– 27 –

Exclusion and Eradication

The most effective method to manage diseases is to exclude them from the field. Quarantine is important in preventing the spread of pathogens across countries, and legislative control is useful to prevent the entry of a pathogen into a new region. If a pathogen has entered into a region, eradication of the established infection is practiced. Because most diseases are spread internationally through seed, seed health testing and seed certification help to prevent the spread of diseases. Various seed health testing methods are described in this chapter.

EXCLUSION

Quarantine services exist in most countries. Plant quarantine is legislative or regulatory control that aims to exclude pathogens from areas where they do not already exist. Legislative control may operate on a national or international level. The legislation prohibits or restricts the introduction of seeds, vegetative propagating materials, plants, or plant parts into a country or a region in a country to exclude pathogens, which may be inadvertently introduced along with those materials. Generally, scientists, the traveling public, and some importers of agricultural products are responsible for the introduction of new pathogens into a region.

Not all pathogens are of quarantine significance. A pathogen species that does not occur in a given country or an exotic strain of a domestic species is of quarantine significance to that country if the pathogen is known to cause economic damage elsewhere or has a life cycle or host/pathogen interaction that shows a potential to cause economic damage under favorable host, inoculum, and environmental conditions (Kahn, 1991). Importation of a pathogen that already occurs in a given country is also of quarantine significance if an ongoing regional or national containment, suppression, or eradication program is directed against that pathogen species.

Actions to be taken to exclude pathogens are authorized by government regulations. In the United States, the Plant Quarantine Act was enacted in 1912. The act provides authority for domestic and foreign quarantines. The

Organic Act of 1944 authorizes the secretary of agriculture to cooperate with states, organizations, and individuals to detect, eradicate, suppress, control, and prevent or retard the spread of plant pests (including pathogens). The Federal Plant Pest Act was enacted in 1957 and regulates the movement (by persons) of plant pests into United States or between states and authorizes emergency actions to prevent the introduction and domestic movement of plant pests not covered by the Plant Quarantine Act of 1912 (Kahn, 1991).

Transport of plant material across international boundaries is regularized by the International Plant Protection Convention of 1951. The convention was organized by the United Nations Food and Agriculture Organization (FAO) with the aim of securing common and effective action to prevent the introduction and spread of pests and diseases of plants and plant products. The convention was signed by 94 nations and conformed to by most other countries. The phytosanitary certificate is an instrument of that treaty. The convention is now regionally organized. In Europe and the Mediterranean, the organizational body is the European and Mediterranean Plant Protection Organization (EPPO), and in North America, it is the North American Plant Protection Organization (NAPPO). These organizations regularly issue bulletins including information on newly identified pests and pathogens and phytosanitary regulations (Parry, 1990).

Most member countries have their own disease legislation with regard to imported plant material. Some countries have formed unions, such as the European Economic Community, and promulgate binding regulations on member countries. The legislation prescribes the form of health certificate to accompany any imported material. It lays down rules for inspection and disposal of material if it contains pathogens. It also provides a list of prohibited imports and a list of restrictions of imports of material from specified areas. In the United Kingdom, Plant Health Order 1987 lists import restrictions into the United Kingdom.

The legislation is normally implemented by customs and excise officers. They check the documentation, and specifically phytosanitary certificates, at ports. Spot checks are carried out by officers of the Plant Health and Seeds Inspectorate on material both entering and leaving the United Kingdom. Suspect materials are put into quarantine for a period of time to detect pathogens, which are present in seed and planting materials.

In spite of quarantine methods, a few pathogens have entered into countries that had not reported the occurrence of such pathogens earlier, shattering the economy of those countries. Rust disease of coffee (*Coffea arabica* and *C. canephora*) wiped out coffee plantations in Sri Lanka in 1880. The disease spread to Central and East Africa by the 1920s and to West Africa in the 1950s. It became severe in Brazil in 1970, and the disease is now prevalent in Mexico, Honduras, Paraguay, Argentina, Peru, Bolivia, Guatemala, Colombia, Costa Rica, India, and almost all countries where coffee is grown. Fire blight of apple is widespread in North America and it is not a quarantine object in the United States. However, in Europe, fire blight of apple is not prevalent in many countries. The disease was first detected in Spain in 1995 and in Hungary in 1996.

Karnal bunt of wheat was first discovered in 1930 at Karnal, a small town in the Haryana state in India. It was subsequently reported in countries around India, Pakistan, Nepal, Afghanistan, and Iraq. It was first reported in Mexico in 1972. In the United States, the disease was first discovered in Arizona in 1996. Subsequently, the disease has been reported in Texas, New Mexico, and California. The Mexican government placed an internal quarantine on Karnal bunt to prevent disease spread within the country in 1984. In 1996, a federal quarantine for Karnal bunt was placed on the states of Arizona, Texas, New Mexico, and California. The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) prohibited importation of seed, grain, straw, and dried plants of wheat, durum, and triticale from Mexico to prevent the entry of Karnal bunt into the United States. Mexico was permanently added to the list of the wheat disease subpart of Quarantine 319 (Babadoost, 2000). A zero-tolerance level of Karnal bunt has been enforced in the United States, Canada, and many other countries. The certification standard in India is zero incidence. APHIS tests the presence of teliospores in wheat seed samples by the centrifuge wash test. Test tubes containing seeds submerged in water are shaken for ten minutes to obtain teliospore suspension, then centrifuged for 20 minutes at 3,000 rpm and the sediment examined under a microscope for the presence of teliospores (Warham, 1992). The size-selective sieving test is also recommended (Peterson et al., 2000). A seed wash of a 50 g seed sample is washed through 50 μM and 20 μM pore size nylon screens to remove unwanted debris and to concentrate and isolate teliospores. The material remaining in the 20 μM pore size is suspended for direct microscopic examination and identified by polymerase chain reaction (PCR) utilizing two pairs of *Tilletia indica*-specific primers.

ERADICATION

National legislation also enforces eradication of exotic pathogens recently introduced along natural or man-made pathways. Canker is the most serious disease in *Citrus* spp. It was first reported in 1913 in Florida, and an extensive citrus canker eradication program was implemented there in 1915. After $6 million had been spent for eradication, Florida was declared free from the disease in 1933. The eradication program was also taken up in Georgia, Alabama, Louisiana, South Carolina, Texas, and Mississippi. In 1947, citrus canker was declared to be eradicated from these states. However, a new form of the disease appeared in 1984 in Florida. Another eradication program was implemented, and by 1991, over 20 million trees had been destroyed at a cost of about $94 million dollars. The Asiatic citrus canker is still prevalent in different parts of the United States (Gottwald et al., 2001). The pathogen of fire blight of apple *(Erwinia amylovora)* entered Hungary in 1996. In 1997 and 1998, further spread of the disease was registered and an eradication program was launched. More than 60,000 trees were uprooted and destroyed across the country. Eradication was performed partly by special brigades and partly with participation of growers. *Erwinia amylovora* was first detected in 1995 in Spain, and several measures were taken to eradicate the bacteria there as well.

In the United Kingdom, national plant disease legislation has been introduced to eradicate specific pathogens. It makes farmers responsible to inform officials about outbreaks of indigenous but geographically localized diseases, known as *notifiable diseases.* Fire blight of apples and pears *(Erwinia amylovora),* wart disease of potatoes *(Synchytrium endobioticum),* brown rot *(Ralstonia solanacearum)* and ring rot (*Clavibacter michiganensis* ssp. *sepedonicus*) of potato, plum pox disease of plums *(Plum pox virus),* red stele disease of strawberries, rhizomania disease of beet *(Beet necrotic yellow vein virus),* and progressive wilt of hops *(Verticillium albo-atrum)* are the important notifiable diseases in the United Kingdom. Occurrence of such diseases must be reported. The diseased material should not be transported or sold and must be destroyed.

LIST OF IMPORTANT SEEDBORNE PATHOGENS

Several fungal, bacterial, viral, and phytoplasmal diseases are transmitted through seeds, including vegetative propagules (Mink, 1993; Johansen et al., 1994; Langerak et al., 1996). The important seedborne pathogens are listed in this section.

Alfalfa—*Alfalfa mosaic virus*
Barley—*Barley stripe mosaic virus, Xanthomonas campestris* pv. *translucens, Xanthomonas campestris* pv. *undulosa, Rhynchosporium secalis, Ustilago segetum* var. *nuda, Pyrenophora teres*
Bean—*Bean common mosaic virus, Bean pod mottle virus, Bean southern mosaic virus, Bean yellow mosaic virus, Pseudomonas savastanoi* pv. *phaseolicola, Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas axonopodis* pv. *phaseoli, Pseudomonas syringae* pv. *syringae, Colletotrichum lindemuthianum*
Beet—*Phoma betae*
Blackgram—*Urd bean leaf crinkle virus, Blackgram mottle virus*
Broad bean—*Broad bean mottle virus, Broad bean true mosaic virus, Broad bean stain mosaic virus, Broad bean wilt virus*
Carrot—*Xanthomonas campestris* pv. *carotae, Alternaria dauci, A. radicina*
Celery—*Septoria apiicola*
Cherry—*Cherry leaf roll virus, Cherry rasp leaf virus, Cherry X-disease*
Corn—*Maize chlorotic dwarf virus, Maize mosaic virus, Erwinia stewartii, Fusarium moniliforme, Peronosclerospora sacchari, Peronosclerospora sorghi, Sclerospora graminicola*
Cotton—*Xanthomonas axonopodis* pv. *malvacearum, Colletotrichum gossypii*
Cowpea—*Blackeye cowpea mosaic virus, Cowpea aphid borne mosaic virus, Cowpea banding mosaic virus, Cowpea mild mottle virus, Cowpea mosaic virus, Cowpea ringspot virus, Cowpea severe mosaic virus*
Crucifers—*Xanthomonas campestris* pv. *campestris, Phoma lingam, Alternaria brassicicola, Leptosphaeria maculans*
Cucumber—*Cucumber mosaic virus, Cucumber green mottle mosaic virus*
Eggplant—*Eggplant mosaic virus*
Flax—*Alternaria linicola*
Grapevine—*Grapevine fan leaf virus, Grapevine Bulgarian latent virus*
Lettuce—*Lettuce mosaic virus*
Melon—*Melon necrotic spot virus, Muskmelon necrotic ringspot virus*
Mung bean—*Mungbean mosaic virus*
Oats—*Oat mosaic virus, Pyrenophora avenae*
Onion—*Onion yellow dwarf virus*
Pea—*Pea early-browning virus, Pea enation mosaic virus, Pea seedborne mosaic virus, Pseudomonas syringae* pv. *pisi, Ascochyta pisi*
Peach—*Peach rosette mosaic virus, Prunus necrotic ringspot virus, Prune dwarf virus, Peach X-disease*
Peanut—*Peanut clump virus, Peanut mottle virus, Peanut stripe virus, Peanut stunt virus*
Pearl millet—*Sclerospora graminicola*
Plum—*Plum pox virus*
Potato—*Potato virus X, Potato virus Y, Potato virus T, Potato spindle tuber viroid*
Raspberry—*Raspberry ringspot virus, Raspberry bushy dwarf virus*
Red clover—*Red clover mottle virus, Red clover vein mosaic virus*

Rice—*Xanthomonas oryzae* pv. *oryzae, X. oryzae* pv. *oryzicola, Burkholderia glumae, Pseudomonas fuscovaginae, Alternaria padwickii, Cochliobolus miyabeanus, Pyricularia oryzae, Tilletia indica, Fusarium moniliforme*
Sorghum—*Peronosclerospora sorghi, Sclerospora sorghi, Sporisorium sorghi, Sporisorium cruentum, Claviceps sorghi*
Soybean—*Soybean mosaic virus, Pseudomonas savastanoi* pv. *glycinea, Cercospora kikuchii, Diaporthe phaseolorum*
Spinach—*Spinach latent virus*
Squash—*Squash mosaic virus*
Strawberry—*Strawberry latent ringspot virus*
Subterranean clover—*Subterranean clover mottle virus*
Sunflower—*Sunflower mosaic virus*
Tobacco—*Tobacco etch virus, Tobacco mosaic virus, Tobacco rattle virus, Tobacco ringspot virus, Tobacco streak virus*
Tomato—*Tomato aspermy virus, Tomato black ring virus, Tomato bushy stunt virus, Tomato ringspot virus, Tomato spotted wilt virus, Clavibacter michiganensis* ssp. *michiganensis, Pseudomonas syringae* pv. *tomato, Fusarium oxysporum* f. sp. *lycopersici*
Turnip—*Turnip yellow mosaic virus*
Watermelon—*Watermelon mosaic virus*
Wheat—*Wheat streak mosaic virus, Wheat striate mosaic virus, Xanthomonas campestris* pv. *translucens, Xanthomonas campestris* pv. *undulosa, Ustilago nuda, Tilletia caries, Tilletia controversa*
White clover—*White clover mosaic virus*

SEED HEALTH TESTING AND CERTIFICATION

Several countries have enacted laws for the certification of seeds (including propagating materials) free from pathogens. For example, in the United Kingdom, it is illegal to sell the seed of major agricultural and horticultural crops unless it has been certified as meeting specified minimum standards of quality, including freedom from disease. Crops are inspected by trained inspectors both in the field and after harvest. However, this is not sufficient to identify the pathogen, which persists in symptomless crops and seeds.

Seed health testing has become important in many countries in recent years. For example, in Canada, until the early 1970s, only visual field inspections of growing potato crops and harvested tubers served to identify *Clavibacter michiganensis* ssp. *sepedonicus*-infected lots that needed to be removed from the seed certification program. In 1979, laboratory testing to detect the possible presence of *C. michiganensis* ssp. *sepedonicus* in seed lots that had passed field inspection was initiated in Canada to facilitate international trade, because it was a pathogen of quarantine significance (De Boer and Hall, 2000). By 1985, the advantage of laboratory testing for detecting incipient ring rot infections had become clear, and testing of domestic seed lots was introduced on a voluntary basis in some provinces. By 1992, laboratory indexing of all seed lots for *C. michiganensis* ssp. *sepedonicus* in Canada became mandatory. With privatization of potato testing in Canada, an accreditation program was implemented to ensure that reliable and uniform results were obtained from multiple laboratories. The quality-assurance program of each private laboratory must follow the criteria set by the International Standards Organization (ISO) in their guide. Analysts in private laboratories are required to complete correctly blind "proficiency panel" samples on a semiannual basis to maintain their certified status, which allows them to conduct the tests in an accredited laboratory. These proficiency tests are administered by the Centre of Expertise for Potato Diseases of the Canadian Food Inspection Agency (De Boer and Hall, 2000). Similar seed health testing laboratories are available in the United States, Europe, and many Asian countries. The major purpose of these laboratories is to ensure the supply of seeds (including propagating materials) free from pathogens to the growers in order to exclude pathogens from fields, farms, regions, and countries.

SEED HEALTH TESTING METHODS

Guidelines for the standardization of seed health testing methods were drafted at the first Workshop on Seed Health Testing of the technical Plant Disease Committee (PDC) of the International Seed Testing Association (ISTA), held in Cambridge in 1958 (Langerak et al., 1996). Since then, numerous plant pathologists have worked on the development and standardization of seed health testing methods. These methods were evaluated in comparative testing programs of the PDC. The evaluated methods were compiled and published by ISTA as working sheets. These working sheets describe seed health testing methods for individual pathogens separately for each host and are included in the ISTA *Handbook on Seed Health Testing.*

Standardization of seed health testing methods is important to provide assurances to the seed user that adequate seed health testing was provided. The International Seed Health Initiative (ISHI) was founded in 1993 to address the immediate need for an efficient standardization process to accommodate the international seed trade as well as the level of testing proficiency required in the private sector for the international movement of seed. ISHI is an international consortium of seed industry and seed health testing plant pathologists from the United States, the Netherlands, France, Japan, and Israel (Maddox, 1998). ISHI supports the accreditation of private laboratories to assure the quality assurance of the testing and provide a means for regulatory testing that is both efficient and acceptable for phytosanitary regulation. The members of ISHI are working for worldwide standards in seed health in conjunction with ISTA and other regulatory agencies to provide a database of acceptable testing methods for world phytosanitary goals (Maddox, 1998).

Common Seed Health Testing Methods

Blotter Tests

Seeds are placed on two to three layers of water-soaked blotter papers in petri dishes and incubated under alternating light, provided by fluorescent white or near ultraviolet (NUV) tube lights, and dark periods. Fructifications of fungal pathogens developing on seeds are identified using stereoscopic and/or light microscopes. In a modified blotter test, seeds are placed between several layers of moist paper and incubated either in darkness or exposed to a 12-hour photoperiod. Specific symptoms may develop on germinating seedlings or characteristic fructifications may develop on the seed coat, which can be identified by microscopic inspection. In the 2,4-D blotter test, seed germination is prevented to create conditions for development of mycelium or spores of the pathogen on the seed itself. The herbicide 2,4-D solution is added to the blotter, and seeds are incubated under alternating fluorescent white or near ultra violet light and dark periods. The developing fructifications on the seed coat can be identified by microscopic inspection. In a modified 2,4-D blotter method, instead of adding 2,4-D, the moist blotter with seeds is frozen at –20°C after pre-imbibition at 20°C to prevent seed germination, allowing development of fungal fructifications on the seed coat. The incubation conditions of various blotter tests can be modified depending on the requirements for development of fructifications of individual pathogens (Langerak et al., 1996).

Seed Washing Method

The seed washing method involves placing individual seeds or portions of seeds in water or water plus detergent to promote release of spores or conidia. Staining techniques are employed to distinguish between closely related species of *Tilletia* in wheat. The repetitive-sequence-based polymerase chain reaction (rep-PCR) method is also useful (McDonald et al., 2000).

Embryo Staining Test

This test is used to detect *Ustilago tritici* in wheat and involves visual inspection of internal parts of the seed after separation, clarification, and staining of mycelium fragments in the seed tissue.

Agar Tests

In this method, seeds are plated on agar media containing nutrients. Selective media are also used to identify some specific pathogens. Surface sterilization of seeds with sodium hypochlorite is needed to avoid development of surface contaminants in the agar medium, but it may also inhibit development of pathogens present on the seed coat. Incubation conditions, such as temperature and exposure to light, also determine the development of pathogens on seeds plated on agar media.

Grow-Out (or Growing-On, Seedling Symptom) Tests

Seeds are grown in agar media in test tubes or in sand/soil in pots and incubated under different light and temperature conditions. Development of disease symptoms on seedlings is assessed.

Seed Extract and Dilution Plating

Seedborne bacteria are separated from seeds by soaking, washing, or extraction after crushing or maceration of the seed. The seed extract is then analyzed for the presence of pathogenic bacteria by dilution plating on selective media.

Serological and Nucleic Acid Probe-Based Methods

Recently, serological techniques and DNA-based methods have been developed. These techniques are mostly used to detect viral and bacterial pathogens. The important tests used are the latex agglutination test, the immuno-diffusion test, the microprecipitin test, enzyme-linked immunosorbent assay (ELISA), the immunoblot test, immunofluorescence, dot-immunobinding assay, enzyme-linked fluorescent assay, immunosorbent electron microscopy, radio immunosorbent assay, polymerase chain reaction, and DNA hybridization on DNA extracted from seeds. All these methods have been described in detail in Chapter 13, "Crop Disease Diagnosis."

The commonly used seed health testing methods to detect various pathogens (Langerak et al., 1996; Maddox, 1998) follow:

Wheat

Tilletia caries and *T. controversa*—washing test, repetitive-sequence-based polymerase chain reaction
Ustilago tritici—embryo staining test
Tilletia indica—NaOH soak test, washing test
Stagonospora nodorum—agar test, blotter test, growing-on test, fluorescence test, agar-fluorescence test
Xanthomonas translucens pv. *translucens*—dilution plating, dot immunobinding assay

Barley

Barley stripe mosaic virus—latex agglutination test, immunodiffusion test, immunosorbent electron microscopy, ELISA
Ustilago segetum var. *nuda*—embryo staining test
Xanthomonas translucens pv. *translucens*—dilution plating, dot-immunobinding assay
Pyrenophora teres—blotter test, agar test, growing-on test, deep-freezing test
Rhynchosporium secalis—PCR (Lee et al., 2001)

Rice

Alernaria padwickii—agar test, blotter test
Cochliobolus miyabeanus—blotter test
Pyricularia oryzae—blotter test
Fusarium moniliforme—agar test and blotter test
Tilletia indica—sodium hydroxide soak test
Xanthomonas oryzae pv. *oryzae*—growing-on test, direct immunofluorescence, dilution plating
Xanthomonas oryzae pv. *oryzicola*—growing-on test, direct immunofluorescence, dilution plating

Tomato

Clavibacter michiganensis ssp. *michiganensis*—immunofluorescence with seedling inoculation test, dilution plating, indicator host inoculation, seed wash/liquid plating, PCR
Pseudomonas syringae pv. *tomato*—growing-on test, plating enriched seed extract
Xanthomonas vesicatoria—dilution plating, immunofluorescence combined with dilution plating, plating enriched seed extract
Fusarium oxysporum—agar test
Tobacco mosaic virus—indicator plants

Soybean

Cercospora kikuchii—agar test, blotter test
Diaporthe phaseolorum—agar test
Peronospora manshurica—washing test
Phomopsis spp.—blotter test, ELISA, immunoblot test
Pseudomonas savastanoi pv. *glycinea*—growing-on test, direct plating, host inoculation, seed wash/liquid plating, immunoassays
Tobacco ringspot virus—ELISA, immunosorbent electron microscopy

Bean

Colletotrichum lindemuthianum—blotter test
Curtobacterium flaccumfaciens—immunofluorescence, seedling inoculation test, growing-on test
Pseudomonas savastanoi pv. *phaseolicola*—dilution plating, immunofluorescence test, immunofluorescence colony staining
Xanthomonas axonopodis pv. *phaseoli*—seed wash and host inoculation, seed wash and dilution plating, immunofluorescence test, immunofluorescence colony staining, DNA hybridization, PCR with seed extract
Bean common mosaic virus—ELISA, dot-immunobinding assay, immunosorbent electron microscopy, microprecipitin test

Crucifers

Xanthomonas campestris pv. *campestris*—direct plating, immunofluorescence test, seed wash/liquid plating plus pathogenicity test

Phoma lingam—deep freezing blotter test
Alternaria brassicicola—seedling symptom test
Leptosphaeria maculans—2,4-D blotter, freezing blotter, PCR with DNA extract from seeds

Cucurbits

Squash mosaic virus—ELISA, grow-out test
Melon necrotic spot virus—ELISA
Cucumber green mottle virus—ELISA
Acidovorax avenae ssp. *citrulli*—grow-out test, PCR, immunomagnetic separation and PCR (Walcott and Gitaitis, 2000)

Lettuce

Lettuce mosaic virus—ELISA, growing-on test, indicator plant test

Sugar beet

Pleospora betae—agar test, blotter test

Peach

Prune dwarf virus—ELISA
Prune necrotic ringspot virus—ELISA

INDEXING PLANT PROPAGATION MATERIALS

Most fruit trees (woody crops) are vegetatively propagated. Use of healthy planting materials will exclude pathogens from the orchard. In recent years, several molecular techniques have been developed to index the plant propagation materials and bud wood materials. Saade and colleagues (2000) have developed techniques for detection of *Prunus necrotic ringspot virus, Prune dwarf virus,* and *Apple mosaic virus* in almond, apricot, cherry, peach, and plum by multiplex reverse-transcription polymerase chain reaction (RT-PCR). This technique will be useful for the analysis of mother plants in certification programs. Merighi and colleagues (2000) developed polymerase chain reaction enzyme-linked immunosorbent assay (PCR-ELISA) for detection of *Erwinia amylovora* in pear. This test will be useful to select nursery stocks and plant propagation materials free from the pathogen. Use of disease-free budwood helps to exclude *Citrus tristeza virus* (CTV) in citrus cultivation. ELISA and double antibody sandwich-ELISA are useful to detect CTV in propagation materials (Terrada et al., 2000). Lin and colleagues (2000) have described an in situ immunoassay for detection of CTV in citrus bud wood.

REFERENCES

Babadoost, M. (2000). Comments on zero-tolerance quarantine of karnal bunt of wheat. *Plant Dis,* 84:711-712.
De Boer, S. H. and Hall, J. W. (2000). Proficiency testing in a laboratory accreditation program for the bacterial ring rot pathogen of potato. *Plant Dis,* 84:649-653.
Gottwald, T. R., Hughes, G., Graham, J. H., Sun, X., and Riley, T. (2001). The citrus canker epidemic in Florida: The scientific basis of regulatory eradication policy for an invasive species. *Phytopathology,* 91:30-34.
Johansen, E., Edwards, M. C., and Hampton, R. O. (1994). Seed transmission of viruses: Current perspectives. *Annu Rev Phytopathol,* 32:363-386.
Kahn, R. P. (1991). Exclusion as a plant disease control strategy. *Annu Rev Phytopathol,* 29:219-246.
Langerak, C. J., van den Bulk, R. W., and Franken, A. A. J. M. (1996). Indexing seeds for pathogens. *Adv Bot Res,* 23:171-215.
Lee, H. K., Tewari, J. P., and Turkington, T. K. (2001). A PCR-based assay to detect *Rhynchosporium secalis* in barley seed. *Plant Dis,* 85:220-225.
Lin, Y., Rundell, P. A., Xie, L., and Powell, C. A. (2000). In situ immunoassay for detection of *Citrus tristeza virus. Plant Dis,* 84:937-940.
Maddox, D. A. (1998). Implications of new technologies for seed health testing and the worldwide movement of seed. *Seed Sci Res,* 8:277-284.
McDonald, J. G., Wong, E., and White, G. P. (2000). Differentiation of *Tilletia* species by rep-PCR genomic fingerprinting. *Plant Dis,* 84:1121-1125.
Merighi, M., Sandrini, A., Landini, S., Ghini, S., Girotti, S., Malaguti, S., and Bazzi, C. (2000). Chemiluminescent and colorimetric detection of *Erwinia amylovora* by immunoenzymatic determination of PCR amplicons from plasmid pEA29. *Plant Dis,* 84:49-54.
Mink, G. I. (1993). Pollen- and seed-transmitted viruses and viroids. *Annu Rev Phytopathol,* 31:375-402.
Parry, D. W. (1990). *Plant Pathology in Agriculture.* Cambridge University Press, Cambridge, U.K.
Peterson, G. L., Bonde, M. R., and Phillips, J. G. (2000). Size-selective sieving for detecting teliospores of *Tilletia indica* in wheat seed samples. *Plant Dis,* 84:999-1007.
Saade, M., Aparicio, F., Sanchez-Navarro, J. A., Herranz, M. C., Myrta, A., Di Terlizzi, B., and Pallas, V. (2000). Simultaneous detection of the three ilarviruses affecting stone fruits by nonisotopic molecular hybridization and multiplex reverse-transcription polymerase chain reaction. *Phytopathology,* 90:1330-1336.

Terrada, E., Kerschbaumer, R. J., Giunta, G., Galeffi, P., Himmler, G., and Cambra, M. (2000). Fully "recombinant enzyme-linked immunosorbent assays" using genetically engineered single-chain antibody fusion proteins for detection of *Citrus tristeza virus*. *Phytopathology,* 90:1337-1344.

Walcott, R. R. and Gitaitis, R. D. (2000). Detection of *Acidovorax avenae* subsp. *citrulli* in watermelon seed using immunomagnetic separation and polymerase chain reaction. *Plant Dis,* 84:470-474.

Warham, E. J. (1992). Karnal bunt of wheat. In U.S. Singh, A. N. Mukhopadhyay, J. Kumar, and H. S. Chaube (Eds.), *Plant Diseases of International Importance, Volume I,* Prentice Hall, Englewood Cliffs, NJ, pp. 1-24.