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Growth, water relations, and CAM induction in *Sedum album* in response to water stress

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Abstract

Growth analysis indicated that carbon gain in the alpine succulent *Sedum album* tended to take place early during the growing season. Leaf water potential remained unchanged for a considerable length of time after the imposition of water stress in the field. Induction of crassulacean acid metabolism (CAM) by protracted water stress occurred before any signs of stress could be observed in the leaves, and appeared to be influenced by a complex interaction of environmental conditions including temperature and duration of sunshine. Increased levels of proline and betaine towards the end of the growing season appeared to reflect seasonal changes.

Introduction

The characteristic morphology, physiology, peculiar stomatal behaviour, and water conservation are fundamental attributes that make constitutive CAM plants the archetype of water stress avoidance. Water use efficiency of CAM plants is superior to C_3 and C_4 plants and is primarily due to their capability for nocturnal CO_2 uptake (Winter 1985). Salinity and water stress are environmental stimuli known to induce the enzymatic machinery of CAM in a number of succulent C_3 species of the *Aizoaceae* (Winter *et al.* 1978, Sayed and Hegazy 1991), and the *Asteraceae* (Earnshaw *et al.* 1987). Induction of facultative CAM by water stress has also been reported in some members of the *Crassulaceae*, of which the genus *Sedum* has received considerable attention (Kluge 1977, Schuber and Kluge 1981, Earnshaw *et al.* 1985, Groenhof *et al.* 1986, Lee and Griffiths 1987). Expression of CAM in *Sedum* spp. has been suggested to involve acceleration by water stress of ontogenetical processes (Schuber and Kluge 1981), and is thought to be influenced by temperature (Earnshaw *et al.* 1985). Moreover, values of $\delta^{13}C$ of -24.4 and -25.4 have also been reported in CAM-performing *Sedum album* suggesting that carbon gain in this species tend to occur early during the growing season (Earnshaw *et al.*

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1985) via C_3 photosynthesis. Work described in this paper was designed to study the effect of protracted water stress on growth, water relations, and the induction of CAM in *Sedum album* under field conditions in order to distinguish between environmental and ontogenetical factors.

Materials and methods

Plant material: Plants of the alpine succulent *Sedum album* were collected from the Spanish Pyrenees in 1983 and were raised under natural conditions in two separate plots of ground (2 m^2 each) at the Firs Experimental Grounds, University of Manchester. Our observations suggest that new shoots initiated during one growing season as vegetative shoots become much elongated flowering shoots during the following year. Senescence of these shoots rapidly ensues following the flowering period.

Experimental design: For growth analysis experiments some plants were transferred to pots (15 cm diameter, 1 kg soil) that were embedded in the above mentioned plots in 1985). Plots were covered by a *Perspex* framework (60 cm above ground) in May 1987 to ensure that they did not receive precipitation. Water stress was imposed in one of these two plots by withholding irrigation while the other plot continued to be irrigated at weekly intervals so as to maintain a stable high soil water potential of about zero MPa. Experiments described herein continued until October 1987.

Methods: Air temperature was measured using a max/min thermometer located in adjacent shade. Duration of daily sunshine was recorded using a *Campbell-Stokes* type sunshine recorder (Casella, UK). Relative growth rate (RGR) based on dry matter of both shoots (vegetative and flowering) and roots of plants growing in pots was estimated by mathematical analysis (Chiariello *et al.* 1989). All other parameters were measured using vegetative shoots of plants growing in the plots. Soil water potential (ψ_{soil}), and leaf water potential (ψ_{leaf}) were measured using the *Wescor C-52 Sample Chamber* and *HR 33 Dew Point Microvoltmeter* (*Wescor*, Logan, USA), as previously described (Earnshaw *et al.* 1985).
Titratable acidity was measured by titration with 0.03M NaOH (Earnshaw *et al.* 1985) and deacidification was presented as the difference between dawn and dusk titratable acidity (ΔH^+). Leaf contents of chlorophyll, proline and betaine were also determined (Arnon 1949, Singh *et al.* 1973, Storey and Wyn Jones 1977, respectively). Three measurements of each parameter were routinely carried out and standard error bars were only omitted when they were too small to apply to data points.

Results

An overall decline in RGR of shoots and roots was observed in both irrigated and non-irrigated plants, and appeared to be much more rapid and dramatic in non-irrigated plants (Fig. 1B, C). Withholding irrigation resulted in a substantial decrease in ψ_{soil} (Fig. 2C). Values of ψ_{leaf} of non-irrigated plants remained more or less unchanged for a considerable length of time and only exhibited a declining trend towards the end of the experiment (Fig. 2D). Total chlorophyll content was initially similar in both irrigated and non-irrigated plants, and showed a gradual increase towards the end of the experiment that coincided with a surge of new vegetative shoots (Fig. 2E). Values of ΔH^+ were similar in both irrigated and non-irrigated plants except on days 21, 42, 56, 63, and 91 when non-irrigated plants exhibited higher values (Fig. 2F). These high ΔH^+ values coincided with a large difference (*ca.* 15 °C) between maximum and minimum temperatures, and long duration of sunshine of 8 - 10 h (Fig. 2A, B). A gradual increase in leaf contents of proline and betaine was observed and was followed by a sizable increase in both irrigated and non-irrigated plants towards the end of the experiment (Fig. 2G, H).

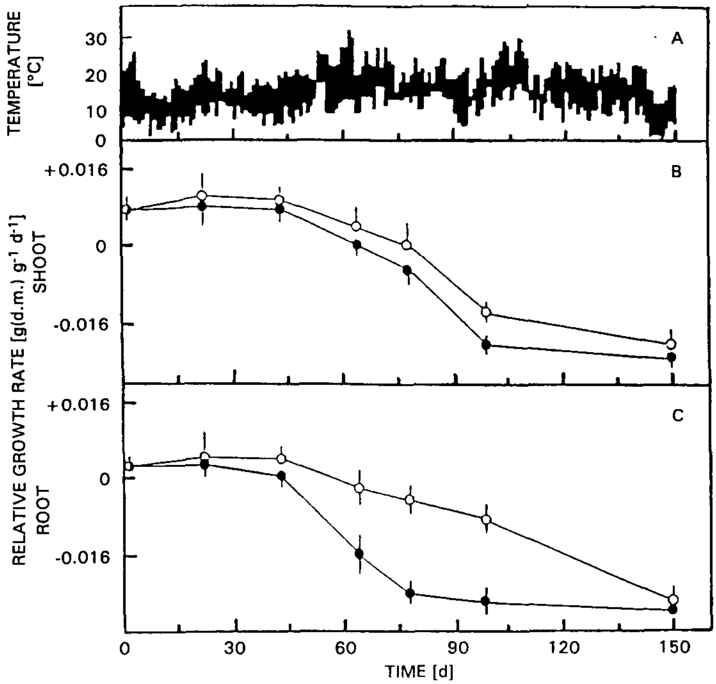

Fig. 1. Daily course of maximum and minimum air temperatures (A), and the effect of protracted water stress in the field on the relative growth rate of shoots (*B*) and roots (*C*) of irrigated plants (*open circles*) and non-irrigated plants (*closed circles*) of *Sedum album* (verical bars represent SE, n = 3).

Discussion

In the growth analysis experiment the biomass of flowering shoots greatly exceeded that of the vegetative shoots. The declining values of RGR of shoots (Fig. 1B) and roots (Fig. 1C) of irrigated *Sedum album* plants reflect, therefore, the onset of senescence in the flowering shoots. Negative values of RGR were due to water loss of senescing flowering shoots and their associated roots towards the end of the

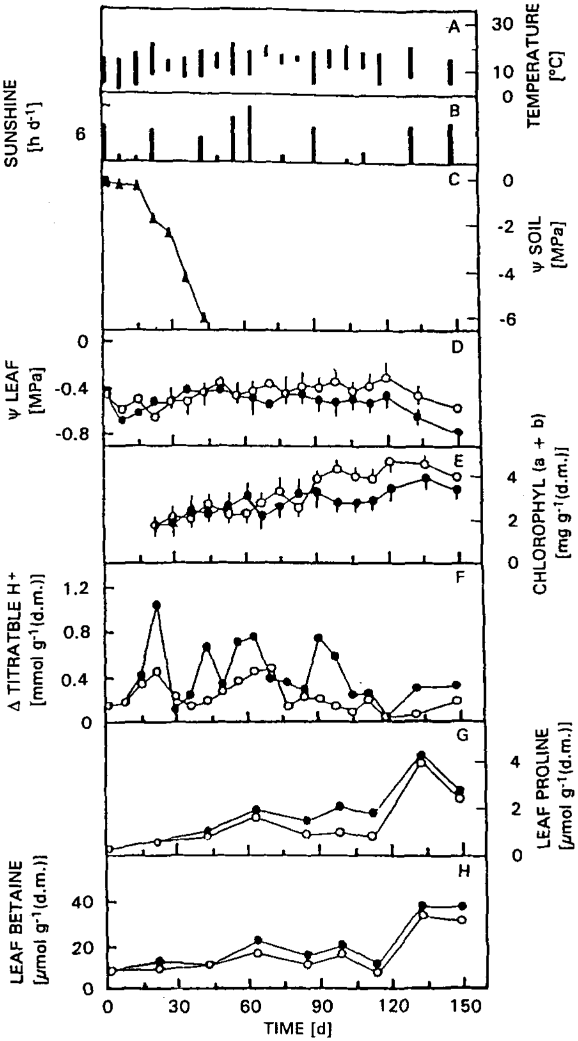

Fig. 2. Air temperature (*A*), daily duration of sunshine (*B*), soil water potential (*C*), and effect of protracted water stress on leaf water potential (*D*), total chlorophyll (*E*), titratable acidity (*F*), and the accumulation of proline and betain (*G, H*) in leaves of irrigated plants (*open circles*) and non-irrigated plants (*closed circles*) of *Sedum album* (verical bars represent SE, n = 3).

experiment. It is worthnoting that shoots of non-irrigated plants exhibited a similar pattern of declining RGR (Fig. 1B). Previous work had shown values of $\delta^{13}C$ of -24.4 and -25.5 in CAM-performing *S. album* (Earnshaw *et al.* 1985) suggesting that CAM makes little contribution to overall carbon gain in this species. A similar pattern has been reported in *S. acre* where carbon is predominantly gained via C_3 photosynthesis (Schuber and Kluge 1981). However, the observed decline in RGR of roots of non-irrigated plants of *S. album* was more rapid and much more pronounced (Fig. 1C) indicating more rapid root senescence.

S. album represented an example of a C_3-CAM intermediate in which CAM could be induced by protracted water stress as indicated by high ΔH^+ values (Fig. 2F). This water stress-induced CAM has previously been reported in *S. album* (Earnshaw *et al.* 1985). However, it was evident that high ΔH^+ values occurred in non-irrigated plants when large differences between day and night temperatures occurred together with long duration of sunshine (Fig. 2A, B, F), indicating that water stress did not act in isolation. Thus low night temperature, high day temperature, and high light represented a complex interaction of environmental conditions that affected CAM in *S. album*. It was also evident that ΔH^+ values occurred in non-irrigated plants before any appreciable increase in leaf contents of proline and betaine could be recorded. These compounds are widespread, appear to possess protective properties, and tend to accumulate in stressed tissues (Stewart 1989). Moreover, the values of ψ_{leaf} remained more or less unchanged for a considerable length of time after ψ_{soil} had dropped significantly. It was clear that the deficit was sensed before any changes in leaf mesophyll turgor could be recorded. Although the mechanisms involved in stress-dependent biosynthesis of root-sourced abscisic acid are not fully understood (Hartung and Davis 1991), the reduction in droughted root cell turgor (Raschke 1982) and/or volume (Ackerson and Radin 1983) may act as triggers. It is, therefore, suggested that amiluration of the stress could be a consequence of transfer of the chemical message to the site of action on the stomata. Furthermore, reduced values of ΔH^+ and increased leaf contents of proline and betaine in both irrigated and non-irrigated plants towards the end of the experiment perhaps reflect seasonal ontogenetical changes in the physiology of *S. album*.

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