RIGENESI

Regione Puglia Action Plan: Sampling (plants and insect vectors); Eradication / containment / compensation

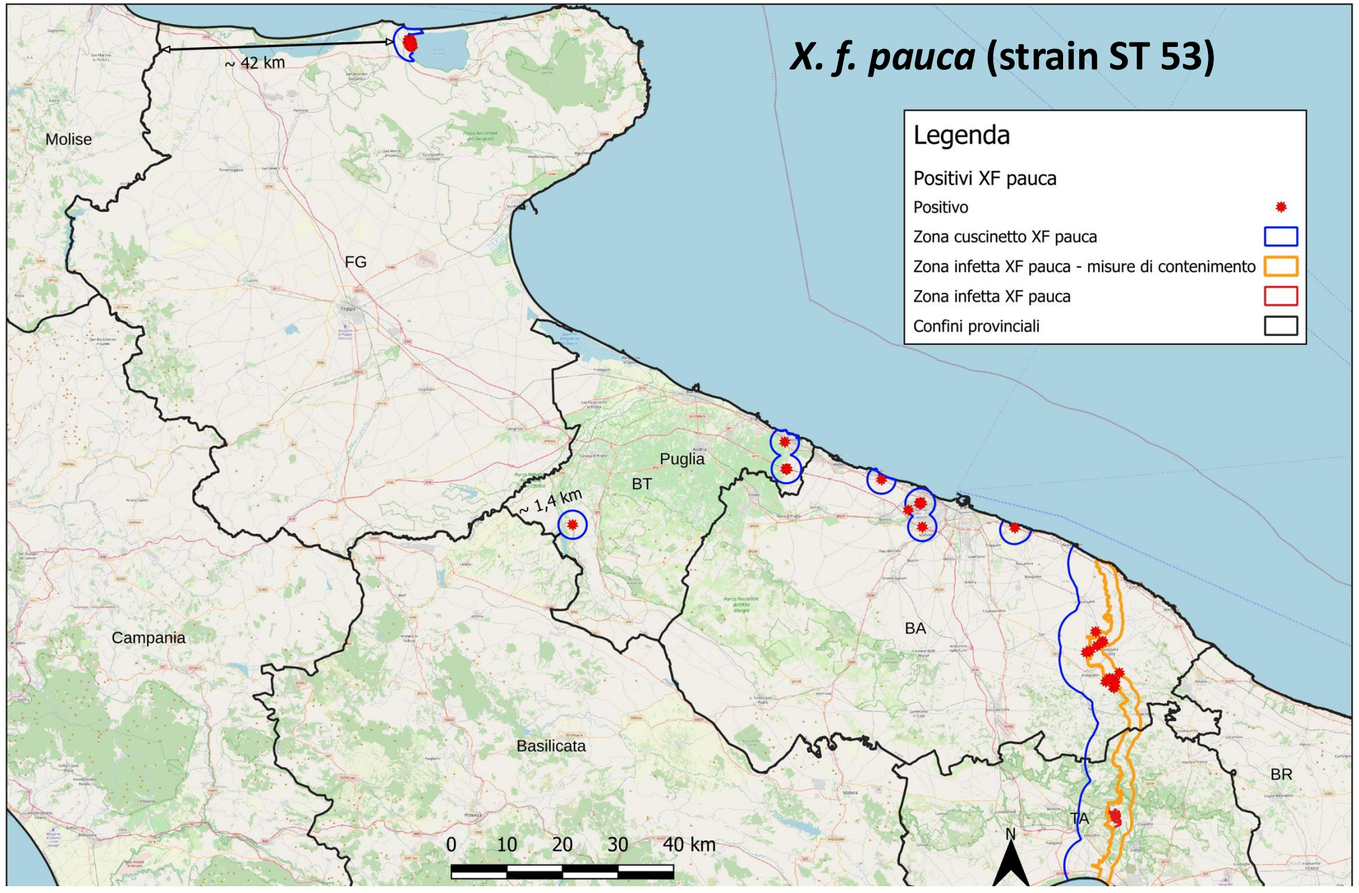
Initiative in the framework of the Cooperation Agreement between Regione Puglia and CIHEAM Bari for the implementation of the "Extraordinary Plan for the Regeneration of Olive Growing in Puglia," Article 18 "Communication and Information" – Action 4.2.

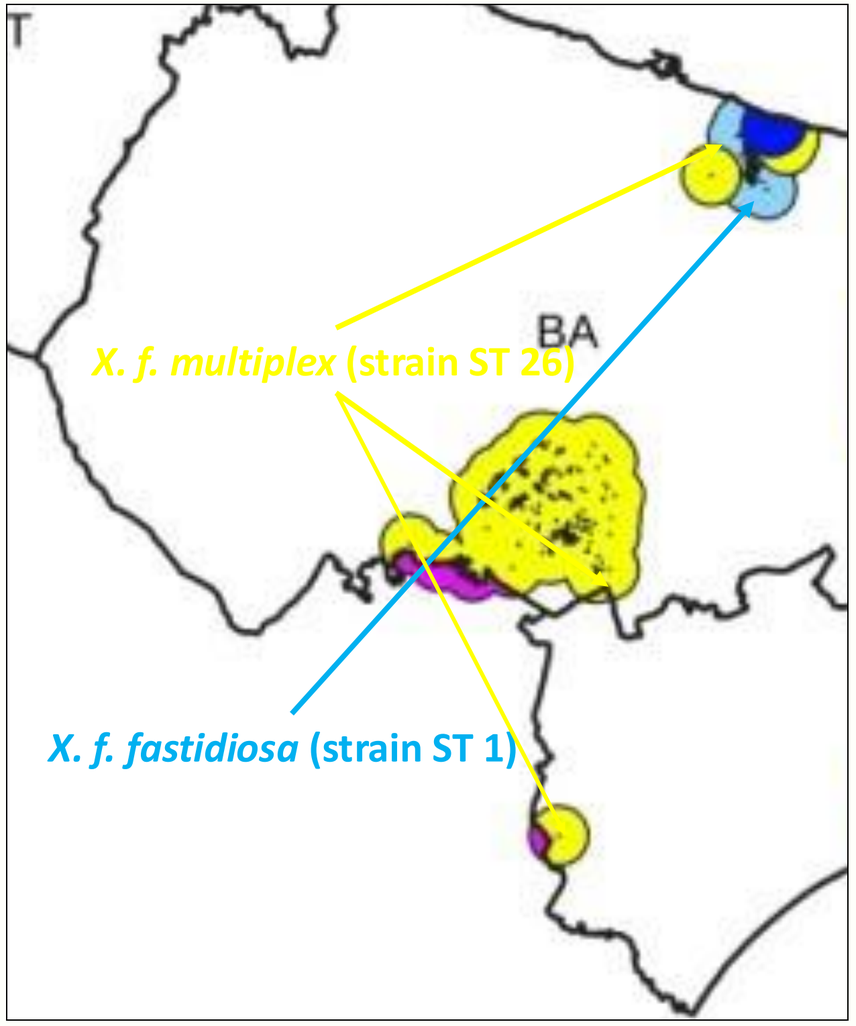
Dott. Francesco Palmisano

E-mail: fra.palmisano@regione.puglia.it

Phytosanitary service of Apulia Region

Three subspecies of *Xylella fastidiosa* were identified in Puglia; *X. f. fastidiosa* (strain ST 1), *X. f. pauca* (strain ST 53) and *X. f. multiplex* (strain ST 26)





REG (EU) 2023/1706 of 7 September 2023 amending Implementing Regulation (EU) 2020/1201 as regards the lists of plants known to be susceptible to *Xylella fastidiosa*

Specified plants susceptible to *Xylella fastidiosa* subspecies *fastidiosa*

Acer L.

Ambrosia artemisiifolia L.

Calicotome spinosa (L.) Link

Cercis occidentalis Torr.

Cistus monspeliensis L.

Citrus limon (L.) Osbeck

Citrus paradisi Macfad.

Citrus reticulata Blanco

Citrus sinensis (L.) Osbeck

Coffea L.

Elaeagnus angustifolia L.

Erysimum L.

Ficus carica L.

Genista lucida L.

Juglans regia L.

Lupinus aridorum McFarlin ex Beckner

Magnolia grandiflora L.

Medicago sativa L.

Metrosideros Banks ex Gaertn.

Morus L.

Myrtus communis L.

Nerium oleander L.

Pelargonium graveolens L'Hér.

Pluchea odorata (L.) Cass.

Polygala myrtifolia L.

Prunus L.

Psidium L.

Rhamnus alaternus L.

Rubus rigidus Sm.

Rubus ursinus Cham. & Schldl.

Ruta chalepensis L.

Salvia rosmarinus Spenn.

Sambucus L.

Spartium junceum L.

Strelitzia reginae Aiton

Streptocarpus Lindl.

Teucrium capitatum L.

Ulex europaeus L.

Ulmus americana L.

Vaccinium corymbosum L.

Vinca L.

Vitis L.

REG (EU) 2023/1706 of 7 September 2023 amending Implementing Regulation (EU) 2020/1201 as regards the lists of plants known to be susceptible to *Xylella fastidiosa*

Specified plants susceptible to *Xylella fastidiosa* subspecies *multiplex*

Acacia Mill.

Acer griseum (Franch.) Pax

Acer pseudoplatanus L.

Acer rubrum L.

Adenocarpus lainzii (Castrov.) Castrov.

Alnus rhombifolia Nutt.

Ambrosia L.

Ampelopsis cordata Michx.

Anthyllis barba-jovis L.

Anthyllis hermanniae L.

Arbutus unedo L.

Argyranthemum frutescens (L.) Sch.Bip.

Artemisia L.

Asparagus acutifolius L.

Athyrium filix-femina (L.) Roth

Baccharis halimifolia L.

Berberis thunbergii DC.

Calicotome spinosa (L.) Link

Calicotome villosa (Poir.) Link

Callistemon citrinus (Curtis) Skeels

Calluna vulgaris (L.) Hull

Calocephalus brownii (Cass.) F.Muell

Carya Nutt.

Celtis occidentalis L.

Cercis canadensis L.

Cercis occidentalis Torr.

Cercis siliquastrum L.

Chionanthus L.

Cistus L.

Clematis cirrhosa L.

Clematis vitalba L.

Convolvulus cneorum L.

Coprosma repens A.Rich.

Coronilla L.

Cytisus Desf.

Dimorphotheca ecklonis (DC.) Norl.

Dimorphotheca fruticosa (L.) Norl.

Dittrichia viscosa (L.) Greuter

Dodonaea viscosa (L.) Jacq.

Echium plantagineum L.

Elaeagnus angustifolia L.

Elaeagnus x submacrophylla Servett.

Encelia farinosa A.Gray ex Torr.

Erica cinerea L.

Erigeron L.

Eriocephalus africanus L.

Erodium moschatum (L.) L'Hérit.

Euryops chrysanthemoides (DC.) B.Nord.

Euryops pectinatus (L.) Cass.

Fallopia japonica (Houtt.) Ronse Decr.

Ficus carica L.

Frangula alnus Mill.

Fraxinus L.

Gazania rigens (L.) Gaertn.

Genista L.

Ginkgo biloba L.

Gleditsia triacanthos L.

Grevillea juniperina Br.

Hebe Comm. ex Juss.

Helianthus L.

Helichrysum Mill.

Hibiscus syriacus L.

Hypericum androsaemum L.

Hypericum perforatum L.

Ilex aquifolium L.

Iva annua L.

Jacobaea maritima (L.) Pelser & Meijden

Koelreuteria bipinnata Franch.

Lagerstroemia L.

Clematis cirrhosa L.

Clematis vitalba L.

Convolvulus cneorum L.

Coprosma repens A.Rich.

Coronilla L.

Cytisus Desf.

Dimorphotheca ecklonis (DC.) Norl.

Dimorphotheca fruticosa (L.) Norl.

Dittrichia viscosa (L.) Greuter

Dodonaea viscosa (L.) Jacq.

Echium plantagineum L.

Elaeagnus angustifolia L.

Elaeagnus x submacrophylla Servett.

Encelia farinosa A.Gray ex Torr.

Erica cinerea L.

Erigeron L.

Eriocephalus africanus L.

Erodium moschatum (L.) L'Hérit.

Euryops chrysanthemoides (DC.) B.Nord.

Euryops pectinatus (L.) Cass.

Fallopia japonica (Houtt.) Ronse Decr.

Ficus carica L.

Frangula alnus Mill.

Fraxinus L.

Gazania rigens (L.) Gaertn.

Genista L.

Ginkgo biloba L.

Gleditsia triacanthos L.

Grevillea juniperina Br.

Hebe Comm. ex Juss.

Helianthus L.

Helichrysum Mill.

Hibiscus syriacus L.

Hypericum androsaemum L.

Hypericum perforatum L.

Ilex aquifolium L.

Iva annua L.

Jacobaea maritima (L.) Pelser & Meijden

Koelreuteria bipinnata Franch.

Lagerstroemia L.

Salvia officinalis L.

Salvia rosmarinus Spenn.

Sambucus L.

Santolina chamaecyparissus L.

Santolina magonica (O.Bolòs, Molin. & P.Monts.) Romo

Sapindus saponaria L.

Scabiosa atropurpurea var. *maritima* L.

Solidago virgaurea L.

Spartium L.

Strelitzia reginae Aiton

Syringa vulgaris L.

Ulex L.

Ulmus L.

Vaccinium L.

Viburnum tinus L.

Vinca L.

Vitex agnus-castus L.

Westringia fruticosa (Willd.) Druce

Xanthium strumarium L.

REG (EU) 2023/1706 of 7 September 2023 amending Implementing Regulation (EU) 2020/1201 as regards the lists of plants known to be susceptible to *Xylella fastidiosa*

Specified plants susceptible to *Xylella fastidiosa* subspecies *pauca*

Acacia Mill.

Amaranthus retroflexus L.

Asparagus acutifolius L.

Catharanthus roseus (L.) G.Don

Chenopodium album L.

Cistus albidus L.

Cistus creticus L.

Citrus L.

Coffea L.

Dimorphotheca fruticosa (L.) Norl.

Dodonaea viscosa (L.) Jacq.

Elaeagnus angustifolia L.

Eremophila maculata (Ker Gawler) F. von Müller.

Erigeron L.

Euphorbia chamaesyce L.

Euphorbia terracina L.

Genista hirsuta Vahl.

Grevillea juniperina Br.

Hebe Comm. ex Juss.

Heliotropium europaeum L.

Hibiscus L.

Laurus nobilis L.

Lavandula L.

Myoporum insulare R.Br.

Myrtus communis L.

Nerium oleander L.

Olea europaea subsp. *europaea* L.

Olea europaea subsp. *sylvestris* (Mill.) Rouy

Pelargonium L'Hér. ex Aiton

Phillyrea latifolia L.

Pistacia vera L.

Polygala myrtifolia L.

Prunus L.

Rhamnus alaternus L.

Salvia rosmarinus Spenn.

Spartium junceum L.

Thymus vulgaris L.

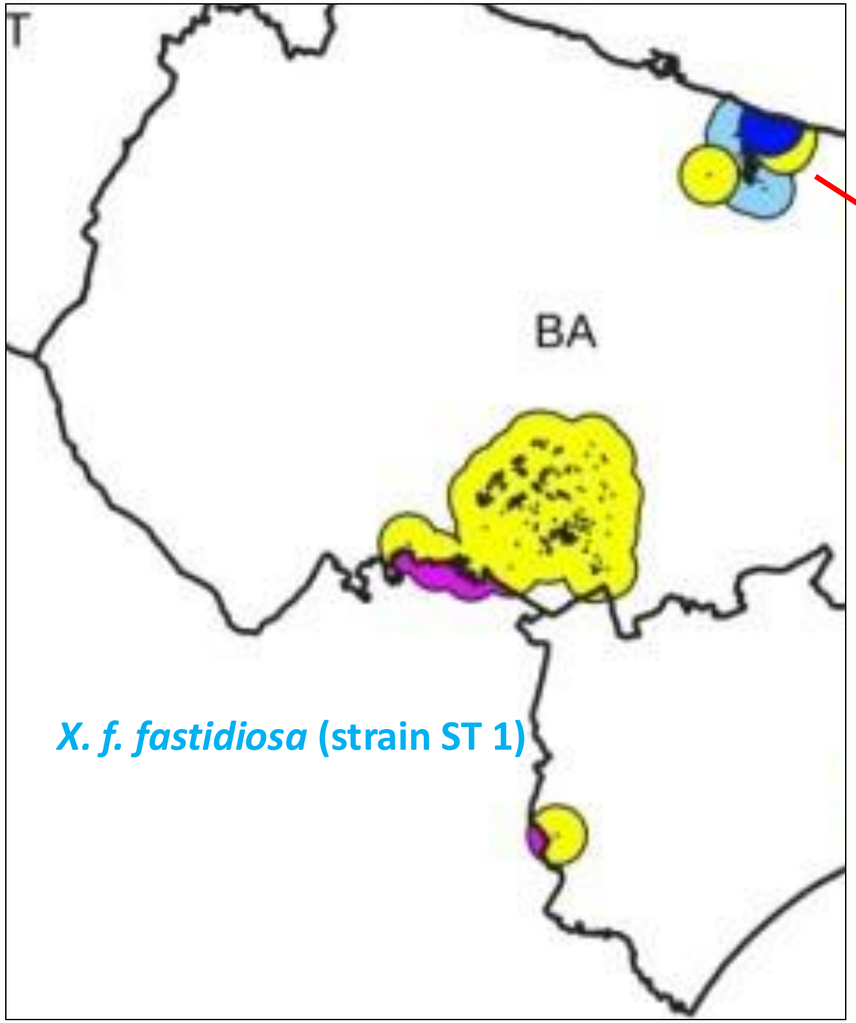
Ulex parviflorus Pourr.

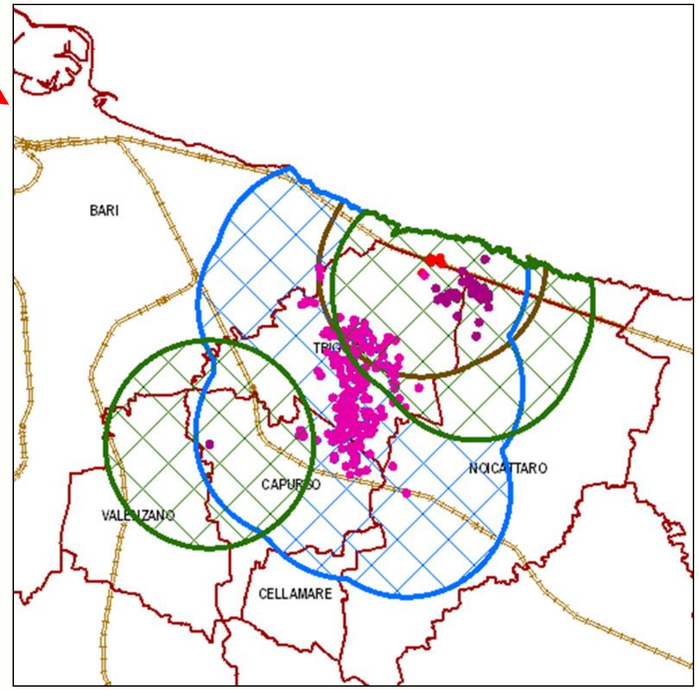
Vinca minor L.

Westringia fruticosa (Willd.) Druce

Westringia glabra R.Br.'

Xylella fastidiosa in Apulia Region - Italy





Occurence of Xf isolates belonging to **3 subspecies**

- Pauca: «olive»
- Multiplex: almond
- Fastidiosa: grapes & almond

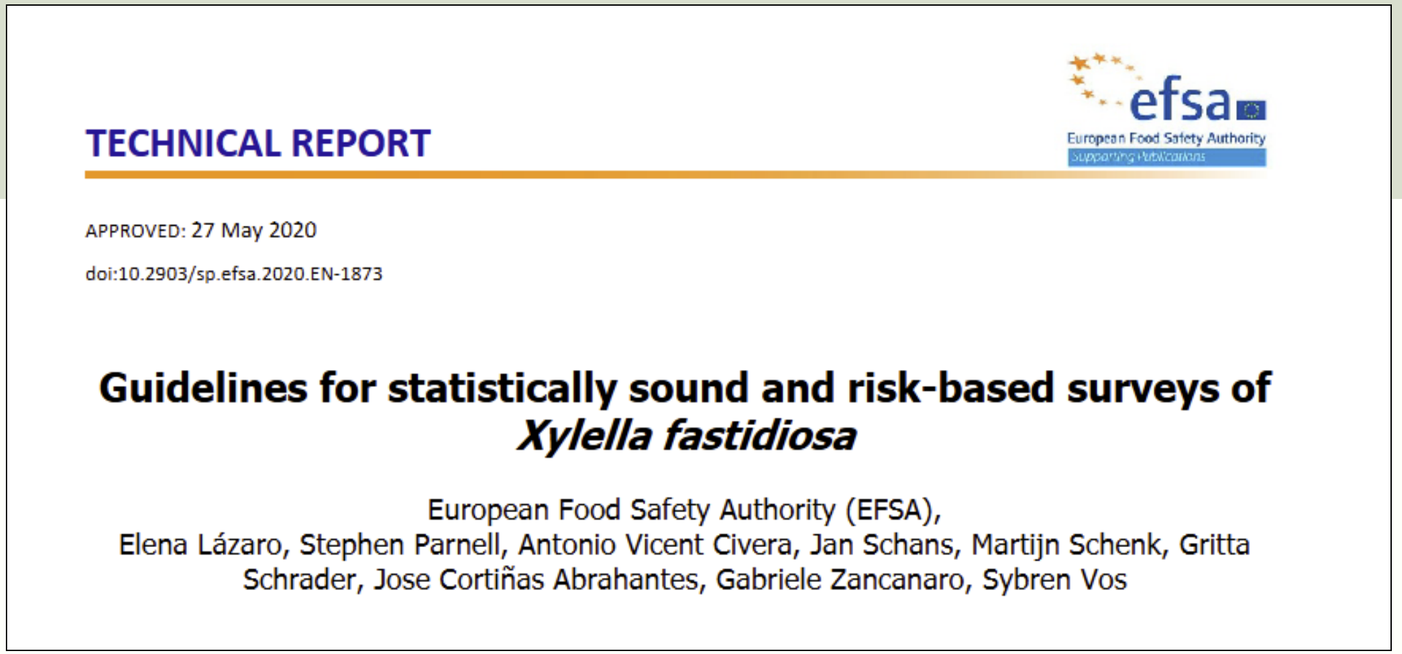
Surveys:

- *Detection/delimiting*
- *Subspecies identification*

Common hosts: eg. Almond, Polygala

SURVEILLANCE

SURVEILLANCE BASED ON



TECHNICAL REPORT

efsa European Food Safety Authority Supporting Publications

APPROVED: 27 May 2020

doi:10.2903/sp.efsa.2020.EN-1873

Guidelines for statistically sound and risk-based surveys of *Xylella fastidiosa*

European Food Safety Authority (EFSA),
Elena Lázaro, Stephen Parnell, Antonio Vicent Civera, Jan Schans, Martijn Schenk, Gritta Schrader, Jose Cortiñas Abrahantes, Gabriele Zancanaro, Sybren Vos

The knowledge acquired in previous plant health surveillance activities performed in accordance with *Article 10 - Reg. (EU)* 1201/2020 and Article 2 - Reg. (EU) 1201/2020.

SURVEILLANCE BASED ON

Article 9

Destruction of plants

1. The Member State concerned shall destroy the plants and parts of plants referred to in Article 7(1), in a manner ensuring that the specified pest is not spread, *in situ* or in a nearby location designated for this purpose within the infected zone or, provided that those plants or parts of plants are covered by net against the vectors, at the shortest distance from that location.

2. The Member State concerned may decide, on the basis of the risk level, to limit the destruction to the branches and foliage only and subject the related wood to phytosanitary treatment as referred to in Article 8(1). The root system of those plants shall be either removed or devitalised, with an appropriate phytosanitary treatment to avoid resprouting.

Article 10

Annual surveillance of the demarcated area

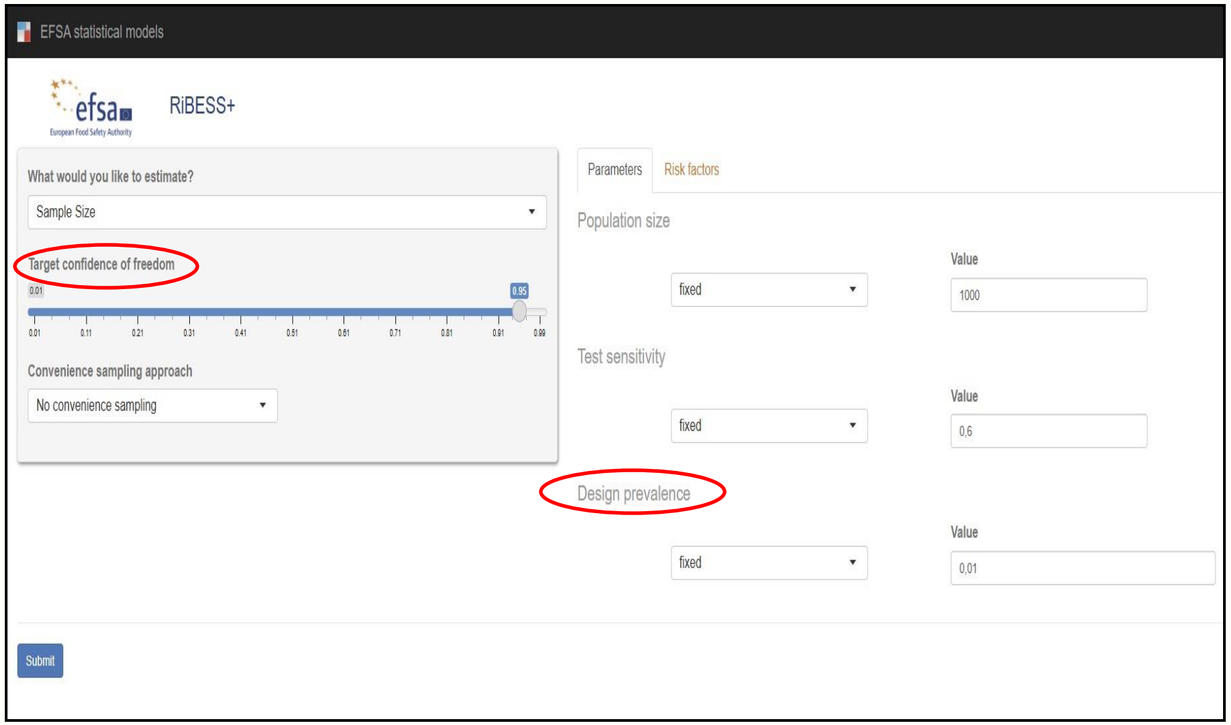
In the entire demarcated area, the Member State concerned shall monitor, at the most appropriate times, the presence of the specified pest by annual surveys, in accordance with Article 2(5) and (6), and taking into account the information referred to in the Authority's Pest Survey Card on *Xylella fastidiosa*.

In the infected zones, the Member State concerned shall sample and test the host plants, including the specified plants which have not been removed pursuant to Article 7(1). For that purpose, and taking into account the Authority's Guidelines for statistically sound and risk-based surveys of *Xylella fastidiosa*, the survey design and sampling scheme shall be able to identify with at least 90 % confidence a level of presence of infected plants of 0,5 %.

In buffer zones, the Member State concerned shall sample and test the host plants, as well as other plants showing symptoms indicating possible infection or suspected to be infected by that pest. For that purpose, and taking into account the Authority's Guidelines for statistically sound and risk-based surveys of *Xylella fastidiosa*, the survey design and sampling scheme shall be able to identify with at least 90 % confidence a level of presence of infected plants of 1 %, taking into account that the first 400 m surrounding the infected zones has a higher risk.

The Member State concerned shall also monitor the presence of the specified pest in the vectors located in the demarcated area in order to determine the risk of further spreading posed by vectors and to evaluate the effectiveness of the phytosanitary control measures applied in accordance with Article 8.

SURVEILLANCE BASED ON

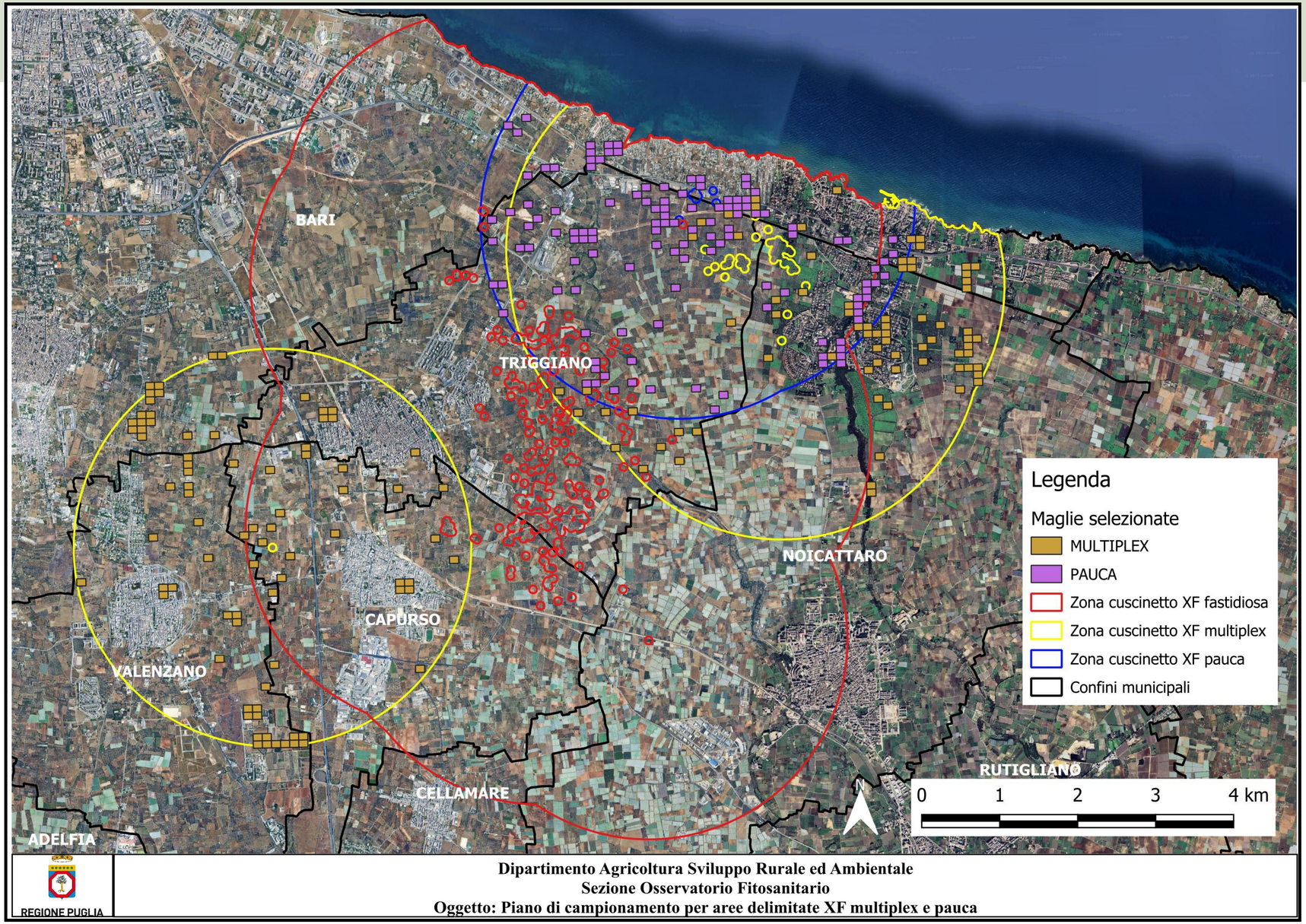


Tab. 5 – Area delimitata multiplex Capurso - Zona cuscinetto. Parametri utilizzati per lo schema di campionamento e Piano di Indagine

		Olivi	Vigneti	Fruttiferi	Altre Aree	TOTALE
Parametri	Livello di confidenza	0,6	0,5	0,6	0,4	**0,95200**
	Prevalenza	0,009	0,05	0,009	0,01	**0,01**
Popolazione Target nell'area delimitata	**Numero di piante specificate**	**175.581**	**239.025**	**11.869**	**924**	**427.399**
	Area a Rischio Alto	7.776	15.855	0	0	23.631
	Area a Rischio Base	167.805	223.170	11.869	924	403.768
	Incidenza sul totale					
	Area a Rischio Alto	0,044287252	0,066331974	0	0	0,047594204
	Area a Rischio basso	0,955712748	0,933668026	1	1	0,952405796
Unità Epidemiologica	Unità epidemiologica	Ettaro				
	Numero di ettari	**585,27**	**159,35**	**18,99**	**3,08**	**766,69**
	Area a Rischio Alto	25,92	10,57	0,00	0,00	36,49
	Area a Rischio Basso	559,35	148,78	18,99	3,08	730,20
	Piante per ettaro	300	1500	625	300	
Piano di indagine	**Piante da campionare**	**146**	**21**	**184**	**93**	**444**
	Area a Rischio Alto	49	7	0	0	56
	Area a Rischio basso	97	14	184	93	388
Allocazione del campione	Piante da campionare per ettaro	**7**	**8**	**4**	**4**	
	Ettari da campionare	**21**	**3**	**46**	**23**	**93**
	Area a Rischio Alto	7	1	0	0	8
	Area a Rischio Basso	13,85714286	1,75	46	23,25	85
	% Area Rischio Alto	27%	8%	0%	0%	22%
	% Area Rischio Basso	2,48%	1,18%	242,23%	754,87%	11,62%

Sampling unit
Grid cells (= 1 hectar)

Number? – defined based on RIBESS+



STAFF FOR SURVEILLANCE ACTIVITIES

The surveillance activities in accordance to EU Regulation 625/2017 falls within the scope of "other official activities" carried out by the competent authorities, or by delegated bodies or natural persons to whom certain other official activities have been delegated in accordance with this Regulation...".

Regional Law 19/2019 assigned ARIF "Regional Agency for Irrigation and Forestry activities" the task of monitoring and eradicating infected plants.

The ARIF staff involved in surveillance activities are appointed "Phytosanitary Assistants" by decree of the MASAF pursuant to Article 20 of Legislative Decree 19/2021:

The "Phytosanitary Assistants":

- are professionally qualified technicians working at facilities or organizations other than regional phytosanitary services,
- respond technically to the directives of the phytosanitary service responsible for the territory;
- comply with the instructions given by the head of the phytosanitary service responsible for the territory;
- carry out all official activities relating to plant protection, with the exception prescribing official measures and issuing phytosanitary certificates;
- operate on the express instructions of the head of the competent phytosanitary service, in relation to the functions assigned to them.
- propose to the phytosanitary inspector the application of a phytosanitary measure or the imposition of a sanction.

The phytosanitary activities are performed also from staff directly assigned to Phytosanitary service: Phytosanitary Inspector or Phytosanitary agent

PHYTOSANITARY ASSISTANTS

Phytosanitary agents are registered in the appropriate section of the National Register of National Phytosanitary Service staff. They have the following skills and experiences:

- Degree in agricultural sectors,

- Work experience in the field of plant health,

- Attendance at a specific training courses in plant health.

SURVEILLANCE ACTIVITIES

Tools to visual inspection and collect samples (equipment):

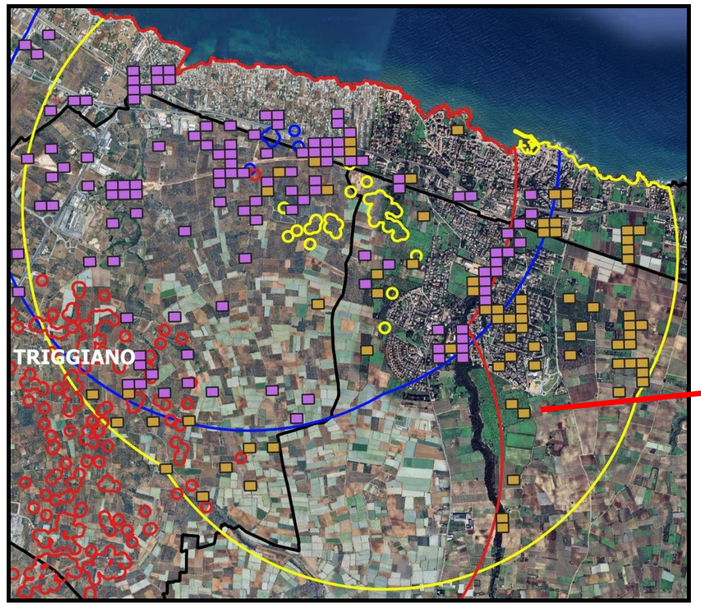
- Specific jacket and ID badge
- telescopic pruning shears;
- pruning shears;
- tablet with specific software (App Xylella);
- Sampling Bag with barcode;
- Ethanol to disinfect the cut tools;
- Cooler bag

SURVEILLANCE ACTIVITIES

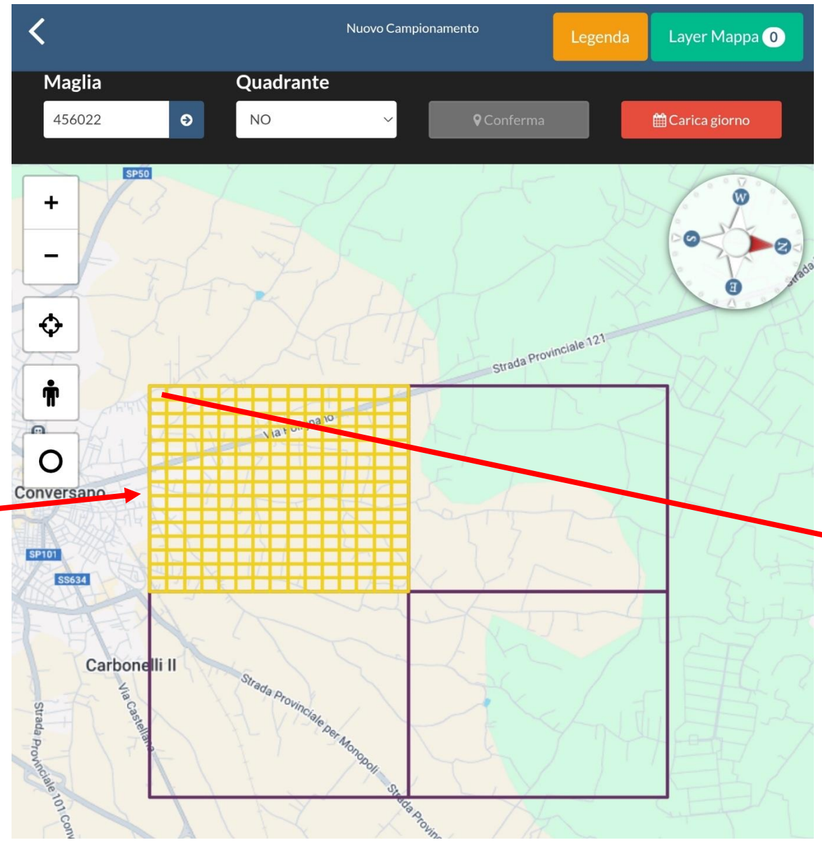
SAMPLING: 1° STEP – Visual inspection and collection of shoots/leaves

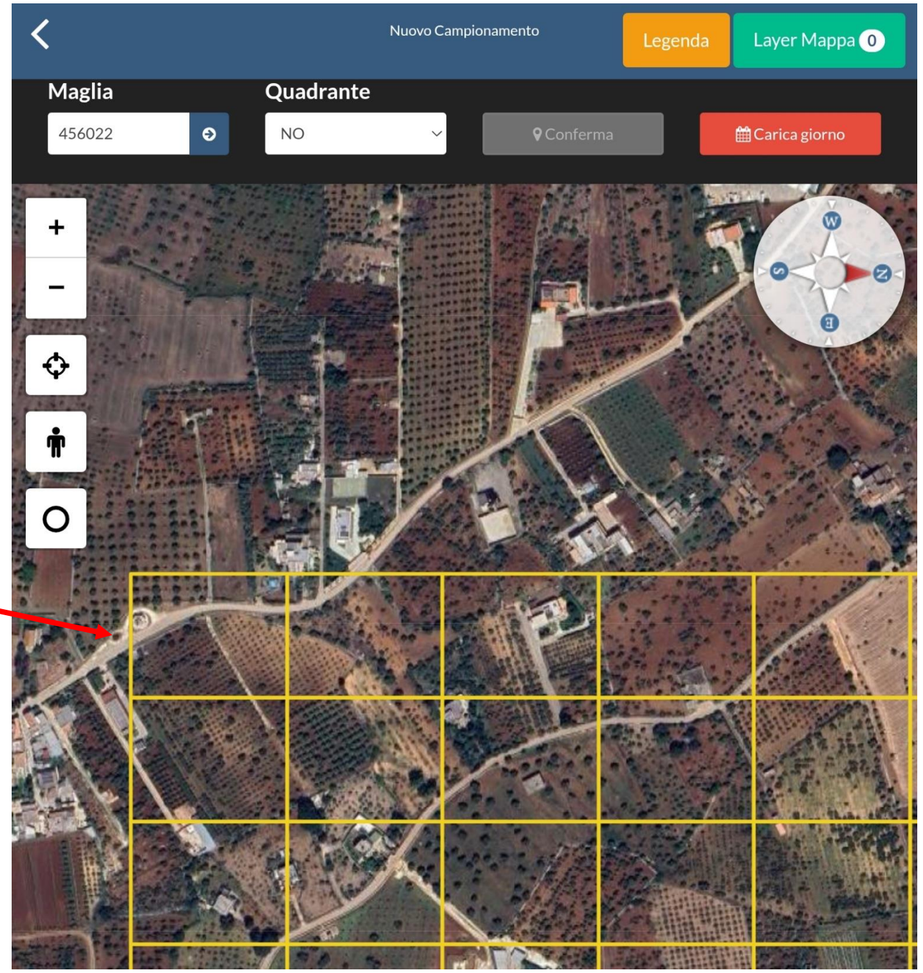
The APP Xylella assign to each team (identified by an ID) the grid cells to sample on a daily basis

Macrocells



Macrocells (15 hectars X 15 hectars)





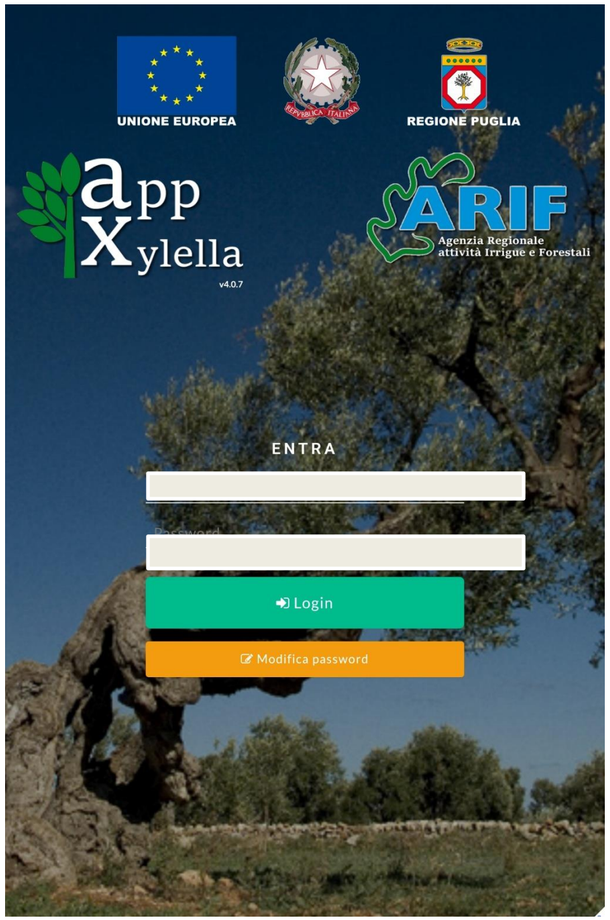
1 pooled sample to each 1 cell assigned (except to grapes in which for each grid are need 2 pooled sample)

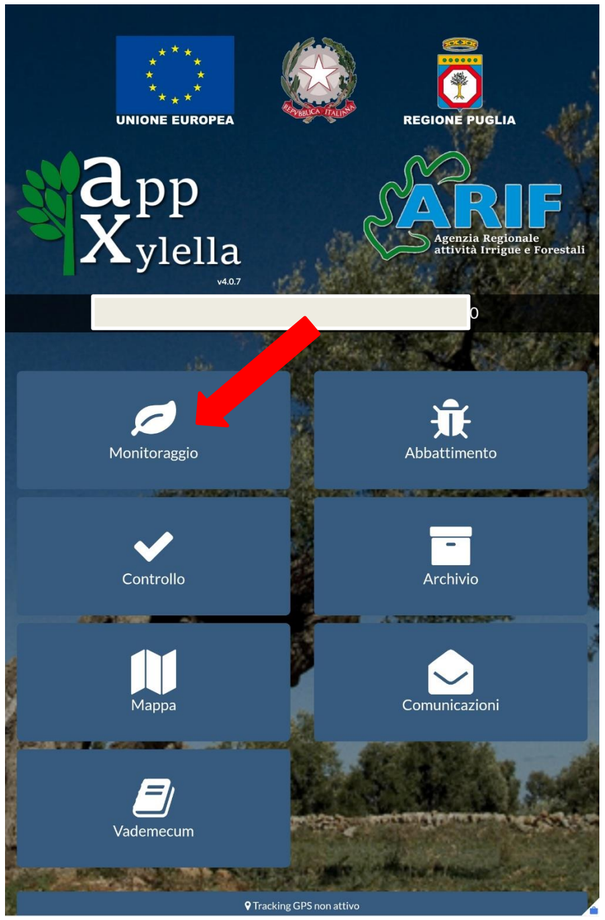
SURVEILLANCE ACTIVITIES

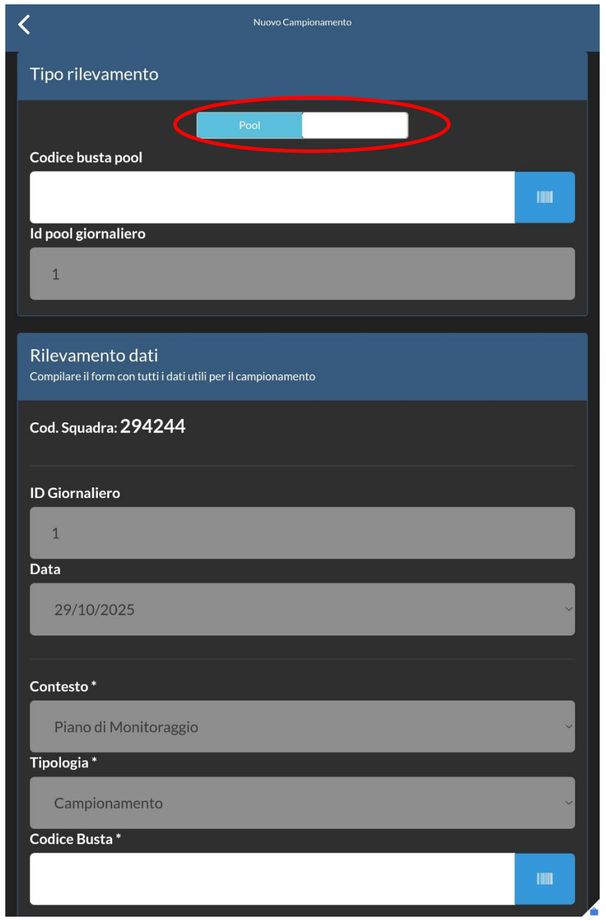
APP Xylella

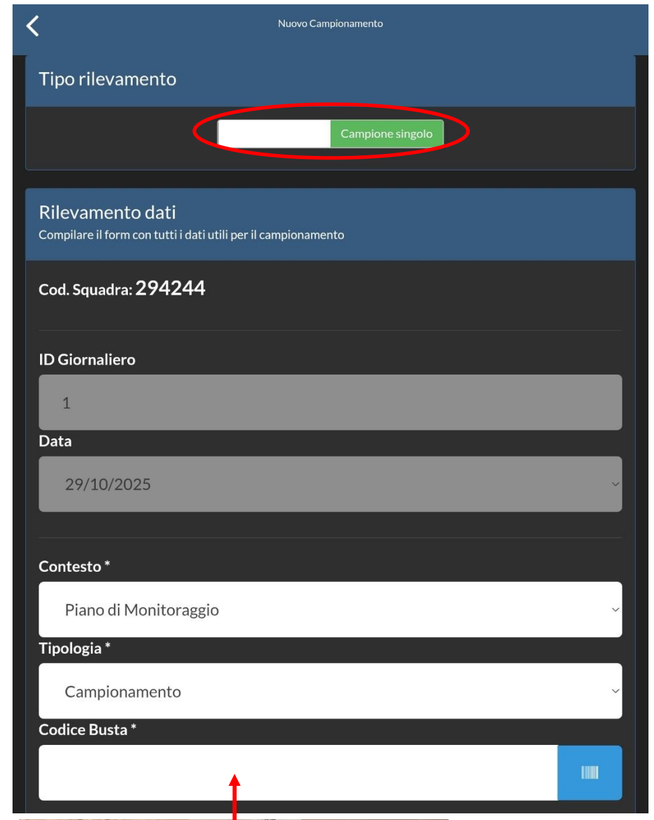
POOLED SAMPLE

FILL THE INFO IN THE APP XYLELLA







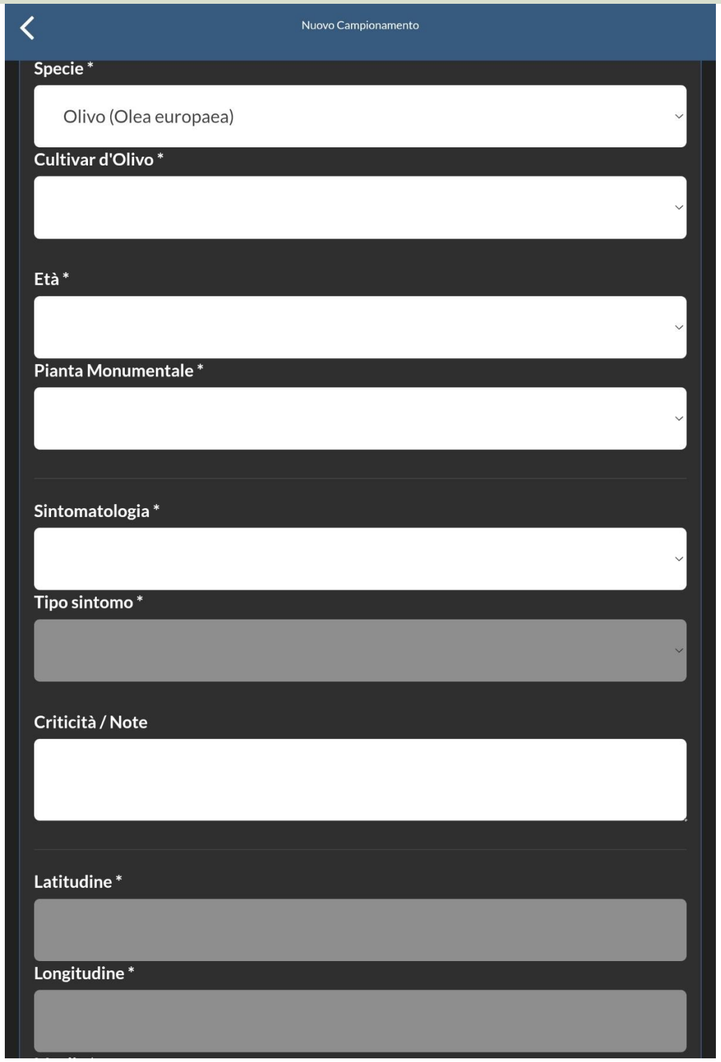


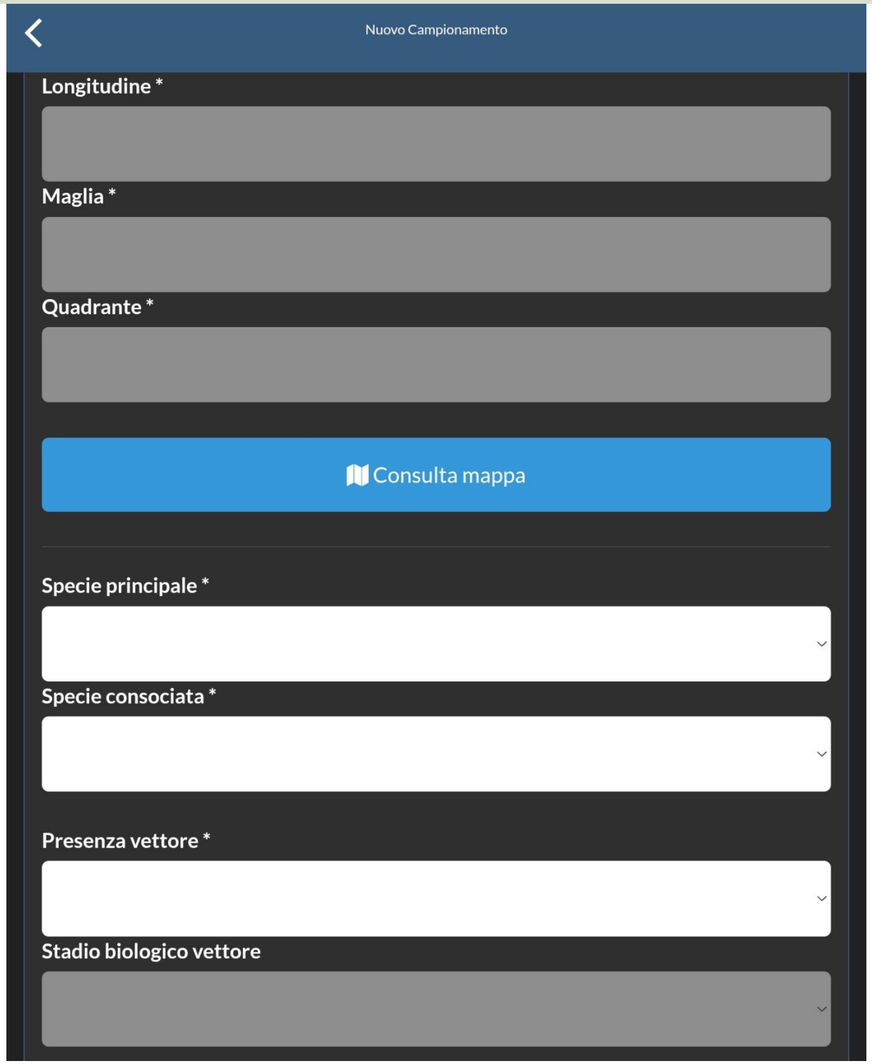
* MANDATORY FIELDS

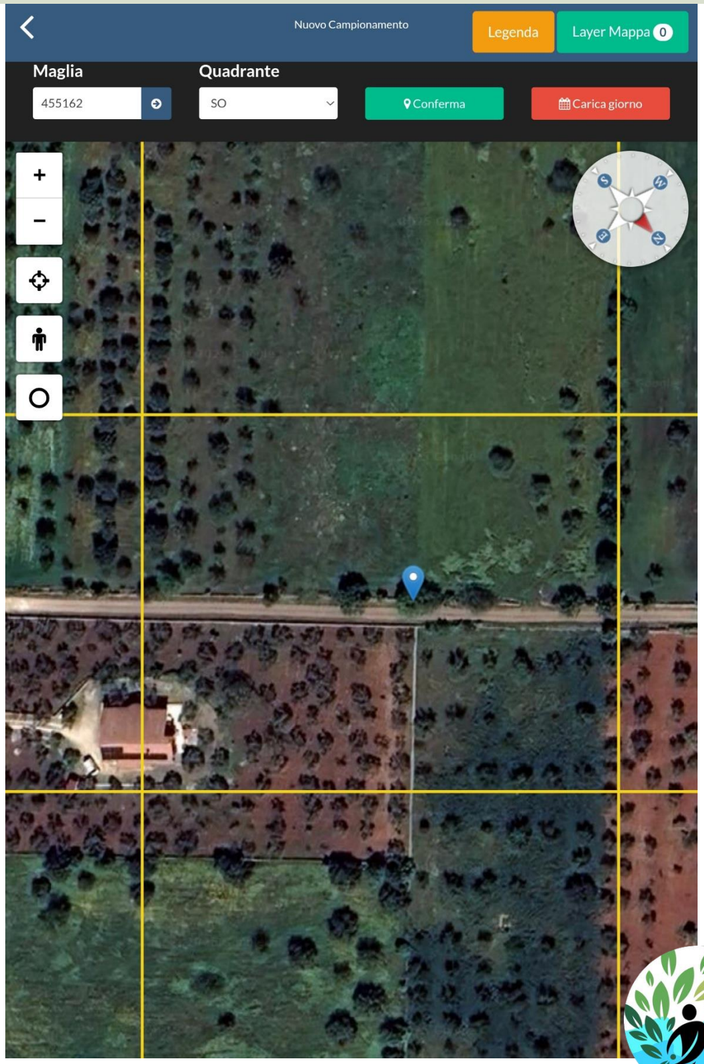
SURVEILLANCE ACTIVITIES

APP Xylella

Possibility to adjust the position of the plant sampled



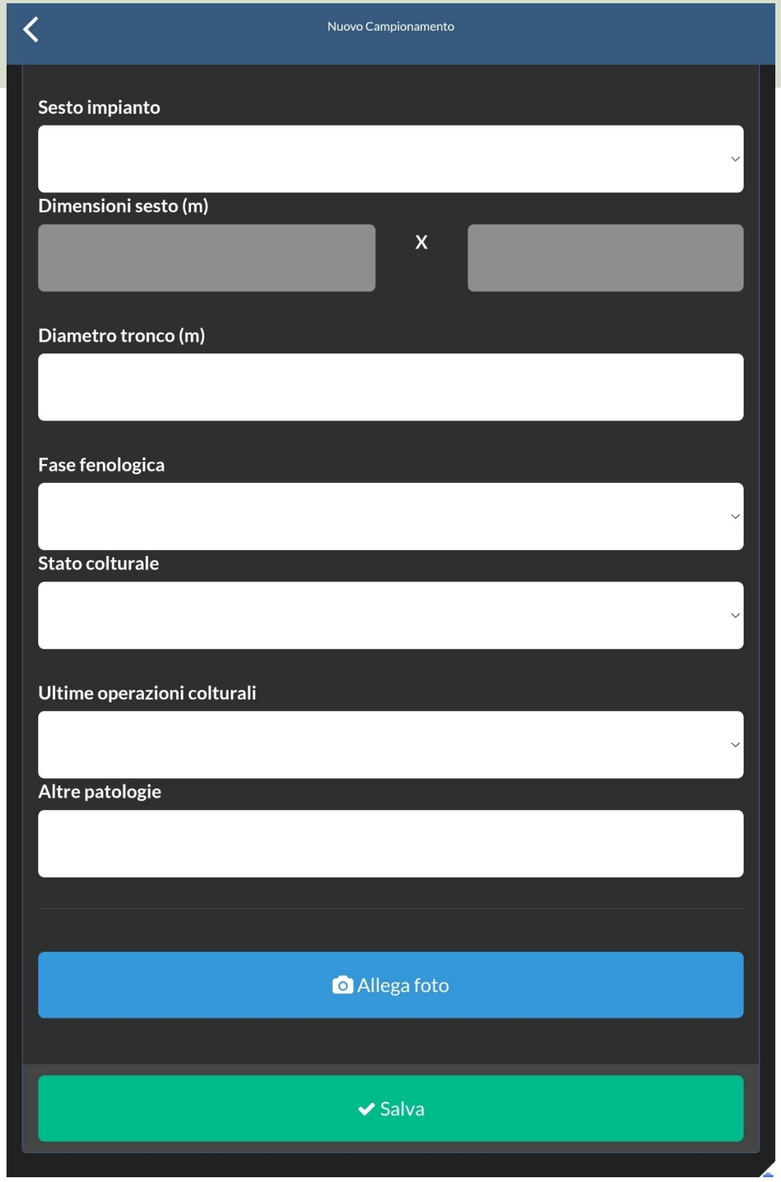




* MANDATORY FIELDS

SURVEILLANCE ACTIVITIES

In the app Xylella is mandatory to attach the picture of the plant sampled or ispectioned



SURVEILLANCE ACTIVITIES

The phytosanitary assistants must visually inspect each assigned grid (“Olive Grove,” “Vineyard,” “Orchard,” or “Other Area”) in order to:

- Verify the presence of the species specified in the subspecies Xylella fastidiosa being monitored (e.g., olive tree in *Xf pauca*, grapevine in *Xf fastidiosa*, or almond tree in *Xf multiplex*);

- Verifity the presence of symptomatic plants.

In presence of symptomatic plants, single samples must be collected

In absence of symptomatic plants, pooled samples must be collected (1 pooled sample consists of several single samples)

SURVEILLANCE ACTIVITIES

Main species inspected and collected

- Olive (*Xylella fastidiosa* sub. *pauca*)
- Grapevine (*Xylella fastidiosa* sub. *fastidiosa*)
- Almond (*Xylella fastidiosa* sub. *multiplex*)

SURVEILLANCE ACTIVITIES

Olive

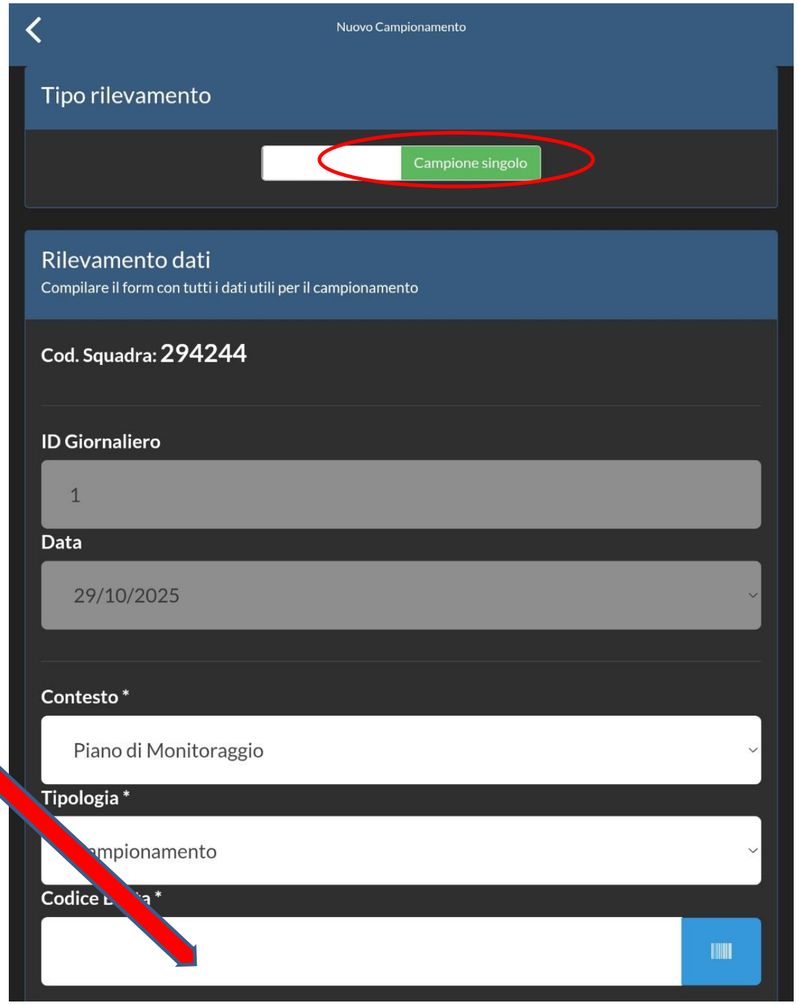
SINGLE SAMPLE

- The samples must be collect in the part of canopy that show symptoms;
- The single sample will not be used to pool sample;
- Collect from well-lignified branches:
  - least 8 twigs of 15-20 cm with leaves;
  - In alternative, 10-12 mature leaves with petioles;
  - Samples must not be taken from suckers and shoots of olive trees.

SURVEILLANCE ACTIVITIES

Olive

SINGLE SAMPLE



Each sample:
Unique ID of the bag

SURVEILLANCE ACTIVITIES

Olive

POOLED SAMPLE

The pooled sample is made by 7 single samples (1 pooled sample each hectar).

Each single sample must collected with the following way:

- Collect from well-lignified branches:
  - least 4 twigs of 15-20 cm with leaves;
  - In alternative, 10-12 mature leaves with petioles;
- Collect the samples in correspondence of the 4 cardinal points (N, E, S, O) and higher part of canopy
- Samples must not be taken from suckers and shoots of olive trees.

SURVEILLANCE ACTIVITIES

Almond and other stone fruit

SINGLE SAMPLE

- The samples must be collect in the part of canopy that show symptoms;
- The single sample will not be used to pool sample;
- Collect from well-lignified branches:
  - least 4 twigs of 15-20 cm;

POOLED SAMPLE

The pooled sample is made by 4 single samples (1 pooled sample each hectar)

SURVEILLANCE ACTIVITIES

Grapes

SINGLE SAMPLE

- The samples must be collect in the part of plant that show symptoms;
- The single sample will not be used to pool sample;
- Collect from well-lignified branches:
  - least 4 twigs of 15-20 cm;

POOLED SAMPLE

The pooled sample is made by 4 single samples (2 pooled sample each hectar)

Once the team complete the field work - all information are sent to the database (accessible by all laboratories and inspectors)

Campione - Data rilevamento: 03/03/2025 - Codice Squadra: 003634 - Id giornaliero: 3

Id	Data rilevamento	Codice Squadra	Id squadra	Id giornaliero	Componenti squadra
1811301	03/03/2025	003634	1	3	Piero Conte

Tipologia	Codice busta	Sub	Codice pool	Zona	Codice ettaro
Campione	273600		438114-24-9-1	ZONA INDENNE	438114-24-9

Specie	Cultivar	Eta	Sintomo	Tipo sintomo	Monumentale
Olivo (Olea europaea)	Altro	Da 50 a 100 anni	Assente		

Note	Specie principale	Specie consociata	Sesto d'impianto	Dimensioni	Fase fenologica
	SI	Frutteto	Non regolare		Stasi vegetativa

Stato colturale	Ultime operazioni colturali	Presenza vettore	Stadio vettore	Altre patologie	Maglia
Coltivato, Discreto					438114

Id

UNIQUE ID n° assigned to the sample

1811301

Quadrante	Data/ora esito laboratorio pool
SO	

Analisi secondo livello pool	Analisi secondo livello

Data/ora esito laboratorio	013)	Esito qPCR (Ouyang et al., 2013)

Sottospecie	Operatore ente accreditato	Note secondo livello	Data/ora esito di secondo livello	Codifica saggio	Data saggio

DDS	Da abbattere	Abbattimento
	No	

Foto

LABORATORY ACTIVITIES

Tissue preparation: example for olive

Tissue preparation: example of almond

UNIQUE ID n° assigned to the sample

Tissue preparation: example of grapes

LABORATORY ACTIVITIES

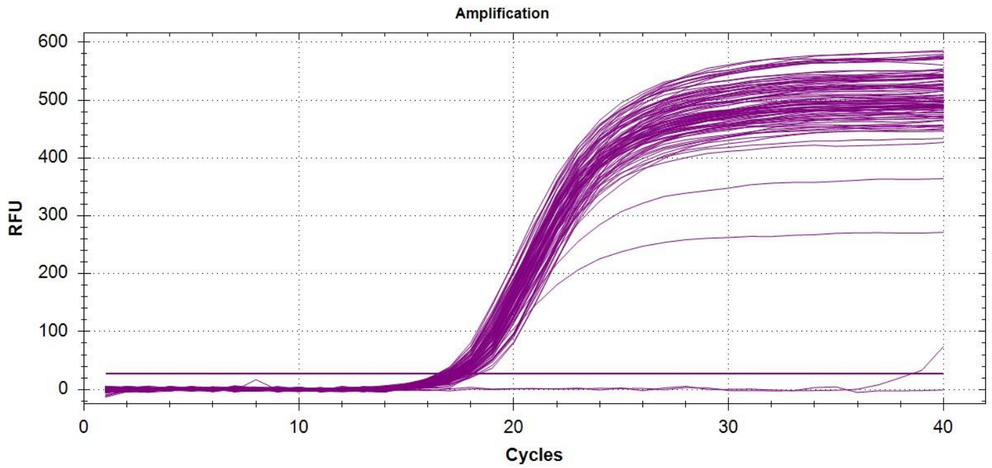
Several protocol to extract DNA

CTAB PROTOCOL

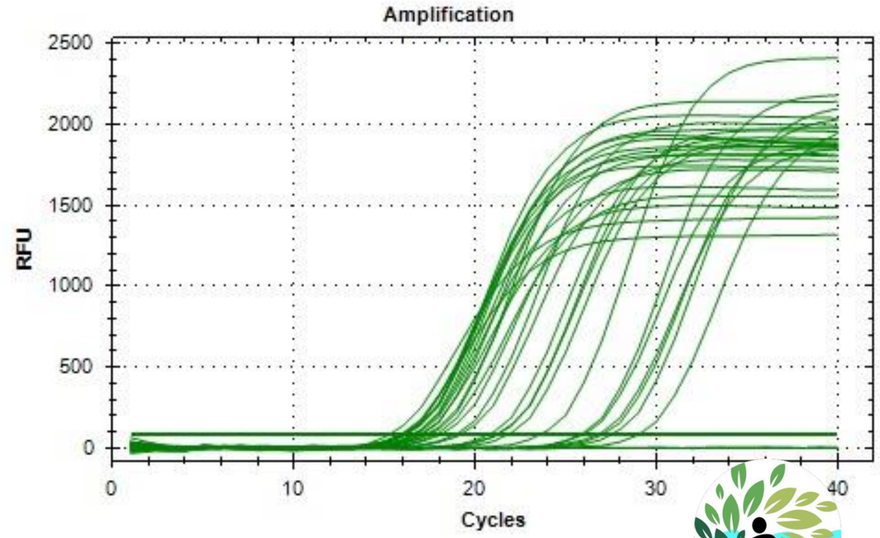
DNA extracts

qPCR

EPPO 7/24 (5)



Simplex or duplex with the internal control «COX»



(Harper *et al.*, 2010)

LABORATORY ACTIVITIES

Campione - Data rilevamento: 03/03/2025 - Codice Squadra: 003634 - Id giornaliero: 3

Id	**Data rilevamento**	**Codice Squadra**	**Id squadra**	**Id giornaliero**	**Componenti squadra**
1811301	03/03/2025	003634	1	3	Piero Conte
Tipologia	**Codice busta**	**Sub**	**Codice pool**	**Zona**	**Codice ettaro**
Campione	273600		438114-24-9-1	ZONA INDENNE	438114-24-9
Specie	**Cultivar**	**Eta**	**Sintomo**	**Tipo sintomo**	**Monumentale**
Olivo (Olea europaea)	Altro	Da 50 a 100 anni	Assente		
Note	**Specie principale**	**Specie consociata**	**Sesto d'impianto**	**Dimensioni**	**Fase fenologica**
	SI	Frutteto	Non regolare		Stasi vegetativa
Stato colturale	**Ultime operazioni colturali**	**Presenza vettore**	**Stadio vettore**	**Altre patologie**	**Maglia**
Coltivato, Discreto	Altro	NO			438114
Quadrante	**Latitudine**	**Longitudine**	**Comune**	**Esito pool**	**Data/ora esito laboratorio pool**
SO	41.0796726228	16.8449431658	Bari		
Analisi secondo livello pool	**Laboratorio**	**Esito laboratorio**	**Operatore laboratorio**	**Note laboratorio**	**Analisi secondo livello**
	CNR				
Data/ora esito laboratorio	**Ente accreditato**	**Esito qPCR(Francis et al., 2006)**	**Esito qPCR(Harper et al., 2010)**	**Esito qPCR (Li et al., 2013)**	**Esito qPCR (Ouyang et al., 2013)**
Sottospecie	**Operatore ente accreditato**	**Note secondo livello**	**Data/ora esito di secondo livello**	**Codifica saggio**	**Data saggio**
DDS	**Da abbattere**	**Abbattimento**			
	No				

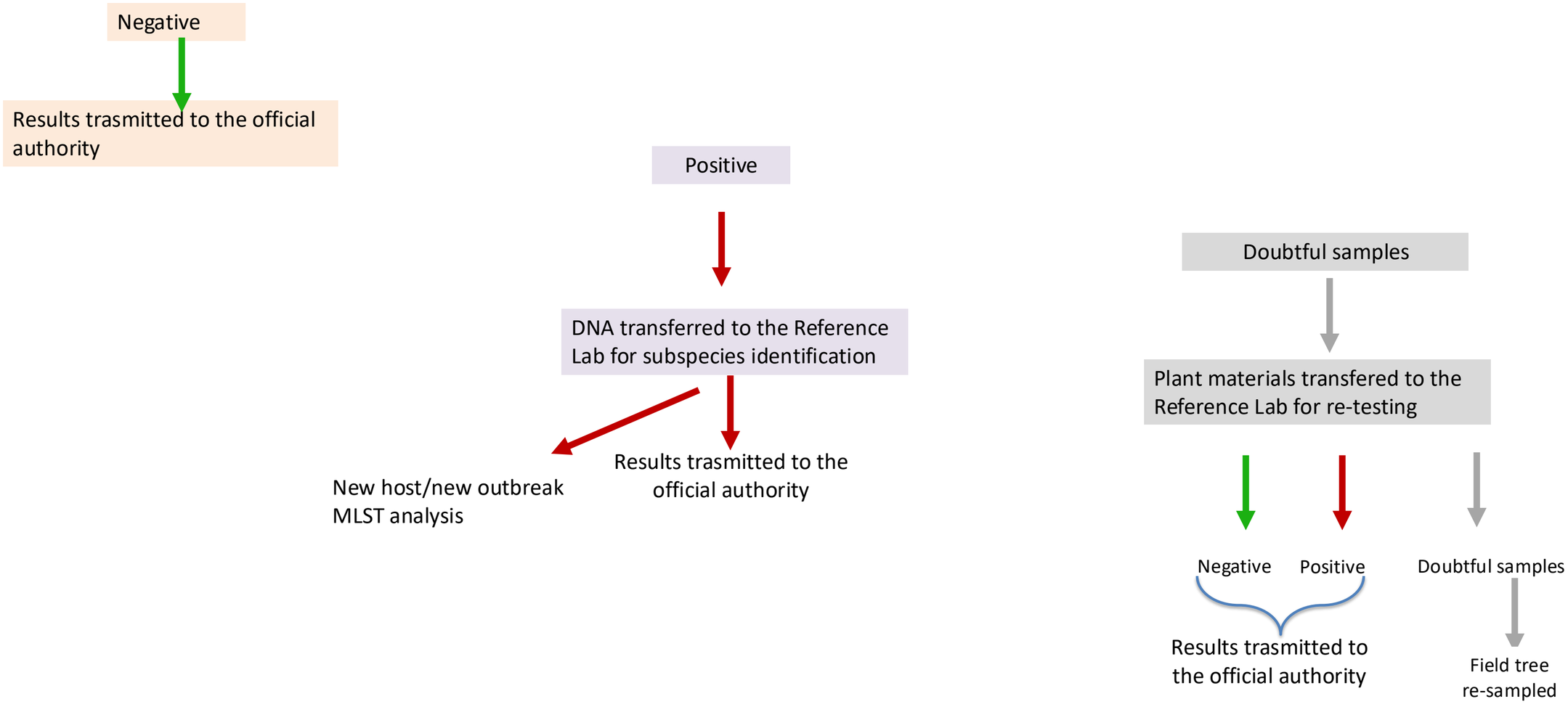
Foto

ALL POSITIVE SAMPLES ARE SUBJECTED TO qPCR for SUBSPECIES IDENTIFICATION WITH METHOD DUPAS ET AL., 2016

LABORATORY ACTIVITIES

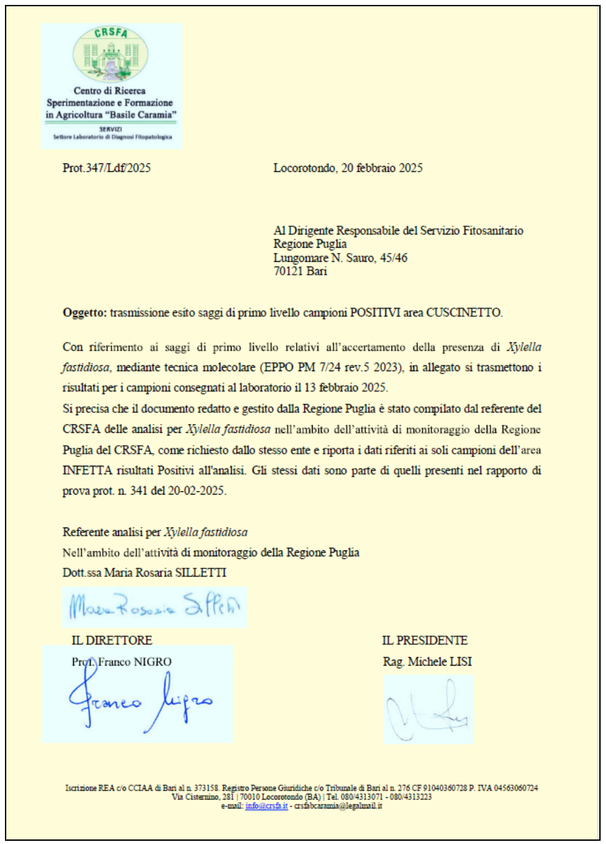
WORKFLOW

Laboratory test results



LABORATORY ACTIVITIES

Official LAB detected a positive (samples are tested within 5-6 days)



Centro di Ricerca Sperimentazione e Formazione in Agricoltura "Basile Caramia"
SERVIZI
Settore Laboratorio di Diagnosi Fitopatologica

Prot.347/Ldf/2025 Locorotondo, 20 febbraio 2025

Al Dirigente Responsabile del Servizio Fitosanitario
Regione Puglia
Lungomare N. Sauro, 45/46
70121 Bari

Oggetto: trasmissione esito saggi di primo livello campioni POSITIVI area CUSCINETTO.

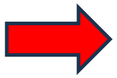
Con riferimento ai saggi di primo livello relativi all'accertamento della presenza di *Xylella fastidiosa*, mediante tecnica molecolare (EPPO PM 7/24 rev.5 2023), in allegato si trasmettono i risultati per i campioni consegnati al laboratorio il 13 febbraio 2025.
Si precisa che il documento redatto e gestito dalla Regione Puglia è stato compilato dal referente del CRSFA delle analisi per *Xylella fastidiosa* nell'ambito dell'attività di monitoraggio della Regione Puglia del CRSFA, come richiesto dallo stesso ente e riporta i dati riferiti ai soli campioni dell'area INFETTA risultati Positivi all'analisi. Gli stessi dati sono parte di quelli presenti nel rapporto di prova prot. n. 341 del 20-02-2025.

Referente analisi per *Xylella fastidiosa*
Nell'ambito dell'attività di monitoraggio della Regione Puglia
Dott.ssa Maria Rosaria SILLETTI

IL DIRETTORE
Prof.Franco NIGRO

IL PRESIDENTE
Rag. Michele LISI

Iscrizione REA c/o CCIAA di Bari n. 373158. Registro Persone Giuridiche c/o Tribunale di Bari al n. 276 CF 91040360728 P. IVA 04563060724
Via Cisternino, 281 | 70010 Locorotondo (BA) | Tel. 080/4313071 - 080/4313223
e-mail: info@crsfa.it - crsfabcaramia@legalmail.it



LABORATORIO CRSFA							
RAPPORTO DI PROVA N° PROT. 347 DEL 20/02/2025							
ID CAMPIONE	DATA RILIEVO	SPECIE	COMUNE	Latitudine	Longitudine	ESITO ANALISI - PCR in tempo reale sulla base di Harper et al., 2010	DATA ANALISI
1804378	13/02/2025	Mandorlo (Prunus dulcis)	Triggiano	41,05178057	16,9514474	POSITIVO	19/02/2025

DOCUMENTO REDATTO E GESTITO DALLA REGIONE PUGLIA
COMPILATO DAL PERSONALE DEL LABORATORIO DI DIAGNOSI FITOPATOLOGICA DEL CRSFA E, COME RICHIESTO DALLO STESSO ENTE, RIPORTANTE I DATI RIFERITI AI SOLI CAMPIONI DELL' AREA INFETTA POSITIVI ALL'ANALISI (GLI STESSI DATI SONO PARTE DI QUELLI PRESENTI NEL RAPPORTO DI PROVA PROT. N. 341 del 20-02-2025)

LABORATORY ACTIVITIES

2 days later - The Reference LAB identified the subspecies

Consiglio Nazionale delle Ricerche | Istituto per la Protezione Sostenibile delle Piante
Laboratorio di diagnosi fitosanitaria

Rapporto di Prova 22F/2025

Bari 21/02/2025

Al Dirigente Responsabile
del Servizio Fitosanitario
Regione Puglia
Lungomare N. Sauro, 45/46
70121 BARI

Oggetto: Trasmissione esito saggio molecolare per l'identificazione della sottospecie di *Xylella fastidiosa* su campioni provenienti dalla zona cuscinetto *Xylella fastidiosa* subspecie *fastidiosa* e zona infetta *Xylella fastidiosa* subspecie

Con riferimento ai campioni di vite europea e *Prunus dulcis* , consegnati in data 20/02/2025 dal tecnico Arif Sig. Michele Prisciandaro da parte del CRSFA, con la presente si comunicano gli esiti delle analisi per l'identificazione della sottospecie utilizzando il protocollo qPCR descritto da Dupas et al. (2019) come previsto nel Regolamento di Esecuzione (UE) 2024/2507 che modifica l'Allegato IV del Regolamento UE 1201/2020.

Restando a disposizione per ulteriori dettagli, si porgono cordiali saluti.

Laboratorio multisito CNR-IPSP
SEDE ISTITUZIONALE Strada delle Cacce 73, 10135 TORINO
SEDE DI BARI - Via Amendola 165/A, 70126 BARI
CF 80054330586 - P.IVA 02118311006

Consiglio Nazionale delle Ricerche | Istituto per la Protezione Sostenibile delle Piante
Laboratorio di diagnosi fitosanitaria

Dettagli ed esiti relativi all'identificazione della sottospecie sui campioni consegnati in data 20/02/25:

Codice accettazione EXP 22/2025

Codice univoco campione	Codice committente	Latitudine	Longitudine	data campionamento	Codice Pool di appartenenza	Specie	DNA/ Tessuto vegetale	Campione pool/singolo	ANALISI MOLECOLARE PER IDENTIFICAZIONE SOTTOSPECIE *Xylella fastidiosa*			
									Esito saggio Dupas et al., 2019			Data prova
									fastidiosa	*multiplex*	*pauca*	
1_EXP22/25_206/54-1	1804248	41,05144201	16,95191138	13/02/2025	438123-28-26-1	Vite europea	DNA	Singolo	Rilevata	Non rilevata	Non rilevata	21/02/2025
2_EXP22/25_206/54-2	1804256	41,0514707	16,95184654	13/02/2025	438123-28-26-1	Vite europea	DNA	Singolo	Rilevata	Non rilevata	Non rilevata	21/02/2025
3_EXP22/25_206/54-3	1804274	41,05147905	16,95161897	13/02/2025	438123-28-26-1	Vite europea	DNA	Singolo	Rilevata	Non rilevata	Non rilevata	21/02/2025
4_EXP22/25_206/55-4	1804378	41,05178057	16,9514474	13/02/2025	438123-27-26-2	*Prunus dulcis*	DNA	Singolo	Rilevata	Non rilevata	Non rilevata	21/02/2025

REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

Example of positive sample in free area to *Xylella fastidiosa* (new outbreak)

Surveillance Xf-free for the olive isolates:
A pool of 7 almond trees tested positive in the Xf-free area

Reference Laboratory confirmed the detection of the bacterium in 2 trees of the pool

REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

Example of positive sample in free area to *Xylella fastidiosa* (new outbreak)

Reference lab reported the results of the subspecies identification: ***multiplex***

Consiglio Nazionale delle Ricerche | Istituto per la Protezione Sostenibile delle Piante Sede Secondaria Bari — IPSP

Il Responsabile

Bari, 02/042/24

Al Dirigente Sezione
Osservatorio Fitosanitario
Regione Puglia
Lung. Nazario Sauro 41
70121 BARI

Oggetto: Identificazione della sottospecie di *Xylella fastidiosa* e del ceppo su campione infetto di *Prunus dulcis* rinveniente da focolaio in zona indenne.

Con riferimento ai due campioni di *Prunus dulcis* (ID-cod.busta 2471663 e 2471668) provenienti dall'in agro di Santeramo (BA) e risultati positivi al saggio qPCR per *Xylella fastidiosa* (Rapporto di Prova CNR-IPSP. 36/24 trasmesso con nota Prot. 105541 del 27/03/24), con la presente si comunica che su un'aliquota del DNA estratto dal campione ID-cod. busta 2471668 è stata effettuata l'analisi molecolare mediante il saggio di 'Tipizzazione MLST (Multi Locus Sequence Typing)', sulla base di Yuan et al. (2010), per la identificazione del ceppo e della sottospecie. Il profilo allelico determinato per i 7 geni amplificati e sequenziati dal suddetto campione è risultato essere il seguente:

Gene	*leuA*	*malF*	*holC*	*cysG*	*gltT*	*nuoL*	*petC*	ST*
Allele	5	3	6	3	5	3	3	26

*sequence type=genotipo

Sulla base delle analisi comparative con i profili allelici sinora noti e disponibili in banca dati (pubmlst.org/organisms/xylella-fastidiosa/), il profilo allelico determinato per il campione ID-cod. busta 2471668 corrisponde al genotipo ST26 appartenente alla sottospecie *multiplex*.

NOTIFICATION OF THE PRESENCE OF A PEST TO THE COMMISSION AND TO OTHER MEMBER STATES — Draft No. 19607

Member State:	2.1 - Notification from:	2.2 - Official contact:
IT - ITALY	SERVIZIO FITOSANITARIO CENTRALE	CARLO FRANCESCO CESARONI cf.cesaroni@politicheagricole.it +39.0646656193

Initial Notification date:	National reference number:
2024-03-29 00:00:00	IT/16/2024/2030 Update No. & Date: 01/2024-03-29

1 - General Information

1.1 - Details of the identity of the pest

1.1.1 - Title: **Update no 1. Presence (confirmed) of Xylella fastidiosa subsp. multiplex in ITALY (Santeramo in Colle)**

1.1.2 - Scientific name of the pest:	1.1.3 - EPPO preferred name:
Xylella fastidiosa subsp. multiplex	Xylella fastidiosa subsp. multiplex
1.1.4 - EU category of pest:	1.1.5 - EPPO category of pest:
Annex II B	A2 list

Further information:

1.2 - Executive summary

1.2.1 - Short summary of the information submitted in points 3-7:

1.2.2 - Summary of information provided in this update: **Confirmation of the presence of a pest.**

1.2.3 - Reason for exceeding the 8 working day deadline from after official confirmation of the presence of a pest to notification (Article 32.1 of Regulation (EU) 2019/1715):

1.3 - Type of presence reported: **Presence (confirmed)**

3 - Location of presence of pest

3.1 - Administrative region of the location of presence of pest

1 -	NUTS year	2021
	NUTS I	Sud (ITF)
	NUTS II	Puglia (ITF4)
	NUTS III	Bari (ITF47)
	Local Administrative Unit	Santeramo in Colle
	Other	

3.2 - Further information about location: "Please refer to boxes 6.1.4 and 7.3.2 where available"

4 - Reason for notification, pest status of the area, and the Member State concerned

4.1 - Reason for the notification

◉ Confirmed or suspected appearance of the pest in part of the territory of the Member State concerned, in which its presence was previously unknown.

4.2 - PEST STATUS of the AREA where the pest has been found to be present, AFTER the OFFICIAL CONFIRMATION.

4.3 - PEST STATUS in the MEMBER STATE concerned BEFORE the OFFICIAL CONFIRMATION of the presence, or suspected presence, of the pest.

4.4 - PEST STATUS in the MEMBER STATE concerned AFTER the OFFICIAL CONFIRMATION of the presence of the pest.

REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

Example of positive sample in free area to *Xylella fastidiosa* (new outbreak)

REGIONE PUGLIA

Dipartimento Agricoltura, Sviluppo Rurale ed Ambientale

Sezione Osservatorio Fitosanitario

ATTO DIRIGENZIALE

Codifica adempimenti L.R. 15/08 (trasparenza)	
Ufficio istruttore	Sezione Osservatorio Fitosanitario
Tipo materia	ALTRO
Materia	ALTRO
Sotto Materia	ALTRO
Riservato	NO
Pubblicazione integrale	SI
Obblighi D.Lgs 33/2013	NO
Tipologia	Nessuno
Adempimenti di inventariazione	NO

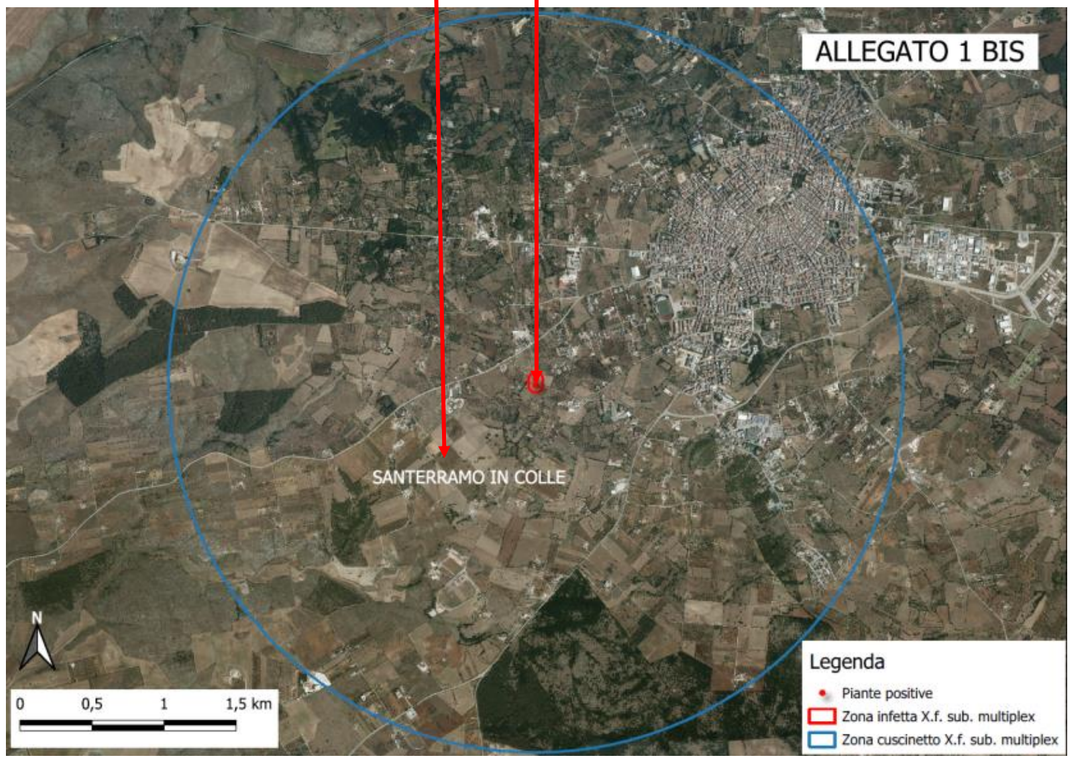
N. 00029 del 08/04/2024 del Registro delle Determinazioni della AOO 181

Codice CIFRA (Identificativo Proposta): 181/DIR/2024/00033

OGGETTO: Individuazione di focolaio di Xylella fastidiosa sottospecie multiplex ST26 in agro di Santeramo in Colle (BA) – Istituzione dell'area delimitata ai sensi dell'art. 4 del Reg. UE 2020/1201.

Regional decree of demarcation area to *Xylella fastidiosa:*

- *Infected zone (50-meter area around the infected plant)*
- *Buffer zone (2,5 Km area around the infected plant)*



REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

Example of positive sample in free area to *Xylella fastidiosa* (new outbreak)

REGIONE PUGLIA

Dipartimento Agricoltura, Sviluppo Rurale ed Ambientale

Sezione Osservatorio Fitosanitario

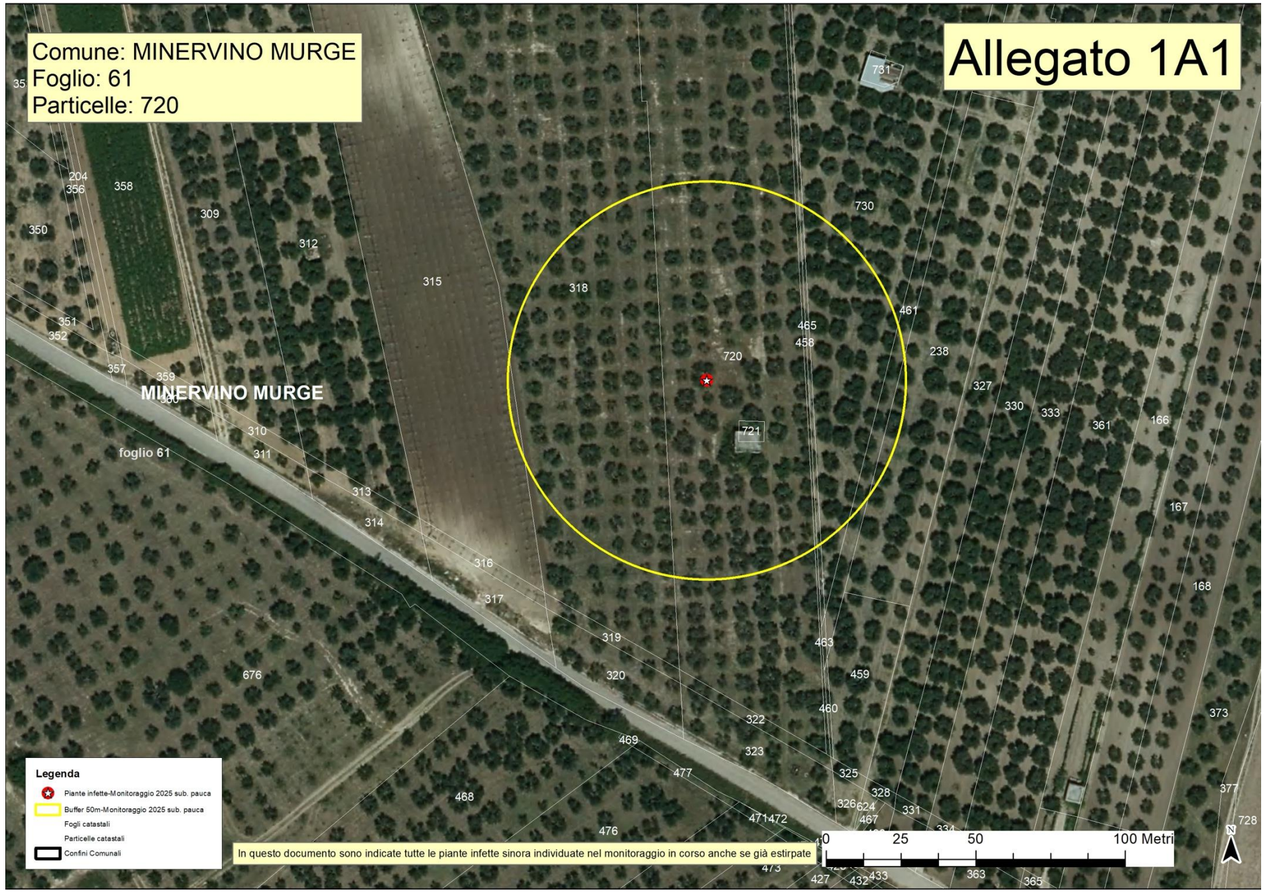
ATTO DIRIGENZIALE

Codifica adempimenti L.R. 15/08 (trasparenza)	
Ufficio istruttore	Sezione Osservatorio Fitosanitario
Tipo materia	ALTRO
Materia	ALTRO
Sotto Materia	ALTRO
Riservato	NO
Pubblicazione integrale	SI
Obblighi D.Lgs 33/2013	NO
Tipologia	Nessuno
Adempimenti di inventariazione	NO

N. 00043 del 22/04/2024 del Registro delle Determinazioni della AOO 181

Codice CIFRA (Identificativo Proposta): 181/DIR/2024/00040

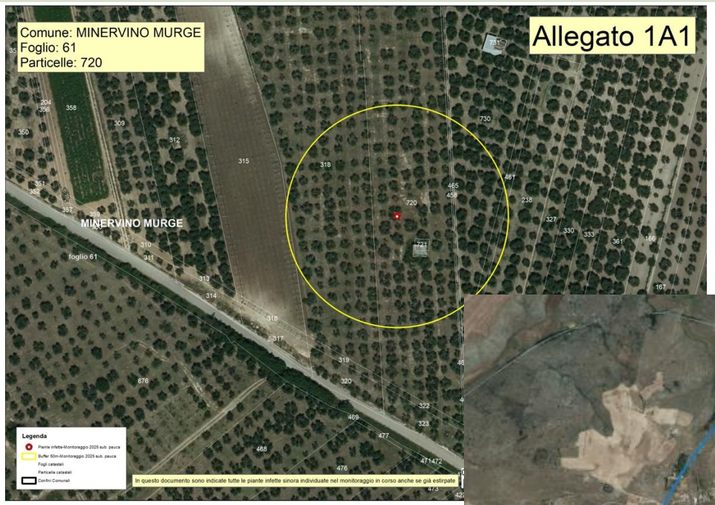
Eradication measures



REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

REGIONE PUGLIA

Example of positive sample in free area to *Xylella fastidiosa* (new outbreak)

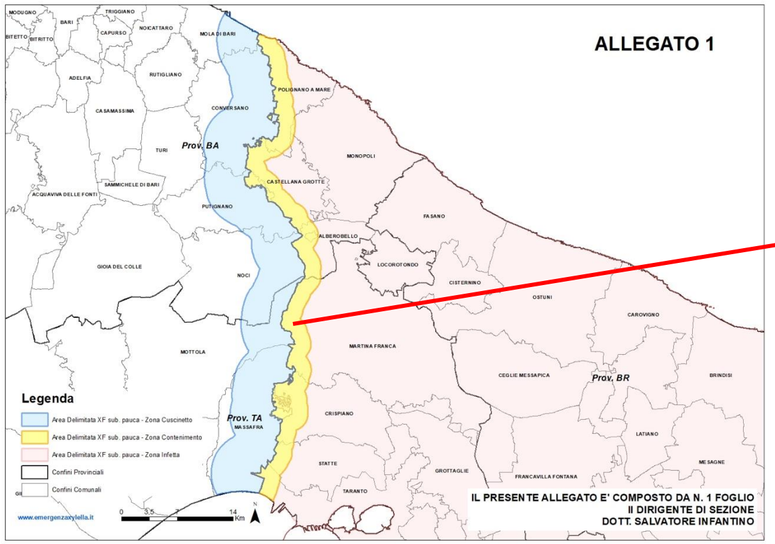


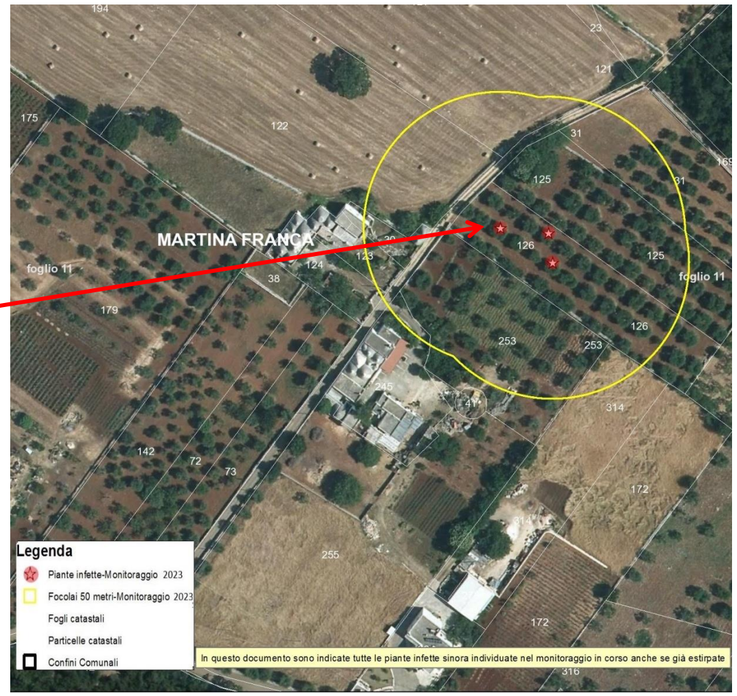
Eradication measures

Uprooted all the plants of specified species in infected zone (except the monumental plants negative at *Xylella fastidiosa*). In case of positive plants in the buffer zone of demarcated area to Xylella fastidiosa are applied the same measures.

REGIONE PUGLIA

Example of positive sample in demarcated area to *Xylella fastidiosa* (zone in which are applied the contaiment measures)





Only positive plants at *Xylella fastidiosa* are uprooted

REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

REGIONE PUGLIA

ARIF Agenzia Regionale attività Irrigue Forestali

ATTIVITÀ DI MONITORAGGIO DIFFUSIONE XYLELLA FASTIDIOSA

VERBALE DI ABBATTIMENTO DA PARTE DEI TECNICI

Data: **15-05-2024**
Codice focolaio: **11200338**
Specie: **Mandorlo (Prunus dulcis)**
Latitudine: **40.78418048**
Ditta esecutrice: [redacted]
Note: **dds n. 043 del 22/04/2024. mandorlo**

Id squadra: **92**
Volontario: **NO**
Proprietario: [redacted]
Longitudine: **16.73659857**
Comune: **Santeramo in Colle**

Prima

Dopo

After the uprooting of the plant an official document is prepared that include the following info:

- date of uprooting
- ID inspectors
- name and surname of Ower
- number of eradication regional act
- GPS coordinate
- Municipality

In addition two pictures are present in the document that shows, respectively, the image of site first and after the uprooting procedure

REGIONAL PHYTOSANITARY SERVICE ACTIVITIES

An aid scheme to farmer who apply the eradication or containment measures were approved with Regional Council Act No. 994 of July 15, 2024.

VITE

Coltura	Indennizzo per danno (€/ha)	Indennizzo per estirpazione di piante (€/ha)
Vite tavola	**58.174**	**3.000**
Vite vino comune	**30.638**	**3.000**
Vite vino qualità	**34.319**	**3.000**

OLIVO

Coltura	Indennizzo per danno (€/pianta)	Indennizzo per estirpazione di piante (€/ha)
Olivo maggiore di 200 piante/ha	**98,00**	**130,5**
Olivo tra 101 e 200 piante/ha	**121,00**	**145**
Olivo minore o uguale 100 piante/ha	**146,00**	**166,75**

CILIEGIO

Coltura	Indennizzo per danno (€/ha)	Indennizzo per estirpazione di piante (€/ha)
Ciliegio	**22.438,00**	**79,75**

MANDORLO

Coltura	Indennizzo per danno (€/ha)	Indennizzo per estirpazione di piante (€/ha)
Mandorlo	**10.323,00**	**69,6**

Monitoring to insect vectors of *Xyella fastidiosa*

Morphological Identification

Philaenus spumarius

- Males: 5.3-6.0 mm
- Females: 5.4-6.9 mm
- Rounded body shape
- Highly variable color morphs

Philaenus italosignus

- Males: 6.4-7.2mm
- Females: 7.0-8.1mm
- Similar *to P. spumarius*
- Genitalia examination required

Neophilaenus campestris

- Males: 5.0-5.3mm
- Females: 5.4-5.7mm
- Slender body shape
- Two light spots on forewing

Important Note: *Xylella fastidiosa* is not pathogenic to the vectors, so no symptoms can be observed on vectors carrying this bacterium.

Life Cycle and Development Stages

February: Egg Hatching

Eggs deposited on dry plant residues on the ground hatch, typically in February

1

Juvenile Phase: 5 Developmental Stages

Nymphs progress through 5 instars (1-5 mm), with color changes from yellowish to greenish and development of wing buds

2

Late Spring: Adult Emergence

Adults emerge around late April to early May in southern Italy, timing varies by region

3

Summer: Host Shifting

Adults move from herbaceous vegetation to woody plants, including olive trees

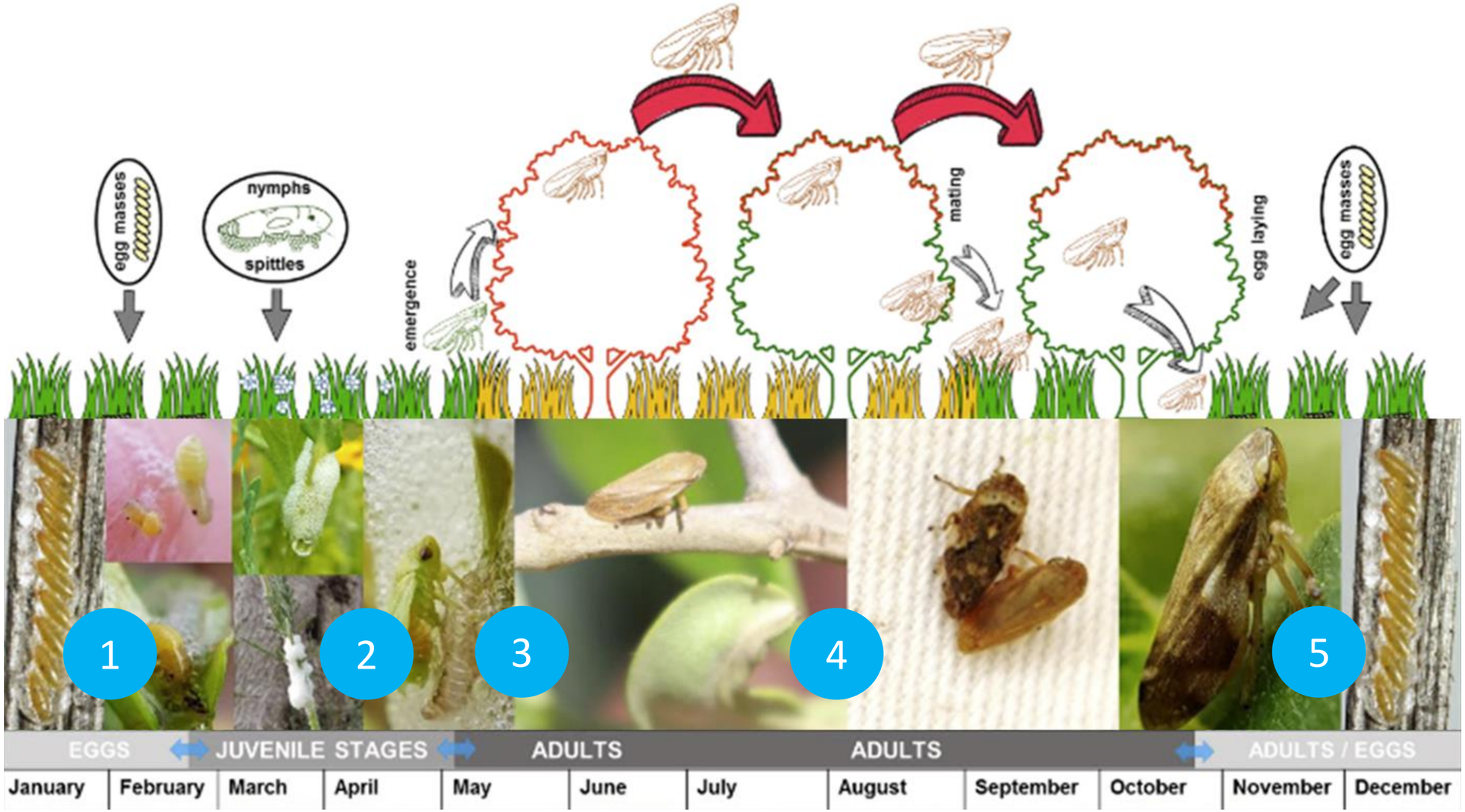
4

Autumn: Oviposition

Adult females return to herbaceous vegetation for egg laying, completing the cycle (1 cycle each year)

5

Life Cycle and Development Stages



Infoxylella®

Monitoring Sites

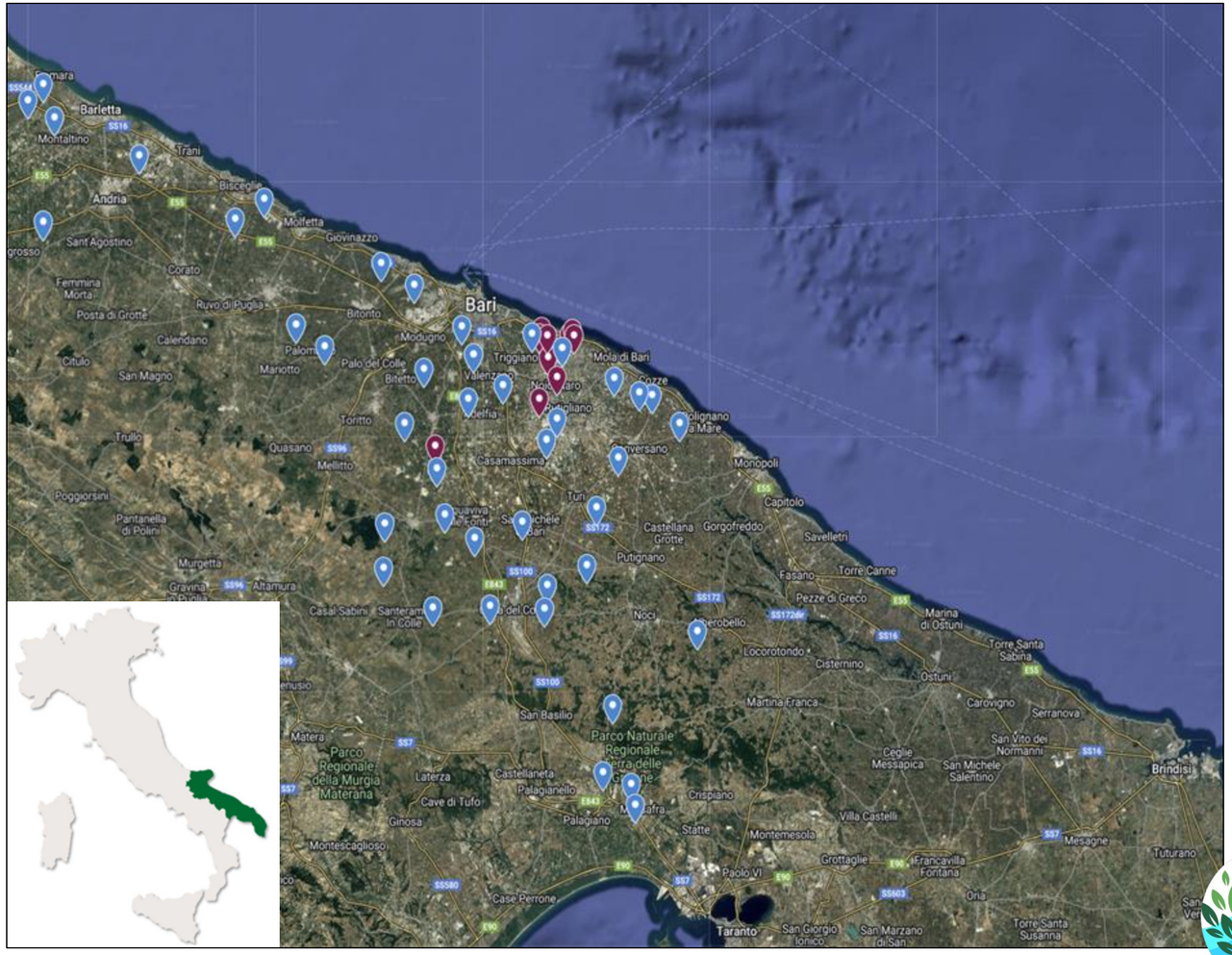
Monitoring sites are selected on the base of a combination of epidemiological and logistical risk factors:

- Spatial distribution of infected plants identified during the previous monitoring campaign;
- Proximity to major transportation infrastructures, such as railways and highways;
- Landscape characteristics and altitude.

The number and selection of monitoring sites are also based on current distribution of different *Xylella fastidiosa* genotypes in Apulia region:

- *Xylella fastidiosa subsp. pauca ST53*
- *Xylella fastidiosa subsp. fastidiosa ST1*
- *Xylella fastidiosa subsp. multiplex ST26*

Map that shows the points in which is made the insects monitoring in Apulia region



The monitoring of juvenile stages

It is carried out from March to May. Five randomly selected 0.25 m^2 transects are placed at each monitoring site. Subsequently, the grass cover is inspected to verify the presence, abundance, and developmental stage of young insects.

This activity provides key data on the seasonal dynamics of vector populations, enabling the identification of optimal timing for implementing tillage

The monitoring of adult stage

It takes place from May to October. In this activity, 18 collection points are adopted at each monitoring site using trawl nets. Subsequently, the collected insects are identified, counted, and used for laboratory analysis by RT-PCR, Dupas *et al.* 2019. This last point is important because permit of individuate eventually contaminated insect at *Xylella fastidiosa*.

This activity provides key data on the seasonal dynamics of vector populations, enabling the identification of optimal timing for chemical or biological control.

The monitoring of adult stage

Olive Groves

Sampling: 18 trees/ha, 10 sweeps per canopy with 30 cm net;

Preservation: Specimens stored in 75% ethanol

Identification: Morphological analysis (EPPO Bulletin 2020)

Analysis: Molecular testing (RT-PCR, Dupas et al. 2019)

THANK YOU!

RIGENESI