Lime-induced chlorosis of grapevine as affected by rootstock and root infection with arbuscular mycorrhiza and Pseudomonas fluorescens

by

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Summary: Grapevine cuttings (Vitis vinifera L. cv. Pinot blanc, clone VCR 5), grafted on 3309 C, a lime-susceptible rootstock, SO 4, a medium lime-tolerant rootstock and 41 B, a lime-tolerant rootstock, were grown in pots containing unsterilized calcareous soil. Before potting, the roots of the grafted plants were inoculated with a suspension of a mutant of Pseudomonas fluorescens and with the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus mossea (Nicol. & Gerd.) Gerd. & Trappe, in order to investigate the effect of these microorganisms on the severity of lime-induced chlorosis. The most significant findings were: a) Pseudomonas fluorescens and Glomus mossea treatments increased Fe and chlorophyll concentrations in the leaves and thus lime tolerance in plants grafted on 3309 C and 41 B; b) the positive effect of VAM treatment was associated with increased levels of root infection and lower root growth; c) the bacterial treatment improved the establishment of VAM fungi; d) fruit quality of the susceptible graft combination raised to the level of untreated Pinot blanc/SO 4 vines due to the root treatments.

Key words: iron chlorosis, grapevine, rootstock, Pseudomonas fluorescens, Glomus mossea, VAM fungi.

Introduction

Vitis species differ in their degree of susceptibility to lime-induced chlorosis (e.g. BAVARESCO et al. 1994). Vitis vinifera L. is ranked as lime-tolerant, but with a range of variation within the varieties (BRANAS 1974). In modern viticulture, V. vinifera is normally grafted on interspecific rootstocks, with a wide range of adaptation to adverse soil conditions. Rootstock tolerance to increasing levels of lime and IPC (index of chlorosis power) has been reported by GALET (1947), quoted by JUSTE and MENCH (1992), and POUGET and JUSTE (1972); resistance to iron-chlorosis is referred to "Strategy I" response mechanisms at the root level (MARSCHNER 1978; BAVARESCO et al.1991; BRANCADORO et al. 1995).

Grapevines growing on calcareous soils can benefit from application with vesicular-arbuscular mycorrhiza (VAM) fungi and with siderophore-producing pseudomonads (BAVARESCO and FOGHER 1992, 1996a and b; BAVARESCO et al. 1995). VAM fungi affect the growth of responsive plants in different ways; the most important is by increasing the uptake of some nutrients, especially phosphorus, in soils with low nutrient level (GEBBING et al. 1977; BOLAN 1991; Li et al. 1991; KOTHARI et al. 1991; GEORGE et al. 1994; KARAGIANNIDIS et al. 1995), due to a mycorrhizal phosphate-uptake transporter (HARRISON and VON BUREN 1995). Other causes for improved growth are changes of the hormonal balance, of the plant water relations and the suppression of root pathogens (MARSCHNER 1995; MORANDI et al. 1984).

The plant growth-promoting activity of fluorescent pseudomonads in the rhizosphere is well documented (KLOEPPEL and SCHROTH 1981); it is related to many factors, including the ability to produce plant hormones such as IAA (DUBEIKOVSKI et al. 1993), the production of hydroxamate siderophores under iron stress conditions (BARKER et al. 1988), the lower availability of iron for pathogenic microorganisms (KLOEPPEL et al. 1980) and the supply of iron to the plant roots (REIO et al. 1986). Recent results of BAR-NESS et al. (1992) and WALTER et al. (1994) do not fully support this latter statement. Other results (WASCHIES et al. 1994) suggest a direct or indirect role of fluorescent pseudomonads in the "replant disease" of grapevine. It is therefore evident that lime tolerance of field-grown plants, including grapevine, is not only related to the plant genome but also to its interaction with rhizosphere microorganisms (MOZAFAR et al. 1992).

Materials and methods

Cultivation of the grapes: Vitis vinifera cv. Pinot blanc, clone VCR 5, was grafted on different rootstock, inducing an increasing degree of lime tolerance: 3309 C (V. riparia Michx. X V. rupestris Scheele), SO 4 (V. berlandieri Planch. X V. riparia Michx.) and 41 B (V. vinifera L. X V. berlandieri Planch.). The plants were grown in pots (9 l) with calcareous soil collected in a vineyard a few days before. The main soil characteristics were: sandy-silt texture; pH 8.9; carbonates 80 %; active lime 32 %; organic matter 0.19 %; P 6 mg kg\(^{-1}\) soil (Olsen method); IPC (indice de pouvoir chlorosant or chlorosis index, according to POUGET and JUSTE 1972) 45. 3309 C and SO 4 rootstocks are referred to be susceptible to lime, while 41 B is ranked tolerant (FREGONI 1980). Before potting, the roots of 6 plants per graft combination were infected by a suspension of a mutant of Pseudomonas fluorescens, and by an inoculum of the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus mossea (Nicol. &
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Gerd. & Trappe; in addition 6 uninoculated (control) plants per rootstock were cultivated. The soil was not sterilized before the experiment in order to simulate field conditions and was maintained near field capacity by drip irrigation. The plants were placed outdoor on a platform with a hail protection net and were trained to two cluster-bearing shoots. The experiment lasted two years and data reported in this paper were recorded in the second year when chlorosis symptoms were more evident, according to previous pot trials (Bavaresco et al. 1993). In the second year, 30 d after bud burst the plants were with 2 g N/pot as calcium nitrate, 0.44 g P/pot as liquid fertilizer, 2.5 g K/pot as potassium nitrate and trace elements without iron.

Preparation of the bacterial inoculum: Strain EM4 RS of Pseudomonas fluorescens, a spontaneous rifampicin- and streptomycin- resistant mutant producing fluorescent pigments, was isolated from roots of maize as used for the experiment by Polinelii et al. (1991). The strain was grown on a nutrient medium (Vitade 1967) at 28 °C for 48 h to a density of about 9.5 x 10^9 colony forming units (CFU)/ml. The root system of the plant to be inoculated was dipped in the bacterial suspension for a few seconds just before potting.

Preparation of the mycorrhizal inoculum: The stock culture was prepared by mixing sterilized soil, sand and peat (1:1:1), the pH corrected to 6.8 by CaCO3. Inoculation was done with isolated spores of Glomus mosseae (original supply: Dr. J. M. Barea, Estacion Experimental del Zaidin, Granada, Spain; Fogher et al. 1986). Spores, mycelium and infected root fragments from stock culture on tomato were used as inoculum, adding ca. 60 ml of culture for each pot underneath the roots.

Leaf sampling: The 3rd and 4th leaf (from the shoot tip) were sampled 40 and 110 d after bud burst (1st and 3rd sampling respectively), while leaves of the same age (20 d after unfolding) were sampled 70 d after bud burst, i.e. at fruit set (2nd sampling). The latter leaves were not located at the same position along the shoot because of different growth rates induced by the rootstocks, but they were all located at the median third of the shoot: one leaf per shoot was sampled.

Chlorophyll: The young leaves sampled at the 1st and 3rd date were analyzed for chlorophyll (Chl) content using the portable Chlorophyll Meter SPAD 502 (Minolta). SPAD units were transformed into Chl values (mg/g DW) using the equation given by Bavaresco (1995).

Mineral elements: After wet digestion macronutrients (N, P, K, Ca and Mg) and some trace elements (Fe, Mn, Zn and B) were analyzed in leaves of the 2nd sampling by flame photometry (K and Ca), atomic absorption spectrometry (Mg, Fe, Mn and Zn) and colorimetry (total N, P and B).

Berry sampling: At berry ripening, 140 d after bud burst, clusters were sampled and berry juice was analyzed for soluble solids (%)Brix).

Root dry weight: At the end of the experiment (in October) the soil was washed off the roots, and the roots of three plants/treatment were oven-dried for 48 h at 80 °C and weighed.

Figure: Shoot growth of Pinot blanc vines as affected by rootstock variety and treatment (LSD only for the final lengths).

Mycorrhizal infection: Before drying, thin lateral roots (φ < 1 mm), cut into ca. 2 cm segments, were analyzed for VA mycorrhizal infection by the slide length method of Giovannielli and Mosse (1980). After initial cleaning in 10% KOH they were stained with trypan blue in acidic glycerol solution, according to Waschkies et al. (1994).
Rhizosphere pseudomonad survey: Soil of the rhizosphere was collected at the end of the experiment and pseudomonad cells were counted on nutrient medium KMB at pH 7.2, after 48 h at 37°C (King et al. 1954). In order to isolate the *P. fluorescens* strain EM4 RS, the KMB medium was added with 50 mg/l rifampicin and 100 mg/l streptomycin. The colony forming units (CFU) were calculated on the basis of 1 g of soil dried overnight at room temperature.

Statistical analysis: A two-way ANOVA with interaction was used, the means were compared by using the LSD test (5 % level). Percent mycorrhizal colonization data were submitted to angular transformation.

Results

Shoot growth of Pinot blanc vines was lowest with the lime-tolerant rootstock 41 B and highest with the lime-susceptible rootstock 3309 C. In tendency, mycorrhizal inoculation increased shoot growth with all three rootstock combinations, but significantly for plants on SO 4 and 41 B rootstocks (Figure).

Root dry weight of control plants was not significantly different between rootstocks (Tab. 1). Root systems of 3309 C and 41 B were reduced by the mycorrhizal inoculum by 21 and 40 %, resp. Morphological changes of the roots were not investigated. The natural VAM infection of uninoculated plants increased, according to the lime tolerance of the rootstock genotypes, from 3309 C to 41 B. The mycorrhizal inoculation increased root infection of 3309 C and 41 B, but not of SO 4. The *Pseudomonas* treatment had a positive effect on VAM infection of all rootstocks.

No *P. fluorescens* colony was found in the microbiological survey, but other *Pseudomonas* spp. occurred. The EM4 RS strain added as inoculum was not recovered either. The data presented in Tab. 1 therefore show no maintenance of *P. fluorescens* inoculation, at least in the investigated rhizosphere under our experimental conditions.

The chlorophyll content was lowest in plants grafted to the lime-susceptible rootstock 3309 C, in particular at the 3rd sampling date, and highest in plants on the lime-tolerant rootstock 41 B (Tab. 2). An inoculation either with *P. fluorescens* or *G. mosseae* resulted in significantly higher chlorophyll concentrations in plants on both rootstocks 41 B and 3309 C, while with 3309 C no plant regreening occurred. No significant effects were observed with the vines grafted on SO 4.

Potassium, manganese, zinc and boron were not significantly affected by the treatments (Tab. 3). Corresponding to the enhanced chlorophyll content due to inoculation with *P. fluorescens* and *G. mosseae* also the Fe concentrations were enhanced, in particular in plants grafted on the rootstock 3309 C. The same was true for phosphorus. It is interesting to note that plants on the rootstock SO 4 had a much higher Ca content in the shoots (+40-50 %) than plants on the other rootstocks.

Table 1
Effect of rootstock and root treatment on root dry weight, root VAM infection, rhizosphere *Pseudomonas* and soluble solids of the fruit at the end of the vegetation period

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Pinot b./3309 C</th>
<th>Pinot b./SO4</th>
<th>Pinot b./41 B</th>
<th>LSD (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>P. fluorescens</td>
<td>G. mosseae</td>
<td>P. fluorescens</td>
<td>G. mosseae</td>
</tr>
<tr>
<td>Root dry weight</td>
<td>53</td>
<td>59</td>
<td>42</td>
<td>51</td>
</tr>
<tr>
<td>Root VAM infection (%)</td>
<td>12.0</td>
<td>20.3</td>
<td>37.9</td>
<td>14.5</td>
</tr>
<tr>
<td>Rhizosphere <em>Pseudomonas</em> (CFU x 10^9/g soil)</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>Fruit soluble solids (°Brix)</td>
<td>20.9</td>
<td>23.3</td>
<td>24.1</td>
<td>23.3</td>
</tr>
</tbody>
</table>

Table 2
Chlorophyll (mg/g DW) of leaves as affected by rootstock and root treatment

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Pinot b./3309 C</th>
<th>Pinot b./SO4</th>
<th>Pinot b./41 B</th>
<th>LSD (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>P. fluorescens</td>
<td>G. mosseae</td>
<td>P. fluorescens</td>
<td>G. mosseae</td>
</tr>
<tr>
<td>1st sampling</td>
<td>1.77</td>
<td>2.17</td>
<td>2.01</td>
<td>2.14</td>
</tr>
<tr>
<td>2nd sampling</td>
<td>1.06</td>
<td>1.99</td>
<td>1.59</td>
<td>2.43</td>
</tr>
</tbody>
</table>
Corresponding to the different chlorophyll content in the shoot, plants on the most lime-tolerant rootstock (41 B) had the highest level of soluble solids in the berries while plants on the most lime-susceptible rootstock (3309 C) had the lowest. But in the latter plants, treatments with *P. fluorescens* and *G. mosseae* caused the highest increase of soluble solids in the berries (Tab. 1).

### Discussion

Shoot growth was strongly affected by the rootstock genotype and, to a lower extent, by root treatments. The lime tolerance of Pinot blanc/41 B is related to a slow vegetative growth; it seems that the plants under stress conditions reduce growth of vegetative organs which have to be supplied with nutrients, while sugar accumulation in the fruit is increased. The positive effect of mycorrhizal treatment on plant growth confirms previous findings with grapevine (*Schubert et al. 1988; Lovato et al. 1992; Karagiannidis et al. 1995; Bavaresco and Fogher 1996a*) and on other species (*Moss et al. 1969; Fortuna et al. 1992*). Root growth did not differ among genotypes, but was depressed by the mycorrhizal treatment in 3309 C and 41 B. In these two rootstocks the mycorrhizal infection increased significantly compared to the untreated plants. According to Marschner (1995), mycorrhization depresses root growth by factors like sink competition and also, assuming that root weight is proportional to root length, by full compensation of the external mycelium of the mycorrhizal fungi for the root’s function, i.e. the uptake of nutrients and water. Another explanation is that mycorrhizal root systems decay faster than non-mycorrhizal roots (*Mossse 1956, quoted by Rajapakse and Miller 1987*). In the case of SO 4, on the other hand, root growth between mycorrhizal and control plants was not different and no significant differences in the degree of VAM infection were recorded. The effect of *Pseudomonas* treatment on root growth was not significant, in contrast to previous results of Bavaresco and Fogher (1992 and 1996b) and Bavaresco et al. (1995) indicating differences due to the rootstock genotype. In fact, microorganisms can stimulate, inhibit, or can be without effect on root growth, depending on many factors including the genotype (*Dubekovsky et al. 1993; Marschner 1995*).

Both, rootstock genotype and root treatment affected the chlorophyll content of young leaves; the rootstocks behaved according to their known degree of lime tolerance and chlorosis symptoms of the susceptible rootstock/plant combination (Pinot blanc/3309 C) increased during the vegetation period, as has been reported earlier (Bavaresco et al. 1993; Bavaresco and Fogher 1996b). In a late stage of growth the effect of the root infection on plants grafted on 3309 C was evident: reduction of the severity of chlorosis without regreening and higher rate of Fe uptake. Nevertheless, the partial recovery from chlorosis was sufficient to enhance grape ripening and to increase sugar concentration in berries (+11 %) for *Pseudomonas* treatment, and +15 % for the mycorrhizal treatment. The lack of a positive effect of the VAM treatment on plants grafted on SO 4 may be due to a failure in establishing artificial root infection. There may exist a positive relationship between the extent of root colonization after VAM infection and the level of chlorophyll in leaves; furthermore it is confirmed by the correlation between the degree of the natural VAM infection of the rootstocks and their lime tolerance (Bavaresco et al. 1995).

Root VAM infection was increased by the *Pseudomonas* treatment, too: in fact, microorganisms may improve the establishment of VAM fungi under field conditions (*Schönbeck and Raschen 1995*). VAM inoculation may have increased chlorosis resistance of the susceptible graft combination by improving the nutritional status of the stressed plants, especially for N, P, K and Fe, while *Pseudomonas* infection may have played an indirect role, or a direct one by siderophore production (not investigated in this experiment).

The failure in *P. fluorescens* EM4 RS recovering from the rhizosphere may be due to an unsuccessful establishment of microorganisms under our experimental conditions,

<table>
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<th>Table 3</th>
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Effect of root treatments on leaf mineral composition and dry matter, 2nd sampling

<table>
<thead>
<tr>
<th></th>
<th>Pinot b./3309 C</th>
<th>Pinot b./SO4</th>
<th>Pinot b./41B</th>
<th>LSD (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N (%)</td>
<td>P (%)</td>
<td>K (%)</td>
<td>Ca (%)</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>2.41</td>
<td>0.25</td>
<td>1.47</td>
<td>0.49</td>
</tr>
<tr>
<td>G. mosseae</td>
<td>2.72</td>
<td>0.34</td>
<td>1.51</td>
<td>0.56</td>
</tr>
<tr>
<td>Control</td>
<td>N (%)</td>
<td>P (%)</td>
<td>K (%)</td>
<td>Ca (%)</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>2.44</td>
<td>0.27</td>
<td>1.53</td>
<td>0.67</td>
</tr>
<tr>
<td>G. mosseae</td>
<td>2.62</td>
<td>0.35</td>
<td>1.60</td>
<td>0.62</td>
</tr>
<tr>
<td>Control</td>
<td>N (%)</td>
<td>P (%)</td>
<td>K (%)</td>
<td>Ca (%)</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>2.68</td>
<td>0.30</td>
<td>1.55</td>
<td>0.65</td>
</tr>
<tr>
<td>G. mosseae</td>
<td>2.65</td>
<td>0.45</td>
<td>1.53</td>
<td>0.45</td>
</tr>
</tbody>
</table>

LSD (Least Significant Difference)
Chlorosis affected by rootstocks, mycorrhiza and pseudomonads

or to a complete endophytic habitus, which can not be identified in a rhizosphere survey. The second hypothesis is more likely, since an appreciable effect of the infection was evident.

Acknowledgements

The authors want to thank G. BRUZZI (viticultural lab crew) and M. DI PARDO (graduate student) for their contribution to this project.

References


Received April 18, 1996