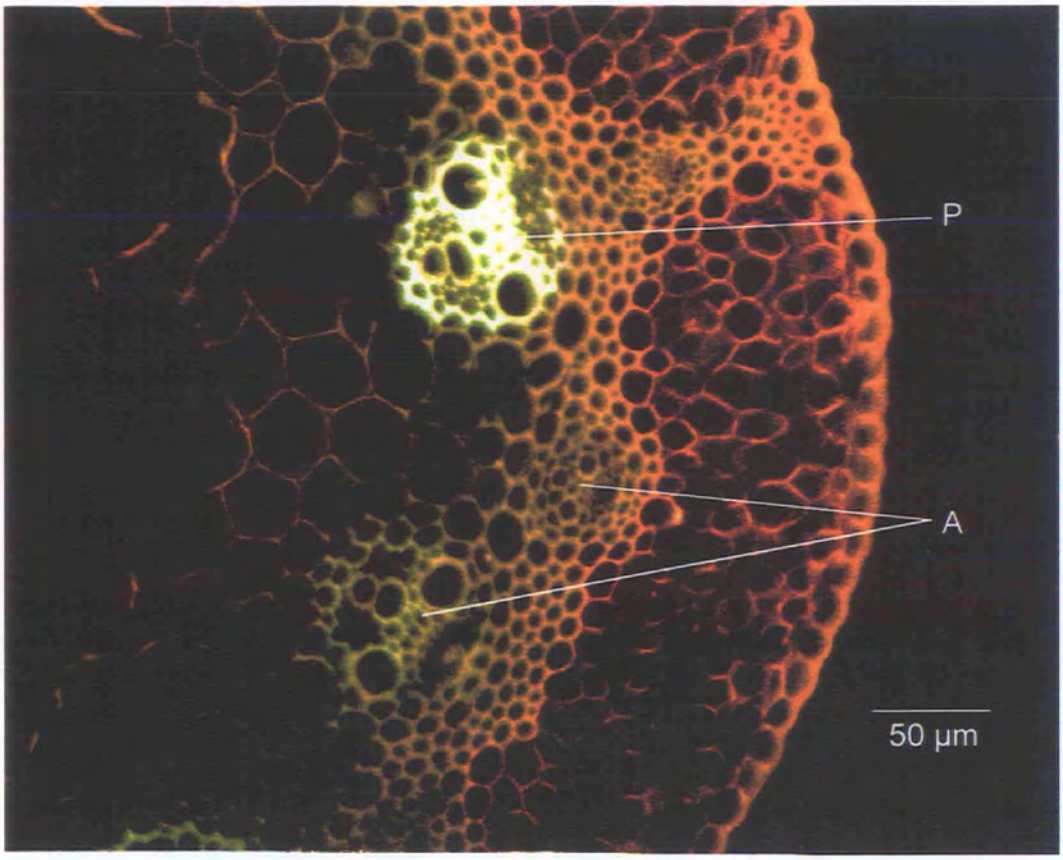


Plate 2.4. Presence (P) or absence (A) of lucifer yellow in vascular bundles. Tissues shown are of the distal internode of the culm at 40 d from exertion.

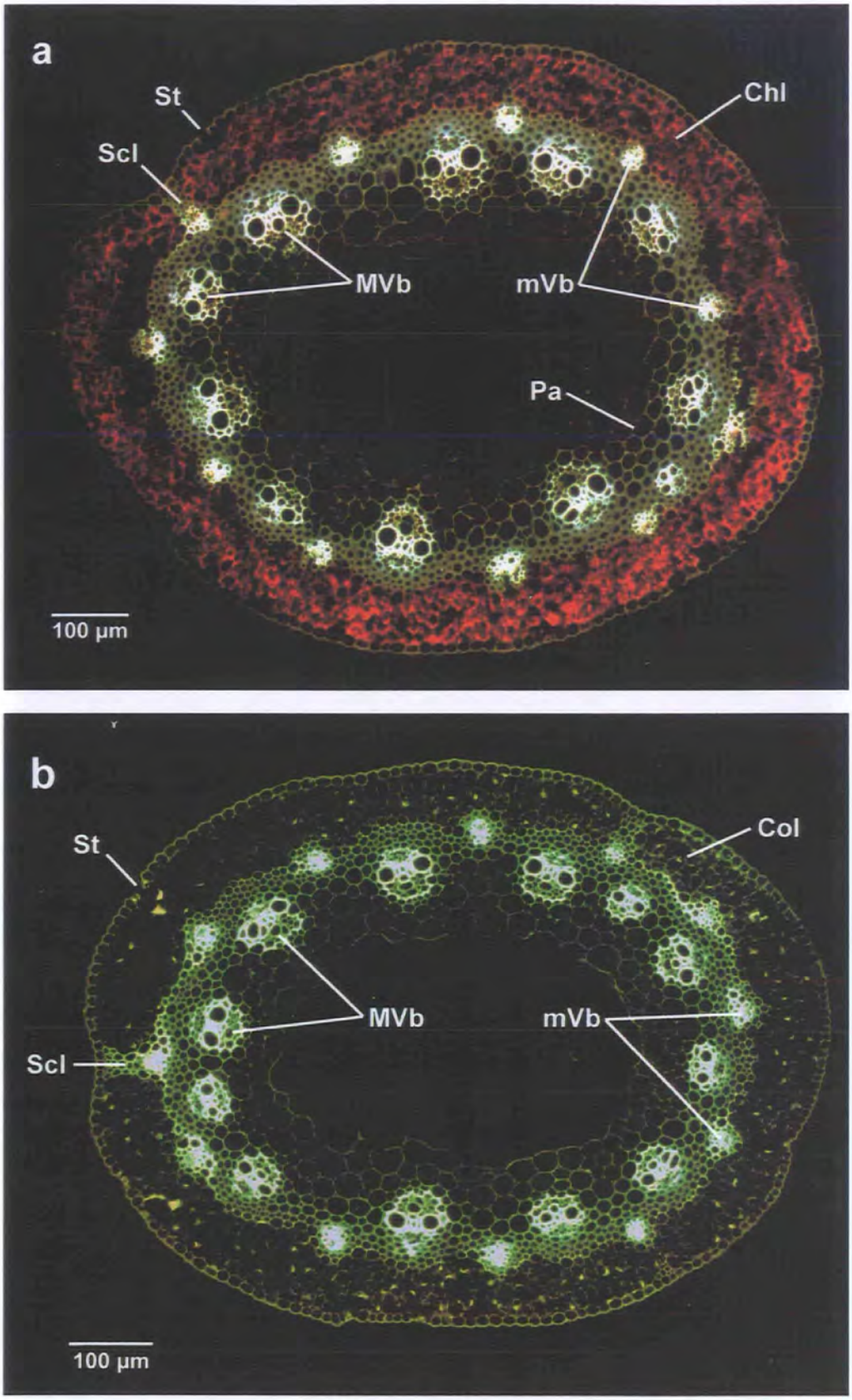


Plate 2.5. Lucifer yellow present in the vascular bundles of the distal internode of the culm at **(a)** 20 d from exertion and **(b)** 40 d from exertion. Chl: chlorenchyma. Col: Collenchyma. MVb: Major vascular bundle. mVb: Minor vascular bundle. Pa: parenchyma. Scl: sclerenchyma. St: Stoma.

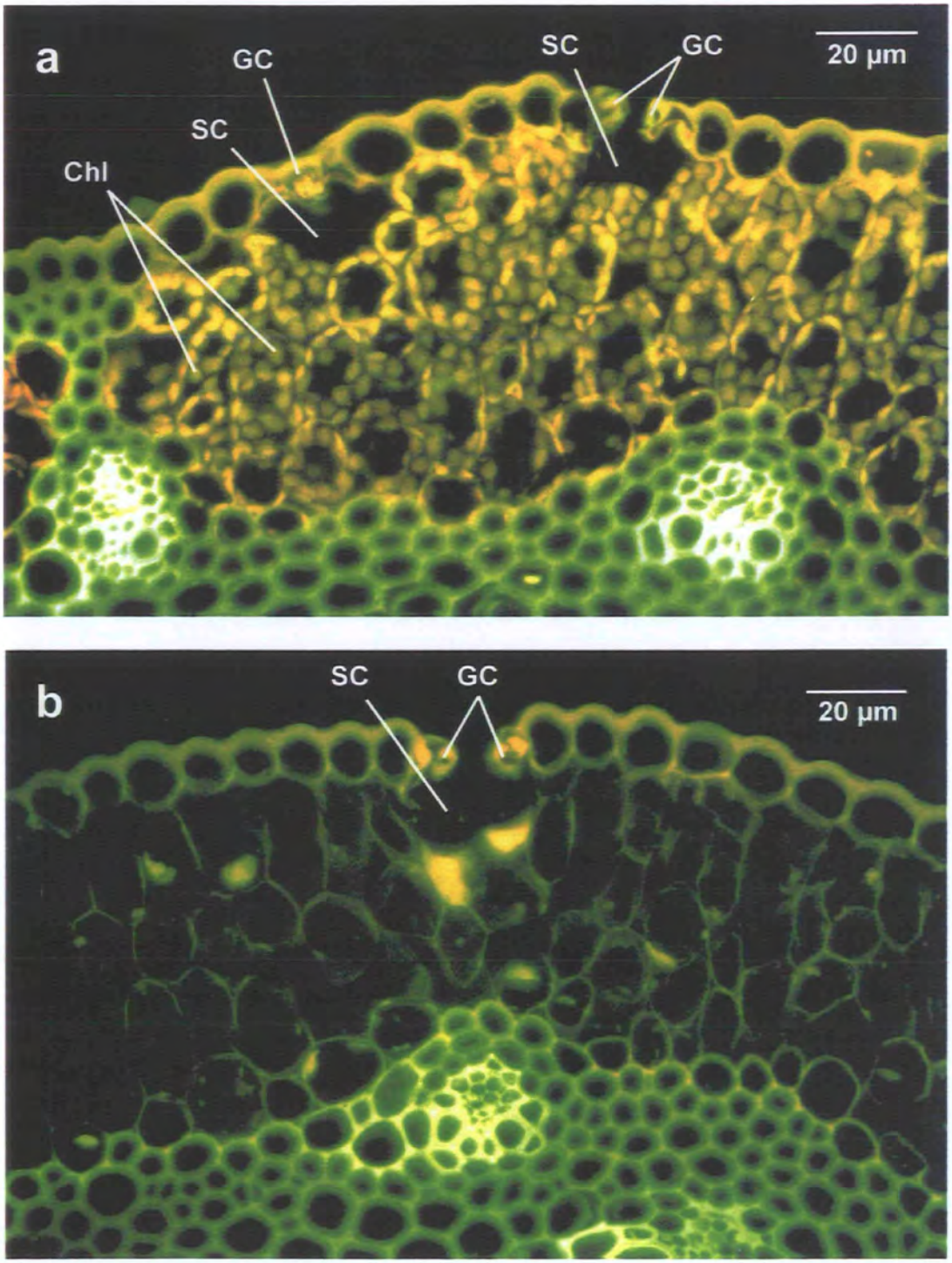


Plate 2.6. Degradation of chloroplasts within culm chlorenchyma during development **(a)** chlorenchyma at 20 d from exertion and **(b)** 40 d from exertion with chloroplasts absent (collenchyma). Chl: Chloroplast. GC: Guard cell. SC: Sub-stomatal cavity.

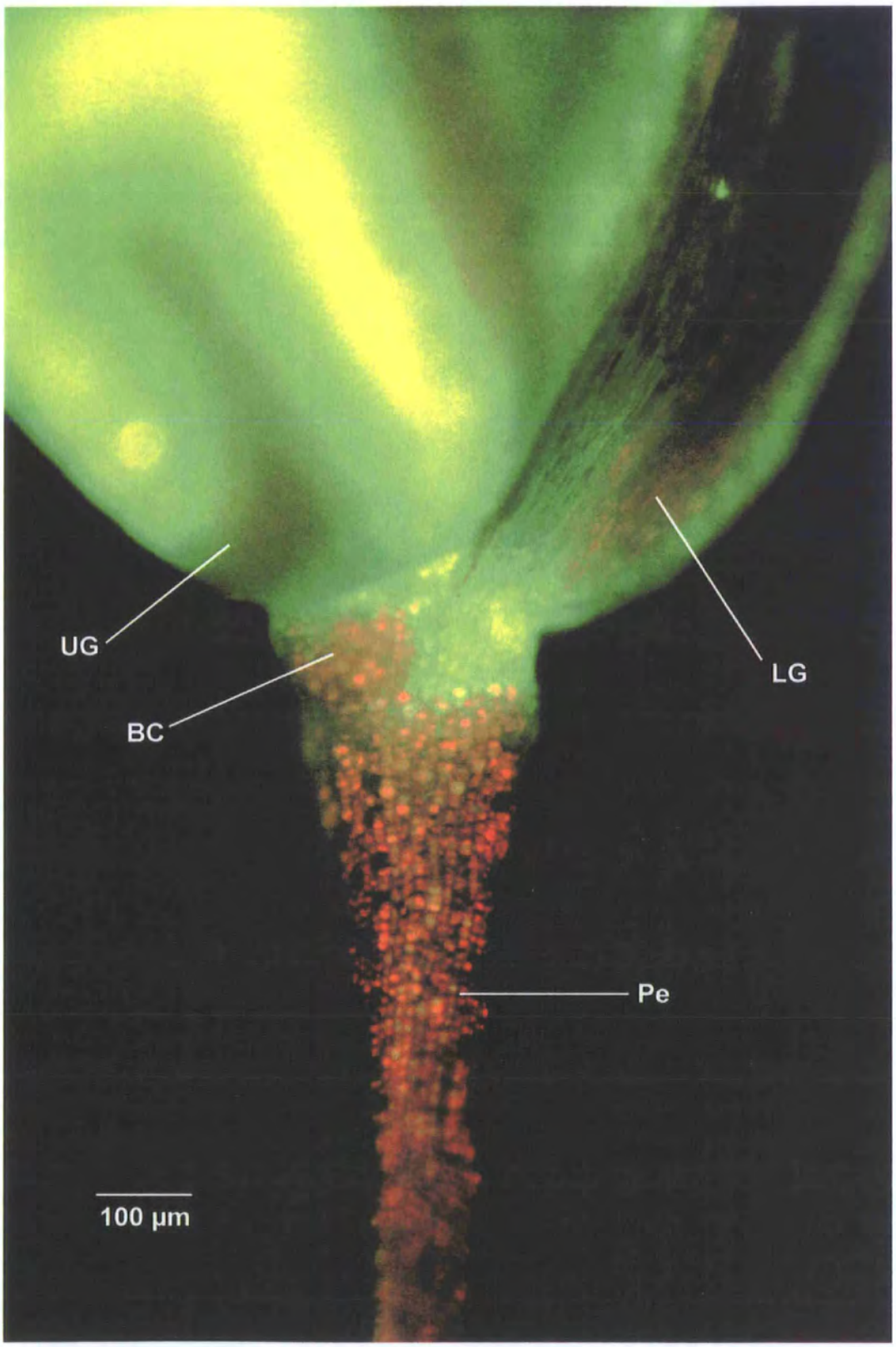


Plate 2.7. Lucifer yellow present in the glumes of a spikelet at 20 d from exertion. The presence of fluorochrome in the pedicel vascular tissues was masked by chlorenchyma. BC: Basal cushion of glume. LG: Lower glume. Pe: Pedicel. UG: Upper glume.

Plate 2.8. Lucifer yellow present in the vascular tissues of the spikelet. Gl: Glume. SP: Spikelet pedicel.

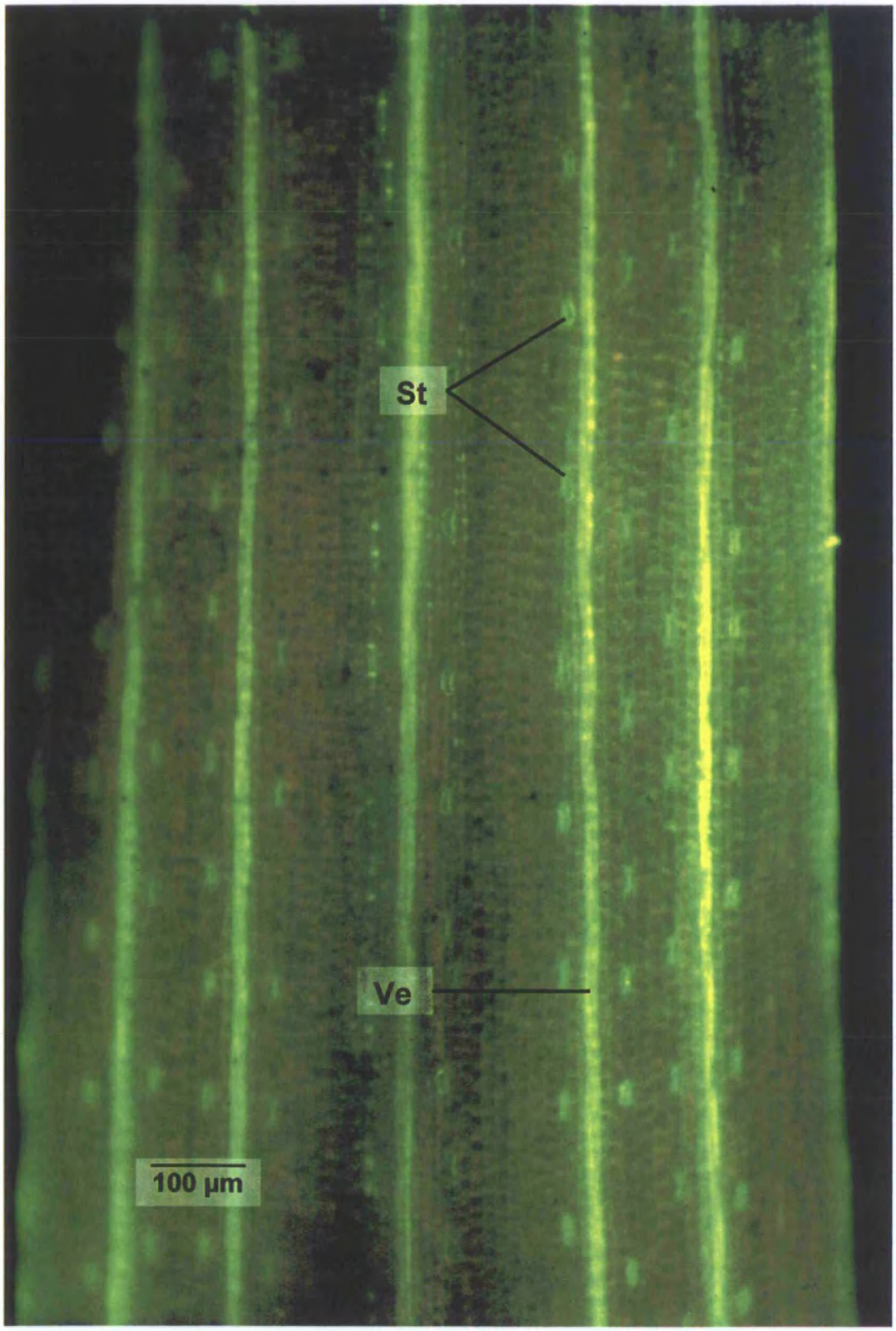


Plate 2.9. The adaxial surface of a plantlet leaf blade with fluorochrome in the transpiration stream revealing the location of stomata and veins. St: Stomata. Ve: Vein.

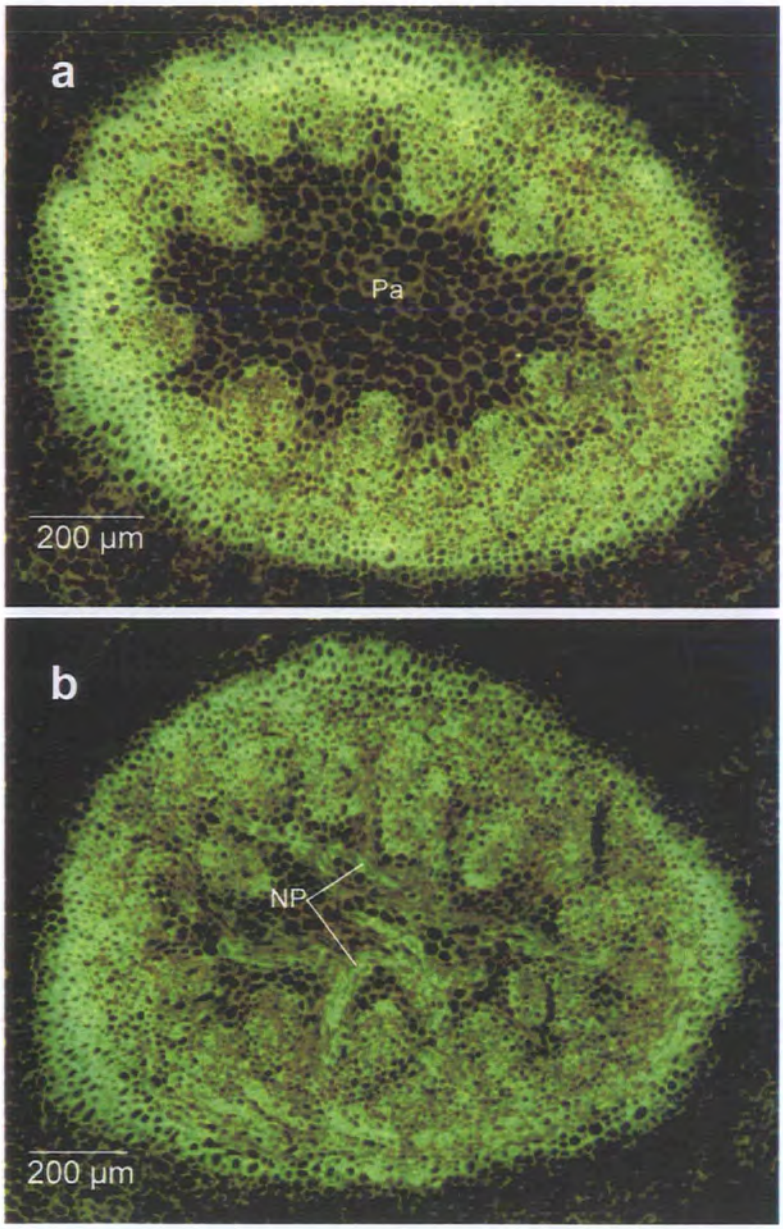


Plate 2.10. Fluorochrome in the basal node of the culm **(a)** the nodal diaphragm 200 µm distal to the nodal plexus and **(b)** in the plane of the nodal plexus. NP: Nodal plexus. Pa: Parenchyma of the nodal diaphragm.

2.3.6 Determination of tylosis

Occlusions were not found in the distal internode and node, or in plantlet material, at any time. Occlusions were occasionally observed in the protoxylem of vascular bundles from the culm internode immediately proximal to the basal node (Plate 2.11). Occlusions infrequently extended into the node, but not into the nodal plexus. These ranged from 40 µm in length (Plate 2.11c), through 500 µm (Plate 2.11a) to 640 µm (Plate 2.11b). These occlusions were not visible 20 d after exertion, and were not the usual state of the vascular bundles at 40 d from exertion (Plate 2.12); at 40 d after exertion occlusions were detected in 21.9 ± 12.7 % of major vascular bundles, and 12.7 ± 7.5 % of minor vascular bundles (Table 2.3). A balanced two-way ANOVA revealed a significant ($P \leq 0.05$) increase in the frequency of occlusion over time (Table 2.4), but not between the two types of vascular bundle. Similar occlusions were observed in the vascular bundles of the leaf sheath at the node at 40 d from exertion (Plate 2.13).

Table 2.3. The proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the basal node in which occlusions could be detected either partially or fully blocking one or more element. Data represent the mean ± one S.E. of four replicates.

Time from exertion (d)	Proportion of vascular bundles partially or fully occluded (%)	
	Major	Minor
20	0.0 ± 0.0	0.0 ± 0.0
40	21.9 ± 12.7	12.7 ± 7.5

Table 2.4. Balanced two-way ANOVA on the proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the basal node in which occlusions could be detected either partially or fully blocking one or more element. Data were arcsin transformed prior to statistical analysis. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Vascular bundle type	n.s.
Time	*
VB type x Time	n.s.

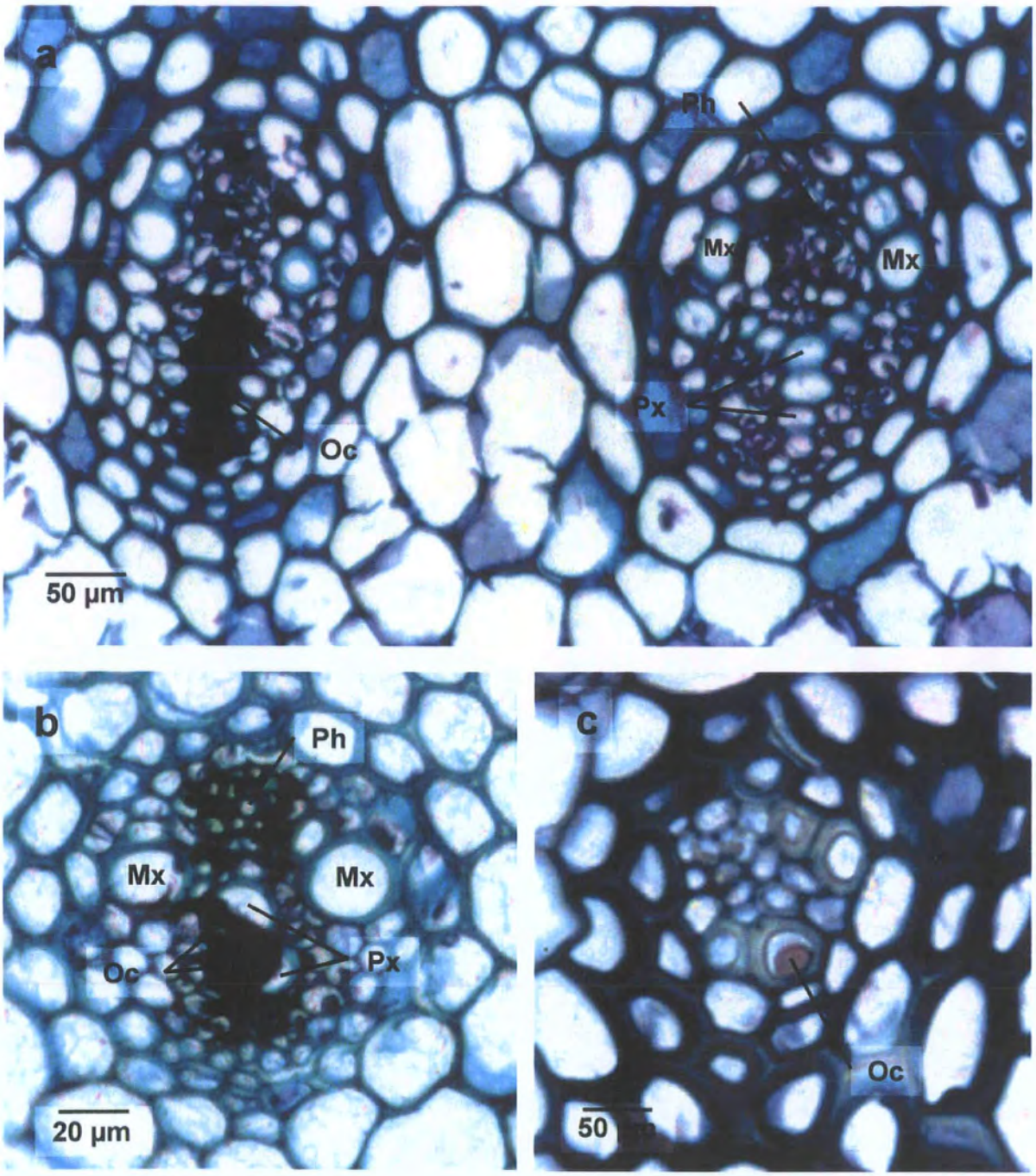


Plate 2.11. Occlusions in the protoxylem of culm vascular bundles situated immediately proximal to the basal culm internode at 40 d from exertion. **(a)** Complete occlusion of the protoxylem of a major vascular bundle (500 µm in length). **(b)** Partial occlusion of the protoxylem of a major vascular bundle (640 µm in length). **(c)** Partial occlusion of xylem vessel in minor bundle (40 µm in length). Mx: Metaxylem. Oc: Occlusion. Ph: Phloem. Px: Protoxylem.

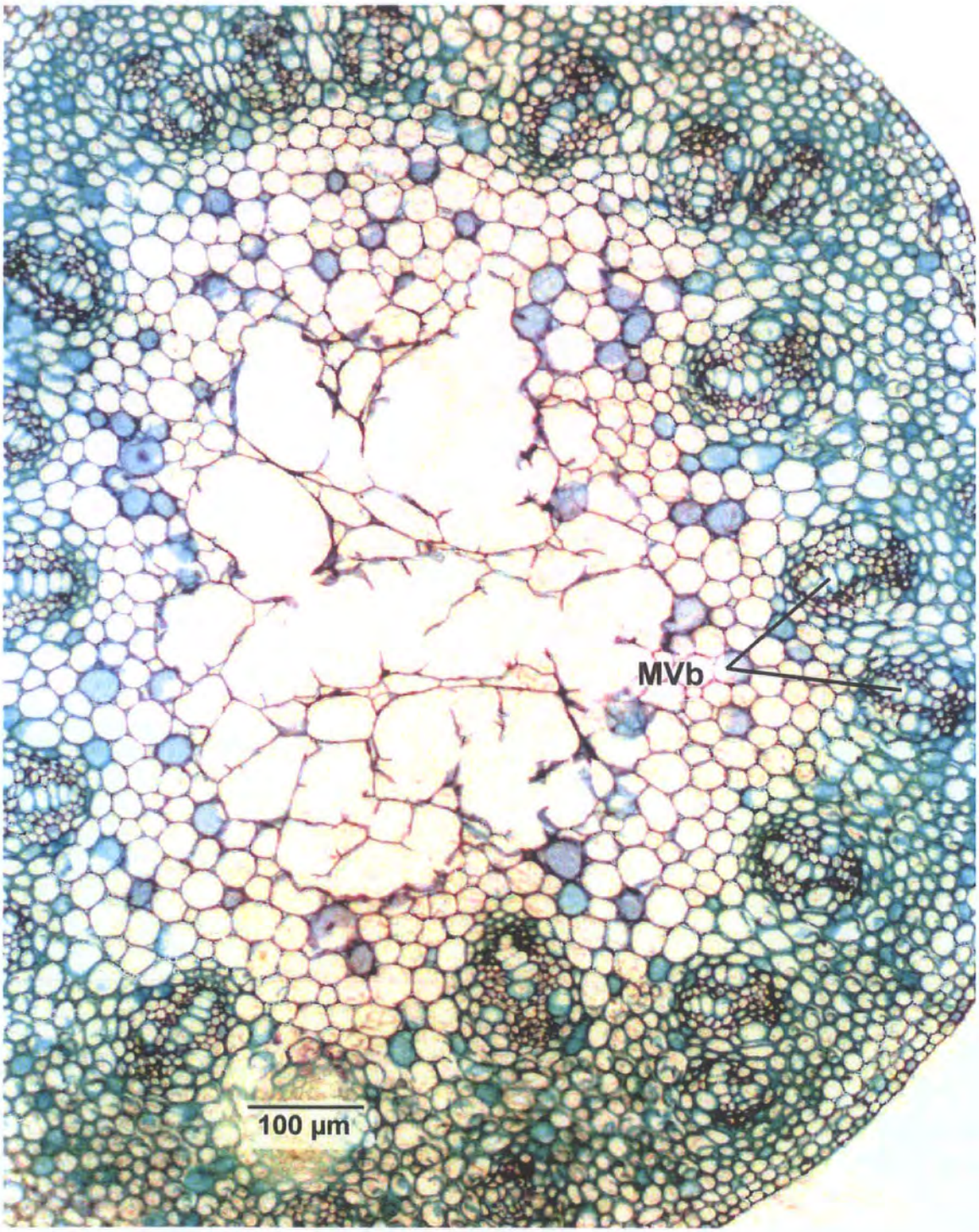


Plate 2.12. Transverse section through a typical culm internode immediately proximal to the basal node at 40 d from exertion. Xylem vessels were free from occlusions. MVb: Major vascular bundle.

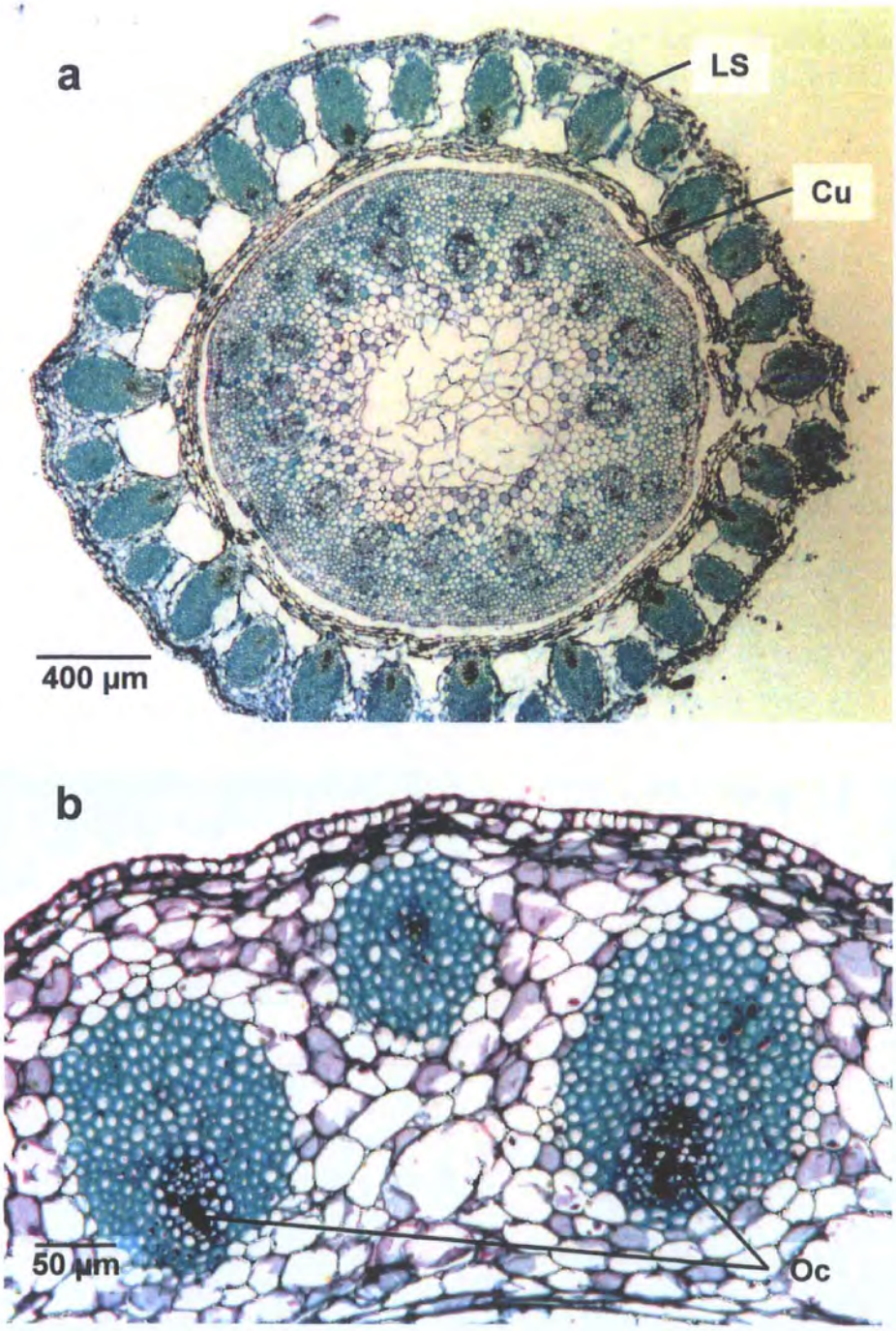


Plate 2.13. Occlusions in the xylem of the leaf sheath at the node at 40 d from exertion: **(a)** the relationship between the culm and the leaf sheath in the distal portion of the node, **(b)** a higher magnification view of the leaf sheath vascular bundles. Xylem vessels of the culm were free of occlusion. Cu: Culm. LS: Leaf sheath. Oc: Occlusion.

2.4 Discussion

Senescence is an active and genetically determined syndrome composed of a variety of individual processes (Noodén 1988). In terms of secondary processes (those peripheral to genetic control), the first outwardly visible signs of senescence in leaves are declining photosynthetic rates and a loss of chlorophyll. This is apparent initially as direct degradation of photosynthetic pigments and, in the later stages of senescence, as degradation of the chloroplast (Gepstein 1988).

Senescence studies of individual organs are often restricted to leaves, as these are particularly amenable to a variety of physiological techniques, and are often the organs that are of direct economic importance or that determine yield (Wareing & Phillips 1990). However, evidence that the processes involved in senescence are similar between leaves and other organs is apparent: for example Steinitz *et al.* (1980) determined that loss of chlorophyll from the flower stalk of Sea Lavender (*Limonium sinuatum* (L.) Mill.) occurred to the same extent as chlorophyll loss in the leaf. In the paracladial zone of rice, chlorophyll content and the activity of succinic dehydrogenase have been shown to decrease during senescence in common with leaves (Debata & Murty 1981, 1982). Debata & Murty (1981, 1982) also determined that different cultivars of rice possessed different rates of senescence within the paracladial zone, that duration of senescence differed from that in leaves, and that the rachillae of spikelets senesced at a higher rate compared to the rachis and caryopses when senescence was judged by these criteria. These results were mirrored by Seo *et al.* (1981), who also determined that the timing of decreased dehydrogenase activity depended on the type of organ. It is therefore likely that chloroplast degradation and protein turnover processes in senescing plant material in general may follow a common pattern, but that the rate and duration of the events involved may differ between organs and biotypes.

The decline in photosynthetic pigment content and the loss of chloroplasts in culm tissues, observed in the present study, can be used as an indication of senescence. Decline in photosynthetic pigment content began from the moment of paracladial exertion, indicating that senescence processes in the culm were in operation from an early stage of development. Also, as chloroplast degradation occurs late in senescence (Gepstein 1988) the complete lack of chloroplasts in culm tissues at 40 d indicates that senescence of the culm was advanced by this stage.

Senescence in the paracladial zone, evident as 'blasting' or desiccated glumes, has been correlated with relative water contents below 50 % and water potential of below -3 MPa in rice (cv. IR36; O'Toole *et al.* 1984) – but is also a general symptom of nutrient stress (Marschner 1995). Water stress is evident in the older plantlets of a variety of pseudoviviparous grasses (including *Poa alpina*) as increased concentrations of proline (Lee & Harmer 1980). Thus the blasting in the paracladial zone observed in the present study is a visual indication of an inadequate supply of resources via the xylem of the culm. As water content of the youngest fully expanded leaf on non-flowering tillers remained at approximately 68 % throughout development, and plants were grown under a strict watering regime, it is evident that the plant as a whole experienced no change in water availability. Thus declining water content and concomitant water stress were inherent to the developing synflorescence.

As transpiration flow was maintained throughout the period studied, and was not greatly hindered by embolism or tylosis, increased transpirational water loss appears to be the most plausible mechanism behind declining water content of plantlet tissues. In the present study fluorochrome was detected in the stomata of plantlet leaves after one hour, and not those of the culm. Also, a characteristic of senescence is the closure of stomata and a lack of transpiration (e.g. *Zea mays*, Aparicio-Tejo & Boyer 1983; in general, Poovaiah 1988). Thus in the pseudoviviparous system studied here it was the green, non-senescent plantlet material that provided the observed transpiration flow, even at a time when plantlets readily dehisced. Data therefore suggest that as plantlets grow their demand for water gradually out-strips the physical capacity of the culm to supply (as suggested by Lee & Harmer 1980). This would result in the observed gradual decline in water content and the increasing water stress of plantlet tissues, whilst maintaining transpiration flow. Due to the continued supply of water, it is likely that water stress of plantlet tissues was not as great as it potentially could have been. Also, a continued physiological connection existed throughout the development of plantlets, supporting the hypothesis that plantlets may acquire more resources (at least in terms of mineral nutrition) from the parent plant the longer they remain attached (Harmer & Lee 1978a).

Declining water content in the culm perhaps represents a lack of demand for water in senescent tissues. Water loss during senescence was observed by Chaffey (1983) in leaves of *Lolium temulentum*. He argued that this could be due to the breakdown of cell membranes, resulting in a decreased ability of cells to retain water. Thus a distinction must be made between water loss from the yellowing culm and from

plantlet leaves which are clearly at different developmental stages, and any similarities in the pattern of water loss may well be coincidental.

The vascular bundles of the grass culm and rachis are continuous between internodes (Joarder & Eunus 1980), with nodal plexi physically connecting these vascular tissues at the node (Arber 1930). Hitch & Sharman (1971) provide a three-dimensional model of these connections for *Poa*. In this study, fluorochrome was demonstrated to move throughout nodal plexi when the base of the culm was 'fed' with Lucifer Yellow fluorochrome (Plate 2.10), demonstrating a physiological connection in terms of water transport. Hence cavitation in proximal internode vessels will not necessarily result in dysfunctional tissue throughout more distal lengths of the culm, as water movement via plexi can bypass dysfunctional vessels. Also, the recovery of xylem vessels from embolism is thought to occur on a regular basis in most vascular plants (Sperry *et al.* 1988a&b), as water transport occurs at negative pressures often bordering on the cavitation threshold (Tyree & Sperry 1988). Therefore vascular plants must frequently address this problem, and are thought to employ root pressure to re-fill embolisms (Kramer & Boyer 1995), or increased local phloem loading and concomitant increases in surrounding tissue osmotic potential may re-fill vessels (Salleo *et al.* 1995). Indeed, xylem recovery of water-stressed maize may occur in a single night (Tyree *et al.* 1986). Xylem recovery, in addition to bypassing via nodal plexi, may therefore decrease the impact of cavitation on transport, although the permanence of individual embolisms was not elucidated in the present study.

Just as cavitation in culm vascular tissues increased over time, so did the related process of tylosis[4]. This, and the occlusions present in the necrosed nodal leaf tissue (Plate 2.13), are indirect evidence that both processes may be more frequent immediately prior to necrosis, and that occlusions may ultimately segregate the necrosed synflorescence from living tillers. Chaffey (1983) states:

> "in the absence of a more detailed study...it is not possible to state whether tyloses may be cause, effect or coincident with initiation of leaf senescence."

[4] N.B., the nature of the occlusions observed is uncertain, i.e. whether they were true tyloses or gels. However, those that stained blue possessed cellulose components and are likely to have been tyloses. Solvents such as 1 % sodium hydroxide can be used to remove gels and identify tyloses (Chattaway 1949), but cannot be applied after coverslipping (which was necessary in order to find the rare examples of occlusion in the tissues studied here).

The complete absence of occlusions from culm tissues at 20 d into senescence precludes tylosis as a primary senesce process in the case of the synflorescence of *Poa alpina*. That occlusions were found only immediately proximal to the basal internode of the culm, and not in the distal internode in which embolism was apparent, is further evidence that tylosis is not necessarily a response to embolism but to declining water content of vascular tissues (Canny 1997). The rare and brief presence of occlusions suggests that the hypothesised role of tyloses as scavengers of metabolites from xylem sap (Chaffey 1983, Chaffey & Pearson 1985) would be of a transient nature in this case.

In conclusion, this chapter provides essential background information concerning culm development and senescence, providing a context within which further work may be considered. Water loss was inherent to the developing synflorescence, and visible indications of water stress were apparent in the paracladial zone (i.e. water content of synflorescence components was dependent on the extent of monocarpic senescence; supporting hypothesis 1). A low frequency of embolism and tylosis/gel formation was apparent in older culms. However, transpiration flow occurred in the majority of vessels, providing a physiological connection between parent and plantlets even when senescence was advanced in culm tissues, as evidenced by a lack of chloroplasts. Also, tylosis did not occur as a direct result of cavitation as predicted by hypothesis 3. Hypothesis 2, that cavitation and tylosis result in extensive xylem dysfunction in older culms, is therefore rejected. Thus the data support the hypothesis that plantlets remaining attached to the parent plant face a trade-off between water stress and the continued acquisition of resources (Harmer & Lee 1987a). Culms possessed machinery (stomata, chloroplasts and photosynthetic pigments) required for photosynthesis. The importance of the culm and paracladial zone as a source of carbon, and the movement of carbon via the culm are considered in the following chapter.

3 Architecture and physiological integration of the paracladial zone

3.1 Introduction

Any investigation of the physiological relationships between organs, such as paracladia, must first of all determine their spatial and temporal relationships. For example Mohapatra & Sahu (1991) and Mohapatra *et al.* (1993) state that in three rice varieties the pattern of development within the paracladial zone was hierarchical, with distal paracladia being further developed than proximal paracladia – there being a gradient in physiological time along the rachis (also observed in grasses in general by Arber 1934). In addition the architecture of the paracladial zone differed spatially, with larger numbers of spikelets being produced by proximal paracladia (and caryopses from these being 'quite poor' [sic]).

An initial aim of this chapter was therefore to describe the architecture and development of the paracladial zone in order to provide a context in which to consider further physiological investigation. A brief description of plantlets is also given as confirmation of true prolification, rather than vivipary or any other process, in this biotype.

In terms of the physiology of plantlets, the occurrence of photosynthesis is perhaps to be expected, as the spikelets of many grasses are capable of fixing carbon. Indeed, the contribution of assimilates from spikelets may be of considerable importance to developing caryopses: Porter *et al.* (1950) noted that photosynthesis within the ear of barley contributed to the growth of caryopses. Thorne (1965) determined that 60 % of the carbohydrate in the caryopses of barley was derived from this source, and that wheat ears contributed 17 – 30 % of caryopsis dry weight (having less spikelet surface area than barley). In the native grass *Poa annua* L., Ong & Marshall (1975) determined that 20 – 25 % of ^{14}C incorporated into the caryopses at 14 days after exertion was initially fixed by the inflorescence. Ong *et al.* (1978) state that the inflorescence of *Poa annua* and *Lolium perenne* L. was the most active assimilatory organ of the reproductive tiller, with all parts of the inflorescence (except caryopses) fixing ^{14}C; 40 – 50 % of which was accounted for by the lemmas and paleas.

It is therefore reasonable to expect that pseudoviviparous plantlets, with their relatively large surface area, may be capable of providing a considerable proportion -

if not all - of their carbon requirements. Unresolved aspects include the extent to which plantlets are reliant on parent tissues in terms of carbohydrate, and also the extent to which plantlets may export photoassimilate – questions posed by Lee & Harmer (1980) after determining that plantlets of *Festuca vivipara* did indeed photosynthesise:

> "to what extent the plantlets are self-sufficient in, or net exporters of, carbohydrates is unknown. It is likely, given the relative rates of photosynthesis of leaves and plantlets observed here, and the slow growth of plantlets ... that at the least plantlets are not an appreciable sink for carbohydrates from the leaves."

The concept of carbon export from plantlets poses further questions: (a) is assimilation by plantlets the sole source of carbon for plantlet development after the exertion of the paracladial zone? (b) if plantlets are net sources of carbon, are physiologically younger plantlets sinks for carbon from older plantlets? and (c) what is the extent of export from the paracladial zone to developing tillers on the parent plant? Further questions posed by the results of the previous chapter concerning the role of the of the culm as a photosynthetic organ were also addressed. All of these questions are embodied in the following hypotheses:

That:

1. the paracladial zone of *Poa alpina* is a carbon source, supplying parental sinks,
2. spatial and temporal heterogeneity exists within the paracladial zone with respect to plantlet development, resulting in a proportion of plantlets that are more able to establish and function as propagules,
3. physiologically older paracladia are sources of carbon for younger paracladia,
4. paracladia do not compete for parental assimilate after exertion,
5. carbon transport basipetally via the culm occurs during later stages of culm development (i.e. at 40 d from paracladial exertion),
6. portions of culm exposed to light and atmosphere are sources of assimilate.

3.2 Methods

3.2.1 Cultivation

The cultivation and inflorescence-induction methods used were identical to those detailed in Chapter 2.

3.2.2 Determination of prolification

A minimum of three plantlets from paracladial zones of various ages (2, 14, 20 and 45 d from exertion) were dissected under a dissection microscope. Typical plantlets were drawn, and the dissections represented by a schematic diagram of the plantlet rendered using CorelDRAW 7 software (Corel Corporation, Orem, Utah, USA).

3.2.3 Architecture of the paracladial zone

Morphological maps were made of the paracladial zone of six replicate synflorescences, the presence and relative position of plantlets and aborted spikelets being noted. The synflorescence of the main axis from separate plants, 20 d after exertion, was used. The paracladial zone of tiller synflorescences may be smaller in physical dimension (*Phleum pratense*; Ryle 1963), and have less spikelets (*Pennisetum americanum* (L.) Leeke; Coldrake & Pearson 1985a&b), due to their production at an earlier developmental age at which the shoot apical dome is relatively small. Thus tiller synflorescences were avoided throughout this study in order to standardise the synflorescences used.

3.2.4 Gas exchange within the paracladial zone

Gas exchange within the paracladial zone was determined by IRGA using an ADC LCA 4 and a re-configured PLC 3 conifer-leaf cuvette (Plate 3.1a). The entire paracladial zone was enclosed within the cuvette, with no culm tissue included. Light was provided using 250 W metal-halide lamps (model HGI/NDL; FGL Lighting Ltd., Pinewood Studios, Bucks., UK), and plant tissue temperature measured using an independent thermistor, placed in direct contact with plant tissue. Instantaneous net photosynthetic rate was measured at a photosynthetic photon flux density (PPFD) of 500 µmol m^{-2} s^{-1} PAR incident at the plant tissue surface, and at a temperature of 20 °C. Air flow within the cuvette was maintained at a constant rate of 300 ml min^{-1}. Respiratory rates were measured by enclosing the cuvette in aluminium foil to exclude light, at 19 °C. Initial measurements were based on an estimated paracladial area. After gas exchange measurements, the paracladial zone was destructively harvested and the actual area determined by dissection followed by scanning of an image of the flattened paracladial zone using a ScanJet 4c flatbed scanner (Hewlett-

Packard Ltd., Bracknell, Berks. UK). This image was then analysed using Delta-T Scan leaf area software (Delta-T Devices Ltd., Burwell, Cambridge, UK) to determine the two-dimensional 'area' of the paracladial zone. Measured net photosynthetic and respiratory rates were then corrected by calculation. Measurements were made from a population of plants at various stages of development up to 21 d after exertion, but not before 7 d as proximal paracladia had not emerged.

3.2.5 Carbon fixation and allocation

The apparatus used for supplying $^{14}CO_2$ to plant parts was similar to that detailed by Farrar (1993). The source of activity was $Na_2{}^{14}CO_3$ (Amersham Life Sciences Ltd., Little Chalfont, Bucks., UK (Code ref. CFA 2)), with an activity per feed of 10 μCi ≡ 370 kBq. Distilled water (25 ml at pH 7.0) containing 4 aliquots of 10 μCi was released from a 25 ml syringe at a rate of 0.25 ml min^{-1} using a Perfusor Pump (FT Scientific Instruments Ltd., Bredon, Nr. Tewkesbury, Glos., UK) into a pear-shaped flask containing 25 ml lactic acid (BDH product no. 101384Q). Air was bubbled through the lactic acid at a flow rate of 1 l min^{-1}, using an air pump (Charles Austin Pumps Ltd., Byfleet, Weybridge, Surrey, UK) and external air supply, regulated by a flowmeter (KDG Instruments, Crawley, West Sussex, UK). Depending on the particular experiment, plant organs were enclosed within either an ADC PLC3 conifer leaf chamber (Plate 3.1a) or a smaller hand-made cuvette (Plate 3.1b), through which flow of air enriched with $^{14}CO_2$ was maintained. A second flowmeter situated beyond the leaf chamber was used to check maintenance of flow rate and system integrity. $^{14}CO_2$ was ultimately trapped within a sodalime column and the remaining gases released into the atmosphere. Containment of spillages was achieved by placing the apparatus within trays lined with paper towelling, and equipment was checked for leaks throughout using a Geiger-Muller tube.

A pulse-feed of 20 minutes duration was applied to the plant part at 20 °C and a PPFD of 700 μmol m^{-2} s^{-1} PAR. The edges of the cuvette were marked on the plant material with fine marker pen to visibly delimit the fed portion. The pulse feed was followed by a 2 hour chase period at 20 °C and a PPFD of 700 μmol m^{-2} s^{-1} PAR. Pulse-fed plants were chased in a separate location to un-fed plants to minimise the risk of cross-contamination from respiratory $^{14}CO_2$, and four un-fed plants from the laboratory were used as control material. After the chase period, pulse-fed plants were dissected into their component organs (depending on the particular experiment) and each component placed in a labelled paper bag in preparation for drying at 70 °C overnight. At a later date samples were oxidised in an OX400 Biological Sample

oxidiser (Lab. Impex Systems, Wimborne, Dorset, UK). The $^{14}CO_2$ evolved was trapped in Oxosol $C^{14\,tm}$ scintillant (National Diagnostics, Hessle, Hull, UK – Order No. LS-211) and counted using a 1414 WinSpectral liquid scintillation counter (EG & G Wallac Ltd., Milton Keynes, UK). A blank containing scintillant was used to remove the background activity from all data.

Experiment 1:

The first leaf (see Fig. 3.1 for a precise definition of plant parts) of plants at 20 d from paracladial exertion (i.e. when paracladial zone, culm and first leaf were all green), and also the entire paracladial zone of further replicate plants were pulse-fed using the conifer leaf chamber (Plate 3.1a).

Experiment 2:

A monochasial unit of three spikelets from the mid portion of the paracladial zone (again at 20 d from exertion) was pulse-fed using the cuvette pictured in Plate 3.1b.

Experiment 3:

The first leaves of plants at 40 d from exertion (i.e. when the culm had ceased elongation growth) were pulse-fed.

Experiment 4:

The distal half (including the mid portion) of the paracladial zone of six replicate plants was removed with a razor blade under water, and the cut rachis sealed with silicon grease. In a further six replicates the paracladia from the proximal two rachis nodes (i.e. proximal half by spikelet number) were removed and stumps sealed. Six control plants were left intact. Treated plants were left for 24 h before pulse-feeding of the first leaf using the conifer leaf chamber (Plate 3.1a).

Experiment 5:

A portion of the distal internode of the culm at 20 d from paracladial exertion was pulse-fed.

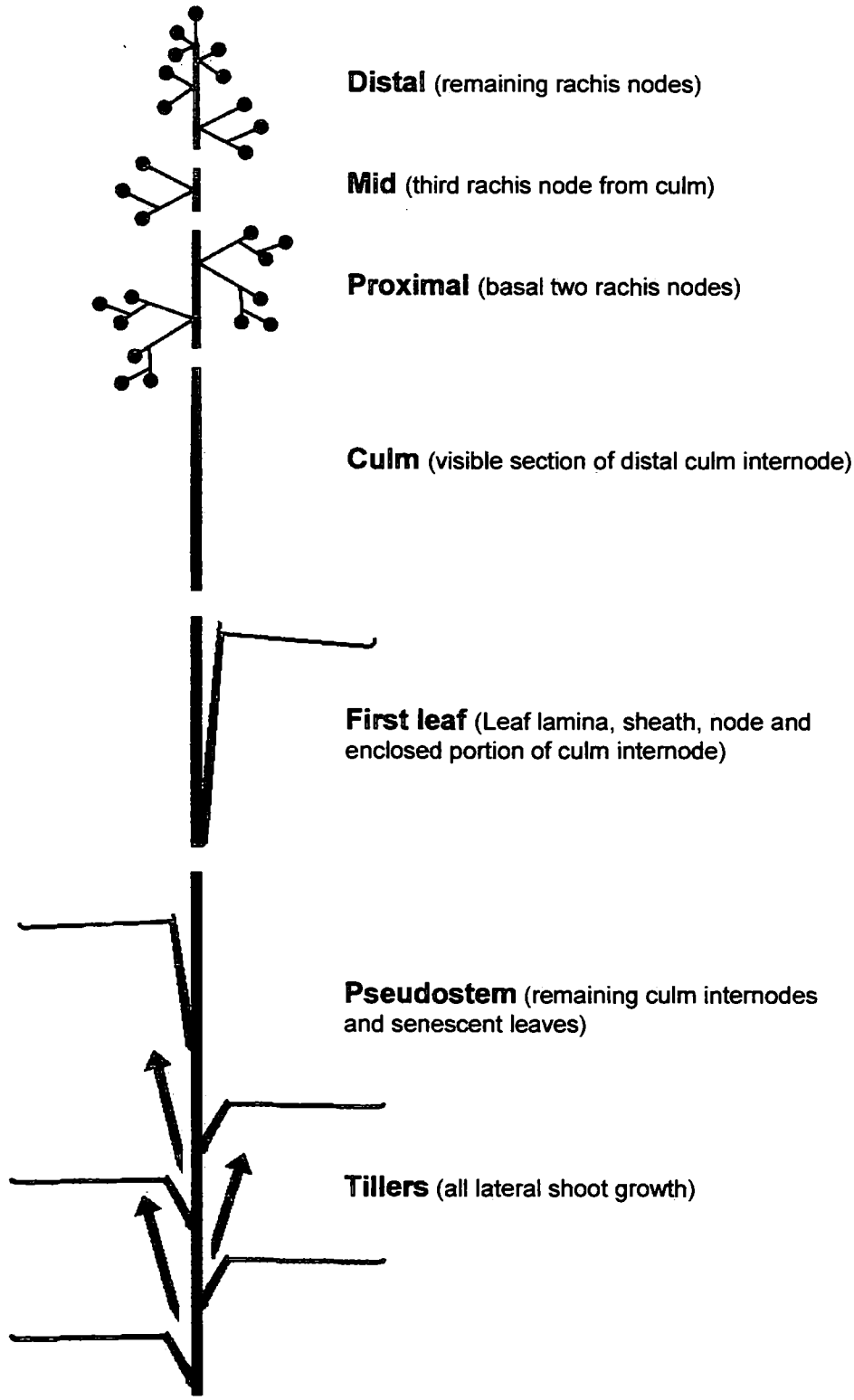


Fig. 3.1. Diagrammatic representation of the zones of the shoot between which the partitioning of ^{14}C was investigated. The portions of the paracladial zone were determined from the architectural investigation of the synflorescence (this chapter).

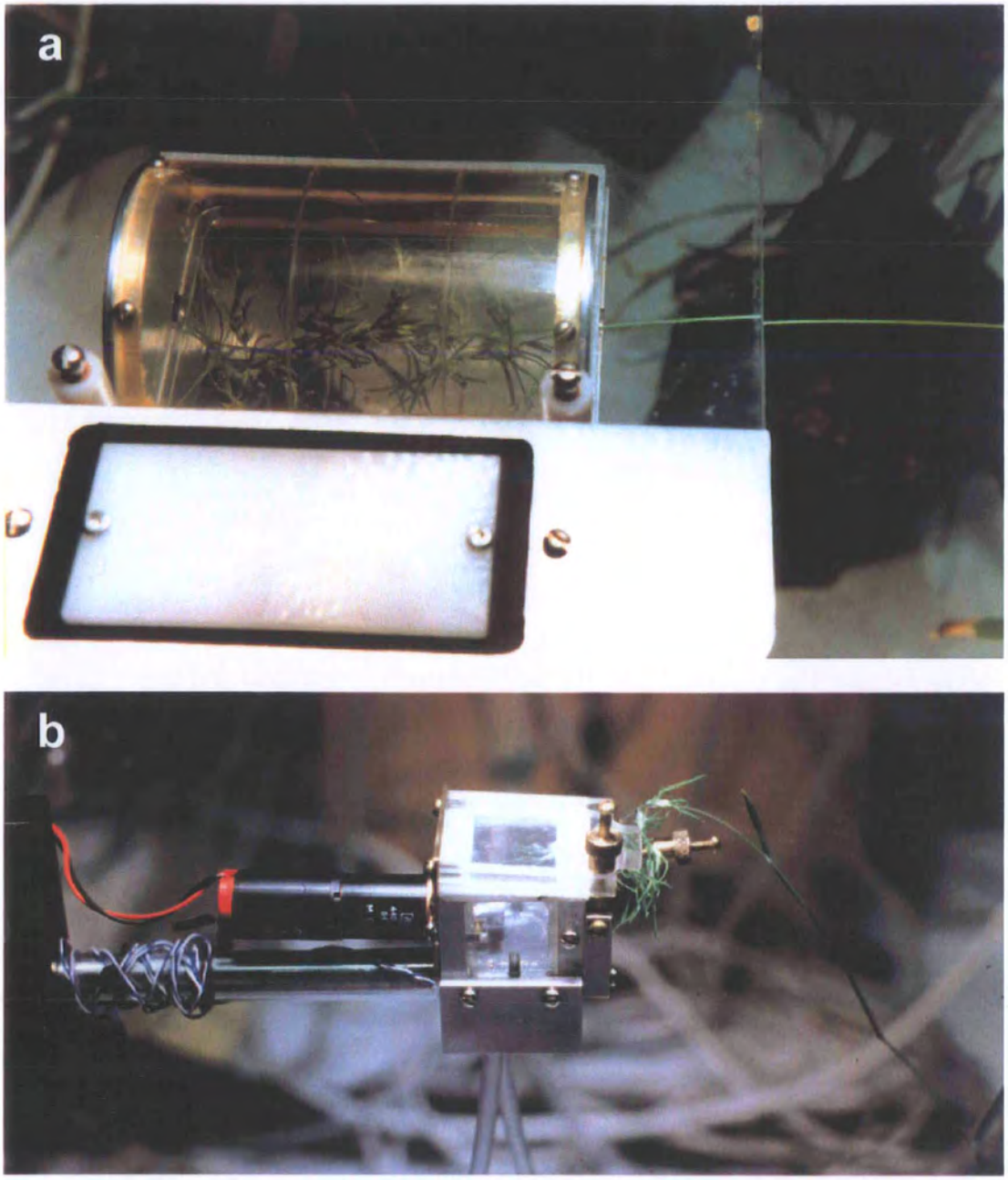


Plate 3.1. Cuvettes used: **(a)** the ADC PLC3 conifer leaf chamber used to feed and measure gas exchange of the entire paracladial zone, **(b)** the small hand-made chamber used to feed small groups of plantlets (photo courtesy of Dr. R. Baxter). The small chamber possessed a battery-operated fan to assist mixing.

3.2.6 Manipulation of sinks

Manipulation treatments (identical to those detailed in Experiment 4 of the previous section) were imposed on the paracladial zone of plants 7 d after paracladial exertion (i.e. when the entire paracladial zone had emerged and proximal paracladia were available for manipulation), with either the distal or proximal half (by spikelet number) removed. Control plants were kept intact. Eight replicate plants of each treatment were harvested at the baseline (i.e. 7 d from exertion), and at two subsequent harvests, each 7 d apart. Relative growth rate was determined using the following equation (Hunt 1990):

$$RGR = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)} \quad [1].$$

Where t_1 to t_2 is a time interval, and W_1 and W_2 are dry weight values for each time point. Units were g g^{-1} d^{-1}.

3.2.7 Establishment

At the time of cessation of culm elongation growth plantlets were removed from twenty plants. From each plant a plantlet from the distal end of the paracladial zone, and a single plantlet from the proximal end were removed; providing twenty plantlets from each position. Ten plantlets from each position were placed immediately in labelled sealed envelopes and dried in an oven at 60 °C for 24 h, and dry weights taken. The remaining ten plantlets from each position were 'sown' (pedicel down) in seed trays containing silver sand, and covered with a sheet of glass to prevent excessive water loss. Trays were placed in an alpine greenhouse (maximum temperature 21.9 ± 0.9 °C, minimum 8.8 ± 0.4 °C averaged over the course of the experiment, with natural illumination of approximately 16 h duration) and watered on alternate days with distilled water. After 30 d of growth all plantlets were removed from the sand, washed and placed in sealed-labelled envelopes. These were then dried as previously. In addition to dry weights the number of live plantlets was recorded in order to give an indication of mortality.

Also, six distal and six proximal plantlets were divided between three petri dishes. Petri dishes each contained ten layers of Whatman No. 1 filter paper (8.5 cm diameter), moistened with distilled water on which plantlets were placed each with their basal end in contact with the filter paper (modified from Harmer & Lee 1978b).

Petri dishes were placed in a growth room at 15 °C with a PPFD of 450 µmol m^{-2} s^{-1} PAR for 20 d with further distilled water added on alternate days using a wash bottle. On the emergence of roots, the oldest root was marked with a spot of red indelible marker ink. Every third or fourth day the number of roots was counted and the length of the oldest root measured with a plastic rule.

3.3 Results

3.3.1 Determination of prolification

Examples of plantlets of four ages (2, 14, 20 and 45 d from exertion) taken from the distal end of the paracladial zone can be seen in Fig. 3.2., with detail of a plantlet from 20 d from paracladial exertion shown in Fig. 3.3, and represented diagrammatically in Fig. 3.4. Plantlets arose via prolification, with true leaves being borne on the rachilla axis (Fig. 3.3, Fig. 3.4). Two florets were consistently present, the proximal floret being fertile and possessing dehiscing anthers and stigma, and the distal floret being sterile and with an extended lemma. The elongation of all other parts of this floret was a frequent occurrence, although not observed in every case. The plantlets dehisced along the rachilla at a point distal to the glumes and proximal to the first floret, which was shed with the plantlet.

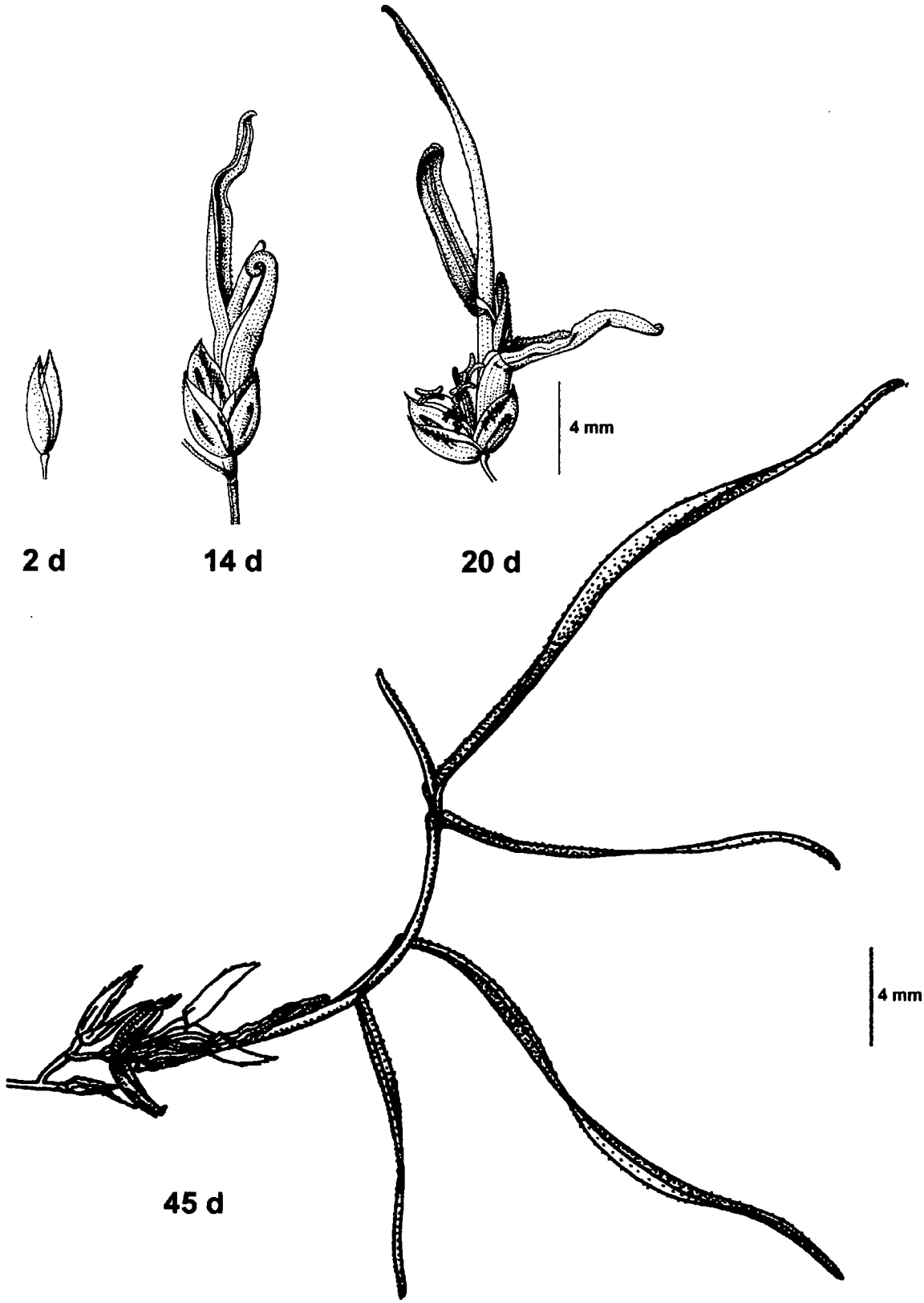


Fig. 3.2. Typical prolificated plantlets of various ages (days from paracladial exertion) from the distal end of the paracladial zone of *Poa alpina*.

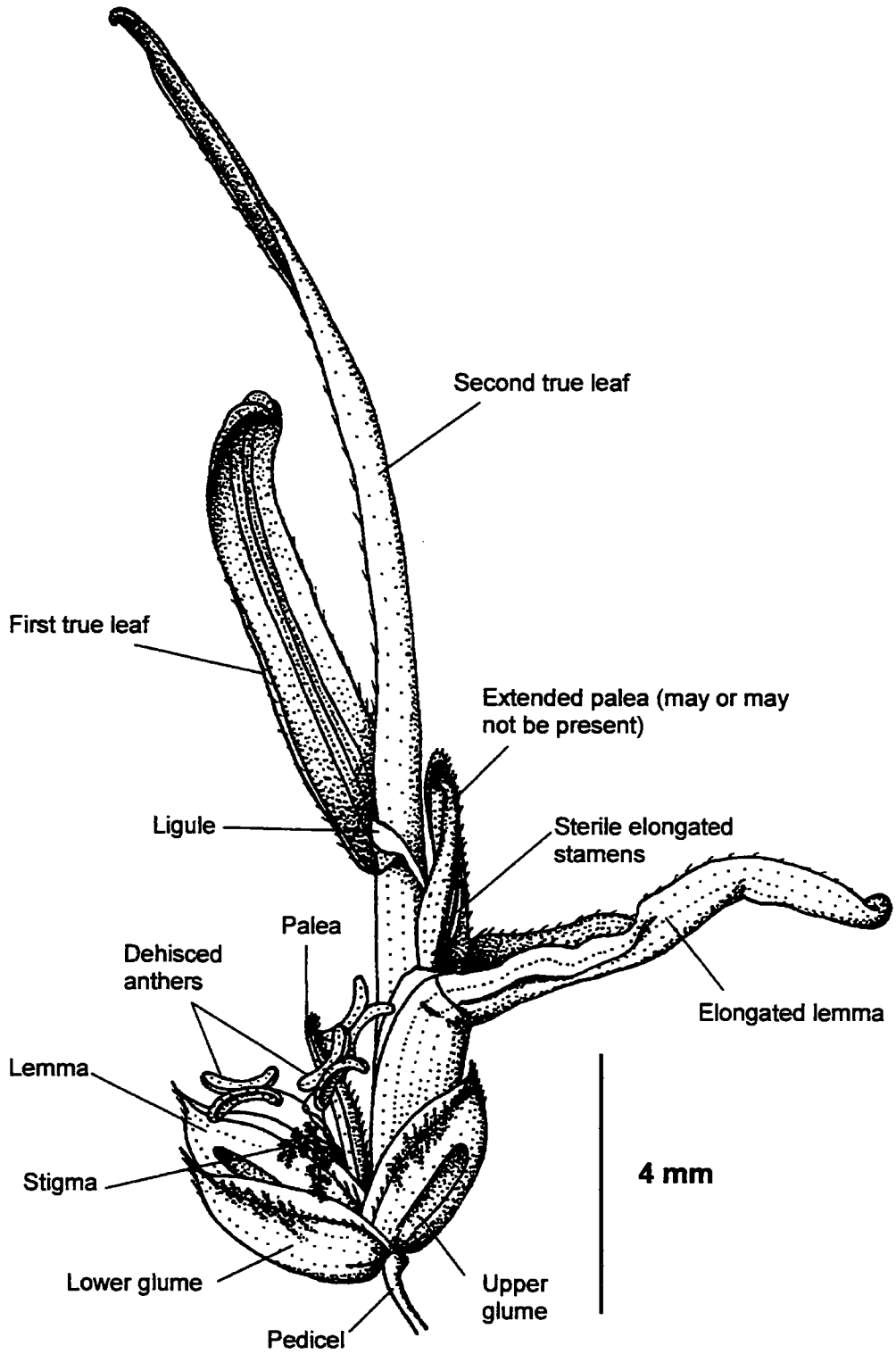


Fig. 3.3. Detail of a typical prolificated plantlet from the distal end of the paracladial zone, 20 d after paracladial exertion.

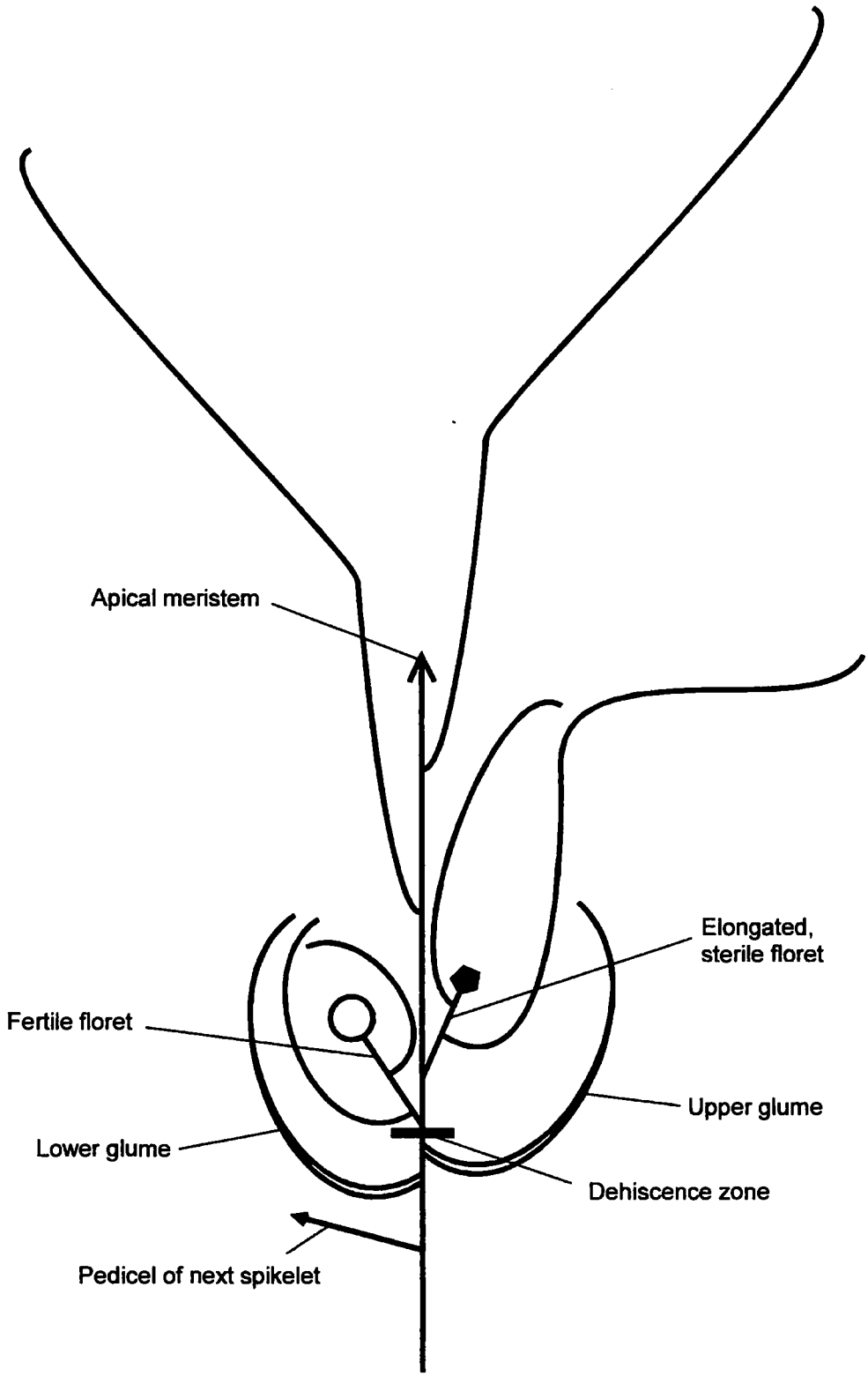


Fig. 3.4. A schematic diagram of a plantlet of the biotype of *Poa alpina* used in this study, showing the position of the dehiscence zone, the fertile floret, the sterile floret and the indeterminate axis.

3.3.2 Architecture of the paracladial zone

An example of the architecture of the paracladial zone is given in Fig. 3.5, with the remaining five replicates shown in Appendix 3. The main axis was hapaxanthic (terminating with the reproductive structures), and paracladia were ultimately monotelic (terminating in a spikelet), i.e. it was a 'closed system' (Cámara-Hernández & Rua 1991). Two paracladia were borne on each node of the rachis. Paracladia were arranged in a sympodial fashion, but were not completely monochasial (i.e. with unbranched chains of units) or dichasial (branching), but a combination of both. In the proximal long paracladia an initial dichasial unit gave rise to either more dichasial units or to monochasial units usually comprised of three or less spikelets. The distal short paracladia were composed of monochasial units of three or less spikelets, with gradation in paracladial structure acropetally along the rachis. There were rare exceptions to this description, with a single rachis node on one replicate bearing four paracladia, and the occasional pleichasial (multiple branching) unit in place of a dichasial unit. Rarely monochasial units were comprised of four spikelets, but never more in the replicates examined. Paracladia were observed to revert to their original un-spread position after the cessation of culm elongation growth.

The proportion of the total number of spikelets borne on each node decreased acropetally along the rachis, with 48.6 ± 1.2 % of spikelets being borne on the proximal two rachis nodes (Fig. 3.6b), defining these two nodes as the proximal half of the paracladial zone by spikelet number. There was a strong exponential relationship between rachis node number and the number of spikelets borne on the node; the number of spikelets decreasing towards the distal end of the rachis (Fig. 3.6; $r^2 = 0.91$, $P \leq 0.001$). An equivalent relationship was seen between internode number and the length of the internode ($r^2 = 0.84$, $P \leq 0.001$), with internodes becoming shorter with distance acropetally along the rachis (Fig. 3.7).

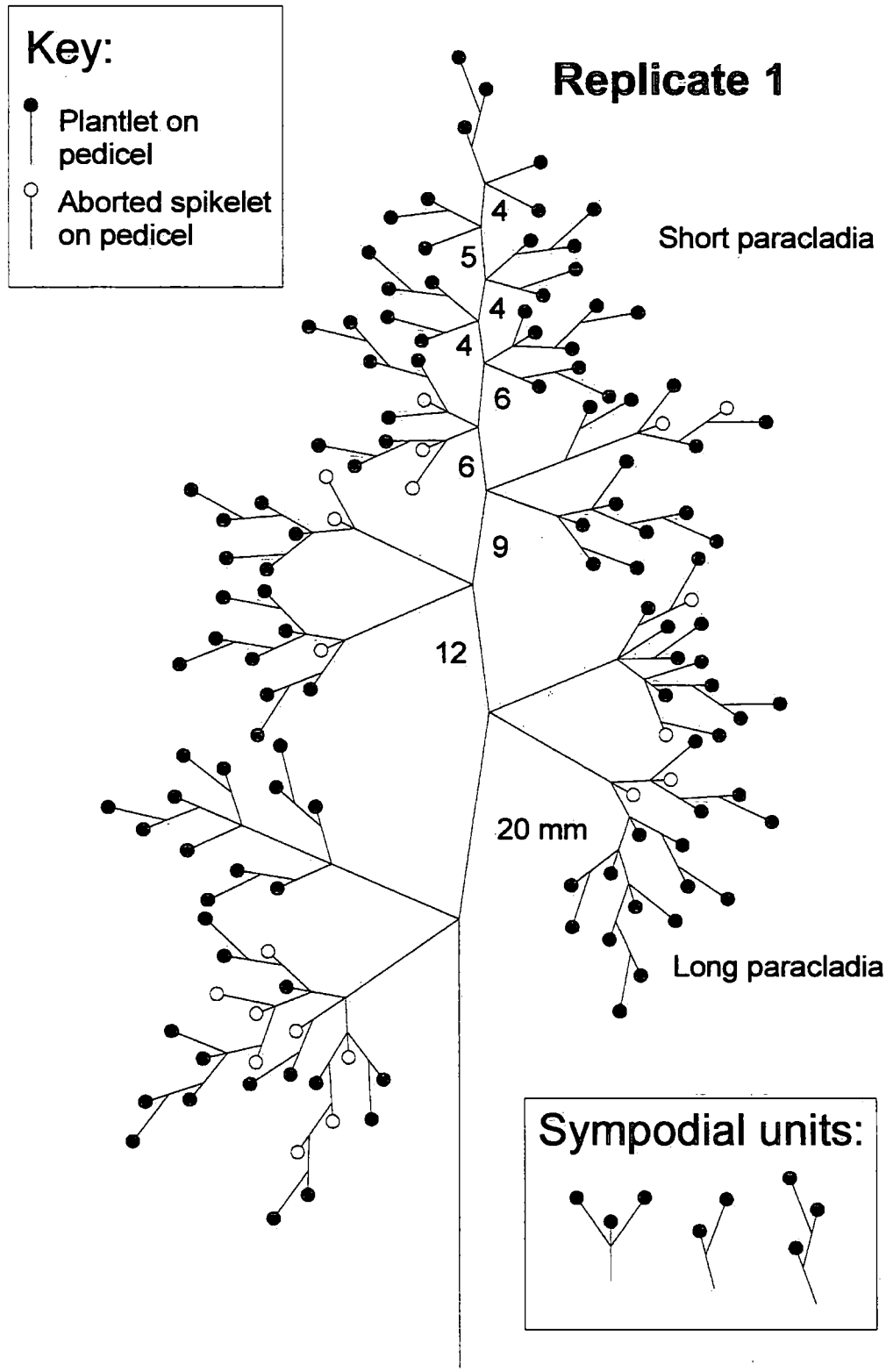


Fig. 3.5. Diagrammatic representation of the paracladial zone of the biotype of *Poa alpina* used in this study. The five other replicates used in this study can be found in Appendix 3. Paracladia possessed monochasial units supported on dichasial units, and the synflorescence was monotelic. Numbers refer to the length of rachis internodes (mm).

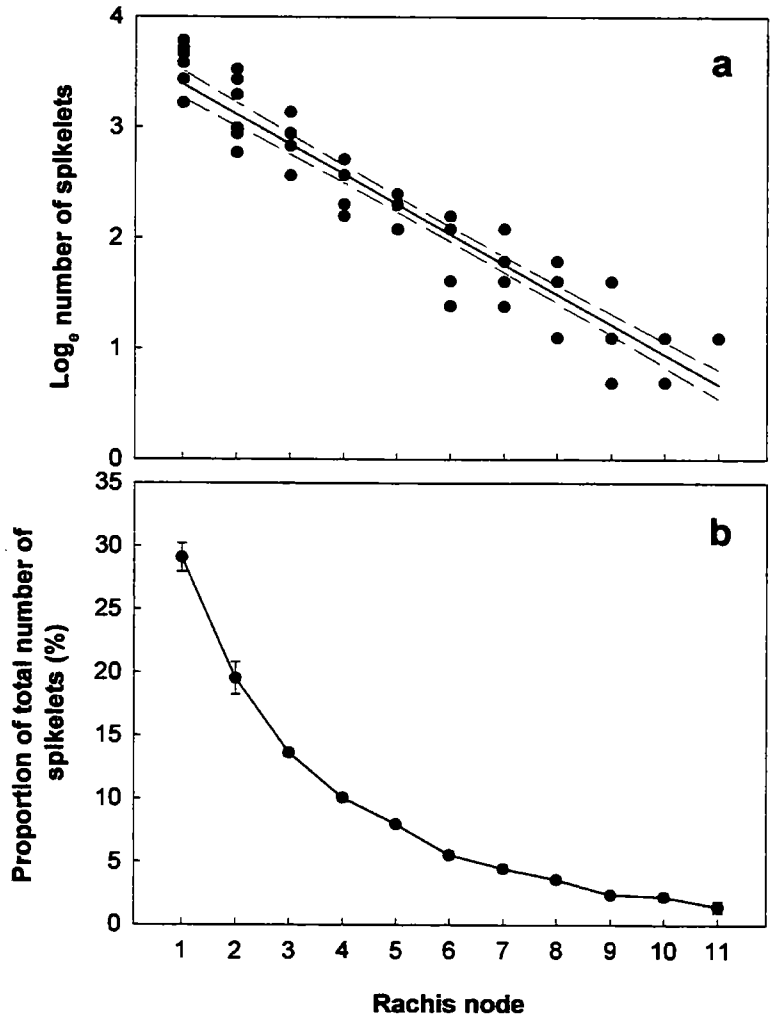


Fig. 3.6. The distribution of spikelets within the paracladial zone. **(a)** The number of spikelets borne on each rachis node (data $\log_e$ transformed) ($r^2 = 0.91$, $b = -0.27$, $P \leq 0.001$; dashed lines represent the 95 % confidence interval) and **(b)** the proportion of spikelets borne on each rachis node expressed as a percentage of the total number of spikelets in the paracladial zone. Rachis node one was the proximal node. Data represent the mean ± one S.E. of six replicates.

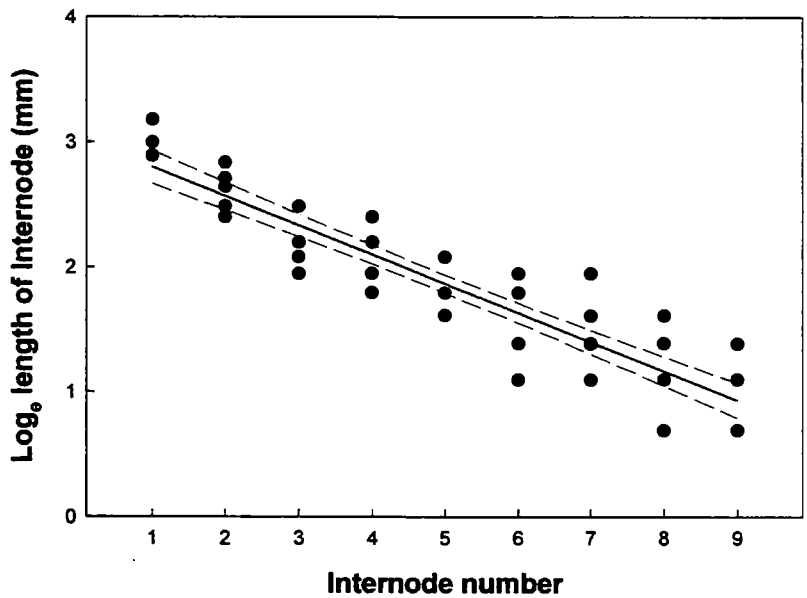


Fig. 3.7. Internode lengths along the rachis of the paracladial zone. Internode one was the proximal internode. Data represent absolute values $\log_e$ transformed from six replicates. $r^2 = 0.84$, $b = -0.23$, $P \leq 0.001$; dashed lines represent the 95 % confidence interval.

3.3.3 Spikelet abortion

A number of spikelets were observed to be aborted – i.e. did not possess florets or plantlets. These were represented by either a pair of green glumes (identical in external appearance to a young spikelet in which florets were not yet visible), a pair of withered brown glumes, or by a withered mass. The proximal node possessed both the highest absolute number (Fig. 3.8a) and proportion (Fig. 3.8b) of aborted spikelets.

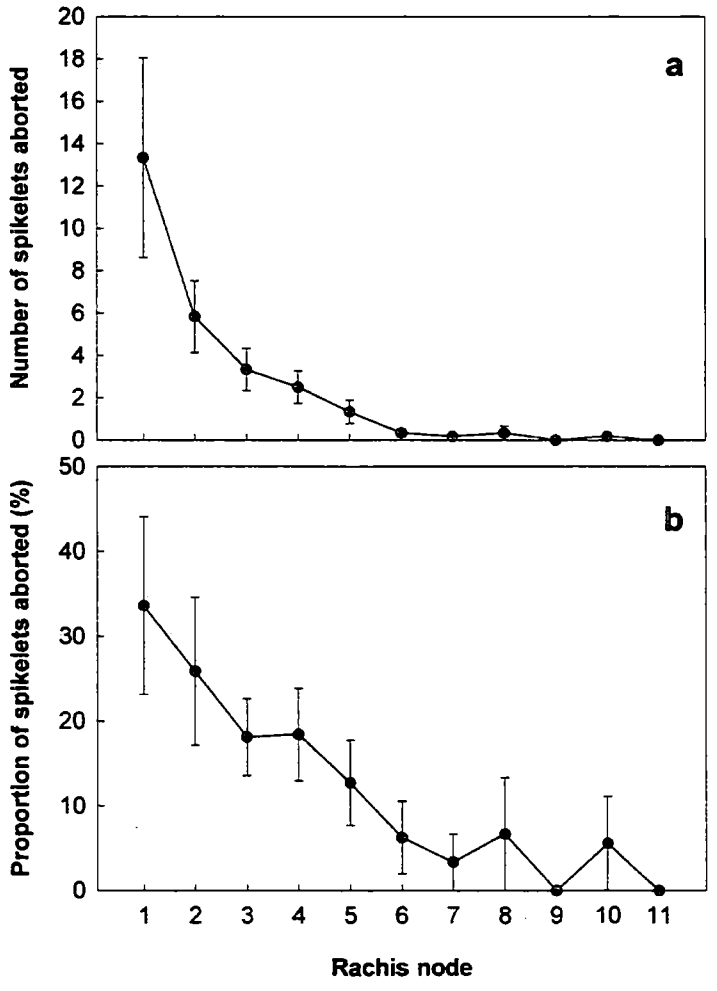


Fig. 3.8. The distribution of aborted spikelets within the paracladial zone. **(a)** The number and **(b)** the proportion of aborted spikelets borne on paracladia at each rachis node (1 = proximal). Data represent the mean ± one S.E. of six replicates.

3.3.4 Gas exchange within the paracladial zone

Net photosynthetic rates were 3 - 4 times respiratory rates ($P \leq 0.001$; Fig. 3.9, Table 3.1), with the difference between these rates increasing over time ($P = 0.018$).

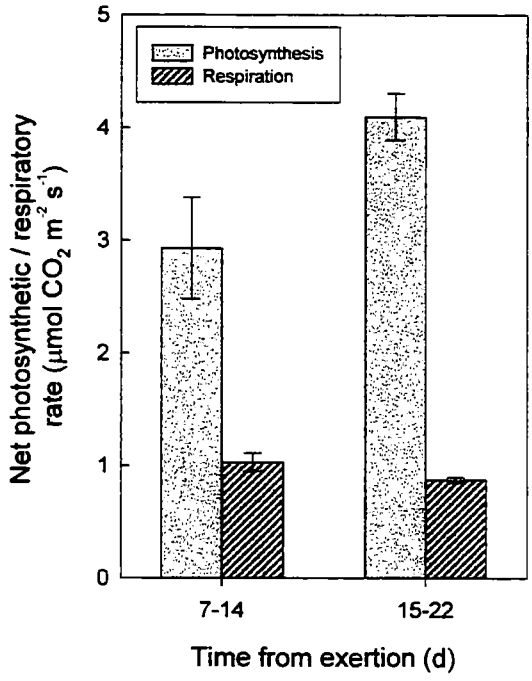


Fig. 3.9. Net photosynthetic (solid bars) and respiratory (hatched bars) rates of the paracladial zone at two and three weeks from exertion. Data represent the mean ± one S.E. of five replicates.

Table 3.1. Balanced two-way ANOVA on the net photosynthetic and respiratory rates of the paracladial zone at two and three weeks from exertion. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Time	n.s.
A/Resp.	***
A/Resp. x Time	*

3.3.5 Carbon fixation and allocation

Un-fed control plants from the laboratory contained a total of 2.2 ± 0.4 Bq above background, resulting in a ^{14}C content of 0.02 ± 0.005 Bq mg^{-1} DW. When the first leaf on the main axis was pulse-fed, 83 % of the ^{14}C fixed was retained in the leaf after a two-hour chase (Fig. 3.10a). Of the remaining ^{14}C, 3.1 % was exported acropetally and 13.9 % was exported basipetally (Fig. 3.10a). When the paracladial zone was pulse-fed, 95.5 % of ^{14}C was retained in the paracladial zone, with the majority of the exported ^{14}C being found in the culm after two hours (Fig. 3.10b). The ^{14}C content of three portions of the fed paracladial zone ranged from 55 to 58 Bq mg^{-1} DW and were not significantly different from each other at the $P \leq 0.05$ level (Fig. 3.11). When a monochasial unit of the mid portion was pulse-fed, 90.5 % of ^{14}C was retained after two hours, with 5 % of exported ^{14}C being shared equally between the remaining portions of the paracladial zone, and the remaining ^{14}C leaving the paracladial zone (Fig. 3.12).

The ^{14}C content of plants pulse-fed via the paracladial zone (4.4 ± 0.6 kBq per plant) was approximately four times that of plants fed via either the first leaf (1.2 ± 0.1 kBq per plant) or the culm (Fig. 3.13a). Per unit dry weight of the fed organ, the amount of ^{14}C fixed by the first leaf was not significantly different (at the $P \leq 0.05$ level) from the amount fixed per unit DW of the paracladial zone (46.2 ± 6.6 Bq fixed per unit fed leaf DW, and 59.6 ± 4.3 Bq fixed per unit paracladial DW; Fig. 3.13b) – i.e. the paracladial zone fixed more ^{14}C due to a larger 'fixing mass'. The culm fixed significantly less (8.1 ± 1.3 Bq fixed per unit culm DW). At 40 d from paracladial exertion a small amount (2.7 ± 1.0 Bq mg^{-1} DW; 7.3 %) of ^{14}C was exported from the fed first leaf basipetally into the pseudostem via proximal culm internodes (Fig. 3.14).

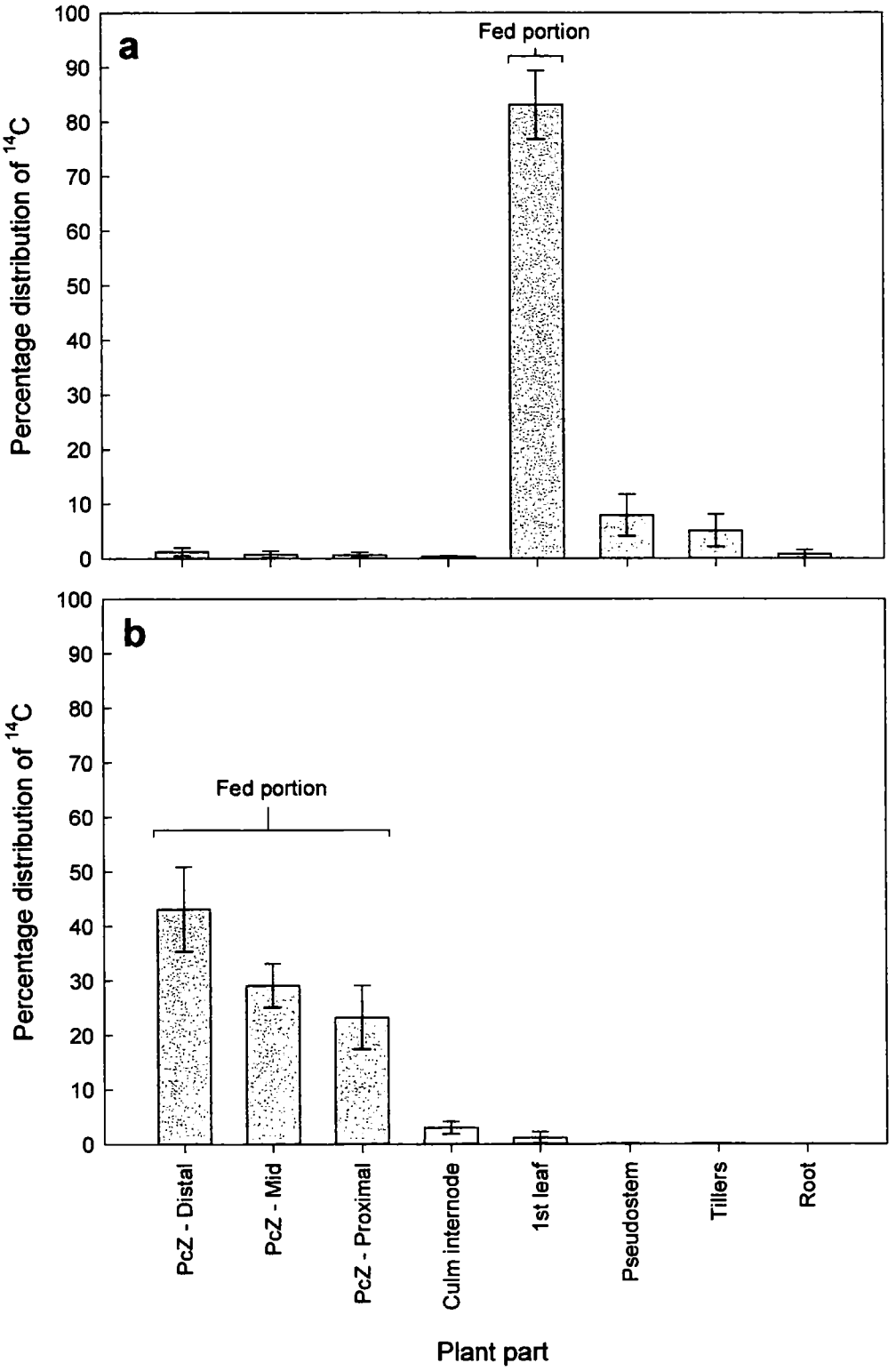


Fig. 3.10. The percentage distribution between plant parts of ^{14}C when **(a)** the first leaf and **(b)** the entire paracladial zone were pulse-fed. Data represent the mean ± one S.E. of eight replicates. PcZ: Paracladial zone.

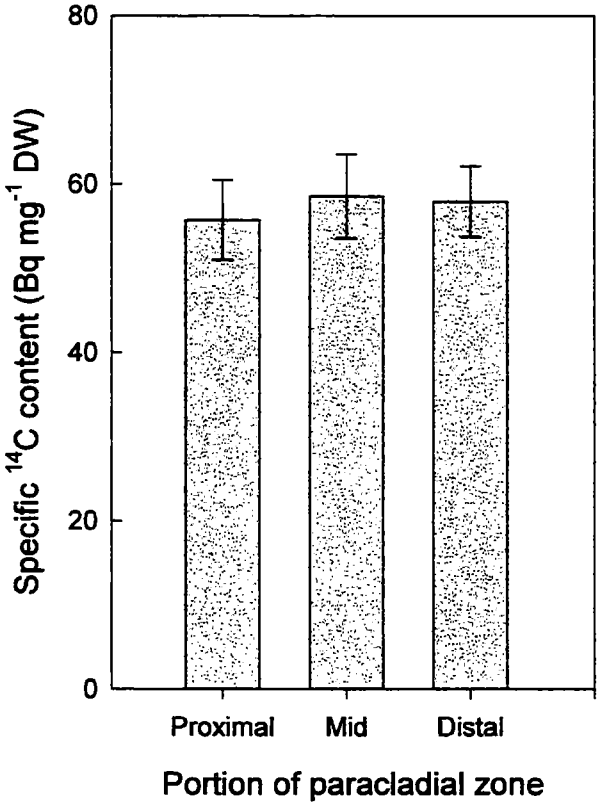


Fig. 3.11. ^{14}C content of different regions of the paracladial zone when the entire paracladial zone was pulse-fed. Data represent the mean ± one S.E. of eight replicates.

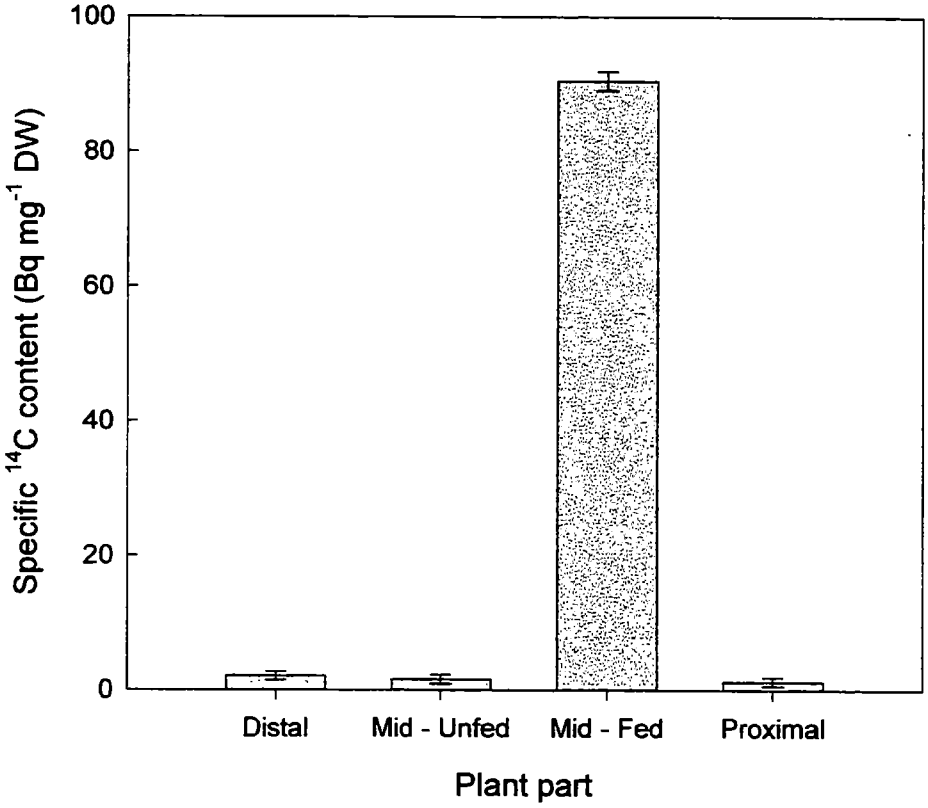


Fig. 3.12. The ^{14}C content of the paracladial zone when a monochasial unit in the mid portion was pulse fed. Data represent the mean ± one S.E. of seven replicates.

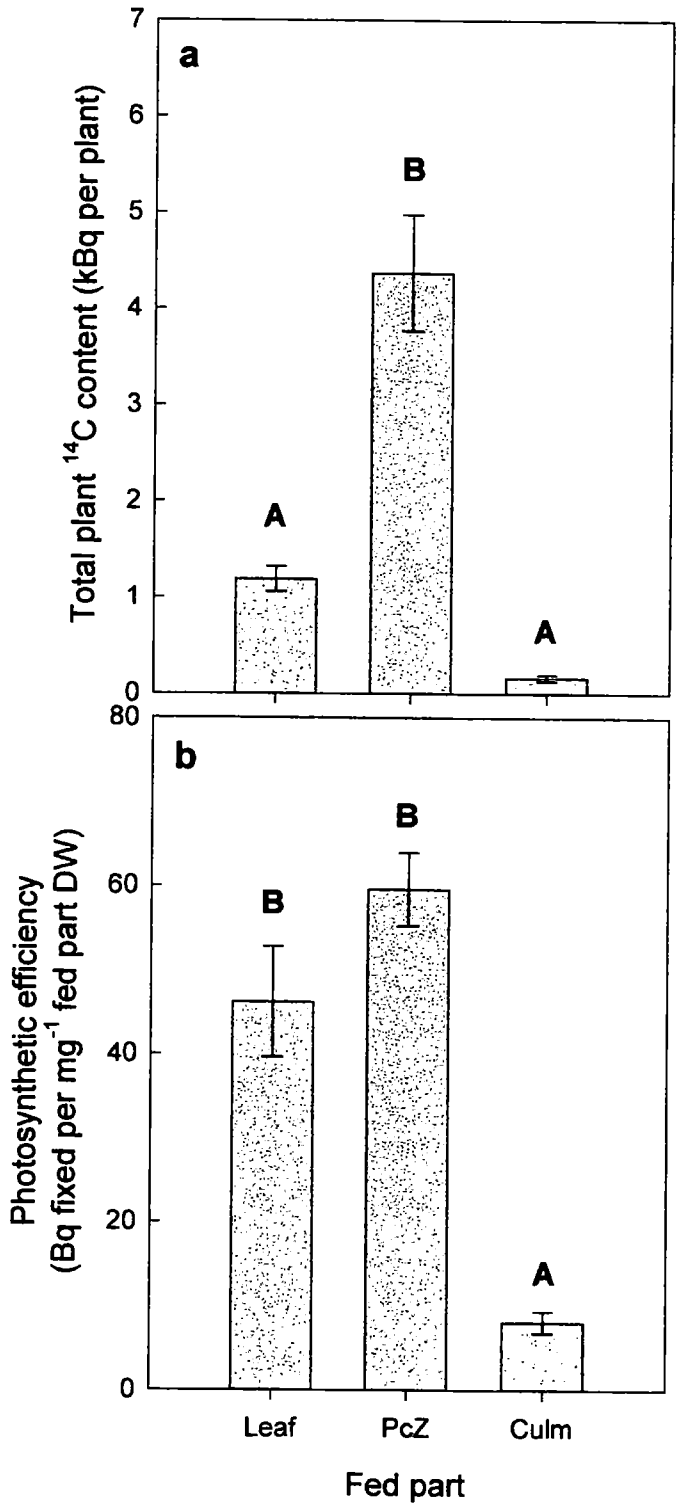


Fig. 3.13. The effects of feeding ^{14}C to the first leaf, paracladial zone and culm on **(a)** total plant ^{14}C content and **(b)** photosynthetic efficiency (total amount of ^{14}C fixed per unit fed organ dry weight) of photosynthetic organs after pulse-chasing. Data represent the mean ± one S.E. of six replicates. Different letters represent significant difference between fed-part means at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure (ANOVA). PcZ: Paracladial zone.

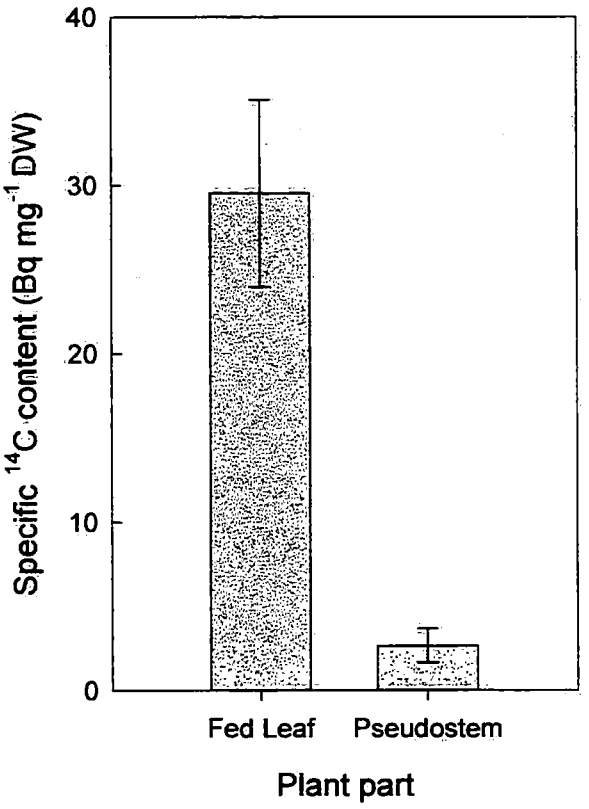


Fig. 3.14. ^{14}C content of the pseudostem after pulse-feeding the first leaf at 40 d after paracladial exertion. Data represent the mean ± one S.E. of five replicates.

3.3.6 Manipulation of sinks

In control plants, distal plantlets attained a higher dry weight than proximal plantlets (Fig. 3.15a), with an arithmetically, but not significantly, higher relative growth rate (Fig. 3.16a; $P > 0.05$). Plantlets from both distal and proximal regions possessed significantly lower RGRs over time (Fig. 3.16a; $P \leq 0.05$). Plants in which the proximal plantlets were removed showed no difference from control plants with respect to dry weight gain or RGR of distal plantlets (Fig. 3.15 and Fig. 3.16 respectively). In plants where the distal plantlets were removed, proximal plantlets maintained a high RGR (Fig. 3.16b) and after 21 d had attained the same dry weight as distal plantlets (Fig. 3.15a). Manipulation did not result in a significant increase in the amount of ^{14}C imported into either half of the paracladial zone from the pulse-fed first leaf after a two hour chase period (Fig. 3.17).

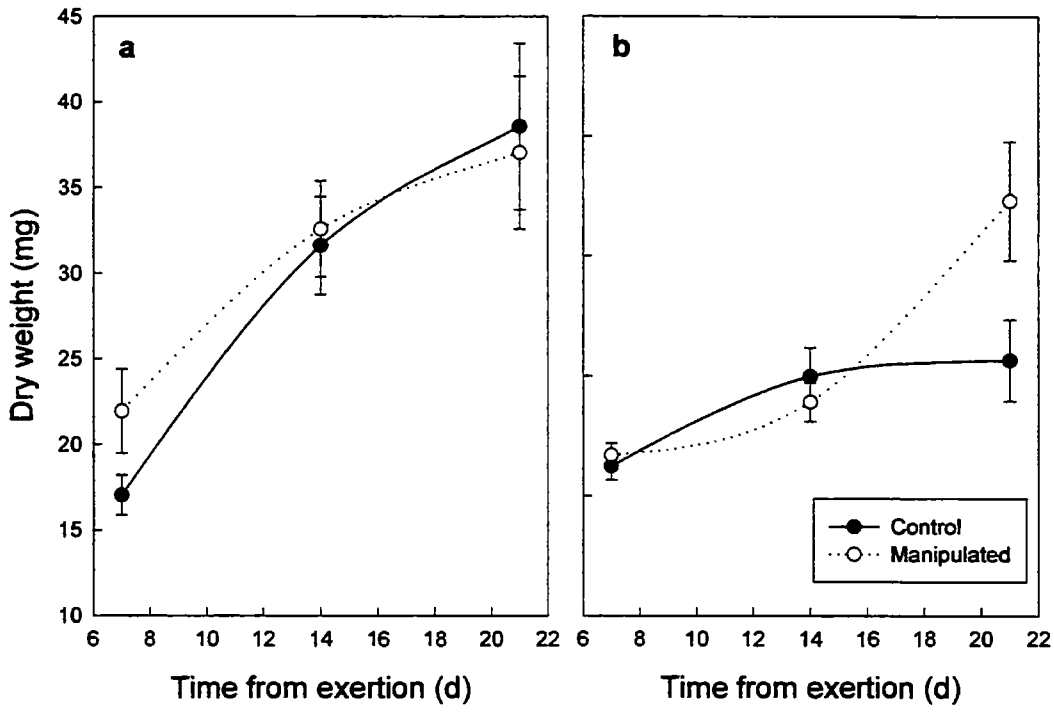


Fig. 3.15. The absolute growth (mg DW basis) of **(a)** distal and **(b)** proximal plantlets from either control (intact paracladial zone; solid line) or manipulated (distal or proximal half of paracladial zone removed; dotted line) plants. Data represent the mean ± one S.E. of ten replicates.

Table 3.2. Balanced two-way ANOVA on the absolute growth of distal and proximal plantlets from either control (intact paracladial zone) or manipulated (distal or proximal half of paracladial zone removed) plants. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Position	***
Manipulation	n.s.
Time	***
Position x Manipulation	n.s.
Position x Time	n.s.
Manipulation x Time	n.s.
Position x Manipulation x Time	*

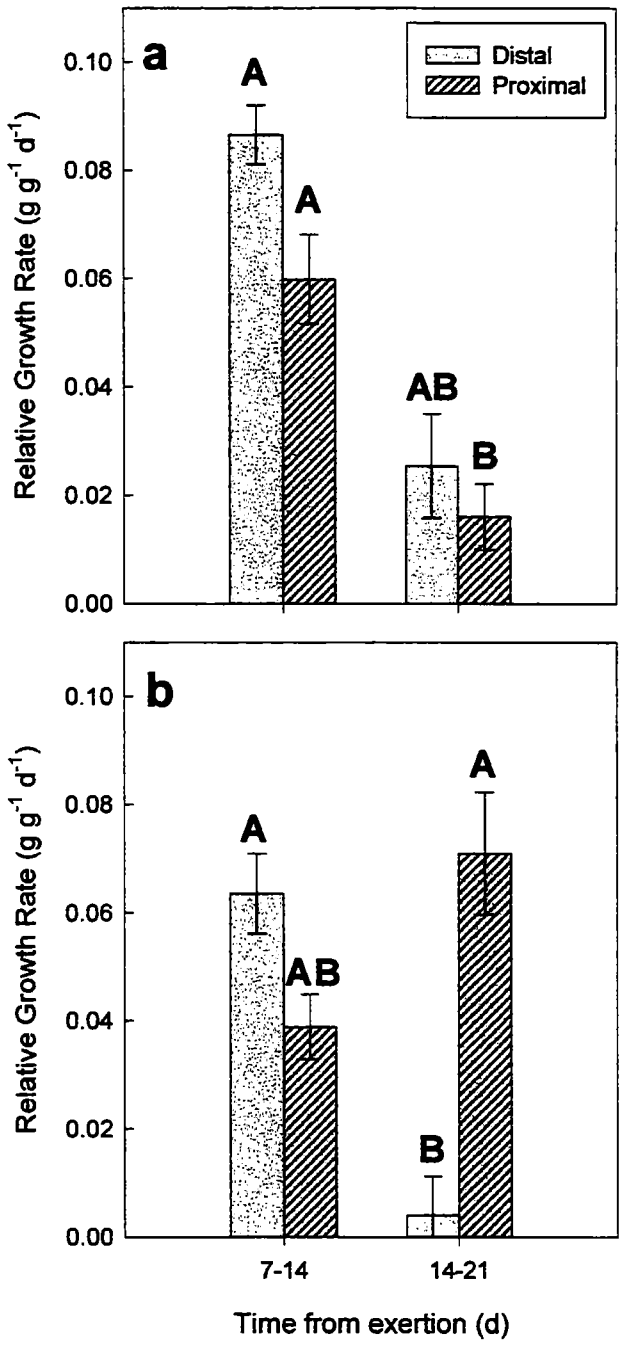


Fig. 3.16. The relative growth rate (RGR) of plantlets from distal (solid bars) and proximal (hatched bars) positions within the paracladial zone; **(a)** control plants and **(b)** manipulated plants. Data represent the mean ± one S.E. of ten replicates. Different letters indicate significant difference between means at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure (ANOVA).

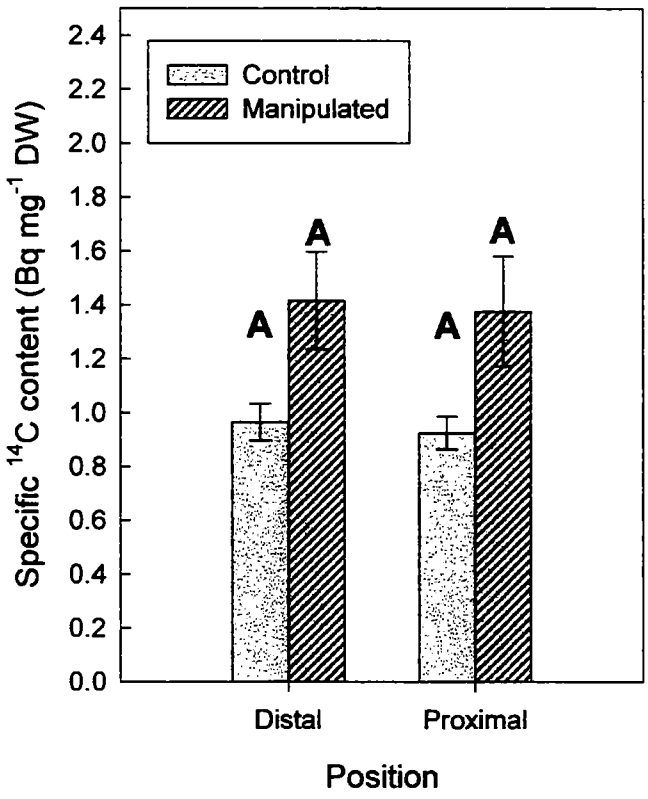


Fig. 3.17. Specific ^{14}C content of distal and proximal halves of the paracladial zone after pulse-feeding the first leaf. The paracladial zone was either intact (control) or manipulated by removal of all spikelets in the reciprocal half of the paracladial zone. Data represent the mean ± one S.E. of five replicates. Different letters indicate significant difference between treatment or position means at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure (ANOVA).

3.3.7 Plantlet establishment post dehiscence

Adventitious roots arose extra-vaginally from the base of plantlets (Plate 3.2). The number of roots produced by distal plantlets was not significantly different from the number produced by proximal plantlets (Fig. 3.18a). However, the length of the oldest root produced by distal plantlets was arithmetically, and at three time points significantly (at the $P \leq 0.05$ level), greater than that of proximal plantlets (Fig. 3.18b; Table 3.2). The final dry weight of these plantlets was 5.3 ± 0.4 mg (distal) and 3.1 ± 0.5 mg (proximal), which was significantly different ($P \leq 0.001$) as determined by Student's t-test. The relative growth rate of distal plantlets (0.042 ± 0.004 g g^{-1} d^{-1}) on establishment in sand culture was over ten times that of proximal plantlets (0.004 ± 0.002 g g^{-1} d^{-1}, Fig. 3.19, $P \leq 0.001$). Also, proximal plantlets possessed a mortality rate of 30 % over this 30 d period, with no distal plantlets dying.

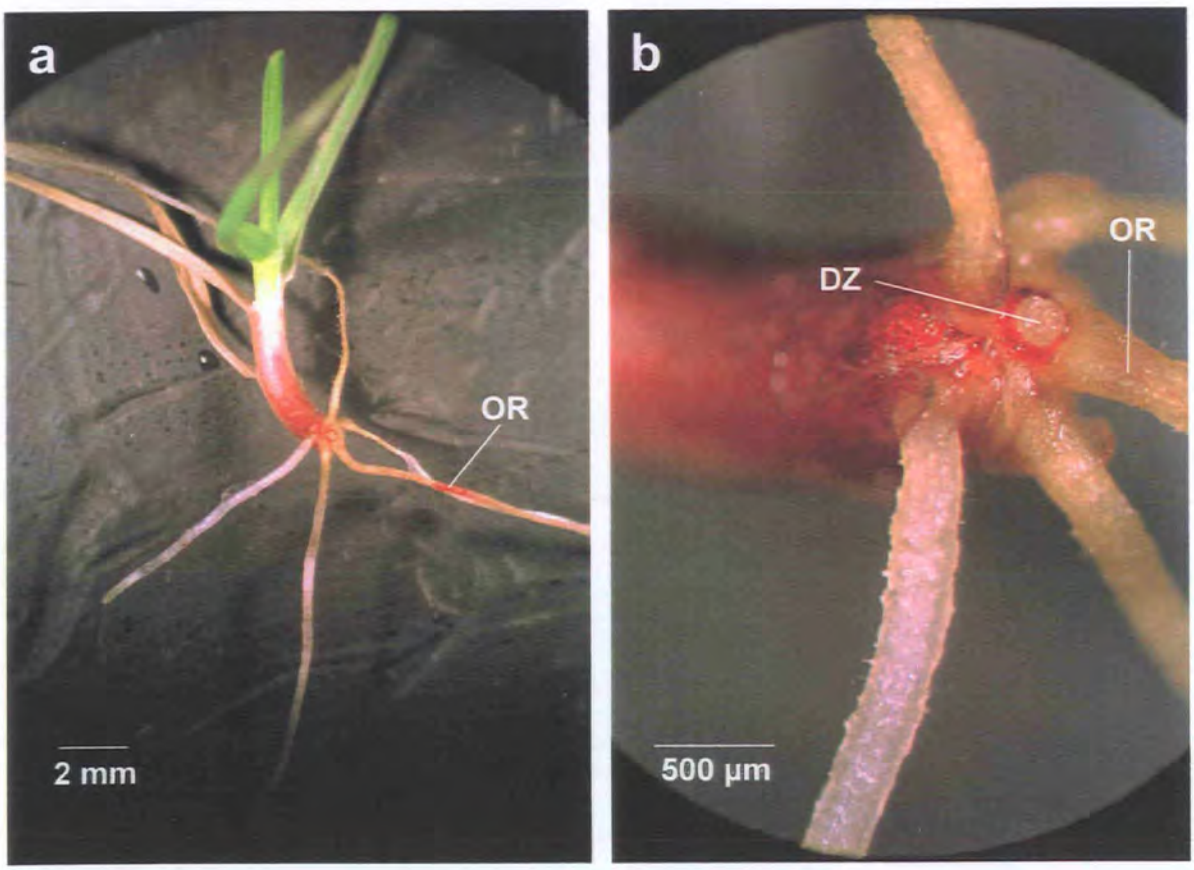


Plate 3.2. The production of roots by plantlets **(a)** a plantlet from the distal end of the paracladial zone and **(b)** a closer view of the base of the same plantlet showing adventitious roots arising extra-vaginally. Note the presence of senesced leaves on plantlet. DZ: Dehiscence zone. OR: Oldest root.

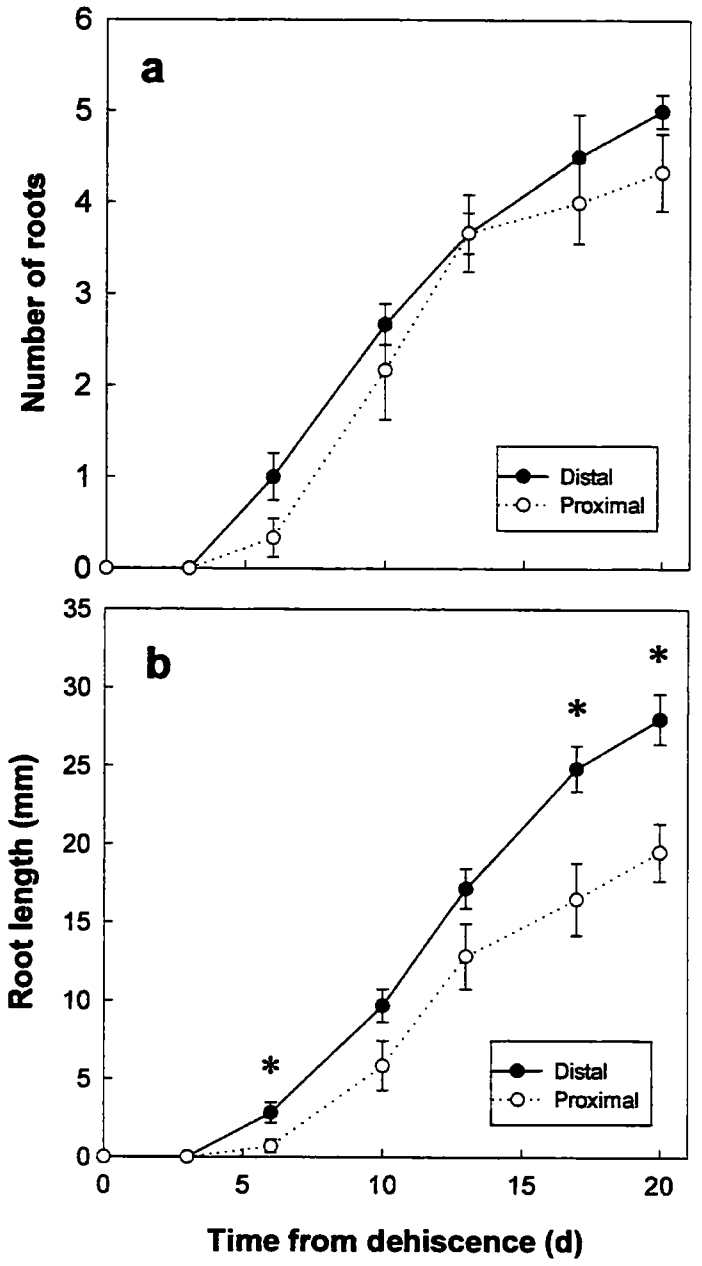


Fig. 3.18. The growth of plantlet roots; **(a)** plantlet root number and **(b)** length of the oldest root after dehiscence of plantlets from either end of the paracladial zone. Data represent the mean ± one S.E. of six replicates. * indicates significant difference between distal and proximal means (at each time point) at the $P \leq 0.05$ level determined by Students t-test.

Table 3.3. Balanced two-way ANOVA on the length of the oldest root after dehiscence of plantlets from either end of the paracladial zone. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Time	***
Position	***
Position x Time	**

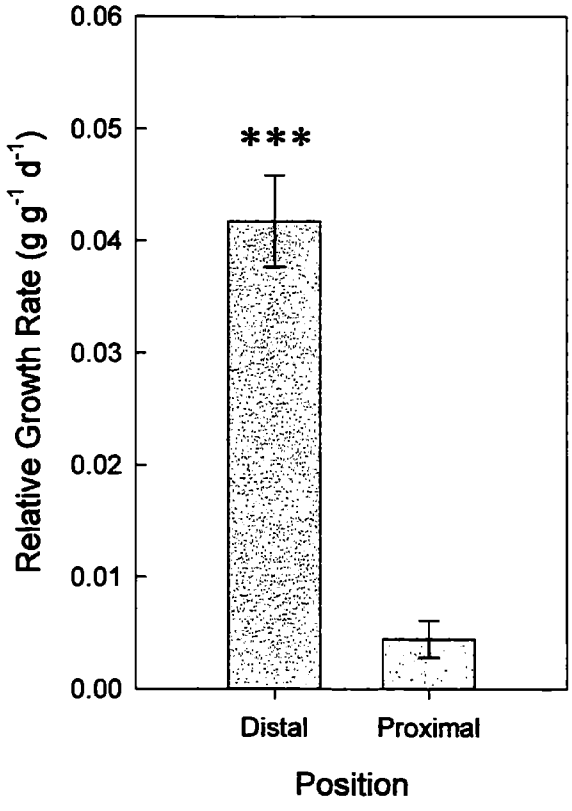


Fig. 3.19. Relative growth rate of plantlets over a thirty day period after dehiscence. Plantlets were originally from either distal or proximal positions within the paracladial zone. Data represent the mean ± one S.E. of seven replicates (due to the death of three replicates from the proximal position). *** indicates significance between position means at the $P \leq 0.001$ level determined by Student's t-test.

3.4 Discussion

Gas exchange data disclose that the carbon requirements of plantlets could be met by photosynthesis within the plantlets themselves. Indeed, the paracladial zone was able to fix nearly four times the amount of ^{14}C as the first leaf at 20 d from exertion, due to the greater dry weight present in the paracladial zone, and was not a major sink for photoassimilate from the first leaf. Although pulse-feeding or gas exchange measurements were not carried out on the entire paracladial zone before 7 d from exertion (due to the lack of exertion of proximal paracladia) it is likely that spikelets began fixing a substantial amount of carbon as soon as they emerged, as net photosynthetic rates exceeded respiratory rates three-fold at least as early as the second week after exertion, and spikelet glumes were green on emergence. Similarly, Lee and Harmer (1980) noted photosynthetic activity in young plantlets of *Festuca vivipara*. However, the development of a fully expanded lemma and true leaf (at approximately 14 - 20 d in distal plantlets) would presumably increase photosynthetic efficiency by increasing the surface area to volume ratio of plantlets, possibly explaining the increase of net photosynthetic rate relative to respiratory rate over time.

The architectural study of the paracladial zone allowed a precise delimitation of its two halves by spikelet number, as 49 % of plantlets were borne on paracladia originating from the proximal two nodes of the rachis. Carbon fixation within the paracladial zone at 20 d was heterogeneous, with more carbon being fixed by the distal half by virtue of the higher dry weight of plantlets in this position. Thus the lower dry weight and higher frequencies of abortion in plantlets from the proximal end resulted in a region in which less carbon was fixed - despite the photosynthetic activity of these plantlets matching that of the distal plantlets. Very little carbon was shown to move between regions of the paracladial zone, which was consistent with plantlets from all regions being sources of carbon.

Clearly the heterogeneity observed in the present study, with proximal plantlets possessing lower relative growth rates (both attached to the parent and also on establishment) and being less likely to establish, is unlikely to be explained purely as a limitation of growth by a lack of carbon, as all plantlets were carbon sources. The data presented in the present chapter do not in themselves rule out competition for nutrients as a cause of paracladial heterogeneity. The unchanged RGR of distal plantlets on the surgical removal of proximal plantlets, rather than indicating a lack of competition, could indicate that the growth of distal plantlets was not limited by

nutrient availability. To this end the effect of nutrient availability on paracladial heterogeneity is examined in more detail in Chapter 4.

The relative lack of translocation of ^{14}C to the roots from either the first leaf or the paracladial zone detected in this study appears to be due to the short chase period employed. For example, although Rattray *et al.* (1995) detected the presence of ^{14}C in the rhizosphere only 30 minutes after pulse-feeding *Lolium perenne* tillers, this was maximal after 3 h. Thus, in the present study, a clearer picture of ^{14}C movement within the whole plant is likely to have emerged had the chase period been longer than it was. However, a primary aim of this study was to investigate carbon fixation by the paracladial zone, and the short chase period used allowed the pattern of ^{14}C fixation within the paracladial zone to be readily discerned; this might not have been the case had the majority of ^{14}C been exported. Also, respiratory loss and re-fixation of ^{14}C was minimised (Atkinson & Farrar 1983; Farrar 1993). The data do show that the paracladial zone as a whole was a source organ, a small amount (4.5 %) of the fixed ^{14}C being exported after two hours. This is in contrast to the first leaf which exported 17 %, mainly basipetally, indicating that the paracladial zone was not in itself as important a source of carbon for parental sinks as the first leaf. In seminiferous *Poa annua* the first leaf mainly allocated ^{14}C acropetally to the developing caryopses, with only older leaves supplying roots and developing tillers (Ong & Marshall 1975). Therefore the data support the hypothesis that photosynthesis in the paracladial zone results in a source-sink hierarchy in which the leaves on the main axis (particularly the most active first leaf; see Chapter 2) provide photoassimilate exclusively for roots and developing tillers rather than for propagules (a situation proposed by Lee & Harmer; 1980).

When the first leaf was pulse-fed ^{14}C at 40 d from paracladial exertion a small amount of ^{14}C was detected basipetally in the proximal portion of the culm and pseudostem after 2 h (2.7 ± 1.0 Bq mg^{-1} DW or 7.3 % of the ^{14}C fixed, *c.f.* 13.9 % at 20 d). Thus the movement of ^{14}C via the culm at 40 d was reduced at this advanced stage of senescence. It is possible therefore that the decline in relative growth rate of both halves of the paracladial zone over time was a result of carbohydrate accumulation and concomitant feedback-inhibition of photosynthesis and growth, a subject investigated and discussed in Chapter 4, although increasing water stress could also account for the decline in relative growth rate. Although the culm was unlikely to fix ^{14}C itself at this late stage (40 d) due to the degradation of chloroplasts (Plate 2.6), at 20 d the culm was confirmed to fix ^{14}C (Fig. 3.13). Despite having a

relatively low activity compared to the first leaf and paracladial zone (Fig. 3.13) again, its value may be in imposing less on source organs by being relatively self-sufficient in terms of carbon. Thus the efficiency of phloem transport is less likely to be detracted from, as the energy required for active processes in the phloem (such as maintenance of membrane integrity) is less likely to be sequestered from the phloem sap.

In conclusion, the paracladial zone of a pseudoviviparous biotype of *Poa alpina* was a source of ^{14}C that was as efficient as the youngest leaf on the axis, capable not only of providing for it's own carbon requirements but also of exporting photoassimilate (supporting hypothesis 1, that paracladia are carbon sources). Distal plantlets fixed a greater amount of ^{14}C due to their larger dry weights. However, as all plantlets were sources of carbon, little movement of ^{14}C between paracladia occurred and hypothesis 3 - that physiologically older paracladia are sources for younger paracladia - was rejected. Not only was carbon exported from the paracladial zone, but carbon allocation from the youngest leaf on the main axis was mainly basipetal; a source of carbon for parental sinks not available to seminiferous grasses, in which the younger leaves allocate carbon mainly acropetally to the paracladial zone. This also provides support for hypothesis 5, that paracladia do not compete for parental photoassimilate. The culm too was able to fix a small amount of ^{14}C (supporting hypothesis 6); potentially making this organ more efficient as a transport system for carbon.

Heterogeneity was observed within the paracladial zone, with distal plantlets possessed higher relative growth rates and hence higher dry weights, resulting - at dehiscence - in more rapid root growth and a higher likelihood of establishment and survival than proximal plantlets (supporting hypothesis 2, that developmental heterogeneity exists and affects the establishment of plantlets from different regions of the paracladial zone). This heterogeneity was not a result of a lack of carbon within the system, as all organs were carbon sources. Further heterogeneity was noted within the paracladial zone with regard to paracladial architecture and the degree of spikelet abortion, which was greater in the proximal region. This aspect of the paracladial zone will be investigated in Chapter 5, with the effect of altered nutrient and atmospheric carbon dioxide availability on the system coming under scrutiny in Chapter 4.

4 The response of the synflorescence to resource availability

4.1 Introduction

The data presented in Chapter 3 indicate that the growth of plantlets in the proximal region of the paracladial zone was limited, but not by a lack of carbon. However, these data do not preclude nutrient availability as a potential limitation to the development of the pseudoviviparous synflorescence system. Also, as is explained in more detail below, an excess of carbon in concert with low nutrient availability may impose potentially greater limitations on growth than low nutrient availability alone (Baxter *et al.* 1997).

Habitually pseudoviviparous grasses are often found in nutrient-poor Arctic-Alpine environments (see Sections 1.3.1 and 1.3.2). In the case of *Poa alpina*, as in many other C_3 plants, sources of limitation to vegetative growth include not only the availability of inorganic nutrients, but also atmospheric carbon dioxide concentration (Baxter *et al.* 1997). Due to industrialisation and burning of organic fuels, ambient atmospheric carbon dioxide concentrations have risen from nineteenth century values of 270 μmol mol^{-1} (Gammon *et al.* 1985) to values of 350 μmol mol^{-1} in 1988 (DOE 1988), and are predicted to rise to 530 μmol mol^{-1} by the year 2050, and to 700 μmol mol^{-1} by the year 2100 (Watson *et al.* 1990). An investigation into the role of CO_2 concentration and nutrient availability on the growth of the synflorescence would provide insight into the physiology of this system not only in terms of the observed heterogeneity, but also the response of the system to a changing environment.

Stitt (1991) reports that elevated concentrations of atmospheric CO_2 increase the rate of carboxylation[1] in C_3 plants, as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) has a low affinity for CO_2, and at present atmospheric CO_2 concentrations Rubisco is limited by this substrate (but not by ribulose-1,5-bisphosphate (Ru1,5bisP), for which Rubisco has a high affinity). Also, under elevated CO_2 conditions, more CO_2 is present to compete against O_2 for the active site of the enzyme, increasing the frequency of carboxylase reactions relative to oxygenase reactions and thus decreasing the proportion of carbon lost to photorespiration (Bazzaz 1990; Stitt 1991). Hence many plants show an initial increase in relative

[1] See Lawlor (1993) for a summary of photosynthetic metabolism.

growth rate and carbohydrate content on exposure to elevated CO_2, as does *Poa alpina* (Baxter *et al.* 1994a, 1995). However, in many plants, including *Poa alpina* (Baxter *et al.* 1994a), this initial increase in growth is not sustained; photosynthetic capacity and growth rates declining as photosynthesis becomes acclimated to the elevated atmospheric CO_2 concentration.

Photosynthetic acclimation occurs as the result of a number of processes. Extra photoassimilate, produced as a result of the initial increased photosynthetic rate, may directly repress the transcription of genes controlling photosynthetic metabolism (van Oosten *et al.* 1994, 1996, 1997). Also, after carbon has been incorporated into photosynthetic metabolism, Ru1,5bisP must be regenerated by enzymes participating in the photosynthetic carbon reduction (PCR) cycle, and if this does not occur at a high enough rate (due to lack of nitrogen or P_i) then Rubisco will become substrate-limited with respect to Ru1,5bisP. Photosynthetic rates will then decline to a point where the regeneration of Ru1,5bisP is once again in equilibrium with its rate of use (Stitt 1991). Also, if excessive amounts of substrate cannot be processed rapidly enough, then P_i will be locked up in the form of phosphorylated intermediate compounds, limiting photophosphorylation in the longer-term (Stitt 1991).

Acclimation of photosynthetic capacity is also thought to be exacerbated by changes in plant source-sink relations. Sink-strength (i.e. the requirement of meristematic/storage tissues for photoassimilate) can be defined as a function of the physical size of the sink and its metabolic activity (Warren-Wilson 1967; Farrar 1993b). Thus nutrient limitation of metabolism, with a concomitant limitation of sink size, limits the strength of sinks and results in decreased flux between source and sink tissues. The continued production of carbohydrates in source tissues results in the accumulation of carbohydrate locally – further promoting the down-regulation of photosynthetic gene-transcription. Thus feedback inhibition of photosynthetic capacity in elevated CO_2 is often greatest in nutrient-limiting conditions (Arp 1991).

There is evidence that nutrient availability mediates the acclimatory response of *Poa alpina* – vegetative plants grown in elevated CO_2 and nutrient-deficient conditions attain a lower dry weight, lower instantaneous net photosynthetic rates and decreased root N and P concentrations (on both a total and structural dry weight basis) compared to plants grown in ambient CO_2 and/or nutrient-sufficient conditions (Baxter *et al.* 1995, 1997). In seminiferous plants, overall sink-strength may increase on flowering (Arp 1991), with seeds and fruit providing additional sink tissue.

However, the synflorescence of *Poa alpina* is a source of carbohydrate (Chapter 3, this study), and is less likely to relieve feedback inhibition. Also, as plantlets are vegetative shoots it is possible that plantlets and tillers of *Poa alpina* exhibit similar physiological responses to elevated CO_2 and low nutrient availability; the investigation of which is a primary aim of this chapter.

The physiological responses of plants to elevated CO_2 and nutrient availability confer profound ecological implications. Studies conducted on a wide range of plant species show a diverse range of responses; for example Baxter *et al.* (1994a) determined that *Festuca vivipara* showed a decline in dry weight as opposed to a dry weight gain exhibited by *Agrostis capillaris* plants in elevated CO_2 (when supplied with 0.2 mol m^{-3} N and 0.05 mol m^{-3} P). Due to the specificity of the response, this aspect of climate change has the potential to alter the pattern of competition between species. Also, Tissue & Oechel (1987) found that the sedge *Eriophorum vaginatum* invested dry matter into a greater number of tillers rather than increasing the productivity of existing tillers. This was also the case for *Poa alpina* which, under elevated CO_2 (when provided with 0.2 mol m^{-3} N) produced on average five more tillers in mature plants (Baxter *et al.* 1994a – or no extra tillers in this treatment in a further study, Baxter *et al.* 1997). A higher nutrient concentration (2.5 mol m^{-3} N) resulted in a 3.5 – 4-fold increase in tillering, with elevated CO_2 at this nutrient level resulting in 11 more tillers than at ambient (Baxter *et al.* 1997). As each tiller has the potential to produce a synflorescence, this behaviour may be regarded as increasing the reproductive potential of the plant. However, whether the increased potential to produce pseudoviviparous synflorescences actually translates into increased numbers of exerted synflorescences or increased reproductive dry weight is not currently known; an investigation of this aspect of growth is a further aim of this chapter.

In addition to the potential for reproduction, the timing of this key event in the life-cycle may also be affected by CO_2 and nutrient availability. Elevated CO_2 affects the initiation, rate of development and longevity of flowers in many species (Garbutt & Bazzaz 1984), with increased soluble carbohydrate content acting directly as a stimulus for floral initiation, although this species-specific response may be mediated by nutrient availability (Lang 1965). Changing patterns of flowering in elevated CO_2 conditions may have a particularly significant impact on community structure, particularly where the growing season is short (Bazzaz 1990). In a pseudoviviparous system - in which asexual reproduction is derived from a seminiferous reproductive system - reproduction must still be initiated and occurs late in the growth season.

Thus the developmental response to elevated CO_2 and nutrient availability may impose time constraints on growth in habitats with a short growing season.

Aside from CO_2 and nutrient affects on growth, the number of spikelets is influenced by a variety of environmental factors including temperature, daylength, and the age of the shoot (i.e. the number of supporting leaves). In *Hordeum vulgare* L. (Garcia del Moral *et al.* 1991), *Pennisetum americanum* (L.) Leeke (Coldrake & Pearson 1985a&b) and *Phleum pratense* L. (Ryle 1963) these factors determine the size of the apical dome during panicle initiation, and hence spikelet number. Thus treatments which promote growth may well be expected to increase the number of spikelets evident in the paracladial zone. As both spikelet number and the number of flowering tillers are key components of the reproductive response (Seneweera & Conroy 1997), these two aspects of development demand investigation. Thus the phenology of flowering and the growth of plantlets were predicted to respond to nutrient availability and atmospheric CO_2 concentration, and these aspects of reproductive growth were investigated by addressing the following hypotheses:

That:

1. the development of pseudoviviparous plantlets shows an identical response to nutrient availability and atmospheric CO_2 concentration as vegetative tillers, with elevated CO_2 in low nutrient conditions resulting in feedback inhibition of photosynthetic capacity and ultimately decreased dry matter production,
2. the availability of nutrients alters the pattern of heterogeneity within the paracladial zone, with lessening of nutrient limitation promoting homogeneity,
3. resource availability determines reproductive dry matter production via both modification of the number of tillers undergoing reproductive development, and the number and dry weight of plantlets produced,
4. the availability of nutrients and atmospheric CO_2 concentration alters the developmental timing of synflorescence production.

4.2 Methods

4.2.1 Plant cultivation and treatment application

Plantlets were initially raised in trays of sand using one-fifth strength Long Ashton nutrient solution (LA), until they possessed eight or nine fully expanded leaves, and were then transferred to the Institute of Terrestrial Ecology Solardome facility (Solardome Industries Ltd., Southampton, Hampshire, UK) at Abergwygregyn, Gwynedd (Plate 4.1). This facility has previously been described in detail by Stirling *et al.* (1997), with the environmental control and monitoring systems detailed in Rafarel *et al.* (1995). Treatments consisted of ambient atmospheric CO_2 (tracking local ambient of approximately 350 μmol mol^{-1} CO_2) and elevated CO_2 (ambient CO_2 concentration plus 340 μmol mol^{-1} CO_2 at any point in time). Within each dome pots were placed in two grid patterns, with each grid receiving either one-fifth strength LA or full-strength LA. This was carefully applied to pots early in the morning, twice a week, and was added to pots until it drained through entirely. Plants were watered twice daily at 6 am and 6 pm using an automated sprinkler system. Following vegetative growth pseudostems were tagged with the date of paracladial exertion, and the length of the culm measured every second day after exertion.

Plate 4.1. The Solardome facility, Institute of Terrestrial Ecology, Bangor Research Station.

4.2.2 Harvests

Plants in each grid were randomly pre-assigned to particular harvests when originally transferred to the Solardomes. Two groups of harvests were conducted, one following the developmental stage of paracladial exertion, and the other following the cessation of culm elongation growth. Harvests conducted after the cessation of culm elongation growth began from two days after this developmental stage (i.e. when a lack of extension could first be detected), and at ten and twenty days from the cessation of culm elongation growth. Harvests directly following paracladial exertion were conducted at zero, ten and twenty days from this event – i.e. before the cessation of culm elongation growth. At all harvests six plantlets were removed from the distal half of the main axis paracladial zone, and six were removed from the proximal half. The remaining paracladial zone of the main axis was removed and divided into green and senescent non-green portions, as were the groups of distal or proximal plantlets. Leaf blades of all tillers including the main axis were removed at the ligule, with necrosed and senescent leaves separated. Areas of all leaf blade and green paracladial zone tissues were taken, with leaf blade material requiring sub-sampling as measurement of the area of all laminae proved impractical. Daughter tiller pseudostems were removed, and their paracladial zones also separated from culms. Roots were also removed and washed, with the sand being sieved to recover fine root material. All plant parts were placed separately in labelled paper bags, sealed, and dried for 48 h at 60 °C in a forced-air oven. Dry weights were then taken.

4.2.3 Carbohydrate content

Dry plant tissue was first extracted in 4 ml of 96 % ethanol at 60 °C using a Dri-Block DB-3H heating block (Jencons Scientific, Leighton Buzzard, Beds., UK; after Farrar 1993a), and sample extracts stored at -20 °C in universal sample bottles until analysis. Following extraction in ethanol, sample material was extracted in 4 ml of water at 80 °C and stored in an identical manner. The remaining material was then solubilised in 0.2 M KOH, and for each millilitre of sample extract, 800 µl of 1N acetic acid (58 ml in a litre) was added to lower the pH back to a value of 4.5. Following this, 1 ml of enzyme solution (8 units ml^{-1} amyloglucosidase and 8 units ml^{-1} α-amylase) then added to each sample. Samples were then left to stand at 37 °C for five hours, after which time they were frozen at -20 °C. At a later date structural material was recovered and oven dried at 60 °C overnight for determination of dry weight.

Starch and soluble carbohydrate contents (combined water- and ethanol-soluble fractions) were determined using the phenol-sulphuric method (Dubois *et al.* 1956;

detailed in Farrar 1993a). 1 ml of sample was pipetted into a dry 9 ml capacity glass test-tube. 1 ml of 5 % (w/v) phenol was then added, followed by 5 ml of analytical grade concentrated sulphuric acid (H_2SO_4). This was then mixed using a glass rod. After cooling to room temperature the absorbance was read at 485 nm using enzyme solution in 0.2 M KOH as a blank for starch, and 50 % (v/v) ethanol as a blank for soluble carbohydrate. Quartz cuvettes were used. Readings were calibrated with glucose at 0 – 100 μg ml^{-1}. Samples found to contain more than 100 μg ml^{-1} glucose equivalent were diluted with either buffered enzyme solution (starch) or 50 % (v/v) ethanol (soluble carbohydrate) before the addition of phenol and sulphuric acid as detailed above, and the absorbance determined a second time.

4.2.4 Nitrogen and phosphorus content of plant tissue

Dry plant material was digested using the Kjeldahl method (Allen 1974; also detailed in Hind 1993). A known dry weight of plant tissue (approximately 0.1 g – the minimum amount of dry plant matter that can be accurately determined via this method; Allen 1974) was digested with 2 ml sulphuric acid in a 50 ml long-necked kjeldahl flask. To this 1 g of catalyst (80 g K_2SO_4, 20 g $CuSO_4 \cdot 5H_2O$, ground to a powder in a mortar) was added, before refluxing for 8 h at 350 °C. After cooling, distilled water was used to wash the digest into a 50 ml volumetric flask, which was then made up to volume with more distilled water. Blank digests, consisting of reagents alone, were also run. Samples were then stored at 4 °C in polypropylene bottles prior to analysis for ammonium nitrogen and phosphate using a SAN^{PLUS} segmented flow autoanalyser (Skalar Analytical, Breda, The Netherlands).

The mean photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE) of both innovation and paracladial zones were calculated following the method of Baxter *et al.* (1994b). These were a function of the mean net assimilation rate (NAR, g m^{-2} d^{-1}), specific leaf area (SLA, m^2 g^{-1}) and mean leaf nitrogen or phosphorus concentration (LN{P}C, g N g^{-1} structural DW):

$$PN\{P\}UE = \frac{NAR}{(LN\{P\}C / SLA)} \qquad [1].$$

where units were g (mean structural dry weight) g^{-1} (tissue nitrogen or phosphorus) d^{-1}. Net assimilation rate was calculated thus:

$$NAR = \frac{(w_2 - w_1)}{(l_2 - l_1)} \times \frac{(\log_e l_2 - \log_e l_1)}{(t_2 - t_1)} \qquad [2].$$

where w_1 and w_2 = total innovation or paracladial zone dry weight (g) and l_1 and l_2 = total leaf blade or plantlet area (m^2) at times t_1 and t_2 (d) respectively.

4.2.5 Gas exchange

A Ciras 1 IRGA (PP systems, Hitchin, Hertfordshire, UK) was used to quantify gas exchange of both photosynthesis and dark respiration of the youngest fully expanded leaf of the main axis, 19 ± 3.4 d prior to paracladial exertion. Measurements were taken in a saturating photosynthetic photon flux density (PPFD) of 1300 µmol m^{-2} s^{-1} PAR and a temperature of 20 °C, from six replicate plants from all nutrient and CO_2 treatments. The cuvettes detailed in Section 3.2.4 were not available for gas exchange measurements of the paracladial zone.

In each nutrient treatment the net photosynthetic capacity of plants grown at ambient and elevated CO_2 was compared at the same internal CO_2 concentration using an index of acclimation (Stirling *et al.* 1997):

$$A_{cc}I = \frac{(A'_e - A_a)}{(A_e - A_a)} \qquad [3].$$

Where A'_e is the net photosynthetic rate per unit leaf area obtained from plants grown in 690 µmol mol^{-1} CO_2 (elevated) and measured at this same CO_2 concentration, A_a is the net photosynthetic rate obtained from plants grown and measured at 350 µmol mol^{-1} (ambient), and A_e the value obtained from plants grown at ambient CO_2 but measured at elevated CO_2 concentrations. An $A_{cc}I$ equal to 1 indicates that no photosynthetic acclimation has occurred; an $A_{cc}I < 1$ indicates a loss of photosynthetic capacity and an $A_{cc}I > 1$ indicates increased photosynthetic capacity.

4.3 Results

Noon temperature and daily photosynthetic photon flux density (PPFD) data from solardomes over the period of study are presented in Fig. 4.1. Data are incomplete due to failure of the automated recording systems on certain dates. Ambient temperature was tracked by all solardomes, with mean mid-day temperature averaging 17.6 °C over the May to August period and ranging between 5.2 and 26.5 °C (Fig. 4.1b). Mean mid-day PPFD in solardome three (ambient CO_2 concentration) averaged 732.7 μmol m^{-2} s^{-1} over the June to August period, and ranged between 190.5 and 1405.4 μmol m^{-2} s^{-1} over this period.

4.3.1 The innovation zone and vegetative response to resource availability

Examples of typical plants 19 ± 3.4 d prior to paracladial exertion are presented in Plate 4.2. Low nutrient availability resulted in lower whole plant dry weights (from 24 – 27 g at high nutrient availability, to 7 – 11 g at low nutrient availability) after the cessation of culm elongation growth (Fig. 4.2), although CO_2 treatments did not significantly affect whole plant dry weight in this study ($P \leq 0.05$). Elevated CO_2 and high nutrient treatments both independently and together increased the amount of senescent leaf blade material present in the innovation zone (Fig. 4.3, Table 4.1), with the amount of senescent material increasing over time in elevated CO_2 treatments. This resulted in a difference of 160 mg in the amount of senescent material at 20 d from cessation of culm elongation growth in the low nutrient treatment, and a difference of 212 mg in the high nutrient treatment ($P \leq 0.001$).

Elevated CO_2 resulted in acclimatory loss of photosynthetic capacity in both nutrient treatments (Table 4.2, Fig. 4.4), but plants grown with low nutrient availability had a significantly lower photosynthetic acclimation index (0.3 ± 0.1 *c.f.* 0.7 ± 0.08, $P \leq 0.01$; Fig. 4.4). Plants grown in elevated CO_2 showed no increase in total non-structural carbohydrate (TNC) content over time - as exhibited by plants grown in ambient CO_2 - ultimately resulting in no difference in TNC between treatments ($P \leq 0.05$; Table 4.3). Over time the tissue nitrogen concentration was increased in elevated CO_2 treatments, with phosphorus concentration being significantly decreased in high nutrient treatments ($P \leq 0.001$; Table 4.4). Nutrient and CO_2 treatments showed an interactive effect on both photosynthetic nitrogen and phosphorus use efficiencies, with elevated CO_2 decreasing PNUE and PPUE in low nutrient conditions (0.02 ± 0.02 and 0.06 ± 0.05 g g^{-1} d^{-1} respectively), and increasing PNUE and PPUE in high nutrient conditions (0.23 ± 0.2 and 0.64 ± 0.04 g g^{-1} d^{-1} respectively; $P \leq 0.001$; Table 4.5).

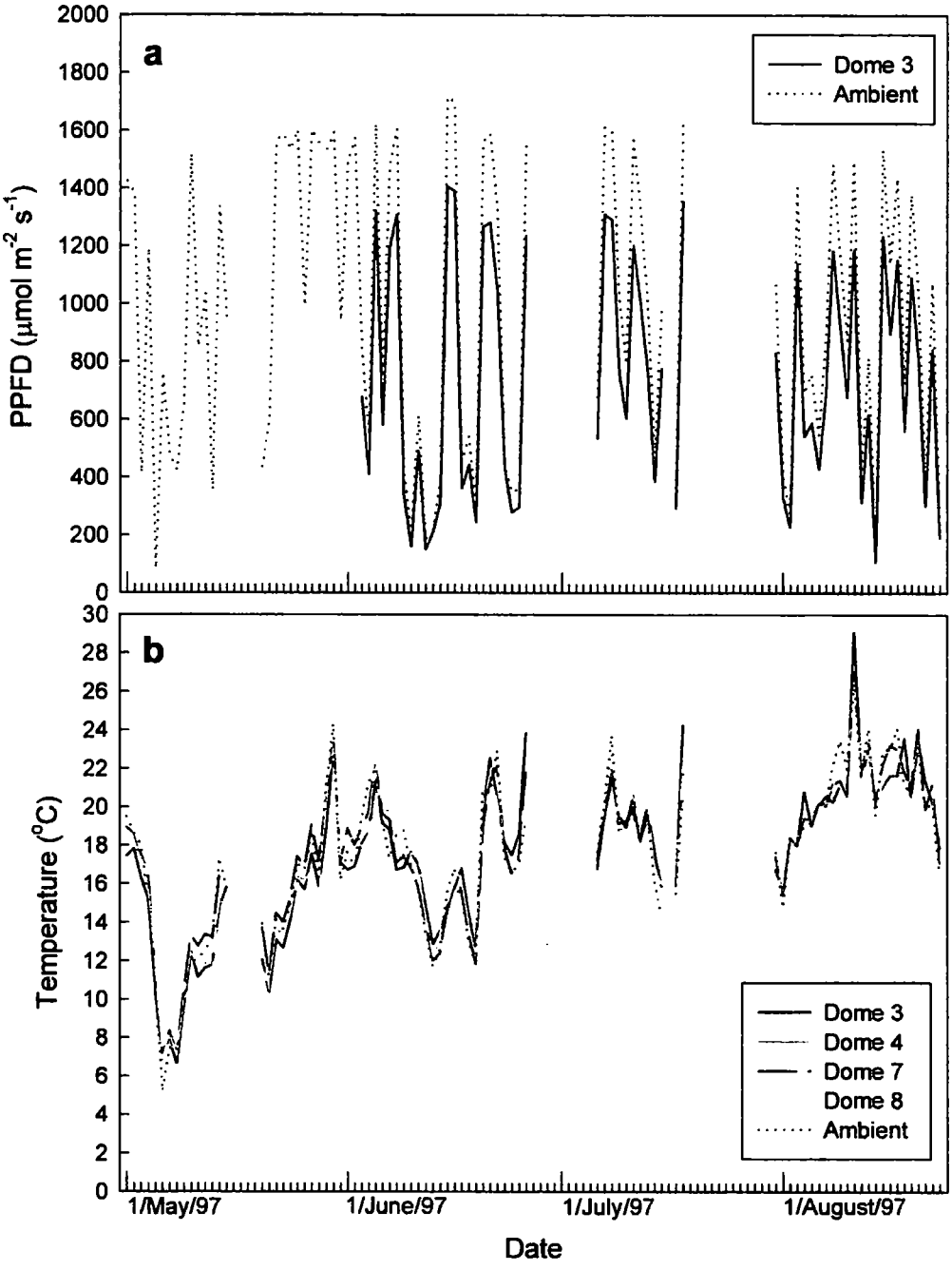


Fig. 4.1. Noon **(a)** photosynthetic photon flux density (µmol m^{-2} s^{-1}) and **(b)** temperature (°C) data from the solardomes over the period of study. Data represent mean mid-day values. Domes 3 & 7 = CO_2 concentration tracking ambient (approximately 350 µmol mol^{-1} CO_2), and Domes 4 & 8 = ambient plus 340 µmol mol^{-1} CO_2 (elevated). Ambient temperature and PPFD data represent environmental conditions outside the solardomes.

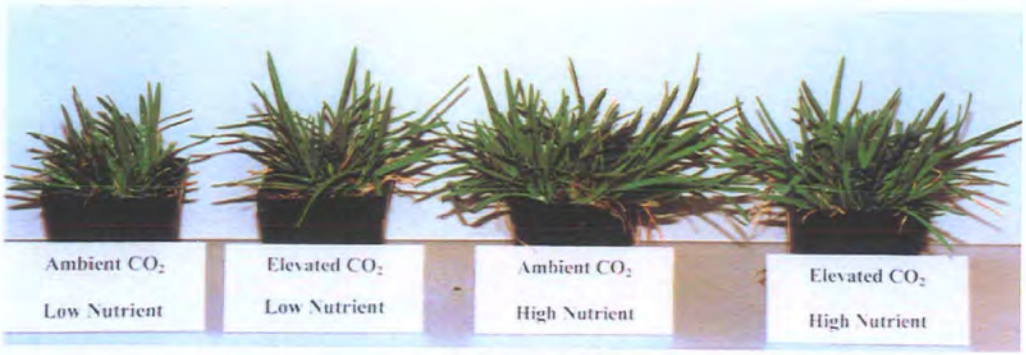


Plate 4.2. Typical *Poa alpina* plants 19 ± 3.4 d prior to paracladial exertion. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton (low) or full-strength Long Ashton (high). For scale, the width of the neck of each pot was 11 cm.

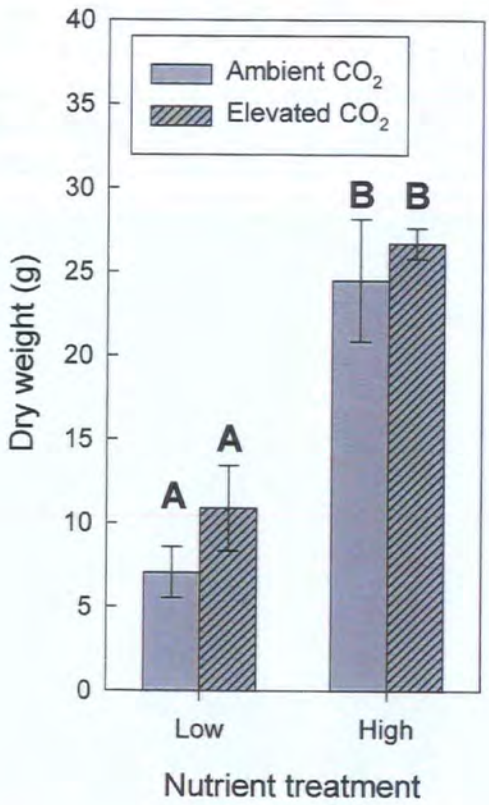


Fig. 4.2. Whole plant dry weight 2 d after cessation of culm elongation growth (after approximately three months of growth from a propagule to a mature plant) in *Poa alpina*. Plants were grown in one-fifth strength Long Ashton nutrient solution (low) and full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. Different letters indicate significance at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure (ANOVA). See Appendix 5 for full results of statistical analysis.

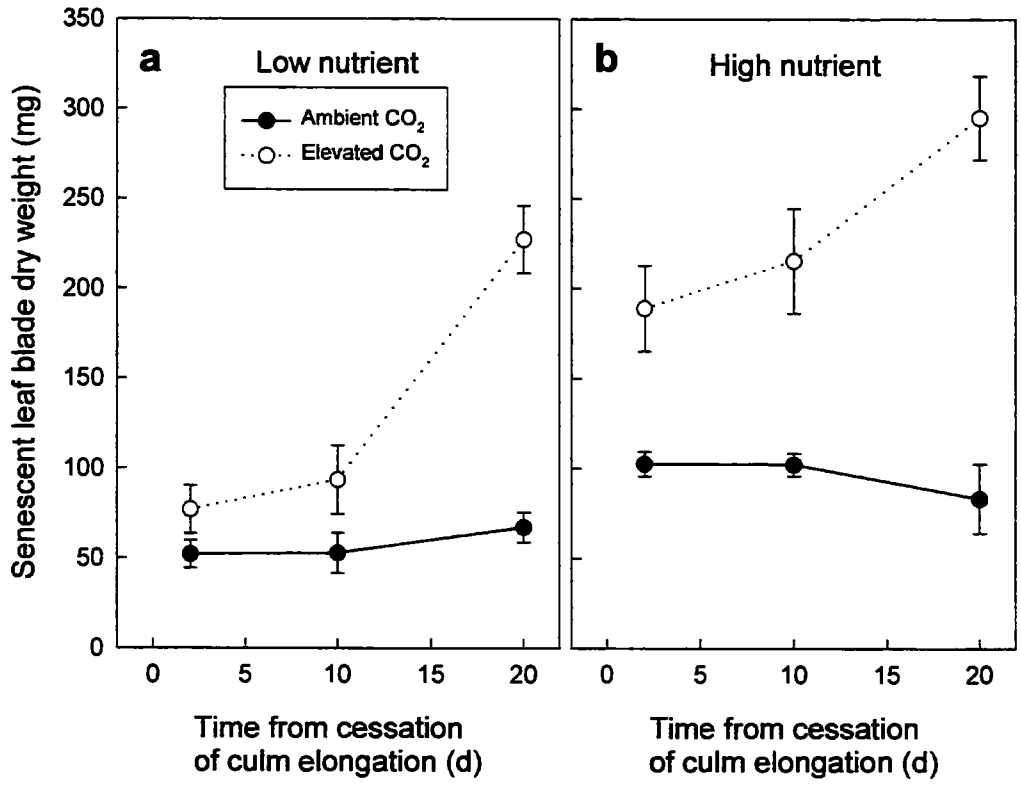


Fig. 4.3. Dry weight of senescent leaf blade material from the innovation zone following cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.1. Balanced three-way ANOVA on the dry weight of senescent leaf blade material from the innovation zone following cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	***
Nutrient	***
Time	***
CO_2 x Nutrient	**
CO_2 x Time	***
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

Table 4.2. Instantaneous net photosynthetic rate (A) (μmol CO_2 m^{-2} s^{-1}) of youngest fully expanded leaf of the main axis of vegetative *Poa alpina* (19 ± 3.4 days before paracladial exertion), grown in one-fifth strength Long Ashton (low) and full-strength Long Ashton nutrient solution (high), and either 350 μmol mol^{-1} (ambient) or 690 μmol mol^{-1} CO_2 (elevated). Net photosynthetic rate was measured at both ambient and elevated CO_2 concentrations. Data represent the mean ± one S.E. of ten replicates. Different letters indicate significant differences between means within the CO_2 concentration at which A was measured, at $P \leq 0.05$ critical level determined by Tukey's multiple comparison procedure (ANOVA).

CO_2 concentration at which A was measured (μmol mol^{-1}).	Nutrient treatment.	CO_2 concentration at which plants were grown (μmol mol^{-1}).	
		350	690
350	Low	16.1 ± 0.9 a	8.9 ± 0.5 b
	High	18.1 ± 1.3 a	11.8 ± 1.1 b
690	Low	27.1 ± 0.6 a	19.4 ± 0.9 b
	High	33.5 ±1.3 c	28.1 ± 1.1 a

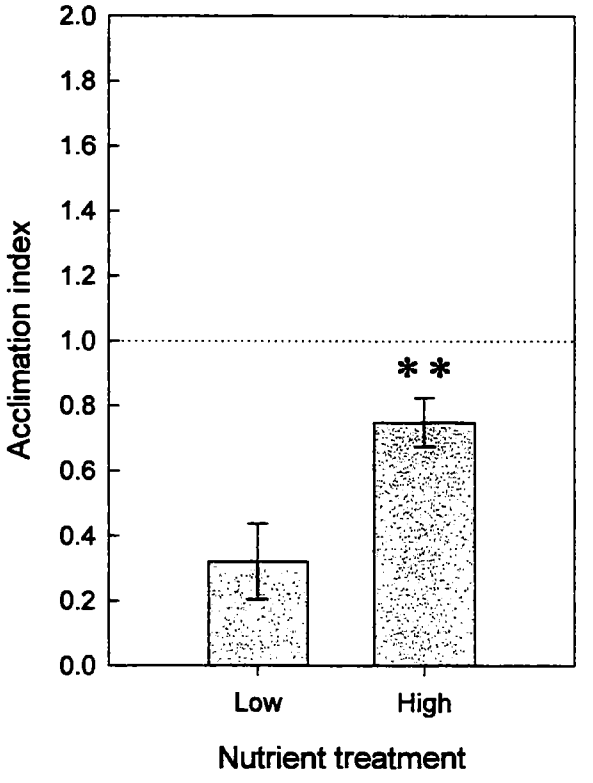


Fig. 4.4. Photosynthetic acclimation to elevated CO_2 of *Poa alpina* plants grown at two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data represent the mean ± one S.E. of six replicates. ** indicates significant difference between nutrient treatment means at the $P \leq 0.01$ level determined by Student's t-test. An A_{cc} < 1 indicates a loss of photosynthetic capacity.

Table 4.3. **(a)** The total non-structural carbohydrate (TNC) content (as a percentage of total dry weight) of innovation zone leaf blade material following cessation of culm elongation growth in *Poa alpina*. Plants were growth in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 μmol mol^{-1} (ambient) or 690 μmol mol^{-1} (elevated) CO_2. Data represent the mean ± one S.E. of four replicates. **(b)** Balanced three-way ANOVA on the TNC content of parent plant leaf blade material. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Time	Treatment			
	Low nutrient		High nutrient	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
2 d	47.5 ± 3.0	56.8 ± 7.2	47.5 ± 7.1	58.0 ± 5.4
20 d	61.2 ± 15.1	56.3 ± 8.1	54.1 ± 8.2	51.3 ± 8.3

(b)

Source	Significance
CO_2	n.s.
Nutrient	n.s.
Time	n.s.
CO_2 x Nutrient	n.s.
CO_2 x Time	*
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

Table 4.4. **(a)** The total nitrogen (N) and phosphorus (P) concentration (mg g^{-1} total DW) of innovation zone leaf blade material following the cessation of culm elongation growth in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. **(b)** Balanced three-way ANOVA on the total nitrogen concentration of innovation zone leaf blade material. **(c)** Balanced three-way ANOVA on the total phosphorus concentration of innovation zone leaf blade material. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Time	Nutrient	Treatment			
		Low nutrient		High nutrient	
		Ambient CO_2	ElevatedCO_2	Ambient CO_2	ElevatedCO_2
2 d	N	22.0 ± 5.1	14.6 ± 2.9	17.5 ± 0.7	12.4 ± 2.1
	P	10.3 ± 0.6	8.5 ± 1.6	6.4 ± 1.6	4.3 ± 0.6
20 d	N	16.0 ± 1.4	20.8 ± 3.4	20.9 ± 1.2	18.2 ± 1.9
	P	9.8 ± 0.5	9.6 ± 0.2	6.2 ± 0.2	6.3 ± 0.3

(b) Nitrogen

Source	Significance
CO_2	n.s.
Nutrient	n.s.
Time	n.s.
CO_2 x Nutrient	n.s.
CO_2 x Time	*
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

(c) Phosphorus

Source	Significance
CO_2	n.s.
Nutrient	***
Time	n.s.
CO_2 x Nutrient	n.s.
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

Table 4.5. **(a)** The photosynthetic nitrogen use efficiency (PNUE; g structural DW g^{-1} N d^{-1}) and photosynthetic phosphorus use efficiency (PPUE; g structural DW g^{-1} P d^{-1}) of innovation zone leaf blade material over an 18 d period following cessation of culm elongation growth in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. **(b)** Balanced two-way ANOVA on the PNUE of innovation zone leaf blade material. **(c)** Balanced two-way ANOVA on the PPNUE of innovation zone leaf blade material. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Nutrient	Treatment			
	Low nutrient		High nutrient	
	Ambient CO_2	ElevatedCO_2	Ambient CO_2	ElevatedCO_2
PNUE	0.13 ± 0.03	0.02 ± 0.02	0.14 ± 0.02	0.23 ± 0.02
PPUE	0.22 ± 0.05	0.06 ± 0.05	0.27 ± 0.01	0.64 ± 0.04

(b) PNUE

Source	Significance
CO_2	n.s.
Nutrient	***
CO_2 x Nutrient	***

(c) PPUE

Source	Significance
CO_2	*
Nutrient	***
CO_2 x Nutrient	***

4.3.2 The flowering response

In low nutrient treatments approximately 28 % of plants flowered, and in high nutrient treatments 62 – 70 % of plants flowered (Table 4.6). Plants pre-designated for harvests immediately post-paracladial exertion did not provide the minimum four plants in flower at each harvest and in all treatments (and in one case no replicates), and so these data were discarded. 'Spare' plants were included in each treatment, but the frequency of flowering in one-fifth strength Long Ashton was far lower than previously observed by the author, and these spare plants were not enough to provide the required replication.

The elevated CO_2 and low nutrient treatment increased the time taken from planting to main axis paracladial exertion (Fig. 4.5a) and also decreased the time taken from exertion to the cessation of culm elongation growth (Fig. 4.5b), whilst other treatments did not affect developmental timing in any measurable way. Additional nutrients independently led to an increase in the number of tillers in flower (Fig. 4.6, Table 4.7). An elevated CO_2 concentration alone increased the number of flowering tillers in the high nutrient treatment; but interacted with low nutrient status to decrease the number of tillers in flower. This interaction was reinforced over time, with all treatments except elevated CO_2 and low nutrient resulting in the continued production of synflorescences (Fig. 4.6). Total reproductive dry weight (i.e. dry weight of all paracladial zones on each plant) was higher in the high nutrient treatment compared to the low nutrient treatment (Fig. 4.7, Table 4.8), and there was no effect or interaction with elevated CO_2 other than an apparent increase in variability in total reproductive dry weight.

Table 4.6. The proportion (%) of the *Poa alpina* population in flower after growth in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 μmol mol^{-1} (ambient) or 690 μmol mol^{-1} CO_2 (elevated). Data represent the absolute percentage.

Nutrient treatment.	CO_2 concentration at which plants were grown (μmol mol^{-1}).	
	350	690
Low	27.3	28.6
High	70.2	62.5

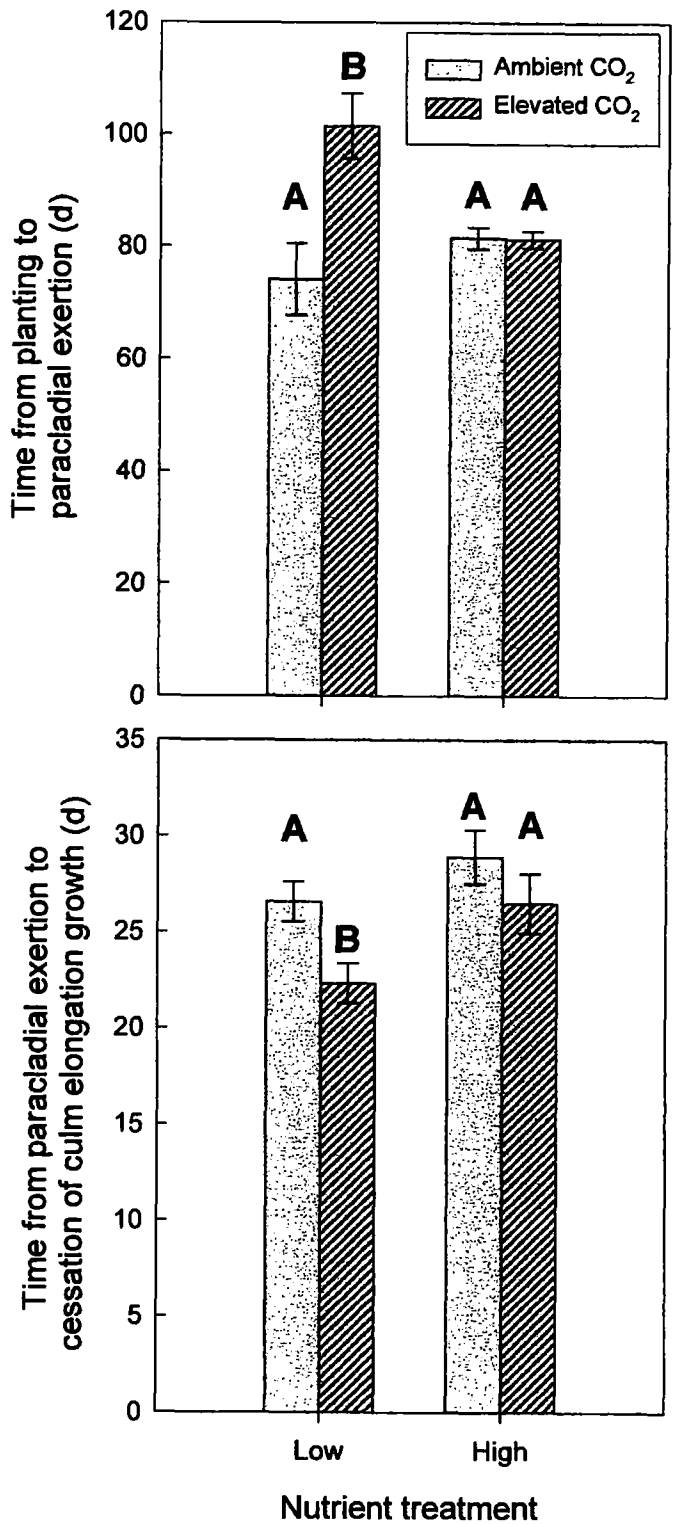


Fig. 4.5. Developmental timing of the culm of *Poa alpina*; **(a)** the time from planting to paracladial exertion, **(b)** the time from paracladial exertion to cessation of culm elongation growth. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data represent the mean ± one S.E. of twelve replicates. Different letters indicate significance at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure (ANOVA).

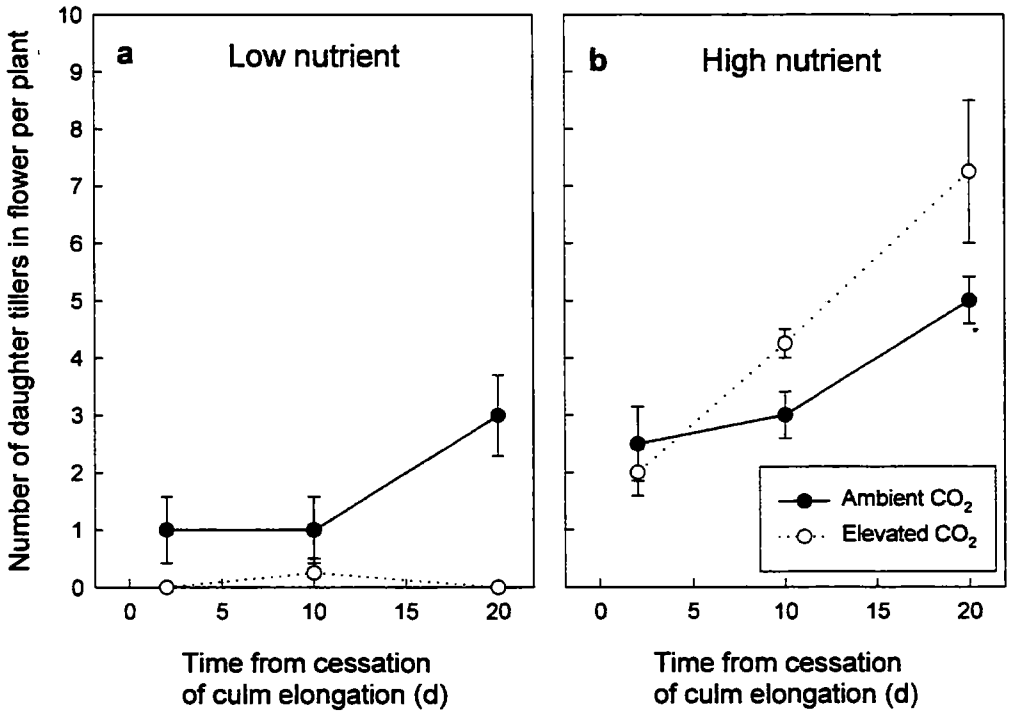


Fig. 4.6. Number of daughter tillers in flower at the time of cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.7. Balanced three-way ANOVA on the number of daughter tillers in flower at the time of cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	**
Nutrient	***
Time	***
CO_2 x Nutrient	***
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	*

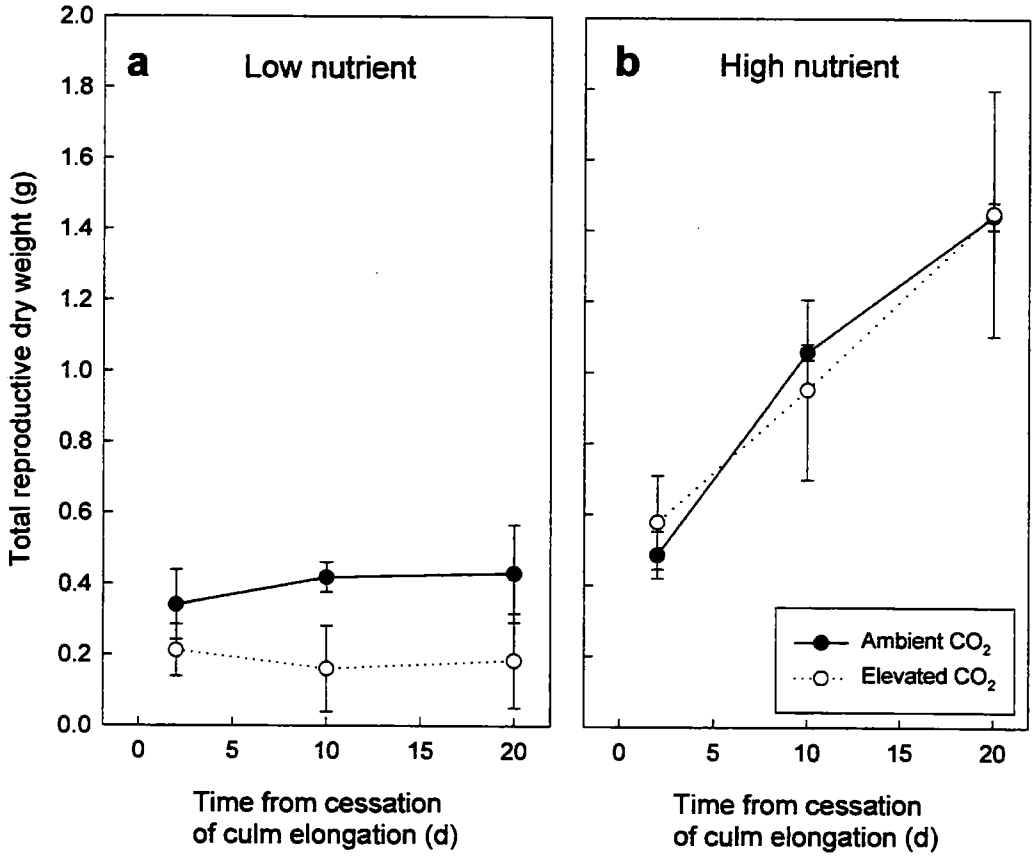


Fig. 4.7. Total reproductive dry weight following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.8. Balanced three-way ANOVA on the total reproductive dry weight following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	***
Time	***
CO_2 x Nutrient	n.s.
CO_2 x Time	n.s.
Nutrient x Time	***
CO_2 x Nutrient x Time	n.s.

4.3.3 The main axis paracladial zone

With regard to the main synflorescence, the final length of the culm was between 73 and 118 mm greater in plants grown in high nutrient availability than in plants grown in low nutrient availability (Table 4.9). The total number of spikelets was not affected by nutrient or CO_2 treatments (Table 4.10), although there was a large amount of variation seen in this measured parameter, particularly in low nutrient conditions - with between 58 and 72 plantlets present.

Total dry weight of the paracladial zone was significantly increased in high nutrient conditions compared to low nutrient conditions, with further increases over time (e.g. a difference of 211 mg in ambient CO_2 and a difference of 505 mg in elevated CO_2 at 20 d; Fig. 4.8, Table 4.11). Elevated CO_2 and low nutrient availability interacted to significantly lower main axis paracladial dry weights ($P \leq 0.001$). The number of fully expanded leaves on plantlets increased over time (Fig. 4.9). Elevated CO_2 interacted with nutrient availability to increase the number of fully expanded leaves in high nutrient and decrease the number of leaves per plantlet in low nutrient treatments (Fig. 4.9, Table 4.12). Plantlets possessed visibly senescent leaves from the time of cessation of culm elongation growth onwards, with the proportion of senescent material in the paracladial zone increasing over time ($P \leq 0.001$; Fig. 4.10, Table 4.13), until approximately 40 % of material in the paracladial zone was senescent at 20 d. High nutrient availability resulted in a higher proportion of senescent material ($P \leq 0.05$), with no effect of - or interaction with - the elevated CO_2 treatment.

Total non-structural carbohydrate (TNC) content of plantlets increased significantly over time, with plantlets from the proximal half of the paracladial zone possessing higher TNC contents than distal plantlets ($P \leq 0.01$; Table 4.14). There were significant reductions in nitrogen concentration within the paracladial zone in the low nutrient and elevated CO_2 treatment at 2 d, which was relieved by 20 d – there being no difference in nitrogen concentration between treatments at this time-point (Table 4.15). Phosphorus concentration was significantly decreased by both the high nutrient treatment and the elevated CO_2 treatment, which acted both independently and together to produce this response. Low nutrient treatments significantly decreased plantlet phosphorus concentration over time from 5.1 – 5.4 mg d^{-1} DW to 3.3 – 4.4 mg g^{-1} DW ($P \leq 0.05$; Table 4.15). Nutrient and CO_2 treatments showed an interactive effect on both photosynthetic nitrogen and phosphorus use efficiencies, with elevated CO_2 decreasing PNUE and PPUE in the low nutrient treatment

(0.04 ± 0.01 and 0.16 ± 0.06 g g^{-1} d^{-1} respectively), and increasing PNUE and PPUE in the high nutrient treatment (0.38 ± 0.13 and 1.38 ± 0.19 g g^{-1} d^{-1} respectively; $P \leq 0.001$; Table 4.16).

Typical examples of main synflorescence paracladial zones from each treatment are presented in Plate 4.3. The dry weight of distal plantlets was significantly greater than that of proximal plantlets in all treatments ($P \leq 0.001$; Fig. 4.11, Table 4.17). Leaf area ratio (LAR) decreased with time only in ambient CO_2, until values at 20 d were equal to the unchanging LAR found in elevated CO_2 (Fig. 4.12, Table 4.18). Distal plantlets had a lower LAR than proximal plantlets.

Table 4.9. The final length (mm) of the distal three internodes of the culm (internode 3 = distal, peduncle) of *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of ten replicates. Different letters indicate significant differences between treatment means within each internode at $P \leq 0.05$ critical level determined by Tukey's multiple comparison procedure (ANOVA).

Internode number	Treatment			
	Low nutrient		High nutrient	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
1	7.9 ± 1.6 a	12.8 ± 1.9 a	31.8 ± 4.9 b	17.8 ± 3.5 a
2	58.2 ± 7.9 a	75.0 ± 7.1 a	109.9 ± 10.2 b	85.3 ± 5.3 a
3	222.0 ± 11.3 a	216.8 ± 7.2 a	264.8 ± 12.0 b	274.3 ± 9.5 b
Total	**288.1 ± 17.7 a**	**304.6 ± 11.8 a**	**406.5 ± 17.9 b**	**377.4 ± 11.6 b**

Table 4.10. Number of spikelets in the main axis paracladial zone of *Poa alpina*, grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of twelve replicates. Data were square-root transformed prior to statistical analysis. Different letters indicate significant differences between means at $P \leq 0.05$ critical level determined by Tukey's multiple comparison procedure (ANOVA).

Nutrient treatment.	CO_2 concentration at which plants were grown (µmol mol^{-1}).	
	350	690
Low	58.8 ± 6.4 a	60.7 ± 6.1 a
High	64.5 ± 3.1 a	72.0 ± 3.3 a

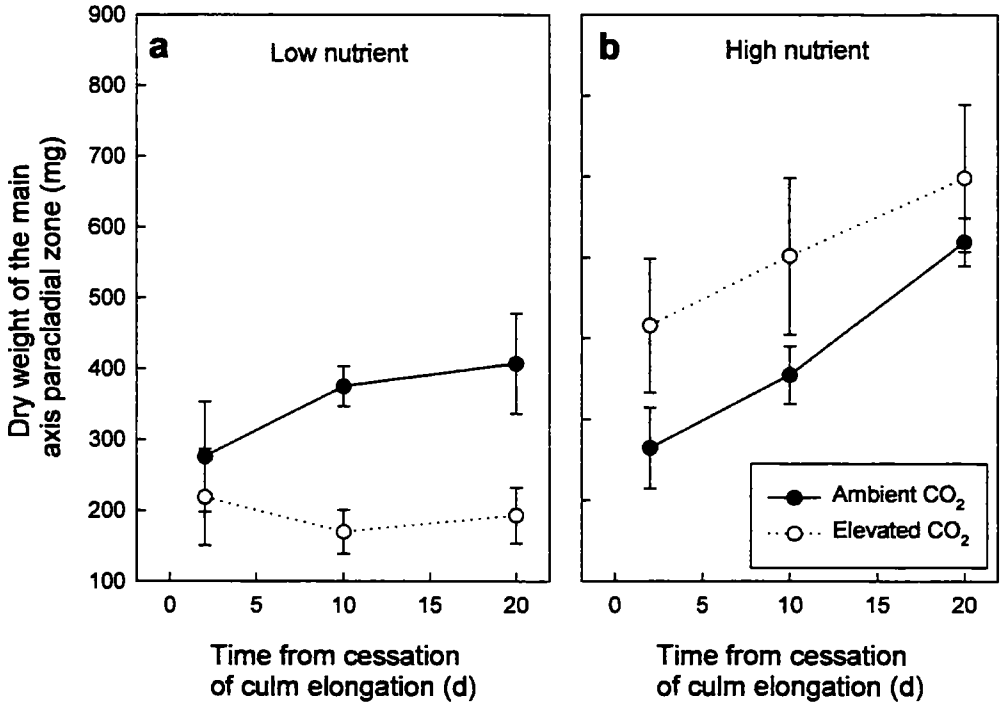


Fig. 4.8. Dry weight of the main axis paracladial zone after the cessation of elongation growth of the culm of *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.11. Balanced three-way ANOVA on the dry weight of the main axis paracladial zone after the cessation of elongation growth of the culm of *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	***
Time	*
CO_2 x Nutrient	***
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

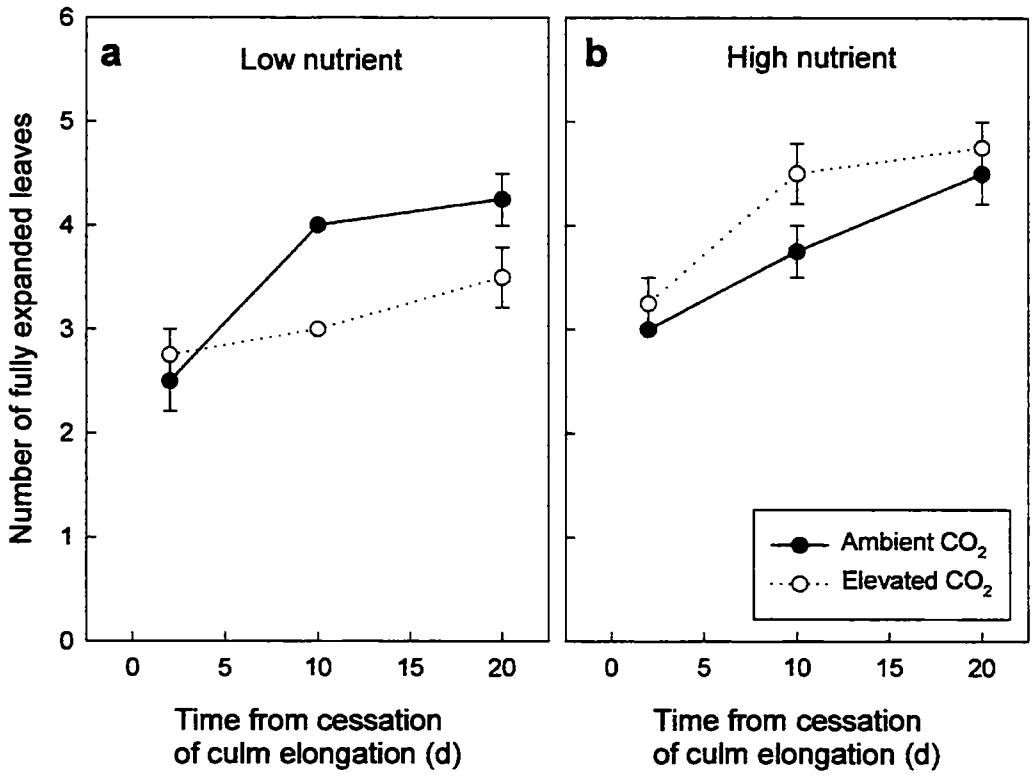


Fig. 4.9. The number of fully expanded leaves of individual plantlets after the cessation of elongation growth of the culm (no distinction was made between distal and proximal). Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.12. Balanced three-way ANOVA on the number of fully expanded leaves on plantlets after the cessation of elongation growth of the culm (no distinction was made between distal and proximal). Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data were square-root transformed prior to statistical analysis. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	***
Time	***
CO_2 x Nutrient	***
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

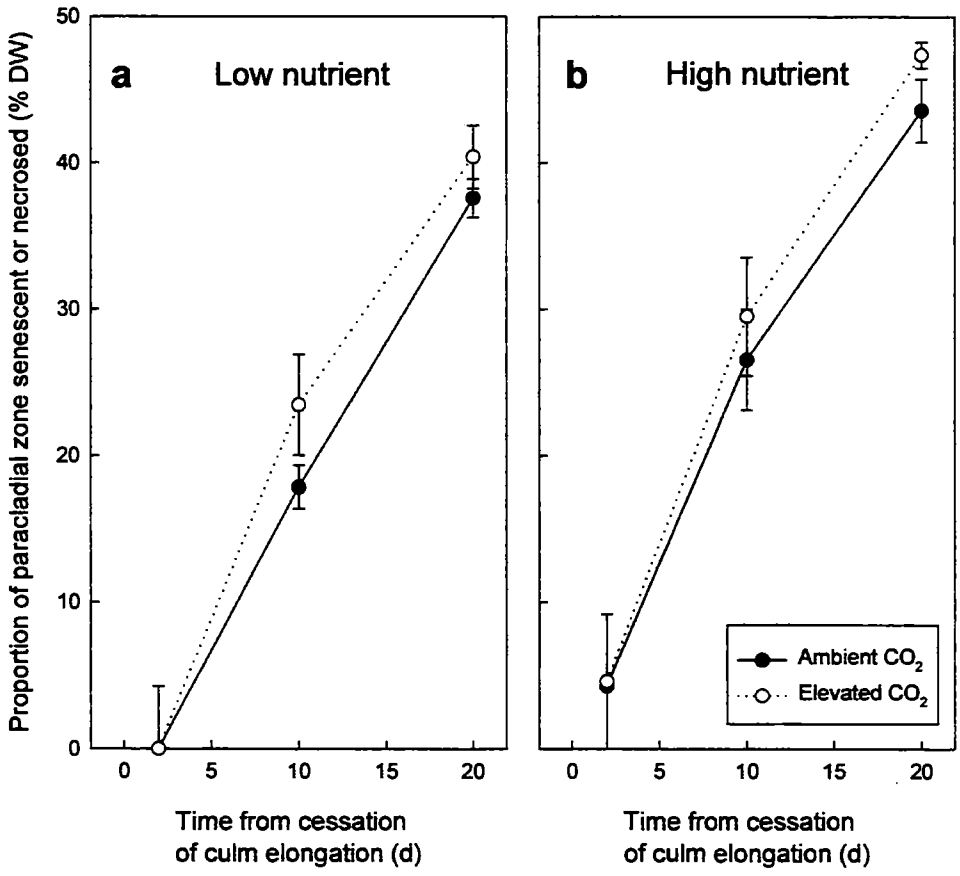


Fig. 4.10. The proportion dry weight in the paracladial zone that was senescent or necrosed following the cessation of elongation growth of the culm in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a**) or full-strength Long Ashton (high; **b**). Data represent the mean ± one S.E. of four replicates.

Table 4.13. Balanced three-way ANOVA on the proportion of dry weight in the paracladial zone that was senescent or necrosed following the cessation of elongation growth of the culm in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data were arcsin-transformed prior to statistical analysis. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	*
Time	***
CO_2 x Nutrient	n.s.
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	n.s.

Table 4.14. **(a)** The total non-structural carbohydrate content (percentage total DW basis) of plantlets from either distal or proximal positions in the paracladial zone at two time points from cessation of culm elongation in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. **(b)** Balanced four-way ANOVA on the total non-structural carbohydrate content of plantlets from either distal or proximal positions in the paracladial zone. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Time	Position	Treatment			
		Low nutrient		High nutrient	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
2 d	Distal	29.3 ± 5.1	22.4 ± 5.2	33.5 ± 1.9	38.7 ± 10.8
	Proximal	52.7 ± 14.3	46.2 ± 13.3	72.3 ± 14.4	40.0 ± 8.5
20 d	Distal	69.9 ± 6.2	67.5 ± 6.9	71.1 ± 13.6	74.3 ± 6.0
	Proximal	82.6 ± 5.3	59.5 ± 12.3	81.0 ± 3.5	82.8 ± 1.6

(b)

Source	Significance
CO_2	n.s.
Nutrient	n.s.
Position	**
Time	***
CO_2 x Nutrient	n.s.
CO_2 x Position	n.s.
CO_2 x Time	n.s.
Nutrient x Position	n.s.
Nutrient x Time	n.s.
Position x Time	n.s.
CO_2 x Nutrient x Position	n.s.
CO_2 x Nutrient x Time	n.s.
CO_2 x Position x Time	n.s.
Nutrient x Position x Time	n.s.
CO_2 x Nutrient x Position x Time	n.s.

Table 4.15. (a) The total nitrogen (N) and phosphorus (P) concentration (mg g^{-1} total DW) of the paracladial zone at two time points from paracladial exertion in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. **(b)** Balanced three-way ANOVA on the total nitrogen content of the paracladial zone. **(c)** Balanced three-way ANOVA on the total phosphorus content of the paracladial zone. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Time	Nutrient	Treatment			
		Low nutrient		High nutrient	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
2 d	N	16.1 ± 3.3	11.4 ± 1.5	14.4 ± 1.0	16.4 ± 0.8
	P	5.4 ± 0.7	5.1 ± 0.7	3.9 ± 02	2.7 ± 0.0
20 d	N	11.9 ± 1.8	19.9 ± 2.3	15.7 ± 1.5	12.8 ± 2.8
	P	4.4 ± 0.1	3.3 ± 0.5	3.5 ± 0.2	2.8 ± 0.1

(b) Nitrogen

Source	Significance
CO_2	n.s.
Nutrient	n.s.
Time	n.s.
CO_2 x Nutrient	n.s.
CO_2 x Time	n.s.
Nutrient x Time	n.s.
CO_2 x Nutrient x Time	**

(c) Phosphorus

Source	Significance
CO_2	**
Nutrient	***
Time	**.
CO_2 x Nutrient	n.s.
CO_2 x Time	n.s.
Nutrient x Time	*
CO_2 x Nutrient x Time	n.s.

Table 4.16. **(a)** The photosynthetic nitrogen use efficiency (PNUE; g structural DW g^{-1} N d^{-1}) and photosynthetic phosphorus use efficiency (PPUE; g structural DW g^{-1} P d^{-1}) of the paracladial zone over an 18 d period following cessation of culm elongation growth in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. **(b)** Balanced two-way ANOVA on the total nitrogen content of the paracladial zone. **(c)** Balanced two-way ANOVA on the total phosphorus content of the paracladial zone. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

(a)

Nutrient	Treatment			
	Low nutrient		High nutrient	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
PNUE	0.22 ± 0.14	0.04 ± 0.01	0.10 ± 0.04	0.38 ± 0.13
PPUE	0.43 ± 0.19	0.16 ± 0.06	0.62 ± 0.26	1.38 ± 0.19

(b) PNUE

Source	Significance
CO_2	n.s.
Nutrient	n.s.
CO_2 x Nutrient	*

(c) PPUE

Source	Significance
CO_2	n.s.
Nutrient	**
CO_2 x Nutrient	*

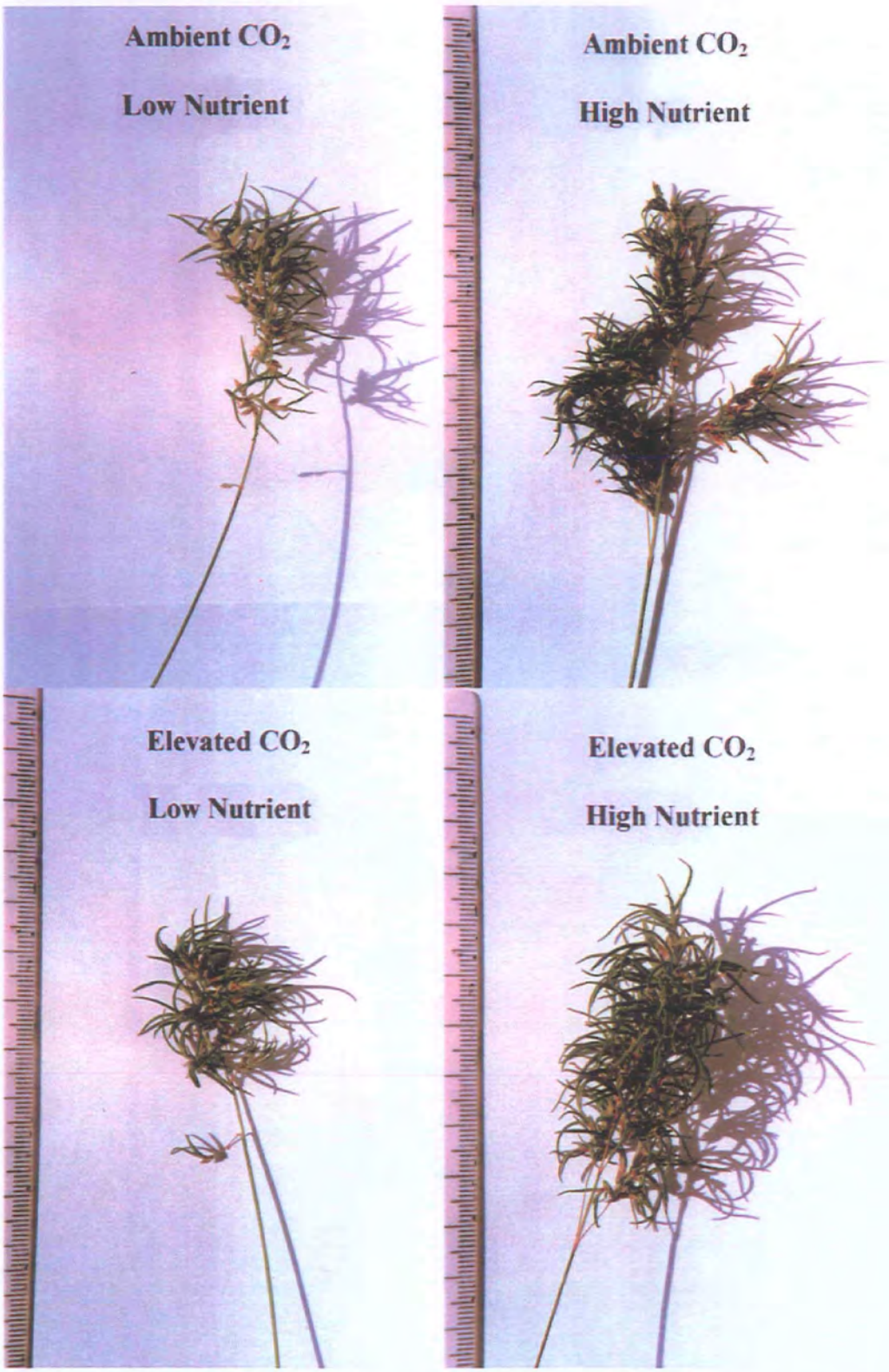


Plate 4.3. Main axis paracladial zones of *Poa alpina* plants grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton (low) or full-strength Long Ashton (high). Rule displayed for scale shows millimetres.

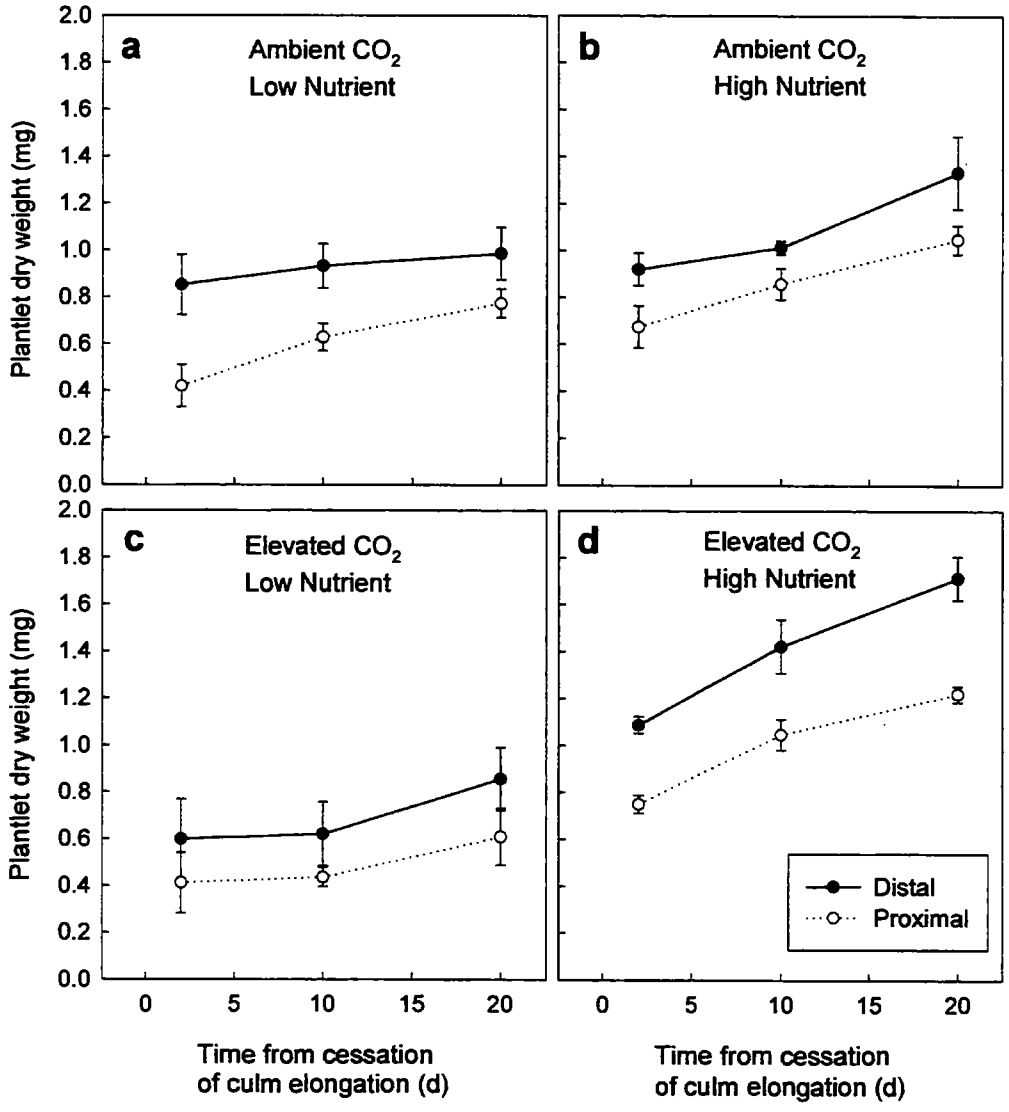


Fig. 4.11. Dry weight of plantlets from distal and proximal halves of the paracladial zone following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient; **a,b**) or 690 µmol mol^{-1} (elevated; **c,d**) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a,c**) or full-strength Long Ashton (high; **b,d**). Data represent the mean ± one S.E. of four replicates.

Table 4.17. Balanced four-way ANOVA on the dry weight of plantlets from distal and proximal halves of the paracladial zone following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	***
Position	***
Time	***
CO_2 x Nutrient	***
CO_2 x Position	n.s.
CO_2 x Time	n.s.
Nutrient x Position	n.s.
Nutrient x Time	*
Position x Time	n.s.
CO_2 x Nutrient x Position	n.s.
CO_2 x Nutrient x Time	n.s.
CO_2 x Position x Time	n.s.
Nutrient x Position x Time	n.s.
CO_2 x Nutrient x Position x Time	n.s.

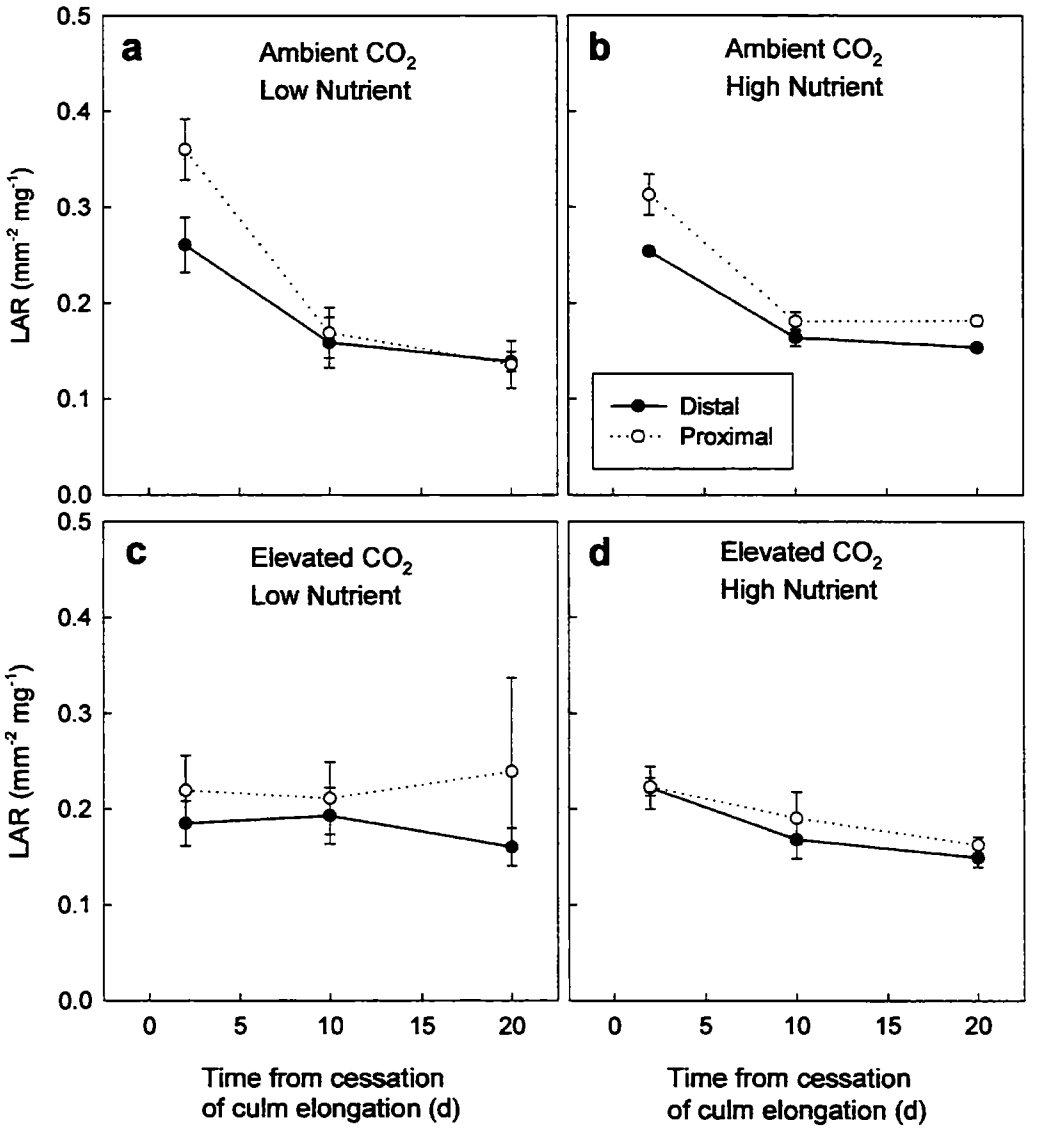


Fig. 4.12. Instantaneous leaf area ratio (LAR) of plantlets from distal and proximal halves of the paracladial zone following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient; **a,b**) or 690 µmol mol^{-1} (elevated; **c,d**) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low; **a,c**) or full-strength Long Ashton (high; **b,d**). Data represent the mean ± one S.E. of four replicates.

Table 4.18. Balanced four-way ANOVA on the leaf area ratio (LAR) of plantlets from distal and proximal halves of the paracladial zone following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
CO_2	n.s.
Nutrient	n.s.
Position	**
Time	***
CO_2 x Nutrient	n.s.
CO_2 x Position	n.s.
CO_2 x Time	***
Nutrient x Position	n.s.
Nutrient x Time	n.s.
Position x Time	n.s.
CO_2 x Nutrient x Position	n.s.
CO_2 x Nutrient x Time	n.s.
CO_2 x Position x Time	n.s.
Nutrient x Position x Time	n.s.
CO_2 x Nutrient x Position x Time	n.s.

4.4 Discussion

The innovation zone of *Poa alpina* exhibited acclimatory loss of photosynthetic capacity after long-term growth in elevated atmospheric CO_2 conditions, which was particularly pronounced with low nutrient availability (Fig. 4.4). Total non-structural carbohydrate (TNC), nitrogen (N) and phosphorus (P) contents[2] of innovation leaf blade material exhibited no difference between treatments that could directly account for this (Tables 4.3 & 4.4). However, lower photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE) indicate that N and P were utilised less efficiently in the production of dry matter in the elevated CO_2 concentration when less nutrients were available (Table 4.5).

Elevated CO_2 has been demonstrated to stimulate cell division in meristems of *Dactylis glomerata*, decreasing the length of the cell cycle by 26 % in shoot meristems and by up to 17 % in root meristems (699 µmol mol^{-1} *c.f.* 400 µmol mol^{-1} CO_2; Kinsman *et al.* 1997). Thus by directly increasing the rate of cell division, elevated CO_2 concentrations may increase the developmental age of an organ. Changes in developmental age and senescence are associated with changes in protein turnover and the size of precursor pools (Peoples & Dalling 1988), often resulting in a lack of correlation between leaf N and Rubisco concentrations (e.g. rice cv. Sasanishiki; Makino *et al.* 1984). In the present study developmental differences were apparent between treatments (such as a three-fold increase in the amount of senescent innovation material under elevated CO_2 and changes in reproductive initiation), indicating that CO_2 concentration and nutrient availability determined the developmental age of organs, and of the main shoot system via monocarpic senescence. This suggests that the initial developmental response of *Poa alpina* to elevated CO_2 may determine the subsequent employment of resources (apparent as PNUE and PPUE), thus governing the plant's response to CO_2 concentration.

Stirling *et al.* (1995) determined that a biotype of *Poa alpina* from Wales, when grown with a large rooting volume and full-strength Long Ashton nutrient solution, showed little or no acclimation of photosynthetic capacity to elevated CO_2 concentration. Aside from being a different biotype, it is possible therefore that the restriction of roots by pots in the present study may have resulted in feedback inhibition and down-regulation of photosynthetic capacity (Arp 1991), which may explain the acclimatory

[2] Nitrogen contents of innovation zone leaf blade material and plantlets from all treatments represented 1.1 – 2.2 % of dry weight; a value of 2 – 5 % being optimal for the growth of a range of plants (Marschner 1995) and values of between 3 and 6 % evident in four other *Poa* species (van Arendonk *et al.* 1997).

loss of photosynthetic capacity observed in plants grown in full-strength Long Ashton in the present study. However, acclimatory loss of photosynthetic capacity in the low nutrient treatment was significantly greater than that experienced in the high nutrient treatment ($P \leq 0.01$) and thus, at least in part, represents acclimation as a result of low nutrient availability. Also, in its natural habitat *Poa alpina* does not have a free root-range, with thin soils and/or the roots of other plants restricting rooting volume and constraining the size of this sink (Baxter pers. comm.). Thus *Poa alpina* is likely to experience acclimatory loss of photosynthetic capacity in future conditions, especially in nutrient-poor habitats.

The reduced total reproductive dry weight observed in the low nutrient and elevated CO_2 treatment was a result of both a lack of flowering of daughter tillers and the subsequent growth response of the paracladial zone(s) produced. Daughter tillers perhaps had not reached a threshold dry-mass ($\approx$ developmental age) above which the apical meristem could be induced into reproductive development (Wareing & Phillips 1990) – which may also explain the general lack of flowering in the low nutrient treatments. Spikelet number was highly variable, but was not shown to differ between treatments; thus dry matter accumulation in the paracladial zone reflected the performance of plantlets, rather than differences in plantlet number between treatments. Photosynthetic acclimation in plantlet tissues was not directly determined as suitable cuvettes for gas exchange were not available at the time of study. Again the TNC, N and P content of tissues cannot directly explain the results of the growth analysis. However, PNUE and PPUE of plantlet tissues showed an identical response to CO_2 concentration and nutrient availability as leaf blade material from the innovation zone, both PNUE and PPUE being decreased in low nutrient and elevated CO_2. This was reflected in the lower dry weight and lower number of fully expanded leaves of plantlets in this treatment relative to the other treatments imposed. Thus the nutrient use efficiency of plantlets was altered in response to CO_2 concentration and nutrient availability, producing the observed growth response and providing evidence that the physiological characteristics of plantlets were similar to those of tillers in the innovation zone.

However, the growth response of plantlets was not necessarily entirely due to the PNUE and PPUE of plantlet tissues. The elevated CO_2 and low nutrient treatment provided the only growth conditions in which the development of the culm was

---

Phosphorus content of *Poa alpina* tissues was generally high, but did not exceed the 1 % of dry weight threshold above which many plants begin to experience phosphorus toxicity (Marschner 1995).

altered, with earlier senescence being promoted. Thus the developing paracladial zone was physiologically connected to the remainder of the plant for a shorter length of time. A decrease in the flux of phloem sap via the culm (although not measured directly), leading to accumulation of carbohydrates and positive feedback inhibition in plantlets did not appear to occur any earlier in this treatment as there was no difference in TNC content in between treatments. Transport in the xylem of the culm is likely to have occurred at this point in time (Chapter 2, this study), providing a route for the delivery of water and growth-promoting cytokinins from the roots. However, changes in the degree of water stress and supply of phytohormones cannot be ruled out as contributing towards the observed behaviour of the paracladial zone, and the degree of water stress and phytohormone content of plantlets in response to CO_2 concentration and nutrient availability warrants further investigation. It is worthwhile noting that the length of the culm was increased by high nutrient availability, the increased path length presumably decreasing flux through this organ in these treatments (Cook & Evans 1983). Although this does not appear to account for any of the observed behaviour within the paracladial zone, any future investigation of phytohormone or water flux via the culm should bear in mind that the physical dimensions of the transport system are apt to change with nutrient availability.

Extensive senescence or 'blasting' within the paracladial zone is a symptom of both nutrient deficiency and water stress (O'Toole *et al.* 1984; see Chapter 2). Earlier senescence of the culm in the elevated CO_2 and low nutrient treatment was also associated with the earlier senescence of plantlet material (as sequential senescence of plantlets began immediately after the cessation of culm elongation growth in all treatments). This sudden and rapid senescence of plantlet material (with 40 % of plantlet material becoming senescent over a 20 d period) indicates that the water economy of plantlets is dependent on the timing of culm senescence, and thus on the effect of CO_2 concentration and nutrient availability on the culm.

It is clear that elevated CO_2 concentrations may prompt earlier senescence, inducing stomatal closure (Mei & Thimann 1984; Kramer & Boyer 1995, respectively). Also, the activity of the enzyme superoxide dismutase (SOD) has been shown to decrease in response to low nutrient availability under elevated CO_2 in the leaves of beech (*Fagus sylvatica* L.; Polle *et al.* 1997), the occurrence of which is thought to facilitate leaf senescence in this species. The effect of nutrient availability and CO_2 concentration on the enzymes involved in senescence processes therefore begs closer inspection. Similarly, the genetic mechanisms involved in senescence are

currently the focus of much work (Smart 1994), and their involvement with carbohydrate metabolism should be divined.

Prior to developmental changes involving the culm, the time from planting to paracladial exertion was increased only in the elevated CO_2 and low nutrient treatment. Changes in timing of floral initiation in elevated CO_2 have been shown to be species - and even biotype - specific, with nutrient-sufficient conditions resulting in either no change or earlier initiation in the species and populations studied by Garbutt & Bazzaz (1984). Hesketh & Hellmers (1973) demonstrated delayed floral initiation in *Sorghum bicolor*, *Zea mays*, *Helianthus annuus* L. and *Gossypium hirsutum* L. in elevated CO_2 (1000 μmol mol^{-1}) when provided with half-strength Hoaglands nutrient solution. This was tentatively attributed to the production of excess photoassimilate, suggestive of feedback-inhibition of photosynthesis. The delay in paracladial exertion observed in the present study is unlikely to have been due to positive feedback inhibition, but could have been the result of the nutrient-use efficiency of a plant growing in elevated CO_2 with a low availability of nutrients.

Distal plantlets, despite being limited by low nutrient availability, consistently possessed greater dry weights than proximal plantlets irrespective of nutrient availability or CO_2 concentration. This indicates that heterogeneity was inherent to the paracladial zone and, as the distal half of the paracladial zone showed no response to the surgical removal of the proximal half in conditions of low nutrient availability (Chapter 3, this study), competition for nutrients or feedback inhibition of photosynthetic capacity in proximal plantlets cannot explain the heterogeneity observed within the paracladial zone (hypothesis 2 – that heterogeneity is altered by resource availability – is rejected). A possible mechanism underlying paracladial heterogeneity is discussed in detail in Chapter 6.

In conclusion, the innovation zone of *Poa alpina* exhibited acclimatory loss of photosynthetic capacity and decreased PNUE and PPUE under long-term growth in elevated CO_2, particularly in low nutrient conditions. This nutrient-use response was also exhibited by plantlets, which attained lower dry weights under elevated CO_2 when low nutrient availability was imposed (supporting hypothesis 1 – that plantlets exhibit the same response to resource availability as innovation tissue). As a result of this - and of a lower number of tillers in flower per plant - the total reproductive dry weight was depressed in plants grown in elevated CO_2 with low nutrient availability when compared with plants grown in high nutrient treatments or ambient CO_2 and low

nutrient availability, despite a lack of difference in the TNC, N and P concentrations of plantlet tissues between treatments. This supports hypothesis 3, with the exception that spikelet number was not part of the plant's reproductive response to resource availability in the present study. Changes in photosynthetic nutrient-use efficiency are probably the result of developmental changes in response to elevated CO_2, as evidenced by an increased proportion of senescent material in the innovation zone and changes in reproductive initiation under elevated CO_2 (supporting hypothesis 4 – that resource availability alters the developmental timing of reproduction). The pattern of heterogeneity observed in the paracladial zone was an inherent feature and occurred irrespective of CO_2 concentration or nutrient availability. The implications of these observations are discussed in a wider context in Chapter 6.

5 Abortion in the paracladial zone

5.1 Introduction

The abortion of spikelets observed in Chapter 3 represents a loss of reproductive potential. Death of spikelet meristematic tissue in this manner also accounts for infertility in seminiferous grasses - although anther sterility (indehiscent anthers; Nishiyama 1983) may also be responsible. Florets may abort early in development, subsequently being represented by knobs of undifferentiated tissue (e.g. *Melica nutans* L.; Arber 1934). In addition, entire spikelets may sometimes be aborted in a similar manner (e.g. *Lolium perenne* L.; Fig. 1.1a).

There is little doubt that in some grasses the abortion of spikelets may be under the direct genetic control of the plant. In addition to abortive spikelets being a consistent and integral component of entire paracladia in grasses such as *Coix lacryma-jobi* L. (Arber 1934), genetic control of spikelet abortion has been proposed in barley (Cottrell *et al.* 1985; Garcia del Moral *et al.* 1991), with three barley cultivars consistently possessing different proportions of spikelet abortion in identical environmental conditions (Kernich & Halloran 1996). Physiological control of spikelet abortion is thought to be mediated via the action of phytohormones, probably as an increase in the ratio of growth suppressers (ABA, ethylene) to growth promoters (IAA, cytokinins; Murty & Murty 1981; Reed & Singletary 1989; Patel & Mohapatra 1992), either within the paracladial zone or within individual spikelets (Lee *et al.* 1988). There is strong evidence that assimilate supply does not determine abortion, but may be subsequently perturbed by the condition (Reed & Singletary 1989; Patel & Mohapatra 1992).

Experimental physical constraint of wheat coleoptiles by plastic collars prevents the growth of tillers (Williams & Metcalf 1975), their growth being aided by surgical incisions in leaf sheaths. This work draws from, and lends credence to, the 'pressure hypothesis' of spikelet abortion (Arber 1934). During the early stages of its development the paracladial zone is enclosed by the sheath of the distal leaf, which in turn may be surrounded by the other sheaths of the pseudostem.

Godron (1879; cited by Arber 1934) observed:

> "The difficulty that is encountered in detaching the leaf-sheaths from a young grass shoot, is an indication of the amount of pressure which they can exert upon the plastic rudiment of the inflorescence; and the effect of the pressure is increased by the fact that this rudiment may be imprisoned for a long time."

Thus the pressure hypothesis suggests that as paracladia develop within the constraining environment of the pseudostem, pressure may develop within the paracladial zone which may cause abortion of meristems. Mechanistically; within each floret pressure between the palea and lemma is thought to crush the enclosed primordium (Arber 1934), a concept which could conceivably be extended to the abortion of entire spikelets by either pressure imposed by the glumes, or by the presence of neighbouring spikelets, acting on the various meristems of the spikelet. Thus it is possible that physical pressure may, at least in part, explain the abortion of spikelets in *Poa alpina* observed in Chapter 3. Thus the following hypotheses are proposed:

That:

1. abortion of organs within the paracladial zone can occur as a result of mechanical pressure on the pseudostem immediately after paracladial initiation,
2. the greater frequency of abortion previously observed in proximal paracladia is a direct result of the greater number of spikelets within this region.

5.2 Methods

The apparatus detailed in Fig. 5.1 was used to apply a firm pressure to the pseudostem during the period of extension growth immediately prior to paracladial exertion. Plants were monitored by measuring interligular distances (see Chapter 2) and treatment imposed at the start of pseudostem elongation. A cane was pushed vertically into the substrate immediately next to the pseudostem of the main axis. A 170 g weight composed of modelling material (Early Learning Centre, South Marston, Swindon, U.K.), tightly wrapped in 30 cm^2 aluminium foil (to limit the exchange of water), was suspended from a 40 cm length of flexible plastisized garden wire. A loop was made at the end of the wire, and this loop placed over the pseudostem and cane so that the weight on the wire compressed the distal leaf sheath, containing the developing inflorescence, against the cane. A 1.5 cm length of 1 mm thick transparent plastic was placed between the wire and the sheath itself, to ensure an even distribution of pressure. The weight and wire were held free of the pot at all times by means of a strong wire arm or spindle borne on a second cane of the same height at the edge of the pot, which also ensured that pressure was applied perpendicular to the pseudostem. Control plants were not compressed in this manner, but canes were pushed into the substrate in an attempt to equalise any potential root damage.

Plants were left to develop under these conditions until the paracladial zone was fully exerted. Treatment was then removed and plants left to develop until the cessation of elongation growth of the culm. At this time the number of spikelets possessing an elongated lemma was used as an indication of the number of viable plantlets within the distal and proximal halves of the paracladial zone. The final length of the culm was also recorded.

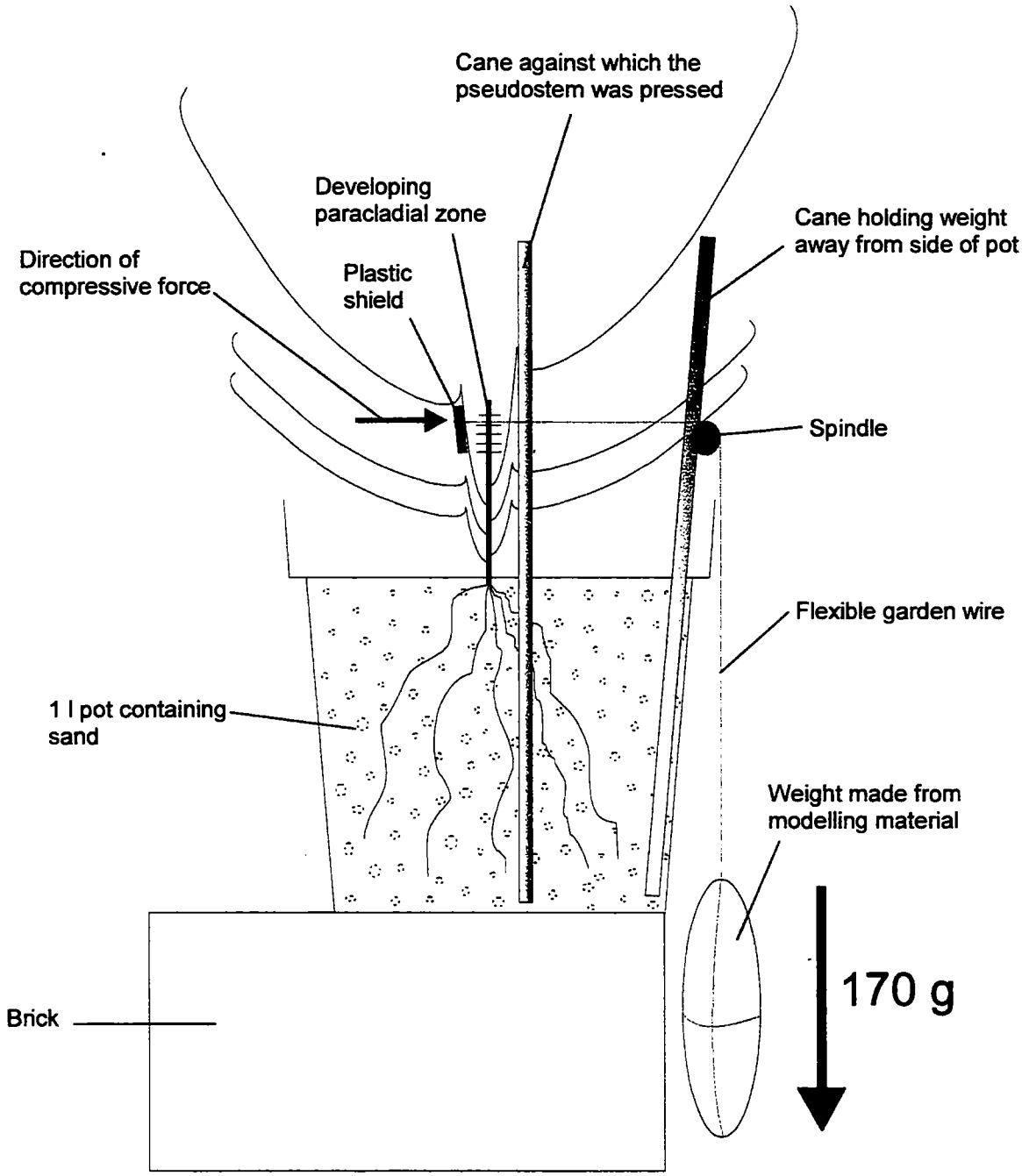


Fig. 5.1. Apparatus used to apply pressure to the pseudostem during the early stages of paracladial development.

5.3 Results

The pressure treatment did not result in any visible mechanical damage to any part of the plant. The number of spikelets possessing an extended lemma was significantly decreased by the application of pressure to the developing paracladial zone ($P = 0.012$; Fig. 5.2). Often entire paracladia were absent, these being represented only by a withered knob of material. The proportion of spikelets possessing an elongated lemma was not significantly different between treatment and control groups ($P = 1.0$), with significant difference maintained between distal and proximal halves irrespective of pressure treatment ($P \leq 0.001$; Fig. 5.3, Table 5.1).

The mean final length of the distal internode of the culm was also observed to be significantly affected by the pressure treatment ($P = 0.034$), with treated plants possessing internodes 135.2 ± 19.6 mm in length, compared to controls of 193.3 ± 10.3 mm (Fig. 5.4).

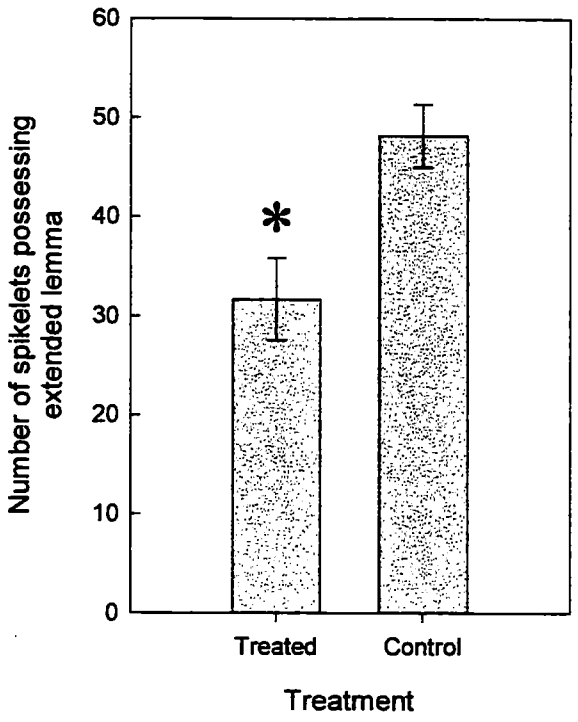


Fig. 5.2. The number of spikelets possessing elongated lemma after the application of increased pressure on the pseudostem (treated). Data represent the mean ± one S.E. of six replicates. Data were square-root transformed prior to statistical analysis. * indicates a significant difference between treatment and control means at the $P \leq 0.05$ critical level determined by Students t-test.

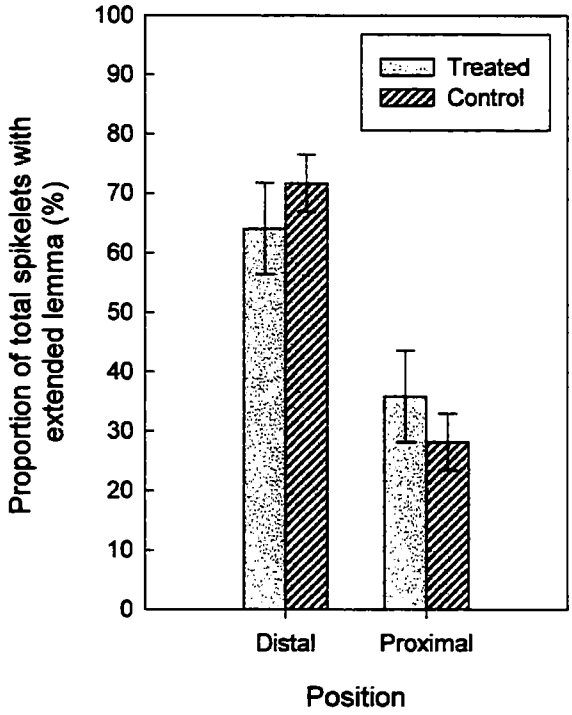


Fig. 5.3. The proportion of the total number of spikelets that possessed an elongated lemma within the two halves of the paracladial zone after the application of increased pressure on the pseudostem (treated). Data represent the mean ± one S.E. of six replicates.

Table 5.1. Balanced two-way ANOVA on the proportion of the total number of spikelets that possessed an elongated lemma within the two halves of the paracladial zone after the application of increased pressure on the pseudostem (treated). Data represent the mean ± one S.E. of six replicates. *, ** and *** indicate significance at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively, and n.s. indicates no significance at the $P \leq 0.05$ level.

Source	Significance
Position	***
Pressure	n.s.
Position x Pressure	n.s.

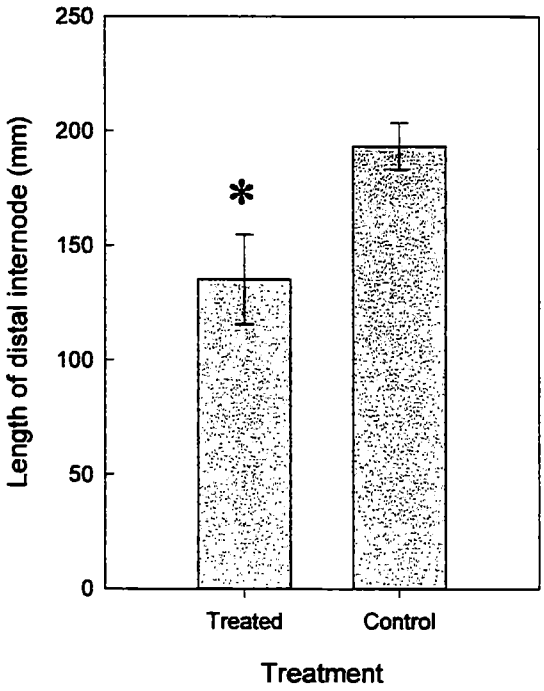


Fig. 5.4. Final length of the distal internode of the culm after the application of increased pressure on the pseudostem (treated). Data represent the mean ± one S.E. of six replicates. * indicates a significant difference between treatment and control means at the $P \leq 0.05$ critical level determined by Students t-test.

5.4 Discussion

The decreased length of the distal culm internode could not have been due to the death of the intercalary meristem, rather a decreased ability of that meristem to produce elongation growth. This, and the lack of visible mechanical damage, indicates that the mechanism behind abortion was not purely physical, such as - for instance - the exceedence of the mechanical threshold of cell walls. Rather, pressure appears to act by mediating a physiological mechanism. As physiological control of abortion rests with phytohormones (Patel & Mohapatra 1992), any proposed mechanism by which pressure modifies spikelet abortion must account for the action of phytohormones. In light of this, a speculative mechanism of pressure modification of abortion in the synflorescence is suggested here:

Changes in water potential result in a measurable change in cell volume (Ferrier & Dainty 1977, 1978; Sovonick-Dunford *et al.* 1982). Conversely, physical restriction of cell volume would lead to restriction of tissue water potential, and so impose a limit on water potential gradient between the developing paracladial zone and parent plant tissues. Thus the transport of cytokinins from root tissues may decline, decreasing the concentration of cytokinins in synflorescence tissues and resulting in relatively little promotion of growth.

In the present study the proportion of abortion remained higher in the proximal half of the paracladial zone with pressure treatment, suggesting that the effect of pressure on the synflorescence was general in nature. Patel & Mohapatra (1992) suggest that the relatively large size of proximal paracladia may result in lower cytokinin concentrations within their tissues, and thus lead to increased abortion. However, an increase in pressure locally by a larger number of spikelets in the proximal region cannot be ruled out.

In conclusion, mechanical pressure, such as that imposed by the constraining pseudostem, may increase the proportion of aborted spikelets in the paracladial zone (supporting hypothesis 1). Leading on from this, experimentation should investigate phytohormone contents (via assays such as those used by Suzuki *et al.*; 1981), aiming to directly determine the relationship between pressure and the balance of phytohormones within tissues. This may also shed more light on the relationship between architecture, phytohormone content and the high proportion of abortion in the proximal region of the paracladial zone. A clear link exists between the frequency of abortion and the position of spikelets within the paracladial zone (supporting

hypothesis 2), but the precise mechanism underlying this heterogeneity remains unclear.

6 General discussion

Resource allocation between the parent shoot and plantlets in the pseudoviviparous system occurs as long-distance transport in the xylem and phloem tissues of the culm. In the present study, water was allocated to plantlets of *Poa alpina* throughout their development on the parent plant, and it is unlikely that the small amounts of cavitation or tylosis observed with the ageing of the culm represented any hindrance to transpiration flow. Thus cessation of allocation via the xylem is only likely to occur with catastrophic breakage of physical connections; either via plantlet dehiscence or collapse of the culm.

The eventual collapse of the culm – or lodging to borrow an agricultural term – is thought to be an advantage to pseudoviviparous grasses as it places the plantlets into contact with the substrate *en masse* and possibly facilitates colonisation via extensive rooting and soil stabilisation (Harmer & Lee 1978b). In the present study lodging was not observed until late in development (i.e. after the cessation of culm elongation) in unsupported plants; hence lodging occurs at a point in time after the culm has reached its maximum length, and does not represent the exceedence of a critical buckling length (Niklas 1992, 1994). In rice, the culm and rachis are strong and flexible cylinders that behave in concert in an identical manner to a cantilevered, tapered, loaded beam (Silk *et al.* 1982) – bending under the weight of the paracladial zone. This bending behaviour presumably allows the culm to remain active as a transport system and platform for propagule dispersal despite increased mechanical loading during development. The curvature is a function of the force acting on the culm (bending moment – a function of gravitational acceleration and mass (Niklas 1994)) and the resistance of the culm to bending (flexural rigidity), more precisely:

$$K = M/C \qquad [1].$$

Where K equals curvature, M equals bending moment, and C equals flexural rigidity (Silk *et al.* 1982). Thus the lodging of culms observed here and in *Festuca vivipara* (Harmer & Lee 1978b) represents the exceedence of a threshold curvature. This could be due to either the development of the paracladial zone (increased bending moment), and/or a decline in the physical competence of the culm (decreased flexural rigidity).

Flexural rigidity of cereal culms depends on many factors. In rice the physical dimensions (Ookawa & Ishihara 1992) and lignin content of cell walls (Ookawa & Ishihara 1993) have been implicated. In barley (*Hordeum vulgare* L.) the proportion of cell wall per cell (Cenci *et al.* 1984; Kokubo *et al.* 1989) and the cellulose content of cell walls (Kokubo *et al.* 1991) are components of flexural rigidity. However, a decline in flexural rigidity also occurs with declining water content, as tissue rigidity is a function of turgor (Falk *et al.* 1958; Nilson *et al.* 1958; Pitt 1982; Lin & Pitt 1986; Niklas 1991, 1992). Niklas (1991) determined that the flexural rigidity of petioles of four angiosperms (two monocots and two dicots) decreased with decreasing water potential. Moreover, different tissues possessed different mechanical responses to dehydration, with the spatial distribution and proportion of structural tissues interacting with turgor to determine flexural rigidity.

Clearly, many factors determine lodging resistance, but central to the lodging process is the water content of tissues. This suggests that it is the declining water content of the culm during senescence in conjunction with the development of the paracladial zone that ultimately determines the timing of lodging in *Poa alpina*, and that lodging is inevitable as the culm senesces. Earlier senescence of the culm, apparent in elevated CO_2 and low nutrient conditions, may well therefore result in earlier lodging, thereby facilitating earlier establishment (N.B. the data support the hypothesis that plantlets remaining attached face a trade-off between increased water stress and the continued acquisition of nutrients via the culm; Harmer & Lee 1978a). Also, as dehiscence is a senescence process, this event would occur earlier in elevated CO_2 and low nutrient conditions.

In natural surroundings loading stresses on the culm will differ from plants grown in the greenhouse in that changing wind speeds produce additional dynamic loads on stems (Niklas 1992). Dynamic loading may perhaps act to cause lodging at an earlier time during culm senescence. However, just as oscillatory movement of grass culms via dynamic loading may facilitate the interception of pollen (Niklas 1992), gusting of wind and oscillatory movement is more effective in providing the shear forces necessary for the dehiscence of fruits than high and constant wind speed (Niklas 1992), and may therefore facilitate the dehiscence of pseudoviviparous plantlets. Thus the senescence of the culm, aside from directly affecting water transport (Chapter 5), may impose limitations on the period of time over which a physiological connection exists between parent and plantlet via the xylem as the biomechanical properties of the organ change during development.

In terms of a physiological connection via the phloem of the culm, radioisotope tracers demonstrated that transport was greatly reduced when the culm was in an advanced state of senescence (i.e. when chloroplasts had been degraded and water content was low). The status of the pseudoviviparous plantlets of *Poa alpina* was confirmed as that of carbon sources; initially supplying parental sinks both directly and indirectly via basipetal allocation from all leaves on the synflorescence axis (as suggested by Lee & Harmer; 1980). However, plantlets showed increases in total non-structural carbohydrate (TNC) over time, which built up to excessive levels - particularly in the smaller proximal plantlets (Chapter 4). This suggests that in addition to water stress the low relative growth rate apparent in plantlets at this point in time could in part be explained by feedback inhibition of photosynthetic capacity as export was stymied.

With regard to the senescence of the culm; chloroplast degradation during senescence is a result of both lipase mediated changes in membrane fatty acid composition (Thompson 1988) and increased lipid peroxidation in cell membranes (Dhinsa *et al.* 1981; Hung & Kao 1997), both of which increase membrane permeability and leakage. Increased frequency of lipid peroxidation is thought to be a result of both the production of endogenous methyl jasmonate (Hung & Kao 1998) and a lack of protective enzymatic machinery (superoxide dismutase and catalase) that would usually limit the action of superoxide radicals and hydrogen peroxide and hence limit lipid peroxidation (Dhinsa *et al.* 1981). A general lack of enzymatic machinery (also resulting in decreased metabolic rates) reflects a change in protein turnover, with degradation exceeding synthesis (Woolhouse 1987) and nitrogenous metabolites being remobilised and exported to physiologically younger organs (Mae & Ohira 1981; Kelly & Davies 1988; Peoples & Dalling 1988); i.e. chloroplast degradation and remobilisation are integral senescence processes.

Remobilisation of resources during senescence is thought to be of advantage to the plant as resources are reallocated to organs that are currently in favoured positions (e.g. un-shaded), or to propagules (Leopold 1961). Indeed, the majority of nitrogen partitioned to the paracladial zone of rice has previously been incorporated into the leaves and subsequently remobilised (Mae & Ohira 1981). Therefore the senescence of the culm and of older plantlet leaves, followed by the remobilisation of nitrogenous metabolites acropetally, could contribute towards the nutrition of developing plantlet tissues. Allocation of remobilised metabolites was not directly investigated in this

study. However, it is clear from the study of the effect of CO_2 concentration and nutrient availability that developmental age may have a profound influence on photosynthetic nutrient-use efficiency (Chapter 3). Thus it is possible that as plants developed, the observed decline in the amount of nitrogen and phosphorus actually used in dry matter production (i.e. decreased PNUE and PPUE) was a reflection of reallocation processes, with N and P being sequestered from photosynthetic metabolism to provide the enzymatic machinery required for degradation and export processes. Remobilisation from senescent tissues and its contribution to developing plantlets is an issue that demands further investigation, unfortunately falling outwith the time constraints imposed on the present study.

Within the paracladial zone itself, a degree of heterogeneity was observed in terms of plantlet dry weight, with distal plantlets possessing greater dry weights and relative growth rates on dehiscence and establishment (Chapter 3). Mohapatra & Sahu (1991) and Mohapatra *et al.* (1993) state that in tall indica rice (cv. Kajalghara) the same progressive basipetal development of paracladia occurs. Similarly, distal spikelets produced 'better quality' caryopses (higher dry weights and starch, carbohydrate and amino acid contents) due to a greater efficiency with regard to converting soluble assimilates into reserve structural matter (Mohapatra *et al.* 1993), i.e. they were more efficient sinks, and the intrinsic concentration of assimilates did not determine the growth of paracladia (Mohapatra & Sahu 1991; Patel & Mohapatra 1992). Similar observations, both within paracladia and within spikelets, have been made in a number of grain crops; *Sorghum bicolor* (L.) Moench cv. DeKalb 'DK 46' and cv. Pioneer '8500' (Heiniger *et al.* 1993), *Triticum aestivum* L. (Arber 1934; Miralles & Slafer 1995) and *Avena sativa* L. (Tibelius & Klinck 1987).

Patel & Mohapatra (1992) determined that patterns of development in the paracladial zone of rice were less heterogeneous with the application of cytokinins, and that heterogeneity could be exacerbated by the application of IAA. They argued that an effective dilution of endogenous cytokinins would occur in proximal paracladia due to the larger number of spikelets in this region, and so growth would be limited in proximal paracladia to a greater extent than in distal paracladia. Thus they suggested that heterogeneity in the paracladial zone of seminiferous grasses might be explained by a hierarchy of phytohormonal dominance (see Wareing & Phillips (1990) for the proposed mechanisms and modes of action of phytohormones involved in apical dominance).

As plantlets are individual shoot systems possessing true leaves, each leaf possessing a suppressed axillary bud (evident as a lack of tillering within plantlets; Chapter 3), apical dominance occurs within each plantlet. On surgical manipulation of the paracladial zone, the relative growth rate of proximal plantlets was higher than un-manipulated controls; behaviour not mirrored by distal plantlets and indicative of the operation of apical dominance (Chapter 3). Also, distal plantlets consistently attained higher dry weights, irrespective of atmospheric CO_2 concentration and/or nutrient availability (Chapter 4). Zollikofer (1939; in German, cited by Gustafsson 1946a) determined that application of growth promoting phytohormones increased the production of dry matter in the paracladial zone of a biotype of *Poa alpina*, although no distinction was made between distal and proximal halves. Thus a system of apical dominance via the action of phytohormones could explain the data presented in the present study, although no experimental work directly concerning plant growth regulators was carried out. In the system suggested here, presumably the first plantlets to be exerted (i.e. distal plantlets) would gain dominance due to their earlier arrival in an environment favourable for photosynthesis and growth. This may also be a cause of the higher proportion of aborted spikelets observed in the proximal paracladia (Patel & Mohapatra 1992), although this issue may be complicated by physical constraints on the growth of the young synflorescence (Chapter 5).

The proposed control of plantlet growth by apical dominance is somewhat analogous to fruit production in many seminiferous plants that may initiate more fruits than can be supported by the available resources. A proportion of these fruits grow 'normally', with the remainder being relatively undeveloped (Lee 1980; Stephenson 1981). Under-developed fruits are viewed as a reserve which may resume development should either more resources become available or the more highly developed fruits dehisce (Lee 1980). Should distal plantlets of *Poa alpina* be lost, perhaps due to dehiscence or grazing, proximal plantlets may then attain an equivalent dry weight (and did so when distal plantlets were experimentally removed; Chapter 3) and presumably become as effective as propagules. There can be little doubt that larger proximal plantlets than actually observed in intact paracladial zones would impose a proportionally larger demand on the culm for xylem sap, which would be expensive in terms of water and nutrients should the distal plantlets still be present. Thus by restricting the growth of proximal plantlets apical dominance may restrict the demand for resources via the culm, indirectly aiding the maintenance of xylem transport evident in older culms (40 d). This results in a proportion of plantlets at dehiscence which are vigorous and well able to establish, with the remainder held in reserve for

quick growth - rather than all of the plantlets being water stressed to a relatively high degree and less likely to establish and compete effectively. Thus apical dominance in the paracladial zone may ensure the most beneficial use of limiting resources, and could be advantageous for pseudoviviparous reproduction.

That spikelet abortion is influenced by pressure in the observed manner (see Chapter 5) suggests that the exertion of the paracladial zone from a constraining pseudostem is not an ideal habit. However, the pseudostem has two advantageous functions which appear to offset the disadvantage of increased spikelet abortion. Firstly, the pseudostem lifts the leaf blades above competitors without lifting the apical meristem into the reach of grazing animals. Also, the young synflorescence is mechanically dependent on the support conferred by the leaf sheaths, as these effectively increase the diameter of culm internodes and hence increase flexural rigidity of the synflorescence (Niklas 1992). The heterogeneity in both plantlet dryweight and abortion suggests that the role of phytohormones in the pseudoviviparous system has perhaps been previously under-estimated. A direct study of these growth regulators following protocols such as that of Suzuki *et al.* (1981) would have been carried out during the present study, had time-constraints permitted.

The architecture of the paracladial zone proved useful not only as a means of delimiting arbitrary zones for physiological investigation, but also in providing further insight into the function and construction of sympodially arranged structures. More precisely, the data presented here describe a system which was composed not simply of a single type of sympodial unit, but by an arrangement of both dichasial and monochasial units. In terms of function, the length of rachis internodes was proportional to the number of spikelets borne on each node. This arrangement in seminiferous grasses would allow for effective spacing of the denser proximal paracladia, presumably facilitating air movement for pollination. The selective advantage of possessing a larger proportion of spikelets on proximal paracladia which requires spacing remains unclear. One possible advantage may be a lower centre of gravity, which would effectively reduce the bending moment acting on the culm and aid the support of the paracladial zone whilst maximising caryopsis production. Another possibility is that spikelets are more evenly distanced from parental sources, acting to equalise flux between the source and numerous sinks and therefore resource allocation between caryopses (flux being partly a function of path length between source and sink; Cook & Evans 1983, Farrar 1993b). Retained by

pseudoviviparous biotypes, this architecture is likely to be equally beneficial - the spacing of paracladia ensuring less shading between plantlets and thus maximising photosynthesis. However, the relationship between space and shading is dynamic, with the high nutrient treatment in the present study resulting in larger plantlets (*c.f.* low nutrient treatment; Chapter 4), younger proximal plantlets being 'leafier' (in terms of LAR) and the number of leaves per plantlet increasing over time.

The plant's response to resource availability determines the competitive abilities of the plant and ultimately the ecological success of the biotype. With regard to the stress-tolerating, competitive and ruderal growth strategies of Grime (1974, 1977), Hunt *et al.* (1991) determined that plants of the competitive growth strategy showed the greatest increase in photosynthetic capacity in elevated CO_2, and predicted that habitats in which plants of different growth strategies are in direct competition are likely to experience a dramatic change in floristic composition with changing atmospheric CO_2 concentration. However, both ruderal and stress tolerating species are also capable of large positive responses (Stirling *et al.* 1997, 1998) with the response being species-specific (e.g. the contrasting response of *Agrostis capillaris c.f. Festuca vivipara*; Baxter *et al.* 1994a) producing variation in the response of members within each growth strategy. Therefore these growth strategies are not a reliable guide to the ability of a species to respond to elevated CO_2 (Stirling *et al.* 1997).

Clearly, the growth of the reproductive phase of the lifecycle of *Poa alpina* is suppressed in conditions of elevated CO_2 concentration and low nutrient availability, as is the vegetative phase (Baxter *et al.* 1997). Therefore *Poa alpina* plants exposed to these growth conditions are at a disadvantage compared to species that show either less acclimatory loss, no change, or a gain in photosynthetic capacity in elevated CO_2. However, a specific prediction as to the ecological performance of this biotype cannot be inferred from the data presented in the present study, as a number of other plant growth responses and also geographical location will determine the relative performance of a species.

The germination and emergence of seeds may be altered by elevated atmospheric CO_2 concentrations, with species-specific responses (Ziska & Bunce 1993). Evidence also suggests that elevated atmospheric CO_2 concentrations may affect the spatio-temporal pattern of root growth and root turnover in ecosystems (Fitter *et al.* 1996). In addition to such direct growth responses to elevated atmospheric CO_2

concentrations, the global climate is predicted to experience increases in temperature and concomitantly higher frequency of heavy precipitation events (Fowler & Hennessy 1995). Perhaps most importantly the frequency, magnitude and temporal characteristics of climate change events are specific to particular regions (Tegart *et al.* 1990). Hence the response of a particular ecosystem to elevated CO_2 depends not only on the species present, but also on the climatic and edaphic factors characteristic of it's geographical location. Therefore, in order to predict the fitness of a species such as *Poa alpina* in future climatic conditions, a microcosm of it's particular habitat must be investigated under predicted future growth conditions for that habitat, and over a time-scale that incorporates repeated reproductive events.

Aside from CO_2 concentration and nutrient use efficiency, climate change may possibly have more fundamental implications for the operation of the prolification process itself. Interactions between daylength and predicted increases in mean global temperature during the next century (1.5 - 4.6 °C; Manabe & Wetherald 1975; Allen 1980; Manabe & Stouffer 1980; Hansen *et al.* 1981) could conceivably result in conditions in which the secondary floral induction requirement is more readily met (see Chapter 1), and complete seminiferous reproduction is favoured. Certainly the biotype of *Poa alpina* examined in the present study produced mature sexual organs - dehiscing anthers and exerted styles (Chapter 3) - although no seed was observed and plantlets are liable to out-compete seeds on initial establishment (Harmer & Lee 1978a; see also Section 1.4.4). Thus, a reversion to purely seminiferous reproduction, and the growth of sexual propagules, could lend the plant an advantage in a changing environment by allowing the expression of greater genetic variation. Therefore, an investigation into the effects of temperature in concert with daylength and changing CO_2 concentration could provide further insight into the relationship between sexual and asexual reproduction, and the potential of the pseudoviviparous grasses to react to a changing climate.

References

Aberg E (1940) Viviparous forms of *Agrostis*. Annals of the Agricultural College of Sweden 8:461-463

Akai S and Fukutomi M (1966) Change in the external morphology of graminaceous plants attacked by *Sclerophthora macrospora*. Phytomorphology 16:291-301

Allen R (1980) The impact of CO_2 on world climate. Environment 22:6-38

Allen SE (1974) Chemical Analysis of Ecological Materials. Blackwell. Oxford

Anton AM and Connor HE (1995) Floral biology and reproduction in *Poa* (Poeae: Graminae). Australian Journal of Botany 43:577-599

Aparicio-Tejo PM and Boyer JS (1983) Significance of accelerated leaf senescence at low water potentials for water loss and grain yield in maize. Crop Science 23:1198-1202

Appalachian Mountain Club (1964) Mountain Flowers of New England. Boston, Mass.

Arber A (1930) Studies in the graminae. IX. i. The nodal plexus. ii. Amphivasal bundles. Annals of Botany XLIV(CLXXV):593-620

Arber A (1934) The Gramineae. A Study of Cereals, Bamboo and Grass. Cambridge University Press. Cambridge

Armstrong SF (1917) British Grasses and their Employment in Agriculture. Cambridge University Press

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant Cell and Environment 14:869-875

Atkinson CJ and Farrar (1983) Allocation of photosynthetically-fixed carbon in *Festuca ovina* L. and *Nardus stricta* L. New Phytologist 95:519-531

Babos K (1993) Tyloses formation and the state of health of *Quercus petraea* trees in Hungary. IAWA Journal 14(3):239-243

Backer CA (n.d.[1930]) The Problem of Krakatao as seen by a Botanist. The Hague

Barry RG (1981) Mountain weather and climate. Methuen. New York

Batey T (1982) Nitrogen cycling in upland pastures of the U.K. Philosophical Transactions of the Royal Society of London Series B. – Biological Sciences 296:551-556

Baxter R, Ashenden TW, Sparks TH and Farrar JF (1994a) Effects of elevated carbon dioxide on three montane grass species. I. Growth and dry matter partitioning. Journal of Experimental Botany 45(272):305-315

Baxter R, Gantley M, Ashenden TW and Farrar JF (1994b) Effects of elevated carbon dioxide on three grass species from montane pasture. II. Nutrient uptake, allocation and efficiency of use. Journal of Experimental Botany 45(278):1267-1278

Baxter R, Bell SA, Sparks TH, Ashenden TW and Farrar JF (1995) Effects of elevated CO_2 concentrations on three montane grass species. III. Source leaf metabolism and whole plant carbon partitioning. Journal of Experimental Botany 46(289):917-929

Baxter R, Ashenden TW and Farrar JF (1997) Effect of elevated CO_2 and nutrient status on growth, dry matter partitioning and nutrient content of *Poa alpina* var. *vivipara* L. Journal of Experimental Botany 48(312):1477-1486

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. Annual Revue of Ecological Systematics 21:167-196

Beadle CL, Ludlow MM and Honeysett JL (1993) Water relations. *In.* Hall DO, Scurlock JMO, Bolhàr-Nordenkampf, Leegood RC and Long SP (eds.) Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. pp. 113-127. Chapman and Hall. London

Bell AD (1991) Plant Form: An Illustrated Guide to Flowering Plant Morphology. Oxford

Beuret E (1976) Viviparie chez *Trisetum* subgen. *Distichotrisetum* chrtek. Bull. Soc. Neuchatel. Sci. Nat. 99:93-96

Bolhàr-Nordenkampf HR and Draxler G (1993) Functional leaf anatomy. *In.* Hall DO, Scurlock JMO, Bolhàr-Nordenkampf, Leegood RC and Long SP (eds.) Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. pp. 91-112. Chapman and Hall. London

Bradshaw AD, Chadwick MJ, Jowett D and Snaydon RW (1964) Experimental investigations into the mineral nutrition of several grass species. IV. Nitrogen level. Journal of Ecology 52(3):665-676

Byrne MC, Nelson CJ and Randall DD (1981) Ploidy effects on anatomy and gas exchange of tall fescue leaves. Plant Physiology 68:891-893

Cain SA (1944) Foundations of Plant Geography. New York and London

Cámara-Hernández J and Rua GH (1991) The synflorescence of Poaceae. Beitr. Biol. Pflanzen 66:297-311

Canny (1997) Tyloses and the maintenance of transpiration. Annals of Botany 80(4):565-570

Casady AJ (1969) Propagation from proliferated sorghum spikelets caused by head smut infection. Crop Science 9:381-382

Chaffey NJ (1983) Structure/function relationships in the Graminaceous leaf. Ph.D. Thesis. University of Durham

Chaffey NJ and Pearson JA (1985) Presence of tyloses at the blade sheath junction in senescing leaves of *Lolium temulentum* L. Annals of Botany 56(6):761-770

Chapin FS III and Bloom A (1976) Phosphate absorption: adaptation of tundra graminoids to a low temperature, low phosphorus environment. Oikos 26:111-121

Chapin FS III and Shaver GR (1985) Individualistic growth response of tundra plant species to environmental manipulations in the field. Ecology 66:564-576

Chapin FS III and Shaver GR (1989) Differences in growth and nutrient use among arctic plant growth forms. Functional Ecology 3:81-89

Chapman GP (1996) The Biology of Grasses. CAB international. Oxon.

Chattaway MM (1949) The development of tyloses and secretion of gum in heartwood formation. Australian Journal of Research 2:227-241

Clay K (1986) Induced vivipary in the sedge *Cyperus virens* and the transmission of the fungus *Balansia cyperi* (Clavicipitaceae). Canadian Journal of Botany 64:2984-2988

Clayton WD and Renvoize SA (1986) Genera Graminum: Grasses of the World. Kew Bulletin Additional Series XIII. HMSO, London

Cochard H and Tyree MT (1990) Xylem dysfunction in *Quercus* – vessel sizes, tyloses, cavitation and seasonal changes in embolism. Tree Physiology 6(4):393-407

Coldrake PD and Pearson CJ (1985a) Panicle differentiation and spikelet number related to size of panicle in *Pennisetum americanum*. Journal of Experimental Botany 36(166):833-840

Coldrake PD and Pearson CJ (1985b) Whole plant development and dry weight accumulation of pearl millet as affected by nitrogen supply. Field Crops Research 11:171-184

Coldrake PD and Pearson CJ (1986) Environmental influences on panicle differentiation and spikelet number of *Pennisetum americanum*. Journal of Experimental Botany 37(179):865-875

Cook MG and Evans LT (1983) The roles of sink size and location in the partitioning of assimilates in wheat ears. Australian Journal of Plant Physiology 10(3):313-327

Cottrell JE, Easton RH, Dale JE, Wasworth AC, Adam JS, Child RD and Hoad GV (1985) A comparison of spike and spikelet survival in mainstem and tillers of barley. Annals of Applied Biology 106:365-377

Culpan G (1995) Pests, Diseases and Common Problems. Hamlyn. London

Debata A and Murty KS (1981) Relation between leaf and panicle senescence in rice. Indian Journal of Experimental Biology 19:1183-1184

Debata A and Murty KS (1982) Panicle senescence in rice. Current Science 51(6):296-298

Dhinsa RS, Plumb-Dhinsa P and Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany 32(126):93-101

DOE (1988) Possible Impacts of Climate Change on the Natural Environment of the United Kingdom. UK Department of the Environment, London

Dubois M, Gilles KA, Hamilton JK, Rebus PA and Smith F (1956) Colorimetric method for the determination of sugars and related substances. Analytic Chemistry 28:330-356

Elmahjoub M, Lepicard D and Moreau M (1984) Origin of tyloses in melon (*Cucumis melo* L.) in response to a vascular *Fusarium*. IAWA bulletin 6(3):269-271

Elmqvist T and Cox PA (1996) The evolution of vivipary in flowering plants. Oikos 77:3-9

Emiliani C (1992) Planet Earth: Cosmology, Geology, and the Evolution of Life and the Environment. Cambridge

Exo A (1916) *Poa alpina* und die Ercheinung der Viviparie bei ihr. Diss. University of Bonn

Falk S, Hertz H and Virgin H (1958) On the relation between turgor pressure and rigidity. I. Experiments on resonance frequency and tissue rigidity. Physiologia Plantarum 11:802-817

Farrar JF (1993a) Carbon partitioning. *In.* Hall DO, Scurlock JMO, Bolhàr-Nordenkampf, Leegood RC and Long SP (eds.) Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. pp. 232-246. Chapman and Hall. London

Farrar JF (1993b) Sink strength: What is it and how do we measure it? Introduction. Plant Cell and Environment 16:1013-1046

Farrar JF and Williams ML (1991) The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. Plant, Cell and Environment 14:819-830

Ferrier JM and Dainty J (1977) A new method for measuring hydraulic conductivity and elastic coefficients in higher plants using an external force. Canadian Journal of Botany 55:858-866

Ferrier JM and Dainty J (1978) The external force method for measuring hydraulic conductivity and elastic coefficients in higher plant cells: application to multi-layer tissue sections and further theoretical development. Canadian Journal of Botany 56:22-26

Fitter AH, Self GK, Wolfenden J, Vuuren MMI van, Brown TK, Williamson L, Graves JD and Robinson D (1996) Root production and mortality under elevated atmospheric carbon dioxide. Plant and Soil 187:299-306

Flovik K (1938) Cytogenetical studies of arctic grasses. Hereditas 24:265-376

Foreman EMF (1971) Growth, flowering and vivipary in arctic & alpine populations of *Deschampsia caespitosa* (L.) Beauv., *Poa alpina* L., and *Trisetum spicatum* (L.) Richt. Ph.D. Thesis. University of Colorado

Fowler AM and Hennessy KJ (1995) Potential impacts of global warming on the frequency and magnitude of heavy precipitation. Natural Hazards 11:283-303

Frederiksen S (1981) *Festuca vivipara* (Poaceae) in the North Atlantic area. Nordic Journal of Botany 1:277-292

Galinat WC and Naylor AW (1951) Relation of photoperiod to inflorescence proliferation in *Zea mays* (L.). American Journal of Botany 38:38-47

Gammon RH, Sundquist ET and Fraser PJ (1985) Atmospheric carbon dioxide and the global carbon cycle. US DOE/ER-0237, National Technical Information Service, Washington, DC

Garbutt K and Bazzazz (1984) The effects of elevated CO_2 on plants. III. Flower, fruit and seed production and abortion. New Phytologist 98:433-446

Garcia del Moral MB, Jimenez Tejada MP, Garcia del Moral LF, Ramos JM, Roca de Togores F and Molina Cano JL (1991) Apex and ear development in relation to the number of grains on the main stem and ears in spring barley. Journal of Agricultural Science 117:39-45

Gepstein S (1988) Photosynthesis. *In.* Noodén LD and Leopold AC (eds.) Senescence and Aging in Plants. pp. 85-104. Academic Press. San Diego

Godron DA (1879) Études Morphologiques sur la Famille des Graminées. Rev. d. sci. nat., Montpellier 7:393-411

Gram K (1961) The inflorescence of the grasses. Bot. Tidsskr. 56:293-313

Grime JP (1974) Vegetation classification by reference to strategies. Nature 250:26-31

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169-1194

Gustafsson Å (1946a) Apomixis in Higher Plants. Lund Universitets Årsskrift N.F. Avd. 2. Bd 42. Nr. 3. Lund

Gustafsson Å (1946b) The plant species in relationship to polyploidy and apomixis. Hereditas 32:444-448

Hansen J, Johnson D, Lacis A, Lebedeff S, Lee P, Rind D and Russell G (1981) Climate impact of increasing carbon dioxide. Science 213:957-966

Harmer R (1978) Some physiological and ecological aspects of reproduction in *F. vivipara*. Ph.D. Thesis. University of Manchester

Harmer R and Lee JA (1978a) The growth and nutrient content of *Festuca vivipara* (L.) Sm. plantlets. New Phytologist 80:99-106

Harmer R and Lee JA (1978b) The germination and viability of *Festuca vivipara* (L.) Sm. plantlets. New Phytologist 81:745-751

Hartung ME (1946) Chromosome numbers in *Poa*, *Agropyron*, and *Elymus*. American Journal of Botany 33:516-531

Haskel G (1951) Plant chromosome-races and their ecology in Great Britain. Nature 167:628-629

Haskel G (1952) Polyploidy, ecology and the British flora. Journal of Ecology 40:265-282

Hawley P (1989) Grasses. Shire Publications. Buckinghamshire

Hegi G (1930) Alpine Flowers. The Most Common Alpine Plants of Switzerland, Austria and Bavaria. Blackie and Son. London

Hegi G (1935) Illustrierte Flora von Mittle-Europa. 2. Aufl., Band 1, Hrsg. Karl Suessenguth, J.F. Lehmanns Verlag, München

Heide OM (1986) Long day control of flowering in *Poa nemoralis* in controlled and natural environments. New Phytologist 104:225-232

Heide OM (1987) Photoperiodic control of flowering in *Dactylis glomerata*, a true short-long-day plant. Physiologia Plantarum 70:523-529

Heide OM (1988) Environmental modification of flowering and viviparous proliferation in *Festuca vivipara* and *F. ovina*. Oikos 51:171-178

Heide OM (1989) Environmental modification of flowering and viviparous proliferation in seminiferous and viviparous arctic populations of two *Poa* species. Arctic and Alpine Research 21(3):305-315

Heiniger RW, Vanderlip RL and Kofoid KD (1993) Caryopsis weight patterns within the sorghum panicle. Crop Science 33(3):543-549

Hiesey WM (1953) Growth and development of species and hybrids of *Poa* under controlled temperatures. American Journal of Botany 40:205-221

Hind G (1993) Thylakoid components and processes. *In.* Hall DO, Scurlock JMO, Bolhàr-Nordenkampf, Leegood RC and Long SP (eds.) Photosynthesis and

Production in a Changing Environment: a Field and Laboratory Manual. pp. 283-297. Chapman and Hall. London

Hitch PA and Sharman BC (1971) The vascular pattern of festucoid grass axes, with particular reference to nodal plexi. Botanical Gazette 132:38-56

Hsiao AI and McIntyre GI (1988) Induction of vivipary in *Avena fatua*. Physiologia Plantarum 73:128-133

Hubbard CE (1992) Grasses: A Guide to their Structure, Identification, Uses and Distribution in the British Isles. Third Edition. Penguin, London

Hung KT and Kao CH (1997) Lipid peroxidation in relation to senescence of maize leaves. Journal of Plant Physiology 150(3):283-286

Hung KT and Kao CH (1998) Involvement of lipid peroxidation in methyl jasmonate promoted senescence in detached rice leaves. Plant Growth Regulation 24(1):17-21

Hunt R, Hand DW, Hannah MA and Neal AM (1991) Response to CO_2 enrichment in 27 herbaceous species. Functional Ecology 5:410-421

Hutson RA and Smith IM (1980) Phytoalexins and tyloses in tomato cultivars infected with *Fusarium oxysporum* f. sp. *lycopersici* or *Verticillium albo-atrum*. Physiological Plant Pathology 17(3):245-257

Irish EE (1997) Class II tassel seed mutations provide evidence for multiple types of inflorescence meristems in maize (Poaceae). American Journal of Botany 84(11):1502-1515

Jenkin TJ (1922) Notes on vivipary in *Festuca ovina*. Rep. Bot. Soc. & EC. 6:418-432

Joarder N & Eunus AM (1980) Correlation of vascular bundle and panicle characters in rice. Kaliksan, Philippine Journal of Biology 9(1):93-95

Johnson AW and Packer JG (1965) Polyploidy and environment in arctic Alaska. Science 148:237-239

Joseph MC, Randall DD and Nelson CJ (1981) Photosynthesis in polyploid tall fescue II. Photosynthesis and ribulose-1,5-bisphosphate carboxylase of polyploid tall fescue. Plant Physiology 68:894-898

Junttila O (1985) Experimental control of flowering and vivipary in timothy (*Phleum pratense*). Physiologia Plantarum 63:35-42

Kelly MO and Davies PJ (1988) The control of whole plant senescence. CRC Critical Reviews in Plant sciences 7:139-173

Kennedy PB (1929) Proliferation in *Poa bulbosa*. Journal of the American Society of Agronomists 21:80-91

Kernich GC and Halloran GM (1996) Temperature effects on the duration of the spikelet growth phase and spikelet abortion in barley. Journal of Agronomy and Crop Science 176:23-29

Kinsman EA, Lewis C, Davies MS, Young JE, Francis D, Vilhar B and Ougham (1997) Elevated CO_2 stimulates cells to divide in grass meristems: a differential effect in two natural populations of *Dactylis glomerata*. Plant Cell and Environment 20:1309-1316

Knox RB (1967) Apomixis: seasonal and population differences in a grass. Science 157:325-326

Körner Ch. and Diemer M (1987) In *situ* photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. Functional Ecology 1:179-194

Koch K (1972) Fluorescence microscopy: instruments, methods and applications. Ernst Leitz GmbH. Wetzlar

Kokubo A, Kuraishi S and Sakurai N (1989) Culm strength of Barley – correlation among maximum bending stress, cell-wall dimensions, and cellulose content. Plant physiology 91(3):876-882

Kokubo A, Sakurai N, Kuraishi S and Takeda K (1991) Culm brittleness of barley (*Hordeum vulgare* L.) mutants is caused by smaller number of cellulose molecules in cell wall. Plant Physiology 97(2):509-514

Kramer PJ and Boyer JS (1995) Water Relations of Plants and Soils. Academic Press. San Diego

Lang A (1965) Physiology of flowering initiation. Encycl. Plant Physiol. 15(1):1380

Larcher W and Bauer H (1981) Ecological significance of resistance to low temperature. *In.* Lange OL, Nobel PS, Osmond CB and Ziegler H (eds.) Encyclopaedia of Physiology. pp. 403-437. Vol. 12A. Springer-Verlag. Berlin

Lawlor DW (1993) Photosynthesis: Molecular, Physiological and Environmental Processes. Second Edition. Longman Group. Essex, U.K.

Lee BT, Martin P and Bangerth F (1988) Phytohormone levels in the florets of a single wheat spikelet during pre-anthesis development and relationships to grain set. Journal of Experimental Botany 39:927-933

Lee JA and Harmer R (1980) Vivipary, a reproductive strategy in response to environmental stress? Oikos 35(2):254-265

Lee TD (1980) Extrinsic and intrinsic factors controlling reproduction in an annual plant. Ph.D. Thesis. University of Illinois, Champaign

Leegood RC (1993) Carbon metabolism. *In.* Hall DO, Scurlock JMO, Bolhàr-Nordenkampf, Leegood RC and Long SP (eds.) Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. pp. 247-266. Chapman and Hall. London

Leopold AC (1961) Senescence in plant development. The death of plant or plant parts may be of positive ecological or physiological value. Science 134:1727-1732

Li LY (1950) Vivipary in some Chinese plants. Botanical Gazette 111:358-359

Lichtenthaler HK and Wellburn AR (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemistry Society Transactions 11:591-592

Lindsey K and Jones MGK (1992) Plant Biotechnology in Agriculture. Wiley. Chichester

Linnaeus C (1737) Flora Lapponica. Amstelaedami

Linnaeus C (1753) Species Plantarum. Stockholm

Linnaeus C (1759) Flora Alpina. Amoenitates Academicae. Vol. 4. Holmiae

Lo Gullo MA and Salleo S (1991) Different methods for measuring xylem cavitation and embolism: a comparison. Annals of Botany 67:417-424

MacGillivray CW and Grime JP (1995) Genome size predicts frost-resistance in British herbaceous plants – implications for rates of vegetation response to global warming. Functional Ecology 9(2):320-325

Makino A, Mae T and Ohira K (1984) Relation between ribulose-1,5-bisphosphate carboxylase in rice leaves from emergence through senescence. Plant and Cell Physiology 25(3):429-437

Manabe S and Stouffer RJ (1980) A CO_2 climate sensitivity study with a mathematical model of the global climate. Nature 282:491-493

Manabe S and Wetherald RT (1975) The effects of doubling the carbon dioxide concentration on the climate of a general circulation model. Journal of Atmospheric Science 32:3-15

Mangelsdorf PC (1930) The inheritance of dormancy and premature germination in maize. Genetics 16:462-494

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press. London

Masters MT (1869) Vegetable Teratology: an Account of the Principal Deviations from the Usual Structure of Plants. The Ray Society. London

McClure F (1966) The Bamboos: a Fresh Perspective. Harvard University Press. Cambridge

Merchand LC and McLean A (1965) Observations on vegetative proliferations in *Agropyron spicatum*. Canadian Journal of Botany 43:1533-1537

Miralles DJ and Slafer GA (1995) Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. Field Crops Research 43:55-66

Mohapatra PK and Sahu SK (1991) Heterogeneity of primary branch development and spikelet survival in rice panicle in relation to assimilates of primary branches. Journal of Experimental Botany 42(240):871-879

Mohapatra PK, Patel R and Sahu SK (1993) Time of flowering affects grain quality and spikelet partitioning within the rice panicle. Australian Journal of Plant Physiology 20(2):231-241

Moore DM and Doggett MC (1976) Pseudo-vivipary in Fuegian and Falkland Island grasses. British Antarctic Survey Bulletin 43:103-110

Müntzing A (1933) Apomictic and sexual seed formation in *Poa*. Hereditas 17:131-154

Müntzing A (1936) The evolutionary significance of autoploidy. Hereditas 21:263-378

Müntzing A (1940) Further studies on apomixis and sexuality in *Poa*. Hereditas 26:115-190

Müntzing A (1946) Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. Hereditas 32:127-129

Müntzing A (1954) The cytological basis of polymorphism in *Poa alpina*. Hereditas 40:459-516

Müntzing A (1980) Mode of propagation and chromosomal peculiarities in Scotch material of *Poa alpina*. Hereditas 92:291-296

Müntzing A and Nygren A (1955) A new diploid variety of *Poa alpina* with two accessory chromosomes at meiosis. Hereditas 41:405-422

Murty PSS and Murty KS (1981) Variation in concentration of growth regulators in high and low sterile rice cultures. Indian Journal of Experimental Biology 19:591-592

Nielson G (1941) Grass studies. V. Observations on proliferation. Botanical Gazette 103:177-181

Niklas KJ (1991) Effects of tissue volume and location on the mechanical consequences of dehydration of petioles. American Journal of Botany 78(3):361-369

Niklas KJ (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press. Chicago

Niklas KJ (1994) Plant Allometry: The Scaling of Form and Process. University of Chicago Press. Chicago

Nikon Corporation ([n.d.]) Fluorescence microscopes: technical bulletin. Nikon, Japan

Nilsson SB, Hertz SH and Falk S (1958) On the relation between turgor pressure and tissue rigidity. II. Theoretical calculations on model systems. Physiologia Plantarum 11:818-837

Nishiyama I (1934) The genetics and cytology of certain cereals. VI. Chromosome behaviour and its bearing on inheritance in triploid *Avena* hybrids. Memoirs of the Coll. of Agricult., Kyoto Imp. Univ. 32:1-157

Nishiyama I (1983) Male-sterility caused by cooling treatment at the young microspore stage in rice plants. 25. The distance between panicle base and spikelet, and the difference in susceptibility to coolness among spikelets on the panicle. Japanese Journal of Crop Science 52(2):200-206

Noodén LD (1988) The phenomena of senescence and aging. *In.* Noodén LD and Leopold AC (eds.) Senescence and Aging in Plants. pp. 2-38. Academic Press. San Diego

Nygren A (1949) Studies on vivipary in the genus *Deschampsia*. Hereditas 35:27-32

Nygren A (1967) Apomixis in angiosperms. *In*: Ruhland W (ed.) Handbuch der Pflanzenphysiologie. Springer-Verlag, Berlin and New York

Nygren A and Almgård G (1962) On the experimental control of vivipary in *Poa*. Annual Revue of the Agricultural College of Sweden 28:27-36

Obst JR, Sachs IB and Kuster TA (1988) The quantity and type of lignin in tyloses of Bur Oak (*Quercus macrocarpa*). Holzforschung 42(4):229-231

Ong CK and Marshall C (1975) Assimilate distribution in *Poa annua*. Annals of Botany 39:413-421

Ong CK, Corvill KE and Marshall C (1978) Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L. Annals of Botany 42(182):855-862

Ookawa T and Ishihara K (1992) Varietal difference of physical characteristics of the culm related to lodging resistance in paddy rice. Japanese Journal of Crop Science 61(3):419-425

Ookawa T and Ishihara K (1993) Varietal difference of the cell-wall components affecting the bending stress of the culm in relation to the lodging resistance in paddy rice. Japanese Journal of Crop Science 62(3):378-384

Ookawa T and Ishihara K (1997) Genetic characteristics of the breaking strength of the basal culm related to lodging resistance in a cross between Koshihikari and Chugoku 117. Japanese Journal of Crop Science 66(4):603-609

Oparka KJ and Read ND (1994) The use of fluorescent probes for studies of living plant cells. *In.* Harris N and Oparka KJ (eds.) Plant Cell Biology: a Practical Approach. pp. 27-50. Oxford University Press. Oxford

O'Toole JC, Hsiao TC and Namuco OC (1984) Panicle water relations during water stress. Plant Science Letters 33:137-143

Patel R and Mohapatra PK (1992) Regulation of spikelet development in rice by hormones. Journal of Experimental Botany 43(247):257-262

Pearce RB and Holloway PJ (1984) Suberin in the sapwood of Oak (*Quercus robur* L.) – its composition from a compartmentalization barrier and its occurrence in tyloses in undecayed wood. Physiological Plant Pathology 24(1):71-81

Peoples MB and Dalling MJ (1988) The interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. *In.* Noodén LD and Leopold AC (eds.) Senescence and Aging in Plants. pp. 182-212. Academic Press. San Diego

Philipson WR (1934) The morphology of the lemma in grasses. New Phytologist 33:359-361

Philipson WR (1935) Abnormal spikelets in the genus *Agrostis* (L.). Journal of Botany 73:65-75

Pitt RE (1982) Models for the rheology and statistical strength of uniformly stressed vegetative tissue. Transactions of the American Society of Agricultural Engineers 25:1776-1784

Polle A, Eiblmeier M, Sheppard L and Murray M (1997) Responses of antioxidative enzymes to elevated CO_2 in leaves of beech (*Fagus sylvatica* L.) seedlings under a range of nutrient regimes. Plant Cell and Environment 20:1317-1321

Poovaiah BW (1988) Calcium and senescence. *In.* Noodén LD and Leopold AC (eds.) Senescence and Aging in Plants. pp. 369-385. Academic Press. San Diego

Pope MN (1949) Viviparous growth in immature barley kernels. Journal of Agricultural Research 78:295

Porter HK, Pal N and Martin RV (1950) Physiological studies in plant nutrition. XV. Assimilation of carbon by the ear of barley and its relation to the accumulation of dry matter in the grain. Annals of Botany 14:55-68

Rafarel CR, Ashenden TW and Roberts TM (1995) An improved solardome system for exposing plants to elevated CO_2 and temperature. New Phytologist 131:481-490

Rattray EAS, Paterson E and Killham K (1995) Characterisation of the dynamics of C-partitioning within *Lolium perenne* and to the rhizosphere microbial biomass using ^{14}C pulse chase. Biology and Fertility of Soils 19(4):280-286

Reed AJ and Singletary GW (1989) Roles of carbohydrate supply and phytohormones in maize kernal abortion. Plant Physiology 91:986-992

Richter K (1890) Plantae Europaeae. 1. Leipzig

Rioux D, Chamberland H, Simard M and Ouellette GB (1995) Suberized tyloses in trees – an ultrastructural and cytochemical study. Planta 196(1):125-140

Rioux D, Nicole M, Simard M and Ouellette GB (1998) Immunocytochemical evidence that secretion of pectin occurs during gel (gum) and tylosis formation in trees. Phytopathology 88(6):494-505

Rothera SL and Davy AJ (1986) Polyploidy and habitat differentiation in *Deschampsia cespitosa*. New Phytologist 102:449-467

Russell RS (1940) Physiological and ecological studies on an arctic vegetation. III. Observations on carbon assimilation, carbohydrate storage and stomatal movement in relation to the growth of plants on Jan Mayen Island. Journal of Ecology 28:289-309

Russell RS and Wellington PS (1940) Physiological and ecological studies on an arctic vegetation. I. The vegetation of Jan Mayen Island. Journal of Ecology 28:153-179

Ryle GJA (1963) Studies in the physiology of flowering timothy (*Phleum pratense* L.). IV. Effects of shoot age and nitrogen level on the size of the inflorescence. Annals of Botany 27(107):467-479

Saitoh T, Ohtani J and Fukazawa K (1992) SEM observations on the occurrence and morphology of tyloses. Mokuzai Gakkaishi 38(3):215-221

Saitoh T, Ohtani J and Fukazawa K (1993) The occurrence and morphology of tyloses and gums in the vessels of Japanese hardwoods. IAWA Journal 14(4):359-371

Salleo S, Gullo MA lo, Paoli D de and Zippo M (1995) Xylem recovery from cavitation – induced embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytologist 132(1):47-56

Sano Y and Fukazawa K (1991) Structural differences of tyloses in *Fraxinus mandshurica* var. *japonica* and *Kalopanax pictus*. IAWA Bulletin 12(3):241-249

Savile DBO (1972) Arctic adaptations in plants. Monograph No. 6. Canadian Department of Agriculture, Ottawa

Schmitt U and Liese W (1994) Wound tyloses in *Robinia pseudoacacia* L. IAWA Journal 15(2):157-160

Schröter C ([n.d.]) Coloured Vade-Mecum to the Alpine Flora. Albert Raustein. Zuerich

Seneweera SP and Conroy JP (1997) Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO_2 and phosphorus nutrition. Developments in Plant and Soil Sciences 78:873-878

Seo SW, Chamura S and Hayakawa T (1981) Panicle senescence and its prevention in rice. 1. A decrease in dehydrogenase activity in spikelet following the progress of ripening and its varietal differences. Japanese Journal of Crop Science 50(2):91-97

Silk WK, Wang LL and Cleland RE (1982) Mechanical properties of the rice panicle. Plant Physiology 70:460-464

Skalinska M (1950) Studies in chromosome numbers of Polish angiosperms. Acta. Soc. Bot. Pol. 20:45-68

Skalinska M (1951) Cyto-ecological studies in *Poa alpina* L. var. *vivipara* L. Bulletin de l'Academie Polonaise des Sciences et des Lettres, Serie B:253-283

Smart C (1994) Gene expression during senescence. New Phytologist 126:419-448

Smith DC, Nielsen EL and Ahlgren HL (1946) Variation in ecotypes of *Poa pratensis*. Botanical Gazette 108:143-166

Solhaug KA (1991) Long day stimulation of dry matter production in *Poa alpina* along a latitudinal gradient in Norway. Holarctic Ecology 14:161-168

Sovonick-Dunford S, Ferrier JM and Dainty J (1982) Water-relations parameters of the phloem. Determinations of volumetric elastic modulus and membrane conductivity using an applied force method and shrinkage and

swelling of tissues in solutions at differing osmotic pressure. Annals of Botany 51:27-37

Sperry JS, Donnelly JR and Tyree MT (1988a) Seasonal occurrence of xylem embolism in sugar maple. American Journal of Botany 75:1212-1218

Sperry JS, Tyree MT and Donnelly JR (1988b) Vulnerability of xylem to embolism in a mangrove vs. an inland species of Rhizophoraceae. Physiologia Plantarum 74:276-283

Steiner AM, Heidenreich SC and Schwarz P (1997) Verification of varieties of alpine meadow-grass (*Poa alpina* L.) – floret morphology, chromosome number and single seed storage protein electrophoresis. Plant Varieties and Seeds 10:129-134

Steinitz B, Cohen A and Leshem B (1980) Factors controlling the retardation of chlorophyll degradation during senescence of detached statice (*Limonium sinuatum*) flower stalks. Zeitschrift fur Pflanzenphysiologie 100(4):343-349

Stirling CM, Davey PA, Williams TG and Long SP (1997) Acclimation of photosynthesis to elevated CO_2 and temperature in five British native species of contrasting functional type. Global Change Biology 3:237-246

Stirling CM, Heddel-Cowie M, Jones ML, Ashenden TW amd Sparks TH (1998) Effects of elevated CO_2 and temperature on growth and allometry of five native fast-growing annual species. New Phytologist 140(2):343-354

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell and Environment 14:741-762

Stoddart JL (1964a) Seed ripening in grasses. I. Changes in carbohydrate content. Journal of Agricultural Science 62:67

Stoddart JL (1964b) Seed ripening in grasses. II. Changes in free amino acid content. Journal of Agricultural Science 62:321

Summerhayes VS and Elton CS (1928) Further contributions to the ecology of Spitsbergen. Journal of Ecology 16:193

Suzuki Y, Kurogochi S, Murofushi N, Ota Y and Takahashi N (1981) Seasonal changes of GA_1, GA_{19} and abscisic acid in three rice cultivars. Plant and Cell Physiology 22(6):1085-1093

Tallowin JRB (1977a) Vegetative proliferation in *Festuca contracta* T. Kirk on South Georgia. British Antarctic Survey Bulletin 45:13-17

Tallowin JRB (1977b) Studies in the reproductive biology of *Festuca contracta* T. Kirk on South Georgia II. The reproductive performance. British Antarctic Survey Bulletin 45:117-129

Tegart WJ McG, Sheldon GW and Griffiths DC (eds.)(1990) Climate Change: the IPCC impacts assessment. Austrialian Government Publication Service. Canberra

Thoenes H (1929) Morphologie und anatomie von *Cynosurus cristatus* und die erscheinung der viviparie bei ihm. Bot. Archiv. Bd. 25

Thompson JE (1988) The molecular basis for memberane deterioration during senescence. *In.* Noodén LD and Leopold AC (eds.) Senescence and Aging in Plants. pp. 52-77. Academic Press. San Diego

Tibelius AC and Klinck HR (1987) Effects of artificial reduction in panicle size on weight of secondary seeds in oats (*Avena sativa* L.). Canadian Journal of Botany 67:621-628

Tincker MAH (1925) The effect of length of day upon the growth and reproduction of some economic plants. Annals of Botany 35:721-754

Tissue DT and Oechel WC (1987) Response of *Eriophorum vaginatum* to elevated CO_2 and temperature in the Alaskan tussock tundra. Ecology 68:401-410

Turesson G (1926) Studien über *Festuca ovina* L. I. Normalgeschlechtliche, halb- und ganz-vivipare typen nordischer herkunft. Hereditas 8:161-206

Turesson G (1930) Studien über *Festuca ovina* L. II. Chromosomenzahl und viviparie. Hereditas 13:177-184

Turesson G (1931) Studien über *Festuca ovina* L. III. Weitere beiträge zur kenntnis der chromosomenzahlen viviparer formen. Hereditas 15:13-16

Tyree MT, Fiscus EL, Wullschlegel SD and Dixon MA (1986) Detection of xylem cavitation in corn under field conditions. Plant Physiology 82:597-599

Tyree MT and Sperry JS (1988) Do plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiology 88:574-580

van Arendonk JJCM, Niemann GJ, Boon JJ and Lambers H (1997) Effects of nitrogen supply on the anatomy and chemical composition of four species belonging to the genus *Poa*, as determined by imaging-processing analysis and pyrolysis-mass spectrometry. Plant Cell and Environment 20:881-897

van Beusekom J (1907) On the influence of wound stimuli on the formation of adventitious buds in the leaves of *Gnetum gnemon* L. Recueil Trav. Bot. Néerl. 4:149-170

van Oosten JJ, Wilkins D and Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates – a mechanism for the acclimation of photosynthesis to high CO_2. Plant Cell and Environment 17(8):913-923

van Oosten JJ, Besford RT (1996) Acclimation of photosynthesis to elevated CO_2 through feedback regulation of gene expression: Climate of opinion. Photosynthesis Research 48(3):353-365

van Oosten JJ, Gerbaud A, Huijser C, Dijkwel PP, Chua NH and Smeekens SCM (1997) An Arabidopsis mutant showing reduced feedback inhibition of photosynthesis. Plant Journal 12(5):1011-1020

van der Pijl L (1972) Principles of Dispersal in Higher Plants. 2nd Edition. Springer-Verlag. New York

Vegetti A and Anton AM (1995) Some evolution trends in the inflorescence of *Poaceae*. Flora 190:255-228

Wager HG (1938) Growth and survival of plants in the Arctic. Journal of Ecology 26:390

Wareing PF and Phillips IDJ (1990) Growth and Differentiation in Plants. Third Edition. Pergamon Press. Oxford

Warner DA, Maurice SBK and Edwards GE (1987) Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C_4 grass *Panicum virgatum*. Plant Physiology 84(2):461-466

Warren-Wilson J (1967) Ecological data on dry-matter production by plants and plant communities. *In.* Bradley EF and Denmead OT (eds.) The Collection and Processing of Field Data. pp. 77-123. Interscience. Sydney

Waters SP, Martin P and Lee BT (1984) The influence of sucrose and abscisic acid on the determination of grain number in wheat. Journal of Experimental Botany 35:829-840

Watson RT, Rohde H, Oeschger H, Siegenthaler U (1990) Greenhouse gases and aerosols. *In.* Houghton JT, Jenkins GJ and Ephraums JJ (eds.) Climate Change the IPCC Scientific Assessment

Williams RF and Metcalf RA (1975) Physical constraints and tiller growth in wheat. Austrialian Journal of Botany 23:213-223

Wilton AC (1963) Vivipary in *Bromus pumpellianus* Scribin. Canadian Journal of Botany 41:1645-1647

Wookey PA, Welker JM, Parsons AN, Press MC, Callaghan TV and Lee JA (1997) Differential growth, allocation and photosynthetic responses of *Polygonum viviparum* to simulated environmental change at a high arctic polar semi-desert. Oikos 70:131-139

Woolhouse HW (1987) Regulation of senescence in the chloroplast. *In.* Thomson WW, Nothnagel EA and Huffaker RC (eds.) Plant Senescence: it's Biochemistry and Physiology. pp. 132-145. The American Society of Plant Physiologists, Rockville, Maryland

Worsdell WC (1915) The Principles of Plant-Teratology. Volume 1. Containing Non-Vascular Plants, and the Root, Stem, and Leaves of Vascular Plants. The Ray Society. London

Worsdell WC (1916) The Principles of Plant-Teratology. Volume 2. Containing the Flower, and concluding the work. The Ray Society. London

Wycherley PR (1953a) Proliferation of spikelets in British grasses. I. The taxonomy of the viviparous races. Watsonia 3:41-56

Wycherley PR (1953b) The distribution of the viviparous grasses in Great Britain. Journal of Ecology 41:275-288

Wycherley PR (1954) Vegetative proliferation of floral spikelets in British grasses. Annals of Botany 18(69):119

Youngner VB (1960) Environmental control of initiation of the inflorescence, reproductive structures and proliferations in *Poa bulbosa*. American Journal of Botany 47:753-757

Ziska LH and Bunce JA (1993) The influence of elevated CO_2 and temperature on seed germination and emergence from soil. Field Crops Research 34:147-157

Zollikofer C (1939) Zur fortpflanzung von *Poa alpina*. Jahrb. St. Gall. Naturw. Gesellsch. 65

Appendix 1: Proliferation and prolification

Table 1. Pooideae in which proliferation has been recorded. Chromosome numbers are included where known (* indicates that chromosome numbers are from Hubbard; 1992).

Grass species	Chromosome number	References
Agrostis canina L. 'Silver Needles'		Personal observation (see Plate 1.2).
Agrostis stolonifera L.	28 or 42*	Personal observation (see Section 1.1.4).
Agrostis stolonifera L. var. *palustris* (Huds.) Farw. (Syn. *A. palustris* Huds.)		Arber (1934).
Agrostis tenuis Sibth.	28*	Personal observation.
Dactylis glomerata L.	28*	Arber (1934); Personal observation.
Eragrostis prolifera (Sw.) Steud.		Beetle (1980).
Holcus lanatus L.	14*	Arber (1934); Personal observation (see Plate 1.1).
Ischaemum muticum L.		Backer (n.d. [1930]).
Phleum pratense L.	42*	Merchand & McLean (1965).
Puccinellia maritima (Huds.) Parl. (Syn. *Glyceria maritima* (Huds.) Wahlb.)	49, 56, 63 or 70*	Arber (1934).
Zea mays L.		Arber (1934).

Table 2. Pooideae species and varieties in which ephemeral prolification has been recorded. Chromosome numbers are included where known (* indicates that chromosome numbers are from Hubbard; 1992).

Grass species	Chromosome number	References
Agropyron cristatum (L.) Gaertn.		Beetle (1980).
Agropyron repens (L.) Beauv.	42*	Wycherley (1952).
Agropyron spicatum (Pursch.) Scribn. & Sm.	14 or 28	Hartung (1946); Marchand & McLean (1965).
Agrostis canina (L.) var. *falklandica* (Hook. f.) Hack.		Moore & Doggett (1976).
Agrostis flavidula Steudel		Moore & Doggett (1976).
Agrostis stolonifera L. var. *palustris* (Huds.) Farw. (Syn. *A. palustris* Huds.)		Arber (1934).
Agrostis stolonifera L.	28 or 42*	Philipson (1935); Aberg (1940).
Agrostis stolonifera L. var. *maritima* Koch.		Philipson (1935).
Agrostis tenuis Sibth.	28*	Philipson (1935); Aberg (1940).
Alopecurus pratensis L.	28	Jenkin (1922); Flovik (1938); Wycherley (1954).
Arrhenatherum elatius (L.) Beauv. ex. J & C Presl. (Syn. *A. avenaceum* Beauv.)	28*	Arber (1934).
Avena sativa L.	14*	Nielson (1941).
Brachypodium sylvaticum Beauv.		Arber (1934).
Briza subaristata Lam.		Beetle (1980).
Bromus inermis Leyss.	56*	Nielson (1941).
Bromus macranthus Desv.		Beetle (1980).
Bromus pumpellianus Scribn.	55 or 56	Wilton (1963).
Bromus purgans L.		Nielson (1941).
Cynosurus cristatus L.	14*	Jenkin (1922); Thoenes (1929); Arber (1934); Wycherley (1952).
Cynosurus echinatus L.	14*	Beetle (1980).
Dactylis glomerata L.	28*	Tincker (1925); Arber (1934); Heide (1987); Personal observations (see Section 1.2.1, Plate 1.3).

Deschampsia caespitosa (L.) Beauv.	26 or 28	Jenkin (1922); Nygren (1949); Foreman (1970).
Deschampsia caespitosa (L.) Beauv. var. *rhenana* Grem.		Arber (1934).
Deschampsia flexuosa (L.) Trin.	28	Flovik (1938); Schultz (1939).
Eleusine coracana (L.) Gaertn.		Li (1950).
Eleusine indica (L.) Gaertn.		Li (1950); Akai & Fukutomi (1966).
Eragrostis virescens Presl.		Beetle (1980).
Festuca contracta T.Kirk.		Tallowin (1977).
Festuca obtusa Spreng.		Nielson (1941).
Festuca purpurascens Banks et Sol.		Beetle (1980).
Festuca rubra L.	56*	Jenkin (1922); Wycherley (1952); Kjellquist (1961).
Festuca rubra L. ssp. *commutata* Gaud.	42	Personal observation (see Section 1.1.5; Plate 1.4)
Glyceria maxima (Hartm.) Holmb. (Syn. *G. aquatica* (L.) Wahlb.)	60*	Harmer (1978).
Hordeum murinum L.	28*	Arber (1934).
Ichnanthus pallens (Sw.) Munro		Beetle (1980).
Koeleria phleoides (Vill.) Pers.		Beetle (1980).
Lolium multiflorum Lam.	14 or 28*	Beetle (1980).
Lolium perenne L.	14 or 28	Jenkin (1922); Arber (1934); Cooper (1951).
Melica papilionaceae L.		Beetle (1980).
Oryza sativa L. var. *Aya-Nishiki*		Akai & Fukutomi (1966).
Panicum virgatum L.		Nielson (1941).
Pennisetum americanum (L.) Leeke		Coldrake & Pearson (1986).
Phleum pratense L.	42*	Arber (1934); Nielson (1941); Langer & Ryle (1958); Juntilla (1985).
Poa chaixii Vill.	14*	Nygren & Almgard (1962).
Poa iridifolia Hauman		Beetle (1980).
Poa trivialis L.	14*	Jenkin (1922).
Puccinellia maritima (Huds.) Parl.	49, 56, 63 or 70*	Wycherley (1953a).

Setaria viridis (L.) Beauv.	18*	Li (1950).
Sorghum bicolor (L.) Moench.		Casady (1969).
Trisetum argenteum (Willd.) Roem. & Schult		Beuret (1976).
Trisetum distichphyllum (Vill.) P.B.		Beuret (1976).
Trisetum flavescens (L.) Beauv.	28*	Arber (1934).
Trisetum spicatum (L.) Richt.	28 or 42	Flovik (1938); Foreman (1970); Clebsh & Billings (1976).
Triticum aestivum L.		Akai & Fukutomi (1966).
Zea mays L.		Galinat & Naylor (1951).

Table 3. Pooideae in which habitual prolification has been recorded. Chromosome numbers are included where known.

Grass species/hybrid	Chromosome number	References
Agrostis alba L. var. *prolifera* Aschers. and Graebn.		Beetle (1980).
Agrostis alba L. var. *vivipara* Sweet		Beetle (1980).
Agrostis canina L. var. *vivipara* Peterm.		Beetle (1980).
Cynosurus elegans Desv. var. *viviparus* Lojac		Beetle (1980).
Deschampsia alpina (L.) Roem. & Schult.	26, 39, 41 or 49	Flovik (1938); Nygren (1949); Wycherley (1953b).
Deschampsia cespitosa (L.) Beauv. ssp. *alpina* (L.) Tzvelev. (Syn. *D. caespitosa* (L.) Beauv. var. *pseudalpina* (Syme) Druce.)	52 in Britain; 39,41,48 or 49 in Arctic	Rothera & Davy (1986).
Deschampsia rhenana Gremli.	39 or 49	Albers (1978).
Deschampsia setacea (Huds.) Hack.		Nygren (1949).
Eragrostis brizoides (L.f.) Nees		Beetle (1980).
Eragrostis capensis (Thumb.) Trin.		Beetle (1980).
Festuca alpina Suter. var. *prolifera* Schur.		Beetle (1980).
Festuca fuegiana Hook. f. forma *vivipara* Hack.		Beetle (1980).
Festuca brachyphylla x *F. vivipara* (L.) Sm. ssp. *vivipara* Frederiksen	35	Frederiksen (1981).
Festuca ovina L. x *F. vivipara* (L.) Sm. ssp. *vivipara* Frederiksen	21	Frederiksen (1981).
Festuca rubra L. x *F. vivipara* (L.) Sm. ssp. *vivipara* Frederiksen	35	Frederiksen (1981).
Festuca rubra L. x *F. vivipara* (L.) Sm. ssp. *hirsuta* (Schol.) Frederiksen stat. nov.	49	Frederiksen (1981).

Festuca rubra L. var. *prolifera* (Piper) Hyl. (Syn. *Festuca prolifera* (Piper) Fern)		Appalachian Mountain Club (1964); Latting (1972).
Festuca subantarctica Parodi.		Moore & Doggett (1976).
Festuca vivipara (L.) Sm. ssp. *glabra* Frederiksen stat. nov. (Syn. *Festuca viviparoidea* s.l. Krajina ex Pavlick)	49 or 56	Frederiksen (1981).
Festuca vivipara (L.) Sm. ssp. *hirsuta* (Schol.) Frederiksen stat. nov.	28	Frederiksen (1981).
Festuca vivipara (L.) Sm. ssp. *vivipara* Frederiksen	28	Frederiksen (1981).
Hierochloe alpina R. & S. var. *vivipara* Scheutz ex Fedtsch.		Beetle (1980).
Koeleria cristata (L.) Pers. var. *vivipara* Opiz		Beetle (1980).
Koeleria glauca var. *typica* f. *vivipara* Domin		Beetle (1980).
Koeleria pyramidata Lam. Domin f. *vivipara* Domin		Beetle (1980).
Molinia coerulea (L.) Moench var. *vivipara* Boenn.		Beetle (1980).
Panicum antidotale Retz. (Syn. *P. proliferum* Lam.)		Beetle (1980).
Panicum viviparum Schumach.		Beetle (1980).
Paspalum proliferum Arech.		Beetle (1980).
Phalaris vivipara Pacluc. (Syn. *P. brachystachys* Link.)		Beetle (1980).
Phleum boehmeri (L.) Wiebel var. *vivipara* Schur		Beetle (1980).
Poa alopecurus (Gaudich) Kunth. ssp. *fuegiana*		Moore & Doggett (1976).
Poa alpigena (E.Fries.) Lindm. var. *colpodea* (Th. Fries.) Scholander	51 + 5ff	Flovik (1938).

Poa alpigena (E.Fries.) Lindm. var. *vivipara* (Malmgr.) Scholander	42 + 4ff	Flovik (1938).
Poa alpina L.	14-60+	Linnaeus (1753); Müntzing (1980).
Poa annua L. var. *vivipara* S.F. Gray		Beetle (1980).
Poa arctica R.Br. var. *vivipara* (Malmgr.) Scholander	56	Flovik (1938).
Poa arctica R.Br. ssp. *stricta* (Lindeb.) Nannf.	38	Nannfeldt (1940).
Poa badensis Haenke ssp. *psammophila* (Schur) Nyar		Beetle (1980).
Poa bulbosa (L.) var. *vivipara* Koel.		Flovik (1938); Müntzing (1940, 1980); Wycherley (1953b).
Poa herjedalica H.Sm.		Nygren (1967).
Poa x *jemtlandica* (Almqu.) Richt.	37	Richter (1890); Wycherley (1953a&b); Harmer (1978); Hubbard (1992).
Poa laxa Haenke var. *flexuosa* (Smith) Hartm.		Beetle (1980).
Poa laxa Haenke var. *vivipara* S.F. Gray		Beetle (1980).
Poa pratensis L. var. *alpigena* Blytt.		Beetle (1980).
Poa pratensis L. var. *colpodea* (Th. Fr.) Schol.	35 or 35 + 4ff	Beetle (1980).
Poa pratensis L. var. *prolifera* Ostenf.		Beetle (1980).
Poa pratensis L. var. *vivipara* Huds.		Beetle (1980).
Poa robusta Steudel		Moore & Doggett (1976).
Poa sinaica Steud. var. *vivipara* V. Tackholm		Beetle (1980).
Poa stricta Lindeb.		Harmer (1978).
Poa sublanata Reverd. var. *vivipara* Tzvelev		Beetle (1980).
Poa tolmatchewii Roshev. var. *stricta* (Lindeb.) Tzvelev		Beetle (1980).

Appendix 2: Long Ashton Nutrient Solution

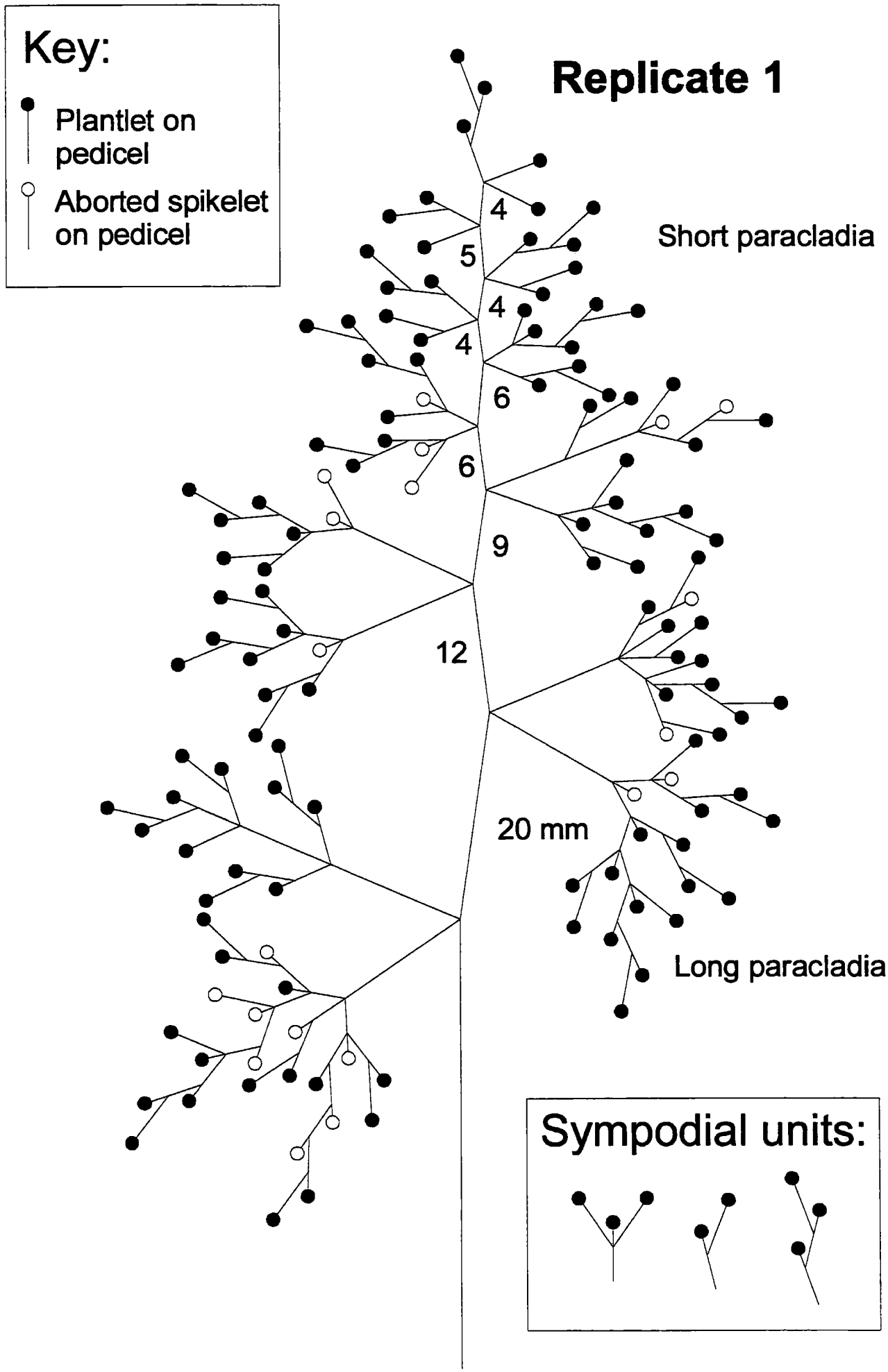
From Hewitt EJ (1966) Sand and Water culture Methods used in the study of Plant Nutrition. (2nd edition). Commonwealth Agricultural Bureaux. The Eastern Press. London and Reading.

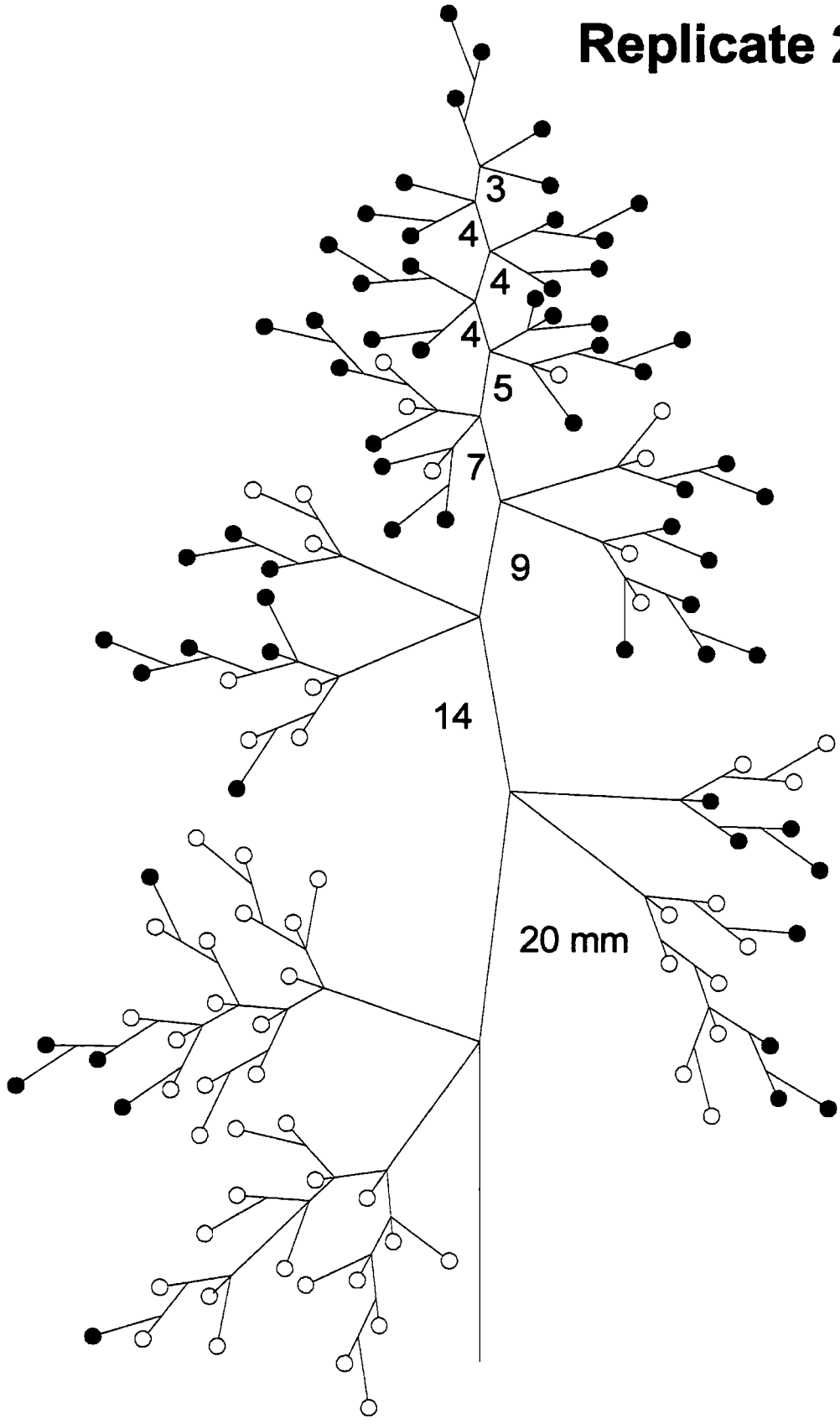
Salt	Conc. (% w/v)	Stock solution (g/l)	Stock solution (g/500 ml)	Vol. of stock (ml) for 1 l of full strength LA	Vol. of stock (ml) for 10 l of fifth strength LA	Milligram equivalents per litre obtained when 1 ml stock is diluted to 1 l.	Final millimolar (mM.) conc. of compound after ten fold dilution to full strength LA
$MgSO_4 . 7H_2O$ Magnesium sulphate 7-hydrate	18.4	184	92	1	2	Mg^{2+} 1.5 SO_4^{2-} 1.5	0.75
$NaH_2PO_4 . 2H_2O$ disodium hydrogen orthophosphate	20.8	208	104	1	2	Na^+ 1.33 PO_4^{3-} 4	1.33
Fe EDTA Ethylenediaminetetra-acetic acid ferric monosodium salt	3.73	37.3	18.65	1	2	Fe^{+++} 0.15	0.05
NH_4NO_3 Ammonium nitrate	16.0	160	80	1	2	NH_4^+ 2 NO_3^- 2	2.0
K_2SO_4 Potassium sulphate	8.7	87	43.5	1	2	K^+ 1 SO_4^{2-} 1	0.5
$CaCl_2 . 6H_2O$ Calcium chloride 6-hydrate	43.8	438	219	1	2	Ca^{2+} 4 Cl^- 4	2.0
Modified in this study:							
$NaH_2PO_4 . 2H_2O$ disodium hydrogen orthophosphate	1.56	15.601	7.8	2	4	Na^+ 0.2 PO_4^{3-} 0.6	0.2
NH_4NO_3 Ammonium nitrate	8	80	40	1	2	NH_4^+ 1 NO_3^- 1	1.0
Monosodium complex						Milligram equivalents per litre or mM. concentration produced when diluted at a rate of 0.1 ml/l.	
$MnSO_4 . 4H_2O$ Manganous sulphate 4-hydrate	2.23	22.3	11.15	0.1	0.2	Mn^{2+} 0.02	mg. equiv./l.
$CuSO_4 . 5H_2O$ Copper sulphate	0.25	2.5	1.25	0.1	0.2	Cu^{2+} 0.002	mg. equiv./l.
$ZnSO_4 . 7H_2O$ Zinc sulphate 7-hydrate	0.29	2.9	1.45	0.1	0.2	Zn^{2+} 0.002	mg. equiv./l.
H_3BO_3 Boric acid	3.1	31	15.5	0.1	0.2	B 0.05	mM
NaCl Sodium chloride	5.85	58.5	29.25	0.1	0.2	Cl^- 0.1	mg. equiv./l.
$Na_2MoO_4 . 2H_2O$ Sodium molybdate 2-hydrate	0.12	1.2	0.6	0.1	0.2	Mo 0.0005	mM

Appendix 3: Paracladial architecture

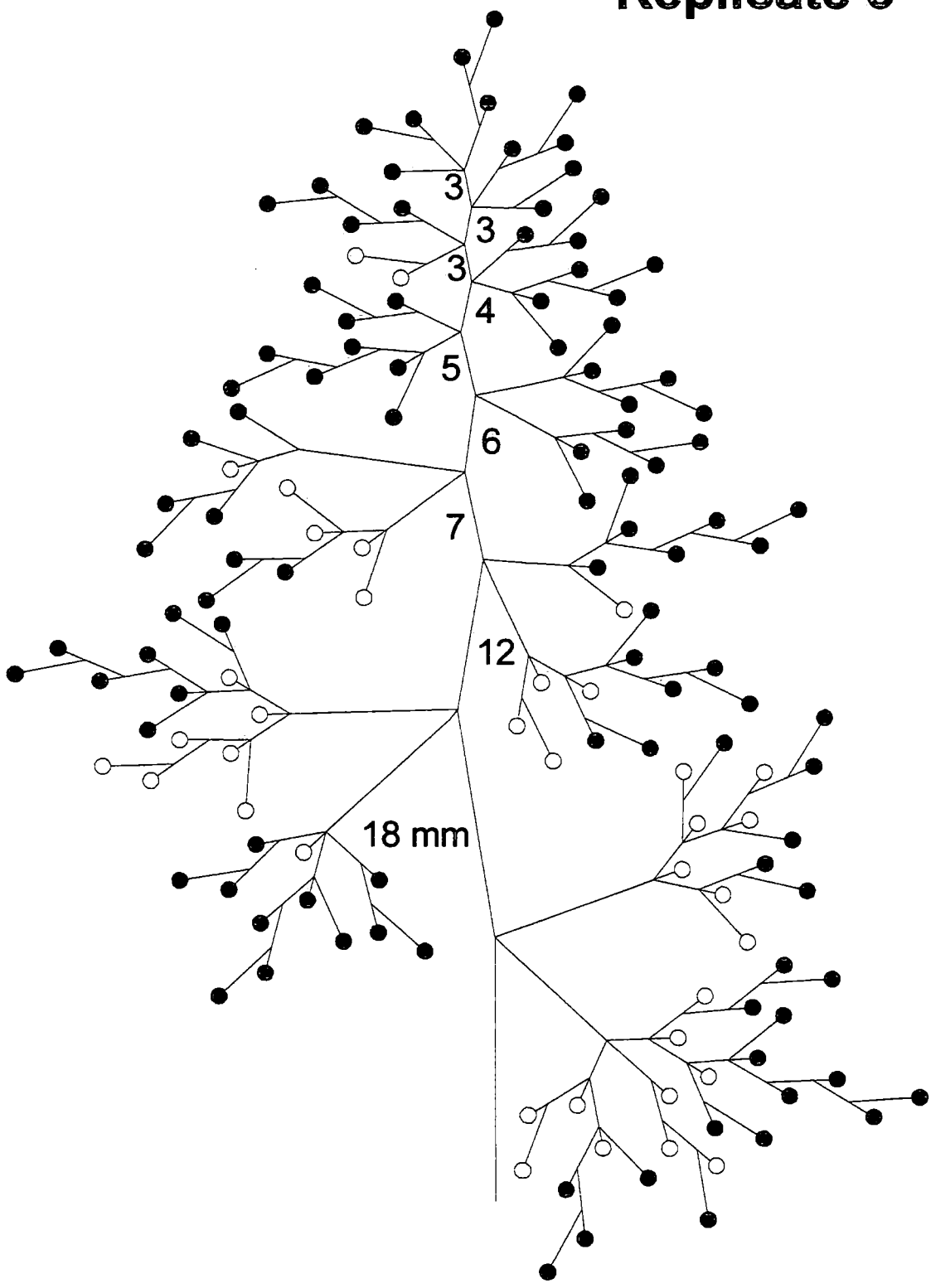
This appendix contains morphological maps of the paracladial zones of the main synflorescences of six *Poa alpina* plants. These were used to gain data on spikelet distribution and the occurrence of spikelet abortion throughout the paracladial zone, which is analysed and presented in Chapter 3.

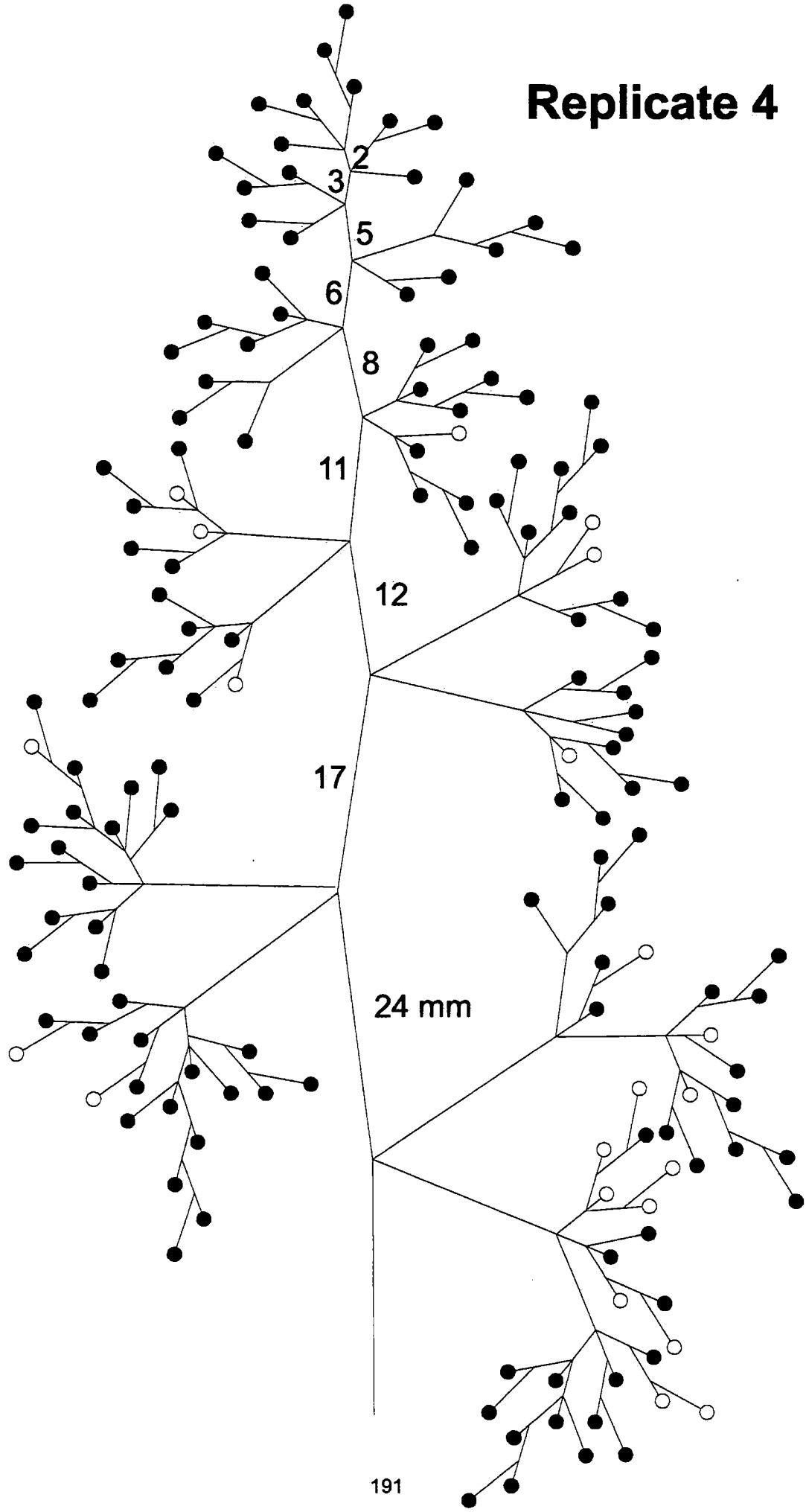
Numbers beside rachis internodes indicate length in millimetres. The angles shown are arbitrary and on live plants change during development (see Section 3.1). Plantlets are represented by filled circles, and aborted spikelets (see Chapters 3 & 5) by open (white) circles.


Key:
Plantlet on pedicel
Aborted spikelet on pedicel
Replicate 1
4
5
4
4
6
6
9
12
20 mm
Short paracladia
Long paracladia
Sympodial units:

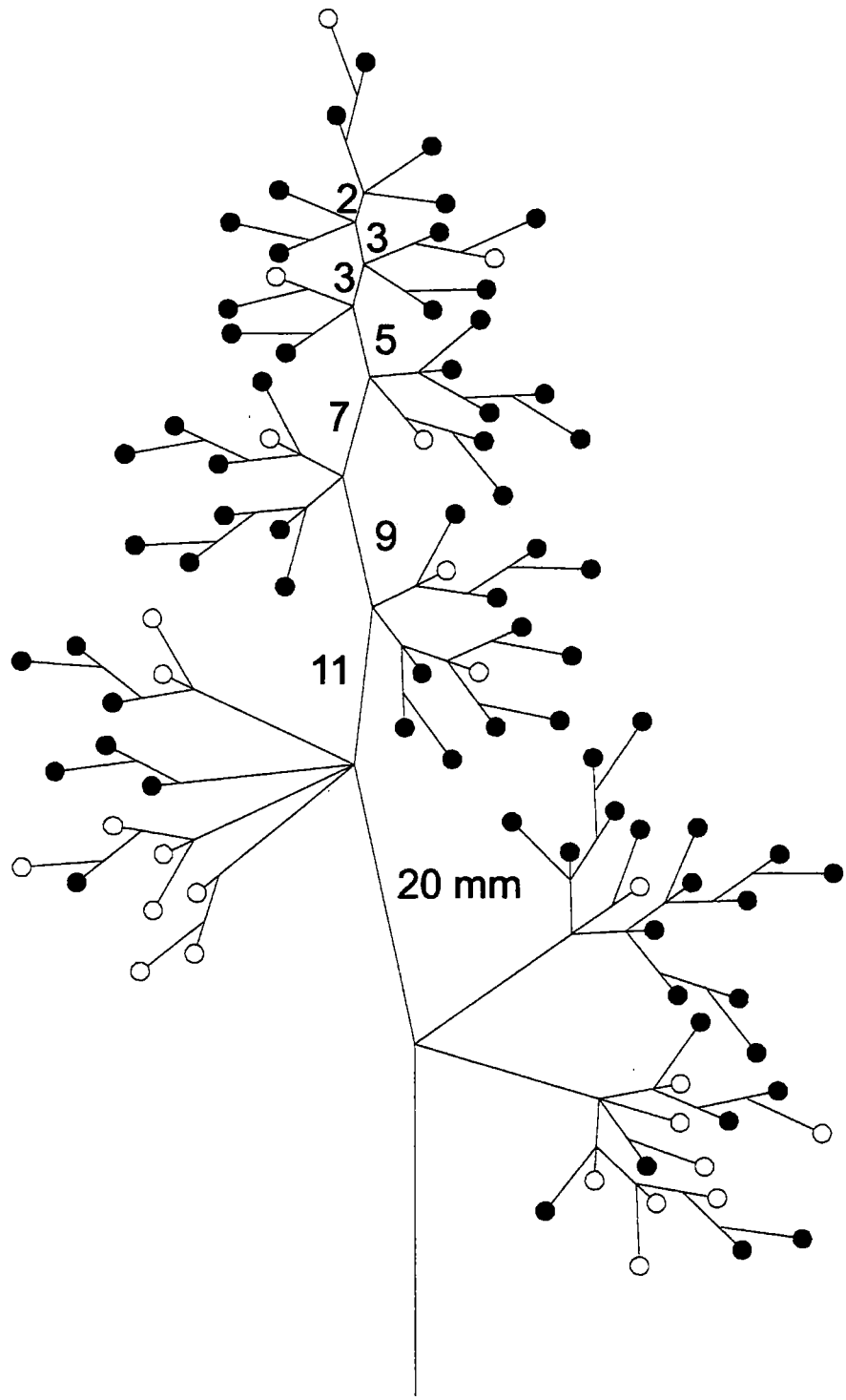

Replicate 2
3
4
4
4
5
7
9
14
20 mm

Replicate 3

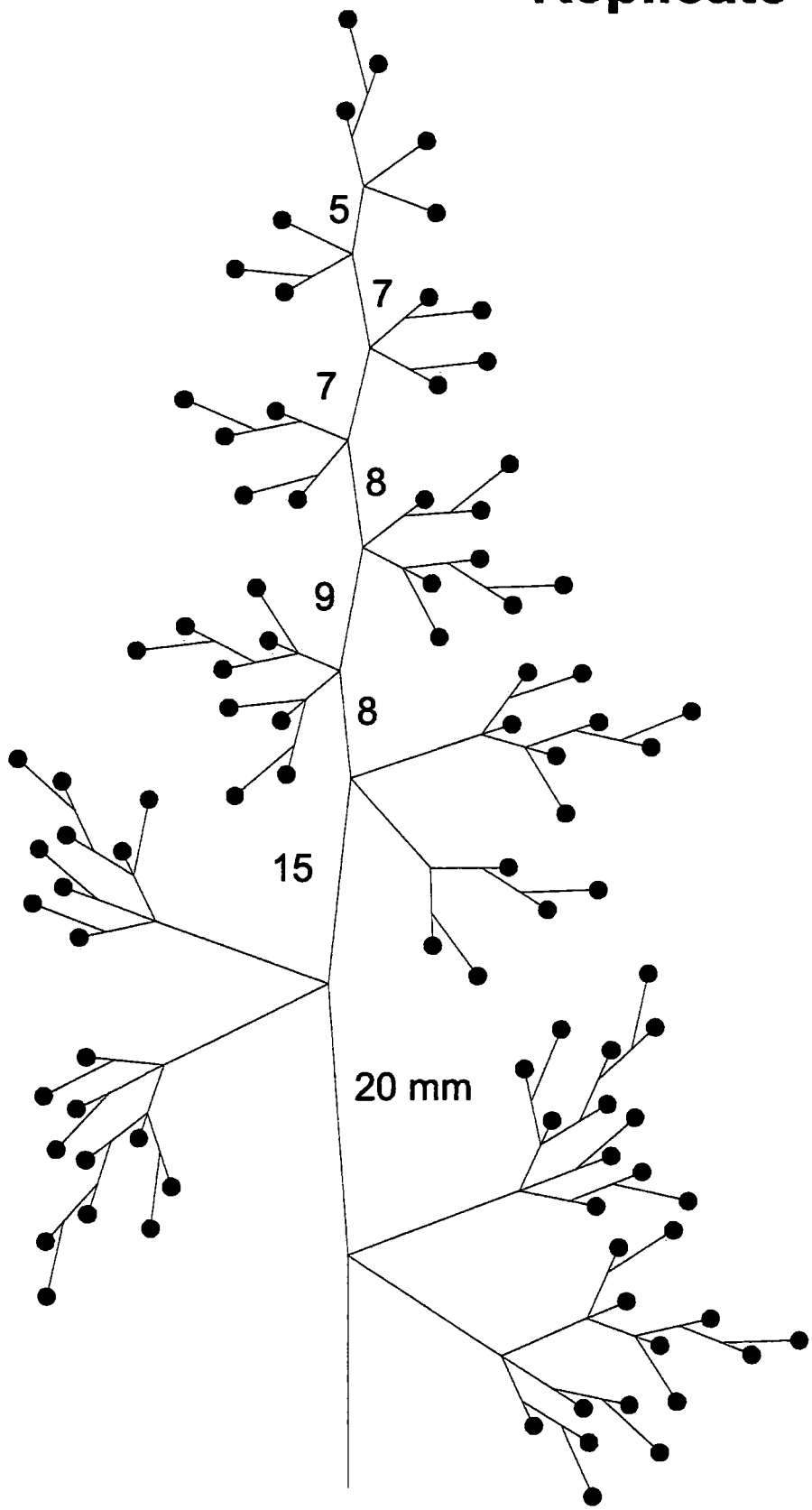



Replicate 4
2
3
5
6
8
11
12
17
24 mm

Replicate 5



Replicate 6



Appendix 4: Suppliers' addresses

Amersham Life Sciences Ltd., Amersham Place, Little Chalfont, Bucks. HP7 9NA, UK. Tel: (orders) 0800 515313, (enquiries) 0800 616928. Fax: 0800 616927. E-mail: uk_custserve@amersham.co.uk

ADC Bioscientific, Unit 12, Spurling Works, Pindar Road, Hoddesdon, Herts. EN11 0DB. Tel: 01992 445995. Fax: +44 (0) 1992 444567. Web: http://www.crowcon.com/adc.htm

BDH (Merck Ltd.), Hunter Boulevard, Magna Park, Lutterworth, Leics. LE17 4XN, UK. Tel: 0800 223344. Fax: 01455 558586. Web: http://www.merck-ltd.co.uk

Charles Austin Pumps Ltd., 100 Royston Road, Byfleet, Weybridge, Surrey KT14 7PB, UK. Tel: 09323 43224/5.

Corel Corporation (USA), 567 East Timpanogos Parkway, Orem, Utah, 84097-6209. Tel: 801 765 4010. Fax: 801 222 4379. Web: http://www.corel.com

Delta-T Devices Co. Ltd., 128 Low Road, Burwell, Cambridge CB5 0EJ, UK. Fax: +44 (1638) 742922. Web: http://www.detla-t.co.uk

Early Learning Centre, South Marston, Swindon, UK.

EG & G Wallac Ltd. Milton Keynes, 20 Vincent Avenue, Crownhill Business Centre, Crownhill, Milton Keynes, MK8 0AB, UK. Tel: +44 1908 265 744. Fax: +44 1908 265 956. E-mail: wallac-uk@wallac.com

Ernst Leitz GmbH, Wetzlar, Germany.

FGL Lighting Ltd., Pinewood Studios, Bucks. UK.

FT Scientific Instruments Ltd., Station Industrial Estate, Bredon, Nr. Tewkesbury, Glos., UK. Tel: 01684 72425.

Hewlett-Packard Ltd., Cain Road, Bracknell, Berks. RG12 1HN, UK. Tel: 344/360 000.

KDG Instruments, Crompton Way, Crawley, West Sussex RH10 2YZ, UK. Tel: +44 (0) 1293 52151. Fax: +44 (0) 1293 533 095. Web: http://www.mobrey.com/kcat.htm

Lab Impex Systems, 15 Riverside Park, Wimborne, Dorset BH21 1QU, UK. Tel: +44 (0) 1202 840685.

National Diagnostics, Unit 4, Fleet Business Park, Itlings Lane, Hessle, Hull HU13 9LX, UK. Tel: +44 01482 646022. Fax: +44 01482 646013. E-mail: 100665.121@compuserve.com Web: http://nationaldiagnostics.com

Nikon UK Ltd., Haybrook, Halesfield 9, Telford, Shropshire, UK.

Osram Ltd., PO Box 17, East Lane, Wembley, Middlesex HA9 7PG, UK. Tel: 181-9 044321. Fax: 181-9 011222. Web: http://www.osram.de

PP Systems, Unit 2, Glovers Court, Bury Meadow Road, Hitchin, Herts. SG5 1RT, UK. Tel: +44 (0) 1462 453090. Fax: +44 (462) 731807. E-mail: uk@ppsystems.com

Sigma-Aldrich Co. Ltd., Fancy Road, Poole, Dorset BH12 4QH, UK. Tel: (orders) 0800 717181, (enquiries) 01202 733114.

Skalar Analytical BV, PO Box 3237, 4800 DE Breda, The Netherlands. Tel: +31 (76) 5 486 486. Fax: +31 (76) 5 486 400.

Solardome Industries Ltd., Southampton, Hampshire, UK.

SPSS Inc., 11th Floor, 233 S. Wacker Drive, Chicago Illinois, 60606 – 6307. Web: http://www.spss.com

TAAB Laboratories Equipment Ltd., 3 Minerva House, Calleva Park, Aldermaston, Berks. RG7 8NA, UK. Tel: +44 (0) 118 981 7775. Fax: +44 (0) 118 981 7881.

Thermoforce Ltd., Camplex Plantcare Division, Bentalls Complex, Heybridge, Maldon, Essex CM9 7NW, UK.

Unicam Ltd., York Street, Cambridge CB1 2PX, UK. Telex: 817331 UNICAM G. Fax: +44 (223) 312764.

William Sinclair Horticulture Ltd., Firth Road, Lincoln, UK.

Appendix 5: Results of statistical tests

The statistical analyses presented here refer to - and are presented in order of - tables and figures presented in the chapters of this thesis. When analysing Tukey's comparisons a pair of figures which are either both positive or both negative indicate significant difference between treatment means at the level stated in each test.

Chapter 2

Fig. 2.3. Absolute water content of green material in both halves of the paracladial zone during reproductive development.

Two sample T for Resp.

Position	N	Mean	StDev	SE Mean
1	31	59.8	16.3	2.9
2	31	61.1	13.3	2.4

95% CI for mu (1) - mu (2): (-8.9, 6.3)
T-Test mu (1) = mu (2) (vs not =): T = -0.35 P = 0.73 DF = 57

Table 2.1. The proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the paracladial zone in which fluorochrome could be detected.

VB = Vascular Bundle

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
VB type	1	648.7	648.7	2.15	0.168
time	1	1790.6	1790.6	5.93	0.031
VB type x time	1	648.7	648.7	2.15	0.168
Error	12	3622.2	301.8		
Total	15	6710.3			

Table 2.2. The proportion (%) of major and minor vascular bundles in the culm internode immediately proximal to the paracladial zone in which occlusions could be detected

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
VB type	1	66.2	66.2	0.33	0.575
time	1	1470.4	1470.4	7.37	0.019
VB type x time	1	66.2	66.2	0.33	0.575
Error	12	2392.6	199.4		
Total	15	3995.5			

Chapter 3

Fig. 3.9. Net photosynthetic and respiratory rates of the paracladial zone at two (C1&2) and three (C3&4) weeks from exertion. Data represent the mean ± one S.E. of five replicates.

Analysis of Variance for Response

Source	DF	SS	MS	F	P
age	1	1.273	1.273	4.01	0.062
A/respiratory	1	32.835	32.835	103.52	0.000
age x A/respiratory	1	2.196	2.196	6.92	0.018
Error	16	5.075	0.317		
Total	19	41.379			

Fig. 3.13a. ^{14}C fixation by the first leaf, paracladial zone, and the culm - ^{14}C content.

1 = leaf
2 = paracladial zone
3 = culm

One-Way Analysis of Variance

Analysis of Variance on C1

Source	DF	SS	MS	F	p
C2	2	57358792	28679396	38.61	0.000
Error	15	11141727	742782		
Total	17	68500520			

Level	N	Mean	StDev
1	6	1205.9	300.4
2	6	4363.2	1460.2
3	6	164.7	77.2

Pooled StDev = 861.8

Individual 95% CIs For Mean
Based on Pooled StDev

```
----+---------+---------+---------+--
        (----*---)
                                (---*----)
(----*----)
----+---------+---------+---------+--
    0       1600      3200      4800
```

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0203

Critical value = 3.67

Intervals for (column level mean) - (row level mean)

	1	2
2	-4449 -1866	
3	-250 2332	2907 5490

Fig. 3.13b. ^{14}C fixation by the first leaf, paracladial zone and culm - photosynthetic efficiency (total amount of ^{14}C fixed per unit fed organ dry weight)

```
1 = leaf
2 = paracladial zone
3 = culm

One-Way Analysis of Variance

Analysis of Variance on C1
Source     DF        SS        MS        F        p
C2          2    7809.5    3904.7    47.85    0.000
Error      15    1224.1      81.6
Total      17    9033.5

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
 Level      N      Mean     StDev  ----------+---------+---------+------
     1      6    45.075    11.617                    (---*--)
     2      6    57.609     9.964                          (---*---)
     3      6     8.511     3.253  (---*---)
                                  ----------+---------+---------+------
Pooled StDev =    9.034                   20        40        60

Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0203
Critical value = 3.67
Intervals for (column level mean) - (row level mean)

              1         2

     2    -26.07
            1.00

     3     23.03     35.56
           50.10     62.63
```

Table. 3.2. The absolute growth (mg DW basis) of plantlets from distal and proximal regions within the paracladial zone - differences between distal and proximal means within control or manipulated plants at each harvest.

```
Analysis of Variance (Balanced Designs)

Source                          DF        SS        MS        F      P
Position                         1   2334.45   2334.45    32.66  0.000
Manipulation                     1    179.31    179.31     2.51  0.117
Time                             2   4468.14   2234.07    31.25  0.000
Position x Manipulation          1     39.78     39.78     0.56  0.458
Position x Time                  2    176.23     88.11     1.23  0.297
Manipulation x Time              2    167.44     83.72     1.17  0.315
Position x Manipulation x Time   2    449.12    224.56     3.14  0.048
Error                           84   6004.36     71.48
Total                           95  13818.83
```

Fig. 3.16a. The relative growth rate of plantlets from distal (1, 3) and proximal (2, 4) positions within the paracladial zone.

```
One-way Analysis of Variance

Source     DF        SS        MS        F        P
Treat2      3  0.020532  0.006844    11.11    0.000
Error      28  0.017241  0.000616
Total      31  0.037773

                                      Individual 95% CIs For Mean
                                      Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1           8   0.02543   0.02711         (-----*-----)
2           8   0.01606   0.01713      (-----*-----)
3           8   0.00402   0.02032   (-----*-----)
4           8   0.07098   0.03197                        (-----*-----)
                                      -----+---------+---------+---------+-
Pooled StDev =  0.02481              0.000     0.030     0.060     0.090
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0108
Critical value = 3.86
Intervals for (column level mean) - (row level mean)

                1           2           3

       2    -0.02449
             0.04324

       3    -0.01246    -0.02183
             0.05527     0.04590

       4    -0.07942    -0.08879    -0.10082
            -0.01169    -0.02106    -0.03309
```

Fig. 3.16b. The relative growth rates of plantlets from distal (1, 3) or proximal (2, 4) positions within the paracladial zone.

```
One-way Analysis of Variance

Source     DF        SS        MS        F        P
Treat1      3  0.009160  0.003053     8.08    0.000
Error      28  0.010578  0.000378
Total      31  0.019738

                                      Individual 95% CIs For Mean
                                      Based on Pooled StDev
Level       N      Mean     StDev  -+---------+---------+---------+-----
1           8   0.08657   0.01537                      (-----*----)
2           8   0.05987   0.02328           (-----*-----)
3           8   0.06356   0.02102             (----*-----)
4           8   0.03888   0.01706   (-----*----)
                                      -+---------+---------+---------+-----
Pooled StDev =  0.01944            0.025     0.050     0.075     0.100
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0108
Critical value = 3.86
```

```
Intervals for (column level mean) - (row level mean)

                1           2           3

2      0.00017
       0.05322

3     -0.00352    -0.03021
       0.04953     0.02284

4      0.02116    -0.00553    -0.00184
       0.07422     0.04752     0.05121
```

Fig. 3.17. ^{14}C content of the two halves of the paracladial zone after pulse-feeding the first leaf. The paracladial zone was either intact (control; 3, 4) or manipulated by removal of all spikelets in the opposing half (1, 2).

```
One-Way Analysis of Variance

Analysis of Variance on C1
Source     DF        SS        MS        F        p
C2          3     1.023     0.341     3.29    0.048
Error      16     1.657     0.104
Total      19     2.680

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
 Level      N      Mean     StDev  ----------+---------+---------+------
     1      5    1.3754    0.4568                  (---------*---------)
     2      5    1.4151    0.4046                   (---------*---------)
     3      5    0.9247    0.1351   (---------*---------)
     4      5    0.9649    0.1538    (---------*---------)
                                    ----------+---------+---------+------
Pooled StDev =   0.3218                  0.90      1.20      1.50
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0113
Critical value = 4.05
Intervals for (column level mean) - (row level mean)

               1         2         3

     2   -0.6226
          0.5431

     3   -0.1322   -0.0924
          1.0336    1.0733

     4   -0.1724   -0.1326   -0.6231
          0.9934    1.0331    0.5427
```

Fig. 3.18b. The growth of plantlet roots; the length of the oldest root after dehiscence of plantlets from either end of the paracladial zone.

```
1, 4, 7, 10, 13 = distal over time
2, 5, 8, 11, 14 = proximal over time

Twosample T for C1 vs C2 - 6 d
    N      Mean     StDev   SE Mean
C1  6      2.83      1.60      0.65
C2  6      0.67      1.03      0.42

95% C.I. for mu C1 - mu C2: ( 0.37,  3.96)
T-Test mu C1 = mu C2 (vs not =): T= 2.78  P=0.024  DF=  8

Twosample T for C4 vs C5 - 10 d
    N      Mean     StDev   SE Mean
C4  6      9.67      4.27       1.7
C5  6      5.83      3.87       1.6

95% C.I. for mu C4 - mu C5: ( -1.5,  9.2)
T-Test mu C4 = mu C5 (vs not =): T= 1.63  P=0.14  DF=  9

Twosample T for C7 vs C8 - 13 d
    N      Mean     StDev   SE Mean
C7  6     17.17      4.36       1.8
C8  6     12.83      5.12       2.1

95% C.I. for mu C7 - mu C8: ( -1.9,  10.5)
T-Test mu C7 = mu C8 (vs not =): T= 1.58  P=0.15  DF=  9

Twosample T for C10 vs C11 - 17 d
     N      Mean     StDev   SE Mean
C10  6     24.83      3.54       1.4
C11  6     16.50      5.68       2.3

95% C.I. for mu C10 - mu C11: ( 2.0,  14.6)
T-Test mu C10 = mu C11 (vs not =): T= 3.05  P=0.016  DF=  8

Twosample T for C13 vs C14 - 20 d
     N      Mean     StDev   SE Mean
C13  6     28.00      3.52       1.4
C14  6     19.50      4.51       1.8

95% C.I. for mu C13 - mu C14: ( 3.2,  13.8)
T-Test mu C13 = mu C14 (vs not =): T= 3.64  P=0.0054  DF=  9
```

Table 3.3. Balanced two-way ANOVA on the length of the oldest root after dehiscence of plantlets from either end of the paracladial zone.

```
Analysis of Variance (Balanced Designs)

Source           DF         SS         MS       F      P
Time              6    7209.57    1201.60  105.43  0.000
Position          1     316.30     316.30   27.75  0.000
Time x Position   6     223.29      37.21    3.27  0.007
Error            70     797.83      11.40
Total            83    8546.99
```

Fig. 3.19. Relative growth rate of plantlets over a period of thirty days after dehiscence. Plantlets were originally from either distal (1) or proximal (2) positions within the paracladial zone.

```
1 = distal
2 = proximal

Twosample T for RGR - difference between distal and proximal plantlets on
establishment
C2  N      Mean     StDev   SE Mean
1   7    0.0417    0.0108    0.0041
2   7   0.00444   0.00435    0.0016

95% C.I. for mu 1 - mu 2: ( 0.0269,  0.0477)
T-Test mu 1 = mu 2 (vs not =): T= 8.47  P=0.0000  DF=  7
```

Chapter 4

Fig. 4.2. Whole plant dry weight 2 d after cessation of culm elongation growth. Plants were grown in one-fifth strength Long Ashton nutrient solution (low) and full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of four replicates. Different letters indicate significance at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure.

```
One-Way Analysis of Variance

Source     DF        SS        MS        F        p
Treat       3     844.5     281.5    16.30    0.000
Error      12     207.2      17.3
Total      15    1051.7

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
 Level      N      Mean     StDev  -------+---------+---------+---------
     1      4     7.047     3.040  (-----*----)
     2      4    21.228     4.752                (-----*----)
     3      4    10.878     5.086       (-----*----)
     4      4    24.798     3.374                      (-----*-----)
                                  -------+---------+---------+---------
Pooled StDev =    4.156              8.0      16.0      24.0
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0117
Critical value = 4.20
Intervals for (column level mean) - (row level mean)

               1         2         3

     2   -22.907
          -5.454

     3   -12.558     1.623
           4.895    19.076

     4   -26.478   -12.297   -22.647
          -9.025     5.156    -5.193
```

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	54.79	54.79	3.17	0.100
Nutrient	1	789.64	789.64	45.73	0.000
CO_2 x Nutrient	1	0.07	0.07	0.00	0.951
Error	12	207.22	17.27		
Total	15	1051.72			

Table 4.1. Balanced three-way ANOVA on the dry weight of senescent leaf blade material from the innovation zone following cessation of culm elongation growth. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	135366	135366	117.91	0.000
Nutrient	1	58211	58211	50.71	0.000
Time	2	36301	18151	15.81	0.000
CO_2 x Nutrient	1	11506	11506	10.02	0.003
CO_2 x Time	2	39081	19540	17.02	0.000
Nutrient x Time	2	4627	2313	2.02	0.148
CO_2 x Nutrient x Time	2	217	108	0.09	0.910
Error	36	41329	1148		
Total	47	326638			

Table 4.2. Instantaneous net photosynthetic rate (A) (µmol CO_2 m^{-2} s^{-1}) of youngest fully expanded leaf of the main axis of vegetative *Poa alpina* (19 ± 3.4 days before paracladial emergence), grown in one-fifth strength Long Ashton (low) and full-strength Long Ashton nutrient solution (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). A was measured at both ambient and elevated CO_2 concentrations. Data represent the mean ± one S.E. of ten replicates.

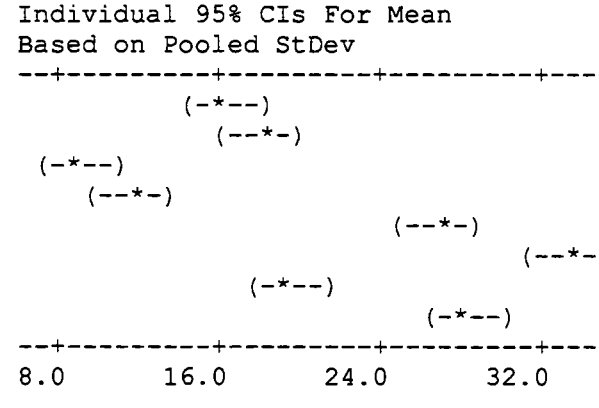
One-Way Analysis of Variance

Analysis of Variance on Photosynthetic rate of youngest fully expanded leaf 19 days prior to anthesis.

Source	DF	SS	MS	F	p
Treat	7	5044.1	720.6	69.80	0.000
Error	72	743.4	10.3		
Total	79	5787.5			

Level	N	Mean	StDev
1	10	16.140	2.774
2	10	18.120	4.154
3	10	8.990	1.414
4	10	11.780	3.446
5	10	27.080	1.961
6	10	33.480	4.249
7	10	19.420	3.110
8	10	28.140	3.493

Pooled StDev = 3.213



Tukey's pairwise comparisons
Family error rate = 0.0500
Individual error rate = 0.00258
Critical value = 4.42

Intervals for (column level mean) - (row level mean)

	1	2	3	4	5	6	7
2	-6.471						
	2.511						
3	2.659	4.639					
	11.641	13.621					
4	-0.131	1.849	-7.281				
	8.851	10.831	1.701				
5	-15.431	-13.451	-22.581	-19.791			
	-6.449	-4.469	-13.599	-10.809			
6	-21.831	-19.851	-28.981	-26.191	-10.891		
	-12.849	-10.869	-19.999	-17.209	-1.909		
7	-7.771	-5.791	-14.921	-12.131	3.169	9.569	
	1.211	3.191	-5.939	-3.149	12.151	18.551	
8	-16.491	-14.511	-23.641	-20.851	-5.551	0.849	-13.211
	-7.509	-5.529	-14.659	-11.869	3.431	9.831	-4.229

Fig. 4.4. Photosynthetic acclimation to elevated CO_2 of plants grown at either 350 μmol mol^{-1} (ambient) or 690 μmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data represent the mean ± one S.E. of six replicates.

Twosample T for C1

C2	N	Mean	StDev	SE Mean
1	10	0.321	0.370	0.12
2	10	0.750	0.239	0.076

95% C.I. for mu 1 - mu 2: (-0.73, -0.131)
T-Test mu 1 = mu 2 (vs not =): T= -3.07 P=0.0077 DF= 15

Table 4.3. Balanced three-way ANOVA on the total non-structural carbohydrate content of innovation zone leaf blade material following cessation of culm elongation. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 μmol mol^{-1} (ambient) or 690 μmol mol^{-1} CO_2 (elevated).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	74.17	74.17	1.04	0.318
Nutrient	1	59.97	59.97	0.84	0.368
Time	1	84.01	84.01	1.18	0.289
CO_2 x Nutrient	1	5.64	5.64	0.08	0.781
CO_2 x Time	1	377.53	377.53	5.29	0.030
Nutrient x Time	1	87.17	87.17	1.22	0.280
CO_2 x Nutrient x Time	1	0.45	0.45	0.01	0.937
Error	24	1712.21	71.34		
Total	31	2401.16			

Table 4.4. **The total nitrogen (ammonium; N) and phosphorus (P) concentration (mg g^{-1} total DW) of innovation zone leaf blade material following cessation of culm elongation. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).**

Analysis of Variance (Balanced Designs) Nitrogen

Source	DF	SS	MS	F	P
CO_2	1	89.92	89.92	3.59	0.070
Nutrient	1	18.53	18.53	0.74	0.398
Time	1	19.65	19.65	0.78	0.385
CO_2 x Nutrient	1	6.06	6.06	0.24	0.627
CO_2 x Time	1	153.83	153.83	6.13	0.021
Nutrient x Time	1	56.39	56.39	2.25	0.147
CO_2 x Nutrient x Time	1	65.45	65.45	2.61	0.119
Error	24	601.76	25.07		
Total	31	1011.60			

Analysis of Variance (Balanced Designs) Phosphorus

Source	DF	SS	MS	F	P
CO_2	1	10.516	10.516	4.03	0.056
Nutrient	1	104.507	104.507	40.06	0.000
Time	1	1.933	1.933	0.74	0.398
CO_2 x Nutrient	1	0.153	0.153	0.06	0.811
CO_2 x Time	1	10.046	10.046	3.85	0.061
Nutrient x Time	1	0.387	0.387	0.15	0.704
CO_2 x Nutrient x Time	1	0.467	0.467	0.18	0.676
Error	24	62.612	2.609		
Total	31	190.619			

Table 4.5. **The photosynthetic nitrogen use efficiency (PNUE; g sDW g^{-1} N d^{-1}) and photosynthetic phosphorus use efficiency (PPUE; g sDW g^{-1} P d^{-1}) of innovation zone leaf blade material over an 18 d period following cessation of culm elongation growth in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).**

Analysis of Variance (Balanced Designs) PNUE

Source	DF	SS	MS	F	P
CO_2	1	0.000476	0.000476	0.20	0.663
Nutrient	1	0.045303	0.045303	18.93	0.001
CO_2 x Nutrient	1	0.044140	0.044140	18.44	0.001
Error	12	0.028724	0.002394		
Total	15	0.118643			

Analysis of Variance (Balanced Designs)PPUE

Source	DF	SS	MS	F	P
CO_2	1	0.04633	0.04633	6.37	0.027
Nutrient	1	0.40361	0.40361	55.50	0.000
CO_2 x Nutrient	1	0.28532	0.28532	39.24	0.000
Error	12	0.08727	0.00727		
Total	15	0.82253			

Analysis of Variance (Balanced Designs)over time

Source	DF	SS	MS	F	P
CO_2	1	0.01871	0.01871	3.87	0.061
Nutrient	1	0.35968	0.35968	74.42	0.000
Time	1	0.22052	0.22052	45.63	0.000
CO_2 x Nutrient	1	0.27695	0.27695	57.31	0.000
CO_2 x Time	1	0.02810	0.02810	5.81	0.024
Nutrient x Time	1	0.08924	0.08924	18.46	0.000
CO_2 x Nutrient x Time	1	0.05251	0.05251	10.86	0.003
Error	24	0.11599	0.00483		
Total	31	1.16169			

Fig. 4.5. Developmental timing of the culm of *Poa alpina*; (a) the time from planting to paracladial emergence, (b) the time from paracladial emergence to cessation of culm elongation growth. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high). Data represent the mean ± one S.E. of twelve replicates. Different letters indicate significance at the $P \leq 0.05$ level determined by Tukey's multiple comparison procedure.

1&3 = low nutrient
2&4 = high nutrient

1&2 = ambient CO_2
3&4 = elevated CO_2

One-Way Analysis of Variance

Analysis of Variance on **(a)** Time from planting to paracladial exertion

Source	DF	SS	MS	F	p
Treat	3	4971	1657	6.44	0.001
Error	44	11322	257		
Total	47	16293			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	12	74.17	22.22	(-----*------)
2	12	81.58	5.95	(-----*------)
3	12	101.50	20.08	(------*-----)
4	12	81.33	9.86	(-----*-----)

Pooled StDev = 16.04 — 75 90 105

Tukey's pairwise comparisons
Family error rate = 0.0500
Individual error rate = 0.0105
Critical value = 3.78
Intervals for (column level mean) - (row level mean)

	1	2	3
2	-24.92 10.09		
3	-44.84 -9.83	-37.42 -2.41	
4	-24.67 10.34	-17.25 17.75	2.66 37.67

One-Way Analysis of Variance

Analysis of Variance on **(b)** Time from exertion to cessation of culm elongation

Source	DF	SS	MS	F	p
Treat	3	270.2	90.1	4.58	0.007
Error	44	865.5	19.7		
Total	47	1135.7			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	12	26.583	3.630	(------*------)
2	12	28.917	4.870	(-------*------)
3	12	22.333	3.627	(-------*------)
4	12	26.500	5.351	(-------*------)

Pooled StDev = 4.435 — 21.0 24.5 28.0 31.5

Tukey's pairwise comparisons
Family error rate = 0.0500
Individual error rate = 0.0105
Critical value = 3.78
Intervals for (column level mean) - (row level mean)

	1	2	3
2	-7.173 2.506		
3	-0.590 9.090	1.744 11.423	
4	-4.756 4.923	-2.423 7.256	-9.006 0.673

Table 4.6. Balanced three-way ANOVA on the number of tillers in flower at the time of cessation of culm elongation growth. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	1.8755	1.8755	10.61	0.002
Nutrient	1	21.8765	21.8765	123.76	0.000
Time	2	4.3508	2.1754	12.31	0.000
CO_2 x Nutrient	1	4.3569	4.3569	24.65	0.000
CO_2 x Time	2	0.4962	0.2481	1.40	0.259
Nutrient x Time	2	0.4880	0.2440	1.38	0.264
CO_2 x Nutrient x Time	2	1.3060	0.6530	3.69	0.035
Error	36	6.3634	0.1768		
Total	47	41.1132			

Table 4.7. Balanced three-way ANOVA on the total reproductive dry weight following the cessation of culm elongation growth. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	135868	135868	1.47	0.233
Nutrient	1	5938731	5938731	64.30	0.000
Time	2	1792164	896082	9.70	0.000
CO_2 x Nutrient	1	130778	130778	1.42	0.242
CO_2 x Time	2	54330	27165	0.29	0.747
Nutrient x Time	2	1569020	784510	8.49	0.001
CO_2 x Nutrient x Time	2	5418	2709	0.03	0.971
Error	36	3324948	92360		
Total	47	12951258			

Table 4.8. The final length (mm) of the distal three internodes of the culm (internode 1 = proximal node). Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of ten replicates.

One-Way Analysis of Variance
Analysis of Variance on distal internode length (internode 3)

Source	DF	SS	MS	F	p
treat	3	30957	10319	8.33	0.000
Error	44	54535	1239		
Total	47	85492			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	12	222.00	39.12	(------*------)
2	12	264.83	41.65	(------*------)
3	12	216.75	24.92	(------*------)
4	12	274.33	32.74	(------*------)

Pooled StDev = 35.21

210 240 270 300

Tukey's pairwise comparisons
Family error rate = 0.0500
Individual error rate = 0.0105
Critical value = 3.78
Intervals for (column level mean) - (row level mean)

	1	2	3
2	-81.2 -4.4		
3	-33.2 43.7	9.7 86.5	
4	-90.7 -13.9	-47.9 28.9	-96.0 -19.2

Analysis of Variance on middle culm internode lengths (internode 2)

Source	DF	SS	MS	F	p
treat	3	16827	5609	7.56	0.000
Error	44	32632	742		
Total	47	49459			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	12	58.17	27.71	(-----*------)
2	12	109.83	35.41	(-----*-----)
3	12	75.00	24.64	(-----*-----)
4	12	85.25	18.39	(-----*-----)

Pooled StDev = 27.23

50 75 100 125

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.0105

Critical value = 3.78

Intervals for (column level mean) - (row level mean)

	1	2	3
2	-81.38 -21.95		
3	-46.55 12.88	5.12 64.55	
4	-56.80 2.63	-5.13 54.30	-39.97 19.47

Analysis of Variance on proximal culm internode lengths (internode 1)

Source	DF	SS	MS	F	p
treat	3	3830	1277	10.13	0.000
Error	44	5544	126		
Total	47	9373			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	12	7.92	5.40	(------*-----)
2	12	31.83	16.85	(------*-----)
3	12	12.83	6.48	(------*-----)
4	12	17.83	12.21	(------*-----)

Pooled StDev = 11.22

10 20 30

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.0105

Critical value = 3.78

Intervals for (column level mean) - (row level mean)

	1	2	3
2	-36.17 -11.67		
3	-17.17 7.33	6.75 31.25	
4	-22.17 2.33	1.75 26.25	-17.25 7.25

```
One-Way Analysis of Variance

Analysis of Variance on Final culm length
Source     DF        SS        MS        F        p
Treat       3    116438     38813    14.25    0.000
Error      44    119882      2725
Total      47    236320

                                      Individual 95% CIs For Mean
                                      Based on Pooled StDev
 Level      N      Mean     StDev  ---------+---------+---------+-------
     1     12    288.08     61.36   (-----*-----)
     2     12    406.50     62.02                          (-----*-----)
     3     12    304.58     40.88      (-----*-----)
     4     12    377.42     40.19                    (-----*------)
                                     ---------+---------+---------+-------
Pooled StDev =    52.20                    300       350       400
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0105
Critical value = 3.78
Intervals for (column level mean) - (row level mean)

              1         2         3

     2    -175.4
           -61.5

     3     -73.5      45.0
            40.5     158.9

     4    -146.3     -27.9    -129.8
           -32.4      86.0     -15.9
```

Table 4.9. Number of spikelets per panicle of *Poa alpina*, grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated). Data represent the mean ± one S.E. of twelve replicates. Data were square-root transformed prior to statistical analysis.

```
One-Way Analysis of Variance
Source     DF        SS        MS        F        p
treat       3      4.80      1.60     1.58    0.210
Error      36     36.42      1.01
Total      39     41.22

                                      Individual 95% CIs For Mean
                                      Based on Pooled StDev
 Level      N      Mean     StDev  -----+---------+---------+---------+-
     1     12     7.556     1.375  (----------*----------)
     2     12     8.010     0.608           (----------*---------)
     3     12     7.710     1.183     (---------*----------)
     4     12     8.465     0.622                  (----------*----------)
                                     -----+---------+---------+---------+-
Pooled StDev =    1.006               7.20      7.80      8.40      9.00
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0107
Critical value = 3.81
```

Intervals for (column level mean) - (row level mean)

	1	2	3
2	-1.666 0.758		
3	-1.365 1.058	-0.911 1.512	
4	-2.120 0.303	-1.666 0.758	-1.967 0.457

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	0.923	0.923	0.91	0.346
Nutrients	1	3.654	3.654	3.61	0.065
CO_2 x Nutrients	1	0.226	0.226	0.22	0.639
Error	36	36.418	1.012		
Total	39	41.222			

Table 4.10. Balanced three-way ANOVA on the dry weight of the main axis paracladial zone after the cessation of elongation growth of the culm. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	3248	3248	0.21	0.653
Nutrient	1	864294	864294	54.85	0.000
Time	2	149050	74525	4.73	0.015
CO_2 x Nutrient	1	242809	242809	15.41	0.000
CO_2 x Time	2	27257	13629	0.86	0.430
Nutrient x Time	2	55915	27957	1.77	0.184
CO_2 x Nutrient x Time	2	10350	5175	0.33	0.722
Error	36	567224	15756		
Total	47	1920148			

Table 4.11. Balanced three-way ANOVA on the number of fully expanded leaves on plantlets after the cessation of elongation growth of the culm (no distinction was made between distal and proximal). Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	0.00069	0.00069	0.04	0.840
Nutrient	1	0.34641	0.34641	20.82	0.000
Time	2	1.11901	0.55950	33.63	0.000
CO_2 x Nutrient	1	0.14849	0.14849	8.93	0.005
CO_2 x Time	2	0.04205	0.02103	1.26	0.295
Nutrient x Time	2	0.00212	0.00106	0.06	0.938
CO_2 x Nutrient x Time	2	0.09829	0.04915	2.95	0.065
Error	36	0.59888	0.01664		
Total	47	2.35593			

Table 4.12. Balanced three-way ANOVA on the proportion of senescent dry weight in the paracladial zone following the cessation of elongation growth of the culm. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	46.9	46.9	0.93	0.342
Nutrient	1	350.4	350.4	6.92	0.012
Time	2	11716.3	5858.1	115.77	0.000
CO_2 x Nutrient	1	4.4	4.4	0.09	0.770
CO_2 x Time	2	28.4	14.2	0.28	0.757
Nutrient x Time	2	15.8	7.9	0.16	0.856
CO_2 x Nutrient x Time	2	15.7	7.8	0.15	0.857
Error	36	1821.7	50.6		
Total	47	13999.5			

Table 4.13. Balanced four-way ANOVA on the total non-structural carbohydrate content of plantlets from either distal or proximal positions in the paracladial zone following cessation of culm elongation. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	952.4	952.4	3.02	0.089
Nutrient	1	1031.7	1031.7	3.27	0.077
Position	1	3024.8	3024.8	9.58	0.003
Time	1	16166.9	16166.9	51.18	0.000
CO_2 x Nutrient	1	66.7	66.7	0.21	0.648
CO_2 x Position	1	862.7	862.7	2.73	0.105
CO_2 x Time	1	91.3	91.3	0.29	0.593
Nutrient x Position	1	8.5	8.5	0.03	0.871
Nutrient x Time	1	3.1	3.1	0.01	0.922
Position x Time	1	1049.4	1049.4	3.32	0.075
CO_2 x Nutrient x Position	1	80.6	80.6	0.26	0.616
CO_2 x Nutrient x Time	1	474.4	474.4	1.50	0.226
CO_2 x Position x Time	1	59.6	59.6	0.19	0.666
Nutrient x Position x Time	1	101.4	101.4	0.32	0.574
CO_2 x Nutrient x Position x Time	1	836.2	836.2	2.65	0.110
Error	48	15161.6	315.9		
Total	63	39971.2			

Table 4.14. Balanced three-way ANOVA on the total nitrogen (ammonium) and phosphorus content of the paracladial zone following cessation of culm elongation. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).

Analysis of Variance (Balanced Designs)N

Source	DF	SS	MS	F	P
CO_2	1	3.40	3.40	0.24	0.626
Nutrient	1	0.00	0.00	0.00	0.988
Time	1	1.80	1.80	0.13	0.722
CO_2 x Nutrient	1	8.56	8.56	0.62	0.440
CO_2 x Time	1	30.87	30.87	2.22	0.149
Nutrient x Time	1	22.34	22.34	1.61	0.217
CO_2 x Nutrient x Time	1	156.66	156.66	11.27	0.003
Error	24	333.61	13.90		
Total	31	557.24			

Analysis of Variance (Balanced Designs) P

Source	DF	SS	MS	F	P
CO_2	1	5.3432	5.3432	9.87	0.004
Nutrient	1	13.3254	13.3254	24.61	0.000
Time	1	4.4316	4.4316	8.18	0.009
CO_2 x Nutrient	1	0.0764	0.0764	0.14	0.710
CO_2 x Time	1	0.0783	0.0783	0.14	0.707
Nutrient x Time	1	2.9576	2.9576	5.46	0.028
CO_2 x Nutrient x Time	1	0.8137	0.8137	1.50	0.232
Error	24	12.9963	0.5415		
Total	31	40.0225			

Table 4.15. The photosynthetic nitrogen use efficiency (PNUE; g sDW g^{-1} N d^{-1}) and photosynthetic phosphorus use efficiency (PPUE; g sDW g^{-1} P d^{-1}) of paracladial zone material over an 18 d period following cessation of culm elongation growth in *Poa alpina*. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).

Analysis of Variance (Balanced Designs) N

Source	DF	SS	MS	F	P
CO_2	1	0.01080	0.01080	0.28	0.605
Nutrient	1	0.05115	0.05115	1.34	0.270
CO_2 x Nutrient	1	0.21820	0.21820	5.70	0.034
Error	12	0.45927	0.03827		
Total	15	0.73942			

Analysis of Variance (Balanced Designs) P

Source	DF	SS	MS	F	P
CO_2	1	0.2467	0.2467	1.75	0.211
Nutrient	1	1.9945	1.9945	14.11	0.003
CO_2 x Nutrient	1	1.0342	1.0342	7.32	0.019
Error	12	1.6959	0.1413		
Total	15	4.9713			

Table 4.16. Balanced four-way ANOVA on the dry weight of plantlets from the two halves of the main axis paracladial zone after the cessation of elongation growth of the culm. Plants were grown at either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} (elevated) atmospheric CO_2, and two nutrient regimes; one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	0.665	0.665	0.54	0.464
Nutrient	1	146.601	146.601	119.37	0.000
Position	1	71.435	71.435	58.16	0.000
Time	2	71.923	35.961	29.28	0.000
CO_2 x Nutrient	1	36.191	36.191	29.47	0.000
CO_2 x Position	1	0.324	0.324	0.26	0.609
CO_2 x Time	2	0.704	0.352	0.29	0.752
Nutrient x Position	1	0.677	0.677	0.55	0.460
Nutrient x Time	2	8.106	4.053	3.30	0.043
Position x Time	2	0.539	0.269	0.22	0.804
CO_2 x Nutrient x Position	1	3.961	3.961	3.23	0.077
CO_2 x Nutrient x Time	2	3.273	1.637	1.33	0.270
CO_2 x Position x Time	2	1.510	0.755	0.61	0.544
Nutrient x Position x Time	2	1.231	0.615	0.50	0.608
CO_2 x Nutrient x Position x Time	2	0.254	0.127	0.10	0.902
Error	72	88.426	1.228		
Total	95	435.819			

Table 4.17. Balanced four-way ANOVA on the leaf area ratio (LAR) of plantlets from either distal or proximal positions in the paracladial zone following cessation of culm elongation. Plants were grown in either one-fifth strength Long Ashton nutrient solution (low) or full-strength Long Ashton (high), and either 350 µmol mol^{-1} (ambient) or 690 µmol mol^{-1} CO_2 (elevated).

Analysis of Variance (Balanced Designs)

Source	DF	SS	MS	F	P
CO_2	1	0.004064	0.004064	1.27	0.263
Nutrient	1	0.000727	0.000727	0.23	0.635
Position	1	0.022216	0.022216	6.95	0.010
Time	2	0.148382	0.074191	23.22	0.000
CO_2 x Nutrient	1	0.001818	0.001818	0.57	0.453
CO_2 x Position	1	0.000223	0.000223	0.07	0.792
CO_2 x Time	2	0.061283	0.030641	9.59	0.000
Nutrient x Position	1	0.001179	0.001179	0.37	0.546
Nutrient x Time	2	0.000053	0.000026	0.01	0.992
Position x Time	2	0.004952	0.002476	0.77	0.465
CO_2 x Nutrient x Position	1	0.001584	0.001584	0.50	0.484
CO_2 x Nutrient x Time	2	0.014982	0.007491	2.34	0.103
CO_2 x Position x Time	2	0.009845	0.004923	1.54	0.221
Nutrient x Position x Time	2	0.002421	0.001210	0.38	0.686
CO_2 x Nutrient x Position x Time	2	0.003118	0.001559	0.49	0.616
Error	72	0.230087	0.003196		
Total	95	0.506935			

Chapter 5

Fig. 5.2. The number of spikelets possessing an elongated lemma after pressure treatment.

```
C1 = treated
C2 = control

Twosample T for spikelet number - difference between pressure treatments
    N      Mean     StDev   SE Mean
C1  6      31.7      10.2       4.1
C2  6     48.17      7.83       3.2

95% C.I. for mu C1 - mu C2: ( -28.3,  -4.7)
T-Test mu C1 = mu C2 (vs not =): T= -3.15  P=0.012  DF=  9
```

Table 5.1. Balanced two-way ANOVA on the proportion of the total number of spikelets that possessed an elongated lemma within the two halves of the paracladial zone after the application of increased pressure on the pseudostem (treated). Data represent the mean ± one S.E. of six replicates.

```
Analysis of Variance (Balanced Designs)

Source              DF         SS         MS       F      P
Position             1     7715.0     7715.0   31.24  0.000
Pressure             1        0.0        0.0    0.00  1.000
Position x Pressure  1      351.1      351.1    1.42  0.247
Error               20     4938.9      246.9
Total               23    13005.0
```

Fig. 5.4. Final length of the distal internode of the culm after pressure treatment.

```
C1 = treated
C2 = control

Twosample T for C1 C2
    N      Mean     StDev   SE Mean
C1  6     135.2      48.1        20
C2  6     193.3      25.2        10
95% C.I. for mu C1 - mu C2: ( -111,  -6)
T-Test mu C1 = mu C2 (vs not =): T= -2.63  P=0.034  DF=  7
```