

An Overview of Distribution, Biology and the Management of Common Bean Anthracnose

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Abstract

Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) is one of the most important seed borne disease of common bean (*Phaseolus vulgaris* L.) in the world. The disease is prevalent in areas that experience cool and wet weather conditions, causing up to 100% yield loss. Besides infecting *Phaseolus vulgaris*, *Colletotrichum lindemuthianum* also attacks other legumes like mung bean (*P. aureus*), cowpea (*Vigna sinensis*), and broad bean (*Vicia faba*). The disease causes symptoms to appear on leaves, stems, pods and seeds. The pathogen can survive in seeds for up to five years, and is also known to overwinter in crop debris. Seed infection is the primary means by which the pathogen spreads. Therefore, the production and the use of certified seeds is one control measure that is effective in dealing with the disease. Fungicidal seed treatment and foliar application as well as cultural and biological methods are very important for bean anthracnose management. Further information on biology and survival of *C. lindemuthianum* is needed to devise more effective management strategies. In this review attention were given to the biology and management options, with an emphasis on the future research priorities.

Introduction

Common bean (*Phaseolus vulgaris* L.) is grown and consumed principally in developing countries in Latin America, Africa, and Asia. It is the most important legume worldwide for direct human consumption. The crop is consumed principally for its dry (mature) beans, shell beans (seeds at physiological maturity), and green pods. It is a major source of dietary protein that complements carbohydrate-rich sources such as rice, maize, and cassava. It is also a rich source of dietary fibers, minerals and certain vitamins [1]. Anthracnose, *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. And Cavi., is the most serious disease attacking bean in cool weathers in Latin America and Africa. Field losses in these regions, due to seedling, leaf, stem and pod infections, are up to 90% under climatic condition favourable to the disease. When *C. lindemuthianum* attack bean leaves, it causes dark brown necrotic lesions and decrease leaf photosynthesis activity [2]. Yield loss is due to early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed that has lesions on its coat [3]. Such beans have a repulsive appearance and are not popular with consumers. This lowers the marketability and thus the income arising from their sale. Management strategies used to minimize seed borne infection in the seed production field include host resistance, cultural and chemical control methods. Harvesting drying and post harvest processing and storage operations can also be used to reduce the spread and to eradicate seed borne pathogens [4].

In addition to the released varieties, a number of common bean breeding lines and landraces available are also susceptible to anthracnose or their reaction to the fungus is unknown, thereby limiting their deployment in programmes for improvement of anthracnose resistance. The fungus is known to have races that vary from, country, region, location, and variety, to another [5]. Despite extensive pathological and molecular studies, the nature and extent of pathogen variability and its biology in *C. lindemuthianum* have not been clearly established. *C. lindemuthianum* pathogen is of particular concern because, unlike others a fungus, a strategy for the management of bean anthracnose disease is inadequate, especially given the limited and antiquated chemical options available. As well, effective bean anthracnose disease management depends on a clear understanding of the biology and survival. Therefore, this review was initiated with

the following objective. To review the distribution, biology and management of common bean anthracnose.

Distribution of Common Bean Anthracnose

Anthracnose was first described from plant specimens obtained in Germany in 1875 [6]. Since then, the disease has become one of the most important and widely distributed throughout the world. It has been reported in USA [7], European countries [8], Canada [9], Latin America [10,11]. In Africa, it is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and D.R. Congo [12]. Although plant residues contribute to pathogen survival and dissemination [13], infected seed plays an important role in the international distribution of pathogen. This is especially true for African countries where farmers continuously use infected seed contributing the distribution of the pathogen.

In Brazil more than 25 different *C. lindemuthianum* races have been identified [14]. In Tanzania yield losses remain very high (40-80%) and are estimated to be worth \$304 million per annum. In Uganda, anthracnose is the most important disease in the high altitude, low temperature areas [15]. In Sudan, field losses in these regions, due to seedling, leaf, stem and pod infections, are up to 90% under climatic condition favourable to the disease. The infected seeds are the most important means of dissemination of this pathogen, which explains its worldwide distribution [16].

Although still regarded as one of the most important bean diseases,

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economic importance of anthracnose in recent years has declined in developed countries through the effective use of clean seed and resistant variety [3,17,18]. However, in developing countries, it remains serious and it is regarded as one of the principal diseases of beans throughout tropical regions including Latin America and Eastern Africa [18].

Taxonomy and Vegetative Cycle of Bean Anthracnose

Taxonomy

Colletotrichum lindemuthianum is considered as hemibiotrophic fungus, its taxonomical classification was a difficult, confused task. This fungus had been named with different synonymous throughout the years. Then, it could be hardly identified through classical taxonomy, because it produces acervuli with or without fruiting body depending of the quality and amount of substrate [19]. Now, the fungal names are given according to principles and rules of the International Code of Botanical Nomenclature, although, there is still some controversy in the designated names to some fungus. The *C. lindemuthianum* classification was made by the [20]. In this case, most authors agreed that *C. lindemuthianum* belongs to: Family, Melanconiaceae; Order, Melanconiales; Sub Class, Coelomycetidae; Class, Deuteromycetes; Sub Division, Deuteromycotina; Division Amastigomycota; Kindom Myceteae; Super Kindom, Eucariota.

Vegetative cycle

Colletotrichum lindemuthianum deploys a complex life cycle which has various development phases and two ways to take food. In every phase may be seen as unique, differentiated stages that let the fungus survive. Independently of the fungus development phase, the spore germination occurs in a similar manner. In the imperfect form of *C. lindemuthianum* the reproduction is asexual, the spores are produced inside acervulus and immerse in water soluble-formed mucilage [21]. The development of fungal spore shows a biphasic behaviour which means two life styles, as a saprophyte and biotroph; therefore, the fungus has been classified as hemibiotroph. In life style saprophytic the fungus growth in any carbon source including crystalline cellulose which may be easily converted into molecules fuel by extracellular lytic enzymes. On the other hand, as a biotroph fungus has the ability to feeding of nutriment outright of living plants.

As a saprophyte fungus, the spore germination process begins with the spore adhesion to the plant surface under adequate humidity conditions; specifically, correct aqueous content in the spore envelope (mucilage). At this level, the spores of the fungus round off by water absorption and active growth. Later, the germinating tube is formed (germinule phase), and the hyphae elongates to colonize the substrate. The aerial mycelia appear; then the fungal reproductive structures are formed where the spores are storage.

Finally, their life cycle is completed and it starts all over again. During the spore adhesion, the hydrophobicity of vegetal surface, the physical-chemistry bidirectional signalization and the mucilage play a major role [22-24]. The mucilage is formed by heavy molecular weight glycoproteins, a variety of enzymes and germination inhibitors; but it does not contain chitin [21,25]. Besides, it acts as a structure that protects the spore of dehydration and even as a protective barrier against environmental toxic and defense plant metabolites. The 6µm length fimbriae structures which are part of the germinule and the appressorium also participate in the adhesion process [21]. The *C. lindemuthianum* hyphae grow constantly reaching a size of 2 or 3 times bigger than their original size spore. It is believed that critical nutrient conditions and chemical bidirectional communication induce

the formation of the dome in the hyphae tip or appressorium. At same time, it initiates the melanin synthesis and the formation of the septum that separates cytoplasm from appressorium and germinule. Also, the plasmatic membrane presents a biochemical differentiation process which separates into two domains, one in the domo or appressorium and the other in the infection peg [26]. The appressorium produces and accumulates melanin an important factor involved in the turgency pressure which is required for the *C. lindemuthianum* to penetrate into the plant cell [27].

A new distribution and localization of plasmatic membrane proteins occur in the base of appressorial dome. It is necessary for the formation of a penetration pore, the synthesis of new cell walls layers, and the secretion of some other materials [21]. The melanin is storage in a cell wall layer which lies very close to the plasmatic membrane [28]. The penetration pore is rounded by an internal wall layer with funnel shape called, «appressorial cone». This structure is contiguous with the peg penetration wall and does not have chitin or melanin. The apical growth starts again with the infection peg emission and the injection of the plasmatic membrane and the citosol through the penetration pore then grows down to the intramural space between plasmatic membrane and cell wall. This will become the infection hyphae which is present in the biotrophic phase [26]. Knowledge about the biotrophical phase between *C. lindemuthianum* and bean cells interaction recently has started. However, during this phase, the primary hyphae growing between the vegetal plasmatic membrane and the cell wall is known. This fungus does not form specialized structures such as haustorium, like the ones seen in some strict biotrophs [29,30]. One characteristic of biotrophic intracellular interaction between plant cell and fungus, it is that, pathogen avoids or suppresses the initiation event of the plant defense responses, those that switch on the «hypersensitivity reaction» or the synthesis and deposition of callose on cell wall [31].

Plant Infection and Symptoms of Common Bean Anthracnose

Leaf infection and symptoms

Lesions are most common on leaf petioles and on the lower surfaces of leaves and leaf veins [32]. Although infection may occur on both sides of the leaf and on the petiole, early signs of infection usually appear on the lower leaf surface along the veins, which show brick red to purplish red discoloration. Later, such discoloration also appears on the upper leaf surface. At the same time, brown lesions of various sizes, with black, brown, or purplish red margins, develop around small veins [33]. During disease progression, vein necrosis appears first, then wilting and bleaching often occurs at the tip of the leaflet before spreading over the margin and finally over the center of the blade [34]. During this stage hyphae proliferate throughout host tissues, inside cells, in walls and through walls and in intercellular spaces. *Colletotrichum lindemuthianum* produces cell wall degrading enzymes and low molecular weight phytotoxins that may, by killing cells in advance of the invading hyphae, contribute to the necrotrophic growth of this pathogen [28,35]. Eventually conidiophores rupture through the host cuticle and form acervuli on the plant surface [28] (Figure 1).

Pod infection and symptoms

Before attacking the pods, the anthracnose fungus will infect the stems first. Stem infection is manifested by dark brown eyespots which develop longitudinally along the stems [9,10]. In the young seedling, if the eyespots enlarge, the stem may break off, but for older stems,

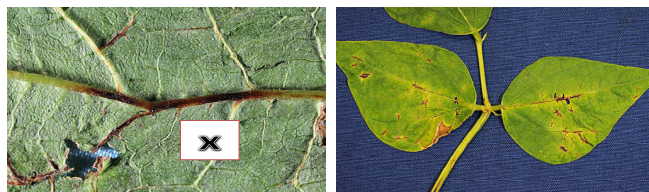


Figure 1: Close-up view of an anthracnose lesion on (X) a leaf vein and early infection of anthracnose on (Y) top side of leaf.



Figure 2: Infection of anthracnose on C) stand plant, B) collection of infected pods and A) collection of anthracnose free pods.

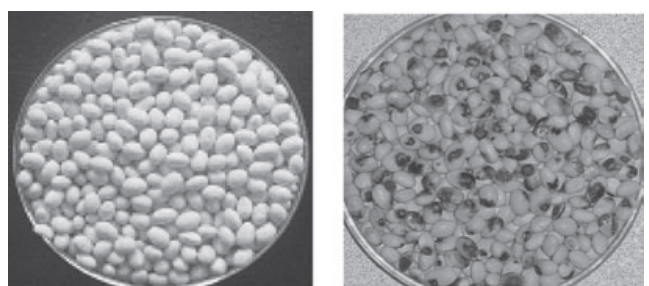


Figure 3: Symptoms of bean anthracnose caused by *Colletotrichum lindemuthianum* on common beans: (left side) healthy seeds; (right side) infected seeds.

the eye-shaped lesion is limited to an approximate length of 5-7 mm, and the lesion often has a sunken cankerous center [33]. After infecting the stems, the infection will then be passed on to the pods on pods, the most striking disease symptoms are small brown specks on rusty brown spots.

As these spots enlarge, their centers turn brown and many tiny black specks appear randomly on the brown area, replacing the brown specks [28]. Each of the tiny black specks contains a mass of pinkish spores, often visible as a viscous droplet in humid conditions. The lesions on the pod usually reach a diameter of 5-8 mm, are slightly sunken at the center and have a dark brown or purplish brown margin [9] (Figure 2).

Seed infection and symptoms

Seed infection is the major source of anthracnose transmission to the next crop generation and provides conditions which enable the fungus to survive unfavourable weather conditions. The fungus will remain alive as long as the seed remains viable, although not all infested and infected seed is capable of transmitting the disease [28]. The variation in seed transmission relates to the degree of infestation as well as the severity and site of infection in the seed [10]. Infection of the

seed is as a result of infections passed on from the pods. The higher the number of pods infected, the higher is the number of seeds infected. On the seed, anthracnose is displayed as brown to light chocolate-colored spots on the seed coats and in highly infected seed, the lesions may extend into the cotyledons [33] (Figure 3).

Epidemiology of Bean Anthracnose

Optimum conditions for disease development

Anthracnose is favoured by cool and wet weather [36]. Temperatures of 13-26°C with an optimum of 17°C, relative humidity above 92% and free moisture favour the germination of spores and initial infection [37]. During favorable environmental conditions typical anthracnose symptoms as lesions develop in all the above ground plant parts [3], from these lesions are washed down with water to other plant parts and serve as secondary sources of inoculum that initiate secondary infection in the field [38]. The spores can spread from infected to healthy plants by rain splash, wind-blown rain and through the movement of insects, animals and man, especially when the foliage is moist [39]. Frequent showers, particularly those accompanied by driving winds and cool temperature, highly favour further disease development in the field and subsequent pod and seed infections that can bring on epidemics [37,40,41].

Survival in seeds and crop debris

The fungus *C. lindemuthianum* over-seasons in infected plant residues and diseased seeds as mycelia or spores [10]. Studies in the USA also have shown that the pathogen can over-season in infected plant residues. The spores can survive for 5 years in infected bean pods and seeds that are air-dried and stored at 4°C and for more than two years in old bean debris under field conditions [36]. Meanwhile, the survival of *Colletotrichum lindemuthianum* in plant debris is adversely affected by the alternate dry and wet conditions of the soil [4]. Therefore, the major primary inoculum sources of bean anthracnose in the field are infected seeds [40]. Seeds also play an important role in the long distance distribution of the pathogen [38].

Host range

Colletotrichum lindemuthianum has been isolated from lima bean (*Phaseolus lunatus* L.), scarlet runner beans (*P. coccineus*), tepany beans (*P. acutifolius* var. *latifolius* L.), Mung bean (*Vigna radiate*), cow pea (*Vignaung viculata*), kudzu beans (*Dolichos bitloris* L.), and broad beans (*Vicia faba* L.), soybean (*Glycine max*), Pea (*Pisum sativum*) and black gram (*Vigna mungo*) [4].

Disease cycle

Between crops, the fungus survives in crop debris and can be spread in seed, air and water. Initial infection can take place anytime during the growing season during cool, wet weather; secondary infections can occur from spores forming on infected plants and spreading in wind and splashing rain, or being transported on equipment [42].

Disease Management

Bean anthracnose is usually introduced to a production field by infected seeds or by machinery during cultivation or harvesting. Prevention is the best way to manage bean anthracnose [43]. The first opportunity for the management of seed-borne diseases is to eradicate or reduce the pathogen inoculum in the seed production field [44]. Management strategies used to minimize seed-borne infection in the seed production field include host resistance, cultural, chemical and biological control methods.

Cultural control

Infested bean debris should be removed or buried in the soil after harvest to reduce winter survival [36]. The spores, present in diseased spots as a sticky mass, are more easily spread from diseased plants to healthy ones when plant parts are wet [36]. Cleaning and bagging stations in areas where anthracnose has been a problem may be sources of contaminated dust. Therefore, these stations should be cleaned of debris between shipments and the shipments isolated [36]. Seed storage facilities should also be disinfected and commonly used agricultural materials (leather, rubber, painted metal and denim) should be disinfected. For this, a 10 percent bleach solution (0.525 percent sodium hypochlorite), followed by chlorine dioxide (Aquacare) and chloroxylenol (Dettol) was found to be the most effective [39]. Seeds produced under wet and humid conditions should not be slowed because in most cases, they harbor the fungus inside their seed coat [19]. Seeds should not be saved for sowing from previously infected fields with anthracnose [45]. Thus, the production of disease free seeds in semi-arid areas, where conditions are not favourable for anthracnose infection could play vital role [40]. A two-year crop rotation is highly recommended to minimize the chance of survival, and it can be done with non-host plants like cereals and *solanaceous* crops [39]. Rotation of non-host crop species may reduce the development of bean anthracnose mainly due to the reduction of initial infection that arises from the initial inoculum source [32].

Others cultural methods like scouting the fields weekly for symptoms of anthracnose is recommended so that seeds from infected plants are not harvested [12]. Ensuring adequate plant spacing which promotes foliar drying [45], weed control will promote proper air circulation and decrease moisture in the foliar canopy [45]. Avoid sowing before the recommended planting dates, because cool conditions favor development of anthracnose [45]. Overhead irrigation practices should also be avoided, since it will wet and liberate fungal spore masses on foliage [39].

Physical methods

Soil solarization through covering the soil with transparent plastic sheeting for one month before sowing resulted in the reduction of both severity and incidence of anthracnose [46]. A hot-water seed treatment by soaking at 64 to 72°F for 15 hours followed by another soaking at 117°F for 25 minutes has been reported to kill the fungus in infested seeds without reducing germination [45].

Biological control

Various workers have reported that seed dressing or application of spore suspension of *Trichoderma viride* as seed dip and soil drench was effective against seed borne infection of *C. lindemuthianum* [47,48]. A strong local protection against bean anthracnose was also obtained when susceptible bean leaves were treated with *Trichoderma harzianum* in a liquid medium [49]. Smearing infected seeds with cultures of *T. harzianum*, *T. viridae*, *T. hamatum* and *Gliocladium virens* for 15min and drying them overnight before sowing significantly inhibited infection of *C. lindemuthianum* and increased seed germination [50]. The main antagonistic activities of these bio-agents were through, mycelial growth inhibition, toxic volatile metabolite production and inhibition of spore germination [51]. Some plant extracts (botanicals) also showed promising results in the control of bean anthracnose of common beans. Neem (*Azadirachta indica*) seed extract effectively inhibited both germination of conidia and mycelial growth of *C. lindemuthianum* [52]. Seed treatment and field spray using the extracts

of *Lawsonia inermis* significantly improved seedling emergence and reduced incidence of bean anthracnose [53].

Host plant resistance

Resistance is the most effective and efficient method of anthracnose management [54]. However, this has been complicated by the presence of several forms or races of the fungus, and the fact that plants resistant to one race may be susceptible to another [36]. In the common bean *C. lindemuthianum* interaction, nine resistance genes have been reported so far in different parts of the world [55,56]. Cultivars AB 136 and G 2333 could be used as sources of resistance in the bean breeding program since they are found to be highly resistant or immune to different races of *C. lindemuthianum* found in Africa, North and Central America [57,58]. Cultivar mixtures containing at least 60% of a resistant cultivar have been reported to offer a good control of anthracnose [38]. Although planting resistant cultivars is the most effective, least expensive, and easiest for farmers to adopt [59], the possible breakdown of resistance due to adaptation of the pathogen is the main drawback for its application [60]. Varieties must be tested where they are to be grown to determine their tolerance to the locally prevalent races [36].

Chemical control

Benlate (500 g a.i./kg WP) as a seed dressing at a rate of 2g/kg seed, difenoconazole (250 ml a.i./EC) at a rate of 87.5 g a.i./ha as a foliar spray and Benlate (500 g a.i./kg WP) seed dressing at a rate of 2 g/kg seed followed by foliar spray of difenoconazole (250 ml a.i./EC) at a rate of 87.5 g a.i./ha effectively reduced anthracnose severity and incidence and increased the yield per plot and 100 g seed weight [%]. Mancozeb seed treatment at a rate of 3g/kg seeds followed by carbendazim foliar spray at a rate of 0.5 kg/ha and Carbendazim seed treatment at a rate of 2g/kg seeds followed by carbendazim foliar spray at a rate of 0.5 kg/ha have been suggested to reduce anthracnose severity and incidence [46].

Integrated disease management

C. lindemuthianum has high pathogenic variability and new races of the pathogen are reported frequently. Thus, integrated disease management is considered the most effective approach to minimize the yield losses to anthracnose [6#]. The integration of soil solarization, Mancozeb seed treatment at a rate of 3 g/kg seeds and Carbendazim foliar spray at a rate of 0.5 kg/ha were found to be effective in reducing bean anthracnose epidemics [46]. Botanicals and biopesticides (10% extracts of *Adenocalymma alliaceae*, *Azadirachta indica* and *Lawsonia inermis*, 0.4% talc formulation of *T. viride* and *Pseudomonas fluorescens* along with fungicides (Carbendazim (0.2%) and Mancozeb (0.4%) were evaluated in a greenhouse and field experiment in India and gave promising results [53].

Conclusions

Management of anthracnose is essential to provide increased and stable bean yields throughout the world. Integrated disease management (IDM), which combines biological, cultural, physical and chemical control strategies in a holistic way rather than using a single component strategy proved to be more effective and sustainable. Recommended bean anthracnose management through IDM practices should include: pathogen free seed, seed treatment with fungicides, practice of crop rotation, deep ploughing of bean fields to bury infested debris, use of disease resistance genotype, and strategic application of foliar fungicides. Moreover, resistance to bean anthracnose cultivars has historically been overcome by new pathotypes of *C. lindemuthianum*;

hence the genotypes intended for release to farmers should be selected based on multi location multi season field trials. Knowledge of the biology and variability of *C. lindemuthianum* is also a prerequisite for breeding program aimed at obtaining durable resistance to bean anthracnose. Further studies on ecology of *C. lindemuthianum* and its epidemiology are required to improve current disease management strategies. Both innovative and conventional approaches should be used to investigate the host-pathogen relationship between *Phaseolus vulgaris* and *C. lindemuthianum*. Intensive research on the management of bean anthracnose using bio-agents and improved cultural practices should be emphasized in the future to enhance the overall efficacy of bean production.

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