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Review

Iron deficiency and chlorosis in orchard and vineyard ecosystems

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Abstract

Several perennial, deciduous, as well as evergreen fruit crops develop symptoms of iron deficiency—interveinal chlorosis of apical leaves—when cultivated in calcareous and alkaline soils. Under these conditions fruit yield and quality is depressed in the current year and fruit buds poorly develop for following year fruiting. This paper reviews the main fundamental and applied aspects of iron (Fe) nutrition of deciduous fruit crops and grapevine and discusses the possible development of sustainable Fe nutrition management in orchard and vineyard ecosystems. Cultivated grapevines and most deciduous fruit trees are made up of two separate genotypes the cultivar and the rootstock, providing the root system to the tree. The effect of the rootstock on scion tolerance of Fe chlorosis is discussed in terms of biochemical responses of the roots to acquire iron from the soil. Symptoms of iron chlorosis in orchards and vineyards are usually more frequent in spring when shoot growth is rapid and bicarbonate concentration in the soil solution buffers soil pH in the rhizosphere and root apoplast. Since the solubility of Fe-oxides is pH dependent, under alkaline and calcareous soils inorganic Fe availability is far below that required to satisfy plant demand, so major role on Fe nutrition of trees is likely played by the iron chelated by microbial siderophores, chelated by phytosiderophores (released into the soil by graminaceous species) and complexed by organic matter. As most fruit tree species belong to Strategy I-based plants (which do not produce phytosiderophores in their roots) Fe uptake is preceded by a reduction step from Fe^{3+} to Fe^{2+}. The role of ferric chelate reductase and proton pump activities in Fe uptake and the possible adoption of these measurements for screening procedure in selecting Fe chlorosis tolerant rootstocks are discussed. In a chlorotic leaf the existence of Fe pools which are somehow inactivated has been demonstrated, suggesting that part of the Fe coming from the roots does not pass the leaf plasmamembrane and may be confined to the apoplast; the reasons and the importance for inactivation of Fe in the apoplast are discussed. The use of Fe chlorosis tolerant genotypes as rootstocks in orchards and vineyards represents a reliable solution to prevent iron chlorosis; in some species, however, available Fe chlorosis resistant rootstocks are not very attractive from an agronomic point of view since they often induce excessive growth of the scion and reduce fruit yields. As most fruit tree crops and grapes are high value commodities, in many countries growers are often willing to apply synthetic Fe chelates to cure or to prevent the occurrence of Fe deficiency. The application of iron chelates does not represent a

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sustainable way to prevent or cure iron chlorosis because of to their costs and of the environmental risks associated with their use. Since Fe chelates were introduced, little research on alternative means for controlling the chlorosis has been performed. Sustainable management of Fe nutrition in orchards and vineyards should include all genetical and agronomical means in order to naturally enhance Fe availability in the soil and in the plant. Special attention should be given to soil analysis and to prevention measures carried out before planting. Alternatives to iron chelates are being developed and in the future they should be included into the routine practices of managing fruit trees and grapevine under Integrated Production and Organic Farming.

Keywords: Leaf chlorosis; Fruit trees; Integrated fruit production; Iron nutrition; Grape; Rootstocks

1. Introduction and scope

Iron (Fe) is one of the most studied element in mineral nutrition of plants. Although its relatively high abundance in the earth's cultivated soils, plant Fe acquisition is often impaired, a fact resulting in severe crop losses. Among the soil properties that impair Fe nutrition problems, calcium carbonate ($CaCO_3$), whose presence is widespread on 30% of total land area (Chen and Barak, 1982), plays a major role (Loeppert et al., 1994).

Cultivated plants differ as to their susceptibility to Fe deficiency in calcareous soils, some being poorly affected while others showing severe leaf chlorotic symptoms. Several perennial, deciduous, as well as evergreen fruit crops belong to the second category. When chlorotic symptoms in orchards and vineyards develop, fruit yield and quality can be severely depressed in the current year and next year fruiting as fruit buds poorly develop. Due to the relatively high profit obtained with some fruit trees and grape, because of the high specialisation of the cultivation techniques, which implies the development of special skills in the growers, orchards and vineyards are also cultivated in areas where soil conditions for Fe nutrition are sub-optimal, providing that the climate is favourable for maximising fruit quality and maintaining satisfactory yields. Under these conditions appropriate strategies, often not very friendly to the environment are, therefore, adopted for preventing the Fe-deficiency symptoms. Clearly, there is a need for designing orchard and vineyard ecosystems more sustainable in terms of Fe nutrition.

Several reviews on iron nutrition of higher plants are available, covering, among others, iron availability for root uptake (Loeppert et al., 1994; Lindsay and Schwab, 1982), Fe nutrition in calcareous soils (Chen and Barak, 1982; Mengel, 1994), plant susceptibility to Fe deficiency and plant adaptation mechanisms (Marschner et al., 1986; Jolley and Brown, 1994) prevention and correction of chlorosis (Chen and Barak, 1982; Wallace, 1991; Tagliavini et al., 2000a). Few reviews on iron nutrition of perennial fruit crops are available (see Wallace and Lunt, 1960). Since the review published by Korcak (1987), a significant amount of literature has been published and seven International Symposia on Fe nutrition held; although a relatively limited number of published papers dealt with perennial fruit crops, new scientific evidences on iron nutrition (e.g. underestimated sources of soil Fe for root uptake, adaptation strategies to Fe deficiency, leaf inactivation of iron) give more insights on iron nutrition of perennial plants.

In this review, we will concentrate on main fundamental and applied aspects of Fe nutrition of deciduous fruit crops and grapevine; while we will not try to be comprehensive of the whole existing literature we will discuss the possible development of sustainable Fe nutrition management in orchard and vineyard ecosystems.

2. Fe nutrition in orchards and vineyards

2.1. Tree physiology and iron chlorosis

Deciduous fruit species (Westwood, 1993) are grown world-wide wherever climate soil and moisture conditions are suitable: temperate deciduous species are confined mostly to the middle latitudes 30–50° in the Northern and Southern Hemispheres, and plantings of European grape (*Vitis vinifera*) are mostly concentrated in areas where isotherms vary between 10 and 20 °C. In the EU, estimates indicate that around 350000 farms are specialised in the production of fruits, of which yields account for 80% of total fruit production, the remaining 20% being accounted for the small-family sized farms (Avermaete, 1999).

A significant part of the fruit tree industry in Europe and especially in the Mediterranean area is located on calcareous or alkaline soils, which favour the occurrence of Fe chlorosis. Fruit trees and grape species differ as to their susceptibility to Fe chlorosis, but it is widely accepted that peach, pear, and kiwifruit are among the most susceptible to Fe chlorosis (Korcak, 1987). *Vitis spp*. also differ as to their susceptibility, some being very tolerant (e.g. *V. vinifera* and *V. rupestris)* others being susceptible (e.g. *V. riparia*).

Cultivated grapevines and most deciduous fruit trees are made up of two separate genotypes: the scion (cultivar), selected for its fruiting performance, and the rootstock (usually from the same species or of a botanically related one), of which root system explores the soil and provides water and nutrient to the canopy. The effect of the rootstock on scion tolerance of Fe chlorosis is well accepted. Within a given fruit species differences in Fe susceptibility among varieties exist: in pear, for example, while cvs. Abbé Fétel' and Bartlett are very susceptible, cv. Conference is quite tolerant: although such different behaviours (different Fe requirements, compatibility with quince rootstocks, different vegetative behaviour) may be explained by hypotheses, the actual causes are still unknown and the phenomenon deserves further studies.

Iron chlorosis is a more complex phenomenon in fruit trees than in annual crops (Tagliavini et al., 2000a). Deciduous fruit trees and grapes exhibit reproductive cycles starting with bud formation in one year and ending with flowering, fruit set and fruit maturity the following year. Estimates of chlorosis severity among pear orchards in the Po Valley (Northern Italy) (Scudellari, 1999, personal communication) suggest that trees bearing a large amount of fruits in one year are more prone to show a severe chlorosis development the following year. This phenomenon, in accordance with findings by Pouget (1974), has been explained considering the fact that fruits represent a strong sink for carbohydrates, of which storage at root level might be insufficient to sustain root growth and activity during growth resumption in spring. In this context it is of interest that Fe is only taken up by root tips (Clarkson and Hanson, 1980) and, therefore, the number of root tips produced by a rootstock in spring may have an influence on Fe uptake.

Another peculiar aspect of Fe nutrition in trees is related to their size and to the fact that, after absorption, Fe has to be transported for a long distance to reach the tree canopy. Problems in Fe transport through the xylem are, therefore, more likely in mature trees. Symptoms of iron chlorosis-typical interveinal yellowing or sometimes atypical uniform chlorosis as in pear-in orchards and vineyards often start as soon as buds open, likely as a result of insufficient storage of Fe, or develop throughout the vegetative season as a consequence of plant demand being excessive in respect to Fe availability. In general, however, chlorosis occurs more frequently in spring when rainfalls cause a raise in soil bicarbonate concentration (Boxma, 1982) in a period of intense Fe demand. If soil conditions then improve, new leaves appear green, but those previously chlorotic unlikely re-green. Fruit yield losses caused by leaf chlorosis also depend on the degree and the period the chlorosis develop and, in general, critical periods coincide with blooming and fruit set: this particularly applies to fruits not easy to set, like pears (*Pyrus communis*) or those like kiwifruit (*Actinidia deliciosa*), of which the final size strongly depends on seed number. A relatively low level of chlorosis is likely more acceptable at other phenological phases, especially if it is confined to parts of the canopy where only vegetative and not reproductive buds are present.

Chlorotic symptoms also vary from year to year as a result of several tree and environmental variables, like yields, temperatures, rains. In soils where shallow layers are less rich in $CaCO_3$ than deeper layers, it is likely that trees and vines develops chlorosis only when they age and roots explore layers with poor conditions for Fe uptake.

Studying the characteristics of soil profile provides a useful tool for understanding such problems. To our experience, soils which had been for many years subjected to ploughing before the plantation may present layers of fine texture, just below the ploughing depth, which could be rich in $CaCO_3$ because of leaching from more shallow layers.

Symptoms of iron chlorosis are not always uniform within a single tree, where chlorotic parts of the canopy are often present together with green branches. Little attention has been given to explaining this phenomenon, but few hypotheses can be made: (1) tree root systems exploit a relatively large volume of soil with heterogeneous characteristics, some roots developing in microsites favourable to Fe uptake while others are located in poor areas. Due to the poor phloem mobility of the iron absorbed by one root, redistribution of iron from green canopy parts to chlorotic ones is unlikely; (2) since iron is mainly transported in a non-ionic form in the xylem (Tiffin, 1970) its transport, driven by the transpiration stream, is likely not uniform. It has been reported (Tagliavini et al., 2000a) that within the same tree or vine, leaf Fe chlorosis is often more severe on second or third order branches than on those directly branching off the trunk.

Problems in transport of Fe may also arise from the fact that in some species (e.g. pear), a certain degree of scion/rootstock (usually quince) incompatibility occurs and is sometimes desirable as it allows a control of the tree size. Graft incompatibility, however, impairs both upward nutrient transportation through the xylem and downward carbohydrates replenishment to the roots through the phloem (Breen, 1975). Under these conditions, root reactions to Fe deficiency, such as the increase of Fe-reduction activity and proton extrusion (Mengel and Malissovas, 1982), may be impaired because of a lack of organic carbon supplied from the shoots.

Iron demands by mature orchards and vineyards are in the range of 650–1100 g Fe ha^{-1} per year (Gärtel, 1993): net removals are mainly accounted by amounts recovered in yields and pruning wood, if not left in the ground and chopped, while the Fe amounts in the perennial framework, with a relatively constant biomass do not significantly vary from year to year and are, therefore, negligible. For kiwifruit, fruit Fe concentrations of 33 $\mu g\ g^{-1}$ (DW) were estimated, resulting in a total removal of Fe in fruits of around 160 g ha^{-1} for a fruit production of 30 t ha^{-1}. For several flesh fruit crops (Tagliavini et al., 2000b) removals are in the range of 1–10 g t^{-1} of harvested yield. Annual removal of Fe by pruning wood were estimated by Abadia et al. (personal communication, 2000) for peach trees in Northern Spain being in the order of 150 g Fe ha^{-1} and similar Fe amounts were estimated returning to the soil through the leaves after their abscission.

2.2. Soil iron availability

As previously described, annual removals of iron in orchards and vineyards are relatively low. Total amounts of iron in cultivated soils, in theory would not justify the development of iron deficiency, which, nevertheless, often occurs as a result of poor availability of iron for plants. Several soil-related characteristics may lead to development of iron chlorosis (Table 1).

The prediction of risks of future development of iron chlorosis in a plantation is of great importance in fruit tree and grape industry and should lead to the correct choice of the rootstock to be used. Mistakes at this stage would make unlikely the achievement of satisfactory yields without agronomic and chemical means for correcting the chlorosis throughout the life span of the orchard or vineyard. Due to the number of soil factors that impair Fe nutrition, it is not always easy to predict the possible chlorosis development of a perennial crop on the basis of a single soil parameter. Soil pH is often a useful but not sufficient parameter: it is well known that fruit crops adapted to acidic soils quickly develop chlorosis at sub-alkaline or alkaline conditions (e.g. blueberry, raspberry, kiwifruit) while other genotypes are more able to cope with high soil pH (likely through an inherent ability to lower root apoplastic pH), unless the soil is also calcareous and, therefore, buffered in the range of 7.5–8.5 (Loeppert et al., 1994). Total lime, however, is not particularly useful for predicting the development

Table 1
List of causes of Iron chlorosis development in orchards and vineyards

Constraint to Fe nutrition	Mechanism
Soil related factors	
High soil pH	Poor availability of Fe Impairment of Fe-reduction
Presence of $CaCO_3$	Buffering effect on rhizosphere and root apoplast pH Possible direct effects of bicarbonate
Poor soil aeration and soil compaction	Poor root development and activity
Low root zone temperature	Limitation of Fe uptake rates
Irrigation with water rich in bicarbonate	Alkalinisation of the soil Increase of bicarbonate in the rhizosphere
Excessive nitrate–N availability	Increase of rhizosphere pH Increase of leaf apoplast pH in species transporting NO_3 to the canopy Stimulation of vegetative growth, increasing needs for iron uptake
Low level of soil organic matter and poor biological fertility of soils	Decrease of the availability of sources of organic-Fe
Tree related factors	
Pathogen infection	Impairment of carbohydrate availability and root Fe uptake Possible effect of sequestration of Fe within the plant by microorganisms
Excessive fruit yield the previous year	Poor winter storage of C skeletons Impairment of root development after growth resumption in spring leading to poor root Fe uptake
Damages to the root system (e.g. caused by soil tillage)	Losses of potential for Fe uptake
Grafting incompatibility between scion and rootstock	Unsufficient replenishment of organic carbon to the root system

of iron chlorosis, while the fine, clay-sized, fraction of $CaCO_3$, active carbonate or active lime (Drouineau, 1942), is more reactive and, therefore, able to build and maintain high levels of HCO_3^- in the soil solution (Inskeep and Bloom, 1986), and is, therefore, often a more reliable indicator. Species are ranked according to the level of active lime at which they start to develop chlorotic symptoms: very susceptible species or genotypes like quinces and kiwifruit, do not tolerate even low concentrations of active carbonate (< 50 g kg^{-1}), while many *Vitis* genotypes can be cultivated in soils with 100–150 g kg^{-1} active carbonate. Evidence obtained in grape suggests that a single genotype may tolerate higher levels of active lime if the amounts of available iron in the soil increase to a certain level. This concept resulted in the 'chlorotic power index' proposed by Pouget (1974) where the amount of active lime is related to the amount of Fe extracted by ammonium oxalate. It must be stressed, however, that the determination of active lime is only an indirect estimate of the amount of fine textured $CaCO_3$, as it actually indicates the amount of free calcium reacting with oxalic acid: caution, therefore, must be taken in using such a method in soils where potential sources of carbonates other than $CaCO_3$, e.g. dolomite, are present.

Iron in soil is largely present in an inorganic form, predominantly as amorphous Fe, goethite, hematite and ferrihydrite and is little available for plant uptake under aerobic conditions. Therefore, to predict soil Fe availability, a series of extraction solutions of soil available iron have been proposed (HCl, DTPA, etc.). Due to the fact that the solubility of Fe-oxides is pH dependent, under alkaline and calcareous soils inorganic Fe availability is far below that required to satisfy plant demand (Lindsay, 1974). One of the main recent experimental evidence concerning major impact on Fe nutrition is the key role played by forms of root available Fe other than inorganic-Fe. At least three main groups of Fe compounds, whose role for iron nutrition is likely underestimated in orchards and vineyards, can be listed: iron chelated by microbial siderophores, iron chelated by phytosiderophores and Fe complexed by organic matter. The role of microbial siderophores on iron acquisition by roots has been demonstrated by studies by Crowley et al. (1991, 1992) and recently Masalha et al. (2000) have shown that destroying soil microflora by sterilisation resulted in severe impairments of Fe nutrition of both strategy I (species which do not produce phytosiderophores in their roots, Römheld and Marschner, 1986). A number of siderophores have been isolated (Yehuda et al., 2000; Crowley, 2001), some of them produced by microorganisms present in cow manure (Chen et al., 1998). Recent experimental data (Chen et al., 2000) indicate that, at least in some Strategy I species, the iron chelated by siderophores (Fe-aerobactin present in the compost) is taken up directly as a complex. Although the study of the role of phytosiderophores in Fe uptake has been almost exclusively restricted to strategy II plants (species synthesising siderophores in their roots), some evidence indicates that they might improve iron uptake of tree roots grown in the same soil volume (Tagliavini et al., 2000a). A third group of natural Fe chelates is that deriving from complexation of Fe by humic substances. As recently shown by Cesco et al. (2000), water extractable humic substances (WEHS) are able to solubilise Fe from hydroxide and make it available for root uptake (Pinton et al., 1999).

2.3. Iron acquisition in perennial fruit plants

Most studies on Fe acquisition mechanisms by plants have been carried out with herbaceous annual plants, whereas this topic in perennial plants has been less investigated and significant advances came only in the last decade. The uptake of Fe is preceded by a reduction step from Fe^{3+} to Fe^{2+} before it can cross the plasmamembrane of outer root cells (Crowley et al., 1991; Fox and Guerinot, 1998). A ferric chelate reductase (FCR) and proton pump activities, along with the release of reductants into the rhizosphere have long been considered as the main components of the Strategy I-based plants (Römheld and Marschner, 1986), therefore, studies on Fe acquisition mechanisms in perennial dicots have been focussed on these physiological and biochemical parameters (Alcántara et al., 2000; Ao et al., 1985; Bavaresco et al., 1991; Brancadoro et al., 1995; Cinelli, 1995; Cinelli et al., 1995; De la Guardia et al., 1995; Egilla et al., 1994; Gogorcena et al., 2000; Manthey et al., 1993, 1994; Manthey and Crowley, 1997; Marino et al., 2000; Romera et al., 1991a,b; Tagliavini et al., 1995a; Treeby and Uren, 1993; Vizzotto et al., 1997, 1999). Many of these studies have indicated that, similarly to herbaceous dicots, some woody genotypes are able to improve Fe acquisition through adaptation mechanisms, like root apoplast acidification and the increase of root enzymatic Fe reduction, whereas the release of reducing compounds (e.g. caffeic acid) plays only a minor role. In general, rootstock tolerance to Fe chlorosis was associated to the ability of reducing external Fe^{3+}; for example, upon induction of Fe deficiency, a clear increase of the root Fe reducing capacity has been observed in tolerant rootstocks used for citrus (Manthey et al., 1994; Treeby and Uren, 1993) and peach (Romera et al., 1991b). On the contrary, FCR activity of Fe-chlorosis sensitive *Citrus* and peach rootstocks was lower in Fe deficient than in Fe sufficient plants. Even in tolerant genotypes, however, it should be noted that the increase of FCR activity recorded in many studies was not as great as that found in some annual plants. Table 2 shows FCR activities of two genotypes used as rootstocks for pear, *Cydonia oblonga* and *Pyrus communis*, the first susceptible, the second tolerant to iron

Table 2
Effects of the presence (+Fe as Fe–EDTA at 50 μM) or absence (−Fe) of Fe and of the presence of $CaCO_3$ (2 g l^{-1}) in the growing medium on root Fe-chelate reductase (FCR) activity of pear and quince genotypes

	FCR (nmol Fe^{2+} g^{-1} FW min^{-1})		
	+Fe	−Fe	$CaCO_3$
Pear C 91	3.18	6.74	1.18
Quince C	2.02	1.13	0.05
Treatment		*	
Genotype		**	
Treatment × genotype		*	
S.E.M.		1.07	

Rombolà, Dallari and Tagliavini, unpublished.
*, ** indicate significance at 5 and 1% levels of probability.
SEM = Standard error of the interaction mean ($n = 5$).

chlorosis. While Fe deficiency increased FCR activity in pear, a similar response was not found in quince plants. The presence of bicarbonate also differently affected FCR activity, which was more depressed in quinces than in pears, an effect likely due to the higher ability of *P. communis* to decrease rhizosphere pH as compared with *C. oblonga* (Tagliavini et al., 1995a).

The determination of the iron-reducing capacity of roots has been used as a screening technique for selecting Fe chlorosis tolerant rootstocks (Gogorcena et al., 2000); recent findings, however, suggest caution when using FCR activity as marker for selecting rootstocks for iron tolerance and according to Alcántara et al. (2000) the Fe^{3+} root reducing activity is not always related to Fe chlorosis tolerance. At least three factors should be taken into account when assessing differences among genotypes for Fe chlorosis tolerance: (1) the time-course variation of FCR after Fe withdrawal, which depends on species (Moog and Brüggemann, 1994); (2) the ability to maintain the induction of FCR for relatively long period (Romera et al., 1991b; Rombolà et al., 1998), as it has been shown that a prolonged exposure to Fe-deficiency decreases Fe reduction capacity also in tolerant genotypes (Tagliavini et al., 1995a); (3) some methodological issues which should be taken into account when comparing studies: as recently shown for the peach/almond GF677 hybrid by Alcántara et al. (2000), FCR is more likely to be induced by very low Fe concentrations than by absolute Fe absence in the nutrient solution, which, however, had been applied in most published studies.

Iron tolerant and sensitive rootstocks for fruit trees and grape also vary as to their ability to decrease nutrient solution or the rhizosphere pH. In a study conducted with different quince and pear genotypes grown in a calcareous soil, it was shown that the root–soil interface was more alkaline with quinces than with pear genotypes (Tagliavini et al., 1995a). Under Fe-stress condition, *M. xiaojinensis*, an Fe-efficient species, decreased the rhizosphere pH by around 2 U in a calcareous soil (Han et al., 1994, 1998). Data indicating higher proton extrusion in Fe chlorosis tolerant than in iron susceptible genotypes have been reported in nutrient solution studies for *Actinidia* (Vizzotto et al., 1999) and blueberry (Brown and Draper, 1980). The rootstock ability to release protons is also affected by the presence of the scion. This phenomenon has been investigated by Mengel and Malissovas (1982) who studied in solution culture the H^+ excretion by roots of the grapevine cultivars Huxel and Faber, both grafted on rootstock Kober 5BB: Huxel showing severe Fe chlorosis when grown on calcareous soils while Faber is chlorosis-resistant. The latter cultivar (Faber) caused a root excretion of 406 μmol H^+ per plant per 12 h compared with only 173 μmol H^+ per plant per 12 h recorded when the scion was Huxel.

Excretion of H^+ by roots into the rhizosphere of calcareous soils has likely no major influence on soil Fe dissolution (Hauter and Mengel, 1988). Even if roots were able to lower the rhizosphere pH at the level of 6.0, inorganic Fe dissolution is still so poor that by far it will not meet the plant Fe demand (Lindsay, 1974). The importance of H^+ release by roots is due to depressing the pH in the root apoplast by neutralizing HCO_3^- and thus providing better conditions for the reduction (Mengel, 1994) of iron, which in a calcareous soil is trapped in the apoplast of root epidermis (Kosegarten and Koyro, 2001).

Adaptative strategies to acquire iron is not confined to proton extrusion and FCR; recent literature (Abadía et al., 2001; De Nisi and Zocchi, 2000; Rombolà, 1998; Rombolà et al., 1998) has suggested a role of the enzyme phosphoenolpyruvate carboxylase (PEPC) within the adaptive mechanisms to cope with Fe deficiency in calcareous soils. Phosphoenolpyruvate carboxylase incorporates bicarbonate, the main causal factor of Fe chlorosis (Mengel, 1994) into phosphoenolpyruvate, generating oxalacetate, which can be easily converted into malate. Oxaloacetate is also a precursor of citrate, which plays an essential role in xylem Fe transport (Tiffin, 1970). In sugar beet, a species tolerant to iron chlorosis, the increase in PEPC activity was 40-fold higher in root tips of plants grown without Fe than in the controls (López-Millán et al., 2000). In *Actinidia*, the differential tolerance to iron chlorosis of two genotypes was associated with a stimulation of the enzyme PEPC in the roots and with an upward flow of organic acids in the xylem (Rombolà et al., 1998). Accumulation of organic acids in Fe deficient plants is often reported for both herbaceous dicots (Abadía et al., 2001), as well as in woody perennials like apple (Sun et al., 1987), grapevine (Brancadoro et al., 1995), kiwifruit (Rombolà et al., 1998) and quinces (Marino et al., 2000).

2.4. Inactivation of iron in the leaves

Although, even in a calcareous soil a significant part of total iron is located at root level, both precipitated outside the roots and trapped in the root apoplast (Kosegarten and Koyro, 2001), it is still unclear what proportion of the absorbed Fe is retained at root level and how much is transported to the canopy where it undergoes a second reduction before entering the mesophyll cells. Iron availability for reduction in the leaf apoplast is a second important step necessary before iron can be available by leaf cells. In a chlorotic leaf, the existence of Fe pools which are somehow inactivated has been proposed by Mengel (1994) and demonstrated by several authors (Kosegarten and Englisch, 1994; Kosegarten et al., 1999a, 2001; Tagliavini et al., 1995b, 2000a), a fact suggesting that part of the Fe coming from the roots does not pass the leaf plasmamembrane and may be confined to the apoplast. Reasons for inactivation of Fe in the apoplast are still being debated. To some authors (Römheld, 2000) Fe inactivation is a side (secondary) effect occurring in a leaf after the occurrence of Fe chlorosis: a high HCO_3^- concentration in the soil would lead to a decrease in the uptake and availability of iron for canopy growth, so the higher Fe concentration in chlorotic leaves would be the final consequence of the leaf growth inhibiting effect of bicarbonate. Other authors consider iron inactivation of major importance for the development of iron chlorosis and have identified some responsible factors. According to Mengel (1994) the poor efficiency of iron in leaf tissues is primary related to the high pH of the leaf apoplast under alkaline conditions that would impair Fe^{3+} reduction by mesophyll cells and consequently depress Fe transport across the plasmalemma.

More insights into apoplast pH and Fe^{3+} reduction derived from studies of Kosegarten et al. (1999a) who, after developing a technique for in vivo measurements, have found that alkaline apoplast pH in different leaf portions may attain values (6.3–7.0) able to depress Fe^{3+} reduction. The relationship between chlorosis and apoplast pH is also confirmed by data of López-Millán et al. (2001) who found that the pH of leaf apoplast from field-grown pear trees increased from 5.5–5.9 to 6.5–6.6. Kosegarten et al. (2001) suggest that nitrate, almost the only form of nitrogen available for uptake in calcareous soils, plays a key role in the alkalisation of leaf apoplast. As many fruit trees reduce the nitrate at root level and transport N as amino acids in the xylem sap (Faust, 1989), other processes than nitrate uptake into leaf cells may be involved in the apoplast alkalisation and in leaf Fe inactivation.

Optimum values of pH for Fe^{3+} reduction in intact leaf apoplast were obtained at apoplastic pH 5 (Kosegarten et al., 1999a,b) and in experiments with leaf protoplasts, which likely relates to optimum pH at the apoplast side of the plasmamembrane-located Fe^{3+} reductase, was estimated at approximately 5.5–6.0 (González-Vallejo et al., 2000; Susin et al., 1996).

The theory of Fe inactivation due to alkalisation of apoplast fits with experimental data obtained under field conditions, showing re-greening of chlorotic leaves as a result of spraying acidic solutions to the canopy (Sahu et al., 1987; Tagliavini et al., 1995b). Kosegarten et al. (2001) spraying chlorotic sunflower leaves with citric acid recovered an apoplastic pH drop from 5.5 to 5.0, and found a re-greening of leaves, whose Fe concentration remained on the same level.

Whether bicarbonate exerts a direct effect on leaf Fe inactivation is still discussed and deserves further attention. Experiments by Rutland (1971) demonstrated that the presence of bicarbonate in the medium alters the normal distribution of ^{59}Fe, which does not reach interveinal laminar areas, while accumulates in veins and the surrounding tissues. Relatively high bicarbonate concentrations (up to 1.8 mM) were found in the xylem sap of woody plants like *Populus deltoides* (Stringer and Kimmerer, 1993). According to other authors, at standard apoplast pH values of 5.5–6.0, however, bicarbonate should not be present (Lucena, 2000).

During the occurrence of iron chlorosis the citrate: Fe ratio in the leaf apoplast significantly increases as found in faba bean (Nikolic and Römheld, 1999) and sugar beet (López-Millán et al., 2000). As FCR activity is adversely affected by increases of citrate: Fe ratio (López-Millán et al., 2000), it is likely that accumulation of Fe in the leaf apoplast may, at least partially, derive from high citrate concentrations in the leaf apoplast (Abadía et al., 2001). At increasing pH values (Spiro et al., 1967) ferric citrate may form large citrate polymers and also could be a cause for the depressed Fe^{3+}-citrate reduction. One may speculate whether the increase of citrate concentration in the xylem sap of chlorotic plants is related to the uptake of HCO_3^- by roots and its reaction with phospho-enol pyruvate leading to oxaloacetate which is a precursor of citrate synthesis.

Using an ecological approach in their experiments, Zohlen and Tyler (2000) suggested that calcicole and calcifuge species have different ability to use the iron taken up by roots. With some exceptions, although iron uptake by calcifuges is not restricted even in calcareous soils, they, in contrast to calcicoles, are not able to retain the Fe in a metabolically active form and leaves turn chlorotic.

3. The choice of tolerant rootstocks to prevent iron chlorosis

The use of Fe chlorosis tolerant genotypes as rootstocks in orchards and vineyards represents a reliable solution to prevent iron chlorosis. This approach has been reviewed by Socias i Company et al. (1995). Although genetical differences in the tolerance to iron chlorosis in fruit tree species resulted in the selection of rootstocks suitable for alkaline and calcareous soils, in many cases Fe chlorosis resistant rootstocks are not very attractive from an agronomic point of view, since they often induce excessive growth of the scion (e.g. *P. communis* seedlings for pear varieties and 'Fercal' for grape) and reduce fruit yield. Classical methods of plant breeding and new biotechnologies are being adopted to incorporate traits of resistance to lime induced iron chlorosis of wild relatives naturally growing in calcareous soils into new, easy to propagate, rootstocks. Table 3 briefly describes the behaviour of the main rootstocks for fruit trees and grape in relation to iron chlorosis. More detailed information on performances of iron chlorosis tolerant rootstocks is available in Rom and Carlson (1987) for fruit trees and in Coombe and Dry (1988) for grape.

4. Agronomic and chemical means to prevent and cure Fe chlorosis

4.1. Synthetic Fe chelates

As most fruit tree crops and grapes are high value commodities, in many countries growers are often willing to apply synthetic Fe chelates to cure or to prevent the occurrence of Fe deficiency. Iron chelates are still expensive, although not as much as in the past and are not affordable for most growers in developing countries. Estimates made in Southern Europe indicate that Fe chelates represent up to 60% of the total fertiliser costs and often amount to more than 250 Euros per ha per year. Several iron chelates are available, those mainly used being ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) characterised by a low stability constant and adopted for foliar applications and ethylenediamine-di(*o*-hydroxy-phenylacetic) acid (EDDHA), ethylenediamine-di(*o*-hydroxy-*p*-methylphenylacetic) acid (EDDHMA) and ethylenediamine-di(*o*-hydroxy-*p*-sulphoxyphenylacetic) acid (EDDHSA), with higher stability constants ranging from 35 to 39 (Lucena et al., 1996) for soil supply. To be effective for improving Fe nutrition, a soil applied Fe-chelate should be stable in calcareous/alkaline soil, however, excessively high stability constants might make the iron poorly available for the FCR and hence impair root Fe uptake (Lucena et al., 2000).

Table 3
Susceptibility to iron chlorosis of main genotypes (rootstocks) for fruit trees and grape

Crop	Rootstock species	Genotype	Degree of susceptibility	Reference
Apple	*Malus baccata*		Susceptible	Han et al., 1994, 1998
	M. pumila	MM 106	Highly tolerant	Socias i Company et al., 1995
		M 9	Moderately tolerant	CTIFL, 1989
		M 27 MM 111	Slightly susceptible	CTIFL, 1989
		M 26	Susceptible	CTIFL, 1989
	M. micromalus		Moderately tolerant	Han et al., 1994, 1998
	M. transitoria		Slightly susceptible	Han et al., 1994, 1998
	M. xiaojinensis		Tolerant	Han et al., 1994, 1998
Apricot	*Prunus armeniaca* (apricot)	Common seedling	Tolerant	Loreti, 1994
	P.cerasifera (Myrobalan)	Myrabolan seedling Myrabolan B	Tolerant	Loreti, 1994
		Ademir (Myzobolan 599 AD)	Tolerant	Moreno et al., 1995a
	P. insititia (St. Julien)	Pixy	Slightly susceptible	Audubert et al., 1994; Loreti 1994
	P. cerasifera × *P. munsoniana* (Marianna)	G.F. 8-1	Highly tolerant	Loreti, 1994
	Prunus domestica × *P. spinosa*	Damas 1869	Highly tolerant	Loreti, 1994
	P. cerasifera × *P. salicina*	G.F. 31	Slightly susceptible	Audubert et al., 1994
	P. cerasifera X (*P. cerasifera* × *P. persica*)	Ishtara	Susceptible	Loreti, 1994
	P. cerasifera × *P. persica*	Myran	Susceptible	Loreti, 1994
	P. insititia × *P. domestica*	Julior	Susceptible	Loreti et al., 2000
	P. salicina × *P. persica*	Citation	Susceptible	Loreti et al., 2000
	P. persica (peach)	P.S.A 5	Susceptible	Loreti et al., 2000
		Montclar	Slightly susceptible	Loreti et al., 2000
Cherry	*Prunus avium* (Sweet cherry)	Common seedling Fercadeau- Mazzard F 12/1	Slightly susceptible	CTIFL, 1990; Loreti, 1994
	P. cerasus (Sour cherry)	Masto de Montañana	Tolerant	Socias i Company et al., 1995
		CAB 6 P CAB 11 E	Moderately tolerant	Loreti, 1994
		Tabel-Edabriz	Slightly susceptible	CTIFL, 1990; Loreti, 1994; Socias i Company et al., 1995

Table 3 (*Continued*)

Crop	Rootstock species	Genotype	Degree of susceptibility	Reference
	P. mahaleb (Mahaleb**)**	Common seedling SL 64	Highly tolerant	CTIFL, 1990; Loreti, 1994; Okie, 1983; Moreno et al., 1995b, 1996
		Ferci-Pontaleb	Tolerant	Loreti, 1994
	P. avium × *P. pseudocerasus*	Colt	Susceptible	CTIFL, 1990; Loreti, 1994; Moreno et al., 1995a,b, 1996; Socias i Company et al., 1995
	P. pandora × *P. subhirtella*	GM 8	Highly susceptible	CTIFL, 1990
	P. incisa × *P. serrula*	GM 9 Inmil	Slightly susceptible	CTIFL, 1990; Socias i Company et al., 1995
	P. avium × *P. mahaleb*	MA × MA 14	Moderately tolerant	CTIFL, 1990; Loreti, 1994
	P. dawyckensis	GM 61/1 Damil	Tolerant	Loreti, 1994
	P. canescens	GM 79 Camil	Moderately tolerant	Loreti, 1994
	P. cerasifera	Adara	Highly tolerant	Moreno et al., 1995b, 1996
Citrus	*Citrus aurantium* (sour orange)		Moderately tolerant	Geraci, 1994; Treeby and Uren, 1993
	C. sinensis (sweet orange)		Susceptible	Geraci, 1994; Treeby and Uren, 1993
	C. limettioides (palestine sweet lime)		Moderately tolerant	Geraci, 1994
	C. limonia (rangpur lime)		Tolerant	Geraci, 1994; Manthey et al., 1994; Treeby and Uren, 1993
	C.macrophylla (Alemow)		Highly tolerant	Geraci, 1994; Manthey et al., 1994
	C. jambhiri (rough lemon)		High tolerant	Geraci, 1994; Manthey et al., 1994; Treeby and Uren, 1993
	C. volkameriana (Volkamer lemon)		Tolerant	Geraci, 1994
	C. reshni (*Cleopatra mandarin*)		Slightly susceptible	Geraci, 1994; Treeby and Uren, 1993
	P. trifoliata (trifoliate orange)		Highly susceptible	Geraci, 1994; Korcak, 1987; Manthey et al., 1994
	P. trifoliata × *C. sinensis* (citrange)	Troyer	Slightly susceptible	Geraci, 1994; Korcak, 1987; Manthey et al., 1994; Treeby and Uren, 1993
		Carrizo	Susceptible	Geraci, 1994; Korcak, 1987; Manthey et al., 1994; Treeby and Uren, 1993
	P. trifoliata × *C. paradisi* (Citrumelo)	Swingle	Highly susceptible	Geraci, 1994; Manthey et al., 1994
Grape	*Vitis berlandieri*		Tolerant	Brancadoro et al., 1995
	V. berlandieri × *V. riparia*	420 A	Tolerant	Chauvet and Reynier, 1979; Saracco, 1992
		Kober 5BB, 225 Ruggeri, SO 4	Moderately tolerant	Chauvet and Reynier, 1979; Saracco, 1992

Table 3 (*Continued*)

Crop	Rootstock species	Genotype	Degree of susceptibility	Reference
	V. berlandieri × *V. rupestris*	140 Ruggeri	Highly tolerant	Chauvet and Reynier, 1979; Saracco, 1992
		775 Paulsen, 1103 Paulsen, 110 Richter	Moderately tolerant	Chauvet and Reynier, 1979 Saracco, 1992
		779 Paulsen	Tolerant	Saracco, 1992
	V. riparia		Highly susceptible	Brancadoro et al., 1995; Chauvet and Reynier, 1979; Saracco, 1992
	V. rupestris		Slightly susceptible	Chauvet and Reynier, 1979; Saracco, 1992
	V. vinifera		Highly tolerant	Brancadoro et al., 1995
	V. vinifera (cv. Chasselas) × *V. berlandieri*	41 B	Highly tolerant	Chauvet and Reynier, 1979; Saracco, 1992
	V. berlandieri × (*V. vinifera fercal* × *V. berlandieri*)		Highly tolerant	Chauvet and Reynier, 1979
Kiwifruit	*Actinidia deliciosa*	Hayward (micro-propagated)	Susceptible	Pelliconi and Spada, 1992; Vizzotto et al., 1997
		Hayward (cuttings)	Highly susceptible	Pelliconi and Spada, 1992
		D1, Bruno	Slightly susceptible	Pelliconi and Spada, 1992; Viti et al., 1990
Peach	*Prunus persica* (peach)	ID 20, Lovell, Montclar	Slightly susceptible	Almaliotis et al., 1995; Byrne et al., 1990; Loreti, 1994; Rashid et al., 1990 Socias i Company et al., 1995
		Seedling, GF 305, Missour, P.S. A5, P.S. A6, P.S. B2, Siberian C	Susceptible	Byrne et al., 1990; Loreti, 1994; Peterlunger et al., 1988; Rom, 1983; Socias i Company et al., 1995
		Higama Nemared Okinawa	Highly susceptible	Byrne et al., 1990; Loreti, 1994; Socias i Company et al., 1995
	P.cerasifera (Myrobalan)	Mr.S. 2/5, Mr.S 1/3, Mr.S 1/6, Mr.S 2/8	Tolerant	Loreti, 1994Loreti and Massai, 1998
	P. domestica (European plum)	Brompton	Moderately tolerant	Byrne et al., 1990; Socias i Company et al., 1995
		GF 43	Slightly susceptible	Byrne et al., 1990; Loreti, 1994; Socias i Company et al., 1995
	P. insititia (St. Julien)	Adesoto 101 (Puebla de Soto AD 101)	Highly tolerant	Byrne et al., 1990; Moreno et al., 1995a; Socias i Company et al., 1995
		GF 655/2, S. Julien A	Susceptible	Byrne et al., 1990; Loreti, 1994; Rom, 1983
	Prunus domestica × *P. spinosa*	Damas 1869	Highly tolerant	Byrne et al., 1990; Loreti, 1994; Socias i Company et al., 1995

Table 3 (*Continued*)

Crop	Rootstock species	Genotype	Degree of susceptibility	Reference
	P. insititia × *P. domestica*	S. Julien 1, S. Julien 2	Moderately tolerant	Byrne et al., 1990; Socias i Company et al., 1995
		Julior	Susceptible	Byrne et al., 1990; Socias i Company et al., 1995
	P. persica × *P. amygdalus*	Adafuel, Adarcias, GF 677, Hansen 536, Hansen 2168, Titan hybrids	Highly tolerant	Almaliotis et al., 1995; Byrne et al., 1990; Cinelli et al., 1996; Loreti, 1994; Moreno and Cambra, 1994; Syrgiannidis, 1985; Socias i Company et al., 1995
		I.S. 5/18, I.S. 5/29	Highly tolerant	Cinelli et al., 1996
		I.S. 5/22 (Sirio)	Tolerant	Cinelli et al., 1996; Loreti, 1994
		I.S. 5/31, I.S. 5/23	Moderately tolerant	Cinelli et al., 1996
		I× Ne 2	Moderately tolerant	Almaliotis et al., 1995
	P. persica × *P. davidiana*	Nemaguard	Highly susceptible	Byrne et al., 1990; Loreti, 1994; Socias i Company et al., 1995
	P. cerasifera × *P. persica*	*Myran*	Slightly susceptible	Byrne et al., 1990; Socias i Company et al., 1995
	P. cerasifera X (*P. cerasifera* × *P. persica*)	Ishtara	Susceptible	Byrne et al., 1990; Socias i Company et al., 1995
Pear	*Pyrus communis* (pear)	Fox 11 (A 28), Fox 16 (B 21), OH × F 51, OH × F 69, OH × F 87, OH × F 333, Pyriam Pyrodwarf (Rhenus 1)	Tolerant	Bassi et al., 1994, 1998; Jacob, 2001; Loreti, 1994; Monney and Eveguoz, 1999; Simard and Michelesi 2001; Socias i Company et al., 1995; Tagliavini et al., 1993, 1992, 1995a; Lombard and Westwood, 1987
		Bartlett (ownrooted)	Moderately tolerant	Tagliavini et al., 1995a
		Abbé Fétel (ownrooted)	Slightly susceptible	Tagliavini et al., 1995a
	Other *Pyrus* species			
	P. amygdaliformis, *P. caucasica*, *P. cordata*, *P. elaegrifolia*, *P. gharbiana*, *P. longipes*, *P. syriaca*		Highly tolerant	Westwood and Lombard, 1983; Lombard and Westwood, 1987; Procopiou and Wallace, 2000
	P. regeli, *P. salicifolia*		Tolerant	Lombard and Westwood, 1987
	P. betulaefolia, *P. nivalis*		Moderately tolerant	Westwood and Lombard, 1983; Lombard and Westwood, 1987; Loreti, 1994
	P. calleriana, *P. dimorphophylla*, *P. fauriei*, *P. pashia*, *P. pyrifolia*, *P. ussuriensis*		Slightly susceptible	Westwood and Lombard, 1983; Lombard and Westwood, 1987; Korcak, 1987; Loreti, 1994

Table 3 (*Continued*)

Crop	Rootstock species	Genotype	Degree of susceptibility	Reference
	P. serotina		Susceptible	Korcak, 1987
	Cydonia oblonga (quince)	Ct.S. 212	Slightly susceptible	Loreti, 1994
		BA 29	Susceptible	Bassi et al., 1998; Marino et al., 2000; Monney and Eveguoz, 1999
		Adams, EMA, EMC, Sydo	Highly susceptible	Bassi et al., 1998; Monney and Eveguoz, 1999
Plum	*Prunus cerasifera* (Myrobalan)	Myrobalan seedlings, Myrobalan B, Myrobalan 29 C	Tolerant	Loreti, 1994
		Ademir (Myrabolan 599 AD)	Tolerant	Moreno et al., 1995b
		Mr.S 2/5	Tolerant	Loreti, 1994
	P. cerasifera × *P. munsoniana* (Marianna)	G.F. 8-1	Highly tolerant	Loreti, 1994; Okie, 1983
	P. cerasifera × *P. salicina*	G.F. 31	Moderately tolerant	Loreti, 1994
	P. cerasifera X (*P. cerasifera* × *P. persica*)	Ishtara	Susceptible	Loreti, 1994
	P. insititia (St. Julien)	Pixy	Moderately tolerant	Loreti, 1994
Walnut	*Juglans regia* (English or Persian walnut)		Susceptible	Catlin and Schreader, 1985
	J. hindsii (Northern California black walnut)		Susceptible	Catlin and Schreader, 1985
	J. hindsii × *J. regia*	Paradox	Moderately tolerant	Catlin and Schreader, 1985

Being soluble and preventing Fe from precipitation, soil applied Fe chelates are easily leached out of the root zone if excessive irrigation regimes are applied or during the autumn-winter period when rains overcome evaporation of water from soils. Iron chelates applied in one year, therefore, usually do not prevent chlorosis in the following year and an annual application of Fe chelates is required.

The fate of chelating agents and Fe chelates in soils has received only little attention, although it is likely that their leaching may rise environmental problems of water pollution. An underestimated problem related to synthetic chelates is their potential to bind heavy metals which may be leached out.

The question related to the quality of the Fe–EDDHA products available on the market has raised significant attention in the recent past and is still being debated. The major point is connected to the technology applied for obtaining the Fe chelate which may result in different concentrations of the *ortho-ortho* isomer (the only form with agronomic value) in the commercial products (Hernández-Apolaza et al., 1997, 2000), a fact that likely explains their differential potential to solve the chlorosis problem. Using ion pair HPLC method, Hernández-Apolaza et al. (1997) reported that none of the tested commercial products reached their nominal or legal composition and several products did not meet the requirements of the 76/116/EC directive, which fixes the parameters that Fe chelates should meet to be commercialised.

Synthetic Fe chelates are either applied to the soil or sprayed on the canopy of the trees. The first approach aims to enhancing Fe availability for uptake at root level and represents an efficient means providing that roots are able to take it up and that Fe transport and utilisation in the leaves is efficient. Soil applied chelates are uneffective when applied too early in the spring when soil temperature is too low or when root uptake is impaired by waterlogging. Foliar applications of Fe chelates are used as alternative to soil Fe supply or to integrate the latter to provide a more rapid leaf Fe availability during special phenological stages. Due to the fact that Fe mobility in the phloem is quite low, during active stage of shoot development, repeated leaf sprays have to be made in order to meet the Fe requirement of developing leaves. Iron chelates are usually more effective for preventing than curing the chlorosis: once the leaves are already chlorotic, the Fe may hardly pass the leaf plasmamembrane, possibly as a result of unfavourable conditions to reduce Fe^{3+}.

Significant enhancement in the efficiency of Fe uptake from chelates, allowing reduction in the amounts distributed, can be achieved by improving the technology related to their distribution and choosing the best application timing. Main developmental stages of the reproductive cycle occur both in summer-autumn in one year and after growth resumption in spring the following year. From the previous considerations it should be clear that enhancing Fe availability for uptake during short period after single application of Fe chelates is not a guarantee for an efficient prevention of Fe chlorosis. Repeated additions of small amounts of Fe chelates with the irrigation water (by drip or microsprinkler systems) maintain Fe availability in the portion of soil where most roots are located sufficiently high to meet tree demand and avoid chlorosis. This system, besides allowing a significant reduction in the total amount of synthetic chelates to be distributed, is flexible, so that rates of Fe supply can be adjusted to the crop needs.

The relative importance of the Fe uptake during growth resumption in spring and the storage of Fe in the perennial framework during the previous vegetative season is shown in Table 4 for *Actinidia deliciosa*. In this experiment we have demonstrated for two years and in two separate orchards that late summer-early fall applications of Fe chelates were more effective than those carried out before bud burst in preventing the occurrence of Fe chlorosis during the first month after bud burst in spring. The reasons for such a phenomenon are not fully understood, but we may speculate that enhancing Fe nutrition in year one has led to more Fe to be stored in the shoots or directly in the roots (Mengel, 1994), and successively remobilised to the shoots during growth resumption in spring.

4.2. Other iron containing compounds

Ferrous sulphate, either applied to the soil or to the tree (leaf treatments, trunk injection), has been the major therapy against Fe chlorosis from the first description of this nutritional disorder until the introduction of Fe synthetic chelates and is still widely used by fruit growers especially in the developing countries due to its low costs. If supplied alone, however, soil applied Fe(II)sulphate is of little or no agronomic value in calcareous soils where the Fe^{2+} is subject to rapid oxidation and insolubilisation as hydroxide. For example, Fe sulphate was not effective for curing Fe chlorosis in *A. deliciosa* in a soil with a high $CaCO_3$ content (32%), while a quite complete recovery was achieved by Fe–EDDHA (Loupassaki et al., 1997).

The effectiveness of soil applied Fe sulfate may be improved by combining iron sulphate with organic substrates able to complex the Fe (e.g. animal manures, sewage sludges, compost, peat, plant residues). Canopy applied Fe sulphate also represents a valuable, inexpensive, alternative to foliar applied synthetic Fe chelates (Tagliavini et al., 2000a) to cure iron chlorosis.

Data of Del Campillo et al. (1998) and Rosado et al. (2000) show that $Fe_3(PO_4)_2 \cdot 8H_2O$, a synthetic iron(II)-phosphate analogous to the mineral vivianite, is a promising compound in preventing iron chlorosis in pear and olive trees grown in the field. When compared with synthetic Fe chelates, soil injection of vivianite was slightly less effective, but showed a more lasting impact. Vivianite application has the advantage of being relatively inexpensive and is directly prepared by growers simply mixing ferrous sulphate with di-ammonium (or mono-ammonium) phosphate (Rosado et al., 2000). The effectiveness of soil applied Fe amorphous minerals is presumably due to the fact that they are more easily mobilised by plants and microorganisms as compared with crystalline Fe forms (Loeppert et al., 1994).

Blood meal is a natural Fe source containing 20–30 g Fe kg^{-1}, chelated as ferrous iron (Fe^{2+}) by the heme group of the hemoglobin molecule. Blood meal is a by-product of industrial slaughter houses and might represent an effective source of Fe for plants (Kalbasi and Shariatmadari, 1993). According to Mori (1999) the incorporation of Fe from hemoglobin into the root cells may follow a similar mechanism as the uptake of iron in animal cells (endocytosis). Under field conditions, the application of blood meal (70 g per tree, equivalent to 180 mg Fe per tree) alleviated Fe chlorosis symptoms of pear plants (Tagliavini et al., 2000a). Blood meal is one of the main fertilisers in organic farming, not only as natural Fe source, but also as slow release N fertiliser.

Injection of Fe salts (mainly Fe^{2+} sulphate and Fe ammonium citrate) in liquid form into xylem vessels has been reported to alleviate Fe chlorosis symptoms in several woody plants like apple, pear, peach, kiwifruit, olive (Wallace and Wallace,

Table 4
Effect of timing of Fe chelate application on shoot Fe concentration and leaf chlorophyll content in *Actinidia deliciosa* in calcareous soils as determined in spring

Treatment	Orchard number 1		Orchard number 2	
	Shoot Fe concentration ($\mu g\ g^{-1}$ DW)	Leaf chlorophyll content ($\mu g\ cm^{-2}$)	Shoot Fe concentration ($\mu g\ g^{-1}$ DW)	Leaf chlorophyll content ($\mu g\ cm^{-2}$)
Control (no Fe supply)	70 b[a]	14 b	50 c	27 b
Autumn supply	86 a	19 a	65 a	31 a
Late winter supply	70 b	13 b	56 b	29 ab
Significance	***	*	***	**

[a] Means within columns followed by different letters are significantly different (LSD test at $P = 0.05$ level of probability); *, **, *** $P < 0.05$, 0.01 and 0.001, respectively.

1986; Wallace, 1991; Fernández-Escobar et al., 1993). In spite of the prompt re-greening and long-lasting effect (2–3 years), this technique is seen as an emergency procedure for curing severely chlorotic trees (Wallace, 1991) and may be only feasible for low density planting systems. Main difficulties of the practise are related to the risk of causing phytotoxicity on leaves when Fe concentration and time injection are not properly chosen. Iron containing compounds in the form of bullets to be inserted in holes made in the trunk have been successfully applied to control iron chlorosis in orchards of Spain.

4.3. The role of organic matter

It is accepted that enhancing soil organic matter content greatly reduces the risk of Fe chlorosis. Animal manure, particularly from cow, have been traditionally used to enhance soil organic matter content and fertility in fruit trees and grapevine ecosystems. The beneficial effect of organic matter on Fe chlorosis prevention does not depend solely on the direct Fe chelating ability of the humic and fulvic substances, but it is also related to the stimulation exerted by organic components on soil microbial activities. Manured fields, for example, are excellent substrates for bacteria (e.g. *Citrobacter diversus*) producing powerful Fe siderophores (Chen et al., 2000). In addition, organic matter improves soil aeration and may prevent the re-crystallisation of ferrihydrite to more crystalline oxides under alkaline conditions (Schwertmann, 1966). Noteworthy, organic matrix components can be taken up by the roots and may stimulate root activity (e.g. proton ATPase) and growth (Pinton et al., 1999). Although organic components of animal manures, sewage sludges, compost, peat, etc. are able of dissolving soil insoluble Fe compounds, their efficiency is likely improved by incubation with Fe salts before application (Tagliavini et al., 2000a).

4.4. Management of the rhizosphere and soil pH

Although alleviating chlorosis by acidification of the entire root zone is impractical (Wallace, 1991), lowering the soil pH in small spots might be feasible by either removing part of the original calcareous soil and replace it with acidified peat (Sommer, 1993) or by the addition of acidifiers to bring about complete neutralisation of the soil in the treated spot (Kalbasi et al., 1986; Wallace, 1991). In calcareous soil, however, long lasting decrease of pH is difficult to achieve by the addition of strong acids to the soil which may raise salinity problems to the roots (Tagliavini et al., 2000a). Although its efficiency has to be proved yet, in the field practice the localised addition of strong acids to drip irrigation water is sometimes adopted by fruit growers to cure iron chlorosis in the Mediterranean areas.

Removing part of the calcareous soil in the root zone and replacing it by a new substrate of acidified peat, supplemented with Fe and ammonium sulphate and other micronutrients, has been proposed to prevent the iron chlorosis in orchards and was developed as ‘Cultan’ System (Sommer, 1993). It is quite feasible that roots growing in the acidified peat face low rhizosphere and apoplast pH due to the ammonium nutrition (Kosegarten et al., 1999b). Since a significant amount of soil is removed by this technique, its adoption should be considered mainly before orchard planting.

Some examples in the literature showed a beneficial effect of an enhanced potassium nutrition on Fe uptake (Barak and Chen, 1984). This beneficial K effect is presumably related to the stimulating impact of K^+ on the root plasma membrane ATPase (Mengel and Schubert, 1985). A still open question is how rapidly such pH changes in the rizosphere are buffered by bicarbonate in calcareous soils. Since nitrogen nutrition in calcareous soils is predominantly based on nitrate, regardless the type of N fertiliser applied (Mengel, 1994), lowering rhizosphere pH by N nutrition can only be achieved by the simultaneous use of ammonium and nitrification inhibitors. This, however, may lead to ammonia toxicity and ammonia loss by volatilisation.

4.5. Management of orchard floor

In orchard and vineyard ecosystems, tree root system shares the soil environment with roots of grasses either naturally present or artificially introduced. Grass sward covers (on the whole soil surface or restricted in the orchard alleys) are a widespread technique largely adopted when water is not a limiting factor, bringing several advantages in the ecosystem mainly linked to soil fertility improvement (lowering soil density, enhancing porosity, organic matter content, water infiltration of soils, etc.). Due to a more shallow root system and less aggressive behaviour, gramineous species are often preferred to dicots. While grasses may compete with the trees for water and nutrients, frequently slowing down the shoot growth rate—a desired effect for reducing vigour and enhancing fruiting—grasses may positively interact with tree or grape roots by enhancing their Fe nutrition. This has been shown by Kamal et al. (2000) who reported Fe chlorosis symptoms of guava seedlings grown in calcareous soils being reduced by intercropping the trees with sorghum or maize, and by Tagliavini et al. (2000a) who showed a promising prevention of Fe chlorosis in a pear orchard under field conditions, by the presence of *Poa* spp., *Lolium* spp. and *Festuca* spp. in the orchard floor along the tree row.

4.6. Foliar applications

Since the existence of inactivated Fe pools in chlorotic leaves has been proposed (Mengel, 1994), the possibility of inducing leaf re-greening under field conditions without applying exogenous Fe (Aly and Soliman, 1998; Tagliavini et al., 1995b), by spraying acidic solutions (*e.g.* citric, sulphuric, ascorbic acids) has been studied. Recently Kosegarten et al. (2001) have reported that spraying citric and sulphuric acid resulted in a decrease of apoplastic pH followed by leaf re-greening. Although re-greening of leaves has often been obtained, especially in kiwifruit and peach, the magnitude of chlorophyll increases following acidic sprays was not so satisfactory for justifying the practical adoption of this means to cure iron chlorosis (Tagliavini et al., 2000a).

5. Conclusions

The knowledge of Fe acquisition mechanisms in fruit tree and grapevine has significantly improved in the last decades and led to advances at both the physiological and practical level. Additional information are needed in order to orienting screening programs for Fe-efficiency. In this respect plasmalemma ATPase activity and regulation may play a major role as they provide favourable apoplastic pH levels and thus improve the FCR activity.

The application of iron chelates does not represent a sustainable way to prevent or cure iron chlorosis because of their costs and of the environmental risks associated with their use. Since Fe chelates were introduced, little research on alternative means for controlling the chlorosis has been performed. Sustainable management of Fe nutrition in orchards and vineyards should include all genetical and agronomical means in order to naturally enhance Fe availability in the soil and in the plant. Special attention should be given to soil analysis and to prevention measures carried out before planting. Alternatives to iron chelates are being developed and in the future they should be included into the routine practices of managing fruit trees and grapevine under Integrated Production and Organic Farming.

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