The essential symbiosis of mycology and plant pathology: present and future needs

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Correct disease diagnosis is essential for plant pathologists striving to combat plant disease. To support them, mycologists are integrating traditional and molecular techniques. Attention must therefore be given to harnessing the power of molecular genetics to provide rapid methods of identification, and to study the adaptability of plant pathogens in terms of virulence and sensitivity to fungicides. To combat African plant diseases effectively, better co-operation between mycologists and plant pathologists is essential.

Fungal diseases of agricultural crops have drastically influenced human history world-wide, and continue to contribute yearly to heavy losses in produce and revenue. Well-documented accounts of disastrous epidemics such as ergotism that killed humans who had consumed ergot-infected rye in the Middle Ages, to Phytophthora late blight of potato that resulted in more than a million deaths in the late 1840s in Ireland are but a few examples of how important these micro-organisms are to our economy and general well-being. Presently in South Africa, no crops are free of fungal diseases, and the over 300 members of the Southern African Society for Plant Pathology focus their professional attention on combating the devastating effects of plant pathogens.

Before management strategies can be defined for a disease, the causal agents must be identified. It is in this grey area, therefore, that the roles of mycologists (who study and identify these organisms) and plant pathologists (who focus on controlling diseases) overlap. Since the classic studies of Doidge[1] earlier this century, there has been a drastic decline in mycological research in southern Africa. This can be attributed largely to the poor economy, as well as to inadequate teaching and research facilities at South African universities, where students are learning more theory while having less contact with the common disease organisms which pose threats to agriculture. In the past, mycology has been driven largely by pathologists needing to determine the identity of the disease organisms they work with. Currently, however, pathologists tend to have a narrower focus, frequently studying only one or two diseases.

This approach is to the detriment of mycology as a science. It is also somewhat alarming, because Hawksworth[2] estimates that 95% of all fungi are still unknown, and amongst these are bound to be numerous pathogens. The future importance of mycology is also guaranteed by the certainty that the disease spectrum will change significantly in years to come as new pathogens are introduced to an area, different cultural practices are applied, pathogens extend their host ranges, and develop resistance to fungicides. In the exciting field of plant pathological mycology, there are many unans-wered questions concerning both new and well-known pathogenic fungi that need to be addressed. The aim of this article is to draw attention to some of these areas of research from my own field of experience, and to identify examples of potential problems in need of urgent attention.

Implications of sexual morphogenesis

Fungi often have more than one form, or morph, and can occur in their asexual (anamorphic) or sexual (teleomorphic) state. Anamorphs usually undergo genetic change through anastomosis or mutation, while teleomorphs have the advantage of genetic recombination through meiotic division. Fungi can either be homo- or heterothallic, and in Ascomycetes the heterothallic habit is usually determined by two alleles (bipolar mating types). Although it is generally accepted that if two similar strains can mate they belong to the same biological species, Donoghue[3] regarded interfertility data to be of secondary importance because of the possibility of reproductive barriers between sister groups. Blackwell[4] stated that sexual compatibility indicates a barrier to gene flow and is informative, but does not rule out the possibility of a clonal relationship. It is in these situations, therefore, that harnessing the power of molecular genetics has become essential for understanding convergence, and indicating distinct populations amongst morphologically conserved, otherwise apparently similar isolates.

There are several examples of morphologically similar, but distinct, biological species that have hitherto been treated as one. One example is that of eyespot disease of wheat. *Ramulispora herpotrichoides* (Fron) Arx is commonly associated with this disease in the Western Cape of South Africa, as well as in most other wheat-producing parts of the world.[5] To prevent serious losses in wheat fields, this disease must be controlled before symptoms become visible. Chemical control with fungicides is costly, and it is therefore essential that farmers know exactly when to spray for disease control. Molecular research on this disease has provided a quick and effective assay kit to detect the pathogen in apparently healthy wheat plants, thereby facilitating effective, economical control.[6]

Two varieties of this pathogen have been described, namely *R. herpotrichoides* var. *herpotrichoides* and *R. herpotrichoides* var. *acuformis* Nirenberg. The two varieties differ in their virulence to wheat and rye, with *herpotrichoides* being more virulent to wheat than to rye, and *acuformis* equally virulent to the two crops.[7] These two varieties are morphologically similar, but can easily be distinguished using molecular techniques or pathogenicity testing.[8] Recently, the teleomorph of *herpotrichoides* was found, and mating studies showed that it exhibits two-allele heterothallism.[5,9] The same mating habit was also observed for *acuformis*.[10] It was shown that, based on the low DNA similarity (25–50%) between the two varieties deduced from comparisons of random amplified polymorphic DNA, as well as the distinct mating populations, these two varieties should be seen as two separate biological species.[11] Further research is now being focused on the effect of recombination on pathogenicity and fungicide sensitivity. These aspects have been found to be important in Europe, as populations of this pathogen have built up resistance there after a single fungicide was sprayed for several consecutive years. The presence of the teleomorph can, therefore, considerably shorten the time needed for resistance to build up in a population. The sexual state adapts by means of meiosis, whereas the anamorph, in the absence of the teleomorph, has to rely on anastomosis, mutation or migration to broaden its diversity.

Another contentious example of two morphologically similar species is that of *Pyrenophora teres* Drechsler and *Pyrenophora japonica* S. Ito & Kurib., that cause net blotch and spot blotch of barley, respectively. In South Africa, these two leaf blotch symptoms have been attributed to these two pathogens.[12] In Denmark, however, Smedegard-Petersen[13] reported that it was possible to mate the spot and the net form of the pathogen, and suggested that they represent the same biolocal species. Recently, it has been shown that, based on A + T-rich DNA restriction fragment length polymorphism profiles, South African spot and net type isolates were similar, but distinct from outgroups such as *P. semeniperda* (Brittleb. & J.F. Adams) Shoemaker and *P. tritici-repentis* (Died.) Drechsler.[14] Furthermore, using isolates from various countries, successful mating with viable progeny was obtained between spot and net type isolates that showed 74–100% DNA similarity.[15] Inoculation of differential barley cultivars with single-ascospore isolates showed that isolates could be grouped as producing either spot, net or intermediate symptoms. This suggests that crossing-over may have occurred between the spot and net type isolates. Present research aims to identify the mating types, and to interpret the effect of recombination between spot and net type isolates on virulence, symptom expression and fungicide sensitivity.

Integrating alpha, beta and gamma taxonomy

Traditional taxonomy has largely been based on morphological criteria (alpha taxonomy), supported by biochemical or physiological criteria (beta taxonomy). A classification system based solely on genome structure and sequence homology (gamma taxonomy) is impractical. This is because only about 17% of the known fungi are available in culture collections.[2] It is therefore generally accepted that the most practical system lies in the integration of all these taxonomic criteria.

Anamorph/teleomorph connections have hitherto been based primarily on the co-occurrence of both morphs, experimental evidence, or constant association of morphs at different times and geographic locations.[16] Because frequently only a few strains of a holomorph may be available for study, the placing of variable isolates may be problematic. A good example to illustrate this is the fungus *Cylindrocladium clavatum* Hodges & L.C. May. Species of *Cylindrocladium* Morgan have teleomorphs that are placed in *Calonectria* De Not. These fungi and in particular *C. clavatum* are notorious nursery pathogens, causing devastating losses under conditions of high humidity and temperature.[17] Crous and Wingfield[18] accepted that *C. clavatum* was the smaller form of *Cylindrocladium gracile* (Bugnic.) Boesew. But when these species concepts were tested using molecular techniques, it was found that *C. clavatum* was synonymous with *C. gracile*, and that the teleomorphs later described from separate collections represented two additional, distinct biological species.[19] In using traditional taxonomy together with molecular techni-ques, mycologists derive a clearer impression of the weight that might be given to certain criteria in a specific genus.[20]

Another problem experienced by traditional mycologists that can be resolved by means of molecular techniques concerns convergent evolution of morphological features. A good example of this phenomenon occurs in Mycosphaerella leaf blotch disease of *Eucalyptus* trees. This disease has been known since 1909 in South Africa, and has caused devastating losses of *E. nitens* (Deane & Maid.) Maid. and *E. globulus* Labill. Currently, only certain provenances of *E. nitens* can be planted, and *E. globulus* has had to be withdrawn from commercial forestry.[21] More than 23 different anamorph genera have been reported to have *Mycosphaerella* Johanson teleomorphs.[22] On *Eucalyptus* species alone, *Mycosphaerella* species with asexual phases ascribable to five distinct anamorph genera have been reported.[23] It would appear that *Mycosphaerella*, which includes some of the most important pathogenic fungi,[22] should be subdivided into several distinct holomorph genera. This would be possible only with the integration of anamorph features supported by molecular data.

It must be stressed, however, that the identity of strains used in molecular studies must be authenticated by specialists in that specific group, as this will ensure that conclusions are not based on contaminated or misidentified strains.

Present and future needs

Mycologists in a plant pathology environment need to address the role the various morphs of fungi play in adaptability with regard to virulence and fungicide sensitivity. Furthermore, it is essential for mycologists to use all the available tools to help elucidate relationships among known and unknown fungi posing potential threats to agriculture. This includes the integration of traditional and molecular techniques, and therefore also stresses the need for a strong interdisciplinary approach, where the work of mycologists will be strengthened by their co-operating with plant pathologists, geneticists, molecular biologists and biochemists.

With the increasing establishment of small-scale farming in southern Africa, local mycologists will be forced once again to play a supportive role to plant pathologists, ranging from advising in disease clinics to systematic revisions of economically important plant pathogenic genera.[24–26] Science in southern Africa is no longer isolated and must therefore liaise more closely with the rest of the continent. For mycologists and plant pathologists, this should entail drawing up a list of all the plant pathogenic fungi of common interest occurring in Africa, and establishing pan-African links in an effort to combat and study these diseases on a wider front. A database of all the plant pathogenic fungi known in South Africa is being established in my laboratory, and it is hoped that this project might also be extended to include the rest of Africa. This would be a first step in identifying plant pathogens of common interest in the region.

My research is supported by the Foundation for Research Development and the South African Wheat Board. I would like to acknowledge my mentor, Mike Wingfield, for his continued support and enthusiasm.

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