

Low air humidity causes non-uniform stomatal closure in heterobaric leaves of *Vitis* species

by

H. DÜRING

Bundesanstalt für Züchtungsforschung im Wein- und Gartenbau, Institut für Rebenzüchtung
Geilweilerhof, Siebeldingen, BR Deutschland

Niedrige Luftfeuchte verursacht uneinheitlichen Stomaschluß bei heterobarischen Blättern von *Vitis*-Arten

Zusammenfassung: Mit Hilfe der Infiltrationsmethode wurde bei Müller-Thurgau sowie verschiedenen *Vitis*-Arten gezeigt, daß Reben einen heterobarischen Blattpolypus besitzen, bei dem das Mesophyll in luftdichte, kammerartige Interzellulardysteme unterteilt ist. Der Stomaschluß dieser heterobarischen Blätter war uneinheitlich, wenn die Blätter einer niedrigen Luftfeuchte ausgesetzt wurden. Die Blattleitfähigkeit korrelierte mit dem Anteil der infiltrierten Blattfläche bzw. mit der infiltrierten Wassermenge. Wurde die Uneinheitlichkeit des Stomaschlusses in trockener Luft bei der Berechnung des interzellulären CO₂-Partialdruckes (p_i) berücksichtigt (vgl. DOWNTON *et al.* 1988 b), ergab sich eine Abnahme von p_i . Die nahezu gleiche Carboxylierungseffizienz bei hoher und geringer Luftfeuchte verdeutlicht, daß die Abnahme der Photosyntheseleistung bei geringer Luftfeuchte ausschließlich über eine stomatär bedingte Verminderung der CO₂-Aufnahme zu erklären ist.

Keywords: leaf, stoma, air humidity, photosynthesis, carboxylation efficiency, leaf conductance.

Introduction

Only a few years ago, leaf gas exchange was evaluated assuming that photosynthetic activity was uniform in all parts of a leaf. However, results of ISHIHARA *et al.* (1971), LAISK *et al.* (1980) and TERASHIMA *et al.* (1988) revealed strong evidence that there exists a wide variation in aperture of stomata even for the small area of leaves of *Oryza sativa* or *Hordeum vulgare*. Direct microscopic analysis of individual stomata indicates that even neighbouring stomata behave differently when subjected to environmental stress (OMASA *et al.* 1985; KAPPEN *et al.* 1987). Also, groups of fully closed stomata were found in the vicinity of groups of fully open stomata (LAISK 1983). Such a non-uniform stomatal behaviour is obviously related to the anatomy of heterobaric leaves. According to NEGER (1918) the mesophyll of heterobaric leaves is separated into intercellular chambers hermetically sealed other than their own stomatal openings, while homobaric leaves have no barriers to gas exchange within the mesophyll.

Since the conventionally used calculation of the internal CO₂ partial pressure (p_i) from gas exchange measurements implies the assumption of uniformly open stomata on the leaf blade, the occurrence of non-uniform stomatal behaviour must lead to erroneous results (VON CAEMMERER and FARQUHAR 1981; TERASHIMA *et al.* 1988; VAN KRAALINGEN 1990). DOWNTON *et al.* (1988 a and b, 1990) were the first to demonstrate non-uniform stomatal behaviour in leaves of grapevines (Cabernet Sauvignon, Sultana) after infiltration of abscisic acid (ABA) and as a consequence of water deficiency or salt stress.

In the present study evidence is presented for the predominance of the heterobaric leaf type in *Vitis* spp. It will be demonstrated that a decrease of the photosynthetic

activity under conditions of low air humidity is caused by stomatal limitation of CO₂ uptake and not - as was previously assumed - by mesophyll factors.

Material and methods

Fully expanded field-grown leaves of *V. berlandieri*, *V. rupestris*, *V. riparia*, *V. amurensis*, *V. slavini*, *V. doaniana*, *V. andersonii* and *V. shuttleworthii* were taken from the grapevine collection of the Institute of Grapevine Breeding Geilweilerhof at Siebeldingen. 3-year-old ungrafted Müller-Thurgau vines grown in pots and cultivated in the substrate 'Einheitserde P' in the glasshouse were transferred to the laboratory 1 week before the experiments were started. They were kept at 24–25 °C (day, 14 h) and 18–20 °C (night), respectively. Light was provided by 2 Osram, HQI-T 400 W/DH lamps with ca. 800 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the apical leaves and 300–350 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the basal leaves. Relative air humidity was about 50 %. Gas exchange was measured using a 'Miniküvetten-System' (Fa. Walz, Effeltrich, BRD) at 35 Pa CO₂, light saturation (750 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and 26 °C leaf temperature. Leaf conductance of fully expanded leaves was determined at various leaf to air water vapour pressure differences (Δ_w) (for details: DURING 1988, 1991). When, after altering Δ_w , photosynthesis and transpiration rates had reached a steady state, the circular leaf area (11.94 cm²) inserted into the cuvette was punched out and immediately weighed (W_1).

Subsequently this leaf disc was subjected to a water infiltration procedure (MOLISCH 1912; MICHAEL 1969; BEYSCHLAG and PFANZ 1990). The leaf disc was placed into a plastic syringe (50 ml) which had been filled before with about 30 ml of distilled water. Remaining air bubbles were removed through the outlet of the syringe. The outlet was then closed and a vacuum was created in the syringe by pulling the piston outward. Simultaneously the syringe was shaken to remove air from internal leaf spaces. After having released this air, the syringe was connected to an electronic pressure meter (DPM 35-G, Penwalt, BRD); the pressure inside the syringe was increased to 0.30 MPa by pushing the piston into the syringe. By this procedure water penetrated the leaf via open stomatal pores. The leaf disc was then blotted dry by filter paper and weighed again (W_2). From W_1 and W_2 the amount of water taken up by the leaf disc can be estimated. The backlit lower leaf surface was photographed and the photograph copied to an overhead foil. Using a leaf area meter (LI-3100, Li-COR, USA), the dark (non-infiltrated) areas of the disc were determined and related to its total area.

The reaction of gas exchange to changes of Δ_w was determined by a stepwise lowering of the dew point in the cuvette to values < 21 °C.

All measurements were performed from 8 to 11 a.m. The gas exchange data were calculated according to VON CAEMMERER and FARQUHAR (1981); the intercellular CO₂ partial pressure derived from gas exchange data (p_i -IRGA) was corrected according to a proposal by DOWNTON *et al.* (1988 b).

The corrected p_i can be obtained approximately by

$$p_i\text{-CORR} = R(p_i\text{-IRGA}) + (1 - R)\Gamma,$$

where R is the ratio of photosynthetic rates (A) at the same p_i ($R = A\text{-stress}/A\text{-control}$), and Γ is the CO₂ compensation point. In our experiment Γ was 4.7 Pa. The carboxylation efficiency (CE) was calculated as follows:

$$\text{CE} = A/(p_i - \Gamma).$$

Results and discussion

In a previous paper, it has already been shown that decreasing air humidity can induce stomatal closure (DURING 1987). Using this dependency in a series of experiments with Müller-Thurgau vines, circular leaf areas were subjected either to $\Delta_w = 14.2 \text{ Pa} \cdot \text{kPa}^{-1}$ (corresponding to 58 % RH at 26 °C) or to $\Delta_w = 26.0 \text{ Pa} \cdot \text{kPa}^{-1}$ (corresponding to 23 % RH at 26 °C). After water infiltration the leaves were photographed using backlight; light areas indicate infiltrated, dark areas non-infiltrated parts. As is demonstrated by the light circle (centre of Fig. 1 A), at high air humidity a large number of open stomata enable water infiltration. The dark ring around the light circle is caused by the India rubber ring which is pressed on the leaf blade to seal the cuvette air from ambient air; stomata situated on this ring are almost entirely closed. At lower air humidity only groups of stomata are open, light areas with open stomata can be clearly distinguished from dark areas, where stomata are closed (Fig. 1 B). Fig. 1 C shows a wilted leaf; most of the stomata are closed ($g_{\text{H}_2\text{O}} = 36 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and subsequently dark areas predominate.

These results clearly demonstrate that the stomatal behaviour of Müller-Thurgau leaves at low air humidity is non-uniform confirming the heterobaric leaf type of *V. vinifera*. Additional experiments on the behaviour of stomata under low air humidity conditions were performed using leaves of *V. berlandieri*, *V. rupestris*, *V. riparia*, *V. amurensis*, *V. slavini*, *V. doaniana*, *V. andersonii* and *V. shuttleworthii*. Leaves of all these genotypes exerted 'patchiness' similar to that shown in Fig. 1. Thus, it appears that the heterobaric leaf type is a widespread character within the genus *Vitis*.

A quantitative evaluation of the photographs of leaf discs after water infiltration indicates that the portion of the infiltrated (light) area increases with leaf conductance determined by porometry (data not shown). In addition, the amount of infiltrated water was positively correlated with leaf conductance (Fig. 2), indicating that the reduction of water infiltration at low air humidity was in fact caused by stomatal closure. To our surprise even at leaf conductance values close to zero about $0.5\text{--}1 \text{ mg H}_2\text{O} \cdot \text{cm}^{-2}$ was infiltrated. In experiments with entire leaf blades (the cut ends of petioles were closed by wax) no water was infiltrated at leaf conductance close to zero. Thus it is assumed that some water may have entered the leaf blade via the peripheral, injured tissue.

If we assume that in the light areas of the infiltrated leaf discs stomata are more or less homogeneously open and closed in the non-infiltrable dark areas, it results that the conventional calculation of the intercellular CO_2 partial pressure (p_i -IRGA) is correct in areas where stomata are open, while in areas where stomata are closed p_i -values are close to the CO_2 compensation point. Non-uniform stomatal closure therefore causes an overestimation of p_i -values (TERASHIMA *et al.* 1988; DOWNTON *et al.* 1988 a and b). This is demonstrated using gas exchange data obtained under different air humidity conditions (Table).

A decrease of air humidity lowered rates of leaf conductance (-22%) and photosynthesis (-27%). From a series of measurements gas exchange data with identical p_i -IRGA values were selected. Calculation of the carboxylation efficiency (A/p_i or CE-IRGA) indicates reduced values at low air humidity. In the past it was deduced that under stress conditions, besides stomatal limitations of photosynthesis, non-stomatal factors would reduce photosynthetic activity (Review: SCHULZE 1986). A more realistic estimation of p_i under stress conditions which takes into consideration the non-uniform stomatal behaviour was proposed by DOWNTON *et al.* (1988 b). At low air humidity this corrected p_i -value (p_i -CORR) decreased compared to p_i -IRGA and CE-CORR at low air humidity differs only slightly from CE-IRGA at high air humidity (Table).

Thus, lowering air humidity obviously did not alter mesophyll factors; rather it is

evident that reductions of photosynthesis are caused by stomatal limitation of CO_2 uptake.

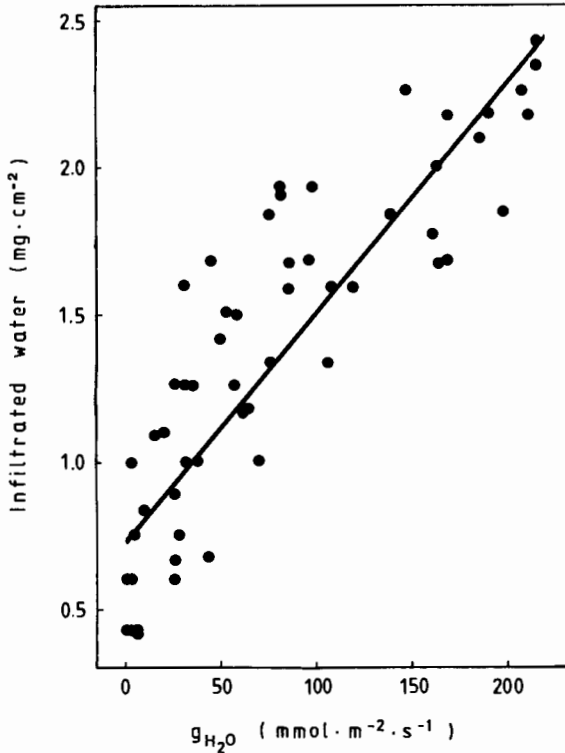


Fig. 2: The amount of infiltrated water related to leaf conductance ($g_{\text{H}_2\text{O}}$) of leaf discs. $r = 0.88$.

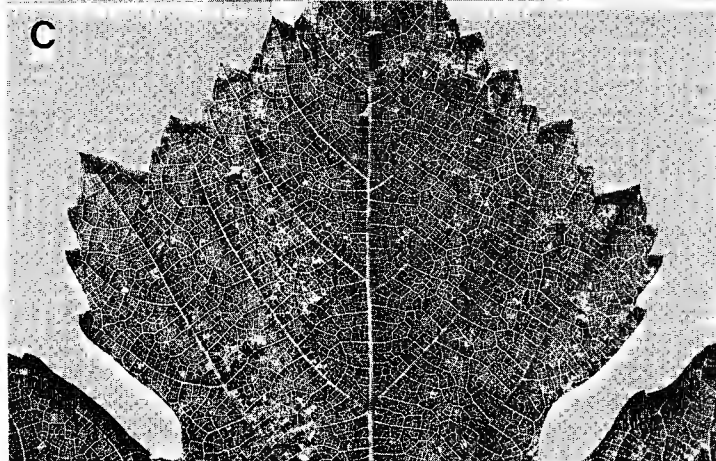
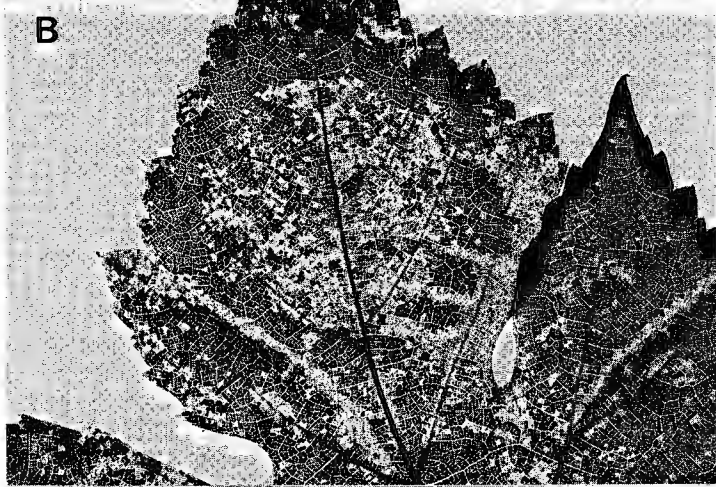
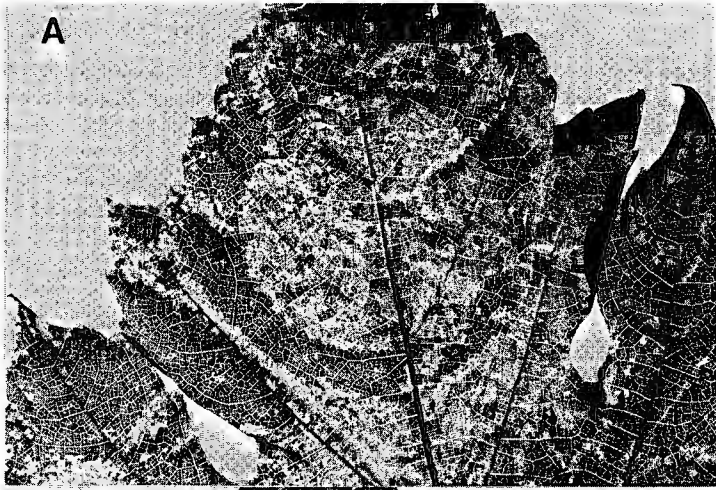
Die Menge an infiltriertem Wasser bezogen auf die Blattleitfähigkeit ($g_{\text{H}_2\text{O}}$) von Blattscheiben. $r = 0,88$.

Conclusion

The results on stomatal behaviour obtained so far in experiments with grapevine leaves have clearly revealed non-uniform stomatal closure due to certain stress factors or infiltrated ABA. As a consequence, estimations of p_i from gas analysis data under stress conditions will lead to erroneous results and values of carboxylation efficiency

Fig. 1: Backlit lower leaf surface of Müller-Thurgau shortly after water infiltration. Within the circular area (center of photographs A and B) leaf conductance was altered by changes of air humidity. — A) At 58 % relative humidity the majority of stomata is open ($g_{\text{H}_2\text{O}} = 112 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Light areas indicate water infiltration, dark areas non-infiltrated parts. — B) At 23 % relative humidity only groups of stomata (patches) are open ($g_{\text{H}_2\text{O}} = 87 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). — C) In wilted leaves almost all stomata are closed ($g_{\text{H}_2\text{O}} = 36 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

Die Unterseite eines Müller-Thurgau-Blattes im Durchlicht kurz nach der Wasserinfiltration. Im Bereich der kreisförmigen Fläche (Bildmitte, A und B) wurde über die Luftfeuchte die Blattleitfähigkeit verändert. — A) Bei 58 % relativer Luftfeuchte sind die Stomata weitgehend geöffnet ($g_{\text{H}_2\text{O}} = 112 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Die hellen Partien lassen Wasserinfiltration erkennen, in dunklen Partien wurde kein Wasser infiltriert. — B) Bei 23 % relativer Luftfeuchte sind nur einzelne Stomata-Gruppen geöffnet ($g_{\text{H}_2\text{O}} = 87 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). — C) Bei welken Blättern sind die Stomata überwiegend geschlossen ($g_{\text{H}_2\text{O}} = 36 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).



are underestimated. This rather new aspect calls in question former results concerning CE but also provokes new questions.

E.g., is the occurrence of patches really a temporary phenomenon which disappears if the ambient conditions remain constant for a sufficient enough time (BEYSCHLAG and PFANZ 1990) or, are gas tight compartments in the mesophyll of leaves of ecological significance for the inhibition of the diffusion of fumes into the entire leaf blade (NEGER 1918)? There are certainly more aspects which would deserve further investigation.

Rate of photosynthesis (A), leaf conductance for CO_2 (g_{CO_2}), intercellular CO_2 partial pressure (p_i) and the carboxylation efficiency (CE) of Müller-Thurgau leaves as affected by low and high leaf to air water vapour pressure difference (Δ_w). Note that a low Δ_w value corresponds to a high relative humidity (RH). IRGA = derived from gas exchange data, CORR = corrected according to DOWNTON *et al.* (1988 b)

Der Einfluß einer niedrigen bzw. hohen Blatt-Luft-Wasserdampfdruckdifferenz (Δ_w) auf die Photosyntheserate (A), die Blattleitfähigkeit für CO_2 (g_{CO_2}), den interzellulären CO_2 -Partialdruck (p_i) und die Carboxylierungseffizienz (CE) bei Müller-Thurgau-Blättern. Ein niedriger Δ_w -Wert entspricht einer hohen relativen Feuchte (RH). IRGA = aus den Gaswechselmeßdaten berechnet, CORR = korrigiert (nach DOWNTON *et al.* 1988 b)

Δ_w , Pa · kPa ⁻¹	14.2	26.0
RH, %	58.0	23.0
A , $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	7.8	5.7
g_{CO_2} , $\text{mmol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	112	87
p_i -IRGA, Pa	23.5	23.5
p_i -CORR, Pa	—	18.4
CE-IRGA, $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$	0.415	0.303
CE-CORR, $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$	—	0.416

Summary

Water infiltration experiments indicate that cv. Müller-Thurgau and several *Vitis* spp. belong to the heterobaric leaf type which is characterised by airtight intercellular chambers in the mesophyll. Stomatal closure of heterobaric leaves was non-uniform if leaves were exposed to low air humidity.

Leaf conductance was correlated with the infiltrated leaf area and the amount of infiltrated water. Taking into account non-uniform stomatal closure of vine leaves in the calculation of intercellular CO_2 partial pressure (p_i) (DOWNTON *et al.* 1988 b), p_i was reduced at low air humidity. The almost identical carboxylation efficiency (A/p_i) under moist and dry air conditions indicates that the decline of the photosynthetic rate caused by dry air is entirely due to stomatal limitation of CO_2 uptake.

Acknowledgement

The author wishes to thank Mr. A. PREISS for skillful technical assistance.

Literature

- BEYSCHLAG, W.; PFANZ, H.; 1990: A fast method to detect the occurrence of nonhomogeneous distribution of stomatal aperture in heterobaric plant leaves. Experiments with *Arbutus unedo* L. during the diurnal course. *Oecologia* **82**, 52—55.
- CAEMMERER, S. VON; FARQUHAR, G. D.; 1981: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376—387.
- DOWNTON, W. J. S.; LOVEYS, B. R.; GRANT, W. J. R.; 1988 a: Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. *New Phytol.* **108**, 263—266.
- — ; — — ; — — ; 1988 b: Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. *New Phytol.* **110**, 503—509.
- — ; — — ; — — ; 1990: Salinity effects on the stomatal behaviour of grapevine. *New Phytol.* **116**, 499—503.
- DURING, H.; 1987: Stomatal responses to alterations of soil and air humidity in grapevines. *Vitis* **26**, 9—18.
- — ; 1988: CO₂ assimilation and photorespiration of grapevine leaves: Responses to light and drought. *Vitis* **27**, 199—208.
- — ; 1991: Determination of the photosynthetic capacity of grapevine leaves. *Vitis* **30**, 49—57.
- ISHIHARA, K.; NISHIHARA, T.; OGURA, T.; 1971: The relationship between environmental factors and behaviour of stomata in the rice plant. 1. On the measurement of the stomatal aperture. *Proc. Japan. Soc. Crop Sci.* **40**, 491—496.
- KAPPEN, L.; ANDRESEN, G.; LÖSCH, R.; 1987: *In situ* observations of stomatal movements. *J. Exp. Bot.* **38**, 126—141.
- KRAALINGEN, D. W. G. VAN; 1990: Implications of non-uniform stomatal closure on gas exchange calculations. *Plant, Cell and Environment* **13**, 1001—1004.
- LAISK, A.; 1983: Calculation of photosynthetic parameters considering the statistical distribution of stomatal aperture. *J. Exp. Bot.* **34**, 1627—1635.
- — ; OJA, V.; KULL, K.; 1980: Statistical distribution of stomatal aperture of *Vicia faba* and *Hordeum vulgare* and Spannungphase of stomatal opening. *J. Exp. Bot.* **31**, 49—58.
- MICHAEL, G.; 1969: Eine Methode zur Bestimmung der Spaltöffnungsweite von Koniferen. *Flora* **159**, 559—561.
- MOLISCH, H.; 1912: Das Offen- und Geschlossenein der Spaltöffnungen, veranschaulicht durch eine neue Methode (Infiltrationsmethode). *Z. Bot.* **4**, 106—122.
- NEGER, F. W.; 1918: Die Wegsamkeit der Laubblätter für Gase. *Flora* **3**, 152—161.
- OMASA, K.; HASHIMOTO, Y.; KRAMER, P. J.; STRAIN, B. R.; AIGA, J.; KONDO, J.; 1985: Direct observation of reversible and irreversible stomatal response of attached sunflower leaves to SO₂. *Plant Physiol.* **79**, 153—158.
- SCHULZE, E.-D.; 1986: Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Ann. Rev. Plant Physiol.* **37**, 247—274.
- TERASHIMA, I.; WONG, S.-C.; OSMOND, C. B.; FARQUHAR, G. D.; 1988: Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant Cell Physiol.* **29**, 385—394.

Received, 17. 9. 1991

Priv. Doz. Dr. H. DURING
 Bundesanstalt für Züchtungsforschung
 im Wein- und Gartenbau
 Institut für Rebenzüchtung
 Geilweilerhof
 D-6741 Siebeldingen
 BR Deutschland