Physiological aspects of stress in Sesleria Caerulea (L) ard

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PHYSIOLOGICAL ASPECTS OF STRESS

IN *SESLERIA CAERULEA* (L) ARD.

M.Sc. Dissertation

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Abstract

Sesleria caerulea is a wide ranging species, growing over a large altitudinal range. It varies spatially in both morphological and physiological characteristics.

Responses to stress are characterised by an increase in proline production, but the ability to respond is not consistent for all populations. Edaphic factors are important in determining the nature of the response to cold stress, but if edaphic factors are standardized, proline production can be correlated with altitude - plants at high elevations accumulate more proline than those from lower elevations.

Edaphic factors are more important than climatic factors in determining the plant's ability to respond to drought stress. Plants growing in shallow soils produce more proline than those of deep soils, and are therefore more drought tolerant.

The ability to produce proline appearsto be maintained throughout the plant's life, with no significant difference between the quantities of proline produced in the apical and basal halves of leaf blades.

In the one case studied, the leaf form of Sesleria caerulea appeared to be an adaptation of the plant to a particular environment, rather than a genotypic characteristic. It is possible that some physiological responses are also not genotypic.

CHAPTER 1

Introduction

A plant that is exposed to any unfavourable environmental factor may be said to be under stress. All plants are subjected to stress at some time during their life cycles, although the nature and intensity of the stress varies. The stress may be that of high or low temperatures, drought or flooding, and salinity. As a result of stress, various metabolic aspects of the plant may be affected, resulting in morphological and physiological changes. Changes in amino acid metabolism and inhibition of protein synthesis may occur (Barnett and Naylor, 1966). The inhibition may cause a change in the amino acid pool of the plant (Routley, 1966).

Work by Singh et al (1973,I) showed that in wilted barley plants, the amino acid concentration doubled. Although amino acids such as asparagine and valine increased, the largest increase occured for proline, whilst other amino acids such as alanine and aspartic acid decreased. Similar results were obtained by Kemble and MacPherson (1954) working with cut perennial rye grass, and by Chen et al (1964) working with citrus seedlings.

At any one time, the amount of free proline in plant tissues depends on it's relative rates of formation and utilisation. Proline is utilised in protein synthesis and in oxidation, the latter occuring within the mitochondria (Barnard and Oaks, 1970). Proline is formed by proteolysis and by "de novo" synthesis. Stewart (1973) proposed that, as the amount produced in stressed plants exceeds that obtained from protein, the proline must be formed by "de novo" synthesis. Accumulation may be due to an increase in proline

synthesis and/or a decrease in protein synthesis. Stewart (1973) showed that when excised bean leaves are water-stressed, there is an increase in proline synthesis and a decrease in protein synthesis.

Conversion of proline to glutamic acid (proline oxidation) occurs readily in turgid tissue. This suggests that proline oxidation could function as a control mechanism for maintaining low cellular levels of proline in turgid tissue. The maintenance of turgidity is apparently the first reaction and response of the plant to stress. Proline accumulation in such a situation could provide a quick mechanism for maintaining osmotic balance (Rajagopal et al, 1971; Stewart et al, 1977). In water-stressed tissue proline oxidation is reduced. Aerobic conditions were found to be necessary for proline accumulation by Singh et al (1973,I) and by Thompson et al (1966).

Singh et al (1973,III) showed a correlation between the amount of chlorophyll in a plant, and it's ability to accumulate proline. Tissues in barley with little chlorophyll accumulated little proline. In some plants, however, neither chlorophyll nor chloroplasts are essential for proline accumulation. Palfi et al (1974) demonstrated that light was necessary for proline accumulation, but studies by Boggess et at (1975) showed that the enzyme proline - 5 - carboxylase may be sensitive to light since proline accumulation occured in the dark.

The effect of drought on plants is complex because plants are then subjected to two stresses - dehydration and overheating (Henckel, 1964). Henckel suggests however, that resistance to both are not correlated.

Plants suffer water-stress when the cells are not fully turgid. It may result from inadequate root absorption of water, or excessive transpiration, or both. Metabolic irregularities occur as a result, and proline accumulation is the most obvious change. Accumulation may be very rapid - a few hours in barley (Chu et al, 1974), and can reach concentrations as high as 1200 μg/gm dry weight in leaf tissue (Barnett and Naylor, 1966). Rajagopal et al (1977) attribute proline accumulation in naturally growing stressed plants to a response to water-stress or reduced relative water content. An immediate response to change in relative water content is shown by wheat which is sensitive to environmental stress.

It is possible that in a water-stressed plant, sudden rehydration may itself impose a stress on the plant, but of a different kind i.e flooding. Results obtained by Stewart (1972) of experiments on wilted excised leaves in the dark, show that accumulation of free proline caused by wilting, ceases when leaves are rehydrated. The fate, and rate of decrease, of proline, depends on the amount of carbohydrate in the leaf. If high levels are present during rehydration, Stewart suggests that the rate of loss of free proline is slow, and this proline is converted to protein. If carbohydrate levels are low, proline is lost rapidly, and is converted to protein proline, other amino acids, organic acids, and carbon dioxide. However, Wample and Bewley (1975) found that on rehydration, proline levels in the aerial parts of sunflower plants doubled, and only began to fall off about twelve hours after rehydration.

The effects of temperature stress are more difficult to elucidate. High temperature can indirectly lower the leaf water potential by it's effect on transpiration. Although the soil maybe at field capacity, water may be unavailable to the plant if the soil temperature is very low. Plants respond to such a temperature stress by producing large amounts of proline (Gates et al, 1971; Palfi and Juhasz, 1970). Whether this is a consequence of the temperature, or due to a correlated change in tissue water potential has not been ascertained.

Chu et al (1974) showed that low temperature treatment (5°C) affected the morphology of barley and radish plants by inhibiting plant height. Accumulation was faster in the barley than in the radish plants.

Many plants grow in a wide range of habitats. Some such as Armeria vulgaris and Plantago maritima are bimodal, with coastal and inland varieties. These varieties are morphologically distinct (Turesson, 1922), and it is likely that biochemical and physiological differences will also exist. Barnett and Naylor (1966) showed that, when water-stressed, coastal varieties of Bermuda grass accumulated more proline than the inland types. Also, different varieties of barley accumulate different amounts of proline, these levels being highest in the drought resistant varieties (Singh et al, 1973 III).

Sesleria caerulea inhabits a variety of habitats at different altitudes, and is commonly found growing on limestone. Morphological differences exist between populations from difference altitudes. West (1975) found that the stomatal index and length of the stomatal aperture could be correlated with altitude, both decreasing with a decrease in altitude. West (1975) attributed these differences to the

plastic response of the plant, although differences in plants of the same species, but of different habitats may be genetic and alterable only by genetic mutation.

This study proposes to investigate the effect of drought and chilling on Sesleria caerulea populations from two altitudes, and on plants from the same altitude where there is local variation. The amino acid proline was chosen as an indicator of stress in Sesleria caerulea because it increases much more in proportion to other amino acids when plants are stressed (Bates et al 1973; Barnett and Naylor, 1966). Proline has been used as an indicator of physiological drought, without any other parameter (Palfi and Juhasz, 1971.)

Description of Sites

Sesleria caerulea grows over a wide range of altitude. It grows on limestone in Northern England, Scotland and Western Ireland.

Three sites were chosen for study; these were considered to represent dissimilar habitat distributions for the species.

Sites A and B, at Cassop Vale (National Grid Reference 340 383[a]) are at an altitude of 121.1 metres. Site A is situated in a frost hollow. It consists of a slope on one side of a gully (Plate 1). Site B is situated on the top of a limestone cliff. (Plate 2).

Site C, on Upper Teesdale (National Grid Reference 824 315[b]) is at an altitude of 484.9 metres. (Plate 3).

	CASSOP VALE SITE A	SITE B	UPPER TEESDALE SITE C
Altitude:	121.2m	121.2m	484.9m
Underlying rock:	Magnesian limestone	Magnesian limestone	Carboniferous limestone
Soil depth:	10-20 cm	<10 cm	<10 cm
Aspect:	Southerly	Southerly	Southerly
pH :	7.9 *	8.2 *	8.0

a Ordinance Survey Map 1:50000 Sheet 92

b Ordinance Survey Map 1:50000 Sheet 93

* as quoted by Darke (1976)

PLATE 1 : Site A at Cassop Vale

(on the right of the photograph)

Gentle slope, soil deep.

PLATE 2 : Site B at Cassop Vale

On top of an exposed limestone cliff.

Shallow, dry soil.

PLATE 1

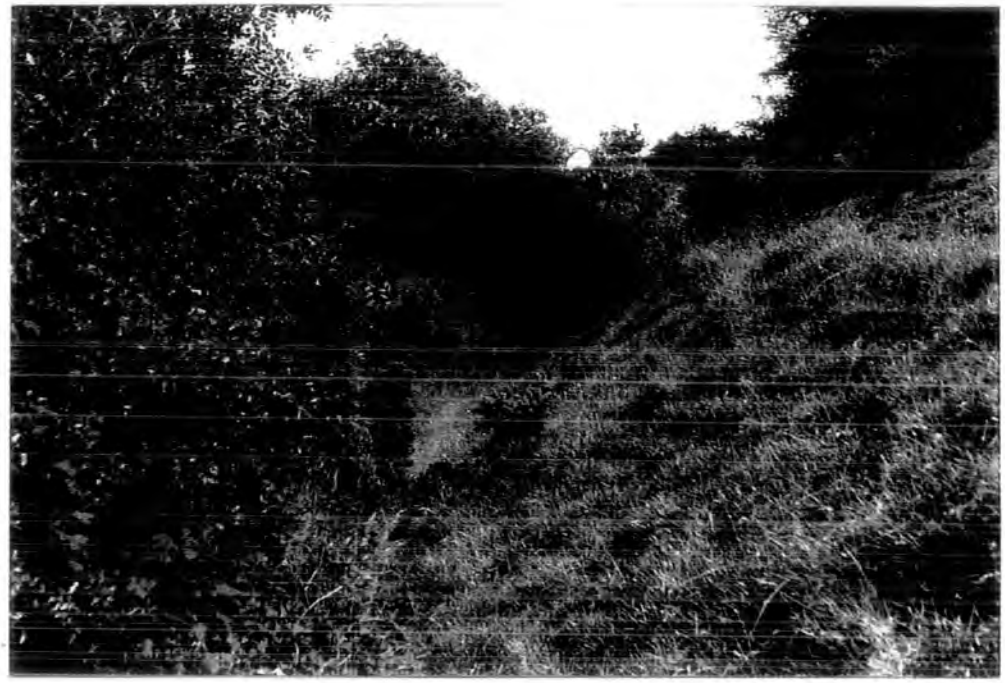


PLATE 2

PLATE 3 : Site C on Upper Teesdale

PLATE 3

CHAPTER 2

Materials and Method

i. Collection of plants.

Sesleria caerulea plants were collected from the three sites in May. Most of the soil around the roots was removed, and the plants were potted in Levington's potting compost, using pots $6cm^3$ in diameter. The soil depth was the same in all pots. Thus, as edaphic factors were standardized, any differences in results could be attributed to the plants themselves.

Plants used for chilling experiments were kept in a constant temperature room at $20^{o}C$ for two days, before subjecting them to the cold stress. During this time they were watered regularly. Plants used for the drought experiments were kept at $20^{o}C$ and watered regularly for three days before they were droughted. Control plants from each site were also kept in the constant temperature room, with a twelve hour photoperiod, and were watered daily.

ii. Morphological Studies.

Thirty plants were chosen at random from each site, and measurements made of their leaf lengths, a total of forty leaves being measured for each site. In addition, plants from Upper Teesdale, which appeared to be the smallest plants of the three sites, were left to grow for a period of eight weeks in the laboratory. At the end of this time, forty leaves were measured, the leaves being obtained from thirty plants. (Appendix D).

iii. Method for Proline Determinations.

Proline determinations were made using the methods described by Bates et al (1973) and Troll and Lindsley (1955).

Acid ninhydrin was prepared by dissolving 1.25 gm ninhydrin in 30cm^3 glacial acetic acid and 20cm^3 6M phosphoric acid. This mixture was warmed to 70°C in a water bath, to ensure that the ninhydrin was completely dissolved. Fresh solutions of acid ninhydrin were prepared for each set of determinations, although the solution is stable for 24 hours at 4°C (Troll and Lindsley, 1955).

Except for the preliminary internal calibration experiments, the quantities of material used for each determination were proportionately less than those quoted by Bates et al (1973). This was done to ensure that there was sufficient plant material for most of the determinations.

0.2 gm of plant material were ground up, for approximately one minute, with 3% sulphosalicyclic acid, using a pestle and mortar. A very small quantity of purified, acid-washed sand was added to achieve more efficient grinding of the mixture. The sulphosalicylic acid is colourless, and therefore it does not affect the colour produced by the reaction mixture. It is also effective in precipitating proteins in aqueous solutions, and does not interfere with the acid ninhydrin (Bates et al, 1973). The mixture was filtered through Whatman #1 filter paper. 2cm^3 of this filtrate were added to 0.15 gm acid permutit in a test tube, and the test tube was shaken vigorously. The permutit negates the effects of some amino acids such as lysine and ornithine which may otherwise interfere with the determination. The acid permutit functions such that the colour yields of these amino acids are then low. To this, 2cm^3 of glacial acetic acid, and an equal quantity of acid ninhydrin were added. The mixture was heated for

one hour in a water bath, set at a temperature of 80°C. At the end of this time, the tubes were cooled in ice to terminate the reactions.

When proline reacts with acid ninhydrin, a pink colour is formed. This occurs at a pH of approximately 1, and the pink product is water-insoluble (Chinard, 1952). The greater the proline concentration, the more intense the colour.

$4cm^3$ of this reaction mixture were added to $4cm^3$ toluene, and the test tube shaken for 20 seconds. Though benzene may be used, it is a less efficient solvent (Bates et al, 1973). The pigment layer with the toluene separated out, and was left until it was at room temperature. The absorbance of this layer was then read at 520 nm, using the "Uvispek" spectrophotometer, and using toluene as a blank. The proline concentration of the reaction mixture was then read off from a standard curve (Appendix A).

The standard curve was plotted using results obtained for the following solutions:

5 $\mu g/cm^3$ hydroxy-proline free L - proline
10 $\mu g/cm^3$ hydroxy-proline free L - proline
25 $\mu g/cm^3$ hydroxy-proline free L - proline
50 $\mu g/cm^3$ hydroxy-proline free L - proline
100 $\mu g/cm^3$ hydroxy-proline free L - proline
200 $\mu g/cm^3$ hydroxy-proline free L - proline
250 $\mu g/cm^3$ hydroxy-proline free L - proline

The value for μmoles proline/gm fresh weight was calculated from the equation (Bates et al, 1973):

$[(\mu gm\ proline/cm^3 \times cm^3\ toluene)/115.5\ \mu gm/\mu mole] /(gm.sample/2)$
= μmoles proline/gm of fresh weight material.

iv. Preliminary experiments.

Preliminary internal calibration experiments were carried out using Dactylis glomerata and Sesleria caerulea, to ascertain the workability of the acid ninhydrin method, and the accuracy of the technique. Proline determinations were done for the leaves, and also for filtrates obtained by grinding the leaves in sulphosalicylic acid to which known amounts of proline had been added (Appendices B and C).

v. Physiological studies.

A leaf blade varies in age along it's length, the oldest part being at the tip, and the youngest part being at the base of the blade. It is possible that different regions of the leaf may respond differently to stress. The younger parts may not have a fully developed mechanism to cope with an imposed stress, or it may be that the efficiency of this mechanism decreases with age, or both. If there is a significant difference that can be correlated with age, then this would have to be taken into account when carrying out the proposed experiments. To test if this was so, seven plants of Sesleria caerulea were allowed to wilt for a period of eight days, at a constant temperature of 20°C. At regular intervals, leaves of the same length were removed and cut into halves. As the leaves for each sample were taken from the same plant, it was assumed that they were of nearly the same age. Separate proline determinations were made for the younger basal halves, and the older apical halves (Appendix E).

The percentage water content for the two halves were compared by taking thirty leaves at random, and oven drying the apical and basal halves for 48 hours at 105°C.

For each cold and drought experiment, three replicates were used for the Cassop Vale sites, and two replicates for the Upper Teesdale site. The plants from Upper Teesdale were much smaller than those from Cassop Vale, and therefore had less plant tissue. In all cases, each experiment was repeated twice.

The plants were subjected to cold stress by placing them in a constant temperature room at 5°C, with a twelve hour photoperiod. The soil was kept at field capacity throughout, by watering the plants, when necessary,with water that was also kept at 5°C. Samples were taken on the first day, and then on every alternate day, for a period of twelve days. Due to lack of plant material towards the end of the experiment, fewer replicates were used on the last sampling day. Samples were collected at the same time of day, to eliminate any source of error that may arise due to diurnal fluctuations which are known to occur in plants (Rajagopal et al, 1977).

Plants were droughted by withholding water for a period of six days, after having initially watered them to field capacity. These plants were kept at 20°C in a constant temperature room with a twelve hour photoperiod. Samples were taken on every alternate day, and on each occasion, they were collected at the same time of day. On day six, after samples were taken, the plants were watered. Samples were collected fifteen and forty hours after rehydration.

The control plants for both the cold and drought experiments were kept at 20°C, with a twelve hour photoperiod, and were watered at regular intervals.

vi. Follow-up experiments.

The plants that were used for the cold experiments were collected from the field during a period of very hot, dry weather.

Therefore, they were already reacting to a natural drought stress. These plants were watered and left for two days before they were subjected to cold stress. It was felt that the two days may have been insufficient for the plants to recover from the droughted conditions they had been exposed to, and the subsequent rehydration in the laboratory, which may have, in itself, been a form of stress. Therefore some plants from the three sites were collected, potted and left for ten days before being cold-stressed. During this time they were watered regularly. The experiment was then carried out as before.

The plants collected from Site B at Cassop Vale appeared to wilt very rapidly when droughted. This suggested that these plants may have been drought stressed before the experiment was carried out. Plants were therefore obtained from this site, and were watered regularly for ten days before wilting them. The experiment was carried out as before.

Results are expressed in terms of % water content, μmoles proline/gm dry weight, and μmoles proline/gm fresh weight.

The experiments were carried out during the months of May, June and July, 1978.

CHAPTER 3

Results.

i. Morphological Studies.

When Sesleria caerulea plants were collected from the three sites, there was an apparent difference in the sizes of the plants. Those from Upper Teesdale (Site C), appeared to be the smallest, those from Site A at Cassop Vale were the largest, and those from Site B at Cassop Vale were intermediate (Plates 4 - 6). Results obtained of leaf lengths indicate that these differences are significant.

TABLE 1.

Length of leaves of Sesleria caerulea populations obtained from Cassop Vale and Upper Teesdale.

Site	Leaf length (mm)
Cassop Vale (A)	103.8 ± 6.14
Cassop Vale (B)	85.5 ± 3.28
Upper Teesdale (C)	36.28 ± 4.04
Upper Teesdale (C_1) (after 8 weeks growth in Durham)	92.65 ± 3.21

Results are expressed as Mean ± Standard Error.

Figure 1.

Significance tests on the morphology of populations from Cassop Vale and Upper Teesdale.

	A	B	C	C_1
A		*	*	*
B	*		*	*
C	*	*		*
C_1	*	*	*	

PLATE 4 : Plants obtained from Site A
at Cassop Vale.

PLATE 5 : Plants obtained from Site B
at Cassop Vale

PLATE 4

PLATE 5

PLATE 6 : Plants from Upper Teesdale - Site C

The plants on the right of the photograph were photographed a few days after collection from the field.

The plant on the left was photographed after 8 weeks growth in Durham.

PLATE 6

* significant at the p= 0.05 level.

Students t-test done

Number of samples = 40

Besides the difference in leaf length, there was a difference in the width of the leaves. The leaves of plants from Upper Teesdale were narrower and more folded than those of plants from Cassop Vale.

These differences may be attributed, in part, to differences in altitude between the Cassop Vale sites and Upper Teesdale. The high altitude plants are smaller than the low altitude plants. However, since there is also a significant difference between the plants from Site A and Site B, factors apart from altitude may be important. Soil depth may be an important determining factor. Site B plants, growing in a shallow soil, are smaller than Site A plants, which grow in a deeper soil. Soil depths for the Sites B and C are comparable, but Site C plants are significantly smaller than the Site B plants. This suggests that edaphic and climatic factors may together determine the morphology of the plant.

Plants from Site C were left to grow in the laboratory for eight weeks. At the end of this time, the leaves were larger and wider than at the start (Plate 6). The plants resembled those of Site B. This change was probably due to the new soil dpeth and the lower altitude at which the plants were growing. The fact that the plants changed in this way indicates that this feature is an adaptation on the part of the plant to a particular environment and is not genotypic.

Differences in length of the inflorescence stalk were also observed, the length decreasing with an increase in altitude.

Plants at the lower elevation flowered earlier than those from the higher elevation.

ii. Physiological Studies.

Table 2

Proline levels in the basal and apical halves of leaves of plants during drought stress.

	Day 0	2	4	6	8
A:	3.9 ± 1.8	4.4 ± 2.3	3.2 ± 0.8	5.9 ± 3.4	17.1 ± 7.3
B:	2.3 ± 0.9	3.8 ± 2.2	2.8 ± 0.4	5.1 ± 3.2	15.5 ± 6.2

A = apical half B = basal half

Total proline produced by A = 5.6 ± 1.3

Total proline produced by B = 4.6 ± 1.2

Results are expressed as Mean ± Standard Error.

Significance tests on the proline levels for the apical and basal halves of leaves of plants under drought stress.

	Day 0	2	4	6	8	Total
Apical and basal half.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Student's t-test done.

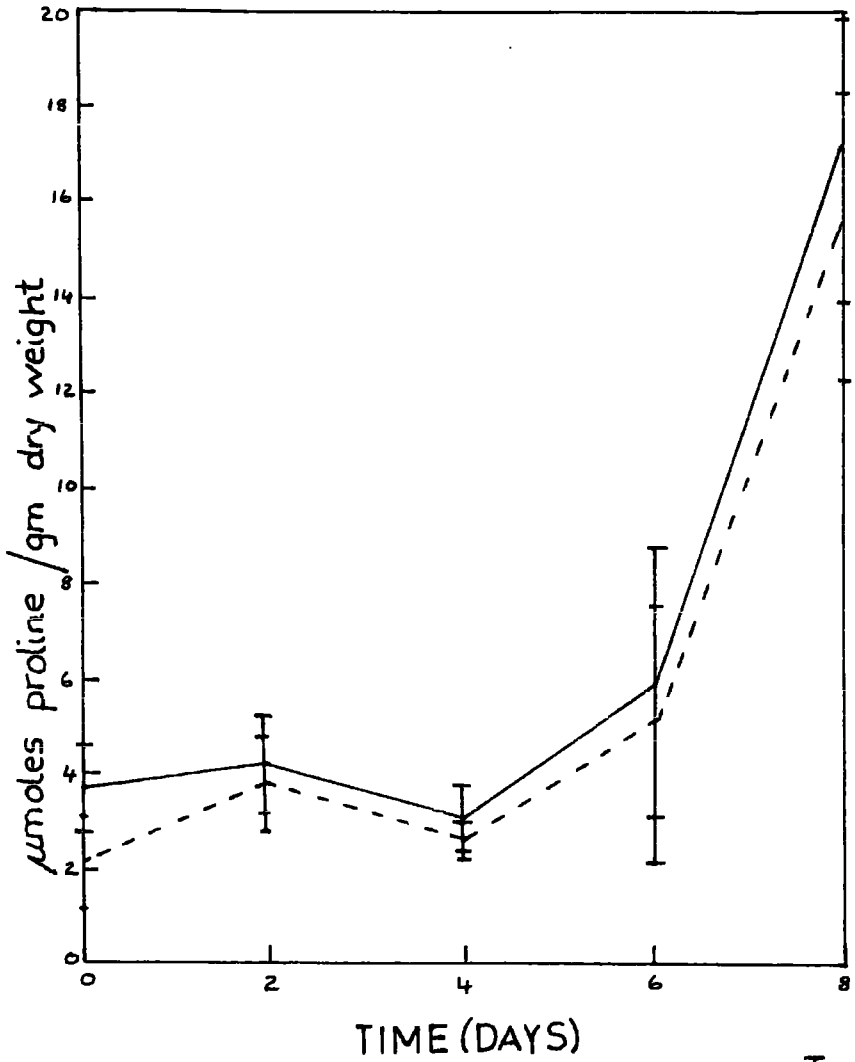
The results show that Sesleria caerulea responds to drought by accumulating proline, but in the plants used, the accumulation was not rapid.

On each sampling occasion, the apical half of the leaf produced more proline than the basal half. However, these differences were not significant at the $p = 0.05$ level, even if the total quantities of proline produced by the two halves are compared. The slightly higher levels in the apical half may be correlated with the slightly lower percentage water content ($\bar{x} = 66.23 \pm 1.01$) in this half.

FIGURE 2

Proline levels in the apical and basal halves of the leaf blades during drought stress.

FIGURE 2



I= Standard error

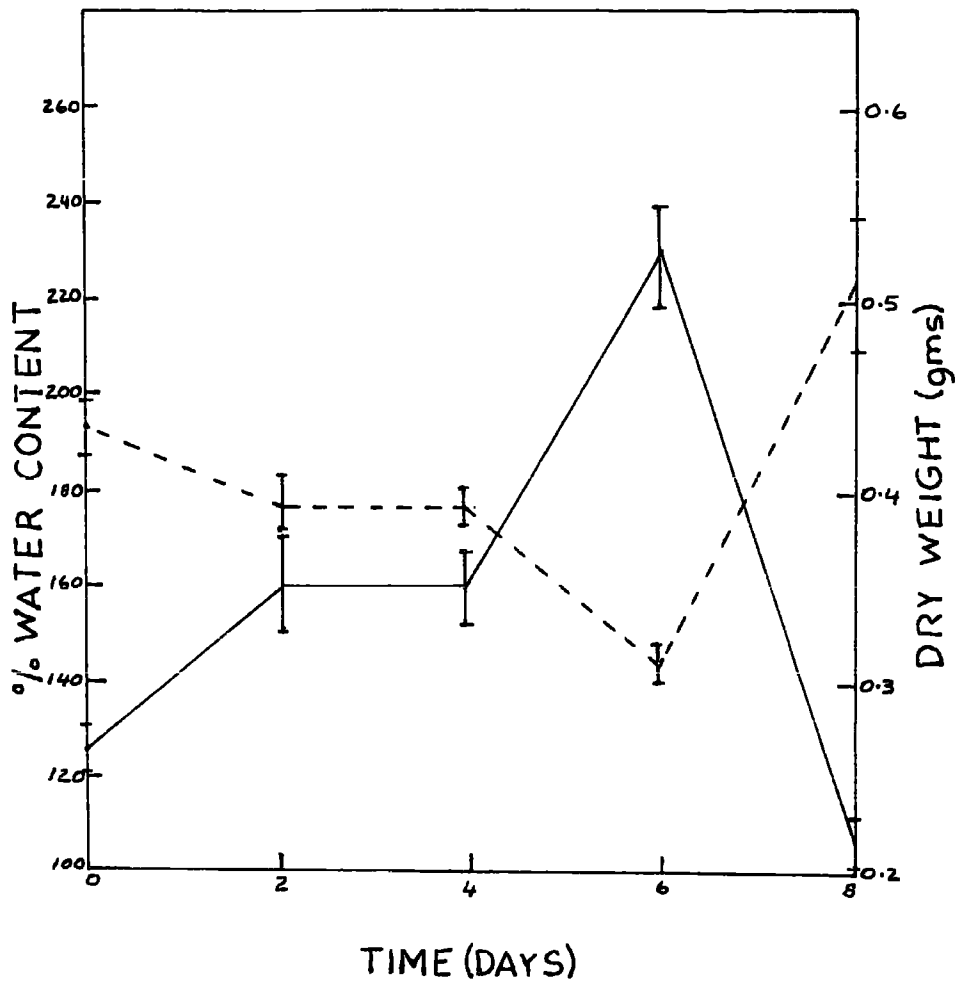
—— Apical half

--- Basal half

FIGURE 3

Change in water content and dry weight during drought stress.

FIGURE 3



I = Standard error

—— % water content

- - - dry weight

The percentage water content in the basal half however, ($\bar{x}$ = 67.23 $\pm$ 0.94) is not significantly different from that of the apical half.

The rates of increase are very similar for the halves. Therefore, within a leaf blade,there appears to be no significant difference in response to drought stress, between the older apical half, and the younger basal half (Figures 2 and 3). During the drought stress, there is a decrease in plant dry weight, and a reciprocal increase in percentage water content of the plant tissue. In all cases the percentage water content is expressed on a dry weight basis, because it was felt that this would be a more accurate measurement that if it was done on a fresh weight basis.

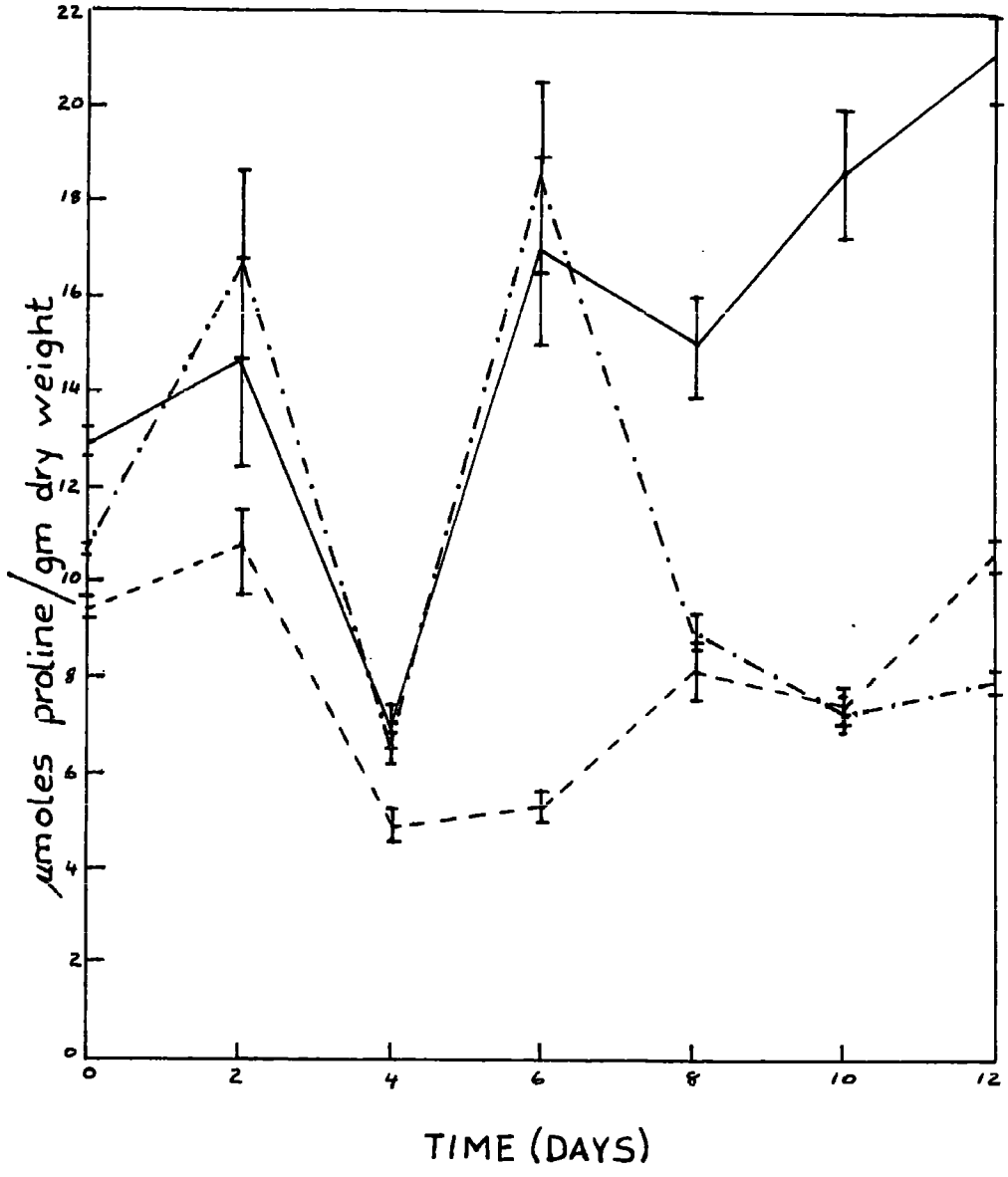
Results for the cold-stress Experiments.

Plants from all three sites showed an initial increase in proline when first subjected to cold stress. However, this was followed by a marked decrease of proline in all plants, so that the lowest levels of proline for the experiment were recorded on day 4 (Figure 4). After this, the proline increased, and this increase was greatest for plants from Sites A and C. This high concentration of proline was not maintained in the Site C plants, but increased further in the Site A plants.

FIGURE 4

Proline levels in *Sesleria* during cold stress.

FIGURE 4



I=Standard error

—— Site A

– – – Site B

·–·–·–Site C

Table 3

Proline levels in Sesleria caerulea during cold stress

μgm proline /gm dry weight

	Site A	Site B	Site C
Day 0	13.91 ± 4.30	9.51 ± 2.29	10.25 ± 1.87
Day 2	14.45 ± 8.71	10.68 ± 4.19	16.73 ± 7.87
Day 4	6.87 ± 1.89	4.71 ± 1.64	6.55 ± 2.45
Day 6	16.81 ± 6.76	5.12 ± 0.70	18.33 ± 9.91
* Day 8	15.03 ± 4.69	8.27 ± 2.78	9.02 ± 1.44
* Day 10	18.61 ± 6.35	7.62 ± 1.34	7.41 ± 1.32
* Day 12	21.14 ± 4.36	10.37 ± 2.21	7.99 ± 1.26

* Site A plants differ significantly from those of sites B and C (Appendix N).

Results are expressed in terms of Mean ± 2 Standard Errors

Site	Lowest $\bar{x}$ value of proline	$\bar{x}$ value of proline after 12 days of cold stress	Fold Increase
A	6.87	21.14	3.08
B	4.71	10.37	2.20
C	6.55	7.99	1.22

Site A plants accumulated far greater quantities of proline than those from Sites B and C, suggesting that they are the most cold tolerant.

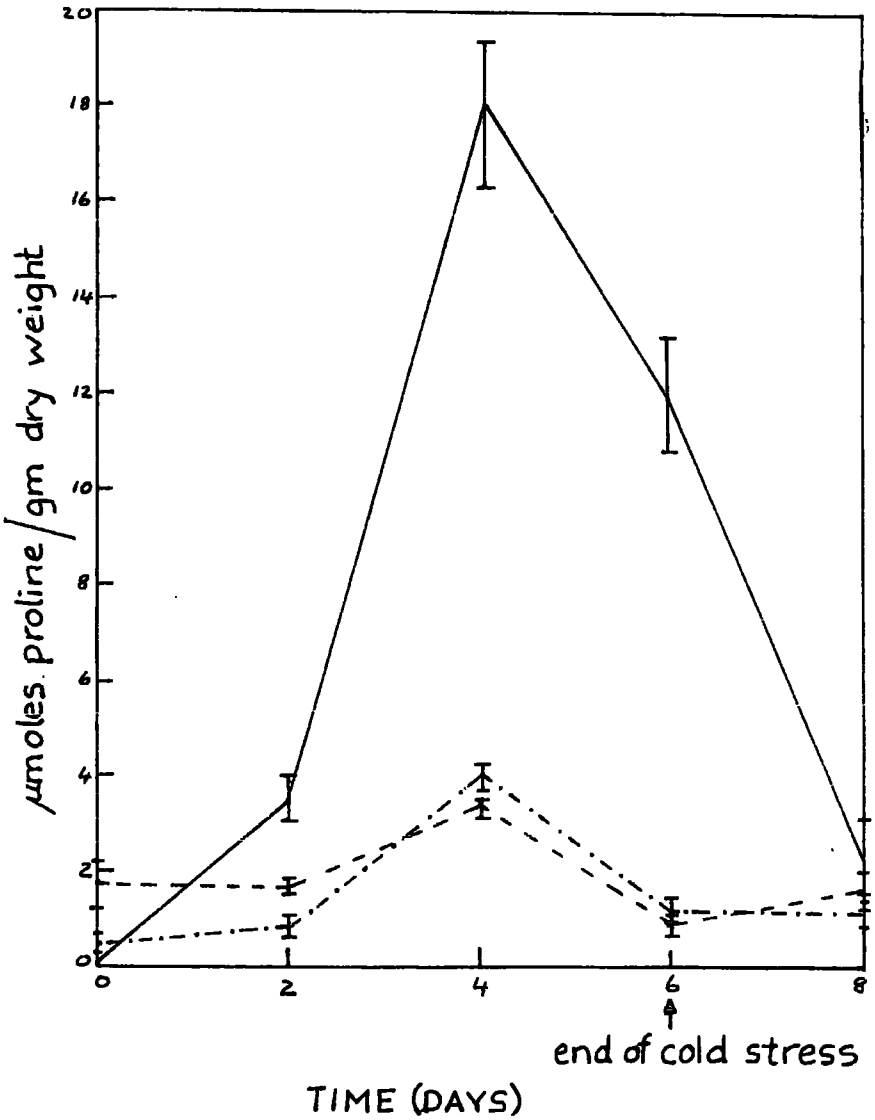
The initial increase and subsequent decrease in proline on day 4 could be due to the fact that these plants were wilted when they were collected from the fields; the effect of rehydration, plus the cold shock could have caused the initial increase in proline. If true, the plants could eventually recover from this,

FIGURE 5

Proline levels in *Sesleria* during cold stress.

(follow-up experiment).

FIGURE 5



I = Standard error

—— Site A

– – – Site B

-.-.- Site C

and then respond solely to the temperature stress.

Results obtained using unstressed plants i.e. with low initial levels of proline, are shown on Figure 5.

Site	Lowest $\bar{x}$ value of proline	Maximum $\bar{x}$ value of proline	Fold Increase
A	0.2	18.0	90.00
B	0.8	3.4	4.25
C	0.4	4.1	10.25

These results do not indicate any initial increase that is immediately followed by a decrease in proline. It is possible, therefore,that the plants in the original experiment, responded in that way because they were partially stressed when the experiment began. This together with the cold shock could account for the initial increase in proline, followed by the decrease after forty eight hours. The effect of cold on it's own does not produce this response. The follow-up experiment also showed that Site A plants were the most cold tolerant, and the most efficient at accumulating proline. Site B plants were the least efficient, while Site C plants were intermediate.

If the results obtained for the original experiment are compared, using the maximum and minimum levels of proline attained, and with day 4 as the starting point, then similar conclusions can be reached. Plants from Site A produced the greatest amounts of proline (fold increase = 3.08), those from Site B produced the least amounts (fold increase = 2.20), and those from Site C were intermediate. (fold increase = 2.8).

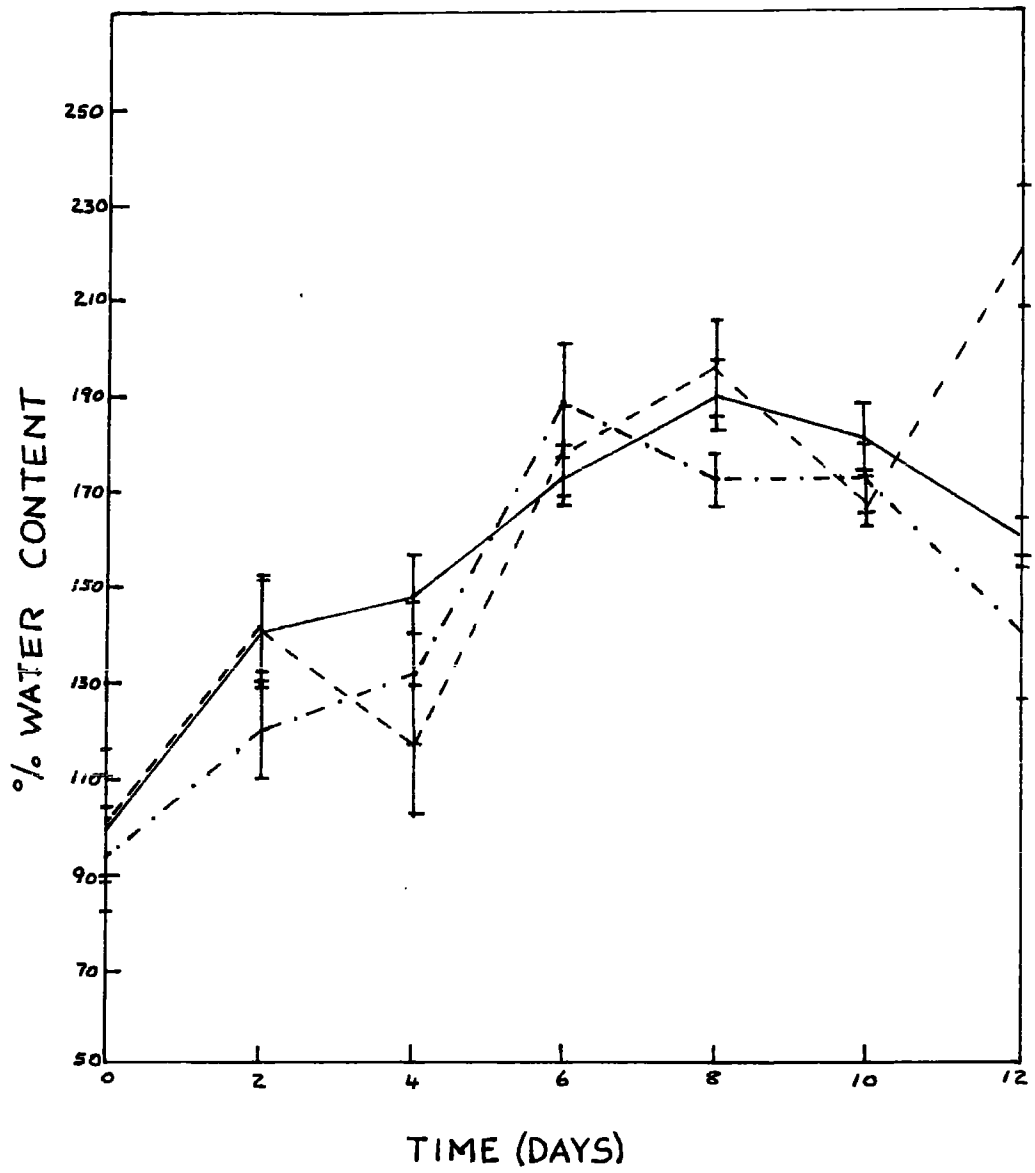
Site A is situated in a frost hollow, and plants here are subjected to great extremes of cold. Therefore, these plants need

FIGURE 6

Water Contents in *Sesleria*

during cold stress.

FIGURE 6


% WATER CONTENT
250
230
210
190
170
150
130
110
90
70
50
0
2
4
6
8
10
12
TIME (DAYS)
= Standard error
Site A
Site B
Site C

to be able to withstand periods of cold stress that they are subjected to i.e. they must be capable of accumulating large quantities of proline. Site C is at a relatively high altitude. Plants from this site suffer the cold stress that is a climatic feature at high altitudes. Therefore, they must also possess a mechanism which will enable them to tolerate the temperature stress. Site B is located at the top of an exposed limestone cliff, at a relatively low altitude. Cold stress here, is not a common occurrence, and plants growing here were incapable of accumulating much proline.

There was a general increase in the percentage water content (based on dry weight) during the period of cold stress (Figure 6). This could be due to either an anomalous gain in water in the tissues as a result of cold stress (physiological drought), or to a decrease in dry weight with no change in water content. The dry weight was found to decrease during the period of cold stress, and this trend was seen in all plants . (Figure 7). The decrease in dry weight was sufficient enough to result in significant differences, in dry weight per gram of fresh tissue, between results obtained for the start, and the end of the experiment.

Table 4

Changes in percentage water content of plant tissue during cold stress

	Site A	Site B	Site C
Day 0	99.21 ± 13.16	101.00 ± 15.90	93.93 ± 15.24
Day 2	138.8 ± 13.48	141.69 ± 21.79	120.67 ± 23.9
Day 4	148.04 ± 8.60	116.94 ± 17.35	131.9 ± 30.58
Day 6	173.27 ± 7.10	177.01 ± 19.98	189.97 ± 15.72
Day 8	190.67 ± 5.57	194.89 ± 14.00	171.48 ± 9.8
Day 10	182.67 ± 6.44	169.13 ± 11.00	171.17 ± 14.05
Day 12	163.6 ± 5.91	223.78 ± 26.46	138.2 ± 29.95

The percentage water content is based on dry weight.

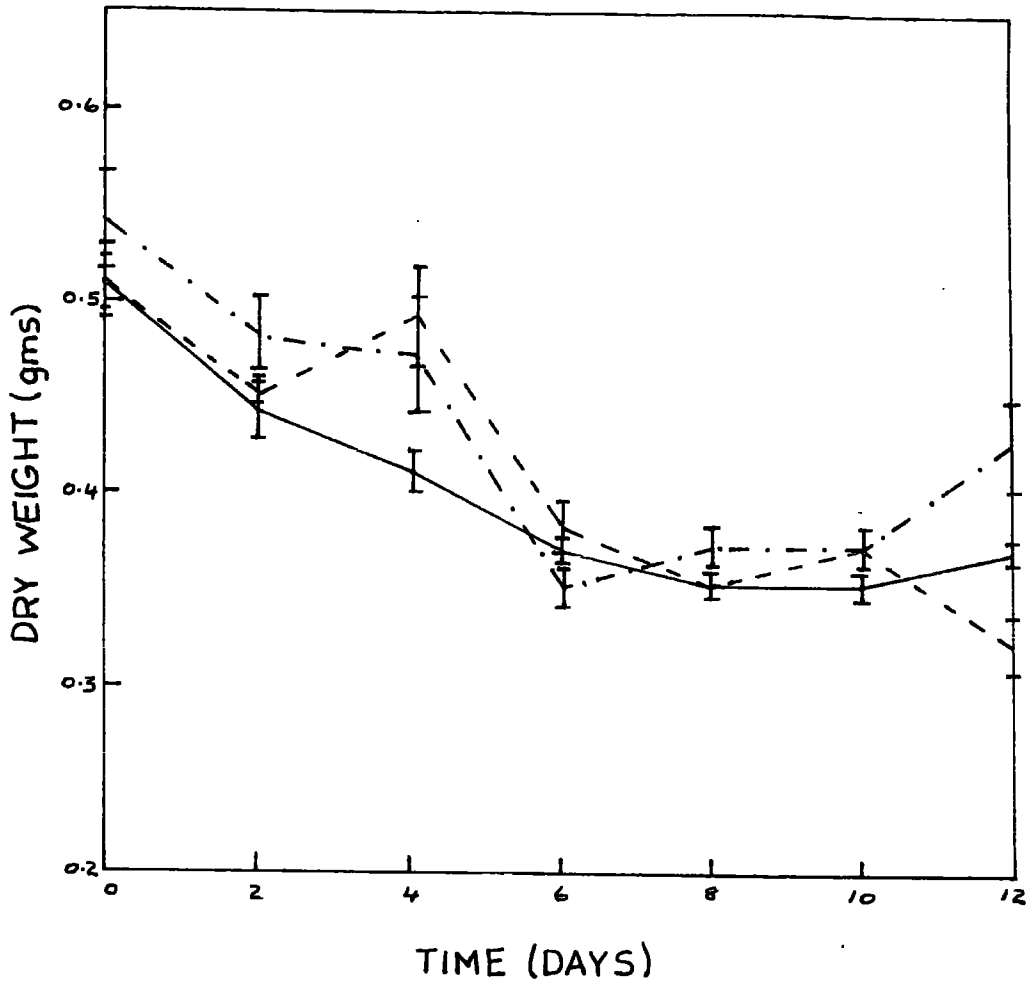
Results are expressed in terms of Mean ± Standard Error.

FIGURE 7

Dry weights of *Sesleria*

during cold stress.

FIGURE 7



I = Standard error

—— Site A

- - - Site B

-·- Site C

Table 5

Changes in dry weight of Sesleria caerulea plants during cold stress.

	Site A	Site B	Site C
Day 0	0.52 ± 0.04	0.52 ± 0.003	0.54 ± 0.05
Day 2	0.44 ± 0.03	0.45 ± 0.01	0.48 ± 0.05
Day 4	0.41 ± 0.02	0.49 ± 0.05	0.47 ± 0.06
Day 6	0.38 ± 0.01	0.38 ± 0.03	0.35 ± 0.02
Day 8	0.35 ± 0.01	0.35 ± 0.02	0.37 ± 0.02
Day 10	0.35 ± 0.01	0.37 ± 0.02	0.37 ± 0.02
Day 12	0.37 ± 0.01	0.32 ± 0.03	0.40 ± 0.05

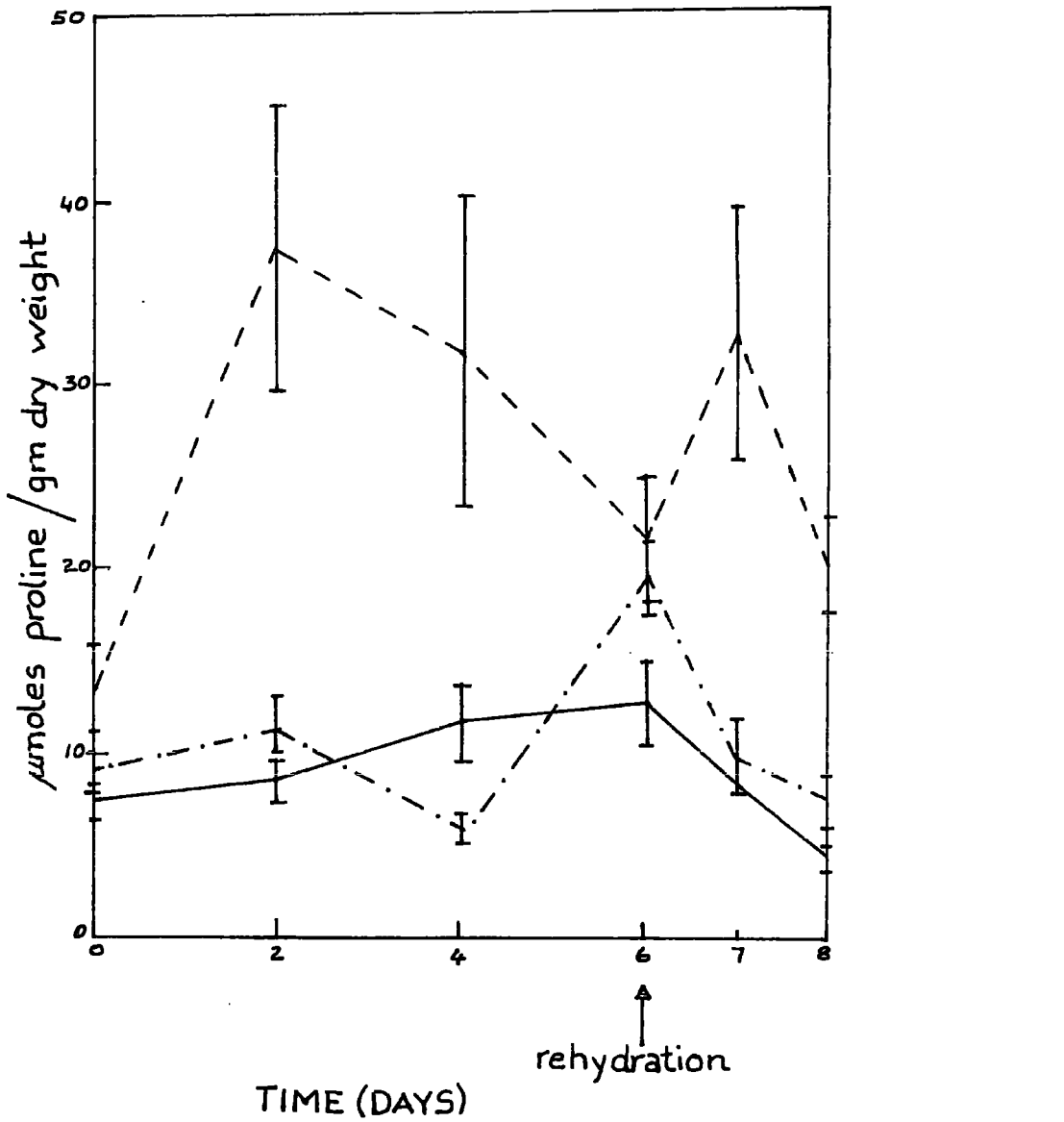
Results are expressed in terms of Mean ± Standard Error.

These results suggest that, as the plant is losing dry matter, the apparent increase in water content is due to decreasing plant material, with the water content probably remaining constant. At low temperatures, absorption of water via the roots is reduced, resulting in physiological drought. Stomatal closure helps to reduce loss of water by transpiration. However, photosynthesis is also reduced. The plant continues to respire, using stored products as substrate. Thus the dry matter decreases. The plant appears to conserve water at the expense of plant tissue, till eventually the drought becomes so intense that water is lost from the plant. This can be seen to occur in plants from Site B and Site C after twelve days of cold stress. Plants from Site A, were still capable of retaining water in the tissues, even after twelve days of cold stress. This is another indication of their ability to tolerate low temperature stress.

FIGURE 8

Proline levels in *Sesleria* during drought stress.

FIGURE 8


50
40
30
20
10
0
µmoles proline /gm dry weight
0
2
4
6
7
8
rehydration
TIME (DAYS)
I = Standard error
Site A
Site B
Site C

When the plants were removed from the stress environment, the proline was removed from the leaves rapidly. In all cases, the levels after forty eight hours, were near the levels obtained at the start of the experiment.

Results for the Drought Stress Experiments.

Plants from all sites responded to drought stress by accumulating proline (Figure 8). Although initial levels for all plants were not significantly different, after forty eight hours of drought stress, plants from Site B produced significantly more proline than plants from either Site A or Site C. (Appendix O) In fact, the maximum level of proline was attained by Site B plants after forty eight hours of drought stress. Plants from the other two sites were slower to attain maximum levels (after eight days of drought stress). These maximum levels were lower than the maximum level reached by plants from Site B. It is possible that tolerance to drought stress is determined by both the rate of proline accumulation, and the ability to accumulate large quantities of proline.

Table 6

Proline levels in Sesleria caerulea plants during drought stress

	Site A	Site B	Site C
Day 0	7.76 ± 2.29	10.16 ± 5.82	9.07 ± 2.02
* Day 2	8.52 ± 2.46	37.22 ± 17.71	11.58 ± 3.08
Day 4	11.95 ± 4.07	32.84 ± 19.17	5.63 ± 1.01
Day 6	12.55 ± 4.91	21.95 ± 7.48	19.78 ± 4.09
* Day 7	8.23 ± 2.30	32.92 ± 15.29	9.84 ± 5.10
Day 8	3.89 ± 0.41	19.81 ± 5.55	7.82 ± 3.11

*Site B plants are significantly different from Site A and Site C plants. (Appendix O).

Results are expressed in terms of Mean ± Standard Error. Samples taken on day 6 were obtained before rehydration. Samples taken on day 7 and day 8 were taken 15 hours and 40 hours, respecively, after rehydration.

Site	Lowest $\bar{x}$ value of proline (before rehydration)	Maximum $\bar{x}$ value of proline	Fold Increase
A	7.76	12.55	1.62
B	10.16	37.22	3.66
C	19.78	9.07	2.18

Plants from Site B showed the largest increase in proline production. The smallest increase occurred in plants from Site A. The soil depth at Sites B and C are comparable (less than 10 cm). The soil depth at Site A is greater than 10 cm. It appears that response to drought stress is dependent more on edaphic conditions than on climatic conditions, with soil depth as an important parameter.

Table 7

Rates of increase in proline production during drought stress.

	Site A	Site B	Site C
Day 2	1.10	3.66	1.28
Day 4	1.40	0.88	0.49
Day 6	1.05	0.67	3.51
Day 7	0.66	1.50	0.50
Day 8	0.47	0.60	0.79
$\bar{x}$ daily increase =	0.94	1.46	1.31

Values <1 indicate a decrease in proline production.

Rates of increase were greatest for the plants from shallow soils. If the rates of increase are calculated for the period of drought stress only i.e. before rehydration, the same conclusion is reached.

When the drought stress was relieved after six days, the proline in plants from Site A and Site C decreased rapidly, so that on day 7 (fifteen hours after rehydration), the levels were similar to those at the start of the experiment. On day 8 (forty hours after rehydration), the proline had decreased further, suggesting that, as with cold stress, once the stress is eliminated, the plant utilises the proline rapidly, presumably synthesising proteins again. However, the rehydration imposed a further stress on the plants from Site B, because proline accumulation increased when the plants were watered. Here, the sudden relief of drought stress resulted in another stress - flooding. The proline level was relatively high fifteen hours after rehydration of these plants, but subsequently dropped rapidly, so that on day 8 (forty hours after rehydration) much of the proline had been utilised. This response to rehydration could explain why, in the original cold-stress experiments, plants from this site accumulated a lot of proline which was rapidly utilised, at the start of the experiment. (Figure 4).

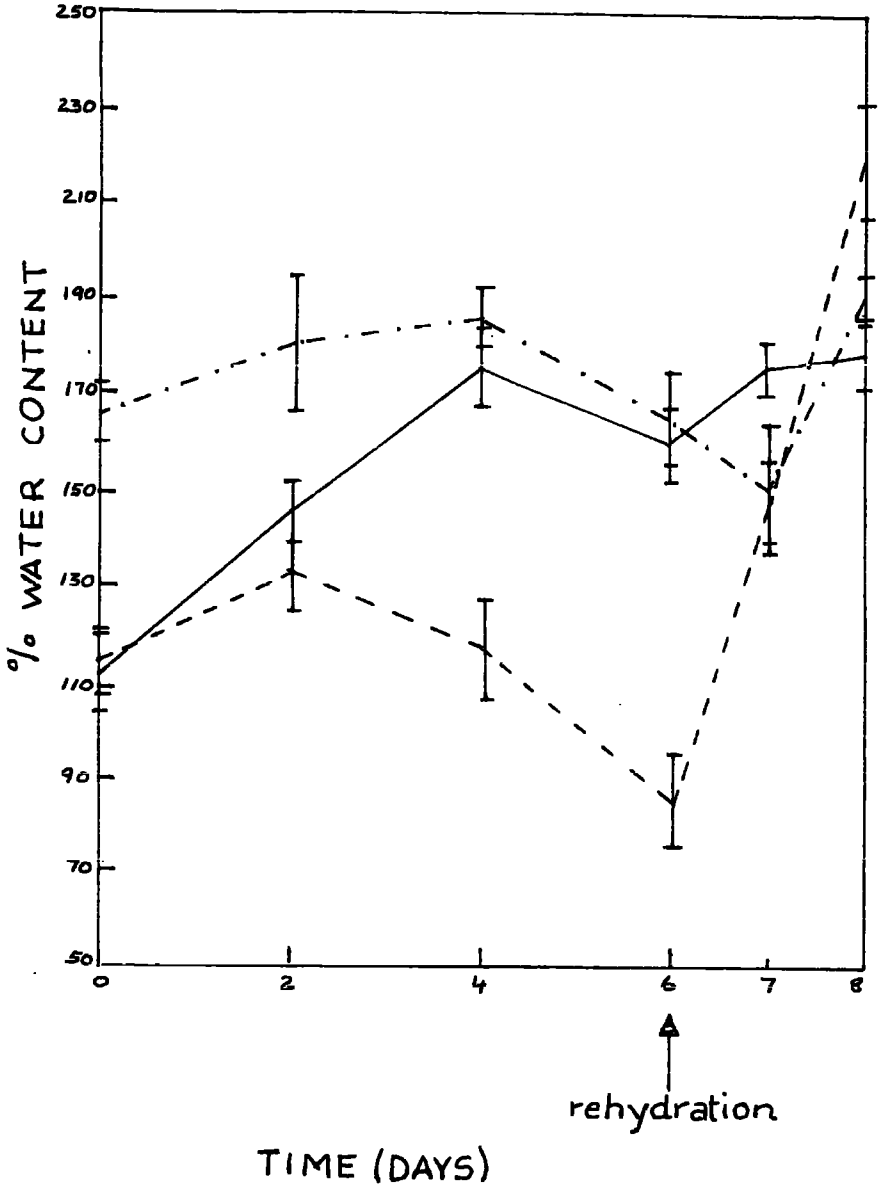
If the percentage water contents are compared, except for Site B plants, there is an apparent increase in water content during drought stress, with a reciprocal decrease in dry weight (Figures 9 and 10). Plants from Sites A and C attempt to conserve water during the stress period, presumably by reduced transpiration.

FIGURE 9

Water Contents in *Sesleria*

during drought stress.

FIGURE 9



I = Standard error

—— Site A

- - - - Site B

·-·-· Site C

Photosynthesis would also be reduced as a result, and fixation of carbon may stop. If this happens, continued respiration would depend on stored products for substrate, resulting in a net decrease in dry weight of the plant.

Table 8

Changes in percentage water content of plant tissue during drought stress

	Site A	Site B	Site C
Day 0	110.85 ± 8.23	113.64 ± 14.13	160.83 ± 13.03
Day 2	147.79 ± 7.55	132.95 ± 14.26	179.10 ± 38.87
Day 4	170.40 ± 16.89	116.35 ± 22.75	186.35 ± 13.47
Day 6	159.67 ± 15.10	85.86 ± 20.25	160.79 ± 19.82
Day 7	175.62 ± 8.01	147.56 ± 18.60	149.75 ± 28.06
Day 8	177.65 ± 8.72	217.20 ± 27.75	190.27 ± 10.72

The percentage water content is based on dry weight.

Results are expressed in terms of Mean ± Standard Error.

Table 9

Changes in dry weight of Sesleria caerulea plants during drought Stress.

	Site A	Site B	Site C
Day 0	0.48 ± 0.02	0.50 ± 0.05	0.39 ± 0.02
Day 2	0.42 ± 0.02	0.44 ± 0.03	0.39 ± 0.05
Day 4	0.37 ± 0.02	0.50 ± 0.06	0.35 ± 0.02
Day 6	0.39 ± 0.02	0.58 ± 0.06	0.37 ± 0.04
Day 7	0.37 ± 0.01	0.43 ± 0.04	0.44 ± 0.08
Day 8	0.36 ± 0.01	0.33 ± 0.02	0.35 ± 0.01

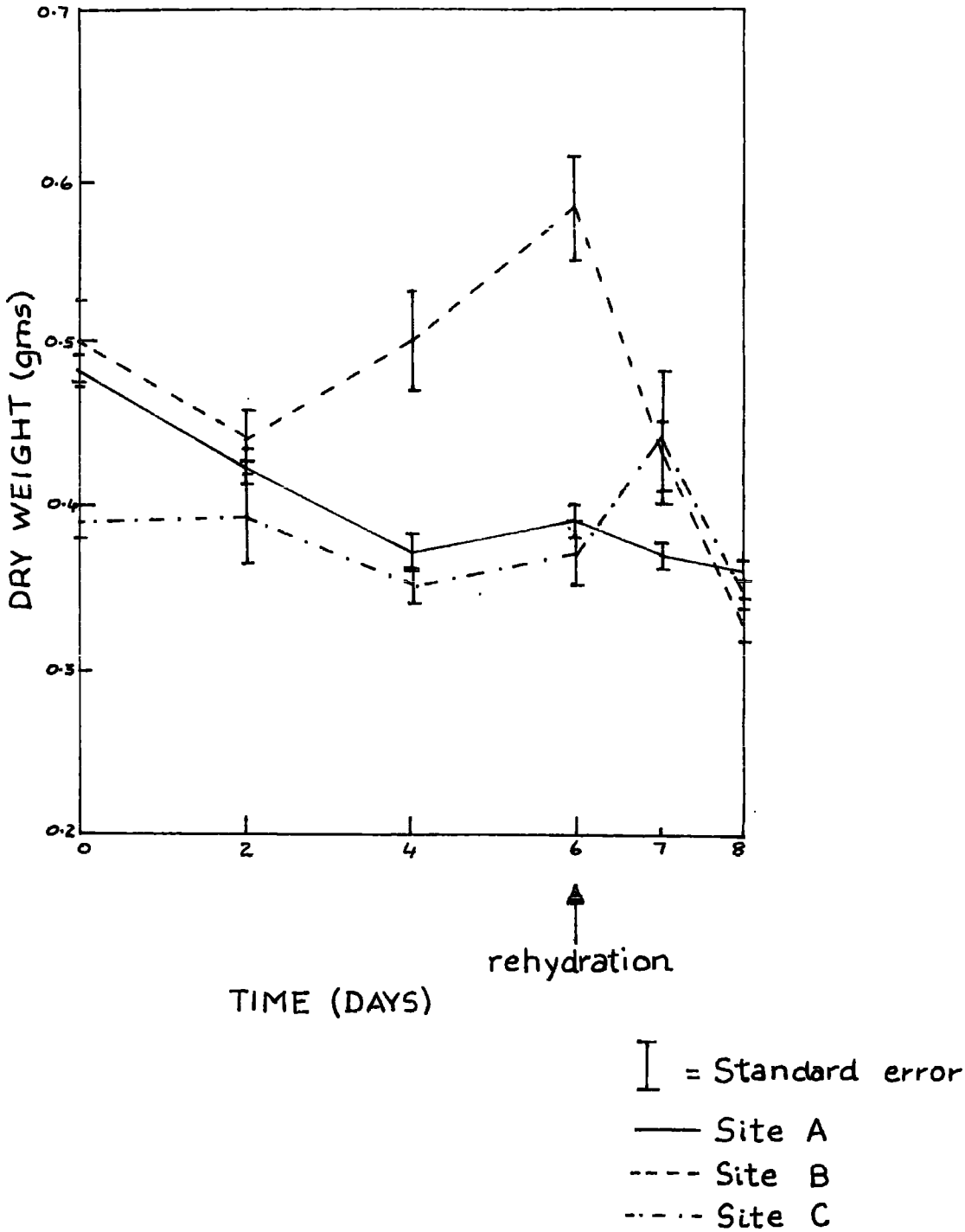
Results are expressed in terms of Mean ± Standard Error.

FIGURE 10

Dry weights of *Sesleria*

during drought stress.

FIGURE 10


0·7
0·6
0·5
0·4
0·3
0·2
DRY WEIGHT (gms)
0
2
4
6
7
8
rehydration
TIME (DAYS)
= Standard error
Site A
Site B
Site C

On rehydration, the plants from Site C began to produce plant material rapidly, so that fifteen hours after rehydration, there was an increase in dry weight in these plants, suggesting immediate recommencement of photosynthesis on rehydration. The plants from the deeper soil at Site A were slower to recover from the drought stress, reflecting their inability to cope with drought stress as efficiently as the plants from the shallow soil. Shallow soils dry out much faster than deeper soils, and plants growing in the shallow soils would need to have an efficient drought resistance mechanism.

Although plants from Site B produced a lot of proline, after two days of drought stress the level began to fall. At this time the plants appeared wilted, and the fact that the water content decreased with a reciprocal increase in dry weight suggests that the plants may have entered the reaction phase proposed by Stocker (1960). After rapid accumulation of proline, utilisation occured more rapidly so that the proline concentration decreased. The proline concentration did not decrease to the initial low level found in unstressed plants. When rehydrated, formation of proline occured more rapidly than the reduction, leading to an increase in proline levels.

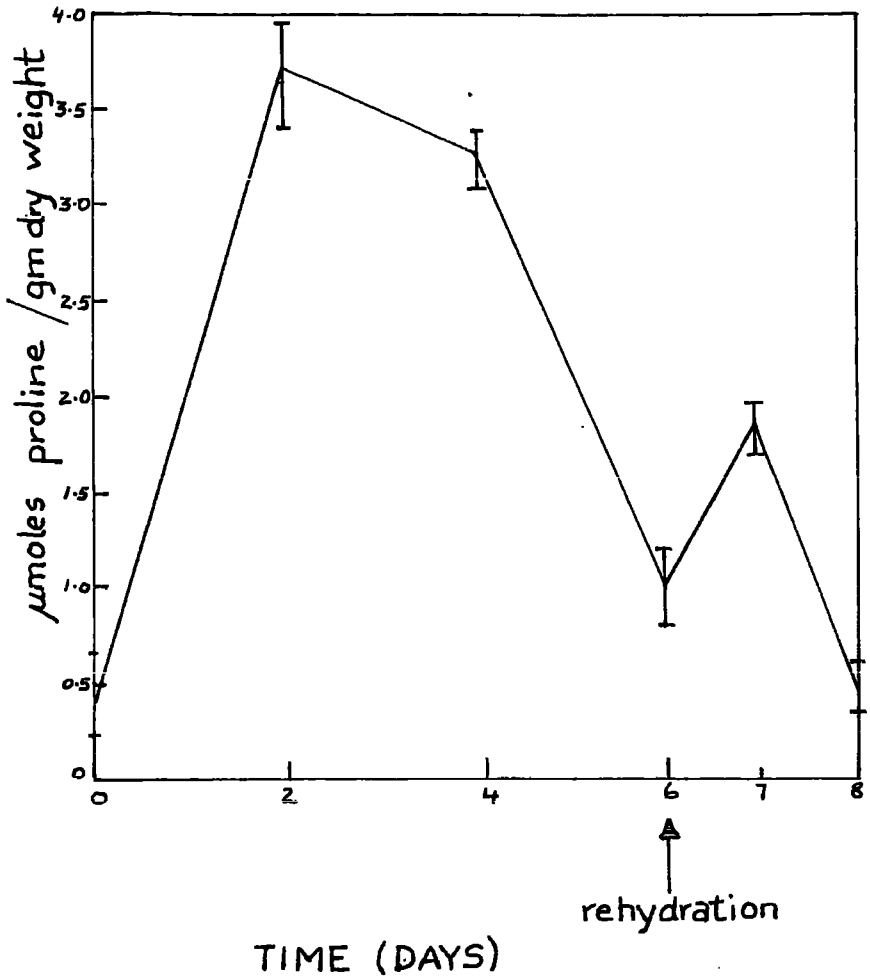
The experiment was repeated using more plants from Site B. These plants were well watered and had very low initial levels of proline. Like the other plants from this site, there was a large increase in proline production after two days of drought stress. (Figure 11). After this initial increase however, the levels fell. On rehydration the plants again showed a stress response, with a large increase in proline accumulation. Forty hours after rehydration,

FIGURE 11

Proline levels in *Sesleria* from Site B

during drought stress.

FIGURE 11

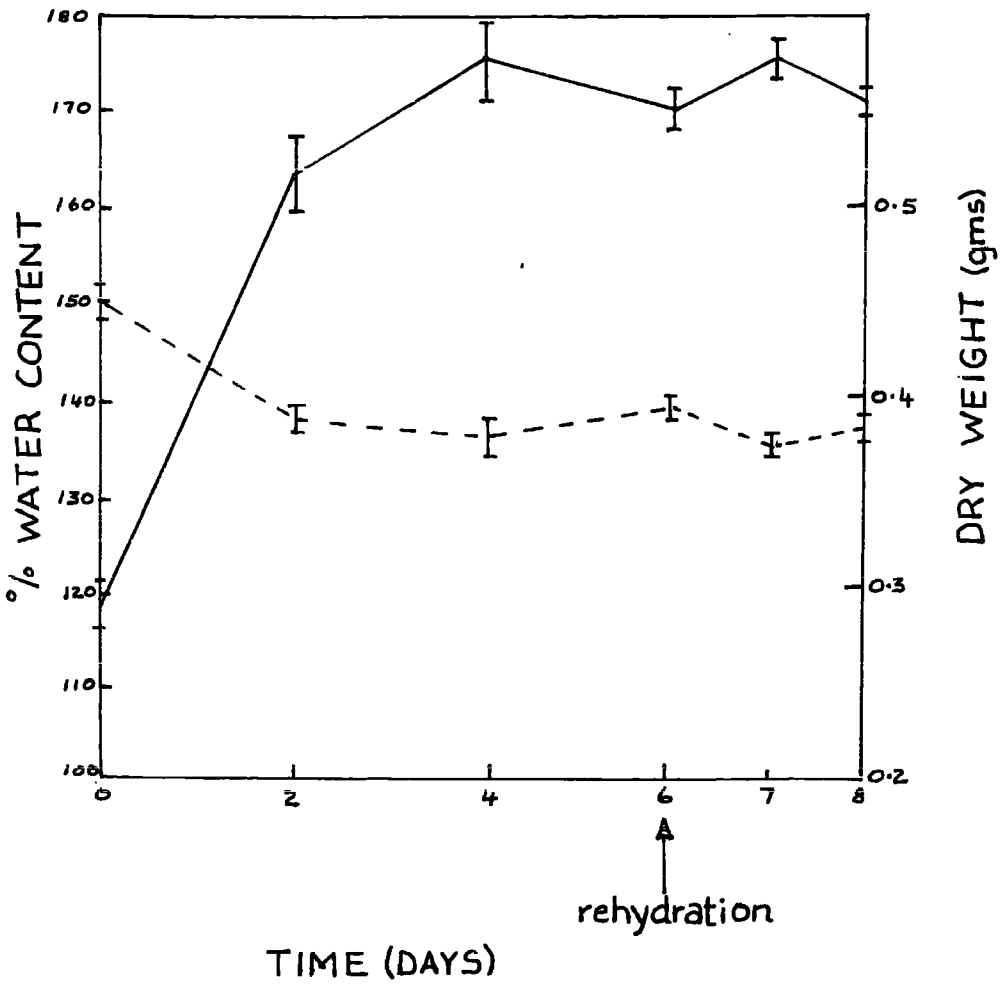


I = Standard error

FIGURE 12

Levels of water and dry weight in *Sesleria* from Site B during drought stress.

FIGURE 12



I = Standard error

—— % water content

---- Dry weight

the proline concentration in the plant was very low. These plants showed a decrease in dry weight with an accompanying increase in water content during drought stress. (Figure 12).

The control plants showed some fluctuations in proline levels, but these were small in comparison to those obtained as a result of stress. (Appendix M).

CHAPTER 4

Discussion.

Turesson (1922) suggested that if a species occurs in a wide range of habitats, it will show a variation in both morphological and physiological features that can be correlated with the habitat. His studies (1922, 1925, 1930) indicate that these differences arose as a result of natural selection, and each of the resulting populations, Turesson called an "ecotype".

Interpopulation variation is therefore determined by the environment, and within the environment climatic, biotic and edaphic factors exert their effects on the plants. If an area has a lot of local variation, numerous populations may exist within it. Bradshaw (1959) suggested that these populations may be separated by small distances - fifty metres or less.

Usually, interpopulation variation is first realised because of differences in morphology that can be correlated with habitat variations. Climatic factors vary along an elevational gradient (Bradshaw, 1960; McNaughton et al, 1974) so that plants of the same species, growing at different altitudes,may show morphological variation along this gradient. A decrease in plant height with an increase in altitude, has been observed by Pearcy and Ward (1972) for Deschampsia caespitosa. In this study on Sesleria caerulea, differences in height were observed for plants from the three sites; the plants from the higher altitude were significantly smaller than those growing at the lower altitude, and the leaves were narrower at the high altitude. However, it appears that edaphic factors are as important as climatic factors - within Cassop Vale, plants growing in a deep, moist soil (Site A) had

larger and wider leaves than those growing in the shallow, dry soil (Site B). West (1975) showed that if edaphic factors are standardized, the effect of altitude becomes more important. This conclusion also holds for this study where it was found that the plants from Upper Teesdale were smaller than those from Site B at Cassop Vale, and the leaves were narrower in the plants from the higher altitude. The significance of this is probably associated with photosynthetic activity which has been shown to vary with altitude (Milner and Hiesey, 1964). Differences in stomatal apparatus have been shown to exist in Sesleria caerulea populations (Lloyd and Woolhouse, 1978; West, 1975).

The physiology of a plant may alter as a plant matures. Gates et al (1971) suggested that proline would increase during maturation. In this study it was found that, when wilted, the older apical, and the younger basal tissues of the leaf blade produced comparable amounts of proline. However, while this indicates that no difference in proline production exists between the oldest and the youngest tissues, it does not prove that proline production is consistent in tissues of all ages. It may be that proline production increases to a maximum when the tissue is mature, but is less in the young and senescing tissue. This could explain the results obtained in this study, but this is only speculation. Experimental work using tissues of various ages is required before any definite conclusions can be drawn.

Proline production in response to stress has been demonstrated by a number of workers (Palfi and Juhasz, 1970; Routley,

1966; Singh et al, 1973 I, 1973 II; Smith, 1975; Stewart, 1973). Proline accumulation does not occur only in stressed tissue. It has been shown to occur in unstressed plants that are kept in the dark, and in this case, was independent of water relations in the plant (Rajagopal et al, 1977). Previous exposure to stress influences the potential of the plant to accumulate proline (Singh et al, 1973 III).

Cold stress may cause physiological drought in the plant. At low temperatures, water absorption is reduced (Kramer, 1942; Palfi and Juhasz, 1970). Water becomes more viscous at low temperatures. This, together with reduced permeability and reduced metabolic activity in the roots results in reduced uptake of water (Kramer, 1969). The ability to absorb water at low temperatures is therefore essential in plants growing in cold soils. Kramer (1942) has shown that some plants differ in their ability to absorb water at low temperatures. The watermelon which normally grows in warm soils absorbed less water at low temperatures than the Georgia collards which grow in cold soils. Watts (1970) disputes the view that cold exerts it's effect on growth through reduced water uptake. He ascribes the growth effects of low root temperatures to cooling of the meristematic regions of the shoot. Chur et al (1974) proposed that the water status of a plant cannot account for proline accumulation, because it was found to alter very little in barley and radish plants that were exposed to cold. When subjected to cold stress, the Sesleria caerulea plants in this study showed an apparent increase in percentage water content. However, this was relative

to the dry weight of the tissue, which was found to decrease.

Noticeable changes in proline accumulation were observed in Sesleria caerulea plants during cold stress. The populations from the three sites differed in their abilities to accumulate proline. If plants from the shallow soil are compared, those from the higher altitude were better able to accumulate proline than those from the lower altitude. Plants growing at high altitudes are subjected to low temperature regimes. Manley (1952) quotes a decrease in temperature of 0.67°C for every 100 metres increase in altitude. Plants from Upper Teesdale are therefore expected to be more tolerant to cold than those from Cassop Vale. (Site B).

Other physiological responses have shown a correlation with altitude (Pearcy and Ward, 1972; Spomer et al, 1968). Hunter and Grant (1971) observed that, in perennial rye grass, development of flowering was delayed by 1.3 days/30.3 metres, and this could be correlated with a temperature lapse of 1°F/90.9 metres

Where edaphic conditions are standardized, response of Sesleria caerulea to cold can be correlated with altitude. Where they differ, edaphic factors may be more important than climatic factors. Plants growing in the deep,moist soil, and situated in a frost hollow (Site A) accumulated more proline, than the plants growing in a shallow, dry soil either at Cassop Vale or on Upper Teesdale. Deep soils warm up more slowly than shallow soils. This may account for the greater tolerance to cold observed in these plants.

It is interesting to note that, although the plants from Site A accumulated more proline than those from the other sites,

the difference becomes significant only after a period of continued stress. Initial responses were similar in all plants.

Once the cold stress was terminated, proline decreased rapidly in all plants, so that the levels were near the normal levels within forty eight hours. When the plants are kept at a relatively high temperature after a period of cold stress, the soil warms up slowly, whereas the aerial parts of the plant experience the higher temperature immediately. Transpiration would therefore increase before water absorption has returned to normal. This would cause a temporary water deficit in the plant and proline accumulation may be expected to increase temporarily. However, this did not occur, and proline was oxidised rapidly after relief of the cold stress.

Wilted Sesleria caerulea plants showed marked increases in proline concentration, but the rate of increase, and the maximum concentrations attained varied. Plants growing in the deep, moist soil accumulated little proline, indicating their poor ability to tolerate drought. Plants growing in the shallow soils accumulated large amounts of proline. Shallow soils dry out rapidly, and plants growing in them must acquire a mechanism to cope with these soil water deficits. However, the plants growing in shallow soil at a high altitude, did not accumulate as much proline as the plants growing in the same soil depth at the lower altitude. Increased rainfall at higher altitudes probably accounts for this, plus the fact that the lower altitude site (Site B) drains more rapidly and to a greater extent than the

other sites. Drought resistance appears to be correlated with edaphic conditions, soil depth being an important parameter. Utilisation of water is more efficient in plants tolerant to drought. McKell et al (1960) observed that for Dactylis glomerata, the subspecies judaica utilised soil moisture more slowly than lusitanica which was less drought resistant. Singh et al (1973 III) observed genotypic differences in the ability to accumulate proline in fourteen varieties of barley. Accumulation was greater in the drought resistant varieties.

Many workers have observed a rapid disappearance of proline on rehydration of wilted plants (Kemble and MacPherson, 1954; Stewart et al, 1977). However, Wample and Bewley (1975) observed that proline accumulation doubled when wilted sunflower leaves were rehydrated. Routley (1966) observed an immediate increase in proline when wilted Ladino clover leaves were watered. Only the Sesleria caerulea plants from Site B showed an increase in proline when the drought stress was relieved. Proline levels fell rapidly in plants from the other sites indicating rapid oxidation. It is possible that plants from Site B are less tolerant of anaerobic conditions which may result from temporary flooding. Site B is a well drained site, and the soil is normally well aerated.

Proline accumulation occurs in response to various forms of stress. This suggests that dissimilar environmental factors can initiate the same metabolic response, probably by affecting the same metabolic process. Levitt (1956) suggested that plant resistance to temperature and drought stress are interrelated.

It has been suggested that during drought stress, proline acts as a storage compound for carbon and nitrogen (Barnett and Naylor, 1966). It's function during cold stress may be of a different nature. It may have a structural role, influencing the resistance of protein molecules to cold. (Gates et al, 1971). Proline may have a role in osmotic control during salinity stress, as it does not affect enzyme systems even if it is present in high concentrations (Stewart and Lee, 1974).

During cold and drought stress, there was a general decrease in dry weight. This can be explained on the basis that, as water absorption decreases (either due to low temperatures or a soil water deficit), stomata close and transpiration is reduced. Veihmeyer and Hendrickson (1955) propose that this occurs only when the permanent wilting point is reached, but this is dubious. Stomatal closure results in reduced photosynthesis and respiration, but the decrease in photosynthesis is more rapid. (Slayter, 1957b). The decrease in dry weight is due to the continued respiration. If the stress continues, carbohydrates and proteins break down. Eventually even more water is lost from the plant. After the relief of stress, there was a decrease in dry weight. This maybe due to respiratory processes recovering faster than photosynthetic processes.

Differences in response to stress exist in Sesleria caerulea, and these can be correlated with environmental variation. These differences may be genetic or adaptive. Plants growing on Upper Teesdale are normally small with narrow, folded leaves. After eight weeks growth in Durham, the plants were large with

wide leaves. When the climatic and edaphic conditions were altered, the plant form changed, indicating that these are not genotypic characteristics. It is likely that the morphological features of the Cassop Vale plants are also adaptive.

If some morphological characteristics of the plants are not hereditary, it is possible that differences in physiological responses are attributable to the plants' plastic response to the environment. Reciprocal transplanting experiments would distinguish between hereditary characteristics and adaptive characteristics in Sesleria caerulea.

APPENDIX

List of Tables

List of Figures

APPENDIX A - Sheet I

Table 1

Data used to obtain the standard Curve.

Concentration of the Proline Solution		Absorbance
500	$\mu g/cm^3$	3.400
250	$\mu g/cm^3$	2.840
100	$\mu g/cm^3$	2.330
50	$\mu g/cm^3$	1.203
40	$\mu g/cm^3$	0.933
30	$\mu g/cm^3$	0.670
25	$\mu g/cm^3$	0.560
20	$\mu g/cm^3$	0.390
10	$\mu g/cm^3$	0.242
5	$\mu g/cm^3$	0.134

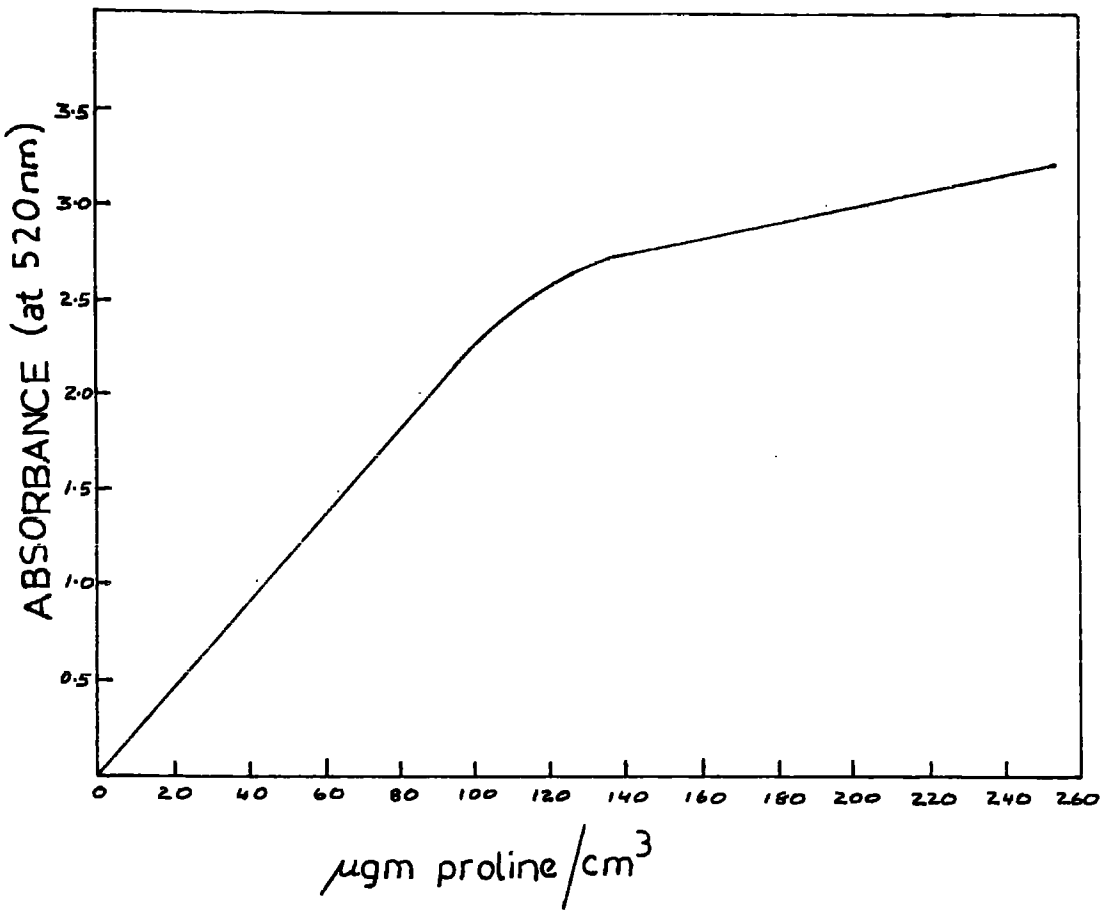
The method followed was that proposed by Bates, Waldren and Teare (1973).
The absorbance was read at 520 nm on an'Uvispek' spectrophotometer, using toluene as a blank.

APPENDIX A - Sheet II

Figure 1

Standard Curve for proline.

FIGURE 1


ABSORBANCE (at 520nm)
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
20
40
60
80
100
120
140
160
180
200
220
240
260
μgm proline/cm3

APPENDIX B

Table 2

Results of spiking experiments using Dactylis glomerata.

	Reaction mixture obtained from:	Absorbance
i	Leaves alone	0.095
ii	Leaves ground with 9cm^3 sulphosalicylic acid + 1 cm^3 25 $\mu g/cm^3$ proline	0.108
iii	Leaves ground with 9cm^3 sulphosalicylic acid + 1 cm^3 50 $\mu g/cm^3$ proline	0.156
iv	Leaves ground with 9cm^3 sulphosalicylic acid + 1 cm^3 100 $\mu g/cm^3$ proline	0.227

The method followed was that proposed by Bates, Waldren and Teare (1973)

The absorbance was read at 520 nm on an 'Uvispek' spectrophotometer, using toluene as a blank.

APPENDIX C

Table 3

Results of spiking experiments using *Sesleria caerulea*.

Reaction mixture obtained from:	Absorbance
i. 25 $\mu g/cm^3$ proline solution	0.500
ii. 50 $\mu g/cm^3$ proline solution	1.271
iii. 100 $\mu g/cm^3$ proline solution	2.258
iv. Leaves alone	0.143
v. Leaves ground with $9cm^3$ sulphosalicylic acid + $1cm^3$ 25 $\mu g/cm^3$ proline	0.187
vi. Leaves ground with $9cm^3$ sulphosalicyclic acid + $1cm^3$ 50 $\mu g/cm^3$ proline	0.232
vii. Leaves ground with $9cm^3$ sulphosalicyclic acid + $1cm^3$ 100 $\mu g/cm^3$ proline	0.345

The method followed was that proposed by Bates, Waldren and Teare (1973).

The absorbance was read at 520 nm on an'Uvispek' spectrophotometer, using toluene as a blank.

APPENDIX D.

Table 4 A

Leaf lengths of Sesleria caerulea from the three sites

Sample No.	SITES A (CASSOP) Leaf Length (mm)	B (CASSOP) Leaf length (mm)	UPPER TEESDALE In the field Leaf length (mm)	After 8 weeks Growth in the Laboratory.
1	77	68	21	72
2	105	105	27	75
3	130	131	28	130
4	70	103	54	75
5	75	99	25	78
6	110	74	42	108
7	95	92	43	101
8	80	90	24	127
9	80	57	36	108
10	133	65	49	112
11	233	78	21	83
12	121	113	23	112
13	113	73	37	72
14	188	90	60	142
15	122	94	34	108
16	62	67	53	81
17	59	65	49	130
18	135	77	28	104
19	74	65	38	102
20	96	65	38	84
21	101	97	55	74
22	180	50	29	84
23	79	95	55	103
24	89	105	26	125
25	190	79	31	86
26	110	95	33	77
27	73	130	70	65
28	92	110	30	83
29	108	80	25	86
30	64	83	21	100
31	74	120	30	73

SITES

Sample No.	A (CASSOP) Leaf length (mm)	B (CASSOP) Leaf length (mm)	UPPER TEESDALE In the field Leaf length (mm)	UPPER TEESDALE After 8 weeks growth in the Laboratory.
32	91	90	26	70
33	96	55	32	92
34	97	55	43	96
35	130	70	28	84
36	122	100	25	50
37	84	103	31	88
38	61	86	24	86
39	89	93	62	80
40	64	53	45	100

Table 4 B

Site	Leaf Length (mm)
A (Cassop)	103.8 ± 6.14
B (Cassop)	85.5 ± 3.28
Teesdale (Field)	36.28 ± 2.04
Teesdale (Laboratory)	92.65 ± 3.21

Sample size = 40

Results expressed as Mean ± Standard Error.

TABLE 5

APPENDIX E Sheet I

Levels of proline and water in Sesleria during drought stress: Results obtained for the apical half of the leaf blade.

	Fresh weight of Sesleria (mg)	µmoles proline/ g.m. F.W.	µmoles proline/ g.m. D.W.	% Water Content on F.W. basis	% Water Content on D.W. basis
Day 0	0.2	0.087	0.23	61.42	159.2
	0.2	0.62	1.62	61.69	161.0
	0.2	0.173	0.32	46.43	86.8
	0.2	0.07	0.173	59.54	147.1
	0.2	6.75	11.02	38.76	63.2
	0.2	1.39	2.97	53.21	113.9
	0.2	4.16	10.66	60.97	156.1
Day 2	0.2	0.173	0.51	66.04	194.49
	0.2	0.173	0.37	53.16	113.2
	0.2	0.173	0.541	68.09	213.1
	0.2	0.52	1.77	70.65	240.9
	0.2	8.76	17.62	50.28	101.1
	0.2	1.21	2.87	57.77	136.9
	0.2	3.12	7.27	57.11	133.1
Day 4	0.2	0.208	0.44	53.16	113.76
	0.2	0.693	1.64	69.24	164.1
	0.2	0.76	2.04	62.80	168.9
	0.2	0.35	0.94	62.78	168.9
	0.2	2.25	5.92	61.99	163.0
	0.2	2.08	5.51	62.28	165.0
	0.2	1.90	5.33	64.36	180.9

Table 5 (Continued)

Table 5 (Continued)

APPENDIX E - Sheet II

Levels of proline and water in Sesleria during drought stress: Results obtained for the apical half of the leaf blades.

	Fresh weight of Sesleria (mg)	µmole proline/ gm F.W.	µmole proline/ gm D.W.	% Water Content on F.W. Basis	% Water Content on D.W. Basis
Day 6	0.2	0.173	0.711	75.64	310.88
	0.2	0.76	2.67	71.53	251.1
	0.2	0.52	1.84	71.78	254.1
	0.2	0.35	1.34	73.91	283.1
	0.2	10.22	26.27	61.10	157.0
	0.2	1.39	3.99	65.19	187.1
	0.2	1.56	4.55	65.73	191.9
Day 8	0.2	14.89	31.69	53.02	112.93
	0.2	5.30	8.28	35.96	56.1
	0.2	4.50	11.12	59.52	147.0

(3 replicates were used on day 8 due to lack of plant material for the other samples. On all other occasions, 7 replicates were used).

APPENDIX E - Sheet III

Table 6.

Levels of proline in Sesleria during drought stress: Results obtained for the basal half of the leaf blades.

	Fresh weight of Sesleria (mg)	μmole proline/ gm F.W.	μmole proline/ gm D.W.
Day 0	0.2	0.087	0.23
	0.2	0.519	1.35
	0.2	0.07	0.13
	0.2	0.45	1.11
	0.2	2.94	4.79
	0.2	0.80	1.71
	0.2	2.56	6.55
Day 2	0.2	0.173	0.51
	0.2	0.116	0.25
	0.2	0.173	0.54
	0.2	0.173	0.59
	0.2	8.14	16.36
	0.2	0.69	1.64
	0.2	3.01	7.01
Day 4	0.2	0.35	0.71
	0.2	0.62	1.47
	0.2	0.31	0.84
	0.2	0.45	1.21
	0.2	1.56	4.10
	0.2	1.04	2.76
	0.2	1.73	5.05
Day 6	0.2	0.173	0.71
	0.2	0.173	0.61
	0.2	0.52	1.84
	0.2	0.173	0.66
	0.2	9.52	24.47
	0.2	0.87	2.50
	0.2	1.73	5.05
Day 8	0.2	13.16	28.03
	0.2	5.54	8.64
	0.2	3.98	9.83

APPENDIX E - Sheet IV

(3 Replicates were used on day 8 due to lack of plant material for the other samples. On all other occasions, 7 replicates were used.)

% Water content on a fresh weight basis and on a dry weight basis is the same as for the apical half (Appendix E - Sheets I and II; and Appendix F.)

APPENDIX F

Table 7.

Water content in Sesleria: Results obtained for the basal and apical halves of the leaf blades.

Sample No.	% Water Content of the Apical Half	% Water Content of the basal half.
1	64.87	65.13
2	62.41	64.45
3	63.30	66.15
4	62.66	63.84
5	69.82	70.60
6	72.92	73.12
7	67.32	70.80
8	63.62	62.34
9	62.32	64.35
10	68.83	69.28
11	62.24	66.75
12	60.99	62.31
13	69.86	71.35
14	72.91	71.86
15	67.45	66.10
$\bar{x} \pm$ S.E.	66.23 ± 1.01	67.23 ± 0.94

% water content is on a fresh weight basis.

Samples were oven dried at 105°C for 48 hours.

APPENDIX G - Sheet I

Table 8

Levels of proline in Sesleria during cold stress:

Results for plants from Cassop Vale - Site A.

	Fresh weight of Sesleria (mg)	µmoles proline/ gm F.W.	µmoles proline/ gm D.W.
Day 0	0.2	3.98	5.90
	0.2	33.25	61.75
	0.2	6.75	9.05
	0.2	1.56	2.99
	0.2	10.91	25.82
	0.2	10.04	19.77
	0.2	0.80	1.88
	0.2	28.40	61.34
Day 2	0.2	0.69	1.51
	0.2	0.14	0.35
	0.2	0.69	2.08
	0.2	5.54	13.26
	0.2	2.08	5.20
	0.2	7.27	16.80
	0.2	1.56	2.73
	0.2	28.12	73.67
Day 4	0.2	3.46	6.50
	0.2	7.10	18.50
	0.2	1.11	2.96
	0.2	1.04	2.54
	0.2	1.25	3.16
	0.2	5.19	14.51
	0.2	2.08	4.86
	0.2	1.56	3.92
	0.2	28.12	73.67

Table 8 (Continued) APPENDIX G - Sheet II

	Fresh weight of *Sesleria* (mg)	μmoles proline/ gm F.W.	μmoles proline gm D.W.
Day 6	0.2	1.28	3.81
	0.2	5.71	14.83
	0.2	1.04	3.14
	0.2	3.29	9.34
	0.2	29.26	67.82
	0.2	7.27	20.86
	0.2	3.12	7.89
	0.2	1.39	3.84
	0.2	7.10	19.79
Day 8	0.2	2.25	7.04
	0.2	16.28	45.19
	0.2	2.91	8.61
	0.2	5.71	16.44
	0.2	3.39	9.15
	0.2	3.57	10.89
	0.2	1.39	4.23
	0.2	1.07	2.83
	0.2	10.39	30.87
Day 10	0.2	2.08	5.91
	0.2	4.50	13.36
	0.2	2.91	8.61
	0.2	6.86	17.06
	0.2	4.61	12.11
	0.2	13.51	40.14
	0.2	1.56	4.53
	0.2	2.08	5.49
	0.2	19.81	60.31
Day 12	0.2	3.29	8.00
	0.2	6.23	16.29
	0.2	11.43	30.54
	0.2	4.50	11.59
	0.2	9.87	26.15

APPENDIX G - Sheet III

Table 9

Water levels in Sesleria during cold stress:
Results for plants from Cassop Vale - Site A.

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry Weight/ 1 gm F.W.
Day 0	59.75	148.2	0.403
	32.56	48.2	0.674
	46.15	85.8	0.539
	25.42	34.1	0.746
	47.8	91.8	0.522
	57.75	136.9	0.508
	49.22	96.96	0.425
	57.48	135.1	0.423
	53.61	115.8	0.464
Day 2	54.16	118.1	0.458
	60.37	152.1	0.396
	66.16	199.1	0.338
	73.42	175.5	0.418
	59.97	149.9	0.400
	56.73	131.0	0.534
	46.60	87.1	0.573
	42.71	74.74	0.433
	61.80	161.9	0.382
Day 4	46.74	87.9	0.533
	61.62	160.8	0.384
	62.45	166.1	0.376
	61.26	149.5	0.387
	60.39	152.2	0.396
	64.24	179.9	0.426
	57.39	134.87	0.398
	60.23	151.2	0.358
	61.80	149.9	0.400

APPENDIX : G - Sheet 1V

Table 9 (Continued)

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry weight/ 1 gm F.W.
Day 6	66.4	186.0	0.336
	61.49	159.9	0.385
	66.91	202.1	0.331
	64.76	183.9	0.352
	56.89	131.9	0.431
	65.14	187.0	0.396
	60.45	152.9	0.362
	63.81	176.8	0.349
	64.12	178.9	0.359
Day 8	68.04	213.0	0.320
	64.47	179.2	0.360
	66.18	195.9	0.338
	65.27	188.0	0.347
	62.95	170.0	0.371
	67.21	205.0	0.329
	67.12	204.0	0.379
	62.07	163.9	0.328
	66.34	197.0	0.337
Day 10	64.82	184.1	0.352
	66.18	196.9	0.337
	66.31	195.9	0.338
	59.78	148.9	0.402
	61.94	162.9	0.381
	66.35	197.1	0.344
	65.57	190.2	0.379
	62.09	163.9	0.337
	67.15	204.1	0.329
Day 12	58.86	143.0	0.411
	61.74	161.1	0.383
	62.57	167.1	0.374
	61.19	157.9	0.388
	62.26	187.1	0.348
	65.20	165.0	0.377

APPENDIX H - Sheet I

Table 10

Levels of proline in Sesleria during cold stress:
Results for plants from Cassop Vale - Site B.

	Fresh weight of Sesleria (mg)	μmoles proline/ gm F.W.	μmoles proline/ gm D.W.
Day 0	0.2	10.04	13.50
	0.2	1.39	2.76
	0.2	0.35	0.55
	0.2	7.62	14.38
	0.2	8.14	20.46
	0.2	5.54	16.08
	0.2	2.53	5.67
	0.2	2.11	3.75
	0.2	4.50	8.45
Day 2	0.2	1.73	5.03
	0.2	0.693	1.71
	0.2	3.90	9.46
	0.2	4.19	11.46
	0.2	14.72	42.24
	0.2	4.16	13.03
	0.2	3.46	8.83
	0.2	1.90	2.52
	0.2	1.39	1.87
Day 4	0.2	2.99	4.01
	0.2	0.87	1.30
	0.2	0.63	1.12
	0.2	1.21	3.28
	0.2	1.28	3.16
	0.2	1.28	2.75
	0.2	2.77	7.57
	0.2	6.93	16.87
	0.2	1.04	2.36

APPENDIX H - Sheet II

Table 10 (continued)

	Fresh weight of *Sesleria* (mg)	µmoles proline/ gm. F.W.	µmoles proline gm D.W.
Day 6	0.2	0.69	2.36
	0.2	1.21	3.23
	0.2	2.08	5.00
	0.2	1.73	6.27
	0.2	1.39	4.59
	0.2	1.73	4.39
	0.2	2.94	8.59
	0.2	2.94	6.53
Day 8	0.2	1.56	5.36
	0.2	1.52	4.55
	0.2	2.77	7.63
	0.2	2.08	6.36
	0.2	2.77	9.85
	0.2	9.52	29.69
	0.2	1.04	3.00
	0.2	1.04	2.65
	0.2	2.42	5.34
Day 10	0.2	4.85	9.7
	0.2	5.71	15.91
	0.2	1.21	3.52
	0.2	1.66	4.02
	0.2	3.29	10.20
	0.2	2.53	7.47
	0.2	1.66	4.45
	0.2	3.39	8.69
	0.2	1.66	4.66
Day 12	0.2	4.57	13.97
	0.2	1.56	3.77
	0.2	3.12	11.49
	0.2	2.77	12.23

(on day 12 four replicates were used due to lack of plant material for the other samples).

APPENDIX H - Sheet III

Table 11

Water levels in Sesleria during cold stress: Results for plants from Cassop Vale - Site B.

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry weight/ 1 gm. F.W.
Day 0	25.64	34.6	0.744
	49.57	98.1	0.504
	36.12	56.7	0.639
	47.03	88.9	0.530
	60.21	151.1	0.398
	65.54	190.1	0.345
	55.28	123.8	0.447
	43.77	77.9	0.562
	46.74	87.9	0.533
Day 2	60.81	155.1	0.344
	65.64	191.0	0.406
	59.38	146.1	0.412
	58.80	142.9	0.366
	63.43	173.2	0.349
	65.15	187.0	0.319
	68.06	213.0	0.392
	24.60	32.7	0.754
	25.63	34.3	0.744
Day 4	25.5	34.17	0.745
	33.33	50.0	0.667
	43.81	78.0	0.562
	63.16	171.2	0.368
	61.70	161.0	0.383
	53.47	115.0	0.465
	63.41	173.1	0 .366
	58.92	143.2	0.411
	55.88	126.8	0.441

APPENDIX H - Sheet IV

Table 11 (continued)

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry weight/ 1 gm F.W.
Day 6	45.16	82.2	0.548
	70.79	242.1	0.292
	62.51	166.9	0.375
	58.36	140.1	0.416
	72.42	262.9	0.276
	69.75	230.9	0.303
	60.63	154.0	0.394
	65.77	192.0	0.353
	54.96	122.0	0.450
Day 8	70.92	244.0	0.291
	66.63	199.9	0.334
	63.67	175.1	0.363
	67.31	206.0	0.327
	71.87	255.9	0.281
	67.27	209.9	0.321
	65.33	188.2	0.347
	60.71	154.2	0.393
	64.36	180.9	0.453
Day 10	50.0	100.0	0.500
	64.11	178.9	0.359
	65.64	191.0	0.344
	58.72	142.1	0.413
	67.75	210.0	0.323
	66.13	195.1	0.339
	62.71	168.1	0.373
	60.97	156.1	0.390
	64.36	180.9	0.356
Day 12	62.5	166.9	0.378
	67.28	205.9	0.327
	58.60	141.8	0.414
	72.86	268.1	0.271
	71.62	315.0	0.226
	71.02	245.0	0.290

APPENDIX 1 - Sheet I

Table 12

Levels of proline in Sesleria during cold stress: Results for plants from Upper Teesdale (Site C).

	Fresh weight of Sesleria (mg)	µmoles proline/ gm F.W.	µmoles proline/ gm D.W.
Day 0	0.2	4.16	6.05
	0.2	3.91	6.61
	0.2	3.81	9,53
	0.2	7.27	13.85
	0.2	9.35	17.50
	0.2	3.81	8.45
Day 2	0.2	0.80	1.27
	0.2	2.08	5.96
	0.2	11.19	25.16
	0.2	16.45	34.51
	0.2	-	-
	0.2	6.75	19.50
Day 4	0.2	1.32	4.50
	0.2	1.73	4.91
	0.2	2.77	4.35
	0.2	2.84	4.70
	0.2	1.21	2.21
	0.2	7.10	18.63
Day 6	0.2	1.39	3.91
	0.2	1.28	3.96
	0.2	6.58	15.40
	0.2	2.08	6.37
	0.2	25.28	66.89
	0.2	3.91	13.46

APPENDIX 1 - Sheet II

Table 12 (continued)

	Fresh weight of *Sesleria* (mg)	μmoles proline/ gm. F.W.	μmoles proline/ gm. D.W.
Day 8	0.2	3.64	9.49
	0.2	3.64	10.42
	0.2	4.16	11.98
	0.2	1.39	4.00
	0.2	4.57	12.74
	0.2	2.42	5.50
Day 10	0.2	1.25	3.27
	0.2	1.39	4.49
	0.2	4.33	9.47
	0.2	2.77	7.55
	0.2	2.60	7.52
	0.2	4.64	12.15
Day 12	0.2	3.12	5.91
	0.2	4.26	7.81
	0.2	14.72	42.67
	0.2	3.53	10.25

(On day 12, four replicates were used due to lack of plant material for the other samples).

APPENDIX 1 - Sheet III

Table 13

Water levels in Sesleria during cold stress:
Results for plants from Upper Teesdale (Site C).

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry Weight/ 1gm. F.W.
Day 0	31.25	45.30	0.750
	40.82	69.0	0.592
	60.00	150.00	0.400
	47.50	90.25	0.525
	46.58	87.10	0.534
	54.89	121.90	0.451
Day 2	37.25	59.20	0.628
	65.08	186.10	0.349
	55.52	124.90	0.445
	52.34	109.90	0.477
	35.48	55.00	0.645
	65.38	188.90	0.346
Day 4	70.70	241.10	0.293
	64.77	183.90	0.352
	36.36	57.10	0.636
	39.56	65.30	0.604
	45.04	82.00	0.550
	61.89	162.20	0.381
Day 6	64.47	67.64	0.355
	57.28	134.00	0.324
	67.33	206.00	0.427
	62.20	164.80	0.327
	70.95	244.10	0.378
	67.64	209.7	0.291

APPENDIX 1 - Sheet IV

Table 13 (continued)

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry weight/ 1gm F.W.
Day 8	61.66	160.90	0.383
	65.07	186.10	0.349
	65.27	188.00	0.347
	65.22	187.80	0.348
	64.12	178.90	0.359
	56.03	127.20	0.440
Day 10	61.80	161.90	0.382
	69.06	223.10	0.309
	54.29	118.90	0.457
	63.29	172.12	0.367
	65.43	189.14	0.346
	61.80	161.93	0.382
Day 12	47.22	89.70	0.528
	45.42	83.10	0.546
	65.51	190.00	0.345
	65.55	190.10	0.345

APPENDIX J - Sheet I

Table 14

Levels of proline in Sesleria during drought stress: Results for plants from Cassop Vale - Site A.

	Fresh weight of Sesleria (mg)	µmoles proline/ gm. F.W.	µmoles proline/ gm D.W.
Day 0	0.2	0.25	0.44
	0.2	7.10	15.42
	0.2	0.55	1.16
	0.2	3.01	7.08
	0.2	5.89	12.56
	0.2	8.66	19.94
	0.2	1.32	2.20
	0.2	3.12	6.67
	0.2	1.49	3.53
Day 2	0.2	1.11	3.24
	0.2	6.93	16.26
	0.2	1.28	3.47
	0.2	1.39	3.41
	0.2	2.42	5.48
	0.2	3.12	7.22
	0.2	1.39	3.13
	0.2	4.09	9.99
	0.2	9.42	24.48
Day 4	0.2	2.25	6.39
	0.2	13.51	29.32
	0.2	1.04	3.98
	0.2	1.90	5.45
	0.2	5.54	15.18
	0.2	1.52	3.14
	0.2	1.56	3.38
	0.2	13.51	35.40
	0.2	1.90	5.30

APPENDIX J - Sheet II

Table 14 (continued)

	Fresh weight of *Sesleria* (mg)	µmoles proline/ gm F.W.	µmoles proline/ gm D.W.
Day 6	0.2	2.35	6.05
	0.2	3.81	9.65
	0.2	1.49	4.34
	0.2	1.73	4.41
	0.2	5.54	15.79
	0.2	2.25	5.99
	0.2	5.89	11.51
	0.2	2.42	4.72
	0.2	14.90	50.46
Day 7	0.2	1.04	2.79
	0.2	1.56	3.92
	0.2	1.04	2.88
	0.2	5.19	13.87
	0.2	4.16	12.58
	0.2	2.25	6.62
	0.2	1.56	3.60
	0.2	1.56	4.55
	0.2	7.79	23.30
Day 8	0.2	0.94	2.77
	0.2	0.94	2.68
	0.2	1.45	4.47
	0.2	1.49	4.08
	0.2	1.73	3.80
	0.2	1.73	4.66
	0.2	0.76	2.00
	0.2	1.56	4.67
	0.2	2.08	5.91

APPENDIX J - Sheet III

Table 14 (continued 2)

Results for Day 6 are those obtained before the plants were watered.

Results for Day 7 and Day 8 are those obtained 15 hours and 40 hours respectively after rehydration.

APPENDIX J - Sheet IV

Table 15.

Water levels in Sesleria during drought stress:

Results for plants from Cassop Vale - Site A

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry weight/ 1 gm F.W.
Day 0	43.02	75.29	0.570
	53.97	117.11	0.460
	52.5	110.78	0.475
	57.5	135.13	0.425
	53.10	111.5	0.469
	56.57	130.11	0.434
	40.10	66.97	0.599
	53.26	113.98	0.467
Day 2	65.78	192.08	0.342
	57.38	134.84	0.426
	63.12	171.06	0.369
	59.28	145.83	0.407
	55.81	126.13	0.442
	56.80	131.21	0.534
	55.53	124.94	0.445
	59.04	144.06	0.410
	61.53	159.98	0.385
Day 4	64.78	183.98	0.352
	53.92	117.01	0.461
	73.86	282.88	0.261
	65.11	186.87	0.349
	63.51	174.02	0.365
	63.94	131.72	0.361
	53.81	116.23	0.462
	61.80	161.92	0.382
	64.14	178.95	0.359

APPENDIX J - Sheet V

Table 15 (continued)

	% Water content on F.W. basis	% Water content on D.W. basis	Dry weight/ 1gm F.W.
Day 6	61.16	157.18	0.388
	60.53	153.14	0.395
	65.70	191.84	0.343
	60.73	154.86	0.396
	64.92	185.02	0.357
	62.38	165.93	0.376
	48.81	95.18	0.512
	48.7	94.97	0.513
	70.47	238.89	0.295
Day 7	62.71	168.06	0.373
	60.19	150.48	0.398
	63.86	176.89	0.361
	62.59	167.12	0.374
	66.94	202.16	0.331
	66.00	194.04	0.340
	56.69	130.95	0.433
	65.71	191.87	0.343
	66.57	199.04	0.334
Day 8	66.10	195.00	0.339
	64.99	185.87	0.350
	67.59	208.85	0.324
	63.48	173.94	0.365
	54.52	119.94	0.455
	62.86	169.09	0.371
	61.99	163.03	0.380
	66.56	199.01	0.334
	64.82	184.09	0.352

Results for Day 6 are those obtained before the plants were watered
Results for Day 7 and Day 8 are those obtained 15 hours and 40 hours respectively after rehydration.

APPENDIX K - Sheet I

Table 16
Levels of proline in Sesleria during drought stress:
Results for plants from Cassop Vale - Site B.

	Fresh weight of Sesleria (mg)	µmoles proline/ gm F.W.	µmoles proline/ gm D.W.
Day 0	0.2	0.52	1.56
	0.2	0.69	1.99
	0.2	2.25	5.83
	0.2	4.68	9.03
	0.2	2.77	5.62
	0.2	26.67	56.23
	0.2	1.21	2.17
	0.2	2.77	5.80
	0.2	1.39	3.23
Day 2	0.2	1.14	3.23
	0.2	4.16	10.69
	0.2	0.69	1.66
	0.2	56.97	113.60
	0.2	2.01	4.30
	0.2	86.58	143.63
	0.2	2.08	5.83
	0.2	13.33	25.00
	0.2	10.04	27.00
Day 4	0.2	2.42	3.16
	0.2	2.42	6.99
	0.2	0.97	2.73
	0.2	86.58	153.97
	0.2	1.39	3.12
	0.2	86.58	110.93
	0.2	1.04	3.30
	0.2	2.60	5.04
	0.2	3.12	6.32

APPENDIX K - Sheet II

Table 16 (continued)

	Fresh weight of *Sesleria* (mg)	μmoles proline/ gm F.W.	μmoles proline/ gm D.W.
Day 6	0.2	2.60	5.58
	0.2	2.70	7.22
	0.2	2.42	6.89
	0.2	48.48	61.70
	0.2	23.90	37.65
	0.2	42.94	48.77
	0.2	1.39	2.00
	0.2	15.58	24.81
	0.2	1.45	2.96
Day 7	0.2	8.68	20.81
	0.2	3.12	8.07
	0.2	2.25	5.99
	0.2	1.39	3.99
	0.2	1.65	2.69
	0.2	2.77	8,15
	0.2	86.58	127.21
	0.2	8.48	23.26
	0.2	31.86	96.14
Day 8	0.2	3.98	14.21
	0.2	2.08	6.25
	0.2	0.69	2.06
	0.2	3.64	7.63
	0.2	6.06	31.07
	0.2	11.08	30.58
	0.2	11.08	35.28
	0.2	15.76	47.84
	0.2	1.21	3.36

Results for Day 6 are those obtained before the plants were watered. Results for Day 7 and Day 8 are those obtained 15 hours and 40 hours respectively after rehydration.

APPENDIX K - Sheet III

Table 17

Water levels in Sesleria during drought stress:
Results for plants from Cassop Vale - Site B.

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry Weight/ 1 gm F.W.
Day 0	15.38	46.14	0.846
	65.38	188.95	0.346
	61.42	159.08	0.386
	48.15	92.93	0.519
	50.67	102.86	0.493
	52.57	110.92	0.474
	44.35	79.83	0.557
	52.26	109.22	0.477
	57.01	132.83	0.430
Day 2	64.65	180.01	0.354
	61.13	87.74	0.389
	58.33	168.99	0.417
	49.85	182.96	0.502
	53.31	157.10	0.467
	39.72	140.00	0.603
	64.29	99.7	0.357
	46.67	114.08	0.533
	62.82	65.94	0.372
Day 4	23.44	30.71	0.766
	65.38	188.95	0.346
	64.56	182.06	0.354
	43.77	77.91	0.562
	55.49	124.85	0.445
	21.95	28.10	0.781
	68.52	217.89	0.315
	48.40	93.90	0.516
	50.64	102.80	0.494

APPENDIX K - Sheet IV

Table 17 (continued)

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry Weight/ 1 gm F.W.
Day 6	53.43	114.87	0.466
	62.59	167.12	0.374
	64.88	184.91	0.351
	21.43	27.22	0.786
	36.53	57.72	0.635
	11.95	13.62	0.881
	30.53	43.96	0.695
	37.21	59.16	0.628
	51.07	104.18	0.489
Day 7	58.28	139.87	0.417
	61.33	158.84	0.387
	62.46	166.14	0.375
	65.19	183.18	0.348
	38.63	62.97	0.614
	66.00	194.04	0.340
	31.94	46.95	0.681
	63.54	174.10	0.365
	66.86	201.92	0.331
Day 8	71.98	256.97	0.280
	66.72	200.16	0.333
	66.52	198.89	0.335
	52.31	109.85	0.477
	80.50	412.97	0.195
	63.77	176.01	0.362
	68.60	218.15	0.314
	67.06	203.86	0.329
	64.00	177.92	0.360

Results for Day 6 are those obtained before the plants were watered
Results for Day 7 and Day 8 are those obtained 15 hours and 40 hours respectively after rehydration.

APPENDIX L - Sheet I

Table 18

Levels of proline in Sesleria during drought stress: Results for plants from Upper Teesdale (Site C).

	Fresh weight of Sesleria (mg)	µmoles proline/ gm F.W.	µmoles proline/ gm D.W.
Day 0	0.2	1.28	3.33
	0.2	3.84	8.83
	0.2	5.02	11.45
	0.2	2.42	6.01
	0.2	5.89	17.54
	0.2	2.42	7.26
Day 2	0.2	4.68	20.59
	0.2	1.14	3.66
	0.2	2.08	5.71
	0.2	10.04	21.06
	0.2	4.16	10.94
	0.2	4.50	7.49
Day 4	0.2	2.60	8.58
	0.2	0.52	1.57
	0.2	1.90	5.57
	0.2	1.90	4.38
	0.2	2.25	6.20
	0.2	2.60	7.46
Day 6	0.2	10.04	31.31
	0.2	10.32	29.37
	0.2	13.51	23.74
	0.2	6.93	16.67
	0.2	2.25	6.58
	0.2	4.24	11.00

APPENDIX L - Sheet II

Table 18 (Continued)

	Fresh Weight of *Sesleria* (mg)	μmoles proline/ gm F.W.	μmoles proline/ gm D.W.
Day 7	0.2	1.39	3.38
	0.2	1.63	2.00
	0.2	1.39	3.75
	0.2	1.73	4.65
	0.2	4.16	10.97
	0.2	10.39	34.28
Day 8	0.2	3.46	8.98
	0.2	1.56	4.92
	0.2	1.39	4.12
	0.2	8.31	22.73
	0.2	1.04	2.81
	0.2	1.04	3.38

Results for Day 6 are those obtained before the plants were watered.

Results for Day 7 and Day 8 are thoseobtained 15 hours and 40 hours respectively after rehydration.

APPENDIX L - Sheet III

Table 19

Water levels in Sesleria during drought stress:
Results for plants from Upper Teesdale (Site C).

	% Water Content on F.W. basis	% Water Content on D.W. basis	Dry Weight/ 1 gm F.W.
Day 0	61.67	160.96	0.383
	66.67	200.01	0.333
	56.54	130.04	0.435
	56.14	128.00	0.439
	59.72	148.11	0.403
	66.40	197.87	0.336
Day 2	77.27	339.99	0.227
	68.81	220.88	0.312
	63.55	174.13	0.365
	52.32	109.87	0.477
	61.99	163.03	0.380
	39.95	66.72	0.601
Day 4	69.69	229.98	0.303
	66.84	201.86	0.332
	65.91	193.12	0.341
	56.62	130.23	0.434
	63.72	175.87	0.363
	65.17	187.04	0.348
Day 6	67.94	211.97	0.321
	64.86	184.85	0.351
	43.10	75.86	0.569
	58.43	140.82	0.273
	65.79	192.11	0.342
	61.44	159.13	0.386

APPENDIX L - Sheet IV

Table 19 (continued)

	% Water Content on F.W. basis	%Water Content on D.W. basis	Dry Weight/ 1gm F.W.
Day 7	58.89	143.10	0.411
	18.37	22.60	0.816
	62.94	169.94	0.371
	62.81	168.96	0.372
	62.09	163.92	0.379
	69.69	229.98	0.303
Day 8	61.48	159.85	0.385
	68.29	215.11	0.317
	66.28	196.85	0.337
	63.44	173.83	0.366
	63.04	170.84	0.370
	69.27	225.13	0.307

Results for Day 6 are those obtained before the plants were watered.

Results for Day 7 and Day 8 are those obtained 15 hours and 40 hours respectively after rehydration.

Table 20

Levels of proline and water in Sesleria: Results for control plants from Cassop Vale - Sites A. and B, and from

Levels of proline and water in Sesleria: Results for control plants from Cassop Vale - Sites A and B and from Upper Teesdale (Site C).

Table 20 APPENDIX M

	Fresh weight of Sesleria (mg)	μmoles proline/ gm F.W.	μmoles proline/ gm D.W.	% Water Content on F.W. basis	Dry weight/ 1 gm F.W.
	CASSOP VALE SITE A				
Day 0	0.2	0.42 ± 0.02	0.96 ± 0.19	58.42	0.446
2	0.2	0.59 ± 0.02	1.90 ± 0.23	65.25	0.347
4	0.2	0.45 ± 0.01	1.27 ± 0.36	66.34	0.337
6	0.2	0.69 ± 0.03	1.56 ± 0.40	65.35	0.346
8	0.2	0.62 ± 0.04	1.42 ± 0.53	64.00	0.344
10	0.2	0.59 ± 0.02	1.75 ± 0.41	65.99	0.320
	CASSOP VALE SITE B				
Day 0	0.2	0.43 ± 0.02	0.80 ± 0.23	62.27	0.477
2	0.2	0.56 ± 0.03	0.99 ± 0.21	64.62	0.354
4	0.2	0.36 ± 0.02	0.61 ± 0.17	62.80	0.372
6	0.2	0.63 ± 0.02	0.98 ± 0.10	67.93	0.321
8	0.2	0.47 ± 0.02	0.73 ± 0.19	63.80	0.347
10	0.2	0.50 ± 0.02	0.85 ± 0.11	64.71	0.340
	UPPER TEESDALE SITE C				
Day 0	0.2	0.56 ± 0.03	0.92 ± 0.23	56.30	0.499
2	0.2	0.52 ± 0.04	0.90 ± 0.31	63.97	0.360
4	0.2	0.62 ± 0.02	1.20 ± 0.40	53.97	0.460
6	0.2	0.61 ± 0.03	0.93 ± 0.33	65.49	0.345
8	0.2	0.53 ± 0.02	1.00 ± 0.20	60.00	0.444
10	0.2	0.57 ± 0.01	0.99 ± 0.21	59.84	0.537

3 Replicates were used for each site. Above values are expressed in terms of Mean ± Standard Error

APPENDIX N

FIGURE 2

Significance tests on Sesleria caerulea: Levels of proline and water in plants from the 3 sites during cold stress.

Sites compared	Day 0	2	4	6	8	10	12
Proline levels							
A and B:	N.S.	N.S	*	N.S.	*	*	*
A and C:	N.S.	N.S	N.S.	N.S.	*	*	*
B and C:	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.
% Water Content							
A and B.	N.S.	N.S.	*	N.S.	N.S.	*	*
A and C:	N.S.	N.S.	*	N.S.	*	N.S.	*
B and C:	N.S.	N.S.	N.S.	N.S.	*	N.S.	*

N.S. = Not significant

* = significant at the p = 0.05 level.

Student's t-test used.

APPENDIX 0

FIGURE 3

Significance tests on Sesleria caerulea: Levels of proline and water in plants from the 3 sites during drought stress.

Sites compared	Day 0	2	4	6	7	8
Proline levels.						
A and B:	N.S.	*	*	*	*	*
A and C:	N.S.	N.S.	*	*	N.S.	*
B and C:	N.S.	*	*	N.S.	*	*
% Water Content.						
A and B:	N.S.	*	*	*	*	*
A and C:	*	N.S.	N.S.	N.S.	*	N.S.
B and C:	*	N.S.	*	*	N.S.	N.S.

N.S. = Not significant

* = significant at the p = 0.05 level

Student's t-test used.

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