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# Changes in Proline Concentration of the Lichen *Pseudevernia furfuracea* during Drought Stress

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With 1 Figure

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## Summary

The lichen *Pseudevernia furfuracea* (L.) Zopf was experimentally subjected to water stress. Decreasing the air humidity (R. H.) gave low values for water potential ($\Psi$) but this was not accompanied by a marked accumulation of free proline. Only a twofold increase was observed. Six other amino acids investigated responded similarily. Proline together with the other amino acids, therefore, do not play an important role as osmotic regulants in the present case.

## Zusammenfassung

Änderungen der Prolin-Konzentration in der Flechte *Pseudevernia furfuracea* unter Wasserstreß

Unter Laborbedingungen wurde der Einfluß von Wasserstreß auf die Konzentration an freiem Prolin in der Flechte *Pseudevernia furfuracea* (L.) Zopf untersucht. Bei niedrigen Luftfeuchten und einem daraus resultierenden starken Abfall des Wasserpotentials wurde eine nur 2-fache Akkumulation des freien Prolins festgestellt. Auch sechs weitere Aminosäuren zeigen unter diesen Wasserstreßbedingungen nur geringe Konzentrationsveränderungen. Dem Prolin und diesen Aminosäuren werden im vorliegenden Fall keine osmoregulatorischen Funktionen zugeschrieben.

## Introduction

One typical reaction of plants to periods of water deficit is the change in the concentrations of free amino acids (Singh *et al.* 1973, Hsiao 1973, Baskin & Baskin 1974, Jäger & Meyer 1977). In particular, several authors have reported an accumulation of free proline (Palfi *et al.* 1974,

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Huber 1974, Jäger & Meyer 1977). Since all reports of proline accumulation have so far dealt with plants which exhibit a homoiohydrous type of water relationship (Walter 1931) it was of interest to investigate a plant able to tolerate periods of water shortage. Lichens are the most characteristic poikilohydrous plants and show significant drought resistance (Lange 1953, 1969, Kappen 1973). This paper reports an investigation of the change in proline content in the lichen *Pseudevernia furfuracea* (L.) Zopf when subjected to increasing drought stress.

## Material and Methods

### 1. Lichen material

The lichen *Pseudevernia furfuracea* (L.) Zopf was collected in the vicinity of Immenstadt (Allgäu; 1,250 m N. N.) in August 1975. The lichen material was air dried (Lange 1953) and stored at 40±2% R. H., 24±0.5° C, 1,200 lux in a photoperiod of 12 : 12 h.

### 2. Exposure to drought stress

Water relations were controlled using saturated salt solutions after Winston & Bates (1960): 0% R. H. = $P_2O_5$; 20% = $CH_3CO_2K$; 33% = $MgCl_2$; 55% = $Ca(NO_3)_2$; 80% = $(NH_4)_2SO_4$; 100% = distilled water. Exposure of the lichen to the various air humidities was by placing small bags of cheesecloth containing the lichen material in a glass vessel (20 l) containing 2 l of the salt solutions at 20±0.5° C and 10,000 lux (Lange 1953). Equilibration of the samples against the atmospheres of various R. H. was complete in 3 days, when a stable fresh weight had been reached. From that time the stress condition was maintained for 5 days.

### 3. Water potential (Ψ), water saturation deficit (WSD) and relative water content (RWC)

Ψ was calculated by the equation of Kramer *et al.* (1966). WSD and RWC were calculated after Slatyer (1967) and Showman & Rudolph (1972).

### 4. Extraction and determination of the free amino acids

Extraction of the free amino acids from lichen material was performed with methanol-chloroform-water according to the method of Jäger (1975). Quantitative determination of the free amino acids was carried out by column exchange chromatography with an amino acid analyzer (LKB 3201).

The experiments were repeated three times.

## Results and Discussion

Table 1 summarizes the water relations of *P. furfuracea* at the end of the five-day drought period. The rapid decrease in water potential with

Table 1

Water relations of *Pseudevernia furfuracea* at different air humidities (R. H.)

| R. H. (%) | 0 | 20 | 33 | 55 | 80 |
|---|---|---|---|---|---|
| Water potential Ψ (bars) | $-10^4$ | −2175 | −1498 | −807 | −301 |
| Rel. water content RWC (%) | 16.7 | 23.2 | 25.9 | 33.6 | 42.7 |
| Water saturation deficit, WSD (%) | 83.2 | 76.6 | 74.0 | 66.3 | 57.2 |

decreasing air humidity is indicative of severe water stress for the lichen. Even a relatively high air humidity (80%) caused a water potential of −301 bars. Similar results were found by BROCK (1975). As a rough guide this author claims that in the range of 100—80% R. H. a decrease in R. H. of 1% is correlated with a decrease in water potential of −15 bars. No marked proline accumulation in *P. furfuracea* was observed in response to

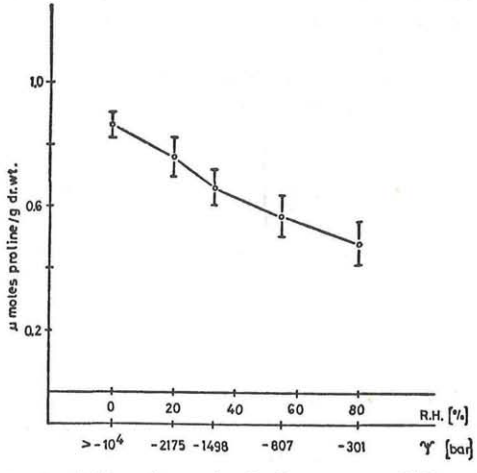


Fig. 1. Proline content of *Pseudevernia furfuracea* at different air humidities

drought stress (Fig. 1). Although its concentration constantly increased over the range 80—0% R. H., the change was only about twofold. This result is not in accord with those of PALFI *et al.* (1974), HUBER (1974) and JÄGER & MEYER (1977), who found up to 100-fold accumulation of proline in higher plants subjected to water stress. These authors consider that proline accumulation functions to encourage C-, N- and energy storage as well as an adaption mechanism by which plants overcome short periods of drought. In contrast, HELLEBUST (1976) and SCHOBERT (1977) see proline

as an osmoregulatory compound. Fungi and other organisms are able to obtain water from environments of particular water potential only if the osmotic pressure of their cytoplasm is higher than that of the environment (ADEBAYO *et al.* 1971). By synthesizing low molecular weight substances, such as amino acids and sugars, the organism's requirement for a high internal osmotic pressure can be met. For instance, this is true for the sugar alcohol mannosidomannitol in the lichen *Lichina pygmea* (FEIGE 1975). As proline has the highest solubility in water of all amino acids it may function together with other amino acids as an osmotic regulant. In the present case, however, proline as well as the amino acids some of which were chosen as representative (Tab. 2) do not make a contribution to osmotic regulation.

Table 2

Amino acid content (μmoles/g dr. wt.) of *Pseudevernia furfuracea* at different air humidities

| R. H. (%) | 0 | 20 | 33 | 55 | 80 |
|---|---|---|---|---|---|
| glutamic acid | 8.34±0.66 | 5.54±0.25 | 7.54±0.77 | 3.76±0.37 | 4.34±0.34 |
| glutamine | 16.60±3.40 | 17.00±3.50 | 14.60±3.00 | 32.20±5.60 | 15.40±3.20 |
| arginine | 0.83±0.07 | 0.99±0.10 | 0.61±0.08 | 1.29±0.16 | 0.55±0.05 |
| aspartic acid | 0.38±0.09 | 0.33±0.07 | 0.44±0.09 | 0.78±0.13 | 0.42±0.09 |
| asparagine | 1.15±0.25 | 0.50±0.13 | 0.28±0.05 | 1.03±0.20 | 0.68±0.15 |
| threonine | 1.06±0.25 | 0.68±0.25 | 0.55±0.14 | 0.34±0.07 | 0.67±0.13 |

This is confirmed by a calculation according to FLOWERS *et al.* (1977) and also by the finding in these experiments that the fully saturated lichen thallus (100% R. H.) contained the same amount of free proline as the severely (0% R. H.) stressed one.

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