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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 cultivar

This is the submitted version (pre peer-review, preprint) of the following publication:

Published Version:

Girelli, C.R., Del Coco, L., Scortichini, M., Petriccione, M., Zampella, L., Mastrobuoni, F., et al. (2017). 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 cultivar. CHEMICAL AND BIOLOGICAL TECHNOLOGIES IN AGRICULTURE, 4(1), 1-9 [10.1186/s40538-017-0107-7].

Availability:

This version is available at: <https://hdl.handle.net/11585/617866> since: 2019-05-10

Published:

DOI: <http://doi.org/10.1186/s40538-017-0107-7>

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Article CopyRight	The Author(s) (This will be the copyright line in the final PDF)	
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Journal Name	Chemical and Biological Technologies in Agriculture	
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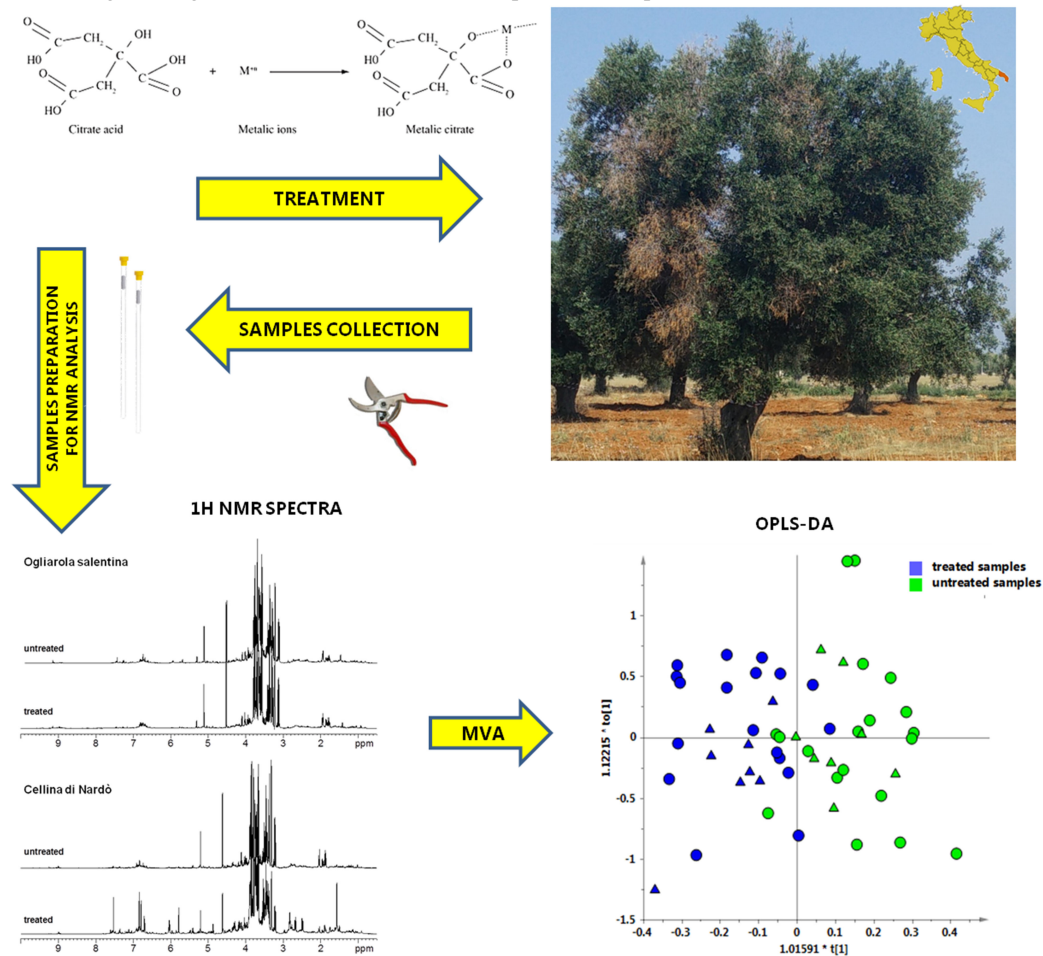
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Schedule	Received	21 March 2017
	Revised	
	Accepted	16 June 2017

Abstract *Xylella fastidiosa* is a Gram-negative bacterium which lives in the xylem of plants, causing its occlusion and other alterations inducing eventually the death of the infected plants. In Salento, the sub-peninsula in the south-eastern of Apulia Region (southern Italy), the infection of *X. fastidiosa* has been associated with the widespread presence of CoDiRO (complex of parasitic agents that constitute the so-called “olive quick decline syndrome”) and currently represents a serious local emergence. The need to adopt specific agronomic measures to contrast the further disease spread has been recently raised. The extensive NMR-based metabolomic approach to study the metabolic effects of CoDiRO on local olive cultivars such as Ogliarola salentina and Cellina di Nardò was used. In this study, the effects of a CE approved fertilizer containing zinc, copper, and citric acid, known as DENTAMET[®], on CoDiRO-exhibiting olive trees infected by *X. fastidiosa* were studied by ¹H NMR spectroscopy. The changes in the metabolomic profiles of aqueous extracts obtained from leaves of the two olive cultivars are reported. Upon the DENTAMET[®] treatments, different and opposite polyphenolic and sugars patterns in the two cultivars, which showed a different incidence and severity of disease before the treatments, were detected. Differences in the sugars and polyphenols content of treated versus untreated trees could potentially contribute to the syndrome

monitoring and might be related to the *X. fastidiosa* presence. Graphical abstract.



Keywords (separated by '-') *Xylella fastidiosa* - CoDiRO - Olive trees - NMR - Metabolomics - DENTAMET®

Footnote Information

Electronic supplementary material The online version of this article (doi:10.1186/s40538-017-0107-7) contains supplementary material, which is available to authorized users.
Chiara Roberta Girelli and Laura Del Coco contributed equally to this work

RESEARCH

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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

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Abstract

Xylella fastidiosa is a Gram-negative bacterium which lives in the xylem of plants, causing its occlusion and other alterations inducing eventually the death of the infected plants. In Salento, the sub-peninsula in the south-eastern of Apulia Region (southern Italy), the infection of *X. fastidiosa* has been associated with the widespread presence of CoDiRO (complex of parasitic agents that constitute the so-called "olive quick decline syndrome") and currently represents a serious local emergence. The need to adopt specific agronomic measures to contrast the further disease spread has been recently raised. The extensive NMR-based metabolomic approach to study the metabolic effects of CoDiRO on local olive cultivars such as Ogliarola salentina and Cellina di Nardò was used. In this study, the effects of a CE approved fertilizer containing zinc, copper, and citric acid, known as DENTAMET[®], on CoDiRO-exhibiting olive trees infected by *X. fastidiosa* were studied by ¹H NMR spectroscopy. The changes in the metabolomic profiles of aqueous extracts obtained from leaves of the two olive cultivars are reported. Upon the DENTAMET[®] treatments, different and opposite polyphenolic and sugars patterns in the two cultivars, which showed a different incidence and severity of disease before the treatments, were detected. Differences in the sugars and polyphenols content of treated versus untreated trees could potentially contribute to the syndrome monitoring and might be related to the *X. fastidiosa* presence.

Keywords: *Xylella fastidiosa*, CoDiRO, Olive trees, NMR, Metabolomics, DENTAMET[®]

Background

Starting from 2010, on the west coast of Salento area (Lecce province, southern Italy), symptoms of the so-called CoDiRO "Olive Quick Decline Syndrome" were observed (i.e., leaf scorching, twig and branch wilting,

tree die-back) [1]. Subsequently, the syndrome spread over many hectares of olive trees causing dramatic effects and currently it represents a serious local emergence [2]. *Xylella fastidiosa* is a Gram-negative bacterium member of the *Xanthomonadaceae* (γ -proteobacteria) which colonizes the xylem of host plants and the foreguts of insect vectors [3]. This plant pathogen was recently found associated with the CoDiRO [4, 5]. *X. fastidiosa* infects a wide range of host plants, such as grapevine, almond, blueberry, cherry, peach, coffee, and citrus trees, causing great economic losses mainly in North, Central and

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Journal : 40538

Article No : 107

MS Code : CBTA-D-17-00011

Dispatch : 23-6-2017

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Pages : 9

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South America [6]. The pathogen is also known to infect landscape and ornamental trees such as oak, maple, and oleander [4]. The introduction of latently infected ornamental plants from Central America was reported as the venue for the subsequent spread of the pathogen in Salento [7]. The main symptoms associated with infections of *X. fastidiosa* are the marginal and or apical leaf scorching, twig and branch die-back and plant death [3]. The symptoms have been attributed to prolonged water stress caused by bacterium growth and biofilm formation in the xylem vessels [8]. The negative socio-economic impact of *X. fastidiosa* infection in North and South America (i.e., USA and Brazil) and now in Italy is well known. In the latter case, the negative impact is due not only to relevant economic losses, but also to the dramatic effect on the typical cultural heritage represented by olive trees. Apulian 1000-year-old olive trees are protected as local patrimony and considered a symbol of the local identity, also for their massive presence in the territory. Moreover, six over a number of 60 mln of olive trees were classified as monumental, as resulted from the first olive tree census in the whole Apulia region [9]. For these reasons, when implementing a specific control method in order to contain the further disease spread, it is important to study also its social and cultural acceptability as well as its socioeconomic impact [10]. Therefore, the need to adopt specific agronomic and phytosanitary measures to improve the vegetative state of the plants has been recently raised. For these reasons, the study of the CoDiRO effects on symptomatic olive trees by using the NMR-based metabolomics approach was carried out. In this study, we analyzed the effects of a CE approved fertilizer, known as DENTAMET® (i.e., a mixture of zinc and copper complex with hydracids of citric acid [11]) sprayed on symptomatic olive trees located in Salento. This fertilizer can be considered as a product with a dual action formulation by which the correction of Zn and Cu deficiencies occurs very quickly, as well as a resistance is induced by the cyclic peptide resembling the chemical structure of several antimicrobial substances released from the plant in response to different stresses. Thanks to the low environmental impact and few restrictions on its use, Dentamet is allowed for organic farming and commercialized in more than 30 countries in the world [11].

The changes in the metabolomic profiles of aqueous extracts obtained from leaves of Ogliarola salentina and Cellina di Nardò olive cultivars assessed by ¹H NMR spectroscopy and MVA are reported.

Methods

Sample collection and treatment procedures

All samples were obtained by collecting, in mid June 2015, leaves from olive trees, strictly located in the same

pedoclimatic areas, which were previously treated with DENTAMET® (i.e., two treatments in April, followed by one in May). This foliar fertilizer based on zinc (4.0% w/w) and copper (2.0% w/w) salt is obtained by an electrolytic process, and then complexed with hydracid of citric acid obtained through a process of fermentation similar to those who occurs in nature by certain soil fungi, [11] (Scheme 1). Although the reported metal ion binding of the citrate molecule in DENTAMET is according to Scheme 1, the possible presence of other coordination mode and/or solvolytic species in the patented commercial product could not be excluded.

In addition, leaves from olive trees showing symptoms of CoDiRO (i.e., twig wilting and branch die-back) with any kind of control measures were collected and served as control samples (i.e., untreated).

A total of 55 leaf samples, each one containing 15–20 apparently healthy leaves, were collected from three different CoDiRO-exhibiting olive tree orchards, located in the districts of Veglie, Galatina, and Galatone (Lecce province, Salento peninsula), where the presence of *X. fastidiosa* was previously ascertained by PCR detection. Briefly, the plants were tested for the bacterium presence using 5 µL of DNA template obtained after extraction with Qiagen kit from 1 g of leaf midribs in PCR with primers and under conditions reported in literature [12]. In the district of Veglie, a total of 16 samples (eight from two treated olive cv. Ogliarola salentina and eight from two untreated cv. Ogliarola salentina trees) were collected. In Galatina district, a total of 19 samples (eight from two treated and eleven from four untreated trees) of olive cv. Cellina di Nardò were processed, while in Galatone district 20 samples (ten from treated and ten from untreated trees) were employed from olive cv. Cellina di Nardò. Where possible, the leaf samples were collected from the four cardinal points in each plant, and, in some cases, from the sucker in the lower part of the plant (Table 1).

Sample preparation for NMR analysis

Olive trees leaves were plunged into liquid N₂ before freeze drying and ground to a fine powder with a stainless steel blender. Freeze-dried plant material (15 mg) was weighted into an autoclaved 2 mL Eppendorf tube.

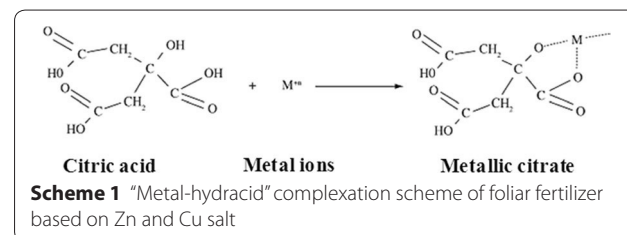


Table 1 Summary of the experimental trials with DENTAMET® Diagro

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	<i>Olea europea</i> cv. Ogliarola salentina	<i>Olea europea</i> cv. Cellina di Nardò	<i>Olea europea</i> 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

137 A D₂O:CD₃OD (1 mL, 80:20) mixture containing 0.05%
138 w/v TSP-*d*4 (sodium salt of trimethylsilylpropionic acid)
139 was added to each sample. The contents of the tube were
140 mixed thoroughly with vortex mixer and then heated at
141 50 °C in a water bath for 10 min. After cooling at room
142 temperature, the samples were spun down in a microcen-
143 trifuge at 10,000g for 5 min; then, 700 µL of the superna-
144 tant were filled into a 5 mm NMR tube.

145 NMR spectroscopy and data processing

146 All measurements were performed on a Bruker Avance
147 III 600 Ascend NMR spectrometer (Bruker, Germany)
148 operating at 600.13 MHz for ¹H observation, equipped
149 with a z axis gradient coil and automatic tuning-match-
150 ing (ATM). Experiments were run at 300 K in automa-
151 tion mode after loading individual samples on a Bruker
152 Automatic Sample Changer, interfaced with the soft-
153 ware IconNMR (Bruker). For each sample, a one-dimen-
154 sional ZGPR and NOESY experiment (referred to as
155 1D-NOESY), including water signal saturation during
156 relaxation, mixing time, and a spoil gradient, was per-
157 formed. All spectra were referenced to the TSP signal
158 (*d* = 0.00 ppm). NMR data were processed using TopSpin
159 2.1 (Bruker) and visually inspected using Amix 3.9.13
160 (Bruker, BioSpin). ¹H-NMR spectra were segmented in
161 rectangular bucket (0.04 ppm width) and integrated. The
162 data table generated with all the spectra was submitted to
163 multivariate statistical analysis, using Simca-P version 14
164 (Umetrics, Sweden).

165 Chemometric data analysis

166 Multivariate analyses were applied to mean-centered
167 data. The Pareto scaling method, which is performed
168 by dividing the mean-centered data by the square root
169 of the standard deviation, was then applied to the

variables (the bucket-reduced NMR spectra). Unsu-
pervised (principal component analysis, PCA) and
supervised (partial least squares discriminant analysis,
PLS-DA and orthogonal partial least squares discrimi-
nant analysis, OPLS-DA) pattern recognition methods
were performed to examine the intrinsic variation in
the data.

Principal component analysis is the mostly used unsu-
pervised dimensionality reduction method to get an
overview of the multivariate profiles and for identifying
patterns in data. PLS-DA and OPLS-DA were used for
the discrimination of samples with different character-
istics (such as cultivars and/or geographical origin) as
shown in several recent studies of metabolomics [13–16].
The robustness and predictive ability of the statistical
models for discrimination purposes were tested by cross-
validation default method (7-fold) and further evaluated
with permutation test (400 permutations) of SIMCA 14
software, Umetrics, Umea, Sweden [17, 18]. The *R*² (cum)
and *Q*² (cum) are the two parameters that describe the
goodness of the models. The former (*R*²) explains the
total variations in the data, whereas the latter (*Q*²) is an
internal cross validation parameter, which indicates the
predictability of the model [19].

194 Results and discussion

195 Multivariate statistical analyses on NMR data

196 Typical 600 MHz ¹H NMR spectra for treated and
197 untreated aqueous extracts obtained from olive leaves
198 are reported in Fig. 1. Sugars and organic acids charac-
199 terized the alkyl and hydroxyl-alkyl region (middle and
200 low frequencies, from 5.5 to 0.5 ppm), whereas phenolic
201 compounds are typical for the aromatic region (high
202 frequencies, 9.0–6.0 ppm). Relevant ¹H NMR data are
203 reported in Table 2. The metabolites were assigned on



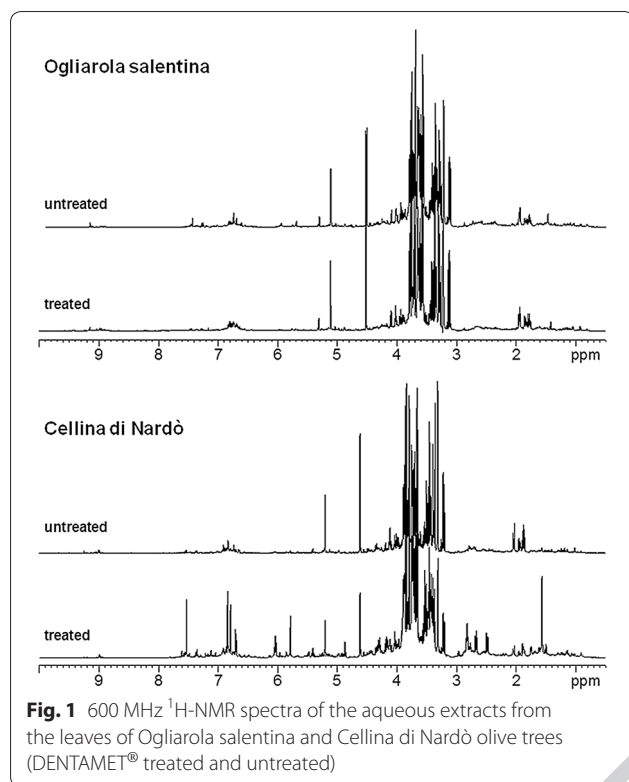


Fig. 1 600 MHz ^1H -NMR spectra of the aqueous extracts from the leaves of Ogliarola salentina and Cellina di Nardò olive trees (DENTAMET[®] treated and untreated)

Table 2 Chemical shifts (δ) and assignment of relevant metabolite resonances in the ^1H NMR spectrum of *X. fastidiosa* leaves extracts

Metabolites	δ (ppm)
Alanine	1.48 (d)
Choline	3.20 (s)
α -Glucose	5.22 (d), 3.50 (dd)
β -Glucose	4.62 (d), 3.22 (t)
Sucrose	5.41 (d), 3.53 (dd)
Hydroxytyrosol	6.90 (d), 6.81 (d), 6.70 (d), 3.78 (t), 2.76 (t)
Tyrosol	6.84 (d), 6.70 (d), 3.78 (t), 2.76 (t)
Oleuropein	6.04 (q), 5.78 (s), 3.89 (sugar protons), 2.68 (d), 2.5 (dd), 1.55 (d)
Other aldehydic and dialdehydic forms of oleuropein and ligstroside	9.22 (s) 9.18 (s), 6.04 (d), 5.74

A typical 600 MHz ^1H -NMR spectrum with the assignment of the metabolite peaks was reported in Additional file 1: Figure S1

Letters in parentheses indicate the peak multiplicities

s singlet, d doublet, t triplet, dd doublet of doublet, q quartet

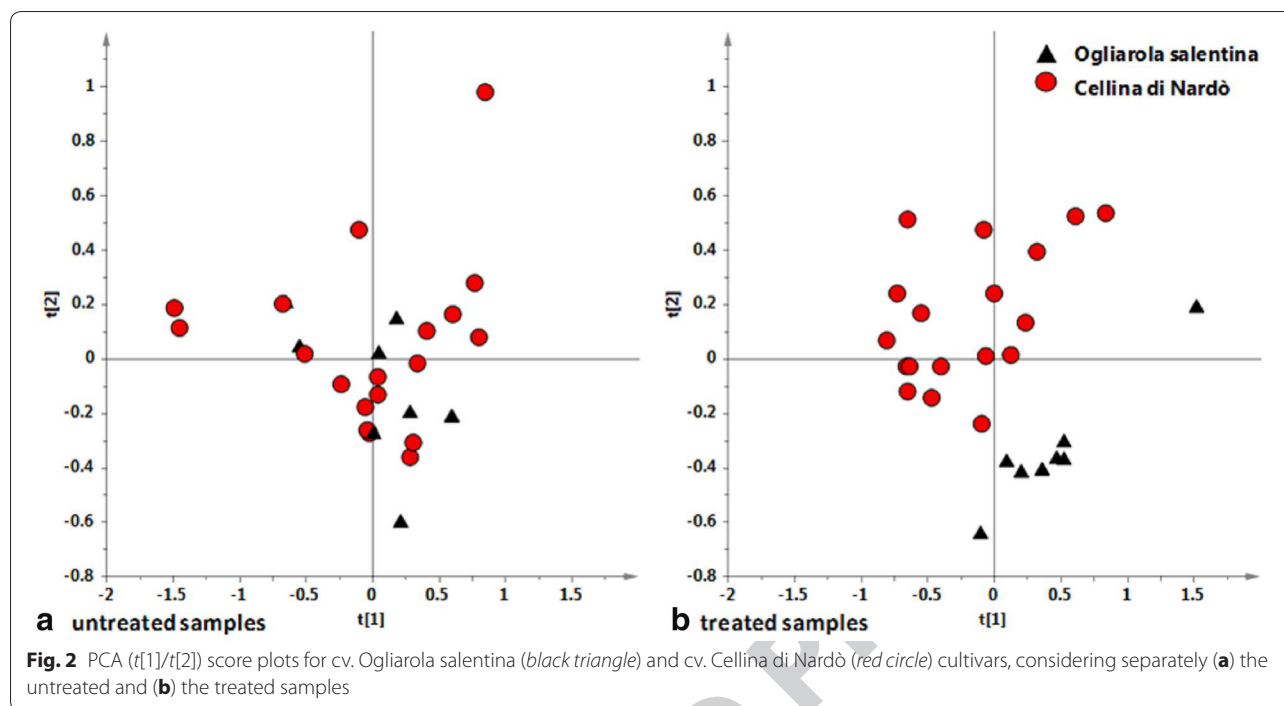
204 the basis of 2D NMR spectra analysis (2D ^1H Jres, ^1H
205 COSY, ^1H - ^{13}C HSQC, and HMBC) and by comparison
206 with published data [20–22].

207 In order to reveal a possible general data group-
208 ing of the samples, an unsupervised PCA analysis was
209 applied considering separately the untreated and the
210 DENTAMET[®] treated samples of the two classes, Ogli-
211 arola salentina and Cellina di Nardò cultivars (Fig. 2).
212 The first PCA model is built with all the untreated Ogli-
213 arola salentina and Cellina di Nardò samples (Fig. 2a).
214 In this model, the first two components give $R^2 = 0.71$
215 and $Q^2 = 0.55$, $t[1]$ and $t[2]$ accounting for 56 and 15%
216 of the explained variance, respectively. The PCA model
217 of Fig. 2b is built with all the treated Ogliarola salentina
218 and Cellina di Nardò samples, and the first two compo-
219 nents give $R^2 = 0.71$ and $Q^2 = 0.59$, describing the sam-
220 ples distribution in the bidimensional space defined by
221 $t[1]$ and $t[2]$ (in this case accounting for 53 and 18% of
222 the explained variance, respectively). Analysis of the
223 PCA ($t[1]/t[2]$) score plots showed that only in the case
224 of samples treated with DENTAMET[®], a clear partition
225 of data was observed with a good grouping according
226 to the original cultivar (Ogliarola salentina and Cellina
227 di Nardò). On the other hand, when all the CoDiRO-
228 exhibiting plants (the untreated samples) were submitted
229 to PCA analysis, no differences of metabolomic profiles
230 appeared among samples. These results suggest that the

effect of the presence of a pathogen, such as *X. fastidi-
osa* on the metabolic profile of CoDiRO symptomatology
exhibiting plants samples, could be predominant with
respect to the differences normally observed among the
olive cultivars. Nevertheless, the presence of other external
factors, abiotic or biotic, responsible for the lack of
discrimination observed in PCA score plot (Fig. 2a) could
not be excluded. On the other hand, the further observed
discrimination after the treatment strongly suggests that
CoDiRO complex could be responsible for the metabolic
uniformity observed in Fig. 2a.

All the CoDiRO-exhibiting plants (considering at the
same time treated and untreated samples and the two
cultivars, Ogliarola and Cellina di Nardò) have been
studied by unsupervised PCA and supervised OPLS-
DA analyses. The explorative unsupervised method
(PCA) used for the whole data did not give clear group
separation (data not shown), while the OPLS-DA model
based on treated vs. untreated category as discriminating
class (Fig. 3) produced a good descriptive but weak
predictive model [one predictive and four orthogonal
components give R^2X (cum) = 0.82, R^2Y (cum) = 0.60,
and Q^2 (cum) = 0.24]. A first level of discrimination
was also observed on the basis of the treatment applied
to the samples (DENTAMET[®] treated vs untreated).
The study of the variables responsible for the class separation
observed in Fig. 3a could be determined by the analysis
of the p (corr) in the S-line plot (Fig. 3b). Interestingly,
by examining the loadings of the original variables a
higher relative content of polyphenols, such as oleuropein
and ligstroside and their derivatives (tyrosol



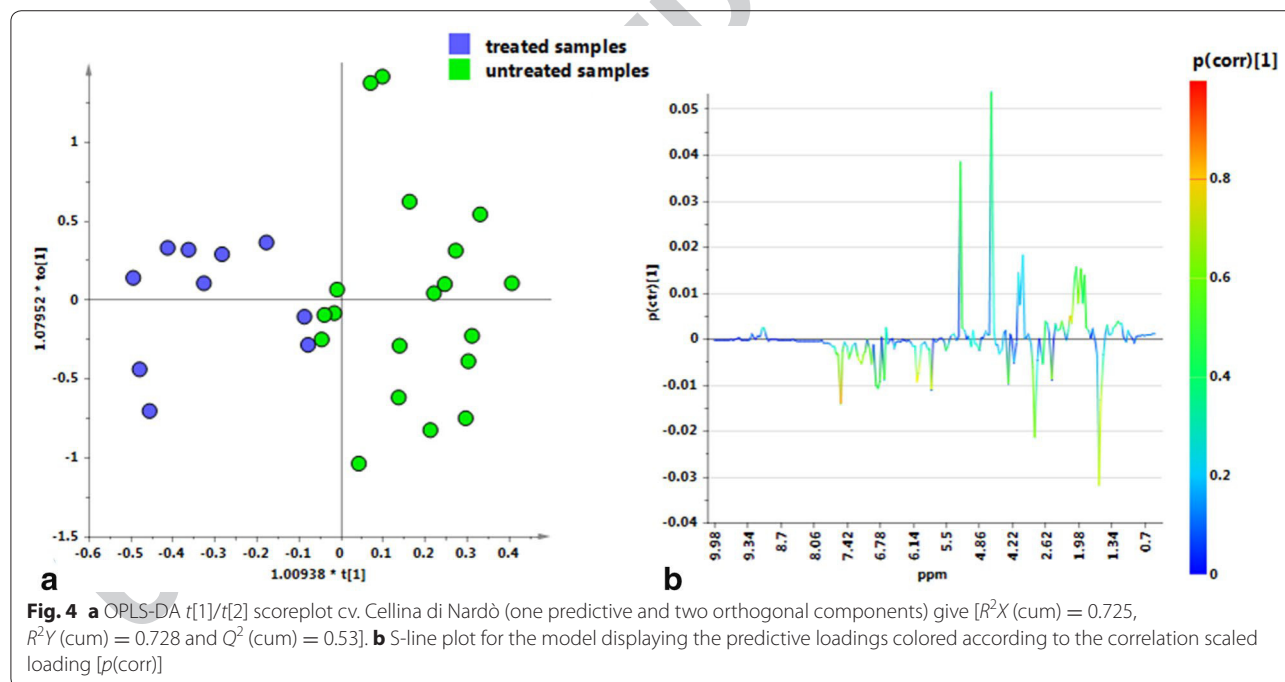
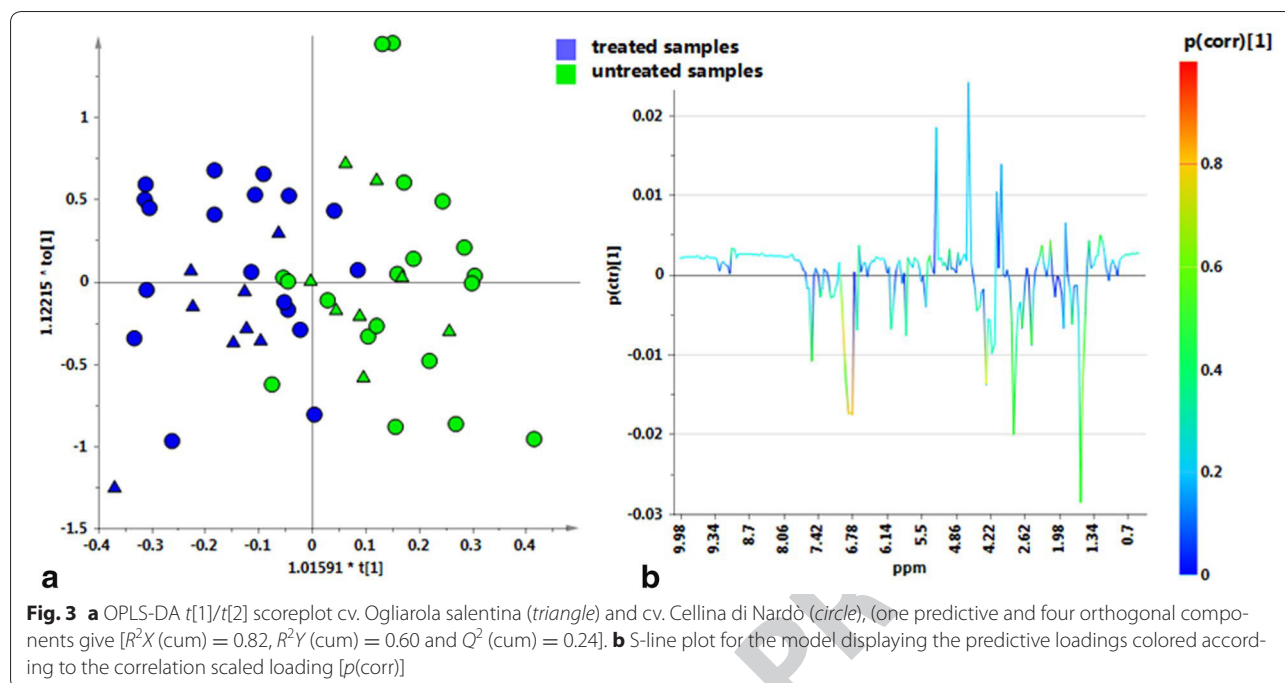


262 and hydroxytyrosol) was observed for the treated sample. This resulted from the presence of signals in the aromatic region, at frequencies corresponding to tyrosol and hydroxytyrosol (6.84, 6.70, 3.78, 2.76 ppm and 6.90, 6.81, 6.70, 3.78, 2.76 ppm) and oleuropein and its aldehydic derivatives (6.04, 5.78, 3.89, 2.68, 2.5, 1.55 ppm and 9.22, 9.18, 6.04, 5.74 ppm). On the other hand, a higher relative content of sugars was observed for the untreated samples. This resulted from the presence of signals of anomeric protons of α - and β -glucose (doublets at 5.22 and 4.62 ppm, respectively) [20–22].

273 In order to deeply analyze the response of the CoDiRO-exhibiting plants to the treatment, the metabolic profile of treated and untreated plants was better characterized for each cultivar. In the first case, the unsupervised PCA analysis, applied to Cellina di Nardò samples resulted in no data clustering observation for the first two components, PC1 and PC2. Indeed, inspection of further components other than the first two was required (PC2 vs. PC4), in order to observe in the scoreplot a certain degree of samples clustering (see Additional file 1: Figure S2). Therefore, the supervised OPLS-DA analysis gave a good model [1 + 2 + 0, R^2X (cum) = 0.725, R^2Y (cum) = 0.728 and Q^2 (cum) = 0.53] with a clear partition between DENTAMET[®] treated and untreated samples (Fig. 4a). By examining the loadings of the original variables, the molecular components distinctive for each class could be determined. CoDiRO-exhibiting samples showed a lower polyphenol content for untreated with respect to

291 treated samples. Interestingly, a relatively higher polyphenol content (with respect to other Salento cultivars) was observed for the for Cellina di Nardò EVOOs samples originating from healthy trees [23]. In the case of Ogliarola salentina samples, the chemometric analysis of a matrix composed by a reduced number of ¹H spectra showed a clear partition between DENTAMET[®] treated and untreated samples, as reported in the OPLS-DA score plot (Fig. 5a). The unsupervised method (PCA) gave unclear results (see Additional file 1: Figure S3), while the OPLS-DA analysis gave a good model [1 + 2 + 0, R^2X (cum) = 0.786, R^2Y (cum) = 0.837 and Q^2 (cum) = 0.489], showing a clear partition between DENTAMET[®] treated and untreated samples along the first predictive component (Fig. 5a). By examining the loadings of the original variables in the S-line plot (Fig. 5b), the molecular components distinctive for each class could be defined. In particular, infected Ogliarola salentina untreated plants showed a metabolic profile characterized by a higher content of polyphenol molecules. On the other hand, the DENTAMET[®]-treated infected plants were characterized by a higher sugar content. In this case, the observed polyphenols decrease, in treated with respect to control trees, is in accord with the polyphenols production associated to drought stress [24, 25], notwithstanding the levels of phenols are characteristic for each cultivar such as abiotic stress responses [26–28]. Interestingly, when unsupervised exploratory method (PCA) was applied a good grouping according

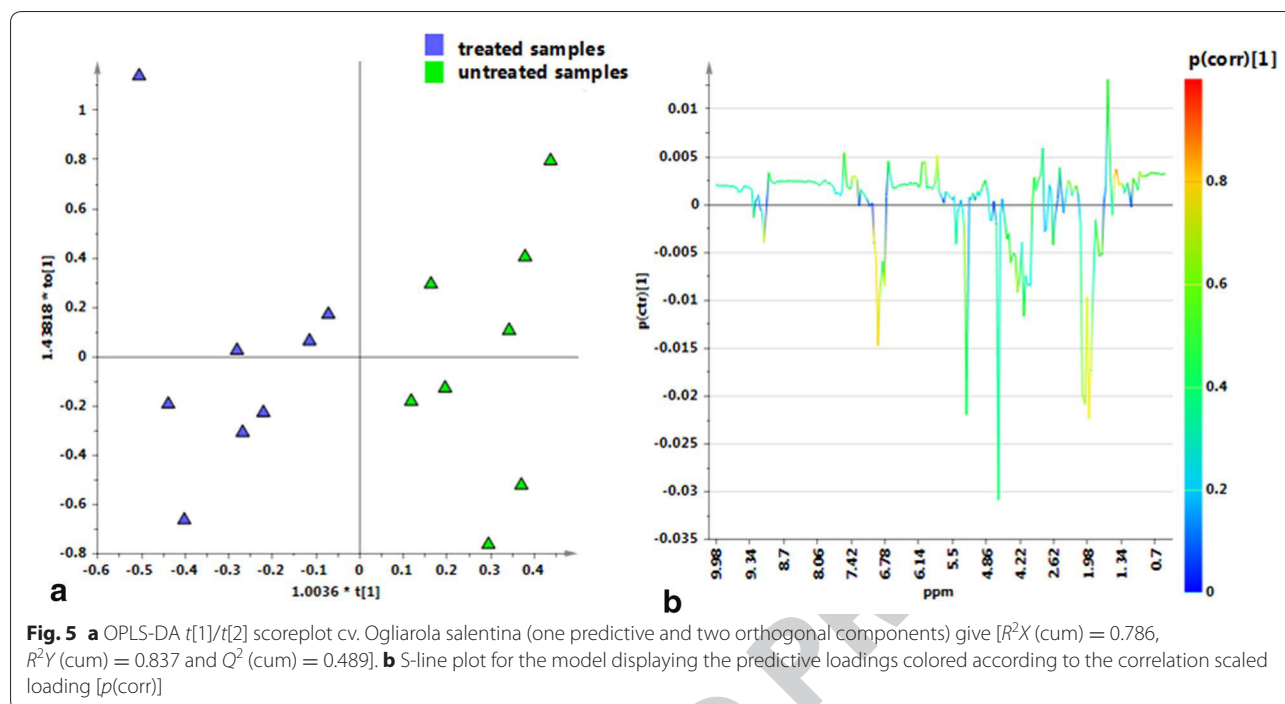




320 to the original cultivar (Ogljarola salentina and Cellina di
 321 Nardò) resulted only in the case of DENTAMET[®]-treated
 322 samples while the differences normally observed accord-
 323 ing to the olive cultivars were not predominant in the
 324 case of untreated CoDiRO-exhibiting plants. Considering
 325 all the CoDiRO-exhibiting samples (obtained from both
 326 the Ogljarola salentina and Cellina di Nardò cultivars),

supervised methods (in particular OPLS-DA analy-
 327 sis) were required to obtain the discrimination between
 328 untreated and DENTAMET[®]-treated samples. The dif-
 329 ferent olive cultivars would seem to differently respond to
 330 the DENTAMET[®] treatments by altering their metabolic
 331 profiles in the sugars and polyphenols content. In partic-
 332 ular, OPLS-DA analyses revealed that Cellina di Nardò
 333





334 CoDiRO samples showed a lower polyphenols and a
 335 higher sugar content for the untreated with respect to the
 336 treated ones. In contrast, in the case of Ogliarola salentina
 337 CoDiRO-exhibiting trees, DENTAMET[®]-untreated
 338 samples showed a higher content of polyphenol molecules
 339 while samples from treated infected plants were characterized
 340 by a higher sugar content. As already reported in literature [24, 25],
 341 physiological and biochemical responses to stress are closely cultivar-dependent.
 342 In the present case, since studied Ogliarola salentina and Cellina di Nardò
 343 cultivar trees were also characterized by a different level of pathogen attack,
 344 the observed changes in metabolic profiles due to DENTAMET[®]
 345 treatment could be also related to such a specific factor.
 346 Moreover in plants, both polyphenols and carbohydrates can play a relevant
 347 role during the pathogen infection. In fact, the downregulation of polyphenol-oxidase
 348 expression dramatically increased the susceptibility of tomato plants to
 349 *Pseudomonas syringae* pv. *tomato* [29]. In olive, polyphenols could also
 350 play a direct and significant role in protecting the tree towards the infection
 351 of phytopathogens. In particular, oleuropein, as extracted from olive waste
 352 water, has been shown to be effective towards *Pseudomonas savastanoi*
 353 pv. *savastanoi*, the causal agent of olive knot disease, by inhibiting its
 354 growth [30]. Also carbohydrate activation can be related to a number of
 355 stress that can disturb or subvert to normal plant metabolism such as a
 356 wound or a pathogen attack [31, 32]. It should be said, however, that the
 357 interplay occurring between

363 polyphenols and carbohydrates during a pathogen infection has not been
 364 studied in detail. The metabolomic approach here preliminary applied to
 365 olive trees showing the CoDiRO symptoms could shed light into their
 366 interrelationships upon a pathogen attack. 367

368 Conclusions

369 In the present work, non-targeted ¹H NMR fingerprinting, in combination
 370 with unsupervised (PCA) and supervised pattern recognition techniques (in
 371 particular OPLS-DA), was used to analyze the response of the CoDiRO-
 372 exhibiting olive trees cvs Ogliarola salentina and Cellina di Nardò, grown
 373 in the Salento peninsula, to the DENTAMET[®] treatments. In both cultivars,
 374 the occurrence of *X. fastidiosa* was ascertained. Metabolic profiles
 375 obtained by ¹H NMR spectra were able to differentiate the two cultivars
 376 for treated with respect to untreated samples. On the other hand, the effect
 377 of DENTAMET[®] treatment resulted specific and different for each of the
 378 two studied cultivars. In particular, treated Cellina di Nardò trees showed
 379 a higher polyphenols content, whereas treated Ogliarola salentina trees
 380 showed a higher sugar content. It should be stressed that a different
 381 incidence and severity of disease was observed for the Cellina di Nardò
 382 with respect to the Ogliarola salentina CoDiRO-exhibiting trees, with the
 383 former showing a more severe infection. Therefore, further research is
 384 needed to determine if the different metabolic responses are correlated to
 385 olive cultivars or to pathogen attack 390



391 levels or both factors. In particular, in order to more
392 precisely assess the relationships between *X. fastidiosa*
393 infection and the polyphenol and carbohydrates trend
394 upon the infection, future studies can be performed by
395 using precise dose(s) of bacterial inoculum to be inocu-
396 lated in pot-cultivated olive plants. Then the relative con-
397 tent of polyphenols and carbohydrates into the leaves
398 together with the multiplication trend of the phytopatho-
399 gen could be monitored during the seasons by the metab-
400 olomic approach here applied.

401 Additional file

Additional file 1: Figure S1. Typical 600 MHz ¹H NMR spectrum of olive leaf extracts. Diagnostic peaks of some metabolites are indicated in the two expansions of the spectrum. **Figure S2.** PCA (a) t[1]/t[2] and (b) t[2]/t[4] score plots for cv. Cellina di Nardò cultivars, considering the DENTAMET® treated and the untreated samples [six components, R²X (cum) = 0.935, Q² (cum) = 0.816]. **Figure S3.** PCA (a) t[1]/t[2] and (b) t[2]/t[4] score plots for cv. Ogliarola Salentina cultivars, considering the DENTAMET® treated and the untreated samples [six components, R²X (cum) = 0.964, Q² (cum) = 0.815].

402 Abbreviations

403 CoDiRO: Olive Quick Decline Syndrome; CE: European community; MVA:
404 multivariate statistical analysis; PCA: principal component analysis; OPLS-DA:
405 orthogonal partial least squares discriminant analysis.

406 Authors' contributions

407 CRG and LDC: prepared the samples for NMR analysis, performed the NMR
408 experiments, analyzed and interpreted the NMR and statistical data, wrote and
409 reviewed drafts of the manuscript, prepared the final writing; MS collected leaf
410 samples, reviewed drafts of the paper, and contributed to the final writing; MP,
411 LZ, FM collected leaf samples and performed the PCR analysis; GC collected
412 leaf samples; AB reviewed drafts of the paper and contributed to the final
413 writing; GD and NC collected leaf samples and performed the PCR analysis;
414 DM contributed to prepare the samples and treated the plant material; FPF
415 supervised NMR experiments and statistical analysis, wrote and reviewed
416 drafts of the paper and contributed to the final writing. All authors read and
417 approved the final manuscript.

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427 Acknowledgements

428 The authors thank the olive farms "La Duchessa" (Veglie, (LE), Italy) and Cosimo
429 Pinca (Galatone, Galatina, (LE), Italy) for hosting the trials.

430 Competing interests

431 The authors declare that they have no competing interests.

432 Availability of data and materials

433 Additional Data attached in the Additional file 1. Other data and materials
434 could be requested from the corresponding author.

435 Consent for publication

436 The authors agreed the publication of the manuscript in this journal.

Ethics approval and consent to participate

This manuscript is an original paper, and has not published in other journals.
The authors agreed to keep the copyright rule.

Funding

This study was supported by Apulia Region, DGR N.2185/2016, Project "Strat-
egie di controllo integrato per il contenimento di *Xylella fastidiosa* in oliveti
pugliesi ed analisi epidemiologica del complesso del disseccamento rapido
dell'olivo (CoDiRO)".

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Received: 21 March 2017 Accepted: 16 June 2017

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