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Free Proline Accumulation in Sapwood, Bark and Leaves of Three Evergreen Sclerophylls and a Comparison with an Evergreen Conifer

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Summary

Free proline content was investigated in sapwood, bark and foliage of *Ceratonia siliqua, Laurus nobilis, Myrtus communis* and *Pinus halepensis* throughout a 12-month period.

Free proline concentration in leaves and needles peaked during the drought and cold periods, respectively, then declined prior to the flushing of the new foliage; the minimum values coincided with the expansion of the young foliage. The results indicate that proline was neither translocated during stress periods, nor accumulated in turgid foliage. In the bark and sapwood of the four species examined, free proline content was generally lower compared with that of the foliage, but the seasonal trends were similar. Thus, free proline accumulation in sapwood and bark decreased during the main and secondary growth periods.

Key words: Ceratonia siliqua, Laurus nobilis, Myrtus communis, Pinus halepensis, proline.

Introduction

It is well known that the level of proline in plants is raised in response to water deficit (Stewart and Hanson, 1980). Although, this amino acid accumulates in every tissue the place of its synthesis is rather uncertain (Stewart, 1981; Ibarra-Caballero et al., 1988; Voetberg and Sharp, 1991; Kohl et al., 1991). In contrast, the translocation of proline has to be considered as an important feature of its metabolism (Karamanos et al., 1983; Pahlich et al., 1983; Venekamp and Koot, 1988; Rhizopoulou et al., 1990).

Proline as a soluble compound can move through the water pathways, i.e. *via* the xylem and phloem streams (Sauter, 1976; 1981). Water moves to the evaporation sites in the foliage from the nearest, most readily available sources such as the branches and sapwood (Jarvis, 1976). To the best of our knowledge, information dealing with proline accumulation in broad-leafed trees is rather limited and refers to foliage and pollen (Tesche, 1987).

Evergreen sclerophyll species grow in nutrient deficient habitats and exhibit low leaf nitrogen content (Margaris et al., 1984). These species are subjected to a prolonged period of drought immediately after a 3 month growth period. However, they exhibit very deep root systems that enable them to exploit water stored deep in the soil (Rhizopoulou and Davies, 1991). Nevertheless, dissolved nutrient movement from the soil to the leaves is greatly reduced as soil dries (Landsberg, 1986). Hence, their «evergreenness» could be an adaptation to such environmental conditions by providing a sink for nutrient storage during the nongrowth period (Mooney and Rundel, 1979). Also, sclerophylly tends to be negatively correlated with nutrient availability (Specht and Rundel, 1990).

In this study we investigated free proline accumulation in the sapwood, bark and leaves of three native evergreen sclerophyll species (*Ceratonia siliqua* L., *Laurus nobilis* L. and *Myrtus communis* L.) over a 12-month period. These species grow under the same environmental conditions, being major components of East Mediterranean vegetation, and are characterized by a different sized foliage. The research site is dominated by the evergreen conifer, *Pinus halepensis* Miller. Measurements of free proline accumulation were also made in *Pinus* sapwood, bark and needles and were compared with those from the evergreen sclerophylls.

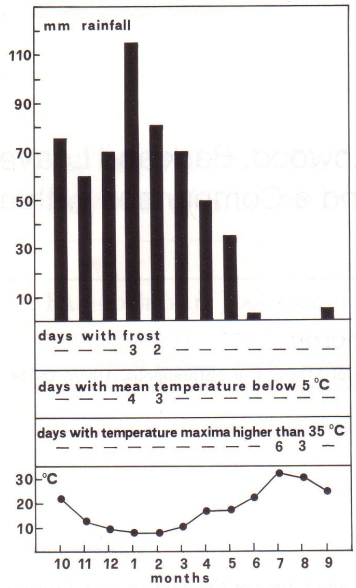

Fig. 1: Climatic diagram of the research area. The order of months is from October to September. Upper diagram: monthly mean rainfall. Low diagram: monthly mean temperature.

Materials and Methods

Ceratonia siliqua L., *Laurus nobilis* L., *Myrtus communis* L., and *Pinus halepensis* Miller plants were grown in the field on mt. Hymettus (latitude 38° 57.5′, longitude 23° 47.8′, altitude about 400 m a.s.l.). The height of the species varied, reaching about 1.5 m for *Myrtus* and about 3.0 m for *Ceratonia* and *Laurus; Pinus* trees reached a height of about 5.0 m. Plant material was collected at 08.00 hours on the 16th day of each month over the period of 1 year (Rhizopoulou et al., 1990).

Free proline content was determined colorimetrically in 4 mL of the condensed fluid in triplicate samples according to a rapid method described by Bates et al. (1973). The sample material was treated as described by Amberger – Oschenbauer and Oberndorfer (1988). Proline content was calculated on a dry weight basis using L-proline (Serva, 33580) for the standard curve.

Values of precipitation and air temperature were obtained from a standard meteorological enclosure about 5.0 km distant from the research site and are given in Fig. 1.

Results and Discussion

The results (Figs. 2–5) give information about: (1) the periodic response of net proline deposition in the tissues and species investigated to seasonal change, (2) the dual (at least) purpose of proline accumulation, (3) the location of proline synthesis and (4) the general features of the mechanism of proline translocation.

In sapwood, proline accumulation exhibits two seasonal periods during the year, i.e. two maxima and two minima. One maximum occurs during the summer drought period (220–260 µmol/g dw) and a somewhat smaller one (180–220 µmol/g dw) during the winter cold period. The minima appear in April–May, i.e. during flushing and development of young expanding leaves (ca. 100 µmol/g dw), and (110–190 µmol/g dw) in October–November, i.e. during the secondary growth period. These fluctuations indicate that saturated permeability of sapwood (Edwards and Jarvis, 1982) responds accordingly to seasonal changes. Another interesting feature is that the periodic patterns of proline accumulation in the three evergreen sclerophylls and the evergreen conifer investigated are generally similar, although there are some difference (*vide infra*).

The fluctuations in proline accumulation are obviously directly related to water availability and reflect the effect of this availability on proline synthesis and translocation. The accumulation of proline in the water-stressed tissues has been

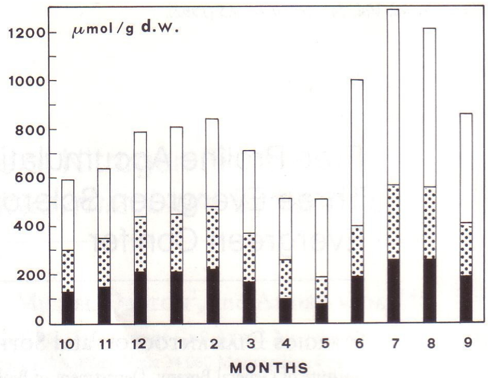

Fig. 2: The seasonal patterns of free proline accumulation in sapwood (dark areas), bark (shaded areas) and leaves (white areas) of *Ceratonia siliqua.*

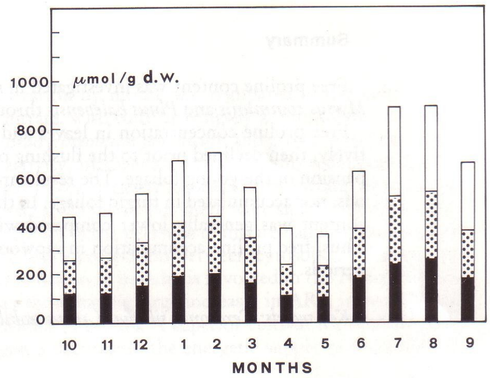

Fig. 3: Seasonal patterns of free proline accumulation in sapwood (dark areas), bark (shaded areas) and leaves (white areas) of *Laurus nobilis.*

creased energy and water resources (Lang, 1983). On the other hand, such a transport might be supported by the mechanism of negative turgor potential (Oertli et al., 1990).

The minimum values for proline accumulation in the leaves of *Ceratonia* (250 µmol/g dw), *Laurus* (145 µmol/g dw) and *Myrtus* (140 µmol/g dw), as well as in needles of *Pinus* (220 µmol/g dw), were recorded at the beginning of the main growth period, which starts in April, coincidently with high water potentials (Rhizopoulou and Mitrakos, 1990). Thereafter, free proline content increased and the maximum values, in *Ceratonia* (720 µmol/g dw), *Laurus* (320 µmol/g dw), *Myrtus* (310 µmol/g dw) and *Pinus* (450 µmol/g dw), were investigated during July. Low values of proline content in leaves and needles were also recorded in October, i.e. at the beginning of a secondary growth period (Rhizopoulou et al., 1991). Thereafter, other shallower maxima were distinguished during winter, i.e. in January for *Ceratonia* (360 µmol/g dw), *Laurus* (260 µmol/g dw) and *Pinus* (300 µmol/g dw) and in February for *Myrtus* (250 µmol/g dw). It is clear that free proline concentration increased rapidly during stress and returned to lower levels after the emergence of leaves and needles. Hence, proline translocation may constitute an essential source of nutrient for the continued growth. Recently, it was reported that proline accumulated to a greater extent with stress that developed slowly rather than rapidly (Naidu et al., 1990). Thus, proline may be a sensitive index of a response dependent on «timing» of environmental stresses.

Considering that proline is a compatible osmotic solute, the presence of high proline levels may be beneficial in postponing leaf senescence (i.e. evergreenness, e.g. by lowering water requirements). Our results confirm that proline was not translocated during the periods of stress, whereas it was mainly accumulated in the above ground tissues of the deep-rooted evergreen sclerophylls. Also, it seems likely that proline retranslocation was independent of nutrients available in the soil. Hence, proline might act as a «buffer» between the deficit of nitrogen and the demand for resource use in growth.

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