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# Physiological aspects of lime-induced chlorosis in some *Vitis* species. I. Pot trial on calcareous soil

by

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S u m m a r y : One-year-old cuttings from eleven *Vitis* spp. (*V. aestivalis* Michx., *V. amurensis* Rupr., *V. andersonii* Rehder, *V. arizonica* Engelm., *V. berlandieri* Planch., *V. californica* Bentham, *V. champini* Planch., *V. cinerea* Engelm., *V. longii* Prince, *V. monticola* Buckl., *V. riparia* Michx.) were grown in pot of a calcareous soil. Leaves selected in the middle of the annual growing season were assayed for total chlorophyll, macronutrients, oligo-elements and ash alkalinity. At the end of the annual growing cycle the whole plants were analyzed for the iron concentration of the dry matter. The most significant findings were: a) *V. berlandieri, V champini* and *V. cinerea* ranked in the high tolerant group; *V. arizonica, V. californica, V. longii* and *V. monticola* in the tolerant group; *V. aestivalis, V. amurensis, V. andersonii* and *V. riparia* in the susceptible group; b) it is likely to exist two different mechanisms of tolerance to lime-induced chlorosis: an adaptive one for *V. berlandieri* and *V. cinerea* and a protective one for *V. champini*

K e y w o r d s : *Vitis* species, lime-induced chlorosis, breeding.

## Introduction

Lime-induced chlorosis affects yield and quality of grapevines growing in lots of calcareous areas world-wide. Breeding efforts to get tolerant rootstocks were successful, and these genotypes are available for grapegrowers who want to establish a commercial vineyard on calcareous soils (Pouget 1980; Fregoni 1980). The genetic variability of *Vitis* species regarding lime tolerance, has already been reported in the main ampelography treatises (Munson 1909; Viala and Vermorel 1910; Galet 1988), and the wild species were at first ranked by Chauzit in 1889 (quoted by Juste and Mench 1992). The main goal of this investigation is to study the physiological mechanisms involved in the tolerance to lime-induced chlorosis of some representative *Vitis* species, and to rearrange the given classification.

## Materials and methods

One-year-old grapevine cuttings from 11 *Vitis* spp. (*V. aestivalis* Michx., *V. amurensis* Rupr., *V. andersonii* Rehder, *V. arizonica* Engelm., *V. berlandieri* Planch., *V. californica* Bentham, *V. champini* Planch., *V. cinerea* Engelm., *V. longii* Prince, *V. monticola* Buckl., *V. riparia* Michx.) were grown in 10 l-pots of a calcareous soil. Before adding basic nutrients (1.5 g N/pot; 1 g $P_2O_5$/pot; 1.5 g $K_2O$/pot) the main soil characteristics were: texture sandy-silt; pH ($H_2O$) = 8.4; total carbonates = 64 %; active lime = 19 %; iron (extracted by $NH_4OAc$ + EDTA) = 137 ppm. The soil was maintained near field capacity by drip irrigation. Each species included 7 plants trained to two shoots and the pots were placed in the open on a platform with a hail-protection net. The shoot growth was checked every 10 d and the 3rd, 4th, 5th and 6th leaf (from the shoot tip) were sampled 75 d after bud-burst; the leaves were washed for 1 min in 1 % NaOCl solution, then rinsed for 3 min in tap water and analyzed as follows:

C h l o r o p h y l l s : Chlorophyll (Chl) a, b and total Chl were expressed in mg/100 g DW and mg/g FW. They were extracted from leaf discs by using 80 % acetone for 72 h in the dark, at 4 °C (Torrecillas *et al.* 1984). Chl concentrations were determined by reading absorbance at 665 and 649 nm and calculated using the equations given by Strain and Svec (1966). Each value is the mean of 3 replicates.

A s h a l k a l i n i t y : The method of Jungk (1968) was used. 0.25 g of oven-dried leaves (the remaining blade after the discs) were ground (< 1 mm) and ashed at 550 °C in a muffle-furnace for about 2 h till the ash got white. After cooling down, the ash was added with 15 ml of 0.1 N HCl and put inside a flask filled up to 100 ml with deionized water. A titration with 0.1 N NaOH followed, by using 0.1 % methyl orange as an indicator. Each value is the mean of 3 replicates.

M i n e r a l e l e m e n t s : N, P, K, Ca, Mg, B, Fe, Mn, Cu and Zn concentrations were assayed after wet destruction of the oven-dried leaves (the remaining blade after the discs). The following methods were used: flame photometry for K and Ca; AAS for Mg, Fe, Mn, Zn and Cu; colorimetry for total N, P and B. Each value is the mean of 3 replicates.

C h l o r o s i s r a t i n g : The scale of Pouget and Ottenwaelter (1978) was used, ranking 0-5 (no symptoms resp. severe chlorosis with >10 % of the blade surface with necrosis). At the end of the growing cycle (162 d after bud-burst) the rating was repeated (2nd chlorosis rating).

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At the end of the growing cycle, the whole plants were oven-dried, weighed and analyzed for their Fe content. The statistical analysis provided for a one-way-ANOVA, and the means were compared by the LSD test, at a 5 % level.

The data presented were recorded during the 2nd growing year, when chlorosis symptoms occur much more than in the first year (BAVARESCO *et al.* 1992 and 1993). *V. aestivalis* grew very little and therefore the data were not recorded.

## Results

The shoot growth is genotype-dependent (Fig. 1), ranking from *V. amurensis* (24 cm) to *V. berlandieri* (234 cm). *V. monticola, V. andersonii* and *V. riparia* developed lots of lateral shoots.

The Chl concentration (Tab. 1) ranked from 0.42 mg/g FW (*V. andersonii,* chlorotic) to 0.99 (*V. cinerea*, green) according to the visual chlorosis score (Tab. 2). *V. amurensis* and *V. riparia* were also chlorotic, while *V. champini* and *V. berlandieri* were within the green plants.

Ash alkalinity was affected by the genotypes in a significant way (Tab. 1) with *V. cinerea* ranking last and *V. champini* first (79 and 147 meq/100 g, resp.). The leaf Fe concentration ranged from 56 ppm (*V. arizonica*) to 95 (*V. amurensis*).

All macronutrient levels and ratios were affected in a significant way by the genotypes (Tab. 3), and the oligo-elements too, except for Cu.

The shoot and root dry matter/plant, checked at the end of the growing cycle, were strongly affected by the genotype, varying from *V. amurensis* (lowest values) to *V. berlandieri* (Fig. 2).

The total amount of Fe taken up by the shoots (including leaves) changed substantially depending on the genotype (Fig. 3), from 0.2 mg/plant (*V. amurensis*) to 19.8 (*V. californica*). Even the Fe efficiency ratio in the shoot was strongly affected by the genotype, ranging from 4.18 (*V. californica*) to 32.14 g DM/mg Fe (*V. champini*).

Table 1

Chlorophyll, ash alkalinity and iron in the leaves of the different genotypes

| | Tot. Chl mg/100 g DW | Tot. Chl mg/g FW | Chl a/Chl b | Ash alkalinity meq/100 g | Fe ppm |
|---|---|---|---|---|---|
| *V. amurensis* | 193 | 0.47 | 3.02 | 127 | 95 |
| *V. andersonii* | 188 | 0.42 | 3.72 | 129 | 92 |
| *V. arizonica* | 185 | 0.43 | 2.77 | 84 | 56 |
| *V. berlandieri* | 291 | 0.72 | 2.57 | 121 | 58 |
| *V. californica* | 287 | 0.64 | 2.70 | 100 | 58 |
| *V. champini* | 393 | 0.91 | 2.64 | 147 | 68 |
| *V. cinerea* | 375 | 0.99 | 2.80 | 79 | 77 |
| *V. longii* | 324 | 0.77 | 2.57 | 98 | 74 |
| *V. monticola* | 223 | 0.43 | 3.38 | 106 | 62 |
| *V. riparia* | 239 | 0.59 | 2.55 | 104 | 59 |
| LSD 0.05 | 37 | 0.09 | 0.25 | 7 | 11.7 |

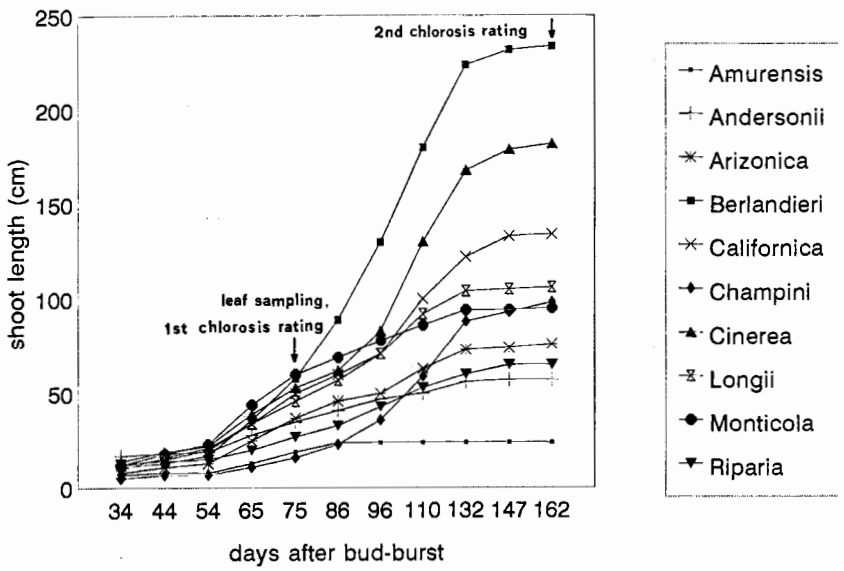


Fig. 1: Shoot growth depending on the genotype.

Table 2

Chlorosis rating of the different genotypes

| | *V. amurensis* | *V. andersonii* | *V. arizonica* | *V. berlandieri* | *V. californica* | *V. champini* | *V. cinerea* | *V. longii* | *V. monticola* | *V. riparia* | LSD 0.05 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st chlorosis rating* | 3.0 | 3.2 | 1.7 | 0.8 | 1.3 | 1.0 | 0.8 | 1.3 | 1.6 | 2.2 | 0.9 |
| 2nd chlorosis rating* | 4.7 | 3.7 | 0.7 | 0.7 | 1.1 | 0.0 | 0.0 | 2.1 | 0.9 | 4.1 | 0.9 |
| Mean* | 4.0 | 3.5 | 1.1 | 0.8 | 1.2 | 0.4 | 0.4 | 1.8 | 1.2 | 3.2 | 0.7 |

*: 0: none; 5: severe

Table 3

Macronutrient and oligo-element leaf (blade) concentration of the different genotypes (% and ppm on the DW basis)

| | N% | P% | K% | Ca% | Mg% | Mn ppm | Cu ppm | Zn ppm | B ppm | K/Ca | P/Fe |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *V. amurensis* | 2.64 | 0.27 | 1.41 | 1.23 | 0.38 | 47 | 10 | 29 | 24 | 1.27 | 28.6 |
| *V. andersonii* | 4.19 | 0.47 | 1.56 | 1.34 | 0.34 | 56 | 14 | 46 | 21 | 1.28 | 52.0 |
| *V. arizonica* | 1.88 | 0.28 | 1.50 | 1.56 | 0.48 | 13 | 8 | 23 | 20 | 0.96 | 49.3 |
| *V. berlandieri* | 2.25 | 0.31 | 1.71 | 1.32 | 0.35 | 28 | 11 | 24 | 15 | 1.30 | 55.1 |
| *V. californica* | 2.72 | 0.31 | 1.59 | 1.35 | 0.35 | 22 | 13 | 25 | 18 | 1.21 | 53.9 |
| *V. champini* | 2.71 | 0.21 | 1.27 | 1.68 | 0.28 | 49 | 10 | 26 | 10 | 0.75 | 31.7 |
| *V. cinerea* | 2.54 | 0.23 | 1.18 | 1.19 | 0.38 | 53 | 9 | 28 | 12 | 0.99 | 29.8 |
| *V. longii* | 2.91 | 0.29 | 1.66 | 1.36 | 0.42 | 33 | 11 | 26 | 14 | 1.24 | 39.0 |
| *V. monticola* | 3.27 | 0.33 | 1.63 | 1.17 | 0.35 | 31 | 11 | 26 | 16 | 1.39 | 53.8 |
| *V. riparia* | 4.04 | 0.42 | 1.33 | 1.32 | 0.33 | 26 | 10 | 28 | 15 | 1.01 | 70.9 |
| *LSD 0.05* | 0.37 | 0.08 | 0.25 | 0.39 | 0.10 | 5 | N.S. | 8 | 4 | 0.48 | 15 |

## Discussion

As was to be expected, the genetic variability of the *Vitis* species tested has been very large. The literature data regarding the known degree of tolerance to lime-induced chlorosis agree for most of the genotypes except for *V. champini*, which is given susceptible by GALET (1988) and tolerant by MUNSON (1909). *V. andersonii*, which is considered by GALET (1988) an hybrid of *V. riparia* x *V. coignetiae* is not classified for the lime response.

*V. berlandieri*, *V. champini* and *V. cinerea* performed best in terms of chlorosis score, with the average chlorosis rating < 1, while *V. amurensis*, *V. andersonii* and *V. riparia* performed worse, with the average chlorosis rating > 3.

According to MENGEL and GEURTZEN (1988), changes in alkalinity of plant tissue have an impact on Fe chlorosis; leaf ash alkalinity, in fact, is related to the apoplast pH (of the leaf), and increasing pH results in the precipitation of Fe (III) oxide hydrate, making Fe less available for Chl synthesis. According to BAVARESCO *et al.* (1993) the differ-

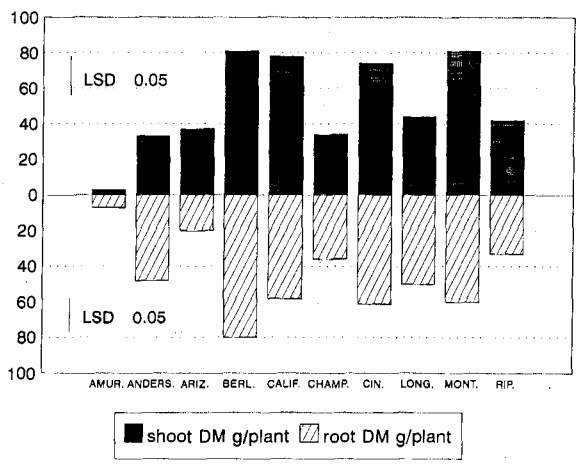


Fig. 2: Shoot and root dry matter of the different genotypes, at the end of the growing cycle.

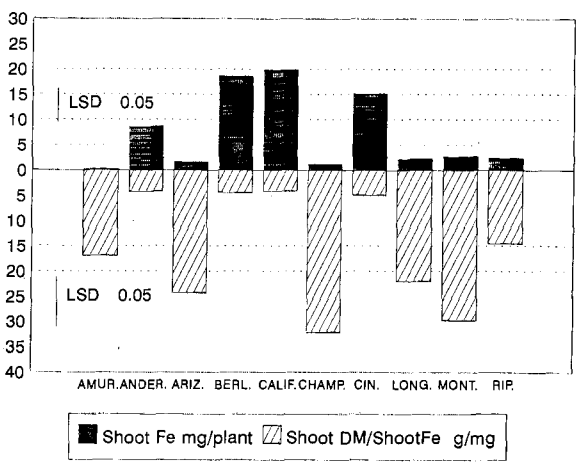


Fig. 3: Total Fe content of the shoots and iron efficiency ratio in the shoot of the different genotypes, at the end of the growing cycle.

ent degree of chlorosis occurrence or Pinot blanc vines with the same leaf Fe concentration was related to the ash alkalinity, which was higher in the more chlorotic vine. Even the cytoplasm pH was assessed by KOLESCH *et al.* (1987) to be related to the chlorosis occurrence of two grape rootstocks growing under bicarbonate stress. In the present study ash alkalinity proved not to be related to chlorosis occurrence or Fe concentration.

Fe concentration and leaf K/Ca ratio of each genotype were not related to chlorosis occurrence. The relationship between chlorosis symptoms and some mineral elements, observed by BAVARESCO (1991) in previous pot trials with the same scion (same leaf apparatus) grafted on different rootstocks and on different soils, does not occur with ungrafted genotypes (different leaf apparatus). It is likely for every species to have its own genetic control of mineral nutrition in terms of nutritional requirements, not related in the same way to Chl metabolism. In other words it does not seem suitable to discriminate the genotypes (for lime tolerance) by their nutritional status, because, for instance, the Fe concentration and the K/Ca ratio have a large range of variation even within the susceptible genotypes.

The susceptibility of some tested species (*V. aestivalis, V. amurensis, V. andersonii, V. riparia*) was better stressed by the very poor growth, while the most tolerant species showed high vigour except for *V. champini.*

It is interesting to point out the different behaviour of the tolerant species. On the one hand *V. berlandieri* and *V. cinerea* seem to have a kind of adaptive mechanism, by growing intensely and by developing a large root system able to take up high Fe quantities. On the other hand *V. champini* shows some kind of protective mechanism, by growing slowly and developing a weak root system, and by producing a lot of dry matter per unit of Fe utilized at a shoot level.

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