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## Studies on the effect of chilling on the photosynthesis of grapevine

by

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### Untersuchungen zur Wirkung des Kältestresses auf die Photosynthese der Rebe

**Zusammenfassung:** Die Wirkung von Kältestreß ( $6 \pm 2^\circ\text{C}$ ) auf die Photosyntheseaktivität von Rebsorten unterschiedlicher Kälteempfindlichkeit wurde untersucht.

1. Der Kältestreß (4 h bei einer Beleuchtungsstärke von  $80 \text{ W m}^{-2}$ ) reduzierte die Kapazität der  $^{14}\text{CO}_2$ -Fixierung um 10 % (Mädchentraube) bzw. 20—70 % (Grüner Veltliner). Das Ausmaß der Beeinträchtigung wurde durch das unterschiedliche Angebot an Nährstoffen beeinflusst. Die Verminderung der Photosynthesekapazität war reversibel. Längere Einwirkung des Kältestresses führte bei beiden Rebsorten zu einer Anpassung.

2. Die tolerantere Sorte Mädchentraube reagierte auf Kältestreß mit Schließen der Stomata, während bei der empfindlicheren Sorte Grüner Veltliner der Stomataschluß beeinträchtigt war.

3. Der Kältestreß wirkte eigentlich als Wasserstreß. Dies konnte anhand einer Verringerung des Wasserpotentials und durch Beeinflussung der Induktionskinetik der Chlorophyll-Fluoreszenz, die auf eine Hemmung des Photosystems II hindeutet, nachgewiesen werden.

**Key words:** cold, resistance, photosynthesis, stoma, hydration, chlorophyll, potassium.

### Introduction

Temperature is one of the most important environmental factors determining the latitudinal and altitudinal limits of viticulture (BECKER 1977). Much research has been done on the optimal temperature conditions (KRIEDEMANN 1968; BUTTROSE 1969; KRIEDEMANN and SMART 1971; ALLEWELDT *et al.* 1982), but data on the effect of sub-, and super-optimal temperatures are scarce (KRIEDEMANN 1968). Although not listed among characteristic chill-sensitive plants (ÖQUIST 1983), the vine grower's practice shows that grapevine is considerably damaged by cold spells often occurring in spring. Such periods of chilling, with diurnal temperatures of  $5\text{—}10^\circ\text{C}$ , retard the development of vineyards. Although the degree of damage depends on varietal characteristics and nutritional conditions, no systematic studies on the genetic potential and physiological background of the chill-sensitivity of grapevine can be found in the literature.

This paper is an attempt to fill in this gap by studying the photosynthetic capacity of different cultivars exposed to chill-temperature and to understand the underlying mechanisms.

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### Materials and methods

#### Plant material

Experiments were carried out with *Vitis vinifera* cv. Leányka and Zöld veltelini (known as 'Mädchentraube' and 'Grüner Veltliner'). For some experiments Zöld veltelini was taken from two vineyards of different mineral supply (see below): 'optimal' with cuttings serving as a parallel for Leányka and 'poor', providing plants for studies on the effect of nutritional conditions.

Properties of the soils (for both optimal and poor mineral supply) before manuring:

Soil type: brown forest soil on loess; pH (H<sub>2</sub>O) = 7.1; CaCO<sub>3</sub> = 1.5 %; clay content = 17 %; humus content = 1.3 %; ammonium lactate soluble K<sub>2</sub>O = 10 mg/100 g; ammonium lactate soluble P<sub>2</sub>O<sub>5</sub> = 5 mg/100 g; MgO = 15 mg/100 g. Rainfall, average of 50 years: 633 mm.

Levels of the soil fertilizers used from 1973 (planting in 1974):

	Poor	Optimal
Potassium – kg K <sub>2</sub> O/ha before planting	0	8000
Phosphorus – kg P <sub>2</sub> O <sub>5</sub> /ha before planting <sup>1)</sup>	540	540
Nitrogen – kg N/ha in autumn every year	400	0

<sup>1)</sup> No subsequent potassium or phosphorus fertilizing took place since the planting (1974).

Grapevine cuttings were collected in December and stored at 4 °C. One-bud cuttings were grown for 5 weeks in distilled water under greenhouse conditions in the period between February to May. Groups of 3–5 plants were transferred for various periods into a chilling cabinet adjusted to 6 ± 2 °C. Control plants were taken from 25 ± 2 °C. Both chilled and control plants were kept under illumination by 80 W m<sup>-2</sup> (400–700 nm) white light and at RH of 90 and 60 %, respectively. The most developed leaves (corresponding in size to about 80 % of full expansion) were used in the experiments.

#### Measurements

Photosynthetic capacity was measured according to MUSTÁRDY *et al.* (1982) or with the method described by LANG *et al.* (1985) using leaf discs cut from the central area of the leaf blade.

The environmental factors during the measurements were: temperature: 25 ± 2 °C; light intensity: 10 W m<sup>-2</sup> (400–700 nm); RH: 100 %.

Stomatal morphology was studied by scanning electron microscopy of leaves, after fixation in Karnovsky's solution (KARNOVSKY 1965) and osmic acid-thiocarbohydrazide staining followed by critical point drying in carbon dioxide (MUSTÁRDY and JÁNOSSY 1979).

Estimation of the stomatal density and of the proportion of open stomata was carried out by using cellulose acetate replicas from silicon rubber impression negatives (SLAVIK 1974).

Stomatal resistance was determined with a diffusion porometer LI-60 Diffusive Resistance Meter (LI-COR, Lambda Instr. Corp.) during 8–12 a. m.

The experimental conditions were, for control:  $25 \pm 2$  °C,  $80 \text{ W m}^{-2}$  (400—700 nm), RH 70 %; for chilled plants:  $6 \pm 2$  °C,  $80 \text{ W m}^{-2}$  (400—700 nm), RH 100 %.

Studies on stomata were made on the lower surface of leaves.

Leaf xylem water potential was estimated with a pressure bomb according to SCHOLANDER *et al.* (1965).

Chlorophyll fluorescence induction kinetics were recorded at 25 °C from the upper surface of detached leaves. After a 3 min dark adaptation, samples were illuminated by a 450 W Xenon lamp through a Corning CS 4-96 glass filter. The intensity of the homogeneous excitation beam was  $10 \text{ W m}^{-2}$ . The fluorescence induction transient was followed for 10 s with a time resolution of 10 ms (DEMETER *et al.* 1985).

### Results and discussion

After several hours of chilling Zöld veltelini plants showed a moderate wilting, while on the Leányka plants no symptoms of chilling occurred. Further treatment led to cold adaptation, resulting in a recovery of the normal turgor of plants. With a more prolonged chilling (4—5 d) leaf blades developed white spots, areas of deteriorated mesophyll. In the present work, we dealt with the effect of brief chilling often occurring in the practice.

Our pilot experiments on chilling under laboratory conditions suggested a difference between the cold tolerance of these cultivars which might play some role in the different performance of Leányka and Zöld veltelini in the field (Table 1).

Differences in the sensitivity to chilling were evident also in the experiments comparing the  $\text{CO}_2$  fixation capacity of the two cultivars after various periods of chilling (Fig. 1). The suppression of the incorporation of  $\text{CO}_2$  was appreciably more severe in Zöld veltelini than in Leányka. Nutritional conditions affected the time course of the injury being much faster in the plants which had earlier a poor nutrient supply. The adaptation became manifest after 8 h of chilling but very likely it started much earlier.

When returning the plants to 25 °C for 24 h, even after 12 h of chilling, each plant recovered completely.

A definitive parallelism found between wilting and the suppression of  $\text{CO}_2$  fixation capacity in grapevine together with earlier data on some other plants (WILSON 1976; MUSTÁRDY *et al.* 1982) suggested a strong relationship between these two symptoms of

Table 1

The cropping level (mean yield of 5 years) and the risk of production in Leányka and Zöld veltelini cultivars (data of Balatonboglár from years 1978—83)

Die Ertragshöhe (Durchschnittsertrag aus 5 Jahren) und das Produktionsrisiko bei den Rebsorten Mädchentraube und Grüner Veltliner (Ergebnisse der Jahre 1978—83, Balatonboglár)

Cultivars	Nutritional conditions	Mean yield t/ha	$\frac{\text{Max. yield}}{\text{Min. yield}}$
Leányka	Optimal	20.0	1.4
Zöld veltelini <sup>1)</sup>	Optimal	18.0	1.7
	Poor	13.0	2.6

<sup>1)</sup> Characterized in Bálo *et al.* (1981)

Table 2

Stomatal density, percentage of open stomata and stomatal resistance ( $\bar{x} \pm SE$ ) in Leányka and Zöld veltelini cultivars (grown in the greenhouse)

Dichte der Stomata, Prozentsatz geöffneter Stomata und stomatäre Resistenz ( $\bar{x} \pm SE$ ) bei den Rebsorten Mädchentraube und Grüner Veltliner (Gewächshauspflanzen)

Cultivars	Nutritional conditions	Number of stomata/ mm <sup>-2</sup> of lower epidermis n=50	% of open stomata n=16	Stomatal resistance s cm <sup>-1</sup> n=3
Leányka	Optimal	178 ± 6	30 ± 2	7.0 ± 2.2
Zöld veltelini	Optimal	187 ± 5	63 ± 4	6.7 ± 1.0
	Poor	205 ± 5	47 ± 3	9.4 ± 0.6

chilling. This means that under chilling evapotranspiration is not properly regulated and, as a result, the photosynthetic apparatus is suffering from drought.

As chilling acts at least partly like a drought stress, stomatal behaviour may be of importance (HOFÁCKER 1976).

Since a comparison between the morphology of stomata of the two cultivars did not offer any explanation of the difference of chilling sensitivities and stomatal densities were also practically the same (Table 2), we concluded that chilling sensitivity may be influenced by the characteristic physiological response of stomata (Fig. 2).

We found with each kind of plant a transitory decrease of the stomatal resistance. This prompt effect was followed by a differential response of stomata which was related to the different chilling sensitivity of the cultivars. In Leányka leaves, the stomatal resistance increased, limiting thereby extensive water loss. This was a normal protection against desiccation of a plant with slow water uptake and translocation at low temperatures. In contrast to the former, in Zöld veltelini leaves evapotranspiration was not limited by stomatal closure, which can be one of the reasons for higher chill-sensitivity.

Table 3

The effect of chilling on the xylem water potential (bar) measured in Leányka leaves ( $\bar{x} \pm SE$ , n = 3)

Der Einfluß von Kältestreß auf das Wasserpotential (bar) des Xylems bei Blättern der Mädchentraube ( $\bar{x} \pm SE$ , n = 3)

Chilling period h	Immediately after chilling	After 24 h of recovery at 25 °C
0	- 1.3 ± 0.3	- 0.5 ± 0
6	- 13.3 ± 1.2	- 3.6 ± 0.3
12	- 10.8 ± 0.8	- 1.3 ± 0.2
24	- 6.8 ± 0.2	- 0.5 ± 0

Stomatal closure, a process known to increase stomatal resistance, was of different extent, depending on the nutritive supply of the plants. Zöld veltelini from optimally supplied cuttings showed a slight increase in stomatal resistance while in plants originating from poor nutritional conditions no stomatal closure occurred. As stomatal movements depend to a large extent on  $K^+$  exchange and transport (ZEIGER 1983), the most important element of the mineral nutrition influencing chilling might be the potassium supply.

In an attempt to understand the chilling effects, we studied the xylem water potential. As expected, the water potential decreased to a considerable extent, but later in the adaptation period the control value was partly recovered (Table 3). This suggests that adaptation to chilling is essentially an osmotic adjustment.

In order to study how chilling inhibits photosynthesis, kinetics of fluorescence induction of chilled plants was studied (Fig. 3). Here we found an effect which was identical with that observed with water stress (DOWNTON 1983). Chilling and water stress did not influence the quickly rising phase of fluorescence induction, but decreased the variable fluorescence known to be emitted from Photosystem II. It is explained as a photoinhibition of the electron transport (POWLES and CRITCHLEY 1980) and is connected with the loss of the turgor (DOWNTON and MILLHOUSE 1983). After

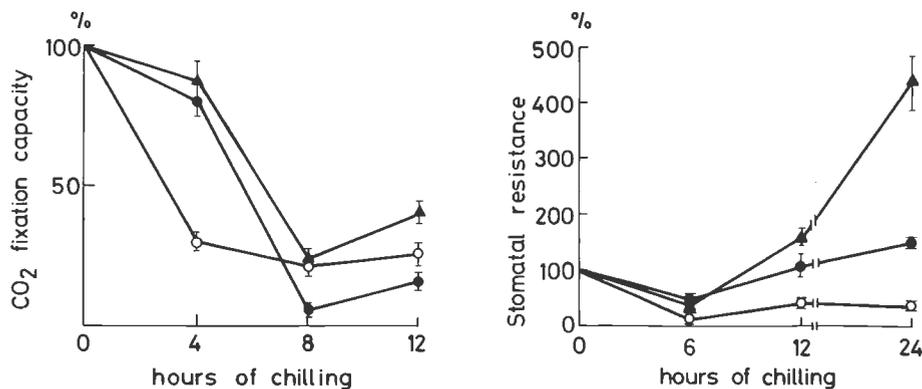


Fig. 1 (left): The effect of chilling on the capacity of  $^{14}CO_2$  fixation of cuttings originating from vineyards with different nutritional conditions. Fixation values of the non-chilled plants (100 %) as  $\mu\text{mol } CO_2 \text{ dm}^{-2} \text{ h}^{-1}$ : Leányka 42.0, Zöld veltelini optimal nutrition 42.0, Zöld veltelini poor nutrition 25.8. Averages of 20 individual values  $\pm$  SE were plotted. Symbols: — $\blacktriangle$ — Leányka; — $\bullet$ — Zöld veltelini optimal nutrition; — $\circ$ — Zöld veltelini poor nutrition.

Die Wirkung von Kältestress auf die Kapazität der  $CO_2$ -Fixierung von Pflanzen aus Weingärten mit unterschiedlichen Nährstoffbedingungen. Werte der Pflanzen ohne Kältestress (100 %) als  $\mu\text{mol } CO_2 \text{ dm}^{-2} \text{ h}^{-1}$ : Mädchentraube 42,0; Grüner Veltliner, optimale Nährstoffbedingungen 42,0; Grüner Veltliner, nährstoffarme Bedingungen 25,8. Die Durchschnittswerte samt Standardabweichungen von jeweils 20 Einzelpflanzen sind dargestellt. Symbole: — $\blacktriangle$ — Mädchentraube; — $\bullet$ — Grüner Veltliner, optimale Nährstoffbedingungen; — $\circ$ — Grüner Veltliner, nährstoffarme Bedingungen.

Fig. 2 (right): The effect of chilling on the stomatal resistance of leaves of cuttings originating from vineyards with different nutritional conditions. Individual values of 100 % are given in Table 2. Averages of 3 plants  $\pm$  SE were plotted. Symbols as in Fig. 1.

Die Wirkung von Kältestress auf die stomatäre Resistenz in den Blättern von Pflanzen aus Weingärten mit unterschiedlichen Nährstoffbedingungen. Die Werte der Kontrollpflanzen (100 %) sind in Tabelle 2 angegeben. Die Durchschnittswerte samt Standardabweichungen von jeweils 3 Pflanzen sind dargestellt. Symbole wie in Abb. 1.

osmotic adjustment, Photosystem II has recovered as shown also by the recovery of the normal induction kinetics of fluorescence.

From our experiments carried out under laboratory conditions, we see only a moderate injury of chilling even in the sensitive plants, due to adaptation phenomena and reversibility of the direct effects of cold. In vineyards, however, indirect damages might be also severe because of intercurrent infections and enhanced sensitivity to a number of unfavourable conditions (SAAYMANN 1977). Both in spring and autumn, cool climates can retard the ripening and, thus, influence the wine quality. Here the photosynthetic processes may be also of importance but the main target is the intermediary carbon metabolism.

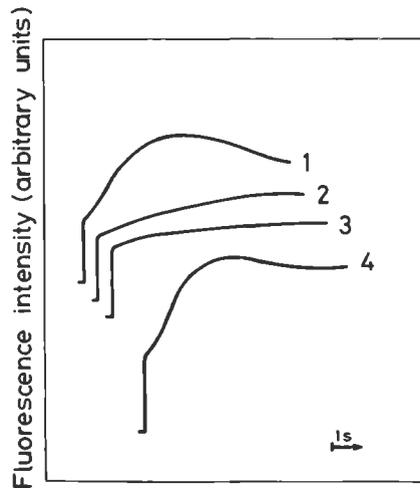


Fig. 3: The effect of chilling on the induction kinetics of fluorescence. Leányka leaves, fluorescence intensity in arbitrary units. 1 = control, no chilling, 2 = 4 h of chilling, 3 = 8 h of chilling, 4 = after chilling treatment of 8 h, kept for 24 h at 25 °C.

Die Wirkung von Kältestreß auf die Kinetik der Fluoreszenzinduktion. Blätter der Mädchentraube, Fluoreszenzintensität in nichtstandardisierten Einheiten. 1 = Kontrolle, kein Kältestreß; 2 = 4 h Kältestreß; 3 = 8 h Kältestreß; 4 = nach 8 h Kältestreßbehandlung 24 h lang bei 25 °C gehalten.

### Summary

The effect of chilling stress ( $6 \pm 2$  °C) on the photosynthetic capacity of grapevine in cultivars of different chilling sensitivity was studied under laboratory conditions.

1. Chilling stress (4 h under  $80 \text{ W m}^{-2}$  illumination) reduced the capacity of  $^{14}\text{CO}_2$  incorporation by 10 % (Leányka) and 20–70 % (Zöld veltelini), respectively. The onset of injury was influenced by the level of nutrients available. This decrease of the photosynthetic capacity was reversible. Longer exposure resulted in a cold adaptation in both cultivars.

2. The more tolerant Leányka responded to chilling by closing stomata while in the more sensitive Zöld veltelini stomatal response was impaired.

3. Chilling acted as a water stress as shown by a decrease of the water potential and by influencing the kinetic properties of fluorescence induction which indicates an inhibition of Photosystem II.

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