

PLANT PATHOLOGY IS LOST WITHOUT TAXONOMY

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Tel: +31 (0)30 2122643 Fax: +31 (0)30 2122601 e-mail: crous@cbs.knaw.nl outlines the current trends in fungal taxonomy and the implications for plant pathology

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Introduction

Taxonomy remains essential for credible plant pathology. Descriptive taxonomy is, however, in danger of disappearing. One only has to consult recent plant pathological and mycological journals to find ample support for this assertion. Taxonomy as science is seen by most plant pathologists as a confusing side-issue that they are forced to deal with, something that they plan to resolve in the first paragraph of the phytopathological paper they are writing. This tendency, taken together with the reality that descriptive taxonomy does not lead to a high impact factor (new names are frequently used without citing the paper in which they are described), also determines journal policy. Editors carefully set criteria for the types of papers that they accept for publication, and would more easily accept a general taxonomic paper than one dealing with the description of new taxa, something frowned upon by many as "stamp collecting". This has forced descriptive taxonomy into journals rarely consulted by mainstream plant pathologists. To be fair, I must also admit that it is not just journals and their resulting policies that are to blame for the current state of affairs: many taxonomists have refused to convert to employing the state-of-the-art techniques and, by so doing, have also made descriptive taxonomy appear passé. This impression can easily be corrected if a polyphasic approach is employed in more taxonomic research laboratories.

The result of this split between taxonomy and plant pathology is that very few plant pathologists actually know the correct taxonomy of the organisms that they study. Or, to put it differently, they prefer to use the names that they are used to, rather than to adopt the correct nomenclature for the organism. This would not be such a problem, but unfortunately the "old household names" frequently turn out to be based on concepts merging several species together. Even worse, the organism under study may belong to a family or order different from the one in which the generic name applies. Most genera of plant pathogenic fungi contain species that are phylogenetically completely unrelated. So does this really matter, you may ask? The answer to that question is an overwhelming yes. We all drive motor vehicles, and several for instance come from Italy, but there

is a fundamental difference between a Ferrari and a Fiat. Knowing exactly what type of organism is causing a disease is equally important, as I will try to illustrate with a few specific examples.

In the latest review written about the estimated number of fungal species that are thought to exist, Hawksworth (2004) retains his opinion that it is around 1.5 million taxa. He states that the current estimate of the number of plant species in existence, 270 000, appears to be too low. This number strongly influences the estimated number of fungal species, so that as the former number rises, so must the latter at a ratio of not less than 5.5 fungal species per plant. The conclusion from these estimates is that we currently know around 7% of the fungi expected to be in existence. One consideration playing a central role in this debate is our definition of species. In brief, what one scientist sees as populations of a single taxon may be accepted by another as different species. Where do we draw the line, and how do we define species? Not easy questions to answer definitively. A final item that we also need to consider is the never-ending anamorph-teleomorph (asexual-sexual) or dual nomenclature debate. Why, in this modern age, do we still need two names for one organism? Here we are faced with numerous arguments. Interestingly, they can be divided into two camps: those made by people who work with these organisms and who have to identify them on a daily basis, and those made by people mainly dealing with theory alone or with a small, static number of study organisms. Although it is impossible to discuss all these matters in the current text adequately, I will try to list some of them below.

Why names change

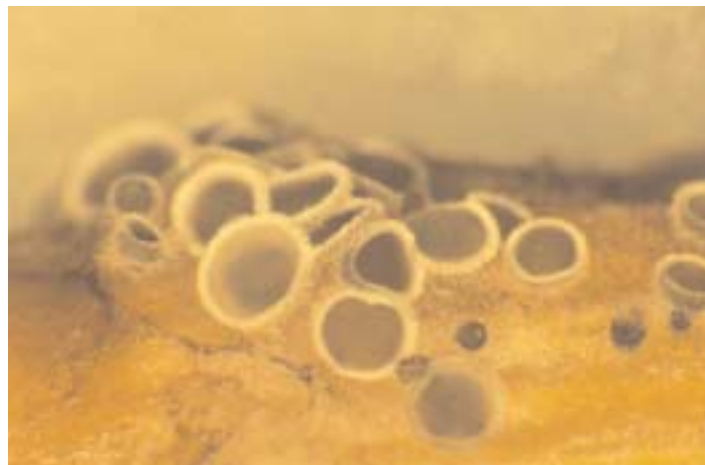
Most fungal names, no matter how attached we are to them, will change with time, unless we conserve them in some legal manner as defined by the International Code of Botanical Nomenclature. This, however, can only be done in a few defined, exceptional situations. Names reflect the current data available about organisms, and thus, it is only logical that as our understanding of the relationships among taxa increases, names will be forced to change so that they do not implicitly contradict the data. Most names are currently based on the phenotype; thus, organisms have a specific epithet or handle based on their appearance. As more data become available, however, we run into various problematic issues, such as convergent evolution, seen as the evolution of the same form in different families and even

orders, so that similar anamorphs may have completely different, unrelated teleomorphs. These names then have to change, as they no longer convey the correct information to the user. Take for instance *Acremonium* Link, a simple anamorph morphology which is known to have affiliations to more than 20 different teleomorph genera (Glenn *et al.* 1999, Rossman *et al.* 1999), or *Cladosporium* Link, which is probably more than 20 different genera (Crous, unpublished data), or *Verticillium* Nees (Zare *et al.* 2000), *Coniothyrium* Corda (Lennox *et al.* 2004, Verkley *et al.* 2004), *Phialophora* Medlar / *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. (Calduch *et al.* 2004, R blov  *et al.* 2004, Vijaykrishna *et al.* 2004), *Fusarium* Link (O'Donnell 1993, Summerbell & Schroers 2002), *Cylindrocarpon* Wollenw. (Halleen *et al.* 2004), *Diplodia* Fr. / *Sphaeropsis* Sacc. (Denman *et al.* 2000, Slippers *et al.* 2004), *Mycosphaerella* Johanson / *Sphaerulina* Sacc. (Crous *et al.* 2003, 2004b), or *Cryphonectria* (Sacc.) Sacc. & D. Sacc. (Gryzenhout *et al.* 2004), to name but a few.

A more specific example is eyespot disease of cereals. The cercosporoid species associated with eyespot disease are rather unusual in resembling leaf spot pathogens accommodated in *Pseudocercospora* Deighton. This has been disputed by some mycologists, who preferred to place these anamorphs in *Ramulispora* Miura (Robbertse *et al.* 1995). The teleomorphs, once found, were revealed to be discomycetes (cup-like sexual structures, apothecia), and described as species of *Tapesia* (Pers.) Fuckel. From the teleomorph association, it was clear that the anamorphs could not be accommodated in *Pseudocercospora*, as species of this genus and *Ramulispora* are anamorphs of *Mycosphaerella*. To add to the confusion, molecular data revealed that *Tapesia* was also not legitimately available for the teleomorph name. The molecularly clarified concept of *Tapesia* was linked to several other anamorph genera, while the eyespot fungi actually represented a unique cluster of lineages for which the name *Oculimacula* Crous & W. Gams (with *Helgardia* Crous & W. Gams anamorphs), was proposed (Crous *et al.* 2003a).

Species, populations, varieties and *formae speciales*

What is a species and what are populations? As discussed above, the Morphological Species Concept is obviously outdated, and no longer works because all names based solely on morphology will have to be re-evaluated. Furthermore, it is clear that the commonly used Biological Species Concept no longer works for many groups, as some isolates within a heterothallic species (requires two opposite mating type strains) can be self-fertile, or worse still, can differ genetically, but still retain the ability to mate with another species, or to form hybrids, making their placement difficult to resolve in phylogenetic trees. For this very reason, the Phylogenetic Species Concept also has limitations, as the decision of where to draw the line between different species is often subjective. A possible solution to this problem is to use the approach of Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (Taylor *et al.* 2000). This approach compares more than one gene genealogy, and



Apothecia of *Oculimacula yallundae*, the causal organism of eyespot disease of cereals

accepts the branches supported by different genes to reveal species. It appears, however, that there is no "golden rule" for fungi, and while DNA sequence data derived from the internal transcribed spacer region (ITS) may adequately reveal species in say *Mycosphaerella*, it is useless in *Fusarium*, *Cylindrocladium* Morgan and *Alternaria* Nees. After sequencing additional genes in accordance with the GCPSR approach, we do, in most cases, find a single gene that adequately supports the species revealed by GCPSR, and that can be used thereafter to position additional new or revised species within the genus (for instance, elongation factor in *Fusarium*, *Botryosphaeria*, ITS in *Mycosphaerella*, β -tubulin in *Cylindrocladium*, etc.).

A lot of time can be spent on arguing about what we see as populations, and where to draw the line between populations, outliers, and species. A good example of this calamity can be found in the *Alternaria* complex occurring on *Citrus*, where Simmons described 10 species from leaf lesions, using concepts based on morphology and cultural characteristics (Simmons 1999). After sequencing five gene loci, however, and taking more of a population genetics approach, Peever and colleagues stated that they considered these species to represent basic variations within *Alternaria alternata* (Fr.) Keissl. (Peever *et al.* 2004). However, in support of Simmons, his concepts for the small-spored species within this complex can be distinguished based on their secondary metabolite profiles, and based on use of some Randomly Amplified Polymorphic DNA (RAPD) primers (B. Andersen, unpublished data). The question of whether these are species or intra-specific variants within *A. alternata* remains unresolved.

In the current age of DNA technology, should we even still refer to varieties and *formae speciales*? A variety is conceived as something that looks somewhat different from a well-known taxon, occurs on a specific host, and, once subjected to DNA analysis, will probably be revealed as a distinct cryptic (closely related) species. A *forma specialis* (special form), however, is more complicated to resolve. *Pyrenophora teres* Drechsler causes net blotch of barley. The fungus is known from two *formae*, namely *P. teres* f.sp. *teres* (net blotch), and *P. teres* f.sp. *maculata* Smed.-Pet. (spot blotch). These two forms can mate, and their progeny



Colletotrichum acutatum sporulating on potato dextrose agar (note typical red pigmentation)

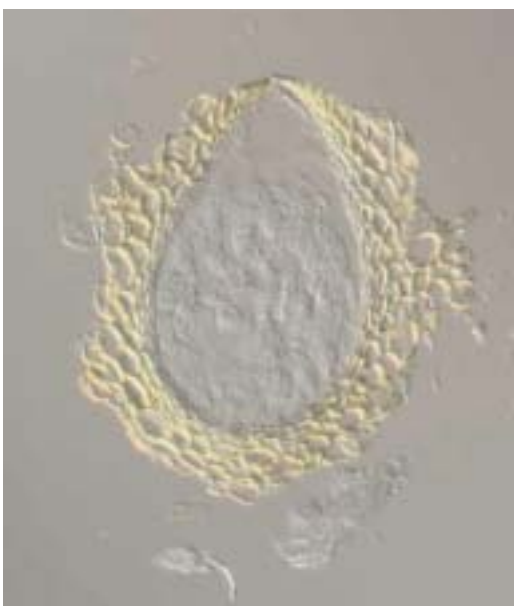
exhibit novel host ranges, symptom expression, and fungicide sensitivity (Campbell *et al.* 1999). It appears, therefore, that speciation is still occurring in *P. teres*, and that the two *formae* will eventually lose the ability to cross sexually, and will evolve as two distinct species (Campbell & Crous 2003).

Colletotrichum acutatum J.H. Simmonds has a wide geographical distribution, and causes anthracnose on a wide range of hosts. In a recent study employing DNA data from two gene loci, as well as morphology, *C. acutatum* f.sp. *hakeae* Lubbe *et al.* could not be distinguished successfully from *C. acutatum* f.sp. *acutatum* (Lubbe *et al.* 2004). However, in nature f.sp. *hakeae* is highly specific in its host range, and does not infect hosts susceptible to f.sp. *acutatum*. In this case, it is premature to conclude that the GCPSR approach failed, as more isolates and gene loci (Taylor *et al.* 2000) need to be sequenced to address species

concepts in this complex adequately. Sufficient to say that we will need to consult all data to enable us to formulate a good working concept of what a species is, and to promote the recognition of genuine species in nature.

Dual nomenclature: the anamorph-teleomorph debate

This rather unusual system originated because it is frequently impossible to say when an asexual state belongs to a specific sexual state without the backup of molecular data. Furthermore, dual nomenclature (two names for one organism) gives us the opportunity to name what we encounter, even if the teleomorph is absent, or may have been lost altogether during evolution. Holomorphic anamorphs do exist (the asexual state being the only state in the life cycle, thus no teleomorph known): though DNA data may reveal a given species of *Diplodia* to belong to *Botryosphaeria*, or a *Cercospora* Fresen. to belong to *Mycosphaerella*, these species actually may lack sexual states altogether. The next question that arises is that if we were to agree that one name should be used per species, would it be the teleomorph generic name or that of the anamorph? In some genera, for instance the anamorph-teleomorph duality *Cylindrocladium-Calonectria* De Not., the informative characters for routine microscopic identification reside in the anamorph (*Cylindrocladium*), and not the teleomorph (*Calonectria*) (Crous *et al.* 2004c). An additional level of complexity is provided by the circumstance that anamorph names often connect to a level of information (spores heat resistant, etc.) different from that associated with teleomorph names. During the International Congress for Plant Pathologists in Christchurch, New Zealand (2003), plant pathologists voted overwhelmingly in favour of retaining dual nomenclature. The adopted concept was, "if it works, do not fix it". However, given the vision of providing unique identifier codes for species, the problem of different names for the same species may disappear spontaneously. Various options have been proposed to derive a



Calonectria asiatica and its anamorph, the teleomorph of *Cylindrocladium asiaticum*. Vertical section through a perithecium of the *Calonectria* teleomorph, and a penicillate conidiophore of the *Cylindrocladium* anamorph

system where one name is to be used for a fungal species. As stated above, though, this should in some cases not be the teleomorph (sexual state) name, but rather that of the anamorph. The situation calls for a careful evaluation of generic names, to make sure that no 'solution' is proposed that causes more confusion than the current system of dual nomenclature, especially since the complexities of the current system are easily handled by means of online databases. Linking species to unique identifier codes (see below), will make the problem of dual nomenclature largely disappear.

Building Species Banks

From the preceding text it is blatantly obvious that names are changing, and will continue to do so. We are currently in the middle of a revolution in fungal taxonomy. Names are linked to data. Old names, however, are limited to insufficiently small data sets (mostly phenotypic), and thus they are subject to change. How will we, as plant pathologists and phytomycologists, deal with this process of continuous change?

The answer to this question lies, I believe, in the taxonomic information backbone. Names are only valuable once linked to meaningful data. These data sets need to be managed in an intelligent fashion, by linking various databases, and making them readily available. One such initiative is MycoBank <www.mycobank.org> (Crous *et al.* 2004a), which will link names to their DNA sequence data, pleomorphic states, herbarium specimens, descriptions, illustrations, publications, etc. Names will also get assigned unique identifier codes that will link them to other data. These codes will in effect keep track of all aspects of the species, providing a bioinformatic structure that will eventually support the formation of species banks. An example of the MycoBank system being linked to a journal can be seen at <<http://www.cbs.knaw.nl/publications/simonline/index.htm>>, in the *Studies of Mycology*, a mycological journal which is now freely available online. In 10 years time, we will probably have reached a stage where we may consider the unique DNA signature and species identifier number to be more relevant and important in species recognition than the name. Field isolates will either fall into the range of DNA variation accepted for a species, or will be detected as new.

Having said all this, I hasten to add that we now face the alarming fact that currently only around 16% of the world's described fungal species are represented by DNA sequence data. If sequences merely representing synonyms and anamorphs were to be removed, this proportion would even be lower. Around 16% of the 100 000 species currently known are represented in culture collections. This is approximately 1.1% of the estimated 1.5 million species on Earth, thus it seems that a very low proportion of the already discovered species are in fact being preserved (Hawksworth 2004). This aspect is a major hurdle standing in the way of all future progress, as species with names unlinked to modern data will have to be recollected and restudied. The fact that interpretation of organisms requires a connection to various types of modern data challenges taxonomists to

employ more sophisticated polyphasic approaches as a standard protocol. In particular, the incorporation of DNA data is needed to ensure stability in names and reliable species recognition. Similarly, plant pathologists are also challenged to deposit the DNA data and voucher strains of the organisms they are working with in appropriate public databanks. This is the only approach, I believe, that will ensure stability – when names are based on verifiable data, not on opinions and statements. As scientists, therefore, we have to lodge our research data and material in public collections, to ensure that we are helping to create a stable environment to practise our science in.

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