Seed Pathology Progress in Academia and Industry

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Abstract

Seed pathology involves the study and management of diseases affecting seed production and utilization, as well as disease management practices applied to seeds. In this paper, three aspects of seed pathology are discussed: research innovations in detection of seedborne pathogens and elucidation of their epidemiology; advances in development and use of seed treatments; and progress toward standardization of phytosanitary regulations and seed health testing methods. The application of nucleic-acid based detection methods in seed health testing has been facilitated by integrating conventional or real-time PCR with other technologies (e.g., BIO-PCR, IMS-PCR, MCH-PCR). PCR-based methods and pathogen marker technologies are being applied to epidemiological research on seedborne pathogens, e.g., seed transmission mechanisms, the influence of external biotic and abiotic factors on seed transmission, and tracking progress of seed-transmitted pathogens. Seed treatment use is discussed in terms of the revolutionary expansion in seed-applied insecticide use, impacts of new fungicide active ingredients, and the effects of some seed treatments on crop physiology. International seed trade has been affected significantly by changing phytosanitary regulations, not always based on science. Efforts are underway to revise phytosanitary regulations to reflect pest risk analysis outcomes and to develop standards for seed health testing methods that facilitate safe and efficient international trade in seeds.

INTRODUCTION

Seed pathology has been recognized as its own specialization for a relatively brief time, and the term itself was probably first used in the 1940s (2). At that time, seed pathology referred almost solely to the detection of microorganisms in or on seeds, a practice that had been conducted already for a century or more. Pathological aspects of seeds initially were considered almost entirely within the context of the seed-testing laboratory. The incorporation of epidemiological concepts and management considerations as components of seed pathology gained momentum with the writings of Kenneth Baker during the 1970s. Baker (7) described events taking place in the seed production field, the postharvest environment, and the crop production field, all in relation to seed pathology. This trend was reflected in the seminal textbook by Paul Neergaard (92), and Baker's ideas were developed and promoted considerably in recent decades by other seed pathologists, particularly Denis McGee. Papers by McGee (78, 79) expanded on Baker's epidemiological concepts, superimposing events in the pathogen life cycle over the environments in which these pathogens interact with seed. Twenty-five years after Baker's pioneering paper, Agarwal & Sinclair (2) still defined seed pathology as the "study of seedborne diseases and pathogens" but included extensive epidemiological components in their *Seed Pathology* textbook. Because of the ideas of Baker and McGee, seed pathology now occupies a broader scope of research and practice; it can be described as "the study and management of diseases affecting seed production and utilization, as well as disease management practices applied to seeds." This broader concept recognizes the inclusion of diseases that affect seed production (but are not necessarily seedborne) under the umbrella of seed pathology, and also recognizes that seed treatments are commonly utilized against diseases and pests that are not associated with seeds. The intent of this review is to underscore developments in seed pathology during the past decade or so in the context of this broader seed pathology paradigm. Because the concept emphasizes the practice of seed pathology as well as the research, I describe important developments in the seed industry and the regulatory environment that have complemented research innovations.

These developments have been numerous, but this review focuses on three areas:

1. Research innovations in the detection of seedborne pathogens and elucidation of their epidemiology;
2. Advances in the development and use of seed treatments;
3. Progress toward standardization of phytosanitary regulations, especially in relation to seed health testing.

RESEARCH INNOVATIONS IN SEED PATHOLOGY

Seed pathology research is typically considered to emphasize detection methods for seedborne pathogens. There have been many innovations in this area since the inception of seed pathology as a unique field. The invention of polymerase chain reaction (PCR) in the 1980s revolutionized biological diagnostics, opening a new era in medical and veterinary pathogen detection, and the potential for detection of pathogens in seed was recognized early on (46, 105). Since that time, many PCR-based detection methods have been developed and applied to seedborne pathogens. Agarwal (1), for example, lists 100 pathogens for which PCR-based seed health tests had been developed through 2005. Agarwal's book also reviews traditional seed health testing methods along with recent developments in immunoassays and nondestructive seed health tests such as ultrasound, optical and infrared analyses, and biopsis (the removal and analysis of tissue cores from seeds). Methods for detection of seedborne pathogens also have been reviewed in several other books during the past decade (4, 49, 73), in a book chapter (131), and at least three recent *Annual Review* papers (5, 37, 119). In this paper, I do not attempt to review method

development comprehensively; instead, the emphasis is on describing the implementation of some of the more innovative methods in seed health testing programs and in research on the epidemiology of seedborne diseases.

Nucleic Acid–Based Detection Methods in Seed Health Testing

The remarkable proliferation of PCR-based methods for detecting pathogens in seeds has provided very useful tools that are available, and have begun to be implemented, in the vegetable seed industry and in some official seed testing laboratories (1) for quality control testing. The overall implementation of these methods, however, has been slow, especially in international seed testing programs. There are three primary organizations that publish standardized seed health test methods for use in international trade: International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System (NSHS). Among the three groups' approved methods, 75 unique tests are represented (**Table 1**). Only three approved methods are PCR-based; 13 are immunology based, and the remaining methods are based on microscopy, incubation methods, indicator plant assays, or grow-out tests.

There are several obstacles that have slowed the adoption of PCR-based methods for seed health testing (134). In the developing world, the capital costs and technical expertise for establishing PCR capabilities can be problematic. Even when costs and expertise are not major barriers, there can be technical impediments in terms of poor quality DNA and PCR inhibitors from seed extracts, leading to false negatives. Poor sensitivity also can result from low sampling intensity for PCR-based methods. One of the major obstacles to the adoption of nucleic acid–based seed health tests has been the potential for false positives due to the detection of remnant DNA from nonviable pathogen propagules (1). Several strategies have been developed to overcome these obstacles, including BIO-PCR, which involves propagation of putative pathogen propagules on a culture medium and subsequent PCR on washes from the culture plates, often using nested PCR primer pairs and sometimes without DNA extraction. Highly sensitive BIO-PCR methods have been developed for several bacterial pathogens from seeds, including *Pseudomonas syringae* pv. *phaseolicola* (117, 118), *Acidovorax avenae* ssp. *avenae* (128), *Xanthomonas oryzae* pv. *oryzae* (116), and *X. campestris* (108); BIO-PCR may be less useful for fungi from seeds, but at least one method has been published (127). Although no BIO-PCR methods are currently approved by ISHI, ISTA, or NSHS, the *X. campestris* method is being reviewed for approval by ISHI and ISTA (108). Flow cytometry approaches have been used for differentiating viable and nonviable cells of bacterial plant pathogens from seed (20); this method could be employed as a prescreening step for PCR. Another approach to ensuring that PCR is detecting DNA from viable pathogen cells is the use of propidium monoazide, which can selectively remove free DNA from dead cells while leaving DNA intact in viable cells (93, 101). This method has potential for use with seedborne pathogens, but there are no published reports.

Improvements in sensitivity of PCR-based assays have come with the development of nested PCR (e.g., 58, 95), real-time PCR, and capture procedures such as immunomagnetic separation and magnetic-capture hybridization. Immunomagnetic separation (IMS) has been used to increase sensitivity of PCR detection of *Acidovorax avenae* subsp. *citrulli* (135). Magnetic beads, coated with polyclonal antibodies against the pathogen, were used to capture bacterial cells from the seed extract. DNA was released from the captured cells by boiling and then subjected to PCR. The IMS-PCR method sensitivity was 100-fold higher than conventional PCR. IMS-PCR has been used for several other pathogens (5, 36). Real-time PCR offers several advantages over conventional PCR that make it preferable for seed health testing. These include its higher sensitivity, ability to quantify pathogen DNA, and

Table 1 Seed health test methods approved as standard methods by the International Seed Health Initiatives (ISHI) (50), the International Seed Testing Association (ISTA) (53), or the National Seed Health System (NSHS) (91)

Pathogen	Host(s)	Type of test	Approval		
			ISHI	ISTA	NSHS
Acidovorax avenae subsp. *citrulli*	*Cucurbitaceae*	Growout	+		+
Acidovorax avenae subsp. *citrulli*	*Cucurbitaceae*	PCR			+
Alternaria dauci	*Daucus carota*	Blotter	+	+	+
Alternaria dauci	*Daucus carota*	Agar	+	+	
Alternaria linicola	*Linum usitatissimum*	Agar		+	
Alternaria padwickii	*Oryza sativa*	Blotter		+	
Alternaria radicina	*Daucus carota*	Blotter	+	+	+
Alternaria radicina	*Daucus carota*	Agar	+	+	
Aphelenchoides besseyi	*Oryza sativa*	Microscopy		+	
Ascochyta pisi	*Pisum sativum*	Agar		+	+
Bean pod mottle virus	*Glycine max*	ELISA			+
Bipolaris zeicola	*Zea mays*	Blotter			+
Botrytis cinerea	*Helianthus annuus*	Blotter		+	
Botrytis cinerea	*Linum usitatissimum*	Agar		+	
Caloscypha fulgens	*Picea*	Agar		+	
Cercospora kikuchii	*Glycine max*	Agar			+
Clavibacter flaccumflaciens pv. *flaccumfaciens*	*Glycine max*	Grow-out			+
Clavibacter michiganensis subsp. *michiganensis*	*Lycopersicon esculentum*	Agar	+		+
Clavibacter michiganensis subsp. *nebraskensis*	*Zea mays*	Agar			+
Cochliobolus heterostrophus	*Zea mays*	Blotter			+
Colletotrichum lindemuthianum	*Phaseolus vulgaris*	Blotter		+	+
Colletotrichum lini	*Linum usitatissimum*	Agar		+	
Didymella bryoniae	*Cucurbitaceae*	Blotter			+
Drechslera oryzae	*Oryza sativa*	Blotter		+	
Fusarium circinatum	*Pinus*	Agar		+	
Fusarium verticillioides	*Zea mays*	Agar			+
Fusarium verticillioides	*Zea mays*	Blotter			+
Gibberella zeae	*Zea mays*	Agar			+
Gibberella zeae	*Zea mays*	Blotter			+
Lettuce mosaic virus	*Lactuca sativa*	ELISA	+		
Lettuce mosaic virus	*Lactuca sativa*	ELISA			+
Lettuce mosaic virus	*Lactuca sativa*	Indicator plant			+
Maize dwarf mosaic virus	*Zea mays*	ELISA			+
Maize chlorotic mottle virus	*Zea mays*	ELISA			+
Microdochium nivale	*Triticum aestivum*	Agar		+	
Neotyphodium spp.	*Festuca; Lolium*	Immunoblot		+	
Pantoea stewartii	*Zea mays*	ELISA			+
Pea early browning virus	*Pisum sativum*	ELISA	+	+	
Pea seedborne mosaic virus	*Pisum sativum*	ELISA	+	+	
Pepino mosaic virus	*Lycopersicon esculentum*	ELISA	+		
Peronospora farinosa f.sp. *spinaciae*	*Spinacia oleracea*	Seed wash			+

(*Continued*)

Table 1 (*Continued*)

Pathogen	Host(s)	Type of test	Approval		
			ISHI	ISTA	NSHS
Peronosclerospora sorghi	*Zea mays*	Grow-out			+
Phoma apiicola	*Apium graveolens*	Grow-out			+
Phoma lingam	*Brassica*	Blotter		+	+
Phomopsis spp.	*Glycine max*	Agar	+	+	+
Phomopsis spp.	*Glycine max*	Blotter			+
Pseudomonas syringae pv. *coriandricola*	*Coriandrum sativum*	Agar			+
Pseudomonas syringae pv. *glycinea*	*Glycine max*	Agar/Serology			+
Pseudomonas syringae pv. *phaseolicola*	*Phaseolus*	Agar	+	+	+
Pseudomonas syringae pv. *syringae*	*Phaseolus*	Agar			+
Pseudomonas syringae pv. *tomato*	*Lycopersicon esculentum*	Agar			+
Pyricularia oryzae	*Oryza sativa*	Blotter		+	
Sclerophthora macrospora	*Zea mays*	Microscopy			+
Sclerotinia sclerotiorum	*Glycine max*	Agar			+
Septoria apiicola	*Apium graveolens*	Blotter	+		
Septoria apiicola	*Apium graveolens*	Seed wash			+
Septoria nodorum	*Triticum aestivum*	Agar		+	
Soybean mosaic virus	*Glycine max*	ELISA			+
Sphacelotheca reiliana	*Zea mays*	Seed wash			+
Stenocarpella maydis	*Zea mays*	Agar			+
Tobacco ringspot virus	*Glycine max*	ELISA			+
Tomato ringspot virus	*Glycine max*	ELISA			+
Tobamoviridae	*Capsicum*	Indicator plant	+		+
Tobamoviridae	*Lycopersicon esculentum*	Indicator plant	+		+
Ustilago maydis	*Zea mays*	Seed wash			+
Ustilago nuda	*Hordeum vulgare*	Microscopy		+	
Verticillium dahliae	*Spinacia oleracea*	Agar			+
Xanthomonas axonopodis pv. *glycines*	*Glycine max*	Agar			+
Xanthomonas axonopodis pv. *phaseoli*	*Phaseolus*	Agar	+	+	+
Xanthomonas axonopodis pv. *phaseoli*	*Phaseolus*	PCR			+
Xanthomonas campestris pv. *campestris*	*Brassica*	Agar	+	+	+
Xanthomonas campestris pv. *campestris*	*Brassica*	Agar (for treated seed)	+		
Xanthomonas campestris pv. *vesicatoria*	*Lycopersicon esculentum*	Agar	+		+
Xanthomonas campestris pv. *vesicatoria*	*Capsicum*	Agar	+		
Xanthomonas hortorum pv. *carotae*	*Daucus carota*	Agar/Pathogenicity or PCR	+	+	+

direct interpretation of amplification results without gel electrophoresis or Southern-blot hybridization. As with conventional PCR, real-time PCR still suffers from interference by inhibitory compounds from seed extracts. To overcome this problem, real-time PCR also has been combined with procedures to separate pathogen DNA from inhibitory compounds such as magnetic capture hybridization (MCH). In this method, single-stranded oligonucleotide probes, conjugated to magnetic beads, hybridize to single-stranded target nucleic acids from DNA preparations. The capture probe-target DNA hybrid can be released from the

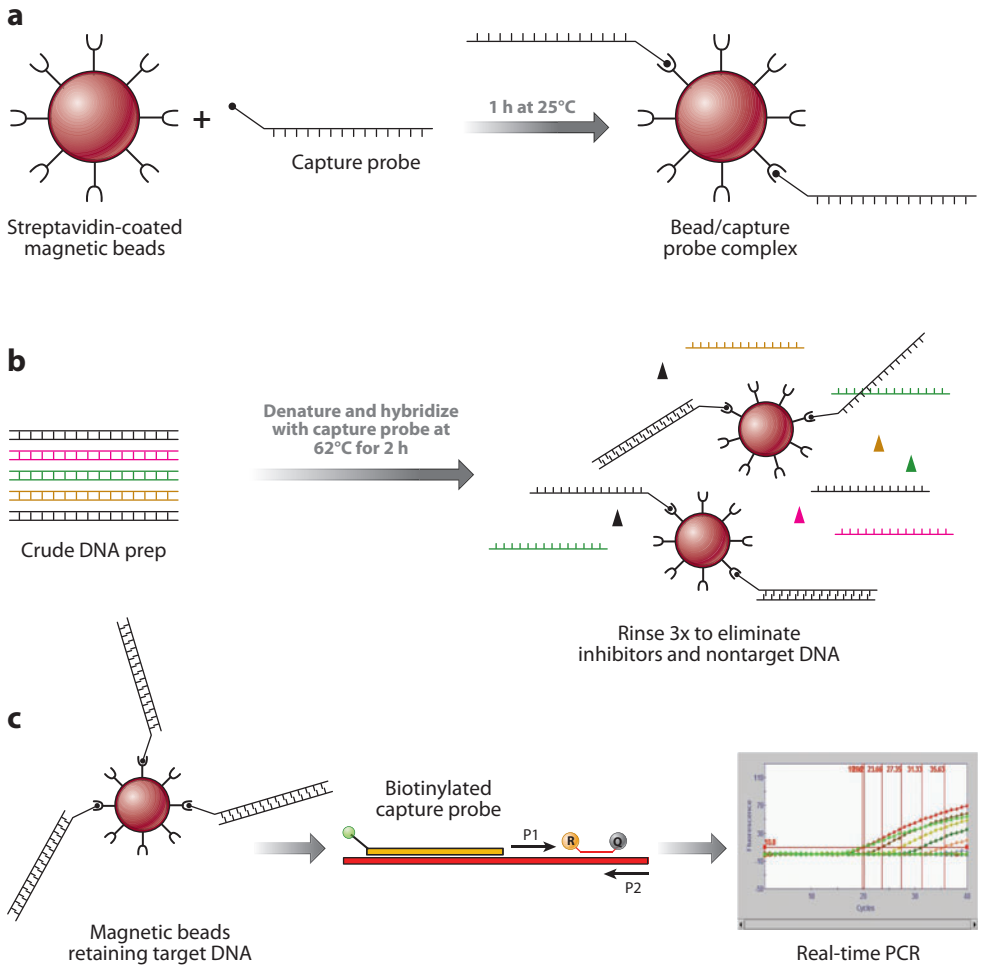

Figure 1

Magnetic capture hybridization PCR. (*a*) Coating of magnetic beads with single-strand oligonucleotide capture probes; (*b*) hybridization of target DNA with capture probes; (*c*) real-time PCR of capture probe-target DNA hybrid (redrawn with permission from A. Fessehaie & R. Walcott).

beads by boiling and detected by real-time PCR (**Figure 1**). MCH can concentrate target DNA and separate it from inhibitory compounds and nontarget DNA, increasing sensitivity at least tenfold compared to direct real-time PCR (138). This method was successfully demonstrated with *Botrytis aclada* from onion seed (137). Multiple hybridization capture probes can be used to target several nucleic acid sequences, enabling simultaneous detection of multiple pathogens (134). This capability capitalizes on of one of the major advantages of real-time PCR: the power to test for multiple pathogens simultaneously. Multiplex assays using MCH-PCR are being developed for simultaneously detecting several seedborne pathogens in cucurbits, including a bacterium (*A. avenae* subsp. *citrulli*), a virus (*Squash mosaic virus*), and a fungus (*Didymella bryoniae*) (69), as well as multiple pathogens (*Botrytis aclada* and *Pantoea ananatis)* from onion seed (43).

Ideally, future seed health tests can be designed as multiplex assays for specific crops, with the capacity to detect all seedborne

pathogens required for quality control or phytosanitary purposes. An approach similar to this would be technically feasible using a platform such as MCH-PCR, although oligonucleotide arrays could potentially include a larger number of target organisms. DNA arrays have been successfully demonstrated for detection of multiple bacterial, viral, and fungal pathogens of potato (3, 32) and for pathogen groups attacking several other crops. Recently developed methods also are capable of quantification of pathogen DNA (68). This platform is well suited to high throughput testing scenarios, which makes it a very powerful approach.

Seed health testing by PCR-based methods is a revolution waiting to happen. Aside from the technical challenges of the methods per se, another barrier to adoption has been the gap in knowledge that relates specific levels of detection by PCR-based methods to outcomes in the field. Are sample sizes used for other seed health tests appropriate for PCR-based tests? Are thresholds established from other tests applicable to PCR results? A significant amount of experience and research is needed to address questions like these before the seed industry and regulatory agencies can become confident in the results of PCR-based seed health tests. Many additional innovative technologies such as electrochemical DNA detection (54), loop-mediated isothermal amplification (65), laser-induced fluorescence (5), electronic noses (119), biomimetic polymer sensors (125), and DNA barcoding (133) are being developed for detection and identification of pathogens. Applications to seedborne pathogens are attractive; however, given the slow pace at which established PCR-based methods have been adopted, it may be a very long process to incorporate these newer technologies into routine seed health testing.

Use of Nucleic Acid–Based Methods in Epidemiology Research on Seedborne Pathogens

Methods used to detect pathogens in seeds can be valuable research tools for tracking the progress of the organisms during disease development. These methods can be applied to understand the sources of seedborne infections, the location of pathogens within seed tissues to confirm the occurrence of seed transmission and its mechanisms, and to understand the influence of external biotic and abiotic factors on seed transmission or other phases of the disease cycle. One example is watermelon fruit blotch (*A. avenae* ssp. *citrulli*), where the use of IMS-PCR facilitated the detection of a high incidence of infection in seeds from symptomless fruit following blossom inoculation (136). This was the first indication that blossoms were an avenue of infection, in the absence of fruit blotch symptoms. Real-time PCR also was used to pinpoint the location of seedborne *A. avenae* ssp. *citrulli* infections to the surface of the perisperm-endosperm layer (28). In rice, a BIO-PCR technique was used to study survival of *Xanthomonas oryzae* pv. *oryza* in rice seed and track its progress in planta following seed transmission (116). In olives, seed transmission of *Verticillium dahliae* was confirmed using a nested PCR assay in seedlings (58). One of the most interesting applications of nucleic acid–based methods in seed pathology has been the elucidation of embryo infection pathways for *Pea early browning virus* and *Pea seedborne mosaic virus* (PSbMV). Using a combination of approaches, including in situ hybridization, Maule and coworkers (74, 75, 114) showed that the two viruses have different routes for embryonic infection. Whereas *Pea early browning virus* reaches the embryo as a result of gamete infection, PSbMV infects the developing embryo after fertilization. The PSbMV pathway is novel among viruses, and the results indicated a symplastic connection between maternal and filial tissues during embryo maturation (74).

Use of Markers in Seedborne Pathogens

This is another approach that has made significant contributions to understanding the epidemiology of seedborne pathogens. For pathosystems involving ubiquitous pathogens or those with multiple infection pathways,

marker use can be critical for differentiating seedborne strains from strains originating from other inoculum sources. The most commonly employed types of markers have been naturally occurring genetic markers, including antibiotic resistance, vegetative compatibility, and molecular markers unrelated to phenotype. Antibiotic resistance in bacterial pathogens, naturally occurring, induced through mutation, or inserted by genetic engineering, has been used effectively as a marker for decades. In maize, seed transmission of *Clavibacter michiganense* subsp. *nebraskense* was first demonstrated using a rifampicin-resistant strain (12); the extremely low frequency of seed transmission of *Pantoea stewartii* was well characterized by Block et al. (13) using a rifampicin and nalidixic acid–resistant strain of the bacterium.

Vegetative compatibility is a genetically controlled trait that describes the ability of fungal isolates to anastomose and form vegetative (asexual) heterokaryons. Strains that are vegetatively compatible are designated as members of the same vegetative compatibility group (67). In fungi with a very diverse vegetative compatibility structure, the trait can be used to differentiate introduced strains (from a rare or unique vegetative compatibility group) from endemic strains. This approach has been used in a number of studies on seed transmission of *Fusarium verticillioides* and related *Fusarium* species. The approach commonly employs nitrogen-utilization mutants (63) of the pathogen as a tool for recognizing compatible reactions. Using this approach, Kedera et al. (60) established the occurrence of seed-to-kernel systemic transmission of *F. verticillioides* and found that a low percentage of kernels was infected by seed-transmitted strains. Munkvold et al. (85) confirmed these results, finding a mean of 2.5% of kernels was infected by seed-transmitted strains, and concluded that seed was a minor source of inoculum for kernel infection in hybrids of dent maize. Further studies indicated that seed-transmitted strains infected higher percentages of kernels (up to 29.4%), but systemic transmission to kernels was suppressed if maize silks were inoculated with other strains of the fungus. Other sources of inoculum continued to be much more important than seedborne inoculum (84). Conversely, using nitrogen-utilization mutants as marked strains, Galperin et al. (34) concluded that seedborne inoculum was a significant source of inoculum for kernel infection of mature sweet corn plants. Seed transmission also has been documented for *Fusarium subglutinans* and *F. proliferatum* by vegetative compatibility tracking of seed-inoculated strains (24). The contribution of seedborne inoculum to epidemics of Stagonospora leaf blotch was characterized by Bennet et al. (10) using *Stagonospora nodorum* strains identifiable by unique AFLP profiles. Their results showed that 57% of *S. nodorum* strains in plots from inoculated seed were identical to the seedborne strains, and disease was more severe in plots from inoculated seed, although disease also developed from other sources of primary inoculum.

Mycotoxin production is another genetic marker used to investigate the importance of seedborne *F. verticillioides*. Desjardins et al. (26) used strains with natural and artificially induced mutations in fumonisin biosynthesis genes to study the role of fumonisins in *F. verticillioides* epidemiology, including seed transmission. The fate of the introduced strains was determined by analysis for genetic markers specific to the wild-type and mutant alleles, as well as by analyzing recovered strains for their ability to produce fumonisin analogs. Seed transmission and other aspects of *F. verticillioides* epidemiology were not significantly different among strains with or without fumonisin production.

Other markers have been employed by transforming pathogen strains with foreign genes that can act as molecular markers or easily distinguishable expression phenotypes. Two examples are genes for beta-glucuronidase (GUS) expression and fluorescent protein expression. Yates et al. (143) confirmed seed transmission and symptomless infection of maize seedlings using GUS-transformed strains of *F. verticillioides*. Further work with *F. verticillioides* and *F. subglutinans* was carried out using strains transformed with a gene from the jellyfish *Aequorea*

victoria to express green fluorescent protein (GFP) (140, 141). The influence of temperature on seed transmission of *F. verticillioides* was studied by Wilke et al. (141), who found that seed transmission frequency was high (>90%) under a wide range of temperature conditions and that the fungus remained primarily in below-ground tissues of the plants during the host vegetative growth stages (**Figures 2**, **3**). In another study, Wilke et al. (140) found that *F. subglutinans* also was seed transmitted at a frequency greater than 90% in maize. In each of these studies, *Fusarium* strains were cotransformed with the reporter gene (GUS or GFP) and a gene for hygromycin resistance to facilitate recovery of the marked strains, which were not identified *in planta* but isolated and propagated on hygromycin-amended media for subsequent observation. Potential pitfalls with these methods are related to inconsistent expression of gene products, low pathogen biomass in plant tissue, autofluorescence or other interference with visualization of fluorescent proteins or staining, and in the case of GUS, accessibility of enzyme substrate to infected plant tissues. There are various strategies to overcome these pitfalls, including the cotransformation of pathogen strains with antibiotic or hygromycin resistance genes, or detection of reporter gene expression by molecular methods rather than expression phenotype. Some of these obstacles can be overcome using other markers for pathogen visualization. Xu et al. (145) used a bioluminescent marker derived from the bacterium *Photorhabdus luminescence* to study the epidemiology of seedborne *Clavibacter michiganense* subsp. *michiganense* in tomato.

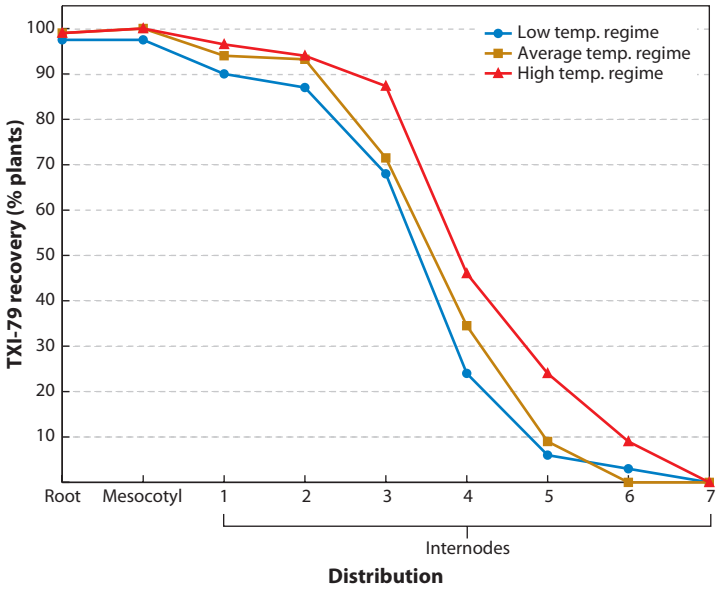

Figure 2

In planta distribution of seed-transmitted GFP-expressing *F. verticillioides* strain TXI-79 among internode tissues of maize plants at growth stage V6, grown from inoculated seeds at three different temperature regimes (adapted from Reference 141).

SEED TREATMENT DEVELOPMENT AND USE

Although Bordeaux mixture is often cited as the first fungicide, in fact substances had been applied to seeds for the control of fungi for more than 200 years prior to 1885 (115). So seed treatments have been used for centuries and used widely on a commercial basis for decades. However, during the past 10–15 years, seed treatment use has rapidly accelerated and evolved. Diverse factors have come together to drive this growth. Pressure to meet global demand for food and fuel has increased the motivation to maximize crop productivity, whereas

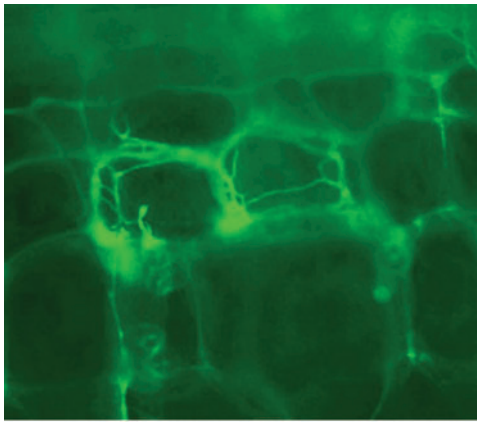

Figure 3

Mycelium of green fluorescent protein labeled *Fusarium verticillioides* growing in maize seedling tissue after transmission from seed (141).

environmental concerns are a disincentive to using foliar and soil applications of crop protection chemicals to meet this objective. Increased global trade and possibly climate change have promoted the emergence or re-emergence of diseases and pests in new locales. For a variety of reasons, crop production practices have moved toward tactics that increase the risks for seed decay, seedling disease, and early-season insect attack (15, 17, 18, 19). These factors and others have resulted in a doubling of the value of the global seed treatment market between 2002 and 2008, which is now more than USD $2 billion annually.

This rapid growth in the popularity of seed treatments also is part of a broader trend in agriculture that emphasizes the value of seed and the potential of seed as a delivery mechanism for crop management inputs. The central role of seeds in agriculture has always been recognized, but the importance of this role has been greatly heightened during the past century and especially the past decade. Several developments have catalyzed the elevation of seed as the most valuable agricultural input. In several of the world's major crops, the development and implementation of hybrids have resulted in a major emphasis on seed production practices. Improved breeding methods, including the use of marker-assisted selection and other so-called molecular breeding techniques, have enhanced the importance of producing and distributing improved cultivars that deliver high levels of yield, quality, and stress tolerance. Intellectual property protection such as the United States Plant Variety Protection Act has contributed to recognition of the value of improved cultivars distributed as seed. The advent of biotechnology has further promoted the value of seed as a result of the incorporation of valuable pest management traits into the seed; this dimension will only accelerate as other traits with high value to the consumer are added to the repertoire of genetically modified crop plants. The role of seed treatments will continue to expand as crop producers seek to protect their growing investment in high-value seed and expect more and more input and output traits to be delivered with the seed.

Growth in the seed treatment market has been accompanied by significant changes in the crop protection chemical industry and the way products are developed for seed treatment use. The 1990s were a period of widespread consolidation in the industry. In 1992, there were 16 major companies in the crop protection chemical industry; that number shrank to 6 by 2002 because of consolidation. In addition, the rise of biotechnology was accompanied by acquisitions or mergers between the seed industry and the crop protection chemical industry. Today, each of the major seed companies operating in the United States is a subsidiary of a crop protection chemical company or has a sister company in crop protection. All these changes have helped alter the way the industry views development of seed treatment products. Traditionally, compounds were screened for activity against major groups of insects or fungi, with the goal of identifying superior products for use in foliar applications. Candidate products that were in the advanced stages were later tested for applicability as seed treatments because the seed treatment market was small and did not justify its own discovery effort. Although there have been numerous significant crop protection chemical manufacturers, only a very few (primarily Bayer CropScience/Gustafson and Syngenta Crop Protection) have dominated the seed treatment market. BASF entered the seed treatment market in 2003 and is a distant third in seed treatment sales. These three companies have all intensified their efforts in seed treatment development. In 2008, Syngenta Crop Protection opened a $7 million Seed Care Institute research and development facility in Stein, Switzerland. It serves as the hub for a global Seed Care network with other major facilities in the United States, Brazil, and China. BASF has increased its investment in seed treatment research and development; entering the market in 2003, BASF offered three products in 2008 but is expected to have 11 seed treatment products on the market in 2009. Bayer CropScience has

maintained its prominent position in the seed treatment market, establishing a new seed treatment business unit in the United States, following the completion of its purchase of Gustafson in 2004. These significant investments all support the expectation in the crop protection industry that seed treatment use will continue to grow.

Seed-Applied Insecticides and Nematicides

The most dramatic change in seed treatment use during the past decade has been the rapid adoption of seed-applied insecticides in several crops (**Table 2**). The popularity of seed-applied insecticides began with the introduction of the neonicotinoid active ingredients, beginning with imidacloprid in 1991. Although some insecticides were approved and marketed as seed treatments prior to the introduction of the neonicotinoid insecticides, their use was very limited. Imidacloprid was introduced as a seed treatment for maize in 1995, followed by thiamethoxam (1997 in New Zealand; 2001 in the United States) and clothianidin (2003). Since 2000, the use of these products as seed treatments has increased dramatically, and currently either thiamethoxam or clothianidin is used as a standard seed treatment for nearly 90% of the maize seed planted in the United States. This trend has occurred in other crops as well. For example, in sugarbeet in the United Kingdom, use of seed-applied insecticides went from zero in 1993 to approximately 75% of the area sown to sugarbeets in 2002 (27). This corresponded with a dramatic 95% drop in overall insecticide use on sugarbeets in the United Kingdom, as seed treatment replaced soil-applied insecticides (16). The same seed-applied insecticides are now also used on a majority of canola seed planted in North America and on increasing percentages of soybean and cotton seed.

Although seed-applied insecticides are used primarily for control of soil-borne insects, they have had important implications from a disease-management perspective. This stems mostly from their systemic properties, which enable the compounds to control above-ground leaf- and stem-feeding insects, including aphids (6). Because several aphid species and other insects controlled by seed-applied insecticides are vectors of plant pathogens, in some cases seed-applied insecticide use has contributed to reductions in disease transmission. In maize, Stewart's wilt, caused by the bacterium *Pantoea stewartii*, is an important quarantined pathogen that can be seed-transmitted. Therefore, it is important to minimize the occurrence of the disease in maize seed production fields. Stewart's wilt also causes economic losses, especially in sweet corn, by prematurely killing plants and blighting leaves. Seed parent plants that are infected early in their development are more likely to produce infected seeds (13, 14), and sweet corn plants infected early in their

Table 2 Insecticides approved for use as seed treatments—2009

Active ingredient	Chemical family	Product	Manufacturer	Major Crops
Acetamiprid	Neonicotinoid		Nippon Soda, Inc.	
Clothianidin	Neonicotinoid	Poncho	Bayer	Maize
Diazinon[a]	Organothiophosphate	Various	Various	Numerous
Imidacloprid	Neonicotinoid	Gaucho	Bayer	Maize, soybean, canola, sorghum
Fipronil	Phenylpyrazole	Regent	BASF	Maize, sunflower, cereals, rice, cotton, vegetables
Lindane[a]	Organochlorine	Various	Various	Numerous
Thiamethoxam	Neonicotinoid	Cruiser	Syngenta Crop Protection	Maize, soybean, canola, sorghum
Thiodicarb	Carbamate	Aeris	Bayer	Cotton

[a] – on-farm application.

development are more likely to die or suffer yield loss through leaf blighting (102, 130), so seedling protection against the insect vector (corn flea beetle) has been an important management component of maize seed production and sweet corn production. Seed-applied neonicotinoid insecticides were shown in several studies to effectively prevent feeding by the corn flea beetle and significantly reduce transmission of *P. stewartii* (6, 66, 86, 103, 104). For example, in field experiments conducted from 2000 through 2003, Pataky et al. (104) showed that average reductions in Stewart's wilt incidence in sweet corn were 75.5% for clothianidin (0.19 to 0.25 mg a.i./seed), 69.6% for imidacloprid (0.34 mg a.i./seed), and 69.3% for thiamethoxam (0.25 to 0.27 mg a.i./seed) (**Figure 4**). In cantaloupe, seed-applied imidacloprid reduced the severity of bacterial wilt, caused by *Erwinia tracheiphila*, through control of its vector, the striped cucumber beetle (33). Seed-applied insecticides also have been effective for reducing aphid transmission of viruses in oats, sorghum, sugarbeet, and wheat (71).

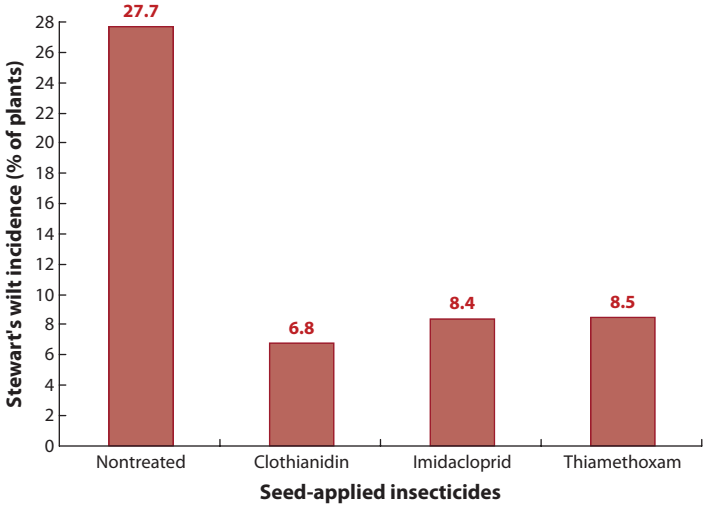

Figure 4

Reductions in Stewart's wilt incidence in sweet corn resulting from insect vector control by seed-applied insecticides. Values are means from 11 field experiments conducted from 2000 to 2003 in Illinois and Delaware. Insecticides were applied to seed at rates of 0.19 to 0.25 mg a.i./seed (clothianidin), 0.34 mg a.i./seed (imidacloprid), or 0.25 to 0.27 mg a.i./seed (thiamethoxam) (data drawn from 104).

Gourmet et al. (42) showed reductions in the spread of *Barley yellow dwarf virus* in oats and wheat when seed was treated with imidacloprid; similarly, Harvey et al. (44) demonstrated reduced incidence of *Sugarcane mosaic virus* strain MDMV-B in sorghum with imidacloprid seed treatment. In sugarbeet, both *Beet mild yellowing virus* and *Beet yellows virus* incidence were reduced by seed treatment with imidacloprid or clothianidin (27). Control of wheat curl mite resulted in reduced incidence of *Wheat streak mosaic virus* in wheat (45). In soybeans, seed-applied insecticides can reduce spread of *Bean pod mottle virus* (BPMV) by overwintering bean leaf beetles (25). In order to achieve significant reductions in seedborne BPMV or symptoms of seed mottling, the duration of bean leaf beetle control must be extended with a foliar insecticide application in addition to seed treatment. Recommendations for integrated control of bean leaf beetle and BPMV call for the use of a seed-applied insecticide or seedling-stage foliar application (112). Seed-applied thiamethoxam can reduce soybean aphid damage (77), which would seem to have potential to reduce spread of *Soybean mosaic virus* by aphid vectors. However, this has not yet been demonstrated.

Another disease-management impact of seed-applied insecticides is related to interactions between target pests and pathogens that attack crop seeds and seedling roots. Although supporting data are limited, it is generally accepted that insect injury facilitates infection of seeds and roots by opportunistic soil-borne pathogens. For example, injury by corn rootworms was associated with increased infection of maize roots by *Fusarium* species in Minnesota (99), and rootworm larvae were found to transport inoculum of pathogenic *Fusarium* species (98). The extent to which seed-applied insecticides can mitigate insect-mediated root infection remains to be seen, but there are indications that corn rootworm control can reduce root infection by fungi (87).

In addition to the dramatic increases in seed-applied insecticides, recent registrations have been approved for seed treatment active ingredients with nematicidal properties.

Although seed-applied chemicals have been tested for nematode control for decades (e.g., 94), their use has been almost nonexistent until recently. Effective nematicidal seed treatment products recently have been introduced for cotton (30) (abamectin and thiodicarb) and maize (abamectin), with other uses and products likely to follow. It is well documented that some parasitic nematodes have important interactions with plant pathogenic fungi; this occurs in various crops including cotton (59), soybeans (144), and maize (55, 97); therefore, it appears likely that seed-applied nematicides may have indirect benefits related to reduced fungal infection.

Seed Treatment Fungicides

Seed treatments were initially used to control fungal plant pathogens, and fungicides continue to be the staple seed treatment active ingredients, notwithstanding the sudden growth in seed-applied insecticides. Without exception, seed-applied insecticides are used in combination with one or more fungicides. Fungicide seed treatment use also has experienced a growth spurt, partially on the coattails of the seed-applied insecticide revolution. However, many new fungicide seed treatments have been adopted on their own merits because of factors already mentioned, along with the development of highly efficacious new active ingredients and the development of precision application equipment. Of the most commonly used fungicide seed treatments (**Table 3**), approximately half have been introduced within the past 15 years, including several of the most widely used active ingredients. I focus on three major developments that have occurred in this area during the past 15 years: the adoption of triazole fungicides (difenoconazole, tebuconazole, and triticonazole) as broad-spectrum, systemic seed treatments for cereals; the introduction of fludioxonil as the first low-rate reduced risk seed

Table 3 Fungicides commonly used as seed treatments—2009

Active ingredient	Chemical family	Product	Year	Manufacturer	Major crops
Azoxystrobin	Strobilurin	Dynasty	2004	Syngenta Crop Protection	Maize
Carbendazim	Benzimidazole	Derosal	1973	BASF; Bayer CropScience; others	Soybeans, vegetables, cereals
Carboxin	Anilide	Vitavax	1969	Bayer CropScience, Chemtura	Cereals, peanuts
Difenoconazole	Triazole	Dividend	1994	Syngenta Crop Protection	Cereals
Fludioxonil	Phenylpyrrole	Maxim	1994	Syngenta Crop Protection	Maize, canola, sorghum, soybeans, peanuts, rice
Mefenoxam	Phenylamide	Apron XL	1996	Syngenta Crop Protection	Numerous
Metalaxyl	Phenylamide	Allegiance	1977	Bayer CropScience	Numerous
Pencycuron	Phenylurea	Monceren	1976	Bayer CropScience; others	Potatoes, cotton
Prothioconazole	Triazole	Redigo, Lombardor	2007	Bayer CropScience	Cereals
Pyraclostrobin	Strobilurin	Stamina	2008	BASF	Maize, soybeans
Tebuconazole	Triazole	Raxil	1986	Bayer CropScience	Cereals, maize
Thiram	Dithiocarbamate	Various	1942	Bayer CropScience	Vegetables, sugarbeets
Triadimenol	Triazole	Baytan	1981	Bayer CropScience	Cotton, cereals
Trifloxystrobin	Strobilurin	Trilex	1999	Bayer CropScience	Maize, soybeans, cotton, peanuts, rice
Triticonazole	Triazole	Charter	1992	BASF	Cereals

treatment, which rapidly replaced older, high-rate compounds such as captan on maize and other crops; and the introduction of the strobilurin fungicides as seed treatment products, with their expanded spectrum of activity and new mode of action.

The significance of difenoconazole, introduced in 1994, was largely due to its excellent control of dwarf bunt (61, 126). Although other systemic fungicides such as carboxin were already available for control of smut diseases in cereals, dwarf bunt control was not adequate (72) and carboxin did not have the broad spectrum of difenoconazole and the other triazoles. Difenoconazole provided a broad range of activity against soil-borne pathogens, including seedling pathogens (23) and take-all (48). Systemic activity combined with this broad spectrum enables difenoconazole to provide protection against foliar pathogens of seedlings, including leaf rust (*Puccinia recondita*), stripe rust (*P. striiformis*), and Stagonospora and Septoria leaf blotches (*Stagonospora nodorum* and *Septoria tritici*). Both infection and sporulation of these pathogens are suppressed in seedlings from treated seeds (81, 129). As a result of its efficacy against *Fusarium* spp. (88), difenoconazole also has been useful for improving germination, stand establishment, and yield from head blight–damaged wheat seed contaminated with *Fusarium graminearum* (120, 139) or *Microdochium* spp. (39). A variety of seed treatments is currently available for small-grain cereals, but difenoconazole, triticonazole, and tebuconazole (combined with metalaxyl or mefenoxam) are still the most widely used. Ipconazole, another triazole active ingredient, has been developed as a seed treatment and will likely experience significant use.

Fludioxonil was rapidly adopted in maize in the United States, largely because of its lower rate and better safety properties compared to captan. Fludioxonil also displayed improved efficacy against some important seedling pathogens such as *Fusarium graminearum* (88). The industry shift from the inexpensive captan products to fludioxonil indicated a willingness to acknowledge greater value in maize seed treatments and set the stage for the subsequent growth in seed treatment investment in maize and other crops. The adoption of fludioxonil as a standard seed treatment in maize has been followed by rapid evolution from a single, nearly universal active ingredient (captan) to diverse seed treatment combinations that include several fungicides and an insecticide. Growth in the use of seed treatments in soybeans also followed the introduction of fludioxonil. Until recently, the economic benefits for the use of seed treatments on soybeans had been questionable, but under current conditions, positive economic returns are being reported (15, 106) (**Figure 5**). According to industry estimates, less than 8% of soybeans planted in 1996 had a seed treatment, but that estimate has grown to more than 30% in 2008, with several seed companies now treating all soybean varieties. This trend is likely to

Figure 5

Dramatic improvement in soybean emergence achieved with a fungicide/insecticide seed treatment combination (left), compared to nontreated control (right) in a field trial in Boone Co., IA, in 2008.

continue, based on decisions by the two largest soybean seed companies (Pioneer Hi-Bred International, Inc., and Monsanto) to require seed treatment on major segments of their soybean product lines beginning in 2009.

Strobilurins have had a very significant impact on the overall fungicide market since their introduction in 1996, and this group now occupies the second-largest fraction of the market (15.3%), exceeded only by the triazoles (20.3%) (83). Their introduction into the seed treatment market has been more recent, but their impact there has been equally dramatic. Azoxystrobin and trifloxystrobin now are widely used as standard seed treatment fungicides, in combination with other active ingredients, on a number of crops including maize and soybeans. Strobilurins bring a uniquely broad spectrum of activity to complement fludioxonil, with activity against ascomycetes, basidiomycetes, deuteromycetes, and oomycetes and improved control of *Rhizoctonia* spp. (8). The systemic activity of azoxystrobin (8), the most widely used strobilurin in seed treatments, may also contribute to improved performance as a result of more complete root tissue protection and a longer duration of control compared to contact fungicides. The addition of azoxystrobin to the maize seed treatment combination has led to improved stands and yields under challenging planting conditions (19). An additional driving force for the adoption of azoxystrobin in maize has been the emergence of isolates of *Pythium* spp. and *Fusarium* spp. with reduced sensitivity to existing standard maize seed treatments (mefenoxam/metalaxyl and fludioxonil) (17, 18). Although the frequency of insensitive isolates is unclear, their detection adds additional priority to development of new seed treatment active ingredients. Azoxystrobin was effective against a wide range of *Pythium* species isolated from soybeans (29) and maize, including all identified *Pythium* strains with low sensitivity to mefenoxam (96). Pyraclostrobin, another strobilurin active ingredient, has been developed as a seed treatment and will likely experience significant use.

Effects of Seed Treatments on Plant Physiology

A major recent development in the use of seed treatments has been their use for purposes other than disease or pest control. Although polymer coatings have been developed and used for various purposes, including the modulation of imbibition or to improve seed flowability, for a number of years, the popularity of these uses has been limited, and in most cases polymers are used in conjunction with crop protection chemicals. A much more striking trend has been the adoption of seed treatments for their direct physiological effects on plants, leading to induction of plant defense responses, increased stress tolerance, or improved growth and yield. Some products sold for these effects are biological control products or chemicals without direct pesticidal properties, but others are insecticides or fungicides.

Physiological effects of strobilurin fungicides have played a major role in marketing of foliar formulations in some crops, and this strategy has begun to impact seed treatments as well. Trademarked phrases such as Plant Health™ (BASF) and Plant Performance™ (Syngenta Crop Protection) have been coined to describe the direct benefits of strobilurin fungicides on plant physiology. Strobilurin fungicides have been shown to induce several physiological changes in plants (8). These include suppression of ethylene biosynthesis, increased levels of abscisic acid (40), and enhanced antioxidative potential (142), resulting in delayed senescence or prolonged leaf greenness (40, 41, 111), increased tolerance to environmental stresses (9, 35, 111), improved CO_2 and nitrogen assimilation (38, 64), and increased water use efficiency as a result of reduced transpiration (40). Additional effects may include induction of plant-defense responses (22, 47), resulting in better resistance to nontarget pathogens (47). Knowledge that fungicides can have a direct impact on plant physiology is not new; similar effects have been documented for triazoles and other fungicides (132, 142), but those characteristics had not been promoted

for the purpose of marketing seed treatments. As strobilurin fungicides are more widely used as seed treatments, it appears that physiological effects will be studied and exploited more extensively.

There also is evidence that some seed treatment insecticides have useful physiological effects on plants. Positive effects on plant establishment, growth, and yield have been associated with the neonicotinoid insecticides, particularly thiamethoxam (89, 110, 124). This property also has been promoted in marketing campaigns, and a "Vigor™ effect" has been patented in relation to the use of thiamethoxam (Cruiser® products from Syngenta Crop Protection), documenting improved emergence and growth of plants including canola, rice, and potatoes (124). Similar effects have been recorded for several other crops, including maize, soybeans, peas, sugarbeet, cotton, sugarcane, and sunflowers (89, 110, 121). Although there is abundant evidence for positive physiological effects of some fungicide and insecticide seed treatments on plants, it remains controversial whether the magnitude of physiological effects is sufficient to have an economic impact on crop productivity (11, 90, 100). However, it is clear that strobilurin and neonicotinoid seed treatments do provide consistent, economically beneficial crop performance gains apparently through the combination of disease/insect control and direct physiological effects. Future research should focus on quantifying and characterizing physiological effects of these chemicals under different environmental conditions and with different crop cultivars.

Seed Treatment Outlook

Growth in the use of seed treatments is likely to continue, as the scope of the seed treatment market expands, both in relation to crops and target pests/diseases. Aside from the developments already mentioned, a small but important market in biological seed treatments also is expected to continue growing. This includes microorganisms and also nonpesticidal chemicals that enhance plant defense responses against multiple pests/pathogens. Also not yet mentioned is the class of seed-applied products sometimes called seed enhancements, which encompasses modulators, polymers, colorants, micronutrients, or beneficial microorganisms applied onto the seed, not necessarily for pest/disease control but to boost the performance of seeds either in processing and planting equipment or to mitigate environmental stress. It is likely that the use of these products will continue to grow.

Strobilurin fungicides are likely to play a very significant role in seed treatments in the foreseeable future, but one of the challenges will be the development of resistance to these active ingredients. Resistance to strobilurins has developed fairly rapidly in a number of pathogens that are subject to foliar applications (8). Although seed treatments are not considered to be high-risk for resistance development, the experience with metalaxyl/mefenoxam suggests that if crops are subject to seed treatment and soil or foliar applications with the same active ingredient (or related active ingredients), insensitivity can develop in the pathogen populations. The popularity of strobilurins as both foliar formulations and seed treatments in maize, soybeans, and other crops indicates that monitoring is warranted; and the coapplication of strobilurin active ingredients with other broad spectrum fungicides should be beneficial. Insecticide seed treatment also is likely to grow, especially with the addition of insecticide/nematicide active ingredients to the portfolio. Emerging data on the economic impact of nematodes on crops such as maize will determine the extent to which these products will be attractive.

There are a number of important pathogens that are not yet controlled by seed treatments but are feasible and attractive targets for seed treatment use. Two examples are soybean cyst nematode, *Heterodera glycines*, and the soybean sudden death syndrome pathogen, *Fusarium virguliforme*. These represent two of the most economically important soybean

pathogens; they both attack seedling roots and could be amenable to control by seed treatment, if efficacious active ingredients can be developed. Development of products targeting these two pathogens should be a high priority. Another promising development is the use of seed treatment fungicides to target mycotoxin-producing *Fusarium* spp. in small grains and maize, with the goal of reducing mycotoxins in grain. Encouraging results have been reported from Italy (82) and France (107), demonstrating reduced levels of deoxynivalenol in wheat grain when seeds were treated with fludioxonil. This tactic may become a useful component of an integrated mycotoxin management strategy.

In the near future, there will be a growing focus on delivering packages of complementary genetic traits and seed treatment combinations. This trend is being fueled by the consolidation of crop protection chemical producers with seed companies. One remarkable current example is the StrigAway® system for control of witchweed (*Striga* spp.). *Striga* is a genus of parasitic plants that causes major losses to African crops, including an estimated USD $1 billion annual loss in maize. In a collaboration among BASF, CIMMYT, AATF (African Agricultural Technology Foundation), local public research systems, and seed companies, the technology was developed, coupling a non-GMO herbicide-resistance trait (Clearfield®) with imazapyr seed treatment. As the maize germinates, it absorbs some of the herbicide. As the parasitic *Striga* attaches to the maize root, it is killed before it causes damage. Imazapyr also diffuses into the soil and kills *Striga* seeds that have not germinated. This system has been very effective in controlling *Striga* in Africa and increasing maize yields (56, 57). Another step toward trait/seed treatment packages occurred in 2008, when Monsanto announced the launch of a proprietary seed treatment line (Acceleron™), to be coupled with SmartStax™ and Roundup Ready 2 Yield™ genetic modification technologies in maize and soybeans.

PHYTOSANITARY REGULATIONS AND SEED HEALTH TEST STANDARDIZATION

Successful international trade in seeds depends on the development and implementation of science-based phytosanitary legislation and regulations. Historically, however, many phytosanitary regulations have lacked a solid scientific foundation. Seed health testing requirements are among the most important and science-intensive aspects of phytosanitary regulations; unfortunately, these requirements have often posed obstacles to safe and efficient seed movement because of insufficient scientific input.

Phytosanitary Regulation Trends

Phytosanitary requirements for seed imports went through a period of rapid increase during the 1990s, largely driven by the approval of NAFTA and other free-trade agreements. For example, prior to 1991, there were no phytosanitary requirements for vegetable seed imports to Mexico from the United States, but by 1994, nearly 60 pathogen restrictions on vegetable seed imports were proposed in Mexico (70). Increased trade in seed and other agricultural products spawned some legitimate concerns about risks of pathogen movement between nations; however, some phytosanitary regulations were not based on pest risk but instead were enacted as substitutes for previously existing trade barriers.

Faced with these new challenges, several organizations with interest in international seed trade began to address the scientific basis of the burgeoning lists of quarantine pests, using pest risk analysis processes. Beginning in the late 1990s and continuing today, a series of workshops has been held in some of the regions of the world with the greatest seed imports (**Table 4**). Organized by the Iowa State University Seed Science Center, and involving various regional and national plant protection organizations, these workshops have the purpose of reviewing national quarantine pest lists and devising

Table 4 Numbers of pathogens and pests on quarantine pest lists for several global regions, before and after application of pest risk analysis (from Reference 122)

Region	Crops selected	Quarantine organisms	
		Before project	After project
Central America	5	82	2
East Central Africa	6	35	7
South America (Mercosur)	11	50	10
Asia Pacific	11	149	38
Andean Pact	7	379	112
Southern Africa	18	87	26

regional quarantine pest lists based on pest risk analysis (122). One of the main obstacles to the development of science-based phytosanitary regulations has been a lack of accessibility to up-to-date information about seedborne aspects of plant pathogens. A major step forward in this area came with the publication of the Crop Protection Compendium (CPC) in 1997 by CAB International. The CABI CPC includes sections describing seedborne aspects of each plant pathogen in the database, and also includes a pest risk analysis function that can be used as a tool to guide phytosanitary regulation development. Now available online (**http://www.cabi.org/compendia/cpc/**) with frequent updates, the CABI CPC has greatly facilitated the increased use of pest risk analysis in the formation and revision of phytosanitary regulations. Information compiled in the CPC was considered along with other information sources in the regional workshops, which resulted in dramatic reductions in numbers of pathogens and pests on quarantine lists (**Table 4**). These reductions occurred primarily because many pathogens and pests appeared on the lists but were not seed-transmitted or likely to be transported with seed, they already occurred throughout the region, or they did not pose an economic risk (122).

Seed Health Test Standardization

Seed health testing methods have been a research focus at some institutions since at least 1918 (2), and, as already discussed, many innovative seed health testing methods have been developed in recent years. However, there has been a disconnection between method development and implementation. Many methods may exist, even for a single pathogen on a single crop, but little effort was made to validate tests under different conditions or to form agreement among trading nations about the acceptability of different methods. For example, Maddox (70) pointed out that methods used to detect *Xanthomonas campestris* pv. *campestris* in crucifers included four different published approaches with nine possible selective media and sample sizes ranging from 10,000 to 60,000 seeds. As a result, there has often been a lack of consistency between exporting and importing nations regarding acceptable methods for documenting phytosanitary compliance. In 1994, a symposium titled "Plant Pathogens and the Worldwide Movement of Seeds" was held at the APS Annual Meeting, and its proceedings were published by APS Press. The status of seed health test standardization was summarized by more than one presenter: "[T]he majority of seed health tests used throughout the world have never been subject to standardization that would ensure accuracy and repeatability" (21); "apart from the ISTA sheets on seedborne diseases, there has been no systematic effort to develop standardized tests that are accepted internationally" (80).

During the past decade, several organizations have begun to address this situation by promoting research, development, implementation, and standardization of meaningful

seed health testing methods. These efforts are guided by the International Plant Protection Convention, especially its International Standards for Phytosanitary Measures (**https://www.ippc.int/IPP/En/default.jsp**). These organizations include the International Seed Testing Association (ISTA), International Seed Federation (ISF), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System (NSHS). Earliest among these was probably ISTA, which formed a Seed Health Committee (SHC) as early as 1928 (1). The committee was alternatively referred to as the SHC or Plant Disease Committee (PDC) through 2002, when the PDC designation was finally dropped. The previously mentioned quote by McGee cites one of the outputs of this committee, but most of the committee effort (during its first several decades) focused on cataloguing seedborne microorganisms (113), rather than the practical aspects of detecting pathogens in a phytosanitary context. This approach evolved and the current Seed Health Committee's objective is to "develop and publish validated procedures for seed health testing, and to promote uniform application of these procedures for evaluation of seeds moving in international trade" (52). The committee has published a handbook on validation of seed health testing methods (123), and ISTA's *International Rules for Seed Testing* now includes a supplement on seed health testing methods (51). The ISTA SHC has approved approximately 28 different seed health test methods (53) (**Table 1**).

During the mid 1990s, ISHI was formed through collaboration between seed companies (primarily vegetable seeds) and the ISF. The objective of ISHI is to facilitate international movement of healthy seeds through collaboration among private seed companies, public and private seed testing labs, and academic and government research institutions. ISHI groups work toward development, assessment, and communication on test protocols for economically important seedborne pathogens. The intent is for these protocols to be generally accepted as internationally standard tests to provide documentation for phytosanitary certification. ISHI efforts are coordinated with ISTA for test validation. The most active area within ISHI has been in vegetable crops (ISHI-Veg), with membership from France, Israel, Japan, the Netherlands, and the United States, representing more than 75% of the world's vegetable seed supply. ISHI-Veg has established an online Manual of Seed Health Testing Methods, with approximately 21 methods approved for seedborne pathogens (**Table 1**). Some ISHI-Veg methods have been accepted as ISTA rules and as standards by the USDA-APHIS National Seed Health System (NSHS).Two more initiatives for herbage (ISHI-H) and field (ISHI-F) crops were established in 1997 and 1998, respectively. However, these areas have not been active, with only a single method approved for field crops. Since 2000, ISF administers the ISHIs with the goals of harmonizing national regulations on phytosanitary issues and eliminating unjustified and unfair barriers to seed trade.

The need for seed health test standardization, along with other challenges related to international seed movement, also led to developments in the United States during the mid-1990s. Discussions among seed industry representatives and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) were held throughout the latter half of the decade; procedures and documentation were developed, culminating in the establishment of the NSHS by publication in the Federal Register in August, 2001 (31). NSHS is an accreditation and coordination system authorized by USDA-APHIS and administered by the Iowa State University Seed Science Center. Its membership consists of accredited entities (both public and private) involved in seed production, testing, and inspection. The mission of NSHS is to facilitate international trade for the U.S. seed industry by providing resources to assist seed companies in meeting phytosanitary regulations. This is accomplished through the pursuit of three primary objectives:

1. To conduct research and develop standardized seed health laboratory tests and phytosanitary inspection procedures,
2. To provide and oversee accreditation to private entities to carry out their own phytosanitary testing activities,
3. To collaborate with other international initiatives to promote international phytosanitary reform and foster fair, equitable trade.

NSHS provides accreditation in four areas: seed health testing, sampling for seed health testing, visual seed inspection, and phytosanitary inspection of seed crops. Entities with accreditation can carry out these processes for their own seed shipments or as a service to others. NSHS has developed a reference manual outlining procedures that must be followed in conducting these processes under accreditation. Accredited entities, however, must develop individual procedures and demonstrate a quality management system that ensures consistent performance and record keeping. A process was developed for seed health testing methods to be approved by NSHS, and the current NSHS reference manual documents 52 approved methods (91) (**Table 1**). NSHS cooperates with ISTA and ISHI to normalize methods approved by the different entities for standard use internationally.

Effective conduct of accurate seed health testing depends on numerous public and private laboratories throughout the world. Significant progress has been made in training personnel and establishing seed testing laboratories in the developing world, through national initiatives (62) and the efforts of organizations such as the Danish Seed Health Center for Developing Countries (DSHC) (109), CIMMYT (76), and the Iowa State University Seed Science Center.

Seed health test method standardization, as a component of the overall harmonization of phytosanitary regulations, remains a long-term goal for the global seed industry and for many organizations and government agencies that are stakeholders in international seed trade. Progress depends on sustained financial support (always inadequate) for the careful scientific evaluation of existing methods and development of innovative new methods. Scientific headway alone, however, will not be sufficient. This goal has critical scientific underpinnings but also has complex economic, social, and political dimensions. Movement toward the goal of international standards also depends on sustained communication and lobbying efforts, coupled with positive and consistent working relationships between seed industry representatives and government regulatory agencies. Organizations such as ASTA, ISTA, ISHI, ISF, and NSHS are attempting to address this challenge from many different angles.

CONCLUDING COMMENTS

The goal of seed pathology research and practice is the production and dissemination of high-quality, disease-free seed that maximizes potential crop productivity and value. Seed pathology occupies a crossroads at the intersection of seed health testing, crop protection chemistry, epidemiology, pathogen taxonomy, and international phytosanitary regulation. As each of these areas evolves, repercussions spread into the other facets of seed pathology, requiring significant and sometimes painful change. The past decade has been witness to remarkable developments in molecular biology that are facilitating rapid expansion of epidemiological knowledge about seedborne pathogens, and driving the evolution in seed health testing that will ultimately result in a landscape very different from today's. Organizations involved in seed health test implementation must redouble their efforts at standardization, explicitly address the questions raised by the implementation of PCR-based seed health tests, and embrace the development of multiplex assays for seed health. This can only occur if appropriate, sustainable resources are available to support these efforts.

Seed-applied crop protection chemicals and enhancements are an important and growing facet of crop management, but their full potential is yet to be realized. Additional

knowledge is needed regarding the effects of multiple active ingredients on the interacting complex of microorganisms to which seeds are exposed in the soil environment. There is potential for seed treatments to contribute to the management of additional pests and diseases that currently are beyond the reach of seed treatments, and this envelope will continue to be pushed. The implications of seed treatment in relation to interpreting seed health tests have never been adequately addressed, but questions persist. Does seed treatment interfere with accuracy of seed testing? Can seed treatments impact appropriate pathogen thresholds? In order to appropriately apply new seed health testing technologies, and to make the most effective use of seed treatments, there continues to be a need for development and refinement of appropriate economic thresholds that can be related to seed health test results. Without this component, progress toward lessening the impact of seedborne pathogens will be hindered.

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Errata

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