Global food and fibre security threatened by current inefficiencies in fungal identification

Pedro W. Crous1,2,*, Johannes Z. Groenewald1, Bernard Slippers3 and Michael J. Wingfield2

1CBS-KNAW Fungal Biodiversity Centre, PO Box 85167, 3508 AD Utrecht, The Netherlands
2Department of Microbiology and Plant Pathology, and 3Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, P. Bag X20, Pretoria 0028, South Africa
4Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

PWC, 0000-0001-9085-8825; JZG, 0000-0002-9474-6246; BS, 0000-0003-1491-3858; MJW, 0000-0001-9346-2009

Fungal pathogens severely impact global food and fibre crop security. Fungal species that cause plant diseases have mostly been recognized based on their morphology. In general, morphological descriptions remain disconnected from crucially important knowledge such as mating types, host specificity, life cycle stages and population structures. The majority of current fungal species descriptions lack even the most basic genetic data that could address at least some of these issues. Such information is essential for accurate fungal identifications, to link critical metadata and to understand the real and potential impact of fungal pathogens on production and natural ecosystems. Because international trade in plant products and introduction of pathogens to new areas is likely to continue, the manner in which fungal pathogens are identified should urgently be reconsidered. The technologies that would provide appropriate information for biosecurity and quarantine already exist, yet the scientific community and the regulatory authorities are slow to embrace them. International agreements are urgently needed to enforce new guidelines for describing plant pathogenic fungi (including key DNA information), to ensure availability of relevant data and to modernize the phytosanitary systems that must deal with the risks relating to trade-associated plant pathogens.

This article is part of the themed issue 'Tackling emerging fungal threats to animal health, food security and ecosystem resilience'.

1. Introduction

Global travel and trade in food and fibre products have become a way of life and underpin the global economy. Current estimates of a world population of 9.1 billion people projected by 2050, changing diets and consumption patterns, and the increasing inability of some regions of the world to produce sufficient food for local consumption (www.fao.org) suggest that (i) intercontinental travel and trade of agricultural and forestry produce will increase and (ii) production of produce will need to become more efficient to meet ever increasing needs. Thus pre- and post-harvest losses, whatever the cause, will be increasingly intolerable.

Importing countries are vulnerable to accidental introductions of new and potentially devastating plant pathogenic fungi [1–3]. Quarantine systems, including trade restrictions from areas where pathogens occur, required treatment of goods and inspections for infected material are all intended to reduce this risk. Given the increase in travel, as well as the volume of trade around the world, including in live plants and fresh produce, the capacity to apply these systems is wholly inadequate, even in the most resourced countries such as the USA [4]. Furthermore, the rate at which goods are being moved
around the world is also increasing. For example, bananas grown in South America are served in European households within days of harvest and this is true for many other plant products globally. This implies that the window of opportunity to intercept, identify and act on a potential new invasion is minute at best.

Currently, applied quarantine systems are complicated by the fact that most well-known fungal plant pathogenic species are primarily known from a specific suite of disease symptoms and general morphology based on only a part of their life cycle [5]. Many pathogens remain undetected as latent infections in apparently healthy tissue and they are unlikely to be detected in routine inspections [6,7]. Furthermore, many of the fungi that cause major problems remain unknown or unnamed until well after they begin to cause major losses and will neither be sought nor detected in quarantine inspections. Once they are studied more intensively, especially where modern molecular tools are applied, many of the fungi that are detected are often found to represent species complexes that would earlier have passed unnoticed [8]. Pathogen detection that relies on visual plant symptoms and pathogen morphology is quite evidently unable to effectively cope with the threats posed by fungi found in traded plants or plant products.

Quarantine systems have traditionally relied on fungal names. These names are increasingly being shown as simplistic and ineffectual as representatives of the relevant information associated with a particular pathogen. Beyond the species level, knowledge relating to mating types, and even clones of particular pathogens, is crucially important when seeking to understand or manage fungal invasions [9,10]. The same is true for the variable presence or absence of small dispensable chromosomes carrying genes involved in pathogenicity, which can influence the ability of a species to infect a specific host, e.g. in *Alternaria alternata* [11] and *Fusarium oxysporum* [12]. To further complicate matters, quarantine lists with names as actionable organisms are often found only in inaccessible national databases or government publications. These are frequently not linked to relevant data; neither are they consistent with modern taxonomic treatments of the species in question.

Although it is unknown how many species of fungi occur on the Earth, and estimates range anywhere from 1.5 to several million [13–15], it is reasonable to conclude that the majority of species have not yet been seen or recorded [16,17]. Communication relating to these species by means of fungal names that remain largely linked to the phenotype, and detached from the genotypic, ecological and other data [16,18], is clearly insufficiently informative to deal with risks associated with increasing volumes and rates of trade in food and fibre. This approach also poses a serious threat to the global security of plant production and the environment alike. In this review, we consider several of these issues and approaches that could help to traverse seemingly unnecessary barriers to efficient identification procedures and management of fungal threats linked to global trade in agricultural and forestry products.

2. **One fungus, but which name?**

Scientific names remain the foundation of how we communicate regarding species of phytopathogenic fungi; also with regards to quarantine. Names are ideally linked to knowledge of the biology, distribution, ecology, host range, control and risks associated with fungal pathogens. The concept of pleomorphism relates to the fact that many ascomycetous fungi are known by either their sexual, asexual or synasexual morphs, to which different names have been attributed based on their morphology; commonly referred to as dual nomenclature [11,19,20].

In practice, this dual nomenclature has meant that a single fungus could be listed on the quarantine list of a country under any one of three valid names (e.g. apple scab caused by *Venturia inaequalis*, asexual morph *Fusccladium poni* or synasexual morph *Spilocaea poni* [21]). This is in a conservative scenario. Where known synonyms are considered, the list of names can be disturbingly long; all of which confuse the literature and they commonly persist in national quarantine lists. Other than having different names to contend with, quarantine officers are also faced with the difficult reality that many reported asexual–sexual relationships have never actually been experimentally confirmed and can also be incorrect.

Following the ‘One Fungus: One Name’ symposium, and the publication of the ‘Amsterdam Declaration on Fungal Nomenclature’, several radical changes were proposed to the code of nomenclature that governs the naming of fungi [19]. This subsequently led to the amendment of the International Code of Nomenclature for algae, fungi and plants (ICN) (Article 59) to abolish the use of dual nomenclature, as well as other sensible changes including registration of nomenclatural details of fungal novelties in databases such as MycoBank [22], the acceptance of electronic publication, and English (as alternative to Latin) descriptions of new names [19,20].

Moving to the application of single names for plant pathogens was strongly supported by the plant pathology community [5], which needed to have meaningful names for species associated with important plant diseases. The concomitant changes to the ICN code, together with the increased understanding of systematic relationships among fungi based on more representative DNA sequence-based phylogenies, have resulted in a large number of taxonomic revisions in recent years. While these name changes might have caused confusion for plant health and quarantine practitioners in the short term, the more accurate application of generic names based on DNA-based data will ensure longer-term stability in the use of names. Appropriate names of fungal pathogens are also important for fundamental plant pathology research, such as various ‘omics’ approaches aimed at understanding the mechanisms of plant–pathogen interactions through comparisons among related species. Past taxonomic treatments lead to confusion in this regard; for example, genome comparisons of *Mycosphaerella* that were later shown to represent members of different genera, e.g. *Zymoseptoria tritici* [23,24], *Pseudocercospora fijiensis* [25,26] and *Dothistroma septosporum* [27,28].

Unfortunately, many genera and species remain to be revised or they are devoid of critical data that would allow for accurate identification and phylogenetic placement. Between 2000 and 2013, 1833 fungal genera were described for which only 155 (8.4%) have type specimens linked to reliably annotated ITS nrDNA sequence data in public databases [29]. This implies that the number of newly described fungi lacking DNA data continues to increase rather than decrease. In an attempt to alleviate this problem, the ‘Genera of Fungi’ project was launched, with the aim of sequencing, restudying and/or recollecting the type species of genera of fungi, focusing on a subset of names that are currently accepted.
[29,30]. Furthermore, to help plant pathologists to know which
generic names they should apply to pleomorphic genera, com-
mittees under the auspices of the International Commission for
the Taxonomy of Fungi have been tasked with preparing lists of
accepted names recommended for use (e.g. [31,32]). These
names will be evaluated by the Nomenclature Committee for
Fungi, and formally accepted or not at the Nomenclature
Session of the 2017 International Botanical Congress to be
held in Shenzhen, Southern China.

Another major constraint to appropriate naming is the
general lack of appropriate, well-characterized reference spec-
imens and/or cultures of quarantine and related species in
publicly accessible collections (see review by [33]). Such bio-
logical resource centres are also under constant threat from
decreasing budgets and increasing costs. These invaluable
reference specimens and cultures are critically important for
the establishment of reliable identification systems. Their loss
would represent a huge impediment for future generations of
mycologists, plant pathologists and other end users.

The above-mentioned efforts to clarify the names of
fungal pathogens and link their phenotypes to genotypic
information are critical to ensure a useful framework for effi-
cient identification and communication of fungi. This is also
essential to ensure the continued discovery and characteriza-
tion of the millions of fungi that are estimated to remain
unnamed. It is, therefore, important that plant pathologists
take note of these efforts and support them with urgency.

3. Cryptic species, mating types and clones

A key question in considering global biosecurity is when to
stop considering a pathogen as an ‘actionable organism’, e.g.
when is it accepted as established in a country? It is important
to recognize that a species is not ‘one dimensional’ as is
suggested by a name on a list. By contrast, it represents a com-
plex or pool of different ‘sexes’ or mating types, virulence
factors and genes. All these influence its response to hosts,
environments and a wide range of other factors. Furthermore,
many pathogens represent cryptic species that pose a particu-
lar problem with regard to understanding invasion and
potential quarantine procedures (e.g. [8,34,35]).

Fungi can reproduce either asexually or sexually. In the later
case, they typically have two or more sexual mating types that
are needed for sexual recombination to occur [36,37]. Under-
standing these cycles is critical for disease management. This
is because it significantly affects the ability of fungal pathogens
to overcome resistance mechanisms of their hosts; with sexually
reproducing strains having an ecological advantage to infect and
invade [9]. Mating types should, therefore, have significant rel-
evance for the status of quarantine organisms. For instance,
invade [9]. Mating types should, therefore, have significant rel-
evance for the status of quarantine organisms. For instance,

4. Latent or endophytic fungal infections

Apart from systematic problems to identify fungal threats in
traded plants and plant products, biosecurity is also currently
unable to deal with the cryptic nature of fungal infections.
There are a great many plant pathogenic fungi that cause
latent infections. These represent a particularly difficult
challenge for international trade and associated quarantine
measures. Latent infections involve a parasitic relationship
between a pathogen and a host that might remain asympto-
matic for some period of time, even years, but that eventually
induces disease symptoms [41]. In this situation, a pathogen
remains latent until environmental or nutritional conditions
or the stage of maturity of the host or pathogen allow it to pro-
duce symptoms of disease [42]. A few pertinent case studies are
provided in figures 1–3.

The key issue illustrated by the three case studies (and
there are many others that could be used) is that many
genera include important plant pathogens that have a latent

bananas, which represent one of the important global staple
food crops, having evolved in the Indo-Malayan archipelago.
Panama disease, which is caused by *F. oxysporum* f.sp. *cubense*
(Foc), appears also to have originated in Southeast Asia [39].
Based on molecular studies, it appears that Foc is a haploid
asexual pathogen with a clonal population structure, and that
temporal and spatial dispersal of devastating disease linked
to Tropical Race 4 is actually due to a single clone [39]. If
additional clones were thus to move from Southeast Asia, the
disease would become even more difficult to manage because
a broader range of cultivars are likely to be affected. A further
complicating factor is that species in the *F. oxysporum* complex
also undergo horizontal gene and chromosome transfer as a
means for lineages to broaden their host range, and this can
influence their pathogenicity [40].

In some genera of phytopathogenic fungi, names mask
variation in host specificity and pathogenicity that is present
below the species level. Although such variation in plant
pathogenic fungi is often found to represent several cryptic
species, the opposite situation also occurs. One case in point
is the *A. alternata* species complex, to which the quarantine
species *A. mali*, causal agent of Alternaria blotch of apple,
and multi-gene analysis to reduce 35 *Alternaria* morpho-
species to synonymy under the older name, *A. alternata*. The
authors concluded that it is the presence or absence of the
gene cluster that codes for a specific toxin that is of quarantine
concern, and not necessarily a specific synonym of *A. alternata*.

McTaggart et al. [10] recently called for ‘gene-based biose-
curity’. They point out that our knowledge of genes that
underlie complex traits such as pathogenicity is growing expo-
nentially. Prediction of lifestyle (e.g. biotrophic versus
saprotophic), the presence of pathogenicity factors and other
elements in the genome are known to be linked to pathogen-
icity. It will still be some time before such information is
known for a sufficient number of fungi to rely solely on
genome scans. But it is relevant to consider the fact that such
an approach would allow much more predictive and preventa-
tive action than any name-based biosecurity system. Until
such information is available for all actionable quarantine
organisms, a more detailed identification system is urgently
needed and in many cases it is already feasible.

Based on molecular studies, it appears that Foc is a haploid
asexual pathogen with a clonal population structure, and that
temporal and spatial dispersal of devastating disease linked
to Tropical Race 4 is actually due to a single clone [39]. If
additional clones were thus to move from Southeast Asia, the
disease would become even more difficult to manage because
a broader range of cultivars are likely to be affected. A further
complicating factor is that species in the *F. oxysporum* complex
also undergo horizontal gene and chromosome transfer as a
means for lineages to broaden their host range, and this can
influence their pathogenicity [40].

In some genera of phytopathogenic fungi, names mask
variation in host specificity and pathogenicity that is present
below the species level. Although such variation in plant
pathogenic fungi is often found to represent several cryptic
species, the opposite situation also occurs. One case in point
is the *A. alternata* species complex, to which the quarantine
species *A. mali*, causal agent of Alternaria blotch of apple,
and multi-gene analysis to reduce 35 *Alternaria* morpho-
species to synonymy under the older name, *A. alternata*. The
authors concluded that it is the presence or absence of the
gene cluster that codes for a specific toxin that is of quarantine
concern, and not necessarily a specific synonym of *A. alternata*.

McTaggart et al. [10] recently called for ‘gene-based biose-
curity’. They point out that our knowledge of genes that
underlie complex traits such as pathogenicity is growing expo-
nentially. Prediction of lifestyle (e.g. biotrophic versus
saprotophic), the presence of pathogenicity factors and other
elements in the genome are known to be linked to pathogen-
icity. It will still be some time before such information is
known for a sufficient number of fungi to rely solely on
genome scans. But it is relevant to consider the fact that such
an approach would allow much more predictive and preventa-
tive action than any name-based biosecurity system. Until
such information is available for all actionable quarantine
organisms, a more detailed identification system is urgently
needed and in many cases it is already feasible.

Based on molecular studies, it appears that Foc is a haploid
asexual pathogen with a clonal population structure, and that
temporal and spatial dispersal of devastating disease linked
to Tropical Race 4 is actually due to a single clone [39]. If
additional clones were thus to move from Southeast Asia, the
disease would become even more difficult to manage because
a broader range of cultivars are likely to be affected. A further
complicating factor is that species in the *F. oxysporum* complex
also undergo horizontal gene and chromosome transfer as a
means for lineages to broaden their host range, and this can
influence their pathogenicity [40].

In some genera of phytopathogenic fungi, names mask
variation in host specificity and pathogenicity that is present
below the species level. Although such variation in plant
pathogenic fungi is often found to represent several cryptic
species, the opposite situation also occurs. One case in point
is the *A. alternata* species complex, to which the quarantine
species *A. mali*, causal agent of Alternaria blotch of apple,
and multi-gene analysis to reduce 35 *Alternaria* morpho-
species to synonymy under the older name, *A. alternata*. The
authors concluded that it is the presence or absence of the
gene cluster that codes for a specific toxin that is of quarantine
concern, and not necessarily a specific synonym of *A. alternata*.

McTaggart et al. [10] recently called for ‘gene-based biose-
curity’. They point out that our knowledge of genes that
underlie complex traits such as pathogenicity is growing expo-
nentially. Prediction of lifestyle (e.g. biotrophic versus
saprotophic), the presence of pathogenicity factors and other
elements in the genome are known to be linked to pathogen-
icity. It will still be some time before such information is
known for a sufficient number of fungi to rely solely on
genome scans. But it is relevant to consider the fact that such
an approach would allow much more predictive and preventa-
tive action than any name-based biosecurity system. Until
such information is available for all actionable quarantine
organisms, a more detailed identification system is urgently
needed and in many cases it is already feasible.
phase in their life cycles. This easily leads to unwanted introductions, further complicated by the fact that these pathogens frequently also have wide host ranges and thus spread throughout local plant communities. The only way to overcome this problem is through the application of molecular-based detection. DNA barcoding technologies and data sharing abilities for such an approach already exist [54,55], but are not used widely yet for quarantine purposes. For this goal to be realized, much work is required to firstly provide a solid taxonomic framework (as discussed above). And there will be a need for human capacity development within quarantine structures to utilize this information.

5. Conclusion
Global trade in plant products faces major challenges related to fungal pathogens that threaten food and fibre security, as well as ecosystem health. Unfortunately, these challenges are exacerbated by inefficiencies in the systematic and physical identification of fungi, which is due to the reliance on outdated taxonomic information and systems, as well as our inability to recognize the cryptic fungal infections. Given the enormity of the risks, it is unfortunate that there is a general lack of global urgency to incorporate already existing tools to deal with them. These tools would make it possible to implement a barcoding-based information and identification system to screen plants and plant products that are traded internationally.

One of the major issues that hamper progress towards an effective DNA-based barcoding system for biosecurity is the present ICN, which governs the naming of fungi, and essentially allows plant pathogenic fungal species to be described without DNA data. This leaves researchers and practitioners trying to play ‘catch-up’ at huge additional cost, having to recollect isolates to provide molecular data for previously
described plant pathogens. A potential remedy would be for the International Commission for the Taxonomy of Fungi to implement a set of guidelines that authors, editors and reviewers could follow to ensure that, wherever possible, relevant genotypic data are provided to supplement novel species descriptions of suspected or known plant pathogens. The current absence of such guidelines hampers both progress and the application of broadly accepted best practices in fungal identification and description. This is not only to the detriment of mycology, but also of global food and fibre production and ecosystem health.

A major constraint to effective plant quarantine is the poor linkage between resources that carry layers of information regarding plant pathogens. Unfortunately, there is a general lack of support to maintain and link databases such as Q-bank (http://www.q-bank.eu/), MycoBank (http://www.mycobank.org/), Index Fungorum (http://www.indexfungorum.org), UNITE (https://unite.ut.ee/), GenBank (http://www.ncbi.nlm.nih.gov/) and the ARS-USDA fungus–host distribution database (http://nt.ars-grin.gov/fungaldata bases/fungushost/fungushost.cfm), to name but a few. Each of these databases includes unique information about species, their identification, strains, hosts and much more. Linking them, and supporting their expansion, appears to be ‘low-hanging fruit’ from a global quarantine and plant health management perspective. Doing so would immediately unlock large volumes of data for important pathogens globally. Only specialists who understand the intricacies of navigating this maze of data resources can currently access much of this information.

As handy as DNA barcodes can be as tools for species recognition, the real value of these data collections will emerge once the fungal genomes have been analysed and linked to function, e.g. using secondary metabolites to infer ecology, the identification of pathogenicity factors, transposable elements, as well as life cycle and population structure [10,56]. There is a growing realization that not only future biological studies but also future quarantine and management systems will be reliant on this information. If we are serious about reducing the impact of fungal pathogens on trade in food and fibre, a fundamental change in how we operate will be required. Names, morphology...
and visual inspection for fungal pathogens are simply not sufficient to deal with the problem.

Competing interests. We declare we have no competing interests.
Funding. We received no funding for this study.

References

5. Blackwell M. 2011 The fungi: 1, 2, 3 and visual inspection for fungal pathogens are simply not sufficient to deal with the problem.
Dothistroma needle blight pathogens. 


