EPPO Datasheet: *Ralstonia pseudosolanacearum*

Last updated: 2021-11-29

IDENTITY

Preferred name: *Ralstonia pseudosolanacearum*
Authority: Safni, Cleenwerck, de Vos, Fegan, Sly & Kappler
Taxonomic position: Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiaceae
Common names: bacterial wilt
view more common names online...
EPPO Categorization: A2 list
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EU Categorization: A1 Quarantine pest (Annex II A)
EPPO Code: RALSPS

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Notes on taxonomy and nomenclature

Ralstonia solanacearum (Smith) Yabuuchi *et al*. (1995) is a species complex (RSSC) that comprises four phylotypes (Fegan and 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). Taxonomy and nomenclature have been reviewed in detail by Paudel *et al*. (2020). This datasheet considers *R. pseudosolanacearum* (RSSC phylotypes I and III).

HOSTS

Various strains of *R. pseudosolanacearum* (RSSC phylotypes I and III) have a wide range of cultivated and wild hosts. Of major importance are the solanaceous crops (tomato, potato, *Capsicum* (sweet/bell/chilli) peppers, aubergine and tobacco) as well as cucurbits, peanut (*Arachis hypogaea*) and ginger (*Zingiber officinale*). Strains pathogenic to the solanaceous crops comprise a very wide range of sequevars belonging to both pathotypes I and III. Strains pathogenic to ginger were historically designated race 4, having a wide host range with additional pathogenicity to ginger, and are now known to comprise several sequevars of phylotype I, so far including: PI-14, PI-15, PI-16, PI-17, PI-18, PI-31, PI-44, PI-47, PI-48 and other as yet unassigned sequevars of phylotype I (Xu *et al*., 2009; Waki *et al*., 2013; Horita *et al*., 2014; Lin *et al*. 2014; Ramesh *et al*. 2014; Wang *et al*. 2017; She *et al*. 2018; Abdurahman *et al*., 2019). Most of the same sequevars of phylotype I have also been found to infect peanut, including: PI-13, PI-14, PI-15, PI-17, PI-18, PI-31, PI-44, PI-48 and PI-54 (Xu *et al*., 2009; Cellier & Prior, 2010; Pan *et al*., 2013; Wang *et al*., 2017; Abdurahman *et al*., 2019), the last of which was incorrectly numbered in China (sequevar 54 being already ascribed to phylotype IIB in Brazil).

R. pseudosolanacearum is essentially absent from the EPPO region, although strains have occasionally been introduced with ornamental/spice plants or plant parts of tropical origin and have caused bacterial wilt disease under heated greenhouse conditions in temperate climates before being successfully eradicated. These include *Curcuma longa* and *C. alismatifolia* (Janse, 2012) and, more recently, *Rosa* spp. (Tjou-Tam-Sin *et al.*, 2016). In the latter case infected plants were distributed amongst several countries (including the Netherlands, Belgium, Portugal and Switzerland) before the pathogen was detected and eradicated.

New hosts of *R. pseudosolanacearum* are still being discovered. Many known hosts are shared with those of the other *Ralstonia* species and phylotypes. Fewer hosts have been reported for strains of phylotype III than for phylotype I, probably due to the fact that phylotype III has been less studied. Hosts listed here are reported natural hosts worldwide, focusing mainly on cultivated plants where isolates of the bacterium have been characterized to

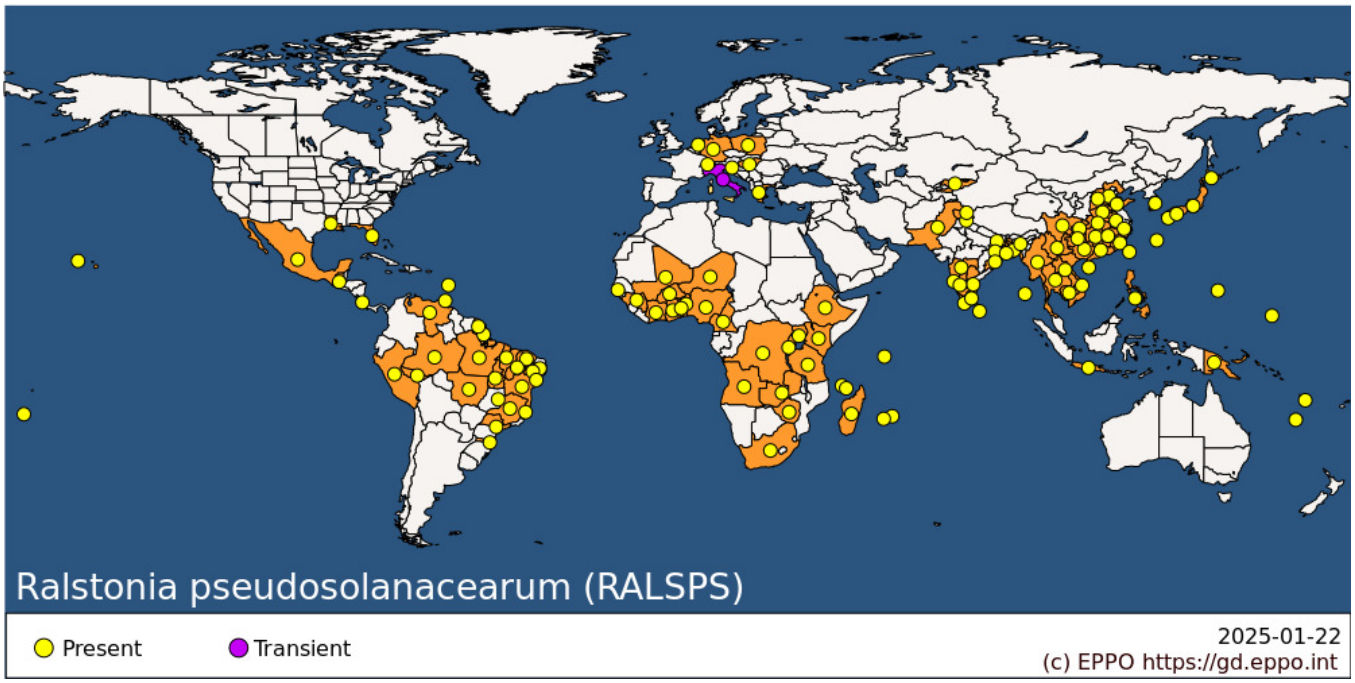
phylotype and sequevar of the RSSC. For historical host lists, see Kelman (1953), Bradbury (1986), Persley (1986) and Hayward (1994). Several other host lists, including wild herbaceous and tree species, have been reported, but for which the pathogen has yet to be fully characterized (e.g. Abdulha, 1993; Supriadi *et al*., 2001; Janse *et al*., 2004; Obregón *et al*., 2008; Paret *et al*., 2008; Mondal *et al*., 2011; Prieto *et al*., 2012; Lopes and Rossato, 2018).

Host list: *Ageratum conyzoides, Amaranthus sp., Amomum subulatum, Angelica keiskei, Annona squamosa, Anthurium andraeanum, Anthurium sp., Arachis hypogaea, Aralia cordata, Artemisia sp., Begonia hybrids, Begonia sp., Beta vulgaris subsp. vulgaris var. cicla, Bidens pilosa, Boehmeria nivea, Bougainvillea sp., Brassica oleracea, Campanula sp., Capsicum annuum, Capsicum frutescens, Casuarina equisetifolia, Cestrum nocturnum, Chaenostoma cordatum, Chrysanthemum sp., Cicer arietinum, Coleus sp., Corchorus olitorius, Cosmos caudatus, Croton hirtus, Cucumis sativus, Cucurbita maxima, Cucurbita moschata, Cucurbita pepo, Curcuma alismatifolia, Curcuma aromatica, Curcuma longa, Curcuma zedoaria, Cyamopsis tetragonoloba, Cyphostemma mappia, Dahlia sp., Delphinium sp., Dimorphotheca ecklonis, Ensete ventricosum, Eruca vesicaria subsp. sativa, Eucalyptus urophylla, Eucalyptus, Eustoma russellianum, Fagopyrum esculentum, Ficus carica, Fragaria x ananassa, Grevillea striata, Hedychium coronarium, Helianthus sp., Hibiscus sabdariffa, Hibiscus sp., Impatiens sp., Ipomoea aquatica, Ipomoea batatas, Justicia adhatoda, Kaempferia galanga, Kalanchoe sp., Lagenaria siceraria, Limonium sp., Ludwigia octovalvis, Luffa aegyptiaca, Mandevilla sp., Manihot esculenta, Maranta arundinacea, Momordica charantia, Morus alba, Nicotiana tabacum, Olea europaea, Pelargonium x hortorum, Pelargonium, Perilla frutescens, Petroselinum crispum, Phaseolus vulgaris, Physalis angulata, Piper hispidum, Platostoma palustre, Plukenetia volubilis, Pogostemon cablin, Portulaca oleracea, Raphanus sativus, Rosa, Salix gracilistyla, Sesamum indicum, Sesbania herbacea, Solanum aethiopicum, Solanum americanum, Solanum campylacanthum, Solanum capsicoides, Solanum incanum, Solanum lycopersicum, Solanum macrocarpon, Solanum melongena, Solanum muricatum, Solanum myriacanthum, Solanum nigrum, Solanum scabrum, Solanum sisymbriifolium, Solanum tuberosum, Spigelia anthelmia, Strelitzia reginae, Symphytum officinale, Syzygium aromaticum, Syzygium samarangense, Tagetes sp., Vaccinium corymbosum, Vaccinium membranaceum, Vicia faba, Vinca major, Zingiber mioga, Zingiber montanum, Zingiber officinale, Zingiber sp., Zinnia sp.*

GEOGRAPHICAL DISTRIBUTION

Within *R. pseudosolanacearum*, Phylotype I is regarded to be of Asian origin, whereas phylotype III is of African origin. Phylotype III has largely remained within its centre of origin, so far being found only in Africa and islands in the Indian Ocean. A large diversity of phylotype I sequevars are widely distributed across South and South-East Asia and some have been further dispersed worldwide, probably through international trade in infected, often asymptomatic, vegetatively propagated crops (e.g. ginger and turmeric rhizomes) and ornamental host plants and plant parts, or possibly in infected peanut seeds.

The worldwide reported distribution of both phylotypes of *R. pseudosolanacearum*, is as follows:



EPPO Region: Germany, Greece (mainland), Hungary, Italy (mainland), Kyrgyzstan, Netherlands, Poland, Slovenia, Switzerland
Africa: Angola, Benin, Burkina Faso, Cameroon, Comoros, Congo, Democratic republic of the, Cote d'Ivoire, Ethiopia, Gambia, Ghana, Guinea, Kenya, Madagascar, Mali, Mauritius, Mayotte, Niger, Nigeria, Reunion, Rwanda, Seychelles, South Africa, Tanzania, Togo, Uganda, Zambia, Zimbabwe
Asia: Bangladesh, Cambodia, China (Anhui, Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Henan, Hubei, Hunan, Jiangxi, Shandong, Shanxi, Sichuan, Yunnan, Zhejiang), India (Andaman and Nicobar Islands, Andhra Pradesh, Bihar, Goa, Himachal Pradesh, Jammu & Kashmir, Jharkand, Karnataka, Kerala, Maharashtra, Meghalaya, Odisha, Tamil Nadu, West Bengal), Indonesia (Java), Japan (Hokkaido, Honshu, Kyushu, Ryukyu Archipelago, Shikoku), Korea, Republic, Kyrgyzstan, Laos, Myanmar, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam
North America: Mexico, United States of America (Florida, Hawaii, Louisiana)
Central America and Caribbean: Costa Rica, Guatemala, Martinique, Trinidad and Tobago
South America: Brazil (Acre, Amapa, Amazonas, Bahia, Ceara, Distrito Federal, Espirito Santo, Maranhao, Mato Grosso, Minas Gerais, Para, Paraiba, Pernambuco, Piaui, Santa Catarina, Sao Paulo, Sergipe, Tocantins), French Guiana, Peru, Venezuela
Oceania: Guam, Micronesia, New Caledonia, Papua New Guinea, Tonga, Vanuatu

BIOLOGY

Although often described as soilborne, survival is usually short lived at low temperature in bare soil but is significant in alternative wild host plants (especially perennial species growing in waterlogged conditions or overwintering volunteers from susceptible crops) (Charkowski *et al*., 2020). Disease is usually most severe at temperatures of 24-35°C. High soil moisture or periods of wet weather or rainy seasons are associated with high disease incidence. Entry into plants is usually through root injuries from where the bacterium moves by colonization of the xylem. Blocking of the vessels by bacterial biofilm is the major cause of wilting. Disease severity generally increases if the bacteria are found in association with root nematodes. In tobacco and other crop hosts, nematode infestation changes the physiology of the plants, causing susceptibility to bacterial wilt (Chen, 1984). Experiments in India showed that the combined pathogenic effects of *R. pseudosolanacearum* and *Meloidogyne javanica* were greater than the independent effects of either pest (Sitaramaiah & Sinha, 1984).

DETECTION AND IDENTIFICATION

Symptoms

In most hosts wilting is a common symptom of infections. The youngest leaves usually wilt first, with symptoms initially appearing at the warmest time of day. Wilting may be visible in only one stem, on one side of a plant or even sectoral in part of a leaf, depending where vascular infections occur (e.g. if they are restricted to sectors of stems and/or leaf petioles. Leaves may become bronzed or chlorotic and epinasty may occur. Wilting of the whole plant may follow rapidly if environmental conditions are favourable for pathogen growth. As the disease develops, a brown discoloration of the xylem vessels in the stem may be observed above the soil line and adventitious roots may develop. A creamy, slimy mass of bacteria exudes from vascular bundles when the stem is cut. Wilting and collapse of whole plants can lead to rapid death.

On potato: Foliar symptoms include rapid wilting of leaves and stems, usually first visible in single stems at the warmest time of day. Eventually, plants fail to recover and become yellow and then necrotic. As the disease develops, a streaky brown discoloration of the stem may be observed on stems above the soil line, and the leaves may have a bronze tint. Epinasty of the petioles may occur. A white, slimy mass of bacteria often exudes from vascular bundles which are cut or broken. If cut stem or tuber vascular tissue is placed in water, threads of bacterial ooze exude, distinguishing this from diseases caused by other wilting pathogens e.g. *Fusarium*, *Verticillium*, *Dickeya* and *Clavibacter*. This test is of presumptive diagnostic value in the field. Plants with foliar symptoms may bear healthy and diseased tubers, while plants that show no signs of the disease may sometimes produce infected tubers. On potato tubers, external symptoms may or may not be visible, depending on the state of development of the disease in relation to the prevailing temperature. Infection eventually results in bacterial ooze emerging from the eyes and stolon end of infected tubers. Soil may adhere to the tubers at the eyes. Cutting the diseased tuber reveals a browning and eventual necrosis of the vascular ring and immediately surrounding tissues up to 0.5 cm each side of the ring, starting from the stolon end. A creamy fluid exudate usually appears spontaneously on the vascular ring of the cut surface a few minutes after cutting. In the case of ring rot the tuber has to be squeezed in order to express a mass of yellowish decayed vascular tissue and bacterial slime. Atypical symptoms on potato (necrotic spots on the epidermis), possibly caused after lenticel infection, have been described by Rodrigues-Neto *et al*. (1984).

On tomato, aubergine and *Capsicum* spp.: The youngest leaves are the first to be affected and have a flaccid appearance, this first occurs usually at the warmest time of day. Wilting of the whole plant may follow rapidly if environmental conditions are favourable for the pathogen. Under less favourable conditions, the disease develops less rapidly, stunting may occur, and large numbers of adventitious roots are produced on the stem. The vascular tissues of the stem show a brown discoloration and, if the stem is cut crosswise, drops of white or cream bacterial ooze may be visible.

On tobacco: One of the main symptoms is unilateral wilting and premature yellowing. Leaves on one side of the plant or even a half leaf may show wilting symptoms. In severe cases, leaves wilt without changing colour and stay attached to the stem. As in tomato, the vascular tissues show a brown discoloration when cut open. The primary and secondary roots may become brown to black (Echandi, 1991).

On ginger: The symptoms are a slight yellowing and wilting of the lower leaves, progressing upward and resulting in complete yellowing and browning of the entire shoot. Under favourable conditions, the entire shoot becomes flaccid and wilts before turning yellow-brown in 3-4 days. Young succulent shoots rot and break off easily from the underground rhizome at the soil level. The underground parts become completely infected. Localised greyish brown discoloration, with a water-soaked appearance in the centre, eventually spreads throughout the rhizomes, resulting in rotting of the entire rhizome. A creamy bacterial exudate oozes from the surface of a cut rhizome or stem (Trujillo, 1964).

On peanut: Wilt symptoms first appear 2-3 weeks after planting. The first sign of disease is a slight drooping or curling of one or more leaves. In more advanced stages, the plants may bend over at the tip, appear dry, and eventually turn brown, wither and die. Infected plants have discoloured and rotten roots and pods with dark brown discolouration in the xylem and pith and streaming of bacterial ooze from cut stems (Mehan *et al*., 1994).

On cucurbits: Symptoms on cucurbits develop rapidly from older to younger leaves that may wilt or not. Leaves turn yellow with necrotic lesions between or along major veins. Plants become flaccid and eventually collapse and die; there are no apparent symptoms on mature fruits (Wicker *et al.* 2007).

On blueberry: Symptoms include bronzing of leaves, marginal leaf necrosis, and bacterial streaming from cut

stems. Symptomatic plants resemble blueberry plants infected with *Xylella fastidiosa* (Norman *et al*., 2018)

On *Anthurium*: Greasy, water-soaked lesions (on the lower leaf surface) turn necrotic with greasy margins (on the upper leaf surface). When the disease becomes systemic, these lesions (generally originating from the insertion point of the leaf with the petiole) develop following the main and secondary veins in a full or partial glove-shape. External infections (disseminated by water) may develop from any natural opening such as hydathodes. Leaves may turn yellow depending on the severity of the systemic invasion, and the stem may rot with abundant bacterial ooze. The plant eventually collapses and dies (Wicker *et al*. 2007).

On geranium: The first symptoms are wilting and subsequent chlorosis (often sectorial yellowing) of leaves. Stems may blacken and eventually become necrotic. Internally, vascular browning is often visible. Leaves later become brown and necrotic as the whole plant desiccates, collapses and dies (Janse *et al*., 2004).

Morphology

Gram-negative rods with a polar tuft of flagella, non-fluorescent, but diffusible brown pigment often produced. Virulent isolates develop pearly (opalescent) cream-white, flat, irregular and fluidal colonies often with characteristic whorls in the centre, which characteristically stain blood red on media containing tetrazolium. Avirulent isolates form small round non-fluidal, butyrous colonies. See also Lelliott & Stead (1987) and Saddler (1994).

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

The main pathway for international spread is via infected vegetative propagating material (e.g. ginger and turmeric rhizomes and ornamental plants for planting). Asymptomatic (latent) infections, which escape visual inspections, are common at low temperatures when the rates of infection and colonization are slower, allowing host resistance mechanisms to be more effective. Latent infections also tend to occur in tolerant varieties. Natural infection of true seed has only been established for *R. pseudosolanacearum* in peanut (*Arachis hypogaea*) in Indonesia and China. There have been findings of contaminated seed of some other hosts (including tomato, *Capsicum*, aubergine and soybean) although seed infection and transmission has not been substantiated. At present, transmission through water, soil or movement of infected vegetative plant parts is considered to be more important for most host plants than transmission via true seed Once infections are established, local spread can occur when the bacterium is transmitted mechanically during pruning operations or when cuttings are taken for propagation. Spread to neighbouring plants can also occur through soil drainage water and by root contact. The bacterium also spreads through surface water.

PEST SIGNIFICANCE

Economic impact

The *R. solanacearum* species complex constitutes a serious obstacle to the culture and export of many crops in both tropical and temperate regions. Recently ranked by international phytobacteriologists as the second most important of all plant pathogenic bacteria after *Pseudomonas syringae* (Mansfield *et al*., 2012), the *Ralstonia* spp. have an extremely wide geographic distribution and host range. On potato alone, it is thought to be responsible for approximately 1 billion USD in losses each year, affecting some 3 million farmers and their families over 1.5 million ha in around 80 countries (Elphinstone, 2005). In many countries in which the pathogen has quarantine status, important losses result from increased surveillance, regulatory eradication measures and restricted further production on contaminated land.

Control

Disease management remains limited and is hampered by the faculty of the pathogens to survive for years in wet environments on plant debris or in asymptomatic weed hosts, which act as inoculum reservoirs. In the absence of any curative chemical control methods, prevention of bacterial wilt largely relies on the availability of pathogen-free planting material and effective surveillance and monitoring to protect areas which are free from the bacteria. For potato, effective disease management mainly relies on the use of limited generation seed multiplication from pathogen-free nuclear stocks with zero tolerances for the disease in official seed certification. Regular post-harvest testing of seed potato tubers is usually also necessary to avoid distribution of latent infections. Similarly, for other vegetatively propagated crops, there is a need to ensure planting material has been tested and found to be free of infection and that there are restrictions on the movement of planting material from affected to disease-free areas.

The effectiveness of strict regulatory control within Europe has been reviewed (EFSA, 2019). In relation to potato brown rot outbreaks in the EPPO region, use of healthy (tested) seed potatoes, early and accurate detection and reporting of the pathogen, quarantine measures on infected fields and farms, rotation with non-host crops for at least two years, control of weed hosts and volunteer plants (and in some cases of nematodes), avoidance prohibition of the use of contaminated surface water for irrigation, and education are key factors in control of *R. solanacearum* (EPPO Standard PM 9/3). For hydroponic glasshouse production systems, disinfection of recirculating water (e.g. using chlorine dioxide) can prevent spread of any introduced bacteria. These approaches have effectively halted international spread of *R. pseudosolanacearum* in turmeric and rose production.

For countries where the pathogen is widespread, various approaches to reduce disease impact through integrated cultural and biological control strategies have been reviewed (Yuliar *et al*., 2015). Disinfection of pruning and harvesting tools, e.g. using 20% solution of household bleach (3.5 % sodium hypochlorite) or less corrosive ammonia-based disinfectants, is important in preventing spread of disease. Drying of peanut seeds to <10% water content has significantly reduced seedborne infection (Zhang *et al*., 1993). Although resistant varieties have been reported for some hosts (e.g. peanut, tobacco, tomato, aubergine) their widespread use is hampered by the broad diversity of the pathogenic strains and the difficulty of introducing resistance from related wild species without losing commercial yield and quality requirements. It is hoped that modern breeding methods will help to increase future availability of acceptable resistant varieties for the wide range of host crops (Huet, 2014).

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 bacteria is an important consideration for countries and pest free areas exporting seed potatoes.

Natural spread may take place if the bacteria are introduced via discharge of poorly or non-treated wastes into surface water, which is then used to irrigate susceptible crops. *R. pseudosolanacearum* has been previously introduced into the EPPO region in infected ornamental plants for planting. *R. pseudosolanacearum* has a wide host range including solanaceous crops and could present a risk if accidentally imported into the EPPO region.

PHYTOSANITARY MEASURES

EPPO Standard PM 9/3 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 solanacearum* species complex 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. pseudosolanacearum*) 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.

Measures for seed are usually not needed except for peanut.

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