EPPO Datasheet: *Curtobacterium flaccumfaciens pv. flaccumfaciens*

Last updated: 2021-09-16

IDENTITY



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Preferred name: *Curtobacterium flaccumfaciens pv. flaccumfaciens*
Authority: (Hedges) Collins & Jones
Taxonomic position: Bacteria: Actinobacteria: Micrococcales: Microbacteriaceae
Other scientific names: *Bacterium flaccumfaciens* Hedges, *Corynebacterium flaccumfaciens pv. flaccumfaciens* (Hedges) Dowson, *Corynebacterium flaccumfaciens* (Hedges) Dowson, *Phytomonas flaccumfaciens* (Hedges) Bergey et al., *Pseudomonas flaccumfaciens* (Hedges) Stevens
Common names: bacterial tan spot of bean, bacterial tan spot of soybean, bacterial wilt of bean, bacterial wilt of common bean, bacterial wilt of dry beans, vascular wilt of bean
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EPPO Categorization: A2 list
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EU Categorization: Quarantine pest ((EU) 2019/2072 Annex II A)
EPPO Code: CORBFL

Notes on taxonomy and nomenclature

Bacterial wilt of common bean (*Phaseolus vulgaris*) was first observed in South Dakota (USA) in 1920; the causal Gram-positive phytopathogenic bacterium was described as *Bacterium flaccumfaciens* by Hedges in 1922 (Hedges, 1922). The disease and its causal agent were further detailed by Hedges in 1926. All phytopathogenic bacteria were placed by Bergey *et al*. (1939) in the genus *Phytomonas*, including the bean bacterial wilt pathogen, as *Phytomonas flaccumfaciens*. Dowson (1942) placed all Gram-positive, club-shaped phytopathogenic bacteria in the genus *Corynebacterium*, including the bacterial wilt pathogen as *C. flaccumfaciens*.

A variant with orange pigmented colonies, inducing orange discoloration of the seed coat, has been observed in Nebraska, USA since 1950, and named *Corynebacterium flaccumfaciens* var. *aurantiacum* (Schuster & Christiansen, 1957). Another variant with yellow colony morphology and producing a blue to purple soluble pigment in culture media, inducing purple discoloration of the seed coat, was also observed in Nebraska in the 1960s and named *Corynebacterium flaccumfaciens* var. *violaceum* (Schuster *et al*., 1968). These two variants, however, were not given taxonomic/nomenclatorial status in the following years, even though the purple variant was much later also discovered in Canada (Huang *et al*., 2006). After further (polyphasic) taxonomic studies on the phytopathogenic members of the genus *Corynebacterium* using DNA:DNA hybridization homology, cell wall composition and biochemical characteristics, a number of subspecies of *C. flaccumfaciens* were recognised, including *Corynebacterium flaccumfaciens* subsp. *flaccumfaciens*, *C. flaccumfaciens* subsp. *betae*, *C. flaccumfaciens* subsp. *oortii* and *C. flaccumfaciens* subsp. *poinsettiae* (Carlson & Vidaver, 1982). This led subsequently to their placement as pathovars (pathogenic varieties) within the species *flaccumfaciens* in a new genus (*Curtobacterium*). The bean bacterial wilt pathogen then became *Curtobacterium flaccumfaciens* pv. flaccumfaciens, the others *C. flaccumfaciens* pv. *betae*, *C. flaccumfaciens* pv. *poinsettiae* and *C. flaccumfaciens* pv. *oortii* (Collins & Jones, 1983). A pathovar *basellae*, affecting spinach, has been described (Chen *et al*., 2000), as well as a pathovar *beticola* affecting sugar beet (Chen *et al*., 2007). These new pathovars have been proposed, but not accepted so far by the ISPP Committee on the Taxonomy of Plant Pathogenic Bacteria. Finally, a new pathovar, *C. flaccumfaciens* pv. *ilicis* (formerly *Arthrobacter ilicis*, causing bacterial blight of American holly (*Ilex opaca*) was named by Agarkova *et al.* (2012).

A variant of *C. flaccumfaciens* pv. *flaccumfaciens* with pink-pigmented colonies, inducing orange-stained seed coats, was described from Nebraska in 2007 (Harveson & Vidaver, 2008; Harveson *et al*., 2015). A red-pigmented variant, inducing deep orange-stained seed coats, was isolated in 2014 in central Iran (Markazi province) from common bean (Osdaghi *et al*., 2016). A further, recent polyphasic study, including pathogenicity tests, MLSA and Box-PCR

studies, showed that there are two lineages within *C. flaccumfaciens* pv. *flaccumfaciens* strains, one with yellow-pigmented colonies and one with red/orange-pigmented colonies. Furthermore, it was found that non-pathogenic strains of *C. flaccumfaciens* pv. *flaccumfaciens* could also be isolated from non-hosts (Osdaghi *et al.*, 2018b).

HOSTS

The main hosts are all Fabaceae, including edible dry beans: *Phaseolus* spp., especially common bean (*P. vulgaris*), but also runner bean (*P. coccineus*) and lima bean (*P. lunatus*); adzuki or red mung bean (*Vigna angularis*), mung bean (*V. radiata*) and black gram (*V. mungo*); *C. flaccumfaciens* pv. *flaccumfaciens* can also attack soybean (*Glycine max*), pea (*Pisum sativum*), cowpea (*Vigna unguiculata*) and dolichos bean (*Lablab purpureus*). On soybean the disease and its causal agent were first reported in the USA in Iowa in 1975 and the disease was named bacterial tan spot (Dunleavy, 1983). In 2013 soybean was also reported as a host from Brazil (Soares *et al*., 2013).

Epiphytic and endophytic colonization can occur in other non-fabaceous crops. Under field conditions in Brazil, *C. flaccumfaciens* pv. *flaccumfaciens* was found to colonize, without causing symptoms, barley (*Hordeum vulgare*), black oat (*Avena strigosa*), canola (*Brassica napus*), common or white oat (*A. sativa*), ryegrass (*Lolium* spp.) and wheat (*Triticum* spp.), when they were cultivated in rotation with common bean. All *C. flaccumfaciens* pv. *flaccumfaciens* strains isolated from these plants were pathogenic to common bean (Gonçalves *et al.*, 2017). In the USA, Harveson *et al*. (2015) isolated dry bean-pathogenic orange and yellow *C. flaccumfaciens* pv. *flaccumfaciens* strains from wheat plants also infected by *Xanthomonas translucens* showing black chaff symptoms, which are typical for infections by the latter bacterium, and from maize leaves also infected with Goss' wilt disease, caused by *Clavibacter michiganensis* subsp. *nebraskensis*. In Iran, dry bean-pathogenic strains of *C. flaccumfaciens* pv. *flaccumfaciens* were isolated from symptomless eggplant (*Solanum melongena*), pepper (*Capsicum* spp.), and tomato (*Solanum lycopersicum*) plants (Osdaghi *et al*., 2018a).

C. flaccumfaciens pv. *flaccumfaciens* has been detected in weeds such as *Lupinus polyphyllus* (Schuster & Sayre, 1967), *Amaranthus retroflexus, Chenopodium album, Vicia villosa,* (Schuster, 1959) and *Ipomoea lonchophylla* (Condé & Diatloff, 1991; Osdaghi *et al*., 2020). Nascimento *et al*. (2020) reported that experiments using artificial inoculation under field conditions showed that the following weeds are potential, symptomless, hosts for *C. flaccumfaciens* pv. *flaccumfaciens*: *Amaranthus viridis, Conyza bonariensis, Commelina benghalensis, Cyperus rotundus, Digitaria insularis, Emilia fosbergii, Galinsoga parviflora, Gnaphalium purpureum, Ipomoea triloba, Lepidium virginicum, Nicandra physalodes, Raphanus sativus, Senna obtusifolia,* and *Solanum americanum*.

For additional information, see Harveson *et al*., 2015 and Osdaghi *et al*., 2020.

Host list: *Amaranthus retroflexus, Amaranthus viridis, Avena sativa, Avena strigosa, Brassica napus, Chenopodium album, Cicer arietinum, Commelina benghalensis, Cyperus rotundus, Digitaria insularis, Emilia fosbergii, Erigeron bonariensis, Galinsoga parviflora, Gamochaeta purpurea, Glycine max, Helianthus annuus, Hordeum vulgare, Ipomoea lonchophylla, Ipomoea triloba, Lablab purpureus, Lepidium virginicum, Lolium, Lupinus polyphyllus, Medicago sativa, Nicandra physalodes, Nicotiana benthamiana, Nicotiana tabacum, Phaseolus coccineus, Phaseolus lunatus, Phaseolus vulgaris, Pisum sativum, Raphanus sativus, Senna obtusifolia, Solanum americanum, Triticum aestivum subsp. aestivum, Vicia faba, Vicia lens, Vicia villosa, Vigna angularis, Vigna mungo , Vigna radiata, Vigna unguiculata subsp. sesquipedalis, Vigna unguiculata, Zea mays, Zornia glabra*

GEOGRAPHICAL DISTRIBUTION

Bacterial wilt was first observed in the USA (South Dakota) in 1920, and in subsequent years in Idaho, Maryland, Michigan, Montana, Virginia and Washington DC (Hedges, 1926). From the 1920s to the 1960s, the disease spread to four more states (Colorado, Nebraska, North Dakota, and Wyoming) with severe outbreaks occurring in the 1930s and 1960s (Harveson, 2013). The disease was subsequently observed in Ontario, Canada in 1954 (Patrick, 1954) and Mexico in 1955 (Yerkes & Crispin, 1956). After a period of low incidence there was a substantial reoccurrence of the disease in North America, especially in Colorado, Wyoming and Nebraska in the USA and Alberta in Canada in the 2000s (Harveson *et al*., 2015).

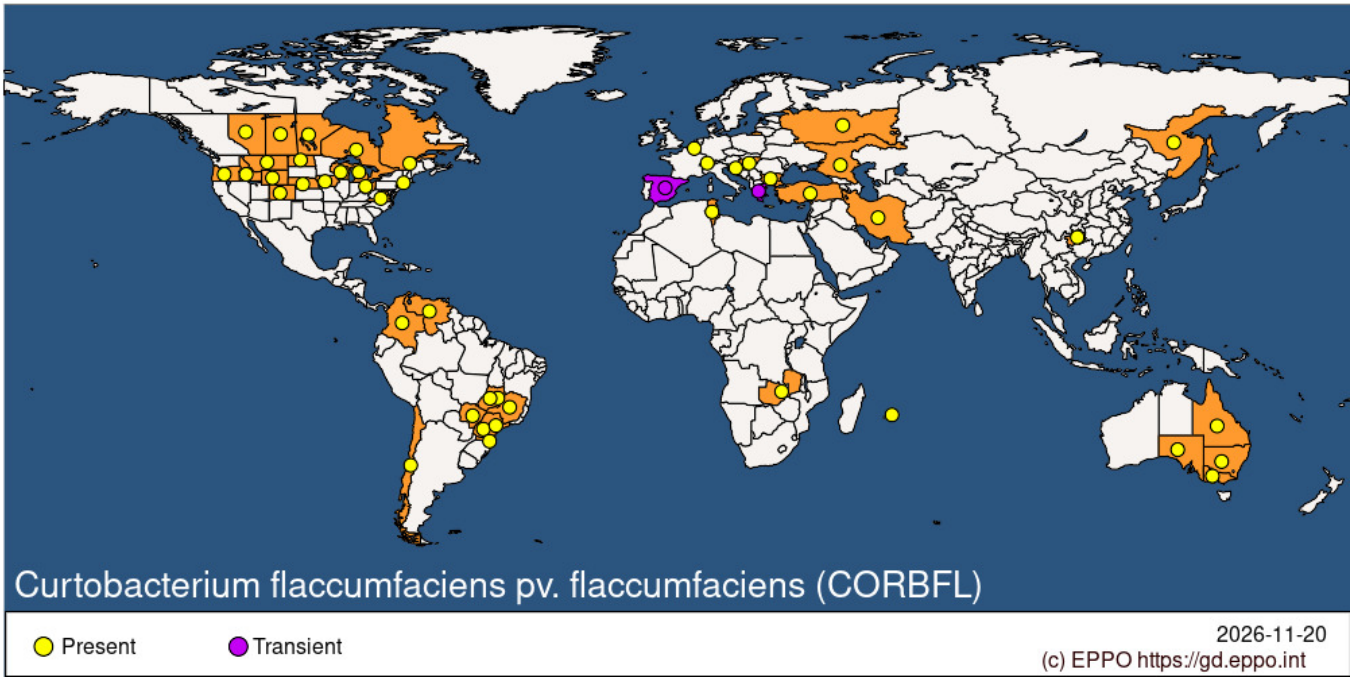
In South America the bacterium was reported on common bean (*Phaseolus vulgaris*) from Colombia (1982), Venezuela (1990) and Brazil (1995), see Osdaghi *et al*. (2020).

The first report outside the Americas was from Australia (Wood & Easdown, 1990), where mung bean (*Vigna radiata*) and cowpea (*Vigna unguiculata*) were the hosts affected.

In the EPPO region, incidental records of *C. flaccumfaciens* pv. *flaccumfaciens* on common bean and soybean have been made and in some of these cases, the disease was later reported to be eradicated. Until now, no economic losses have been reported in bean or soybean crops (Bastas & Sahin, 2017; CABI, 2020; González *et al*., 2005; Ishimaru *et al*., 2005; O'Leary & Gilbertson, 2020; Sammer & Reiher, 2012; Sonmezalp, 1966).

The first finding in the Middle East was in Iran in 2013 in common bean and cowpea (Osdaghi *et al*., 2015a and 2015b).

In Africa, the first confirmed case is from Zambia in soybean (Pawlowski and Hartman, 2019).



EPPO Region: Belgium, Bulgaria, Croatia, Greece (mainland), Hungary, Russian Federation (Central Russia, Far East, Southern Russia), Spain (mainland), Switzerland, Tunisia, Türkiye
Africa: Mauritius, Tunisia, Zambia
Asia: China (Guizhou), Iran, Islamic Republic of
North America: Canada (Alberta, Manitoba, Ontario, Québec, Saskatchewan), United States of America (Colorado, Connecticut, Idaho, Iowa, Michigan, Montana, Nebraska, North Dakota, Ohio, Oregon, Virginia, Wisconsin, Wyoming)
South America: Brazil (Distrito Federal, Goias, Mato Grosso do Sul, Minas Gerais, Parana, Santa Catarina, Sao Paulo), Chile, Colombia, Venezuela
Oceania: Australia (New South Wales, Queensland, South Australia, Victoria)

BIOLOGY

C. flaccumfaciens pv. *flaccumfaciens* is seedborne and can be transmitted both within and on the seed; it is very resistant to drying and has been found to remain viable for up to 24 years in seed stored under laboratory conditions (Burkholder, 1945). In soil, survival is much shorter and, in the case of bean crops rotated with wheat, survival does not exceed two winters. The bacterium is able to survive longer in plant debris and non-hosts, including non-leguminous crops and weeds. The practice of minimum tillage or no-tillage farming for soil moisture conservation therefore enhances survival of *C. flaccumfaciens* pv. *flaccumfaciens* and favours new infections in bean crops (Gonçalves *et al*., 2017 and 2018; Silva Júnior 2012; Urrea & Harveson, 2014).

The bacterium may enter roots and above-ground plant parts through wounds, usually under windy, hailstorm type of weather. Entry through stomata is rare, unlike other bacterial bean pathogens. There are no reports of vectors, but the nematode *Meloidogyne incognita* may assist entry by wounding roots (Schuster, 1959).

Inside the plant the bacterium spreads mainly via the xylem, where it is often present in biofilms.

Disease development and expression is stimulated by temperatures above 30°C. Latent infection and colonization of seeds and plants is possible, especially at lower temperatures. *C. flaccumfaciens* pv. *flaccumfaciens* can infect plants in the absence of rain; however, disease progression is associated with warm moist conditions and spread of the bacterium is favoured by overhead sprinkler irrigation. The more recent outbreaks of *C. flaccumfaciens* pv. *flaccumfaciens* in North America, observed after some 20 years of non-detection, may result from changed cultural practices (such as increased sprinkler irrigation), climate change to warmer and moister summers, spread from alternative hosts and lack of experience with the disease because of the long period in which it has not been recorded (Harveson *et al*., 2006; Harveson & Vidaver, 2008; Harveson, 2013; Osdaghi *et al*., 2016). Host species/cultivar susceptibility, weather conditions and agricultural practices are therefore important factors in disease outbreaks and their severity (Harveson *et al*., 2015).

There is no information on race variation. Yellow colony variants, however, are more virulent than the red/orange ones on a number of hosts including cowpea, lima bean, broad bean (*Vicia faba*) and pea and only these yellow colony variants cause disease on hairy vetch (*Vicia villosa*), a major weed in dry bean growing areas of Iran (Osdaghi *et al.* 2015b).

In a comparative genomics study of *C. flaccumfaciens* pv. *flaccumfaciens* with actinobacterial plant pathogens, a set of unique low G+C% content genomic islands were detected in the *C. flaccumfaciens* pv. *flaccumfaciens* genome. Homologous sequences of pathogenicity-determining loci found in these islands were those responsible for production of 1,4-beta-xylanase (*xylA*), pectate lyase (*pelA1* and *pelA2*), serine protease (*chpC*, *chpG*, and *pat-1*), and sortase (*srtA*) (Chen *et al*., 2020).

For additional information, see also Harveson *et al*. (2015), Hedges (1926), Osdaghi *et al*., (2020), Zaumeyer (1932), Zaumeyer & Thomas (1957).

DETECTION AND IDENTIFICATION

Symptoms

The most severe symptoms occur on young *Phaseolus* plants just emerged from seed. When infected, they usually die. If plants survive an early attack, or are infected at a later stage of growth, they may survive throughout the season and bear mature seed. All the developmental stages of the plant are susceptible. The disease is characterized by initial interveinal chlorosis that becomes later necrotic due to systemic vascular infection, leading to wilting of leaves or parts of leaves during the heat of the day and recovery as the temperature drops in the evening. Bacterial plugging of the vessels cuts off the water supply and the leaves turn brown and fall prematurely. Wilting may eventually cause plant death. During hailstorms or under strong winds necrotic tissues may easily rupture; symptomatic leaves will then have a ragged appearance and may drop down.

Occasionally these typical wilting symptoms may be absent, but instead there are golden-yellow necrotic leaf lesions, closely resembling those of common blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (EPPO/CABI, 1996). The lesion margin, however, is more irregular in *C. flaccumfaciens* pv. *flaccumfaciens* infections. In general, there is no water-soaking of stems and leaves, as found in common blight and halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) infections.

On pods the disease is much more conspicuous than common blight. All the seeds in a pod may be infected, while the pod remains apparently healthy. This is due to the pathogen infecting the seed via the vascular system, following the sutures of the pods, which may be discoloured. On young pods, water-soaked spots occasionally appear, the area turning to either a yellowish-green or darker colour than the rest of the pod. On ripe pods lesions are more conspicuous, being an olive-green colour in contrast to the yellow colour of the normal pod. It should be noted that

seemingly vigorous plants may bear one or more shrivelled shoots or infected pods which are hidden by healthy foliage.

When older plants are heavily infected, flowers may also be blighted and seed set severely reduced (Wood & Easdown, 1990).

Seeds of white-seeded bean cultivars, when infected systemically, may be discoloured and appear yellow, orange, pink, or purple; however, in cultivars with coloured seed coats the discolouration is less conspicuous. There may be a little bacterial slime present at the hilum, and seeds may be shrivelled.

On cowpea the necrotic areas on infected leaves tend to have chlorotic margins; this is less common in other hosts. On mung bean, as well as soybean, severe wilting of infected plants rarely occurs, and the intercostal necrosis has a pale brown to tan colour, leading to the name 'tan spot' for the disease in these two crops.

For additional information, see Hedges (1926), Zaumeyer (1932), Zaumeyer & Thomas (1957), Schuster *et al.* (1968), Harveson (2013), Harveson *et al*. (2015); Osdaghi *et al.* (2020).

Morphology

C. flaccumfaciens pv. *flaccumfaciens* is an aerobic, motile, Gram-positive, non-sporing rod, occurring singly or in pairs, 0.3-0.5 x 0.6-3.0 µm with one to three lateral or polar flagella.

Preliminary identification is hampered by the occurrence of at least five different colony colour variants on culture media: yellow, orange, pink, red and purple (yellow colony, but with purple diffusible pigment). *C. flaccumfaciens* pv. *flaccumfaciens* strains possessing different pigmentation produce bacteriocins against the other strains in culture media (Osdaghi *et al*., 2018a).

On non-selective media, such as yeast-peptone-glucose agar (YPGA) or nutrient broth yeast extract agar (NBY), after 48-72h of growth at 25-27°C colonies of *C. flaccumfaciens* pv. *flaccumfaciens* are circular, 2–4 mm in diameter, smooth and with entire margins, more often convex and translucent, but sometimes also flat and semi-opaque. As stated above, their pigmentation is variable, also depending on temperature and pH, and vary from creamy to bright yellow or red/orange. Therefore, in case of doubt, several colonies should be selected.

For further information, see EPPO (2011); Osdaghi *et al*., (2020).

Detection and identification methods

Inspection in the field

Visual inspection of plants with symptoms should be followed, when possible, by laboratory diagnosis.

Bacteria may be detected beneath the seedcoat by means of a combined cultural and immunofluorescence (IF) test (see below). Bean seed from countries where the disease is known to occur should be visually inspected for discolouration of the seed coat.

Laboratory detection and diagnosis

Direct isolation, IF and/or PCR can be used as screening tests. IF protocols have been described for seed tests, using polyclonal (Calzolari *et al*., 1987) or monoclonal antibodies (Diatloff *et al*., 1993). Specificity (false-negatives) and sensitivity, however, are less than PCR-based tests (McDonald & Wong, 2000).

Semi-selective media for in vitro growth of *C. flaccumfaciens* pv. *flaccumfaciens* were developed by Mizuno & Kawai, 1993; Tegli *et al*., 1998; Maringoni & Camara, 2006. The medium of Tegli *et al*. (1998), is detailed in EPPO (2011).

Two polymerase chain reaction (PCR) tests with different specific primer sets have been described for the identification of *C. flaccumfaciens* pv. *flaccumfaciens* isolated colonies and for its detection in bean seed extracts:

one according to Guimara?s *et al*. (2001) and the other according to Tegli *et al*. (2002). They are detailed in EPPO (2011). A method improving the isolation of bacteria and DNA from bean leaves, using the primers of Tegli *et al*. (2002) was developed by Puia *et al*. (2021b).

A sensitive Loop-Mediated Isothermal Amplification (LAMP) based detection method for *C. flaccumfaciens* pv. *flaccumfaciens* has recently been developed by Tegli *et al*. (2020), and could be, after further validation, useful for on-site testing of seeds and plants.

When positive PCR results on plant/seed samples are obtained, direct isolation of viable *C. flaccumfaciens* pv. *flaccumfaciens* cells on non-specific and semi-selective agar media should be performed.

BOX-PCR and multilocus sequence analysis (MLSA) with the housekeeping genes *atp*D, *gyr*B, ppk, *rec*A and *rpo*B can be used for identification; both methods were able to discriminate between yellow-pigmented strains and red/orange pigmented strains. Non-pathogenic strains of *C. flaccumfaciens* pv. *flaccumfaciens* could be discriminated from pathogenic strains on the basis of sensitivity to sodium arsenate and sodium arsenite (Osdaghi *et al*., 2018b). Rep PCR, including Box-PCR, is described in detail in EPPO (2010).

Details about presumptive diagnosis with rapid tests, detection and identification methods (including methods for extraction of bacterial cells and DNA), biochemical, serological and molecular and pathogenicity tests (using inoculation of bean plantlets or hilum injury/seed inoculation) for latent and symptomatic infected material, flow chart, culture media, chemicals and reference material) are provided in the EPPO Standard PM 7/102 (1), 2011 on *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*.

PATHWAYS FOR MOVEMENT

Spread of the bacterium over short and long distances is mainly through movement of infected seeds (Hedges, 1926; Zaumeyer, 1932; Zaumeyer & Thomas, 1957; Hsieh *et al*., 2006; Camara *et al*., 2009; Bastas & Sahin, 2017, Osdaghi *et al*., 2020). Other parts of infected plants or their residues, however, are also potential inoculum sources (Silva Júnior *et al*., 2012b; Gonçalves *et al*., 2017). Especially sprinkler irrigation and windy/hailstorm type of weather may enhance survival and dispersion of *C. flaccumfaciens* pv. *flaccumfaciens* within fields where infected plants and/or their residues are present. Dispersal via machinery/humans/animals/surface water has been little investigated and is poorly understood. Persistence and continuation of infection can be due to colonization (epiphytic and endophytic) of symptomless-host plants such as crops (e.g., barley, canola, ryegrass, oat) grown in rotation with fabaceous hosts, or weeds (e.g., *Amaranthus retroflexus, Chenopodium album*).

In conclusion, contaminated/infected seeds are the most likely pathway of spread to cause infection of host plants in other areas of the world. Symptomless weed and crop hosts play a local role. No insect vectors have been found.

PEST SIGNIFICANCE

Economic impact

Economic losses due to bacterial wilt disease result from lower crop yields as well as lower marketability as a result of quality loss due to discoloured and shrivelled seeds (Huang *et al*., 2009).

Following the first report of its occurrence in 1920, bacterial wilt became one of the most important bacterial diseases of beans in the USA, causing almost total losses in some years, but the disease gradually declined in later years. By the 1970s the disease had become manageable by planting pathogen-free/tested seeds, crop rotation, and sanitation measures. In the early 2000s, however, bacterial wilt re-emerged (first in North Dakota in 1995 and later in Colorado, Nebraska, and Wyoming from 2004 to 2007) and became an economically-damaging disease in those states, as well as in Canada where the yellow and orange variants were found in 2002, followed by the purple variant in 2006 (Harveson *et al*. 2015; Huang *et al*., 2009). In soybean, yield losses were reported to be very variable depending on the year; losses were often only minor, but could reach 18.5% in the USA (Dunleavy *et al*., 1983; Dunleavy, 1984).

Over the past ten years outbreaks with economically important yield losses have been reported from Australia, Brazil, Canada, Iran and the central high plains in the USA (Osdaghi *et al*, 2020; Puia *et al.*, 2021a). In most of these countries the disease is sporadic, and its incidence varies greatly. In *C. flaccumfaciens* pv. *flaccumfaciens* endemic areas in the USA and Australia (on mung bean), disease incidence has reached more than 90% in the past (Harveson *et al*., 2015; Wood & Easdown, 1990).

There is practically no information on yield losses or economic impact of the outbreaks or introductions recorded in Europe, including the Russian Federation.

Control

The most effective measure to prevent the entry, establishment and spread of *C. flaccumfaciens* pv. *flaccumfaciens* is the use of healthy seeds. Seed grown in dry climates is usually free from infection and therefore whenever possible it is advisable to grow seed crops in these dry areas.

Chemical control

Chemical (seed) treatments, including NaOCl 5% for 10 min, copper compounds and antibiotics have not been found effective against *C. flaccumfaciens* pv. *flaccumfaciens*. Antibiotics eliminated bacteria only from the seed surface and showed phytotoxicity (Estefani *et al*., 2007; Harveson, 2019; Tripepi & George, 1991). The use of antibiotics against plant pathogens is not permitted in many EPPO countries.

Soares *et al*. (2004) found that treatment with acibenzolar-S-methyl was ineffective in inducing resistance of the plant to the bacterium.

However, Harveson (2019) reported the results of a 7-year (2010-2016) field study investigating treatments with hydrogen peroxide and peroxyacetic acid and plant-based fatty acids. These consistently resulted in higher seed yields than copper/antibiotic treatments, although disease incidence was not reduced. This positive yield effect held true especially for heavily-infested crops.

Heat treatments

No suitable heat treatments have been developed for use in practice. Dry heat treatments (at 52°C for 20 h and 85°C for 5 h or at 60°C and even at 70ºC for more than 3 h) alone, or in combination with pre-soaking seeds in water for more than 3 h, significantly reduced the vigour of the seeds but usually did not eliminate the bacterium (Zaumeyer & Thomas, 1957; Estafena *et al*., 2007).

Biological control

No biological control method for *C. flaccumfaciens* pv. *flaccumfaciens* is operational in practice to date (Osdaghi *et al*., 2020). Under greenhouse conditions and artificial seed inoculation with *C. flaccumfaciens* pv. *flaccumfaciens*, a treatment of seeds with a strain of *Bacillus subtilis* (ALB629rif) gave a disease reduction of 71% and 75%, at 20 and 30°C respectively, (Martins *et al*., 2014). Soaking seeds of great northern bean cv. US1140 in a suspension of the bacterium *Pantoea agglomerans* gave good endophytic colonization of the entire bean seedling and in combination with artificial infection with *C. flaccumfaciens* pv. *flaccumfaciens*, gave reduction in disease severity of up to 70% and better emergence and seedling growth (Hsieh *et al*., 2005). Seed treatment under greenhouse conditions with *Rhizobium leguminosarum* biovar *viceae* R2 had a mild protective effect for seeds that showed no or light symptoms only (Huang *et al.*, 2007a).

Plant resistance

In addition to the use of healthy, tested seed, the other main control measure is the broader use of resistant cultivars. However, resistant, commercially attractive varieties are still scarce. For example, the common bean cv. Emerson is resistant, but has only a small (Europe-targeted) market. True resistance was found in a germplasm collection accession PI 325691, a wild common bean (*P. vulgaris*) from near Tzitzio, Michoacán, Mexico (Urrea & Harveson, 2014). In greenhouse tests, Huang *et al*. (2007b) determined resistance in some varieties and a line of common bean

against the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens*.

A high level of resistance to three variants of *C. flaccumfaciens* pv. *flaccumfaciens* (yellow, orange, and purple) was observed in the light red kidney bean cultivars AC Litekid, Chinook 2000, and Redkanner as well as dark red kidney bean cultivars Cabernet and Red Hawk in Canada and could be useful in breeding programmes (Conner *et al*., 2008).

Under greenhouse conditions in Brazil, Maringoni *et al.* (2015) observed resistance in a number of local, varieties and lines of common bean. A rapid method for screening of resistance against *C. flaccumfaciens* pv. *flaccumfaciens* has been described by Hsieh *et al*. (2003).

Cultural control

Common bean should preferably be grown in a rotation with non-hosts and not in succession (in the rotation scheme) with barley, black oat, oilseed rape, maize, ryegrass, sunflower, wheat and white oat when the disease is prevalent (Gonçalves *et al*., 2017 and 2021; Nascimento *et al*., 2020). Crop debris, weed hosts and volunteer plants should be carefully removed when the disease is already present.

Phytosanitary risk

From its existing distribution and biology, the disease seems most likely to be important in the southern part of the EPPO region where dry bean species and soybean are widely grown. A risk evaluation of *C. flaccumfaciens* pv. *flaccumfaciens* for the European Union has been made by the European Food Safety Authority which concluded that the pathogen had the potential to establish, spread and have an impact on its host crops (EFSA, 2018).

C. flaccumfaciens pv. *flaccumfaciens* is most likely to re-enter pest-free areas or spread further via infected host seeds. No records of interception of this bacterium were made in the European Union database between 2005 and 2018 (Europhyt, 2019). Past records of the disease in Spain and Germany could possibly be linked to the import of infected seeds, although the origin of these seeds was not reported (González *et al*., 2005; Sammer & Reiher, 2012).

Based on the various literature sources cited here, *C. flaccumfaciens* pv. *flaccumfaciens* can establish itself, climate-wise, in the EPPO region. Host plants are widely grown throughout the EPPO region. In 2019, dry pulses were grown on 2.17 million hectares in the European Union territory (about 2% of the total arable land), with a production of about 4.75 million tonnes. France, the United Kingdom, Poland and Spain were the largest producers in 2019 (Eurostat, 2021).

The production of soybean has been recently increasing in Europe. In 2018, 10 million tonnes of soybean were produced from the cultivation of 4.3 million ha. The acreage of soybean has doubled over the last seven years (Donausoja, 2021). The relatively steady growth in soybean production in the European Union halted in 2019 there was a decline in the area harvested (-5.0 %) and production (-3.4 %). Nevertheless, the 2.8 million tonnes of soybean produced in the European Union in 2019 was 1.9 million tonnes more than a decade earlier (Eurostat, 2021).

The disease caused by *C. flaccumfaciens* pv. *flaccumfaciens* has an erratic character, prevalent in some years, absent or very minor in others. Its present distribution in Europe may therefore may well be underestimated, as in many EU/EPPO region countries no systematic surveys for this bacterium are carried out. Declarations of eradication and absence should therefore be regarded with caution.

PHYTOSANITARY MEASURES

Phytosanitary (quarantine) measures can be implemented to reduce the risk of long-distance dissemination of the pathogen. It can be recommended that consignments of host seeds should have been produced from pest-free areas, or from pest-free places or sites of production.

Seed inspections of dry beans in intra- and international transport will assist in preventing the pathogen’s spread to areas with no history of the disease. However, visual inspection of imported seeds is not very reliable due to the occurrence of latent infections and therefore, when material is imported from areas where the disease is known to occur, field inspections and laboratory testing are necessary (EPPO 2011, 2021). For the European Union, measures

on certification and inspections of dry bean seeds and soybean to guarantee the health status of seeds before marketing in the EU are provided by Council Directive 2002/55/EC.

Management of bacterial blight can also be achieved by rapid, reliable detection and identification of *C. flaccumfaciens* pv. *flaccumfaciens* (EPPO 2011) and, the use of pathogen-free/tested seeds.

The EPPO diagnostic standard (EPPO 2011), whose wide and systematic application is another essential way to prevent entry and spread of the pathogen, should be used for diagnosis.

The presence and possible importance of *C. flaccumfaciens* pv. *flaccumfaciens* needs to be checked in countries or regions where it was previously reported. This would include awareness campaigns for stakeholders. Over the past forty years, disease outbreaks/introductions have been sporadic; many years may pass between outbreaks in an infected area without any noticeable symptoms being observed (Harveson, 2013; Harveson *et al*., 2015).

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