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FINAL REPORT – SRDC PROJECT BSS215
TAXONOMY OF THE DOWNY MILDEW PATHOGEN
PERONOSCLEROSPORA SACCHARI

by

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SD04010

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SUMMARY

Downy mildew is an important sugarcane disease in Pacific-rim countries. The disease has been present in Australia, but was eradicated in 1957. There are several species of the pathogen *Peronosclerospora* (*P. sacchari*, *P. spontanea*, *P. miscanthi*, *P. philippinensis*, etc) that lead to downy mildew, and there is evidence to suggest the existence of races within species; downy mildew (*P. sacchari*) in PNG had a variable effect on several Queensland varieties in the late 1980s. It is important to know what variation in the pathogen occurs and the likely effect of this on the resistance of commercial varieties. Funding was sought for research into the variation in *Peronosclerospora* species in the hope that tools could be developed to identify species and strains of *Peronosclerospora* using molecular diagnostic techniques.

A PhD student studying at the University of Reading, UK, undertook molecular and traditional taxonomic research into fungi within this pathogen group. Several issues arose during the course of the project that negatively affected project outcomes. These were: (i) poor health of the PhD student; (ii) retirement mid-project of the PhD supervisor; (iii) poor communication skills of the PhD student; (iv) lack of suitable disease specimens; and (v) an absence of interim project reports. Some significant outcomes were achieved, but these related less to specific *Peronosclerospora* species issues and more to relationships between fungi within the broader fungal group.

Outcomes were:

(i) A major revision of the Peronosporomycetes group was proposed based on traditional and molecular techniques.

(ii) Certain molecular assays were shown to be useful in distinguishing genera and species within the Peronosporomycetes group.

(iii) Only one *Peronosclerospora* species was included in the study (*Peronosclerospora sorghii*); this affected sugarcane-related outcomes.

(iv) Using BSES isolates, the study confirmed the very close association of the sugarcane pathogens *Pythium arrhenomanes* and *P. graminicola.*

(v) Fungi in the group should be called Peronosporomycetes, not Oomycetes, and considered ‘true’ fungi.

(vi) The student was awarded his PhD in early 2004 – supplied to SRDC. Further papers will be written on thesis research in the coming year.

1.0 BACKGROUND

Downy mildew disease of sugarcane is caused by several species within the genus *Peronosclerospora* (*P. sacchari, P. miscanthi, P. philippinensis, P. spontanea*). The disease is of economic importance in Pacific-rim countries such as Papua New Guinea (PNG), Fiji, Taiwan, and Philippines. The disease has been present in Australia (*P. sacchari*) and was probably introduced with early sugarcane introductions. A concerted eradication program based on resistant varieties, selective plough out of diseased crops and legislation to prevent the growth of the alternative, highly susceptible host maize close to cane crops, led to eradication. The last known diseased commercial crop was located in the Bundaberg district in 1957. PNG is most likely the home of downy mildew (caused by *P. sacchari*) and it is here that pathogen variability is most likely to exist, if it does.

Downy mildew is the economically most important disease in PNG, with up to 50% of imported germplasm discarded because of disease susceptibility. Many of the most important Australian cultivars cannot be grown in PNG because of susceptibility to downy mildew. BSES-bred clones are more susceptible than those from other breeding programs, making the disease an important quarantine consideration for the Australian industry. Concern was raised into variation in the pathogen when two Australian-bred cultivars (Cassius and Q107) suddenly became susceptible to the disease at Ramu Sugar in the late 1980s. Variation in *P. sacchari* had not been examined previously and there was little evidence on pathogen variability in existence elsewhere.

There are few traditional taxonomists with expertise in this group of fungi. However, a PhD student at the University of Reading, UK, began a project to compare traditional and molecular techniques as a means of comparing the taxonomy of downy mildew-type fungi. His supervisor was Dr Michael Dick, well known to BSES pathologists as he assisted with the description of the sugarcane root pathogen *Pachymetra chaunorhiza.* Funding was obtained from SRDC to support research by the PhD student.

Several issues arose that negatively affected sugarcane-related outcomes from this research. These were: (i) poor health of the PhD student; (ii) retirement mid-project of the PhD supervisor; (iii) poor communication skills of the PhD student; (iv) lack of suitable disease specimens; (v) lack of interim reporting by the student to keep BSES up-to-date with research outcomes and directions. The poor student communication skills hindered timely writing of this final report; this report could only be written by extracting information from the student's thesis. The scope of the fungi examined by the student was much broader than anticipated and included many fungi within the Oomycete group including other sugarcane pathogens - *Pachymetra chaunorhiza, Sclerophthora macrospore, Pythium arrhenomanes* and *Pythium graminicola*. Most of these pathogens had originally been sent to the UK by BSES in the 1980s.

Although these problems arose, there were significant outcomes from the work that are likely to have a major impact on the taxonomy of the broader group of fungi containing the downy mildew pathogens. These are summarised in this report.

2.0 OBJECTIVES

SRDC funding objectives

- Utilise biotechnological and traditional taxonomy to develop assays for different species and strains of *Peronosclerospora*.
- Provide a resource for the proposed ACIAR-funded project for determining the distribution of species and strains of *Peronosclerospora* (sugarcane downy mildew pathogens) within the centre of origin of *Saccharum officinarum*.

Neither objective was addressed by the student in the depth expected.

Stated PhD research objectives

The key objectives quoted in the thesis were:

- The evaluation of the validity and utility of dividing the Peronosporomycetes into subclasses Peronosporomycetidae, Rhipidiomycetidae and Saprolegniomycetidae.
- Evaluating the placement of both the dicotyledonous downy mildews and graminaceous downy mildews within the same taxon. Generic concepts within the Pythiales and Saprolegniaceae were re-appraised.

These were not entirely the objectives envisaged when funding was provided, but they still addressed some useful concepts.

3.0 METHODS

The student set out to examine the relationships between various fungal species and groups in the Peronosporomycetes group. He included a number of species of fungi (165), used traditional taxonomy to separate species and groups, and compared these groupings to those suggested by molecular approaches. The techniques allowed the comparison of some closely related species within genera. The student then made suggestions for regrouping genera and species (with some new genera suggested). This provided further information on relationships between fungi in the general group of downy mildew fungi.

3.1 Traditional taxonomy

Traditional (morphological, cytological, ultra-structural) methods to distinguish species of fungi were used to determine the identity of the fungal species within the Peronosporomycetes group. Details of the techniques used in the research are given in Spencer (2004) - PhD thesis titled *Peronosporomycetes: molecular phylogenies and organism evolution*. The fungal specimens used in the study had largely been collected and identified some time previous to the PhD research and stored in UK collections.

3.2 Molecular methods

The molecular approaches for separating species, genera and groups of fungi are detailed below. It is included here to provide ready and convenient access by molecular biologists at a later time. Tree analyses were used to determine the 'relatedness' of various fungal groups, genera and species.

The molecular studies utilised rDNA cistron as a means to distinguish genetic variation. SSU rDNA is part of the rDNA cistron and occurs in tandem arrays of multiple copies (Hershkovitz *et al*. 1999). There is a high degree of conservation with coding regions of the rDNA cistron. SSU rDNA is the precursor, prior to transcription, translation and processing of SSU rRNA, which alongside approximately 30 proteins make up the 40S subunit of eukaryotic ribosomes. The other ribosomal unit (60S) is derived from LSU, 5.8S rDNAs and is approximately 45 proteins. Additionally, there is a 5S subunit that is genomically unrelated to the other ribosomal units. Additional to the coding regions, there are spacer regions, the external transcribed spacer (ETS) and two internal transcribed spacers (ITS1 and ITSII). There is a considerable degree of conservation in rRNA secondary structure, which correlates with rDNA sequence conservation, the functionality of which is largely unresolved. Regions of considerable variability (ITI and ITII) contrasts markedly with conserved, coding parts of the rDNA. The range in the level of variation within the rDNA cistron was the basis for its usage as a phylogenetic tool in these studies.

The construction of robust phylogenies often requires data from more than one locus. In this study, the classification previously proposed for the Peronosporomycetes was tested using SSU rDNA and combined multi-loci sequence data. 168 Peronosporomycetes were used in the study. Sequence data from three gene loci were considered: two nuclear (SSU rDNA and LSU rDNA) and one mitochondrial (COX2). The isolates represent approximately 25% of all Peronosporomycetes.

4.0 RESULTS

Full results are given in Spencer (2004). Outcomes related to sugarcane pathogens were hindered for the reasons stated in section 1.0; significant sugarcane-related outcomes are detailed briefly below.

4.1 Name of the downy mildew group of fungi

The fungal group containing the downy mildew pathogens (both dicot and monocot pathogens) was years ago called 'phycomycetes' and later 'oomycetes'; a recommendation from the thesis research is that they be called 'Peronosporomycetes'. Dr Spencer also argued that it was entirely appropriate that they be considered fungi rather than close relatives to algae, as otherwise suggested (Cavalier-Smith 1987).

4.2 Breadth of study into sugarcane downy mildews

Only one *Peronosclerospora* species was included in the study, *Peronosclerospora sorghii,* a non-sugarcane downy mildew pathogen. This had direct consequences on the sugarcane-related outcomes from the research. Unfortunately, no direct information on how the sugarcane downy mildew pathogens relate to each other, or to the other fungi in the same pathogen group, was obtained in this PhD research. However, the molecular methods employed by Dr Spencer may well be useful in future sugarcane studies. Some other sugarcane pathogens (not downy mildews) in this group of fungi (*Pachymetra chaunorhiza, Sclerophthora macrospora, Pythium arrhenomanes* and *Pythium graminicola*) were included and some useful information was obtained on these species. Molecular assays suggested *Pachymetra chaunorhiza* should remain in the group where it was previously placed (Order Sclerosporales).

4.3 Classification of groups within the Peronosporomycetes

Regrouping of the fungi within the Peronosporomycetes was suggested in this study. Previous groupings of the fungi are outlined in Table 1. The suggested regrouping is detailed in Table 2.

In addition, changes to the species contained within the genus *Pythium* were also suggested. A new genus is proposed and the details are outlined in Table 3.

4.4 Other notable findings

- *Sclerophthora* maybe an intermediary between the grass downy mildews and *Peronospora* (dicot downy mildews).
- *Pachymetra* oospores are truly plerotic and the oospores may be separated from the oogonial wall.
- The Verrucalvaceae (including *Pachymetra)* are biochemically intermediate between the Peronosporomycetidae and the other Saprolegniomycetidae in terms of response to the fungicidal isoxazoles (Hymexazole) and the phenylamides (Metalaxyl). The Verrucalvaceae are relatively resistant to Metalaxyl (as are Saprolegniaceae and Leptomitaceae, unlike the susceptible Phytophthora).
- Grass downy mildews are primarily associated with the highly evolved tropical grazing grasses of the subfamilies Chloridoideae and Panicoideae. The latter family has its centre in South East Asia.
- There are two apparent centres of diversity for *Peronosclerospora*:
  Sub-continental India: *P. dichanthiicola, P. heteropognis* and *P. westonii.*
  Eastern Melanesia and Australasia: *P. globosa, P. maydis, P. miscanthi, P. noblei, P. sacchari, P. spontanea.*
  The other species (*P. philippinensis and P. sorghii)* are of widespread occurrence (Figure 1).
- *Sclerophthora* spp have no predisposition to grasses of the Panicoideae or the Chloridoideae. Hosts include over 140 taxa throughout the Poaceae.
- *P. northi* has been transferred out of the *Peronosclerospora* and into *Sclerospora.*

Table 1 Original classification of the Peronosporomycetes

	Peronosporomycotina											
Class	**Peronosporomycetes**											
Subclass	Peronosporomycetidae			Rhipidiomycetidae	Saprolegniomycetidae							
Order	Peronosporales		Pythiales	Rhipidiales	Saprolegniales		Sclerosporales		Salilagenidiales		Leptomitales	
Family	Peronosporaceae	Albuginaceae	Pythiaceae	Rhipidiaceae	Saprolegniaceae	Leptolegniaceae	Sclerosporaceae	Verrucalvaceae	Salilagenidiaceae	Haliphthoraceae	Leptomitaceae	Apodachlyellaceae
Genera	*Bremia* *Peronospora* *Plasmospara*	*Albugo*	*Cystosiphon* *Halophytophthora* *Lagenidium* *Peronophythora* *Phytophthora* *Pythium*	*Sapromyces*	*Achlya* *Aplanes* *Aplanopsis* *Brevilegnia* *Calyptralegnia* *Dictyuchus* *Isoachlya* *Newbya* *Protoachyla* *Pythiopsis* *Saprolegnia* *Scoliolegnia* *Thraustotheca*	*Aphanomyces* *Leptolegnia* *Plectospira*	*Peronosclerospora* *Sclerospora*	*Pachymetra*	*Salilagenidium*	*Atkinsiella* *Haliphthorus* *Halodaphnea*	*Apodachlya* *Leptomitus* *Plerogone*	*Apodachlyella*

Table 2 Revised classification of the Peronosporomycetes following molecular research conducted by Dr Spencer

	Peronosporomycotina										
Class	**Peronosporomycetes**										
Subclass	Peronosporomycetidae					Rhipidiomycetidae	Saprolegniomycetidae				
Order	Albuginales		Peronosporales	Pythiales		Rhipidiales	Saprolegniales			Leptomitales	
Family	Albuginaceae	Peronosporaceae	Phytophthoraceae	Sclerosporaceae	Pythiaceae	Rhipidiaceae	Saprolegniaceae	Leptolegniaceae	Verrucalvaceae	Leptomitaceae	Apodachlyellaceae
Genera	*Albugo*	*Bremia* *Peronospora* *Plasmospara*	*Phytophthora*	*Peronosclerospora* *Sclerospora* *Sclerophthora*	*Halophytophthora* *Lagenidium* *Pythium* *Ampulla* *Helodoxa* *Salilagenidium*	*Sapromyces*	*Achlya* *Racema* *Aplanes* *Aplanopsis* *Brevilegnia* *Calyptralegnia* *Dictyuchus* *Isoachlya* *Newbya* *Pythiopsis* *Scoliolegnia* *Thraustotheca*	*Leptolegnia*	*Aphanomyces* *Pachymetra* *Plectospira* *Verrucalvus*	*Leptomitus*	*Apodachlya* *Apodachlyella* *Plerogone*

Table 3 **Details of the revised grouping of species that all formerly belonged to the genus *Pythium***

Pythium	***Ampulla*** (new genus)
adhaerens	*australe*
aphanidermatum	*debaryanum*
aquatile	*echinulatum*
arrhenomanes	*heterothallicum*
coloratum	*hypogynum*
deliense	*irregulare*
diclinum	*iwayamae*
dissimile	*mamillatum*
graminicola	*middletonii*
indigoferae	*minor*
insidiosum	*multisporum*
lutarium	*nagae*
monospermum	*opalinum*
myriotylum	*ostracodes*
pachycaule	*paddicum*
papillatum	*parvum*
pyrilobum	*pleroticum*
sulcatum	*prolatum*
tardicrescens	*pulchrum*
tenue	*pyrilobum*
torulosum	*rostratum*
vanterpoolii	*salinum*
volutum	*spinosum*
	splendens
	sylvaticum
	ultimum
	violae

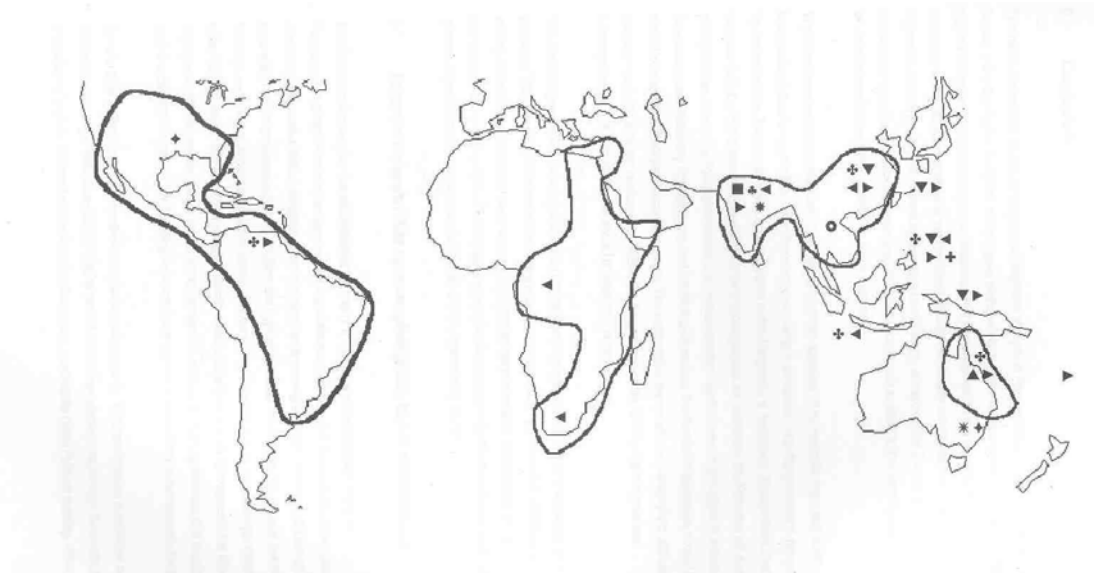

Fig. 26: **Known distribution of *Peronosclerospora* species**. *Peronosclerospora*: ■ - *dichanthiicola*, ✳ - *eriochloae*, ✦ - *globosa*, ♣ - *heteropogonis*, ✣ - *maydis*, ▼ - *miscanthi*, ▲ - *noblei*, ◄ - *philippinensis*, ► - *sacchari*, ━━ - *sorghi*, ✚ - *spontanea*, ✸ - *westonii*, ✪ - *zeae*.

Figure 1 Known world distribution of *Peronosclerospora* species

5.0 OUTPUTS

The absence of communication from the PhD student during the course of his PhD candidature meant that BSES was unaware of some of the directions of his research. This hindered sugarcane-related outcomes and the results were not as applicable to sugarcane downy mildews as expected. There remains a need therefore for research into molecular tools for separating the various species causing downy mildew in sugarcane.

However, high quality research was undertaken by the student, a reflection of the standards of his supervisor, Dr Michael Dick, and a thesis was produced (Appendix 1). The re-grouping of the Peronosporomycetes is likely to have some far-reaching implications, not least of which is the suggested splitting of *Pythium* species into two genera. Some of the reclassified species are of great economic importance and altered names could lead to some confusion in the literature. The regrouping will not directly affect the classification of the root-infecting *Pythium* species affecting sugarcane. The research undertaken by Dr Spencer confirms the close morphological association of the species *Pythium arrhenomanes* and *P. graminicola,* as noted by BSES pathologists.

The findings by the student, coupled with the results from previous studies, confirmed that the downy mildews affecting grasses should be separated from those affecting dicots; the grass downy mildews are in the Order Sclerosporales, while the dicot downy mildews are in the Order Peronosporales. The sugarcane root pathogen, *Pachymetra chaunorhiza,* was previously placed close to the grass downy mildews, but this study suggested it is more closely related to the Saprolegniomycetidae (including species of *Aphanomyces*).

The major outputs of the thesis were:

1. The group of fungi causing downy mildew diseases is now called the Peronosporomycetes (in contrast to the phycomycetes or oomycetes).
2. This group of organisms should be considered true fungi, not close relatives of the algae.
3. Significant changes are suggested for the taxonomy of the Peronosporomycetes, with new genera created for some previous *Pythium* species. The Verrucalvaceae (including *Pachymetra)* have been transferred from the Peronosporales to the Saprolegniales.
4. Molecular tools, coupled with traditional methods, enabled changes to the understanding of the phylogenies of various groups of fungi within the Peronosporomycetes. Molecular tools proved useful in separating related groups of fungi.
5. *Pythium arrhenomanes* and *P. graminicola*, root pathogens of sugarcane, were shown to be very closely related as found in morphological studies conducted by BSES pathologists.
6. Little research was undertaken into species differences in the *Peronosclerospora* genus; further research is needed to develop molecular tools to separate sugarcane downy mildew pathogens.

6.0 OUTCOMES AND FURTHER RESEARCH

The molecular methods used by Dr Spencer are outlined in depth in his thesis and, while they do not address differences between *Peronosclerospora* species, are likely to be useful in any further studies in species variation in this fungal group. There is a definite need for further research into molecular tools for separating the *Peronosclerospora* species - *P. sacchari*, *P. philippinensis*, *P. spontanea* and *P. miscanthi.* This will require the collection of specimens from various parts of south east Asia and adaptation of the molecular tools outlined in the thesis. As there are not many pathologists in the region where these downy mildew species are found, and the morphological features of these fungi are ephemeral, it would be ideal if disease surveys were to be carried out through south east Asia, downy mildew specimens collected at the same time and preserved appropriately. Follow up ACIAR projects to the current project in PNG, Indonesia and northern Australia could provide this opportunity.

There is no doubt Dr Spencer's research has contributed significantly to our understanding of the taxonomy of Peronosporomycetes fungi in general, but not much to the differences between species of *Peronosclerospora*. Further research is required if molecular biologists are to contribute to species identification of sugarcane downy mildew pathogens. The re-grouping of taxa, as suggested in Dr Spencer's thesis, will not take effect until the results are fully published in refereed journals; this may take some time to accomplish.

7.0 PUBLICATIONS

Spencer MA. 2004. *Peronosporomycetes: molecular phylogenies and organism evolution*. PhD thesis, The University of Reading, Reading, UK. 175 pp.

Spencer MA and Dick MW. 2002. Aspects of graminicolous downy mildew biology: perspectives for tropical plant pathology and Peronosporomycetes phylogeny. In: Tropical Mycology, Vol. 2, Micromycetes (eds Watling R, Frankland JC, Ainsworth AM, Isaac S, Robinson CH), pp 63-81. CABI Publishing, Wallingford, UK.

Spencer MA, Vick MC and Dick MW. 2002. Revision of *Aplanopsis*, *Pythiopsis* and 'subcentric' *Achlya* (Saproleniaceae) using SSU rDNA and morphological data. *Mycological Research* 106: 549-560.

8.0 ACKNOWLEDGEMENTS

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9.0 REFERENCES

Cavalier-Smith T. 1987. The origin of fungi and pseudofungi. In: *Evolutionary Biology of the Fungi* (eds Rayner ADM, Brasier CM and Moore D) pp. 339-353. Cambridge University Press, Cambridge, UK.

Hershkovitz MA, Zimmer EA and Hahn WJ. 1999. Ribosomal DNA sequences and angiosperm systematics. In: Molecular systematics and plant evolution (eds Hollingsworth PM, Bateman RM and Gornall RJ), pp. 268-326. Taylor and Francis, London, UK.

Spencer MA. 2004. *Peronosporomycetes: molecular phylogenies and organism evolution*. PhD thesis, The University of Reading, Reading, UK. 175 pp.