BIOSECURITY NEW ZEALAND

Import Risk Analysis:

Miscanthus × *giganteus* (Poaceae) plants *in vitro* from the United Kingdom and the United States of America

18 December 2007

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Te Manatū Ahuwhenua, Ngāherehere

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Import risk analysis: Miscanthus × giganteus (Poaceae) plants *in vitro*
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Every effort has been made to ensure that the information provided in this document is true and accurate at the time of publication. A number of factors may affect the accuracy or completeness of this information. These factors include changes in hazard organism status, scientific information, and material continually being reviewed by Biosecurity New Zealand or otherwise provided that is relevant to the final import risk analysis.

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1 EXECUTIVE SUMMARY

1.1 INTRODUCTION

Miscanthus, a perennial C4 rhizomatous grass with stems that emerge from a rhizome complex annually in spring, is a genus comprised of approximately 25 species. *Miscanthus x giganteus* is a hybrid of two *Miscanthus* species, *M. sinensis* and *M. sacchariflorus*, and is considered to have the capacity for substantial biomass development that has high-energy potential. Biomass obtained from *M. x giganteus* can be used for construction materials such as particleboard; as a solid fuel for use in coal fired power stations; pelletised as a low emission fuel for domestic and industrial burners; and as a source of cellulose for industrial ethanol fermentation for use as a bio-fuel. *M. x giganteus* is cold tolerant and thus considered suitable to New Zealand conditions, and is considered a non-invasive and environmentally benign plant because it cannot produce viable seed.

There are currently no import health standards for nursery stock of any species of *Miscanthus*. New Zealand industry has requested approval for the importation of tissue culture (plants *in vitro*) initials of *M. x giganteus* from the United Kingdom (UK) or the United States of America (USA). The overall objective of this project, therefore, is to complete an analysis of the biosecurity risks of importing *Miscanthus* plants *in vitro* into New Zealand from the UK and USA, and identify measures that appropriately mitigate the identified risks. The identified options for measures will then form the basis of a new import health standard for importing *Miscanthus* plants *in vitro* into New Zealand.

1.2 PROPOSED MEASURES

Based on the risk analyses completed for each identified pest or pest group listed in Chapters 4 and 5, the following measures are proposed:

1.2.1 GENERAL MEASURES

The following or equivalent general measures should be required for all consignments of *Miscanthus* plants *in vitro* being imported into New Zealand:

- Mother plants (from which the *in-vitro* plantlets were excised) should be free of obvious signs of organism or disease contamination. Where possible, efforts should be made to isolate mother plants from potential sources of inoculum such as other contaminated plants.
- Mother plants should, where possible, be subjected to an antimicrobial and insecticide spray program 3 to 14 days prior to explant removal.
- Explant material should, where possible, be the material least likely to be contaminated by hazard organisms or diseases. Material such as small young actively

growing meristems would be preferable to older plant material such as leaves or stems.

- Explant material should be surface sterilised using standard procedures such as a dip in 0.5% sodium hypochlorite. The duration of exposure and concentration of active ingredient will depend on the explant material, softer material usually being less tolerant.
- Nutrient concentrations in the tissue culture media used during quarantine inspections should be kept at similar rates to those used during the multiplication (sub-culturing) process.

As hazard organism growth *in vitro* is the principle mechanism for detection and subsequent risk management, the growing medium should not contain chemicals that may have significant antibiotic properties. The *in-vitro* growing medium should therefore be free of antibiotics, biocides or chemicals having antimicrobial properties (e.g. activated charcoal).

To maintain as far as possible the axenic condition of plants *in vitro* and facilitate inspection during quarantine, rigid clear-sided (including base) tissue culture vessels that are sealable should be used.

1.2.2 Organism specific measures

From areas or places of production that are unable to obtain suitably supported area freedom declarations for the specified hazard organisms or organism types, the following alternative risk management options are proposed:

Hazard Organisms	Proposed Options for Measures
Acidovorax avenae subsp. *avenae*	*Miscanthus* plants *in vitro* should be inspected over two 30-day periods for abnormal growth or other such disease symptoms. Inspections should be of plantlets[1] in normal growing conditions[2]; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for 3 hours per treatment; or *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *A. avenae* subsp. *avenae*.
Leifsonia xyli subsp. *xyli*	*Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *L. xyli* subsp. *xyli*; or *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to fluorescent-antibody staining of sap extracts and then concentrated on membrane filters by filtration before observation with epifluorescence microscopy to confirm freedom from *L. xyli* subsp. *xyli*; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for 3 hours per treatment.

1 no sub-culturing should occur during each inspection period
2 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation

Hazard Organisms	Proposed Options for Measures
Puccinia melanocephala	*Miscanthus* plants *in vitro* should be inspected over two 30-day periods for abnormal growth or other such disease symptoms. Inspections should be of plantlets[3] in normal growing conditions[4]; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for 3 hours per treatment; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 52°C or greater for 1 hour per treatment.
Magnaporthe salvinii and other filamentous facultative fungi	*Miscanthus* plants *in vitro* should be inspected over two 28-day periods for disease symptoms of mycelial growth on the medium. Inspections during each of the 28-day growing periods should be of growing plantlets[3] in normal growing conditions[4].
Schizotetranychus celarius	*Miscanthus* plants *in vitro* should be inspected over two 30-day periods for mites, webbing or bacterial growth on the growing medium. Inspections should be of plantlets[3] (no sub-culturing) in normal growing conditions[4]; or Prior to *in-vitro* culture, explants should be subjected to a hot-water treatment at 50°C or greater for a minimum of 30 minutes.
Ustilago scitaminea	*Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *U. scitaminea*; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for 3 hours per treatment; or Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at 52°C or greater for 1 hour per treatment.
Miscanthus streak virus and Sugarcane mosaic virus	*Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR testing procedure to confirm freedom from MiSV and SCMV

Any plant material found to be contaminated by hazard organisms should not be planted into the New Zealand environment but rather disposed of in a suitable manner. In all cases measures that provide an equivalent level of protection to those listed above against the target organisms or organism groups should also be considered appropriate. Care should be taken to test any proposed treatments on *Miscanthus* samples before widespread application to ensure treated plant material is not adversely affected.

1.2.3 ASSESSMENT OF RESIDUAL RISK

1.2.3.1 OBJECTIVES FOR PROPOSED MANAGEMENT OPTION(S)

The objective of these general measures is to ensure that any imported *Miscanthus* plants *in vitro* are free of hazard organisms or diseases prior to biosecurity clearance into New Zealand.

3 no sub-culturing should occur during each inspection period
4 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation

2 BACKGROUND AND PROCESS

2.1 INTRODUCTION

Miscanthus, a perennial C4 rhizomatous grass with stems that emerge from a rhizome complex annually in spring, is a genus comprised of approximately 25 species. Most of these species are indigenous to South-East Asia with a few extending into Africa (Barkworth 2002).

Miscanthus x giganteus is a hybrid of two *Miscanthus* species, *M. sinensis* and *M. sacchariflorus*, and is considered to have the capacity for substantial biomass development that has high-energy potential. Biomass obtained from *M. x giganteus* can be used for construction materials such as particleboard; as a solid fuel for use in coal fired power stations; pelletised as a low emission fuel for domestic and industrial burners; and as a source of cellulose for industrial ethanol fermentation for use as a bio-fuel (Lewandowski *et al.* 2000). *M. x giganteus* is cold tolerant and thus considered suitable to New Zealand conditions, but is not particularly frost tolerant until established after its first season (Jorgensen & Schwarz 2000). It is considered a non-invasive and environmentally benign plant (DEFRA 2001) because it cannot produce viable seed (Lewandowski *et al.,* 2000), however it can be propagated vegetatively from rhizomes.

There are currently no import health standards for nursery stock of any species of *Miscanthus*. New Zealand industry has requested approval for the importation of tissue culture (plants *in vitro*) initials of *M. x giganteus* from the UK or the USA. The applicant intends to develop the tissue culture plantlets and plant them out in the field for initial evaluation and later production trials.

The overall objective of this project, therefore, is to complete an analysis of the biosecurity risks of importing into New Zealand *Miscanthus* plants *in vitro*, and identify appropriate measures to mitigate the identified risks. The identified options for measures will then form the basis of a new import health standard for importing *Miscanthus* plants *in vitro* into New Zealand.

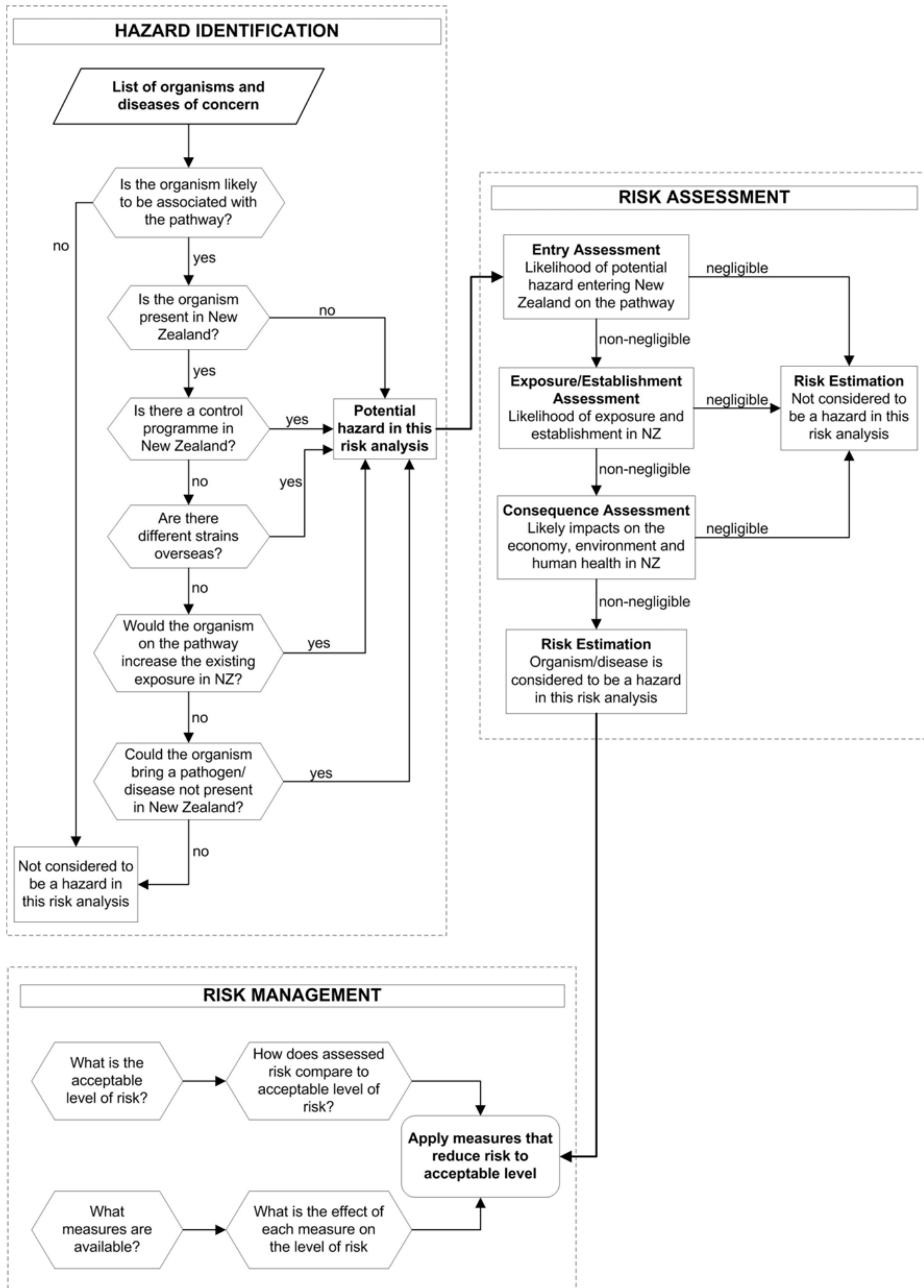
2.2 THE RISK ANALYSIS PROCESS

The following briefly describes the MAF Biosecurity New Zealand process and methodology for undertaking import risk analyses. For a more detailed description of the process and methodology please refer to the Biosecurity New Zealand Risk Analysis Procedures (Version 1 12 April 2006) which is available on the Ministry of Agriculture and Forestry web site[5].

5 http://www.biosecurity.govt.nz/files/pests-diseases/surveillance-review/risk-analysis-procedures.pdf

The risk analysis process leading to the final risk analysis document is summarised in Figure 2.1.

Figure 2.1: Diagrammatic representation of the risk analysis process



The process outlined in figure 2.1 is further supported by the following:

2.2.1 ASSESSMENT OF UNCERTAINTIES

In this aspect of the risk analysis process the uncertainties and assumptions identified during the preceding hazard identification and risk assessment stages are summarised. An analysis of these uncertainties and assumptions can then be completed to identify which are critical to the outcomes of the risk analysis. Critical uncertainties or assumptions can then be considered for further research with the aim of reducing the uncertainty or removing the assumption.

Where there is significant uncertainty in the estimated risk, a precautionary approach to managing risk may be adopted. In these circumstances the measures should be reviewed as soon as additional information becomes available[6] and be consistent with other measures where equivalent uncertainties exist.

2.2.2 ASSESSMENT OF RESIDUAL RISK

Residual risk can be described as the risk remaining after measures have been implemented. Assuming:

- **a)** the measures have been implemented in a manner that ensures they reduce the level of risk posed by the hazard(s) to a degree anticipated by the risk analysis; and
- **b)** the level of risk posed by the hazard(s) was determined accurately in the risk assessment;

the remaining risk while being acceptable may still result in what could be interpreted as failures in risk management.

The residual risk information then becomes the basis for developing a monitoring protocol that may, for instance, interpret interception data to determine if risk thresholds are being exceeded. The residual risk information also ensures the risk management decision maker understands the nature of the risk remaining should the measures achieve their objectives. Should monitoring activities then determine that the risk threshold has been exceeded for any particular hazard or group of hazards, either the risk analysis can be reviewed to determine what aspects of the risk(s) or management option(s) have altered or were assessed incorrectly, or the implementation audited to ensure adequate compliance.

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6 Article 5.7 of the SPS Agreement states that “a Member may provisionally adopt sanitary …. measures” and that “Members shall seek to obtain additional information …. within a reasonable period of time.” Since the plural noun “Members” is used in reference to seeking additional information a co-operative arrangement is implied between the importing and exporting country. That is the onus is not just on the importing country to seek additional information.

2.2.3 Review and consultation

Peer review is a fundamental component of a risk analysis to ensure the analysis is based on the most up to date and credible information available. Each analysis must be submitted to a peer review process involving appropriate staff within those government departments with applicable biosecurity responsibilities, and recognised and relevant experts from New Zealand or overseas. The critique provided by the reviewers is reviewed and where appropriate, incorporated into the analysis. If suggestions arising from the critique are not adopted the rationale must be fully explained and documented.

Once a risk analysis has been peer reviewed and the critiques addressed it is then published and released for public consultation. The period for public consultation is usually 6 weeks from the date of publication of the risk analysis.

All submissions received from stakeholders will be analysed and compiled into a review of submissions. Either a document will be developed containing the results of the review or proposed modifications to the risk analysis or the risk analysis itself will be edited to comply with the proposed modifications.

2.2.4 References

Barkworth M E (2002) Grass manual on the web; 26.03 *Miscanthus* Anderson. Intermountain Herbarium. Utah State University. http://herbarium.usu.edu/webmanual/default.htm

DEFRA (2001) Planting and growing *Miscanthus*. Best Practice Guidelines for Applicants for DEFRA's Energy Crops Scheme. Page 11. Department for the Environment, Food and Rural Affairs (DEFRA), United Kingdom. Available online at http://www.defra.gov.uk/erdp/pdfs/ecs/miscanthus-guide.pdf

Jorgensen U, Schwarz K U (2000) Why do basic research? A lesson from commercial exploitation of *Miscanthus*. New Phytologist 148: 190-193.

Lewandowski I, Clifton-Brown J C, Scurlock J M O, Huisman W (2000) *Miscanthus*: European experience with a novel energy crop. Biomass and Bioenergy 19: 209-227.

MAF (2006) Biosecurity New Zealand risk analysis procedures. Ministry of Agriculture and Forestry, New Zealand, 201 pp. Available online at http://www.biosecurity.govt.nz/files/pests-diseases/surveillance-review/risk-analysis-procedures.pdf

3 COMMODITY AND PATHWAY

This chapter provides information on the commodity and pathway that is relevant to the analysis of biosecurity risks, and common to all organisms or diseases potentially associated with the pathway and commodity. Organism or disease-specific information is provided in subsequent chapters (4 and 5).

3.1 DESCRIPTION OF THE COMMODITY

Miscanthus, a perennial C4 rhizomatous grass with stems that emerge from a rhizome complex annually in spring, is a genus comprised of approximately 25 species. Most of these species are indigenous to South-East Asia with a few extending into Africa (Barkworth 2002). *Miscanthus x giganteus* is a hybrid of two *Miscanthus* species, *M. sinensis* and *M. sacchariflorus*, and is considered to have the capacity for substantial biomass development that has high-energy potential.

The applicant has requested approval to import *M. x giganteus* plants *in vitro* into New Zealand for development and later field trials. For the purposes of this risk analysis biosecurity risks will be assessed for hazard organisms recorded as being associated with all species of the *Miscanthus* genus.

3.1.1 HOST MATERIAL

The genus *Miscanthus* is a member of the Poaceae family, order Cyperales, class Liliopsida. Some species of *Miscanthus* hybridise with *Saccharum* spp., from which *Miscanthus* differs in its non-disarticulating branches and unequally pedicellate, rather than sessile-pedicellate spikelets (Barkworth 2002). *M. x giganteus* is a sterile hybrid of *M. sinensis* and *M. sacchariflorus* (Linde-Laursen 1993, Lewandowski *et al.* 2000). This is because the combination of a tetraploid species (*M. saccchariflorus*) with a diploid species (*M. sinensis*), results in a triploid hybrid that confers innate sterility on the hybrid.

New Zealand currently has four species of *Miscanthus* listed as being present: *M. floridulus*, *M. nepalensis*, *M. sinensis*, and *M. zebrinus* (MAF 2007). *M. saccchariflorus*, one of the parent species of the hybrid *M. x giganteus*, is currently considered absent from New Zealand. New triploid cultivars of *M. x giganteus* have been created by crossing tetraploid *M. sacchariflorus* with diploid *M. sinensis* all of which proved to be sterile (Heaton *et al.* 2004). According to Linde-Laursen (1993) *M. x giganteus* has no known natural distribution. Although hybrids between parental species appear to occur frequently, these species co-exist (Kayama 2001). *M. x giganteus* is sterile and as such does not appear to be invasive (Lewandowski *et al.* 2000, DEFRA 2001), thus it will not out compete parental species in their natural habitat. It therefore appears that *M. x giganteus* only exists in cultivation.

Controlled field experiments have shown that rhizomes begin growth after winter dormancy when soil temperatures reach 10-12°C. Once growth has commenced, temperatures then need to be between 5 and 10°C for leaf expansion to occur (Lewandowski *et al.* 2000). *M. x giganteus* is limited by its lack of frost tolerance especially in the first winter where mortality rates of up to 100% have been reported. Laboratory freeze tests have shown that lethal soil temperature for rhizomes is −3.4°C (Jorgensen & Schwarz 2000). Although in the field these temperatures are not always reached, the effect is believed to be compounded by a secondary infection of damaged buds formed on the rhizomes in late autumn. This damage facilitates the entry of pathogenic species such as *Fusarium*, which cause further damage to the plant (Jorgensen & Schwarz 2000).

As *M. x giganteus* is a sterile triploid hybrid and thus is incapable of producing viable seed, the only available mechanisms for propagation are either micro-propagation *in vitro* or vegetative propagation of rhizomes (Lewandowski *et al.* 2000). Once established, *Miscanthus* is capable of continuing to be grown on the same site for at least 15 years (Ireland Department of Agriculture and Food (*undated*)).

3.1.2 A DESCRIPTION OF PLANTS *IN VITRO*

"Plants *in vitro*" is defined under the International Plant Protection Convention as "*a commodity class for plants growing in an aseptic medium in a closed container*" (ICPM 2002). The use of this term replaced the "plants in tissue culture" which essentially has the same meaning. Other definitions include "*the* in-vitro *culture of plant cells, tissues or organs*" (Cassells & Gahan 2006) and "*the maintenance or growth of tissues,* in vitro*, in a way that may allow differentiation and preservation of their architecture and/or function*" (Schaffer 1990).

Plants in vitro culture or "micro-propagation" allows the production of large numbers of plants from small pieces of the stock plant in relatively short periods. Depending on the species in question, the original tissue piece may be taken from shoot tip, leaf, or lateral bud, stem or root tissue. In most cases, the original plant is not destroyed in the process - a factor of considerable importance to the owner of a rare or unusual plant. Once the plant is placed in tissue culture, proliferation of lateral buds and adventitious shoots or the differentiation of shoots directly from callus results in tremendous increases in the number of shoots available for rooting. Rooted "micro-cuttings" or "plantlets" of many species have been established in production situations and have been successfully grown on either in containers or in field plantings (Lineberger 2007).

Micro-propagation therefore offers several distinct advantages not possible with conventional propagation techniques:

- A single explant can be multiplied providing several thousand plants in less than one year;
- With most species, the taking of the original tissue explant does not destroy the parent plant;

- Once established, actively dividing cultures are a continuous source of year-round micro-cuttings which can result in plant production under greenhouse conditions without seasonal interruption.

Micro-propagation allows for the rapid production of selected superior clones in sufficient quantities to have an impact on the plant market (Lineberger 2007).

The process of creating plants *in vitro* requires that a small piece of the plant to be cloned (the explant) is removed from a healthy, well-maintained stock plant and sterilized in a dilute bleach solution. The sterilized explant is rinsed with sterile water, and placed in aseptically prepared containers on a specially formulated medium. The explant may produce shoot proliferating cultures directly by enhanced lateral bud break, or the tissue may undergo a certain period of unorganized growth (callus) prior to shoot differentiation. The pattern of growth of the cultures is principally determined by the plant growth regulator content of the tissue culture medium (the auxin and cytokinin concentration). Most cultures are established within 4 to 12 weeks depending on the species or cultivar. A shoot proliferating culture is one which can be subdivided (subcultured) to produce divisions which will continue rapid multiplication (Lineberger 2007).

3.2 DESCRIPTION OF THE PATHWAY

For the purposes of this risk analysis, it is assumed that *Miscanthus* plants *in vitro* will be sourced from mother plants that may be growing anywhere in the UK or USA (e.g. open ground, enclosed nursery or laboratory areas). United Kingdom countries at the time of drafting this risk analysis include England, Northern Ireland, Scotland and Wales. The plant material may be established *in vitro* either before shipping to New Zealand or after arrival in New Zealand. In the latter case all plant material not included in the micro-propagation process will need to be either reshipped or destroyed, or held under suitable containment conditions.

3.2.1 SUMMARY OF EXISTING IMPORT REQUIREMENTS FOR PLANTS *IN VITRO*

The current standard biosecurity requirements (or "basic" requirements) for importing plants *in vitro* are provided in MAF standard 155.02.06[7]. These standard or "basic" requirements that are applied to all plants *in vitro* require that any such shipments:

- Are accompanied by a phytosanitary certificate. Phytosanitary certificates are issued by the national plant protection organisation of the exporting country in compliance with ISPM No. 12 (FAO 2001). All phytosanitary certificates must contain (in relation to biosecurity) a description of the commodity being exported including the botanical name, and a certifying statement

  "*that the plants … described herein have been inspected and/or tested according to appropriate official procedures and are considered to be free from the quarantine pests specified by the importing contracting party and to*

7 Standard 155.02.06 is available on the MAF web site at http://www.biosecurity.govt.nz/files/imports/plants/standards/155-02-06.pdf

conform with the current phytosanitary requirements of the importing contracting party, including those for regulated non-quarantine pests".

For plantlets removed from *in-vitro* culture before arrival in New Zealand, the phytosanitary certificate must be endorsed that:

"*These plantlets were removed from the original culture container(s) in which they were grown, not more than 48 hours before export, and have not been in contact with any other growing media*".

- o Must have been grown in the vessel in which they are imported. The container must be pest-proof, rigid, and either clear plastic or glass. The tissue culture media must not contain fungicides or antibiotics. Plants in tissue culture must be produced in a facility under conditions that prevent contamination with regulated pests.
- o Visually inspected on arrival in New Zealand to determine if the plants *in vitro* are showing any signs of contamination (e.g. cloudy agar, fungal spores or bacterial growth). If contamination is observed the importer is given the option of reshipment or destruction of the consignment.

For a subset of plants there are additional conditions for tissue-cultured material. These additional requirements may include:

- o inspection at a specialist laboratory (rather than at the port of entry);
- o that the media must not contain charcoal;
- o a period in post entry quarantine;
- o binocular microscope inspections for mite contamination;
- o testing for bacteria, viruses, diseases of unknown aetiology, or phytoplasmas;
- o area freedom declarations for mites, fungi, bacteria, viruses, diseases or unknown aetiology, or phytoplasmas;
- o declarations that the tissue cultures have been derived from parent stock tested or inspected and found free of viruses or fungi;
- o treatment (dipping) in a fungicide for potential fungal contamination;

3.3 REFERENCES

Barkworth ME (2002) Grass manual on the web; 26.03 *Miscanthus* Anderson. Intermountain Herbarium. Utah State University. http://herbarium.usu.edu/webmanual/default.htm

Cassells A, Gahan P (2006) Dictionary of plant tissue culture. The Haworth Press Inc, New York. 243 pp

DEFRA (2001) Planting and growing *Miscanthus*. Best Practice Guidelines for Applicants for DEFRA's Energy Crops Scheme. Page 11. Department for the Environment, Food and Rural Affairs (DEFRA), United Kingdom. Available online at http://www.defra.gov.uk/erdp/pdfs/ecs/miscanthus-guide.pdf

FAO (2001) International Standards for Phytosanitary Measures number 12 (ISPM No. 12): *Guidelines for phytosanitary certificates*. FAO, 15 pp.

Heaton EA, Clifton-Brown J, Voigt TB, Jones MB, Long SP (2004) *Miscanthus* for renewable energy generation: European Union experience and projections for Illinois. Mitigation and Adaptation Strategies for Global Change 9: 433-451.

ICPM (2002) Report of the 4th meeting of the Interim Commission on Phytosanitary Measures, 2002. FAO, Rome.

Ireland Department of Agriculture and Food (undated). Best Practice Manual for *Miscanthus*. http://www.agriculture.gov.ie/schemes/bioenergy/BestPracticeManual_Miscanthus.doc

Jorgensen U, Schwarz KU (2000) Why do basic research? A lesson from commercial exploitation of *Miscanthus*. New Phytologist 148: 190-193.

Kayama M (2001) Comparison of the aluminium tolerance of *Miscanthus sinensis* Anderss. and *Miscanthus sacchariflorus* Bentham in hydroculture. International Journal of Plant Sciences 162: 1025–1031.

Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W (2000) *Miscanthus*: European experience with a novel energy crop. Biomass and Bioenergy 19: 209-227.

Linde-Laursen I (1993) Cytogenetic analysis of *Miscanthus* 'Giganteus', an interspecific hybrid. Hereditas 119: 297-300.

Lineberger DR (2007) The many dimensions of plant tissue culture research. Texas A&M University, College Station, TX 77843. Available online at http://aggie-horticulture.tamu.edu/

MAF (2007) Biosecurity Index: Database of plants and import conditions for New Zealand. Ministry of Agriculture and Forestry, New Zealand. Available online at http://www1.maf.govt.nz/cgi-bin/bioindex/bioindex.pl

MAF standard 155.02.06: Importation of Nursery Stock. Ministry of Agriculture and Forestry, New Zealand. Available online at http://www.biosecurity.govt.nz/files/imports/plants/standards/155-02-06.pdf

Schaffer WI (1990) Terminology associated with cell, tissue and organ culture, molecular biology and molecular genetics. In vitro Cell and Developmental Biology 26: 97-101

4 POTENTIAL HAZARDS

In this chapter the organisms and diseases potentially associated with *Miscanthus* plants *in vitro* are identified and assessed for their potential to be considered as hazards on this pathway. A discussion on potential measures for mitigating biosecurity risks is also provided.

4.1 HAZARD IDENTIFICATION

The first step in this process is to identify as many organisms and diseases as reasonably possible that could potentially be associated with the pathway in question, *Miscanthus* plants *in vitro* from the UK or the USA. There are a number of limitations on the information that is available for the development of such a list of organisms or diseases. These limitations include:

- The information must be considered at least reasonably reliable and therefore be sourced from the scientific literature rather than the popular media or other such sources.
- Many organisms and diseases associated with a commodity will not have been identified in any scientific (or other) sources of information. This will vary depending on how well the commodity in question has been studied, which itself is most often a reflection of the commodities economic importance to a region or country.
- Many organisms have yet to be discovered or identified and as such may not be reported. Crous & Groenewald (2005) estimated that only 7% of the fungal species thought to exist are known to science.
- Organisms or diseases that are considered insignificant on the commodity in question may be under-reported, even though they may be significant for other commodities.

One factor in favour of organism or disease identification is that any significant organisms or diseases on the commodity in question are more likely to have been reported.

4.1.1 ORGANISMS AND DISEASES ASSOCIATED WITH *MISCANTHUS*

Several sources of information are routinely referenced when developing hazard lists for most plant-based commodities. CAB International (CABI CPC 2006) provides a web-based compendium of crop pests and diseases that can be used to compile hazard information on any plant host included in the supporting database. "Plant Viruses Online" (Brunt *et al.* 1996) provides a web-based interface into a database containing information on viruses including host association. The "Fungal Databases" (Farr *et al.* 2007) is provided online by the United States Department of Agriculture, and contains extensive information on fungal and host associations.

M. x giganteus has also been the subject of plant risk assessments (PRA) in the UK, which were carried out for the importation of *M. x giganteus* rhizomes from the Dominican

Republic in August 1999 and a global pest risk analysis (PRA) in December 1999 (Sansford & McLeod 2000). The organism association information included in these PRAs is also considered in this risk analysis.

From these sources a list of organisms and diseases recorded as being associated with *Miscanthus* plants was developed. This list is provided in the Appendix, and contains 226 organisms including 2 bacteria, 166 fungi, 55 invertebrates (including insects, mites and nematodes), and 3 viruses.

4.1.2 ORGANISM ASSOCIATION WITH PLANTS *IN VITRO*

In general, tissue culture contamination frequently originates with the introduction into culture of explants contaminated with obligate or surface sterilisation-resistant micro-organisms e.g. in biofilms (Cassells 2001). These include pathogens of the plant and common environmental micro-organisms, both of which may become pathogenic in culture ('vitropaths'). Heterotrophic plant tissue media are capable of supporting the growth of many common environmental micro-organisms. The latter may provide a food source for micro-arthropods, which can act as vectors in the spread of laboratory contamination (Cassells 2001). Cultivatable micro-organisms may over-run the cultures killing the explants; or inhibited by media components, may remain latent until the medium is changed to become vitropaths; yet other expressed micro-organisms may not visibly affect the growth of the cultures (Cassells 2001).
General publications on the contamination of plants *in vitro*, combined with an understanding of the material itself and a review of current phytosanitary requirements for such material, allow the following conclusions to be drawn:

- Bacteria and fungi are common contaminating organisms of plants *in vitro* (Bunn & Tan 2002);
- Endophytic micro-organisms associated with plants *in-vivo* (such as viruses, viroids, phytoplasmas, diseases of unknown aetiology, and fungi or bacteria that are obligate organisms) are unlikely to be eliminated from the material at the time of culturing;
- Mite contamination can originate from the excised plant material or directly onto the culture medium from the surrounding environment;
- Properly prepared tissue cultures should not be expected to be contaminated by invertebrate organisms other than mites and nematodes.

The contaminants may be associated with just the plant material or with both the plant material and the culture medium. These two types of contaminants can be described as:

- Facultative organisms (saprophytic and pathogenic) that will visually manifest themselves on the culture media, sometimes within days; and
- Obligate organisms that may cause disease or growth effects on plant material only.

From a plant tissue culturist's perspective, micro-organisms such as fungi and bacteria become problematic by virtue of their prolific growth under high nutrient *in-vitro* conditions (Bunn & Tan 2002). From a biosecurity perspective this "prolific growth" offers an effective opportunity for the detection and management of the risks of many of these organisms.

4.1.3 POTENTIAL HAZARD ORGANISMS ON *MISCANTHUS* PLANTS *IN VITRO*

From the web-based sources listed above and using subject-specific searches of CAB Abstract databases, there is little direct evidence in the literature of any of the listed organisms (Appendix) being associated with *Miscanthus* plants *in vitro*. This lack of recorded information does not indicate that no such organisms could be associated with *Miscanthus* plants *in vitro* as there would be little reason for industry or research institutes to record such information. From the list of organisms recorded in association with *Miscanthus* plants (Appendix), and with reference to the discussion in the preceding section, it is proposed that the organisms or groups of organisms listed in table 4.1 be considered further in this risk analysis.

Particular examples were selected from each organism group based primarily on their recorded absence from New Zealand and the availability of relevant biological and epidemiological information. The exceptions are Miscanthus streak monogeminivirus, which has only been recorded in Japan but may be distributed more widely, and *Cochliobolus lunatus*, which has been chosen to represent other filamentous facultative fungi on the list even though it is believed to be present in New Zealand.

The following references were used to verify the presence or absence of the listed organisms in New Zealand:

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (eds.) (1996) Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. Available on line at http://image.fs.uidaho.edu/vide/refs.htm

CABI CPC (2006) The Crop Protection Compendium, 2006 Edition. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

NZFungi (2007) New Zealand fungi (and bacteria). Landcare Research New Zealand Limited. Available on line at http://nzfungi.landcareresearch.co.nz/html/mycology.asp

Manson DCM (1987) A list of New Zealand mites and their host plants. Science Information Publishing Centre; Wellington, New Zealand; DSIR Bulletin No. 240.

Pearson MN; Clover GRG; Guy PL; Fletcher JD; Beever RE (2006) A review of the plant virus, viroid and mollicute records for New Zealand. Australasian Plant Pathology 35 (2): 217-252

Pennycook, SR (1989) Plant diseases recorded in New Zealand. Vol. 2 & 3. Plant Diseases Division, DSIR, Auckland, New Zealand.

PPIN (2007) Plant Pest Information Network. New Zealand Ministry of Agriculture and Forestry

Ramsay GW (1980) Common and scientific names of New Zealand mites. DSIR Information Series No. 139.

Table 4.1: Organisms present in the United Kingdom and the United States of America potentially associated with *Miscanthus*.

Scientific name	Common name	In New Zealand? (reference if yes)	Strains, hosts or genetic differences from offshore?	Under official control or notifiable in NZ?	Potential Hazard?	Other comments
Bacteria (see section 5.1 for risk analysis)						
Acidovorax avenae* subsp. *avenae	Leaf stripe	No	N/A	N/A	**Yes**	**Included in section 5.1**
Leifsonia xyli* subsp. *xyli	Sugarcane ratoon stunting	No	N/A	N/A	**Yes**	**Included in section 5.1**
Fungi (or fungi like) (see section 5.2 for risk analysis)						
Acremonium sp.		Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Alternaria alternata	Alternaria leaf spot	Yes (NZFungi 2007)	None known	No	No	
Apiospora montagnei	Ascomycete	Yes (NZFungi 2007)	None known	No	No	
Claviceps purpurea	Ergot	Yes (NZFungi 2007)	None known	No	No	
Cochliobolus cynodontis	Browning	Yes (NZFungi 2007)	None known	No	No	
Cochliobolus lunatus	Mould	Yes (NZFungi 2007)	None known	No	No	**Included in section 5.2***
Cochliobolus sativus	Leaf spot	Yes (NZFungi 2007)	None known	No	No	
Cochliobolus spicifer	Spring dead spot	Yes (NZFungi 2007)	None known	No	No	
Colletotrichum graminicola	Leaf spot	Yes (NZFungi 2007)	None known	No	No	
Colletotrichum sp.	Leaf spot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Davidiella tassiana	Black mould	Yes (NZFungi 2007)	None known	No	No	
Diaporthe sp.	Canker	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Diplodia sp.	Blight	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Drechslera gigantea	Eyespot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Epicoccum purpurascens	Red blotch	Yes (NZFungi 2007)	None known	No	No	
Fusarium culmorum	Rot	Yes (NZFungi 2007)	None known	No	No	
Fusarium miscanthi	Rot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Fusarium moniliforme	Rot	Yes (NZFungi 2007)	None known	No	No	
Fusarium pallidoroseum	Rot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Gibberella avenacea	Rot	Yes (NZFungi 2007)	None known	No	No	
Glomerella sp.	Leaf spot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi

Scientific name	Common name	In New Zealand? (reference if yes)	Strains, hosts or genetic differences from offshore?	Under official control or notifiable in NZ?	Potential Hazard?	Other comments
Glomerella tucumanensis	Leaf spot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Helminthosporium sp.	Eyespot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Khuskia oryzae	Rot	Yes (NZFungi 2007)	None known	No	No	
Leptosphaeria sp.	Canker	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Lophiostoma tetraploa	Leaf spot	Yes (NZFungi 2007)	None known	No	No	
Magnaporthe salvinii	Stem rot	No	N/A	N/A	**Yes**	**Included in section 5.2**
Meliola panici	Sooty Mould	Yes (NZFungi 2007)	None known	No	No	
Mycosphaerella recutita	Leaf blight	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Mycosphaerella striatiformans	Leaf spot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Nigrospora sp.	Stalk rot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Nigrospora sphaerica	Stalk rot	Yes (NZFungi 2007)	None known	No	No	
Paraphaeosphaeria michotii	Canker	Yes (NZFungi 2007)	None known	No	No	
Passalora koepkei	Yellow spot	No	N/A	N/A	**Yes**	Filamentous facultative fungi
Peronosclerospora sp.	Downy mildew	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Phlyctema sp.	Canker	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Phoma sp.	Blight	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Phomopsis sp.	Blight	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Phyllachora sp.	Leaf spot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Puccinia melanocephala	Rust	No	N/A	N/A	**Yes**	**Included in section 5.2**
Ramularia sp.	Leaf spot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Rhizoctonia sp.	Root rot	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Sclerophthora macrospora	Downy mildew	Yes (NZFungi 2007)	None known	No	No	
Stagonospora sp.	Scorch	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Pleospora tarda	Leaf blight	Yes (NZFungi 2007)	None known	No	No	
Thanatephorus cucumeris	Blight	Yes (NZFungi 2007)	Many physiological strains	No	Yes	Filamentous facultative fungi
Trichothecium roseum	Saprophyte	Yes (NZFungi 2007)	None known	No	No	

Scientific name	Common name	In New Zealand? (reference if yes)	Strains, hosts or genetic differences from offshore?	Under official control or notifiable in NZ?	Potential Hazard?	Other comments
Ustilago scitaminea	Sugarcane smut	No	N/A	N/A	**Yes**	**Included in section 5.2**
Verticillium sp.	Verticillium wilt	Some species in NZ	N/A	N/A	Yes/No	Filamentous facultative fungi
Invertebrates (see section 5.3 for risk analysis)						
Chilo suppressalis	Rice stem borer	No	N/A	N/A	No	Not found on plants *in vitro*
Helicotylenchus dihystera	Common spiral nematode	Yes (PPIN 2007)	None known	No	No	Not found on plants *in vitro*
Locusta migratoria	Migratory locust	Yes (PPIN 2007)	None known	No	No	Not found on plants *in vitro*
Melanaphis sacchari	Yellow sugarcane aphid	No	N/A	N/A	No	Not found on plants *in vitro*
Meloidogyne hapla	A root knot nematode	Yes (PPIN 2007)	None known	No	No	Not found on plants *in vitro*
Mesapamea secalis	Common rustic moth	No	N/A	N/A	No	Not found on plants *in vitro*
Noctua pronuba	Common yellow underwing	No	N/A	N/A	No	Not found on plants *in vitro*
Saccharicoccus sacchari	Grey sugarcane mealybug	No	N/A	N/A	No	Not found on plants *in vitro*
Schizotetranychus celarius	Bamboo spider mite	No	N/A	N/A	**Yes**	**Included in section 5.3**
Sesamia inferens	Purple stem borer	No	N/A	N/A	No	Not found on plants *in vitro*
Sitobion miscanthi	Indian grain aphid	Yes (PPIN 2007)	None known	No	No	Not found on plants *in vitro*
Viruses (see section 5.4 for risk analysis)						
Barley yellow dwarf luteovirus	BYDV	Yes (Pearson *et al.* 2006)	None known	No	No	
Miscanthus streak monogeminivirus	MiSV	No	N/A	N/A	**Yes**	**Included in section 5.4**
Sugarcane mosaic potyvirus	SCMV	No	N/A	N/A	**Yes**	**Included in section 5.4**

* *Cochliobolus lunatus* has been included as a representative of filamentous facultative fungi as the required information about this fungus is more readily available than any other example

4.1.4 POTENTIAL RISK MITIGATING MEASURES

For plant material to survive in culture for even a short time some reasonably successful attempt at rendering the material clean of contaminating external micro-organisms will have to have been attempted. While these methods will not necessarily be effective against invertebrate organisms, if any such organisms survive or the decontaminating process was ineffective, it would be expected that either the plants *in vitro* or the culture medium would show symptoms or signs of contamination. This contamination would then be expected to become visible under inspection given sufficient time.

Internally contaminating micro-organisms or diseases may not necessarily be expected to show symptoms on the plants *in vitro* for a number of reasons. Disease symptoms may only be expressed:

- On plant organs not found on plantlets *in vitro* e.g. fruit, flowers, secondary wood;
- In environmental conditions that plants *in vitro* are not normally exposed;
- On plant material older than the usual life cycle of plants *in vitro*.

In these circumstances mother plant, explant or *in-vitro* plantlet treatment or testing may be the only effective way of ensuring the material is free of such organisms or diseases.

4.1.5 GENERAL RISK MITIGATING MEASURES

The following or equivalent general measures should be required for all consignments of *Miscanthus* plants *in vitro* being imported into New Zealand (based on Bunn & Tan 2002 with additions):

- Mother plants (from which the *in-vitro* plantlets were excised) should be free of obvious signs of organism or disease contamination. Where possible, efforts should be made to isolate mother plants from potential sources of inoculum such as other contaminated plants.
- Mother plants should, where possible, be subjected to an antimicrobial and insecticide spray program 3 to 14 days prior to explant removal.
- Explant material should, where possible, be the material least likely to be contaminated by hazard organisms or diseases. Material such as small young actively growing meristems would be preferable to older plant material such as leaves or stems.
- Explant material should be surface sterilised using standard procedures such as a dip in 0.5% sodium hypochlorite. The duration of exposure and concentration of active ingredient will depend on the explant material, softer material usually being less tolerant.
- Nutrient concentrations in the tissue culture media used during quarantine inspections should be kept at similar rates to those used during the multiplication process.

As hazard organism growth *in vitro* is the principle mechanism for detection and subsequent risk management, the growing medium should not contain chemicals that may have

significant antibiotic properties. The *in-vitro* growing medium should therefore be free of antibiotics, biocides or chemicals having antimicrobial properties (e.g. activated charcoal).

To maintain as far as possible the axenic condition of plants *in vitro* and facilitate inspection during quarantine, rigid clear-sided (including base) tissue culture vessels that are sealable should be used.

4.2 REFERENCES

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (eds.) (1996) Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. Available at: http://biology.anu.edu.au/Groups/MES/vide/

Bunn E, Tan B (2002) Microbial contaminants in plant tissue culture propagation. In Sivasithamparam K, Dixon K W, Barrett R L (eds) 2002. Microorganisms in plant conservation and biodiversity. Pp 307-335. Kluwer Academic Publishers.

CABI CPC (2006) The Crop Protection Compendium, 2006 Edition. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

Cassells AC (2001) Contamination and its impact in tissue culture. ISHS ACTA Horticulturae 560: IV International Symposium on *In Vitro* Culture and Horticultural Breeding.

Crous PW, Groenewald JZ (2005): Hosts, species and genotypes: opinions versus data. Australasian Plant Pathology 34: 463-470.

Farr DF, Rossman AY, Palm ME, McCray EB (2007) Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA. Retrieved August 2, 2007, from http://nt.ars-grin.gov/fungaldatabases/

NZFungi (2007) New Zealand fungi (and bacteria). Landcare Research New Zealand Limited. Available on line at http://nzfungi.landcareresearch.co.nz/html/mycology.asp

Pearson MN, Clover GRG, Guy PL, Fletcher JD, Beever RE (2006) A review of the plant virus, viroid and mollicute records for New Zealand. Australasian Plant Pathology 35 (2): 217-252

Pennycook SR (1989) Plant diseases recorded in New Zealand. Vol. 2 & 3. Plant Diseases Division, DSIR, Auckland, New Zealand.

PPIN (2007) Plant Pest Information Network. New Zealand Ministry of Agriculture and Forestry

Sansford C, McLeod A (2000) PRA for *Miscanthus*: Summary of PRAs for 1. Import of Rhizomes from Dominica and 2. Global Imports. Central Science Laboratory PPP8860

5 ANALYSIS OF HAZARDS

5.1 BACTERIAL HAZARDS

5.1.1 HAZARD IDENTIFICATION

5.1.1.1 AETIOLOGIC AGENT

Acidovorax avenae subsp. *avenae* (Manns 1909)

Leifsonia xyli subsp. *xyli* (Davis *et al*. 1984)

5.1.1.2 NEW ZEALAND STATUS

Acidovorax avenae subsp. *avenae* and *Leifsonia xyli* subsp. *xyli* have not been recorded in New Zealand (Pennycook 1989, PPIN 2005, NZFungi 2007).

5.1.1.3 BIOLOGY AND EPIDEMIOLOGY

Information on these organisms has been collated from the CABI CPC 2006[8] with available or supplementary references provided.

Acidovorax avenae subsp. *avenae*

The bacterium *A. avenae* subsp. *avenae* causes several important plant diseases including bacterial stripe of rice, bacterial stalk rot of corn, bacterial leaf blight of oats, and red stripe of sugarcane and millet (Song *et al.* 2004). It typically infects primary leaves of rice seedlings and, in most instances, the seedlings later outgrow the disease. It appears to be transmitted by rain and wind. Entry into the plant is through stomata and hydathodes. Although bacterial exudate is not seen, large numbers of bacteria can emerge onto wet leaf surfaces, especially on damaged leaves (Bradbury 1986). It can attack and rot young, unfolded leaves which may lead to stunting or death of the seedling. *A. avenae* subsp. *avenae* occurs sporadically on maize in India, but is of minor economic importance on this crop. *A. avenae* subsp. *avenae* also causes red stripe disease of sugarcane. During rainy seasons in the 1970s, the disease appeared on several commercially important sugarcane cultivars in Central America. Incidence was favoured by high and frequent rainfall suggesting that the pathogen was spread in warm, humid weather by dispersal of bacteria exuded from stomata and hydathodes (CABI CPC 2006).

The bacterium is not thought to survive well in soil or in plant debris; however, it survives in association with ditch-bank and roadside weeds. Contaminated farm equipment has been

8 The Crop Protection Compendium, 2006 Edition. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

mentioned as implicated as a primary means by which the bacterium disseminates within a field of sweetcorn. Alternative hosts such as *Paspalum urvillei* in Florida, USA, have been noted as an inoculum source in outbreaks of bacterial leaf blight of maize (CABI CPC 2006).

The bacterium can be transmitted internally from plant to seed in latently infected plants. Seed transmission has been confirmed by germination of artificially inoculated rice seeds in test tubes. Bending symptoms (coleoptiles and mesocotyls of infected seedlings showed abnormal elongation) appeared on rice seedlings after incubation for 3 days. Various other assays have been applied in the isolation of the pathogen from seeds, namely seedling and liquid assays. Immunomagnetic separation and PCR assays have been used in the detection of the pathogen from pearl millet seeds (CABI CPC 2006). Specific PCR primers are available for the identification of the pathogen (Schaad *et al*. 2003). A semi-selective liquid medium based on d-sorbitol and l-pyroglutamic acid (SP medium), and two sets of polymerase chain reaction (PCR) primers were also designed for use in a BIO-PCR assay for detection of *A. avenae* subsp. *avenae* (Song *et al.* 2004).

Recorded hosts of *A. avenae* subsp. *avenae* include *Oryza sativa* (rice), *Saccharum officinarum* (sugarcane), *Sorghum bicolor* (sorghum), *Zea mays* (maize), *Avena sativa* (oats), *Camellia sinensis* (tea), *Eleusine coracana* (finger millet), *Hordeum vulgare* (barley), *Panicum miliaceum* (millet), *Pennisetum glaucum* (pearl millet), *Setaria italica* (foxtail millet), *Setaria lutescens*, *Setaria viridis* (green foxtail), *Triticum aestivum* (wheat), *Agropyron* (wheatgrass), *Bromus catharticus* (prairiegrass), *Bromus inermis* (awnless brome), *Bromus marginatus* (Mountain brome(grass)), *Caryota*, *Digitaria sanguinalis* (large crabgrass), *Echinochloa crus-galli* (barnyard grass), *Paspalum*, *Poaceae* (grasses), *Zea mexicana* (teosinte) (CABI CPC 2006).

Leifsonia xyli subsp. *xyli*

Ratoon stunting disease caused by this bacterium has been found in most sugarcane growing areas of the world and can cause yield losses of up to 50% in susceptible and intolerant varieties. Although sugarcane is reported as the only known natural host of the pathogen (*Saccharum* spp. and *Saccharum* interspecific hybrids), numerous grasses have been determined to be hosts after experimental inoculation. The experimental hosts include *Zea mays*, *Sorghum* spp., *Brachiaria mutica*, *Brachiaria miliiformis*, *Chloris gayana*, *Cynodon dactylon*, *Echinochloa colonum*, *Imperata cylindrica*, *Panicum maximum*, *Pennisetum purpureum* and *Rhynchelytrum repens* (CABI CPC 2006). *Leifsonia xyli* subsp. *xyli* was reported occurring naturally on *Miscanthus* in Indonesia (Tew *et al*. 1991).

The pathogen has no known insect vectors with infection taking place through wounds. The pathogen can be mechanically transmitted from sugarcane to sugarcane on the blades of equipment used to cultivate and harvest crops and can be spread by propagation with infected cuttings. No evidence exists for transmission in true seed. The pathogen can remain viable and infectious for several months apparently in either moribund plant debris or the soil itself, contributing to the persistence of ratoon stunting disease in areas where the disease is common (CABI CPC 2006).

The pathogen systemically invades plants through the xylem. It has been detected in most vegetative parts of sugarcane where mature xylem exists. It was recovered readily from

mature stalks and the leaf sheaths and lamina of the lower leaves of infected plants, but not from the midrib and lamina of upper leaves. Large populations existed in mature stalks, and smaller populations were found in the growing point, leaf lamina, leaf midrib and leaf sheath (CABI CPC 2006).

Ratoon stunting disease is widely regarded as causing greater economic loss to the cane sugar industries throughout the world than any other disease; yet paradoxically, few other diseases of sugarcane are less conspicuous. Yield losses have frequently been estimated at 5 to 10% overall. Yield reduction is caused by slower growth of diseased crops with the accompanying production of thinner and shorter stalks and sometimes a reduction in the number of stalks when the disease is severe. In stubble or ratoon crops, diseased plants are slower to initiate growth, and death of individual plants of extremely susceptible cultivars may occur (CABI CPC 2006).

Although there may be no externally conspicuous symptoms of the disease, internally there is usually an orange-red discoloration of the vascular bundles containing the water-conducting tissues (xylem) at the basal nodes of the stalk. Although it can be isolated from diseased cane, it is very difficult since it is slow growing and must be grown on specialized culture media (Comstock & Lentini 2005). Historically, diagnosis of ratoon stunting disease has been difficult because there are no definitive external symptoms and internal symptoms do not develop adequately in all varieties (CABI CPC 2006).

The pathogen is extremely fastidious in its nutritional requirements and can only be grown in axenic culture on specialized culture media and aerobically at 29°C for 2-3 weeks. Pathogenicity tests for routine identification are rarely conducted because of the long incubation period (usually at least 3-6 months or longer) required for symptom development or detection of the pathogen in the host plant (CABI CPC 2006).

A tissue-blot DNA hybridization technique and polymerase chain reaction (PCR) procedures have been reported for the sensitive and specific detection of the pathogen in sugarcane (CABI CPC 2006). Hot-water treatment at ca 50°C for 2-3 hours has been the most commonly used method for treatment of infected material. Under practical conditions, however, heat treatment is often not completely curative for ratoon stunting disease. To enhance effectiveness, heat therapy has to be repeated to ensure disease-free seed cane (CABI CPC 2006).

General bacterial information

Latent infections of tissue-cultured *Anthurium andraeanum* caused by the blight pathogen, *Xanthomonas campestris* pv. *dieffenbachiae*, were examined by Norman & Alvarez (1994). The pathogen survived in or on callus for over 4 months without producing symptoms in callus or turbidity in the medium. The pathogen survived for more than 1 year on or within stage II shoots without producing symptoms and was successively transferred three times as latently infected shoots were multiplied. The pathogen did not grow or survive for more than 2 weeks in Murashige and Skoog medium lacking plant material. The addition of coconut water enhanced bacterial growth and produced turbidity in culture media. As a result it was considered by the authors that latently infected *in vitro* anthuriums may be inoculum sources for subsequent outbreaks of *Xanthomonas campestris* pv. *dieffenbachiae* (Norman & Alvarez

1994). No equivalent studies could be found on the persistence of *Acidovorax avenae* subsp. *avenae* or *Leifsonia xyli* subsp. *xyli* on plants *in vitro*.

5.1.1.4 HAZARD IDENTIFICATION CONCLUSION

Based on:

- The accepted absence of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* in New Zealand;
- The potential ability of these bacteria to be vectored by *Miscanthus* plants *in vitro*; and
- The potential ability of these bacteria to cause disease symptoms on commercially important plants in New Zealand;

it is proposed that these bacteria be considered potential hazards requiring further assessment.

5.1.2 RISK ASSESSMENT

5.1.2.1 ENTRY ASSESSMENT

Should *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* be associated with the *Miscanthus* mother plants at the time the *in-vitro* material is excised, there is a high likelihood that any plants *in vitro* will also be contaminated. There is little available information, however, on the prevalence of these bacteria within infected *Miscanthus* populations. The likelihood of survival of these bacteria during long-distance transport in infected propagation material is high as long as the propagated material remains viable.

It is therefore considered that the likelihood of entry of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* into New Zealand with *Miscanthus* plants *in vitro* from infected populations is moderate and therefore non-negligible.

5.1.2.2 ASSESSMENT OF EXPOSURE AND ESTABLISHMENT

Should *A. avenae* subsp. *avenae* or *L. xyli* subsp. *xyli* be associated with imported *Miscanthus* plants *in vitro* either bacterium could be expected to use the infested material as a vehicle to allow their exposure and establishment in the New Zealand environment. Provided these bacteria manifest themselves then any *in-vitro* material showing disease symptoms would usually be discarded during the micro-propagation process reducing the level of contamination within the imported material prior to or during the transfer of explants to the field.

The likelihood of exposure and establishment should be considered low to moderate and therefore non-negligible.

5.1.2.3 CONSEQUENCE ASSESSMENT

Spread

A. avenae subsp. *avenae* appears to be transmitted naturally by rain and wind. The bacterium is not thought to survive well in soil or in plant debris however it survives in association with ditch-bank and roadside weeds. Contaminated farm equipment has been implicated as a primary means by which the bacterium disseminates within a field of sweetcorn. The bacterium can be transmitted internally from plant to seed in latently infected plants. Seed transmission has been confirmed in rice by germination of artificially inoculated rice seeds in test tubes. The rate of natural or assisted dispersal within New Zealand should therefore be considered moderate to high in areas where hosts are grown commercially.

L. xyli subsp. *xyli* has no known insect vectors with infection taking place through wounds. The pathogen can be mechanically transmitted from sugarcane to sugarcane on the blades of equipment used to cultivate and harvest crops and can be spread by propagation with infected cuttings. No evidence exists for transmission in true seed. The pathogen can remain viable and infectious for several months apparently in either moribund plant debris or the soil itself, contributing to its persistence in areas where the disease is common. Given the narrow host range and limited availability of hosts within New Zealand, *L. xyli* subsp. *xyli* spread is likely to be limited to *Miscanthus* and as such should be considered low.

Economic consequences

A. avenae subsp. *avenae* causes several important plant diseases including bacterial stripe of rice, bacterial stalk rot of corn, bacterial leaf blight of oats, and red stripe of sugarcane and millet (Song *et al.* 2004). The potential economic impact of *A. avenae* subsp. *avenae* on the New Zealand agricultural sector should therefore be considered moderate to high.

L. xyli subsp. *xyli* can cause yield losses of up to 50% in susceptible and intolerant varieties of sugarcane, however the likely impacts on *Miscanthus* production are unknown. While this bacterium had been found associated with *Miscanthus* in the field, there is no evidence that resulting impacts to the *Miscanthus* plants were high. The potential economic impact of *L. xyli* subsp. *xyli* on the New Zealand agricultural sector should therefore be considered low.

Environmental consequences

It should be considered possible (though a low likelihood) that, either through a known host or via a new and as yet unidentified host, *A. avenae* subsp. *avenae* or *L. xyli* subsp. *xyli* could have a low-level environmental impact.

Human health consequences

A. avenae subsp. *avenae* and *L. xyli* subsp. *xyli* are not known to be of any significance to human health.

5.1.2.4 CONCLUSION OF CONSEQUENCE ASSESSMENT

From the assessment above it is possible to conclude that:

- *A. avenae* subsp. *avenae* could cause moderate to high economic consequences and low environmental consequences to New Zealand.
- *L. xyli* subsp. *xyli* has a low likelihood of causing low economic consequences and low environmental consequences to New Zealand.

5.1.2.5 RISK ESTIMATION

The likelihood estimate is moderate that *A. avenae* subsp. *avenae* or *L. xyli* subsp. *xyli* would be associated with *Miscanthus* plants *in vitro* on entry into New Zealand, and low to moderate that any such bacteria that do enter would successfully establish in New Zealand. The likelihood estimate is considered moderate to high that the establishment of *A. avenae* subsp. *avenae* in New Zealand would result in moderate to high economic consequences and low environmental consequences to New Zealand. The likelihood estimate is considered low that the establishment of *L. xyli* subsp. *xyli* in New Zealand would result in low economic and environmental consequences to New Zealand.

As a result the risk estimate for *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* associated with imported *Miscanthus* plants *in vitro* is non-negligible and these bacteria should be considered hazards.

5.1.2.6 ASSESSMENT OF UNCERTAINTY

There is significant uncertainty around the association of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* with *Miscanthus*. As such this risk assessment should be reviewed once further relevant information becomes available.

5.1.3 RISK MANAGEMENT

5.1.3.1 RISK EVALUATION

Since the risk estimate for *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* associated with imported *Miscanthus* plants *in vitro* is non-negligible, phytosanitary measures should be employed to reduce the risks to an acceptable level.

5.1.3.2 RISK MANAGEMENT OBJECTIVE

The risk management objective is to ensure any *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* infecting imported *Miscanthus* plants *in vitro* is neither:

- transplanted into the New Zealand environment on the imported *Miscanthus* plants *in vitro*; or

- o transmitted to a host plant in the New Zealand environment from the imported *Miscanthus* plants *in vitro*.

5.1.3.3 OPTION EVALUATION

There are conceivably a number of points on the importation pathway that measures could be implemented to meet the aforementioned management objectives. The following risk management options should be assessed:

- **a.** Pest free area (PFA): *Miscanthus* plants *in vitro* are established from mother plants growing in areas that are free of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli*;
- **b.** Pest free place of production (PFPP): *Miscanthus* plants *in vitro* are imported from a place of production that is free of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli*;
- **c.** Treatment, for any infecting *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli*, of mother plants before explants are taken or explants before culturing;
- **d.** Inspections for symptom expression of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* either prior to export to New Zealand or on arrival in New Zealand in a post entry quarantine facility.

Pest free area (PFA)

The International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas* (ISPM No 4) describes the requirements for the establishment and use of PFAs as a risk management option for meeting phytosanitary requirements for the import of plants. The standard identifies three main components or stages that must be considered in the establishment and subsequent maintenance of a PFA:

- o systems to establish freedom
- o phytosanitary measures to maintain freedom
- o checks to verify freedom has been maintained.

Normally PFA status is based on verification from specific surveys such as an official delimiting or detection survey. Visual examination may reveal infected plants in the field, but the absence of symptoms does not exclude latent infection of *Miscanthus*. It therefore should be considered that a reliable PFA determination may not be possible in areas where disease presence may be suspected.

Pest free place of production (PFPP)

The International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites* (ISPM No 10) describes the requirements for the establishment and use of pest free places of production as a risk management option for meeting phytosanitary requirements for the import of plants. A pest free place of production is defined in the standard as a "place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period". Pest freedom is established by surveys and/or growing season inspections and maintained as necessary by other systems to prevent the entry of the pest into the place of production. As with PFA

above, it should be considered that a reliable PFPP determination may not be possible in areas where disease presence may be suspected.

Treatment options

Hot-water treatment at 50°C or greater for 2-3 hours has been the most commonly used method for treatment of sugarcane material infected with *L. xyli* subsp. *xyli* (Comstock & Lentini 2005). Under practical conditions, however, heat treatment is often not completely effective (Damann & Benda 1983). To enhance effectiveness the heat treatment should be repeated (CABI CPC 2006, Comstock & Lentini 2005).

It should be noted that this treatment has been reported to reduce germination in some sensitive sugarcane clones (Frison & Putter 1993), and no information could be found to indicate how sensitive *Miscanthus* material may be. Care should therefore be taken to test these or other sugarcane treatments to ensure plant material is not adversely affected.

Detection methods

Acidovorax avenae subsp. *avenae*

Bending symptoms (coleoptiles and mesocotyls of infected seedlings showing abnormal elongation) appeared on rice seedlings after incubation (*in vitro*) for 3 days. Various other assays have been applied in the isolation of the pathogen from rice seeds, namely seedling and liquid assays. Immunomagnetic separation and PCR assays have been used in the detection of the pathogen from pearl millet seeds (CABI CPC 2006). Specific PCR primers are available for the identification of the pathogen (Schaad *et al*. 2003). A semi-selective liquid medium based on d-sorbitol and l-pyroglutamic acid (SP medium), and two sets of polymerase chain reaction (PCR) primers were also designed for use in a BIO-PCR assay for detection of *A. avenae* subsp. *avenae* (Song *et al.* 2004)

Leifsonia xyli subsp. *xyli*

The pathogen is extremely fastidious in its nutritional requirements and can only be grown in axenic culture on specialized culture media and aerobically at 29°C for 2-3 weeks. Pathogenicity tests for routine identification are rarely conducted because of the long incubation period (usually at least 3-6 months or longer) required for symptom development or detection of the pathogen in the host plant (CABI CPC 2006). The disease is reported to be able to cause a 5 to 15% loss in crop yield without the grower even knowing their fields have been infected (Comstock & Lentini 2005).

A tissue-blot DNA hybridization technique and polymerase chain reaction (PCR) procedures have been reported for the sensitive and specific detection of the pathogen in sugarcane (CABI CPC 2006). Considerable sensitivity has also been obtained by using fluorescent-antibody staining to detect bacterial cells with epifluorescence microscopy. Staining of the bacterium in dried extracts on glass slides was at least tenfold more sensitive than phase-contrast microscopy for detection. At least another tenfold increase in sensitivity was obtained when the bacterium was first stained while still suspended in sap extracts and then concentrated on membrane filters by filtration before observation (CABI CPC 2006).

5.1.3.4 RISK MANAGEMENT PROPOSALS

From areas or places of production that are unable to obtain suitable supported area freedom declarations, the following alternative risk management options are proposed:

For *A. avenae* subsp. *avenae*:

- *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *A. avenae* subsp. *avenae*; or
- *Miscanthus* plants *in vitro* should be inspected over two 30-day periods for abnormal growth or other such disease symptoms. Inspections should be of plantlets (no sub-culturing should occur during each 30-day period) in normal growing conditions e.g. 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation; or
- Prior to *in-vitro* culturing, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for at least 3 hours per treatment.

For *L. xyli* subsp. *xyli*:

- *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *L. xyli* subsp. *xyli*; or
- *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to fluorescent-antibody staining of sap extracts and then concentrated on membrane filters by filtration before observation with epifluorescence microscopy to confirm freedom from *L. xyli* subsp. *xyli*; or
- Prior to *in-vitro* culturing, explants should be subjected to two consecutive hot-water treatments at 50°C or greater for 3 hours per treatment.

Any plant material found to be contaminated by *A. avenae* subsp. *avenae* or *L. xyli* subsp. *xyli* should not be planted into the New Zealand environment but rather disposed of in a suitable manner.

5.1.4 ASSESSMENT OF RESIDUAL RISK

5.1.4.1 OBJECTIVES FOR PROPOSED MANAGEMENT OPTION(S)

The objective of these general measures is to ensure that any imported *Miscanthus* plants *in vitro* are free of *A. avenae* subsp. *avenae* and *L. xyli* subsp. *xyli* prior to biosecurity clearance into New Zealand.

5.1.5 REFERENCES

Bradbury JF (1986) Guide to plant pathogenic bacteria. CAB International, Wallingford, Oxon, UK.

CABI CPC (2006) Crop Protection Compendium on Internet. CAB International, Wallingford, UK; http://www.cabi.org/compendia/CPC/

Comstock JC, Lentini RS (2005) Sugarcane Ratoon Stunting Disease. Florida Sugarcane Handbook, SS-AGR-209. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available on the EDIS website at http://edis.ifas.ufl.edu.

Damann KE, Benda GTA (1983) Evaluation of commercial heat-treatment methods for control of ratoon stunting disease of sugarcane. Plant Disease, 67(9); pp 966-967

ISPM No. 4 (1995) International Standards for Phytosanitary Measures number 4: Requirements for the establishment of pest free areas. FAO, 15 pp.

ISPM No. 10 (1999) International Standards for Phytosanitary Measures number 10: Requirements for the establishment of pest free places of production and pest free production sites. FAO; 15 pp.

Frison EA, Putter CAJ (1993) FAO/IBPGR Technical Guidelines for the Safe Movement of Sugarcane Germplasm. Food and Agricultural Organisation of the United Nations; pp 44.

Norman DJ, Alvarez AM (1994). Latent infections of *in vitro Anthurium* caused by *Xanthomonas campestris* pv. *dieffenbachiae*. Plant Cell, Tissue and Organ Culture 39; 55-61.

NZFungi (2007) New Zealand fungi (and bacteria). Landcare Research New Zealand Limited. Available on line at http://nzfungi.landcareresearch.co.nz/html/mycology.asp

Pennycook SR (1989) Plant diseases recorded in New Zealand. Plant Diseases Division, DSIR, Auckland, New Zealand; 180pp.

PPIN (2007) Plant Pest Information Network, MAF database (date of search indicated).

Song WY, Kim HM, Hwang CY, Schaad NW (2004) Detection of *Acidovorax avenae* subsp. *avenae* in rice seeds using BIO-PCR. Journal of Phytopathology 152 (11-12): 667-676(10)

Schaad, N. W., Song, W. Y., Sechler, A. J., Hatziloukas, E., Kim, H. M. Use of PCR for rapid identification of *Acidovorax avenae* and *A. avenae* subsp. *citrulli*. Presentations from the 6th International Conference on *Pseudomonas syringae* pathovars and related pathogens, Maratea, Italy, September 15-19, 2002., 2003

Tew TL, Purdy LH, Lamadji S, Irawan (1991) Indonesian sugarcane germplasm collecting expedition – 1984 Hawaiian Planters' Record 61(2): pg 25-43

5.2 FUNGAL HAZARDS

5.2.1 HAZARD IDENTIFICATION

5.2.1.1 AETIOLOGIC AGENT

1. *Cochliobolus lunatus* R.R. Nelson & Haasis

This is a representative example of the following potential hazard organisms (see table 4.1):

Drechslera gigantea	Eyespot	*Mycosphaerella recutita*	Leaf blight
Acremonium sp.	Black bundle disease	*Mycosphaerella striatiformans*	Leaf spot
Colletotrichum sp.	Leaf spot	*Passalora koepkei*	Yellow spot
Diaporthe sp.	Canker	*Phlyctema* sp.	Canker
Glomerella sp.	Leaf spot	*Phyllachora* sp.	Leaf spot
Glomerella tucumanensis	Leaf spot	*Ramularia* sp.	Leaf spot
Helminthosporium sp.	Eyespot	*Stagonospora* sp.	Scorch
Leptosphaeria sp.	Canker	*Verticillium* sp.	Verticillium wilt

2. *Magnaporthe salvinii* (Catt.) R.A. Krause & R.K. Webster

This is a representative example of the following potential hazard organisms (see table 4.1):

Diplodia sp.	Blight	*Phoma* sp.	Blight
Fusarium miscanthi	Rot	*Phomopsis* sp.	Blight
Fusarium pallidoroseum	Rot	*Rhizoctonia* sp.	Root rot
Nigrospora sp.	Stalk rot	*Thanatephorus cucumeris*	Blight

3. *Puccinia melanocephala* Syd. & P. Syd.

This is a representative example of the following potential hazard organisms (see table 4.1):

Peronosclerospora sp.	Downy mildew

4. *Ustilago scitaminea* Syd.

5.2.1.2 NEW ZEALAND STATUS

Magnaporthe salvinii, *Puccinia melanocephala* and *Ustilago scitaminea* have not been recorded in New Zealand (Pennycook 1989, PPIN 2007, NZFungi 2007). *Cochliobolus lunatus* is recorded as being present in New Zealand (NZFungi 2007), however this fungus has been included in the analysis as a representative of other filamentous facultative fungi (refer chapter 4).

5.2.1.3 Biology and epidemiology

Information on these organisms has been collated from the CABI CPC 2006[9] with available or supplementary references provided.

Cochliobolus lunatus

C. lunatus is pathogenic to numerous hosts, primarily attacking the floral structures or leaves. The anamorph, *Curvularia lunata*, is the form usually recorded in nature. This species is considered part of a complex of fungi that produce grain mould of many hosts under conditions of high rainfall and high relative humidity (CABI CPC 2006).

Magnaporthe salvinii

This fungus mainly causes stem tissue rot, with only the epidermis remaining intact. This interferes in the transport of materials to the grain so that commercial yields of the affected crop decrease. The milling quality is also lowered because of partial grain filling and chalkiness of grains when infection occurs at an early stage. Rice yield losses of 5-80% caused by stem rot have been reported from different countries. The highest percentage loss per plant was observed at the 20th day after infection (CABI CPC 2006).

In culture, the mycelium is white at first, later becoming smoky to black at the surface of medium. The optimum pH and temperature for chlamydospore germination are pH 5-8 and 25-30°C, respectively, with sugar and light acting as promoters (CABI CPC 2006).

Aside from rice, the stem rot pathogen has also been found on the following natural hosts: *Zizaniopsis miliacea*, three species of Cyperaceae, one species of Liliaceae and one species of Juncaeae. Other host species identified by wound inoculation are *Eleusine indica*, *Leptochloa chinensis* and *Setaria pallide-fusca* (CABI CPC 2006). Lu *et al*. (2000) recorded this pathogen as a saprophyte on the culms of *Miscanthus floridulus* from Hong Kong.

Puccinia melanocephala

The life cycle of sugarcane common rust is simple with the urediniospore being the only known infectious spore. Urediniospores are produced in, and are released from, pustules that develop on the underside of sugarcane leaves. The wind and rain dispersed urediniospores land on sugarcane leaves, germinate, develop appressoria, and penetrate the leaf via stomatal openings. The development of sub-stomatal vesicles, infectious hyphae, haustoria, and subsequent infection processes are similar to other *Puccinia* spp. Urediniospore production occurs 8-18 days after the initial urediniospore lands on a leaf, depending on varietal susceptibility and environmental conditions. The majority of urediniospores are dispersed short distances either within or between adjacent fields. However, the spores may also be dispersed over much longer distances. Introduction into the Americas is believed to be from transoceanic transport of spores from Cameroon (CABI CPC 2006).

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9 The Crop Protection Compendium, 2006 Edition. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

Rust development is favoured by cooler weather, since optimum urediniospore germination occurs at 21-26°C and sporulation and germination of urediniospores are inhibited at high temperatures. In Florida, USA, the severity of rust symptoms decreases in late May as summer approaches and the temperature rises. Environmental conditions that promote longer periods of leaf moisture favour rust development. Leaf moisture for 8 hours or longer is required to allow the germ tube to penetrate the stomatal opening and initiate infection. If the germ tube dries prior to penetration it dies. The relatively short urediniospore reproductive cycle (8-18 days) allows the rapid disease build-up in susceptible fields. Within 5 to 6 weeks green fields can appear reddish due to the massive pustule formation that develops on the foliage (CABI CPC 2006). *P. melanocephala* has caused direct yield losses and indirect economic costs in various sugarcane industries of the world and is continuing to cause an economic impact to sugarcane industries throughout the world (CABI CPC 2006).

The initial symptoms of sugarcane common rust are elongate yellowish leaf spots, 1-4 mm long. On susceptible plants, the spots increase in size with a reddish-brown change in colour. As uredinia formation occurs, the leaf epidermis ruptures giving rise to typical rust pustules. Uredinia form primarily on the lower leaf surface and erupt, releasing masses of orange-to-orange-brown urediniospores. The elongate pustules are parallel to the leaf venation and measure 2-20 mm by 1-4 mm. Multiple pustules on leaves give a reddish appearance to plants from a distance. Pustules coalesce causing large areas of necrotic leaf tissue usually on the lower leaves (CABI CPC 2006).

Normal heat treatments used in quarantine (50°C or greater for 2-3 hours) is likely to kill urediniospores contaminating sections of stalks used to propagate sugarcane vegetatively (CABI CPC 2006). The main hosts of *P. melanocephala* are in the genus *Saccharum* (*S. officinarum* (sugarcane), *S. robustum*, and *S. spontaneum* (wild sugarcane)) (CABI CPC 2006), while it is recorded on *Miscanthus* sp. in China by Zhuang (2001).

Ustilago scitaminea

Sugarcane smut, *U. scitaminea* is transmitted by means of airborne teliospores and by vegetative propagation of systemically infected stalks. Airborne spores that are deposited on healthy cane germinate on wet surfaces to produce an infectious mycelium that penetrates the base of the bud scales, infecting the meristem tissue. Thus, certain characteristics of the buds affect sugarcane resistance to *U. scitaminea*. Infection takes place within 24 hours of inoculation at high humidity and an optimum temperature of 30-31°C. The fungal hyphae remain viable in the dormant buds until stalks are cut, planted and the buds germinate. The new developing plants are thus systemically infected by the mycelium that continues to grow along with meristematic tissue. There is no evidence that spores remain viable for extended periods in soil, or other environments, outside the living plant tissue (CABI CPC 2006).

Infected sugarcane may continue to grow for months with no external symptoms of the disease. After some time (from 1 or 2 months to more than a year), the host growing point begins to produce a long terminal sorus, often called a 'whip', that is composed of plant tissue surrounded by a gelatinous layer of fungal tissue and begins to produce more teliospores. In susceptible varieties this stage occurs sooner, and in a higher percentage of stools, than in non-susceptible varieties. Spore production may continue for as long as 3 to 4

months from a single sorus and around 100 spores per day can be released (CABI CPC 2006).

When susceptible varieties are planted, the inoculum level in the field increases more rapidly than with resistant varieties. Infected lateral buds on the sugarcane plant may also germinate and produce smaller sori. Extensive germination of lateral shoots or 'lalas' on canes is one symptom of *U. scitaminea* infection. In heavily infected fields, a large number of small, weak canes are produced giving the stools a grassy appearance. During the plant crop cycle and through each successive ratoon, the percentage of infected canes continues to increase. This occurs more rapidly with susceptible varieties and in regions with hot, moist environments. If infected cane is cut for seed, the new field will also be infected and the disease cycle continues (CABI CPC 2006).

Even though sugarcane smut is worldwide in distribution, the existence of different *U. scitaminea* races makes quarantine regulations important (CABI CPC 2006). In most sugarcane-growing countries of the world, strict quarantine regulations govern the importation of sugarcane vegetative propagation materials or true seed. Most of these countries require proof of hot water treatment of stalk pieces followed by treatment with a fungicide and insecticide. This is to ensure that a number of bacterial, viral and fungal diseases, including *U. scitaminea*, will not be brought in. Some countries, Australia and the USA in particular, require additional quarantine in sequestered locations until the cane has been grown for one generation before releasing it for commercial propagation (CABI CPC 2006).

It is difficult to make a precise assessment of the economic importance of *U. scitaminea* since most estimates of yield loss are based on observation and experience rather than rigorous experimentation. It is certain, however, that losses may be quite severe in susceptible varieties under conditions suitable for disease development. There are reports of yield losses of 50-73%. In addition to cane yield losses, *U. scitaminea* also appears to reduce cane quality. Decreases in both sugar extractability and recovery, as estimated by reductions in juice purity, have been reported. *U. scitaminea* is also known to cause decreases in the number of millable stalks as well as in stalk diameter. In Hawaii, highly susceptible varieties showed cane yield losses of 10-15% in severely infected commercial ratoon fields, while losses in sugar processing were an additional 5-7% (CABI CPC 2006).

The most obvious symptom of *U. scitaminea* infection is the long, whip-like sorus that emerges from the growing point and frequently extends above the tops of the infected plant. Sori may also be produced from side shoots originating from lateral buds. For much of its life cycle, however, the fungus is systemic in the plant and produces no identifiable symptoms except for a 'grassy' appearance in severe cases. This appearance results from the production of numerous weak, spindly stalks in place of the usual vigorous canes. The systemic infection may be present for months without producing a sorus (CABI CPC 2006).

Polymerase chain reaction (PCR) has been used successfully to identify *U. scitaminea* in infected host tissue. The primers for this assay amplify a product from a *U. scitaminea* mating type allele (bE gene) which is specific to *U. scitaminea* (CABI CPC 2006).

Hot-water dip of cane pieces before planting is effective in ensuring clean seed. A short hot-water treatment of 52°C for 30 minutes, or a longer hot-water treatment of 50°C for 2 hours, are both adequate in eliminating *U. scitaminea* from sugarcane pieces. This practice is now standard procedure in many plantations (CABI CPC 2006). *Saccharum officinarum* (sugarcane) is the most significant economic host of *U. scitaminea* (CABI CPC 2006), while Vanky (2000) records it as being found on *Miscanthus* and being present in most sugarcane-growing areas of the world.

5.2.1.4 HAZARD IDENTIFICATION CONCLUSION

Based on:

- The accepted absence in New Zealand of *Magnaporthe salvinii*, *Puccinia melanocephala* and *Ustilago scitaminea*, and the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*;
- The potential ability of these fungi to be vectored by *Miscanthus* plants *in vitro*; and
- The potential ability of these fungi to cause disease symptoms on commercially important plants in New Zealand;

it is proposed that these fungi be considered potential hazards requiring further assessment.

5.2.2 RISK ASSESSMENT

5.2.2.1 ENTRY ASSESSMENT

Should *Magnaporthe salvinii*, *Puccinia melanocephala*, or *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, be associated with the *Miscanthus* mother plants at the time the *in-vitro* material is excised, there is a high likelihood that any plants *in vitro* will also be contaminated. There is little available information, however, on the prevalence of these fungi within infected *Miscanthus* populations. The likelihood of survival of these fungi during long-distance transport in infected propagation material is high as long as the propagated material remains viable.

It is therefore considered that the likelihood of entry into New Zealand of *Magnaporthe salvinii*, *Puccinia melanocephala* and *Ustilago scitaminea*, and the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, with *Miscanthus* plants *in vitro* from infected populations is moderate and therefore non-negligible.

5.2.2.2 ASSESSMENT OF EXPOSURE AND ESTABLISHMENT

Should *Magnaporthe salvinii*, *Puccinia melanocephala* or *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, be associated with imported *Miscanthus* plants *in vitro* they would be expected to use the infested material as a vehicle to allow exposure and establishment in the New Zealand environment. As *in vitro* material showing disease symptoms is usually discarded during the micro-propagation process, the level of contamination within the imported material would be expected to be reduced during the transfer of explants to the field.

The likelihood of exposure and establishment should be considered low to moderate and therefore non-negligible.

5.2.2.3 CONSEQUENCE ASSESSMENT

Spread

Cochliobolus lunatus, like many of the other filamentous facultative fungi listed in table 4.1, is pathogenic to numerous hosts and is more active under conditions of high rainfall and high relative humidity. The rate of dispersal within New Zealand should therefore be considered moderate to high in northern areas where climate is more suitable for disease expression.

Magnaporthe salvinii has a relatively narrow host range and while acting as a saprophyte as well as a pathogen, requires relatively high temperatures (25-30°C) for optimum germination and disease expression. The rate of dispersal within New Zealand should therefore be considered moderate in northern areas where climate is more suitable for disease expression.

Puccinia melanocephala and *Ustilago scitaminea* both have very narrow host ranges, a common characteristic of rust and smut diseases. In both cases the main infective agents are wind dispersed with *P. melanocephala* also being rain dispersed. The optimum urediniospore germination for *P. melanocephala* occurs at 21-26°C, while *U. scitaminea* infection occurs in optimum temperatures of 30-31°C in high humidity. Given the narrow host range and limited availability of hosts within New Zealand, *P. melanocephala* and *U. scitaminea* spread is likely to be limited to *Miscanthus* and as such should be considered low between areas of *Miscanthus* cultivation. *U. scitaminea* spread should be further restricted to northern areas where climate is more suitable for disease expression.

Economic consequences

Cochliobolus lunatus, like many of the other filamentous facultative fungi listed in table 4.1, is pathogenic to numerous hosts producing grain mould under conditions of high rainfall and high relative humidity. The likely impacts of *C. lunatus* on *Miscanthus* production are unknown. Given that any significant disease expression will more than likely to be restricted to northern areas, the potential economic impact of *C. lunatus* on the New Zealand agricultural sector should therefore be considered low to moderate.

Magnaporthe salvinii has a relatively narrow host range and requires relatively high temperatures (25-30°C) for optimum germination and disease expression. Given that significant disease expression will more than likely be restricted to northern areas, and the limited availability of hosts, the potential economic impact of *M. salvinii* on the New Zealand agricultural sector should therefore be considered low.

P. melanocephala has caused direct yield losses and indirect economic costs in various sugarcane industries of the world and is continuing to cause an economic impact. Given the very narrow host range and the limited availability of hosts, the potential economic impact of *P. melanocephala* on the New Zealand agricultural sector should therefore be considered low.

Even though sugarcane smut is worldwide in distribution, the existence of different *U. scitaminea* races makes quarantine regulations important. In most sugarcane-growing countries of the world, strict quarantine regulations govern the importation of sugarcane vegetative propagation materials. While losses of 50-73% in sugarcane have been reported, the likely impacts of *U. scitaminea* on *Miscanthus* production are unknown. Given the very narrow host range and the limited availability of hosts, the potential economic impact of *U. scitaminea* on the New Zealand agricultural sector should therefore be considered low.

Environmental consequences

It should be considered possible (though a low likelihood) that, either through a known host or via a new and as-yet unidentified host, *Cochliobolus lunatus*, *Magnaporthe salvinii*, *Puccinia melanocephala* or *Ustilago scitaminea* could have a low-level environmental impact.

Human health consequences

Magnaporthe salvinii, *Puccinia melanocephala*, *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, are not known to be of any significance to human health, however it is possible that exposure to fungal spores may cause allergic reactions in some people and as such have a low level human health impact.

5.2.2.4 CONCLUSION OF CONSEQUENCE ASSESSMENT

From the assessment above it is possible to conclude that:

- *Cochliobolus lunatus* has a moderate to high likelihood of causing low to moderate economic consequences and low environmental and/or human health consequences in New Zealand.
- *Magnaporthe salvinii* has a moderate likelihood of causing low economic consequences and low environmental and/or human health consequences in New Zealand.
- *Puccinia melanocephala* and *Ustilago scitaminea* have a low likelihood of causing low economic consequences and low environmental and/or human health consequences in New Zealand.

5.2.2.5 RISK ESTIMATION

The likelihood estimate is moderate that *Magnaporthe salvinii*, *Puccinia melanocephala*, *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, would be associated with *Miscanthus* plants *in vitro* on entry into New Zealand, and low to moderate that any such fungi that do enter would successfully establish in New Zealand. The likelihood estimate is considered moderate to high that the establishment of *Cochliobolus lunatus* in New Zealand would result in low to moderate economic consequences and low environmental and/or human health consequences to New

Zealand. The likelihood estimate is considered moderate that the establishment of *Magnaporthe salvinii* in New Zealand would result in low economic, environmental and/or human health consequences to New Zealand. The likelihood estimate is considered low that the establishment of *Puccinia melanocephala* or *Ustilago scitaminea* in New Zealand would result in low economic, environmental and/or human health consequences to New Zealand.

As a result the risk estimate for *Magnaporthe salvinii*, *Puccinia melanocephala* or *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, associated with imported *Miscanthus* plants *in vitro* is non-negligible; these fungi should be considered hazards.

5.2.2.6 ASSESSMENT OF UNCERTAINTY

There is significant uncertainty around the association with *Miscanthus* of *Magnaporthe salvinii*, *Puccinia melanocephala*, *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*. As such this risk assessment should be reviewed once further relevant information becomes available.

5.2.3 RISK MANAGEMENT

5.2.3.1 RISK EVALUATION

Since the risk estimate for *Magnaporthe salvinii*, *Puccinia melanocephala* or *Ustilago scitaminea*, or the filamentous facultative fungi represented by *Cochliobolus lunatus* and *Magnaporthe salvinii*, associated with imported *Miscanthus* plants *in vitro* is non-negligible, phytosanitary measures should be employed to reduce the risks to an acceptable level.

5.2.3.2 RISK MANAGEMENT OBJECTIVE

The risk management objective is to ensure any hazardous filamentous facultative fungi (see section 5.2.1.1), *Puccinia melanocephala* or *Ustilago scitaminea* infecting imported *Miscanthus* plants *in vitro* is neither:

- transplanted into the New Zealand environment on the imported *Miscanthus* plants *in vitro*; or
- transmitted to a host plant in the New Zealand environment from the imported *Miscanthus* plants *in vitro*.

5.2.3.3 OPTION EVALUATION

There are conceivably a number of points on the importation pathway that measures could be implemented to meet the aforementioned management objectives. The following risk management options should be assessed:

a. Pest free area (PFA): *Miscanthus* plants *in vitro* are imported from areas that they are free of hazardous filamentous facultative fungi, *P. melanocephala* or *U. scitaminea*;

b. Pest free place of production (PFPP): *Miscanthus* plants *in vitro* are imported from a place of production that is free of hazardous filamentous facultative fungi, *P. melanocephala* or *U. scitaminea*;

c. Treatment, for any infecting hazardous filamentous facultative fungi, *P. melanocephala* or *U. scitaminea*, of mother plants before explants are taken or explants before culturing;

d. Inspections for symptom expression of hazardous filamentous facultative fungi, *P. melanocephala* or *U. scitaminea* either prior to export to New Zealand or on arrival in New Zealand in a post entry quarantine facility.

Pest free area (PFA)

The International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas* (ISPM No 4) describes the requirements for the establishment and use of PFAs as a risk management option for meeting phytosanitary requirements for the import of plants. The standard identifies three main components or stages that must be considered in the establishment and subsequent maintenance of a PFA:

- systems to establish freedom
- phytosanitary measures to maintain freedom
- checks to verify freedom has been maintained.

Normally PFA status is based on verification from specific surveys such as an official delimiting or detection survey. Visual examination may reveal infected plants in the field, but the absence of symptoms does not exclude latent infection of *Miscanthus*. It therefore should be considered that a reliable PFA determination may not be possible in areas where disease presence may be suspected.

With particular regard to the hazardous filamentous facultative fungi potentially associated with *Miscanthus* in PFAs, the list includes a number of organisms identified to genus level only. As providing area-freedom declarations for unspecified species is not possible, this options should not be considered for the hazardous filamentous facultative fungi.

Pest free place of production (PFPP)

The International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites* (ISPM No 10) describes the requirements for the establishment and use of pest free places of production as a risk management option for meeting phytosanitary requirements for the import of plants. A pest free place of production is defined in the standard as a "place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period". Pest freedom is established by surveys and/or growing season inspections and maintained as necessary by other systems to prevent the entry of the pest into the place of production. As with PFA above, it should be considered that a reliable PFPP determination may not be possible in areas where disease presence may be suspected.

As described for PFA above, the list of hazardous filamentous facultative fungi potentially associated with *Miscanthus* includes a number of organisms identified to genus level only. As providing PFPP declarations for unspecified species is not possible, this options should not be considered for the hazardous filamentous facultative fungi unless the PFFP area is within a contained area such as a tissue culture laboratory.

Treatment options

Hot-water treatment at 50°C or greater for 2-3 hours has been the most commonly used method for treatment of sugarcane material infected with *P. melanocephala* and *U. scitaminea* (CABI CPC 2006). There is no information however on the actual level of efficacy of this hot-water treatment on *Miscanthus*. The texts available relate to sugarcane only and make various claims about the level of efficacy: from “disinfection” and “elimination” to “control”. BSES (2006) refers to a control level of 99.5% for *Ustilago scitaminea* infection of sugarcane treated at 52°C for 45 minutes. It is not clear from the paper whether this control level relates to organism or disease control (e.g. survival of the organism or expression of disease after treatment), or the level of confidence supporting this stated efficacy. Alfieri (1979) refers to a similar treatment (52°C for 60 minutes) providing 70%-100% sugarcane stools free of a downy mildew disease.

If we assume that the level of efficacy is 99.5% (1 in 200) from a single treatment, two treatments would provide a combined efficacy of 99.9975% or 1 in 40,000 infected units. While 1 in 200 survivors would seem a little to many given the volumes of imported material could be higher than 200, 1 in 40,000 would seem to be more than sufficient when considering the uncertainty associated with the effectiveness of this treatment on *Miscanthus*. To enhance effectiveness therefore, the heat therapy should be repeated.

It should be noted that while these treatments have been reported to improve germination and growth rate in seed canes of sugarcane (Alfieri 1979), no information could be found to indicate their suitability for *Miscanthus* material. Care should therefore be taken to test these or other sugarcane treatments to ensure plant material is not adversely affected.

Detection methods

The hazardous filamentous facultative fungi including *Magnaporthe salvinii* can act as saprophytes and as such are likely to become visible on the *in-vitro* media within a relatively short time (21 days). An inspection after each of two 28-day periods of continuous culturing (no sub-culturing should occur during the 28-day period) in normal growing conditions (20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation[10]) should be completed to provide adequate assurance that the *in-vitro* material is free of these fungi.

The relatively short urediniospore reproductive cycle (8-18 days) of *P. melanocephala* allows the rapid disease build-up in susceptible fields. Within 5 to 6 weeks green fields can appear reddish due to the massive pustule formation that develops on the foliage (CABI CPC 2006). Vinagre *et al*. (2006) transferred *in vitro*-grown sugarcane plantlets into a greenhouse and

10 Nutrient concentrations should be maintained at rates similar to those used during the micro-propagation process.

inoculated 15 days later with an approximately 106-spore solution of *P. melanocephala*. The material was harvested 15 days later, with the inoculated plants showing rust disease symptoms (Vinagre *et al*. 2006). This suggests that plants in vitro are likely to express detectable disease symptoms within the normal period between micro-propagation (ca 1 month).

For *U. scitaminea* the most recognizable diagnostic feature of a smut-infected plant is the emergence of a "smut whip". Whips begin emerging from infected cane by 2-4 months of age with peak whip growth occurring at the 6th or 7th month. Plants grown under stress conditions (dry and hot) are more likely to show symptoms (Comstock & Lentini, 2005). Plantlets growing *in vitro* will not be exposed to suitable stress conditions and are likely to be micro-propagated within 1 to 2 months of active growth. Disease symptoms of *U. scitaminea* are therefore unlikely to be reliably expressed on the plantlets during *in vitro* propagation. Plantlets removed from *in vitro* containers and potted in glasshouses (to provide more suitable conditions for disease symptom expression) would need to be held in containment conditions to prevent smut spores spreading onto to hosts in the environment.

Polymerase chain reaction (PCR) has been used successfully to identify *U. scitaminea* in infected host tissue. The primers for this assay amplify a product from a *U. scitaminea* mating type allele (bE gene) which is specific to *U. scitaminea*.

5.2.3.4 RISK MANAGEMENT PROPOSALS

From areas or places of production that are unable to obtain suitable supported area freedom declarations, the following alternative risk management options are proposed:

For *Magnaporthe salvinii* and hazardous filamentous facultative fungi in general:

- *Miscanthus* plants *in vitro* should be inspected over two 28-day periods for disease symptoms of mycelial growth on the medium. Inspections should be of plantlets (no sub-culturing should occur during each 28-day period) in normal growing conditions e.g. 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation.

For *Puccinia melanocephala*:

- *Miscanthus* plants *in vitro* should be inspected over two 30-day periods for disease symptoms. Inspections should be of plantlets (no sub-culturing should occur during each 30-day period) in normal growing conditions e.g. 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation; or
- Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at either 50°C or greater for 3 hours or 52°C or greater for 1 hour per treatment.

For *Ustilago scitaminea*:

- *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR or BIO-PCR testing procedure to confirm freedom from *U. scitaminea*; or

- Prior to *in-vitro* culture, explants should be subjected to two consecutive hot-water treatments at either 50°C or greater for 3 hours or 52°C or greater for 1 hour per treatment.

Any plant material found to be contaminated by *Magnaporthe salvinii*, *Puccinia melanocephala*, *Ustilago scitaminea* or hazardous filamentous facultative fungi should not be planted into the New Zealand environment but rather disposed of in a suitable manner.

5.2.4 ASSESSMENT OF RESIDUAL RISK

5.2.4.1 OBJECTIVES FOR PROPOSED MANAGEMENT OPTION(S)

The objective of these measures is to ensure that any imported *Miscanthus* plants *in vitro* are free of *Magnaporthe salvinii*, *Puccinia melanocephala*, *Ustilago scitaminea* and hazardous filamentous facultative fungi prior to biosecurity clearance into New Zealand.

5.2.5 REFERENCES

Alfieri, SA (1979) Downy Mildew of Sugarcane. Plant Pathology Circular No. 205. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

BSES (2007) Information Sheet ISO6052: Sugarcane Smut. BSES Ltd; www.nses.org.au

CABI CPC (2006) *Crop Protection Compendium on Internet.* CAB International, Wallingford, UK; www.cabi.org/compendia/CPC/

Comstock JC, Lentini RS (2005) Sugarcane Smut Disease. Florida Sugarcane Handbook, SS-AGR-208. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available on the EDIS website at http://edis.ifas.ufl.edu.

ISPM No. 4 (1995) International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas*. FAO, 15 pp.

ISPM No. 10 (1999) International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites*. FAO; 15 pp.

Lu B, Hyde KD, Ho WH, Tsui KM, Taylor JE, Wong KM, Yanna, Zhou D (2000) Checklist of Hong Kong Fungi. Fungal Diversity Press, Hong Kong; 207 pp.

Pennycook SR (1989) Plant diseases recorded in New Zealand. Vol. 3. Plant Diseases Division, DSIR, Auckland, New Zealand; 180pp.

PPIN (2007) PPIN (Plant Pest Information Network), MAF database (date of search indicated).

Vanky K (2000) The smut fungi on *Saccharum* and related grasses. Australasian Plant Pathology 29: 155-163

Vinagre F, Vargas C, Schwarcz K, Cavalcante J, Nogueira EM, Baldani JI, Ferreira PCG, Hemerly AS (2006) SHR5: A Novel Plant Receptor Kinase involved in Plant–N2-Fixing

Endophytic Bacteria Association. Journal of Experimental Botany, Volume 57, No. 3; pp. 559–569

Zhuang W-Y Ed. (2001) Higher Fungi of Tropical China. Mycotaxon, Ithaca, NY, 485 pp.

5.3 INVERTEBRATE HAZARDS

5.3.1 HAZARD IDENTIFICATION

5.3.1.1 AETIOLOGIC AGENT

Schizotetranychus celarius (Banks) (Acari: Prostigmata: *Tetranychidae*)

5.3.1.2 NEW ZEALAND STATUS

Schizotetranychus celarius has not been recorded in New Zealand (Manson 1987, PPIN 2007, Ramsay 1980, Zhang & Martin 2001).

5.3.1.3 BIOLOGY AND EPIDEMIOLOGY

Information on these organisms has been collated from the CABI CPC 2006[11] and Gerdeman & Tanigoshi (2004) with available or supplementary references provided. No recorded instance could be located of this particular mite species being found on plants *in vitro*.

Bamboo spider mites are cosmopolitan being found virtually everywhere bamboos exist. These mites are easily recognized by their characteristic large and densely woven web nests, primarily found on the underside of bamboo leaves. Bamboo spider mites initially infest leaves along the midvein or edge of the leaf where a linear depression suitable for nest building is typically found. A newly formed web nest is composed of a single translucent layer and the mites can be seen beneath the webbing. The thick silken webbings protect the mites from pesticides and predators. Bamboo spider mites spend most of their time under this webbing but occasionally venture out and scuttle about on the underside of the leaves. Eventually all life stages exist beneath a single web nest which also help protect the mite from spray treatments (Gerdeman & Tanigoshi 2004).

Bamboo spider mites pass through 5 stages: egg, larva, protonymph, deutonymph and adult. Following each of the motile stages, the immature mites pass through an inactive quiescent stage. The average generation time for *S. celarius* is 26 days, with females producing an average of 85 eggs during their lifetime.

Bamboo spider mites are active during the warm months of the year and have been reported from greenhouses year round. Red-coloured adult bamboo spider mites have been observed in September in Portland, Oregon. This suggests outdoor populations may over-winter as adults in a diapause state in protected sites and resume activity in the spring when day length and temperatures become optimal.

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11 The Crop Protection Compendium, 2006 Edition. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

Spider mite feeding damage on bamboo is lighter in colour than the surrounding leaf tissue, permanent and aesthetically displeasing. Feeding damage may first appear as light speckling or blotches on the upper leaf surface. As the leaf becomes more infested, damage may acquire a chequered pattern sometimes resembling leaf variegation. The mites pierce individual plant cells on the underside of the leaf and suck out the cell contents causing the discoloration on the upper leaf surface. Heavy infestations can cause green bamboos to appear yellow-green in colour. Leaf damage can impair photosynthesis and reduce plant vigour. Damaged plants may lose their leaves more frequently. In summary these mites can be easily spread by humans, are difficult to control, and cause irreparable damage to ornamental bamboo leaves.

***General comments* (from Cassells 1977)**

Tissue culture contaminating mites require a high relative humidity, conditions normally found in tissue culture containers. Several mite genera have been observed in tissue cultures, all of which were plant-specific rather than house or dust mites. A normal mite life cycle under *in vitro* conditions takes 10 to 20 days, with each female producing 100 to 200 eggs of which 98% are female. Nine days after their birth the first mites of the next generation will brood. Theoretically a single mite in a tissue culture container could give rise to 25,000 descendents after 30 to 40 days and more than 5 million after 60 days. Mite contamination is often accompanied by bacterial contamination which will become apparent on the surface of the culture medium. Young mites will spread between containers as they can penetrate containers through the smallest cracks or through parafilm seals.

5.3.1.4 HAZARD IDENTIFICATION CONCLUSION

Based on:

- The accepted absence of *Schizotetranychus celarius* in New Zealand;
- The potential ability of this mite to be vectored by *Miscanthus* plants *in vitro*; and
- The potential ability of this mite to cause symptoms on commercially important plants in New Zealand;

it is proposed that *Schizotetranychus celarius* be considered a potential hazard requiring further assessment.

5.3.2 RISK ASSESSMENT

5.3.2.1 ENTRY ASSESSMENT

Should *Schizotetranychus celarius* be associated with the *Miscanthus* mother plants at the time the *in-vitro* material is excised, there is a moderate likelihood that any plants *in vitro* will also be contaminated if normal surface sterilisation processes are followed. There is little available information, however, on the prevalence of *Schizotetranychus celarius* within infested *Miscanthus* populations. The likelihood of survival of *Schizotetranychus celarius* during long-distance transport in infected propagation material is high as long as the propagated material remains viable.

It is therefore considered that the likelihood of entry of *Schizotetranychus celarius* into New Zealand with *Miscanthus* plants *in vitro* from infected populations is moderate and therefore non-negligible.

5.3.2.2 ASSESSMENT OF EXPOSURE AND ESTABLISHMENT

Should *Schizotetranychus celarius* be associated with imported *Miscanthus* plants *in vitro* it would be expected to use the infested material as a vehicle to allow exposure and establishment in the New Zealand environment. As *in vitro* material showing symptoms of pest infestation is usually discarded during the micro-propagation process, the level of contamination within the imported material would be expected to be reduced during the transfer of explants to the field.

The likelihood of exposure and establishment should be considered low to moderate and therefore non-negligible.

5.3.2.3 CONSEQUENCE ASSESSMENT

Spread

Bamboo spider mites are active during the warm months of the year and have been reported from greenhouses year round. These mites can be easily spread by humans, are difficult to control, however have relatively narrow host ranges on bamboo species and a few other related grasses. Outdoor populations may over-winter as adults in a diapause state in protected sites and resume activity in the spring when day length and temperatures become optimal. The rate of natural or assisted dispersal within New Zealand should therefore be considered moderate to high in areas where hosts are available.

Economic consequences

Heavy infestations can cause green bamboos to appear yellow-green in colour. Leaf damage can impair photosynthesis and reduce plant vigour. Damaged plants may lose their leaves more frequently. In summary these mites are difficult to control and cause irreparable damage to ornamental bamboo leaves. The potential economic impact of *Schizotetranychus celarius* on the New Zealand agricultural or ornamental sector should therefore be considered moderate.

Environmental consequences

It should be considered possible (though a low likelihood) that, either through a known host or via a new and as-yet unidentified host, *Schizotetranychus celarius* could have a low-level environmental impact.

Human health consequences

Schizotetranychus celarius is not known to be of any significance to human health.

5.3.2.4 CONCLUSION OF CONSEQUENCE ASSESSMENT

From the assessment above it is possible to conclude that *Schizotetranychus celarius* has a moderate to high likelihood of causing moderate economic consequences and low environmental consequences to New Zealand.

5.3.2.5 RISK ESTIMATION

The likelihood estimate is moderate that *Schizotetranychus celarius* would be associated with *Miscanthus* plants *in vitro* on entry into New Zealand, and low to moderate that it would successfully establish in New Zealand. The likelihood estimate is considered moderate to high that the establishment of *Schizotetranychus celarius* in New Zealand would result in moderate economic consequences and low environmental consequences to New Zealand.

As a result the risk estimate for *Schizotetranychus celarius* associated with imported *Miscanthus* plants *in vitro* is non-negligible and this mite should be considered a hazard.

5.3.2.6 ASSESSMENT OF UNCERTAINTY

There is significant uncertainty around the association of *Schizotetranychus celarius* with *Miscanthus*. As such this risk assessment should be reviewed once further relevant information becomes available.

5.3.3 RISK MANAGEMENT

5.3.3.1 RISK EVALUATION

Since the risk estimate for *Schizotetranychus celarius* associated with imported *Miscanthus* plants *in vitro* is non-negligible, phytosanitary measures should be employed to reduce the risks to an acceptable level.

5.3.3.2 RISK MANAGEMENT OBJECTIVE

The risk management objective is to ensure any *Schizotetranychus celarius* infecting imported *Miscanthus* plants *in vitro* is neither:

- transplanted into the New Zealand environment on the imported *Miscanthus* plants *in vitro*; or
- transmitted to a host plant in the New Zealand environment from the imported *Miscanthus* plants *in vitro*.

5.3.3.3 OPTION EVALUATION

There are conceivably a number of points on the importation pathway that measures could be implemented to meet the aforementioned management objectives. The following risk management options should be assessed:

a. Pest free area (PFA): *Miscanthus* plants *in vitro* are imported from areas that they are free of *Schizotetranychus celarius*;

b. Pest free place of production (PFPP): *Miscanthus* plants *in vitro* are imported from a place of production that is free of *Schizotetranychus celarius*;

c. Treatment, for any infecting *Schizotetranychus celarius*, of mother plants before explants are taken or explants before culturing;

d. Inspections for symptom expression of *Schizotetranychus celarius* either prior to export to New Zealand or on arrival in New Zealand in a post entry quarantine facility.

Pest free area (PFA)

The International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas* (ISPM No 4) describes the requirements for the establishment and use of PFAs as a risk management option for meeting phytosanitary requirements for the import of plants. The standard identifies three main components or stages that must be considered in the establishment and subsequent maintenance of a PFA:

- systems to establish freedom
- phytosanitary measures to maintain freedom
- checks to verify freedom has been maintained.

Normally PFA status is based on verification from specific surveys such as an official delimiting or detection survey. Visual examination may reveal infected plants in the field, but the absence of symptoms does not exclude latent infection of *Miscanthus*. It therefore should be considered that a reliable PFA determination may not be possible in areas where mite presence may be suspected.

Pest free place of production (PFPP)

The International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites* (ISPM No 10) describes the requirements for the establishment and use of pest free places of production as a risk management option for meeting phytosanitary requirements for the import of plants. A pest free place of production is defined in the standard as a "place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period". Pest freedom is established by surveys and/or growing season inspections and maintained as necessary by other systems to prevent the entry of the pest into the place of production. As with PFA above, it should be considered that a reliable PFPP determination may not be possible in areas where mite presence may be suspected.

Treatment options

Hot-water treatment at greater than 50°C for 10 minutes is a reported method for the treatment of bamboo material infested with *Schizotetranychus celarius* (Young & Haun 1961). Under practical conditions, however, heat treatment is often not completely effective. To enhance effectiveness, heat therapy should to be repeated or the duration increased to ensure pest-free explants.

Detection methods

Schizotetranychus celarius is likely to become visible on the *in-vitro* media within a relatively short time (30 days) either directly, through the development of webbing, or through the transference of bacterial contaminants to the growing medium. Inspections during two 30-day periods of continuous culturing (no sub-culturing should occur during each 30-day period) in normal growing conditions (20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation[12]) should be completed to provide adequate assurance that the *in-vitro* material is free of this mite.

5.3.3.4 RISK MANAGEMENT PROPOSALS

From areas or places of production that are unable to obtain suitably supported area freedom declarations from *Schizotetranychus celarius*, the following alternative risk management options are proposed:

- *Miscanthus* plants *in vitro* should be inspected over two 30-day periods for mites, webbing or bacterial growth on the growing medium. Inspections should be of plantlets (no sub-culturing should occur during each 30-day period) in normal growing conditions e.g. 20-26°C temperatures and 8-16 hour light periods on media suitable for micro-propagation; or
- Prior to *in-vitro* culture, explants should be subjected to a hot-water treatment at 50°C or greater for a minimum of 30 minutes.

Any plant material found to be contaminated by *Schizotetranychus celarius* or other hazardous invertebrates should not be planted into the New Zealand environment but rather treated or disposed of in a suitable manner.

5.3.4 ASSESSMENT OF RESIDUAL RISK

5.3.4.1 OBJECTIVES FOR PROPOSED MANAGEMENT OPTION(S)

The objective of these general measures is to ensure that any imported *Miscanthus* plants *in vitro* are free of *Schizotetranychus celarius* prior to biosecurity clearance into New Zealand.

12 Nutrient concentrations should be maintained at rates similar to those used during the micro-propagation process.

5.3.5 REFERENCES

CABI CPC (2006) *Crop Protection Compendium on Internet.* CAB International, Wallingford, UK; http://www.cabi.org/compendia/CPC/

Cassells AC (1977) Pathogen and microbial contamination management in micropropagation. ISHS ACTA Horticulturae 560: IV International Symposium on *In Vitro* Culture and Horticultural Breeding.

Gerdeman BS, Tanigoshi LK (2004) Bamboo spider mites: biology and management in the Pacific northwest. Extension Bulletin 1992E. Washington State University, College of Agricultural, Human, and Natural Resource Sciences; 4 pp

ISPM No. 4 (1995) International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas*. FAO; 15 pp.

ISPM No. 10 (1999) International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites*. FAO; 15 pp.

Manson DCM (1987) A list of New Zealand mites and their host plants. Science Information Publishing Centre; Wellington, New Zealand; DSIR Bulletin No. 240.

PPIN (2007) PPIN (Plant Pest Information Network), MAF database (date of search indicated).

Ramsay GW (1980) Common and scientific names of New Zealand mites. DSIR Information Series No. 139.

Young RA, Haun JR (1961) Bamboo in the United States : description, culture, and utilization. Agriculture handbook 193. U.S. Department of Agriculture, Crops Research Division, Agricultural Research Service; 74 pp

Zhang, ZQ & Martin, NA (2001) A review of *Schizotetranychus*-like mites (Acari: Tetranychidae) from New Zealand. Journal of the Royal Society of New Zealand 31 (2): 307-325

5.4 VIRAL HAZARDS

5.4.1 HAZARD IDENTIFICATION

5.4.1.1 AETIOLOGIC AGENT

Miscanthus streak virus (MiSV), Family *Geminiviridae,* Genus *Monogeminivirus* (Brunt *et al*. 1996)

Sugarcane mosaic virus (SCMV), Family *Potyviridae,* Genus *Potyvirus* (Brunt *et al*. 1996)

5.4.1.2 NEW ZEALAND STATUS

MiSV and SCMV have not been recorded in New Zealand (Pennycook 1989, PPIN 2007, Pearson *et al*. 2006).

5.4.1.3 BIOLOGY AND EPIDEMIOLOGY

Miscanthus streak virus (MiSV)

Reported only from the wild grass, *Miscanthus sacchariflorus* from Chiba, Japan. MiSV has not been transmitted mechanically and while its vector is unknown it may be a leafhopper like other geminiviruses occurring in the Poaceae. It has not yet been recorded on any species other than its sole natural host, *M. sacchariflorus* (Yamashita & Doi 2007). MiSV causes leaf mosaic or streaking, sometimes 'ragged leaf' symptoms and stunting, but is considered of minor economic importance (CABI CPC 2006). Virions are found in leaves, in mesophyll, in cytoplasm, in nuclei, and in cell vacuoles (Brunt *et al*. 1996).

Sugarcane mosaic virus (SCMV)

SCMV causes mosaic diseases in sugarcane (*Saccharum* spp.) and some other graminaceous plants. The natural host range is restricted to members of the Poaceae, with the exception of the abaca mosaic strain, which infects *Musa textilis,* a monocotyledon in the family Musaceae. Sorghum, maize and some wild grasses growing near infected sugarcane may be infected naturally (Teakle 2007). *Miscanthus* sp. was reported as a host by Yamashita & Doi (2007). It occurs in most parts of the world where sugarcane is grown, while strains adapted to perennial hosts other than sugarcane may occur in areas remote from sugarcane plantations. Although sap transmission tests have shown that many graminaceous species are susceptible, cultivated cereals such as wheat, barley, rye and rice are rarely infected naturally (Teakle *et al.* 2007).

There are three principal modes of spread of SCMV: (1) by aphid vectors, (2) by infected seed cane and (3) by mechanical inoculation. Only aphid vectors and infected seed cane are important in the field. Mechanical transmission, for the most part, is important only in greenhouse and laboratory research. There are at least 12 species of aphids that can transmit

SCMV from diseased sugarcane to healthy sugarcane, including *Dactynotus ambrosiae, Hysteroneura setariae, Rhopalosiphum maidis*, and *Toxoptera graminum*. The virus is more readily transmitted to or from hosts such as maize and sorghum than to or from sugarcane (Teakle *et al.* 2007). Control of mosaic through heat treatment of cuttings is partially effective but is only practical in quarantine situations (Comstock & Lentini 2005).

Estimated yield losses due to the disease vary greatly depending on the time period and sugarcane growing area involved. Mosaic, superimposed on already established diseases in Louisiana, caused a near collapse of the industry in the mid-1920s (Comstock & Lentini 2005).

Mosaic is identified primarily by its leaf symptoms. As with most sugarcane diseases, the symptoms may vary in intensity with the cane variety, growing conditions, and the strain of the virus involved. The most distinctive symptom is a pattern of contrasting shades of green, often islands of normal green on a background of paler green or yellowish chlorotic areas on the leaf blade. Generally, the chlorotic areas are diffuse, but they may be sharply defined in some clones infected with certain strains of the virus. The infection may be accompanied by varying degrees of leaf reddening or necrosis. Chlorotic areas are most evident at the base of the leaf. Chlorotic areas may also be present on the leaf sheath, but rarely on the stalk. Young, rapidly growing plants are more susceptible to infection than more mature, slower growing plants (Comstock & Lentini 2005).

5.4.1.4 HAZARD IDENTIFICATION CONCLUSION

Based on:

- The accepted absence of MiSV and SCMV in New Zealand;
- The potential ability of MiSV and SCMV to be vectored by *Miscanthus* plants *in vitro*; and
- The potential ability of MiSV and SCMV to cause disease symptoms on commercially important plants in New Zealand; and
- The possibility that MiSV may be more widely spread than is officially recorded;

it is proposed that MiSV and SCMV be considered potential hazards requiring further assessment.

5.4.2 RISK ASSESSMENT

5.4.2.1 ENTRY ASSESSMENT

Should MiSV or SCMV be associated with the *Miscanthus* mother plants at the time the *in-vitro* material is excised, there is a high likelihood that any plants *in vitro* will also be contaminated. There is little available information, however, on the prevalence of MiSV or SCMV within infected *Miscanthus* populations. The likelihood of survival of MiSV or SCMV during long-distance transport in infected propagation material is high as long as the propagated material remains viable.

It is therefore considered that the likelihood of entry of MiSV and SCMV into New Zealand with *Miscanthus* plants *in vitro* from infected populations is moderate and therefore non-negligible.

5.4.2.2 ASSESSMENT OF EXPOSURE AND ESTABLISHMENT

Should MiSV or SCMV be associated with imported *Miscanthus* plants *in vitro* it would be expected to use the infested material as a vehicle to allow exposure and establishment in the New Zealand environment. As *in-vitro* material showing disease symptoms is usually discarded during the micro-propagation process, the level of contamination within the imported material would be expected to be reduced during the transfer of explants to the field. However should *Miscanthus* become a widely grown production crop within New Zealand, micro-propagation and large-scale planting are likely to be common increasing the likelihood of establishment.

The likelihood of exposure and establishment should be considered low to moderate and therefore non-negligible.

5.4.2.3 CONSEQUENCE ASSESSMENT

Spread

The mode of transmission of MiSV is currently unknown, but it is considered that it may be a leafhopper like other geminiviruses occurring in the Poaceae. MiSV is recorded as spreading on *Miscanthus sacchariflorus* in Japan and as such should be considered able to spread on *Miscanthus* in New Zealand. No other hosts have been recorded. The rate of natural or assisted dispersal of MiSV within New Zealand should therefore be considered low to moderate in areas where hosts (*Miscanthus*) are grown.

There are three principal modes of spread of SCMV: (1) by aphid vectors, (2) by infected seed cane and (3) by mechanical inoculation. Only aphid vectors and infected seed cane are considered important in the field. Mechanical transmission, for the most part, is important only in greenhouse and laboratory research. There are at least 12 species of aphids that can transmit SCMV from diseased sugarcane to healthy sugarcane, including *Dactynotus ambrosiae, Hysteroneura setariae, Rhopalosiphum maidis,* and *Toxoptera graminum*. It is likely that effective aphid vectors are present in New Zealand. The virus is more readily transmitted to or from hosts such as maize and sorghum than to or from sugarcane. The natural host range of SCMV is restricted to members of the Poaceae, with the exception of the abaca mosaic strain, which infects *Musa textilis,* a monocotyledon in the family Musaceae. Sorghum, maize and some wild grasses growing near infected sugarcane may be infected naturally. The rate of natural or assisted dispersal of SCMV within New Zealand should therefore be considered moderate to high in areas where hosts (*Miscanthus*) are grown.

Economic consequences

SCMV impacts in the most part have been restricted to sugarcane, a crop not currently commercially grown in New Zealand due to climate constraints. SCMV has a wider host range including sorghum and maize, however few disease symptoms are apparent on these alternative hosts. At this time the host range of MiSV is restricted to *Miscanthus sacchariflorus*. The potential economic impact of MiSV and SCMV on the New Zealand agricultural sector should therefore be considered low.

Environmental consequences

It should be considered possible (though a low likelihood) that, either through a known host or via a new and as-yet unidentified host, MiSV and SCMV could have a low-level environmental impact.

Human health consequences

MiSV and SCMV are not known to be of any significance to human health.

5.4.2.4 CONCLUSION OF CONSEQUENCE ASSESSMENT

From the assessment above it is possible to conclude that:

- MiSV has a low likelihood of causing low economic and environmental consequences to New Zealand.
- SCMV has a moderate to high likelihood of causing low economic and environmental consequences to New Zealand.

5.4.2.5 RISK ESTIMATION

The likelihood estimate is moderate that MiSV and SCMV would be associated with *Miscanthus* plants *in vitro* on entry into New Zealand, and low to moderate that any such viruses that do enter would successfully establish in New Zealand. The likelihood estimate is considered low that the establishment of MiSV in New Zealand would result in low economic and environmental consequences to New Zealand. The likelihood estimate is considered moderate to high that the establishment of SCMV in New Zealand would result in low economic and environmental consequences to New Zealand.

As a result the risk estimate for MiSV and SCMV associated with imported *Miscanthus* plants *in vitro* is non-negligible and should be considered hazards.

5.4.2.6 ASSESSMENT OF UNCERTAINTY

There is significant uncertainty around the association of MiSV and SCMV with *Miscanthus*. As such this risk assessment should be reviewed once further relevant information becomes available.

5.4.3 RISK MANAGEMENT

5.4.3.1 RISK EVALUATION

Since the risk estimate for MiSV and SCMV associated with imported *Miscanthus* plants *in vitro* is non-negligible, phytosanitary measures should be employed to reduce the risks to an acceptable level.

5.4.3.2 RISK MANAGEMENT OBJECTIVE

The risk management objective is to ensure any MiSV and SCMV infecting imported *Miscanthus* plants *in vitro* is neither:

- transplanted into the New Zealand environment on the imported *Miscanthus* plants *in vitro*; or
- transmitted to a host plant in the New Zealand environment from the imported *Miscanthus* plants *in vitro*.

5.4.3.3 OPTION EVALUATION

There are conceivably a number of points on the importation pathway that measures could be implemented to meet the aforementioned management objectives. The following risk management options should be assessed:

a. Pest free area (PFA): *Miscanthus* plants *in vitro* are imported from areas that they are free of MiSV and SCMV;

b. Pest free place of production (PFPP): *Miscanthus* plants *in vitro* are imported from a place of production that is free of MiSV and SCMV;

c. Treatment, for any infecting MiSV and SCMV, of mother plants before explants are taken or explants before culturing;

d. Inspections for symptom expression of MiSV and SCMV either prior to export to New Zealand or on arrival in New Zealand in a post entry quarantine facility.

Pest free area (PFA)

The International Standards for Phytosanitary Measures number 4: *Requirements for the establishment of pest free areas* (ISPM No 4) describes the requirements for the establishment and use of PFAs as a risk management option for meeting phytosanitary requirements for the import of plants. The standard identifies three main components or stages that must be considered in the establishment and subsequent maintenance of a PFA:

- systems to establish freedom
- phytosanitary measures to maintain freedom
- checks to verify freedom has been maintained.

Normally PFA status is based on verification from specific surveys such as an official delimiting or detection survey. Visual examination may reveal infected plants in the field, but the absence of symptoms does not exclude latent infection of *Miscanthus*. It therefore should be considered that a reliable PFA determination may not be possible in areas where disease presence may be suspected.

Pest free place of production (PFPP)

The International Standards for Phytosanitary Measures number 10: *Requirements for the establishment of pest free places of production and pest free production sites* (ISPM No 10) describes the requirements for the establishment and use of pest free places of production as a risk management option for meeting phytosanitary requirements for the import of plants. A pest free place of production is defined in the standard as a "place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period". Pest freedom is established by surveys and/or growing season inspections and maintained as necessary by other systems to prevent the entry of the pest into the place of production. As with PFA above, it should be considered that a reliable PFPP determination may not be possible in areas where disease presence may be suspected.

Treatment options

The control of SCMV through heat treatment of cuttings is partially effective but is only practical in quarantine situations. No details on the heat treatment used or the level of effectiveness are available.

Detection methods

Little has been published on the nature and reliability of disease expression of MiSV in *Miscanthus*. It is therefore not possible at this time to determine if visual inspection would be effective enough to provide enough assurance that imported material is free of MiSV. Specific PCR primers are however available for the identification of MiSV (CABI CPC 2006).

SCMV is visually identified primarily by its leaf symptoms which may vary in intensity with the sugarcane variety, growing conditions, and the strain of the virus involved (Comstock & Lentini 2005). Visual detection us therefore unlikely to be suitably effective for ensuring imported material is free of SCMV, especially given the lack of information on the expression of disease symptoms in *Miscanthus*. Putra *et al*. (2003) used a reverse transcription polymerase chain reaction (RT-PCR) assay to detect SCMV distribution in infected sugarcane. The virus was found in samples from the leaves, roots and tillers 7 weeks after inoculation, young leaves proving to be the most suitable tissue for testing (Putra *et al.* 2003).

5.4.3.4 RISK MANAGEMENT PROPOSALS

From areas or places of production that are unable to obtain suitable supported area freedom declarations, *Miscanthus* mother plants, explants or *in-vitro* plantlets should be subjected to a suitable PCR testing procedure to confirm freedom from MiSV and SCMV. Any plant material found to be contaminated by MiSV or SCMV should not be planted into the New Zealand environment but rather treated or disposed of in a suitable manner.

5.4.4 ASSESSMENT OF RESIDUAL RISK

5.4.4.1 OBJECTIVES FOR PROPOSED MANAGEMENT OPTION(S)

The objective of these general measures is to ensure that any imported *Miscanthus* plants *in vitro* are free of MiSV and SCMV prior to biosecurity clearance into New Zealand.

5.4.5 REFERENCES

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (eds.) (1996). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Available at: http://biology.anu.edu.au/Groups/MES/vide/

CABI CPC (2006) Crop Protection Compendium on Internet. CAB International, Wallingford, UK; http://www.cabi.org/compendia/CPC/

Comstock JC, Lentini RS (2005) Sugarcane Mosaic Virus Disease. Florida Sugarcane Handbook, SS-AGR-209. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available on the EDIS website at http://edis.ifas.ufl.edu.

ISPM No. 4 (1995) International Standards for Phytosanitary Measures number 4: Requirements for the establishment of pest free areas. FAO; 15 pp.

ISPM No. 10 (1999) International Standards for Phytosanitary Measures number 10: Requirements for the establishment of pest free places of production and pest free production sites. FAO; 15 pp.

Pearson MN, Clover GRG, Guy PL, Fletcher JD, Beever RE (2006) A review of the plant virus, viroid and mollicute records for New Zealand. Australasian Plant Pathology 35 (2): 217-252

Pennycook SR (1989) Plant diseases recorded in New Zealand. Vol. 3. Plant Diseases Division, DSIR, Auckland, New Zealand; 180pp.

PPIN (2007) Plant Pest Information Network, MAF database (date of search indicated).

Putra LK, Ogle HJ, James AP, Whittle PJL (2003) Distribution of Sugarcane mosaic virus in sugarcane plants. Australasian Plant Pathology 32(2), pg 305 - 307

Teakle DS, Shukla DD, Ford RE (2007) Sugarcane mosaic virus. Descriptions of Plant Viruses. Available online at http://www.dpvweb.net/dpv/showadpv.php?dpvno=348

Yamashita S, Doi Y (2007) Miscanthus streak virus. Descriptions of Plant Viruses. Available online at http://www.dpvweb.net/dpv/showadpv.php?dpvno=348

6 GLOSSARY OF TERMS

Area — An officially defined country, part of a country or all or part of several countries, as identified by the competent authorities (SPS agreement 1994)

Biosecurity — The exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and human health (Biosecurity Strategy 2003).

Commodity — A good being moved for trade or other purposes. Packaging, containers, and craft used to facilitate transport of commodities are excluded unless they are the intended good (MAF 2006).

Consequences — The adverse effects or harm as a result of entry and establishment of a hazard, which cause the quality of human health or the environment to be impaired in the short or longer term (MAF 2006).

Disease — A finite abnormality of structure or function with an identifiable pathological or clinicopathological basis, and with a recognizable syndrome of clinical signs. Its cause may not be known, or may be from infection with a known organism (MAF 2006)

Ecosystem — A dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit (Convention on Biological Diversity 1992)

Entry (of a organism or disease) — Movement of an organism or disease into a risk analysis area (MAF 2006).

Environment — (Biosecurity Act 1993) Includes:

(a) Ecosystems and their constituent parts, including people and their communities; and
(b) All natural and physical resources; and
(c) Amenity values; and
(d) The aesthetic, cultural, economic, and social conditions that affect or are affected by any matter referred to in paragraphs (a) to (c) of this definition

Establishment — Perpetuation, for the foreseeable future, of an organism or disease within an area after entry (MAF 2006).

Exposure — The condition of being vulnerable to adverse effects (MAF 2006).

FAO — Food and Agriculture Organization, United Nations.

Hazard organism — Any disease or organism that has the potential to produce adverse consequences (MAF 2006).

Import health standard (IHS) — A document issued under section 22 of the Biosecurity Act 1993 by the Director General of MAF, specifying the requirements to be met for the effective management of risks associated with the importation of risk goods before those goods may be imported, moved from a biosecurity control area or a transitional facility, or given a biosecurity clearance (MAF 2006).

Note: An import health standard is also an “import permit” as defined under the IPPC

Import risk analysis	A process to identify appropriate risk-mitigating options for the development of import health standards. These risk analyses can focus on an organism or disease, a good or commodity, a pathway, or a method or mode of conveyance such as shipping, passengers or packaging (MAF 2006).
IPPC	International Plant Protection Convention (1997), FAO
MAF	New Zealand Ministry of Agriculture and Forestry
Measure	A measure may include all relevant laws, decrees, regulations, requirements and procedures including, *inter alia*, end product criteria; processes and production methods; testing, inspection, certification and approval procedures; quarantine treatments including relevant requirements associated with the transport of risk goods, or with the materials necessary for their survival during transport; provisions on relevant statistical methods, sampling procedures and methods of risk assessment; and packaging and labelling requirements directly related to biosecurity (MAF 2006)
Micro-organism	A protozoan, fungus, bacterium, virus or other microscopic self-replicating biotic entity (ISPM No. 5 2007)
Nursery stock	Whole plants or parts of plants imported for growing purposes, e.g. cuttings, scions, budwood, marcots, off-shoots, root divisions, bulbs, corms, tubers and rhizomes (MAF 2006).
Organism	(Biosecurity Act 1993) (a) Does not include a human being or a genetic structure derived from a human being: (b) Includes a micro-organism: (c) Subject to paragraph (a) of this definition, includes a genetic structure that is capable of replicating itself (whether that structure comprises all or only part of an entity, and whether it comprises all or only part of the total genetic structure of an entity): (d) Includes an entity (other than a human being) declared by the Governor-General by Order in Council to be an organism for the purposes of this Act: (e) Includes a reproductive cell or developmental stage of an organism: (f) Includes any particle that is a prion.
Pathway	Any means that allows the entry or spread of a potential hazard (MAF 2006).
Pest	Any species, strain or biotype of plant, animal or pathogenic agent, injurious to plants or animals (or their products) or human health or the environment (MAF 2006). Note: the definition given for "pest" here is different from that used in the Biosecurity Act 1993 "an organism specified as a pest in a pest management strategy". The Biosecurity Act 1993 deals more with "risks" and "risk goods".
Pest risk assessment	A process to measure the level and nature of biosecurity risk posed by an organism. A pest risk assessment can be used to inform biosecurity surveillance activities or identify pests of high risk to New Zealand (MAF 2006).
Plants *in vitro*	A commodity class for plants growing in an aseptic medium in a closed container (ISPM No. 5 2007)
Post-entry quarantine (PEQ)	Quarantine applied to a consignment after entry (ISPM No. 5 2007)
Residual risk	The risk remaining after risk management requirements have been implemented (MAF 2006).

Risk	The likelihood of the occurrence and the likely magnitude of the consequences of an adverse event (MAF 2006).
Risk analysis	The process composed of hazard identification, risk assessment, risk management and risk communication (MAF 2006).
Risk analysis area	The area in relation to which a risk analysis is conducted (MAF 2006).
Risk assessment	The evaluation of the likelihood, and the biological and economic consequences, of entry, establishment, or exposure of an organism or disease (MAF 2006).
Risk good	(Biosecurity Act 1993) Means any organism, organic material, or other thing, or substance, that (by reason of its nature, origin, or other relevant factors) it is reasonable to suspect constitutes, harbours, or contains an organism that may: (a) Cause unwanted harm to natural and physical resources or human health in New Zealand; or (b) Interfere with the diagnosis, management, or treatment, in New Zealand, of pests or unwanted organisms
Risk management	The process of identifying, selecting and implementing measures that can be applied to reduce the level of risk (MAF 2006).
Spread	Expansion of the geographical distribution of a potential hazard within an area (MAF 2006).
Tissue culture	See "Plants *in vitro*"
Treatment	Official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalization (ISPM No. 5 2007)

REFERENCES

ISPM No. 5 (2007) International Standards for Phytosanitary Measures number 5: Glossary of phytosanitary terms. FAO: 15 pp.

MAF (2006) Biosecurity New Zealand risk analysis procedures. Ministry of Agriculture and Forestry, New Zealand: 201 pp. Available online at http://www.biosecurity.govt.nz/files/pests-diseases/surveillance-review/risk-analysis-procedures.pdf

SPS Agreement (1995) World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures. World Trade Organization, Geneva

Biosecurity Strategy (2003) The Biosecurity Strategy for New Zealand. Available online at http://www.biosecurity.govt.nz/bio-strategy/

APPENDIX: ORGANISMS RECORDED ON *MISCANTHUS*

The following organisms have been recorded as being associated with *Miscanthus* species:

Scientific name	Common name	Reference: Host Association	Reference: In UK or USA*
Bacteria			
Acidovorax avenae subsp. *avenae*	Leaf stripe	Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Leifsonia xyli subsp. *xyli*	Ratoon stunting	Sansford & McLeod (2000)	CABI CPC (2006)
Fungi (or fungi like)			
Acremonium sp.		Farr *et al*. (2007)	CABI CPC (2006)
Alternaria alternata	Alternaria leaf spot	Farr *et al*. (2007)	CABI CPC (2006)
Amphisphaerella saccharicola		Farr *et al*. (2007)	
Amphisphaeria saccharicola		Farr *et al*. (2007)	
Annulatascus triseptatus		Farr *et al*. (2007)	
Anthostomella miscanthea		Farr *et al*. (2007)	
Anthostomella punctulata		Farr *et al*. (2007)	
Apiospora montagnei		Farr *et al*. (2007)	CABI CPC (2006)
Arecophila nypae		Farr *et al*. (2007)	
Arecophila sp.		Farr *et al*. (2007)	
Arthrinium euphorbiae		Farr *et al*. (2007)	
Arthrinium sp.		Farr *et al*. (2007)	
Arthrobotrys foliicola		Farr *et al*. (2007)	
Articulospora ozeensis		Farr *et al*. (2007)	
Bactrodesmium longisporum		Farr *et al*. (2007)	
Balansia andropogonis		Farr *et al*. (2007)	
Balansia claviceps		Farr *et al*. (2007)	
Cochliobolus sativus	Leaf spot	Farr *et al*. (2007)	Farr *et al*. (2007)
Brachysporiella gayana		Farr *et al*. (2007)	
Ceratosporella compacta		Farr *et al*. (2007)	
Ceratosporella disticha		Farr *et al*. (2007)	
Cercospora miscanthi	Leaf spot	Farr *et al*. (2007)	
Cerebella paspali	Ergot	Farr *et al*. (2007)	
Claviceps miscanthi	Ergot	Farr *et al*. (2007)	
Claviceps panicoidearum	Ergot	CABI CPC (2006), Sansford & McLeod (2000)	
Claviceps purpurea	Ergot	Farr *et al*. (2007)	CABI CPC (2006), Farr *et al*. (2007)
Cochliobolus cynodontis	Browing	Farr *et al*. (2007)	Farr *et al*. (2007)
Cochliobolus lunatus	Mould	Farr *et al*. (2007)	CABI CPC (2006), Farr *et al*. (2007)
Cochliobolus spicifer	Leaf spot	Farr *et al*. (2007)	CABI CPC (2006)
Colletotrichum graminicola	Leaf spot	Farr *et al*. (2007), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Colletotrichum sp.	Leaf spot	Farr *et al*. (2007)	CABI CPC (2006)
Curvularia intermedia	Mould	Farr *et al*. (2007)	

Scientific name	Common name	Reference: Host Association	Reference: In UK or USA*
Davidiella tassiana	Black mould	Farr *et al*. (2007)	CABI CPC (2006)
Dendryphion nanum		Farr *et al*. (2007)	
Diaporthe sp.	Canker	Farr *et al*. (2007)	CABI CPC (2006)
Diplodia sp.	Blight	Farr *et al*. (2007)	CABI CPC (2006)
Drechslera gigantea	Eyespot	Farr *et al*. (2007)	Farr *et al*. (2007)
Ellisembia vaga		Farr *et al*. (2007)	
Endophragmiella dimorphospora		Farr *et al*. (2007)	
Epicoccum purpurascens	Red blotch	Farr *et al*. (2007)	CABI CPC (2006)
Exserohilum longisporum	Leaf blight	Farr *et al*. (2007), Sansford & McLeod (2000)	
Exserticlava vasiformis		Farr *et al*. (2007)	
Fusariella sarniensis		Farr *et al*. (2007)	
Fusarium culmorum	Rot	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Fusarium incarnatum	Rot	Farr *et al*. (2007)	
Fusarium miscanthi	Rot	Farr *et al*. (2007)	Farr *et al*. (2007)
Fusarium moniliforme	Rot	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Fusarium pallidoroseum	Rot	Farr *et al*. (2007)	CABI CPC (2006)
Gibberella avenacea	Rot	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Glomerella sp.	Leaf spot	Farr *et al*. (2007)	CABI CPC (2006)
Glomerella tucumanensis	Leaf spot	Farr *et al*. (2007), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Helicomyces lilliputeus		Farr *et al*. (2007)	
Helminthosporium sp.	Eyespot	Farr *et al*. (2007)	CABI CPC (2006)
Hyphodontia pilaecystidiata		Farr *et al*. (2007)	
Hypocrella sp.		Farr *et al*. (2007)	
Janetia synnematosa		Farr *et al*. (2007)	
Khuskia oryzae	Rot	Farr *et al*. (2007)	CABI CPC (2006)
Kramasamuha sibika		Farr *et al*. (2007)	
Leptosphaeria macrospora	Canker	Farr *et al*. (2007)	
Leptosphaeria sacchari	Leaf spot	Sansford & McLeod (2000)	
Leptosphaeria sp.	Canker	Farr *et al*. (2007), Sansford & McLeod (2000)	Farr *et al*. (2007)
Linocarpon angustatum	Rot	Farr *et al*. (2007)	
Linocarpon pandani	Rot	Farr *et al*. (2007)	
Lophiostoma tetraploa	Leaf spot	Sansford & McLeod (2000)	Ellis *et al*. (1997)
Lophodermium arundinaceum		Farr *et al*. (2007)	
Lophodermium arundinaceum f. vulgare		Farr *et al*. (2007)	
Lophodermium miscanthi		Farr *et al*. (2007)	
Magnaporthe salvinii	Stem rot	Farr *et al*. (2007)	CABI CPC (2006)
Massarina purpurascens		Farr *et al*. (2007)	
Meliola andropogonis	Sooty Mould	Farr *et al*. (2007)	
Meliola panici	Sooty Mould	Farr *et al*. (2007), Sansford & McLeod (2000)	Sansford & McLeod (2000)
Meliola panici var. *major*	Sooty Mould	Farr *et al*. (2007)	
Meliola panici var. *panici*	Sooty Mould	Farr *et al*. (2007)	

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Meliola setariae	Sooty Mould	Farr *et al.* (2007)	
Metasphaeria miscanthi	Leaf scald	Farr *et al.* (2007)	
Microsphaeropsis sp.		Farr *et al.* (2007)	
Monodictys paradoxa		Farr *et al.* (2007)	
Monodictys putredinis		Farr *et al.* (2007)	
Monodictys sp.		Farr *et al.* (2007)	
Mycosphaerella recutita	Leaf spot	Farr *et al.* (2007)	CABI CPC (2006)
Mycosphaerella striatiformans	Leaf spot	Farr *et al.* (2007), Sansford & McLeod (2000)	Sansford & McLeod (2000)
Nigrospora sp.	Stalk rot	Farr *et al.* (2007)	CABI CPC (2006)
Nigrospora sphaerica	Stalk rot	Farr *et al.* (2007)	CABI CPC (2006)
Ophiobolus miscanthi	Foot rot	Farr *et al.* (2007)	
Ophiobolus sp.	Foot rot	Farr *et al.* (2007)	
Ophioceras filiforme		Farr *et al.* (2007)	
Ophioceras sorghi		Farr *et al.* (2007)	
Ophioceras sp.		Farr *et al.* (2007)	
Ophioceras tenuisporum		Farr *et al.* (2007)	
Ornatispora taiwanensis		Farr *et al.* (2007)	
Oxydothis miscanthicola		Farr *et al.* (2007)	
Oxydothis sp.		Farr *et al.* (2007)	
Paraphaeosphaeria michotii		Farr *et al.* (2007)	Farr et al. (2007)
Passalora koepkei	Yellow spot	Farr *et al.* (2007), Sansford & McLeod (2000)	CABI CPC (2006)
Peronosclerospora miscanthi	Downy mildew	Farr *et al.* (2007), Sansford & McLeod (2000)	
Peronosclerospora sp.	Downy mildew	Farr *et al.* (2007)	CABI CPC (2006)
Peronosclerospora spontanea	Spontaneum downy mildew	CABI CPC (2006)	
Petrakia sp.		Farr *et al.* (2007)	
Phaeoisaria clematidis		Farr *et al.* (2007)	
Phaeosaccardinula javanica		Farr *et al.* (2007)	
Phaeosphaerella miscanthi		Farr *et al.* (2007)	
Phlyctema sp.		Farr *et al.* (2007)	Farr *et al.* (2007)
Phoma sp.	Blight	Farr *et al.* (2007)	CABI CPC (2006)
Phomopsis sp.	Blight	Farr *et al.* (2007)	CABI CPC (2006)
Phyllachora graminis	Leaf spot	Farr *et al.* (2007)	
Phyllachora miscanthi	Leaf spot	Farr *et al.* (2007)	
Phyllachora miscanthidii	Leaf spot	Farr *et al.* (2007)	
Phyllachora miscanthi-japonici	Leaf spot	Farr *et al.* (2007)	
Phyllachora sacchari	Leaf spot	Farr *et al.* (2007)	
Phyllachora sp.	Leaf spot	Farr *et al.* (2007)	Farr et al. (2007)
Pithomyces chartarum		Farr *et al.* (2007)	
Pithomyces maydicus		Farr *et al.* (2007)	
Pleospora miscanthi	Leaf spot	Farr *et al.* (2007)	
Pleospora tarda	Leaf blight	Farr *et al.* (2007)	Farr et al. (2007)

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Pleurophragmium simplex		Farr *et al.* (2007)	
Pseudospiropes miscanthi		Farr *et al.* (2007)	
Puccinia daisenensis	Rust	Farr *et al.* (2007), Sansford & McLeod (2000)	
Puccinia erianthi	Rust	Farr *et al.* (2007)	
Puccinia erythropus	Rust	Farr *et al.* (2007), Sansford & McLeod (2000)	
Puccinia eulaliae	Rust	Farr *et al.* (2007)	
Puccinia kuehnii	Rust	Sansford & McLeod (2000)	
Puccinia melanocephala	Rust	Farr *et al.* (2007)	CABI CPC (2006)
Puccinia miscanthi	Rust	Farr *et al.* (2007), Sansford & McLeod (2000)	
Puccinia miscanthicola	Rust	Farr *et al.* (2007), Sansford & McLeod (2000)	
Puccinia miscanthidii	Rust	Farr *et al.* (2007), Sansford & McLeod (2000)	
Puccinia rufipes	Rust	Farr *et al.* (2007)	
Ramularia sp.	Leaf spot	Farr *et al.* (2007)	CABI CPC (2006)
Rhinocladiella sp.		Farr *et al.* (2007)	
Rhizoctonia sp.	Root rot	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Rosellinia formosana	Root rot	Farr *et al.* (2007)	
Roussoella serrulata		Farr *et al.* (2007)	
Saccardoella miscanthi		Farr *et al.* (2007)	
Sarocladium sp.	Rot	Farr *et al.* (2007)	
Sclerophthora macrospora	Downy mildew	Farr *et al.* (2007)	CABI CPC (2006)
Sclerospora miscanthi	Downy mildew	Farr *et al.* (2007)	
Septoria miscanthina	Leaf spot	Farr *et al.* (2007)	
Spadicoides sp.		Farr *et al.* (2007)	
Sphacelotheca macrospora	Smut	Farr *et al.* (2007)	
Sphacelotheca miscanthi	Smut	Farr *et al.* (2007)	
Spiropes capensis		Farr *et al.* (2007)	
Sporidesmium sp.	Leaf spot	Farr *et al.* (2007)	
Sporisorium kusanoi	Smut	Farr *et al.* (2007)	
Sporisorium macrosporum	Smut	Farr *et al.* (2007)	
Sporisorium masseeanum	Smut	Farr *et al.* (2007)	
Sporisorium miscanthi	Smut	Farr *et al.* (2007)	
Stachybotrys kampalensis		Farr *et al.* (2007)	
Stachylidium sp.		Farr *et al.* (2007)	
Stagonospora hachijoensis		Farr *et al.* (2007)	
Stagonospora sacchari	Sugarcane scorch	Farr *et al.* (2007), Sansford & McLeod (2000)	
Stagonospora sp.	Scorch	Farr *et al.* (2007), Sansford & McLeod (2000)	Farr et al. (2007)
Telimena graminis		Farr *et al.* (2007)	
Tetranacrium gramineum		Farr *et al.* (2007)	
Tetraploa aristata		Farr *et al.* (2007)	
Thanatephorus cucumeris	Blight	Farr *et al.* (2007)	CABI CPC (2006)

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Torulomyces macrosporus		Farr *et al*. (2007)	
Trichothecium roseum	Saprophyte	Farr *et al*. (2007)	CABI CPC (2006)
Tubeufia miscanthi		Farr *et al*. (2007)	
Tubeufia vermicularispora		Farr *et al*. (2007)	
Uredo miscanthi-floriduli	Rust	Farr *et al*. (2007)	
Uredo miscanthi-sinensis	Rust	Farr *et al*. (2007)	
Ustilago kusanoi	Smut	Farr *et al*. (2007), Sansford & McLeod (2000)	
Ustilago kusanoi f. *anomala-ovariicola*	Smut	Farr *et al*. (2007)	
Ustilago morobiana	Smut	Farr *et al*. (2007)	
Ustilago scitaminea	Sugarcane smut	Farr *et al*. (2007)	CABI CPC (2006)
Verticillium sp.	Verticillium wilt	Farr *et al*. (2007)	CABI CPC (2006)
Zygosporium oscheoides		Farr *et al*. (2007)	
Invertebrates			
Aleurocybotus miscanthus	A whitefly	Sansford & McLeod (2000)	
Atherigona bonensis	Sugarcane stem maggot	Sansford & McLeod (2000)	
Bryobia pritchardi	A mite	Sansford & McLeod (2000)	
Cavelerius saccharivorus	Oriental chinch bug	Sansford & McLeod (2000)	
Ceratovacuna lanigera	An aphid	Sansford & McLeod (2000)	
Chilo christophi	-	Sansford & McLeod (2000)	
Chilo hyrax	Amur silvergrass stem borer	Sansford & McLeod (2000)	
Chilo suppressalis	Rice stem borer	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Chorthippus latipennis	A grasshopper	Sansford & McLeod (2000)	
Chryschraon japonicas	A grasshopper	Sansford & McLeod (2000)	
Cnaphalocrocis latimarginalis	-	Sansford & McLeod (2000)	
Criconema miscanthi	A nematode	Sansford & McLeod (2000)	
Criconemella hawangiensis	A nematode	Sansford & McLeod (2000)	
Gampsocleis buergeri	A grasshopper	Sansford & McLeod (2000)	
Helicotylenchus dihystera	Common spiral nematode	CABI CPC (2006), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Hemiberlesia palmae	A scale	Sansford & McLeod (2000)	
Laelia coenosa	-	Sansford & McLeod (2000)	
Lissorhoptrus oryzophilus	American rice water weevil	Sansford & McLeod (2000)	
Locusta migratoria	Migratory locust	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Melanaphis formosana	An aphid	Sansford & McLeod (2000)	
Melanaphis koreana	An aphid	Sansford & McLeod (2000)	
Melanaphis sacchari	Yellow sugarcane aphid	CABI CPC (2006), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Melanaphis yasumatsui	An aphid	Sansford & McLeod (2000)	
Meloidogyne hapla	A root knot nematode	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Mesapamea secalis	Common rustic moth	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Metrioptera hime	A grasshopper	Sansford & McLeod (2000)	

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Mimophantia maritima	Flatid bugs	Sansford & McLeod (2000)	
Mogannia minuta	-	Sansford & McLeod (2000)	
Mongolotettix japonicus	Locust	Sansford & McLeod (2000)	
Neomaskellia bergii	Sugarcane whitefly	CABI CPC (2006), Sansford & McLeod (2000)	
Noctua pronuba	Common yellow underwing	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Oligonychus shinkajii	A mite	Sansford & McLeod (2000)	
Paractinolaimoides hawangensis	A nematode	Sansford & McLeod (2000)	
Parapleurus alliaceus	A grasshopper	Sansford & McLeod (2000)	
Parnara guttata	Rice skipper butterfly	CABI CPC (2006), Sansford & McLeod (2000)	
Pelopidas mathias	Rice skipper	CABI CPC (2006)	
Phenacaspis susukicola	A scale insect	Sansford & McLeod (2000)	
Pilococcus miscanthi	A scale	Sansford & McLeod (2000)	
Podisma mikado	A grasshopper	Sansford & McLeod (2000)	
Pseudoregma alexanderi	An aphid	Sansford & McLeod (2000)	
Pygalataspis miscanthi	A scale insect	Sansford & McLeod (2000)	
Radopholus sanoi	A nematode	Sansford & McLeod (2000)	
Saccharicoccus sacchari	Grey sugarcane mealybug	CABI CPC (2006)	CABI CPC (2006)
Salurnis marginellus	A flatid bug	Sansford & McLeod (2000)	
Schizotetranychus celarius	Bamboo spider mite	Sansford & McLeod (2000)	Sansford & McLeod (2000)
Schizotetranychus miscanthi	A mite	Sansford & McLeod (2000)	
Sericothrips marginalis	A Thrips	Sansford & McLeod (2000)	
Sesamia inferens	Purple stem borer	CABI CPC (2006), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Sitobion miscanthi	Indian grain aphid	CABI CPC (2006), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Stenchaetothrips albicornis	A thrips	Sansford & McLeod (2000)	
Tetramoera schistaceana	Sugarcane gray borer	CABI CPC (2006), Sansford & McLeod (2000)	
Tetraneura radicola	An aphid	Sansford & McLeod (2000)	
Tetraneura yezoensis	An aphid	Sansford & McLeod (2000)	
Tetranycopsis borealis	A mite	Sansford & McLeod (2000)	
Verutus mesoangustus	A nematode	Sansford & McLeod (2000)	
Viruses			
Barley yellow dwarf *luteovirus*	BYDV	CABI CPC (2006), Sansford & McLeod (2000)	CABI CPC (2006), Sansford & McLeod (2000)
Miscanthus streak *monogeminivirus*	MiSV	Brunt *et al.* (1996), Sansford & McLeod (2000)	
Sugarcane mosaic *potyvirus*	SCMV	Yamashita & Doi (2007)	Brunt *et al.* (1996)

* References indicate that these organisms may be in these countries; however there may not be any record of these organisms being associated with *Miscanthus* in these countries.

REFERENCES

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (eds.) (1996). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. Available online at http://image.fs.uidaho.edu/vide/refs.htm

CABI CPC (2006) *The Crop Protection Compendium, 2006 Edition*. © CAB International, Wallingford, UK, 2006. http://www.cabicompendium.org/cpc/home.asp

Ellis MB, Ellis JP (1997) Microfungi on land plants. Richmond Publishing

Farr DF, Rossman AY, Palm ME, McCray EB (2007) Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA. Retrieved August 2, 2007, from http://nt.ars-grin.gov/fungaldatabases/

Sansford C, McLeod A (2000) PRA for *Miscanthus*: Summary of PRAs for 1. Import of Rhizomes from Dominica and 2. Global Imports. Central Science Laboratory PPP8860

Yamashita S, Doi Y (2007) Miscanthus streak virus. Descriptions of Plant Viruses. Available online at http://www.dpvweb.net/dpv/showadpv.php?dpvno=348