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Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera* L.)

by

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Nachweis einer osmotischen Anpassung an Trockenheit bei Reben (*Vitis vinifera* L.)

Z u s a m m e n f a s s u n g. — Die Induktion einer osmotischen Anpassung wurde unter Freiland- und Gewächshausbedingungen bei Reben der Sorten Silvaner, Riesling und Bacchus untersucht.

1. Zyklische Trocken- und Bewässerungsphasen ließen bei ausgewachsenen Silvanerblättern, nicht jedoch bei Rieslingblättern, das osmotische Potential (ψ_s) entsprechend der Streßintensität um bis zu 4 bar absinken. Mit dieser osmotischen Veränderung gingen in den Blättern beider Sorten ein Abbau der Stärke und bei Silvaner Zunahmen an Glucose und Fructose einher. Eine ähnliche Abnahme des ψ_s wurde auch in den Wurzeln gestreßter Silvaner- und Rieslingreben nachgewiesen.
2. Im Gegensatz zu den ausgewachsenen Blättern von Bacchus-Reben im Freiland war in den Sproßspitzen ein diurnaler Rhythmus des ψ_s mit einem Minimum um 11 h zu erkennen. Die ψ_s-Werte der Sproßspitzen von Topfpflanzen der Sorten Silvaner und Riesling nahmen mit ansteigenden Temperaturen und zunehmendem Dampfdruckdefizit der Luft ab und mit abnehmenden Temperaturen und sinkendem Dampfdruckdefizit der Luft zu; ein schwacher Wassermangelstreß im Boden verstärkte diese Reaktionen des ψ_s.
3. Zyklische Trocken- und Bewässerungsphasen führten bei ausgewachsenen Blättern der Sorten Silvaner und Riesling zu einer Abnahme der Zellelastizität.

Introduction

As in many other crops, water deficiency in the soil or atmosphere can lead to a decrease of turgor in the leaves of grapevines due to transpirational water loss (SMART 1974, DÜRING and LOVEYS 1982, SMART and COOMBE 1983). As even small changes of the turgor potential have been regarded "as the most likely means whereby a plant transduces changes in metabolism" (TURNER and JONES 1980), processes leading to a maintenance of turgor during alterations of the plant water status are assumed to be essential for the formation of yield and quality under drought conditions, i.e. for drought tolerance. A low osmotic potential (ψ_s), a high cell elasticity and a small cell size contribute to maintain a constant turgor as the water content of the plant decreases (TURNER 1979).

In a previous paper, it had been shown that under the semiarid conditions of South Australia the ψ_s values of the leaves of field-grown grapevines were lower than those of leaves grown under the humid-temperate conditions of Germany; it had been argued that to a certain degree grapevines are able to lower their ψ_s values actively under stress conditions (DÜRING and LOVEYS 1982).

In order to study the adaptation processes in grapevines more in detail, the effects of water stress on ψ_s, some carbohydrates and the cell elasticity of the leaves were examined using three *V. vinifera* cultivars. Moreover, the ψ_s of the roots and apices of stressed vines was recorded.

Material and methods

a) Stress treatments

In two subsequent years, 1-year-old ungrafted Riesling and Silvaner vines were cultivated under glasshouse conditions.
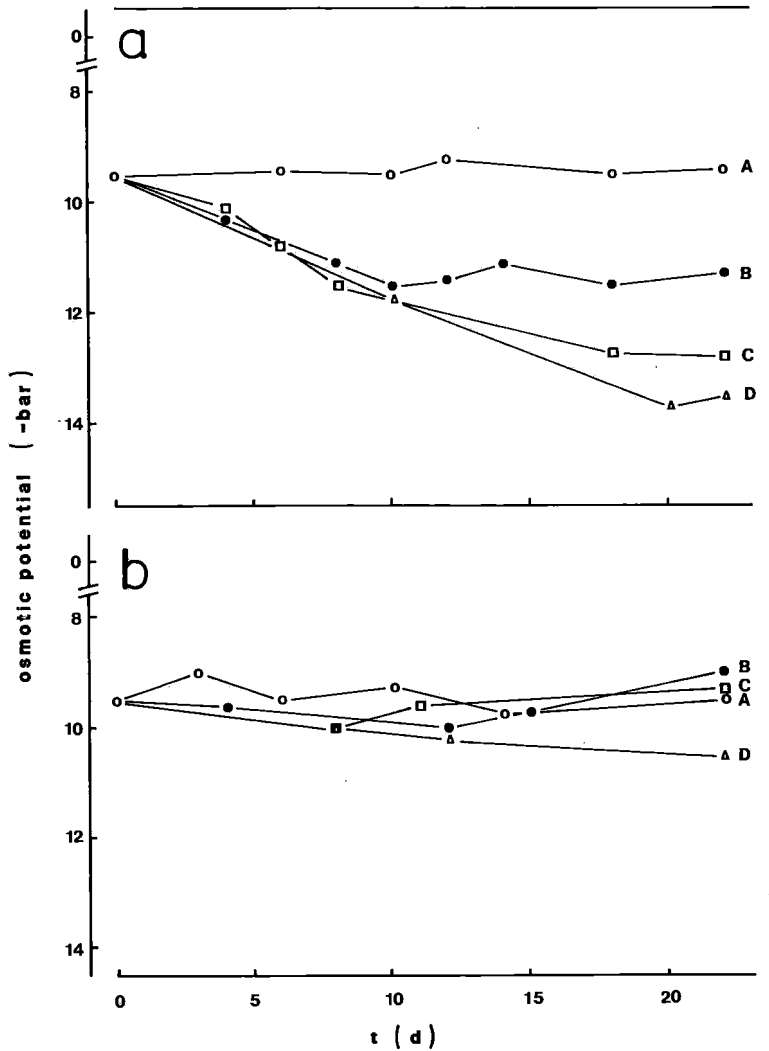

Fig. 1: The osmotic potential at RWC = 100 % during a 22-d cyclic drought stress and irrigation experiment under glasshouse conditions. Water stress increases from A (control) to D. For details see Table 1. — a) Silvaner, b) Riesling.

Das osmotische Potential bei RWC = 100 % im Verlaufe eines 22tägigen zyklischen Trockenstreß- und Bewässerungsexperimentes im Gewächshaus. Der Wassermangelstreß nimmt von A (Kontrolle) nach D zu. Nähere Einzelheiten s. Tabelle 1. — a) Silvaner, b) Riesling.

Table 1

Water stress and irrigation cycles of a 22-d experiment in 1982, water stress increasing from A (control) to D

Trockenstreß- und Bewässerungszyklen eines 22tägigen Versuches im Jahre 1982 · Der Trockenstreß nimmt von A (Kontrolle) nach D zu

Group	Water potential (-bar) at 14.00 h (stress maximum)	Number of irrigations during the experiment
A	7—10	22
B	10—13	6
C	13—16	3
D	16—19	2

In 1982, the vines were selected for uniformity at a height of 145 cm. For 22 d the plants were subjected to several water stress and irrigation cycles. Corresponding to changes of the climatic conditions daily irrigated vines exerted at the time of the stress maximum in the early afternoon a leaf water potential (ψ_w) of -7 to -10 bar (group A = control). Plants kept unirrigated for several days, until a stress maximum of -10 to -13 bar was reached, are referred to as group B, etc. The ψ_w of mature leaves as well as the ψ_s of mature leaves and roots were measured just before irrigating the plants (Figs 1, 2; Tables 1, 2, 3).

In 1983, which turned out to be an unusual hot year, selected Riesling and Silvaner vines were divided in two groups, the daily irrigated group A (control, field capacity = 100 %) and the group B (field capacity between 60 % (Riesling) or 69 % (Silvaner) and 100 %). The plants of group B were left unirrigated (days 0 and 1), then irrigated for 2 d

Table 2

The starch, fructose and glucose content (mg · g^{-1} dry weight) of fully expanded Silvaner and Riesling leaves after different stress treatments (for details see Table 1)

Der Stärke-, Fructose- und Glucosegehalt (mg · g^{-1} Tr. Gew.) ausgewachsener Silvaner- und Rieslingblätter nach unterschiedlicher Streßbehandlung (Einzelheiten s. Tabelle 1)

Cultivar	Carbohydrate	Control A	Stress B	C	D
Silvaner	Starch	10.2[a]	5.0[b]	1.8[b]	1.2[b]
	Fructose	32.1[a]	38.5[b]	37.3[b]	44.3[b]
	Glucose	32.5[a]	38.2[b]	36.5	41.3[b]
Riesling	Starch	10.9[a]	5.1[b]	2.1[b]	1.5[b]
	Fructose	33.3	33.7	35.8	33.9
	Glucose	34.5	34.9	36.3	34.4

Figures followed by different letters within rows differ significantly at the 5 % level.
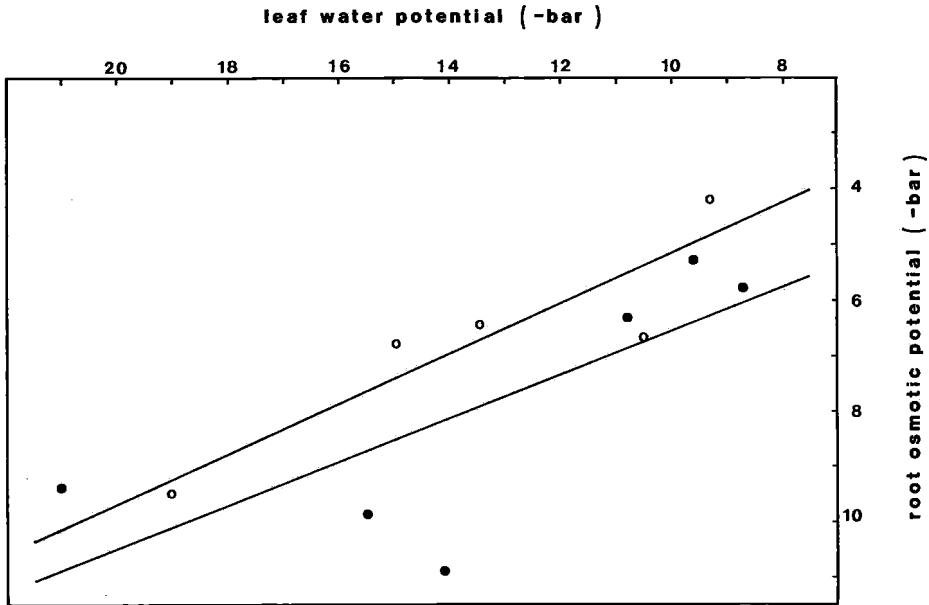
leaf water potential (-bar)

Fig. 2: The osmotic potential of roots at RWC = 100 % related to the water potential of the leaves.
● = Riesling; y = 0.44 x − 0.77. ○ = Silvaner; y = 0.39 x − 2.71.

Das osmotische Potential der Wurzeln bei RWC = 100 % bezogen auf das Blattwasserpotential.
● = Riesling; y = 0,44 x − 0,77. ○ = Silvaner; y = 0,39 x − 2,71.

(days 2 and 3) and again left unirrigated (days 4 to 7). The field capacity of the soil was estimated by weighing the pots daily. The ψ_s of the apices, which were defined as the upper 10 cm of the stem, was recorded daily between 13.00 and 14.00 h (Figs 4 and 5). The diurnal changes of ψ_s were measured on 19 July 1983 on field-grown Bacchus vines. That day the maximum temperature was 30.0 °C, the maximum vapor pressure deficit (VPD) was 2.51 kPa.

b) Water and osmotic potential

The ψ_w of mature leaves was measured by the pressure bomb technique according to SCHOLANDER et al. (1965). Pressure increase was 0.5 bar · min⁻¹, the manometer used enabled readings of 0.25 bar. The ψ_s was determined by an osmometer (Vogel OM 801) after freezing fully hydrated leaf blades by liquid N_2 and pressing out the cell sap at ca. 100 bar. After centrifugation, 50 µl of the cell sap was used for the osmometer operation (reproducibility 0.5 %). All ψ_s values are given at relative water content (RWC) = 100 % and are not corrected for the dilution of cell sap with apoplastic water that occurs during freezing (ACKERSON and HEBERT 1981).

c) The elastic modulus

The elastic modulus ε was determined according to TYREE and HAMMEL (1972) and CHEUNG et al. (1975). Detached leaves were fully hydrated overnight in the dark; the relationship between changes of volume (or fresh weight) and those of the water poten-
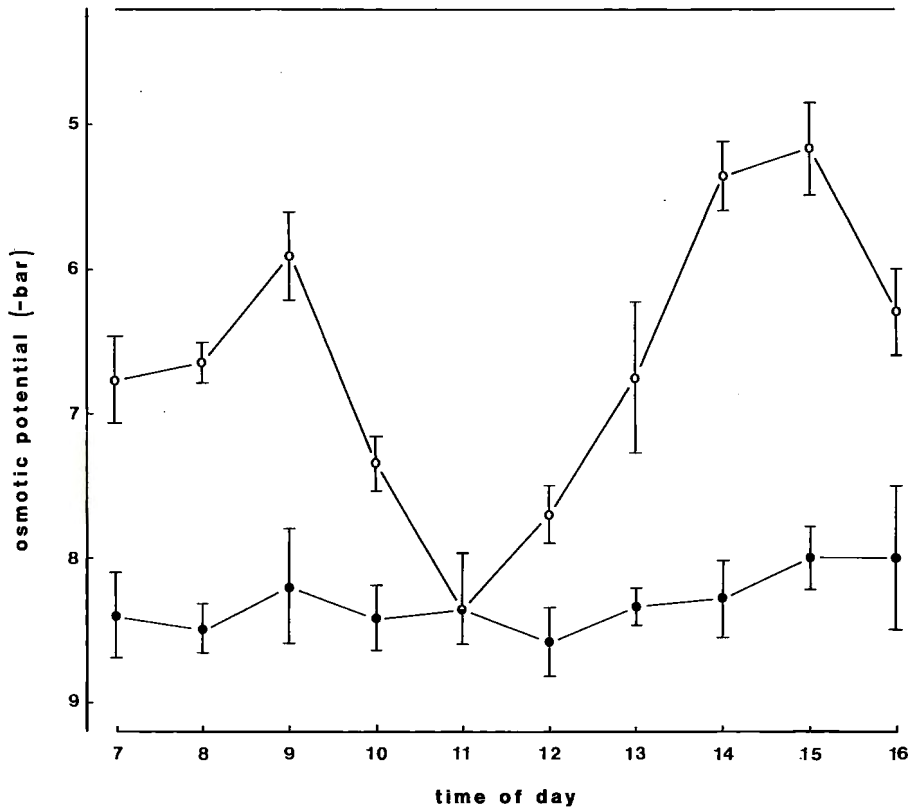
Fig. 3: Diurnal changes of the osmotic potential in apices (O) and fully expanded leaves (●) at RWC = 100 % of field-grown Bacchus vines.

Der Tagesgang des osmotischen Potentials in Sproßspitzen (O) und ausgewachsenen Blättern (●) bei RWC = 100 % von Bacchus-Reben im Freiland.

tial of the wilting leaves gives a pressure-volume curve, from which the water loss and the elastic modulus, both at turgor 0, can be calculated as follows:

$$\varepsilon = \frac{dP \cdot V_o}{dV}$$

where dP represents the change in turgor, V_o the volume or fresh weight of the fully turgid leaves and dV the change of volume or fresh weight.

d) A n a l y s i s   o f   c a r b o h y d r a t e s

For quantitative analysis of starch, glucose and fructose leaf segments (2.5 g fresh weight) were homogenized in 20 ml MeOH by an Ultraturrax (Janke & Kunkel). The material was centrifuged and stirred up twice with hot MeOH. The combined solutions (ca. 80 ml) were adjusted to 100 ml by MeOH, 20 ml of which was evaporated to dryness and solved by 1 ml H_2O dest. After centrifugation, 20 µl of the extract was separated by HPCL for quantitative analysis of glucose and fructose (Milton Roy; column: LiChro-

sorb NH₂, 7μ; mobile phase: acetonitrile : H_2O 75 : 25 (v/v), pH 4.5; flow rate: 1.6 ml · min⁻¹; detector: RI). The insoluble residue after centrifugation (see above) was dried at 110 °C and used thereafter for the quantitative starch analysis according to Boehringer instructions (ANONYMOUS 1973).

Abbreviations: ψ_w = water potential
                 ψ_s = osmotic potential
                 ε = elastic modulus
                 RWC   = relative water content
                 VPD   = vapour pressure deficit

Results

1. Responses of leaves and roots to soil water deficiency

In 1982, Silvaner and Riesling vines in a 22-d experiment were subjected to periodic soil water stress in order to study the conditions which induce osmotic adjustment in the fully expanded leaves (for details concerning stress see Table 1). As can be seen from Fig. 1, the osmotic potential of both Silvaner and Riesling control plants (A) remains close to −9.5 bar during the experiment, whereas in Silvaner, but not in Riesling vines, drought stress induces a decrease of the osmotic potential to ca. −11.5 bar (B), −12.5 bar (C) or −13.5 bar (D), respectively, corresponding to the stress intensity. An analysis of the leaf carbohydrate content (Table 2) shows that in both varieties the starch content of leaves decreases during the experiment, while the fructose and glucose content of the leaves is higher in the majority of the stressed Silvaner, but not of the Riesling leaves. This indicates that the depression of the osmotic potential in stressed Silvaner vines is at least in part due to an active accumulation of fructose and glucose in the mature leaves. It can be speculated that the increases of glucose and fructose may not only derive from the starch metabolism observed during stress in both varieties, as the starch metabolism in Silvaner, but not in Riesling coincides with an increase of sugars. At the end of the experiment, after several cyclic drought

Table 3

Effects of cyclic drought stress on water loss at turgor 0 and on the elastic modulus (ε) of detached Silvaner and Riesling leaves

Der Einfluß zyklischer Wassermangelbedingungen auf die Wasserabgabe bei Turgor 0 und den Elastizitätsmodulus ε bei abgeschnittenen Silvaner- und Rieslingblättern

Cultivar	Treatment	Water loss (%) at turgor 0	ε (bar)
Silvaner	Control	3.64 (0.58)	210.30 (23.34)
	Stress	2.34 (0.52)	298.94 (41.43)
Riesling	Control	4.98 (0.72)	161.63 (23.03)
	Stress	3.28 (0.52)	230.16 (49.04)

Figures in parentheses: Standard deviation.

periods, the cell elasticity of fully expanded Riesling and Silvaner leaves was studied. As can be seen from Table 3, the water loss at turgor 0 of stressed plants turned out to be lower and the elastic modulus to be higher compared to the irrigated control plants, which indicates a lowering of the leaf elasticity due to the stress treatments.

Besides the osmotic potential of the leaves that of the roots was also measured. As is shown in Fig. 2, in both cultivars the leaf water potential is positively correlated to the osmotic potential of the roots under increasing stress conditions. Compared to the
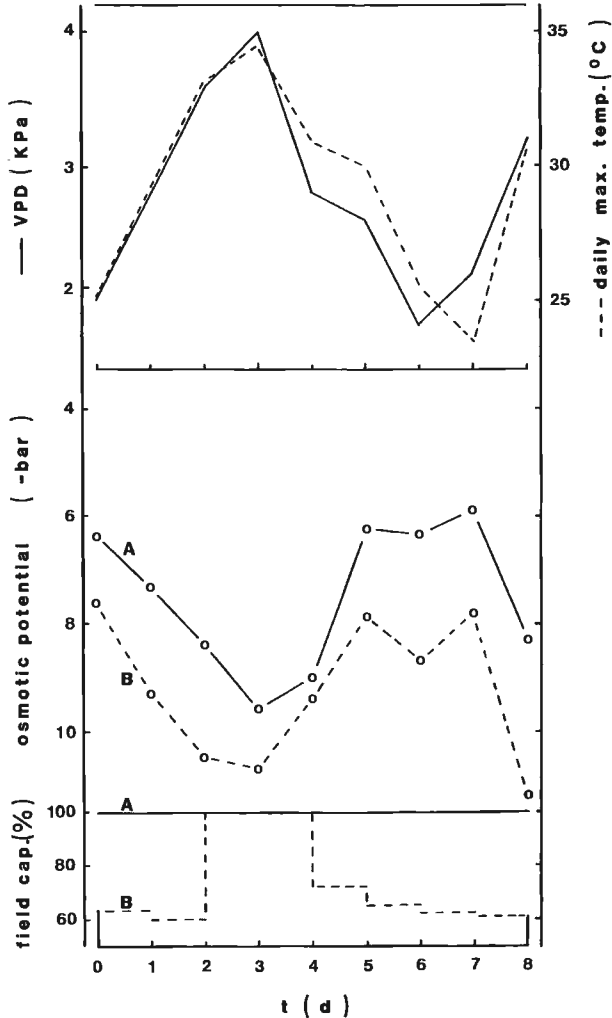

Fig. 4: Effects of the air temperature, the vapor pressure deficit of the air and of different soil water regimes (A: field capacity = 100 %; B: field capacity = 60—100 %) on the osmotic potential of apices at RWC = 100 %. Variety Riesling.

Der Einfluß der Lufttemperatur, des Dampfdruckdefizites der Luft und verschiedener Bodenwasserbedingungen (A: Feldkapazität = 100 %; B: Feldkapazität = 60—100 %) auf das osmotische Potential von Sproßspitzen bei RWC = 100 %. Sorte Riesling.

unstressed control, the osmotic potential of stressed roots was decreased at a rate of approximately 4 bar. These results indicate that cyclic drought stress can induce osmotic adjustment in both leaves and roots in the range of about 4 bar.

2. Responses of leaves and apices to atmospheric stress conditions

On 19 July 1983, the diurnal cycle of the osmotic potential of fully expanded leaves and apices was studied using field-grown Bacchus vines (Fig. 3). This day turned out to be hot and dry (see "Material and methods"). The osmotic potential of fully expanded leaves remained fairly constant during the day between -8 and -8.5 bar. In the apices the osmotic potentials were in general higher ($=$ less negative) compared to the fully expanded leaves, but a decrease was observed during 9 and 14 h to a minimum of -8.37 bar at 11 h and a subsequent increase in the afternoon. From these results it was concluded that under our experimental conditions the apical, i.e. physiologically younger tissues under atmospheric stress conditions were able to accumulate actively solutes during the day while expanded leaves no longer showed diurnal oscillations of ψ_s. This may be part of an adaptation system by which the apical tissue with its relatively high ψ_s can actively lower its ψ_s during the period of high water loss (from 9 to 15 h) in order to maintain a positive turgor.

The fact that ψ_s of the apices is able to react to changes of ambient air humidity and/or temperature is also shown in Fig. 4 (variety Riesling). The air temperature changed during the experiment in the glasshouse for more than 10 °C and the VPD for more than 2 kPa within a few days. Compared to the atmospheric changes, the alterations of the soil water conditions appear to be of less importance. ψ_s of the apices of both, group A and B, declines for 3 to 4 bar at increasing temperature and VPD and vice versa, the ψ_s of the plants of group B being always somewhat lower than those of the group A. Parallel experiments with Silvaner gave similar results (no figure).

These results indicate that at a moderate soil water stress, but also at a sufficient water supply in the soil (100 % field capacity), a high evaporative demand of the atmosphere and/or high temperatures are able to induce osmotic changes in apices.

Discussion

The results presented in this paper clearly show that the changes of ψ_s observed during our experiments can not be explained by a (passive) concentration effect as the osmotic potential was always determined using fully hydrated leaves. The active net accumulation of solutes indicates that grapevines generally are able to adjust osmotically when subjected to stress situations. As far as these results show, glucose and fructose are involved in the osmotic changes, a fact, which in the case of Silvaner corresponds well with the degradation of starch observed after a prolonged stress. At the moment it remains open if in addition other osmotically active compounds, such as amino acids or ions, participate in the osmotic adjustment of grapevines (TURNER and JONES 1980). A distinct difference in sensitivity towards stress was found between the fully expanded mature leaves and the apices. While in mature Silvaner leaves osmotic adjustment becomes visible only after several drought and irrigation cycles, in the apices alterations of ψ_s due to changes of the atmospheric conditions were observed even within hours. Similar results were obtained from apple leaves by DAVIES and LAKSO (1979). Apices are known to delay or stop their growth in response to water defi-

cits in a very sensitive way (HSIAO 1973, BARLOW *et al.* 1980); thus the attraction and accumulation of osmotically active compounds in apices during a stress period may be due to the fact that these compounds are used transitorily to stabilize the water status of this organ. There is no clear answer to the question why osmotic adjustment in fully expanded leaves was observed in Silvaner but not in Riesling. Obviously no active net accumulation of solutes, e.g. sugars, took place in Riesling (Table 2), although the starch content declined. Referring to TURNER and JONES (1980) many factors such as the rate of development of stress, the degree of stress, the environmental conditions and genetic differences affect osmotic adjustment. Under field conditions Riesling was shown to lower its osmotic potential of the fully expanded leaves when subjected to a prolonged water stress in the soil and atmosphere (DÜRING and LOVEYS 1982). Moreover, ALLEWELDT and GEISLER (1958) observed increased cell sap concentrations in Riesling after a period of wilting. Thus the missing reaction with Riesling in our experiments may possibly be due to experimental implications, as the induction of osmotic adjustment in potted plants seems to be more delicate than under field conditions. The rate of osmotic adjustment found in these experiments ranges from 4 to 5 bar; this is somewhat more than the rate reported by FREEMAN (unpublished, quoted by SMART and COOMBE 1983) who found maximum changes of ψ_s of only 1 bar in vines.

The adaptive response of mature leaves to water stress due to osmotic adjustment seems to be counteracted by decreases of the cell elasticity after stress treatments. A similar decrease in mature leaves was noticed also during the hot and dry summer in 1983 in several field-grown *Vitis vinifera* cultivars (DÜRING, in preparation) and is reported to occur also in other species (e.g. JONES and TURNER 1978). According to TURNER and JONES (1980) water stress generally reduces cell size in young expanding tissues. From this it can be speculated that contrary to the mature leaves in apices of grapevines a prolonged drought stress may increase cell elasticity via reduction of the cell size (ELSTON *et al.* 1976, quoted by TURNER and JONES 1980). Further research to this is required.

Summary

The induction of osmotic adjustment was studied in the grapevine varieties Silvaner, Riesling and Bacchus in the field and in the glasshouse.

1. After cyclic drought and irrigation periods the osmotic potential (ψ_s) of fully expanded Silvaner, not of Riesling leaves, declined corresponding to stress intensity by up to 4 bar. These osmotic changes were accompanied by a degradation of starch in both cultivars and an increase of fructose and glucose in Silvaner. A similar decrease of ψ_s was observed also in the roots of stressed Silvaner and Riesling vines.

2. The apices, not the mature leaves, of field-grown Bacchus vines showed a diurnal cycle of ψ_s with a minimum ψ_s at 11 h. The ψ_s of apices of potted Riesling and Silvaner vines increased and decreased inversely to changes of the vapor pressure deficit of the atmosphere and temperature; a moderate water stress in the soil intensified these reactions.

3. Cyclic drought and irrigation periods led to a decrease of the cell elasticity in mature leaves of Silvaner and Riesling.

Literature

ACKERSON, R. C. and HEBERT, R. R., 1981: Osmoregulation in cotton in response to water stress. I. Alterations in photosynthesis, leaf conductance, translocation and ultrastructure. Plant Physiol. **67**, 484—488.

ALLEWELDT, G. and GEISLER, G., 1958: Untersuchungen über die Zellsaftkonzentration bei Reben. I. Modifikabilität und Variabilität des osmotischen Wertes. Vitis 1, 181—196.

ANONYMOUS, 1973: Enzymatische Analysen für die Lebensmittelchemie. Boehringer, Mannheim.

BARLOW, E. W. R., MUNNS, R. E. and BRADY, C. J., 1980: Drought responses of apical meristems. In: TURNER, N. C. and KRAMER, P. J. (Eds.): Adaptation of plants to water and high temperature stress, 191—205. J. Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore.

CHEUNG, Y. N. S., TYREE, M. T and DAINTY, J., 1975: Water relation parameters on single leaves obtained in a pressure bomb and some ecological interpretations. Canad. J. Bot. **53**, 1342—1346.

DAVIES, F. S. and LAKSO, A. N., 1979: Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees. Physiol. Plant. **46**, 109—114.

DÜRING, H. and LOVEYS, B. R., 1982: Diurnal changes in water relations and abscisic acid in field grown *Vitis vinifera* cvs. I. Leaf water potential components and leaf conductance under humid temperate and semiarid conditions. Vitis **21**, 223—232.

HANSON, A. D. and HITZ, W. D., 1982: Metabolic responses of mesophytes to plant water deficits. Ann. Rev. Plant Physiol. **33**, 163—203.

HSIAO, TH. C., 1973: Plant responses to water stress. Ann. Rev. Plant Physiol. **24**, 519—570.

JONES, M. M. and TURNER, N. C., 1978: Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol. **61**, 122—126.

SCHOLANDER, P. F., HAMMEL, H. T., BRADSTREET, E. D. and HEMMINGSEN, E. A., 1965: Sap pressure in vascular plants. Science **148**, 339—346.

SMART, R. E., 1974: Aspects of water relations of the grapevine (*Vitis vinifera*). Amer. J. Enol. Viticult. **25**, 84—91.

— and COOMBE, B. G., 1983: Water relations of grapevines. In: KOZLOWSKI, T. T. (Ed): Water deficits and plant growth, Vol. **VII**, 138—196. Academic Press, New York, London, Paris, San Diego, San Francisco, Sao Paulo, Sydney, Tokyo, Toronto.

TURNER, N. C., 1979: Drought resistance and adaptation to water deficits in crop plants. In: MUSSELL, H. and STAPLES, R. C. (Eds.): Stress physiology in crop plants, 343—372. J. Wiley and Sons, New York, Chichester, Brisbane, Toronto.

— and JONES, M. M., 1980: Turgor maintenance by osmotic adjustment: A review and evaluation. In: TURNER, N. C. and KRAMER, P. J. (Eds.): Adaptation of plants to water and high temperature stress, 87—103. J. Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore.

TYREE, M. T. and HAMMEL, H. T., 1972: The measurement of the turgor pressure and the water relations of plants by the pressure bomb technique. J. Exp. Bot. **23**, 267—282.

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