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Research Note

Physiological aspects of lime-induced chlorosis in some *Vitis* species. II. Genotype response to stress conditions

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Summary: One-year-old cuttings from 7 *Vitis* species (*V. amurensis* Rupr., *V. berlandieri* Planch., *V. californica* Bentham, *V. cinerea* Engelm., *V. longii* Prince, *V. monticola* Buckl., *V. riparia* Michx.) were grown in pots containing non-calcareous and calcareous soil. Leaves selected in the middle of the second year's growing season were assayed to test the iron concentration of the dry matter and the total iron uptake. The most significant findings of the trial were: a) shoot growth of *V. amurensis*, *V. longii*, *V. monticola* and *V. riparia* was strongly depressed by the calcareous soil; b) *V. berlandieri* and *V. californica* took up higher amounts of iron when growing in the calcareous soil; c) a high ash alkalinity occurred in chlorotic leaves of *V. riparia* compared to non-chlorotic leaves at the same iron concentration.

Key words: Vitis species, lime-induced chlorosis, ash alkalinity, iron.

Introduction: Some physiological parameters of 10 *Vitis* species growing in calcareous soil have been reported in the first part of this paper (Bavaresco *et al.* 1994). The species behaved according to the degree of tolerance/susceptibility to lime-induced chlorosis as indicated by the main ampelographic treatises, except for *V. champinii*, which is considered to be susceptible by Galet (1988), while it behaved like tolerant. This paper deals with seven species out of the above mentioned 10, comparing their behaviour in two different soils.

Materials and methods: One-year-old grapevine cuttings from the *Vitis* species *V. amurensis* Rupr., *V. berlandieri* Planch., *V. californica* Bentham., *V. cinerea* Engelm., *V. longii* Prince, *V. mon-ticola* Buckl., *V. riparia* Michx. were grown in 10-l-pots containing non-calcareous and calcareous soil. The non-calcareous soil was prepared by mixing one part of peat, one part of soil and two parts of sand, while the calcareous soil was collected from the field: pH (H_2O) = 8.4; 64 % total carbonates; 19 % active lime. Soil moisture was maintained near field capacity by drip irrigation. 14 plants of each species (7 plants for each soil) were trained to two shoots and the pots were placed open-air at a platform with a hail protection net. Young leaves (3rd to 6th leaf from the shoot tip) were sampled 75 d after bud burst; the leaves were washed for 1 min in a 1 % NaOCl solution, then rinsed for 3 min in tap water and analysed as follows.

Chlorophylls: Chlorophyll (Chl) a, b and total Chl were extracted from leaf discs by using 80 % acetone for 72 h in the dark at 4 °C (Torrecillas *et al.* 1984). The Chl concentrations were determined by reading the absorbance at 665 and 649 nm and calculated using the equations given by Strain and Svec (1966).

Ash alkalinity: Ash alkalinity was assayed by the method of Jungk (1968). 0.25 g of oven-dried leaves (the remaining blades) 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, 15 ml of 0.1 N HCl was added to the ash, this was transferred into a flask and filled up to 100 ml with deionized water. A titratation with 0.1 N NaOH followed using 0.1 % methyl orange as indicator.

Iron concentration: The concentration of Fe was assayed after wet destruction of the oven-dried leaves (the remaining blades), using atomic absorption spectrometry.

Chlorosis rating: The scale of Pouget and Ottenwaelter (1978) was utilized, ranking from 0 (no symptoms) to 5 (severe chlorosis, with more than 10 % of the leaf blade being necrotic). At the end of the growing cycle (162 d after bud burst) the rating was repeated.

At the end of the growing cycle the aerial parts of the plants were oven-dried, weighed and the iron concentration was analysed.

Statistical analysis: One-way-ANOVA was applied comparing the behaviour of the genotypes growing in the two soils.The data were recorded in the 2nd year, when the chlorosis symptoms were more distinct (Bavaresco *et al.* 1992 and 1993 b).

Results: The shoot dry matter (DM) was depressed significantly by the calcareous soil except for *V. californica* (Table). *V. amurensis*, *V. longii*, *V. riparia*, *V. monticola* showed the strongest effects. The calcareous soil depressed the leaf chlorophyll concentration of each genotype significantly, except for *V. californica* and *V. cinerea*.

Ash alkalinity of all species, save *V. cinerea*, was higher in the calcareous soil (Table) and this parameter was related negatively to the Chl concentration (Figure).

The iron concentration was affected significantly by the soil type only in leaves of *V. amurensis*, *V. berlandieri* and *V. longii* (Table), with higher values in the calcareous soil for *V. amurensis* and *V. longii*.

In the most susceptible species (*V. amurensis* and *V. riparia*) the content of iron strongly decreased when growing on the calcareous soil (Table), while *V. berlandieri* and *V. californica* took up more iron under the same stress conditions.

Discussion: The responses of the species to calcareous soil varied strongly. The most susceptible species, *V. amurensis* and *V. riparia*, have been very impaired by the calcareous soil; they produced 91 % and 63 % less shoot dry matter. On the other hand the shoot dry matter of the tolerant species was decreased only by 35 % (*V. berlandieri*) and 41 % (*V. cinerea*). The different behaviour of potted *V. riparia* and *V. berlandieri* with respect to iron chlorosis is related to modifications of some physiological parameters at the root level, according to Brancadoro *et al.* (1995). *V. riparia* showed chlorosis also when cultured *in vitro* on a medium containing high bicarbonate concen-

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Table

Percentage of variation of some leaf and shoot parameters of plants growing on calcareous versus non-calcareous (=100 %) soil (DM: dry matter; Chl: chlorophyll)

	V. amurensis	*V. berlandieri*	*V. californica*	*V. cinerea*	*V. longii*	*V. monticola*	*V. riparia*
Shoot DM g/plant	-91% **	-35% **	+5%	-41% **	-65% **	-49% **	-63% **
Tot. Chl mg/100g DM	-19% *	-28% **	-10%	-7%	-12% *	-47% **	-45% **
Ash Alkalinity meq/100g	+31% **	+29% **	+28% **	+7%	+18% **	+19% **	+51% **
Fe (leaf) ppm	+61% **	-18% **	-2%	+0%	+30% **	-7%	+7%
Shoot Fe mg/plant	-98% **	+46% *	+43% *	-25% *	-89% **	-90% **	-88% **

* statistical difference ($p \leq 0.05$) between the two soils
** statistical difference ($p \leq 0.01$) between the two soils

tration (BAVARESCO *et al.* 1993 b). It is interesting to note that the medium-tolerant species *V. californica* increased its shoot dry matter by 5 % when growing in calcareous soil.

The chlorophyll concentration of the leaves of the two susceptible species (*V. riparia* and *V. amurensis*) did not decrease under stress conditions to the same extent. In fact, *V. riparia* had the highest chlorophyll concentration when growing in the non-calcareous soil, while *V. amurensis* even under normal soil conditions was not able to synthesize as much chlorophyll.

A parameter which emphasizes the differential response of the genotypes to stress conditions was the shoot iron content or, in other words, the capability of the plant to take up iron from the soil. *V. berlandieri* showed a high degree of tolerance by taking up 46 % more iron when growing on calcareous soil, while *V. cinerea* took up 25 % less iron. *V. riparia* and *V. amurensis* showed susceptibility by taking up 88 % and 98 % less iron, respectively. Thus, the iron content of the shoots was stronger related to the occurrence of chlorosis than the leaf iron concentration.

Ash alkalinity seems to be responsible for different chlorophyll concentrations at the same iron level, as was already observed in a pot experiment with grafted plants (BAVARESCO *et al.* 1993 a). In fact, *V. riparia* had the same iron concentration in both soils, but different chlorophyll and alkalinity levels. The plants showing chlorosis symptoms had a higher ash alkalinity than the healthy, green ones. The ash alkalinity of leaves is related to the pH of the apoplast, increasing pH values of the leaf apoplast inducing a precipitation of Fe (III) oxide hydrate (MENGEL and GEURTZEN 1988). This may lead to a decrease of the iron uptake from the leaf apoplast into the mesophyll cells, resulting in decreased chlorophyll synthesis and chlorosis, while at the same time the iron concentration of the leaf does not change.

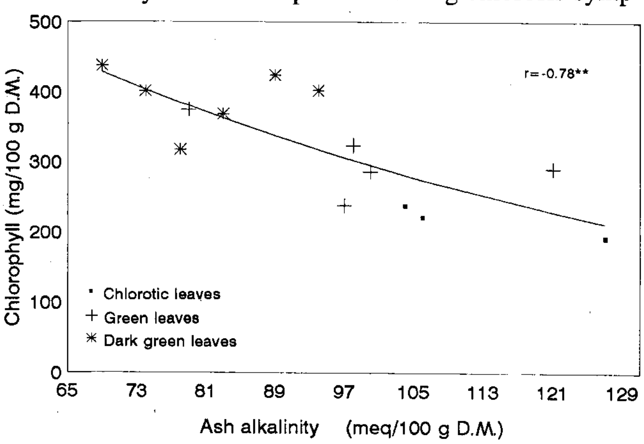

Figure: Correlation between leaf chlorophyll concentration and ash alkalinity.

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