Table 2. Incidence of *Phytophthora* symptoms in the avocado orchard.

Tree condition	Canopy area (ha)		Percentage	
	Block L16	Block L17	Block L16	Block L17
Healthy	2.50	3.83	57.5	56.7
Stressed	1.85	2.91*	42.5	43.2*

*Mean difference significant at $P < 0.001$.

and no. 24 were rated 8 on a scale of 1–10 when checking for disease on the ground and this compared closely with the 89.4% and 83.8% values from image processing.

Figure 3 shows the processed image of the avocado trees. The two classes created distinguished significantly between the disease affected and healthy parts. From Table 2, which presents the percentage of the tree canopy showing symptoms of *Phytophthora cinnamomi*, it is evident that about 43% of the canopy area of the trees

Table 3. Observations on the fungicide control of rootrot disease in a cashew nut orchard.

Treatment	Healthy canopy area (m^2)	(%)	Rootrot-affected canopy area (m^2)	(%)
Untreated trees (10)	612.0	60.0	404.4	40.0
Treated trees (10)	907.1	81.3*	207.6	18.6*

*Mean difference significant at $P < 0.001$.

in both blocks L16 and L17 were stressed. These areas differed significantly from the healthy parts of the tree, which gives the farmer a good indication of how large an area has to be treated against the disease.

The processed image of the cashew nut orchard showed that the trees were affected to some degree with rootrot disease (Fig. 4). Diseased parts showed up as a red colour whereas healthy areas appeared yellow. From the figure it is clear that the upper seven rows of the fungicide-treated trees on the image show less disease-induced stress. Treated trees (10 trees in row no. 7, counted from the top) showed a significantly lower incidence of disease stress (18.6%) than the 10 untreated trees (39.9%) in row no. 8 (Table 3).

Conclusions

Plants under stress, whether affected by disease or nutrient deficiency, produce changes in their physiological status which results in predictable shifts in the spectral reflectance of the canopy. Inexpensive 35-mm CIR photographs taken by remotely controlled aircraft can be scanned into a desktop computer for image processing. Given the relatively low cost of image processing software (such as TNT MIPS), screening and monitoring of stress conditions in crops with colour enhancement can be done by any agricultural scientist even without a strong background in image processing. We have shown that this technology gives the agri-cultural field adviser a tool to quantify the prevalence of rootrot disease in citrus, avocado and cashew nut orchards. Data can be filed and the management of the disease studied over the course of the seasons.

1. Myers V.I., Bauer M.E., Gausman H.W., Hart W.G., Schmugge T.J. and Westin F.C. (1983). Remote sensing applications in agriculture. In *Manual of Remote Sensing*, ed. Robert N. Colwell, pp. 2111–2229. Amer. Soc. Photogrammetry, Falls Church, Virginia.

2. Hoffer R.M. (1989). Color and color infra-red photography for vegetation assessment. In *Proc. 12th Biennial Workshop on Color Aerial Photography and Videography in Plant Sciences*, pp. 1–5. Amer. Soc. Photogrammetry and Remote Sensing, Falls Church, Virginia.

3. Stutte G.W., Stutte C.A. and Newell M.J. (1988). Quantification of water and nitrogen stress in peach trees using ICAS computer video image analysis. In *Proc. First Workshop on Videography*, pp. 137–144. Amer. Soc. Photogrammetry and Remote Sensing, Falls Church, Virginia.

4. Blazques C.H. (1989). Computer-based image analysis and tree counting with aerial color infra-red photography. In *Proc. 12th Biennial Workshop on Color Aerial Photography and Videography in the Plant Sciences*, pp. 149–163. Am. Soc. Photogrammetry and Remote Sensing, Falls Church, Virginia.

5. Fouché P.S. and Booysen N.W. (1991). Remotely piloted aircraft for low altitude aerial surveillance in agriculture. *Appl. Plant Sci.* 5(2), 53–59.

6. Darvas J.M., Toerien J.C. and Milne D.L. (1984). Control of avocado rootrot by trunk injection with phosethyl-Al. *Plant Disease* 68, 691–693.

The author is in the Department of Soil Science, University of the North, Private Bag X1106, Sovenga, 0727 South Africa.

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

P.W. Crous

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 biological 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.

1. Doidge E.M. (1950). The South African fungi and lichens to the end of 1945. *Bothalia* 5, 1–1094.
2. Hawksworth D.L. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.* 95, 641–655.
3. Donoghue M.J. (1985). A critique of the biological species concept and recommendations for a phylogenetic alternative. *Bryologist* 88, 172–181.
4. Blackwell M. (1993). Phylogenetic systematics and Ascomycetes. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*, eds D.R. Renolds and J.W. Taylor, pp. 93–103. CAB International, Oxon.
5. Robbertse B., Crous P.W. and Holz G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathology* 125, 23–28.
6. Unger J.G. and Wolf G. (1988). Detection of *Pseudocercosporella herpotrichoides* (Fron) Deighton in wheat by indirect ELISA. *J. Phytopathol. (Berl.)* 122, 281–286.
7. Hollins T.W., Scott P.R. and Paine J.R. (1985). Morphology, benomyl resistance and pathogenicity to wheat and rye of isolates of *Pseudocercosporella herpotrichoides*. *Plant Pathol.* 34, 369–379.
8. Nicholson P., Hollins T.W., Rezanoor H.N. and Anamthawat-Jonsson K. (1991). A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides*. *Plant Pathol.* 40, 584–594.
9. Dyer P.S., Nicholson P., Rezanoor H.N., Lucas J.A. and Peberdy J.F. (1993). Two-allele heterothallism in *Tapesia yallundae*, the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. *Physiol. molec. Plant Pathol.* 43, 403–414.
10. Dyer P.S., Papaikonomou M., Lucas J.A. and Peberdy J.F. (1994). Isolation of R-type progeny of *Tapesia yallundae* from apothecia on wheat stubble in England. *Plant Pathol.* 43, 1029–1044.
11. Robbertse B., Campbell G.F. and Crous P.W. (1995). Revision of *Pseudocercosporella*-like species causing eyespot disease of wheat. *S. Afr. J. Bot.* 61, 4–48.
12. Scott D.B. (1991). Identity of *Pyrenophora* isolates causing net-type and spot-type lesions on barley. *Mycopathologia* 116, 29–35.
13. Smedegard-Petersen V. (1971). *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. *Yrbk. R. Vet. Agric. Univ. (Copenhagen)*, 124–144.
14. Louw J.P.J., Victor D., Crous P.W., Holz G. and Janse B.J.H. (1995). Characterization of *Pyrenophora* isolates associated with spot and net type lesions on barley in South Africa. *J. Phytopathol. (Berl.)* 143, 129–134.
15. Crous P.W., Janse B.J.H., Tunbridge J. and Holz

16. Nag Raj T.R. (1979). Some coelomycetous anamorphs and their teleomorphs. In *The Whole Fungus, the Sexual-Asexual Synthesis*, ed. W.B. Kendrick, pp. 183–200. Mycologue Publications, Univ. of Waterloo, Ontario.
17. Crous P.W., Phillips A.J.L. and Wingfield M.J. (1993). New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Plant Pathol.* 42, 302–305.
18. Crous P.W. and Wingfield M.J. (1994). A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. *Mycotaxon* 51, 341–435.
19. Crous P.W., Korf A. and Van Zyl W.H. (in press). Nuclear DNA polymorphisms of *Cylindrocladium* species with 1-septate conidia and clavate vesicles. *Syst. appl. Microbiol.*
20. Crous P.W., Janse B.J.H., Victor D., Marais G.F. and Alfenas A.C. (1993). Molecular characterization of *Cylindrocladium* spp. with three-septate conidia and ovoid-like vesicles. *Syst. appl. Microbiol.* 16, 266–273.
21. Crous P.W., Wingfield M.J. and Park R.F. (1991). *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. *Mycol. Res.* 95, 628–632.
22. Arx Von, J.A. (1983). *Mycosphaerella* and its anamorphs. *Proc. K. Ned. Akad. Wet. Ser. C: Biol. Med. Sci.* 86, 15–54.
23. Crous P.W., Carnegie A.J. and Keane P.J. (in press). IMI descriptions of fungi and bacteria, Set 121. *Mycosphaerella cryptica*, *M. delegatensis*, *M. gracilis*, *M. heimii*, *M. marksii*, *M. molleriana*, *M. parkii*, *M. suberosa*, *M. swartii*, *M. walkeri*. *Mycopathologia*.
24. Crous P.W. and Braun U. (1994). *Cercospora* species and similar fungi occurring in South Africa. *Sydowia* 46, 204–224.
25. Crous P.W. and Braun U. (1995). *Cercospora* species and similar fungi of South Africa. *Mycol. Res.* 31–36.
26. Crous P.W. and Braun U. (in press). Cercosporoid fungi from South Africa. *Mycotaxon*.

G. (in press). DNA homology of *Pyrenophora japonica* and *P. teres*. *Mycol. Res.*

Prof. Pedro W. Crous is the mycologist in the Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602.

In forthcoming issues

- Research at the National Botanical Institute
- Extreme sea levels around South Africa
- Threats to Namaqualand flowers
- X-ray diffraction study of fossils
- Alternative approaches to health care
- Anti-malarial drugs
- Research policy in South Africa
- The Iron Age in Botswana
- Atmospheric ozone
- Land-cover classification
- Symmetries of ordinary differential equations
- Lupins in Africa
- Water quality criteria