A physio-ecological study on sesleria caerulea (L) ard.: at Thrislington common (Durham)

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A PHYSIO-ECOLOGICAL STUDY ON SESLERIA CAERULEA (L) ARD. AT THRISLINGTON COMMON (DURHAM)

M. Sc. Dissertation

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CONTENTS

		Page
Dedication		ii
Acknowledgements		iii
List of Figures		iv
List of Plates		v
List of Tables		vi
Chapter 1:	Introduction	1
	Description of sites	4
Chapter 2:	Materials and Methods	
	I. Plant Collection	8
	II. Soil Depth Measurements	9
	III. Soil Moisture Determination	9
	IV. pH Determination	10
	V. Determination of Exchangeable cations	10
	VI. Determination of the Angle of Slope	10
	VII. Methods for Proline Determination	10
Chapter 3:	Results	
	1. Morphological Studies	12
	2. Physiological Studies	12
	I. Results for proline produced by Sesleria under uniform conditions of temperature and water	12
	II. Investigation of proline produced by young and old leaves	16
	III. Investigation of proline produced by flowering and non-flowering plants	19
	IV. Proline production in the water stress experiments	24
	V. Proline produced in the low temperature stress experiment	28
Chapter 4:	Discussion	33
Appendices:		37
Bibliography		75

DEDICATION

This work is dedicated to my wife and daughter "Asma" for their encouragement and inspiration.

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LIST OF FIGURES

Figure 1: Map of the area.

Figure 2: Proline levels in Sesleria under the uniform conditions of temperature and water.

Figure 3: Proline levels in Sesleria leaves from flowering and non-flowering plants.

Figure 4: Proline levels in Sesleria under water stress.

Figure 5: Proline levels in Sesleria from different points under water stress.

Figure 6: Proline levels in Sesleria under low temperature stress.

Figure 7: Levels of proline in Sesleria from different points under low temperature stress.

LIST OF PLATES

Plate 1: Site A at Thrislington Common.

Plate 2: Site B at Thrislington Common.

Plate 3: Sites A and B with the frost depression in between.

Plate 4: Site C at Thrislington Common.

LIST OF TABLES

Table 1: Soil characteristics of sites A, B and C.

Table 2: Mean leaf length from non-flowering Sesleria plants.

Table 3: Mean leaf lengths from flowering Sesleria plants.

Table 4: Mean inflorescence stalk lengths of Sesleria from different points within the sites.

Table 5: Mean proline accumulated in plants from different points under the uniform conditions of temperature and water.

Table 6: Differences in proline levels between young and old leaves.

Table 7: Proline levels in leaves from flowering and non-flowering Sesleria under field conditions.

Table 8: Correlation tests between the levels of proline accumulated in flowering and non-flowering Sesleria and the soil characteristics.

Table 9: Proline levels in Sesleria under water stress.

Table 10: Significance tests on the proline levels in Sesleria, from different points within the three sites, under water stress.

Table 11: Proline levels in Sesleria under low temperature (5°C) treatment.

Table 12: Significance tests on the proline levels in Sesleria plants, from different points within the three sites, under low temperature (5°C) treatment.

Chapter 1

INTRODUCTION

The work of Klebs (1903, 1906) and Goebel (1908, 1913) has increased the knowledge about the possible reactions by plants when exposed to different environmental factors. It is expected that if changes in the genotypical composition of a species result in response to climatic or edaphic factors, these changes would be clearly noticed in species which have an extended and uninterrupted distribution running through areas of different climatic and edaphic character (Turesson, 1922).

The general effect of altitude within an area has already been described by Pearsall (1950). From observations made on Juncus squarrosus, Pearsall concludes

> ...the effects of altitude are differential, affecting the seed-production most, flower-production less and vegetative growth least. The analysis of these effects show that they vary little between districts receiving great differences in rainfall and they can thus be attributed mainly to the diminution of mean temperature with increasing altitude.

Differences are also found, although on a smaller scale, between populations originating from less contrasting environments. Such physiological differentiation is not necessarily accompanied by morphological differentiation. It is likely that this is because the two types of differentiation are in relation to unrelated factors of the environment.

The simplest means by which physiological characteristics may be investigated, is by transplant experiments, where samples of a population are grown in a series of contrasting natural environments. This is, from a physiological standpoint an exceedingly crude method, since the plants concerned will be subjected to all the many variations of natural conditions, and it may never be possible to know exactly which factor of the environment is the most important in determining the performance of the plants (Bradshaw, 1959).

The present study aims at investigating the physiological differentiation occurring within a species, since population originating from contrasting habitats differed considerably in their ability to tolerate different extreme conditions of temperature, dehydration, salinity, etc. Supra-optimal temperatures can lead to rapid transpiration and a consequent lowering of tissue water potential in the leaves. Furthermore, low environmental temperature can lower the availability of water in the soil and its movement to the plant roots, also resulting in a lowering of leaf water potential. In both cases, it is difficult to separate the direct effects of temperature on metabolism from those mediated through the concomitant change in water potential.

It has been suggested that plant resistance to cold, heat and water stress are interrelated (Levitt, 1956) which is easily understood if each is a manifestation of response to a similar change in tissue environment.

The most striking metabolic consequence of lowered water potential in many plants is a rapid and extensive accumulation of amino acid proline (Singh, et al. 1973). Accumulation of proline has also been reported to occur in plants subjected to low temperatures (Shvedskaya and Kruzhilin, 1966; Bendo, 1968; Palfi and Juhasz, 1970; Gates et al. 1971) and in desert plants exposed to high temperature (Oshanina, 1972). In neither case is it known whether the accumulation of proline was a consequence of the temperature regime or due to a correlated change in tissue water potential. It has been reported by Goas in 1965, that halophytes such as Aster tripolium contain high levels of the amino acid proline when grown under saline conditions. It is suggested that proline functions as a source of solute for intercellular osmotic adjustments under saline conditions. Barnett and Naylor (1966) found that in water stressed plants of Bermuda grass there was a rapid increase in free proline which accumulated to a level of 1.2 mg/g dry weight. Similar observations have been made for other species including ladino clover (Routley, 1966), broad bean (Stewart et al; 1966) and barley (Singh et al; 1972).

While this accumulation of proline may be a stress response resulting from a decreased rate of protein synthesis or an increase in protein turn over. Two groups of workers have been able to correlate the potential for proline accumulation with drought resistance. Singh et al, (1972) found that barley varieties having different degrees of drought resistance also differed in their capacity to accumulate proline under stress, resistant varieties accumulating higher levels of proline under water stress than non-resistant varieties. Similarly in a comparison of two Carex sp. Hubac and Guerrier (1972) found that the drought resistant Carex pachystylis accumulated higher proline than the non-resistant species C. setifolia. In the case of the non-resistant species, its resistance was found to be increased by the exogenous application of proline.

Sesleria caerulea is widely spread, mainly over different open habitats at different altitudes but commonly grow on basic soils.

Round-Turner (1968), Lloyd (1974) studied the anatomy, growth and mineral relationships of two populations from different sites, postulated the existence of edaphic and climatic ecotypes of Sesleria caerulea. The utilization of proline production as an indication of the genetic plasticity of stress resistance in Sesleria in relation to its ecological amplitude, was indeed the approach followed in all the studies carried out by Darke (1976) and Ferreira (1978) in Cassop Vale in Durham. On the other hand, West (1975) discovered considerable variations in the morphology of Sesleria populations from various selected sites at Cassop Vale.

Such variations encountered by the species stimulated the desire for the present study of the plant in Thrislington Common in Durham. It is meant to examine whether the grass represents a cline (i.e. genetically based, continuously graded variations which can be correlated with an observable environmental gradient). To evaluate this, morphological and physiological variations within the population were investigated through the three selected sites.

SITE DESCRIPTION

Thrislington Common, the studied area (Fig. 1) is a magnesium limestone area. Three sites were selected for the study. Site 'A' (Plate 1), south facing, and Site 'B' (Plate 2), north facing, were on two opposite slopes with a frost depression in between (Plate 3). Whereas Site 'C' (Plate 4) was a flat exposed area.

Seven points were chosen along Slope 'A' (A_1 to A_7 with A_1 at the bottom, and A_7 on top of this slope), and three along Slope 'B' (B_1 to B_3 with B_1 at the bottom, and B_3 on top of the slope), with successive points approximately 3.5 metres apart. Samples were collected from these points for comparison, whereas in Site 'C', samples were randomly taken.

The soil characteristics of each point within a site is as shown in Table 1.

Table 1

Soil Characteristics of the Sites "A", "B" and "C"

Site		pH	Soil depth in "cm"	% Moisture	Soil Exchangeable Cations in "ppm"			
					Ca	Mg	Na	K
	A_1	7.27	24.5	37.2%	226	18.95	2.65	3.70
	A_2	7.23	15.7	34.7%	143	29.05	2.75	4.30
	A_3	7.30	17.2	32.9%	154	30.9	2.55	5.55
A	A_4	7.30	19.1	32.2%	140	29.5	2.60	3.60
	A_5	7.43	20.5	32.9%	139	29.55	2.45	3.95
	A_6	7.30	19.8	32.9%	145	29.75	2.06	4.95
	A_7	7.27	19.3	34.3%	166	28.75	2.12	5.25
	B_1	7.53	22.3	26.7%	235	6.60	1.94	2.25
B	B_2	7.60	33.0	29.2%	284	6.10	2.11	2.00
	B_3	7.47	17.2	31.2%	294	10.75	2.14	3.15
	C	7.50	22.0	26.4%	141	23.75	2.01	3.50

* The high calcium content in site "B" is attributed to the ballast laid for the railway line which was previously built there.

Figure 1: <u>Map of the Area</u>

Reproduced from the Ordnance Survey Map - Sheet NZ 33 SW; scale 6 inches to 1 mile; published in 1966 - by permission of the Ordnance Survey.

A : Site A.

B : Site B.

C : Site C.

PLATE 1: Site A at Thrislington Common
(Gentle slope)

PLATE 2: Site B at Thrislington Common
(Sharp slope within the frost depression)

PLATE 3: Sites A and B with the frost depression in between.

PLATE 4: Site C at Thrislington Common
(Driest, drained and exposed)

Chapter 2

MATERIALS AND METHODS

I. Plant Collection

Plants were dug from each site in the field with as little damage to the root system as possible, and transplanted into plastic seed trays of 13 x 8.5 x 2 ins. dimensions, kept in the glasshouse and were regularly watered. Leaf samples for proline production determination as regards the basic experiments, old and young leaves, were taken from these plants, kept at the same conditions of temperature and watering. The cut leaves were wrapped with muslin and dropped immediately into liquid air to stop any further proline production. They were then stored at -20^{o}C.

Leaf samples from the flowering and non-flowering plants were cut directly in the field every fortnight, wrapped, and put into liquid air, later stored as before in the -20^{o}C room prior to the proline assay being carried out.

Plants for the low temperature (5^{o}C) experiment, were transplanted into plastic pots filled with John Innes Compost No. 2. They were left in the glasshouse and regularly watered for two weeks to establish themselves before being transferred to the cold room (5^{o}C) where they were watered whenever necessary with cold water kept in the same room. Leaf samples were taken every other day for proline determination.

As before, plants were also transplanted into plastic pots and left for two weeks before the water stress was imposed. The experiment was designed in such a way that while one set of plants was kept under water stress, the other was watered whenever necessary, and in the meantime to function as a control for the low temperature experiment since the former were kept in the glasshouse at 23°C.

The morphological investigations were performed by randomly taking eighty plants (half flowering and half non-flowering) every fortnight from the field. The lengths of randomly selected eighty leaves (forty from flowering and forty from non-flowering plants) and forty inflorescence stalks were determined.

Since the flowering head differs in plants, it was found most convenient to take the distance between the first node and the basal part of the flowering head as a measure of the stalk length.

II. Soil Depth Measurement

An auger was used, screwed into the soil until it encountered the hard bed rock. The depth to which it was pushed was then measured in cms. as a measure of soil depth. An average of three readings was recorded every time from each point within the site.

III. % Soil Moisture Determination

A soil corer was used, to extract a soil clod, 5 - 10 cms. in length. As in II. three samples were taken every time. 10 gms. from each sample were put in separate crucibles and left in the oven set at 55°C to maintain steady water loss while the integrity of the calcareous soil remained unchanged. When no further loss in weight was encountered, which indicated that all the water was evaporated, the crucibles were then kept in a dessicator. The percentage moisture content was then calculated using the following equation:

$$\% \text{ moisture} = \frac{\text{weight of fresh soil} - \text{weight of the dried soil}}{\text{weight of the fresh soil}} \times 100$$

IV. pH Determination

15 gms. were taken from the soil samples in III, shaken into 30 cm^3 of distilled water (i.e. ratio of 1:2). It was then left for twenty minutes to set before the pH was read using a pH-meter.

V. Determination of Exchangeable Cations

A wad of cotton wool was placed in a leaching tube and 5 gms of air-dried, sieved soil (wire mesh No. 40 was used) was added and covered with a second wad of cotton wool.

A volumetric flask, filled with 250 cm^3 of 1M ammonium acetate buffered to pH 7, was then inverted into the leaching tube and all together placed in the leaching rack. The leachate was collected in a conical flask.

The amount of exchangeable Ca, Mg, Na and K cations in the leachate, were determined in "ppm" by means of the atomic adsorption spectrophotometer which is more accurate than the flame photometer.

VI. Determination of the Angle of Slope

A surveyor clinometer was used to measure the angle of slope in degrees.

VII. Methods for Proline Determinations

Methods described by Bates et al. (1973), Troll and Lindsley (1955) were used in proline determinations. 0.2 gm of plants material were mixed with a little purified acid-washed sand and ground in 25 cm^3 of 3 per cent sulphosalicylic acid for one minute using a pestle and mortar. The purified acid-washed sand is meant to assure thorough grinding. The colourless sulphosalicylic acid is effective in precipitating proteins in aqueous solution and does not interfere with the acid ninhydrin (Bates et al., 1973). The mixture was filtered through Whatman # 1 filter paper. 2 cm^3 of the filterate were added to 0.15 gm acid permutit in a test tube which was shaken vigorously. The permutit removes the interfering basic amino acids lysine, hydroxylysine and ornithine.

To the 2 cm^3 of the filterate were added 2 cm^3 of glacial acetic acid and an equal quantity of acid ninhydrin (prepared by dissolving 1.25 gm ninhydrin in 30 cm^3 glacial acetic acid and 30 cm^3 6M phosphoric acid. The mixture was warmed to 70^oC 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 table for 24 hrs at 4^oC (Troll and Lindsley, 1955).

The mixture was heated in a water bath at 80^oC for one hour after which time the tubes were cooled in ice-bath to terminate the reactions.

A pink colour was formed when the proline reacted with acid ninhydrin. It occurred at a pH of approximately 1 and the pink product was water-insoluble (Chinard, 1952). 4 cm^3 of this reaction mixture were added to 4 cm^3 toluene, and the test tube was shaken for 20 seconds. The pigment layer with the toluene separated out, was allowed to stand until it was at room temperature. The absorbance of this layer was then read at 520 nm, in a 1 cm cuvette, using the "Uvispek" spectrophotometer and toluene was used as a blank. The proline concentrations (μ gm proline/cm^3) of the reaction mixtures were read off from a standard curve prepared using sig ma proline. The value for μ moles proline/gm fresh weight was calculated from the equation (Bates, et al., 1973):

$$[(\mu gm\ proline/cm^3 \times cm^3\ toluene)/115.5\ \mu gm/\mu mole]/(gm\ sample/2)$$

$$= \mu\ moles\ proline/gm\ of\ fresh\ weight\ material.$$

Chapter 3

RESULTS

1. Results of the Morphological Studies

In the majority of samples taken (Tables 2, 3), leaves from non-flowering plants were longer than the leaves from flowering plants. It was early in the study that leaves from flowering plants were longer, however, later on, the reverse was true. It was interpreted that flowering plants, as they grew, put more material into the flowering process than in increasing leaf length.

No consistent variation was found in either leaf or inflorescence stalk length between points along the slopes as it would be expected that both lengths decrease with altitude. It therefore followed that the least morphological variations encountered could well mean that plants in the three sites were of the same ecological races, or that the characteristics of the habitats (Table 1) in which the plants were growing, though differing slightly, nevertheless were still within the acceptable limits of environmental conditions conducive for the plant growth.

2. Results of the Physiological Studies

I. Results for proline produced by plants under uniform conditions of temperature and water

The plants were kept under constant conditions of temperature and water as described in method. Consequently any difference in the amount of proline accumulated could be attributed to differences in soil and plant characteristics, since all plants were kept intact in their original field soil.

Table 2

Mean Leaf Length from Non-flowering Sesleria Plants

Site	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
A1	54.75 ± 3.71	118.75 ± 5.90	123.50 ± 6.13	100.60 ± 5.28	119.50 ± 4.58	133.30 ± 3.88
A_2	41.38 ± 3.22	135.10 ± 5.92	131.93 ± 5.71	108.58 ± 5.08	140.22 ± 6.16	142.30 ± 6.40
A_3	74.20 ± 7.45	104.18 ± 6.40	118.30 ± 6.04	88.40 ± 6.61	142.73 ± 4.94	112.28 ± 6.95
A_4	77.18 ± 6.33	113.28 ± 5.91	131.78 ± 6.19	96.78 ± 6.92	152.53 ± 6.71	154.48 ± 5.15
A_5	72.10 ± 5.24	129.38 ± 4.96	116.78 ± 5.99	98.75 ± 6.55	151.33 ± 5.22	128.20 ± 6.21
A_6	71.98 ± 5.39	125.28 ± 7.10	131.53 ± 7.15	90.25 ± 6.88	155.78 ± 4.64	159.85 ± 6.65
A_7	95.95 ± 7.74	126.50 ± 7.91	140.93 ± 4.17	84.03 ± 7.17	125.20 ± 6.97	159.93 ± 7.30
B_1	62.78 ± 5.19	124.08 ± 7.63	120.90 ± 5.22	99.25 ± 7.25	143.18 ± 5.81	137.10 ± 5.51
B_2	56.58 ± 4.71	113.95 ± 5.67	127.93 ± 8.99	96.48 ± 5.38	125.58 ± 6.18	135.68 ± 5.75
B_3	87.43 ± 5.47	90.10 ± 6.78	122.55 ± 6.98	102.33 ± 5.95	136.03 ± 5.99	127.53 ± 4.97
C	70.10 ± 5.37	118.45 ± 7.40	117.28 ± 5.66	100.55 ± 5.64	146.33 ± 6.69	175.08 ± 7.00

Table 3

Mean Leaf Length from Flowering Sesleria Plants

Site	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
A_1	65.08 ± 4.44	96.15 ± 4.50	118.38 ± 5.34	88.03 ± 5.98	114.85 ± 3.24	116.43 ± 4.20
A_2	76.25 ± 5.02	101.68 ± 5.51	119.58 ± 4.70	83.80 ± 5.39	112.75 ± 4.62	124.23 ± 4.96
A_3	82.73 ± 7.98	94.10 ± 3.71	96.33 ± 4.76	72.30 ± 4.88	132.80 ± 6.87	104.83 ± 5.35
A_4	90.23 ± 5.76	99.45 ± 6.33	118.38 ± 6.13	74.80 ± 5.83	128.00 ± 5.73	154.05 ± 3.78
A_5	70.48 ± 5.00	105.68 ± 5.38	105.80 ± 5.29	89.05 ± 4.86	118.30 ± 5.43	115.90 ± 7.56
A_6	63.93 ± 4.73	110.65 ± 5.38	106.95 ± 4.81	81.38 ± 5.56	128.48 ± 5.44	130.53 ± 7.01
A_7	68.55 ± 6.56	104.95 ± 6.10	131.03 ± 5.64	73.20 ± 5.62	131.73 ± 4.16	126.15 ± 7.37
B_1	57.65 ± 4.23	114.00 ± 4.97	104.75 ± 5.32	91.45 ± 6.15	118.33 ± 4.67	133.93 ± 4.42
B_2	51.43 ± 2.82	98.03 ± 4.99	99.35 ± 4.47	66.38 ± 3.86	111.75 ± 4.27	113.60 ± 5.57
B_3	89.70 ± 5.35	98.73 ± 7.38	101.28 ± 4.82	77.48 ± 5.55	128.15 ± 5.63	124.18 ± 3.91
C 1	69.10 ± 4.71	99.48 ± 6.18	84.83 ± 5.29	76.88 ± 3.73	127.78 ± 5.66	143.03 ± 6.93

Table 4

Mean Inflorescence Stalk Length of Sesleria from Different Points within the Sites

Site	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
A_1	209.63 ± 7.61	263.93 ± 12.89	319.65 ± 19.03	205.18 ± 7.94	303.88 ± 11.86	256.08 ± 15.39
A_2	175.75 ± 6.02	262.70 ± 13.27	392.85 ± 15.20	194.63 ± 7.93	374.20 ± 14.56	266.95 ± 18.87
A_3	272.00 ± 6.55	334.93 ± 20.06	347.55 ± 17.47	251.88 ± 8.69	346.30 ± 15.03	266.40 ± 21.56
A_4	211.10 ± 6.90	296.60 ± 17.63	391.95 ± 15.93	243.38 ± 9.61	398.08 ± 18.86	314.25 ± 16.50
A_5	195.73 ± 8.28	267.18 ± 13.07	294.58 ± 13.58	226.80 ± 11.43	376.98 ± 24.81	262.68 ± 11.63
A_6	240.03 ± 10.01	315.75 ± 19.30	343.75 ± 17.59	196.40 ± 8.66	340.43 ± 13.45	283.55 ± 18.91
A_7	229.98 ± 7.79	320.90 ± 31.56	405.93 ± 23.19	256.13 ± 7.64	421.10 ± 18.02	216.33 ± 13.58
B_1	186.90 ± 5.44	327.93 ± 12.78	378.33 ± 14.66	239.70 ± 7.47	425.58 ± 20.55	288.53 ± 16.10
B_2	154.43 ± 6.21	332.32 ± 13.21	391.78 ± 13.75	203.95 ± 6.91	372.55 ± 13.86	244.10 ± 16.89
B_3	215.73 ± 7.27	318.58 ± 21.25	296.70 ± 13.65	206.13 ± 7.60	332.43 ± 14.86	263.98 ± 15.26
C	216.55 ± 6.77	313.93 ± 18.67	329.03 ± 24.23	200.55 ± 7.03	245.28 ± 12.28	201.08 ± 11.41

The highest mean proline accumulated (0.969 $\pm$ 0.05) was by plants from Site C, the driest, most drained and exposed site. The least proline (0.812 $\pm$ 0.04) was produced by plants from point B_2, half-way within Site B. Whereas plants from Site A achieved proline levels ranging between 0.814 $\pm$ 0.02 for point A_7 (top of the slope) and 0.862 $\pm$ 0.03 for A_2 (second from bottom of the slope).

The statistical tests showed no significant difference at P = 0.5 between points within the same slope and between points from different sites, (see Table 5).

Since water and temperature were stable, it could be seen from the results that temperature and water were of profound importance. To verify this observation, separate experiments (IV and V) were designed to investigate how plants from different sites responded to water and temperature stress.

II. Investigation of Proline produced by Young and Old Leaves (μ mole proline/gm fresh weight)

Leaves with fully expanded blades were considered as old whereas those with their blades rolled or partially expanded were regarded as young.

It was found (Ferreira, 1978) that there were no significant differences between the apical and basal portions of Sesleria leaf as regards the amount of proline accumulated when the plants were under stress. It was then found more convenient, in the present study to base the work on the whole leaf. A further test was carried out to look into the difference in the level of proline accumulated in young and non-senescing old leaves under field conditions.

Table 5

Mean Proline accumulated in Plants from Different Points under the Uniform Conditions of Temperature and Water (μ mole proline/gm fresh weight)

SITE "A":

A_1	A_2	A_3	A_4	A_5	A_6	A_7
0.815 ± 0.03	0.862 ± 0.03	0.818 ± 0.03	0.819 ± 0.03	0.835 ± 0.04	0.848 ± 0.05	0.814 ± 0.02

SITES "B" AND "C":

B_1	B_2	B_3	C
0.841 ± 0.04	0.812 ± 0.04	0.829 ± 0.04	0.969 ± 0.05

Figure 2: Proline levels in Sesleria plants, from different points, under the uniform conditions of temperature and water.

figure 2

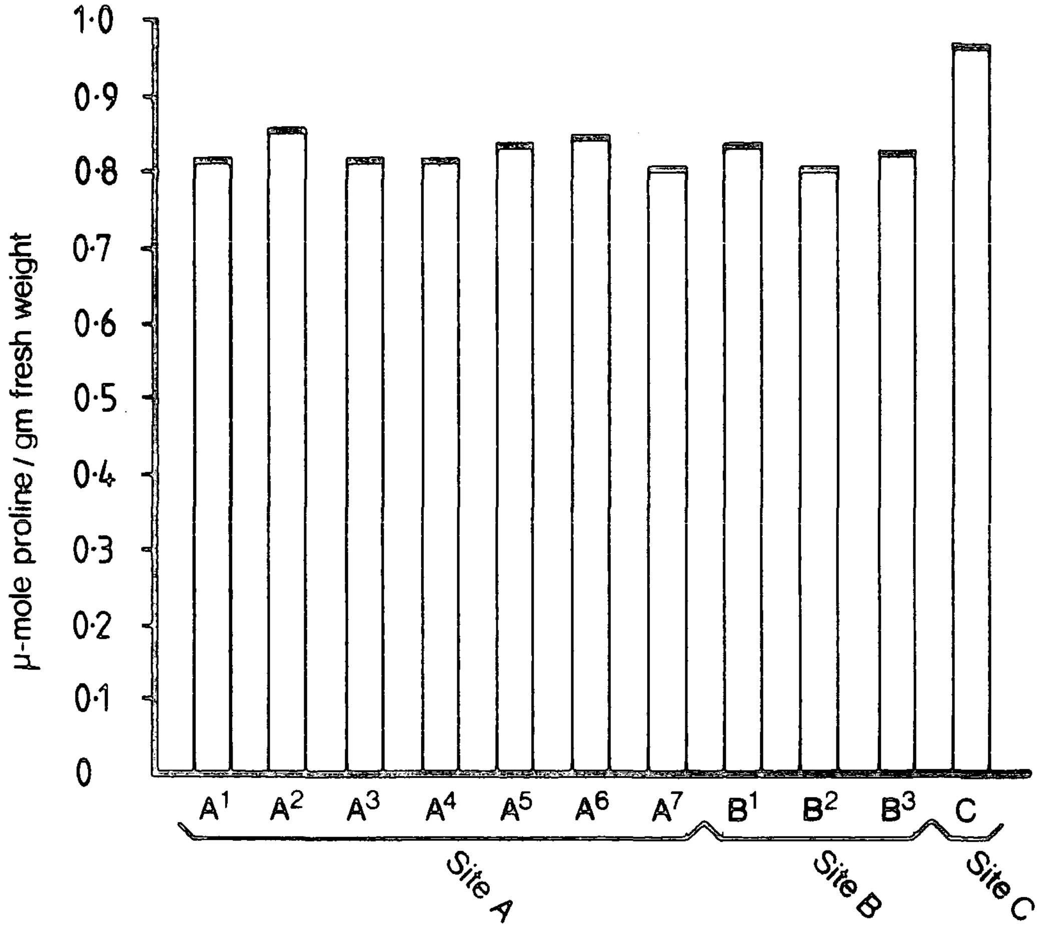

1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
μ-mole proline / gm fresh weight
A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
C
Site A
Site B
Site C

Table 6

Differences in Proline Levels between Young and Old Leaves
(μ mole proline/gm fresh weight)

	Site A	Site B	Site C
Young leaves	1.642 ± 0.06	1.721 ± 0.15	2.159 ± 0.16
Old leaves	1.672 ± 0.06	1.607 ± 0.13	1.719 ± 0.06

Sample size was 36

Difference in proline accumulated was found to be insignificant at P = 0.05 between young and old leaves drawn from plants in all the three sites studied. In Site A, old leaves produced a negligible higher amount of proline than young leaves. Opposite results were obtained from Site B. In Site C young leaves accumulated 1.3-fold more proline than old leaves.

Results from Site A and B could indicate that old and young leaves resist drought in a more or less similar manner. The more proline produced by young leaves from Site C (Drier site with mean percentage moisture 26.2 - Table 1), could be explained by the fact that old and young leaves respond differentially to drought with young leaves expected to be more resistent, and since they were more actively growing they were capable of synthesising more proline. Results from this experiment were made use of in experiments IV and V, where equal numbers of old and young leaves were taken in each sample for the proline determination, thus minimizing errors which might result from differential response by old and young leaves to water and temperature stress.

III. Investigation of Proline Accumulated by Flowering and Non-flowering Plants (μ mole proline/gm fresh weight)

Similar investigations comparable to those in the previous Section II were made to test proline levels accumulated in leaves taken from flowering and non-flowering plants (Fig. 3), since it was observed in the field that some plants were flowering and others were not.

A t-test was done which revealed insignificant differences (at $P = 0.05$) in the amount of proline produced by leaves from flowering and those from non-flowering plants, (see Table 7).

It was the rate of proline accumulation with time which was of most interest. And it could be concluded from the results that as the plants got older, the more proline they accumulated once growth and protein systhesis slows, the more proline will be available for stress resistance.

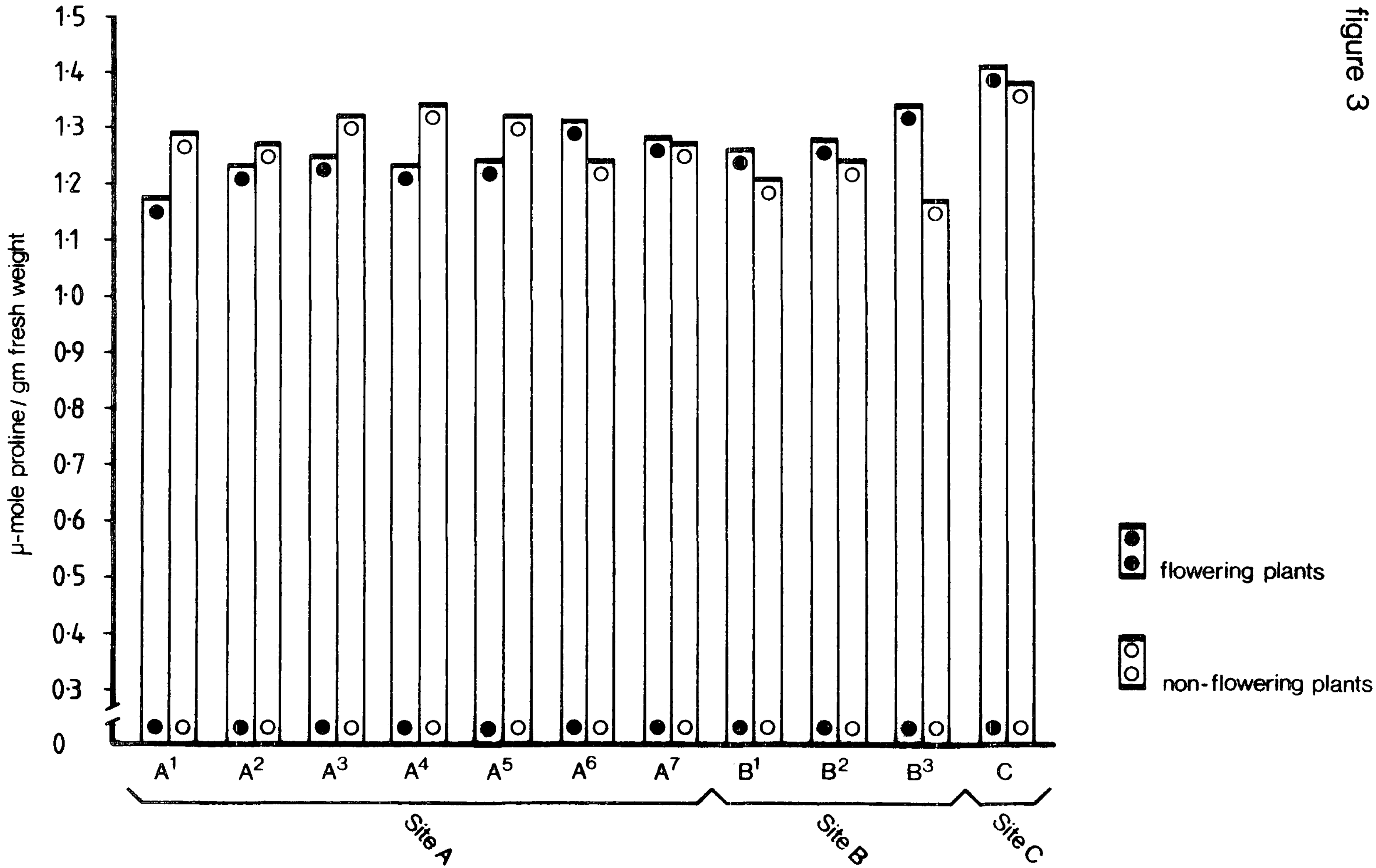
An investigation was carried out to see whether there was correlation between the dependent variables (proline levels in flowering and non-flowering plants), and the independent variables (Table 1) such as pH, soil depth, moisture content and soil exchangeable cations from the different sites. Results were as shown in Table 8.

Proline levels in flowering plants were more negatively correlated with the soil moisture content as the later decreases the former increases. It could otherwise mean that flowering plants were more sensitive to water stress than non-flowering plants.

Calcium and magnesium were found to be more positively correlated with the level of proline in non-flowering plants.

Sutcliffe (1962) pointed out that, though Sesleria can not be termed a true calcicole, nevertheless its physiology must be at least comparable to calcicolous plants because it has the ability of either suppressing calcium absorption or rapidly transporting it to inactive centres before enzyme systems are blocked. So Sesleria with such characteristics could tolerate levels of calcium non-tolerable by other plant species as indeed the case for Sesleria growing on Site B where the exchangeable calcium ranges between 235 ppm and 294 ppm.

Figure 3: Proline levels in Sesleria leaves from flowering and non-flowering plants.



μ-mole proline / gm fresh weight
0
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2
1·3
1·4
1·5
A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
C
Site A
Site B
Site C
flowering plants
non-flowering plants

figure 3

Table 7

Proline levels in Leaves from Flowering and Non-flowering Plants under Field Conditions

(μ mole proline/gm fresh weight)

	Site "A"		Site "B"		Site "C"	
	F	N	F	N	F	N
Day 0	0.810 ± 0.03	0.790 ± 0.02	0.793 ± 0.05	0.780 ± 0.01	0.877 ± 0.01	0.818 ± 0.01
Day 15	0.941 ± 0.03	0.961 ± 0.01	0.903 ± 0.01	0.903 ± 0.02	0.898 ± 0.02	1.006 ± 0.02
Day 30	1.164 ± 0.02	1.165 ± 0.03	1.467 ± 0.12	1.036 ± 0.02	1.298 ± 0.01	1.279 ± 0.08
Day 45	1.387 ± 0.04	1.368 ± 0.06	1.574 ± 0.02	1.169 ± 0.02	1.624 ± 0.04	1.297 ± 0.06
Day 60	1.500 ± 0.06	1.581 ± 0.04	1.387 ± 0.03	1.391 ± 0.08	1.519 ± 0.03	1.579 ± 0.01
Day 75	1.719 ± 0.03	1.949 ± 0.06	1.915 ± 0.06	2.217 ± 0.12	2.307 ± 0.05	2.439 ± 0.14

F = flowering plants
N = non-flowering plants

Each sample size was 18

Table 8

Correlation Tests between the Level of Proline (dependent variable) in Flowering and Non-flowering Plants, and the Soil Characteristics (independent variables)

	pH	Soil depth	% Moisture	Ca	Mg	Na	K
Flowering plants	0.489	-0.023	-0.664	+0.437	0.129	-0.129	-0.336
Non-flowering plants	-0.162	-0.234	-0.249	+0.547	0.539	0.344	0.275

IV. Proline Production in the Water Stress Experiments
(μ mole proline/gm fresh weight)

The proline accumulated in the plants from the three sites fluctuated enormously during water stress treatment (Fig. 4), with all plants from the three sites A, B and C achieving their highest levels of 8.099 ± 1.0, 6.931 ± 2.04 and 6.616 ± 0.26 respectively in Day 6 after actually responding to the water stress treatment. And plants from Site A accumulated significantly higher proline than those from Sites B and C. Whereas in Day 12, plants from Site B accumulated a significantly higher proline, being most sensitive to drought; for the fact they were growing on a very steep slope (angle of slope was 26°). However, plants from Site C, the driest and exposed site, were expected to accumulate the highest levels of proline. And the relatively lower levels achieved could be explained on the ground that the magnitude of the stress imposed was beyond the threshold necessary to justify the accumulation of higher levels of proline in plants from site C which seemed to have a wider range of drought tolerance.

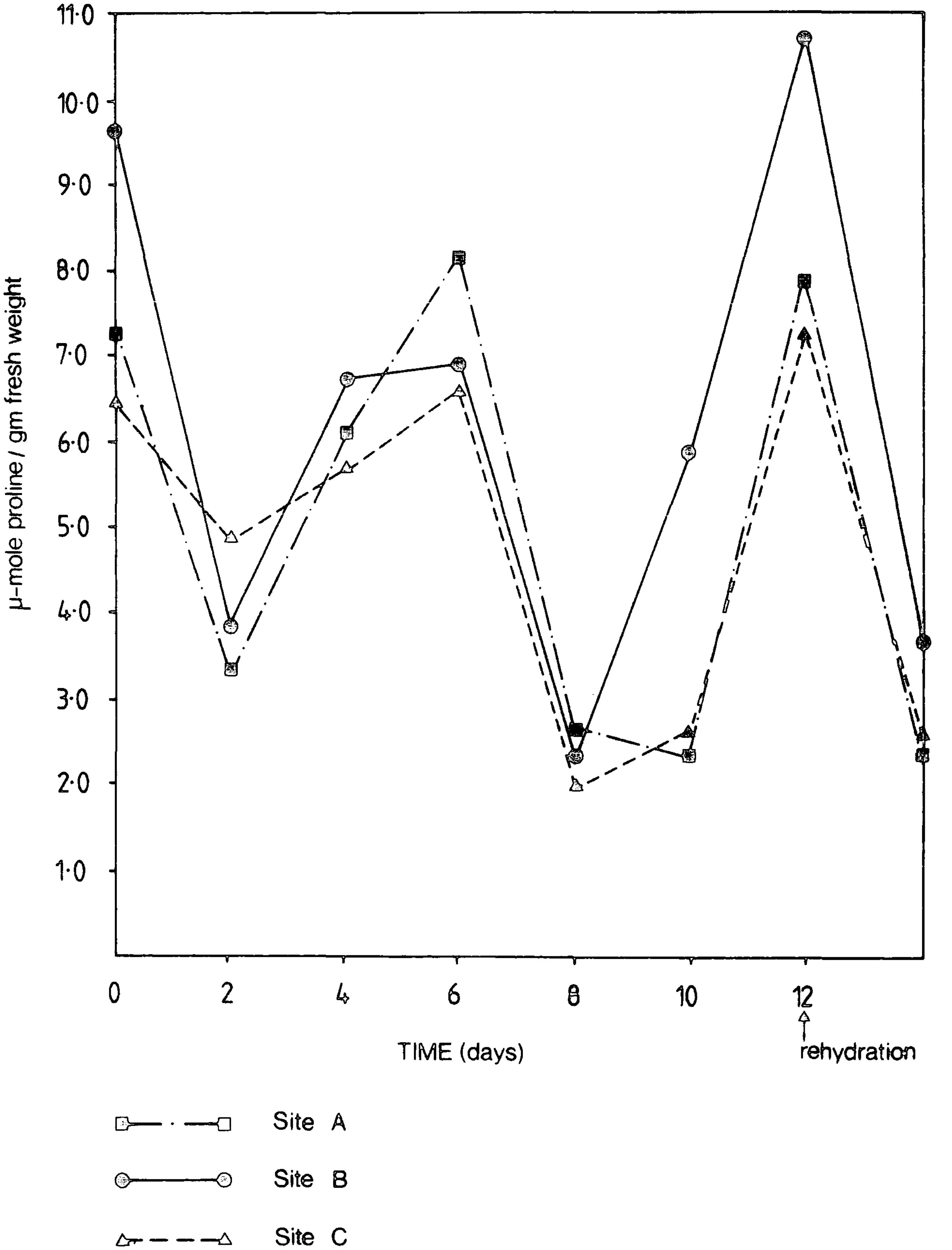
Table 9

Proline Levels in Sesleria under Water Stress
(μ mole proline/gm fresh weight)

	Site A	Site B	Site C
Day O	7.248 ± 0.54	9.613 ± 1.28	6.469 ± 0.23
Day 2	3.320 ± 0.25	3.848 ± 0.11	4.861 ± 0.13
Day 4	6.034 ± 0.95	6.738 ± 0.81	5.713 ± 0.11
Day 6	8.099 ± 1.00	6.931 ± 2.04	6.616 ± 0.26
Day 8	2.651 ± 0.43	2.298 ± 0.54	1.910 ± 0.10
Day 10	2.335 ± 0.39	5.865 ± 0.85	2.618 ± 0.10
Day 12	7.858 ± 0.67	10.742 ± 0.38	7.296 ± 0.22
Two weeks after rehydration	2.343 ± 0.29	3.642 ± 0.24	2.603 ± 0.33

Figure 4: Proline levels in Sesleria plants, from the three sites, under water stress treatment.

figure 4


11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
µ-mole proline / gm fresh weight
0
2
4
6
8
10
12
TIME (days)
rehydration
Site A
Site B
Site C

The initial high levels of proline in all plants was not expected because the soil was moist in the first two days after the water was withheld. It then appeared as though the stressed plants were unable to establish themselves properly in spite of the fact that they were left, after being transferred into the plastic pots, for more than two weeks before the water stress was imposed. Hence, such high initial levels of proline could be due to that reason.

Differences in proline accumulated by plants within the different sites were investigated and it was found that plants from points A_1 (bottom); A_4 (mid) and A_7 (top point); B_1 (bottom), B_2 (mid) and B_3 (top) within slopes A and B respectively, were insignificantly different at $P = 0.05$ (Table 10).

It then followed that plants within the same site were of the same magnitude of resistance to water stress whereas plants from different sites differed.

Table 10

Significance Tests on the Proline Levels in Plants from Different points within the Three Sites under Water Stress

	A_4	A_7	B_1	B_2	B_3	C
A_1	N.S	N.S	N.S	-	-	N.S
A_4	-	N.S	-	-	-	-
A_7	-	-	-	-	N.S	N.S
B_1	-	-	-	N.S	N.S	N.S
B_2	-	-	-	-	N.S	-
B_3	-	-	-	-	-	N.S

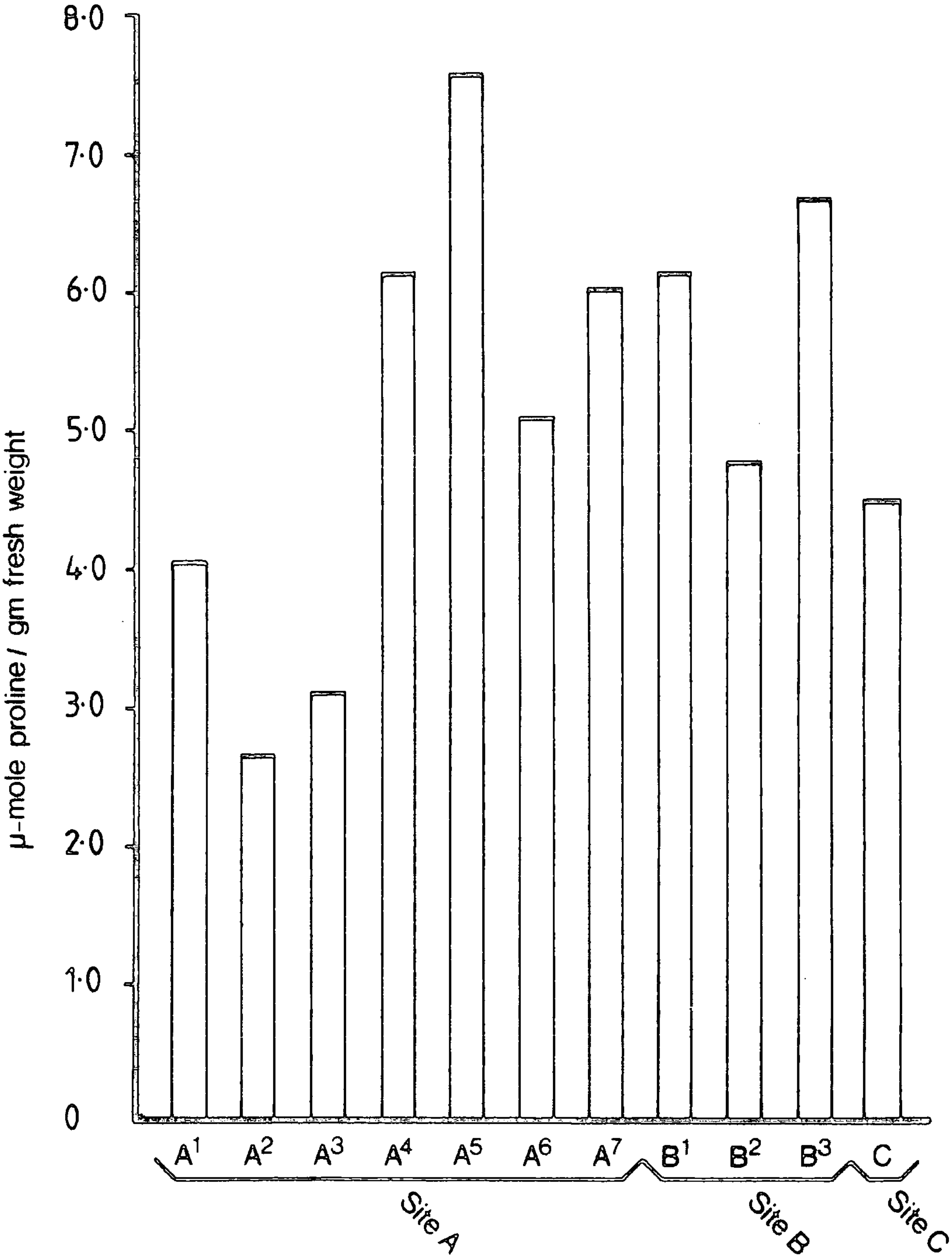
N.S = not significant

Sample size was 18

A student t-test was done

Figure 5: Proline levels in Sesleria plant, from different points within the sites, under water stress treatment.

figure 5



8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
μ-mole proline / gm fresh weight
A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
C
Site A
Site B
Site C

V. Proline produced in the Low Temperature Stress
(μ mole proline/gm fresh weight)

As in the previous experiment IV, the plants from the three sites accumulated high levels of proline when the low temperature stress was first imposed (Table 11). Such high levels could be explained as a result of the combined effect of both temperature and water. The reason mentioned previously in experiment IV could be a third factor.

Table 11

Proline Levels in Sesleria under Low Temperature ($5^{o}C$) Stress
(μ mole proline/gm gresh weight)

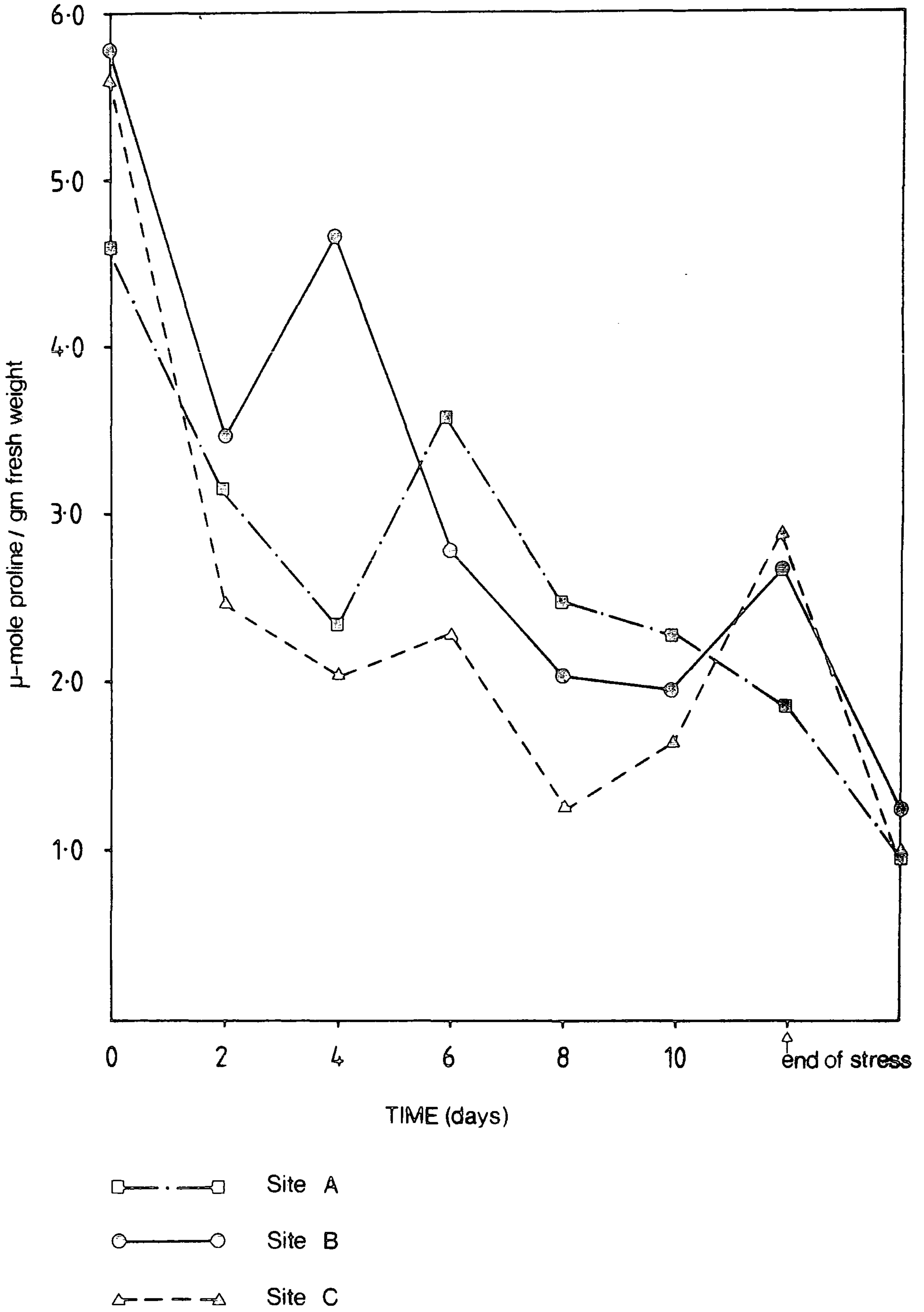
	Site A	Site B	Site C
Day 0	5.591 ± 0.51	5.454 ± 0.48	5.691 ± 0.10
Day 2	2.143 ± 0.24	3.459 ± 0.33	2.456 ± 0.10
Day 4	2.344 ± 0.14	4.658 ± 0.75	2.021 ± 0.10
Day 6	3.573 ± 0.22	2.754 ± 0.20	2.305 ± 0.20
Day 8	2.455 ± 0.20	2.026 ± 0.20	1.228 ± 0.02
Day 10	2.267 ± 0.14	1.929 ± 0.38	1.596 ± 0.09
7 days after the stress was over	1.832 ± 0.09	2.658 ± 0.18	2.916 ± 0.13
15 days after the stress was over	0.984 ± 0.09	1.237 ± 0.10	0.942 ± 0.03

The levels of proline accumulated were slightly fluctuating and all plants soon adjusted themselves to the stress by steadily achieving lower levels of proline (Fig. 6).

The relatively high temperature in the glasshouse ($23^{o}C$) imposed some kind of stress upon plants from Sites B and C which responded by the increased level of proline, when transferred from the $5^{o}C$ room.

Figure 6: Proline levels in Sesleria plants, from the three sites, under low temperature (5^oC) treatment.

figure 6


6·0
5·0
4·0
3·0
2·0
1·0
μ-mole proline / gm fresh weight
0
2
4
6
8
10
end of stress
TIME (days)
Site A
Site B
Site C

As before in experiment IV, statistical tests were performed, and significant difference at P = 0.05 was found to be between plants from A_1 and C; B_3 and C in the amount of proline accumulated under the stress, with plants from A_1 and B_3, being within the frost depression area, accumulating relatively more proline than Site C which was well exposed.

Table 12

Significance tests on the Proline Levels in Plants from Different Points within the Three Sites under Low Temperature Stress

	A_4	A_7	B_1	B_2	B_3	C
A_1	N.S	*	N.S	-	-	*
A_4	-	N.S	-	-	-	-
A_7	N.S	-	-	-	N.S	N.S
B_1	-	-	-	N.S	N.S	N.S
B_3	-	-	-	-	-	*

* = significant at 0.05

N.S = not significant

Sample size was 18

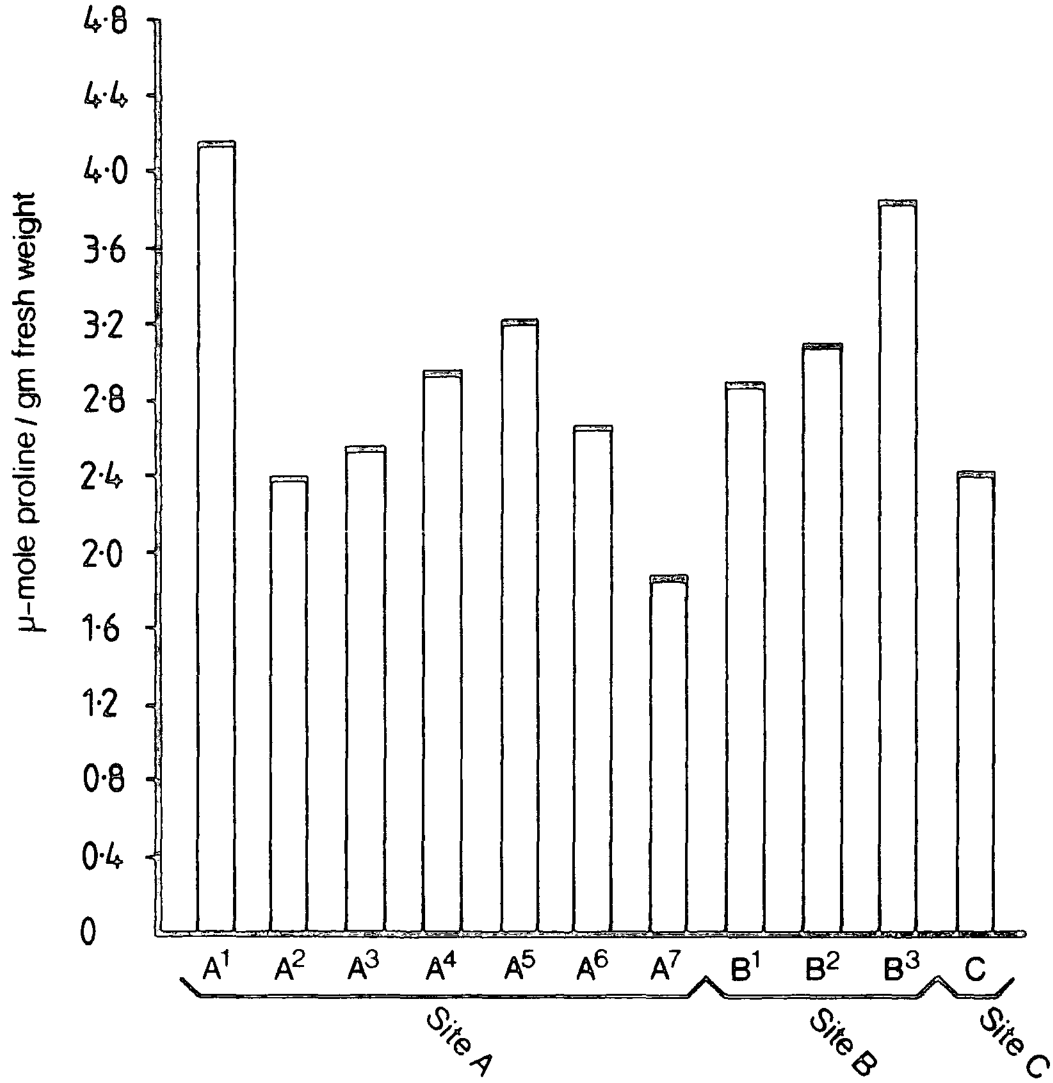
Student t-test was done

Plants from A_1 were significantly different from those from A_7 with the former accumulating more proline, thus being more cold resistant. Such attitude was attributed to the location of A_1 at the bottom of slope "A" within the frost depression where the plants usually encounter lower temperatures.

Although the plants studied responded to both water and temperature stress, however, under low temperature stress, plants in different points, differentially responded. A conclusion was drawn that the temperature factor

Figure 7: Levels of proline in Sesleria plants, from different points within the three sites, under the low temperature (5°C) treatment.

figure 7



μ-mole proline / gm fresh weight
4·8
4·4
4·0
3·6
3·2
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0
A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
C
Site A
Site B
Site C

was of more critical effect, and the differential plant response could explain the wide spread of Sesleria caerulea over the British Isles.

Chapter 4

DISCUSSION

It has been suggested by Turesson, 1930, that climatic conditions enormously affect the nature of the biotype group from different habitats, in such a way that some particular species of plant may consist of a variety of ecotypes genetically dissimilar selected by the nature of the environmental conditions within which the population is growing.

Observations made by Clausen, Keck and Hiesey (1948) revealed differences in the height and flowering of Achillea landulosa over an altitudinal range in the Sierra Nevada. These differences were largely maintained when seeds were collected and grown under uniform conditions. Leaf morphology in Abies balsamina was found to vary with altitude (Meyers and Bormann, 1963). Ward (1969), Pearcy and Ward (1972) found changes in the phenology and growth of Deschampsia caespitosa with plants from high elevations having shorter growth period.

This present performed work aimed at exploring the relationship between the morphology and physiology of Sesleria caerulea and its environment, and to what extent such variations were due to the plastic response of the plant or the expression of the genotype. The approach was to look into the response when plants from different selected sites were brought under uniform growth conditions.

Indeed West (1975), Ferreira (1978) confirmed the existence of considerable variations in the morphology and physiology of Sesleria caerulea populations from various selected sites within a small area of Gassop Vale in County Durham. But in this study although the area was of the same size as that investigated by West and Ferreira in Gassop Vale, still there were no great variations between plants from the selected sites within Thrislington Common as encountered in Gassop Vale. Factors of the environment, especially the edaphic ones, were less variable between the sites within Thrislington Common which indeed explained the results observed.

The effects of a period of lowered tissue water potential on plant growth include decreased accumulation of dry matter, decreased extension growth and changes in morphology (Gates, 1968). Such responses have been ascribed to decreased photosynthesis (Brix, 1962), decreased turgor inhibiting cell expansion (Ordin, 1960) and effects of lowered cell water potential on metabolism (Barnett and Naylor, 1966). Of these three possibilities, effects of lowered water potential on metabolism appear to be the most likely cause of such specific effects of water stress on plant morphogenesis as the inhibition of floral induction (Aspinall and Husain, 1970) and of gametogenesis in cereals (Skazkin and Lukomskaya, 1962).

Several aspects of metabolism have been shown to be affected by water deficit, including inhibition of proline synthesis and changes in amino acid metabolism (Barnett and Naylor, 1966). Inhibition of protein synthesis and hydrolysis of existing proteins result in profound changes in the concentrations of free amino acids in the tissues (Barnett and Naylor, 1966; Routley, 1966; Saurier, et al., 1968).

Although the concentration of some amino acids declines during water stress, there is an overall increase in the concentration of soluble nitrogenous compounds (Chen et al., 1964). The most pronounced component is the amino acid proline.

However, Sesleria caerulea, in this study responded to the water stress by accumulating the amino acid proline, yet differences between plants from the different sites were indeed very small. Such differences encountered, though small, were due to the slightly different habitats from which the plants were taken. Site A was a gentle slope (26^{o}) partially exposed, Site B steep, sharp slope (11^{o}) almost within the frost depression and Site C was the driest, fully exposed site. It was interpreted that plants from the drier site C seemed to have a wider range of drought tolerance and responded by accumulating a comparatively lower proline to stand the level of stress imposed.

Another important limiting factor that plants may encounter along altitudinal gradients is low soil temperature, and altitudinal races may differ in their ability to grow in cold soils (Spomer and Salisbury, 1968; Anderson, 1971).

One mechanism by which low soil temperature might limit plant growth is by decreasing the permeability of root membrane to water (Kramer, 1942, 1969) resulting in decreased photosynthesis, either through direct effects on photochemical capacity (Nir and Poljakoff, Mayber, 1967), or indirect effects through stomatal aperature (Troughton, 1969).

The nutrient uptake at the root surface is an active process depending upon metabolic energy. As a consequence cold soils might have a more severe effect on nutrient uptake than on water uptake, where the chilling response is primarily physical (Kramer, 1969).

In this particular study, the response of Sesleria caerulea under the cold stress was found to be differential, with some plants accumulating higher levels of proline than others. It was interpreted that the temperature factor was more crucial and plants at the bottom of slopes A and B - within the frost depression - have accumulated the highest mean proline.

It appeared as though the habitat characteristics of the sites selected were not variable enough to justify the existence of different ecotypes within the population, contrary to what was found by West (1975) and Ferreira (1978) among *Sesleria* population growing in a comparable sized area within Cassop Vale in County Durham.

APPENDIX A

1. Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 0.
2. Ditto - on Day 15.
3. Ditto - on Day 30.
4. Ditto - on Day 45.
5. Ditto - on Day 60.
6. Ditto - on Day 75.
7. Leaf Length (mm) measurements, of Flowering Plants from Site "A", on Day 0.
8. Ditto - on Day 15.
9. Ditto - on Day 30.
10. Ditto - on Day 45.
11. Ditto - on Day 60.
12. Ditto - on Day 75.
13. Inflorescence Stalk Length (mm) of Plants from Site "A", on Day 0.
14. Ditto - on Day 15.
15. Ditto - on Day 30.
16. Ditto - on Day 45.
17. Ditto - on Day 60.
18. Ditto - on Day 75.
19. Leaf Length (mm) measurements, of Non-Flowering Plants from Sites "B" and "C" on Day 0.
20. Ditto - on Day 15.
21. Ditto - on Day 30.
22. Ditto - on Day 45.
23. Ditto - on Day 60.
24. Ditto - on Day 75.

25. Leaf Length (mm) measurements, of Flowering Plants from Sites "B" and "C" on Day 0.

26. Ditto - on Day 15.

27. Ditto - on Day 30.

28. Ditto - on Day 45.

29. Ditto - on Day 60.

30. Ditto - on Day 75.

31. Inflorescence Stalk Length (mm) of Plants from Sites "B" and "C" on Day 0.

32. Ditto - on Day 15.

33. Ditto - on Day 30.

34. Ditto - on Day 45.

35. Ditto - on Day 60.

36. Ditto - on Day 75.

Table 1: Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 0.

	A1	A2	A3	A4	A5	A6	A7
1.	47	25	85	50	65	139	138
2.	41	37	145	15	85	63	176
3.	39	60	136	94	90	53	155
4.	45	26	75	96	31	160	150
5.	64	41	77	33	125	45	40
6.	32	78	109	87	112	69	145
7.	130	14	20	59	135	78	73
8.	20	27	110	88	90	29	102
9.	34	36	17	71	112	85	90
10.	35	80	35	43	98	85	145
11.	70	115	98	68	90	80	160
12.	60	21	129	97	49	42	69
13.	87	38	72	61	70	54	147
14.	51	51	62	60	21	35	265
15.	47	25	17	55	58	31	88
16.	57	37	76	114	22	22	100
17.	105	37	65	28	51	25	82
18.	60	25	93	58	60	120	76
19.	38	36	23	70	46	95	60
20.	42	25	50	24	38	80	67
21.	73	35	48	35	46	63	97
22.	74	40	43	65	23	55	106
23.	25	39	20	25	42	73	120
24.	97	66	27	48	55	103	39
25.	73	22	28	113	44	99	83
26.	33	29	24	19	26	65	115
27.	60	39	19	36	40	130	100
28.	44	30	23	37	75	82	140
29.	69	47	45	77	46	40	70
30.	79	58	62	145	100	105	98
31.	36	28	38	132	103	83	100
32.	33	36	56	121	112	86	52
33.	83	63	115	125	117	55	40
34.	43	36	99	58	86	59	44
35.	41	34	169	138	90	85	39
36.	50	30	52	102	136	101	110
37.	37	52	55	146	99	30	36
38.	42	30	59	163	87	29	36
39.	64	35	56	120	37	25	49
40.	30	18	25	111	73	121	63

Table 2: Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 15.

	A1	A2	A3	A4	A5	A6	A7
1.	40	156	157	157	120	190	56
2.	136	155	37	105	123	110	133
3.	70	72	165	165	115	85	141
4.	85	115	100	115	175	150	79
5.	136	145	76	157	124	42	133
6.	110	164	160	56	143	166	110
7.	75	103	137	156	160	65	136
8.	146	109	40	99	97	146	194
9.	85	153	86	139	195	170	106
10.	140	140	145	73	150	97	169
11.	83	110	117	155	160	125	100
12.	120	104	132	62	129	110	113
13.	95	123	85	159	184	57	60
14.	135	147	120	126	109	63	154
15.	103	171	134	56	155	146	135
16.	107	117	115	95	174	52	143
17.	76	157	130	76	155	182	175
18.	135	145	99	152	113	122	55
19.	187	149	65	154	151	147	151
20.	164	80	66	120	117	170	215
21.	144	80	95	148	116	205	81
22.	107	185	74	141	139	160	102
23.	156	167	100	129	57	92	167
24.	160	150	43	136	74	74	192
25.	165	168	71	149	113	123	155
26.	118	84	62	127	94	90	114
27.	100	122	53	117	85	150	166
28.	103	205	60	141	111	130	230
29.	137	146	195	95	152	96	119
30.	190	80	89	93	125	127	190
31.	118	70	138	82	120	195	63
32.	117	95	125	85	158	73	172
33.	110	139	146	161	160	132	207
34.	184	207	71	80	141	143	71
35.	120	165	121	118	120	206	96
36.	187	130	58	50	142	125	107
37.	70	90	183	57	135	165	103
38.	83	186	76	36	120	100	90
39.	68	197	137	129	108	65	27
40.	85	123	104	80	60	171	50

Table 3: Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 30.

	A1	A2	A3	A4	A5	A6	A7
1.	160	76	130	160	142	160	150
2.	110	180	52	150	79	133	140
3.	130	111	116	90	103	205	152
4.	70	175	159	72	150	130	129
5.	160	155	88	175	40	79	110
6.	105	125	151	155	110	153	142
7.	86	96	135	85	123	93	149
8.	134	110	107	170	181	150	163
9.	157	184	87	210	90	180	117
10.	89	108	46	143	165	125	150
11.	58	203	90	172	170	101	123
12.	155	143	61	110	90	169	190
13.	73	92	160	190	50	96	80
14.	90	85	163	124	85	137	120
15.	141	145	104	130	130	168	160
16.	159	183	82	97	130	80	120
17.	180	184	166	80	112	136	186
18.	103	81	71	103	113	115	140
19.	81	185	60	124	162	210	141
20.	176	130	142	192	108	113	180
21.	125	125	90	110	190	186	145
22.	143	116	129	155	159	206	95
23.	133	160	142	170	108	223	130
24.	84	115	86	85	165	123	162
25.	85	135	172	115	100	113	125
26.	180	99	176	63	132	152	173
27.	50	121	108	123	167	107	112
28.	75	160	98	176	118	122	132
29.	75	85	140	180	125	140	157
30.	135	130	160	96	57	80	190
31.	132	62	122	153	80	145	150
32.	133	96	213	52	79	146	89
33.	167	183	138	103	156	70	129
34.	172	115	106	120	83	76	180
35.	132	163	100	122	90	95	150
36.	94	145	111	183	90	160	116
37.	179	103	72	132	135	190	150
38.	135	137	141	105	110	75	125
39.	103	117	122	147	139	116	130
40.	189	159	136	149	55	93	155

Table 4: Leaf Length (mm) Measurements, of Non-Flowering Plants from Site "A", on Day 45.

	A1	A2	A3	A4	A5	A6	A7
1.	170	190	103	35	15	175	46
2.	75	156	120	115	90	87	125
3.	25	130	150	170	155	93	95
4.	122	91	90	150	113	62	54
5.	99	144	100	133	60	61	130
6.	160	42	93	125	85	70	120
7.	80	105	69	65	57	145	50
8.	129	66	45	123	90	130	86
9.	56	110	80	140	125	60	129
10.	150	115	57	86	150	140	95
11.	92	123	110	105	100	80	42
12.	80	124	30	139	50	160	66
13.	72	113	17	150	160	165	99
14.	110	104	30	60	133	110	56
15.	60	67	48	110	140	190	145
16.	30	50	50	73	122	60	27
17.	135	129	48	140	86	90	49
18.	110	95	165	69	80	150	26
19.	60	120	140	102	130	80	39
20.	50	125	65	95	140	40	36
21.	90	123	143	103	100	100	35
22.	96	79	160	88	38	150	57
23.	93	107	129	15	90	70	50
24.	100	66	165	70	50	40	56
25.	63	122	60	45	40	105	72
26.	94	140	49	90	33	75	100
27.	110	115	93	60	156	103	90
28.	100	142	80	25	156	70	121
29.	102	71	100	115	110	145	139
30.	125	120	86	50	110	59	53
31.	81	115	35	105	152	120	82
32.	112	130	120	30	140	65	54
33.	156	76	118	75	86	70	50
34.	129	132	130	63	110	51	101
35.	120	33	105	54	130	32	45
36.	110	135	50	130	25	35	159
37.	115	123	120	153	123	130	205
38.	112	80	17	130	40	90	175
39.	139	116	70	145	76	95	120
40.	112	119	96	140	100	80	30

Table 5: Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 60.

	A1	A2	A3	A4	A5	A6	A7
1.	80	80	132	163	170	136	85
2.	120	143	111	110	51	215	105
3.	112	142	154	185	200	136	145
4.	145	165	135	138	180	175	217
5.	101	117	136	161	163	175	96
6.	61	153	145	190	140	116	100
7.	139	202	101	131	196	186	143
8.	180	121	176	125	111	171	150
9.	104	143	141	200	170	183	100
10.	111	163	221	228	145	149	93
11.	160	125	136	142	220	141	142
12.	103	145	54	181	160	142	200
13.	95	108	160	135	110	193	50
14.	103	82	114	150	180	145	113
15.	142	130	171	112	125	120	143
16.	101	105	120	201	176	153	160
17.	113	160	156	225	216	180	176
18.	171	109	155	205	171	108	126
19.	110	141	60	80	133	113	200
20.	132	120	76	106	135	190	82
21.	130	81	182	137	170	163	80
22.	150	160	111	100	157	171	161
23.	72	205	152	170	130	170	218
24.	93	132	160	240	192	143	121
25.	140	165	165	140	130	142	151
26.	142	103	141	145	137	130	60
27.	113	172	130	160	135	80	175
28.	110	126	140	116	145	155	86
29.	130	81	163	121	125	130	170
30.	111	128	104	145	120	160	140
31.	80	173	166	181	135	190	110
32.	157	125	132	206	145	169	151
33.	192	161	131	155	155	124	140
34.	115	120	158	119	160	140	50
35.	130	160	181	155	150	161	80
36.	96	141	173	70	170	140	120
37.	110	145	121	202	181	175	119
38.	130	165	153	185	150	180	75
39.	86	162	125	95	103	160	100
40.	120	150	177	91	110	221	75

Table 6: Leaf Length (mm) measurements, of Non-Flowering Plants from Site "A", on Day 75.

	A1	A2	A3	A4	A5	A6	A7
1.	175	210	150	105	170	123	165
2.	90	145	105	134	140	130	173
3.	152	89	230	185	160	184	146
4.	130	131	96	143	155	185	130
5.	155	130	99	233	170	203	174
6.	123	106	100	181	120	115	175
7.	115	163	192	185	129	200	210
8.	98	183	64	75	114	140	160
9.	131	115	131	122	85	199	140
10.	140	190	113	180	81	100	139
11.	100	146	129	176	172	98	110
12.	95	75	156	133	145	63	151
13.	130	159	81	170	73	49	105
14.	125	140	62	180	109	105	180
15.	130	100	170	173	105	88	145
16.	167	200	103	210	133	163	163
17.	140	131	220	160	65	224	130
18.	115	192	198	185	145	165	181
19.	180	152	130	165	175	200	91
20.	155	138	60	120	126	210	191
21.	99	52	155	60	170	250	
22.	127	170	72	115	123	183	193
23.	131	105	100	120	105	160	135
24.	163	86	116	140	186	110	145
25.	164	133	111	180	131	240	155
26.	122	220	75	195	165	142	140
27.	130	100	72	120	176	110	290
28.	170	200	110	146	149	211	185
29.	165	191	120	135	71	213	170
30.	120	160	120	153	135	201	110
31.	105	72	90	125	81	192	67
32.	105	200	115	133	110	150	153
33.	152	136	71	170	180	190	150
34.	113	125	106	164	203	175	170
35.	120	110	67	161	89	137	100
36.	140	173	70	120	43	212	150
37.	145	107	53	175	124	182	213
38.	95	185	129	101	142	180	149
39.	169	130	140	181	113	165	220
40.	113	95	113	175	170	130	190

Table 7: Leaf Length (mm) measurements, of Flowering Plants from Site "A", on Day 0.

	A1	A2	A3	A4	A5	A6	A7
1.	45	113	152	170	125	24	173
2.	41	18	147	142	90	85	98
3.	50	90	165	130	97	77	118
4.	60	130	123	130	79	101	96
5.	86	44	193	105	135	80	110
6.	25	89	175	116	32	86	94
7.	55	80	53	126	132	56	70
8.	100	98	49	139	92	66	55
9.	31	110	36	110	39	63	41
10.	61	75	47	100	73	72	100
11.	62	45	152	115	52	55	53
12.	38	69	45	88	68	24	63
13.	102	70	33	47	84	53	40
14.	28	73	61	55	44	92	30
15.	40	88	99	133	68	67	48
16.	15	87	30	53	70	67	24
17.	117	21	75	116	64	33	60
18.	46	69	139	95	65	80	80
19.	28	104	61	99	35	40	42
20.	97	88	129	68	135	76	53
21.	46	112	39	115	55	173	73
22.	64	35	87	69	113	63	75
23.	96	73	27	100	38	36	23
24.	60	126	66	96	83	85	103
25.	127	47	118	50	75	29	20
26.	68	50	32	90	63	74	23
27.	78	22	20	47	47	40	45
28.	110	19	28	66	70	62	43
29.	52	90	62	112	59	40	62
30.	72	49	38	46	59	118	23
31.	67	115	56	47	24	65	22
32.	69	60	115	60	92	34	50
33.	29	51	99	42	49	30	39
34.	91	116	169	64	74	28	29
35.	57	117	52	39	56	85	47
36.	41	114	55	30	30	21	108
37.	90	60	59	116	32	66	175
38.	103	82	56	50	47	87	146
39.	67	46	25	78	34	36	20
40.	89	105	142	155	140	84	138

Table 8: Leaf Length (mm) measurements, of Flowering Plants from Site "A", on Day 15.

	A1	A2	A3	A4	A5	A6	A7
1.	120	63	98	133	150	115	95
2.	118	114	105	39	71	176	110
3.	157	80	126	92	55	135	94
4.	136	137	102	119	149	94	153
5.	77	180	64	129	55	110	33
6.	107	138	50	139	97	70	140
7.	132	78	117	115	39	113	155
8.	79	140	127	113	96	140	120
9.	114	140	110	97	139	150	24
10.	115	62	98	81	56	156	107
11.	118	75	91	100	161	116	138
12.	78	91	62	110	100	50	56
13.	110	121	37	111	130	137	29
14.	76	140	114	98	132	125	50
15.	113	97	120	95	74	135	97
16.	60	76	123	96	126	172	123
17.	102	57	60	118	122	70	120
18.	81	62	81	58	129	82	159
19.	87	60	113	170	66	70	106
20.	153	60	103	25	44	160	133
21.	80	60	107	53	98	90	80
22.	88	132	115	45	115	122	160
23.	87	76	68	140	90	80	89
24.	115	119	107	60	75	125	90
25.	68	131	135	170	89	123	96
26.	47	62	106	120	92	87	55
27.	70	120	63	35	139	90	150
28.	110	122	111	89	175	40	53
29.	39	62	93	120	115	114	146
30.	95	114	95	126	103	125	72
31.	82	163	67	100	132	120	102
32.	60	96	74	103	140	62	129
33.	40	123	110	183	82	105	130
34.	120	53	77	51	100	49	123
35.	105	105	68	65	72	131	122
36.	98	150	93	50	136	120	145
37.	136	83	107	110	125	165	69
38.	86	117	102	30	125	106	56
39.	116	143	91	165	140	109	153
40.	71	65	74	119	93	87	120

Table 9: Leaf Length (mm) measurements, of Flowering Plants from Site "A", on Day 30.

	A1	A2	A3	A4	A5	A6	A7
1.	125	152	45	83	80	153	153
2.	100	98	104	130	49	73	121
3.	160	154	78	210	132	148	210
4.	86	123	83	130	132	143	106
5.	65	113	145	125	123	128	98
6.	67	73	60	80	121	76	60
7.	160	159	125	147	96	34	95
8.	160	140	52	72	126	111	172
9.	115	68	130	161	93	140	111
10.	135	129	51	122	133	135	135
11.	83	148	137	137	152	67	89
12.	91	131	103	112	150	118	67
13.	58	75	54	100	94	100	56
14.	125	143	92	155	145	133	50
15.	113	130	147	117	54	140	165
16.	106	140	94	147	70	82	123
17.	91	111	69	130	44	93	95
18.	180	106	147	142	113	106	130
19.	160	107	106	110	142	153	93
20.	120	105	132	67	140	140	150
21.	110	90	93	140	82	60	74
22.	160	125	125	70	142	91	165
23.	103	131	132	141	63	93	86
24.	142	152	126	103	150	100	170
25.	160	141	114	66	57	132	76
26.	65	60	120	170	103	110	45
27.	145	120	106	76	106	95	132
28.	132	116	90	84	66	142	105
29.	57	110	80	162	80	137	46
30.	145	48	107	60	105	140	81
31.	132	75	82	122	55	73	149
32.	145	109	70	95	149	60	80
33.	113	138	103	142	80	75	155
34.	160	124	101	70	95	110	85
35.	80	157	84	175	155	86	140
36.	120	163	109	91	100	119	80
37.	133	106	38	180	113	97	99
38.	77	119	96	60	152	88	180
39.	151	174	51	171	98	125	75
40.	105	120	72	80	92	72	145

Table 10: Leaf Length (mm) measurements, of Flowering Plants from Site *A* on Day 45.

	A1	A2	A3	A4	A5	A6	A7
1.	100	50	125	120	100	95	68
2.	92	81	100	118	160	52	100
3.	110	139	120	90	90	83	55
4.	13	86	55	50	130	65	82
5.	90	75	130	122	90	84	71
6.	80	110	80	93	93	45	120
7.	55	50	30	120	145	106	130
8.	35	25	65	110	94	158	40
9.	38	57	70	125	29	90	153
10.	50	91	40	85	60	45	160
11.	85	112	79	60	90	50	90
12.	123	29	45	35	55	115	27
13.	62	43	50	121	110	70	31
14.	130	55	90	160	90	60	71
15.	120	100	100	02	53	58	40
16.	120	96	50	100	80	90	85
17.	92	115	60	83	58	72	36
18.	118	49	20	60	96	160	27
19.	89	65	31	83	76	43	70
20.	59	100	37	90	115	64	36
21.	35	25	36	60	130	46	90
22.	120	70	45	40	70	49	54
23.	41	120	70	45	50	60	63
24.	132	33	69	26	59	93	110
25.	90	127	63	51	143	60	49
26.	125	70	67	70	56	47	103
27.	85	115	80	92	143	70	123
28.	93	30	110	87	40	42	31
29.	165	115	38	76	72	85	64
30.	130	100	100	72	90	26	105
31.	99	103	120	85	75	120	65
32.	87	136	82	21	130	96	30
33.	65	124	81	40	88	70	92
34.	80	132	61	30	111	60	80
35.	85	81	65	53	70	90	161
36.	43	126	123	37	113	90	33
37.	140	86	70	25	79	62	54
38.	105	98	105	18	80	101	70
39.	40	91	110	29	83	80	60
40.	100	42	20	118	56	80	93

Table 11: Leaf Length (mm) measurements, of Flowering Plants from Site *A*, on Day 60.

	A1	A2	A3	A4	A5	A6	A7
1.	125	94	110	211	120	122	156
2.	133	79	70	125	63	103	100
3.	151	134	132	81	110	160	150
4.	148	61	175	125	123	92	170
5.	132	100	110	102	108	130	160
6.	72	66	131	130	129	130	141
7.	117	125	153	196	150	110	121
8.	111	107	185	132	83	110	110
9.	110	154	144	115	120	170	160
10.	130	80	112	102	129	180	76
11.	122	70	172	156	140	92	110
12.	100	63	151	155	52	153	141
13.	92	123	170	100	172	170	162
14.	142	110	120	110	60	95	150
15.	120	94	90	141	120	100	101
16.	143	152	130	130	151	110	142
17.	120	151	140	200	140	120	95
18.	106	106	120	170	152	96	103
19.	127	140	150	142	62	92	110
20.	116	87	130	139	60	165	172
21.	80	131	84	141	130	115	142
22.	118	121	140	135	125	111	
23.	70	80	170	109	101	172	142
24.	108	133	92	126	160	80	169
25.	110	95	184	130	90	155	151
26.	113	130	180	81	73	104	131
27.	140	66	112	143	120	131	120
28.	82	120	160	145	172	120	145
29.	108	160	122	155	65	110	131
30.	141	122	110	140	160	120	140
31.	131	140	106	100	142	70	140
32.	140	125	186	95	125	77	81
33.	120	161	115	200	180	169	142
34.	110	100	110	100	100	230	95
35.	110	80	136	36	123	150	115
36.	97	113	80	120	141	160	91
37.	91	130	170	121	140	150	120
38.	130	154	130	82	101	153	160
39.	122	116	166	123	150	96	159
40.	90	140	133	72	120	152	160

Table 12: Leaf Length (mm) measurements, of Flowering Plants from Site "A", on Day 75.

	A1	A2	A3	A4	A5	A6	A7
1.	143	90	62	160	46	145	240
2.	120	80	160	126	154	47	130
3.	102	166	110	147	148	180	171
4.	150	111	135	150	153	125	125
5.	120	90	72	170	162	155	75
6.	60	119	91	142	200	46	145
7.	111	123	45	170	80	135	161
8.	80	167	85	141	52	130	155
9.	86	133	96	105	151	135	141
10.	150	131	153	140	155	165	140
11.	140	155	158	130	117	103	65
12.	63	191	107	160	141	45	125
13.	126	130	146	161	190	75	80
14.	155	190	110	181	120	124	171
15.	125	142	89	172	155	118	150
16.	156	182	120	140	60	145	110
17.	133	135	140	190	55	135	145
18.	120	68	128	144	19	141	129
19.	140	85	82	173	181	120	200
20.	93	70	76	145	120	150	145
21.	122	115	65	176	100	253	60
22.	162	85	137	169	136	140	65
23.	120	155	75	127	115	195	119
24.	125	122	105	141	100	196	110
25.	130	140	163	172	119	95	65
26.	120	153	135	130	90	140	190
27.	130	90	74	138	135	145	59
28.	110	160	65	195	55	160	155
29.	90	110	133	150	61	135	210
30.	105	125	56	140	34	125	155
31.	95	125	117	120	80	55	42
32.	140	106	100	120	60	190	170
33.	56	120	100	185	132	110	140
34.	146	130	60	180	48	115	155
35.	120	92	160	147	183	53	60
36.	120	120	90	142	135	140	112
37.	85	117	145	122	162	180	123
38.	113	97	85	203	150	140	82
39.	90	140	60	160	120	140	80
40.	80	109	103	198	162	95	91

Table 13: Inflorescence Stalk Length (mm) of Plants from Site "A" on Day 0.

	A1	A2	A3	A4	A5	A6	A7
1.	151	153	3-3	226	245	311	218
2.	194	190	302	241	181	270	214
3.	161	250	321	217	210	270	230
4.	246	235	315	191	177	233	312
5.	146	182	288	149	304	185	201
6.	240	202	293	182	226	272	204
7.	250	192	228	170	241	230	208
8.	163	217	269	226	217	211	218
9.	180	220	250	267	191	201	229
10.	263	205	285	205	149	226	270
11.	153	172	297	163	182	229	339
12.	182	166	267	150	170	223	176
13.	222	206	244	186	226	244	310
14.	185	153	282	216	267	229	215
15.	234	153	270	165	205	237	241
16.	226	205	315	163	163	205	162
17.	237	164	230	136	150	247	168
18.	145	211	259	183	186	234	195
19.	222	177	252	226	275	251	234
20.	250	120	299	197	228	207	238
21.	210	163	234	225	310	262	235
22.	230	110	170	287	156	285	253
23.	265	114	228	195	233	216	185
24.	94	117	256	226	145	215	176
25.	215	196	282	253	215	204	218
26.	258	198	285	270	279	260	350
27.	266	152	277	246	188	255	203
28.	181	155	315	231	198	253	204
29.	293	180	335	249	256	252	249
30.	212	138	220	255	167	232	174
31.	251	192	296	251	202	200	250
32.	244	172	305	195	229	229	295
33.	232	281	284	138	97	164	234
34.	213	174	327	245	112	192	170
35.	178	163	310	154	145	250	225
36.	185	159	287	245	173	300	330
37.	220	66	283	181	117	261	292
38.	165	170	189	210	139	251	196
39.	180	177	151	177	204	195	168
40.	143	230	277	304	235	310	210

Table 14: Inflorescence Stalk Length (mm) of Plants from Site "A", on Day 15.

	A1	A2	A3	A4	A5	A6	A7
1.	160	360	280	463	200	470	502
2.	162	282	201	483	219	493	478
3.	285	270	415	210	483	472	430
4.	95	373	360	211	277	533	267
5.	125	161	372	182	343	352	458
6.	145	223	336	270	243	390	107
7.	345	160	337	423	241	435	431
8.	266	340	465	442	245	201	451
9.	297	330	409	234	483	190	403
10.	320	210	216	256	235	255	460
11.	141	366	272	166	261	324	340
12.	246	467	425	312	301	203	390
13.	380	280	402	410	319	407	425
14.	320	304	403	245	289	231	107
15.	330	360	432	372	370	327	82
16.	366	174	212	250	285	241	143
17.	205	221	322	145	245	394	164
18.	185	250	313	426	249	446	76
19.	277	183	450	232	277	441	140
20.	260	260	417	413	232	500	92
21.	410	192	305	493	223	368	87
22.	330	200	245	425	177	335	54
23.	356	192	335	459	238	303	132
24.	150	240	368	200	276	380	146
25.	150	180	250	223	126	177	371
26.	302	110	155	246	206	435	560
27.	205	223	280	223	250	207	99
28.	220	272	161	442	235	235	247
29.	346	150	237	446	194	210	224
30.	271	170	316	170	243	160	555
31.	355	285	295	205	212	200	443
32.	352	286	452	211	132	187	531
33.	270	412	446	160	156	100	401
34.	180	196	415	310	330	230	447
35.	311	205	204	169	372	369	420
36.	303	175	133	346	480	239	427
37.	320	371	430	370	245	241	422
38.	330	320	482	172	217	420	366
39.	150	390	493	176	273	310	437
40.	236	265	265	273	305	219	516

Table 15: Inflorescence Stalk Length(mm) of Plants from Site "A", on Day 30.

	A1	A2	A3	A4	A5	A6	A7
1.	165	290	350	486	293	542	540
2.	239	198	283	490	420	405	163
3.	300	361	335	221	392	250	508
4.	235	342	389	230	220	436	360
5.	210	335	369	512	505	340	455
6.	320	350	212	455	245	235	300
7.	476	300	280	236	165	272	195
8.	391	260	280	468	263	140	363
9.	185	220	374	485	340	181	384
10.	215	298	472	192	403	270	540
11.	176	213	460	430	180	430	370
12.	133	455	322	500	393	170	485
13.	172	505	379	440	310	453	420
14.	403	490	333	325	281	420	286
15.	177	437	587	352	460	285	549
16.	495	490	245	428	251	295	455
17.	260	443	628	440	266	534	170
18.	307	357	215	341	335	503	510
19.	521	340	443	232	265	542	407
20.	502	450	492	390	416	427	352
21.	463	533	448	400	172	352	517
22.	485	377	370	383	215	207	473
23.	519	184	280	435	360	201	430
24.	472	253	514	397	280	380	405
25.	500	419	410	320	290	470	502
26.	280	367	202	210	192	442	303
27.	340	510	300	400	279	444	396
28.	388	399	300	456	343	468	476
29.	292	440	175	424	340	327	411
30.	320	510	290	360	234	300	442
31.	472	500	240	465	210	263	360
32.	204	306	360	200	325	372	387
33.	353	398	460	416	322	194	457
34.	190	443	230	449	263	223	430
35.	246	222	400	500	453	230	480
36.	240	509	370	513	223	225	527
37.	393	559	475	560	207	369	330
38.	421	280	479	345	210	393	380
39.	294	398	240	500	242	315	436
40.	182	472	415	292	210	345	363

Table 16: Inflorescence Stalk Length (mm), of Plants from Site *A*, on Day 45.

	A1	A2	A3	A4	A5	A6	A7
1.	230	135	190	205	281	170	260
2.	183	295	260	320	360	150	308
3.	152	180	243	310	350	170	410
4.	193	242	185	170	385	145	320
5.	162	220	167	160	148	160	306
6.	252	215	205	250	259	183	269
7.	290	275	297	190	250	126	210
8.	340	205	345	310	293	150	200
9.	171	262	329	300	170	176	283
10.	239	223	230	315	270	96	205
11.	129	194	150	253	317	220	240
12.	290	65	249	135	211	130	220
13.	202	150	295	340	325	90	271
14.	206	260	180	283	210	130	362
15.	153	202	232	250	211	274	234
16.	230	195	367	310	261	150	254
17.	169	212	283	245	192	153	285
18.	211	130	221	210	212	250	274
19.	130	185	279	290	185	268	270
20.	204	109	305	260	190	210	233
21.	230	166	271	305	310	182	270
22.	183	163	254	310	285	280	232
23.	123	180	211	315	260	170	240
24.	190	183	320	273	164	272	171
25.	300	210	285	149	186	262	260
26.	270	132	259	133	207	298	263
27.	210	202	220	240	163	245	240
28.	215	189	242	270	154	203	293
29.	240	160	270	208	300	215	230
30.	153	182	160	175	180	152	279
31.	263	160	211	172	142	220	283
32.	205	149	147	293	213	192	243
33.	160	148	232	223	183	213	250
34.	180	201	265	276	121	250	248
35.	196	175	176	193	152	240	280
36.	190	245	195	183	160	211	150
37.	150	280	318	285	195	190	208
38.	259	205	330	129	129	290	250
39.	171	296	315	232	227	173	271
40.	183	205	235	265	161	203	170

Table 17: Inflorescence Stalk Length (mm) of Plants from Site *A*, on Day 60.

	A1	A2	A3	A4	A5	A6	A7
1.	370	367	227	485	540	370	537
2.	192	123	195	487	512	396	422
3.	326	450	196	275	535	386	340
4.	420	342	324	225	340	510	280
5.	325	360	340	240	272	216	476
6.	203	356	229	505	170	387	475
7.	385	396	367	523	220	412	420
8.	362	525	221	542	424	265	565
9.	270	477	336	563	440	260	386
10.	350	285	225	350	183	470	460
11.	283	319	497	455	415	430	320
12.	342	392	331	341	500	420	202
13.	397	376	563	200	459	360	515
14.	162	215	327	401	389	175	392
15.	284	440	540	390	445	230	228
16.	323	370	260	492	394	341	272
17.	166	468	341	346	390	460	360
18.	315	428	322	285	293	280	560
19.	390	291	178	334	450	273	480
20.	415	352	401	598	413	300	482
21.	409	390	300	310	250	221	471
22.	181	300	341	530	403	293	522
23.	210	509	199	305	495	253	508
24.	291	410	351	351	359	430	450
25.	270	290	362	508	220	350	242
26.	220	252	411	489	380	370	586
27.	233	140	380	345	210	385	571
28.	241	370	344	395	330	360	510
29.	270	386	334	119	202	390	385
30.	236	412	387	595	220	160	383
31.	342	500	453	510	429	270	351
32.	300	425	352	340	335	375	356
33.	320	460	313	320	522	420	433
34.	291	340	510	400	420	265	523
35.	242	465	396	332	413	320	260
36.	333	400	496	532	470	200	245
37.	372	243	357	555	480	392	523
38.	373	472	383	404	456	482	510
39.	295	490	362	342	478	300	582
40.	446	382	401	240	223	352	161

Table 18: Inflorescence Stalk Length (mm) of Plants from Site "A", on Day 75.

	A1	A2	A3	A4	A5	A6	A7
1.	500	475	222	250	236	191	210
2.	430	352	430	192	251	241	200
3.	197	330	380	250	155	293	210
4.	130	297	140	325	275	159	167
5.	305	120	170	261	189	141	215
6.	171	215	65	155	271	195	405
7.	303	175	180	252	273	515	330
8.	240	350	165	290	440	159	190
9.	220	420	125	345	290	220	170
10.	165	91	121	190	270	261	159
11.	195	181	246	110	250	250	250
12.	325	265	118	270	210	199	105
13.	255	115	42	260	162	200	101
14.	142	160	102	281	392	342	270
15.	134	309	271	375	360	169	287
16.	140	101	403	281	310	241	181
17.	270	155	160	290	260	239	119
18.	180	410	340	290	217	213	175
19.	133	132	320	343	360	259	80
20.	455	223	352	240	268	251	185
21.	240	370	522	405	226	142	231
22.	220	286	451	570	280	310	161
23.	405	179	392	300	303	485	250
24.	230	308	110	463	200	282	188
25.	145	545	205	251	225	523	200
26.	295	290	251	256	110	162	160
27.	391	182	302	410	173	302	231
28.	205	270	272	295	205	320	280
29.	216	190	295	395	220	395	116
30.	270	210	203	390	210	187	117
31.	230	445	212	270	230	515	123
32.	356	221	420	115	225	242	112
33.	270	240	290	415	325	291	280
34.	195	252	363	595	325	515	283
35.	230	353	600	355	215	355	171
36.	420	155	120	455	310	481	330
37.	161	150	235	445	320	165	248
38.	235	560	565	380	465	512	270
39.	380	375	206	215	260	230	362
40.	259	227	290	240	241	190	481

APPENDIX D

Table 19 : Leaf length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 0

	B_1	B_2	B_3	C
1.	128	94	29	86
2.	99	70	47	109
3.	50	60	80	50
4.	69	53	21	95
5.	66	65	150	45
6.	70	39	80	56
7.	37	43	52	115
8.	30	44	78	60
9.	40	28	141	45
10.	42	68	99	100
11.	23	76	75	77
12.	86	74	110	84
13.	24	94	103	45
14.	27	48	93	80
15.	104	81	78	118
16.	115	19	67	32
17.	93	27	45	29
18.	28	82	91	78
19.	94	36	92	84
20.	34	80	48	33
21.	20	95	77	40
22.	114	55	55	34
23.	29	21	117	60
24.	25	25	54	34
25.	28	36	75	50
26.	55	30	80	19
27.	20	20	53	64
28.	70	42	68	42
29.	139	13	75	16
30.	74	92	160	34
31.	52	85	145	109
32.	80	60	145	133
33.	35	55	125	115
34.	80	121	117	120
35.	58	15	140	65
36.	90	23	114	119
37.	73	140	78	126
38.	70	39	90	75
39.	42	52	55	99

Table 20 : Leaf length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 15

	B_1	B_2	B_3	C
1.	161	145	143	56
2.	150	125	45	142
3.	44	155	200	139
4.	180	40	55	161
5.	165	155	190	76
6.	81	130	87	184
7.	125	115	157	166
8.	187	89	170	96
9.	182	158	110	152
10.	173	139	90	157
11.	68	170	92	184
12.	120	115	90	136
13.	183	84	145	108
14.	97	167	102	145
15.	144	50	105	34
16.	163	83	120	71
17.	160	142	47	125
18.	141	139	70	152
19.	143	110	183	81
20.	140	90	130	86
21.	172	105	150	80
22.	93	110	140	75
23.	72	85	141	105
24.	140	65	105	55
25.	158	133	153	152
26.	43	116	153	81
27.	119	115	82	196
28.	37	83	159	195
29.	46	132	136	179
30.	37	111	156	160
31.	109	164	101	73
32.	146	109	190	110
33.	105	91	171	111
34.	175	76	143	22
35.	122	30	160	151
36.	89	140	130	155
37.	30	144	149	76
38.	145	86	152	79
39.	150	165	150	69
40.	165	77	99	163

Table 21 : Leaf length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 30

	B_1	B_2	B_3	C
1.	134	110	69	155
2.	102	138	60	46
3.	85	59	157	150
4.	118	192	168	120
5.	100	130	83	72
6.	70	213	71	100
7.	106	150	190	110
8.	76	80	219	94
9.	110	70	147	107
10.	126	105	171	142
11.	144	107	23	113
12.	54	150	108	135
13.	69	123	163	92
14.	188	186	144	55
15.	81	63	103	80
16.	145	183	55	101
17.	108	163	130	90
18.	123	76	90	121
19.	145	119	65	123
20.	156	52	145	132
21.	100	192	127	136
22.	132	116	138	140
23.	132	142	185	147
24.	185	145	146	140
25.	140	171	50	172
26.	153	136	150	36
27.	150	192	141	120
28.	92	63	115	154
29.	153	35	150	70
30.	109	145	103	176
31.	147	124	113	152
32.	140	120	96	107
33.	80	91	149	172
34.	155	47	123	115
35.	170	159	152	160
36.	115	170	156	85
37.	90	146	52	140
38.	156	132	102	135
39.	132	136	163	56
40.	130	166	130	140

Table 22 : Leaf length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 45

	B_1	B_2	B_3	C
1.	150	101	89	140
2.	180	76	119	119
3.	78	30	85	200
4.	160	105	57	127
5.	120	35	66	108
6.	120	90	68	150
7.	70	66	99	115
8.	170	75	120	145
9.	85	125	65	190
10.	87	105	49	80
11.	91	139	176	194
12.	180	55	45	125
13.	112	90	82	140
14.	73	55	85	100
15.	110	135	105	220
16.	120	69	110	130
17.	43	53	65	120
18.	80	145	41	100
19.	96	133	180	85
20.	30	73	120	15
21.	36	126	110	90
22.	92	145	103	75
23.	95	92	44	130
24.	93	119	122	63
25.	30	77	178	70
26.	42	83	89	90
27.	32	46	130	80
28.	60	95	113	48
29.	115	90	170	27
30.	50	130	70	45
31.	23	71	69	46
32.	170	140	170	52
33.	69	60	136	54
34.	145	151	156	63
35.	95	92	108	70
36.	90	83	120	39
37.	110	89	82	54
38.	162	146	106	153
39.	170	133	105	70
40.	136	136	150	103

Table 23 : Leaf length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 60

	B_1	B_2	B_3	C
1.	105	110	172	80
2.	205	181	71	191
3.	163	83	187	84
4.	112	72	156	130
5.	140	185	92	226
6.	123	113	165	110
7.	118	107	142	145
8.	174	103	152	87
9.	135	90	148	129
10.	95	137	108	170
11.	75	105	110	150
12.	160	165	215	170
13.	130	60	160	103
14.	157	134	100	201
15.	203	120	140	121
16.	130	93	190	150
17.	126	113	100	146
18.	150	75	150	180
19.	210	144	140	180
20.	230	190	192	100
21.	125	225	83	160
22.	205	80	167	133
23.	133	71	150	156
24.	100	123	100	150
25.	120	152	118	103
26.	123	140	150	184
27.	160	150	91	141
28.	210	120	112	193
29.	155	165	66	129
30.	120	115	147	230
31.	143	153	185	180
32.	84	123	90	95
33.	173	115	150	150
34.	141	152	115	143
35.	145	94	110	215
36.	103	155	132	190
37.	170	165	75	110
38.	120	155	190	126
39.	121	80	166	150
40.	135	110	154	43

Table 24 : Leaf Length (mm) measurements of non-flowering plants from sites "B" and "C" on Day 75

	B_1	B_2	B_3	C
1.	146	141	175	167
2.	91	193	155	280
3.	80	145	170	210
4.	240	202	71	107
5.	145	160	132	140
6.	131	96	150	200
7.	121	97	121	161
8.	140	110	103	150
9.	200	133	170	186
10.	195	142	180	145
11.	130	80	125	150
12.	67	155	119	190
13.	145	213	135	221
14.	128	151	170	247
15.	155	220	150	178
16.	125	151	160	180
17.	120	135	105	201
18.	150	109	141	160
19.	165	65	170	120
20.	150	115	100	145
21.	66	101	120	185
22.	133	115	91	150
23.	156	96	114	180
24.	144	81	138	210
25.	116	151	100	200
26.	130	120	145	235
27.	122	121	75	153
28.	85	160	131	202
29.	190	156	96	155
30.	168	105	170	110
31.	100	95	160	70
32.	141	170	103	255
33.	131	117	115	153
34.	120	148	195	200
35.	156	163	95	174
36.	120	119	142	155
37.	120	159	132	150
38.	150	133	100	260
39.	142	125	70	153
40.	170	182	77	115

APPENDIX E

Table 25 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 0

	B_1	B_2	B_3	C
1.	104	51	136	84
2.	34	77	125	45
3.	69	78	135	63
4.	105	35	148	140
5.	70	51	98	52
6.	26	48	73	53
7.	83	54	95	75
8.	85	94	105	110
9.	98	53	125	75
10.	76	65	123	100
11.	100	92	78	24
12.	50	46	43	68
13.	81	55	121	60
14.	26	49	66	60
15.	47	36	130	48
16.	57	35	111	81
17.	70	44	85	39
18.	40	20	136	20
19.	43	55	70	54
20.	33	57	95	60
21.	29	59	105	105
22.	50	56	84	25
23.	47	21	120	54
24.	60	40	50	136
25.	80	10	37	96
26.	112	34	97	42
27.	90	34	68	46
28.	35	29	55	102
29.	54	59	32	78
30.	34	50	60	74
31.	63	49	110	110
32.	54	64	40	105
33.	21	72	83	85
34.	17	78	45	90
35.	26	58	115	80
36.	46	59	130	48
37.	32	51	65	65
38.	30	47	110	49
39.	61	46	49	24
40.	68	46	35	29

Table 26 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 15

	B_1	B_2	B_3	C
1.	149	95	170	100
2.	130	75	100	75
3.	115	136	36	103
4.	104	111	120	46
5.	87	93	140	80
6.	86	115	90	80
7.	59	74	120	160
8.	160	76	70	55
9.	111	126	183	136
10.	109	170	161	108
11.	64	47	150	110
12.	106	110	175	58
13.	107	128	110	70
14.	100	125	93	123
15.	82	95	90	120
16.	63	175	60	155
17.	122	120	145	110
18.	70	116	150	43
19.	100	126	180	140
20.	116	91	36	23
21.	152	87	58	53
22.	120	40	85	117
23.	123	70	42	162
24.	130	163	80	105
25.	137	113	104	26
26.	120	50	31	100
27.	172	91	36	60
28.	90	110	46	162
29.	72	71	63	154
30.	100	93	150	72
31.	183	85	46	152
32.	121	89	52	80
33.	109	73	43	130
34.	106	83	130	103
35.	100	110	143	97
36.	175	108	80	37
37.	95	70	116	60
38.	156	43	100	139
39.	99	86	132	55
40.	160	82	43	150

Table 27 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 30

	B_1	B_2	B_3	C
1.	122	86	66	88
2.	115	105	71	64
3.	157	145	122	68
4.	53	110	120	83
5.	85	115	58	53
6.	93	70	117	43
7.	125	119	105	120
8.	128	120	89	75
9.	55	120	110	85
10.	123	150	106	113
11.	71	125	90	93
12.	65	120	131	85
13.	133	75	145	81
14.	53	120	62	40
15.	110	133	100	152
16.	132	100	110	79
17.	102	80	115	165
18.	140	65	121	43
19.	155	55	95	70
20.	156	90	90	78
21.	135	73	106	50
22.	107	130	91	115
23.	72	79	140	185
24.	105	110	92	108
25.	162	103	115	94
26.	123	43	163	35
27.	102	60	110	71
28.	125	93	65	70
29.	40	120	121	100
30.	118	100	143	100
31.	100	70	60	103
32.	60	79	55	70
33.	56	60	88	43
34.	112	114	81	74
35.	70	80	140	60
36.	92	75	145	86
37.	64	92	122	95
38.	142	95	43	72
39.	103	85	112	133
40.	141	120	34	51

Table 28 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 45

	B_1	B_2	B_3	C
1.	170	68	135	119
2.	115	50	44	42
3.	110	25	103	102
4.	195	90	36	80
5.	145	125	40	103
6.	155	28	73	100
7.	80	53	100	78
8.	66	67	105	48
9.	55	55	130	80
10.	105	20	33	63
11.	115	37	110	70
12.	40	105	140	85
13.	38	85	90	80
14.	79	55	60	82
15.	55	87	85	79
16.	33	83	53	92
17.	72	80	50	92
18.	90	81	58	63
19.	102	110	140	80
20.	116	90	35	99
21.	75	63	98	86
22.	90	40	29	94
23.	90	93	39	80
24.	62	84	145	46
25.	88	72	60	41
26.	96	59	60	48
27.	165	78	110	35
28.	65	53	90	60
29.	132	70	26	115
30.	145	69	70	90
31.	61	56	30	43
32.	56	50	95	70
33.	122	80	80	93
34.	92	95	55	30
35.	60	25	115	65
36.	73	55	80	115
37.	64	65	91	110
38.	76	40	55	63
39.	60	63	110	56
40.	50	51	41	98

Table 29 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 60

	B_1	B_2	B_3	C
1.	110	146	145	169
2.	180	80	115	90
3.	162	115	160	90
4.	113	84	153	86
5.	125	120	115	131
6.	71	117	135	105
7.	150	80	230	130
8.	125	103	201	132
9.	130	120	85	72
10.	140	145	135	152
11.	130	94	120	111
12.	120	117	121	95
13.	85	116	89	90
14.	91	120	72	35
15.	110	144	126	85
16.	56	117	110	110
17.	110	85	130	170
18.	103	62	80	143
19.	145	85	72	92
20.	113	130	97	116
21.	125	133	122	152
22.	78	146	115	50
23.	171	119	90	103
24.	119	95	120	171
25.	94	128	135	145
26.	97	120	150	130
27.	163	65	82	192
28.	95	91	151	180
29.	140	63	115	140
30.	142	103	160	163
31.	80	126	110	120
32.	140	143	130	120
33.	112	111	135	124
34.	78	13	200	200
35.	140	165	90	131
36.	135	66	150	140
37.	100	125	190	81
38.	142	160	140	147
39.	70	135	125	180
40.	134	83	125	151

Table 30 : Leaf length (mm) measurements of flowering plants from sites "B" and "C" on Day 75

	B_1	B_2	B_3	C
1.	185	91	152	165
2.	151	62	121	105
3.	120	110	115	145
4.	145	77	130	160
5.	107	110	127	161
6.	82	77	138	140
7.	140	100	137	105
8.	120	154	129	165
9.	140	120	161	86
10.	160	172	129	130
11.	170	126	110	166
12.	95	160	132	110
13.	130	77	110	82
14.	100	120	122	150
15.	75	73	129	215
16.	175	131	86	205
17.	175	126	110	113
18.	150	70	115	171
19.	123	165	171	131
20.	120	175	145	103
21.	139	121	93	122
22.	112	160	90	60
23.	130	61	190	220
24.	110	111	101	105
25.	130	151	103	55
26.	135	141	162	116
27.	103	153	130	210
28.	121	176	115	208
29.	123	113	170	220
30.	183	140	98	125
31.	154	110	132	121
32.	123	120	130	215
33.	140	73	115	125
34.	160	95	76	137
35.	182	89	121	135
36.	146	94	131	175
37.	151	41	116	107
38.	139	112	84	191
39.	82	75	110	123
40.	131	112	131	143

APPENDIX F

Table 31 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 0

	B_1	B_2	B_3	C
1.	148	124	150	235
2.	120	105	171	254
3.	220	225	228	305
4.	174	205	255	230
5.	173	156	288	175
6.	193	190	269	185
7.	163	235	185	145
8.	196	136	221	170
9.	181	168	181	189
10.	113	113	287	193
11.	168	152	128	225
12.	240	114	251	253
13.	166	195	179	221
14.	170	150	222	157
15.	230	215	265	181
16.	151	129	193	189
17.	165	160	201	270
18.	149	127	294	224
19.	181	201	224	220
20.	213	112	247	146
21.	188	193	103	280
22.	158	161	160	164
23.	193	162	231	277
24.	165	230	227	223
25.	196	170	232	256
26.	188	135	210	250
27.	167	120	181	100
28.	192	90	172	270
29.	196	120	290	186
30.	187	150	260	261
31.	184	203	232	250
32.	225	145	212	252
33.	136	115	220	282
34.	210	111	182	253
35.	205	110	192	190
36.	276	97	185	192
37.	210	135	301	163
38.	193	133	186	192
39.	263	173	199	147
40.	230	182	215	207

Table 32 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 15

	B_1	B_2	B_3	C
1.	297	410	161	263
2.	227	403	233	210
3.	327	470	187	162
4.	400	460	493	187
5.	317	420	570	225
6.	250	425	282	221
7.	345	331	480	479
8.	470	303	443	416
9.	202	442	293	380
10.	342	460	492	178
11.	344	390	251	415
12.	141	376	287	492
13.	280	362	435	120
14.	253	368	277	271
15.	329	330	215	420
16.	313	386	471	430
17.	388	350	463	490
18.	458	350	403	241
19.	355	456	445	306
20.	360	343	470	503
21.	302	310	411	450
22.	376	296	251	180
23.	376	283	340	220
24.	265	305	183	221
25.	330	240	200	192
26.	222	215	190	452
27.	332	162	190	510
28.	225	285	176	300
29.	480	202	150	283
30.	442	420	133	150
31.	255	288	370	184
32.	404	316	260	480
33.	445	308	203	291
34.	385	330	459	384
35.	403	325	426	340
36.	358	345	511	207
37.	333	318	490	368
38.	362	112	174	388
39.	210	220	145	210
40.	214	225	130	338

Table 33 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 30

	B_1	B_2	B_3	C
1.	150	403	202	203
2.	380	495	210	420
3.	356	430	173	340
4.	383	403	179	482
5.	421	213	225	400
6.	401	352	260	205
7.	432	276	250	203
8.	199	290	305	222
9.	640	165	155	487
10.	503	490	343	524
11.	360	421	420	605
12.	322	409	335	565
13.	295	336	403	565
14.	378	287	450	212
15.	466	436	271	410
16.	322	416	110	457
17.	237	420	210	180
18.	316	452	393	180
19.	280	435	216	399
20.	476	452	186	450
21.	265	422	320	339
22.	460	400	355	152
23.	441	520	450	582
24.	375	505	232	502
25.	381	578	433	309
26.	331	256	261	161
27.	336	269	243	145
28.	340	342	256	223
29.	480	481	339	479
30.	433	370	380	372
31.	390	350	390	370
32.	252	603	220	195
33.	416	370	169	220
34.	440	415	202	225
35.	392	367	170	545
36.	400	332	160	280
37.	445	375	190	93
38.	370	400	250	162
39.	497	395	119	190
40.	270	440	140	103

Table 34 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 45

	B_1	B_2	B_3	C
1.	270	166	210	302
2.	180	80	339	282
3.	305	126	186	140
4.	302	250	231	340
5.	208	175	223	205
6.	210	193	249	265
7.	185	190	260	155
8.	202	259	230	295
9.	223	155	235	260
10.	280	213	236	303
11.	260	184	242	199
12.	221	270	212	256
13.	160	255	240	211
14.	230	196	206	201
15.	191	203	223	300
16.	232	227	191	332
17.	190	164	263	345
18.	260	206	155	300
19.	190	183	243	230
20.	250	182	180	194
21.	340	213	173	340
22.	272	152	170	273
23.	185	230	186	260
24.	263	250	280	330
25.	240	196	151	273
26.	199	297	240	81
27.	240	170	120	240
28.	210	186	123	120
29.	224	140	210	235
30.	166	190	196	229
31.	235	193	153	340
32.	262	262	285	230
33.	373	190	135	221
34.	285	280	160	385
35.	260	269	220	221
36.	233	250	170	169
37.	212	220	252	406
38.	300	260	135	276
39.	290	190	140	235
40.	260	243	120	320

Table 35 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 60

	B_1	B_2	B_3	C
1.	310	202	250	169
2.	400	396	420	90
3.	410	330	390	90
4.	285	475	370	86
5.	581	412	255	131
6.	410	490	322	105
7.	470	466	440	130
8.	345	351	392	132
9.	495	531	562	170
10.	504	390	405	152
11.	470	411	404	111
12.	480	461	370	95
13.	465	453	340	90
14.	525	439	382	135
15.	405	362	335	85
16.	330	360	490	110
17.	485	355	455	170
18.	450	437	420	143
19.	510	445	420	92
20.	385	417	410	116
21.	450	280	285	152
22.	330	325	334	50
23.	315	282	400	133
24.	386	462	340	161
25.	415	301	275	145
26.	403	375	280	240
27.	365	293	283	420
28.	450	392	340	200
29.	225	409	231	210
30.	385	270	170	420
31.	578	390	395	215
32.	360	186	295	242
33.	475	420	150	203
34.	462	223	305	100
35.	405	191	216	280
36.	416	370	352	230
37.	515	340	300	140
38.	340	440	152	290
39.	510	355	260	320
40.	223	425	196	180

Table 36 : Inflorescence stalk length (mm) of plants from sites "B" and "C" on Day 75

	B_1	B_2	B_3	C
1.	305	170	202	140
2.	410	265	210	237
3.	560	360	173	250
4.	410	370	179	180
5.	375	250	225	235
6.	368	142	260	265
7.	245	111	250	230
8.	510	313	305	355
9.	297	310	155	167
10.	510	131	343	204
11.	180	150	420	122
12.	180	195	335	307
13.	230	111	403	81
14.	283	140	450	135
15.	304	220	271	96
16.	379	260	110	149
17.	332	160	210	231
18.	177	185	393	252
19.	257	290	210	170
20.	210	160	186	190
21.	352	232	320	166
22.	263	170	355	369
23.	242	260	440	155
24.	191	180	232	238
25.	181	313	433	261
26.	221	210	261	180
27.	200	260	243	193
28.	463	175	256	285
29.	280	185	339	220
30.	250	231	380	273
31.	362	375	390	133
32.	225	270	220	67
33.	142	386	169	301
34.	210	210	202	106
35.	163	251	170	130
36.	220	432	160	150
37.	280	375	190	265
38.	256	401	250	210
39.	232	440	119	218
40.	205	215	140	110

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