Vitis **45** (1), 45–46 (2006)

Research Note

Lime-induced chlorosis and physiological responses in grapevine (*Vitis vinifera* L. cv. Pinot blanc) leaves

L. Bavaresco[1)], M. Bertamini[2)] and F. Iacono[2)]

[1)]Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italy
[2)]Dipartimento di Produzione Agricola e Alimentare, Istituto Agrario di San Michele all'Adige (Trento), Italy

Key words: *Vitis vinifera*, lime-induced chlorosis, Chl fluorescence, photochemical quenching, stomatal conductance, net photosynthetic rate.

Abbreviations: Chl: chlorophyll; E: transpiration rate; ETR: apparent electron transport; Fo: minimal fluorescence; Fm: maximum fluorescence; Fv: variable fluorescence; gs: stomatal conductance; PPFD: photosynthetic photon flux density; Pn: leaf net photosynthetic rate; qp: photochemical quenching.

Introduction: Lime-induced iron chlorosis is a major problem of grapevine and high value fruit trees growing especially in the mediterranean region or in other semi-arid areas. Chlorotic plants are characterized by the development of pronounced interveinal yellowing, occurring first in the youngest leaves, and by yield reduction.

Most of the knowledge of iron chlorosis was obtained with annual plants grown in hydroponics. These plants were usually grown in greenhouses or chambers under controlled environmental conditions. Relatively few studies have focused on the consequences of iron deficiency on the photosynthetic performance of perennials growing outside (Nedunchezhian *et al*. 1997, Morales *et al*. 2000, Bertamini *et al*. 2002).

Iron plays a fundamental role in several physiological processes; *e. g.* iron is a constituent of electron transport chains in mitochondria and chloroplasts. Therefore, it may be concluded that a shortage of physiologically active iron leads to a decrease of the electron transport rate, the photosynthetic pigments (Val *et al*. 1987), as well as to a lowering of the efficiency of PSII Fv/Fm photochemistry (Morales *et al*. 2000, Bertamini *et al*. 2002).

The objective of this study was to determine possible changes in parameters of photosynthesis as affected by iron chlorosis of grapevine leaves inserted at different positions.

Material and Methods: Plant material: Three-year-old *Vitis vinifera* L. cv. Pinot blanc vines grafted on the lime-susceptible rootstock 3309 C (*V. riparia* Michx. x *V. rupestris* Scheele) were potted (pot volume = 10 l) in a non-calcareous and a calcareous soil. The main soil characteristics were as follows (non calcareous *vs.* calcareous soil, respectively): sandy clay-loam (for both soils), pH 7.3/8.2; 3/67 % total carbonates; 1.5/16.5 % active lime, 38/6 mg·kg^{-1} Olsen P, 80/14 mg·kg^{-1} available Fe (extracted by DTPA 0.005 M + $CaCl_2$ 0.01 M + triethanolamina 0.1 M). 10 pots per soil type with one plant each were placed outside on a platform covered by a hail-protection net. During summer, when the average shoot length was about 150 cm, one representative shoot per plant was choosen and three leaves in the basal, medial and apical part were tested. These "old", "intermediate" and "young" leaves were green when grown on non-calcareous soil, while they were chlorotic when grown on calcareous soil.

Leaf net CO_2 exchange rates (*Pn*), stomatal conductance (*gs*) and transpiration rates (*E*) of test leaves were measured at 11:00 a.m. with a portable photosynthesis system (CI-310, CID Inc., Camas, WA 98607, USA).

Chlorophyll fluorescence was measured using a PAM 2000 fluorometer (H. Walz, Effeltrich, Germany). Fo was determined with modulated light (0.6 kHz); PPFD was less than 0.1 µmol·m^{-2}·s^{-1} at the leaf surface. Fm was measured at 20 kHz with a one s pulse of 6000 µmol m^{-2} s^{-1} (white light). Induction kinetics of fluorescence quenching coefficient qp (photochemical quenching) was determined by the saturation pulse method according to Schreiber *et al*. (1986) using 200 µmol·m^{-2}·s^{-1} of actinic radiation at ambient CO_2 concentration. Apparent electron transport rate (*ETR*, µmol·m^{-2}·s^{-1}) was calculated as described by Genty *et al*. (1989).

Leaf area, leaf weight and iron content: After the above mentioned measures, leaves were sampled, weighed, and leaf area was assessed according to the gravimetric method of Sestàk *et al*. (1971). Leaf blades were then oven dried (70 °C, 3 d) and after wet digestion (H_2SO_4/H_2O_2) of the dry matter iron was analysed by atomic absorption spectrometry (Cottenie 1980).

Results and Discussion: The iron content of control and chlorotic leaves was similar for apical and medial leaves while basal chlorotic leaves had a lower iron content than control leaves. According to Häussling *et al*. (1985), only leaves of the same insertion (same physiological age) can be compared and considering a lower leaf number of chlorotic shoots due to growth inhibition, apical leaves of chlorotic shoots have to be compared with medial leaves of green shoots. Such a shift results in a lower Fe content in chlorotic leaves.

The leaf area, as well as fresh and dry matter of chlorotic plants were lower than those of control plants. The marked reduction of leaf dry matter in chlorotic plants was due to a reduction of leaf thickness and area indicating that cell division and expansion were significantly impaired assumably as a consequence of reduced CO_2 fixation.

In fact, chlorotic plants had lower rates of leaf net photosynthesis (*Pn*), stomatal conductance (*gs*) and transpiration (*E*) compared to the control; this confirms previous results with table grapes (Bavaresco and Poni 2003). A significant reduction of *Pn* was noticed in apical chlorotic leaves as compared to the control (-56 %), while *Pn* of medial and basal chlorotic leaves was reduced by 47 % and 30 %, re-

Correspondence to: Prof. Dr. L. Bavaresco, Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy. Fax: +39-0523-599-268. E-mail: luigi.bavaresco@unicatt.it

T a b l e

Morphological and physiological parameters of leaves as affected by chlorosis and shoot position. Mean values ± SE; n = 6

	Apical		Medial		Basal	
	Control	Chlorotic	Control	Chlorotic	Control	Chlorotic
Fe (μg·leaf $^{-1}$)	25 ± 1	27 ± 1	63 ± 4	60 ± 4	77 ± 4	57 ± 3
Leaf area (cm^2)	56 ± 2	34 ± 3	95 ± 10	68 ± 3	94 ± 6	77 ± 7
Fresh weight (g·leaf $^{-1}$)	1.04 ± 0.04	0.60 ± 0.08	1.76 ± 0.22	1.12 ± 0.08	1.60 ± 0.14	1.16 ± 0.18
Dry weight (g·leaf $^{-1}$)	0.35 ± 0.04	0.22 ± 0.04	0.59 ± 0.03	0.42 ± 0.03	0.52 ± 0.05	0.42 ± 0.05
Pn (μmol CO·m^{-2}·s^{-1})	9.45 ± 0.54	4.18 ± 0.27	10.26 ± 1.10	5.41 ± 0.54	8.23 ± 0.54	5.94 ± 0.41
E (mmol·m^{-2}·s^{-1})	2.34 ± 0.18	1.35 ± 0.18	2.40 ± 0.12	1.47 ± 0.12	2.11 ± 0.12	1.59 ± 0.24
gs (mmol·m^{-2}·s^{-1})	11.5 ± 1.5	7.6 ± 0.5	10.9 ± 1.3	7.9 ± 0.5	12.7 ± 1.5	10.2 ± 0.5
Fo	0.28 ± 0.01	0.27 ± 0.03	0.25 ± 0.01	0.28 ±0.02	0.29 ± 0.01	0.36 ± 0.01
Fm	1.22 ± 0.05	0.70 ± 0.14	1.20 ± 0.02	0.72 ± 0.04	1.24 ± 0.04	0.84 ± 0.14
Fv/Fm	0.76 ± 0.03	0.60 ± 0.03	0.79 ± 0.05	0.67 ± 0.02	0.79 ± 0.03	0.69 ± 0.03
qp	0.62 ± 0.04	0.41 ± 0.04	0.55 ± 0.03	0.39 ± 0.04	0.46 ± 0.03	0.38 ± 0.01
ETR (μmol·m^{-2}·s^{-1})	78 ± 4	37 ± 2	73 ± 3	42 ± 2	52 ± 2	32 ± 2

spectively. Decreased leaf *Pn* due to iron chlorosis was closely related to lower stomatal conductance and transpiration rates. Leaf *gs* of chlorotic plants was decreased by 42 %, 39 % and 25 % respectively for apical, medial and basal leaves. As expected leaf net photosynthetic rate, transpiration rate and stomatal conductance were positively related. In order to investigate whether the decreased *Pn* of chlorotic leaves was associated with changes of PSII activity and *Fv/Fm*, reflecting the quantum yield of PSII photochemistry, leaves were dark-adapted for 30 min by moving the plants to a dark room. Control leaves showed a high *Fv/Fm* ratio while the ratio was decreased for chlorotic leaves. The effect of iron chlorosis was prominent on variable fluorescence without altering *Fo* in apical leaves. This is characteristic for inhibition of the donor side of PSII in chlorotic leaves. In contrast, the *Fv/Fm* ratio of dark-adapted chlorotic leaves was always lower than that of control leaves, while a substantial (poor) increase of the *Fo* level was observed for chlorotic basal (medial) leaves, respectively. Iron chlorosis reduced the apparent electron transport (*ETR*) and photochemical quenching (*qp*).

The authors wish to thank Mr. G. BRUZZI (Univ. Cattolica lab crew) for his contribution to the project.

BAVARESCO, L.; PONI, S.; 2003: Effect of calcareous soil on photosynthesis rate, mineral nutrition, and source-sink ratio of table grape. J. Plant Nutr. **26**, 2123-2135.

BERTAMINI, M.; MUTHUCHELIAN, K.; NEDUNCHEZHIAN, N.; 2002: Iron deficiency induced changes on the donor side of PS II in field grown grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Plant Sci. **162**, 599-605.

COTTENIE, A.; 1980: Soil and Plant Testing as a Base of Fertilizer Recommendations. FAO Soils Bull. **38** (2). FAO, Roma, Italy.

GENTY, B.; BRIANTAIS, J. M.; BAKER, N. R.; 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87-92.

HÄUSSLING, M.; RÖMHELD, V.; MARSCHNER, H.; 1985: Beziehungen zwischen Chlorosegrad, Eisengehalten und Blattwachstum von Weinreben auf verschiedenen Standorten. Vitis **24,** 158-168.

MORALES, F.; BELKHOJA, R.L.; ABADIA, A.; ABADIA, J.; 2000: Photosystem II efficiency and mechanism of energy dissipation in iron-deficient, field grown pear trees (*Pyrus communis* L.). Photosynth. Res. **63**, 9-21.

NEDUNCHEZHIAN, N.; MORALES, F.; ABADIA, A.; ABADIA, J.; 1997: Decline in photosynthetic electron transport activity and changes in thylakoid protein pattern in field grown iron deficient Peach (*Prunus persica* L.). Plant Sci. **129**, 29-38.

SCHREIBER, U.; SCHLIWA, U.; BILGER, W.; 1986: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. **10**, 51-62.

SESTÀK, Z.; CATSKY, J.; JARVIS, P. G.; 1971: Plant Photosynthetic Production. Manual of Methods. Dr. W. Junk N.W. Publ. The Hague.

VAL, J.; MONGE, E.; HERAS, L.; ABADIA, J.; 1987: Changes in photosynthetic pigment composition in higher plants as affected by iron nutrition status. J. Plant Nutr. **10**, 995-1001.

Received April 4, 2005