Chapter 19

SEED PATHOLOGY

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Introduction

Dry or common beans (*Phaseolus vulgaris* L.) are not vegetatively propagated and therefore depend on seed production for the perpetuation of the crop. The quality of common bean seeds used for planting by farmers in developing countries is usually low, especially among smallholders. Farmers in developed regions usually give priority to high-quality seeds and use them for production.

Seeds provide an efficient method for the transfer of plant pathogenic microorganisms between locations and seasons. More than 50% of the major bean diseases can be seed-borne (Ellis et al., 1977; Hampton, 1983). As a farmer plants infested seeds, he also sows the potential for future disease problems. Seed transmission of plant pathogens is of concern in developing countries because most farmers plant seeds saved from previous harvests (Gutiérrez-P. et al., 1975), thereby perpetuating diseases. The effect of seed-borne organisms upon germination of bean seeds is not well documented. However, many internally borne fungi are known to decrease seed germination (Dhingra, 1978; Ellis et al., 1976d) and field emergence (Figures 143-146). The halo-blight bacterium (*Pseudomonas syringae* pv. *phaseolicola* (Burk.) Young et al.) is seed-borne. Severely infected seeds germinate at a low rate, producing deformed seedlings (Katherman et al., 1980; Saettler et al., 1981; Weller and Saettler, 1980). Seed viability, germination, and contamination by microorganisms also can be affected by mechanical damage which may occur during harvesting, threshing, and/or planting (Dickson and Boettger, 1976; Schweitzer, 1972; Weller and Saettler, 1980).

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The extent of transmission from seed to crop or of development of seed-borne disease depends on various factors such as the amount or rate of seed-borne inoculum; extent or rate of transmission of this inoculum to the seedling at any stage of its plant development; subsequent rate of inoculum or disease increase until harvest; and rate of re-establishment of seed-borne inoculum during the next seed generation. Seed pathology programs must also consider those biological factors which influence pathogen development, detection, and management. These are inoculum potential, infection probability, other means of transmission, variation in pathogen virulence and host susceptibility, accuracy and reliability of testing methods, and efficacy of seed disinfection (Neergaard, 1977).

Seed-borne Fungi

Many fungi can be borne internally or as surface contaminants in seeds of *Phaseolus vulgaris* (Table 1). Many of these microorganisms are also seed-borne in other members of the Leguminosae such as soybean (*Glycine max* (L.) Merr.), pigeonpea (*Cajanus cajan* (L.) Millsp.), and cowpea (*Vigna unguiculata* (L.) Walpers ssp. *unguiculata*) (Ellis et al., 1976d). Most internally borne fungi are located inside the seed coat and some infection may occur in the cotyledon or embryo (Bolkan et al., 1976; Dhingra and Asmus, 1983; Ellis et al., 1976a; Menten et al., 1979). The anthracnose fungus (*Colletotrichum lindemuthianum* (Saccardo *et* Magnus) Briosi *et* Cavara) can become seed-borne after penetrating pod walls (Figure 147). Angular leaf spot (*Isariopsis griseola* Sacc.) is usually found in the hilum area of the seed coat (Correa-Victoria, 1984).

Date of harvest is important in producing high-quality and pathogen-free seeds (Ellis et al., 1976b; Rena and Vieira, 1971). Weed management also reduces seed infection by some pathogens such as web blight (*Rhizoctonia solani* Kühn) and pod decay (*Fusarium semitectum* Berk. *et* Rav.) (Chagas and Dhingra, 1979). Seed infection by fungi increases (Gomes and Dhingra, 1981) and seed germination decreases if harvesting is delayed (Figures 148 and 149) (Ellis et al., 1976b). It is, therefore, important that seed be

Table 1. Examples of seed-borne and seed-contaminating microorganisms associated with common beans (*Phaseolus vulgaris* L.).

Microorganism	Common name	Source[a]
FUNGI		
Acrostalagmus spp.		26
Alternaria spp.	Leaf-and-pod spot	61
Ascochyta boltshauseri Saccardo	Leaf-and-pod spot	38
Ascochyta phaseolorum Saccardo	Leaf-and-pod spot	38
Aspergillus candidus Link ex Fries	Storage rot	43
Aspergillus glaucus Link ex S.F. Gray	Storage rot	43
Aspergillus niger van Tieghem	Storage rot	26
Aspergillus repens de Bary	Storage rot	43
Aspergillus restrictus Smith	Storage rot	43
Botryodiplodia theobromae Patonillard	Seed decay	26
Botrytis cinerea Persoon ex Fries	Gray mold	26
Cercospora canescens Ellis *et* Martin	Leaf spot	26
Cercospora cruenta Saccardo	Leaf blotch	76
Chaetoseptoria wellmanii Stevenson	Leaf spot	13
Cladosporium herbarum (Persoon) Link	Cladosporium spot	68
Colletotrichum dematium (Persoon ex Fries) Grove		26
Colletotrichum lindemuthianum (Saccardo *et* Magnus) Briosi *et* Cavara	Anthracnose	76
Colletotrichum truncatum (Schweinitz) Andrus *et* Moore	Stem anthracnose	41
Curvularia spp.	Leaf spot	18
Dendrophoma spp.		3
Diaporthe phaseolorum (Cooke *et* Ellis) Saccardo	Pod-and-stem blight	26
Diplodia natalensis Pole-Evans	Seed contaminant	76

(Continued)

Table 1. (Continued).

Microorganism	Common name	Source[a]
Erysiphe polygoni DC.	Powdery mildew	76
Fusarium equiseti (Corda) Saccardo	Damping-off	26
Fusarium moniliforme Sheldon		54
Fusarium oxysporum f.sp. *phaseoli* Kendrick *et* Snyder	Fusarium yellows	76
Fusarium roseum Link		18
Fusarium semitectum Berkeley *et* Ravenel	Pod decay	74
Fusarium solani f.sp. *phaseoli* (Burkholder) Snyder *et* Hansen	Root rot	52
Fusarium sulphureum Schlechtendahl		26
Isariopsis griseola Saccardo	Angular leaf spot	55
Macrophomina phaseolina (Tassi) Goid.	Ashy stem blight	76
Monilia spp.		26
Mucor spp.		18
Nematospora coryli Peglion	Yeast spot	74
Nigrospora spp.		22
Penicillium spp.	Storage rot	43
Pestalotiopsis spp.		26
Peyronellaea spp.		26
Phomopsis phaseolina	Leaf-and-pod spot	26
Rhizoctonia solani Kühn	Root rot	42
Rhizopus spp.	Soft rot	3

(Continued)

Table 1. (Continued).

Microorganism	Common name	Source[a]
Sclerotinia sclerotiorum (Libert) de Bary	White mold	76
Sclerotium rolfsii Saccardo	Southern blight	3
Sporotrichum spp.		61
Stemphylium spp.	Leaf spot	61
Thanatephorus cucumeris (Frank) Donk	Web blight	76
BACTERIA		
Achromobacter spp.		61
Aerobacter aerogenes (Kruse) Beijerinck		61
Agrobacterium radiobacter (Beijerinck *et* van Delden) Conn		61
Alcaligenes viscosus Weldin		61
Bacillus cereus Frankland *et* Frankland		61
Bacillus megatherium Schroeter		61
Bacillus polymyxa (Prazmowshi) Macé		61
Bacillus sphaericus Neide		61
Bacillus subtilis (Ehrenberg) Cohn		61
Corynebacterium flaccumfaciens pv. *flaccumfaciens* (Hedges) Dowson	Bacterial wilt	76
Corynebacterium helvolum (Zimmermann) Kisskalt *et* Berend		61

(Continued)

Table 1. (Continued).

Microorganism	Common name	Source[a]
Micrococcus spp.		61
Pseudomonas fluorescens (Trevisan) Migula		61
Pseudomonas syringae pv. *phaseolicola* (Burk.) Young et al.	Halo blight	76
Pseudomonas syringae pv. *syringae* van Hall	Bacterial brown spot	76
Xanthomonas campestris pv. *phaseoli* (Smith) Dye	Common and fuscous bacterial blights	76
VIRUSES		
Bean common mosaic virus	BCMV	76
Bean southern mosaic virus	BSMV	76
Bean western mosaic virus	Strain of BCMV	76
Cherry leafroll virus		31
Cucumber mosaic virus	CMV	48
Tobacco streak virus	Red node strain	76

a. Numbers refer to sources' order in list of references.

harvested immediately after plant maturity. In some cultivars, pod contact with the soil may cause significantly higher levels of seed infection by various soil-borne fungi such as web blight (*Rhizoctonia solani*), southern blight (*Sclerotium rolfsii* Saccardo) (Figure 150), and ashy stem blight (*Macrophomina phaseolina* (Tassi) Goid.) (Figure 151). This may result in a significantly lower seed germination than in seeds collected from pods of the same plant but free from soil contact (Ellis et al., 1976c; Zaumeyer and Thomas, 1957). When harvesting seed-production fields care must be taken to prevent pods coming into contact with the soil. Subsistence farmers, in particular, must take care when handpicking desirable pods to supply seeds for future plantings.

Seed treatment is relatively inexpensive and can improve germination and field emergence of seed lots that are moderately infected. Protective fungicides such as captan, Ceresan (now discontinued), and thiram, diffuse into the seed coat where many seed-borne fungi are found, without entering the cotyledons (Ellis et al., 1976a and 1977). The recommended application rate for most seed treatment is 1-2 g/kg of seed. Systemic fungicides such as metalaxyl and benomyl, penetrate both seed coat and cotyledons, providing a degree of control (Bolkan et al., 1976; Dhingra and Muchovej, 1980; Ellis et al., 1976b and 1977; Muchovej and Dhingra, 1980).

The most efficient method of producing seeds free of a specific pathogen is to use a cultivar that is immune or resistant to that pathogen. Variation exists among cultivars for susceptibility to specific pathogens (Asmus and Dhingra, 1985). Cultivars which are tolerant to a specific pathogen may still allow limited development of the pathogen and therefore potential seed transmission. Seed from such cultivars must be assayed carefully to determine whether seed-borne fungi are present.

Seed-borne Bacteria

At least 95 species and varieties of bacteria are seed-borne in crops (Coyne and Schuster, 1974). Various bacterial pathogens are internally seed-borne in *Phaseolus vulgaris* (Table 1). Common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye) and bacterial wilt (*Corynebacterium flaccumfaciens* pv.

flaccumfaciens (Hedges) Dows.) can remain viable for 2-10 and 5-24 years, respectively, in seeds (Schuster and Coyne, 1974).

Seeds with visible symptoms of *Xanthomonas campestris* pv. *phaseoli* infection are found in visibly infected pods. However, symptomless seeds can still be internally contaminated and so provide inoculum for disease outbreaks. Infected seed symptoms vary from a slightly darkened spot in the hilum region to discoloration and shrivelling of the seed coat. Weller and Saettler (1980) reported that seed-surface populations can exceed 40,000 bacteria per seed and that a minimum population of 1,000-10,000 per seed was needed to produce an infected plant under field conditions. External infection of seeds occurs during threshing when bacteria from dried bean tissue (especially stems and pods) become air-borne in bean dust (Weller and Saettler, 1980).

There are no satisfactory methods of seed treatment that completely control internally borne bacteria of common beans. Several methods and compounds have been tested with varying results. External seed contamination can be reduced by application of streptomycin (Taylor and Dudley, 1977).

The most reliable method of producing seeds free from bacterial pathogens is to select production areas where environmental conditions and cultural practices do not favor bacterial growth and development (Guthrie et al., 1975). Copeland et al. (1975) state that additional control can be achieved by long rotations of nonhost crops, planting different cultivars in alternating seasons, and sequential planting of adjacent fields to reduce large acreages of susceptible plants uniformly mature at one point during a growing season.

Most certification programs rely upon laboratory tests for cleanliness or as a routine complement of field inspections for bacterial diseases. Traditional seed tests rely upon seed-soak bioassays and usually require large quantities of seeds and testing resources to detect a minimal threshold of infection in any given seed lot (Sheppard, 1983a; Webster et al., 1983; Weller and Saettler, 1980). Many programs are investigating newer procedures and combinations which may be more precise and efficient such as ELISA (enzyme-linked immunosorbent assay) and other serological procedures; immunosorbence; immunofluorescence; electron

microscopy; selective growth media; and dilution plating (Klement, 1983; Kulik, 1984a and 1984b; Kulik and Stanwood, 1984; Lahman and Schaad, 1985; Sheppard, 1983a; van Vuurde and van Henten, 1983; van Vuurde et al., 1983). Halo blight and common bacterial blight detection varies from 100-1,000 to 10,000-100,000 colony forming units/ml, according to the method used. For example, immunofluorescence (Malin et al., 1983 and 1985; van Vuurde and van Henten,1983; van Vuurde et al., 1983) is more sensitive than other methods such as ELISA (Barzic and Trigalet, 1982). However, low levels of seed-borne pathogenic bacteria cannot yet be reliably detected by any method (Malin et al., 1985). The sensitivity, specificity, reliability, and expense of each method varies considerably. Seed pathology laboratories have not yet standardized testing procedures or threshold levels for certification.

At present, no commercial cultivar is immune to infection by the common bacterial blight (Cafati-K. and Saettler, 1980) or halo blight pathogens. However, resistance to infection occurs and differential pod susceptibility can be used to further reduce seed contamination by the common bacterial blight pathogen and others (Coyne and Schuster, 1974; Webster et al., 1983).

Seed-borne Viruses

Of the 70 or more viruses which infect *Phaseolus vulgaris*, only seven are known to be transmitted in bean seed (Table 1). Bean common mosaic and bean southern mosaic viruses are considered as the most significant economically. The seed transmission properties of bean common mosaic virus have been the subject of various studies since 1919 (Ekpo and Saettler, 1974; Hampton, 1983; Reddick and Stewart, 1919). In general, the virus is transmitted in a high but variable proportion (often more than 50%) of seeds produced by susceptible plants. Seed transmission varies according to the cultivar infected, time of infection (for example, little seed transmission occurs after flowering), and virus strain involved (Hamilton, 1983; Zaumeyer and Thomas, 1957). There are also susceptible bean genotypes which restrict seed transmission of bean common mosaic virus to less than 1% (F.J. Morales and M. Castaño-J., unpublished data).

Bean southern mosaic virus can be internally transmitted through infected bean embryos (Uyemoto and Grogan, 1977). However, the virus is mainly a seed-coat contaminant since seed transmission is low and, furthermore, considerably reduced by dehydration associated with seed maturity (Cheo, 1955). Nevertheless, bean southern mosaic virus can be efficiently transmitted (10%-20%) in seeds of some cultivars and cause economically significant yield losses (Hamilton, 1983; Morales and Castaño-J., 1985).

Other seed-transmitted viruses are currently considered of minor economic significance in the tropics and other regions. Cucumber mosaic virus is perhaps internally seed-borne (1%-30%) in *P. vulgaris* (Bos and Maat, 1974; Davis et al., 1981; Hamilton, 1983), because it is stable and survives seed storage periods of more than two years. Soybean mosaic virus infects *P. vulgaris*, including seeds, under natural conditions (Castaño-J. and Morales, 1983). Seed transmission, however, is low and many bean cultivars are not susceptible to infection. Bean mild mosaic virus is apparently seed-borne as a seed-coat contaminant (Jayasinghe, 1982). However, the virus is highly infectious and not easily inactivated by desiccation. Tobacco streak virus transmission reportedly varies from 1%-26% (Hamilton, 1983), but neither it nor the cherry leafroll virus are significant problems in tropical bean-producing regions.

The main recommendation for virus-free seed production is field multiplication of seeds obtained from virus-free plants grown under greenhouse conditions. Multiplication fields need to be planted in areas free of seed-borne virus and, if possible, of insect vectors. Roguing seed-infected seedlings or plants in the field is recommended only in the absence of insect vectors. Chemical control of insect vectors is not worthwhile in the case of aphid-borne viruses such as the bean common mosaic, soybean mosaic, or cucumber mosaic, because they are acquired and transmitted by aphids in a few seconds. Insecticides can reduce seed transmission of beetle-borne viruses such as bean southern mosaic and bean mild mosaic.

Virus detection must be simple, rapid, specific, sensitive, and inexpensive (Carroll, 1979; Hamilton, 1983; Kulik and Stanwood, 1984). Bean seed-transmitted viruses are most effectively detected by ELISA because other conventional serological techniques are

affected by nonspecific reactions. A polyclonal antiserum containing antibodies to several seed-borne viruses is desirable.

In the absence of antisera, the "growing on" test is recommended. That is, a representative seed sample (at least 100 seeds for advanced lines or cultivars, or 50 seeds for segregating materials) is sown in trays or pots. Fifteen to 30 days after sowing the health of the seedlings is visually assessed. Since some viruses may not induce visible symptoms in all genotypes or under certain environmental conditions, the "indexing" of bean seedlings with indicator plants is necessary.

Seed Certification

Benefits derived from the use of clean seeds have been demonstrated in temperate regions such as the United States (Copeland et al., 1975; Guthrie et al., 1975), Canada (Sheppard, 1983b), and Australia (Lovelady, 1974), and in tropical regions such as Africa and Latin America (Douglas, 1980; Issa et al., 1964; Sánchez-M. and Pinchinat, 1974). Clean-seed production has been difficult in Brazil (Issa et al., 1964; Wetzel et al., 1972), but programs are being developed. Clean-seed production fields must be located in areas where the environment is unfavorable for the survival of, infection by, and spread of pathogenic microorganisms. An ideal production site has an annual rainfall of less than 30 cm, a daily relative humidity of less than 60%, a daily temperature regime between 25-35 °C, and gravity-irrigation facilities. Production sites also must be located in regions where common beans or other legumes are not grown commercially in order to avoid contamination by insect-transmitted viruses that have wide host ranges. Ideally, a seed-production program is coordinated by a national seed policy (Douglas, 1980) that requires a form of inspection and certification that will ensure seed cleanliness and purity.

Plants must be inspected weekly during their growth to detect and eliminate infected plants. Critical evaluation times after germination are 30-45 days to detect bean common mosaic virus, and 30-60 days to detect common bacterial blight, angular leaf spot, anthracnose, and web blight. The ideal tolerance is 0% infection by any bean pathogen which may be transmitted by seed. However, this

tolerance may have to be raised when seed is produced under those tropical conditions which are marginal for successful clean-seed production.

Successful production of clean seeds also needs proper field management during maturation and harvest. Chemical applications may be required to prevent or reduce plant infection by pathogens or the buildup of insect vectors. Foliar applications of chemicals 7-10 days after flowering and again before plant maturity, will reduce pod infection by plant pathogens and/or saprophytes, and improve seed viability. Mature pods which are not in contact with the soil must be harvested immediately.

A windrow inspection is necessary if beans are not harvested and threshed immediately. Pods must be carefully threshed and cleaned to avoid mechanical damage and cracking. They should be stored under proper conditions. Subsequent laboratory (serology or other detection procedures) and greenhouse tests are carried out to verify that the seeds are indeed pathogen-free or within established standards.

It is not possible to determine if a seed lot is free from infected or infested seeds, but it is possible to certify that a seed lot contains less than a specified level of infection. Seed testing must use controlled conditions (especially for temperature and moisture) and detailed procedures which maximize the probability of recovering the pathogen of interest. Tests vary from simple seed grow outs on media or in pots to complicated laboratory schemes which involve washing, soaking, grinding, infiltration, and state-of-the-art physical and chemical techniques (Schaad, 1982).

Proper seed storage conditions are vital for maximizing the survival of high-quality seeds for long periods and for minimizing storage losses inducted by various seed-borne saprophytes and pathogens (Table 1). Proper storage conditions are also critical for minimizing health threats from fungal byproducts such as aflatoxin which has been recovered from beans inoculated with storage rot (*Aspergillus parasiticus* Speare) (Seenappa et al., 1981). López-F. and Christensen (1962) report that the seed moisture content must be less than 15%, preferably 13%, and seed must be stored in conditions of less than 75% relative humidity. López-F. and

Crispín-Medina (1971) report that cultivars vary in their resistance to seed-storage-disease microorganisms. Also, storage temperatures lower than 10 °C will extend the viability of bean seeds.

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