"Proline Production in Relation to

Stress in Sesleria caerulea L.."

M.Sc. Dissertation.

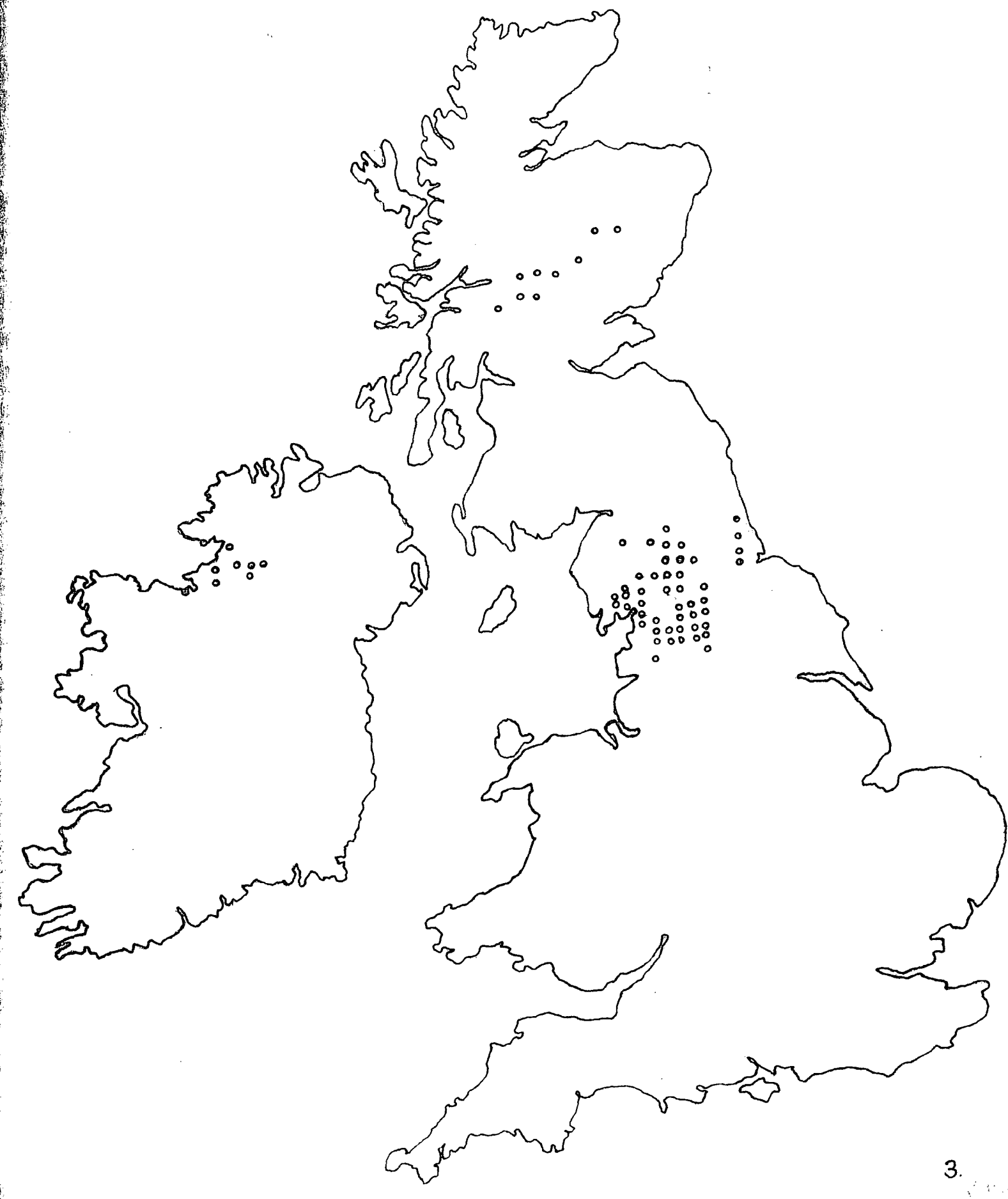
Stuart Darke

September 1976.

FIG I.

DISTRIBUTION OF *SESLERIA*

CAERULEA IN BRITAIN + N. IRELAND.



ACKNOWLEDGEMENTS

I wish to acknowledge Dr. A. Pearson for his help and consideration during the course of this study.

CONTENTS

ABSTRACT

Leaves of plants grown under optimum conditions contain a very low proportion of proline amongst their free amino acids. However, after having water withheld for a period of 24 hours or more, the proline content of the leaves becomes many times that observed as being normal.

Large scale accumulation of proline also takes place in response to low temperature treatment, increased calcium ion concentrations and developmental stress.

Inter-population variation with regard to stress induced proline production has been observed as well as intro-population variation.

Chapter 1

INTRODUCTION

Stress and stress resistance in plants is concerned with their resiliance to the possible injurious effects of a multitude of environmental factors. The organism involved may exhibit physical strain or chemical strain incorporating a shift in metabolism. It is the latter possibility that has provided the impetus for this and previous studies on proline production.

Numerous authorities (1, 8, 9, 10, 12, 17, 18, 21, 22, 23, 25, 34, and 47) have noted that when subjected to stress, plants cease to synthesise proteins and instead accumulate high levels of free amino acids. Such a reaction appears to take place in response to low temperature (17, 23), high light intensity and high temperature (13), salinity changes (32), drought (1, 45) and flooding (47).

Of these amino acids proline is of major significance in quantitative terms. Barnett (1966) observed a 10 to a 100 fold increase in free proline in the shoots of Cynodon dactylon under water stress, compared with only a 2 to 6 fold accumulation of free asparagine. Similar results having been obtained by a number of other research workers.

The significance of such increases in proline remains unanswered. Wilding et al (1960) obtained a correlation between amino acid content and hardiness in red clover, and proline in particular has been shown to accumulate with increasing hardiness and vice versa.

Le Saint (1966) actually induced hardening in cabbage plants by providing them with an exogenous solution of $5g/l^{-1}$ proline.

High proline levels appear to be correlated with a number of forms of tolerance. Light resistant sun leaves of the copper beech have, according to Haas (1969), higher proline levels than light sensitive ones, and in Carex pachystylis proline and drought tolerance show concomitance.

Durzan (1969) discovered a diurnal periodicity in the proline levels of white spruce and concluded that because of the wide range of fluctuation that a causal relationship between the mere presence of proline and tolerance must at least be questioned. This view suggests that proline may simply be a less toxic amino acid and that its synthesis is a convenient way of producing a less noxious store of NH_3.

In either event there are two widely accepted generalizations that can be made with regard to the production of proline in plants.

Firstly that proline accumulates as a result of bioligical stress and secondly that at least a non causal correlation exists between proline production and tolerance.

The present study is thus concerned with the utilization of proline production as an indication of the genetic plasticity of stress resistance in Sesleria caerulea in relation to its ecological amplitude.

Turesson (1930) investigating the relationship between biotypes and climatic conditions considered that climate strongly influences the nature of the biotype group at any particular site, such that any particular species of

plant may consist of variety of ecotypes with genetic dissimilarities selected by the nature of the specific environmental conditions within which the population is growing.

Morphological variation of ecotypes is now well documented (40, 41, 42), indeed West (1975) discovered considerable variations in the morphology of Sesleria populations from various selected sites. However, little work has to date been conducted with regard to the genetic plasticity of ecotypes as exemplified in the variability of the metabolic pathways in relation to biological stress phenomena.

The phenomena under consideration by the present author are chilling and water stress. Waldren and Teare (1974) observed that soybean plants accumulated proline some 60 fold when the leaf water potential (Ψ_L) reached -14 Bars and Sorghum showed 200 fold increases in proline at a Ψ_L of -24 Bars. Barnett and Naylor (1966) found that in Bermuda grass at -30 Bars free proline levels increased up to 125 times that in the controls. These findings have been confirmed by numerous authorities.

In all cases the extent of proline production was directly related to the Ψ_L as summarised by Chu et al (1974) who, working on radish plants, found a correlated increase in proline with lowering water potential. The effects of chilling are at least in part the same as drought stress in that the water potential is altered. Chilling at 5^{o} C. in barley leads to the production of proline after 24 hours which continues for at least a further 4 days at a rate of 74 g/g dry wt./hr.$^{-1}$ Indeed Levitt (1956) suggests that a

plant's resistance to cold, heat and water stress are inter-related. In addition, however, further indirect stress through chilling might be brought about by increased membrane permeability or starvation, as respiration could conceivably proceed faster than the production of carbohydrates by photosynthesis.

Whilst the latter remains unproven the relationship between sugar content and proline production is well established, its role probably being the furnishing of a ketoglutarate and N.A.D.P.H. which are required for proline synthesis. The process requires oxidation and highlights the importance of aerobic conditions in proline production as indicated by Thompson et al. (1966).

With this in mind the synthesis of proline in Sesleria caerulea in response to environmental stress was investigated for four populations at Cassop Vale, Durham.

CHAPTER II

SITE DESCRIPTIONS

The four study sites at Cassop Vale, Sites A, B C and D are located as indicated in Fig. II, page 11(a).

SITE A - located towards the base of a limestone cliff with a soil depth of 23cm and a pH of 8, as measured by an "E.I.L." model 23A glass electrode direct reading pH meter.

Soil water content varied between 10.7% when first recorded and 0.71% after field drought stress conditions.

Calcium content was between 5 and 12% by weight.

The Sesleria plants bore mature leaves which averaged 0.041 g D.W. and produced spikes of 19.15 cm in length.

SITE B - located in a gully overshadowed by small trees, with a soil depth of 12 cm. and a pH of 7.9.

Soil water content varied between 21.87% when first recorded and 8.25% after field drought stress conditions.

Calcium content was between 5 and 12% by weight.

The Sesleria plants bore mature leaves which averaged 0.054 g D.W. and produced spikes of 27.99 cm in length.

SITE C - located at the top of a limestone cliff with a soil depth of 4 cm and a pH of 8.2.

Soil water content varied between 19.56% when first recorded and 5.96% after field drought stress conditions.

Calcium content was between 5 and 12% by weight.

The Sesleria plants bore mature leaves which averaged 0.011 g D.W. and produced spikes of 19.75 cm. in length.

SITE D - located at a roadside verge overshadowed by a dense cover of shrubs and trees, with a soil depth of 17 cm. and a pH of 7.9.

Soil water content varied between 24.84% when first recorded and 16.89% after field drought stress conditions.

Calcium content was between 5 and 8% by weight.

The Sesleria plants bore mature leaves which averaged 0.057 g D.W. and produced spikes of 18.85 cm. in length.

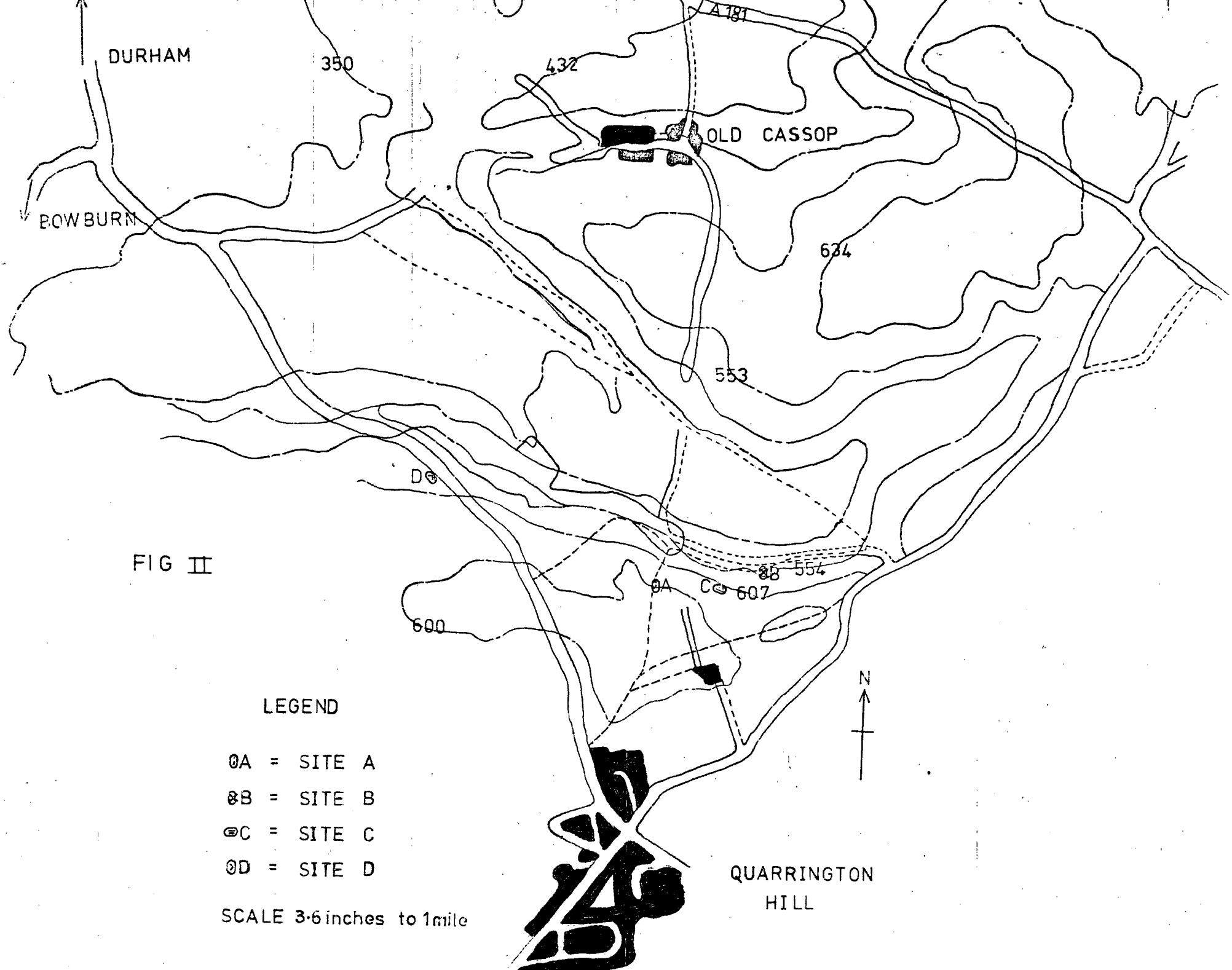

DURHAM
350
A181
432
OLD CASSOP
BOWBURN
634
553
DG
554
0A
607
600
N
LEGEND
0A = SITE A
0B = SITE B
0C = SITE C
0D = SITE D
SCALE 3·6 inches to 1 mile
QUARRINGTON
HILL

FIG II

Chapter III

METHODS AND MATERIALS

Sesleria caerulea was collected from four sites at Cassop Vale, National grid reference number NZ 341 383, and transplanted into John Innes No. 2 potting compost in the laboratory. The plants were subjected to two soil depth regimes comparable to the two extremes which were observed at the field sites, that is 5 and 10 cm respectively. All plants were kept well watered for about four weeks until new young leaves had been formed and were then removed to a constant temperature room at 20°C with a 14 hour photoperiod of 1,400 Lux intensity for a further week to equilibrate soluble carbohydrate content.

Subsequently Sesleria plants at both soil depths were either subjected to drought stress by witholding water or chilling stress by removing them to 1 and 5°C rooms with comparable photoperiods.

In order to confirm whether proline content fluctuated under natural conditions Sesleria leaves were removed in the field and placed immediately into 25 cm^3 of 3% aqueous sulfosalicyclic acid. These samples being assayed on returning to the laboratory.

Proline determinations were made using a modification of the acid Ninhydrin technique described by Chinard (1952) and Troll and Lindsley (1955). The acid ninhydrin was prepared by adding 125mg ninhydrin to 30 cm^3 glacial acetic acid and 20 cm^3 6M phosphoric acid. This mixture was then warmed

and stirred until the ninhydrin was dissolved. When stored at 5°C the reagent remained stable for 48 hours.

The procedure involved homogenising between 5 and 20mg of Sesleria in 25cm^3 of 3% aqueous sulfosalicyclic acid. This was carried out on a "virtis" homogeniser at 45,000 r.p.m. for 90 seconds. The homogenate was then filtered through Whatman # 1 filter paper.

10 cm^3 of the filtrate was mixed 2cm^3 of acid ninhydrin reagent and 2cm^3 glacial acetic acid in a boiling tube. At pH values between 1 and 7 only Ornithine is an effective contaminant at 520nm and to alleviate this problem 1g of Permutit acid ion exchange material was added to the reagent mixture and shaken.

The colour change was developed by heating in a waterbath at a temperature in excess of 80°C for an hour, a full colour change being developed in 30 minutes at 100°C. The reaction was terminated by partly immersing the boiling tube in an ice bath.

The chromophore was extracted and concentrated by adding 4cm^3 toluene and stirring electronically for about 20 seconds. Then warmed to room temperature the extent of the colour change was determined by pipetting off the tinctorial phase and reading its optical density against a blank of toluene in a 'Uvispek' spectrophotometer at 520nm.

Quantitative values of proline content were obtained by comparison against a standard curve obtained from hydroxy-proline free L. Proline, as supplied commercially by the Sigma chemical company.

Because of the importance of sugar and changes on proline production, both these criteria were measured for each set of experiments.

The problems of field measurements and the unavailability of a portable thermocouple psychrometer, as advocated by Boyer (1968), meant that an estimation of ψ_L was obtained by using density method determinations, nevertheless values measured by the density or dye method generally agree to within 3 Bars of those measured with the thermocouple psychrometer if left to stabilise.

The method was to place thirteen .5mg leaf samples into a series of solutions of known water potential, produced according to Ursprung and Blum (1916)*1, and to which a few drops of methylene blue had been added. Each test solution totalling .5cm^3 of liquid. The Sesleria leaf samples were then left to stabilise for 4 hours, whereupon a drop of coloured test solution was removed and placed into an uncoloured control tube containing 5ml of test solution of identical water potential. The drops fall if the test solutions have been concentrated and rise if the solutions have been diluted.

The ψ_L being assumed to equal the test solution that brings about no change in the position of the transferred methylene blue drop. This method is accurate at detecting leaf sucrose concentration differences of 0.0005M.

Soluble sugar content was estimated by homogenising 0.2g of Sesleria in 25cm^3 distilled water and further diluting the homogenate with 175cm^3 distilled water in a graduated flask, which was then shaken for one hour. The homogenate

*1 see Appendix (II)

was then filtered through 12.5 cm # 1 filter paper, discarding the first few cm^3 of the filtrate. The soluble carbohydrate was determined immediately by adding $2cm^3$ of filtered extract to $10cm^3$ of anthrone reagent in a loosely covered Pyrex test tube. This was then placed in a waterbath at $100^{\circ}C$ for 20 minutes. On cooling to room temperature the absorbance was measured in a 10 mm optical cell at 620nm on the 'Uvispek' spectrophotometer calibrated with a filament lamp. The blank consisted of untreated extract.

Anthrone reagent was produced by stirring $760cm^3$ of 98% sulphuric acid in $330cm^3$ of water, cooling and adding 1g of thiourea and 1g of anthrone. The reagent can be stored in a refrigerator.

Quantitative values of soluble carbohydrate were obtained by comparison against a standard curve produced by reacting anhydrous D. glucose standards with the reagent mixture. The glucose standards being derived by serial dilution of a glucose stock solution consisting of 0.4g of anhydrous D. glucose in 500ml of water.

The percentage soluble carbohydrate in the sample, as measured in terms of glucose, was finally calculated by multiplying the difference between blank and test sample values by 50.

The nature of the experiments involving drought stress and chilling stress necessitated removing individual plants at daily intervals and estimating their proline content. As the glucose reagents involved required being used immediately and the acid ninhydrin was also only stable for a short period of time, the Sesleria plants were

frozen with liquid Nitrogen and stored at -20°C until required for proline determinations.

Soil calcium content was discovered by acid digestion of 1g of soil that had been sifted through a 2mm sieve. The acid used for this purpose being 4N.Hnoz. After filtering through Whatman 9cm #1 filter paper the filtrate was diluted to produce a concentration detectable within the scope of an "Eel" flame photometer. Absolute values of calcium content were obtained by reference to a standard curve produced from galvanometer deflection readings in relation to known concentrations of calcium in p.p.m. The calcium content of the soils was used as a guideline in determining a range of suitable Ca concentrations for an experiment designed to make preliminary investigations with the relationship between soil Ca content and Sesleria proline production. Calcium, in the form of calcium corbonate, was added to John Innes number 2 potting compost to produce 3 test conditions consisting of 0.14, 5 and 12% Ca by weight. Sesleria, Phleum pratense L., Agropyron caninum and a sedge species were transferred to the test conditions and allowed to acclimatise for 14 days. Subsequently approximately 1 mg of mature leaf tissue from each test pot was used for proline determinations according to the Ninhydrin method.

Chapter IV

R E S U L T S

Proline Production

Sesleria samples were taken from the field sites A to D on three occasions which correspond to:-

i) the start of the investigation

ii) a lengthy period of field drought stress,

iii) after relief of the drought stress by 0·57 inches of rain.

The climatic data during the periods of sampling were obtained from the Durham University Meterological Station, located at National Grid Reference No. NZ 267416 - (see Appendix (I)).

The null hypothesis for all field experiments was that given comparable periods of stress there would be no significant variation in proline production between sites.

Prior to such an investigation of the nature of between site variation it was considered important to study within site variation of Sesleria specimens. Preliminary research by the present author on chinese cabbage indicated that young growing leaves exhibited proline levels several fold higher than those observed in mature leaves.

The Sesleria plants were thus subjectively divided into easily recognisable age classes in the field namely non flowering mature plants, immature plants and mature plants bearing inflorescences. Maturity being determined in relation to the mean height of the leaves of flowering plants. Thus at each site three series of

proline determinations were made, incorporating leaves from non flowering and flowering mature plants, along with the infloresences themselves.

TABLE (I) *1

DETERMINATION OF WITHIN SITE AGE VARIATION IN RELATION TO PROLINE PRODUCTION

Site Description	Test used	Test Statistic	Df = K - 1 Degrees of freedom	Level of Significance P
A	Friedman two way analysis of variance	44.46143	2	$< .001$
B	Friedman two way analysis of variance	62.20508	2	$< .001$
C	Friedman two way analysis of variance	47.12817	2	$< .001$
D	Friedman two way analysis of variance	43.12817	2	$< .001$

Having thus established significant differences within sites it was important to locate the particular age class that gave the most consistent results involving the smallest standard error of the mean, as in this way a more reliable estimate of any between site variation that might exist could be obtained.

As an ordinal ranking scale had been produced for the related samples of Sesleria plants at any one sight, the Wilcoxon signed rank test allowed a closer investigation of the nature of within site variation. *2

*1 Data for this test is located in Appendix (III)
*2 See Appendix (III) for Wilcoxon signed rank test data.

In all cases a significant difference was located between the chosen age groups at each site, the group having the smallest standard error of the mean being the leaves of the mature, but not flowering plants.

TABLE (II)

LOCATION OF THE AGE CLASS CONTAINING LEAST WITHIN-SITE VARIABILITY IN FREE PROLINE PRODUCTION

SITE	AGE CLASS TESTED	MEAN VALUE OF FREE PROLINE PRODUCED, mm/g	S.E. OF THE MEAN
A	Leaves from mature non-flowering plants	9.697	0.771
A	Leaves from mature flowering plants	10.403	1.556
A	Spikes from flowering plants	29.665	2.56
B	Leaves from mature non-flowering plants	13.451	0.272
B	Leaves from mature flowering plants	12.093	0.303
B	Spikes from flowering plants	38.055	1.557
C	Leaves from mature non-flowering plants	7.616	0.470
C	Leaves from mature flowering plants	8.140	0.631
C	Spikes from flowering plants	37.94	2.617
D	Leaves from mature non-flowering plants	19.534	2.191
D	Leaves from mature flowering plants	33.732	0.95
D	Spikes from flowering plants	51.316	3.32

When these mature leaves were compared between sites a result was obtained which indicated a probability $<.001$ that the plants concerned came from independent populations. A further breakdown of the data with paired values indicated significant differences between the sites studied.

TABLE (III)

NON FLOWERING PLANTS FROM SITES A - D

Site samples compared	Statistical test	Test Statistic	Df = k-1 Degrees of freedom	Significant level ρ
A,B,C & D	Kruskal-Wallis one way analysis of variance	56.01987	3	$<.001$
A,B.	" " "	40.08443	1	$<.001$
A, & C	" " "	1.88816	1	.2
A & D	" " "	18.94220	1	$<.001$
B & C	" " "	38.39085	1	$<.001$
B & D	" " "	1.74014	1	.20
C & D	" " "	17.07545	1	$<.001$

Leaves from flowering plants and the inflorescences themselves were subsequently compared between the four test sites to determine whether the observed variability of Sesleria populations in response to environmental stress remains unaltered when the plants are subjected to the additional suspected morphological stress involved in sexual reproduction.

In both cases the tests showed continued dissimilarly in the 4 populations response to superimposed morphological stress; but closer investigation revealed

that the test statistic was being influenced by one site in particular, that is, site D.

TABLE (IV)

FLOWERING PLANTS FROM SITE A - D

Site samples compared	Statistical test used	Test Statistic	Degrees of freedom (Df = k-1)	Significance level p
A, B, C & D	Kruskal Wallis one way analysis of variance	95.95595	3	$<.001$
A & B	" " "	0.25509	1	.70
A & C	" " "	0.51393	1	.50
A & D	" " "	58.50142	1	$<.001$
B & C	" " "	27.50444	1	$<.001$
B & D	" " "	58.50212	1	$<.001$
C & D	" " "	57.60887	1	$<.001$

TABLE (V)

INFLORESCENCES FROM SITES A - D COMPARED FOR PROLINE PRODUCTION

Site samples compared	Statistical test used	Test Statistic	Degrees of freedom Df = k - 1	Significance level
A,B,C & D	Kruskal Wallis one way analysis of variance	24.91850	3	$<.001$
A & B	" " "	5.74815	1	.02
A & C	" " "	6.13949	1	.02
A & D	" " "	17.52075	1	$<.001$
B & C	" " "	1.53851	1	.03
B & D	" " "	8.19866	1	.01
C & D	" " "	11.86694	1	$<.001$

The physical stress the Sesleria population had to endure

during reproductive activity seemed therefore to obscure the underlying environmentally induced differences in proline production previously observed to be prevalent at the test sites. This idea being supported by the much higher proline levels which were found in inflorescenses in particular and flowering plants in general.

TABLE (VI)

Age class tested	Mean value of Proline in mm/g F.W. for sites A,B,C & D combined
Leaves from non-flowering mature plants	12.57
Leaves from flowering mature plants	16.09
Spikes of flowering plants	39.24

For subsequent considerations of Seslerias response to environmnetally induced stress it was considered important to use data obtained from proline determinations on the leaves of mature non flowering plants. This assumption was based

a) on the fact that the confidence limits of this particular age group were in general much narrower involving considerably less within site variation and thereby allowing more accurate comparisons between the various sites and,

b) that the results from preliminary experiments indicated the enhancing of the sress response by the flowering activities of the Sesleria populations.

Field measurements for the chosen Sesleria age class were not taken on Sunday, 19th July, after a prolonged

period of drought stress lasting 22 days. In all cases free proline content had increased considerably with individual sites ranging between 3.34 and 135.01 fold increases.

TABLE (VII)

Lowest $\bar{x}$ value of proline	$\bar{x}$ value of proline after (22) days of drought stress	fold increase	site number	soil water content %
6.67	35.28	5.29	A	.71
11.74	39.16	3.34	B	8.25
7.47	1008.53	135.01	C	5.96
6.71	30.36	4.53	D	16.89

A simple correlation between soil water content and proline production provides an inadequate explanation for the pattern observed in the Sesleria plants taken from Cassop Vale. Indeed when other readily observable site variables are superimposed upon the drought stress response the intricate network of environmentally important regulating factors becomes more apparent.

TABLE (VIII)

Site	Fold increase in proline	Soil water content in %	Soil depth in cm	PH
A	3.66	.71	23	8
B	2.91	8.25	12	7.9
C	132.35	5.96	4	8.2
D	1.49	16.89	17	7.9

In the field the drought stress was relieved on Monday, 12th July by the precipitation of 0·57 inches, of water. Laboratory work had shown that immediately on application of water Sesleria produced a stress response.

TABLE (IX)

THE EFFECT OF WATERING ON SESLERIA IN THE LABORATORY

Site	Free proline level before watering in mm/g	Free proline level after watering in mm/g
A	108	288
B	97	302
C	36	278
D	86	652

Consequently samples were not collected until 24 hours after the relief of the drought stress. The subsequent proline determinations when compared between sites indicated that the sampling areas did not include Sesleria plants from a single continuous population ($p < .001$). Closer investigation reveals that in fact a single site, site B, is the only area in which free proline levels are significantly different.

TABLE (X)

(this follows on next page)

TABLE (X)

Site Number	Test used	Test Statistic	Degrees of freedom	Level of Significance ρ
A,B,C & D	Kruskal-Wallis one way analysis of variance	58.11728	3	$<.001$
AB	" " "	36.77705	1	$<.001$
AC	" " "	4.76584	1	.05
AD	" " "	0.00006	1	$>.99$
BC	" " "	36.77174	1	$<.001$
BD	" " "	36.77351	1	$<.001$
CD	" " "	1.84505	1	.20

Whilst field observations suggested that under natural environmental conditions proline production in Sesleria varied according to the specific nature of the local population, nevertheless, it was considered important to be able to eliminate the wide number of site variables before drawing anything more than tentative conclusion with regard to ecotype variation.

In the laboratory Sesleria plants from all four field sites and a further site in Lipinia, Yugoslavia, were planted in 10 cm and 4 cm of John Innes potting Compost No. 2. These plants were then subjected to either drought stress by withholding water or cold stress by subjecting them to $5^{o}C$ for 10 days. The choice of two soil depths in the laboratory was based on the findings of the initial field experiments which showed that the depth of soil appeared important in connection with proline production.

When subjected to drought stress all plants exhibited the same response pattern that is, an increase : decrease cycle throughout the 10 days of stress and subsequent

period of watering. (See Fig. III page 26(a)).

Whilst the overall pattern of proline production was similar, absolute values with regard to the amount of free proline contained in the Sesleria leaves varied considerably according to the plants location in the field.

TABLE (XI)

Site	Maximum value of proline in mm/g	Time when maximum value reached
A	862 ± 80	After 198 hours
B	520 ± 43	After 198 hours
C	380 ± 58	After 98 hours
D	662 ± 63	After 272 hours (i.e. 32 hours after the relief of drought stress by watering)

Table (XI) also shows that the timing of peak proline values varies between sites. At site C where the plants were growing in extremely shallow soil with poor rooting conditions, proline levels were highest after 98 hours of drought stress, whilst the deeper rooting sites such as A and B contained plants that reached maximum proline values after a much longer period of sustained drought stress; a feature which adds support to the possible casual relationships between proline production and stress resistance as obtained from the Ca stress experiments.

Site D which, although having comparitively deep soil, reached its maximum value of free proline after a considerably longer period of time than the comparably deep rooting sites of A and B. In fact 317 hours experimental

time means that the highest proline levels were obtained not during the drought stress period, but 77 hours after the relief of drought stress by thorough watering. This is the site in the field which appeared to contain most moisture, both from soil water determinations and by specia composition observations.

The rate of proline production may, therefore, be as important, if not more important, than the absolute value of the maximum level of free proline, as this value might be super-optimal or merely contain a quantity of proline.which is superfluous in terms of survival value.

A more detailed study of the rate of production was therefore undertaken by considering the rate of increase between each observation and the next, in terms of fold increases in proline, where 1 represents the maintainance of the status quo and the values <1 a decrease in free proline levels.

TABLE (XII)

RATES OF INCREASE IN PROLINE LEVELS OF PLANTS SUBJECTED TO DROUGHT STRESS AT 20°C

TIME IN HOURS	SITE A	SITE B	SITE C	SITE D
24	29.41	22.53	18.59	20.99
50	0.74	0.61	0.68	1.13
72	0.52	0.45	1.47	0.70
98	0.87	1.74	2.71	0.54
120	2.46	1.4	0.67	0.93
144	1.56	1.3	0.73	1.02
170	1.92	1.27	0.6	1.0
198	1.80	1.80	0.63	1.02
219	0.12	0.22	1.06	1.48
240 watered	0.95	0.87	1.08	2.36
272	2.88	0.95	0.94	3.23
317	0.22	3.24	3.32	0.09
$\bar{x}$ daily increase	3.62	3.03	3.54	2.87

See fig IV Page 28(a)

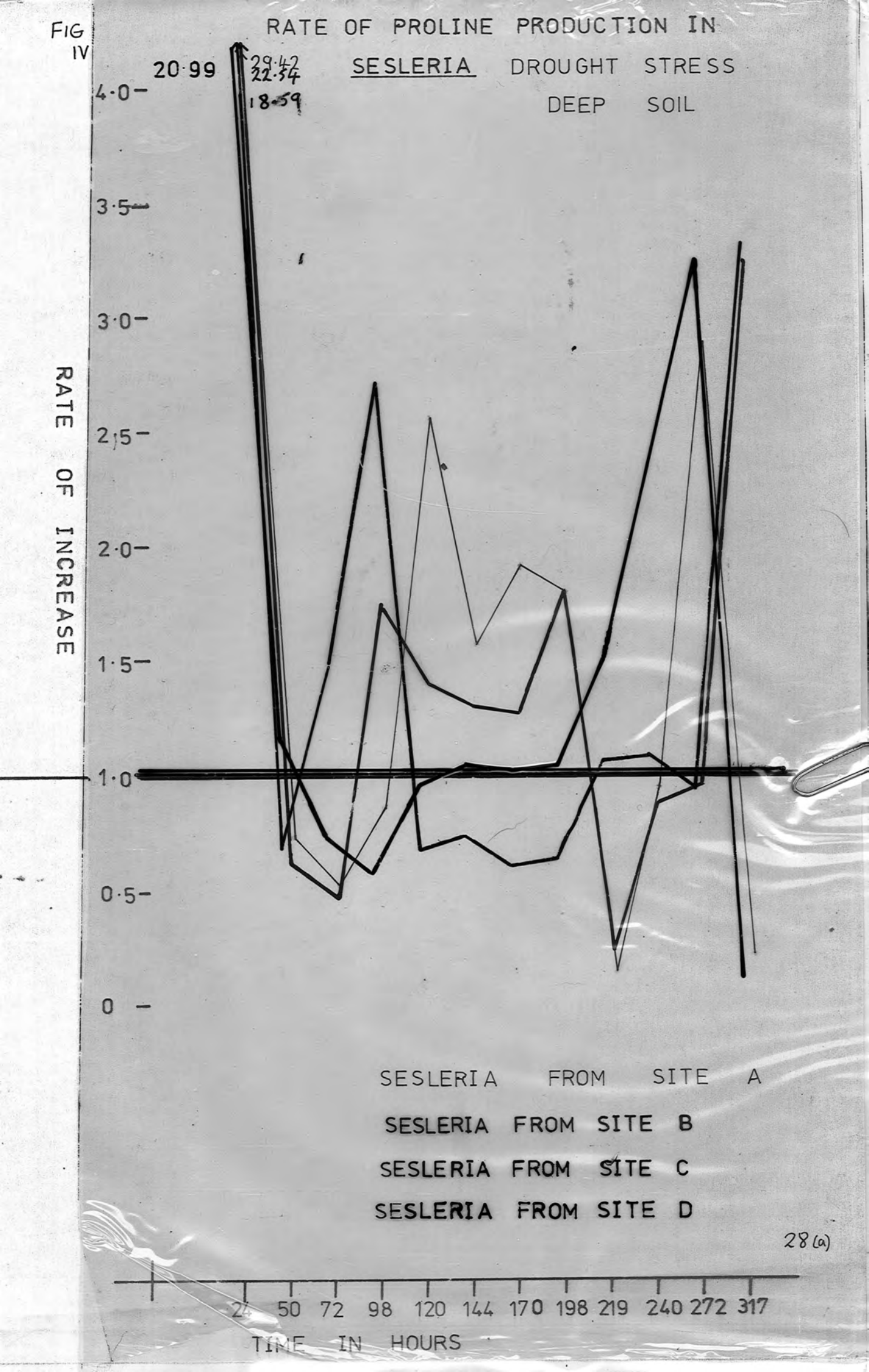
Figure IV clearly shows the relative occurences of the increase/decrease cycle of proline production located in Sesleria caerulea and indicates how a simple mathematical consideration of the $\bar{x}$ daily increase values is inadequate in explaining the presence of any interspecific variation in site response to drought stress.

An interesting picture is also obtained when the rates of increase in cold stressed plants from comparable sites are viewed in relation to the results obtained under drought stress conditions. When Sesleria plants are maintained at a temperature of 5°C for a sustained period of time the cyclical nature of proline production becomes far less pronounced and at the same time total free proline for the experimental period is considerably reduced, ranging between 37% and 50% of the levels obtained during drought stress at 20°C. The temperature discrepancy between drought and cold stress conditions is assessed at 15°C, with this in mind, a temperature coefficient calculation for Sesleria would indicate that under cold stress conditions approximately 66.67% reduction in free proline production would be expected in relation to the drought conditions if proline content was regulated solely by temperature dependent metabolic processes. Data obtained during these experiments showed that the reduction ranged between 4% and 63% according to the site under consideration.

TABLE (XIII)

Site	Reduction in proline production during the course of the experiment.
A	50.95%
B	57.24%
C	62.15%
D	58.34%

FIG IV


RATE OF PROLINE PRODUCTION IN
SESLERIA DROUGHT STRESS
DEEP SOIL
20·99
29·42
22·54
18·59
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
RATE OF INCREASE
SESLERIA FROM SITE A
SESLERIA FROM SITE B
SESLERIA FROM SITE C
SESLERIA FROM SITE D
28(a)
24 50 72 98 120 144 170 198 219 240 272 317
TIME IN HOURS

Site C therefore represents the least restricted temperature dependent metabolic process, whilst Sites A and B, in particular, have higher proline levels in the cold treatment than would be expected on this basis, presumably because under drought conditions they are more susceptable to the water deficit being endured and that this is restricting the temperature dependent process.

A detailed study of the rates of increase in proline production under cold stress reveals more accurately the depressed proline production cycle with decreased temperature.

TABLE (XIV)

RATES OF INCREASE IN PROLINE LEVELS OF PLANTS SUBJECTED TO COLD STRESS AT 5°C

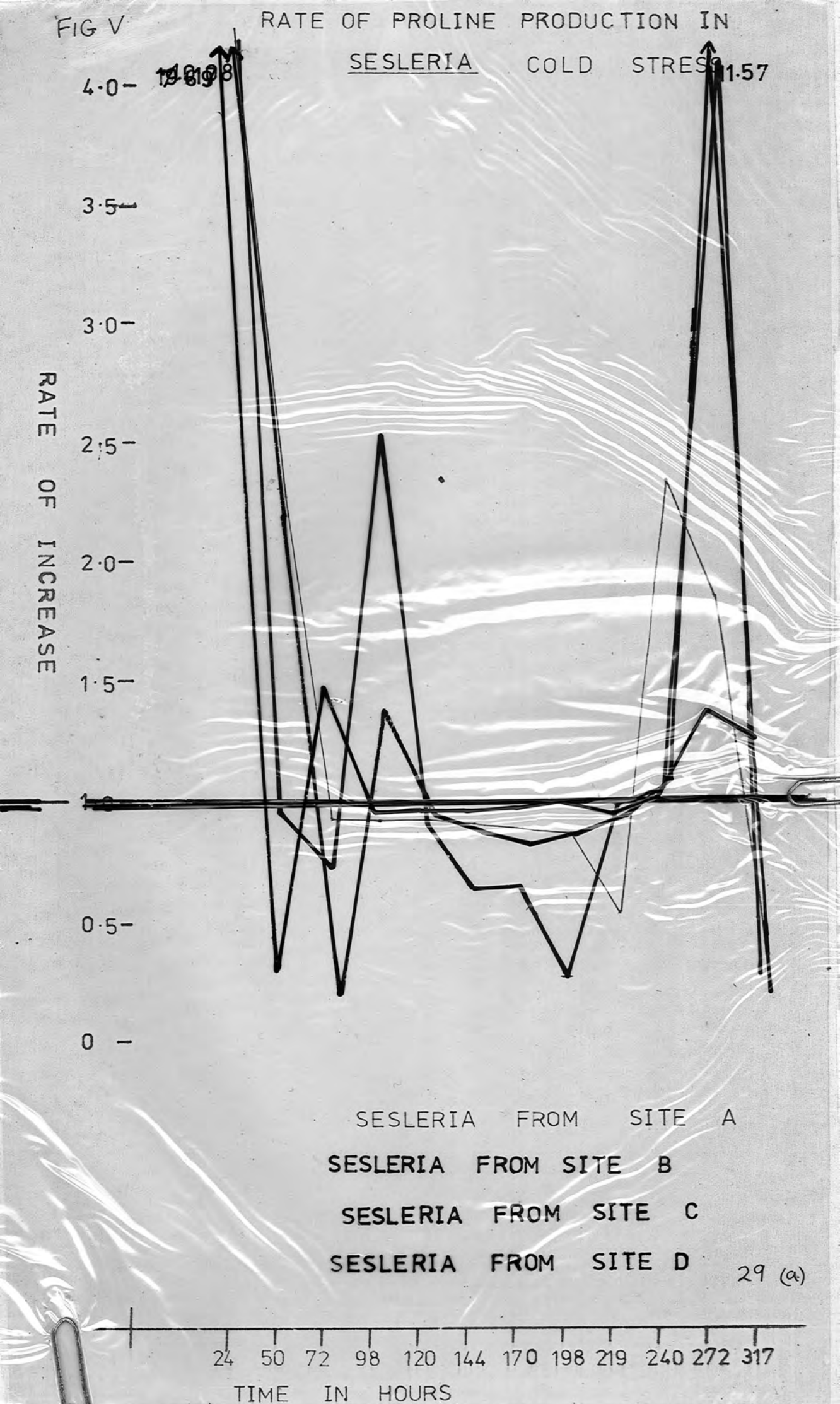
Time in hours	Site A	Site B	Site C	Site D
24	10.33	10.05	7.63	19.10
50	2.28	0.95	2.25	0.30
72	0.93	0.71	0.20	1.47
98	0.92	2.54	1.4	0.96
120	0.93	0.90	0.94	0.98
144	0.91	0.63	0.91	0.96
170	0.90	0.64	0.8	0.96
198	0.87	0.26	0.88	1.0
219	0.53	1.0	0.95	0.96
240 returned to 20°C	2.34	1.05	1.1	1
272	1.86	4.15	11.68	1.36
317	0.40	0.28	0.19	1.27

See fig V - page 29(a)

A similar depression of the cyclical nature of proline production is obtained from the drought stress experiments involving shallow soils. Here it is suspected that the

FIG V

RATE OF PROLINE PRODUCTION IN SESLERIA COLD STRESS



SESLERIA FROM SITE A

SESLERIA FROM SITE B

SESLERIA FROM SITE C

SESLERIA FROM SITE D

29 (a)

reduced rooting capacity combined with the lower total moisture content in the soil combine to limit the metabolic processes involved more severely than occurs in deeper rooted Sesleria plants.

TABLE (XV)

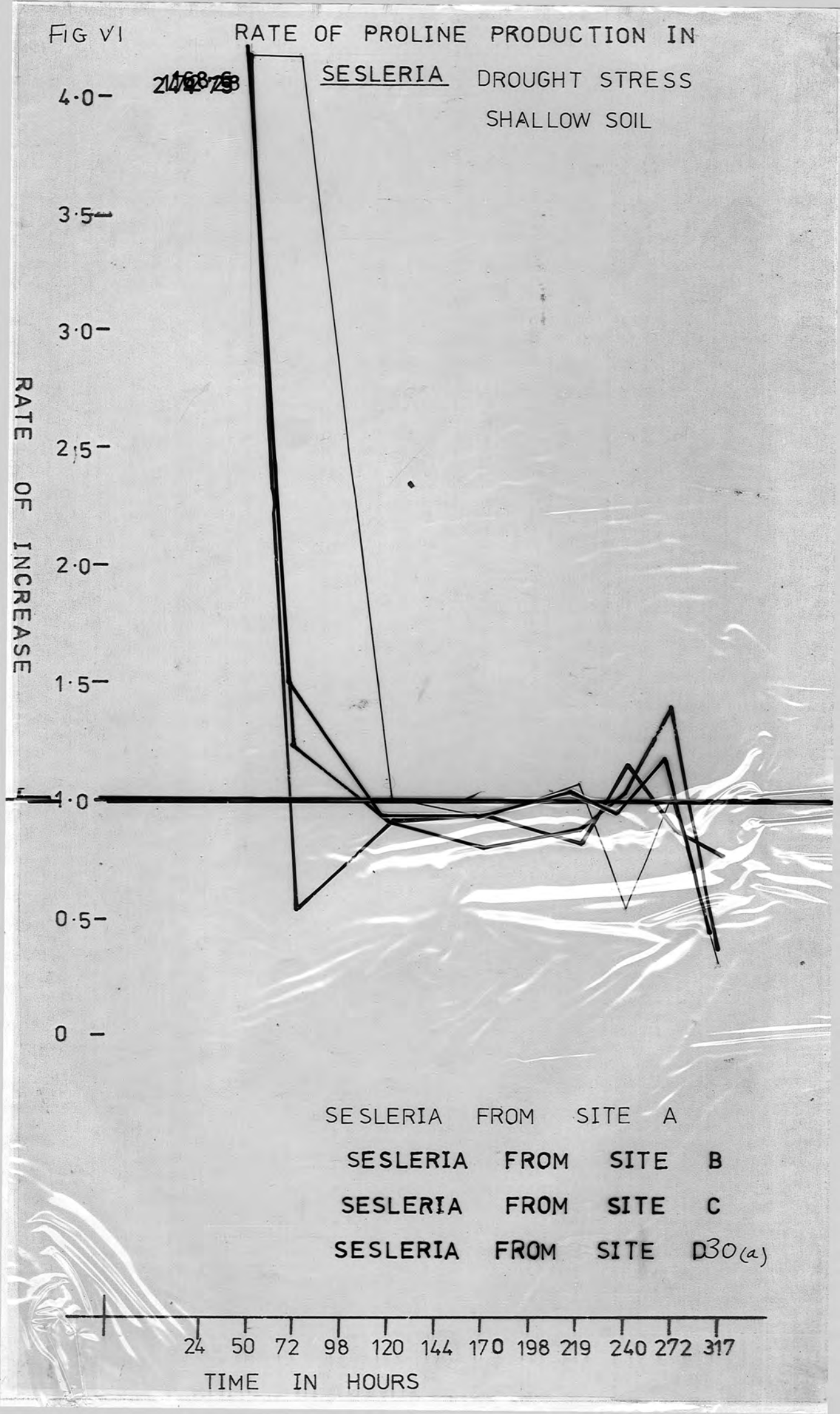
RATES OF INCREASE IN PROLINE LEVELS OF PLANTS SUBJECTED TO DROUGHT STRESS IN SHALLOW SOIL AT 20°C.

Time in hours	Site A	Site B	Site C	Site D
50	53.08	244.75	172.28	168.6
72	5.97	0.56	1.24	1.51
120	1.03	0.93	0.91	0.93
170	0.94	0.95	0.80	0.93
219	1.09	0.82	0.88	1.03
240 (watered)	0.54	1.16	1.03	0.95
276	1.03	0.89	1.41	1.18
317	0.31	0.76	0.37	0.42

see Fig. (VI) page 30(a)

The location of the peaks and troughs in proline production indicates a differential response of Sesleria plants from the four sites to continued stress. This is particularly noticeable when the levels of free proline are plotted in connection with ψ_L potential as shown in Fig. (V) page 29(a). This information clearly shows that Sesleria plants taken from Site C reach their maximum proline level at a lesser stage of dessication than the other sites, even though this maximum is less than that eventually obtained by Sites A, B and D. A result which lends support to the hypothesis that it is possible for the rate of proline

FIG VI


RATE OF PROLINE PRODUCTION IN
SESLERIA DROUGHT STRESS
SHALLOW SOIL
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
RATE OF INCREASE
SESLERIA FROM SITE A
SESLERIA FROM SITE B
SESLERIA FROM SITE C
SESLERIA FROM SITE D30(a)
24 50 72 98 120 144 170 198 219 240 272 317
TIME IN HOURS

production to be of more importance in terms of stress avoidance and/or resistance than the absolute value of proline obtained after a sustained stress period.

In an attempt to discover preliminary evidence to indicate the causal or non-causal relationship between proline and resistance, the results from the Ca stress experiments have proved enlightening.

TABLE (XVI)

MEAN VALUES OF PROLINE PRODUCED BY 4 PLANT SPECIES IN RELATION TO CALCIUM STRESS

Plant species	Ca soil content in % by weight	$\bar{x}$ level of free proline in mm/g F.W.
Phleum pratense L.	0.14	11.48
" "	5	10.50
" "	12	9.03
Agropyron caninum	0.14	25.09
" "	5	16.22
" "	12	18.38
Sedge species	0.14	5.71
	5	6.58
	12	6.03
Sesleria caerulea	0.14	29.30
" "	5	253.14
" "	12	138.39

CHAPTER V

DISCUSSION

Genecological diversity within a species population depends upon the three Turessonian propositions:

1) That a plant species with an extensive distribution pattern exhibits spatial variation in morphological and physiological characters
11) This variation shows a strong correlation with habital differences
111) That such ecologically related variability within the species is not simply due to genetic plasticity inherent in the species population as a whole - Turesson (1922) (1925) (1930)

Morphological variation was observed in Sesleria plants studied at thefour sites in Cassop Vale, although the conclusions of the present author were similar to those proposed by West (1975). That is edaphic conditions of the habitat were of major importance in creating morphological differences between sites rather than the development of particular ecotypes. Sesleria morphology was closely related to soil depth with stunted plants bearing shorter inflorescences being found on the shallow soil associated with Site C. This particular population also bore narrower leaves than any of those growing on deeper soil, a feature suggested as being important on dry limestone soils by Turesson (1925) and given wider appreciability to Xerophitic conditions in general by Stocker (1960).

Soil water content however did not give as strong a correlation with certain morphological features such as plant height as was obtained when considering soil depth. It is therefore considered that rooting depth is a more

important factor influencing morphological expression than absolute values of the soil water content.

This is confirmed by the findings of Todd, Ingram and Stutte (1962), who observed that soil moisture levels between 8 and 30% caused no significant changes in the relative turgidity of a wide range of cereal plants. Such changes only taking place when the soil moisture content dropped below 6%.

Stomatal structure on the other hand bore a close relationship to soil water content with plants from drier areas having a greater frequency of occurrence of Stomata to wetter areas, i.e. mean values of 380 stomata /mm^2 and 250 stomata /mm^2 respectively.

The morphological variability as observed in new leaves was depressed when the Sesleria plants from all sites were cultured under uniform laboratory conditions. Thus, there is insufficient evidence from anatomical observation to confirm possible genotypic variation between sites.

This study therefore concerned itself with a more detailed analysis of the physiological response of the Sesleria populations in relation to stress and specifically with regard to their accumulation of free Proline. The relationship between possible ectotypic variation and proline production was based on the two a priori

i) that proline levels are closely correlated to the severity of the imposed stress factor

ii) that the range of possible proline production is governed genetically.

WATER STRESS AND SESLERIA CAERULEA

The water balance in the field is summarised by Hillel and Rawitz (1972)*1 as consisting of the following elements.

$$(P + I) - [R + D + C + (E + T)] = \Delta s$$

where P is equal to precipitation; I, irrigation; R, runoff; D, flow through the root zone; C, the amount of water incorporated in the plants; E, direct evaporation from the soil; T, the water lost in transpiration; and Δs, the change in water stored in the root zone. The shallow soil present at site C created a potentially more intensive drought area for the vegative cover as it was located on the top of a porous limestone ridge where loss from drainage would be high, and flow through the root zone in the form of capillary rise would be low. Consequently when the plant had been subjected to drought stress in the field those plants at site C exhibited larger increases in free proline levels per unit weight. This was still evident when proline values were corrected for dry weight values of Sesleria to compensate for the differential loss of leaf water content at the four sites.

The large increases in free amino acids under field drought stress were not suspected to be primarily due to leaf protein hydrolysis, as the changes in free amino acids is not uniform. Barnett and Naylor (1966), Routley (1966), Steward et al (1966), Also the work of Steward et al (1966) showed that proline accumulation in wilting plant material could be prevented by the addition of arsenate, arsenite flouracetate, iodoacetate and iodoacetamide which blocks new synthesis

*1 in Kozlowski

or proline by inhibiting glycolysis and the tricarboxylic acid cycle.

The synthesis of free proline in plants maintained both in the laboratory and in the field corroborated such a hypothesis of free proline accumulation being closely correlated with the sugar content of the Sesleria plants. This result is comparable to that obtained by Steward et al (1966) who observed such a relationship in a wide range of plant species.

Drought stress thus seems to result in the synthesis of up to 383 fold increases in free proline levels; the exact level for a particular site being determined largely by the ψ leaf (leaf water potential). As the cell water content drops there is a corresponding increase in the amount of detectable free proline. Increase in free proline during drought stress has been well documented. Wample and Bewley (1975) observed that sunflower plants accumulated proline in both its aerial and subterranean parts when subjected to water stress. Chu et al (1974) discovered that Radish plants, which had water withheld for up to 72 hours, accumulated proline as the ψ L declined.

Period of Stress in hours	Water Stress in (bars).	Proline in mg/g dry weight
0	-5.8	0.4
48	-9.4	2.9
60	-24.2	10.0
72	-33.9	17.2

Barnett (1966) detected 10 to a 100 fold increases in proline in Cynodon dactylon when subjected to water stress of between -15 and -30 bars, and Singh (1973) observed 16 fold increases in leaves of barley plants at - 16 bars ψ L.

In these experiments ψ L measurements indicated increased proline production to $\simeq$ -32 bars. The lack of continued proline production beyond this value may largely be described by referring to the correlation obtained at this point between dropping sugar content and decrease in proline. Indeed such a correlation has already been described by Stewart et al (1966) working on excised bean leaves, who proposed that the role of sugar was to furnish α Ketoglutanate and NADPH essential for proline production to proceed.

Such an hypothesis can be easily summarised diagramatically, see Fig. VII, page 36

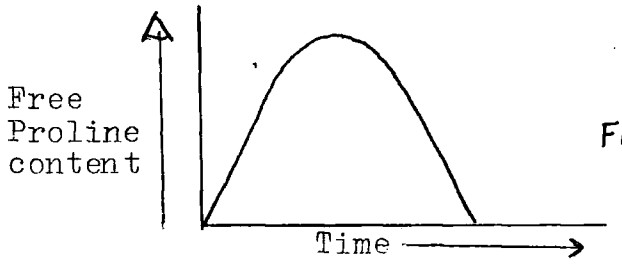


FIG VII

This general trend has been observed by a number of other researchers. Thompson et at (1966) whilst working on turnips recorded a similar relationship during a sustained stress period. In fact some years before, this trend had already been documented by Kemble and Macpherson (1954) when working with ryegrass.

A simple schematic representation as outlined in Fig. VII is, however, inadequate in explaining the

results obtained during the laboratory investigations into drought stress on Sesleria. The present author's findings indicated a more cyclical pattern with regard to proline production utilization and removal.

This pattern coincides with that expected in Stocker's (1960) advocation of activation, reaction and restitution phases of drought response, and upheld by Chen et al (1964) in their work on rough lemon and sweet lime citrus fruits.

In these terms Sesleria when subjected to drought stress enters an activation phase or period of rapid increase in proline synthesis which is detectable within 24 hours. Subsequently utilization and removal processes proceed more rapidly and there is a net reduction in proline content - the reaction phase. Even during this phase it is important to note that there is only a loss in proline relative to the highest values obtained and not a return to the low levels of proline observed in the absence of stress conditions. The restitution phase brings with it increasing levels of free amino acid with decreasing ψ_L until this process is apparently limited by a sugar shortage brought about by depressed photosynthetic activity through shortage of water.

At this point in time if the stress conditions continue then there is a progressive reduction in proline as the reduction processes proceed more rapidly than the now sugar limited rate of synthesis. Such reduction appears to continue steadily.

If the stress conditions are relieved by watering

them, in a seemingly anomalous fashion, proline synthesis is accelerated or conversely the reduction processes decelerated such that the net result is an increase in free proline. A similar result was obtained by Wample and Bewley (1975), who found that when sunflower plants were wilted in the light and then watered and allowed to recover, proline accumulation doubled in the aerial parts within the first 12 hours after watering. It seems that this is yet another indication of the non-specific response of proline production to stress phenomena.

Waldren and Teare (1974) maintain that proline production is a poor indicator of stress conditions as its accumulation takes place after critical conditions have been reached. This is contradictory to the results obtained in this investigation. Both in the field and under laboratory conditions the present author observed large and frequently rapid increases in proline levels in plants subjected to stress conditions. Furthermore, even after a sustained period of 10 days drought stress in shallow soils there was a 64% recovery of the total number of experimental Sesleria plants, a clear indication that the high proline levels produced under these conditions did not take place after the critical survival period had been reached.

In addition plants from the four sites under consideration exhibited a slight staggering of their response to drought stress, by producing proline at differing ψ_L values. Sesleria plants from site C, potentially the most drought susceptible site, produced

their highest levels of free amino acid at relatively lower ψ_L values than plants from sites A, B and D. If the relationship between proline production and drought stress resistance was causal then it would be easy to see how the Sesleria plants at Site C had developed increased survival potential by accelerated proline production. See FIG VIII P 39(a)

Singh et al (1973) experimenting with 14 different barley varieties grown in a controlled soil environment recorded that those which accumulated larger concentrations of free proline tended to have leaves which survived extreme water stress more readily and which grew faster on the relief of stress. Even so he was unable to confirm whether this was a precise causal relationship. Similarly Stewart and Lee (1974) could not confirm the causal relationship between salinity stress and proline production in Armeria maritinia, when they observed that this species developed higher proline levels in coastal regions than in mountain ones.

If a causal relationship between proline production and stress resistance could be confirmed then the faster rate of production of plants from Site C in the laboratory, and the higher levels of proline produced by Sesleria in the field at Site C would suggest a survival advantage of this possible ecotype to drought stress conditions. The calcium stress experiments give some clearer indications with regard to the relationship of proline synthesis and stress avoidance/resistance.

CALCIUM STRESS AND SESLERIA CAERULEA

In the British Isles Sesleria grows on calcareous soils only in the North and extreme West (in Ireland) see

FIG VIII

PROLINE PRODUCTION IN RELATION TO

LEAF WATER POTENTIAL AT SITES A-D

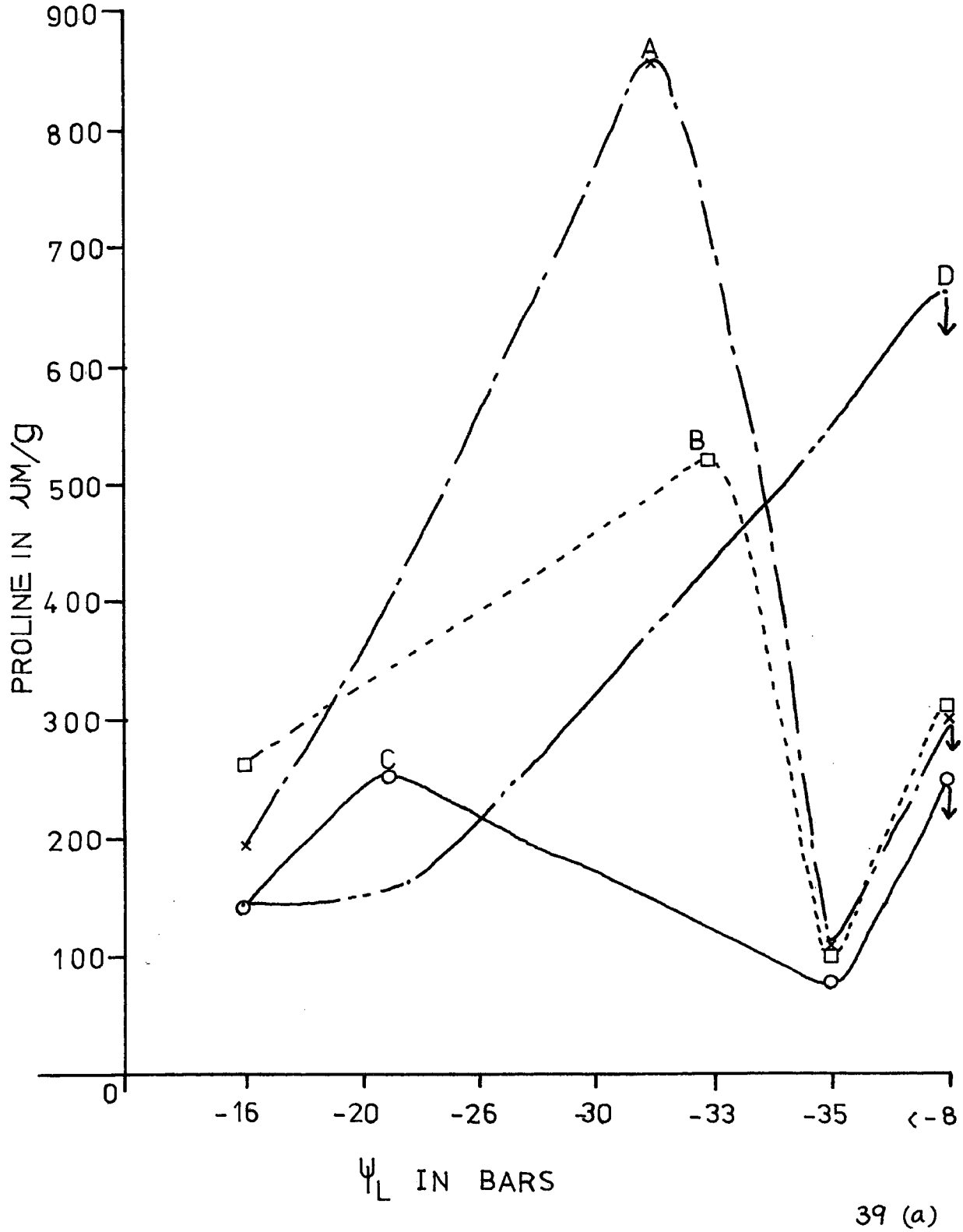


Fig. I - page 3 . Thus it seems that Sesleria's tolerance of calcium ions combined with the colder weather and therefore slower growing conditions give it a competitive advantage in this particular habitat type. Whilst Sesleria cannot be termed a true calcicole, nevertheless as Sutcliffe (1962) points out its physiology must be at least comparable to calcicolous plants in that it must possess the ability to either suppress calcium absorption or have the capacity to transport the calcium rapidly to inactive centres before enzyme systems are blocked.

A plant species with these advantages would therefore be tolerant of Calcium ions at levels which would produce severe stress to non tolerant calcium susceptible plants or calcifuges. If proline was produced in accordance with the degree of stress being sustained, i.e. if a non-causal relationship between proline and stress tolerance existed, then one might expect higher proline levels with increasing Ca concentration in the plants not regularly associated with the magnesian limestone belt.

Alternatively if a causal relationship existed and proline provided a survival davantage to the plant then one might expect higher proline levels with increasing Ca concentration in Sesleria.

The results show the latter possibility to be the case. Sesleria plants produced over an 8 fold increase in proline when grown in soil enriched with Calcium to 5% by weight as opposed to when grown in soil containing 0.14% Ca by weight ($P < 0.001$). Phleum pratense L and Agrophyron caninum actually exhibited decreased proline levels with increased Calcium levels whilst the sedge, a fringe species

on magnesium limestone, increased its proline by a mere 13%.

Whilst this experiment gives a useful insight into the probable causal relationship of proline to stress tolerance, further studies in calcium ion availability must be undertaken as the present study only dealt with total concentrations.

COLD STRESS AND SESLERIA CAERULEA

A certain degree of controversy surrounds the question of low temperature stress. Chu et al (1974) maintain that in their experiments the accumulation of proline during cold temperature treatment at 5°C was unrelated to changes in ψ_L. Whilst the work of Palfi and Juharz (1970) is based solely on the assumption that the effect of low temperature treatment is to create a physiological drought situation by restricting water uptake and transport to intensely transpiring shoots.

It is undoubtedly true that low environmental temperature can lower the availability of water in the soil and restrict its movement, thereby resulting in a lowering of the ψ_L. Levitt (1956) referring to the difficulty in isolating the effect of temperature on metabolism from the concommitant changes in the ψ_L suggested the possible interrelated nature of the plant's resistance to cold, heat and water stress.

The present author observed similar findings to Chu et al, however, that is a temperature of 5°C had an inconsistent affect on ψ_L, producing leaf water

deficit values of only approximately 3.8 to -8 bars. Thus it appeared in this study that temperature was the major contributing stress factor.

The Sesleria plants in response to the low temperature conditions produced up to 23.5 fold increases in proline with the highest value reached being 203 mm/g, there being a significant difference ($P < .001$) in the rates of proline production between Sesleria plants from different sites.

Proline production increased on being returned to 20°C a comparable result to that obtained by Chu et al (1974)working on barley. Barley plants subjected to a temperature of 5°C accumulated proline after 24 hours and continued for at least the following four days at a rate of 74 mg $(g \text{ dry wt})^{-1}$ hr^{-1}. On returning to 20°c increased proline production was observed for a further 24 hours.

CHAPTER VI

SUMMARY

Proline production is Sesleria appears to take place in response to a wide variety of stress phenomena. Within any one species population there is a considerable dggree of variability in connection with proline production as a stress response. Despite this, however, it is still possible to detect a noteable variation in stress response between different populations in the field. These differences in physiological response to stress may be genetically maintained in the form of ecotypes or may be a further expression of the plants genetic plasticity, which takes a longer period of time to be nullified than the morphological plasticity observed in Sesleria populations.

Proline production in Sesleria was found to alter continually both in laboratory experiments and under field conditions and preliminary experiments have indicated that the relationship between proline and stress tolerance may be causal, proline being of significance in terms of survival, during periods of stress. It is still uncertain, however, whether the rate of proline production or the level of proline produced is of more significance in this respect.

Morpholdgical development may be considered as a more unusual stress phenomenon which initiates a proline response along with the more conventional factors of drought and low temperature. The withholding of water from Sesleria rooted in deep soil produces a cycle of

proline production and reduction, this cycle being depressed when low temperature treatment or drought stress in shallow soil is experienced by the plant. It is not known whether there is a daily periodical cycle of proline production in Sesleria.

The present author has indicated the nature of proline production in Sesleria caerulea and pointed to the possible role of proline as being of competitive advantage in the magnesium limestone environment where it flourishes.

APPENDIX 1 : CLIMATIC DATA FOR DURHAM 1.4.1976 TO 31.7.1976.

	RAINFALL IN mm		RAINFALL IN mm
APRIL 1st	11.3	MAY 1st	2.3
" 2nd	6.1	" 2nd	1.6
" 3rd	2.0	" 3rd	2.6
" 4th	0	" 4th	3.1
" 5th	0	" 5th	0.2
" 6th	0	" 6th	0.7
" 7th	0	" 7th	1.9
" 8th	1.1	" 8th	0
" 9th	0	" 9th	0.1
" 10th	0	" 10th	Tr
" 11th	4.0	" 11th	3.1
" 12th	0.1	" 12th	3.6
" 13th	3.1	" 13th	0.1
" 14th	2.0	" 14th	0
" 15th	Tr	" 15th	2.9
" 16th	0	" 16th	0
" 17th	0.6	" 17th	1.3
" 18th	0.2	" 18th	4.4
" 19th	0	" 19th	1.6
" 20th	0	" 20th	4.6
" 21st	Tr	" 21st	0
" 22nd	0	" 22nd	0.2
" 23rd	0	" 23rd	0.1
" 24th	Tr	" 24th	0
" 25th	0	" 25th	2.3
" 26th	Tr	" 26th	15.4
" 27th	0	" 27th	Tr
" 28th	0	" 28th	0.2
" 29th	Tr	" 29th	14.9
" 30th	0.2	" 30th	1.8
		" 31st	6.8

	RAINFALL IN mm		RAINFALL IN mm
JUNE 1st	17.4	JULY 1st	0
" 2nd	Tr	" 2nd	0
" 3rd	0	" 3rd	Tr
" 4th	0	" 4th	0
" 5th	0	" 5th	0
" 6th	0	" 6th	0
" 7th	Tr	" 7th	0
" 8th	0	" 8th	0
" 9th	Tr	" 9th	0.2
" 10th	0.1	" 10th	0
" 11th	1.3	" 11th	Tr
" 12th	0	" 12th	14.2
" 13th	0	" 13th	0.1
" 14th	0.5	" 14th	1.1
" 15th	1.9	" 15th	11.5
" 16th	1.1	" 16th	Tr
" 17th	1.0	" 17th	0
" 18th	1.4	" 18th	0
" 19th	0.2	" 19th	0.1
" 20th	0	" 20th	1.7
" 21st	0	" 21st	Tr
" 22nd	0	" 22nd	0
" 23rd	0	" 23rd	0
" 24th	0	" 24th	0
" 25th	0	" 25th	0
" 26th	0	" 26th	0
" 27th	0	" 27th	0
" 28th	0	" 28th	0
" 29th	0	" 29th	0
" 30th	0	" 30th	1.1
		" 31st	0

NOTE : Tr = Trace.

APPENDIX II

OSMOTIC PRESSURES OF CANE SUGAR SOLUTIONS, CONVERTED FROM URSPRUNG AND BLUM (1916)

Mol cane sugar in 1 litre of solution	Osmotic pressure at 20° C in bars	Mol cane sugar in 1 litre of solution	Osmotic pressure at 20°C in bars
0.010	0.268	0.29	7.942
0.020	0.535	0.3	8.236
0.030	0.804	0.31	8.531
0.040	1.071	0.32	8.826
0.050	1.338	0.33	9.121
0.060	1.607	0.34	9.416
0.070	1.874	0.35	9.711
0.080	2.142	0.36	10.005
0.090	2.410	0.369	10.271
0.098	2.624	0.37	10.303
0.1	2.678	0.38	10.941
0.12	3.211	0.4	11.259
0.13	3.477	0.41	11.578
0.14	3.744	0.42	11.896
0.15	4.008	0.43	12.215
0.16	4.278	0.44	12.534
0.17	4.544	0.45	12.852
0.18	4.811	0.452	12.916
0.19	5.077	0.46	13.181
0.192	5.131	0.47	13.511
0.2	5.360	0.48	13.841
0.21	5.646	0.49	14.172
0.22	5.932	0.5	14.502
0.23	6.218	0.51	14.831
0.24	6.504	0.52	15.162
0.25	6.791	0.53	15.492
0.26	7.076	0.533	15.591
0.27	7.362	0.54	15.843
0.28	7.649	0.55	16.204
0.282	7.705	0.56	16.565

Mol cane sugar in 1 litre of solution	Osmotic pressure at 20° C in bars	Mol cane sugar in 1 litre of solution	Osmotic pressure at 20° C in bars
0.570	16.926	0.740	23.357
0.580	17.286	0.750	23.754
0.590	17.646	0.757	24.030
0.600	18.007	0.760	24.159
0.610	18.367	0.770	24.587
0.620	18.742	0.780	25.017
0.630	19.118	0.790	25.446
0.640	19.493	0.800	25.874
0.650	19.868	0.810	26.304
0.660	20.243	0.820	26.732
0.670	20.619	0.826	26.990
0.680	20.994	0.878	29.109
0.685	21.181	1.229	47.813
0.690	21.379	1.580	73.417
0.700	21.775	1.931	109.588
0.710	22.170	2.195	145.435
0.720	22.566	2.485	198.993
0.730	22.962		

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE NON-FLOWERING PLANTS

SITE A

Wt of Sesleria in mg.	µm Proline	µm Proline/g F.W.
0.265	1.8	6.79
0.193	6.4	33.16
0.162	2.4	14.8
0.192	1.1	5.72
0.122	1.5.	12.29
0.32	2.3	7.18
0.24	2.2	9.16
0.313	2.3	7.34
0.326	2.2	6.75
0.281	2.2	7.82
0.177	1.5	8.45
0.118	1.1	9.3
0.237	1.8	7.6
0.190	1.6	8.4
0.281	2.3	8.2
0.211	1.3	6.16
0.229	1.7	7.42
0.252	2.4	9.5
0.242	5.6	23.14
0.242	2.5	10.3
0.225	2.3	10.2
0.209	1.9	9.1
0.236	2.0	8.45
0.291	2.1	7.21
0.215	1.6	7.44
0.183	1.5	8.2
0.238	2.3	9.65
0.23	2.4	10.4
0.142	1.2	8.45
0.246	1.8	7.31

SITE A - continued

Wt of Sesleria in mg.	µm Proline	µm Proline/g F.W.
0.121	1.1	9.1
0.206	2.4	11.65
0.145	1.5	10.32
0.283	2.2	7.77
0.244	2.3	9.42
0.248	1.9	7.65
0.173	1.7	9.8
0.190	1.5	7.92
0.265	2.3	8.66
0.293	2.2	7.5

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE NON-FLOWERING PLANTS

SITE B

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.314	4.6	14.64
0.238	3.1	13.02
0,32	3.6	11.25
0.206	3.3	16.01
0.224	3.5	15.62
0.328	4.2	12.8
0.222	2.4	10.81
0.278	2.9	10.41
0.299	3.4	11.35
0.281	4.1	14.6
0.233	3.6	15.45
0.211	3.4	16.1
0.290	3.7	12.8
0.279	3.2	11.44
0.211	3.1	14.67
0.314	3.9	12.4
0.280	4.0	14.31
0.238	3.5	14.66
0.265	3.7	24.0
0.237	3.2	13.51
0.252	2.8	11.12
0.340	4.1	12.06
0.272	3.9	14.31
0.264	3.7	14.01
0.274	3.2	11.65
0.227	3.5	15.36
0.267	4.1	15.31
0.254	3.7	14.6
0.180	2.7	14.98
0.225	3.5	16.55
0.326	3.6	11.02

SITE B continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.201	2.5	12.41
0.257	3.8	14.8
0.280	4.2	15.01
0.238	2.9	12.17
0.231	3.3	14.24
0.215	2.3	10.66
0.280	3.5	12.48
0.230	3.0	13.01

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE NON-FLOWERING PLANTS

SITE C

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.301	1.5	4.98
0.269	1.6	5.94
0.17	2.1	12.35
0.222	1.2	5.40
0.19	2.0	10.52
0.27	2.9	10.74
0.20	0.7	3.5
0.272	0.9	3.31
0.201	2.2	10.98
0.233	2.8	12.02
0.296	1.7	5.76
0.273	1.5	5.51
0.317	1.6	5.04
0.217	2.0	9.21
0.396	2.1	5.31
0.369	1.5	4.07
0.195	2.2	11.25
0.233	2.5	10.71
0.224	1.8	8.04
0.187	2.1	11.21
0.200	1.3	6.51
0.281	1.7	6.06
0.267	2.0	7.5
0.264	0.9	3.42
0.226	2.3	10.16
0.238	1.2	5.05
0.321	1.4	4.37
0.223	1.6	7.2
0.250	2.5	10.02
0.168	0.8	4.76
0.144	1.7	11.81

SITE C continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.349	1.8	5.16
0.318	1.3	4.09
0.211	2.7	12.77
0.279	2.1	7.52
0.288	1.9	6.6
0.199	1.7	8.52
0.232	2.1	9.04
0.255	2.7	10.6

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE NON-FLOWERING PLANTS

SITE D

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.21	0.3	1.42
0.276	11.3	40.94
0.135	0.3	2.22
0.229	7.8	34.06
0.304	14.4	47.36
0.28	4.2	15.00
0.21	2.2	10.47
0.19	2.1	11.05
0.258	3.7	14.33
0.223	8.4	37.6
0.198	7.8	39.35
0.193	2.3	11.91
0.273	4.1	15.01
0.212	3.9	18.35
0.186	1.5	8.06
0.39I	0.9	2.3
0.243	4.0	16.43
0.168	1.9	11.29
0.133	2.2	16.46
0.270	10.8	40.01
0.318	1.7	5.35
0.117	1.5	12.72
0.225	6.8	30.18
0.185	7.5	40.35
0.310	0.8	1.61
0.218	2.7	12.36
0.255	3.8	14.85
0.233	4.6	19.7
0.231	8.4	36.3
0.249	11.1	44.48
0.227	2.3	10.12

Site D continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.129	4.7	36.26
0.308	3.4	11.04
0.268	0.7	2.61
0.14I	1.6	11.35
0.12I	1.9	15.69
0.157	2.2	14.01
0.159	2.6	16.34
0.189	9.1	48.19
0.166	6.5	39.21

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE FLOWERING PLANTS

SITE A

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.27	1.1	4.07
0.224	1.2	5.35
0.284	0.8	2.81
0.15	3.3	22.0
0.21	4.5	21.42
0.12	2	16.66
0.193	0.7	3.62
0.173	2.6	15.02
0.389	1.7	4.36
0.399	2.2	5.51
0.185	3.4	18.33
0.171	3.7	21.61
0.297	0.7	2.36
0.181	3.6	19.84
0.256	1.1	4.3
0.246	1.5	6.1
0.193	1.8	9.35
0.288	2.9	10.06
0.205	3.1	15.11
0.164	2.7	16.49
0.178	1.3	7.31
0.362	1.1	3.04
0.215	4.1	19.1
0.164	3.6	22.01
0.187	3.9	20.91
0.307	1.3	4.24
0.388	1.2	3.09
0.099	1.6	16.1
0.360	0.9	2.5
0.115	2.1	18.3
0.120	2.6	21.6

Site A continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.196	1.1	5.61
0.140	2.3	16.42
0.238	0.8	3.36
0.235	0.7	2.98
0.184	3.4	18.44
0.127	0.3	2.36
0.253	2.3	9.08
0.162	2.5	15.41
0.138	2.4	17.37

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE FLOWERING PLANTS

SITE B

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.264	2.7	10.22
0.29	2.6	8.96
0.26	3.5	13.46
0.254	3.5	13.77
0.246	3.0	12.19
0.236	3.6	15.25
0.235	3.2	13.61
0.39	4.7	12.56
0.338	4.6	13.6
0.257	2.7	10.49
0.198	2.3	11.61
0.238	3.I	13.04
0.285	2.I	7.36
0.265	2.7	10.21
0.271	3.I	11.46
0.308	3.3	10.73
0.273	2.8	10.24
0.263	2.3	8.76
0.243	2.9	11.95
0.251	3,1	12.36
0.273	3.4	12.47
0.224	2.7	12.03
0.267	3.6	13.48
0.227	2.6	11.46
0.297	3.4	11.44
0.267	3.3	12.36
0.242	2.8	11.55
0.285	3.7	12.98
0.240	3.6	15.01
0.237	3.4	14.36
0.25	2.1	8.4

SITE B - continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.222	2.3	10.36
0.187	2.7	14.44
0.251	3.1	12.36
0.235	3.0	18.76
0.246	2.8	11.39
0.245	3.3	13.45
0.252	3.8	15.06
0.215	3.1	14.46
0.199	2.6	13.07

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE FLOWERING PLANTS

SITE C

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.263	0.2	0.76
0.176	1.4	7.95
0.273	2.2	8.05
0.25	1.2	4.8
0.137	2	14.5
0.121	1.2	9.91
0.14	2.7	19.28
0.125	1.1	8.8
0.270	1.9	7.05
0.213	2.2	10.31
0.209	2.5	11.97
0.265	1.1	4.15
0.388	1.3	3.35
0.218	2.6	11.93
0.251	2.1	8.36
0.325	2.4	7.39
0.201	1.8	8.97
0.190	1.6	8.43
0.165	0.9	5.46
0.138	0.8	5.81
0.252	1.1	4.36
0.349	0.3	0.86
0.203	1.7	8.36
0.285	2.2	7.71
0.281	1.3	4.63
0.293	1.7	5.8
0.230	1.8	7.83
0.154	1.3	8.46
0.293	2.2	9.23
0.156	2.7	17.36
0.173	1.4	8.08

Site C - continued

Wt of *Sesleria* mg	µm proline	µm proline/g F.W.
0.209	1.7	8.13
0.275	1.2	4.36
0.189	2.6	13.76
0.185	2.6	14.03
0.214	1.6	7.49
0.203	1.7	8.36
0.297	1.3	4.37
0.179	1.4	7.05
0.194	1.3	6.69

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM THE LEAVES OF MATURE FLOWERING PLANTS

SITE D

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.197	5.3	26.90
0.273	6.5	23.8
0.26	7.5	28.84
0.294	10.1	34.35
0.34	9.1	26.76
0.253	11.5	44.92
0.242	10.4	42.97
0.300	10.3	34.37
0.285	7.6	26.66
0.256	10.1	39.42
0.278	7.5	25.98
0.278	7.9	28.37
0.274	8.3	30.24
0.293	9.1	31.06
0.239	10.1	42.29
0.249	10.4	41.73
0.228	7.6	33.39
0.218	7.5	34.46
0.202	5.6	27.73
0.222	6.3	28.43
0,280	11.5	41.06
0.249	9.6	38.49
0.282	11.1	39.37
0.335	9.1	27.16
0.341	9.4	27.55
0.260	8.9	34.30
0.247	9.3	37.61
0.260	10.1	38.9
0.220	10.8	40.02
0.309	8.6	27.84
0.281	7.5	26.69

Site D -continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.279	9.3	33.3
0.296	10.1	34.09
0.262	10.3	39.37
0.266	11.0	41.4
0.271	11.1	40.9
0.296	7.9	26.73
0.266	7.8	29.37
0.309	9.3	30.13

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM FLOWERING PARTS OF THE PLANTS

SITE A

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.13	3.6	27.67
0.05	1.6	32
0.144	2.7	18.75
0.105	5.7	54.28
0.071	4.2	59.15
0.066	1.6	24.24
0.06	1.1	18.33
0.079	1.4	17.72
0.065	1.7	26.3
0.096	5.6	58.5
0.069	1.3	18.79
0.064	1.1	17.21
0.099	2.4	24.15
0.083	4.6	55.2
0.089	2.3	25.87
0.109	2.4	22.05
0.073	1.3	17.71
0.087	1.6	18.45
0.087	5.1	58.61
0.084	2.3	27.75
0.102	2.9	28.48
0.091	5.1	56.21
0.13	3.6	27.66
0.124	2.4	19.37
0.084	4.9	58.3
0.101	2.3	22.7
0.010	2.1	21.05
0.106	1.9	17.93
0.085	5.0	59.01
0.098	2.6	26.68
0.104	2.7	26.04

Site A continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.09	2.3	25.6
0.093	1.7	18.31
0.097	1.9	19.6
0.086	1.5	17.44
0.088	5.2	59.12
0.106	3.0	28.37
0.111	2.1	18.9
0.086	4.6	53.34
0.089	2.6	29.06

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM FLOWERING PARTS OF THE PLANTS

SITE B

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.168	8.1	48.21
0.12	6.0	50
0.143	3.2	22.37
0.134	3.0	22.38
0.10	3.8	38
0.127	5.6	44.09
0.123	5.1	41.46
0.176	7.5	42.6
0.135	3.1	22.7
0.134	3.0	22.4
0.163	3.6	22.06
0.120	5.2	43.21
0.095	3.7	38.97
0.125	6.1	49.01
0.092	3.9	42.36
0.094	3.7	39.44
0.126	5.6	44.37
0.125	5.8	46.29
0.117	5.2	44.36
0.123	5.7	46.21
0.096	3.7	38.66
0.091	3.6	39.4
0.112	5.0	44.7
0.150	3.4	22.7
0.170	7.7	45.24
0.164	7.9	48.06
0.164	8.1	49.35
0.169	7.5	44.37
0.165	6.3	38.29
0.151	5.8	38.33
0.158	3.6	22.83

Site B continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.118	5.5	46.61
0.112	5.1	45.54
0.144	3.3	22.91
0.135	3.1	23.01
0.133	5.5	41.39
0.099	3.9	39.40
0.132	3.0	22.65
0.125	5.0	40.01

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM FLOWERING PARTS OF THE PLANTS

SITE C

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.08	3.1	38.75
0.156	4.5	37.64
0.07	4.0	57.14
0.117	1.1	9.4
0.139	2.9	20.86
0.094	3.5	37.23
0.07	4.5	64.28
0.072	4.6	64.34
0.091	3.50	38.4
0.078	4.4	56.48
0.134	1.3	9.69
0.099	2.5	25.38
0.068	4.1	60.28
0.103	2.5	24.21
0.062	2.3	37.34
0.094	3.6	38.21
0.102	3.8	37.2
0.101	4.0	39.46
0.073	1.7	23.22
0.093	3.2	34.36
0.106	4.1	38.81
0.075	4.3	57.43
0.171	1.6	9.37
0.105	3.8	36.04
0.116	1.1	9.45
0.073	4.3	58.61
0.105	4.1	39.23
0.095	3.6	38.07
0.085	3.2	37.64
0.083	4.7	56.51

Site C - continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.092	2.1	22.86
0.085	3.3	38.85
0.098	3.9	37.66
0.077	4.2	54.44
0.073	4.6	62.21
0.088	3.4	38.66
0.087	3.3	37.74
0.094	2.9	30.78
0.026	2.1	24.35

PROLINE LEVELS IN SESLERIA : RESULTS TAKEN FROM FLOWERING PARTS OF THE PLANTS

SITE D

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.057	3.7	66.007
0.138	6.0	43.47
0.164	6.6	40.24
0.07	1.6	22.85
0.11	6.7	60.90
0.163	15.3	93.86
0.113	3.0	26.54
0.085	3.9	45.88
0.079	3.5	44.36
0.126	6.1	48.47
0.098	6.4	65.09
0.064	3.9	60.81
0.159	14.8	92.87
0.067	1.7	25.56
0.105	2.3	21.83
0.082	3.8	46.18
0.075	3.4	45.37
0.077	3.1	40.18
0.096	2.3	23.97
0.096	6.5	67.94
0.160	15.1	94.12
0.076	3.4	44.53
0.074	3.0	40.79
0.095	6.0	63.19
0.101	6.2	61.28
0.086	3.7	43.17
0.067	3.1	46.61
0.162	15.2	93.71
0.057	1.5	26.47
0.086	1.9	22.01
0.07	3.2	45.58

Site D - continued

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.08	3.3	41.27
0.092	6.0	65.39
0.063	3.8	60.22
0.072	3.1	43.36
0.059	2.5	42.1
0.042	2.0	47.76
0.067	3.1	45.98
0.169	15.4	91.27
0.079	1.6	20.23

PROLINE LEVELS IN SESLERIA AFTER FIELD DROUGHT STRESS

SITE A

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.06	1.66	27.66
0.064	1.77	27.66
0.06	2.91	48.5
0.06	1.75	29.16
0.07	1.95	27.85
0.061	1.69	27.70
0.06	1.65	27.5
0.07	3.55	50.75
0.06	2.9	48.33
0.07	2.48	35.42
0.064	1.84	28.75
0.063	1.75	27.77
0.06	1.67	27.83
0.062	1.71	27.58
0.07	2.48	35.42
0.072	2.61	36.25
0.063	3.09	49.2
0.06	3.03	50.5
0.061	3.09	50.6
0.058	1.9	34.4
0.06	1.67	27.84
0.055	1.59	28.88
0.07	1.95	27.86
0.06	1.65	27.5
0.063	3.2	50.79

PROLINE LEVELS IN SESLERIA AFTER FIELD DROUGHT STRESS

SITE - B

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.086	2.37	27.56
0.052	1.33	25.58
0.093	2.78	29.89
0.084	2.32	27.62
0.071	2.0	28.16
0.066	1.83	27.72
0.062	2.95	47.58
0.075	4.88	65.06
0.057	3.56	62.46
0.061	1.5	24.59
0.075	1.9	25.33
0.072	1.88	26.11
0.061	2.4	39.34
0.062	3.06	49.35
0.064	1.46	22.81
0.062	1.73	27.9
0.061	3.39	66.47
0.058	1.58	27.24
0.079	4.42	55.94
0.083	5.02	60.48
0.081	2.27	28.02
0.054	3.59	66.48
0.092	2.56	27.82
0.066	1.51	22.88
0.058	3.86	66.55

PROLINE LEVELS IN SESLERIA AFTER FIELD DROUGHT STRESS

SITE C

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.041	122.18	2980.0
0.053	7.90	149.06
0.04	4.81	120.35
0.044	5.94	135.0
0.058	8.12	140.0
0.062	9.18	148.07
0.048	124.85	2601.04
0.052	148.2	2850.0
0.05	6.77	135.4
0.041	6.13	149.51
0.061	7.42	121.64
0.044	125.84	2860.0
0.042	5.23	124.52
0.038	97.13	2556.05
0.032	72.62	2269.37
0.046	6.84	148.70
0.051	6.92	135.68
0.055	6.73	122.36
0.043	5.99	139.3
0.052	7.62	146.54
0.033	65.69	1990.61
0.046	6.86	149.13
0.057	164.16	2880.0
0.031	4.36	140.65
0.054	109.1	2020.91

PROLINE LEVELS IN SESLERIA AFTER FIELD DROUGHT STRESS

SITE D

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.07	2.2	31.43
0.038	1.23	32.37
0.052	1.45	27.88
0.13	3.45	26.54
0.064	1.85	28.91
0.063	2.03	32.22
0.066	2.14	32.42
0.081	2.58	31.85
0.053	1.69	31.89
0.048	1.33	27.71
0.061	1.69	27.71
0.066	1.72	26.06
0.048	1.3	27.08
0.061	1.76	28.85
0.072	2.33	32.36
0.058	1.86	32.07
0.066	2.1	31.82
0.06	1.91	31.83
0.13	4.2	32.31
0.038	1.46	30.53
0.065	2.10	32.46
0.069	2.2	31.88
0.062	2.01	32.42
0.064	2.04	31.87
0.06	1.59	26.5

PROLINE LEVELS IN SESLERIA, 24 HOURS AFTER THE RELIEF OF FIELD DROUGHT STRESS BY RAIN

SITE A

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.05	0.34	6.67
0.06	0.39	6.5
0.072	0.49	6.81
0.075	0.49	6.54
0.068	0.46	6.77
0.054	0.37	6.85
0.05	0.32	6.4
0.062	0.41	6.61
0.055	0.37	6.73
0.058	0.38	6.55
0.064	0.42	6.56
0.044	0.29	6.59
0.075	0.51	6.8
0.061	0.42	6.89
0.048	0.32	6.66
0.043	0.29	6.74
0.051	0.33	6.47
0.049	0.32	6.53
0.058	0.39	6.72
0.052	0.34	6.54
0.046	0.31	6.74
0.041	0.28	6.83
0.061	0.42	6.89
0.062	0.41	6.61
0.074	0.49	6.62

PROLINE LEVELS IN SESLERIA, 24 HOURS AFTER THE RELIEF OF FIELD DROUGHT STRESS BY RAIN

SITE B

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.061	0.78	12.79
0.053	0.61	11.51
0.072	0.80	11.11
0.074	0.82	11.08
0.054	0.61	11.30
0.051	0.65	12.75
0.061	0.75	12.30
0.049	0.56	11.43
0.066	0.74	11.21
0.061	0.68	11.15
0.078	0.90	11.54
0.077	0.98	12.73
0.072	0.83	11.53
0.06	0.76	12.67
0.053	0.67	12.64
0.066	0.75	11.36
0.064	0.73	11.41
0.059	0.65	11.02
0.057	0.65	11.40
0.063	0.77	12.22
0.077	0.88	11.43
0.072	0.82	11.39
0.064	0.73	11.41
0.055	0.65	11.82
0.063	0.78	12.39

PROLINE LEVELS IN SESLERIA, 24 HOURS AFTER THE RELIEF OF FIELD DROUGHT STRESS BY RAIN

SITE C

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.041	.42	10.24
0.044	.33	7.5
0.04	.19	4.75
0.051	.25	4.90
0.043	.23	5.35
0.042	.2	4.76
0.049	.25	5.10
0.047	.23	4.89
0.053	.25	4.72
0.055	.26	4.73
0.054	.40	7.41
0.041	.28	6.83
0.046	.28	6.09
0.048	.35	7.29
0.044	.34	7.73
0.057	.46	8.07
0.055	.45	8.18
0.043	.33	7.67
0.047	.48	10.21
0.05	.5	10.00
0.057	.56	9.83
0.058	.57	9.83
0.042	.43	10.24
0.047	.47	10.00
0.041	.38	9.27

PROLINE LEVELS IN SESLERIA, 24 HOURS AFTER THE RELIEF OF FIELD DROUGHT STRESS BY RAIN

SITE D

Wt of Sesleria mg	µm proline	µm proline/g F.W.
0.061	0.35	5.74
0.065	0.5	7.69
0.066	0.44	6.67
0.071	0.41	5.78
0.059	0.4	6.78
0.068	0.46	6.79
0.074	0.55	7.43
0.077	0.57	7.70
0.069	0.4	5.80
0.052	0.31	5.96
0.054	0.41	7.59
0.063	0.41	6.51
0.073	0.53	7.26
0.056	0.43	7.68
0.062	0.36	5.81
0.074	0.56	7.57
0.06	0.35	5.83
0.057	0.34	5.97
0.062	0.4	6.45
0.073	0.49	6.71
0.07	0.48	6.86
0.069	0.44	6.38
0.068	0.44	6.47
0.054	0.38	7.04
0.074	0.53	7.16

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY DROUGHT STRESS EXPERIMENTS IN DEEP SOIL

SITE A

Time in Hours	$\bar{x}$								
24	194.2	170.61	188.3	219.49	208.05	197.17	180.21	196.33	193.44
50	144.44	140.7	155.39	139.06	133.88	144.61	150.09	147.4	142.08
72	75.1	79.43	85.28	63.4	72.2	68.61	80.47	78.63	72.78
98	65.42	69.64	67.6	58.43	70.18	61.8	60.71	70.84	64.16
120	160.2	151.34	158.44	169.12	171.4	159.61	163.60	149.05	159.04
144	250.15	220.7	238.11	291.02	237.35	261.4	268.33	244.36	239.93
170	480.2	440.24	455.2	493.02	498.34	502.43	475.6	496.71	490.06
198	862.5	782.3	846.41	879.4	890.31	865.22	891.6	887.07	857.69
219	105.3	120.2	136.3	99.6	87	128.4	86.5	78.42	105.98
240	100	72.31	84.3	87.9	122.41	131.86	127.5	70.03	103.69
270	288.91	297.12	284.35	327.6	242.61	246.42	279.4	355.38	278.4
317	62.46	68.81	69.5	62.4	76.8	57.4	53.21	49.2	62.36

SITE B

Time in Hours	$\bar{x}$								
24	264.56	279.41	288.3	234.31	298.4	246.5	248.21	219.38	301.97
50	161.76	160.94	173.5	188.46	151.49	154.3	159.71	160.02	145.66
72	72.43	79.8	89.31	60.05	62.25	73.49	78.07	75.19	61.28
98	125.13	122.41	106.89	144.37	136.5	119.39	100.1	124.4	146.98
120	175.24	150.16	148.41	185.37	190.16	142.4	168.91	203.61	212.9
144	228.30	220.44	197.08	184.99	247.36	244.85	261.1	205.51	265.07
170	289.61	298.81	326.4	221.49	233.6	314.67	317.49	276.35	328.07
198	520.09	563.09	548.38	517.46	484.18	493.61	525.6	477.75	550.65
219	115.31	114.37	88.1	89.47	134.6	121.71	120.05	117.4	136.78
240	100.09	97.08	121.15	134.65	84.79	77.83	101.49	120.91	72.82
272	95.46	72.27	87.41	121.36	130.59	116.45	82.48	80.94	72.18
317	308.2	300.46	287.4	293.66	317.46	322.53	318.9	330.24	294.95

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY DROUGHT STRESS EXPERIMENTS IN DEEP SOIL

SITE C

Time in Hours	$\bar{x}$								
24	138.88	127.31	120.48	143.85	151.91	121.16	147.08	141.71	157.54
50	95.21	72.25	108.23	121.43	101.66	88.41	82.81	115.83	71.06
72	140.4	121.41	156.52	161.61	163.09	124.21	130.34	141.33	124.7
98	380.61	419.86	370.67	438.71	376.42	384.5	364.42	369.31	320.99
120	254.95	228.1	251.12	280.6	241.61	247.75	203.84	280.52	306.06
144	185.86	199.06	160.2	158.23	217.4	220.52	195.58	187.81	148.08
170	111.73	90.96	121.04	110.42	136.32	95.13	88.81	127.97	123.19
198	70.81	59.67	71.74	74.71	68.58	77.44	80.64	78.9	54.8
219	74.60	62.9	70.81	71.82	79.4	83.46	68.21	69.46	90.74
240	80.51	73.82	84.75	83.6	80.37	79.37	67.06	85.3	89.81
272	75.71	72.61	70.48	64.46	71.3	80.81	81.29	79.51	85.22
317	249.88	230.71	218.81	274.6	251.72	240.3	243.9	259.08	279.92

SITE D

Time in Hours	$\bar{x}$								
24	140.62	120.42	136.02	148.63	152.41	160.22	141.47	144.16	121.63
50	159.30	142.31	136.41	159.71	168.28	175.41	160.63	157.32	179.33
72	112.21	98.02	111.53	130.86	121.37	87.53	95.46	112.47	140.44
98	61.48	69.94	58.69	46.76	70.4	73.41	66.19	60.65	45.8
120	57.2	73.22	63.72	40.73	66.63	75.43	41.41	51.79	44.67
144	58.16	65.16	60.66	57.41	54.58	50.72	63.82	61.86	51.07
170	58,43	63.68	57.43	50.31	51.41	63.98	61.66	53.24	65.73
198	59.27	63.21	67.07	58.33	53.78	69.67	75.41	41.98	44.71
219	87.44	99.43	118.72	121.74	68.93	69.44	75.24	68.47	77.55
240	205.21	191.1	226.92	250.68	175.81	193.23	185.13	201.22	217.59
272	662,78	678.6	693.01	599.41	601.62	675.03	670.91	681.19	701.95
317	62.5	65.26	63.44	55.52	69.47	71.92	63.84	58.61	51.94

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY DROUGHT STRESS EXPERIMENTS IN SHALLOW SOIL

Site A

Time in Hours	$\bar{x}$								
50	350.3	378.61	350.46	386.33	321.12	301.62	337.28	364.68	362.3
72	2080.9	2099.47	2336.11	1859.26	2236.34	2347.14	2010.13	1829.23	1929.52
120	2150.4	2051.63	2436.9	2361.34	1892.61	1849.99	2450.61	1690.07	2470.05
170	2010.3	2090.07	1869.04	2106.47	1973.49	2065.81	2154.81	2081.91	1740.8
219	2190.6	2238.91	2316.17	2189.82	2051.78	1928.6	1991.49	2204.44	2603.59
240	1180.8	1091.28	1117.62	998.19	1354.32	1279.33	1191.58	984.39	1429.69
272	1220.41	1301.31	1115.97	1279.24	1441.29	1301.45	1014.66	1097.08	1212.28
319	380.5	392.22	320.44	412.07	345.54	338.51	361.2	429.79	444.23

Site B

Time in Hours	$\bar{x}$								
50	2790.1	2841.46	2779.21	2651.40	2668.47	2743.01	2889.28	2901.43	2846.54
72	1562.5	1369.21	1721.91	1654.19	1826.61	1643.43	1321.14	1406.51	1557.0
120	1458.24	1441.78	1769.84	1587.08	1450.32	1327.62	1098.13	1214.67	1776.48
170	1380.81	1247.99	1569.12	1671.47	1422.44	1018.38	1279.69	1375.22	1462.17
219	1125.8	1364.04	1279.62	1124.88	1097.56	1018.74	1065.92	1229.10	826.54
240	1300.13	1401.32	1526.35	1324.91	1307.21	1229.05	1117.31	1301.98	1197.71
272	1160.3	1421.69	1306.76	1098.93	1217.99	1143.57	1018.72	1244.77	829.97
319	880.2	921.45	1019.22	779.64	861.87	784.69	871.62	994.6	868.51

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY DROUGHT STRESS EXPERIMENTS IN SHALLOW SOIL

Site C

Time in Hours	$\bar{x}$								
50	1286.9	1471.06	1306.9	1217.42	1149.28	1037.41	1241.28	1275.97	1595.88
72	1598.12	1484.21	1497.61	1584.51	1591.7	1749.43	1606.34	1714.62	1556.54
120	1453.3	1603.73	1541.33	1309.67	1347.06	1414.47	1464.21	1497.4	1448.53
170	1161.26	1098.42	1146.15	1227.98	1349.45	1406.96	1021.24	1098.72	941.16
219	1018.24	1049.63	1161.72	1213.54	908.95	817.18	1017.99	1143.93	832.98
240	1047.2	1228.28	987.66	779.36	1006.29	1341.48	1223.81	1030.89	779.83
272	1480.68	1343.47	1691.98	1643.12	1228.19	1147.41	1515.97	1481.64	1793.66
319	550.8	487.23	621.13	614.49	574.13	481.32	499.63	575.88	552.59

SITE D

Time in Hours	$\bar{x}$								
50	1129.73	1423.46	1234.98	1119.41	987.69	991.72	1014.89	1174.61	1091.08
72	1708.6	1563.37	1622.21	1894.21	1549.82	1631.07	1876.43	1581.77	1949.92
120	1587.84	1483.32	1698.45	1722.9	1414.78	1379.84	1766.21	1659.8	1577.42
170	1481.4	1372.41	1622.19	1221.59	1391.61	1719.44	1592.46	1246.87	1684.63
219	1530.1	1761.32	1391.28	1274.86	1189.44	1629.61	1362.99	1801.64	1829.66
240	1460.22	1531.74	1398.63	1621.23	1715.28	1229.4	1346.31	1621.45	1217.72
272	1716.3	1698.49	1751.69	1764.08	1682.19	1794.61	1756.99	1842.91	1439.44
319	720.61	699.41	747.60	710.49	739.89	729.66	701.4	742.09	694.34

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY LOW TEMPERATURE STRESS EXPERIMENTS

Site A

Time in Hours	$\bar{x}$								
24	68.18	42.41	59.68	81.69	50.2	76.71	84.02	80.19	70.54
50	155.17	135.22	172.61	168.43	140.23	177.51	149.46	158.43	139.47
72	144.2	130.61	161.34	141.08	157.21	167.2	138.61	129.55	128.0
98	133.31	127.47	148.38	143.21	130.2	131.8	129.37	119.3	136.75
120	124.46	141.24	137.5	121.5	120 .43	119.72	134.7	109.7	110.88
144	113.71	133.24	127.06	113.9	110.81	85.7	93.41	121.29	124.27
170	101.5	90.43	126.3	116.27	111.71	84.8	79.64	105.38	97.47
198	88.43	70.29	93.31	97.46	79.38	84.8	85.76	101.34	95.1
219	47.19	40.77	53.21	48.34	60.4	39.51	44.45	47.27	43.57
240	110.8	90.36	131.71	130.84	87.92	93.28	89.43	141.9	140.96
272	205.36	229.06	233.1	201.5	187.4	194.07	189.21	219.06	189.48
317	81.27	84.36	87.71	101.4	77.27	83.2	85.34	73.08	57.8

Site B

Time in Hours	$\bar{x}$								
24	118.27	116.64	99.81	127.08	119.72	130.41	106.44	100.61	145.45
50	112.34	89.21	95.56	127.81	121.4	118.76	115.68	98.73	131.57
72	80.71	70.36	96.73	85.26	71.33	75.26	83.21	90.01	73.52
98	203.6	184.49	175.28	220.37	219.23	206.38	218.37	171.5	233.18
120	183.71	197.52	206.9	173.41	180.18	195.02	177.21	179.4	160.04
144	115.5	130.27	127.41	101.06	98.7	112.1	101.4	111.37	133.69
170	74.41	68.68	59.53	78.51	84.09	87.99	68.29	77.66	70.53
198	19.1	18.97	17.72	21.22	23.44	14.82	19.56	21.27	15.8
219	19.01	17.11	16.82	15.79	23.63	25.12	17.83	19.38	16.4
240	20.2	17.04	19.45	19.87	18.72	18.5	21.37	20.45	26.2
270	83.45	75.6	87.1	93.61	81.09	79.01	86.04	81.69	83.46
317	23.27	20.91	17.05	30.44	28.21	18.61	22.5	19.81	23.63

PROLINE PRODUCTION IN SESLERIA : RESULTS FROM LABORATORY LOW TEMPERATURE STRESS EXPERIMENTS

Site C

Time in Hours	$\bar{x}$								
24	57.41	54.08	63.61	71.3	48.07	49.24	51.27	54.66	67.05
50	128.24	119.91	135.74	127.73	131.02	121.68	124.14	133.27	132.43
72	25.13	20.64	17.98	29.82	33.21	18.97	21.96	26.01	32.45
98	35.26	33.56	45.14	27.49	29.86	48.24	29.88	36.58	31.33
120	33.7	30.49	38.12	41.51	28.93	37.13	41.4	33.46	28.56
144	30.04	21.47	28.37	23.77	34.75	38.28	41.21	22.55	29.91
170	24.96	20.76	27.29	31.96	22.61	27.06	21.06	24.37	24.57
198	21.82	19.21	27.44	28.43	20.2	17.19	16.47	18.58	27.04
219	20.43	18.8	17.81	23.22	22.49	21.24	20.58	22.64	16.66
240	22.27	19.43	21.07	25.16	28.78	19.56	21.39	20.83	21.94
272	257.69	239.38	261.63	241.37	264.17	273.39	251.62	281.92	248.04
317	49.41	38.33	41.29	53.41	51.26	57.81	60.48	39.31	53.39

Site D

Time in Hours	$\bar{x}$								
24	128.42	108.76	139.42	140.62	117.18	146.03	99.6	132.71	143.04
50	38.37	31.61	48.4	51.06	22.71	21.49	29.44	59.07	43.18
72	56.86	49.31	71.84	75.95	39.64	64.51	31.68	51.89	70.16
98	54.94	41.92	38.68	76.12	69.81	43.88	39.71	56.41	72.99
120	53.37	51.64	43.69	71.6	74.49	32.12	36.11	77.71	39.6
144	51.24	39.39	73.41	33.51	59.16	63.49	37.04	70.96	42.96
170	49.66	38.28	71.83	73.37	38.32	51.76	54.13	44.42	45.17
198	49.54	31.71	29.07	51.29	54.71	63.98	41.36	38.38	65.82
219	47.07	41.24	66.69	28.99	51.55	40.6	41.42	39.21	66.86
240	47.61	39.51	35.18	56.44	61.06	43.13	38.81	43.3	63.46
272	64.58	86.38	59.31	79.26	53.46	49.28	71.69	66.01	51.25
317	81.33	101.72	96.41	63.81	79.31	89.14	76.97	73.68	69.6

PROLINE PRODUCTION : RESULTS OBTAINED FROM CALCIUM STRESS EXPERIMENTS

Phleum pratense L.

% calcium in soil (by weight)	Weight of plant material in g	µm proline	µm proline/g F.W.
0.14	0.1329	1.46	10.99
0.14	0.1307	1.5	11.48
0.14	0.1326	1.49	11.24
0.14	0.1347	1.55	11.51
0.14	0.1389	1.6	11.52
0.14	0.1381	1.67	12.09
0.14	0.1356	1.56	11.50
0.14	0.1337	1.53	11.44
.5	0.1376	1.24	9.01
.5	0.1381	1.45	10.50
.5	0.1392	1.69	12.14
.5	0.1344	1.54	11.46
.5	0.1321	1.32	9.99
.5	0.1373	1.12	8.16
.5	0.1366	1.6	11.71
.5	0.1389	1.59	11.45
.12	0.1396	1.38	9.89
.12	0.1362	1.23	9.03
.12	0.1362	1.36	9.99
.12	0.1376	1.33	9.67
.12	0.1309	1.11	8.48
.12	0.1399	1.3	9.29
.12	0.1374	1.16	8.44
.12	0.1398	1.28	9.16

PROLINE PRODUCTION : RESULTS OBTAINED FROM CALCIUM STRESS EXPERIMENTS

Agropyron caninum

% calcium in soil (by weight)	Weight of plant material in g	µm proline	µm proline/g F.W.
0.14	0.1347	3.32	24.65
0.14	0.1355	3.4	25.09
0.14	0.1369	3.79	27.68
0.14	0.1372	3.71	27.04
0.14	0.1336	3.35	25.07
0.14	0.1342	3.13	23.32
0.14	0.1357	3.19	23.50
0.14	0.1361	3.39	24.91
.5	0.1384	1.72	12.43
.5	0.1356	2.2	16.22
.5	0.1321	1.94	14.69
.5	0.1373	2.46	17.92
.5	0.1361	2.53	18.59
.5	0.1322	2.56	19.37
.5	0.1337	2.2	16.46
.5	0.1398	1.94	13.88
.12	0.1399	2.31	16.51
.12	0.1333	2.45	18.38
.12	0.1327	2.55	19.22
.12	0.1339	2.62	19.57
.12	0.1346	2.67	19.84
.12	0.1387	2.62	18.89
.12	0.1379	2.29	16.61
.12	0.1366	2.43	17.79

PROLINE PRODUCTION : RESULTS OBTAINED FROM CALCIUM STRESS EXPERIMENTS

Sedge sp

% calcium in soil (by weight)	Weight of plant material in g	µm proline	µm proline/g F.W.
0.14	0.1317	0.81	6.15
0.14	0.1328	0.8	5.71
0.14	0.1362	0.86	6.31
0.14	0.1347	0.76	5.64
0.14	0.1361	0.76	5.58
0.14	0.1337	0.78	5.83
0.14	0.1333	0.73	5.48
0.14	0.1340	0.82	6.12
.5	0.1306	0,88	6.47
.5	0.1368	0.9	6.58
.5	0.1399	0.93	6.65
.5	0.1322	0.89	6.73
.5	0.1341	0.88	6.56
.5	0.1352	0.89	6.58
.5	0.1366	0.89	6.52
.5	0.1371	0.92	6.71
.12	0.1369	0.82	5.99
.12	0.1327	0.8	6.03
.12	0.1344	0.84	6.25
.12	0.1389	0.86	6.19
.12	0.1327	0.77	5.8
.12	0.1331	0.79	5.94
.12	0.1342	0.82	6.11
.12	0.1367	0.83	6.07

PROLINE PRODUCTION :: RESULTS OBTAINED FROM CALCIUM STRESS EXPERIMENTS

Sesleria

% calcium in soil (by weight)	Weight of plant material in g	µm proline	µm proline/g F.W.
0.14	0.0907	2.47	27.23
0.14	0.0966	2.83	29.30
0.14	0.0862	2.79	32.37
0.14	0.0857	2.71	31.62
0.14	0.0992	2.85	28.73
0.14	0.0873	2.6	29.78
0.14	0.0922	2.65	28.74
0.14	0.0847	2.42	28.57
.5	0.0879	20.85	237.20
.5	0.0877	22.2	253.14
.5	0.0931	24.65	264.77
.5	0.0942	23.44	248.83
.5	0.0869	21.88	251.78
.5	0.0871	21.75	249.71
.5	0.0924	24.16	261.47
.5	0.0833	20.27	243.34
.12	0.0913	16.1	176.34
.12	0.0896	12.4	138.39
.12	0.0872	12.56	144.04
.12	0.0864	10.49	121.41
.12	0.0888	11.3	127.25
.12	0.0919	12.33	134.17
.12	0.0926	11.95	129.05
.12	0.0847	11.62	137.19

REFERENCES

1. Barnett N.M. and Naylor A.W. (1966)

   Amino acid and protein metabolism in bermuda grass during water stress.

   Plant Physiol. 41 pp 1222-1230

2. Bates N.S. (1973)

   Rapid determination of free proline for water stress studies

   Plant soil 39 pp 205-207

3. Edited by Black C.A., Evans D.D., White J.L., Ensminger L.E. and Clark F.E. (1965)

   Methods of soil analysis Part 2 Pub, Madison Wisconsin U.S.A. pp 770

4. Boyer J.s. (1966)

   Isopiestic technique : measurement of accurate leaf water potentials

   Soience 154 pp 1459-1460

5. Boyer, J.S. (1968)

   Relationship of water potential to growth of leaves.

   Plant Physiol. 43 pp 1056-1062

6. Chen D, Kessler B and Monselise I.P. (1964)

   Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress.

   Plant Physiol. 39 pp 379-386

7. Chinard F.P. (1952)

   Photomatric estimation of proline and ornithine.

   J. Biol. Chem. 199 pp 91-95

8. Chu T.M., Aspinall D and Paleg L.G. (1974)

   Stress metabolism : VI temperature stress and the accumulation of proline in barley and radish.

   Aust J. Plant Physiol. 1 pp 87-97

9. Gates C.T., Williams W.T. and Court R.D. (1971)

Effect of droughting and chilling on Maturation and chemical composition of Townsville stylo (stylosanthes humilis)

Aust J. Agric. Res. 22
pp 369-381

10. Henckel P.A. (1964)

Physiology of Plants under drought

Ann. Rev. Plant. Physiol. 15
pp 363-386

11. Itai C. and Vaadia Y. (1971)

Cytokinin activity in water stressed shoots

Plant physiol. 47, pp 87-90

12. Kemble A.R. and Macpherson H.T. (1964)

Liberation of amino acids in perennial rye grass during wilting

Biochem. J. 58 pp. 46-49

13. Klieiver W.M. and Kider L.A. (1970)

Effects of day temperature and light intensity on growth and composition of Vitis vinifera fruits.

J. AM. Soc. Hortic. Sci. 95
pp 766-769

14. Knipling E.B. (1967)

Measurement of leaf water potential by the dye method.

Ecology. Vol. 48, No. 6.
pp. 1038-1040

15. Kozlowski T.T. (1972)

Water deficits and plant growth. Volume III plant responses and control of water balance.

Pub. Academic Press, New York and London.

16. Kreeb K. and Abdelall R. (1973)

Water relations measured as electrical resistance of leaves, and its relation to plant growth and production.

Proc. Uppsala Symp. 1970 (Unesco) pp. 497-503

17. Levitt J. (1972)

Responses of plants to environmental stresses.

Pub. Academic Press, New York and London pp 697

18. McKell C.M., Perrier E.R. and Stebbins G.L. (1960)

Responses of two subspecies of orchard grass Dactylis glomerata subsp. lusitanica and judaica to increasing soil moisture stress.

Ecology 41 pp 772-778

19. Ministry of Agriculture, Fisheries and Food (1973)

Technical bulletin 27, the analysis of agricultural materials.

Pub. London : H.M.S.O.

20. Molisch H (1896)

Das erfrieren von pflanzen bei temperaturen über dem Eispunkt.

Sitzber, Kaiserlichen Akad. Wiss. Wien-Math Naturwiss Kl. 105

pp 1 - 14

21. Palfi G. (1965)

The effect of sodium salts on the nitrogen, phosphorous, potassium, sodium and amino acid content of rice shoots.

Plant and Soil 22. pp 122-135

22. Palfi G., Bito M. and Palfi Z. (1973)

Free proline and water deficit in plant tissues.

Fisiol. Rast. 20. pp 233 -238

23. Palfi G., and Juharz J. (1970)

Increase of the free proline level in water deficient leaves as a reaction to saline or cold root media.

Acta. Agron. Acad. Sci. Hung. 19
pp 79-88

24. Round-Turner N.L. (1968)

Some aspects of the ecology of Sesleria caerulea L. Ard. Subsp. Calcarea.

M.Sc. Dissertation Durham University.

25. Routley D.G. (1966)

Proline accumulation in wilted Ladino clover leaves. Crop. Sci. 6

pp 358-361

26. Singh T.N., Paleg L.G. and Aspinall D. (1973)

Stress metabolism III. Variations in response to water deficit in the barley plant.

Aust. J. Biol. Sci. 26
pp 65 - 76

27. Singh T.N., Aspinall D., Paleg L.G. and Boggess S.F. (1973)

Stress metabolism II. Changes in proline concentration of excised plant tissues.

Aust. J. Biol. Sci. 26
pp 57 - 63

28. Singh T.N., Paleg L.G. and Aspinall D. (1973)

Stress metabolism I. Nitrogen metabolism and growth in the barley plant during water stress.

Aust. J. Biol. Sci. 26
pp 45-56

29. Smith J. L. (1975)

Levels of free proline in a number of higher plants on collection from the field and after wilting.

M.Sc. Dissertation Durham University.

30. Stewart C.R. (1972)

Proline content and metabolism during rehydration of wilted excised leaves in the dark.

Plant Physiol. 50.
pp. 679-681

31 Stewart C.R. (1972)

The effect of wilting on proline metabolism in excised bean leaves in the dark.

Plant Physiol. 51
pp. 508 - 511

32. Stewart C. R. and Lee J.A. (1974)

The role of proline accumulation in halophytes.

Planta 120. pp. 279 - 289

33. Stewart C. R., Morris C. J. and Thompson J.F. (1966)

Changes in amino acid content of excised leves during incubation II. Role of sugar in the accumulation of proline in wilted leaves.

Plant Physiol. 41
pp 1585 - 1590

34. Stocker O. (1960)

Physiological and morphological changes in plants due to water deficiency.

U.N.E.S.C.O. arid Zone Res. 15
pp. 63 - 104

35. Sutcliffe J.F. (1962)

Mineral salts absorption in plants.

Pub. Pergamon Press

36. Thompson J.F. and Morris C.J. (1959)

The determination of amino acids by paper chromatography.

Anal. Chem. 31
pp. 1031 - 1037

37. Thompson J.F., Stewart C.R and Morris C.J. (1966)

Changes in amino acid content of excised leaves during incubation I. The effect of water content of leaves and atmospheric oxygen level.

Plant Physiol. 41
pp. 1578 - 1584

38. Todd G.W., Ingram F.W. and Stutte C.A. (1962)

Relative turgidity as an indicator of drought stress in cereal plants.

Proc. Oklahoma Acad. Sci. 42
pp. 55 - 60

39. Troll W. and Lindsley J. (1955)

A photometric method for the determination of proline.

J. Biol. Chem. 215
pp. 655 - 660

40. Turesson G. (1922)

The genotypical response of the plant species to the habitat.

Hereditas 3. pp. 211 - 350

41. Turesson G. (1925)

The plant species in relation to habitat and climate.

Hereditas 6. pp. 147 - 236

42. Turesson G. (1930)

The selective effect of climate upon the plant species.

Hereditas 14. pp. 99 - 152

43. Ursprung A. and Blum G. (1916)

Zur Kenntris der saugkraft

Ber. Deut. Bot. Ges. 34
pp. 525 - 539

44. Vratny P., Stefl M. and Troka I. (1975)

Automatic determination of Proline in plant material.

Chem. Listy 69 (4)
pp. 379 - 383

45. Waldren R.D. and Teare I.D. (1974)

Free proline accumulation in drought stressed plants under laboratory conditions.

Plant Soil 40. 3
pp. 689 - 692

46. Walter H. (1931)

Die Hydratur der pflanze

Pub, Gustav Fisher, Jena
pp. 161

47. Wample R.L. and Bewley D.J. (1975)

Proline accumulation in flooded and wilted sunflower and the effect of benzyladenine and abscicic acid.

Canadian Journal of Botany 53/23
pp. 2893 - 2896

48. West I. (1975)

An ecophysiological study of Sesleria caerulea L.

M.Sc. dissertation Durham University