Seed-borne Diseases in Seed Production

Marlene Diekmann

International Center for Agricultural Research in the Dry Areas (ICARDA)

WANA Seed Network

	Network activities	Lead countries
1	Regional referee testing: purity and germination, seed health	Morocco
2	WANA seed newsletter	Secretariat
3	WANA seed directory	Egypt
4	Undergraduate and post-graduate study in seed science; overview of university training in WANA	Jordan, Iraq, Lebanon, Algeria
5	WANA variety catalogue	Morocco
6	Standardized seed certification procedures	Turkey
7	Catalogue of seed standards of all WANA countries	Syria
8	Develop uniform national seed policy	Sudan
9	Seed movement across national borders; develop regional between-country seed trade - plant quarantine measures - variety and seed registration regulations - laws governing investment, forming companies, joint ventures - seed import and trade regulations	Egypt, Sudan, Secretariat, Ethiopia
10	Exchange information on programs, seed; regional seed meeting; tours	Yemen, Pakistan, Saudi Arabia
11	Exchange descriptions, information on weed seed	Cyprus
12	Studies of seed industry costs and economic benefits	Egypt, Algeria, Iran, Saudi Arabia
13	Develop and share technical publications	Algeria, Egypt, Sudan
14	WANA seed health testing lab; cooperation in seed health testing - list of seed health equipment and supplies - list of seed testing equipment and supplies	Pakistan, Saudi Arabia Secretariat Secretariat
15	Emergency seed stocks	Pakistan
16	WANA directory of available seed (yearly)	Iran
17	WANA consultative group, to assist with problems	Iran, Saudi Arabia

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Foreword

The WANA (West Asia and North Africa) Seed Network was established during a workshop which took place from 22-25 June 1992 in Amman, Jordan. Twenty senior seed production program managers from 12 different WANA countries participated. The workshop elected a Steering Committee for the WANA Seed Network: (1) Mr S. Abd El Wanis, Under-Secretary for Seed, Director Central Administration for Seed, Egypt; (2) Mr Abdulsattar Abdullah Kirukchi, Exec. Director, Iraqi Seed Production Company, Iraq; (3) Mr M. Tourkmani, Chef, Service du Contrôle des Semences et des Plantes, DPVCTRF, Morocco; (4) Dr Omar Abdul Yousif, Coordinator, Sudan Seed Project, National Seed Administration, Sudan, and (5) Dr T. Dabakoglu, Dep. Director General, General Directorate of Production and Development, Ministry of Agriculture and Rural Affairs, Turkey. ICARDA's Seed Unit serves as the Secretariat.

The Network has not only made recommendations for improvement of the seed sector in WANA, but also has embarked on implementation of a number of important activities. Each country participating in the network is responsible for implementation of an activity.

One of the most important aims of the network is to disseminate information on seed programs, seed production and seed related topics. The present publication fills an important gap in the seed health literature by presenting material derived from several seed pathology publications in one volume. The material was first published as WANA Seed Network Publication No. 3/93.

This publication was written on the basis of material used in numerous seed technology and seed health training courses. In addition to general background information on the importance of seed-borne diseases in seed production, it contains methods relevant for seed health testing in the context of seed certification, on seed treatment and on other topics pertinent to seed health in seed production. A list of relevant literature for further reading is given at the end. We hope this publication will serve as a useful tool in seed health testing stations as well as for future seed health training courses.

Dr A.J.G. van Gastel
Secretariat
WANA Seed Network

Important Seed-borne Fungal Diseases in Cereals and Food Legumes

Introduction

Most crops are subject to attacks by a variety of organisms: viruses, bacteria, fungi, nematodes, insects, weeds, etc. Hereafter the term pest will be used for any of these organisms, including viruses. The term pathogen is used for anything able to cause a disease, such as viruses, bacteria, fungi and, to some extent, nematodes. Diseases are deviations from the normal growth and function of a plant. The vast majority of plant diseases are caused by fungal pathogens. In most cases crops tolerate the attacks to a certain extent or react only with slight yield losses. Sometimes, however, attacks result in severe yield losses and even destruction of a crop. In general, plant diseases are recognized by certain characteristic symptoms such as yellowing, necrosis, stunting, wilt and root rot.

Similar symptoms, however, may be caused by factors other than pathogens. For example, mineral deficiency is often expressed by yellowing, pollution (acid rain) may cause necrosis in many plants, and lack of water results in wilting of the plants.

In some cases, the structures of the pathogen itself are clear signs of disease, e.g., teliospores of rust and conidia of mildew. Any part of the plant is subject to diseases, and they may occur at any stage: seed, seedling, growing plant, etc. The infection may be systemic (invading the whole plant) or restricted to the attacked parts of the plant. Pathogens are disseminated in many ways:

- wind (mildew, rust)
- water (nematodes)
- plants (viruses through *Cuscuta*)
- insects (viruses)
- man or man-made tools (nematodes)
- seeds (bunt, smut)
- cuttings (viruses).

It is generally accepted that a pathogen which is disseminated does not necessarily cause a disease. If it does, we use the term "transmitted" which includes infection. For example, spores of barley powdery mildew (*Erysiphe graminis*) are easily disseminated by wind. Transmission of this pathogen occurs, however, only if the spores meet with a susceptible barley plant and if the conditions are favorable for infection. The same holds true for pathogens that are disseminated by seeds (seed-borne pathogens). If wheat seeds are contaminated with *Tilletia contraversa* by spores of this pathogen being carried on the seed surface, this pathogen will be disseminated with the seeds to wherever they are planted; it is a seed-borne pathogen. Again we would use the term "transmitted" only if an infection actually takes place. If these seeds are planted in Egypt, most likely the pathogen will not be transmitted, because the environmental conditions there are not favorable for the germination of the spores.

For some pathogens, transmission with seed is the most important, or the only, means of transmission. An example of exclusively seed-transmitted pathogens is *Ustilago nuda*. *Tilletia* spp. are potentially seed-transmitted, the other possibility being through soil. Bean yellow mosaic virus may be transmitted in seeds or by aphids.

A seed may be a "victim" of a pathogen, so that we can say the seed itself is diseased. This is the case with many storage fungi, e.g., *Aspergillus* spp. or *Penicillium* spp., which do not cause plant diseases in the field. They need a high relative humidity (RH). In seed stored under optimum (low RH and low temperature) conditions they do not play a role. In a wider sense, storage pests such as *Callosobruchus* spp. are in this category. Seed may also be a "vehicle" of a pathogen, by carrying the pathogen without any effect on seed quality, e.g., *Tilletia caries* as a surface contamination. In other cases seed-borne pathogens may cause symptoms on the seeds (broad bean stain virus, *Ascochyta* spp.) or affect seed germination, so that the seed is both victim and vehicle for the pathogens. Usually control measures are taken against storage pests and pathogens, because they may quickly destroy a seed lot and because they are easily detected. Optimum storage conditions help in suppressing this group.

Seed-borne diseases can be looked at from different viewpoints:

- The farmer, unless he is reusing his own seed, is concerned only if yield losses result from seed-borne diseases.
- The producer of certified seed has to expect rejection of a seed lot if a certain level of infection or contamination is exceeded.
- In the international transfer of seeds, for specific pathogens a complete zero-tolerance if required, because they might not occur in all countries (quarantine).

It is difficult to obtain reliable data on the economic importance of seed-borne diseases. In some crops (cereals, soybeans) about half of the total losses due to diseases are attributed to seed-borne pathogens. We have to distinguish two types of seed infection:

1. The pathogen is present on or in the seeds and will only attack the seedlings derived from them. This presents a relatively simple relationship between percentage infected seeds, disease incidence in the field, and crop loss. Loose smut (*Ustilago nuda*) is an example.
2. The pathogen is present on or in the seeds, but may also infect the plants by other means during the vegetative stages. In this case the relationship between seed infection and crop loss is much more complicated since it depends largely on conditions during the growing season. Ascochyta blight (*Ascochyta* spp.) is an example of such a pathogen.

The number of seed-borne diseases is very large. In the literature some 500 different plant species are reported to be hosts for at least one of the more than 1300 different pathogens that are potentially seed-transmitted. There are probably only very few species that are not hosts for one or more seed-transmitted pathogens. No seed-transmitted pathogens were reported for coffee, tea and rubber. Cereals and legumes are among the

crops susceptible to many seed-borne diseases. For wheat at least 40 different pathogens are listed, for barley 31, for faba beans 23. Only those that are economically important are mentioned here.

Wheat

Tilletia caries and *T. foetida* (common bunt)

Symptoms and epidemiology. The two species have the same life cycle and cause similar symptoms; they can be controlled in the same way. They differ slightly in spore morphology: teliospores of *T. caries* are globose with reticulate walls, and measure 15 to 23 µ. Those of *T. foetida* are the same size and globose to elongated with smooth walls. Infected plants are slightly reduced in height. Diseased spikes are green-blue, and remain so into the maturity stage. The gray-brown bunt balls replacing the kernels contain masses of black teliospores, which are easily released when the bunt balls are crushed. Particularly those caused by *T. caries* are thicker than the kernels and therefore cause the glumes to spread apart slightly. The fishy odor is characteristic of bunt-infected plants. Teliospores are released from bunt balls during harvest and processing. They contaminate the surface of other seeds and the soil. They germinate if moisture becomes available. Basidiospores are formed from the promycelium; they fuse and develop secondary sporidia, which infect the coleoptile of seedlings or the growing point of young tillers. The fungus grows with the seedling and invades meristematic tissue. Yield losses correspond to the disease incidence. The disease can be serious, particularly on winter wheat in temperate climates. In addition to yield losses there is also a loss in grain quality; the strong odor can make flour unsuitable for baking.

Control. Because the disease has only one generation per year, it cannot be controlled by fungicide application in the field. In seed treatment, contact fungicides are effective. Some examples of fungicides registered for the control of common bunt are Abavit (prochloraz), Baytan (triademonol), Vincit (flutriafol) and Vitavax (carboxin + thiram). Liquid or slurry treatment is preferable over dust treatment.

The disease is considered in many seed certification schemes. Tolerances are set for field inspection (Egypt: 0.05% in fields for the production of certified seed) or for laboratory testing (Sweden: 500 spores/g seed). A cultural measure to control common bunt in Europe is early planting so that seedlings pass the susceptible stage faster.

Tilletia indica (Karnal bunt)

Symptoms and epidemiology. Kernels are typically only partially (in the embryo area) converted to bunt sori and only a few grains per ear are affected. Only in a severe infection are all kernels totally bunted. The teliospores are often covered by the intact pericarp and in these cases do not contaminate other seeds. Teliospores are 22 to 49 µ in size and globose and dark-brown. They persist in soil and on seed, and germinate in response to moisture. From the promycelium, which reaches the soil surface, many sporidia are formed. They are spread by wind and directly infect flowering wheat plants. Free water during flowering is required for infection. Bread wheat is more susceptible than durum wheat.

Control. The disease is difficult to control. The spore wall is not easily penetrated by fungicides. Teliospores in partially bunted seeds are well protected from the fungicides by the pericarp. Moreover, spores remain viable in the soil for up to 5 years. The pathogen is quarantined in Europe, North America and some countries of the Near East.

Tilletia contraversa (dwarf bunt)

Symptoms and epidemiology. *Tilletia contraversa* causes the seeds to be transformed into bunt balls like those of common bunt, and in addition causes a marked stunting. Infected plants reach only half the size of healthy ones. The fungus requires low temperatures (0-5°C) over long periods of time (1 to 3.5 months) for infection; therefore it occurs only in parts of the USA, Canada and Northern Europe. Areas that are covered by snow on unfrozen soil show particularly heavy infection. Because of the prolonged germination period, plants are infected after the seedling stage. Spring wheat is normally not affected.

The teliospores resemble closely those of *T. caries*, but are surrounded by a fragile sheath, which may be detected by staining.

Control. Because older plants rather than seedlings are infected, seed treatment fungicides are not as effective as against common bunt. Moreover, soil-borne inoculum plays a much more important role than in the case of *T. caries* and *T. foetida*. Seed certification schemes often consider this disease. Because of the dwarfing, infected plants are not easily detected in field inspection. In lab testing, the standard is usually lower than for *T. caries* and *T. foetida* (100 spores/g seed in Sweden). The pathogen is quarantined in many countries.

Ustilago tritici (loose smut)

Symptoms and epidemiology. Infected plants can be recognized after heading. They reach the heading stage slightly earlier than healthy ones. The spikelets of diseased plants are transformed into black spore masses. At first the spores are covered by a gray membrane, which ruptures soon after heading to release the spores. After a few days, only the rachis is left. Spores are dispersed by wind over a distance of up to 200 m. They germinate after landing on flowering plants of the same species and the mycelium infects the ovaries and eventually the embryo. Infections are favored by wet weather and temperatures of 16-22°C. The mycelium remains dormant in the embryo until the seed germinates. Then the fungus grows systemically and changes the flowers to masses of black teliospores. Yield losses correspond to the disease incidence. The percentage of infected seeds can increase dramatically if no measures are taken.

Control. The disease has only one generation per year, and thus it cannot be controlled by fungicide application in the field. In seed treatment, only systemic fungicides and not contact fungicides are effective because the fungus is inside the embryo. Some examples for fungicides registered for the control of loose smut are Abavit, Baytan, Vincit and Vitavax. Liquid or slurry treatment is preferable to dust treatment.

The fungus survives only in the seed; spores that do not infect flowering plants will lose their viability quickly. The isolation distance from other wheat or barley fields should be at least 150 m. Because infected seed is the only source of infection, seed certification using field inspection and/or laboratory testing can effectively control the disease.

Urocystis agropyri (flag smut)

Symptoms and epidemiology. When plants approach the heading stage the smut sori are clearly visible as long, gray-black streaks on leaves and sheaths. Like the sori of yellow rust (*Puccinia striiformis*), they develop between the veins. When the sori erupt the teliospores are liberated and dispersed by wind. Additional, less specific symptoms are stunting and excessive tillering. The teliospores are reddish-brown, 10 to 20 µ in size and often surrounded by hyaline sterile cells. They contaminate soil and seed and remain viable for up to 4 years. Germination occurs in the temperature range of 5-25°C. Subsoil coleoptiles are infected by sporidia. Infection is favored by low soil moisture (10-15%) and temperatures between 10 and 20°C.

Control. Crop rotation and seed treatment with Dividend or Baytan can control the disease. The pathogen is quarantined in many countries.

Fusarium spp. (head blight, scab, foot and root rot, snow mold)

Symptoms and epidemiology. Many different species of *Fusarium* occur on cereals. Their prevalence and the diseases they cause depend largely on climatic conditions. While in cool, humid climates *Fusarium nivale*, *F. graminearum* and *F. avenaceum* are common and result in mold and head scab, the latter two species and also *F. culmorum* cause root rots in dry climates. All of them may be seed borne. However, seed-borne inoculum is not very important compared with soil-borne inoculum.

Control. Crop rotation is a more important means of control than seed treatment. Most of the seed treatment fungicides are also effective in controlling *Fusarium* species.

Septoria nodorum (glume blotch)

Symptoms and epidemiology. The pathogen may occur jointly on the same plant with *Septoria tritici*, and together they cause the "Septoria complex". Only *S. nodorum* is transmitted by seed. It is generally considered the cause of glume blotch, while *S. tritici* causes leaf blotch. Glumes as well as leaves show elongated lesions. Characteristic are the pycnidia, which measure 100-200 µ. They can be seen as dark spots in the lesions. They contain numerous hyaline 1 to 3-septate pycnidiospores measuring 2-4 x 15-32 µ. The perfect stage is *Leptosphaeria nodorum*, with asci and ascospores in globose perithecia which develop mostly on affected glumes. The fungus survives on seeds as well as on straw and stubble. Being a facultative parasite, it can even multiply there. For infection, wetness is required. Wet and windy weather is ideal for disease development. Since normally the heading of wheat in semiarid climates occurs in the dry period, *S. nodorum* is not important there, in contrast to *S. tritici*, which causes leaf blotch.

Control. Apart from crop rotation and stubble burning, seed treatment can control the disease. No-till practice increases the disease risk.

Helminthosporium tritici-repentis (tan spot)

Symptoms and epidemiology. Initial symptoms are tan-brown flecks, which expand into lens-shaped, tan blotches up to 12 mm long. In a moist chamber (see Isolation of Pathogens section) olive-black conidiophores with cylindrical, 4 to 7-septate conidia measuring 12-21 x 45-200 µ develop. Later, on stubble, the perfect stage develops in the form of pseudothecia with asci and ascospores. This stage is called *Pyrenophora tritici-repentis*. Infections may occur from conidia or ascospores, or even from mycelia. Leaf wetness is required. Compared with inoculum on host debris, the seed-borne inoculum is less important.

Control. Stubble management and fungicide application during the season reduce the disease incidence.

Helminthosporium sativum

Symptoms and epidemiology. This pathogen causes a wide range of symptoms — spot blotch, root rot, seedling blight — that depend, among other factors, on the time of infection. Seed-borne inoculum often leads to the death of seedlings or to extensive brown lesions. Olive-brown conidia develop on conidiophores and are 12-20 x 60-120 µ large with 3 to 9 septa. The perfect stage, which is rarely found, is called *Cochliobolus sativum* and is characterized by pseudothecia of 300-400 µ diameter. The fungus survives on plant debris and seeds.

Control. Seed treatment reduces seedling infection, but does not prevent infections at later growth stages. The disease is frequently associated with stressed crops, e.g., water stress. Crop rotation minimizes the inoculum from previous crops.

Barley

Ustilago nuda (loose smut)

Symptoms, epidemiology and control are the same as for loose smut of wheat. It is a distinct species, which means that spores from diseased wheat plants will not infect barley or vice versa.

Ustilago hordei (covered smut)

Symptoms and epidemiology. As with the true loose smut, infected plants can be recognized after heading. They reach the heading stage later than healthy ones and may fail to emerge completely because they are trapped in the flag leaf sheath. Occasionally, the sori may develop on leaf blades, resembling the symptom of flag smut on wheat. The spikelets of diseased plants are transformed into black spore masses. In contrast to loose smut, the gray membrane remains intact until harvest. The diseased plants are therefore

easily recognized at maturity. The disease cycle resembles that of common bunt of wheat (*Tilletia caries* and *T. foetida*) in that the seeds are contaminated on the surface with teliospores. The soil also may be contaminated. The olive-brown to brown teliospores measure 5-8 µ in diameter and have a smooth surface. When they germinate they form a promycelium and four sporidia, which produce numerous secondary basidiospores. From these the dicaryotic mycelium originates, which infects the germinating seed through the coleoptile. The mycelium remains in the growing point until flowering, then penetrates the ovary and forms the teliospore mass in place of the seed.

Control. The disease is rare in areas where seed treatment is normally practised. As with most other smut diseases, yield losses correspond to the disease incidence if no control measures are taken. All standard seed treatment fungicides are effective in controlling *Ustilago hordei*. Liquid or slurry treatment is preferable to dust treatment.

Helminthosporium sativum (spot blotch)

It is the same pathogen as already described for wheat.

Helminthosporium teres syn. *Drechslera teres* (net blotch)

Symptoms and epidemiology. Net blotch is named for the typical netlike symptoms that develop from the initial spots or streaks. Eventually, the affected parts of the leaf turn brown and the surrounding tissue becomes chlorotic. Conidia are 1 to 11-septate, cylindric with round apical cells and measure 15-23 x 30-175 µ. Globose pycnidia also are formed with numerous small pycnidiospores. The perfect state with pseudothecia is called *Pyrenophora teres* and is formed toward the end of the season. They overwinter on the host tissue. Also important is the seed-borne mycelium. Secondary infection occurs by conidia, which are formed after a period of darkness, relative humidity of close to 100%, and temperature between 15 and 25°C. Conidia are dispersed by wind, and infection requires leaf wetness.

Control. Stubble management reduces initial inoculum, particularly if seed-borne inoculum is eliminated by seed treatment. Most fungicides are effective against *H. teres*.

Helminthosporium gramineum syn. *Drechslera gramineum* (stripe disease)

Symptoms and epidemiology. Typical yellow stripes may appear on seedling leaves and on the newly formed leaves. Infected tissue turns necrotic and dies. Infected plants are usually dwarfed, and often ears do not develop at all or are deformed. If seeds are formed, they are often shriveled. Conidia resemble those of *H. teres* and measure 11-24 x 30-110 µ. The perithecia of the perfect state *Pyrenophora graminea* are rarely formed on barley straw in autumn. The fungus survives only in seeds, and has only one generation per year (no secondary spread). Infection of the seedling occurs at temperatures below 12°C. Seed infection can occur from before head emergence to almost maturity. Since high relative humidity is necessary for sporulation, the disease is a problem only in areas with rainfall or sprinkler irrigation during heading.

Control. Because of the strictly seed-borne nature of this disease, healthy seed is extremely important. It is achieved either by seed production in semiarid areas without sprinkler irrigation, or by seed treatment. Vitavax is not as effective as newer fungicides like Baytan Universal.

Rhynchosporium secalis (scald)

Symptoms and epidemiology. Lesions of scald are easily recognized. The center is dry and white-gray, as if scalded. Margins are dark brown and may be surrounded by chlorotic areas. Conidia are small (2-4 x 12-20 μ), hyaline, 1-septate and cylindric to ovate. The fungus survives in infected seed and plant debris.

Control. Stubble management and seed treatment control the disease. Foliar treatments also are effective.

Legumes (Faba Bean, Chickpea, Lentil, Pea)

Among the legumes, lentil is the crop which has relatively few diseases. Important seed-transmitted pathogens are *Ascochyta lentis, Fusarium* spp., and some viruses. Yield losses due to these pathogens are usually less than in other legumes.

Ascochyta fabae, A. rabiei, A. lentis, A. pisi, A. pinodes, A. pinodella (blight, leaf and pod spot)

Symptoms and epidemiology. The first symptoms are elongated lesions, in faba beans up to 10 mm long, on the primary foliage leaves of seedlings developing from infected seed, with characteristic chestnut-brown margins and grayish centers. Lesions develop first on the tips and margins of leaves, gradually spreading toward the main veins of the compound leaves and petioles. Pycnidia are easily seen in older lesions. In severe attacks, foliage is totally destroyed. Elongated red-brown stem lesions develop which weaken the stem and may cause lodging. Pod and stem lesions are usually darker and more deeply sunken than those on leaves. Infected seeds may be covered with circular dark brown lesions.

The pathogens belong to the imperfect fungi, order Sphaeropsidales. The conidia are hyaline, straight or slightly curved, mono- to triseptate and measure 16-24 x 3.5-6 μ. They are formed in yellow-brown pycnidia of 200-250 μ with a papillate ostiole.

The pathogens can remain viable on seed for up to 3 years and for 3-4 years on infected crop residue. Short-range spread occurs by rain splash, which transmits the pycnidiospores that ooze from pycnidia in wet weather. The disease can spread at least 120 m. The UK Seed Certification Scheme therefore recommends that seed crops should be isolated by at least 50 m from other bean crops and from fields that carried a faba bean crop the previous year. A disease cycle from infection to pycnidia formation takes 12-18 days. As with many fungi, the rate of epidemic development from infected seed and re-establishment of seed infection from diseased plants varies considerably from year

to year and from site to site depending on environmental conditions, particularly moisture.

In a greenhouse study it was found that more than 2 days of high humidity following inoculation are required for a significant infection. In this study, older leaves seemed to be more resistant than younger leaves. For successful infection of seed there must be rain early in the development of a crop so that the pathogen is carried sufficiently high in the leaf canopy to re-infect the pods and seeds as they develop. The time of harvesting also may be important, with an early harvest preventing high seed infection rates. In Germany, samples from areas with consistently dry climatic conditions showed a lower infection rate. A strong correlation of seed infection with both foliar and pod infection was found.

Control. In the UK, the introduction of a seed certification scheme considerably reduced the incidence of *A. fabae*. The cultivar Minor was freed from the disease. Seed treatment with benomyl and thiram slurries reduced the infection to less than 0.1%, but affected seed germination.

The application of chlorothalonil (Bravo 500 with 50% a.i.) at 0.1% a.i. in water, using 2.5 kg a.i./ha was found effective. Also five sprays with Benlate 50 (0.1%) or Dithane M-45 (0.25%) reduced the disease incidence on pods and increased yield. Spraying a standing crop, however, has only limited success compared with seed treatment.

Although some authors reported that *Ascochyta* species could be eradicated by seed treatment, others have proved the contrary. Captan, benomyl and thiram did not significantly affect the amount of seedling or adult plant infection. Thiram was found to be ineffective on *Ascochyta* beneath the seed coat. Also, benomyl, captafol, captan, chlorothalonil, metiram and thiabendazole failed to eradicate the fungus. Although soaking of the seeds in a 0.2% a.i. suspension of benomyl-thiram for 8 hours and subsequent air-drying of 48 hours gave complete control in laboratory tests, up to 2.9% infected seedlings emerged in the field. Often, various seed treatments were effective in the laboratory, but did not provide complete control in the field.

Botrytis fabae and *Botrytis cinerea* (chocolate spot and gray mould)

Symptoms and epidemiology. The chocolate spot disease causes rust-colored to dark-brown or gray, more or less circular spots up to several mm in diameter on the leaves, petals and pods, but elongated on the stems in dry conditions. Under wet or humid conditions, the fungus spreads rapidly, both inter- and intracellularly, producing both pectolytic enzymes and non-enzymic phytotoxins. Lesions blacken, increase in size and coalesce, leading to a destructive blight. This is referred to as the aggressive stage of the disease when most of the leaves drop off and the stems bend, causing the plants to fall over. The fungus sporulates on the dead tissues. Under alternately wet and dry conditions, concentric rings may be formed as the growth of a lesion starts and stops. These lesions are distinguished from those of *A. fabae* by the absence of pycnidia. Infected young pods are stunted and the ovules develop irregularly or not at all. Mature pods become blackened, with the discoloration extending to the seeds. Black sclerotia are

formed in stem tissues when *B. fabae* has become well established, but are mostly found in culture.

Botrytis fabae belongs to the Ascomycetes; no teleomorph has been described. It is easily distinguished from *B. cinerea*, which also attacks faba beans, by its larger conidia, measuring 15-24 x 11-18 μ compared with 9-12 x 7-10 μ. In contrast to *B. cinerea, B. faba* produces sclerotia abundantly in culture. Their size is variable; mostly 1-1.7 mm and rarely up to 3 mm.

The pathogen mainly overwinters as sclerotia in plant debris. Conidia produced in humid weather infect the seedlings. Other possible ways of overwintering are as hyphae living saprophytically, or as resting mycelia. Alternative sources of inoculum are volunteer faba bean and *Vicia sativa* plants. The disease is spread by wind-borne conidia that may travel over large distances. Both wind and rain play a role in the dispersal, with wind being more important. Infection requires a relative humidity (RH) of at least 86%; a water film is not necessary. Lesion growth below 70% RH is very slow, and is directly proportional to RH between 70 and 100%. The optimum temperature ranges between 15 and 22°C, with the minimum and maximum around 4 and 30°C respectively. The minimum RH for conidia production is 80-90%. Older leaves are more susceptible and produce more spores. Pods, however, become more resistant with age. The "honeydew" excretion of aphids favors the disease.

In China, a highly significant correlation between disease severity and the days of continuous rainfall (> 0.1 mm/day) during early to mid-April (at the flowering stage of the crop) was found.

In England, *B. fabae* was isolated from commercial seed lots; however, most infected seeds gave rise to healthy plants. Moreover, the pathogen could not be detected after seed storage for 9 months. It seems that seed-borne infection does not play an important role in the transmission of chocolate spot.

Botrytis cinerea causes gray mould in many crops and contributes to the chocolate spot syndrome on faba beans, but is apparently less aggressive than *B. fabae* and therefore less important. Gray mould can be easily identified in many host plants (other vegetables, strawberries, grapes) by the abundant off-white to gray mycelium covered with dark conidia. It can cause severe pod rotting, with the fungus infecting through flower parts.

Botrytis cinerea is the conidial (imperfect) state of *Sclerotinia fuckeliana*. Sclerotia are black and their size varies between 1 and 5 mm, depending on the isolate.

The colorless to brown macroconidia are ellipsoidal to ovoid and measure 6-18 x 4-11 μ. They are produced on dark, branched conidiophores of up to 2 mm long and 16-30 μ thick. Microconidia develop from clusters of phialides.

Conidia are disseminated by wind or in rain splashes. Saprophytic mycelia or sclerotia on plant debris can overwinter. Spores and mycelia may be seed borne on 46 different species.

Control. Foliar sprays of benomyl are more effective than iprodione, prochloraz or thiabendazole. Weather conditions play an important role. Benomyl sprays are likely to be beneficial only in wet years with severe damage of chocolate spot. The continued use of benomyl will encourage the spread of benomyl-resistant strains.

The effect of benomyl seed treatment (0.8 g a.i./kg seed) is largely attributed to the protection against early infection and, to a lesser extent, to the control of seed-borne inoculum.

Removal of debris from a previous bean crop, either by burning or soil cultivation, will also reduce the initial inoculum.

Many fungicides are effective in controlling *B. cinerea* (benomyl, carbendazim, captan, dichlofluanid, iprodione, thiram); however, attention should be paid to the development of resistance. Cultural measures such as planting in wind-exposed sites, early and not-too-dense sowing, increasing pH to at least 6.5 and burying plant residues of the previous crop are the most important means of control.

Fusarium wilt and other *Fusarium* diseases

Symptoms and epidemiology. The symptoms of Fusarium wilt are a light vein-clearing on the older leaves, accompanied by chlorosis of the lamina and/or wilting, which then progresses to younger leaves. Often the symptoms start unilaterally on some leaves. Affected parts of the plant turn brown; longitudinal necrotic streaks appear on the stems and spread toward the stem apex. Browning of the vascular tissue can be seen in sections of roots or stems. Typically the disease starts in a crop as foci which spread gradually. Affected plants may die prematurely.

Fusarium oxysporum is an abundant saprophyte in soil and on organic matter. Some strains have specific pathogenic activity. Some are poorly specialized and cause seedling blight, necrosis or rot. About 80 formae speciales (pathotypes specific to species) have been identified, and several are subdivided into races (specific to cultivars within a species). Except for the Gramineae, most families are attacked. Chickpeas and lentils are the legumes suffering most from wilt.

In culture, microconidia are always present. They are oval-ellipsoid, mono- or bicellular, and formed on short, unbranched phialides. Their size is 5-12 x 2.2-3.5 μ. Macroconidia are abundant and usually 3- to 5-septate, fusoid, slightly curved and often with a foot-shaped basal cell. They form first on individual phialides, then form sporodochia. According to septation, their size varies from 27-66 x 3-5 μ. Chlamydospores are solitary or in short chains. No teleomorph is known. Growth on potato dextrose agar (PDA) is rapid, and colony colors may vary from dark blue or dark purple when sclerotia are abundant to cream or tan or orange when sporodochia are abundant.

Fusarium wilts are classic soil-borne diseases, with infected plant debris being the main source of inoculum. Chlamydospores can persist in an inactive form for several years. The pathogens also can be spread in irrigation water, with infected plants, but rarely by

seed. Disease development is favored by acid soil, potassium deficiency, ammonium nitrogen fertilizer and high temperatures.

The symptoms caused by *Fusarium solani* are similar to those caused by *Rhizoctonia solani*, namely a brown-black discoloration of roots and stem bases, followed by yellowing of tips and borders of older leaves, which then turn brown. Occasionally young leaves die without showing disease symptoms. High temperature and water stress aggravate the disease. Occasionally, dusty white or pinkish spore masses can be found on the stem base and upper part of the root.

Fusarium solani has a very wide host spectrum. Micro- and macroconidia are produced on 45-80 x 2.5-3 μ long, irregularly branched phialides. The oval to cylindrical microconidia measure 8-12 x 2-4 μ and are sometimes 1-septate. The macroconidia are 1- to 6-septate and measure 35-55 x 4.5-6 μ. Their dorsal and ventral surfaces are parallel for most of their length, and the apical cell is blunt and rounded. The size of the globose chlamydospores is 9-12 x 8-10 μ. Typical colors of a colony on PDA are grayish-white to bluish-brown, but never orange. The perfect state is *Nectria haematococca* Berk & Br.

Other species that are reported to occur on faba beans include *F. equiseti*, *F. moniliforme*, *F. solani*, *F. acuminatum*, *F. avenaceum*, *F. culmorum* and *F. graminearum* f.sp. *fabae*. They cause mostly root rots, but *F. graminearum* f.sp. *fabae* is reported to cause wilt in China.

Control. Fusarium wilt is difficult to control. *Fusarium oxysporum* rapidly developed resistance to benzimidazole fungicides. Crop rotation of 4-5 years was found to be an efficient control measure.

Fusarium species causing seedling damping-off can be controlled with benomyl + thiram, carboxin + captan, or pentachloronitrobenzene (PCNB) at 4 g/kg seed. A crop rotation avoiding all legume species for 6-8 years is the main means of control.

Peronospora viciae, *P. lentis*, *P. pisi* (downy mildew)

Symptoms and epidemiology. Younger leaves show light gray, greenish spots which enlarge into lesions covering more than half of the leaf area. Older lesions turn brown, desiccate, and lead to leaflet distortion. Light gray sporangiophores and sporangia can be observed on the undersurface, occasionally also on the upper surface. No infection on pods and stems of faba beans is reported. A systemic infection leads to dwarfing and distortion of either the entire plant or of only a few nodes.

Besides faba bean, *P. viciae* attacks vetch, pea and sweet pea (*Lathyrus odoratus*). Sporangiophores measure 160-750 x 3-13 μ and are unbranched for at least two-thirds of their height. Each end bears a single oval to elliptical sporangium of 15-30 x 15-20 μ. Oospores are spherical, light brown to deep yellowish pink with a reticulate surface and measure 25-37 μ in diameter.

Spread of the pathogen is via rain and wind-transported sporangia for which optimal germination occurs at 4-8°C. Formation of sporangia requires 90% RH for more than 12 hours and temperatures below 15°C. Therefore it is not surprising that the pathogen is mostly found in cool and moist climates. Oospore production is favored by temperatures in excess of 20°C. Oospores may survive in the soil for 10-15 years.

Seed transmission is doubtful; however, there seems to be evidence that long-range transport is likely to be by seed infection.

Control. Control of *P. viciae* on peas can be achieved by treatment with metalaxyl at rates of 35-70 g a.i./100 kg seed. Sprays of metalaxyl, alone or with mancozeb, considerably reduce disease incidence and severity. Furthermore, deep tillage and extended crop rotations are recommended.

Uromyces viciae-fabae (rust)

Symptoms and epidemiology. The disease can be easily recognized by rust-colored pustules on leaves and stems of faba bean and other legumes, which are formed by urediospores. Later in the season, lesions of darker teliospores are produced mainly on stems and petioles.

Uromyces viciae-fabae is a macrocyclic autoecious rust with spherical hyaline aeciospores (18-16 μ) and elliptical yellowish urediospores (22-28 x 19-22 μ). Urediospores have a finely echinulate wall of yellow to sienna color. The teliospores are similar in shape (25-40 x 18-26 μ), but with chestnut-colored walls and yellow, 100-μ-long pedicels. A number of races have been recorded. *Uromyces viciae-fabae* also infects pea, lentil and wild and cultivated species of *Vicia* and *Lathyrus*.

Aeciospores may overwinter only in Mediterranean climates, but not in northern regions. Urediospores and/or teliospores may remain viable for 1-2 years and can be disseminated with the seeds. High atmospheric humidity favors infection. Late irrigations may promote the severity of the disease.

Control. Benomyl at 1 kg a.i./ha at 14-day intervals from emergence to flowering prevented natural infection of faba bean rust. Benodanil, fenpropimorph, maneb, mancozeb, maneb + mancozeb, propiconazole, thiram, triadimefon and zineb poly + tridemorph also were found effective against rust. The best results for control of a combined infection of rust and chocolate spot on faba bean were achieved with maneb + mancozeb, which also gave the highest yield increase.

Production of Healthy Seed

Healthy seed — seed that is pathogen and pest free — is a prerequisite for a high-yielding crop. Seed health is a component of quality seed, as are viability, vigor and purity. In seed production schemes usually all efforts are made to supply the farmers with pure seeds of a high germination capacity. There are different ways to achieve healthy seed:

- seed production in areas with low pressure from pests and diseases
- seed production under effective pest control
- field inspection schemes
- seed treatment
- seed health testing.

Normally a combination of these methods will be used, since it is not feasible to rely on only one method. In many cases it will not be possible to select areas that are completely free from pests, but problems can be avoided by careful site selection. Production of seed potatoes is recommended for windy areas, where important vectors of viruses, namely aphids, do not play a significant role. During multiplication effective control can be exercised against a number of pests such as *Ascochyta* spp. (blight), *Ditylenchus dipsaci* (stem nematode), *Bruchus* spp. (bruchid weevils) and others. For viruses, there is only the possibility of vector control, mostly with insecticides. Seed-transmitted pathogens with only one generation per year, such as *Tilletia caries* and *T. foetida* (bunt) and *Ustilago* spp. (smut), cannot be controlled in the field.

Field inspection is carried out regularly for varietal purity, but is also very important for seed health. Usually diseases express symptoms in the field, and so an idea about the health status of the seeds to be expected can be obtained. However, some experience is required, especially when dealing with low incidence and severity levels. Plants with viral diseases may be completely symptomless in the field, but the virus can be transmitted in the seeds. Some other diseases, such as Karnal bunt (*Tilletia indica*), might be difficult to detect in the field. For seed health testing a number of laboratory methods can be used to determine the health status of seeds.

Seed treatment is suitable to control a number of fungal diseases, and to some extent also insects, nematodes and bacteria. Care must be taken to chose a dosage sufficient to eradicate the pathogens, but not to kill the seeds.

Seed-borne Bacteria

Introduction

Bacteria are single-celled organisms with no chlorophyll. Those causing plant diseases are about 0.5 μm wide and 1-3 μm long and rod-shaped. Many have flagella; either one or more at one end, or at points over the surface. They can be seen with the help of a microscope with 100X objective and oil immersion. The disease symptoms they cause in plants include mainly wilt, yellowing, leaf spot, rot, scab and galls. A preliminary test for bacteria is the Gram stain. Bacteria will react either positively (retain the dye applied and have a dark violet color) or negatively (lose the dye when washed with alcohol and have a pale red color). All plant-pathogenic bacteria except *Corynebacterium* are Gram negative. Almost all crops are host to one or more pathogenic bacteria, exceptions are cocoa, coffee, and rubber.

Spread of Bacteria

Many bacteria are seed borne (Table 1). Other means of dissemination are by tools (*Corynebacterium michiganense* in tomatoes by cutting side branches, or *Agrobacterium tumefaciens* in grapes and many trees by pruning), by rain (*Pseudomonas syringae* syn. *Ps. mors-prunorum* in cherries during flowering), by cuttings (*Xanthomonas begoniae* and *X. pelargoniae* in horticultural plants), or by insects (*Xanthomonas campestris* pv. *translucens* with aphids). Bacteria cannot enter plants actively. They need wounds, or they enter through natural openings like stomata or nectaries. They need free water on the host's surface.

Table 1. Important seed-borne bacteria.

Pathogen	Disease	Host
Pseudomonas phaseolicola	Halo blight	Beans
Ps. lachrymans	Angular leaf spot	Cucumber
Ps. syringae pv. *pisi*	Bacterial blight	Peas
Xanthomonas campestris pv. *campestris*	Black rot	*Brassica*
X. c. pv. *translucens*	Bacterial leaf streak, black chaff	Wheat, barley
X. c. pv. *oryzae*	Bacterial leaf blight	Rice
Xanthomonas malvacearum	Angular leaf spot	Cotton
Corynebacterium michiganense	Bacterial canker	Tomato

Detection of Bacteria in Seeds

1. Growing-on test (e.g., for *Xanthomonas malvacearum* in cotton): seeds are planted in sterile sand or vermiculite and seedlings are evaluated for symptoms.

2. Plating of washing suspension (e.g., for *Xanthomonas campestris*): seeds are immersed in sterile saline solution in refrigerator, then dilutions are plated on XTS medium.

3. Plating of ground seed in water (e.g., for *Pseudomonas syringae* pv. *pisi*): surface-sterilized seeds are ground in sterile water and dilutions are plated on King's B medium.

Control of Seed-borne Bacteria

As for other bacteria, or even other pathogens, the use of resistant varieties can be quite successful, if they are available. Seed can be disinfected with NaOCl, or other surface sterilizers. Production of "pathogen-free" seed is important. The use of antibiotics, although effective, is restricted in many countries.

Seed-borne Nematodes

Introduction

Nematodes are also called eelworms because of their long, threadlike body. Most of them live in the soil, and they are the most abundant multicellular organisms in soil. In 100 ml of arable soil, usually several thousand nematodes can be found. At present, about 17 000 different species are known and new species are described every year. Fortunately, fewer than 1000 of them are economically significant plant pests; several thousand are parasites of man and animals, and the vast majority live as predators on microorganisms. Some species are being tested for use in biological control of plant-parasitic fungi, others as parasites in insects (*Neoaplectana carpocapsae*).

Most plant-parasitic nematodes are about 1 mm long. They all have a stylet, which is used to pierce plant cells and to suck out their contents. On the basis of plant parts that are parasitized, we can distinguish root nematodes (ectoparasites and endoparasites), stem nematodes and leaf nematodes.

Plants are affected in different ways: by direct withdrawal of nutrients, which leads to poor growth and lower yields, and also by nematode transmission of viruses, bacteria and fungi.

Plant-parasitic nematodes are widely distributed in all agricultural areas of the world. There is hardly a crop which is not attacked by at least one nematode species. Many of them are extremely polyphagous, i.e., they occur on many different host plants, for example *Ditylenchus dipsaci* or *Meloidogyne javanica*, the latter with over 700 host plants. Some nematodes are known to form races that specialize in one or only a few crops, e.g., *Ditylenchus dipsaci*.

Many of the nematode pests are indigenous species, but despite precautions being taken in quarantine, some have moved from their original area of distribution to new areas. Some examples of recent introductions of nematodes are given in Table 2.

Table 2. Spread of plant-parasitic nematodes to new areas.

Nematode	Common name	Introduced to
Aphelenchoides besseyi	White tip nematode	East Africa, Central Africa
Globodera rostochiensis	Potato nematode	Japan, India, New Zealand, Philippines
Heterodera glycines	Soybean nematode	North America

Quarantine for nematodes poses special problems:

- They are too small to be detected by a visual inspection
- Species are difficult to identify
- They may come as concomitant contamination, e.g., potato nematode cysts may be found in soil mixed with wheat seeds.

Spread of Nematodes

Most nematodes are capable of active movement. In soil, this depends largely on the size of soil pores and on the water content of the soil. On average, nematodes move 0.1-0.5 cm/day; *Ditylenchus dipsaci* is a fast "runner", covering 10 cm in 3 hours. The spread from one field to another occurs most likely by adhesion to farm implements, or in flood and irrigation water. For long-distance spread, soil is the most important vehicle. Nematodes are moved unintentionally in the soil of potted plants, or in other media such as Peruvian guano, which probably transported the potato nematode to Japan in the 1960s. The unintentional movement of soil occurs in many ways, for example with bulbs, seeds, and other planting material, and also on car tires and the shoes of travellers. For most nematodes, e.g., *Xiphinema* or *Pratylenchus* spp., soil is the only effective way of dispersal. Important seed-borne nematodes are listed in Table 3.

Table 3. Important seed-borne nematodes.

Nematode	Common name	Host
Ditylenchus dipsaci	Stem nematode	Faba bean, alfalfa, clover, narcissus, onion, strawberry, oats, many others
Aphelenchoides besseyi	White tip nematode	Rice, millet, maize
Aphelenchoides ritzemabosi	Chrysanthemum leaf nematode	Chrysanthemum
Anguina tritici	Wheat gall nematode	Wheat, rye

Seed-borne nematodes are more likely to become established in new areas than others. This is primarily because a suitable host exists in the new environment, whereas nematodes moved with soil may not find a suitable host for a long time. Some ectoparasitic nematodes, however, are known to have an extremely wide host range. Furthermore, seed-borne nematodes are known to survive in seeds for several years, e.g., *Anguina tritici* for 32 years at 5°C.

Whether or not a nematode will become established in an area depends also on the prevailing temperature. *Radopholus similis*, the burrowing nematode, occurs mostly in tropical and subtropical countries on banana, citrus, black pepper, citrus, sugar cane,

maize and vegetables. Although some of these host plants are grown in temperate areas, the chance of introduction of *R. similis* is small because of its temperature requirements of 24-32°C. It has been found in glasshouses in Europe, but could be eradicated from there because of the contained conditions. *Pratylenchus penetrans*, on the other hand, is largely confined to temperate climates.

Detection of Nematodes in Seeds

Ditylenchus dipsaci

The nematode may survive externally as well as inside faba bean, clover or alfalfa seeds (seed transmission in cereals is doubtful).

Method Augustin (qualitative)

1. Keep seeds at 10°C covered with water for 24 hours.
2. Remove seed coat, cut seeds open and leave at 10°C for 72 hours.
3. Check for extracted nematodes.
4. For negative samples: crush seeds in blender with water (approximately 700 ml/100 seeds), let stand and stain approximately 20 ml sediment with 5 ml 1% lactophenol-cotton-blue solution. After 12-14 hours, sieve sample over 25 µm sieve and examine for stained nematodes.

Misting Method (ISTA, only active specimens are recovered)

1. Set up two funnels as in Figure 1 and place nematodes on a sieve in the top funnel.
2. Spray with a mist of water.
3. After 48 hours, remove glass tube and examine for nematodes.

Anguina tritici

Galls are separated from other seeds either by calibration (they are smaller than healthy seed) or by floating on water (they are lighter than healthy seed). They can be distinguished from bunt balls by the lack of fishy odor and by the harder endosperm.

Control of Seed-borne Nematodes

As for most nematodes, the best control method is crop rotation. *Anguina* also can be controlled by seed cleaning or by hot water treatment (54°C for 10 min). Attempts to control *D. dipsaci* in faba bean seeds by fumigation failed, since the dose required for fumigation is higher than the seeds can tolerate without loss in viability.

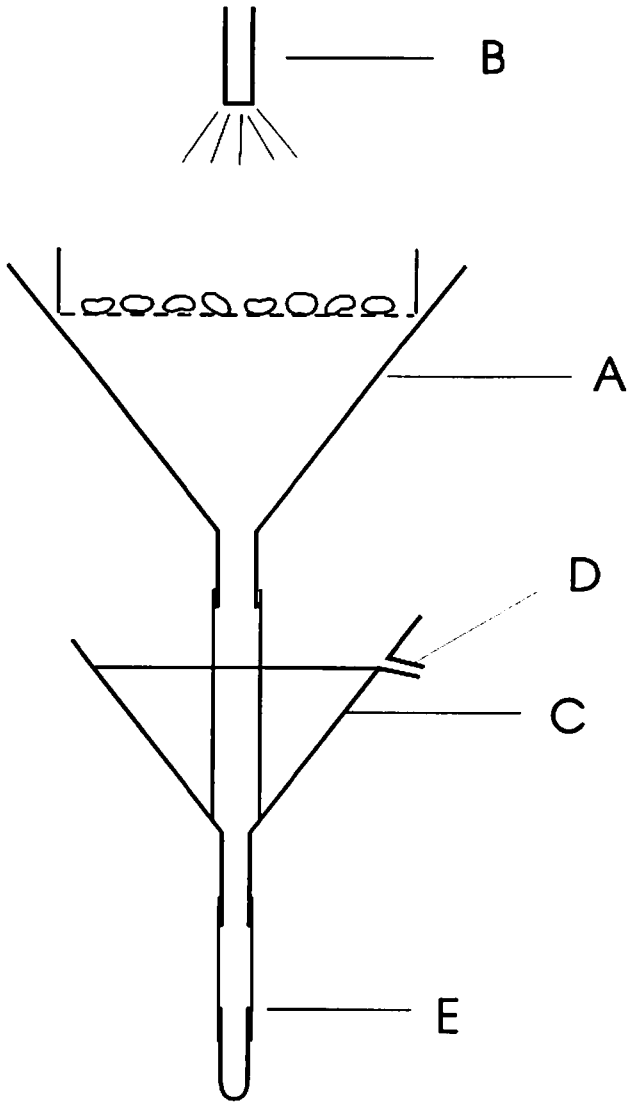


Fig. 1. Modified misting method for extraction of stem nematode in seeds. A, funnel with sample on the sieve; B, mister; C, funnel with overflow pipe (D); E, glass tube fitted by rubber tubing. (Source: ISTA Handbook of Seed Health Testing, Working Sheet No. 57, by G. Caubel.)

Seed Health Testing

Introduction

There are different methods available for testing seed for pathogens. Most of them are destructive to the seed. For reliable, reproducible results a sufficiently large sample is required. For different pests/pathogens, different methods are appropriate. Inspection and testing of soil and plant debris may give information on the general health status of the crop, but not necessarily on the inoculum in the seeds. A brief description of some commonly used test methods is given below.

Direct Inspection

This is a non-destructive method of health testing. The seeds are checked under a low-power stereoscopic microscope at a magnification of 40 to 60X. Fungal structures such as bunt balls (*Tilletia* spp.) or sclerotia of ergot (*Claviceps purpurea*) can be identified easily. In some cases pathogens such as *Ascochyta* spp. may cause discoloration or deformation of the seed. However, because the symptoms are generally non-specific, other tests are required to confirm the diagnosis. Moreover, symptomless seeds are not necessarily free from pathogens. The direct inspection method can be considered only a preliminary test, which has to be supplemented by other tests.

Other pests/pathogens that can be detected by this method include Broad Bean Stain Virus, parasitic weed seeds (*Orobanche* spp. and *Cuscuta* spp.), other weed seeds, nematode cysts (*Heterodera* spp.) and storage insects.

Examination of Washing Suspension

This test reveals contamination with spores adhering to the seed surface. A seed sample is placed in a container (reagent tube or flask) and covered with water. For a quantitative analysis the seed/water relation must be constant, e.g., for 50 g seeds, 50 ml water, or for 200 g seeds, 200 ml water. The sample is shaken vigorously so that all spores will be suspended in the liquid. This can be facilitated by adding a few drops of detergent. Depending on the contamination level expected and the required sensitivity of the test, the liquid is examined directly at 100-400X magnification under a compound microscope, or first centrifuged at 2000 rpm for 10 min. After centrifugation, the sediment is diluted in 0.5 ml water. For the evaluation a counting chamber (Fuchs-Rosenthal Hemacytometer) can be used. In some cases a rough idea about the degree of contamination (zero, low, medium or high) will be sufficient and the area of a cover glass (18 × 18 mm) can be evaluated for spores.

With this method mainly spores of *Tilletia* spp. and *Urocystis* spp. can be detected. For *T. indica* (Karnal bunt) testing, the seeds are first soaked in 0.2% sodium hydroxide for 24 hours. This will allow checking of the seeds for shiny black symptoms of partial infection (teliospore masses) in the embryo area. The liquid is treated as indicated above.

Blotter Test

Staff from seed testing laboratories will be familiar with this method, since it is similar to the standard germination test. Seeds are placed on moistened absorbent paper in petri dishes, germination boxes, or any other container that can be sealed to prevent evaporation of water. Paper towels, filter paper or cotton pads can be used as the substratum. For routine testing it is advisable to have standard paper cut to a suitable size, and thick enough to absorb moisture for the duration of the test (Schleicher & Schüll filter paper No. 8272 for large-seeded crops (legumes) or pleated strips for cereals). A constant amount of distilled water, depending on the substratum used, size of container, crop, etc. will be added.

The seeds are incubated for 7 to 10 days at 20°C. Near-ultraviolet light at a cycle of 12/12 hours will stimulate sporulation. It should be operated only after 4-5 days incubation at 12/12 hours white light/darkness.

Evaluation is done under the stereoscopic microscope; confirmation under a compound microscope will be necessary, especially until the examinor is familiar with the appearance of the different pathogen species on seeds. Not every fungus growing on seeds is a pathogen, in fact most of them are not! Surface sterilization suppresses saprophytes but also affects the pathogens. It is not generally recommended.

Evaluation is easier when germination is suppressed. Some fungi, e.g., *Helminthosporium* spp., tolerate very low temperatures. In tests for these pathogens seeds are incubated for 24 hours at 20°C, then placed overnight in a deep-freezer at -18°C, and afterwards incubated again at 20°C for 7 days.

Agar Plate Test

This method requires some equipment and skills for working under sterile conditions. With specific media, reliable results can be obtained. It is essential to avoid contamination with saprophytes. Proper sterilization of petri dishes, media, working area, seeds and tools is important.

Petri dishes. If glassware is used, it has to be thoroughly cleaned and sterilized. The petri dishes can be placed in special metal containers, or simply wrapped in aluminum foil. Either dry hot air (oven, 180°C for 20 min) or moist heat (autoclave or pressure cooker, 15 psi for 15 min) can be applied. Disposable plastic petri dishes come pre-sterilized, packed in plastic bags of 10.

Media. Sterilized in autoclave as indicated above. Use distilled water for media preparation. **Flasks should be filled only to 50% of capacity.** At about 45-55°C the media can be poured into plates. Care should be taken that this is done in a clean working area and that the rim of the flask is sterilized over a flame. Open the petri dishes carefully to avoid breathing on them. Media should be evenly distributed in the petri dish. After the medium has solidified, store the petri dishes upside down, preferably in a refrigerator.

Working area. A laminar air flow bench is ideal because it gives a perfectly clean area. If that is not available, a corner away from drafts, thoroughly cleaned with 70% alcohol, will do.

Seeds. Surface sterilization of seeds in sodium hypochlorite (3-5 min in 10% solution of commercial Clorox) suppresses saprophytes.

Tools (needles, tweezers, etc.) can be sterilized by dipping into 70% alcohol and then heating to redness over a Bunsen burner or alcohol burner flame.

Suitable media for seed health testing

Potato-Dextrose Agar (PDA) and Malt Extract Agar (MEA). Dehydrated culture media are available from Merck, Difco, etc., who make all-round media for isolation of fungi. Follow instructions on the label.

Ox-gall agar is suitable mainly for *Fusarium* testing. After autoclaving and cooling 1 L of PDA, add 2 g of ox-gall and 100 mg streptomycin sulfate.

Host media (legumes) is preferred for *Ascochyta* testing. Mix 40 g of ground seeds with 1 L of distilled water, autoclave for 40 min, filter the suspension through cheesecloth and add 20 g of dextrose, 20 g agar, and water to 1 L, autoclave again for 15 min at 15 psi.

Technique

After surface sterilization of the seeds, a number of seeds are placed on the media, with sufficient space between seeds to allow development of distinct colonies (i.e., 10 chickpea seeds, 15-20 lentil seeds etc. in one 9-cm petri dish). Seeds are incubated for 7 days. Exposure to near-ultraviolet light after 3-4 days is recommended to increase sporulation. For evaluation, some colonies can be identified easily by characteristic growth, color or fruiting bodies. For others, checking at high magnification under the compound microscope is necessary.

Embryo Method

This method has been developed for testing for *Ustilago* spp. It has been modified by several authors. At least 2000 seeds are soaked in 5% sodium hydroxide (NaOH) with 200 ppm aniline blue or trypan blue for 18 hours. Then about the same amount of 5% sodium chloride (NaCl) is added, the mixture well stirred and embryos separated over a set of sieves (2.5 mm mesh to collect endosperm and chaff, 1 mm mesh to collect embryos). Lots of warm water is added and constantly stirred. Alternatively, a Fenwick can (as used in Nematology) may be helpful to speed up the procedure. The sample is transferred to a funnel closed with rubber tube and stopcock and covered with a mixture of lactophenol and water (50:50). If the specific gravity is correctly adjusted, the embryos will float and the chaff will sink down and can be removed by releasing the stopcock.

The embryos are transferred to fresh lactophenol and boiled for approximately 30 sec, then inspected in glycerol for mycelia in the scutellum at about 20 × magnification with substage illumination.

Growing-on Test

This method is close to field conditions. Greenhouse or growth-chamber facilities are required. Plants are grown in sterile media (sand, soil, vermiculite). Environmental conditions should be favorable for the pathogens to be detected, i.e., low temperature for *Tilletia contraversa* and *Helminthosporium gramineum*, high humidity for bacteria.

A major disadvantage of this method is the time required for growth and expression of the symptoms (several weeks or even months). An advantage is the possibility of using seedlings from the routine germination tests, thus saving on seeds of valuable germplasm. Moreover, the pathogens recorded in this test are transmitted with the seeds, whereas the tests mentioned above give information only on seed-borne pathogens.

Methods for Bacteria and Viruses

There are very specific serological methods to identify bacteria and viruses. The ELISA (enzyme-linked immunosorbent assay) test has been developed as a routine test, and ready-to-use kits are available for many different pathogens.

Bacteria also can be isolated directly from the seed. Either the seeds are ground and soaked in water (recommended method for *Pseudomonas phaseolicola*) or they are soaked intact in sterile saline until the bacteria ooze into the saline (recommended method for *Xanthomonas campestris* pv. *translucens*). In either case, dilutions of 1:10, 1:100, etc. are plated with an L-shaped glass rod on plates of selective or differential media (e.g., XTS medium for *Xanthomonas*, King's B medium for *Pseudomonas*, see agar plate test above for general instructions on media preparation).

XTS agar (for isolation of *Xanthomonas campestris* pv. *translucens* from seeds)
23 g nutrient agar
5 g glucose
After autoclaving, add:
2 ml of Cycloheximide stock solution (1 g in 10 ml 75% ethanol)
0.5 ml of Gentamycin stock solution (50 mg in 5 ml 75% ethanol)
1 ml of Cephalexin stock solution (50 mg in 5 ml 75% ethanol)

Equipment Required for a Basic Laboratory (Testing for Fungi Only)

The items marked * are essential, the others are required for certain tests.

* Compound microscope, preferably with phase-contrast
* Stereomicroscope, magnification up to 50X, preferably with substage illumination
* Incubator, preferably with NUV-light ("black light")
* Refrigerator
* Deep freezer

* Water-still or deionizer
* Autoclave or pressure cooker
* Seed treater for small samples
Centrifuge
Reagent tube shaker
pH meter
UV lamp (366 nm)
Water bath
Shaker
Oven
Analytical mill
* Glassware
    Petri dishes
    Flasks (up to 2000 ml size)
    Beakers (up to 1000 ml size)
    Microscope slides
    Cover glasses
    Alcohol burners
    Reagent bottles, different sizes
    Counting chamber (hemacytometer)
    Funnels
    Reagent tubes
Miscellaneous items
Tweezers
Dissection needles
Inoculation loops
Scissors
Rubber tubes, stopcock clips
Set of sieves, 1 and 2.5 mm mesh
Filter paper
Germination boxes, plastic with transparent covers
Aluminum foil
Labels

Seed-borne Diseases in Seed Certification

Introduction

Seed certification schemes usually prescribe different aspects of seed quality to be tested or inspected. These include: trueness to variety, physical purity, content of other seed, germinability, health and moisture. Some of these can be checked only or mostly in the field (e.g., trueness to variety), others such as germinability and moisture require laboratory testing. Assessment of the health status of seeds needs both field inspection and laboratory testing.

Certain diseases such as loose smut of wheat and barley can be readily detected in the field, but detection in laboratory tests is difficult or time-consuming. Other diseases are difficult to detect in the field, unless the inspector is well trained for detection of these diseases or they appear at a high incidence and severity. Karnal bunt (*Tilletia indica*), for example, is hard to recognize in the field, but a centrifuge wash test reveals spore contamination even at a low level. Viruses usually produce distinct symptoms in the field. However, they also may be latent, that is infected plants do not show symptoms, but produce infected seeds. Laboratory testing for viruses requires antisera and special equipment for serological tests.

For some pathogens, transmission with seed is the most important, or even the only, means of transmission. An example for exclusively seed-transmitted pathogens is *Ustilago nuda*. *Tilletia* spp. are potentially seed-transmitted, the other possibility being through soil.

To allow an objective evaluation of the seed quality, tolerances have to be established. This will allow rejecting a field or a seed lot that does not have the expected standard. Tolerances are not fixed data; they have to be set according to the situation in a particular area, country, or region where the seed will be planted. A seed certification scheme just starting in a country will probably set lower standards than those used by well-established organizations. Standards for seed-borne pathogens are even more variable than those for other quality aspects.

Field Inspection

Field inspectors must know which plant diseases may be seed transmitted, and to what extent. In wheat, for example, a field may be severely affected by one or more of the rust diseases. In the case of yellow rust, even glumes and seeds could be covered with rust pustules. Yet, the pathogen is not transmitted by seed, and is therefore not of primary concern in seed certification, although it may reduce the yield considerably. This will affect producers of breeder seed and foundation seed, who would like to supply a particular variety in larger quantities, and of course any seed grower because his return will be less than expected because of the yield loss. Also the percentage of shrivelled seed will increase.

A general scheme which could be followed is shown below. For identification of the pest or pathogen, pictorial keys such as those listed in the references could be used. When in doubt, the help of an experienced plant pathologist/entomologist/plant protection specialist should be sought.

Key for decisions concerning pests and diseases in seed increases (for field inspectors).

1	Identify the pest/pathogen, then:	2
2	The pest/pathogen is not known to be seed-transmitted:	3
2*	The pest/pathogen is known to be seed-transmitted: **Take records and compare with standards, test seeds after harvest.** In addition:	5
3	The pest/pathogen is not likely to affect yield or quality: **Do not do anything and hope for the best.**	
3*	The pest/pathogen is likely to affect yield or quality:	4
4	The causal agent is a fungus, insect, or nematode: **Select an appropriate pesticide and advise application at recommended dosage.**	
4*	The causal agent is a virus or bacterium: **Advise grower to rogue infected plants to prevent further spread (if feasible) and to spray insecticide if potential vectors are present.**	
5	The causal agent is a virus:	6
5*	The causal agent is not a virus:	7
6	The virus is not known to be insect transmitted: **Advise grower to rogue infected plants (if feasible), test harvested seeds.**	
6*	The virus is known to be insect transmitted: **Advise grower to rogue infected plants (if feasible), and to spray insecticide to prevent further spread; test harvested seeds.**	
7	The pathogen has only one generation per year, e.g., smut, bunt: **Advise grower to rogue infected plants (if feasible), test seeds after harvest, treat infected seed lots.**	
7*	The pathogen has several generations per year, e.g., Ascochyta blight: **Advise application of appropriate pesticides at early stages of disease development, test seeds after harvest, treat infected seed lots.**	

Sampling of inspection areas is done the same way as in field inspection for purity. Timing of inspection is crucial; some diseases show only at certain stages, e.g., common bunt after development of ears. In some cases, neighboring fields also have to be inspected, for example in case of export to countries requesting on additional declaration on the phytosanitary certificate stating that a particular "disease does not occur at the place of production or in its immediate vicinity." Field inspectors should be familiar with the pests and diseases endemic in their region. Cooperation with plant pathologists, entomologists and plant protection specialists should be sought. A short meeting with these specialists from extension services, universities and other research institutions each year before field inspection starts will help to familiarize field inspectors with the current problems, and to put them in a position to advise the farmer. Field guides with pictorial keys should be available, as well as herbaria specimen of diseased plants. For the final identification, isolation of pathogens may be necessary, and for this normally the cooperation of a specialized laboratory is required.

Examples of field standards from various countries are given in Table 4. However, this is not meant as a guideline because each country has to set the required standards individually, considering the following:

seed generation: the strictest standards are required for early generations, i.e., breeder and foundation seed.
available methods of control, either by seed treatment or another way. For a pathogen that can be easily controlled with a fungicide seed treatment (common bunt) the standard may be much higher than for one with no control method available (Lettuce Mosaic Virus).
epidemiology and pathogenic potential: a country in which environmental conditions are conducive to the development of a particular disease, e.g., bacterial leaf streak of wheat or barley, may set high standards for the pathogen, in this case *Xanthomonas campestris* pv. *translucens*. Generally for pathogens with only one generation per year (monocyclic pathogens, for example *Tilletia caries* or *Ustilago nuda*) the epidemic potential is less than for polycyclic pathogens with several generations per year, such as *Xanthomonas campestris* or *Ascochyta rabiei*. In this context, the seeding rate may be important. A 1% infection in a crop for which 25 000 seeds/ha are planted brings more initial inoculum to a field than the same percentage in a crop where 5000 seeds/ha are planted.
other means of transmission: if a pathogen is mostly transmitted by means other than seed, e.g., *Alternaria brassicae* in cabbage, the seed-borne inoculum is not important in the development of epidemics, and strict tolerances will not reduce the incidence. The situation is different with exclusively or almost exclusively seed-transmitted pathogens, such as Barley Stripe Mosaic Virus.
relation between seed inoculum and field infection, i.e., the number of spores, bacteria, etc. per seed required to cause an infection. Whereas in ideal conditions (for the pathogen!) as little as one spore can infect a plant, normally more spores are required (for common bunt, for example, about 3000/seed). This is a matter of probability and varies according to varietal resistance, environmental conditions, virulence of the pathogen and other factors.
quarantine: if seeds are produced for export, the phytosanitary import regulations of the respective country have to be observed.

Table 4. Examples of field standards for seed-borne diseases.

Disease	Tolerance	Country
Loose smut (wheat and barley)	1 in 10 000 ears	England
Loose smut (wheat)	0.1%, foundation seed 0.5%, certified seed	India
Loose smut (wheat and barley), common bunt, barley stripe	0.02%, basic seed 0.05%, certified seed (1) 0.1%, certified seed (2)	Morocco
Ashy stem blight (cowpea)	0.1%, foundation seed 0.2%, certified seed	India
Bean blight	0.005% of plants	USA
Pea early browning	1 plant/100 m^2	Holland
Cowpea mosaic	0.1%, foundation seed 0.2%, certified seed	India
Soybean mosaic	20 plants/line, tested by ELISA	USA
Barley stripe mosaic	Zero in seed production fields	USA
Pea seed-borne mosaic	Zero in seed production fields	USA (Idaho, Washington)

Laboratory Seed Health Testing

The main purpose of laboratory testing in seed production and seed certification is the evaluation of seed quality. The different aspects of seed quality that can be evaluated in the laboratory are physical purity, germination, moisture, health, and to some extent varietal purity and vigor. The test results of all the components together give an indication of the planting value. A seed lot free from pathogens and meeting high purity standards, but with a poor germination, is of low planting value, as is a lot with high purity and good germination but infected with seed-borne pathogens. The actual stand in the field also depends on a number of other factors such as soil conditions, weather, planting techniques, etc. Standards for pathogens are even more variable from one country to another than those on germination or purity. It is of utmost importance that the correct method for the respective pathogens is used. If a tolerance for *Ustilago nuda* is set, there is **only one possible test method**: the embryo test. In the case of *Tilletia* spp. a visual inspection can reveal bunted seeds; however, the lack of bunted seeds does not mean the seed sample is free from *Tilletia* spp. The presence of a spore contamination can be revealed only in the washing test.

As in the case of field standards, laboratory standards have to be set by each seed program according to the local conditions. Some examples are given in Table 5. However, copying other countries' standards without considering possible differences could lead to unnecessarily strict standards, resulting in too many rejections without a benefit to the national seed program or to the farmers. It also may result in a situation where more stringent standards could prevent severe yield losses. Furthermore, it is important to review the standards periodically.

Table 5. Examples of laboratory standards for seed-borne diseases.

Pathogen	Tolerance	Country
Tilletia caries	500 spores/g seeds	Sweden
Tilletia contraversa	100 spores/g seeds	Sweden
Tilletia indica	0.1%, foundation seed 0.5%, certified seed	India
Neovossia horrida (rice)	0.1%, foundation seed 1.5%, certified seed	India
Ustilago nuda (wheat, barley)	0.2%, basic seed 0.5%, certified seed	UK
Ustilago nuda (wheat)	0.5% without treatment 2% with treatment	India
Botrytis spp. (linseed, sunflower)	5%	France
Claviceps purpurea (rye)	1 piece/500 g (basic seed) 3 pieces/500 g (certified seed)	France
Colletotrichum lindemuthianum	0.1%, basic seed 0.5%, certified seed	France
Phoma lingam (cabbage)	Zero in 1100 seeds	Denmark
Pseudomonas phaseolicola	Zero in 1000 seeds (prebasic and basic seed)	England
Xanthomonas phaseoli	Zero in 5 kg seed	Canada
Lettuce Mosaic Virus	Zero in 30 000 seedlings	USA (Calif.)
Pea Seed-borne Mosaic Virus	Zero in 200 seeds/seed lot (ELISA)	USA (Idaho, Washington)
Barley Stripe Mosaic Virus	Zero in foundation seed (latex flocculation method)	USA (N Dakota)
Barley Stripe Mosaic Virus	5%-1964, 3%-1968, 0%-1972 (200 embryos, SDS disk test)	USA (Montana)

Isolation of Pathogens Found in Field Inspection

Introduction

Identification of the causal agent of a plant disease is of utmost importance before control measures are applied. Only after correct diagnosis can the appropriate treatments be selected. For example, leaf yellowing may be caused by a number of viruses, or could be the first symptom of a bacterial or fungal disease, or may even have nonbiotic causes such as nitrogen or iron deficiency.

For some diseases detected in field inspection, identification is fairly easy, particularly for those where signs of the pathogen are present, such as spores of powdery mildews or rusts. A quick look for spore morphology under the microscope can immediately confirm a diagnosis.

Most fungi and bacteria, however, cause leaf spots or other rather unspecific symptoms. In this case, isolation of the propagules helps in finding the correct diagnosis.

Steps to Follow if the Pathogen is Suspected to be a Bacterium

Typical symptoms of a bacterial disease are water-soaked lesions (lesions are abnormal developments in plant growth such as leaf spots and stripes). They are well differentiated from the healthy areas. A quick check can be made by placing a small piece of the affected plant in a drop of distilled water on a microscope slide and cutting with a scalpel through the margin area between the lesion and healthy tissue. After covering the sample with a cover glass, observe the oozing of bacteria into the water at approximately 40X magnification. It looks as if clouds of bacteria are discharged into the water. However, there is still a chance that the bacteria observed are not pathogenic, but are growing saprophytically on tissue destroyed by other pathogens. For identification of the bacteria, culturing of single colonies is necessary.

1. Cut either small lesions or marginal parts of larger lesions from the infected plant and wash in a surface disinfectant (e.g., 0.5% sodium hypochlorite) for 1 to 3 min.
2. Move tissue with sterile tweezers to sterile distilled water and rinse, then dry on sterile blotter paper.
3. Transfer tissue to a tube with 10 ml sterile distilled water and macerate with a sterile glass rod.
4. Transfer 1 ml of the suspension to a tube with 9 ml sterile distilled water, mix well. Repeat this step 2-3 times to obtain a serial dilution (1:10, 1:100, 1:1000, etc).
5. Place 0.5 ml of each concentration on a petri dish with nutrient agar or, if you suspect a certain bacteria, on a selective medium such as King's B agar for fluorescent pseudomonads or XTS for *Xanthomonas campestris*. Disperse suspension with an L-shaped glass rod (a turntable will speed up this procedure).
6. Incubate plates at 28°C for 2-5 days. Single colonies will appear at the correct dilution. They can be identified immediately by morphological characteristics, or

used for biochemical tests if needed. New rapid and sensitive techniques include immunofluorescence and ELISA.

Steps to Follow if the Pathogen is Suspected to be a Fungus

If no fruiting bodies, such as spores, are present, the first step is to encourage sporulation. The diseased plant parts are washed if necessary and placed in a moisture chamber, i.e., any container in which a saturated atmosphere can be maintained. This could be for instance a petri dish with moistened filter paper. After incubation for 12-24 hours, the samples can be checked for appearance of spores. This method works very well with *Helminthosporium* spp., *Botrytis cinerea* and downy mildews. Other species may have to be isolated on an artificial medium, following the steps indicated below.

1. Cut either small lesions or marginal parts of larger lesions from the infected plant and wash in a surface disinfectant (e.g., 0.5% sodium hypochlorite) for 1-3 min.
2. Move tissue with sterile tweezers to sterile distilled water and rinse, then dry on sterile blotter paper.
3. Place tissue on prepared petri dishes with suitable medium and incubate for 7-10 days at 20°C and 12-hour light cycle. Near-UV light after 3-4 days enhances sporulation.
4. Check plates for pure culture of the pathogen.

Care must be taken not to confuse saprophytes with the pathogens. Many fungi and bacteria live on decayed plant material and destroy it, but do not cause the decay. Examples are: *Alternaria alternata*, *Cladosporium* spp., *Rhizopus* spp., *Penicillium* spp. and *Aspergillus* spp.

Recipes for commonly used media

Potato Dextrose Agar
200 g potatoes, unpeeled, cleaned, and cut into cubes of about 1 cm
20 g dextrose
20 g agar
1 liter water
Boil and mash potatoes, put through fine sieve, add agar and boil again until dissolved, add dextrose, stir and add water to make 1 L. Sterilize at 121°C for 20 min.

King's B medium
20 g protease peptone #3 (Difco)
1.5 g K_2HPO_4
1.5 g $MgSO_4 \times 7H_2O$
15 g agar
15 ml glycerol
water to make 1 liter

YDC agar (for *Xanthomonas campestris*)
10 g yeast extract
20 g dextrose
20 g finely ground $CaCO_3$
15 g agar
Autoclave at 115°C for 1 hour. When pouring plates or tubes make sure $CaCO_3$ is well suspended.

XTS agar (for isolation of *Xanthomonas campestris* pv. *translucens* from seeds)
23 g nutrient agar
5 g glucose
After autoclaving add:
2 ml of Cycloheximide stock solution (1 g in 10 ml 75% ethanol)
0.5 ml of Gentamycin stock solution (50 mg in 5 ml 75% ethanol)
1 ml of Cephalexin stock solution (50 mg in 5 ml 75% ethanol)

Crop Loss Assessment

Introduction

Devastating plant diseases and pests have been known since biblical times, when they were regarded mostly as God's punishment. "Famous" epidemics, which resulted in very high losses, are those of potato late blight (*Phytophthora infestans*) in Ireland from 1845 to 1849, the Helminthosporium disease of rice (*Helminthosporium oryzae*) which caused "the great Bengal famine" in 1942/43, the Dutch elm disease (*Ceratocystis ulmi*) in the USA in the 1930s and in England in the early 1970s, and the southern corn leaf blight (*Helminthosporium maydis*) in the USA in 1970/71. Some of the most devastating epidemics resulted from the introduction of new pathogens, e.g., grape downy mildew (*Plasmopara viticola*) in France in 1878, potato late blight (*Phytophthora infestans*) in Ireland in 1842, and, to name a more recent example, tobacco blue mold (*Peronospora tabacina*) in Europe in 1957. The introduction of a new pathogen strain may be responsible for the development of an epidemic. The Dutch elm disease (*C. ulmi*) was well established in England since about 1930, when it had killed about 10-20% of the tree population. In 1970, the period of stabilization was disturbed by an aggressive strain, apparently introduced from North America.

Yield and loss figures may be used in a retrospective (by analyzing past events) or a prospective (by predicting future events) manner. The latter is particularly important in timing pesticide applications (e.g., forecast systems for downy mildews), planning large-scale control measures (e.g., locust control in Africa), and organizing production logistics (e.g., pesticides, equipment, storage).

Definition of Terms

Crop loss assessment is based on various disciplines: epidemiology, agronomy and economics. Epidemiology basically relates the relation between weather and disease development; agronomy affects how the crop reacts to the infection; in the economical considerations the yield losses are quantified and related to inputs.

One of the keys to crop loss assessment is the measurement of disease and pathogen. Also important is the growth stage at which a disease occurs. An attack at the seedling stage most likely will be more damaging than an attack just before maturity. Calendar data are not suitable because comparisons between locations and different years are impossible. Growth stage keys have been developed by various authors.

Some terms frequently used in quantifying diseases are **incidence** (the percentage of infected plants in a field) and **severity** (the amount of tissue affected by the disease). For some diseases the **incidence** gives sufficient indication of the expected yield loss: a wheat or barley plant infected with loose smut (*Ustilago* spp.) will not give any yield, so 10% infected plants will result in 10% yield loss. For most diseases, however, the degree of infection, or the amount of damaged tissue, will determine the yield loss. This is the case with downy and powdery mildews, rusts, blights, etc. It should be noted, however, that

plants have the ability to compensate for an attack, and that a low disease level may even result in a slight yield increase. To help in assessing the disease severity, standard diagrams have been developed for many crops. Computer programs are available to train pathologists in correctly estimating affected leaf or fruit areas.

In some cases it may be necessary to measure the pathogen, either directly as in the case of fungus spores, or indirectly by trapping aphid vectors of viruses. This, however, is more important in disease forecasting than in crop loss assessment. Various techniques have been developed, e.g., a volumetric spore trap or yellow traps to catch aphids.

A relatively new technique to measure both incidence and severity is **remote sensing.** Healthy and diseased tissues have different reflectance, which can be measured in the near-infrared wavelength region (700-950 nm). Use of false color infrared film also is possible. Remote sensing may be done by using planes (including remotely controlled model planes), kites or satellites.

Crop loss assessment seems an easy task: you just compare the yield of a disease-free or healthy plot with that of plots showing various disease severities. However, there are a few pitfalls involved. If, for example, data sets on yield losses from two years are compared, and since yield potential varies from one year to another, the expression of yield as percentage of the control plot is misleading. If percentages are given, the control plot has to be kept disease-free, but otherwise exposed to the same conditions. This is difficult: fungicide sprays may have side effects on other than the target pathogen, caging of plots to keep out virus vectors may give excessive shadow, and may keep out other pests.

Definitions of frequently used terms

The biological agents (viruses, bacteria, fungi, nematodes, insects) injuring the crop are called **harmful organisms.** The "measurable produce of economic value from a crop is the **yield.**" In a cereal or legume crop, the straw is considered part of the yield in Syria (for feeding sheep), whereas in Europe and the USA it is considered a problematic waste product. Visible symptoms caused by a harmful organism are called **injury.** The reduction in yield is called **damage.** The reduction in financial returns is called **loss.** So **injury** may cause **damage, damage** may cause **loss.** The **economic injury level** (mostly used for insect pests) or the **damage threshold** (mostly used for pathogens) marks "the lowest population density that will cause economic damage." In many cases treatment has to be applied before the population density has reached the damage threshold, for example because there may not be an eradicative treatment available. This level is called **action threshold.**

Unfortunately, little is known about these thresholds. They are specific to crop, pest, climate and market situations. Farmers may believe they have to spray insecticides when only a few insects are present in their crop. Misidentifications also may occur and may be followed by inappropriate treatment (insect damage treated with a fungicide or vice versa). The lack of systematic monitoring of yield-loss relationship is largely responsible for this situation.

Crop Loss Assessment for Seed-borne Diseases

According to Agarwal and Sinclair (1987) overall losses due to plant diseases are estimated at 30% in Asia, 25% in Europe and 15% in North America. They also cited examples for specific seed-borne diseases: 100% yield loss due to loose smut in wheat in Georgia, USA in 1954; more than 50% yield loss due to blast disease (*Pyricularia oryzae*) in rice in the Philippines; $1 billion loss in maize due to southern leaf blight (*Drechslera maydis*) in the USA in 1970; 64% yield loss in barley and 75% yield loss in wheat due to Barley Stripe Mosaic Virus (BSMV) in Canada, and $25 million loss due to black rot (*Xanthomonas campestris* pv. *campestris*) and black leg (*Phoma lingam*) in the USA.

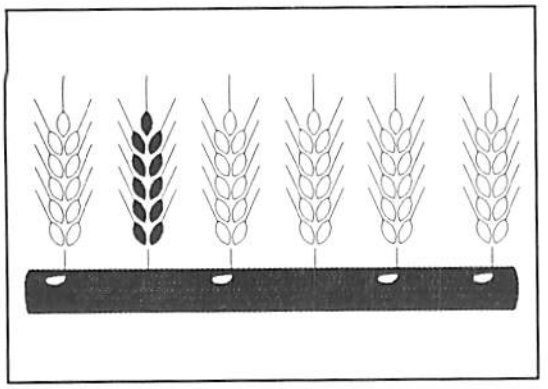
Seed-borne pathogens cause crop losses in various ways.

Reduced germination. A number of pathogens cause complete failure in germination, e.g., *Ascochyta rabiei* in chickpea or alfalfa mosaic virus in alfalfa. Others cause a seedling decay at early stages, maybe even before emergence, e.g., *Alternaria padwickii* in rice or *Phytophthora parasitica* in tomato. While neighboring plants can make up for the missing plants to some extent, a high number of missing plants will inevitably result in yield loss.

Distribution of inoculum to as-yet-uninfested fields, areas or countries. Since seeds are a very effective vehicle for pathogens (pathogens survive longer than in other means of transportation and they can be transported over great distances), there is a risk of introducing pathogens to fields, areas or countries which otherwise would not have a chance to get there. This is particularly true for exclusively seed-borne pathogens such as loose smut of wheat and barley, or barley stripe mosaic virus. For other pathogens, it is often difficult to establish whether the inoculum came with the seed or by wind, plant debris in the field, or with human activities such as pruning, plowing or even walking in the field.

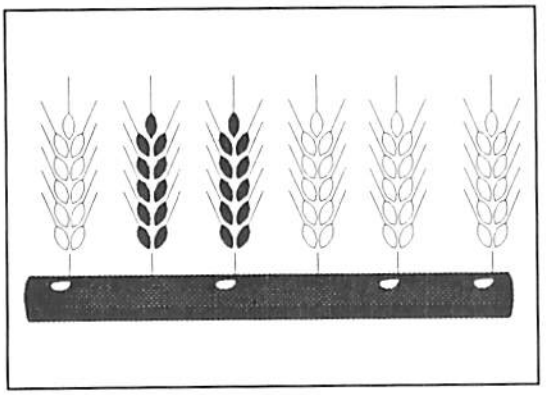
Losses in seed and grain quality. Many pathogens cause shriveling of seeds, e.g., *Alternaria triticina*, *Fusarium nivale*, *Septoria* spp. and *Drechslera sorokiniana* on wheat, or *Ascochyta* spp. on legumes. Small seeds do not meet standards for high quality seed and have to be removed in the seed-cleaning process. Toxin-producing fungi such as *Aspergillus flavus* may be harmful to animals fed with infected grain. *Claviceps purpurea* or ergot produces a toxic alkaloid.

Losses due to seed-borne pathogens are difficult to estimate. They depend on biology and epidemiology of the pathogen, as well as external factors. Different types of seed infection are illustrated in Figure 2. With a simple relationship such as type 1 (loose smut), expected losses can be estimated relatively easily. When other sources of inoculum, such as soil-borne spores of *Tilletia* spp. in type 2, or even several generations of the pathogen (type 3) are involved, the estimate becomes very difficult. For type 3 for example, yield losses could vary between 0 and 100%. With the example of Ascochyta blight of chickpeas, when a resistant variety is grown, the weather is relatively dry, sprinkler irrigation is avoided and fungicides are applied, so the incidence (the percentage of infected plants in a field) will be low. The time of disease outbreak is important. Late in the season, there might be no effect on yield. Moreover, a disease like



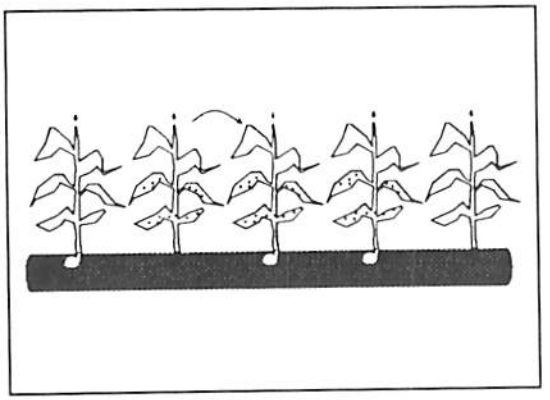
Type 1

Ustilago nuda (loose smut):
exclusively seed-borne,
one generation per year



Type 2

Tilletia caries (common bunt):
seed- and soil-borne,
one generation per year



Type 3

Helminthosporium maydis (southern corn leaf blight): seed-, soil- and air-borne, several generations per year

Fig. 2. Different types of seed-borne diseases: black seeds = infected or contaminated seeds; black ears = diseased ears; dots in soil = contaminated soil or plant debris; dots on plants = lesions.

Ascochyta blight of legumes is known to affect yield, whereas others like bacterial leaf blight of wheat, caused by *Pseudomonas atrofaciens*, have hardly any influence on yield.

These different types of seed infection and their implications on yield and pathogen carry-over to the new generation have to be considered when standards are set and control measures are taken. Pathogens with only one generation per year, such as smuts and bunts, can be controlled by seed treatment, and not by foliar sprays in the field. In addition, for some of them roguing can be a means of control, such as common bunt or covered smut, whereas others will have infected neighboring plants before they are detected by the farmer or field inspector, such as loose smut. In contrast, seed-borne pathogens with more than one generation per year, such as *Ascochyta* spp. (type 3 in Fig. 2) may be controlled by foliar treatments in the field. Field inspectors can advise the farmers on frequency of sprays and appropriate chemicals.

Epidemiology of Seed-borne Diseases

Introduction

Plant disease epidemiology as a science started when van der Plank (in subsequent publications Vanderplank), published his well-known book on "Plant Diseases: Epidemics and Control" in 1963. He defined epidemiology as "the science of disease in populations."

Occasionally, the words "epiphytotic" or "epiphytology" are used instead. The question of whether to use epidemic or epiphytotic has been discussed at length. The Encyclopaedia Britannica (1973) defined epidemiology as: "Although originally limited to disease in human populations, epidemiology has been applied to the study of disease in animal herds and in plant life. Such usage is justified by the derivation of the word epidemic, which literally translated from the Greek means 'upon the population'. The population may consist of human beings, animals, plants or whatnot." The word epidemia means "stay in a place", and the word "demos" can mean "people" or "district." Moreover, the word "epiphytology" is related to the word epiphyte, which describes a plant growing on another plant, usually not as a parasite. Both the origin and usage in international journals such as Phytopathology or the Annual Review of Phytopathology, offer ample justification for the use of epidemiology rather than epiphytology.

Epidemiology entails the collection of vast amounts of data, particularly those on weather, disease incidence and severity, yield loss, etc. Factors affecting development of an epidemic are: the pathogens' life cycles, weather, host plant resistance, availability of susceptible host plants, availability of vectors and management practices including control measures.

Practical applications of plant disease epidemiology include various aspects of disease management such as sanitation, resistance breeding, crop loss assessment, forecast systems and geophytopathology. Often mathematical models are used as a tool in plant disease epidemiology. Mathematical models may be regarded by some scientists with a certain suspicion.

Particular Points Regarding Seed-borne Diseases

The epidemiology of seed-borne diseases follows the same principles as that of other plant diseases. Seed-borne diseases, however, have some additional characteristics in common. The following factors are important:

- amount of seed-borne inoculum
- rate of seed transmission (seed to plant)
- rate of subsequent disease development in the field
- rate of re-establishment of seed-borne inoculum (plant to seed).

The importance of any of these factors is largely determined by pathogens, environmental conditions and ultimate use of the crop.

Amount of Seed-borne Inoculum

The amount of seed-borne inoculum in a seed sample can be determined either as percentage of infected seeds, or quantitatively with regard to the pathogen propagules in the seeds. The former is common in seed health testing, whereas the latter may prove particularly difficult. Occasionally, seed lots are required to be free from a certain pathogen, e.g., when a zero tolerance is required for quarantine pathogens. This may be desirable in certain cases; however, such statements are unrealistic. All that can be reasonably testified is that the level of infection or infestation is less than a specified percentage. The sensitivity of the method used in seed health testing and the sample size are very important factors. Tables 6 and 7 give some indication of the number of seeds to be tested for a given standard and of the confidence level for a given number of seeds tested and found pathogen-free.

Table 6. Number of seeds to be tested to meet given standards, according to Poisson distribution.

	Number of seeds to be tested	
Standard	95% probability	99% probability
5%	60	92
2%	150	230
1%	300	460
0.3%	1000	1533
0.1%	3000	4600

Table 7. Infection level that may be assumed if a given number of seeds are tested and found healthy, according to Poisson distribution.

	95% probability	99% probability
No. seeds to be tested	Infection level less than	
30	10%	15%
50	6%	9.2%
100	3%	4.6%
400	0.75%	1.15%
2000	0.15%	0.23%

Although the importance of quantifying the inoculum per seed and its relation to disease expression has been recognized, there are still considerable difficulties with many pathogens. Few problems are encountered with pathogens that are exclusively surface-borne, such as *Tilletia* spp. on wheat. The spores can be washed off the seeds and counted. A similar method can be employed for bacteria, such as *Xanthomonas campestris*, where internally seed-borne bacteria ooze into sterile saline, which then can be plated on a selective medium in dilutions so that colonies can be counted after incubation. Seeds infected with *Ustilago* spp. may be seen as "a simple unit of inoculum potential."

With other pathogens such as *Fusarium* and *Ascochyta* spp., the percentage of infected seeds as a unit of measurement does not do justice to the complicated host-pathogen relationship. A differentiation between superficial and deep-seated inoculum can be made by sterilizing the seed surface before the test. Little is known about the importance of the surface-borne inoculum in disease epidemiology. The relation between seed infection and disease incidence of the emerging plants was studied for *Drechslera maydis* on maize and a good correlation was found if the seed infection was recorded in the freezing blotter test with 10 min pretreatment with 2% sodium hypochlorite. In some samples a very high incidence of seed infection was found in tests without pretreatment, indicating the presence of surface-borne inoculum, which apparently did not play a role in disease development. Generally, in tests without surface sterilization of the seeds a higher percentage of pathogens is recorded. Such considerations are particularly important when it comes to the establishment of tolerances in quarantine regulations.

While in tests that require incubation only viable inoculum is considered, in washing tests it is difficult to differentiate between spores that are able to germinate and those that are dead, e.g., killed by seed treatment. A spore germination test on water agar could provide such information, but such a test may be time consuming and particularly difficult if the spore load is small.

Most detection methods in virology — latex agglutination and passive hemagglutination assays, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunoelectron microscopy (IEM) — depend on the detection of virus antigens (coat protein), whereas various "blot tests" permit the detection of specific viral nucleic acids by the use of isotope-labelled complementary DNA. With immunosorbent assays, the detection of 1-2% seed infection is possible. A clear disadvantage is the fact that there is no discrimination between infective and non-infective virus particles. Furthermore, it is important to distinguish between viruses located in the embryo, which may cause seedling infection, and those in or on the seed coat, which most likely will not infect the seedling. Thus, serological tests should be conducted on germinated seedlings rather than on seeds.

The importance of the initial inoculum depends on the maximum infection rate. Some examples: 0.07/day for *Ascochyta fabae*, 0.11/day for *Pseudomonas phaseolicola*, 0.44/day for *Septoria apiicola*. Obviously, in the latter case a small amount of initial inoculum may lead more easily to an epidemic than in the first two examples. For quarantine diseases,

even an otherwise insignificant level of initial inoculum may have drastic effects when the conditions in the new area are favorable for disease development.

Seed Transmission Rate (Seed to Plant)

The seed transmission rate can be expressed as the percentage of infected seeds that result in infected seedlings or plants. It varies greatly, depending on pathogen, host and environmental factors. A simple relationship between seed infection and percentage infected plants in the field was found for loose smut of barley (*Ustilago nuda*), where the transmission rate is close to 100%. On the other hand, faba bean seeds infected with *Ascochyta fabae* produced only 2-15% of seedlings with primary infection. A similarly low transmission rate of approximately 10% was reported for *Pseudomonas phaseolicola*. The transmission rate often depends on the severity of seed infection. Seeds showing severe symptoms of bacterial infection (wrinkled, discolored seeds with lesions) may have a higher number of bacteria per seed than those with slight to moderate symptoms or those without symptoms. However, since many of the heavily infected seeds failed to germinate, their contribution to the total disease transmission is relatively low compared with that of moderately infected and symptomless seeds.

The effect of the host was demonstrated for several viruses. The transmission rate for bean yellow mosaic virus (BYMV) is 0.1-2% in faba beans and 6% in lupins. Pea Seed-borne Mosaic Virus (PSbMV) is seed-transmitted in pea at a rate of up to 90%, but not in faba bean.

Monocyclic pathogens, i.e., pathogens with only one generation per year, cause "simple-interest diseases." Examples are *Tilletia caries* or *Ustilago nuda*. The initial or seed-borne inoculum is closely correlated with disease incidence. Sanitation either by using seed treated with an eradicative fungicide or by preventing seed infection is an effective way of disease control. In countries where cereal seed is regularly treated, loose smut and common bunt of wheat have almost disappeared.

However, there may be significant differences among the monocyclic pathogens. Although *Ustilago nuda* has an extremely high seed to plant transmission rate (approximately 100%), it failed to maintain the infection level in seed lots resown for several years without control measures. In contrast, *Drechslera graminea*, with a transmission rate of less than 15%, maintained the initial level of 30-90% fairly well over several years.

The rate of transmission from seed to plant, along with the first of the abovementioned factors — the amount of seed-borne inoculum — are important in quarantine. The critical, although somewhat theoretical, question is: can a single spore on, in or with the seed cause a disease? The answer is clearly no if the spore is not viable, but it may not be easy to verify that. The answer is also negative if seeds are planted in an area with adverse environmental conditions; it is unlikely that dwarf bunt would be established in a country like Egypt, since *Tilletia contraversa* spores require a temperature of about 5°C for several weeks. The susceptibility of the host is also important. In a susceptible variety,

104 spores of *Tilletia tritici* per grain resulted in bunted heads, whereas a resistant variety remained healthy even with 542 spores per grain.

One "dispersal unit" can establish one "infection unit"; however, the infection efficiency is generally lower, for example in the range of 10%.

Subsequent Disease Development in the Field

The more life cycles a pathogen completes in a season, the greater the potential for causing an epidemic. In the case of such "compound-interest diseases", the relationship between initial inoculum and disease development in the field is complicated and affected by many factors, particularly those related to the environment. Here the amount of initial inoculum may be very small and yet cause an epidemic, because there are chances to multiply throughout the year.

Bacteria or viruses are good examples. For *Xanthomonas campestris*, as little as 0.05% infected cabbage seedlings, that is 5 in 10 000, resulted in a considerable incidence of black rot. A tolerance level of 1 in 20 000 was established for *Pseudomonas phaseolicola*, causing halo blight of beans. Effective control of Lettuce Mosaic Virus in California required that the seed infection had to be less than 0.003%. In New Zealand, 15% diseased faba bean plants resulted from an initial inoculum of 0.2%.

If all other factors are constant, seed-borne pathogens will develop at more rapid rates than other types of pathogens. The reason is that the initial inoculum, if seed-borne, is more evenly distributed throughout the field, so that the number of infections or cycles required to cover a given crop area is less than with a disease that develops from a localized primary focus. Seed-borne inoculum also makes an early start of disease development and a higher initial disease intensity more likely. Moreover, we can assume that the cultivar is susceptible to the pathogen race, because otherwise there would be no seed-borne inoculum.

In the case of pathogens with several generations per year it is, however, difficult to verify whether the disease incidence results from seed-borne inoculum that has been multiplied, or whether other sources of inoculum, such as from neighboring fields, also contributed to the disease.

The disease incidence in plots that had been planted with chickpea seeds infected with *Ascochyta rabiei* at a rate of 7.7% and those inoculated with infected plant debris was compared. In both cases the disease spread in the main wind direction. The infected seeds resulted in 31% infected plants, almost randomly distributed in the field. Within 6 weeks the disease incidence had reached 100%. In the plots infected with plant debris the first symptoms were observed more than 3 weeks after those resulting from infected seeds. Originating from only one focus, the final disease incidence was eventually 16%.

Another factor determining the epidemic potential of a pathogen is whether or not there are other means of transmission. If a pathogen is exclusively transmitted by seed, such as *Ustilago nuda* or Barley Stripe Mosaic Virus, measures such as production of

"pathogen-free" seed or seed treatment may control the disease satisfactorily. However, additional means of transmission, such as the soil-borne inoculum of a *Tilletia* species, are generally much more difficult to control.

Rate of Re-establishment of Seed-borne Inoculum (Plant to Seed)

This point is particularly important if the harvested crop is to be used as seed. For *Ascochyta fabae* a strong correlation of seed infection with both foliar and pod infection was reported. Furthermore, the infection of newly formed seed depends largely on weather conditions, particularly rainfall. For successful infection of faba bean seed with *A. fabae* there must be rain early in the development of a crop so that the pathogen is carried sufficiently high in the leaf canopy to re-infect the pods and seeds as they develop. This is why short-strawed faba bean cultivars generally show a higher incidence of pod infection and why faba bean samples from areas with consistently dry climatic conditions have a lower infection rate of *A. fabae*. The same is true for the three rice pathogens *Trichoconis padwickii*, *Drechslera oryzae* and *Pyricularia oryzae*. Seeds harvested from the relatively dry region of Ludhiana (India) generally show a low pathogen incidence in seeds, even if a moderate infection (in the case of *D. oryzae*) was recorded in the field.

In soybean, the highest incidence of Phomopsis seed decay was found in wet years and in areas bordering major waterways. The distribution of the disease was primarily influenced by the rainfall patterns during the maturation period of the crop. Correlation between disease incidence and temperature was low.

Reinfection of loose smut of wheat and barley (*Ustilago nuda*, *U. tritici*) is greatly influenced by weather. Since spore transfer occurs only during the few weeks of flowering, the pathogen is particularly vulnerable during this relatively short period of time. If moisture conditions are favorable, the rate of increase may be 10 to 20 times. For this monocyclic disease, isolation of a crop grown from clean seed from sources of inoculum is therefore a valuable control measure. The amount of reinfection in a plot is largely determined by the level of inoculum in this plot.

Cultural practices also may affect seed infection. Sprinkler irrigation, for example, is to be avoided where cereal seed production may be affected by the bacterial stripe disease caused by *Xanthomonas campestris* pv. *translucens*. Many pathogens, such as *Pseudomonas phaseolicola* on beans or *Cercospora beticola* on beets, do not infect seeds when planting of the crops can be timed so that flowering and maturity phases are in a dry season.

For viruses, infection after flowering often does not result in seed transmission. The time of harvesting also may be important, with an early harvest preventing high seed infection rates with *Ascochyta fabae*.

Quarantine for Seed

Introduction

Plant pests have been spreading to new areas ever since plant propagation material has been moved. Some of them are disseminated directly with this material, others do not need the vehicle of seeds or cuttings. Live Colorado beetles, for example, were found in imported spare car parts. Examples for long-distance spread of spores by wind are sugarcane rust (from Cameroon to the Dominican Republic and the USA) and coffee rust (from West Africa to South America). Other important natural vectors are animals and waterways. However, particularly for pathogens with short-lived propagules, such as viruses and bacteria, plants and plant parts including seeds are among the most important vehicles.

New or Endemic Pests and Diseases?

It is most important to distinguish between reports of new diseases/pests in a country and the actual interception (isolation from seeds) of the pathogen/pest. In many countries there is not sufficient knowledge of the pest/disease situation. Systematic surveys are frequently lacking; reports are usually published only if a pest/disease causes substantial yield losses. Information on pests/pathogens that are endemic and inconspicuous is scarce. However, changed agricultural practice could promote epidemics, which may then be blamed on newly introduced pests/pathogens. An example is the bacterial stripe disease, or black chaff, caused by *Xanthomonas campestris* pv. *translucens* in Syria. The symptoms are rather inconspicuous at a low disease incidence and severity. Symptoms on leaves may be confused with those of Barley Stripe Mosaic Virus, Barley Yellow Stripe Virus or the stripe disease caused in barley by *Pyrenophora graminea*. A black discoloration of the glumes of wheat or barley also can be caused by *Alternaria* spp., *Cladosporium* spp. or by *Septoria nodorum*. In Syria the disease was first observed in the late 1970s in the areas of Raqqa/Hassakeh, and some scientists suspected that the pathogen had been introduced with seeds. However, the occurrence of the disease coincided with the implementation of extended irrigation in this area. Irrigation enhances infection with *Xanthomonas* spp., and to date the disease does not play a role in rain-fed cereals in Syria. In this case the possibility of a newly introduced pathogen cannot be excluded, but is unlikely.

A clear affirmation is usually only possible when the pest/pathogen is intercepted, i.e., isolated from imported seeds. This is especially true for most pests, which have their own ways of distribution, and many pathogens, which may be transmitted by means other than seed (e.g., soil and wind). Even with exclusively seed-borne pathogens, such as *Ustilago nuda* or *Pyrenophora graminea*, infected plants in a field sown with imported seed could result from volunteer seed in the field originating from a crop in which the disease remained unnoticed, or from inadvertent seed mixtures.

Interception versus Transmission

On the other hand, direct interceptions have to be looked at carefully. Pests/pathogens introduced into new areas will not necessarily find the right environmental conditions to attack host plants, to multiply, or to survive and become endemic. An example is the introduction of the dwarf bunt pathogen *Tilletia contraversa* into warm agroecological zones. The pathogen requires temperatures of 1-5°C for germination and grows best if plants are under snow cover for extended periods of time. Although the longer persistence of the spores in the soil, compared with those of *Tilletia caries* and *T. foetida*, increases the risk of a disease outbreak in a year with favorable conditions, it seems quite unlikely that the pathogen will become established in a country like Egypt. The same is true for many insect pests from warmer areas. They may multiply during the summer in temperate climates, but usually do not survive the winter. An example is the introduction of the larger grain borer *Prostephanus truncatus* to Iraq in 1969, which did not get established there. However, pests and pathogens may come in strains with different requirements for temperature and it is therefore possible that such a strain will become established in an area previously not considered suitable for the survival of the pest/pathogen.

If a pest/pathogen is intercepted in a shipment from a particular country, the assumption is that the pest/pathogen occurs in that country. However, insect pests may infest shipments en route, and the common practice of reforwarding seeds with a new phytosanitary certificate makes it difficult to find out the country from which the seeds came originally. Between 1970 and 1980 the Indian quarantine service intercepted *Tilletia indica* in seeds received from, among other countries, Lebanon, Syria, Turkey and Sweden. These countries are now frequently listed as host countries. An extensive survey of material from Syria, however, did not give any indication that the pathogen occurs there, and the disease was not reported from any of the other countries mentioned. In the case of ICARDA it has been confirmed that the seeds intercepted in India originated from a country where the disease occurs and were repacked and dispatched from Syria. The seeds from Lebanon, Turkey and Sweden also were dispatched from research institutes/ genebanks and possibly did not originate from these countries either.

New Pests/Diseases in the WANA Region

With the *caveat* resulting from the paragraphs above, some examples for pests and pathogens that have spread to the WANA region from elsewhere or within the region from one country to another are given in Table 8.

Successful Quarantine Efforts

Undoubtedly it is difficult to measure the success of quarantine. The UK is free from the Colorado beetle (*Leptinotarsa decemlineata*) because of surveillance of imports and eradication campaigns that controlled a total of 143 outbreaks since 1877. Measures taken in the UK included a temporary ban on parsley imports from Italy in 1984.

Table 8. Examples of pathogens and pests newly reported from countries of West Asia and North Africa.

Pest/pathogen	Introduced to	Reference
Xanthomonas campestris pv. *translucens*	Arab Republic of Yemen	Dickmann and Aalders du Bois, 1988
Ascochyta fabae	Egypt	Omar, 1986
Orobanche crenata	Ethiopia	Telaye and Saxena, 1986
Prostephanus truncatus	Iraq	Laborius, 1988

Many of the Pacific Islands, including Australia and New Zealand, are still free from a number of potential pests and pathogens. Islands are generally in a more advantageous position for eradicating pests and pathogens.

At least temporarily successful were the eradication campaigns for the Mediterranean fruit fly (*Ceratitis capitata*) in Florida and California which were carried out at great cost.

Conclusion

Whenever material is moved from one region to another, there is a risk of pest movement along with it. In many cases this risk may be negligible, in others the expected benefit from introducing new material may outweigh a possible risk. The cost of running an efficient quarantine service has to be taken into consideration, too. In order to reduce such cost, it is important to concentrate on important, "high-risk" pests. Kahn (1989) summarized the justification for quarantine as follows:

An exotic organism may be considered as high risk (or of quarantine significance) to an importing country if it:

- does not occur in the importing country, *and*
  - is known to cause economic damage elsewhere, *or*
  - has a life cycle and host/pest interaction indicating that the organism is capable of causing damage under favorable host, environment and pest population or pathogen inoculum circumstances that occur in the importing country, *OR*

- occurs in the importing country, *and*
  - is not widely distributed in the ecological range of its hosts in that country, *or*
  - is under a national suppression, containment, or eradication program, *or*
  - has exotic strains of quarantine importance that do not occur in the country, *or*
  - causes economic damage, or has the potential to cause such damage, in important crops of the importing country, *OR*

- is a common pest already established in the importing country, but government or industry agreement requires that all commercial growers use pathogen-tested nursery stock, and the government has taken steps to ensure that imported stocks meet domestic standards.

Some suggestions that could be considered:

- consider agroecological zones rather than countries. It is quite likely that the pest population does not change at borders. Only in special cases will the freedom from a pest/pathogen in one country justify extensive routine testing of seeds coming from a neighboring country.

- list as pests of quarantine significance only those that are not already present in the country and could be of potential economic importance. Ubiquitous and saprophytic fungi such as *Cladosporium* spp. should not be considered in quarantine.

Seed Treatment

Introduction

Seed health is an important attribute of quality seed. A seed lot that meets high standards of germination, vigor and purity, but is contaminated with seed-borne pathogens, may not be suitable for planting because it may result in severe yield losses or even total crop failure in a large area. Seed may be affected by viruses, bacteria, fungi, nematodes and insects. Only certain groups can be controlled effectively by seed treatment. There are good pesticides available to control fungal pathogens and insects; for viruses, bacteria and nematodes, there are still problems to be solved. Seed treatment can be carried out through application of heat, mixing chemicals with seeds (seed dressing), and fumigation. Each treatment is described here in some detail.

Application of Heat

Heat can be applied using hot water or hot air, or as solar heat. Hot water, the most widely used, was first applied at the end of the 19th century to control cereal smuts. Until the introduction of systemic fungicides in the late 1960s, hot water treatment was the only way to control loose smut (*Ustilago nuda*, *U. tritici*). It is currently used by the Australian quarantine authorities to control exotic pathogens. The procedure for wheat is to presoak the seeds for 4-5 hours at 37-40°C, then treat them for 10 minutes at 54°C followed by air drying. Hot air is sometimes applied to eradicate viruses from seed, and solar heat treatment is used in countries with hot climates such as Egypt, but is being replaced by chemical treatment. Dry heat treatments (71°C for 7 days) have been used to eradicate *Xanthomonas campestris* pv. *translucens* from infected barley seeds.

Mixing of Chemicals with Seeds (Seed Dressing)

This is now a standard seed treatment procedure for many crops such as cereals, canola and cotton. The wide range of chemicals available is ever increasing. Equipment has been developed to ensure a safe, exact dosage.

Chemicals

Tables 9 and 10 give an overview of chemicals used for seed dressing. These lists are certainly not complete, nor do they imply a recommendation of the chemicals listed over others. It should also be noted that chemicals may not be registered in some countries, or may be registered under different trade names. Chemicals may be available in different formulations; e.g., dust, wettable powder for slurry treatment, or liquid concentrates. In general, dusts are applied at a rate of approximately 2 g/kg seed and slurries or liquids at 5-10 ml/kg seed. The latter formulations are frequently preferred over dust, because they enable easier exact measurement, allow better coating of the seeds, and cause less hazard to operators by avoiding dustiness. Different admixtures may be used in dust treatment to avoid the last two problems. A cheap additive is a 0.2% dextrine solution, added at a rate of 3-5 ml/kg seed. More effective, but also more

Table 9. Chemicals used in seed treatment to control important seed-borne diseases. ALWAYS read the label before applying any pesticides! ALWAYS follow the instructions given on the label!

Trade name (manufacturer)	Active ingredient	Target disease/pathogen	Crop
Abavit (Schering)	Prochloraz	Broad spectrum	Various
Agricultural streptomycin (Gustafson)	Streptomycin sulfate	Halo blight of beans (*Pseudomonas phaseolicola*)	Beans
Apron (Ciba Geigy)	Metalaxyl	Downy mildews	Various
Apron FL (Gustafson)	Metalaxyl	Downy mildews, *Pythium*, *Phytophthora* spp.	Peanuts, vegetables
Arbosan (Ciba Geigy)	Carboxin + imazalil	Loose smut, stripe disease	Barley
Baytan (Bayer)	Triadimenol	Loose and covered smut, common bunt, *Urocystis* spp., also early infections with rust or mildew	Cereals
Baytan F (Bayer)	Triadimenol + fuberidazole	Loose and covered smut, common bunt, *Urocystis* spp., snow mold, also early infections with rust or mildew	Cereals
Baytan Universal (Bayer)	Triadimenol + fuberidazole + imazalil	Loose and covered smut, common bunt, *Urocystis* spp., snow mold, *Helminthosporium* spp., also early infections with rust or mildew	Cereals
Captan 300 (Gustafson)	Captan	Seed decay, damping-off, and seedling blights	Numerous

Trade name (manufacturer)	Active ingredient	Target disease/pathogen	Crop
Epic 30 (Gustafson)	Iprodione	Seed decay, damping-off, and seedling blights	Ornamentals, turfgrass
Etilon GW (Ciba Geigy)	Imazalil	Stripe disease	Barley
Ferrax (ICI)	Flutriafol + thiabendazole + ethirimol	Broad spectrum	Cereals
Flo-Pro IMZ (Gustafson)	Imazalil	*Helminthosporium* spp., *Fusarium* spp., *Septoria nodorum*	Wheat, barley
		Fusarium spp., *Thielaviopsis* spp.	Cotton
4-Way (Gustafson)	Captan + maneb + PCNB	Seed decay, damping-off, root-rot and seedling blights	Peanuts
Funginex, Saprol (Marshall Thomas)	Triforine	Powdery mildew	Cereals
Galbas (Ciba Geigy)	Fenpiclonil	Snow mold, common bunt, *Urocystis occulta*	Rye, wheat
Germate Plus (Gustafson)	Lindane + diazinon + carboxin	Wireworms, Hessian fly, common bunt and smuts	Cereals
Kocide SD (Griffin Agr.)	Copper hydroxide	Seed-borne bacteria	Rice, wheat, barley
Mertect, Tecto (Gustafson, MSDAGVET)	Thiabendazole	*Ascochyta* spp.	Legumes
		Common bunt, dwarf bunt	Wheat
Panoctin 35 (Ciba Geigy)	Guazatine	Snow mold	Rye

Trade name (manufacturer)	Active ingredient	Target disease/pathogen	Crop
Panogen (Ciba Geigy)	Guazatine + propiconazol	Snow mold Common bunt	Wheat, rye Wheat
Prelude Universal (Schering)	Prochloraz + carboxin	Broad spectrum	Various
Pyrol (Ciba Geigy)	Fenpiclonil + imazalil	Stripe disease Snow mold, common bunt, *Urocystis occulta*, *Fusarium culmorum*, *Septoria nodorum*	Barley Wheat, rye, oats
Raxil (Bayer)	Tebuconazole	Smuts and bunt	Cereals
Rovral UFB (Ciba Geigy)	Iprodion + carbendazim	Loose smut, snow mold, common bunt, *Urocystis occulta*	Oats, wheat, rye
Sibutol (Bayer)	Bitertanol + fuberidazole	Loose smut, common bunt, snow mold, *Urocystis occulta, Tilletia contraversa, Fusarium nivale*	Wheat, rye
Vincit (ICI)	Flutriafol + thiabendazole	Smut, common bunt, *Septoria* spp., *Fusarium* spp., *Pyrenophora graminea*	Wheat, barley
Vitavax 200 (Gustafson), Vitavax 200FF (Uniroyal)	Carboxin + thiram	*Rhizoctonia solani, Helminthosporium* spp. *Fusarium* spp., smuts, common bunt, many others	Cereals Various

This table is based on manufacturer's information brochures. It is not an exclusive list, nor does it recommend listed chemicals in preference to others available. Trade names might be different in some countries. Chemicals listed are not registered in all countries, and some are registered for use only by commercial seed treaters.

Table 10. Insecticides used in seed treatment to control important insect pests (*not for control of storage pests*). ALWAYS read the label before applying any pesticides! ALWAYS follow the instructions given on the label!

Trade name (manufacturer)	Active ingredient	Target pest/pathogen	Crop
Contur (Ciba Geigy, Bayer)	Cyfluthrin	Wheat bulb fly (*Delia coarctata*)	Wheat, rye, oats
Di-Syston (Bayer, Mobay)	Disulfoton	Aphids, thrips, mites	Cotton
Flowable Lindane 40% (Gustafson)	Lindane	Wireworms	Various
Germate Plus (Gustafson)	Lindane + diazinon + carboxin	Wireworms, Hessian fly, common bunt, smuts	Cereals

This table is based on manufacturer's information brochures. It is not an exclusive list, nor does it recommend listed chemicals in preference to others available. Trade names might be different in some countries. Chemicals listed are not registered in all countries.

expensive, are special "incrusters;" e.g., Sacrust. Selection of the proper chemicals depends on the target organisms. In general, we can distinguish between three types of pathogens:

1. Pathogens contaminating the seed superficially and infecting the seedling after planting, e.g., common bunt (*Tilletia* spp.) and flag smut (*Urocystis agropyri*). They have only one generation per year and can be controlled with a wide range of chemicals applied as seed treatment. Because the spores may survive several years in the soil, healthy seeds planted in contaminated soil can give rise to diseased plants. In such cases, the treatment of healthy seeds may be advisable.
2. Pathogens infecting the embryo during flowering, e.g., loose smuts (*Ustilago* spp.). They also have only one generation per year and can be controlled only with systemic fungicides. Infected seeds do not show any symptoms.
3. Pathogens infecting many parts of the plant (leaves, stems, pods, seeds), e.g., *Ascochyta* spp. They have many generations per year, depending on environmental conditions. The seed infection is mostly internal, so systemic fungicides are recommended.

Control of insect storage pests is usually more effective with fumigation. In some cases, however, seed treatment may be preferable, especially to protect the seedling against soil-borne insects such as wireworms.

Even with excellent chemicals available, it is difficult, if not impossible, to achieve 100% control. During the treatment process, some seeds almost always escape proper treatment, whereas others receive more than the recommended dosage. By choosing the right equipment and calibrating it properly these problems can be minimized.

Equipment

The simplest way to mix seed with chemicals is with a shovel. This method, however, does not meet the requirements of even mixing, avoiding under- and overdosage, and operator safety. Better results are achieved by using a concrete mixer or a hand- or motor-driven drum, preferably in a diagonal position. Care must be taken to ensure that these devices are operated long enough to distribute the chemicals evenly.

A major disadvantage is that the required amount of chemicals is measured by hand. Frequently an unknown quantity of chemicals is added to an unknown quantity of seed, usually resulting in overtreated seed, which is not only quite expensive, but also could affect seed germination. For these reasons, machines have been developed that ensure automatic measuring of chemicals. In the Gustafson treaters, for instance, the weight of the seed, measured in a weigh pan, is used to operate the chemical measuring system. By adjusting a counterweight, a fixed quantity of seed is treated with a fixed quantity of chemical, e.g., 1 kg seed with 5 ml chemical. The chemicals are measured in standard cups which are operated with the trip of the weigh pan. Sizes appropriate for the required treatment capacity are available for this device and for similar devices from other manufacturers. **Automatic seed treaters only work reliably when properly calibrated.** The manufacturer's instructions should be followed.

Safety

There is a general tendency to use chemicals that are safe for user and environment. Very toxic substances, such as organic mercurials (Ceresan and others), and very persistent fungicides, such as Hexachlorobenzene (HCB), are being replaced by new chemicals. In the past, these chemicals have caused severe cases of poisoning, some resulting in death. Most, if not all, occurred because treated seed was used for human consumption and/or livestock feeding instead of for planting. Even with the new, less toxic chemicals, the following safety precautions must be taken:

1. Treated seed must be clearly labelled and under no circumstances be used for feed or food.
2. Seed treatment should be carried out in a well-aerated area. Contact with chemicals through breathing of dusts and skin contact must be avoided. Protective clothing (overalls, boots, gloves, masks) should be worn.
3. As with all pesticides, empty containers should be properly disposed of and never reused in the household or on the farm.

Control of Storage Pests

Introduction

There is a wide range of storage pests. In general, any organism that reduces the quantity and/or quality of stored grain or other food is a storage pest. This includes rodents, birds, insects, mites, fungi and bacteria.

The losses due to storage pests, sometimes referred to as post-harvest losses, vary according to climatic conditions, crops and storage facilities available. Quantitative losses are estimated to reach up to 30% world-wide. Qualitative losses, such as toxins produced by fungi and losses in viability of seed, are more difficult to determine or even to estimate. In absolute figures the losses in food or feedstuff are much higher than in seeds, but the consequences of losses in stored seeds are or might be more drastic. If the seed quantity is reduced considerably, the country concerned might have to import seeds and become dependent on the world market. The varieties available might not be suitable for growing under the country's specific conditions. Varieties unfit for the conditions inevitably result in poor harvests. For these reasons the prevention of storage losses in stored seed deserves special attention. We will concentrate here on cereals and food legume seed, because of their great importance. Special cases, such as specific pests of seed potatoes, will be mentioned briefly.

Rodents and their Control

Rodents, mainly rats and mice, can be very destructive, not only to seeds but also to buildings and electrical wiring (which may result in fire). They can consume substantial amounts of seeds and may also damage bags, boxes and other containers. The different species of rats may differ in their susceptibility to control measures.

A very effective but expensive way of control is to keep them away from stored products by using rodent-proof stores. However, because of the high costs of concrete buildings and silos, much of the seed stock is still stored easily accessible for rodents.

Traps are cheap and quite efficient if the population to be controlled is small. Biological control by predators (cats, hawks, etc.) or pathogens (causing rats' diseases) depends on too many factors to be reliable.

Still the most widely used method of rodent control is poisoning. A wide range of rodenticides is available from different companies. Those that are extremely toxic to human beings have been replaced by less hazardous ones (anticoagulants). Poisoning of rodents can be effective in the long run only if reinfestation is prevented by rat-proof buildings or by rodent control campaigns in larger areas.

Insects and Mites and their Control

While mites play a role mainly by transmitting spores of storage fungi, and by causing skin irritation and allergies in persons handling infested seed, insects can be very destructive to the seed. For seeds, those species of storage pests that develop inside the grain are the most important. Other pests, like flour beetles (*Tribolium* spp.) and mealworms, are more important in stored food grain.

Different species feed on cereals and legumes, and their different life cycles affect the appropriate control measures. In legumes, particularly faba beans, *Bruchus* spp. infest the seeds in the field and the insects complete their life cycle in the store. There is only one generation per year and no reinfestation in the store. However, *Callosobruchus* spp. and *Bruchidius* spp. are typical storage pests. The eggs are laid on the dry seeds and several generations develop in the store through reinfestation. In cereals, only the latter type, the true storage pest type, occurs. There are more than 20 different species, most of them belonging to the Coleoptera (weevils, beetles, grain borers) and some to the Lepidoptera (grain moths, mealworms). Most frequently encountered are the lesser grain borer (*Rhizopertha dominica*) and the granary weevil (*Sitophilus granarius*). Some of the storage insects are considered quarantine pests in some countries, namely the Khapra beetle (*Trogoderma granarium*).

In general, development of insects is faster with higher temperatures. About 35°C is considered the maximum for development and about 38°C the maximum for survival. There are differences between the different species, *Rhizopertha dominica* being an example of a heat-tolerant species. Below 0°C most of the insects cannot survive for more than 2-3 weeks. Also, a certain moisture level is required for insect development: 10-11% seed humidity is in general considered the minimum. The number of insects increases with increasing moisture up to a level where too many microorganisms develop (at about 15-16%).

The sources of infestation are important to know when it comes to control measures. As mentioned already, for *Bruchus* spp. infestations the source is in the field. Spraying the fields with insecticides during the time of oviposition can prevent infestation, but is expensive unless other insects have to be controlled at the same time. Care should be taken to select insecticides that are not toxic to bees, since some plants could be at the flowering stage. Visual inspection of the seeds after harvest can give an indication of the level of infestation. The seeds show characteristic dark spots, which are the holes through which the newly hatched larvae penetrate the seeds. If high infestation levels are detected, fumigation might be applied. This helps to stop larval feeding and thus will retain germinability. Moreover, the source of infestation for the next season, the adults that are carried with the seeds to the fields, will be reduced.

For the "true storage pests" there are many sources of infestation. A very important one is contamination from stored infested seeds. Frequently, the stores have a hidden focus, where a stock of insects survives attempts at control, such as crevices, corners, spilled seeds outside the store, or empty sacks with some leftover seeds. It is extremely important to control these areas. It is highly desirable to detect infestation at early stages before the insect population increases too much. Careful inspection of the stored

material at regular intervals helps. There are different methods to detect infestations before they become clearly visible. Some of them are simple (flotation of grains), others very sophisticated (X-ray). However, insect infestation is detected mainly by visual inspection. Storage insects are mostly controlled chemically. Unlike with grain stored for food or feed purposes the problem is not with residues hazardous to warm-blooded animals, but with the effect of chemicals on seed viability. Nevertheless, a low toxicity to humans is important, in order to protect the workers. In addition, a high and long-lasting effectivity against a wide range of insects is required. Last, but not least, it should also be cheap. We have to distinguish between insecticides that kill the insect population and have a longer lasting effect (pyrethroids, organophosphorous insecticides) and those which only kill the insects and have no residual effect (fumigants). These types are not true alternatives, but should be used in complementary treatments and in combination with storage sanitation.

Protective Insecticides

There is a wide range of products on the market. Table 11 gives some examples, but is not exhaustive. Insecticides may be applied in different ways: Dusting does not require much equipment: a powder formulation is either mixed with the seed, applied in layers ("sandwich method") or dusted over stacks. The latter can only prevent reinfestation (after fumigation), since most powder insecticides do not penetrate well enough into the stacks to control internal infestation.

For spraying, either a suspension (solid insecticide suspended in water, usually a wettable powder formulation) or a solution (liquid insecticide diluted in water) can be used. They can be applied with knapsack sprayers. For suspensions care should be taken that the particles remain suspended and do not sink to the bottom of the sprayer. This can be achieved by special stirring devices or by shaking the container frequently. Fogging is a technique used especially in stores. The droplets are much finer than in spraying. Special equipment is required. Evaporation can be used in special cases to control flying insects such as moths. This technique requires volatile insecticides and well-closed stores.

Fumigation

In many countries, fumigation is a routine treatment, carried out mainly against storage insects like grain weevils and bruchids. In general, the procedure is to apply a volatile insecticide in a confined area (silo, warehouse, or fumigation chamber). The main advantage of fumigation is that all insect stages including eggs, larvae and pupae are controlled.

Chemicals

Two chemicals are widely used: phosphine and methyl bromide. Others are dichlorvos, carbon dioxide, ethylene oxide, and HCN (Table 12). They have the following features in common: they are active in the gas phase, they are very hazardous to human beings, and they have a good penetration capacity into piles of sacks or seed stored in silos. It is therefore most important to hermetically seal the area under fumigation.

Table 11. Insecticides for control of storage pests in buildings (formulations for spraying, dusting and fogging).

Active ingredient	Trade name	Manufacturer
Bromophos	Nexion	Shell
Cyfluthrin	Baythroid, Solfac	Bayer
Dichlorvos	Nuvan, Nogos, Mafu, Vapona	Ciba Geigy, Bayer, Shell
Fenitrothion	Folithion, Sumithion	Bayer, Sumitomo
Jodfenphos	Nuvanol N	Ciba Geigy
Lindane	Lindagrain	Rhone Poulenc
Malathion	Malathion, Cythion	American Cyanamid
Methoprene (growth regulator)	Diacon	Gustafson
Phoxim	Baythion	Bayer
Pirimiphos-methyl	Actellic, Actellifog	ICI
Tetrachlorvinphos	Gardona	Shell

Table 12. Fumigants for control of storage pests.

Active ingredient	Trade name	Manufacturer
Ethylene dibromide (EDB)	Dowfume	Dow Chemicals
Methyl bromide (MB)	Dowfume MC Haltox	Dow Chemicals Degesch
Magnesium phosphide	Magnophos, Magtoxin	Degesch
Aluminum phosphide, phosphine (PH_3)	Phostoxin	Degesch

Phosphine. Available in a solid form (0.6 g pellets, 3 g tablets). The active ingredient is aluminum phosphide, mixed with ammonium carbonate and paraffin (trade name: Phostoxin). After exposure to the atmosphere, the pellets decompose and release the active substance, hydrogen phosphide (PH_3), which has the same specific weight as air,

and is thus evenly distributed in the fumigated material or chamber. Phosphine can penetrate bags, carton boxes, and other containers. The recommended dosage depends on the temperature (Table 13). Sometimes an extra day is recommended when tablets are used. Tablets or pellets are placed on cardboard that is spaced sufficiently wide apart to prevent spontaneous ignition. A powdery inert material remains after fumigation. This should be buried. The major advantages of Phostoxin are that it does not accumulate in seeds, does not affect flavor or germination, and is easy to handle.

Table 13. Exposure time to aluminum phosphide† depending on temperature.

	Temperature			
	12-15°C	16-20°C	21-25°C	> 26°C
Days	7	6	5	4

† The recommended dosage is 2.5 g phosphine/m^3, generated by 2.5 Phostoxin tablets of 3 g or 12 Phostoxin pellets of 0.6 g (Bond 1984).

Methyl bromide. Above 5.6°C, methyl bromide is in the gas phase, and is available in cylinders similar to those used for cooking gas. Since it is odorless, other gases such as chloropicrin are sometimes added to facilitate detection of leaks. Because methyl bromide is 3.5 times heavier than air, care has to be taken that it is properly distributed within the goods to be fumigated, by using a fan, for example. At a given temperature the product of concentration and time required to kill all stages of insects is constant. That means a high concentration requires a short exposure time and a low concentration requires a longer exposure time. The recommended dosage is 24 g/m^3 for 24 hours at 10-19°C and 16 g/m^3 above 20°C. Fumigation with methyl bromide is not advised above 25°C. Special safety measures are required because methyl bromide is absorbed through the skin. It tends to accumulate in commodities, therefore repeated fumigation with methyl bromide should be avoided. For the same reason it is also more hazardous for operators.

Equipment

Gas-proof plastic sheets with at least 50 cm overlap, firmly pressed to the ground with sand, iron bars or other weights, are frequently used. Gas escape results in reduced insecticidal effect and is a hazard to users. A cement floor is necessary to prevent gas escape through soil. Care must be taken that the fumigation area is properly aerated; fans may help. If a store's doors and windows can be hermetically sealed, fumigation of the entire store is possible. Most stores, however, allow gas to escape through other openings. Silos are usually good fumigation facilities. When large quantities must be fumigated within a short time, a vacuum fumigation chamber is appropriate. These

chambers are available in sizes between 1 and 50 m^3, and sometimes as a plant of up to 6 x 50 m^3, equipped with common fans, pumps and other equipment. The insecticides used are methyl bromide or ethylene oxide.

Safety

Face masks with a proper canister should be used, especially during the aeration process (gray canister for Phostoxin, brown canister for Methylbromide). When handling Phostoxin, cotton gloves should be worn. Gas concentration can be checked with a Halide gas detector for methyl bromide and with a tube detector (Draeger) for Phostoxin. A warning sign should be clearly visible to prevent people from inadvertently removing plastic sheets or entering a building under fumigation.

A more recent development is the storage of grain and seed in controlled atmosphere. This method is well known from the storage of fruits, particularly apples. The air in stores is replaced by an inert atmosphere: less than 1% O_2, about 9% CO_2 and the balance N. This inert gas is generated by combustion of air with propane in special devices. In this environment no storage pests can survive. The seeds retain their viability over years, much superior to storage in normal air. However, this method is quite expensive. In addition to the equipment for changing the atmosphere, it is essential to have airtight stores in order to retain the inert atmosphere over the whole storage period.

Special case: Potato Tuber Moth

This pest attacks potato plants in the field as well as seed potatoes in the store. The damage in the field is minor and usually does not result in severe yield losses. The main problem with this pest is losses in quality of the tubers for consumption. The insect has several generations per year, depending on the temperature. Above all, stores with their constantly high temperatures offer ideal conditions. Control of the pest in the seed store breaks its cycle and prevents planting of infested tubers, and thus helps to control the pest in the field. For effective pest control in the store a combination of different measures is required:

1. Only uninfested tubers should be stored.
2. Fumigation should be carried out if there is infestation.
3. Doors and windows should be protected with insect-proof mesh to prevent moths from invading the stores.
4. Reusable containers (bags, etc.) should be treated with malathion.
5. Traps (pheromone traps, light traps) should be installed for monitoring infestation; to some extent they will reduce the population.
6. As with the control of other storage pests, cleanliness of the stores and their surroundings is very important.

Fungi and Bacteria and their Control

Here we deal only with those fungi that infect seeds in the store, and not the seed-transmitted fungi, which cause plant diseases in the field and are carried on or in seeds. Bacteria normally do not affect stored seeds, unless the moisture content is very high and the temperature is already raised by fungal infection. Fungi require a high moisture content to grow (minimum about 14% seed moisture) so they do not play a very important role in dry climates.

The most important genera are *Aspergillus* and *Penicillium*. They are mostly saprophytes, which means they are unable to attack living tissue. They grow on dead cells of the seed surface, where they produce toxins. They cause seed decay and may kill the embryo. Heavily infected seed should not be used for feeding because of the toxins many of these fungi produce. The best way to control storage fungi is to maintain low moisture in seeds and store. Low temperature slows down the growth of fungi. Fungicidal seed treatment often does not give the expected results, because of lack of free water, which is required for many fungicides to become effective. The use of propionic acid to control storage fungi is restricted to grain for feeding purposes. In summarizing the control of storage pests, it has to be stressed that the best method of control is with solid buildings and a rigid storage hygiene.

International Activities in Seed Health Testing

Introduction

Seed health testing is often a matter of international relevance, for example when seeds are exported. A number of institutions either prescribe methods or standards, or offer assistance to laboratories. Below is a selection of such institutions, particularly those with activities in the area of West Asia/North Africa.

International Seed Testing Association (ISTA)

ISTA is the organization prescribing rules for seed quality testing. The rules are regularly updated. Accredited laboratories are authorized to issue the "International Seed Lot Certificate" and they have to follow the rules for sampling and testing. The ISTA certificates normally contain the results of purity, germination and sometimes moisture analysis. Results of seed health tests may be given on request in the section "other determinations."

ISTA also publishes a journal, *Seed Science and Technology*, which contains scientific articles related to seed, proceedings of ISTA Congresses and the ISTA rules.

The *ISTA News Bulletin* is distributed free of charge and contains useful information on activities related to seed testing, seed trade, etc., and on relevant publications. For more information contact:

ISTA Secretariat
P.O. Box 412
CH-8046 Zürich
Switzerland

The ISTA Plant Disease Committee

This committee organizes workshops and training courses, and publishes the *ISTA Handbook on Seed Health Testing*, which consists of a regularly revised *Annotated List of Seed-borne Diseases*, a publication *Introduction to Methods of Seed Health Testing* and a loose-leaf collection of *Working Sheets* that describe important seed-borne pathogens and methods for their detection. These publications are designed as concise information for seed pathologists, but they are not part of the ISTA rules. The committee consists of different working groups: bacteriology, nematology, etc. They work out new methods and organize referee testing between laboratories. For more information contact:

Dr. Cees Langerak
Chairman, ISTA Plant Disease Committee
CPRO-DLO
Postbus 16
6700 AA Wageningen
The Netherlands

FAO and the Phytosanitary Certificate

In 1951 the International Plant Protection Convention was founded by FAO and most countries are members of this convention. One of the achievements was a standard quarantine certificate, which is recognized by all members. The Phytosanitary Certificate has received some criticism. It is rather vaguely phrased, e.g., "plants, parts of plants or plant products described below or representative samples of them": it makes a great difference whether all plants, seeds, etc. or a representative sample was considered, moreover, what is supposed to be a representative sample? No details on test methods are given, only "thoroughly examined." It goes on "to the best of his knowledge" and "substantially free from injurious diseases and pests." Eventually, the "consignment is believed to conform with the current phytosanitary regulations of the importing country." Many developing countries in particular have great faith in these certificates, and may even request that they be authenticated by their embassies.

On the other hand, the "current phytosanitary regulations" referred to above often leave a lot to be desired. They are frequently outdated, irrelevant or not available to those who sign that they believe the consignment is in agreement with them.

Regional Plant Protection Organizations

They have been established on the basis of biogeographical areas and are active in areas of plant protection, including quarantine. Regarding activities in the Near East region contact:

Dr. Mahmoud M. Taher
Regional Plant Protection Officer
Regional Office for the Near East
P.O. Box 100
Cairo - Dokki
Egypt
Tlx: 21055 FAONE
Fax: 3497232

The North African countries are members of EPPO, the European and Mediterranean Plant Protection Organization. EPPO has been particularly active in quarantine matters. They publish the *EPPO Bulletin* and *Data Sheets on Quarantine Organisms*. For more information contact:

EPPO
Rue LeNotre
75000 Paris
France

The Danish Government Institute of Seed Pathology for Developing Countries

The institute was founded in 1967 by Dr Paul Neergaard and is now directed by Dr S.B. Mathur. The institute organizes workshops, seminars and training courses world-wide and trains staff at the institute near Copenhagen. Through association with the Agricultural University in Copenhagen it can award academic degrees. Scholarships are available through DANIDA, the Danish Development Aid Institution. The institute publishes a newsletter, *Seed Pathology News*, which is distributed free of charge, and a series of *Technical Bulletins*, dealing for example with seed-borne diseases of rice.

For more information write to:

Danish Government Institute of Seed Pathology for Developing Countries
Ryvangs Allé 78
DK-2900 Hellerup
Denmark

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Glossary*

Acervulus, pl. **acervuli:** a cushion-like mass of hyphae and palisade-like conidiophores and conidia, characteristic of Melanconiales.
Adhesion: sticking or adhering to the surface of treated seed.
Agar: powder made from seaweed that when dissolved in water and heated results in a gelatinous base for culturing microorganisms.
Antagonist: microorganism counteracting physiologically another microorganism; an agent of physiological antagonism.
Antibiotic: a substance produced by a microorganism capable of inhibiting in low concentrations the growth of other microorganisms.
Antibody: any substance or body in tissues or fluids of an organism, as blood or serum, which acts in antagonism to specific foreign bodies.
Antigen: a substance that induces production of an antibody when it is introduced into the body of a living warm-blooded aninmal.
Antiserum: a serum containing antibodies.
Apothecium: a discoid or cup-like ascus-containing fruiting body.
Appressorium: a swelling on a germ tube or hypha, attaching it to a host tissue in an early stage of infection.
Ascocarp: a structure producing asci.
Ascospore: spore produced in an ascus (perfect state).
Ascus, pl. **asci:** a sac-like structure containing usually eight ascospores characteristic of Ascomycetes.
Asexual: non-sexual; produced vegetatively.
Avirulent: lacking virulence.

Basidiospore: a spore produced on the outside of a basidium (perfect state).
Basidium, pl. **basidia:** the spore-producing hypa, generally club-shaped, on which are produced usually four basidiospores, characteristic of the Basidiomycetes.
Bioassay: a test based on response of living organisms.

Carrier: an infected plant that shows no marked symptoms.
Certification of seed: organized quality control of seed for marketing with regard to germination capacity, freedom from seed-borne diseases, and maintenance of varietal purity.
Certified Seed: seed tested and found to conform with certain requirements as to origin, purity, germination, freedom from disease, and produced for seed merchants and farmers.
Chlamydospore: a thick-walled, non-sexual resting spore, developed directly from hyphal cells.
Cleistothecium: a completely closed ascocarp, as in powdery mildew fungi.

* Adapted from: Neergaard, P. 1977. Seed Pathology Vol.II The Macmillan Press Ltd., London
Johnston, A. and C. Booth (eds.) 1983. Plant Pathologist's Handbook, 2nd edition. Commonwealth Agricultural Bureaux, Slough, UK.

Closed quarantine: post-entry control of plants for pests and diseases carried out by growing polants kept under surveillance, under conditions excluding contaminations from the surroundings.
Colony: a circumscribed mass of microorganisms, usually grown out of a single propagule.
Concomitant contamination: presence of inoculum as admixture with seed, as sclerotia or infected plant debris mixed with seed.
Conidiophore: a specialized hypha on which conidia are produced.
Conidium, pl. conidia: any asexual fungus spore, except chlamydospores and sporangiospores.
Contamination: passive contact, presence of a microorganism or pathogen with any material (soil, implement, etc.) or any host.
Control: 1. to prevent or retard the development of disease; 2. untreated subject for comparison with experimental treatment.
Cultivar: a variety of cultivated plant, short for cultivated variety.
Culture: a growth of an organism for the purpose of experiment, especially on laboratory media (culture media) often used in the sense of isolate or strain.
Cyst: a closed sac, a resting structure containing a juvenile stage of an organism, such as a cyst of nematodes containing larvae.

Diagnosis: determination of a pathogen or a disease.
Disease: an impairment of the normal state of a living plant or animal that interferes with structure, functions or economic value.
Disinfect: to free diseased plants or plant parts from infection.
Disinfest: to kill or inactivate disease organisms as a precaution against infection of plants, as in the soil or on surfaces of seed.
Disorder: a harmful nonpathogenic deviation from normal growth.
Dispersal: the scattering, e.g., of seed or of pathogenic units, such as fungus spores or bacteria by wind, animals, etc.
Dissemination: the transportation of inoculum from one location to another.
Dormant: in a resting condition, as in dormant mycelium (mycelium in an inactive persistent stage).
Dressing: 1. the process of covering seeds with a fine coating of fungicide; 2. the fungicide applied to seed.

Embryo: the young sporophyte of a seed plant; the rudimentary plant as formed in a seed, a germ.
Endemic: native to one region, country or part of the earth.
Epidemic: widespread temporary increase in the incidence of an infectious disease.
Eradicant fungicide: 1. a fungicide applied to a substratum in which the fungus is already present; 2. a fungicide used in disease control after infection has been established.
Etiology: the causes and the study of the causes of a disease or abnormal condition, and the nature of the causal agents.

Facultative parasite: one able to live as a saprophyte and to be cultured on laboratory media; not obligate.
Focus: local concentration of disease.

Forecasting: prediction of development of disease as a result of rational study of available pertinent data.
Forma specialis, pl. **formae speciales:** a group of biotypes classified on the basis of physiologic properties, particularly those of pathogenicity as established in relation to a defined host range of plant genera, species or (botanical) varieties.
Foundation Seed: primary source of seed of highly selected standard in regard to qualities of a cultivar, and used for seed production.
Fungicide: a chemical agent that inhibits or kills fungi.
Fungistatic: arresting fungal growth but not killing the fungus concerned.
Fungus: any of a major group of saprophytic and parasitic lower plants that lack chlorophyll, such as molds, mildew, smuts, rusts.

Germination: active growth by the embryo of a seed; the process of growth from a seed or a spore. For germination in seed testing these are considered: normal seedlings (plant-producing seeds), hard (dormant) seeds, abnormal seedlings (regarded not to produce normal plants), dead seeds and blind or empty seeds.
Growing-on test: test on plants grown from seed beyond the seedling stage in a greenhouse or an environment-controlled room.

Haustorium: a special hyphal branch, especially one within a living cell of the host, for absorption of food.
Hemacytometer: a special glass slide devised for microscopic recording of units such as red blood cells or fungal spores suspended in a known quantity of liquid; counting chamber.
Host: a living organism harboring a pathogen, a parasite.
Hyaline: colorless, transparent.
Hypersensitivity: reaction of a host to infection resulting in prompt death of invaded tissue.
Hypha, pl. **hyphae:** a single thread of a fungus mycelium.

Immune: not susceptible, having absolute resistance to a disease.
Imperfect fungus: a fungus for which a sexual reproductive stage is lacking or unknown, a state of a fungus producing non-sexual spores only.
Imperfect state: reproductive asexual state of a fungus.
Incubation: in seed health testing, maintaining seeds in an environment favorable to development of symptoms or structures of pathogens.
Indexing: testing disease condition of a seed stock by preplanting portions of the stock or by inoculating from the seed stock into indicator plants.
Indicator plant: one that reacts to certain viruses or environmental factors with specific symptoms, used for identification of the viruses or the environmental factors.
Inert matter: as used in seed testing (purity analysis), any foreign matter that are not seeds, such as stones, chaff, pieces of seed less than one-half of the original seed, sclerotia, etc.
Infect: to invade and establish a pathogenic relationship in a host or to persist in a carrier.
Infection: the act or result of invading and getting established as a pathogen in a host or a carrier.

Infestation: contamination; presence of a pathogen in material, from which it may infect plants, as in soil or on the surface of seed.
Inoculate: to place inoculum in order to establish infection.
Inoculum: infective material; pathogens or part of them.
Intercept: to interrupt communication, as in withholding a quarantine object in controlling plants in international trade; to prevent introduction of a pathogen.
In vitro: carried out under laboratory conditions, in test tubes, petri dishes, flasks, etc.
In vivo: under natural conditions in contrast to artificial, laboratory conditions.
Isolate: 1. (verb) to separate a microorganism from host or substrate and establish it in pure culture; 2. (noun) a single spore or pure culture and the subcultures derived from it. Also applicable to viruses.

Koch's postulates: three criteria proposed by R. Koch for proving pathogenicity, namely: 1. isolation of the pathogen; 2. inoculation of healthy plants, resulting in the same symptoms, 3. re-isolation of the pathogen.

Latent period: time between infection and appearance of disease symptoms.
Lesion: a usually well-defined abnormal change in structure of an organ due to disease or injury.
Local lesion reaction: response of inoculation of leaves by a suspension of bacteria or virus or some other preparation of a pathogen, on which a quantitative estimation of the infectivity can be recorded.

Macroconidium, pl. macroconidia: a larger conidium, as distinguished from a smaller type of condiia in the same species, a microconidium, e.g., in *Fusarium* spp.
Macroscopic: visible by the naked eye.
Medium, pl. media: culture medium, a substance or solution for the culture of microorganisms.
Microconidium: a type of small conidia.
Microflora: vegetation of microorganisms on a restricted area of substrate, as of a plant or in a seed sample after incubation; also that of a geographic region.
Morphology: the study of the form and structure of, for example, seeds.
Mycelium, pl. mycelia: a mass of hyphae forming the thallus (body) of a fungus.
Mycoplasma: genus of microorganisms, which have a unit membrane and not a rigid cell wall, such as bacteria.
Mycostatic: fungistatic.

Necrosis: death of a living cell; dead part of living tissue.

Obligate parasite: one capable of living only as a parasite.
Oidium: spore produced by simultaneous segmentation of hyphae, e.g., in powdery mildew.
Oospore: a thick-walled resting spore produced by sexual reproduction, as in Oomycetes.
Ostiole: opening of a true perithecium or a pycnidium, ending in a pore.

Parasite: an organism that lives in or on another living organism of a different species, from which it draws its nourishment.

Pathogen: a causal agent or factor of disease.
Pathogenic: capable of causing disease in a host or range of hosts.
Pathogenicity: the ability of a pathogen to cause disease.
Pathogenesis: the sequence of processes in disease development from initial contact between pathogen and host to completion of syndrome.
Pathology: the science of the nature and control of diseases.
Perfect state: reproductive sexual state of a fungus.
Pericarp: the mature ovary wall.
Period of incubation: time between placing seed on agar, blotters, etc. and recording of infections or health condition.
Perithecium: a flask-like or globose, hollow fruiting body that produces asci and usually opens by a pore at the top, a true ostiole.
Pest: any pathogen or other organism such as insects, mites, nematodes that injure plants and plant products.
Petri dish: a flat, circular glass or plastic container with vertical sides and slightly larger cover that fits over it.
Phialide: a usually bottle-shaped terminal cell from which conidia are produced.
Phytosanitary certificate: a certificate of health of plants or plant products to be exported.
Pionnotes: a slimy or gelatinous, effuse sporodochium, especially of *Fusarium*.
Plant quarantine: official precautions against introduction of plant pathogens and pests from one area into another, usually uninfested area. Applied usually between countries, and may include: provision for inspection of plants and parts of plants in the exporting country; issue of plant health certificates and plant health certification schemes in the exporting country; inspection of plants and parts of plants and post-entry control in the importing country; embargo.
Post-emergence: seedlings after appearance above the soil surface; post-emergence killing of seedlings, post-emergence damping-off.
Post-entry control: quarantine control, inspection of plants or parts of plants after arrival of the consignment to the country of importation.
Pre-emergence: the condition of ungerminated seed and nearly germinated seed below the soil surface; pre-emergence killing.
Pre-entry control: quarantine control inspection of plants or parts of plants in the exporting country.
Pretreatment of seed: any physical or chemical laboratory treatment of seed preceding incubation, given solely to facilitate seed health testing, such as pretreatment with sodium hypochlorite (Clorox).
Primary infection: the first infection following a resting or dormant period of a pathogen.
Promycelium, pl. **promycelia:** a hypha of restricted growth bearing basidiospores, the epibasidium of the rusts and smuts.
Propagule: any unit in service of propagation, such as spore, sclerotium, mycelial fragment, etc.
Protective fungicide: one used as a protectant.
Pycnidium, pl. **pycnidia:** an asexual, hollow fruiting body in which conidia are produced, characteristic of the Sphaeropsidales, e.g., *Ascochyta* spp.

Range (host): the series of hosts of different species, varieties and cultivars of a certain pathogen.
Registered Seed: seed of a standard between Foundation Seed and Certified Seed, usually used for production of seed for farmers and seed merchants.
Resistant: able to suppress or retard the effect of a pathogen or other injurious factor; in a broader sense, also to escape or exclude infection.
Resting spore: a thick-walled spore, usually formed as a result of a sexual process, which germinates after a resting period (frequently over winter).

Sampling: selection of a minor quantity of seeds or other material from a larger bulk, with the purpose of evaluating the lot.
Saprophyte: an organism that feeds on dead or decaying organic matter.
Sclerotium: a rounded or irregular firm mass of fungus tissue which usually serves as a resting body.
Secondary infection: infection in a period of active spread of a disease.
Seed-borne: of a pathogen or any microorganism or pest, carried on or with the seed, but not necessarily transmitted.
Seed dressing: chemical seed treatment.
Seed pathology: the science and technology dealing with 1. seed-borne plant diseases, 2. seed diseases, 3. the mechanisms of their transmission, 4. factors influencing their development, 5. techniques for their detection in seed, 6. the methods for preventing and controlling these diseases in the field and during seed storage, and 7. the assessment of seed-borne inoculum and seed disease for seed certification schemes, quarantine, planting value, and quality for consumption or processing.
Seed sample: a small quantity of seed taken as representative of a seed lot.
Seed treatment: any process, physical or chemical, to which seeds are submitted.
Sensitive: reacting with severe symptoms to the attack of a given pathogen.
Single spore isolate: a pure fungus culture grown from one spore.
Slurry: a thin mixture of seed treatment chemical powder and water.
Sorus: a fruiting structure or spore mass of certain fungi, particularly spore masses of rust and smuts.
Source of inoculum: the place harboring inoculum and from which it may be disseminated and cause infection.
Sporangiophore: a specialized hypha on which a sporangium is produced.
Sporangium: an organ producing asexual spores endogenously.
Spore: a minute, reproductive unit, as of fungi, which may germinate and develop into a new individual.
Sporodochium, pl. sporodochia: cushion-like mycelial masses on which conidia are produced, as particularly in *Fusarium* spp.
Sporulate: to produce spores.
Storage fungi: fungi particularly adapted to development in stored seed, usually requiring for development a high osmotic pressure, such as species of *Aspergillus* and *Penicillium*.
Strain: a group of biotypes presumed to have common ancestry, usually distinguishable not morphologically, but physiologically from other strains; a strain of an isolated fungus.
Stroma, pl. stromata: a cushion-like tissue of fungal cells or of mixed fungus cells and host tissue, in or on which fructifications are developed.

Substrate: the material, dead or living, on which an organism lives and draws nourishment.
Summer spore: a spore germinating without resting, frequently living only a short time.
Susceptible: unable to withstand attack of a pathogen or influence of an injurious factor; non-immune.
Symptom: evidence of a disease; any reaction of a host to disease.
Symptomless: showing no observable signs of disease; usually of a carrier.
Systemic: (as infection of a host) - affecting the host generally; general presence of a pathogen in the host, originating from the spread from a single infection or a few points of invasion.

Teliospore: a thick-walled resting spore; in the rust fungi (state III) and smut fungi.
Tolerance: 1. the capacity of a host to endure an infection without being seriously affected, particularly in terms of agricultural yield. [Note: not to be used in the sense of intermediate resistance]; 2. maximum level of infection acceptable in a crop or a seed lot according to agreement, as may be established in a seed health certification scheme; 3. evitable (permitted statistical deviation from standard).
Tracheomycosis: a fungal disease in which the pathogen is mainly confined to the xylem.
Transmission: the acts of disseminating and inoculating inclusively; the act of perpetuating a disease from one generation to another, as through seed or vegetative propagation. There is some difference of opinion among plant pathologists in regard to the use of the phrase 'disease transmission.' Some maintain that a disease, not being a concrete body, cannot be transmitted, only the pathogen or infectious agent being subject to transmission. The term 'dissemination' is used only in connection with a pathogen. This restricted use is based on the concept that the pathogen may be disseminated widely but the disease is not transmitted until inoculation and infection have occurred. Thus, an insect may disseminate a pathogen without transmitting the disease.

Uredospore: a binucleate spore (state II) of the Uredinales (rusts).

Vector: an organism that transmits inoculum.
Viable: capable of living.
Virulence: the degree of ability to produce disease; often used to denote qualitative rather than quantitative differences of pathogenicity.
Virus: any of a large group of submicroscopic, filterable, infective agents, capable of multiplication only in living cells and causing various diseases.

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