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CENTRO DE DOCUMENTACION

# Chapter 16 Seed Pathology

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## Chapter 16 Seed Pathology Introduction

Dry beans (*Phaseolus vulgaris* L.) are not vegetatively propagated. Therefore, they depend upon seed production for perpetuation of the crop. The quality of dry bean seeds used for planting by Latin American farmers generally is low, especially among those with small land holdings.

Sánchez and Pinchinat (36) conducted a survey of seed used by farmers in Costa Rica and found an average germination of 68%. Ellis *et al.* (16) conducted a similar survey of farmers with small land holdings in Colombia and reported that germination was as low as 8% with 100% seed infected by fungi. Certified seed is difficult to obtain and rarely used by farmers in Latin America, since less than 3% of all seed sown is certified (44).

### Seed Transmission of Pathogens

Seeds provide an efficient method for the transfer of plant pathogenic organisms between locations. More than 50% of the major bean diseases are seed-borne (14). As a farmer plants infested seed, he also sows the potential for future disease problems. Seed transmission of plant pathogens is of concern in Latin America because most farmers plant seed saved from previous harvests (20). The effect of seed-borne organisms upon seed germination is not well documented, but internally-borne fungi are associated with decreased seed germination and field emergence of dry beans (Figs. 1-4, p 304). Ellis *et al.* (16) found a correlation of -0.88 between percentage recovery of internally-borne fungi and seedling emergence. Seed viability, germination and contamination by microorganisms also can be affected by mechanical damage which may occur during harvesting, threshing and/or planting (9, 39).

### Seed Storage Problems

Conditions for seed storage are critical to the survival of high quality seed for long periods and to the degree of storage losses incited by various

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Fig. 1- Sample of seed relatively free of seed-borne organisms.



Fig. 3- Sample of seed severely contaminated by seed-borne organisms.



Fig. 2- Seed from clean seed sample surface disinfected and incubated on potatodextrose agar.



Fig. 4- Seed from contaminated seed sample surface disinfected and incubated on potato-dextrose agar.

seed contaminants and seed-borne pathogens (see Table 1). López and Christensen (26) report that the seed moisture content should be less than 15%, preferably 13%, and seed should be stored in conditions with less than 75% relative humidity. López and Crispin (27) report that cultivars vary in their resistance to storage rot organisms. Also, storage temperatures lower than 10°C should extend the viability of dry bean seed.

### **Control of Seed-Borne Fungi**

Numerous fungi are reported to be borne internally or as surface contaminants in seed of *Phaseolus vulgaris* (Table 1). Many of these organisms also are seed-borne in other members of the Leguminoseae, such as soybeans, pigeon peas and cowpeas (16). Figure 5 illustrates the manner by which *Colletotrichum lindemuthianum* may become seed-borne in dry beans. Most internally-borne fungi are located inside the seed coat and some infection may occur in the cotyledon or embryo (1, 15).

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Fig. 5- (right) Pod and seed infection by the anthracnose fungus.

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Fig. 6- (below) Seed sample harvested at maturity, surface disinfected and incubated on potato-dextrose agar.

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Fig. 7-(lower right) Seed sample harvested two weeks after maturity, surface disinfected and incubated on potato-dextrose agar.





Protectant fungicides such as Captan (Orthoside), Ceresan and Arasan or Thiram diffuse into the seed coat where many seed-borne fungi are located but do not enter dry bean cotyledons (14, 15, 40). Recommended application rates for most seed treatments is 1-2 g per kg seed. Seed treatment is relatively inexpensive and can improve germination and field emergence of seed lots with moderate levels of infected seed.

Systemic fungicides such as Benomyl can penetrate the seed coat and cotyledons of beans to provide some degree of control (1, 14). Investigations are being conducted with chemicals such as ethylene oxide (34) which has excellent biocidal and penetrative properties and may prove to be practical in removing seed-borne contaminants with little reduction in seed viability.

Systemic fungicides were foliarly applied beginning 40 days after planting, with four applications made at 9-day intervals by Ellis and coworkers. Benomyl (1 kg/ha) significantly reduced seed infection by *Colletotrichum lindemuthianum* when compared to the non-sprayed treatment (11, 13). A protectant fungicide such as Difolatan or Captafol was not as effective, because heavy rainfalls consistently washed the chemical off the plants. Fungicides may be useful for clean seed production in Latin America. However, they may not be economical for regular production operations unless growers are willing to pay for the increased production costs.

Date of harvest is important in the production of high quality and pathogen-free seed (13, 35). The percentage of seed infection by fungi increases and the percentage of seed germination decreases with prolonged time in the field after plant maturity (Fig. 6 and Fig. 7)(13). Therefore, it is



Fig. 8- Seed infection by Sclerotium rolfsii.



Fig. 9- Seed infection by *Macrophomina* phaseolina (black mycelia) and *Phomopsis* species (white mycelia).

important that seed production fields be harvested immediately after plant maturation. Foliar applications of Benomyl during the growing season can reduce the incidence of seed-borne fungi and low seed gerinination commonly associated with delayed harvest. Similar results are reported for soybean production (10).

In some dry bean cultivars, pod contact with the soil may cause significantly higher levels of seed infection by various soil-borne fungi, such as *Rhizoctonia solani*, *Sclerotium rolfsii* (Fig. 8), and *Macrophomina phaseolina* (Fig. 9). This may result in a significantly lower seed germination than in seeds collected from pods of the same plant free from soil contact (12, 47). When harvesting seed production fields, it would be beneficial to avoid pods which have soil contact, especially for farmers who can hand-pick desirable pods with seeds destined for future plantings.

The most efficient method of producing clean seed free from a specific pathogen is to use a cultivar that is immune or resistant to infection by that pathogen. For example, York *et al.* (46) have studied resistance to Pythium seed decay intensively. Cultivars which are tolerant to a specific pathogen may allow limited development of the pathogen and its potential to be transmitted within the seed. Therefore, seed from such cultivars must be assayed carefully to determine whether seed-borne fungi are present.

### **Control of Seed-Borne Bacteria**

It is reported that 95 species and varieties of bacteria may be seed-borne in numerous crops (38). Various bacterial pathogens are reported to be internally seed-borne in *Phaseolus vulgaris* (Table 1). Xanthomonas phaseoli and Corynebacterium flaccumfaciens can remain viable for two to 10 and five to 24 years, respectively, in seeds (38).

No satisfactory method of seed treatment will completely control internally-borne bacteria of dry beans. Several methods and compounds have been tested with varying results, but the general conclusion is still negative. External seed contamination can be controlled by application of Streptomycin or Kasugamycin (41).

The most reliable method of producing seed free from bacterial pathogens is to select production areas where environmental conditions and cultural practices do not favor bacterial growth and development (19). Copeland *et al.* (4) state that additional control can be achieved by long rotations of different crops, planting different cultivars in alternating seasons and sequential planting of adjacent fields to reduce large acreages of susceptible plants at one point during a growing season.

At present, no commercial cultivar is immune to infection by the common blight pathogen. However, resistance to infection has been reported and differential pod susceptibility (5, 6) may be used to further reduce seed contamination.

### **Control of Seed-Borne Viruses**

Viruses are reported to be seed-borne in *Phaseolus vulgaris* (Table 1). Bean common mosaic virus is transmitted internally in cotyledons and embryos but not in seed coats, while southern bean mosaic virus is transmitted in embryos and seed coats (17). Once seeds are infected, no seed treatments available currently will eliminate the virus from bean seed. The most effective procedure is to produce clean seed in an area where the virus-infected plants can be eliminated and where vectors which transmit the virus can be controlled or do not exist.

Development of resistant cultivars also will allow the production and use of clean seed. However, research still is needed to determine if low levels of virus can persist in resistant or tolerant cultivars and serve as reservoirs of inoculum for infection of susceptible cultivars by insects or other vectors.

### **Production of Pathogen-Free Seed**

Benefits derived from the use of clean seed have been demonstrated in temperate regions such as the United States (4, 19) and in Australia (28) and Latin America (2, 3, 18). Clean seed production has been difficult in Brazil (23), but programs still are being developed. Clean seed production fields should be located in areas where the environment is unfavorable for survival, infection and spread of pathogenic organisms. An ideal production site should have an annual rainfall of less than 300 mm, a daily relative humidity less than 60%, a daily temperature regime between 25°-35°C, and gravity irrigation facilities. These production sites also should be located in regions where dry beans or other legumes are not grown commercially in order to avoid contamination by insect transmitted viruses with wide host ranges. A seed production program will require a form of inspection and certification to ensure seed cleanliness and purity.

Seed production programs often are provided with a limited seed quantity. The CIAT bean production program has used the following glasshouse and/or screen house technique (Fig. 10) to produce small quantities (10-100 g) of pathogen-free seed:

- Seed of each entry is planted (2 seeds/pot measuring 15-20 cm in diameter by 25 cm in depth) in sterilized soil in a glasshouse or finemeshed screen house.
- Seedlings are carefully irrigated to avoid physical contact between plants and observed daily to identify the expression of bean disease symptoms. When an infected plant is identified, the data is recorded and the plant + soil + pot are immediately sterilized.
- Surviving plants are protected from outside contamination and observed daily for symptom expression.



Fig. 10- Clean seed production in screen house facilities at CIAT.

- Seedlings and/or mature plants may be assayed serologically and harvested separately to avoid contamination, especially from latent seed-borne viruses.
- Pathogen-free seed then is stored in sealed containers at less than 10°C and 13% relative humidity.

Field production and increase of pathogen-free seed should be undertaken in the proper production zone. Seed should be planted 25-30 cm apart within rows spaced 1 m apart. Plants must be inspected frequently (weekly) during their growth to detect and eliminate plants infected with diseases. Critical evaluation times after germination include 15 days to detect bean common mosaic virus; 30 days to detect common bacterial blight, angular leaf spot, and web blight; 45 and 60 days to detect common bacterial blight, angular leaf spot and anthracnose. Chemical applications may be required to prevent plant infection by pathogens or the buildup of insect vectors.

It is ideal to tolerate 0% infection by any bean pathogen which may be transmitted by seed. However, this tolerance may have to be raised to 0.5-1% infection when seed is produced in tropical environmental conditions which are marginal for successful clean seed production.

Successful production of clean seed also is dependent upon proper field management during maturation and harvest. Foliar applications of chemicals seven to 10 days before plant maturity may reduce pod infection by plant pathogens and/or saprophytes and ensure good seed viability. Mature pods which are not in contact with the soil should be harvested immediately.

A windrow inspection is advised if beans are not harvested and threshed immediately. Pods must be carefully threshed and cleaned to avoid mechanical damage and cracking, and they should be stored under proper conditions. Subsequent laboratory (serology or other detection procedures) and greenhouse tests may be conducted to verify that the seed is indeed pathogen-free (21, 29, 45). Certified seed should be planted in pathogen-free commercial production regions or protected with chemicals to assure improved production. Additional yield advances may be possible by utilization of clean seed practices for newly developed high-yielding and disease resistant cultivars.

Organism	Common Name	Literature Cite
FUNGI		
Acrostalagmus spp.	-	16
Alternaria spp.	Leaf and Pod Spot	37
Ascochyta spp.	Leaf and Pod Spot	1
Aspergillus candidus	Storage Rot	27
Aspergillus glaucus	Storage Rot	27
Aspergillus niger	Storage Rot	16
Aspergillus repens	Storage Rot	27
Aspergillus restrictus	Storage Rot	27
Botryodiplodia theobromae	Seed Decay	16
Botrytis cinerea	Gray Mold	16
Cercospora cruenta	Leaf Blotch	47
Chaetoseptoria wellmanii	Leaf Spot	7
Cladosporium herbarum	Cladosporium Spot	42
Colletotrichum dematium	-	16
Colletotrichum lindemuthianum	Anthracnose	47
Colletotrichum truncatum	Stem Anthracnose	25
Curvularia spp.	Leaf Spot	8
Dendrophoma spp.	-	1
Diaporthe phaseolorum	Pod and Stem Blight	16
Diplodia natalensis	Seed Contaminant	47
Erysiphe polygoni	Powdery Mildew	47
Fusarium equiseti	Damping Off	16
Fusarium moniliforme	-	32
Fusarium oxysporum		
f. sp. phaseoli	Fusarium Yellows	47
Fusarium roseum	-	8
Fusarium semitectum	Pod Decay	43
Fusarium solani	Root Rot	31
Fusarium sulphureum		16
Isariopsis griseola	Angular Leaf Spot	33
Macrophomina phaseolina	Ashy Stem Blight	47
Monilia spp.	-	16
Mucor spp.	-	8
Nematospora coryli	Yeast Spot	43
Nigrospora spp.	-	12
Penicillium spp.	Storage Rot	27
		(continued)

 Table 1. Examples of seed-borne and seed-contaminating organisms associated with dry beans (Phaseolus vulgaris L.).

Organism	Common Name	Literature Cited
Pestalotiopsis spp.	-	16
Peyronellaea spp.	-	16
Phomopsis phaseolina	Leaf and Pod Spot	16
Rhizoctonia solani	Root Rot	24
Rhizopus spp.	Soft Rot	1
Sclerotinia sclerotiorum	White Mold	47
Sclerotium rolfsii	Southern Blight	1
Sporotrichum spp.	-	37
Stemphylium spp.	Leaf Spot	37
Thanatephorus cucumeris	Web Blight	47
ACTERIA		
Achromobacter spp.		37
Aerobacter aerogenes		37
Agrobacterium radiobacter	-	37
Alcaligenes viscosus	-	37
Bacillus cereus	-	37
Bacillus megatherium	-	37
Bacillus polymyxa	-	37
Bacillus sphaericus	-	37
Bacillus subtilis	-	37
Bacterium globiforme		37
Corynebacterium flaccumfaciens	Bacterial Wilt	47
Corynebacterium helvolum	-	37
Micrococcus spp.		37
Pseudomonas fluorescens		37
Pseudomonas phaseolicola	Halo Blight	47
Pseudomonas syringae	Bacterial Brown Spot	47
Xanthomonas phaseoli	Common Bacterial Blight	47
Xanthomonas phaseoli var. fuscans	Fuscous Bacterial Blight	47
TRUSES		
Bean Common Mosaic Virus	BCMV	47
Bean Western Mosaic Virus	Strain of BCMV	47
Bean Southern Mosaic Virus	BSMV	47
Tobacco Streak Virus	Red Node Strain	47
Cucumber Mosaic Virus	CMV-PR	30
Cherry Leaf Roll Virus		22

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