BIOSECURITY NEW ZEALAND

Import Risk Analysis:

Wollemia nobilis (Wollemi Pine) Araucariaceae Nursery Stock from Australia

14 March 2008

Ministry of Agriculture and Forestry
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Biosecurity New Zealand

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The picture on the front cover is of the foliage of a small (2 metre) *Wollemia nobilis* tree growing in the Royal Botanic Gardens at Kew, London, England. The picture was taken by the author, Dr Mike Ormsby in July 2006.

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 MAF Biosecurity New Zealand or otherwise provided that is relevant to the final import risk analysis.

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TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

1.1 Synopsis

The Ministry of Agriculture and Forestry (MAF) has evaluated the nature and possible effect on people, the New Zealand environment, and the New Zealand economy of any organisms that may be associated with *Wollemia nobilis* (Wollemi pine) nursery stock imported from Australia.

Wollemi pine is the world's newest known conifer, having been discovered in an isolated area in Australia only 10 years prior to commencing this analysis. Information on associations with hazard organisms is limited by both the lack of research and the lack of opportunity for contamination. To ensure as far as possible that this risk analysis will be relevant into the future, available information on organisms associated with all members of the *Wollemia* family of plants (Araucariaceae) have been considered together with organisms known to associate with *Wollemia nobilis*.

The recommended management options contained in this risk analysis take account of existing industry practices and systems established in Australia and New Zealand to manage biosecurity risks associated with nursery stock material in international trade. Therefore while around 25 separate biosecurity measures are recommended for Wollemi pine nursery stock imported from Australia, the bulk of these can be easily incorporated into existing industry practices and should be seen as enhancements to the current biosecurity system.

1.2 Introduction

Wollemia nobilis (Wollemi pine) is the plant equivalent of the New Zealand tuatara, being a species that appears to be most closely related to fossils over 100 million years old. It a monotypic genus and the world's newest known conifer, having been discovered 10 years ago by a New South Wales National Parks & Wildlife Service officer.

There currently is no import health standard for *Wollemia nobilis* nursery stock. The overall objective of this project therefore, is to complete an analysis of the biosecurity risks of importing *Wollemia nobilis* nursery stock into New Zealand 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 *Wollemia nobilis* nursery stock into New Zealand.

The stated risk management objectives of this risk analysis are to ensure that no unwanted organisms potentially associated with *Wollemia nobilis* germplasm in Australia are:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

1.3 Summary of Risk Assessments

The import risk analysis assessed the biosecurity risks to New Zealand of a number of organisms potentially associated with this pathway. The organisms were in the most part grouped into organism types to help simplify the assessment and subsequent analysis. The organism types or "hazard categories" are listed in the following table:

Hazard Category	Chapter	Hazard Description	Significant Examples
Invertebrates		(Beetles, nematodes, moths, aphids)	
Surface Feeding Invertebrates	4	Pollen feeders, Seed eaters, Shoot feeders, Moths, Scales, Aphids.	*Agathiphaga queenslandensis, Chrysomphalus dictyospermi, Hyblaea puera, Neophyllapis araucariae*
Wood Boring Insects	5	Borers, Longicorn beetles, Ambrosia beetles, Bark beetles, Weevils	*Hylurdrectonus araucariae, Platypus froggatti, Xyleborus perforans, Hoplocerambyx severeus, Eurhamphus fasciculatus*
Micro-organisms		(Fungi, bacteria)	
Foliage Diseases	6	Rusts, leaf spots, mildews.	*Aecidium fragiforme*
Root Diseases	7	Root rots	*Phellinus noxius, Sclerotium rolfsii*
Wood Decay Fungi	8	Wood rot and heart rot	*Fomitopsis pinicola, Phellinus noxius,*
Canker Fungi	9	Stem cankers and die back	*Macrophoma araucariae*
Mycorrhizal fungi	10	Ectomycorrhizae, Arbuscular mycorrhizae	*Endogone sp*

A small number of organisms have been isolated from *Wollemia nobilis* nursery stock in Australia and were considered in this analysis. These organisms are listed in the following table:

Chapter	Scientific name	In NZ?	Vector of a hazard	More virulent strains on goods overseas	In NZ but not associated with goods	In NZ but not in region.
10	Arbuscular mycorrhizae	Y/N				
10	Ectomycorrhizae	Y/N				
11	*Botryosphaeria* sp.	Y/N				
12	*Phytophthora cinnamomi*	Y	N	Y	N	N

Scientific name	In NZ but different host associations	Under official control or notifiable	No or little information on organism	Potential Hazard?
Arbuscular mycorrhizae				Y
Ectomycorrhizae				Y
Botryosphaeria sp.				Y
Phytophthora cinnamomi	Y	N	N	Y

1.4 Recommended Measures

Based on the risk analyses completed for each identified pest or pest group listed in Chapters 4 to 12 the following measures are recommended for *Wollemia nobilis* whole plants and root-less cuttings from Australia to achieve the stated risk management objectives:

i) Pre-export measures during nursery growth and before export to New Zealand.

ii) Treatment and inspection either shortly before export from Australia or after arrival in New Zealand.

iii) A period in a Level 2 post-entry quarantine facility in New Zealand.

Aside from ensuring plants *in vitro* are indeed axenic, there are no phytosanitary measures required for *Wollemia nobilis* nursery stock imported from Australia as plants *in vitro*.

It is important to note that these are recommended measures only, and any alternative measures that provide an equivalent level of protection and meet the specified management objective will be accepted by MAF. The following sections summarise the three measures for *Wollemia nobilis* whole plants and root-less cuttings from Australia.

1.4.1 Pre-Export Nursery Management and Plant Inspection Requirements

Referring to the existing management practices provided in section 3.6.1 and Appendix 2, and the risk assessments completed in Chapters 4 to 12, the following nursery management practices should provide appropriate assurances that the levels of organism infestation on exported *Wollemia nobilis* nursery stock have been lowered sufficiently to allow for the adequate management of biosecurity risks on arrival in New Zealand.

i) To ensure plant material is free of wood-decay fungi before export to New Zealand, it is recommended that consignments of whole plants or root-less cuttings should be limited to specimens that have a maximum stem diameter of less than 12 centimetres (5 inches).

ii) It is recommended that the following nursery management practices be undertaken to provide assurances of adequate quality control and crop hygiene, particularly disease, pest and weed control and nursery hygiene in the production of plants for export to New Zealand:

- Accreditation under the Nursery Industry Accreditation Scheme, Australia (NIASA).

Additional or more specific requirements to the NIASA scheme include:

- To achieve an outcome that reduces the likelihood of exported plants being contaminated by a hazard organism, it is recommended that plants in the consignment to be exported be raised from seeds or cuttings in soil-less media in containers maintained out of contact with the soil;

- To achieve an outcome that improves the likelihood of any infesting hazard organisms developing a visible life stage or symptoms during pre-export inspections, it is recommended that a minimum pre-export inspection of one growing season in mild daytime temperatures (above 15^{o}C)[1] be undertaken;

- To achieve an outcome that reduces the likelihood of exported plants being contaminated by canker fungi, it is recommended that at least one month prior to export to New Zealand, plants should be subjected to a period of water stress. The application of successful water stress shall be measured by a visible response from the plant foliage such as leaf wilting;

- To achieve an outcome of ensuring pesticides and fungicides do not mask symptom expression and significantly reduce the effectiveness of pre-export inspections, it is recommended that only pesticide or fungicide treatments approved by New Zealand Ministry of Agriculture and Forestry should be applied;

- To achieve an outcome that reduces the likelihood of exported plants being contaminated by a hazard organism, it is recommended that plant stock is continually (regularly) inspected for pest and disease attack by qualified nursery staff;

- To achieve an outcome that reduces the likelihood of exported plants being contaminated by a hazard organism, it is recommended that mother-stock plants should be monitored regularly for pests and diseases, maintained in a suitable environment (e.g. where mother stock is in-ground, good drainage should be maintained.), and treated as appropriate with plant protection chemicals;

- To achieve an outcome that reduces the likelihood of exported plants being contaminated by a hazard organism, it is recommended that all access to the site is via a series of footbaths containing an antibiotic agent effective against soil-borne diseases such as *Phytophthora* species (e.g. 128 g/l of benzalkonium chloride);

- To reduce the likelihood of plant stock of greater than 5 centimetres (2 inches) stem diameter becoming infested by wood boring insects, it is recommended that stock becoming larger than this size in the nursery should be held in containment conditions that restrict the access of adult wood-boring insects.

1 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15^{O}C on any one day.

1.4.2 Plant Inspection Requirements

To provide adequate assurance of freedom from hazard organisms such as foliage, root, and canker diseases, surface or wood boring insect pests, *Botryosphaeria* species, and *Phytophthora cinnamomi*, it is recommended that the following plant inspection requirements be undertaken either as close to the packing and export of the plants to New Zealand as possible or soon after arrival in New Zealand (e.g. one week may be an appropriate time frame):

i) Each plant in the consignment:

- should have their above ground material (leaves and stems) inspected by an appropriately trained person for indications of foliage disease expression or evidence of surface feeding insect infestation;
- with a stem diameter greater than 5 centimetres (2 inches) should be inspected by an appropriately trained person for evidence of wood boring insect infestation;
- should have their roots cleaned (e.g. washed) of potting media and the roots examined by an appropriately trained person for symptoms of root diseases.

ii) Any plant showing indications of foliage or root disease, symptoms of *Phytophthora cinnamomi* infection, or surface feeding or wood boring insect infestation should not be included in the consignment for export to New Zealand. If more than 5% of the plants to be exported to New Zealand are found to have evidence of surface feeding or wood boring insect infestation, the entire consignment should be rejected for export to New Zealand until adequate treatments have been applied and subsequent inspections indicate the infestation rate has dropped below the 5% threshold.

1.4.3 Plant Treatment Requirements at time of Shipment

Based on the review of treatments provided in Appendix 3 and the analysis of risk assessment of hazards provided in chapters 4 to 12 of this document, it is recommended that one of the following treatment options is required for all plants, either as close to the packing and export of the plants to New Zealand as possible or soon after arrival in New Zealand.

The following chemical pesticides (table 1.1), when used as dips, are considered in Appendix 3 to be effective against infestations of surface feeding insects.

Table 1.1: Approved insecticide treatments for surface feeding insects.

Active ingredient (Chemical Group)	Treatment Specification[2]	Residue Persistence[3]
Acephate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 8 g a.i. per litre of dip. Treatment of non-dormant plant material only.	3 days (aerobic)
Carbaryl (Carbamate)	Dip at room temperature for 2-5 minutes at 18 g a.i. per litre of dip.	6 days (aerobic)
Chlorpyrifos (Organophosphorous)	Dip at room temperature for 2-5 minutes at 10 g a.i. per litre of dip. A non-ionic surfactant is required for dipping	113 days* (aerobic)
Dimethoate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 1.1 g a.i. per litre of dip. Treatment of non-dormant plant material only.	2 days (aerobic)
Imidacloprid (Neonicotinoid)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip. Treatment of non-dormant plant material only.	997 days* (aerobic)
Spinosad (Spinosyns)	Dip at room temperature for 2-5 minutes at 2 g a.i. per litre of dip.	17 days (aerobic)
Tebufenozide (Diacylhydrazine)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip.	405 days* (aerobic)

* It is likely that these periods of residue persistence are not reflected as equivalent periods of residual activity on the treated plants. These "worse case" figures have been listed in the absence of information on the actual period of residual activity *in-vivo*.

A combination of pesticides from two different chemical groups (organophosphorous, carbamate, neonicotinoid, spinosyns, or diacylhydrazine) should be applied to maximize the efficacy and mitigate potential issues with insect resistance. The consignment of whole plants or root-less cuttings should be spray treated 10-14 days after the initial treatment to manage any potentially surviving insects or insect life stages that the treatments may have failed to adequately treat. As mentioned above, care should be taken to ensure phytotoxicity levels are acceptable before applying any chemical treatments to plant material.

1.4.4 Post-Entry Quarantine Requirements

The principle measure available for detecting and removing infested germplasm before the consignment is released into New Zealand is through inspection while in post-entry quarantine. Contamination of nursery plants by many pests and diseases may not become apparent when conditions for the expression, such as humidity, temperature, and water levels, are not suitable or the infected plants are symptom-less hosts. Based on the review of treatments provided in Appendix 4 and the analysis of risk assessment of hazards provided in chapters 4 to 12 of this document, the following post-entry quarantine conditions are recommended to ensure as far as is possible that disease expression will become apparent on infected plants that are not symptom-less hosts, and that the infecting pests and diseases will remain contained within the quarantine facility:

- Post entry quarantine equivalent to Level 2 quarantine is considered appropriate, with the following additional structural and management requirements:

2 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.
3 Based on the aerobic soil half life (where provided, hydrolysis or anaerobic half life where not provided) provided by the PAN Pesticides Database at http://www.pesticideinfo.org

Plant Management Requirements

- The period of quarantine for each consignment of *Wollemia nobilis* whole plants or root-less cuttings should be one growing season, in conditions suitable for disease expression e.g. mild daytime temperatures (above $15^{o}C$)[4], drought stress, over watering, excess humidity, and varying day length and temperature. Temperature and day length variations should simulate as far as possible natural temperate-climate seasonal variations. The quarantine period shall commence after the listed half-life period of any applied pest management chemicals has passed;

Physical Containment and Hygiene Requirements

It is recommended that the implementation of and physical containment or hygiene requirements for post-entry quarantine facilities be considered as part of the review currently underway of the MAF Biosecurity New Zealand post-entry quarantine facility standard (PBC-NZ-TRA-PQCON: Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator (November 1999)[5]). Until that review has been completed the existing post-entry quarantine Level 2 physical containment and hygiene requirements should be accepted.

- To improve the level of general plant hygiene within the post-entry quarantine facilities it is recommended that a number of improvements to the management of post-entry quarantine be made. Examples include the spacing and segregation of containers, construction and design of benches and floor coverings, and general hygiene practices;

- To limit the likelihood of pests or diseases present on the imported plants escaping into the New Zealand environment, it is recommended that measures be taken to improve the level of containment provided by the post-entry quarantine facilities. Post entry quarantine measures to achieve this include:

    - The level of security provided by the facility should be sufficient to contain such propagules as airborne fungal spores. Currently the only measures known to provide this level of containment are to ensure all outward flowing air vents are filtered with a High-Efficiency Particulate Air (HEPA) filter, and a negative air pressure (15 Pa) is maintained within the facility. MAF recognises the likely costs associated with such measures may not be cost-effective and will review potential alternatives to managing this identified risk;

Plant Inspection Requirements

- It is recommended that the following plant inspections be undertaken during the period of post-entry quarantine:

    - A plant inspection is undertaken one month after treatment residues have adequately dissipated, but before any plants receive biosecurity clearance, to

4 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds $15^{O}C$ on any one day.

5 http://www.biosecurity.govt.nz/border/transitional-facilities/plants/pbc-nz-tra-pqcon.htm

confirm the level of treatment efficacy was appropriate. Should live insects be detected, the reason for treatment failure should also be determined and steps taken to ensure such failures are unlikely to re-occur.

- By the conclusion of the post-entry quarantine period, all parts of each plant (roots, stems and leaves) should have been examined by an appropriately authorised and experienced person for evidence of contamination or infestation by surface feeding insects, foliage, canker and root disease-causing fungi, *Botryosphaeria* species, and *Phytophthora cinnamomi*. Plants in the consignment with maximum stem diameters greater than 5 centimetres (2 inches) shall also be examined for evidence of live wood boring insect infestation. Root inspections in this instance should not require any cleaning of potting mix residues and may be completed through clear-sided plant containers.

Any plants that have indications of infestation or contamination by living hazard organisms should be treated (if appropriate), reshipped or destroyed. Depending on the type and nature of the hazard organism, host plants remaining in the quarantine facility may need to be treated and/or placed back under quarantine conditions (for a period determined by treatment type and organism biology), reshipped or destroyed.

1.4.5 General Requirements

The following additional general requirements are recommended to manage uncertainties associated with the performance of the risk mitigation measures recommended by this risk analysis.

- To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

- To ensure residues of any applied chemicals on the imported germplasm do not interfere with pest or disease detection in New Zealand post-entry quarantine, it is recommended that records of all added pesticides (insecticides, fungicides, miticides, nematicide etc) applied within 6 months of export to New Zealand, during transport to the New Zealand post-entry quarantine facility, or during the post-entry quarantine period in New Zealand, be maintained and reported as appropriate to the Ministry of Agriculture and Forestry.

1.4.6 Expected Performance of Measure(s)

Insect Infestations

The pre-export nursery management measure is expected to ensure no more than 5% of the consignment is infested with surface feeding or wood boring insects on export to New Zealand. Any inspection of the imported plants prior to treatment should not detect surface feeding or wood boring insect infestation levels above 5% of the consignment.

The pesticide treatment requirements should ensure that no *Wollemia nobilis* nursery stock is infested by live insect life stages. Therefore no live insects should be found on inspection of the imported plants in post-entry quarantine. The post-entry quarantine inspection should detect, with a 95% level of confidence, all treatment failures. A failure in this measure will only be detected if an unwanted insect establishes in New Zealand from *Wollemia nobilis* nursery stock imported from Australia.

Micro-organism Contamination

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are contaminated with unwanted micro-organisms. There should therefore be no detections of diseased plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export contamination. In reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through long term monitoring or targeted research.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no imported *Wollemia nobilis* plants infected with unwanted micro-organisms are released into the New Zealand environment. As *Wollemia nobilis* plants entering the New Zealand environment would be expected to become infested with already established unwanted micro-organisms, some of which may not have been described by science, identifying a success measure for this option is more problematic. Therefore it is expected that a high level of assurance is obtained that the post-entry quarantine inspections were undertaken appropriately before any plants are released into the New Zealand environment. As above, in reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through targeted research.

2. ANALYSIS BACKGROUND AND PROCESS

2.1 Risk Analysis Background

Wollemia nobilis Jones, Hill & Allen 1995 (Wollemi pine) is the plant equivalent of the New Zealand tuatara, being a species that appears to be most closely related to fossils over 100 million years old. It a monotypic genus and the world's newest known conifer, being discovered 10 years ago by a New South Wales National Parks & Wildlife Service officer (Jones *et al.* 1995). The Wollemi pine is a relict species currently known to occur in only two sites located about 1 km apart in Wollemi National Park on the Central Tablelands of New South Wales in south eastern Australia, 200 km northwest of Sydney. Less than 40 adult and 200 juvenile plants are known in the wild (NPWS 1998). *Wollemia nobilis* is a member of the Araucariaceae family, which also includes the genera *Agathis* and *Araucaria*. The common name of "Wollemi pine" is misleading, as it is not a member of the "pine" family Pinaceae.

There currently is no import health standard for *Wollemia nobilis* nursery stock. The overall objective of this project, therefore, is to complete an analysis of the biosecurity risks of importing into New Zealand *Wollemia nobilis* nursery stock from Australia, 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 *Wollemia nobilis* nursery stock into New Zealand from Australia.

As *Wollemia nobilis* is a new species to science there is a lack of published technical information about associated pests. The import risk analysis will therefore review the literature for pests associated with the *Wollemia nobilis* family, namely Araucariaceae, in an attempt to ensure any future or as yet unidentified potential risks are assessed.

The objective of this project therefore is to conduct an import risk analysis, which will be used as the platform for the development of an import health standard for *Wollemia nobilis* nursery stock from Australia.

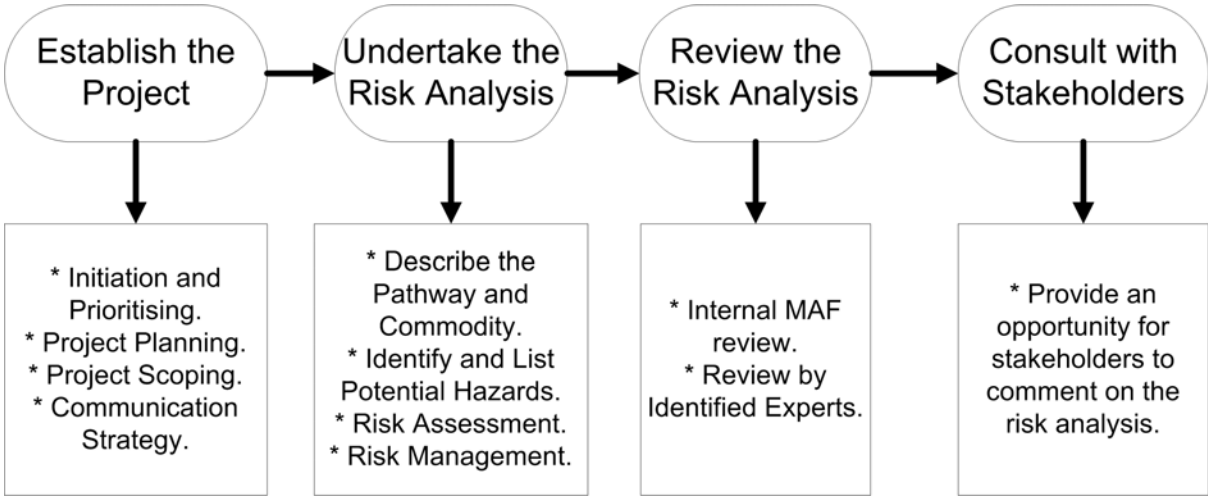
2.2 Risk Analysis Process and Methodology

The following chapter briefly describes the 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[6].

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

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

Figure 2.1: A summary of the Biosecurity New Zealand risk analysis development process



The "Establishing the Project" phase is an internal project management process undertaken within Biosecurity New Zealand for all risk analysis projects and as such is not described further here.

2.2.1 Commodity and Pathway Description

The first step in the risk analysis process once the project gets underway is to describe all the relevant attributes of the pathway within the scope of the risk analysis. This includes relevant information on:

- the country of origin, including such things as climate, relevant agricultural practices, phytosanitary system;
- pre-export processing and transport systems;
- export and transit conditions, including packaging, mode and method of shipping;
- nature and method of transport and storage on arrival in New Zealand;
- the risk analysis area in New Zealand, including such things as climate, relevant agricultural practices.

Care should be taken to ensure no measures are included as default conditions on the pathway unless these are included in the scope of the analysis. Once the risk analyst has a thorough understanding of the setting or context for the risk analysis, the risk analysis proper can begin.

2.2.2 Hazard Identification

Hazard identification is an essential step that must be conducted prior to a risk assessment where the hazard is not itself defined in the scope of the risk analysis (e.g. a pest risk assessment). To effectively manage the risks associated with imported risk goods, unwanted organisms or diseases which could be introduced by the risk goods into New Zealand and are capable of, or potentially capable of, causing unwanted harm must be identified.

A hazard is any organism or disease that has the potential to produce adverse consequences. Under section 22 of the Biosecurity Act (1993), a Chief Technical Officer must have regard to the following matters before recommending that an import health standard be issued:

> The nature and possible effect on people, the New Zealand environment, and the New Zealand economy of any organisms that the goods specified in an import health standard may bring into New Zealand.

The environment can be further defined as including ecosystems and their constituent parts, including people and their communities; and all natural and physical resources; and amenity values; and the aesthetic, cultural, economic, and social conditions that affect or are affected by any matter referred to above.

A list of organisms and diseases likely to be associated with the pathway (i.e. associated with the commodity) should be assembled. The list may include organisms or diseases for which the biosecurity hazard is not clear. It is important to also consider organisms or diseases that might be associated with material that is contaminating the risk good, if that contaminating material can not be easily separated from the goods on import.

When considering whether an identified organism or disease should be included in the hazard list for a particular risk analysis, the following questions should be considered:

- Is the organism or disease associated with the commodity or conveyance?
- Is the organism or disease absent from New Zealand but likely to be present in the exporting country?
- Is the organism or disease present in New Zealand and likely to be present in the exporting country, and meets one of the following criteria?
    - The organisms are vectors of pathogens or parasites, but whose populations in New Zealand are free of the pathogen or parasite of concern.
    - The organisms have strains that do not occur in New Zealand, though the overall species does occur in New Zealand.
    - The organisms differ genetically from those that occur in New Zealand in a way that may present a potential for greater consequences in New Zealand, either from the organism itself or through interactions with existing organisms in New Zealand.
    - The organisms or diseases are already in New Zealand however the nature of the imports would significantly increase the existing hazard.
    - The organisms or diseases are already in New Zealand however their presence is geographically bounded.

Continued next page:

    - The organisms or diseases have host associations different to those currently present in New Zealand.
    - The organisms or diseases have only minimal information available and as such should be considered a hazard at this stage, as the more detailed risk assessment process will

determine the level of likely risk.

- The organisms or diseases have free zones or zones of low prevalence in New Zealand that are established under a national or regional pest management strategy or small-scale program and where the movement of host products into the zone is under statutory control.

- The organisms or diseases are listed on the unwanted organisms register as a notifiable organism.

2.2.3 Risk Assessment of Hazards

Risk assessment is the evaluation of the likelihood and environmental, economic, and human health consequences of the entry, exposure and establishment of a potential hazard within New Zealand. The aim of risk assessment is to identify hazards which present an unacceptable level of risk, for which risk management measures are required. A risk assessment consists of four inter-related steps:

- Assessment of likelihood of entry
- Assessment of likelihood of exposure and establishment
- Assessment of consequences
- Risk estimation.

The uncertainties and assumptions identified during the preceding stages are also summarised and considered for further research with the aim of reducing the uncertainty or removing the assumption.

2.2.3.1 Assessment of Risk of Entry

The aim of this step is to assess the likelihood of movement of a potential hazard from its country of origin into a risk analysis area via an import pathway, in this case *Wollemia nobilis* nursery stock from Australia. A conclusion should be stated on the likelihood of entry of each potential hazard identified in the risk analysis. The risk analysis may be concluded at this point if the likelihood of the potential hazard being able to enter into New Zealand is negligible.

2.2.3.2 Assessment of Risk of Exposure and Establishment

The aim of this step is to assess the likelihood of the potential hazard or group of hazards, having entered a risk analysis area, gaining exposure to and becoming established in it, and/or having the potential to cause an adverse consequence. A potential hazard or group of hazards may cause an adverse consequence through exposure without necessarily being established, for example, spiders on grapes can adversely affect vulnerable consumers. Each potential hazard, group of hazards, should be dealt with separately with a reasoned, logical and referenced discussion of its relevant epidemiology and/or biology to:

i) describe the biological mechanisms necessary for the potential hazard or group of hazards to gain exposure and/or become established;

ii) describe the mechanism for the exposure of the environment or other receptors in New Zealand to the potential hazard or group of hazards;

iii) estimate the likelihood of establishment and/or exposure occurring.

A conclusion will be stated on the likelihood of exposure and establishment of each potential hazard or group of hazards. The risk analysis may be concluded at this point if the likelihood of exposure and establishment in New Zealand is negligible.

2.2.3.3 Assessment of Consequences

The aim of this step is to assess the potential consequences associated with the entry, exposure and establishment of the potential hazard or group of hazards, and to estimate the likelihood of such consequences occurring. The Biosecurity Act (1993)[7] requires that the nature and possible effect on people, the New Zealand environment, and the New Zealand economy be considered in developing risk management measures.

Each potential hazard or group of hazards should be dealt with separately with a reasoned, logical and referenced discussion to:

i) identify the likely spread within the risk analysis area;

ii) identify the potential biological, environmental, economic and human health consequences associated with the entry, establishment, and exposure of the potential hazard;

iii) estimate the likelihood of these potential consequences.

A conclusion of the consequences of the entry, establishment, and exposure of the potential hazards should be given. The areas of New Zealand where potential consequences may occur should be stated, as appropriate. Hazards for which the potential consequences are very high (high consequence hazards) should be flagged as such to assist in prioritising other work such as incursion response preparedness.

The risk assessment of the hazard organism or hazard group may be concluded at this point if potential consequences are not identified or the likelihood of the potential consequences is negligible.

7 Section 22 part 5; of the Biosecurity Act (1993)

2.2.3.4 Risk Estimations

The aim of this step is to summarise the conclusions arising from the entry, exposure and establishment, and consequence assessments, to estimate the likelihood of the potential hazard or hazard group entering the risk analysis area and resulting in adverse consequences. Each potential hazard or hazard group should be dealt with individually. If the estimated risk is not negligible the potential hazard is classified as an actual hazard and risk management measures may be required (while the level of risk may be non-negligible it may still be considered acceptable).

2.2.3.5 Assessment of Uncertainties

The purpose of this section of the risk analysis process is to summarise the uncertainties and assumptions identified during the preceding hazard identification and risk assessment stages. 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[8] and be consistent with other measures where equivalent uncertainties exist.

2.2.4 Analysis of Measures to Mitigate Biosecurity Risks

Risk management in the context of risk analysis is the process of deciding measures to effectively manage the risks posed by the hazard(s) associated with the commodity or organisms under consideration. It is not acceptable to identify a range of measures that might reduce the risks. There must be a reasoned relationship between the measures chosen and the risk assessment so that the results of the risk assessment support the measure(s).

Since zero-risk is not a reasonable option, the guiding principle for risk management should be to manage risk to achieve the required level of protection that can be justified and is feasible within the limits of available options and resources. Risk management (in the analytical sense) is the process of identifying ways to react to a risk, evaluating the efficacy of these actions, and identifying the most appropriate options.

The uncertainty noted in the assessments of economic consequences and probability of introduction should also be considered and included in the consideration of risk management options. Where there is significant uncertainty, a precautionary approach may be adopted. However, the measures selected must nevertheless be based on a risk assessment that takes account of the available scientific information. In these circumstances the measures should

8 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.

be reviewed as soon as additional information becomes available. It is not acceptable to simply conclude that, because there is significant uncertainty, measures will be selected on the basis of a precautionary approach. The rationale for selecting measures must be made apparent.

Each hazard or group of hazards should be dealt with separately using the following framework:

2.2.4.1 Risk evaluation

- If the risk estimate, determined in the risk assessment, is non-negligible, measures can be justified.

2.2.4.2 Option evaluation

a) Identify possible options, including measures identified by international standard setting bodies, where they are available.

b) Evaluate the likelihood of the entry, exposure, establishment or spread of the hazard according to the option(s) that might be applied.

c) Select an appropriate option or combination of options that will achieve a likelihood of entry, exposure, establishment or spread that reduces the risk to an acceptable level. The following guidelines must be taken into account when selecting option(s):

- ensure that the option(s) are based on scientific principles.
- ensure that measures identified by international standard setting bodies are considered. If there is a scientific justification that an international measure does not effectively manage the risks, measures that result in a higher level of protection may be applied. Alternatively less stringent measures than those recommended in international standards may be applied where there is sufficient justification that the risks can be effectively managed.
- ensure that the option(s) are applied only to the extent necessary to protect human, plant or animal life or health, or the environment.
- ensure that negative trade effects are minimised.
- ensure that the option(s) do not result in a disguised restriction on trade.
- ensure that the option(s) are not applied arbitrarily e.g. ISPM 1: *Principle of "non-discrimination"* - If the pest under consideration is established in the risk analysis area but of limited distribution and under official control, the measures in relation to import should not be more stringent than those applied within the risk analysis area.
- ensure that the option(s) do not result in discrimination between exporting countries where similar conditions prevail.
- ensure that the option(s) are feasible by considering the technical, operational and economic factors affecting their implementation.

The result of the risk management procedure will be either that no measures are identified which are considered appropriate, or the selection of one or more management options that have been found to lower the risk associated with the hazard(s) to an acceptable level. These management options form the basis of regulations or requirements specified with an import health standard.

2.2.5 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.

2.2.6 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 recommended modifications to the risk analysis or the risk analysis itself will be edited to comply with the recommended modifications.

2.3 References

Jones W G, Hill K D & Allen J M, 1995, '*Wollemia nobilis*, a new living Australian genus and species in the Araucariaceae', *Telopea*, 6: 173–176

NPWS, 1998, 'Wollemi Pine Recovery Plan', NSW National Parks and Wildlife Service, Sydney.

3 IMPORT RISK ANALYSIS

The following 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 to 12).

3.1 Commodity Description

As already mentioned in Chapter 2, the scope of this risk analysis is the potential hazard organisms or diseases associated with nursery stock of *Wollemia nobilis* imported from Australia. For the purposes of this analysis "nursery stock" means "*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.*" The three forms of nursery stock being considered in this analysis include:

- "**plants *in vitro***" which is defined as "*a commodity class for plants growing in an aseptic medium in a closed container*";
- "**root-less cuttings**" which is defined as "*Plant cuttings that may have leaves and shoots, but no roots*"; and
- "**whole plants**" which is defined as "*a nursery stock commodity sub-class for rooted cuttings and plants with roots and leaves*".

The first officially published record of *Wollemia nobilis* was by Jones, Hill & Allen (1995). The *Wollemia* genus is part of the Araucariaceae family of the Gymnospermae (a plant which has naked seeds). As this genus has not existed in New Zealand for over 100 million years it was considered a "new organism" and was prohibited entry into New Zealand under the Hazardous Substances and New Organisms Act (1998) until October 2005, when the New Zealand Environmental Risk Management Authority (ERMA) gave an approval[9] for its import subject to the development of an import health standard under the Biosecurity Act (1993).

3.1.1 Basic description of **Wollemia nobilis**

Wollemia nobilis is an erect conifer with attractive, dark green foliage and unusual bubbly bark ("resembling bubbling chocolate"), growing up to 40 metres high in the wild with a trunk diameter of over one metre. The leaves on adult lateral shoots are one of the most distinctive features, being arranged in four ranks, with two ranks at about 150-175° and the other two ranks lying between the first two at about 50-90°. Adult and juvenile shoots of *Wollemia* differ in leaf arrangement, leaf shape, and cuticular features: in these features they are most similar to *Araucaria*. An unusual characteristic is its habit of shedding whole branches rather than individual leaves (NPWS 1998).

9 ERMA document NOR04003: at http://www.ermanz.govt.nz/appfiles/execsumm/word/NOR04003-002.doc

Photo of *Wollemia nobilis* foliage in Kew Gardens, London, July 2006.

Courtesy of Dr Mike Ormsby

3.1.2 Life history and life cycle information

The *Wollemia nobilis* is bisexual (monoecious), like its closest living relatives, with both male and female cones on the same tree. Male cones concentrate low down but also grow on upper branches. In Australia pollen is cast in spring (October) or thereabouts, and wind drafts take it to female cones that generally grow on higher branches. It is thought that cone development takes about 14 months but this is still being studied. Cones shatter in late summer to early autumn (March). It is thought that only the oldest trunks are able to produce seeds (NPWS 1998).

Only about 5% of seeds are viable which is not unusual in Araucarias but probably lower in the *Wollemia nobilis* than in most species, due to inbreeding. It is probably self-compatible but this is difficult to ascertain in the wild. Seeds of the *Wollemia nobilis* are light and winged and most probably dispersed by wind although not for long distances (NPWS 1998).

Growth of seedlings in the wild appears to be very slow. From current observations they appear to increase by more than one growth segment per year, but this may depend on the site and season of germination. In cultivation growth is much faster. Two-year-old cultivated seedlings are nearly one metre tall (NPWS 1998). Although mycorrhizal associations have been identified, as yet no mycorrhizal association appears necessary for seedling growth and survival so that plants can be grown without any associate fungi being needed (McGee, 1999).

Vegetative reproduction occurs through rudimentary buds which are carried in the axils of leading vertical shoots. Initially, these buds can replace the leading shoot if it is damaged. If they do not replace the leading shoot they become buried under the thickening bark. These buds may remain dormant for long periods of time until they sprout from older trunks or from

the base of the trunks. This coppicing leads to a number of trunks of various ages in a mature tree. In the wild, most trunks arise from a common base but some may derive from a suckering of larger roots. Trunks have also developed from the epicormic shoots of fallen branches (NPWS 1998).

Adult trees have been observed to increase by one additional growth unit (referred to as stem segments) per year from orthotropic shoots (vertical growing shoots), and no more than one segment from plagiotropic reproductive shoots (lateral growing shoots) (NPWS 1998).

Researchers have developed methods for propagating the *Wollemia nobilis* by cuttings, and the Queensland Forestry Research Institute is developing mass propagation techniques. Cuttings taken from the lateral branches produce a prostrate (low growing) plant suitable as a spreading pot plant or ground cover. After an initial slow start, cultivated seedlings grow to about one metre after three years with a diameter near the base of about 30 millimetres. *Wollemia nobilis* specimens planted out in other locations are also being monitored for their growth rate and their growth habits. They respond well to light and favour acid soils (NPWS 1998).

On average cultivated specimens grow around half a metre a year, although growth in the wild is much slower. They start growing in early spring and grow upwards for around two months. After that they concentrate their energy on growing outwards. *Wollemia nobilis* plants respond well to fertiliser. A range of potting mix requirements and levels and types of fertilisers is being trialled. Young plants in cultivation need some protection from strong light provided by shade cloth or the shelter of other trees (NPWS 1998).

Research is also being conducted to test the viability of utilising micro propagation methods for commercial production.

3.2 Occurrence of Araucariaceae in New Zealand

There are two members of the Araucariaceae family in New Zealand: *Agathis* and *Araucaria*. New Zealand has one native member of the Araucariaceae, *Agathis australis* (kauri). All the other species of the family Araucariaceae currently believed to be present in New Zealand have been introduced since European settlement.

Species in the *Agathis* genus are distributed from Malaysia, Brunei and Indonesia, through New Guinea, Queensland and the eastern Solomon's, to Vanuatu, New Caledonia, Fiji and New Zealand. There are more than 20 species, with approximately half having been introduced to New Zealand, although most are probably in glasshouses or warmer environments as they are generally from climates warmer than New Zealand.

Table 3.1 *Agathis* species present in NZ

Agathis australis	*Agathis macrophylla*	*Agathis ovata*
Agathis brownii	*Agathis montana*	*Agathis robusta*
Agathis corbassonii	*Agathis moorei*	*Agathis vitiensis*
Agathis lanceolata	*Agathis palmerstonii (= Agathis robusta)*	

There are about 20 species in the genus *Araucaria*, more than half endemic to New Caledonia. The genus is more widely distributed than *Agathis* and is indigenous to Chile, Argentina, southern Brazil, New Caledonia, Norfolk Island, Australia, and New Guinea.

The most commonly cultivated species in NZ is the Norfolk pine, *Araucaria heterophylla*. Several other species are not uncommon in cultivation and do reasonably well, but the rest are likely to be in specialist collections only.

Table 3.2 *Araucaria* species present in NZ

Araucaria angustifolia	*Araucaria excelsa*	*Araucaria montana*
Araucaria araucana	*Araucaria heterophylla*	*Araucaria muelleri*
Araucaria bernieri	*Araucaria humboldtensis*	*Araucaria nemorosa*
Araucaria bidwillii	*Araucaria hunsteinii*	*Araucaria rulei*
Araucaria biramulata	*Araucaria imbricata*	*Araucaria schmidii*
Araucaria columnaris	*Araucaria klinkii*	*Araucaria scopulorum*
Araucaria cookii	*Araucaria laubenfelsii*	*Araucaria subulata*
Araucaria cunninghamii	*Araucaria luxurians*	

3.3 Description of the Import Pathway

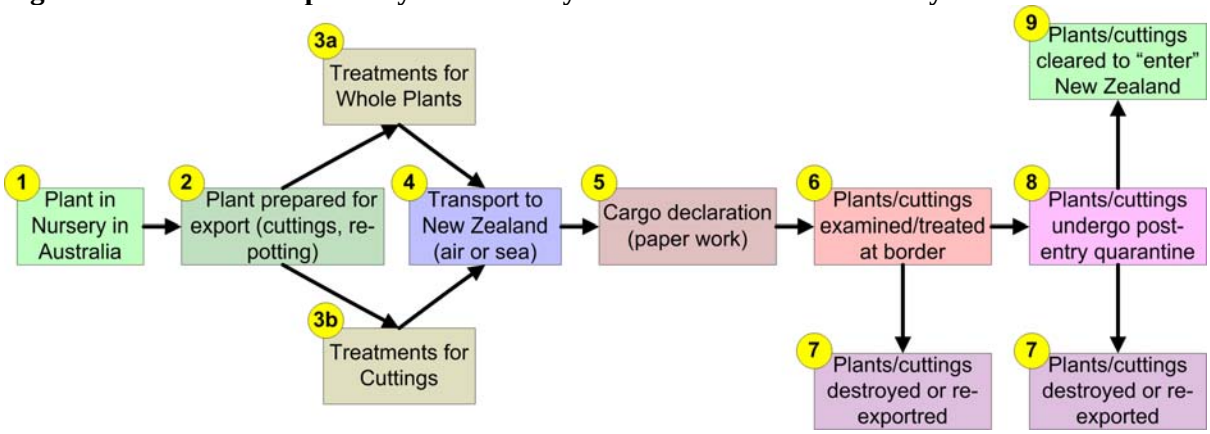
For the purpose of this risk analysis, it is assumed that *Wollemi nobilis* nursery stock will be sourced from anywhere in Australia (open ground, enclosed nursery or laboratory areas). To comply with existing New Zealand import requirements for soil, plants would need to be prepared for export to New Zealand by removing all visible soil from the roots and subsequently re-potted in a soil-less medium. Viable nursery stock could then be carried (personal luggage), air or sea-freighted to New Zealand before being distributed to homes or nurseries within New Zealand in the absence of any other import controls.

3.3.1 Summary of existing nursery stock import pathways

The current standard requirements (or "basic" requirements) for importing plant nursery stock are provided in MAF standard 155.02.06[10] and are more involved than that mentioned above. These standard or "basic" requirements that are applied to almost all nursery stock require a number of pre-entry treatments and a period in post-entry quarantine[11] for a minimum of 3 months. Assuming that the standard or "basic" requirements are applied to the importation of *Wollemia nobilis* nursery stock, the import pathway would be as indicted in the following diagram (figure 3.1) and explanatory text.

10 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
11 The standard for the registration of a plant quarantine facility is available on the MAF web site at http://www.biosecurity.govt.nz/border/transitional-facilities/plants/pbc-nz-tra-pqcon.htm

Figure 3.1: The "basic" pathway for the entry into New Zealand of nursery stock.



1) Mother plants in Australia are growing:
- In a quarantine nursery;
- In a commercial nursery;
- In a home nursery or greenhouse;
- In a cultivated garden; or
- "Naturally" in a park or wilderness area.

2) Plants or plant parts are prepared for export in Australia by:
- Removing whole plants from potting mix or soil, treating (see 3a) and re-potting in soil-less potting mix;
- Taking root-less cuttings from mature plants;
- Taking seeds from mature trees (not included in this risk analysis);
- Taking cuttings from mature plants or seedlings and placing into sterile culture (plants *in vitro*);
- Germinating seeds in sterile culture (plants *in vitro*).

3a) Whole plants may be treated pre-export as per MAF pre-entry treatment requirements. For whole plants this includes root washing and a number of chemical treatments.

3b) Cuttings may be treated pre-export as per MAF pre-entry treatment requirements[12].

4) The nursery stock is packaged and transported to New Zealand either:
- Unaccompanied by airfreight or sea cargo, or
- Accompanied by air or sea passengers.

5) Each shipment or accompanied luggage must arrive at the New Zealand border (a place of first arrival) with the appropriate biosecurity-related papers e.g. phytosanitary certificate attesting to the identity of the nursery stock, any treatments completed, or any other information required to help mitigate biosecurity risks.

6) Nursery stock is examined at the port of entry to ensure packaging is compliant and the shipment or luggage is not contaminated with organisms that can be detected on

12 Seed import requirements are excluded from consideration in this analysis. Tissue cultures do not normally require any form of pre-entry treatment.

visual inspection. Any of the required pre-entry treatments not completed prior to export to New Zealand will need to be completed at this point.

7) Any nursery stock not complying with New Zealand's biosecurity requirements is either reshipped or destroyed.

8) Whole plants or root-less cuttings undergo a period of inspection and/or testing in post-entry quarantine within New Zealand. The extent of quarantine will depend on the pre-export condition of the nursery stock, but is usually 3 months of active growth. Plants *in vitro* do not usually require a period in post-entry quarantine.

9) Nursery stock that has complied with all of the biosecurity requirements may be given clearance to enter New Zealand without further biosecurity-related restrictions.

3.4 Hazard Identification

Wollemia nobilis is currently known to occur in only two sites located about 1 kilometre apart in Wollemi National Park on the Central Tablelands of New South Wales in south eastern Australia. Less than 100 plants are known in the wild. Since 1994 the plants have been studied in the wild and seeds have been collected and propagated in a number of nurseries within Australia. The distribution of plants of *Wollemia nobilis* is still very limited and it is likely that many organisms that will infest or infect this plant species have yet to be identified or have not as yet had the opportunity to associate with the plants growing in Australia.

To account for the uncertainty around the type and nature of the potential hazards associated with future *Wollemia nobilis* nursery stock imported from Australia, the hazard identification process has been separated into two main approaches:

1) Listing potential hazard organisms that have been recorded on *Wollemia nobilis* nursery stock in the wild or in nurseries in Australia.

2) Listing potential hazard organisms that are present in Australia and have been recorded on other species within the Araucariaceae (the family of *Wollemia nobilis*). These family-associated hazards will then be grouped into hazard-types (based on epidemiological characteristics).

3.4.1 Potential Hazard Organisms

So far more than 50 species of fungi living on or near the trees have been identified, at least one third of which are new to science. One of the species isolated from the foliage, *Pestalotiopsis*, produces taxol, a cancer-controlling drug, though not in quantities useful for medicine. Two types of mycorrhizal fungi have been found with the roots of the *Wollemia nobilis*: *arbuscular mycorrhizae* and *ectomycorrhizae* (NWPS 1998). Finding two types of mycorrhizal fungi was unexpected as previously only the arbuscular type has been found in the Araucariaceae. Of all the fungi found living on or near the trees at least one third are new to science and probably specific to the particular habitat of *Wollemia nobilis* (NPWS 1998)[13].

13 http://www.rbgsyd.nsw.gov.au/information_about_plants/wollemi_pine/research_projects?p=424

There is, however, no evidence that any of the fungi noted are essential to the survival of *Wollemia nobilis* and so do not need to be imported with this species (McGee, 1999).

Tests have shown that, like many other Australian plant species, *Wollemia nobilis* is susceptible to two common and easily transmitted pathogens: *Phytophthora cinnamomi* and *Botryosphaeria* sp. (Bullock *et al.* 2000). A promotional video produced by Wollemi Pine International Pty Ltd describes the likely pest and diseases of *Wollemia nobilis* as follows:

> "Like most ornamental plants the Wollemi pine is susceptible to common garden pests. These include caterpillars and sap-sucking insects, such as aphids, scales and mealybugs. The Wollemi pine has been found to be relatively resistant to most diseases."

The hazard assessment of the four organisms known to be associated with *Wollemi nobilis* nursery stock at the time of undertaking the risk analysis is provided in chapters 10, 11 and 12. A summary of these hazard assessments is provided in table 3.3.

Table 3.3: Potential hazard organisms recorded on *Wollemia nobilis* nursery stock

Chapter	Scientific name	In NZ?	Vector of a hazard	More virulent strains on goods overseas	In NZ but not associated with goods	In NZ but not in region.
10	Arbuscular mycorrhizae	Y/N				
10	Ectomycorrhizae	Y/N				
11	*Botryosphaeria* sp.	Y/N				
12	*Phytophthora cinnamomi*	Y	N	Y	N	N

Scientific name	In NZ but different host associations	Under official control or notifiable	No or little information on organism	Potential Hazard?
Arbuscular mycorrhizae				Y
Ectomycorrhizae				Y
Botryosphaeria sp.				Y
Phytophthora cinnamomi	Y	N	N	Y

3.4.2 Potential hazard groups

It is likely that *Wollemia nobilis* nursery stock will be shown to be susceptible to infestation or infection by many more potentially unwanted organisms as plants are distributed and become more widely grown. In an attempt to anticipate the full range of organisms potentially able to become associated with *Wollemia nobilis* nursery stock, a list was generated of organisms that are:

a) Recorded as being associated with other plant species that are members of the Araucariaceae; and

b) Currently believed to be established in Australia.

The list of these potential hazard organisms is provided in Appendix 1.

As the association of the list of organisms with *Wollemia nobilis* nursery stock is at this stage hypothetical, detailed examination of each species of organism individually would seem unnecessary. To simplify the analysis, each species or organism was allocated a group corresponding to the basic nature of the organism association with its plant host. The species

to group allocations are provided in Appendix 1 and summarised in table 3.4 below. As indicated further information about each hazard group is provided in chapters 4 to 10 of this document.

Table 3.4: List of Potential Hazard Groups

Hazard Category	Chapter	Hazard Description	Significant Examples
Invertebrates		(Beetles, nematodes, moths, aphids)	
Surface Feeding Invertebrates	4	Pollen feeders, Seed eaters, Shoot feeders, Moths, Scales, Aphids.	*Agathiphaga queenslandensis, Chrysomphalus dictyospermi, Hyblaea puera, Neophyllapis araucariae*
Wood Boring Insects	5	Borers, Longicorn beetles, Ambrosia beetles, Bark beetles, Weevils	*Hylurdrectonus araucariae, Platypus froggatti, Xyleborus perforans, Hoplocerambyx severeus, Eurhamphus fasciculatus*
Micro-organisms		(Fungi, Bacteria)	
Foliage Diseases	6	Rusts, leaf spots, mildews.	*Aecidium fragiforme*
Root Diseases	7	Root rots	*Phellinus noxius, Sclerotium rolfsii*
Wood Decay Fungi	8	Wood rot and heart rot	*Fomitopsis pinicola, Phellinus noxius,*
Canker Fungi	9	Stem cankers and die back	*Macrophoma araucariae*
Mycorrhizal fungi	10	Ectomycorrhizae, Arbuscular mycorrhizae	*Endogone sp*

3.4.3 Other risk characteristics of Wollemia nobilis

In a paper published in 2000 and titled "Threats to New Zealand's indigenous forests from exotic pathogens and pests", Ridley *et al.* (2000) came to the following conclusions from the analysis of a number of cases studies in great epiphytotics:

> ".. pathogenic fungi do not tend to jump great taxonomic distances."
> "Therefore to be able to predict the source of a threat it is necessary to understand the biogeography of the host."

The conclusion drawn by the authors in the context of New Zealand biosecurity was that:

> "… the fungi most likely to penetrate native forests (*in New Zealand*) whether they are pathogenic or not (*in there natural ecosystems*) will come from forests of a similar floristic and climatic composition."

Wollemia nobilis and its constituent ecosystem should be considered similar to native ecosystems in New Zealand that include the closely related *Agathis australis* (Kauri). Organisms that themselves originate from ecosystems of similar floristic and climatic composition and are associated with *Wollemia nobilis* should therefore be considered to represent an intrinsically higher risk to the New Zealand native environment than other imported nursery stock.

3.4.4 Association of Organisms of Negligible Risk

It is accepted in the scientific community that it is usual for plants to form associations with micro-organisms that are considered to be endophytes or saprobes (saprophytes). In the case of endophytes these organisms live symbiotically within the plant tissue and, in return for a safer environment and perhaps some nutrition, it is believed can in some circumstances provide limited protection to the plant from other disease-causing organisms. In some studies endophytes were found to be relatively host specific. Saprophytes live on or around the plant and survive on dead organic material. In contrast to endophytes, saprophytes are not usually host specific. While neither type of micro-organism are likely to cause diseases on plants, it is likely that the majority of disease-causing micro-organisms were at one stage saprophytes or endophytes as the mechanisms for plant invasion by these disease-causing micro-organisms are modified from those used by endophytes and saprophytes.

From a biosecurity risk-perspective therefore, these endophytic or saprophytic organisms are very unlikely to have any negative or unwanted impact on the New Zealand economy, environment or human health. While it is possible that a plant endophyte or saprophyte may act as a plant pathogen or parasite on an alternative plant host, there is little indication at this time that an event such as this would happen more than very rarely.

Currently the risk from these organisms is considered negligible, as no phytosanitary measures are taken to ensure imported nursery stock is free of them. Should the biosecurity risks from these types of organisms be considered non-negligible, the options for managing the risks are limited to either:

a) Importing axenic (sterile) nursery stock only, such as plants *in-vitro*; or

b) Testing and treating all plant material for these organisms either pre-export or during post-entry quarantine in New Zealand.

While importing only plants *in-vitro* is possible for some species of plants, many plant species have yet to be successfully placed into *in-vitro* culture, and the use of *in-vitro* culture techniques is more costly than normal nursery systems. It is also difficult to envisage how nursery stock could be effectively inspected or tested for the presence of such endophytic or saprophytic organisms, as these organisms do not cause symptoms on the host plant material. A number of blind tests would need to be performed to detect such organisms, and one would never know if the correct testing methods had been used and whether or if all of the infesting organisms had been detected. Crous and Groenewald (2005) stated that only an estimated 7% of the fungal species thought to exist are known. While tests may detect endophytic or saprophytic organisms, in the majority of cases we would not be able to identify, for example, the fungi to species level to allow any decisions to be made on the significance of the organism to New Zealand.

There is also no single treatment available to sterilise nursery stock *in-vivo* while maintaining plant viability.

The following principles should therefore be applied to the likely infestation of nursery stock by endophytic or saprophytic organisms:

- As a group, endophytic or saprophytic organisms associated with imported nursery stock represent a negligible risk to the New Zealand economy, environment or human health;

- Tests for organisms associated with plant nursery stock should be limited, where possible, to detecting hazard organisms that may potentially be associated with the pathway. So called "blind" tests that detect non-specific organisms should be avoided.

3.4.5 Association of latent or asymptomatic organisms of non-negligible risk

"Latent or asymptomatic organisms" are organisms that are capable of acting as a pest or causing diseases on one plant or group of plants, but can form an association with another plant or group of plants on which they do not act as a pest or cause a disease. For example *Botryosphaeria* species in Australia can act as both endophytes and stress-related pathogens of various woody hosts (Slippers *et al*., 2005). On an inspection-based phytosanitary system such as that currently in use for many organism types potentially associated with imported nursery stock, risks from latent or asymptomatic organisms are unlikely to be managed adequately. It is also significant that this group of latent or asymptomatic organisms includes organisms that have yet to spread outside their natural distribution and become associated with plant species or groups on which they would act as a significant pest or cause a significant disease.

From a risk management perspective latent or asymptomatic organisms pose a significant problem as their association with a plant in all likelihood is unknown, making their biosecurity risk unmeasured. The current risk analysis procedures provided in the International Standard for Phytosanitary Measures (ISPM) 11: *Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms*[14] focus on known organism and commodity or pathway associations, therefore not taking account of these other undetected risks.

The New Zealand standards for importing nursery stock, namely MAF standard 155.02.06[15], mandates as "basic" requirements treatments for insects and mites, and a period of inspection in post-entry quarantine. Assuming the mandatory treatment for insects is effective against the great majority of insect pests; this would seem to be an effective way of managing the unknown risks from latent or asymptomatic insects. The reliance on quarantine inspection for latent or asymptomatic micro-organisms, however, would seem to be an inadequate phytosanitary measure, as latent micro-organisms are by definition "latent or asymptomatic" and will not show the symptoms required for their detection.

Options for managing these latent or asymptomatic micro-organisms are much the same as those listed previously in section 3.4.4 for organisms of negligible risk, namely allowing the import of axenic (sterile) material only, or testing and/or treating all imported nursery stock. As with the organisms of negligible risk, neither of these options are favoured or possible at this time for all but a few examples. A third option is to require significant improvements to systems used to produce the plant nursery stock prior to it being exported to New Zealand. These improvements, such as requiring periods of plant stressing and improving plant hygiene management, would be designed to greatly reduce the likelihood of these latent or

14 Available at http://www.ipp.fao

15 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

asymptomatic organisms being associated with the imported nursery stock on its arrival in New Zealand. Nursery stock found to harbour organisms or families of organisms of potential non-negligible phytosanitary risk would then be treated or excluded from import into New Zealand based on the assumption that any such contamination indicates a failure in the pre-export production system.

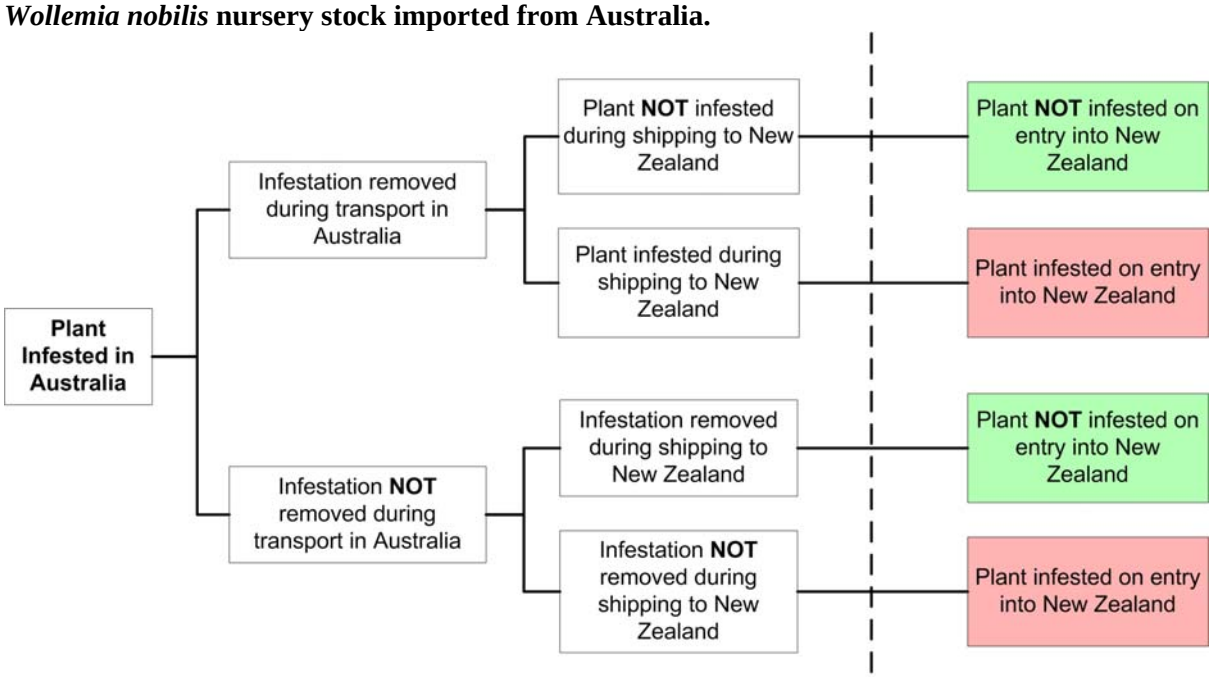
3.5 Risk Assessment of Hazards

The risk assessment for each hazard or hazard group is contained in chapters 4 to 12 of this risk analysis. The following sections describe aspects of the risk assessment of imported *Wollemia nobilis* nursery stock from Australia that are common to all of the identified hazards or hazard groups.

3.5.1 Assessment of Risk of Entry

Figure 3.2 below provides a simple overview of the risk attributes of the pathway for the entry of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.

Figure 3.2: Overview of the pathway for the entry of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.



In essence for the nursery stock being exported to New Zealand to be a biosecurity risk to New Zealand, hazard organisms would have to become associated with the nursery stock in Australia before production or during transport, and survive the journey to New Zealand.

3.5.2 Assessment of Risk of Exposure and Establishment

Exposure is considered the point where the contaminating organism becomes associated with a host in New Zealand in a manner that provides the contaminating organism with the opportunity to infest that host. Exposure in the context of imported nursery stock is complicated by the fact that the plant material on which the contaminating organism has been imported will itself become an established host if it is taken out of its packaging and planted into the New Zealand environment. Exposure is therefore guaranteed unless the imported nursery stock is:

a) not planted into the environment and the contaminating organism is unable to spread from the imported plant material into the environment;

b) planted into the environment and the contaminating organism is unable to infest another host;

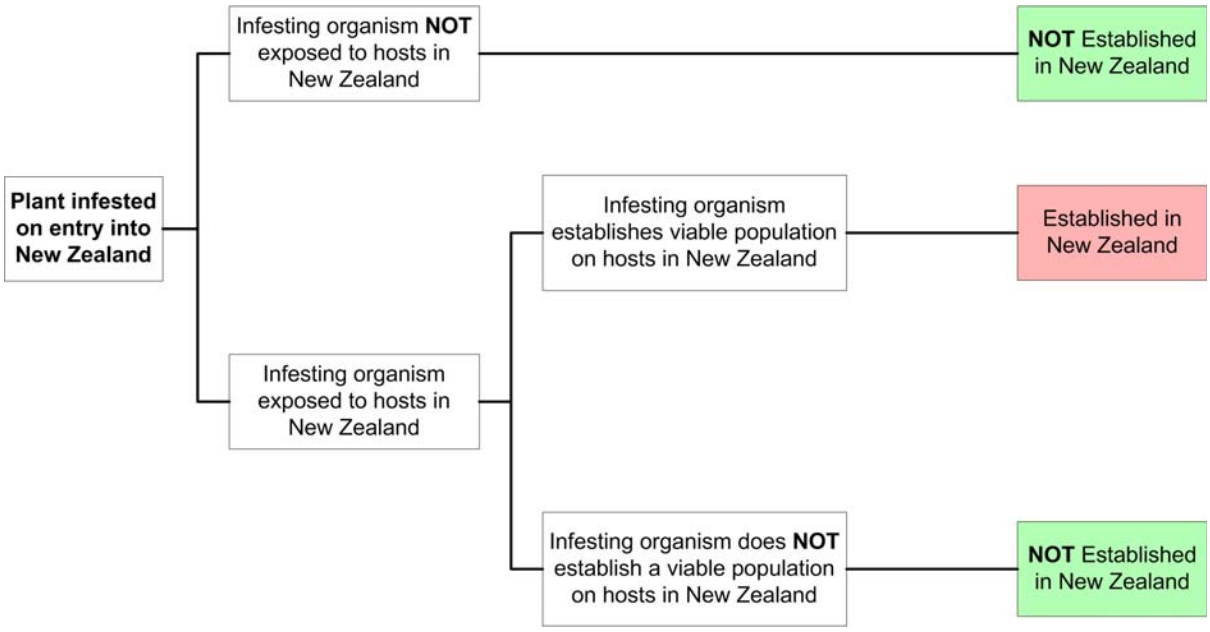
c) killed by the contaminating organism and appropriately disposed of before being moved into the environment;

d) decontaminated through treatment or unassisted necrosis of the contaminating organism.

Establishment is considered the point when the contaminating organism has established a viable population on hosts or host material in New Zealand such that the contaminating organism would be expected (if possible and at some stage in the future) to spread to its maximum possible distribution within New Zealand. As with exposure above, establishment in the context of imported nursery stock is complicated by the fact that the plant material on which the contaminating organism has been imported will itself become an established host if it is taken out of its packaging and planted into the New Zealand environment. This would then allow the contaminating organism to establish a viable population on the imported plant material in the New Zealand environment.

Figure 3.3 provides a simple overview of the risk attributes of the pathway from entry to exposure and establishment of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.

Figure 3.3: Overview of the pathway from entry to exposure and establishment of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.



Hazard organisms on *Wollemia nobilis* nursery stock that enters New Zealand from Australia would need to become exposed to a suitable host within the New Zealand environment and successfully establish a viable population on that host such that, over time, the organism could spread to its maximum possible distribution within New Zealand.

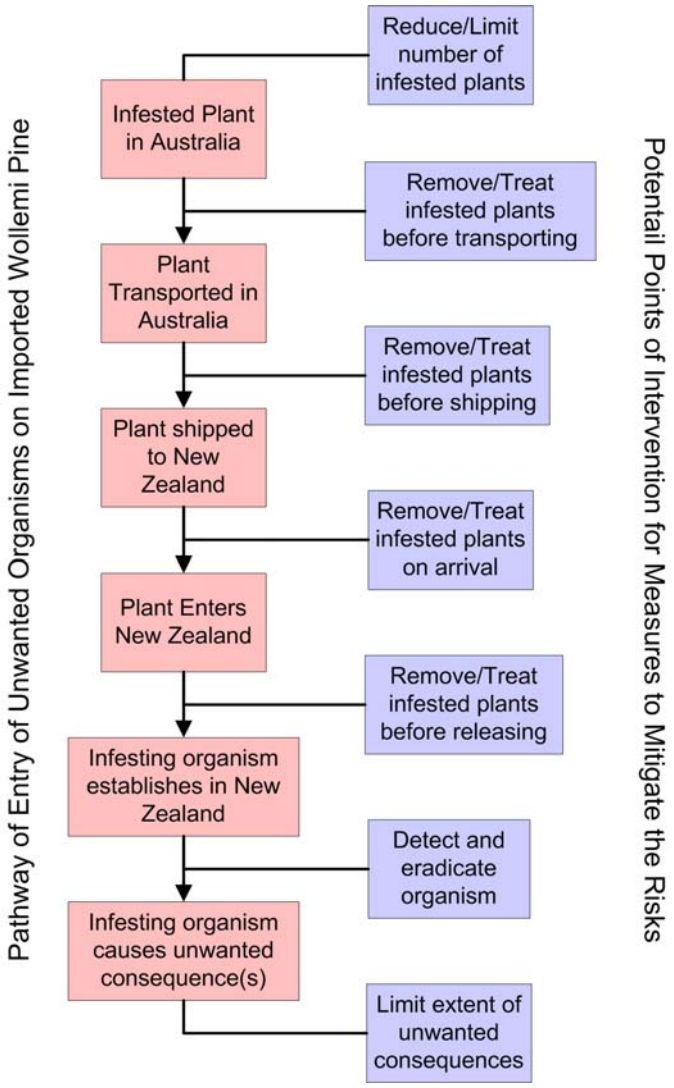
3.5.3 Assessment of consequences

An assessment of the potential consequences associated with the entry, exposure and establishment of each potential hazard or groups of hazards, and the estimate of the likelihood of such consequences occurring through the spread of the hazard, is provided within each of chapters 4 to 12 of this risk analysis.

3.6 Analysis of Measures to Mitigate Biosecurity Risks

Figure 3.4 below provides a simple overview of the potential points of intervention for risk mitigating measures on the pathway of entry, exposure and establishment of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.

Figure 3.4: Overview of the potential points of intervention for risk mitigating measures on the pathway of entry, exposure and establishment of hazard organisms associated with *Wollemia nobilis* nursery stock imported from Australia.



The following sections describe relevant attributes of the existing pathway in the context of their actions as potential risk mitigating options for the identified hazards.

3.6.1 Existing nursery-based pre-export conditions

A Nursery & Garden Industry Association in Australia[16] reportedly provides professional network, industry representation and business development initiatives for Australian growers, wholesalers, retailers and allied traders in the nursery industry. This association has established a set of standards and accreditation programmes to provide nursery businesses with a benchmark to become recognised providers of a standardised high level of quality. Through the accreditation scheme businesses and business practices are independently assessed to ensure they meet the required standards. The intention is for accreditation to provide customers and the industry with an assurance that the nursery business they are dealing with are committed to the highest quality business practices, consistency and

16 http://www.ngia.com.au

reliability in delivering service, professional standards and dedication to continuous improvement.

The Nursery & Garden Industry manages two business accreditation programs:

a) For nurseries and growing media suppliers - *Nursery Industry Accreditation Scheme Australia (NIASA)*

b) For garden centres - *Australian Garden Centre Accreditation Scheme (AGCAS)*

In addition, individuals who work in the nursery and garden industry can gain professional recognition through the *Certified Nursery Professional (CNP) Program* scheme.

For the purposes of this risk analysis, the Nursery Industry Accreditation Scheme Australia (NIASA) is the most applicable for potential use as a pre-export phytosanitary measure for imported *Wollemia nobilis* nursery stock. The currently implemented nursery-based propagation system in Australia for *Wollemia nobilis*, being overseen by the Queensland Government Department of Primary Industries (DPI) Forestry, is based on the NIASA system. A description of the DPI system was extracted from an official document titled "*Information on the propagation and production of Wollemi Pine plants for quarantine and export authorities*" (August 2005) and is provided in Appendix 2.

3.6.2 New Zealand's existing border clearance requirements

The New Zealand Ministry of Agriculture and Forestry, operating under the powers of the Biosecurity Act 1993, has in place an import health standard and clearance procedures that provides two main forms of general risk mitigation measures for nursery stock:

1. Basic conditions to be met by all imported nursery stock (from MAF Standard 155.02.06 (1 March 2005): *Importation of Nursery Stock*[17]);

2. Post Entry Quarantine for the great majority of imported nursery stock (from "PBC-NZ-TRA-PQCON: *Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator* (November 1999)[18]" and "PP42: *Inspection and Clearance of Plant Products held in Post-Entry Quarantine* (June 2003)[19]").

For reasons of ease of implementation it would be most ideal if these general risk mitigation measures were applied to the *Wollemia nobilis* nursery stock pathway. In consideration of this the general efficacy of the basic conditions provided in standard 155.02.06 and the applicable post-entry quarantine facility requirements provided in PBC-NZ-TRA-PQCON and PP42 have been reviewed in Appendix 3 and Appendix 4 respectively for consideration in this risk analyses.

17 http://www.biosecurity.govt.nz/files/imports/plants/standards/155-02-06.pdf
18 http://www.biosecurity.govt.nz/border/transitional-facilities/plants/pbc-nz-tra-pqcon.htm
19 This document is not available outside MAF.

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4. SURFACE FEEDING INVERTEBRATES

4.1 Hazard Identification

4.1.1 *Aetiologic agent*

A full list of surface feeding invertebrates recorded as being associated in Australia with plants in the Araucariaceae is provided in Appendix 1.

Examples of surface feeding insects that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known or can not be confirmed to be present in New Zealand include:

Agathiphaga queenslandensis	*Basiliorhinus araucariae*	*Eutactobius puellus*	*Notomacer zimmermani*
Aragomacer leai	*Bunyaeus eutactae*	*Neophyllaphis araucariae*	*Oxythrips agathidis*
Aragomacer uniformis	*Bunyaeus monteithi*	*Nipaecoccus* sp.	*Palophagus australiensis*
Basiliogeus prasinus	*Chrysomphalus dictyospermi*	*Notomacer eximius*	
Basiliogeus striatopunctatus	*Coniferococcus agathidis*	*Notomacer reginae*	

It should be emphasised that at the time of undertaking the risk analysis none of these species have necessarily been found on *Wollemia nobilis* nursery stock, and their inclusion in this analysis is as representative examples only.

4.1.2 *New Zealand Status*

There are many indigenous and introduced surface feeding insect species established in New Zealand. There are a great many more surface feeding insects existing overseas that have never established in New Zealand, a portion of which exist in Australia.

4.1.3 *Epidemiology*

The description of the epidemiology of surface feeding insects is based on examples from the following genera or species: *Chrysomphalus dictyospermi*, *Nipaecoccus sp. (viridis)*, and *Hyblaea puera*. Information on these organisms has been collated from the CABI Crop Protection Compendium 2006[20] with available or supplementary references provided. As these organisms are included to provide an indication of the possible biological nature of the risk posed by surface feeding insects, the epidemiological descriptions have been summarised to include only relevant and general organism characteristics.

***Chrysomphalus dictyospermi:* Scale**

Chrysomphalus dictyospermi is a highly polyphagous species having been recorded from hosts belonging to 73 plant families, but its host range is probably wider than this. Favoured hosts are citrus and other trees such as olives (*Olea europaea* subsp. *europaea*) and palms. *C. dictyospermi*, a sap sucking insect, preferentially feeds on leaves, but in heavy infestations it is sometimes found on fruit and occasionally on branches. In many countries all stages of the insect can be seen feeding throughout the year. *C. dictyospermi* was probably spread on

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

citrus planting material and fruit a long time ago, so there is no traceable history of its spread in the literature.

There are two immature, feeding stages in the female and four immature stages in the male; the last two of which (pre-pupa and pupa) are non-feeding and are spent beneath the scale cover secreted by the second-instar male. After moulting to adult, the male spends some time sitting beneath this scale while his flight muscles mature, before flying away to seek females.

The adult male is very short-lived because he cannot feed, while the adult female lives for several months and feeds throughout her life. In California, *C. dictyospermi* has three or four overlapping generations each year (Gill, 1997) while (Salama, 1970) recorded only two generations per year in Egypt. Reproduction is sexual in most *C. dictyospermi* populations. The adult male flies to locate the sessile adult female and the long genitalia are used to mate with the female beneath her scale cover. It is likely that the male locates an unmated female by smell, although details of the pheromone secretion mechanism are not known. However, both uniparental (parthenogenetic) and biparental (sexual) populations of this species have been recorded in the USA. Each female lays 1 to 200 eggs beneath her scale cover, where they are sheltered until they hatch and the first-instar crawlers disperse.

Crawlers, which are quite short-lived in the absence of a suitable feeding site, are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Dispersal of sessile adults and eggs occurs through human transport of infested plant material. *C. dictyospermi* requires warm temperatures and does not multiply much in cold weather. In Egypt, optimal conditions for *C. dictyospermi* were found to be 22 to 25°C, and mean relative humidity of 50 to 58% (Salama, 1970).

On citrus, heavy infestations of *C. dictyospermi* can cover the tree. The toxic saliva injected while feeding causes leaf chlorosis, and feeding by many scale insects causes drying and death of the branches. Infestation decreases plant growth and development and disfigures the fruit, reducing their market value. The species has been reported as a significant pest of citrus in a number of countries in the South Pacific region; it is also very destructive to rose trees (Williams and Watson, 1988).

***Hyblaea puera:* Moth**

Hyblaea puera, which has earned the common name 'teak defoliator' and is mainly a pest of teak plantations, has been recorded on approximately 45 host plants, including some shrubs. Most host plants belong to the families Bignonaceae and Lamiaceae, with some representatives from Verbenaceae, Rhizophoraceae, Oleaceae, Juglandaceae and Araliaceae. It is believed that during non-outbreak periods, small populations of the insect thrive on alternative host plants but data are not available on the periods of infestation or population levels on most other hosts. Outside the natural distribution range of teak, where *H. puera* outbreaks are not common, *H. puera* occurs on *Vitex parviflora* and *Tabebuia pentaphylla* in the Caribbean and on the straggling shrub (*Vitex trifolia*) in Australia. *H. puera* is widely distributed across the tropics and subtropics, covering Asia, Africa, Central America, the Caribbean, South America and Oceania including Australia.

The moth lays eggs on tender new leaves of teak or other host plants, attaching them singly near the veins, and usually on the lower surface. Each female lays approximately 500 eggs, with a recorded maximum of 1000. The pre-mating period of both sexes was 1 day and the

mean oviposition period was 7 days, with a mean fecundity of 434 eggs. *H. puera* has five larval instars; the first- and second-instars feeding mainly on the leaf surface and protecting themselves in a shallow depression on the leaf covered with strands of silk. Starting with the third-instar, the larva feeds from within leaf folds. The entire leaf, excluding the major veins, is eaten. Early instars cannot feed successfully on old, tough leaves. Under optimal conditions, the larval period lasts 10 to 12 days, but an average of 21 days has been recorded in the cooler climates.

Mature larvae usually descend from the tree crown to the ground on silk threads and pupate under a thin layer of leaf litter or soil, within a loosely built cocoon made of dry leaf pieces or soil particles held together with silk. During the rainy season, when the ground is wet, or in mangroves, pupation may occur within folded or juxtaposed green leaves of the host or non-host plants in the undergrowth. Under optimal conditions the average pupal period is 6 to 8 days, but it may extend to 20 to 25 days in cooler climates. There is no evidence of hibernation or aestivation of the pupae.

The development from first-instars to adult is completed in a minimum of 18 to 19 days and a maximum of 36 days. A new batch of eggs can be produced in approximately 2 days, thus giving a minimum generation time of 20 to 21 days together with the egg stage. In areas where there is a distinct winter season with chances of occasional frost, the number of generations is reduced to ten, with a partial eleventh. Here the moths are believed to hibernate for a period of approximately 3 months over the coldest period, but no details of the hibernation behaviour or the places of hibernation have been reported.

***Nipaecoccus* sp.*(viridis):* Mealybug (pseudococcid)**

The true mealybugs are the second most injurious family of Coccoidea. Most of these sap sucking insects feed on aerial plant parts but some occur on roots or under bark. About 2000 species have been described. They are characterized by a covering of white, mealy wax over the body of the sessile stages. The ephemeral males are up to 3 mm long; females are slightly longer and are sessile unless disturbed; they live for several months. Eggs may be laid under white woolly wax secreted from the female's abdomen. Crawlers of a few species have been shown to be inefficient virus vectors.

Mature females lay eggs that hatch one to two months later. The crawlers migrate and settle mainly in protected areas, under the sepals of the fruitlets when they are pea-sized or larger. Adults often settle in cryptic places on plant material, such as under sepals of citrus fruits, and can easily be distributed on exported plants or plant products (Hattingh *et al*., 1998).

Hattingh *et al*. (1998) described and illustrated the effect of *Nipaecoccus viridis* on citrus in South Africa. Feeding on young twigs causes bulbous outgrowths, and heavy infestations may severely stunt the growth of young trees. Occasionally, this mealybug becomes so abundant on citrus that the branches and leaves become covered with white cottony threads. Also, the leaves and other parts of the tree become shining wet with honeydew which becomes a substrate for sooty mould growth. Citrus fruits infested with *N. viridis* may develop lumpy outgrowths or raised shoulders near the stem end. Such swellings are already present on fruit from the size of a pea, and they enlarge with the growth of the fruit. Frequently, fruits turn yellow and then partly black around the stem end, finally dropping off the tree. Late infestations on large green fruits result in congregations of young mealybugs in

clumps over the face of the fruit. Each colony produces a raised spot which turns yellow. Whole plants can undergo distortion and resetting in response to a heavy infestation.

Summary of hazard assessment

Crawlers of *Chrysomphalus dictyospermi*, which are quite short-lived in the absence of a suitable feeding site, are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Dispersal of sessile adults and eggs occurs through human transport of infested plant material. *C. dictyospermi* requires warm temperatures and does not multiply much in cold weather. The toxic saliva injected while feeding causes leaf chlorosis, and feeding by many scale insects causes drying and death of the branches.

Hyblaea puera, while mainly being a pest of teak plantations, has been recorded on approximately 45 host plants, including some shrubs. The first- and second-instars feed mainly on the leaf surface and protect themselves in a shallow depression on the leaf covered with strands of silk. Starting with the third-instar, the larva feeds from within leaf folds.

Nipaecoccus sp. *(viridis)* can feed on young twigs causing bulbous outgrowths, and heavy infestations may severely stunt the growth of young trees. Individuals often settle in cryptic places on plant material, such as under sepals of citrus fruits, and can easily be distributed on exported plants or plant products.

4.1.4 *Hazard Identification Conclusion*

From the information above it can be seen that surface feeding insects could become associated with *Wollemia nobilis* nursery stock in a manner that could allow for their entry and establishment in New Zealand, and potentially result in unwanted impacts. Surface feeding insects are therefore considered a potential hazard requiring further assessment.

4.2 Risk Assessment

4.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. A whole plant or root-less cutting infested with a surface feeding insect at a less vulnerable life stage such as eggs or larvae should be considered highly likely to survive the packaging and transport to New Zealand as conditions that would effect their survival would also be detrimental to the whole plant or root-less cutting.

It is not considered possible that surface feeding insects would infest *Wollemia nobilis* plants *in vitro*.

4.2.2 *Conclusion of Entry Assessment*

The likelihood of a surface feeding insect entering New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* is high and should be consider non-negligible.

The likelihood of a surface feeding insect entering New Zealand on plants *in vitro* is considered negligible and the risks from this commodity will not be considered further in this assessment.

4.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

When considering limitations on the ability of a surface feeding insect to move from the infested imported plant to a host on which it can establish, it should be considered unlikely that a surface feeding insect will kill the imported plant directly before it would spread to another host. As the intention of any importer would be to plant the imported infested plant into the New Zealand environment, it should be considered highly likely that surface feeding insects on an infested plant would be placed in close proximity to host plants in the New Zealand environment. It is, however, possible that due to climate limitations the infesting insect will not be able to develop to a stage that allows spread from the infested material. It is also possible that the emergent adults of species with a more limited host range will be unable to find an established host before mortality.

4.2.4 *Establishment Assessment*

Establishment in New Zealand of surface feeding insects imported on *Wollemia nobilis* whole plants or root-less cuttings should be considered to be limited only by the ability of an infestation to survive and produce life stages (e.g. crawlers) that themselves infest other hosts. For some species of surface feeding insects their ability to produce further life stages may be limited in New Zealand's colder climate. It is expected, however, that *Wollemia nobilis* whole plants or root-less cuttings exported to New Zealand may originate in areas of Australia that have similar climatic conditions to New Zealand and therefore potentially be infested by organisms adapted to the New Zealand climate.

4.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that any surface feeding insect entering New Zealand within *Wollemia nobilis* whole plants or root-less cuttings would have a high likelihood of developing motile life stages and establishing a viable population in New Zealand, the likelihood of exposure and establishment should be considered non-negligible.

4.2.6 *Consequence Assessment*

In the context of the pathway for importing nursery stock contaminated with surface feeding insects, any potential consequences to people, the New Zealand environment, and the New Zealand economy will only become apparent after establishment and some degree of spread.

Potential for spread

Surface feeding insects can be highly polyphagous, greatly increasing their ability to find hosts in natural environments. In the case of the scale example used, *Chrysomphalus dictyospermi*, crawlers, which are quite short-lived in the absence of a suitable feeding site, are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Dispersal of sessile adults and eggs can occur through human transport of infested plant material. Assuming the surface feeding insect first establishes in a part of New Zealand with a suitable climate, the likelihood of spread should be considered high.

Likely consequences

Surface feeding insects can act as significant pests on important horticultural plant species, and can be highly polyphagous. Infestations can reduce or severely stunt plant growth, development and productivity, and disfigure fruit resulting in a significant reduction in their market value. The effects of such infestations on endemic plant species could result in environmental stress and decline.

4.2.7 *Conclusion of Consequence Assessment*

The establishment and spread in New Zealand of a surface feeding insect imported on *Wollemia nobilis* whole plants or root-less cuttings has the potential to cause significant (low to high) unwanted consequences to the economy and the environment. The potential consequence of the establishment of surface feeding insects imported on *Wollemia nobilis* whole plants or root-less cuttings in New Zealand should therefore be considered non-negligible.

4.2.8 *Risk Estimation*

The likelihood is high that *Wollemia nobilis* whole plants or root-less cuttings would become infested with a surface feeding insect while growing in Australia, be transported to and enter into New Zealand still infested with the insect, and form an established population of the surface feeding insect once the whole plant or root-less cutting is grown in the New Zealand environment. There is also a high likelihood that, given sufficient time, the contaminating insect will spread throughout New Zealand wherever hosts are growing. There is a moderate likelihood that resulting from the spread of these surface feeding insects the unwanted consequences to the environment and to the economy will be low to high. As a result the risk estimate for surface feeding insect associated with *Wollemia nobilis* whole plants or root-less cuttings imported from Australia is non-negligible and it is considered a hazard.

4.2.9 *Assessment of Uncertainty*

As the subject of this assessment is a group of insects that could potentially have a very wide variety of biological and epidemiological characteristics. The assessment is further complicated by the fact that no members of this insect group have been recorded as associated with the commodity in question, namely *Wollemia nobilis* nursery stock from Australia. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative insect species included in the assessment. It is possible however, that should an actual surface feeding insect be recorded as being associated with *Wollemia nobilis*

nursery stock, the results of this assessment would have significantly under or over estimated the level of risk posed by the organism.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

4.3 Risk Management

4.3.1 *Risk Evaluation*

Since the risk estimate for surface feeding insects associated with *Wollemia nobilis* whole plants or root-less cuttings imported from Australia is non-negligible, phytosanitary measures will need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for surface feeding insects associated with *Wollemia nobilis* plants *in vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

4.3.2 *Option Evaluation*

4.3.2.1 *Risk Management Objective*

To ensure that no surface feeding insects associated with *Wollemia nobilis* nursery stock in Australia are:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

4.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of surface feeding insects establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of surface feeding insects;

b) Inspecting all *Wollemia nobilis* whole plants or root-less cuttings before packaging and transport to New Zealand to ensure they are free of surface feeding insects;

c) Treating all imported *Wollemia nobilis* whole plants or root-less cuttings before release into the New Zealand environment to ensure they are free of surface feeding insects;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* whole plants or root-less cuttings are released into the New Zealand environment.

Inspection

Options b) and d) above both rely on an effective inspection process to identify infested plants or cuttings. The three most important variables of any inspection process are (see Appendix 3 section App 3.2 for further details):

i) how detectable the organism or infestation is during visual inspection;

ii) the ability of the inspector to detect the organism or infestation; and

iii) the level of infestation of the consignment that is considered acceptable (the detection level that is required).

Dealing with the latter first (the level of infestation of the consignment that is considered acceptable), in the case of *Chrysomphalus dictyospermi* and *Nipaecoccus* sp. the potential for parthenogenesis and the high likelihood of establishment and spread in warmer areas or enclosures indicates that a single female or cluster of eggs on a single plant should pose an unacceptable risk. *Hyblaea puera* is less likely to establish in New Zealand and as such a higher tolerance level could be accepted. Any inspection undertaken would therefore need to have a 95% confidence level of detecting a small group (3 or less) of larvae or adults or a single egg group on a single plant within the consignment.

The second inspection variable, the ability to detect the organism or infestation, is influenced by the distribution and physical characteristics of the larvae, adults or a single egg group on the plant. For *Chrysomphalus dictyospermi*, a close examination is required of the leaves of the plants in strong light, looking for circular, flat, greyish or reddish-brown scale covers. For *Hyblaea puera*, each female moth lays approximately 500 eggs on tender new leaves of teak or other host plants, attaching them singly near the veins, and usually on the lower surface. The first- and second-instars feed mainly on the leaf surface and protect themselves in a shallow depression on the leaf covered with strands of silk. Starting with the third-instar, the larva feeds from within leaf folds. Low-level infestations can be detected by the presence of leaf-folds within which the larvae can be seen when the fold is partially eaten. In both examples, a person inspecting large volumes of material in a relatively short time would find detecting low-level infestations difficult.

A single inspection would therefore not provide sufficient confidence that the level of consignment infestation by surface feeding insects is acceptable. Extending the inspection period by holding the plants within post-entry quarantine in New Zealand may increase the size of the infestation as the insects complete a generation or two. *Chrysomphalus dictyospermi* can complete 6 generations per year (2 months per generation) whereas mealybugs may take up to 3 months for egg hatching. As a three month inspection period may not be sufficient to allow a single generation of a pseudococcid (mealybug) to develop,

at least six months of inspections would be required in appropriate climatic conditions (e.g. mild or warmer conditions (15^{O}C minimum)). It is also possible that a single generation may not produce any more than a few extra surviving individuals thereby not improving inspection efficacy to any significant degree.

The final inspection variable, the ability of the inspector to detect the organism or infestation (inspector proficiency), would be strongly influenced by the frequency at which the inspectors undertake these types of inspections and encounter similar types of infestations. Inspectors acting pre-export and in post-entry quarantine in New Zealand would be considered to have a high level of inspection proficiency for these types of detections.

Without specific inspection efficacy information the level of inspection efficacy can not be determined accurately. While it can be assumed that the efficacy level would be high, given the likelihood that only a low number of infestations would occur within a single plant it should be considered likely that the level of inspection efficacy would be less than the desired level of detection confidence. Unless the actual level of inspection efficacy can be determined and shown to be greater than the desired level of confidence, this measure should not be considered sufficiently efficacious to act as the only mitigation against the risk of surface feeding insects.

Treatment

Based on the review of insecticide treatments provided in Appendix 3, the following chemical and fumigation treatments are considered effective against all life stages likely to be found in infestations of surface feeding insects.

a) Methyl bromide fumigation

The following methyl bromide treatment schedule (table 4.1) has been derived from schedules for surface insects provided in the FAO Manual of Fumigation Control and the USDA Treatment Manual (see Appendix 3, table App 3.2). The listed C/T values are the highest levels provided in table App 3.2 for surface feeding insects (e.g. the USDA treatment for mealybugs etc) and have been extrapolated across all temperatures. The actual level of efficacy of this treatment against all but a few insect species has yet to be determined with any accuracy.

Table 4.1: Methyl bromide fumigation schedule for surface feeding insect infestations (foliated dormant plants under atmospheric conditions).

Rate (g/m^3)	Temperature (^{O}C)	Treatment Duration (hours)	C/T Value (g h/m^3)
64 g/m^3	4 to 10^{O}C	3	114
64 g/m^3	11 to 15^{O}C	2.5	102
64 g/m^3	16 to 20^{O}C	2	90
48 g/m^3	21 to 25^{O}C	2	76
40 g/m^3	26 to 29^{O}C	2	56
32 g/m^3	30 to 32^{O}C	2.5	48

At the time of completing this analysis the level of phytotoxicity of methyl bromide against *Wollemia nobilis* nursery stock was unknown. Care should be taken to ensure phytotoxicity levels are acceptable before applying any chemical treatments to plant material.

While this methyl bromide treatment is considered suitably efficacious, New Zealand's commitment to the Montréal Protocol necessitates that we minimise the use of substances that damage the ozone layer where possible. Methyl bromide is considered an ozone-damaging chemical and in line with New Zealand's protocol commitments methyl bromide treatments will not be accepted where alternative treatments are available.

b) Chemical treatments

The following chemical pesticides (table 4.2), when used as dips, are considered in Appendix 3 to be effective against infestations of surface feeding insects. The actual level of efficacy of this treatment against all but a few insect species has yet to be determined with any accuracy.

Table 4.2: Approved insecticide treatments for surface feeding insects.

Active ingredient (Chemical Group)	Treatment Specification[21]	Residue Persistence[22]
Acephate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 8 g a.i. per litre of dip. Treatment of non-dormant plant material only.	3 days (aerobic)
Carbaryl (Carbamate)	Dip at room temperature for 2-5 minutes at 18 g a.i. per litre of dip.	6 days (aerobic)
Chlorpyrifos (Organophosphorous)	Dip at room temperature for 2-5 minutes at 10 g a.i. per litre of dip. A non-ionic surfactant is required for dipping	113 days* (aerobic)
Dimethoate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 1.1 g a.i. per litre of dip. Treatment of non-dormant plant material only.	2 days (aerobic)
Imidacloprid (Neonicotinoid)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip. Treatment of non-dormant plant material only.	997 days* (aerobic)
Spinosad (Spinosyns)	Dip at room temperature for 2-5 minutes at 2 g a.i. per litre of dip.	17 days (aerobic)
Tebufenozide (Diacylhydrazine)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip.	405 days* (aerobic)

* It is likely that these periods of residue persistence are not reflected as equivalent periods of residual activity on the treated plants. These "worse case" figures have been listed in the absence of information on the actual period of residual activity *in-vivo*.

A combination of pesticides from two different chemical groups (organophosphorous, carbamate, neonicotinoid, spinosyns, or diacylhydrazine) should be applied to maximize the efficacy and mitigate potential issues with insect resistance. The consignment of whole plants or root-less cuttings should be spray treated 10-14 days after the initial treatment to manage any potentially surviving insects or insect life stages that the treatments may have failed to adequately treat. As mentioned above, care should be taken to ensure phytotoxicity levels are acceptable before applying any chemical treatments to plant material.

Additional measures

As the actual level of efficacy for these treatments has yet to be determined with any accuracy, the additional measures should be taken to ensure the conditions of treatment

21 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.

22 Based on the aerobic soil half life (where provided, hydrolysis or anaerobic half life where not provided) provided by the PAN Pesticides Database at http://www.pesticideinfo.org

application are maximised as far as possible, and failures in treatment application are detected and managed. Pre-treatment inspections of the whole plants or root-less cuttings should be undertaken to ensure that the likely level of plant contamination by surface feeding insects at the time of treatment is low, thereby enhancing the overall treatment efficacy. Post-treatment inspections should be undertaken to detect any populations of surface feeding insects that manage to survive the treatment, or infest the whole plants or root-less cuttings after the treatment has been completed.

a) Pre-export and shipment inspections

Pre-export and shipment inspections should be undertaken to ensure the level of likely contamination is minimized before treatment. The following specifications for pre-export and shipment inspection should therefore be required:

- To improve the likelihood that any infesting surface feeding insects will develop a visible life stage or symptoms during pre-export inspections, it is recommended that a minimum pre-export inspection of one growing season be undertaken, in mild daytime temperatures (above 15^{o}C)[23] and varying day length and temperature;

- To ensure pesticides do not mask symptom expression and significantly reduce the effectiveness of pre-export inspections, it is recommended that only pesticide treatments approved by New Zealand Ministry of Agriculture and Forestry should be applied;

- To reduce the likelihood of exported plants being contaminated by surface feeding insects, it is recommended that plant stock is continually (regularly) inspected for pest and disease attack by qualified nursery staff;

- To reduce the likelihood of exported plants being contaminated by surface feeding insects, it is recommended that mother-stock plants should be monitored continually (regularly) for pests and treated as appropriate with plant protection chemicals;

- To ensure the contamination levels of any exported plants are minimal prior to treatment, each plant in the consignment should be inspected by an appropriately trained person for evidence of surface feeding insect infestation.

Any plants showing evidence of surface feeding insect infestation should not be included in the consignment for treatment either prior to or just after export to New Zealand. If more than 5% of the plants to be treated are found to have evidence of surface feeding insect infestation, the entire consignment should be rejected for treatment until further treatments have been applied and subsequent inspections indicate the infestation rate has dropped below the 5% threshold.

The level of protection provided by these pre-export and shipment measures would be affected by the following attributes of surface feeding insects:

- *Infestation within the nursery:* many of the surface feeding insects spread easily through air-borne life stages. It is unlikely that a nursery in Australia could provide

23 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15^{O}C on any one day.

sufficient protection from surface feeding insect infestation given the extent of pest pressure within the surrounding environment.

- *Delay in symptom expression:* visually detectable symptoms or life stages may not become apparent for an extended period after infestation.

- *Post inspection infestation:* plants may become infested with surface feeding insects after the inspections have been completed but before the plants are treated.

These attributes of surface feeding insects suggest that these measures alone (without treatment) would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants and root-less cuttings from Australia would not vector surface feeding insects into New Zealand.

b) Post-entry quarantine inspection

Post-treatment (post-entry quarantine) inspections should be undertaken to detect any populations of surface feeding insects that have managed to survive the treatment or have infested the whole plants or root-less cuttings after treatment has been completed. As this inspection will be to determine the existence of live infestations only, inspections will need to be for evidence of live surface feeding insects. The following specifications for post-treatment inspection should therefore be required, based in part on the review of the current post-entry quarantine requirements provided in Appendix 4:

- The post-entry quarantine inspections should be completed in Level 2 post-entry quarantine facilities with the following added requirements:

  - A period of quarantine for each consignment of *Wollemia nobilis* whole plants or root-less cuttings should be one growing season, in conditions suitable for disease expression e.g. mild daytime temperatures (above 15^{o}C)[24] and varying day length and temperature. Temperature and day length variations should simulate as far as possible natural temperate-climate seasonal variations. The quarantine period shall commence after the listed half-life period of any applied pesticides has passed;

  - An inspection of all plants in the consignment for evidence of a live surface feeding insect infestation at the conclusion of the post-entry quarantine period;

  - Treatment (or removal or destruction) of the consignment if a live surface feeding insect infestation is found that indicates a failure in the previous measures. Where possible the reason for treatment failure should also be determined and steps taken to ensure such failures are unlikely to re-occur.

The level of protection provided by this post-entry measure would be affected by the following attributes of surface feeding insects:

- *Escape from the post-entry quarantine facility:* many surface feeding insects spread easily through air-borne life stages. The current containment requirements in Level 2

24 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15^{O}C on any one day.

post-entry quarantine facilities as specified in MAF standard 155.02.06 should be considered adequate to ensure air-borne life stages of the majority of surface feeding insect species do not escape from the facility.

- *Delay in symptom expression:* visually detectable symptoms or life stages may not become apparent for a period that exceeds the duration of post-entry quarantine or requires environmental conditions not provided in post-entry quarantine.

- *Masking of symptom expression by other insects:* The current containment requirements in Level 2 post-entry quarantine facilities as specified in MAF standard 155.02.06 should be considered adequate to ensure air-borne life stages of the majority of surface feeding insect species do not enter the facility from the New Zealand environment.

It should be considered that the risks associated with the delay in symptom expression would be minimised for plants growing in an environment that is optimal for symptom expression. Optimal symptom expression would, however, considerably enhance the likelihood of insect escape from the facility.

4.3.2.3 *Recommended Management Options*

It is recommended that the following three measures are applied to reduce, to an acceptable level, the risk of surface feeding insects establishing in New Zealand and causing unwanted consequences.

i) One growing season of pre-export inspections before treatment and/or export to New Zealand, in conditions suitable for disease expression e.g. mild daytime temperatures (above 15^{o}C)[24] and varying day length and temperature.

ii) A pesticide treatment as close to export as possible (with appropriate re-infestation management in place) or as soon as possible after arrival in New Zealand. A second pesticide treatment should be applied as a spray 10 to 14 days after the first.

iii) One growing season in a Level 2 post-entry quarantine facility, in conditions suitable for disease expression e.g. mild daytime temperatures (above 15^{o}C)[25] and varying day length and temperature and that includes an inspection of all plant material before biosecurity clearance.

4.4 Assessment of Residual Risk

4.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure the proportion of infested whole plants or root-less cuttings within any consignment of *Wollemia nobilis* nursery stock treated prior to or just after export to New Zealand is less than 5%.

25 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15ºC on any one day.

The objective for the pesticide treatments is to ensure no live surface feeding insect life stages enter the post-entry quarantine facility on *Wollemia nobilis* whole plants or root-less cuttings imported from Australia.

The objective for inspection during post-entry quarantine is to have a 95% level of confidence that all treatment failures will be detected.

4.4.2 *Expected Performance of Measure(s)*

The pre-export nursery management measure is expected to ensure no more than 5% of the consignment is infested with surface feeding insects prior to treatment just before or after export to New Zealand. Inspection of the imported plants in post-entry quarantine should not therefore detect surface feeding insect infestation levels above 5%.

Treatment requirements should ensure the no *Wollemia nobilis* nursery stock is infested by live surface feeding insect life stages. Therefore no live surface feeding insects should be found on inspection of the imported whole plants or root-less cuttings in post-entry quarantine.

The post-entry quarantine inspection should detect, with a 95% level of confidence, all treatment failures. A failure in this post-entry quarantine measure will only be detected in a new or unwanted surface feeding insect establishes in New Zealand from *Wollemia nobilis* nursery stock imported from Australia.

It is expected that a combination of these three measures will ensure that the number of surface feeding insects establishing in New Zealand from this pathway, and causing unacceptable consequences to the New Zealand economy or environment, should be considered acceptable.

4.5 References

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5. WOOD BORING INSECTS

5.1 Hazard Identification

5.1.1 *Aetiologic agent*

A full list of wood boring insects recorded as being associated in Australia with plants in the Araucariaceae is provided in Appendix 1.

Examples of wood boring insects that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known or can not be confirmed to be present in New Zealand include:

Aesiotes notabilis	*Euwallacea barbatus*	*Pachycotes minor*	*Treptoplatypus australis*
Araucariana queenslandica	*Euwallacea destruens*	*Pachycotes* sp.	*Tyrtaeosus microthorax*
Coptocorynus araucariae	*Hyleops glabratus*	*Platypus froggatti*	*Xenocnema* sp
Coptocorynus sp	*Hylurdrectonus corticinus*	*Platypus omnivorus*	*Xyleborus affinis*
Dihammus australis	*Hylurdrectonus pinarius*	*Platypus queenslandi*	*Xyleborus emarginatus*
Diotimana undulata	*Hylurdrectonus* sp	*Platypus semigranosus*	*Xyleborus perforans*
Dysthaeta anomala	*Ilacuris laticollis*	*Platypus subgranosus*	*Xyleborus similis*
Euplatypus parallelus	*Mallus costatus*	*Prospheres aurantiopictus*	*Xylosandrus pseudosolidus*
Eurhamphus fasciculatus	*Mitrastethus australiae*	*Strongylurus decoratus*	
Euthyrrhinus meditabundus	*Orthorhinus cylindrirostris*	*Strongylurus* sp	

It should be emphasised that at the time of undertaking the risk analysis none of these insects have been found on *Wollemia nobilis* nursery stock, and their inclusion in this analysis is as representative examples only.

5.1.2 *New Zealand Status*

There are a number of indigenous and introduced wood boring insect species established in New Zealand. There are a great many more wood boring insects existing overseas that have never established in New Zealand, a portion of which exist in Australia.

5.1.3 *Epidemiology*

The description of the epidemiology of wood boring insects is based on examples from the following genera: *Platypus, Xyleborus,* and *Coptotermes*. Information on these organisms has been collated from the CABI Crop Protection Compendium 2006[26] with available or supplementary references provided. As these organisms are included to provide an indication of the possible biological nature of the risk posed by wood boring insects, the epidemiological descriptions have been summarised to include only relevant and general organism characteristics.

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

***Coptotermes*: Termite**

Coptotermes are wood-feeding termites that can attack both living and dead wood. The genus is notorious for its habit of colonizing living trees and hollowing out the heartwood to the extent that the trunk can be 'piped' and replaced with nest material and soil, without the tree showing external signs of their presence. Due to their feeding habits they are unlikely to colonise young trees or saplings.

Coptotermes colonies can produce functional replacement reproductives (called neotenics) and thus rapidly exploit available food resources by establishing satellite colonies. This ability also allows fragmented colonies to thrive in the absence of the primary founding queen, and this is seen as possibly the most important factor in the success of *Coptotermes* in colonization of new areas when introduced by the activities of man (Lenz *et al*., 1986). *C. formosanus* is now established in many countries and has become a serious pest.

***Platypus (quercivorus)*: Ambrosia beetles**

The genus *Platypus* is exceptionally large within the Platypodidae, with several hundred recognized species. These are distributed throughout the world's temperate and tropical forests, and attack both broadleaf and coniferous trees.

Members of the genus *Platypus* are ambrosia beetles and breed in the wood of host trees. A unique feature of many species of *Platypus* (and its close relatives) are the white splinters produced by the adults however the larger larvae, when they start extending the nest system, produce granular frass that is much like fine frass produced by non-platypodine ambrosia beetles. Unlike other ambrosia beetles, the galleries of *Platypus* spp. often penetrate into the heartwood. The larvae and adults feed on ambrosia fungi, which are stored and disseminated by the adult female. The fungal associates of many species of *Platypus* are members of the genus *Raffaelea* and *Ophiostoma*, species of which can act as plant pathogens.

Male *P. quercivorus* initiate the attacks on the boles of host trees (mainly Fagaceae and especially *Quercus* species) and excavate galleries for mating from June to October (Soné *et al*., 2000). A single female joins the male and constructs the oviposition gallery after mating. This is kept clean by the male who expels the residues to the outside of the tree. During gallery construction, the females inoculate the gallery surface with spores of the ambrosia fungus, which the larvae feed on. The adult females begin to deposit eggs at the terminal parts of the tunnels, 2 to 3 weeks after gallery construction begins. An average of 50 to 60 larvae develop in a single gallery system but the number of larvae can be as high as 161. The larvae feed on the ambrosia fungus that develops on the walls of the galleries and may over-winter in pupal chambers. Pupation occurs in the larval galleries and emergence is through entry holes made by the parents (Soné *et al*., 1998).

The external symptoms of infestation include copious amounts of white, splinter-like boring dust near the base of infested trees, and late summer wilting of the foliage.

Xyleborus (perforans)

Members of *Xyleborus* and the related genera *Ambrosiodmus, Euwallacea, Xyleborinus and Xylosandrus* are all ambrosia beetles that feed and breed in a variety of forest trees and shrubs under a considerable range of climatic conditions. Depending on the species, they may be found in plant material from small branches and seedlings to large logs. All are potentially damaging to agriculture and/or forestry under suitable conditions. Many species, previously considered of only minor importance, may become important pests in agriculture and forestry as a result of the continuing destruction of natural forests and the expansion of forest and tree crop plantations, agroforestry and agriculture.

In the example of *X. perforans*, living trees are normally only attacked through injuries or diseased areas. It is not very size-selective, attacking stems from about 5 centimetres (2 inches) in diameter to the largest logs, but is not found in small shoots and twigs. The gallery system consists of branching tunnels, without enlargements, and penetrates deeply into the wood. The parent female and the larvae feed on the ambrosia fungus growing on the walls of the galleries. In most conditions a generation would be expected to take 4-6 weeks.

Adult females fly readily and flight is one the main means of movement and dispersal to previously uninfected areas. The attacks result in numerous pinholes in the wood and fungal staining around them, and can render the timber unusable for furniture or veneer. Visual inspection of suspected infested material is required to detect the presence of ambrosia beetles. Infestations are most easily detected by the presence of entry holes made by the attacking beetles, and the presence of frass produced during gallery construction.

Species in *Xyleborus* and related genera are inbreeding, with the males generally mating with their sisters within the parental gallery system before dispersal. Thus the introduction of only a few mated females may lead to the establishment of an active population if suitable host plants can be found and environmental conditions are satisfactory. A very wide range of host plants have been recorded for many species of *Xyleborus* and related genera. Any woody material of suitable moisture content and density may be all that is required.

Summary of epidemiology

Coptotermes colonies can produce functional replacement reproductives (called neotenics) and thus rapidly exploit available food resources by establishing satellite colonies. This ability also allows fragmented colonies to thrive in the absence of the primary founding queen, and this is seen as possibly the most important factor in the success of *Coptotermes* in colonization of new areas when introduced by the activities of man. However, due to their feeding habits *Coptotermes* are highly unlikely to colonise young trees or saplings.

Members of the genus *Platypus* are ambrosia beetles and breed in the wood of host trees, producing white splinters and fine sawdust (frass) during gallery construction. The larvae and adults feed on ambrosia fungi, which must be stored and disseminated by the adult females for colony establishment. It is possible, though less likely, that *Platypus* ambrosia beetles could infest trucks of large seedlings or small trees.

Members of *Xyleborus* and the related genera *Ambrosiodmus, Euwallacea, Xyleborinus and Xylosandrus* are all ambrosia beetles that feed and breed in a variety of forest trees and shrubs under a considerable range of climatic conditions. Depending on the species, they may be

found in plant material from small branches and seedlings to large logs (greater than 5 centimetres (2 inches) stem diameter). All are potentially damaging to agriculture and/or forestry under suitable conditions.

5.1.4 *Hazard Identification Conclusion*

Based on the wood boring insects considered as potential hazards it is considered highly unlikely that any would be found infesting seedlings or cuttings of the size likely to be imported for propagation. It is possible however, that these wood boring insects could establish in New Zealand from larger (greater than 5 centimetres (2 inches) stem diameter) specimens of imported *Wollemia nobilis* nursery stock and cause an unwanted impact within New Zealand. Wood boring insects are therefore considered a potential hazard requiring further assessment.

5.2 Risk Assessment

5.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. A whole plant or root-less cutting infested with a wood boring insect should be considered highly likely to survive the packaging and transport to New Zealand as conditions that would effect their survival would also be detrimental to the whole plant or root-less cutting. It is very unlikely that a wood boring insect would infest a small plant or cutting as they prefer larger diameter stems and wood. Infestation of larger plants (greater than 5 centimetres (2 inches) stem diameter) should also be considered low if the plants are healthy and showing vigorous growth.

It is not considered possible that wood boring insects would infest *Wollemia nobilis* plants *in vitro*.

5.2.2 *Conclusion of Entry Assessment*

While it should be considered highly likely that an infestation of a wood bring insect within *Wollemia nobilis* whole plants or root-less cuttings would enter New Zealand, the likelihood that stems of diameters less than 5 centimetres (2 inches) becoming infested is very low and increasing to low for stems of diameters greater than 5 centimetres (2 inches). The likelihood of a wood boring insect will enter New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* originating from Australia should therefore be considered negligible for material with maximum stem diameters of less than 5 centimetres (2 inches) and non-negligible for material with maximum stem diameters of greater than 5 centimetres (2 inches).

The likelihood of a wood boring insect entering New Zealand on plants *in vitro* is considered negligible and the risks from this commodity will not be considered further in this assessment.

5.2.3 *Exposure Assessment*

The pathway for exposure of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.2.

When considering limitations on the ability of a wood boring insect to move from the infested imported whole plant or root-less cutting to a host on which it can establish, it should be considered unlikely that a wood boring insect will kill the host directly (it is possible that an associated fungus may result in host mortality) and the intention of the importer applicant is to plant the imported *Wollemia nobilis* into the New Zealand environment. It is, however, possible that due to climate limitations the infesting insect will not be able to develop to a stage that allows spread from the infested material. It is also possible that the emergent adults of species with a more limited host range will be unable to find an established host before mortality.

While there is no information on the success rate of insect development within infested wood, it is likely that, given the small size of the stems of even the larger imported whole plants or root-less cuttings, the infestation would only be expected to contain a few larvae. It would therefore be expected that the few adults that did emerge would more likely be all males or unmated females and be unable to find an established host before mortality. That being said, a very wide range of host plants have been recorded for many species of *Xyleborus* and related genera. Any woody material of suitable moisture content and density may be all that is required. It is therefore very likely that any mated females that do emerge will be able to find a suitable host in the environment.

The combination of the low density infestations and high host ranges in many species of wood boring insects provides a moderate to low likelihood that any such insects entering New Zealand on imported *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter would successfully become exposed to a suitable host that could lead to establishment.

5.2.4 *Establishment Assessment*

As species in *Xyleborus* and related genera are inbreeding, with the males generally mating with their sisters within the parental gallery system before dispersal, the introduction of only a few mated females may lead to the establishment of an active population if suitable host plants can be found and environmental conditions are satisfactory. Other species of wood boring insects do not inbreed, or reproduce by parthenogenesis, and may require both sexes for colony establishment, significantly reducing the likelihood of establishment from low-density infestations. The likelihood of establishment after successful exposure should therefore also be considered moderate to low.

5.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that any wood boring insect entering New Zealand within *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter would have a moderate to low likelihood of developing emergent adults capable of infesting hosts and establishing a viable population in New Zealand, the likelihood of exposure and establishment should be considered non-negligible.

5.2.6 *Consequence Assessment*

In the context of the pathway for importing nursery stock contaminated with wood boring insects, any potential consequences to people, the New Zealand environment, and the

New Zealand economy will only become apparent after establishment and some degree of spread.

Likely consequences

Direct damage caused by wood boring insects is associated with galleries constructed in the wood of host trees during breeding attacks. This can result in the loss of structural integrity in the wood and loss of lumber quality. The New Zealand forest industry contributes approximately 3.2 billion export dollars to the New Zealand economy annually, around 50% of which is high grade timber, finished timber and structural timber (MAF Statistics 200527) all of which would be negatively impacted by wood boring damage. While industry management processes do currently mitigate the impacts from wood boring insects already existing within New Zealand, more aggressive species would add significant costs to these management practices.

Wood boring insects, in combination with associated ambrosia fungus, are capable of causing extensive tree mortality in forests dominated by host plants. This could result in major environmental impacts such as the loss of biodiversity, changes in the species composition of forests, and the resultant adverse impacts on wildlife species that depend on these host trees for food.

There are unlikely to be any significant direct health impacts from the establishment of wood boring insects.

Potential for spread

Adults of some species of wood boring insects are capable of sustained flight for at least one kilometre and may also be dispersed on air currents. All life stages are subjected to human-assisted dispersal. The localized spread of newly established infestations could be facilitated via the transport of nursery stock, logs and firewood. A very wide range of host plants have been recorded for many types of wood boring insects ensuring widespread host availability.

5.2.7 *Conclusion of Consequence Assessment*

The establishment in New Zealand of new species of wood boring insects imported on *Wollemia nobilis* nursery stock has a moderate likelihood of causing high unwanted consequences to the New Zealand environment and moderate unwanted consequences to the New Zealand economy. The potential consequence of the establishment of wood boring insects imported on *Wollemia nobilis* nursery stock in New Zealand should therefore be considered non-negligible.

5.2.8 *Risk Estimation*

The likelihood is low that *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter would become infested with a wood boring insect while growing in Australia, be transported to and enter into New Zealand still infested with the insect, and form an established population of the wood boring insect once the plant is grown in the New Zealand environment. There is a high likelihood that, given sufficient

27 http://www.maf.govt.nz/statistics/primary-industries/index.htm

time, the contaminating insect will spread throughout New Zealand wherever trees are growing. There is a moderate likelihood that resulting from the spread of these wood boring insects the unwanted consequences to the environment will be high and to the economy will be moderate. As a result the risk estimate for wood boring insect associated with *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter imported from Australia is non-negligible and should be considered a hazard.

5.2.9 *Assessment of Uncertainty*

As the subject of this assessment is a group of insects that could potentially have a very wide variety of biological and epidemiological characteristics. The assessment is further complicated by the fact that no members of this insect group have been recorded as associated with the commodity in question, namely *Wollemia nobilis* nursery stock in Australia. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative insect species included in the assessment. It is possible however, that should an actual wood boring insect be recorded as being associated with *Wollemia nobilis* nursery stock, the results of this assessment could have significantly underestimated the level or risk posed by the organism.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

5.3 Risk Management

5.3.1 *Risk Evaluation*

Since the risk estimate for wood boring insects associated with *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter imported from Australia is non-negligible, phytosanitary measures will need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for wood boring insects associated with *Wollemia nobilis* whole plants or root-less cuttings of less than 5 centimetres (2 inches) stem diameter, or plants *in vitro*, imported from Australia is negligible and as such phytosanitary measures will not be required.

5.3.2 *Option Evaluation*

5.3.2.1 *Risk Management Objective*

To ensure that no wood boring insects associated with *Wollemia nobilis* nursery stock in Australia are:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

5.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of wood boring insects establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings of greater than 5 centimetres (2 inches) stem diameter before packaging and transport to New Zealand to ensure they are free of wood boring insects;

b) Inspecting all *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter before packaging and transport to New Zealand to ensure they are free of wood boring insects;

c) Treating all imported *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter before release into the New Zealand environment to ensure they are free of wood boring insects;

d) Detecting and treating infested plants before any *Wollemia nobilis* whole plants or root-less cuttings of greater than 5 centimetres (2 inches) stem diameter is released into the New Zealand environment.

Inspection

Options b) and d) rely on an effective inspection process to identify infested plants or cuttings. The three most important variables of any inspection process are (see Appendix 3 section App 3.2 for further details):

i) how detectable the organism or infestation is during visual inspection;

ii) the ability of the inspector to detect the organism or infestation; and

iii) the level of infestation of the consignment that is considered acceptable (the detection level that is required).

Infestations are most easily detected by the presence of entry holes made by the wood boring insects, and the presence of frass produced during gallery construction. Both of these features can be difficult to detect if the wood has a covering of contoured bark and only a very few (one or two) entry holes are made. Due to the relatively small size of any imported nursery stock, the number of entry holes would be expected to be low.

The level of inspector proficiency, assuming their appropriate physical and mental attributes are at least average, would be strongly influenced by the frequency at which they undertake these types of inspections and encounter similar types of infestations. Inspectors acting pre-export and in post-entry quarantine in New Zealand would be considered to have a high level of inspection proficiency for these types of detections.

Without specific inspection efficacy information the level of inspection efficacy can not be determined accurately. While it can be assumed that the efficacy level would be high, given the likelihood that only a low number of infestations would occur within a single plant it should be considered likely that the inspection efficacy level would be less than the desired level of detection confidence. Unless the actual level of inspection efficacy can be determined and shown to be greater than the desired level of confidence, this measure should not be considered sufficiently efficacious to act as the only mitigation against the risk of wood boring insects.

Pre-export Containment

Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings of greater than 5 centimetres (2 inches) stem diameter before packaging and transport to New Zealand could be a feasible measure if production facilities in Australia could provide adequate containment conditions. Restricting the access of adult wood-boring insects should be possible with minor enhancements to the NIASA programme.

Treatment

If an active infestation of wood boring insects is detected, control using insecticides is possible but is of limited effectiveness. Chemical control is not generally effective because the adult beetles bore deep into the host material. The following methyl bromide treatment schedule (table 5.1), based on the review of insecticide treatments provided in Appendix 3, is considered to be effective against "internal infestations" of insects. The actual level of efficacy of this treatment against all but a few insect species has yet to be determined with any accuracy.

Table 5.1: Methyl bromide fumigation schedule for internal insect infestations (foliated dormant plants under atmospheric conditions).

Rate (g/m^3)	Temperature (^OC)	Treatment Duration (hours)	C/T Value ($g\ h/m^3$)
64 g/m^3	4 to 10^OC	3.5	126
64 g/m^3	11 to 15^OC	3	114
64 g/m^3	16 to 20^OC	2.5	102
64 g/m^3	21 to 25^OC	2	90
48 g/m^3	26 to 29^OC	2.5	84
40 g/m^3	30 to 32^OC	2.5	80

At the time of completing this analysis the level of phytotoxicity of methyl bromide against *Wollemia nobilis* nursery stock was unknown. Care should be taken to ensure phytotoxicity levels are acceptable before applying any chemical treatments to plant material.

While this methyl bromide treatment is considered suitably efficacious, New Zealand's commitment to the Montréal Protocol necessitates that we minimise the use of substances that damage the ozone layer where possible. Methyl bromide is considered an ozone-damaging chemical and in line with New Zealand's protocol commitments methyl bromide treatments will not be accepted where alternative treatments are available.

Additional measures

Inspections should be undertaken to detect any populations of wood boring insects that manage to infest the whole plants or root-less cuttings should there be a failure in containment.

a) Pre-export and shipment inspection

The following specifications for pre-export and shipment inspection should therefore be required:

- To improve the likelihood that any infesting wood boring insects will develop a visible life stage or symptoms during pre-export inspections, it is recommended that a minimum pre-export inspection of one growing season be undertaken, in mild daytime temperatures (above 15°C)[28] and varying day length and temperature;

- To reduce the likelihood of plant stock of greater than 5 centimetres (2 inches) stem diameter becoming infested by wood boring insects, it is recommended that stock becoming larger than this size in the nursery should be held in containment conditions that restrict the access of adult wood-boring insects;

- To ensure pesticides do not mask symptom expression and significantly reduce the effectiveness of pre-export inspections, it is recommended that only pesticide treatments approved by New Zealand Ministry of Agriculture and Forestry should be applied;

- To reduce the likelihood of exported plants being contaminated by a hazard organism, it is recommended that plant stock of greater than 5 centimetres (2 inches) stem diameter is continually (regularly) inspected for wood boring insects attack by qualified nursery staff;

- To reduce the likelihood of exported plants being contaminated by wood boring insects, it is recommended that mother-stock plants should be monitored continually (regularly) for pests and treated as appropriate with plant protection chemicals;

Any plants showing evidence of wood boring insect infestation should not be included in the consignment for treatment prior to or after export to New Zealand. If more than 5% of the plants with greater than 5 centimetres (2 inches) stem diameter are found to have evidence of wood boring insect infestation, these plants should be rejected until appropriate treatments have been applied.

The level of protection provided by these pre-export and shipment measures would be affected by the following attributes of wood boring insects:

- *Infestation within the nursery:* many of the wood boring insects spread easily through air-borne life stages. It is possible that a nursery in Australia would not be able to provide sufficient protection from wood boring insect infestation given the extent of pest pressure within the surrounding environment.

28 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15°C on any one day.

- *Delay in symptom expression:* visually detectable symptoms or life stages may not become apparent for an extended period after infestation.

- *Post inspection infestation:* plants may become infested with wood boring insects after the inspections have been completed.

These attributes of wood boring insects suggest that this pre-export measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants and root-less cuttings from Australia of greater then 5 centimetres (2 inches) stem diameter would not vector wood boring insects into New Zealand.

b) Post-entry quarantine inspection

Post-entry (post-entry quarantine) inspections should be undertaken to detect any populations of wood boring insects that have not been detected during pre-export inspections. As the post-entry inspection will be to determine the existence of live infestations only, inspections will need to be for evidence of live wood boring insects. The following specifications for post-entry inspection should therefore be required, based in part on the review of the current post-entry quarantine requirements provided in Appendix 4:

- The post-entry quarantine inspections should be completed in Level 2 post-entry quarantine facilities with the following added requirements:

  - A period of quarantine for each consignment of *Wollemia nobilis* whole plants or root-less cuttings should be one growing season, in conditions suitable for disease expression e.g. mild daytime temperatures (above $15^{o}C$)[29] and varying day length and temperature. Temperature and day length variations should simulate as far as possible natural temperate-climate seasonal variations. The quarantine period shall commence after the listed half-life period of any applied pesticides has passed;

  - An inspection of all plants of greater than 5 centimetres (2 inches) stem diameter in the consignment for evidence of a live wood boring insect infestation at the conclusion of the post-entry quarantine period;

  - Treatment (or removal or destruction) of plants of greater than 5 centimetres (2 inches) stem diameter if a live wood boring insect infestation is found. Where possible the reason for failure should also be determined and steps taken to ensure such failures are unlikely to re-occur.

The level of protection provided by this post-entry measure would be affected by the following attributes of wood boring insects:

- *Escape from the post-entry quarantine facility:* many wood boring insects spread easily through air-borne life stages. The current containment requirements in Level 2 post-entry quarantine facilities as specified in MAF standard 155.02.06 should be

29 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds $15^{O}C$ on any one day.

considered adequate to ensure air-borne life stages of the majority of wood boring insect species do not escape from the facility.

- *Delay in symptom expression:* visually detectable symptoms or life stages may not become apparent for a period that exceeds the duration of post-entry quarantine or requires environmental conditions not provided in post-entry quarantine.

- *Masking of symptom expression by other insects:* The current containment requirements in Level 2 post-entry quarantine facilities as specified in MAF standard 155.02.06 should be considered adequate to ensure air-borne life stages of the majority of wood boring insect species do not enter the facility from the New Zealand environment.

It should be considered that the risks associated with the delay in symptom expression would be minimised for plants growing in an environment that is optimal for symptom expression. Optimal symptom expression would, however, considerably enhance the likelihood of insect escape from the facility.

5.3.2.3 *Recommended Management Options*

It is recommended that three measures are applied to *Wollemia nobilis* whole plants and root-less cuttings from Australia of greater then 5 centimetres (2 inches) stem diameter to reduce, to an acceptable level, the risk of wood boring insects establishing in New Zealand and causing unwanted consequences.

i) One growing season of pre-export inspections before export to New Zealand, in conditions suitable for disease expression e.g. mild daytime temperatures (above 15^{o}C)[30] and varying day length and temperature.

ii) Pre-export containment of any plants reaching 5 centimetres (2 inches) in maximum stem diameter.

iii) A one growing-season period in a Level 2 post-entry quarantine facility in conditions suitable for disease expression e.g. mild daytime temperatures (above 15^{o}C)[30] and varying day length and temperature and that includes a final inspection of all nursery stock of greater than 5 centimetres (2 inches) stem diameter before biosecurity clearance.

5.4 Assessment of Residual Risk

5.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure that the proportion of infested nursery stock within any consignment of *Wollemia nobilis* is less than 5%.

The objective for the pre-export containment is to ensure no live wood boring insects enter the New Zealand environment on *Wollemia nobilis* nursery stock imported from Australia.

30 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15ºC on any one day.

The objective for inspection during post-entry quarantine is to have a 95% level of confidence that all failures in pre-export containment are detected.

5.4.2 *Expected Performance of Measure(s)*

The pre-export nursery management measure is expected to ensure no more than 5% of the consignment is infested with wood boring insects. Inspection of the imported plants in post-entry quarantine should not detect wood boring insect infestation levels above 5%.

The post-entry quarantine inspection should detect, with a 95% level of confidence, all failures in pre-export containment. A failure in this post-entry quarantine measure will only be detected in a new or unwanted wood boring insect establishes in New Zealand from *Wollemia nobilis* nursery stock imported from Australia.

It is expected that a combination of these measures will ensure that the number of wood boring insects establishing in New Zealand from this pathway, and causing unacceptable consequences to the New Zealand economy or environment, should be considered acceptable.

5.5 References

Lenz M, Barrett RA, Miller LR, 1986. The capacity of colonies of Coptotermes acinaciformis from Australia to produce neotenics (Isoptera: Rhinotermitidae). Sociobiology, 11(3):237-242

Soné K, Mori T, Ide M, 1998. Life history of the oak borer, Platypus quercivorus (Murayama) (Coleoptera: Platypodidae). Applied Entomology and Zoology, 33(1):67-75

Soné K, Uto K, Fukuyama S, Nagano T, 2000. Effects of attack time on the development and reproduction of the oak borer, Platypus quercivorus (Murayama). Japanese Journal of Applied Entomology and Zoology, 44(3):189-196

6. FOLIAGE DISEASES

6.1 Hazard Identification

6.1.1 *Aetiologic agent*

Examples of foliage diseases that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known to be or can not be confirmed as being present in New Zealand are listed in Appendix 1. Examples from the list of fungi or fungi-like organisms include *Aecidium fragiforme*, *Alternaria* sp, *Leptosphaeria* sp, *Meliola* sp, *Phytophthora boehmeriae* and *Servazziella longispora*. For the purposes of this analysis *Phytophthora boehmeriae*, a chromist or fungi -like organism, will be used as an example due to lack of available information on the other foliage diseases.

It should be emphasised that at the time of undertaking the risk analysis none of the diseases listed above have been found on *Wollemia nobilis* nursery stock, and their inclusion in this analysis is as representative examples only.

6.1.2 *New Zealand Status*:

There are many indigenous and introduced foliage diseases established in New Zealand. There are a great many more foliage diseases existing overseas that have never established in New Zealand, a portion of which exist in Australia.

6.1.3 *Epidemiology*

The description of the epidemiology of foliage diseases is based on an example species: *Phytophthora boehmeriae*. Information on this organism has been collated from the CABI Crop Protection Compendium 2006[31] with available or supplementary references provided. As this organism has been included to provide an indication of the possible biological nature of the risk posed by foliage diseases, the epidemiological description has been summarised to include only relevant and general organism characteristics.

Phytophthora boehmeriae

Phytophthora boehmeriae is a semi-aquatic oomycete, a facultative saprophyte that is favoured by high humidity and warm temperatures. As no information could be found on the expression of diseases caused by *P boehmeriae* on members of the Araucariaceae, aspects of the life cycle and epidemiology are provided from descriptions of disease on other hosts.

Life cycle

When the tissues (leaves, bolls, etc.) infected by *P. boehmeriae* decompose, oospores formed in the diseased tissue are released into the soil. Under suitable conditions they germinate by germ tubes to form sporangia or mycelia, which may produce sporangia (Zheng *et al*., 1992; Ho *et al*., 1993). The germination mode of sporangia is affected mostly by temperature. It

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

has been observed that at ca 18-20°C all sporangia germinated indirectly to produce zoospores, whereas at 22-24°C most sporangia germinated directly, and terminated with secondary sporangia capable of releasing zoospores (Shan *et al*., 1995). All the spore forms may be carried long distances by water or soil, and all germinate in water. When the spores (mostly zoospores) lodge against a stem or root of a certain host, such as ramie or pine, or splash onto a leaf or fruit of a suitable host plant, such as cotton, they germinate and produce appressoria or mycelia that infect the plant. The oomycete invades the plant either through the cuticle or via stomata or wounds. Incubation periods vary with host type and temperature: from 24 h on cotton leaves at 20-22°C to 2-3 days on cotton bolls at 22-24°C (Shan *et al*., 1995).

During warm, wet weather the oomycete may fruit near the surface of the ground or on wet, diseased leaves, stems or fruits (bolls). Under these conditions the zoospores germinate to produce mycelia with sporangia and a new crop of zoospores within 72 hours. Successive generations of sporangia and zoospores quickly produce large amounts of inoculum that infects plants and causes the disease epidemic so often seen in cotton fields in low regions of China under rainy conditions. Darkness stimulates mycelial growth and oospore formation, whereas illumination inhibits both (Ho *et al*., 1993; Gao *et al*., 1998). However, illumination stimulates the germination of oospores and the formation of sporangia (Zheng *et al*., 1992).

In diseased tissue (leaves, stems, bolls, etc.), abundant oospores are formed. Infected detached tissues become an important primary source of infection by *P. boehmeriae* in the field in the following season or year (Zheng *et al*., 1992; Shan *et al*., 1995).

There is some discussion on the role of chlamydospores in the life cycle of the fungus. Zhang and Ma (1980) reported that chlamydospore survival on infested boll cuticle in the soil was the major form of the pathogen to over-winter and serve as a source of infection; whereas other researchers (Zheng *et al*., 1992; Ho *et al*., 1993; Ma and Shen, 1994) suggest that chlamydospores are seldom (or not at all) present in *P. boehmeriae*, and that the oospores can over-winter and survive in soil while the hyphae cannot (Zheng *et al*., 1992). The sporangia could survive in boll residues in the soil for about 3-4 months with a survival rate of 58%, but could not over-winter because their viability was affected by low temperature; no viable sporangia were observed at 0°C in January (Ma and Shen, 1994).

Disease Characteristics

In nature, *P. boehmeriae* can infect and damage the seedlings and bolls of cotton, the leaves and stems of ramie, the leaves of paper mulberry (*Broussonetia papyrifera*), the fruits of citrus and the roots of pine, causing blights.

On cotton seedlings, it produces round or irregular, water-soaked, dark-green spots or lesions on cotyledons or true leaves, resulting in premature leaf fall or wilting of some or all leaves under cold, humid conditions. On the roots and root-stem transition zones, the disease first appears as brown streaks and then as a brown rot, resulting in wilting and death of whole seedlings. On cotton bolls, dark-green, water-soaked lesions generally form at basal cracks or tips, causing rotting of the tissues within, with a layer of downy mildew on the surface under humid conditions. As the spots develop, whole diseased bolls may rot away.

On ramie, the pathogen attacks leaves and stems. The leaf spots are round or irregular, water-soaked and light green at first, then dark brown or dark green. At later stages the spots

turn yellowish brown or grey at the centre, with a brown margin. Diseased leaves tend to fall early. Elliptical, dark-brown stem lesions are present, mainly at the bases, resulting in rotting of whole bases. In addition, the pathogenic organism causes brown fruit rot and root stock gummosis of citrus, and root rot of pine and *Pterocarya stenoptera*.

Latent infection of *P. boehmeriae* has been found in cotton bolls at resistant stages (with bolls younger than 25 days post-anthesis; Shan and Li, 1992). The pathogen can complete infection within hours when zoospores are used as inoculum, and remain dormant in necrotic spots. Diverse physical conditions, such as high humidity on rainy days, are responsible for bolls at resistant stages becoming susceptible to colonization by the pathogen.

P. boehmeriae is commonly spread by soil and water. Rainwater or drainage water transports infested soil and spores along rows, terraces and ditches, and from infested fields into disease-free fields, drainage ponds or streams. When water from these infested sources is used for irrigation it may carry the oospores to new locations and cause disease. The spores may be dispersed by rain splashing or by wind during wet weather.

The danger of spreading the pathogen in soil is much greater when the soil is wet: soil on farm tools, feet of farm animals, labourers' shoes and the wheels of vehicles may be responsible for transferring the fungus from field to field, and road-working machines may carry infested soil over long distances. Deposition of spores through contact with diseased plants can also occur in the field.

Summary of hazard assessment

P. boehmeriae can complete infection within hours when zoospores are used as inoculum, and remain dormant in necrotic spots. Under optimal conditions the zoospores can germinate to produce mycelia with sporangia and a new crop of zoospores within 72 hours. All the spore forms may be carried long distances by water or soil, and all germinate in water. *P. boehmeriae* can infect and damage leaves, stems and roots causing root rots and leaf blights.

6.1.4 *Hazard Identification Conclusion*

From the epidemiological information provided above it should be considered possible that foliage disease-causing organisms could establish in New Zealand from imported *Wollemia nobilis* nursery stock and cause an unwanted impact. Foliage disease-causing organisms are therefore considered a potential hazard requiring further assessment.

6.2 Risk Assessment

6.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. Aerial parts of a plant that have become infected by foliage disease-causing organisms would be considered an almost guaranteed pathway for the entry of these organisms into New Zealand, as their survival in-transit is linked to the health of the plants and the intention of any importer of nursery stock is to import healthy plants.

Wollemia nobilis nursery stock not otherwise infected before packaging and transportation to New Zealand could become infected by some types of foliage disease-causing organisms if

they were stored for a length of time immediately adjacent to other plants that have developed foliage disease symptoms.

As foliage disease-causing organisms are associated with all aerial plant parts, whole plants and root-less cuttings should be considered a pathway for the entry into New Zealand of these organisms. Plants *in vitro* would not be considered a pathway for the entry assuming the cultures in question are sterile (axenic).

6.2.2 *Conclusion of Entry Assessment*

The likelihood of foliage disease-causing organisms entering New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* that have become infected in Australia is very high and therefore is consider non-negligible.

The likelihood of foliage disease-causing organisms entering New Zealand on plants *in vitro* is considered negligible and this commodity will not be considered further in this assessment.

6.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

When considering limitations on the ability of foliage disease-causing organisms to move from the infested imported plant to a host on which it can establish, it would be considered possible that foliage disease-causing organisms could kill the host; however it is unlikely that all plants infected by these types of organisms will suffer to this extent. It is also unlikely that the organisms infecting a susceptible host would themselves become deceased. The only remaining option for avoiding exposure is if the plants are not moved into the New Zealand environment. As the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, this final limitation to exposure is removed.

6.2.4 *Establishment Assessment*

As indicated in the exposure assessment, the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, therefore allowing the foliage disease-causing fungus to establish a viable population.

6.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that the imported and contaminated *Wollemia nobilis* plants themselves can act as the agent for exposure and establishment, and the intention of any importer would be to plant the imported *Wollemia nobilis* into the New Zealand environment, the likelihood of exposure and establishment is high and therefore non-negligible.

6.2.6 *Consequence Assessment*

In the context of the pathway for importing nursery stock contaminated with foliage disease-causing organisms, any potential consequences to people, the New Zealand environment, and

the New Zealand economy will only become apparent after establishment and some degree of spread.

Potential for spread

P. boehmeriae is commonly spread by soil and water. Rainwater or drainage water transports infested soil and spores along rows, terraces and ditches, and from infested fields into disease-free fields, drainage ponds or streams. When water from these infested sources is used for irrigation it may carry the oospores to new locations and cause disease. The spores may be dispersed by rain splashing or by wind during wet weather.

Likely consequences

Phytophthora boehmeriae has been reported to damage ramie, cotton, citrus, pine, paper mulberry (*Broussonetia papyrifera*) and Chinese wingnut (*Pterocarya stenoptera*). In China, *P. boehmeriae* is the principal agent causing cotton blight and ramie blight. Cotton blight, including cotton seedling blight and cotton boll blight, is one of the main diseases in cotton in mainland China. Cotton seedling blight attacks leaves, stems and roots, resulting in lesions, premature leaf fall, wilting or death of whole seedlings. A severe attack can kill 30-50% of seedlings in the field in cool, wet weather. Seedling blight mortality up to 60% has been recorded in fields (Tang, 1990). Cotton boll blight injures bolls, resulting in rotting: the rotting rate of bolls is between 10 and 30% in ordinary years, with a maximum over 50% in rainy years. In addition to yield loss, the disease affects cotton quality resulting in reduced fibre length and decreased ginning outturn (Ji and Fan, 1988; Tan and Li, 1988). Ramie blight, also called leaf spot, attacks leaves and stems resulting in premature leaf fall and rotting. In China, *P. boehmeriae* occasionally attacks citrus, paper mulberry (Zheng and Lu, 1989) and *P. stenoptera* (Ho and Lu, 1997).

In Australia it has been recorded to attack citrus and pine, causing rot of fruit and roots, respectively (Gerrettson-Cornell, 1989).

As indicated in section 3.4.3, the association of pathogens with plants that are closely related to important native species and grow in similar climatic conditions offshore, increases the potential that those pathogens will be able to invade New Zealand's native forests.

There are no indications that foliage disease-causing organisms could cause unwanted consequences to human health.

Summary of Consequences

Mortality rates from foliage disease-causing organisms can be significant in susceptible plants, and these diseases may be able to spread easily through the New Zealand environment. In many instances disease expression and subsequent economic impact can be controlled on commercial crops through the use of chemicals such as fungicides, but these management options would not be available to control impacts in natural environments within New Zealand.

6.2.7 *Conclusion of Consequence Assessment*

From the assessment of a single example species, it is possible to conclude that foliage disease-causing organisms could cause mortality and/or reduction in growth or form of plants of environmental or economic importance. These impacts have the potential to cause moderate to high unwanted consequences to the New Zealand environment and economy should therefore be considered non-negligible.

6.2.8 *Risk Estimation*

The likelihood is high that *Wollemia nobilis* whole plants and root-less cuttings could become associated with a foliage disease-causing organism while growing in Australia, be transported to and enter into New Zealand still infected with the organism, and form an established population of a new foliage disease-causing organism once the plant is grown in the New Zealand environment. There is also a high likelihood that, given sufficient time, the contaminating organism will spread throughout New Zealand wherever host shrubs or trees are growing.

It is also possible that resulting from the spread of a new foliage disease-causing organism the unwanted consequences to the environment and economy will be moderate to high. As a result the risk estimate for foliage disease-causing organisms associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is non-negligible and it is considered a hazard.

The likelihood that a new foliage disease-causing organism would be associated with *Wollemia nobilis* plants *in vitro* is considered negligible, and as such on this pathway foliage disease-causing organisms are not considered a hazard.

6.2.9 *Assessment of Uncertainty*

The subject of this assessment is a group of organisms that could potentially have a very wide variety of biological and epidemiological characteristics. The assessment is further complicated by the fact that no members of this group of organisms have been recorded as being associated with the commodity in question, namely *Wollemia nobilis* nursery stock from Australia. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative fungal-like species included in the assessment. It is possible however, that should an actual foliage disease-causing organism be recorded as being associated with *Wollemia nobilis* nursery stock, the results of this assessment would significantly underestimate the level or risk posed by the organism.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

6.3 Risk Management

6.3.1 *Risk Evaluation*

Since the risk estimate for foliage disease-causing organisms associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is non-negligible, phytosanitary measures will need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for foliage disease-causing organisms associated with *Wollemia nobilis* plants *in vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

6.3.2 *Option Evaluation*

6.3.2.1 *Risk Management Objective*

To ensure any unwanted foliage disease-causing organisms associated with *Wollemia nobilis* whole plants and root-less cuttings in Australia are neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* whole plants and root-less cuttings imported from Australia.

6.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of foliage disease-causing organisms establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of foliage disease-causing organisms;

b) Inspecting all *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of foliage disease-causing organisms;

c) Treating all imported *Wollemia nobilis* whole plants and root-less cuttings before release into the New Zealand environment to ensure they are free of foliage disease-causing organisms;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* nursery stock are released into the New Zealand environment.

Given that there are potentially a number of different genera and species of organisms in Australia that could act as foliage disease-causing organisms on *Wollemia nobilis* nursery stock, some of which may not as yet have been described, it is unlikely that a treatment will be identified that could decontaminate already infested plants (see Appendix 3). It is also unlikely, due to the difficulties detecting and delimiting an established population of some types of foliage disease-causing organisms, that an effective method of eradication or control could be developed for post-entry management of any unwanted consequences.

Management options therefore are limited to either ensuring *Wollemia nobilis* nursery stock is free of foliage disease-causing organisms before packaging and transport to New Zealand, or detecting and removing all infested plants before any *Wollemia nobilis* nursery stock is released into the New Zealand environment.

Appendix 4 (section 4.3) suggests an inspection period of two growing seasons is required to provide an adequate level of inspection efficacy. The two growing season requirement could potentially be met in this instance through a one growing season pre-export inspection period combined with a one growing season post-entry inspection period.

Pre-export Inspection Requirements

The principle measure available for obtaining foliage disease-causing organism freedom prior to export to New Zealand is through nursery management in Australia. Referring to the existing management practices provided in 3.6.1 and Appendix 2, the following nursery management practices would be necessary to provide an assurance of pre-export freedom from foliage disease-causing organisms:

- Accreditation under the Nursery Industry Accreditation Scheme, Australia (NIASA). Accreditation is based on adherence to guidelines and recommendations to ensure quality control and crop hygiene, particularly disease, pest and weed control and nursery hygiene.

Additional or more specific requirements to the NIASA scheme include:

- To improve the likelihood that any contaminating foliage disease-causing organisms will develop a visible life stage or symptoms during pre-export inspections, it is recommended that a minimum pre-export inspection of one growing season in mild daytime temperatures (above 15^{o}C)[32] be undertaken;

- To ensure fungicides do not mask symptom expression and significantly reduce the effectiveness of pre-export inspections, it is recommended that only fungicide treatments approved by New Zealand Ministry of Agriculture and Forestry should be applied;

- To reduce the likelihood of exported plants being contaminated by foliage disease-causing organisms, it is recommended that plant stock is continually (regularly) inspected for disease symptoms by qualified nursery staff;

32 Temperature recorded over the majority of the period between sunrise and sunset (daylight hours) exceeds 15^{O}C on any one day.

- To reduce the likelihood of exported plants being contaminated by foliage disease-causing organisms, it is recommended that mother-stock plants should be monitored continually (regularly) for pests and diseases, maintained in a suitable environment (e.g. where mother stock is in-ground, good drainage should be maintained), and treated as appropriate with plant protection chemicals;

- To reduce the likelihood of exported plants being contaminated by foliage disease-causing organisms, it is recommended that all access to the site is via a series of footbaths containing an antibiotic agent effective against soil-borne diseases such as *Phytophthora* species (e.g. 128 g/l of benzalkonium chloride);

Shipment Inspection Requirements

To provide adequate assurance of freedom from foliage disease-causing organisms, it is recommended that the following plant inspection requirements be undertaken either just prior to export or soon after arrival in New Zealand (e.g. one week may be an appropriate time frame):

- Each plant in the consignment should have their above ground material (leaves and stems) inspected by an appropriately trained person for indications of foliage disease expression;

Any plant showing signs of foliage disease shall not be included in the consignment for export to or post-entry quarantine in New Zealand.

The level of protection provided by these pre-export and shipment measures would be affected by the following attributes of foliage disease-causing organisms:

- *Infection within the nursery:* many of the foliage disease-causing organisms spread through air-borne spores. It is unlikely that a nursery in Australia could provide sufficient protection from foliage disease-causing fungal infection given the extent of propagule pressure within the surrounding environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for an extended period after infection.

- *Post inspection infection:* plants may become infected with foliage disease-causing organisms after the inspections have been completed but before the plants arrive in New Zealand.

These attributes of foliage disease-causing organisms suggest that this measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants and root-less cuttings from Australia would not vector foliage disease-causing organisms into New Zealand.

Post Entry Quarantine

The principle measure available for detecting and removing infested plants before the consignment is released into New Zealand is through inspection while in post-entry quarantine. Contamination of nursery plants by foliage disease-causing organisms may not

become apparent when conditions for the expression, such as humidity, temperature, and water levels, are not suitable or the infected plants are symptom-less hosts. The following post-entry quarantine conditions shall apply to ensure as far as is possible that disease expression will become apparent on infected plants that are not symptom-less hosts, and that the infecting organisms will remain contained within the quarantine facility:

- Post entry quarantine equivalent to Level 2 quarantine is considered appropriate (see Appendix 4), with the following additional structural and management requirements:

  Plant Management Requirements

  - The period of quarantine for each consignment of *Wollemia nobilis* whole plants or root-less cuttings should be for one growing season in conditions suitable for disease expression to ensure diseases with seasonal symptoms will be detected. The quarantine period shall commence after the listed half-life period of any applied pest management chemicals has passed (see Appendix 3);

  - Conditions required to enhance disease expression shall include drought stress, over watering, excess humidity, varying day length, and temperature variation during the growing season. Temperature and day length variations should simulate as far as possible natural temperate/climate seasonal variations;

  Physical Containment and Hygiene Requirements

  - To improve the level of general plant hygiene within the post-entry quarantine facilities it is recommended that a number of improvements to the management of post-entry quarantine be made (see Appendix 4). Examples include the spacing and segregation of containers, construction and design of benches and floor coverings, and general hygiene practices;

  - To limit the likelihood of foliage disease-causing organisms present on the imported plants escaping into the New Zealand environment, it is recommended that measures be taken to improve the level of containment provided by the post-entry quarantine facilities. Post entry quarantine measures to achieve this include:

    - The level of security provided by the facility should be sufficient to contain such propagules as airborne fungal spores. Currently the only measures known to provide this level of containment are to ensure all outward flowing air vents are filtered with a High-Efficiency Particulate Air (HEPA) filter, and a negative air pressure (15 Pa) is maintained within the facility. MAF recognises the likely costs associated with such measures may not be cost-effective and will review potential alternatives to managing this identified risk;

  Plant Inspection Requirements

  - At the conclusion of the post-entry quarantine period, each plant should be examined for evidence of foliage disease-causing organism contamination. Any plants that have indications of contamination by hazard organisms should be treated (if appropriate), reshipped or destroyed. Depending on the type and nature of the hazard organism, host plants remaining in the quarantine facility may need to be treated and/or placed

back under quarantine conditions (for a period determined by treatment type and organism biology), reshipped or destroyed.

The level of protection provided by this post-entry measure would be affected by the following attributes of foliage disease-causing organisms:

- *Propagule escape from the post-entry quarantine facility:* many organisms spread through air-borne spores. It may be difficult for a quarantine glass/greenhouse facility in New Zealand to limit the exit of fungal spores into the environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for a period after infection that exceeds the duration of post-entry quarantine or requires environmental conditions not provided in post-entry quarantine.

- *Masking of symptom expression by other diseases:* while the recommended enhancements to facility containment requirements related to micro-organism escape could be considered sufficient, the same level of containment is not currently recommended for micro-organism entry into the facility (e.g. HEPA filters are not recommended for inward flowing air). Foliage diseases entering the facility from the New Zealand environment and infesting plants may result in the expression of diseases symptoms that mask symptoms of diseases that the plants have carried in from Australia.

It should be considered that the risks associated with the delay in symptom expression would be minimised for plants growing in an environment that is optimal for disease expression. Optimal disease expression would, however, considerably enhance the likelihood of propagule escape from the facility. This would occur if plants were not showing symptoms from some types of fungal infection and therefore would not be removed as propagule pressure increased. The specifications for the facility are therefore optimised for fungal containment (HEPA filters and negative air pressure).

To manage the potential risks associated with the masking of disease expression by diseases found in New Zealand, plants showing foliage disease symptoms should not be given biosecurity clearance. To limit as far as possible the level of fungal propagule pressure within post-entry quarantine, it is recommended that pre-export measures are also implemented.

6.3.2.3 *Recommended Management Options*

It is recommended that two measures are applied to reduce, to an acceptable level, the risk of foliage disease-causing organisms establishing in New Zealand and causing unwanted consequences.

i) Pre-export nursery management and pre-export and shipment inspection.

ii) Inspection during post-entry quarantine.

6.4 Assessment of Residual Risk

6.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with foliage disease-causing organisms.

The objective for inspection during post-entry quarantine is to ensure that any plants in a consignment of *Wollemia nobilis* whole plants or root-less cuttings imported into New Zealand from Australia that have become infested just prior to or during transport to New Zealand will be detected and prevented from being released into the New Zealand environment.

6.4.2 *Expected Performance of Measure(s)*

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with foliage disease-causing organisms. There should therefore be no detections of infested plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export infestation. In reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through long term monitoring or targeted research.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no imported *Wollemia nobilis* plants infected with foliage disease-causing organisms are released into the New Zealand environment. As *Wollemia nobilis* plants entering the New Zealand environment would be expected to become infested with already established foliage disease-causing organisms, some of which may not have been described by science, identifying a success measure for this option is more problematic. Therefore it is expected that a high level of assurance is obtained that the post-entry quarantine inspections were undertaken appropriately before any plants are released into the New Zealand environment. As above, in reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through targeted research.

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7. ROOT DISEASES

7.1 Hazard Identification

7.1.1 *Aetiologic agent*

Examples of root diseases that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known to be or can not be confirmed as being present in New Zealand are listed in Appendix 1. Examples from the list include *Armillaria* sp., *Pythium* sp. and *Athelia rolfsii*. For the purposes of this analysis *Pythium vexans* and *Athelia rolfsii* will be used as examples of this group of plant diseases.

It should be emphasised that at the time of undertaking the risk analysis only one of these root diseases, *Phytophthora cinnamomi*, has been found on *Wollemia nobilis*, and the inclusion of the others in this analysis is as representative examples only. The analysis of *Phytophthora cinnamomi* has been completed separately in Chapter 12.

7.1.2 *New Zealand Status*

There are many indigenous and introduced root diseases established in New Zealand. There are a great many more root diseases existing overseas that have never established in New Zealand, a portion of which exist in Australia.

It has latterly been confirmed that *Athelia rolfsii* has been recorded in New Zealand and as such measures may not be required for this organism. For the purposes of establishing generic measures, however, the relevant biological characteristics will be analysed here.

7.1.3 *Epidemiology*

The description of the epidemiology of root diseases is based on two example species: *Pythium vexans* and *Athelia rolfsii*. Information on these organisms has been collated from the CABI Crop Protection Compendium 2006[33] with available or supplementary references provided. As these organisms are included to provide an indication of the possible biological nature of the risk posed by root diseases, the epidemiological descriptions have been summarised to include only relevant and general organism characteristics.

Athelia rolfsii

The mycelial fungus also known as *Sclerotium rolfsii* may be an aggregate of geographical races differing appreciably in the criteria exhibited in the teleomorph stage (Watkins, 1981). *A. rolfsii* infects more than 500 species of monocotyledonous and dicotyledonous plants, but is especially severe on legumes, solanaceous crops, cucurbits and other vegetables grown in rotation with beans (Hall, 1991).

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

Life cycle

The main inoculum source of *A. rolfsii* for a broad range of hosts is small, black microsclerotia in soil and crop debris of hosts. Sclerotia survived in high numbers from November 1977 to August 1978 in groundnut fields in North Carolina, USA. The viability of sclerotia produced in field soil was 56-73% in field microplots after 8-10 months' incubation. In growth chambers, survival was less at temperatures above 20°C in moist field soil than at 20°C or below. No adverse effect of temperature on survival was observed in dry field soil. Mycelia rapidly died in moist field soil, but survived for at least 6 months in dry soil. *C. rolfsii* grew on groundnut stems buried in field soil and produced new sclerotia (Beute *et al*., 1981). In another study, sclerotia of *A. rolfsii*, isolated from groundnut, remained viable in soil at low temperature, but high temperatures (greater than 70°C) were lethal. Sclerotia remained viable at up to 80% soil moisture level, with 60-70% being optimal, but viability was adversely affected at saturated soil moisture levels (Sahu and Narain, 1995). Survival of mycelium of *A. rolfsii* was 5% after 7 months on stem segments of *Phaseolus vulgaris* placed in plastic net bags buried at 25 cm depth in the soil, while sclerotia lost viability after 60 days (Herrera-Isla *et al*., 1986). In a mixture of 2% inoculum of *A. rolfsii* isolated from *Piper betle*, the optimum soil temperature for growth in sterile soil (indicated by colonization of sorghum seed) was 25-30°C, and no growth occurred above 50°C. Survival of the pathogen was best at low soil moisture levels (20-40%) (Palakshappa *et al*., 1989).

Disease Characteristics

Disease expression of *A. rolfsii* is characterised by early yellowing and dropping of upper leaves; this is usually accompanied by girdling of the stem at the soil line, resulting in wilting and death of the plant. On rice, dying or white seedlings may be observed in patches or in short strips along a drill row. On maize, infected ears display white to pink mycelial growth, usually with sclerotia.

In a study of the process of infection, hyphae from germinating sclerotia of *A. rolfsii* ramified over various host tissues (sugarbeet leaves, bean (*Phaseolus vulgaris*) hypocotyls and carrot roots or petioles) within 24-48 h of inoculation and frequently coalesced to form infection cushions. Penetration occurred after death and collapse of cells beneath infection cushions, and from appressoria that formed at the tips of individual hyphae. Severe infection of sugarbeet by *A. rolfsii* occurred in the pre-emergence stage at a range of soil moistures, but was especially high in soil of 60% and 75% water-holding capacity. Low or high soil moisture was less favourable for post-emergence infection (Fahim *et al*., 1981). Foot rot of *Piper betle*, caused by *A. rolfsii*, was related more to mean minimum temperature (greater than 22°C) than to rainfall (Maiti and Sen, 1982). In rice, the disease was favoured by continuous cloudy weather, intermittent rainfall and temperatures of 25-32°C (Amin, 1976). When surface sterilized, green and ripe tomato fruits were inoculated with *A. rolfsii* sclerotia, a positive linear relationship between incubation time and rate of rotting was observed at 20, 25 and 30°C, but ripe fruits rotted more rapidly. The optimum temperature for rotting was 25-30°C (Prasad *et al*., 1988).

Relationships between numbers of sclerotia in soil and plant infection have been recorded. Regression equations were developed to relate populations of viable sclerotia in field soil to the number of sugarbeet roots infected by *A. rolfsii* in Uruguay (Backman *et al*., 1981). The number of lentil seedlings emerging decreased linearly with increasing inoculum densities of *A. rolfsii* sclerotia (Canullo and Rodriguez Kabana, 1992).

Summary of Hazard Potential

Sclerotia of *A. rolfsii* are expected to survive in infected plant tissue and soil for over 12 months, therefore infected plants and associated soil should be considered a potential pathway for the entry of this disease into New Zealand. Disease expression of *A. rolfsii* is characterised by early yellowing and dropping of upper leaves; this is usually accompanied by girdling of the stem at the soil line, resulting in wilting and death of the plant.

Pythium vexans

Life cycle

Little has been documented concerning diseases caused by *Pythium vexans*. Probably, like other species of Pythium, *P. vexans* survives in soil by means of zoospores and sporangia for short and intermediate periods and by means of oospores for longer periods (Hendrix and Campbell, 1973). The oospores germinate indirectly by releasing zoospores. The zoospores swim around in water and infect the roots of host plants. Mycelial growth occurs between 5 and 35°C (optimum 30°C) (Plaats-Niterink, 1981).

Disease Characteristics

P. vexans is known to cause diseases on a number commercially important plant species, such as *Eucalyptus*, *Hevea*, *Vitis*, *Vigna*, *Prunus*, *Pyrus*, *Persea*, *Malus*, *Lycopersicon*, *Citrus*, and *Ananas*. Peach stunting induced by *P. vexans* is more severe under high moisture conditions (Biesbrock and Hendrix, 1970). Symptoms caused by *P. vexans* are similar to those caused by other soil-borne pathogens. Isolation of *P. vexans* is the only way to confirm its presence.

Summary of Hazard Potential

Infested plant material and associated soil should be considered a potential pathway for the entry of *P. vexans* into New Zealand. Symptoms caused by *P. vexans* are similar to those caused by various other soil-borne pathogens, namely early yellowing and dropping of upper leaves; this is usually accompanied by girdling of the stem at the soil line, resulting in wilting and death of the plant.

7.1.4 *Hazard Identification Conclusion*

From the epidemiological information provided above it should be considered possible that a species of root disease-causing fungi could establish in New Zealand from imported *Wollemia nobilis* nursery stock and cause an unwanted impact. Root disease-causing fungi are therefore considered a potential hazard requiring further assessment.

7.2 Risk Assessment

7.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. The great majority of root diseases are transmitted through infested plant material and soil. In the case of *P. vexans*, zoospores of this fungus can also swim in open water for a short distance. Roots of a plant that has become infected with a root disease-causing fungus would be considered an almost guaranteed pathway for the entry of the fungi into New Zealand either through the roots themselves or through any contaminated soil surrounding the roots.

Wollemia nobilis whole plants not otherwise infected before packaging and transportation to New Zealand could become infected by root disease-causing fungi if they were stored for a length of time immediately adjacent to other plants that have developed root disease symptoms.

As root disease-causing fungi can be associated with woody plant parts, root-less cuttings should also be considered a pathway for the entry into New Zealand of these fungi, though the likely incidence of disease would be considerably lower (low to moderate). Plants *in vitro* would not be considered a pathway for the entry assuming the cultures in question are indeed sterile (axenic).

7.2.2 *Conclusion of Entry Assessment*

The likelihood of root disease-causing fungi entering New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* that has become infected in Australia is very high and low-moderate respectively and therefore is consider non-negligible.

The likelihood of root disease-causing fungi entering New Zealand on plants *in vitro* is considered negligible and this commodity will not be considered further in this assessment.

7.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

While some types of root disease-causing fungi can kill the host in a relatively short period, many can survive in plant tissues for an extended period. As the intention of any importer would be to plant the imported *Wollemia nobilis* whole plants or root-less cuttings into the New Zealand environment, any limitation there may be to exposure is removed.

The transfer into the environment of contaminated soil that has been associated with infected plants should also be considered a possible pathway to exposure.

7.2.4 *Establishment Assessment*

As indicated in the exposure assessment, the intention of any importer would be to plant the imported *Wollemia nobilis* whole plants or root-less cuttings into the New Zealand

environment, therefore allowing any contaminating root disease-causing fungi to establish a viable population.

7.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that the imported and contaminated *Wollemia nobilis* whole plants or root-less cuttings themselves can act as the agent for exposure and establishment, and the intention of any importer would be to plant the imported *Wollemia nobilis* whole plants or root-less cuttings into the New Zealand environment, the likelihood of exposure and establishment is very high and therefore non-negligible.

7.2.6 *Consequence Assessment*

In the context of the pathway for importing whole plants or root-less cuttings contaminated with root disease-causing fungi, any potential consequences to people, the New Zealand environment, and the New Zealand economy will only become apparent after establishment and some degree of spread.

Direct impacts to plants affected by root disease-causing fungi include: failure of seedling emergence after planting; dark necrosis of emergent shoots or roots; the seedling emerges from the soil but dies soon afterwards. For some hosts, once the plant has reached a certain stage after emergence, infections by root disease-causing fungi are no longer lethal, but they can still have a significant impact on plant growth and yield. Apart from stunting of plant growth there may not be any overt symptoms of infection other than necrotic roots. In cases where root infection is heavy, wilting of plants may be observed in warm or windy weather. Foliar symptoms of nutrient deficiency also may be observed due to extensive root rotting preventing the uptake of nutrients. Crop losses caused by sublethal infections of the root system reduce the growth and vigour of the host with a concomitant effect on crop yield especially in the warmer regions of the world.

7.2.7 *Conclusion of Consequence Assessment*

From the assessment of a single example species, it is possible to conclude that root disease-causing fungi could cause mortality and/or reduction in growth or form of plants of environmental or economic importance. These impacts have the potential to cause moderate to high unwanted consequences to the New Zealand environment and economy.

7.2.8 *Risk Estimation*

The likelihood is high and moderate to low respectively that *Wollemia nobilis* whole plants or root-less cuttings could become associated with a root disease-causing fungal species while growing in Australia, be transported to and enter into New Zealand still infected with the fungus, and form an established population of the root disease-causing fungus once the whole plants or root-less cuttings are grown in the New Zealand environment. There is also a high likelihood that, given sufficient time, the contaminating fungus will spread throughout New Zealand wherever host shrubs or trees are growing. There is a moderate likelihood that resulting from the spread of a root disease-causing fungus the unwanted consequences to the environment and economy will be moderate to high. As a result the risk estimate for root disease-causing fungi associated with *Wollemia nobilis* whole plants or root-less cuttings imported from Australia is non-negligible and it is considered a hazard.

The likelihood that a root disease-causing fungus would be associated with *Wollemia nobilis* plants *in vitro* is considered negligible, and as such on this pathway root disease-causing fungi are not considered a hazard.

7.2.9 *Assessment of Uncertainty*

As the subject of this assessment is a group of fungi that could potentially have a very wide variety of biological and epidemiological characteristics. The assessment is further complicated by the fact that to date only one member of this fungal group, namely *Phytophthora cinnamomi*, has been recorded as associated with *Wollemia nobilis* nursery stock in Australia. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative fungal species included in the assessment. It is possible however, that should another root disease-causing fungus be recorded as being associated with *Wollemia nobilis* nursery stock, the results of this assessment would significantly underestimate the level or risk posed by the organism.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

7.3 Risk Management

7.3.1 *Risk Evaluation*

Since the risk estimate for root disease-causing fungi associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is non-negligible, phytosanitary measures will need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for root disease-causing fungi associated with *Wollemia nobilis* plants *in-vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

7.3.2 *Option Evaluation*

7.3.2.1 *Risk Management Objective*

To ensure any root disease-causing fungi associated with *Wollemia nobilis* whole plants and root-less cuttings in Australia are neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* whole plants and root-less cuttings imported from Australia.

7.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of root disease-causing fungi establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of root disease-causing fungi;

b) Inspecting all *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of root disease-causing fungi;

c) Treating all imported *Wollemia nobilis* whole plants and root-less cuttings before release into the New Zealand environment to ensure they are free of root disease-causing fungi;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* whole plants and root-less cuttings is released into the New Zealand environment.

Given that there are potentially a number of different genera and species of fungi in Australia that could act as root disease-causing fungi on *Wollemia nobilis* nursery stock, some of which may not as yet have been described, it is unlikely that a treatment will be identified that could decontaminate already infested plants. It is also unlikely, due to the difficulties detecting and delimiting an established population of some types of root disease-causing fungi, that an effective method of eradication or control could be developed for post-entry management of any unwanted consequences.

Management options therefore are limited to either ensuring *Wollemia nobilis* whole plants and root-less cuttings are free of root disease-causing fungi before packaging and transport to New Zealand or detecting and destroying infested plants before any *Wollemia nobilis* whole plants and root-less cuttings are released into the New Zealand environment.

Inspection efficacy

Roots infected by *A. rolfsii* become covered with thick, white strands of cottony mycelium bearing numerous spherical sclerotia. Sclerotia are white at first, later becoming tan to dark-brown. Roots infected by *P. vexans* have a water-soaked, necrotic appearance. The root tips are often attacked, although other areas of the root may also be infected. Infected plants may be smaller and have a less well-developed root system than healthy plants. Root inspections after allowing for disease development should provide an adequate indication of the presence of many root disease-causing fungi.

Pre-export and Shipment Inspection Requirements

The principle measure available for obtaining root disease-causing fungi freedom prior to export to New Zealand is through nursery management in Australia. Referring to the existing

management practices provided in 3.6.1 and Appendix 2, the following nursery management practices would be necessary to provide adequate assurance of root disease-causing fungi freedom:

- Accreditation under the Nursery Industry Accreditation Scheme, Australia (NIASA) as detailed in for foliage diseases in Chapter 6 section 6.3.2.2. Additional or more specific requirements to the NIASA scheme above those required for foliage diseases include:

    - To achieve an outcome that reduces the likelihood of exported plants being contaminated by root disease-causing fungi, it is recommended that plants in the consignment to be exported be raised from seeds or cuttings in soil-less media in containers maintained out of contact with the soil;

    - To provide adequate assurance of freedom from root disease-causing fungi, it is recommended that either prior to export or soon after arrival in New Zealand (e.g. one week may be an appropriate time frame) each plant should have their roots cleaned (e.g. washed) of potting media and the roots examined by an appropriately trained person. Any plants that have indications of root disease-causing activity shall not be included in the consignment for export to New Zealand.

The level of protection provided by this pre-export measure would be affected by the following attributes of root disease-causing fungi:

- *Infection within the nursery:* some of the root disease-causing fungi spread through air-borne spores. It is unlikely that a nursery in Australia could provide sufficient protection from root disease-causing fungal infection given the extent of propagule pressure within the surrounding environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for an extended period after infection.

- *Post inspection infection:* plants may become infected with root disease-causing fungi after the inspections have been completed but before the plants arrive in New Zealand.

These attributes of root disease-causing fungi would suggest that this measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants and root-less cuttings from Australia would not vector root disease-causing fungi into New Zealand.

Post Entry Quarantine

The principle measure available for detecting and removing infested plants before the consignment is released into New Zealand is through inspection while in post-entry quarantine. Contamination of nursery plants by root disease-causing fungi may not become apparent when conditions for the expression, such as humidity, temperature, and water levels, are not suitable or the infected plants are symptom-less hosts. The following post-entry quarantine conditions shall apply to ensure as far as is possible that disease expression will become apparent on infected plants that are not symptom-less hosts, and that the infecting fungi will remain contained within the quarantine facility:

- Post entry quarantine equivalent to Level 2 quarantine is considered appropriate as detailed in for foliage diseases in Chapter 6 section 6.3.2.2.

Symptoms of root disease-causing fungi are not necessarily easily visible on aerial portions of the plant as they are only likely to be associated with below-ground roots. Infections may be seen by carefully washing roots or using extraction procedures and placing under a dissecting microscope at a medium level of magnification. Given that plants can be physically damaged through excessive handling of root material, and the processes of root extraction and examination would require considerable resource, inspections should be limited to looking for more significant disease symptoms in plants grown in conditions that favour disease expression.

The following additional post-entry quarantine conditions should therefore apply:

- During the post-entry quarantine period, each plant should have their roots examined by an appropriately authorised person. Root inspections in this instance should not require any cleaning of potting mix residues and may be completed through clear-sided plant containers. Any plants that have indications of root disease activity should be treated (if appropriate), reshipped or destroyed. Depending on the type and nature of the hazard organism, host plants remaining in the quarantine facility may need to be treated and/or placed back under quarantine conditions (for a period determined by treatment type and organism biology), reshipped or destroyed.

The level of protection provided by this pre-export measure would be affected by the following attributes of root disease-causing fungi:

- *Propagule escape from the post-entry quarantine facility:* some root disease-causing fungi spread through air-borne spores. It may be difficult for a nursery facility in New Zealand to limit the exit of fungal spores into the environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for a period after infection that exceeds the duration of post-entry quarantine.

- *Masking of symptom expression by other diseases:* while the recommended enhancements to facility containment requirements related to micro-organism escape could be considered sufficient, the same level of containment is not currently recommended for micro-organism entry into the facility (e.g. HEPA filters are not recommended for inward flowing air). Root diseases entering the facility from the New Zealand environment and infesting plants may result in the expression of diseases symptoms that mask symptoms of diseases that the plants have vectored in from Australia.

It should be considered that the risks associated with the delay in symptom expression would be minimised for plants growing in an environment that is optimal for fungal growth. Optimal fungal growth would, however, considerably enhance the likelihood of propagule escape from the facility. This would occur if plants were not showing symptoms from some types of fungal infection and therefore would not be removed as propagule pressure increased. The recommended specifications for the facility are therefore to provide optimal conditions for fungal containment (e.g. HEPA filters and negative air pressure or equivalent).

To manage the potential risks associated with the masking of disease expression by diseases found in New Zealand, plants showing foliage disease symptoms should not be given biosecurity clearance. To limit as far as possible the level of fungal propagule pressure within post-entry quarantine, it is recommended that pre-export measures are also implemented.

7.3.2.3 *Recommended Management Options*

It is recommended that two measures are applied to reduce, to an acceptable level, the risk of root disease-causing fungi establishing in New Zealand and causing unwanted consequences.

i) Pre-export nursery management and pre-export and shipment inspection.

ii) Inspection during post-entry quarantine.

7.4 Assessment of Residual Risk

7.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with root disease-causing fungi.

The objective for inspection during post-entry quarantine is to ensure that any plants in consignment of *Wollemia nobilis* nursery stock imported into New Zealand from Australia that have become infested just prior to or during transport to New Zealand will be detected and not released into the New Zealand environment.

7.4.2 *Expected Performance of Measure(s)*

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with root disease-causing fungi. There should therefore be no detections of infested plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export infestation. In reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through long term monitoring or targeted research.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no imported *Wollemia nobilis* plants infected with root disease-causing fungi are released into the New Zealand environment. As *Wollemia nobilis* plants entering the New Zealand environment would be expected to become infested with already established root disease-causing fungi, some of which may not have been described by science, identifying a success measure for this option is more problematic. Therefore it is expected that a high level of assurance is obtained that the post-entry quarantine inspections were undertaken appropriately before any plants are released into the New Zealand environment. As above, in reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through targeted research.

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8. WOOD DECAY FUNGI

8.1 Hazard Identification

8.1.1 *Aetiologic agent*

Examples of wood decay fungi that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known to be or can not be confirmed as being present in New Zealand are listed in Appendix 1. Examples from the list include *Fomitopsis pinicola*, *Ganoderma lucidum* and *Phellinus noxius*. For the purposes of this analysis *Fomitopsis pinicola* and *Phellinus noxius* will be used as examples of this group of plant diseases.

It should be emphasised that at the time of undertaking the risk analysis none of these diseases have been found on *Wollemia nobilis* nursery stock, and their inclusion in this analysis is as representative examples only.

8.1.2 *New Zealand Status*

There are many indigenous and introduced wood decay fungi established in New Zealand. There are a great many more wood decay fungi existing overseas that have never established in New Zealand, a portion of which exist in Australia.

8.1.3 *Epidemiology*

The description of the epidemiology of wood decay fungi is based on example species: *Fomitopsis pinicola* and *Phellinus noxius*. Information on these organisms has been collated from the CABI Crop Protection Compendium 2006[34] with available or supplementary references provided. As these organisms are included to provide an indication of the possible biological nature of the risk posed by wood decay fungi, the epidemiological descriptions have been summarised to include only relevant and general organism characteristics.

Fomitopsis pinicola

Life cycle

The main habitat for *F. pinicola* is dead wood and its fruitbodies are reported to be very abundant both in managed and old-growth forests on logging slash and dead trees, whether standing or down (Sinclair and Lyon, 2005). The life cycle of *F. pinicola* begins when a basidiospore, produced by a perennial basidiocarp, comes into contact with a woody substrate. The basidiospore germinates to form a germ tube, which further expands into a hypha. Mating is accomplished by hyphal fusion (anastomosis), and can be achieved only between homokaryons of different mating types. The heterokaryotic mycelium continues to develop within a substrate, selectively removing cellulose and hemicellulose from wood. Following wood colonization, the fungus fruits, forming a basidiocarp of a new generation to complete the life cycle. Fruiting usually takes place when the substrate has been successfully

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

colonized and conditions are not optimal for further mycelial expansion (Cooke and Rayner, 1984).

In living trees, *F. pinicola* is presumed to act slowly (Sinclair and Lyon, 2005). Following natural infection to wounds, decay (reported to be mainly caused by *F. pinicola*), usually extended less than 1 metre in young *Tsuga heterophylla* and *Picea sitchensis* (Hennon and DeMars, 1997), and old-growth *Pinus sylvestris* (Storozhenko, 2003).

Epidemiology

Many different types of injury have been reported to be associated with *F. pinicola* decay: fire scars; logging wounds; scars made by falling trees; resin tapping injuries; broken and dead tops; sunscald lesions; stem cracks due to extensive radial growth; cankers that develop at sites of dwarf mistletoe infection; and injuries caused by longhorned beetles (Coleoptera: Cerambicydae). Branch stubs are also reported as possible entry points of *F. pinicola* (Sinclair and Lyon, 2005). However, the possibility cannot be excluded that it enters young trees through dead twigs or some other pathway and remains latent until a wound triggers fungal growth and wood decay (Sinclair and Lyon, 2005). It also has been reported that the fungus more readily attacks trees that are stressed while growing in disturbed environments.

Substrate size is an important environmental factor for fruiting of *F. pinicola*. The fungus prefers to form basidiocarps on large logs, trunks and stumps and is seldom observed on wood less than 12 cm in diameter (Stepanova, 1973). A significant positive correlation is observed between substrate diameter and occurrence of *F. pinicola* basidiocarps (Vasiliauskas *et al*., 2002).

Summary of Hazard Potential

It is possible that *F. pinicola* may enter a young tree through dead twigs or some other pathway and remain latent until a wound triggers fungal growth and wood decay. *F. pinicola* decay can result in many different types of injury to living trees. The minimum size of the young tree required for this to occur would be relatively large for the usual international trade in nursery stock.

Phellinus noxius

Life cycle

Phellinus noxius is spread in two main ways. The first is by windborne spores which can infect freshly cut tree stumps or fresh wounds (Sujan-Singh and Pandey, 1989). The second is by root-to-root contact (Lewis and Arentz, 1988). The leading edge of the mycelial sleeve will infect healthy roots of other trees if they touch. Infected root pieces can remain viable for many years in the soil. Long-term survival in soil is mainly through infected woody debris and 80-90% survival in soils of lower moisture content has been recorded (Chang, 1996). The fungus is confined mainly to tropical areas. In Taiwan at the limit of the northern tropics it is found mostly at lower altitudes on sandier soils in the southern areas, but not in the north (Chang and Yang, 1998).

Epidemiology

P. noxius attacks a wide range of tropical plants, although mostly trees. The leaves of an infected tree yellow and wilt and typical dieback symptoms result. Symptoms may develop slowly or the tree may wilt and become defoliated in only a few days.

The most characteristic symptom of this disease is the brown encrustation covering the surface of the diseased roots. This consists of brown mycelium in which soil and small stones are firmly embedded. The fungus moves towards the collar of the tree and occasionally the encrustation may be visible above ground level. In the diseased wood, dark lines are visible due to the presence of the fungal hyphae. In advanced stages of decay, the wood becomes light, dry and friable and honeycombed. It is one of several fungi associated with heart or butt rots of forest and timber trees.

There are only two risks to consider. Firstly, infection by spores is through freshly cut stumps. Therefore, preventing stumps being susceptible to infection by either poisoning the stump or removing it eliminates this risk. The second risk is from infected root fragments which may harbour viable fungus for up to 4 years in buried roots 3 inches in diameter. The accidental movement of such fragments in soil poses a risk of spreading the disease, and soil should not be removed from infested areas.

Summary of Hazard Potential

The movement of infected root fragments in soil poses the greatest risk of spreading the disease through the nursery stock pathway, although relatively large root pieces (7.5 cm) are required. *P. noxius* attacks a wide range of tropical plants, although mostly trees. The fungus is confined mainly to tropical areas.

8.1.4 *Hazard Identification Conclusion*

From the epidemiological information provided above it should be considered possible that wood decay fungi could establish in New Zealand from imported *Wollemia nobilis* nursery stock and cause an unwanted impact within New Zealand. Wood decay fungi are therefore considered a potential hazard requiring further assessment.

8.2 Risk Assessment

8.2.1 *Entry Assessment*

The potential for wood decay fungi to enter, establish and cause unwanted consequences in New Zealand is limited to *Wollemia nobilis* whole plants and root-less cuttings with a maximum stem diameter greater than 12 centimetres (5 inches). Infected plants would be considered an almost guaranteed pathway for the entry of these organisms into New Zealand, as their survival in-transit is linked to the health of the plants and the intention of any importer of nursery stock is to import healthy plants.

Plants *in vitro* would not be considered a pathway for the entry assuming the cultures in question are sterile (axenic).

8.2.2 *Conclusion of Entry Assessment*

The likelihood of wood decay fungi entering New Zealand on *Wollemia nobilis* whole plants or root-less cuttings with a maximum stem diameter greater than 12 centimetres (5 inches) that have become infected in Australia is very high and therefore is consider non-negligible. The likelihood of *Wollemia nobilis* whole plants or root-less cuttings with a maximum stem diameter less than 12 centimetres (5 inches) becoming infected with wood decay fungi in Australia is very low and therefore is consider negligible. As there is very unlikely to be any demand to import *Wollemia nobilis* whole plants or root-less cuttings with maximum stem diameters greater than 12 centimetres (5 inches), in the context of this pathway the likelihood of wood decay fungi entering New Zealand should be considered negligible.

The likelihood of wood decay fungi entering New Zealand on plants *in vitro* is considered negligible and this commodity will not be considered further in this assessment.

8.2.3 *Assessment of Uncertainty*

As the subject of this assessment is a group of fungi that could potentially have a very wide variety of biological and epidemiological characteristics. The assessment is further complicated by the fact that no members of this fungal group have been recorded as associated with the commodity in question, namely *Wollemia nobilis* nursery stock from Australia. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative fungal species included in the assessment. It is possible however, that should an actual wood decay fungus be recorded as being associated with *Wollemia nobilis* nursery stock, the results of this assessment would significantly underestimate the level or risk posed by the organism.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

8.3 Risk Management

8.3.1 *Risk Evaluation*

Since the risk estimate for wood-decay fungi associated with *Wollemia nobilis* whole plants or root-less cuttings imported from Australia with a maximum stem diameter greater than 12 centimetres (5 inches) is non-negligible, phytosanitary measures will be required to manage the risks to an acceptable level.

The risk estimate for wood-decay fungi associated with *Wollemia nobilis* whole plants or root-less cuttings with a maximum stem diameter less than 12 centimetres (5 inches) and plants *in vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

8.3.2 *Option Evaluation*

8.3.2.1 *Risk Management Objective*

To ensure any new wood-decay fungi associated with *Wollemia nobilis* nursery stock in Australia are neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

8.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of wood-decay fungi establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings with a maximum stem diameter greater than 12 centimetres (5 inches) before packaging and transport to New Zealand to ensure they are free of wood-decay fungi;

b) Inspecting all *Wollemia nobilis* whole plants or root-less cuttings with a maximum stem diameter greater than 12 centimetres (5 inches) before packaging and transport to New Zealand to ensure they are free of wood-decay fungi;

c) Limiting the size of *Wollemia nobilis* nursery stock exported to New Zealand to stem diameters of less than 12 centimetres (5 inches);

d) Treating all imported *Wollemia nobilis* whole plants or root-less cuttings with a maximum stem diameter greater than 12 centimetres (5 inches) before release into the New Zealand environment to ensure they are free of wood-decay fungi;

e) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* nursery stock is released into the New Zealand environment.

Given that there are potentially a number of different genera and species of fungi in Australia that could act as wood-decay fungi on *Wollemia nobilis*, some of which may not as yet have been described, it is unlikely that a treatment will be identified that could decontaminate already infested plants (see Appendix 3). It is also unlikely, due to the difficulties detecting and delimiting an established population of an introduced wood-decay fungus, that an effective method of eradication or control could be developed for post-entry management of any unwanted consequences.

As indicated earlier it is considered very unlikely that there will be any demand to import *Wollemia nobilis* whole plants or root-less cuttings with maximum stem diameters greater than 12 centimetres (5 inches). Restricting the consignments of *Wollemia nobilis* nursery

stock to whole plants or root-less cuttings with maximum stem diameters less than 12 centimetres (5 inches) would seem to be the most simple and cost-effective measure.

8.3.2.3 *Recommended Management Options*

It is recommended that consignments of nursery stock should be limited to specimens that have maximum stem diameters less than 12 centimetres (5 inches).

8.4 Assessment of Residual Risk

8.4.1 *Objectives for Recommended Management Option(s)*

The objective of limiting plant size to specimens that have maximum stem diameters less than 12 centimetres (5 inches) is to ensure no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with wood-decay fungi.

8.4.2 *Expected Performance of Measure(s)*

The plant stem diameter restriction on consignments is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with wood-decay fungi. There should therefore be no detections of infested plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export infestation.

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9. CANKER FUNGI

9.1 Hazard Identification

9.1.1 *Aetiologic agent*

Examples of canker-causing fungi that are associated with plants in the Araucariaceae and are known to be present in Australia but are not known to be or can not be confirmed as being present in New Zealand are listed in Appendix 1. Examples from the list include *Macrophoma araucariae* and *Pestalosphaeria gubae*.

The *Fusicoccum* genus and *Macrophoma araucariae* are both believed to be anamorphic to *Botryosphaeria* and as such can be considered under Chapter 11 of this risk analysis.

9.1.2 *New Zealand Status*

There are many indigenous and introduced canker-causing fungi established in New Zealand. There are a great many more canker-causing fungi existing overseas that have never established in New Zealand, a portion of which exist in Australia.

10. MYCORRHIZAL FUNGI

10.1 Hazard Identification

10.1.1 *Aetiologic agent*

Fungi of the Zygomycete order Glomales for the arbuscular mycorrhizae, and largely Basidiomycetes for ectomycorrhizae, forming associations with plant roots.

10.1.2 *New Zealand Status*

There many species of mycorrhizal fungi world wide. It is highly likely that there will be species of mycorrhizal fungi in Australia that are not currently present or established in New Zealand.

10.1.3 *Epidemiology*

Mycorrhizal associations form when host roots and compatible fungi are both active in close proximity and the soil conditions are favourable[35]. Unlike fungal pathogens, mycorrhizal fungi form symbiotic relationships with their hosts, gaining food and nutrients from the plant in return for specific nutrients from the fungi. A diverse range of fungi can form mycorrhizal associations with terrestrial plants. Phyla include the Zygomycotina, Ascomycotina, Basidiomycotina, and Fungi Imperfecti.

Harley J. L. and Harley E. L. (1987) divide mycorrhizae into three broad groups: ectomycorrhizae, ectendomycorrhizae and endomycorrhizae. Arbuscular mycorrhizae fall within the endomycorrhizae, and form by far the most common kind of mycorrhizae. Endomycorrhizae are characterised by hyphae that penetrate both between and into the living cells of the cortex and epidermis of plant roots.

Arbuscular mycorrhizal fungi are all members of the Endogonaceae (Zygomycotina) and are characterised by the formation of arbuscules, in which penetrating hyphae branch repeatedly in a dichotomous manner to form a complex branched haustorium, and vesicles, which are swollen multinucleate bodies enclosed in a thick wall and formed in cells or intercellular spaces (Harley and Harley 1987). Ectomycorrhizae are characterised by hyphae that penetrate between the living cells of the cortex and epidermis of plant roots and form fan-like systems within the walls that are referred to as the Hartig net. Ectomycorrhizae are common in roots of shrubs and trees, but are not common or very rare in herbaceous plants (Harley and Harley 1987).

Arbuscular mycorrhizal fungi form associations with angiospermous and gymnospermous herbs, shrubs and trees, pteridophytes and bryophytes. The fungi are virtually non-specific, infecting a very wide range of host plants (Harley and Harley 1987). Unlike arbuscular mycorrhizal fungi, ectomycorrhizal fungi have differing levels of host specificity. A given species of ectomycorrhizal fungus is usually only able to establish mutualistic symbiosis with a number of species of plants from the same bio-geographical realm (Diez J. 2005). Most forest trees form ectomycorrhizae with a polyphyletic group of basidiomycetes and

35 http://www.ffp.csiro.au/research/mycorrhiza

ascomycetes (Diez 2005). Although uncommon among gymnosperms, ectomycorrhizae are found in the *Pinaceae* forming specific and obligatory associations with a small number of fungi (McGee *et. al.* 1999). This specificity can mean that new plant introductions, such as pine into the southern hemisphere, can be delayed until a suitable ectomycorrhizal fungi are introduced into the area. Some of these pines subsequently invaded a wide range of systems with the introduction of their ectomycorrhizal fungi (Diez 2005).

Two types of mycorrhizal fungi have been found with the roots of the *Wollemia nobilis*: *arbuscular mycorrhizae* and *ectomycorrhizae* (NWPS 1998). Finding two types of mycorrhizal fungi was unexpected as previously only the arbuscular type has been found in the Araucariaceae. Of all the fungi found living on or near the trees at least one third are new to science and probably specific to the particular habitat of *Wollemia nobilis*. There is, however, no evidence that any of the fungi noted are essential to the survival of *Wollemia nobilis* and so do not need to be imported with this species (NPWS 1998).

Only three New Zealand native woody plant genera, *Kunzea*, *Leptospermum*, and *Nothofagus*, are known to form ectomycorrhizal associations in the field (Orlovich and Cairney 2004). A total of 76 genera of known or probable ectomycorrhizae fungi have been recorded as associated with ectomycorrhizal plants in New Zealand. This includes 1 Zygomycete, 11 Ascomycete, and 64 Basidiomycete genera. 10 of these genera have been reported in association with only introduced tree species, while 24 genera occur in association with either native or introduced plant species. Species from a number of genera have been collected under both native and introduced ectomycorrhizal hosts (Orlovich and Cairney 2004).

The hyphae in the root are connected to an external mycelium which may penetrate the soil for several centimetres. This external mycelium bears a variety of reproductive organs according to genus and species (sporocarps, azygospores or chlamydospores etc), which may act as sources of infection. Ectomycorrhizae that are basidiomycetes can form obvious hypogeous or epigeous fruiting bodies. Spores can remain viable in soil for some months (Harinikumar and Bagyaraj 1994), whereas hyphae resulting from spore germination have a limited capacity to grow and will die if they do not encounter a susceptible root within one to two weeks. Hyphae in root fragments may also be viable. Plants potted into soil, non-sterile soil or media may be infected by mycorrhizal fungi. Viable spores or root fragments may be transferred from the ground to a potted plant or one plant to another via gardening tools or equipment, such as trowels or gloves.

Mycorrhizae are not easily visible to the naked eye. Spores and hyphae may be seen by carefully washing roots or using extraction procedures and placing under a dissecting microscope at a medium level of magnification. More detailed observation requires specific treatment and use of a compound microscope. The fruiting bodies of ectomycorrhizal fungi are usually conspicuous, such as the epigeous *Amanita's* (a common toadstool) or hypogeous *Tuber's* (truffles)[36].

Many species of Glomalean fungi have worldwide distribution patterns and have apparently adapted to diverse habitats. However, it is known that soil factors such as pH restrict the distribution of some taxa (Abbott & Robson 1991) and some of these widespread taxa are now known to comprise more than one species (Morton 1988). Much of the functional

36 http://www.ffp.csiro.au/research/mycorrhiza

diversity of Glomalean fungi occurs at the isolate level rather than species level (Brundrett 1991, Morton & Bentivenga 1994). Consequently, habitat information is as important as knowledge of the taxonomic identity of fungi, for comparing the results of experiments, or the selection of isolates for practical use.

Changes in populations of Glomalean fungi have been observed when ecosystems are converted to monocultures or severely disturbed, providing indirect evidence for habitat preferences by these fungi (Abbott & Robson 1991, Brundrett 1991). There is also experimental evidence that the performance (measured as increased host plant growth) of fungal isolates is related to environmental factors (Brundrett 1991). Kilronomos (2003) goes further to say that arbuscular mycorrhizal fungi can function along a continuum from parasitism to mutualism.

10.1.4 *Hazard Identification Conclusion*

It is feasible that mycorrhizal fungi could establish in New Zealand from imported *Wollemia nobilis* nursery stock and cause an unwanted impact within New Zealand. Mycorrhizal fungi are therefore considered a potential hazard requiring further assessment.

10.2 Risk Assessment

10.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. Roots of a plant that has formed an association with mycorrhizal fungi would be considered an almost guaranteed pathway for the entry of the fungi into New Zealand. Hyphae from mycorrhizal fungi penetrate both between and into the living cells of the cortex and epidermis of plant roots (Harley and Harley 1987) and are therefore protected by the plant from external or physical treatments such as soil removal or root washing. Whole plants of *Wollemia nobilis* cultivated in soil would be expected to form mycorrhizae associations within a relatively short period, a few weeks or months (Bellgard 1992), after germination or transplanting.

Whole plants of *Wollemia nobilis* not otherwise infected before packaging and transportation to New Zealand could become associated with mycorrhizal fungi if they were stored for a length of time immediately adjacent to other plants or soil products that have developed or contain mycorrhizae.

As mycorrhizal fungi are only likely to be associated with plant roots, cuttings with no roots should not be considered a pathway for the entry into New Zealand of these fungi. Plants *in vitro* would also not be considered a pathway for the entry assuming the cultures in question are indeed sterile.

10.2.2 *Conclusion of Entry Assessment*

The likelihood of mycorrhizal fungi entering New Zealand on whole plants of *Wollemia nobilis* that have been exposed to contaminated soil in Australia is very high and therefore is consider non-negligible.

The likelihood of mycorrhizal fungi entering New Zealand on root-less cutting or plants *in vitro* is considered negligible and these two commodities will not be considered further in this assessment.

10.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

When considering limitations on the ability of mycorrhizal fungi to move from an infested imported plant to a host on which it can establish, mycorrhizal fungi will not kill the host (they form symbiotic relationships) and are themselves unlikely to become necrotic once associated with the host. The only remaining option for avoiding exposure without intervention is to keep the plants from being exposed to the New Zealand environment. As the intention of any importer would be to plant any imported *Wollemia nobilis* nursery stock into the New Zealand environment, this final limitation to exposure is removed.

10.2.4 *Establishment Assessment*

As indicated in the exposure assessment, it is the intention of any importer would be to plant any imported *Wollemia nobilis* nursery stock into the New Zealand environment, therefore allowing any associated mycorrhizal fungi to establish a viable population.

10.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that the imported and contaminated *Wollemia nobilis* whole plants themselves can act as the agent for exposure and establishment of mycorrhizal fungi and the intention of the applicant is to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, the likelihood of exposure and establishment is very high and therefore non-negligible.

10.2.6 *Consequence Assessment*

In the context of the pathway for importing nursery stock contaminated with mycorrhizal fungi, any potential consequences to people, the New Zealand environment, and the New Zealand economy will only become apparent after establishment and some degree of spread. As mycorrhizal fungi are symbiotic, by their nature they are only likely to cause positive impacts on plant growth of their natural hosts. Potential unwanted consequences could occur should a member of the mycorrhizal fungi family become more widespread within the New Zealand environment. Examples of potentially unwanted impacts include:

- Displacing an existing indigenous or endemic mycorrhizal fungus from an area or ecological niche and altering the relative competitiveness of a native or introduced host plant such that the existing environment is adversely affected. Displacing endemic mycorrhizal fungi may itself be considered an adverse consequence to ecological values;

- Displacing or out competing an economically important mycorrhizal fungus. Examples of economically important mycorrhizal fungi include truffles and some fungi forming mycorrhizae with important agricultural or forestry crops;

- Acting as a parasite (pathogen) on a host within New Zealand that is not a natural host of the mycorrhizal fungus;

- Becoming available to an introduced host such that the host becomes invasive and causes unwanted consequences where it would otherwise not have been able to.

Evidence for these potential unwanted consequences is already available in New Zealand and offshore examples. *Amanita muscaria* (fly agaric) probably entered New Zealand in the 1800's and was noticed to be mycorrhizal on native beech *Nothofagus* species about the mid 1900's, mostly on tracks through forest but quite distant from exotic trees. There are a large number of native mycorrhizal species on *Nothofagus* so it is surprising an exotic species can take the niche, which suggests species displacement (P. Buchanan, pers. comm., 22 Dec 2005). *Chalciporus piperatus* has also been recorded as forming mycorrhizal associations with New Zealand native *Nothofagus* species (Jackson and Buchanan, 1997 in Orlovich and Cairney, 2004). The association of the eucalypt ectomycorrhizal fungus *Laccaria fraterna* with *Cistus ladanifer*, a shrub native to Spain in the Iberian Peninsula (Diez 2005) is also a warning sign that mycorrhizal fungi can be invasive, even if challenging to quantify currently (P. Buchanan, pers. comm. 3 Apr 2006).

Marler *et al* (1999) found that *in situ* mycorrhizal fungi increase the ability of an exotic plant invader to suppress a native competitor. Exotic mycorrhizal fungi could find suitable exotic hosts and enhance their competitiveness to the detriment of native plant species. Kilronomos (2003) concluded from experiments exposing large numbers of hosts to different mycorrhizal fungi that mycorrhizal inoculations within an ecosystem can range from highly parasitic to highly mutualistic.

As indicated, however, while the available evidence does suggest that exotic mycorrhizal fungi can spread into existing ecosystems within New Zealand, further research would need to be undertaken to determine whether this invasiveness is resulting or would result in significant unwanted consequences to the New Zealand environment or economy.

While it is possible that fruiting bodies or spores produced by any fungus could, through being toxic or by inducing an allergic reaction, cause unwanted consequences to human health within New Zealand, there is no evidence to date suggesting that mycorrhizal fungi have done or could do so. Some species of basidiomycete mycorrhizal fungi produce poisonous fruiting bodies (mushrooms) that can be mistaken for edible mushrooms. While the impacts to human health from these fungi are limited in frequency, they can be significant when they occur.

Potential for spread of arbuscular mycorrhizal fungi

Initial and continued dispersal of mycorrhizal fungi is likely via a number of vectors. Many mycorrhizal fungi sporulate in the soil and depend on external agents for long range dispersal, initial and continued dispersal is dependent on these vectors (Gerdeman and Trappe, 1974 in Gange 1993). Soil fauna activity is thought to bring mycorrhizal propagules to the surface where wind and rain may disperse them (Gange 1993). Earthworms and other soil invertebrates (e.g. ants) are also considered to disperse mycorrhizal fungi by ingestion of spores or hyphal fragments that remain viable after expulsion in castings or faeces (Gange, 1993; Klironomos and Moutoglis 1999; Harinikumar and Bagyaraj, 1994). Many mycorrhizal

fungi sporulate from above ground dispersal structures allowing for effective and long distance aerial dispersal. Human disturbance may mediate spore dispersal by exposure of spores to wind or by carrying spores on footwear or equipment. Gardening implements such as secateurs, wheelbarrows, trowels, forks, or spades, and protective garments such as overalls, gloves and boots can retain infected potting medium or root fragments and be reused in another area.

There is also evidence suggesting that sporocarpic fungi are adapted for dispersal by rodents and small mammals. Sporocarps and spores of fungi from the suborder Glomineae were found in faecal samples from *Proechimys* (spiny rats) and *Oryzomys* (Mucidae: rice rats) (Janos and Sahle, 1995). In New Zealand, Cowan (1989) recorded possums feeding on the sporocarps of *Glomus macrocarpum*, an arbuscular mycorrhizal fungus established in New Zealand. Prior to the introduction of exotic mammals into New Zealand, insects and ground feeding birds are presumed to have aided dispersal (McKenzie 2004).

The low specificity of some mycorrhizal fungi relationships and the easy acquisition of mutualistic symbionts by herbs and shrubs in any ecosystems are important reasons for so many ecosystems being susceptible to invasion by alien plants (Diez J. 2005). Many species of Glomalean fungi have worldwide distribution patterns and have apparently adapted to diverse habitats (Abbott & Robson 1991).

It is therefore highly likely that, given:

- the high number and variety of vectors available within New Zealand for the distribution of mycorrhizal fungi; and

- the assumption that many of the non-endemic plant species would form symbiotic associations with these mycorrhizal fungi; and

- the assumption that these mycorrhizal fungi would be immediately suited to or relatively quickly adapt to habitats present in the greater part of New Zealand;

should mycorrhizal fungi become established in New Zealand on or from imported *Wollemia nobilis* nursery stock, they would spread over an extended period throughout New Zealand wherever herbs, trees and/or shrubs are growing. Once widely distributed these fungi could not be removed from the environment although in cultivated areas it may be possible to mitigate through management some of the unwanted consequences should they occur.

10.2.7 *Conclusion of Consequence Assessment*

The establishment in New Zealand of mycorrhizal fungi imported on whole plants of *Wollemia nobilis* has a low likelihood of indirectly causing minor but wide ranging and irreversible unwanted consequences to the New Zealand environment and economy. The potential consequence of the establishment of mycorrhizal fungi imported on whole plants of *Wollemia nobilis* in New Zealand should therefore be considered non-negligible.

10.2.8 *Risk Estimation*

The likelihood is high that whole plants of *Wollemia nobilis* would become associated with a mycorrhizal fungi while growing in Australia, be transported to and enter into New Zealand

still infected with the fungus, and form an established population of the mycorrhizal fungus once the plant is grown in the New Zealand environment. There is also a high likelihood that, given sufficient time, the contaminating fungus will spread throughout New Zealand wherever herbs, trees and/or shrubs are growing. There is a low likelihood that resulting from the spread of these mycorrhizal fungi the unwanted consequences to the environment will be low to moderate and to the economy will be moderate. As a result the risk estimate for mycorrhizal fungi associated with *Wollemia nobilis* whole plants imported from Australia is non-negligible.

The likelihood that mycorrhizal fungi would be associated with *Wollemia nobilis* root-less cuttings or plants *in vitro* is considered negligible, and as such on these pathways the mycorrhizal fungi are not considered a hazard.

10.2.9 *Assessment of Uncertainty*

The subject of this assessment is a group of fungi that could potentially have a very wide variety of biological and epidemiological characteristics. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative fungal species included in the assessment.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored. Research should also be undertaken to determine whether the invasiveness of mycorrhizal fungi is resulting or would result in significant unwanted consequences to the New Zealand environment or economy

10.3 Risk Management

10.3.1 *Risk Evaluation*

While the risk estimate for mycorrhizal fungi associated with *Wollemia nobilis* whole plants imported from Australia is non-negligible, phytosanitary measures are not considered necessary for the following reasons:

- The likelihood of any minor but wide-ranging potential impacts is considered low;
- The overall level of risk will be further reduced by phytosanitary measures being targeted at other types or organisms;
- There is no effective and justifiable measures available to manage the risk adequately (see the following paragraphs).

The risk estimate for mycorrhizal fungi associated with *Wollemia nobilis* root-less cuttings and plants *in vitro* imported from Australia is negligible and as such no phytosanitary measures will be required.

While no actions are recommended for mycorrhizal fungi associated with imported *Wollemia nobilis* whole plants, this applies to unintentional organism associations only. Any importer that intends to deliberately or knowingly import into New Zealand *Wollemia nobilis* whole

plants infected by species of mycorrhizal fungi that are new to New Zealand must first apply to the Environmental Risk Management Authority New Zealand for permission to do so.

10.3.2 *Option Evaluation*

10.3.2.1 *Risk Management Objective*

Should measures be required they would need to ensure that mycorrhizal fungi associated with *Wollemia nobilis* nursery stock in Australia are neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

10.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of mycorrhizal fungi establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants before packaging and transport to New Zealand to ensure they are free of mycorrhizal fungi;

b) Inspecting all *Wollemia nobilis* whole plants before packaging and transport to New Zealand to ensure they are free of mycorrhizal fungi;

c) Treating all imported *Wollemia nobilis* whole plants before release into the New Zealand environment to ensure they are free of mycorrhizal fungi;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* whole plants are released into the New Zealand environment.

Given that there are potentially a number of different genera and species of fungi in Australia that could act as mycorrhizal fungi on *Wollemia nobilis*, many of which may not as yet have been described, it is unlikely that a treatment will be identified that could decontaminate already infested plants. It is also unlikely, due to the difficulties detecting and delimiting an established population of introduced mycorrhizal fungi, that an effective method of eradication or control could be developed for post-entry management of any unwanted consequences.

Management options therefore are limited to either ensuring *Wollemia nobilis* whole plants are free of mycorrhizal fungi before packaging and transport to New Zealand, or infested plants are detected and destroyed before any *Wollemia nobilis* nursery stock is released into the New Zealand environment. Mycorrhizal fungi are not easily visible to the naked eye and are only likely to be associated with below-ground roots. Spores and hyphae may be seen by carefully washing roots or using extraction procedures and placing under a dissecting microscope at a medium level of magnification. Given that plants can be physically damaged

through excessive handling of root material, and the processes of root extraction and examination would require considerable resource, inspections would be limited to looking for evidence of mycorrhizae in plants grown in conditions that optimise mycorrhizal development.

Available pre and post arrival clearance options are provided in Chapter 7 for Root Diseases except as otherwise stated below:

Pre-export Requirements

- To provide adequate assurance of freedom from mycorrhizal fungi, it is recommended that either just prior to export or soon after arrival in New Zealand (e.g. one week may be an appropriate time frame) each plant would need to have their roots washed of potting media and the roots examined by an appropriately trained person. Any plants that have indications of mycorrhizal activity shall not be included in the consignment for export to or post-entry quarantine in New Zealand.

The level of protection provided by this pre-export measure would be affected by the following attributes of mycorrhizal fungi:

- *Infection within the nursery:* many mycorrhizal fungi spread through air-borne spores. It is unlikely that a nursery in Australia could provide sufficient protection from mycorrhizal fungal infection given the extent of propagule pressure within the surrounding environment.

- *Delay in symptom expression:* visually detectable symptoms may never become apparent in the host plants.

- *Post inspection infection:* plants may become infected with mycorrhizal fungi after the inspections have been completed but before the plants arrive in New Zealand.

These attributes of mycorrhizal fungi suggest that this measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants from Australia would not vector foliage disease-causing organisms into New Zealand.

Post Entry Quarantine

As it has not been recommended that the containment requirements of post-entry quarantine facilities in New Zealand are sufficient to exclude the entry of micro-organisms beyond what can be achieved through plant and facility hygiene practices, it is likely that plants within quarantine facilities will become infected with mycorrhizal fungi originating from the New Zealand environment. Distinguishing these established mycorrhizal fungi from any introduced species is not possible at this time in the great majority of instances. It is therefore recommended that other than the hygiene and facility management recommendations made in chapter 7 for root diseases, no further measures could be applied in post-entry quarantine for mycorrhizal fungi not otherwise causing disease symptoms on *Wollemia nobilis* nursery stock.

10.3.3 *Recommended Management Options*

No measures are recommended for mycorrhizal fungi on *Wollemia nobilis* nursery stock.

10.4 Assessment of Residual Risk

10.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management would be to ensure no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with mycorrhizal fungi.

The objective for inspection during post-entry quarantine would be to ensure that any plants in consignment of *Wollemia nobilis* nursery stock imported into New Zealand from Australia, that have become infested just prior to or during transport to New Zealand with mycorrhizal fungi that are pathogenic on *Wollemia nobilis*, will be detected and treated before being released into the New Zealand environment.

10.4.2 *Expected Performance of Measure(s)*

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with mycorrhizal fungi. In reality the effectiveness of this measure would be less than 100%, but it is probable that this actual level will only be determined through long term monitoring or targeted research.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock infected with mycorrhizal fungi that act as pathogens on *Wollemia nobilis* are released into the New Zealand environment. As *Wollemia nobilis* plants entering the New Zealand environment would be expected to become infested with already established mycorrhizal fungi, many of which may not have been described by science, identifying a success measure for this option is more problematic. Therefore it is expected that a high level of assurance is obtained that the post-entry quarantine inspections were undertaken appropriately before any plants are released into the New Zealand environment. As above, in reality the effectiveness of this measure would be less than 100%, but it is probable that this actual level will only be determined through targeted research.

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11. *BOTRYOSPHAERIA* SPECIES

11.1 Hazard Identification

11.1.1 *Aetiologic agent*

Botryosphaeria sp.

11.1.2 *New Zealand Status*

There are species of *Botryosphaeria* that are present in Australia and New Zealand (e.g. *B. ribis*), and species of *Botryosphaeria* that are present in Australia and not present in New Zealand.

11.1.3 *Epidemiology*

For the purposes of this hazard identification, the epidemiological characteristics of a well researched species of *Botryosphaeria*, *Botryosphaeria ribis*, will be described. Information on this organism has been collated from the CABI Crop Protection Compendium 2006[37] with available or supplementary references provided. As this organism has been included to provide an indication of the possible biological nature of the risk posed by *Botryosphaeria* sp., the epidemiological description has been summarised to include only relevant and general organism characteristics.

Botryosphaeria ribis

Taxonomy

Most taxa in *Botryosphaeria* are referred to as a representative of the *B. dothidea*/*ribis* complex. Science has only recently been able to distinguish *B. ribis* and *B. dothidea*, as well as closely related *B. parva* and *B. lutea* (Slippers *et al*., 2004). Isolates from *Wollemia nobilis* included two *Botryosphaeria* species; the first being closely related to *B. ribis* but with some similarities to *B. parva* while the other grouped with *B. australis*, a new species from Australia. All the taxa identified on *Wollemia nobilis* had *Fusicoccom* anamorphs (Slippers *et al*., 2005).

Life Cycle

Under suitable conditions (wet periods) *B. ribis* produces pseudoparenchymatous structures that may contain either ascogenous (ascostromata) or pycnidial (pycnidial stromata or conidiomata) locules. Conidiomata are produced abundantly on necrotic tissues of infected plant parts, ascostromata are sometimes observed in field conditions but are rarely produced on artificial growth media. Both structures may occur on the same general area of an affected plant part at the same time. These ascogenous and conidial locules may also occur in the same stroma (Luttrell, 1950). Discharge of conidia and ascospores is accelerated by a period of wetness. Conidia are sticky and are produced abundantly on the surface of infected stems

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

or fruits. The majority of the ascospores and conidia are dispersed by air and rainwater, respectively. Viable conidia, ascospores or hyphal fragments that come in contact with a susceptible host will germinate or grow and colonize tissue. Spore germination requires adequate moisture and a suitable temperature range. For example, at 100% ambient humidity, a temperature range of 25-35°C supported over 90% germination of macroconidia within a 4 hour incubation period. During the same humidity and incubation period, germination of macroconidia was arrested at 5°C and rendered non-viable at 45°C (Rayachhetry *et al*., 1996c).

Disease Characteristics

The genus *Botryosphaeria* are commonly associated with stem cankers, leaf spots and fruit rots of many hosts (Crous and Groenewald, 2005). The epidemiology of *B. ribis*, however, has not been fully determined for most susceptible hosts. All three forms of *B. ribis* inocula (hyphal fragments, conidia and ascospores) are capable of initiating infection on host plants. *B. ribis* over winters in dead stems and perennial cankers as active mycelia, as well as pseudoparenchymatous stroma on bark (Maas and Uecker, 1984; Michailides, 1991). The transmission of *B. ribis* within and among adjacent trees may occur through stem run-off, raindrop splashing (Pusey, 1989) and pruning equipment (Schreiber, 1964). Similarly, the long-distance transmission of this fungus may occur through air, pruning equipment, insects and birds. Colonization of tissues of susceptible hosts by *B. ribis* occurs through wounds, lenticels, stomata, branch/twig stubs, frost cracks and sun-scorched bark. Stems of healthy plants callus rapidly but the fungus may perpetuate underneath callus and may result in perennial canker when such plants are stressed (Rayachhetry *et al*., 1996b). In general, extreme temperature, drought and repeated defoliation predispose woody plants to invasion by *B. ribis* (Rayachhetry *et al*., 1996a). Disease development is generally rapid among stressed plants. Terminal portions of the affected twigs or branches of walnut die soon after they are girdled (Rumbos, 1987). Winter and spring infection of the healthy petioles, leaves and fruits is initiated by the inoculum from dead plant parts from the previous season and the perennial stem cankers. Current-season plant parts die following rapid expansion of lesions (Michailides, 1991).

Perennial cankers and lesions on stems and fruit-rot can be easily detected under field and storage conditions. Such disease symptoms may also be caused by other fungal pathogens such as *Botryosphaeria obtusa, B. quercum, B. rhodina* and species of *Botryodiplodia* (depending on the host plant) (Sinclair *et al*., 2005). Dead tissues around the diseased areas should be examined for signs of conidiomata or ascostromata. If found, these can be used for preliminary identification of agents associated with the disease. When conformation is needed, aseptically obtained segments of tissue from lesions should be cultured in appropriate media to induce sporulation for positive identification.

Botryosphaeria species in Australia act as endophytes and stress-related pathogens of various woody hosts (Slippers *et al*., 2005). *Botryosphaeria* species were also found to be highly pathogenic to *Wollemia nobilis*, killing plants in the glasshouse within 4 weeks (Bullock *et al*., 2000).

Summary of hazard assessment

Botryosphaeria species have been recorded on *Wollemia nobilis* in Australia and have been found to be highly pathogenic to their host. The genus *Botryosphaeria* are commonly associated with stem cankers, leaf spots and fruit rots of many hosts. Long-distance transmission of this fungus may occur through air, pruning equipment, insects and birds as well as the trade in nursery stock.

11.1.4 *Hazard Identification Conclusion*

Based on the epidemiological information provided above it is considered that species of *Botryosphaeria* present in Australia could establish in New Zealand from imported *Wollemia nobilis* germplasm, and cause an unwanted impact. *Botryosphaeria* species are therefore considered a potential hazard requiring further assessment. It should also be noted that, given the uncertainties around what is considered species complexes of the more widely distributed *Botryosphaeria* species, the potential hazards should be considered for all representatives of the *Botryosphaeria* including those otherwise considered established in New Zealand.

11.2 Risk Assessment

11.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. Aerial parts of a plant that have become infected by a *Botryosphaeria* sp. would be considered an almost guaranteed pathway for the entry of the fungi into New Zealand. *Wollemia nobilis* nursery stock not otherwise infected before packaging and transportation to New Zealand could become infected by *Botryosphaeria* sp. if they were stored for a length of time immediately adjacent to other plants that have developed *Botryosphaeria* sp. disease symptoms.

As *Botryosphaeria* sp are associated with all aerial plant parts both whole plants and root-less cuttings should be considered potential pathways for the entry into New Zealand of these fungi. Plants *in vitro* would not be considered a pathway for the entry assuming the cultures in question are indeed sterile (axenic).

11.2.2 *Conclusion of Entry Assessment*

The likelihood of *Botryosphaeria* sp entering New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* that has become infected in Australia is very high and therefore is consider non-negligible.

The likelihood of *Botryosphaeria* sp. entering New Zealand on plants *in vitro* is considered negligible and this commodity will not be considered further in this assessment.

11.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

It is possible that a serious infection of a *Botryosphaeria* species could kill the host and effectively prevent the host and the associated disease from establishing in New Zealand. This would only be the case for the most pathogenic species of *Botryosphaeria* found on *Wollemia nobilis* nursery stock. It is unlikely that a *Botryosphaeria* infection would become necrotic in the host and therefore not be able to spread from the host into the New Zealand environment, as species of *Botryosphaeria* can act as an endophyte. The only remaining option for avoiding exposure is if the host plants are not moved into the New Zealand environment. As the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, this final limitation to exposure is removed.

11.2.4 *Establishment Assessment*

As indicated in the exposure assessment, the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, therefore allowing any *Botryosphaeria* infection to establish a viable population.

11.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that the imported and contaminated *Wollemia nobilis* plants themselves can act as the agent for exposure and establishment, and the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, the likelihood of exposure and establishment is very high and therefore non-negligible.

11.2.6 *Consequence Assessment*

In the context of the pathway for importing nursery stock contaminated with *Botryosphaeria* sp., any potential consequences to people, the New Zealand environment, and the New Zealand economy will only become apparent after establishment and some degree of spread.

Potential for spread

Botryosphaeria species can produce abundant conidia on the surface of infected stems or fruits. The majority of the ascospores and conidia are dispersed by air and rainwater, respectively. Usually, spore discharge occurs just after periods of surface wetness. Waterborne ascospores and conidia may be present in rainwater run-off from diseased and pruned limbs. The transmission of *Botryosphaeria* within and among adjacent plants may occur through stem run-off, raindrop splashing and pruning equipment. Similarly, the long-distance transmission of this fungus may occur through air, pruning equipment, insects and birds. Species of *Botryosphaeria* are considered in many cases to have a wide potential host range, making potential hosts widely available. A species of *Botryosphaeria* introduced into the New Zealand environment from *Wollemia nobilis* nursery stock would be expected to spread relatively rapidly over its maximum potential range.

Likely consequences

Stem blight of blueberry caused by a *Botryosphaeria* species starts with leaf browning or reddening due to rapid wilting which is often followed by mortality of entire plant as the fungus progresses through the vascular tissue. The fungus can reduce the fruit yield of thornless blackberry to an uneconomic level (Maas and Uecker, 1984). A 40-80% crop loss has also been reported for pistachio in commercial orchards in California (Michailides, 1991). If plant health is not properly maintained, *B. ribis* may cause significant economic damage to any susceptible plant listed under the host range (and others yet to be discovered). There are no known direct consequences to human health.

From an environmental perspective, *Botryosphaeria* species that originate from ecosystems of similar floristic and climatic composition to New Zealand should be considered to represent an intrinsically higher risk to the New Zealand native environment (Ridley *et al.*, 2000) (see section 3.4.3). Because of this association it is more likely than otherwise expected that a *Botryosphaeria* species associated with *Wollemia nobilis* nursery stock from Australia will be pathogenic to related plants in New Zealand. The iconic New Zealand native species of *Agathis australis* (kauri) is one such closely related species. From an environmental perspective, disease-related impacts to *Agathis australis* in New Zealand would be considered very significant.

Summary of consequences

An introduced *Botryosphaeria* species would rapidly spread throughout its potential distribution in New Zealand and be moderately likely to cause significant unwanted indirect consequences on the New Zealand environment and economy. The nature of these consequences, namely the potential widespread mortality of plants of environmental or economic importance or the potentially significant damage to fruit production in the horticultural sector, has the potential to have wide ranging and irreversible consequences on the New Zealand environment and economy.

11.2.7 *Conclusion of Consequence Assessment*

From the assessment of a single example species, it is possible to conclude that *Botryosphaeria* species could cause mortality and/or reduction in growth or form of plants of environmental or economic importance to New Zealand. These impacts have the potential to cause moderate to high unwanted consequences to the New Zealand environment and economy should therefore be considered non-negligible.

11.2.8 *Risk Estimation*

The likelihood is very high that *Wollemia nobilis* whole plants and root-less cuttings would become associated with a *Botryosphaeria* species while growing in Australia, be transported to and enter into New Zealand still infected with the fungus, and form an established population of the new *Botryosphaeria* species once the plant is grown in the New Zealand environment. There is also a high likelihood that, given sufficient time, the contaminating fungus will spread throughout New Zealand wherever host plants are growing. There is a moderate likelihood that resulting from the spread of these *Botryosphaeria* species the unwanted consequences to the environment and economy will be moderate to high. As a result the risk estimate for *Botryosphaeria* species associated with *Wollemia nobilis* whole

plants and rootless cuttings imported from Australia is non-negligible and it is considered a hazard.

The likelihood that new *Botryosphaeria* species would be associated with *Wollemia nobilis* plant *in vitro* is considered negligible, and as such on this pathway *Botryosphaeria* species are not considered a hazard.

11.2.9 *Assessment of Uncertainty*

The subject of this assessment is a group of fungi that could potentially have a very wide variety of biological and epidemiological characteristics. To accommodate as far as possible these significant uncertainties the assessment has been undertaken at a relatively generic level, focusing on general attributes of the representative fungal species included in the assessment. Given the uncertainties around what are considered species complexes of the more widely distributed *Botryosphaeria* species, the potential hazards should be considered for all representatives of the *Botryosphaeria* including those otherwise considered established in New Zealand.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

11.3 Risk Management

11.3.1 *Risk Evaluation*

The risk estimate for *Botryosphaeria* species associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is considered non-negligible. Phytosanitary measures may need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for *Botryosphaeria* species associated with *Wollemia nobilis* plants *in vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

11.3.2 *Option Evaluation*

11.3.2.1 *Risk Management Objective*

To ensure any *Botryosphaeria* species associated with *Wollemia nobilis* nursery stock in Australia are neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

11.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of *Botryosphaeria* species establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of *Botryosphaeria* fungi;

b) Inspecting all *Wollemia nobilis* whole plants or root-less cuttings before packaging and transport to New Zealand to ensure they are free of *Botryosphaeria* fungi;

c) Treating all imported *Wollemia nobilis* whole plants or root-less cuttings before release into the New Zealand environment to ensure they are free of *Botryosphaeria* fungi;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* nursery stock is released into the New Zealand environment.

It is unlikely, due to the difficulties detecting and delimiting an established population of an introduced *Botryosphaeria* fungus, that an effective method of eradication or control could be developed for post-entry management of any unwanted consequences.

Treatments

There are potentially a number of treatments types that could be used to reduce the incidence of *Botryosphaeria* infection in nursery stock. As the requirement for the treatment is freedom from infection, any treatment selected would need to have a very high level of efficacy. Treatments would also need to be undertaken immediately prior to shipping to New Zealand or after arrival in New Zealand but before release into the New Zealand environment.

The only treatment type likely to provide a high level of efficacy without also damaging the host plant is a fungicide. Any treatment programme would need multiple applications (6 or more) of relatively high concentrations of the fungicide over an extended period. Application of benomyl-kaolin has been shown to be reasonably effective, but high concentrations may result in phytotoxicity (Cline and Milholland, 1992). Use of indole butyric acid with benomyl or thiabendazole has been reported to enhance rooting and reduce disease impact on Rhododendron (Punithalingam and Holliday, 1973). It is unlikely; however, that any of these treatments alone would provide sufficient confidence that a *Botryosphaeria* species infecting nursery stock would be removed.

Inspection

Botryosphaeria species in Australia can act as endophytes and stress-related pathogens of various woody hosts (Slippers *et al*., 2005). It is therefore expected that symptom expression will either require or be enhanced by the stressing of the plants during their inspection period.

Pre-export Requirements

The principle measure available for obtaining *Botryosphaeria* fungi freedom prior to export to New Zealand is through nursery management in Australia. Referring to the existing management practices provided in 3.6.1 and Appendix 2, the following nursery management practices would be necessary to provide adequate assurance of *Botryosphaeria* fungi freedom:

- Accreditation under the Nursery Industry Accreditation Scheme, Australia (NIASA) as detailed in for foliage diseases in Chapter 6, with the following additional requirements:

    - To improve the likelihood that any infesting hazard organisms will develop visible symptoms during pre-export inspections, it is recommended that, at least one month prior to export to New Zealand, plants should be subjected to a period of water stress to release any infecting *Botryosphaeria* fungi from quiescence. The application of successful water stress shall be measured by a visible response from the plant foliage such as leaf wilting.

The level of protection provided by this pre-export measure would be affected by the following attributes of *Botryosphaeria* species:

- *Infection within the nursery: Botryosphaeria* species spread aerially, or through stem run-off, raindrop splashing and pruning equipment. It is unlikely that a nursery in Australia could provide sufficient protection from *Botryosphaeria* fungal infection given the extent of propagule pressure within the surrounding environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for an extended period after infection even when the plants are placed under stress. Stressing plants will, however, significant reduce the rate of pathogen dormancy.

- *Post inspection infection:* plants may become infected with *Botryosphaeria* fungi after the inspections have been completed but before the plants arrive in New Zealand.

These attributes of *Botryosphaeria* fungi suggest that this measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants and root-less cuttings from Australia would not vector *Botryosphaeria* fungi into New Zealand.

Post Entry Quarantine

The principle measure available for detecting and removing infested plants before the consignment is released into New Zealand is through inspection while in post-entry quarantine. Available post-entry quarantine options are provided in Chapter 6 for Foliage Diseases; however the following measure should also be included:

- To improve the likelihood that any infesting hazard organisms will develop visible symptoms during post-entry quarantine inspections, it is recommended that, at least two months prior to biosecurity clearance, plants should be subjected to a period of water stress to release any infecting *Botryosphaeria* fungi from quiescence. The application of

successful water stress shall be measured by a visible response from the plant foliage such as leaf wilting.

The level of protection provided by this post-entry measure would be affected by the following attributes of *Botryosphaeria* fungi:

- *Propagule escape from the post-entry quarantine facility: Botryosphaeria* fungi can spread through air-borne spores. It may be difficult for a quarantine glass/greenhouse facility in New Zealand to limit the exit of fungal spores into the environment.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for a period after infection that exceeds the duration of post-entry quarantine or requires environmental conditions not provided in post-entry quarantine. Stressing plants will, however, significant reduce the rate of pathogen dormancy.

- *Masking of symptom expression by other diseases:* while the recommended enhancements to facility containment requirements related to micro-organism escape could be considered sufficient, the same level of containment is not currently recommended for micro-organism entry into the facility (e.g. HEPA filters are not recommended for inward flowing air). *Botryosphaeria* fungi entering the facility from the New Zealand environment and infesting plants may result in the expression of diseases symptoms that mask symptoms of diseases that the plants have vectored in from Australia.

It should be considered that the risks associated with the delay in symptom expression would be minimised for plants growing in an environment that is optimal for disease expression such as plant stressing. Optimal disease expression would, however, considerably enhance the likelihood of propagule escape from the facility. This would occur if plants were not showing symptoms from some types of fungal infection and therefore would not be removed as propagule pressure increased. The specifications for the facility are therefore optimised for fungal containment (HEPA filters and negative air pressure).

To manage the potential risks associated with the masking of disease expression by diseases found in New Zealand, plants showing *Botryosphaeria* disease-like symptoms should not be given biosecurity clearance. To limit as far as possible the level of fungal propagule pressure within post-entry quarantine, it is recommended that pre-export measures are also implemented.

11.3.2.3 *Recommended Management Options*

It is recommended that two measures are applied to reduce to an acceptable level the risk of *Botryosphaeria* species establishing in New Zealand and causing unwanted consequences.

i) Pre-export nursery management.

ii) Inspection during post-entry quarantine.

11.4 Assessment of Residual Risk

11.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure no individual plants within a consignment of *Wollemia nobilis* whole plants or root-less cuttings being exported to New Zealand are infested with *Botryosphaeria* species. The objective for inspection during post-entry quarantine is to ensure that any plants in consignment of *Wollemia nobilis* nursery stock imported into New Zealand from Australia that have become infested just prior to or during transport to New Zealand will be detected and not released into the New Zealand environment.

11.4.2 *Expected Performance of Measure(s)*

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with *Botryosphaeria* species. There should therefore be no detections of infested plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export infestation.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock are released into the New Zealand environment. There should therefore be no detections of *Botryosphaeria* species on plants within New Zealand that can be attributed directly or indirectly to imported *Wollemia nobilis* whole plants or root-less cuttings.

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12. *PHYTOPHTHORA CINNAMOMI*

12.1 Hazard Identification

12.1.1 *Aetiologic agent*

Phytophthora cinnamomi (Rands)

12.1.2 *New Zealand Status*

Present and widely distributed.

12.1.3 *Epidemiology*

The epidemiological descriptions have been summarised to include only organism characteristics relevant to the assessment of biosecurity risk. *Phytophthora* is a member of the Oomycete family now considered part of the Stramenopiles and more closely related to the algae and dinoflagellates rather than fungi. The similarity in morphology to fungi is believed to be a result of convergent evolution.

Life Cycle

Sporangia of *P. cinnamomi* germinate either by the formation of a germ tube(s) that eventually form a mycelium, or by release motile zoospores into water in the soil which swim to small roots (a chemotactic response to root exudates), encyst and within 20 to 30 minutes germinate on the root surface (Hardman, 2005). Penetration occurs within 24 hours of germination (Ribeiro O. K. 1983) and sporangia may appear on the root surface within 2 to 3 days in susceptible plants (Hardman, 2005). The fungus then spreads in the young feeder roots causing a rot which may extend into the base of the stem. Propagules may also be splashed onto and infect aerial parts of the plant (Ribeiro O. K. 1983). The latent period (the time between pathogen penetration and sporulation) can be as short as 24 hours on eucalyptus seedlings (Weste, 1983).

P. cinnamomi can survive for long periods in dead plant material. This saprophytic phase can allow an increase in the population of the pathogen. The fungus may also survive in the soil as mycelium, sporangia, zoospore cysts, chlamydospores and oospores, and survival can be extended in the presence of an organic substrate. Mycelium of *P. cinnamomi* can survive for at least 6 years in moist soil while zoospore cysts can survive for at least 6 weeks. Varying germination periods may help to maintain a low but continuing population. Chlamydospores can survive for at least 6 years if soil moisture exceeds 3% (Weste, 1983).

Chlamydospores form in soil, gravel or plant tissue during dry periods, germinate under favourable (moist) conditions and grow to form mycelia and sporangia or more chlamydospores. The latter may, in turn, remain dormant until conditions become suitable, then germinate to produce infective mycelia, sporangia and zoospores, or more chlamydospores. This cycle may continue for at least 5 years, provided there is a nutrient source (organic matter) and a non-competitive soil micro-flora (Weste, 1983).

Disease Characteristics

P. cinnamomi is currently considered the most widely distributed species of Phytophthora, with more than 1000 host species recorded in more than 90 different countries (Hardman, 2005). In Western Australia it has been estimated that about 2000 of the 9000 locally indigenous plant species area susceptible to *P. cinnamomi* (Wills, 1993 in Huberli *et al*., 2001). Bullock *et al* 2000 found that inoculations of *P. cinnamomi* caused the death of *Wollemia nobilis* plants over 5 days. It is also considered one of the most ubiquitous and destructive plant pathogens, causing great devastation in forests and production systems (Zentmyer, 1983). Although *P. cinnamomi* was originally mainly reported in tropical and subtropical countries and is believed to have originated from tropical Asia, it can apparently survive and develop in cooler countries, and does not seem to be obviously restricted by growing season or winter temperatures. Optimal temperatures for growth are in the range of 21-27^{O}C, with no growth below 6^{O}C or above 32-35^{O}C soil temperatures. *P. cinnamomi* does not survive well or spread under conditions of low soil moisture (Weste, 1983). Moisture is clearly a key factor in the establishment, spread and longevity of *P. cinnamomi* diseases.

P. cinnamomi causes a rot of fine feeder roots, leading to dieback and death of host plants. Larger roots are only occasionally attacked. Other symptoms include wilt, stem cankers (with sudden death of tree), decline in yield, decreased fruit size, gum exudation, collar rot (if infected through grafts near soil level) and heart rot (e.g. pineapple) (Zentmyer, 1983). The reported level of severity of disease resulting from *P. cinnamomi* is considerably less in New Zealand than in Australia (Johnston, 2004). This is likely to be due to a number of environmental factors, such as New Zealand's lower soil temperatures, different soil type and pH, and different floristic composition, and also to differences in pathogen populations (Johnston, 2004).

Certain ectomycorrhizal fungi are antagonistic to *P. cinnamomi*. It is believed that differences in soil symbiotic relationships between different species of trees, or trees in different localities, probably exert differing effects on the pathogenicity of *P. cinnamomi* populations (Weste, 1983. Johnston, 2004. Marx 1973).

Strains

P. cinnamomi is heterothallic with two known mating types, A1 and A2. The A2 mating type is the dominant strain world wide, while the A1 mating type has a limited distribution and host range (Zentmyer, 1983). Both A1 and A2 mating types have been recorded in Australia (Zentmyer, 1983, Huberli *et al*., 2001).

P. cinnamomi in California and Australia shows considerable intraspecific variability in colony morphology, pathogenicity, response to environmental stimuli, and other physiologic responses (Zentmyer, 1983, Huberli *et al*., 2001). Even when both mating types are present it appears that genetic diversity arises asexually rather than as a result of sexual recombination. Population studies of indicate that there are three clonal lineages in many areas around the world (Hardman, 2005).

In New Zealand, 6 isolates sampled from diverse sites around NZ and sequenced in a study on *Phytophthora* diversity had identical ITS sequences. In contrast, authentic sequences from

overseas showed modest variation, suggesting that the New Zealand isolates comprise a limited gene pool consistent with this species having being introduced (Beever *et al.*, 2006).

12.1.4 *Hazard Identification Conclusion*

From the epidemiological information provided above it should be considered possible that a strain or isolate of *P. cinnamomi* present in Australia but not in New Zealand could establish in New Zealand from imported *Wollemia nobilis* nursery stock and cause an unwanted impact. *P. cinnamomi* is therefore considered a potential hazard requiring further assessment.

12.2 Risk Assessment

12.2.1 *Entry Assessment*

The pathway for entry of *Wollemia nobilis* nursery stock, and any associated pests, has been summarised in section 3.5.1. *P. cinnamomi* could be vectored to New Zealand through infested plant material or contaminated soil or water. Roots of a plant that has become infected with *P. cinnamomi* would be considered an almost guaranteed pathway for the entry of this organism into New Zealand as the survival of the infesting organism is linked to the survival of the host plant. *Wollemia nobilis* nursery stock not otherwise infected before packaging and transportation to New Zealand could become infected by *P. cinnamomi* if stored for a length of time immediately adjacent to other plants that have developed disease symptoms.

As *P. cinnamomi* can be associated with woody plant parts, root-less cuttings should also be considered a pathway for the entry into New Zealand of these fungi though the likely incidence of disease would be considerably lower (low to moderate). Plants *in vitro* would not be considered a pathway for the entry assuming the cultures in question are indeed sterile (axenic).

12.2.2 *Conclusion of Entry Assessment*

The likelihood of *P. cinnamomi* entering New Zealand on whole plants or root-less cuttings of *Wollemia nobilis* that has become infected in Australia is very high and low-moderate respectively and therefore is consider non-negligible.

The likelihood of *P. cinnamomi* entering New Zealand on plants *in vitro* is considered negligible and this commodity will not be considered further in this assessment.

12.2.3 *Exposure Assessment*

The pathway for exposure and establishment of organisms associated with *Wollemia nobilis* nursery stock has been summarised in section 3.5.2.

While some strains or isolates of *P. cinnamomi* can kill the host in a relatively short period under optimal conditions for disease expression, many can survive in plant tissues or soil for an extended period when conditions are less that optimal for disease expression. As the intention of any importer would be to plant the imported *Wollemia nobilis* into the New Zealand environment, any limitation there may be to exposure is removed.

The transfer into the environment of contaminated soil that has been associated with infected plants should also be considered a possible pathway to exposure.

12.2.4 *Establishment Assessment*

On a local scale, the pathogen can be moved naturally by soil-splash, by wind-blown soil or debris, or by water movement and run-off in drainage/irrigation ditches. The most likely means of more distant movement is in contaminated soil or plant debris. Propagules can also be carried on machinery used for cultivation or harvesting and on seed. Movement of contaminated soil with container-grown ornamentals can spread the pathogen to disease-free areas, and this is the most probable pathway for international spread (Weste, 1983).

As indicated in the exposure assessment, the intention of any importer would be to plant the imported *Wollemia nobilis* nursery stock into the New Zealand environment, therefore allowing any infesting *P. cinnamomi* to establish a viable population.

12.2.5 *Conclusion of Exposure and Establishment Assessment*

Given that the imported and contaminated *Wollemia nobilis* whole plants and root-less cuttings themselves can act as the agent for exposure and establishment, and the intention of any importer would be to plant the imported *Wollemia nobilis* into the New Zealand environment, the likelihood of exposure and establishment is high and therefore non-negligible.

12.2.6 *Consequence Assessment*

In the context of the pathway for importing whole plants or root-less cuttings contaminated with *P. cinnamomi*, any potential consequences to people, the New Zealand environment, and the New Zealand economy will only become apparent after establishment and some degree of spread.

Consequences

P. cinnamomi is currently considered the most widely distributed species of Phytophthora, with more than 1000 host species recorded in more than 90 different countries (Hardman, 2005). It is also considered one of the most ubiquitous and destructive plant pathogens, causing great devastation in forests and production systems. *P. cinnamomi* causes a rot of fine feeder roots, leading to dieback and death of host plants. Other symptoms include wilt, stem cankers (with sudden death of tree), decline in yield, decreased fruit size, gum exudation, collar rot (if infected through grafts near soil level) and heart rot (e.g. pineapple) (Zentmyer, 1983).

P. cinnamomi is now widely distributed in New Zealand's indigenous forests but rarely causes conspicuous disease symptoms. Disease associated with Phytophthora is only noticeable in seasons of unusual weather that results in significant stress on infested plants. The reported level of severity of disease resulting from *P. cinnamomi* is considerably less in New Zealand than in Australia (Johnston, 2004). This is likely to be due to a number of environmental factors, such as New Zealand's lower soil temperatures, different soil pH, and different floristic composition, and also to differences in pathogen populations. In kauri forests in the far north on the north island of New Zealand, soil temperatures and moisture

content were considered important factors in affecting seedling mortality from *P. cinnamomi* infection (Johnston, 2004).

In *Nothofagus* forests in New Zealand, the spread of *Nothofagus* is believed to be dependent on the formation of the mycorrhizal association (*Nothofagus* is obligatory ectomycorrhizal). Seedling establishment is only successful within a few meters of the forest margin, within the root zone of established trees (Baylis 1980). Alternatively it has been suggested that the presence of *P. cinnamomi* may be the factor limiting the successful establishment of *Nothofagus* seedlings in soil lacking ectomycorrhizal inoculum, with ectomycorrhizal fungi offering protection from *P. cinnamomi* infection (Johnston, 2004). Based on this hypothesis it has been suggested that the impact of *P. cinnamomi* may have been to reduce the effective expansion rate of *Nothofagus* forests from 700 to 6 meters per century (Johnston, 2004). The introduction into New Zealand of a more cold-tolerant or virulent strain or isolate of *P. cinnamomi* could overcome the protection afforded by the associated ectomycorrhizal fungi leading to direct impacts on established *Nothofagus* forests.

As discussed in section 3.4.3 of this document, *Wollemia nobilis* and its constituent ecosystem should be considered similar to native ecosystems in New Zealand that include the closely related *Agathis australis* (Kauri). Association of more pathogenic strains or isolates of *P. cinnamomi* with imported *Wollemia nobilis* nursery stock would represent an intrinsically higher risk of causing unwanted consequences to the New Zealand native environment than imported nursery stock of many other plant species.

Spread

For a new strain of *P. cinnamomi* to spread within the New Zealand environment it would need to either:

a) out compete existing strains of the pathogen already widespread in New Zealand; or

b) be able to colonise or invade environments or hosts not currently occupied by New Zealand's existing strains.

In both examples the increased level or range of pathogenicity would be expected to lead to greater impacts within the New Zealand environment. However it would be expected that many of the strains or isolates of *P. cinnamomi* that are introduced from Australia would not necessarily be able to meet these criteria, limiting successful spread and subsequent impacts to a smaller number only.

12.2.7 *Conclusion of Consequence Assessment*

From the assessment above it is possible to conclude that new strains or isolates of *P. cinnamomi* could cause a greater level of mortality and/or reduction in growth or form of plants of environmental or economic importance in New Zealand. These impacts have a low likelihood of causing moderate to high unwanted consequences to the New Zealand environment and economy.

12.2.8 *Risk Estimation*

The likelihood is high that *Wollemia nobilis* whole plants and moderate to low that root-less cuttings could become associated with a new strain or isolate of *P. cinnamomi* while growing in Australia, be transported to and enter into New Zealand still infected with the organism, and form an established population of the new strain *P. cinnamomi* once the plant is grown in the New Zealand environment. There is also a low likelihood that, given sufficient time, the new strain *P. cinnamomi* will spread throughout New Zealand wherever host shrubs or trees are growing. Resulting from the successful spread of a new strain *P. cinnamomi* the unwanted consequences to the environment and economy will be moderate to high. As a result the risk estimate for *P. cinnamomi* associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is non-negligible and it is considered a hazard.

The likelihood that *P. cinnamomi* would be associated with *Wollemia nobilis* plants *in vitro* is considered negligible, and as such on this pathway *P. cinnamomi* is not considered a hazard.

12.2.9 *Assessment of Uncertainty*

The significant assumption underpinning this assessment is that the strains or isolates being generated in Australia would represent greater level of risk to New Zealand than the strains or isolates being generated in New Zealand. While there has been work completed on the genetic and pathogenic variability of *P. cinnamomi* in parts of Australia, very little of such work has been completed on isolates in New Zealand. The work completed by Beever *et al.* (2006), while indicating that New Zealand isolates had lower levels of genetic variation, looked at small representative sample only and as such could not be considered conclusive. Research should be undertaken to consider the potential for strain development within the Australian and New Zealand environments based on the distribution of mating types and the significant environmental influences.

To ensure that the risk management measures resulting from this assessment remain appropriate to the risk posed by the pathway, organism associations recorded with the commodity in the place of origin and during import into New Zealand or other countries should be monitored.

12.3 Risk Management

12.3.1 *Risk Evaluation*

Since the risk estimate for *P. cinnamomi* associated with *Wollemia nobilis* whole plants and root-less cuttings imported from Australia is non-negligible, phytosanitary measures will need to be employed to effectively manage the risks to reduce them to an acceptable level.

The risk estimate for *P. cinnamomi* associated with *Wollemia nobilis* plants *in vitro* imported from Australia is negligible and as such phytosanitary measures will not be required.

12.3.2 *Option Evaluation*

12.3.2.1 *Risk Management Objective*

To ensure any *P. cinnamomi* associated with *Wollemia nobilis* nursery stock in Australia is neither:

- transplanted into the New Zealand environment with *Wollemia nobilis* nursery stock imported from Australia; or

- transmitted to a host plant in the New Zealand environment from *Wollemia nobilis* nursery stock imported from Australia.

12.3.2.2 *Options Available*

Referring to figure 3.4 in section 3.6, there are conceivably a number of points on the *Wollemia nobilis* nursery stock import pathway at which measures could be applied to reduce to an acceptable level the risk of *P. cinnamomi* establishing in New Zealand and causing unwanted consequences. The following management options should be assessed:

a) Limiting exposure of the *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of *P. cinnamomi*;

b) Inspecting all *Wollemia nobilis* whole plants and root-less cuttings before packaging and transport to New Zealand to ensure they are free of *P. cinnamomi*;

c) Treating all imported *Wollemia nobilis* whole plants and root-less cuttings before release into the New Zealand environment to ensure they are free of *P. cinnamomi*;

d) Detecting and treating infested plants within New Zealand before any *Wollemia nobilis* nursery stock is released into the New Zealand environment.

Treatment Efficacy

Currently there are no known treatments for the successful eradication of *P. cinnamomi*. A number of treatments of strategies have been successfully applied to control the disease in the environment (Hardham, 2005). Hygienic precautions can be applied to exclude *P. cinnamomi* from a place of production (Smith, 1988). Unsterilized soil, water or growing medium, or farm machinery, should be excluded from production sites. Introduced plants should be kept apart until their phytosanitary status has been checked. All propagation should be done from healthy plants or seed. Cultural measures can be taken to reduce the risk of spread in case of introduction.

Inspection efficacy

Roots infected by *P. cinnamomi* have a water-soaked, necrotic appearance. The root tips are often attacked, although other areas of the root may also be infected. Infected plants may be smaller and have a less well-developed root system than healthy plants. Root inspections after allowing for disease development should provide an adequate indication of the presence of *P. cinnamomi*.

Management options are therefore limited to either ensuring *Wollemia nobilis* whole plants or root-less cuttings are free of *P. cinnamomi* before packaging and transport to New Zealand, or infested plants are detected and destroyed before any *Wollemia nobilis* nursery stock is released into the New Zealand environment.

Pre-export Requirements

The principle measure available for obtaining *P. cinnamomi* freedom prior to export to New Zealand is through nursery management in Australia. Referring to the existing management practices provided in 3.6.1 and Appendix 2, the following nursery management practices would be necessary to provide adequate assurance of *P. cinnamomi* freedom:

- Accreditation under the Nursery Industry Accreditation Scheme, Australia (NIASA) as detailed in for root diseases in Chapter 7 section 7.3.2.2, with the following additional requirement:

    - To reduce the likelihood of exported plants being contaminated by *P. cinnamomi*, it is recommended that all access to the site is via a series of footbaths containing an antibiotic agent effective against soil-borne diseases such as *Phytophthora* species (e.g. 128 g/l of benzalkonium chloride). Benzalkonium chloride, a quaternary ammonium compound shown by Smith & Clements (2006) to be effective at sterilising soil and surfaces of *Phytophthora cinnamomi* at the rate stipulated.

The level of protection provided by this pre-export measure would be affected by the following attributes of *P. cinnamomi*:

- *Infection within the nursery:* to date there have been no recorded instances of *P. cinnamomi* on *Wollemia nobilis* plants within nurseries in Australia managed under these requirements.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for an extended period after infection.

- *Post inspection infection:* plants may become infected with *P. cinnamomi* after the inspections have been completed but before the plants arrive in New Zealand.

These attributes of *P. cinnamomi* would suggest that this measure alone would not provide a sufficient level of confidence that consignments of *Wollemia nobilis* whole plants or root-less cuttings from Australia would not transfer *P. cinnamomi* into New Zealand.

Post Entry Quarantine

The principle measure available for detecting and removing infested plants before the consignment is released into New Zealand is through inspection while in post-entry quarantine. Contamination of nursery plants by *P. cinnamomi* may not become apparent when conditions for the expression, such as humidity, temperature, and water levels, are not suitable or the infected plants are symptom-less hosts. The following post-entry quarantine conditions shall apply to ensure as far as is possible that disease expression will become

apparent on infected plants that are not symptom-less hosts, and that the infecting fungi will remain contained within the quarantine facility:

- Post entry quarantine equivalent to Level 2 quarantine is considered appropriate as detailed in for root diseases in Chapter 7 section 7.3.2.2.

The level of protection provided by this pre-export measure would be affected by the following attributes of *P. cinnamomi* diseases:

- *Propagule escape from the post-entry quarantine facility:* it is likely that, with these additional controls on hygiene and containment, propagule escape from post-entry quarantine would not occur.

- *Delay in symptom expression:* visually detectable symptoms may not become apparent for a period after infection that exceeds the duration of post-entry quarantine.

12.3.2.3 *Recommended Management Options*

It is recommended that two measures are applied to reduce, to an acceptable level the risk of *P. cinnamomi* establishing in New Zealand and causing unwanted consequences.

i) Pre-export nursery management.

ii) Inspection during post-entry quarantine.

12.4 Assessment of Residual Risk

12.4.1 *Objectives for Recommended Management Option(s)*

The objective of pre-export nursery management is to ensure no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with *P. cinnamomi*.

The objective for inspection during post-entry quarantine is to ensure that any plants in consignment of *Wollemia nobilis* nursery stock imported into New Zealand from Australia that have become infested just prior to or during transport to New Zealand will be detected and not released into the New Zealand environment.

12.4.2 *Expected Performance of Measure(s)*

Pre-export nursery management is expected to be 100% effective at ensuring no individual plants within a consignment of *Wollemia nobilis* nursery stock being exported to New Zealand are infested with *P. cinnamomi*. There should therefore be no detections of infested plants during post-entry quarantine inspections in New Zealand that can be attributed to pre-export infestation. In reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through long term monitoring or targeted research.

Inspection during post-entry quarantine is expected to be 100% effective at ensuring no imported *Wollemia nobilis* nursery stock infected with *P. cinnamomi* are released into the

New Zealand environment. As *Wollemia nobilis* plants entering the New Zealand environment would be expected to become infested with already established *P. cinnamomi*, identifying a success measure for this option is more problematic. Therefore it is expected that a high level of assurance is obtained that the post-entry quarantine inspections were undertaken appropriately before any plants are released into the New Zealand environment. As above, in reality the effectiveness of this measure will be less than 100%, but it is probable that this actual level will only be determined through targeted research.

12.5 References

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13. GLOSSARY OF TERMS

a. i.	Active ingredient
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[38])
Biosecurity	The exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and human health." (Biosecurity Strategy 2003[39])
Biosecurity clearance	A clearance under section 26 of this Act for the entry of goods into New Zealand (Biosecurity Act 1993)
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.
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 (DOE, 1995).
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. (Blood & Studdert 1990)
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.
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
Exposure	The condition of being vulnerable to adverse effects
FAO	Food and Agriculture Organization, United Nations.
Growing season	An extended period of plant growth that includes environmental conditions equivalent to spring (longer wetter days and cold temperatures), summer (longer dryer days and warm temperatures), and autumn (shorter wetter days and warm but cooling temperatures)
Hazard organism	Any disease or organism that has the potential to produce adverse consequences

38 Agreement on the Application of Sanitary and Phytosanitary Measures, 1994. World Trade Organization, Geneva.
39 The Biosecurity Strategy for New Zealand. 2003. http://www.biosecurity.govt.nz/bio-strategy/

HEPA filter	A 'high-efficiency particulate air' (HEPA) Type 1, Class A filter as specified in AS 1324.1 with metal separators and elastomeric compression seals, which meets all requirements of AS 4260 with a minimum performance of Grade 2 and complies with US Military Specification MIL-F-51079-D or an equivalent specification (AS/NZS 2243.3, 2002).
Hitchhiker organism	An organism that is carried by or with a commodity and is not a pest of the commodity.
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 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.
Inspector	Person authorized by a National Plant Protection Organization to discharge its functions [FAO, 1990]
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
Micro-organism	A protozoan, fungus, bacterium, virus or other microscopic self-replicating biotic entity (ISPM No. 3, 1996)
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
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

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. 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.
Plants *in vitro*	A commodity class for plants growing in an aseptic medium in a closed container (FAO, 1990; revised CEPM, 1999; ICPM, 2002; formerly plants in tissue culture)
Post-entry quarantine (PEQ)	Quarantine applied to a consignment after entry (FAO, 1995)
Residual risk	The risk remaining after risk management requirements have been implemented.
Risk	The likelihood of the occurrence and the likely magnitude of the consequences of an adverse event.
Risk analysis	The process composed of hazard identification, risk assessment, risk management and risk communication.
Risk analysis area	The area in relation to which a risk analysis is conducted.
Risk assessment	The evaluation of the likelihood, and the biological and economic consequences, of entry, establishment, or exposure of an organism or disease.
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.
Root-less cuttings	Plant cuttings that may have leaves and shoots, but no roots.
Spread	Expansion of the geographical distribution of a potential hazard within an area
SPS Agreement 1995	World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (1995)
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 [FAO, 1990, revised FAO, 1995; ISPM No. 15, 2002; ISPM No. 18, 2003; ICPM, 2005]

Unwanted organism	(Biosecurity Act 1993) Means any organism that a chief technical officer believes is capable or potentially capable of causing unwanted harm to any natural and physical resources or human health; and (a) Includes: (i) Any new organism if the Authority has declined approval to import that organism; and (ii) Any organism specified in the Second Schedule of the Hazardous Substances and New Organisms Act 1996; but (b) Does not include any organism approved for importation under the Hazardous Substances and New Organisms Act 1996, unless: (i) The organism is an organism which has escaped from a containment facility; or (ii) A chief technical officer, after consulting the Authority and taking into account any comments made by the Authority concerning the organism, believes that the organism is capable or potentially capable of causing unwanted harm to any natural and physical resources or human health.
Whole plants	A nursery stock commodity sub-class for rooted cuttings and plants with roots and leaves

APPENDIX 1: HAZARD LIST

App 1.1 Organisms recorded on Wollemia nobilis *nursery stock in Australia*

Scientific name	In NZ?	Vector of a hazard	More virulent strains on goods overseas	In NZ but not associated with goods	In NZ but not in region.
Arbuscular mycorrhizae	Y/N				
Ectomycorrhizae	Y/N				
Botryosphaeria sp.	Y/N				
Phytophthora cinnamomi	Yes	No	Yes	No	No

Scientific name	In NZ but different host associations	Under official control or notifiable	No or little information on organism	Potential Hazard?	Reference (Host Association)
Arbuscular mycorrhizae				Yes	NWPS 1998
Ectomycorrhizae				Yes	NWPS 1998
Botryosphaeria sp.				Yes	Bullock *et al.* 2000
Phytophthora cinnamomi	Yes	No	No	Yes	Bullock *et al.* 2000

App 1.2 Organisms associated with Araucariaceae in Australia

The following organisms have been recorded as being associated with Araucariaceae in Australia and either are not believed to be or can not be confirmed to be present in New Zealand, or if a listed genus only have species that are not present in New Zealand.

Scientific name	Common name	Hazard Group	Reference: In Australia	Reference: Host Association
Aecidium fragiforme	Rust	Foliage diseases	Ramsden *et al*. 2002	Ridley *et al*. 2000
Aesiotes notabilis	Bark beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005
Agathiphaga queenslandensis	Kauri moth	Surface feeding invertebrates	Whitmore 1977	Whitmore 1977
Alternaria sp	Blights and Leaf Spots	Foliage diseases	AFFA, IRA:Sawn timber 2001	Farr *et al*. 1989
Aragomacer leai	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Aragomacer uniformis	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Araucariana queenslandica	Bark beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005
Armillaria sp	Armillaria root rot	Root diseases	AFFA, IRA :Sawn timber 2001	Pennycook 1989
Basiliogeus prasinus	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Basiliogeus striatopunctatus	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Basiliorhinus araucariae	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Botryosphaeria rhodina	Cankers and Diebacks	Root, foliage and canker diseases	CABI CPC 2006	Ramsden *et al*. 2002
Bunyaeus eutactae	Pine Flower Snout Beetles	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Bunyaeus monteithi	Pine Flower Snout Beetles	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al*. 2005
Chrysomphalus dictyospermi	Spanish red scale	Surface feeding invertebrates	www.ento.csiro.au	Martin-Mateo 1983
Coniferococcus agathidis	kauri coccid	Surface feeding invertebrates	Whitmore 1977	Whitmore 1977
Coptocorynus araucariae	Weevil	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Coptocorynus sp	Weevil	Wood boring insects	Schneider 1999	Schneider 1999
Dihammus australis	Longicorn beetle	Wood boring insects	Schneider 1999	Schneider 1999
Diotimana undulata	Longicorn beetle	Wood boring insects	Schneider 1999	Schneider 1999
Dysthaeta anomala	Marbled Longicorn	Wood boring insects	Mecke *et al*. 2005	Schoenherr 1991
Euplatypus parallelus	Wood Borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Eurhamphus fasciculatus	Giant pine weevil	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Eutactobius puellus	Weevil	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al* 2005
Euthyrrhinus meditabundus	Boring beetle	Wood boring insects	www.botanik.uni-bonn.de	Whitmore & Page 1997
Euwallacea barbatus	Ambrosia beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005

Scientific name	Common name	Hazard Group	Reference: In Australia	Reference: Host Association
Euwallacea destruens	Ambrosia beetle	Wood boring insects	Wood & Bright 1992	CABI CPC 2006
Fomitopsis pinicola	Red belt fungus	Wood decay fungi	Gilbertson & Ryvarden 1986	www.worldagroforestry.org
Ganoderma lucidum	Root & butt rots, and Trunk decay	Wood decay fungi	AFFA, IRA:Sawn timber 2001	Ramsden *et al.* 2002
Hyleops glabratus	Hoop pine stitch beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Hylurdrectonus corticinus	Pin-shot hole borer	Wood boring insects	Sequeira & Farrell 2001	Sequeira & Farrell 2001
Hylurdrectonus pinarius	Pin-shot hole borer	Wood boring insects	Sequeira & Farrell 2001	Sequeira & Farrell 2001
Hylurdrectonus sp	Pin-shot hole borer	Wood boring insects	Sequeira & Farrell 2001	Sequeira & Farrell 2001
Ilacuris laticollis	Pin-shot hole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Leptosphaeria sp	Blights and Leaf Spots	Foliage diseases	Sosnowski *et al*. 2001	Farr *et al*.1989
Macrophoma araucariae	Cankers and Diebacks	Canker fungi	Ramsden *et al*. 2002	Ramsden *et al.* 2002
Mallus costatus	Boring beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Meliola sp	Black mildew	Foliage diseases	Old & Yuan 1990	Ramsden *et al.* 2002
Mitrastethus australiae	Pine stump weevil	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Neophyllaphis araucariae	Araucaria aphid	Surface feeding invertebrates	www.ento.csiro.au	Schneider 1999
Nipaecoccus sp.	Mealybug	Surface feeding invertebrates	Sequeira & Farrell 2001	Ridley *et al*.2000
Notomacer eximius	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al* 2005
Notomacer reginae	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al* 2005
Notomacer zimmermani	Beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Mecke *et al* 2005
Orthorhinus cylindrirostris	Elephant weevil	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Oxythrips agathidis	Kauri thrip	Surface feeding invertebrates	Sequeira & Farrell 2001	Whitmore & Page 1997
Pachycotes minor	Borer	Wood boring insects	Mecke *et al*. 2005	Sequeira & Farrell 2001
Pachycotes sp.	Borer	Wood boring insects	Sequeira & Farrell 2001	Sequeira & Farrell 2001
Palophagus australiensis	Leaf beetle	Surface feeding invertebrates	Mecke *et al*. 2005	Sequeira & Farrell 2001
Pestalosphaeria gubae	Cankers and Diebacks	Canker fungi	Yuan ZiQing, 1996	CABI CPC 2006
Phellinus noxius	Brown root rot	Wood decay fungi	Ramsden *et al*. 2002	Ramsden *et al.* 2002
Phytophthora boehmeriae	Ramie leaf spot	Foliage diseases	D'Souza *et al*. 1997	Ramsden *et al.* 2002
Phytophthora sp.		Root and foliage diseases	www.science.murdoch.edu.au 2001	Farr *et al*.1989
Platypus froggatti	Large ambrosia beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Platypus omnivorus	Pinhole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Platypus queenslandi	Pinhole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Platypus semigranosus	Pinhole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Platypus subgranosus	Mountain pinhole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Prospheres aurantiopictus	Hoop pine jewel beetle	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Pythium sp		Root diseases	AFFA, IRA:Sawn timber 2001	Nair 2000
Servazziella longispora		Foliage diseases	CABI CPC 2006	Farr *et al*.1989
Strongylurus decoratus	Hoop pine branchcutter	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Strongylurus sp	Hoop pine branch pruner	Wood boring insects	Schneider 1999	Schneider 1999
Treptoplatypus australis	Pin-shot hole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Tyrtaeosus microthorax	Weevil	Wood boring insects	Mecke *et al*. 2005	Mecke *et al* 2005
Xenocnema sp	Weevil	Wood boring insects	Sequeira & Farrell 2001	Sequeira & Farrell 2001
Xyleborus affinis	Borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005
Xyleborus emarginatus	Borer	Wood boring insects	Wood & Bright 1992	CABI CPC 2006
Xyleborus perforans	Island pinhole borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005
Xyleborus similis	Borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005
Xylosandrus pseudosolidus	Borer	Wood boring insects	Mecke *et al*. 2005	Mecke *et al*. 2005

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APPENDIX 2: NIASA BEST PRACTICE GUIDELINES

The following description of a specific nursery-based propagation system in Australia for Wollemi Pine has been extracted from the Department of Primary Industries (DPI) Forestry, Queensland Government: "*Information on the propagation and production of Wollemi Pine plants for quarantine and export authorities*", August 2005.

3.6.1.1 METHODS OF PROPAGATION

There are two commercial methods currently being used by DPI Forestry to propagate Wollemi Pine:

- Tissue culture; and
- Vegetative propagation.

a) Tissue culture

Tissue culture plants have been successfully produced and small number of these plantlets have been transferred to pots and growing media. It is anticipated that future production of Wollemi Pine may be entirely through tissue culture. These will principally be produced for export.

b) Vegetative propagation

Currently, the production of Wollemi Pine is largely through vegetative propagation. All cuttings, taken from container mother plants, are set in high humidity igloos. Once rooted, cuttings are transferred from the igloo to 50% shade for conditioning.

There are two forms of Wollemi Pine plants produced, depending on the type of cutting taken: an orthotropic cutting produces a plant with a shoot with apical dominance, producing a tall, upright plant and; a plagiotropic cutting produces a plant without apical dominance and the shoots grow as branches.

The following sections outline the procedures adhered to for all Wollemi Pine stock raised by DPI Forestry.

3.6.1.2 NURSERY ACCREDITATION (NIASA)[40]

DPI Forestry nurseries are accredited under The Nursery Industry Accreditation Scheme, Australia (NIASA). Accreditation is based on adherence to guidelines and recommendations to ensure quality control and crop hygiene, particularly disease, pest and weed control and nursery hygiene. NIASA accreditation is available to production nurseries and growing media manufacturers. The Nursery Garden Industry Association (NGIA) also has a national scheme for retail nurseries. DPI Forestry obtains all growing media from NIASA accredited manufacturers.

3.6.1.3 THE PREVENTION OF ROOT DISEASES

DPI F nurseries have a comprehensive program in place to prevent introduction of root rot diseases, particularly those caused by *Phytophthora* spp. and monitoring and corrective programs.

The following principals reduce the risk of contamination:

40 Details of NIASA accreditation requirements are available at http://www.ngia.com.au

a) Access

Access to propagation and growing facilities is restricted to all but essential traffic as the facility is surrounded by a 2.4 metre x 24 wire alarmed electric fence. Key access to the site is limited. Casual nursery staff are assessed and participate in a training course before access is permitted. Propagation areas are not thoroughfares for staff or materials involved in materials and plant handling operations unrelated to propagation.

b) Footbaths

All access to the site is via a series of footbaths containing Phytoclean®. Foot baths are drained and refilled daily or as required.

Phytoclean® contains 128g/l of the active ingredient Benzalkonium chloride, a quaternary ammonium compound shown by Smith & Clements (2006) to be effective at sterilising soil and surfaces of *Phytophthora cinnamomi* at 0.1% a.i (12.8 g/l Benzalkonium chloride).

c) Vehicles

Vehicle access to the site is also restricted. All approved vehicles are thoroughly washed down and treated with Phytoclean® prior to entry through vehicle bath area.

d) Propagating and on-growing facilities

Propagating and growing on facilities are designed and prepared to ensure no contact with underlying soil base or puddling. Underlying soil is a free drained sandy loam. The site has been graded ensuring run off is away from the nursery compound. A subsurface drainage system at five metre spacings has been constructed over the total site. Weed mat is then placed over the soil base. The site is then covered with 100 mm of pathogen free 20 mm crushed blue metal. All deliveries to the site is via wash down/sterilisation bays. Upon completion newly constructed sites including dedicated access roads are treated with a copper drench. All design and construction fully complies with NIASA guidelines subject to external audit biannually.

Cuttings are initially grown in a Queensland Native Tube (QNT). QNTs (220 cc volume by 125 mm length) are arranged in 50 cell black polypropylene trays at a density of 277/m^2. The set trays are placed on 1 m high, galvanised pipe benches. The surface is free draining and easily disinfested. Propagation is carried out in dedicated plastic/shade covered igloos under mist.

Once rooted, plants are potted up into larger sized (140mm, 420mm or 500mm) pots and placed on crushed blue metal under 50% shade 4 metre high structures. Gravel areas are sprayed with copper treatment before pot placement. Pathways throughout and adjacent to containerised plant production facilities are constructed of 100 mm deep over weed mat, 20 mm crushed blue metal on a consolidated graded drained surface. All pathways are sprayed monthly with a copper treatment.

e) Water

Water for production of Wollemi Pine is obtained from the local creek and bore. All water is chlorinated to 5ppm before use. Retention time for treatment is 28 minutes. The chlorination process is checked daily. Records of water disinfestation treatments are maintained. The pH and EC of water sources are checked and recorded at least once per month. The facilities for the subsequent storage of treated water does not allow for contamination by untreated water, soil, plant debris, dust and animal movement.

f) Growing media/propagating media

All potting media is supplied from NIASA accredited sources and is certified free of soil, sand, diseases and pests. All potting mixes are composed of either composted or boiled pine bark and perlite but may also include blends with peat moss or coir peat. Delivery trucks are washed down and sterilised before loading. Undercarriage and wheels are again washed down and sterilised immediately prior to unloading. Potting media is stored on a clean raised, covered concrete surface. Slabs are washed down and sterilised between loads.

g) Motherstock plants

Shoots/cuttings are collected from hedge plants raised in 140mm pots in the growing on facilities described above.

h) Working surfaces and tools

Working surfaces used to plant or prepare plant propagules are constructed of non-porous materials and are cleaned and sterilised frequently using a standard Phytoclean® solution. Secateurs are cleaned regularly throughout collection operations using methylated spirits. All tools including shovels are treated between batches of media or after use.

i) Removal of plant and media wastes

Discarded plants and spilt media are accumulated off site and removed on a frequent basis.

j) Washing facilities

Access to hand washing facilities is provided to all staff and their routine use is encouraged. Latex gloves are also provided at all times. Sterile containers including pots and trays are stored on a sheltered concrete pad on sterilised pallets and are isolated from other areas by footbaths.

k) Sterilisation

The following sterilising techniques are used by DPI Forestry for the materials indicated in **Table 3.1**.

The preferred method for sterilisation is the use of steam. However, when unavailable chlorine is recommended. Phytoclean® is recommended for use in footbaths and for vehicle/equipment wash-down, but not for pots and trays due to high cost. All pots and trays are either new or sterilised before re-use.

Table 3.1: Sterilising techniques

Materials	**Steam** 20 minutes at 65°C	**Chlorine[1]** 1 litre 12% fresh sodium hypochlorite to 29 litre water (4000 ppm chlorine).	**Phytoclean®2** Footbaths (10g/l a.i.) Wash-down (20g/l a.i.)
Polypropylene trays/pots	OK	OK	No
Footbaths, vehicle & equipment wash-down	No	No	OK
Tools, workbenches and plastic bins	OK	No	OK

3.6.1.4 DISEASE, PEST AND WEED CONTROL

a) Weed Control

Growing media and media components supplied to the nursery are free of weeds and weed propagules. Media storage and mixing areas, propagation areas and production areas are maintained as weed free by hand weeding and regular spraying. Stored potting mix is covered. Weeds are suppressed on the general nursery site, outside of the immediate production area, by slashing or chemical control.

b) Pest and disease monitoring

Training and awareness programs ensure stock is continually inspected for pest and disease attack by qualified nursery staff. A formal check is done daily.

DPI Forestry also employs qualified pathologists and entomologists to inspect Wollemi Pine propagation and growing areas every 3 months for pests and diseases. A walk through survey is conducted daily.

c) Prevention

Stock is sprayed with insecticide and fungicide on a fortnightly rotational basis using a tractor mounted blower mister, powered sprayers or by hand. The chemicals chosen minimises the risk of drift and environmental impact. Equipment is calibrated regularly and kept in good working order.

Records of calibration and maintenance of all equipment are kept. Staff operating the equipment have access to adequate and properly used measuring devices and safety equipment.

Complete records of the chemicals used, the rates, the dates of application, the approximate volumes (or weights) applied, the section of the nursery sprayed and/or the crops treated, and the name of the spray operator are maintained.

d) NIASA

Strict adherence to NIASA guidelines ensures timely and effective control of pest and diseases.

3.6.1.5 CROP MANAGEMENT PRACTICES

a) Infrastructure

All Wollemi Pine are produced in secure facilities, with adequate protection from bad weather and air-borne contaminants. Generally, this is in the form of large open shade-houses, covered by 50% shade cloth, which provides good air-flow and protection from frost and excessive light. Structures are regularly maintained and provide a safe and comfortable place to work in during periods of peak staff activity. Buildings, fences, roadways and parking areas are appropriate for the purpose and in good repair.

b) Water, irrigation and humidity

Plants are irrigated with treated water depending on level of development and environmental conditions to maximise growth. Overhead irrigation provides a uniform distribution of water via an automatic watering computer system.

Waste water is minimised and good surface and sub-surface drainage ensures waste water,

potentially containing fertilisers and/or plant protection chemicals is disposed of by an approved discharge system.

The growing media has a range of physical properties in terms of water holding capacity, aeration and drainage to optimise irrigation results and plant growth. The humidity of atmospheres maintained in nursery structures do not continually exceed levels beyond which plant health becomes a persistent problem (over 85%, except in propagation houses).

c) Nutrition and fertilising

Slow release fertilisers are applied in the potting mix at rates of 2.5 kg to 5kg per cu metre. In addition foliage fertiliser is applied fortnightly via a fertigation system. Foliage and potting mix nutrient levels are tested and analysed regularly by experts.

Fertiliser treatments are selected to maximise benefit to the plant and minimise nutrient leaching and run-off. Staff operating the application equipment have access to adequate and properly used measuring devices and safety equipment.

Complete records of the chemicals used, the rates, the dates of application, the approximate volumes (or weights) applied, the section of the nursery sprayed and/or the crops treated, and the name of the spray operator are maintained.

App 2.1 References

Smith, I W; Clements, P A (2006) Assessment of quaternary ammonium compounds as disinfectants for control of *Phytophthora cinnamomi* in washdown situations. Centre for Forest Tree Technology, Department of Natural Resources and Environment.

APPENDIX 3: ANALYSIS OF CLEARANCE REQUIREMENTS

The New Zealand Ministry of Agriculture and Forestry, operating under the powers of the Biosecurity Act 1993, has in place an import health standard providing general risk mitigation measures for nursery stock. MAF Standard 155.02.06 (1 March 2005): *Importation of Nursery Stock*[41] provides a set of "*basic*" conditions to be met by all nursery stock imported into New Zealand. For the purpose of developing risk management measures for imported *Wollemia nobilis* nursery stock, the following appendix describes these general risk mitigation measures, reviews their expected efficacy against the target organisms, and provides recommendations on the measures in light of the analysis of efficacies. This appendix also reviews a number of general risk mitigation measures for potential fungi contamination of imported *Wollemia nobilis* nursery stock, which are recommended in a report prepared for the Ministry of Agriculture and Forestry by Lester and Lunn (2003).

App 3.1 Phytosanitary Certification

App 3.1.1 Description of measure

Consignments must be accompanied by a phytosanitary certificate certifying that the nursery stock has been inspected in the exporting country in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests, and conforms with New Zealand's current import requirements. If visually detectable pests are found which are not listed in the import health standard, the certifying NPPO must establish their regulatory status prior to issuing the certificate. This information is available in MAF's "Biosecurity Organisms Register for Imported Commodities"[42]. If a visually detectable pest is not listed in this register, the certifying NPPO must contact MAF to establish the regulatory status of the pest.

The phytosanitary certificate must also have one of the following additional declarations:

> "The plants were raised from seed/cuttings in soil-less rooting media in containers maintained out of contact with the soil".
>
> OR
>
> "The roots of the plants have been dipped in fenamiphos at 1.6g a.i. per litre of water for 30 minutes".

App 3.1.2 Analysis of measure efficacy

The requirement that a consignment of *Wollemia nobilis* nursery stock be accompanied by a phytosanitary certificate certifying that "*the nursery stock has been inspected in the exporting country in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests, and conforms with New Zealand's current import requirements*" would seem to mitigate to some degree risks from "*any visually detectable regulated pests*". However, as the inspections are carried out "*in accordance with*

41 http://www.biosecurity.govt.nz/files/imports/plants/standards/155-02-06.pdf
42 http://www.biosecurity.govt.nz/pests-diseases/registers-lists/boric/

appropriate official procedures", and these "*official procedures*" are not themselves specified, there is no way to determine how effective these inspections would be at detecting infestations of "*visually detectable regulated pests*".

The two alternative additional declarations would seem to be targeting the potential risks from nematodes on *Wollemia nobilis* plant roots. It is expected that the vast majority of plant species have the potential to have their roots associated with nematode species. While no specific nematode species have been recorded on *Wollemia nobilis* nursery stock to date, general measures against these potential hazard organisms on *Wollemia nobilis* nursery stock would therefore seem appropriate as the likelihood that nematodes would be associated with *Wollemia nobilis* nursery stock should be considered very high.

The requirement that the plants are "*raised from seed/cuttings in soil-less rooting media in containers maintained out of contact with the soil*" would be an effective measure if adequate quarantine were maintained around the treatment and maintenance of the *Wollemia nobilis* plants during the conditioning period. There is, however, no further information provided on the quarantine requirements of this measure and as such it is not possible to determine how effective the declaration would be for imported *Wollemia nobilis* nursery stock. That being said, it would be expected that due to conflicting economic incentives, such a declaration alone would have limited value in mitigating the risk of contamination from nematodes if the quarantine controls were maintained by the exporting nursery only rather than the NPPO.

The alternative requirement that the "*the plants have been dipped in fenamiphos at 1.6g a.i. per litre of water for 30 minutes*" suggests that a single treatment of fenamiphos will adequately mitigate the risk from any species of nematode on *Wollemia nobilis* nursery stock. Fenamiphos is an organophosphate that acts as a systemic insecticide and cholinesterase inhibitor (a nerve toxin) with a contact action. The active ingredient is absorbed into the tissues of the plant or taken up by the roots and the toxic effects can be expressed in all parts of the plant. The pesticide also has secondary activity against other invertebrates such as sucking insects and spider mites. Field efficacy (crop yield studies for nematode control) has been demonstrated on at least the following nematodes:

Table App 3.1: List of nematodes showing field based susceptibility to Fenamiphos

Belonolaimus longicaudatus	*Heterodera schachtii*	*Pratylenchus crenatus*
Caenorhabditis elegans	*Hoplolaimus columbus*	*Pratylenchus penetrans*
Criconemella ornata	*Hoplolaimus galeatus*	*Pratylenchus vulnus*
Criconemella spp.	*Meloidogyne arenaria*	*Rotylenchulus reniformis*
Ditylenchus destructor	*Meloidogyne incognita*	*Tylenchorhynchus dubius*
Globodera rostochiensis	*Meloidogyne javanica*	*Tylenchorhynchus vulgaris*
Heterodera carotae	*Paratrichodorus christiei*	

Research completed on the toxicity of a single application of fenamiphos to nematode cultures or plant roots infected with nematode species indicated the following:

a) That by acting as a nerve toxin, fenamiphos causes paralysis in juvenile or adult nematodes that may not result in death (Opperman and Chang 1991). The recovery

rate of *Caenorhabditis elegans* from paralysis after a single 24 hour application of fenamiphos at 0.1 grams of active ingredient (g a.i.) per litre was recorded in (Opperman and Chang 1991) as around 10%. The authors concluded that a combination of concentration and time (C/T) was required to achieve complete toxicity, and that exposure times in excess of at least 24 hours (at 0.1g a.i. per litre) would be required.

b) Kimpinski *et al*. (1983) demonstrated that at fenamiphos concentrations of 24 mg a.i. per litre over a 24 hour exposure period, *Pratylenchus penetrans* and *Pratylenchus crenatus* survival levels were as high as 40%.

c) Steele (1976) demonstrated that fenamiphos concentrations of 1 g a.i. per litre over a 1 week exposure period permanently suppressed egg hatching from cysts of *Heterodera schachtii*.

d) The half life of fenamiphos in soil (anaerobic) is recorded as 88 days[43] with no activity found up to 10 weeks (70 days) after application (Melton *et al*. 1995).

e) Melton *et al*. (1995) and Kimpinski *et al.* (1983) found indications that different nematode species in the *Meloidogyne* and *Pratylenchus* genera respectively show varying levels of susceptibility to fenamiphos treatments.

A summary of these results indicate that a single short-duration application of fenamiphos at a relatively high concentration (1.6 g a.i. per litre) may not be 100% effective against all nematode species and nematode life stages that could potentially be associated with *Wollemia nobilis* nursery stock. The research also indicates that a nematode infestation that is not adequately treated by the single application of fenamiphos may remain suppressed by residue nematicide activity over the majority of a concurrent short duration (3 month) post-entry quarantine period.

App 3.1.3 Recommendations for measure

From the description of the aforementioned measures and the analysis of their potential efficacy, the following actions are recommended for *Wollemia nobilis* nursery stock:

a) The requirement that any consignment of *Wollemia nobilis* nursery stock be accompanied by a phytosanitary certificate certifying that "*the nursery stock has been inspected in the exporting country in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests, and conforms with New Zealand's current import requirements*" should be retained as a general "good practice" measure. However, in the absence of clear directions on the minimum standard of inspection required, this measure should be considered ineffective at mitigating risk to any significant degree. Specifications should therefore be developed around the application of this measure for imported *Wollemia nobilis* nursery stock to ensure both adequate compliance and an adequate level of risk mitigation.

43 Provided by the PAN Pesticides Database at http://www.pesticideinfo.org

b) The alternative additional declaration that the *Wollemia nobilis* plants are "*raised from seed/cuttings in soil-less rooting media in containers maintained out of contact with the soil*" should have specifications developed around the application of this measure to ensure both adequate compliance and an adequate level of risk mitigation. This measure (with improvements) should be made mandatory for imported *Wollemia nobilis* nursery stock.

c) Treatment with fenamiphos within 10 weeks of export of *Wollemia nobilis* nursery stock to New Zealand should be either prohibited or the period of post-entry quarantine extended to accommodate the likely residue effects of this treatment. Treatment with fenamiphos on arrival in New Zealand or during quarantine should only occur as part of a treatment programme in response to an identified nematode infestation, and the potential residue nematicide activity (up to 10 weeks) should be taken into consideration when extending the post-entry quarantine after treatment.

d) Any visibly diseased or infested *Wollemia nobilis* plant parts should be removed and appropriately reshipped or destroyed as high concentrations of an infesting organism will reduce treatment efficacy.

e) Research should be completed on determining a sufficiently effective nematicide treatment schedule for imported *Wollemia nobilis* nursery stock. The hot water dip treatment provided for nematodes in the USDA treatment manual[44], namely 47.8^{o}C for 30 minutes, is one possible candidate for assessment.

App 3.2 Inspection of nursery stock on arrival in New Zealand

App 3.2.1 Description of measure – Sampling Inspection

All nursery stock must be inspected at the first port of entry (airport, wharf, mail centre) or at specifically approved transitional facilities designed for nursery stock inspections. The nursery stock will be inspected using a randomly selected minimum 600 unit sample, to ensure that it complies with the following entry conditions:

> Infestation by visually detectable quarantine pests on inspection at the border must not exceed the Maximum Pest Limit (MPL) which is currently set at 0.5%.

To achieve 95% level of confidence that the maximum pest limit will not be exceeded, no infested units are permitted in a randomly drawn sample of 600 units (i.e. acceptance number = 0). For lines of less than 600 units, 100% inspection is required. The sample must be drawn from the entire consignment and not, for example, from just the front of the container. If organisms are detected that cannot be identified, they will be treated as regulated organisms. If the number of units infested with quarantine pests exceeds the acceptance number, the nursery stock will be treated, reshipped or destroyed as directed by the inspector.

Only inert/synthetic material may be used for the protection, packaging and shipping materials of the nursery stock. Consignments contaminated with soil shall be treated, reshipped or destroyed. The interception of other extraneous matter, where it cannot be

44 http://www.aphis.usda.gov/ppq/manuals/port/Treatment_Chapters.htm

readily removed, may result in reshipment or destruction of the consignment. Packaging used to transport plants or plant products must also be inspected for contaminants. Where any contaminants are found the packages are to be treated or reshipped/destroyed at the importers option and expense.

App 3.2.2 Analysis of measure efficacy

In the context of *Wollemia nobilis* nursery stock, the "*600 sample*" inspection requirement to achieve a "*95% level of confidence that the MPL will not be exceeded*" makes the following assumptions:

a) That the *Wollemia nobilis* nursery stock consignment is homogenous (the pests are randomly distributed through the consignment). Heterogeneous or non-randomly distributed consignments would require a higher sampling rate to achieve the same confidence levels. The level of sampling required depends on the degree of heterogeneity;

b) That the samples are chosen randomly from the *Wollemia nobilis* nursery stock consignment;

c) That the inspector is 100% likely to detect the pest if it is present in the sample. It is highly unlikely that an inspector would be as likely to detect small or camouflaged organisms;

d) That the risk posed by a pest contamination level of 0.5% (at the 95% confidence level and given the above assumptions) for imported *Wollemia nobilis* nursery stock is acceptable to New Zealand. This would mean that it would be considered acceptable in 5% of cases that a *Wollemia nobilis* nursery stock consignment of say 1000 plants could contain 5 or more live pests of a given species, and in 95% of cases that a consignment would contain less than 5 live pests.

e) That it is acceptable that for *Wollemia nobilis* nursery stock the sampling system is based on a level (percentage) of contamination rather than a level of surviving individuals. In practice this means that using the current sampling system the acceptable number of live pests entering on *Wollemia nobilis* nursery stock consignments will increase with increasing consignment size (5 in a consignment of 1000 plants, 10 in a consignment of 2000 plants).

f) That because "*for lines of less than 600 units, 100% inspection is required*", it is therefore acceptable that the effective level of confidence gained by the sampling method significantly increases as the consignment size moves below 10,000. This is because a sample of around 590 provides 95% confidence that a contamination level of 1 in 200 (0.5%) will be detected in consignments larger than about 25,000 individuals. From table App 3.1 below it can be seen that as the consignment size moves below 25,000, a smaller sample size is required to achieve the same level of confidence.

Table App 3.1: Calculated sample size to detect a contamination level of 0.5% with 95% confidence, assuming 100% efficacy in detection and consignment homogeneity (from ISPM: Guidelines for Sampling of Consignments – draft May 2006)

Number of units in consignment (lot)	Sample Size	Number of units in consignment (lot)	Sample Size
25	(all)	6 000	569
50	(all)	7 000	573
100	(all)	8 000	576
200	190	9 000	579
300	285	10 000	581
400	311	20 000	589
500	388	30 000	592
600	379	40 000	594
700	442	50 000	595
800	421	60 000	595
900	474	70 000	596
1 000	450	80 000	596
2 000	517	90 000	596
3 000	542	100 000	596
4 000	556	200 000+	597
5 000	564		

It is unlikely that many (if any) of the organisms that could potentially be associated with imported *Wollemia nobilis* nursery stock would meet the requirements of the current sampling standard. It is highly unlikely that:

- A consignment of *Wollemia nobilis* nursery stock would be homogenous, as the distribution of pests within a nursery would be influenced by variations in environmental factors such as air movement rates and directions, light and temperature levels, and water distribution;

- An inspector would be able to detect a contaminating organism 100% of the time. It is important to note that when sampling for an acceptance level of zero contaminants when the acceptable level of contamination is low, the efficacy of detection has to be above the required level of confidence. In other words, if you are only 80% likely to detect an organism on an infested unit you can not ever be 95% confident of detecting low levels of contamination; and

- The number of contaminating organisms in a consignment can increase as the consignment size increases, and maintain an equivalent level of biosecurity risk to New Zealand.

The requirement that "*only inert/synthetic material may be used for the protection, packaging and shipping materials of the nursery stock*" and "*packaging used to transport plants or plant products must also be inspected for contaminants*" would seem appropriate as a general hygiene measure for imported *Wollemia nobilis* nursery stock. Combined with the supplementary requirements that "*the interception of other extraneous matter, where it cannot be readily removed, may result in reshipment or destruction of the consignment*" and "*consignments contaminated with soil shall be treated, reshipped or destroyed*" and "*any contaminants are found the packages are to be treated or reshipped/destroyed*" reinforces the general hygiene requirement.

App 3.2.3 Recommendations for measure: Sampling Inspection

If sampling and inspection is considered an appropriate measure for one or more organisms potentially associated with imported *Wollemia nobilis* nursery stock, the appropriate rate of sampling will need to be determined for each organism species or group based on:

1) the ability of the inspector to detect the contaminating organism under normal operating conditions;

2) the likely degree of homogeneity of the consignment in relation to pest distribution; and

3) the likely size of the consignments being imported.

It may be possible to manage variable and unknown consignment sizes by limiting a consignment to a certain size or by allowing larger consignments to be split into smaller groups for sampling purposes.

App 3.3 Pesticide (insecticide) treatments for whole plants and root-less cuttings

On arrival in New Zealand or before export to New Zealand (pre-export), all whole plants and root-less cuttings must be treated for insects and mites as follows:

Either **(1)** Methyl bromide treatment for mite and/or insect infestations on dormant material only:

Or **(2)** Hot water treatment/chemical treatment for insect infestations on dormant material only:

Or **(3)** Chemical treatment for insect or mite infestations.

or a combination thereof to ensure both mites and insects are treated against.

App 3.3.1 Description of measure: Methyl Bromide Fumigation

The measure is described as a "*Methyl bromide treatment for mite and/or insect infestations on dormant material only*".

Fumigation for 2 hours at atmospheric pressure at one of the following combinations of rate (g/m^3) and temperature (^OC):

Rate (g/m^3)	Temperature (^OC)
48	10 – 15
40	16 – 20
32	21 – 27
28	28 – 32

App 3.3.2 Analysis of measure efficacy: Methyl Bromide Fumigation

For methyl bromide fumigation the most important variables to consider during any treatment are as follows:

- Temperature, as methyl bromide activity and therefore toxicity declines with decreasing temperatures until about 3.4^{O}C when the gas condenses into a liquid;
- The concentration of methyl bromide over the exposure time, otherwise referred to as the C/T value (concentration over time); and
- Duration of exposure to allow adequate diffusion of the gas into the product or consignment being fumigated.

The description of the methyl bromide treatment provided in the standard defines the initial rate of methyl bromide, the duration of the treatment and the required temperature at a given rate, but does not provide any C/T value or any requirements that would ensure an adequate C/T value over the duration of the treatment. It is also likely that a 2 hour treatment of methyl bromide at atmospheric pressure would not penetrate to a significant extent stems of plants that have undergone secondary thickening and bark production.

Available treatment schedules for methyl bromide fumigations of nursery stock are provided in table App 3.2.

Table App 3.2: Available treatments schedules for methyl bromide fumigations of nursery stock.

Reference	Treatment Schedules and Comments		
FAO Manual of Fumigation for Insect Control[45]	Mite fumigation under atmospheric conditions and repeat after 10 to 14 days:		
	15 to 21^{O}C for 2.5 hours	48 g/m^3	
	21 to 27^{O}C for 2 hours	48 g/m^3	
	27^{O}C and above for 2 hours	40 g/m^3	
FAO Manual of Fumigation for Insect Control	Insect fumigation for foliated dormant plants under atmospheric pressures:		
	External Infestations:		
	4 to 10^{O}C for 3.5 hours	40 g/m^3	80 C/T (g h/m^3)
	11 to 15^{O}C for 3 hours	40 g/m^3	72 C/T (g h/m^3)
	16 to 20^{O}C for 2.5 hours	40 g/m^3	64 C/T (g h/m^3)
	21 to 25^{O}C for 2 hours	40 g/m^3	56 C/T (g h/m^3)
	26 to 29^{O}C for 2 hours	32 g/m^3	48 C/T (g h/m^3)
	30 to 32^{O}C for 2 hours	24 g/m^3	40 C/T (g h/m^3)
	Internal Infestations:		
	4 to 10^{O}C for 3.5 hours	64 g/m^3	126 C/T (g h/m^3)
	11 to 15^{O}C for 3 hours	64 g/m^3	114 C/T (g h/m^3)
	16 to 20^{O}C for 2.5 hours	64 g/m^3	102 C/T (g h/m^3)
	21 to 25^{O}C for 2 hours	64 g/m^3	90 C/T (g h/m^3)
	26 to 29^{O}C for 2.5 hours	48 g/m^3	84 C/T (g h/m^3)
	30 to 32^{O}C for 2.5 hours	40 g/m^3	80 C/T (g h/m^3)

45 www.fao.org/docrep/X5042E/x5042E0t.htm

Reference	Treatment Schedules and Comments		
USDA Treatment Manual (from Davis and Venette 2004)	Insects and Mites on various commodities at atmospheric pressures: (Thrips, aphids, scales, leafminers, spider mites, lygaeid bugs, ants, earwigs and surface-feeding caterpillars)		
	4.4 to 4.9^{O}C for 2 hours	64 g/m^3	90 C/T (g h/m^3)
	10 to 15^{O}C for 2 hours	48 g/m^3	76 C/T (g h/m^3)
	15.6 to 20.6^{O}C for 2 hours	40 g/m^3	56 C/T (g h/m^3)
	21.1 to 26.1^{O}C for 2 hours	32 g/m^3	48 C/T (g h/m^3)
	26.6 and above for 2 hours	24 g/m^3	40 C/T (g h/m^3)
	(Mealybugs, Scirtothrips dorsalis and Halotydeus destructor)		
	15.6 to 20.6^{O}C for 2 hours	64 g/m^3	90 C/T (g h/m^3)
	21.1 to 26.1^{O}C for 2 hours	48 g/m^3	76 C/T (g h/m^3)
	26.6 and above for 2 hours	40 g/m^3	56 C/T (g h/m^3)

Methyl bromide fumigation has been found by Biosecurity New Zealand to be less effective against mite eggs. A second fumigation or alternative mite treatment should be undertaken 10 to 14 days after the first treatment to kill any mites that emerge from surviving eggs. The level of efficacy of these treatments is not stated but is assumed to be probit 9 (less than 1 in 30,000 survivors).

While a methyl bromide treatment may be considered suitably efficacious, New Zealand's commitment to the Montréal Protocol necessitates that we minimise the use of substances that damage the ozone layer where possible. Methyl bromide is considered an ozone-damaging chemical and in line with New Zealand's protocol commitments methyl bromide treatments will not be accepted where alternative treatments are available.

App 3.3.3 Recommendations for measure: Methyl Bromide Fumigation

The following recommendations for *Wollemia nobilis* nursery stock are provided in relation to "*Methyl bromide treatment for mite and/or insect infestations on dormant material only*":

- The treatment description should include specifications that ensure adequate control is maintained over the critical aspects of the fumigation, namely the C/T value (concentration over time) and temperature;

- Research should be completed on determining a sufficiently effective methyl bromide treatment schedule for all organisms of concern associated with imported *Wollemia nobilis* nursery stock;

- The following methyl bromide treatment schedule should be used as a generic insect and mite treatment on imported *Wollemia nobilis* nursery stock unless an alternative non-methyl bromide treatment is available:

Rate (g/m^3)	Temperature (^{O}C)	Treatment Duration (hours)	C/T Value (g h/m^3)
64 g/m^3	4 to 10^{O}C	3.5	126
64 g/m^3	11 to 15^{O}C	3	114
64 g/m^3	16 to 20^{O}C	2.5	102
64 g/m^3	21 to 25^{O}C	2	90
48 g/m^3	26 to 29^{O}C	2.5	84
40 g/m^3	30 to 32^{O}C	2.5	80

- Due to a lower than acceptable efficacy of this treatment against mite eggs, a second fumigation or alternative mite treatment is required 10 to 14 days after the first treatment to kill any mites that emerge from surviving eggs.

- Research should be completed to identify any phytotoxicity issues with the plant material requiring this treatment.

App 3.3.4 Description of measure: Hot Water/Chemical Treatment

This measure is described as a "*Hot water treatment/chemical treatment for insect infestations on dormant material only*" and consists of:

- Immersion in hot water at a constant temperature of 24 ^{O}C for at least 2 hours, followed by immersion in hot water at a constant temperature of at least 45$_{o}$C for at least 3 hours (period required at the stated temperatures excluding warm-up times).

- Immersion in chlorpyrifos dip (2.4 g a.i. per litre of dip or as per manufacturer's recommendations) containing a non-ionic surfactant for 2 minutes with agitation. The treatment time must be increased to 5 minutes if bubbles remain present on the bulb surface. The dip solution must be used no more than twice or as per manufacturer's recommendations. The chlorpyrifos dip may be incorporated in the hot water treatment.

App 3.3.5 Analysis of measure efficacy: Hot Water/Chemical Treatment

App 3.3.5.1 Heat Treatment

The critical aspects of a temperature treatment for killing insects are heating rate, heating level (throughout the plant) and heating duration. The preconditioning treatment of "*24 ^{O}C for at least 2 hours*" immediately followed by the heat treatment should provide the appropriate heating rate. No other specifications are provided for the effective application of the treatment to the plant material and the standard suggests the heat treatment is effective against all insects.

For comparison the USDA treatment manual[46] provides the following water bath heat treatment schedules:

46 http://www.aphis.usda.gov/ppq/manuals/port/Treatment_Chapters.htm

Table App 3.3: A selection of USDA water bath treatments.

Water Bath Treatment	Target Organisms (and host)
47.8oC for 30 minutes	1) Nematodes (from cold temperatures) 2) Leaf miner, *Eurytoma* spp., infesting *Rhynchostylis* (followed by a cool water bath).
43.3 °C for 30 minutes as pre-treatment followed by 48.9 °C for 60 minutes	External feeders on banana roots
43.3-43.9 °C for 1 hour	*Steneotarsonemus laticeps* (bulb scale mite)

The USDA treatments are clearly targeted at surface feeders suggesting the water bath treatments are not expected to penetrate plant material to any great degree.

The conclusion therefore is that the stated heat treatment for insects, 45oC for at least 3 hours, will only be effective if the contaminating insects are on the surface of the plant and is unlikely to be effective against all possible insect contaminants of imported *Wollemia nobilis* nursery stock.

App 3.3.5.2 Chlorpyrifos dip

Foliar spray of chlorpyrifos at 6.0 g a.i. per litre caused 100% mortality of adults (after 8 hours), and eggs and larvae (10 days) of the gall-forming midge *Contarinia nasturtii* (Keiffer) (Diptera: Cecidomyiidae) (Wu *et al*. 2006). Galvan *et al*. (2005) directly sprayed the different life stages of the lady beetle *H. axyridis* at a rate of 2.9 g a.i. per litre, leading to varying mortality rates that ranged from 20% for eggs to 100% for 1st instars and pupae.[47]

Diet bioassays giving chlorpyrifos at 10.0 g a.i. per litre caused 100% mortality of the storage mite *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) adults after 3 days (Sanchez-Ramos *et al*. 2003). For the tetranychids *T. urticae* and the European red mite *Panonychus ulmi* Koch, the highest LC_{50} for adults directly sprayed with chlorpyrifos (it varied according to strains) were 40 and 54 mg a.i. per litre, respectively (Nauen *et al*. 2001).

As chlorpyrifos is neurotoxic, acting as a contact pesticide and via ingestion (Armstrong-Fay, 2004), dipping would enhance the effectiveness of the rates described. Based on the evidence from the above studies a rate of 10 g a.i. per litre of chlorpyrifos should be applied, which is likely to be effective both as an insecticide and acaricide for non-dormant feeding insect or mite life stages. As egg or non-feeding life stages may survive a single treatment, a second treatment should be applied 10 days later if an actual infestation of insects or mites is detected. Chlorpyrifos is recorded as having an aerobic half life of 113 days[48].

App 3.3.6 Recommendations for measure: Hot Water/Chemical Treatment

From the description of the aforementioned measures and the analysis of their potential efficacy, the following actions are recommended:

47 Based on the assumed average of 250 L/ha, the AI concentration used of 0.73 kg AI/ha equals ca. 2.9 g a.i. per litre.
48 Based on the aerobic soil half life provided by the PAN Pesticides Database at http://www.pesticideinfo.org

- As a standard measure *Wollemia nobilis* nursery stock could be dipped in 10 g a.i. per litre of chlorpyrifos and a non-ionic surfactant for 2 minutes with agitation. The treatment time must be increased to 5 minutes if bubbles remain present on the plant surface. If this treatment is used against knowingly infested plant material, second treatment should be applied 10 days later;

- Any visibly diseased or infested plant parts should be removed and appropriately reshipped or destroyed as high concentrations of an infesting organism will reduce treatment efficacy;

- It is unlikely a hot water treatment will be an effective treatment for all insect species potentially associated with nursery stock, however combining the chlorpyrifos dip with a 48^{o}C 30 minute hot water treatment would considerably improve overall treatment efficacy;

- Neither treatment alone or in combination would be expected to be effective against insects or mites that are contained within (internally) the host plant material e.g. boring insects;

- Nursery stock entering a post-entry quarantine facility should not begin their quarantine period for insect contamination until the chemical residues have sufficiently dissipated or become inactive. As a rule the quarantine period for plants treated with chlorpyrifos should not begin within 113 days of the last treatment.

- Research should be completed on determining a sufficiently effective insect treatment schedule for imported *Wollemia nobilis* nursery stock.

App 3.3.7 Description of measure: Chemical Treatment for Insect Infestations

Plants must be sprayed or dipped with agitation using two active ingredients chosen from the table below, one belonging to the organophosphorous chemical group and the other from a different group. For dipping, the treatment time is normally 2 minutes (except fenvalerate and deltamethrin) but must be increased to 5 minutes if bubbles remain present on the plant surface. Dip solutions must be used no more than twice or as per manufacturer's recommendations. All treatments must be carried out in accordance with manufacturer's recommendations using either the recommended label rate or the rates shown in table App 3.4.

Table App 3.4: Chemical treatments for insect infestations currently approved in the "basic" requirements of MAF standard 155.02.06.

Active ingredient	Chemical group	Dip time	Notes
Acephate (0.75 g per litre of dip/spray)	Organophosphorous	2-5 mins	Non-dormant material only
Carbaryl	Carbamate	2-5 mins	
Chlorpyrifos (2.4 g per litre of dip/spray)	Organophosphorous	2-5 mins	Non-ionic surfactant required for dipping
Deltamethrin	Pyrethroid	15 mins	
Dimethoate	Organophosphorous	2-5 mins	Non-dormant material only
Fenvalerate	Pyrethroid	15 mins	

Active ingredient	Chemical group	Dip time	Notes
Imidacloprid (0.16 g per litre of dip/spray)	Neonicotinoid	2-5 mins	Non-dormant material only
Pirimiphos-methyl (0.475 g per litre of dip/spray)	Organophosphorous	2-5 mins	Non-ionic surfactant required for dipping
Spinosad	Spinosyns	2-5 mins	Dip/spray at room temperature
Tebufenozide	Diacylhydrazine	2-5 mins	
Thiacloprid (0.16 g per litre of dip/spray)	Neonicotinoid	2-5 mins	Non-dormant material only

App 3.3.8 Analysis of measure efficacy: Chemical Treatment for Insect Infestations

App 3.3.8.1 Acephate

Very little information could be found on the efficacy of acephate against plant pests. According to Wu *et al*. (2006) the application of acephate as a foliar spray at a high rate 7.8 g a.i. per litre was 100% effective against the midge *Contarinia nasturtii* (Diptera: Cecidomyiidae) after 24 hours. Mortality of eggs and larvae of *C. nasturtii* at the same rate was 99.5% after 10 days. Another experiment gave low efficacy against a sharpshooter species (Hemiptera: Cicadellidae) at approximately 40% the label rate (Bethke *et al*. 2001).

App 3.3.8.2 Carbaryl

Larval dipping of 4th instars of the diamondback moth *Plutella xylostella* (L.) (Plutellidae) on 18,000 mg a.i. per litre lead to 100% mortality over 72 hours (Hill *et al*. 2000). The same rate applied as contact and ingestion toxicity (leaf dip) lead to between 85 and 93% mortality of *P. xylostella* instars and adults. The LC_{90} for field resistant strains of the grape berry moth *Endopiza viteana* (Clemens) (Tortricidae) was estimated at 15,000 mg a.i. per litre, while LC_{90} for the susceptible strain was 2,319 mg a.i. per litre (Nagarkatti *et al*. 2002).

Laboratory experiments on eggs, 1st and 3rd instars, and adults of the multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) (Coccinellidae) lead to 100% mortality after 5 days, when these were directly sprayed upon with a 7,800 mg a.i. per litre solution (Galvan *et al*. 2005)[49]. However, it is important to point out that no mortality was observed for pupae at the same rate, all of which successfully hatched into adults. Although 40% of the latter appear to have died soon after, no information on the adult mortality observed in the controls was given.

Diet bioassays on two coccinellid species *Hippodamia convergens* Guérin-Menev and *H. axyridis* lead to ~95 and 100% mortality, respectively, at ca. 2.6 mg a.i. per litre via foliar spray (Tenczar *et al*. 2006). Diet bioassays at the same rate lead to 100% mortality of cottonwood leaf beetles *Chrysomela scripta* F. (Chrysomelidae) (Tenczar *et al*. 2006).

49 Galvan *et al*. (2005) used "kg a.i./ha". The manufacturer's rate for the application of Sevin XLR Plus on corn is at least 220-275 l/ha. It has therefore been assumed an average of 250 l/ha to calculate the a.i. concentration used, so 1.96 kg a.i./ha equals ca. 7.8 g a.i. per litre.

Direct dipping of bulb mite (*Rhizoglyphus echinopus* (Fum. & Robin), Acaridae) and two-spotted spider mite (*Tetranychus urticae* Kock, Tetranychidae) adults for 5 seconds suggested that LC_{90} were 38 and >1,000 mg a.i. per litre, respectively (Knowles *et al*. 1988). The same rate of carbaryl (7,800 mg a.i. per litre) used as foliar spray against the sharpshooter *Homalodisca coagulate* (Say) (Hemiptera: Cicadellidae) led to 71% mortality (1 day) (Bethke *et al*. 2001).

App 3.3.8.3 Deltamethrin

Very little information is available on this product or its efficacy.

App 3.3.8.4 Dimethoate

Bostanian *et al*. (2004) obtained 100% mortality of the predatory bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and the parasitoid wasp *Aphidius colemani* Viereck (Hymenoptera: Braconidae) with 0.19 g a.i. per litre via contact toxicity within 48 hours. Dimethoate was also described as very toxic to the bumble bee *Bombus terrestris* (L.) (Hymenoptera: Apidae) with mortality within the range of 75-100% resulting from direct contact toxicity with a pesticide solution at 0.5 g a.i. per litre (Sterk *et al*. 2003). Relatively low LC_{50} values were also obtained for adults and nymphs of the predacious mirid *Hyaliodes vitripennis* (Say) (Hemiptera: Miridae), whose rates were 0.9 and 1.7 mg a.i. per litre (Bostanian *et al*. 2001).

Knowles *et al*. (1988) assessed the LC_{50} of the organophosphorous dimethoate on the *T. urticae* and *R. echinopus*, whose adults were directly dipped into pesticide solutions. The obtained rates were 0.39 and 0.34 g a.i. per litre, respectively, over 48 and 72 hours, respectively.

App 3.3.8.5 Fenvalerate

The USA's Environmental Protection Agency has cancelled the registration of this product, and "*any distribution, sale or use of the products (…) is only permitted in accordance with the terms of the existing stocks provisions of this cancellation order*"[50]. There seems to be no clear justification for this action, which appears to have been requested by the manufacturers themselves.

App 3.3.8.6 Imidacloprid

Efficacy data are available for the diamondback moth *P. xylostella* and the Indian meal moth *Plodia interpunctella* (Hübner) (Pyralidae). Larval dip of *P. xylostella* 4th instars for 3 seconds obtained mortality of approximately 20% when exposed to 600 mg a.i. per litre over 72 hours (Hill *et al*. 2000). Similar mortality rates were obtained for residual contact toxicity at the same rate for both 4th instars and adults. Diet bioassays against the various life stages of *P. interpunctella* showed imidacloprid to be effective against this species in the long term

50 See: http://www.epa.gov/fedrgstr/EPA-PEST/2004/August/Day-05/p17881.htm

(Yue *et al*. 2003). All exposed stages from 2nd to mature 5th instars experienced 100% mortality when exposed to rates from 313 to 938 mg a.i. per litre. However, this rate was only obtained in the long term and, in the case of 5th instars, after 32 days. Exposure of 5th instars of the European corn borer *Ostrinia nubilalis* (Hübner) (Pyralidae) to 938 mg a.i. per litre via diet bioassay let to 58 % mortality over 6 days (Yue *et al*. 2003).

Approximately 93% mortality over 24 hours was obtained with 600 mg a.i. per litre for the parasitoid wasp *Diadegma insulare* (Cresson) (Ichneumonidae) adults via residual contact toxicity (Hill *et al*. 2000). Sterk *et al*. (2003) exposed pupae and adults of *Encarsia formosa* Gahan (Aphelinidae) to 200 mg a.i. per litre via residual contact toxicity and obtained mortality rates in the range of 51-75% and 75-100%, respectively. The same rate and form of exposure also led to 75-100% mortality in adult bumble bees. The efficacy of imidacloprid was tested against another parasitoid wasp *Diaeretiella rapae* (M'Intosh) (Braconidae) via residual contact toxicity (Stark *et al*. 2004). Over an undisclosed period of time mortality rates were 50% and 100% for larvae and adults, respectively, exposed to an estimated 570 mg a.i. per litre[51].

Exposure of larvae and adults of the cottonwood leaf beetle *C. scripta* via diet bioassay at 0.7 ml a.i. per litre led to 100% mortality over 7 days (Tenczar *et al*. 2006). Adults of two coccinellid species *H. convergens* and *H. axyridis* exposed to the same imidacloprid concentration experienced approximately 38 and 70% mortality, respectively, over 7 days (Tenczar *et al*. 2006). Diet bioassays at 160 mg a.i. per litre against two cerambycid species were carried out by Poland *et al*. (2006). None of the cottonwood borer *Plectrodera scalator* (Fab.) larvae were killed after 4 weeks. In contrast, 100% of exposed adults of Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) were killed within 5 days, although the rate for larvae was considerably lower at 20% after 4 weeks.

Adult males and females of the hemipteran predator *O. insidiosus* were exposed to approximately 290 mg a.i. per litre via residual contact toxicity, and experienced mortality rates of 98-100% and 93-100%, respectively (Studebaker *et al*. 2003). Nymphs exposed to ca. 580 mg a.i. per litre were killed in the range of 98-100%. Two other predatory bugs *Orius laevigatus* (Fieber) (Anthocoridae) and *Macrolophus caliginosus* (Wagner) (Miridae) exposed to imidacloprid, experienced 75-100% mortality of 1st and 2nd instars when exposed to 200 mg a.i. per litre via residual contact toxicity (Sterk *et al*. 2003). Another predacious bug exposed *H. vitripennis* was found to be highly susceptible to imidacloprid, with LC_{50} estimated to be 2.3 and 1.1 mg a.i. per litre for nymphs and adults, respectively (Bethke *et al*. 2001). Residual contact toxicity tested against 1st and 2nd instars of *Macrolophus caliginosus* (Wagner) (Miridae) lead to 75-100% mortality (Sterk *et al*. 2003). 100% mortality of the sharpshooter *H. coagulate* adults was obtained via foliar spray at 380 mg a.i. per litre (Bethke *et al*. 2001).

Wu *et al*. (2006) assessed the toxicity of imidacloprid against the gall-forming midge *C. nasturtii* using different methods. 100% of adults died after exposure to treated soil drenched at 420 mg a.i. per litre (within 8 days). Foliar spray led to 82% mortality of adults after 24 hours, and 92% of eggs and larvae within 10 days. Diet bioassay on adult females of the apple maggot *Rhagoletis pomonella* (Walsh) (Tephritidae) led to 53% mortality after 48 hours at 11 mg a.i. per litre (Reissig 2003).

51 Estimated based on the manufacturer's rate of 3.8 fl oz/acre for brassica.

Immature and adult females of the predatory mite *Neioseiulus fallacies* (Garman) (Acari: Phytoseiidae) were exposed via residual contact toxicity to 60 mg a.i. per litre (Villanueva *et al*. 2005). After 24 hours no mortalities occurred among immatures, and only 1.3% of adult females died (although the later rate increased to 20% after 96 hours). The flea *Oropsylla montana* (Baker) (Siphonaptera: Ceratophyllidae), a vector of plague, was exposed via residual contact (bedding treatment) to 10,000 mg a.i. per litre (Metzger *et al*. 2002). All eggs and adults were killed within one week.

App 3.3.8.7 Pirimiphos-methyl

Very little information is available on this product or its efficacy.

App 3.3.8.8 Spinosad

Dipping eggs of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Noctuidae) for 3 seconds on a 10 mg a.i. per litre spinosad solution led to 100% mortality within 3 days (Pineda *et al*. 2004). However, topical application of 1 µl of 1000 mg a.i. per litre to the pupae caused only 13% mortality after 12 days. Efficacy tests carried out by Hill *et al*. (2000) on *P. xylostella* found spinosad to cause 100% mortality of adults and 4th instars at 300 mg a.i. per litre after 72 hours. The former were exposed to foliage dipped in spinosad, and the larvae were dipped for 3 seconds or exposed to leaves dipped in the solution. Spinosad was also very efficient against the gypsy moth *Lymantria dispar* L. (Lymantriidae) (Wanner *et al*. 2000). Foliage spray and consequent exposure of 2nd instars to 50 mg a.i. per litre lead to 100% mortality over 5 days.

Spinosad at very low concentrations (in comparison to dose tested against lepidopterans) seemed to have some effect against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Tephritidae). Adults exposed via residual contact to 10 mg a.i. per litre had 100% mortality after 48 hours, while the LC_{90} obtained via oral treatment was 19.5 mg a.i. per litre after 14 hours (Adán *et al*. 1996). Diet bioassay for *C. capitata* adults gave a LC_{50} value of 4.2 mg a.i. per litre over 24 hours (Stark *et al*. 2004). Similar values for LC_{50} (24 h) were also obtained for adults of the Tephritidae melon fly *Bactrocera cucurbitae* Coquillett and oriental fruit fly *B. dorsalis* Hendel at 5.5 and 3.3 mg a.i. per litre, respectively (Stark *et al*. 2004). However, considerably higher resistance to spinosad was observed for the midge *C. nasturtii* (Wu *et al*. 2006). Exposure of adults via soil drench to 936 mg a.i. per litre for 24 hours led to 28% mortality. For eggs and larvae exposed to the same rate after foliar spray the mortality rate was 92% after 10 days (Wu *et al*. 2006). For adult females of the apple maggot *R. pomonella* the obtained mortality was circa 89% via diet bioassay at 316 mg a.i. per litre.

The value of LC_{95} for adults of the eggplant flea beetle *Epitrix fuscula* Crotch (Chrysomelidae) was 208 mg a.i. per litre after 2 days via diet bioassay (McLeod *et al*. 2002). Larvae and adults of another chrysomelid *C. scripta* were found to be considerably more susceptible to spinosad, with diet bioassays leading to 100 and 97% mortality, respectively, after 7 days (Tenczar *et al*. 2006). In contrast, mortality under the same experiment (at the same rate) for the coccinellids *H. convergens* and *H. axyridis* was nil (Tenczar *et al*. 2006).

First and 2nd instars of the predators' *O. laevigatus* and *M. caliginosus* exposed to 200 mg a.i. per litre suffered mortalities in the 0-25% range (Sterk *et al*. 2003). Adults of *O. insidiosus* experienced 54% mortality (54 hours) via direct contact toxicity to 60 mg a.i. per litre (Jones *et al*. 2005).

Residual contact toxicity against the parasitoid wasp *D. insulare* caused 100% mortality at 300 mg a.i. per litre over 24 hours (Hill *et al*. 2000). For *Encarsia formosa* resistance levels varied according to authors. Jones *et al*. (2005) obtained 95% mortality of adults exposed via residual contact toxicity to 60 mg a.i. per litre after 48 hours. In contrast the same method of exposure induced mortality in the range of 0-25% and 51-75% for pupae and adults, respectively, exposed to 200 mg a.i. per litre (Sterk *et al*. 2003). *Bombus terrestris* were comparatively more susceptible to spinosad with mortality in the range of 75-100% obtained with 96 mg a.i. per litre via direct contact toxicity (50 µl per specimen) (Sterk *et al*. 2003). Two other parasitoid wasps were assessed on their tolerance to spinosad via residual contact toxicity (Stark *et al*. 2004). Mortality rates for the braconids *Fopius arisanus* (Sonan) and *Pysttalia fletcheri* (Silvestri) were 77 and 57% (24 hours), respectively, after exposure to 2000 mg a.i. per litre (Stark *et al*. 2004).

The western flour thrip *Frankliniella occidentalis* Pergande (Thripidae) was found to be susceptible to spinosad (Jones *et al*. 2005). Larvae and adults exposed to 60 mg a.i. per litre via direct contact toxicity were 97% (48 h) and 100% (24 h), respectively (Jones *et al*. 2005).

Adult females of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae) were resistant to spinosad with exposure to 200 mg a.i. per litre causing mortality in the 0-25% range (Sterk *et al*. 2003). Adults of another phytoseiid mite (*Amblyseius cucumeris* Oudemans) exposed to 60 mg a.i. per litre via contact toxicity suffered 17% mortality after 48 hours (Jones *et al*. 2005). A further phytoseiid species (*Neioseiulus fallacies* (Garman)) was exposed to 60 mg a.i. per litre spinosad via residual contact toxicity (leaf dip) (Villanueva *et al*. 2005), and mortality rates were approximately 25% for the immature stage (24 h) and 58% for female adults (96 h). However, spinosad at very high concentrations were largely ineffective against the storage mite *T. putrescentiae* (Sanchez-Ramos *et al*. 2003), as a diet bioassay at 10,000 mg a.i. per litre caused only 9% mortality.

App 3.3.8.9 Tebufenozide

Hill *et al*. (2000) experimented on diamondback moths using the label rates of ca.1.5 g a.i. per litre on 4th instars larvae and adults. Larval dips led to approximately 90% mortality after 72 hours, while exposure to leaves dipped in the solution was ca. 92%. In contrast, no adult mortality was observed after exposure to 1.5 g a.i. per litre after 72 hours, with the same results obtained for the parasitoid wasp *D. insulare* after 24 hours. In contrast, Argentine *et al*. (2002) also targeted diamondback moth larvae and obtained a LC_{90} of 27 mg a.i. per litre, considerably lower than the figure obtained by Hill *et al*. (2000). Argentine *et al*. (2002) obtained values on the toxicity of tebufenozide for the larvae of a number of other Noctuidae species exposed to diet bioassays: tobacco budworm *Heliothis virescens* (F.), soybean looper *Pseudoplusia includens* (Walker), beet armyworm *Spodoptera exigua* (Hübner), fall armyworm *Spodoptera frugiperda* (JE Smith) and the cabbage looper *Trichoplusia ni* (Hübner). The LC_{90} values were 27.1, 2.6, 7.8, 2.1 and 0.24 mg a.i. per litre, respectively.

App 3.3.8.10 Thiachloprid

Very little information is available on the efficacy of this chemical compound, which is not included in the manual on phytosanitary treatments for nursery stock (Lester and Lunn 2003). The little information gathered on its efficacy showed that thiachloprid failed to induce high rates of mortality.

App 3.3.8.11 General Comments

The literature examined highlights that it is not possible to extrapolate the effects of chemical treatments between:

a. Life stages – Galvan *et al*. (2005) studied the efficacy of carbaryl on different life stages of the diamondback moth using spray treatments. While, the rate of 1.96 kg a.i. per hectare lead to 100% mortality of eggs, 1st and 3rd instars and adults, it lead to practically nil mortality among pupae, almost all of which successfully hatched into adults. The same study also showed that 0.11 kg a.i. per hectare of carbaryl killed ca. 92% of first instars but no eggs of the multicoloured Asian lady beetle *Harmonia axyridis*.

b. Taxa that are apparently closely related – Argentine *et al*. (2002) evaluated the efficacy of tebufenozide in diet assays against a number of Lepidoptera species. The observed LC_{90} after 6 days for the tobacco budworm *Helioth virescens* (F.) and the cabbage looper *Trichoplusia ni* (Hübner) were 27.1 and 0.24 µg/ml, respectively. Although both species are in the family Noctuidae, the LC_{90} for the former species was 113 times higher than that of the latter.

There are numerous references in the literature to insecticide resistance developed by numerous pests. Many studies have compared insecticide efficacy between resistant and susceptible strains indicating considerable differences. For example, Shimada *et al*. (2005) compared the effect of fenvalerate treatments via topical application or dipping on different strains of the beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). The LC_{50} over 24 hours for 5th instars of the susceptible and resistant strains were 2.6 and 336 µg/larva, respectively, i.e. a 129-fold difference.

App 3.3.9 Recommendations for measure: Chemical Treatment for Insect Infestations

An examination of the efficacy information is provided in table App 3.5 for each of the active ingredients listed.

Table App 3.5: Derived efficacies for chemical treatments for insect infestations

Active ingredient	Comment on Efficacy
Acephate (see App 3.3.8.1)	It is not possible to compare the efficacy of the dipping method against foliar spray. Acephate is said to have "contact and systemic action, and also acts as a stomach poison when ingested" (Armstrong-Fay 2004). Therefore, dipping is most likely to be considerably more effective than foliar spray alone, which means that the rate adopted would be lower. However, in the absence of efficacy data and any information on the above, it is not possible to extrapolate on the dipping rate that would be as effective as the foliage rate. Therefore, it is recommended that dipping with acephate at approximately 10 times the label rate, at 8.0 g a.i. per litre should achieve the necessary quarantine requirements. A foliar spray application should not be permitted.
Carbaryl (see App 3.3.8.2)	Carbaryl has neurotoxic effects, and acts via direct contact or ingestion (Armstrong-Fay 2004). As a result, a dipping treatment is likely to be considerably more effective than a foliar spray. It is clear however, that the label rate of 1200 mg a.i. per litre would not be an effective quarantine measure against many pests. Therefore the rate of 18 g a.i. per litre of carbaryl should be used and a foliar spray application should not be permitted.
Chlorpyrifos (see App 3.3.5.2)	As chlorpyrifos is neurotoxic, acting as a contact pesticide and via ingestion (Armstrong-Fay 2004), dipping would enhance the effectiveness of the rates described. Based on the available evidence a rate of 10 g a.i. per litre of chlorpyrifos should be applied, which is likely to be effective both as an insecticide and acaricide for non-dormant feeding insect or mite life stages. As egg or non-feeding life stages may survive a single treatment, a second treatment should be applied 10 days later if an actual infestation of insects or mites is detected. A foliar spray application should not be permitted.
Deltamethrin (see App 3.3.8.3)	Until adequate information can be obtained, this chemical compound should no longer considered for quarantine purposes in New Zealand.
Dimethoate (see App 3.3.8.4)	Dimethoate is an organophosphorous pesticide with insecticidal and acaricidal properties (Bostanian *et al*. 2004), being neurotoxic and acting via contact or ingestion (Armstrong-Fay 2004). Assuming the effects of dimethoate on the very few species listed here are a good representation of the susceptibilities to this pesticide, the recommended label rate (1.1 g a.i. per litre) would likely be effective as a quarantine treatment.
Fenvalerate (see App 3.3.8.5)	Until adequate information can be obtained, this chemical compound should no longer considered for quarantine purposes in New Zealand.
Imidacloprid (see App 3.3.8.6)	The available data suggests that the label rate of 3.0 g a.i. per litre is likely to be effective against a wide range of organisms.
Pirimiphos-methyl (see App 3.3.8.7)	Until adequate information can be obtained, this chemical compound should no longer considered for quarantine purposes in New Zealand.
Spinosad (see App 3.3.8.8)	The recommended label rate (192 mg a.i. per litre) is clearly not appropriate to be adopted for quarantine purposes. It seems that in order to err on the side of caution, approximately 10 times that concentration should be adopted, which should be effective against the vast majority of insects. For example, the mortality rate induced on cotton leafwork at 1,000 mg a.i. per litre (Pineda *et al*. 2004) would likely have been much higher if the pupae had been dipped, in contrast to the few droplets used in the test. However, it is important to point out that even the high dose recommended here is unlikely to be effective against all insects, such as the braconids that suffered only 57% mortality after exposure to 2000 mg a.i. per litre (Stark *et al*. 2004). In addition, spinosad does not seem to be effective as an acaricide as implied by the tests on *T. putrescentiae* (Sanchez-Ramos *et al*. 2003). It is therefore recommended that spinosad is used as an insecticide (but not as an acaricide) at the suggested rate of 2 g a.i. per litre. A foliar spray application should not be permitted.

Active ingredient	Comment on Efficacy
Tebufenozide (see 3.3.8.9)	The studies above provided large discrepancies on the toxicity of tebufenozide to *P. xylostella*. However, since 90% mortality was obtained for the label rate in one of the studies, it seems advisable to use 2x label rate to add a safety margin based on the available information. Since all other lepidopteran pests had LC_{90} values that were considerably lower, a tebufenozide concentration of 3.0 g a.i. per litre is likely to be effective against lepidopterans. Nonetheless, it is advisable to carry out complementary treatment with a pesticide from another chemical group. A foliar spray application should not be permitted.
Thiacloprid (see 3.3.8.10)	Until adequate information can be obtained, this chemical compound should no longer considered for quarantine purposes in New Zealand.

It is recommended that no single insecticide is adopted for treatment, and that the more efficacious dip treatment be the standard method of application. In addition, ideally the risk analysis should indicate whether certain resistant strains of high risk pests occur in the country of origin of a particular commodity, so that the recommended measures may exclude particular pesticides as possible mitigating measures. It is also likely that insecticide dipping would have reduced efficacy against insects such as wood borers that have life stages deep within plant tissue. A research project is also recommended to address issues relating to the lack of efficacy data underpinning the currently prescribed treatments for nursery stock.

In summary therefore:

- Deltamethrin, pirimiphos-methyl and thiachloprid should not be used at this stage for quarantine purposes, until adequate efficacy information is available;
- Fenvalerate should no longer be used as a prescribed chemical treatment, since its permit has been revoked by the EPA (USA);
- The remaining pesticides, namely acephate, chlorpyrifos, and dimethoate (organophosphorous); carbaryl (carbamate); imidacloprid (neonicotinoid); spinosad (spinosyns); and tebufonized (diacylhydrazine), are adopted in chemical treatments of nursery stock for quarantine purposes, but using the revised rates until adequate efficacy data are available;
- A combination of pesticides from two different chemical groups (organophosphorous, carbamate, neonicotinoid, spinosyns, or diacylhydrazine) should be applied to maximize efficacy and mitigate potential issues with insect resistance;
- Nursery stock should be treated again 10-14 days after the initial treatment to manage any potential lower treatment efficacy against untested insects or life stages;
- Nursery stock entering a post-entry quarantine facility should not begin their quarantine period for insect contamination until the chemical residues have sufficiently dissipated or become inactive (considered here to be equivalent to the aerobic soil half life - see table App 3.6). Care should be taken to ensure that adequate account is taken in the quarantine period of nursery stock subjected to a pre-quarantine application of insecticides with extended residual activity.
- Scientific research is required to address issues relating to:

- the lack of efficacy data underpinning the currently prescribed treatments for nursery stock;
- any potential phytotoxicity effects from the off-label application rates recommended in this analysis; and
- the actual period of *in-vivo* residual activity of the insecticides.

Table App 3.6: Details of Recommended General Treatments for Insects on Nursery Stock

Active ingredient (Chemical Group)	Treatment Specification[52]	Residue Persistence[53]	Target Insect Pests
Acephate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 8 g a.i. per litre of dip. Treatment of non-dormant plant material only.	3 days (aerobic)	*Contarinia nasturtii*, *Homalodisca coagulata*
Carbaryl (Carbamate)	Dip at room temperature for 2-5 minutes at 18 g a.i. per litre of dip.	6 days (aerobic)	*Plutella xylostella*, *Diadegma insulare*, *Harmonia axyridis*, *Chrysomela scripta*, *Hippodamia convergens*, *Harmonia axyridis*, *Endopiza viteana*, *Dendroctonus rufipennis*, *Homalodisca coagulate*. *Tetranychus urticae* (Acari), *Rhizoglyphus echinopus* (Acari)
Chlorpyrifos (Organophosphorous)	Dip at room temperature for 2-5 minutes at 10 g a.i. per litre of dip. A non-ionic surfactant is required for dipping	113 days* (aerobic)	*Contarinia nasturtii*, *Harmonia axyridis*
Dimethoate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 1.1 g a.i. per litre of dip. Treatment of non-dormant plant material only.	2 days (aerobic)	*Orius insidiosus*, *Aphidius colemani*, *Bombus terrestris*, *Hyaliodes vitripennis*
Imidacloprid (Neonicotinoid)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip. Treatment of non-dormant plant material only.	997 days* (aerobic)	*Anoplophora glabripennis*, *Bombus terrestris*, *Chrysomela scripta*, *Contarinia nasturtii*, *Diadegma insulare*, *Diaeretiella rapae*, *Encarsia formosa*, *Harmonia axyridis*, *Harpalus pennsylvanicus*, *Hippodamia convergens*, *Homalodisca coagulate*, *Hyaliodes vitripennis*, *Macrolophus caliginosus*, *Neioseiulus fallacies*, *Orius insidiosus*, *Orius laevigatus*, *Oropsylla montana*, *Ostrinia nubilalis*, *Oulema melanopus*, *Plectrodera scalator*, *Plodia interpunctella*, *Plutella xylostella*, *Rhagoletis pomonella*, *Phytoseiulus persimilis* (Acari)

52 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.

53 Based on the aerobic soil half life (where provided, hydrolysis or anaerobic half life where not provided) provided by the PAN Pesticides Database at http://www.pesticideinfo.org

Active ingredient (Chemical Group)	Treatment Specification[52]	Residue Persistence[53]	Target Insect Pests
Spinosad (Spinosyns)	Dip at room temperature for 2-5 minutes at 2 g a.i. per litre of dip.	17 days (aerobic)	*Bactrocera cucurbitae, Bactrocera dorsalis, Bombus terrestris, Ceratitis capitata, Chrysomela scripta, Contarinia nasturtii, Cryptolestes ferrufineus, Diadegma insulare, Encarsia formosa, Epitrix fuscula, Fopius arisanus, Frankliniella occidentalis, Harmonia axyridis, Hippodamia convergens, Lymantria dispar, Macrolophus caliginosus, Orius insidiosus, Orius laevigatus, Oryzaephilus mercator, Oryzaephilus surinamensis, Plodia interpunctella, Plutella xylostella, Pysttalia fletcheri, Rhagoletis pomonella, Rhyzopertha dominica, Sitophilus oryzae, Spodoptera littoralis, Tribolium castaneum, Tribolium confusum, Trogoderma variabile*
Tebufenozide (Diacylhydrazine)	Dip at room temperature for 2-5 minutes at 3 g a.i. per litre of dip.	405 days* (aerobic)	*Chrysoperla carnea, Diadegma insulare, Heliothis virescens, Orius insidiosus, Plutella xylostella, Pseudoplusia includens, Spodoptera exigua, Spodoptera frugiperda, Trichoplusia ni*

* It is likely that these periods of residue persistence are not reflected as equivalent periods of residual activity on the treated plants. These "worse case" figures have been listed in the absence of information on the actual period of residual activity *in-vivo*.

App 3.3.10 Description of measure: Chemical Treatment for Mite Infestations

Plants must be sprayed or dipped with agitation using two active ingredients chosen from the table below, using either Abamectin or two active ingredients belonging to different chemical groups. For dipping, the treatment time is normally 2 minutes but must be increased to 5 minutes if bubbles remain present on the plant surface. Dip solutions must be used no more than twice or as per manufacturer's recommendations. All treatments must be carried out in accordance with manufacturer's recommendations using either the recommended label rate or the rates shown in the table App 3.7 below.

Table App 3.7: Chemical treatments for mite infestations currently approved in the "basic" requirements of MAF standard 155.02.06.

Chemical group	Active ingredient	Dip time	Notes
Organochlorine	Abamectin (0.009 g per litre of dip/spray)	2-5 mins	Non-ionic surfactant required for dipping
Organochlorine	Dicofol	2-5 mins	
Organophosphorous	Acephate (0.75 g per litre of dip/spray)	2-5 mins	Non-dormant material only
Organophosphorous	Chlorpyrifos (2.4 g per litre of dip/spray)	2-5 mins	Non-ionic surfactant required for dipping
Organophosphorous	Dimethoate	2-5 mins	Non-dormant material only
Organophosphorous	Pirimiphos-methyl (0.475 g per litre of dip/spray)	2-5 mins	Non-ionic surfactant required for dipping

App 3.3.11 Analysis of measure efficacy: Chemical Treatment for Mite Infestations

App 3.3.11.1 Abamectin

Abamectin, a contact and stomach acaricide, belongs to the organochlorine chemical group. Lester and Lunn (2003) indicated that this acaricide has shown efficacy on motile stages only of *Eutetranychus banksi* (Texas citrus mite), *Tetranychus kanzawai* (Desert spider mite), *Tetranychus neocaledonicus*, and *Tetranychus urticae* (Two spotted spider mite), and recommended an application rate of 180 mg a.i. per litre of water with a 10-14 days re-treatment to manage the lack of efficacy on mite eggs.

Knowles *et al*. found that abamectin and the other organochlorines tested were inactive against the bulb mite (*Rhizoglyphus echinopus*). Herron *et al.* (1996) determined that the $LC_{99.9}$ against *Polyphagotarsonemus latus* (banks) (Acari: Tarsonemidae) adults was 0.1 mg a.i. per litre. Humeres and Morse (2005) found maximum LC_{90} rates for *Oligonychus perseae* (Acari: Tetranychidae) adults at the upper 95% confidence limit as 3.3 mg a.i. per litre of dip. Nauen *et al.* (2001) found maximum LC_{95} rates for *Tetranychus urticae* (Acari: Tetranychidae) first-motility stage larvae at the upper 95% confidence limit as 0.21 mg a.i. per litre of spray.

App 3.3.11.2 Dicofol

Dicofol, a non-systemic acaricide with contact action, belongs to the organochlorine chemical group. Lester and Lunn (2003) noted that this acaricide has shown efficacy in field situations on *Eutetranychus banksi* (Texas citrus mite), *Tetranychus kanzawai* (Desert spider mite), *Tetranychus neocaledonicus*, and *Tetranychus urticae* (Two spotted spider mite). Lester and Lunn (2003) also noted that resistance to dicofol is present in some mite populations. If the mite species is likely to be resistant in the country of origin, an alternative treatment should be used instead of, or in combination with dicofol.

Herron *et al.* (1996) determined that the $LC_{99.9}$ against *Polyphagotarsonemus latus* (banks) (Acari: Tarsonemidae) adults was 1 g a.i. per litre. Dagli and Tunc (2001) demonstrated that resistance to dicofol in agricultural populations of *Tetranychus cinnabarinus* (Acari: Tetranychidae) could, depending on the bioassay, range from as much as 17 to 59 fold. Maximum LC_{95} results obtained at the upper 95% confidence limit were as high as 8.9 g a.i. per litre of the applied acaricide. Similar levels of resistance have also been reported for *Tetranychus urticae*. Kumar and Singh (2004) found that dicofol was effective on *Tetranychus urticae* eggs at relatively low concentrations.

App 3.3.12 Recommendations for measure: Chemical Treatment for Mite Infestations

Table App 3.8 below examines the efficacy information for each of the active ingredients listed above.

Table App 3.8: Derived efficacies for chemical treatments for insect infestations

Active ingredient	Comment on Efficacy
Abamectin (see App 3.3.11.1)	As abamectin acts as a contact pesticide and via ingestion, dipping would enhance the effectiveness of the rates described. Based on the available evidence a rate of 180 mg a.i. per litre of abamectin should be applied, which is likely to be effective on non-dormant feeding mite life stages. As egg or non-feeding life stages may survive a single treatment, a second treatment should be applied 10 days later if an actual infestation of mites is detected. A foliar spray application should not be permitted.
Dicofol (see App 3.3.11.2)	As dicofol acts as non-systemic acaricide with contact action, dipping would enhance the effectiveness of the rates described. Based on the available evidence a rate of 10 g a.i. per litre of dicofol should be applied, which is likely to be effective on all mite life stages. A foliar spray application should not be permitted.
Acephate (see App 3.3.8.1)	This treatment should not be adopted as an acaricide (miticide) until adequate efficiency data on mites can be obtained.
Chlorpyrifos (see App 3.3.5.2)	As chlorpyrifos is neurotoxic, acting as a contact pesticide and via ingestion (Armstrong-Fay 2004), dipping would enhance the effectiveness of the rates described. Based on the available evidence a rate of 10 g a.i. per litre of chlorpyrifos should be applied, which is likely to be effective both as an insecticide and acaricide for non-dormant feeding insect or mite life stages. As egg or non-feeding life stages may survive a single treatment, a second treatment should be applied 10 days later if an actual infestation of insects or mites is detected. A foliar spray application should not be permitted.
Dimethoate (see App 3.3.8.4)	Dimethoate is an organophosphorous pesticide with insecticidal and acaricidal properties (Bostanian *et al.* 2004), being neurotoxic and acting via contact or ingestion (Armstrong-Fay 2004). Assuming the effects of dimethoate on the very few species listed here are a good representation of the susceptibilities to this pesticide, the recommended label rate (1.1 g a.i. per litre) would likely be effective as a quarantine treatment.
Pirimiphos-methyl (see App 3.3.8.7)	This treatment should not be adopted as an acaricide (miticide) until adequate efficiency data can be obtained.

It is recommended that no single insecticide is adopted for treatment, and that the more efficacious dip treatment be the standard method of application. In addition, ideally the risk analysis should indicate whether certain resistant strains of high risk pests occur in the country of origin of a particular commodity, so that the recommended measures may exclude particular miticides as possible mitigating measures. A research project is also recommended to address issues relating to the lack of efficacy data underpinning the currently prescribed treatments for nursery stock.

In summary therefore it is recommended that:

- Acephate and pirimiphos-methyl should not be used at this stage as a mite treatment for quarantine purposes, until adequate efficacy information is available;
- The remaining miticides, namely abamectin (avermectin), dicofol (organochlorine), chlorpyrifos, and dimethoate (organophosphorous), are adopted in chemical treatments for mites on nursery stock for quarantine purposes, but using the revised rates until adequate efficacy data are available;
- A combination of miticides from two different chemical groups (organochlorine and organophosphorous) should be applied to maximize efficacy and mitigate potential issues with mite resistance;

- Nursery stock should be treated again 10-14 days after the initial treatment to manage potentially lower treatment efficacy against mite eggs and other untested mite species and life stages;

- Nursery stock entering a post-entry quarantine facility should not begin their quarantine period for mite contamination until the chemical residues have sufficiently dissipated or become inactive (considered here to be equivalent to the aerobic soil half life - see table App 3.9). Care should be taken to ensure that adequate account is taken in the quarantine period of nursery stock subjected to a pre-quarantine application of miticides with extended residual activity.

- A research project is required to address issues relating to the lack of efficacy data underpinning the currently prescribed treatments for nursery stock.

Table App 3.9: Details of Recommended General Treatments for Mites on Nursery Stock

Active ingredient (Chemical Group)	Treatment Specification[54]	Residue Persistence[55]	Target Pest (Mites)
Abamectin (Organochlorine)	Dip at room temperature for 2-5 minutes at 180 mg a.i. per litre of dip. A non-ionic surfactant is required for dipping	143 days (anaerobic)	*Eutetranychus banksi*, *Tetranychus kanzawai*, *Tetranychus neocaledonicus*, *Tetranychus urticae*, *Polyphagotarsonemus latus*, *Oligonychus perseae*
Dicofol (Organochlorine)	Dip at room temperature for 2-5 minutes at 10 g a.i. per litre of dip.	66 days (aerobic)	*Eutetranychus banksi*, *Tetranychus kanzawai*, *Tetranychus neocaledonicus*, *Tetranychus urticae*, *Polyphagotarsonemus latus*, *Tetranychus cinnabarinus*
Chlorpyrifos (Organophosphorous)	Dip at room temperature for 2-5 minutes at 10 g a.i. per litre of dip. A non-ionic surfactant is required for dipping	113 days (aerobic)	*Tyrophagus putrescentiae*, *Tetranychus urticae*, *Panonychus ulmi*
Dimethoate (Organophosphorous)	Dip at room temperature for 2-5 minutes at 1.1 g a.i. per litre of dip. Treatment of non-dormant plant material only.	2 days (aerobic)	*Tetranychus urticae*, *Rhizoglyphus echinopus*

App 3.4 Chemical treatment for fungal infestations

App 3.4.1 Description of measure: Chemical Treatment for Fungal Infestations

Currently no fungicide treatments are approved for use on nursery stock in the "basic" requirements of the nursery stock standard 155.02.06 (there are a number of fungicide treatments approved for specific pests in the plant schedules within the standard). The following fungicide treatments (table App 3.10) were recommended for use in the report developed by Lester and Lunn (2003) for the Ministry of Agriculture and Forestry.

54 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.

55 Based on the aerobic soil half life (where provided, hydrolysis or anaerobic half life where not provided) provided by the PAN Pesticides Database at http://www.pesticideinfo.org

Table App 3.10: Details of Recommended General Treatments for Fungi on Nursery Stock

Active ingredient (Chemical Group)	Treatment[56]	Notes	Target Diseases
Azoxystrobin (Strobilurin)	Dip at room temperature for 15 minutes at 0.95 g a.i. per litre of dip	Protective, curative, eradicant, translaminar and systemic properties. Inhibits spore germination and mycelial growth, and also shows antisporulant activity.	Wide range of activity. Label claims on various crops have been made for species from the following genus: *Sclerotium, Monilia, Cladosporium, Mycosphaerella, Puccinia, Rhizoctonia, Colletotrichum, Septoria, Alternaria, Phytophthora, Pythium* spp.
Carbendazim (Benzimidazole)	Dip for 20 minutes at 1 g a.i per litre of dip.	Systemic fungicide with protective and curative action. Absorbed through roots and green tissues, with translocation acropetally. Acts by inhibiting development of the germ tubes, formation of appressoria and growth of mycelia.	Label claims for *Septoria, Fusarium, Erysiphe, Psuedocercosporella, Sclerotinia, Alternaria, Cylindrosporium, Cercospora, Uncinula, Botrytis, Cladosporium, Venturia, Podosphaera,* and *Monilia*. Efficacy shown on *Botryodiplodia palmarum, Macrophoma* sp., *Microsphera* spp., *Ovulina azaleae, Uredinales, Fusarium oxysporum* f. sp. *vasinfectum*. Likely to be resistant: *Botrytis, Monilinia* spp., *Penicillium* spp.
Chlorothalonil (Chloronitrile)	Dip at room temperature for 15 minutes.	Non systemic foliar fungicide with protective action.	Wide spectrum of activity. Field efficacy shown on *Apiosporina morbosa*
Fosetyl-aluminium (Phosphonates: ethyl phosphonates)		Systemic fungicide, absorbed through leaves and roots, with translocation both acropetally and basipetally. Inhibits germination of spores or by blocking development of mycelium or sporulation.	Phytomycetes (*Phytophthora, Pythium, Plasmopara, Bremia* spp. etc)
Iprodione (Dicarboximide)	Dip for 30 minutes at 2 g a.i. per litre of dip	Contact fungicide with protective and curative action. Inhibits germination of spores and growth of fungal mycelium	Likely to be effective against Ascomycetes and Hyphomycetes. The NZ label has approved claims for *Botrytis, Stemphylium, Alternaria, and Sclerotinia* on flowers and ornamentals. US label claims are for *Fusarium* spp., *Botrytis, Sclerotinia, Phoma* spp., *Rhizoctonia* spp., *Helminthosporium* spp. etc. Likely to be resistant: *Botrytis, Monilinia* spp., *Penicillium* spp.
Pencycuron (Phenylurea)	Dip for 15 minutes.	Non-systemic fungicide with protective action. Probably acts on cell division.	*Rhizoctonia* spp.

56 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.

Active ingredient (Chemical Group)	Treatment[56]	Notes	Target Diseases
Propiconazole (Triazole)	Dip for 15 minutes at 0.5 g a.i. per litre of dip.	Systemic foliar fungicide with protective and curative action, with translocation acropetally in the xylem. Steroid demethylation (ergosterol biosynthesis) inhibitor.	Broad range of activity. Propicanozole is most likely to be effective against a wide range of Basidiomycetes and some Ascomycetes and Hyphomycetes. Approved label claims against *Cochliobolus sativus, Erysiphe graminis, Leptosphaeria nodurum, Puccinia spp., Pyrenophora teres, Pyrenophora tritici-repentis, Rynchosporium secalis, Septoria* spp., *Mycosphaerella musicola, Mycosphaerella fijiensis var. difformis, Sclerotinia homeocarpa, Rhizoctonia solani, Erysiphe graminis, Helminthosporium* spp., *Hemileia vastatrix, Cercospora* spp., *Monilia* spp., *Podosphaera* spp., *Spaerotheca* spp., *Tranzschelia* spp.. Dip on sugarcane pieces effective against *Ceratocystis* or *Thievaviopsis paradoxa*. Current NZ Biosecurity treatment for *Cronatium flaccidum, Ceratocystis fagacearum, Cronatium queruum* and *Cryphonectria parasitica, Gymnosporangium, Uredinales, Chysomyxa ledi, Microsphaeria spp*. Likely to be effective on *Phellinus noxius, Phymatotrichopsis omnivora, Phytophthora meadii and Gymnosporangium asiaticum*. Likely to be effective as a protectant only against *Phytophthora lateralis* and *Phellinus punctatus* (infects xylem tissue).
Pyrimethanil (Anilinopyrimidine)	Dip for 15 minutes.	Protectant fungicide. Inhibits methionine biosynthesis leading to inhibition of the secretion of enzymes necessary for infection.	*Botrytis* spp., *Venturia* spp., *Monilinia* spp.

Active ingredient (Chemical Group)	Treatment[56]	Notes	Target Diseases
Thiophanate-methyl (Benzimidazole)	Dip at 27-29.5^{O}C for 10-15 minutes at 0.75 g a.i. per litre of dip.	Systemic fungicide with protective and curative action.	Most likely to be effective against Ascomycetes, Hyphomycetes and a few Basidiomycetes. US labels have claims for *Botrytis, Cylindrocladium, Fusarium, Gliocladium, Myrothecium, Penicillium, Pamularia, Rhizoctonia, Sclerotinia, and Thieviopsis*. NZ Biosecurity recommends this for use on *Microsphera* spp., *Ovulina azaleae* and Uredinales on *Rhododendron*. Likely to be effective on *Cronartium flaccidum.* Likely to be resistant: *Botrytis, Monilinia* spp., *Penicillium* spp.
Thiram (Dimethyldithio-carbamate)	Dip at room temperature at 11.2 g a.i. per litre of dip	Contact fungicide with multi-site activity and protective action.	Efficacy has been tested against *Pythium* and *Fusarium* spp. Likely to be suitable for most fungal diseases but efficacy has not been tested. Wide spectrum, but less likely to be effective against *Botrytis, Phycomycetes* or *Sclerotium* than specific treatments. Field efficacy has been shown on *Phellinus noxius*.

App 3.4.2 Analysis of measure efficacy: Chemical Treatment for Fungal Infestations

Some considerable extrapolations and assumptions are made in this report. Therefore, the recommendations regarding the appropriate treatment rates made in this report must be considered with great caution. It should be noted that resistance to chemicals is commonly observed for fungi and it is therefore advisable that, in the absence of comprehensive efficacy data, two fungicides from different chemical groups should be adopted for quarantine purposes.

App 3.4.2.1 Azoxystrobin

Azoxystrobin appears to be one of the commonly used fungicides overseas. The research done on this particular chemical clearly illustrates the major differences in susceptibility to fungicides that occur. Sudisha *et al.* (2005) showed *in vitro* that azoxystrobin at 1 mg a.i. per litre lead to 100% suppression of zoospore release and sporangia formation for *Sclerospora graminicola*, the causative agent of pearl millet downy mildew disease. Perez *et al.* (2002) and Perez *et al.* (2004) obtained 100% inhibition of ascospore germination of *Mycosphaerella fijiensis* (black Sigatoka) *in vitro* at 5 and 1 mg a.i. per litre, respectively. In contrast, azoxystrobin at 1000 and 1200 mg a.i. per litre were used to induce 71% and 95% inhibition of mycelial growth *in vitro*, respectively, for *Armillaria mellea* (Aguín *et al.* 2006) and *Alternaria alternata* (Reuveni *et al.* 2002), respectively. Note that for *A. alternata* 95% inhibition of conidial germination was observed at 720 mg a.i. per litre.

Matheron *et al.* (2000) assessed *in vitro* the efficacy of azoxystrobin against three *Phytophthora* species (*P. capsici*, *P. citrophthora* and *P. parasitica*). For *P. capsici* and *P. parasitica* 1000 mg a.i. per litre inhibited 100% of sporangium formation and approximately 70% and 75% of cyst germination, respectively. In contrast, the EC_{50} values for azoxystrobin to inhibit mycelial growth of both species were >3000 mg a.i. per litre. For *P. citrophthora*, 100 and 1000 mg a.i. per litre inhibited 100% of sporangium formation and ca.85% of cyst germination, respectively, with EC_{90} value for mycelial growth being >3000 mg a.i. per litre (Matheron *et al.* 2000).

App 3.4.2.2 Carbendazim

Different fungal pathogens have shown contrasting susceptibility *in vitro* to carbendazim, with ED_{100} (effective dose) varying in some cases by three orders of magnitude (Table 1). For example, 0.5 and 1.0 mg a.i. per litre lead to 100% suppression of conidial germination and spore germination, respectively, for *Phomopsis theae* (Ponmurugan *et al.* 2006) and *Ascochyta rabiei* (Demirci *et al.* 2003), respectively. In contrast, at 550 mg a.i. per litre carbendazim lead to only 59% reduction in lesions caused by *Botrytis narcissicola* (O'Neill *et al.* 2004). Similarly, 500 mg a.i. per litre suppressed 85% of radial growth of *Paecilomyces fumosoroseus* (Er *et al.* 2004).

App 3.4.2.3 Chlorothalonil

Chlorothalonil also has contrasting efficacy against different fungal pathogens, with the effective dose varying by more than three orders of magnitude. The least susceptible pathogens to chlorothalonil were *Ustilago scitaminea* (sugarcane smut) with 53% reduction in disease incidence after dipping (Wada 2003) and *Botrytis narcissicola* (narcissus smoulder) with 64% reduction in extent of lesions *in vitro* (O'Neill *et al.* 2004) at the rates of 3400 and 1500 mg a.i. per litre, respectively. There was also no observed reduction in fruit disease caused by *Alternaria alternata* pathovar *citri* after foliar spray at 1250 mg a.i. per litre (Solel *et al.* 1997), and *in vitro* experiments with chlorothalonil against *Nectria galligena* lead to 70% suppression of colony formation at 1250 mg a.i. per litre (Xu *et al.* 1996).

It should be pointed out that one study on chlorothalonil (Demirci *et al.* 2003) showed the efficacy of this fungicide clearly varies depending on the targeted stage of fungal development. Chlorothalonil at 2 mg a.i. per litre caused 100% suppression of *Ascochyta rabiei* spore germination, but the same efficacy on mycelial growth would only be obtained using an undetermined rate above 128 mg a.i. per litre (Demirci *et al.* 2003).

App 3.4.2.4 Fosetyl-aluminium

There were a few studies found where the efficacy of fosetyl-aluminium as a fungicide was assessed, only one of which carried out the test *in vitro*. In the latter (Aguín *et al.* 2006), at 1000 mg a.i. per litre fosetyl-aluminium lead to 70% suppression of mycelial growth in *Armillaria mellea*. The remaining studies mostly assessed the consequent reduction in disease incidence. Foliar spray at 4000 mg a.i. per litre lead to 49% reduction in disease incidence caused by *Peronospora sparsa* (O'Neill *et al.* 2002).

A study using plant collar treatment against *Phytophthora cryptogea* lead to variable efficacy rates depending on trial ranging from 23-97% reduction in disease incidence at rates of 2400 and 3000 mg a.i. per litre (Benigni *et al.* 2006; Benigni *et al.* 2004). However, *in vitro*

experiments assessing the efficacy of fosetyl-aluminium against three *Phytophthora* species (Matheron and Porchas 2000) yielded much better results than those obtained by Benigni *et al.* (2006) and Benigni *et al.* (2004) using field trials. Fosetyl-aluminium at 1000 mg a.i. per litre lead to 100% inhibition of mycelial growth, sporangium formation, and cyst germination for *P. capsici*, *P. citrophthora* and *P. parasitica* (Matheron *et al.* 2000).

Another study using foliar sprays in the field crops of garlic at 2000 mg a.i. per litre, fosetyl-aluminium was ineffective against *Stemphylium vesicarium* with a 5% reduction in disease incidence observed in comparison to the control (Basallote-Ureba *et al.* 1998).

It should be noted that since the experimental methods vary, it is difficult to compare the results of different studies. Regarding in particular the Basallote-Ureba *et al.* (1998) field experiment, the authors have also obtained unusually poor results for thiram and other fungicides indicating that their methods are likely to have been inadequate.

App 3.4.2.5 Iprodione

Iprodione's efficacy has been tested against a range of *Alternaria* spp. The EC_{50} values for iprodione causing *in vitro* inhibition of radial mycelial growth of *Alternaria japonica*, *A. alternate* and *A. brassicae* were 6.1, 5.3 and 2.2 mg a.i. per litre, respectively (Iacomi-Vasilescu *et al.* 2004). The same EC_{50} value against *A. brassicicola* was highly variable depending on the isolate, being as low as 0.95 mg for some but identified to be above 100 mg a.i. per litre for at least four of the 19 isolates tested (Iacomi-Vasilescu *et al.* 2004). Further tests done by (Iacomi-Vasilescu *et al.* 2004) on *A. brassicicola* lead to 25-98% suppression of spore germination (depending on isolate) at 100 mg a.i. per litre. Solel *et al.* (1997) tested iprodione against *Alternaria alternata* pathovar *citri* using foliar spray and obtained a 64% reduction in disease incidence at 500 mg a.i. per litre.

For *Colletotrichum acutatum* (Kososki *et al.* 2001) and *Rhizoctonia solani* (Silveira *et al.* 2003) 100% suppression of mycelial growth was obtained with iprodione at 100 and 10 mg a.i. per litre, respectively. Paredes *et al.* (2002) also tested the efficacy of iprodione against *Colletotrichum acutatum*, and found it to be effective at considerably higher rates than that obtained by Kososki *et al.* (2001), with the rate necessary to yield 100% suppression of mycelial growth and colony formation found to be >500 mg a.i. per litre. Note that ED_{50} values for the latter were 10 and 100 mg a.i. per litre, respectively (Paredes *et al.* 2002).

Iprodione lead to 100% suppression of conidial germination and radial growth of *Paecilomyces fumosoroseus* at 500 and 5000 mg a.i. per litre, respectively (Er *et al.* 2004). Note that in the latter study the rates used varied with orders of magnitude, and for radial growth ca.50% reduction was obtained at 500 mg a.i. per litre (Er *et al.* 2004). A relatively small dose was tested against *Nectria galligena* with approximately 25% suppression of colony formation obtained with 80 mg a.i. per litre (Xu *et al.* 1996). Labuschagne *et al.* (1996) tested iprodione *in vitro* against *Chalara elegans* and did not obtain 100% suppression of radial growth at 1,000 mg a.i. per litre, although no other details were given.

App 3.4.2.6 Pencycuron

Pencycuron is listed in Armstrong-Fay (2004) under the trade name Monceren®, which is described as a fungicide for the control of *Rhizoctonia solani* in potatoes (apparently specifically so). In fact, the single study found on the efficacy of this chemical observed 100% suppression of mycelial growth of *Rhizoctonia solani in vitro* at 1 mg a.i. per litre (1 ppm) (Silveira *et al.* 2003).

App 3.4.2.7 Propiconazole

A number of studies have assessed the efficacy of propiconazole against several fungal pathogens. Propiconazole at 1 mg a.i. per litre lead to approximately 90% suppression *in vitro* of mycelial growth of *Colletotrichum acutatum* (Kososki *et al.* 2001), with 100% suppression *in vitro* obtained by Paredes *et al.* (2002) at 5 mg a.i. per litre. The latter authors also obtained 100% suppression of *C. acutatum* colony formation at the same rate of 5 mg a.i. per litre. Against *Colletotrichum capsici* (Gopinath *et al.* 2006) obtained 100% suppression of spore germination, mycelial growth and sporulation with propiconazole at 5, 10 and 5 mg a.i. per litre, respectively.

Aguín *et al.* (2006) also obtained 100% *in vitro* suppression of mycelial growth of *Armillaria mellea* at 1000 mg a.i. per litre. Against the leek rust *Puccinia allii* (Clarkson *et al.* 1997) obtained 100% suppression of spore germination (*in vitro*) and formation of lesions (via foliar spray) at 50 and 750 mg a.i. per litre, respectively. Kenyon *et al.* (1997) observed *in vitro* 100% suppression of sporulation of *Erysiphe* sp. at 10 mg a.i. per litre, while Labuschagne *et al.* (1996) obtained 100% inhibition of radial growth of *Chalara elegans* at 100 mg a.i. per litre. At a rate of 125 mg a.i. per litre propiconazole foliar spray against *Phakopsora euvitis* lead to 72% decrease in incidence of infection and 88% decrease in the extent of lesions (Naruzawa *et al.* 2006). Finally, van den Berg *et al.* (2002) obtained 81% decrease in incidence of infection with propiconazole at 188 mg a.i. per litre.

App 3.4.2.8 Pyrimethanil

No studies were found on the efficacy of this fungicide although Armstrong-Fay (2004) mentions that pyrimethanil (sold in NZ as Scala®) is for example active on *Botrytis* strains that are resistant to benzimidazole.

App 3.4.2.9 Thiophanate-methyl

Very few studies appear to have been published on the efficacy of thiophanate-methyl. (Labuschagne *et al.* 1996) obtained 100% inhibition of radial growth of *Chalara elegans* at 1000 mg a.i. per litre. Thiophanate-methyl at 100 mg a.i. per litre lead to approximately 73% *in vitro* suppression of mycelial growth of *Colletotrichum acutatum* (Kososki *et al.* 2001), with ~37% *in vitro* suppression of conidial germination obtained against *C. gloeosporioides* at 490 mg a.i. per litre by (Haddad *et al.* 2003).

App 3.4.2.10 Thiram

Demirci *et al.* (2003) assessed the *in vitro* efficacy of thiram against *Ascochyta rabiei*, which clearly varied with fungal development, as 8.0 mg a.i. per litre caused 100% suppression of spore germination in comparison to the same efficacy on mycelial growth which was only

obtained at 128 mg a.i. per litre. Against *Chalara elegans* thiram lead to 100% inhibition of radial growth at 1000 mg a.i. per litre (Labuschagne *et al.* 1996). Silveira *et al.* (2003) obtained 96% suppression *in vitro* of mycelial growth of *Rhizoctonia solani* at 10 mg a.i. per litre, with 100% suppression observed at 100 mg a.i. per litre. Against *Fusarium oxysporum* Song *et al.* (2004) found EC_{50} for thiram to be 26 mg a.i. per litre to suppress mycelial growth *in vitro*. In contrast to the above studies, Basallote-Ureba *et al.* (1998) obtained very poor results for thiram against *Stemphylium vesicarium*, with 2 g a.i. per litre leading to just 12% reduction in disease incidence. However, as previously discussed, in this experiment the authors have also obtained unusually poor results for fosetyl-aluminium and other fungicides tested, indicating that their results are unlikely to be reliable.

App 3.4.3 Recommendations for measure: Chemical Treatment for Fungal Infestations

The table App 3.11 below examines the efficacy information for each of the active ingredients listed above.

Table App 3.11: Derived efficacies for chemical treatments for fungal infestations

Active ingredient (Chemical Group)	Comment on Efficacy
Azoxystrobin (Strobilurin) (see App 4.4.2.1)	Based on the contrasting susceptibility to azoxystrobin (of more than 3 orders of magnitude) and the results obtained by Matheron and Porchas (2000) for *Phytophthora*, it is recommended that azoxystrobin is used at the rate of 5000 mg a.i. per litre. Based on the published evidence, this rate would likely be effective against the above organisms and also chrysanthemum white rust *Puccinia horiana* (Cook 2001), grapevine rust *Phakopsora euvitis* (Naruzawa *et al.* 2006), *Cercospora beticola* (Anesiadis *et al.* 2003), powdery mildew *Erysiphe betae* (Anesiadis *et al.* 2003), *Phytophthora cryptogea* (Benigni *et al.* 2004; Benigni *et al.* 2006), corky root of tomato *Pyrenochaeta lycopersici* (Bubici *et al.* 2006), *Verticillium dahliae* (Bubici *et al.* 2006), chickpea blight *Ascochyta rabiei* (Demirci *et al.* 2003) and *Fusarium oxysporum* (Song *et al.* 2004). Based on the EC_{50} values obtained for azoxystrobin to inhibit mycelial growth of *P. capsici* and *P. parasitica*, it is possible that azoxystrobin at 5000 mg a.i. per litre may not induce 100% suppression of all *Phytophthora* species. It is therefore advisable to also use another fungicide from a different chemical group, in particular fosetyl-aluminum, which is the only other fungicide assessed with efficacy data against *Phytophthora* spp.
Carbendazim (Benzimidazole) (see App 3.4.2.2)	Based on the available information it seems therefore advisable to adopt this treatment at four times the label rate, i.e. 2000 mg a.i. per litre. Apart from the above cited species the suggested rate is likely to be effective also against *Colletotrichum gloeosporioides* (Haddad *et al.* 2003), *Colletotrichum capsici* (Gopinath *et al.* 2006), *Cladobotryum mycophilum* (Grogan 2006), *Fusarium oxysporum* (Song *et al.* 2004) and *Nectria galligena* (Xu *et al.* 1996). Resistance to benzimidazole has been observed in fungi, with certain strains being considerably less susceptible to this fungicide (e.g. (Grogan 2006). Therefore, as previously suggested, two fungicides from different chemical groups should be used for quarantine treatment.
Chlorothalonil (Chloronitrile) (see App 3.4.2.3)	Based on the evidence gathered it seems advisable to use chlorothalonil at twice the highest recommended rate described in (Armstrong-Fay 2004). Application of this fungicide at 7000 mg a.i. per litre should be effective against all the above cited organisms, and also against *Colletotrichum acutatum* (Paredes *et al.* 2002), *Colletotrichum gloeosporioides* (Haddad *et al.* 2003), *Rhizoctonia solani* (Silveira *et al.* 2003), *Cercospora beticola* (Anesiadis *et al.* 2003) and *Peronospora sparsa* (O'Neill *et al.* 2002). Nonetheless, again, two fungicides from different chemical groups should be used for quarantine treatment.

Active ingredient (Chemical Group)	Comment on Efficacy
Fosetyl-aluminium (Phosphonates: ethyl phosphonates) (see App 3.4.2.4)	Fosetyl-aluminium at 10,000 mg a.i. per litre (twice the highest recommended rate by (Armstrong-Fay 2004) might be effective as a dipping treatment for *Phytophthora* fungi only. However, caution should be exercise and fosetyl-aluminum should only be used as a supplement with another fungicide from another chemical group, for which the efficacy of the recommended rate is better defined.
Iprodione (Dicarboximide) (see App 3.4.2.5)	Based on the above studies it seems that the rate recommended by (Lester and Lunn 2003) of 2000 mg a.i. per litre, which is four times that of the label rate, would likely be appropriate to be used for quarantine purposes. Some uncertainties do remain, for example regarding the actual impact that 1,000 mg a.i. per litre had on *Chalara elegans* (Labuschagne *et al.* 1996). However, such uncertainties would be likely to be compensated by the use of another fungicide from a different chemical group.
Pencycuron (Phenylurea) (see App 3.4.2.6)	As a result of the apparent specificity of this chemical to control *Rhizoctonia solani* it should not be adopted as a fungicide for general quarantine purposes.
Propiconazole (Triazole) (see App 3.4.2.7)	Based on the rather positive results obtained with propiconazole in the published studies described above, this chemical should be effective as a fungicide for quarantine purposes at 1,000 mg a.i. per litre, which is twice the rate recommended by Lester and Lunn (2003).
Pyrimethanil (Anilinopyrimidine) (see App 3.4.2.8)	In the absence of any published evidence of this chemical's efficacy its adoption for quarantine purposes in New Zealand is not recommended.
Thiophanate-methyl (see App 3.4.2.9)	Since considerably more information is available on carbendazim, another benzimidazole fungicide, it is advisable to use the latter chemical rather than thiophanate-methyl for quarantine purposes.
Thiram (Dimethyldithio-carbamate) (see App 3.4.2.10)	In contrast to the other fungicides discussed, the recommended rates by Lester and Lunn (2003) seem unusually high. There were few studies published on the efficacy of thiram, but since this chemical appear to have high toxicity (Armstrong-Fay 2004), the use of such high dose (11,200 mg a.i. per litre) does not appear to be justified. It seems that the adoption of thiram at 2000 m g a.i. per litre should be sufficient for quarantine purposes, especially if another fungicide from a different chemical group is to be used as well.

It is recommended that no single fungicide is adopted for treatment, and that the more efficacious dip treatment be the standard method of application. In addition, ideally the risk analysis should indicate whether certain resistant strains of higher risk fungi occur in the country of origin of a particular commodity, so that the recommended measures may exclude particular fungicides as possible mitigating measures. A research project is also recommended to address issues relating to the lack of efficacy data underpinning these recommended treatments for nursery stock.

In summary therefore:

- Fosetyl-aluminium, Pencycuron, Pyrimethanil, and Thiophanate-methyl should not be used at this stage general for quarantine purposes, until adequate efficacy information is available;

- The remaining fungicides Azoxystrobin (strobilurin); Carbendazim (benzimidazole); Chlorothalonil (neonicotinoid); Iprodione (dicarboximide); Propiconazole (triazole); and Thiram (dimethyldithio-carbamate) may be adopted as chemical treatments of nursery stock for quarantine purposes, but using the revised rates until adequate efficacy data are available;

- A combination of fungicides from two different chemical groups (strobilurin, benzimidazole, neonicotinoid, dicarboximide, triazole or dimethyldithio-carbamate) should be applied to maximize efficacy and mitigate potential issues with fungal resistance;

- Nursery stock entering a post-entry quarantine facility should not begin their quarantine period for fungi contamination until the chemical residues have sufficiently dissipated or become inactive (considered here to be equivalent to the aerobic soil half life - see table App 3.12). Care should be taken to ensure that adequate account is taken in the quarantine period of nursery stock subjected to a pre-quarantine application of fungicides with extended residual activity.

- A research project is required to address issues relating to the lack of efficacy data underpinning these recommended treatments for nursery stock.

Table App 3.12: Details of Recommended General Fungicide Treatments for Nursery Stock

Active ingredient (Chemical Group)	Treatment Specification[57]	Residue Persistence[58]	Target Fungal Diseases
Azoxystrobin (Strobilurin)	Dip at room temperature for 15 minutes at 5 g a.i. per litre of dip	112 days (hydrolysis)	*Puccinia horiana*, *Phakopsora euvitis, Armillaria mellea*, *Cercospora beticola*, *Erysiphe betae*, *Cercospora beticola*, *Erysiphe betae*, *Phytophthora cryptogea*, *Pyrenochaeta lycopersici*, *Verticillium dahliae*, *Phytophthora cryptogea*, *Ascochyta rabiei*, *Mycosphaerella fijiensis*, *Alternaria alternate*, *Fusarium oxysporum*, *Sclerospora graminicola*, *Mycosphaerella fijiensis*, *Phytophthora citrophthora*
Carbendazim (Benzimidazole)	Dip for 20 minutes at 2 g a.i per litre of dip.	320 days (aerobic)	*Phomopsis theae*, *Sclerotinia minor*, *Colletotrichum gloeosporioides*, *Phakopsora pachyrhizi*, *Ascochyta rabiei*, *Paecilomyces fumosoroseus*, *Colletotrichum capsici*, *Cladobotryum mycophilum*, *Botrytis narcissicola*, *Colletotrichum acutatum*, *Fusarium oxysporum*, *Nectria galligena*
Chlorothalonil (Chloronitrile)	Dip at room temperature for 15 minutes at 7 g a.i per litre of dip.	35 days (aerobic)	*Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Cercospora beticola*, *Ascochyta rabiei*, *Botrytis narcissicola*, *Peronospora sparsa*, *Colletotrichum acutatum*, *Alternata alternata* pathovar *citri*, *Ustilago scitaminea*, *Nectria galligena*
Iprodione (Dicarboximide)	Dip for 30 minutes at 2 g a.i. per litre of dip	64 days (aerobic)	*Colletotrichum acutatum*, *Rhizoctonia solani*, *Paecilomyces fumosoroseus*, *Alternaria brassicicola*, *Alternaria japonica*, *Alternaria alternate*, *Alternaria brassicae*, *Chalara elegans*, *Colletotrichum acutatum*, *Alternaria alternata* pathovar *citri*, *Nectria galligena*
Propiconazole (Triazole)	Dip for 15 minutes at 1 g a.i. per litre of dip.	71 days (aerobic)	*Colletotrichum acutatum*, *Phakopsora euvitis*, *Armillaria mellea*, *Puccinia allii*, *Colletotrichum capsici*, *Erysiphe* sp., *Chalara elegans*, *Colletotrichum acutatum*, *Alternaria cassiae*

57 The dip solution must be used with agitation according to the prescribed conditions and no more than twice or as per manufacturer's recommendations.

58 Based on the aerobic soil half life (where provided, hydrolysis half life where not provided) provided by the PAN Pesticides Database at http://www.pesticideinfo.org

Active ingredient (Chemical Group)	Treatment Specification[57]	Residue Persistence[58]	Target Fungal Diseases
Thiram (Dimethyldithio-carbamate)	Dip at room temperature at 2 g a.i. per litre of dip	24 days (aerobic)	*Rhizoctonia solani, Stemphylium vesicarium, Ascochyta rabiei, Chalara elegans, Fusarium oxysporum*

App 3.5 References

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APPENDIX 4: ANALYSIS OF PEQ REQUIREMENTS

Imported *Wollemia nobilis* nursery stock may have quarantine pests associated with it that are not sufficiently detectable by visual inspections at the point of entry, and hence require a period in quarantine during which inspections and/or tests are conducted to confirm pest status.

The current minimum quarantine period for the majority of imported plant nursery stock is specified to be 3 months. The nursery stock must be actively growing throughout this period. The quarantine period may be extended if material is slow growing, pests and diseases are detected, or tests or treatments are required.

The following post-entry quarantine specifications have been extracted for review from the following MAF standards or procedures:

- PBC-NZ-TRA-PQCON: Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator (November 1999)[59]; and

- PP42: Inspection and Clearance of Plant Products held in Post-Entry Quarantine (June 2003)[60].

App 4.1 Levels of Registration of Quarantine Facilities

The current standards specify that post-entry quarantine facilities can be registered into one of the following three levels:

(i) Level 1 Quarantine Facility:

for plant propagating material which may be infected/infested with risk group 1 pests which cannot be detected by visual inspections at the point of entry and are highly unlikely to be spread by wind, water, insects or other vectors.

(ii) Level 2 Quarantine Facility:

for plant propagating material which may be infected/infested with risk group 1 pests which cannot be detected by visual inspections at the point of entry and can be spread by wind, water, insects or other vectors/means.

(iii) Level 3 Quarantine Facility:

for plant propagating material which may be infected/infested with:

- risk group 1 pests which require specific tests for detection
- risk group 2 pests.

59 http://www.biosecurity.govt.nz/border/transitional-facilities/plants/pbc-nz-tra-pqcon.htm
60 This document is not available outside of MAF

App 4.2 Analysis of Quarantine Facility Levels

The following chapters review the current specifications for post-entry quarantine facilities and make recommendations on improvements to these specifications for use in importing *Wollemia nobilis* nursery stock.

App 4.2.1 Description of measure: General Requirements

The descriptions of the three levels of quarantine facilities provided in the current standards (see section App 4.1) include references to pest "risk groups" (1, 2 and 3). These "risk group" categorisations are no longer used by MAF to describe organisms the pose a biosecurity risk and as such should be removed from any specifications for quarantine facilities. The levels or types of quarantine facilities should relate to the stated roles and performance expectations of each subsequent facility rather than an arbitrary pest designation.

The following sub-sections describe related general and specific conditions for the different levels of quarantine facilities that are provided in the current standards and procedures.

App 4.2.1.1 Security of quarantine/containment facility

The quarantine or containment facility must be secured during the quarantine or containment period so that material cannot be removed without the prior approval of a Chief Technical Officer, an authorised representative or the Supervisor. The quarantine or containment operator must have adequate procedures for controlling access to the quarantine or containment facility.

In the case of Level 1 or Level 2 quarantine facilities, the facility shall only be used for the purpose of screening imported plant propagative material under quarantine. Should another consignment of imported plant propagating material be added to the quarantine facility, the quarantine period for the first consignment shall be extended to coincide with the release of the second import, unless it can be shown that there can be no cross infection/infestation by all pests common to both consignments.

In the case of Level 3 quarantine facilities mixing of consignments may occur, however regard shall be taken for the consequences of cross contamination, and plant movement within facilities should not occur. These facilities may also be used for the purpose of holding indicator plants for tests being completed on plants within the facility. Indicator plants used as positive controls for quarantine pests shall be held in a Level 4 Containment facility only.

App 4.2.1.2 Reporting of pests

The quarantine operator shall report the detection of any pests or disease symptoms found in the quarantine facility to a Chief Technical Officer or the Supervisor within 48 hours of observing the pest(s).

App 4.2.1.3 Treatments applied to plants in quarantine

All forms or types of treatment applied to plants in quarantine shall be approved by a Supervisor or Chief Technical Officer prior to application or use. Records shall be kept of all treatments applied to plants during quarantine. Treatments specifically targeting regulated pests shall only be applied to plants in quarantine after written approval has been granted by a Chief Technical Officer or the Supervisor.

Waste material shall be collected in a robust container (plastic bag) that can be sealed. Waste containers are to be kept in the quarantine or containment facility until examined by a Supervisor and cleared for disposal by an approved method.

App 4.2.2 Analysis of measure efficacy: General Requirements

These general requirements are sound in principle but require some detail to ensure they are applied in a manner that would ensure an adequate level of risk mitigation for imported *Wollemia nobilis* nursery stock. For instance the statement that "*The quarantine operator must have adequate procedures for controlling access to the quarantine facility*" does not provide any guidance on the level of control required or the outcome the "control" would be aiming to achieve. As an example it may be expected that the "control" would ensure only personnel who have completed adequate quarantine training can enter the facility unsupervised. The outcome would be that all personnel accessing the facility would have a clear understanding of the quarantine requirements of that facility.

More guidance should also be provided on the approval of treatments to be used in the facility to ensure adequate account is taken of the likely treatment efficacy for each circumstance and any effects of treatment residues on the extension of the subsequent quarantine period.

App 4.2.3 Recommendations for measure: General Requirements

It is recommended that, where possible, quarantine requirements for imported *Wollemia nobilis* nursery stock are provided with adequate guidance to ensure those implementing the specifications clearly understand the performance expectations (the required outcomes).

App 4.2.4 Description of measure: Level 1 Quarantine Facility (Open Ground Sites)

Some plant species that only have risk group 1 pests which are not likely to be transmitted by wind; water; insects or other vectors may be grown in an open ground quarantine facility. Open ground facilities are subject to the isolation requirements below, and those prescribed in the relevant import health standard or Permit to Import. All open ground quarantine sites shall:

- have ready access for quarantine inspections,
- provide good growing conditions for the imported plant material,
- not be subject to flooding.

The following minimum isolation requirements must be met unless otherwise stated in the relevant import health standard or Permit to Import.

For herbaceous plants in quarantine:

- 50 metre distance from plants of the same genus;
- 20 metre distance from all other herbaceous plants (excluding lawn);
- 5 metre distance from woody plants.

For woody plants in quarantine:

- 50 metre distance from plants of the same genus;
- 20 metre distance from all other woody plants;
- 5 metre distance from herbaceous plants (excluding lawn).

Barriers such as a waterway, sealed road, or solid fence may allow for reduced isolation distances.

"Buffer zones" may be established around quarantine crops under the following conditions:

- the plants within the "buffer zones" (buffer plants) are included in the quarantine facility and are therefore subject to quarantine requirements regarding treatment or destruction;

- the isolation requirements from other plants outside the quarantine area shall apply to the quarantine plants only, as specified above (i.e. the area within the 50 metre isolation from plants of the same genus may be occupied by buffer plants);

- intensive (sampling) inspections of the plants within the quarantine facility by the Supervisor shall be restricted to the quarantine plants only;

- the quarantine area (including buffer zone) shall be clearly delineated on all sides.

Operators' of Level 1 quarantine facilities shall make arrangements for the supply of diagnostic services for the following pest categories:

- Plants (weeds), fungal, bacterial, viral/phytoplasma.

App 4.2.5 Analysis of measure efficacy: Level 1 Quarantine Facility

The primary purpose of a plant quarantine facility is to hold plant material for sufficient time to allow adequate inspections and/or testing to detect potentially infesting organisms, while ensuring those same contaminating organisms do not establish in New Zealand from the plant material before the inspections and/or testing is completed. Given by definition the nature of invasive organisms is to be "invasive", and the level of uncertainty around the nature and types of organisms associated with the great majority of nursery stock entering New Zealand, Level 1 quarantine facilities would seem to offer such a low level of containment that they would be of little use for managing the risks associated with imported *Wollemia nobilis* nursery stock.

App 4.2.6 Recommendations for measure: Level 1 Quarantine Facility

No recommendations are provided for the specifications for Level 1 quarantine facilities, as the author considers such facilities are of little use for the effective management of biosecurity risks associated with imported *Wollemia nobilis* nursery stock.

App 4.2.7 Description of measure: Level 2 Quarantine Facilities

Plant species with risk group 1 pests which are likely to be transmitted by wind, water, insects or other vectors shall be grown under conditions which minimises the risk of these quarantine pests becoming established in New Zealand. There are three types of Level 2 Quarantine Facilities, that is, Quarantine Aquarium for aquatic plants, Greenhouse/Screenhouse for terrestrial plants, and tissue culture facilities for *in-vitro* plants or plant material.

Operators of Level 2 Quarantine Facilities shall make arrangements for the supply of diagnostic services for all pest categories (see MAF standard 155.04.03).

The critical service and structural requirements/specifications are as follows:

- the quarantine facility shall be constructed and operated in a manner that shall contain, in isolation, the plant material and any associated pests;

- the structure shall be completely enclosed in glass, polythene, or other continuous material except for the entry/exit and ventilation requirements. The requirement for plastic film cladding is a minimum of 200 microns thick (heavy duty) polyfilm;

- all windows, louvres or vents shall be effectively screened with insect-proof mesh with a maximum aperture of 0.6mm or a 30 X 30 (per sq. inch) mesh;

- the vents and doors shall be tight fitting and constructed of material which shall maintain rigidity at all times;

- the structure shall have a concrete floor, be strong enough to withstand the normal range of weather conditions and not be subject to flooding;

- the structure shall have an insect-proof anteroom or porch with a double door for entrance/exit. There must be sufficient space to permit the entry of people and planting material with one door being closed at all times;

- a gully or soil trap connected to sewage, septic tank or a suitable rubble drain shall be used. The quarantine operator shall ensure that all material released into the sewage or waste water system is in compliance with local or regional by-laws/regulations;

- a foot bath utilising an effective disinfectant shall be used;

- appropriate plant hygiene measures (e.g. disinfection of cutting tools) shall be maintained at all times;

- the facility shall be maintained free of weeds, lichen and moss;

- yellow sticky insect traps shall be appropriately installed in each quarantine house at a minimum rate of one per 15 square metres of planted area and replaced for every new consignment after inspection by the Supervisor;

- all plants must be grown in sterilised or inert media and be easily accessible for inspection by the Supervisor;

- during the quarantine period the quarantine facility shall only be used for the registered purpose.

App 4.2.8 Analysis of measure efficacy: Level 2 Quarantine Facilities

As stated earlier, the primary purpose of a plant quarantine facility would be to hold imported *Wollemia nobilis* nursery stock for sufficient time to allow adequate inspections and/or testing to detect potentially infesting organisms, while ensuring those same contaminating organisms do not establish in New Zealand from the plant material before the inspections and/or testing is completed.

The first and most obvious limitation of Level 2 quarantine facilities is that "*all windows, louvres or vents shall be effectively screened with insect-proof mesh with a maximum aperture of 0.6mm or a 30 X 30 (per sq. inch) mesh*". As indicated by the description of the mesh as being "*insect-proof*", a "*0.6mm or a 30 X 30 (per sq. inch) mesh"* would not prevent airborne micro-organisms (e.g. fungal spores) from leaving the facility and as such could not be used to quarantine plant material that could potentially be contaminated by micro-organism with airborne life stages (e.g. fungi that produce air-borne spores).

There are also a number of specifications that fail to provide adequate guidance on what the specifications are attempting to achieve. For example, "*a foot bath utilising an effective disinfectant shall be used*" does not indicate the types or organisms the disinfectant will need to be effective against. The use of "*appropriate plant hygiene measures*" that do not indicate the elements of hygiene that are necessary would seem to be of little use.

There is no mention in the current specifications for level 2 quarantine facilities of the environmental conditions required for growing the imported *Wollemia nobilis* nursery stock. While it is likely that requirements for environmental stressing and definitions of optimal growth or other growing conditions will be stated on a plant-by-plant basis in the respective import health standards, some guidance needs to be given as to the requirement that these facilities are able to provide some appropriate control over the environmental conditions within.

App 4.2.9 Recommendations for measure: Level 2 Quarantine Facilities

It is recommended that for the post-entry quarantine of imported *Wollemia nobilis* nursery stock:

- the description of the Level 2 quarantine facility include guidance on the types and nature of organisms such a facility is considered able or unable to contain during the quarantine period;

- as standard requirements for all Level 2 quarantine facilities, venting systems should be specified to adequately prohibit (where possible and practical) the movement out of the facility of micro-organisms as well as insect pests;

- specifications are developed to provide guidance on the nature and extent of environmental control required to deliver appropriate growing conditions;

- any specifications for the facility provide adequate guidance on both the delivery of the specification and the performance requirements for that specification.

App 4.2.10 Description of measure: Level 3 Quarantine Facilities

Plant material which may be infected/infested with risk group 1 quarantine pests, the identity of which can only be determined by conducting specific tests, and risk group 2 quarantine pests shall be inspected and tested in a Level 3 Quarantine Facility.

Operators of Level 3 quarantine facilities shall make arrangements for the supply of diagnostic services for all pest categories.

The critical service and structural requirements/specifications for a Level 3 Quarantine Greenhouse are as follows:

- the quarantine facility shall be constructed and operated in a manner that shall contain, in isolation, the plant material and any associated pests;

- the structure shall be completely enclosed in glass, polythene, or other impact-resistant material except for entry/exit and ventilation requirements;

- all windows, louvres or vents shall be effectively screened with insect-proof mesh with a maximum aperture of 0.6mm or 30 X 30 (per sq. inch) mesh;

- the vents and doors shall be tight fitting and constructed of material which shall maintain rigidity at all times;

- the structure shall have concrete floor, be strong enough to withstand the normal range of weather conditions and not be subject to flooding;

- the structure shall have an insect-proof anteroom or porch with a double door for entrance/exit. There must be sufficient space to permit the entry of people and planting material with one door being closed at all times;

- a gully or soil trap connected to sewage, a septic tank or a suitable rubble drain shall be used. The quarantine operator shall ensure that all material released into the sewage or waste water system is in compliance with local or regional by-laws/regulations;

- a foot bath utilising an effective disinfectant shall be used;

- appropriate phytosanitary measures (e.g. disinfection of cutting tools) should be maintained at all times;

- the facility shall be maintained free of weeds, lichen and moss;

- all plants shall be grown in sterilised or inert media;

- all plants shall be grown on raised benches and be easily accessible for inspection by the Supervisor;

- yellow sticky insect traps shall be appropriately installed in each quarantine house at a minimum rate of one per 15 square metres of planted area and replaced for every new consignment after inspection by the Supervisor;

- during the quarantine period the quarantine facility shall only be used for the registered purpose;

- the operator shall provide evidence that all testing for quarantine pests can be conducted in accordance with the requirements stipulated in the import health standard. Tests may be undertaken, on the quarantine operator's behalf, by a testing laboratory approved by a Chief Technical Officer;

- the operator shall provide a monthly report summary to the Supervisor listing the plants in the facility (number and type) and their current status (e.g. under treatment, awaiting disposal). The report shall also detail what plants have been given biosecurity clearance, destroyed, transferred, or otherwise removed from the facility since the last report.

- the operator shall establish and maintain procedures and instructions for the following NZS 5602 or ISO 9002 quality system requirements:

    - management responsibility (the qualifications, experience and responsibilities of all persons involved in pest diagnosis shall be included in this)
    - quality system
    - document control system
    - process control
    - inspection and testing
    - control of non-conforming product
    - corrective action
    - handling, storage, packaging and delivery
    - quality records
    - internal audits
    - training

App 4.2.11 Analysis of measure efficacy: Level 3 Quarantine Facilities

As stated earlier, the primary purpose of a plant quarantine facility would be to hold imported *Wollemia nobilis* nursery stock for sufficient time to allow adequate inspections and/or testing to detect potentially infesting organisms, while ensuring those same contaminating organisms do not establish in New Zealand from the plant material before the inspections and/or testing is completed.

The limitations of the specifications for the Level 3 quarantine facilities are therefore much the same as those stated for Level 2 quarantine facilities in sections App 4.2.8 and App 4.2.9.

App 4.3 Inspections within Post Entry Quarantine

The two main activities undertaken within a quarantine facility, aside from maintaining the facility and the plants therein, are the inspections and testing required to ensure the imported *Wollemia nobilis* nursery stock meets New Zealand's biosecurity standards. Specifications for testing requirements should be provided on a case-bay-case basis as part of the development of the import health standard. Inspection requirements are more generic and as such are included in the quarantine specifications. The following sub-sections review the current specifications related to the inspection activities within quarantine facilities.

App 4.3.1 Description of measure: Inspection Frequency

The current quarantine facility specifications provided a number of different inspection frequency requirements.

App 4.3.1.1 Plants required to be held in a PEQ facility for one growing season.

Unless otherwise stated on the IHS/Permit to Import, plants requiring one growing season in quarantine must be inspected three times. The first post-entry quarantine inspection should be carried out within four weeks of the consignment entering the quarantine facility.

If foliage is present on 95% or more of plants at the first inspection then only one further inspection (final inspection) is required after a period of 2½ to 3 months of quarantine. If no foliage is present on any plants at the first inspection, or if foliage is present on less than 95% of plants at the first inspection, then two further inspections are required; one of these occurring when 60% or more of plants have foliage and the final inspection when 95% or more of the plants have foliage.

(a) The first inspection for Level 1 materials (e.g. Paeonies) can be delayed until the tubers/bulbs have sprouted and the first leaves are fully expanded.

(b) A final inspection of plants from accredited offshore facilities and Level 3 New Zealand facilities needs to be conducted by the NPPRL.

(c) At the final inspection, if 5% or more plants of each line are not actively growing with foliage, a sample should be sent to the NPPRL to test for the presence of fungi, bacteria, insects, or nematodes.

If no regulated organisms are detected, the whole consignment may be released. If a regulated organism is detected, the whole consignment should be treated, destroyed or reshipped.

App 4.3.1.2 Plants required to be held in a PEQ facility for two growing seasons

Complete two inspections during the first growing season as per above and two further inspections during the second growing season. Plants must remain in the post-entry quarantine facility for at least six months.

App 4.3.1.3 Plant inspection procedures

Stage 1 Inspection

On each visit, visually inspect plants pertaining to the particular consignment without the use of aids for obvious evidence of pest or disease symptoms. Special attention will be taken for pests listed in the MAF Biosecurity Authority Reference Index 155.02.06 pertaining to the crop being inspected and any regulated organisms identified and treated from the border inspection.

(i) If pests/diseases are found during the first inspection, inspect affected and surrounding material thoroughly, take appropriate action, (refer below) and also carry out sampling as specified in Stage 2 Inspection.

(ii) If no pests/diseases are detected during the first inspection proceed to the Stage 2 Inspection.

Stage 2 inspection

The inspector shall select samples for a more intensive inspection in accordance with the sampling regime:

Consignment (homogenous) size	Sample size
0-50	All
51-100	30
101 upwards	60

The inspector shall thoroughly inspect the selected samples with the naked eyes, using a 10x hand lens if required, for disease symptoms and insect pests.

App 4.3.2 Analysis of measure efficacy: Inspection Frequency

The requirement that plants within quarantine are inspected three (for 1 season) or 4 times (for 2 seasons), unless adequate foliage is present on 95% or more of plants at the first inspection, would seem to be appropriate for imported *Wollemia nobilis* nursery stock. It would seem important; however, that all imported *Wollemia nobilis* nursery stock develop foliage for inspection prior to completing quarantine, as in many circumstances a single infected plant could act as a successful vector for establishing a hazard organism. This risk is not adequately mitigated by the requirements that "*At the final inspection, if 5% or more plants of each line are not actively growing with foliage, a sample should be sent to the*

NPPRL to test for the presence of fungi, bacteria, insects, or nematodes" as these samples may not themselves contain the organism causing the infestation.

More significantly there seems to be no expectation that the roots or underground portions of the quarantined plants are inspected for disease symptoms. Plants pests or diseases that affect underground portions of imported *Wollemia nobilis* nursery stock are as significant as above ground pests and diseases, and regard should be taken of them.

The "stage 1" non-intensive inspection would be of little use in detecting organisms in low population densities or as yet not causing the plants to express disease symptoms. The "stage 2" more intensive inspection is only targeted at a small sample of the plants in quarantine and as such only offers a limited opportunity to detect low-density infestations.

An analysis of the sample size (60), assuming the efficacy of detection of the infesting organism is 100%, would provide 95% confidence that a 5% (1 in 20) level of infestation would be detected for a large consignment. This acceptable level of infestation would seem rather high for many of the infesting organisms of potential concern on imported *Wollemia nobilis* nursery stock.

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ): *Postentry Quarantine Manual for State Inspectors* (2006) provides some valuable guidance on the requirements for plant inspections to detect pests and diseases. The PPQ manual states the following on page 3-7 under "procedures" (note relevance to northern hemisphere seasons in text):

> "Plant disease symptoms caused by viruses, bacteria, and fungi do not necessarily appear in the same season. Hence, inspect post-entry quarantine material two or more times (if possible) during each growing season. For example, the majority of leaf spots and leaf diseases are not well developed before summer. Virus diseases, in general, are more conspicuous in the spring. (Some virus symptoms are masked or tend to disappear in hot weather.) Cankers of woody plants are usually visible throughout the year. Thus, it is obvious that while late spring and early summer are most satisfactory for virus inspection, mid-summer to fall will give the best results for most fungus diseases.
>
> The principle of seasonal occurrence holds for insects also. Leaf-feeding insects in larval and adult stages may appear in May and June and be entirely absent in mid-summer and later. Insects with a long season of hibernation (many sawflies, scarabs, weevils) spend a relatively short season on the above ground portions of plants and may be missed unless two or more inspections are made."

Within the PPQ manual under Appendix I: *Inspection Aid for Plants Growing in Postentry Quarantine*, the following table is provided as general guidance:

Use Table I-1 (below) to determine when to look for symptoms of infections caused by bacteria, viruses, fungi, and cankers.

TABLE I-1: Causal agent and onset of symptoms

If the likely causal agent is:	**Then look for symptoms at this time:**
Bacteria	Spring and early summer
Viruses	Cool weather (when leaves are first expanding)
Cankers	Year long
Fungi including leaf spots	Mid-summer to fall (autumn)

This guidance suggests that as a standard requirement two growing seasons of quarantine and inspection are required to detect infesting pests or diseases with sufficient confidence, and that special care needs to be taken to the time of the inspections during the growing season. The two growing season requirement is likely to reflect the fact that in the natural environment a season may not necessarily provide appropriate conditions for disease expression. Where quarantine is undertaken in a more controlled environment that ensures the growing conditions each season are appropriate for disease expression, only one growing season of quarantine would be required.

This guidance also suggests that when growers use glasshouse conditions to replicate growing seasons (and thus reduce the quarantine period), care should be taken to ensure that any such replication includes appropriate combinations and sequencing of environmental factors such as day length, soil and air moisture content, and temperature. For example any one season would start with longer wetter days and cold temperatures, move to longer dryer days and warm temperatures, then move back to shorter wetter days and warm but cooling temperatures.

The definition of "growing season" would therefore be:

An extended period of plant growth that includes environmental conditions equivalent to spring (longer wetter days and cold temperatures), summer (longer dryer days and warm temperatures), and autumn (shorter wetter days and warm but cooling temperatures).

App 4.3.3 Recommendations for measure: Inspection Frequency

It is recommended that for imported *Wollemia nobilis* nursery stock:

- plants in quarantine that have not developed foliage when the majority of other similar plants have should be removed from the quarantine facility and reshipped or destroyed as required;

- more intensive inspection that includes both above and below ground portions of the plants be conducted on all plants in quarantine unless a risk analysis has determined that less intensive inspections can be undertaken;

- the minimum quarantine period be established as two growing seasons unless the growing environment is controlled sufficiently to ensure conditions are appropriate for disease expression in the first season;

- guidance be provided in the post-entry quarantine standards on when inspections should take place for the various organisms of concern;

- care should be taken to ensure that when growers use glasshouse conditions to replicate growing seasons (and thus reduce the quarantine period), any such replication includes appropriate combinations and sequencing of environmental factors such as day length and temperature.

App 4.3.4 Description of measure: Hygiene

The following sections review the facility and operator hygiene requirements for quarantine facilities.

App 4.3.4.1 Handling Plants within the PEQ facility

After each visit the inspector is to wash hands thoroughly with soap and water.

App 4.3.4.2 Handling during plant inspections

Plants should not be touched with bare hands during inspections or brushed against with clothing. Disposable gloves and lab coats are to be worn when handling plants, a new pair of disposable gloves for plants from different permits or for different species.

App 4.3.4.3 Handling during sample collection

Disposable gloves are to be worn when taking samples to avoid touching plants with bare hands. Each sample is to be collected using a gloved hand and while still holding the sample, the glove is turned inside-out leaving the sample inside. A new disposable glove is to be worn for each sample.

Wash hands thoroughly with soap and water and decontaminate any equipment (e.g. secateurs) with 1% Sodium hypochlorite solution (one part Janola to three parts water).

App 4.3.5 Analysis of measure efficacy: Hygiene

The hygiene measures seem appropriate for non-motile organisms that may be associated with imported *Wollemia nobilis* nursery stock. For motile organisms or air-borne inoculate hygiene measures should ensure that the person, their clothing, or any equipment they may be using does not vector the organism or inoculate outside the quarantine facility.

App 4.3.6 Recommendations for measure: Hygiene

It is recommended that post-entry quarantine hygiene measures for imported *Wollemia nobilis* nursery stock include steps to manage the risks from motile organisms or air-borne inoculate.

App 4.3.7 Description of measure: Treatment of risk organisms

An appropriate knock down spray should be applied immediately if mobile insects and/or pests are observed during the plant inspections.

When the facility Inspector receives from the registered laboratory the results of the identification and a regulated pest/disease has been found, the inspector shall liaise with the importer on whether the consignment shall be destroyed, reshipped or treated. If the importer wishes the consignment to be treated, the importer or registered lab (at the importers cost), or an expert in the crop or pest concerned may formulate a technically sound treatment program to be approved by the inspector.

After two applications, a period of "no spray" will occur for six weeks if it is a fungi/bacteria related problem and four weeks if an entomological problem prior to any re-inspection by the inspector.

App 4.3.8 Analysis of measure efficacy: Treatment of risk organisms

As it is possible, or in many cases likely that an infestation of an airborne or mobile organism would not be removed by the application of a "knock down" spray, it would seem likely that organisms from that infestation would become airborne or motile again within the facility prior to the diagnostic result becoming available and a suitable treatment being identified. If the existence of an airborne or mobile organism is considered of significant risk to warrant the immediate application of a knock down spray, it would seem appropriate that the suppression of the organism continue until the diagnostic result has become available and a suitable treatment has been identified if needed.

The requirement that a "*technically sound treatment program*" for any identified pests or diseases be formulated is a laudable one; however the treatment program needs to be reviewed by a suitably qualified independent treatment expert familiar with the goals and expectations of the biosecurity system, prior to the use of the treatment programme being approved. It should also be clearly stated that the application of any such treatment programme requires a following period where no treatments are performed. This "resting" period would need to be equivalent to the length of time required for any significant residual effects of the treatment to have dissipated. Following the resting period the plant nursery stock would have to undergo another period of quarantine equivalent to the normal period of quarantine required for that organism.

App 4.3.9 Recommendations for measure: Treatment of risk organisms

It is recommended that for imported *Wollemia nobilis* nursery stock in post-entry quarantine:

- a temporary spray programme be developed for the control of any airborne or mobile organisms detected in the quarantine facility, prior to reshipping or destroying the contaminated material, or the development of a technically sound treatment program for the eradication of the identified organism;

- any treatment programmes developed to manage an identified contamination should be reviewed by a suitably qualified independent treatment expert familiar with the goals and expectations of the biosecurity system;

- following the completion of the treatment programme a resting period be completed where no treatments are performed. The duration of the resting period should be equivalent to the length of time required for any significant residual effects of the treatment to have dissipated;

- following the resting period the plant nursery stock should undergo another period of quarantine equivalent to the normal period of quarantine required for that contaminating organism.