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

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## Abstract

Proline levels and percentage water content of the leaves were measured for a wide range of plant species from several habitats classified as either drought-prone or drought-free. Little differences in initial proline levels or maximum proline levels after wilting intact in the laboratory were found between species taken from the two habitat types.

Similar measurements were made for six species of each of the families, Compositae and Cruciferae. Little differences in initial or maximum proline levels after wilting were found between species of the two families.

Results indicate that proline accumulation is genetically controlled by species. Species which accumulate proline may be more frequent in habitats liable to drought.

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## Introduction

Free proline accumulation has been observed in several higher plants under conditions of stress induced by environmental and laboratory conditions. Drought, salinity, cold temperature and application of solutions of high osmotic potential have been used to induce stress. In addition to proline measurements of plants under stress, measurements have been made of changes in proline content with temperature (Chu et al., 1974) with time of day (Waldren and Teare, 1974), with season and stage of development (Dabrowska, 1974) and of differences in proline content in different plant tissues (Palfi et al., 1974). This study, however, is concerned with the phenomenon of proline accumulation during wilting.

The mechanism producing proline accumulation during wilting is not well understood. A correlation between drought-resistance and potential for proline accumulation has been found by Singh et al., (1973, II). Both Thompson et al. (1966) and Singh et al. (1973, I) found that aerobic conditions were necessary for proline accumulation whereas Palfi et al., (1974) showed that light also was required. Organs containing chlorophyll show higher proline accumulation (Palfi, et al., 1974), yet a number of plants with a chlorophyll deficiency caused by virus showed higher proline accumulation (Perdrizet, 1974). However, this accumulation may be related to the changes in water balance caused by the virus rather than to the chlorophyll deficiency. Stewart et al., (1966, II) found that proline accumulation was greater and most prolonged in wilted leaves with higher sugar and starch content.

Palfi et al., (1974) states that during the development of water-deficit in plants, the synthesis of starch, protein and nucleic acid

in the leaves is reduced and accordingly the growth is arrested. However, photosynthesis occurs and produces mainly essential amino acids, amides and large amounts of proline which are stored and accumulated in organs containing chlorophyll.

Stewart and Lee (1974) suggest, however, that the accumulation of proline may be a stress response resulting from a decreased rate of protein synthesis or due to an increased protein turnover.

The mechanism of proline accumulation is therefore not clearly understood. However a number of observations have been made. Kemble and MacPherson (1964) used excised shoots of perennial rye grass and allowed them to wilt on the laboratory bench. They found that free proline occurred in wilting shoots in amounts greatly in excess of expectation, but only if the shoots were permitted to lose moisture during starvation.

Barnett and Naylor (1966) found that water stress induced a 10 - 100 times accumulation of free proline in the shoots of Bermuda grass.

Ladino clover leaves, of wilting plants from both the field and the greenhouse, accumulated large quantities of proline (Routley, 1966) as did barley under wilting conditions in the greenhouse (Singh et al., 1972). Cynodon dactylon, when stressed osmotically, yielded proline levels 10 - 100 times as high as irrigated controls (Palfi and Juhasz, 1970). In a later study Palfi et al. (1974) surveyed 60 cultivated species from 14 families. Water deficit produced proline accumulation in the entire Solanaceae family and most species of the Leguminosae, Cruciferae, Umbelliferae, Compositae (Tubuliflorae) and Graminae.

Proline levels in leaves of intact sorghum and soybean plants under field conditions of drought stress and of adequate moisture were measured (Waldren and Teare, 1974 and Waldren et al., 1974). Free proline accumulated in drought-stressed plants, but did not increase significantly until plants were visibly drought stressed.

Stewart and Lee (1974) also made measurements in the field. They proposed that high proline levels may occur in plants, such as halophytes, exposed to physiological drought. Salt marsh plants were found to have higher initial proline levels; for example, coastal populations of Armeria maritima Willd. had higher proline levels than inland populations. They hypothesized that this increase is not merely due to stress, but that it is adaptive. Populations of A. maritima were treated in the laboratory with varying salt concentrations. In media of higher salt concentrations the coastal populations accumulated more proline and survived the treatment longer.

This study proposes:

i) to investigate how general is this phenomenon of high proline accumulations under conditions of wilting

ii) to test whether there is a correlation between the degree of proneness to drought of certain habitats and either the initial proline content of the leaves of plants growing in that habitat or the highest levels of proline accumulated during wilting of the plants growing there.

## Materials and Methods

### 1. Choice of habitats

Six habitats were chosen; three were chosen to represent habitats prone to drought and three to represent drought-free habitats. The actual sites used were chosen primarily for their proximity to Durham. As a salt marsh had been examined by Stewart and Lee (1974) and there is no salt marsh near Durham, it was not chosen as one of the habitats for study.

The three drought-susceptible habitats were:

i) a sandy waste ground - an area surrounding the tarmac car park of the Science Site of the University

ii) Lead mine waste - a site near Rookhope in the northern Pennines

iii) sand dunes - a site near Seal Sands at Teesmouth.

The three habitats not prone to drought were:

i) woodland - Little High Wood of the University

ii) river banks - those of the River Wear in Durham City

iii) wet heathland - a site near Quickcleugh in the northern Pennines

### 2. Collection of species

Approximately 6 species were chosen from each habitat. Common species were chosen and an effort was made to include species from a wide variety of families.

Plants were collected with the roots nearly intact. A minimum of approximately 8gms fresh weight of leaf material was needed for each species. This quantity permitted three tests for proline to be run with one replicate on each occasion.

Plants were collected in the morning and brought into the laboratory. Leaf material of each species was tested for proline; for each species, comparable leaves, generally the upper leaves, were chosen. A similar sample was dried in order to obtain the percentage water content of the leaves.

Plants were then allowed to wilt intact. They were tested for proline on subsequent days, the choice of day depending on the rate of wilting and the availability of plant material and laboratory time. Plants varied considerably in the time taken to wilt; the time varying with the species, the habitat of origin and the amount of soil clinging to the roots. An attempt was made to choose leaves in successive stages of wilt. Due to the quantity of plant material required, it was not possible to follow one plant through wilting.

These preliminary experiments were carried out during the month of July, 1975.

3. Method to test for proline

Approximately 600 - 1000 mg of leaf material was used for each species. This sample was divided into two parts and each was weighed. One was dried in an oven for 48 hours at 105°C in order to obtain an estimate of the percentage of water content of the leaves and an estimate of dry weight of the sample. The other part was tested for proline.

Initially proline was measured using the method of Singh (1973, I). The total amino acids were extracted from fresh tissues by homogenizing samples (weight 150 - 200 mg) with 2 ml of methanol-chloroform water (MCW 12:5:1/V) at room temperature.

The homogenate was briefly centrifuged and the clear supernatant collected. The residue was then shaken with a further 2 ml of MCW for 5 minutes and centrifuged. The supernatants were combined and separated into a lower chlorophyll-containing chloroform layer and an upper methanol-water phase by adding water (1.5 ml) and chloroform (1 ml). The upper was dried and used to obtain the proline estimate (Troll and Lindsay, 1955). It was first diluted with 10 ml water and shaken for 10 minutes with Permutit resin. The solution was decanted off the resin into a boiling tube, and 5 ml glacial acetic and 5 ml acidic ninhydrin reagent (125 mg ninhydrin:3 ml glacial acetic: 2 ml 6M orthophosphoric acid) were added. The mixture was held in a boiling water bath for 45 minutes, cooled to room temperature and shaken with a known amount of benzene (5-15 ml). The optical density of the ninhydrin product dissolved in the benzene was measured at 515 nm and the proline concentration estimated from a standard curve.

This method was used extensively with Balsam impatiens, but was found time consuming. In addition, some difficulty in obtaining a good calibration curve arose. For these reasons, a second method was tried and used for all subsequent proline measurements (Bates, Waldren and Teare, 1973).

About 500 mg of leaf material was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and filtered through a Whatman # 2 filter paper. Two ml of the filtrate was combined with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin[1] in a test tube for 1 hour at 100°C. The reaction was terminated in an ice bath, the mixture was extracted with 4 ml toluene (or 8 ml if required) and stirred vigorously. The upper phase was warmed to room

[1]See Appendix A

temperature, and the optical density read at 520 nm using toluene for a blank. The proline content was determined from a standard curve. The concentration was calculated from the equation:

$$[(\text{g proline/ml} \times \text{ml. toluene})\ 115.5\ \text{g}/\mu\text{mole}] / [(\text{g sample})/5] =$$

$\mu$moles proline/g of fresh weight material.

4. Follow-up experiments

The preliminary investigations of the 6 habitats suggested there may be little correlation between the proneness to drought of the habitat and either the initial levels of proline or the levels accumulated after wilting.

For this reason, two families were chosen to be studied in greater detail; Compositae and Cruciferae. Bellis perennis and Sinapis arvensis were used to determine the better technique. Two experiments were tried. In the first instance, plants were allowed to wilt intact and the leaves were tested for proline on days 1, 2, 3, 5, and 7. In the second instance, leaves were detached from the plant and allowed to wilt on the laboratory bench. Proline levels were measured on days 1, 2, 3 and 4. Ten replicates were used on each occasion and the proline was measured using the method of Bates, Waldren and Teare (1973).

Wilting and subsequent death occurred so rapidly during the second treatment that, for the remaining species, the plants were wilted intact.

The other species tested were:

Compositae 1. Sonchus arvensis

2. Cirsium arvense
3. Senecio aquaticus
4. Senecio jacobaea
5. Latuca saligna

Cruciferae
1. Capsella bursa
2. Cakile maritima
3. Brassica chinensis (Var. Chihili)
4. Alliaria officinalis
5. Brassica campestris

The results were expressed as percentage water content of the leaves, μmoles proline/g fresh weight of leaf, μmoles proline/g estimated dry weight, and mg proline/g fresh weight. An accumulation factor was calculated for each species by taking the maximum level of proline attained, expressed in terms of dry weight, and dividing it by the level measured on the first day

## Results

Although the levels of proline determined are expressed both as quantity per unit fresh weight and per unit dry weight, only the quantity expressed per unit dry weight will be discussed. It gives a more realistic figure, although based on an estimate of dry weight; for the dry weight remains nearly constant throughout the experiment, in contrast to the fresh weight which decreases, often considerably, as water loss continues.

Data obtained for the species in drought-prone habitats are given in Tables 1, 2 and 3. Table 1 represents the results for species collected from waste ground. Initial levels of proline ranged from 3.5 to 5.5$\mu$moles/gdw of leaf. Only Sinapis arvensis accumulated large amounts of proline (accumulation factor = 29.2).

Table 2 lists the proline levels of species from lead mine waste. Initial levels range between 3.3 and 15.0$\mu$moles/gdw of leaf. Only Lotus corniculatus showed a conspicuous increase (accumulation factor = 11.4).

Levels for sand dune species are given in Table 3. Initial levels ranged from 9.4 to 33.9$\mu$moles/gdw, with the largest increase observed for Atriplex laciniata.

Tables 4, 5, and 6 list the data obtained for species of habitats not subject to drought. Woodland species are listed in Table 4. Initial levels of proline ranged from 3.6 to 6.8$\mu$moles/gdw. Milium effusum showed the highest accumulation of proline (accumulation factor = 8.2).

Table 5 represent proline levels for heathland species. Proline levels ranged from 3.6 to 17.5$\mu$moles/gdw of leaf. The highest proline levels after wilting were obtained with Juncus effusus (accumulation factor = 8.9).

Table 1

Proline levels, initially and after wilting, of plants collected from waste ground (a drought-prone habitat).

| Species (Family) | | Number of days from collection 1 | 3 | 4 | 6 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Plantago lanceolata (Plantoginaceae) | % water content of leaves | *80.9 | *75.5 | *72.5 | *40.0 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 5.5 | 6.7 | 6.2 | 6.9 | 1.2 |
| | - μmoles/gfw of leaf | 1.1 | 1.6 | 1.9 | 4.1 | |
| | - mg/gfw of leaf | 0.12 | 0.19 | 0.21 | 0.47 | |
| Impatiens glandulifera (Balsaminaceae) | % water content of leaves | *84.4 | *88.6 | *83.5 | *78.0 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 5.0 | 6.7 | 8.6 | 4.9 | 1.8 |
| | - μmoles/gfw of leaf | 0.8 | 0.8 | 1.3 | 1.1 | |
| | - mg/gfw of leaf | 0.09 | 0.08 | 0.15 | 0.13 | |
| Lamium purpureum (Labiatae) | % water content of leaves | *78.9 | *77.8 | *72.1 | 32.9 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 3.5 | 4.9 | 6.2 | 11.3 | 3.2 |
| | - μmoles/gfw of leaf | 0.8 | 1.1 | 1.7 | 7.6 | |
| | - mg/gfw of leaf | 0.08 | 0.13 | 0.20 | 0.9 | |
| Sinapis arvensis (Cruciferae) | % water content of leaves | *77.3 | *22.4 | - | - | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 4.6 | 133.6 | | | 29.2 |
| | - μmoles/gfw of leaf | 1.1 | 104.1 | | | |
| | - mg/gfw of leaf | 0.12 | 12.0 | | | |

[1]Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Two replicates were used.

Table 2

Proline levels, initially and after wilting, of plants collected from lead mine waste (a drought-prone habitat).

| Species (Family) | | Number of days from collection 1 | 2 | 5 | Accumulation Factor [1] |
|---|---|---|---|---|---|
| Nardus stricta (Gramineae) | % water content of leaves | *44.1 | - | *16.3 | |
| | proline content | | | | |
| | - μmoles/g dry weight | 5.7 | | 4.8 | - |
| | - μmoles/gfw of leaf | 3.1 | | 4.3 | |
| | - mg/gfw of leaf | 0.36 | | 0.50 | |
| Festuca ovina (Gramineae) | % water content of leaves | *59.7 | 20.7 | 18.7 | |
| | proline content | | | | |
| | - μmoles/g dry weight | 6.6 | 11.8 | 16.5 | 2.5 |
| | - μmoles/gfw of leaf | 2.7 | 9.4 | 13.4 | |
| | - mg/gfw of leaf | 0.31 | 1.1 | 1.5 | |
| Veronica officinalis (Scrophulariaceae) | % water content of leaves | 62.3 | 37.7 | | |
| | proline content | | | | |
| | - μmoles/g dry weight | 15.0 | 9.3 | | - |
| | - μmoles/gfw of leaf | 5.5 | 5.8 | | |
| | - mg/gfw of leaf | 0.63 | 0.66 | | |
| Lotus corniculatus (Papilionaceae) | % water content of leaves | *81.9 | 17.7 | | |
| | proline content | | | | |
| | - μmoles/g dry weight | 9.0 | 102.4 | | 11.4 |
| | - μmoles/gfw of leaf | 1.6 | 84.2 | | |
| | - mg/gfw of leaf | 0.19 | 9.69 | | |
| Thymus scrphyllum | % water content of leaves | 50.0 | *47.0 | | |
| | proline content | | | | |
| | - μmoles/g dry weight | 3.3 | 10.2 | | 3.1 |
| | - μmoles/gfw of leaf | 1.7 | 5.4 | | |
| | - mg/gfw of leaf | 0.19 | 0.62 | | |

/Cont'd...

Table 2 Continued

[1] Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

* Two replicates were used.

Table 3

Proline levels initially and after wilting, of plants collected from sand dunes (a drought-prone habitat).

| Species (Family) | | Number of days from collection 1 | 2 | 3 | 4 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Salicornia europaea (Chenopodiaceae) | % water content of leaves | *91.0 | *87.7 | *86.6 | *77.3 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 9.4 | 13.5 | 10.6 | 14.9 | 1.6 |
| | - μmoles/gfw of leaf | 0.9 | 1.6 | 1.4 | 3.4 | |
| | - mg/gfw of leaf | 0.10 | 0.19 | 0.17 | 0.39 | |
| Atriplex patula (Chenopodiaceae) | % water content of leaves | *90.6 | *89.9 | *89.2 | *81.6 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 33.9 | 22.6 | 54.0 | 10.7 | 1.6 |
| | - μmoles/gfw of leaf | 3.1 | 2.3 | 5.8 | 2.0 | |
| | - mg/gfw of leaf | 0.36 | 0.27 | 0.67 | 0.23 | |
| Atriplex lacinata (Chenopodiaceae) | % water content of leaves | 81.3 | *69.8 | *63.8 | *47.6 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 12.8 | 11.0 | 54.3 | 19.2 | 4.2 |
| | - μmoles/gfw of leaf | 2.5 | 3.3 | 19.7 | 10.1 | |
| | - mg/gfw of leaf | 0.29 | 0.38 | 2.27 | 1.2 | |
| Honkenya peploides (Caryophyllaceae) | % water content of leaves | *87.7 | *83.5 | *83.7 | *75.8 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 20.1 | 19.3 | 26.6 | 20.2 | 1.3 |
| | - μmoles/gfw of leaf | 2.5 | 1.6 | 4.3 | 4.9 | |
| | - mg/gfw of leaf | 0.29 | 0.18 | 0.50 | 0.57 | |

[1] Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Two replicates were used.

Table 4

Proline levels initially and after wilting, of plants collected from woodland (drought-free habitat).

| Species (Family) | | Number of days from collection 1 | 3 | 4 | 6 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Milium effusum (Gramineae) | % water content of leaves | *77.3 | *76.7 | *74.8 | *63.5 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 6.8 | 8.5 | 11.4 | 55.8 | 8.2 |
| | - μmoles/gfw of leaf | 1.0 | 2.0 | 2.9 | 20.2 | |
| | - mg/gfw of leaf | 0.12 | 0.23 | 0.33 | 2.32 | |
| Tussilago farfara (Compositae) | % water content of leaves | *90.3 | 91.5 | *84.7 | *83.7 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 6.8 | 15.8 | 12.1 | 6.1 | 2.3 |
| | - μmoles/gfw of leaf | 0.65 | 1.5 | 2.0 | 1.3 | |
| | - mg/gfw of leaf | 0.08 | 0.17 | 0.10 | 0.15 | |
| Chamaenerion angustifolium (Onagraceae) | % water content of leaves | *78.4 | *69.8 | *12.0 | - | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 4.8 | 6.6 | 6.5 | | 1.4 |
| | - μmoles/gfw of leaf | 1.0 | 2.0 | 5.9 | | |
| | - mg/gfw of leaf | 0.12 | 0.23 | 0.68 | | |
| Ranuculus acris (Ranunculaceae) | % water content of leaves | *81.6 | *79.4 | *78.0 | 60.7 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 3.6 | 5.9 | 8.9 | 4.2 | 2.5 |
| | - μmoles/gfw of leaf | 0.6 | 1.2 | 2.0 | 1.6 | |
| | - mg/gfw of leaf | 0.08 | 0.14 | 0.23 | 0.19 | |
| Rumex sanguineus (Polygonaceae) | % water content of leaves | *85.9 | *83.9 | *47.0 | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 4.6 | 10.1 | 126.0 | | 27.4 |
| | - μmoles/gfw of leaf | 0.6 | 1.7 | 66.8 | | |
| | - mg/gfw of leaf | 0.08 | 0.19 | 7.7 | | |

[1]Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Two replicates were used.

Table 5

Proline levels, initially and after wilting, of plants collected from wet heathland (a drought-free habitat)

| Species (Family) | | Number of days from collection 1 | 5 | 7 | 9 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Eriophorum angustifolium (Gramineae) | % water content of leaves | 59.5 | 42.5 | 18.9 | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 5.0 | 6.5 | 1.6 | - | 1.3 |
| | - μmoles/gfw of leaf | 2.0 | 3.7 | 1.3 | | |
| | - mg/gfw of leaf | 0.23 | 0.43 | 0.15 | | |
| Juncus effusus (Juncaceae) | % water content of leaves | 76.7 | 63.5 | *38.7 | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 4.5 | 40.1 | 18.2 | - | 8.9 |
| | - μmoles/gfw of leaf | 1.0 | 14.6 | 11.2 | | |
| | - mg/gfw of leaf | 0.12 | 1.68 | 1.29 | | |
| Carex echinata (Cyperaceae) | % water content of leaves | *64.1 | 77.1 | *72.6 | 56.7 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 5.2 | 4.8 | 8.2 | 10.1 | 1.9 |
| | - μmoles/gfw of leaf | 1.9 | 1.2 | 2.3 | 4.2 | |
| | - mg/gfw of leaf | 0.21 | 0.14 | 0.26 | 0.48 | |
| Polytrichum commune (Musci) | % water content of leaves | *54.0 | 44.9 | | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 3.6 | 2.4 | - | - | - |
| | - μmoles/gfw of leaf | 1.6 | 1.3 | | | |
| | - mg/gfw of leaf | 0.19 | 0.15 | | | |
| Campidium stellatum (Musci) | % water content of leaves | *88.4 | | 23.1 | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 7.2 | - | 10.9 | - | 1.5 |
| | - μmoles/gfw of leaf | 0.8 | | 8.8 | | |
| | - mg/gfw of leaf | 0.09 | | 1.01 | | |

Table 5 Continued

| Species (Family) | | Number of days from collection 1 | 5 | 7 | 9 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Calium saxatile (Rubiaceae) | % water content of leaves | *78.8 | 78.8 | | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 17.5 | 41.2 | - | - | 2.4 |
| | - μmoles/gfw of leaf | 3.7 | 8.9 | | | |
| | - mg/gfw of leaf | 0.43 | 1.01 | | | |
| Potentilla erecta (Rosaceae) | % water content of leaves | 74.0 | 77.6 | | 57.1 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 6.3 | 7.1 | - | 10.8 | 1.7 |
| | - μmoles/gfw of leaf | 1.1 | 1.6 | | 4.2 | |
| | - mg/gfw of leaf | 0.13 | 0.18 | | 0.48 | |

[1]Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Two replicates were used.

Table 6

Proline levels, initially and after wilting, of plants collected from river banks (a drought-free habitat).

| Species (Family) | | Number of days from collection 1 | 2 | 3 | 4 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| Cardamine pratensis (Cruciferae) | % water content of leaves | *78.0 | | 69.7 | | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 11.5 | - | 38.8 | - | 3.4 |
| | - μmoles/gfw of leaf | 2.6 | | 11.3 | | |
| | - mg/gfw of leaf | 0.30 | | 1.29 | | |
| Circaea lutetiana (Onagraceae) | % water content of leaves | *81.1 | *80.0 | *67.8 | *35.7 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 11.9 | 12.9 | 31.7 | 16.7 | |
| | - μmoles/gfw of leaf | 2.2 | 2.5 | 10.0 | 10.7 | |
| | - mg/gfw of leaf | 0.26 | 0.29 | 1.1 | 1.2 | |
| Epilobium hirsutum (Onagraceae) | % water content of leaves | *80.2 | *64.9 | *31.9 | *6.7 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 16.3 | 12.3 | 11.0 | 9.8 | - |
| | - μmoles/gfw of leaf | 3.3 | 4.3 | 7.6 | 9.1 | |
| | - mg/gfw of leaf | 0.37 | 0.49 | 0.87 | 1.1 | |
| Bellis Perennis (Compositae) | % water content of leaves | *83.2 | | *83.5 | *38.1 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 4.9 | - | 11.7 | 70.5 | 14.5 |
| | - μmoles/gfw of leaf | 0.8 | | 2.0 | 46.3 | |
| | - mg/gfw of leaf | 0.09 | | 0.23 | 5.3 | |

[1]Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Two replicates were used.

Initial levels for riverside species appear quite high, ranging from 4.9 to 16.3 μmoles/gdw of leaf. The high levels may be due to the wilt-susceptibility of the species; Cardamine pratensis, Circaea lutetiana and Epilobium hirsutum. They wilt quickly and may have accumulated proline during the time taken to bring them to the laboratory. They should have been kept damp. Bellis perennis, on the other hand, wilts more slowly and does not show the high initial level of proline. However, the highest accumulation factor, of 14.5, was seen for Bellis perennis.

In order to better compare proline levels initially and after wilting for species of the two habitat types, a summary is given in Table 7. Means and standard errors are given. The results are inconclusive. The means, for both the initial levels of proline and the highest levels accumulated, are much higher for species of drought-prone habitats; however, the accumulation factor is only slightly higher (drought-prone, 4.7; drought-free, 3.4). These higher initial proline results may be accounted for by the presence of more species of a wilt-susceptible nature as suggested above. If so, then it is possible that a higher accumulation factor would be determined if the proline levels of these species were measured prior to the development of any substantial water saturation deficit.

Percentage water contents of the leaves at the time proline was measured initially and at the time of highest proline levels are also included in Table 7. The water content initially was similar for species of both types of habitats; it was 77.7% for species of drought-free habitats and 74.6% for species of drought-prone habitats. However,

Table 7

Summary of proline levels and water content, initially and after wilting, of plants of drought-prone and drought-free habitats.

| Species | Amoles proline/gdw | | Accumulation Factor | % water content | |
|---|---|---|---|---|---|
| | Initial level | Highest level | | On collection | During Highest Proline |
| **Drought-prone** | | | | | |
| Sinapis arvensis | 4.6 | 133.6 | 29.2 | 77.3 | 22.4 |
| Impatiens glandulifera | 5.0 | 8.6 | 1.8 | 84.0 | 78.0 |
| Lamium purpureum | 3.6 | 11.3 | 3.2 | 78.9 | 32.9 |
| Plantago lanceolata | 5.5 | 6.9 | 1.2 | 80.9 | 40.0 |
| Nardus stricta | 5.7 | 5.7 | 0.0 | 44.1 | 44.1 |
| Festuca ovina | 6.6 | 16.5 | 2.5 | 59.7 | 18.7 |
| Veronica officinalis | 15.0 | 15.0 | 0.0 | 62.3 | 62.3 |
| Lotus corniculatus | 8.9 | 102.4 | 11.4 | 81.9 | 17.7 |
| Thymus serphyllum | 3.3 | 10.2 | 3.1 | 49.5 | 47.0 |
| Salicornia europaea | 9.4 | 14.9 | 1.6 | 91.0 | 77.3 |
| Atriplex patula | 33.9 | 54.0 | 1.6 | 90.6 | 89.2 |
| Atriplex laciniata | 12.8 | 53.4 | 4.2 | 81.3 | 63.8 |
| Honkenya poploides | 20.1 | 26.6 | 1.3 | 87.7 | 83.7 |
| MEANS | 17.5±3.0 | 45.5±6.0 | 4.7±2.2 | 74.6±4.3 | 52.1±7.0 |
| **Drought-free** | | | | | |
| Milium effusum | 6.8 | 57.8 | 8.2 | 77.3 | 63.5 |
| Tussilago farfara | 6.8 | 15.8 | 2.3 | 90.3 | 91.5 |
| Chamaenerion angustifolium | 4.8 | 6.6 | 1.4 | 78.4 | 69.8 |
| Ranuculus acris | 3.6 | 8.9 | 2.5 | 81.6 | 78.0 |
| Rumex sanguineus | 4.6 | 10.1 | 2.4 | 85.9 | 83.9 |
| Potentilla erecta | 6.3 | 10.8 | 1.7 | 74.0 | 57.1 |
| Galium saxatile | 17.5 | 41.2 | 2.4 | 74.8 | 78.8 |
| Juncus effusus | 4.5 | 40.1 | 8.9 | 76.7 | 63.5 |
| Carex echinata | 5.2 | 10.1 | 1.9 | 64.1 | 56.7 |
| Polytrichum commune | 3.6 | 3.6 | 0.0 | 54.0 | 54.0 |
| Campidium stellatum | 7.2 | 10.9 | 1.5 | 88.4 | 23.1 |
| Eriophorum angustifolium | 5.0 | 6.5 | 1.3 | 59.5 | 42.5 |
| Cardamine pratensis | 11.5 | 38.8 | 3.4 | 78.0 | 69.7 |
| Circaea latetiana | 11.9 | 31.7 | 2.7 | 81.1 | 67.8 |
| Epilobium hirsutum | 16.3 | 16.3 | 0.0 | 80.2 | 80.2 |
| Bellis perennis | 4.9 | 70.5 | 14.5 | 83.2 | 38.1 |
| MEANS | 8.6±0.9 | 20.3±4.0 | 3.4±1.0 | 77.0±1.5 | 63.8±4.5 |

Table 7 (continued)

Analysis of Variance Results

i) Initial levels of proline

| | Source of variation | Sum of squares | Degrees of freedom | Mean squares |
|---|---|---|---|---|
| a) | Between sites | 1352.672 | 1 | 1352.672 |
| b) | Residual | 11888.156 | 68 | 174.826 |
| c) | Total | 13240.828 | 69 | |

Variance Ratio = 7.74

ii) Highest levels of proline

| | Source of variation | Sum of squares | Degrees of freedom | Mean squares |
|---|---|---|---|---|
| a) | Between sites | 9617.188 | 1 | 9617.188 |
| b) | Residual | 51157.500 | 59 | 867.076 |
| c) | Total | 60774.688 | 60 | |

Variance Ratio = 11.09

at the time of highest proline content, the water content of species of drought-prone habitats was lower (52.1% for species of drier habitats and 63.8% for species of the moist habitats).

There may be a relationship between proline content and percentage water content of the leaves, but the results presented here are inadequate to suggest the nature of the relationship.

Two analyses of variance were carried out to assess the significance of the habitat with regard to both the initial and accumulated proline levels (Appendices B and C, Table 7). When the initial proline levels of species of the two habitat types were compared, an 'F' value of 7.74 was obtained (df = 1,68). Hence there is a significant difference between the initial proline levels of the two habitat types ($p = 0.05$). An 'F' value of 11.09 was obtained when the maximum proline levels of species of the two habitat types were compared (df = 1,59). This value is significant at the $p = 0.01$ level, indicating there is a significant difference between the maximum levels of proline of the two habitat types.

Although the results for proline levels of species of the two habitat types were significantly different, the arbitrary nature of the choice of species prevents any definite conclusions. For that reason, it was decided to investigate two families to test for the significance of family in contrast to habitat. Both Bellis perennis and Sinapis arvensis had been seen to accumulate proline and hence Compositae and Cruciferae were chosen.

Initially, proline accumulation during wilting both in excised leaves and in leaves attached to wilting plants was tried with Bellis perennis and Sinapis arvensis (Tables 8 and 9, Figures 1, 2, 3 and 4). However,

Table 8

Proline levels, initially and after wilting, of excised leaves of Bellis perennis and Sinapis arvensis

| Species | | Number of days from collection 1 | 2 | 3 | 4 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|
| B. perennis | % water content of leaves | 88.9 ± 0.3 | 87.0 ± 0.8 | 9.0 ± 3.8 | 5.6 ± 2.8 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 10.1 ± 1.0 | 18.2 ± 3.3 | 15.8 ± 4.5 | 8.4 ± 0.6 | 1.8 |
| | - μmoles/gfw of leaf | 1.1 ± 0.1 | 15.0 ± 2.7 | 15.8 ± 4.3 | 7.9 ± 0.7 | |
| | - mg/gfw of leaf | 0.13 ± 0.0 | 1.7 ± 0.3 | 1.8 ± 0.5 | 0.9 ± 0.1 | |
| S. arvensis | % water content of leaves | 86.4 ± 0.3 | 8.7 ± 1.3 | 0.0 | 0.9 ± 0.4 | |
| | proline content | | | | | |
| | - μmoles/g dry weight | 7.7 ± 0.5 | 14.5 ± 1.3 | 14.0 ± 1.9 | 10.8 ± 1.2 | 1.9 |
| | - μmoles/gfw of leaf | 1.1 ± 0.1 | 13.1 ± 1.8 | 14.0 ± 1.9 | 11.0 ± 1.3 | |
| | - mg/gfw of leaf | 0.12 ± 0.0 | 1.5 ± 0.2 | 1.6 ± 0.2 | 1.1 ± 0.3 | |

[1] Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

*Ten replicates were used.

Table 9

Proline levels, initially and after wilting, of leaves of
Bellis perennis and Sinapis arvensis

| Species | | Number of days from collection 1 | 2 | 3 | 5 | 7 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|---|
| B. perennis | % water content of leaves | 86.6 ± 0.4 | 85.7 ± 0.3 | 83.2 ± 2.4 | 86.0 ± 1.6 | 75.0 ± 1.0 | |
| | proline content | | | | | | |
| | - µmoles/g dry weight | 9.7 ± 0.3 | 6.8 ± 0.4 | 15.0 ± 0.6 | 15.4 ± 4.2 | 30.8 ± 3.6 | 3.2 |
| | - µmoles/gfw of leaf | 1.3 ± 0.1 | 1.0 ± 0.4 | 2.2 ± 0.1 | 2.5 ± 0.4 | 7.6 ± 0.8 | |
| | - mg/gfw of leaf | 0.15 ± 0.0 | 0.11 ± 0.0 | 0.26 ± 0.0 | 0.29 ± 0.1 | 0.88 ± 0.1 | |
| S. arvensis | % water content of leaves | 84.8 ± 0.3 | 83.0 ± 2.6 | 71.0 ± 2.9 | 12.5 ± 0.8 | 17.1 ± 0.5 | |
| | proline content | | | | | | |
| | - µmoles/g dry weight | 3.2 ± 0.6 | 47.5 ± 9.8 | 129.5 ± 13.9 | 120.6 ± 16.8 | 149.9 ± 10.3 | 47.4 |
| | - µmoles/gfw of leaf | 0.5 ± 0.0 | 9.3 ± 3.2 | 39.0 ± 6.4 | 105.1 ± 14.3 | 120.6 ± 9.4 | |
| | - mg/gfw of leaf | 0.61 ± 0.0 | 1.07 ± 0.4 | 4.5 ± 0.7 | 12.1 ± 1.7 | 13.9 ± 1.1 | |

[1]Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

Ten replicates were used.

Figure 1

PERCENTAGE WATER CONTENT OF EXCISED LEAVES ON SUCCESSIVE DAYS

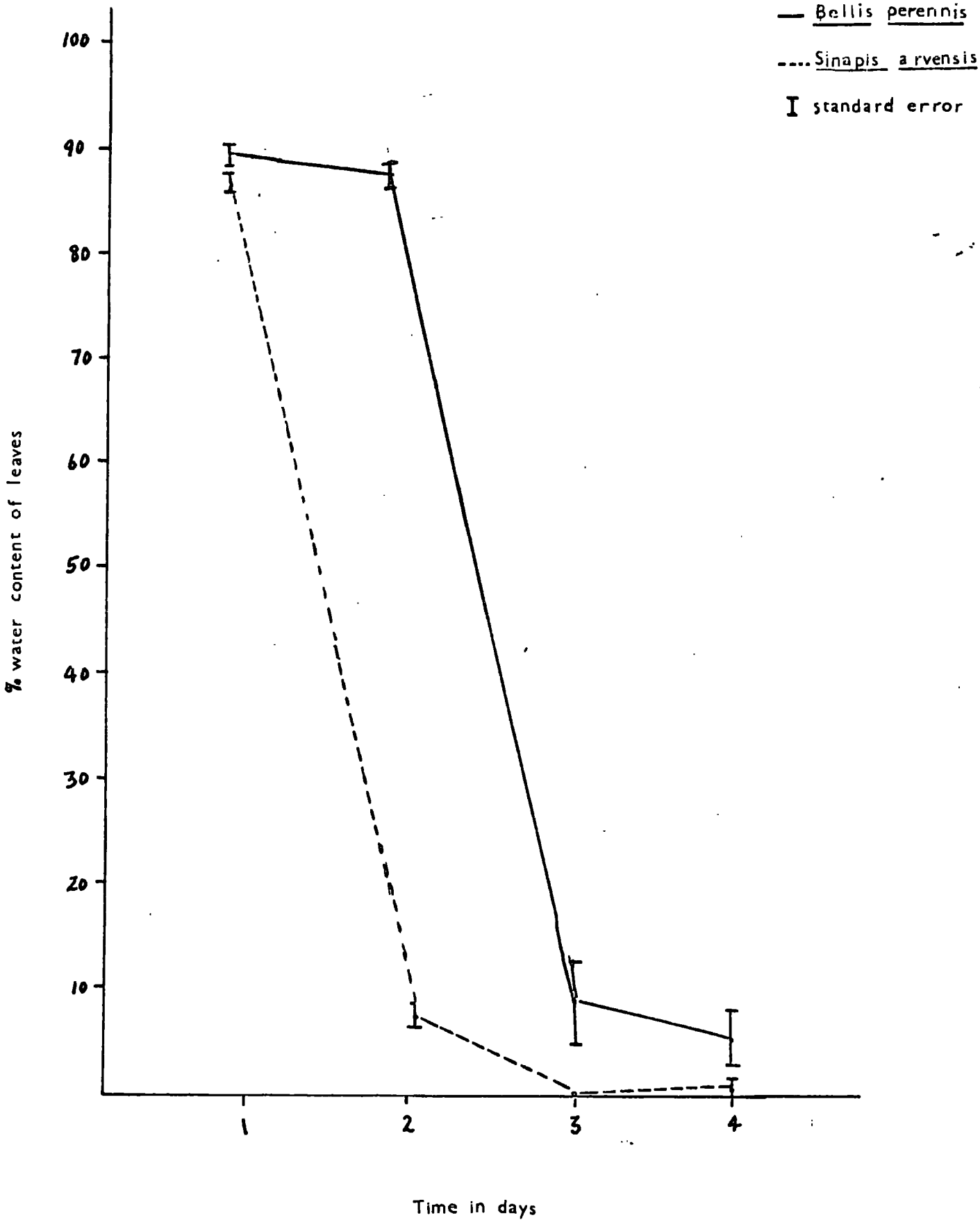


Figure 2

PROLINE LEVELS OF EXCISED LEAVES OF BELLIS PERENNIS AND SINAPIS ARVENSIS

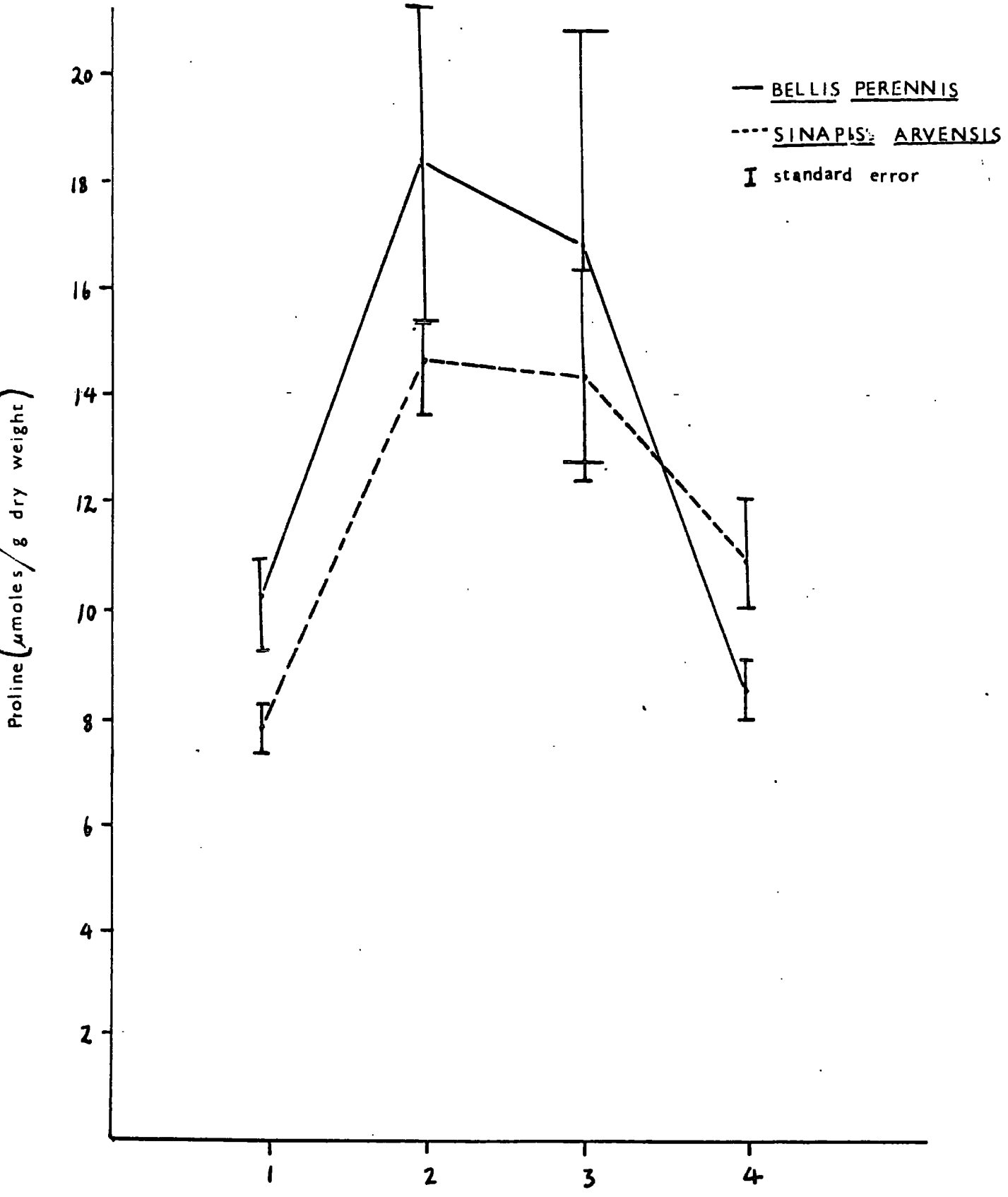


Figure 3

PERCENTAGE WATER CONTENT OF LEAVES DURING WILTING

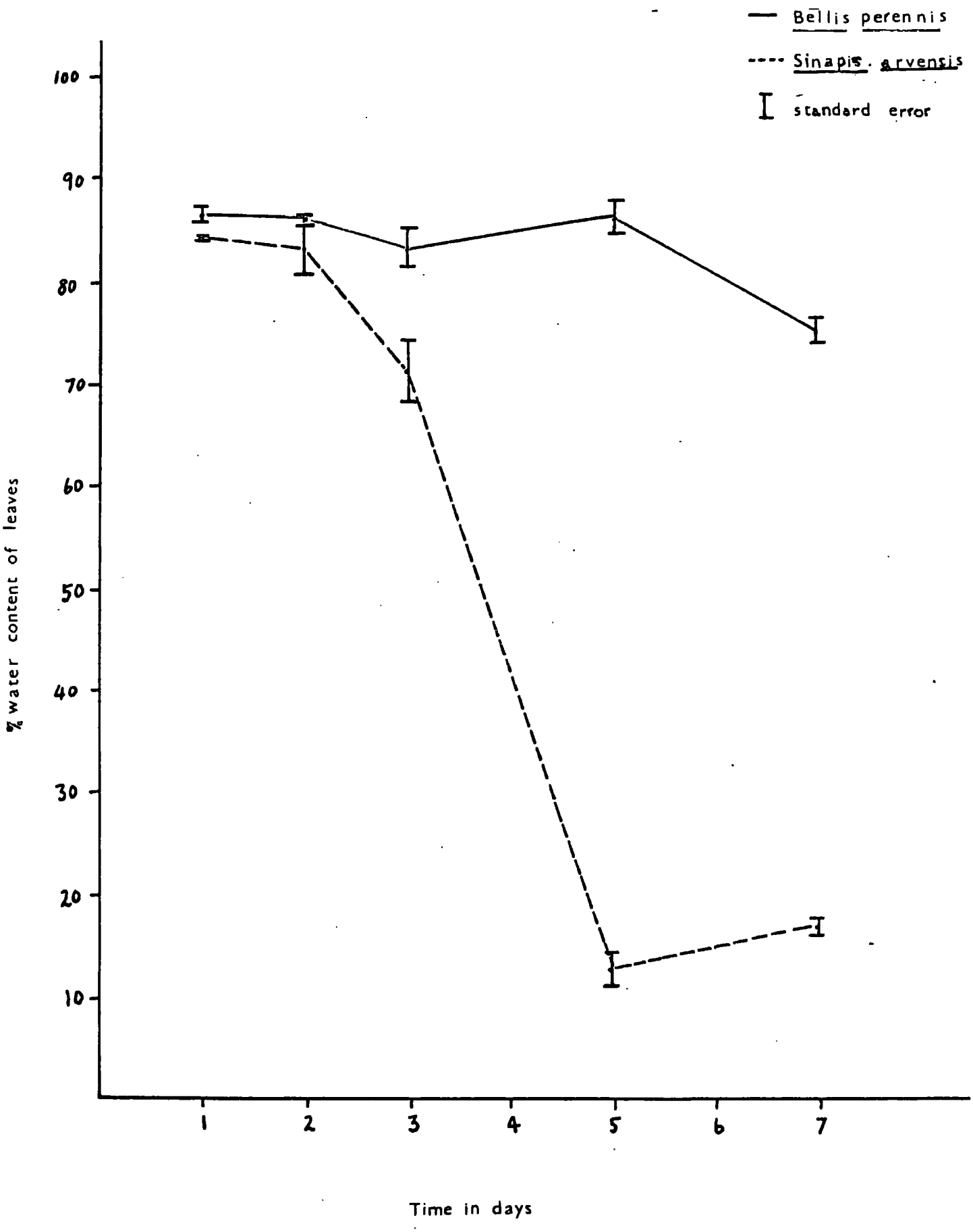
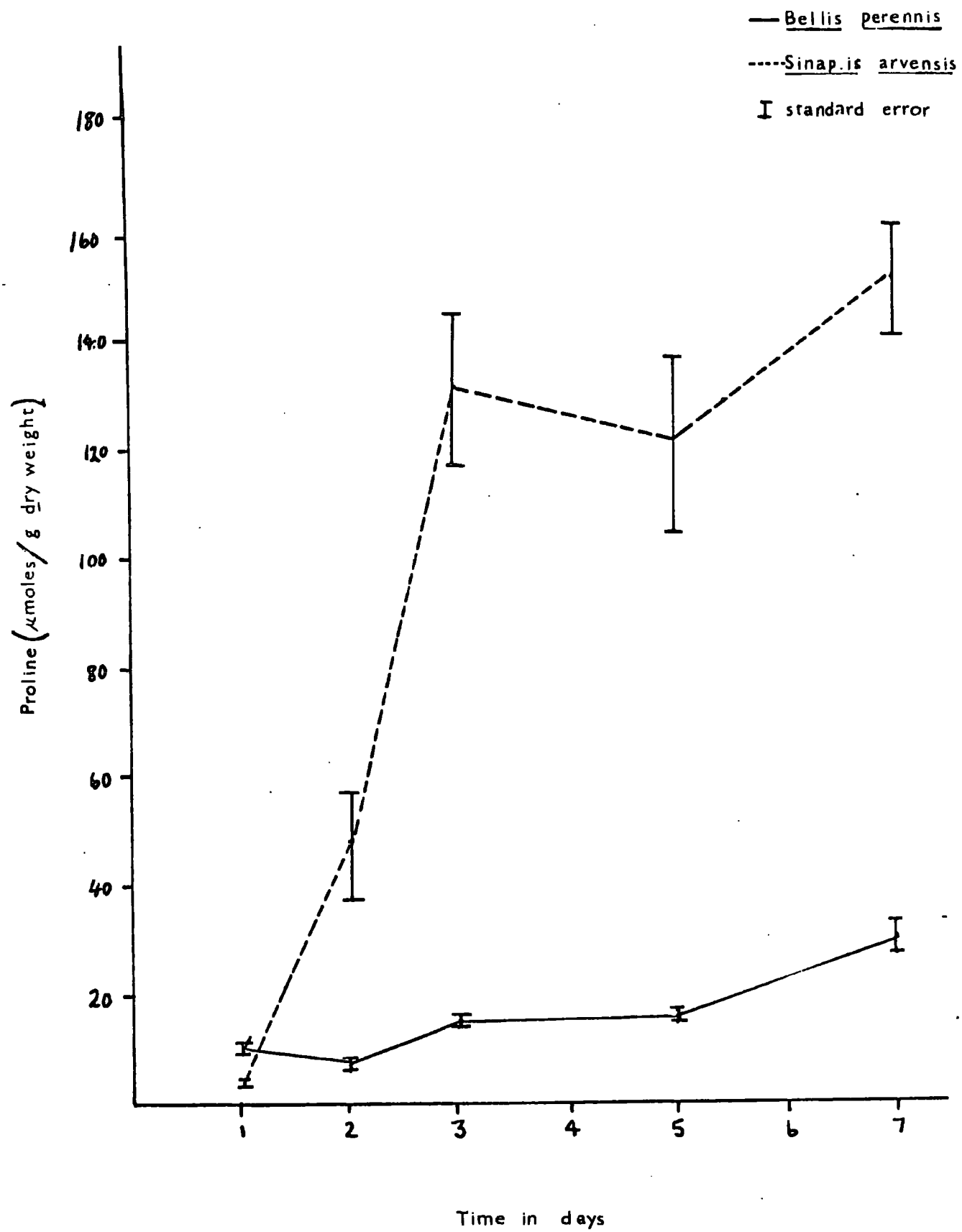


Figure 4

PROLINE LEVELS IN LEAVES OF BELLIS PERENNIS AND SINAPIS ARVENSIS



the results for excised leaves indicated that death occurred very rapidly. The water content of the leaves of Bellis perennis dropped from 87.0% to 9.0% between day 2 and 3, during which time the leaves died (Figure 1). The water content of Sinapis arvensis dropped from 86.4% to 8.7% between day 1 and 2, during which time the leaves died (Figure 1). In addition, the accumulation factors for both species were not at all as high as those attained previously for these species (Table 8, Figure 2). Hence, the use of excised leaves was abandoned, as wilting and desiccation occurred too rapidly for proline to accumulate significantly and for its increase to be monitored by the experimenter.

When leaves were left attached to the plant, wilting occurred more easily and could be monitored more easily (Table 9, Figures 3 and 4). Figure 3 illustrates the changes in percentage water content of leaves and Figure 4, the proline content.

Bellis perennis attained the highest proline levels on day 7 (30.8 $\mu$moles/gdw), at which time the percentage water content of the leaves was 75.0. The accumulation factor is 3.2. This is not as high as that obtained previously for B. perennis (Table 6), at which time the percentage water content of the leaves was 38.1. Therefore, had it been possible to continue this experiment, a higher level might have been reached.

Sinapis arvensis produced the maximum amounts of proline also on day 7 (149.9 $\mu$moles/gdw), when a percentage water content of 17.1 was recorded. The accumulation factor is 47.7. However, even on day 3 when the percentage water content was 71.0, proline had increased 43.2 times the initial level. With both species, a high proline content was evident at percentage water contents below 75.

Data for the other Compositae are given in Table 10 (Figures 5

Table 10

Proline levels, initially and after wilting of five Compositae

| Species | | Number of days from collection 1 | 2 | 3 | 5 | 7 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|---|
| Sonchus arvensis | % water content of leaves | 87.6 ± 0.5 | 90.5 ± 4.3 | 90.1 ± 1.6 | 33.2 ± | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 9.2 ± 1.9 | 29.4 ± 1.3 | 29.0 ± 3.9 | 18.0 ± 0.6 | | 3.2 |
| | - μmoles/gfw of leaf | 1.0 ± 0.1 | 2.8 ± 0.2 | 2.8 ± 0.3 | 12.0 ± 0.3 | | |
| | - mg/gfw of leaf | 0.12 ± 0.0 | 0.33 ± 0.0 | 0.32 ± 0.0 | 1.4 ± 0.0 | | |
| Circium arvense | % water content of leaves | 81.4 ± 0.1 | 83.6 ± 0.6 | 82.8 ± 0.6 | 81.2 ± 0.3 | 62.4 ± 5.0 | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 12.4 ± 2.1 | 61.7 ± 6.1 | 74.4 ± 5.4 | 42.9 ± 3.0 | 54.3 ± 2.3 | 6.0 |
| | - μmoles/gfw of leaf | 2.3 ± 0.4 | 10.4 ± 1.2 | 12.8 ± 2.0 | 8.1 ± 0.6 | 20.1 ± 2.0 | |
| | - mg/gfw of leaf | 0.27 ± 0.0 | 1.2 ± 0.1 | 1.5 ± 0.1 | 0.9 ± 0.1 | 2.31 ± 0.2 | |
| Senecio aquaticus | % water content of leaves | 80.5 | 59.5 ± 0.2 | 62.3 | 48.0 ± 3.5 | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 17.6 ± 1.8 | 13.0 ± 4.6 | 139.2 ± 11.3 | 87.5 ± 5.0 | - | 7.9 |
| | - μmoles/gfw of leaf | 3.4 ± 0.4 | 17.4 ± 1.9 | 52.5 ± 4.3 | 45.3 ± 2.1 | | |
| | - mg/gfw of leaf | 0.4 ± 0.0 | 2.0 ± 0.2 | 6.0 ± 0.5 | 5.2 ± 0.3 | | |
| Senecio jacobaea | % water content of leaves | 89.5 ± 0.7 | 89.4 ± 0.5 | 90.4 ± 0.1 | 88.9 ± 1.0 | 18.6 ± 0.1 | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 14.9 ± 1.7 | 17.4 ± 2.0 | 22.4 ± 2.9 | 49.2 ± 6.1 | 34.5 ± 3.2 | 3.3 |
| | - μmoles/gfw of leaf | 1.6 ± 0.2 | 1.8 ± 0.2 | 2.2 ± 0.3 | 5.4 ± 0.5 | 28.2 ± 2.6 | |
| | - mg/gfw of leaf | 0.18 ± 0.0 | 0.21 ± 0.0 | 0.26 ± 0.0 | 0.62 ± 0.1 | 3.2 ± 0.3 | |

Table 10 (Continued)

| Species | | | | | | | | | Accumulation Factor[1] |
|---|---|---|---|---|---|---|---|---|---|
| Lactuca saligna | % water content of leaves | 74.5 | ± 0.5 | 69.1 | ± 0.9 | 64.3 | ± 1.0 | 27.8 ± 12.6 | |
| | proline content | | | | | | | | |
| | - μmoles/g dry weight | 5.0 | ± 0.6 | 12.6 | ± 4.0 | 13.9 | ± 1.0 | 27.9 ± 2.8 | - 5.6 |
| | - μmoles/gfw of leaf | 1.3 | ± 0.2 | 4.5 | ± 1.3 | 5.0 | ± 0.4 | 21.2 ± 3.7 | |
| | - mg/gfw of leaf | 0.15 | ± 0.0 | 0.52 | ± 0.2 | 0.58 | ± 0.1 | 2.41 ± 0.4 | |

[1] Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

Six replicates were used.

Figure 5

PERCENTAGE WATER CONTENT OF LEAVES OF COMPOSITAE DURING WILTING

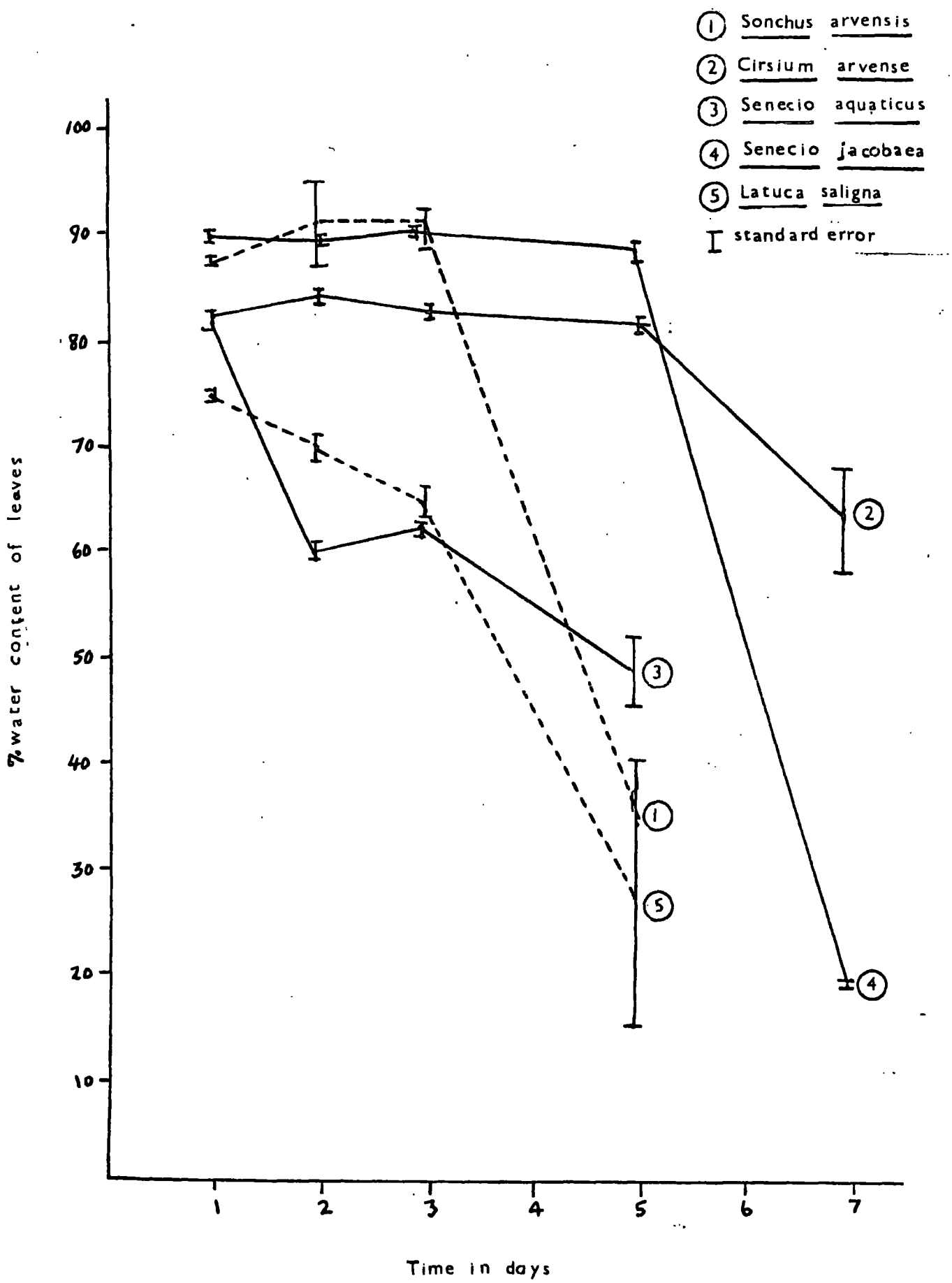
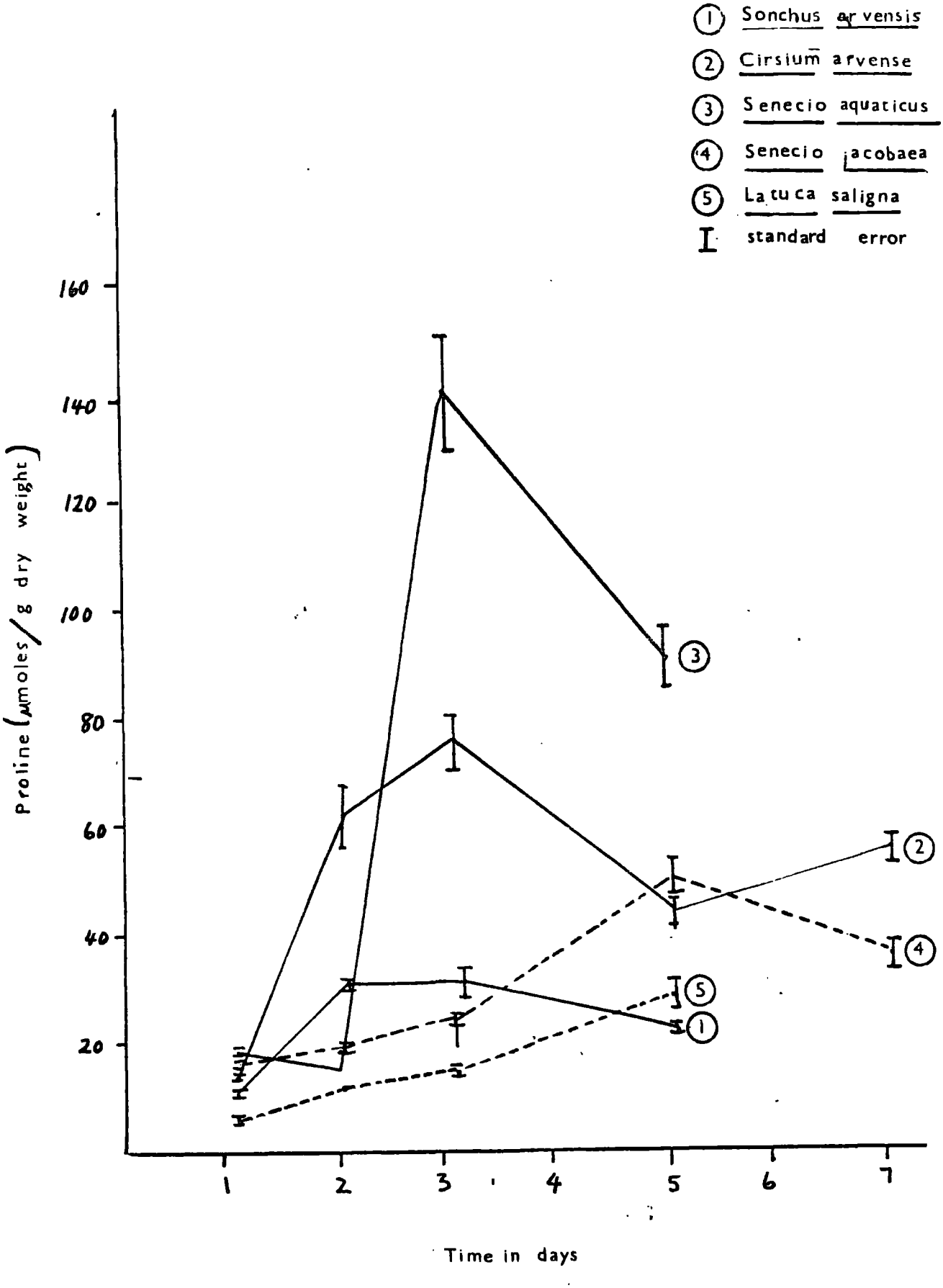


Figure 6

PROLINE LEVELS IN LEAVES OF COMPOSITAE DURING WILTING



and 6). The initial levels of proline ranged between 5.0 and 17.6$\mu$moles proline/gdw of leaf and the water content of the leaves between 74.5 and 89.5%. All showed increases in proline during wilting; the accumulation factors ranging between 3.2 and 7.9. The day of highest proline levels and the percentage water content of the leaves at this time varied with species: S. arvensis (day 2, 90.5), C. arvense (day 3, 82.8), S. aquaticus (day 2, 62.3), S. jacobaea (day 5, 88.9) and L. saligna (day 5, 17.9). Proline levels may have risen higher in between the times of testing. All species had less proline at death, which had occurred by the last test.

Data for the Cruciferae studied are given in Table 11 (Figures 7 and 8). The initial levels of proline ranged from 10.0 to 78.4$\mu$moles/gdw of leaf and the percentage water content from 76.5 to 92.7. These initial levels appear higher than those of the Compositae. Cakile maritima, the sand dune species, showed the highest water content and the highest proline content.

The accumulation factors for Cruciferae are similar to those of the Compositae studied, ranging from 1.4 to 7.5. All species showed an increase in proline, though the increase observed in Cakile maritima was small. None of the others accumulated proline to the extent exhibited by Sinapis arvensis.

The percentage water content, at the time when the proline levels were highest, varied. Capsella bursa, Cakile maritima and Brassica chinensis (var. Chihili) yielded highest proline contents after death, when the water contents were 51.5%, 43.4% and 82.0% respectively. Alliaria officinalis and Brassica campestris produced highest levels prior to death, when the water contents of the leaves were 84.6% and

Table 11

Proline levels, initially and after wilting, of five Conciferae

| Species | | Number of days from collection 1 | 2 | 3 | 5 | 7 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|---|
| Capsella bursa | % water content of leaves | 76.5 ± 0.9 | 80.9 ± 0.6 | 86.7 ± 2.1 | 51.5 | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 31.4 ± 1.9 | 116.4 ±16.1 | 180.2 ±55.5 | 205.4 ± 7.8 | | 6.5 |
| | - μmoles/gfw of leaf | 7.5 ± 0.5 | 22.0 ± 2.6 | 22.6 ± 6.3 | 99.7 ± 3.8 | | |
| | - mg/gfw of leaf | 0.86± 0.1 | 2.53± 0.3 | 2.6 ± 0.7 | 11.24± 0.6 | | |
| Cakile maritima | % water content of leaves | 92.7 ± 3.4 | 90.9 ± 0.6 | 87.6 ± 1.3 | 43.4 ± 1.2 | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 78.4 ±15.6 | 88.7 ±17.1 | 62.6 ± 9.0 | 111.0 ±15.4 | | 1.4 |
| | - μmoles/gfw of leaf | 5.6 ± 0.8 | 8.6 ± 1.5 | 6.9 ± 0.8 | 16.8 ± 2.2 | | |
| | - mg/gfw of leaf | 0.55± 0.1 | 1.00± 0.2 | 0.80± 0.1 | 1.91± 0.3 | | |
| Brassica chinensis (var.Chihili) | % water content of leaves | 90.9 ± 0.6 | 93.0 ± 0.2 | 93.0 ± 0.9 | 92.6 ± 0.5 | 81.0 ± 2.5 | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 10.0 ± 1.5 | 30.0 ± 3.9 | 35.7 ± 1.6 | 39.4 ± 2.5 | 75.1 ± 6.2 | 7.5 |
| | - μmoles/gfw of leaf | 0.9 ± 0.1 | 2.1 ± 0.3 | 3.0 ± 0.1 | 3.2 ± 0.3 | 13.9 ± 2.1 | |
| | - mg/gfw of leaf | 0.11± 0.0 | 0.25± 0.0 | 0.35± 0.0 | 0.37± 0.0 | 1.60 ± 0.3 | |
| Alliaria officinalis | % water content of leaves | 86.5 ± 1.0 | 84.7 ± 1.5 | 87.6 ± 1.0 | 30.9 ± 3.9 | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 14.6 ± 0.7 | 47.2 ± 9.9 | 30.5 ± 3.2 | 26.1 ± 6.4 | | 3.3 |
| | - μmoles/gfw of leaf | 2.0 ± 0.1 | 7.2 ± 1.6 | 3.2 ± 0.6 | 17.6 ± 3.9 | | |
| | - mg/gfw of leaf | 0.23± 0.0 | 0.83± 0.2 | 0.43± 0.0 | 2.02± 0.5 | | |

Table 11 (Continued)

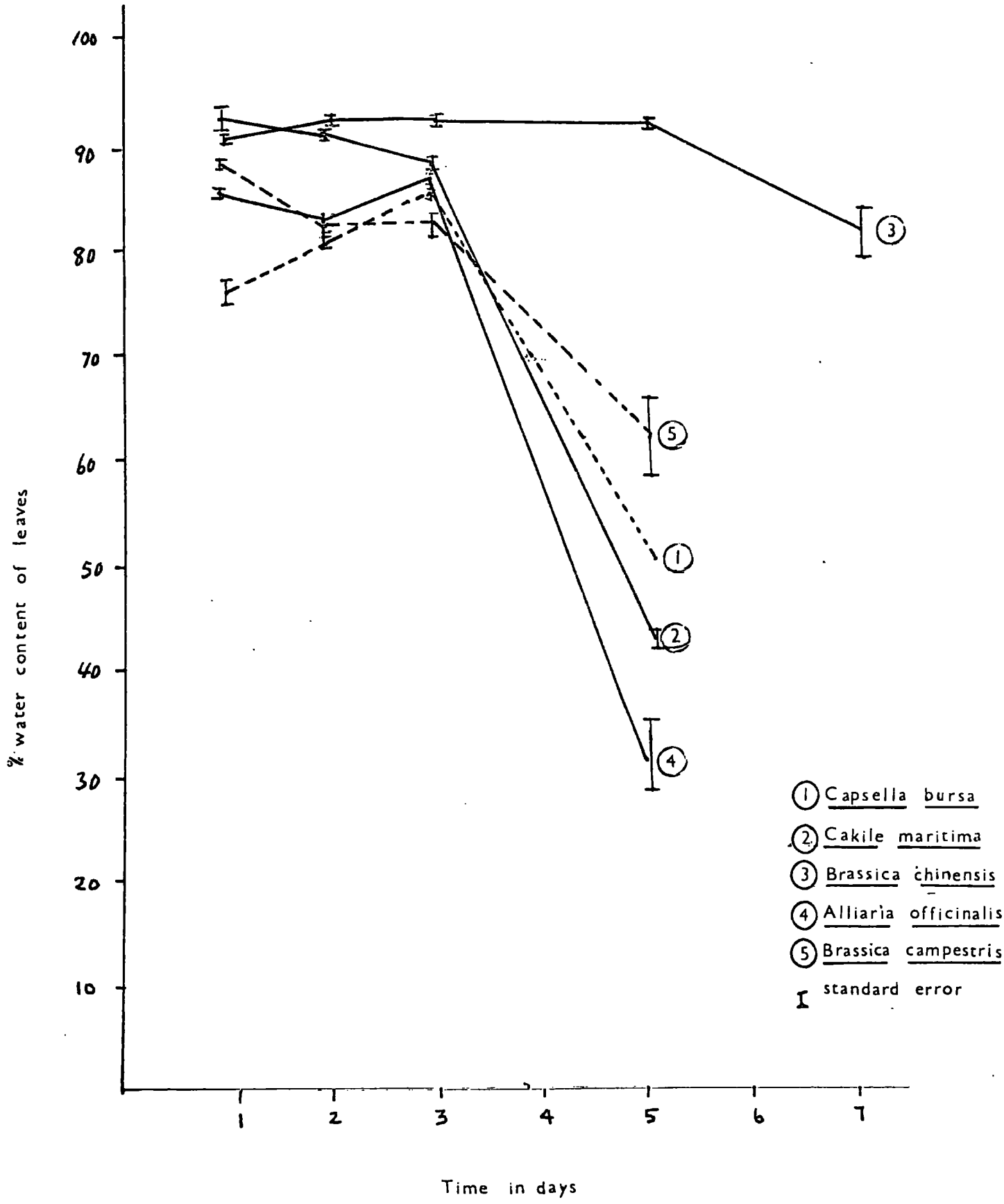
| Species | | Number of days from collection 1 | 2 | 3 | 5 | 7 | Accumulation Factor[1] |
|---|---|---|---|---|---|---|---|
| Brassica campestris | % water content of leaves | 89.9 ± 0.1 | 83.0 ± 0.8 | 83.5 ± 1.1 | 62.6 ± 3.7 | | |
| | proline content | | | | | | |
| | - μmoles/g dry weight | 14.7 ± 0.8 | 106.1 ± 9.6 | 87.9 ± 8.8 | 39.6 ± 2.9 | - | 7.2 |
| | - μmoles/gfw of leaf | 1.5 ± 0.1 | 18.0 ± 1.2 | 14.3 ± 0.9 | 14.7 ± 1.0 | | |
| | - mg/gfw of leaf | 0.17 ± 0.0 | 2.08 ± 0.13 | 1.64 ± 0.1 | 1.69 ± 0.1 | | |

[1] Calculated by dividing the maximum level of proline obtained by the level obtained on Day 1.

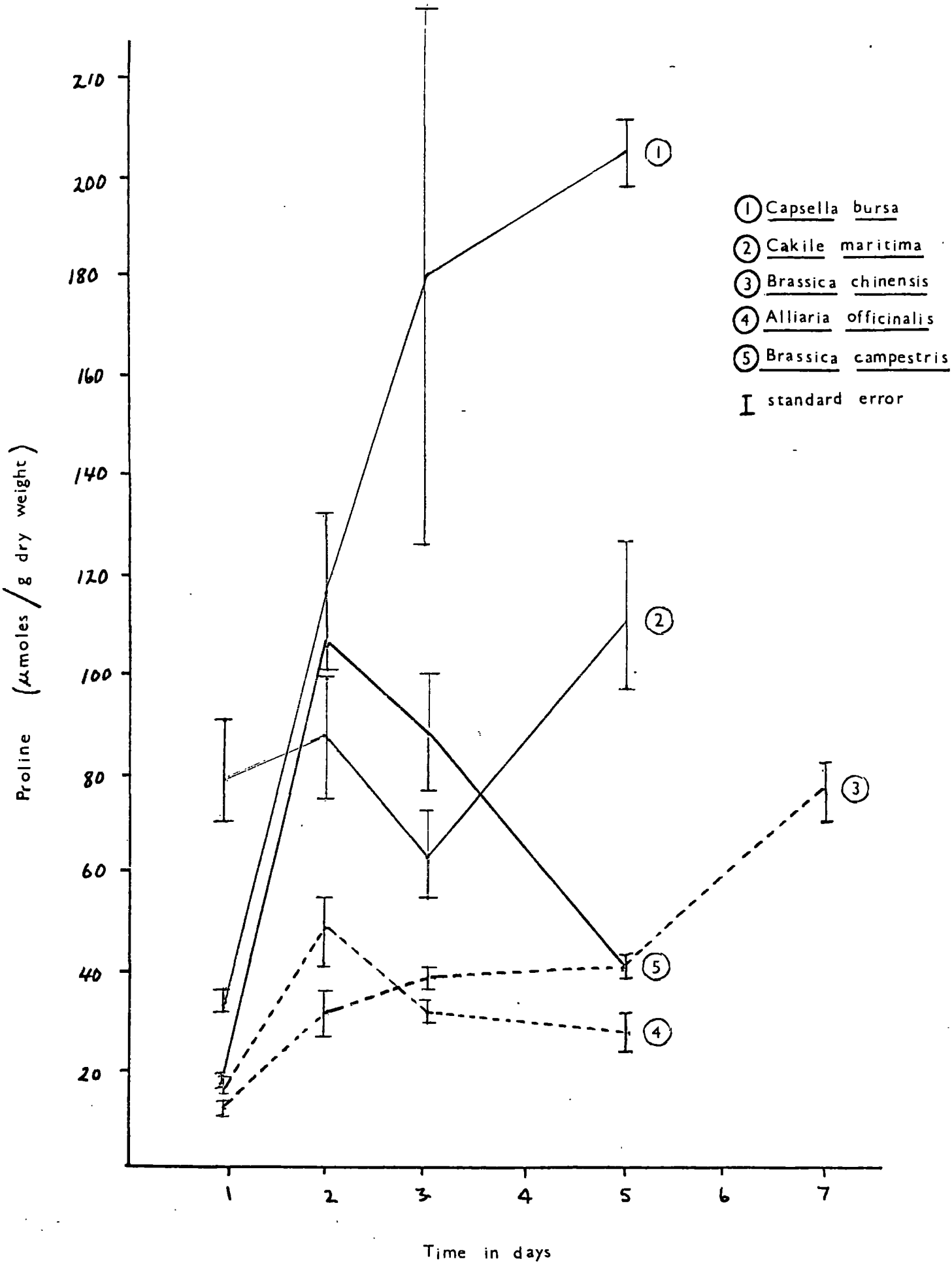
Six replicates were used.

Figure 7

PERCENTAGE WATER CONTENT OF LEAVES OF CRUCIFERAE DURING WILTING



PROLINE LEVELS IN LEAVES OF CRUCIFERAE DURING WILTING



83.0% respectively.

Two analyses of variance were tried to test for the significance of family as a factor accounting for both the initial level of proline and the highest level attained (Appendices D and E, Table 12). When the initial levels of proline of species of the two families were compared, an 'F' value of 9.71 was obtained (df = 1,57) indicating a significant difference between the levels of the two families (p = 0.01). An 'F' value of 39.08 was obtained when the highest proline levels of species of the two families were compared (df = 1,78). This value indicates a significant difference (p = 0.01) between the maximum levels of proline in the leaves of species of Compositae and Cruciferae.

Again the arbitrary nature of the choice of species must be emphasized. The very high levels of proline produced by Sinapis arvensis will have greatly influenced the results obtained for Cruciferae.

Table 12

Summary of proline levels and water content, initially and after wilting, of selected Compositae and Cruciferae.

| Species | μmoles proline/gdw | | Accumulation Factor | % water content | |
|---|---|---|---|---|---|
| | Initial level | Highest level | | On collection | During Highest Proline |
| Compositae | | | | | |
| Bellis perennis | 9.7 | 30.8 | 3.2 | 86.6 | 75.0 |
| Sonchus arvensis | 9.2 | 29.4 | 3.2 | 87.6 | 90.5 |
| Circium arvense | 12.4 | 74.4 | 6.0 | 81.4 | 82.8 |
| Senecio aquaticus | 17.6 | 139.2 | 7.9 | 80.5 | 62.3 |
| Senecio jacobaea | 14.9 | 49.2 | 3.3 | 89.5 | 88.9 |
| Latuca saligna | 5.0 | 27.9 | 5.6 | 74.5 | 27.8 |
| MEANS | 11.8±1.1 | 47.9±4.0 | 4.9 | 83.4 | 71.2 |
| Cruciferae | | | | | |
| Sinapis arvensis | 3.2 | 149.8 | 47.4 | 84.8 | 17.1 |
| Capsella bursa | 31.4 | 205.4 | 6.5 | 76.5 | 51.5 |
| Brassica chinensis | 10.0 | 75.1 | 7.5 | 90.9 | 82.0 |
| Alliaria officinalis | 14.6 | 47.2 | 3.3 | 86.5 | 84.7 |
| Brassica campestris | 14.7 | 106.1 | 7.2 | 89.9 | 83.0 |
| Cakile maritima | 78.4 | 111.0 | 1.4 | 92.7 | 43.4 |
| MEANS | 29.7±5.7 | 113.8±9.7 | 12.2 | 86.9 | 60.3 |

Analysis of Variance Results

i) Initial levels of proline

| Source of variation | Sum of squares | Degrees of freedom | Mean squares |
|---|---|---|---|
| a) Between sites | 4730.457 | 1 | 4730.457 |
| b) Residual | 27780.141 | 57 | 487.371 |
| c) Total | 32510.598 | 58 | |

Variance ratio = 9.71

ii) Highest levels of proline

| Source of variation | Sum of squares | Degrees of freedom | Mean squares |
|---|---|---|---|
| a) Between sites | 86961.625 | 1 | 86961.625 |
| b) Residual | 17357.563 | 78 | 2225.366 |
| c) Total | 260540.188 | 79 | |

Variance ratio = 39.08

## Discussion

The phenomenon of proline accumulation during water stress is widespread throughout families and habitats. This statement is supported by the present study, as well as by studies of barley by Singh et al (1973,I), sorghum and soybean by Waldren et al (1974), rye grass by Kemble and MacPherson (1954), clover by Routley (1966) and by the study of many cultivated species by Palfi et al (1974). Accumulation factors vary. The highest factor obtained during this study was a 47 times increase in the amount of proline; this factor is based on a dry weight basis. Others have obtained higher factors, but their values for proline have been based on quantity expressed per wet weight.

Results for proline content would be better expressed in relation to total amino acid content as was done by Stewart and Lee (1974). Expressing proline content as a percentage of the total amino acid content permits a better comparison of the proline content of various species, as amino acid content varies considerably with species. To obtain substantial data, an automatic analyser for amino acids would be required and the use of this equipment was not possible for this project. An attempt was made to estimate total amino acids colourimetrically using a ninhydrin reagent. However it yielded too crude a measure and was abandoned. To have gained a good calibration curve for total amino acids would have necessitated the use of a mixture of amino acids in similar

proportion to those found in the species of leaf studied.

Whether there are critical levels of drought stress at which proline accumulates more rapidly is not known. My results are inconclusive. Waldren and Teare (1974) and Singh et al (1973, I) did suggest there may be a concentration level above which proline accumulates more rapidly.

As to the fate of the accumulated proline on death of the plant, only speculation exists. Routley (1966, p.360) states: 'the fate of proline after watering (to relieve stress) or prior to death of the leaves is not known'.

Neither Singh et al (1973,III), Waldren and Teare (1974), Kemble and MacPherson (1954), Routley (1966) nor Palfi et al (1974) report on a subsequent decline in proline content on death, as their experiments were not carried out until death. However, this present study indicates that decline is likely. Four of the six Compositae and two of the six Cruciferae studied had proline levels on the last day of testing that were lower than the maximum levels attained.

Thompson et al (1966) in their experiments with turnips noted a rise in proline content and then a subsequent decline. Thompson suggested that this disappearance of proline (which occurs in turnips far earlier than in ryegrass studied by Kemble and MacPherson, 1954) might be due to the low water content in ryegrass which reduces the enzymatic activity which would break down proline. When making this suggestion, he drew attention to a correlation between sugar decline and proline decrease.

A critical question which has not been answered is whether the high proline levels are adaptive. Two approaches are helpful in answering this question. The first approach is to compare levels in species grown or found in different types of habitats subject to a range of water stress conditions. For instance, Stewart and Lee (1974) suggest that high proline levels may be adaptive with salt marsh plants. Armeria maritima from coastal populations had higher levels of proline than populations from mountains where drought stress due to salt does not occur.

The experiments of Singh et al (1973, III) also point to the possible adaptive advantages of increased proline levels. Varieties of barley which accumulated large concentrations of free proline tended to have leaves which survived extreme water stress and grew more rapidly following stress relief.

In contrast to the work of Stewart and Lee (1974) and Singh et al (1973, III), results for two varieties of Bermuda grass showed no significant difference in proline content between varieties growing in two areas widely different in respect of water availability (Barnett and Naylor, 1966). My study did not investigate the same species from different habitats, except for the preliminary investigations with Balsam impatiens which showed no difference in initial proline levels or in amounts accumulated. Certainly a far more sophisticated method is needed to assess the drought stress status of the plant. As an index I used percentage water content of the leaves which is useful for within-species comparisons, but not to compare species.

Observations on leaf water potential or stomatal diffusive resistance, both used by Singh et al (1973,III), would be appropriate; they were, however, beyond the scope of this project.

The second approach to investigate whether high proline levels are adaptive is to determine whether there is a correlation between the level of drought stress of the habitat and the levels of proline in plants found there. Plants of each habitat would have to be classified in three ways:-

i) according to their initial levels of proline on collection

ii) According to their maximum level of proline attained under drought stress

iii) according to their proline accumulation factor.

On this basis they could be characterised as proline 'accumulators' or not. Palfi et al (1974) gives an empirical definition of a plant which he terms 'proline accumulating'. He would apply that term:

> 'if the amount of free proline in the leaves (in stage of microsporogenesis, with illumination) at times of strong water deficit attains at least 1% of its dry weight'.

When the plants have been characterised in this way, one could look for correlations with the water stress situation of the habitats.

To obtain plants for inclusion in such a site comparison, it would be necessary to resort to the use of phytosociological data to obtain species of high frequency.

To obtain a measure of the drought stress of the habitat is not easy. I used only a subjective assessment, but objective quantitative determinations are called for.

In conjunction with this approach, it would be useful to record wilt-susceptibility of the plants. Also more extreme habitats than those observed in this study might, with advantage, be included.

From this project only a tentative conclusion can be suggested. Capacity for proline accumulation seems to be genetically controlled for a particular species. Species which accumulate proline may be more frequent in drought-prone habitats than species which do not have this ability, but a more extensive study is still needed.

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# Appendix A

## Reagent - Ninhydrin

1.25g ninhydrin was warmed in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid, with agitation until dissolved.

## Appendix B

Initial levels of proline found for each replicate of each species of drought-susceptible and drought-resistant habitats

Proline levels expressed as μmoles/g dry weight of leaf for:

1) Drought-susceptible habitats

| | | | | | | |
|---|---|---|---|---|---|---|
| 4.3 | 5.6 | 4.1 | 3.0 | 4.1 | 6.9 | 3.9 |
| 5.3 | 6.4 | 6.8 | 8.7 | 4.8 | 6.6 | 15.0 |
| 6.7 | 11.2 | 3.3 | 10.4 | 8.3 | 81.1 | 60.7 |
| | | | | 33.7 | 33.7 | 16.4 |
| 24.6 | 37.7 | 57.2 | 20.8 | 19.5 | 11.6 | 13.9 |
| 19.6 | 23.0 | 21.2 | 16.3 | 12.8 | | |

Mean = 17.5 ± 3.0 n = 36

2) Drought-resistant habitats

| | | | | | | |
|---|---|---|---|---|---|---|
| 6.8 | 7.4 | 6.1 | 5.4 | 4.2 | 2.7 | 4.6 |
| 5.2 | 4.0 | 6.3 | 14.9 | 20.2 | 3.2 | 3.9 |
| 4.5 | 5.0 | 5.4 | 3.6 | 2.2 | 5.3 | 9.1 |
| 9.1 | 13.8 | 5.0 | 10.0 | 9.2 | 14.3 | 10.6 |
| 12.7 | 14.8 | 14.8 | 17.5 | 17.4 | 16.0 | |

Mean = 8.6 ± 0.9 n = 34

Analysis of variance results

Variance ratio = 7.74

| Source of Variation | Sum of Squares | Degrees of freedom | Mean Squares |
|---|---|---|---|
| i) Between sites | 1352.672 | 1 | 1352.672 |
| ii) Residual | 11888.156 | 68 | 174.826 |
| iii) Total | 13240.828 | 69 | |

## Appendix C

Maximum levels of proline found for each replicate of each species of drought-susceptible and drought-resistant habitats.

Proline levels expressed as μmoles/g dry weight of leaf for:

1) Drought-susceptible habitats

| | | | | | | |
|---|---|---|---|---|---|---|
| 5.91 | 11.24 | 11.24 | 7.26 | 68.34 | 65.21 | 8.80 |
| 5.52 | 6.46 | 5.38 | 4.27 | 15.00 | 102.39 | 11.49 |
| 8.92 | 105.91 | 91.86 | 69.48 | 91.36 | 83.00 | 83.09 |
| | | | | 74.54 | 105.11 | 14.89 |
| 65.77 | 65.77 | 41.31 | 44.83 | 35.11 | 71.33 | 63.67 |
| 45.00 | 34.63 | 24.28 | | | | |

Mean = 45.5 ± 6.0 n = 34

2) Drought-resistant habitats

| | | | | | | |
|---|---|---|---|---|---|---|
| 56.93 | 54.61 | 20.36 | 6.30 | 7.21 | 8.29 | .44 |
| 10.36 | 70.50 | 66.83 | 7.16 | 41.22 | 3.23 | 3.87 |
| 40.06 | 16.41 | 10.24 | 6.21 | 10.10 | 10.06 | 38.83 |
| 10.61 | 6.47 | 5.16 | 10.81 | 11.26 | 6.46 | |

Mean = 20.3 ± 4.0 n = 27

Analysis of variance results

Variance ratio = 11.09

| Source of Variation | Sum of Squares | Degrees of freedom | Mean Squares |
|---|---|---|---|
| i) Between sites | 9617.188 | 1 | 9617.188 |
| ii) Residual | 51157.500 | 59 | 867.076 |
| iii) Total | 60774.686 | 60 | |

## Appendix D

Initial levels of proline found for each replicate of each species of Compositae and Cruciferae

Proline levels expressed as μmoles/g dry weight of leaf for:

1) Compositae

| | | | | | |
|---|---|---|---|---|---|
| 20.4 | 8.8 | 16.5 | 17.8 | 14.8 | 10.9 |
| 4.9 | 4.9 | 6.5 | 6.9 | 2.9 | 3.8 |
| 11.6 | 13.9 | 19.6 | 23.0 | 21.2 | 16.3 |
| 6.0 | 6.4 | 13.5 | 12.7 | 7.0 | 9.8 |
| 16.3 | 16.3 | 13.2 | 16.3 | 6.2 | 6.0 |

Mean = $11.8 \pm 1.1$ n = 30

2) Cruciferae

| | | | | | |
|---|---|---|---|---|---|
| 8.8 | 8.1 | 9.3 | 8.5 | 7.9 | 17.2 |
| 13.2 | 11.8 | 15.3 | 16.8 | 14.5 | 15.9 |
| 81.1 | 60.7 | 122.2 | 122.2 | 25.5 | 58.8 |
| 24.5 | 37.0 | 28.8 | 34.3 | 30.6 | 14.3 |
| 15.9 | 10.9 | 14.9 | 15.2 | 16.9 | |

Mean = $29.7 \pm 5.7$ n = 29

Analysis of variance results

Variance ratio = 9.71

| Source of Variation | Sum of Squares | Degrees of freedom | Mean Squares |
|---|---|---|---|
| i) Between sites | 4730.457 | 1 | 4730.457 |
| ii) Residual | 27780.141 | 57 | 487.371 |
| iii) Total | 32510.598 | 58 | |

## Appendix E

Maximum levels of proline found for each replicate of each species of Compositae and Cruciferae

Proline levels expressed as μmoles/g dry weight of leaf for:

1) Compositae

| | | | | | |
|---|---|---|---|---|---|
| 30.7 | 20.2 | 30.6 | 41.9 | 31.9 | 18.5 |
| 68.3 | 93.6 | 73.6 | 60.1 | 86.6 | 64.1 |
| 105.9 | 91.9 | 69.4 | 91.3 | 83.0 | 83.1 |
| 53.6 | 53.6 | 29.8 | 34.3 | 70.8 | 93.1 |
| 24.5 | 17.0 | 26.7 | 28.6 | 35.1 | 35.1 |
| 39.2 | 47.0 | 24.2 | 24.2 | 39.0 | 45.2 |
| 30.5 | 27.3 | 19.4 | 12.4 | | |

Mean = $47.9 \pm 4.0$ n = 40

2) Cruciferae

| | | | | | |
|---|---|---|---|---|---|
| 228.8 | 232.5 | 187.2 | 198.8 | 184.5 | 207.6 |
| 164.9 | 150.4 | 88.7 | 82.2 | 74.5 | 105.1 |
| 36.1 | 35.1 | 33.3 | 40.4 | 41.1 | 50.3 |
| 29.7 | 33.6 | 58.1 | 78.3 | 67.2 | 16.0 |
| 102.5 | 152.2 | 87.3 | 94.7 | 104.7 | 95.2 |
| 138.0 | 94.2 | 196.2 | 202.1 | 131.8 | 133.2 |
| 132.0 | 156.6 | 169.6 | 145.2 | | |

Mean = $113.8 \pm 9.7$ n = 40

Analysis of variance results

Variance ratio = 39.08

| Source of Variation | Sum of Squares | Degrees of freedom | Mean Squares |
|---|---|---|---|
| i) Between sites | 86961.625 | 1 | 86961.625 |
| ii) Residual | 173578.563 | 78 | 2225.366 |
| iii) Total | 260540.188 | 79 | |