



Preliminary results from

Sustainable strategies to contain the Olive Quick Decline Syndrome in south-east Italy

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Olive Quick Decline Syndrome (OQDS)



This definition comes from the fact that the symptoms observed were initially confused and not-specific, since abiotic and biotic factors were thought to be involved. The biotic factors responsible include lignicolous fungi, insects, and especially the bacterium ***Xylella fastidiosa***.

Olive Quick Decline Syndrome (OQDS)

Subsequently, fungi and insects were considered as 'aggravators', in other words not primary pathogens responsible for the damage to olive trees, while *X. fastidiosa* bacterium is considered solely responsible for the serious damage to olive trees.



Olive Quick Decline Syndrome (OQDS)

The Salento olive trees affected by OQDS were seen to be massively infested by *Zeuzera pyrina*, or Wood Leopard Moth.



In fact, the presence of *Z. pyrina*, whose larvae develop in tunnels bored into branches and twigs, is generally regarded as a factor predisposing the plant to attack by secondary pests, including beetles, bacteria and fungi.



The main aims of experimental trial

- **To set up** of the best " agronomic " and " protection " practices able to contain the olive leaf desiccation, and/or control the microorganisms inhabiting the infected plants tissues;
- **To test** the ability of the olive trees to tolerate the presence of the bacterium;
- **To allow to** olive trees to contrast/tolerate the bacteria, by use of products with to zero/low environmental impact, so that they themselves could synthesize the natural antimicrobial compounds in each, i.e. "phytoalexins";
- **To understand** if the "coexistence" between the bacterium and the olive tree, as well as between the bacterium and the territory is possible.

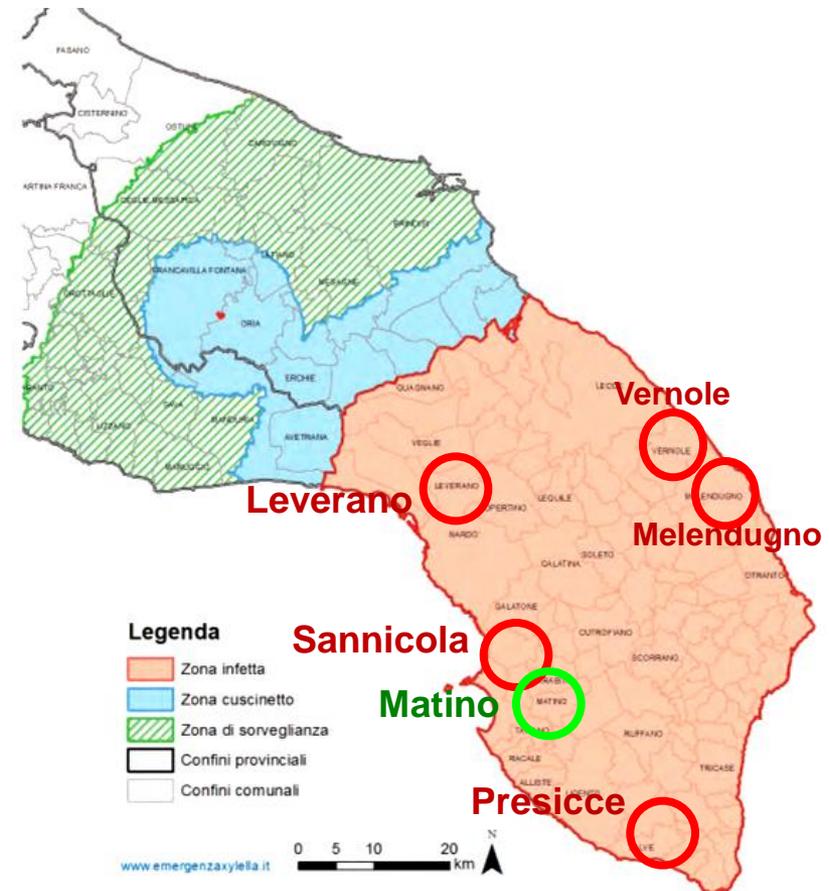
Farms where the experimental trials were carried out

1. Comparing treatments: 18 (12 products, 5 combinations and Control)

- Romano and Rotino Farms, both located in **Matino** (LE)

2. Experimental trials with eight different products used individually and in combination

- Luceri-Garzia Farm, **Sannicola** (LE)
- Nestola Farm, **Leverano** (LE)
- Monte Farm, **Presicce** (LE)
- Vizzino Farm, **Melendugno** (LE)
- Tondolongo Farm, **Vernole** (LE)
- Rotino Farm, **Matino** (LE)
- Romano Farm, **Matino** (LE)





The personnel involved in the experimental trials

The trial has been possible thanks to the cooperation and collaboration of the following persons:

Dept. SAFE of Univ. of Foggia

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Scientific support in the planning and execution of the open-field and laboratory treatments

COPAGRI of Lecce province

F. Ingrosso (president)
S. Faggiano
G. Scorrano
F. Pennetta
L. Romano
S. Romano

Technical support in the planning and execution of the treatments in open-field

CIHEAM-IAM of Bari

A.M. D'Onghia
F. Valentini
G. Cavallo

Collecting leaf samples for the assessment of the *X. fastidiosa* bacterium in their laboratories

Collecting leaves for for the assessment of the *X. fastidiosa*



Experimental trial in open field in Salento province

In 2015 a trial was conducted on olive groves affected by rapid desiccation in order to assess the possibility of containing the symptoms using products with different activities (**fertilisers, resistance inducers** and **agrochemicals**) combined with good farming practices (Table 1).

TABLE 1 - Products used in tests

Product (manufacturer)	Composition	Function
Agroallium Terra (Domca)	Alliaceae extracts	Plant Protector
Radicon (Fertek)	Humic acids	Natural soil improvers
Rhizosum Max (Biosum tech.)	Oligominerals	Growth promoter
Overground (Overtis)	Oligominerals and trace elements	Fertiliser
Keos Cu 15 (Green Fertilizer)	Copper hydroxide	Fertiliser
Kodens (Iko-Hydro)	Copper complex	Fertiliser
IRF-230 (Isagro) ⁽¹⁾	Copper complex	Fungicide
Remedier (Isagro)	Trichoderma gamsii and T. harzianum	Biological antagonist
Bion (Syngenta)	Acibenzolar-S-methyl	Resistance inducer
Prodeo 80 WG (Syngenta)	Fosetyl-Al	Fungicide
Biobacter (IG-Italia)	Copper and peracetic acid	Antimicrobial
Kuprum Red (IG-Italia)	Copper chloride oxide	Fungicide

⁽¹⁾ Product pending registration.

1. Comparing of 18 treatments (12 products, 5 combinations and Control)

SETTING- UP

- The trial started on 21 May 2015 in two different olive farms located both in Matino area.

Previously:

- The olive trees were subjected to a hard pruning, in order to eliminate all infected/dead leaves and stems;
- the soil was plowed at about 20-30 cm deep in order to eliminate all weeds such as hosts for leafhoppers vectors of *X. fastidiosa*



1. Comparing treatments: 18 (12 products, 5 combinations and Control)

... Ongoing operations

- Each product was used for 7-10 olive trees on the base of availability more than 70 years old showing clear symptoms of desiccation in leaves and branches.

Eighteen tests were performed: one untreated control, 12 in which the olive trees were treated with only one of the 12 products available and five in which the olive trees were treated by combining two or three of those products.

The olive groves in the experimental trial were neither fertilised nor irrigated.





1. Comparing treatments: 18 (12 products, 5 combinations and Control)

... Ongoing operations

- The products were used by foliar and/or radical applying on the base of manufacturer instructions, at intervals 25-35 days apart.
- The number of treatments was 6 from 21 May to 06 October 2015, except for two products as their availability was not immediate.





2. Experimental trial with eight different products used individually and in combination

SETTING- UP

- The trial started from 21 to 30 May 2015 in seven different olive farms located both in Lecce province.

Previously:

- the olive trees were subjected to a hard pruning, in order to eliminate all infected/dead leaves and stems;
- the soil was plowed at about 20-30 cm deep in order to eliminate all weeds such as hosts for leafhoppers vectors of *X. Fastidiosa*.

<u>Olive farms (location)</u>	<u>Product</u>
Az. Nestola (Leverano)	Agroallium
Az. Tondo Longo Gianfranco (Vernole)	Keos
Az. Rotino Elisabetta (Matino)	IRF-230
Az. Romano Elio (Matino)	Keos + Radicon + Bion
Az. Romano Elio (Matino)	Keos + Radicon
Az. Vizzino (Melendugno)	Keos + copper + Kendal
Az. Garzia Pasquale (Sannicola)	Microphyt + Fosal
Az. Monte Carmelo (Presicce)	Keos + copper + sulphur

2. Experimental trials with eight different products used individually and in combination

... Ongoing operations

- Each product was used for at least 30 – 100 olive trees on the base of availability more than 70 years old showing clear symptoms of desiccation in leaves and branches.
- The products were used by foliar and/or radical applying on the base of manufacturer instructions, at intervals 25-35 days apart.
- The number of treatments was 6 from 21 May to 06 October 2015.

The olive groves were subjected to standard agronomic management applied by the person in charge of the olive farms, including fertilization and irrigation.

Measurements carrying out in trials and olive groves

As it was not possible to collect plant samples (leaves, stems, etc.) in order to study the possible traslocation and/or effectiveness of products used to cointain the symptoms,

The observations carried out consisted of:



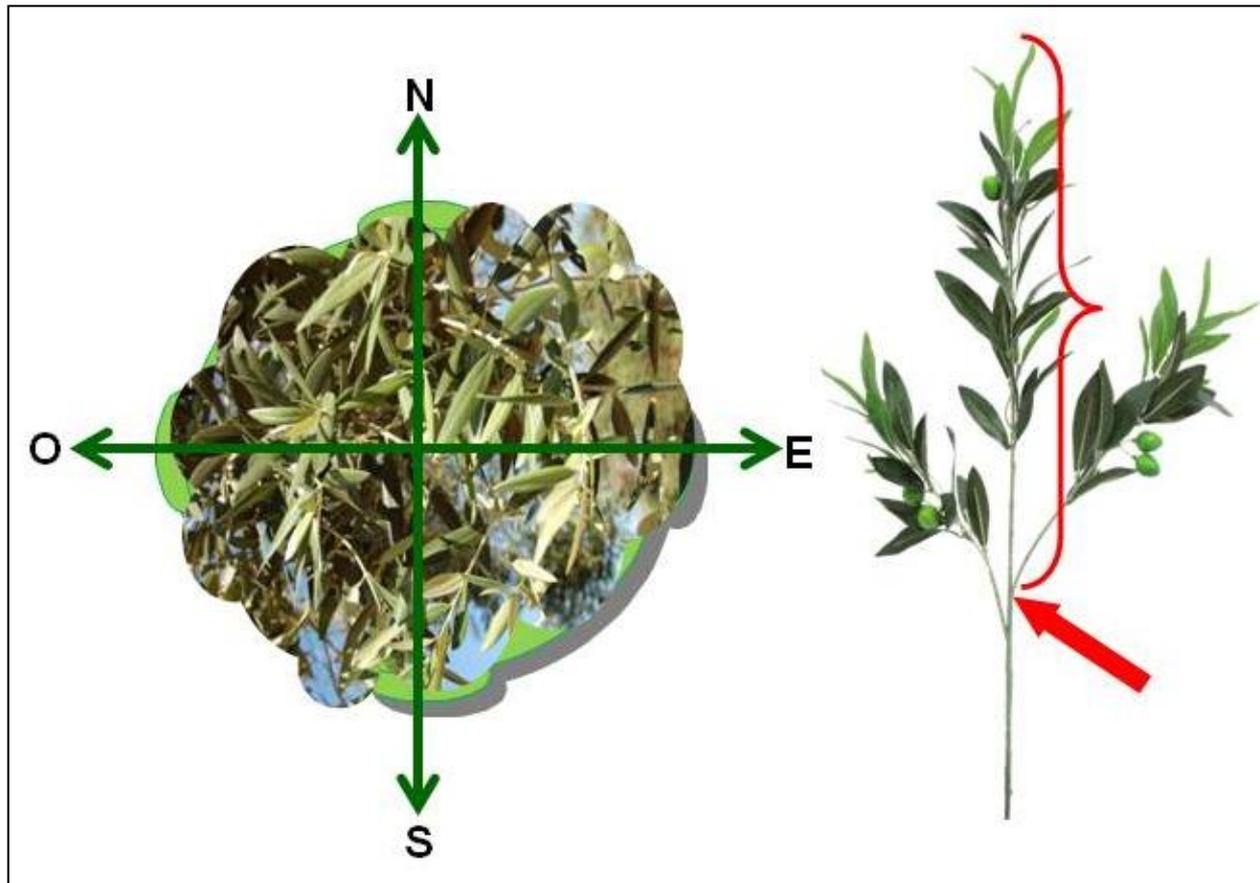
1. Assessment the vegetative development of the plants treated by determining the number of leaves that developed on the last twig produced after the first treatment starting from the point of insertion.

On each plant, in all tests, we took into account the leaves of three twigs on the four cardinal points;



Measurements carrying out in trials and olive groves

1. Assessment of the vegetative development of the plants treated by **determining the number of leaves that developed on the last twig** produced after the first treatment starting from the point of insertion. **On each plant, in all tests, we took into account the leaves of three twigs on the four cardinal points;**





Results

Leaves grown on twigs of the current year.

The products that seemed to produce the greatest leaf development were the new formulation currently being registered (IRF 230) and acibenzolar-S-methyl (Bion), either when used alone or in combination with each other or with other products, with the average of leaves produced ranging from 23.67 (about 60 days after the first treatment) to 34.9 (about 180 days after the first treatment) (Table 2).

By and large, all other treatments produced good leaf development in the treated plants, particularly bearing in mind that some products contain nutritional components. In particular, **plants treated with Alliaceae extracts (Agroallium) did not show good vegetative growth, but were less susceptible to knot disease (*Pseudomonas syringae* pv. *savastanoi*)** (data not shown).

Drapes also ripened better on such plants.



Results: Leaves grown on twigs of the current year.

Products	09/07/2015	30/07/2015	06/10/2015	27/10/2015	19/11/2015
Radicon+Keos+Bion	12,00A	15,83A	24,30CDE	27,40DEF	31,86FG
Agroallium	14,00AB	17,67AB	20,17B	20,83AB	27,22C
Radicon+ Keos	14,67B	18,33AB	24,67DE	27,78DEF	30,86EFG
Keos	15,17B	20,50BC	24,67DE	26,50DEF	28,21CD
Kodens+Bion	16,00BC	19,67B	22,50BCD	24,00CD	28,41CD
Radicon	17,00C	23,50D	27,00EF	27,83EF	29,54E
Remedier	17,67C	21,00BC	26,17EF	28,32EF	31,20F
Rhizosum	18,00D	22,00C	25,00DE	26,00DE	29,35D
Overground	18,33D	21,67C	24,67DE	26,33DEF	29,22D
Kodens	18,67DE	22,50CD	26,67EF	26,67DEF	31,34F
Keos+Bion	18,67DE	20,00B	24,67DE	26,50DEF	33,85G
IRF-230	18,83DE	21,67C	25,33DEF	27,87DEF	32,03FG
Fosetyl-Al	21,0F	23,67D	27,33EF	28,67EF	30,33EFG
Bion	22,00G	25,00F	28,67F	29,54F	33,83G
IRF-230+Bion	23,67H	24,67DE	26,67EF	28,45EF	34,9G
Biobacter	ND	ND	15,33A	19,21A	24,56B
Kuprum Red	ND	ND	14,67A	20,33AB	22,42A
Control	13,17AB	15,67A	12,17A	18,33A	20,03A

The data collected were subjected to variance analysis (ANOVA) and the averages were compared using the Duncan test ($P < 0.05$). * Data from young leaves.

Results: Chlorophyll index

Products	30/07/2015*	30/07/2015	06/10/2015*	06/10/2015	27/10/2015*	27/10/2015	19/11/2015*	19/11/2015
Rhizosum	76,9CD	75,2C	56,2A	90,7F	51,1C	89,2F	54,2B	80,5C
Radicon	69,6A	74,2C	57,1A	63,9BC	41,4B	85,4CD	55,3B	85,7D
IRF-230+Bion	74,5B	79,7E	60,6B	70,9C	49,4C	89,1F	47,8 A	79,5BC
Radicon+Keos+Bion	83,0G	77,5CD	60,9B	61,0BC	47,3BC	88,6F	52,5AB	82,8C
Remedier	76,0C	80,1E	64,8C	51,9A	51,2C	89,4F	56,4B	80,8C
Kodens	77,2D	79,8E	65,2C	92,6F	44,9BC	82,2B	55,3B	83,2CD
Keos	79,8E	65,9B	65,3C	68,3C	51,1C	61,1A	57,4B	78,5B
Bion	76,3CD	80,7E	67,0CE	87,7E	48,3C	87,7EF	45,9A	81,1C
Fosetyl-AI	82,2F	72,4C	68,8CE	85,2E	47,2BC	84,4C	52,8AB	80,8C
Agroallium	74,4B	84,5F	71,2F	79,4DE	57,7C	89,0F	62,3 C	90,8E
Radicon+Keos	75,0B	78,9E	71,2F	62,0BC	46,3BC	95,6I	49,7A	75,9B
Kodens+Bion	75,1B	78,3E	71,3F	67,2C	45,3BC	88,5F	49,9A	80,8C
Overground	74,3B	62,3A	71,4F	71,7C	48,9C	86,4DE	45,3A	76,8B
Keos+Bion	74,6B	75,2C	90,4G	76,0D	37,4A	81,7B	57,6BC	83,3C
IRF-230	82,3F	79,2E	94,8G	79,2DE	50,9C	89,1F	67,3D	85,8D
Biobacter	ND	ND	62,0C	60,0BC	44,3BC	93,0H	54,9 B	87,9D
Kuprum Red	ND	ND	58,0B	63,0BC	76,9D	97,9L	68,6D	92,9E
Control	79,9E	78,6E	54,9A	59,5B	52,4C	61,2A	50,8A	58,7A

The data collected were subjected to variance analysis (ANOVA) and the averages were compared using the Duncan test (P <0.05). * Data from young leaves.



Results

2. **Assessment of the chlorophyll index** (using a Minolta Chlorophyll Meter SPAD-502), i.e. the total chlorophyll content in plant tissues, which provide an indirect marker of the nutritional status of the plant.

Chlorophyll index. The values obtained were essentially encouraging, apart from some sporadic cases in which they were lower than those of untreated plants. The values were higher in plants treated with products and combinations of products with a high nutrient content (fertilizer).

In the last measurement particularly, the products containing Alliaceae extracts and copper oxychloride (Agroallium and Kuprum Red) produced the highest chlorophyll content in absolute values compared to all other treatments, with values of 90.8 and 92.9, respectively.

Observations on good plant growth of olive trees treated on 30 July 2015



Results: Stomatal conductance

Products	09/07/2015	30/07/2015*	30/07/2015	06/10/2015*	06/10/2015	27/10/2015*	27/10/2015	19/11/2015*	19/11/2015
Rhizosum	31,8 A	61,4C	58,1D	52,2B	68,4BC	42,4A	64,9AB	53,2A	94,2C
Remedier	53,6B	70,4D	59,3D	44,1A	62,5B	54,8B	69,6AB	52,4A	89,8B
Radicon	57,6B	39,6A	20,3A	55,3B	68,4BC	51,9B	62,8AB	50,5A	87,8B
Radicon+Keos +Bion	60,2BC	136,0H	103,6G	58,4B	64,1B	88,4D	68,3AB	64,7B	100,3CD
Radicon+ Keos	60,9BC	127,5G	54,8D	60,9BC	52,1A	68,4C	58,2A	63,6B	89,2B
IRF-230+Bion	62,7C	70,9D	20,2A	65,7C	56,7B	75,7CD	86,4B	78,3CD	98,4C
Agroallium	62,9C	99,5EF	46,8C	64,7C	86,9C	74,5CD	92,6C	75,1CD	102,7CD
Bion	64,6CD	44,5B	146,5H	69,2C	135,1F	99,6E	121,7E	90,8DE	109,9D
Keos+Bion	65,9CD	76,7DE	67,83E	67,8BC	97,8D	97,5E	107,8D	98,2E	127,4E
IRF-230	69,5D	60,5C	103,7G	70,2BCD	112,6E	79,2D	99,4CD	81,6D	121,8E
Fosetyl-Al	74,16DE	41,0AB	73,7EF	73,3CD	88,6C	87,2D	81,3B	84,8D	101,5CD
Kodens	77,1DEF	42,3AB	48,9C	74,4D	68,6BC	94,9E	63,2AB	99,3E	99,6C
Kodens+Bion	81,4EF	94,5E	31,8B	85,1D	91,6D	95,8E	101,5D	105,6F	99,8C
Keos	85,1G	71,7D	52,4CD	82,3D	82,4C	93,1E	86,9B	98,8E	100,2CD
Overground	85,4G	92,1E	46,8C	90,3E	86,8C	88,4D	82,2B	92,8DE	97,92C
Biobacter	ND	ND	ND	65,6C	80,2C	71,3CD	98,2CD	92,9DE	109,5D
Kuprum red	ND	ND	ND	78,5DE	110,5E	88,8D	105,4D	98,4	114,2D
Control	95,1H	61,6C	81,1E	77,4D	68,3B	58,3BC	55,3A	69,9C	67,7A



Results

3. **Assessment of leaf stomatal conductance** (using a Leaf Porometer), i.e. an assessment of the plant's water potential (indicating the capacity for sap to travel from the roots to the leaves).

Stomatal conductance. Compared to untreated plants, all the products were seen to react with the plant by increasing water potentials, which are an indirect indication of the movement of sap in the plant tissues.

Particular attention should be paid to all plants treated with acibenzolar-S-methyl, either alone or in combination with other products. Indeed, this product almost always induced a higher water potential than the others.

Results: Experimental trials with eight different products used individually and in combination (30-100 trees)

This table shows the results obtained from a one-way ANOVA trial in which the data of the number of leaves, from the Spad index and stomatal conductance of each plant tested, were compared to each other.

The table shows that the best results were obtained from plants tested using a combination of the products.

Olive holding (location)	Product	New leaves (n)	Chlorophyll index (I. SPAD)	Conductance stomatal
az. Nestola (Leverano)	Agroallium	4,67A	72,63A	62,67A
az. Tondo Longo (Vernole)	Keos	14,33F	76,43A	107,09B
az. Rotino (Matino)	IRF-230	7,00B	76,88A	105,55B
az. Romano (Matino)	Keos + Radicon+Bion	10,83EF	80,47AB	108,29B
az. Romano (Matino)	Keos + Radicon	10,17E	82,95B	104,73B
az. Vizzino (Melendugno)	Keos + rame + Kendal	8,00C	85,80BC	116,19B
az. Garzia (Sannicola)	Microphyt + Fosal	9,33D	86,81C	96,20B
az. Monte (Presicce)	Keos + copper+ S	8,17C	88,59C	162,56C

CONCLUSIONS

- Generally, the results obtained show that the treatments performed on olive trees suffering from **OQDS** were all effective, regardless of the numeric values.



- In particular, treated plants showed increased vegetative vigour (by number of leaves), higher chlorophyll index and stomatal conductance values, and were thus statistically significant.

- In deed, the results of the field tests highlighted the capacity of olive plants to react to pathogenic attack when standard agricultural practices (ploughing, milling, pruning) and protection treatments were put in place.





CONCLUSIONS

- Moreover, the best results seem to have been achieved in plants treated with combined applications of two or more products with different characteristics.

-This would indicate the desirability of a strategic approach meeting all the plant's requirements, ranging from nutrition to protection and defence, particularly in situations such as those prevalent in Salento.

-Therefore, while the results suggest that no product was more effective than the other, this result should not be overstated at this stage, particularly since the results were obtained over a short five-month period;

- in any case, the results of analyses on the assessment of the bacterium *X. fastidiosa* were always positive in all samples taken from all plants.

This means that the bacterium is present in the tissues of the treated plants, but on 06 April 2016 they had shown no symptoms.

CONCLUSIONS

To this end, the trials set up in 2015 will be repeated again in 2016.

On the basis of these experiments, we believe, at least in the **'infected' area** considered in Salento, **that it is possible for olive trees to coexist with the bacterium**, and for the bacterium to coexist with the Lecce province, since the olive plants' productivity was not compromised by the presence of the bacterium.

This situation was also helped by performing good agricultural practices, i.e. proper ordinary agricultural and phytosanitary management.

CONCLUSIONS

Moreover, the olive yield in 2015, although the plants were infected by *X. fastidiosa* was substantial and of excellent quality.

These preliminary, partial results of experimental activities of just one year need to be confirmed and validated by subsequent experiments.



CONCLUSIONS

Furthermore, we believe that more time is needed for scientific verification and confirmation, and that treatment cycles should be repeated for at least another two years (in 2016 and 2017) to validate the encouraging results obtained scientifically.





Summarized Conclusions

1. It seems that the actions undertaken have produced excellent results, in view of the short time elapsed since the beginning of the trial;
2. It seems that the olive tree is able to tolerate / contrast the bacteria despite its presence, as it has been growing, blooming and fruiting. To date there are no symptoms of Leaf Scorch;
3. Ordinary management, agronomic and 'phytoiatric' actions were useful to "stimulate" the ability of plants to counteract the effects of pathogenic fungi and bacteria.
4. To date, no obvious symptoms.

Thanks for attention

