MONOGRAPH

FERRILENE

Cures and prevents iron chlorosis

Valagro®

Where science serves nature

Company profile

MISSION

To create a sustainable future for people and nature.

VISION

At Valagro we live by the third way mindset pursuing excellence and applying science to agriculture to produce more and healthier food with sustainability at heart.

VALUES

Integrity - as we act with honesty, respecting people, and nature

Passion - as we are responsible for the sustainable future, we are building

Trust - as we create relationships based on doing what we say

Connections - as we see the bigger picture while recognizing individual contribution

Innovative mindsets - as we create possibilities with courage, curiosity, and determination.

COMPANY CULTURE

We focus on challenging what has already been done.

We match expertise and business acumen with great results in the constant pursuit of excellence.

We work for the impossible to become reality.

For those who are curious and never stop learning, those who courageously bring change and openly listen to new ideas.

For those who can transfer their values to others always respecting diversity while leveraging inclusion.

GEAPOWER

Using science to harness the potential of nature with an eye toward environmental sustainability: this is the principle on which GeaPower is based. It is the **exclusive technology** platform developed by Valagro to turn potential active ingredients into high quality nutrient solutions.

Thanks to GeaPower, we can offer solutions based on sustainable innovation that, by using the **most modern technologies**, borrow valuable active ingredients from nature and return them to nature itself to obtain **healthier**, **richer**, **better harvests**.

GeaPower reduces the cost of taking a solution to market while ensuring consistent efficacy.

DEEP KNOWLEDGE OF ACTIVE INGREDIENTS AND RAW MATERIALS

This enables Valagro to identify, characterize and preserve specific active ingredients that can achieve targeted physiological responses in plants

PROPRIETARY EXTRACTION PROCESSES

Customized extraction processes help maintain the correct ratio of each ingredient in complex natural mixtures

ADVANCED SCREENING AND INVESTIGATION TECHNOLOGIES

- Genomics, phenomics and other “omic” sciences allow Valagro to decipher the genetic and molecular triggers for specific physiological responses in plant systems
- Screening of hundreds of samples per experiment

PROVEN ABILITY TO PROVIDE COMMERCIALLY VIABLE SOLUTIONS

- Extensive experience with field experiments
- Commercial function and research function are closely integrated
- Allows Valagro to fast-track product candidates with the best chance of attaining commercial viability

1

Iron

Alberto Stivaletta, Alessandro De Marco
- *Global Research*

1.1 Iron in nature

Depending on the redox state, Iron is found in nature in either the Fe3+ (ferric) or Fe2+ (ferrous) form. After Si, O and Al, iron is the most abundant element in the earth's core at around 5.1%, while in the soil an average level of 3.8% is estimated to be found (Linsay 1979). Iron is usually found in the soil in sufficient quantities to form various compounds, most usually as oxides and hydroxides. Soil color is primarily based on the presence of free oxides. The yellow-brown colors in cool/temperate zones are due to the presence of hydroxides such as goethite. The red colors in arid regions are due to non-hydrated oxides such as hematite.

1.2 Iron in the soil

Most of the iron is found in the crystalline structures of numerous minerals and in the water table. Beginning with the weathering of primary minerals, soluble iron is released that can be used by organisms, bind itself to different organic ligands or even be transformed into secondary minerals such as sulphides, carbonates and clay minerals. Oxides and hydroxides of various compositions and degrees of crystallization primarily control the solubility of this element in the soil (Murad and Fischer, 1988: Lindsay 1979). Goethite is the most frequent iron oxide in the soil because it has the most stability under the environmental conditions found in soils. Goethite is found in many types of soil and climatic regions and is responsible for the ochre color of many soils. Goethite is often found associated with the second most common form hematite however the former does not seem to be restricted to a specific climatic region, while hematite is found in tropical areas where the temperature conditions and pH favor its formation.
It is red and has high pigmentation strength (Schwertman and Taylor, 1989). Due to the extremely low solubility of the Fe3+ oxides in soil's normal pH range, the iron released will quickly precipitate as oxide or hydroxide. Only a small part of oxidized iron is incorporated into secondary laminar silicates (clays) and/or is complexed by organic matter (Schwertman and Taylor, 1989). Under conditions of anaerobiosis, microorganisms can use the Fe3+ oxides as final electron acceptors to achieve the oxidative decomposition of the organic matter. This process causes the reduction of Fe3+ to Fe2+, which is generally more soluble.

LEGEND

1 Weathering
2 absorption
3 decomposition after death
4 complexing
5 hydrolysis
6 reduction
7 oxidation
8 reduction by organisms

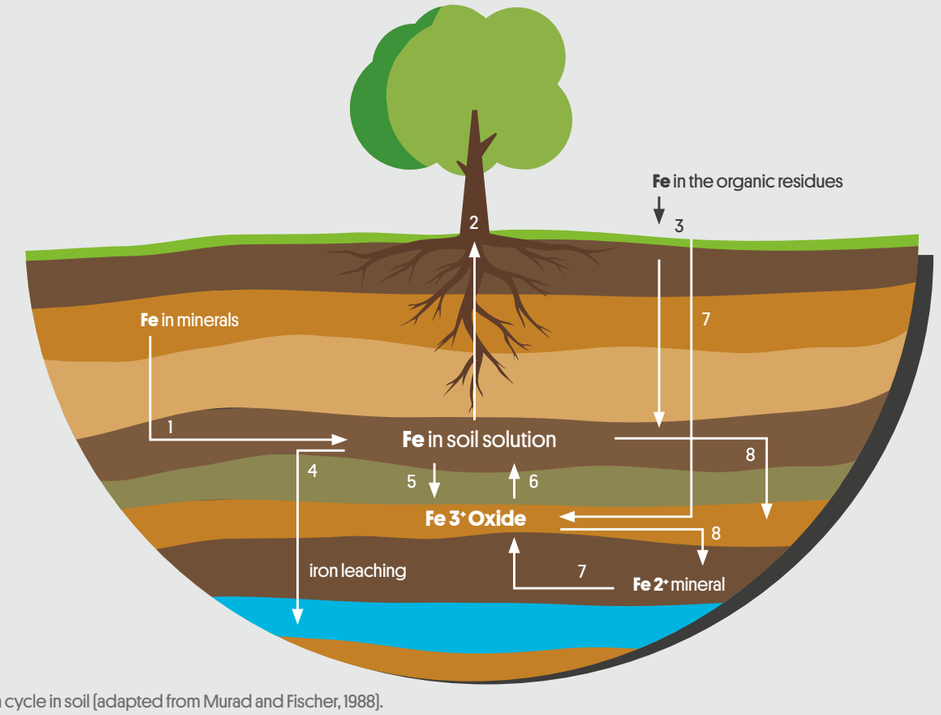


Fig. 1 Iron cycle in soil (adapted from Murad and Fischer, 1988).

1.3 The chemistry of iron

Iron compounds can exist as ferrous (Iron (II)), or ferric forms (Iron (III)).

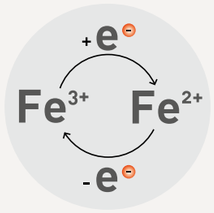


Fig. 2 Iron oxidation-reduction reaction.

Iron is a transition element characterized by the relative ease with which it can change its oxidation state and by its ability to form octahedral compounds with various ligands, with a large variation in the redox potential depending on the ligand. This variability gives it special importance in biological redox systems and in soil (Schwertman and Taylor,1989; Schwertman 1991).

1.4 General aspects of iron deficiency

Iron (Fe) is an essential micronutrient for plants, humans, and other animals. In the early 17th century, this element was exploited as a medicine by some communities, including Egyptians, Hindus, Greeks, and Romans (Guggenheim, K.Y.). The World Health Organization (WHO) states that the lack of sufficient micronutrients, such as Fe and Zn, represents a major threat to the health and development of the world population.

Vitamin and mineral deficiencies remain as a serious health problem globally, affecting more than two billion people or one in three individuals (FAO; IFAD; WFP.) and mostly in developing countries, the search for solutions that can reduce the harmful effects of these nutritional disorders is of paramount significance (Black, R.E.; Allen). This approach aims to place the micronutrient dense trait, such as iron, in staple crops and food products, using different procedures such as agronomic practices to obtain a final food product with a higher iron content, considering it as an important strategy to reduce Fe deficiency in people (Cakmak 2002).

1.5 Principal factors that affect the availability of iron

Fe deficiency is typically found in crops grown on calcareous or alkaline soils, in arid and semi-arid regions of the world; these soils cover over 30% of the earths' land surface (Figure 1) (Alvarez-Fernandez, et al., 2006). The causes of iron deficiency should be not just linked to Iron unavailability, but it may depend on numerous situations and have a distinct nature. The following are the most relevant factors that affect iron availability.

1.5.1 Solubility of iron in soil

The studies carried out by Lindsay (1979, 1991) regarding the various solubilities of the different iron oxides and hydroxides in soil (Fig. 3) highlight the fact that:

1. Amorphous iron oxide would be the most soluble while goethite would be the least soluble.

2. The solubility of Fe3+ oxides and hydroxides present in the environment is closely related to the soil's pH; thus, the solubility decreases 1000 times for each pH unit increase, reducing the concentration of soluble iron to values lower than 10 E-10 M in environments with a pH value of 7.5.

3. The region with the least iron solubility corresponds to a pH range between 7.5 and 8.5 (Fig.4), which coincides with calcareous soils. The concentration of iron for this pH interval is approximately below 10 E-10 M, an insufficient amount for optimal plant growth, which requires a range of soluble iron of 10 E-4 and 10 E-9 in the environment (Guerinot and Yi, 1994). Studies carried out by Römheld and Marschner (1986) showed that in well aerated soils, the amount of dissolved iron at pH values greater than 4 is less than the amount required for most vegetation, which results in the iron deficiencies in plants cultivated in these soils.

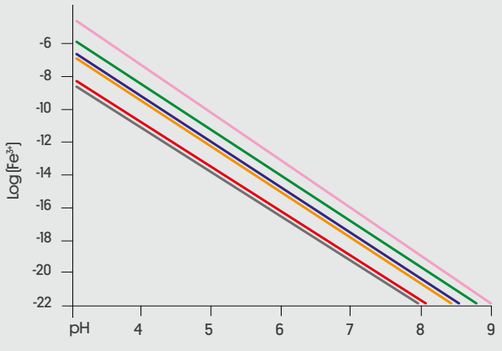


Fig. 3 Fe3+ activity in various ferric oxides and hydroxides present in soils according to pH (Lindsay, 1979).

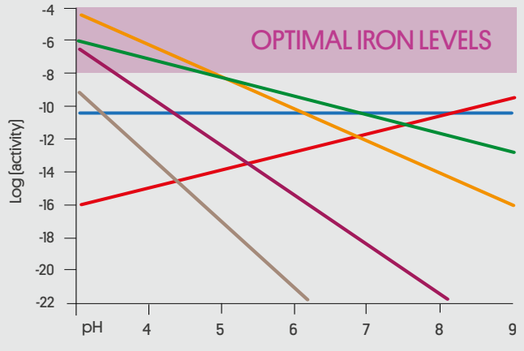


Fig. 4 Hydrolyzed species of Fe3+ in balance with Fe soil (Lindsay, 1979).

Since iron can be present in two conditions, the redox potential of the soil is another factor that influences the content of this nutrient. In well-aerated soils--a condition that is normally met by cropland--Fe3+ is not altered by the redox potential. Fe2+ can be present in different forms at different soil's pH: at pH lower than 6,7 the predominant species is Fe2+, while at higher pH values the principal species are FeOH+ (Fig. 5).

Under aerobic conditions these species are unstable; however, if there are reducing conditions, these two species are the most abundant and are the ones that control the amount of iron available for plants (Lindsay, 1991). In soils that have recently been reduced, iron solubility will seemingly be controlled by siderite ($FeCO^3$). Increments in the concentration of CO in the soil will result in a decrease of iron solubility for plants (Lindsay and Schwab, 1982).

Consequently, the general rule for iron solubility is that under aerobic conditions and with a neutral or alkaline pH the iron will precipitate in insoluble forms, while with acidic pH and reducing conditions the concentration of soluble iron will be higher.

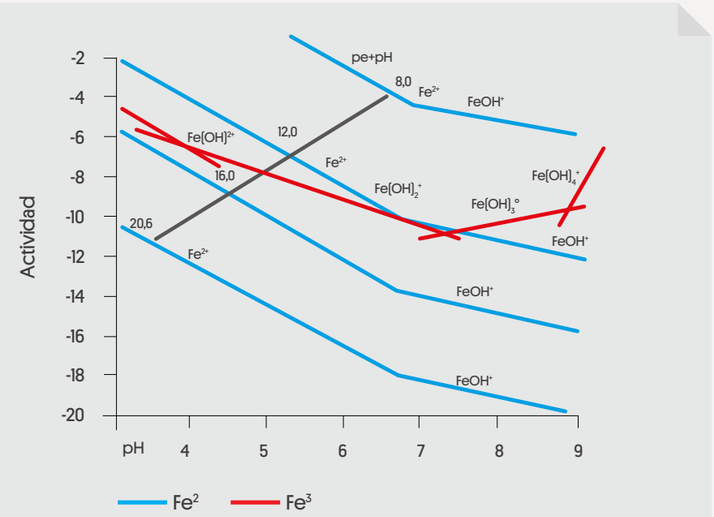


Fig. 5 Effect with respect to the redox potential for the forms Fe2+ and Fe (OH) + compared with the hydrolysed forms of Fe3+ in equilibrium with the system Fe-soil (Lindsay and Schwab, 1982).

1.5.2 Presence of bicarbonates (pH)

The hydrogencarbonate ion in calcareous soil, shift the pH in the range to 7-5-8,5, and in extreme condition up to 9. At this pH, the concentration of soluble iron is low (Lindsay, 1979,1991; Lindsay and Schwab, 1982); thus, it is difficult for crops to get the ferric nutrition they need. Furthermore, the plant's principal response mechanisms are negatively impacted by the iron deficiency: the protons released by the vegetation are neutralized, alkalinization reduces the secretion of phenolic compounds and makes the reduction of Fe3+ difficult in the plasma membrane (Römheld and Marschner, 1986). All of this influences the bioavailability of iron, causing large alterations and inhibiting the crop's response to the iron deficiency (Susín et al. 1996). Table 1 reports the characteristics of some alkaline soils.

SOIL	HORIZON, CHARACTERISTICS	FERTILITY	pH	DISTRIBUTION
Alfisols	Differentiated, particularly the clay horizon	Deficient, needs fertilizers	7.5 / 7.8	Humid and temperate regions of North America and Europe
Aridisols	Differentiated, particularly the clay horizon	Good with risks	7.8 / 8.1	In desert regions all over the world
Mollisols	Differentiated, with thick, dark, organic surface horizon	Excellent, particularly for cereals	6.1 / 8.6	Large meadows, Argentine Pampa, Russian steppe
Vertisols	None, high content of expansive clay	Good	7.6 / 9.5	Pastures in regions that are seasonally dry, for example India, Sudan, Texas

Tab. 1 Main characteristics of the soils (alfisols, aridisols, mollisols, vertisols).

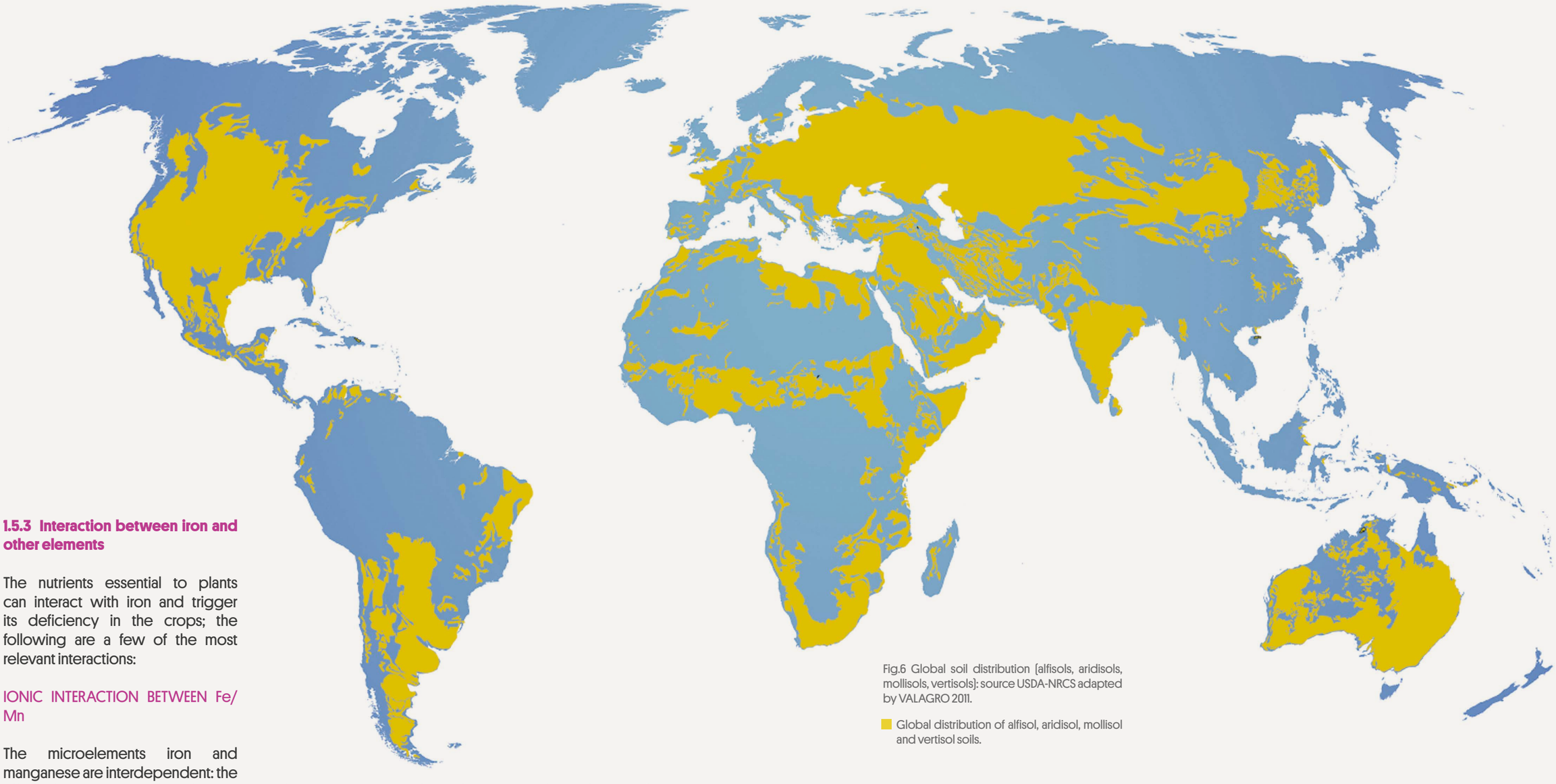


Fig.6 Global soil distribution (alfisols, aridisols, mollisols, vertisols): source USDA-NRCS adapted by VALAGRO 2011.

Global distribution of alfisol, aridisol, mollisol and vertisol soils.

1.5.3 Interaction between iron and other elements

The nutrients essential to plants can interact with iron and trigger its deficiency in the crops; the following are a few of the most relevant interactions:

IONIC INTERACTION BETWEEN Fe/Mn

The microelements iron and manganese are interdependent: the effect of the presence of one in plant tissue coincides proportionally with the amount of the other. The reactivity of iron depends on its oxidation state, but manganese has better oxidation potential than iron; if there is a balance between the two, Mn oxidizes the excess iron and transforms it into inactive iron, which is immobilized by phosphates, forming an iron-phosphate precipitate (phosphoprotein called phytoferritin). If there is an imbalance between the two, two different conditions may occur:

1. The excess iron in the solution causes the loss of dynamic balance between the two elements, resulting in secondary symptoms of excess iron with symptoms similar to manganese deficiency.

2. If there is excess manganese, the plants become chlorotic in their new leaves with symptoms similar to iron deficiency. Excess manganese causes excess oxidation of Fe2+ to Fe3+, the latter being insoluble and thus unusable for the cells.
High levels of soluble Mn2+ in the tissue are related to low levels of soluble Fe and vice versa, (Somers and J. W. Shive 1942).
In outdoor cultivation situations, iron chlorosis occurs at the same time, masking the chlorosis triggered by manganese deficiency. The correction of the latter in many cases causes the visual manifestation of the second and exacerbates it. This has been confirmed on numerous occasions by researchers (Warden et al. 1991). This means that the Fe/Mn ratio in nutrient solutions is more important than the concentrations of Fe and Mn (Somers and Shive 1942, and Warden 1991).

IONIC INTERACTION BETWEEN Fe/K

The role of K+ is very important concerning the functioning of the proton pump; thus, the plants are not able to respond to the iron stress in the absence of K+, even if said ion is substituted by Na (Jolley et al 1988), meaning that sufficient potassium content is related to a better response to the iron deficiency, both in Strategy I and II plants. (Hughes et al, 1992).
Therefore, the presence of potassium is important to the availability of iron; this is considered useful in agronomic terms.

PHYTOSIDEROPHORES

Many authors demonstrated that phytosiderophores (PS) released by graminaceous species can mobilize Fe from sparingly soluble soil sources. (Römheld and Marschner 1990). The amount of phytosiderophores present in the environment is related to the degree of chlorosis (Kawai et al, 1988).

TEMPERATURE

Extreme temperatures are another factor that induces iron chlorosis; low temperatures cause a reduction in root development and thereby a reduction in the ability to absorb iron (Chaney, 1984).
At the same time, high temperatures reduce the stability of the phytosiderophores, reducing iron's availability (Awad et al. 1988).

2

Iron in plants

Giustino Sirolli, Gianluca Nicolini - *Global Research*

2.1 Absorption

In the face of iron deficiency, plants can remain indifferent, can develop mild response (inefficient plants), or develop adaptation mechanisms that allow them to increase their capacity for absorbing iron from the soil solution (Fig. 7) (efficient plants) (Mengel and Kirby, 2001).
In each group, the degree of response is different, even between species and genotypes (Brown and Jolley, 1988; Shi et al., 1993).
Regarding the oxidation states in which iron presents itself in the soil-ferric Fe3+ and ferrous Fe2+ - it is accepted that the plant prefers Fe2+, although there are some plants capable of absorbing iron as chelated Fe3+. Fe2+ is preferably absorbed via the roots through an active absorption process (Uren, 1984), in, well-aerated soils, which is normally the type of soil in which one finds cropland.

Under these conditions, it is necessary to take a prior step in the reduction of Fe3+ to Fe2+. This process is carried out by a reductase enzyme located in the plasma membrane of the root (Römheld, 1987). Fe2+is also absorbed through the foliar epidermis and the surface of the branches.

2.2 Absorption Strategies

In situations in which there is an iron deficiency in the environment, superior plants have developed a series of mechanisms to increase the availability of iron in the soil solution. Said plants are divided into two groups based on the model of response that they develop with said deficiency: strategy I and strategy II plants (Marschner et al 1986; Brown and Jolley, 1988; Hopkins et al 1992).

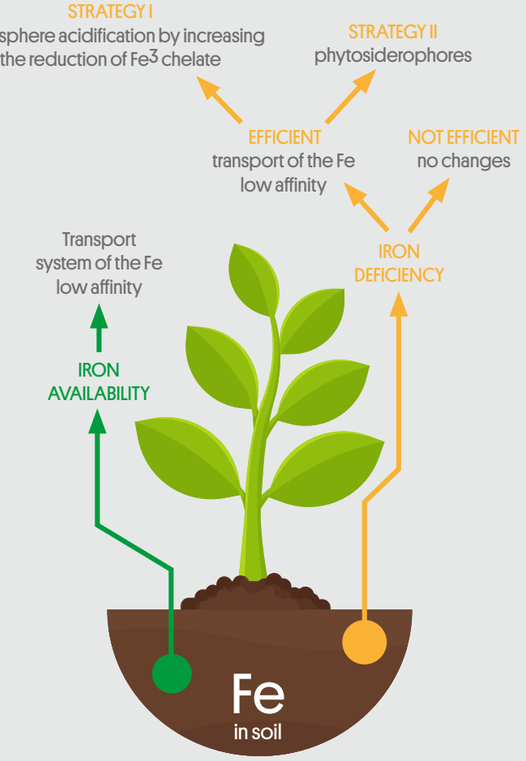


Fig. 7 Reactions of the plant in iron deficiency.

LEGEND
→ UNIDIRECTIONAL
↔ MUTUAL

ANTAGONISM

POTASSIUM (K)	→	BORON (B)
MAGNESIUM (Mg)	↔	POTASSIUM (K)
MOLYBDENUM (Mo)	→	COPPER (Cu)
COPPER (Cu)	→	MANGANESE (Mn)
		IRON (Fe)
PHOSPHORUS (P)	→	ZINC (Zn)
		POTASSIUM (K)
		COPPER (Cu)
		CALCIUM (Ca)
		IRON (Fe)
ZINC (Zn)	→	IRON (Fe)
BORON (B)	→	POTASSIUM (K)
IRON (Fe)	→	PHOSPHORUS (P)
NITROGEN (N)	→	POTASSIUM (K)
		COPPER (Cu)
		BORON (B)
CALCIUM (Ca)	↔	POTASSIUM (K)
		MAGNESIUM (Mg)
		NH4+
CALCIUM (Ca)	→	MANGANESE (Mn)
		ZINC (Zn)
		BORON (B)
		PHOSPHORUS (P)
		IRON (Fe)

SYNERGISM

NITROGEN (N)	→	MAGNESIUM (Mg)
MAGNESIUM (Mg)	→	PHOSPHORUS (P)
MOLYBDENUM (Mo)	→	NITROGEN (N)
POTASSIUM (K)	→	MANGANESE (Mn)
		IRON (Fe)
SULPHATE (SO_2)	→	NITROGEN (N)
		POTASSIUM (K)
		COPPER (Cu)
		MANGANESE (Mn)
		MAGNESIUM (Mg)
IRON (Fe)	→	MANGANESE (Mn)

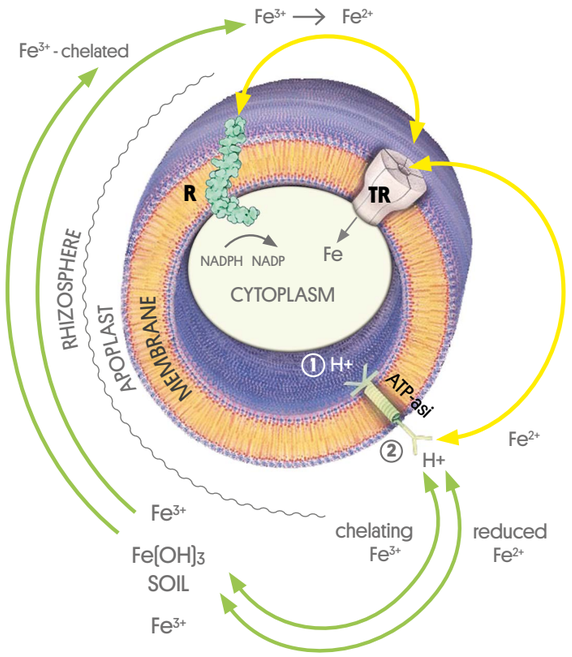
Tab.2 Ionic interaction of nutrients in the soil.

2.2.1 STRATEGY I

Dicotyledonous and mono-cotyledonous plants except gramineous develop this type of response. In circumstances in which there is an iron deficiency, these plants improve their absorption through three reactions:

1. an increase in the activity of a reductase bonded to the plasma membrane in the cells of the rhizodermis, responsible for the reduction of Fe3+, which causes an increase in the decomposition speed of the reduction of Fe3+, which in turn causes an increase in the decomposition speed of the chelated Fe3+ and consequently absorbs the reduced iron for the plant.
This dissociation of the chelates is the principal mechanism in strategy I. This causes root cells from the plants in strategy I to possess two systems for reducing Fe3+: standard reductase and turbo reductase, its reducing capacity is 20 times greater than the standard system (Moog and Brüggeman, 1994).

2. Consists of the expulsion of H+ through the roots to the rhizosphere. This sub-strategy is less frequent; only some dicotyledonous demonstrate it (Marschner et al., 1986; Zocchi and Cocucci, 1990; Toulon et al., 1992). This effect is due to the induction of a proton pump, which depends on the ATP of the plasma membrane (Zocchi and Cocucci, 1990; Toulon et al., 1992). A decrease in the pH of the rhizosphere takes place once the capacity to excrete protons is increased, which makes it possible to increase the solubility of the iron present in the soil and turbo reductase activity because this enzyme achieves its maximum activity at pH 6.5 (Holden et al., 1991; Jolley et al., 1988), emphasizing the role of K+ in the functioning of the proton pump.



LEGEND:
R- induced reductase
TR- transporter or channel for iron
1- proton stimulated by pump flow
2- increase in the release of reducers/chelates

Fig. 8 Strategy I: model of response from the root to the iron deficiency in dicotyledonous and non-gramineous monocotyledonous.

3. This third sub-strategy is found in some species; the plants that demonstrate strategy I are characterized by the induction of an iron transport system in the plasma membrane (Young and Terry, 1982; Fox et al., 1996; Fox and Guerinot, 1998). Studies carried out with cucumber plants showed that there are two different iron transport systems (one with high affinity and one with low affinity). In situations where there is sufficient iron, the low-affinity system transports the iron to the plant, while the high-affinity system is activated if there are iron deficiencies (Zaharieva and Römheld, 2000).

MORPHOLOGICAL CHANGES IN THE ROOT

In addition to these three reactions, the plants that develop this strategy can generate another type of response to increase the soil's ability to absorb iron (Abadía 1998; López-Millán, 2000). They tend to increase the contact surface between the roots and the soil. Said changes consist of the formation of root hairs, thickening of the subapical regions and the development of transfer cells in thickening (Welkie and Miller, 1993).

EXCRETION OF COMPOUNDS WITH LOW MOLECULAR WEIGHT

The roots of the plants can excrete a large variety of organic compounds, including reducing sugars, caffeic acid, amino acids, organic acids, phenolic compounds and flavins. The rate of exudation and its composition depend on the pH, the temperature and the type of soil, the intensity of light, the age and nutritional state of the plant, and the presence of microorganisms (Jones, 1998).

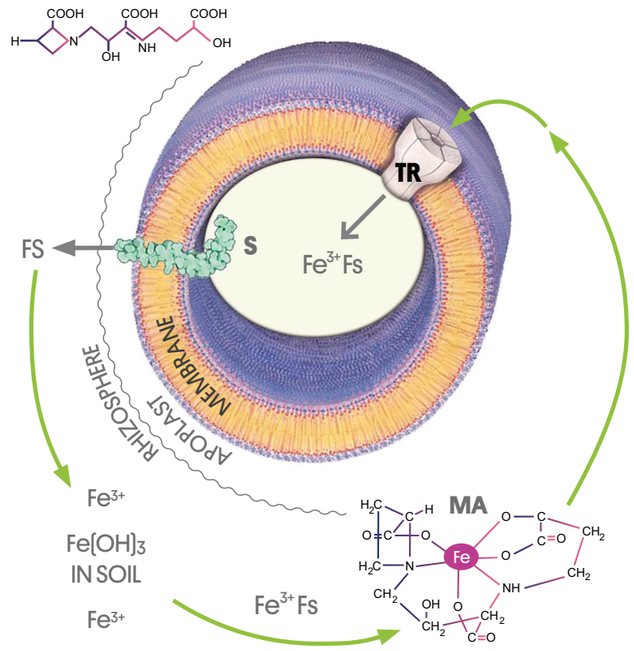
PROTEIN CHANGES IN THE ROOT

Various works realized using electrodeposition and in vitro transcription of root mRNA (Bienfait, 1988b; Herbik et al, 1996).

2.2.2 STRATEGY II

Only gramineous plants develop this type of response; the response of strategy II plants to situations of iron deficiencies is characterized by the release in the root zone of proteinogenic amino acids with low molecular weight, the so-called phytosiderophores, which chelate the Fe3+ present in the soil and are subsequently absorbed without prior reduction via a transport system for Fe3+ phytosiderophores with high affinity (Römheld and Marschner, 1986; Takagi, 1976).
The release of phytosiderophores follows a characteristic diurnal rhythm (Fig 9) and is quickly reduced with the iron supply (Marschner, 1995). The diurnal rhythm in the release of phytosiderophores in the plants with iron deficiencies is inversely related to the volume of a particular type of vesicle in the cytoplasm of the cortical cells (Nishizawa and Mori, 1987).
Phytosiderophores (PS) is the name given to the mugineic acid and its derivatives. They are released through the roots and form highly stable complexes with Fe3+, thereby increasing the amount of iron in dissolution. The number of PS released is related to the degree of resistance to chlorosis (Kawai et al., 1988; Hansen et al., 1996). The biosynthesis of the PS in the root increases if the deficiency increases, with an increase of up to 20 times with respect to the control plants (Takagi et al., 1984). Transport of the complex to the cell cytoplasm is done via a specific protein that was recently identified (Curie et al., 2001) and localized in the apical zone of the root and the stem and allows for the transport of the complex Fe2+ -NA (NA= nicotianamine) and possibly other metals (Hell and Stephan, 2003). Once the complex is in the cytosol, the Fe3+ is released and the PS degrades or is excreted to the exterior.

It has been proven that the released PS and the iron complex are absorbed through the plant's roots (Kawai and Alam, 2005). Both demonstrate different absorption, which suggest that the cells in the root could differentiate between the released PS and the complex so that the released PS could then be excreted into the rhizosphere.



LEGEND:
S - increased synthesis and release of phyto-siderophores
TR - translocator of Fe (III) phytosiderophores in the membrane plasma;
MA - structure of the phytosiderophore

Fig.10 Strategy II response pattern of the root in front to iron deficiency in gramineous.

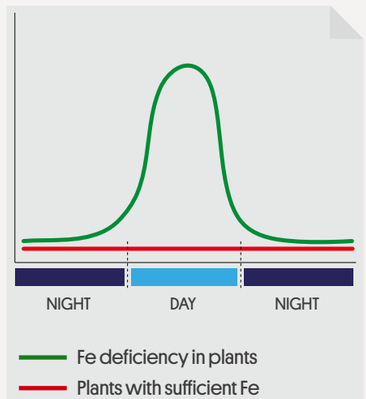


Fig.9 Performance of the release of phytosi-derophores by barley.

Methionine › nicotianamine › 2-deoxymugineic acid › mugineic acid › 3-hydromugineic acid.
The synthesis cycle of methionine (Fig.11) experiences substantial activation in the roots of the iron-deficient plants caused by the methionine for the synthesis of phytosiderophores; this fact has been established in roots but not in the part of the plant that is aboveground (Kobayashi et al, 2006).

2.3 Iron transport in plants

The ferrous ion is transported through the root cortex via the symplast by means of the plasmodesmata when it appears in the form of Fe2+ - nicotianamine (Pich et al., 1997; Stephan, 2002]. Subsequently and still in the root system's symplast, the Fe2+ undergoes oxidation to become Fe3+. Once in the Fe3+ form, it is transported to the superior parts of the plant via the xylem in the form of a soluble dicitrate complex (Tiffin, 1970; Cambell and Redinbaugh, 1984; López- Millán et al., 2000[a]; Mengel and Kirby, 2001; Stephan, 2002). Once the iron reaches the leaves, it should re-cross the plasma membrane of the foliar cells. This step once again requires the reduction of Fe3+ to Fe2+, a process that is carried out by a reductase enzyme like that of the root (Brüggemann et al., 1993; de la Guardia and Alcántara, 1996; González-Vallejo et al.; 1998, 2000; Rombolà et al., 2000). Although transport is realised via the xylem, iron was found in the phloem. The ability to transport this ion is related to the plants' response to iron deficiencies. The transport of Fe3+ in the phloem is done as Fe3+ - nicotianamine (Becker et al., 1992; Stephan and Scholz, 1993). It must be considered that high concentrations of Fe2+ in the cellular cytoplasm have toxic effects (Mengel and Kirby, 2001). Therefore, the iron should be rapidly oxidized to Fe3+.

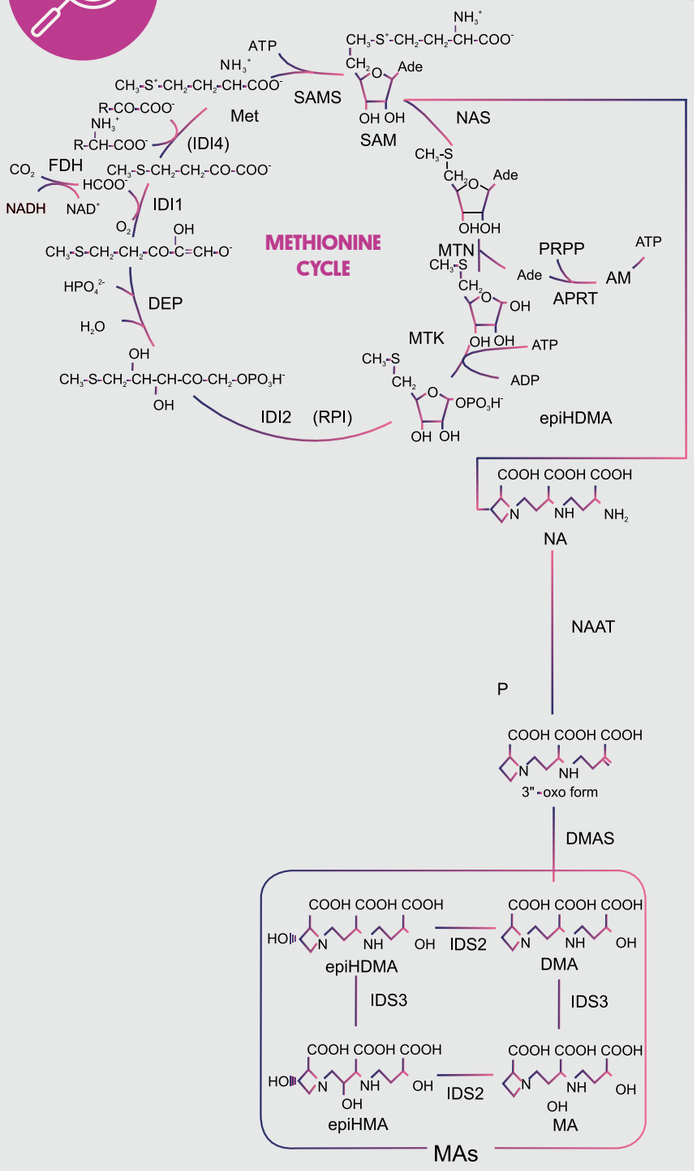


Fig.11 Synthesis of phytosiderophores and the methionine cycle in Gramineae (Kobayashi et al., 2005).

Thus, the ferrous ion is transported to the chloroplast, where it is oxidized to Fe (OOH) and accumulated in the form of a phosphoprotein known as phytoferritin, a multi-numeric protein that secludes more than 4- 103 iron atoms in the form of a stable mineral within the protein coating (Theil, 1987; Andrews et al., 1992; Laulhere and Briat, 1993), creating a non-toxic iron reserve in the cell. The phytoferritin is mainly found in the chloroplasts, but it is not confined solely to this organelle, as it has also been found in the xylem and phloem (Smith, 1984).

In situations where there are iron deficiencies, the ferric ion is once again reduced to a ferrous ion, which could be transported in the form of Fe-nicotianamine through the cells (Mengel and Bübl, 1983; Laulhere and Briat, 1993). The transport of iron in strategy II plants- -through the roots and the xylem - is realized in the form of Fe- phytosiderophore (Mori et al., 1991; Alam et al., 2001; Kawai et al., 2001).

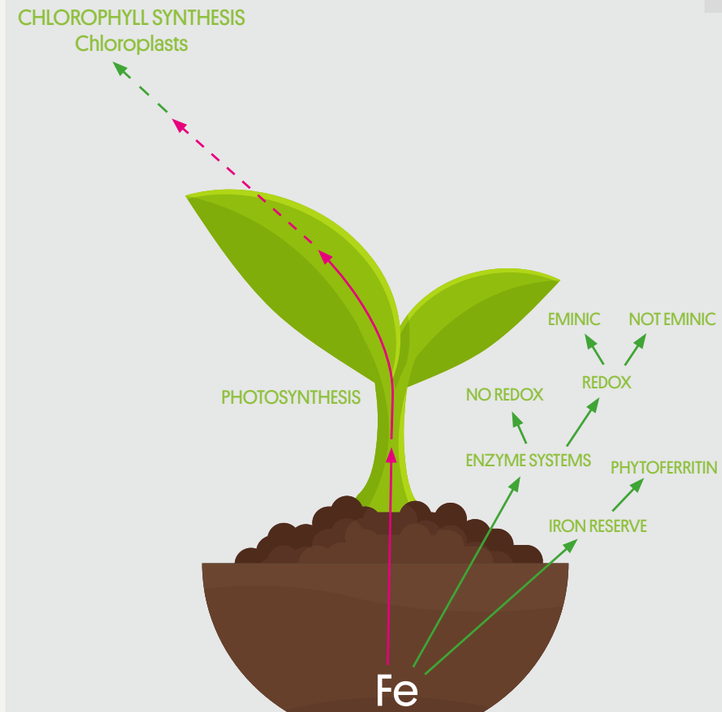


Fig. 12 Functions of iron in plants.

2.4 Biological functions of iron in plants

Fe deficiency is a worldwide problem in crop production, affecting yield both qualitatively and quantitatively (Mortvedt, 1991]; plants do not reach their full growth potential, and the nutritional value is compromised, leading to economic losses and limitations in crop selection (Chaney, 1984). In extreme cases, Fe deficiency may result in complete crop failure (Chen and Barak, 1982). The list of plant species affected is vast and includes apple, citrus, grapevine, peanut, dryland rice, sorghum, and soybean (Marschner, 1995).

As a critical component of proteins and enzymes, iron plays a significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation, uptake mechanisms (Kim and Rees, 1992), it is essential for the maintenance of chloroplast structure and DNA synthesis function through the action of the ribonucleotide reductase (Reichard, 1993). Iron is also an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase,1-aminocyclopropane acid-1-carboxylic oxidase (Siedow,1991), or abscisic acid (compounds that are active in many plant development pathways and their adaptive responses to fluctuating environment conditions). (Rout and Sahoo, 2015).

The most well-known of the components of the non-heme systems is ferredoxin, a ferric protein that acts as a final acceptor of electrons in many metabolic processes such as photosynthesis, the reduction of nitrate and sulphate (Mengel and Kirby, 2001; Marschner, 1995). Its high redox potential allows it to reduce substances such as NADP+, nitrate, oxygen, and sulphate. The aconitase is an enzyme with non-heme iron that catalyzes the isomerization of citrate to isocitrate in the cycle of tricarboxylic acids (Hsu and Miller,1968; Beinert and Kennedy, 1989; Marschner, 1995). Other enzymes with non-heme iron are riboflavins; they accumulate in plants with iron deficiencies 200 times more than in plants that grow in environments with enough of the element (Welkie and Miller, 1989).

Said accumulation of riboflavin is produced because the iron deficiency causes alterations in the metabolism of the purines since the enzyme xanthine oxidase is seriously damaged (Schlee et al., 1968).

The superoxide dismutase enzymes constitute another non-heme iron system that eliminates superoxide anionic free radicals: it involves common isoenzymes in the chloroplasts but can also be found in mitochondria, peroxisomes, and cytoplasm (Droillar and Paulin, 1990), or xanthine oxidase, which has functions in metabolic processes such as photosynthesis, mitochondrial breathing, N2 fixation, the reduction of SO42+ to SO32-, etc. There are series of less known enzymes in which iron also acts as metallic component in redox reactions or as a connecting element between enzyme and substrate (Marschner, 1995). This includes the lipoxygenases that regulate lipid peroxidation, which means they are involved in cellular and tissue senescence and in the incompatible host-pathogen combinations and thus in the resistance to diseases (Nagarathana et al., 1992). Iron plays an important role in photosynthesis not just because of its involvement in the synthesis of chlorophyll (Miller et al., 1984), but also because of its influence on chloroplast morphology (Terry and Abadía, 1986; Marschner 1995).

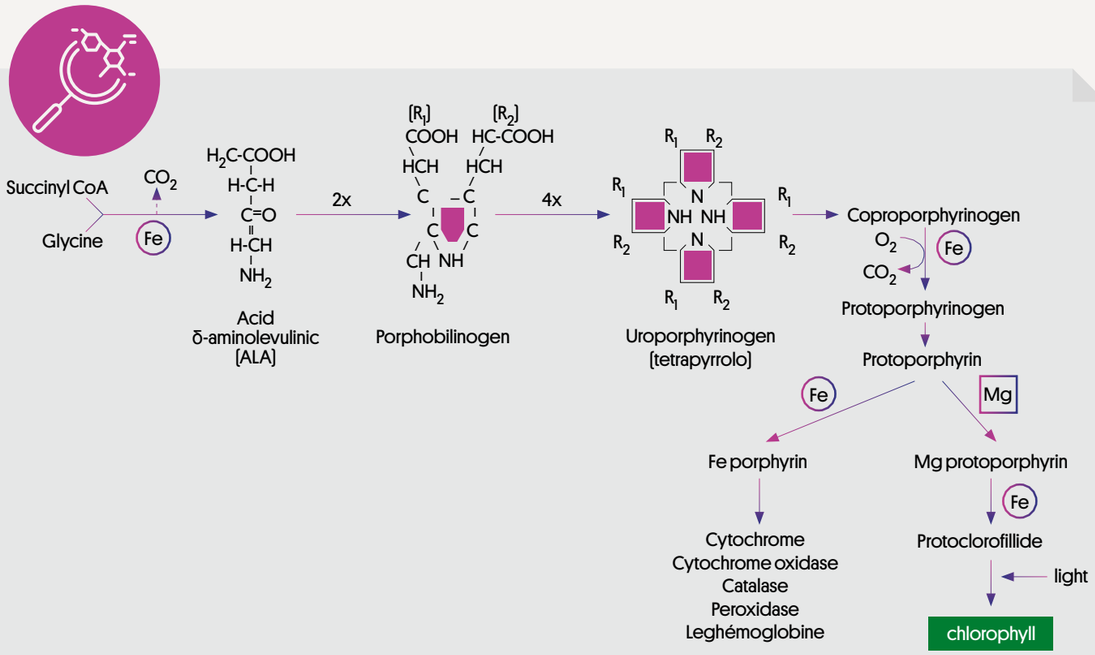


Fig.13 Iron's role in chlorophyll synthesis (Marschner, 1995).

3

Iron chlorosis

Giustino Sirolli - *Global Research*

3.1 IRON CHLOROSIS

Iron chlorosis is considered one of the most complex nutritional deficiencies (Pestana et al., 2003) and is caused by diverse factors related to the availability of iron in the soil, its absorption through the roots and its distribution in the plant tissues (Lucena, 2000[a]: Alvarez-Fernadez, 2000).

Iron chlorosis generally manifests itself through the loss of green color in the leaves due to a decrease in the concentration of photosynthetic pigments, fundamentally chlorophyll (Abadia and Abadia, 1993). It generally occurs in the interveinal area of young plants, but the veins remain green because the iron is scarcely distributed in the growth zones (Chaney, 1984).

An important factor in iron chlorosis is that it provokes a decrease or inactivation of all the physiological processes in which iron is involved, in particular chlorophyll synthesis, which is produced in a reduction of the production and quality of the crops, including the premature death of the plant.

The methods used up to know to resolve this problem are localized application of iron salts and chelates to the plants (application in the soil or via the foliar pathway), artificial modification of the pH of the soil solution (application of organic and inorganic acids) and the use of cultivars of the crop variety with the ability to absorb iron from soils in which the element is not readily available (Olsen et al., 1987; Chen and Barak, 1982; Emery, 1982).

3.2 VISUAL SYMPTOMS

Iron deficiency is the easiest symptomatology to recognize of those caused by other micronutrients because it produces a type of characteristic chlorosis. The symptoms will vary depending on:

- The age of the leaf;

- The severity of the deficiency;

- The environmental conditions.

In the case of gramineous plants, the symptoms are more difficult to identify because they can be confused with magnesium because they occur in the form of yellow bands between nerves alternating with green nerves. It must be considered that in many cases chlorosis is more a problem of iron mobility than of an iron deficiency; thus, it is not unusual to find that in a single plant there are areas with iron chlorosis and others without it. The characteristic visual symptoms are:

- Old leaves are green, while young leaves start to turn yellow. Various studies show that there is a correlation between the amount of iron and the chlorophyll content in the leaves.

- As the state of deficiency advances, one sees an interveinal chlorosis characteristic; only the veins remain green, in contrast with the yellow or off-white color of the limb.

- In cases of extreme deficiency, yellowing may become total and appear in necrotic areas in the edges of the limb, causing the leaves to fall early and, in extreme cases, total defoliation.

- Shoots remain fine and curved, causing significant growth reduction.

- In annual plants one observes a decrease in growth, a rickety appearance, and a decrease in production. Trees become defoliated and start to dry out on the periphery; fruits are small and mature early.

3.3 CORRECTING IRON CHLOROSIS

Iron chlorosis is considered complex, which means to correct it one should consider diverse prevention and treatment techniques. The first solution is genetic crop selection, thereby improving the iron absorption mechanisms and decreasing its susceptibility to iron chlorosis (Charlson et al., 2003). The use of tolerant cultivars is considered the better solution to combat this deficiency in limed soils, although this technique is far from being a solution for fruit trees and other species (Álvarez-Fernández et al., 2003a). It involves a preventative measure that is taken prior to planting. Nevertheless, once a crop has been established, the application of correct agricultural practices is crucial in the prevention of iron chlorosis (Chen, 1997).
These practices consist in eliminating the risks of compaction, alkalinization and flooding of the soil, giving preference to good drainage and controlling the frequency of risks. Another practice, regularly used with fruit trees and vineyards, is joint cultivation with other annual plants, thereby reducing compaction and increasing the porosity, filtration, and organic material content of the soil.
It involves a preventative measure that is taken prior to planting. Nevertheless, once a crop has been established, the application of correct agricultural practices is crucial in the prevention of iron chlorosis (Chen, 1997). In situations of iron deficiency, it should be corrected with the application of fertilizers with or without iron in the soil or on the plant. Inside the compounds that contain iron one finds ferric complexes; iron chelates and ferric or ferrous organic salts that increase the iron content in the plant and in the soil. And, inside those that do not contain iron, there are acidifiers and organic material that favor solubilization of non-available native iron. These treatments can be applied to the soil in solid or liquid state, in fertigation or in foliar applications.

HIGH SENSITIVITY	AVERAGE SENSITIVITY	LOW SENSITIVITY
CITRUS FRUITS, VINE	ALFALFA	APPLE TREE
SORGHUM	BARLEY	POTATO
PEACH	CORN	BEET
WALNUT, PEAR TREE	RICE	
SOYBEAN, ROSE	WHEAT	
STRAWBERRY, TOMATO	OAT	
KIWI		

Tab.3 Sensitivity of different crops to iron deficiency.

Fig.14-19 visual symptoms of iron chlorosis in plants

Fig.14 Iron chlorosis in corn

Fig.16 Iron chlorosis in rose

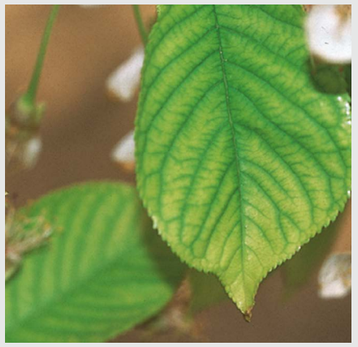


Fig.18 Iron chlorosis in cherry

Fig.15 Iron chlorosis in vine

Fig.17 Iron chlorosis in peach

Fig.19 Iron chlorosis in Azalea

4

Chelates

Angelo Squadrone, Luciano Di Sciullo, Antonio Menna, Giuseppe Iezzi, Antonio Coccia, Giulio Maria Cocco - *Global Operations*

4.1 CHELATING MOLECULES

Chelating agents or ligands are organic molecules with two or more functional groups capable of sharing pairs of electrons with a central metallic ion. In this way, a cyclical structure is formed in which the metal is retained, thereby preventing its precipitation in specific environmental conditions.

Iron chelates represent amore soluble form of iron and consequently the uptake from roots increase. There are two groups of chelating agents, both of which are derivatives of polyamino carboxylic acids, depending on the presence or absence of phenolic groups in their structure. On the one hand, there are the chelating agents that have a structure like that of EDTA, which are called non-phenolic, and on the other, there are those that contain phenolic groups (EDDHA, EDDHSA...). Figure 26 shows the structure of o,o-EDDHA molecules.

The structure of the phenolic chelating agents provides six electron-donor functional groups: two carboxylic acids, two secondary amines, and two phenols. The strength of the coordination of the donor groups is related to the acidity of the metal and the basicity of the oxygen proton and the donor nitrogen.

The better capacity of the phenolic chelating agents is due to the presence of two phenolic groups, which are more basic, and allows to effectively maintain Fe3+ in solution in limed soils (Lindsay, 1979).

	R1	R2
o,o-EDDHA	H	H
EDDHSA	SO_3K	SO_3K

Tab.4 Radicals of phenolic chelating agents.

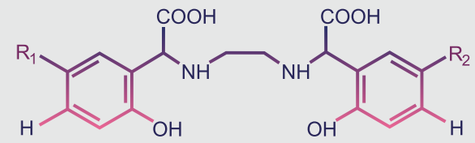


Fig.20 The structure of the phenolic chelating agent.

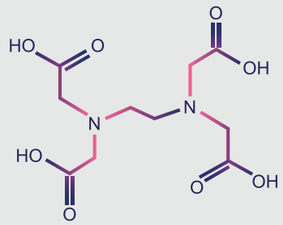


Fig.21 Structure of EDTA.

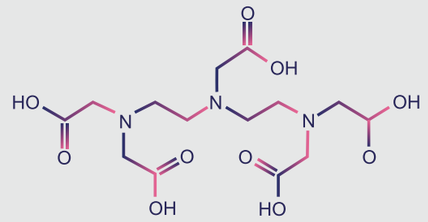


Fig.22 Structure of DTPA.

4.2 STABILITY OF IRON CHELATES

The factors that affect the stability of iron chelates are the structure of the chelating agent, the metal, and the environmental conditions (light, microorganisms, temperature, ionic force, pH, the partial pressure of CO2, redox potential, and presence of other ions) (Álvarez-Fernández, 2000).
The stability constants of different chelating agents with Fe3+ are reported in Table 5. One can see that the stability constants of phenolic iron chelates are similar to one another and considerably superior to those of the non-phenolic ones, except for o,p-EDDHA/Fe3+.

These differences in stability are fundamentally attributed to the structure of the ligands. The stability of iron chelates made of hexadentate ligands increases with the number of phenolic groups available in the coordination. Thus, the stability of the iron chelate increases if the carboxylates are replaced with two phenolates to create o,o-EDDHA, or EDDHSA (Table 5). Iron chelates also increase their stability once the number of donor groups available to coordinate is in an octahedral disposition with Fe3+. Hence, the lower stability constant for o,p-EDDHA/ Fe3+ cIn the o,p-EDDHA/Fe3+, the phenolic hydroxyl group is in para position to the aliphatic carbon, and as a consequence no bond is made with iron, and the position is occupied by a water molecule (Yunta et al., 2003a).

EDTA	25,0
DTPA	27,3
o-o EDDHA	35,9
o-p EDDHA	28,72
EDDHSA	32,79
HBED	39,01

Tab. 5 Logarithm of the stability constants ([FeL]/[Fe]•[L]) with EDTA Fe3+ and DTPA Fe3+ (Martell and Smith, 1974) of the regioisomer and diastereomers of o,o-EDDHA and EDDHSA (Yunta et al., 2003 a and b).

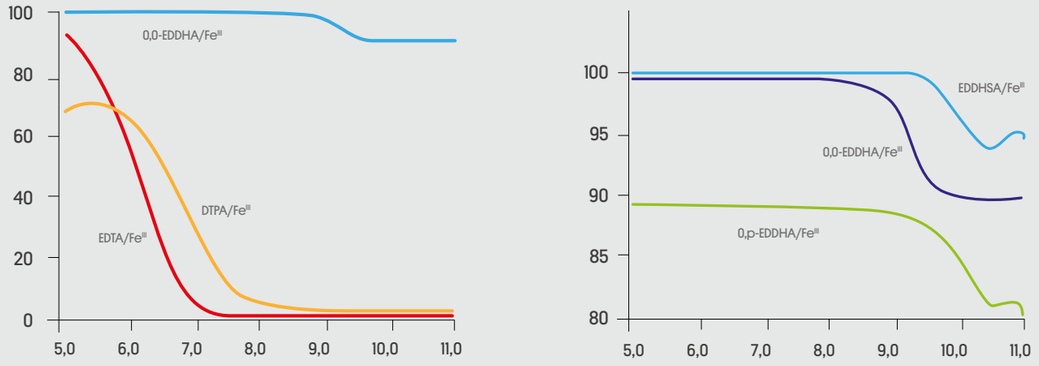


Fig.23 Stability diagrams of different ferric chelates in soil. (A) Comparison of iron chelates and the phenolic or not, or- EDDHA/Fe3+; Fig.24 Chelates of iron phenolic. Conditions: concentration of chelated iron = 100 mM, Cu2+ concentration maximum = 10 M.

4.3 QUALITY OF CHELATES

The amount of coordination between the metal and the aromatic chelating compounds varies from 4 to 6 based on the position of the phenolic oxygen I the molecules. Thus, the o-o-EDDHA forms six bonds, the o-p EDDHA stabilizes the iron with five bonds, and the p-p-EDDHA with only four bonds (like what happens with EDTA iron). The number of bonds is a fundamental point in influencing the stability of the above-mentioned chelated molecules, i.e., their inability to keep iron from being insoluble in an alkaline environment. Consequently, commercial products based on EDDHA always contain ortho-ortho, ortho-para or para-para isomers; the quality of the final product is mainly linked to the percentage of ortho, ortho isomer, the most stable complex.

4.4 IRON CHELATES AND THEIR USAGE IN AGRICULTURE

Iron chelates are used in agriculture to satisfy the nutritional needs of the crops. If the crops are in unfavorable pedoclimatic conditions, chelates can be used according to two strategies:

- Preventative, when the visible symptoms have not manifest and the objective is to avoid their manifestation.

- Curative, when iron chlorosis is visible, and the objective is to regain the correct nutritional state of the plant. Based on the application modality, we can distinguish between iron chelates primarily for radical use (phenolics: EDDHA, EDDHSA, HBED), and foliar use (non-phenolics: EDTA, DTPA). Phenolic chelates are primarily used in radical pathways because they are more stable than non-phenolic chelates in the pH ranges that are normally found in terrains that favor the occurrence of iron deficiency.

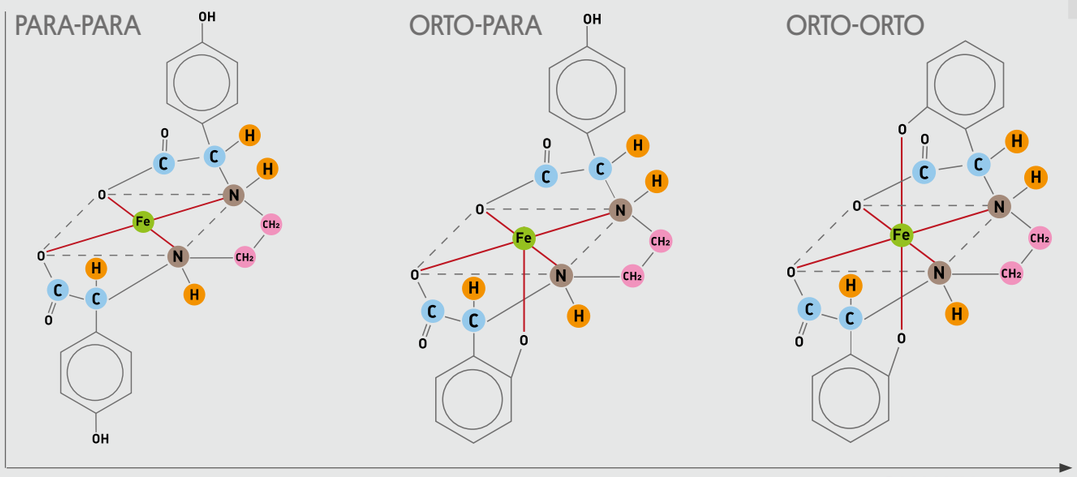


Fig.25 Quality of the different isomers of EDDHA chelated.

4.5 THE CHELATING AGENTS IN THE FERRILENE LINE PRODUCED BY VALAGRO:

Fe-EDDHA

• Is a complex in which iron is bonded to an organic molecule through more coordination bonds (preferably 6) in the ortho-ortho isomer.

• In this complex, iron is fully available for active absorption through the roots.

• Agronomically, the o-o fraction of the EDDHA complex gives Fe stability and persistence in the soil and is thus useful for curing iron deficiencies in extreme conditions.

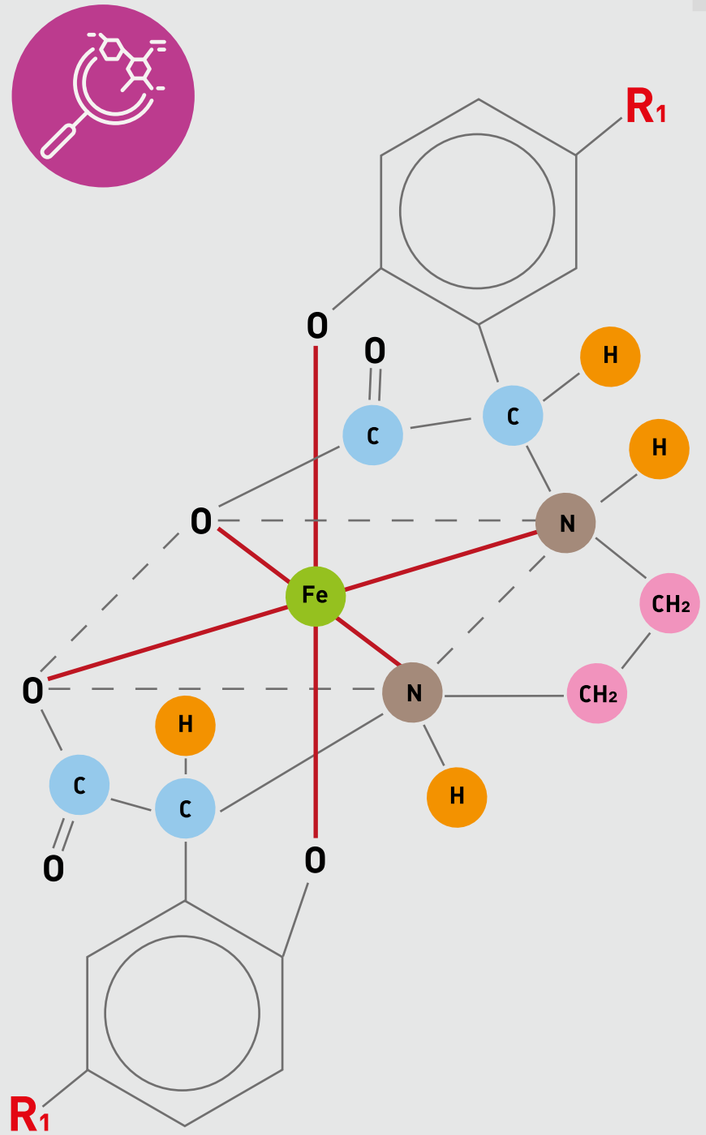
Fe-EDDHSA

• Differs from the EDDHA molecule due to the presence of the sulphonic group in the para position in the benzoic ring of the structural phenol.

• Potassium salt is used in the synthesis; sufficient amounts of potassium optimize iron absorption in the vegetal tissue.

• Agronomically, EDDHSA complex gives Fe stability and rapid action; this increases the regreening of the plants.

Furthermore, the solubility of Fe-EDDHSA is about 3.4 times greater than that of Fe-EDDHA. The presence of sulfo groups makes the phenolic groups more acidic, which results in an increase in the affinity of iron for this chelator. (Klem-Marciniak Molecules 2021, 26, 1933.).



Fe-EDDHA	R1= H
Fe-EDDHSA	R1 = SO_3K

Fig. 26 Structure of the chelating agents Fe-EDDHA, Fe-EDDHSA.

5 Ferrilene line

Rita Basile - *Global Marketing & Development*

5.1 CHELATING AGENT HISTORY

WE MAKE CHELATION!

Valagro is one of the most important companies in the world that manufactures Iron chelated based products in its own chelating plant.

The ability to manufacture iron chelates directly is important, as it enables Valagro to check and guarantee the quality of the final products. Chelated products are always checked and analyzed by Valagro internal laboratory before being packed and marketed.

Ferrilene Line is a full line of the best available iron chelates (EDDHSA; EDDHA) to suit specific adverse conditions in the soil where iron availability is impaired. These chelates can provide plants with iron in the pH range 4 -10. The chelate increases iron solubility and transports the iron to the root of the plant, where it releases the iron quickly.The line includes Ferrilene Trium, which due to its diverse components has the characteristic of having triple action against iron chlorosis; the Ferrilene line also includes Ferrilene 4.8 and Ferrilene.

"We are chemists, that is, hunters ... nature is immense and complex, but it is not impermeable to intelligence; we must circle around it, pierce and probe it, look for the opening or make it."

Primo Levi

CHELATING PLANT HISTORY

At the begging of the 90s, at Valagro (at that time called Farmer) the construction of the new factory building for the chelates facility begun, in which Valagro Chemist work (Leo Giannantonio currently Global Q-EHS & Compliance Director) played a decisive role, thus covering all the needs of farmlands, their owners, and more. Hailing from that specific sector, Leo understood chelate micronutrients were manufactured by just a handful of industries worldwide, and while constituting a niche business, even small productions guaranteed high economic margins. He also knew the properties of those molecules that imprisoned the nutrient, like the claws (or chelae) of a crab, conveying it undamaged to the plant and avoiding its solidification and dispersion in the soil. The expiration of Ciba's patent for chelated micronutrients meant that they were no longer expensive to manufacture, yet very few industries were doing so. Giuseppe and Ottorino realized that it would be more economical to produce their own, considering that they already had the facilities. Indeed, they would not only be able to package and sell the chelates, but could also use them to make other fertilizers, for example a water-soluble type, and of course manufacturing their own ingredients would ensure the quality that had become the company's mantra, as it was the only aspect that could make it stand apart. The gamble on chelates soon paid off. The facility managed by Pino Codagnone, who immediately became its personification having overseen the entire installation process, was operating at full capacity after a couple of years, significantly boosting turnover. This was aided by the fact that it widened the firm's potential clientele to other types of industries using micronutrients in other manufacturing sectors, leading Farmer to take over the Milan-based Siso, the very factory where Leo formerly worked, and become the only Italian company to manufacture chelating agents. More investments, more research and development, more revenue, more profits: it was a virtuous circle, a clearly defined path that continued to strengthen the entire structure.

Source: 2020 Valagro Book- Creating the future. Since 1980

5.2

FERRILENE 4,8 AND FERRILENE
(cure and prevent iron chlorosis)

CHARACTERISTICS

In this last classification we find different concentrations of ortho-ortho chelated iron as well as the most important iron chelating agents, Fe-EDDHA (Ferrilene 4,8) and Fe-EDDHSA (Ferrilene).
This group of stable chelates efficiently provide iron for crops in difficult conditions and represent important tools in the cure and prevention of iron chlorosis.
Currently included in this group of products are the ortho-ortho and ortho-para isomers.
In summary, within the Ferrilene line we find products that are stable in the face of hydrolysis, ensure the absorption of iron in difficult situations, have a rapid and long term effect, the metal is not easily substituted by other metal, they are not phytotoxic, are formed in soluble microgranules that do not generate dust and ensure rapid and total solubility.

COMPOSITION

PRODUCT	FERRILENE 4.8	FERRILENE
CHELATING AGENT	EDDHA	EDDHSA
IRON SOLUBLE IN WATER	6%	6%
CHELATED FRACTION	100%	100%
% ORTHO-ORTHO IRON	4.8%	3.8%

MODALITY OF USE AND DOSAGE

PRODUCT	FERRILENE 4.8	FERRILENE
POME FRUITS Kg/ha	5-30	10-30
STONE FRUITS Kg/ha	5-30	10-30
STRAWBERRY Kg/ha	5-15	5-15
CITRUS FRUITS Kg/ha	5-30	10-30
TABLE GRAPE Kg/ha	5-30	10-30
KIWI Kg/ha	5-30	10-30
VEGETABLES/ INDUSTRIAL CROPS Kg/ha	5-15	5-15
FLOWER CROPS Kg/ha	5-15	5-15
POTTED PLANTS g/plant	0,5 - 2	0,5 - 2

POWERED BY GEAPOWER

5.3

FERRILENE TRIUM
(Triple action against chlorosis)

CHARACTERISTICS

Ferrilene Trium is the latest technological innovation within the line: the result of research and development, marking a turning point in the treatment of iron chlorosis through a multi-strategy approach. Ferrilene Trium bases its effect on a series of interrelated elements that make it an integral and unique product amongst the products that focus on correcting iron chlorosis. FERRILENE TRIUM contains different components that are interrelated in the soil-plant complex as shown in the figure 27. All of this inside a tiny, complex, highly soluble microgranules obtained through a chemical reaction specifically designed for VALAGRO, fruit of the vision of GEAPOWER, which once again manifests Valagro's knowledge and technology in the production and development of solutions tailored to agriculture.

COMPOSITION

1. In FERRILENE TRIUM there are two types of chelated iron, Fe-EDDHA and Fe-EDDHSA in ortho-ortho position; the first Fe-EDDHA is distinguished by its stability and persistence in the soil. The second Fe-EDDHSA is a stable chelate that is simultaneously highly soluble, which gives it the characteristic of rapid action. Potassium salt is used in the product synthesis; potassium administrated in adequate amounts optimizes iron absorption, which makes the presence of potassium in this form a positive characteristic of the product.

2. FERRILENE TRIUM contains an equal ratio of iron and manganese; thus, in this section we will briefly discuss the importance of manganese in the plant. This micronutrient is absorbed by the plant primarily in a divalent form (Mn^{2+}). In this form, it rapidly combines with organic ligands in which it can rapidly be oxidized to Mn (III) and Mn (IV). Manganese has various functions in the plant's metabolism. The most documented and exclusive studies on manganese in green plants are those that break the water molecule and the evolution system of O_2 from the photosynthesis that occurs in the chloroplasts and which is called the Hill reaction. As a result, the manganese deficiency mainly affects photosynthesis and the evolution of O_2. Furthermore, it is a catalyst of the synthesis of chlorophyll and participates in the oxidation reduction reactions in the plants. It forms a structural part of manganin, a constituent in the synthesis of glutathione, activation of methionine, hormone control and protein synthesis. Manganese (Mn) also acts as an important cofactor for various fundamental enzymes in the biosynthesis of secondary metabolites in the plant associated with the shikimic acid pathway, including phenolic aromatic

Fig. 27 FERRILENE TRIUM's components.

amino acids, coumarins, lignin and flavonoids. The current presence of iron and manganese in Ferrilene Trium in the form of highly stable chelates ensures that an optimum reciprocal relation is maintained between the two elements, avoiding the phenomenon of secondary chlorosis.

3. The presence of biologically active ingredients makes Ferrilene Trium a unique product that offers an integral response when curing and preventing iron chlorosis. The triple action of Ferrilene Trium is:

ACTION	RESULT
1. Facilitates the absorption of iron through two distinct chelating agents, Fe-EDDHA/Fe-EDDHSA.	Has rapid action (EDDHSA) and is persistent (EDDHA) in the cure and prevention of iron chlorosis.
2. Gives the plant the right balance of Fe/Mn	Ensures an optimum relation between the two elements, improving the metabolic functions in the plant and curing it of the iron chlorosis cased by Fe and Mn deficiency.
3. Biological active ingredients improve the activity of the proton gates.	Improves the plant's absorption ability, making it stronger and more vigorous.

The biologically active components with biostimulant activities act directly on the root increasing levels of absorption of iron and Manganese and improving the chlorophyl content of the leaves.

5.2.3 MODALITY AND DOSAGE (FERTIGATION)

PRODUCT	FERRILENE TRIUM
POMACEOUS	10-30 Kg/ha
DRUPACEOUS	10-30 Kg/ha
STRAWBERRY	5-15 Kg/ha
CITRUS FRUITS	10-30 Kg/ha
TABLE GRAPE	10-30 Kg/ha
KIWI	10-30 Kg/ha
VEGETABLES/INDUSTRIAL CROPS	5 -15 Kg/ha
FLOWERS	5-15 Kg/ha
POTTED PLANTS	0.5 - 2 g/plant

PRODUCT	FERRILENE TRIUM
CHELATING AGENT	EDDHA/ EDDHSA
IRON SOLUBLE IN WATER	6%
CHELATED FRACTION	100%
% ORTHO-ORTHO IRON	4.0%
MANGANESE (Mn-EDTA)	1%
POTASSIUM (K_2O)	6%

6

Phenomic approach

Stefano Di Russo - *Plant phenotyping associate*

6. PHENOMIC APPROACH ON KIWIFRUIT

Using the Scanalyzer 3D System, we obtain images at different wave lengths (RGB, UV, NIR) and observe the effects of the prototype under analysis on the phenotype of the plant.

Standardization of the plants' growth conditions (irrigation, micro-climate, soils) and computerized data management enable us to efficiently analyze the results of more than 3,000 plants a day.

Fig. 28 Scanalyzer 3D system.

We start to see the trial on kiwifruit, hayward variety, In this trial 7 different chelates were tested compared to untreated test. 1 application for eachone when chlorosis appeared was done.

General information

Location	Metaponto (Southern Italy)
Variety	Hayward
Invest (p/ha)	-
Trial level	II

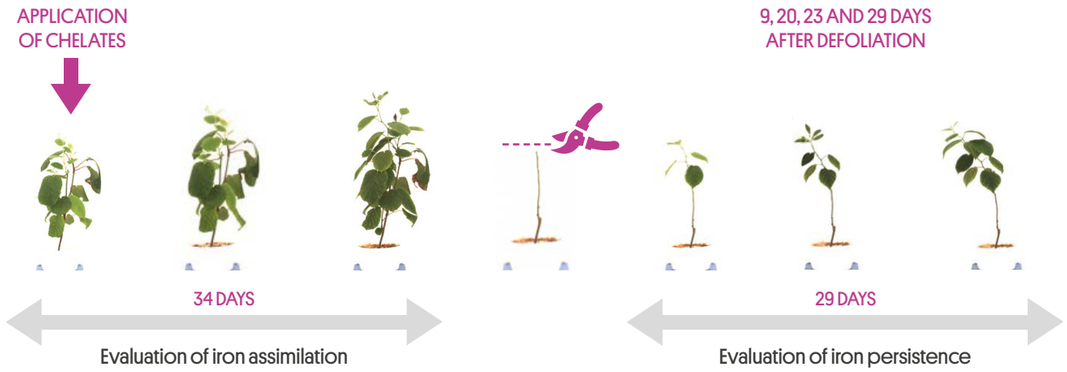
Date and growth stage of the applications

N°	Treatment	Date	Growth stage
1°	A	04/07/2012	When chlorosis appeared

Treatments

Treatment	Rate	N° of applications	Volume of water	Date of treatments
FERRILENE 4,8	5 g/plant	1	100 ml/plant	A
TRIUM	5 g/plant	1	100 ml/plant	A
Untreated	-	-	-	-

In these trials we have evaluated the iron assimilation until 34 days after application of chelates. Then we have removed the leaves to evaluate the iron persistence in the soil until 29 days after defoliation.
In both cases, digital biomass, increase of colour classes and low florescense were detected. We start to see the evaluation of iron assimilation.

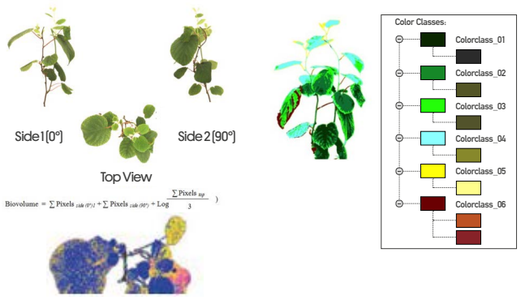


1. RGB (Red-Green-Blue) → growth and colour

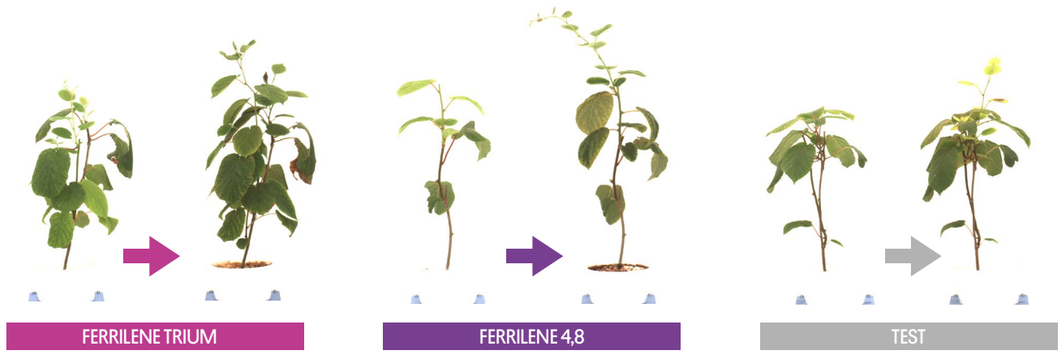
- Digital biomass
- Increase of color classes

2. UV (ultra violet) →photosynthetic activity

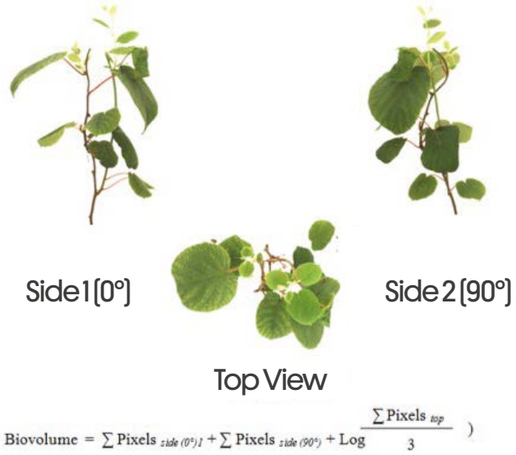
- Low florescence



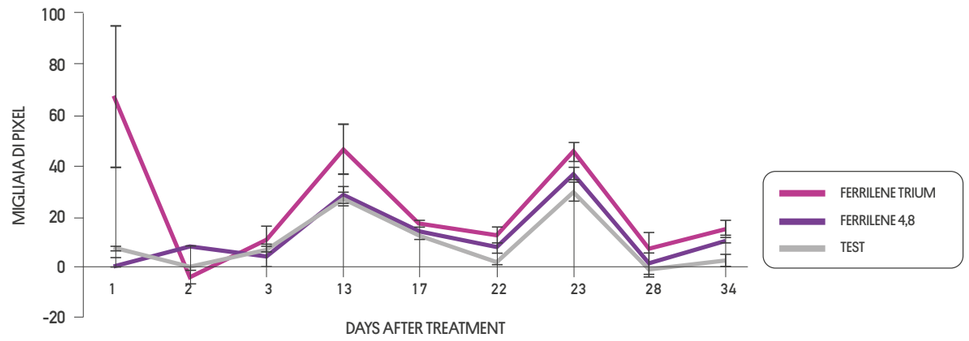
RGB: EVALUATION OF IRON ASSIMILATION – DIGITAL BIOMASS

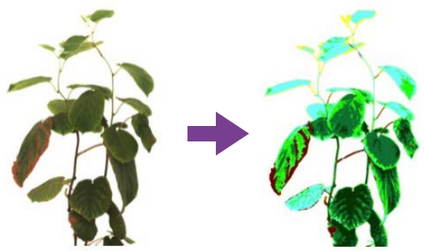


They are images taken in RGB chamber before application. Let's see the plant growth after the application of chelates. This is the situation 34 days after application. As you can see the untreated test showed some yellow apical leaves compared to other treatments.

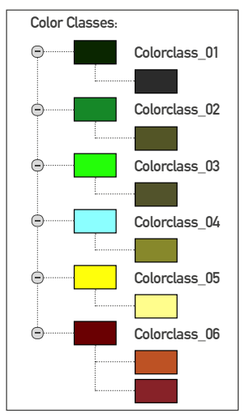


In RGB chamber we have taken for each treatment 3 different images: In side, top view and side 90 degrees; than throught this formula the computer station has calculated the digital biomass.

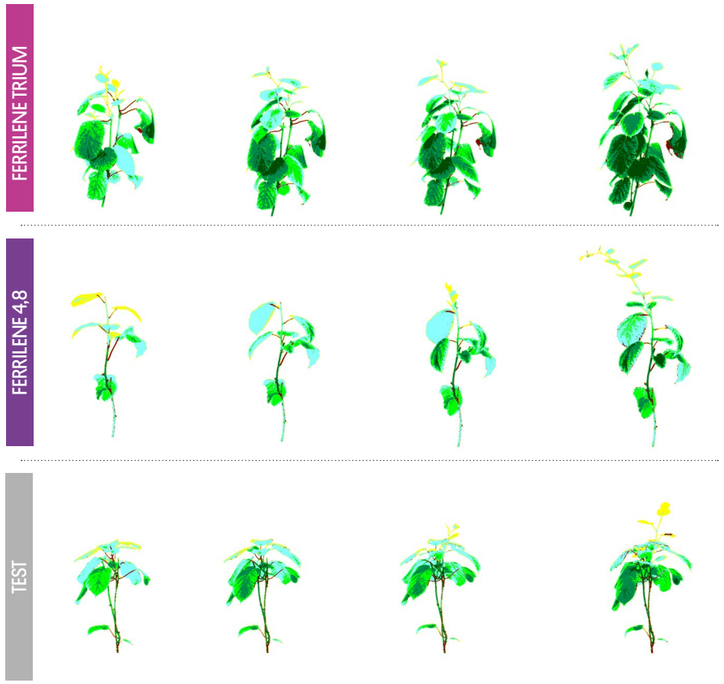


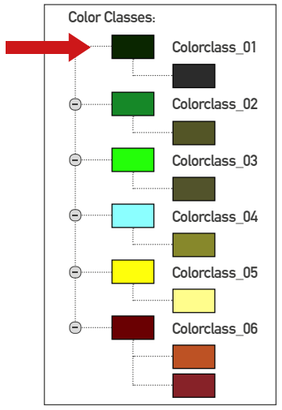


The prototype Ferrilene 4,8 showed the best result in terms of digital biomass. Then we have evaluated the increase of color classes from dark green to brown.



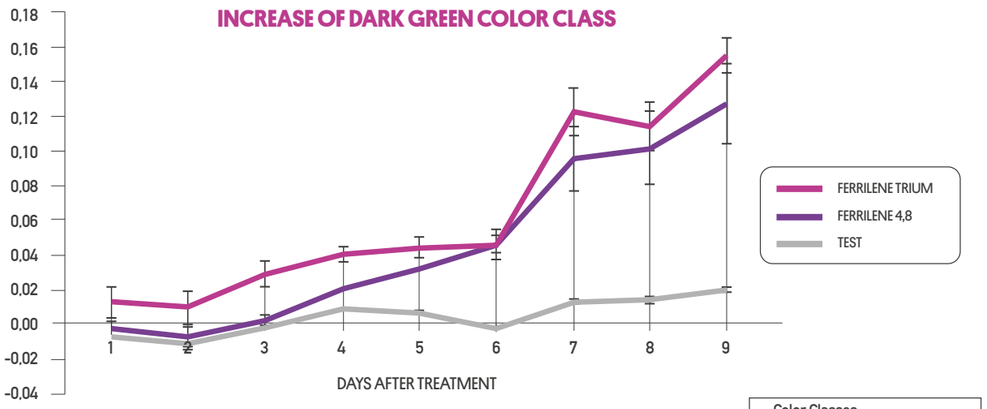
INCREASE OF COLOR CLASSES

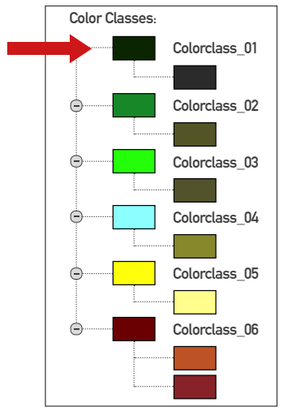




This is the situation before treatmets... while they were taken 34 days after application. Now we are going to see the results about the increase of dark green and yellow colors that they are the most rappresentative color classes.

INCREASE OF DARK GREEN COLOR CLASS

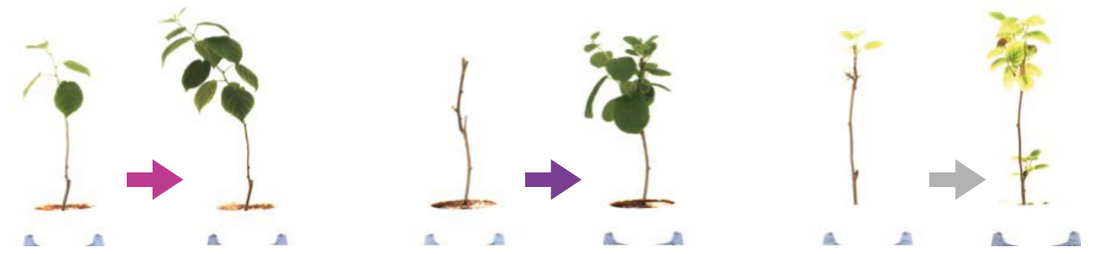




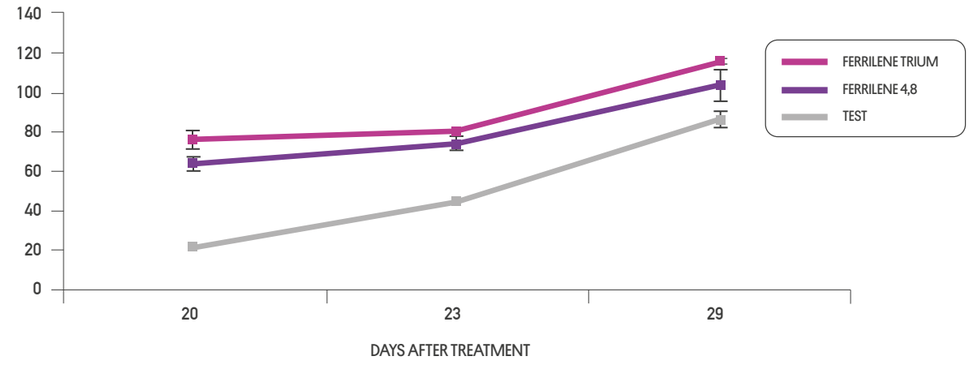
There was the first group of chelate which includes Ferrilene 4,8 and Ferrilene Trium that showed the best results.

RGB: EVALUATION OF PERSISTENCE - DIGITAL BIOMASS

Now we are going to see the iron persistence in the soil after plant defoliation.



This is in RGB chamber... where it is possible to see clear difference beetween treated plants and untreated test that showed a lot of chlorosis.



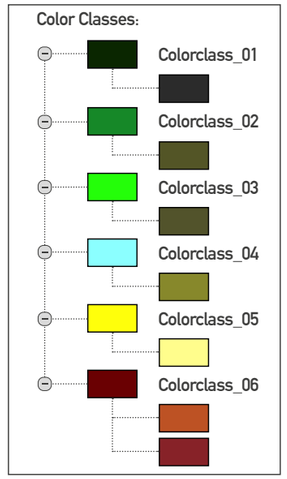
$$\text{Biovolume} = \sum \text{Pixels}_{\text{side (0°)1}} + \sum \text{Pixels}_{\text{side (90°)}} + \text{Log}\left(\frac{\sum \text{Pixels}_{\text{top}}}{3}\right)$$

INCREASE OF COLOR CLASSES

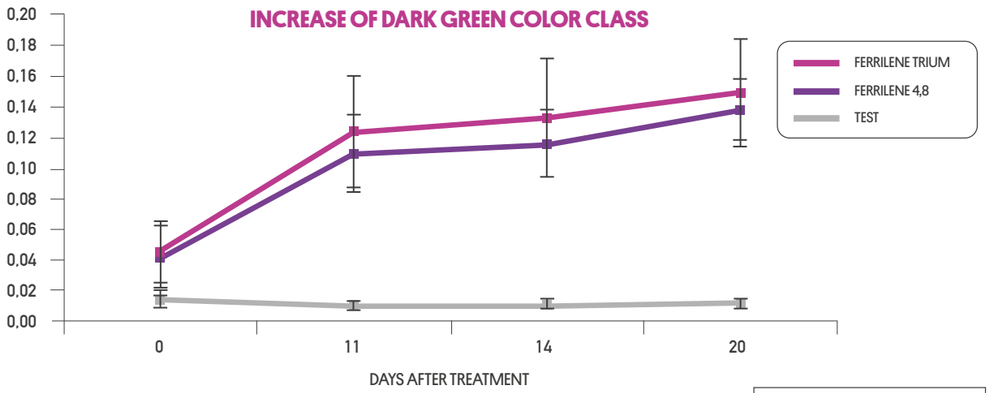
FERRILENE TRIUM

FERRILENE 4,8

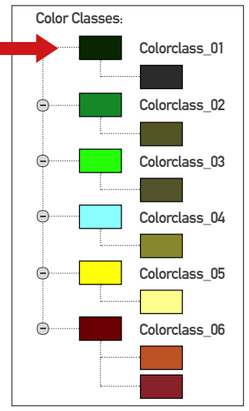
TEST



INCREASE OF DARK GREEN COLOR CLASS



All prototype have increased the dark green class.



7

Agronomic trials

FERRILENE 4,8

Peach

LOCATION
Catania - Italy

VARIETY
Baby gold 7

APPLICATION METHOD
Localized fertigation

Crop info & soil description

Trial ID:
FERRILENE 12.OPEAC.01ITA_X

Distance between rows	4,5 m
Distance on rows	2,0 m
p/ha	1000

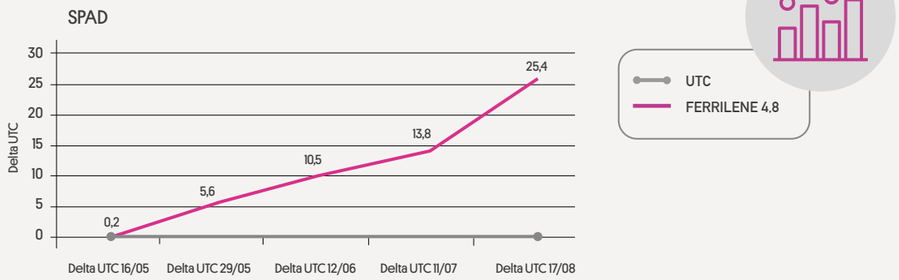
Soils with a high carbonates content and high pH, where Iron (Fe) is naturally unavailable for the plant, and is needed to be supplemented in the soil with iron chelate fertilisers.

Trial protocol

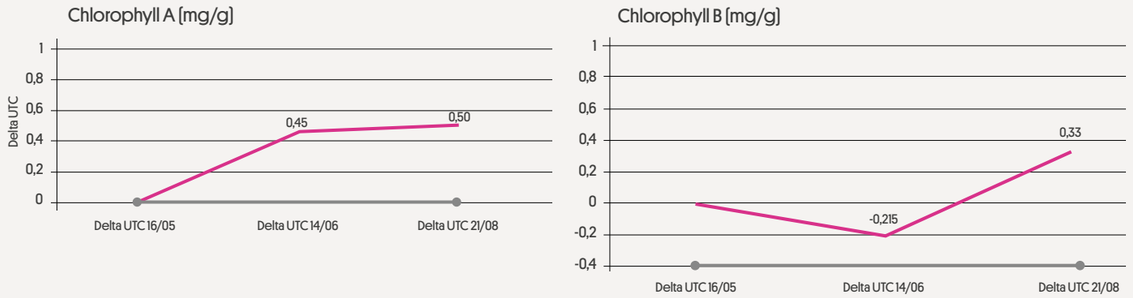
PRODUCT	# of applications	Dosages (kg/ha)	1° application	2° application	3° application
UTC	-	-	-	-	-
FERRILENE 4,8	3	5	16/05 Fruit development	29/05	26/06

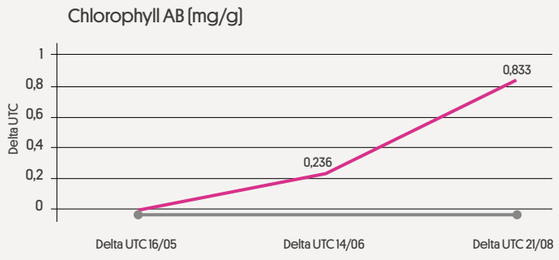
Broadcast fertilizer applications were made at standard production levels. Pre- and post-emergence herbicide maintenance applications were made across the trial area as needed.

Results SPAD vs UTC

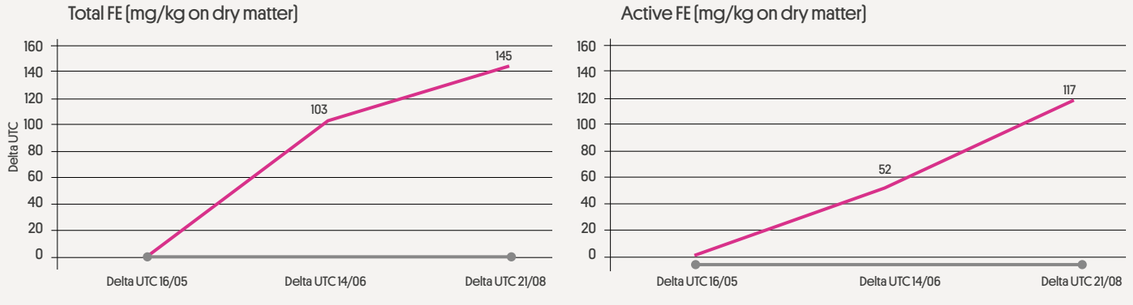


Results CHLOROPHYLL vs UTC





Results FE (mg/kg) vs UTC



FERRILENE TRIUM

Tomato - greenhouse

LOCATION
Catania - Italy

VARIETY
Icram

APPLICATION METHOD
Localized fertigation

Crop info & soil description

Trial ID:
FERRILENE12.VTOFR.01ITA_X

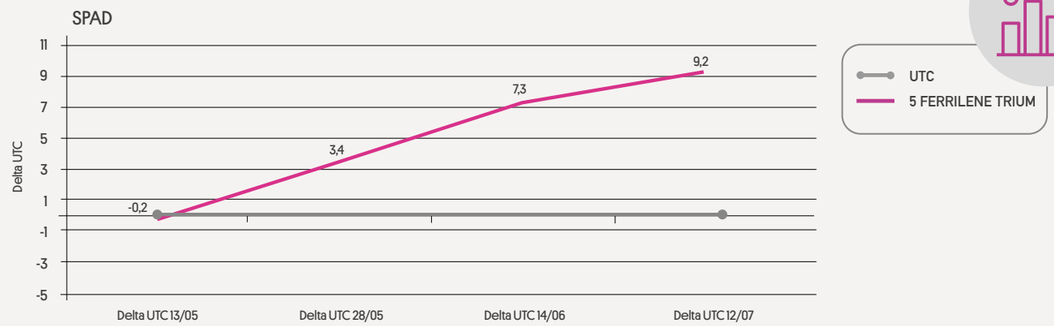
Distance between rows	1,00 m
Distance on rows	0,33 m
p/ha	30000

Soils with a high carbonates content and high pH, where Iron (Fe) is naturally unavailable for the plant, and is needed to be supplemented in the soil with iron chelate fertilisers.

Trial protocol

PRODUCT	# of applications	Dosages (kg/ha)	1° application	2° application
UTC	-	-	-	-
FERRILENE TRIUM	2	5	13/05 Flowering - 6° Bunch	16/06 Ripening - 1° Bunch

Results SPAD vs UTC



FERRILENE

Table Grape

LOCATION
Catania - Italy

VARIETY
Italia

APPLICATION METHOD
Localized fertigation

Crop info & soil description

Trial ID:
FERRILENE12.OGRTA.01ITA_X

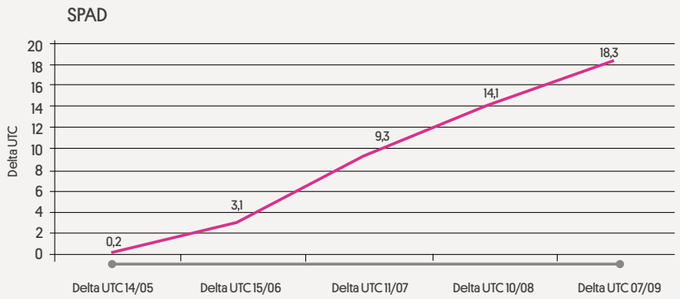
Distance between rows	3,00 m
Distance on rows	3,00 m
p/ha	1000

Soils with a high carbonates content and high pH, where Iron (Fe) is naturally unavailable for the plant, and is needed to be supplemented in the soil with iron chelate fertilisers.

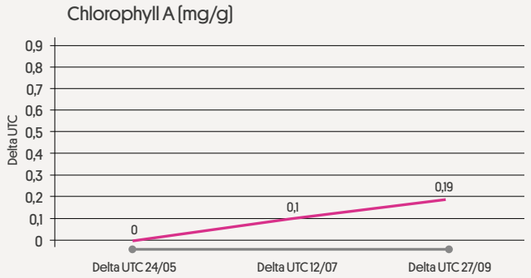
Trial protocol

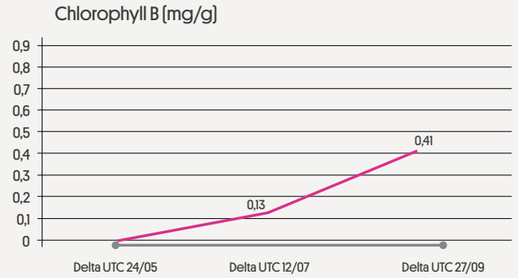
PRODUCT	# of applications	Dosages (kg/ha) 1° application	Dosages (kg/ha) 2° application	1° application	2° application
UTC	-	-	-	-	-
FERRILENE	2	5	10	24/05 Flowering	11/07 Fruit development

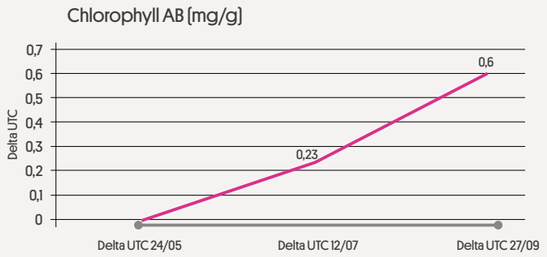
Results SPAD vs UTC



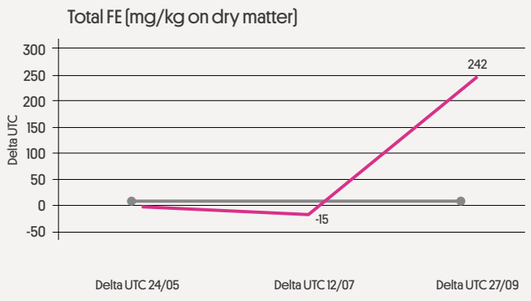
Results CHLOROPHYLL vs UTC

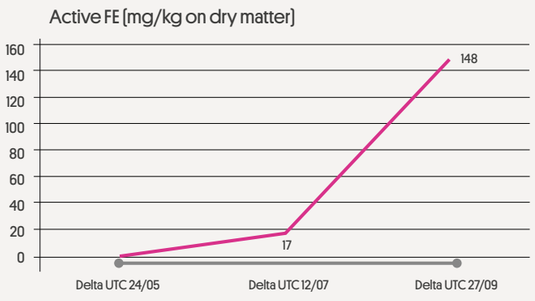






Results FE (mg/kg) vs UTC





FERRILENE 4,8

Pear

LOCATION
Ferrara - Italy

VARIETY
Abate Fétel

APPLICATION METHOD
Localized fertigation

Crop info & soil description

Trial ID:
FERRILENE12.OPEAR.01ITA_X

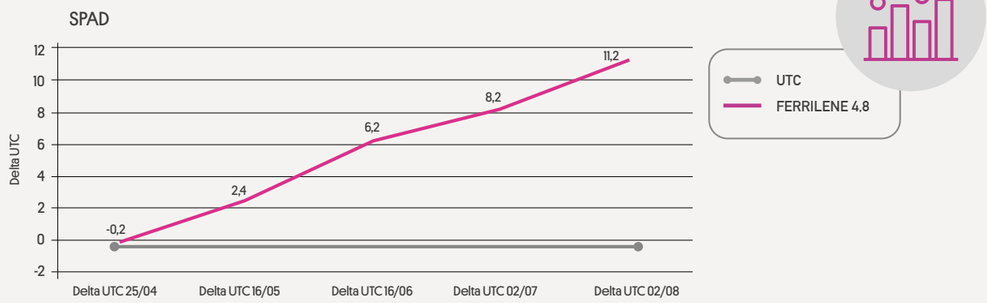
Distance between rows	3,30 m
Distance on rows	0,70 m
p/ha	4329

Soils with a high carbonates content and high pH, where Iron (Fe) is naturally unavailable for the plant, and is needed to be supplemented in the soil with iron chelate fertilisers.

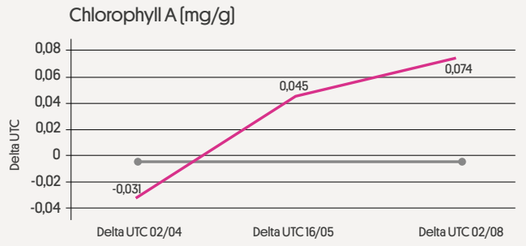
Trial protocol

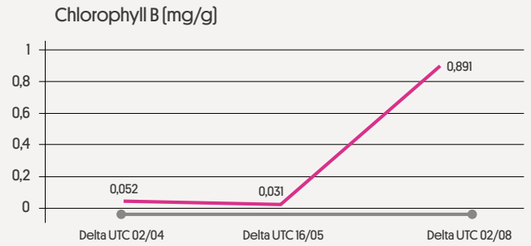
PRODUCT	# of applications	Dosages (kg/ha) 1° application	1° application
UTC	-	-	-
FERRILENE 4,8	1	20	24/04 Post-flowering

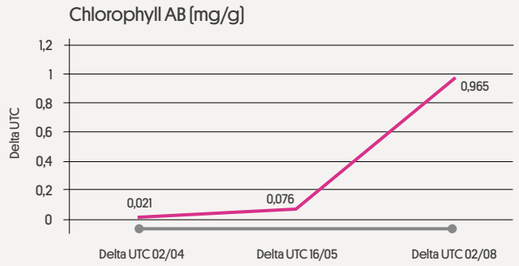
Results SPAD vs UTC



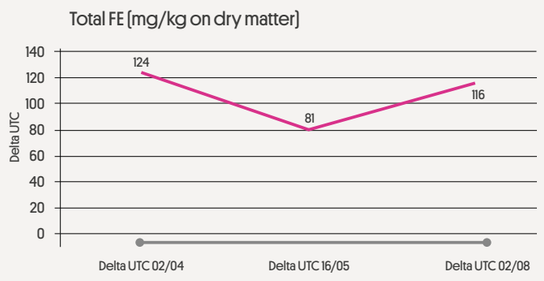
Results CHLOROPHYLL vs UTC

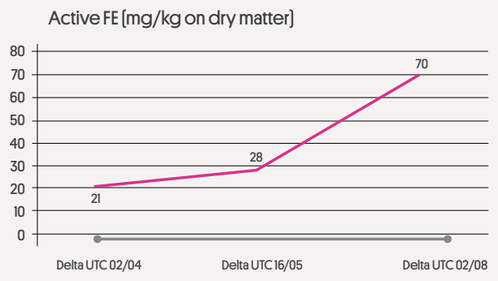






Results FE (mg/kg) vs UTC





FERRILENE TRIUM

Wine Grape

LOCATION
San Michele all'Adige - Italy

VARIETY
Cabernet franc

APPLICATION METHOD
Localized fertigation

Crop info & soil description

Trial ID:
FERRILENE12.OGRWI.01ITA_MACH_X

Distance between rows	2,2 m
Distance on rows	0,9 m
p/ha	5050

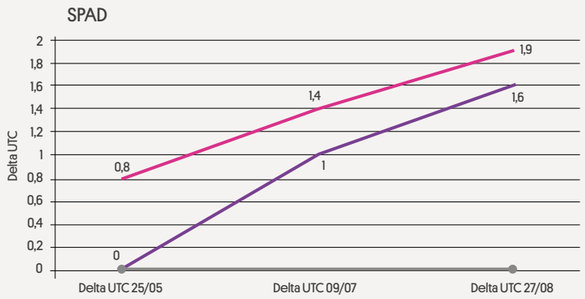
Soils with a high carbonates content and high pH, where Iron (Fe) is naturally unavailable for the plant, and is needed to be supplemented in the soil with iron chelate fertilisers.

% Sand	58	P_2O_5 ppm	80
% Silt	34	K_2O ppm	75
% Clay	8	MgO ppm	230
pH	7.8	$CaCO_3$%	60
Organic Substance %	2.8	CEC meq/100 gr	13

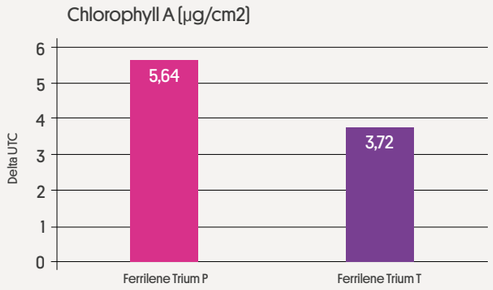
Trial protocol

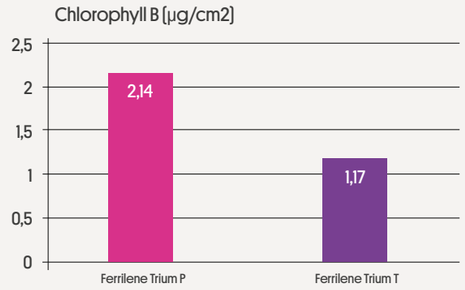
PRODUCT	# of applications	Dosages (kg/ha)	1° application
UTC	-	-	-
FERRILENE TRIUM P (Previous treatment)	1	10	26/04
FERRILENE TRIUM T (Tardive treatment)	3	10	07/06

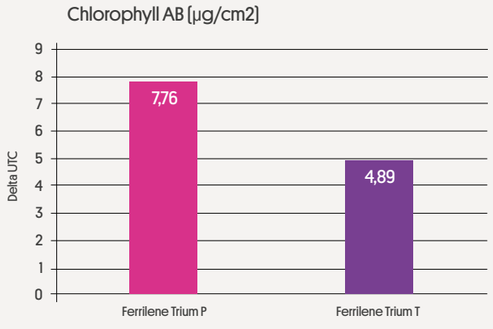
Results SPAD vs UTC



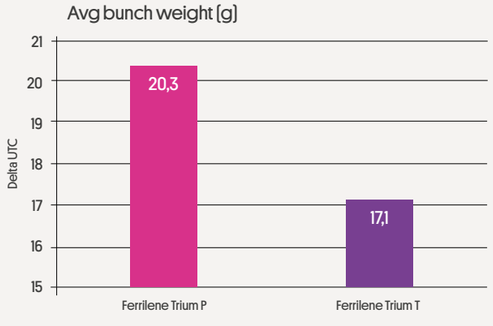
Results CHLOROPHYLL vs UTC

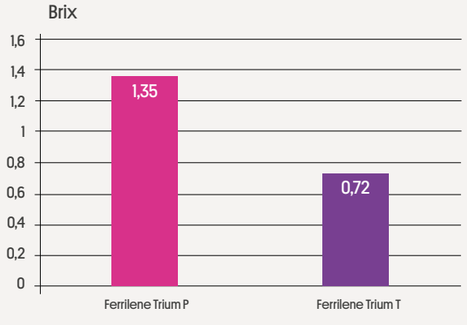


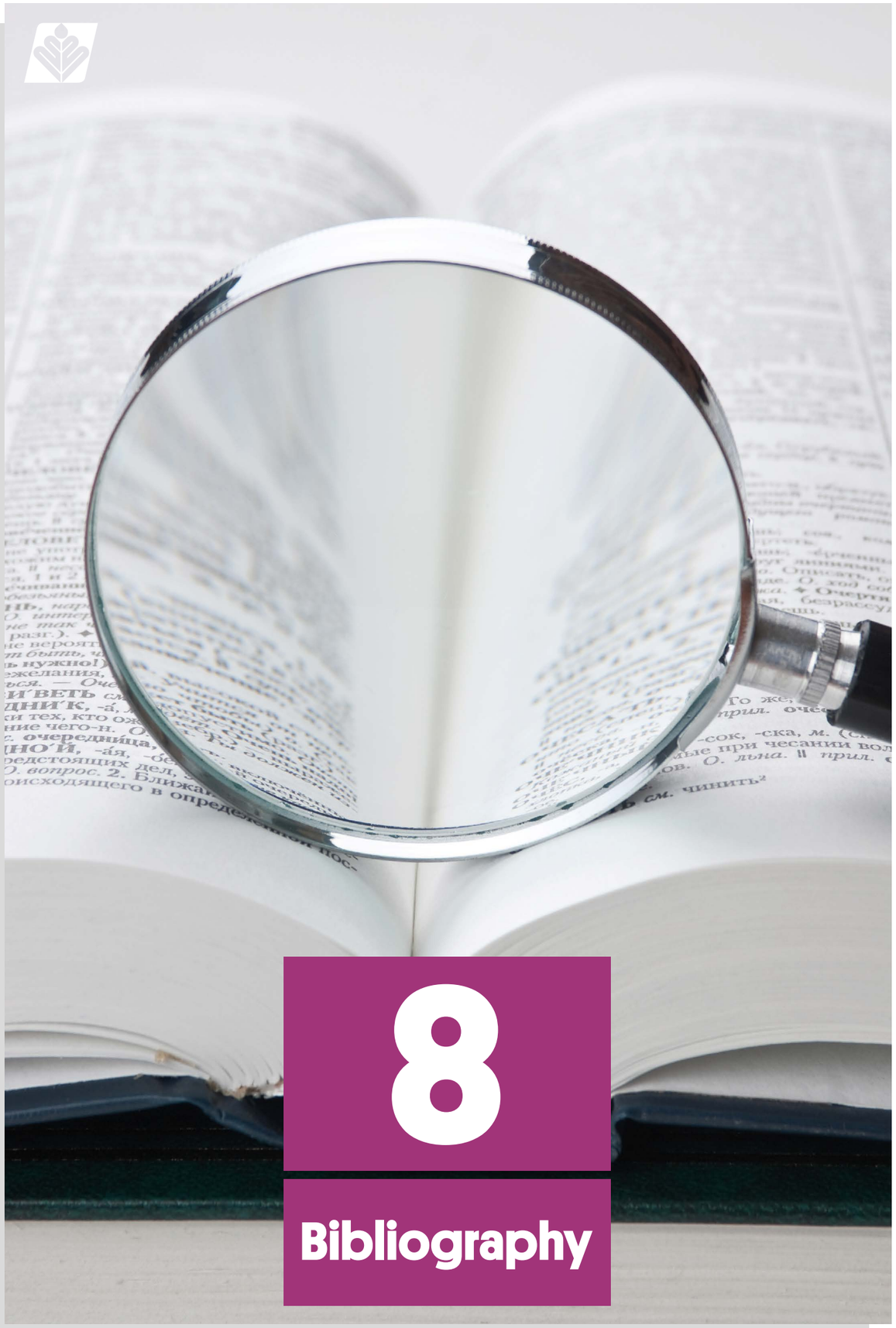




Results







8

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