EPPO Datasheet: *Ralstonia syzygii*

Last updated: 2021-11-29

IDENTITY

Preferred name: *Ralstonia syzygii*
Authority: (Roberts et al.) Vaneechoutte et al.
Taxonomic position: Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiaceae
Common names: Sumatra disease, banana blood disease
view more common names online...
EPPO Categorization: A1 list
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EPPO Code: RALSSY

Notes on taxonomy and nomenclature

Ralstonia solanacearum (Smith) Yabuuchi *et al*. (1995) is a species complex (RSSC) that comprises four phylotypes (Fegan & Prior, 2005). Each phylotype includes multiple phylogenetic and pathogenic variants differing in barcoding markers (including the 16S-23S rRNA gene intergenic spacer region and the, *hrpB*, *mutS* and *egl* genes), known as sequevars. Safni *et al*. (2014) reclassified the four phylotypes of the RSSC into three distinct species: *R. solanacearum* (Smith, 1896) Yabuuchi *et al*., 1996 emend. Safni *et al*., 2014 (Phylotype II), *Ralstonia pseudosolanacearum* Safni *et al*., 2014 (Phylotypes I and III) and *Ralstonia syzygii* (Roberts *et al*., 1990) Vaneechoutte *et al*., 2004 emend Safni *et al*., 2014 (Phylotype IV). *R. syzygii* comprises three subspecies: subsp. *syzygii*, subsp. *celebesensis* and subsp. *indonesiensis*. Phylotype IV comprises 4 sequevars (PIV-8, PIV-9, PIV-10 and PIV-11) but the three subspecies of *R. syzygii* are not clearly delineated according to sequevar. Taxonomy and nomenclature have been reviewed in detail by Paudel *et al*. (2020).

HOSTS

As a relatively recently classified species, new hosts of *R. syzygii* are likely still to be discovered. Two host-specific subspecies of *R. syzygii* (subsp. *syzygii* and subsp. *celebesensis*) respectively cause the spittlebug-transmitted Sumatra disease of cloves and the pollinator-transmitted banana blood disease. Following inoculation, *R. syzygii* subsp. *syzygii* has been shown to infect other related species of the family Myrtaceae, including some indigenous species native to Indonesian forests, such as *Syzygium aqueum* (Safni *et al*., 2018). *R. syzygii* subsp. *indonesiensis* has a wider host range, including some solanaceous crops as well as clove and arrowroot (*Canna indica*). It has also been detected on various wild spp., including *Asclepias currassiva* (tropical milkweed), *Datura stramonium* (jimsonweed), and *Solanum nigrum* (black nightshade). Natural hosts reported so far are listed below. Other potential hosts, including tree species, have been reported (e.g. by Supriadi *et al*., 2001) but the pathogen has yet to be fully characterized.

Host list: *Asclepias curassavica*, *Canna indica*, *Capsicum annuum*, *Datura stramonium*, *Eucalyptus urophylla*, *Heliconia*, *Musa acuminata*, *Musa balbisiana*, *Musa textilis*, *Musa x paradisiaca*, *Musa*, *Solanum lycopersicum*, *Solanum nigrum*, *Solanum tuberosum*, *Strelitzia reginae*, *Syzygium aromaticum*

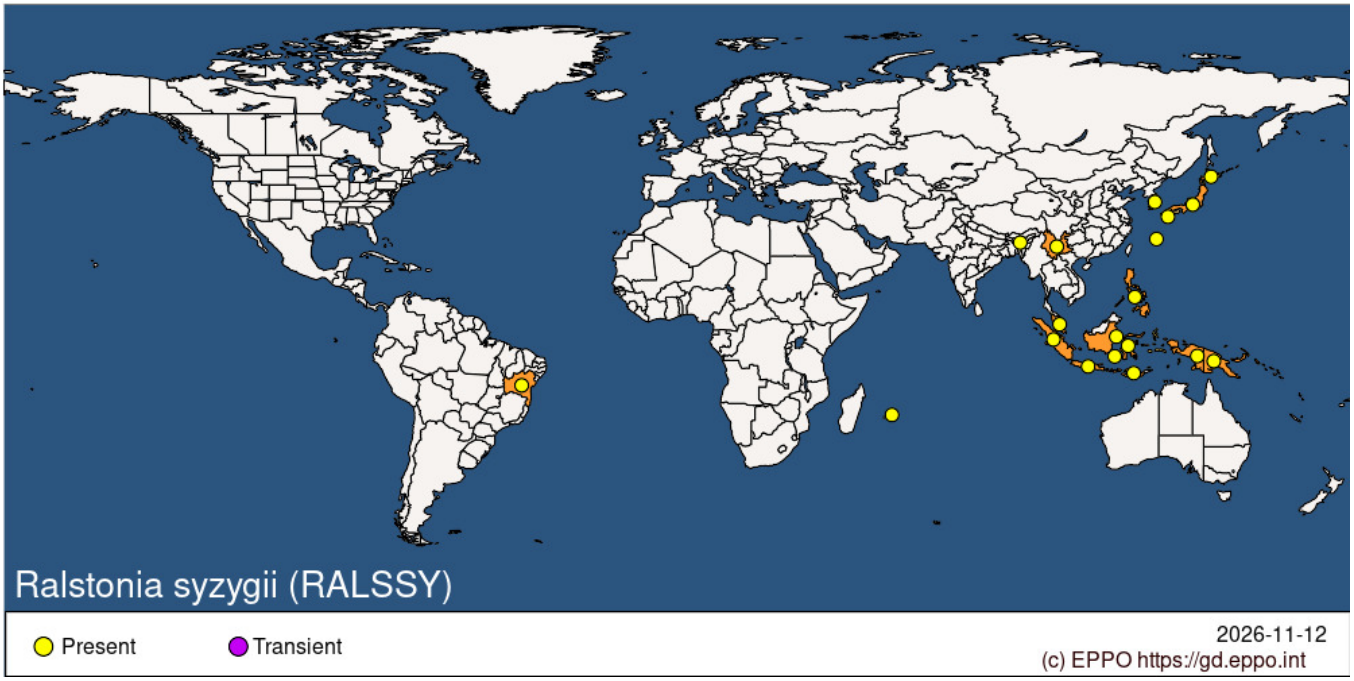
GEOGRAPHICAL DISTRIBUTION

First found in 1975 in the Indonesian provinces of Sumatra and West Java, *R. syzygii* subsp. *syzygii* has now spread to Central and East Java (Safni *et al*., 2018). Lomer *et al*. (1992) suggested that Sumatra disease of cloves may have been transferred to clove trees from wild forest hosts because of the localized initial distribution of the disease and the corresponding localized distribution of the vector species, indicating that the pathogen may have a wider host range than has so far been identified (Purcell and Hopkins, 1996).

Banana blood disease was first reported in Indonesia on Selayar Island in the early 1900s after the introduction of dessert banana. The disease spread to Java in the late 1980s and has become common on local *M. paradisiaca* cultivars in Sulawesi. *R. syzygii* subsp. *celebesensis* has since spread to most of the larger Indonesian islands (Safni *et al*., 2018), and has also been reported on the island of New Guinea (Davis *et al*., 2001) and more recently in Malaysia (Teng *et al*., 2016).

R. syzygii subsp. *indonesiensis* causes disease on solanaceous hosts in Indonesia and some other countries, and has also been isolated from clove in Indonesia (Safni *et al*., 2018).

The currently known worldwide distribution of *R. syzygii* is as follows:



Africa: Mauritius
Asia: China (Yunnan), India (Meghalaya), Indonesia (Irian Jaya, Java, Kalimantan, Nusa Tenggara, Sulawesi, Sumatra), Japan (Hokkaido, Honshu, Kyushu, Ryukyu Archipelago), Korea, Republic of, Malaysia (West), Philippines
South America: Brazil (Bahia)
Oceania: Papua New Guinea

BIOLOGY

Ralstonia syzygii subsp. *syzygii* is a xylem-limited bacterium, living in xylem cells or tracheary elements of plants (Purcell and Hopkins, 1996). It is transmitted by insect vectors, tube building cercopoids (Hemiptera), that feed on xylem sap. Of these, *Hindola fulva* was found to be a natural insect vector in Sumatra whereas *H. striata* is a primarily vector in Java (Eden-Green *et al*., 1992). Vector transmission of *R. syzygii* subsp. *syzygii* is persistent with a short latent period between acquisition and transmission of the pathogen (Eden-Green *et al*., 1992). Blocking of the xylem vessels by bacterial biofilm is the major cause of wilting.

R. syzygii subsp. *celebesensis* can survive in soil for at least a year in infested plant residues and infected fruits and can be disseminated through plantations in soil and water or on tools and vehicles. It enters the plant through its roots, eventually colonizing and blocking the xylem. Various pollenating insects, including the stingless bee (*Trigona minangkabau*) and the banana skipper butterfly (*Erionota thrax*) also transmit this subspecies (Safni *et al*. 2018). Plantain (type ABB) cultivars with dehiscent bracts are particularly susceptible to insect transmission since the bacterium can reach the vascular tissue through wounds created when the bracts fall off. The high sugar content of the male flower nectar of the highly susceptible cultivar ‘Pisang Kepok’ appears to be particularly attractive to

insects such as wasps, bees and flies (Setyobudi and Hermanto, 1999).

Less is known of the biology of *R. syzygii* subsp. *indonesiensis* (Safni *et al*., 2018). As for *R. pseudosolanacearum*, it appears to persist in the environment for long periods, probably associated with its wild hosts. Unlike *R. solanacearum* potato brown rot strains, *R. syzygii* subsp. *indonesiensis* only causes disease of potato in tropical but not temperate conditions (Cellier and Prior, 2010); it was highly pathogenic to potato at 26°C (Habe, 2016).

DETECTION AND IDENTIFICATION

Symptoms

On clove: Sumatra disease usually affects productive trees over 10 years of age (Hadiwijaya, 1983). Externally the initial symptom is unseasonal yellowing of leaves followed by leaf-drop from the tips of branches high in the crown (Safni *et al*., 2018). However, the leaves may also wilt suddenly and turn brown, but stay attached to the branch. Affected twigs turn reddish brown and progressively die back. Symptoms typically progress to lower branches until the whole crown is affected, and the tree dies within 6-18 months (Bennett *et al*., 1985). Symptoms are usually more severe and disease spread is more rapid as elevations increase and temperatures decrease. Artificial inoculation of *R. syzygii* subsp. *syzygii* on *S. aromaticum* seedlings leads to leaf yellowing and drying after 28 days and death of the seedling after 56 days (Danaatmadja *et al*., 2009). Internally, the newly formed wood adjacent to the cambium becomes discoloured a pale greyish-brown and the xylem discoloration can be traced down the trunk into one or more major roots (Bennett *et al.*, 1985). When cut, infected branches ooze a milky white to pale brown bacterial exudate from the cut surface. Although *R. syzygii* subsp. *indonesiensis* strains can be isolated from the roots and lower trunk of *S. aromaticum* trees only *R. syzygii* subsp. *syzygii* can systemically colonize and kill these trees.

On banana and plantain: Symptoms of blood disease are quite similar to Moko disease caused by insect-transmitted strains belonging to RSSC phylotype II. The male flower bud and peduncle discolour and shrivel, the fruit pulp shows a reddish dry rot, and the vascular tissue throughout the plant exhibits a reddish discoloration, which oozes reddish-brown bacterial exudate when cut (Sequeira and Averre, 1961; Buddenhagen, 1962). The older leaves of blood disease-infected *Musa* spp. become yellow, followed by wilting, necrosis and collapse; younger leaves turn bright yellow before becoming necrotic and dry. The pathogen rapidly colonizes the entire plant, and suckers also wilt and die (Eden-Green, 1994b).

On potato, tomato and *Capsicum*: The disease symptoms caused by *R. syzygii* subsp. *indonesiensis* strains on solanaceous crops are no different from those described for *R. solanacearum* and *R. pseudosolanacearum* (see relevant EPPO data sheets). The external symptoms of infected plants are wilting, stunting and yellowing of the foliage with the disease progressing until the plant completely collapses. Internally the vascular tissue and portions of the pith and cortex become progressively discoloured eventually leading to complete necrosis and plant death.

Morphology

Morphology and phylogeny of *R. syzygii* subspecies is fully described by Safni *et al*. (2014). Cells of *R. syzygii* are Gram-negative, non-sporulating, non-capsulated, motile or non-motile, rods, approximately 0.5-0.6 x 1.0-2.5mm, occurring singly, in pairs or occasionally in short chains. Aerobic growth. Catalase- and oxidase-positive. Growth is observed on MacConkey agar without NaCl, but not on this media with 5 % NaCl.

Growth on casamino peptone glucose (CPG) agar at 28 °C is very sparse or absent after 6 days for *R. syzygii* subsp. *syzygii*, whereas *R. syzygii* subsp. *celebesensis* typically produces small (0.5-2 mm), round, mucoid, and non-fluid colonies after 4-5 days. *R. syzygii* subsp. *indonesiensis* colonies vary from fluidal to butyrous, irregular and convex, white in colour with a diameter of approximately 0.5 mm after 2-3 days of incubation at 28 °C on CPG.

Detection and inspection methods

EPPO standard PM 7/21 describes sampling methods, screening and identification tests for inspection and detection of the *R. solanacearum* species complex relevant for symptomatic and asymptomatic plant samples, and water samples. For field diagnosis from symptomatic tissues, bacterial slime oozing into clean water (as described above) is a simple test and lateral flow serological tests are commercially available. Suspected infections should be

confirmed by laboratory testing. For testing asymptomatic plant material, it is advised to bulk sample and prepare extracts of vascular tissues from up to 200 stem base pieces, or in the case of potato tubers, up to 200 tissue cores from the heel ends at the point of stolon attachment. A range of screening tests are available that include isolation on semi selective and elective media, immunofluorescence microscopy (EPPO Standard PM 7/97) and a range of DNA-based tests that include conventional PCR, real-time PCR and LAMP tests. It is recommended to use more than one screening test to safeguard against false positive and false negative results. These tests can also be used to confirm the identity of bacterial colonies isolated on agar media. It may also be useful to conduct a pathogenicity test on a susceptible host, especially if the pathogen is found in a location for the first time. For accurate pathogen identification, phylotypes and sequevars are differentiated by DNA sequencing of 16S-23S rRNA gene intergenic spacer region, egl, mutS and hrpB barcodes. Conventional PCR (Opina *et al*., 1997) or TaqMan qPCR (Weller *et al*., 2000) tests universally identify strains in all phylotypes whereas multiplex PCR tests identify each individual phylotype (Fegan and Prior, 2005) or host-specific strains within phylotypes (e.g. Cellier *et al*., 2015). See EPPO Standard PM 7/21 for detailed information on the available tests.

PATHWAYS FOR MOVEMENT

In Indonesia, spread of Sumatra disease of clove appears to be largely through transmission by the insect vectors of the order Hemiptera (*Hindola* spp.) resulting in a distinct pattern of disease expression and distribution in the field (Eden-Green *et al.*, 1992). Seedlings less than 2 years old are rarely affected. The disease advances on a broad front, at an estimated rate of 1-2 km per year and then disappears for years until young trees mature and the cycle repeats (Bennett *et al*., 1985). The disease spreads in all directions uphill, downhill, and across rivers.

Banana blood disease is also vectored by a variety of pollenating insects. Dispersion by insects at over 25 km per annum was shown on cooking and dessert bananas (Eden-Green and Seal, 1993). Mairawita *et al*. (2012) reported that the flying insect, *Trigona minangkabau*, often carried the blood disease pathogen in Sumatra. In Sulawesi, various large wasps, *Oncopsia* spp., *Trigona* bees and flies have been observed in contact with ooze discharging from peduncles and male buds of blood bacterial wilt-affected plants. *Erionota thrax*, is also known to carry the bacterium in Java (Suharjo *et al*., 2008). Within plantations, spread also occurs via surface water, contaminated tools and vehicles. However, it is likely that the transmigration of people from Java to less populated islands in the country was associated with the spread *R. syzygii* subsp. *celebesensis* in infected planting material (especially suckers of cooking banana ABB and BBB types, but also dessert banana and related ornamental plants), initially from Selayar Island to Sulawesi, Java and eventually to most of the larger Indonesian islands as well as to New Guinea and Malaysia.

Long distance spread of *R. syzygii* subsp. *indonesiensis* is probably also due to movement of infected planting material. Spread of this subsp. outside of Indonesia has mainly been of a single sequevar (PIV-8) and is limited to potato, suggesting possible movement in the international potato trade. As with the other *Ralstonia* spp., local spread can occur through mechanical transmission during cultivation or via soil drainage or surface water used for irrigation or by root contact.

PEST SIGNIFICANCE

Economic impact

In Indonesia, the reduction in banana production due to blood disease was estimated to be approximately 36% in 1991 (Muharam and Subijanto, 1991). In Southern Sumatra, losses due to blood disease have been estimated to reach 64% (Cahyaniati *et al*., 1997). With spread of the pathogen to most of the larger Indonesian islands, the average yield loss has been estimated to exceed 35% (Supriadi, 2005). Production of *S. aromaticum* has decreased rapidly since 1996, mainly due to Sumatra disease (Safni *et al*. 2018). The disease, which was initially confined to the Indonesian provinces of Sumatra and West Java, has now spread to Central Java and East Java and causes economic losses of up to 5-10% per year (Safni *et al*., 2018). In all, plant diseases caused by *Ralstonia* spp. in Indonesia were estimated to cause the 6th highest pre-harvest losses of all pests, including mammals and insects (Geddes, 1992).

Control

All clove varieties appear susceptible to Sumatra disease, so local agricultural departments in Indonesia recommend that agricultural tools used for field work should be disinfected, infected plants should be eradicated, and insecticides should be applied to minimize the spread of the disease by insect vectors (Safni *et al*., 2018). As an alternative to insecticides, different natural enemies of *Hindola* spp. have been identified, such as parasitizing species of *Stylops* and *Acmopolynema* and predatory species of the family Tettigoniidae. Some potentially antagonistic bacterial endophytes and rhizobacteria which are antagonistic to *Ralstonia syzygii*, such as *Bacillus subtilis*, have also been identified (Fina Dwimartina *et al*., 2017) However, biological control strategies are difficult to apply on a sufficiently large scale.

Similarly, all edible *Musa* spp. are thought to be susceptible to blood disease. The cultivar 'Pisang Puju', an acceptable resistant *Musa x paradisiaca* plantain variety from Sulawesi, and 'Pisang Sepatu Amora' appear to be less susceptible since these cultivars abort the male bud, blocking insect transmission (Hermanto *et al*., 2013). Similarly, removal of the male flower bud of susceptible varieties, as practiced for Moko disease control, has been an effective cultural control practice (Safni *et al*. 2018). Removal of infected plants is an essential part of control but disposal of infected plant material can be difficult. Indonesian farmers managed to effectively control banana blood disease by burning uprooted material (Setyobudi and Hermanto, 1999). In commercial plantations, strict and sustained preventive management measures, based on the use of healthy planting material, male fluorescence de-budding or bagging to prevent insect transmission and strict hygiene and quarantine measures can be very effective (Blomme *et al*., 2017). However, in the case of small scale farmers, facing banana blood disease in cooking bananas, adoption of simple measures is not always easy. Many cooking varieties are grown in small informal plantings that continuously provide sources of inoculum. Restricting movement of planting material from infected areas remains the most effective way to limit spread of disease.

Control of *R. syzygii* subsp. *indonesiensis* relies on the same approaches as for *R. solanacearum* and *R. pseudosolanacearum*. Use of pathogen-free planting material, prevention of movement of infected potato tubers or other host plants for planting, regular use of inspection and diagnostics to identify and eliminate infections and careful hygiene during cultivation, pruning and harvesting activities are key control measures.

Phytosanitary risk

The R. solanacearum species complex (RSSC) has quarantine status in many countries. The occurrence around the world of different strains of the pathogen presents an ongoing risk of the introduction of new variants capable of affecting potato and tomato production in the EPPO region. Absence of the bacterium is an important consideration for countries and pest free areas exporting seed potatoes.

There are no reports of *R. syzygii* being introduced into, or intercepted in, the EPPO region. Introduction of some strains of *R. syzygii* may present a risk for the warmer southern member countries or for host plants grown under protected cultivation. Due to the host specificity of *R. syzygii* subsp. *syzygii*, it should only present a risk to clove-producing countries. Similarly, *R. syzygii* subsp. *celebesensis* is mainly a risk to banana production and should be given the same phytosanitary consideration as Moko disease-causing strains of phylotype II of *R. solanacearum*. *R. syzygii* subsp. *indonesiensis* has already spread internationally within Asia on potato, but seem to be lower risk for the EPPO region than the phylotype IIB-1 strain of *R. solanacearum* because it only causes disease of potato in tropical but not temperate conditions (Cellier and Prior, 2010; Habe, 2016).

PHYTOSANITARY MEASURES

EPPO Standard PM 9/3 (under revision) describes a national regulatory control system for the *Ralstonia solanacearum* species complex (RSSC) that provides guidance on surveillance for the pathogen and its containment and eradication if found with a focus on potato. Seed potato tubers, and other plants for planting of known hosts, should have been grown in areas found free from RSSC strains during the growing season and during the previous two growing seasons. Since the bacteria can also contaminate water courses, the irrigation of host plants with water from contaminated waterways should be prohibited.

Visual inspections should be performed routinely upon export and import of known host plants for planting. Laboratory checks are necessary to detect asymptomatic (latent) infections. EPPO Standard PM 8/1 recommends the phytosanitary measures which EPPO countries should use or require for seed and ware potatoes moving in

international trade to prevent the introduction and spread of *Ralstonia* and other quarantine pests. EPPO Standard PM 3/21 *Post entry quarantine for potato* describes inspection and tests for the detection of pests (including *R. solanacearum*) infecting *Solanum* species or hybrids imported for germplasm conservation, breeding or research purposes, in post-entry quarantine. Plants for planting of known host plants may be placed in post-entry quarantine to observe any symptoms and if relevant to test them to ensure their freedom from RSSC strains.

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Datasheet history

This datasheet was first published online in 2021. It is maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', ‘Hosts’, and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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