EPPO Datasheet: *Xanthomonas hortorum pv. gardneri*

Last updated: 2024-05-15

This datasheet covers the four bacterial species and pathovars that are associated with the bacterial spot of tomato and pepper: ***Xanthomonas euvesicatoria* pv. *euvesicatoria, Xanthomonas euvesicatoria* pv. *perforans, Xanthomonas hortorum* pv. *gardneri, Xanthomonas vesicatoria***.

IDENTITY

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Preferred name: *Xanthomonas hortorum pv. gardneri*
Authority: (Jones et al.) Morinière et al.
Taxonomic position: Bacteria: Proteobacteria: Gammaproteobacteria: Lysobacterales: Lysobacteraceae
Other scientific names: *Xanthomonas cynarae pv. gardneri* (Timilsina et al.), *Xanthomonas gardneri* (ex ?uti?) Jones et al.
Common names: bacterial leaf spot of pepper, bacterial leaf spot of tomato, bacterial spot of pepper, bacterial spot of tomato, leaf spot of tomato, stem canker of tomato
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EPPO Categorization: A2 list
view more categorizations online...
EU Categorization: RNQP (Annex IV)
EPPO Code: XANTGA

Notes on taxonomy and nomenclature

Bacterial spot of tomato and pepper was first reported in the early 1920s and, since then, the taxonomy of bacterial spot pathogens has been extensively revised. The causal agent was initially named *Bacterium vesicatorium* (Doidge, 1920, 1921; Gardner & Kendrick, 1921, 1923), which later changed to *Xanthomonas vesicatoria* and subsequently *Xanthomonas campestris* pv. *vesicatoria* (Dowson, 1939; Young *et al*., 1978). Decades later, three phenotypically and phylogenetically distinct bacterial populations were established (Stall et al., 1994; Jones et al., 1995), which were associated with *Xanthomonas axonopodis* pv. *vesicatoria* (strains from groups designated A and C) and *Xanthomonas vesicatoria* (strains from group B) (Vauterin *et al*., 1995). Independently in 1957 a bacterial pathogen was isolated from tomato in the former Yugoslavia and named as *Pseudomonas gardneri* (Šutic, 1957). The pathogen was later proposed to be reclassified as *Xanthomonas gardneri* and considered to be group D of the bacterial spot pathogens (Jones *et al*., 2000). In 2004, DNA:DNA hybridization analysis led to a new taxonomic revision considering four distinct species: *Xanthomonas euvesicatoria* (group A), *Xanthomonas vesicatoria* (group B), *Xanthomonas perforans* (group C) and *Xanthomonas gardneri* (group D) (Jones *et al*., 2004a). Later, new molecular analysis showed that *X. euvesicatoria* and *X. perforans* were not clearly differentiated as stand-alone species and were hence reclassified as pathovars of the same species, *X. euvesicatoria* pv. *euvesicatoria* and *X. euvesicatoria* pv. *perforans*, respectively (Constantin *et al*., 2016). Finally, Morinière *et al.* (2020) reclassified *X. gardneri* as *X. hortorum* pv. *gardneri*. Currently, the bacterial spot *Xanthomonas* falls into four lineages within three validly described species: *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans*, *X. hortorum* pv. *gardneri* and *X. vesicatoria.*

HOSTS

The main hosts of bacterial spot xanthomonads are tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.). *X. euvesicatoria* pv. *euvesicatoria* and *X. hortorum* pv. *gardneri* are reported as pathogens for both tomato and pepper. Meanwhile, *X. vesicatoria* primarily infects tomato and, until recently, *X. euvesicatoria* pv. *perforans* strains had only been isolated from tomato (Timilsina *et al*., 2015). However, over in recent years *X. euvesicatoria* pv. *perforans* has been isolated from pepper fields in Florida and Alabama (USA) (Potnis *et al*., 2015; Newberry *et al*., 2019).

Three pathotypes of strains have been distinguished among the bacterial spot causative agents: those exclusively infecting tomato (T races), those exclusively affecting pepper (P races), and those infecting both tomato and pepper. Several races have been identified based on the hypersensitive reaction (HR) triggered by effector proteins in *Xanthomonas* strains delivered via the type III secretion system into host cells and recognition by specific resistance proteins in tomato or pepper (Stall *et al*., 2009). Currently 11 pepper races and five tomato races have been documented (Bouzar *et al*., 1994; Stall *et al*., 2009; Adhikari *et al*., 2020; Jibrin *et al*., 2022). Pathogenic races are determined by the presence or absence of HR in the susceptible *C. annuum* cultivar Early Calwonder (ECW), its near-isogenic lines and *Capsicum pubescens* PI235047, or in different *S. lycopersicum* genetic lines. Nevertheless, future studies may reveal unknown races and potential novel dynamics in pathogen-host interaction.

A range of solanaceous and non-solanaceous plants, mainly weeds, have also been recorded as incidental hosts (Osdaghi *et al*., 2021).

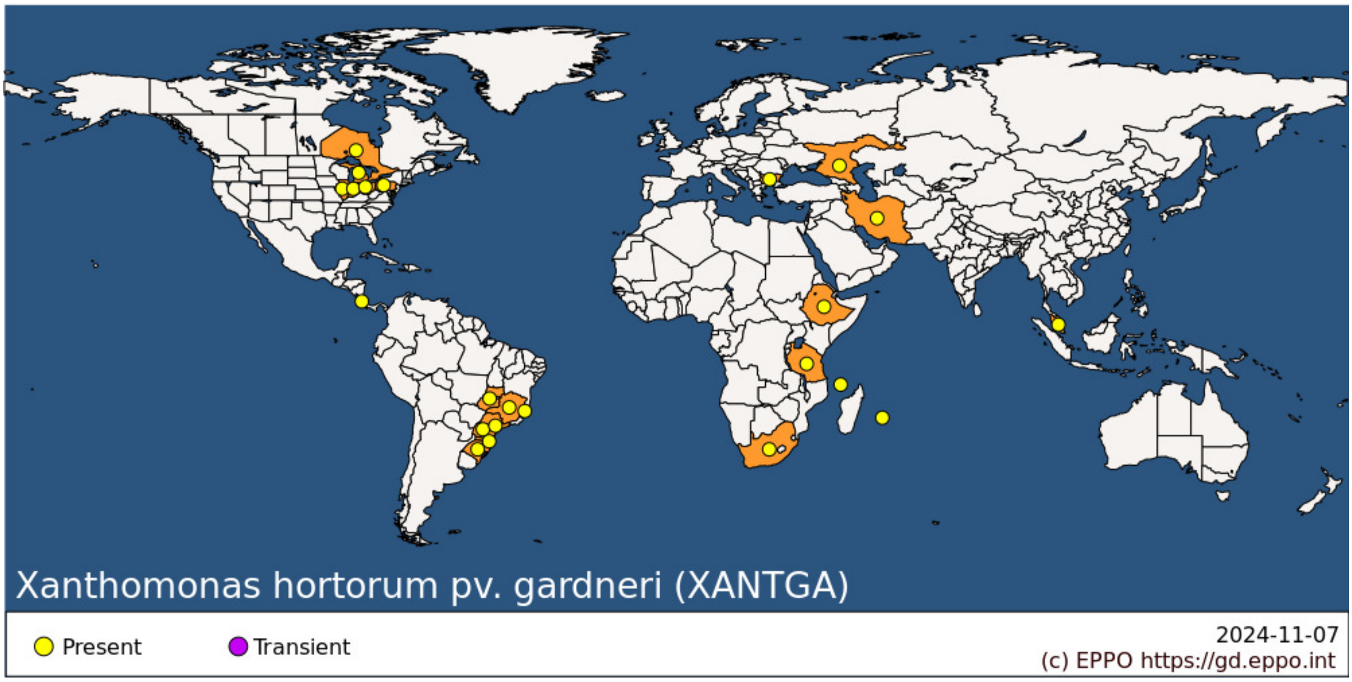
Host list: *Arctium lappa*, *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum pubescens*, *Euphorbia heterophylla*, *Solanum lycopersicum*, *Tubocapsicum anomalum*

GEOGRAPHICAL DISTRIBUTION

Bacterial spot causative agents occur widely in tomato and pepper-growing areas, especially in tropical and subtropical regions with moderate or higher rainfall. The disease has primarily been observed in field crops but can also occur in greenhouses. The environmental conditions in Southern Europe are particularly favorable for disease expression in the field, given that the optimal growth temperature for xanthomonads is between 25 and 30°C (Holt, 1994).

The classification of the four lineages of bacterial spot xanthomonads has experienced several revisions due to the intricate taxonomic relationships within this group (see Notes on taxonomy and nomenclature). This has led to uncertainties regarding the specific species present in various geographical areas. As reported by Timilsina *et al.* (2015), shifts in the species composition of bacterial spot pathogens populations have occurred due to the global spread of dominant genotypes, and recombination between species has generated genetic diversity in these populations. Therefore, the global distribution and genetic diversity of bacterial spot xanthomonads are poorly understood. Strains belonging to *X. euvesicatoria* pv. *euvesicatoria* and *X. vesicatoria* were historically considered the dominant bacterial lineages with a worldwide distribution (Jones *et al*., 2004b). However, more recently, *X. euvesicatoria* pv. *perforans* and *X. hortorum* pv. *gardneri* were increasingly isolated in North and South America, the Middle East, East Africa, and regions bordering the Indian Ocean (Vancheva *et al*., 2021). Dramatic changes in the dominant lineages and population structure of bacterial spot in different local areas have been documented over the past few decades. For example, prior to 1991, *X. euvesicatoria* pv. *euvesicatoria* was the only species isolated from tomato in Florida (USA), while this taxon has been entirely replaced by *X. euvesicatoria* pv. *perforans* on this host since then (Klein-Gordon *et al*., 2021). Similar changes are also reported in Taiwan (Burlakoti *et al*., 2018).

The map includes only records where the *Xanthomonas* species has been identified. Articles mentioning '*Xanthomonas* spp.' or 'bacterial spot of tomato' without mentioning the species are not included in the database. A map showing the records of *Xanthomonas axonopodis* pv. *vesicatoria* is available as an archive in **EPPO Global Database**.



EPPO Region: Bulgaria, Russia (Southern Russia)
Africa: Comoros, Ethiopia, Reunion, South Africa, Tanzania
Asia: Iran, Malaysia (West)
North America: Canada (Ontario), United States of America (Illinois, Indiana, Michigan, Ohio, Pennsylvania)
Central America and Caribbean: Costa Rica
South America: Brazil (Espirito Santo, Goias, Minas Gerais, Parana, Rio Grande do Sul, Santa Catarina, Sao Paulo)

BIOLOGY

The primary source of inoculum for bacterial spot xanthomonads are infected seeds and planting material (Potnis *et al* ., 2015). Bacteria can survive from one season to another, mainly on tomato and pepper seeds, for at least 10 years (Bashan *et al*., 1982b). A positive correlation was observed between the inoculum concentration of the bacterium on pepper blossoms and the percentage of infested seeds (Dutta *et al*., 2013). These pathogens can also survive in infected debris and the soil to some extent, possibly in the rhizosphere of non-host plants (Bashan *et al*., 1982a). Diverse solanaceous and non-solanaceous weeds may be alternative hosts (Osdaghi *et al*., 2021).

Once introduced in an area, the spread of bacteria is primarily by rain-splash or overhead irrigation, with the handling of young plants also playing an important role (Goode & Sasser, 1980). Viable bacteria have been detected in aerosols over commercial fields, indicating a putative aerial dispersal (McInnes *et al.*, 1988; Bernal & Berger, 1996). Leaves are infected through stomata, while fruits are infected through small wounds, such as abrasions, and insect punctures. Young leaves and fruits are more sensitive to infection and the bacteria can multiply epiphytically on young plants in the absence of symptoms. Thinning of directly seeded tomato seedlings (practice of selectively removing weaker seedlings to improve the health and productivity of the plants) is reported to promote spread of the disease, and it is recommended to thin in the afternoon, when plants are dry, and to use prophylactic hand washes (Pohronezny *et al.*, 1990). The disease is favored by heavy rainfall, high humidity (Diab *et al.*, 1982) and temperatures between 25°C and 30°C (EFSA, 2014).

DETECTION AND IDENTIFICATION

Symptoms

All bacterial spot xanthomonads can induce a wide variety of symptoms on their host plants, including angular lesions that later become brown and necrotic on the leaves, fruits, petioles and stems. Some symptoms may be mistaken for those caused by other organisms (see below). Pathogen aggressiveness and the development of symptoms often depend on the host-pathogen combination, with *X. euvesicatoria* pv. *euvesicatoria* appearing to be significantly more aggressive on bell pepper than on tomato (Ignjatov *et al*., 2010).

Fruits of tomato show superficial corky spots or scabs, with water-soaked margins, oval or irregular in shape and with a diameter of around 2-10 mm. The 'flecks' caused by *Pseudomonas syringae* pv. *tomato* are distinctly smaller (diameter <1 mm), black, circular and elevated. Differences are also observed with symptoms of *Clavibacter michiganensis* on fruits, consisting of brown spots, slightly raised, and surrounded by a white halo (a distinctive 'bird's eye' appearance). Scabbing of fruit (but without water-soaking) may also be a symptom of the phytotoxicity of plant protection products. Bacterial spot xanthomonads fruit lesions in pepper are scab-like, raised and rapidly necrotising. Lesions on tomato or pepper leaves appear as irregular water-soaked areas, initially green and later becoming brown and necrotic. Speck lesions caused by *P. syringae* pv. *tomato* look similar in a first stage, but are surrounded by a more distinct yellow halo; lesions are often in streaks and the yellow haloes run together to give large chlorotic areas (Goode & Sasser, 1980). Severe infections in pepper cause defoliation, favoring sunscald of the fruits on hot and sunny days.

Bacterial spot xanthomonads can cause canker-like splits in stems, but their presence alone is not diagnostic as similar symptoms may also be caused by *P. syringae* pv. *tomato*, *C. michiganensis* and *Alternaria solani*. Pith necrosis has been associated with the presence of *X. euvesicatoria* pv. *perforans*, but this symptom could be confused with those of *Pseudomonas mediterranea* and *Pseudomonas corrugata* (Aiello *et al*., 2013).

Morphology

Xanthomonads causing bacterial spot of tomato and pepper are aerobic, mobile, Gram-negative rods, occurring singly or in pairs, 0.6 x 1.0-1.5 µm, with a single polar flagellum. Like other species of the genus, they produce characteristic yellow pigments (xanthomonadins). On general media, such as yeast-glucose-calcium carbonate agar (YGCA) or yeast peptone glucose agar (YPGA), colonies are mucoid-fluidal, convex, and yellow with entire edges. Unlike *P. syringae* pv. *tomato*, bacterial spot xanthomonads are non-fluorescent on King's B medium.

Detection and inspection methods

Symptoms of bacterial spot xanthomonads can be confused with those caused by other pathogens. Confirmation through diagnostic analysis is necessary when the presence of spot-causing bacteria is suspected. The EPPO Diagnostic protocol Standard PM 7/110 (2) (EPPO, 2023) offers comprehensive guidelines for the preliminary screening of plant material or seeds. The Standard includes isolation and molecular tests and, if required, confirmatory pathogenicity tests in susceptible cultivars of tomato and pepper plants. The EPPO Standard recommends multiplex real-time PCR (Strayer *et al*., 2016) and real-time PCR tests for specific identification of *Xanthomonas* species causing bacterial spot disease (Baldwin *et al*., 2023). Serological methods are not recommended in the Standard; few antibodies are commercially available for immunofluorescence and ELISA, and no validation data could be retrieved.

The International Seed Health Initiative (ISHI) advises using a minimum of 10 000 tomato or pepper seeds for seed detection purposes to ensure effective screening (ISF, 2017). This approach, with subsamples capped at 10 000 seeds, aims to identify contamination levels as low as 0.03% with a 95% confidence interval. In instances where there is a high likelihood of saprophytic bacteria overshadowing the presence of *Xanthomonas* spp., opting for smaller subsample sizes, such as five sets of 2 000 seeds each, is recommended.

A procedure by which consignments of tomato seeds should be subjected to phytosanitary import inspection, including sampling and identification, is provided in EPPO Standard PM 3/80 (2) (EPPO, 2021).

PATHWAYS FOR MOVEMENT

Trade of infected seeds and plants for planting (transplants) are associated with long-distance dissemination of *Xanthomonas* spp. which are responsible of causing bacterial spot. Bacteria may escape from infected plants as exudates, and short-distance dispersal is then facilitated by splashing water, such as irrigation or rain. This becomes particularly concerning during transplant in greenhouse production when several thousands of transplants are growing closely together, as well as in the field, especially in the case of sprinkler irrigation. Short-distance-spread of bacteria is also possible through contaminated tools (EFSA, 2014).

PEST SIGNIFICANCE

Economic impact

Bacterial spot xanthomonads are widespread and are considered significant pathogens of tomato and pepper in field-grown crops in warm-temperate and tropical countries, especially under overhead irrigation. They can also occur in greenhouses. Fruit yield losses are most substantial when infection occurs early, as observed in tomato in USA, and pepper in Israel (Dougherty, 1978; Bashan *et al*., 1985). Damage to the leaves tends to expose fruits to the sun, increasing the risk of sunscald. Although fruit lesions are often only superficial, they result in loss of marketability.

Bacterial spot has been recognized as a severe disease accompanied by significant damages in production in countries of the EPPO region, such as Serbia (Ignjatov et al., 2010; Vlaji? et al., 2017), Bulgaria and the Republic of North Macedonia (Kizheva *et al*., 2011), or Türkiye (Aysan & Sahin, 2003). Although no recent data are available on economic losses caused by these pathogens in the European Union (EU), infections resulting in up to 30 % losses have been reported (EFSA, 2014). Yield losses of up to 66% have been reported in the USA (Pohronezny & Volin, 1983). Tomato yield loss estimated to be 7 413 USD per ha have been reported in Florida (USA) (Vallad *et al*., 2013). Furthermore, outbreaks in 2009-2010 in 2000 ha of processing tomatoes in Northwest Ohio and Southeast Michigan resulted in total losses of up to 7.8 USD million (Ma *et al*., 2011).

Control

Due to the seedborne nature of bacterial spot xanthomonads, management of the disease has been a major challenge since its original description. As no effective methods or chemical control agents are available for infected crops, disease control requires the adoption of integrated management measures, primarily focused on prevention and exclusion. Pathogen-free seeds and transplants are crucial to avoid the introduction and spread of bacteria. Tomato seed extraction from fruits using appropriate fermentative or acid treatments to reduce xanthomonads population is required in the EU (Anonymous, 2019). For both tomato and pepper seeds, hot water soak, dry heat therapy or selected chemicals have been recommended (EFSA, 2014).

Once infection occurs, control of disease in the field is particularly difficult. Cultural practices, such as four-year crop rotations, are recommended. Avoiding the handling of wet plant material and minimizing free moisture on foliage helps prevent disease development and spread. The application of protective chemicals or biological treatments is advised to reduce the severity and spread during transplant production.

The most common approach for managing bacterial spot pathogens is the preventive application of copper-based bactericides, but their success is limited as bacterial resistance to such chemicals has appeared worldwide (Lamichhane *et al*., 2018). Additionally, in the EU a new legislation limits the use of copper compounds (Anonymous, 2018). Several biological control approaches have been studied, including bacteriophages (Jones *et al*., 2012; Balogh *et al*., 2018; Gaši? *et al*., 2018; Ríos-Sandoval *et al*., 2020), plant growth-promoting rhizobacterium (Naue *et al*., 2014; Liu *et al*., 2018) and antagonistic bacteria. Integration of biological control agents, such as tailocins (phage-tail-like bacteriocins produced by *Pseudomonas fluorescens* SF4c (Príncipe *et al*., 2018) and *Bacillus velezensis* GF267 (de Paula Kuyat Mates *et al*., 2019), and SARS inducers (harpin and acibenzolar-S-methyl) enhances the effectiveness of bacterial spot management (Obradovic *et al*., 2005; Abo-Elyours & El-Hendawy, 2008). The application of bacteriophages alone or in combination with biocontrol agents or copper hydroxide reduces disease incidence.

Efforts have been made to develop tomato and pepper lines with resistance to bacterial spot xanthomonads, and sources of resistance have been identified and incorporated into breeding programs and varieties (Stall *et al*., 2009). However, the persistence of resistance can change rapidly due to the evolving geographical distribution of the pathogen and the rapid emergence of new pathogenic variants (Potnis *et al*., 2015).

Phytosanitary risk

Xanthomonas spp. are important bacterial pathogens that affect tomato and pepper production. Environmental conditions in Southern Europe are particularly favorable for bacterial spot expression in the field, as the optimal growth temperature for xanthomonads is between 25°C and 30°C, but the disease also occurs in greenhouses (EFSA, 2014).

Long-distance spread of *Xanthomonas* spp. causing bacterial spot of tomato and pepper, is commonly related to the movement of infected seeds and planting material (transplants). Once introduced into a production area, such as a cultivation plot or a greenhouse, the pathogens disperse easily, and short-distance dispersal is facilitated by splashing water (from irrigation and rain) or contaminated tools (EFSA, 2014).

PHYTOSANITARY MEASURES

As the main means of spread of bacterial spot xanthomonads are with seeds and plants for planting, the use of healthy seed and plantlets are a key phytosanitary measure (Picard *et al*., 2018).

Currently *Xanthomonas euvesicatoria* pv. *euvesicatoria*, *Xanthomonas euvesicatoria* pv. *perforans*, *Xanthomonas hortorum* pv. *gardneri* and *Xanthomonas vesicatoria* are classified as regulated non-quarantine pests (RNQP) in many EPPO countries, including those within the EU (Anonymous, 2019), and measures to prevent their presence on seeds and planting material are mandatory. The EU measures are as follows: the presence of these bacteria is not allowed in propagating material of ornamental plants or other plants for planting intended for ornamental purposes, vegetable seeds, and vegetable propagating and planting material other than seeds (threshold level 0%) (Anonymous, 2019 Annex IV, Parts D, F, and I). For tomato seeds, an appropriate extraction method for bacterial elimination is required (there is no indication whether or not pepper seeds should be treated). Tomato and pepper seeds should originate in areas known to be free from these pathogens where no symptoms of bacterial spot have been observed during the complete cycle of vegetation of the plants at site of production. Alternatively, seeds should have been subjected to official testing on a representative sample using appropriate methods and have been found free of the pathogens (Anonymous, 2019 Annex V, Parts C, E, and H).

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