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BEYOND XYLELLA

Identification of new germplasm resistant to *Xylella fastidiosa* in olive, almond and other important crops

Deliverable 4.1

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1. INTRODUCTION

Identification of disease-resistant germplasm is a key component of integrated disease management, particularly for bacterial pathogens for which curative field treatments are not available. In the case of *Xylella fastidiosa*, the identification and deployment of resistant or tolerant plant material is especially relevant because disease management relies mainly on prevention, surveillance, vector control, removal of infected plants and the use of plant genotypes with reduced susceptibility.

Within BeXyl WP4, collections of major crop species were screened for resistance to *X. fastidiosa* using the methods described below. The objective of this deliverable is to provide a dataset summarising crop genotypes and cultivars that showed notable resistance or moderate resistance to *X. fastidiosa* infection under the experimental conditions tested. Susceptible genotypes are not reported in the main dataset, except where needed as reference controls.

Particular attention was given to olive because of the severe impact of Olive Quick Decline Syndrome (OQDS) in Apulia, southern Italy. OQDS, associated with *X. fastidiosa* subsp. *pauca* ST53, represents the most damaging *X. fastidiosa*-associated disease reported in Europe and has caused extensive decline and mortality of olive trees. Due to the biological, epidemiological and socio-economic relevance of this pathosystem, and because olive resistance assessment requires long-term field observations under high inoculum pressure, an extended technical report is provided in Annex 1. This Annex complements the dataset by describing in greater detail the olive germplasm screening programmes, the criteria used to classify cultivar response, the search for resistant genotypes in wild germplasm and the breeding activities aimed at generating new resistant olive material.

2. METHODOLOGY

Germplasm screenings were conducted on different crop species across multiple countries and experimental settings. Some trials used artificial inoculation, mainly by mechanical inoculation using the pin-prick method, whereas others relied on natural infection in open fields where *X. fastidiosa* was already present. A detailed description of the pin-prick inoculation procedure is provided in Deliverable 1.3, together with additional information on some of the experiments summarised in this dataset.

Resistance or reduced susceptibility was assessed using a combination of symptom severity and molecular detection of *X. fastidiosa* by quantitative PCR (qPCR). Depending on the crop, partner and experimental design, qPCR results were used to determine infection status and, where possible, to estimate relative bacterial load. The response categories reported in this deliverable therefore integrate phenotypic observations and pathogen detection data.

Because the screenings were conducted across different crops, countries, plant ages, inoculation procedures, pathogen subspecies, sequence types and strains, full standardisation of resistance assessment across all experiments was not possible. For this reason, resistance classes should be interpreted within each experimental context and in

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comparison, with the susceptible and resistant controls included in the same screen. The dataset therefore identifies genotypes showing notable resistance or moderate resistance under the specific conditions tested, rather than providing a universal ranking across crops or experiments.

For olive, the methodology required additional detail because resistance assessment to OQDS is strongly influenced by long incubation periods, uneven symptom development, natural vector-mediated transmission, plant age and prolonged exposure to inoculum pressure. Therefore, an extended methodological description is provided in Annex 1. This Annex reports the structure of the olive field plots established in Apulia, the number and origin of cultivars and selections tested, the criteria used to classify plant response, the sampling and qPCR procedures, and the complementary activities aimed at identifying resistant wild genotypes and selecting new cross-bred olive progenies.

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3. RESULTS

Table 1, summarizes the germplasm screenings conducted in the frame of WP4. The table provides details on the crop species evaluated, the relevant institute that performed the screening, the total number of genotypes screened, the inoculation method used, whether the experiment was carried out indoors or outdoors, the *X. fastidiosa* strains used for inoculation, and the criteria applied for resistance assessment.

Table 1. Description of crop plant germplasm collection screening for *X. fastidiosa* resistance

Crop plant	Screened by Partner	No. of genotypes screened	Greenhouse/ outdoor	Inoculation type	*X. fastidiosa* subsp. (ST) and strain*	Resistance assessment
Olive	P1-CSIC	13	Greenhouse	Mechanical	Xfp (ST53, CoDiRo Xfp (ST81), XYL 1966/18 Xfm (ST80), XYL 1961/18	Symptoms + qPCR
	P2-IVIA	4	Greenhouse	Mechanical	Xfm (ST6), IVIA 5901 and ESVL	Symptoms + qPCR
	P5-CNR/ P18-IFAPA**	402 (olive cultivars and selections)	Outdoor	Natural	Xfp (ST53)	Symptoms + qPCR
		20	Greenhouse	Mechanical and/or vector mediated	Xfp (ST53, De Donno)	Symptoms + qPCR
	P21-IRFAP	75	Outdoor	Natural	Xfm (ST81)	Symptoms + qPCR
		26	Outdoor	Natural	Xfp (ST53)	Symptoms + qPCR
	P27-INTA	2	Greenhouse	Mechanical	Xfp (ST69), OLI17A INTA	Symptoms + qPCR
Almond	P2-IVIA	8	Greenhouse	Mechanical	Xfm (ST6), IVIA 5901 and ESVL	Symptoms + qPCR
	P11-ARO-VOL	49	Greenhouse	Mechanical	Xff (ST1), SN17-4	Symptoms + qPCR
	P21-IRFAP	94	Outdoor	Natural	Xff (ST1) Xfm (ST81)	Symptoms + qPCR

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Crop plant	Screened by Partner	No. of genotypes screened	Greenhouse/ outdoor	Inoculation type	*X. fastidiosa* subsp. (ST) and strain*	Resistance assessment
Grapevine	P21-IRFAP	14	Outdoor	Natural	Xff (ST1)	Symptoms + qPCR
Coffee	P16-UCR	70 varieties (accessions) belonging to 3 species (*C. canephora*, *C. liberica*, *C. arabica*) and hybrids.	Outdoor	Natural	Xff (Not determined)	Symptoms + qPCR
Psidium guajava **(Guava)**	P16-UCR	1	Greenhouse	Mechanical	Xff (Not determined)	Symptoms + qPCR
Psidium friedrichsthalium **(Sour guava)**	P16-UCR	1	Greenhouse	Mechanical	Xff (Not determined)	Symptoms + qPCR
Peach	P16-UCR	1	Outdoor	Natural	Xff (Not determined)	Symptoms + qPCR

*Xff, *X. fastidiosa* subsp. *fastidiosa*; Xfm, *X. fastidiosa* subsp. *multiplex*; Xfp, *X. fastidiosa* subsp. *pauca*; ST, sequence type; ND, not determined

**A detailed report of the experiments is in the Annex 1

In Table 2, we list the genotypes/cultivars that exhibited notable level of resistance to *X. fastidiosa* infection. Because these data were generated by multiple partners under diverse experimental conditions, including differences in plant age, cultivation practices, inoculation methods and *X. fastidiosa* strains, it was not possible to fully standardize plant response assessments across all crops and trials. We therefore categorized responses into four classes, as described herein:

Susceptible – plants showing clear symptoms and high bacterial loads (low C_T values).

Moderately resistant – plants with reduced symptom severity and/or bacterial load (high C_T values).

Resistant – plants with no symptoms and reduced or no pathogen detection by qPCR.

These classifications should be interpreted as relative within each experimental context.

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Table 2. List of genotypes/cultivars showing notable resistance to *X. fastidiosa* infection.

Crop plant	Screened by Partner	*X. fastidiosa* strain	Moderately resistant	Resistant	With preliminary evidence of resistance (prolonged observations needed)
Olive	P1-CSIC	Xfp (ST53), CoDiRo Xfp (ST81), XYL 1966/18 Xfm (ST80), XYL 1961/18	-	-	-
	P2-IVIA	Xfm (ST6) IVIA 5901 and ESVL	**Cultivars:** • Arbequina • Arbosana • Cornicabra • Gordal	-	-
	P5-CNR**	Xfp (ST53)- naturally occurring isolates	**Cultivars:** • Pasola • Dolce di Cassano • Toscanina • Frantoio **New selections (from P19-IFAPA)** • Koroneiki o.p. • Arbequina o.p. • Changlot x Dolce Agogia • Frantoio o.p. • Koroneiki x Empeltre • PicualxJeac9	**Cultivars:** • Leccino • FS17 • Cipressino • Ascolana Tenera • Leccio del Corno **New selections to be registered**: • S215 • S105 • GM9 • GM25 • F10P30 • F12P98 • F8P17 • F6P43 • F8P9 • F6P5	**Cultivars:** • Aurina di Venafro • Cornarella • Leccino • Olivastro di Montenero • Tonda di Filadelfia • Correggiolo • Lazzero • Madre Mignola • Verzola • Mari • Gordal Sevillana • Medjhol • Memeçik • Myrtolia • Agristigna • Aitana • Ascolana semitenera

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Crop plant	Screened by Partner	*X. fastidiosa* strain	Moderately resistant	Resistant	With preliminary evidence of resistance (prolonged observations needed)
			New pre-selections from Uni. of Seville: • US 07-83 • US 08-24	**Other subspecies:** • *O. europaea* subsp. *cerasiformis*	• Blanqueta • Campanita • Cassanese • Cerasa di Montenero • Changlot Real • Cornilar • Dolce D'andria • Dolce Rossano • Galega • Gaydourelia • Grappolo • Lastovka • Less • Lumiaro • Maarri • Manzanilla de Sevilla • Maurino • Miniol • MorrutGrossane • Nocellara Messinese • Oliva Nera di Colletorto • Olivastra Seggianese • Olivastro di Montenero • Palmareccia • Pasola • Redondal • Rosciola Marche • Rossina • Sirole

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Crop plant	Screened by Partner	*X. fastidiosa* strain	Moderately resistant	Resistant	With preliminary evidence of resistance (prolonged observations needed)
					• Sperone di Gallo • Toffahi • Uovo di Piccione • Uslu • Verdella • Verdial De Huevar
	P27-INTA	Xfp (ST69), CE1	**Cultivars:** • Frantoio		
Almond	P11-ARO-VOL	Xff (ST1), SN17-4	**Cultivars:** • Ferraduel • Butte • Glorieta • 48-35 • 43-83 • Guara • Ferragnes • 144 • Sde 1	**Cultivars:** • Lauranne • 44-1 • Usha • Bab al Hawa • Harmon • 44-18	-
	P2-IVIA	Xfm (ST6) IVIA 5901 and ESVL	**Cultivars:** • Antoñeta • Avijor (Lauranne) • Constantí • Ferragnes	-	-
	P21-IRFAP	Xff (ST1) Xfm (ST81)	-	**Cultivars:** • Varyo • Marinada • Mare de Deu	-

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Crop plant	Screened by Partner	*X. fastidiosa* strain	Moderately resistant	Resistant	With preliminary evidence of resistance (prolonged observations needed)
				• Soleta	
Grapevine	P21-IRFAP	Xff (ST1)	-	-	-
Coffee	P16-UCR	Xff (Not determined)	-	-	-
***Psidium guajava* (Guava)**	P16-UCR	Xff (ND)	-	-	-
***Psidium friedrichsthalium* (Sour guava)**	P16-UCR	Xff (ND)	• Unknown genotype	-	-
Peach	P16-UCR	Xff (ND)	-	• Unknown genotype	-

*Xff, *X. fastidiosa* subsp. *fastidiosa*; Xfm, *X. fastidiosa* subsp. *multiplex*; Xfp, *X. fastidiosa* subsp. *pauca*; ST, sequence type; ND, not determined

**Based on the results reported in Annex I, cultivars/selections have been categorized as resistant (classes 6 and 5), moderately resistant (classes 5 and 4); indeed, those referred to the category "with preliminary evidence of resistance" include the cultivars categorized within the classes 6-5-4, but whose data cannot be considered definitive either due to the limited incubation period of the infections or due to the limited number of replicates.

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4. GENERAL REMARKS

Across WP4, BeXyl partners screened germplasm from several crop species of agronomic relevance, including olive, almond, grapevine, coffee, guava, sour guava and peach. The screenings combined greenhouse inoculation assays and field evaluations under natural infection pressure. The resulting dataset identifies genotypes and cultivars that showed moderate resistance or resistance to *X. fastidiosa* infection under the experimental conditions tested.

The dataset presented here has some limitations. The experiments differed in crop species, plant age, number of genotypes, number of replicates, inoculation method, environmental conditions, pathogen subspecies, sequence type and strain. In addition, artificial inoculation with *X. fastidiosa* can be technically difficult and some experiments require long periods before clear conclusions can be drawn. Therefore, the resistance categories reported here should be interpreted as context-specific and should not be directly compared across independent screens without considering the experimental design.

Despite these limitations, the genotypes classified as resistant or moderately resistant showed a clearer resistance phenotype than susceptible reference material within the same experimental screen. This provides a solid basis for prioritising candidate germplasm for further validation, multi-environment testing and breeding.

The results also underline the need to evaluate resistance against different *X. fastidiosa* subspecies, sequence types and strains. Resistance observed against one strain or subspecies may not necessarily confer broad-spectrum resistance.

The largest and most detailed screening effort concerned olive, reflecting the major relevance of OQDS in Europe. Long-term field trials in Apulia provided a unique opportunity to evaluate olive germplasm under high natural inoculum pressure of *X. fastidiosa* subsp. *pauca* ST53. These trials confirmed that reliable phenotypic classification in olive requires prolonged observation because symptom onset and full disease expression may take several years, even in susceptible cultivars.

The olive results confirmed the resistant or highly resistant behaviour of known reference cultivars such as Leccino and FS17 in several experimental contexts. In addition, the work identified further olive cultivars and selections with promising resistance profiles, including material derived from field-selected genotypes and breeding programmes. These materials represent valuable candidates for further agronomic evaluation, cultivar registration, resistance breeding and future genetic studies.

The large field screening trials carried out in Apulia, confirmed that none of the olive genotypes/subspecies/cultivars proved to be immune to Xfp-ST53. However, as indicated, some cultivars showed lower bacterial titre and restricted host colonization whose infections did not progressed into symptoms. Conversely, other cultivars showed high bacterial titre and severe symptoms, indicating a positive correlation between bacterial load in the trees and development of symptoms. In few cases, medium–high bacterial load did not correlate with the development of significant symptomatology and thus these trees were putatively categorized as tolerant (class 4 – Annex 1).

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The almond screenings also identified several genotypes and cultivars with reduced susceptibility or resistance to *X. fastidiosa*, depending on the pathogen subspecies, sequence type and experimental conditions tested. These results provide useful candidate material for further validation and for breeding programmes aimed at reducing the impact of *X. fastidiosa* in almond production systems.

Overall, this deliverable provides a structured dataset of candidate resistant germplasm generated through BeXyl WP4. The results support the development of resistant cultivars, the identification of resistance-associated traits and markers, and the design of more sustainable management strategies for *X. fastidiosa*-associated diseases in Europe and other affected regions. The extended Annex on olive strengthens the deliverable by providing the biological and methodological context needed to interpret the OQDS-related data.

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ANNEX 1: IDENTIFICATION OF NEW GERMPLASM RESISTANT TO *X. FASTIDIOSA* IN OLIVE

1. Background

This task took advantage of the possibility to work directly under field conditions and pressure of inoculum in the Region of Puglia, in the infected demarcated area for the *Xylella fastidiosa* subsp. *pauca* ST53, the causal agent of the olive quick decline syndrome. The Partner P5-CNR and the linked third-party Az. Melcarne had direct access to field plots and natural resources exposed since the beginning of the epidemics to high inoculum pressure. Given the long incubation period of the infections and the onset of the symptoms (a critical parameter for the categorization of the olive genotypes), having access to these resources has been critically to reach the project outcomes.

To expand the availability of resistant olive germplasm, BeXyl project pursued to evaluate two different types of olive germplasm:

- Exploiting the long-term screening programs started in the previous research initiatives (POnTE and XF-ACTORS);
- Searching for resistant olive genotypes in the wild germplasms
- Supporting the selection of the progenies resulting from the breeding programs and already available at the start of the project.

2. Experimental plots and olive germplasm analyzed

2.1 Experimental plots for screening olive cultivars

These plots have been set during the past 10 years in the framework of several European initiatives, including the Pilot project NP.EFSA.ALPHA.2014.07 funded by EFSA and then the two afore mentioned Horizon H2020 projects (POnTE and XF-ACTORS). The materials under testing include more than 400 different cultivars, encompassing major olive cultivars of the Mediterranean Basin, either from commercial stocks or provided by the Partner P19-IFAPA, which owns the worldwide core collection of Córdoba (Spain). Some of these plots were planted before the start of the project BeXyl, whereas two new ones were planted in the framework of BeXyl (Table 1).
In each plot, every year the plants were scored for the occurrence of symptoms and individually sampled and tested annually or every 2 years. This was not the case of plot F, that was planted recently (less than 1year ago).

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Table A1. Experimental plots for the screening of the olive germplasm in the region of Puglia

ID plot	N. of cultivars/ selections*	Total number of trees**	Year of plantation	Location
Parcel A	10	144	2015	Parabita https://maps.app.goo.gl/ujUTHyaDk4gitaJr9 GPS: 40.0369149793454, 18.079309922247045
Parcel B	25	365	2016 (April)	
Parcel C	15	144	2016 (November)	
Parcel D	41	303	2023	
Plot E	283	820	2018-2022***	Pastene https://maps.app.goo.gl/JXgLvLS1bJxEBTpQ8 GPS: 39.92886342369036, 18.18703209256858
Plot F	28	140	2025	Restinco https://maps.app.goo.gl/5mT3n8QqCsf6H1Jq5 GPS: 40.643438911917364, 17.86462303947314
Total	**402**	**1916**		

*The numbers include the cultivars used as control (Cellina di Nardò, Ogliarola salentina and Leccino) planted in each parcel/plot.
** The number of trees represents the actual number of plants grown per plot, excluding those lost due to mortality occurred during the experiment.
*** The plot was established in 2018; however, in 2022, it became necessary to relocate the plants from their original rows to the border of the plot. To minimize transplant shock, the plant canopy was significantly reduced.

Experimental plot in Parabita (Parcel A, B, C)

Three different parcels were planted between 2015 and 2016 in the municipality of Parabita (LE), at that time the plot was surrounded by olive trees showing severe OQDS symptoms, thus trees were exposed to high natural pressure of inoculum; for their management insecticide treatments were avoided and pruning interventions were minimal.

Although some trees died due to waterlogging and flooding in one part of the plot, at least 10 replicates were successfully maintained over the years for 29 out of the 35 cultivars grown in Parcels A and B. For the remaining cultivars, at least three trees survived, ensuring at least three biological replicates. For Parcel C, the number of replicates varied between 5 and 11. The first round of sampling was performed the first year after planting and then the trees testing negative were tested in the subsequent years.

With regard to symptoms, although some suspicious symptoms were observed starting at 3-4 years after planting, these were not consistent among the replicates, between the infected and not-infected trees, and throughout the following years. At 8-10 years after planting, when

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more consistent symptoms were recorded on the susceptible controls (Cellina di Nardò and Ogliarola salentina), symptoms were scored on the entire plots and cultivars categorized according to the severity of the symptoms, and a database of images for representative classes of symptoms and cultivars was prepared.

Experimental plot in Parabita (Parcel D)

For the majority of the cultivars the number of replicates grown in this parcel ranges from 6 to 12, Sampling and testing were performed twice, in 2024 and in early 2026. The lack of clear symptoms on the infected plants of this parcel, did not allow the full categorization of the cultivars; however, a preliminary categorization of the cultivars was performed based on the % of infected replicates and the bacterial titre estimated by qPCR.

Experimental plot E Pastene

In this plot in 2018 approximately 1000 plants were planted, these were from two distinct batches: one lot of plants was provided by P19-IFAPA and derived from the propagation of cultivars maintained in their international core collection (10-12 replicates were planted) (Lot IFAPA). The second batch included spare plants from a CNR collection, thus the number of replicates was much lower, in some cases the variety was represented by a single plant.

In 2022, all the plants had to be transferred from the original rows to the border of the plot. This implied a severe reduction of the canopies and the failure of some transplanted trees. As reported in Table 1, after the transplant 820 plants survived and are currently grown in this plot.

Parameters, criteria and classes used to categorize the cultivars under field testing

Cultivars responses to infections were classified by using a combination of phenotypic descriptors: (i) severity of the symptoms, (ii) bacterial load in the infected trees and (iii) frequency of the infections among the replicates of each cultivar.

Given the long incubation period of the infections (i.e. the time needed for the symptom onset and full manifestation) the classes of severity of the symptoms (from 0 to 5) were calibrated on the degree of severity of the symptoms showed by the highly susceptible control included in each plot.

As an illustrative example, in the oldest plots in Parabita (Parcels A and B), the cultivars Cellina di Nardò and Ogliarola salentina developed severe desiccation, with some replicates of both cultivars reaching the score values of 3.5-4.0; which for the assessment carried out in these parcels represented the peak symptom severity for a highly susceptible cultivar (Table 2). Conversely, in Parcel C—the youngest of the three parcels—symptoms on both cultivars peaked at 2.0–2.5. Consequently, for this specific parcel, these were the values used as reference for the most severe class (Table 3).

For plot E, these values were even lower, given that the plants were younger and during the experiment it was necessary to have a severe pruning intervention that most likely reduced the vegetation colonized by the bacterium from the canopies. For such reason it was not possible to use the same empirical scores (0-5) but the severity of the symptoms was

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expressed as qualitative incidence of desiccation on canopy: intense = desiccated shoots were observed across several portions of the canopy; medium= desiccated shoots were observed on a single branch; mild=withering and shoot dieback; very mild= suspicious shoot dieback (Table 4). Given that the symptomatology is not yet fully expressed on the plants in this plot, the intermediate class '4' could not be clearly associated with tolerance (i.e., high bacterial populations coupled with a lack of symptoms). Thus, it was precautionarily defined as putatively tolerant or moderately resistant.

About the bacterial titre in the plants, this consisted of an indirect estimation of the bacterial cells in the sap used for the purification of the total DNA. Briefly, the bacterial population of each positive tree was expressed as CFU/ml, calculated from Cq values generated in real time PCR (Harper et al., 2010) plotted on the standard curves generated by processing plant sap spiked with a bacterial cell suspension with known concentrations ranging from 10^5 to 10^3 CFU/ml.

Ultimately, the percentage of infected plants was based on the detection of the bacterium in the hardwood twigs of the olive trees by qPCR assay using the primers and probe developed by Harper et al. (2010). Sampling usually took place in late winter (February–March) or early May, meaning the tissues were tested after the summer spittlebug season (i.e. the peak of the natural transmission events). This guaranteed an incubation period of at least 3-4 months after transmission occurred. Samples consisted of 8 twigs representative of the canopies and/or of the symptomatic branches. DNA was extracted from 0.5gr of tissues using the Automated Nucleic Acid Purification platform Maxwell® RSC 48 (Promega, USA) and the dedicated PureFood GMO and Authentication Kit (AS1600). Samples were categorized as positive if the generated Cq value was lower than 32, doubt or negative if Cq was >32 or absent.

Using these three descriptors 6 different classes were defined, with class 1 grouping the highly susceptible cultivars and class 6 the highly resistant cultivars, as reported in the following tables. The use of these six numeric classes allows the integration of these phenotypic assessment into genome-wide association studies ongoing in Task 4.3.

Table A2. Criteria used to categorize the cultivars in Parcel A and B

CLASS	Development of symptoms	Score of the symptoms (0-5)	Bacterial load CFU/ml (corresponding average Cq)	% prevalence of the infections	Qualitative assessment
1	Yes	3,5-4	>10^5 (<25)	100	Highly susceptible
2	Yes	2-3	10^5-10^4 (29>Cq<25)	>80	Susceptible
3	Yes	1-1,5	10^5-10^4 (29>Cq<25)	>50	Moderately susceptible
4	Very mild	0-0,5	10^5-10^4 (29>Cq<25)	<50	Tollerant
5	No	0	<10^3 (Cq>29)	<50	Resistant
6	No	0	0	0	Highly resistant

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Table A3. Criteria used to categorize the cultivars in Parcel C

CLASS	Development of symptoms	Score of the symptoms (0-5)	Bacterial load CFU/ml (corresponding average Cq)	% prevalence of the infections	Qualitative assessment
1	Yes	2,5-2	>10^5 (<25)	100	Highly susceptible
2	Yes	1-1,5	10^5-10^4 (29>Cq<25)	>80	Susceptible
3	Yes	1	10^5-10^4 (29>Cq<25)	>50	Moderately susceptible
4	Very mild	0-0,5	10^5-10^4 (29>Cq<25)	<50	Tollerant
5	No		<10^3 (Cq>29)	<50	Resistant
6	No		0	0	Highly resistant

TableA4. Criteria used to categorize the cultivars in Plot E

CLASS	Development of symptoms	Severity of the symptoms	Bacterial load CFU/ml (corresponding average Cq)	% prevalence of the infections	Qualitative assessment
1	Yes	Intense	>10^5 (<25)	>80	Highly susceptible
2	Yes	Medium	10^5-10^4 (29>Cq<25)	>80	Susceptible
3	Yes	Mild	10^5-10^4 (29>Cq<25)	>80	Moderately susceptible
4	Very mild	Very mild	10^5-10^4 (29>Cq<25)	>80	Putatively tolerant/moderately resistant
5	No		<10^3 (Cq>29)	>80	Resistant
6	No		<10^3 (Cq>29) or negative	<50	Highly resistant

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2.2. Searching for resistant olive genotypes in the wild germplasms

As the previous task, this activity was built on an ongoing program of prospections carried out in the epidemic area to select adult open pollinated seedlings showing traits of resistance to OQDS and promising productive features. Within this task, P5-CNR finalized the work on previous selected genotypes (mainly recovered from the regional project ResiXO) and extended the prospections by identifying 40 more open pollinated genotypes (namely from GM1 to GM40). Prior to propagate and assess their features, a paternity test was performed and those harbouring 'Leccino' as one of the two parentals and showing promising production traits were retained for further investigations, i.e. propagated and subjected to pathogenicity tests.

The criteria used to categorize these genotypes have been previously published in the work of La Notte et al (2024), and hereafter reported (Table 5).

For the most promising genotyping the incompatibility group has been determined so that they could be included as resistant parental in the new set of controlled crosses.

Table A5. Criteria used to categorize the field-selected genotypes (from La Notte et al., 2024)

Classification of the genotype	Symptom score	Estimation of the bacterial population	Distribution of bacterium in the canopy (% of the subsamples testing positive)
Highly susceptible (HS)	>3.5	10^5–10^6 CFU/mL	>80%
Susceptible (S)	2.5–3.5	10^5–10^6 CFU/mL	>80%
Tolerant (T)	<2	10^4–10^5 CFU/mL	>50%
Resistant (R)	<2	10^3–10^4 CFU/mL	<40%
Highly resistant (HR)	<1	10^3–10^4 CFU/mL	<25%

2.3. Breeding for generating new olive germplasm

Given that breeding programs in olives require more than a decade for the selection of new genotypes, Partner P5-CNR and the Third-Party Az. Melcarne have made available cross-bred material obtained prior the start of the BeXyl project. Thus, the implementation of BeXyl project supported the preselection and selection steps on these progenies, while new combinations of crosses were performed during the project or in some cases, open pollinated seeds were collected from putative resistant cultivars other than 'Leccino' and 'FS17', widely used in the initial part of the breeding program.

For each combination at least 100-150 seeds are generally generated/collected and germinated *in vitro*, prior to be transferred in the soil and ultimately transplanted in field.

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TableA6. Information on the progenies available for the project or related to the crosses performed during the project implementation

Parental 1	Parental 2	Year of cross	Notes
Leccino	Cellina di Nardò	2018	Used as segregating progeny for task 4.3; **currently genotypes ID F10P30 and F12P98 are under evaluation as potential new resistant cultivars**
Leccino	Ogliarola salentina	2018	Used as segregating progeny for task 4.3
Leccino	FS17	2018	Used to select new putative cultivars; **currently genotypes ID F8P17 and F6P43 are under evaluation as potential new resistant cultivars**
Leccino	Oliana	2021	**Currently genotypes ID F8P9 and F6P5 are under evaluation as potential new resistant cultivars**
Leccino	Coratina	2021	Only 1 (ID F6P19) out of 160 individuals currently shows promising agronomic features and has been propagated for further evaluations.
Leccino	FS17	2021	Since most genotypes exhibit a highly wild phenotype, we are currently waiting for them to reach the adult, productive stage before initiating propagation and evaluating their potential
Leccino	Frantoio	2022	Planted in field in May 2025 (a total of 900 seedlings)
Leccino	Cipressino	2022	
Fs17	Arbequina	2022/2023	
Leccio del Corno	S215*	2022	
S105*	Arbosana	2022	

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Parental 1	Parental 2	Year of cross	Notes
Ascolana tenera	Open pollination	2023	
Frantoio	Open pollination	2023	
F6P5*	Coratina	2024/2025	In pots
F8P9*	Coratina	2024/2025	
CHIQUITITA	S215	2025	
F7P53*	F6P19*	2025	
F12P19*	F8P9*	2025	
ARBEQUINA	F6P5*	2025	
F6P19*	F6P5*	2025	

*Preselected genotypes crossed based on the resultant incompatibility group assignment

Following seed germination, once the potted plants reached a height of 40–50 cm, they were transplanted into the open field. These experimental plots are located in the demarcated infected area, so that the genotypes are exposed to the natural pressure of inoculum since the early stage of selection process.

Phenotyping of these progenies was carried out according to the following steps:

1) Field scoring and selection of those with short juvenile phase;
2) Propagation (by grafting and self-rooted cuttings) of those with promising agronomic and productive traits;
3) Inoculation of the grafted plants (at least 10 replicates) with bacterial suspensions, followed by testing and symptom assessment for the following 12-18 months;
4) Periodic field assessment on the preselected genotypes: (i) visual inspections for symptoms, and pomological traits (growth habitus, flowering intensity, and productivity); (ii) sampling and diagnostic tests. It is worth noting that in this case having a single individual for each genotype, diagnostic tests were performed by collecting multiple samples, at least one from 4 distinct portions of the canopy. Thus, the parameters evaluated to assign the phenotypic class to each pre-selected genotype were the following: (i) frequency of the infections on the canopy (i.e. number of subsamples testing positive; (ii) bacterial load estimated by qPCR; (iii) presence/absence and severity of the symptoms.

In addition to these sets of progenies, selected genotypes from controlled and open pollinated crosses deriving from the cultivars Ascolana tenera and Uovo di Piccione, provided by the University of Seville were inoculated (with insect-mediated transmission) in greenhouse to assess their response to *X. fastidiosa* subsp. *pauca* ST53.

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Table A7. List of the genotypes received from the University of Seville (Spain) (4-8 reps each)

ID pre-selection	Parentals
US 06-1173	Ascolana Tenera' open pollination
US 06-1176	Ascolana Tenera' open pollination
US 07-038	'Ascolana Tenera' x 'Uovo di Piccione'
US 07-78	'Ascolana Tenera' x 'Uovo di Piccione'
US 07-83	'Ascolana Tenera' x 'Uovo di Piccione'
US 08-24	Ascolana Tenera' open pollination
US 08-49	Ascolana Tenera' open pollination

3. Results

3.1 Screening programs for olive germplasm

Observations carried out in the experimental fields confirmed that, under high inoculum pressure, young plants are susceptible to infections, but symptom onset may take several years to become fully manifest, as clearly observed on the control trees of the susceptible olive cultivars Cellina di Nardò and Ogliarola salentina included in all experimental plantations.

On both cultivars, initial symptoms consisting in few or scattered desiccated shoots could be observed 3-4 years after planting, but these were not consistent on all replicates, even if the majority of them tested positives; indeed, in some plants the symptoms did not show a typical expansion on the affected branches, with in some case a regression of the symptoms in the following year. The lack of consistent symptom expression in the infected susceptible controls, and thus the absence of a consolidated symptomatic response (most likely due to the young age of the trees), prevented comparative analyses from being performed for the cultivars under testing until the desiccation phenomena extended and severely impacted the majority of the canopies of the susceptible controls.

In 2025, 10 years after the first setting of the experimental plantation (Parcel A), all the replicates of Cellina were infected and showed symptoms (scores >2.5 in 21 trees and two trees with 0.5). In the same parcel, ‘Leccino’ trees remained mostly asymptomatic, with only 7 symptomatic trees out of 24, with a very low score (0.5), and less than 50% of the replicates testing positive in qPCR. Under this experimental scenario, given that the controls exhibited the typical and expected phenotypic response to *Xfp* ST53, the categorization of the cultivars grown under the same conditions was considered robust (Table A8).

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Table A8. Phenotypic data collected in Parcel A

Cultivar	N. of trees*	%positive trees	Estimated bacterial population		N. of symptomatic trees	Highest score for symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value			
Leccino	24	41,67	8,36E+03	28,57	6	0,5	6
FS17	3	100,00	4,21E+04	26,24	1	1	5
Frantoio	22	90,91	3,71E+04	26,42	16	2	3-4
Cima di Melfi	21	100,00	3,19E+05	23,32	21	2,5	2
Don Carlo	3	100,00	1,29E+05	24,62	2	2,5	2
Koroneiki	22	95,45	4,70E+04	26,08	21	2,5	2
Arbequina	21	100,00	7,12E+04	25,48	20	4	1
Cellina di Nardò	23	100,00	3,61E+05	23,14	23	4	1
Coratina	22	100,00	2,73E+05	23,54	20	4	1
Arbosana	24	100,00	3,49E+05	23,19	24	3	1

*For FS17 and Don Carlo high mortality occurred after planting due to transplant shock, a consequence of the smaller size of these plants compared to those of the other cultivars.

Table A9. Phenotypic data collected in Parcel B

Cultivar	N. of trees*	%positive trees	Estimated bacterial population		N. of symptomatic trees	Highest score for symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value			
Leccino	13	38,46	5,22E+03	29,25	2	0,2-0,5	6
Cipressino	23	73,91	1,74E+04	27,51	4	0,5	5
Ascolana Tenera	21	80,95	1,98E+04	27,33	8	0,5	5
Dolce di Cassano	14	85,71	7,96E+04	25,32	2	0,5	4
Toscanina	20	95,00	2,43E+05	23,71	8	1	4
Pasola	3	100,00	2,33E+05	23,77	1	0,5	4
Bella di spagna	22	86,36	2,22E+05	23,84	18	1,5-2	3
Picholine Languedoc	12	66,67	5,63E+04	25,82	8	1,5-2	3
Nociara	15	93,33	2,28E+05	23,8	14	1,5-2	3
Oliastro	19	94,74	2,91E+05	23,45	10	1,5	3
Picholine	13	92,31	2,77E+04	26,84	8	1,5-2	3
Termite di Bitetto	22	81,82	4,70E+04	26,08	8	1,5	3
Carolea	15	86,67	1,68E+05	24,24	11	2,5-3	2
Koroneiki	18	100,00	8,77E+04	25,18	18	3	2
Peranzana	15	86,67	4,63E+05	22,78	5	3	2
Nolca	11	100,00	3,66E+05	23,12	10	4,5-5	1
Ogliarola salentina	23	100,00	1,97E+05	24,01	23	3,5-4	1
Simone	18	100,00	9,80E+04	25,02	18	3,5-4	1

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Parcels B and C were established in the same year (2016). However, because parcel B was planted in April, its plants were exposed to an additional season of vector-mediated transmission compared to those in plot C, which were planted in early winter 2016, thereby yielding more robust results (Tables A9 and A10).

Table A10. Phenotypic data collected in Parcel C

Cultivar	N. of trees	%positive trees	Estimated bacterial population		N. of symptomatic trees	Highest score for symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value			
Leccino	3	33,33	6,94E+02	32,16	0	0	6
O. cerasiformis	6	66,67	1,67E+04	27,57	0	0	5
Koroneiki o.p.	11	72,73	2,29E+04	27,12	1	1	4
Arbequina o.p.	10	80,00	6,83E+04	25,54	1	0,5	4
Changlot x Dolce Agogia	10	100,00	4,12E+05	22,95	1	2	4
Frantoio o.p.	5	100,00	4,35E+04	26,19	2	1	4
Koroneiki x Empeltre	15	100,00	1,49E+05	24,42	6	1	4
PicualxJeac9	17	88,24	2,70E+05	23,56	7	1	4
Frantoio x Arbosana	28	100,00	1,61E+05	24,3	26	2	2
Cellina di Nardò	6	100,00	1,54E+05	24,37	5	2,5	1
subsp. europaea var. sylvestris	23	82,61	1,37E+05	24,54	17	3,5	1
subsp. guanchica	7	71,43	2,61E+05	23,61	5	3	1

The analysis and categorization of the cultivars in parcel D (established in 2023), relied primarily on diagnostic test results: frequency of infected replicates and estimated bacterial load. No overt symptoms were observed, although suspicious desiccation was recorded on a few plants. Consequently, the combination of a high bacterial load and putative symptoms was used to define the most susceptible class (Table A11).

Table A11. Phenotypic data collected in Parcel D

Cultivar	N. of trees	% positive trees	Estimated bacterial population		Appearance of initial sign of symptoms (Yes/no)	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Aurina di Venafro	9	0,00	0		no	6
Cornarella	12	16,67	32	7,75E+02	no	6
Leccino	23	8,70	28,76	7,33E+03	no	6
Olivastro di Montenero	8	12,50	32,08	7,34E+02	no	6
Tonda di Filadelfia	12	8,33	31,34	1,23E+03	no	6
Correggiolo	7	57,14	26,52	3,46E+04	no	5

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Cultivar	N. of trees	% positive trees	Estimated bacterial population		Appearance of initial sign of symptoms (Yes/no)	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Lazzero	4	75,00	27,27	2,06E+04	no	5
Madre Mignola	11	45,45	26,47	3,59E+04	no	4
Verzola	6	33,33	28	1,24E+04	no	4
Americana	9	66,67	24,9	1,07E+05	no	3
Nera di Gonnos	10	70,00	25,12	9,14E+04	no	3
Redal	7	42,86	24,82	1,13E+05	no	3
Sargano di Fermo	10	30,00	23,8	2,28E+05	no	3
Tonda di Cagliari	10	60,00	25,56	6,74E+04	no	3
Verdello	8	12,50	24,06	1,91E+05	no	3
Limongella	5	40,00	21,02	1,57E+06	no	2
Marzio	7	71,43	24,14	1,80E+05	yes	2
Nera di Villacidro	9	22,22	24,75	1,18E+05	no	2
Nero di Colletorto	12	33,33	22,26	6,64E+05	no	2
Nocellara del Belice	9	55,56	22,6	5,25E+05	no	2
Pegaso	8	62,50	23,95	2,06E+05	no	2
Roggianella	5	20,00	23,52	2,77E+05	no	2
Semidana	6	33,33	21,99	8,01E+05	no	2
Tonda Iblea	5	60,00	22,7	4,90E+05	no	2
Bosana	10	50,00	21,56	1,08E+06	yes	1
Calatina	10	50,00	21,12	1,46E+06	no	1
Cerasuola	6	83,33	24,04	1,93E+05	yes	1
Coroncina	3	100,00	22,44	5,86E+05	yes	1
Ghiacciolo	3	66,67	22,92	4,20E+05	yes	1
Koroneiki	7	14,29	21,28	1,31E+06	yes	1
Nebbia del Menocchia	2	100,00	21,36	1,24E+06	no	1
Nostrale di Rigali	11	27,27	22,36	6,20E+05	yes	1
Raggiola	11	81,82	24,57	1,34E+05	yes	1
Razzo	6	83,33	23,37	3,08E+05	yes	1
Rossellino Cerretano	12	50,00	22,4	6,03E+05	yes	1

For Plot E (established in 2018), results are presented for a subset of 139 cultivars (Table A12 and A13); those with only a single replicate were excluded from the current analysis. However, once these plants reach a larger size, more in-depth investigations will be performed to obtain general insights into the host response to *X. fastidiosa*-infections. The limited number of replicates for several cultivars combined with the severe pruning intervention carried out on these plants in 2022, have weakened the robustness of the data collected so far, thereby requiring a longer period of experimental observation. Even so, it is worth noting that the onset of symptoms on susceptible controls indicates that the current experimental conditions are adequate to differentiate highly susceptible cultivars.

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Conversely, a discrepancy was noticed for the cultivar Giulia, that although it is known to be highly susceptible in commercial olive groves of the same area, it was scored to date as resistant in this plot. This may be a consequence of the long incubation period characterizing some cultivars, likewise 'Coratina', for which discrepancies were encountered in its classification among the different experimental plots.

Table A12. Phenotypic data collected in Plot E from Cultivars from IFAPA germplasm collection

Cultivar	N. of trees*	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Mari	8	12,50	2,38E+03	30,38	-	6
Uovo di Piccione	2	0,00	3,31E+03	29,91	-	6
FS17	3	0,00			-	6
Gordal Sevillana	3	0,00			-	6
Medjhol	2	0,00			-	6
Memeçik	5	20,00			-	6
Myrtolia	2	0,00			-	6
Changlot Real	7	28,57	2,48E+04	27	-	5
Ascolana Tenera	9	22,22	7,86E+03	28,66	-	5
Grappolo	6	66,67	2,50E+04	26,99	-	5
Maarri	6	66,67	5,14E+04	25,95	-	5
Toffahi	4	25,00	4,97E+04	26	-	5
Coratina	10	70,00	3,54E+04	26,49	-	5
Leccino	17	23,53	7,69E+03	28,69	-	5
Leccio del Corno	6	33,33	6,51E+04	25,61	Mild	4
Frantoio	8	50,00	1,18E+04	28,07	Mild	4
Giulia	**4**	**75,00**	**2,89E+04**	**26,78**	**-**	**4**
Uslu	3	66,67	7,59E+03	28,71	Very mild	4
Galega					Very mild	4
Maurino	5	40,00	4,33E+03	29,52	Very mild	4
Morrut	9	33,33	1,53E+04	27,7	Very mild	4
Abou Kanani	7	100,00	1,03E+05	24,95	Very mild	3
Barri	10	100,00	7,03E+04	25,5	Mild	3
Chetoui	9	100,00	8,18E+05	21,96	Mild	3
Empeltre	5	40,00	4,97E+04	26	Mild	3
Ravece	7	85,71	3,30E+05	23,27	Mild	3
Lastovka	10	60,00	6,12E+04	25,7	Mild	3
Moraiolo	9	100,00	7,22E+04	25,46	Medium	3

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Cultivar	N. of trees*	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Picholine	12	50,00	3,25E+04	26,61	Medium	3
Picual	8	87,50	7,17E+04	25,47	Medium	3
Arbequina	8	100,00	2,38E+04	27,06	Intense	2
Canino/caninese	9	100,00	1,14E+04	28,12	Intense	2
Chemial de Kabylie	11	100,00	5,29E+04	25,91	Intense	2
Chiquitita	9	100,00	1,51E+05	24,4	Medium	2
Forastera Detortosa	5	100,00	5,14E+04	25,95	Intense	2
Kalamon	5	80,00	2,68E+05	23,57	Medium	2
Koroneiki	9	44,44	3,54E+04	26,49	Medium	2
Manzallinera do Huercal	9	100,00	2,33E+05	23,77	Medium	2
Hojiblanca	11	54,55	1,00E+05	24,99	Medium	2
Picudo	10	80,00	6,92E+05	22,2	Medium	2
Arbosana	5	100,00	2,93E+05	23,44	Intense	1
Barnea	7	85,71	3,08E+05	23,37	Intense	1
Llumeta	8	100,00	3,89E+05	23,03	Intense	1
Manzanilla de Sevilla	10	70,00	5,95E+05	22,42	Intense	1
Mastoidis	3	100,00	8,12E+05	21,97	Intense	1
Meski	5	60,00	1,28E+05	24,64	Intense	1
Picholine marrocaine	9	77,78	3,10E+05	23,36	Intense	1
Pinonera	8	87,50	9,27E+04	25,1	Intense	1
Cellina di Nardò	11	100,00	7,27E+04	25,45	Intense	1

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Table A13. Phenotypic data collected in Plot E from CNR germplasm collection

Cultivar	N. of trees	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Grossane	2	0,00	-	-	-	6
Leccino	3	0,00	-	-	-	6
Lumiaro	2	0,00	-	-	-	6
Less	2	0,00	-	-	-	5
Agristigna	4	25,00	2,40E+03	30,37	-	5
Ascolana semitenera	2	100,00	1,51E+04	27,72	-	5
Cassanese	3	66,67	7,91E+03	28,65	-	5
Cipressino	2	100,00	7,69E+03	28,69	-	5
Cornilar	2	100,00	6,16E+04	25,69	-	5
Dolce D'andria	3	100,00	4,12E+04	26,27	-	5
Miniol	2	50,00	9,68E+02	31,68	-	5
Rosciola Marche	2	50,00	3,06E+04	26,7	-	5
Sirole	3	33,33	2,43E+04	27,03	-	5
Uovo di Piccione	3	33,33	1,29E+04	27,95	-	5
Verdella	2	100,00	6,43E+03	28,95	-	5
Aitana	2	100,00	1,26E+05	24,66	-	4
Blanqueta	2	50,00	7,48E+04	25,41	-	4
Campanita	2	100,00	1,67E+05	24,25	-	4
Cerasa di Montenero	2	100,00	2,02E+05	23,98	-	4
Dolce Rossano	2	100,00	1,84E+05	24,11	-	4
Gaydourelia	2	100,00	3,76E+04	26,4	-	4
Lastovka	3	100,00	2,29E+04	27,12	-	4
Leccio Del Corno	2	100,00	1,66E+04	27,58	-	4
Manzanilla de Sevilla	3	100,00	1,82E+05	24,13	-	4
Nocellara Messinese	4	50,00	4,32E+04	26,2	-	4
Oliva Nera di Colletorto	2	50,00	3,56E+05	23,16	-	4
Olivastra Seggianese	4	0,00	8,59E+04	25,21	-	4
Palmareccia	2	100,00	7,28E+03	28,77	-	4
Pasola	2	100,00	1,58E+05	24,33	-	4
Redondal	2	100,00	1,38E+05	24,53	-	4
Rossina	2	50,00	1,66E+05	24,26	-	4
Sperone di Gallo	2	50,00	2,89E+05	23,46	-	4
Verdial De Huevar	2	100,00	6,60E+04	25,59	-	4

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Cultivar	N. of trees	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Olivastro di Montenero	8	12,50	4,54E+04	26,13	Very mild	4
Maurino	2	100,00	3,90E+04	26,35	Very mild	4
Pendolino	2	100,00	3,25E+04	26,61	Medium	3
Nasitana	4	25,00	2,63E+04	26,92	Medium	3
Gordal Sevillana	8	87,50	1,16E+05	24,78	Mild	3
Moraiolo	14	71,43	1,04E+05	24,94	Mild	3
Frantoio	38	73,68	1,91E+04	27,38	Mild	3
Virdisa	2	100,00	1,87E+04	27,41	Mild	3
Galega	2	100,00	1,47E+04	27,76	Mild	3
Pizzutella	2	50,00	3,03E+05	23,39	Very mild	3
Cerasuola	2	100,00	1,46E+05	24,45	Very mild	3
Coratina	3	100,00	1,16E+05	24,78	Very mild	3
Borgiona	3	100,00	1,02E+05	24,96	Very mild	3
Castiglionese	2	100,00	8,24E+04	25,27	Very mild	3
Dritta	7	100,00	5,44E+04	25,87	Very mild	3
Maremmano	2	100,00	4,64E+04	26,1	Very mild	3
Calatina	6	100,00	1,10E+05	24,85	Very mild	3
Capolga	9	88,89	8,36E+04	25,25	Intense	2
Carbò	2	100,00	7,17E+04	25,47	Medium	2
Piangente	2	100,00	1,50E+04	27,73	Intense	2
Bosana	2	100,00	6,15E+05	22,37	Medium	2
Olivone Di Viterbo	2	50,00	3,71E+05	23,1	Medium	2
Grappuda	2	100,00	2,83E+05	23,49	Medium	2
Brandofino	2	100,00	2,62E+05	23,6	Medium	2
Olivo San Lupo	2	100,00	1,24E+05	24,68	Medium	2
Nostrale di Brisighella	2	100,00	1,07E+05	24,89	Medium	2
Leucocarpa	4	75,00	1,07E+05	24,9	Medium	2
Bottone Di Gallo	3	100,00	1,03E+05	24,95	Medium	2
Zaituna	2	100,00	1,01E+05	24,97	Medium	2
Domat	2	100,00	8,89E+04	25,16	Medium	2
Cellina	2	100,00	8,07E+04	25,3	Medium	2
Ogliarola Messinese	2	50,00	7,85E+04	25,34	Medium	2
Minuta	2	100,00	6,20E+04	25,68	Medium	2
Ramigiana	3	66,67	3,64E+04	26,45	Medium	2

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Cultivar	N. of trees	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value		
Picholine Marocaine	3	100,00	2,21E+05	23,85	Mild	2
Erbano	2	100,00	3,98E+04	26,32	Intense	2
Meski	2	100,00	2,35E+05	23,76	Medium	2
Biancolilla	2	100,00	1,80E+05	24,14	Medium	2
Nociara	2	100,00	5,59E+04	25,83	Intense	2
Olivastra	2	100,00	3,49E+04	26,51	Intense	2
Peranzana	3	100,00	7,53E+05	22,08	Intense	1
Cobrançosa	2	100,00	5,66E+05	22,49	Intense	1
Palomar	2	100,00	2,00E+05	23,99	Intense	1
Manzanilla De Jean	2	100,00	1,14E+05	24,8	Intense	1
Ogliarola salentina	3	100,00	1,01E+05	24,98	Intense	1
Passalunara	2	100,00	6,69E+04	25,57	Intense	1
Itrana	5	60,00	2,97E+05	23,42	Intense	1
Picudo	2	100,00	6,88E+05	22,21	Intense	1
Intosso	3	100,00	3,51E+05	23,18	Intense	1
Chetoui	5	100,00	3,39E+05	23,23	Intense	1
Favarol	2	100,00	2,15E+05	23,89	Intense	1
Hojiblanca	2	100,00	1,60E+05	24,31	Intense	1
Lechin De Sevilla	3	100,00	1,49E+05	24,42	Intense	1
Fontana Del Pino	3	66,67	1,38E+05	24,53	Intense	1
Picual	6	100,00	9,40E+04	25,08	Intense	1
Morrut	2	100,00	9,27E+04	25,1	Intense	1
Pallara	2	100,00	2,53E+05	23,65	Medium	1

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3.2 Searching for resistant olive genotypes in the wild germplasms

Field observations and results of the inoculations on selected propagated genotypes allowed to select a restricted panel of genotypes, descending from 'Leccino' as one of parental, that confirmed to harbour resistant traits against the bacterium as reported in the published work where a more comprehensive description of the results can be found (La Notte et al., 2024).

Agronomic and pomological assessments have been initiated for all varieties to evaluate their productive traits. In the framework of the project, replicates of these selections, have been planted in different plots to evaluate their habitus and the fruit/oil quality. The yields and the profile of the fatty acids was determined for at least 1 or 2 years and will continue in order to complete the examination for *Distinctness, Uniformity and Stability* (DUS) by the official authorities.

Overall, by combining the preliminary data on production and the level of resistance, the following four genotypes have been identified as potential new olive cultivars and thus currently under examination for the pomological traits: **"S215"; "S105"; "GM9"; "GM25".**

3.3. Screening new cross-bred olive progenies

Given the long juvenile period of olive trees, pre-selection has progressed only for the progenies generated in 2018. Indeed, the individuals from most of the remaining progenies are still in the juvenile phase and exhibit a wild growth habit, making them not yet suitable for evaluation.
Within the progenies of the crosses 'Leccino' x 'Cellina di Nardò'/'Ogliarola salentina', investigations were focused on those that showed promising habitus and productive features. Among these, only those with the highest degree of resistance (mild or no symptoms, limited bacterial colonization of the canopy and limited symptoms developed on the replicated plants artificially inoculated.
Two of these pre-selected genotypes, both deriving from 'Leccino' x 'Cellina di Nardò', "**F10P30**" and "**F12P98**" have been propagated and planted in field to further observe their habitus and production, prior to extend the evaluations to a higher number or replicated trees.

Two additional promising pre-selections have been identified within the progeny derived by crossing the two-resistant parental ('Leccino' x 'FS17'), plants ID "**F8P17**" and "**F6P43**" have been propagated and planted in the field along with the previous two.

More recently, within the progeny derived from 'Leccino' x 'Oliana', plants ID "**F8P9**" and "**F6P5**" have been propagated both for artificial inoculation and for planting (during the current year) in the field for agronomic evaluations.

If these pre-selected genotypes prove to be promising new cultivars after additional rounds of agronomic observation, the registration process will be initiated.

With regard to the progenies obtained from the breeding programs carried out from the University of Seville, all the lines resulted susceptible, with inoculated plants systemically colonized, with large bacterial populations, and extensive shoot dieback, with the exception of two genotypes that although infected did not develop symptoms (Table A14).

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Table A14. Phenotypic data collected on the table olive selection from University of Seville (Spain)

ID PRE-SELECTION	**Parentals**	**Symptoms reproduced on artificially inoculated plants**
US 06-1173	Ascolana Tenera' open pollination	Higly susceptible
US 06-1176	Ascolana Tenera' open pollination	Higly susceptible
US 07-038	'Ascolana Tenera' x 'Uovo di Piccione'	Higly susceptible
US 07-78	'Ascolana Tenera' x 'Uovo di Piccione'	Higly susceptible
US 07-83	'Ascolana Tenera' x 'Uovo di Piccione'	Moderately resistant
US 08-24	Ascolana Tenera' open pollination	Moderately resistant
US 08-49	Ascolana Tenera' open pollination	Higly susceptible

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