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Attempts to Reduce the Systemic Spread of *Xylella fastidiosa* in Olive Trees by Pruning

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Abstract: *Xylella fastidiosa* subsp. *pauca* (*Xfp*) is the plant pathogenic bacterium causing the epidemic of olive quick decline syndrome decimating olive trees in the Apulia region (southern Italy). The lack of any effective therapeutic application for the control of this pathogen and its categorization as a regulated quarantine pathogen in many countries worldwide, impose mandatory eradication and containment measures. Based on current EU legislation, containment measures apply in those areas where the bacterium is widely established, such as in the Apulia region, and thus containment strategies to mitigate and cope with the infections are needed. We set up a field trial to assess if pruning interventions could limit and/or recover *Xfp*-infected trees by reducing the systemic spread of the bacterium and the severity of the desiccation phenomena typically compromising the crown of the highly susceptible cultivars, e.g., cv. Cellina di Nardò. Trees subjected either to major or light pruning interventions, including the removal of all the symptomatic branches, did not demonstrate a reduced bacterial colonization or development of symptoms. After two years of targeted pruning interventions, no significant amelioration of the sanitary status of the infected olive trees was recorded, suggesting that the sole application of these interventions is not effective to counteract the impact of the bacterium in the susceptible olive trees.

Keywords: disease management; olive; heading; thinning; pruned biomass

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1. Introduction

Xylella fastidiosa (*Xf*) is a vector-borne bacterial agent able to colonize and multiply in the xylem vessels of more than 650 plant species [1]. Although the majority of the susceptible host plants may not display symptoms upon infection, the bacterium is the causal agent of some of the most detrimental diseases affecting important agricultural crops. Notably, the bacterium has a long history in the American continent [2], being the causal agent of severe diseases in grapes (Pierce's disease) and citrus (citrus variegated chlorosis), as well as being associated with leaf-scorching syndromes reported on almond, oleander and several different host species (i.e., landscape trees). The wide host range and the different biological features of the bacterial strains are linked to their large genetic variability. Actually, at least three different subspecies are widely recognized based on genomic sequences [3], with strains falling in the same subspecies sharing, in principle, a common set of host plants. Even so, the capability of a given strain to infect or not to infect a plant species cannot be fully predicted based on genetic signatures, still requiring *in planta* biological tests. The subspecies *multiplex* accounts for the largest host range, including numerous crops, ornamentals and landscape trees, while the main host plants of the subspecies *fastidiosa* are *Vitis* spp. and *Prunus amygdalus*; conversely, citrus and coffee plants are notoriously susceptible to strains of the subsp. *pauca* (*Xfp*). In the last decade, strains of this subspecies have been reported to cause a severe disease affecting olives, the olive quick decline syndrome (OQDS), firstly described in the Apulia region (southern

Italy) [4] and then reported in Argentina [5] and Brazil [6]. Olive quick decline syndrome is characterized by leaf scorching and the scattered desiccation of twigs and small branches, which in the early stages of the infection are mainly observed on the upper part of the canopy [7]. Over time, symptoms become increasingly severe and extend to the rest of the crown, causing the death of the entire tree.

The discovery of the OQDS epidemic in southern Italy represented the first report of bacterial infection established in an open field in Europe and in the Mediterranean Basin [4], thus raising major concerns for the whole Old Continent and the Mediterranean countries. Although this represents the most severe epidemic of *Xf* in the EU, several outbreaks are nowadays reported in France, Spain and Portugal, with infections occurring in several host plants other than olives, and mainly associated with strains of the subspecies *multiplex* and *fastidiosa*. Investigations to unravel the European xylem feeders responsible for the spread of the bacterium unambiguously indicated spittlebugs, in particular *Philaenus spumarius* [8]. The cultivars Cellina di Nardò and Ogliarola salentina are highly susceptible to *Xfp* [9]; unfortunately, these are the most diffused cultivars in the infected area and they are characterized by very large century-old trees, hard pruning and high alternate bearing [10–13].

At the EU level, *Xf* is currently regulated as a quarantine and priority pest (EU Regulations 2019/1702 and 2020/1201), implying that mandatory surveillance programs are in place in all Member States. Legislative provisions include eradication and containment measures, which are implemented according to the pest risk assessment carried out in the different outbreaks. For example, where outbreaks occur at limited extension the infected areas are under eradication measures (i.e., in mainland France and Spain; in Portugal), whereas in the Apulia region, Corsica and the Balearic Islands, where infections are well established (i.e., affect a large number of host species and a vast territory, and a high density of vector populations occurs) the contaminated territories are under containment measures. These include all actions and interventions aiming at reducing the rate of spread of the infections and the pressure of inoculum in order to mitigate the negative impact of the infections, i.e., by using resistant or immune plant species, by applying formulations to improve plant response to infections and by controlling vector populations. In these few past years, the need for effective containment strategies has prompted the development of numerous experimental research programs aiming at developing practical solutions to cope with the detrimental impact of OQDS [14].

In this context, we tested the efficacy of different types of pruning interventions as a tool for (i) reducing the prevalence of the infected branches on the olive canopies, eventually (ii) recovering infected trees at the early stage of the infections, and ultimately (iii) to create unfavorable conditions on the olive canopies, which serve as a refuge for the adult *P. spumarius* throughout the entire summer season. The evidence that (i) *Xfp* is a slow-growing bacterium, (ii) the bacterial movement in the xylem vessels is mainly upward with the transpiration flow, (iii) symptoms appear first on the upper part of the canopies and (iv) sites of infections correspond to the apical portions of the shoots, i.e., those suitable for insect feeding, suggested that removing branches from infected trees at the early stage of the infections could help to (i) reduce the efficiency of downward movement and thus the systemic spread of the bacterium, (ii) impair the successful establishment of novel infections following insect transmission and (iii) determine a reduction of the bacterial population in the infected trees, which is proven to be positively correlated with symptom expression [15,16].

Targeted pruning interventions (i.e., removing symptomatic branches) have been tested in the past, alone or in combination with other treatments, in oleander [17], grapevine [18], almond [19], coffee [20] and citrus trees [21]. However, the efficacy of pruning as a means to recover *Xf*-infected plants is highly uncertain, depending on the host plant, the stage of the infections and the application of other management strategies to protect plants from new infections. Indeed, pruning interventions may result in the significant production of new vegetative shoots, attracting insect vectors and increasing the risk of re-infection [22].

In this two-year work, we applied different pruning interventions on naturally *Xfp*-infected mature olive trees to assess if removing diseased branches would restrain tree colonization, and thus reduce the prevalence and impact of the infections. Our results experimentally documented that pruning interventions had no significant impact on the recovery of the olive trees located in the infected area of the Apulia region, in olive groves under high *Xfp* pressure of inoculum.

2. Materials and Methods

2.1. Olive Orchard

The olive orchard selected for the study consisted of 20-year-old trees of the cv. Cellina di Nardò, spaced 7 m × 5 m apart (285 trees/ha), located in the infected zone (Lequile, Lecce, southern Italy; 40°14′39″ N; 18°10′16″ E; 54 m a.s.l.). Trees were irrigated and grown on sandy clay loam soil, with good content of potassium and low content of nitrogen and phosphorus. Climate conditions were those typical of the Mediterranean climate, with an average annual temperature of 17.5 °C and a total rainfall of 645 mm, 40% concentrated during fall–winter period.

A total of 30 *Xfp*-symptomatic olive trees were selected for the trials (Figure 1) and subjected to three different types of pruning procedures (10 for each procedure) for two consecutive years (2017 and 2018). The 10 trees selected for each treatment were fully randomized in the orchard; moreover, they were at the initial stage of symptom development, with symptom severity scores in the range of 1–1.5; the score was based on an empirical scale ranging from 0 to 5 referring to the % of the canopy affected by desiccation: 0 = absence of symptoms; 1 = up to 10–20%; 2 = up to 30%; 3 = 40%; 4 = up to 60%; 5 = majority of the canopy with desiccated branches.

Figure 1. The experimental olive orchard. Note the initial symptoms (red arrows) on the trees before pruning treatments started.

In February of both years, three pruning procedures were applied (Figure 2):

- heading cuts of the secondary and tertiary branches with symptoms (HEADN);
- thinning cuts of the secondary and tertiary branches with symptoms (THINN);
- thinning cuts of the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN).

Figure 2. Pruning treatments applied (from left to right): heading (HEADN) and thinning (THINN) the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN).

In the heading treatment, the branches were shortened by about 50% in length, whereas thinning treatments consisted in a complete removal of the vegetative axes. The CLEAN procedure represented the conventional pruning intervention, and thus was used as a reference procedure (EU Regulations 2019/1702 and 2020/1201).

2.2. Diagnostic Tests

Prior to start of the trials in February 2017, all experimental trees were sampled and tested for the detection and quantification of *Xfp*. Trees were then retested in November 2018. A total of eight mature asymptomatic shoots were collected from N, S, E and W orientation and xylem tissues subjected to DNA extraction and quantitative (q) real-time PCR [23]. Trees were retested at the end of the experiment with the same procedure.

The estimation of the bacterial population in the sampled shoots was determined by plotting the quantification cycle (Cq) recorded for each tree on the linear standard curve generated using 10-fold serial dilutions prepared using artificially contaminated samples containing known bacterial concentration: from 10^3 to 10^7 CFU/mL (colony forming unit/milliliter).

2.3. Vegetative and Production Assessment

After each pruning intervention, the fresh biomass (kg tree^{-1}) and the median diameter (cm) of the removed axes were measured for each tree; the fresh biomass was weighed by means of a dynamometer.

At the end of the vegetative season, in July of both experimental years, the vegetative growth (cm), the number of nodes (n) and the average internode length (cm) were determined. Measurements were carried out on 4 shoots/tree selected at the four cardinal points of the canopy.

Yield (kg tree^{-1}) was assessed for each tree by harvesting all the fruits in November of both years, whereas fruit oil content (% fresh weight) was assessed as the average of the olives collected from the 10 trees of each treatment.

2.4. Data Analysis

The data were analyzed by ANOVA, followed by post hoc testing (Student–Newman–Keuls protected test) using R 2.15.0 software (R Foundation for Statistical Computing, Free Software Foundation, Boston, MA, USA). Trees that tested negative by qPCR in 2017 were excluded from statistical analysis.

3. Results

3.1. Symptoms on Pruned Trees

Using the THINN procedure, all symptomatic branches were completely removed from the trees during both years of application. Following the first pruning interventions (in February 2017), the trees subjected to HEADN and THINN cuts developed a higher vegetative mass but lower reproductive organs than the CLEAN pruning system, which conversely did not allow the removal of all the symptomatic branches (Figure 3).

Figure 3. The CLEAN tree once pruned (thinning the suckers and the shoots placed at a distance $\leq$2 m from the soil) at the end of the first vegetative season and just before the second pruning intervention (**left**). The focus shows clear symptoms on the crown (**right**).

Unfortunately, after the second pruning treatments (2018), the trees subjected to HEADN and THINN cuts developed only vegetative shoots, which later on started to desiccate, even if all the symptomatic materials had been removed. The trees subjected to the CLEAN pruning system did not develop new vegetation in 2018. At the end of 2018, symptoms were recorded on all trees, regardless of the pruning procedure used, with an average severity score between 3.5 and 4.

3.2. Infection Status of Pruned Trees

As expected, almost all the symptomatic trees tested at the start of the trial tested positive (Table S1). Only in four trees could the bacterium not be detected, most likely due to an erratic and low bacterial concentration determining the failure of the detection;

these four trees were removed from the trial. The bacterial population estimated in the trees of each treatment was in the range of 10^5–10^6 CFU/mL, indicating that in some trees (those harboring 10^6 CFU/mL) the infections were well established, and the bacterium had systemically colonized these trees. As the infections progressed from the first to the second year, an increase in bacterial concentration was detected in all the trees that in 2017 had the lowest CFU/mL values. It should be remarked that in highly susceptible and symptomatic olive trees the highest detected concentrations under field conditions range from 10^6 to 10^7 at the maximum (M. Saponari IPSP-CNR, personal communication).

As shown in Table 1, no statistical differences were detected among the bacterial concentrations harbored in the trees subjected to the different pruning treatments within each year, with CFU/mL values being 2.4 and 3.5 times higher in the second year.

Table 1. Bacterial concentration expressed in CFU/mL estimated by qPCR in two subsequent years in the experimental trees subjected to: heading cuts (HEADN) and thinning cuts (THINN) of the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN). Trees that tested negative by qPCR in 2017 were excluded from statistical analysis. The mean values of two years and standard errors are reported. No significant differences among treatments for each year were found. Letters denote significant differences between years for each treatment (SNK test; $p = 0.05$).

Pruning Treatment	Bacterial Concentration (Cq)	
	2017	**2018**
HEADN	$5.45 \pm 0.6 \times 10^5$ b	$1.92 \pm 0.4 \times 10^6$ a
THINN	$6.00 \pm 1.1 \times 10^5$ b	$1.14 \pm 0.3 \times 10^6$ a
CLEAN	$6.42 \pm 0.9 \times 10^5$ b	$1.60 \pm 0.3 \times 10^6$ a
Mean	$5.96 \pm 1.3 \times 10^5$ b	$1.64 \pm 0.7 \times 10^6$ a

3.3. Vegetative and Reproductive Parameters

As reported in Table 2, the fresh weight of the biomass removed applying CLEAN cuts was the lowest (10.1 kg tree^{-1}), followed by the HEADN cuts (14.4 kg tree^{-1}), while the highest amount of biomass was removed upon THINN cuts (19.4 kg tree^{-1}). These values positively correlated with the diameters of the pruned branches, corresponding to 2 cm diameter for CLEAN cuts, 3.1 cm for HEADN and 3.5 cm for THINN cuts.

Table 2. Fresh weight and diameter of the biomass removed by different pruning treatments: heading (HEADN) and thinning (THINN) the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN). The mean values of two years and standard errors are reported. Letters denote significant differences among treatments (SNK test; $p = 0.05$).

Pruning Treatment	Fresh Weight (kg Tree^{-1})	Diameter (cm)
HEADN	14.4 ± 1.2 b	3.09 ± 0.19 a
THINN	19.4 ± 0.9 a	3.46 ± 0.16 a
CLEAN	10.1 ± 0.5 c	1.87 ± 0.06 b

With regard to the vegetative regrowth, Table 3 reports the values recorded after the first pruning intervention, as during the second year almost all the new shoots became symptomatic and died. The HEADN cuts allowed the development of the longest shoots (10.6 cm) with the highest number of nodes (4.6) compared to the THINN and CLEAN pruning cuts, both producing similar values in terms of the length of the shoots (8.3 cm and 8.8 cm, respectively) and the number of nodes (3.8 and 4.2, respectively). No influence of the pruning system was recorded on the length of the internodes.

Table 3. Sprout growth, node number and internode length induced by different pruning treatments: heading (HEADN) and thinning (THINN) the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN). The mean values and standard errors refer to the first year. Letters denote significant differences among treatments (SNK test; $p = 0.05$).

Pruning Treatment	Sprout Growth (cm)	Node Number (n)	Internode Length (cm)
HEADN	10.6 ± 0.6 a	4.6 ± 0.4 a	2.3 ± 0.08 a
THINN	8.3 ± 0.3 b	3.8 ± 0.2 b	2.2 ± 0.07 a
CLEAN	8.8 ± 0.4 b	4.0 ± 0.2 b	2.1 ± 0.09 a

Olive yield and oil content were determined only during the first experimental year (Table 4) because in the second year the trees were not bearing. The trees subjected to CLEAN interventions yielded the highest production values, both in terms of fruits (32.3 kg/tree) and oil content (16.4%). Intermediate yields were obtained from the trees subjected to HEADN interventions, with 19.4 kg of olives per tree, and 14.5% of oil content, while the lowest yields were obtained from the trees subjected to THINN interventions, with 14.7 kg of olives per tree and 14.4% of oil content.

Table 4. Olive yield and oil content resulting from differently pruned trees: heading (HEADN) and thinning (THINN) the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance ≤2 m from the soil (CLEAN). The mean values and standard errors refer to the first year. Letters denote significant differences among treatments (SNK test; $p = 0.05$).

Pruning Treatment	Olive Yield (kg tree^{-1})	Oil Content (% Fresh Weight)
HEADN	19.4 ± 1.9 b	14.5
THINN	14.7 ± 2.5 c	14.4
CLEAN	32.3 ± 3.0 a	16.4

4. Discussion

Given the knowledge gaps in the *Xfp*-olive pathosystem and the high uncertainty of the effectiveness of using pruning as an OQDS management strategy, in this work we attempted to gather experimental data on the impact of three different types of pruning interventions for recovering symptomatic olive trees. Pruning is an important part of the management strategy to control key olive pathogens such as *Venturia oleaginea* (Castagne) Rossman & Crous (=*Spilocaea oleaginea*) [24] and an efficient control practice for non-systemic bacteria, such as olive knot (*Pseudomonas savastanoi* pv. *savastanoi*), minimizing the effects of the infections [25]. Moreover, pruning plays a pivotal role in reducing *Pseudomonas syringae* pv. *actinidiae* populations, by removing symptomatic shoots and reducing the vegetation density and thus the relative humidity inside the trees [26].

Conversely, the systemic bacteria results are controversial or not straightforwardly reproducible: this is the case with *Xf* [27] and Candidatus *Liberibacter asiaticus* [28]. For *Xfp*, pruning at the early stage of the infection was effective in reducing bacterial prevalence in citrus trees in Brazil [29], while it did not reduce the bacterial populations in *Xfp*-infected blueberries [30]. On the other hand, very severe pruning has been reported as effective to cure *Xfp*-infected grapevines and coffee plants [20,31]. Overall, pruning as a management strategy for *Xf* has only been shown to be effective in a limited number of cases, on very early symptoms and together with vector control and the use of healthy plants for new plantations [27].

In olives, the recent emergence of *Xfp* affecting century-old trees has raised the question of whether removing secondary or tertiary branches can help recover symptomatic trees, and in particular to save century-old trees. Empirical field observations in the OQDS epidemics in the Apulia region have shown that the severe pruning of infected olive trees resulted in the emission of new sprouts from the base of the trees [32], but, so far, this has

not been shown to cure the plants and prevent them from dying; severe pruning caused the death of the olive tree during the following months [12].

An updated estimation of the pruning biomass in the olive groves in the Apulia region indicates an average value of 4.5 kg per tree [33]. A more circumstantiated study carried out in Spain under the same agronomical conditions, such as tree vigor, tree age, tree density, irrigation and yield level, indicated an average pruning biomass of 14–16 kg per tree for regular pruning, and over 20 kg per tree when pruning was performed for rejuvenation and secondary branches were pruned [34]. These values are in line with those reported in our study, given that the cv. Cellina di Nardò is a high-vigor genotype [35].

Under our experimental conditions, HEAD cuts determined the highest rate of shoot regrowth. This is in line with previous work showing that olive trees subjected to heading cuts produce, over time, a high rate of shoot regrowth [36]. On the other hand, tree regrowth depends on non-structural carbohydrate (NSC) reserves in the remaining organs and recovery of trees after pruning depends on pruning intensity [37]. Most likely, this is the reason why THINN cuts resulted in the lowest regrowth rate, as a consequence of the reduced NSC reserves, energy for the growth of new shoots in the early growing season [38] and starch storage [39].

The removal of peripheral branches decreases canopy volume and increases sylleptic bud breaking and sprout growth, while the competition between vegetative and reproductive shoots reduces total fruit yield, especially for high-vigor cultivars such as Cellina di Nardò [40]. Moreover, it is well documented in the literature that light pruning interventions in olives promote a dominant reproductive expression in the following vegetative season; on the contrary, intense pruning can induce vegetative expression and a delay in production [41,42]. Our results are consistent with these observations: the olive yield and oil content recovered from trees subjected to CLEAN cuts (9.2 t per hectare and 16.4%) were in line with what was reported for the olive orchards in the area under study [13,35]. The HEAD and THINN cuts significantly reduced olive production to 5.5 t and 4.2 t per hectare, respectively, and about 14.4% of oil yield as mean. On the other hand, both pruning systems did not result in any reduction of the infection prevalence and bacterial population in the *Xfp*-infected trees. Thus, the removal of substantial portions of the tree canopies through the HEAD and THINN pruning systems is likely to have negative and multi-season impacts on olive regrowth, yield and quality, as reported for vineyard *Xf*-infected and for C*Las*-affected sweet orange trees, where severe pruning resulted in fruit of low quality [28,43]. Indeed, the traditional high-vigor cultivars have a low number of branches, but are thicker and longer, with fruiting shoots mostly concentrated in the periphery of the canopy; thus, intense pruning on such cultivars causes a severe drop in fruit yield [40].

Under controlled conditions and in potted plants, it was demonstrated that the bacterium upon artificial inoculation in the shoots is able to efficiently colonize the roots of grafted plants of highly susceptible plants [44] and thus spread systemically. The capability of the bacterium to move downward in the xylem tissues may thus impair the effectiveness of the pruning interventions carried out at the initial stage of the symptom development of the canopy. The incubation period (latency) of infections in olives has been shown to be more than 1 year [45]. A recent work by Dos Santos et al. [46] on olive trees in Brazil showed a broad dispersion of the bacterium into the canopy and the root system, independent of the severity of symptoms, with the bacterium detected frequently in the asymptomatic shoots. It is then probable that when the first symptoms appear on field trees, the bacterium has already colonized the main organs of the trees, impairing the effectiveness of the pruning of the symptomatic branches to recover the trees from the infections. This is also confirmed by our data showing that in most of the trees at the start of the experiment the bacterial population had already reached high population levels and had most likely colonized a large part of the trees.

5. Conclusions

To our knowledge, this is the first experimental study testing the effectiveness of pruning interventions as a strategy to reduce the impact of *Xfp*. Under the epidemic conditions occurring in the infected area of Apulia, light or more severe pruning interventions did not reduce the olive bacterial colonization in infected trees.

Despite the results obtained in our work showing that pruning cannot recover OQDS-affected trees, it should be remarked that performing annual light pruning in traditional olive groves is a recommended good agronomic practice to preserve tree productivity and to prevent the development of several diseases, including OQDS. As such, regular pruning and the removal of suckers is a recommended practice in those olive-growing areas under high risk for *Xfp*, being able to reduce the attractiveness of the trees for spittlebugs, while removing insect feeding sites, i.e., asymptomatic shoots at the early stage of the infection. An effective strategy for the prevention and control of *Xfp* epidemics remains the use of pathogen-resistant cultivars [47].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12122917/s1, Table S1: Bacterial concentration (Cq) expressed in CFU/mL estimated by qPCR in two subsequent years in the experimental trees subjected to: heading cuts (HEADN) and thinning cuts (THINN) of the secondary and tertiary branches with symptoms; thinning the suckers and the shoots placed at a distance $\leq$2 m from the soil (CLEAN).

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