A Holistic Model Can Be Used to Explain the Symbiotic Mitigation of the Olive Quick Decline Syndrome

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Abstract. The introduction of a bio-fertilizer (BF), based on symbiotic micro-organisms as agents to promote the yield and health of crops, into the soil is aimed at inducing modifications in the rhizosphere as well as in the plant phenotype. It is here shown that, in *Olea europaea* cv. Ogliarola di Lecce groves affected by Olive Quick Decline Syndrome (OQDS, involving *Xylella fastidiosa subsp. Pauca*): i) the vegetative responses to the disease appeared highly variable, but the symptoms were significantly mitigated in two groves out of six and aggravated in only one; ii) the NIR-tomoscopy of hay-litter-bags from non-inoculated soils can be used to forecast the outcome of BF inoculation; iii) a holistic model that gathers differential and compositional analyses of the leaf (pH, crude protein, water) and of the soil (respiration) can explain over 95% of the average mitigation response to BF inoculation. The two keys for a successful inoculation have been identified as a high degree of variability of the soil conditions, which is favorable for welcoming the guest BF (lowering the fingerprint of the control litter-bags) and for an enhancement of the homogeneity of the leaves (with increases in the fingerprint of the leaves treated with BF). However, inoculation of BF consortia is far from being the ultimate remedy to mitigate OQDS. Further studies are needed, at a field level, to clarify the soil hosting capacity and to define the mycorrhizal and / or endophytic * plant * pathogen interactions using rapid methods (litter-bags, foliar pH, near-infrared tomoscopy).

Extended abstract. Upon an attack by pathogens or insects, plants can "enlist" the help of protective microorganisms and increase their microbial activity to contrast pathogens [1]. However, the delivery of a complex BF, based on microbial consortia (Micosat F ®) [2], can act by modifying the plant's physiology and lowering the in-vivo raw leaf pH, which is a concrete and easy endpoint to measure. Apart from accelerating the metabolism, BF acts on the induction of the genes of resistance present in plants, but which are not expressed without prior contact with pathogens. As a result of the inoculum, a consequent activation or suppression of otherwise silent genes is obtained, which recent studies on the genome of plants have identified as being closely related to contrast and alarm activities toward several phyto-pathologies. Demonstrations of this were pertaining to the recovery of pears heavily affected by *Erwinia amylovora* fire blast [3], and resolving strong outbreaks in coffee Nicaraguans plantations affected by *X. fastidiosa subsp. pauca* [4]. Since the above considerations, the present work has been conducted with three objectives: i) to revitalize the root microbiome of the infected plants, that is, to reactivate the symbiotic interactions between the root system of the olive tree and the Arbuscular Mycorrhizae network; ii) to strengthen the defense capabilities of the olive trees by increasing their resilience to the pathogen, through an

activation of the latent gene pool; iii) to evaluate simple and accessible techniques to measure the health status of the olive trees as well as the biological status of the soil. This study has involved the use of a BF, which has been defined as "*symbiotic*" because it contains arbuscular mycorrhizal spores, *propaguli*, and other microbial species. The BF was used at a dose of 20 kg ha^{-1} also falling into the framework of precision agriculture, because the inoculum is distributed precisely in the proximity of the secondary roots of adult olive trees affected by OQDS. After three months, treated Symbiotic (S) and non-inoculated Control (C) plants (436 as total) logged in six farms located near Ugento (LE, Italy) were compared to establish their disease severity, by means of a visual appraisal of the Disease Severity Degree (DSD) [0-healty; 1- One dry branch; 2- two÷five dry branches; 3 => five dry branches; 4- plant almost dried; 5- plant totally dry] (Tab. 1). Complementary rapid tests were applied: the published *litter-bags* coupled to NIR-SCIO evaluation [5] (differential and respiratory), and the foliar pH [6, 2], and the new foliar NIR scanning (differential and compositional)[7] (Tab. 1). The Lab-SCIO™ software used the Random Forest algorithm to fingerprint the four classes (CC, CS, SC, SS).

Table 1. Summary table of the disease severity evolution (DSD) observed in the plants (Y) and the results of independent analytical determinations (X) of the foliar pH, foliar NIRS and litter-bags: values of the BF symbiotic effect d_S/C = Ln(S/C).

Farm	Disease Severity	Foliar NIRS fingerprint		Leaf H^+	*Litter-bags* fingerprint		Soil Respiration	Leaf Water	Leaf Protein
	d_DSD	F_CC	F_SS	d_S/C	L_CC	L_SS	d_S/C	d_S/C	d_S/C
A	-3.4%	72.0%	79.0%	-14%	65.0%	87.0%	-1.0%	-0.1%	0.3%
B	0.0%	67.0%	68.0%	-7%	63.0%	70.0%	-11.3%	0.8%	0.7%
C	**-40.4%**	67.0%	93.0%	16%	61.0%	85.0%	3.0%	0.9%	-3.4%
D	**-11.4%**	72.0%	72.0%	5%	73.0%	70.0%	-6.4%	0.0%	-0.7%
E	3.8%	65.0%	73.0%	16%	100.0	100.0	37.9%	0.5%	-0.4%
G	**7.9%**	57.0%	61.0%	5%	75.0%	77.0%	-17.3%	0.5%	-0.1%

[1] The one significant non-favorable value is bold red and the two significant favorable values are in bold blue.

A descending parabolic curve (Fig. 1) shows that the disease decreased (favourable) when the fingerprint of the S leaves recognized as S was high, and vice versa. Therefore, the symbiotic treatment increased the homogeneity of the leaves.

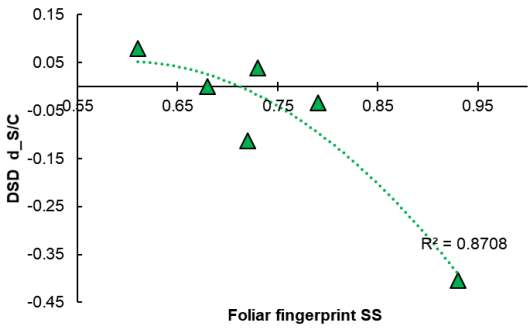
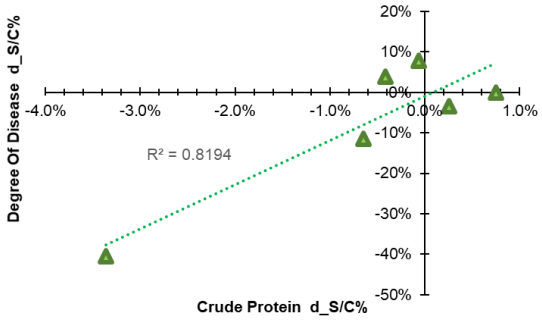


Figure 1. Regression of the variation in the disease severity degree (DSD) (Y = d_S/C = Ln(S/C)) on the NIRS fingerprinting of the S-symbiotic olive leaves (X= fingerprint_SS).

Among all the variables, the crude protein in the leaf emerged because of its high correlation (+0.90) with the variation in the disease severity degree, as clearly shown in Fig. 2, where a positive relationships linked the two traits, which means that a decrease in the Ln(S/C) of protein favored a reduction in the disease.

Figure 2. Regression of the variation in the disease severity degree (Y=DSD d_S/C = Ln(S/C)) on the variation of the mean crude protein content of the leaf (X= CP d_S/C = Ln(S/C).



The litter-bags fingerprint appeared very different from the foliar NIRS fingerprint. In fact, an ascending parabolic trend is shown in Fig. 3: when the disease decreased, the fingerprint of the Control litter-bags was lower. Vice versa, when the fingerprint of the Control litter-bags increased, the incidence of the disease increased. Therefore, the symbiotic treatment increased the heterogeneity of the litter-bags.

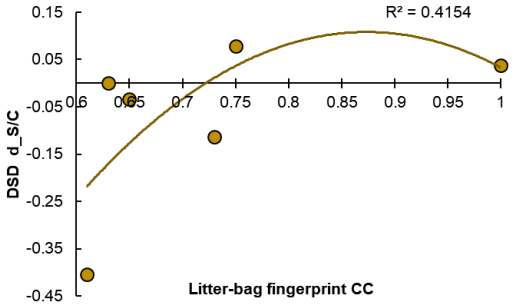


Figure 3. Regression of the degree of variation of the disease severity (Y, d_DSD) on the fingerprint value of the Control litter-bags (X, Litter-bags_CC).

The average spectra of the litter-bags from the control plots in the six groves were calibrated directly with the effective size Ln(S/C) of the plant response to the disease caused by the pathogen from the soil inoculation. For this purpose, the spectra imported into the WinISI II v1.04 chemometric software were math-treated as 2nd derivates (code SNV, 2,8,8,2), and the observed responses were then fitted to spectra using the modified partial least squares (MPLS) method, in which two latent variables were admitted, and the model was cross-validated. A valuable result was obtained (Fig. 4)

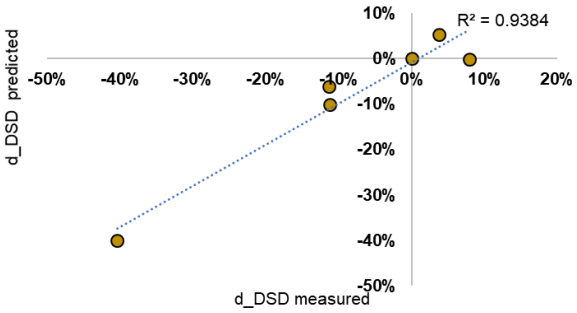
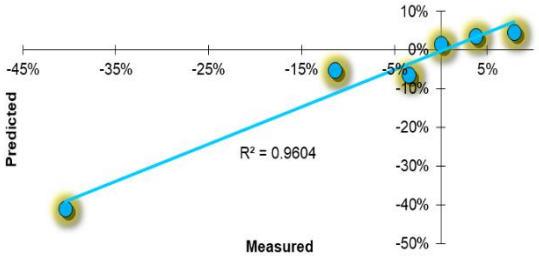


Figure 4. Fitting of the symbiotic evolution of the disease severity degree (d_DSD) from the average NIR spectra of the Control litter-bags.

A final holistic elaboration, using the Partial Least Squares method (StatBox 6.5, Grimmer Soft, Paris), gathered the most characteristics results from the three main information tools, concerning the plant-soil-BF interactions, in a model [1] that was then used to explain a possible symbiotic mitigation process of the disease:

[1] Ln(S/C) DSD = d_H^+ (-0.155); d_R (-0.209); F_SS (-0.301); L_CC (0.281); Leaf water (-0.133); Leaf crude protein (+0.350). R^2 0.96 (Fig.5) R^2 cross-validated 0.87.

Figure 5. Linear regression scatterplot of the holistic model solutions for the symbiotic evolution of the disease severity degree (DSD).



In the model [1] there were 6 characteristic factors: 1) the acidity differential, with standardized factor d_H^+ = -0.155, had a negative sign as the factors were opposite: symbiotic BF lowered the pH, raised the H+ and therefore reduced the disease; 2) the fingerprint of the CC litter-bags (L_CC = +0.281) had a positive sign: when the value was reduced, the pathological degree diminished, a sign that the BF had produced some effects; 3) the fingerprint of the SS leaves (F_SS= -0.301) had a high value and a negative sign: when the value was increased, the disease was reduced; 4) the soil respiration had a favorable negative sign (-0.209): when the respiration increased, the incidence of the disease decreased; 5) the water content of the leaves accounted for -0.133 units, which means that a greater quantity of water flowed and remained in the olive leaves during mitigation and recovery; 6) the crude protein accounted for +0.350 units, the highest contribution to the fitting.

Since *X. fastidiosa subs. pauca* damages the vessels and the leaves of plants, why should we pay attention to the roots of these plants? In this work, we have shown that an initial factor (like an *original sin*) can be found in the soil biota. Although we are unaware of the exact etiology of such a favorable response to the inoculation of a small quantity of selected BF, we have described and tested a simple method - litter-bags – which are useful to evaluate the hospitality of a plant-soil complex to the foreign but beneficial BF.

Moreover, we have described and tested a set of rapid analyses to monitor the evolution of the disease, not by means of remote sensing, but through friendly contact with the plant and its earthly world.

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