



Technical study and field tests

www.acting-herbagreen.com

Introduction.....	p.3
I. An innovative technology: tribomechanical activation.....	p.4
II. Chemical and physical analysis of HerbaGreen.....	p.5
1. qualitative and quantitative composition.....	p.5
2. granularity.....	p.5
3. standards.....	p.6
4. report of analysis from ENSCMU..... (Chemical Superior National College of Mulhouse)	p.6
5. solubility and suspension stability.....	p.16
 <i>Summary p.21</i>	
III. Study of HerbaGreen properties.....	p.23
1. improvement of photosynthesis.....	p.23
2. antioxidant potential effect of HerbaGreen, SADEF Laboratory.....	p.32
3. roles of Calcium in the vegetal reinforcement.....	p.34
- study on young vine suffering from powdery mildew..... (SADEF laboratory)	p.34
- alternative solutions against powdery mildew of strawberries.....	p.43
4. phytotoxicity test of HerbaGreen (on lettuce).....	p.46
5. HerbaGreen and hydrous stress.....	p.50
 <i>Summary p.54</i>	
IV. Experimentation results of HerbaGreen.....	p.57
1. on field crops.....	p.57
2. on consumption potatoes.....	p.68
3. on potatoes for industrial transformation.....	p.72
4. in market gardening.....	p.74
5. in viticulture.....	p.97
 <i>Summary p.114</i>	
V. Technical data.....	p.117
Conclusion.....	p.119
Appendix 1.....	p.122
Abbreviations list.....	p.123

INTRODUCTION

Since 2004, Tribo Technology develops interesting agricultural and zootechnic solutions coming from the research of different European laboratories.

Our job: we set up the necessary researches to improve agricultural products with agronomic and environmental added value.

The team of Tribo Technologies is composed of scientists and professionals who want to go into service with a lasting agriculture.

The invention of a new micro grinding technology, which is the subject of an international patent, allows to create HerbaGreen, made of a particular calcite which acts on the vegetal physiology.

Experimentations made in France and in many European countries, in China and in South America show that HerbaGreen has a strong potential as an innovative solution for the development of a lasting agriculture.

HerbaGreen is a 100 % natural product, no polluting, made of calcite micro particles.

HerbaGreen is a foliar treatment which acts on the plant reinforcement and on the activation of the primary and secondary metabolisms. It favours a better resistance of the plant against the biotic and abiotic stresses. Moreover, it improves quality and yield of cultivations, while the environment is always respected.

I. AN INNOVATIVE TECHNOLOGY : TRIBOMECHANICAL ACTIVATION

For more than one century, many researchers tried to improve the activation techniques of minerals to get finer and more reactive particles. The discovery of Tihomir Lelas allows to get a more superior granularity by mechanical and no chemical process. The mineral particles became more reactive.

In the fifties, a tribomechanical disintegrator was conceived and used to activate coal. The mineral goes through two discs which turn from the opposite direction with a very high speed. The discs have steel rods which collide with the mineral particles. This technique allows to increase the calorific power by 30%, but the collision would wear away the steel rods and would destroy the machine.

Tihomir Lelas always tried to improve the process; he perfected the tribomechanical activation by using ailerons as substitute for the steel rods. It allowed the aerodynamic driving of the micro particles. In this new activator, the mineral particles are driven by an air flux and collide with each other (shocks mineral/mineral and no steel/mineral) with a very intense rhythm: in average, 3 collisions per millisecond. Therefore, the surface of the particles will be extremely irregular and fragmented; this changes the properties of the contact surface, destabilizes the mineral structure and increases mineral reactivity and solubility.

II. CHEMICAL AND PHYSICAL ANALYSIS OF HERBAGREEN

1. qualitative and quantitative composition

HerbaGreen is made of calcite only. Calcite is a sedimentary rock which emerges from calcareous seaweed. Calcite is mainly made of carbonate calcium, of silica, of magnesium and trace elements.

Main components:

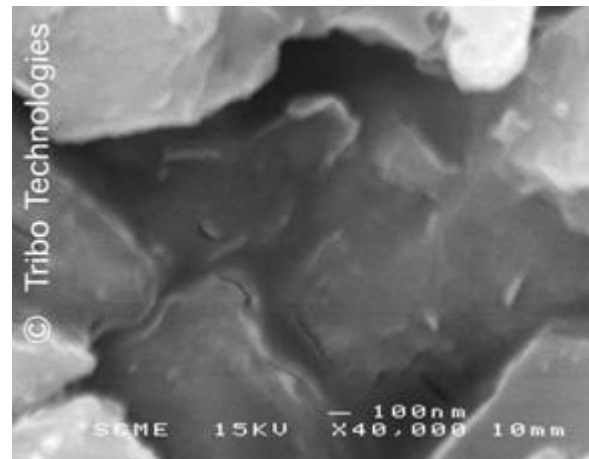
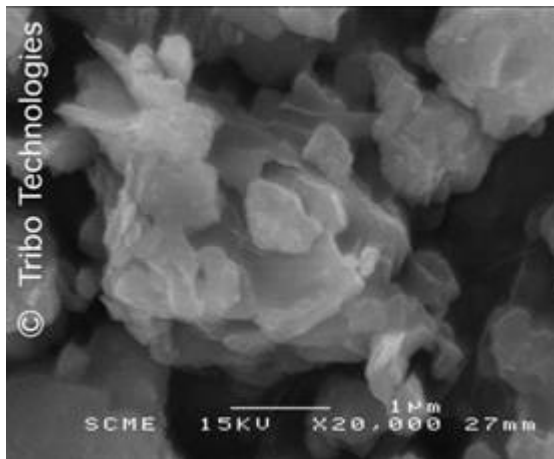
- CaCO_3 (carbonate calcium) : 82.3%
- SiO_2 (silicium dioxide) : 8.56%
- MgO (magnesium oxide) : 3.02%
- CaO (calcium oxide) : 41.7%
- Iron: 8783 mg/kg
- Mn (manganese) : 156 mg/kg
- Selenium: 0.24 mg/kg

Carbonic solubility: 65

Neutralizing value: 47

2. granularity

HerbaGreen has a granularity in the region of the micron.



Pictures taken by transmission electronic microscope at CIRAD

The mineral surface has a fragmented structure with a big exchange surface.

3. Standards

HerbaGreen corresponds to the NFU 44-001 standard and can be used in organic agriculture, in conformity with the modified CEE n° 2092/91 regulation of 24th July 1991.

4. REPORTS OF ANALYSIS FROM THE ECOLE SUPERIEURE DE CHIMIE DE MULHOUSE LABORATORY

Material and methods

X-rays diffraction analysis

Parent calcite sample and TMA sample

The powder was grinded in a mortar before studied by X-rays diffraction.

Nitrogen absorption results in the calcite samples

Two samples have been studied:

- parent calcite
- TMA calcite

Nitrogen absorption isotherms were made thanks to an apparatus called Tristar 3000 of MICROMERITICS; it is adapted to mesoporous and no porous samples. A masse of 100 to 200 mg was taken and the gas which was in it was removed, with a temperature of 90° for one hour first and then of 300° for 15h, before making measurements on anhydrous components.

Electronic microscope observation

Samples were observed with electronic microscope. Qualitative analyses were made on TMA calcite with an EDX microprobe.

Chemical analysis made with X fluorescence

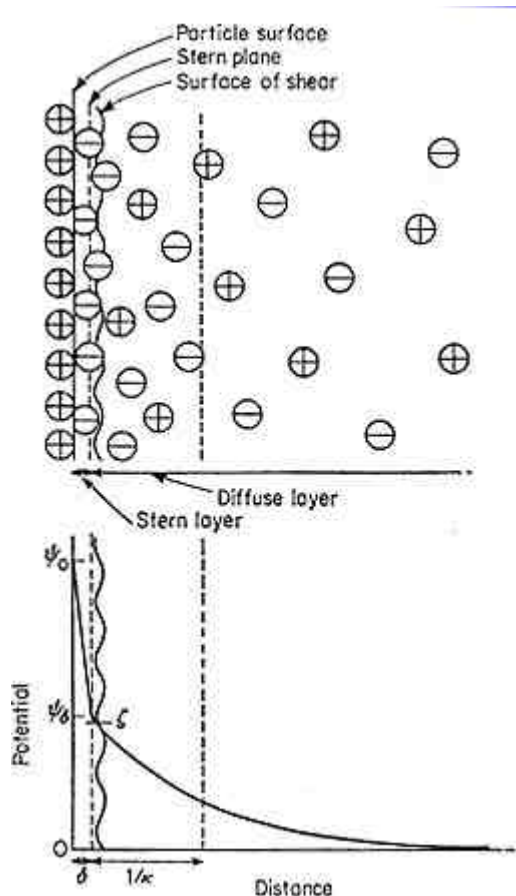
Two samples were studied by X fluorescence spectrometry.

The samples were compacted under a pressure of 20 tonnes for 10 minutes to form pastilles of 40 mm diameter. The analysis of those pastilles was made under vacuum (5 Pascals) with the wavelength dispersion X fluorescence spectrometer.

dynamic diffusion of the light and zeta potential

ZETA POTENTIAL

WHAT IS ZETA POTENTIAL?



The charge which develops at the interface between a colloidal particle and the liquid medium in which it is suspended may arise by any of several mechanisms. Among these are the dissociation of ionogenic groups in the particle surface and the differential adsorption from solution of ions of different charges into the surface region; in clays, ion exchange mechanisms may also be important.

The development of a net charge at the particle surface affects the distribution of ions in the neighbouring interfacial region, resulting in an increased concentration of counter ions - ions of charge opposite to that of the particle - close to the surface. Thus an electrical double layer is formed in the region of the particle-liquid interface.

The double layer (see figure above) may be considered to consist of two parts: an inner region which includes ions bound relatively strongly to the surface (including specifically adsorbed ions) and an outer, or diffuse, region in which the ion distribution is determined by a balance of electrostatic forces and random thermal motion. The potential in this region,

therefore, decays as the distance from the surface increases until, at sufficient distance, it reaches the bulk solution value, conventionally taken to be zero.

When subjected to an electric field as in micro electrophoresis, each particle and its most closely associated ions move through the solution as a unit and the potential at the boundary between this unit i.e. at the surface of shear between the particle with its ion atmosphere and the surrounding medium, is known as the zeta potential ζ . When a layer of macromolecules, whether a polyelectrolyte or an uncharged polymer, is adsorbed on the surface of the particle, this can alter the zeta potential simply because it shifts the location of the shear plane further from the actual surface.

Zeta potential is therefore a function of the surface charge of the particle, any adsorbed layer at the interface and the nature and composition of the surrounding medium in which the particle is suspended. It is usually, but not necessarily, of the same sign as the potential actually at the particle surface but, unlike the surface potential, the zeta potential is readily accessible by experiment. Moreover, because it reflects the **effective** charge on the particles and is therefore related to the electrostatic repulsion between them, zeta potential has proven to be extremely relevant to the practical study and control of colloidal stability and flocculation processes.

The principal of determining zeta potential by micro electrophoresis is very simple. A controlled electric field is applied via electrodes immersed in the sample suspension and this causes the charged particles to move towards the electrode of opposite polarity. Viscous forces acting upon the moving particle tend to oppose this motion and equilibrium is rapidly established between the effects of the electrostatic attraction and the viscous drag. The particles therefore reach a constant "terminal" velocity.

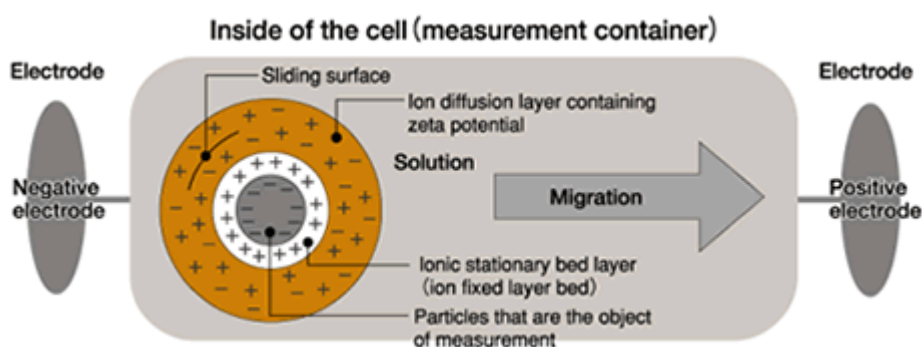
This velocity is dependent upon the electric field strength or voltage gradient, the dielectric constant and viscosity of the liquid - all of which are known - and **the zeta potential**. It is usually expressed as the particle mobility which is the velocity under unit field strength. For all practical purposes, the relationship between mobility, μ , and zeta potential, ζ , is quite simple and, for instance, in water at 25 ° C can be expressed **as: $\zeta = 12.85 \mu$**

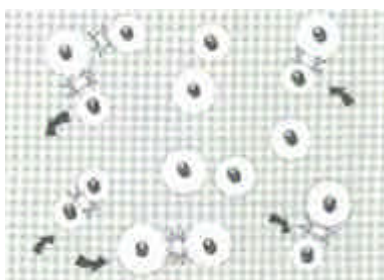
In practice, zeta potentials are usually negative, i.e. the surface is negatively charged, but they can lie anywhere in the range from -100 to +100 mV.

Colloidal particles dispersed in a solution are electrically charged due to their ionic characteristics and dipole attributes.

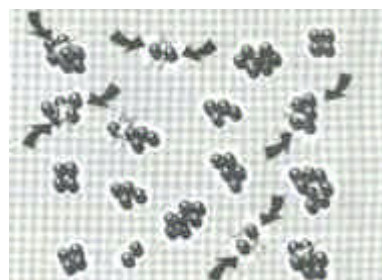
Each particle dispersed in a solution is surrounded by the opposite ions called the fixed layer. Outside of the fixed layer are of varying composition of ions of opposite polarities, forming the cloud-like area. The area is called the diffuse double layer, and the whole area is electrically neutral.

When voltage is applied to the solution in which particles are dispersed, particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the diffuse double layer, which is the internal side of the sliding surface.





Dispersed particles



Aggregated particles

Zeta potential is considered to be the electric potential of this inner area including the sliding surface. As this electric potential approaches zero, particles tend to aggregate.

Zeecom uses an electrophoresis microscope for observing particle migration on the monitor while performing image processing to obtain the zeta potential results.

To avoid error due to electro osmotic flow caused by electrostatic charge of the measurement cell, zeta potential is measured at the Static Layer, which can be calculated from the width and depth of the cell. (The software carries out the above calculation automatically)

The Static Layer is computed using the following formula

$$\frac{hs}{b} = \sqrt{\frac{1}{3} + \frac{128}{\pi^5 K}} \quad \left| \begin{array}{l} K = (\text{Width of Cell})/(\text{Thickness of Cell}) \\ b = \text{A Half of Thickness of Cell} \\ hs = \text{Static Layer (Distance from center of Cell)} \end{array} \right.$$

Zeta Potential (Smoluchowski's Formula)

$$\zeta = \frac{4\pi\eta}{\varepsilon} \times U \times 300 \times 300 \times 1000$$

ζ = Zeta Potential (*mV*)

η = Viscosity of Solution

ε = Dielectric Constant

$U = \frac{v}{V/L}$: Electrophoretic Mobility

v = Speed of Particle (*cm/sec*)

V = Voltage (*V*)

L = The distance of Electorode

Samples preparation is the same than the one which is used for the dynamic diffusion of the light.

Measurement is made at $T=25^\circ$ (floating liquid) on the apparatus Zetasizer NanoZS (Malvern instrument). Measurement is made 5 times on each sample, every 3 minutes

Dynamic diffusion of light

2 to 3 mg of the sample are placed in 20 ml of distilled water. The sample is shaken for 10 min and undergoes ultra sonic sounds for 30 min and another shaking for one night. The measurement is made on the solution obtained at $T=25^\circ$ in a static medium with the apparatus Zetasizer NanoZS (Malvern instrument). Detection limit of the apparatus is between 0.6 nm to 6 μm . Measurement is repeated 4 times on each sample, every 3 minutes.

Results

X-rays diffraction analysis

Parent calcite

- CaCO_3 calcite is the main phase. Reference card 85-1108 is first given by the software, as we can see on the additional spectrum. This experimental intensity is not totally explained by the intensity of the card. Moreover, the positions indicated by the card in the graphs are lightly out of line with the experimental vertex.

The trials with many cards of the CaCO_3 do not allow to get a better coincidence for all the vertices of the experimental peaks.

- Presence of quartz is proved by the comparison with the reference card 46-1045
- Presence of Ankerite is proved by the comparison with the reference card 41-586.

The chemical composition indicated on the card is:



It does not correspond to the formula of the sample which is studied but it explains the presence of elements which are in very low concentration by X fluorescence spectrometry.

Differences observed between the different cards of the Ankerite are too low to get more precisions about the chemical composition of this phase. Moreover, some dolomite cards have some positions near of the one of the Ankerite cards.

- As you can see on the attached documents, which compare the diffractogramm of the selected reference cards, there are low density peaks which are not assigned for a card. Some peaks have vertices which are wide with epaulements.
- The choice of the cards is not indifferent and modifies the half quantitative approach given by the software, through the RIR factor (reference intensity ratio). This approach has no sense here because the cards do not correspond to the diffractogramm, according to the position and the intensity.

TMA Calcite sample

TMA calcite and parent calcite diffractogramms are almost super imposable.

The search for the phases on the TMA calcite diffractogramm leads to a quartz calcite and an Ankerite, but the numbers of the cards are different than the ones found for the parent calcite.

Calcite is the isotype of: the giobertite MgCO_3
the smithsonite ZnCO_3
the siderite FeCO_3

Those products are miscible between each other.

The most frequent dolomite formula is CaCO_3 , MgCO_3 , but CaCO_3 , 2MgCO_3 and 2CaCO_3 , MgCO_3 exist too.

In the Ankerite iron is replaced by magnesium of the dolomite. ($\text{Fe}/\text{Mg} > 1$).

Calcite can contain Mn, Fe, Zn, Co in low quantity and Ba, Sr, Mg and traces of Cu, Al, Ni, V, Cr, Mo and rare earths. In the Ankerite, Mn can replace Mg, that product can contain Co, Pb, Zn.

Nitrogen absorption results in the calcite samples

The isotherms of the two samples have the same appearance. They are mainly of type II, which is a characteristic of the no porous solids, with a very light hysteresis at

the desorption. The solids have a very low mesoporosity, limited to big mesopores superior of 10 nm. Those solids have no microporosity.

Comparative table of the textural characteristics of the studied calcites

Samples-results	Parent calcite	TMA calcite
Total surface (m ² /g)	1.8 +/- 0.1	3.9 +/- 0.1
Total porous volume (cm ³ /g)	0.005 +/- 0.001	0.016 +/- 0.002

Dynamic diffusion of light

In any case, there is a formation of sediment and there are only few particles in suspension. Thus, measurement is made on the floating liquid.

? Parent calcite

Calcite parent sample has 2 types of distribution of the particles size. The first one is between 100 to 120 nm and the second one is between 300 to 340 nm. Measurements are very reproducible

? TMA calcite

TMA calcite sample has two types of distribution of particles size. The first one is between 90 to 140 nm and the second one is between 350 to 450 nm. TMA product seems to have a narrower distribution of the particles size. (peak at 90-140 nm less intense)

Zeta potential

? parent calcite

Parent calcite sample has a zeta potential of -20.34 mV. So the particle's surface has a negative charge. Measurements are very reproducible.

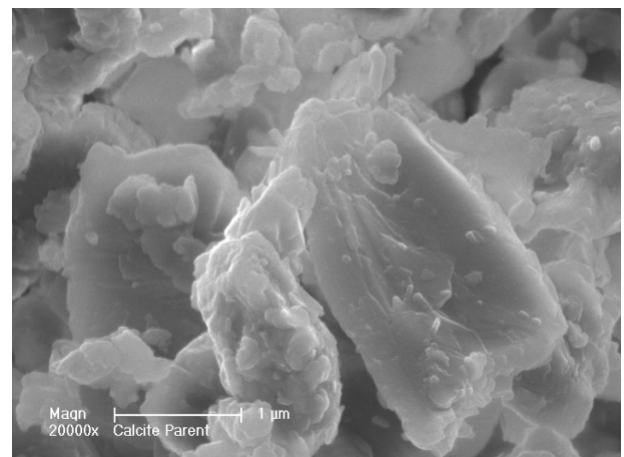
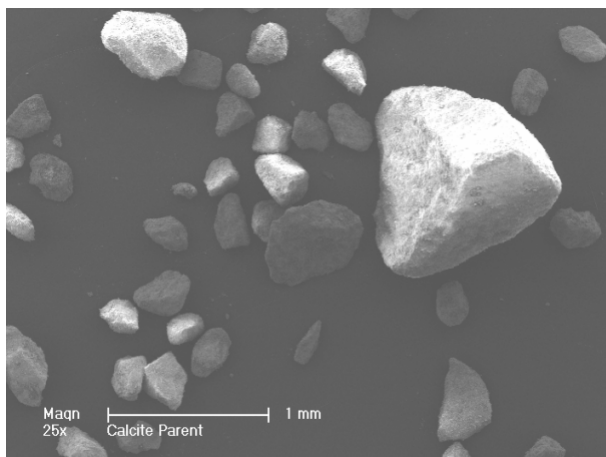
? TMA calcite

TMA calcite sample has a zeta potential of -19.28 mV. So the particle's surface has a negative charge. Measurements are very reproducible.

Electronic microscope observation

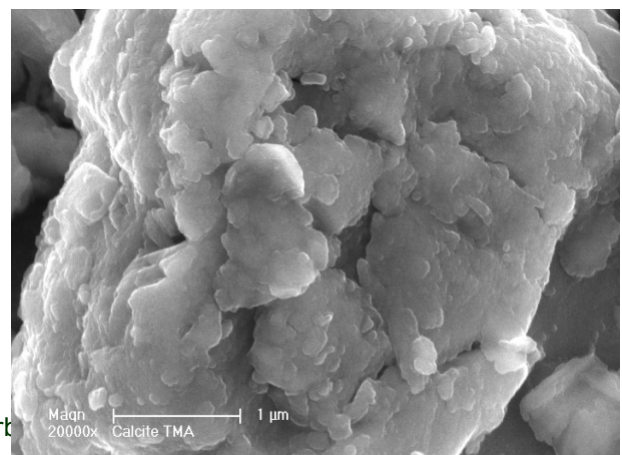
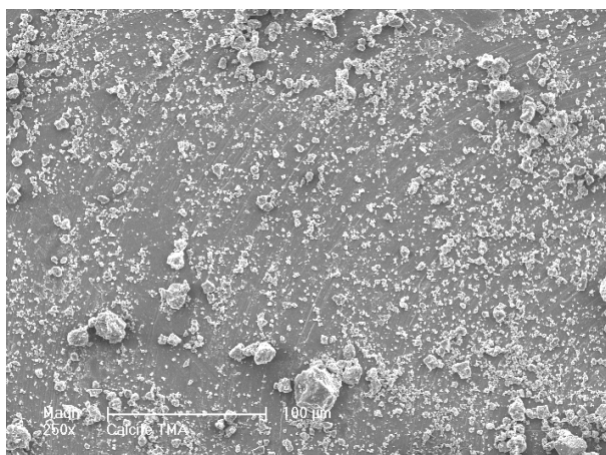
? Parent calcite

This sample is made of many types of forms. The biggest dimensions are between 0.1 and 1.5 mm (picture 5). The surface of those blocks is irregular. They are covered by small particles (less than 10 μm) without any particular geometric form. (Picture 6).



? TMA calcite sample

This sample is made of particles aggregate, without any particular forms. Those aggregates have a size between 25 μm and less than 1 μm (picture 7). With a bigger magnification, we can see the same particles than in the parent calcite sample. No change occurred.



Qualitative analyses with EDX microprobe

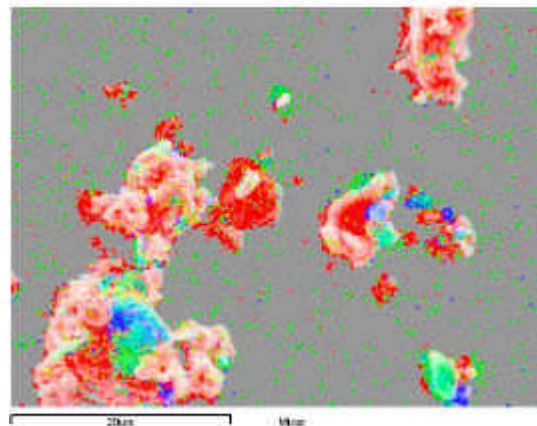
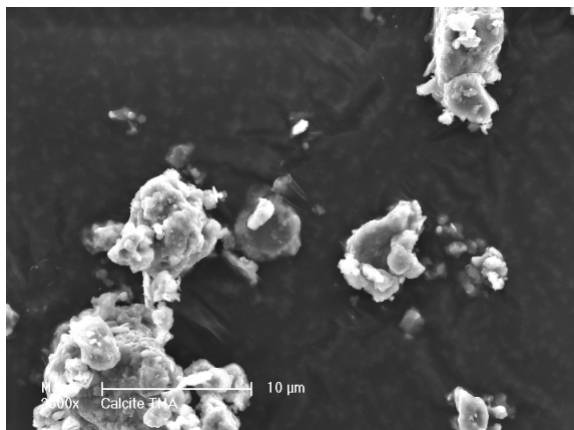
Qualitative analyses were made on TMA calcite with an EDX micro probe. Cartography of the main elements (Ca, O, Mg, Si, Al) was obtained. The carbon, present in the sample, was not studied at the time of the EDX analysis.

Results:

- calcium in CaCO_3 is the main component of the sample
- magnesium is with calcium when it is present. They are associated together in mixed carbonate form
- silicium is present in silicate form with or without aluminium
- aluminium must be in alumina form but this has to be confirmed by the X-rays diffractions analysis.

For instance, EDX cartography was made on a calcite sample (picture 9). We observe:

- calcium in red
- aluminium in green
- silicium in blue



Conclusion

X rays diffraction analysis

The parent calcite is made of a 3 phases blend:

- calcite CaCO_3
- quartz (SiO_2)
- Ankerite $\text{Ca (Fe, Mg) (CO}_3)_2$

Comments:

- 89-1304 card corresponding to the formula $(\text{Mg}_{0.042}\text{Ca}_{0.958})\text{CO}_3$ shows many experimental vertices
- A card like the reference 86-2340 show positions which correspond to epaulements on the peaks of the calcite. This card corresponds to the formula $(\text{Mg}_{0.002}\text{Ca}_{0.998})\text{CO}_3$.
- Certain peaks attributed to the Ankerite have a width which could be explained by the presence of two products with the same composition, for instance, 31 degrees peak in 2?. Its width could be explained by the presence of dolomite (75-1654 cards). The distinction between ankerite and dolomite was difficult. X image may explain the presence of calcium, iron and magnesium crystals.

Nitrogen absorption

Tribomechanical treatment has a determining effect on the textural characteristics because the total specific surface (determined by the BET technique) has doubled and the one of the total porous volume has tripled after the treatment. By applications of the geometrical relations for the determination of the external surface of the divided solids with pseudo spherical grains, a doubling of the surface corresponds to a double reduction of the average diameter of the grains. It is possible that the tribomechanical treatment is responsible for this effect.

Electronic microscope observation

For the TMA calcite, the mechanical grinding is only responsible for the bursting of the big blocks of the parent calcite and it is not responsible for the small particles morphology.

EDX analysis of the TMA calcite allows to show the chemical heterogeneity of this sample. Moreover, it is interesting to notice that the chemical heterogeneity is not translated by a morphological heterogeneity of the particles.

Dynamic diffusion of light

There are two types of particles size for each sample (100-120 nm and 300-340 nm for the parent calcite and 90-140 and 350-450 nm for the TMA calcite sample). In this case, grinding does not seem to induce a reduction of the particles size; however, the size distribution seems to be narrower.

Zeta potential

Both samples have almost the same zeta potential (-20.34 mV for the parent calcite sample and -19.28 mV for the TMA calcite sample). Those samples have almost the same number of negative charges on the surface.

- Parent calcite

This sample is made of many types of forms. The biggest dimensions are between 0.1 and 1.5 mm

- TMA calcite sample

This sample is made of particles aggregate, without any particular form. Those aggregates have a size between 25 µm and less than 1 µm

5. Solubility and Suspension stability

Material and methods

QUANTITATIVE SOLUBILITY

The solubility of TMA-C Calcite sample has been measured in soft acid solution, in order to model the vegetal leaf cuticle pH, the material used as a fertilizer under trade mark HerbaGreen® being sprayed on the plants during the vegetative period, resulting in dissolving the carbonated phases of the mineral.

ATOMIC ABSORPTION ANALYSIS OF SOLUBLE ELEMENTS IN SOLUTION:

The requested nutritional soluble elements were analysed in the solution of dissolved product by atomic absorption (Ca, Mg, Si, Fe, Mn, Zn, and Cu)

Results

Soluble elements / HerbaGreen®					
Elements	Ca	Mg	Fe	Mn	Si
Mass %	32.8 %	2.89%	0.025%	0.025 %	0.033%

X-RAY FLUORESCENCE SPECTROSCOPY: ANALYSIS OF INITIAL AND RESIDUAL MATERIAL

Analysis of sample before dissolution

Elements	Mass %	Elements	Mass %
C	9.39	Ti	0.076
O	47.4	Cr	0.005
Na	0.160	Mn	0.015
Mg	2.16	Fe	0.76
Al	2.53	Ni	0.005
Si	4.44	Zn	0.003
P	0.011	Rb	0.001
S	0.079	Sr	0.10
K	0.55	Zr	0.003
Ca	32.4		

Analysis of insoluble residue, 14.8% in mass of the sample after dissolution, the table below shows the disappearance of soluble elements:

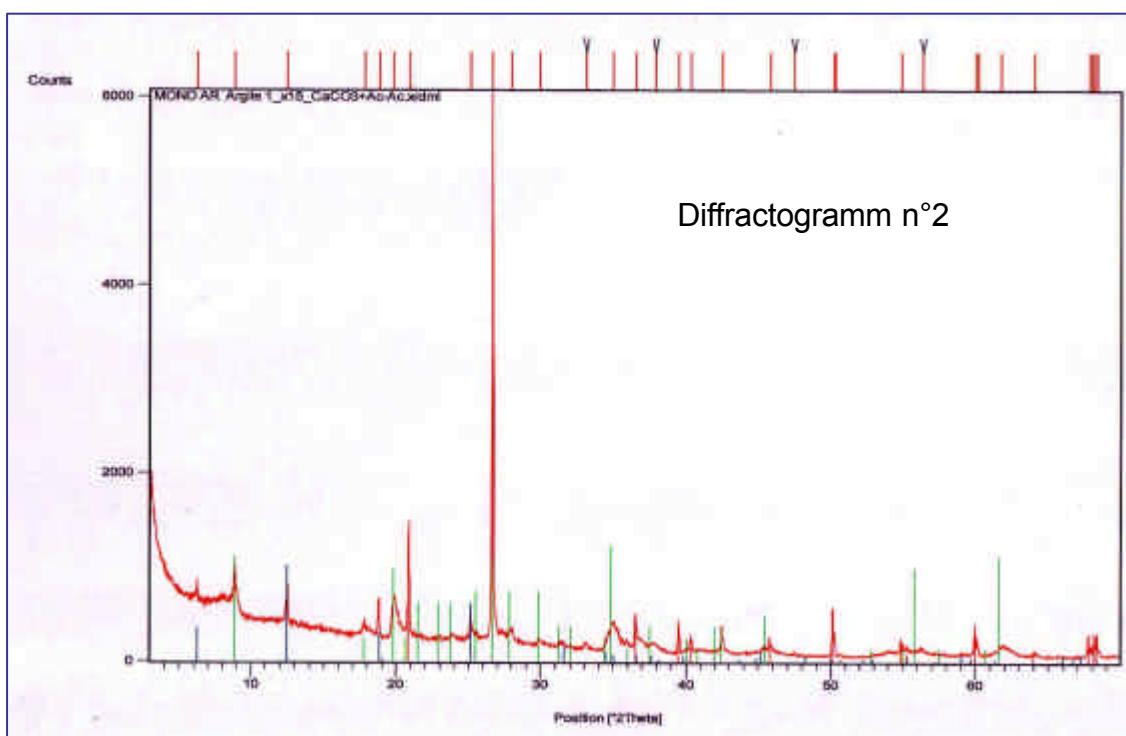
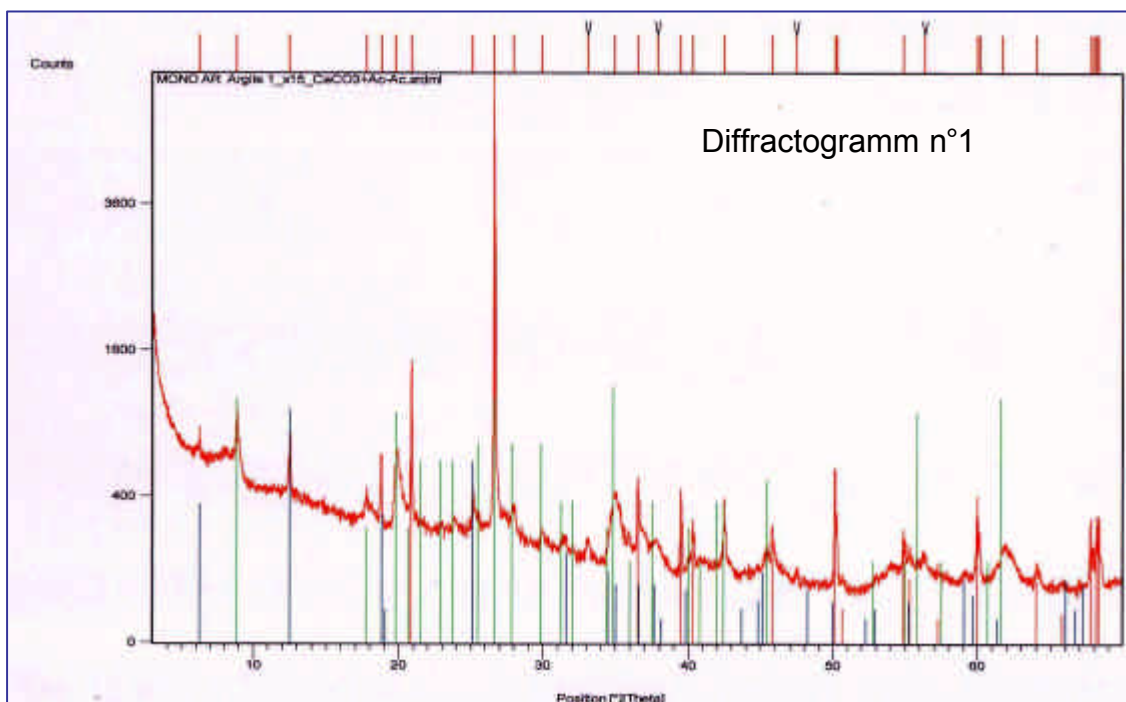
Elements	Mass %	Elements	Mass %
C	0.00	Ti	0.435
O	48.0	Cr	0.012
Na	0.60	Mn	0.004
Mg	1.55	Fe	4.82
Al	12.5	Ni	0.007
Si	27.8	Zn	0.008
P	0.073	Rb	0.009
S	0.443	Sr	0.020
K	3.24	Zr	0.022
Ca	0.435		

MINERALOGY OF SOLUBLE MATERIAL:

The mineral calcite under trade mark HERBAGREEN is divided in four mineralogical phases: Calcite, Quartz, Ankerite and Dolomite (see diffractogramm n°1). The

diffractogramm n°2 shows the mineralogy of insoluble residue: it shows the Quartz as main phase in the insoluble phase, due to the disappearance of all soluble elements, the soluble carbonated phase and of many other peaks.

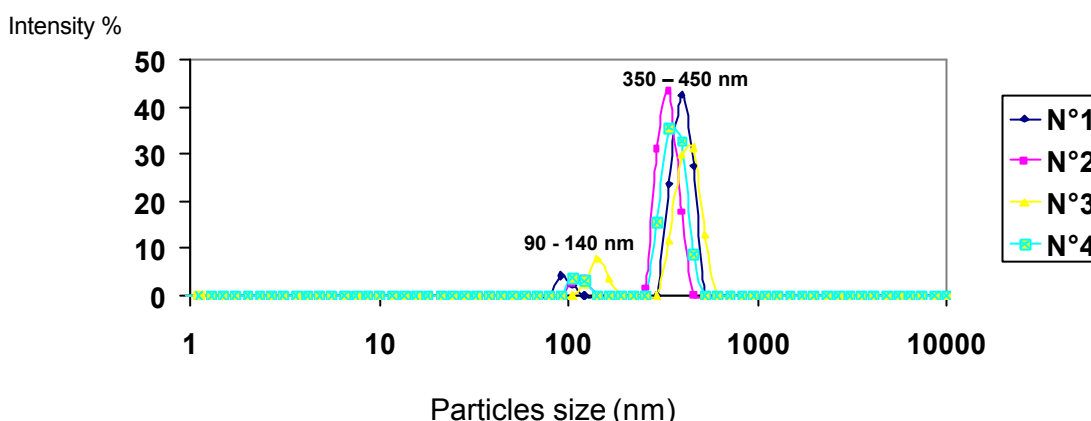
Diffractograms of the Calcite HerbaGreen before and after dissolution of soluble elements:



Discussion

The calcite particles in the products blend form a colloidal suspension. HerbaGreen is not soluble in neutral pH water. Mineral particles sediment in few seconds because of their very small size. But they are too big to create a physical and chemical interaction with the water molecules.

When HerbaGreen is blend with pulverization pulp, the finest particles phase is still in suspension during a longer period, even in water at rest. The surface liquid is made of nanoparticles. The granularity of those nanoparticles has been measured at the laboratory “Laboratoire de Matériaux à Porosité Contrôlée de l’Ecole de Chimie de Mulhouse (68, Haut Rhin, France). “Calcite TMA/HerbaGreen” sample measured in laboratory presents two types of distribution of particles size. The first one is included in 90 to 140 nm and the second one is included in 350 to 450 nm.



Many systems can be used for HerbaGreen pulverisations:

- a carried jet
- a projected jet
- a return in tank agitation system
- a mechanical agitation

All those systems allow the suspension setting of HerbaGreen micro particles.

There are 1 to 2 % of coarse quartz particles which could be found during the cleaning of the tank. They never block up the filter even with reduced volume pulverisation and very low volume pulverisation.

Suspension carrying induces a shaking and thus the suspension stability of microparticles. Moreover, the product can be associated with other specialities without changing the good suspension-keeping.

Conclusion

The quantitative solubility of the sample TMA-C is 85.2%

The tribomechanical technology does not modify the general characteristics of the solubility of natural calcium carbonate, which depends on the acidity of the pulp.

The calcium carbonate contained in HerbaGreen will be dissociated at pH 5.8 (pH of the leaf) during the foliar application. The plant will be able to have a continuous contribution of calcium ions and carbonates ions.

Summary

HerbaGreen characteristics

1. **main components** : calcite (CaCO_3 , SiO_2 , MgO , CaO , Iron, Mn, Selenium)
2. **granularity** : in the region of the μm
3. **standards**: HerbaGreen corresponds to the NFU 44001 standards
4. **Suspension keeping of HerbaGreen**: the tribomechanical technology does not modify the general characteristics of the solubility of the carbonate calcium, which is soluble only at acid pH. It is in the region of 5.8, which is the pH of the leaf. The particles finest phase is still in suspension in a blend. The granularity of those particles was measured by the laboratory "Laboratoire de Matériaux à porosité contrôlée de l'Ecole de Chimie de Mulhouse" (Haut Rhin, France)

5. Report of X rays diffraction analysis

The analysis shows the presence of quartz and ankerite in the sample. The chemical composition of this calcite is:



Samples	Parent calcite	TMA calcite
Total surface (m^2/g)	1.8 +/- 0.1	3.9 +/- 0.1
Total porous volume (cm^3/g)	0.005 +/- 0.001	0.016 +/- 0.002

The tribomechanical treatment has a determining effect on the textural characteristics because the total specific surface has doubled and the one of the total porous volume has tripled after the treatment.

6. Report of the electronic microscope observation

TMA calcite is made of particles aggregate, without any particular form. The size of the aggregate is between 25 μm and less than 1 μm .

Calcium in CaCO_3 is the main component.
Mg and Ca are associated together.

*Si is present in silicate form.
Al is present in alumina form.*

For the TMA calcite, the mechanical grinding is only responsible of the bursting of the big blocks of the parent calcite and it is not responsible of the small particles morphology. EDX analysis of the TMA calcite allows to show the chemical heterogeneity of this sample. Moreover, it is interesting to notice that the chemical heterogeneity is not translated by a morphological heterogeneity of the particles.

7. Expert's report by dynamic diffusion of the light and zeta potential

dynamic diffusion of the light

There are two types of distribution of mineral particles size:

- between 90 to 140 nm*
- between 350 to 450 nm*

zeta potential

The zeta potential of TMA calcite is -19.28 mV; so there are negative charges on the surface.

8. Solubility of TMA calcite

The quantitative solubility of the sample TMA-C is 85.2%

The tribomechanical technology does not modify the general characteristics of the solubility of natural calcium carbonate, which depends on the acidity of the pulp.

The calcium carbonate contained in HerbaGreen will be dissociated at pH 5.8 (pH of the leaf) during the foliar application. The plant will be able to have a continuous contribution of calcium ions and carbonates ions.

III. STUDY OF HERBAGREEN PROPERTIES

1. Improvement of photosynthesis

Introduction

The objective: improvement of photosynthesis to allow improvement of quantity and quality of the harvests.

Material and methods

This study was made on vine leaves, tomato stems and leaves and leaves of **wheat**. They were coloured with a double coloration. It allows a differentiation of the different biochemical components of the vegetal tissue. Schiff reagent shows polysaccharides whereas naphthol blue black reagent shows organites with proteins.

Leaves samples were observed with an optical microscope.

The comparison of organites (chloroplasts, polyphenols and starch) was made far away from the conducting vessel of the sap, thus the observation was not distorted.

2 places of taking:

- SADEF laboratory: comparison of treated and untreated leaf and stem (tomatoes and vine). Fluorimetry measurements (vine).

- St Emilion: Comparative study of vine leaves treated with HerbaGreen and control leaves

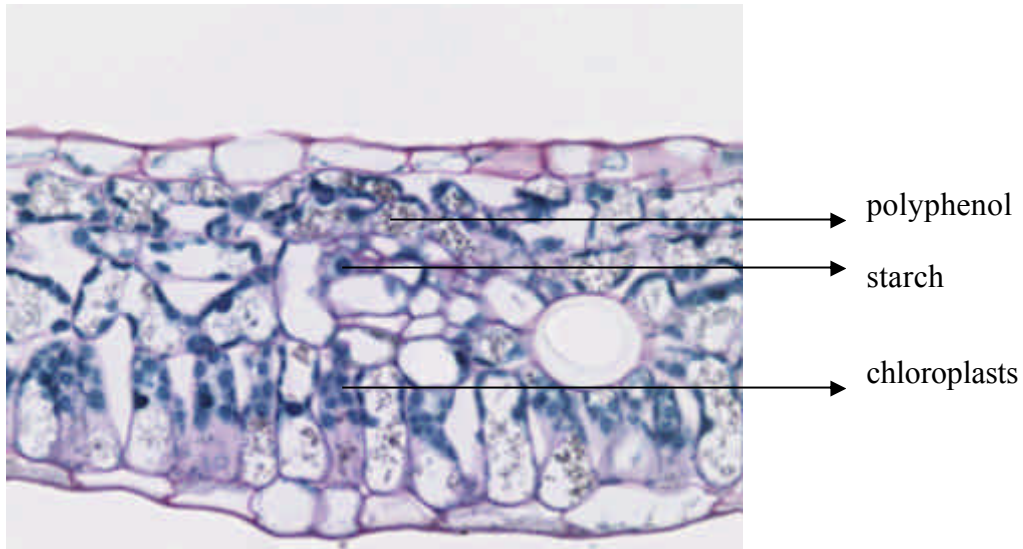
Place of observation: CIRAD cellular imagery department (Montpellier)

Leaves taken at St Emilion after 3 HerbaGreen applications of 2 kg/ha every 15 days were young and old. They are placed in a preservative before observation. The differentiation was obtained by comparison of samples which come from the same parcel.

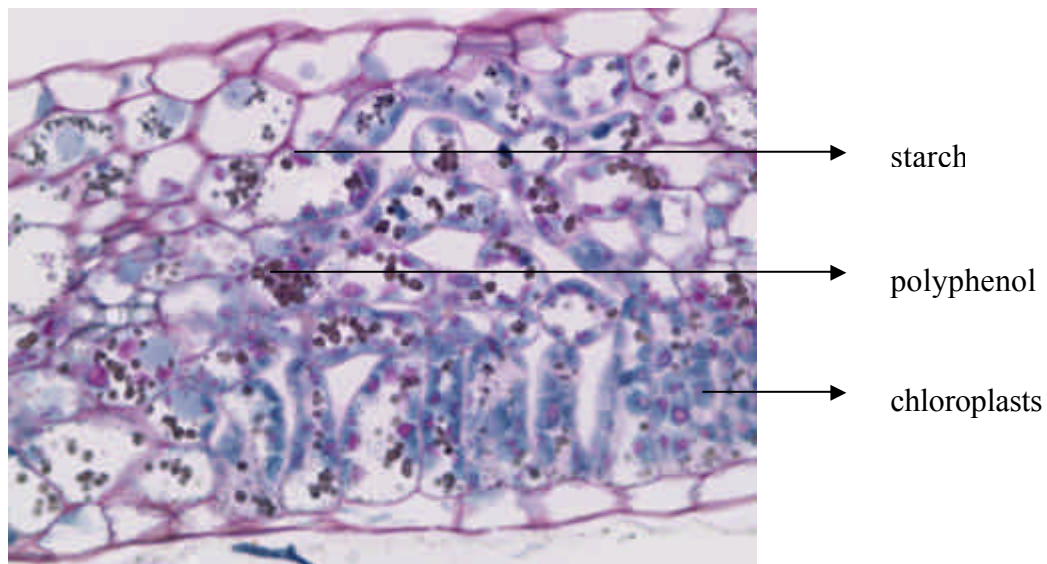
For wheat, two applications of HerbaGreen were made at last leaf stadium and node stadium. Fluorimetry measurements were made to observe the effects of HerbaGreen on the photosynthetic process.

Results

Control vine leaf (X40)



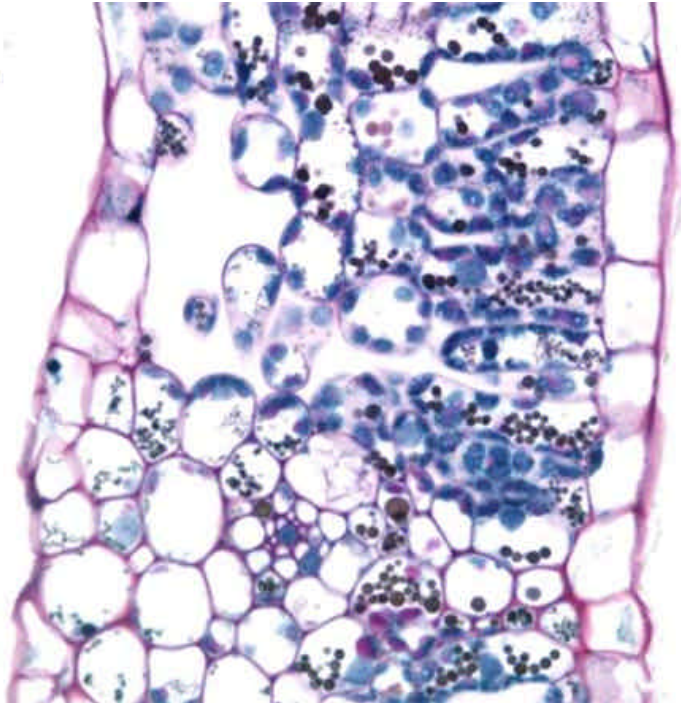
Treated vine leaf with HerbaGreen (X40)



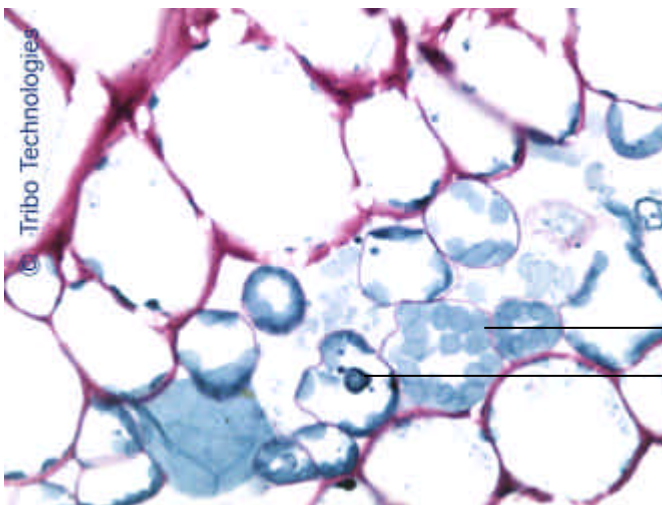
The treated leaf has a greater thickness because it is taller and contains more cells. The cuticle seems to be thicker, that observation requires experimentation. The treated leaf contains more polyphenols

**Treated vine leaf with Megagreen
(x40)**

© Tribo Technologies



Tomato stem (X40)



Chloroplasts

starch

We can see starch while the plant has already produced its fruits. The fact that there is still a lot of starch in the stem means that there was a strong activation of the

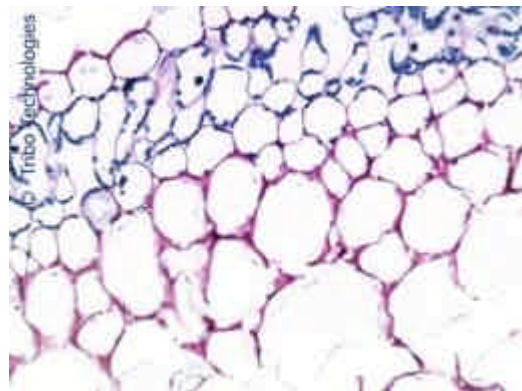
primary metabolism. We can see a lot of chloroplasts; it confirms a strong photosynthetic activity.

Tomato stem (X20)



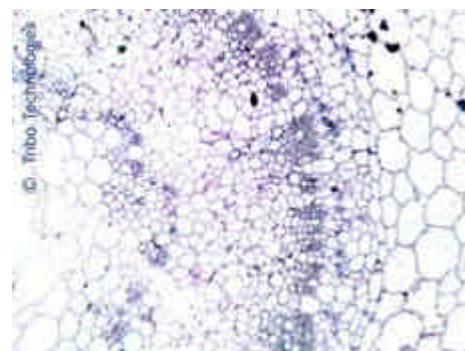
←→
Cortical zone

Tomato stem (X20)



↑↓
Cortical zone

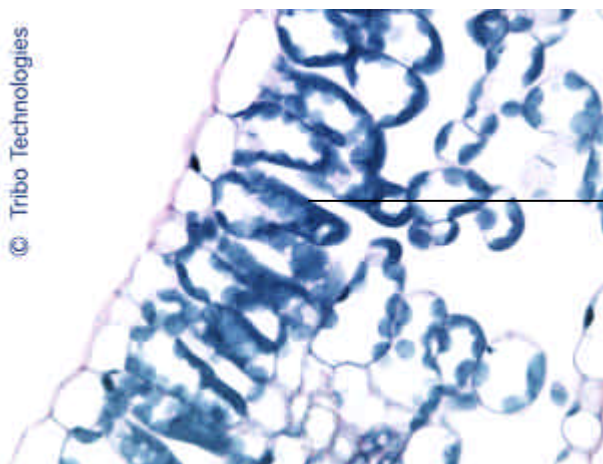
Tomato stem (X5)



←→ ←→
wood Cortical zone

We can notice
wood thickness on
this picture.

Treated tomato leaf (X40)



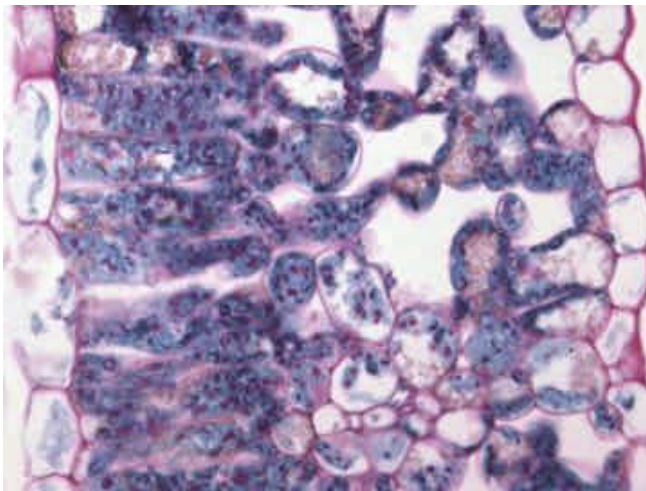
Chloroplasts

Untreated tomato leaf (X40)

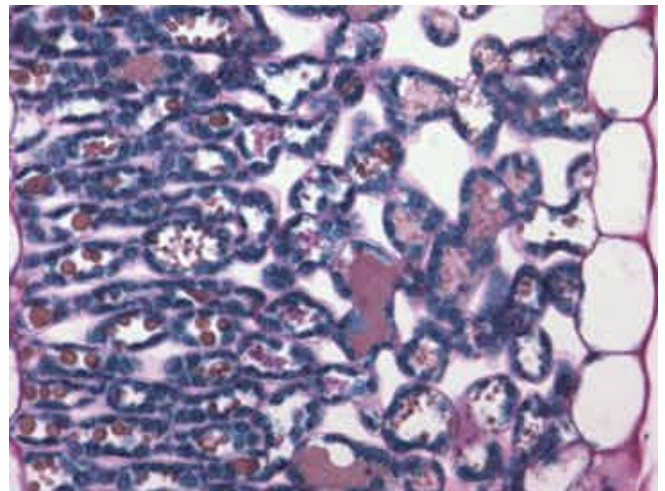


Comparison: we can notice a less important quantity of chloroplasts for the sample of untreated tomato.

St EMILION



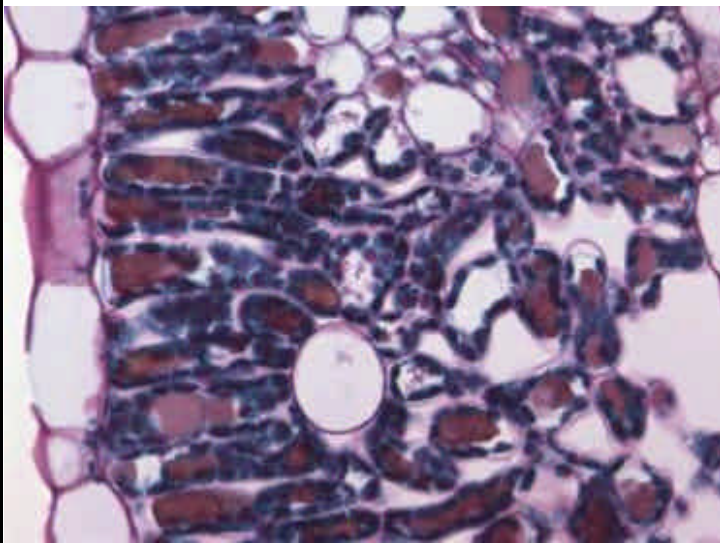
Control leaf parcel 1



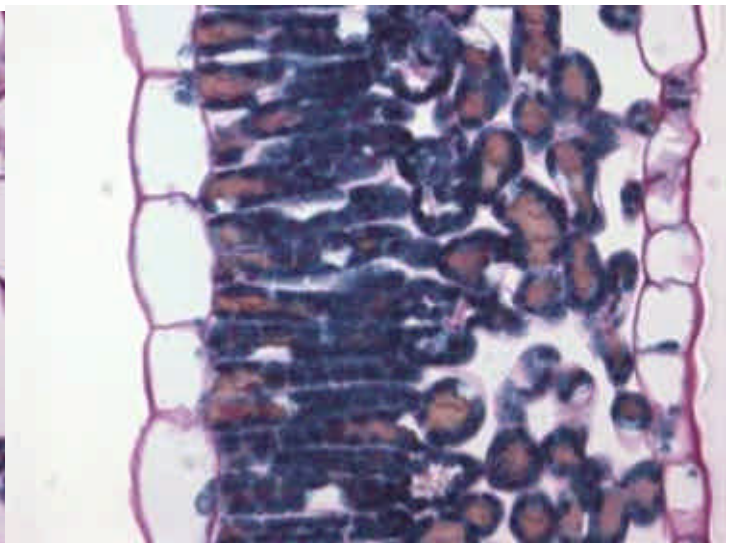
Treated leaf Megagreen parcel 1

Observation:

Chloroplasts are better formed and bigger in the treated leaves; it shows a better functioning of the photosynthesis. A higher export of polyphenols to the berries seems to be confirmed by the maturity controls.

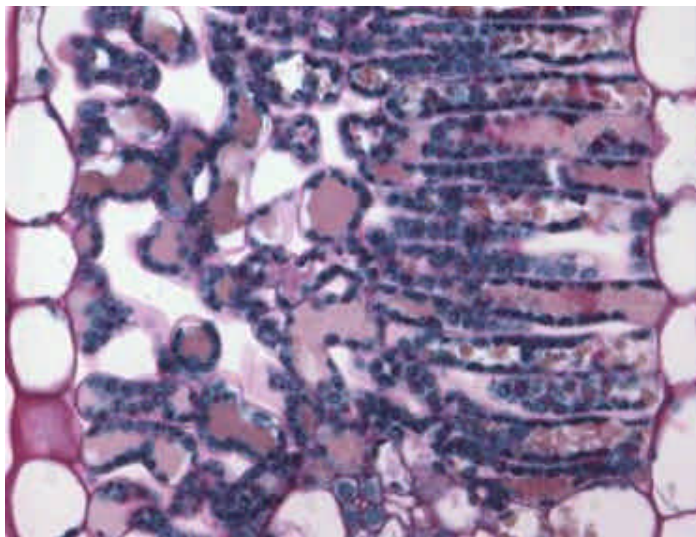


Control leaf parcel 2
2

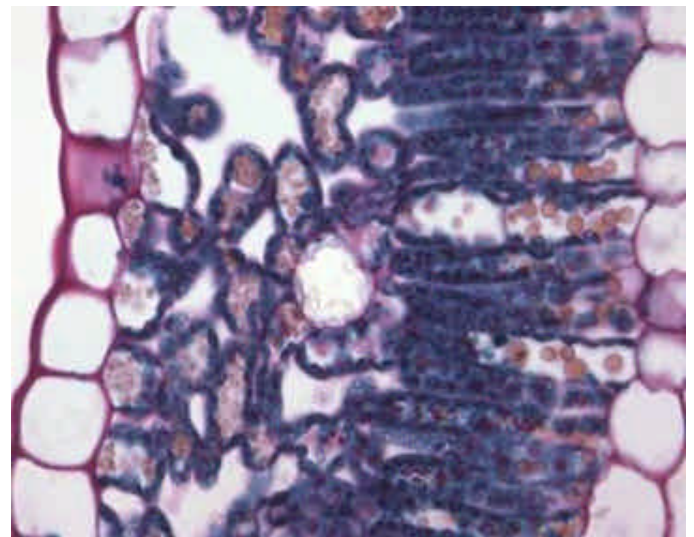


treated leaf HerbaGreen parcel

The treated leaf has a more important activity because of the many chloroplasts with a very deep colour on the picture. This shows the high number of proteins in their structure, which indicates a more important metabolic activity, confirmed by the chloroplasts structure. **The treated leaf has a better metabolism**



Control leaf parcel 3
3



treated leaf HerbaGreen parcel

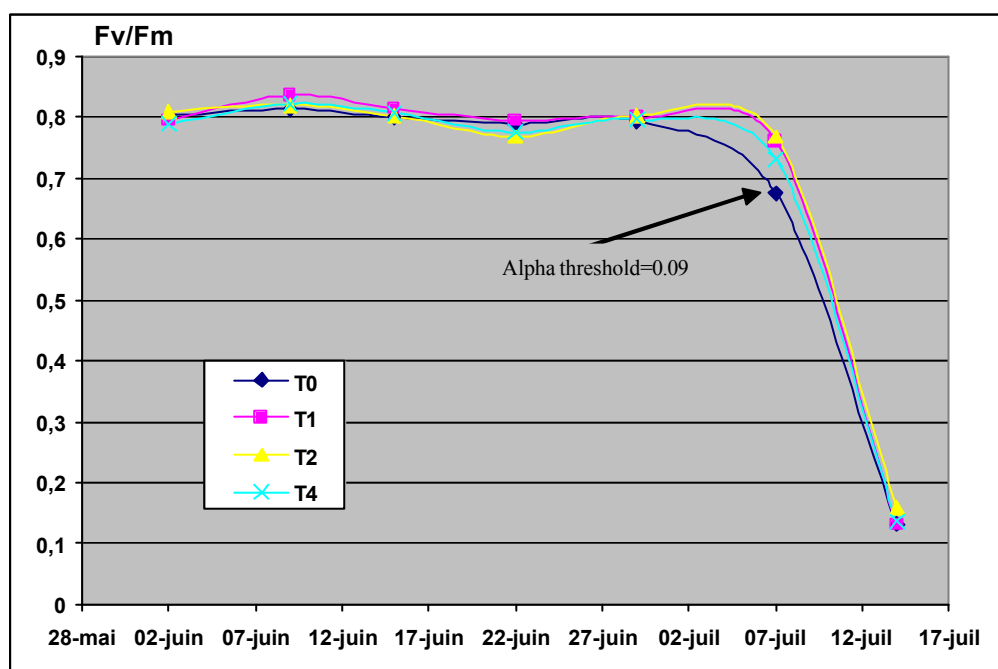
More starch reserve in the non-treated part. Metabolism is more active in the treated part; the presence of many cellular nucleuses indicates a high protein synthesis activity. Chloroplasts are bigger and more numerous in the treated leaf, it indicates a high photosynthetic activity.

Chloroplasts: chloroplasts are better formed and bigger in the treated leaves; it shows a better functioning of the photosynthesis.

Polyphenols : A higher export of polyphenols to the berries seems to be confirmed by the maturity controls.

Proteins : Treated leaves have more proteins in their structure, which indicates a higher metabolic activity, confirmed by the chloroplasts structure. Metabolism is more active in the treated part; the presence of many cellular nucleuses indicates a high protein synthesis activity

Fluorimetry measurements (vine)



The measurement made on the 7th of July 2005 has almost detected a significant standard deviation. (Threshold $\alpha = 9\%$ instead of 5%). That result shows that the control plants have a lower activity.

Discussion

The treated leaf contains more chloroplasts. The photosynthesis takes place in the chloroplasts, so it shows an activation of the primary metabolism.

The treated leaf contains more polyphenols, which are chemical products, precursor of the secondary metabolism. They have a part in natural defence reactions

Metabolism and particularly photosynthesis are more active in the treated leaves. Better functioning of the photosynthesis can increase earlier and better migration of the starch and polyphenols to the berries.

The carbonate calcium would be dissociated progressively to bring calcium and CO₂ continuously to the plant treated leaf, according to the reaction as follows:



The photosynthetic process will be improved thanks to carbon dioxide contribution in the intra cellular medium.

Atmospheric carbon dioxide is responsible of the RuBp carboxylation on the 4th carbon of the molecule; that reaction is catalysed by RUBISCO enzyme. It can fix CO₂ or oxygen if the CO₂ content is reduced. If the RUBISCO fixes oxygen, the photo breathing process occurs; it is a competing phenomenon of the photosynthesis process. The photosynthesis yield is reduced of 25 to 50 % by the photobreathing process for the C3 plant. CO₂ which comes from the dissociation of calcium carbonate of HerbaGreen saturates the intra cellular medium of the plant with CO₂; this will turn the RUBISCO activity to the photosynthesis process. Photosynthesis activity will be favoured.

In 2005, the wheat ripened very fast because of the heat occurred at the beginning of June and at the end of July. The phase from the end of flowering to the beginning of senescence lasted one and a half of weeks, whereas in general, it lasts 3 or 4 weeks. This difference of photosynthetic activity is minimised because of the senescence acceleration. It is possible to conclude that the pulverisations had an effect on the lengthening of the vegetation cycle.

Conclusion

The difference which can be put in a prominent position by the data analysis is about the metabolic activity. The structure and the number of chloroplasts show that 3 applications of HerbaGreen launched a high activation of the photosynthetic activity. Starch and polyphenols must be put in a dynamic context, thus, conclusions about the increase of the photosynthetic system in the leaf can be drawn when the wine harvest is analysed.

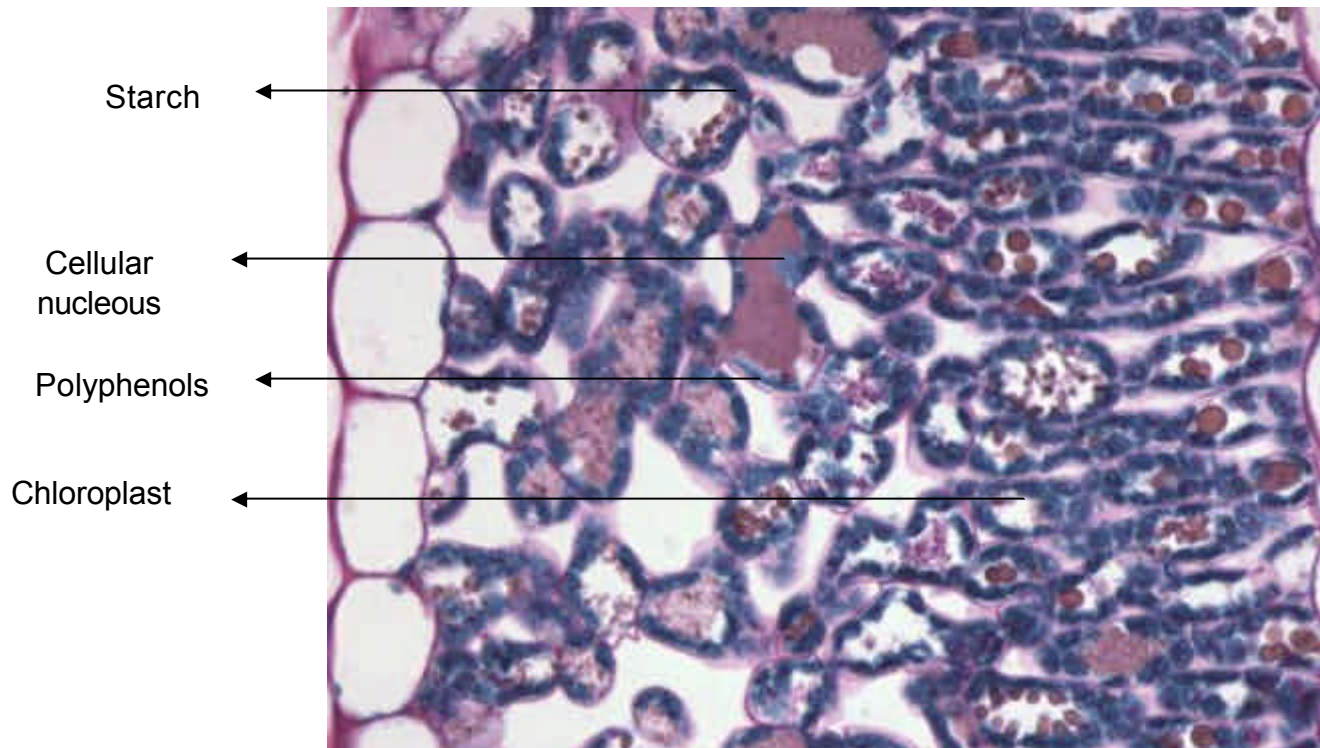
HerbaGreen treatment allows different physiologic and metabolic changing:

Chloroplasts: chloroplasts are better formed and bigger in the treated leaves; it shows a better functioning of the photosynthesis.

Polyphenols: A higher export of polyphenols to the berries seems to be confirmed by the maturity controls.

Proteins: Treated leaves have more proteins in their structure, which indicates a more important metabolic activity, confirmed by the chloroplasts structure. Metabolism is more active in the treated part; the presence of many cellular nucleuses indicates a high protein synthesis activity.

If photosynthetic process is increased, primary and secondary metabolisms will be increased too. Thus, primary and secondary metabolites rate will be more important (starch, polyphenol...). It will have an incidence in different parameters: yield will be increased and harvests organoleptic qualities will be improved. Moreover, if photosynthesis activity is increased, photobreathing will be inhibited which allow to avoid an important water loss, limiting the watering.



2. Antioxidant potential effect of HerbaGreen

Material and methods

Tomato samples come from SADEF laboratory. Tomato plants were photographed in November 2004 after appearance of the fruits.

Results



Tomatoes



Treated plants



Vine



We notice that the treated plant has a significant green coloration and the untreated one presents senescence signs. Conditions of culture were the same for all plants but not for plants treated with HerbaGreen. That effect occurred on every plant of the experimentation.

Discussion

Green effect was noticed in many cases, in field and in laboratory, in spite of the non significant presence of nitrogen (<0.18 %) or sulphur (<0.07 %) in the product.

Senescence of the vegetal cells is bound to the free radicals emitted during the cellular breathing. The main free radicals are the peroxides. Those chemical species become very reactive after an electron loss. The free radicals will pick up the missing electron in the cellular membrane components or in the DNA. That process is responsible of the destruction of the cells plant.

After the very high speed collisions between the calcite particles, the electrons distribution changes on the surface of the mineral. The electrons of the chemical bound get a new distribution like a half availability state. Those electrons can be given up to the free radicals to neutralize them.

Conclusion

After HerbaGreen treatment, the treated plants have a more important coloration and the control plants present senescence signs. That green effect was noticed in many field and laboratory experimentation.

The senescence of vegetal is due to the peroxides emitted during the cellular breathing. They become very reactive after an electron loss. They pick up the missing electron in the cellular components or in the DNA. That process induces the plant degeneration.

Increase of plant activity traduces by enhancement of primary and secondary metabolite could activate senescence effect on plant, which will be faster. Thanks to anti oxidant potential of HerbaGreen senescence will slow down and plant activity will be better and maintained all along crop period.

3. Role of Calcium in the vegetal reinforcement

Example of calcium role in defence process: study on young vine suffering from powdery mildew - research of alternative solutions for protection against powdery mildew of strawberries



study on young vine suffering from powdery mildew (SADEF laboratory)

Material and methods

Study was made at SADEF laboratory. An experimentation was made on vine leaves first infected by powdery mildew. As our low level of investigation, those results cannot be considered as a proof of the efficiency of HerbaGreen on this disease. They can be use for the development of HerbaGreen use.

Study plan:

	Healthy plants	Contaminated plants
Without HerbaGreen	3 pots	3 pots
With HerbaGreen	3 pots	3 pots

HerbaGreen pulverisations at 0.5 % were made.

3 parameters were measured:

- leaves number evolution to check if pulverisations do not modify plant characteristics while vegetative cycle.
- evolution of contaminated leaves number
- contaminated leaves percentage.

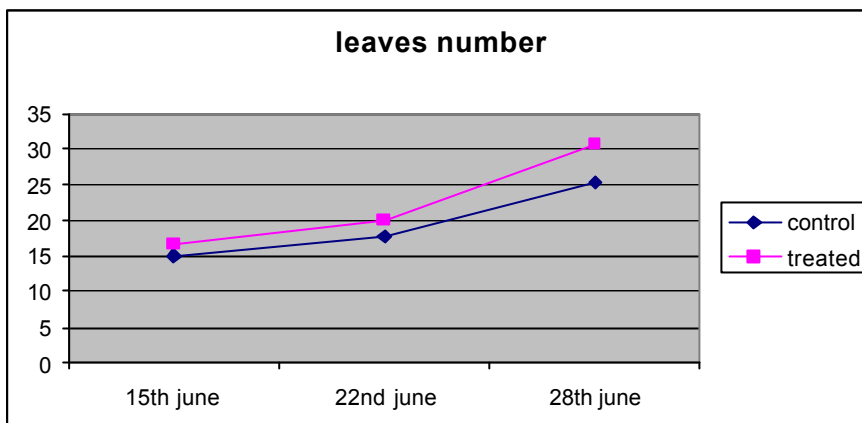
Cultural calendar:

Dates	Operations	Dates	Operations
15th June	test beginning	15th July	treated plant rinse
16th June	0,5 % HerbaGreen pulverisation	16th July	treated plant rinse
17th June	counting	19th July	treated plant rinse
18th June	0,5 % HerbaGreen pulverisation	20th July	counting
19th June	0,5 % HerbaGreen pulverisation	03rd August	0,5 % HerbaGreen pulverisation
20th June	0,5 % HerbaGreen pulverisation	18th August	0,5 % HerbaGreen pulverisation
21st June	0,5 % HerbaGreen pulverisation	25th August	0,5 % HerbaGreen pulverisation
22nd June	counting	31st August	powdery mildew attack on controls and on treated plants
23rd June	0,5 % HerbaGreen pulverisation	01st September	0,5 % HerbaGreen pulverisation
24th June	0,5 % HerbaGreen pulverisation	07th September	0,5 % HerbaGreen pulverisation
25th June	0,5 % HerbaGreen pulverisation	10th September	0,5 % HerbaGreen pulverisation
26th June	0,5 % HerbaGreen pulverisation	15th September	0,5 % HerbaGreen pulverisation
27th June	0,5 % HerbaGreen pulverisation	16th September	counting
28th June	counting	22nd September	0,5 % HerbaGreen pulverisation
29th June	0,5 % HerbaGreen pulverisation	23rd September	counting
30th June	0,5 % HerbaGreen pulverisation	29th September	0,5 % HerbaGreen pulverisation
01st July	0,5 % HerbaGreen pulverisation	06th October	0,5 % HerbaGreen pulverisation
02nd July	0,5 % HerbaGreen pulverisation		
03rd July	0,5 % HerbaGreen pulverisation		
04th July	0,5 % HerbaGreen pulverisation		
05th July	counting		
06th July	treated plant rinse		
07th July	treated plant rinse		
08th July	treated plant rinse		
09th July	treated plant rinse		
12th July	counting/treated plant rinse		
13th July	counting/treated plant rinse		

Results

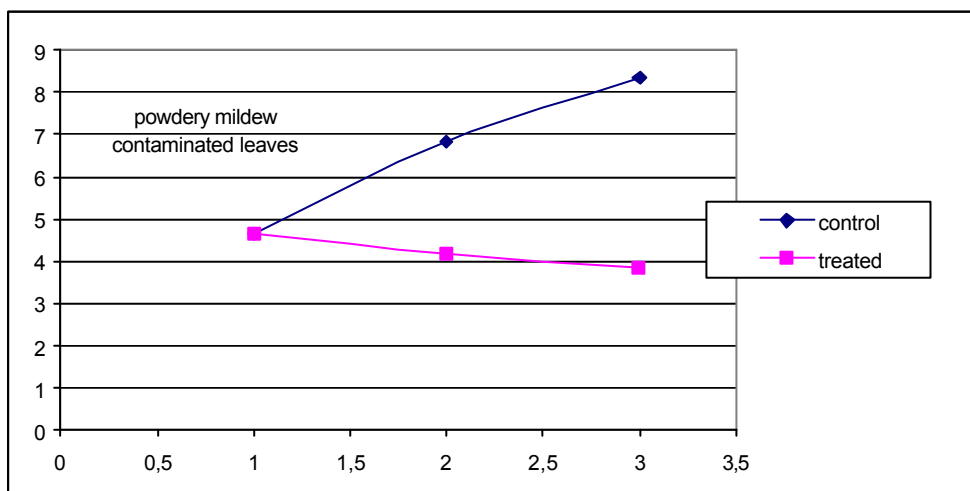
Leaves number evolution

Leaves number and senescence were not modified by powdery mildew attack and by repeated pulverisations.



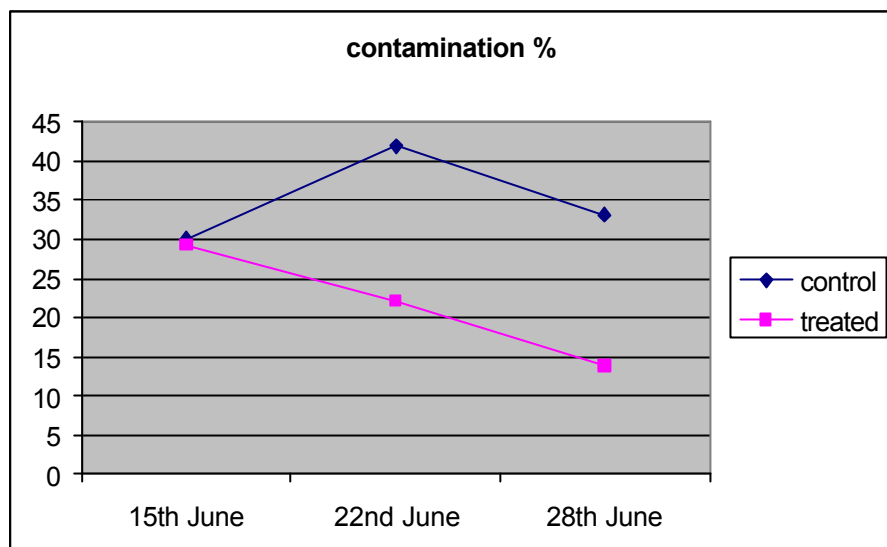
✍ Counting of contaminated leaves with powdery mildew

- if contaminated leaves number is the same at the beginning of the test for diseased plants, this number decreases on treated plants, whereas it increases on control plants. So HerbaGreen has a direct effect on the disease.
- HerbaGreen contribution repetition allows to hold back the attack risk



✍ Percentage of contaminated leaves

There were two powdery mildew attacks and HerbaGreen answer was the same for both. Second attack occurs after a period without HerbaGreen treatment; it confirms that HerbaGreen seems to be persistent.



Leaves and woods observations



Control plant



Treated plant



After the treatment, the powdery mildew was less developed. The effect is visible on wood and on leaves as one can see on those pictures. There are less crackles, the wood seems to be healthier. After many testimonies of growers, HerbaGreen helps to dry the powdery mildew when it is used alone or with other approved products.

Discussion

That propriety must be checked thanks to others tests to certificate the efficiency. If that efficiency is confirmed, the product would be a very interesting way to fight the powdery mildew.

Calcium contained in HerbaGreen must be responsible for those effects on the plant; from an agronomic point of view, the calcium contribution favours the healing, improves the fruits and vegetables preserving and increases the resistance against the biotic and abiotic stresses, like diseases. It is one of the most important element for plant nutrition:

 **Calcium has an important role among the necessary nutriments for the plant.**

Its chemical properties allow it to be bound to many ions. Calcium has a privileged part in the preservation of the structural and functional integrity of the membrane, in the stabilization of the cellular wall and in the regulation, the transport and the control of the ionic exchanges. The mobility of the essential element is low, in the plant and in the ground. The quantity of calcium is one the most frequent restrictive factor for the development and the resistance of the plant. A calcium contribution by a foliar treatment allows overcoming the deficiencies which can occur; this kind of treatment allows the plant to get an optimal development and a good stress resistance capacity.

HerbaGreen releases calcium as the carbonate calcium dissociation. Then the plant will be supplied continuously, contrary to massive calcium contribution in liquid form; the calcium will be fixed very fast and will not be available for the cellular mechanisms.

 **Calcium is an element involved in many cellular mechanisms: structural and functional effect.**

A calcium gradient allows the control of the polarised growth of the apical cells. The plant development takes place at the extremity of the stem, which is a characteristic of the vegetal. This zone is called vegetative point or apical zone. The meristem is the top of this zone. The meristem represents the cells which are at the extremity of the stem and which can split up to generate the different parts of the vegetal : leaf, stem, root, reproductive organ...A calcium gradient (difference of

concentration between the intra cellular and extra cellular medium) allows the control of this process of the specific development of the plant.

The integrity of the membrane is maintained thanks to calcium ions; the cells will have a better resistance to the turgescence and the plant will be able to retain more water and more nutriments for a better growth. By that physiologic process, the cell can increase its volume. This swelling will be responsible for the membrane deformation because of the water entries in the cell. This process occurs in period of cellular growth or when the cell is placed in medium less concentrated than the intra cellular medium.

 **Calcium is an essential element in the nitrogen metabolism, because it stimulates the ammonium absorption.**

The nitrogen contained in ammonium ion is essential for the vegetal growth. It is a component of the amino acids, which are the basis molecules of the proteins. Nitrogen is a component of the nucleotides of the DNA.

In addition, ammonium ion is an essential component of the breathing cycle of the plant. The plant cannot use the atmospheric nitrogen; it is used by the roots in ammonium ion form, which comes from the decomposition of the nitrogenous organic matter in the ground. Its increase in the plant is bound to calcium contribution which will activate the photosynthesis activity.

 **Calcium variations are responsible for the stomata opening and closing.**

The stoma is present on the inferior face of the leaf and allows the gases exchanges with the atmosphere, which is essential for the photosynthesis activity. An external contribution through the leaves will be responsible for the stomata closing (Schroeder and al., 2001) and can inhibit the stomata opening because of the light. There is a synergy effect between the carbon dioxide and the calcium when the stomata are closed. This induces a saving of water. The water need of a vegetal is different throughout the day, after the opening and the closing of the stomata and because of climatic conditions. During the day, the mature leaves need more water. At night, their breathing is reduced and the growth of the stocking organs will be faster. There are bigger quantities of water and calcium in young leaves. If the plants get enough water during the dry days and during the night where breathing is low, the calcium contribution increases in stocking organs and in the leaves with a low breathing.

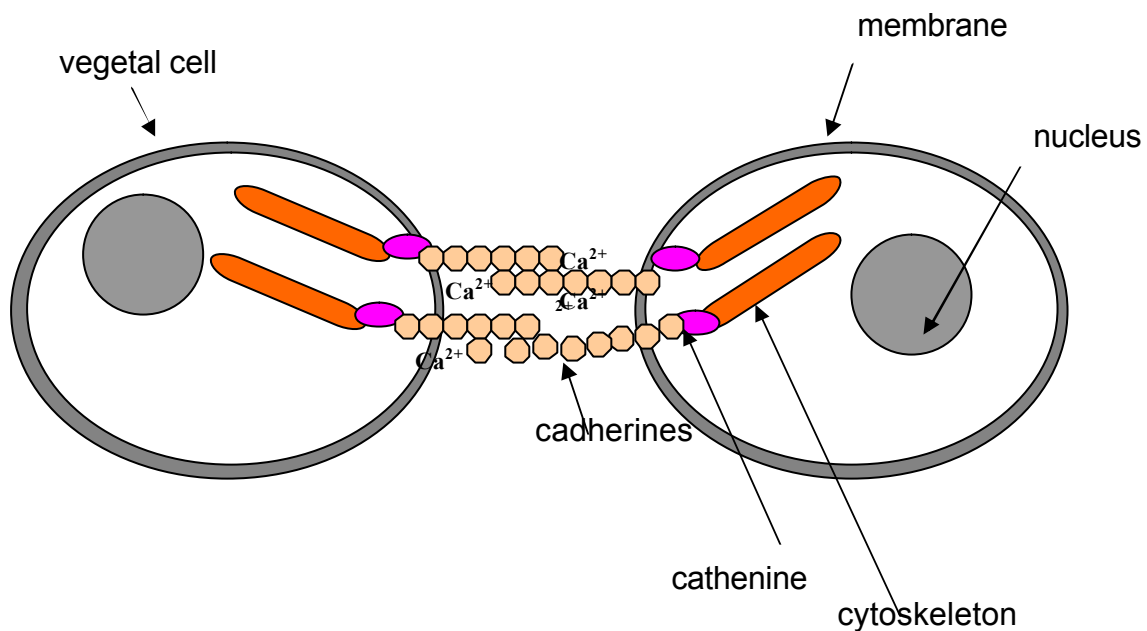
 **Calcium is important for the cellular communication.**

Indeed, cells must be able to stick together to communicate. Cells can do it thanks to calcium dependant proteins.

Cells of many vegetal tissues are linked by adherent junctions. At this place, there is an important concentration of cadherines, link proteins which allow the connection

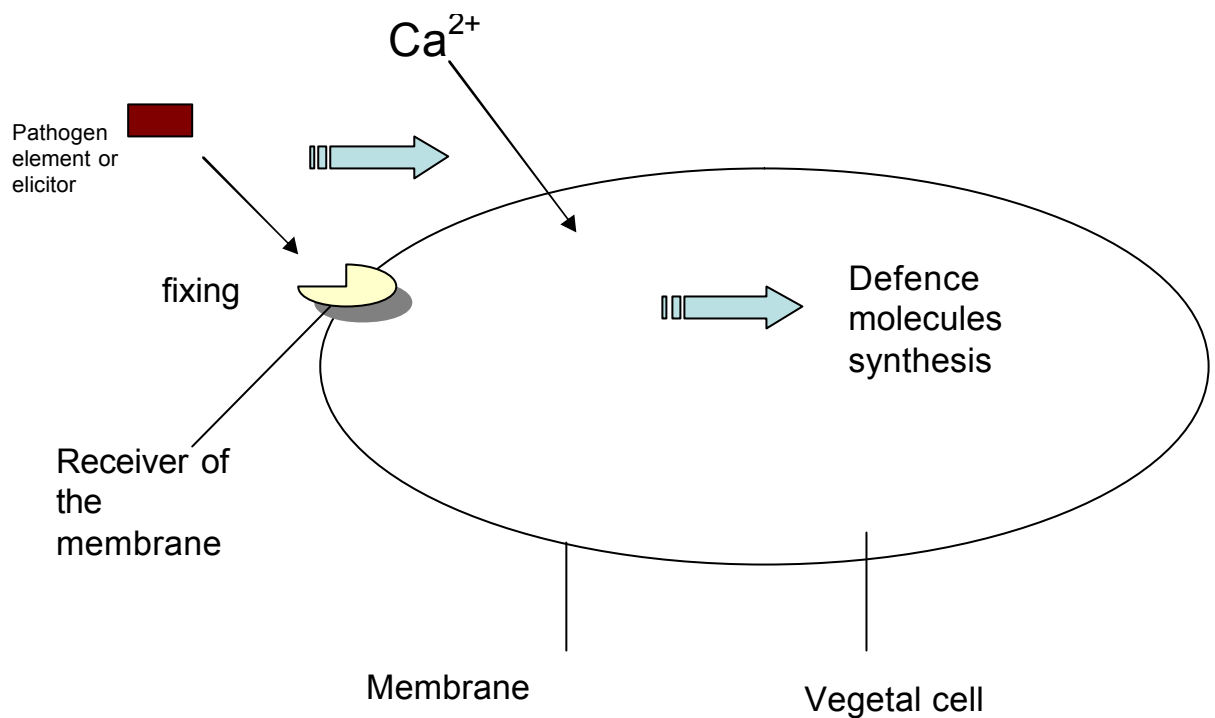
between the cells. Their functionality depends on the calcium. This connection can be blocked if there is not enough calcium in the medium.

Diagram of cadherins structure



Calcium is important for the cellular communication by regulation of the plasmodesms and the cell size. (Hake and al., 1997). Plasmodesm is a biologic continuity between two adjacent vegetal cells through the medium of perforations in the walls.

- ✍ The plant is always exposed to negative environmental conditions; it must develop suitable strategies to survive. Defence reactions need calcium.



Many kinase proteins have a catalytic activity, dependant of the calcium. Those proteins have an important part in the transmission of the defence signal when the plant detects a pathogen agent. This agent can induce a defence reaction in the plant. Signal transmission responsible of the plant reaction is bound to many biochemical cellular and molecular modifications. This process is bound to calcium influx, which is combined with proteins to activate the signalisation processes due to the wounds and the pathogen agent. The dosage of the intra cellular calcium shows its implication in many environmental stress responses in different plants. (Harsh 2003; Malho 1999). The free cytoplasmic calcium increases to 1 mol/l, whereas in general it is around 1 nmol/l.

A membrane has an electronic polarization when it is in rest. Thanks to an electrode placed in the cell, we can notice a difference of potential: there is a positive charge inside the cell and a negative charge outside the cell. This difference of charge is due to a continue ions flux (like the calcium) through the membrane. It induces a reduction of the electrostatic attraction of the membrane. So calcium can reduce the copper, aluminium, zinc and sodium toxicity.

Effect of a calcium deficiency

The apical necrosis occurs when there is a calcium deficiency at the apical extremity of the fruit. (Taylor and al., 2004). This phenomenon occurs also in sufficient calcium content ground and in plants with a lot of calcium in the tissues, because this element does not move a lot in the plant. The apical necrosis comes from a variation of the water contribution in the plant; it interferes with the calcium contribution in the fruit.

That local calcium deficiency is responsible for many quality problems of the tomato harvest.

The apical necrosis can get worse when certain conditions prejudice the calcium absorption and its carrying. Its absorption is reduced when the roots are injured at the time of ground ploughing, if the roots are poor, when there is a hydrous stress, when the magnesium, potassium or ammonium contents are too big in the ground, when the temperatures and ground pH are too low.

Conclusion

Calcium has an important part among the necessary nutriments. Its main parts are:

- preservation of the structural and functional integrity of the membrane
- stabilisation of the cellular wall
- transport and control of the ionic exchanges.

A calcium contribution for the plant thanks to a foliar pulverisation can overcome the frequent Ca deficiency.

From an agronomic point of view, Ca has many important functions:

- it favours the healing
- it improves the fruits and vegetables preserving
- increases the resistance to the biotic and abiotic stresses

HerbaGreen released Ca as the carbonate calcium dissociation; so the plant will be supplied continuously.

A calcium gradient allows the control and the polarised growth of the apical cells. This zone is called vegetative point or apical zone. It can split up to generate the different parts of the vegetal (leaf, stem, root, reproductive organs).

The integrity of the membrane is maintained thanks to the calcium ions; the cell will leave a better resistance to the turgescence and the plant will retain more water and more nutrients for a better growth.

Ca is an essential element in the nitrogen metabolism because it stimulates the ammonium absorption. The nitrogen contained in NH_4^+ is essential for the vegetal growth.

Calcium variations are responsible for the stomata opening. That process is essential for the photosynthetic activity.

Calcium is important for inter cellular communication. The functionality of the cadherins (link proteins) depends on the presence of the calcium.

Moreover, calcium is important for the natural defence reactions. Many kinase proteins have a catalytic activity, dependant of the calcium. Those proteins have an important part in the transmission of the defence signal when the plant detects a pathogen agent.

According to the study made at SADEF laboratory, HerbaGreen pulverisations were efficient to stop the development of the disease and to stop it later, surely thanks to many calcium roles in the plant. **HerbaGreen releases calcium as the carbonate calcium dissociation.** Then the plant will be supplied continuously, contrary to massive calcium contribution in liquid form; the calcium will be fixed very fast and will not be available for the cellular mechanisms.

Thanks to HerbaGreen treatment, there was a diminution of 54 % of contaminated leaves with powdery mildew.

These experimentations have shown that *HerbaGreen* has a potentially preventive effect on the one hand, reducing the possible contamination of healthy grapevines, and, on the other hand, a healing effect on the grapevines already contaminated with powdery mildew.

Research of alternative solutions for protection against powdery mildew of strawberries

Introduction

Different alternative solutions were used to struggle against powdery mildew (*Sphaerotheca macularis*) of strawberries, considering stimulation of the plant's natural defences. Strawberries are cultivated under greenhouse and out of ground.

Experimentation objective consists in the reinforcement of the plant thanks to HerbaGreen pulverisation, alternated with a chemical fungicide.

Material and methods

Plan of experimentation: Fischer blocks with 4 repetitions, except control and no treated plants.

Methods	Active molecules	Use dose
Control		
Alternated chemical reference: Topaze Nimrod Ortiva	Penconazole (100 g/l) Bupirimate (250 g/l) Azoxystrobine (250 g/l)	0.5 l/ha 1.0 l/ha 0.8 l/ha
HerbaGreen Anvil	Hexaconazole (50 g/l)	500 g/100 l 0.6 l/ha

Elementary parcel seize: 28 plants (2 plants x 14 pots)

Density under greenhouse: 91 plants/m²

Variety: Elsanta

Transplanting date on exterior tree nursery: 03/08/05

Plants transplanting: 2 plants per 3 liter pot, on blonde peat.
Entry under greenhouse: 23/08/05

Treatments carrying out

Modalities	A	D	B
Products	Control	Reference	HerbaGreen/Anvil
Transplanting 03/05/05; under greenhouse putting			
26 th August		Topaze	HerbaGreen
02 nd September		Nimrod	Anvil
09 th September		Nimrod	HerbaGreen
16 th September		Nimrod	Anvil
Harvest beginning 22/09/06			
23 rd September		Nimrod	HerbaGreen
30 th September		Ortiva	HerbaGreen
06 th October		Ortiva	HerbaGreen
14 th October		Ortiva	HerbaGreen

First treatments are preventive; they begin 3 weeks after plants transplanting made on from naked roots. Then, they are made every week to keep on the initial program. First powdery mildew symptoms occur in the region of 10/09/05, without contamination.

Efficiencies notations

Symptoms notations on foliage: 5 notations from 14/09 to 12/10:

- taking of one leaf per pot (14 leaves x 4 repetitions per modality)
- contaminated leaves number counting per elementary parcel
- attack intensity notation according to AQUITAINE SRPV protocol for each taken leaf (% of contaminated surface according to a scale: 1-2.5-5-10-20-35-55-75-90)

Results

Notations of 12/10/05	% contaminated leaves by powdery mildew	Attack intensity % average contaminated surface
Control	100	45
Reference	46	8
HerbaGreen + Anvil	71	46

Discussion

For both first notations made on 14th and 21st September, there is a very good efficiency for both methods including alternated applications of chemical fungicide (Topaze, Nimrod and Ortiva). Percentage of contaminated leaves is very low and attack intensity is almost equal to zero.

HerbaGreen modality including an Anvil protection induces 50 to 65 % of contaminated leaves, against 90 % for control plants.

For next notations, powdery mildew pressure becomes stronger, and there are just modalities with chemical references which are less contaminated by the disease until the end of cultivation:

Moreover, HerbaGreen treatment allows to increase fruits preserving; no treated strawberries will be in decomposition state faster than the treated strawberries, as we can see on this picture below. Control strawberries release more decomposition juice.

We notice the same preserving aspect on salad; many tests indicate that the treated ones have a longer preserving time.



On the left: treated strawberries; on the right, non treated strawberries

Conclusion

In comparison with chemical reference (alternation of 3 products: Topaze, Nimrod, Ortiva), modality HerbaGreen + hexaconazole (2 applications) allows an important reduction of attack frequency. However, it does not allow a satisfying protection against powdery mildew.

From a physiologic point of view, we can notice a thickening and a stronger coloration of foliage on HerbaGreen modality. We have to check if this foliage reinforcement can allow to reduce the number of treatments with traditional fungicides and pesticides.

4. Phyto toxicity tests of HerbaGreen on lettuce

Introduction

The objective is to define the innocuity of HerbaGreen for its agronomic use

Material and methods

Product definition: HerbaGreen is made of 100 % natural calcite, which corresponds to NFU 44001 standard. It is mainly made of carbonate calcium and it is used by foliar pulverisation. The recommended doses are 0.3 to 0.5 %.

Study object: To determine the toxicity threshold and to show an eventual phytotoxicity. Lettuce was chosen as the reference plant for its high sensibility, its facility of implementation and its growth speed.

Pulverisation of HerbaGreen on young lettuce plants with a concentration of 1 to 3 %. Fluorimetry rate and weight of salads were determined. Fluorimetry analysis allows the measurement of activity yield; it allows to know if the plant finds suitable physiologic conditions for its development. For a good working of the plant, this parameter must be superior to 0.80

Tfm: for a good working of the plant, this parameter must be in the region of 300.

Results

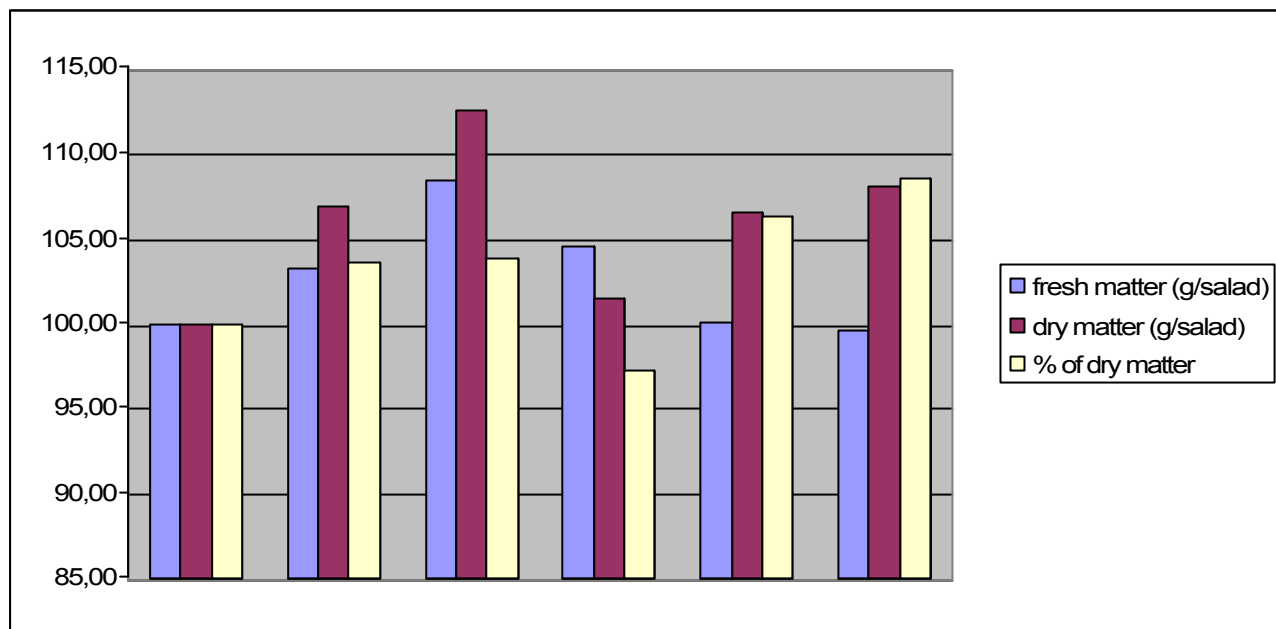
Weight of salads

	Real weights		
	Fresh matter (g/salad)	Dry matter (g/salad)	% of dry matter
TO	18.94	1.70	8.96
T 1 %	19.54	1.82	9.29
T 1.3 %	20.52	1.91	9.31
T 1.5 %	19.79	1.72	8.70
T 2 %	18.96	1.81	9.53
T 3 %	18.86	1.84	9.73
	19.70	1.81	9.21

Observation: there was no phytotoxicity; but you could notice a greyish layer.

	Weights in comparison to T0		
	Fresh matter (g/salad)	Dry matter (g/salad)	% of dry matter
TO	100	100	100
T 1 %	103.20	106.92	103.61
T 1.3 %	108.37	112.52	103.83
T 1.5 %	104.50	101.47	97.10
T 2 %	100.12	106.48	106.35
T 3 %	99.62	108.10	108.52
	104.05	106.85	102.72

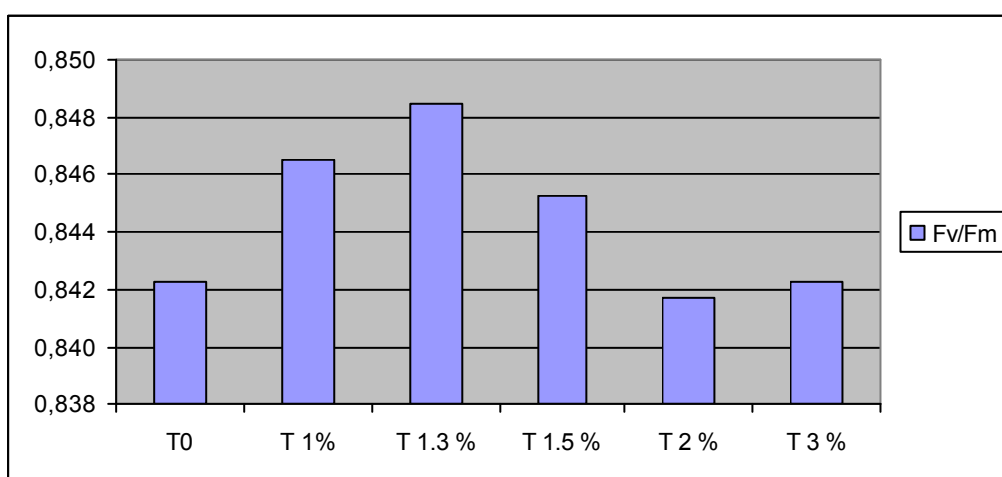
Graphic result of different dosage on the salad global weight and dry matter level.
Better production on T 1.3 % (+12%), the stronger the product concentration is, the more the content of dry matter is high, but for T 1.5 %.



Fluorimetry study

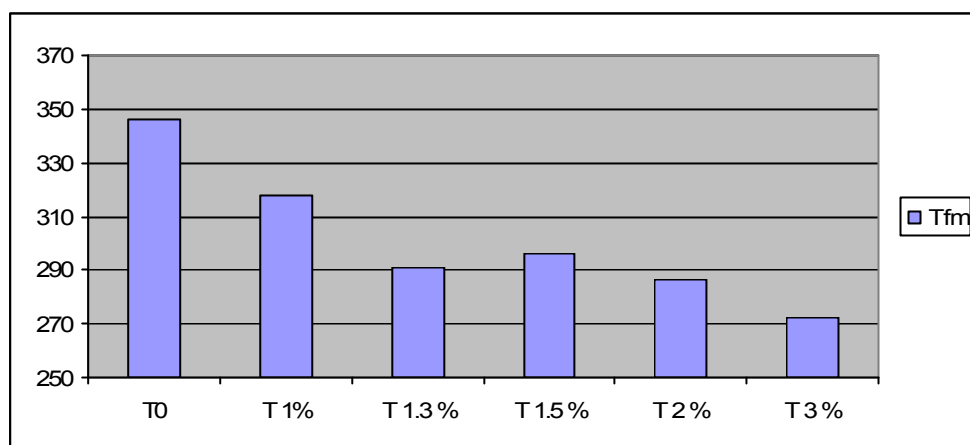
✍ Measurement of Fv/Fm:

Better effect on T 1.3 % but no significant.





Measurement of Tfm

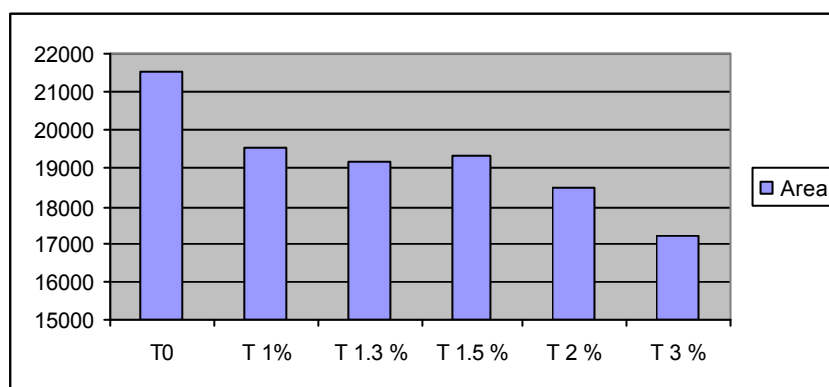


The treatment had a strong effect, but no significant one. The stronger the concentration, the more the negative effect is important.

Measurement of "Area"

The optimal value of this parameter is 30 000.

	Area	
T0	21545	a
T 1%	19523	ab
T 1.3 %	19182	ab
T 1.5 %	19341	ab
T 2 %	18500	b
T 3 %	17182	b



The treatment had a very strong and significant effect. The stronger the concentration, the more the negative effect is important.

Discussion

HerbaGreen has no phytotoxicity even at very important doses. The using of HerbaGreen by foliar pulverisation was tested with doses 6 to 10 times more concentrated than the recommended dose. Application of carbonate calcium microparticles, obtained by tribomechanical, does not create marks on the leaves which are used for the phytotoxicity tests. You can notice a positive effect up to 1.5 %, with a gradation of the effects.

Conclusion

The using of HerbaGreen by foliar pulverisation was tested with doses 6 to 10 times more concentrated than the recommended dose. There was no phytotoxicity even with these very important doses.

Application of carbonate calcium microparticles obtained by Tribo mechanical does not create marks on the leaves which are used for the phytotoxicity tests.

You can notice a positive effect only up to 1.3%, with a gradation of the effect.

5. HerbaGreen and hydrous stress

Material and methods

This study was made on vine leaves, tomato stems and leaves and leaves of wheat. It were coloured with a double coloration. It allows a differentiation of the different biochemical components of the vegetal tissue. Schiff reagent shows polysaccharides whereas naphtol blue black reagent shows organites with proteins.

Leaves samples were observed with optical microscope.

The comparison of organites (chloroplasts, polyphenols and starch) was made far away from the conducting vessel of the sap, thus the observation was not distorted.

2 Places of taking:

- SADEF laboratory: comparison of treated and untreated leaf (tomatoes and vine).
- Carignan Douzans (11): Comparative study of vine leaves treated with HerbaGreen and control leaves

Place of observation: CIRAD cellular imagery department (Montpellier)

Results



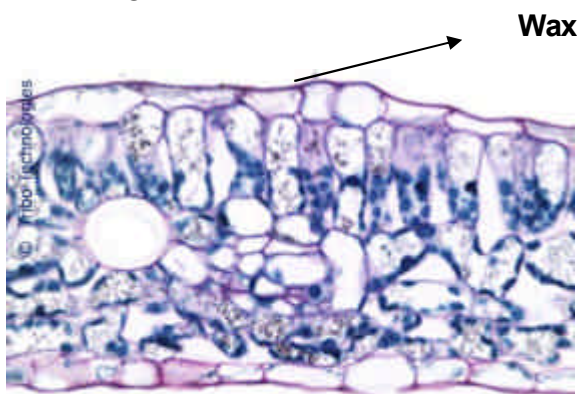
A better use of the water

During the SADEF experimentation, different observations were made: leaves of the treated vine had a particular different surface, in comparison with control leaves. This was due to a change of structure of the cuticular wax, as you can see on the pictures below

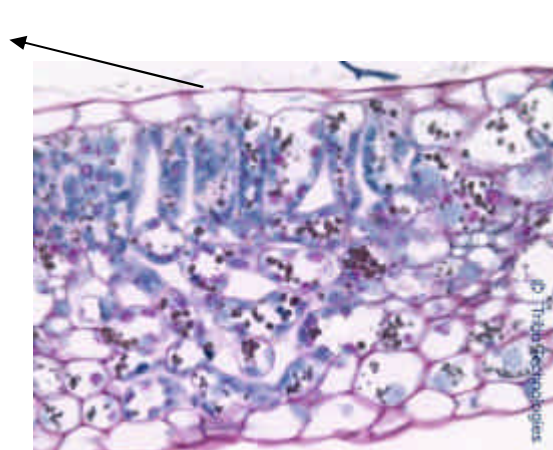


The specific surface of the wax allows to hold water and to create a superficial tension. HerbaGreen modified the tensio active properties of the treated leaves.

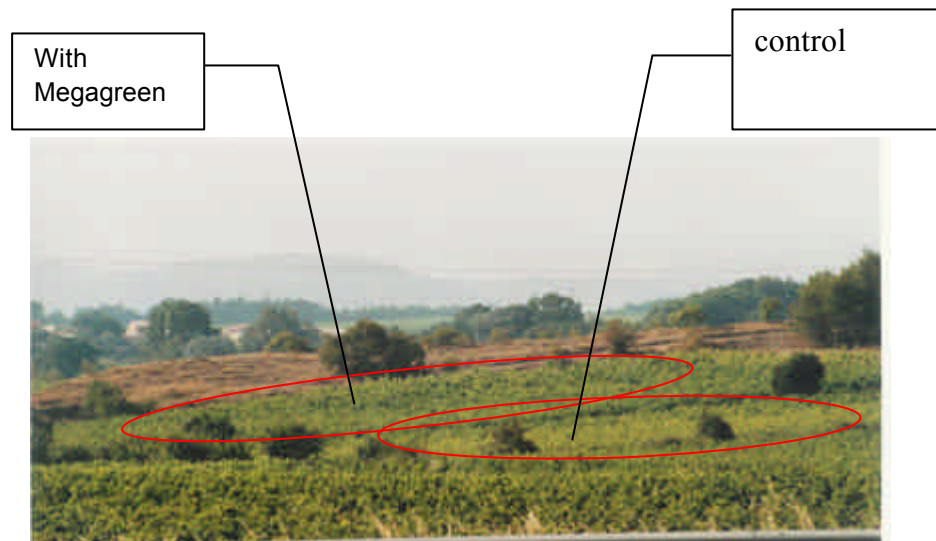
Picture :
CIRAD



Control vine leaf (cross section, x20)



Treated vine leaf (cross section, x20)



Essai Carignan Douzens (11) – Photo du 26/07/2005

The treated parcel needs less water and is greener.

Discussion

This phenomenon was observed on tomato plant to; this could explain many observations made by Sicilian growers who reduced the water quantity thanks to this phenomenon. According to the water distribution on the surface of the leaf, we can conclude that:

- ✍ Control leaf : presence of droplets hold on the surface
- ✍ Treated leaf: drops are not visible because the surface of the leaf is covered by a water film. The entire surface of the leaf is in contact with the water, which induces a high hydration.

HerbaGreen particles dissociation induces a CO₂ contribution, in gaseous and dissolved form, which is responsible of the stomata closing. It will limit the water loss by sweating. Sweating is the essential mechanism which allows the hydrous balance preservation.

Dehydration of the plant is due to hydrous loss by the stomata. A treatment of the plant with HerbaGreen does not modify the sweating process, but would avoid the

useless evaporation. Intracellular medium saturated with CO₂ is responsible of the stomata closing; this will induce a reduction of the evaporation and water loss.

Conclusion

SADEF laboratory experimentations show that HerbaGreen applications induce a change of structure of the cuticular wax. The entire surface of the leaf is in contact of the leaf; this induces a high hydration.

HerbaGreen modifies the tensio active properties of the treated leaves.

HerbaGreen particles dissociation induces a CO₂ contribution responsible of the stomata closing; it will limit the water loss.

Summary

1. Improvement of photosynthesis

The treated leaf has a more important activity because of the many chloroplasts. This shows the high number of proteins in their structure, which indicates a more important metabolic activity, confirmed by the chloroplasts structure. **The treated leaf has a better metabolism.** The difference which can be put in a prominent position by the data analysis is about the metabolic activity. The structure and the number of chloroplasts show that **3 applications of HerbaGreen launched a high activation of the photosynthetic activity.**

Starch and polyphenols must be put in a dynamic context, thus, conclusions about the increase of the photosynthetic system in the leaf can be drawn when the wine harvest is analysed.

HerbaGreen treatment allows different physiologic and metabolic changing:

Chloroplasts: chloroplasts are better formed and bigger in the treated leaves; it shows a better functionary of the photosynthesis.

Polyphenols: A higher export of polyphenols to the berries seems to be confirmed by the maturity controls.

Proteins: Treated leaves have more proteins in their structure, which indicates a more important metabolic activity, confirmed by the chloroplasts structure. Metabolism is more active in the treated part; the presence of many cellular nucleuses indicates a high protein synthesis activity.

If photosynthetic process is increased, primary and secondary metabolisms will be increased too. Thus, primary and secondary metabolites rate will be more important (starch, polyphenol...). It will have an incidence in different parameters: **yield will be increased and harvests organoleptic qualities will be improved.**

Moreover, if photosynthesis activity is increased, photobreathing will be inhibited which allow to avoid an important water loss, limiting the watering.

2. Antioxidant potential effect of HerbaGreen

After HerbaGreen treatment, the treated plants have a more important coloration and the control plants present senescence signs. That green effect was noticed in many field and laboratory experimentation.

The senescence of vegetal is due to the peroxides emitted during the cellular breathing. They become very reactive after an electron loss. They pick up the missing electron in the cellular components or in the DNA. That process induces the plant degeneration.

✍ **Increase of plant activity traduces by enhancement of primary and secondary metabolite could activate senescence effect on plant, which will be faster. Thanks to anti oxidant potential of HerbaGreen senescence will slow down and plant activity will be better and maintained all along crop period.**

3. Role of Calcium in the reinforcement of the vegetal

HerbaGreen released Ca as the carbonate calcium dissociation; so the plant will be supplied continuously.

Calcium has an important part among the necessary nutriments. Its main parts are:

- *preservation of the structural and functional integrity of the membrane*
- *stabilisation of the cellular wall*
- *transport and control of the ionic exchanges.*

A calcium contribution for the plant thanks to a foliar pulverisation can overcome the frequent Ca deficiency.

From an agronomic point of view, Ca has many important functions:

- *it favours the healing*
- *it improves the fruits and vegetables preserving*
- *increases the resistance to the biotic and abiotic stresses*

A calcium gradient allows the control and the polarised growth of the apical cells. This zone is called vegetative point or apical zone. It can split up to generate the different parts of the vegetal (leaf, stem, root, reproductive organs).

The integrity of the membrane is maintained thanks to the calcium ions; the cell will leave a better resistance to the turgescence and the plant will retain more water and more nutrients for a better growth.

Ca is an essential element in the nitrogen metabolism because it stimulates the ammonium absorption. The nitrogen contained in NH_4^+ is essential for the vegetal growth.

Calcium variations are responsible for the stomata opening. That process is essential for the photosynthetic activity.

Calcium is important for inter cellular communication. The functionality of the cadherins (link proteins) depends on the presence of the calcium.

Moreover, calcium is important for the natural defence reactions. Many kinase proteins have a catalytic activity, dependant of the calcium. Those proteins have an important part in the transmission of the defence signal when the plant detects a pathogen agent, like powdery mildew:

- vine powdery mildew:

According to the study made at SADEF laboratory, HerbaGreen pulverisations were efficient to stop the development of the disease and to stop it later, surely thanks to many calcium roles in the plant. HerbaGreen releases calcium as the carbonate calcium dissociation. Then the plant will be supplied continuously, contrary to massive calcium contribution in liquid form; the calcium will be fixed very fast and will not be available for the cellular mechanisms.

Thanks to HerbaGreen treatment, there was a diminution of 54 % of contaminated leaves with powdery mildew.

These experimentations have shown that HerbaGreen has a potentially preventive effect on the one hand, reducing the possible contamination of healthy grapevines, and, on the other hand, a healing effect on the grapevines already contaminated with powdery mildew.

- strawberry powdery mildew:

In comparison with chemical reference (alternation of 3 products: Topaze, Nimrod, Ortiva), modality HerbaGreen + hexaconazole (2 applications) allows an important reduction of attack frequency. However, it does not allow a satisfying protection against powdery mildew.

From a physiologic point of view, we can notice a thickening and a stronger coloration of foliage on HerbaGreen modality.

4. Phyto toxicity tests of HerbaGreen on lettuce

The using of HerbaGreen by foliar pulverisation was tested with doses 6 to 10 times more concentrated than the recommended dose. There was no phytotoxicity even with these very important doses.

Application of carbonate calcium microparticles obtained by Tribo mechanical does not create marks on the leaves which are used for the phytotoxicity tests.

You can notice a positive effect only up to 1.3%, with a gradation of the effect.

5. HerbaGreen and hydrous stress

HerbaGreen particles dissociation induces a CO₂ contribution, in gaseous and dissolved form, which is responsible of the stomata closing. It will limit the water loss by sweating. Sweating is the essential mechanism which allows the hydrous balance preservation.

*Dehydration of the plant is due to hydrous loss by the stomata. **A treatment of the plant with HerbaGreen does not modify the sweating process, but would avoid the useless evaporation.** Intracellular medium saturated with CO₂ is responsible of the stomata closing; this will induce a reduction of the evaporation and water loss.*

SADEF laboratory experimentations show that HerbaGreen applications induce a change of structure of the cuticular wax. The entire surface of the leaf is in contact of the leaf; this induces a high hydration.

HerbaGreen modifies the tensio active properties of the treated leaves.

IV. EXPERIMENTATIONS RESULTS WITH HERBAGREEN

1. EXPERIMENTATIONS ON FIELD CROPS (WHEAT, BEET and BARLEY)

Material and methods

General protocol:

- ✍ Cereals: one application/ last leaf/ dose 1.5 kg/ha
- ✍ Beet: one application/ dose 1.5 kg/ha
Band experimentation with 3 repetitions. There are 45 cm between each row. The dose is 1.5 kg/ha, applied in once. The treatment was made at 4-6 leaves stadium. After the treatments, beets are taken on 3x3 m.

Application can be different according to conditions of the medium .

Two places of experimentation for wheat

- ✍ Groupe coopératif région centre, France (service technique)

Wheat experimentation type: micro parcel/3 repetitions. HerbaGreen is used by foliar pulverisation with a dose of 1.5 kg/ha, last leaf stage

- ✍ EARL Scharrenberger, route de Gunstett, Surbourg, France

Seedling at the beginning of October

- ? 80 nitrogen unities at the spring
- ? Hussard (herbicide) 1.25 l/ha, Courtex T 1.5 l/ha
- ? 80 unities of nitrate ammonium
- ? HerbaGreen (2 kg/ha), application second node and last leaf stadium
- ? No phosphorus and no potassium hydroxide
- ? Type of ground: silt laden clay

Place of experimentation for beet (department 28, France)

Results

Wheat

Average of results, 3 repetitions groups. Coopératif région centre

	HerbaGreen 1.5 kg	Control
Nitrogenized nutrition	130 unities in 3 contributions	130 unities in 3 contributions
Humidity	16.5	16.5
Proteins	12.1	11.7
Specific weight	73.7	73.1
Yield	95.2	90

On average, one application of HerbaGreen allowed **an increase yield of 5 q/ha**.

EARL Scharrenberger

	Wheat	
Mode	1	2
	HerbaGreen	Control
Surface (m ²)	540	540
Weight (kg)	469	402
Humidity (%)	11.3	11.5
Wet yield	86.9	74.4
Norm yield	90.6	77.5

One application of HerbaGreen allowed **an increase yield of 13,1 q/ha**

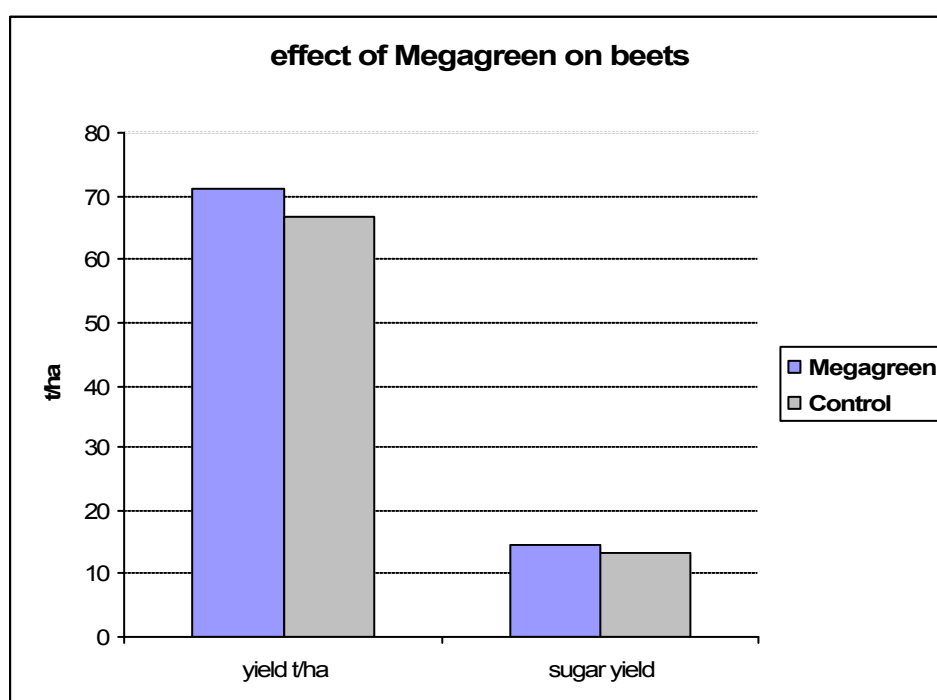
Beet

	Treated beets	Control beets
Yield (t/ha)	71.1	66.7
Gain	7 %	

Thanks to HerbaGreen treatment, yield of treated beets increased of 7 %, in comparison of control beets.

	Treated beets	Control beets
Sugar content (%)	20.3	20.1
Sugar content (t/ha)	14.44	13.40
Gain	8 %	

Results showed that one treatment of HerbaGreen can increase the sugar content of the beets by 1.44 t/ha, which corresponds to a **sugar improvement of 8 %**.



Discussion

Different tests in field confirm the fact HerbaGreen has a positive indirect effect on the syndrome of low wealth in sugar.

HerbaGreen has a positive action on crop fields (wheat and beet) yields, proteins and sugar contents.

Conclusion

We have noticed specific effects:

- ✍ Increase of the yield and of the photosynthesis
- ✍ Reinforcement of the plant
- ✍ Improvement of sugar content in the beet

So it is possible to conclude that HerbaGreen can be used to improve crop fields

✍ **Barley (RITTMO laboratory)**

Introduction

The objective of the experimentation consists in the study of HerbaGreen pulverisation on barley. Treated barley plants are cultivated on a water retenting ground and control plants are cultivated on the draining ground.

Material and methods

HerbaGreen is applied by foliar pulverisation.

Different parameters are evaluated:

- 1. After 4 or 5 weeks of barley growth, measurement of dry and fresh foliar biomass - measurement of roots biomass.**

Plants are harvested according to phytosanitary state.

- 2. Evaluation of rhizospheric activity index (RAI)**

Objective: RAI is the link between the compressed ground mass on the roots and the roots mass. It is an indication of the good health of the plant. If the index is high, the plant is in good health. If RAI is high, it means that roots quantity is higher; quantity of proteins and sugar increase in the roots. Bacteria of the roots will degrade those molecules and plants will feed on the products which come from this degradation.

- Barley variety: Hordeum vulgare, Scarlett variety, no treated seeds.

- Solutions: Hoagland complete nutritive solutions, ethanol aqueous solution, distilled water.

Physical and chemical properties of grounds:

Granularity (for 1000)

A	LF	LG	SF	SG
133	182	109	147	427
	Free alluvium		Total sands	
	291		574	

A: silt

LF: sandy silt, with a poor drainage

LG: sandy silt, with a very poor drainage

SF: silty sand, with a poor drainage

SG: silty sand, with a very poor drainage

Organic matter : 22.1

Nitrogen/carbon : 9.8

pH : 7.9

Total limestone : 16 (for 1000)

Cationic exchange capacity: 104

Retention capacity: 47.3 %

Fertility:

P ₂ O ₅	K ₂ O	CaO total	MgO	Na ₂ O	Fe	Mn	Cu	Zn	B
0.18	0.15	9.11	0.21	0.015	74.3	11.3	2.6	1.7	0.16

25 barley seeds are planted in one pot. Pots are placed in a greenhouse.

Four days after the sowing, there are 15 plants per pot. HerbaGreen pulverisations are made 2 weeks of growth. HerbaGreen is pulverised until the streaming limit. The control plants get water pulverisation.

All treatments are repeated three times. The treated plants are placed at one extremity of the greenhouse, the control plants at the other. This is necessary to avoid the interactions between the plants.

HerbaGreen treatment is repeated three times.

Experimentation calendar:

01/08/2005	Pots preparation
04/08/2005	Barley sowing
	pots watering
	Put under greenhouse
10/08/2005	Reduction of the number of barley to 15
19/08/2005	HerbaGreen pulverisation
	Diseased barley plants uprooting
26/08/2005	Treatment against thrips
31/08/2005	Problem of nitrogen deficiency : new contribution of nutritive solution
	Contribution of nutritive solution
05/09/2005	harvesting

Watering:

Dates	Water quantities+NS (ml)	NS contribution
29/07/2005	150	
05/08/2005	150	
12/08/2005	180	
16/08/2005	100	
19/08/2005	80	
22/08/2005	150	
25/08/2005	80	NS
29/08/2005	200	
31/08/2005	30	NS

Results

Results in the table below correspond to fresh matter values, of dry matters of the plants, of fresh matter of the roots, dry matter of the ground for the calculation of the rhizospheric activity index (RAI). Each value was taken on control plants and on treated plants.

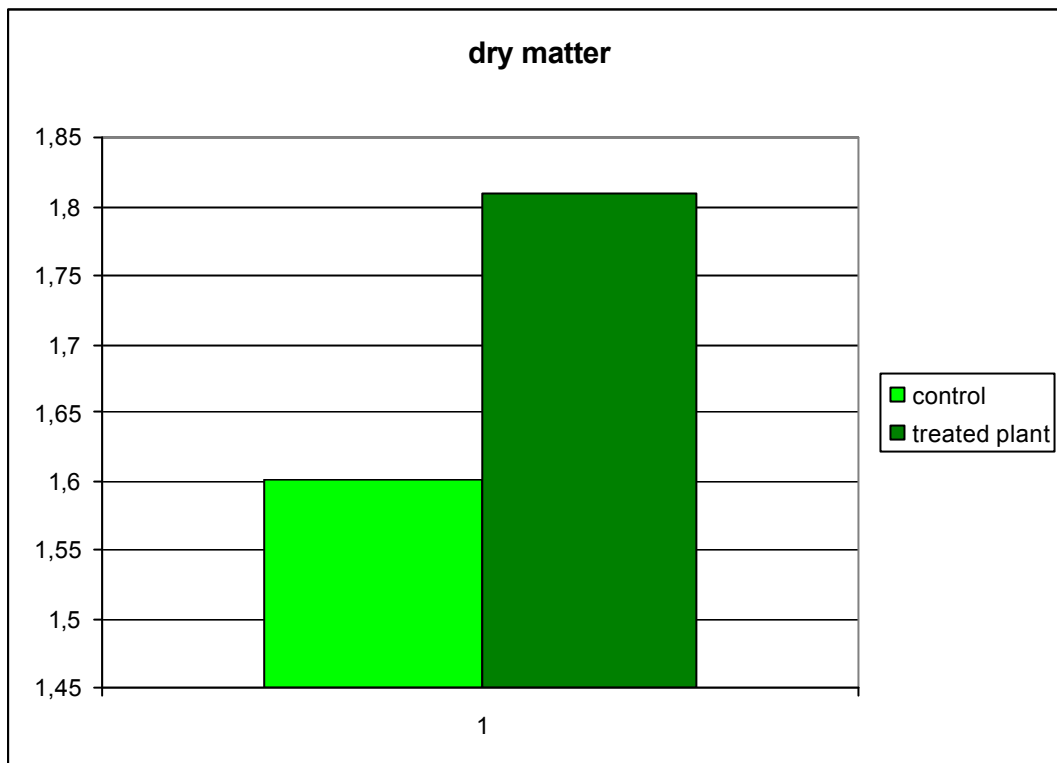
		Plants		RAI		
		FM	DM	Roots FM	Dry matter ground	RAI
Control plants	1015	9,2	1,71	3,1	6,22	2,01
	1016	9,4	1,56	3,2	5,34	1,67
	1017	8,6	1,54	2,4	5,42	2,26
	Average	9,07	1,60	2,90	5,66	1,98
	Standard deviation	0,42	0,09	0,44	0,49	0,30
Treated plant	1018	9,3	1,91	2,3	4,84	2,10
	1019	8,8	1,8	2,2	4,91	2,23
	1020	9,1	1,72	3,3	7,45	2,26
	Average	9,07	1,81	2,60	5,73	2,20
	Standard deviation	0,25	0,10	0,61	1,49	0,08

Values of each parameter are presented in those graphics as hereunder:

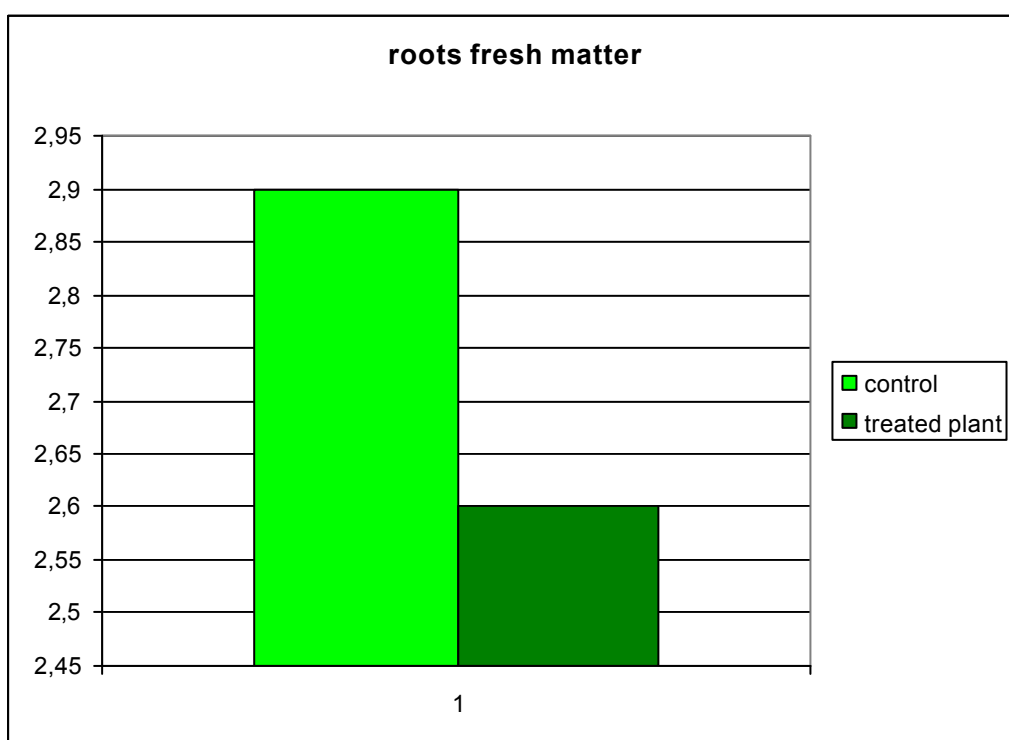
Average fresh matter



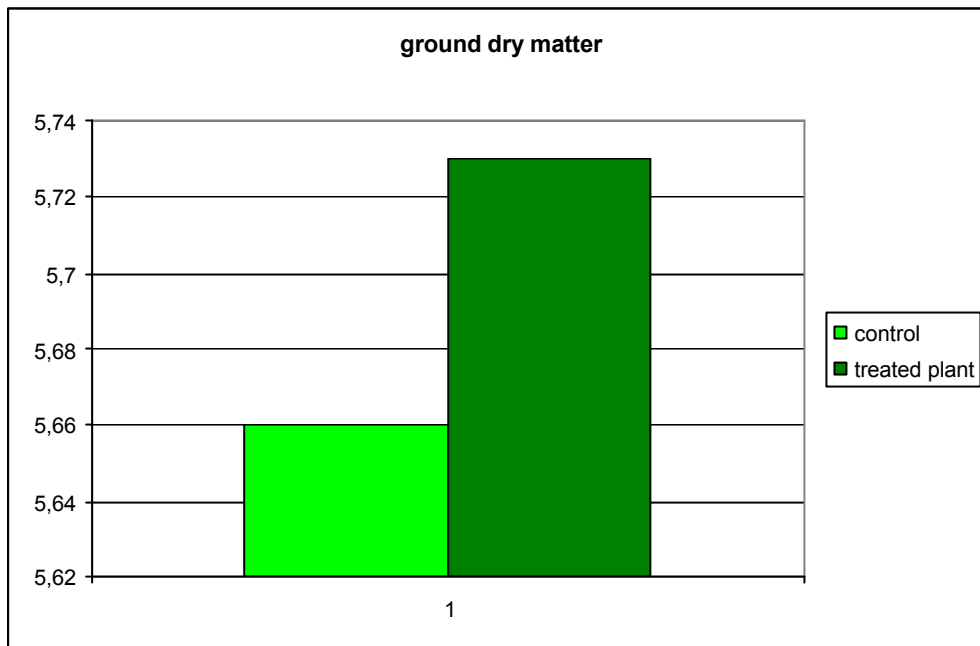
Average dry matter



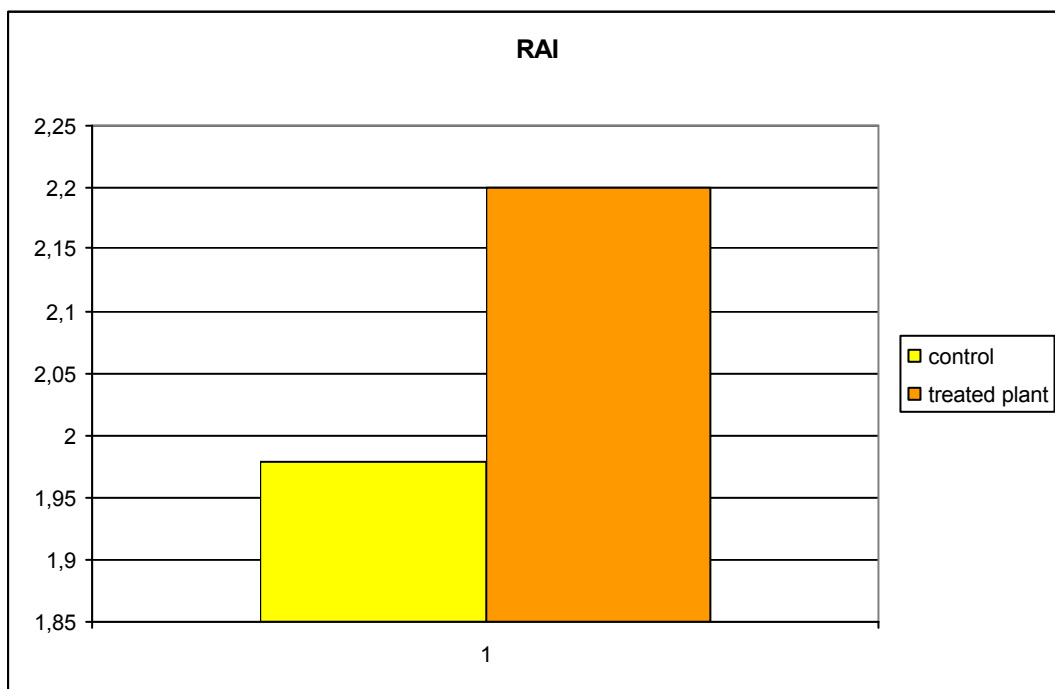
Average roots fresh matter



Average ground dry matter



average rhizospheric activity index



Leaves observations



Figure 1: control plants



Figure 2: treated plants



Figure 3 - comparative picture. On the left: control plant; on the right: treated plants

On average, fresh matter rate of control plants and of treated plants is the same: 9.07. Dry matter rate of treated plants (1.81) is higher than the one of the control plants (1.60)

Moreover, roots fresh matter rate of control plants (2.90) is higher than the one of the treated plants (2.60). Ground dry matter of the control plant is lower than the one of the treated plants. So the rhizospheric activity index will be higher for the treated plants.

Discussion

According to the results, we can notice a difference of leaves posture. The treated plants have leaves without posture and with a descending sprouting. At the contrary, the control plants have a better posture than the treated plants. The treated plants and the control plants have the same number of leaves.

It is possible that HerbaGreen treatment allows an increase of ligneous tissues, which contain less water than others tissues. Those ligneous tissues correspond to dry matter; that increase is maybe responsible of the better strength of the plants.

Conclusion

According to the results, action of HerbaGreen allows a beneficial effect on the growth of the barley and on the posture of the leaves.

HerbaGreen allows dry matter increase and rhizospheric activity index increase. It indicates that barley plants treated with HerbaGreen are in a better global health.

2. USE ON CONSUMPTION POTATOES

Introduction

Objectives:

- ? Increase of the photosynthesis and the yield
- ? Indirect effect on fungi diseases by a general reinforcement of the plant
- ? Effect on quality : increase dry matter and reduce the burnishing because of Maillard reaction at the time of transformation
- ? Indirect effect on doryphore by increase of the thickness of the leaf and reinforcement of cellular wall thanks to calcite treatment.

Material and methods

Potato (consumption and early product)



2 applications:

T1: potatoes in formation + 15 days dose 1.5 kg/ha

T2: T1 + 15 days dose 1.5 kg/ha

? 4 varieties and 6 experimentations

number	Varieties	Under cover	Planting date	Harvesting date	repetitions	place
1	Ostara	yes	03/02/05	15/06/05	3	29
2	Ostara	yes	03/02/05	15/06/05	3	29
3	Agatha	No	-	08/05/05	3	28
4	Charlotte	No	-	08/05/05	3	28
5	Charlotte	No	-	30/08/05	3	67
6	Noirmoutier	no	-	-	3	Noirmoutier

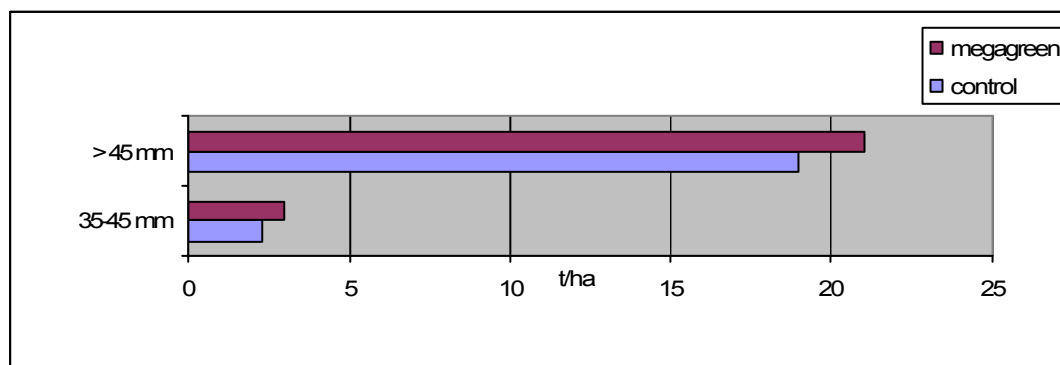
Results

number	Yield (T/ha)			Dry matter content (%)			Yield increase (%)
	control	HerbaGreen	difference	control	HerbaGreen	difference	
1	22,4	26,4	+ 4,1	15,1	15,6	+ 0,5 pt	18
2	22,4	24,8	+2,4	15,1	15,5	+ 0,4 pt	11
3	67,1	69	+1,9				3
4	50,3	55,1	+4,8				10
5	45,9	55,7	+9,8				21
6	See the results below						

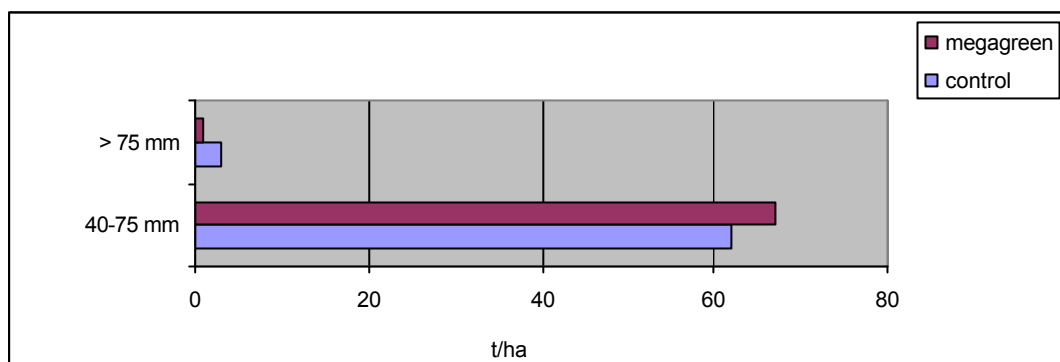
HerbaGreen improves the yield (from 1,9 to 9,8 T/ha) and the dry matter (3 to 21 %) in each case.

Size and yields of potatoes

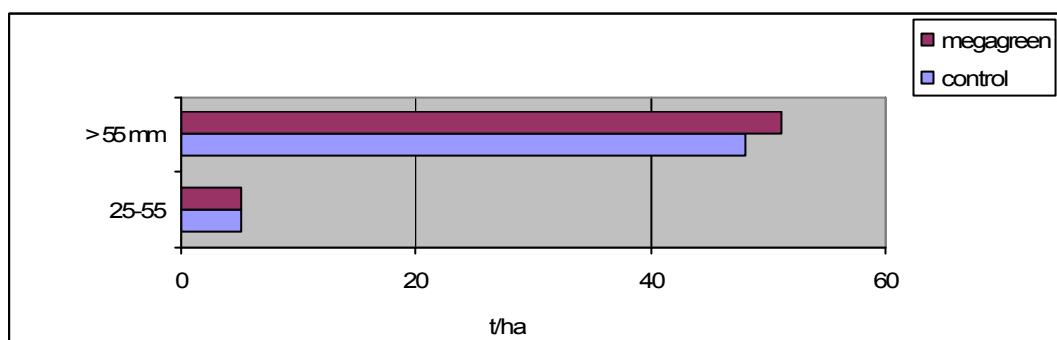
Essay n° 1



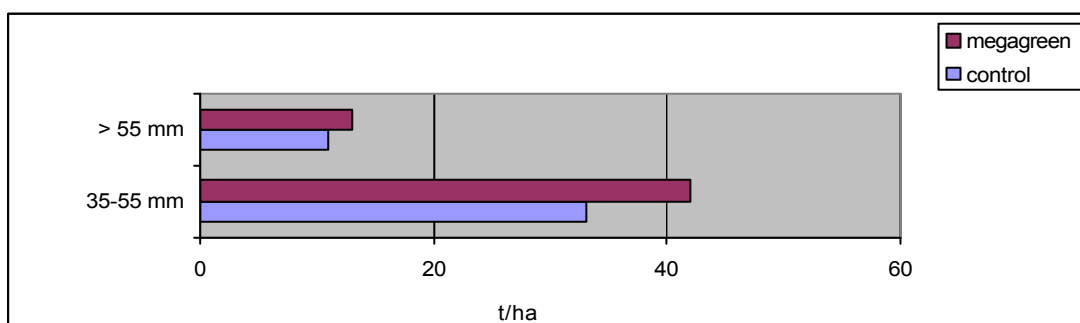
Essay n° 2



Essay n° 3



Essay n° 4



HerbaGreen improves the proportion of marketable potatoes by increasing potatoes' size, and the homogeneity size in marketable range, as observed in all field trials: see experimentation number 3 showing the lessening of potatoes exceeding 75 mm calibre.

	Number of tuber/ha	Average weight of tubers (kg)	yield T/ha	Yield of tuber 30-55mm
control	609877	79	46,6	33,7
HerbaGreen	703704	81	57,0	42,1
Gain in comparison of the control	15%	+ 2 g	+ 21 % 10T/ha	+ 25 % 8,5T/ha

For experimentation 5 we observed an interesting increase of the number of tuber (+ **15%**) and of yield (+ **21%**) with a big proportion of marketable tuber from 30 to 55mm (**85%**)

Experimentation 6

On early product potatoes (Noirmoutier)

	Gross weight (kg) / 6m	Number of tubers	Number of marketable tubers (without rhizoctone)	Marketable net weight	Dry matter
<i>parcel 1</i>					
% /control	+13,4%	+10,1%	+27,0%	+21,9%	idem
<i>parcel 2</i>					
% /control	+4,1%	+24,4%	+33,5%	+8,7%	0,5pt

For each parcel the number of marketable tubers increases tremendously (27 to 33 %)

Discussion

Effects on potatoes are:

- increase of the photosynthesis and of the yield
- indirect effect on fungi diseases by a general reinforcement of the plant
- effect on quality: increase of the dry matter and reduction of the burnishing because of Maillard reaction at the time of transformation
- indirect effect on doryphore by increase of the thickness of the leaves and reinforcement of cellular wall thanks to calcite treatment.

Conclusion

Thanks to HerbaGreen, yields, homogeneity size in marketable range, number of tuber and dry matter increase.

3. HERBAGREEN ON POTATOES FOR THE INDUSTRIAL TRANSFORMATION

Introduction

Dry matter corresponds to starch, cellulose and others chemical elements present in the tuber.

Dry matter is different according to cultivation characteristics, ground, climate, and fertilisation. Indeed, diurnal temperature conditions lower than 27 ° are better for the photosynthesis and the product which comes from it will be more easily transferred and accumulated in starch form in the tubers when night temperatures are lower than 15 °. That is why the same culture made in the north of Europe or in the mountains will give tubers with a dry matter content higher than average of 2-3 %. Moreover, excessive precipitations at the time of agronomic maturation of the tuber are responsible for the reduction of the dry matter content even if water, suitable luminosity and temperatures allow the accumulation of dry matter in growth phases of the tubers. Finally, the dry matter content is higher in clayey grounds than in sandy grounds and the dry matter content in the tuber of potatoes is reduced because of the nitrogen fertilisation, whereas potassium attenuates the negative effects of nitrogen.

The nitrogenous fertilisation is a very important indication. When it increases, we get higher yields but the quality of tubers will be worst. For the last years, nitrogenous fertilisation indications were reduced, from 300 kg/ha to 160-180 kg.ha.

During the growth phases of the cultivation in the field, the dry matter content increases until agronomic maturation. It is maximum in 45-55 grades and diminishes in bigger tubers. The dry matter concentration is very different within a same population and in one tuber, where it is maximum near of the peel.

Material and methods

Evaluation of the yield and of the dry matter content.

Experimentation on the potatoes for industrial transformation was made in a farming society associated with "l'APPA" (Association des Producteurs de pommes de terre de la Vénétie)

Potatoes variety which is used is Hermès, with Scottish class E tubers. The experimentation was made on two 4000 m² parcels: one control parcel and one treated parcel.

HerbaGreen was used with a dosage of 6 kg/ha twice, the first one in the full phase of the formation of the potatoes (20 days after and the second one 10 days after.)

Dry matter content was evaluated by drying on the sample and indirectly thanks to the Simmonds method which allows the dry matter and starch determination thanks to those formulas:

$\% \text{ dry matter} = 4.13 + 0.204 * (1000 * (\text{specific weight} - 1))$

$\% \text{ starch} = 1.39 + 0.196 * (1000 * (\text{specific weight}))$

Chromatographic readings were made on chlorophyll content through the Spad.

It was not possible to appreciate the economy of water need thanks to HerbaGreen treatment because the society was not able to differentiate hydrous contribution between the different treatments.

We took product samples at agronomic maturation to get visible, productive and qualitative evaluations of the tubers.

Results

Successive behaviour of the treated cultivation vegetation was observed when treatments were applied. For the parcel treated with HerbaGreen, there was a bigger equilibrium, that is to say a higher homogeneity of growth. However, the cover of the treated parcel was 5 days later in total.

Treated sample was more pigmented than the control sample. The photosynthetic activity is more pronounced in the treated plants.

Yields are higher in the treated lot (41 t/ha) than in the control lot (39.5 t/ha)

Dry matter content is more important in the treated lot (23.5 %) than in the control lot (20.5 %)

Discussion

Yields, dry matter and photosynthetic activity are higher in the treated lot than in the control lot, so these 3 different points are very important for the industrial transformation: the treated sample had no enzymatic burnishing because of phenolic substances. There was no unenzymatic burnishing because of the formation of brown blackish pigments of transformed products (chips and sticks). That reaction is called Maillard reaction, where sugars react with amino acids to form blackish compounds. Burnishing intensity is bound to the concentration of sugar in the paste.

Conclusion

HerbaGreen can exercise using synergies over by a direct action on the vegetal physiology. That is why yield, dry matter and photosynthetic activity are higher for potatoes treated with HerbaGreen. Those 3 points are very important for potatoes which are used for industrial transformation; they must contain enough dry matter, not much reducing sugar because it is responsible of burnishing of fried products. HerbaGreen treatment allows to get potatoes with those specific characteristics.

4. USE IN MARKET GARDENING

Salads

Introduction

Objectives:

- ✍ Yield increase
- ✍ Plant reinforcement
- ✍ Quality improvement (rate, dry matter, coloration, grade)

Material and methods

varieties	Under cover	Planting date	Harvesting date	repetitions
Atria	yes	01/04/05	25/05/05	4
Oak leaf	no	23/05/05	12/07/05	3
Estelle	no	03/05/05	22/06/05	3
Loyale	no	01/06/05	12/07/05	3
Cigale	yes	01/06/05	12/07/05	4
Boogie	yes	Adapted protocol, see below		

Average weight: The average weight was measured on each variety.

Preserving aspect: They were evaluated (rate and useful matter) on Cigale variety.

Boogie

This experimentation took place at Challan. It consists in the measurement of average weight of treated salads on control salad and of density of plant number per hectare, of average yield estimation and useful matter percentage on treated salads and on control salads. Parameters are as follow:

- control : fertilisation + phytosanitary protection
- treated plants : fertilisation + phytosanitary protection + HerbaGreen

Three repetitions were made, 10 salads were taken and analysed by repetitions.

	Planting	T1	T2
date	02/06/05	13/06/05	20/06/05
Products		HerbaGreen	HerbaGreen
Volume		1000l/ha	1000l/ha
dose kg/ha		1,5	1,5
Concentration %	0,00	0,15	0,15

Applications frequency

2 to 3 applications

T1: resumption of vegetation. Dose: 1 kg/ha

T2: T1 + 15 days. Dose: 1.5 kg/ha

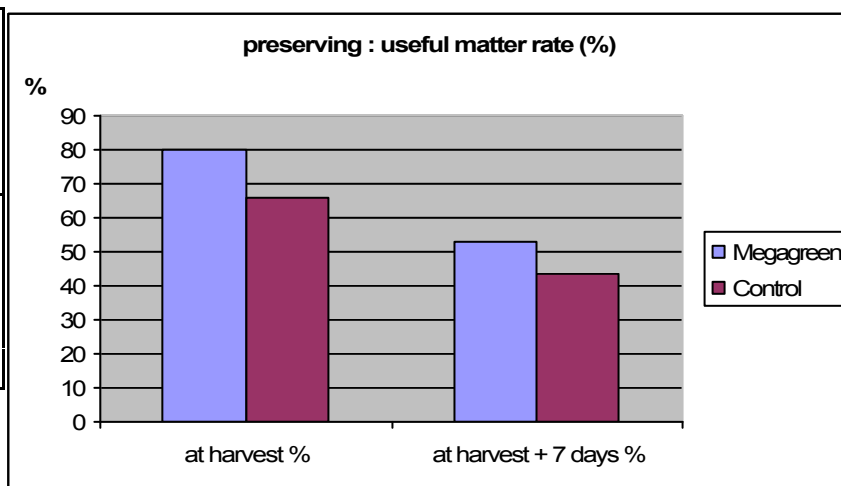
T3: T2 + 15 days. Dose: 1.5 kg/ha

Results

	Average weight of salads (g)				
varieties	<i>Atria</i>	<i>Oak leaf</i>	<i>Estelle</i>	<i>Loyale</i>	<i>Cigale</i>
Control	726	389	865	571	514
HerbaGreen	814	450	948	595	576
difference	88	61	83	24	62
Weight increase	12%	16%	10%	5%	12%

For each variety, HerbaGreen improve average weight of salads from 5 to 16 %

	Useful matter rate At harvest (%)	Useful matter rate 7 days after harvest
control	66	43
HerbaGreen	80	53
% increase	21	23



We note a bigger useful mater rate (21%) and better salad conservation (23%) after 7 days thanks to HerbaGreen.

Boogie variety

	Average weight (g)	Density number of plants	Average yield (t/ha)	Useful matter (%)
Control	571	90000	51,4	5,8
Treated	595	90000	53,6	6,1
%/control	4,2%		+ 2 T/ha	5,2%

HerbaGreen treatment allows an increase yield of 2 t/ha and an increase dry matter rate of 5.2 %.

	Control		HerbaGreen	
	Weight (g)	Useful matter (%)	Weight g	Useful matter (%)
salads				
1	638	5,82	781	5,55
2	731	5,51	693	6,43
3	789	6,07	570	6,34
4	558		890	
5	565		656	
6	629		832	
7	854		660	
8	670		700	
9	721		707	
10	700		661	
Total	6855	17,4	7150	18,32
average	685	5,8	715	6,1

With HerbaGreen useful matter rate increase of 4,37 %

Conclusion

HerbaGreen treatment allows:

- an increase of average yield
- an increase of dry matter rate

- bigger useful matter rate
- better salad conservation after 7 days

HerbaGreen test on struggle against tip burn of salad - SERAIL/ADABIO 2005

Material and methods

Experimentation location: St Blaise du Buis
Variety from Grenoble de Gautier, no coated seed

Seeding: 03rd May 2005

Full field planting: 26/05/05
Harvest: 12/07/05

Applications and treatments

Stage of salad	Date	HerbaGreen quantities
Seeding + 10 days	13th May	Dosage 5g/l eau Using 1 g to treat 81 salads
seeding + 7 days rabbit damage	2 nd June	Dose 5g/l eau Using 25 cl
seeding + 21 days	15th June	Dose 5g/l Using of 0.5 l

Results

One salad of HerbaGreen modality it is not much attacked, which reduced the global result. If this value is taken off, necrosis average is around 62 %. In spite of a reduction of tip burn symptoms with HerbaGreen, damages are still important (on a sensitive variety)

Repetition	HerbaGreen				control			
	Healthy leaves	Necrosed leaves	Totality	% necrosed	Healthy leaves	Necrosed leaves	Totality	% necrosed
1	17	33	50	66	7	32	39	82
2	9	36	45	80	9	29	38	76
3	22	11	33	33	12	30	42	71
4	12	30	42	71	8	21	29	72
5	17	23	40	58	7	34	41	83
6	13	33	46	72	6	29	35	83
7	21	2	23	9	7	36	43	84
8	15	29	44	66	8	36	44	82
9	15	23	38	61	13	39	52	75
10	23	23	46	50	8	33	41	80
	57				79			

The result which is presented in a percentage rate of necrosed leaves seems to support the positive influence of HerbaGreen. It can be noticed that a salad treated with HerbaGreen is less attacked, thus reducing the total result heavily. If one deducts this value, the medium necrosis climbs up to 62%. This value is still a good result.

Discussion

In spite of a symptoms reduction with HerbaGreen, damages are still important on a sensitive variety.

Conclusion

HerbaGreen can reduce tip burn on salad.

Experimentation on celery

Material and methods

Place: Haut Rhin

Harvesting date: 19/10/05

Protocol: 4 applications of HerbaGreen

Taking: 4x15 plants for each modality

Sanitary effect: nothing to report

Results

- Control parcel: 4 x 15 plants. Total weight: 65.50 kg
- Treated parcel: 4 x 15 plants. Total weight: 72.35 kg

Weight was put up by 10 %.



Conclusion

HerbaGreen allows a weight increase of celeriac.

Experimentation on cucumber

Material and methods

Place: “Jardins de Cocagne” (Cocagne Gardens) 25

Varieties: smooth cucumber + gherkin cucumber

Under tunnel. 6 lines = 240 m² (30x8)

3 control lines + 3 HerbaGreen lines (1.5 kg/ha)

Enrichment: N = 100 unities brought with guano containing 10 % N. No phosphor and potassium enrichment. Same enrichment for the whole cultivation.

Daily spraying for 5 to 10 minutes.

No optimized hygrometry: water contribution with punched pipe according to the ground humidity.

Cultivation calendar: 01/08/05: planting; 19/08/05: 1st HerbaGreen treatment (plant height: 40 cm); 31/08/05: 2nd HerbaGreen treatment. (Plant height: 120 cm) No 3rd treatment.

Results



On the left: 3 control lines; on the right: 3 treated lines

Observations: treatments had an important effect on the foliage colour and the foot height. Mildew attack at the beginning of September. Treated plants are less attacked. On 23/09/05, less affected leaves on the treated plants. No estimation of the yield was made.

Treated plants: on 23/09/05, there was a persistent flowering on the plants treated with HerbaGreen.



Conclusion

HerbaGreen increases foliage colour and foot height of cucumber. Treated plants are less attacked by mildew. There are less affected leaves and a longer flowering period on treated plants.

Experimentation on courgette

Material and methods

Place: **“Jardins de Cocagne” (Cocagne Gardens) 25, France**

On plastic: 2 rows = $2 \times 150 \times 0.8 = 240 \text{ m}^2$ (30x8)

One control row + one HerbaGreen row.

Enrichment: N = 100 unities/ha brought with guano containing 10 % of N. No enrichment of phosphor and potassium. Same enrichments on the whole cultivation.

No optimized hygrometry: water contribution with pipes according to ground humidity.

There were just two applications of HerbaGreen every 15 days, with a concentration of 1.5 kg/ha.

Results



On the left: treated rows with HerbaGreen; on the right: control line
Treatments have important effects on the colour of foliage.

On 23/09/05: date of shooting: treatment effect always very visible. No estimation of the yield was made.

Conclusion

HerbaGreen increases colour of foliage by improvement of photosynthetic process.

Experimentation on melon

Material and methods

Place: ANSOUIS (84), France

Producer programme

Producer programme + 3 HerbaGreen applications

1.5 Kg/ha at the resumption of the vegetation.

1.5 kg/ha at the beginning of the node formation

Notation:

Notation date: 12/07/05

On 5 places of 6 plants (4.5 m) – total 30 plants or 22.5 m

Number of melons per place.

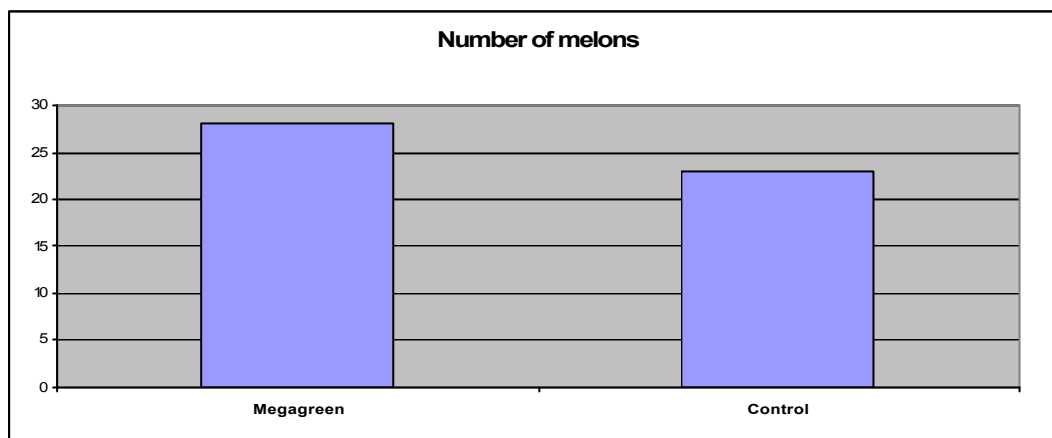
Minimum grade (mm)

Maximum grade (mm)

Results

Notation results n°1

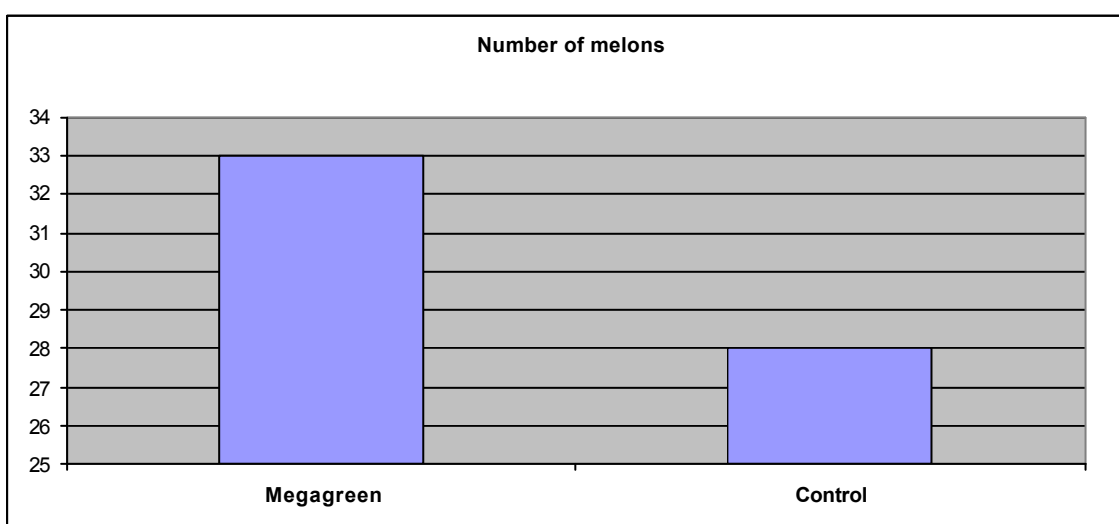
		Average	Standart deviation	IdC
Number of melon	Treated	28,60	4,980	6,183
	Control	22,40	2,608	3,238
Minimum grade	Treated	103,00	7,714	9,578
	Control	111,00	6,000	7,450
Maximum grade	Treated	139,00	6,964	8,647
	Control	138,20	8,871	11,015



We can observe a 22 % increase of the average number of melons.

Notation results n°2

		Average	Standart deviation	IdC
Number of melons	Treated	33,00	1,225	1,521
	control	29,00	3,240	4,023
Minimum grade	Treated	94,20	12,276	15,243
	control	91,60	6,229	7,734
Maximum grade	Treated	133,20	7,430	9,225
	control	132,20	4,970	6,171



We can observe a 12 % increase of the average number of melons

Conclusion

HerbaGreen treatment increases average number of melons

Experimentation in China

CUCUMBER

Summary

HERBAGREEN EXPERIMENTATION CHINA 2004/2005 – Cucumber

Truck farming station, Chendaoping's, North Zhanglou village, Heguan town, Qingzhou city,

Agrotype "SHAJIANG black soil" of medium fertility.

pH MO% Ca Mg K N P S B Cu Fe Mn Zn

7.6 1.0 2070 1258 86 14 11 12 0.47 2.5 8.0 6.3 4.5

3 modalities TNT control with fertilisation 100%, water fertilisation 100% and water foliar pulverisation, MGG fertilisation 50% and foliar pulverisation of HerbaGreen

6 applications of HerbaGreen every 14 days, 2kg/ha

For each experimentation 4 repetitions, random placement

HERBAGREEN EXPERIMENTATION CUCUMBER N°1

	Notations				Average	Yield	difference
	I	II	III	IV	Kg/20m2	Kg/667m2	%/TNT
TNT	159	154	164	161	159.0	5315.8	
MGG	187	191	188	182	187.0	6252.0	17.6
TW	161	165	162	161	162.3	5424.5	2.0

Yield increased of 17.6% in comparison with 100% fertilisation, and of 15.6% in comparison with "water" modality.

Average yield on control, for one hectare: 69.7 tonnes/ha

HERBAGREEN EXPERIMENTATION CUCUMBER N°2

	Notations				average	Yield	difference
	I	II	III	IV	Kg/20m2	Kg/667m2	%/TNT
TNT	170	165	175	172	170.5	5683.6	
MGG	205	215	211	209.8	209.8	6992.0	23.0
WT	172	174	167	179	173.0	5767.0	1.5

Yield increased of 23.0% in comparison with 100% fertilisation, and of 21.5% in

comparison with “water” modality.

Average yield on control, for one hectare: 88.2 tonnes/ha

Observations in fields showed an improvement of vegetal growth on HerbaGreen parcels and on leaves physiology. Cucumbers were more commercially interesting according to the visual and organoleptic qualities. Necessary irrigation level lowered a lot and parasitism reinforcement is improved.

Introduction

HerbaGreen nutrition is from the biologic mineral sediment in the benthal of the Mediterranean Sea, sanforized into foliar improving nutrition which should be sparged on the leaves of the crops. The product contains many kind of nutritious ingredients (please check table1), and it has visible impacts on increasing production of cucumber. To validate its useful effects, there are some practical experiments that have been taken in the vegetable field of Chendaoping's, North Zhanglou village, Heguan town, Qingzhou city during July to December 2004.

Material and methods

Experiment administrator: Shandong Province Fertilization Centre (with STAMP)

Functionary GAO, Ruijie (with signature)

Date: January 12th 2005

Table1: nutritious ingredients (%Unit)

Category	OM%	N%	P ₂ O ₃	K ₂ O	CaO	MgO	Na	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃
Proportion	2.91	0.18	0.03	0.528	43	1.65	0.112	8.56	0.65	1.16

Material

Fertilizer provided: HerbaGreen nutrition, Net Weight 165g/bag

Crop provided: Cucumber, which is the main vegetable specie locally.

Condition provided: agrotype is brown soil, with complanate hypsography, medium fertility, and same planting administration with other crops soil nutrition ingredients : Organic Material 1.57%, Nitrogen 0.94%, hydrolyzed Nitrogen 143.1ppm, Phosphorus 160.3ppm, Kalium 231ppm, PH=7.1

Methods

The experiment has been designed for 3 different treatments. 20 ? for every section, every treatment repeated 4 times, randomly arranged. In Chinese Measuring unit 667 ? means 1 'Mu', same as hereinafter :

Treatment 1 (TNT) : 100 % local chronic fertilization (Organic Fertilizer 400kg/Mu, Diammonium Phosphate (DAP) 20kg/Mu, 45% multiple manure in 25kg/Mu, Calcium Superphosphate 12kg/Mu) per unit as the Vacant Comparison (CK);

Treatment 2 (MGG): 50% local chronic fertilization plus HerbaGreen nutrition, to fertilise as 0.3% every 15 days, and 55 kg every time, 6 times in total.

Treatments 3(WT) : local chronic fertilization plus pure water, to fertilise every 15 days, and 55 kg every time, 6 times in total.

Experiment requirement

One person specially assigned in charge of the experiments, strictly according to the experimental scheme, 20 ? acreage in sections to arrange logically, protective rows around each section, with signs in between the sections, and to make into the map of collocation for the experimental field. To construct the profiles of the sections, with making detailed notes on the situation of the experiment, and the administration in the fields. As the experiment is going on, keep the other agricultural measurements, conditions and administration consistent in every treatment, except the fertilization.

Investigation method

- ✍ growing period investigation : To observe the growing situation of cucumbers in each section
- ✍ every section harvest output calculation: calculating the output as 667 m² (1 'Mu').
- ✍ Analysing Economic benefits: calculating and compare costs and income proportion.

Experimental results statistics

Fertilization efficiency:

Productivity increasing effects (%) = $\frac{\text{Treatment Section Result} - \text{Contrast Section Result}}{\text{Contrast Section Result}} \times 100$

Results

Experimentation 1

Effects of HerbaGreen nutrition on cucumber's Generational Period

The observation in fields has indicated that: the treatment with using HerbaGreen nutrition has hearty growing, large and plump leaves, and a fresh tender taste. The produced crops are thickset with high market value. On the other hand, the treatment with HerbaGreen nutrition led to the following effects: **need of irrigation has decreased distinctly, and its resistance for insects and diseases has enhanced, basically without using any pesticide, to save the producing costs.**

Effects of HerbaGreen nutrition on cucumber's yield

Please refer to table 2 for the actual harvest production, the results have indicated that: the treatment with HerbaGreen nutrition, the production has increased 23.0% more than the one as local chronic fertilization, and 21.5% higher than the treatment as local chronic fertilization plus pure water, which means that HerbaGreen nutrition has better effects on increasing cucumber's production.

Table 2: HerbaGreen's influence on cucumber production

Treatment	Sections	Average Value		Production	Percentage
?	?	?	?	KG/20 ? (kg/667 ?)	increase(%)
TNT	170 165 175 172	170.5	5683.6	--	
MGG	205 215 211 208	209.8	6992.0	23.0	
WT	172 174 167 179	173.0	5767.0	1.5	

Statistics Analysis of Variance for effects of HerbaGreen on cucumber production

Table 3 indicated that the difference between repeating procedures is not remarkable, but greatly obvious among the treatments.

Table 3: Statistics Analysis of Variance for effects of HerbaGreen on cucumber production

Variation elements	df	SS	MS	F	F _{0.05}	F _{0.01}
Alternation of Treatment	2	986.17	493.09	213.95	3.44	5.72
Alternation of Repetition	3	1.67	0.56	3.26	5.41	
Error	6	13.83	2.3			
Overall Variation	11	1001.67				

Economic Profit Analyse

Table 4 compares investment for HerbaGreen nutrition with the sales income of the products, it shows that income from cucumbers produced with HerbaGreen nutrition

increased ? 470/Mu more than the traditional fertilization, and ? 329/Mu higher than WT. Memo(prices): 45% Multiple Manure ? 2.8/kg, Diammonium Phosphate(DAP) ? 2.6/kg, Organic Fertilizer ? 0.6/kg, Calcium Superphosphate? 1.00/kg, HerbaGreen nutrition ? 300/kg. Cucumber ? 1.00/kg

Table 4: HerbaGreen's effect on cucumber production income

Treatment	Investment (RMB/Mu)			Output	Profit	Increase than CK
	45% Multiple HerbaGreen Soil			(RMB/Mu)	(RMB/Mu)	(RMB/Mu)
	Manure	Nutrition	TOTAL			
CK	140	-	140	3624.2	3484.2	--
TF	70	300	370	4359.3	3989.3	505.1
WT	140	-	140	3671.9	3531.9	47.7

Experimentation 2

Effects of HerbaGreen nutrition on cucumber's yield

Table 5: for actual harvest production, HerbaGreen allows an increased of 17.6% compared with local chronic fertilization. HerbaGreen allows too an increased of 15.6% compared with fertilization plus pure water, which means that HerbaGreen nutrition increases cucumber's production.

Table 5 : Influence of HerbaGreen on cucumber's production

Treatment	Sections				Average Value	Production	Increase
	?	?	?	?	(kg/20 ?)	(kg/667 ?)	(%)
CK	159	154	164	161	159.0	5315.8	--
TF	187	191	188	182	187.0	6252.0	17.6
WT	161	165	162	161	162.3	5424.5	2.0

Statistics Analysis of Variance for effects of HerbaGreen on cucumber production

Table 6 indicated that the difference between repeating procedures is not remarkable, but greatly obvious between the treatments.

Table 6: Statistics Analysis of Variance for effects of HerbaGreen on cucumber production

Variation elements	df	SS	MS	F	F _{0.05}	F _{0.01}
Alternation of Treatment	2	3863.16	1931.58	73.58	5.14	10.92
Alternation of Repeatment	3	24.25	8.08	0.31		

Error	6	157.50	26.25
Overall Variation	11	4044.91	

Economic Profit Analysis:

The investment for HerbaGreen nutrition with the sales income of the products (table 7) shows that the income from the cucumber's produced with HerbaGreen nutrition increased ? 824.0/Mu more than the traditional fertilization, and ? 715.0/Mu higher than WT. Memo(prices) : 45% Multiple Manure ? 2.8/kg, Diammonium Phosphate (DAP) ? 2.6/kg, Organic Fertilizer ? 0.6/kg, Calcium Superphosphate ? 1.00/kg, HerbaGreen nutrition ? 300/kg. Cucumber ? 1.00/kg

Table 7: HerbaGreen's effect on cucumber production income

Treatment	Investment (RMB/Mu)			Output	Profit	Increase than CK
45% Multiple	HerbaGreen	Soil		(RMB/Mu)	(RMB/Mu)	(RMB/Mu)
	Manure	Nutrition	Total			
CK	362	-	374	5315.0	4941.0	--
TF	187	300	487	6252.0	5765.0	824.0
WT	362	-	374	5424.0	5050.0	109.0

These data prove the economic advantage in using HerbaGreen in cucumber cultivation, while reducing the conventional soil fertilizer – 50%, in the present economic context of China.

The statistics shows that: HerbaGreen nutrition can promote cucumber's growth, increase its production and improve its quality. Thus it can be seen that HerbaGreen nutrition is a high-efficient and new foliar reinforced nutrition of plants, for a better marketing value.

There is a yield increased of 23.0% in comparison with 100% fertilisation, and of 21.5% in comparison with “water” modality.

Average yield on control, for one hectare is 88.2 tonnes/ha

Discussion

Observations in fields showed an improvement of vegetal growth on HerbaGreen parcels and on the physiology of the leaves. Commercially, cucumbers were more interesting according to the visual and organoleptic qualities. Necessary irrigation level lowered a lot and parasitism resistance is improved.

Conclusion

HerbaGreen treatments in this test cause an important yield increase of cucumber, with a high qualitative value.

LEEK

Summary

EXPERIMENTATION HERBAGREEN CHINA 2004/2005 - Leek							
Location	Truck farming station, Chendaoping's, North Zhanglou village, Heguan town, Qingzhou city,						
Ground	agrotype "SHAJIANG black soil" of medium fertility. pH OM% Ca Mg K N P S B Cu Fe Mn Zn 7.6 1.0 2070 1258 86 14 11 12 0.47 2.5 8.0 6.3 4.5						
Protocols	3 modalities TNT control with fertilisation 100%, water fertilisation 100% and water foliar fertilisation, MGG fertilisation 50% and HerbaGreen foliar pulverisations.						
Applications	6 applications of HerbaGreen every 14 days, 2kg/ha						
Experimentation types	For each experimentation 4 repetitions, random placement						
HERBAGREEN EXPERIMENTATION LEEK N°1							
	Notations				Average	Yield	difference
	I	II	III	IV	Kg/20m2	Kg/667m2	%/TNT
TNT	108	105	104	106	105.8	3525.2	
MGG	126	128	127	126	126.8	4225.2	19.9
WT	109	111	111	109	110.0	3666.9	4.0
Result experimentation n°1	Yield increased of 19.9 % in comparison with the 100 % fertilization and of 15.7 % with water modality. Average yield on the control for one hectar : 52.8 t/ha						
HERBAGREEN EXPERIMENTATION LEEK N°2							
	Notations				Average	Yield	difference
	I	II	III	IV	Kg/20m2	Kg/667m2	%/TNT
TNT	108	107	111	109	108.8	3624.2	
MGG	130	132	131	131	131.0	4359.3	20.2
WT	111	110	110	110	110.3	3671.9	4.8
Experimentation result n°2	Yield increased of 20 %, in comparison with the 100 % fertilisation and of 15.4 % in comparison with the water modality. Average yield on the control for one hectare: 54.3 %.						
	Observations in field showed an improvement for the HerbaGreen parcels and very interesting parameters. Necessary irrigation level lowered a lot and the parasitism resistance is improved.						

Introduction

HerbaGreen nutrition is from the biologic mineral sediment in the benthal of Mediterranean Sea, sanforized into foliar improving nutrition which should be sparged on the leaves of the crops. The product contains many kinds of nutritious ingredients (please check table1), and it has visible impacts on increasing production of cucumber. To validate its useful effects, there are some practical experiments that have been taken in the vegetable field of Chendaoping's, North Zhanglou village, Heguan town, Qingzhou city during July to December 2004.

Material and methods

Experiment administrator: Shandong Province Fertilization Centre (with STAMP)

Functionary GAO,Ruijie (with signature)

Date: January 12th 2005

Table1: nutritious ingredients (%Unit)

Category	OM%	N%	P ₂ O ₃	K ₂ O	CaO	MgO	Na	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃
Proportion	2.91	0.18	0.03	0.528	43	1.65	0.112	8.56	0.65	1.16

Material

Fertilizer provided: HerbaGreen nutrition, Net Weight 165g/bag

Crop provided: Leek, which is the main vegetable species locally.

Condition provided: The agrotpe is SHAJIANG black soil, with complanate hypsography, medium fertility, and same planting administration with other crops, please check table 2 for soil composition.

Table 2: Soil Ingredients of Experimental field in Hejia Village Units (mg/L)

pH	OM%	Ca	Mg	K	N	P	S	B	Cu	Fe	Mn	Zn
7.6	1.0	2070	1258	86	14	11	12	0.47	2.5	8.0	6.3	4.5

Methods

The experiment has been designed for 3 different treatments. 20 ? for every section, every treatment repeated 4 times, randomly arranged. In Chinese Measuring unit 667 ? means 1 'Mu', same as hereinafter :

Treatment 1 (TNT) : 100 % local chronic fertilization (Organic Fertilizer 400kg/Mu, Diammonium Phosphate (DAP) 20kg/Mu, 45% multiple manure in 25kg/Mu, Calcium Superphosphate 12kg/Mu) per unit as the Vacant Comparison (CK);

Treatment 2 (MGG): 50% local chronic fertilization plus HerbaGreen nutrition, to fertilise as 0.3% every 15 days, and 55 kg every time, 6 times in total.

Treatments 3(WT) : local chronic fertilization plus pure water, to fertilise every 15 days, and 55 kg every time, 6 times in total.

Experiment requirement

One person specially assigned for being in charge of the experiments, strictly according to the experimental scheme, 20 ? acreage in sections to arrange logically, protective rows around each section, with signs in between the sections, and to make into the map of collocation for the experimental field. To construct the profiles of the sections, with making detailed notes on the situation of the experiment, and the administration in the fields. As the experiment is going on, keep the other agricultural measurements, conditions and administration consistent in every treatment, except the fertilization.

Investigation method

- ✍ growing period investigation : To observe the growing situation of leek in each section
- ✍ every section harvest output calculation: calculating the output as 667 m² (1 'Mu').
- ✍ Analysing Economic benefits: calculating and compare costs and income proportion.

Experimental results statistics

Fertilization efficiency:

Productivity increasing effects (%) = (Treatment Section Result – Contrast Section Result) X 100/ Contrast Section Result

Results

Experimentation 1

Effects of HerbaGreen nutrition on leek's Generational Period:

The observation in the fields has indicated that: the treatment when using HerbaGreen nutrition, has hearty growing, large and plump leaves, and a fresh and tender taste. The produced crops are thickset with high market value. On the other hand, the treatment with HerbaGreen nutrition, its need of irrigation has decreased

distinctly, and its resistibility for insects and diseases has enhanced, basically without using any pesticide, to save producing costs.

The effects of HerbaGreen nutrition on leek's yield:

Please refer to table 3 for the actual harvest production, the results have indicated that: the treatment with HerbaGreen, the production has increased 20.2% more than the one as local chronic fertilization, and 15.4% higher than the treatment as local chronic fertilization plus pure water, which means that HerbaGreen has better effects on increasing leek's production.

Table 3: HerbaGreen influence on leek's production

Treatment	Sections	Average Value	Production per Mu	increase
?	?	?	?	?
		(kg/20 ?)	(kg/667 ?)	%
CK	108 107 111 109	108.8	3624.2	--
TF	130 132 131 131	131.0	4359.3	20.2
WT	111 110 110 110	110.3	3671.9	4.8

Statistics Analysis of Variance for effects of HerbaGreen on leek production

Table 4 is the Statistics Analysis of Variance for the effects of HerbaGreen nutrition on leek's production, the results has also indicated that the difference between repeating procedures is not remarkable, but greatly obvious among the treatments.

Table 4: Statistics Analysis of Variance effects of HerbaGreen on leek production

Variation elements	df	SS	MS	F	F0.05	F0.01
Alternation of Treatment	2	986.17	493.09	213.95	3.44	5.72
Alternation of Repeatment	3	1.67	0.56		3.26	5.41
Error	6	13.83	2.3			
Overall Variation	11	1001.67				

Economic Profit Analyse

The following Economic Profit Analysis was communicated by the experimental station:

HerbaGreen nutrition with the sales income of the products shows that the income from the leek's produced with HerbaGreen nutrition increased ? 505.1/Mu more than the traditional fertilization, and ? 457.4/Mu higher than WT.

Table 5: HerbaGreen's effect on production income

Treatment	Investment (RMB/Mu)			Output	Profit	Increase than CK
	45% Multiple HerbaGreen Soil			(RMB/Mu)	(RMB/Mu)	(RMB/Mu)
	Manure	Nutrition	TOTAL			
CK	140	-	140	3624.2	3484.2	--
TF	70	300	370	4359.3	3989.3	505.1
WT	140	-	140	3671.9	3531.9	47.7

Memo(prices): 45% Multiple Manure ? 2.8/kg, HerbaGreen nutrition 300/kg. Leek ? 1.00/kg.

The statistics show that: HerbaGreen nutrition can promote the growth of leek, increase its production and improve its quality. Thus it can be seen that HerbaGreen nutrition is a high-efficient and new foliar reinforced nutrition for plants, which leads to a better marketing value.

experimentation 2

The effects of HerbaGreen nutrition on leek's Generational Period

The observation in fields has indicated that: the treatment when using HerbaGreen nutrition, has hearty growing, large and plump leaves, and fresh tender taste. The produced crops are thickset with high market value. On the other hand, the treatment with HerbaGreen nutrition, its need of irrigation has decreased distinctly, and its resistibility against insects and diseases has enhanced, basically without using any pesticide, to save producing costs.

The effects of HerbaGreen nutrition on leek's yield

Please refer to table 1 for the actual harvest production, the results have indicated that: the treatment with HerbaGreen nutrition, the production has increased 19.9% more than the one as local chronic fertilization, and 15% higher than the treatment as local chronic fertilization plus pure water, which means that HerbaGreen nutrition has better effects on increasing the leek production.

Table 1: HerbaGreen's influence on leek production

Treatment	Sections				Average Value	Production	increase
	?	?	?	?	KG/20 ?	(kg/667 ?)	(%)
CK	108	105	104	106	105.8	3525.2	--
TF	126	128	127	126	126.8	4225.2	19.9
WT	109	111	111	109	110.0	3666.9	4.0

Statistics Analysis of Variance for effects of HerbaGreen on leek production

Table 2 is the statistics Analyses of HerbaGreen's nutrition effects on leek harvest production, the results also indicated that the difference between repeating procedures is not remarkable, but greatly obvious during the treatments.

Table 2: Statistics Analysis of Variance effects of HerbaGreen on leek production

Variation elements	df	SS	MS	F	F _{0.05}	F _{0.01}
Alternation of Treatment	2	986.17	493.09	213.95	3.44	5.72
Alternation of Repetition	3	1.67	0.56		3.26	5.41
Error	6	13.83	2.3			
Overall Variation	11	1001.67				

5.3 Economic Profit Analyse

Please check table 3 to compare the investment for HerbaGreen nutrition with the sales income of the products. It shows that the income from the leek's produced with HerbaGreen nutrition increased ? 470/Mu more than the traditional fertilization, and ? 329/Mu higher than WT.

Table 3: HerbaGreen effect on production income.

Treatment	Investment (RMB/Mu)			Output	Profit	Increase than CK
	45% Multiple	HerbaGreen Soil		(RMB/Mu)	(RMB/Mu)	(RMB/Mu)
	Manure	Nutrition	Total			
CK	140	-	140	3525.2	3385.2	--
TF	70	300	370	4225.2	3855.2	470.0
WT	140	-	140	3666.9	3526.9	141.7

Memo(prices): 45% Multiple Manure ? 2.8/kg, HerbaGreen nutrition ? 300/kg. Leek ? 1.00/kg

The statistics show that: HerbaGreen nutrition can promote the growth of leek, increase its production and improve its quality. Thus it can be seen that HerbaGreen nutrition is a high-efficient and new foliar reinforced nutrition for plants, leading to a better marketing value.

Yield increased by 20 %, in comparison with the 100 % fertilisation and by 15.4 % in comparison with the water modality. Average yield on the control for one hectare is 54.3 %.

Discussion

Observations in field showed an improvement for the HerbaGreen parcels and very interesting parameters. Necessary irrigation level lowered a lot and the parasitism resistance is improved.

Conclusion

HerbaGreen treatments allow to get an important yield increase of leeks, with a high qualitative value.

5. USE IN VITICULTURE

Introduction

The 3 main objectives of the experimentations in viticulture are:

1. to validate laboratory and field experimentation results about the activation of cellular metabolism of vine leaves
2. to validate interests of HerbaGreen pulverisations on oenological qualities of the harvest in the field
3. to validate the practical applications of HerbaGreen with a dose of 1 to 2 kg/ha with different type of sprays, different volumes of water and different blends according to the agronomic practices.

- reinforcement of the vegetal
- better resistance to the hydrous stress
- improvement of the wine harvest quality
- better efficiency of the photosynthesis

Methods and material

Saint Emilion, on Merlot and Cabernet Francs and Sauvignon

The treated rows were adjacent to the control rows to limit the interactions due to the medium and analysis of maturity in laboratory.*

Different parameters were measured to evaluate the impact of HerbaGreen on quality: total polyphenol index (TPI), sugar rate, weight of berries, alcoholic degree, total acidity, pH, assimilable nitrogen, anthocyan rate and acid malic rate.

To test HerbaGreen reinforcement of the plant, 2 treatments were made, at 1.5 kg

To test the impact of HerbaGreen on quality and vegetative aspect, 2 to 3 treatments were made, at different states:

- separated grapes
- node formation
- grapes closing

CAVES COLLI BERICI, Soc. Coop. Arl, in Lonigo, ITALY

HerbaGreen's effect on the optimization of the yield was evaluated.

Use of HerbaGreen with the dose of 7 kg/ha according to the application protocol:

2 kg/ha before the flowering

2.5 kg/ha grapes closing

2.5 kg/ha colour changing

Tests were made on the parcels:

- n° 3 irrigated hill, P. Grigio type vine
- n°4 irrigated hill, Chardonnay type vine
- n°5 irrigated hill, Cabernet Sauvignon

With a control on each treated parcel.

Experimentation Vallée du Rhône (granges gontardes) 2004

Different parameters were measured:

- grapes weight
- sugar rate
- total acidity (H₂SO₄)
- pH
- % alcohol

? Grafted Grenache on sets planted in 1968

? Gravel ground with rolled pebble .pH 6.7

? AOC (label guaranteeing the quality of wine), Tricastin hill

? Pulverisation:

- 18/06/04 500g
- 05/07/04 500g
- 29/07/04 500g
- 25/08/04 1.5 kg

Sugar rate determination was made thanks to 10 takings with a manual spectrometer and the determination of the production thanks to a taking on 400 m² per parcel.

Experimentation Vallée du Rhône (granges gontardes) 2005

Different parameters were measured:

- grapes weight
- sugar rate
- total acidity (H₂SO₄)
- pH
- % alcohol

- ? Grafted Grenache on sets planted in 1968
- ? Gravel ground with rolled pebble.pH 6.7
- ? AOC (label guaranteeing the quality of wine), Tricastin hill
- ? Pulverisation:
 - 20/05/05 500g
 - 18/06/05 500g
 - 11/08/05 1.5 kg (rain in the day)
 - 18/08/05 1.5 kg

Experimentation on Syrah, 2005

Magnesium and calcium concentrations were measured in the control leaves and treated plants. This analysis was made to check calcium and magnesium contribution in the plant.

Efficiency of HerbaGreen on the vegetative development of young vine stock.

It consists in the observation of the control roots and treated vine stocks.

In top of the right, at the bottom: plants which got 3 applications of HerbaGreen, 2 kg/ha; young vine stocks, St Emilion, France, 2005



Effect of HerbaGreen on wood weight

Wood weight of vine stocks was measured to evaluate HerbaGreen's effect on the wood.

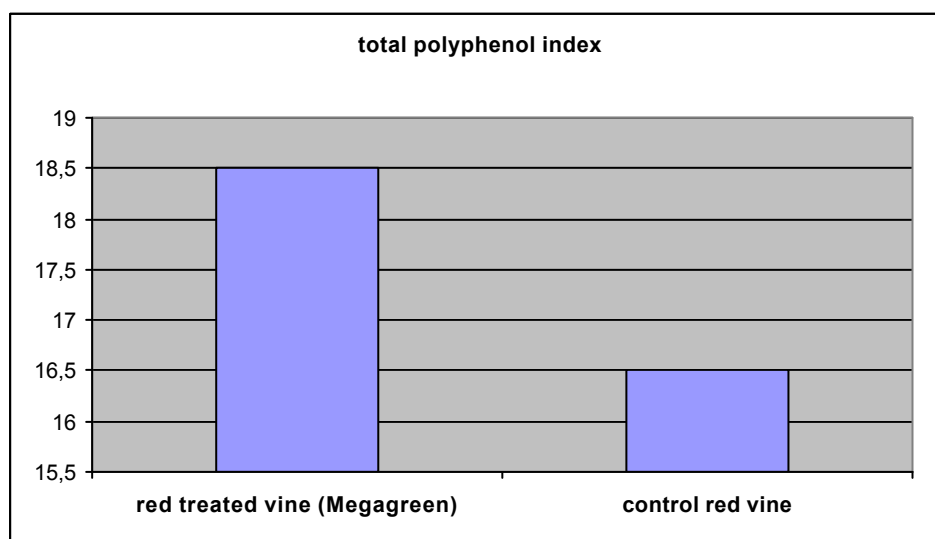
3 applications of 2 kg every 14 days from flowering separated buds stadium to nouaison.

Results

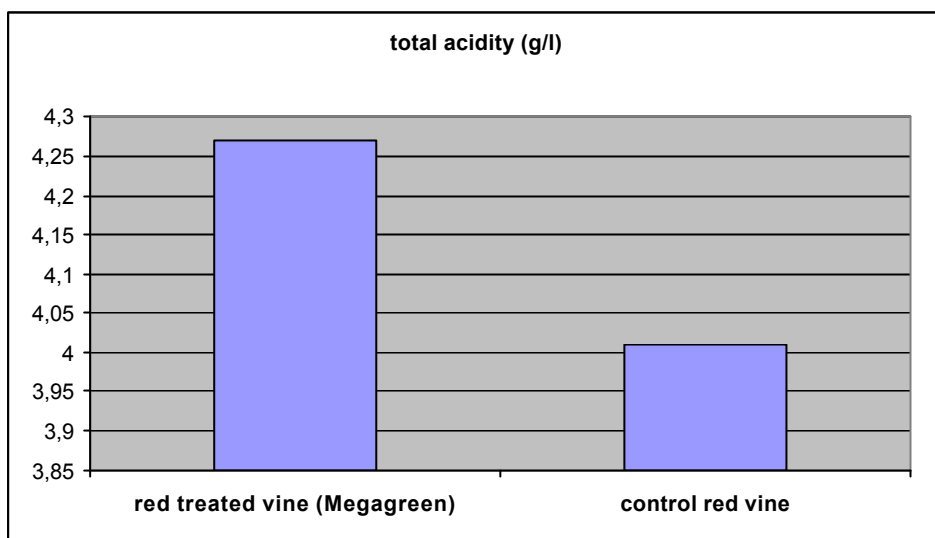
SAINT EMILION, on Merlot

Parameters	Measurement unity	Treated red vine 2005	Control red vine 2005
TPI		18,5	16,5
Total acidity	g/l	4,27	4,01
Sugar	g/l	214	212
pH		3,23	3,27
Assimilable nitrogen	mg/l	122	110
Berries weight	g per 100 berries	173	150
Anthocyanes		205	228
Potential degree	% vol	12,59	12,47
Malic acid	g/l	1,7	1,7

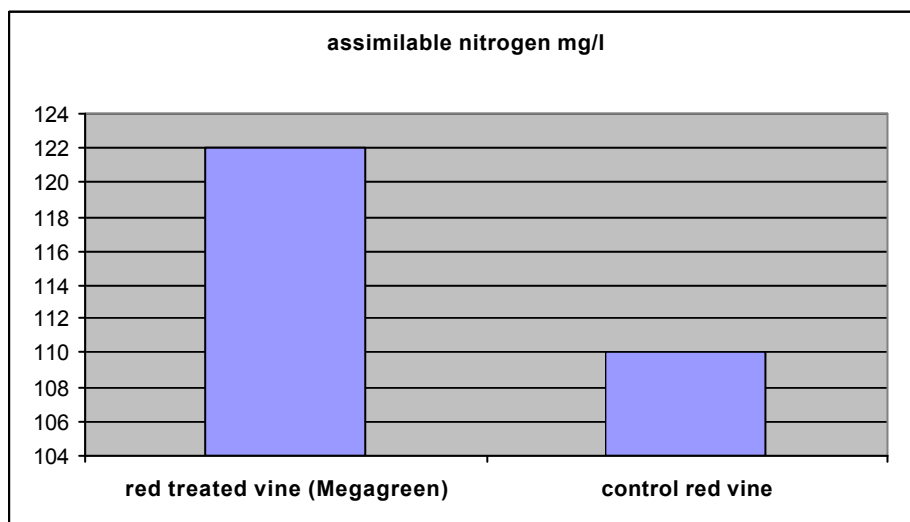
Total polyphenol index



We can notice an increase of polyphenol and anthocyanes rate in the treated parcels which can improve the tannin structure and a better extraction of the colour at the time of wine making



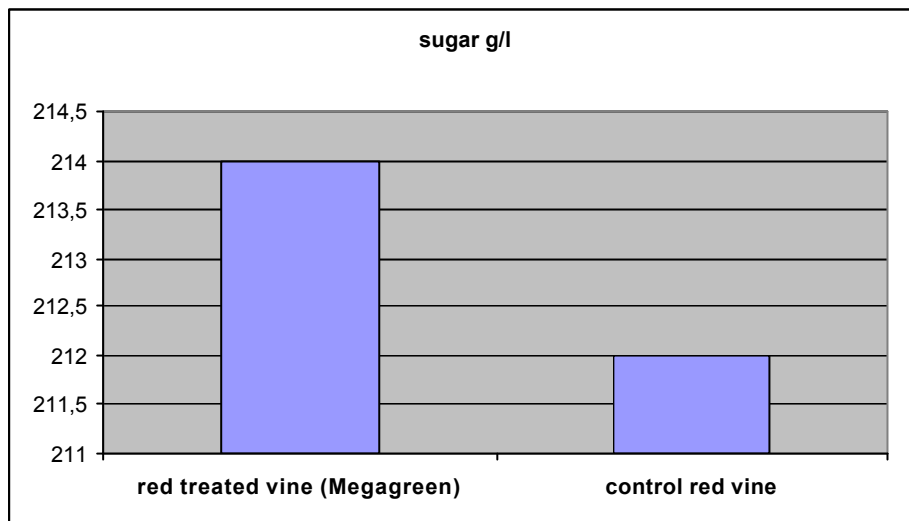
Results show a better total acidity rate in the treated parcels.
This element is important because of the high temperatures in the year 2005.
HerbaGreen applications allows to get a better pH.
pH brings a better freshness to the wine and dynamics.
Acidity guarantees a better stability of the colour.



We notice that there is more assimilable nitrogen in the parcels which are treated with HerbaGreen.
Nitrogen is the essential nutritive substance for bacteria and yeasts.
The presence of assimilable nitrogen is very important for the success of wine making.

Improvement of assimilable nitrogen is important for vine with grass because of the competition between grass and vine.

Sugar rate

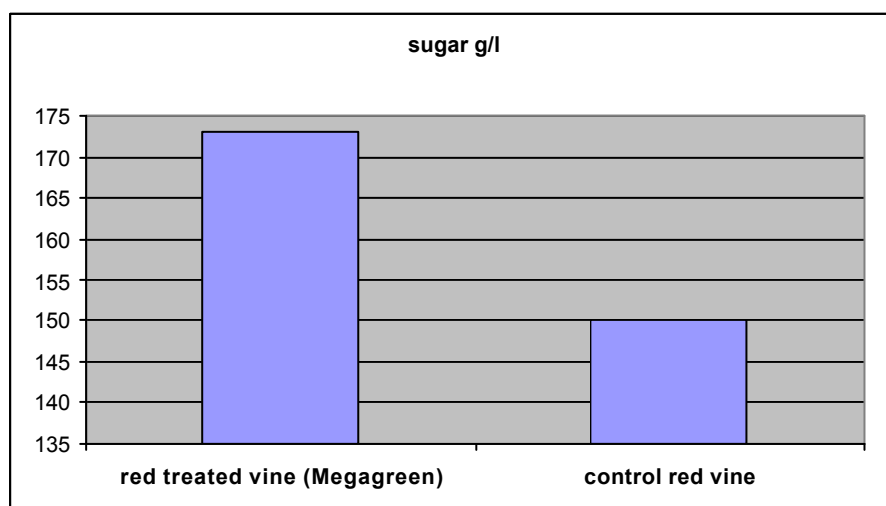


Sugars come from the photosynthesis.

- directly from the synthesised saccharose in the herbaceous parts of the plant, especially in the leaves
- indirectly from the starch and in this case it is put in reserve when the plant does not make sugar any more that it does not consume anymore and migrates it to the fruits.

HerbaGreen applications allow to increase the sugar rate without extending the vegetative cycle and to improve the gustative qualities.

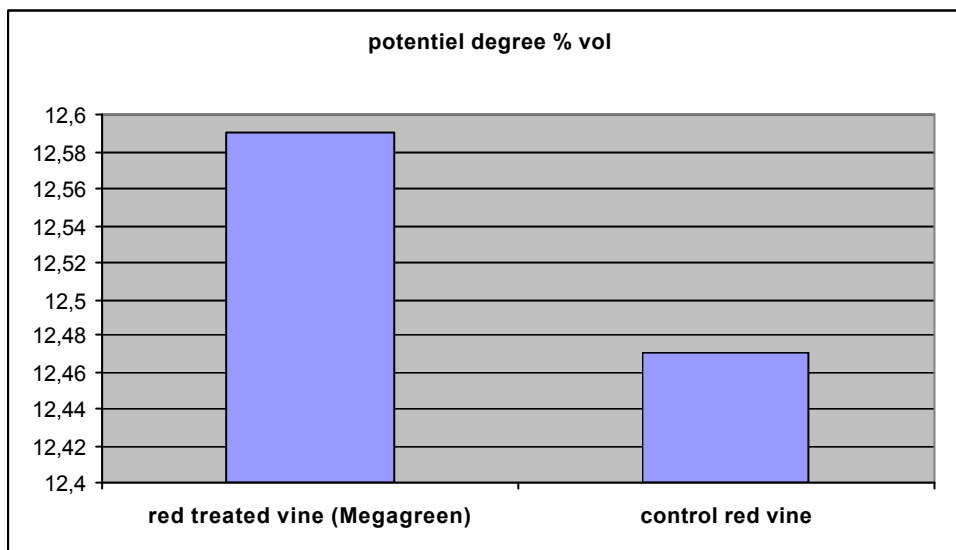
Equilibrium between sugar/alcohol and the important concentration of the sugar are important for the quality.



Weight of the berries

Increase of weight of the berries with HerbaGreen by optimizing the whole gustative and qualitative elements for the wine making.

Alcoholic degree



Analyses results show a better alcoholic degree of the vine

The treated parcels, for all the tests made in 2004 and 2005 in France and in Italy. Directly bound to the sugar and alcohol rate contributes to qualitative of a wine increasing its natural flavour of the other components and increasing the natural viscosity of the wine.

Other analyses results on vine stocks taken on different type of grounds:

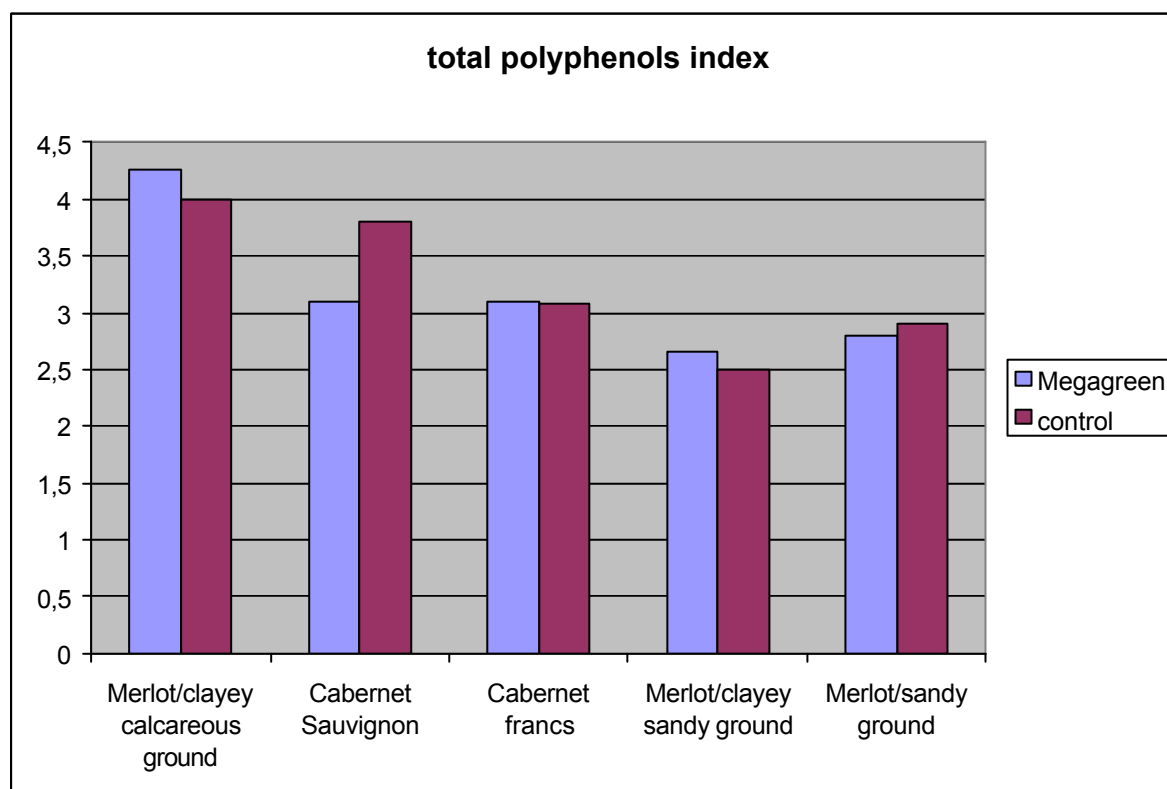
Comparatives results

Analyses averages (Results details are presented in the appendix 1)

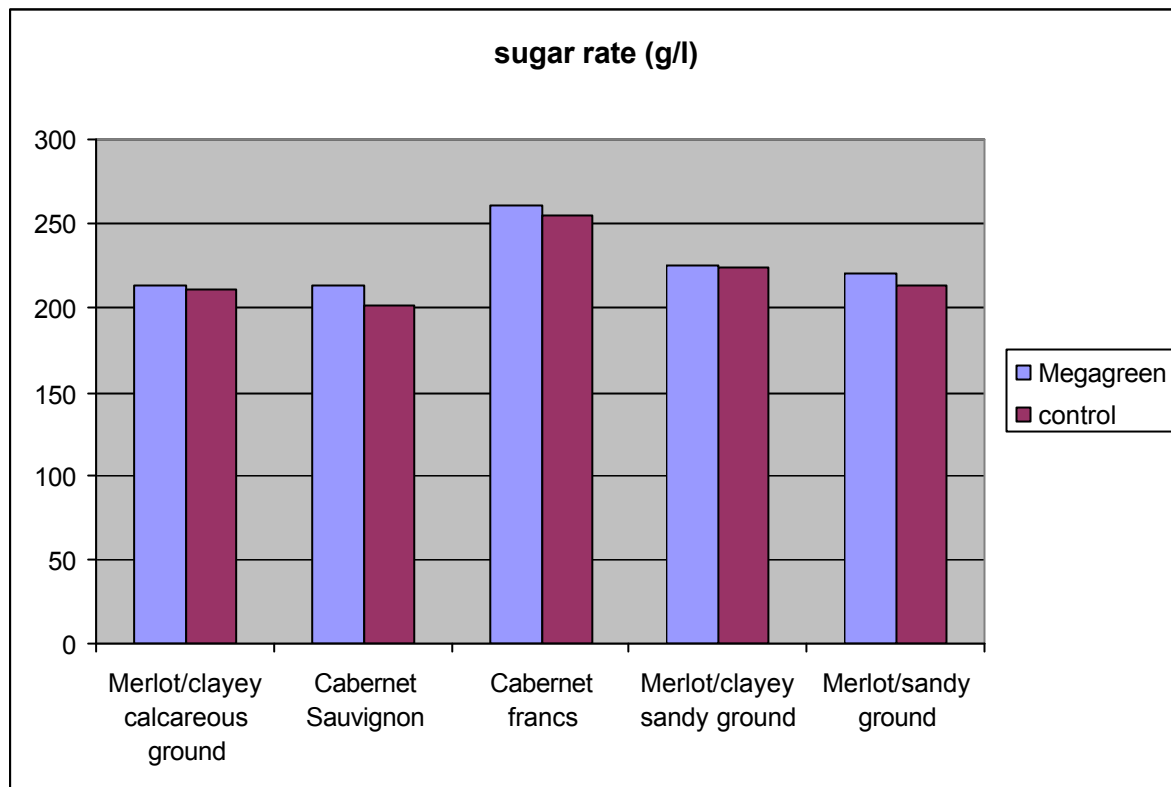
3 applications of 2 kg/ha every 15 days; applications are made at flowering stadium, nouaison and grapes closing.

Parameters	Measurement unity	Treated red vine 2005	Control red vine 2005
Total weight index		20.1	18.2
Total acidity	g/l	3.2	3.2
Sugar	g/l	226.8	221.4
Berries weight	g/100 berries	147.20	142.40
Anthocyanes		214.8	191.4
Potential degree	% vol	13.34	13.02

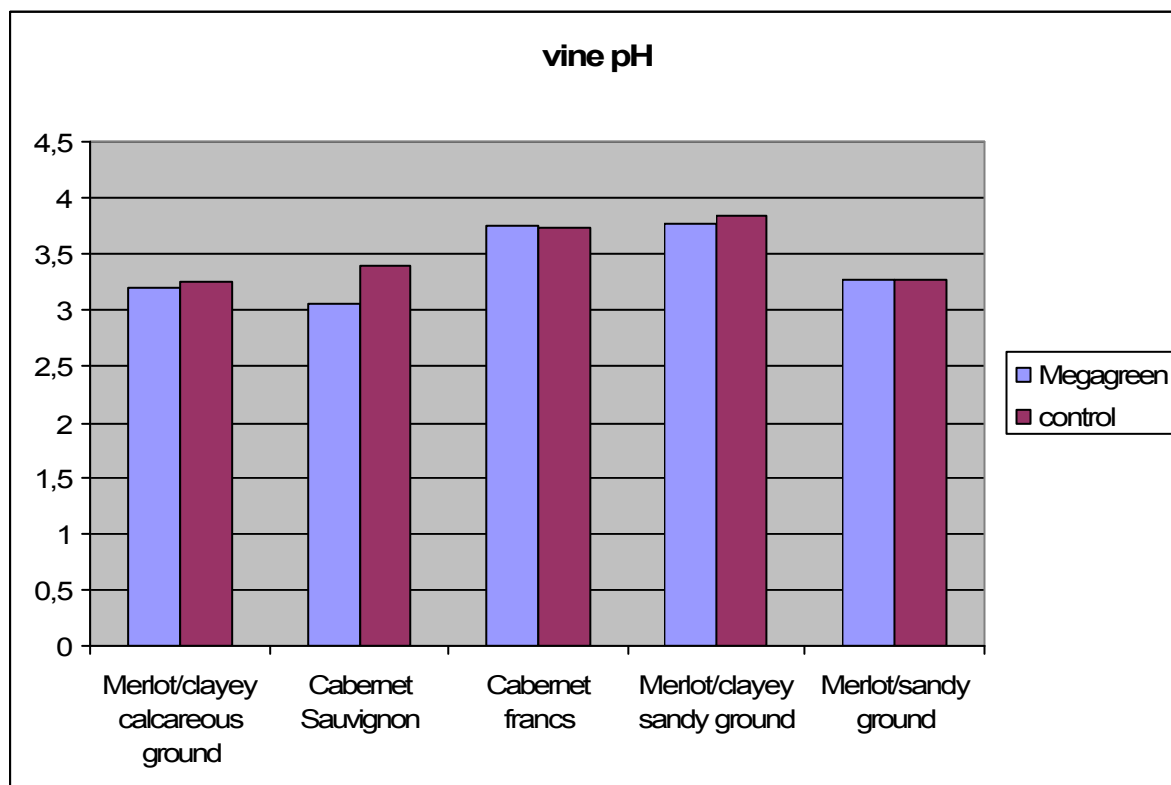
Total polyphenols index



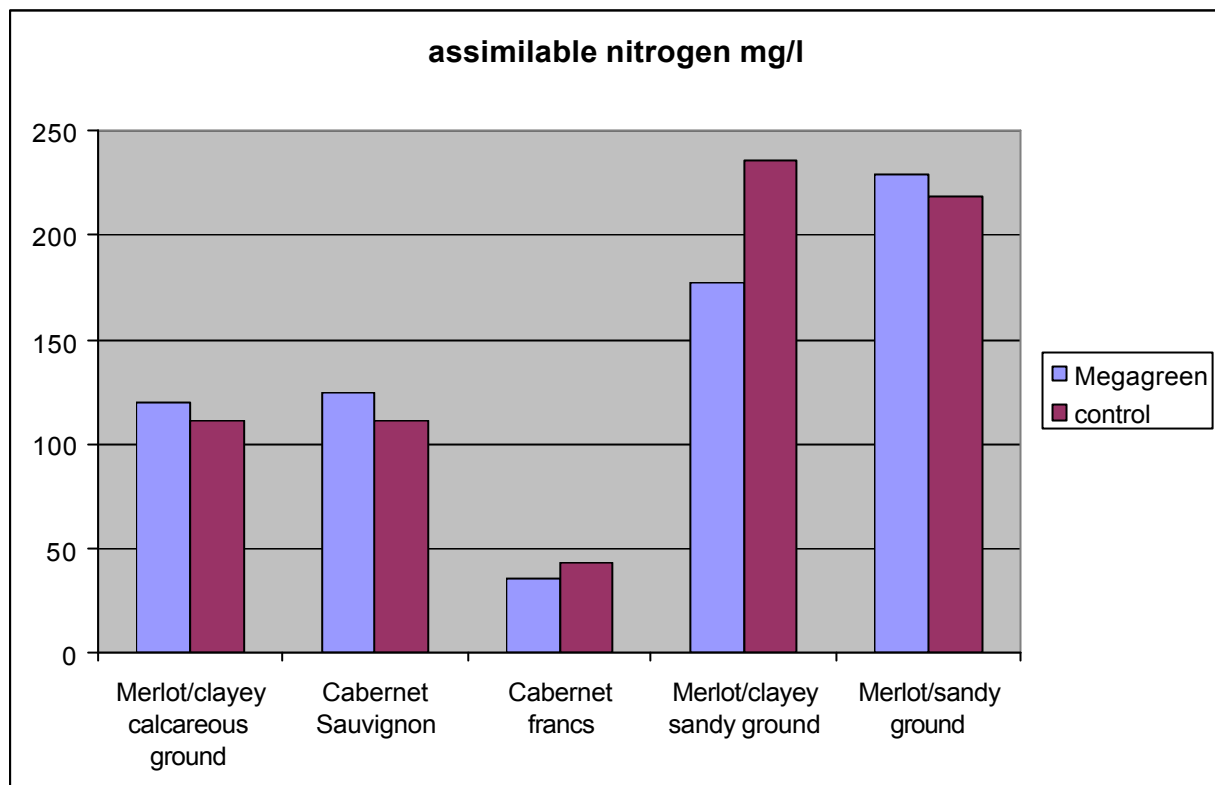
Sugar rate



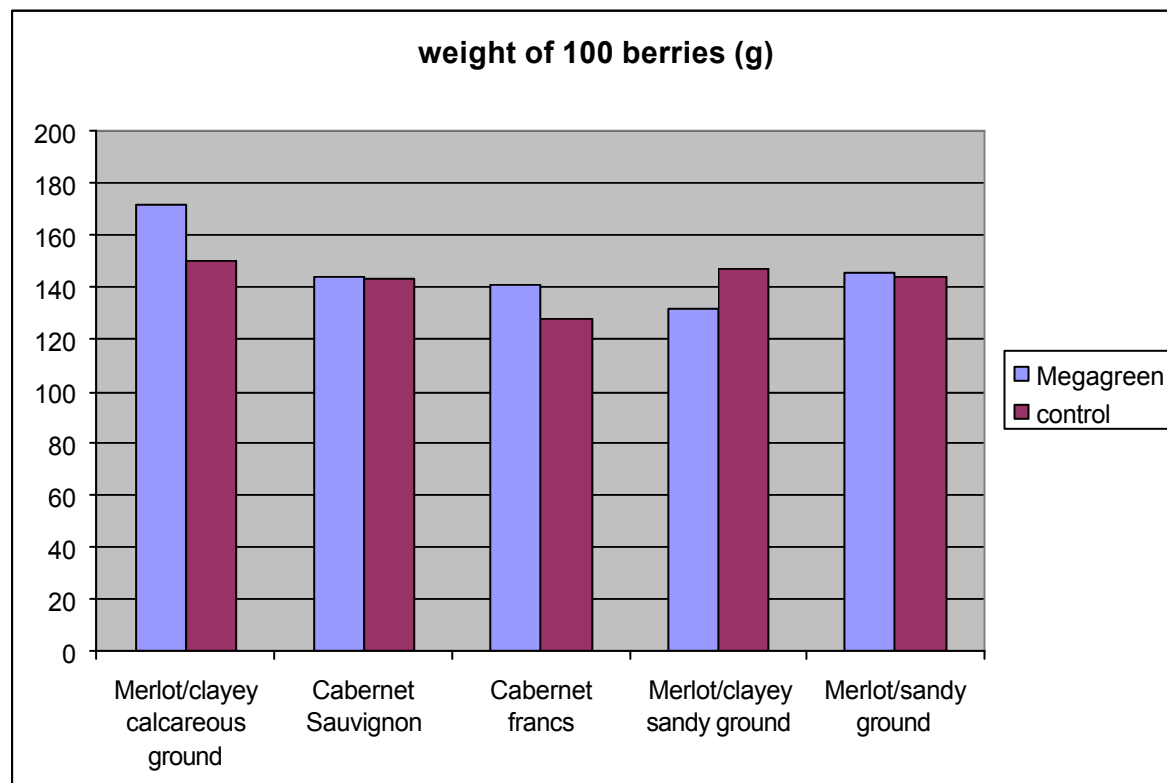
pH



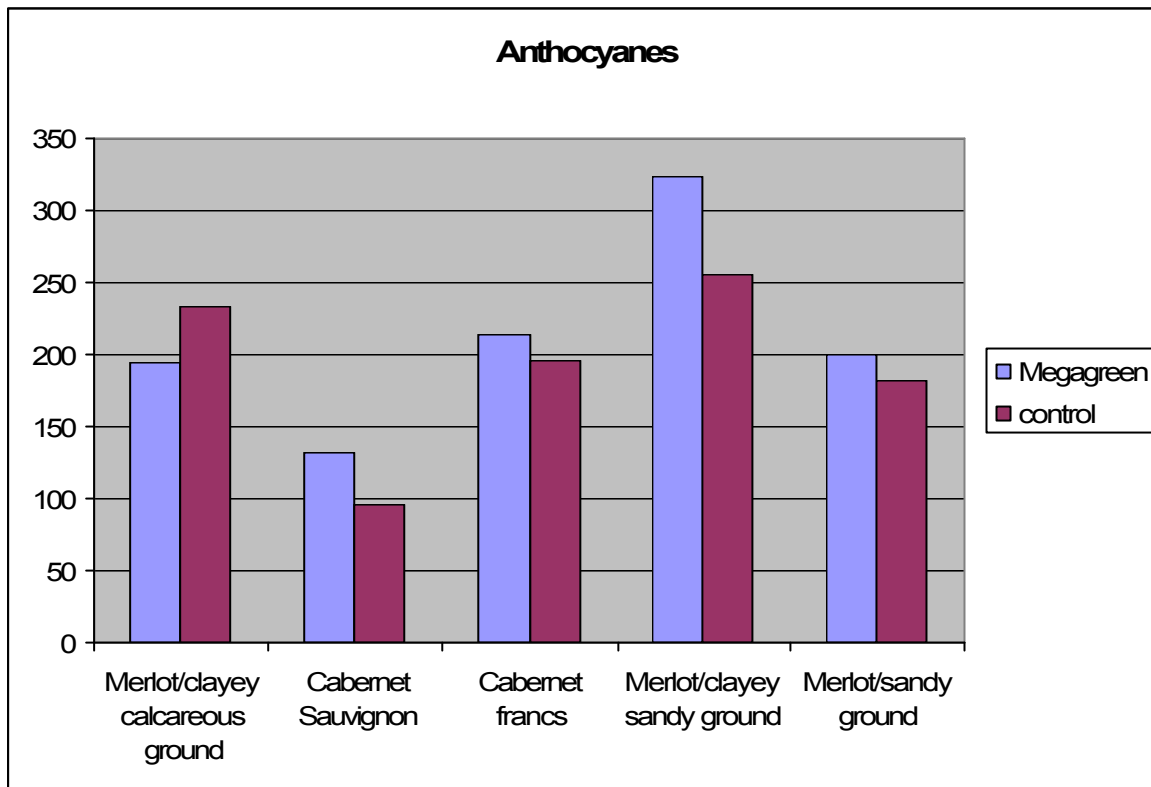
Assimilable nitrogen



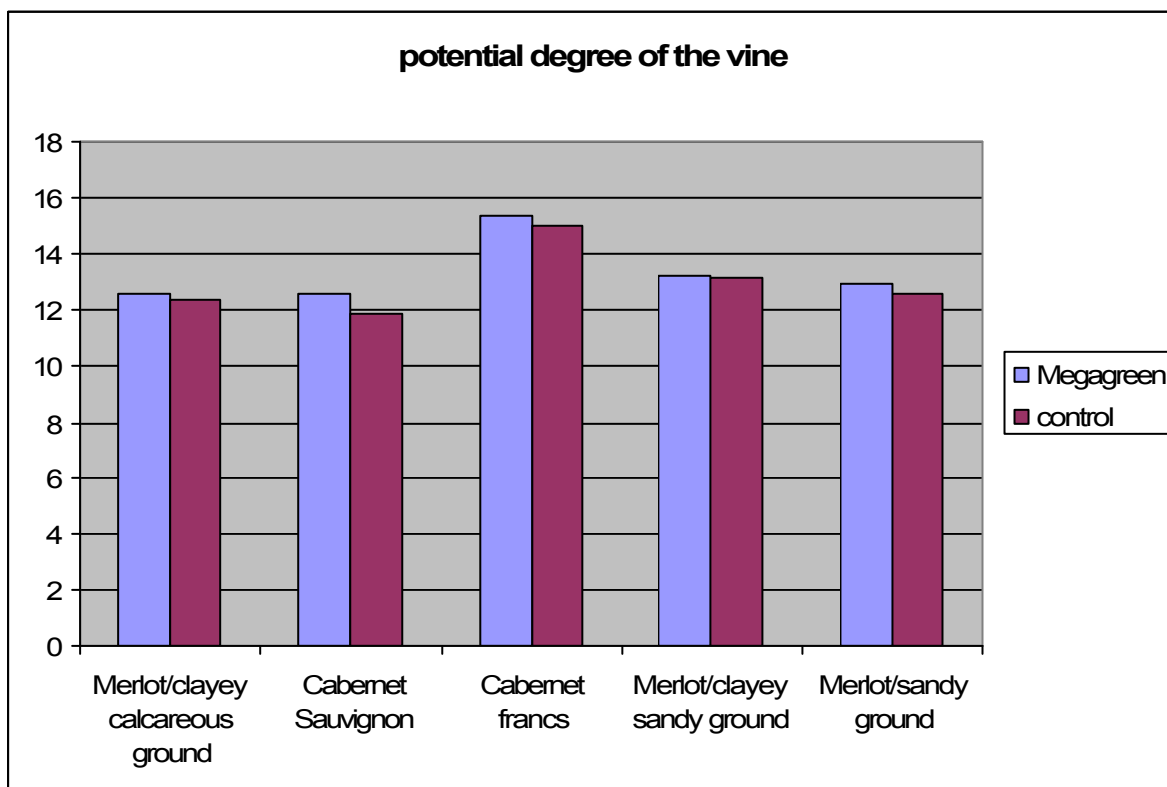
Weight of 100 berries



Anthocyanes



Potential degree



On each type of grounds and for each type of vine, we can notice an increase of:

- ITP rate
- Total acidity
- Sugar rate
- pH
- assimilable nitrogen
- anthocyanes
- potential degree
- berries weight
- acid malic

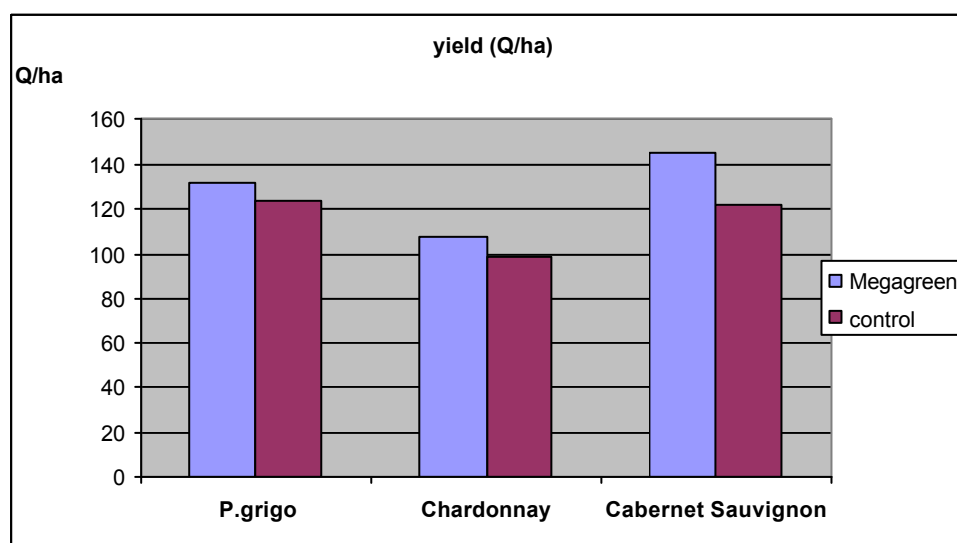
RESULTS CAVES COLLI BERICI

A first evaluation data shows the data about the parcels 3-4-5 and the control.

Parcel	Production Qx/ha	Sugar rate (%)
3 P. Grigio	132	17.20
Control	124	17.10
4 Chardonnay	108	20.10
Control	99	19.60
5 Cabernet Sauvignon	145	18.20
Control	122	18.10

According to those results, we can see that HerbaGreen treatment allows a yield increase and a sugar rate increase.

Yield (Qx/ha):



The vegetative development on the treated parcels shows a significant increase of the branches' length and the total foliar surface.

Results Vallée du Rhône (granges Gontardes) 2004

	Grape weight	Sugar g/l	Total acidity g H ₂ SO ₄ /l	pH	Probable alcohol Must % Vol
G68 east Control 4 rows	450	206.8	3.4	3.41	12.29
G68 west Treated 4 rows	445	216.3	3.5	3.46	12.85

Results Experimentation Vallée du Rhône (granges gontardes) 2005

	Grape weight	Sugar g/l	Total acidity g H ₂ SO ₄ /l	pH	Probable alcohol Must % Vol
G68 east Control 4 rows	405	246.3	3.60	3.49	14.63
G68 west Treated 4 rows	400	249.5	3.80	3.45	14.82

HerbaGreen treatment allows an increase of sugar rate, of total acidity, of pH and of the potential alcohol degree.

Results on Syrah

	Control	Treated HerbaGreen
Magnesium	0.50	0.60
Calcium	2.51	2.88

Samples taken at colour change stadium
Analyses seem to confirm calcium and magnesium contribution to the plant.
There are just punctual analyses; results must be taken as a tendency but not as a quantitative indicator.

Results of efficiency of HerbaGreen on the vegetative development of young vine stock.

Better photosynthesis and development of plants treated with HerbaGreen.

On the roots:



On each picture on the left: control structure of the roots
On the right: structure of the roots treated with HerbaGreen

Plants treated with HerbaGreen have a more important development of the roots for a better implanting at the time of the plantation and at the time of the co plantations.



Results on wood weight

Wood weight of 10 vine stocks

Control: 2.46 kg

Treated with HerbaGreen: 4.08 kg

HerbaGreen applications increase the wood weight by a better synthesis of starch, for a better ripening and wood maturity of 65 %.

If the starch concentration in wood is really superior, an interesting effect on the gel at the time of a rough winter would be confirmed.



Discussion

✍ According to the results, we can notice an increase of polyphenol and anthocyanes rate in the treated parcels which can improve the tannin structure and a better extraction of the colour at the time of wine making

✍ Results show a better total acidity rate in the treated parcels. HerbaGreen applications allow to get a better pH, which brings a better freshness to the wine and dynamics. Moreover, acidity guarantees a better stability of the colour.

✍ We notice that there is more assimilable nitrogen in the parcels which are treated with HerbaGreen. Nitrogen is the essential nutritive substance for bacteria and yeasts; so its presence is very important for the success of wine making and for vine with grass because of the competition between grass and vine.

✍ HerbaGreen applications allow to increase the sugar rate without extending the vegetative cycle and to improve the gustative qualities. Sugars come from the photosynthesis :

- directly from the synthesised saccharose in the herbaceous parts of the plant, especially in the leaves
- indirectly from the starch and in this case it is put in reserve when the plant does not make sugar any more that it does not consume anymore and migrate to the fruits.

As the sugar rate increases, alcohol potential degree will increase too; equilibrium between sugar/alcohol and the important concentration of the sugar are important for the quality.

Directly bound to the sugar and alcohol rate contributes to qualitative of a wine increasing its natural flavour of the other components and increasing the natural viscosity of the wine.

✍ The vegetative development on the treated parcels with HerbaGreen shows a significant increase of the branches length and the total foliar surface. Moreover, we can notice an increase of the yield. Increase of weight of the berries with HerbaGreen by optimizing the whole gustative and qualitative elements for the wine making.

Analyses seem to confirm calcium and magnesium contribution to the plant.

✍ Plants treated with HerbaGreen have a more important development of the roots for a better implanting at the time of the plantation and at the time of the co plantations. HerbaGreen applications increase the wood weight by a better synthesis of starch, for a better ripening and wood maturity of 65 %. If the starch concentration in wood is really superior, an interesting effect on the gel at the time of a rough winter would be confirmed.

Conclusion

According to the results, HerbaGreen treatment induces:

- a significant difference for the total weight index
- a significant difference for the anthocyanes
- a significant difference for berries weight
- a significant difference for sugar rent
- a significant difference for potential degree
- a good maintenance of the pH
- an equivalent level in total acidity

✍ HerbaGreen applications allow an optimization for all the constituents of vine increasing significantly the weight of berries and by improving the qualitative criterions for the vine making and for breeding.

✍ HerbaGreen application allows a better physiologic stress management of the vine and allows a better synthesis of the polyphenols, of the anthocyanes by increasing the berries weight, the potential degree and the sugar rate. All the qualitative components are improved by 3 HerbaGreen pulverisations.

✍ On vine which is in stress state (physiologic and hydrous), HerbaGreen applications allow a significant correction of the maturity controls and allow an optimisation of the maturity allowing a good extraction of the phenolic and aromatic components and an improvement of the coloration.

3 applications between flowering and grapes closing will give better results to optimize the stress of the vine after the colour change of the berry to allow a better concentration in the berries.

HerbaGreen applications allowed:

- **An effect on the vegetation by increase of the photosynthesis activity**
- **Improvement of the water absorption and increase of sugar synthesis which resulted in an improvement of the foliage quality and a better ripening of the woods.**
- **Increase of weight berries by increasing the qualitative components of the grapes.**
- **A better extraction of the polyphenols and anthocyanes**
- **Optimisation and homogenization**

Summary

1. USE ON FIELD CROPS (WHEAT AND BEET)

Different tests in field confirm the fact HerbaGreen has a positive indirect effect on the syndrome of low wealth in sugar.

HerbaGreen has a positive action on crop fields (wheat and beet) yields, proteins and sugar contents.

We have noticed specific effects:

- ✍ **Increase of the yield and of the photosynthesis***
- ✍ **Reinforcement of the plant***
- ✍ **Improvement of sugar content in the beet***

So it is possible to conclude that HerbaGreen can be used to improve crop fields

3. USE ON POTATOES

ON CONSUMPTION POTATOES :

Effects on potatoes are:

- *increase of the photosynthesis and of the yield*
- *indirect effect on fungi diseases by a general reinforcement of the plant*
- *effect on quality: increase of the dry matter and reduction of the burnishing because of Maillard reaction at the time of transformation*
- *indirect effect on doryphore by increase of the thickness of the leaves and reinforcement of cellular wall thanks to calcite treatment.*

Thanks to HerbaGreen, yields, homogeneity size in marketable range, number of tuber and dry matter increase.

ON POTATOES FOR THE INDUSTRIAL TRANSFORMATION:

HerbaGreen can exercise using synergies over by a direct action on the vegetal physiology. That is why yield, dry matter and photosynthetic activity are higher for potatoes treated with HerbaGreen. Those 3 points are very important for potatoes which are used for industrial transformation; they must contain enough dry matter, not much reducing sugar because it is responsible of burnishing of fried products.

HerbaGreen treatment allows to get potatoes with those specific characteristics.

4. USE IN MARKET GARDENING

on salads

HerbaGreen treatment allows:

- *an increase of average yield*
- *an increase of dry matter rate*
- *bigger useful matter rate*
- *better salad conservation after 7 days*

Moreover, according to tests results, HerbaGreen can reduce tip burn on salad.

on celery

HerbaGreen allows a weight increase of celeriac.

on cucumber

HerbaGreen increases foliage colour and foot height of cucumber. Treated plants are less attacked by mildew. There are less affected leaves and a longer flowering period on treated plants. HerbaGreen treatments causes an important yield increase of cucumber, with a high qualitative value.

on courgette

HerbaGreen increases colour of foliage by improvement of Photosynthetic process.

on melon

HerbaGreen treatment increases average number of melons

on leek

HerbaGreen treatments allow to get an important yield increase of leeks, with a high qualitative value.

5. USE IN VITICULTURE

According to the results, HerbaGreen treatment induces:

- a significant difference for the total weight index
- a significant difference for the anthocyanes
- a significant difference for berries weight
- a significant difference for sugar rent
- a significant difference for potential degree
- a good maintenance of the pH
- an equivalent level in total acidity

✍ HerbaGreen applications allow an optimization for all the constituents of vine increasing significantly the weight of berries and by improving the qualitative criterions for the vine making and for breeding.

✍ HerbaGreen application allows a better physiologic stress management of the vine and allows a better synthesis of the polyphenols, of the anthocyanes by increasing the berries weight, the potential degree and the sugar rate. All the qualitative components are improved by 3 HerbaGreen pulverisations.

✍ On vine which is in stress state (physiologic and hydrous), HerbaGreen applications allow a significant correction of the maturity controls and allow an optimisation of the maturity allowing a good extraction of the phenolic and aromatic components and an improvement of the coloration.

3 applications between flowering and grapes closing will give better results to optimize the stress of the vine after the colour change of the berry to allow a better concentration in the berries.

HerbaGreen applications allowed:

- An effect on the vegetation by increase of the photosynthesis activity***
- Improvement of the water absorption and increase of sugar synthesis which resulted in an improvement of the foliage quality and a better ripening of the woods.***
- Increase of weight berries by increasing the qualitative components of the grapes.***
- A better extraction of the polyphenols and anthocyanes***
- Optimisation and homogenization***

V. TECHNICAL DATA

HerbaGreen characteristics

Main components: calcite (CaCO_3 , SiO_2 , MgO , CaO , Iron, Mn, Selenium)

Granularity: in the region of the μm

Standards: HerbaGreen corresponds to the NFU 44001 standards

Suspension keeping: soluble only at acid pH. It is in the region of 5.8, which is the pH of the leaf. The particles finest phase is still in suspension in a blend.

Recommended standard doses

HerbaGreen must be used by foliar pulverization on the whole aerial part and diluted in water. 1 to 3 kg must be used per hectare and per application. The number of applications is different according to the culture type. The using protocol is different according to the desired effect and the climatic and sanitary conditions.

HERBAGREEN ON FIELD CROPS

- ? Cereals: 1 application/last leaf. Dose : 1.5 kg/ha
- ? Beet : 1 application/dose : 1.5 kg (4-6 leaves stage)

HERBAGREEN IN TRUCK CULTIVATION

- ? Salad, melon, leek
2 to 3 applications
T1: resumption of vegetation. Dose: 1 kg/ha
T2: T1 + 15 days. Dose: 1.5 kg/ha
T3: T2 + 15 days. Dose: 1.5 kg/ha
- ? Tomato, courgette, carrot, sweet pepper
3 to 4 applications/dose 1.5 kg/ha
- ? Strawberry and small fruits
3 applications
T1: beginning of flowering. Dose: 1 kg/ha
T2: first white fruits. Dose: 1.5 kg/ha
T3: T2 + 15 days. Dose: 1.5 kg/ha
- ? Potato (consumption and early product)

2 applications:

T1: potatoes in formation in the ground + 15 days dose 1.5 kg/ha

T2: T1 + 15 days dose 1.5 kg/ha

HERBAGREEN IN VITICULTURE

Reinforcement of the plant: 2 treatments 1.5 kg

Impact on vegetative aspect and quality: 2 to 3 treatments:

HerbaGreen has two different actions on vine:

1. **global action on vine physiology**
2. **reinforcement action on certain vegetal diseases**

Leaves exit to flag leaf expended: 1 kg/ha

Visible grapes: 1.5 kg/ha

Grapes and separated flowering buds: 1.5 kg/ha

HerbaGreen applications bring:

- **A booster effect on growth at the beginning of vegetation**
- **An important effect on general physiology of vine to:**
- ? **Allow a resistance against diseases in addition to approved specialities**
- ? **Put vine in an optimal situation for flowering**

Applications are made during the second part of the cycle to improve grapes qualities and yield.

Nouaison: 1.5 kg/ha

Grape closure: 1.5 kg/ha

Veraison: 1.5 kg/ha

CONCLUSION

HerbaGreen made of calcite comes from tribomechanical technology, which allow to get finest granularity (granularity in the region of micrometer) and new physicochemical characteristics with a higher reactivity in the plant.

Phytotoxicity tests were made at SADEF laboratories to show HerbaGreen innocuity on vegetal. The product is not toxic, even with important doses. Fluorimetry and % dry matter measurements confirms this fact.

Different laboratories analysis and many fields experimentations were made to show particularities and specificities of calcite micronised by this new tribomechanical technology.

Analyses made at Chemical Superior National College of Mulhouse show that tribomechanical treatment has a determining effect on the textural characteristics: total specific surface has doubled and the one of the total porous volume has tripled. Moreover, calcite microparticles have a negative charge which gives us a lot of particularities.

Cuticular pH of the leaf is in the region of 5.8. At this acid pH, 85.2 % of the TMA calcite is soluble on leaf. Calcium is the main soluble element is (32.8 %). So calcium carbonate dissociation on the plant allows an important calcium contribution. Calcium is involved in many biochemical mechanisms and in cellular functioning. Many kinase proteins have a catalytic activity, calcium dependant. Those proteins have an important part in the transmission of the defence signal when plant detects a pathogen agent.

From an agronomic point of view, calcium contribution favours the healing, improves fruits and vegetables preserving and increases resistance to biotic and abiotic stress. Photosynthetic process is enhanced by HerbaGreen applications. Calcium carbonate dissociation allows an intra cellular carbon dioxide contribution. So photobreathing process is inactivated thanks to a photosynthetic activation

Microscope analysis (CIRAD) of treated leaves shows an increase:

- ? Of chloroplasts, photosynthesis takes place in the chloroplasts, so it shows an activation of the primary metabolism.
- ? Of polyphenol and starch rate, which are chemical products, precursor of the secondary metabolism. They have a part in natural defence reactions.

This Increase of starch quantity, polyphenol quantity and the number of chloroplasts thanks to HerbaGreen treatment induces an improvement of harvest quantity and a yield increase.

In addition to the photosynthetic activity increase, HerbaGreen has a powerful antioxidant effect. So, after HerbaGreen treatment, plants have a more important coloration with less oxidation and less plants senescence signs. That green effect was noticed in many field and laboratory experimentation.

Moreover, HerbaGreen applications induce a change of structure of the cuticular wax (SADEF). The entire surface of the leaf is in contact with water, inducing a high continue hydration and limiting hydrous stress.

Many results of field experimentation are very positive; they confirm laboratories results which show the improvement of harvest qualities.

✍ **Results on wheat:** yield and proteins rate increase.

✍ **Results on truck farming:**

- on potatoes: yield, homogeneity size, tuber number and dry matter increase by a direct action on vegetal physiology
- on beet: yield and sugar rate increase.
- on salad: yield, dry matter, useful matter and preserving time increase. Tip burn contamination is reduced thanks to HerbaGreen treatment
- on celery: weight increase
- on cucumber: foliar colour, foot height, flowering period increase, mildew attack reduction
- on melon: average melon number increase
- on leek: yield increase

○ **Results on vine:**

According to the results, HerbaGreen treatment induces a significant difference:

- for the total weight index
- for the anthocyanes
- for berries weight
- for sugar rent
- for potential degree
- a good maintain of the pH
- an equivalent level in total acidity

✍ HerbaGreen applications allow an optimization for all the constituents of vine increasing significantly the weight of berries and by improving the qualitative criterions for the vine making and for breeding.

✍ HerbaGreen application allows a better physiologic stress management of the vine and allows a better synthesis of the polyphenols, of the anthocyanes by increasing the berries weight, the potential degree and the sugar rate. All the qualitative components are improved by 3 HerbaGreen pulverisations.

✍ On vine which is in stress state (physiologic and hydrous), HerbaGreen applications allows a significant correction of the maturity controls and allows an optimisation of the maturity allowing a good extraction of the phenolic and aromatic components and an improvement of the coloration.

3 applications between flowering and grapes closing will give better results to optimize the stress of the vine after the colour change of the berry to allow a better concentration in the berries.

HerbaGreen has a promising role in the defences alternative strategies based on nutrition and in the research of natural solutions to improve qualitative factor of cultivation and harvests.

APPENDIX 1: Other analyses results on vine stocks taken on different type of grounds

SAINT EMILION on Merlot

	Sandy clayey ground		Sandy ground	
	Control	HerbaGreen	HerbaGreen	control
ITP	19	26	13	13
Total acidity (g/l)	2.51	2.64	2.79	2.94
Sugar (g/l)	224	225	220	214
pH	3.77	3.85	3.27	3.27
Assimilable nitrogen (mg/l)	177	236	229	219
Berries weight (g/100 berries)	147	132	146	144
Anthocyanes	255	323	200	182
Potential degree	13.18	13.24	12.94	12.59
Malic acid	0.9	0.8	0.9	0.8

SAINT EMILION on Cabernet Francs and Sauvignon

	Sandy silt laden ground		Chalky clayey ground	
	HerbaGreen	control	HerbaGreen	control
ITP	15.5	14.5	27.5	28
Total acidity (g/l)	3.18	3.44	3.14	3.12
Sugar (g/l)	214	202	261	255
pH	3.052	3.40	3.76	3.74
Assimilable nitrogen (mg/l)	125	111	36	43
Berries weight (g/100 berries)	144	143	141	128
Anthocyanes	132	96	214	196
Potential degree	12.59	11.88	15.35	15
Malic acid	2.1	1.4	0.7	0.8

ABBREVIATIONS LIST

Appa : Association des Producteurs de Pommes de Terre de Vénétie (Potatoes Producers Association of Venetia)

CEE : Communauté Economique Européenne (ECC : European Economic Community)

CIRAD : Centre International de Recherche Agronomique pour le Développement (Agronomic Research International Center for Development)

cl : centiliter

cm : centimeter

CO₂ : carbon dioxide

DNA : desoxyribonucleic acid

ENSCMU : Ecole Supérieure de Chimie de Mulhouse (Chemical Superior National College of Mulhouse)

Fv/Fm : quantic efficiency of photosynthesis

g : gram

ha : hectar

kg : kilogram

m : meter

mg : milligram

min : minute

ml : milliliter

mm : millimeter

mol : mole

mV : millivolt

N : nitrogen

nm : nanometer

nmol : nanomole

OM : organic matter

pH : hydrogen potential

q/Qx: quintal

RUBISCO : ribulose 1,5- biphosphate carboxylase/oxygénase

RuBP : ribulose 1,5 biphosphate

TMA: tribomechanical activation

TPI: total polyphenol index

° c: degree Celsius

µm: micrometer