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Reduced levels of calcium and other mineral elements in leaves of grapevines infected with Bois noir

Wolfgang Schweigkofler, Anna Cassar and Elmar Stimpfl

Research Centre for Agriculture and Forestry Laimburg
I-39040 Auer/Ora
E-Mail: Wolfgang.Schweigkofler@provinz.bz.it

The concentrations of seven mineral elements (Ca, K, N, Mg, Mn, Fe and P) were analyzed in leaves of healthy grapevines and of grapevines infected with Bois noir (BN). The levels of calcium of all five varieties used in the study ('Chardonnay', 'Müller Thurgau', 'Pinot Noir', 'Lagrein' and 'Zweigelt') were significantly lower in leaves infected with BN. The other six elements also showed a tendency towards decreased contents, but the effects were less consistent. A distinctive varietal influence on both visual symptoms of BN and mineral contents was observed, 'Chardonnay' and 'Zweigelt' being the most susceptible cultivars.
Keywords: Grapevine yellows, Bois noir, minerals, physiology, *Vitis vinifera*

Reduzierte Gehalte von Calcium und anderen Mineralstoffen in mit Schwarzholzkrankheit (Bois Noir) infizierten Traubenblättern. *Die Konzentrationen von sieben Mineralstoffen (Ca, K, N, Mg, Mn, Fe und P) wurden sowohl in Blättern von gesunden als auch in Blättern von mit der Schwarzholzkrankheit (Bois Noir) infizierten Rebstöcken analysiert. Die Calciumgehalte aller fünf in dieser Studie verwendeten Sorten ('Chardonnay', 'Müller Thurgau', 'Pinot Noir', 'Lagrein' und 'Zweigelt') waren in mit Bois Noir infizierten Blättern deutlich reduziert. Auch die anderen sechs Mineralstoffe zeigten einen deutlichen Trend zu reduzierten, jedoch weniger beständig niedrigeren Gehalten. Ein deutlicher Einfluss der Sorte auf sowohl den visuellen Eindruck als auch die Gehalte an Mineralstoffen wurde beobachtet, wobei sich 'Chardonnay' und 'Zweigelt' als die empfindlichsten Sorten herausstellten.*
Schlagwörter: Vergilbungskrankheiten, Bois Noir, Mineralstoffe, Physiologie, *Vitis vinifera*

Les niveaux réduits de calcium et d'autres substances minérales dans les feuilles de vignes infectées par le bois noir. *Les concentrations de sept éléments minéraux (Ca, K, N, Mg, Mn, Fe and P) ont été analysées dans les feuilles de vignes saines et de vignes infectées par le bois noir (BN). Les teneurs en calcium de toutes les cinq variétés étudiées ('Chardonnay', 'Müller Thurgau', 'Pinot Noir', 'Lagrein' et 'Zweigelt') étaient significativement plus basses dans les feuilles infectées par BN. Les autres six éléments montraient également une tendance vers des contenus réduits, mais les effets étaient moins consistants. Une influence distincte des variétés, tant sur les symptômes visuels du BN que sur les teneurs en minéraux, a été observée, 'Chardonnay' et 'Zweigelt' étant les cépages les plus sensibles.*
Mots clés: Jaunisses de la vigne, bois noir, substances minérales, physiologie, *Vitis vinifera*

Bois noir (BN) is a grapevine yellows disease caused by phytoplasmas of the Stolbur-group (16SrXII-A) (proposed name: *Ca. Phytoplasma solani*). The disease is widespread in different wine growing areas in Southern and Central Europe and can cause significant economic losses. Typical symptoms include yellowing and downward curling of leaves, stunted shoots, and reduced ripening of the grapes, making them unsuitable for quality wine production. The same symptoms appear on grapevines suffering from Flavenscence dorée (FD), caused by phytoplasmas of the elm-yellows-group (16SrV-C, -D) (proposed name: *Ca. Phytoplasma vitis*). The two diseases differ by their vector insects: the polyphagous *Hyalesthes obsoletus* Signoret (Cixiidae) in the case of BN (Maixner, 1994), and the strictly monophagous *Scaphoideus titanus* Ball (Cicadellidae) in the case of FD (Schvester et al., 1961).
Relatively little is known about the pathogenesis of BN. Phytoplasma growth is limited to the sieve tubes of the phloem, which can be physically blocked when

Table 1. Origin of analyzed grapevine leaves

Variety	Geographic area	Elevation (masl)	Pruning system	Soil type	BN infection rate 2007 (%)	Drip irrigation
Chardonnay	Unterland	230	Guyot	silicate	1.7	yes
Müller-Thurgau	Eisacktal	700	Guyot	silicate	7.3	yes
Pinot Noir	Meran	320	Guyot	silicate	15.6	yes
Lagrein	Unterland	300	Guyot	calcareous	2.9	yes
Zweigelt	Eisacktal	650	Guyot	silicate	8.9	no

the pathogen titers are very high (CHRISTENSEN et al., 2005). Low phytoplasma concentrations and more indirect effects, however, seem to be typical for many phytoplasma diseases (SIDDIQUE et al., 1998). Inhibition of photosynthetic activities was shown in leaves of *Vitis vinifera* cv. 'Chardonnay' (BERTAMINI et al., 2002); alterations of phytohormon levels, enzymatic reactions (e.g. of the peroxidases) and phytotoxin activity have been discussed for a number of phytoplasma diseases, but no data are available for grapevine.

The leaf symptoms of white and red varieties of *V. vinifera* infested by the BN phytoplasma show some similarities to those caused by some mineral element deficiencies (e.g. chlorosis, reddening, curling, patchy necrosis). In order to determine if these visual similarities could be corroborated by analytical data, contents of seven elements (Ca, K, Mg, Mn, N, P and Fe) were determined in healthy and BN-infected field-grown grapevines. The varieties investigated were 'Chardonnay', 'Müller Thurgau', 'Pinot Noir', 'Lagrein', and 'Zweigelt', all vines came from vineyards in South Tyrol (Northern Italy).

Material and methods

Leaves from field grown grapevines showing severe symptoms of BN were sampled in South Tyrol's grape-growing areas in late September 2007. Leaves from neighboring healthy grapevines were used as a control. Four to ten samples of each of the five varieties were taken from infected and healthy grapevines. Each sample included six to eight leaves of similar size and development stage from each grapevine. Additionally, leaves from 'Chardonnay' and 'Pinot Noir' grapevines were sampled at monthly intervals from the end of May till the end of September to study the dynamics of mineral changes. The presence of the BN phytoplasma in selected symptomatic grapevines was confirmed by PCR analysis (BARIC and DALLA VIA, 2007), FD phytoplasmas were not present (data not shown). Details on the origin of the leaves are given in Table 1.

Leaves were washed with distilled water and oven-dried with air circulation at 60 °C until constant weight. After drying, samples were ground with the Cyclotec^{TM} (Foss, Denmark), using a 0.45 mm sieve. Nitrogen concentration was determined by the Dumas combustion method at 1050 °C (VDLUFA, 1995), using the Truspec N Elemental Determinator (Leco Corp., St-Joseph, MI, USA). To determine the content of the mineral elements calcium, magnesium, potassium, phosphorous, manganese and iron, leaves were mineralized with nitric acid (65 % (w/w)) and hydrogen peroxide (30 % (m/m)) by the microwave digestion labstation Ethos TC (Pro 24 Rotor, 30 bar, Milestone Inc., USA) according to the SW-846 EPA

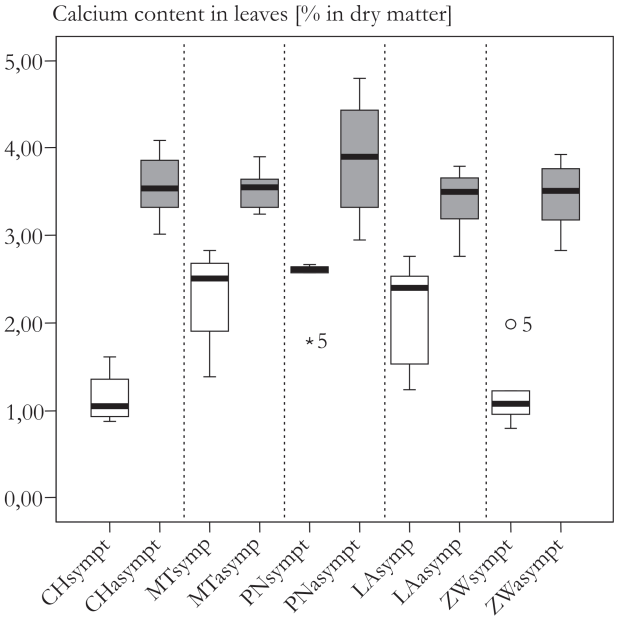

Fig. 1: Calcium content (expressed as % of dry matter) in leaves of BN-infected (white boxes) and healthy grapevines (grey boxes) (CH = 'Chardonnay', MT = 'Müller Thurgau', PN = 'Pinot Noir', LA = 'Lagrein', ZW = 'Zweigelt')

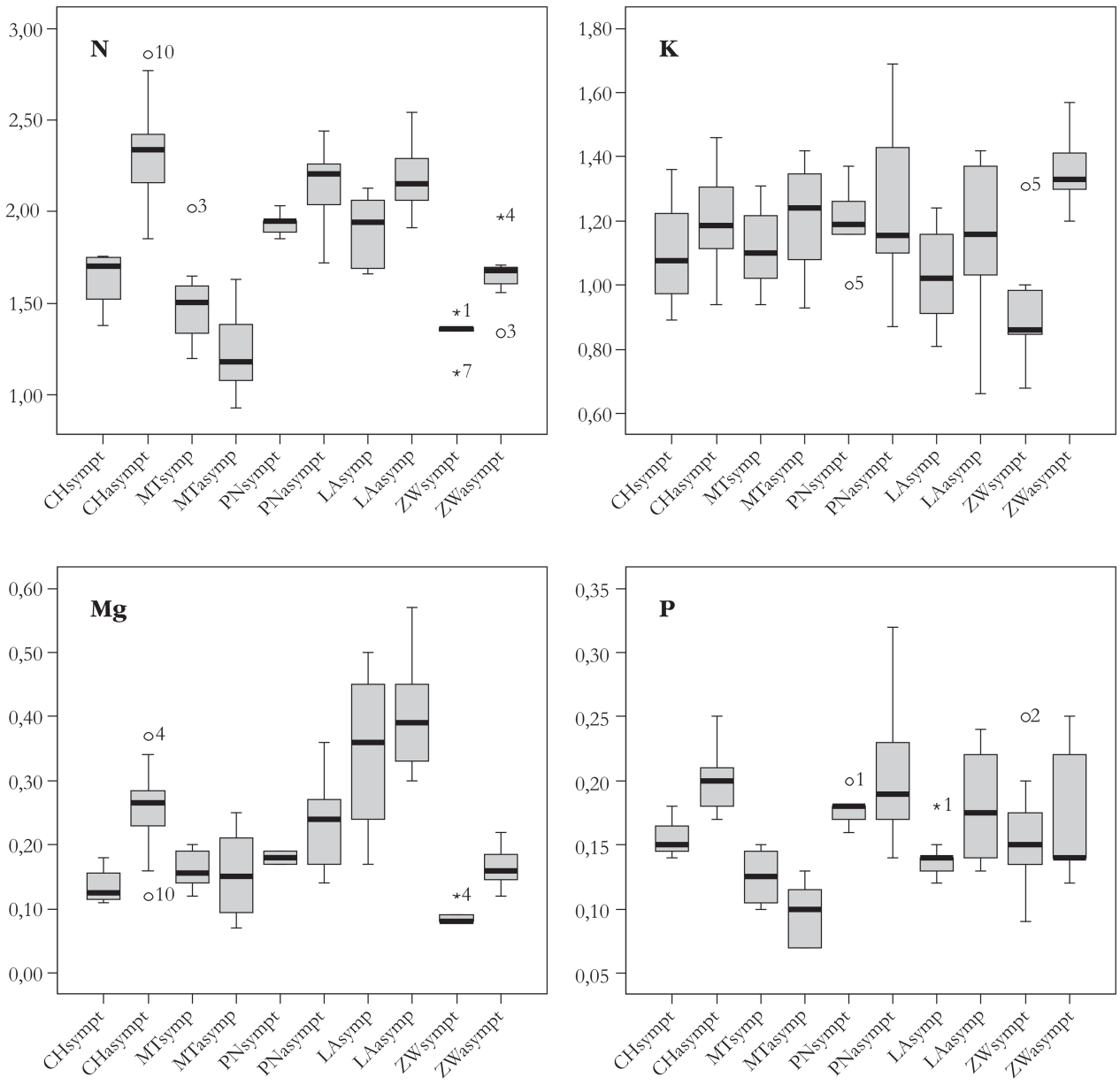

Fig. 2a: Levels of nitrogen, magnesium, phosphorus and potassium in leaves of BN-infected (sympt) and healthy grapevines (asympt) (CH = 'Chardonnay', MT = 'Müller Thurgau', PN = 'Pinot Noir', LA = 'Lagrein', ZW = 'Zweigelt') (concentrations of N, Mg, P and K are expressed as % of dry matter)

Method 3052 (EPA, 1996). The elements were analyzed by the ICP-OES Vista MPX (Varian Inc., USA) according to VDLUFA (2003).

For statistical analysis average values of the data were compared by means of the T-test (p = 0.05) using the software SPSS 12.0.

Results and discussion

Compared to healthy leaves, those infected with BN showed reduced levels of several mineral elements. The extent of these differences, however, varied depending on grapevine cultivar and element. Calcium levels were significantly reduced in symptomatic leaves compared

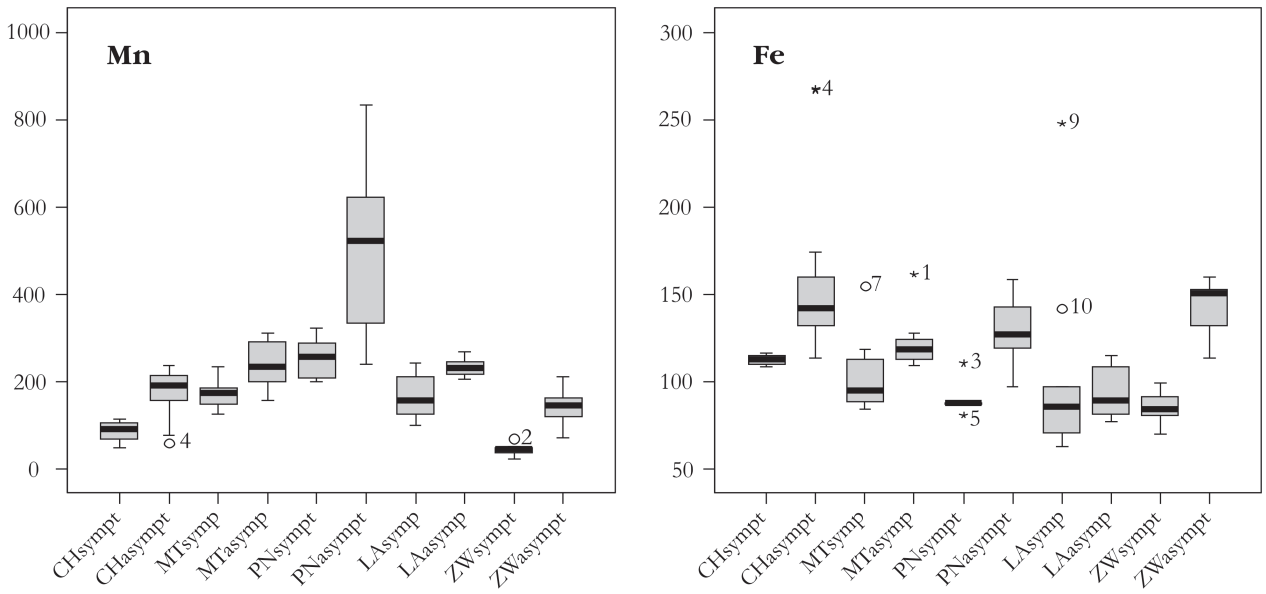

Fig. 2b: Levels of manganese and iron in leaves of BN-infected (sympt) and healthy grapevines (asympt) (CH = 'Chardonnay', MT = 'Müller Thurgau', PN = 'Pinot Noir', LA = 'Lagrein', ZW = 'Zweigelt') (concentrations of Mn and Fe are expressed as mg/kg)

to symptom-free ones in all five grape varieties (T-test, $p < 0.05$) (Fig. 1). Similar results had been obtained in previous experiments in 2005 and 2006 using only leaves from cv. 'Chardonnay' (data not shown). Whereas median contents of calcium were very similar for healthy leaves in the five varieties (about 3 % of plant dry weight), greater variations were observed in the symptomatic leaves (ranging from approximately 1 % with 'Chardonnay' and 'Zweigelt' to approximately 2.7 % with 'Müller Thurgau', 'Pinot Noir' and 'Lagrein'). Most leaf samples had been obtained from plants growing on silicate sands on slope debris, with the exception of the site planted with the variety 'Lagrein' (calcareous sands). In comparison with the neighboring healthy grapevines, it seems plausible that the low calcium levels in BN-vines were not caused by poor availability of soil calcium, but rather by poor translocation of calcium to the leaves. It is well known that calcium accumulation in plants can be affected by seasonal differences in vapor pressure gradients and thus transpiration (Marschner, 1995). The decline in accumulation of calcium, manganese and zinc in grape berries was also explained by a diminished xylem flow (Rogiers et al., 2006). The typical downward curling of the leaves infected with BN resembles the calcium deficiency symptoms in older leaves, where growth at the margins of the leaves is slower than that of the rest of the leaf (Taiz and Zeiger, 2006).

Alterations in the contents of the other elements were less consistent (Fig. 2a and 2b). Nitrogen levels were significantly lower in 'Chardonnay' and 'Zweigelt' leaves, lower (but not significantly) in 'Pinot Noir' and 'Lagrein' leaves, and unchanged in 'Müller Thurgau' leaves. Magnesium, manganese and potassium levels were slightly reduced in BN-infected leaves (significantly reduced only in 'Zweigelt'). Similar results were obtained with iron, which showed significantly reduced levels in 'Zweigelt' and 'Pinot Noir'. Phosphorus levels in BN-infected leaves were unchanged compared to healthy leaves with the exception of 'Chardonnay' (reduced levels in BN-infected leaves). The theory that potassium deficiency might play an essential role for the inward leaf curl disorder in grapes (Sharma et al., 2003) was not corroborated by our data. Whereas Sharma et al. (2003) found significantly lower K:Ca-ratios in symptomatic plants than in healthy ones, the opposite effect was found in our study (data not shown). Magnesium, phosphorus, potassium and sulfur are elements with high mobility in the phloem, whereas calcium, nitrogen and manganese are mainly transported through the xylem. The phloem mobility of iron depends on plant species, tissue and environmental factors. Phytoplasma growth is restricted to the phloem, and one of the mechanisms by which they damage their host plants might be conduit blockage in the sieve tubes (Christensen et al., 2005). The effect on the elements

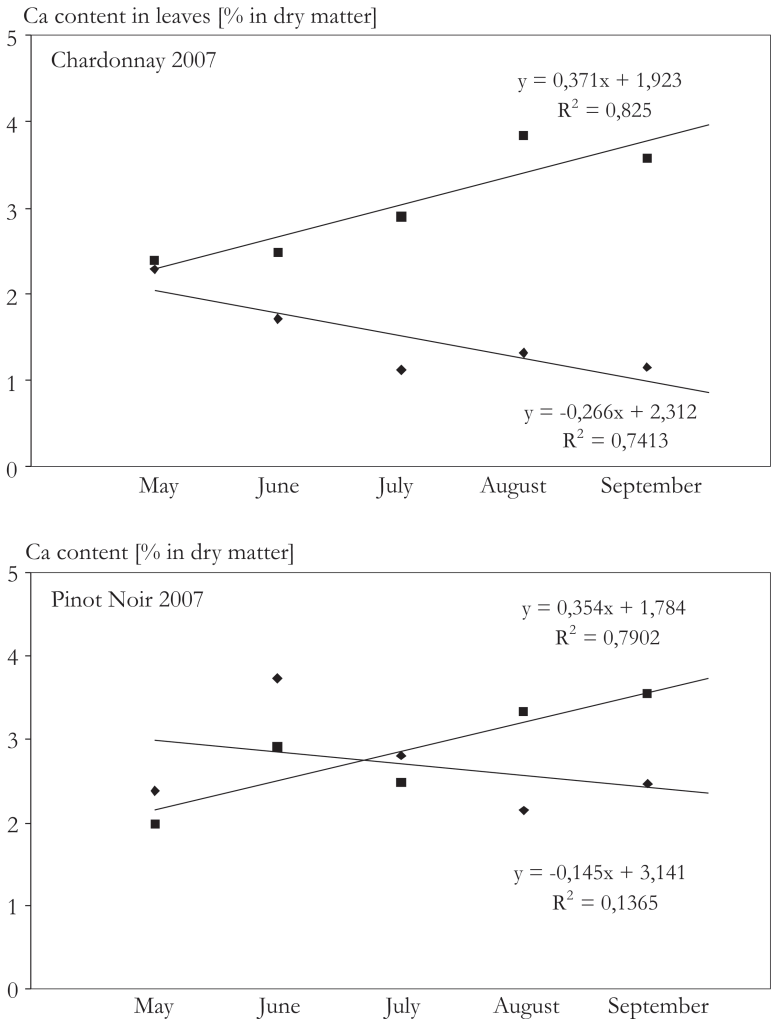

Fig. 3: Changes of the calcium contents in leaves of BN-infected (✦) and healthy grapevines (■) cv. 'Chardonnay' and 'Pinot Noir' during a five-months period from end of May till end of September

transported through the xylem, however, has to be more indirect. Some BN symptoms resemble that of water stress, and it is probable that flow rates of mineral elements along the transpiration stream are lowered in BN-infected grapes. Moisture levels in the soil were not measured for this study, but most vineyards (with the exception of the one planted with 'Zweigelt' grapes) were equipped with drip irrigation systems and no general water deficit was observed in neighboring healthy grapevines. While the average precipitation rate in South Tyrol's winegrowing areas is around 800 mm per year, 2007 was a relatively dry year with a precipitation of 635.9 mm (measured at the Research Station Laimburg). Water deficits might enhance the development of BN symptoms, as has been suggested for Pierce's disease (Thorne et al., 2006). The symptoms of Pierce's disease (PD), widespread in North America but not yet present in Europe, show some similarities to BN, but are caused by a bacterium (*Xylella fastidiosa)*, which does not inhibit the phloem, but the xylem. Whereas leaf drop occurs in phases of water deficiency and with PD infections, leaves showing typical symptoms of BN often remain attached to the stem longer than asymptomatic leaves of the same vine or healthy neighboring vines.

Symptoms of BN are expressed differently in the grape cultivars, ranging from nearly absent (e.g. 'Cabernet-Sauvignon' - not used in this study) to moderate ('Müller Thurgau', 'Pinot Noir', 'Lagrein') to very strong ('Chardonnay', 'Zweigelt'). Whereas the first leaf symptoms with 'Chardonnay' in South Tyrol are visible around mid-June, normally they do not appear on 'Pinot Noir' leaves before early August. First studies into the dynamics of mineral elements in the leaves over a five-month period from May until September also show that differences in calcium levels between healthy and BN-infected leaves can be detected with 'Chardonnay' as early as June/July and increase till the end of September (Fig. 3), whereas leaves of 'Pinot Noir' show the decrease of calcium levels only later in the season and at a lower rate. The increase of calcium levels in healthy grapevine leaves throughout the summer months is in accordance with data published by Conradie (1981), who found that calcium uptake by the vine occurs mainly between budburst and pre-veraison, during the time of fastest vegetative and fruit development, and slows down after veraison as root growth slows down. Weather conditions, especially water availability, during this time are important for calcium accumulation in the plant.

Our results indicate that BN-infected grape leaves suffer from severe malnutrition of several mineral elements, especially calcium. Analysis of the biochemical pathways leading from phytoplasma infection to these leaf symptoms might help gain a better understanding

of this complex pathosystem and eventually establish strategies for symptom reduction.

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