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# Diurnal variations in abscisic acid content and stomatal response to applied abscisic acid in leaves of irrigated and non-irrigated *Arbutus unedo* plants under naturally fluctuating environmental conditions

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**Summary.** Endogenous abscisic acid content (ABA) of *Arbutus unedo* leaves growing under natural conditions in a macchia near Sobreda, Portugal, was very high (0.25 to 2.3 μg $g^{-1}$ fresh weight). Highest concentrations were found during the very early morning hours and at midday. During the late morning hours and in the late afternoon ABA concentrations decreased to between one-third and one-fourth of peak values. The samples for ABA content were obtained from both irrigated ($\psi$ between $-10$ and $-25$ bar) and non-irrigated plants experiencing natural water stress during the dry season ($\psi$ of $-50$ bar). During the course of the measurement day, stomatal conductance was relatively constant and conductance of watered plants was 50 to 100% greater than that of unwatered plants. No clear correlations between ABA content and stomatal conductance and/or xylem water potential were observed. Despite large differences in water potential and differences in degree of stomatal opening, absolute concentrations of ABA were not found to differ.

Small quantities (8–14 pmoles $cm^{-2}$ leaf area) of ABA were applied to leaves of irrigated and non-irrigated *Arbutus unedo* plants by injection into the petiole. These extremely small ABA doses resulted in transient reductions in stomatal conductance. The effectiveness with which injected ABA closed stomata was highest during the morning and decreased substantially at midday. Increased sensitivity to injected ABA may again occur in the late afternoon but recent measurements suggest that this may depend on long-term drought experience of the plants. The characteristics of the response to injected ABA were similar in irrigated and non-irrigated plants although irrigated plants responded in general more strongly.

## Introduction

The plant hormone abscisic acid is an important plant factor interacting with environmental controls to determine leaf stomatal aperture (see review by Raschke 1979). Synthesis is induced in the leaves when reduction in plant water potential reduces leaf turgor pressure to approximately zero (Pierce and Raschke 1981). We have recently investigated the stomatal behavior and water relations of plants growing under arid conditions in an evergreen macchia near Lisbon, Portugal (Tenhunen et al. 1980, 1981, 1982; Lösch et al. 1982). While studies have been conducted which were designed to elaborate the role which ABA plays in determining stomatal aperture of mesophytes under field conditions (Kannangara 1982, Xiloyannis 1980), no similar measurements have been undertaken with xerophytes which are differently adapted to stress conditions. During the summer dry season of 1981 in Portugal, we examined possible correlations in leaf endogenous ABA, leaf response to applied ABA, and leaf stomatal behavior of naturally water stressed and irrigated plants of *Arbutus unedo* growing at the same site.

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## Materials and methods

Experiments were performed with *Arbutus unedo* shrubs as well as with shrubs of other species growing in a natural macchia at the Research Station Quinta São Pedro, Sobreda, Portugal during the dry season in August 1981. The shrubs were approximately two meters in height. The irrigated shrub of *Arbutus* was given 25 l of water daily beginning on August 3, 1981. Samples of leaf material were collected at different times of the day and immediately frozen in liquid nitrogen. ABA was extracted, purified and identified according to the technique of Lenton et al. (1971) and as modified by Hartung and Abou-Mandour (1981). The ratio of leaf fresh weight to leaf dry weight was not found to change substantially during the day. For GLC-analysis, a Siemens L 402 gas chromatograph equipped with a 2 m long glass column packed with 1% OV 17+1% carbowax 20 M on gaschrom Q, 80–100 mesh, and a $^{63}$Ni-electron capture detector was used. Purification of extracts of *Arbutus* leaves was extremely difficult because of the release of large amounts of Ericaceous slime from the materials (see Esdorn and Schanze 1954). Therefore, only a maximum of 0.5 g fresh weight per sample could be used in the purification procedure.

One to three μl portions of aqueous solutions of ABA ($10^{-4}$ Molar) were injected with a Hamilton syringe into the petiole of *Arbutus* leaves close to the lamina. Autoradiographic experiments with $^{14}$C-ABA showed that with transpiration rates between 0.5 and 3.1 mmole $m^{-2}$ $s^{-1}$, the injected ABA was distributed over the entire leaf surface within 30 min. The change in stomatal conductance with time after injection was monitored with a Li-Cor 1600 portable steady-state porometer (Lincoln, Nebraska) and water potential was determined with a pressure chamber as described by Tenhunen et al. (1981). Control injections with water were conducted to establish that the stomatal aperture did not change in response to treatment of this type. At the end of each experiment, ABA treated leaves were harvested and leaf area was determined. Applied ABA can thus be expressed on a leaf area basis. All experiments were repeated three times.

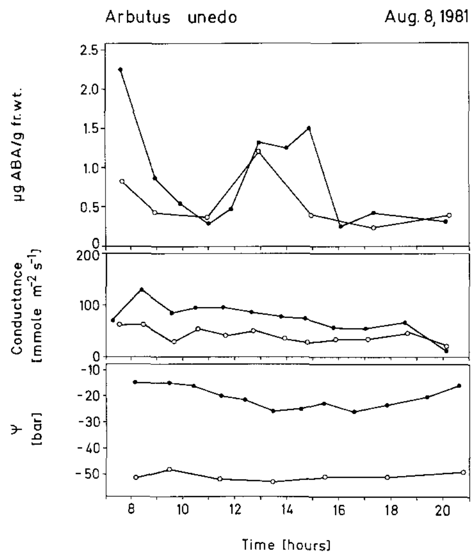


**Fig. 1.** Diurnal changes of endogenous ABA content, stomatal conductance and water potential of mature leaves of irrigated (—●—) and non-irrigated (—○—) *Arbutus unedo* plants, growing in the macchia at the Quinta São Pedro, Sobreda, Portugal

## Results

### *Endogenous ABA content of Arbutus leaves*

In Fig. 1 the diurnal changes in endogenous leaf ABA content, of leaf stomatal conductance and of leaf water potential are shown for an irrigated and a non-irrigated *Arbutus unedo* shrub. Further information on atmospheric environmental conditions during this relatively cool summer day were presented by Tenhunen et al. (1982). The leaves harvested at 8:00 h and those harvested between 12:00 and 15:00 h had extremely high ABA contents of up to 2.3 µg per gram fresh weight of material. While midday stomatal closure did not occur and stomatal conductance and water potential of each plant remained relatively constant, large changes in ABA content were found. A similar daily pattern in ABA content occurred in both plants and the overall concentration of ABA was the same despite extremely large differences in water potential. While ABA fluctuations in the whole leaf did not differ, stomatal function did such that maximal conductance of the naturally water stressed plants was in general approximately 50% of that in the well-watered plants. Obviously there is not a correlation between leaf total endogenous ABA content and stomatal conductance and/or xylem water potential.

### *Effect of injected ABA on stomatal conductance*

Species comparison: Abscisic acid which is applied exogenously by injection into the petiole of the leaf, induced

**Table 1.** Effect of exogenously applied ABA on stomatal conductance of leaves from different non-irrigated shrubs growing in a macchia near Sobreda, Portugal. Stomatal conductance was measured immediately before (*a*) and 30 min after (*b*) ABA injection during the morning between 8:00 and 10:00 h

| Species | Conductance ($\text{mmole m}^{-2}\,\text{s}^{-1}$) | | Applied ABA ($\text{pmole cm}^{-2}$) |
|---|---|---|---|
| | Control | ABA-treated | |
| *Quercus coccifera* | a) 62.7 | 152.3 | 11.27 |
| | b) 29.7 | 0 | |
| *Quercus suber* | a) 51.3 | 61.3 | 19.80 |
| | b) 63.3 | 23.2 | |
| *Myrtus communis* | a) 151.5 | 196.2 | 19.69 |
| | b) 349.4 | 77.7 | |
| *Phillyrea angustifolia* | a) 69.9 | 59.5 | 16.42 |
| | b) 45.5 | 44.9 | |
| *Pistacia lentiscus* | a) 208.9 | 213.7 | 15.53 |
| | b) 177.5 | 129.5 | |

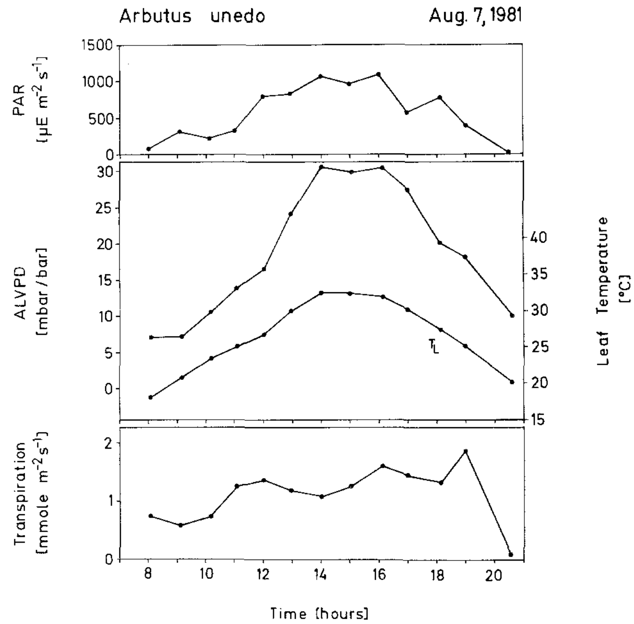


**Fig. 2.** Daily time course of light intensity (PAR) incident on leaves, leaf temperature ($T_L$), air to leaf vapor pressure difference (ALVPD) and transpiration rate (Tr) for the control leaf (see also Fig. 3) of an irrigated *Arbutus unedo* plant growing in a macchia at the Quinta São Pedro, Sobreda, Portugal

a transient stomatal closure during the morning in leaves of several sclerophyll shrub species.

With *Arbutus unedo*, the very low concentration of 8 to 14 pmoles $\text{cm}^{-2}$ induced a strong stomatal closure within 30 min which was followed in one and a half to two hours by stomatal reopening and increase in leaf conductance such that values equal to those of the control leaves were again attained. Cowan et al. (1982) have predicted, based on a mathematical analysis of the half time of reequilibration of ABA from the apoplast into the mesophyll cells, that a similar length of time is necessary to overcome the effects of an ABA pulse. According to Cowan's calculation,

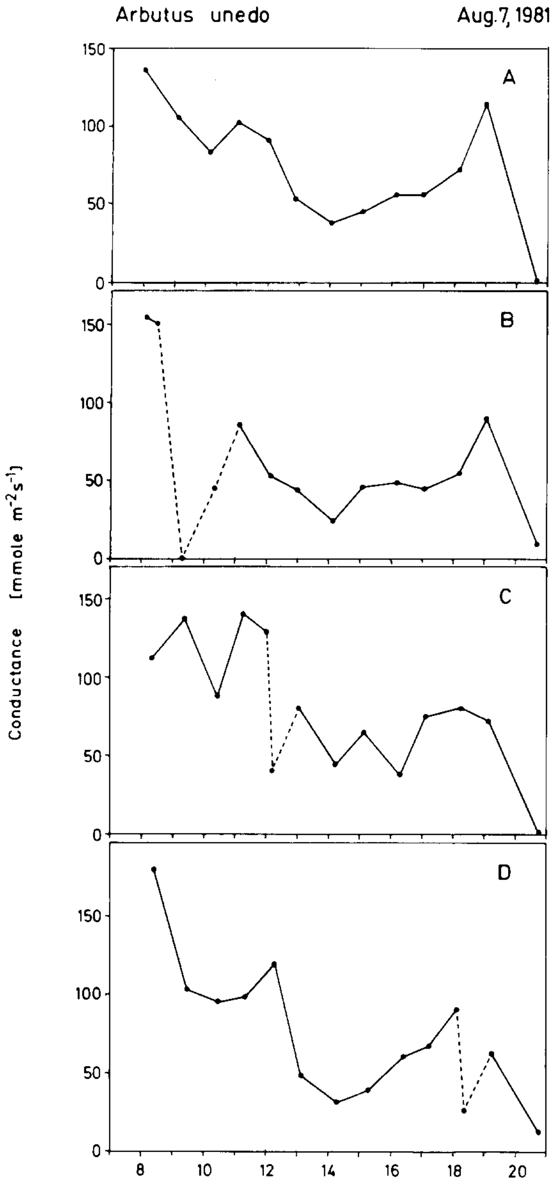


**Fig. 3A–D.** Effect of ABA on stomatal conductance of mature leaves of an irrigated *Arbutus unedo* plant growing in a macchia at the Quinta São Pedro, Sobreda, Portugal. **A** Control, **B** ABA injection at 8:18 h, **C** ABA injection at 12:02 h, **D** ABA injection at 18:07 h. The dotted lines show the time intervals of the transient ABA response. Responses shown for individual leaves

the half time of reequilibration should be approximately 27 min. While in other sclerophyll species applied concentrations varied slightly due to differences in leaf size which could not be compensated for in the field, these also responded sensitively to low concentrations of ABA, e.g. *Quercus coccifera, Quercus suber*, and *Myrtus communis* (Table 1). *Phillyrea angustifolia* and *Pistacia lentiscus* did not show strong stomatal closure after ABA injection when compared to control leaves.

Daily time course: In a series of experiments, injections of ABA were made at different times of the day to determine whether endogenous sensitivity to ABA changes. The results obtained on August 7, 1981 are shown in Figs. 2 and 3. Leaf temperature and air to leaf vapor pressure difference (ALVPD) were high enough to induce midday stomatal closure in leaves of irrigated *Arbutus unedo* plants. When ABA was injected during the early morning hours into the petioles of the leaves, the strongest reaction of stomata to ABA treatment was observed. Only at this time (Fig. 3) was it possible to observe complete stomatal closure as indicated by failure to measure any loss of water from the leaf, a situation which otherwise occurred only during the night with this plant during the dry season. At midday, the sensitivity to injected ABA decreases in both irrigated and non-irrigated plants as indicated by the maximal change in stomatal conductance measured after injection greater than that of the control leaf (Figs. 3 and 4). The decreased effectiveness of ABA to close stomata during the afternoon did not occur at a time when transpiration was decreased, thus decreased transport of ABA to the guard cells cannot explain the response. The estimated time course of sensitivity to injected ABA (Fig. 4) is similar for both irrigated and non-irrigated plants, although the degree of response appears to be affected by plant water status which also changes maximal conductance. Some tendency is seen in these results to indicate that sensitivity to injected ABA increases again in the very late afternoon. Recent measurements in 1982 suggest that this may be a function of drought experience as well which was more extreme in 1981.

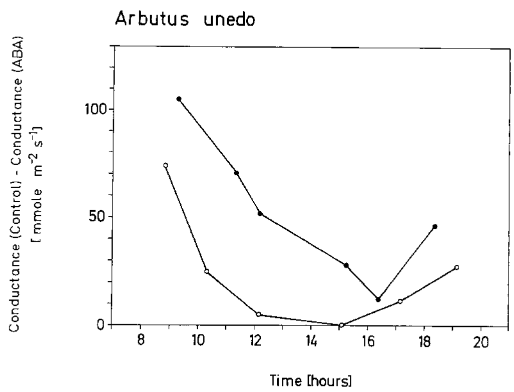


**Fig. 4.** Maximum observed differences of stomatal conductance between control and ABA treated leaves depending on the time of ABA injection into the petioles of irrigated (—●—) and non-irrigated (—○—) *Arbutus unedo*. Responses shown for individual leaves

## Discussion

Endogenous ABA levels of the macchia plant *Arbutus unedo* are very high (up to 2.3 $\mu g\ g^{-1}$ fresh weight). No correlation was found between the fluctuations in ABA content and the daily course of leaf stomatal conductance or xylem water potential. Furthermore, no differences were found between leaves of irrigated ($\psi$ between $-10$ and $-25$ bar) and non-irrigated plants ($\psi$ of $-50$ bar). Other determinations of diurnal fluctuations in endogenous ABA have been conducted by McMichael and Hanny (1977) with cotton plants, Kannangara et al. (1982) with soybeans, Henson et al. (1982) with pearl millet, and Xiloyannis et al. (1980) with peach trees. In all cases, endogenous ABA content was much lower than found here with *Arbutus* leaves. Only Kannangara et al. were able to establish a correlation between ABA content and plant xylem water potential. In each set of experiments, two peak values in endogenous ABA were found, one in the morning and one in the afternoon. Kobriger et al. (1982) determined a peak in ABA content at noon for pea plant leaves, but changes over time were very small.

Raschke and Zeevaart (1976) reported that a high ABA content in *Xanthium* leaves did not decrease stomatal aperture. They proposed that large changes in the concentration of ABA in the mesophyll do not necessarily reflect changes of ABA in the apoplast which is the only compartment directly in communication with the guard cells. We determined in our experiments that stomata of a number of sclerophyll species are sensitive to very small quantities (in the case of *Arbutus unedo* only 1% of leaf total ABA content) injected into the transpiration stream, i.e. apoplastic ABA. Henson et al. (1982), in order to explain noncorrelation of leaf ABA content of *Sorghum* with plant water potential or stomatal conductance have also postulated either a change in stomatal sensitivity to ABA or a change in accessability of ABA to the stomata.

Several sclerophyll species, e.g. *Arbutus unedo, Quercus suber,* and *Quercus coccifera,* have been shown to respond to high leaf temperature and high air to leaf vapor pressure difference at midday with strong stomatal closure (Lange et al. 1982). In this context, it remains quite intriguing that leaf ABA content peaks at midday and one is curious to know whether some mechanisms may control release of this ABA into the apoplast. If ABA is released on days when midday closure is observed, then it may be possible to interpret the decrease in sensitivity to injected ABA found at midday. Low sensitivity to an ABA pulse could indicate that a high ABA concentration is already in the apoplast and added ABA can only produce a small increase in the degree of response. Important in the future is to determine whether specific ABA fractions are set free in the apoplast in response to specific environmental signals.

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