# Depressed growth of non-chlorotic vine grown in calcareous soil is an iron deficiency symptom prior to leaf chlorosis

**Bernd Gruber**[1] and **Harald Kosegarten**[2*]

[1]Department of Viticulture, State Research Institute Geisenheim, Blaubachstr. 19, D-65366 Geisenheim, Germany
[2]Present address: Dipartimento di Colture Arboree, Via Filippo Re 6, I-40126 Bologna, Italy

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## Summary – Zusammenfassung

The development of iron deficiency symptoms (growth depression and yellowing of the youngest leaves) and the distribution of iron between roots and leaves were investigated in different vine cultivars (Silvaner, Riparia 1G and SO4) grown in calcareous soils. As a control treatment all cultivars were also grown in an acidic soil. Only the cultivars Silvaner and Riparia 1G showed yellowing of the youngest leaves under calcareous soil conditions at the end of the cultivation period. All cultivars including SO4 showed severe shoot growth depression, by 50 % and higher, before yellowing started or without leaf yellowing in the cultivar SO4. Depression of shoot growth occurred independently from that of root growth. In a further treatment the effect of Fe-EDDHA spraying onto the shoot growth of the cultivar Silvaner after cultivation in calcareous soil was investigated. Prior to Fe application plants were non-chlorotic, but showed pronounced shoot growth depression. Spraying led to a significant increase in shoot length, though leaf growth was not increased. Accordingly, depression of shoot growth of non-chlorotic plants under calcareous soil conditions and with ample supply of nutrients and water has been evidenced to be at least partly an iron deficiency symptom. We suggest that plant growth only partially recovered because of dramatic apoplastic leaf Fe inactivation and/ or a high apoplastic pH which may directly impair growth. Since growth was impaired before the youngest leaves showed chlorosis we assume that meristematic growth is more sensitively affected by Fe deficiency than is chlorophyll synthesis and chloroplast development.

In spite of high Fe concentrations in roots and leaves of the vines grown in calcareous soils plants suffered from Fe deficiency. The finding of high Fe concentrations also in young, but growth retarded green leaves is a further indication that iron deficiency chlorosis in calcareous soils is caused by primary leaf Fe inactivation. However, in future, only a rigorous study of the dynamic changes of iron and chlorophyll concentration, leaf growth and apoplastic pH at the cellular level during leaf development and yellowing will provide causal insights between leaf iron inactivation, growth depression, and leaf chlorosis.

**Key words:** Growth depression / iron deficiency / leaf chlorosis / primary Fe inactivation / symptoms / Vitis sp.

## Gehemmtes Wachstum von Reben auf Karbonatböden ist ein empfindlicheres Eisenmangelsymptom als die Chlorose junger Blätter

Es wurde die Entwicklung von Eisenmangelsymptomen (Wachstumshemmung und Chlorose bei jungen Blättern) unter dem Aspekt der Eisenverteilung zwischen Wurzel und Blättern bei verschiedenen Rebsorten (Silvaner, Riparaia 1G, SO4) auf Karbonatböden untersucht. Die Kontrollpflanzen wurden auf einem Boden mit niedrigem pH angezogen. Nur die jüngsten Blätter der Sorten Silvaner und Riparia 1G wurden am Ende der Vegetationsperiode nach Anzucht auf den Karbonatböden chlorotisch. Alle Sorten, einschließlich SO4, zeigten auf den Karbonatböden eine erhebliche Hemmung des Sprosswachstums, um 50 % und höher, bevor sich Chlorosesymptome zeigten (Silvaner, Riparia 1G) bzw. ohne dass sich eine Chlorose ausbildete (SO4). Die Wachstumshemmung des Sprosses erfolgte unabhängig von der Hemmung des Wurzelwachstums. In einem weiteren Versuch wurde die Applikation von Fe-EDDHA auf das Sprosswachstum bei der Sorte Silvaner untersucht. Die Gabe von Fe-EDDHA führte zu einer signifikanten Verbesserung des Längenwachstums von Pflanzen, die nicht-chlorotisch, aber im Wachstum gehemmt waren; das Blattwachstum wurde nicht signifikant gefördert. Damit wurde gezeigt, dass das gehemmte Wachstum von nicht-chlorotischen Pflanzen auf Karbonatböden bei ausreichender Nährstoff- und Wasserzufuhr, zumindestens teilweise auf einen Eisenmangel zurückzuführen ist. Das gehemmte Wachstum beruht sehr wahrscheinlich auf einer massiven Eiseninaktivierung im Blattapoplasten und/oder auf einem direkten pH-Effekt und wurde insofern durch Eisenspritzung nur teilweise wieder hergestellt. Da die Wachstumshemmung vor der Chlorose auftrat, nehmen wir an, dass das meristematische Wachstum empfindlicher durch Eisenmangel gestört wird als die Chlorophyllsynthese und die Chloroplastendifferenzierung.

Trotz hoher Eisenkonzentrationen in Blatt und Wurzel bei allen Weinsorten nach Anzucht auf den Karbonatböden zeigten die Pflanzen Eisenmangel. Hohe Eisenkonzentrationen wurden nicht nur in chlorotischen Blättern, sondern auch in jungen grünen, im Wachstum gehemmten Blättern nachgewiesen. Dieser Befund spricht für eine primäre Eiseninaktivierung in Blättern als direkte Ursache des Eisenmangels. Allerdings wird nur eine konsequente Untersuchung der dynamischen Veränderungen der Eisen- und Chlorophyllkonzentration, des Blattwachstums und des apoplastischen pH auf zellulärer Ebene während der Blattentwicklung und der Ausbildung von Chlorose die kausalen Beziehungen zwischen der Eiseninaktivierung im Blattapoplasten, der Hemmung des Blattwachstums und der Chlorose klären.

* Correspondence: PD Dr. Harald Kosegarten;
E-mail: Harald.Kosegarten@ernaehrung.uni-giessen.de

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## 1 Introduction

Iron deficiency chlorosis is one of the major problems affecting a variety of crop species grown in calcareous soils. In severe cases iron deficiency chlorosis leads to failure of whole crop stands which in cropping systems with perennial plants results in high economical losses (e.g. *Schaller*, 1983). It is well-known that Fe-deficiency is characterized by yellowing of young leaves, whereas more mature leaves are frequently green. From several older observations in the literature it is also known that plant growth is often considerably depressed and, interestingly, independent of whether young leaves are chlorotic or green (*Mengel* and *Malissiovas*, 1981; *Mengel* et al., 1984a). *Mengel* et al. (1984a) working with the chlorosis-susceptible *Vitis* cultivar "Huxelrebe" grown in a calcareous soil found that the dry weight of the above-ground matter was depressed by 28 %, but plants were still green. Also *Bavaresco* et al. (1993) have reported growth depression by 50 % with the chlorosis-susceptible *Vitis* cultivar "Pinot blanc", even before the youngest leaves became chlorotic. Since many rootstocks for vines are derived from the chlorosis-susceptible American *Vitis* species (*Mullins* et al., 1992), they also may suffer from iron deficiency on calcareous soils without showing leaf yellowing. *Wutscher* et al. (1970) working with grapefruit trees on 16 different rootstocks have shown that shoot growth of the chlorosis-susceptible rootstocks can be impaired by up to 40 %, while staying green or showing only slight traces of leaf chlorosis.

As yet it still remains to be proved that growth depression of plants cultivated in calcareous soils is due to iron deficiency. In solution culture experiments mimicking calcareous soil solutions *Kosegarten* et al. (1998) suggested that the impaired formation of new leaves and restricted leaf growth is a typical and more sensitive symptom of Fe-deficiency than is leaf chlorosis. These authors showed that by increasing external Fe concentrations at low external pH, leaf Fe concentrations increased and depression of leaf formation and growth was overcome. In the present work the development of growth depression and leaf chlorosis was investigated in three different *Vitis* genotypes grown in calcareous soils. Control plants were grown in an acidic soil under the same conditions of ample supply of nutrients and water. In an additional experiment the shoots of non-chlorotic vine plants were sprayed with Fe chelate (Fe-EDDHA) to investigate the causal relationship between insufficient Fe supply and growth depression on calcareous soils before the onset of leaf chlorosis.

According to the hypothesis of *Mengel* (1994) iron deficiency chlorosis on calcareous soils is not caused by low Fe acquisition by the roots, but by restricted Fe translocation from the root apoplast into the root symplast and from leaf apoplast into the leaf symplast. As recently shown by *Kosegarten* et al. (1999, 2001) Fe chlorosis under alkaline conditions is primarily induced by nitrate nutrition leading to an increase in apoplastic pH in young green leaves. High apoplastic pH impairs $Fe^{III}$ reduction, an essential step for Fe transport into the symplast (primary leaf Fe inactivation). *Römheld* (2000) holds the view that Fe translocation to the leaves is low, thus leading to leaf yellowing. Yellow leaves may lack sufficient energy for the plasmalemma $H^+$-ATPase at low photosynthetic rates (*Kosegarten* et al., 1998) and as suggested by *Römheld* (2000) apoplastic pH may increase (secondary leaf Fe inactivation). If the above mentioned hypothesis of *Mengel* (1994) is valid also young, but growth retarded green leaves should show high Fe concentrations, but suffer from iron deficiency. We have therefore analyzed the Fe distribution between the roots and chlorotic and non-chlorotic shoots of different cultivars grown in calcareous soils and for comparison in an acidic soil.

## 2 Materials and methods

### 2.1 Plant cultivation and foliar application of iron

Green cuttings of the ungrafted *Vitis* cultivar Silvaner (*Vitis vinifera* L.) and of the rootstock cultivars Riparia 1 Geisenheim (Riparia 1G) (*Vitis riparia* Michaux) and Selection Oppenheim 4 (SO4) (*Vitis berlandieri* Planchon x *Vitis riparia* Michaux) were cultivated in perlite for four weeks until the secondary shoots began to develop. The roots by then were approximately 6–8 cm in length and the cuttings were transferred into pots. Plants were grown both in a calcareous and in an acidic soil (control) (Tab. 1). Cultivation was carried out in pots filled with 6.5 kg of soil in the greenhouse and the plants were grown for 12 weeks until the end of the vegetation period (experiment 1). The experiment was repeated in the second year with the genotype Silvaner (*Vitis vinifera* L.), using another calcareous soil, but with the same acidic soil (as control) (Tab. 1). In experiment 2 half of the plants grown in the calcareous soil were sprayed twice with 10 µM Fe-EDDHA (approximately 30 ml per plant) *via* the leaf

**Table 1:** Most important physico-chemical soil properties and fertilization rates (in brackets) to assure a sufficient nutrient level in the soils. (1): experiment 1; (2): experiment 2.

**Tabelle 1:** Übersicht über die wichtigsten physiko-chemischen Eigenschaften der verwendeten Böden und die vorgenommene Aufdüngung (in Klammern) zur Gewährleistung einer ausreichenden Nährstoffversorgung. (1): Experiment 1; (2): Experiment 2.

| | extraction method | acidic soil (mg $kg^{-1}$); (g $(100g)^{-1}$)* | calcareous soil (1) (mg $kg^{-1}$); (g $(100g)^{-1}$)* | calcareous soil (2) (mg $kg^{-1}$); (g $(100g)^{-1}$)* |
|---|---|---|---|---|
| pH | 0.02N $CaCl_2$ | 4.22 | 7.28 | 8.6 |
| $CaCO_3$ | | – | 19.6* | 23.0* |
| sand | | 80.7* | 35.6* | 66.3* |
| silt | | 3.9 | 39.0* | 20.2* |
| clay | | 15.4* | 25.5* | 13.5* |
| N | 0.0125M $CaCl_2$ | 16.5 | 7.1 | – |
| | | (+40) | (+50) | (+60) |
| Mg | 0.0125M $CaCl_2$ | 32 | 305 | 110 |
| | | (+90) | – | |
| P | CAL | 120 | 24 | 24 |
| | | – | (+80) | (+100) |
| K | CAL | 35 | 46 | 65 |
| | | (+150) | (+150) | (+150) |
| Cu | DTPA | 1.7 | 0.4 | 0.4 |
| Zn | DTPA | 10.7 | 0.7 | 0.2 |
| | | – | (+20) | (+20) |
| Fe | DTPA | 60.9 | 4.5 | 4.2 |
| Mn | DTPA | 16.1 | 2.3 | 4.6 |
| | | – | (+20) | (+20) |

and stem surface. Iron spraying was carried out for the first time after four weeks and then again after eight weeks in order to investigate growth responses. Shoot length was measured at the end of the vegetation period. All treatments were watered to 70 % water holding capacity and fertilized to the same nutrient level at rates as shown in Tab. 1 (in brackets). In both years seven plants of each treatment were cultivated with one plant per pot.

### 2.2 Collection and treatment of samples

The roots and leaves were harvested 12 weeks after transplantation into soils. Root and shoot fresh weight was determined. The roots were thoroughly washed under running water and afterwards again washed three times for five minutes with 0.5 mM $CaCl_2$ in order to exchange non-specifically bound Fe from the root apoplast. Young leaves were collected from the shoot tip downwards (leaf position 1 to 4, see Figs. 1–3) for the determination of chlorophyll and iron. Due to the differences in leaf growth between treatments the leaf samples of the three varieties comprised a different span of leaf developmental stages. For iron determination, roots and leaves were dried at 70 °C (48 hours). Leaves for chlorophyll analysis were stored at –20 °C.

### 2.3 Chlorophyll and iron analysis

Chlorophyll and iron determinations were conducted according to the methods of *Arnon* (1949) and *Schaller* (1988), respectively. For chlorophyll analysis 1 g FW and for Fe analysis 0.5 g DW of plant material, respectively, were analyzed with seven replicates per treatment.

### 2.4 Statistical treatment

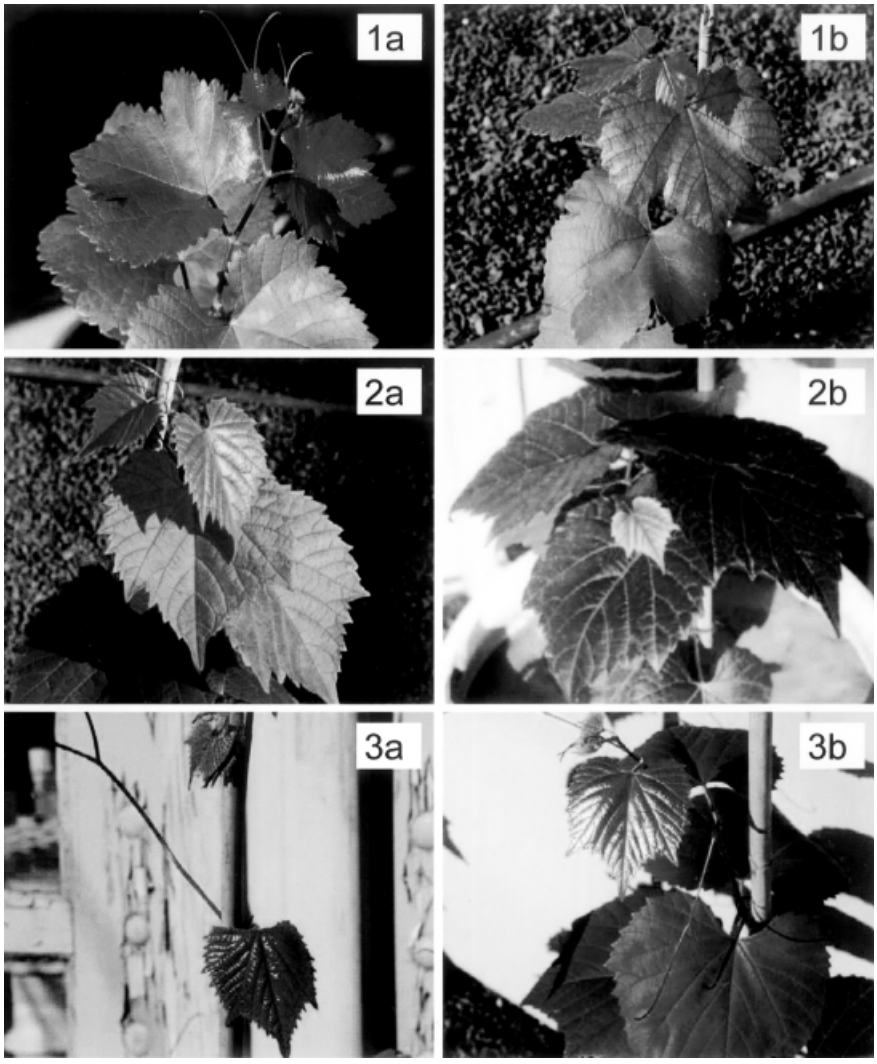
Significant differences were assessed using the *t*-test (*Köhler* et al., 1984). Variation is indicated by SD.

## 3 Results

### 3.1 Plant Growth

In the experiment of the first year shoot growth of all three genotypes cultivated on the calcareous soil was significantly depressed as compared to the control plants grown in the acidic soil (Tab. 2). Growth reduction started with the onset of plant development and after 12 weeks shoot fresh weight was reduced by at least 50 % as compared with the control. From the data in Tab. 2 it is evident that only *Vitis riparia*, the variety with the strongest shoot growth depression, also showed significant depression of root development (50 %).

As a consequence of the strong inhibition of shoot growth the ratio of shoot FW to root FW was significantly decreased in plants grown in the calcareous soil and was lowest in the




**Figs. 1a + b, 2 a + b, and 3 a + b:** Shoot tips with youngest leaves of the genotypes Silvaner (Fig. 1), Riparia 1G (Fig. 2), and SO4 (Fig. 3) at the end of the vegetation period (after 12 weeks).
(a) after growth in the acidic soil; (b) after growth in the calcareous soil (experiment 1). Notice: only Silvaner (Fig. 1b) and Riparia 1G (Fig. 2b) show (interveinal) chlorosis of the youngest leaves. The red-brownish color of the youngest leaves (Fig. 3a) and their low chlorophyll concentrations (Tab. 2) are typical for the cultivar SO4 being indicative for rapid plant growth (corresponding to an increased number of young leaves) on the acidic soil.
**Abbildungen 1a + b, 2a + b und 3a + b:** Triebspitzen mit den jüngsten Blättern der verschiedenen Genotypen Silvaner (Abb. 1), Riparia 1G (Abb. 2) und SO4 (Abb. 3) am Ende der Vegetationsperiode (nach 12 Wochen).
(a) nach Anzucht auf einem sauren Boden; (b) nach Anzucht auf einem Karbonatboden (Experiment 1). Man beachte: nur bei Silvaner (Abb. 1b) und bei Riparia 1G (Abb. 2b) weisen die jüngsten Blätter (Interkostal-) Chlorose auf. Die rotbraune Farbe (Fig. 3a) sowie die niedrigen Chlorophyllkonzentrationen (Tab. 2) der jüngsten Blätter sind typisch für die Sorte SO4 und spiegeln das schnelle Pflanzenwachstum (welches mit einer erhöhten Anzahl junger Blätter korreliert) auf dem sauren Boden wider.

**Table 2:** Shoot and root fresh weight and chlorophyll concentration of the youngest leaves (leaf position 1 to 4 from the shoot tip downwards) of the different genotypes of *Vitis* after 12 weeks growth in the acidic and calcareous soils, respectively (experiment 1). Means (± SD), ** and ***: significant differences at $^{**}P \leq 0.01$ and at $^{***}P \leq 0.001$.

**Tabelle 2:** Spross- und Wurzelfrischmasse und Chlorophyllkonzentration der jüngsten Blätter (Blattposition 1 bis 4 von der Sprossspitze abwärts) der verschiedenen Genotypen von *Vitis* nach 12-wöchigem Wachstum im sauren Boden und im Karbonatboden (Experiment 1). Alle Angaben sind Mittelwerte (± Standardabweichung; n = 7); ** und ***: signifikante Unterschiede bei $^{**}P \leq 0{,}01$ bzw. bei $^{***}P \leq 0{,}001$.

| treatment | shoot fresh weight (g) | root fresh weight (g) | shoot FW / root FW | chlorophyll concentration (mg (g FW)$^{-1}$) |
|---|---|---|---|---|
| acidic soil | | | | |
| Silvaner | 37.8 ± 10.1 | 33.9 ± 10.0 | 1.1 ± 0.2 | 1.3 ± 0.1 |
| Riparia 1G | 38.7 ± 6.6 | 37.8 ± 7.2 | 1.0 ± 0.1 | 1.2 ± 0.1 |
| SO4 | 62.3 ± 19.2 | 36.3 ± 3.1 | 1.7 ± 0.5 | 1.0 ± 0.2 |
| calcareous soil | | | | |
| Silvaner | 20.2 ± 5.1*** | 27.3 ± 5.6 | 0.8 ± 0.2** | 0.8 ± 0.1*** |
| Riparia 1G | 10.9 ± 3.3*** | 18.7 ± 4.9*** | 0.6 ± 0.04*** | 1.3 ± 0.4 |
| SO4 | 31.4 ± 3.5*** | 37.4 ± 4.6 | 0.8 ± 0.1** | 1.3 ± 0.2*** |

most chlorosis-susceptible cultivar Riparia 1G. However, the differences in the ratio of shoot FW to root FW between the two soil treatments showed no clear pattern in relation to chlorosis susceptibility (Tab. 2).

Depression of shoot growth of the cultivar Silvaner on the calcareous soil (Tab. 2) was confirmed in the second year; however, root growth was also severely impaired by 55 % in the second experiment (root fresh weight (g): acidic soil: 37.8 ± 7.0; calcareous soil: 16.6 ± 4.2). In comparison with the plants on the acidic soil, the shoot length of the plants grown in the calcareous soil was reduced by 67 % (Tab. 3). Shoot sprayings with 10 µM Fe-EDDHA solution resulted in a significant increase of shoot length by 22 % compared with the plants grown in the same calcareous soil, but without Fe-chelate supply (Tab. 3); however, in comparison with the acidic soil, shoot length recovery was not pronounced and growth of comparable leaves was not increased (data not shown).

## 3.2 Chlorophyll concentration

Despite of the highly significant growth depression under calcareous soil conditions for the cultivar SO4 there was no evidence of chlorosis of the youngest leaves of this cultivar at the end of the vegetation period (Fig. 3b). Yellowing of the youngest leaves only became apparent at the very end of the cultivation period of the two chlorosis-susceptible cultivars Silvaner and Riparia 1G (Figs. 1b and 2b) and hence after growth depression had occurred in plants grown in the calcareous soil. Leaf chlorosis of the cultivar Silvaner was also evident from the chlorophyll concentration of the youngest leaves which was significantly depressed for plants grown in the calcareous soil (Tab. 2).

The leaf chlorophyll concentrations of the cultivars Riparia 1G and SO4 (Tab. 2) did not reflect the visual appearance of the youngest leaves (Figs. 2 and 3). Because of the differences in leaf growth between the cultivars it was not possible to collect young leaves at the same stage of development. Accordingly, the chlorosis of the very small youngest leaf of *Vitis riparia* grown in calcareous soil (Fig. 2b) was not reflected by the chlorophyll concentration shown in Tab. 2; here, the sample of the youngest leaves inevitably comprised a relatively high amount of green leaf material from lower leaf positions (position 2 to 4). In the case of the vigorously growing SO4 plants on the acidic soil (Tab. 2) the low chlorophyll concentrations of 1.0 mg (g FW)$^{-1}$ was obviously not indicative of leaf chlorosis (Fig. 3a), but for increased plant growth (corresponding to an increased number of young leaves) under acidic conditions compared with the slower development of the plants grown in calcareous soil (Tab. 2). It is typical for the cultivar SO4 that young leaves show a red-brownish color (Fig. 3a) before the onset of chlorophyll synthesis (Fig. 3b).

**Table 3:** Shoot length of the cultivar Silvaner in experiment 2 at the end of the vegetation period after 12 weeks growth in the acidic and calcareous soil (with and without application of Fe-EDDHA), respectively. Means (± SD), *, ** and ***: significant differences at $^{*}P \leq 0.05$, at $^{**}P \leq 0.01$, and at $^{***}P \leq 0.001$.

**Tabelle 3:** Sprosslänge der Rebsorte Silvaner in Experiment 2 am Ende der Vegetationsperiode nach 12-wöchiger Anzucht im sauren Boden und im Karbonatboden (mit und ohne Blattapplikation von Fe-EDDHA). Alle Angaben sind Mittelwerte (± Standardabweichung; n = 7), *, ** und ***: signifikante Unterschiede bei $^{*}P \leq 0{,}05$, bei $^{**}P \leq 0{,}01$ bzw. bei $^{***}P \leq 0{,}001$.

| treatment | shoot length (cm) |
|---|---|
| acidic soil | 49.0 ± 12.8*** |
| calcareous soil | 15.7 ± 2.4 |
| calcareous soil + Fe-EDDHA | 19.2 ± 2.2* |

## 3.3 Fe concentration in the roots and in the leaves

The root Fe concentrations in both soil treatments of experiment 1 were at the same level in the cultivar Riparia 1G and were even significantly increased by 64 % and 31 %, respectively, in the calcareous soil-grown plants of the cultivars Silvaner and SO4 (Tab. 4).

The leaf Fe concentrations amounted to approximately one third of the Fe concentrations found in the roots (Tab. 4). Also the leaf Fe concentrations were in the same range in both soil treatments or significantly increased for the plants grown in the calcareous soils. Interestingly, high leaf Fe concentrations were found not only in the cultivars with chlorosis, but also in the non-chlorotic cultivar SO4 at the end of the vegetation period (Tab. 4). Also, interestingly, the leaf Fe concentration in the cultivar Silvaner grown in the calcareous soil in the experiment of the second year was lower in plants sprayed with Fe-EDDHA than in plants without Fe chelate application. Because the plants on the calcareous soils showed relatively high standard deviations in leaf as well as in root Fe concentrations (Tab. 4) none of the three varieties revealed a correlation between the Fe concentrations in roots and leaves (data not shown).

**Table 4:** Root and leaf total Fe concentrations of the different genotypes of *Vitis* after growth in acidic and calcareous soil (with and without application of Fe-EDDHA), respectively (experiment 1 and 2). Means (± SD), *, ** and ***: significant differences at $*P \leq 0.05$, at $**P \leq 0.01$ and at $***P \leq 0.001$.

**Tabelle 4:** Gesamteisenkonzentration in Wurzel und Blatt der drei verschiedenen Genotypen von *Vitis* nach Anzucht im sauren Boden bzw. Karbonatboden (mit und ohne Blattapplikation von Fe-EDDHA, Experiment 1 und 2). Alle Angaben sind Mittelwerte (± Standardabweichung; n = 7); *,** und ***: signifikante Unterschiede bei $*P \leq 0{,}05$, bei $**P \leq 0{,}01$ und bei $***P \leq 0{,}001$.

| treatment | root Fe concentration (µg (g DW)$^{-1)}$ | leaf Fe concentration (µg (g DW)$^{-1}$) |
|---|---|---|
| experiment 1 | | |
| acidic soil | | |
| Silvaner | 297.8 ± 51.3 | 108.8 ± 12.6 |
| Riparia 1 G | 396.6 ± 76.0 | 109.3 ± 9.3 |
| SO4 | 301.4 ± 66.4 | 95.8 ± 15.9 |
| calcareous soil | | |
| Silvaner | 487.4 ± 121.9** | 117.3 ± 7.6 |
| Riparia 1 G | 342.5 ± 78.3 | 180.4 ± 49.5** |
| SO4 | 393.3 ± 68.3* | 88.2 ± 11.8 |
| experiment 2 | | |
| Silvaner | | |
| acidic soil | 268.5 ± 52.1 | 71.4 ± 9.8 |
| calcareous soil | 307.0 ± 58.9 | 226.7 ± 41.0*** |
| calcareous soil + Fe-EDDHA | 282.6 ± 39.8 | 167.9 ± 18.1*** |

## 4 Discussion

### 4.1 Growth inhibition is an iron deficiency symptom prior to leaf yellowing

The vine genotypes Silvaner and Riparia 1G grown in the calcareous soil showed significant growth depressions prior to the yellowing of the youngest leaves in comparison to plants grown in the acidic soil (Tab. 2) under conditions of ample supply of nutrients and water in both treatments (Tab. 1). It is suggested that these growth depressions were directly caused by an insufficient supply of physiologically available Fe in leaves beause of Fe trapping in the apoplast (*Kosegarten* et al., 1999, 2001). This phenomenon is characterized by relatively high Fe concentrations in leaves and is frequently found in plants grown in calcareous soils (*Mengel* and *Malissiovas*, 1981; *Mengel* et al., 1984b; *Römheld*, 2000). Our assumption that the observed growth depression was a direct effect of an insufficient Fe supply is supported by several findings:

1. Spraying the vine shoot (cultivar Silvaner) with Fe-EDDHA increased shoot length by about 20 % relative to the non-sprayed plants (Tab. 3).
2. *Kosegarten* et al. (1998) cultivating *Helianthus annuus* in a nutrient solution which mimicked the situation of calcareous soils ($NO_3^-$, $HCO_3^-$) found that leaf growth and leaf formation were retarded. In a parallel treatment high Fe supply alleviated these growth depressions.
3. *Masalha* et al. (2000) growing plants in a sterile and in a non-sterile soil medium found that the sterile-grown plants (sunflower and maize) ran into an absolute iron deficiency because of a lack of microbial produced siderophores in the soil. The iron deficiency was evidenced by very low Fe concentrations in the plants, poor growth and in the case of sunflowers also by chlorotic leaves. Maize leaves, however, were not chlorotic, but severely restricted in growth.

From these observations it is clear that poor growth of plants grown in calcareous soils is at least partly caused by an insufficient supply of physiologically available Fe in the plant. Under such conditions poor leaf growth may be followed by leaf chlorosis as was the case with sunflowers (*Masalha* et al., 2000) and also with the vine cultivars Silvaner and Riparia 1G of which the youngest leaves became yellow at the end of the vegetation period (Figs. 1b and 2b). In contrast, the SO4 cultivar which is known to be resistant to Fe chlorosis (*Fleuchhaus*, 1987) showed no leaf yellowing (Fig. 3b), but severe growth depression (Tab. 2). Accordingly, these results show that restriction of growth is a more sensitive symptom of iron deficiency than the more spectacular symptom of chlorotic leaves. In this context it should be emphasized that depressed growth rates of green leaves may be frequently overlooked in particular under field conditions when there is no possibility of a direct comparison with plants grown in a non-calcareous soil. In several older, but also in new studies with perennials, e.g. vine (*Mengel* and *Malissiovas*, 1981; *Mengel* et al., 1984a; *Bavaresco* et al.,1993), citrus (*Wutscher* et al., 1970), and peach (*Shi* et al., 1993) growth depression was also observed under alkaline conditions, before leaf chlorosis developed, but was not investigated in relation to iron deficiency. From these findings it follows that low growth rates (small leaves) are not caused by an insufficient photosynthate provision as claimed e.g. by *Barbaresco* et al. (1999). In addition, as found by *Kosegarten* et al. (1998) small green leaves with normal chlorophyll concentrations also show high photosynthetic rates.

The impairment of growth could also be caused by a high apoplastic pH *per se*. According to the *acid growth theory*, a high apoplastic pH decreases cell wall elasticity thus restricting cell expansion (*Rayle* and *Cleland*, 1992). It was found that under the conditions of Fe chlorosis ($NO_3^-$, $HCO_3^-$) the apoplastic pH in roots was raised more (Kosegarten, unpublished) than in leaves (*Kosegarten* et al., 1999) because of the permanent presence of $HCO_3^-$ in the root apoplast. For this reason root growth should have been more affected than leaf growth. The finding of *Shi* et al. (1993) and our own results are to the contrary and from this we speculate that decreased cell wall elasticity was not the overriding factor causing the depressed leaf growth.

It has been shown by *Kosegarten* et al. (1999) that under alkaline conditions as a consequence of high leaf apoplastic pH, $Fe^{3+}$ reduction is inhibited causing insufficient Fe supply to the symplast thereby impairing most sensitively the development of leaf primordia (*Kosegarten* et al., 1998). We therefore suggest that insufficient Fe availability in the plant cell restricts the synthesis of ribonucleotide reductase which is an Fe containing enzyme producing deoxyribonu-

cleotide diphosphate (*Reichard*, 1993; *Jordan* and *Reichard*, 1998). Deoxyribonucleotide diphosphate is a precursor of DNA and a lack of DNA will severely affect cell division and thus meristematic growth.

Shoot growth of Silvaner and SO4 in the first experiment was depressed by approximately 50 % without affecting root fresh weight of plants grown in the calcareous soil (Tab. 2), and thus inhibition of shoot growth may occur independently from that of root growth. This observation is in agreement with results of *Shi* et al. (1993) who reported that insufficient Fe supply impaired shoot growth more strongly than root growth. Only the cultivar Riparia 1G, the most susceptible cultivar to Fe chlorosis, was significantly affected in root growth in experiment 1 (Tab. 2). In the experiment of the second year root growth of Silvaner was also clearly depressed and as suggested by *Römheld* (2000) shoot growth in these cases may have been additionally affected because of secondary effects by impaired root development, as is shown by Riparia 1G where shoot fresh weight was depressed by 70 % (Tab. 2). The depression of root growth of Silvaner in the second year is probably related to the higher bicarbonate buffer capacity of the calcareous soil in experiment 2 showing a higher soil pH (Tab. 1) and probably leading to a higher apoplastic pH in the roots. The bicarbonate buffer capacity of soils is not only related to the $CaCO_3$ concentration of soils which was rather similar for both calcareous soils (Tab. 1), but also to the proportions of crystalline and amorphous $CaCO_3$. Root growth depression of Riparia 1G is probably related to a direct bicarbonate effect (*Yang* et al., 1994) and we speculate to increased root apoplastic pH of this most chlorosis-susceptible cultivar.

### 4.2 Fe inactivation in roots and leaves

Iron concentrations in roots were much higher than in leaves (Tab. 4), a finding which has been frequently reported (*Mengel* and *Geurtzen*, 1988; *Mengel*, 1994; *Kosegarten* et al., 1998). Root Fe concentrations of plants grown in the calcareous soil were as high (Riparia 1G) or even significantly higher (Silvaner, SO4) than in roots from the acidic soil, although the DTPA-Fe solubility (Tab. 1) was more than ten times higher in the acidic soil as compared with the calcareous soil. This shows that the physicochemical solubility of the soil Fe had no major impact on the bioavailability of Fe in soils. As shown recently by use of EDX analysis (*Kosegarten* and *Koyro*, 2001) high Fe concentrations are accumulated in the epidermal root apoplast under alkaline conditions, and thus Fe transport from the soil to the root apoplast is not blocked in calcareous soils.

Vine not cultivated under the conditions of calcareous soils ($NO_3^-$, $HCO_3^-$) is sufficiently supplied with Fe if the leaf Fe concentration is in a range of 80 µg $(g\ DW)^{-1}$ (*Mengel* and *Malissiovas*, 1981). Plants grown in the calcareous soil showed much higher leaf Fe concentrations (Tab. 4), but suffered from iron deficiency as evidenced by a restricted leaf growth (Tab. 2) and at the end of the experiment also by chlorosis of the youngest leaves of the cultivars Silvaner and Riparia 1G (Figs. 1b and 2b). Therefore, Fe in young leaves is inefficient under alkaline growth conditions and according to the hypothesis of *Mengel* (1994) a substantial proportion of Fe in the roots and leaves was trapped in the apoplast (*Kosegarten* et al., 1999; *Kosegarten* and *Koyro*, 2001) inducing leaf chlorosis (*Kosegarten* et al., 2001). Iron trapped in the leaves can be mobilized by acidic sprays as shown by *Tagliavini* et al. (1995, 2000) and *Kosegarten* et al. (2001). Spraying resulted in a decrease of apoplastic pH followed by leaf re-greening without increasing the leaf Fe concentration (*Kosegarten* et al., 2001). From this it follows that Fe was translocated into the leaf cells from the apoplast into the symplast there stimulating chlorophyll synthesis and chloroplast development.

This Fe trapping may frequently lead to high Fe concentrations in vine leaves (*Mengel*, 1994; *Mengel* et al., 1984b; *Römheld*, 2000). *Römheld* (2000) found that Fe concentrations in vine leaves increased with time in plants grown in calcareous soils and suggests that the phenomenon of depressed leaf growth and high leaf Fe concentrations is related to root growth and phytohormones. We hold the view that on calcareous soils leaf growth is reduced because of insufficient Fe in the symplast, while Fe is still translocated from roots and other plant parts into the leaf apoplast. Here, Fe may accumulate with time since $Fe^{3+}$ reduction and hence Fe uptake into the symplast is much depressed because of increased apoplastic pH of young, still green leaves (*Kosegarten* et al., 1999, 2001). We share the opinion of *Häussling* et al. (1985) that Fe accumulation in chlorotic leaves is caused by restricted leaf growth which in particular is shown for Riparia 1G and for Silvaner in experiment 2 (Tab. 4). In these cases, as suggested by *Römheld* (2000) also secondary effects due to impaired root development on the inhibition of leaf growth (Tab. 2) may have occurred.

Because plant growth can also be impaired directly by insufficient Fe supply under alkaline growth conditions, the finding of high Fe concentrations not only in young chlorotic leaves, but also in young, growth retarded green leaves (Tab. 4) is a further indication for primary leaf Fe inactivation under alkaline growth conditions. However, we wish to emphasize that at least only a rigorous study (e.g. by use of a combination of different subtile optical techniques) of the dynamic changes between apoplast pH, chlorophyll concentration, and leaf growth at the cellular level in conjunction with leaf Fe concentrations during leaf yellowing will provide final conclusive evidence for the causal relationships of leaf Fe inactivation, growth depression and leaf chlorosis.

Leaf Fe concentrations under alkaline conditions found in the second experiment were also very high (Tab. 4). However, the Fe concentrations found in the plants sprayed with Fe-EDDHA were lower than that of the non-sprayed plants (Tab. 4). Since the sprayed plants recovered shoot length by 20 % some Fe dilution may have occurred. Fe-EDDHA applied via the leaf surface probably gained access into the leaf apoplast *via* stomata (*Eichert* et al., 1998) and we suggest that Fe-EDDHA molecules which reached apoplastic sections of high pH presumably will not improve leaf growth because of depressed $Fe^{3+}$ reduction (*Kosegarten* et al., 1999). However, as reported by *Kosegarten* et

al. (1999) the leaf apoplast pH is not homogenous and the apoplast of xylem veins shows lower pH values where $Fe^{III}$ reduction occurs. One may speculate that some Fe-EDDHA molecules will be reduced at these sites, and $Fe^{2+}$ taken up into the symplast, then translocated *via* plasmodesmata into sieve cells and from here to the shoot apex (*Stephan* and *Scholz*, 1993) where it may improve meristematic growth. This interpretation is in accord with our observation that Fe-EDDHA spraying increased partially shoot length (Tab. 3), but not leaf growth (data not shown). However, from these results it is not clear in the present experiment whether Fe inactivation in leaves under alkaline conditions is the major process which prevented a clear recovery of growth or whether secondary effects due to restricted root growth were responsible for severely restricted stem and leaf growth so that Fe application led only to a partial recovery of upper plant growth (Tab. 3).

## 5 Conclusions

Growth depression as a symptom of Fe deficiency has until now frequently been overlooked, in particular when plants were non-chlorotic and growth depression was relatively low. Such plants appear to be healthy, but are actually already suffering from Fe deficiency. Because growth depression of non-chlorotic plants in calcareous soils can be pronounced, by 50 % and higher, this phenomenon is of high importance for crop production.

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