PHYSIOLOGIA PLANTARUM 114: 380–386. 2002
Printed in Denmark – all rights reserved

ISSN 0031-9317

Effect of drought and high solar radiation on 1-aminocyclopropane-1-carboxylic acid and abscisic acid concentrations in *Rosmarinus officinalis* plants

Sergi Munné-Bosch[a], Marta López-Carbonell[a], Leonor Alegre[a,*] and Harry A. van Onckelen[b]

[a]*Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 645, E-08028 Barcelona, Spain*
[b]*Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, Antwerp, B-2610 Belgium*
**Corresponding author, e-mail: leonor@porthos.bio.ub.es*

Received 2 March 2001; revised 10 July 2001

The endogenous concentrations of ACC and ABA were measured, at predawn and at maximum solar radiation, during a summer drought, and recovery after autumn rainfalls, in rosemary (*Rosmarinus officinalis* L.), a drought-tolerant species, growing under Mediterranean field conditions. During the summer, plants were subjected to both water deficit and high solar radiation. Plants showed severe reductions in shoot water potential to −3 MPa, which were associated with drastic stomatal closure (73%), a decrease in net photosynthesis, reaching almost zero, and a severe chlorophyll loss (74%). Despite the severity of the stress, plants recovered after the autumn rainfalls. The concentration of ACC was not enhanced by drought, and at predawn these concentrations remained constant at approximately 600 pmol ACC^{-1} DW throughout the experiment. Thus, ethylene did not regulate the response of rosemary to drought. However, a sharp increase in ACC levels between predawn and midday was observed. This increase was positively correlated to the intensity of the incident solar radiation. ACC levels recorded in June at midday reached 16 000 pmol g DW and in October values of 1000 pmol g^{-1} DW were observed. In contrast, in drought-stressed plants predawn concentrations of ABA were up to 130-fold those of recovered plants, and the levels of ABA scored at midday were double of those scored at predawn. In conclusion, although drought-stressed rosemary plants showed a relatively moderate ABA accumulation (approximately 500 pmol g^{-1} DW, at predawn), it seems to be an essential factor for the regulation of the plant response to stress, thereby enabling a rapid recovery after stress release, although other mechanisms can not be excluded. As drought stress did not induce ACC accumulation, it was concluded that ethylene production was not a major factor in the drought stress resistance of rosemary plants. The increased ACC and ABA concentrations at midday were correlated with day length and light intensity and not with the water status of the plant.

Introduction

Among native Mediterranean shrubs, rosemary (*Rosmarinus officinalis* L.) is drought resistant and can survive at relative water content (RWC) of below 35% (Munné-Bosch et al. 1999, Munné-Bosch and Alegre 2000). The mechanisms by which these plants withstand such a low RWC and high radiation for 3 months during summer, and the relationship between drought and oxidative stress have been studied at the level of lipophilic nonenzymatic antioxidants (Munné-Bosch et al. 1999, Munné-Bosch and Alegre 2000, 2001) and photoprotection (Munné-Bosch and Alegre 2000). However, there are few studies on the function of the plant hormones abscisic acid (ABA), ethylene, and its immediate precursor 1-aminocyclopropane-1-carboxylic acid (ACC) as modulators of plant responses to drought in Mediterranean shrubs and, even fewer studies about the significance of the magnitude and amplitude of fluctuations of hormones in drought-stressed plants.

Many abiotic environmental factors elicit the production of 'stress-ethylene' in higher plants and 'stress ethylene' has frequently been reported (Abeles 1973, Wang et al. 1990, Hyodo 1991, Abeles et al. 1992, Morgan and

Abbreviations – ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; G_s, stomatal conductance; H, hydration of leaves; PPFD, photosynthetically, active photon flux density; RWC, relative water content; T, air temperature; ψ, shoot water potential.

Drew 1997). ACC accumulation is stimulated by several stress factors (Yu and Yang 1980, Wang and Adams 1982, Hyodo et al. 1985). Thus, ACC concentrations can indicate plant stress (Wang et al. 1990). The topic of whether drought stress will induce ACC synthesis is complex. Based on studies with detached leaves (Appelbaum and Yang 1981, Morgan et al. 1990), it was shown that rapid desiccation of these leaves promoted ethylene biosynthesis. However, when intact plants were subjected to water-stress by slowly decreasing soil water content, neither ACC nor ethylene production rates increased (Morgan et al. 1990, Xu and Qui 1993). The complexity of the response to drought depends on the speed and intensity with which it is imposed. In addition, daily changes in ethylene evolution have been found (Rikin et al. 1984, Finlayson et al. 1998, 1999). Machácková et al. (1997) concluded that the photoperiodic regime affects ACC concentrations and that the diurnal fluctuations observed in ethylene production were caused primarily by changes in the levels of ACC. Furthermore, several environmental factors, especially sunlight, induce the gene expression of ACC synthase, and it has been suggested that ACC synthase occurs as a multiple family and that each gene is regulated independently by stress (Ge et al. 2000).

Abscisic acid (ABA) is a well-known stress hormone that plays an important role in plant response to water stress (Zeevaart and Creelman 1988, Davies and Zhang 1991, Jia and Zhang 2000). In addition, diurnal variations in endogenous levels of ABA have been described (Henson et al. 1982, Loveys 1984, Jackson et al. 1995). Leecoq et al. (1983) showed that daily changes in ABA concentrations were only partially related to leaf water potential.

In a previous study on the variations in endogenous concentrations of abscisic acid in Mediterranean shrubs cultivated in growth chambers (López-Carbonell et al. 1996), we concluded that water-stressed rosemary plants showed a much lower increase in ABA levels than water-stressed lavender plants. Moreover, Nogués et al. (2001) showed severe leaf abscission in drought-stressed rosemary growing in a practically closed chamber. These experiments with rosemary plants subjected to a rapid water stress led us to perform more realistic experiments using rosemary plants growing in natural field conditions.

The aim of this study was to analyse the diurnal variations in the concentrations of ACC and ABA in drought-stressed, and recovered rosemary plants grown in Mediterranean field conditions to assess the contribution of these compounds to drought stress tolerance and recovery upon stress release.

Materials and methods

Plant material and growth conditions

Rosemary (*Rosmarinus officinalis*) plants obtained from cuttings were grown for 2 years in a greenhouse under controlled environmental and water regime conditions as described by Munné-Bosch et al. (1999). Plants were transplanted in the Experimental Fields of the University of Barcelona (Barcelona, NE Spain) on 11 May 1998 and were watered with 20 mm d^{-1} on 11 and 18 May 1998. Thereafter, they grew under Mediterranean field conditions, receiving water exclusively from rainfall. Once a month samples (16 plants, approximately the same size) were taken during the summer drought (June–August 1998), and during recovery after autumn rainfall (October 1998).

Environmental conditions were monitored using a weather station (Delta-T Devices, Newmarket, UK). Measurements of photosynthetically active photon flux density (PPFD) and air temperature were taken at 1-min intervals, and 5 min means were logged. PPFD was measured with a Quantum Sensor (Li-Cor, Lincoln, NE, USA), air temperature with a Vaisala thermocouple (Vaisala, Helsinki, Finland) and precipitation (mm) with a standard rain gauge.

Plant water status

Plant water status was followed by predawn measurements (1 h before sunrise) of shoot water potential (ψ) and leaf hydration (H). ψ was determined by using a Scholander-type pressure chamber (model ARIMAD-2, Ari Far Charuv-Water Supply Accessories, Ramat Hagolan, Israel). H (g_{water} g^{-1} DW) was calculated as (FW-DW)/DW, where FW is the fresh weight of the sample, and DW is the dry weight after oven drying at 80°C for 24 h.

Leaf gas exchange

Leaf gas exchange rates of 10-cm apical non-woody shoots were measured at 2-h intervals throughout the day using an Li-6200 portable gas-exchange system (Li-Cor, Lincoln, NE, USA). Net CO_2 assimilation rates (P_n) and stomatal conductance (G_s) were estimated from leaf gas exchange data using the equations developed by von Caemmerer and Farquhar (1981).

Chlorophyll content

The chorophyll a + b (Chl) content of leaves was determined spectrophotometrically in 80% (v/v) acetone extracts as described by Lichtenthaler and Wellburn (1983).

ABA and ACC analysis

Fully developed young leaves were collected at predawn (1 h before sunrise) and mid-day (at maximum incident PPFD), immediately frozen in liquid nitrogen and stored at −20°C until analysis. Endogenous ABA and ACC were determined as described (López-Carbonell et al. 1996, Child et al. 1998) with slight modifications. After grinding samples in liquid nitrogen, they were extracted overnight at −20°C in 80% (v/v) methanol. After centrifugation, 1–^{18}O-ABA and deuterated ACC were added as internal standards. The extract was passed through an RP-C18 cartridge (500 mg, Varian) and ABA and ACC were eluted with 80% (v/v) methanol.

For further purification, ABA samples were eluted with 6% (v/v) formic acid from DEAE-Sephadex A25 and concentrated on an RP-C18 cartridge with diethyl ether and then evaporated in vacuo. A preparative ion suppression RP-HPLC (50/49/0.5, v/v/v, methanol/water/acetic acid; Lichrospher-60, 100×4.6 mm, 5 μm; 0.5 ml/min) coupled to a fluorimetric detector (Shimadzu RF551, λ_{ex} 285 nm, λ_{em} 360 nm) was used to collect fractions corresponding to ABA by using ^{3}H-ABA as a reference. The ABA samples were methylated with diazomethane prior to GC-MS analysis.

Derivatization of ACC was carried out with pentafluorobenzylbromide in acetone. Samples were then further purified by elution with acetonitrile from an RP-C18 cartridge, prior to GC-MS.

The derivatized forms of ABA and ACC were analysed by GC-MS separative chemical ionization in selective ion mode (CI-SIM) HP5890 Series II, coupled to a VG TRIO 2000 quadrupole mass spectrometer (column 15 m, 0.25 mm ID; gas phase He, temperature gradient from 150 to 300°C for 11 min, injection temperature 325°C, CI gas methane).

Results

Plant water relations

The experimental period was characterized by a severe drought during the summer and rainfall during autumn, typical Mediterranean climatic conditions (Fig. 1). Drought occurred from mid June to mid August, with maximum diurnal PPFD ranging from 2000 μmol m^{-2} s^{-1} (June) to 1750 μmol m^{-2} s^{-1} (August), and midday temperatures of around 28°C. Heavy rains fell in autumn, the maximum diurnal PPFD decreased to 1200 μmol m^{-2} s^{-1} and midday temperature reached 23.5°C (October). During drought plants showed a severe decrease in predawn shoot water potential, which reached values of approximately −3 MPa in July and August; however, this potential recovered in October ($\psi = -0.4$ MPa). Concomitant with the water potential, predawn measurements of leaf hydration decreased to approximately 0.5 g water g^{-1} DW in July and August and again increased to approximately 1.7 g water g^{-1} DW in October. Therefore, in spite of severe predawn water stress during the summer, plants were able to recover after the autumn rainfalls (Fig. 1).

Drought and photosynthesis

The decrease in predawn water potential and plant hydration was associated with a decrease in net photosynthesis, which reached values near 0 μmol CO_2 m^{-2} s^{-1} in July and August. However, net photosynthesis recovered after the autumn rainfalls to 9 μmol CO_2 m^{-2} s^{-1} in October. Simultaneously, a strong chlorophyll loss (74%) was observed in severely stressed plants. Chlorophyll content increased once the plant water relations were completely restored after the autumn rainfalls (Fig. 1).

The diurnal changes in net CO_2 assimilation rates and stomatal conductance of water-stressed, and recovered plants are shown in Fig. 2. At the beginning of the study period (June), rosemary showed a maximum peak of CO_2 assimilation of approximately 5 μmol CO_2 m^{-2} s^{-1} and stomatal conductance rates of approximately 0.08 mol m^{-2} s^{-1} early in the morning. Net photosynthesis decreased progressively with increasing drought, reaching values near zero throughout most of the day in August. The low values observed in stomatal conductance during the summer indicated that stomata were practically closed throughout the day in stressed plants. This

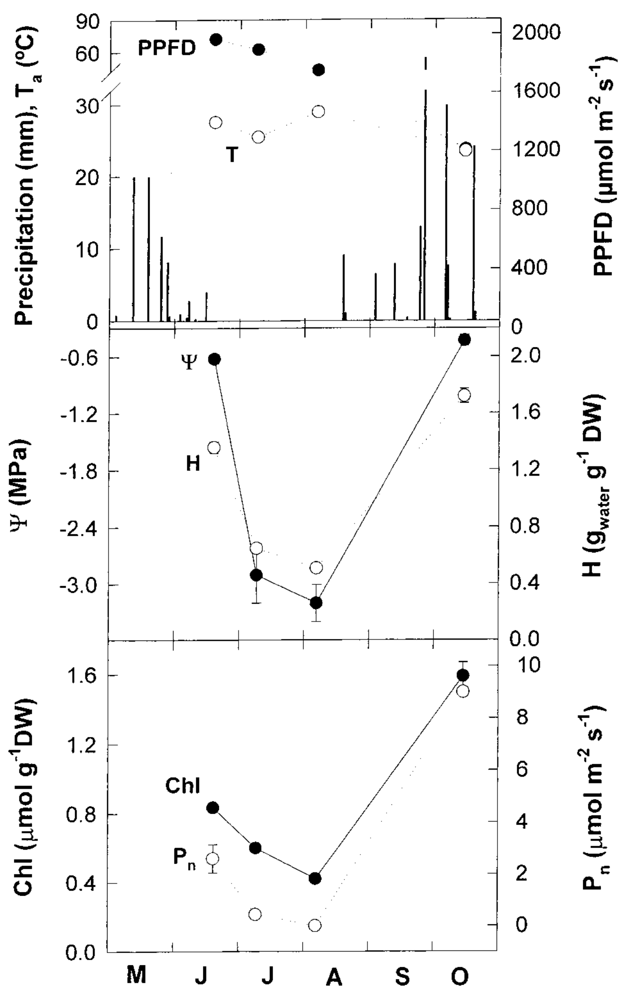

Fig. 1. Precipitation, photosynthetically active photon flux density (PPFD) and air temperature (T) at midday in the experimental fields of the University of Barcelona (NE Spain) from June to October 1998, and variations in the shoot water potential (ψ), leaf hydration (H) at pre-dawn and leaf net photosynthetic rates (P_n), and leaf chlorophyll a + b (Chl) content at midday in *R. officinalis* plants throughout the study period. ψ, H, Pn and Chl data are the means ± SE for $n = 6$.

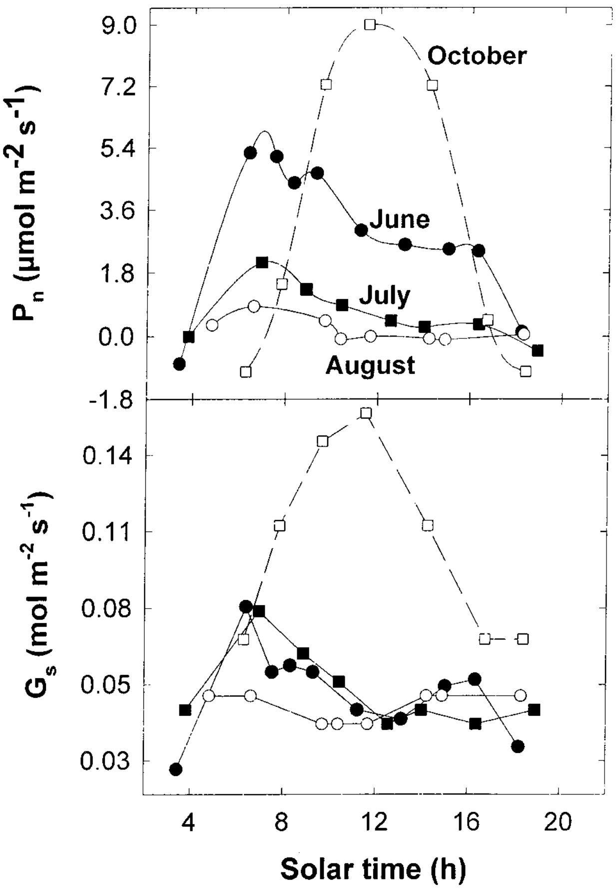

Fig. 2. Diurnal variations in leaf net photosynthetic rates (P_n), and stomatal conductance (G_s) in *R. officinalis* plants from June to October 1998. Data are the means for n = 6. Standard errors were less than 10% of the mean in all cases. Symbols: June (black circles), July (black squares), August (white circles), October (white squares).

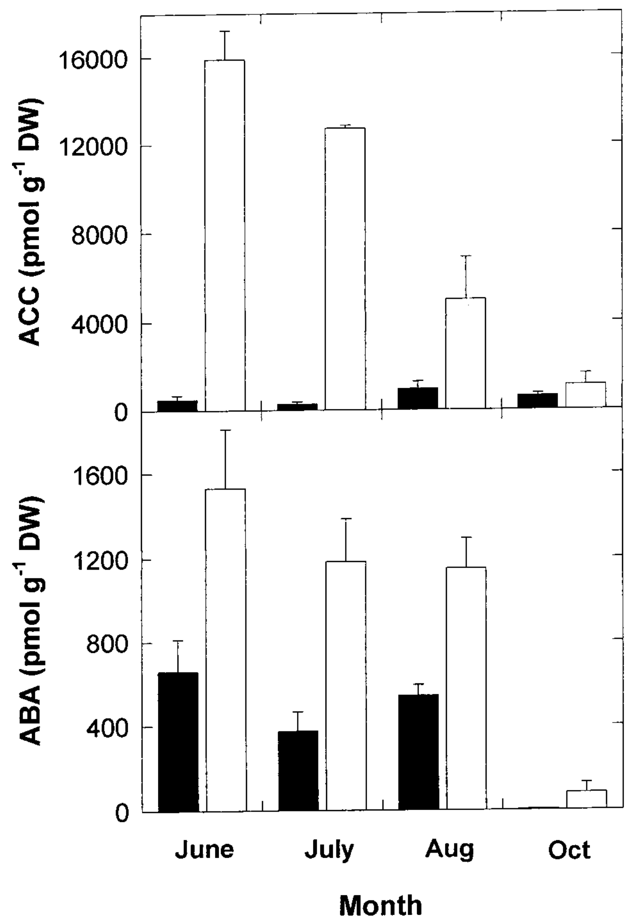

Fig. 3. Diurnal variations in 1-aminocyclopropane-1-carboxylic acid (ACC) and abscisic acid (ABA) in *R. officinalis* leaves from June to October 1998. Data are the means ±SE for n = 3. Values obtained at predawn (closed bars) and midday (open bars) are given.

was more evident in August when stomatal conductance reached 0.05 mol m^2 s^{-1} throughout the day. Decreases in net photosynthesis were associated with stomatal conductance in drought. However, net photosynthesis decreased progressively from June to August, while stomatal conductance did not, thus indicating a non-stomatal limitation of photosynthesis in July and August. In October, photosynthesis peaked at midday at 9 μmol m^{-2} s^{-1}, which was associated with the stomatal conductance of leaves.

ACC and ABA during drought and recovery

At predawn there were no differences in the levels of ACC, between drought-stressed and recovered plants, and levels of this compound remained almost constant at approximately 600 pmol g^{-1} DW throughout the experiment. However, a sharp increase in ACC levels was observed at mid-day. A maximum concentration of this compound of 16 000 pmol g^{-1} DW, was observed in June at mid-day, when the PPFD was at its highest. This midday maximum was up to 33-fold that observed at pre-dawn. In the following months, PPFD and photoperiod decreased progressively, simultaneously with the levels of ACC at midday; however, in October, the midday concentrations of this compound were still up to 2-fold those at pre-dawn (Fig. 3).

In contrast, in June at pre-dawn, drought-stressed plants showed, on average, 525 pmol ABA g^{-1} DW of endogenous ABA, up to 130-fold the levels observed in recovered plants. This increase was mirrored by the low Gs, which, at predawn, was 66% lower than in October. Nevertheless, the stress-induced increase in ABA may serve a function other than affecting stomatal movement. Although ψ and leaf hydration decreased progressively as drought progressed, the ABA concentration did not increase further. After the autumn rainfalls and once plant status was completely restored, concentrations of ABA decreased to as low as 4 pmol g^{-1} DW, concomitantly with an increase in stomatal conductance. Furthermore, midday ABA concentrations were up to 2-fold greater than predawn measurements (Fig. 3). The highest levels of ABA were observed in June at midday (1530 pmol g^{-1} DW), as occurred with ACC.

The relationship between ACC and ABA levels at pre-dawn and mid-day is shown in Fig. 4. A positive asymptotic relationship between midday levels was observed. In contrast, there was no relationship between the pre-dawn levels of ABA and ACC. Thus, while sunlight affected the concentrations of both compounds, drought increased only the levels of ABA.

Discussion

Stresses trigger a wide range of plant responses, and some of them clearly enable plants to acclimatize to stress. Failure to compensate for a severe stress can lead to plant death. In previous studies, we have shown that rosemary plants have a great ability to withstand prolonged and severe droughts by minimizing water loss and increasing photo- and antioxidative protection (Munné-Bosch et al. 1999, Munné-Bosch and Alegre 2000). In this study, we investigated hormonal regulation in the acclimation of rosemary plants to severe drought. Greenhouse-grown plants were transferred to Mediterranean field conditions, and ACC and ABA contents were measured in plants subject to severe stress during summer and in recovered plants during autumn.

Rosemary plants showed symptoms of stress in June shown by comparing some stress parameters between plants grown under drought and those recovered after the autumn rainfalls. Thus, plants grown in June showed lower values of leaf hydration (21%), chlorophyll content (47%) and net photosynthesis (71%) than recovered plants. Furthermore, ABA concentrations were higher (up to 164-fold). These increased levels of ABA were responsible for the low stomatal conductance observed throughout the day, although it can serve a function other than affecting stomatal movement.

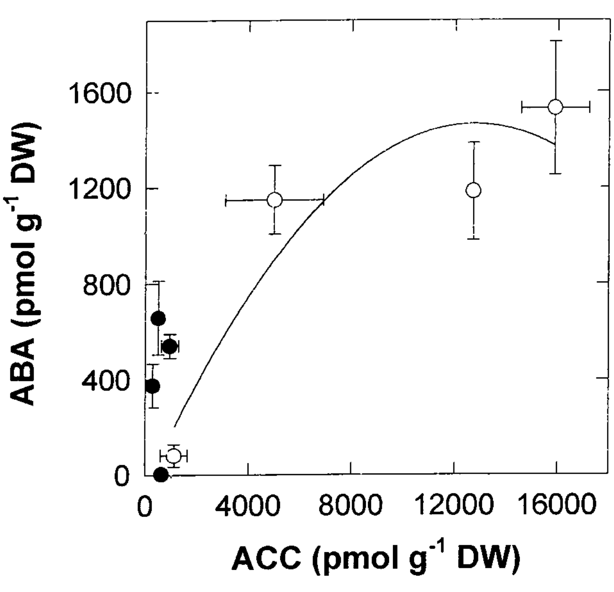

Fig. 4. Relationship between abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) levels in the leaves of *R. officinalis* plants growing in Mediterranean field conditions. Data are the means ± SE for n = 3. Values obtained at predawn (closed bars) and midday (open bars) are given. The curve was obtained by fitting the data to a second order function.

As drought progressed during July and August, sharp decreases in pre-dawn ψ (to -3 MPa) and in leaf hydration (57% lower than in June) were observed. Furthermore, from the point of view of gas exchange, in August, plants were below the CO_2 compensation point, and the concentration of chlorophyll was 39% lower than that in June. It is evident that rosemary plants were under severe stress during the summer. These conditions have generally been considered indicative of irreversible damage (Kaiser 1987), but some studies have already shown that Mediterranean shrubs growing in the field can withstand such low water contents for several months (Kyparisis et al. 1995, Munné-Bosch and Alegre 2000). Thus, it is of great interest to elucidate the mechanisms by which plants recover completely from severe drought.

In a previous experiment (Nogués et al. 2001), rosemary plants were subjected to rapid (10 d) and severe water stress (RWC = 35%) in small chambers (0.125 m^3 volume per plant) day/night temperature 28/18°C, maximum PPFD 1500 μmol m^{-2} s^{-1} and 14 h photoperiod. Under these conditions plants shed a third of their leaves because of stress-ethylene accumulation. However, no leaf abscission was observed in rosemary plants grown in the field and subjected to progressive but severe drought stress (Munné-Bosch et al. 1999, Munné-Bosch and Alegre 2000). We therefore decided to study the levels of the immediate ethylene precursor, ACC. Also in the present study, despite severe chlorophyll loss, no leaf abscission was observed in plants growing in field conditions. Accordingly, it seems that drought did not affect the levels of the immediate ethylene precursor (ACC), which showed constant predawn values of approximately 584 pmol g^{-1} DW throughout the experiment. Our data show that plants react differently to rapid drying (Nogués et al. 2001) than to slow progressive water stress induced in the field conditions described in this paper. These observations are consistent with those of Morgan et al. (1990), Narayana et al. (1991), which showed no significant production of ethylene when plants were subjected to natural drought. Furthermore, Chen and Kao (1990) found that ethylene production was strongly inhibited by water stress through the inhibition of ACC synthesis.

Nonetheless, a strong influence of sunlight and photoperiod on ACC levels was observed, the higher was the incident solar radiation and the longer the photoperiod, higher were the levels of ACC. Thus, in June, with a day/night photoperiod of 16/8 and a maximum PPFD of 1900 μmol m^{-2} s^{-1}, midday concentrations of ACC were up to 33-fold those at pre-dawn. Thereafter, ACC levels decreased concomitantly with maximum diurnal PPFD and photoperiod throughout the following months (Fig. 3). These results are consistent with those of Ge et al. (2000), which showed that sunlight is the main factor regulating gene expression of ACC synthase. Moreover, it has been shown that ACC concentrations increased in response to light as a result of regulation by the photoperiodic regime of cyclic ACC synthase activity (Macháčková et al. 1997, Finlayson et al. 1999). However, it is difficult to separate drought and light intensity effects because of the light–drought interaction in this study. The regulation of ACC synthase both by drought and circadian rhythms cannot be excluded. Further studies are needed to explain the role of the midday increase of ACC in plants.

Special attention should be given to the effect of high solar radiation on ACC concentrations, particularly when working with drought-stressed plants in Mediterranean field conditions, in which large diurnal fluctuations of environmental parameters are common.

Previously, we observed that the predawn levels of ABA showed small fluctuations in native Mediterranean plants subjected to rapid water stress. Likewise, the ABA levels in water-stressed rosemary plants were lower than those in lavender, another Mediterranean shrub (López-Carbonell et al. 1996). However, even if the bulk leaf ABA content did not reach extremely high concentrations (approximately 500 pmol g^{-1} DW), rosemary plants growing in the field showed much more resistance to drought stress than lavender (Munné-Bosch et al. 1999). The data suggest that the sensitivity to increased ABA levels rather than the absolute values determine drought tolerance in rosemary plants. Thus, ABA levels at predawn seem to be an essential factor controlling the capacity of the plant to react to drought-stress. It is well known that stomatal opening is strongly inhibited by ABA (Zeevaart and Creelman 1988, Mansfield and McAinsh 1995), and our results showed that, in June, ABA increases were mirrored by a decrease in stomatal conductance and photosynthesis.

The increases in the mid-day concentrations of ABA, which were concomitant with the increase in incident solar radiation, may affect functions other than stomatal movement.

In summary, ethylene did not regulate the response to drought in rosemary plants and the increase in ACC concentrations were dependent on incident solar radiation but were independent of drought. The relative increases in pre-dawn concentrations of ABA in drought-stressed plants conferred resistance to tolerate drought stress, whereas, the combined effect of ethylene and ABA may lead to the regulation of plant responses to photoperiod and radiation intensity.

Acknowledgements – The authors are very grateful to Sevgi Ôden (University of Antwerp) and Servei de Camps Experimentals (Universitat de Barcelona) for their valuable technical help. The authors also acknowledge the financial support given to LA by MCT (BOS2000–0560).

References

Abeles FB (1973) Ethylene in Plant Biology Academic Press, New York, NY

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology, 2nd edn, Academic Press, New York, NY

Appelbaum A, Yang SE (1981) Ethylene biosynthesis of stress ethylene induced by water deficit. Plant Physiol 68: 74–79

Chen CT, Kao CH (1990) Comparative study of the metabolism of 1-aminocyclopropane-1-carboxylic acid and senescence of water-stressed and ABA-treated excised rice leaves. Plant Cell Physiol 31: 463–468

Child RD, Chavaux N, John K, Ulvskov P, van Onckelen HA (1998) Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. J Exp Bot 49: 829–838

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Physiol Plant Mol Biol 42: 55–76

Finlayson SA, Lee IJ, Morgan PW (1998) Phytochrome B and the regulation of circadian ethylene production in sorghum. Plant Physiol 116: 17–25

Finlayson SA, Lee Ij Mullet JE, Morgan PW (1999) The mechanism of rhythmic ethylene production in sorghum. The role of phytochome B and simulated shading. Plant Physiol 119: 1083–1089

Ge L, Liu JZ, Wong WS, Hsiao LW, Chong K, Xu ZK, Yang SF, Kung SD, Li N (2000) Identification of a novel environmental factor responsive 1-aminocyclopropaane-1-carboxylate synthase gene, NT-ACS2, from tobacco. Plant Cell Environ 23: 1169–1182

Henson IE, Alagarswamy G, Mahalakshimi V, Bidinger FR (1982) Diurnal changes in endogenous abscisic acid in leaves of pearl millet (*Pennisetum americanum* (L) Leeke) under field conditions. J Exp Bot 33: 416–425

Hyodo H (1991) Stress/wound ethylene. In: Mattoo AK, Sutlle JC (eds) The Plant Hormone Ethylene CRC Press, Boca Raton, FL, pp 43–63

Hyodo H, Tnanaka K, Yoshiaka J (1985) Induction of 1-aminocyclopropaane-1-carboxilic acid synthase in wounded mesocarp tissue of Winter Squash and the effects of ethylene. Plant Cell Physiol 26: 161–167

Jackson GE, Irvine J, Grace J, Khalil AAM (1995) Abscisic acid concentrations and fluxes in droughted conifer samplings. Plant Cell Environ 18: 12–22

Jia W, Zhang J (2000) Water stress-induced abscisic acid accumulation in relation to reducing agents and sulfhydryl modifiers in maize plants. Plant, Cell Environ 23: 1389–1395

Kaiser WM (1987) Effect of water deficit on photosynthetic capacity. Physiol Plant 71: 142–149

Kyparissis A, Petropoulou Y, Manetas Y (1995) Summer survival of leaves in a soft-leaved shrub (*Phlomis fructicosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot. 46: 1825–1831

Leecoq CJ, Koukkari WL, Brenner ML (1983) Rhythmic changes in abscisic acid (ABA) content of soybean leaves. Plant Physiol 72: S52

Lichtenthaler K, Wellburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 11: 591–592

López-Carbonell M, Alegre L, Pastor A, van Onckelen H (1996) Variations in abscisic acid, indole-3-acetic acid and zeatin riboside concentrations in two mediterranean shrubs subjected to water stress. Plant Growth Regul 20: 271–277

Loveys BR (1984) Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars. III. The influence of xylem-derived abscisic acid on leaf exchange. New Phytol 98: 563–573

Macháčová I, Chavaux N, Dewitte W, van Onckelen H (1997) Diurnal fluctuations in ethylene formation in *Chenopodium rubrum*. Plant Physiol 113: 981–985

Mansfield TA, McAinsh MR (1995) Hormones as regulators of water balance. In: Davies PJ (ed) Plant Hormones, Kluwer Academic Publisher, Dordrecht, pp 598–616

Morgan PW, He CJ, De Greef JA, Proft MP (1990) Does water stress promote ethylene synthesis by intact plants? Plant Physiol 94: 1616–1624

Morgan PG, Drew MC (1997) Ethylene and plant responses to stress. Physiol Plant 100: 620–630

Munné-Bosch S, Nogués S, Alegre L (1999) Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions. New Phytol 144: 109–119

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210: 925–931
Munné-Bosch S, Alegre L (2001) Subcellular compartmentation of the diterpene carnosic acid and its derivatives in the leaves of rosemary. Plant Physiol 125: 1094–1102
Narayana I, Lalonse S, Saini HS (1991) Water-stress-induced ethylene production in wheat. Plant Physiol 96: 406–410
Nogués S, Munné-Bosch S, Casadesús J, López-Carbonell M, Alegre L (2001) Daily time course of whole-shoot gas-exchange rates in two drought-exposed Mediterranean shrubs. Tree Physiol 21: 53–60
Rikin A, Chalutz E, Anderson JD (1984) Rhytmicity in ethylene production in cotton seedlings. Plant Physiol 104: 1139–1149
von Caemmerer S, Farquhar GD (1981) Some relations between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387
Wang CY, Adams DO (1982) Chilling-induced ethylene production in cucumbers (*Cucumis sativus* L.). Plant Physiol 69: 424–427
Wang SY, Wang CY, Welburn AR (1990) Role of ethylene under stress conditions. In: Alscher R, Cumming J (eds) Stress Responses in Plants Adaptation and Acclimation Mechanisms. Wiley-Liss, New York, NY, pp 147–173
Xu CC, Qui Z (1993) Effect of drought on lipoxygenase activity, ethylene and ethane production in leaves of soybean plants. Acta Bot Sin 35: 31–37
Yu YB, Yang SF (1980) Biosynthesis of wound ethylene. Plant Physiol 66: 281–285
Zeevaart JAD, Creelman RA (1988) Metabolism and physiology of abscisic acid. Annu Rev Plant Mol Biol 39: 439–473

Edited by T. C. Vogelmann