Analysis of Scortichini et al. 2018

A critical reappraisal

21-03-2018

In vitro experiments

- All *in vitro* experiments were conducted on *X. fastidiosa* **subsp. *fastidiosa***
- However, copper response might be very different in *X. fastidiosa* subsp. *pauca,* for example because:

"Sequencing results indicate specific characteristics for several genes analyzed which represent novel findings. In agreement with previous reports (85), the pglA frameshift mutation seen in X.fastidiosa subsp. pauca isolates is absent in the non-X. fastidiosa subsp. pauca isolates analyzed in this study. There is a 1-bp insertion in the GB514 strain copB sequence in GenBank that likely represents a sequencing error. The insertion produces a stop codon that results in truncation of the gene, whereas the gene is translated in other complete genome sequences and X. fastidiosa isolates from grape in this study (including another isolate from Texas). copB functions in copper homeostasis in X. fastidiosa (68), and inactivation of the homologous copAB operon in Xanthomonas axonopodis pv. citri rendered the bacterium incapable of growing in medium containing copper (82), so this gene is probably necessary for X. fastidiosa survival. Also, copB contains a region with a 15-bp (minisatellite)-coding variable number tandem repeat (VNTR). When oriented in the coding direction, a repeat of 5'-ACACCCAGATGGATC-3' occurs (described here for the first time), followed by a repeat which is a previously described variation of this sequence: 5'-ACACAGGGATGGATC-3' (48). The two substitutions that account for the difference in the two repeats represent a nonsynonymous mutation, whereby glutamine (CAG) versus glycine (GGG) is translated in the first versus second repeat, respectively. It is also notable that xadA contains a 21-bp indel. The deletion is present in all unique X. fastidiosa subsp. fastidiosa isolate groups except for haplotype groups GR_GAFL (9 Georgia grape and 2 Florida grape isolates) and EB_LUPINE (1 Florida elderberry isolate and 1 Florida lupine isolate). The insertion is present in all other isolates examined here."

J. K. Parker, J. C. Havird, and L. De La Fuente, "Differentiation of Xylella fastidiosa strains via multilocus sequence analysis of environmentally mediated genes (MLSA-E)," *Appl. Environ. Microbiol.*, vol. 78, no. 5, pp. 1385–1396, Mar. 2012.

Only 1 out of 3 experimental fields was reported

C. R. Girelli, L. Del Coco, M. Scortichini, M. Petriccione, L. Zampella, F. Mastrobuoni, G. Cesari, A. Bertaccini, G. D'Amico, N. Contaldo, D. Migoni, and F. P. Fanizzi, "Xylella fastidiosa and olive quick decline syndrome (CoDiRO) in Salento (southern Italy): a chemometric 1H NMR-based preliminary study on Ogliarola salentina and Cellina di Nardò cultivars," *Chem. Biol. Technol. Agric.*, vol. 4, no. 1, p. 25, Dec. 2017.

Table 1 Summary of the experimental trials with DENTAMET® Diagro

Farm	La Duchessa	Cosimo Pinca	Cosimo Pinca
Location	Veglie (LE), C.da Duchessa	Galatone (LE), C.da 3 Pietre	Galatina (LE)
GPS point	N 40°20′50.31″ E 17°54′24.55″	N 40°7′22.92″ E 18°1′27.91″	N 40°9′55.96″ E 18° 6,48.26″
Crop	*Olea europea* cv. Ogliarola salentina	*Olea europea* cv. Cellina di Nardò	*Olea europea* cv. Cellina di Nardò
Age of plants	~70 years old	~60 years old	~60 years old
Plant distance	~10 m × 10 m	~10 m × 10 m	~10 m × 10 m
Dose of product and time of spray treatment	5 kg/ha (3.9 L of product) for foliar application by atomizer in April (2) and May	5 kg/ha (3.9 L of product) for foliar application by atomizer in April (2) and May	5 kg/ha (3.9 L of product) for foliar application by atomizer in April (2) and May
Plot area (blocks of trees)	20 treated and 20 untreated trees	15 treated and 15 untreated trees	20 treated and 20 untreated trees
Mean incidence and severity of disease (as recorded before the treatment)	20% of trees showing symptoms with a severity of 10%	50% of trees showing symptoms with a severity of 20%	60% of trees showing symptoms with a severity of 20%

All the plant material was made inert by chemical and physical processes, including 200 °C overnight treatment, according to standard procedures

Discarded (70 out of 110 trees)

Reported (40 out of 110 trees)

M. SCORTICHINI, J. CHEN, M. DE CAROLI, G. DALESSANDRO, N. PUCCI, V. MODESTI, A. L'AURORA, M. PETRICCIONE, L. ZAMPELLA, F. MASTROBUONI, D. MIGONI, L. DEL COCO, C. R. GIRELLI, F. PIACENTE, F. PIACENTE, N. CRISTELLA, P. MARANGI, F. LADDOMADA, M. DI CESARE, G. CESARI, F. P. FANIZZI, and S. LORETI, "A zinc, copper and citric acid biocomplex shows promise for control of Xylella fastidiosa subsp. pauca in olive trees in Apulia region (southern Italy)," Phytopathol. Mediterr., vol. 0, no. 0, Mar. 2018.

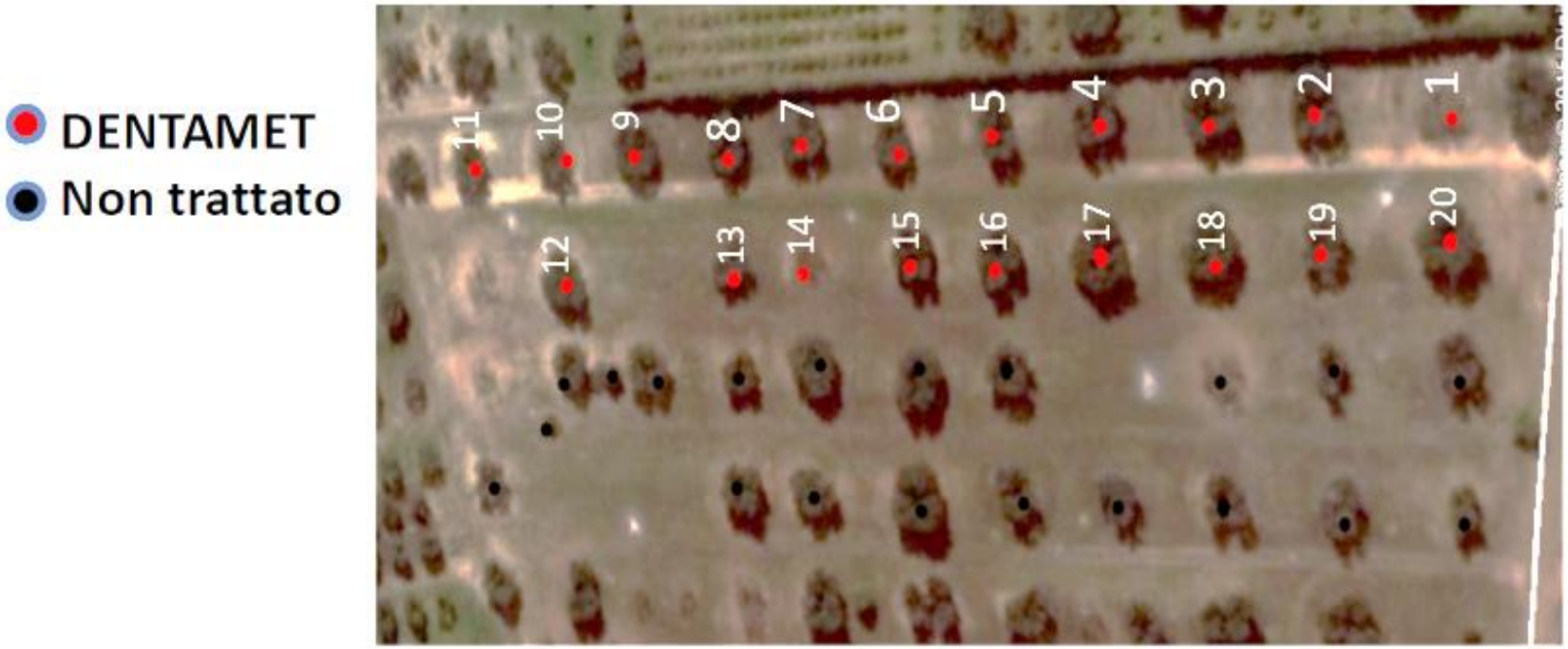
Experimental field in Galatone (discarded)

2015 **GALATONE** **2017**

Experimental field in Galatina (discarded)

Reported data (Veglie)

- 40 olive trees were divided in 2 groups, each including 10 trees of cv. Ogliarola and 10 of cv Cellina.



Taken from: Marco Scortichini, IOC-CIHEAM International Workshop - Xylella fastidiosa: a serious worldwide challenge for the safeguard of Olive trees - Valenzano (BA): 20-30 November, 2016

Confounding factors

- A bordure of *Cupressus sp.* flanked the treated trees

  This bordure might divert insect vectors to plants other than olive trees, partially screening the treated group from infections

- Compounds routinely applied for controlling the main diseases and pests (olive peacock spot, olive knot, olive fruit fly) were used on the control plants, but not on treated plants

  The chemical treatments applied selectively on control plants included many more chemicals other than Dentamet®, thus introducing an array of confounding factors in the study

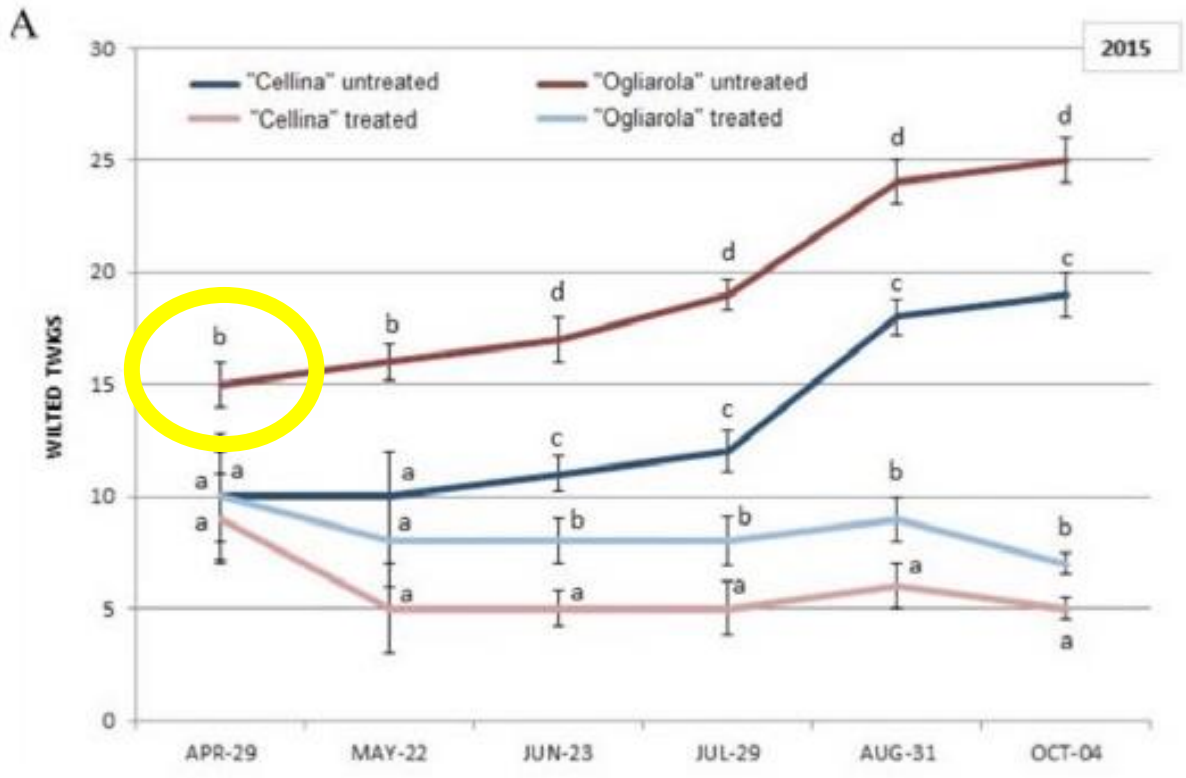
- In authors' words, the orchard soil is characterized by **very low zinc** and boron contents (Supplementary Table 1)

  In a zinc-poor soil, Dentamet® confers an obvious advantage to the treated trees, connected to its action as a fertilizer

Selection bias (fig. 4)

As evident from figure 4A of the paper, the original selection of the Ogliarola trees to be used as control was wrong: the amount of wilted twigs was significantly different from that of the group selected for the treatment.

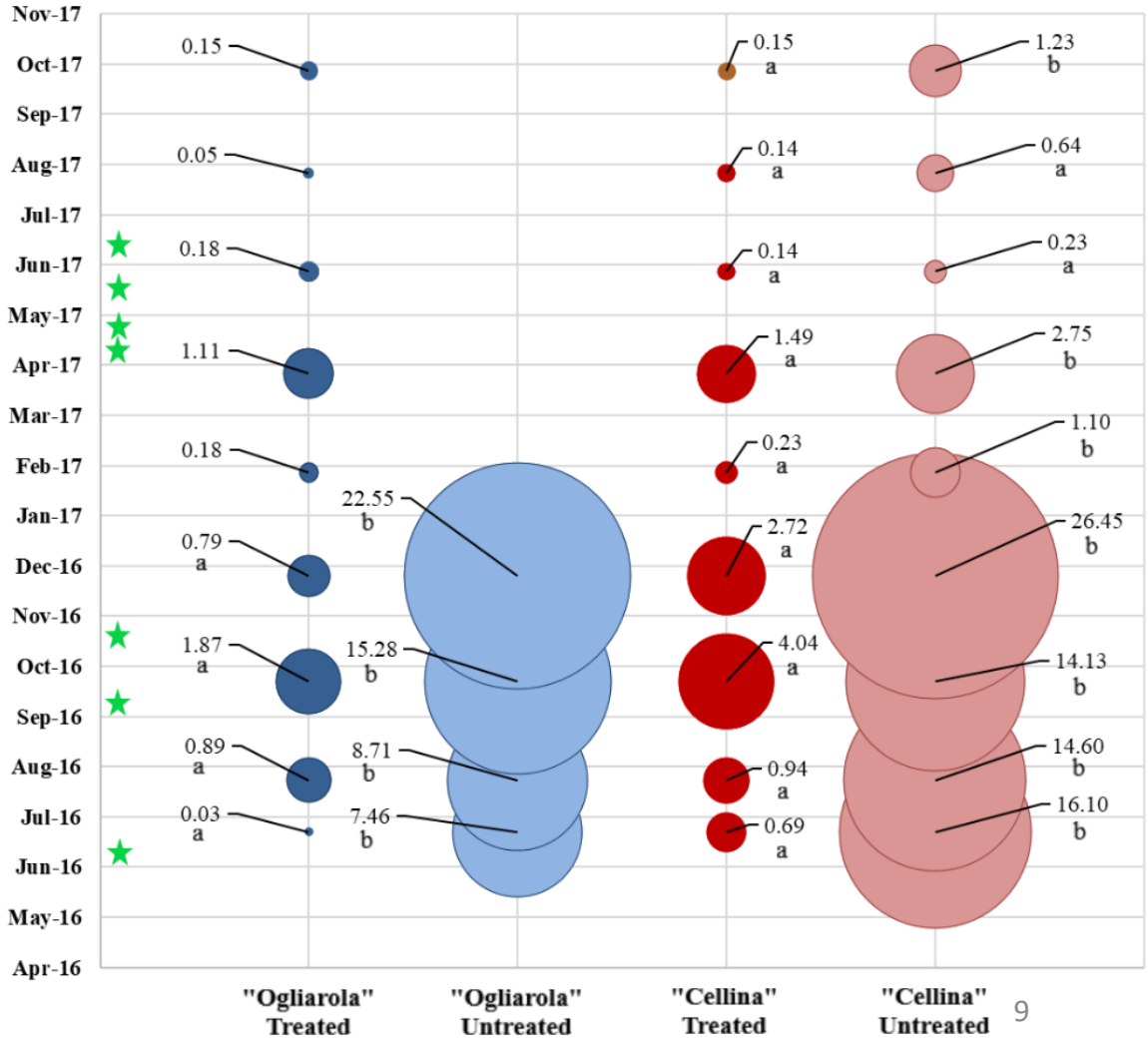
This selection bias impairs any further analysis for the Ogliarola trees (20 out of 40 plants), reducing the overall statistical power of the remaining sample.



Insignificant effects on bacterial loads (fig.7)

- Only 4 trees (2 controls and 2 treated plants) were selected. This sample is too small to draw any meaningful conclusion.
- Bacterial loads of controls and treated trees at the start of the monitoring period were very large and very small respectively; thus the experiment cannot be used to assess any effect occurring during the monitoring period (since controls and treated trees are by far too different).
- After a random freezing event in 2017 killed one control and reduced the bacterial load of the other one to levels comparable to the treated trees, difference due to treatment became irrelevant; intraplant random fluctuations in the control are of the same magnitude of differences between the control and the treated trees.

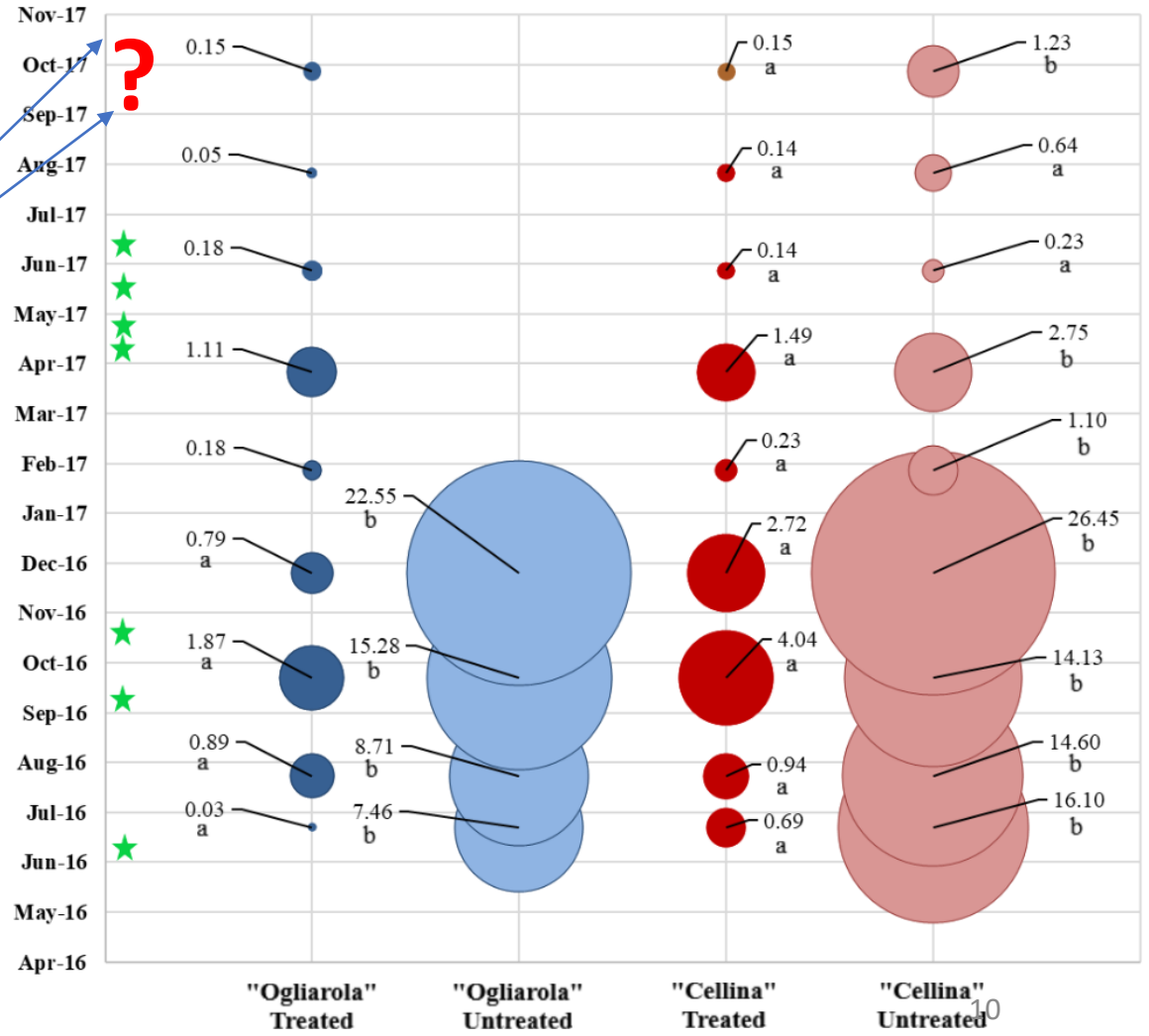
BRIEFLY, AVAILABLE DATA DO NOT SUPPORT ANY EFFECT OF DENTAMET®; IF THEY ARE ASSUMED AS SIGNIFICANT (AND THEY ARE NOT), THEY SHOW THAT RANDOM FLUCTUATIONS OF BACTERIAL LOADS ARE COMPARABLE IN TREATED AND UNTREATED TREES, WITH NO SIGNIFICANT DIFFERENCES AMONG THE TWO GROUPS WHEN TREES HAVING SIMILAR BACTERIAL LOADS ARE COMPARED



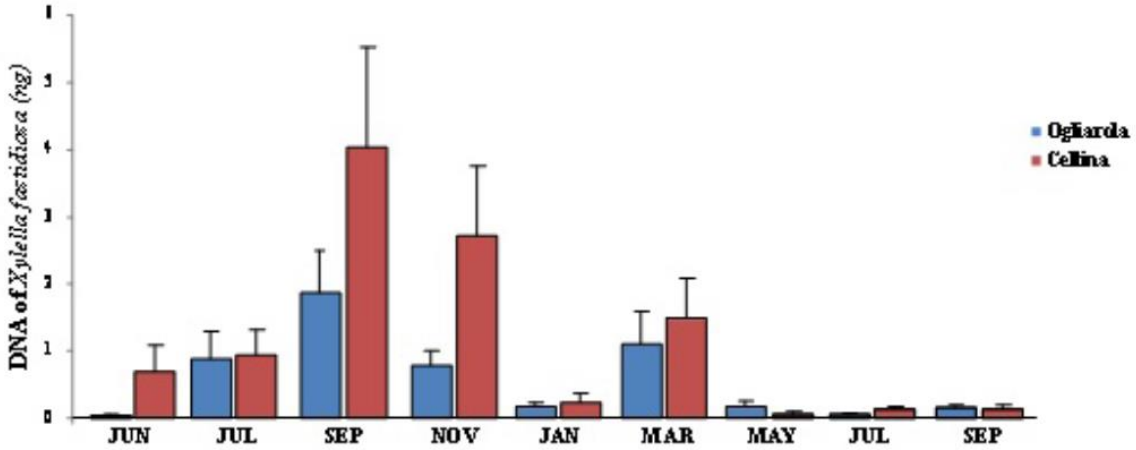
Inconsistent reporting of treatments

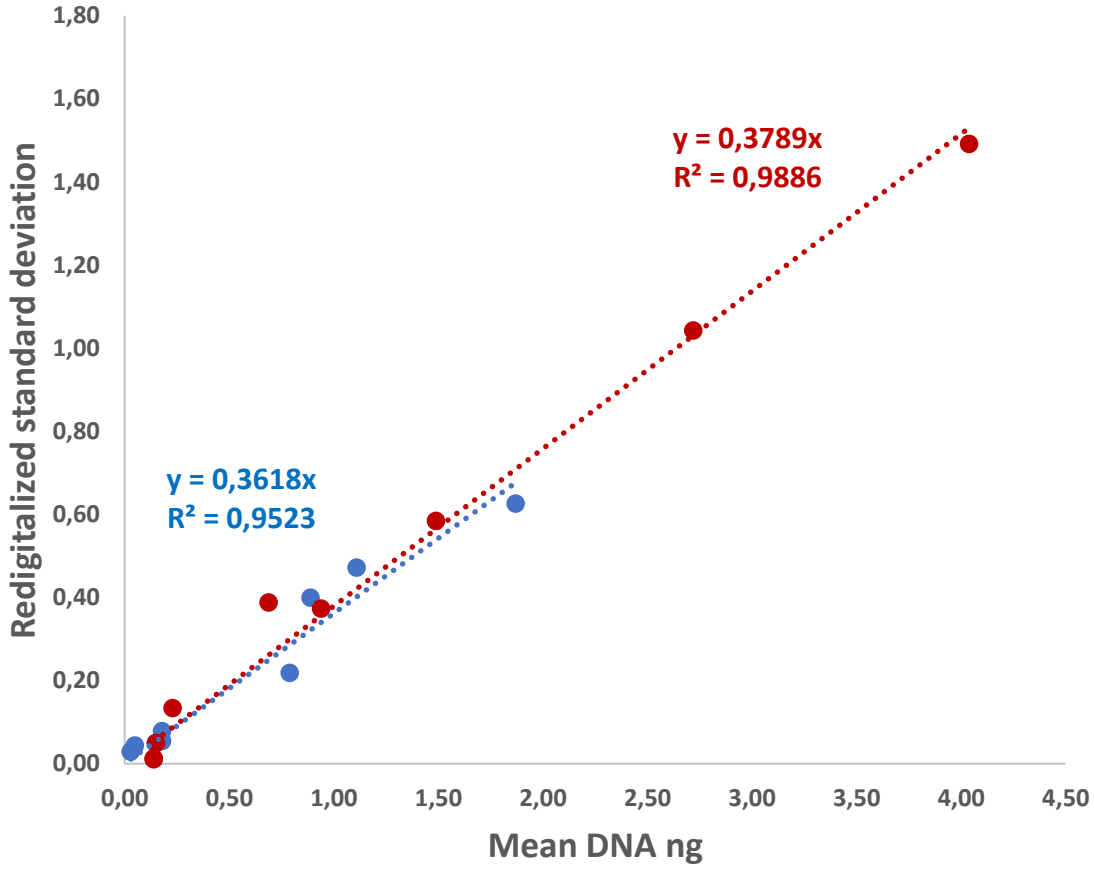
Supplementary Table 2. Day of the Dentamet® spray treatment on experimental olive orchard of Veglie (Lecce province, Apulia region) during the three years of efficacy assessment.

Month	2015	2016	2017
April	2nd	2nd	4th
April	22th	24th	26th
May	14th	12th	14th
June	4th	3rd	5th
September	2nd	4th	5th
October	22th	20th	18th



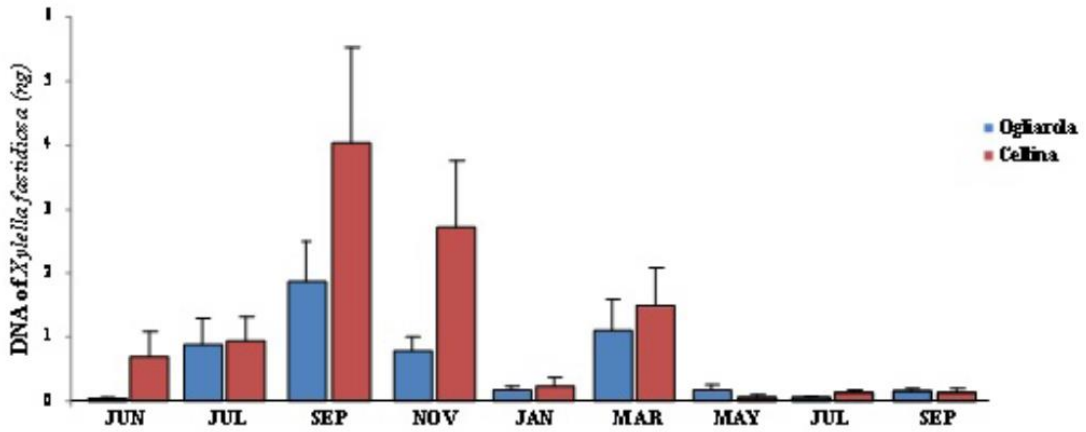
Strange data (fig.S8)



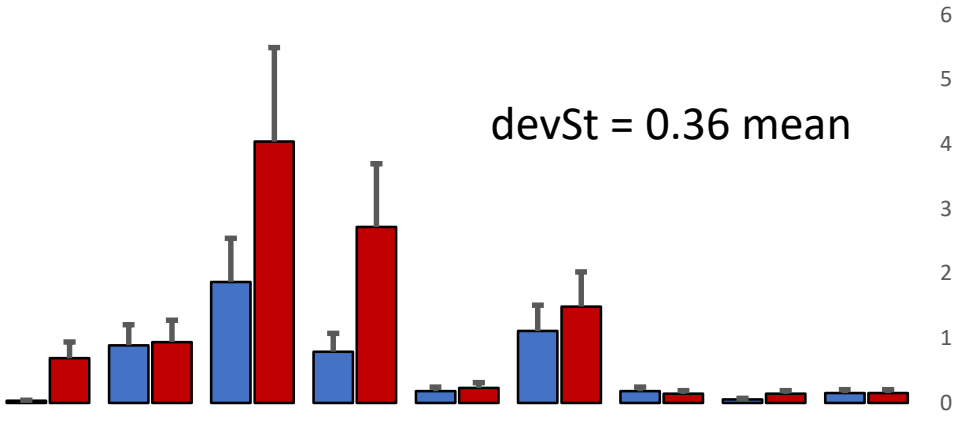


THE STANDARD ERRORS IN FIGURE S8 ARE LINEARLY CORRELATED TO THE MEAN DNA NG PER TREE, I.E. THE VARIABILITY IS PROPORTIONAL TO THE SIGNAL!

Strange data (fig.S8)



- Figure S8 – Xf Dna amount (mean ng per tree) in treated trees.



- Graph obtained from the mean values reported in fig. 7 of the paper and a standard deviation linearly proportional to the value of each bar.

THE STANDARD ERRORS IN FIGURE S8 ARE LINEARLY CORRELATED TO THE MEAN DNA NG PER TREE, I.E. THE VARIABILITY IS PROPORTIONAL TO THE SIGNAL!