Dossier on sugarcane downy mildew (species of the genus PERONOSCLEROSPORA) as a disease of sugarcane

Sugar Research Australia

DOSSIER ON SUGARCANE DOWNY MILDEW (SPECIES OF THE GENUS *PERONOSCLEROSPORA*) AS A DISEASE OF SUGARCANE

Disease & Cause	Downy mildew is a systemic fungal disease of sugarcane that has significant economic consequences in susceptible varieties. Sugarcane downy mildew can be caused by four species: *Peronosclerospora sacchari*, *P. miscanthi*, *P. spontanea* and *P. philippinensis*. In Papua New Guinea the causal agent might include an unclassified member of the *Peronosclerospora* (SRA final report).
Host range	Hosts in the *Saccharum* genus include: *S. officinarum*, *S. robustum*, *S. spontaneum* and *S. edule*. Other hosts include the following: *Zea mays* (maize), teosinte (*Euchlaena mexicana*), *Sorghum halapense* (Johnson grass), *S. sudanense* (Sudan grass), *Tripsacum dactyloides* (Gama grass), *Sorghum bicolor* (broom corn). Other studies suggest there are 18 species that are systemically infected in the sub-family Panicoideae; these include species of *Andropogon*, *Bothriochloa*, *Eulalia*, *Schizachyrium* and *Sorghum* (Bonde & Peterson 1981).
Distribution	The disease is restricted to the Pacific, South Asia and South East Asian regions: Fiji, India, Indonesia, Japan, Papua New Guinea, Philippines, Taiwan and Thailand (Suma & Magarey 2000).
Key Signs	The most identifiable symptoms are creamy white leaf stripes that turn red with age and stunting of infected stools. After warm humid nights, down may be seen on the underside of leaves showing stripe symptoms. Less common symptoms include elongated, thin stalk growth (jump ups); brown lesions on external stalk surfaces and leaf shredding (Suma & Magarey 2000).
Spread	The disease is principally spread via infected planting material and also by wind-borne conidia. Spores are fragile and last only for several hours after formation. Spores generally can only travel a few hundred metres. Long distance spread is almost exclusively via infected planting material.
Persistence of the fungus	The pathogen persists in the cane plant (it is systemic) but can be eliminated by hot water treatment of infected stalks (50^0 C for 2 hours) or by dipping infected material in metalaxyl (1.25 g a.i./ litre water). Conidial spores are fragile and only survive for a few hours after release. Oospores are produced which possibly can survive for longer periods but their importance in spread of the disease is unknown.
Control strategy	The main disease control strategy is the cropping of resistant varieties coupled with the use of disease-free plant sources (Suma & Magarey 2000). If susceptible crops are eliminated and disease-free sources of more resistant varieties planted, the disease is fairly rapidly brought under control. The disease can be eliminated from seed cane by hot water treatment (50°C for 2-3 hours) or by dipping in the fungicide metalaxyl. Metalaxyl is also effective at controlling infection when applied as a foliar spray or soil application.

NATURE OF THE DISEASE

Aetiology

Sugarcane downy mildew can be caused by one of several fungi in the *Peronosclerospora* genus, these include *P. sacchari* (T. Miyake) Shirai & Hara (1927), *P. spontanea* (Weston) C.G. Shaw, *P. miscanthi* (T. Miyake) C.G. Shaw and *P. philippinensis* (Weston) C.G. Shaw. *P. sacchari* and *P. philippinensis* are considered to be the most important pathogens of commercial sugarcane crops. In Papua New Guinea there is evidence that an unclassified member of the *Peronosclerospora* can cause downy mildew: this member is suspected to be closely related to the known sugarcane-infecting species (SRA Final Report).These pathogens invade the sugarcane plant via conidia landing on young buds and via young leaf tissue at the base of the leaf spindle in young shoots. The pathogen invades the stalk tissue and moves through the cane plant to infect newly-developed leaves. With time, these show the characteristic leaf striping symptoms.

Host Range

P. sacchari

Hosts in the *Saccharum* genus include: *S. officinarum*, *S. robustum*, *S. spontanea* and *S. edule* Hughes & Robinson, 1961). Other hosts include the following: *Zea mays* (maize), teosinte (*Euchlaena mexicana*), *Sorghum halapense* (Johnson grass), *S. sudanense* (Sudan grass), *Tripsacum dactyloides* (Gama grass), *Sorghum bicolor (*broom corn). Other studies suggested 18 species became systemically infected (when artificially inoculated) in the sub-family Panicoideae: these include species of *Andropogon*, *Bothriochloa*, *Eulalia*, *Schizachyrium* and *Sorghum* (Bonde& Peterson, 1981).

P. philippinensis

Hosts include species of *Zea*, *Saccharum, Euchlaena* and *Sorghum*. *P. philippinensis* causes one of the serious diseases of maize, known as Philippine downy mildew or sleepy disease. This species is a common cause of downy mildew in sugarcane in the Philippines (Sivanesan & Waller 1986).

P. miscanthi

Hosts include species of *Saccharum* and *Miscanthus* (Sivanesan & Waller 1986).

P. spontanea

Hosts include *S. officinarum, S. spontaneam, Zea mays, Euchlaena luxurians* and *Miscanthus japonicus* (Sivanesan & Waller 1986).

Distribution

P. sacchari

The disease is found in different parts of South Asia, South East Asia and the Pacific including the following countries: Fiji, India, Indonesia, Papua New Guinea, Philippines, Taiwan and Thailand (Suma & Magarey 2000; Leu, 1996; Kuniata *et al.* 2006; Wang *et al.* 1994; Magarey *et al.* 2008a & b; Shieh *et al.* 2006). In some of these countries the disease is rarely seen (for instance, Fiji, Tamanikaiyaroi & Johnson 1996) while in others the disease is widespread. In Papua New Guinea, four species of *Saccharum* have a wide distribution, both in the wild and in indigenous gardens: downy mildew can be seen throughout the country (Magarey 1996;

Magarey *et al.* 2008). Several pathogen species are thought to occur in various parts of PNG (Hughes & Robinson 1961) but insufficient study of the disease have meant that pathogen and species distribution information lacks detail. A molecular assay is available for detection of the pathogen and species/taxa in PNG. Downy mildew distribution in PNG includes the Eastern Highlands,, Morobe, and Madang Provinces (SRA Project BSS331 Final Report). There is an unconfirmed report of a sugarcane downy mildew pathogen (either *P. sacchari* or *P. philippinensis*) in mainland China (Wang *et al.* 1994).

P. philippinensis
Philippines. There is one report of *P. philippinensis* on *S. spontaneum* in India (Chona & Suryanarayana 1955).

P. miscanthi
Fiji, Philippines, Taiwan and likely Papua New Guinea.

P. spontanea
Thailand and Philippines.

***Peronosclerospora* species (unclassified)**
Evidence that an unclassified *Peronosclerospora* is present in PNG. The species is most likely to be closely related to *P. sacchari* and *P. miscanthi* (SRA Final Report)

Diagnosis
Plant symptoms
All four pathogens cause similar symptoms. The following descriptions are for downy mildew caused by *P. sacchari*, the disease most thoroughly studied.

Typical symptoms of downy mildew are leaf streaks of 1-3mm in width which are separated by normal green leaf tissue. Streaks vary in length and may be just a few centimetres or run the length of the leaf blade. There may be up to 30-40 leaf streaks on individual leaves, though in some varieties there may be relatively few streaks of much wider dimension. Streaks can occur on the lower surface of the mid-rib but not usually on the leaf sheath (Hughes & Robinson 1961, Leu & Egan 1989, Suma & Magarey 2000). Cooler conditions may lead to a narrowing of leaf streaks.

Initially leaf streaks are pale creamy-yellow in colour, but with age streaks turn yellow and then to a mottled brick-red colour (Hughes & Robinson 1961; Leu & Egan 1989). In some varieties, streaks may be a consistent red colour. When symptom development is intense, the whole shoot may appear mottled-red and discoloured.

On warm humid nights, the pathogen produces a soft white velvety down on the underside of leaves (Hughes & Robinson 1961; Leu & Egan 1989). With time, the down may appear like a grey powder as the conidiophores and conidia dry and age.

Initial symptoms in young systemically-infected plant cane are a mottled paleness of the young spindle leaf (Leu & Egan 1989). Surviving stalks and stools are characterised by narrow discoloured leaves, and upright habit, abnormally thin stalks and varying degrees of stunting (Hughes & Robinson, 1961; Leu & Egan 1989).

Mature stalks may develop side-shoots in autumn and early winter leading to a witches broom effect (Leu & Egan 1989). Not all lateral buds will side-shoot and it appears that it is the infected buds that germinate. Oospores (sexual spores of the fungus) develop as winter approaches and as these form they may lead to leaf splitting; the spores cause a sideways force on the interveinal leaf tissue causing the tissues to separate. Leaf splitting can be a quite spectacular symptom. Often the production of oospores is also accompanied by the rapid elongation of infected stalks which leads to thin, brittle stalks referred to as 'jump ups' (Hughes & Robinson 1961). These weakened stalks may bend; when they do, they are often higher than the remaining healthy stalks in the canopy. The stunting associated with systemic infection of susceptible varieties can cause losses of up to 40% or more (Rauka *et al.* 2005b; Suma & Pais 1996).

Figure 1: young leaf symptoms (white leaf stripes) in sugarcane affected by downy mildew, Gusap, PNG

Figure 2: more mature downy mildew leaf symptoms on sugarcane; note the reddening starting to appear in the otherwise light-coloured leaf stripes

Figure 3: reddening associated with older downy mildew symptoms in sugarcane growing at Gusap, PNG

Figure 4: extensive reddening on leaves affected by downy mildew

Figure 5: elongated stalks (jump-ups) caused by downy mildew growing among flowering stalks in a crop affected by the disease.

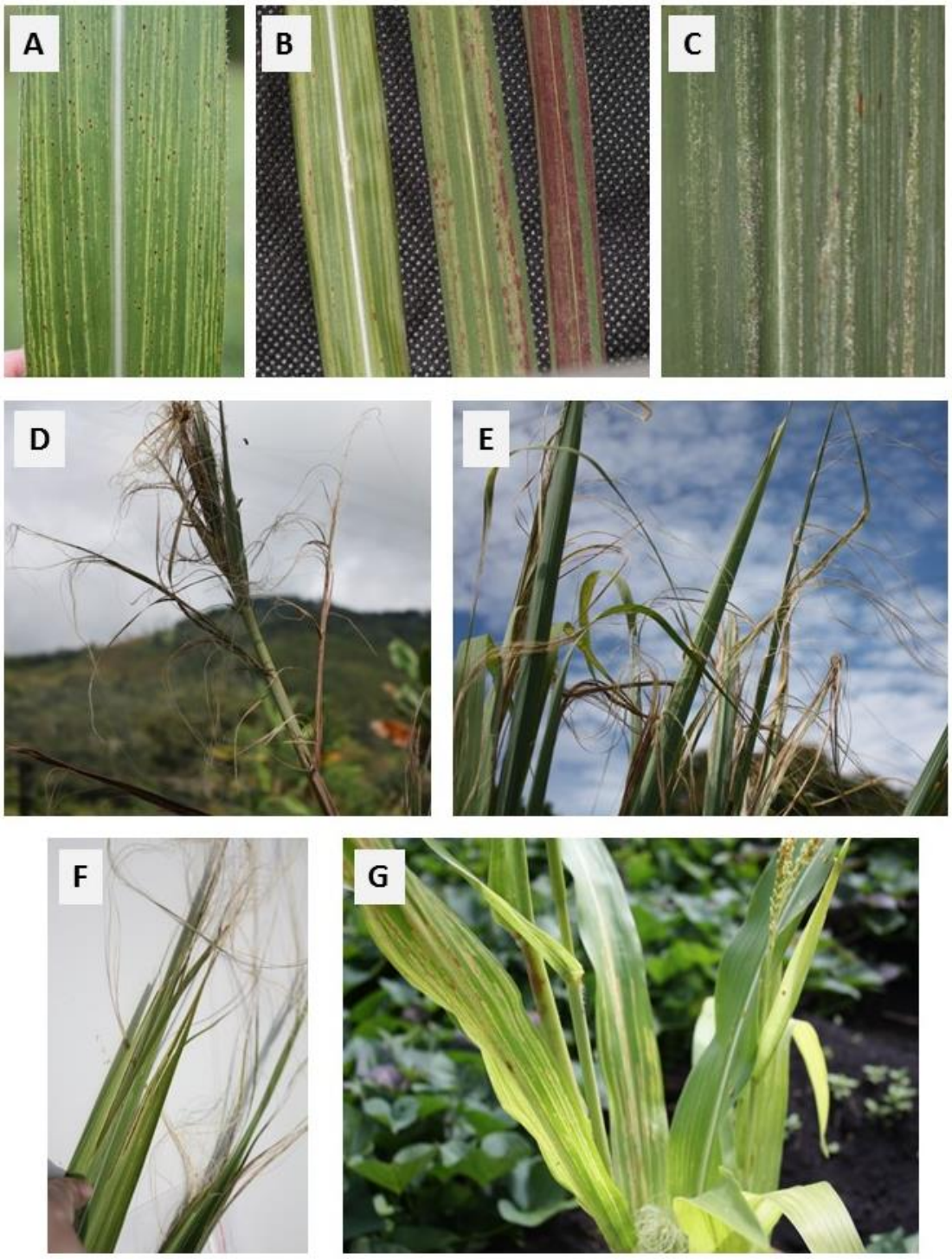

Figure 6: Leaf symptoms of downy mildew and leaf shredding in Papua New Guinea. A) Leaf streaking on commercial sugarcane; B) Reddened leaf streak symptoms on *S. edule*; c) Close-up of down formation along the leaf streaks on commercial sugarcane; D) Leaf shredding symptoms on *Miscanthus*; E) Leaf shredding symptoms on *S. robustum*; F) Leaf shredding on commercial cane; G) Downy mildew leaf symptoms on corn (*Zea mays*) (Thompson et al., 2013).

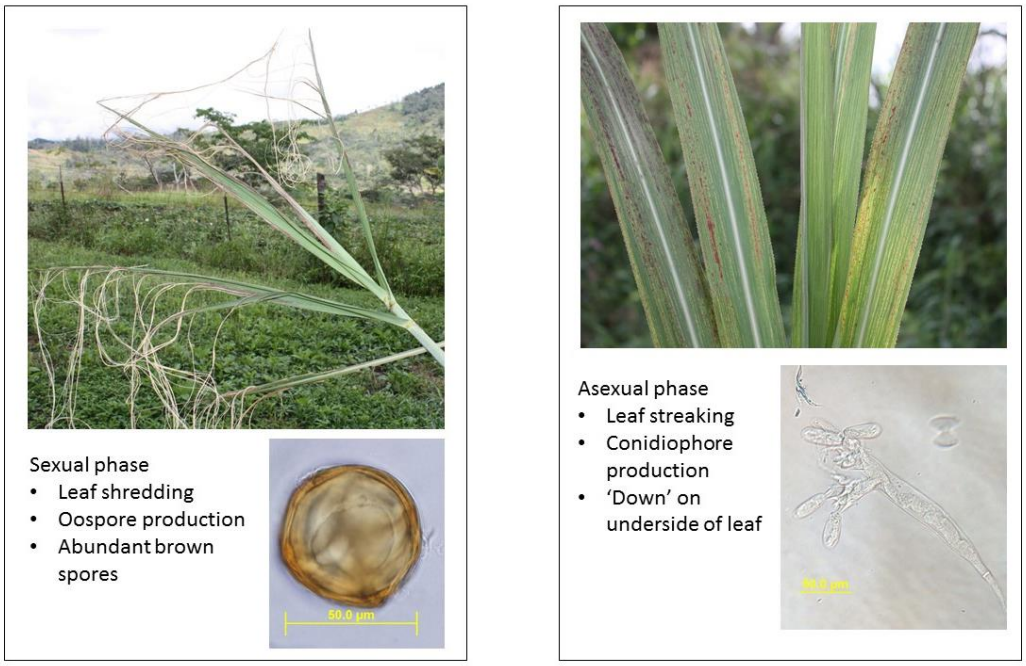

Figure 7: Typical leaf symptoms of downy mildew for both the sexual and asexual phases of *Peronosclerospora* species in PNG (SRA Final Report).

Fungal taxonomy

Descriptions of the four species of *Peronsclerospora* that infect sugarcane can be found in Sivanesan & Waller (1986). Drawings and descriptions of *P. sacchari* and *P. philippinensis* are also given in CMI Descriptions of Pathogenic Fungi and Bacteria, Set 46 No 453 and 454 (1975).

Key to the species (Sivanesan & Waller 1986):

Conidia 25-55 x 15-25 µm…….....… *P. sacchari*
Conidia 37-48.5 x 14-30 µm….....…… *P. miscanthi*
Conidia 27-39 x 17-21 µm…….…… *P. philippinensis*
Conidia 39-45 x 15-17 µm……...… *P. spontanea*

P. sacchari

Conidia of *P. sacchari* may vary in size but are usually between 25-55 µm in length and 15-25 µm in width (Sivanesan & Waller 1986). Conidia are elliptical or oblong, rounded at the apex, and rounded or slightly apiculate at the base with a thin, smooth, hyaline wall. The conidiophores arise singly or in groups from stomata and are erect, thin, smooth, have a hyaline wall and the apex is branched several times (Leu & Egan 1989). Oospores are embedded in the interveinal leaf tissue, are globular and yellow with a wall thickness of 3.8-5um. Their diameter is 40-59um (Leu & Egan 1989).

Sivanesan & Waller 1986 give the following description; Haustoria bulbous. Conidiophores hypophyllous, 125-190 x 18-25 µm, dichotomously branched twice or thrice at the apex with 2-4 sterigmata on ultimate branches. Conidia ellipsoid to ovoid, base pendunculate or rounded,

25-55 x 15-25 µm. Oospores yellow to brown, average 50 µm diameter with a wall 3.5-5 µm thick.

P. philippinensis
Mycelium in all parts except in roots, irregularly constricted and inflated. Haustoria simple, vesicular to subdigitate (Sivanesan & Waller 1986). Conidiophores always produced in night dew, with 2-4 branches which in turn may branch twice, 150-400 x 15-26 µm. Conidia elongate ellipsoid to ovoid, 27-39 x 17-21 µm. Oogonia average 23 µm diameter, smooth, with remanants of oogonial stalk or antheridial cell frequently adhering. Oospores regularly spherical, central to eccentric, 15.5-22.5 x 2-4 µm.

P. miscanthi
Conidiphores hypophyllous, 97-300 x 12-37 µm, branched twice at the apex (Sivanesan & Waller 1986). Conidia ovoid, elongate, 37-48.5 x 14-30 µm. Oogonia brown, angular, 51-81 x 43-64 µm. Oospores 32.5-56.5 µm diameter.

P. spontanea
Conidiophores more or less dichotomously branched, 350-550 x 22-32 µm (Sivanesan & Waller 1986). Conidia with round apex lacking papilla and a rounded base with an apiculus, 39-45 x 15-17 µm with fine granular contents. Oogonia and oospores similar to *P. sacchari.*

There is considerable overlap in the size of conidia of the four species of *Peronosclerospora* which would make positive identification of the species involved in an incursion by traditional taxonomy difficult. A molecular assay is available for *Peronosclerospora* detection. The PCR test was designed to amplify across a deletion in the Cox1 gene to give amplicon size differences based on the *Peronosclerospora* species (SRA Final Report). This test can be performed by SRA This assay will greatly assist in rapid identification of the pathogen if an incursion occurs.

Varietal Resistance
Varietal resistance is a key control measure for downy mildew caused by *P. sacchari* (Hughes & Robinson 1961; Leu & Egan 1989; Suma & Magarey 2000; Rauka *et al.* 2005). There are sources of resistance available in most plant improvement programs and this has been utilised to assist with disease control in most places where the disease occurs (Suma & Magarey 2000). Downy mildew was a serious disease in Australia but the last known infested commercial field was in Bundaberg in 1957 (Hughes & Robinson 1961). The disease was eradicated by planting resistant varieties and roguing or ploughout of infested crops (Hughes & Robinson 1961). Restrictions were placed on planting alternative hosts such as corn in close proximity to sugarcane crops. Resistance screening has relied on natural transmission from diseased spreader rows, accompanied by the regular inspection of test plots (Leu & Egan 1989). Research has shown that resistance is strongly heritable and breeding for resistance has been successful (Hsu & Lee 1999).

Screening of Australian commercial varieties in PNG suggests that up to 50% of varieties are susceptible to the disease. The Australian sugarcane industry is vulnerable to an incursion of downy mildew (Magarey 1996). A cooperative research program between SRA and Ramu Agri-Industries commenced in 2009 to obtain ratings for current Australian commercial varieties.

Varietal resistance is also employed to manage downy mildew caused by *P. philippinensis* in the Philippines. Quantitative trait loci (QTLs) have been identified for *P. philippinensis* in maize (George *et al.* 2003).

Epidemiology

Infection

The four species that cause downy mildew have similar requirements for infection. The following is a description of infection for *P. sacchari*:

Downy mildew infects the sugarcane plant via conidia infecting lateral buds or the basal portions of the young spindle leaf (Leu & Egan 1989). With the latter, the conidia germinate and the resulting mycelium grows down into the base of the leaves and up toward the growing point. From here, the pathogen infects the plant systemically leading to symptoms in developing leaves. Infected lateral buds may germinate to produce infected side-shoots on standing stalks or they can produce systemically infected plants if the buds are planted. Infection from oospores has been reported for *P. miscanthi* (Chu 1965) but the role of oospores in the infection cycle of the other species is not clear and some authors consider that they play no role (Hughes & Robinson 1961; Leu & Egan 1989; Suma & Magarey 2000).

Reproduction and dispersal

The pathogen has two forms of sporulation; asexual conidia produced under high humidity on leaf surfaces and sexual oospores which are produced in infected leaves during the cooler months (Hughes & Robinson 1961; Leu & Egan 1989). Conidia are the primary infective propagule and constitute one of the important transmission mechanisms. The conidia are very fragile and not long lasting. Conidia are produced on humid nights but they lose viability within a matter of hours after sunrise (Hughes & Robinson, 1961). Studies suggest the conidia are dispersed by air currents and that they rarely move more than 400m from an infested crop (Hughes & Robinson 1961). Oospores are not released from leaf tissue until the leaves begin to decay on the soil surface. Except for *P. miscanthi,* there has been only limited evidence of oospores leading to infection of sugarcane plants (Suma & Magarey 2000). SRA Project BSS331 reseach showed that high populations of oospores may infect young sugarcane plants (SRA Project BSS331 Final Report). This research will be repeated in follow up research. .

The primary mechanisms for disease spread are through conidial dispersal and the transport of infected planting material (cane stalks). Conidia generally can only travel up to 400m therefore they are not likely to be a source of spread between countries or between districts. Long distance dispersal is most likely to occur from the movement of infected sugarcane stalks.

Survival

The survival of the pathogen via conidia is very limited; these fragile spores rarely last longer than a few hours and are very susceptible to low relative humidity. Oospores are produced which possibly can survive for longer periods but their importance in spread of the disease is unknown. The disease primarily persists in living diseased plants. In Australia, planting of resistant varieties, supply of disease-free plant sources and the ploughing out of diseased crops and rouging of any remaining diseased plants lead to the eradication of downy mildew. The growing of maize in sugarcane areas was restricted to prevent maize acting as an alternative host for the pathogen. Fiji has also been successful in eliminating the disease from commercial crops and

the disease is now restricted to experimental plots. In countries such as Papua New Guinea, where there are relatively high populations of alternative hosts, disease survival is much more likely and it is very unlikely that the disease can be eradicated.

The conidia of downy mildew are unlikely to be spread by harvesters or other machinery unless they are operating at night time. Infected stalk material or leaves that remain turgid in protected parts of machinery could potentially release spores at night time but it is unlikely that this would occur for more than one or two days. This constitutes a very low risk of spreading the disease.

The most likely way that the disease could spread on machinery is for infected stalks pieces with viable buds (carried on machinery) to become dislodged and germinate to produce infected plants. The highest risk would be if infected stalk pieces were carried in planting machines or cane transporters used to carry cane for planting. Thorough cleaning and the removal of any leaf and stalk material should be sufficient to prevent spread on machines such as harvesters, transporters and planters.

Risk of introduction

The introduction of planting material is the highest risk for entry of downy mildew into Australia (Magarey *et al.* 2002; Magarey *et al.* 2008). Sugarcane and its relative *S. edule* (pit pit or duruka) are popular in Pacific island and Asian cuisine and illegal movement of sugarcane cuttings or plants is a real risk. Sugarcane is a popular garden plant in coastal areas of Australia and it is possible that an incursion could be detected in a domestic garden. It remains essential to strictly enforce border quarantine measures if we are to keep the disease from entering Australia. It is impossible for a disease incursion to occur from wind-blown conidia. Contaminated soil or machinery are unlikely to carry the disease.

Principles of control and eradication

Sugarcane and its relative, *Saccharum edule,* are widely grown throughout the Torres Strait and in home gardens in northern Australia and as far south as Melbourne. If downy mildew was found in isolated plants in a non-crop area, eradication would have a high probability of success. An eradication program would involve:

- Immediate isolation and destruction of all *Saccharum* species and alternative hosts within 1km of the outbreak and follow-up destruction of any regrowth.
- Trace-back to determine whether any plant material of sugarcane or alternative hosts have been moved from the affected site.
- If it is not feasible to remove all hosts, a spray program of hosts with metalaxyl may be warranted.
- Public awareness campaign to alert all Department of Agriculture (Biosecurity) staff, SRA staff, State Departments of Primary Industries, Cane Productivity Service Company staff, cane farmers and the general public to report any symptoms resembling downy mildew.
- Spore trapping and molecular detection can be used to study the spread of the disease into new areas or to detect the presence of spores in an incursion.

Resistant varieties
The known infested fields and those close-by should be planted with resistant varieties after the prescribed fallow period.

Varieties with high levels of resistance to downy mildew have been bred in several overseas sugar industries. Some of these varieties are held in variety collections at SRA. Some Australian varieties are resistant to the disease. In the case of an incursion, a selection of these resistant varieties should be multiplied for use on infested farms and for possible introduction into the area if eradication is unsuccessful or is not possible.

Screening for downy mildew resistance should not commence in Australia until the disease has been declared established and widespread or should only occur at an isolated site with strict quarantine procedures.

Screening of Australian varieties for resistance has been undertaken in PNG. The resistance of a number of current Australian commercial varieties has been assessed and these data will be used to manage the outbreak if an incursion occurred.

Hot water treatment and fungicides
The pathogen may be eliminated from systemically infected planting material by hot water treatment at 50^0 C for two hours (Hughes and Robinson, 1961, Leu and Egan, 1989) or by dipping stalks or stalk pieces (setts) in metalaxyl at 1.25g (a.i.) / litre water for five seconds (James 1983; Eastwood & Malein 1998). Metalaxyl may also be applied to setts in commercial cane planters and to both the soil and foliage of infested crops. In ratoon crops, metalaxyl has been applied at 1.8-2.0kg (a.i.) / ha using a granular formulation (Malein 1993; Eastwood & Malein 1998). The fungicide has a prophylactic effect in the growing crop, protecting the crop from infection for up to 20 weeks post application (Eastwood & Malein 1998).

Approved Seed Plots
Approved seed plots provide growers with disease-free seed cane in most districts of the Australian sugarcane industry. Approved seed will play a key role in control of downy mildew, by providing disease-free plant sources for commercial crops. Approved seed is produced from hot water-treated cane and is regularly inspected to ensure freedom from serious diseases. Growers should plant their own farm seed cane at least 400 m from any known infested crops.

Abandoned sugarcane and alternative hosts
Abandoned or volunteer sugarcane could be infected with downy mildew. It would be important to inspect and destroy any infected abandoned or volunteer sugarcane.

One of the most important alternative hosts of downy mildew is corn (maize), and there is evidence to suggest that it is transmitted through maize seed (SRA Final Report). Some varieties of corn are extremely susceptible to *P. sacchari* and *P. philippinesis* and control of the disease in corn would be essential in an eradication program. The sugarcane and grains industry would need to work in close cooperation if eradication was to be successful. In the previous outbreak of downy mildew in Australian cane fields, legislation was introduced that ensured a minimum distance was maintained between sugarcane and corn crops; this effectively reduced disease

transfer between the two crops (Hughes & Robinson 1961). Sorghum and other grasses may also be significant alternative hosts of some species of *Peronosclerospora* that infect sugarcane and management of the disease in these other hosts may be essential in an eradication campaign.

In some areas the wild sugarcane relative, *S. spontaneum*, has established as a weed (eg banks of the Mulgrave River near Cairns, the lower reaches of the Herbert River, Cardwell Range, and near Feluga). Attempts should be made to destroy these plants or treat them with metalaxyl if they are found to be infected with downy mildew. This would need to be discussed with the Department of Natural Resources to determine the environmental impacts of any control program. Sugarcane grown in backyards should be inspected in the area near any incursion and any infected plants should be destroyed.

Feasibility of control in Australia

There is a high probability of successfully eradicating downy mildew if it was found in Australia. Eradication was successfully achieved in Australia in the 1950-1960 period. Ultimately, if eradication is not achieved, the disease can be successfully controlled with resistant varieties but this will involve the loss of valuable commercial varieties and potentially significant yield losses in the period of changeover from susceptible to resistant varieties.

APPENDIX 1

CONTACTS FOR TAXONOMY OF THE PATHOGEN

Specimens suspected of being infected with an exotic *Peronosclerospora* species should be sent immediately to:

Dr Roger Shivas
Curator Plant Pathology Herbarium (BRIP)
Biosecurity Queensland
Department of Agriculture and Forestry
Eco Sciences Precinct
Boggo Road, Dutton Park AUSTRALIA
GPO Box 267, Brisbane, Qld 4001
P: 07 3255 4378 F: 07 3844 4529
Email: roger.shivas@daff.qld.gov.au

DNA diagnosis of sample can be done by:

Dr Nicole Thompson
Sugar Research Australia
50 Meiers Rd
Indooroopilly Q 4068
P: 07 3331 3333 F: 07 3871 0383
Email nthompson@sugarresearch.com.au

Specimens should consist of leaves showing the suspect symptoms. Select leaves that are still green and if down is present select leaves showing down. Leaf pieces 20-30 cm in length should be taken from a range of plants that are showing symptoms.

Specimens should be placed in a sealed heavy duty plastic bag and then sealed in a second bag with a security seal. The plastic bags should be placed in a sealed courier bag. A sheet with the details of the sample should be included in the bag as per appendix 2.

The sample should be transported by express overnight or same day courier or in person to Dr Shivas and marked URGENT Quarantine sample.

Dr Shivas and Dr Thompson should be advised by telephone that the samples have been sent and the consignment note and expect time of delivery.

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