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Coexistence between *Xylella fastidiosa* Subsp. *pauca* and Susceptible Olive Plants in the Salento Peninsula (Southern Italy)

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Abstract: Olive Quick Decline Syndrome (OQDS) associated with *Xylella fastidiosa* subsp. *pauca* is one of the most destructive diseases of olive trees in the Salento Peninsula (Southern Italy), particularly on the cultivars Cellina di Nardò and Ogliarola Salentina. This study proposes the NuovOlivo protocol as a management strategy to permit coexistence between *X. fastidiosa* subsp. *pauca* and olive drupes and extra-virgin oil production. Thirty-two private olive orchards affected by OQDS and cultivated following the standard agronomic techniques in use in the area were surveyed during the 2019–2023 olive-growing seasons. Tested cultivars included Cellina di Nardò, Ogliarola Salentina, Coratina, Ascolana Tenera, Nociara, Leccino, and Bella di Cerignola. At the beginning of the protocol application, the susceptible plants showed OQDS symptom severity of 40–80% and did not produce olives or oil, while the resistant(?)/tolerant cultivars exhibited a 2–8% leaf scorch and a drupe production less than 1–2 kg/plant. After the removal of dry branches in January–February, plants were sprayed two times per year (preferably in March and October) with NuovOlivo®, a mixture of aqueous botanical extracts esterified in the presence of sodium hydroxide with vegetable oils and activated at the time of use with sodium bicarbonate. In all the orchards, a slow-release fertilizer was distributed, and weeds were controlled by mowing or chopping. Upon eventual appearance, the dry twigs were removed. Treated olive trees produced new vegetation, rebuilt their foliage, reduced OQDS symptoms, and turned out cluster inflorescence and drupes. The drupes yield was 6.67–51.36 kg per plant, with an average of 13.19% in extra-virgin olive oil (free acidity 0.01–0.2%). Plants used as controls showed OQDS symptoms and were unproductive, and newly formed shoots were desiccated. The proposed protocol promotes, supports, and restores new vegetation, flowers, fruits, and oil production of the treated olive plants affected by OQDS without losing susceptible olive plants. The Apulian landscape and economy, based on olive presence and production, could be also safeguarded.

Keywords: resilience; disease management strategy; botanical extracts; natural plant detergent; recovery; olive oil yield

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1. Introduction

The olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*) is the symbol of the Mediterranean landscape, where it has been cultivated for its fruits and wood since the prehistoric period [1–3]. Phoenicians, Greeks, and Romans introduced olive cultivation into the Mediterranean basin, especially in Italy, Greece, Tunisia, Spain, and Portugal. The Roman Empire favored olive oil production in Puglia, including Salento [1,4]. The cultivars Cellina di Nardò (≡ Olivo di Nardò ≡ Saracena) and Ogliarola Salentina (≡ Ogliarola di Lecce) produce high-quality oil and represent the history of Salento. Cato (234 BC–149 BC) refers to the cv Ogliarola Salentina, while the Saracens spread the cv Cellina di Nardò during the IX and XVIII centuries [1–4]. At the end of 1700, the brusca disease slowed down the cv Ogliarola Salentina diffusion, preferring the brusca-resistant cv Cellina di Nardò [5].

Since 2013, in the Salento peninsula, olive trees have been affected by Olive Quick Decline Syndrome (OQDS), showing leaf scorch (necrosis of leaf margins) along with the wilting and then drying of leaves and the scattered desiccation of twigs and small branches, mainly on the upper part of the canopy. Over time, symptoms develop progressively more severity and spread to the canopy. Drupes mummify. Leaves with leaf scorch and mummified drupes remain attached to shoots [6–8]. Stunted growth and withering of canopy parts are additionally present [9]. Twigs, branches, and trunks show irregular discoloration of xylem vessels [10]. The affected trees die off [9].

Associated with OQDS is the gram-negative, strictly aerobic, non-motile, slow-growing, fastidious, non-flagellate bacterium *Xylella fastidiosa* Wells, Raju, Hung, Weisburg, Parl and Beemer (*Proteobacteria*, *Gammaproteobacteria*, *Xanthomonadales*, *Xanthomonadaceae*) [6,11,12]. This bacterial species is one of the most destructive plant pathogens worldwide and affects many taxonomically different cultivated botanical species (e.g., grape, citrus, almond, oleander, pecan, coffee), forest trees, and wild and ornamental plants [8,11,13–15]. This bacterium colonizes the xylem vessel network of its host plants, restricts water movement, blocks water transport, and triggers the drying out of the related parts of the tree canopy [16,17]. Involved in *X. fastidiosa* symptom development are formations of bacterial aggregates and production and deposition of biofilms, in conjunction with plant tyloses and gum produced as defense responses [16–19]. Because of xylem clogging and decreased water and nutrient flow, *Xylella*-affected plants have declined yields and reduced fruit quality, and then die [12,20]. The polyphagous *Philaenus spumarius* L. (*Hemiptera*, *Aphrophoridae*) spread the subspecies *pauca* from plant to plant and among the susceptible cultivars in Puglia [7,8,21,22]. *Neophilaenus campestris* Fallén and *Philaenus italosignus* Drosopoulos and Remane are indicated as potential *Xylella*-vectors in southern Italy [23].

In the Salento infected area, the *X. fastidiosa* subsp. *pauca* eradication, at the time of the first official report, was considered impossible due to (i) the presence of about 1 million olive trees affected by OQDS; (ii) the continuum of olive orchards cultivated in the area; (iii) the feeding activity of the vector insect; and (iv) the possibilities of transporting the vector through cars, motorcycles, bicycles, tractors, buses, trains, and other means of locomotion, including farmers' shoes [21,24]. Moreover, the rapid spread of OQDS in the Puglia region was associated with drought events accompanying climate change, which altered plant physiology, triggering a general weakening of defense mechanisms and increasing their susceptibility to wilting vascular pathogens comprising *X. fastidiosa* subsp. *pauca* [24–29]. The roles of *Pseudomonas savastanoi* (E.F. Smith) Stevens pv *savastanoi* (etiological agent of olive knot); *Venturia* (*Spilocaea*) *oleaginea* (Castagne) Rossman and Crous (causing peacock leaf spot); *Glomerella* (*Colletotricum*) spp. (the causal agent of olive anthracnose); and fungal species of the genera *Phaeoacremonium*, *Phaeomoniella*, *Pleurostomophora*, and *Neofusicoccum*, usually present in olive trees affected by OQDS, remain not completely clarified [4,16,21,24,30,31].

Devastating economic losses, landscape damage, and socio-cultural and ecological injury are associated with OQDS in the Salento Peninsula [32]. The infected olive orchards covered 8000–10,000 ha in 2013, which increased to 750,000 in 2018. Approximately 22 million olive trees were affected by OQDS in 2018 [33,34], reducing olive oil production to 329 and 208 million kg in the 2021–2022 and 2022–2023 olive-growing seasons, respectively.

In California and Brazil, the management of *X. fastidiosa* subsp. *fastidiosa* on the grapevine (Pierce's disease) and *X. fastidiosa* subsp. *multiplex* on citrus fruits (citrus variegated chlorosis) provides an integrated approach to limiting its spread using resistant or tolerant plants, inoculum, vector population containment, and appropriate cultivation/hygiene practices [35]. Microbial endophytes, fungal natural products, bacteriophages, weakly virulent strains, and avirulent *X. fastidiosa* strains are promising in grapevine defense [36,37].

In Puglia, due to the limited effects of vector control and the eradication of infected plants, different measures have been utilized to manage *X. fastidiosa* subsp. *pauca*. Different pruning strategies did not restore olive plants affected by OQDS [38]. Agrochemicals did not appear to be the right action to limit pathogen spread [8,16,39,40]. Agronomical management of olive orchards associated with bioactive compounds and resistance inducers (e.g., fosetyl aluminum and acibenzolar S-methyl), treatments with COS-OGA (a complex of oligochitosans and oligopectates as active substances), Harpin proteins σ–β, Cerevisane (cell wall derivatives from the *Saccharomyces cerevisiae* strain LAS117), and N-acetylcysteine (NAC) showed increases in olive vegetation and decreases in symptoms of OQDS, but were not enough to cure the infected plants [41,42]. The mucolytic NAC, used primarily for treating human diseases, showed inhibitory effects on *X. fastidiosa* subsp. *pauca* strain 9a5c associated with Citrus Variegated Chlorosis [43], but, in Puglia, has a significant impact on the in vitro biofilm formation and lowers OQDS progression without significant bacterial population reduction [42,44]. Copper hydroxide and silver nitrate inhibit the in vitro growth of the bacterium at 0.05 and up to 0.005 ppm, respectively [45]. Nine treatments to the canopy, performed every two weeks from April to September, with Kopper® (Menfin, s.r.l., Grottaminarda, Italy) as well as a formulation containing silver nitrate reduced the OQDS symptom intensity [45]. Antimicrobial plant-derived phenolic compounds (e.g., 4-methylcatechol, catechol, veratric acid, caffeic acid, and oleuropein) showed in vitro inhibitory activity against *X. fastidiosa* subsp. *pauca* isolated from olive trees [46,47]. A family of short-chain fatty acid molecules, formally known as Diffusible Signaling Factors (DSFs), is under investigation to modulate cell–cell signaling and reduce *X. fastidiosa* infections in olive trees [48]. Fungal toxins (e.g., ophiobolin A and gliotoxin), *Trichoderma citrinoviride* Bissett culture extracts, and microfiltered Olive Mill Wastewater (OMW) fractions exhibited bactericidal properties [46]. *Methylobacterium mesophilicum* (Austin and Goodfellow) Green and Bousfield and *M. radiotolerans* (Ito and Iizuka) Green and Bousfield, endophyte-inhabiting *X. fastidiosa*-infected olive trees, and *Bacillus* spp. strains inhibiting the *X. fastidiosa* subsp. *pauca* De Donno growth [47,49,50] mitigated its dangerous effects [47] and contributed to the expression of resistance traits [47,51,52]. The onion root endophyte *Paraburkholderia phytofirmans* PsJNn reduces Pierce's disease symptom severity and *X. fastidiosa* subsp. *fastidiosa* Temecula1 in grapevines [47,53], but was not effective in the olive pathosystem in Italy [47,54]. The olive ionome, i.e., the relative content of mineral elements present in a specific tissue, interfered with OQDS symptoms expression in olive trees [55]. Ammonium chloride reduced OQDS symptoms development without significant differences in *X. fastidiosa* subsp. *pauca* populations [56]. Transmission electron microscope observations showed a bacterial cell wall alteration following calcium–carbonate–nanocarriers interactions [47,57]. None of these strategies has, so far, had extensive practical effort. Two olive cultivars, FS-17® (≡ Favolosa®) and Leccino, showing reductions in symptom expression, were considered resistant/tolerant and used to replace the susceptible olive cultivars in the infected zone according to EC decision 2020/1201 [58] and an Olive Regeneration Plan managed by the Puglia Region and the Ministry of Agriculture, Food Sovereignty and Forestry. 'Cellina di Nardò' and 'Ogliarola Salentina' plants were pollarded and grafted or uprooted and replaced with cvs Leccino or Favolosa® [4,7,24,40,59]. New olive orchards utilize intensive (400–600 cv Leccino plants/ha) and super-intensive (1250 and more cv Favolosa® plants/ha) cultivation systems [39].

Many farmers in the Salento peninsula appear oriented towards the cultivation of new crops suitable for (i) resistance to biotic factors and immunity to *X. fastidiosa* subsp. *pauca*; (ii) xerophyte habitus and adaptability to dry, arid, and drought climates; and (iii) high economic importance. Almond, pistachio, fig, hazelnut, pomegranate, citrus fruits, avocado, and carob are useful crops for replanting olive trees.

The application of NuovOlivo® (patent no. 102017000109094 of the Ministry of Economic Development; Antica Saponeria del Salento, Andrano, Italy) [60] or Dentamet® (Diagro s.r.l., Albano Sant'Alessandro, Italy) protocols [4,28,29,47,61–63] reduces OQDS symptom severity and *X. fastidiosa* concentration in treated olive plants.

In the previous trial [60], NuovOlivo® was tested during a two-year experiment considering disease index, total phenolic content, and cell membrane damage between treated and untreated cvs Cellina di Nardò and Ogliarola Salentina plants. In this paper, the effects of the NuovOlivo protocol applications on adult olive trees affected by OQDS were assessed according to drupes production and extra-virgin olive oil yield. The numbers of plants and orchards used were implemented. Lastly, the OQDS-susceptible olive cultivars were improved and the resistant(?)/tolerant cvs Leccino and Bella di Cerignola were considered. The proposed management approach could: (i) support *X. fastidiosa* subsp. *pauca* susceptible olive cultivars, (ii) maintain high drupes and oil production even in the presence of pathogen inoculum, (iii) safeguard the Apulian natural landscape associated with the two historical olive cultivars Cellina di Nardò and Ogliarola Salentina, and (iv) mitigate the effects of a quarantine pathogenic microorganism established in a large territory according to the EC recommendation.

2. Materials and Methods

2.1. Olive Orchards

The 32 private olive orchards selected for the study (Figure 1) are in the Lecce province, where *Xylella* infection is endemic.

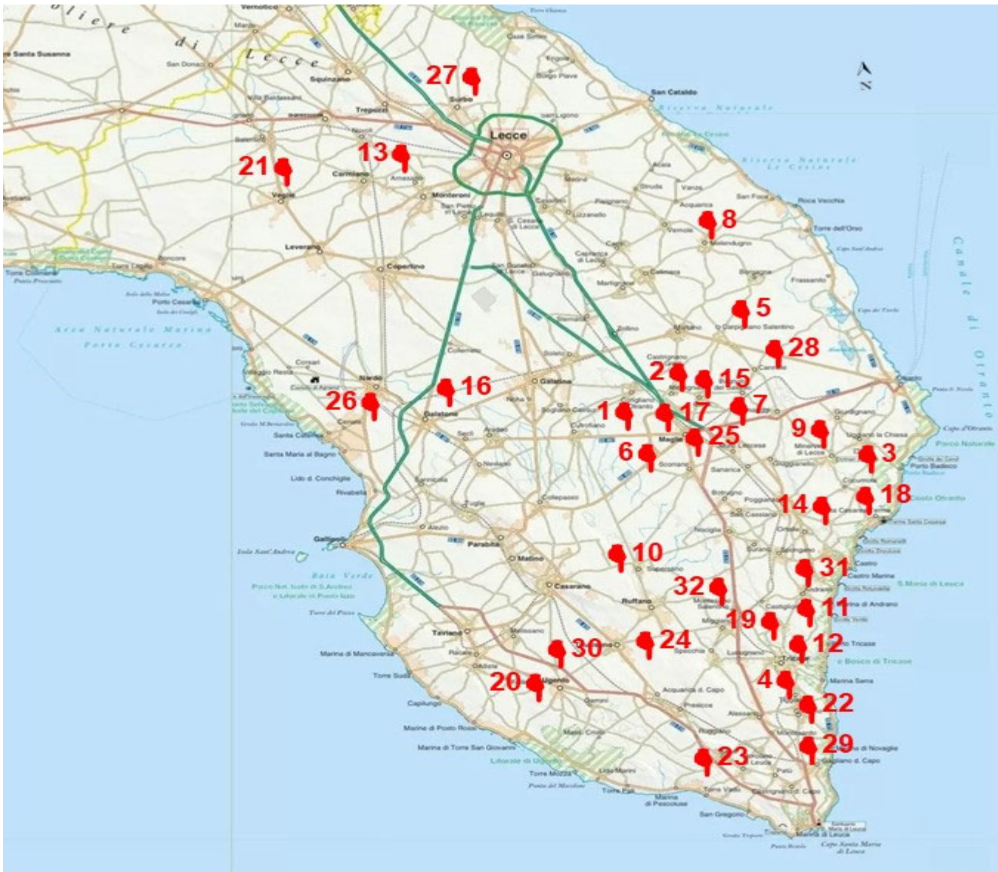

Figure 1. Distribution of the 32 private olive orchards (📍) considered in this study in the province of Lecce. Details of location and cultivar composition in the orchards are reported in Table 1.

Orchards were managed following the standard agronomic techniques in use in the area. Trees were not irrigated and were grown on reddish, clayey, silty soil that covered limestone and/or carbonate bedrock. Climate conditions were those typical of the Mediterranean climate, with an average annual temperature of 17.5 °C and a total rainfall of about 645 mm, 40% concentrated during the fall–winter period. Olive trees were grown in a polyconic vase form. Mono- and multi-cultivar olive orchards were considered

(Table 1). Cultivar composition in the orchards (Table 1) included the susceptible Cellina di Nardò (2434 plants), Ogliarola Salentina (1020 plants), Coratina (50 plants), Ascolana Tenera (65 plants), and Nociara (250 plants), as well as the resistant(?)/tolerant Leccino (251 plants) and Bella di Cerignola (35 plants). A total of 4105 plants were considered.

Table 1. Plant number and age in the different cultivars consociated and first application date in the olive orchards considered in this study.

N	Orchard Location (Lecce Province)	Olive Cultivars [a]			Age (Years)	NuovOlivo Spraying Since
		CN	OS	Others		
1	Maglie 2	80	20	0	40	April 2022
2	Melpignano	88	0	0	100	April 2022
3	Santa Cesarea Terme 2	85	5	0	200	April 2022
4	Tiggiano	10	80	0	85–90	April 2022
5	Carpignano Salentino	400	200	0	30–70	April 2021
6	Maglie 4	100	0	0	50	April 2021
7	Maglie-Otranto	250	30	0	90–100	April 2021
8	Melendugno	100	18	0	25–70	April 2021
9	Minervino di Lecce	60	20	0	50–250	April 2021
10	Supersano	130	35	L35	40–100	April 2021
11	Tricase 2	120	30	0	40	April 2021
12	Tricase 3	40	10	0	60–65	April 2021
13	Arnesano	60	40	L50	50–>200	April 2020
14	Casamassella	80	20	0	40–60	April 2020
15	Cursi	76	4	0	55–60	April 2020
16	Galatone	12	11	0	>90	April 2020
17	Maglie 3	0	2	AT35 BC35	10	April 2020
18	Santa Cesarea Terme 1	130	70	0	50	April 2020
19	Tricase 1	38	2	0	60–70	April 2020
20	Ugento 2	40	25	AT5	60–80	May 2020
21	Veglie	0	0	L86	35–40	October 2019
22	Corsano	20	20	0	80–100	October 2019
23	Salve	0	12	0	>200	October 2019
24	Taurisano	20	2	0	50	October 2019
25	Maglie 1	30	15	A25	80	October 2019
26	Nardò	250	50	L80 K50 N250	50–150	October 2019
27	Surbo	40	40	0	70–80	April 2019
28	Cannole	30	100	0	>200	April 2019
29	Gagliano del Capo	80	100	0	70–80	April 2019
30	Ugento 1	20	50	0	70–80	April 2019
31	Andrano	22	5	0	80–90	April 2017
32	Montesano Salentino	23	4	0	80–90	April 2016
33	Untreated Control	30	30	AT30 K30 N30	10–40	NT [b]

[a] CN = Cellina di Nardò, OS = Ogliarola Salentina, L = Leccino, AT = Ascolana Tenera, BC = Bella di Cerignola, K = Coratina, N = Nociara. [b] NT = not treated.

Moreover, outside the selected olive orchards, 150 plants (30 for each susceptible cultivar) were chosen as untreated controls (Table 1, Figure 2).

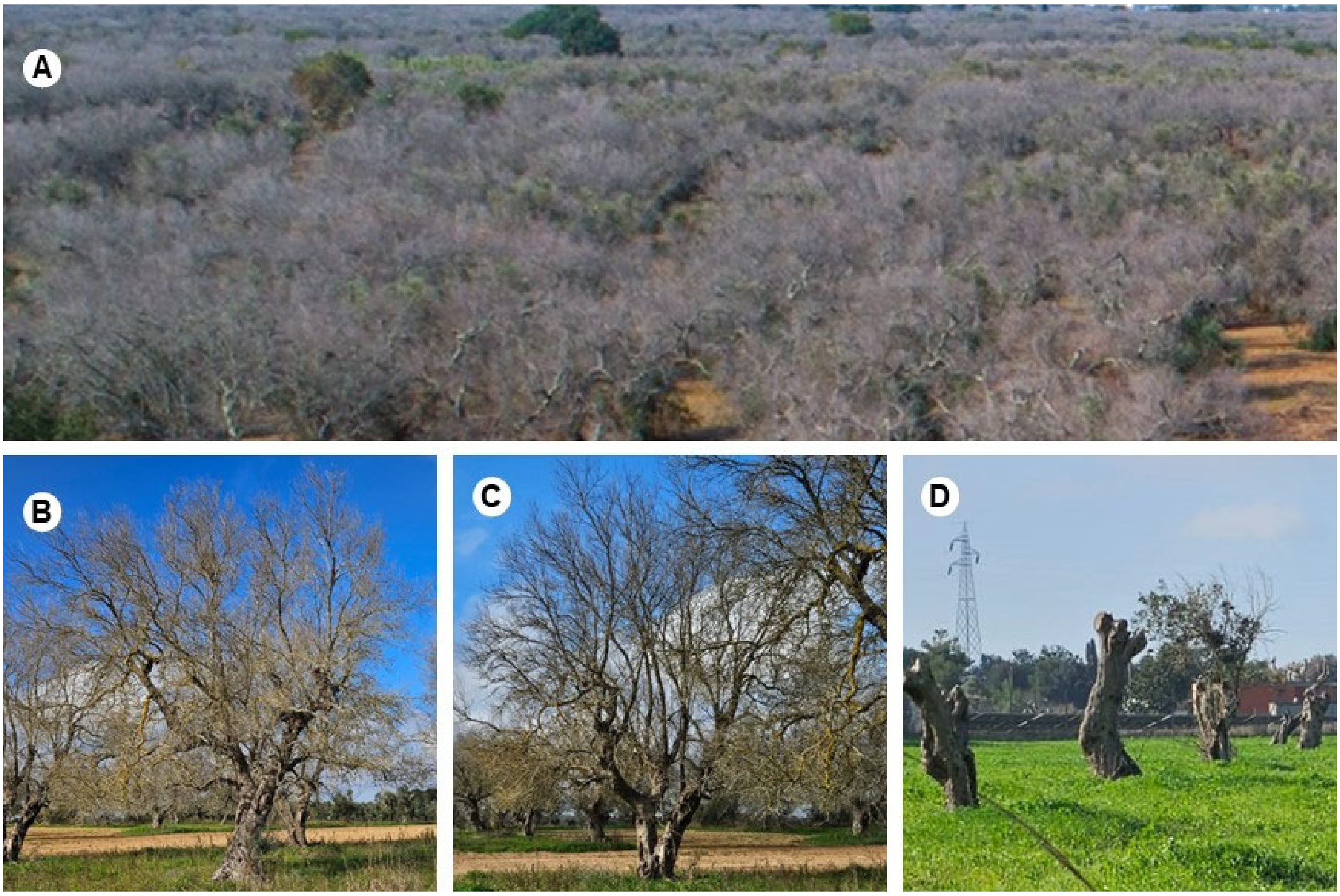

Figure 2. Examples of cvs Cellina di Nardò and Ogliarola Salentina olive plants affected by Olive Quick Decline Syndrome in the province of Lecce: Montesano Salentino (**A**), San Donato (**B**), Cavallino (**C**), and San Cesario di Lecce (**D**).

2.2. Description of the Applied Protocol

All the selected plants were observed at the beginning of the experiment and every four months. The percentage of diseased canopy was visually assessed and recorded. *Xylella*-sensitive olive cultivars (Figure 3A–E) showed canopies with OQDS symptoms (leaf scorch, desiccation of twigs and branches) of 40–80%. The resistant(?)/tolerant cultivars (Figure 3F) exhibited a 2–8% leaf scorch. Canopy dry parts were removed in January–February. A slow-release (organic, mixed organic, or chemical) fertilizer (e.g., Nitrophoska® special, EuroChem Agro Spa, Cesano Maderno, Italy) was distributed at the end of winter (January–February). Weeds were controlled by mowing or chopping according to EU Regulation 2020/1201 [58]. No treatments were performed on untreated control plants.

The first NuovOlivo® distribution was performed in April, May, or October (Table 1), while the others were carried out every March and October. When the first application was conducted in October, the second was performed in December of the same year. The canopy and trunk of each plant were sprayed with a 22 ± 2 bar hydraulic sprayer equipped with a high-pressure lance (1.8 mm diameter nozzle). Individual trees received [59] about 10 L of water containing NuovOlivo® (1%) and 0.5% commercial baking soda (Eurospin Italia S.p.A., San Martino Buon Albergo, Italy) as an activator added to the solution just before use. The scorched twigs were cut as they appeared during the vegetative season. To optimize farm organization, the recovered plants were subjected annually to suckering and pruned every two years following the standard agronomic techniques in the area.

In the Cannole, Andrano, and Montesano Salentino orchards, after the harvest in 2020, the plants were headed (topping cuts) to control the overall vertical size of the trees, reduce their height, and facilitate the management of pruning and harvesting operations. It is worth remembering the weather in May–September 2023 over several municipalities in the province of Lecce: there were extreme rainfall events (rain bombs), often accompanied by hailstorms. These weather conditions zeroed the production of olives and oil in the Cannole orchard.

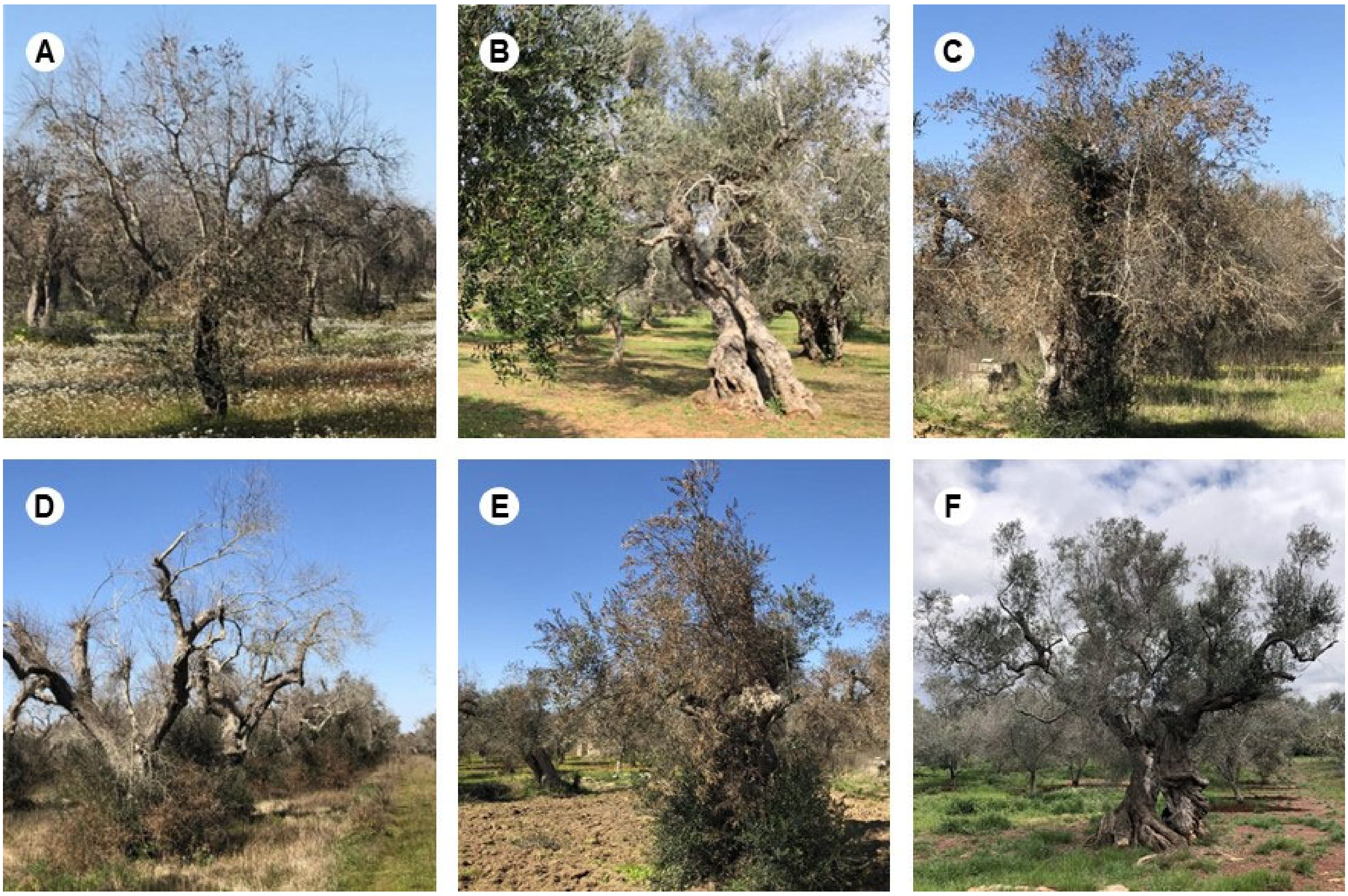

Figure 3. Pictures of *Xylella*-susceptible (**A**–**E**) or resistant(?)/tolerant (**F**) olive trees before protocol application in Montesano (**A**), Arnesano (**B**), Tricase 1 (**C**), Tricase 2 (**D**), Tricase 3 (**E**), and Veglie (**F**). The pictures show the situation in October 2016 (**A**), 2019 (**F**), 2020 (**B**), and 2021 (**C**–**F**).

2.3. Production Assessment

In the growing season preceding the survey, *Xylella*-sensitive plants did not produce drupes, while a low value (<1–2 kg/plant) was recorded for the resistant(?)/tolerant cultivars. Between the end of October and November of the growing seasons 2019–2020, 2020–2021, 2021–2022, and 2022–2023, with the olive fruits in the phase of veraison already complete, the drupes were disarticulated with manual or pneumatic combs (LISAM s.r.l., Imola, Bo, Italy) and dropped onto nets placed on the ground. Daily, the harvested olives were transported to the mill and cold-milled, and the oil was separated by centrifugation. The degree of acidity of the oil was determined as a percentage of oleic acid following the method described in the European Commission Regulation 2022/2104 [64].

2.4. Data Analysis

For each orchard, plants were divided into three groups to ensure reproducibility for drupes collection and oil yield and quality. Replicate values (n = 3) were used to calculate the means and standard deviations (sd). The statistical analysis was conducted with SAS/STAT version 9.0 (SAS Institute Inc., Cary, NC, USA). Normal distributions and homoscedasticity were assessed using the Shapiro–Wilk and Bartlett's tests, respectively. Data were subjected to a general linear analysis of variance. The percentage values were transformed to arcsine before analysis. Means were compared using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$. Drupe production was analyzed against the olive orchard, treatments, and their interactions. Analyses of olive yield against cultivar composition, treatments, and their interactions were performed. The Pearson correlation coefficient (r) between the number of treatments and olive production was estimated. The variables were considered [65] uncorrelated (r = 0), positively correlated (r > 0), or negatively (r < 0) correlated. The correlation was considered weak (r < |0.3|), moderate (|0.31| < r < |0.7|), or strong (r > |0.71|).

3. Results

3.1. Vegetative Assessment

During the surveyed growing seasons, the olive plants used as untreated controls produced few shoots and basal suckers in spring, which developed leaf scorch and dried in the summer season. If leaves on shoots and basal suckers remained green during June–August, they dried up in September or in winter. Each spring, new leaf development was reduced and the whole canopy dried. Untreated olive plants used as controls showed neither flowers nor drupes.

Olive trees subjected to the protocol produced new vegetation and turned out cluster inflorescence and drupes (Figure 4) formation.

Figure 4. Olive production in *Xylella fastidiosa* subsp. *pauca*-susceptible olive cv Cellina di Nardò Mignola (cluster inflorescence) in May 2023; (**A**) Ogliarola Salentina mature (**B**) and yielded (**C**) drupes.

X. fastidiosa subsp. *pauca*-sensitive plants (cvs Cellina di Nardò, Ogliarola Salentina, Coratina, Ascolana Tenera, and Nociara) resumed vegetation, reduced OQDS symptoms, and rebuilt their canopies (Figures 5–7). The initial 40–80% of diseased canopy with OQDS symptoms (e.g., leaf scorch, desiccation of twigs and branches, and dry sectors) decreased to 15–18, 10–15, 7–10, 5–8, up to 3, or 1–2% after 3, 5, 7, 9, 13, or 15 treatments, respectively. OQDS symptoms usually occur in the hot period (June–August) and, with slight intensity, in September. Resprouting was observed 1–2 months after the first treatment. After two treatments, trees had numerous young shoots along the main branches, indicating that severely diseased trees can recover from the disease.

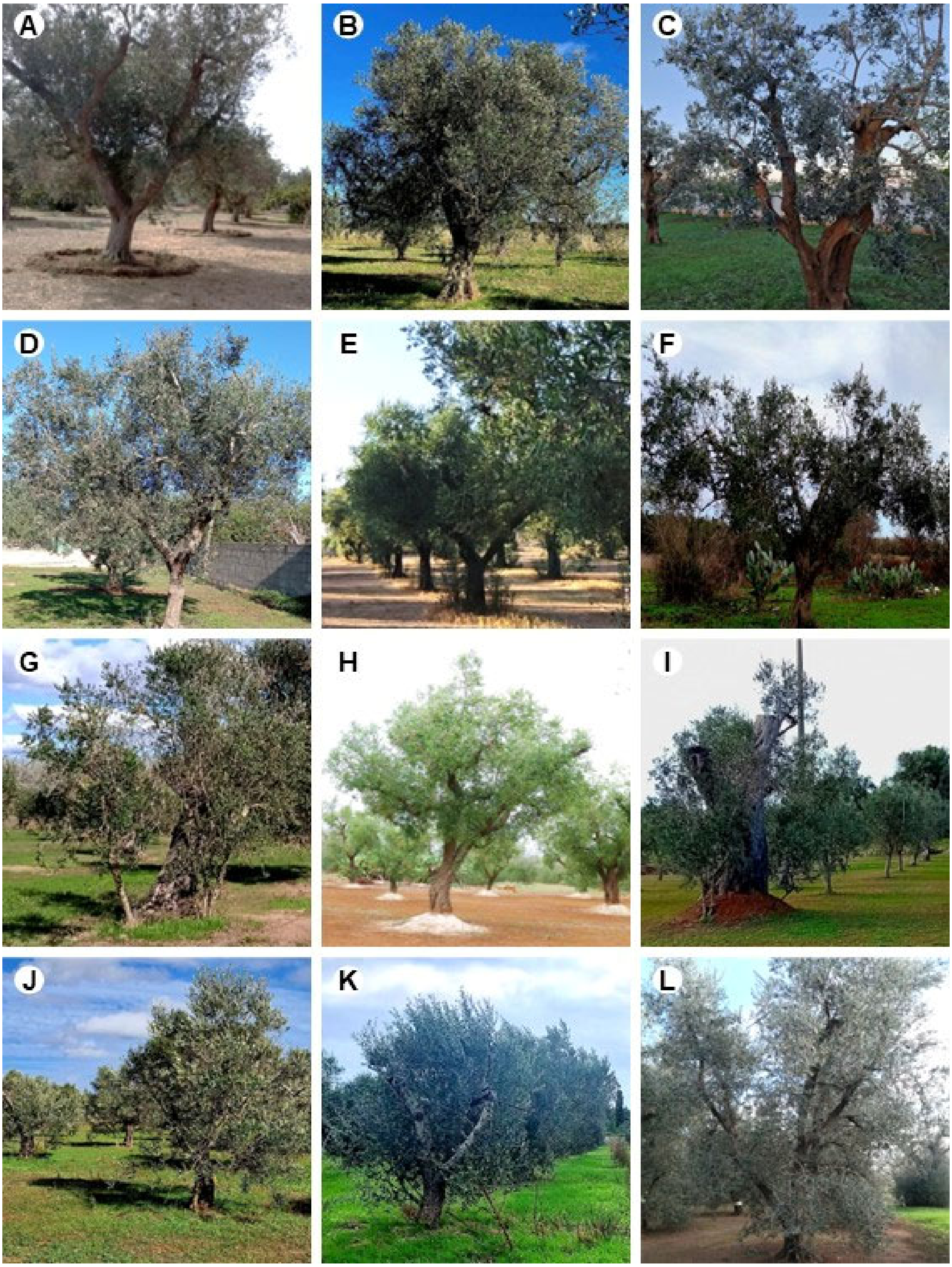

Figure 5. Recovery of *Xylella fastidiosa* subsp. *pauca*-susceptible olive trees subjected to the protocol since April 2022 (**A**–**D**) or 2021 (**E**–**L**) in Melpignano (**A**), Santa Cesarea Terme 2 (**B**), Tiggiano (**C**), Maglie 2 (**D**), Carpignano Salentino (**E**), Tricase 3 (**F**), Supersano (**G**), Maglie-Otranto (**H**), Minervino di Lecce (**I**), Tricase 2 (**J**), Maglie 4 (**K**), and Melendugno (**L**). Details of cultivar composition in the orchards are reported in Table 1. The pictures show the situation in November 2023.

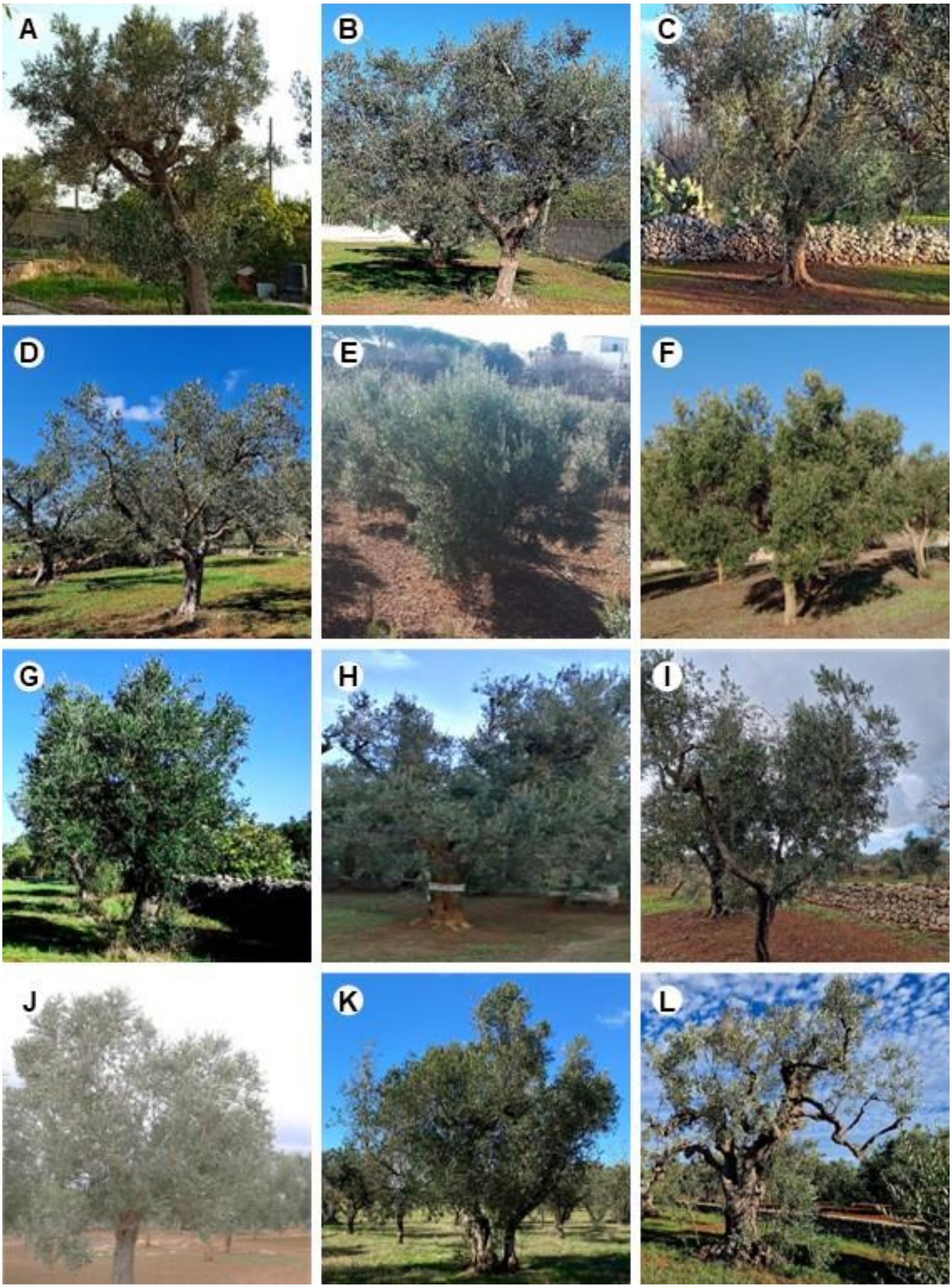

Figure 6. Selection of *Xylella fastidiosa* subsp. *pauca*-susceptible olive trees subjected to the protocol since April 2020 (**A**–**H**) or October 2019 (**I**–**L**) in Ugento 2 (**A**), Casamassella (**B**), Cursi (**C**), Tricase 1 (**D**), Maglie 3 (**E**), Santa Cesarea Terme 1 (**F**), Galatone 4 (**G**), Arnesano (**H**), Corsano (**I**), Maglie 1 (**J**), Nardò (**K**), and Salve (**L**). Details of cultivar composition in the orchards are reported in Table 1. The pictures show the situation in November 2023.

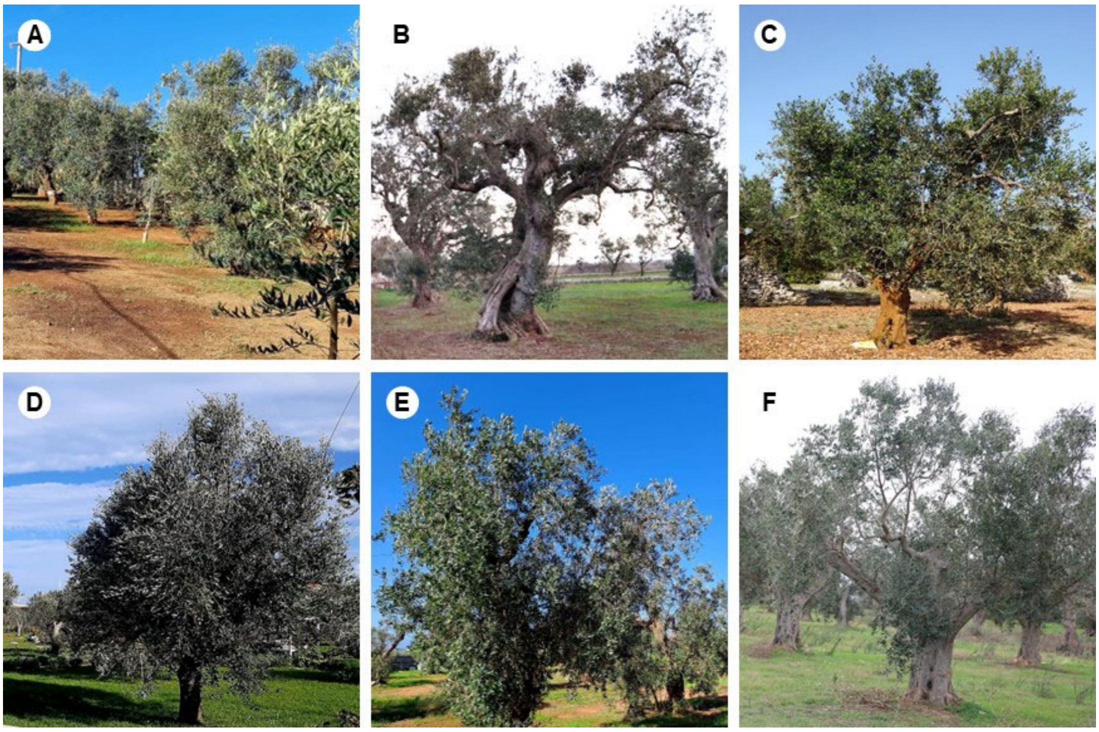

Figure 7. Olive trees of *Xylella fastidiosa* subsp. *pauca*-susceptible cv Cellina di Nardò or Ogliarola Salentina subjected to the protocol since October 2019 (**A**) or April 2019 (**B**–**E**) or 2017 (**F**) in Taurisano (**A**), Cannole (**B**), Gagliano del Capo (**C**), Surbo (**D**), Ugento 1 (**E**), and Andrano (**F**). Details of cultivar composition in the orchards are reported in Table 1. The pictures show the situation in November 2023.

The orchards in Veglie, Supersano, and Maglie 3 recorded the absence of dry twigs on the resistant(?)/tolerant 'Leccino' (Figure 8) and 'Bella di Cerignola' plants.

Figure 8. 'Leccino' plants in the Veglie countryside subjected to the protocol since October 2019. The pictures show the situation in November 2023.

3.2. Olive Production

No drupes were produced from untreated plants used as controls. Based on the initial OQDS symptom, after 3–5 NuovOlivo® applications, plants of all the selected olive orchards subjected to the protocol produced cluster inflorescence (Figure 4) and olives (Figure 9).

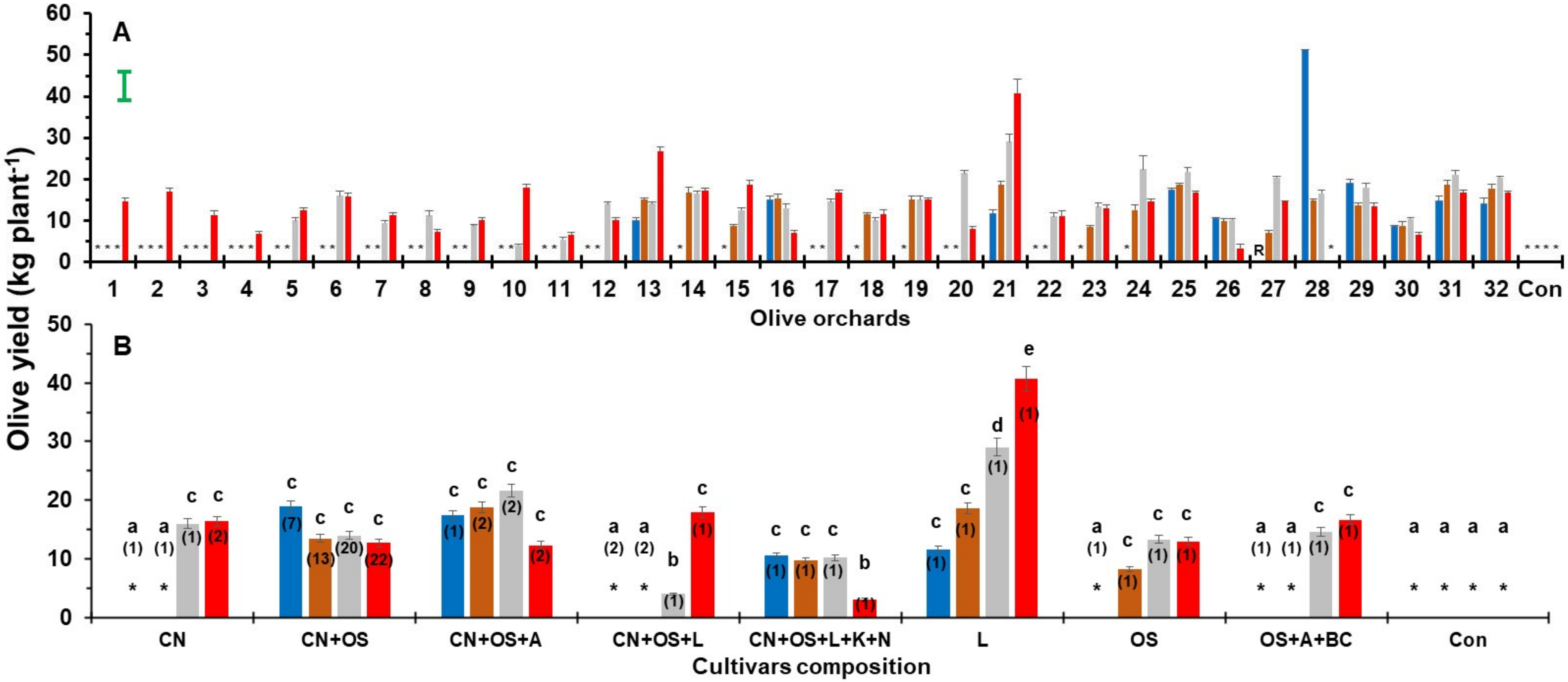

Figure 9. Drupes yield in olive orchards (**A**) or from different cultivar compositions (**B**) during the 2019–2020 (■), 2020–2021 (■), 2021–2022 (■), and 2022–2023 (■) olive-growing seasons subjected to different NuovOlivo® treatments. In section (**A**): each value is the means of three different drupes collections per orchard ± sd. * = No production. R = Rain and hailstorms destroyed olive production. The green vertical bar indicates the Fisher's LSD ($p \leq 0.05$). In section (**B**): numbers in round brackets represent the orchard considered. Each histogram refers to the means of considered orchards and drupes collections ± sd. Cultivar acronyms: CN = Cellina di Nardò, OS = Ogliarola Salentina, L = Leccino, A = Ascolana Tenera B = Bella di Cerignola, K = Coratina, N = Nociara. Con = untreated control. Values accompanied by the same letters are not significantly different ($p \leq 0.05$) according to Fisher's LSD test. Numbers indicate the orchards considered. Details of cultivar compositions in the orchards are reported in Table 1.

As shown in Figure 9, olive yield (kg plant^{-1}) ranged from 3.11 ± 0.21 (Nardò in 2020) to 51.36 ± 0.47 (Cannole in 2020). The Shapiro–Wilk (0.961942; p < 0.0001) and Bartlett's (X^2 36.7410; p < 0.0001) tests indicated normal distributions and homogeneity of the data.

Drupes yield was strongly affected by the number of treatments, orchards, and cultivar composition (Figure 9, Table 2). The r coefficient (0.49563; p < 0.0001) indicates a positive moderate correlation between the number of treatments and olive production. As expected, cv Leccino (Figure 8) was the most productive, with an average of 25.00 ± 3.3 kg plant^{-1} during the four growing seasons surveyed, followed by the mixed cultivations with cultivars Cellina di Nardò and Ogliarola Salentina (about 15.00 ± 0.13 kg plant^{-1}).

Table 2. Results of general linear analyses of variance considering effects of orchard locations (OL) and treatments (T) or cultivar compositions (Cv) and T, and their interactions with olive production.

Effects of OL, T				Effects of Cv, T			
Sources of Variation	**Df**	**F Values**	***p***	**Sources of Variation**	**df**	**F Values**	***p***
OL	32	383.20	<0.0001	Cv	8	15.47	<0.0001
T	8	1835.37	<0.0001	T	8	37.19	<0.0001
OL × T	72	317.47	<0.0001	Cv × T	24	5.12	<0.1084

3.3. Oil Production and Quality

Oil content (Table 3) ranged from 8.67% (Ugento 2, in 2022) to 26.62% (Ugento 1, in 2023). The r coefficient (0.45416; p < 0.0001) indicates a positive moderate correlation between the number of treatments performed and oil production.

Table 3. Oil production (assessed as % olive fresh weight; w/w) and related acidity (expressed as % of oleic acid) during the 2020–2023 olive-growing seasons.

Cultivars Composition (1) (Orchards)	Olive-Growing Seasons (2)							
	2020		2021		2022		2023	
	Oil	Acidity	Oil	Acidity	Oil	Acidity	Oil	Acidity
CN [2]	0 d		0 d		12,51 ± 0.01 bc	0.3	11.70 ± 0.01 bc	0.2
CN + OS [23]	13.03 ± 0.03 b	0.1-0.2	12.05 ± 0.01 bc	0.1-0.8	12.84 ± 0.01 b	0.1–0.7	13.23 ± 0.02 b	0.1–0.8
CN + OS + A [2]	11.75 ± 0.02 bc	0.2	11.11 ± 0.02 bc	0.2	9.83 ± 0.02 bc	0.3	11,75 ± 0.02 bc	0.2
CN + OS + L [1]	0 d		0 d		10.00 ± 0.01 c	0.3	10.00 ± 0.01 c	0.08
CN + OS + L + K + N [1]	11.36 ± 0.03 bc	0.3	13.85 ± 0.01 b	0.2	11.83 ± 0.01 d	0.2	10.22 ± 0.01 c	0.2
L [1]	11.00 ± 0.01 c	0.2	10.63 ± 0.01 c	0.2	10.00 ± 0.01 c	0.2	13.71 ± 0.02 b	0.2
OS [1]	0 d		20.00 ± 0.01 a	0.1	15.00 ± 0.01 b	0.1	14,10 ± 0.02 b	0.2
OS + A + B [1]	0 d		0 d		10.86 ± 0.01 c	0.3	10.00 ± 0.01 c	0.3
Untreated Control	0 d		0 d		0 d		0 d	

(1) Cultivar acronyms: CN = Cellina di Nardò, OS = Ogliarola Salentina, L = Leccino, A = Ascolana Tenera, BC = Bella di Cerignola, K = Coratina, N = Nociara. In the square brackets, the number of orchards involved is shown. (2) Values are the means ± sd; data accompanied by the same letters are not significantly different ($p \leq 0.05$) according to Fisher's LSD test. Details on the orchards involved are reported in Table 1.

Extra-virgin olive oil (Table 3) was produced along the orchards subjected to the protocol. The degree of oil acidity was in the range of 0.1–0.3%, except for olive oil milled in Melendugno and Supersano during the olive oil season of 2023 and in Casamassella during 2021 and 2023, which reached an acidity value of 0.8%.

The 'Leccino' orchard in Veglie (Figure 8) responded to the applied protocol, allowing, in 2023, for production of 40.7 ± 3.46 kg per plant (Figure 9), as well as average yields of 11.33% of extra-virgin olive oil with 0.1% free acidity (Table 3).

4. Discussion

Since their appearance on Earth, plants have co-evolved with their pathogens, becoming susceptible, tolerant, or resistant. A susceptible plant develops extensive infections, severe disease symptoms, and damage when interacting with one of its pathogens [66–68]. Diseased plants reduce yields and increase production costs or die. Therefore, economic losses, landscape, socio-cultural and ecological damage, biodiversity loss, ecosystem degradation, and environmental imbalances subdue susceptible plants. A resistant plant prevents the development of a pathogen, while a tolerant plant withstands the dangerous effects of a pathogen without suffering severe injury or crop loss [66,68]. In a tolerant diseased plant, the undamaged portions compensate for the functional and productive losses of the damaged parts. Tolerance to the pathogen occurs when increases in the amount of pathogen in the plant do not correspond to equally high increases in the symptom severity [66,68]. Plants with partial resistance that can be colonized by the pathogen but with reduced disease severity have been characterized [66,68]. Furthermore, the climate, abiotic components, and biodiversity (microbiota) in the soil and plant rule the plant, pathogen, and vector interaction [66].

Apart from cvs Cellina di Nardò and Ogliarola Salentina, the other cultivars susceptible to *X. fastidiosa* subsp. *pauca* were Carolea, Nocellara del Belice, Cima di Melfi, Nociara, Bianco Lilla, Ascolana Tenera, and Sant'Agostino. Olive cultivars that, despite having *X. fastidiosa* subsp. *pauca* inside them, did not show symptoms or had few desiccated branches, such as Leccino, Frantoio, Coratina, Pendolino, Bella di Cerignola, Cipressino, and Favolosa® [https://www.italiafruit.net/ulivi-sei-varieta-resistenti-alla-xylella (accessed on 30 June 2024)], are considered 'tolerant' as defined by Agrios [66] or 'partially resistant' in the sense of Tai et al. [68]. Lignin concentration in the xylem vessel [69], secondary metabolite production and concentration [70], the antioxidant defense system [71], and the xylem vessel size [16] were the bases for 'Leccino' tolerance/resistance [24]. Moreover, a decline in adult cv Leccino trees is frequently reported in *Xylella*-infected areas [24]. Similarly, young Favolosa® planted in the infected area of Salento showed leaf scorch and canopy desiccation.

OQDS-diseased cvs Ogliarola Salentina and Cellina di Nardò also collapsed, yielding collard suckers that may wilt in weeks [24]. Diseased plants neither grow nor fructify. They are abandoned and inexorably die (Figure 3). Several experimental findings demonstrate that keeping the olive plants in a "good state of health" and pruning strategies alone could slow down OQDS, but were not enough to reduce the impact of *X. fastidiosa* subsp. *pauca* on production [38,41]. Here, untreated olive plants died in two to three growing seasons, confirming the conclusions reported by other authors [4,6–9,61].

The findings explored in Section 3 provide a clear depiction of susceptible cvs Cellina di Nardò, Ogliarola Salentina, Ascolana Tenera, and Nociara plants affected by OQDS, which are able to produce new vegetation, flowers, and drupes and yield excellent extra-virgin olive oil after the application of the NuovOlivo protocol. Although the presence of OQDS symptoms did not lead to fatal consequences, leaf scorch and desiccation of branches were reduced to 1–2% of the canopies.

The protocol herein discusses associated good agricultural practices (i.e., regular pruning, suckering, soil fertilization, weed mowing, *P. spumarius* control, and removal of dry twigs) and the effects of NuovOlivo® distribution, which could enhance natural defenses inside the plant tissue, triggering defense pathways and possible *X. fastidiosa* growth inhibition (e.g., aggregate formation in the plant xylem and self-production of polymeric biofilms). Indeed, in the interaction of *X. fastidiosa* vs. susceptible olive plants, the treatment with NuovOlivo® confirms previous observations [60]: a reduction in the severity of OQDS foliar symptoms, production of new vegetation, cluster inflorescence, flowers, and drupes. Because of plant growth and development, the density of the pathogen within the olive vessels and leaves was decreased [60].

NuovOlivo® (patent no. 102017000109094 of the Ministry of Economic Development) is a detergent based on vegetable oils and aqueous botanical extracts (e.g., *Thymus vulgaris* L., *Petroselinum crispum* (Mill.) Fuss, *Crataegus monogyna* Jacq., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Origanum vulgare* L., *Matricaria chamomilla* L., *Malva sylvestris* L., *Salix babylonica* L., *Capsicum annuum* L., *Piper nigrum* L.) esterified in the presence of sodium hydroxide "activated" at the time of use with sodium bicarbonate [60]. In other words, this product is a mixture of plant extracts: primary and secondary plant metabolites, phytohormones, enzymes, growth promoters, antioxidants, and surface-active agents. Plant extracts have well-characterized antifungal and antimicrobial properties [72–74] and plant bio-stimulant effects [74]. Control strategies of plant pathogens, including quarantine species, require new tools involving sustainable agrochemicals. Among these, plant extracts are natural sources of biocides, and other substances to support and sustain plant growth and development. Different plant extracts with antibacterial activity represent safe alternatives for plant disease management, including phytobacteria [74]. In this context, thymol was effective in vitro against *X. fastidiosa* subsp. *pauca* strain CFBP 8402, as the major phenolic constituent of thyme and oregano essential oils, in the form of nanoparticles with $CaCO_3$ nanocrystals [75]. Also, a garlic-powder-based solution has demonstrated bacteriostatic activities against *X. fastidiosa* subsp. *pauca.* Phenolic extract from cv Coratina olive leaves in Puglia's naturally infected area increased the leaf area index and density and the growth of newly formed healthy shoots due to the bacteriostatic effect shown in an in vitro test [76]. In the NuovOlivo® formulation, in addition to the functioning extract mixture, surface-active agents could lower the surface tension, facilitate waxy layer penetration on leaves, and allow other chemicals to interface with plant cells and enter the cell-free space [77].

Considering the ages of the plants used in these experiments, i.e., 25–250 years, the spry treatments conceived in this trial were suitable for all ages of plants, including secular monumental olive trees.

If a diseased plant reduces yields and increases damage, the management strategies of plant diseases increase the quantity and the quality of plant products available for use and reduce the dangerous impact of plant diseases [66]. Thus, NuovOlivo® applications stimulate new vegetation development, promote intrinsic flower bud quality, and support the plant in fruits and oil production, leading them to agronomic restoration. This potential makes NuovOlivo® a promising tool in the disease management strategy for olives and, very likely, other crops.

Landscape restoration in the *Xylella*-infected zone consists of removal of damaged olive plants, replanting resistant olive cultivars in an intensive production system, conversion to other crops (e.g., vineyards, pomegranate, stone fruits), and preservation of monumental olive trees via grafting with resistant cultivars [4,7,24,40,59]. As resistant(?)/tolerant olive cultivars, Favolosa®, Leccino, Lecciana, and Leccio del Corno are suggested to replant the most susceptible Ogliarola Salentina and Cellina di Nardò according to EC decisions.

New intensive plantations of cvs Favolosa® and Leccino fail in seedling rooting, probably due to drought stress and lack of irrigation [24] or the application of brackish water. As reported here, cvs Leccino and Bella di Cerignola have proven a 2–8% leaf scorch and a short (<1–2 kg/plant) drupe production. The bacterium presence inside the xylem vessels could stimulate the activation of induced defenses and "distract" the plant from its ordinary metabolism aimed at growth (new vegetation) and reproduction (flowers and fruits). NuovOlivo® could restore plant physiological function and stimulate flower and fruit differentiation.

In the same way, grafting is not a cure and does not respect quarantine legislation on plant disease agents. In fact, with grafting, the canopy of grafted olive trees affected by OQDS is reconstituted; it produced, after three years, drupes and oil of the *Xylella* resistant(?)/tolerant cultivar. In this way, diseased plants remain a reservoir of *Xylella* inoculum, which is useful for new infections. In addition, grafted plants did not produce the typical and unique oil of cvs Cellina di Nardò and Ogliarola Salentina, the flagship of olive tradition and culture in the Salento.

Based on the results obtained, the protocol described herein has proved to be an effective method for the treatment of *X. fastidiosa* subsp. *pauca* on olive plants affected by OQDS. Furthermore, this protocol would reduce the need to replace local cultivars with cvs Leccino and Favolosa® [21,24,47,59,77], which are less susceptible to *X. fastidiosa* subsp. *pauca*, preserving the Apulian olive biodiversity.

The effects of the discussed protocol agree with the current concept of plant disease management, which avoids the goal of zero disease but promotes the reduction in disease damage to below acceptable levels by decreasing (i) the initial inoculum, (ii) the rate of infection, and (iii) the duration of the epidemic.

For the plants affected by OQDS and belonging to the susceptible cultivars Cellina di Nardò (2434 plants), Ogliarola Salentina (1020 plants), Ascolana Tenera (65 plants), and Nociara (250 plants) presented herein, and subjected to the tested protocol, the phenomenon of "recovery" could be invoked. Recovery is the healing of symptoms and the restoration of the vegetative and productive normality of the diseased plant, associated with compartmentalization mechanisms that allow the trees to overcome the occlusions caused by the pathogen in the vascular system. Recovery events have already been studied in elms affected by Dutch elm disease caused by *Ophiostoma ulmi* (Buisman) Nannf. [78,79]; oaks with symptoms of wilt caused by *Bretziella* (*Ceratocystis*) *fagacearum* (Bretz) Z.W. de Beer, Marinc., T.A. Duong and M.J. Wingf [80]; several phytoplasmoses [81–83], almond, peach, apricot, ash, Norwegian maple, pistachio, cocoa, avocado, and olive trees infected by the fungus *Verticillium dahliae* Kleb. [28,84–90]; and grapevines with Esca symptoms on leaves [91].

The protocol proposed here could help olive plants to restore their xylem network transport capacity, stimulate new vessel production, neutralize water scarcity perception, and increase drought tolerance. Usually, in vascular diseases, including grapevine Pierce's disease, xylem occlusion occurs via cells proliferation and/or exopolysaccharides production by the pathogen, as well as tyloses and gels developed as plant response [8,92–94]. In plants affected by vascular diseases, the clogged xylem vessels (i) limit the pathogen spread through the vascular system, (ii) reduce water conductivity, (iii) activate water stress and adaptive responses to drought [74], (iv) increase wilting symptoms [27,92], (v) influence the social behavior of the plant microbiome [95,96], and (vi) favor/promote the downward and lateral colonization of the plant host. These effects are demonstrated for the xylem colonization by *Dickeya* sp. (Biovar 3), *Pseudomonas syringae* Van Hall pv. *Syringae* Young, and *X. fastidiosa* [92,96–100]. The transcriptional responses observed in the 'Ogliarola Salentina' and 'Leccino' plants agree with this hypothesis: the presence of *X. fastidiosa* subsp. *pauca* is perceived in both cultivars, and up-regulating genes encode receptor-like kinases (RLK) and receptor-like proteins (RLP) [99]. Moreover, in 'Leccino' plants, higher expression levels are reported for genes belonging to ROS-scavenging systems and for genes involved in pathogen stress (pathogenesis-related, PR, and leucine-rich repeat genes, LRR-RLK) and drought response (dehydrin, DHN) [69].

The data discussed here could represent successful experiments with the *X. fastidiosa* subsp. *pauca* management and coexistence approach, supporting susceptible olive cultivars in returning/continuing to vegetate and produce olives and oil. In particular, 'Cellina di Nardò' and 'Ogliarola Salentina' will provide their unique oil to the world and continue to be natural monuments, symbols of the landscape, history, cultural identity, and tourist attraction for Puglia and the other olive-growing regions of Italy, even if they are affected by OQDS.

Finally, the strategies proposed here to reduce the economic impact of pathogens on olive plants; maintain high plant productivity even in the presence of pathogen inoculum; and agree with the EC recommendation, which suggests containment and mitigation measures when a quarantine pathogenic microorganism is established in a large territory, cannot be eradicated like the *X. fastidiosa* subsp. *pauca* in the olive groves in Puglia.

5. Conclusions

Under the epidemic conditions in the *X. fastidiosa*-infected area of Puglia, the presented protocol recovers OQDS-affected trees in traditional olive orchards. The NuovOlivo protocol associates good agricultural practices and treatments with plant extracts, vegetable oils, and surface-active agents.

Good agronomic practices, e.g., regular pruning, suckering, soil fertilization, weed mowing, and removal of dry twigs, preserve tree productivity. The removal of suckers reduces spittlebug feeding sites and trees' attractiveness for *P. spumarius*. The mixture of primary and secondary plant metabolites, phytohormones, enzymes, growth promoters, and antioxidants could explain the bio-stimulant properties inside treated plants. Bactericidal and/or antibiofilm activity on *X. fastidiosa* could be also hypothesized.

The applied protocol stimulates new vegetation development and promotes, supports, and restores flower, fruit, and oil production without biodiversity alteration, environmental imbalances, ecosystem modification, or landscape change in the *X. fastidiosa*-overrun areas of Puglia. This practice could have significant importance for the ancient olive trees, which are indissolubly linked with the culture, the nutrition, the ecosystem's biodiversity, the landscape, and the economy of this region. The ancient olive ecosystems contribute to the conservation of the soils, improve the sustainability of natural resources, and represent an element of identity in the Mediterranean basin. Loose olive cultivation using traditional cultivation strategies has devastating consequences for the environment and the economy, especially in Puglia's marginal, fragile, and poor areas.

This promising strategy for managing *X fastidiosa* subsp. *pauca* in olive orchards will be assessed on a larger scale and on centenary olive trees.

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