Tropical Plant Pathology (2022) 47:457–469
https://doi.org/10.1007/s40858-022-00504-1

REVIEW: DISEASE PROFILE

Common bacterial blight of beans: an integrated approach to disease management in Brazil

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Received: 17 September 2021 / Accepted: 24 February 2022 / Published online: 14 March 2022

Abstract
Common bacterial blight (CBB), caused by *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans*, is the main bacterial disease of common bean (*Phaseolus vulgaris*) in Brazil and in the world. The disease affects the entire aerial part of the plants, especially in conditions of high temperature and humidity. Yield losses of up to 70% have been recorded in susceptible bean cultivars. The bacteria that cause CBB are seed-transmitted, which facilitates spread over long distances. Furthermore, they survive for long periods in crop residues, and large amounts of secondary inoculum are produced in crop fields. Despite the control tactics used by farmers, CBB is still frequent in some regions. In this review, we provide an overview of the main management practices utilized for managing the disease. As so far there is no single effective control measure against the bacteria that cause CBB, the information presented here will help in the development of an integrated management program to reduce crop damage and yield losses in the bean crop.

Keywords *Phaseolus vulgaris* · Genetic control · Crop control · Chemical control · Seed health

Introduction

The common bean (*Phaseolus vulgaris* L.) is a crop of great importance for human consumption throughout the world, being a component in the diet of many developing countries. In Brazil, *P. vulgaris* cultivation is widespread throughout the country, and due to its wide edaphoclimatic adaptation, the crop can be grown throughout the year (Myers and Kmiecik 2017; CONAB 2021). In 2019, the world production of dry beans was 28.9 million tons (mt) in a cultivated area of 33 million hectares (FAOSTAT 2021). Brazil is the third largest producer with 2.9 mt, behind Myanmar (5.8 mt) and India (5.2 mt), and has an average productivity of 1.1 t/ha (FAOSTAT 2021). Diseases are among the main factors associated with low Brazilian productivity of common bean (Wendland et al. 2016; De Mio 2018). Common bacterial blight (CBB), caused by *Xanthomonas phaseoli* pv. *phaseoli* (Xpp) and by *X. citri* pv. *fuscans* (Xcf) (Bradbury 1986; Bull et al. 2010; Constantin et al. 2016) is one of the main bacterial diseases of the bean crop (Schwartz et al. 2005; Wendland et al. 2016). Due to the economic importance of CBB for the bean crop in Brazil, this review aims to update the main recommended practices for CBB management, highlighting the main aspects of genetic, crop, chemical, alternative, biological control, and bean seed health.

Etiology, history, and symptomatology

Initially, CBB was associated with *X. axonopodis* pv. *phaseoli* and its variant *X. axonopodis* pv. *phaseoli* var. *fuscans*, but genetic studies showed that these were two distinct species (Rademaker et al. 2005; Schaad et al. 2005), which led Constantin et al. (2016) to propose the reclassification of these pathogens as Xpp and Xcf. One of the first reports on the causal agent of CBB was published in 1897 in the USA (Rava and Sartorato 1994). In Brazil, CBB was first described by Caldeira Travassos, in the state of Pará, but Robbs (1954) was the one who isolated the pathogen from symptomatic bean material in the state of Rio de Janeiro.

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Later, Kimati and Mascarenhas (1967) found CBB in bean varieties in the São Paulo state.

Currently, CBB occurs in the main bean-producing areas of the world, except for dry tropical regions. The most recent outbreak was reported in Belgium in 2019 (Schwartz et al. 2005; Bultreys and Gheysen 2020; Chen et al. 2021). In Brazil, the disease is present in the states of Paraná, Minas Gerais, São Paulo, Santa Catarina, Espírito Santo, and Rio Grande do Sul, in addition to producing states in the Midwest region (Maringoni and Komori 1989; Wendland et al. 2016). So far, it is not known which bacterium (Xcf or Xpp) is prevalent in these states, but both are present in most producing regions (Almeida et al. 2015; Torres et al. 2005). In general, Xcf strains are more aggressive than Xpp (Mutlu et al. 2008). Xcf strains also have a greater negative impact on plant emergence, disease incidence, transmission rate from seed to seedlings, and seedling infection (He and Munkvold 2013).

During periods of high humidity and temperature, CBB can be highly destructive, causing losses in crop productivity and seed quality (Schwartz et al. 2005), with losses of up to 70% in productivity (Wendland et al. 2016). In Brazil, the highest CBB incidence occurs during the rainy season due to the high temperatures and frequency of rainfall (Wendland et al. 2016). As these are warm-weather pathogens, Xpp and Xcf cause greater damage at 28 °C (Patel and Walker 1963). In addition to favorable climatic conditions, a higher incidence of CBB occurs when contaminated bean seeds are used for sowing and when planting is carried out in areas with a history of disease occurrence (Schwartz et al. 2005).

Symptoms of CBB manifest throughout the aerial part of the bean plant, affecting leaves, stems, pods, and seeds. Both Xpp and Xcf infections cause similar symptoms. The initial lesions on the leaves are small, waterlogged areas, and as they develop the lesions coalesce and the tissues become dry and brittle, surrounded by yellowish, chlorotic halos. In older lesions, which take up much of the leaf blade, the necrotic center and yellow halo are more evident, characterizing the typical symptom of leaf blight (Rava and Sartorato 1994; Schwartz et al. 2005; Wendland et al. 2016). Lesions on the stems of young plants can be depressed and start in the form of watery patches, which gradually increase in size. Later, they take on the appearance of red streaks that extend along the stem, which may present cracks and bacterial exudate. Small watery spots appear in the pods, which progressively increase in size and become covered with yellowish encrustations due to the desiccation of the bacterial exudate. The affected tissue loses its watery appearance as it gets older, becoming dry, depressed, and reddish. Infection in the pods usually occurs in the vascular elements of the dorsal suture of the pod, penetrating the seed through the funiculus (Rava and Sartorato 1994; Wendland et al. 2016). In seeds, the infection can be asymptomatic, but when it occurs, there are malformed, wrinkled, and yellowish spots in those with light tegument, with reduced germination and vigor (Schwartz et al. 2005; Wendland et al. 2016).

Xpp and Xcf penetrate the aerial part of bean plants through stomata, hydathodes, and wounds. They colonize the intercellular spaces of host plant tissues and reach the xylem vessels (Torres and Maringoni 1997; Wendland et al. 2016). Bacteria survival occurs in crop residues in the soil, in seeds, and in alternative hosts (Schwartz et al. 2005; Torres et al. 2009a; Wendland et al. 2016). In addition, several crops and weed species are reported to host CBB causing bacteria, especially in the Fabaceae (Bradbury 1986; Rava and Sartorato 1994; Wendland et al. 2016).

Genetic control

Within the genus *Phaseolus*, there are species with resistance to CBB such as *P. acutifolius* (Schuster 1955; Coyne et al. 1973) and *P. coccineus* (Scharen 1959; Coyne et al. 1973), and many genotypes of these two species showed resistance to CBB (Yoshii et al. 1978; Mohan 1982; Zaiter et al. 1989; Rava et al. 1990). Interspecific crosses between *P. acutifolius* and *P. vulgaris* gave rise to the variety G. N. Nebraska #1 in the USA (Honma 1956) and many cultivars with foliar resistance to CBB were obtained (Coyne et al. 1963; Coyne and Schuster 1969, 1970; Mohan and Mohan 1983). These North American cultivars and genotypes gave rise to bean cultivars with different levels of resistance to CBB in Brazil (Rava et al. 1990; Maringoni et al. 1993; Maringoni 1998).

However, genotypes with foliar resistance do not always have pod resistance to CBB, since these reactions are independent (Valladares-Sanchez et al. 1983; Park and Dhanvantari 1987; Rava et al. 1990; Maringoni 1998). In some cases, it has been possible to combine these two reaction characteristics in the same genotype (Yoshii et al. 1978; Mohan and Mohan 1983; Maringoni et al. 1993; Maringoni 1998; Torres and Maringoni 1999). The inheritance of bean leaf and pod resistance to CBB is quantitative (Pompeu and Crowder 1972; Coyne et al. 1973; Valladares-Sanchez et al. 1983; Valladares-Sanchez et al. 1983). In leaves, resistance is conditioned by dominant genes (Pompeu and Crowder 1972; Coyne and Schuster 1974). Transgressive segregation has also been reported, as the level of foliar resistance increased between crosses of resistant strains, or between resistant and susceptible parents (Pompeu and Crowder 1972), resulting in segregants with higher levels of resistance than their parents (Mohan 1982; Mohan and Mohan 1983). Research has identified 22 quantitative trait loci (QTLs—*quantitative trait loci*), distributed in 11 linkage groups, associated with resistance to CBB (Kelly et al. 2003; Miklas et al. 2006; Liu et al. 2008), with some of these QTLs conferring resistance to

leaves or pods, but not both (Arnaud-Santana et al. 1994; Singh and Muñoz 1999).

The expression of bean resistance to CBB may be influenced by the origin of bacterial isolates and by environmental and crop factors. In general, isolates of Xpp and Xcf from tropical regions are more aggressive compared to isolates from temperate regions. Schuster and Coyne (1971) and Schuster et al. (1973) found that isolates from Colombia and Uganda were more aggressive to beans than an isolate from the United States. This fact was also observed among Xpp and Xcf isolates from Colombia (Rava 1984), Guatemala (Ekpo and Saettler 1976), Brazil (Rava 1984; Maringoni and Lauretti 1999), and the Dominican Republic (Schuster and Smith 1983). Bean cultivars considered resistant to CBB in some regions may be susceptible in others. Yoshii et al. (1978) found susceptibility in some genotypes under Colombian conditions, while the same genotypes were classified as resistant in the USA (Coyne et al. 1973). Recent transcriptome analyzes of resistant and susceptible cultivars showed that the bacteria manipulate the transcriptome of the susceptible host (Foucher et al. 2020). Downregulation of resistance genes and upregulation of the ethylene pathway and genes involved in cell wall modification are linked to the successful colonization of beans by Xpp. In resistant cultivars, in which the plant adapts its metabolism for defense purposes, there is upregulation of the salicylic acid pathway and downregulation of photosynthesis and sugar metabolism (Foucher et al. 2020). Simons et al. (2021) evaluated 852 common bean genotypes from the North Dakota State University breeding program, identifying genotypes with high levels of resistance and eight regions associated with resistance. These candidate genes for resistance to CBB are an important tool in the development of new bean cultivars.

Studies of bean resistance against CBB have focused more on Xpp than Xcf (Mutlu et al. 2008; Paiva et al. 2018; Monteiro et al. 2020). Some resistance components, however, are more effective against only one of these pathogens and, since both can occur in the same regions, it is interesting to obtain genotypes with resistance to both bacteria (Mutlu et al. 2008; Paiva et al. 2018). In Brazil, many cultivars susceptible to Xpp, were considered resistant to Xcf (Silva et al. 2009; Rezende et al. 2011; Azevedo et al. 2015; Assis et al. 2018; Zagonel 2018; Fedrigo 2019; Monteiro et al. 2021). BRS Radiante and IAPAR 16 showed resistance to Xcf and to six Xpp isolates from different geographic regions, demonstrating broad-spectrum resistance (Monteiro et al 2020).

Currently, several Brazilian bean cultivars are available on the market, from the carioca, special and black groups, with different levels of resistance to CBB (Table 1) (Embrapa 2017; IAC 2020; IDR-Paraná 2020). New progenies from Embrapa's recurrent selection program for resistance to CBB are also being selected. The achievement of resistant progenies, not only to this disease but also to other diseases of economic importance for the bean crop, allied to favorable agronomic characteristics, such as greater seed production, stands out (Melo et al. 2019).

Table 1 Common bean cultivars from groups "carioca," "special," and "black" with resistance levels to common bacterial blight available in Brazil

Group	Cultivar	Reaction to common bacterial blight
Carioca	BRS 10,408 Notável	Resistent
	BRS Ametista	Moderately resistant
	BRS Pontal	
	IPR Campos Gerais	
	IPR Sabiá	
	IPR Tangará	
	IPR Curió	
	IAC Milênio	
	IAC Imperador	
	IAC Alvorada	
	IAC 1850	
	IAC Sintonia	
	BRS FC402	Intermediate
Special	Jalo Precoce (jalo)	Moderately resistant
	IAC Boreal (rajado)	
	IAC Tigre (rajado)	
	BRS Realce (rajado)	Intermediate
Black	BRS Esplendor	Resistant
	IPR Gralha	Moderately resistant
	IPR Urutau	

Source: EMBRAPA 2017; IAC 2020; IDR-PARANÁ 2020

Cropping practices

Crop control includes practices that act on the three vertices of the disease triangle, with the objective of creating unfavorable conditions for the pathogen. The recommended practices for CBB management are adequate fertilization and irrigation, crop rotation, eradication of alternative hosts, and elimination of crop residues. These practices usually interfere in the survival, production, and dissemination of the bacterial inoculum and, consequently, contribute to the reduction of the disease incidence (Saettler 1989; Belete and Bastas 2017; Bedendo et al. 2018).

The susceptibility of plants to bacterial attack may be related to their nutritional status (Ogle and Dale 1997). The deficiency or excess of nutrients favors diseases by compromising the normal development of the plant and decreasing its resistance to pathogen attack (Bedendo et al. 2018). Excess nitrogen, for example, leaves plant tissues tender, prolongs the vegetative period, and causes delay

in maturation. On the other hand, the lack of this nutrient leaves the plant with slow growth and rapid aging, contributing to the host's predisposition to diseases (Agrios 2005). In a study carried out in Brazil, the effect of fertilization with nitrogen and calcium on the susceptibility of common beans to CBB was evaluated. The results showed that the increase in nitrogen doses reduced disease symptoms, while the calcium fertilization had no effect on CBB severity (Biazon et al. 2004). The use of manganese (Mn) and zinc (Zn) phosphites also helps in the management of CBB when applied preventively. In addition to significantly reducing the progress of the disease, inducing plant defense responses, phosphites attenuate the photochemical dysfunctions caused by bacterial infection (Costa et al. 2020). In any case, for disease management, it is necessary that nutrients are supplied in adequate amounts, according to the fertilization and liming recommendations contained in the soil analysis (Agrios 2005; Bedendo et al. 2018).

Irrigation is important to avoid water deficit and obtain better yields in the bean crop; however, attention must be paid to the irrigation system used (Silveira et al. 2015). A study conducted in Iran compared the effects of furrow and sprinkler irrigation on epiphytic Xpp populations in beans and on the severity of CBB. Sprinkler irrigation significantly increased the bacterial population as well as disease severity (Akhavan et al. 2009). Based on studies with other species of phytopathogenic bacteria, the authors argued that the bean leaf surface provided an ideal environment for bacterial multiplication due to the presence of a thin layer of water because of sprinkler irrigation. Furthermore, the increase in severity may also have occurred due to the spread of the bacteria from infected to uninfected leaves provided by this type of irrigation. The furrow method, on the other hand, did not favor the increase in the Xpp population or CBB severity (Akhavan et al. 2009) and can be indicated for bean cultivation in areas with low rainfall (Akhavan et al. 2013).

Alternative hosts are crops and weed that play an important role in the disease occurrence, as they ensure the survival and multiplication of the pathogen during the absence of the main host (Bedendo et al. 2018). In addition to *P. vulgaris*, natural infections have been reported for several other crops belonging to the genus Fabaceae (*Phasolus* and *Vigna* species), as well as in weeds (Table 2). Some hosts were also described after artificial inoculation (Table 2).

In Brazil, experiments were carried out under field conditions to evaluate the survival of Xpp in the phyllosphere and rhizosphere of 21 weed species. In the phyllosphere, Xpp survived for up to 14 days in *Cyperus rotundus* (purple nutsedge), *E. heterophylla* (wild poinsettia), *Commelina benghalensis* (bengal dayflower), *Senna obtusifolia* (sicklepod), *Alternanthera tenella* (joyweed), and *Bidens pilosa* (hairy beggartick), and for shorter periods in the other species (Tadeu A. F. da Silva Júnior, unpublished data). In the rhizosphere, Xpp survived for 14 days in *Lepidium virginicum* (Virginia pepperweed) and 7 days in *Gnaphalium spicatum* (gray everlasting) (Tadeu A. F. da Silva Júnior, unpublished data). Despite the low survival of Xpp in weeds, eradication is recommended in areas with bean cultivation, especially of species already described as hosts, to interrupt the pathogen's cycle in the field (Akhavan et al. 2013; Bedendo et al. 2018).

Bacteria pathogenic to the aerial part of the plants are poorly adapted to survival in the soil, but when associated with crop debris they can survive for long periods (Karavina et al. 2011). In the United States, Xpp survived for up to seven months in bean crop debris on the soil surface in the no-tillage system, but when incorporated into the soil at a depth of 20–40 cm, the survival decreased to four months (Gilbertson et al. 1990). In the Dominican Republic, Xpp survived for 150 days in bean leaf debris on the soil surface, and less than 30 days when the debris were buried at a depth of 15 cm (Arnaud-Santana et al. 1991). This reduction in the survival period of the bacteria in crop debris buried in the soil can be explained by their exposure to the microbial community, which rapidly decomposes the plant material, and exposes bacterial populations to unfavorable conditions (Belete and Bastas 2017). A study conducted in Brazil showed that under mild temperatures and little rain, Xcf survived for up to 180 days in bean leaflets kept on the soil surface, and for up to 120 days in those incorporated at a depth of 15 cm. On the other hand, under greater volumes of rainfall and higher temperatures, Xcf survived for 60 days on the soil surface, and 45 days in leaflets buried at a depth of 15 cm (Torres et al. 2009a). The management of infected crop debris is an effective strategy and must be carried out to reduce the inoculum in the field, either by deep plowing or removal of debris left on the ground (Belete and Bastas 2017).

Crop rotation is the practice of planting different crops on the same plot of land across a sequence of growing season. This practice is recommended in disease management, as it promotes the elimination of the substrate that favors the pathogen (Karavina et al. 2011; Bedendo et al. 2018). CBB epidemics can be reduced by crop rotation, but attention must be paid to the crops used (Belete and Bastas 2017). In Colorado, USA, epiphytic Xpp populations were recovered from asymptomatic onion plants in commercial fields after growing beans, but not from onions after growing corn, sugar beet, or wheat. The onion-bean rotation scheme should be avoided to reduce the survival of Xpp in the crop fields (Gent et al. 2005). In Brazil, experiments were carried out under field conditions to evaluate the survival of Xpp in the phyllosphere and rhizosphere of 14 crops. In the phyllosphere, Xpp survived for 70 days on beans, 49 days on black oat, 35 days on pearl millet, and 21 days on velvet bean. In the rhizosphere, Xpp survived for 42 days on pigeon pea,

Table 2 Alternative hosts (crops and weeds) described for *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans* by natural infections and after artificial inoculation

Family	Species	English name	Portuguese name
Natural infection			
Acanthaceae	*Ruellia tuberosa*	Meadow weed	Ruélia
Amaranthaceae	*Acanthospermum hispidum*	Hispid starbur	Carrapicho-de-carneiro
	Amaranthus spp.	Amaranth	Amaranto
Euphorbiaceae	*Acalypha alopecuroidea*	Foxtail copperleaf	
	Euphorbia heterophylla	Mexican fireplant	Leiteiro
Fabaceae	*Aeschynomene americana*	Shyleaf	Angiquinho
	Calopogonium sp.	Calopo	Calopogônio
	Lablab purpureus	Hyacinth bean	Lab-lab
	Macroptilium lathyroides	Wildbush bean	Feijão-do-campo
	Phaseolus acutifolius	Tepary bean	
	Phaseolus coccineus	Scarlet runner bean	Feijão-da-espanha
	Phaseolus lunatus	Sieva bean	Feijão-de-lima
	Pisum sativum	Pea	Ervilha
	Pueraria sp.	Kudzu	Kudzu
	Rhynchosia minima	Least snout-bean	Favinha-brava
	Senna hirsuta	Hairy senna	
	Strophostyles helvola	Trailling fuzzy bean	
	Vicia sativa	Garden vetch	Ervilhaca
	Vicia villosa	Winter vetch	Ervilhaca-peluda
	Vigna aconitifolia	Moth bean	Feijão-de-orvalho
	Vigna angularis	Adzuki bean	Feijão-azuqui
	Vigna mungo	Black gram	Feijão-preto
	Vigna radiata	Mung bean	Feijão-mungo
	Vigna umbellata	Rice bean	Feijão-arroz
	Vigna unguiculata	Cowpea	Feijão-caupi
Malvaceae	*Malachra alceifolia*	Yellow leafbract	
Poaceae	*Digitaria sclalarum*	Fingergrass	
	Echinochloa colona	Watergrass	Capim-arroz
	Leptochloa filiformis	Red sprangletop	Capim-mimoso
Portulaceae	*Portulaca oleracea*	Common purslane	Beldroega
Solanaceae	*Physalis* sp.	Physalis	Fisális
Artificial inoculation			
Amaranthaceae	*Amaranthus retroflexus*	Red-root amaranth	Caruru gigante
	Beta vulgaris	Beet	Beterraba
Asteraceae	*Ambrosia artemisiifolia*	Annual ragweed	Cravo-da-roça
Chenopodiaceae	*Chenopodium album*	Lambsquarters	Fedegoso
Cyperaceae	*Cyperus rotundus*	Nutgrass	Tiririca
Fabaceae	*Glycine max*	Soybean	Soja
	Lupinus polyphyllus	Bigleaf lupine	Lupinus
	Mucuna deeringiana	Velvet bean	Mucuna-anã
Poaceae	*Cenchrus echinatus*	Southern sandbur	Capim-carrapicho
	Echinochloa crus-galii	Cockspur	Meã
Solanaceae	*Solanum nigrum*	Black nightshade	Erva-moura

35 days on common bean, and 21 days on black oat and forage radish. These crops were considered potential hosts of the bacterium and should be avoided in succession or rotation with beans (Tadeu A. F. da Silva Júnior, unpublished data).

In addition to these practices, it is also recommended for the management of CBB to consider the bean cultivation site, keeping it at a minimum distance of 30 m from other crops; use healthy seeds free of Xpp and Xcf, which can be purchased from lots that were inspected during production,

and tested for the presence of these bacteria; eliminate voluntary bean plants from crop fields; and avoid the movement of machines and people in the crop when the plants are wet, in order to avoid the spread of the bacteria from diseased to healthy plants (Rava and Sartorato 1994; Akhavan et al. 2013; Bedendo et al. 2018).

Chemical control

Chemical control is one of the tools in integrated disease management (Belete and Bastas 2017). For the treatment of bean seeds to control CBB, the use of antibiotics streptomycin, tetracycline, and chlorotetracycline conveyed to polyethyleneglycol and glycerol is well known (Liang et al. 1992). According to Saettler & Anderson (1978), the use of streptomycin sulfate in bean seed treatment was effective in eradicating Xpp and Xcf from the surface of the seeds. However, Liang et al. (1992) found that the mixture of streptomycin sulfate + polyethylene glycol did not completely eradicate populations of Xpp and Xcf that were infecting bean seeds. Sprays of the aerial part of bean plants with copper sulfate or copper hydroxide are indicated in the CBB management (Belete and Bastas 2017). However, the effectiveness in controlling the disease is variable and depends on the environmental conditions, the level of resistance of the cultivar, and the frequency of spraying of the products (Belete and Bastas 2017). However, studies conducted in Brazil evaluating the effect of different groups of chemical compounds showed low efficacy in controlling CBB (Maringoni 1990).

The use of acibenzolar-S-methyl (ASM) resistance inducer has reduced CBB symptoms in common bean leaves under field conditions (Navarini et al. 2009) and in snap bean leaves in a greenhouse (Vigo et al. 2012). Furthermore, recent studies have shown that progeny from a mother plant treated with ASM was able to suppress the disease by up to 11% (Akkopru 2020). When these progenies were treated with a low dose of ASM (20 µM), the suppression capacity was raised to 60%. These data show that seed production using this method can increase the resistance of later progenies. The fungicide pyraclostrobin also showed potential to control CBB in snap bean leaves in a greenhouse (Vigo et al. 2012). Currently, in Brazil, three active ingredients are registered for spraying on bean crops to control CBB: ASM, copper hydroxide, and cuprous oxide (Agrofit 2021).

Biological and alternative control

Despite the potential of biological control for the management of CBB in beans, there are no commercial products registered in Brazil (Agrofit 2021). However, biocontrol agents compatible with *Rhizobium leguminosarum* bv. *phaseoli*, isolated from soil, bean leaves, and seeds, showed potential to control CBB after seed treatment, and some of these isolates controlled the disease up to 100% (Zanatta et al. 2007). In Iran, treatment of bean seeds resistant and susceptible to CBB with an isolate of *R. leguminosarum* bv. *phaseoli* resulted in a reduction in the severity of CBB, and an increase in plant growth (Osdaghi et al. 2011). In Egypt, in vitro experiments demonstrated the potential for biocontrol of the saprophytic bacterium *Rahnella aquatilis* to Xpp. In greenhouse and field conditions, bean plants sprayed with a suspension of *R. aquatilis* and inoculated with Xpp presented a reduction in the severity of CBB, which was attributed to the reduction in the multiplication of the bacterium. Furthermore, bean plants treated with *R. aquatilis* exhibited higher concentrations of phenolic compounds and higher activity of the peroxidase enzyme (Sallam 2011).

In Brazil, bean seeds were treated with suspensions of *Bacillus cereus*, *Pseudomonas veronii*, *P. fluorescens*, and *Rhodococcus fascians* and the plants obtained were inoculated with Xpp. Most of the biocontrol agents evaluated reduced the severity of CBB in bean plants, highlighting the association between *B. cereus* and *P. fluorescens*, showing that the combination of more than one agent increased the efficiency of biocontrol (Corrêa et al. 2017). Isolates of *B. cereus* and *P. fluorescens* also showed potential to induce resistance in plants due to the accumulation of phytoalexin phaseolin (Sangiogo et al. 2018). In addition, it has been demonstrated that copper hydroxide was compatible with a biocontrol agent; plants sprayed with *B. subtilis* and copper hydroxide showed a reduction in the CBB infection rate (Belete et al. 2021).

With regard to biological control, there are still no commercial products registered for alternative control of CBB in Brazil. However, some studies with plant extracts, tinctures, and essential oils showed their potential for disease management. Greenhouse experiments compared the efficiency of the BioZell-2000 B product, consisting of 50% *Thymbra spicata* etheric oil, 20% corn oil, 20% fennel oil, and 10% sesame oil, with ASM in the CBB control (Abo-Elyousr 2006). Four days after inoculation of the bean plants with Xpp, BioZell-2000B reduced the multiplication of Xpp cells inside the plant tissue by 45%, while in treatments with ASM, the reduction reached 50%. As for disease severity, bean plants treated with ASM showed a 68% reduction in CBB severity, and a 50% reduction in treatments with BioZell-2000 B, compared to the control treatment. In in vitro experiments, none of the products evaluated had an inhibitory effect on Xpp. In addition, components of essential oils, such as ketones, aldehydes, ethers, phenols, and alcohols, inhibited the growth of Xcf in culture medium. Furthermore, the treatment of bean seeds, with high populations of Xcf on their surface, with different concentrations of eugenol (clove oil), significantly reduced the bacterial

population compared to control treatments (Cantore et al. 2009). Extracts of *Azadirachta indica* (neem), *Acalypha wilkesiana* (copperleaf), and *Carica papaya* (pawpaw) reduced the incidence and severity of CBB and increased cowpea production (Ganiyu et al. 2017). Lastly, aqueous and methanolic extracts of *Mentha piperita* (peppermint), *Syzygium cumini* (jambolan), and *Datura metel* (horn of plenty) inhibited the growth of Xpp in culture medium. The aqueous and methanolic extracts of *Spinacia oleracea* (spinach) and *Catharanthus roseus* (vinca) had no inhibitory effect on Xpp growth (Kulshrestha et al. 2015). Aqueous extracts of *Althea officinalis*, *Origanum vulgare* (oregano), *Satureja hortensis*, *Solanum dulcamara*, and *Quercus robur* also showed an inhibitory effect on the growth of Xpp in culture medium (Babu et al. 2007).

In Brazil, experiments evaluated the action of tinctures and essential oils from medicinal plants on CBB and the inducing resistance. The tinctures of *Lippia alba* (bushy matgrass) and *L. sidoides* (pepper-rosmarin), in concentrations above 50%, and the essential oils of *Rosmarinus officinalis* (rosemary) and *Cinnamomum zeylanicum* (cinnamon), in concentrations between 1 and 5%, inhibited the growth of Xpp in culture medium. In the greenhouse, all the plant tinctures when sprayed on bean plants, before inoculation with Xpp, reduced the severity of CBB. Essential oils did not show this action in controlling the disease. The tincture of *L. alba* also provided increased concentrations of polyphenoloxidase and peroxidase in bean plants, showing a possible induction of resistance (Vigo et al. 2009). In addition, mycelial extracts of the fungus *Pycnoporus sanguineus* stimulated the growth of Xpp in culture medium; however, the filtrate from the 20% fungus extract reduced bacterial growth by 21%, when compared to that in culture medium in the absence of treatments. In the greenhouse, bean plants treated with *P. sanguineus* culture filtrate at 5 and 10% presented a reduction in CBB severity of up to 33% for the first treated leaf, and up to 90% for the second treated. The peroxidase and polyphenoloxidase enzymes also had their activities increased in plants treated with the fungus extracts, which may explain the reduction in the severity of CBB (Toillier et al. 2010). These studies show that several genera of biocontrol agents, especially bacteria, have potential for the development of commercial products to be used in the biological and alternative control of CBB.

Seed health

Bean seeds infected with Xpp are primary sources of inoculum, being responsible for introducing the disease into a new field (Gilbertson and Maxwell 1992). In white seed varieties, yellow or brown spots may appear on the integument, especially near the hilum. In dark seed varieties, this discoloration is not visible. Infected seeds can also be asymptomatic (Yoshii 1980). Plants grown from these seeds often show damage to the cotyledons or primary leaves. These lesions increase and, under favorable conditions of humidity and temperature, viscous masses of bacteria accumulate on the leaf surface. They can then be spread to uninfected plants leading to secondary infection (Vidaver 2012). The most effective survival mechanism for Xpp is to colonize bean seeds (Cafati and Saettler 1980; Gilbertson et al. 1990; Arnaud-Santana et al. 1991), in which bacterial cells can survive for up to 36 years (Allen et al. 1998). Marques et al. (2005) demonstrated that Xpp survival was reduced during the first six months of seeds storage. However, seeds stored at − 18 and 5 °C maintained the contamination rate, showing that the optimal temperatures for seed storage are like favorable conditions for the longevity of Xpp.

The presence of Xpp and Xcf in bean seeds can be internal (infection) or external (infestation) (Sheppard et al. 1989; Allen et al. 1998), which has implications for seed certification programs. According to Weller & Saettler (1980), approximately 1000 to 10,000 bacterial cells per seed are sufficient to give rise to infected plants in the field. However, under Brazilian conditions, it was found that the development of the CBB outbreaks depended on the level of cultivar resistance and climatic conditions, and not on the population of Xpp and Xcf present in the bean seeds used for planting (Maringoni et al. 1995). Bean seeds from asymptomatic plants play an important role in the epidemiology of CBB, once the bacterium is difficult to detect (Mabagala, 1997) and can occur at low frequency in commercial lots (Maringoni et al. 1993). In Brazil, surveys of commercial bean seed lots showed that of 34 lots analyzed in the Paraná state, 50% were infected with Xpp and Xcf, with incidences ranging from 0.1 to 1.7% (Torres et al. 2009b).

Experiments have demonstrated the success of hot water (52 °C for 20 min) or dry heat treatments (60 °C for 23 to 32 h) in eradicating Xpp and Xcf from seeds (Grondeau et al. 1994). Treatments with streptomycin controlled the bacterial infestation, and the treatment with streptomycin + polyethylene glycol, reduced, but not eliminated internal populations of Xpp from the seeds (Liang et al. 1992). It was also shown that tetracycline and chlorotetracycline in solutions containing polyethylene glycol reduced Xpp populations but were phytotoxic to seeds (Liang et al. 1992).

The control of Xpp and Xcf by antibiotics in Brazil is not regulated. The development of chemical resistance (Romeiro et al. 1998), the costs involved, and the effectiveness limit the use of chemical control, which may be feasible in certain circumstances, such as seed production, or as a component of an integrated management strategy (Allen et al. 1998). In addition, antibiotics also inhibit the growth of soil microorganisms, which act as biological control or perform vital processes maintaining the soil health and quality, influencing

the microbial community and altering the ecological functionality of the soil (Varma and Buscot 2005; Cycoń et al. 2019).

Considering this, some studies have evaluated the effectiveness of biological control, such as the application of *R. leguminosarum* bv. *phaseoli* for bean seeds, which reduced CBB severity and improved plant growth, both in the field and greenhouse (Osdaghi et al. 2011). The use of bacteria-free seeds, combined with a strict seed certification program, is important to reduce the amount of initial inoculum. In this sense, it is recommended that the bean fields for seed production should be cultivated in isolated areas from the production fields. There is zero tolerance for infected seeds in samples of 4000–45,000 in USA corporate certification programs to ensure that Xpp is not transmitted by commercial seed lots (Maddox 1998).

The efficiency of the bacterial detection method in bean seeds depends mainly on the sensitivity and location of the pathogen in the seeds (Darrasse et al. 2018). Several techniques were developed and adapted to detect the presence of Xpp and Xcf in bean seeds. The methodologies for the detection of Xpp and Xcf can be found in the International Rules for Seed Testing (IRST) from the International Seed Testing Association (ISTA) (Grimault et al. 2021). In general, the methods developed, such as semi-selective media, serology, molecular tests, and cytometry, have different levels of sensitivity for the detection and identification of Xpp and Xcf (Van Vuurde et al. 1983; Valarini and Menten 1992; Remeeus and Sheppard 2006; Sheppard et al. 2007; Tebaldi et al. 2007; Torres et al. 2009b; Popović et al. 2010; Tebaldi et al. 2010). However, for routine seed diagnosis and testing, two semi-selective culture media are recommended (MT and XCP1). The MT medium is less selective, but more sensitive, and can be used to detect several bacteria that infect seeds (Remeeus and Sheppard 2006). In this sense, XCP1 is more efficient in quantifying and detecting Xpp in seed extracts (Tebaldi et al. 2007).

Serological methods have been successfully used in the detection of Xpp (Van Vuurde et al. 1983). Polymerase chain reaction(PCR)-based methods are also available, commonly used primers (X4c and X4e) allow the detection of both Xpp and Xcf (Audy et al 1994). Xpp can be detected by PCR after DNA extraction from intact or crushed seeds. Studies conducted by He and Munkvold (2012), to evaluate the influence of different extraction methods on Xpp sensitivity, indicated that vacuum extraction and centrifugation of seed extracts increased the sensitivity of Xpp detection (He 2010). The specificity of the assay was tested and confirmed against DNA from various *Xanthomonas* species and *X. axonopodis* pathovars (He 2010). Primers Am1F/R and Am2F/R can be used together with X4c and X4e in multiplex-PCR reactions to favor the distinction between pathogenic and non-pathogenic isolates, reducing the risk of false positives (Boureau et al. 2013). Among the DNA-based techniques, BIO-PCR, which consists of the selection of bacterial colonies obtained in a selective culture medium and subsequent PCR reaction, has provided reliable results (Silva et al. 2013). Recently, the Loop-mediated isothermal amplification (LAMP) technique was developed for CBB. Comparing LAMP with PCR, LAMP was more sensitive (limit detection of 10 CFU.mL^{-1} and 1 fg of DNA against 10^5 CFU.mL^{-1} and 10 ng of DNA for LAMP and PCR, respectively), fast (detection in 4 h) and Xpp and Xcf had a seed detection rate of 100% (de Paiva et al. 2020).

Another method for the detection of plant pathogenic bacteria in seeds is flow cytometry. This technique is a fast, accurate, and reliable method for detecting and evaluating the viability of microorganisms. A fluorescent probe capable of exploring different properties of the target cell is used, such as enzymatic activity, cytoplasmic membrane permeability, cytoplasmic membrane potential, respiratory activity, relative DNA content, and pH gradient (Barrocas et al. 2009). Tebaldi et al. (2010) evaluated flow cytometry to distinguish live and dead Xpp cells, also allowing its use after seed treatments. The method was able to distinguish viable cells from dead cells in pure cultures, but not in seed extract, due to a high number of contaminants. For the detection of Xpp and Xcf in seeds, it is up to each laboratory, based on its structure and available conditions, to choose the best techniques to be used. In most cases, to obtain a higher level of reliability, it is necessary to apply more than one technique to reach a correct diagnosis.

Concluding remarks

CBB is one of the main diseases for common beans in Brazil and other countries. Correct diagnosis of the disease in the crop fields is the first step to make CBB management more efficient, thus reducing the impact of disease incidence and severity. The successful management of CBB must be based on the integration of available management techniques, mainly the planting of Xpp and Xcf free seeds and cultivars with levels of resistance. Crop rotation is a recommended practice for managing CBB, and with recent studies on the host range of Xpp in Brazil, it was possible to identify crops such as black oat, millet, pigeon pea, and millet as potential hosts of these bacteria, and their planting in succession or rotation to common beans is not recommended. However, further studies of the host range of Xpp and Xcf should be carried out to identify new potential hosts for these bacteria.

Despite the registration of active ingredients for the chemical control of CBB in Brazil, mainly copper-based products, their effectiveness has shown to be variable, being influenced mainly by the environment, level of resistance of cultivars, and spraying frequencies. Therefore, biological

control is shown as a new option for the management of the disease. The research carried out has shown its importance in the control of CBB and the registration of new biological products will allow the improvement of the management of this bacterial disease in Brazil.

Author contribution ACM and TAFSJ coordinated and wrote the review; DMN, JCS, JMS, and RMG wrote, commented on, and improved the review.

Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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