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Field survey guidance for *Xylella fastidiosa*



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The guidance provided in this document was prepared by subject matter experts from USDA APHIS and reviewed by technical officers at the IPPC Secretariat.

The IPPC implements APP in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Commission on Agriculture, through its technical unit- the African Union Inter-African Phytosanitary Council (AU-IAPSC). The IPPC Secretariat and the IPPC community are grateful to all the institutions that contributed to the production of this technical resource.

Abbreviations

APHIS	Animal and Plant Health Inspection Service
APP	Africa Phytosanitary Programme
AU-IAPSC	African Union Inter-African Phytosanitary Council
CNR	National Research Council (Consiglio Nazionale delle Ricerche)
EPPO	European Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographic Information System
IPPC	International Plant Protection Convention
NPPO	National Plant Protection Organization
USDA	United States Department of Agriculture

Introduction

Active and regular monitoring and surveillance are critical for countries to establish the presence or absence of plant pests, especially those with severe impacts on food security, the environment, trade, and agricultural productivity.

This field survey guidance provides easy-to-follow guidelines for technical personnel of national plant protection organizations (NPPOs) to survey for *Xylella fastidiosa*, a major plant disease.

This document provides a protocol to aid in the monitoring, detection, sample collection and diagnostics for *Xylella fastidiosa*, ensuring effective phytosanitary decision-making to manage the pest risk and protect cross-border trade.

This field survey guidance complements the digital tools available through the Africa Phytosanitary Programme (APP) mobile application and Geographic Information System (GIS) platforms.

APP is an initiative of the International Plant Protection Convention (IPPC), designed to transform pest management across Africa by enhancing the capabilities of phytosanitary personnel within NPPOs, to leverage advanced science and modern digital technology for effective and timely pest surveillance, detection, diagnostics, control, and prevention. APP aims to strengthen the resilience of Africa's phytosanitary systems against plant pests of regulatory, economic and environmental significance. Some of the countries involved in APP listed *Xylella fastidiosa*, as a priority pest in their countries, requiring effective surveillance.

The IPPC implements APP in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Department of Agriculture, Rural Development, Blue Economy and Sustainable Development, through the African Union Inter-African Phytosanitary Council (AU-IAPSC).

Figure 1. Symptoms of *Xylella fastidiosa* on grape leaf



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Field survey guidance for *Xylella fastidiosa*

Scientific name

Xylella fastidiosa (Wells et al.)

Common names

Pierce's disease of grapevine, citrus variegated chlorosis, coffee leaf scorch, olive leaf scorch and quick decline, almond leaf scorch, bacterial leaf scorch of shade trees, bacterial leaf scorch of blueberry, phony peach disease, plum leaf scald, alfalfa dwarf.

Type of pest

Bacterium

Taxonomic position

Xanthomonadales: Xanthomonadaceae

Known hosts

Xylella fastidiosa is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as olives (*Olea* spp.), coffee (*Coffea* spp.), grapes (*Vitis* spp.), stone fruits such as almond, peach or plum (*Prunus* spp.), citrus (*Citrus* spp.), elm (*Ulmus* spp.), oak (*Quercus* spp.), blueberry (*Vaccinium* spp.), alfalfa (*Medicago sativa*), various flowers and other fruit tree hosts. The pathogen has a wide, expanding host range and comprehensive lists of susceptible hosts are available (see "Identification and diagnostic resources" for comprehensive host lists).

Survey protocol

Survey-site selection

Surveys should be carried out wherever hosts of concern are present.

Time of year to survey

Samples should be collected during a plant's period of active growth.

Visual survey

Symptoms

Although host plants infected with *X. fastidiosa* may not show symptoms, some display leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing. The bronzing may intensify before browning and drying. Symptoms are usually more pronounced in stressed plants (e.g. by temperature or drought) and can vary between plant species and environmental conditions.

Examples of characteristic symptoms on key hosts include:

PIERCE'S DISEASE OF GRAPEVINE (*Vitis*)

Chlorotic spots form on leaves, especially along the margins, with a sudden drying of leaf edges often surrounded by a yellow or red halo (see Figure 1, Figure 2(a)). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but it remains firm and may shrivel and drop while the petiole remains attached to the branch (as the so-called "match sticks", Figure 2(b)). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants.

Figure 2. Symptoms of *X. fastidiosa* on grapevine: (a) leaf showing marginal necrosis surrounded by chlorotic halo; (b) persistent petioles ("match sticks")



2(a) © M. Scortichini / Istituto Sperimentale per la Frutticoltura

2(b) © J. Clark & A.H. Purcell / University of California, Berkeley

Figure 3. Symptoms of citrus variegated chlorosis caused by *X. fastidiosa*: (a) typical spots on sweet orange leaves; (b) twig dieback and reduction of production; (c) fruits ripen early and have reduced dimensions



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CITRUS VARIEGATED CHLOROSIS (*CITRUS*)

Mottled variegations appear on leaves, with small chlorotic spots on the upper surface that correspond to small gummy brown spots on the underside of the leaf (see Figure 3(a)). Symptoms are most obvious on three-to-six-year-old trees, especially on cultivars of *C. sinensis*. The pattern of chlorosis between leaf veins is asymmetrical on opposite sides of the leaf. Symptoms may only appear on portions of newly infected trees, whereas the entire canopy may be symptomatic in older infections. Stunting occurs along with a thin canopy as a result of the defoliation and dieback of twigs and branches (see Figure 3(b)). Flowering is abnormal. Fruits ripen early without filling and are much smaller and very firm (see Figure 3(c)).

COFFEE LEAF SCORCH (*COFFEA*)

Symptoms appear on young flushes of field plants as large, scorched zones on the edges and ends of recently matured leaves (see Figure 4). Other symptoms include premature leaf drop, shoot stunting, small and chlorotic apical leaves, followed by shoot dieback and overall plant stunting. Fruit size and yield are reduced. Side branches have no leaves or fruit except for a tuft of leaves at the branch tip.

Figure 4. Symptoms of coffee leaf scorch caused by *X. fastidiosa*



Xylella fastidiosa (XYLEFA) - <https://gd.eppo.int>

© Maria Bergsma-Vlami / NPPO, Kingdom of the Netherlands

OLIVE LEAF SCORCH AND QUICK DECLINE (*Olea*):

Symptoms include leaf scorching (see Figure 5(a)) and randomly distributed desiccation of twigs and small branches (see Figure 5(b)), mainly in the upper part of the canopy in the early stages of infection. Leaf tips and margins turn dark yellow to brown, eventually leading to desiccation. Symptoms become increasingly severe over time and give a blighted appearance to the entire crown. Desiccated leaves and mummified fruits remain attached to shoots (see Figure 5(c)). Wood in cross-section shows irregular discolouration.

Figure 5. Symptoms of olive leaf scorch and quick decline caused by *X. fastidiosa*: (a) leaf scorching; (b) desiccation of branches; (c) mummified fruits



(a) © Donato Boscia /CNR – Institute for Sustainable Plant Protection, Italy; Franco Nigro / Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di, Italy; Antonio Guarino / Plant Protection Service, Regione Puglia, Italy; (b) © Camille Picard / DGAL-SDQPV, France; (c) FAO

ALMOND LEAF SCORCH (*Prunus dulcis*)

The most characteristic symptoms in almond trees infected with *X. fastidiosa* are leaf scorching followed by decreased productivity and general decline. Leaf scorching occurs along the edges of leaves and may be surrounded by a narrow band of yellow (see Figure 6). As the disease progresses, twigs may die back starting at the tip and nut production is severely reduced.

Figure 6. Symptoms of almond (*P. dulcis*) leaf scorch caused by *X. fastidiosa*



© Donato Boscia / CNR, Institute for Sustainable Plant Protection, UOS, Bari, Italy

BACTERIAL LEAF SCORCH OF SHADE TREES

Symptoms are similar on different shade tree hosts (e.g. *Acer*, *Platanus*, *Quercus*, *Ulmus*). Scorching at leaf margins may be surrounded by a yellow or red halo (see Figure 7). Symptoms generally progress from older to younger leaves as diseased branches die and the tree declines.

Figure 7. Symptoms of bacterial leaf scorch caused by *X. fastidiosa* on maple (*Acer* sp.)



© John Hartman, University of Kentucky, USA

BACTERIAL LEAF SCORCH OF BLUEBERRY (*VACCINIUM*)

Scorching at leaf edges may be bordered by a darker band (see Figure 8(a)). Over time, symptoms may become uniformly distributed throughout the foliage. New shoots may be thin with fewer flower buds. Leaves drop and twigs and stems develop a “skeletal” yellow appearance (see Figure 8(b)).

Figure 8. Bacterial leaf scorch of blueberry caused by *X. fastidiosa*:
(a) leaf scorch; (b) twigs and stems with “skeletal” yellow appearance



© Phillip M. Brannen / University of Georgia, USA

PHONY PEACH DISEASE AND PLUM LEAF SCALD (*PRUNUS* spp.)

Young shoots are stunted with greener, denser foliage compared to healthy trees (see Figure 9). Side branches grow horizontally or droop, so the tree appears uniform, compact and rounded. Leaves and flowers appear early and remain on the tree longer, and fruit production is reduced in quantity and size.

Figure 9. Symptoms of phony peach disease caused by *X. fastidiosa*



© M. Scortichini / Istituto Sperimentale per la Frutticoltura, Rome, Italy

ALFALFA DWARF (*MEDICAGO SATIVA*)

The main symptom on alfalfa is stunted regrowth after cutting (see Figure 10). Leaflets are smaller and often slightly darker in colour, but not distorted, cupped, mottled or yellow. Internal lignified tissue is yellowish with fine dark streaks of dead tissue.

Figure 10. Symptoms of alfalfa dwarf caused by *X. fastidiosa* on alfalfa



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OTHER HOSTS

Most symptomatic plants display typical leaf scorching (see Figure 11).

Figure 11. Leaf scorch symptoms caused by *X. fastidiosa* on examples of other hosts:
(a) milkwort (*Polygala* sp.); (b) oleander (*Nerium* sp.)

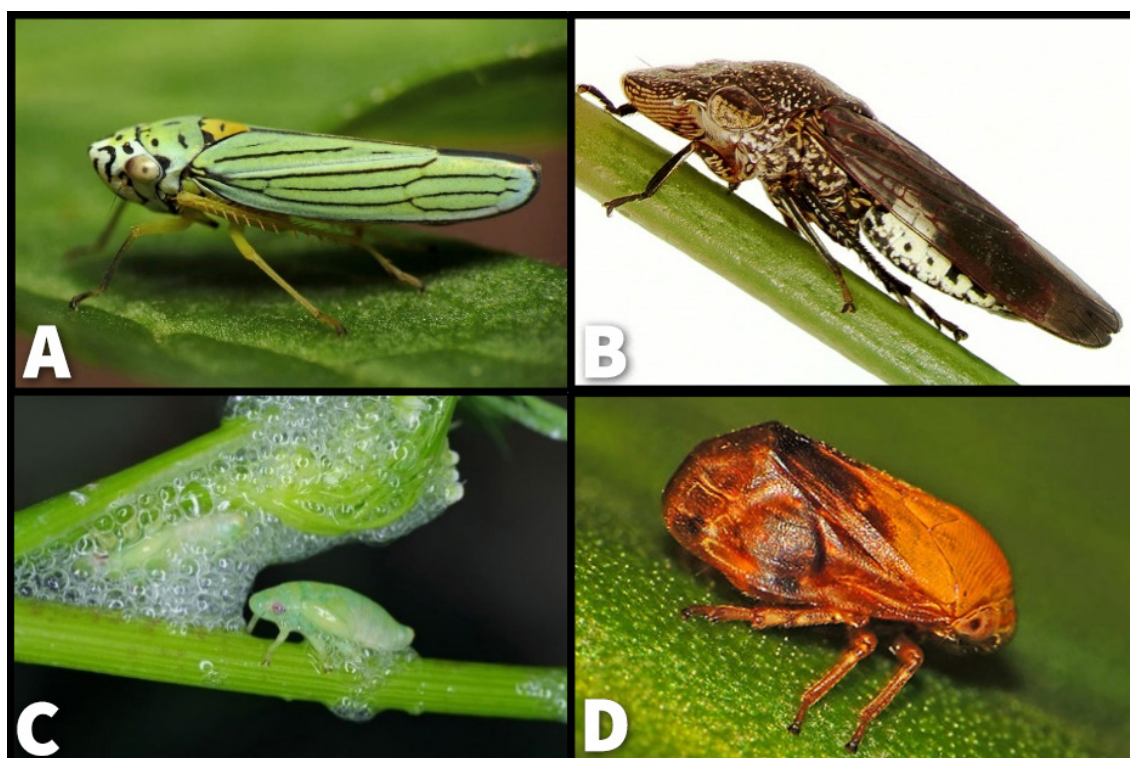


11(a) © Céline Vidal / European and Mediterranean Plant Protection Organization; 11(b) © Donato Boscia / CNR, Institute for Sustainable Plant Protection, Italy

INSECT VECTORS

Xylella fastidiosa is spread by insect vectors that feed on the xylem of plants. Many known vectors belong to the families Aphrophoridae, Cercopidae and Cicadellidae; however, any xylem-feeding insect has the potential to transmit the disease when feeding on infected plants and subsequently feeding on healthy plants (see Figure 12).

Figure 12. Examples of insects that are vectors for *X. fastidiosa*: (a) *Graphocephala atropunctata*; (b) *Homalodisca vitripennis*; (c) *Philaenus spumarius*; (d) *Clastoptera achatina*.



(a) © Katja Schulz / Flickr; (b) © Rodrigo Krugner / USDA; (c) © Tomasz Klejdysz / Shutterstock; Lisa Powers / CABI; (d) © Lisa Powers

Sample collection

Samples of branches or canes with attached leaves that include mature leaves generally provide the most reliable results. Young growing shoots should be avoided. For small plants, the entire plant can be sent to the laboratory.

The petiole and midrib of leaves are the best sources for diagnosis; however, other sources of living tissue can include small twigs and roots of stone fruits, stems and roots of blueberry and citrus fruit petioles.

The sample should consist of branches or cuttings representative of the symptoms seen on the plant or plants – preferably from a single plant, although a pooled sample may also be collected. See Table 1 for guidelines on sample type and size.

Table 1. Number of leaves (including the petioles) to be used and approximate weight of the laboratory sample

Type of sample	Host plants and type of tissue	Minimum number of leaves per lab sample	Approximate weight of lab sample
Sample from individual plants with leaves	Petioles or midribs, or both, of large-sized leaves (e.g. <i>Coffea</i> spp., <i>Ficus</i> spp., <i>Vitis</i> spp., <i>Nerium</i> spp.)	5	0.5 g–1 g
	Petioles or midribs, or both, of small-sized leaves (e.g. <i>Polygala myrtifolia</i> , <i>Olea</i> spp.)	25	0.5 g–1 g
	Plant species without petioles or with small petiole and midrib	25	0.5 g–1 g
Dormant plant or cuttings	Xylem tissue	Not applicable	0.5 g–1 g
Composite sample from several coffee plants from a single lot with leaves	Samples of asymptomatic plants (e.g. collected from imported consignments or nursery monitoring)	100–200	10 g–50 g

Source: IPPC, Standards Committee on behalf of the Commission on Phytosanitary Measures. 2018. [ISPM 27: Diagnostic protocols for regulated pests](#), DP 25: *Xylella fastidiosa*. Rome, Italy. FAO.

IMPORTANT: After collection, keep samples cool (4 °C–15 °C) to avoid deterioration. Send samples to the laboratory as soon as possible.

Pest identification and diagnostics

Laboratory diagnosis is best achieved with serological methods such as double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) or molecular methods such as conventional polymerase chain reaction (PCR), real-time PCR or loop-mediated isothermal amplification (LAMP). Isolation methods are not recommended for detection because of the difficulty of isolating *X. fastidiosa* from plant tissue.

Pest description

Xylella fastidiosa is a xylem-limited bacterium with fastidious growth requirements (i.e. it is difficult to isolate and culture outside of a host plant). It is inoculated into the water-transporting (xylem) elements of its host plants by xylem-sap-feeding insects.

Identification and diagnostic resources

ISPM 27. Annex 25. 2018. *Diagnostic protocol for Xylella fastidiosa*. IPPC Secretariat, Rome, FAO. <https://www.ippc.int/en/publications/86498/>

EPPO (European and Mediterranean Plant Protection Organization). 2019. EPPO Standard on Diagnostics. PM 7/24 (4) Diagnostics for *Xylella fastidiosa*. *EPPO Bulletin*, 49(2): 175–227. <https://doi.org/10.1111/epp.12575>

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Easily mistaken species and conditions

Symptoms can be confused with those of other diseases (e.g. several fungal diseases, depending on the host) or physiological causes (environmental stress, water deficiency, salt, air pollutants, nutritional problems, etc.).

Reference

Much of the content of this datasheet was taken directly from the IPPC Diagnostic Protocol for *X. fastidiosa*:

ISPM 27. Annex 25. 2018. *Diagnostic protocol for Xylella fastidiosa*. IPPC Secretariat, Rome, FAO. <https://www.ippc.int/en/publications/86498/>

IPPC

The International Plant Protection Convention (IPPC) is an international plant-health agreement that aims to protect global plant resources and facilitate safe trade. The IPPC vision is that all countries have the capacity to implement harmonized measures to prevent pest introductions and spread, and minimize the impacts of pests on food security, trade, economic growth, and the environment.

Organization

- » There are over 180 IPPC contracting parties.
- » Each contracting party has a national plant protection organization (NPPO) and an official IPPC contact point.
- » Ten regional plant protection organizations have been established to coordinate NPPOs in various regions of the world.
- » The IPPC Secretariat liaises with relevant international organizations to help build regional and national capacities.
- » The secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

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