

The impact of invasive fungi on agricultural ecosystems in the United States

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Abstract Invasive fungi and other non-indigenous plant pathogens have had a significant effect on American agriculture for hundreds of years. At present crop loss due to invasive plant pathogens, especially fungi, is estimated at \$21 billion per year in the United States, greater than the loss caused by non-indigenous insects. Plant pathogenic fungi are difficult to detect and identify. Thus knowledge of which fungi pose a threat is essential to prevent their entry by means other than inspection. In this paper, examples are presented of invasive fungi on agricultural commodities introduced into the United States. In all cases two factors have been crucial: first, the pathway through which these fungi have entered, and second, systematic knowledge to prevent and respond to the new invasive species. Historically important plant pathogens such as black stem rust of wheat still cause considerable damage while others such as late blight of potato appear to be having a resurgence. Known previously in Australia, then moving to Africa and South America, the virulent species of soybean rust appeared in the U.S. in 2004 but has not been as devastating as anticipated. Plant pathogenic fungi on specialty crops such as daylily, gladiolus and chrysanthemum are threatened by rust fungi recently found in the U.S.

apparently brought in on infected germplasm. A crisis in the export of U.S. wheat occurred in the late 1990's when the molecular diagnostic test for Karnal bunt gave a false positive response to a closely related but previously unknown species. Many potentially dangerous plant pathogens of crop plants have not yet been introduced into the U.S. It is critical that meticulous surveillance be conducted as plant material enters the country as well as where crops are grown prior to shipment. In addition, the scientific infrastructure is needed to be able to respond quickly to new invasive fungi. This requires sound systematic knowledge of plant pathogenic fungi both in the U.S. and around the world and a cadre of systematic experts who can characterize invasive fungi.

Keywords Black stem rust · Karnal bunt · Non-indigenous pathogens · Plant pathogens

Invasive fungi and other non-indigenous plant pathogens have had a significant effect on American agriculture for hundreds of years. With the increase in international trade the rate of introduction of these destructive organisms is increasing. Some reports estimate that up to 65% or an estimated \$21 billion per year of crops are lost in the United States (U.S.) due to introduced plant pathogens (Pimental et al. 2002, 2005). With control costs included, this translates into about \$23.5 billion annually in the U.S. Surprisingly, the crop loss due to non-indigenous

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plant pathogens, most of which are fungi, is greater than that caused by non-indigenous insects.

For most of their life histories, plant pathogenic fungi cannot be seen on the plant surface and thus it is difficult to determine their presence and prevent their movement on agricultural commodities. Effective measures to prevent the introduction of invasive fungi must take these factors into account. Many potentially dangerous plant pathogens of crop plants have not yet invaded the U.S. Thus, it is critical that meticulous surveillance be conducted as plant material enters the U.S. at ports of entry as well as where this material is grown prior to shipment of agriculture commodities.

Why are fungi such a big problem as invasive species?

Fungi are organisms that obtain their nutrition via absorption. As a result of this mode of nutrition they are always associated with other organisms, often plants, but also insects and humans, as parasites, symbionts, or saprobes. One reason that fungi pose such a problem as invasive species is that most fungi are composed of hyphae, which are very narrow threadlike structures only 5–10 μm diameter, thus the body of the fungus itself occurs inside the substratum and may not be visible from the outside. Only when the fungus is sporulating, often after the plant starts dying, can a fungal pathogen be detected by those who are inspecting agricultural commodities.

A second reason the fungi are such a big problem as invasive species is that there are so many different kinds of them, most of which are unknown. At present it is estimated that about 1.5 million species of fungi exist, second only to the insects in total estimated number of species. Of the total number of fungi thought to exist, only 7–20% or about 150,000 fungal species have been described and illustrated (Hawksworth 2001; Hawksworth and Rossman 1997). Even fewer are known in the modern literature with only about 22,000 species or 1.5% of the known fungi having been sequenced. Thus, it is not surprising that about half of the invasive fungi in North America were not described prior to their appearance as invasive species. Given this lack of knowledge, it is often difficult to determine if a new disease outbreak is caused by an invasive fungus or is simply a fungus encountering a stressed host plant

or a new climatic situation. For example, a fungus that is known primarily as a weak parasite or harmless endophyte in a living plant may become aggressive once the plant is weakened or stressed such as when an agricultural plant is grown in a monoculture or climatic conditions change (Manter et al. 2005; Ortiz-Garcia et al. 2003).

Because fungi are relatively unknown and difficult to detect, it is also difficult to anticipate and prevent the arrival of fungal pathogens that have not been adequately described and characterized. One would think that molecular diagnostic tools might be the answer to the detection of plant pathogenic fungi in agricultural commodities and, indeed, progress has been made in that regard. However, such molecular diagnostic tools are only as good as the systematic underpinnings upon which these tools are based. Molecular tests are developed by finding a unique sequence for the organism to be detected, thus it is necessary to know what is the most closely related species in order to determine if a sequence is truly unique to the target species. The consequences of an inaccurate molecular diagnostic tool are exemplified by the test for the Karnal bunt fungus of wheat that gave false positive responses and resulted in a serious agricultural trade crisis as discussed below.

Although rapid progress is being made with the use of molecular tools, the identification of fungi has only recently moved from being based on microscopic characteristics to molecular diagnostics that are the result of phylogenetic studies. Within the major groups of fungi, species are now being reevaluated based on the use of multigene phylogenies with some unexpected results. In some groups of fungi, sexual state characters that have traditionally been used for species definition are found to be misleading, masking a number of cryptic or similar looking species. For some groups the morphological variability exhibited by the asexual state, rather than the sexual state, may reflect the phylogeny. For example, in the powdery mildews or Erysiphales the sexual states have morphologically variable appendages that have served as the basis for their identification. Recent phylogenetic studies have confirmed that the morphological characteristics that correlate with phylogeny are those of the conidial or asexual states (Takamatsu 2004). Such is also the case with the leaf parasites in the relatively non-descript sexual genus *Mycosphaerella*, many of which have highly variable asexual states in such



Fig. 1 *Phomopsis* sp. on agar plate. Species of *Phomopsis* had been considered host specific but molecular data have shown that one species can occur on several hosts or one host can harbor more than one fungal species. Photo courtesy of USDA-ARS

genera as *Cercospora*, *Phloeospora*, *Septoria* and others (Hunter et al. 2006). Conversely, species defined based on differences in their plant hosts such as in the asexual genus *Phomopsis* with over 1,000+ described taxa (Fig. 1) may or may not be host-specific (Castlebury et al. 2001). Many species of *Phomopsis* defined based on host differences have been discovered actually to be one species that may attack numerous hosts (Black et al. 1996; Li et al. 2001). Conversely, a single plant host may harbor numerous species of *Phomopsis* (Farr et al. 2002). Lack of accurate systematic knowledge hinders the ability to develop the accurate molecular diagnostic tests needed to detect invasive fungi on agricultural commodities.

In this paper I will focus on examples of invasive fungi of agricultural commodities introduced into the U.S. that have been historically important as pathogens, fungi that have recently been introduced, and those that have influenced the exchange of agricultural commodities. In all cases two factors have been crucial to each situation. One factor is the pathway through which these fungal pathogens have invaded. The second factor is the critical role that knowledge of systematics has played in responding to invasive fungi and the even greater role that systematics could play in preventing the entry of new invasive fungal species. Finally, a discussion is presented of the actions that could be taken to prevent the continuing entry of invasive fungi into the U.S. on agricultural commodities.

Historically important diseases caused by introduced fungi: most are still a problem

Black stem rust of wheat

Historically, many fungi have negatively affected agricultural ecosystems in the U.S. Wheat rusts are mentioned in the literature from antiquity with a festival, the Robigalia, practiced for over 1,700 years, dedicated to pleasing the forces that control rust diseases (Schumann 1991). Given the close association of rust fungi with their host plants, it is not surprising that the early European settlers who brought wheat seed to the North American continent also introduced the fungus that causes black stem rust. The disease is caused by the rust fungus, *Puccinia graminis* Pers. (Uredinales), which has a complex life history with five different spore types that infect hosts in two diverse plant families. The uredinal and telial states occur on 77 genera of grasses (Poaceae, primarily subfamily Pooideae), while the aecial state occurs on more than 70 species of barberry and related plants (*Berberis* and *Mahonia*, family Berberidaceae).

In the early 1900s black stem rust was an especially damaging disease of wheat in the U.S. Steps were taken to eliminate the alternate barberry host, which continued until 1990 (Peterson 2001). A concomitant drop in wheat rust epidemics has been documented (Roelfs 1982). At the same time plant breeders were discovering genes in wheat for resistance to black stem rust; this program has been successful although the research continues to this day. The combination of barberry eradication in the major wheat-growing states and the constant inclusion of black stem rust resistant genes has resulted in limited damage from this disease in recent years. Although the barberry eradication program was successful in reducing the amount of wheat disease, one wonders what impact eliminating these plant species from the Mid-West has had on the existence of native species such as *Berberis canadensis* P. Mill. used as a cure for diarrhea by the Cherokee Indians as well as the other organisms, some possibly beneficial, associated with these plants.

Despite the worldwide occurrence of *P. graminis*, the systematics of this species is still relatively unknown. Over the decades many segregate taxa have been described, particularly segregate species and biologically specialized *formae specialis*, which are taxa defined by their occurrence on a specific host.

Subdivisions of *P. graminis* into subspecies, varieties and *formae speciales* have been proposed based on the morphology of the urediniospores and the host range. Crossing studies support the separation of at least two subspecies, but not the proposed separation based on spore size. Recent molecular sequence data have been used to determine that *P. graminis* is truly one species with broad genetic diversity that attacks many genera of plant hosts. Its high genetic variability suggests that its center of origin is in Asia. This confirms previous studies demonstrating that this disease was introduced into the U.S. at least twice (Abbasi et al. 2005). These data suggest that strains from diverse hosts may be undergoing genetic exchange at a low level. Recently a new strain (Ug99) of this fungus was detected in eastern Africa, has spread to the Middle East, and is projected to move eastward to India and beyond (Stockstad 2007). Thus, black stem rust is still a problem with new races evolving and new introductions occurring that result in a continuous need to develop resistant wheat cultivars (Leonard 2001).

Late blight of potatoes

Late blight of potatoes caused by *Phytophthora infestans* (Mont.) de Bary was introduced into Europe in the 1840's. In 1845 it moved into Ireland where 25% of the potato crop was lost. The next year the disease was even more severe when 80% of the potato crop was destroyed. With their food staple gone about a quarter of the Irish population, or two million people, died of starvation and one million Irish immigrated to the U.S. Although copper fungicides are now used to protect potatoes from this organism and resistant potato cultivars have been developed, losses due to late blight of potatoes still occur when the weather is unusual or new strains of the organism are introduced.

In the last decade a new mating type of *P. infestans* was found to occur in the U.S. and this disease has re-emerged as a serious problem for potato growers (Fry et al. 1993; Fry and Goodwin 1997). In addition to increased genetic diversity due to recombination, changes in weather patterns have also been implicated in the increased disease severity. As a result there is rekindled interest in this historically important disease and the threats posed by genetic variants of this organism. Using specimens deposited in herbaria over the past one hundred years, Jean Ristaino and her

associates have been able to determine the routes and approximate times in which *P. infestans* was transported around the globe. May and Ristaino (2004) determined that this organism was moved with potatoes from South America to Europe in about 1830 and then to North America in 1910. Recent data suggest that *P. infestans* originated in the Andes (South America) and was transported to Europe, Mexico, and North America (Gómez-Alpizar et al. 2007). Several genetic variants reside outside the U.S., the most threatening in Mexico; thus, actions to prevent the introduction of invasive isolates of *P. infestans* are still needed. These recent molecular analyses, especially of historical specimens, have contributed to the ability to track the spread of this pathogenic organism. Although not known for certain, it seems likely that the pathogen was spread with diseased germplasm that was transported for planting into new geographic areas.

Phytophthora infestans belongs to a group of organisms that has traditionally been considered fungi but recently has been determined to be phylogenetically distinct. *Phytophthora*, *Pythium* and the downy mildews (*Peronosporales*) are now known to belong to the Chromista, more closely related to the yellow-brown algae than to the true Fungi (Rossman and Palm 2006). Many species of *Phytophthora*, *Pythium* and downy mildews are obligate parasites of living plants and cause diseases throughout the world. New invasive species of *Phytophthora* are being discovered regularly, such as *P. ramorum* S. Werres, A.W.A.W. de Cock & W.A. Man in't Veld which causes sudden oak death, a disease that has recently killed hundreds of trees in the western U.S., and ramorum blight that affects numerous woody ornamental hosts and has had a significant impact on trade.

Southern corn leaf blight

Southern corn leaf blight is of considerable significance in the history of American agriculture. This disease reached epidemic proportions in 1970 when it devastated much of the corn crop at an estimated monetary loss of \$1 billion (Schumann 1991). Southern corn leaf blight is caused by an ascomycetous fungus with the asexual state *Bipolaris maydis* (Nisikado) Shoemaker and the sexual state or teleomorph *Cochliobolus heterostrophus* Drechsler. The fungus causing southern corn leaf blight occurs on several hosts including corn, sorghum and teosinte.

The fungus overwinters on crop debris, primarily on the soil surface, as mycelium, conidia and chlamydospores. Conidia are windblown or splashed by water to fresh plant tissue in the spring. The spores germinate on the leaf surface and infect the host directly and through stomata. Disease development is favored by warm moist weather and the presence of free moisture on the host tissue surface. The fungus is very prolific and is able to complete an entire life cycle in 60–72 h under favorable weather conditions.

The fungus causing southern corn leaf blight exists in two distinct races each of which produces phytotoxins: Race O (old race) and Race T (virulent on corn containing Texas male sterile cytoplasm). The phytotoxin produced by Race O is nonspecific to cytoplasm types and is produced in small amounts. The more aggressive Race T produces a phytotoxin that specifically affects corn containing Texas male sterile cytoplasm. Although present in the U.S. prior to that time, Race T became prevalent in 1970 in the Midwest where almost all of the corn was genetically uniform and contained Texas male sterile cytoplasm, a desirable trait that was, unfortunately, correlated with susceptibility to this pathogen. Following the 1970 disaster corn was bred with single gene and polygenic resistant to Race T. Today corn is resistant to both races of the pathogen, the primary means of controlling the plant's health. In this example the new race of the pathogen was noticed but the impact was not appreciated. The pathogen appears to have been introduced through natural means. However, the genetic uniformity of the crop resulted in extreme susceptibility to this disease (Ullstrup 1972).

New invasive fungi arriving daily

Soybean rust

One of the most recent invasive fungi to arrive in the U.S. is soybean rust caused by two fungi, *Phakopsora pachyrhizi* Syd. & P. Syd. and *P. meibomia* (Arthur) Arthur. *Phakopsora pachyrhizi* is by far the more aggressive of these two species and the disease it causes is referred to as Asian soybean rust. Native to Asia and present in Australia for some time where it has had significant economic impact, the fungus has recently been found in Africa, South America, Hawaii, and along the Gulf Coast in the continental U.S. Fungal

spores of *P. pachyrhizi* carried by wind currents over long distances may have led to its introduction into South America from Africa and then into the U.S. from South America. Schneider et al. (2005) suggest that spores of soybean rust were blown northward from northern South America into the U.S. by Hurricane Ivan. Spores of soybean rust have been found high in the atmosphere (Barnes et al. 2006). Both species of *Phakopsora* infect a broad range of leguminous hosts; thus, control of the disease may not be easy.

The arrival of this invasive fungus was long anticipated following an outbreak of a *Phakopsora* disease on legumes in Puerto Rico in the mid-1970's (Bromfield 1984). In this situation the fungus on soybean was the less harmful *P. meibomia*, but the government was alerted to the more serious potential problem posed by *P. pachyrhizi*. At that time a research program was begun anticipating the introduction of the more virulent species. In 2001 *P. pachyrhizi* was found in South America, causing serious damage to the soybean crop in Brazil. It was considered inevitable that the disease would arrive in North America and plans were made for actions to be taken when/if the fungus was found in the U.S. A molecular test was developed to provide rapid diagnosis. State diagnosticians were alerted and trained in the morphological identification of the disease. Finally in the fall of 2004 following Hurricane Ivan, Asian soybean rust caused by *P. pachyrhizi* was detected in Louisiana (Levy et al. 2005) (Fig. 2). The response plan was put into place. Soon thereafter Asian soybean rust was also detected in a number of southeastern states (Krupa et al. 2006).



Fig. 2 Soybean rust (*Phakopsora pachyrhizi*) infecting living leaves. Photo courtesy of Dr. Jose Hernandez

After three years the virulent soybean rust fungi has spread north as far as Illinois although damage to soybean has not been as devastating as anticipated. Grower readiness may be a factor as well as weather conditions not conducive to the disease.

Citrus diseases

Citrus black spot, caused by the ascomycetous fungus *Guignardia citricarpa* Kiely, is a disease that has been considered of plant quarantine importance for Europe and the U.S. for several decades. Detection of this fungus is difficult because it often remains latent until late in the growing season. Identification of this fungus is difficult because of confusion with a similar species that occurs on the same host. Recently this systematic puzzle was unraveled after the look-alike fungus was mistaken for real black spot on citrus fruits exported to Europe from the U.S., where Citrus black spot does not occur. This potential trade issue was averted when the fungus was determined to be the non-pathogenic species. Additional systematic research showed that, while similar morphologically to the citrus pathogen *G. citricarpa*, an endophytic species, *G. mangiferae* A.J. Roy, can be distinguished upon close scrutiny and using molecular sequence data (Baayen et al. 2002). Once the taxonomic and biological distinction was made between these two species, a molecular test was developed to distinguish them (Meyer et al. 2006). In order to protect U.S. agriculture from invasive fungi, knowledge of the fungi normally associated with crop plants as well as the biological characteristics of the plant pathogen is essential.

Another potentially harmful pathogen on *Citrus* causes a tracheomycotic disease called *mal secco*. It is caused by *Phoma tracheiphila* (Petri) Kantachveli & Gikachvili and is known from the Mediterranean countries from which the U.S. imports significant amounts of *Citrus* products (Smith et al. 1992). The systematics of this group is relatively unknown as they generally are non-descript and lack distinctive morphological characteristics. In fact, *P. tracheiphila* may be more closely related to genera other than *Phoma*. A molecular diagnostic test has been developed for the cause of *mal secco* disease (Licciardello et al. 2006) but this test was not based on accurate systematic knowledge of species closely related to the causal organism. The most likely pathway by which pathogens of *Citrus* may enter the U.S. is via infected

nursery stock. Because a fungal pathogen may not be visible on the plant surface, nursery stock must be inspected prior to shipment or held on quarantine for extended periods of time after arrival in the U.S. Only then will fungal pathogens such as *P. tracheiphila*, the cause of *mal secco* disease, be detected.

Diseases of specialty crops—daylily rust, chrysanthemum white rust, and gladiolus rust

Specialty crops that include many beloved horticultural plants are extremely valuable to commercial growers and home gardeners. New varieties and cultivars are continuously developed based on novel, often exotic germplasm. With importation of germplasm from outside the U.S. comes the risk of new invasive fungi. One recently introduced unwanted fungus is the cause of daylily rust, *Puccinia hemerocallidis* Thüm., on daylily (*Hemerocallis* spp.) (Wise et al. 2004). Nursery stock of daylily is grown in some Central American countries during the winter. In early spring daylilies are imported for distribution to nurseries throughout the U.S. The plant material is usually trimmed to include only the basal living rootstock and may appear to be free of disease. In 2001 *P. hemerocallidis* was found on daylily in the U.S. The rust fungus was previously known mainly in eastern Asia where most daylilies are resistant to that fungus. It is difficult to know how a fungus known only from Asia came to infect rootstocks of daylilies in Central America and why it was not detected in situ prior to export. Unfortunately, infected nursery stock was distributed throughout the U.S. before the disease was detected and the fungus was spread quickly. Initially there was a question of whether the rust on daylily in the U.S. was the same as that in Asia. Systematic research demonstrated that the fungus in the U.S., Costa Rica, and Japan is the same (Hernandez et al. 2002). At that time, so little was known about the life history of daylily rust that it was unclear if an alternate host existed. Recent research demonstrates that *P. hemerocallidis* and a closely related species develop additional spore states on the horticultural plant *Patrinia* (Chatasiri et al. 2006). Knowing the complete life history, specifically that daylily rust has an alternate host on which this fungus could have been transported from Asia to the western hemisphere, provides a clue to the mysterious appearance of this invasive species. Although daylily growers enamored of these lovely

flowers are worried about the future of their favorite plant, resistance exists in cultivars of daylily.

Other rust fungi pose a significant threat to specialty crops especially because their pathways of entry continue to elude detection. Outbreaks of Chrysanthemum white rust caused by *Puccinia horiana* P. Henn. have occurred repeatedly in greenhouses throughout the U.S. apparently brought in with infected germplasm. This fungus was transported from its native range in Asia to Europe, especially England and The Netherlands, in the 1960's and more recently to South America. Despite the recurring outbreaks around the U.S., Chrysanthemum white rust has been contained and eradicated every time it has been encountered. Given its widespread occurrence in other parts of the world and the vast quantities of imported germplasm, continuous vigilance and rigorous inspection of plants and cut flowers prior to shipping is required to keep this invasive fungus from becoming established in the U.S. (Wise et al. 2004).

Rust fungi also threaten gladiolus and other Iridaceae. An outbreak of the rust *Uromyces transversalis* (Thümen) Winter in April–May 2006, then again in 2007, occurred in Florida and later in California (Blomquist et al. 2007; Schubert et al. 2007) as well as in Mexico (Rodríguez-Alvarado et al. 2006). Prior to discovery in the U.S. this rust fungus was widely distributed throughout the world but not known from the northern half of the western hemisphere. In this case both federal and state plant quarantine officials were aware of the potential problem and responded quickly when the disease was detected. This awareness resulted in containment of this invasive fungus. A second rust fungus on gladiolus, *U. gladioli* P. Henn., also poses a threat (Wise et al. 2004) but is not as widespread nor as close to U.S. borders as *U. transversalis*. Despite knowledge of the existence and threat posed by the rust fungi on horticultural commodities, these invasive fungi continue to be introduced.

Fungal diseases influence exchange of agricultural commodities

Karnal bunt

Karnal bunt is a disease primarily of wheat (*Triticum aestivum* L.) caused by the obligately parasitic fungus *Tilletia indica* Mitra (Ustilaginales-smut and bunt

fungi). This fungus was described in Karnal, India, in the 1930's and spread to Mexico. For decades the U.S. was concerned that the disease might be detected in this country. Just one diseased kernel can generate hundred of spores that can be carried by wind or on equipment and humans. Although the disease does little to damage crop yield, the quality of the grain is degraded due primarily to a fishy odor. Because of the decrease in seed quality and proximity of this disease to the U.S., the federal government in the 1980's placed a zero tolerance for Karnal bunt on imported wheat. Other countries throughout the world followed the U.S.'s lead and also placed a zero tolerance for Karnal bunt on imported wheat.

Knowing that this invasive fungus was close to our borders, the USDA developed a response plan in case Karnal bunt was discovered in the U.S. Identification of most bunt fungi is difficult based on the patterns of ornamentation as seen on the outside of the microscopic teliospores (Fig. 3). In addition to detecting the teliospores by visual inspection of seed washes, a molecular test was developed for rapid identification of the germinated teliospores (Smith et al. 1996). The basic systematics of bunt fungi had not been well studied partly because most agricultural crops have been bred for resistance to bunt and smut diseases. At the time that Karnal bunt was initially recognized as a potentially invasive fungus, there was only one scientist working on the systematics of bunt and smut fungi in the U.S.

In 1996, the Arizona Department of Agriculture detected Karnal bunt on durum wheat planted in Arizona, California, New Mexico and Texas (Ykema et al. 1996). Because the fungus can spread rapidly, a survey plan was put into effect and government agencies including APHIS (Animal and Plant Health Inspection Service) issued a quarantine for these states. A survey of wheat for Karnal bunt throughout the U.S. was initiated by visual inspection of teliospores in seed washes and using the molecular test to verify the identity of purported Karnal bunt teliospores. To the surprise of everyone, both the inspection and molecular test indicated that Karnal bunt was present throughout much of the U.S. including the Pacific Northwest, the southwestern, and the southeastern states. What a shock! Countries that imported wheat from the U.S. immediately closed their ports to U.S. wheat and the annual \$5.5 billion wheat export market was threatened.

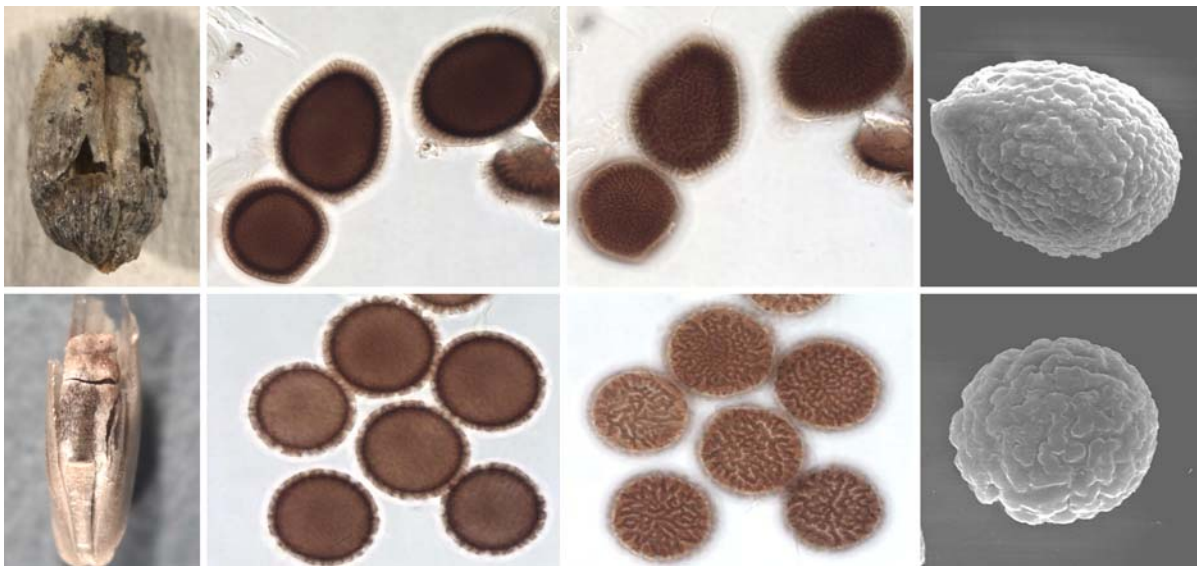


Fig. 3 Karnal bunt (*Tilletia indica*) (top) and a similar looking species (*Tilletia walkeri*) (bottom) that were confused causing a trade crisis in the export of U.S. wheat. Photo courtesy of Dr. Lisa Castlebury

Dr. Mary Palm, the APHIS National Mycologist who examined and verified the identification of the teliospores of Karnal bunt, noticed that some of the teliospores were very slightly different and could not be identified with certainty as *T. indica*. With Drs. Lori Carris and Lisa Castlebury and others it was discovered that these odd teliospores were not Karnal bunt but were those of a previously unknown bunt species that occurred on annual and perennial rye grass (*Lolium multiflora* L. and *L. perenne* L.), often inadvertently mixed with wheat upon harvest. After detailed examination with light and scanning electron microscopes, it was determined that these similar-appearing teliospores had a slightly different ornamentation that could be distinguished by an experienced, practiced eye (Fig. 3). The slightly different teliospores that gave the positive test to the molecular test for Karnal bunt represented a new species described as *Tilletia walkeri* and now known on rye grass from Australia as well as the U.S. (Castlebury and Carris 1999).

Throughout the survey the molecular test was giving a false positive response to the rye grass bunt fungus because the test had been developed based on a wrong assumption about the relationship of the Karnal bunt fungus to its next closest relative. The test was developed assuming that rice bunt (*Tilletia horrida* Takah.) was the next closest relative. Later molecular studies demonstrated that the newly discovered

T. walkeri on perennial ryegrass was more closely related to Karnal bunt than rice bunt, thus resulting in the false positive response (Levy et al. 2001). This situation illustrates the dire need for accurate phylogenetic information upon which to base molecular diagnostic tests. Such tests are not accurate without the essential underpinning of systematic knowledge.

Once the ryegrass bunt fungus was discovered, new criteria were established for positive identification of Karnal bunt. In reality the Karnal bunt disease had spread to only a few limited locations. The U.S. could prove that most wheat for export was grown in areas without Karnal bunt and importing countries throughout the world were again willing to accept wheat from the U.S. This crucial agricultural export market was saved. The U.S. has contained the spread of Karnal bunt and most U.S. wheat is grown in areas free of this disease.

Given the tremendous challenge posed by invasive fungi, how can we combat them?

Legal recognition of the seriousness of invasive organisms was demonstrated in the Plant Quarantine Act of 1912 (Campbell et al. 1999; Palm 2001). Since then, scientists and policy makers have worked to prevent entry and damage caused by invasive species.

Some progress has been made in the last decade given modern tools for communication and molecular tools for identification of cryptic organisms such as fungi.

One of the most basic requirements is to know which organisms threaten U.S. agriculture. For fungal pathogens this is not an easy task because, as mentioned above, most fungi have not yet been discovered and are difficult to detect. APHIS has regulated a number of plant pests through inclusion in federal regulations. The fungi on this Regulated Plant Pest List (RPPL) were recently reviewed to determine the accuracy of the systematic information about them including their host range and geographic distribution (Cline and Farr 2006). Once current knowledge was synthesized, each fungal species on the RPPL was evaluated for the threat it posed to U.S. agriculture (Rossman et al. 2006). About half of the fungi on the RPPL were determined to already exist in the U.S., occur on crops not grown in the U.S., or be too unknown to evaluate their threat e.g., known only from their type specimen. This exercise demonstrates the need to periodically evaluate which fungi pose a threat to U.S. agriculture. As new fungal pathogens outside the U.S. are described on agricultural commodities, their threat to U.S. agriculture should be evaluated immediately.

Knowledge of the plant pathogenic organisms that already occur in the U.S. is also essential. A comprehensive account of the fungi reported in the U.S. was published (Farr et al. 1989) including about 12,000 fungal species on 6,000 plant hosts. Since then, the number has almost doubled to 23,162 species of fungi now reported in the U.S. (D.F. Farr, pers. comm. 2007). In addition, progress has been made in providing access to reports of fungi on plant hosts from throughout the entire world. Farr et al. (2007) currently maintains a database of literature reports of fungi on plant hosts throughout the world that includes over 94,000 fungal species. Most plant quarantine officials agree there is a need for increased knowledge of potentially invasive organisms that exist throughout the world including their movement in real-time. Such knowledge was essential in preparing for the invasion by Asian soybean rust and Karnal bunt.

In the last decade the emphasis has begun to shift from quarantine policies centered on individual invasive species to determining the most dangerous pathways through which invasive organisms enter the country. For fungi these pathways appear to be

primarily nursery stock and raw logs (Ivors et al. 2006; Palm and Rossman 2003). The heightened awareness of the need to prevent the introduction of invasive species through greater scrutiny of most likely pathways of entry has resulted in new regulations governing the import of wood packing material. Nursery stock as a pathway has been implicated in many serious diseases including daylily rust, gladiolus rust, and dogwood anthracnose. Plans are in progress to increase scrutiny of nursery stock as a pathway for invasive species. For agricultural, horticultural and forest pathogens, this pathway should include all germplasm for planting including seeds and tubers.

Given the vast quantities of agricultural goods that are shipped around the world, the most efficacious approach to detect invasive species is to inspect commodities prior to shipment. This would be especially useful for agricultural goods growing outside of their natural environment. Ideally, such inspections would be done by local authorities trained in plant pathology and able to detect potential invasive fungi. This requires that the scientific infrastructure in developing countries be created or enhanced. Developing these capabilities in the countries that ship agricultural commodities to the U.S. will be expensive but will pay off in preventing the entry of invasive species. Continuous scrutiny by a local plant pathologist who would notice diseased plants in the field prior to harvesting will be more effective than sending a U.S. inspector to look superficially at the commodity prior to shipment.

All of the above approaches require a strong systematics safety net. By safety net I mean a body of knowledge about invasive species useful for their identification, developing accurate methods of detection, and determining pathways of entry for pathogenic fungi outside and inside the U.S. Such knowledge will serve as the basis for quarantine policy decisions to prevent the entry of invasive fungi and respond when they are found in the U.S. Knowledge needed about invasive fungi includes accurate scientific names, with descriptions and illustrations; biology such as host range and pathogenicity; geographic distribution and the ability to track their movement; and phylogeny to predict the disease potential. This information must be readily available on the Internet. In addition, rapid molecular diagnostic tools for invasive fungi should be developed. As mentioned in the Karnal bunt example, these tools must be based on sound systematic knowledge of the phylogenetic relationships among

species. While molecular diagnostics may seem to be the solution for rapid identification of invasive fungi, the Karnal bunt situation dramatically exemplifies the crucial need for accurate systematic understanding of potentially invasive fungi.

The organisms most destructive to agricultural systems are fungi, insects and nematodes. Ironically, these are the groups of organisms that are still relatively unknown. The systematics safety net must include a body of experts who know these organisms well. For example, scientists who are experts in the microscopic fungi are crucial for identification of invasive species once they are detected in the U.S. These experts also are essential for determining relationships among species essential for designing accurate molecular diagnostic tools.

Conclusion

Historically, plant pathogenic fungi have caused considerable damage to agricultural crops. Many of these fungi still pose serious problems. Because of the enormous damage caused by invasive fungi, great effort is made to identify and understand these organisms once they have entered the country. However, knowledge of invasive fungi that threaten the U.S. is still relatively meager. Often even the most rudimentary systematic understanding required for their identification is lacking. More emphasis should be placed on (1) knowing specifically what species of invasive fungi exist on agricultural plants outside the U.S., (2) preventing their entry through inspection of agricultural commodities before export, and (3) being able to detect and eradicate invasive fungi once they are found within our borders. In addition, there is a need for the scientific infrastructure able to respond quickly to invasive fungi especially those not previously described. This requires a cadre of systematic experts who can characterize newly discovered invasive fungi, whether previously known or new to science, not just in the U.S. but in countries around the world.

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