EPPO Datasheet: *Pseudomonas syringae pv. actinidiae*

Last updated: 2021-06-02

IDENTITY

Preferred name: *Pseudomonas syringae pv. actinidiae*
Authority: Takikawa, Serizawa, Ichikawa, Tsuyumu & Goto
Taxonomic position: Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae
Common names: bacterial canker of kiwi fruit
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EPPO Categorization: A2 list
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EU Categorization: Emergency measures (formerly), RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: PSDMAK



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Notes on taxonomy and nomenclature

Comparative analysis of *Pseudomonas syringae* pv. *actinidiae* strains isolated in different geographical areas worldwide revealed that this pathovar is characterized by a number of distinct genetic lineages, giving rise to 5 biovars (biovars 1, 2, 3, 5, and 6) (Chapman *et al.*, 2012; Sawada *et al.*, 2014; Sawada *et al.*, 2016). Biovar 1 and 2 are described as moderately aggressive and were both reported affecting *Actinidia* spp. in the 1980-90s, the former in Japan, South Korea and Italy, the latter in South Korea (Serizawa *et al.*, 1989; Scortichini, 1994; Sawada and Fujikawa, 2019). Biovar 3, which is highly pathogenic, is the lineage responsible for the worldwide pandemics; biovar 3 has been diversifying for a long time in China and, in addition to the pandemic lineage, it exists in diverse native strains in several Chinese provinces (Butler *et al.*, 2013; McCann *et al.*, 2017). Biovar 5 (Sawada *et al.*, 2014) and biovar 6 (Sawada *et al.*, 2016) are described as weakly pathogenic bacteria and reported in two Japanese Prefectures. The formerly known biovar 4 of *P. syringae* pv. *actinidiae*, has since been transferred into a new pathovar, named *P. syringae* pv. *actinidifoliorum* (Cunty *et al.*, 2015).

HOSTS

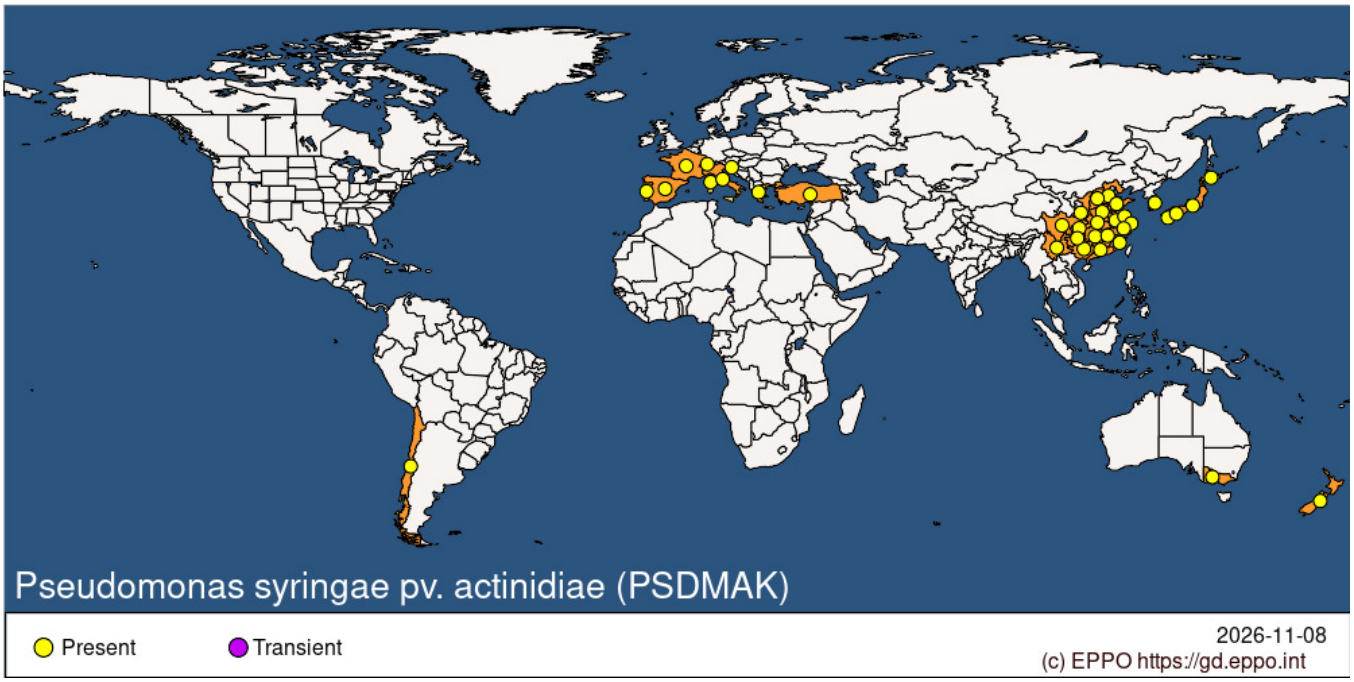
The most important host plants affected by *P. syringae* pv. *actinidiae* belong to the genus *Actinidia*. In particular, the cultivated *A. chinensis* and *A. deliciosa* cultivars are considered as major hosts (Serizawa *et al. 1989*; Fang *et al.*, 1990). Differences in host plant susceptibility are reported for different *Actinidia* species, or different cultivars belonging to the same species (Perez *et al.*, 2019; Donati *et al.*, 2020). In general, *A. chinensis* (the yellow-fleshed kiwifruit) is far more susceptible than *A. deliciosa* (the green-fleshed kiwifruit). Other wild or ornamental *Actinidia* species, such as *A. arguta* or *A. kolomikta*, are considered as minor host plants (Ushiyama *et al.*, 1992a, 1992b). Recently, three non-kiwifruit species, *Alternanthera philoxeroides*, *Paulownia tomentosa* and *Setaria viridis*, have been reported as incidental host plants for *P. syringae* pv. *actinidiae*. These plant species displayed necrotic spots on leaves and were grown in proximity to kiwifruit orchards severely affected by bacterial canker (Liu *et al.*, 2016).

Host list: *Actinidia arguta*, *Actinidia chinensis*, *Actinidia deliciosa*, *Actinidia kolomikta*, *Actinidia*, *Alternanthera philoxeroides*, *Broussonetia papyrifera*, *Paulownia tomentosa*, *Setaria viridis*

GEOGRAPHICAL DISTRIBUTION

The bacterial canker of kiwifruit was first observed in Japan in the late 1980s on *Actinidia* spp. (Serizawa *et al.*, 1989; Takikawa *et al.*, 1989) and, later, in South Korea (1988) (Koh *et al.*, 1994): in both countries, it was considered as a limiting factor for the production of kiwifruits. A few years later, the pathogen was reported in China (Wang *et al.*, 1992). In the EPPO region, *P. syringae* pv. *actinidiae* was observed for the first time in 1992 in Central Italy (Scortichini, 1994). More than a decade later, severe disease outbreaks were repeatedly observed in Italy in the

summer 2007 and in the following years, giving rise to massive crop losses (Balestra *et al.*, 2009; Scortichini *et al.*, 2012). The bacterial populations causing such outbreaks were genetically different from those previously recorded in Italy, Japan, South Korea and China. Later, several outbreaks of the disease were reported in Turkey in 2009, in France and Portugal in 2010, in Spain and Switzerland in 2011, in Slovenia and in Georgia in 2013, in Greece in 2014. Outside the EPPO region, the pathogen continues to be present in several provinces of China, in many prefectures of Japan and in South Korea. In New Zealand *P. syringae* pv. *actinidiae* was first detected in 2010, then rapidly spread throughout the country, whereas in Australia the pathogen, first detected in 2011, still has a very limited distribution in Victoria (EPPO, 2011). Finally, the bacterium has a restricted distribution in Chile, where it was first recorded in 2010 (ProMed, 2010). In Argentina, the pathogen was found on kiwifruit pollen produced in the Mar del Plata region (Balestra *et al.*, 2018), but it has not been detected in kiwifruit orchards (Sánchez *et al.*, 2018).



EPPO Region: France (mainland, Corse), Greece (mainland), Italy (mainland), Portugal (mainland), Slovenia, Spain (mainland), Switzerland, Türkiye
Asia: China (Anhui, Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Shanghai, Shanxi, Sichuan, Yunnan, Zhejiang), Japan (Hokkaido, Honshu, Kyushu, Shikoku), Korea, Republic of
South America: Chile
Oceania: Australia (Victoria), New Zealand

BIOLOGY

Bacterial canker is the most important limiting factor in the cultivation and production of kiwifruit (Kim *et al.*, 2017). *P. syringae* pv. *actinidiae* overwinters in cankers that are formed on trunks, along the leaders (cordons) and on canes. In winter, symptomless plants may also harbour the pathogen latently inside the vascular tissue (Minardi *et al.*, 2019). In late winter or early spring (February to March in the Mediterranean area), bacteria start to multiply in diseased tissues and pale, milky droplets of bacterial ooze start to exude from cankers or other lesions, such as pruning cuts. Bacterial exudates are the primary inoculum in infected orchards and these start the first seasonal infection cycle. Sap from infected, but symptomless plants exuding from pruning cuts in springtime also represent a pathway for pathogen spread inside the orchards (Biondi *et al.*, 2013). High humidity, rain and showers favour the dispersal of bacterial cells that may contaminate the developing buds, shoots, leaves, and flowers. Frost events correlate positively with the occurrence of bacterial canker: indeed, frost injuries provide the pathogen with additional penetration sites and enable colonization, multiplication and dispersal of inocula (Serizawa *et al.*, 1989; Ferrante *et al.*, 2012; Ferrante and Scortichini, 2014). Penetration into the host plants happens via natural openings (stomata and lenticels) or lesions (mainly hail wounds and pruning cuts). Flowers are very prone to infections and pollen is easily contaminated by the pathogen, thus serving as an additional pathway for pathogen dispersal (Stefani

and Giovanardi, 2011; Vanneste *et al.*, 2011). *P. syringae* pv. *actinidiae* has an optimum temperature range between 15-22°C: therefore, the disease rapidly progresses until early summer (Serizawa and Ichigawa, 1993). Then, the pathogen aestivates in the vascular tissue of its hosts. In non-conducive conditions, vascular colonization of Actinidia plants may also proceed for some years, without the development of symptoms (Minardi *et al.*, 2019). As is the case for several other *P. syringae* pvs, *P. syringae* pv. *actinidiae* easily survives as an epiphyte in infected orchards during spring and summer, both on its host plants and on several weeds, among them the stinging nettle (*Urtica dioica*), amaranths (*Amaranthus* spp.) or the common mallow (*Malva sylvestris*) (Stefani and Giovanardi, 2011; Tontou *et al.*, 2013). On kiwifruits, *P. syringae* pv. *actinidiae* may also survive epiphytically until summer: populations then decrease, being already undetectable a few weeks before harvesting (Stefani and Giovanardi, 2011; Minardi *et al.*, 2011). Thus, kiwifruits do not represent a pathway for pathogen dissemination.

DETECTION AND IDENTIFICATION

Symptoms

P. syringae pv. *actinidiae* may cause symptoms on any aerial part of its host plants: trunk, leaders, canes, leaves, flowers, fruits. Cankers are formed on lignified plant parts following the penetration of the pathogen through lenticels or lesions, such as pruning cuts or hail wounds. In late winter, cankers are moist and exude bacterial ooze together with plant sap; plant exudates are initially creamy whitish, later turning yellowish or yellow-orange, then reddish to brown (Serizawa *et al.*, 1989; Balestra *et al.*, 2009). Saprophytes (bacteria and yeasts) may develop on exudates, thus producing colour variations even in the same orchard. Active cankers may also appear along canes: diseased canes also exude bacterial ooze when cut. After debarking, the affected wood appears reddish-brown, with diseased areas developing through the healthy tissue. Infected canes develop shoots that, later, wilt and desiccate. Extensive dieback of twigs and vines are common, with abundant leaf fall, whereas the developing fruits remains tenaciously attached to the vines, eventually rotting and/or drying. On leaves, tiny angular and water-soaked lesions may develop early in the season, later necrotizing and developing confluent necrosis: chlorotic haloes may surround the developing lesions. Flowers and flower buds may darken, dry and fall off (Serizawa *et al.*, 1989; Balestra *et al.*, 2009). On flower buds, flowers and leaves similar lesions are also produced by other phytopathogenic pseudomonads, such as *P. syringae* pv. *syringae*, *P. syringae* pv. *actinidifoliorum* and *P. viridiflava*. In other cases, wilting and death of growing shoots, together with the development of necrotic cores at the base of the sprouting buds, are not caused by *P. syringae* pv. *actinidiae*, but may be due to frost injuries caused by the presence of ice nucleating bacteria or due to some physiological disorder.

Affected fruitlets are misshapen, smaller in size than healthy fruits and may develop a necrotic apex; they usually fall during late spring or early summer or are manually detached and thrown away during fruit thinning. Fruits may collapse as a consequence of wilting of branches; wilted fruits are not marketable.

Morphology

P. syringae pv. *actinidiae* is a Gram negative, aerobic, motile, rod-shaped bacterium with polar flagella and it is approximately 2-2.5 x 0.5-0.8 µm in size. It forms small, smooth pearly-whitish, circular colonies that are elevated or convex on nutrient-sucrose-agar medium (NSA) and flat on King's B medium (KB). *P. syringae* pv. *actinidiae* colonies usually do not produce a fluorescent pigment on KB, although Everett *et al.* (2011) reported that some isolates fluoresce on that medium. Fluorescence production appears quite a useful tool to discriminate *P. syringae* pv. *actinidiae* from *P. syringae* pv. *syringae*, a fluorescent phytopathogenic bacterium that may be also found on diseased and healthy *Actinidia* spp. as well. *P. viridiflava* is easily discriminated from *P. syringae* pv. *actinidiae*, since its colonies produce a distinctive blue-green pigment when grown on NSA medium.

Detection and inspection methods

P. syringae pv. *actinidiae* can be detected on both symptomatic and asymptomatic plant material. The EPPO Standard 7/120 (2) describes the diagnostic protocol to detect, isolate, identify and characterize the pathogen in plant various material, including pollen.

Inspections are necessary to monitor the presence of *P. syringae* pv. *actinidiae* in nursery stocks, in pollen lots, in kiwifruit orchards. EFSA described the key elements to design a pest survey on the pathogen, defining the target

population, the epidemiological unit and the inspection unit for EU countries (EFSA, 2020). Inspections are planned to enable detection of typical disease symptoms and/or to collect plant material for analysis. The most suitable periods to perform inspections in orchards are: i) late winter/early spring, in order to easily identify and collect tissues from oozing cankers; ii) early summer, in order to observe the disease developing on leaves and shoots and, consequently, collect plant material for analysis. Inspection planned in late winter/early spring may be useful to observe and collect plant sap bleeding from pruning cuts. In orchards, where the pathogen has not been observed, sampling and analysis of plant sap might help enable early detection of the pathogen, therefore allowing immediate action prior to the first disease cycle. Inspections should also be conducted in orchards of male plants for pollen production: in such a case, a late-winter inspection is needed to confirm the absence of any symptom that might indicate the possible presence of the pathogen, e.g. cankers. Finally, inspections and sample collection should also be conducted to check the phytosanitary status of nursery stock and issue phytosanitary certificates and/or plant-passports for propagation material. In such a case, an aggregate sample for analysis is composed of 100 vitroplants, representing a lot up to 10 000 plants, taken prior their acclimatization period, or 30 plantlets from acclimatization premises.

PATHWAYS FOR MOVEMENT

Two main pathways are recognized for short to long distance movement of *P. syringae* pv. *actinidiae*: nursery stock (i.e. plants for planting excluding seed), such as rooted micropropagated cuttings, and pollen (Stefani and Giovanardi, 2011; Tontou *et al.*, 2013; Kim *et al.*, 2017; Balestra *et al.*, 2018). Micropropagation is, in general, an effective technique to ensure that plant material produced is free of this pest; nevertheless, rooted cuttings may become infected at a later stage during the production cycle, e.g. during their acclimatization under tunnels or in the open. *Actinidia* spp. are dioecious species and, therefore, the male and female reproductive structures are on separate plants. Mechanical pollination is a common practice during the management of kiwifruit orchards to improve fruit weight and quality, and approx. 400-500 grams of pollen are applied per ha through dusting or spraying under the canopy (Galliano *et al.*, 2008). Additionally, flower colonization by *P. syringae* pv. *actinidiae* from infected pollen has been proven to be very effective (Donati *et al.*, 2018). Since Actinidia pollen is a marketed commodity worldwide, this pathway should not be neglected (MAF, 2011; EPPO 2012) and might have the same pathogen dissemination potential as the micropropagated cuttings (Stefani and Giovanardi, 2011; Kim *et al.*, 2017). Seeds and fruits are not a pathway.

Short distance movement of *P. syringae* pv. *actinidiae* is ensured by infected pollen, wind driven rain splash, showers, irrigation, pruning tools, and several human activities inside the kiwifruit orchard (e.g. curving down canes and tying them onto trellis, fruit thinning and picking, pruning). Pollinators appear to have a negligible role in pathogen dissemination.

PEST SIGNIFICANCE

Economic impact

In 2019, China was the leading producer of kiwifruit in terms of production volume (2 035 160 tonnes), followed by Italy (562 190 tonnes) and New Zealand (414260 tonnes) (Shahbandeh, 2020). *Actinidia chinensis* and *A. deliciosa*, when infected with *P. syringae* pv. *actinidiae* biovar 3 in particular, develop abundant lesions and cankers and eventually die. In particular, some yellow-fleshed cultivars, such as Hort16A and JinTao that are recognized to be highly susceptible, may die within 1-2 seasons. Therefore, this disease is considered the greatest challenge in kiwifruit production (Vanneste, 2017; CABI, 2019). Potential crop losses in New Zealand were estimated to be 310 to 410 million EUR, from 2013 to 2018 (Khandan *et al.*, 2013). In Italy, in 2010, crop losses exceeded 60 million EUR and, during the following years, yield reduction dropped by approximately 43%. The prompt introduction of specific phytosanitary measures, together with an increased knowledge of disease epidemiology, the replacement of the most susceptible cultivars of *A. chinensis* by new tolerant genotypes, and a tailored disease management reduced the economic impact of the disease which is, nowadays, of much less concern than a few years ago.

Control

The official definition of areas with different phytosanitary status, together with the implementation of inspections and certification schemes, allowed the risk associated with both recognized pathways (*i.e.* nursery stock and pollen) to be reduced. The enormous influence that *P. syringae* pv. *actinidiae* pandemic had on the kiwifruit industry in the main production areas worldwide activated several research programmes devoted to developing and implementing control strategies based on different approaches. These are: i) orchard management through the optimization of cultural practices; ii) chemical and biological control options; iii) breeding programmes for the selection of tolerant/resistant cultivars.

Cultural control

The bacterial canker of kiwifruit is a polycyclic disease: therefore, reduction of primary and secondary inocula are key to successful management. Additionally, *Pseudomonas syringae* pvs. infections are strongly influenced by external environmental conditions, such as air humidity, temperature and microbiota that live on healthy plants (Xin *et al.*, 2018). Good hygiene practices play a pivotal role in reducing bacteria populations, e.g. through removal and destruction of any symptomatic plant material, pruning excess vegetation, regular disinfection of any pruning tools, weed management and reduction of relative humidity inside orchards (especially those under hail nets) through pruning of green vines (Vanneste *et al.*, 2011). Large pruning cuts (over 2-3 cm) should be treated with a disinfection paste (e.g. containing copper salts). Drip irrigation should be preferred in place of sprinkler irrigation, or any other irrigation system that causes a prolonged wetting of the canopy. Efficient soil drainage should be ensured. Finally, excessive nitrogen fertilization should be avoided, since it increases the susceptibility of kiwiplants to this pathogen (Monchiero *et al.*, 2015). Since mechanical pollination is a common practice to produce high quality fruits, dust pollination is preferable to wet pollination, since the use of water to suspend and spray pollen in kiwifruit orchards creates micro-climatic conditions under the canopy that favour pathogen survival and its penetration into the host plants through stomata and lenticels. Although kiwifruit cultivars with known resistance to *P. syringae* pv. *actinidiae* are not yet available, a few tolerant varieties are currently present on the market; furthermore, a number of breeding programmes are currently devoted to developing new cultivars with additional tolerance/resistance traits (Tahir *et al.*, 2019). Possible sources of tolerance/resistance that might be exploited in breeding programmes are currently being sought in *A. arguta* germplasm (Nunes da Silva *et al.*, 2020).

Chemical control

Chemical control of *P. syringae* pv. *actinidiae* is difficult, especially in rainy and humid areas, and should be done together with cultural control, as described above. Chemical options for effective control are based on copper formulations and, where allowed, antibiotics, such as streptomycin or kasugamycin (Vanneste *et al*., 2011). Copper compounds are recommended after fruit harvest and winter pruning, to disinfect wounds on plants, and at bud break, to limit the quantity and the dissemination of primary inoculum. Post-flowering sprays are suggested before major rain events (Vanneste *et al.*, 2011; Monchiero *et al.*, 2015). To reduce the input of copper in orchards, treatments with acybenzolar-S-methyl may also be used in combination with reduced copper quantities (Monchiero *et al.*, 2015).

Biological control

The need to reduce copper inputs into agricultural environments and the development of isolates resistant to copper (Colombi *et al.*, 2017) or to streptomycin (Han *et al.*, 2004), led to the implementation of biological control with microbial biocontrol agents. These comprise: yeasts (de Jong *et al.*, 2019), bacteria (Tontou *et al.*, 2016), bacteriophages (Frampton *et al*., 2014) or natural substances (Balestra, 2007). The yeast *Aureobasidium pullulans* significantly reduced the disease, especially in combination with acybenzolar-S-methyl (de Jong *et al.*, 2019). Several bacterial epiphytes and endophytes proved to be active *in vitro* and *in vivo* against *P. syringae* pv. *actinidiae* (Tountu *et al.*, 2016): among the several bacterial species studied, *Lactobacillus plantarum* and *Bacillus amyloliquefaciens* had the best performance (Biondi *et al.*, 2012; Daranas *et al.*, 2018; Purahong *et al.*, 2018). Products based on microbial antagonists are commercially available and are currently authorized as biocontrol agents during flowering.

Phytosanitary risk

P. syringae pv. *actinidiae* is considered the major pest threat for *Actinidia* spp., especially for *A. chinensis* (the

yellow-fleshed kiwifruit). After its introduction into the EPPO region, it rapidly spread and is now established in all kiwi-producing areas and its impact was high in the first decade the pest was present. Countries where kiwifruit is grown, and this pathogen is not present should avoid its introduction. International movement of the pathogen is associated with trade of plants for planting and pollen. There is no risk of introduction with kiwifruits or seeds.

P. syringae pv. *actinidiae* was the object of EU emergency measures until March 31st, 2020 (EU, 2017). Later, following an official exchange of views at the Standing Committee on Animals, Plants, Food and Feed on the need of prolongation of the Commission Implementing Decision mentioned above, and pending a decision whether *P. syringae* pv. *actinidiae* qualifies as RNQP (EU, 2016), a new Commission Implementing Regulation was approved, thus extending the emergency measures in force until December 31st, 2021 (EU, 2020).

PHYTOSANITARY MEASURES

EPPO (2012) recommends the following phytosanitary measures: plants for planting (except seeds) and pollen should originate from a pest-free place of production or a pest-free area. Tissue culture should be produced from mother plants produced in a pest-free place of production or a pest-free area. Additionally, EPPO strongly recommends that surveys are conducted in all kiwifruit growing countries.

Heat treatment has been suggested as a method to reduce the bacterial load of pollen lots (Everett *et al.*, 2016).

Emergency measures have been implemented in the EU since 2012 (EU, 2020) to prevent the introduction and spread of the pathogen within the Union. Such measures include following specific points: i) specified plant material originating in third countries shall be accompanied by a phytosanitary certificate; ii) rigorous inspections shall be implemented at the border control posts and, where appropriate, such material shall be tested; iii) specified plants shall be moved inside the EU territory only when accompanied by a plant passport.

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