Determining the Pathogenicity of 13 Fungal Species with Respect to Their Required Containment Measures

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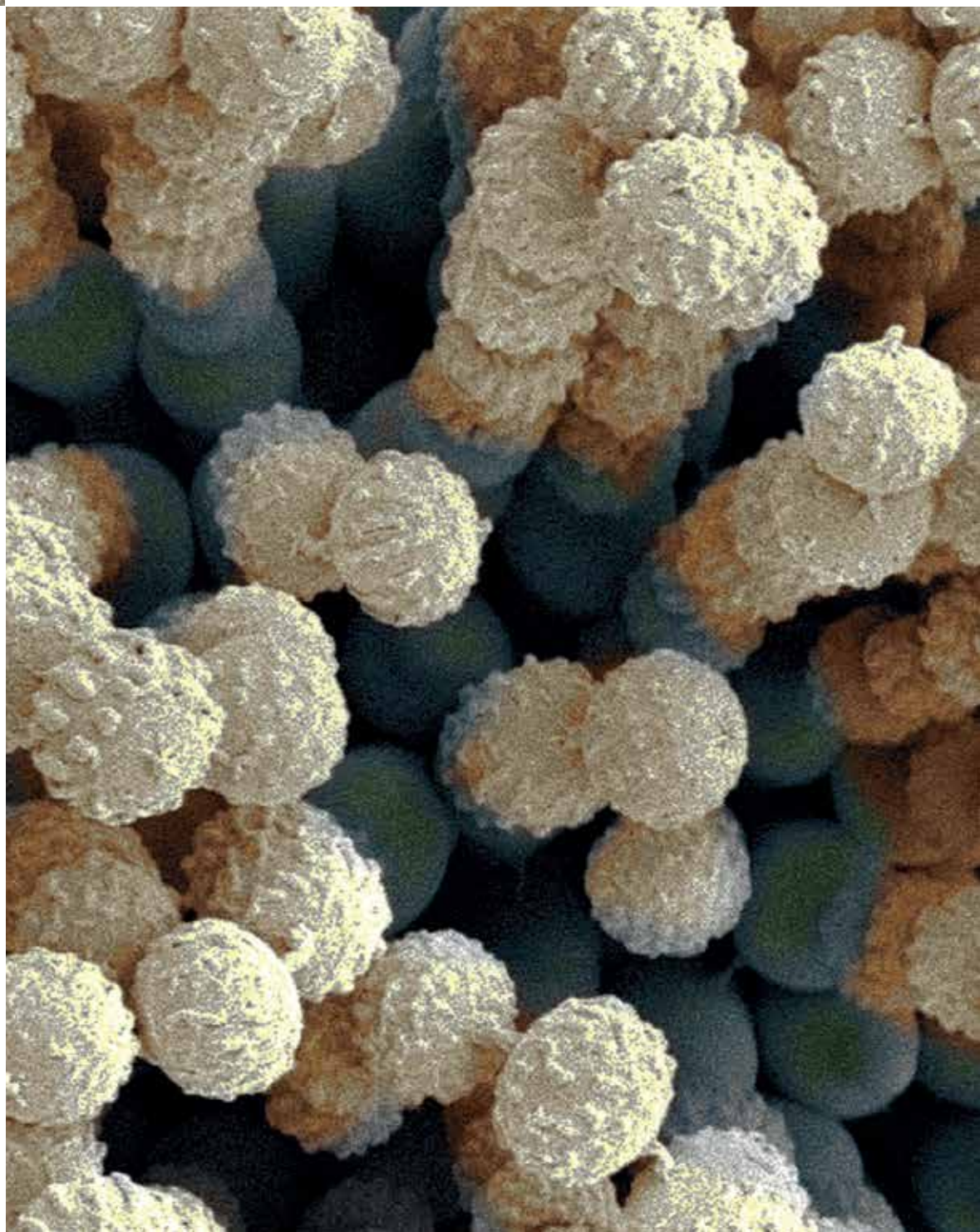

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ONDERZOEKSRAPPORT

Determining the pathogenicity of 13 fungal species with respect to their required containment measures

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Cover Photo

This is a close-up of a spore carrier from the fungus *Aspergillus niger*. The spores are the round yellow-brown coloured cells. The blue coloured cells are phialides, specialized spore-forming cells. This picture has been taken with a cryo-scanning electron microscope. The raw black-white picture has been artistically coloured to create this image. A spore (conidium) has a diameter of about 3-4 µm (one thousandth millimetre).

Photographer and designer: Jan Dijksterhuis, Westerdijk Fungal Biodiversity Institute, Utrecht

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Preface

Fungi are important biological resources for enzymes and secondary compounds with a potential application in the bio-based economy. Several fungal species are however pathogenic to man, animals, plants or other microorganisms and so the research on these species requires containment measures in order to protect laboratory workers and the external environment. COGEM advices the Dutch Government on the classification of fungi with respect to the required containment measures for organisms that are genetically modified. To do so properly, information on the inherent pathogenicity of fungal species is necessary. In previous COGEM reports a large number of species were screened for their possible ill-effects to human health, and so-called postharvest diseases were screened for their potential to cause disease in plants before harvest. In these studies, a number of fungal species remained for which a possible negative effect on plants, invertebrates or other fungi (especially mycorrhizal fungi and mushrooms) could not be excluded. The present report considers these thirteen species.

The authors have conducted a thorough review of the scientific literature, ending in a scoring table for each species. This approach demonstrates how information from the primary literature can be conveyed in a succinct yet very transparent manner. At the same time, this provides an excellent basis for weighting the various arguments underlying pathogenicity classification. The methodology applied in this report is worthwhile to consider as a general model for similar future studies.

A factor complicating this study is the rapidly changing classification. Many fungi are known under more than one taxonomic name, depending on their life stage. In some cases a group of species has been assigned to new genera or a species was split into several new ones. Due to such taxonomic revisions the correspondence between older and modern literature is sometimes equivocal. In this report the authors have ruled out any confusion by noting the old names as well as the new ones and by consulting mycologists specialized in certain groups.

The supervisory committee for this project trusts that the report constitutes an excellent scientific basis for COGEM to classify the thirteen species of fungi. It also provides an interesting new methodology for evaluating pathogenicity of microorganisms that has a possible wider relevance.

Nico M. van Straalen
Chair of the supervisory committee
Chair of the Agriculture Subcommittee of COGEM
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Summary

It is important to regulate the application of fungi used in relation to genetic modification on the basis of sound knowledge about their potential pathogenicity. The commission on genetic modification (COGEM) is the primary body in the Netherlands to advise on the pathogenicity of GMOs, and this advice forms the basis for the GMO office to determine the containment level of working in the laboratory with these fungi. Thus, COGEM requires the key information on which to base the pathogenicity class into which the organism is placed, and if required, commissions research to gain this information.

The objective of this study is to determine the pathogen class into which 13 fungal species should be placed, on the basis of a thorough literature review. These fungi came from an earlier report, commissioned by COGEM (CGM 2015-06), on the potential of fungi and bacteria that are present on the list of non-pathogenic organisms (CGM/141218-03 and CGM/141218-01 resp.) of COGEM to cause post-harvest disease. These 13 fungi were indicated to be potentially pathogenic, and it was determined that further research was necessary.

We report on the results from a literature search on the potential for pathogenicity on plants, as well as on other fungi (in particular mushroom- and mycorrhiza-forming fungi), arthropods and nematodes. Since a review of fungal pathogens of humans and animals was conducted in 2011 (CGM 2011-08), these target hosts are not included in this review, although - when we came across information on these groups - we did briefly report on them. In addition, large changes in fungal taxonomy have occurred in recent years, and several of the fungi on the list have been subjected to name changes. We also investigated this and report on the most current names for these fungi as well as the reasons behind these changes.

Three fungi have been renamed, and for one there were contradictory reports regarding its nomenclature. One of the species consisted of four varieties, which have recently been redefined as four different species. Taking on the new taxonomy, we found sufficient evidence for ten fungi to consider them to be pathogenic on one or multiple hosts. In this group of ten fungi, pathogenicity towards plants was found eight times, towards fungi two times, and once each towards nematodes and arthropods. For the remaining three fungal species, we found either no or insufficient evidence to consider them pathogens.

Samenvatting

Het is belangrijk om de biologische soorten die gebruikt worden als genetisch gemodificeerde organismen (GGOs) te reguleren. In Nederland is de commissie genetische modificatie (COGEM) het primaire orgaan dat advies geeft over de pathogeniteit van GGOs. Het advies dat gegeven wordt vormt de basis voor het bureau genetisch gemodificeerde organismen (GGO) om het inperkingsniveau van deze organismen te bepalen. Hiervoor heeft de COGEM goed onderbouwde informatie nodig en, indien nodig, schrijft zij hiervoor onderzoek uit.

Het doel van deze literatuurstudie is het bepalen van de pathogeniteitsklasse van 13 schimmelsoorten. Deze soorten kwamen voort uit een eerder onderzoek, uitgevoerd in opdracht van de COGEM (CGM 2015-06), waarin beoordeeld werd of schimmels en bacteriën die op de lijst van niet-pathogene organismen (CGM/141218-03 en CGM/141218-01 respectievelijk) voorkwamen in staat waren om bewaarziektes in plantmateriaal te veroorzaken. Uit dit onderzoek kwamen 13 schimmels naar voren waarbij er indicaties waren dat ze mogelijk pathogeen waren, maar waarvan beoordeeld werd dat hiervoor verder onderzoek nodig was om uitsluitsel te kunnen geven.

We vermelden de resultaten van een literatuurstudie naar de eerder genoemde schimmels over hun mogelijke pathogeniteit voor planten, andere schimmels (met name paddestoelen en mycorrhiza), nematoden en arthropoden. In 2011 is een lijst met schimmels die pathogeen zijn voor mens en dier opgesteld (CGM 2011-08), en derhalve zijn deze groepen niet in deze literatuurstudie meegenomen. Indien er aanwijzingen waren voor pathogeniteit voor mens en dier is dit echter wel gemeld. Aangezien er de afgelopen jaren een grote verandering in de taxonomie en classificatie van schimmels heeft plaatsgevonden is er eveneens gekeken naar de huidige stand van zaken met betrekking tot de naamgeving van deze schimmels en zijn de redenen voor eventuele naamsveranderingen vermeld.

Bij drie schimmels heeft er een naamsverandering plaatsgevonden, voor één waren er tegenstrijdige rapporten ten aanzien hiervan. Één soort bestond uit vier variëteiten, die recentelijk tot verschillende soorten benoemd zijn. Van tien schimmels werd er voldoende bewijs gevonden om te stellen dat ze ziekteverwekkers zijn op ten minste één gastheer. Binnen deze groep van tien schimmels werd pathogeniciteit voor planten achtmaal, voor schimmels tweemaal, voor nematoden eenmaal en voor arthropoden eenmaal gevonden. Van de overige drie schimmels is er geen of onvoldoende bewijs gevonden voor pathogeniteit.

List of abbreviations and definitions

COGEM	Commission on Genetic Modification
EPPO	European and Mediterranean Plant Protection Organization
GMO	Genetically modified organism
gpdh:	Glyceraldehyde 3-phosphate dehydrogenase *Gene encoding the glyceraldehyde 3-phosphate dehydrogenase protein, sometimes used as a secondary marker for fungal identification*
ICTF:	International Commission on the Taxonomy of Fungi, www.fungaltaxonomy.org/
IF:	Index Fungorum, http://www.indexfungorum.org
ITS:	Internal Transcribed Spacer (1 and 2) *The regions between the genes encoding ribosomal RNA. These regions are located between the small subunit and 5.8S (ITS1) and between the 5.8S and large subunit (ITS2) RNA genes. ITS1 and ITS2 are highly variable, and serve as a primary marker for fungal identification (Fig. 1)*
MB:	Mycobank, http://www.mycobank.org
LSU:	Large subunit of the ribosome, containing ribosomal RNA *LSU rRNA gene: gene encoding the RNA that combines with proteins to form the large (60S) ribosomal RNA subunit (Fig. 1)*
OTA	Ochratoxin A
RPB1:	RNA polymerase II *DNA-directed RNA polymerase II subunit rpb1, the RPB1 gene is sometimes used as a secondary marker for fungal identification*
rRNA:	Ribosomal RNA *Ribosomal RNA complex that combines with proteins to form the two subunits of the ribosome, i.e. the large subunit (LSU) and small subunit (SSU) (Fig. 1)*
SSU:	Small subunit of the ribosome, containing ribosomal RNA *SSU rRNA gene: gene encoding the RNA (18S) that combines with proteins to form the small (40S) ribosomal RNA subunit (Fig. 1)*
tef-1:	Translation Elongation Factor EF-1 α *tef-1 gene: gene encoding the translation elongation factor protein, sometimes used as a secondary marker for fungal identification*
UAFD:	United States Department of Agriculture, Agricultural Research Service, Fungal Database
WFBI:	Westerdijk Fungal Biodiversity Institute, Royal Netherlands Academy of Arts and Science (formerly known as the CBS-KNAW)
WoS	Web of Science, scientific citation database website link

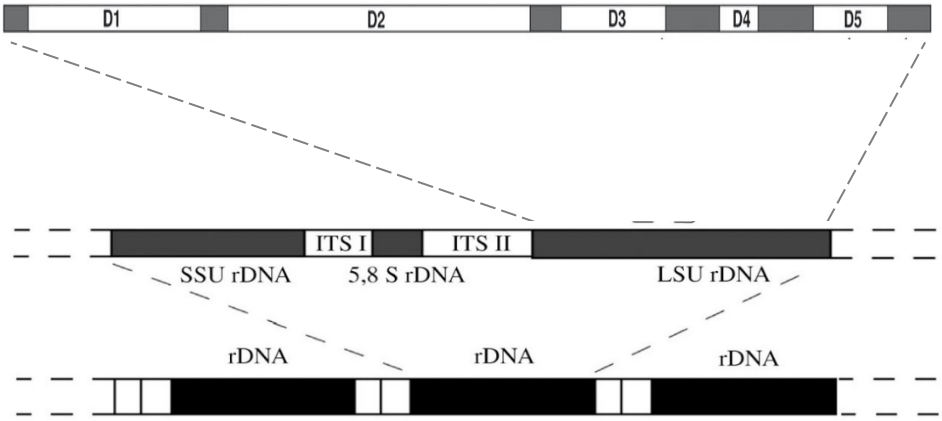

Figure 1: Ribosomal RNA gene region, including the small subunit (SSU), large subunit (LSU, with the D-regions D1 through D5) and the 5.8S RNA genes and the internal transcribed spacer regions ITS 1 and ITS 2 (Adapted from Wylezich *et al*. 2010 and Markmann and Tautz 2005). rDNA: ribosomal RNA gene.

1 Introduction

1.1 Background

To allow to work safely with genetically modified organisms (GMOs), containment measures may be necessary. The level of containment is dependent on the degree of risk that organisms pose towards human health and the environment and an important aspect of this is the determination of the pathogenicity of the organisms which will be genetically modified. In the Netherlands, the Commission on Genetic Modification (COGEM) is the primary body to advise on the pathogenicity and the pathogen classes into which GMOs should be placed. This advice in turn guides the decision by other government agencies that determine the level of containment necessary for working with GMOs. The roles of COGEM are laid down in the Environmental Protection Act (http://wetten.overheid.nl/BWBR0003245/2017-01-01).

COGEM conducts and commissions research to assess the pathogen status of organisms and publishes lists of non-pathogenic and pathogenic organisms. A number of lists of pathogenic and non-pathogenic bacteria and fungi were published in 2011, and the last actualisation of these lists was published in 2014 (CGM/141218-01 and CGM/141218-03).

The current report has been drafted following the results from a previous COGEM study (CGM 2015-06), whereby a screening was conducted on the classification of (post-harvest disease /plant pathogenicity) of bacteria and fungi classified as non-pathogenic by COGEM in 2014. Several fungi were reclassified as a result of that study, but the committee was unable to draw a firm conclusion based on the presented evidence, for 13 of these fungi (Table 1.1). There were, however, indications that these fungi might be pathogenic towards plants, other fungi, nematodes and/or arthropods. Therefore, to be able to place these fungi into either a pathogen or non-pathogen class, COGEM commissioned the current literature research. The pathogenicity of these fungi towards humans and animals was investigated in an earlier study (CGM 2011-08).

Hence, the goal of the current study was to determine whether these 13 fungi have the potential to cause disease in plants, fungi (emphasis on mushrooms and mycorrhiza), nematodes and arthropods.

In this report, we first give a brief introduction into pathogenicity and an overview of methods used to determe pathogenicity, followed by the approaches we used to find and assess the literature. We then present the results from the literature review, per fungal species, and finally we present an overview of our findings.

Table 1.1: List of fungi assessed in the current study. This list was based on group B in the table in COGEM report CGM/151126-01, and gives the fungi along with the groups of organisms to which they were deemed to be potentially pathogenic in the aforementioned COGEM report.

Species	Potentially pathogenic towards:
Acremonium strictum	plants, fungi
Aspergillus niger	plants
Aureobasidium pullulans	plants
Bipolaris spicifera	plants, humans
Bjerkandera adusta	plants
Cladosporium herbarum	plants
Clonostachys rosea	insects, nematodes
Dichotomophthora portulacae	plants
Nigrospora sphaerica	plants
Phoma herbarum	plants
Plectosporium tabacinum	plants
Trichoderma koningii	plants
Trichoderma viride	plants, fungi, arthropods, nematodes

1.2 Identification and characterisation of fungi

Accurate characterization of fungal pathogens relies on correct identification. Confusion about naming can cause unnecessary control measures or, alternatively, insufficient control. Fungal taxonomy and nomenclature has been subjected to major changes in the past number of years. Since the asexual (anamorph) and sexual (teleomorph) stages of the same fungal species can differ morphologically, it has been common practice to assign different Latin binomial names to these different stages. At the 18th International Botanical Congress in Melbourne, Australia, in 2011, it was agreed that this dual naming system would be discontinued per 2013, and the "one fungus, one name" principle was introduced into the International Code of Nomenclature for algae, fungi and plants (http://www.iapt-taxon.org/nomen/main.php). An important consequence of this change is the merging of anamorph and teleomorph names. The code stipulates that teleomorph names are given preference, but priority can be given to anamorph names if they are better known or belong to a genus with a larger number of species.

There is currently a strong drive to use molecular (DNA-based) methods for identification and taxonomical goals. However, before molecular analysis became common practice, fungi were generally identified and classified based on morphological features. Current practice is to employ a polyphasic approach, by using a combination of morphological identification, DNA sequencing and ecological data (Crous *et al*., 2015). In fact, several of the plant pathology journals, such as "Plant Disease" or the "Australian Journal of Plant Pathology", will no longer publish descriptions of new diseases or disease-causing organisms without molecular identification (Prof. P. Crous, pers. comm.). Often, sequencing data from a number of different genes or gene regions (markers), such as those for (mitochondrial or nuclear) ribosomal RNA and their intervening regions, as well as protein-encoding genes, are used to infer phylogenies. These markers are ideally short standardized regions of between 400 and 800 base pairs which are identical/similar within a species, but sufficiently different from those of other species, to allow differentiation (Kress and Erickson, 2008). Based on research by Schoch *et al*. (2012), the International 'Barcode of Life' project has selected the internal transcribed spacer (ITS) region of the rRNA gene cluster to be the primary marker for fungal identification. However, there is sometimes insufficient species-level resolution using the ITS region, and therefore a secondary marker is used to completely separate species within a genus or clade. Commonly-used markers include the D1/D2 region of the large subunit (LSU) ribosomal RNA (Fig. 1), the gene *tef-1* which encodes the translation elongation factor EF-1 α protein and the gene *rpb1* which encodes the largest subunit of RNA polymerase II (RPB1) (for a comprehensive overview, see Xu *et al*. 2016).

1.3 Pathogenicity

1.3.1 Definition and use of the criterion

Pathogenicity is generally defined in the scientific literature as the ability of an organism to produce disease in a previously healthy host. However, this is a broad concept, and, in reality, determining pathogenicity is not always straightforward. In this section, we give an overview on how the pathogenic nature of an organism is determined.

Determining pathogenicity

The determinative method that defines whether an organism is a pathogen or not is classically based on the fulfillment of Koch's postulates. These consist of four different criteria an organism must comply with before it can be considered to be pathogenic:

1. The microorganism must be found in abundance in all host organisms suffering from the disease, but should in principle not be found in similar numbers in healthy organisms.

2. The microorganism must be isolated from a diseased organism and grown in pure culture.

3. The cultured microorganism should cause disease when introduced onto the healthy host organism.

4. The microorganism must be reisolated from the inoculated, diseased host organism and identified as being identical to the original specific causative agent.

Although Koch's postulates provide, in most cases, a robust way to determine whether a microorganism is causally related to a specific disease, a number of problems is associated with them. First, pathogenicity is not always clear-cut and so its unequivocal detection is difficult. For example, pathogens can be latently present in apparently healthy hosts and become virulent only under particular environmental conditions, or in combination with other species (so-called [disease-causing] species complexes). Second, it is difficult to determine the causal relationship of organisms with disease for certain obligate pathogenic fungi, as these are often not culturable. In those cases, this makes fulfillment of Koch´s second postulate impossible. On the other hand, there are so-called opportunists, which appear to be pathogens, but can cause disease only in hosts with an impaired or weakened immune system. Another particular case is formed by opportunists that can thrive and be deleterious once inside a plant, but do not have the capacity to access it. We here use the definition that such organisms, sensu strictu, are not to be considered pathogenic on the respective host.

So, while meeting all of Koch's postulates indicates whether an organism can cause disease, failure to meet all of them does not necessarily preclude this. Therefore, in this report, we will consistently indicate it when all of Koch's postulates have been met, and the manner in which they were met (i.e. on intact or injured plants). However, for reasons of precaution, we will not consider it to be the only method that can be used to determine the pathogenicity of a target fungus (see section 2.3 for a full description of the methods we used).

Pathogen classes

COGEM adheres to the pathogen classes described in the Dutch regulations on genetically modified organisms (Regeling genetisch gemodificeerde organismen milieubeheer 2013, http://wetten.overheid.nl/BWBR0035072/2017-01-01), which places micro-organisms into four different classes, denoted class I through IV, based on their (progressively increasing) pathogenicity and the level of threat:

Class I: A micro-organism is placed in this class if it complies at least with one of the following conditions:

a. it does not belong to a species of which representatives are known to be pathogenic for humans, animals or plants

b. it has a long history of safe use under conditions without any containment measures

c. it belongs to a species that includes representatives of class 2, 3 or 4, but the particular strain does not contain the genetic material that is responsible for the virulence

d. it has been shown to be non-virulent through adequate tests

Class II: This class includes micro-organisms that can cause disease in plants or those that can cause disease in humans or animals but whereby it is unlikely to spread within the population while an effective prophylaxis, treatment or control strategy exists.

Class III: The micro-organisms in this class can cause a serious disease in humans or animals and are likely to spread within the host population but where an effective prophylaxis, treatment or control strategy exists.

Class IV: A micro-organism is grouped in Class 4 when it can cause a very serious disease in humans or animals whereby it is likely to spread within the population, while no effective prophylaxis, treatment or control strategy exists.

Since the current report focuses solely on whether the 13 listed fungi are pathogenic to plants, arthropods, nematodes or fungi (with emphasis on mushrooms and mycorrhiza), we will primarily focus on determining whether these fungi are to be considered pathogenic on these hosts, whilst refraining from allocating them into the aforementioned classes. In addition, potential pathogenicity towards humans or animals will be noted if encountered.

1.3.2 Variation in pathogenicity

Fungal species are generally placed into three discrete ecological functional groups: (1) saprotrophs, (2) mutualists or (3) pathogens. However, many species do not fall into just a single group. Often, their life style spans two (or occasionally all three) groups, depending on external factors. One example of this are the necrotrophs, which kill their hosts (=pathogen) in order to feed on the dead organic matter that becomes available (=saprotroph). As a result of these dual roles, there can be considerable variation in the expression and initiation of pathogenicity. In addition, while many fungi are obviously pathogenic on particular hosts, there are many others whose putative pathogenicity is less well defined. This can be for a multitude of reasons and can depend on many different factors. For instance, a species can contain both non-pathogenic and pathogenic strains, the latter – if understood - based on extra virulence factors. Novel research, e.g. with *Fusarium*, shows that within such fungal species, whole choromosomes can move horizontally and such chromosomes often harbour virulence and host specificity genes. In addition, potential pathogenicity can become evident only in particular conditions or in particular (susceptible) hosts. In the following section, we give an overview of the variability in pathogenicity in fungi.

Opportunistic fungi

Opportunistic fungi are not strictly pathogenic, but rather rely on strategies that allow them to become invasive in or on other organisms in cases where such potential hosts offer colonisable interior parts by damage, are immunocompromised or are already diseased by other organisms.

A particular type of opportunist is exemplified by organisms that cause post-harvest disease in plant tissue or fruits. Post-harvest disease is generally defined as an infection of, and growth in, plant material that results in spoilage, caused by microorganisms. Post-harvest diseases are responsible for large financial losses all over the world, with between 19 and 38 % of global production of fresh fruit and vegetables lost yearly (FAO, 2011). In a previous COGEM report (CGM 2015-06), the authors argued that organisms that cause (true) postharvest disease, i.e. if they do not attack live plants, should be considered to be non-pathogenic, since this behaviour does not interfere with the life cycle of the plant.

The interplay between host and pathogen in determining pathogenicity

Disease occurs as a result of the interplay between pathogen and host and, as such, both determine to a degree the outcome of their interaction. Host specificity is an important aspect to consider when determining the pathogenic nature of organisms. Many pathogens do not cause disease in all hosts they come into contact with, but rather are limited to just one or a few species, or to a functional group of species. This can make it difficult to determine pathogenicity, as not all potential hosts can be tested in a study, or have been studied. In addition, pathogens often only cause disease during particular growth stages. While some diseases are common in seedlings or juveniles, others are typical of mature organisms.

The immune status of a host is also important in determining the expression of pathogenicity. Plants, fungi, insects and basal multicellular organisms rely primarily on their innate immune system, which provides immediate defence against infection, but occurs only at the cellular level and offers no long-term protection against disease. When an organism's immune system is compromised, this affects its ability to defend itself against pathogens, leading to an increased susceptibility to harmful organisms, including those that do not normally cause disease. However, fungi that critically depend on host damage to invade tissues (observed only incidentally) and that otherwise are mere 'outsiders', are defined here as being intrinsically non-pathogenic.

An interesting group of fungi that have a close relationship with their hosts are the so-called endophytes. These organisms reside inside healthy individual plants without causing apparent symptoms of disease. In fact, in many cases endophytes can offer benefits to their hosts, for instance by changing disease expression and/or progression (Busby *et al*. 2016), and there are many examples whereby endophytes are proposed as biocontrol agents, in particular for plants. However, many species described as endophytes may be opportunistic or latent pathogens tolerated by the host (Sanz-Ros *et al*. 2015) and many of the mechanisms required for endophytic behaviour are shared with pathogens (Kogel *et al*. 2006). Interestingly, the same fungal species is sometimes listed as a pathogen, in a particular context, while at other times it is described, or even used, as a (beneficial) endophyte.

The effect of environmental conditions on pathogenicity

Another factor that can make it difficult to determine pathogenicity is that different environmental conditions can lead to different ways in which fungi function. Thus, under one set of environmental conditions, a particular fungus may be harmless, while under a different set of conditions it will cause disease. Often, suboptimal environmental conditions may lead to reduction in the host defence status, resulting indirectly in disease. However, environmental conditions can also directly result in a change of fungal behaviour. An interesting case study is that of the common tropical tree *Iriartea deltoidea* and its associated fungus *Diplodia mutila* (Álvarez-Loayza *et al*. 2011). This fungus is generally found as an endophyte in mature plants, causing no negative effects, but it can on occasion cause disease in seedlings. Seedlings were found to occur primarily in shaded areas. The authors showed that high light conditions triggered pathogenicity of the endophyte, while low light favoured it to remain endosymbiotic. As a result, recruitment of endophyte-infested seedlings was restricted to the shaded understory by reducing seedling survival in direct light. This example highlights the influence that the environment can have in triggering infection.

Disease complexes

Disease is not always due to a single causal agent acting in isolation, but rather to a complex of organisms that work synergistically to cause harm to the host (Lamichhane and Venturi, 2015). One example of fungal complexes is given by the co-occurrence of up to six fungal types (*Trichoderma* sp, *Penicillium* sp., *Pyrenochaeta indica, Fusarium moniliforme, F. graminearum* and *F. oxysporum*) in root and stalk rot of maize (Lamichhane and Venturi, 2015; Ramsey 1990). In this example, the increase in

the level of complexity of pathogenicity confounds the adherence of potential pathogens to Koch's postulates greatly.

1.3.3 Molecular basis of pathogenicity

With the advent of new sequencing technologies, entire genomes of virtually any organism can now be sequenced relatively quickly and cheaply, adding to our understanding of their ecological and lifestyle capabilities. Following the sequencing of the *Saccharomyces cerevisiae* genome in 1996 (Goffeau *et al*. 1996), the full genomes of around 850 fungi have been, or are in the process of being, sequenced to date (GOLD database, https://gold.jgi.doe.gov/). The species that have been sequenced tend to be biased towards those utilised in the biotechnological, pharmaceutical and food industries.

Knowledge of whole fungal genomes enables a better classification if they can be linked to lifestyle determinations, but there are still large knowledge gaps both in our understanding of the functioning of genes and how they relate to the host's ecological role. Thus, we are still far from being able to reliably distinguish between pathogenic and non-pathogenic species using this method, but we foresee that in the future it could be an excellent way to make this distinction.

However, identification of fungal pathogenicity does not necessarily require whole-genome sequencing, as knowledge about particular genes or gene systems in fungi also allows us to identify potential pathogenicity. These pathogenicity genes can be broadly subdivided into two main categories: virulence genes and genes coding for mycotoxin production. While the presence of such virulence or mycotoxin genes does not necessarily guarantee that a species is or will be pathogenic, it does give an indication of its potential for it and as such can provide us with additional evidence for determining pathogenicity.

Virulence or pathogenicity genes

Virulence genes encode factors that enable organisms to become invasive to susceptible host organisms and thus contribute to disease. Often, pathogenic organisms carry whole arrays of virulence genes that together allow it to be optimally invasive. This has been thoroughly studied in bacterial pathogens like *Escherichia coli*. Interestingly, we find similarities in fungi. Van der Does and Rep (2007) indicated that the ability of fungi to cause disease in plants may have arisen multiple times during evolution. Often, it depends on specific genes that distinguish virulent fungi from their sometimes closely related non-virulent relatives (e.g. the *PEP* and *PDA* genes in *Nectria haematococca [Fusarium solani]* are required for pathogenicity towards pea). These genes thus encode host-determining "virulence factors," including small, secreted proteins and enzymes involved in the synthesis of toxins. These virulence factors are often involved in evolutionary arms races between plants and their pathogens. Thus, there are cases of organisms in which one type is a commensal, whereas others are clearly pathogenic based on a change, which can even be a mutation to virulence in a single gene (Freeman and Rodriguez, 1993).

Mycotoxin genes

Mycotoxins are biologically-active secondary metabolites that exhibit toxic properties, leading to suppression of the immune system. They can cause fetal abnormalities and stillbirths and have been linked to various forms of cancer. Species within the genera *Aspergillus*, *Penicillium* and *Fusarium* are responsible for the production of the approximately 400 currently known mycotoxic compounds (Xiong *et al*. 2017). Of these, the most toxic for mammals are aflatoxin B1, ochratoxin A and fumonisin B1 (Reddy *et al*. 2009). Considering that many mycotoxins are chemically stable and do not degrade during food processing, when they occur on or in crops and food products they can cause serious harm in humans and animals. The COGEM report "Mycotoxins and assessment of environmental risks in laboratory conditions in The Netherlands" (CGM/ 2013-01) gives a comprehensive overview of many of the aspects of mycotoxin production, including the genetic background, and the level of containment necessary for safe work with mycotoxin producing organisms.

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2. Methodology

2.1 Goal

The goal of the current study is to determine whether the fungi listed in table 1.1 have the potential for causing disease. Specifically, we reviewed the pathogenic potential of these 13 fungi towards:

- plants,
- arthropods,
- nematodes or
- other fungi (emphasis on mushroom-, mycorrhiza-forming fungi)

The examination of pathogenicity towards humans and animals other than arthropods and nematodes was beyond the scope of this report, as this has in part been performed previously (CGM 2011-08, Boekhout, 2011).

In addition, given the recent changes in fungal nomenclature mentioned in section 1.2 and the uncertainties in former fungal classifications, we also included a study into the current and past names of these 13 fungi, so that we could fully incorporate all regularly used names in the literature into our searches.

2.2 Fungal nomenclature

All of the species examined in this report were discovered a long time ago, and the majority of them have been subjected to numerous name changes over the years. In this report, we mainly focus on the recent changes (if any) in nomenclature that have occurred, and list only the most commonly used synonyms.

To examine the current nomenclature for all 13 species, we used two of the databases assigned by the Nomenclature Committee for Fungi to be a repository of fungal names: Index Fungorum (IF) and Mycobank (MB). IF is an international project, which is currently based at the Royal Botanic Gardens, Kew (UK) and aims to provide an index of all scientific names in the Fungal Kingdom (http://www.indexfungorum.org). MB is an on-line database provided by the International Mycological Association and is aimed as a service to the mycological and scientific community by documenting new names and name combinations of fungi (http://www.mycobank.org). Since new species descriptions or renaming of old ones can be submitted to either database, we submitted each of the 13 fungal species' names to each database. We also used Google and Google Scholar searches and the web-based literature databases Web of Science (WoS) and PubMed to gather background information on current standards in nomenclature and to gauge what the scientific support is for any name changes. Several of the fungi in this study have either been renamed, or are in the process of being renamed, as a consequence of the new naming strategy (see section 1.2) or as a result of recent research on the classification of these species. When we performed literature searches we took these changes into account (see section 2.3 for further information).

2.3 Pathogenicity

The majority of the literature on the pathogenicity of each fungus was found through Web of Science (WoS) as well as PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), whereas some references were found using Google scholar and Google. While information that was obtained from the main Google page often consists of literature that has not gone through peer review, and therefore needs to be viewed with caution, it can yield important additional information that the more formal channels can not.

The search terms used were as follows:

- fungal species name, either alone or in combination with
- pathogen*, parasit*, disease, virulence, predator, insect, arthropoda, nematod*, mushroom, agaricus, mycoparasit*, mycotoxi*, mycorrhi*

In cases where large numbers of search results were obtained, we limited our search to the most recent five years. We also consulted Prof. P. Crous, leader of the Phytopathology Research group and Dr. J. Houbraken, leader of the Applied and Industrial Mycology group, both experts from the Westerdijk Fungal Biodiversity Institute (WFBI, http://www.cbs.knaw.nl) for assessment of the pathogenicity of the fungi. Prof. Crous and Dr. Houbraken also recommended to consult the USDA-ARS Fungal Database (UAFD, Farr and Rossman, 2017), which contains a comprehensive list of fungal-host pairings and the literature in which they are mentioned, and is up to date in terms of nomenclature. It is important to note that this database does not specifically list *pathogen*-host combinations, and therefore we used it as a general starting point to further examine the literature relating to the pathogenicity of each fungus. Since we often found large numbers (> 100) of publications for each species, we generally conducted a quick scan to find appropriate literature, and also used this to get an impression of how often the literature related to disease compared to how often it was merely found to be associated with a particular host. This provided us with a good indication of how likely it was that a given fungus might be a pathogen. In addition, we examined two lists published by the European and Mediterranean Plant Protection Organization (EPPO); the EPPO Study on Pest Risks Associated with the Import of Tomato Fruit (EPPO, 2015) and Forest pests on the territories of the former USSR (EPPO, 2004), for occurrence of the 13 fungal species. We also examined the EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests (EPPO, 2016), but none of the fungal species were listed.

For background information on occurrences, ecological niches, host preferences and culturing conditions, we consulted the Compendium of Soil Fungi (Domsch *et al*., 2007). Following consultation with Prof. Crous and Dr. Houbraken, we came to the conclusion that any reports of pathogenicity where identification was not conducted using molecular methods should be viewed with skepticism. Therefore, when reviewing the literature, we paid particular attention to the method by which species were identified. In the case of reports on plant pathogens, we also adhered strongly to whether or not Koch's postulates were met. Since we came across many instances where Koch's postulates were examined

only on wounded plants, we list the manner in which this was tested, and consider those tests to only indicate proof of opportunism, not of true pathogenicity. We also report on the potential of any of the 13 fungal species to take part in disease complexes. Where available, we list specific varieties, strains or isolates of fungi. We also examined the credibility of the journal, for instance whether it was peer-reviewed or not. Finally, we searched for evidence that points to the presence of mycotoxin or virulence genes which indicate the potential for a fungus to become virulent or pathogenic. However, since these genes only indicate a potential for pathogenicity, and give no information on whether they are expressed or not, we regard their presence only as supplementary evidence.

The results from these literature reviews are presented in the next section, separately for each species. We present a brief background on nomenclature and any recent changes, a brief summary on their ecological niche and results from the literature in detail in the text. In addition, we summarize the results in a table, which details - amongst other issues - disease type and host, methods used to determine pathogenicity and a score indicating pathogenicity.

2.4. References

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3. Results and discussion from literature review

3.1 Acremonium strictum

Taxonomy

Acremonium strictum was renamed *Sarocladium strictum* in 2011 and this name change has been incorporated by IF, MB and the UAFD. The change in nomenclature occurred as a result of a phylogenetic analysis by Summerbell *et al*. (2011) using the ribosomal large subunit (LSU) and small subunit (SSU) RNA gene regions of a large number of different *Acremonium* species and related taxa. Their analyses showed that taxa within the *A. strictum* clade were closely related to *Sarocladium* species; they thus placed this species within the *strictum* clade of *Sarocladium*. This clade includes *A. strictum* as well as *A. kiliense*, a medically-important opportunistic pathogen, and *A. zeae*, a protective endophyte of maize, all of which were renamed to *Sarocladium* (Giraldo *et al*., 2015). Since the reclassification of *A. strictum* to *S. strictum* is generally accepted, we will from this point forward exclusively refer to this species as *S. strictum*.

Ecology

S. strictum is a soil fungus with a worldwide distribution. It is reported by Domsch *et al*. (2007) to be the most common of all (using the old wording) *Acremonium* species, occurring in a wide variety of soil types. It is frequently isolated from the rhizosphere and leaf surfaces of a number of different vascular plants and is often found as an endophyte (see for instance Clay *et al*., 2016). It is known as a producer of cellulases (Goldbeck *et al*., 2012) and has the ability to oxidize Mn(II), giving it potential in bioremediation and water cleansing (Chang *et al*., 2013).

Pathogenicity

Prof. Crous and Dr. Houbraken of the WFBI do not consider *S. strictum* to be a true pathogen, rather they indicate that it is a saprophytic soil fungus. However, it is listed as a potential pathogen on tomato plants in the European Union (EPPO, 2015). The UAFD (Farr and Rossman, 2017) indicates that *S. strictum* is the causal agent of leaf spot and wilt on various hosts. The database lists 70 unique fungus-host combinations, with 39 literature references, the majority of which relate to its occurrence as an endophyte.

A WoS search using the name “Sarocladium strictum” yielded 9 hits, while in PubMed it resulted in 6 hits, therefore no additional search terms were necessary. Searches with “Acremonium strictum” yielded 181 and 86 hits, respectively. This was reduced considerably by the additional search terms mentioned in section 2.3 (Table 3.1.1), yielding 0-31 hits. A selection of the literature pertaining to the potential pathogenicity of *S. strictum* is presented below and is summarized in table 3.1.2.

Table 3.1.1: Results from Web of Science and PubMed search for *Acremonium strictum*. When too many results were found using the main search term alone, the search was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Acremonium strictum"		181	86
	patho*	31	0
	parasit*	7	4
	disease	30	11
	virulence	2	2
	predator	0	0
	insect	3	1
	arthropod*	0	2
	nematod*	6	3
	mushroom	3	1
	agaricus	1	0
	mycorrhiza*	5	1
	mycotoxin*	7	1
	mycoparasit*	8	2

S. strictum has been associated with opportunistic infections in immunocompromised patients, causing infections of lungs, skin and brains (Guarro *et al*., 1997), although molecular analysis by Perdomo *et al*. (2011) indicated that its involvement in human infections was uncertain since it was frequently erroneously identified from clinical isolates.

There is a number of studies that indicate pathogenicity of *S. strictum* towards plants. Tagne *et al*. (2002) demonstrated that *S. strictum* caused disease in several maize cultivars (*Zea mays*) in Cameroon. Symptoms included chlorosis of leaves and stem which resulted in barren plants and wilting symptoms. As a consequence, a reduction in growth and yield was found. Identification of the fungus occurred by morphological means and Koch's postulates were met.

In Argentina, *S. strictum* was found to be the causal agent of a wilt disease in a number of cultivars of strawberries (*Fragaria* x *ananassa*) (Racedo *et al*., 2013). The symptoms included necrotic spots in the leaves and petioles, which increased in number and size as the disease progressed and necrotic areas that expanded over petioles and leaves causing strangulation of petioles and plant wilt. Molecular analysis of the ITS1 and ITS2 regions confirmed the identity of the fungus and Koch's postulates

established it as the causal agent. The same fungus was also able to cause disease in two sorghum (*Sorghum bicolor*) varieties, but not in the four varieties of maize they also tested. In Pakistan, Anjum and Akram (2014) described a wilting disease of currant tomatoes (*Lycopersicon esculentum*). The lower leaves of plants turned yellow following necrosis and were subsequently shed. In addition, roots of diseased plants were dark brown and vascular browning was observed in stems. *S. strictum* was identified through morphological and molecular (ITS 1 and ITS 2) means and Koch's postulates confirmed it as the causal agent.

S. strictum has been reported as a mycoparasite on several different fungi. Rivera-Varas *et al*. (2007) examined the effect of growing *Helminthosporium solani* with *S. strictum* (identified morphologically) *in vitro* and found that *S. strictum* reduced sporulation, spore germination and mycelial growth of *H. solani* considerably. *H. solani* is the causal agent of silver scurf of potato and as a result of the antagonism of *S. strictum* towards *H. solani* the incidence of this disease on potato tubers was reduced significantly. The authors concluded that *S. strictum* could be considered a mycoparasite of *H. solani*. Choi *et al*. (2008) conducted dual culture tests between the strain BCP of *S. strictum* and the causal agent of gray mold disease, *Botrytis cinerea*. Strain BCP dominated over *B. cinerea* and caused severe lysis of the host hyphae. Microscopic examination revealed frequent penetration and hyphal growth of strain BCP inside the hyphae of *B. cinerea*, which also suffered from morphological abnormalities such as granulation and vacuolation of the cytoplasm in its hyphae. *S. strictum* was additionally found to be an inhibitor of several other plant- pathogenic fungi.

In addition, several studies have shown parasitic activity of *S. strictum* on nematodes. Nigh *et al*. (1980) describes *S. strictum* as a fungal parasite of *Heterodera schachtii* eggs, and Verdejo-Lucas *et al*. (2009) found that a filtrate of *S. strictum* consistently inhibited the motility of second-stage juveniles of *Tylenchulus semipenetrans*. *S. strictum* was also found - in *in vitro* tests - to possess egg-parasitic capabilities against the root-knot nematode *Meloidogyne incognita* (Singh *et al*. 2010), while field tests showed that *S. strictum* in combination with *Trichoderma harzianum* could greatly reduce *M. incognita* populations (Goswami *et al*., 2008).

Conclusions/Recommendations

We recommend that from now on the new name *Sarocladium strictum* is used instead of *A. strictum*, while ensuring a link to older literature is maintained by retaining *A. strictum* as a synonym. We suggest the following: "*Sarocladium strictum* (syn. *Acremonium strictum*)".

We found many studies where *S. strictum* was reported to be pathogenic to plants, several of which presented enough solid evidence to indicate that *S. strictum* is a pathogen on plants. We also came across evidence – in which the fungus was identified by morphological criteria - indicating that *S. strictum* could potentially be a mycoparasite and a parasite of certain nematodes. We therefore conclude that this species is a pathogen for plants. Its potential pathogenicity to nematodes and fungi needs further scrutiny.

Table 3.1.2: Scoring table for *Sarocladium strictum*.

Source, strain information and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogen's identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence/observations**	**Conclusion**
Anjum and Akram 2014	FCBP 1099	Wilt in currant tomatoes (*Lycopersicon esculentum*)	Morphological and molecular identification. Koch's postulates were fully met	3 (P)
Choi *et al.* 2008	BCP	Mycoparasite of *Botrytis cinerea*	Morphological identification	2
Goswami *et al*. 2008	none listed	Egg parasitie of the root knot nematode (*Meloidogyne incognita*)	No clear identification, high toxicity and inhibition of egg hatching	2
Racedo *et al*. 2013	SS71	Wilt disease in strawberries (*Fragaria* x *ananassa*) and sorghum (*Sorghum bicolor*)	Morphological and molecular identification. Koch's postulates were fully met	3 (P)
Tagne *et al.* 2002	IMI 363644	Various symptoms on maize (*Zea mays*)	Uncertain morphological identification. Koch's postulates were fully met	2
Rivera-Varras *et al*. 2007	none listed	Mycoparasite of *Helminthosporium solani* (causal agent of silver scurf of potato)	Morphological identification, in vitro test showed reduced sporulation, spore germination and mycelial growth	2

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3.2 Aspergillus niger

Taxonomy

The genus *Aspergillus* contains 339 species subdivided into four subgenera and 20 sections. *Aspergillus niger* is a member of the *niger* clade, which is one of five clades in the *nigri* section (Samson *et al*., 2014). A full list of the members of this clade and an overview of the recent history of its taxonomy are presented in Chiotta *et al*. (2016). Since the *Aspergillus* name is derived from the anamorph form, strictly speaking it does not have naming priority, but in 2012 the members of the International Commission of *Penicillium* and *Aspergillus* decided to preserve the *Aspergillus* genus rather than maintain the different teleomorph names spread over several smaller genera (Samson *et al*., 2014).

The primary identification marker for *Aspergillus* is the ITS1-5.8S-ITS2 DNA region. However, the ITS region will not distinguish species in, amongst others, the *nigri* section, therefore Samson *et al*. (2014) proposed the use of the calmodulin gene, which codes for a calcium-binding messenger protein and is present in all eukaryotic cells, as a secondary marker. They examined the calmodulin gene in the section *nigri* and showed that calmodulin sequences have fixed, unique variations that vary from species to species, making the gene suitable for identifying isolates/strains in this section.

Ecology

A. niger is a cosmopolitan species (Farr and Rossman, 2017), occurring in both temperate and tropical regions (Domsch *et al*., 2007). It is found on, amongst others, fresh litter, seeds, senescent leaves and a wide variety of soils (Domsch *et al*., 2007). Furthermore, it has been reported as endophytic in a number of different lichen species (Tripathi and Joshi, 2015).
Species within the *nigri* section are also known as black Aspergilli. These species are amongst the most common fungi responsible for food spoilage and bio-deterioration of other materials. *A. niger* displays a large degree of phenotypic variation and has been extensively used in biotechnological applications; it is used to produce citric and other organic acids through fermentation and polysaccharide-degrading enzymes, such as amylases, pectinases and xylanases (Andersen *et al*., 2011).

Pathogenicity

The UAFD (Farr and Rossman, 2017) views *A. niger* as the causal agent of post-harvest disease, primarily causing fruit rots and other types of food spoilage. However, Prof. Crous and Dr. Houbraken from the WFBI do not consider *A. niger* to be a plant pathogen and it is not listed on the ITCF list of accurate scientific names of plant pathogenic fungi (Anon, not dated). However, *A. niger* is named on two EPPO lists: as a potential pathogen on tomato plants in the European Union (EPPO, 2015) and as occurring on diseased seeds in the forest in the territories of the former USSR (EPPO, 2004).

A WoS search using the term “Aspergillus niger” yielded 16,116 hits. Further refinement reduced this considerably (see Table 3.2.1). Because the terms “patho*” and “disease” still yielded a large quantity of hits, only the results from the previous 5 years were examined. The PubMed search resulted in 7,811

hits, further refinement yielded similar reduction to that of the WoS search. Very few of the search results referred to any kind of pathogenicity, and the main results were related to the industrial uses of *A. niger*. A selection of the literature pertaining to pathogenicity of *A. niger* is presented in the following paragraphs and is summarized in Table 3.2.2.

Table 3.2.1: Results from Web of Science and PubMed search for *Aspergillus niger*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Aspergillus niger"		16,116	7,811
	patho*	1,116	576
	parasit*	42	16
	disease	576	246
	virulence	73	113
	predator	0	0
	insect	15	79
	arthropod*	2	1
	nematod*	27	13
	mushroom	68	45
	agaricus	29	12
	mycorrhiza*	94	0
	mycotoxin*	0	0
	mycoparasit*	10	5

In industrial uses, *A. niger* has been reported to have a long history of safe use (Andersen *et al*., 2011; Schuster *et al*., 2002) and the USDA considers it to fall in the GRAS class ("generally recognised as safe"). However, it has also been linked to pathogenicity, as outlined below.

A. niger has been implicated in a number of human infections in immunocompromised patients and is a known allergen and one of the causal agents of aspergillosis, an infection caused by air-borne species of *Aspergillus*. In addition to causing opportunistic infections in humans, *A. niger* has also been found to be an opportunistic pathogen in fish, birds and mammalian livestock (Hurst, 2016). We did not investigate to what extent this pathogenicity/virulence is specific or unique per strain of *A. niger* or whether it is widespread in this species.

Some members of the Aspergillus section *nigri* have the potential to form the toxin ochratoxin A (OTA), a mycotoxin that has been has been found in a number of products, including wine, coffee, beer, grapes and cereals. OTA has been shown to be able to cause kidney failure, and it has carcinogenic, immunotoxic, genotoxic, teratogenic and possibly neurotoxic properties in humans (Chiotta *et al*., 2016). However, in a review on the safety of *A. niger*, Schuster *et al*. (2002) note that only 3–10% of the *A. niger* strains examined for OTA production have tested positive under favourable conditions. The authors suggested that new and unknown isolates should be checked for OTA production before they are developed as production organisms. When keeping to these restrictions, *A. niger* was a safe production organism. Frisvad (2011) found that *A. niger* strains were capable of producing several different forms of the mycotoxin fumonisin, which is toxic to animals and linked to esophageal cancer and birth defects in humans (http://www.apsnet.org/edcenter/intropp/topics/mycotoxins/pages/Fumonisins.aspx). The authors tested a large number of different strains of black *Aspergilli* and found that 81% of 180 *A. niger* strains were capable of producing fumonisin forms B2, B4 and B6. This included strains used in industry, of which 83% produced fumonisin. In addition, the production of OTA was also tested, revealing that the tested *A. niger* strains also produced this mycotoxin.

A. niger has been linked to a range of rot diseases in phytopathological studies. It was indicated as a possible causal agent of lint and boll rot disease on cotton (*Gossypium hirsutum*) in Iran (Mirzaee *et al*., 2013). This study aimed to isolate and identify the causal agents of this disease, which causes damage to the cotton bolls on the plant. While *A. niger* was frequently isolated, the authors only briefly summarized its potential pathogenicity, noting that it occurred only on mature bolls and grew slowly. Morphological characteristics of the fungus were not described, nor was there a description of how Koch's postulates were tested. Pawar *et al*. (2008) found necrotic leaf spot on ginger (*Zingiber officinale*) plants, which in severe cases resulted in defoliation of the plant. Isolated fungi from the leaf spots were morphologically identified as *A. niger*. Reinfection of healthy leaves confirmed the fungus as a causal agent. In addition to morphological identification, the authors also indicated that the fungus was identified using molecular techniques. However, they list the region examined as the 16S rRNA gene, which is found exclusively in prokaryotes and archaea, or in the mitochondrial DNA of eukaryotes, but is not used as a marker for fungal identification. Using the sequence the authors deposited, we performed a BLAST search, which resulted in several close matches to the 18S rRNA gene of *A. niger*, but also to other species within the *nigri* section and therefore it could not confirm the species identity to *A. niger*. In a recent study, Xu *et al*. (2015) isolated and re-inoculated *A. niger* on wounded peanut plants (*Arachis hypogaea*), showing that it was able to cause root rot on damaged seedlings, but not necessarily on intact ones. Liaquat *et al*. (2016) demonstrated that *A. niger* was able to cause fruit rot on lemons (*Citrus* × *limon*) and grapefruit (*Citrus* × *paradisi*), but again Koch's postulates were only confirmed through wound inoculation. Finally, Zhang *et al*. (2016) connected *A. niger* to leaf spot of field bindweed (*Convolvulus arvensis*), again by using wounded plants. In all three studies, identification was performed on the basis of both morphological and DNA-based criteria.

Examination of the nematidicidal potential of several fungi, including *A. niger*, was performed by Singh and Mathur (2010) in India. Fungi were isolated from egg masses of the root-knot nematode (*Meloidogyne incognita*) collected from vegetable fields that showed patchy growth and whose roots showed clear galls, but the authors did not describe the way in which they identified the fungus. *A. niger* was able to reduce egg hatching, and increased inactivity and death of the nematodes. Filtrates of *A. niger* were found to be toxic to the root knot nematode *Meloidogyne javanica*, by killing the 2nd stage of juveniles (Qureshi *et al*., 2012). In addition, Jang *et al*. (2016) found that filtrate of *A. niger* F22 was highly active against *M. incognita,* resulting in a high mortality of second-stage juveniles (J2s) and inhibition of egg hatching of the nematodes. The authors were able to identify the nematicidal component as oxalic acid. Field-applied dried fungus, as well as oxalic acid, showed a moderate reduction in galling on watermelon roots. Given that release of oxalic acid is very common in fungi, the clear identification of oxalic acid as the causal compound of nematode death in this study precludes *A. niger* from being considered as a pathogen on nematodes in this study.

Conclusions/Recommendations

The identification of *A. niger* was unsatisfactory in all of the literature that we examined, but no name changes have been proposed for this species. Several references did not indicate how they came to identify the fungus they used/discovered and none identified the fungus in the manner described in Samson *et al*. (2014). We also note that because of the extremely large volume of literature available for this species, we cannot be completely certain that there may have been reports of true pathogenicity that we did not find.

Nevertheless, from the review of the literature, it is apparent that *A. niger* is an agent of post-harvest disease and could be toxic towards nematodes. However, we did not find any studies in which *A. niger* was shown to be a true pathogen, i.e. that it could cause disease on healthy and undamaged individuals. Therefore, we conclude that *A. niger* is not pathogenic for plants, fungi, nematodes or arthropods.

Table 3.2.2: Scoring table for *Aspergillus niger*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Liaquat *et al*. 2016	F7 (from ITS sequence)	Fruit rot of lemon (*Citrus* × *limon*) and grapefruit (*Citrus* × *paradisi*)	Morphological and molecular identification (ITS). Koch's postulates on damaged fruit	2
Mirzae *et al*. 2013	none listed	Boll and lint rot on cotton (*Gossyphium hirsutum*)	Unclear morphological identification, Koch's postulates indicate post-harvest disease	1
Pawar *et al*. 2008	none listed	Necrotic leaf spot on ginger (*Zingiber officinale*)	Morphological and questionable molecular identification (16S), Koch's postulates	2
Singh and Mathur 2010	none listed	Parasite on root knot nematode (*Meloidogyne incognita*)	No clear identification. Reduction of hatching and death of nematodes, but no penetration by the fungus	2
Xu *et al*. 2015	none listed	Root rot of peanut (*Arachis hypogaea*)	Morphological and molecular identification (ITS), Koch's postulates on damaged seedlings	2
Zhang *et al*. 2016	none listed	Leaf spot of field bindweed (*Convolvulus arvensis*)	Morphological and molecular identification (ITS and β-tubulin), Koch's postulates on wounded leaves	2

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3.3 Aureobasidium pullulans

Taxonomy

Based on full genome sequencing, four varieties of *A. pullulans* were redefined as separate species in 2014 (Gostinčar *et al.*, 2014). The varieties *pullulans*, *subglaciale*, *namibiae* and *melanogenum* were elevated to the species level and named *A. pullulans*, *A. subglaciale*, *A. namibiae* and *A. melanogenum* respectively. These changes are confirmed by both IF and MB.

As a result of these name changes, the species definition of *A. pullulans* has become narrower. However, the majority of the literature referring to *A. pullulans* makes no mention of variety name or strain, and most likely will include species that presently no longer fall within this narrower range. Therefore, throughout the remainder of this report, *A. pullulans* s.l. (sensu lato) will refer to the broader pre-2014 concept of *A. pullulans*, while *A. pullulans* s.s. (sensu stricto) will refer to the narrower post-2014 definition.

Ecology

A. pullulans s.l. is a ubiquitous black yeast, with saprophytic capabilities, occurring most commonly on the leaf surfaces of plants (Domsch *et al*., 2007). The UAFD lists it as a cosmopolitan, saprophytic species that occurs on leaf surfaces and other plant parts and is a common contaminant (Farr and Rossman, 2017).

A. pullulans s.l. (including four member organisms) is of importance in the biotechnology industry, producing a large number of different extracellular enzymes and carbohydrates (Chi *et al*., 2009) and biopolymers (Kim *et al*., 2015). It is also listed as an endophyte with known biocontrol potential (Vero *et al*., 2009). For example, *A. pullulans* s.l. strains DSM14940 and 14941 are used as a commercial biocontrol product for management of a bacterial disease (fire blight) involving *Erwinia amylovora* that can affect pome fruit (http://www.bio-ferm.com/en/products/blossom-protect/blossom-protect/).

Pathogenicity

A. pullulans s.l. is listed on the "Accurate scientific names of plant pathogenic fungi" by the ICTF as causal agent of leaf spots and blights (Anon, n.d.). The UAFD lists *A. pullulans* (and its synonyms) as a saprophyte that also causes post-harvest fruit rot (Farr and Rossman, 2017). References from the database generally indicate an association with various hosts, but few indicate disease. Prof. Crous and Dr. Houbraken from the WFBI do not consider *A. pullulans* s.l. to be a pathogen.

A WoS search using the term "Aureobasidium pullulans" yielded 1,503 hits, while PubMed yielded 586 hits. Further refinements reduced this number considerably, but still resulted in a large number of hits for several terms (Table 3.3.1), therefore these were limited to references from the past 5 years. Searches for the new *Aureobasidium* species in WoS and PubMed yielded 10 and 8 hits for *A. melanogenum*, 0 and 3 for *A. namibiae* and 1 for *A. subglaciale,* in each database.

Table 3.3.1: results from Web of Science and PubMed search for *Aureobasidium pullulans*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Aureobasidium pullulans"		1503	586
	patho*	190	0
	parasit*	148	0
	disease	16	26
	virulence	9	14
	predator	2	0
	insect	11	9
	arthropod*	1	1
	nematod*	2	0
	mushroom	15	4
	agaricus	7	0
	mycorrhiza*	7	0
	mycotoxin*	17	12
	mycoparasit*	1	0

While most of the reports related directly either to post-harvest disease or to biotechnological products, we did come across reports of pathogenicity. A selection of the literature pertaining to pathogenicity of *A. pullulans* (both s.l. and s.s.) is presented in the following paragraphs and summarized in Table 3.3.2.

A. pullulans s.l. has been previously reported to cause infections in humans, but this was found to occur only within the new species *A. melanogenum* (Gostinčar *et al.,* 2014). Since human diseases are beyond the scope of this report we did not research this further.

In terms of plant pathogenicity, *A. pullulans* s.l. was found to be one of the causal agents in sooty blotch on apples (*Malus* x *domestica*) in Poland (Mirzwa-Mróz and Wińska-Krysiak, 2011). Sooty blotch is a disease complex that occurs worldwide on apples and similar fruits and results in superficial damage to the cuticle of the fruit. Identification of the causal agent occurred by morphological and molecular (ITS) means and Koch's postulates were met, but only on the fruit, proving a potential for post-harvest disease but not plant pathogenicity.

A. pullulans s.s. has also been found as part of a black mould complex that occurred on baobab trees (*Adansonia digitata*) in Africa (Cruywagen *et al*., 2015). This disease begins as orange brown spots, mostly on the undersides of branches, which then turn black and can coalesce to form larger patches. In this study, the fungus was isolated from infected plant tissue and identified using morphological characteristics and sequencing (ITS). However, Koch's postulates were not tested and the authors describe the infection as being merely superficial in nature and suggested that - overall - the complex was in all likelihood opportunistic on these trees.

A recent study reports *A. pullulans* s.l. as the causal agent of stem and fruit spot of pitaya (*Hylocereus* spp.) in China (Wu *et al*., 2017). In this study, morphological and molecular identification confirmed the identity of the fungus, but only at the level of *A. pullulans* s.l., unfortunately, the novel classification in four species was not taken into account. Koch's postulates were met, but only on wounded plants. This disease was not reported to affect pitaya until 2014, therefore it is the only report in the literature of this disease in combination with *A. pullulans* s.l..

A study into the fungal endophytic communities of buckwheat (*Fagopyrum* spp.) seeds and their effects on germination and seedling development showed that *A. pullulans* had potential for pathogenicity (Kovečec *et al*., 2016). The study showed that *A. pullulans* s.l. had high cellulolytic activity (meaning that it is able to degrade the cell walls in the seeds) and amylase secretion (allowing it to break down starches). The presence of *A. pullulans* s.l. also decreased seed germination and seedling development, potentially as a result of the decrease in storage resources of the seeds.

Although several papers were found showing an attraction of insects to *A. pullulans* s.l. volatiles (Hung *et al.,* 2015), there are no instances of *A. pullulans* s.l. infecting or causing disease on insects. In addition, no instances of *A. pullulans* s.l. infecting or causing disease on other fungi were found.

Conclusions/Recommendations

Due to the recent changes within the taxonomy of *Aureobasidium pullulans,* we recommend a strict consideration is given of the taxonomic assignment of fungi named *A. pullulans,* guided by the fact that in reports before 2014, *A. pullulans* is to be read as *A. pullulans* 'sensu lato' and after this time point as *A.pullulans* sensu stricto, the latter when taken into account by authors. We also recommend the critical consideration of the three newly defined species, with reference to their previous synonym, with respect to their potential pathogenicity.

While we found many reports that claimed pathogenicity of *A. pullulans* (both s.l. and s.s.) on plants, they either examined, or indicated, that *A. pullulans* was an agent of post-harvest disease, or that it was an opportunist. We also did not find any reports that *A. pullulans* (both s.l. and s.s.) was able to cause disease in other fungi, nematodes and insects. We therefore do not consider this species as pathogenic towards plants, fungi, nematodes and arthropods.

We also did not come across mentions of pathogenicity in other fungi, nematodes and insects for the new species *A. namibiae*, *A. subglaciale* and *A. melanogenum.* Therefore, we also do not consider these species to be pathogenic towards plants, fungi, nematodes and arthropods. However, we note the potential pathogenicity towards humans of *A. melanogenum*, which may warrant further investigation.

Table 3.3.2: Scoring table for *Aureobasidium pullulans*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence/observations**	**Conclusion**
Cruywagen *et al*. 2015	none listed, *A. pullulans* s.s.	Black mould complex on baobab (*Adansonia digitata*)	Morphological and molecular identification (ITS), Koch's postulates not tested	2
Kovečec *et al*., 2016	none listed, *A. pullulans* s.l.	Isolated from buckwheat (*Fagopyrum* spp.) seeds	Morphological and molecular identification (ITS), cellulase and lipolytic activity on seeds	1
Mirzwa-Mróz and Wińska-Krysiak 2011	none listed *A. pullulans* s.l.	Sooty blotch on apples (*Malus* x *domestica*	Morphological and molecular (ITS) identification, Koch's postulates on fruit	1
Wu *et al*. 2017	none listed *A. pullulans* s.l.	Stem and fruit spot of pitaya (*Hylocereus* spp.)	Morphological and molecular identification (ITS and β-tubulin), Koch's postulates met on wounded plants	2

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3.4 Bipolaris spicifera

Taxonomy

The genus *Bipolaris* was subjected to a recent revision based on DNA sequencing of living cultures of fresh isolates (Manamgoda *et al.,* 2012). Using a multi-locus analysis of the ribosomal RNA region ITS and LSU, GPDH (glyceraldehyde 3-phosphate dehydrogenase) and *tef-1* (translation elongation factor 1-α) genes, the researchers showed that many *Bipolaris* species were closely related to those of the genus *Curvularia*, with which it shares the same teleomorph (*Cochliobolus*) name. As a result, the suggestion to change the name *Bipolaris spicifera* to *Curvularia spicifera* was made.

However, the name *C. spicifera* is listed as the current name in IF and the UAFD, and recent (post-2012) medical literature also uses it. In MB, this species is still listed as *Bipolaris spicifera,* although this might be because older names are currently not updated in this database (Prof. P. Crous, pers. comm.).

There is sufficient evidence to support the name change, and therefore we will be referring to this fungus as *Curvularia spicifera* from now on.

Ecology

Curvularia spicifera is a fungus occurring commonly in soil, air and on a wide range of plant hosts. Although it has a world-wide occurrence, it tends to be more prevalent in tropical and sub-tropical areas (Domsch *et al.,* 2007). The UAFD lists it as a cosmopolitan species occurring on the leaves of a variety of different host plants (Farr and Rossman, 2017). It was shown to be an endophyte on the stems of *Artemisia subulata* (Cosoveanu *et al*., 2016).

Pathogenicity

Neither *B. spicifera* nor *C. spicifera* is listed in the ICTF list of accurate scientific names of plant pathogenic fungi (Anon, n.d.). The UAFD lists 112 unique host-fungus combinations for *B. spicifera* and its eight synonyms (including *C. spicifera* and *C. spicifer*) from a large range of different countries and from different continents (Farr and Rossman, 2017). Of the 82 literature references listed, 36 refer directly to diseases caused by *C. spicifera* and its synonyms. Prof. Crous and Dr. Houbraken from the WFBI identified *B. spicifera* as a plant pathogen and as an opportunist of immunocompromised patients.

Searches using “Bipolaris spicifera”, “Curvularia spicifera” or “Cochliobolus spicifer” did not yield enough results to warrant further refinement. WoS yielded 58 hits for “Bipolaris spicifera”, of which 15 referred to disease/pathogenicity, and 8 and 34 for “Curvularia spicifera” or “Cochliobolus spicifer” respectively, with 5 and 2 referring to disease/pathogenicity. PubMed search for “Bipolaris spicifera” yielded 61 hits, but only 1 pertaining to a study on plant pathogenicity. However, this study was observational and did not test for pathogenicity. “Curvularia spicifera” and “Cochliobolus spicifer” yielded 6 and 5 hits respectively. A selection of the literature pertaining to pathogenicity of *C. spicifera* is presented in the following paragraphs and is summarized in Table 3.4.1.

A large number of literature references report *C. spicifera* as the causal agent of a variety of human and animal diseases, primarily in immunocompromised patients. Furthermore, there is also a large number of reports of pathogenicity on the organismal groups that fall within the scope of this report.

Lin *et al.* (2012) obtained three isolates of *C. spicifera* from leaves of sugarcane (*Saccharum officinarum*) in China, which showed symptoms of leaf spot disease. The identity of the isolates was confirmed morphologically and by sequencing of the rRNA gene - ITS region. Pathogenicity of each isolate to sugarcane leaves was confirmed by artificial inoculation tests based on Koch's postulates. *B. spicifera* has additionally been reported to cause seedling blight of sugarcane in Algeria (Narendra *et al*., 1978), although here the identification method was not mentioned. In addition, it was found to cause leaf spot on maize (*Zea mays*) in China (Li *et al.,* 2016), leaf spot on switchgrass (*Panicum virgatum*) in the USA (Vu *et al.,* 2011), leaf spot and necrosis on watermelon (*Citrullus lanatus*) in Morocco (El Mhadri *et al.,* 2009), and leaf blight on buffalograss (*Buchloë dactyloides*) in the USA (Amaradasa and Amundsen, 2014).

Only one reference was found indicating some measure of pathogenicity against other fungi. In dual culture assays Cosoveanu *et al.* (2016) found that *C. spicifera* reduced the growth of the fungal pathogen *Sclerotinia sclerotiorum* and its extracts additionally exhibited antifungal activity against *S. sclerotiorum* and *Fusarium oxysporum*. However, no other references were found that indicated pathogenicity against fungi.

One reference was also found that showed potential pathogenic activity of *C. spicifera* against larvae of the mosquitos *Aedes caspius* and *Culex pipiens* (Abutaha *et al.,* 2015). This study examined the effect of *C. spicifera* extracts on these larvae and on zebrafish embryos, on which it was not toxic, but it was exclusively a laboratory study.

No further references were found to indicate pathogenicity to arthropods and none were found for nematodes.

Conclusions/Recommendations

There are strong arguments to indicate that the name of this species should be changed to *C. spicifera*. Since the majority of literature is still based on the *Bipolaris* genus, we recommend changing the name to "*Curvularia spicifera* (syn. *Bipolaris spicifera)*".

We conclude that there are enough reliable studies indicating pathogenicity of *B. spicifera* to plants to consider this species a pathogen for plants, but we do not consider it to be a pathogen for fungi, nematodes and/or arthropods.

Table 3.4.1: Scoring table for *Curvularia spicifera*/*Bipolaris spicifera*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Abutaha *et al.* 2015	none listed	Larvicidal activity of fungal extract, *Aedes caspius* and *Culex pipiens*	Morphological identification	2
Amaradasa *et al.* 2014	CCTU 245	Leaf blight, buffalo grass (*Buchloë dactyloides*)	Koch's postulates met, morphological and molecular (ITS and *gpd* gene) identification	3 (P)
El Mhadri *et al.* 2009	none listed	Leaf spot, watermelon (*Citrullus lanatus*)	Koch's postulates, morphological identification	2
Li *et al.* 2016	none listed	Leaf spot, maize (*Zea mays*)	Koch's postulates, morphological and molecular (ITS) identification	3 (P)
Lin *et al.* 2012	CGMCC 3.14921 CGMCC3.14922 CGMCC 3.14923	Leaf spot disease, sugarcane (*Saccharum officinarum*)	Koch's postulates, morphological and molecular (ITS) identification	3 (P)
Vu *et al.* 2011	NRRL 47508	Leaf spot, switchgrass (*Panicum virgatum*)	Koch's postulates, morphological and molecular (ITS) identification	3 (P)

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3.5 Bjerkandera adusta

Taxonomy

Binder *et al*. (2013) used a full genome sequencing approach to conduct a review of the phylogeny of the Polyporales, to which the genus *Bjerkandera* belongs. This included a full genome sequencing of *B. adusta*. They showed that *B. adusta* is placed firmly in the Polyporales and no changes to the name *Bjerkandera adusta* were suggested.

Several synonyms for *B. adusta* are reported by IF and the UAFD (Farr and Rossman, 2017); the mostly commonly reported one is *Polyporus adustus*.

Ecology

Bjerkandera adusta is a bracket fungus and is widely distributed over all continents, predominantly occurring as an endophyte in living trees, and as saprophyte producing white rot in wood of almost all broad-leaved trees (Chowdhary *et al*., 2014; Domański, 1982). It is not listed in the Compendium of Soil Fungi (Domsch *et al*., 2007). *B. adusta* is widely used in the biotechnology industry, mainly in the treatment of textile wastewater (Anastasi *et al.*, 2010) and as a bioremediation agent because of its ability to degrade organic pollutants (Gao *et al*., 2010).

Pathogenicity

The UAFD records 291 host-fungi combinations, of which the majority are deciduous trees, although occurrence on evergreen trees is also listed (Farr and Rossman, 2017). It lists *B. adusta* as the causal agent of white fibrous heart-rot, which is a decay disease in living trees (https://www.forestpathology.org/dis_decay.html). Due to the long-lived nature of the hosts of *B. adusta,* it is difficult to conduct Koch's postulates to confirm pathogenicity. Prof. Crous and Dr. Houbraken from the WBFI list *B. adusta* as a plant pathogen causing white rot. However, white rot is a disease that can occur on live or dead wood, depending on the organism involved, therefore it does not necessarily indicate pathogenicity. *B. adusta* is not listed on the accurate scientific names of plant pathogenic fungi of the ICFT (Anon, n.d.), but is named in the EPPO list 'Forest pests on the territories of the former USSR' (EPPO, 2004) as pathogenic on wood of deciduous trees with a low to medium financial impact in the area.

A WoS search on the term "Bjerkandera adusta" yielded 351 hits and PubMed returned 138 hits, of which the majority was related to the biotechnological uses of *B. adusta*. Refining with the terms listed in section 2.3 reduced the hits considerably (Table 3.5.1). However, none of these related to studies on *B. adusta* pathogenicity. Use of the term "Polyporus adustus" yielded only 3 hits. A selection of the literature pertaining to pathogenicity of *B. adusta* is presented in the following paragraphs and is summarized in Table 3.5.2.

Table 3.5.1: results from Web of Science and PubMed search for *Bjerkandera adusta*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Bjerkandera adusta"		355	138
	patho*	11	2
	parasit*	3	0
	disease	7	6
	virulence	1	0
	predator	0	0
	insect	3	1
	arthropod*	0	0
	nematod*	0	0
	mushroom	12	8
	agaricus	0	0
	mycorrhiza*	3	2
	mycotoxin*	0	0
	mycoparasit*	2	1

B. adusta has been reported to be an opportunistic human pathogen. There are many reports linking *B. adusta* to fungus-associated chronic cough and also to bronchial asthma and hypersensitivity pneumonitis, all occurring in Japan (reviewed in (Chowdhary *et al*., 2014).

In a survey on necrotrophic and heart-rot fungi in living trees in China, Dai *et al*. (2007) recorded *B. adusta* as causal agent of white trunk rot on deciduous trees. The authors note that Koch's postulates were not fulfilled for most of the species that they listed, but that all fungi were recorded on living trees, with some trees apparently being killed by these fungi, although they do not list which.

In a new disease of horse chestnuts (*Aesculus* x *carnea*) *B. adusta* was one of several fungi identified from affected tissue predominantly in the center of the damage and reaction zones (Müller-Navarra *et al*., 2014). However, this study was observational in nature and several different fungi co-occurred on diseased tissue, making it difficult to ascertain whether *B. adusta* was a causal agent or an opportunist.
B. adusta is described as a wound parasite infecting wood of living trees either through wounds or dead bark. Domański (1982) reported on the intensity of the occurrence of fungi on frost-damaged *Quercus rubra* and *Q. robur* trees. The study showed that *B. adusta* occurred on almost 25% of diseased *Q. rubra*

trees, and less on *Q. robur*. The degree of wood decay was additionally examined *in vitro* using sapwood blocks and *B. adusta* inoculation led to a high intensity of decay of sapwood of both tree species.

In a survey of fungal endophytes associated with the xylem of apparently healthy trees, Oses *et al*. (2008) found that *B. adusta* was a frequent isolate, although occurring in less than 5%. Fungi were isolated from wood cores from several different trees and these isolates were introduced onto sterilized wood cores: decay and ultrastuctural changes were observed. *B. adusta* was observed to completely cover the wood fragments following incubation. The authors suggested that *B. adusta* was latently present, enabling it to be present in the early stages of wood decay.

No reports of pathogenicity of *B. adusta* towards other fungi, nematodes or insects were found.

Conclusions/Recommendations

No name changes have been reported for this species.

While there have been reports of *B. adusta* on living trees, there is insufficient evidence to conclude that it is a plant pathogen in the strict sense rather than an opportunist taking advantage of wounds. Therefore, we consider *B. adusta* as non-pathogenic for plants, nematodes, arthropods and other fungi.

Table 3.5.2: Scoring table for *Bjerkandera adusta*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Dai *et al*. 2007	none listed	White trunk rot	Morphological identification	2
Dománski 1982	none listed	Frost damaged *Quercus robur* and *Q. rubra* trees	Morphological identification	2
Müller-Navarra *et al.* 2014	none listed	Unknown disease of horse chesnut (*Aesculus* x *carnea*)	Molecular identification, *B. adusta* occurred in diseased tissue	2
Oses *et al*. 2008	none listed	Endophyte on several different trees	Morphological identification	1

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3.6 Cladosporium herbarum

Taxonomy

Cladosporium herbarum is part of the *C. herbarum* complex, which comprises five different *Cladosporium* species. No name changes have been proposed for *C. herbarum* and a recent investigation into *Cladosporium* taxonomy and diversity confirmed its position within the genus *Cladosporium* (Schubert *et al.* 2007). Schubert *et al.* (2007) list *Davidiella tassiana* as the teleomorph name of *C. herbarum*, but its synonym *Mycosphaerella tassiana* seems to be more common.

Ecology

Cladosporium herbarum is a cosmopolitan fungus that occurs on a large number of different hosts (Domsch *et al*., 2007). It is one of the most common environmental fungi to be isolated worldwide (Schubert *et al*., 2007). The UAFD lists approximately 850 unique plant hosts, ranging from mosses, grasses and forbs to crop species and trees (Farr and Rossman, 2017). It is an early colonizer of dead and dying plant substrates. In addition, it is readily airborne, dominating air spore content, and occurs frequently in a range of different types of soil (Domsch *et al.,* 2007).

Pathogenicity

The UAFD lists *C. herbarum* as the causal agent of various spots and rots (Farr and Rossman, 2017). This database lists 240 literature references for the plant-host combinations, but only around 70 of these relate to the term disease. A random selection of these revealed that they refer primarily to *C. herbarum* as agent of post-harvest and seed storage diseases. Prof. Crous and Dr. Houbraken from the WFBI do not consider *C. herbarum* to be pathogenic, rather they classify it as a saprophyte. In addition, *C. herbarum* is not listed as a pathogen on the ICFT list (Anon, n.d.). The EPPO report into forest pests on the territories of the former USSR (EPPO, 2004) lists *Mycosphaerella tassiana* (syn: *C. herbarum*) as a pathogen on *Pinus* and deciduous tree seedling, but considers it of low economic significance.

The search term “Cladosporium herbarum” yielded 443 and 280 hits in WoS and PubMed respectively. Refinement with the terms listed in section 2.3 reduced this considerably (Table 3.6.1). The search term “Mycosphaerella tassiana” yielded only 4 and 3 hits resp. in WoS and PubMed. A selection of the literature pertaining to pathogenicity of *C. herbarum* is presented in the following paragraphs and is summarized in Table 3.6.2.

Table 3.6.1: Results from Web of Science and PubMed search for *Cladosporium herbarum*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Cladosporium herbarum"		443	280
	patho*	57	0
	parasit*	8	0
	disease	77	28
	virulence	1	12
	predator	0	0
	insect	8	10
	arthropod*	0	0
	nematod*	6	0
	mushroom	4	2
	agaricus	4	1
	mycorrhiza*	4	1
	mycotoxi*	18	11
	mycoparasit*	0	0

Cladosporium herbarum is known to be a common allergen and readily contaminates samples in clinical laboratories (Crous *et al.,* 2007). Schubert *et al*. (2007) list *C. herbarum* as occurring on living leaves (phylloplane fungus) and to be a secondary invader and an endophyte.

The Washington State University postharvest information network (http://postharvest.tfrec.wsu.edu/pages/J4I1F) lists *C. herbarum* as the causal agent of *Cladosporium* rot, a widespread disease in orchards but classifies this as a postharvest disease, as the fungus only enters the fruit through breaks in the skin. Kwon *et al.* (2001) reported on strawberry scab in Korea caused by *C. herbarum*. Fungi were collected from an outbreak in greenhouse-grown strawberries that were not severely infected. *C. herbarum* was identified using morphological characteristics, but not using molecular methods. Koch's postulates were met, but only when grown under cool, moist conditions. Scab disease did not cause mortality and only superficially infects strawberries (*Fragaria ananassa*).

Only a few studies showed potential plant pathogenicity of *C. herbarum*. Johnson *et al*. (2008) reported on leaf spot found in marsh marigold (*Caltha leptosepala* ssp. *howellii*) being caused by a specialized

biotype of *C. herbarum*. This strain was not able to cause symptoms on spinach or lettuce under conditions analogous to those for marsh marigold. Berner *et al.* (2007) described an aggressive strain of *C. herbarum* on yellow starthistle (*Centaurea solstitialis*) in a greenhouse experiment. The fungus mainly affected bolted plants and developing flowers and readily spread to non-inoculated plants. Necrosis developed within several days of inoculation on leaves and stems and then spread upwards on the stems and often led to death of the plant. Finally, severe brown spots caused by *C. herbarum* were observed on the leaves of Egyptian henbane (*Hyoscyamus muticus*) grown in a greenhouse in Japan (Abdel-Motaal *et al.,* 2009). Koch's postulates were met and identification of the fungus was conducted both morphologically and using molecular markers. The authors reported that the fungus penetrated the leaf tissue through open or damaged stomata, increased in size over time and eventually led to leaf curling and defoliation.

One study reported on pathogenicity of *C. herbarum* towards insects. Carvalho *et al.* (1972) reported that *C. herbarum* killed all stages of the cashew whitefly (*Aleurodicus cocois*) on a single cashew-nut tree (*Anacardium occidentale*) in Brazil. However, attempts to control the whitefly by the application of suspensions of the fungus in the field were unsuccessful. The authors concluded that the fungus was parasitic on the whitefly only under optimal conditions. Unfortunately, this paper was not readily accessible in complete form and therefore we could not ascertain the reliability of this study. No further studies were found indicating pathogenicity on arthropods, nor were any found for nematodes, mushrooms or mycorrhizae.

Conclusions/Recommendations

No changes in nomenclature are necessary for *C. herbarum.*

There are credible reports of potentially aggressive strains of *C. herbarum* on plants, even though the overwhelming literature refers to *C. herbarum* as either a saprophyte, a leaf epiphyte or as a causal agent of post-harvest diseases. We did not find any reports of pathogenicity towards nematodes and other fungi and we do not consider the single report we found on pathogenicity towards arthropods to be of sufficient weight. We therefore consider this species pathogenic only for plants.

Table 3.6.2: Scoring table for *Cladosporium herbarum*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	Strain	Disease and host	Evidence	Conclusion
Abdel-Motaal *et al.* 2009	none listed	Cladosporium leaf spot on Egyptian henbane (*Hyoscyamus muticus*)	Morphological and molecular (ITS and D1/D2) identification, Koch's postulates	3 (P)
Berner *et al*. 2007	BPI 863446	Leaf spot, yellow starthistle (*Centaurea solstitialis*)	Morphological and molecular (ITS) identification, Koch's postulates	3 (P)
Johnson *et al*. 2008	ATCC 201852 ATCC 201853 ATCC 201854 ATCC 201855	Leaf spot, marsh marigold (*Caltha leptosepala* ssp. *howellii*)	Morphological identification, Koch's postulates	2
Kwon *et al*. 2001	none listed	Strawberry scab, strawberry (*Fragaria ananassa*)	Morphological identification, Koch's postulates	2

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3.7 Clonostachys rosea

Taxonomy

The currently accepted name for this species remains *Clonostachys rosea* and both IF and MB lists this species as such. It has two main synonyms, namely *Gliocladium roseum*, and *Bionectria ochroleuca*, its former teleomorph name. IF lists two forms: *C. rosea* f. *rosea* and *C. rosea* f. *catenulate*. We included both synonyms in our searches.

Ecology

C. rosea is a common saprotroph with a cosmopolitan distribution. It is versatile in terms of its ecological niches and is isolated from numerous habitats, including various soil types and decaying plant material, and is reported to occur on a wide variety of hosts, such as fungi, nematodes and plants (Gan *et al*., 2007; Schroers, 2001). Domsch *et al.* (2007) indicate it is a common soil fungus with a cosmopolitan distribution.

Pathogenicity

UAFD lists *C. rosea* as occurring on a variety of plant parts, which include living and newly killed parts, and as the causal agent of dieback, pod rot, crown and root rot diseases as well as being a saprobic and endophytic fungus (Farr and Rossman, 2017). The database lists 223 fungus-host associations, and 128 literature references, which include mentions of disease, biocontrol and endophytic occurrences. Prof. Crous and Dr. Houbraken indicated that *C. rosea* is not a plant pathogen, but that it is used as a biocontrol agent of other fungi and nematodes. The joint genome institute JGI reports that *C. rosea* is known as an aggressive parasite of other fungi (mycoparasitism) and that it has potential as a biocontrol agent of plant-pathogenic fungi and that it has occasionally been found on dead insects and is associated with living nematodes and slime molds.

The search term “Clonostachys rosea” yielded 211 hits in WoS and 77 in PubMed. Refinement with the additional search term patho* in WoS reduced this to 103 (Table 3.7.1). “Gliocladium roseum” yielded 308 and 52 hits, with refinement in WoS it was 113. The PubMed searches were not further refined. The majority of results related to the biocontrol potential of *C. rosea*. Additional search terms yielded between 1 and 78 hits. A selection of the literature pertaining to pathogenicity of *C. rosea* is presented in the following paragraphs and is summarized in Table 3.7.2.

C. rosea was reported to cause root rot in soybean (*Glycine max*) (Bienapfl *et al*., 2012). The authors isolated *C. rosea* from diseased soybean roots, confirmed its identity using morphological and molecular methods, and successfully confirmed its infectivity in healthy soybeans. However, no other papers were found indicating pathogenicity in plants.

Table 3.7.1. Results from Web of Science and PubMed search for *Clonostachys rosea and Gliocladium roseum*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Clonostachys rosea"		211	77
	patho*	103	n.d.
	parasit*	29	n.d.
	disease	76	n.d.
	virulence	14	n.d.
	predator	2	n.d.
	insect	12	n.d.
	arthropod*	1	n.d.
	nematod*	26	n.d.
	mushroom	2	n.d.
	agaricus	0	n.d.
	mycorrhiza*	2	n.d.
	mycotoxi*	25	n.d.
	mycoparasit*	44	n.d.
"Gliocladium roseum"		308	52
	patho*	113	n.d.
	parasit*	20	n.d.
	disease	78	n.d.
	virulence	2	n.d.
	predator	1	n.d.
	insect	10	n.d.
	arthropod*	2	n.d.
	nematod*	13	n.d.
	mushroom	0	n.d.
	agaricus	1	n.d.
	mycorrhiza*	4	n.d.
	mycotoxi*	8	n.d.
	mycoparasit*	39	n.d.

Mycoparasitism has been widely reported for *C. rosea* (Domsch *et al*., 2007). Barnett and Lilly (1962) screened different isolates of *Gliocladium roseum* and found that it attacked and destroyed spores and vegetative cells of many fungal species, possibly through secretion of non-mobile substances. *G. roseum* was reported to parasitize *Rhizoctonia solani* by penetrating its mycelia (Jager *et al*., 1979). In an *in vitro* interaction assay between *C. rosea* and *Trichoderma* spp., *C. rosea* was found to be an aggressive mycoparasite while resistant to mycoparasitism by the *Trichoderma* species (Krauss *et al*., 2013).

C. rosea is widely reported to be nematophagous. In a faecal bioassay in sheep, Baloyi *et al*. (2012) showed that an isolate of *C. rosea* caused a 66% reduction of mixed infection of parasitic nematodes, predominantly *Haemonchus contortus*. One of the ways in which fungi can penetrate nematodes is through the production of extracellular enzymes. *C. rosea* has been found to be able to produce one such enzyme (a subtilisin-like extracellular serine protease, PrC). Crude enzyme extracts from *C. rosea* resulted in 100% mortality of the nematode *Panagrellus redivivus*, and purified PrC resulted in 80% mortality (Li *et al*., 2006). The presence of the *prC* gene was also shown to be important in *C. rosea* virulence in an assay against *P. redivivus* (Zou *et al*., 2010). Mutant *C. rosea* strains carrying a disrupted copy of the *prC* gene were demonstrated to have reduced virulence compared to wild-type *C. rosea* which caused almost 100% mortality.

C. rosea, identified by morphological criteria (and confirmed by CBS deposit) was also found to be entomopathogenic towards the Cicadellid leafhoppers, *Sonesimia grossa* and *Oncometopia tucumana*, in Argentina (Toledo *et al*., 2006). In this experiment, spraying insects with conidial suspensions of *C. rosea* resulted in increased mortality of the insects, although only a proportion could be attributed to the fungus.

Conclusions/Recommendations

No name changes have been proposed for this fungus.

We found one report of plant pathogenicity, which we consider to be robust and reliable. In addition, we found sufficient evidence to indicate that *C. rosea* can be pathogenic on insects. Although a number of studies were found that provided evidence of mycoparasitic and nematophagous behavior, they did not provide robust evidence of identification. Therefore, we conclude that *C. rosea* is a pathogen of plants and/or arthropods, but that there is insufficient robust evidence to conclude that it is a pathogen of nematodes or other fungi.

Table 3.7.1: Scoring table for *Clonostachys rosea*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows:
1 = non-pathogenic, 2 = potentially pathogenic (inconclusive identification or Koch's postulates), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Baloyi *et al*. 2012	none listed	Biocontrol of sheep nematodes (mixture of different species, dominated by *Haemonchus contortus*)	Fungi were isolated from pasture soils, but no clear identification. Inoculation led to up to 60% nematode mortality	2
Barnett and Lilly 1962	different isolates of *G. roseum*	Preliminary experiment on mycoparasitism on five fungi	Morphological identification only. Spores and cells were destroyed through direct contact without cell penetration	2
Bienapfl *et al*. 2012	none listed	Root rot on soybean (*Glycine max*)	Morphological and molecular (ITS) identification, Koch's postulates were fully met	3 (P)
Krauss *et al.* 2013	8 different strains	Experiment on interaction between *C. rosea* and *Trichoderma* spp.	*Clonostachys rosea* was identified as an aggressive mycoparasite, but no identification was presented	2
Li *et al.* 2006	CGMCC 0806	Bioassay of *C. rosea* extract on the nematode *Panagrellus redivivus*	Morphological identification. Crude enzyme extracts from *C. rosea* killed 100% of nematodes withinin 48 hours	2
Toledo *et al*. 2006	none listed	Entomopathogenic fungus of *Oncometopia tucumana* and *Sonesimia grossa*	Morphological identification, CBS deposit (CBS115883) and Koch's postulates were fully met	3 (A)

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3.8 Dichotomophthora portulacae

Taxonomy

Both IF and MB list this species as *Dichotomophthora portulacae* with no synonyms. However, according to the USDA fungal database, previous records of this species have been subjected to confusion and *D. portulacae* has previously been confused with *D. lutea*, the only other species within the same genus.

Ecology

D. portulacae is not listed in the Compendium of Soil Fungi (Domsch *et al*. 2007). Eken (2003) regards it as a soil-borne fungus, but due to previous misidentification (de Hoog and van Oorschot 1983) there is uncertainty regarding hosts and distribution ranges. In the literature, this species is often mentioned as a possible biocontrol agent of the invasive plant common purslane (*Portulaca oleracea*). It has been found as an endophyte in Malabar spinach (*Basella rubra*) in China (Jin *et al.* 2008).

Pathogenicity

The UAFD lists four host-fungus combinations and 18 literature records of which the majority list it as a pathogen on purslane (Farr and Rossman, 2017). *D. portulacae* is listed as an introduced plant pathogen into the United Kingdom (Jones and Baker 2007). Prof. Crous and Dr. Houbraken of the WFBI indicate that *D. portulacae* (identified on the basis of traditional and/or marker-based taxonomy) is a plant pathogen.

The search term “Dichotomophthora portulacae” yielded four hits in WoS and none in PubMed. All four results list *D. portulacae* as a pathogen of purslane.

D. portulacae (identified using the standards of the 80’s) was reported on purslane (*Portulaca oleracea*) in the USA (Klisiewicz *et al.* 1983). The fungus caused disease primarily on stems, which showed dark discoloration and constriction, but also on roots and the main stem and branches, which resulted in subsequent death of some plants, particularly when young. Koch’s postulates confirmed that *D. portulacae* was the causal agent.

Mitchell (1986) similarly reported on the pathogenicity of *D. portulacae* in the USA. The fungus was isolated from diseased purslane plants and was able to reinfect purslane seedlings, causing rapid death. The fungus was also tested on a different plant species, carpetweed (*Mollugo verticillata*), where it caused only mild disease symptoms. No method of identification was mentioned in this study.

Klisiewicz (1985) examined the growth, reproduction and biological activity of *D. portulacae* to determine if this fungus was suitable to use as a biological control agent. The study revealed rapid germination and infection of *D. portulacae* on purslane. Initial infection occurred only under moist conditions, but environmental conditions were not relevant for further progression of the disease.

Eken (2003) observed a crown and root rot of cultivated purslane in Turkey. Pathogenicity was assessed using both an agar plate assay developed by the authors and by using Koch's postulates, during which all plants died.

Conclusions/Recommendations

No changes in nomenclature are necessary for this species.

Although there are only a few reports of *D. portulacae* and pathogenicity in the literature, all indicate that this species (identified using traditional and/or marker-based criteria) is a pathogen of purslane. We did not find any literature referring to pathogenicity of *D. portulacae* towards other plants, fungi, nematodes or arthropods. We mark this species as a pathogen of plants (purslane) and alert the reader to the need to distinguish it from the related species *D. lutea*.

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Table 3.8.1: Scoring table for *Dichotomophthora portulacae*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Eken 2003	none listed	Crown and root rot, common purslane (*Portulaca oleracea*)	Morphological identification; Koch's postulates were fully met	3 (P)
Klisiewicz *et al*. 1983	none listed	Black stem, common purslane (*Portulaca oleracea*)	Morphological identification; Koch's postulates were fully met	3 (P)
Klisiewicz 1985	none listed	No disease name listed, common purslane (*Portulaca oleracea*)	Morphological identification, Koch's postulates were not examined	2
Mitchell 1986	none listed	Black stem rot, common purslane (*Portulaca oleracea*)	No identification was mentioned, Koch's postulates were fully met	2

3.9 Nigrospora sphaerica

Taxonomy

The UAFD gives the current name for this species as *Nigrospora sphaerica* (Farr and Rossman, 2017). In addition, the UAFD notes that Standen (in 1943) considered *Nigrospora sphaerica* to be a synonym of *Nigrospora oryzae*, but that others (Mason in 1927 and Hudson in 1963) considered it to be a distinct species due to differences in conidium size. The current name in MB is also *N. sphaerica*, but IF considers the current name to be *Nigrospora oryzae* and names *N. sphaerica* as a synonym. However, no references are listed and it remains unclear what this renaming is based on.

Since there is no clear evidence to assume that *N. sphaerica* and *N. oryzae* are the same species, we have not considered the latter species in this review, and therefore only present results from *N. sphaerica*.

Ecology

N. sphaerica is a cosmopolitan species (Farr and Rossman, 2017), although it seems to prefer warmer regions and is generally recorded in tropical and sub-tropical regions (Domsch *et al*., 2007). It occurs on living and dead plant parts and fruits from a variety of different plant families (Farr and Rossman, 2017) as well as various other non-plant substrates such as soils and mangrove swamps (Domsch *et al*., 2007). It has been reported to be an endophyte in - amongst others - grasses (White and Backhouse, 2007) and lichens (Tripathi and Joshi, 2015).

Pathogenicity

The UAFD views *N. sphaerica* as a saprophyte that can be weakly parasitic on a large range of plant hosts (Farr and Rossman, 2017). It names the following diseases with which *N. sphaerica* is associated: squirter and black end disease of banana, elm wilt, damping-off in red pine, inflorescense rot of cauliflower, bark necrosis of apples, and ear and stalk rot of grasses. Prof. Crous and Dr. Houbraken from the WFBI also consider *N. sphaerica* to be a plant pathogen.
N. sphaerica in not listed on the ITCF list of accurate scientific names of plant-pathogenic fungi, nor does it occur on any of the EPPO lists (see section 2.3).

A WoS search using the term “Nigrospora sphaerica” yielded 65 hits and the PubMed search resulted in 23 hits, neither were further refined. A selection of the literature pertaining to pathogenicity of *N. sphaerica* is presented in the following section and is summarized in Table 3.9.1.

N. sphaerica was found to be an opportunistic pathogen on blueberry (*Vaccinium corymbosum*) by infecting wounded fruit but not intact ones (Wright *et al.*, 2008). It has also been implicated in banana crown rot (Krauss *et al*., 1998) as a secondary invader. However, Abd-Alla *et al*. (2016) were unable to induce *N. sphaerica* to produce banana crown rot disease under artificial inoculation conditions *in vitro.*

Xu and Li (2017) reported an experiment in which *N. sphaerica* was isolated from diseased leaves of Chinese fir (*Cunninghamia lanceolata*). Initially the leaves had yellow to brown, irregular-shaped lesions, which developed into dark brown spots. Leaves eventually withered, and severely affected young plants died. Confirmation of the infection occurred using only artificially wounded leaves. Hernández-Cubero *et al*. (2017) similarly only fulfilled Koch's postulates by using injured leaves when confirming *N. sphaerica* as an infection agent on Jatropha (*Jatropha curcas*) in a study on the resistance of this plant against a wide range of potential pathogens.

Dutta *et al*. (2015) reported that *N. sphaerica* was the causal agent of leaf blight on tea. The symptoms started with the occurrence of irregular shaped lesions in young leaves, but later expanded to affect the entire leaf and included older leaves. This eventually resulted in the drying out of the tissue, leading to defoliation. Although the bushes did not die, it caused considerable damage to the plants. The identity of the fungus was confirmed by sequencing of the ITS1-2 region and Koch's postulates were fully met on intact plants.

N. sphaerica was found to be the causal agent of leaf spot of Calabash (*Lagenaria siceraria*) by Li *et al*. (2016). Initially the leaves had yellow round or irregular spots, and these became brown as the disease progressed. On occasion, the spots coalesced to form larger lesions. The fungus was isolated from leaves that showed disease symptoms, and its identity was confirmed by morphological and molecular means. Re-inoculation occurred on intact healthy leaves, which began to show the original symptoms within 15 days. Re-isolation confirmed that *N. spaerica* was the causal agent of the disease.

Conclusions/Recommendations

There remains inconsistency regarding the nomenclature of *N. sphaerica* between the different databases, and - as a result - there is uncertainty regarding its correct name. Therefore, we recommend keeping the name *Nigrospora sphaerica* for this species.

While many of the studies conducted on determining pathogenicity of *N. sphaerica* used damaged plants to re-inoculate, there is also reliable evidence that *N. sphaerica* is a true plant pathogen. We did not find any evidence to indicate that *N. sphaerica* is pathogenic towards nematodes, arthropods or other fungi. We therefore conclude that *N. sphaerica* is a pathogen towards plants.

Table 3.9.1: Scoring table for *Nigrospora sphaerica*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Dutta *et al*. 2015	none listed	Leaf blight on tea (*Camellia sinensis*)	Morphological and molecular identification (ITS1-5.8S-ITS2), Koch's postulates were fully met	3 (P)
Hernández-Cubero *et al*. 2017	isolate 4IL	Leaf disease on Jatropha (*Jatropha curcas*)	Morphological and molecular identification (ITS1-5.8S-ITS2). Koch's postulates on punctured leaves	2
Li *et al.* 2016	none listed	Leaf spot on Calabash (*Lagenaria siceraria*)	Morphological and molecular identification (ITS1-5.8S-ITS2), Koch's postulates were fully met	3 (P)
Liu *et al*. 2016	none listed	Red-brown leaf spots on Pitaya (*Hylocereus undatus*)	Morphological and molecular identification (ITS1-5.8S-ITS2), Koch's postulates on wounded plants only	2
Xu *et al*. 2017	Ns-12	Leaf blight on Chinese fir (*Cunninghamia lanceolate*)	Morphological and molecular identification (ITS1-5.8S-ITS2), Koch's postulates on punctured leaves	2

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3.10 Phoma herbarum

Taxonomy

Phoma herbarum is the type species for the highly polyphyletic *Phoma* genus in the family *Didymellaceae* (de Gruyter *et al.*, 2010). It has several synonyms, primarily based on host plant identity, but none are used regularly. In a comprehensive review of the *Didymellaceae*, Chen *et al*. (2015) used a multi-locus phylogenetic approach, using the ITS and LSU regions and the *rpb2* and *tub2* genes, to resolve the phylogeny within this group. They placed *P. herbarum* in clade 12 of this family and, although they introduced new genera, species and reclassification of species, no name changes were proposed for *P. herbarum.* In our searches, we thus used this name, and accepted the inherent potential polyphyly.

Ecology

P. herbarum is a cosmopolitan species that occurs on a wide range of plant substrates, as well as soil and water (Domsch *et al*., 2007). In addition, it is found on dead plants, animals and on nutritional and inorganic material (Farr and Rossman, 2017). *P. herbarum* is often reported as a plant endophyte on - amongst others - turmeric (*Curcuma longa*) (Gupta e*t al*., 2016) and a fast-growing variety of scots pine in Spain (Sanz-Ros *et al.*, 2015).

Pathogenicity

The list of accurate scientific names of plant pathogenic fungi by the ICTF does not include *P. herbarum* (Anon, n.d.), nor does it occur on any of the EPPO lists (see section 2.3). However, Prof. Crous and Dr Houbraken from the WFBI consider it to be a plant pathogen. The UAFD (Farr and Rossman, 2017) lists 189 fungus-host combinations and 88 literature references, a large number of which relate to disease.

The WoS search for the term “Phoma herbarum” yielded 126 hits, which was reduced to between 0 and 31 when refined with the terms in section 2.3 (Table 3.10.1). PubMed search for “Phoma herbarum” resulted in 40 hits which were not further refined. A selection of the literature pertaining to pathogenicity of *P. herbarum* is listed in the following section and is summarized in Table 3.10.2.

Several studies have shown that *P. herbarum* is the causal agent of disease in fish, such as chinook salmon (*Oncorhynchus tshawytscha*) (Faisal *et al*., 2007) and nile tilapia (*Oreochromis niloticus*) *(*Ali *et al*., 2011). In addition, there are a few reports of *P. herbarum* infections occurring in humans, causing infections of the skin, hair and nails (Tullio *et al*., 2010).

Table 3.10.1. Results from Web of Science and PubMed search for *Phoma herbarum*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Phoma herbarum"		126	40
	patho*	32	n.d.
	parasit*	3	n.d.
	disease	19	n.d.
	virulence	1	n.d.
	predator	1	n.d.
	insect	0	n.d.
	arthropod*	0	n.d.
	nematod*	1	n.d.
	mushroom	1	n.d.
	agaricus	0	n.d.
	mycorrhiza*	1	n.d.
	mycotoxi*	0	n.d.
	mycoparasit*	1	n.d.

However, the majority of the literature on pathogenicity of *P. herbarum* relates to plants. Kumla *et al*. (2016) identified *P. herbarum* as the causal agent of a spot disease on leaves of the cherry palm (*Pseudophoenix sargentii*) in Thailand. They isolated and cultured the fungus from leaves that were affected by dark spots that gradually increased in size and turned dark brown to black. Identification of the causal agent using molecular identification (ITS) confirmed its identity as *P. herbarum*. While Koch's postulates were met, it was not performed on intact plants, but on leaves separated from the plant. Zheng *et al*. (2017) reported on the occurrence of a leaf spot disease on oil palm. Leaves initially presented round or irregular dark brown spots, which became oval or irregular in shape and brown to grey in the centre and dark brown at the edge. Then, eventually scattered black specks appeared in the centre of the spot. The fungus isolated from these leaves was morphologically and molecularly identified as *P. herbarum*. Re-introduction on damaged leaves resulted in the same symptoms, but on undamaged leaves no infection occurred. Similarly, Hernández-Cubero *et al*. (2017) only confirmed *P. herbarum* as an infectious agent on Jatropha (*Jatropha curcas*) by fulfilling Koch's postulates on injured leaves of this plant.

In Australia, *P. herbarum* was shown to be pathogenic on pea plants (*Pisum sativum*) and the legume Tedera (*Bituminaria bituminosa*) (Li *et al*., 2011, 2012). In both reports, symptoms included pale brown lesions on leaves with distinct dark brown margins, occasionally with a chlorotic halo. Identification using the ITS region confirmed the identity of the fungus and Koch's postulates were fully met in both cases.

Conclusions/Recommendatio0ns

No name changes have occurred for *P. herbarum* and we did not find any proposals to do so.

There are many reports of *P. herbarum* pathogenicity to plants. While the majority of these did not fully fulfill Koch's postulates, we did find evidence where that was the case. In combination with the advice of Prof. Crous and Dr. Houbraken and the large number of reports of pathogenicity, we consider this species to be a plant pathogen. We did not find evidence that *P. herbarum* was pathogenic towards fungi, nematodes or arthropods. We note that two reports named it as a pathogen of fish, as previously reported in CGM 2015-06.

Table 3.10.2: Scoring table for *Phoma herbarum*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
Hernández-Cubero *et al*. 2017	none listed	Disease on leaves of Jatropha (*Jatropha curcas*)	Morphological and molecular (ITS) identification. Koch's postulates were tested on wounded leaves	2
Kumla *et al*. 2016	isolate CMU-PP05	Leaf spot on cherry palm (*Pseudophoenix sargentii*)	Morphological and molecular (ITS) identification. Koch's postulates were met on separated leaves	2
Li *et al*. 2011	none listed	Black spot disease on field pea (*Pisum sativum*)	Morphological and molecular (ITS) identification. Koch's postulates were fully met	3 (P)
Li *et al*. 2012	WAC13435	Leaf spot on Tedera (*Bituminaria bituminosa*)	Morphological and molecular (ITS) identification. Koch's postulates were fully met	3 (P)
Zheng *et al*. 2017	isolate YZ-8	Leaf spot on oil palm (*Elaeis guineensis*)	Morphological and molecular (ITS) identification. Koch's postulates were met only on wounded leaves, not intact ones	1

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3.11 Plectosporium tabacinum

Taxonomy

Plectosporium tabacinum was named in 1995 by Palm *et al.* (1995) as the anamorph name for *Plectosphaerella cucumerina*. Carlucci *et al*. (2012) used molecular techniques (rRNA gene) to assess *Plectosphaerella* species associated with root and collar rots and proposed a reassignment of *P. tabacinum* to the genus *Plectosphaerella*. Réblová *et al*. (2016) recommended this name change based on its economic importance and because it is an older name and the teleomorph name for this species. This change has been incorporated in SF and the UAFD and both list the current name for this species as *P. cucumerina*, with *P. tabacinum* as a synonym. MB still lists this species as *P. tabacinum.* Prof. Crous from the WFBI indicated that this database may not be fully up to date regarding current names of this species. Other former names for this species are *Fusarium tabacinum* and *Microdochium tabacinum*, but neither name is common in the literature (Farr and Rossman, 2017).

Ecology

P. tabacinum is considered to be a common soil fungus, occurring primarily in temperate zones (Domsch *et al*., 2007). It is often present in agricultural settings where it survives in soil and on plant debris between crops (https://ipm.illinois.edu/diseases/rpds/946.pdf). The UAFD lists *P. tabacinum* as a cosmopolitan species that occurs on different parts of a wide range of plants (Farr and Rossman, 2017). *P. cucumerina* was reported as a root endophyte on *Arabidopsis thaliana* plants growing in their natural environment (Junker *et al*., 2012).

Pathogenicity

P. cucumerina is listed on the ICFT list of accurate scientific names of plant-pathogenic fungi (Anon, n.d.), although no details of disease are given. However it is not listed on any of the EPPO lists (see section 2.3). The UAFD considers it to be the causal agent of disease of various plants, and notes that it has also been listed as a parasite of crayfish (Farr and Rossman, 2017). In this database, 65 fungus-host combinations are listed, with 51 literature records, the majority of which indicate disease. Prof. Crous and Dr. Houbraken of the WFBI indicated that *P. tabacinum* is a plant pathogen.

A WoS search using the terms "*Plectosporium tabacinum"* and "*Plectosphaerella cucumerina"* yielded 39 and 96 hits respectively and in PubMed 7 and 49 literature references were found respectively. Refinement of "Plectosphaerella cucumerina" in WoS with the search terms listed in section 2.3 reduced the number to between 61 and 0 (Table 3.11.1). A selection of the literature pertaining to pathogenicity of *P. tabacinum* is presented in the following section and is summarized in table 3.11.2.

Table 3.11.1. Results from Web of Science and PubMed search for *Plectosphaerella cucumerina*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.
n.d. = not determined.

Main search term	Additional search term	Web of Science	PubMed
"Plectosphaerella cucumerina"		96	49
	patho*	61	n.d.
	parasit*	8	n.d.
	disease	49	n.d.
	virulence	2	n.d.
	predator	0	n.d.
	insect	0	n.d.
	arthropod*	0	n.d.
	nematod*	6	n.d.
	mushroom	0	n.d.
	agaricus	0	n.d.
	mycorrhiza*	40	n.d.
	mycotoxi*	0	n.d.
	mycoparasit*	0	n.d.

P. cucumerina was reported as the causal agent of fruit and collar rot, and vine collapse of several crops including melons (Carlucci *et al*., 2012). These authors sampled diseased cucurbit, tomato and bell pepper roots and collars and isolated their associated fungi. *P. cucumerina* was found on all three species. However, the study did not explicitly examine disease.
Quesada-Ocampo *et al.* (2015) reported on *Plectosporium* blight on pumpkins and squash (*Cucurbita* spp.). Symptoms included lesions on the stems, petioles and fruit. Identification of the causal agent occurred using morphological and molecular (ITS) methods. However, Koch's postulates were not examined.

P. cucumerina was reported as the causal agent of sunflower wilt on Sunflower (*Helianthus annuus*) roots (Zhang *et al.*, 2015). In this study, the fungus was isolated from diseased tissue and identified using morphological and molecular (ITS) methods. Koch's postulates were fulfilled, but only on damaged roots. Xu *et al*. (2014) reported on root rot and plant wilt on greenhouse grown tomato plants. At the beginning of fruit set, symptoms were chlorosis of lower leaves and lack of turgidity in young leaves. Severely affected plants were wilted and stunted as fruit approached maturity. Primary and secondary

roots became necrotic with few fine feeder roots. The fungus was isolated from symptomatic roots and identified using morphological and molecular (ITS) means. Koch's postulates were fully met by sowing seeds in soil that received a conidial suspension of the fungus. D'Amico *et al*. (2008) conducted a study into the fungal endophytes of commercial crops in Italy (fennel, lettuce, chicory and celery) and identified the most commonly occurring one (both morphologically and by WFBI standards) as *P. tabacinum*. Reinoculation of this isolate on lettuce plants showed pathogenic activity that included causing obvious root necrosis and leaf chlorosis.

P. cucumerina has also been reported as pathogenic to nematodes. In experiments to determine its applicability as a biocontrol agent against the potato cyst nematode (*Globodera* spp.), Jacobs *et al*. (2003) showed that application of *P. cucumerina* significantly reduced the number of nematode eggs. However they considered it to be of poor value as a biocontrol agent, due to its poor competitiveness against other fungi, indicating a lack of mycoparasitic potential.

Conclusions/Recommendations

We conclude that there is sufficient evidence to recommend changing the name of *P. tabacinum* to *Plectosphaerella cucumerina* (*syn. Plectosporium tabacinum*), and note that this name is already in common use in the recent literature.

There are several reliable reports that indicate that *P. cucumerina* is a plant pathogen, and it is listed as such on both the ITCF and UAFD. This was confirmed by Prof. Crous and Dr. Houbraken from the WFBI. We also found reports that *P. cucumerina* exhibits pathogenicity towards nematodes, and that it is widely listed as such, but none provided sufficient evidence to considered it unequivocally so. We did not find any evidence for pathogenicity towards fungi and arthropods. We therefore consider this species to be a pathogen for plants.

Table 3.11.2: Scoring table for *Plectosporium tabacinum/Plectosphaerella cucumerina*.
Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
D'Amico *et al*. 2008	none listed	root necrosis and leaf chlorosis of fennel, lettuce, chicory and celery plants	Morphological and identification by the WFBI. Koch's postulates were fully met	3 (P)
Jacobs *et al*. 2003	IMI 380408	Parasite on potato cyst nematodes (*Globodera* spp.)	Morphological identification only, application reduced egg numbers	2
Quesada-Ocampo *et al*. 2015	none listed	Plectosporium blight on Pumpkin and Squash (*Cucurbita* spp.)	Morphological and molecular identification (ITS), Koch's postulates were not examined	2
Xu *et al*. 2014	isolate HLDT15	Tomato wilt on tomato (*Lycopersicon esculentum*)	Morphological and molecular identification (ITS), Koch's postulates were fully met	3 (P)
Zhang *et al*. 2015	none listed	Wilt on sunflowers (*Helianthus annuus*)	Morphological and molecular identification (ITS), Koch's postulates only tested on damaged roots	2

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Zhang, Y.Y., Li, M., Liang, Y., Zhou, H.Y., Zhao, J., 2015. First Report of Sunflower Wilt Caused by *Plectosphaerella cucumerina* in China. *Plant Disease* **99**, 1646–1646. doi:10.1094/PDIS-02-15-0135-PDN

3.12 Trichoderma koningii

Taxonomy

The genus *Trichoderma* has been subjected to a number of revisions historically, primarily due to difficulty in morphological identification of the different species. The species concept of *T. koningii* has been narrowed considerably over the years (Samuels *et al*. 2006). As a consequence, Domsch *et al*. (2007) suggested treating reports of *T. koningii* occurrence before 1969 with caution and the UAFD mentions that *T. koningii* is often confused with *T. hamatum*. However, since the development of molecular identification techniques *T. koningii* can be reliably identified. The international subcommission on *Trichoderma* and *Hypocrea* taxonomy (www.isth.info) suggests using the ITS1-ITS2 region in combination with intron 4 of the *tef-1* gene to identify *T. koningii*.
T. koningii is proposed as the protected name for this species, with the teleomorph name of *Hypocrea koningii* as its synonym (Bissett *et al.*, 2015).

Ecology

T. koningii was previously reported to be a common cosmopolitan soil fungus, but, since the narrowing of the definition of *T. koningii*, confirmed occurrences of *T. koninigii* are now limited to Europe and North America (Samuels *et al*. 2006). *T. koningii* is listed by the UAFD as occurring in soil, while its synonym *H. koningii* is reported to be present on decorticated wood. Domsch *et al*. (2007) indicate that *T. koningii* is a common soil fungus, and occurs frequently in the litter layer but also on a variety of plants.

Pathogenicity

T. koningii is not listed on any of the EPPO lists (see section 2.3), nor on the ICTF list of accurate scientific names of plant-pathogenic fungi (Anon, n.d.). The UAFD lists 71 unique fungus-host associations, but these are primarily based on occurrences, rather than on reports of disease. Prof. Crous and Dr. Houbraken of the WFBI indicate that *T. koningii* is a pathogen of sweet potato (*Ipomoea batatas*), and it is also listed as such on the American Phytopathological Society website (https://www.apsnet.org/publications/commonnames/Pages/Sweetpotato.aspx), but no references were presented to back up this assertion. In Korsten and Wehner (2005), *T. koningii* is listed as the causal agent of punky rot of sweet potatoes, but they indicate that the main mode of infection is through wounds.

A WoS search for “Trichoderma koningii” yielded 276 hits, and PubMed yielded 112 hits, with the majority referring either to its potential as a biocontrol agent or use in bioremediation. Due to the large number of hits, the additional search terms listed in section 2.3, were used in combination with “Trichoderma koningii” (Table 3.12.1). A selection of the literature pertaining to pathogenicity of *T. koningii* is presented in the following paragraphs and is summarized in table 3.12.2

Table 3.12.1. Results from Web of Science and PubMed search for *Trichoderma koningii*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.

Main search term	Additional search term	Web of Science	PubMed
"Trichoderma koningii"		276	112
	patho*	40	0
	parasit*	7	0
	disease	30	8
	virulence	1	3
	predator	0	0
	insect	3	3
	arthropod*	0	1
	nematod*	1	0
	mushroom	4	8
	agaricus	1	0
	mycorrhiza*	8	1
	mycotoxi*	6	6
	mycoparasit*	19	10

Trichoderma species are frequently studied for their biocontrol potential of fungal pathogens. *T. koningii* has been found to inhibit *Sclerotium cepivorum*, the fungus responsible for *Allium* white rot, potentially through the production of chitin-degrading enzymes (Metcalf and Wilson, 2001). Similarly, Gajera *et al.* (2016), showed that *T. koningii* strain MTCC 796 was a potent inhibitor of the test pathogen *Rhizoctonia solani*, the causal agent of root rot in cotton. The potential for mycoparasitism also has negative effects. *T. koningii* is also implicated in green mould disease on commercial mushroom (amongst others *Agaricus bisporus*) farms, although generally *T. aggressivum* is thought to be responsible for most outbreaks (Kredics *et al*., 2010). Other studies have shown yield reduction (Górski *et al.*, 2014) and moderately severe disease symptoms (Kosanović *et al*., 2013) of *T. koningii* on *A. bisporus*, although neither study used sufficiently robust methods for identification of the fungus.

There is also evidence that *T. koningii* may have a negative effect on nematodes. Windham *et al*. (1989) showed that the presence of *T. koningii* strongly reduced the effect that the peanut root knot nematode *Meloidogyne arenaria* had on maize plant growth and seemed to lead to a decline in *M. arenaria* reproduction. However, it was not clear if this effect was due to a parasitic action of *T. koningii* or whether the fungus merely improved plant vigor against the nematode. Similarly, El-Shennawy *et al*. (2012) showed the potential of *T. koningii* for biocontrol of a disease complex on potatoes caused by a

mixed population of root knot nematodes (*Meloidogyne javanica* and *M. incognita*) and *Fusarium oxysporum*. They showed that *T. koningii*, both alone or in combination with the plant-growth-promoting rhizobacterium *Bacillus megaterium*, significantly reduced levels of nematodes and pathogen, and increased potato yield. However, in this study the authors did not report on the identification of the fungus.

Conclusions/Recommendations

No name changes have been reported for *T. koningii*.

The majority of evidence suggest that *T. koningii* is not a pathogen of plants. There is limited, but insufficient, evidence for pathogenicity on nematodes. Despite the fact that most studies did not show clear evidence of *T. koningii* identity, there is sufficient evidence that it is a pathogen of fungi. In conclusion, *T. koningii* is considered as pathogenic for fungi, although we recommend that this should not influence its use as a fungal disease biocontrol agent. It is not considered to be pathogenic for plants, arthropods or nematodes.

Table 3.12.2: Scoring table for *Trichoderma koningii*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	**Strain**	**Disease and host**	**Evidence**	**Conclusion**
El-Shennawy *et al*. 2012	none listed	Control of a disease complex by root-knot nematodes (*Meloidogyne spp*) and *Fusarium oxysporum*	No clear confirmation of *T. koningii* identity. Significant reduction in disease incidence and nematode infection	2
Gajera *et al*. 2016	MTCC 796	No disease, host is *Rhizoctonia solani* which causes root rot in cotton	Strain obtained from a culture collection. 88% *in vitro* growth inhibition and disintegration and degradation of pathogen mycelia	3 (F)
Górski *et al*. 2014	none listed	Experiment determining the effect of *T. koningii* on the yield of *Agaricus bisporus*	No clear confirmation of *T. koningii* identity. Significant reduction in yield of *A. bisporus*	2
Kosanović *et al*. 2013	Isolate T39	Green mould on *A. bisporus*	Morphological and molecular (ITS1/ITS4) identification, moderately severe symptoms in virulence assay	3 (F)
Metcalf and Wilson 2001	Tr5	Biocontrol of *Sclerotium cepivorum* causing white rot on onion roots	No clear confirmation of *T. koningii* identity. *T. koningii* exudates caused detachment of *S. cepivorum* hyphae and dissolving of cell walls	2

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3.13 Trichoderma viride

Taxonomy

Trichoderma viride is the type species of the genus *Trichoderma*, which was first described in 1794 by Persoon. The genus *Trichoderma* has been subject to a number of revisions over the years (most recently by Samuels, 2006a), primarily due to difficulties in the morphological identification of the different species. The teleomorph name of *T. viride* is *Hypocrea rufa*, but it should be regarded as a synonym only, because the International Subcommission on the Taxonomy of *Trichoderma* and *Hypocrea* indicated a strong preference to use *Trichoderma* rather than *Hypocrea* (Rossman *et al*., 2013).

T. viride has often been confused with other *Trichoderma* species. Because of this, Domsch *et al.* (2007) indicated that all data on *T*. *viride* have to be treated with considerable reserve. *T. viride* can only be reliably identified using a combination of two molecular markers. The International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy recommend firstly using the ITS marker to classify *T. viride* to the genus/species complex level, followed by identification using the *tef-1* marker to separate out *T. viride* from other *Trichoderma* species (www.isth.info).

Ecology

Domsch *et al*. (2007) indicated that *T. viride* in a broad sense (i.e. including closely related species that have been misidentified as *T. viride*) is one of the most commonly reported species of soil fungi, and occurs on a wide variety of substrates, such as plants, soil, litter and water. The UAFD (Farr and Rossman, 2017, citing Samuels *et al*. 2006b), also notes that many previous reports of *T. viride* occurrence actually refer to other spp. of *Trichoderma*. In fact, they consider *T. viride* to be an uncommon species occurring primarily on wood, roots, fruits and seeds in North America and Europe.

T. viride is widely listed as a biocontrol agent against a variety of fungal diseases, although this tenet also should be treated with caution. For example, Hermosa *et al*. (2004) used molecular identification using ITS and *tef-1* markers on strains of *T. viride* commercially used for biocontrol, and showed that, in fact, out of 7 strains examined only one was correctly identified as *T. viride*. The remaining strains were identified as other *Trichoderma* species.

Pathogenicity

The list of accurate scientific names of plant-pathogenic fungi given by the ICTF indicates that *T. viride* is the causal agent of dieback, root rot and post-harvest fruit rot (Anon, n.d.). However, Prof. Crous and Dr Houbraken from the WFBI do not consider *T. viride* to be pathogenic, rather they indicate that it is a biocontrol agent. The UAFD (Farr and Rossman, 2017) considers *T. viride* to be the causal agent of dieback, root rot, post-harvest fruit rot, and lists 313 fungus-host associations which gives 140 references, although the majority of these do not indicate disease, rather they refer to occurrences of *T. viride* in diseased plants and crops.

A WoS search for the term “Trichoderma viride” yielded 1779 hits, while in PubMed it resulted in 689 hits. Further refinement reduced this number considerably (Table 3.13.1). However, for the terms patho* and disease there were still too many results to take account of in WoS, therefore only the results from the last 5 years were examined. Overall, most of the results from this search did not imply pathogenicity of *T. viride*, but rather its use as a biocontrol agent. A selection of the literature pertaining to pathogenicity of *T. viride* is presented in the following paragraphs and is summarized in table 3.13.2.

Table 3.13.1. Results from Web of Science and PubMed search for *Trichoderma viride*. When too many results were found using the main search term alone, it was refined using the terms named in section 2.3, and those results were used to determine pathogenicity.

Main search term	Additional search term	Web of Science	PubMed
“Trichoderma viride”		1779	689
	patho*	249	83
	parasit*	25	12
	disease	166	26
	virulence	5	6
	predator	2	1
	insect	14	19
	arthropod*	1	1
	nematod*	15	7
	mushroom	11	11
	agaricus	7	2
	mycorrhiza*	26	3
	mycotoxi*	11	10
	mycoparasit*	30	9

We encountered one paper describing *T. viride* as a plant pathogen. Li Destri Nicosia *et al*. (2015) examined four different isolates of *T. viride*, isolated from black pine (*Pinus nigra*) trees. The trees showed signs of dieback, characterized by leaf chlorosis, dark brown discoloration of cortical and external vascular tissues and occasionally led to death. Morphological and molecular identification of the ITS region and *tef-1* marker confirmed the species to be *T. viride*. Testing of Koch’s postulates was performed using bark plugs on 10-year-old trees and on damaged leaves of 2-year-old seedlings, but were not performed on undamaged trees/seedlings. Plants (*P. nigra*, *Citrus* spp.) developed disease (die-back).

In contrast, we encountered many different reports of mycoparasitism. Gajera *et al*. (2016) showed that *T. viride* strain NBAII Tv23 was highly inhibitory to the growth of the test pathogen *Rhizoctonia solani*, the causal agent of root rot in cotton, in a dual culture assay. Wang *et al*. (2016) isolated different *Trichoderma* spp. from green mold on Shiitake (*Lentinula edodes*) mushrooms. One of the species recovered was identified as *T. viride* using the ITS and *tef-1* marker. A dual culture assay showed that *T. viride* was strongly inhibitory and deleterious to the mycelial growth of *L. edodes*, causing swelling and distortion of its hyphae.

In addition to mycoparasitism, *T. viride* has also been found as a pathogen on nematodes. Singh and Mathur (2010) examined the effect of *T. viride* and several other fungi on the rates of egg infection and hatching and the mobility and mortality of the root-knot nematode *Meloidogyne incognita*. They showed that all measures were significantly affected by exposure to *T. viride* cultures and filtrates in comparison to the control values. However, *T. viride* performed less well than several of the other fungi tested, making it less effective as a control agent. Al-Hazmi and TariqJaveed (2016) examined the effect of an isolate of *T. viride* from Saudi Arabia on *M. javanica*. They applied fungal inoculum at four different densities to pots containing nematodes affecting tomato roots. The isolate was obtained from a different group that used morphological and molecular (ITS1-2 region) methods for identification. The effectiveness of the fungus increased with increasing inoculum size; the tomato roots showed a reduction in galling, and an increase in growth, while nematode reproduction decreased. The molecular component of the nematode parasitic ability of *T. viride* on *M. incognita* was examined by Rajinikanth *et al*. (2016). They examined the expression of the gene *chi18-5* in *T. viride* following exposure to *M. incognita* egg masses. This gene encodes the chitinolytic enzyme chi18-5 which plays a major role in egg parasitism by breaking down chitin, a component of the cell walls of fungi and the exoskeleton of some animals. The authors showed that *chi18-5* was highly up-regulated between 2 and 5 hours after inoculation and indicated that the *chi18-5* gene encoded one of the lytic enzymes required by *T. viride* to parasitise nematode eggs.

Conclusions/Recommendations

No name changes for this species have been proposed and are unlikely to occur in the foreseeable future, given its priority status.

Due to the frequent misidentification of this species, any report where identification was not based on a two-phase molecular analysis (using ITS and a secondary marker) should be viewed with caution. However, we consider that we found sufficient evidence to suggest that *T. viride* is a mycopathogen and pathogenic towards nematodes, but not that it is a plant pathogen or pathogenic towards arthropods.

Table 3.13.2: Scoring table for *Trichoderma viride*.

Source, strain information, and disease name and host on which it occurred are listed. The evidence on which the author's conclusion was based is listed and the method by which they ascertained the pathogens identity. The overall conclusion is rated based on the identification methods and overall evidence presented and is scored as follows: 1 = non-pathogenic, 2 = inconclusive evidence (inconclusive identification or Koch's postulates not met), 3 = pathogenic (pathogen of P=plant, F=fungi, N=nematodes, A=arthropods).

Source	Strain	Disease and host	Evidence	Conclusion
Al-Hazmi and TariqJaveed 2016	isolate-08	Root-knot nematode (*Meloidogyne javanica*) on tomato plants (*Solanum lycopersicum*)	Morphological and molecular (ITS1-2) identification. Inoculum suppressed nematode reproduction	3 (N)
Gajera *et al*. 2016	NBAII Tv23	Mycoparatism on *Rhizoctonia solani*, causal agent of root rot in cotton	Strain obtained from a culture collection, but no direct evidence of identification. Reduced mycelial growth	2
Li Destri Nicosia *et al*. 2015	four isolates	Dieback of black pine (*Pinus nigra*)	Morphological and molecular (ITS and *tef-1*) identification. Koch's postulates were performed on wounded plant only	2
Singh and Mathur 2010	none listed	Root-knot nematode (*Meloidogyne incognita*)	Isolate was identified at the Indian Type Culture Center, but no indication of how. Culture filtrate affected nematode hatching and mortality	2
Wang *et al*. 2016	none listed	green mold on Shiitake (*Lentinula edodes*)	Morphological and molecular (ITS and *tef-1*) identification. Dual culture assay showed distortion/swelling of 'prey' hyphae and inhibition of mycelial growth	3 (F)

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4. Summary of recommendations and conclusions

Following an in-depth literature search and review of the literature, we are able to make recommendations with respect to the potential for pathogenicity for all 13 fungal species (Table 4.1). Of these, we consider that for three of them, evidence is pointing towards their non-pathogenicity on the targeted host organismal groups. Of the remaining ten species, we consider the evidence strong enough to suggest that (some with dual or multiple activity): eight are pathogenic to plants, one to arthropods, one to nematodes and two to other fungi.

We alert the reader to the new species *Aureobasidium melanogenum* with respect to of its potential pathogenicity towards humans, and *Phoma herbarum* for its potential pathogenicity towards fish, and recommend an in-depth examination of both.

We recommend name changes for four fungal species, as indicated by experts and listed in Table 4.1. However, these and other changes in nomenclature and phylogeny are still very much in progress and we expect it will take some time before this issue is fully resolved. Therefore, we consider it possible that name changes for some of the species in this report could (again) occur in the near future.

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Table 4.1 Summary of recommendations for each fungal species. Pathogenicity on plants, nematodes, (other) fungi and arthropods was assessed, whereas that on humans and animals was not the focus of this study. Listed are original fungal names and suggested name changes, pathogenicity, including host organism (if applicable). Superscripts at X: P= plant, F= fungi, N = nematode and A= arthropod

Species	Name change	Pathogenicity
Acremonium strictum	*Sarocladium strictum* (syn. *Acremonium strictum*)	X^{P}
Aspergillus niger		-
Aureobasidium pullulans sensu lato	*A. pullulans* s.s. *A. subglaciale* (syn. *A.pullulans* s.l.) *A. namibiae* (syn. *A.pullulans* s.l.) *A. melanogenum* (syn. *A.pullulans* s.l.)	-
Bipolaris spicifera	*Curvularia spicifera* (syn. *Bipolaris spicifera*)	X^{P}
Bjerkandera adusta		-
Cladosporium herbarum		X^{P}
Clonostachys rosea		$X^{P,A}$
Dichotomophthora portulacae		X^{P}
Nigrospora sphaerica	currently uncertain	X^{P}
Phoma herbarum		X^{P}
Plectosporium tabacinum	*Plectosphaerella cucumerina* (syn. *Plectosporium tabacinum*)	X^{P}
Trichoderma koningii		X^{F}
Trichoderma viride		$X^{F,N}$