Review of Phytophthora Root Rot of Chestnuts

Dr Fiona Giblin
University of the Sunshine Coast

Project Number: CH14002

CH14002

This report is published by Horticulture Innovation Australia Ltd to pass on information concerning horticultural research and development undertaken for the chestnut industry.

The research contained in this report was funded by Horticulture Innovation Australia Ltd with the financial support of the chestnut industry.

ISBN 0 7341 3465 7

Published and distributed by:
Horticulture Innovation Australia Ltd
Level 8
1 Chifley Square
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

Horticulture Innovation Australia

Contents

Summary

The Australian chestnut industry is expected to increase production over the next few years. However, an important issue which has not been sufficiently addressed is the susceptibility of the chestnut tree (*Castanea sativa*) to infection by the root rot pathogen *Phytophthora*.

The objectives of this project were to review the relevant literature related to *Phytophthora* root rot of chestnuts in Australia and overseas and to provide recommendations for the chestnut industry and for future RD&E investment.

Project outputs were a literature review on *Phytophthora* root rot of chestnuts and a Project Final Report and the outcomes will assist to guide future funding priorities on *Phytophthora*-related R,D&E by the Chestnut Industry Advisory Committee (IAC).

This review presents important existing knowledge of the biology and epidemiology of *Phytophthora cinnamomi*, gives practical advice on how to integrate components of disease control and suggests promising avenues for research. An understanding of the basic biology of *Phytophthora* is essential when developing an integrated management program. The ability of the 'water mould' to increase inoculum density rapidly, is the basis for its success as a plant pathogen, and helps explain why effective control is rarely achieved by a single disease control measure. There is a need for a number of different approaches, several of which are sensible agronomic/horticultural practices, to be used in an integrated manner. Drainage control is fundamental to success. Shallow, poorly drained soils, containing a large number of host roots that are water-saturated for some time, provide ideal conditions for zoospore formation and dispersal, infection and predisposition to infection. Disease-free nursery trees are a key element in any horticultural enterprise. Besides preventing the spread of the pathogen to new areas, it is well understood that when nursery trees are destined for areas where pathogens are already present, disease control is increased greatly if plants are free of pathogens at time of establishment. High soil organic matter is a factor commonly implicated in *P. cinnamomi* suppression. It is a key factor in horticultural soils known to be suppressive to *Phytophthora* root rot. There are many examples where organic matter has been added to soils to minimise the impact of root rot and induce suppressive conditions in the soil. A major role of organic matter is related to microbial antagonism of the pathogen. The use of chemicals requires knowledge of optimum timing of sprays or injections, rates of application, and methods of application to be applied effectively. With phosphonates, timing of applications in relation to growth events (relative sink strength), maximises root phosphonate concentrations thus maximising protection. The planting of resistant rootstocks, is perhaps the most important type of control measure, but with many horticultural crops resistance is not always attainable. Resistant trees will still

benefit from well-drained and aerated soils and mulching to produce good soil health.

Although the review did in part concentrate on increasing the understanding of the biology of *P. cinnamomi*, its major role is to determine how this knowledge can be translated into practical control measures for the chestnut industry. The integrated control program mentioned in the review is largely derived from research undertaken for the Australian avocado and pineapple industries. Research findings were successfully adopted by these industries – adoption by industry is the ultimate test of research. Much of the research is also applicable to the chestnut industry and there is no need to try to reconstruct the wheel, but to be aware of crop and regional differences.

Recommendations for future R&D include:

- Assessment of *Phytophthora*-conducive soils in growing areas, diagnosis of species of *Phytophthora* involved, and impacts on orchards.
-Development of robust tests for disease resistance evaluation of local chestnut selections, particularly varieties or individuals showing tolerance to disease pressure.
- Evaluation of phosphonate usage, both injection and sprays, and the development of a relevant phenology model for chestnuts growing in Australia.
- Encouragement of collaboration with international *Phytophthora* resistant rootstock R&D programs for future screening of promising resistant varieties.

Recommendations for practical application to industry include:

- Review of nursery practices and the initiation of an industry self-regulated accreditation scheme
- Review of phosphonate usage by growers – methods, timing and rates of injections and sprays.

Key Words

Chestnuts; *Castanea sativa*; *Phytophthora* root rot; disease management; phosphonate; nursery practice, rootstock; resistance; soil health; *Phytophthora cinnamomi*

Introduction

Limited research has been conducted on Phytophthora root rot of chestnuts in Australia. Phytophthora is an oomycete that can result in serious diseases of a range of plant species. *Phytophthora cinnamomi* is a species of Phytophthora that is particularly insidious and is often associated with root rot diseases in different hosts, including chestnut, avocado and macadamia.

Control options for Phytophthora root rots are often limited to cultural practices that result in conditions that are less favourable for Phytophthora growth or dispersal, good farm hygiene to limit the spread of the pathogen, the use of planting material that may exhibit a level of tolerance to the pathogen, or limited chemical options for management of the disease, including the use of phosphorus acid as trunk injections, or in some cases, as foliar applications.

The chestnut industry in Australia has an estimated production value of $9 million from approximately 1000 hectares of crop based on 2011 figures, with expected industry growth to increase production by 250 hectares by 2016 (Australian Chestnut Industry Strategic Investment Plan 2011-2016). Access to appropriate pest and disease management technology is seen as a constraint on future growth of the industry and Phytophthora root rot is a key disease that can impact on this projected industry growth. The 2013/14 estimated R&D Chestnut Levy income is $55,000 (Chestnut Annual Investment Plan July 2013 – June 2014) covering all R&D related activities for the industry.

During 2012 in North East Victoria where approximately 70% of the chestnut crop is grown, it was estimated that up to 6700 trees died from Phytophthora infection (Australian Chestnut Growers Handbook, 2013). Considerable tree impacts due to Phytophthora have been experienced in other chestnut production areas also. Whilst environmental parameters which can be beyond the control of growers plays a key role in the incidence and severity of Phytophthora root rot, a better understanding of the disease and how it impacts on chestnut production in Australia, in addition to access to management options, would assist to ensure this disease does not further constrain growth of the chestnut industry into the future.

Methodology

The project reviewed relevant literature related to Phytophthora root rot of chestnuts in Australia including disease epidemiology, control/management options (both current and potential), information from overseas sources that may inform the situation in Australia and recommendations for future R,D&E investment in this area by the Australian Chestnut Industry. Such recommendations take into account consideration for the level of available chestnut R&D levy funding, or potential alternative funding options in these areas of interest.

Outputs

There were two outputs to this project:

1. A literature review on Phytophthora root rot of chestnuts in Australia; and
2. A Project Final Report

Outcomes

This project will assist to guide future funding priorities on Phytophthora-related R,D&E by the Chestnut Industry Advisory Committee (IAC).

Evaluation/Discussion

This has been captured in the attached literature review.

Recommendations

The following lines of investigation should be of value to chestnut industry and are worthy of industry support:

- **Nursery practices**

All *Phytophthora* species are primarily dispersed in contaminated soil and water and less frequently in infected planting material. For this reason nursery practices designed to prevent the spread of the pathogen with planting material should focus on preventing infested soil and water from entering the nursery.

It is suggested that the chestnut industry initiate some form of industry self-regulated nursery accreditation scheme. The aim of this scheme would be to foster sound nursery practices for the production of pathogen tested, true-to-type chestnut trees. This may also include the accreditation of seed trees. Keith Bodman (2014) has recently reviewed the avocado nursery voluntary accreditation scheme (ANVAS) (HAL Project AV13020) and has proposed to partner this scheme with the Nursery Industry Accreditation Scheme, Australia (NIASA) system.

He suggests having 'a national self-regulated process for the development, continuing improvement, and adherence to guidelines for the production of pathogen tested, true-to-type avocado plants suitable to transplant'. Such a scheme may require an enlistment process for chestnut nurseries some of which may already be NIASA accredited. Accredited nurseries should not be allowed to use chemicals to suppress pathogens. This will not eradicate a pathogen or eliminate disease and could lead to serious problems further down the supply chain.

- **Evaluation of phosphonate applications**

Research for the avocado industry found that the most acceptable formulation for use by the industry was 0.5% mono-dipotassium phosphonate buffered to pH 7.2 and applied without surfactants. Sprays produced similar root phosphonate levels as the trunk injection treatment and gave similar protection against the disease.

As sink strength at the time of application is the dominant factor influencing the translocation of phosphonate in the tree, phenological activity of the chestnut

tree needs to be identified. There is a need to know the timing of phenological development phases of roots, shoots, flowers and nuts to changing source and sink strengths. As phosphonate is required in the trunk and roots for *Phytophthora* control, root growth can be based on relatively superficial measurements by placing newspaper under a mulch layer. Root growth can then be determined by lifting the paper at regular intervals. Foliar sprays should be applied to coincide with root flushes. A phenology model will be more accurate if a trunk starch concentration curve is superimposed on the phenology model. The distribution and persistence of phosphonate applications can be monitored using root sampling and analysis to determine the most appropriate times for spraying or injecting.

- **Resistant rootstocks**

Rootstocks may change the situation dramatically in the future. As plant improvement programs require a long term investment of funds and research effort, the industry should encourage collaboration with international programs that have identified sources of resistance to *Phytophthora*. Material can then be introduced and screened to identify useful rootstocks. Meanwhile, there is a need to develop robust tests for disease resistance evaluation of local selections.

Focus on the implementation and adoption of technologies based on sound principles of integrated disease management.

Be guided by what has been adopted by other industries.

Scientific Refereed Publications

None to report

IP/Commercialisation management

Not applicable

Appendices

Literature Review – Review of Phytophthora Root Rot of Chestnuts

Review of *Phytophthora* Root Rot of Chestnuts

Project Number: CH14002

Dr Fiona Giblin

University of the Sunshine Coast

Contents

Background

Limited research has been conducted on *Phytophthora* root rot of chestnuts in Australia. *Phytophthora* is an Oomycete that can result in serious diseases of a range of plant species. *Phytophthora cinnamomi* is a species of *Phytophthora* that is particularly insidious and is often associated with root rot diseases in different hosts, including chestnut, avocado and macadamia.

Control options for *Phytophthora* root rots are often limited to cultural practices that result in conditions that are less favourable for *Phytophthora* growth or dispersal, good farm hygiene to limit the spread of the pathogen, the use of planting material that may exhibit a level of tolerance to the pathogen, or limited chemical options for management of the disease, including the use of phosphorus acid as trunk injections, or in some cases, as foliar applications.

The chestnut industry in Australia has an estimated production value of $9 million from approximately 1000 hectares of crop based on 2011 figures, with expected industry growth to increase production by 250 hectares by 2016 (Australian Chestnut Industry Strategic Investment Plan 2011-2016). Access to appropriate pest and disease management technology is seen as a constraint on future growth of the industry and *Phytophthora* root rot is a key disease that can impact on this projected industry growth. The 2013/14 estimated R&D Chestnut Levy income is $55,000 (Chestnut Annual Investment Plan July 2013 – June 2014) covering all R&D related activities for the industry.

During 2012 in North East Victoria where approximately 70% of the chestnut crop is grown, it was estimated that up to 6700 trees died from *Phytophthora* infection (Australian Chestnut Growers Handbook, 2013). Considerable tree impacts due to *Phytophthora* have been experienced in other chestnut production areas also. Whilst environmental parameters which can be beyond the control of growers plays a key role in the incidence and severity of *Phytophthora* root rot, a better understanding of the disease and how it impacts on chestnut production in Australia, in addition to access to management options, would assist to ensure this disease does not further constrain growth of the chestnut industry into the future.

The objectives of this project are:

A. To review relevant literature related to *Phytophthora* root rot of chestnuts in Australia including:

- Disease epidemiology in Australian growing areas
- Information from overseas sources that may inform the situation in Australia
- Control/management options (current and potential)

B. To provide recommendations for future RD&E investment with budget, taking into account the level of available levy funding or potential for alternative funding options.

There are three essential components to achieve a successful outcome in this project:

i. Review of the literature both in Australia and overseas, including phenology of chestnut species, assessment of *Phytophthora* species and origin, epidemiology, and orchard management practices.

ii. Engagement with the chestnut industry via teleconferencing to discuss all aspects of the disease problem in Australia to gain information (data and/or anecdotal feedback) not reported in the literature.

iii. Reference to research gained from other industries, such as avocado, to determine the most effective avenues for future work in chestnuts to achieve appropriate management of disease problems.

Introduction: Chestnut Production

The Australian chestnut industry is only about 20 years old. There are approximately 300 growers, most of which are small farms with a few larger producers. The majority (70%) of commercial orchards are in NE Victoria (Bright, Myrtleford, Buckland Valley, Harrietville, Beechworth, Stanley). The

remaining orchards are in South West Victoria (High Country of Gippsland and the Macedon Ranges), New South Wales (Batlow, Orange, Canberra, Sassafras, and Tenterfield), South Australia (Adelaide Hills), South West Western Australia and throughout Tasmania. Chestnuts prefer a climate of hot summers and cold winters and over 800m above sea level.

Currently there are approximately 200,000 trees over 1000 hectares with new plantings expected to increase that number to 250,000 trees in the next few years. The 2013 production was valued at $9 million for approximately 1200 tonnes per year of fresh chestnuts. Production is expected to increase to 2000 tonnes by 2020.

On a global scale, Australian production is very small with a farm gate value of approximately $12 million per annum. Estimated 2012 chestnut production (http://faostat.fao.org/) for USA, South America, and Turkey was approximately 60,000 tonnes each. Italy produced 52,000 tonnes, and China produced over 1.5M tonnes. Comparing other nut producers in Australia, almond production was over 140,000 tonnes and walnuts 2800 tonnes in 2012.

Chestnut Species, Cultivars, and Rootstocks

Chestnut, oak and beech trees all belong to the family Fagaceae. There are thirteen species of chestnut worldwide, but the four main species are:

- *Castanea sativa* (European) – probably originated in Asia minor/Turkey
- *Castanea crenata* (Japanese)
- *Castanea dentata* (American)
- *Castanea mollissima* (Chinese)

European chestnut is the most common species grown in Australia with small numbers of other species, as well as some hybrids. When different species are growing in close proximity to each other natural hybrids between the species can occur readily. This has occurred in several countries around the world including Australia, where today hybrid chestnut trees are common. Most of the chestnuts grown in New Zealand are *C. crenata x C. sativa* hybrids, which

grow well under New Zealand conditions (Klinac *et al.,* 1999). Chestnut breeding programmes around the world have deliberately hybridised the various species to create superior varieties for fruit and/or timber production. European/Japanese hybrids are now common commercial fruiting varieties in France, Australia, New Zealand and western USA. Japanese/Chinese hybrid varieties are now found in South Korea and Japan. American/Chinese hybrid varieties are now found in the eastern USA along with even more complex hybrids.

Chestnut trees require cross pollination by wind or insects from a different compatible variety to ensure good nut production. Therefore, orchards must contain a few pollen producing varieties. Asexually reproduced cultivars of chestnut can be propagated by various methods such as grafting, rooted cuttings, root collar division, or tissue culture (Serdar *et al.,* 2005). The most successful propagation is by grafting or budding onto a seedling rootstock, preferably of the same variety to minimise graft incompatibility (Oraquzie *et al.,* 1998). Interestingly, however, it has just been shown that there is graft compatibility using European chestnut (*C. sativa*) scion on oak (*Quercus vulcanica*) rootstock (Ada & Ertan, 2013).

There are hundreds of chestnut varieties or cultivars around the world and in Australia the most popular have included De Coppi Marone, Buffalo Queen, Red Spanish, Purton's Pride. Bouche de Betizac is promoted in Europe as a *Phytophthora* resistant rootstock but in Australia Menzies is the current favoured rootstock due to its reported resistance to *Phytophthora*. Growers have observed that some rootstocks appear to have greater resistance than others. In one orchard Buffalo Queen were less affected than other rootstocks. In another orchard where 50% of trees were dead the immediately adjacent Menzies rootstock was not affected even though they were growing in a similar landscape. On the other hand, another orchard with 4% disease incidence was all Menzies rootstock. Growers have also noted that older trees generally have higher incidences of *Phytophthora* than younger trees which may be related to rootstock but is more likely to involve root to shoot ratios.

A goal of the Australian chestnut industry is to develop industry standards in relation to varieties and best practice management of orchards.

Pathogenic *Phytophthora* Species

Introduction

The history of *Phytophthora* in chestnuts began in the 19th century when chestnut trees (*Castanea dentata*) were found dying in southeastern United States forests. It was not until 1932 that a *Phytophthora* sp. was found to be the cause of the disease which had killed thousands of trees over a wide area (Milburn & Gravatt, 1932). It was later identified as *Phytophthora cinnamomi*. The pathogen was later recovered from chestnuts in Portugal in 1942. Affected chestnut trees were reported to gradually die back, and leaves were reduced in size or turned yellow, or wilted severely. Lesions on the roots showed an inky-blue exudate that stained the soil near the roots (thus the name ink disease); larger roots, the taproot, and the lower trunk in the collar region were invaded to form irregular wedge-shaped streaks.

In Australian chestnuts *Phytophthora cinnamomi* appears to be the major species of concern but *Phytophthora cryptogea* was found attacking chestnuts in South Australia (Wicks & Volle, 1976) and has been reported in Greece (Perlerou *et al*., 2010).

Phytophthora cambivora and *P. cinnamomi* are co-implicated as the cause of ink disease in Europe (Prospero *et al.,* 2012. *P. cambivora i*s favoured by cooler temperatures than *P. cinnamomi. Phytophthora citricola* also infects chestnuts in Europe but it is only weakly pathogenic. Uchida (1967) reported a trunk rot of *Castanea crenata* caused by *Phytophthora katsurae* in Japan. He found lesions on trunks of mature trees about 10 to 100 cm above ground. The bark around the lesions became soft and fissures developed around the infected area. There was a black exudate from the fissures and a smell of fermentation. Diseased trees were killed within four years of infection. *P. heveae* has been found in rainforest soils of north Queensland (Brown, 1976).

It would be interesting to know what other species of *Phytophthora* are associated with ink disease in Australia, but the industry clearly has the more immediate task of managing *P. cinnamomi* in existing plantings. However it needs to be aware of potential Biosecurity threats. Recently *Phytophthora ramorum* has killed over one million oak trees in the wildlands of western USA. When first detected it was a minor foliar blight of ornamentals in nurseries, but in trees it causes a fatal trunk canker disease. The oak (*Quercus* spp.) belongs in the same plant family (*Fagaceae*) as chestnut, and chestnut has been reported as a natural host of *P. ramorum* in the UK. Symptoms reported were a foliar blight and shoot dieback. *P. ramorum* is a cool temperature pathogen (20ºC optimum for growth). Long distance spread is by movement of infected plants and soil, infested with chlamydospores, on footwear. *Phytophthora kernoviae* shares many characteristics with *P. ramorum,* but has a more limited host range. It produces bleeding cankers on beech trees and is very aggressive on *Rhododendron.* It is reported from Wales, England (on a range of hosts including Castanea sativa) and New Zealand (on custard apple). The exclusion of these soilborne pathogens from Australia is critical.

Phytophthora cinnamomi

Phytophthora cinnamomi belongs to the class Oomycetes within the Kingdom Chromista. It is not a true fungus but a fungal-like organism and is known as a water mould. It is distinguished from the true fungi by its non-septate hyphae and production of motile zoospores that emerge from sporangia, which play a key role in its life cycle. It is the zoospores that enable the rapid spread of disease in wet weather.

The following factors make *P. cinnamomi* such an effective plant pathogen, with over 3000 plant hosts:

- It is favoured by wet conditions
- Sporangia release zoospores which are attracted to plant tissue by chemical stimuli as well as root generated electric fields

- They encyst and germinate to produce germ tubes which penetrate the host cell wall
- Zoospores will travel short distances (centimetres) under their own power or can be carried a long distance in flowing water.

If the infection is successful another generation of sporangia and zoospores is produced on the host surface within 3 to 5 days, which will result in an explosive epidemic if environmental conditions are favourable.

It may persist in soil for years as chlamydospores (resting spores) or sometimes saprophytically.

Factors predisposing roots to infection

Factors affecting root growth are soil temperature and moisture, soil aeration, soil carbon dioxide, pH, mineral elements and salt concentration. Roots can die from a lack of oxygen even when *P. cinnamomi* is not present. This is called anoxia. Roots must be able to breathe. Respiration, which supplies the energy necessary for roots to function, relies on soil pores to provide oxygen and remove carbon dioxide. The proper amount of pore space in the soil is essential to achieve good drainage and aeration. As oxygen levels fall roots become more leaky; they leak greater amounts of soluble metabolites which attract zoospores. If the soil is water-saturated as well as oxygen deficient the roots will also leak ethanol which is also attractive to zoospores. *Phytophthora* is an aerobic organism and in water-saturated soils will attack roots and trunk near the soil surface where there is sufficient oxygen.

Poor soil drainage also restricts leaching and leads to salt accumulation. Salinity will interfere with the growth of plants and will predispose plants to infection by *Phytophthora.* Drought is another stress factor that predisposes roots to infection.

Management strategies

As *P. cinnamomi* has a short generation time and high reproductive capacity under favourable conditions (soil temperature 12-30°C; temporary flooding), which allows for a massive increase in the inoculum load in a very short time, no one disease management strategy is likely to be completely satisfactory. Managing water moulds like *P. cinnamomi* requires a combination of practical management procedures designed to reduce pathogen activity and increase host resistance. Ink disease management in Italy largely relies on pathogen epidemiology (Vettraino *et al.,* 2001, 2010) via integrated control methods including water management, copper sulphate and metalaxy applications and potassium phosphite trunk injections.

The most important procedures will be discussed in detail later in the document.

They include:

1. **Exclusion and orchard hygiene**

In many areas *P. cinnamomi* may not be endemic and the simplest approach will be to prevent its introduction. Even if it is introduced orchard hygiene will help prevent its spread. The water mould spreads via contaminated water, infested soil usually containing small pieces of infected plant tissue and by nursery plants. The pathogen rarely forms spores above the ground and sporangia are not deciduous, so it cannot be dispersed by wind. The pathogen may be present in an orchard long before symptoms appear. The most appropriate way to test for its presence is by soil sampling and baiting samples with New Zealand blue lupins.

2. **Disease-free nursery trees**

Nursery trees should be propagated under best practice guidelines as outlined in The Nursery Industry Accreditation Scheme, Australia (NIASA). There is an opportunity to partner NIASA and form a national chestnut industry (self-regulated) nursery accreditation scheme for the development, continued improvement, and observance of guidelines for the production of pathogen

tested true-to-type chestnut nursery trees. The industry may need to include other soilborne pathogens besides *Phytophthora.* Soilborne diseases need to be managed successfully in nurseries to provide pathogen-free planting material and reduce the risk of spreading *Phytophthora* and other pathogens to all production areas.

3. Cultural control

Good soil drainage is essential to minimise temporary flooding. Good internal drainage and aeration, as well as uniformity of structure are required to reduce the impact of *P. cinnamomi.* The water mould requires saturated soil pores for infection by zoospores.

A well designed irrigation system should be set up so that excessive water does not exacerbate the effects of root and trunk rot.

Addition of calcium (usually gypsum, unless pH correction required) can be effective as it acts as a mild fungicide.

4. Biological control

Poor soil drainage and low organic matter are conducive to disease development; good soil drainage and high organic matter suppress disease. This includes suppressive soils, addition of mulches, composts and manures to stimulate the activity of soil microbes.

5. Chemical control

One of the more effective chemical controls is phosphonate which should be applied when roots are a strong metabolic sink. Timing of phosphonate applications in relation to growth events (relative sink strengths) maximises root phosphonate concentrations. A phenological model may need to be developed.

6. Resistance

Resistant or tolerant rootstocks, where available, are a vital component of the integrated management program. It is often quite difficult to find high

resistance to pathogens like *P. cinnamomi* which have a large host range. Resistance may not be adequate to cope with severe disease pressure.

Phytophthora management: major components of an integrated program

Soil selection

Key factors influencing disease development:

- *Soil temperature*
  The range 12° to 30°C favours disease, with an optimum temperature of 24° to 28°C.

- *Soil pH*
  Disease occurs between pH 4.5 to 7.5.

- *Soil aeration*
  P. cinnamomi does not tolerate low oxygen conditions and is not favoured by waterlogged soils.

- *Soil moisture*
  P. cinnamomi requires free water for the production of sporangia and release of zoospores which use tail-like flagella to swim out of the sporangium into saturated soil pores. Free water is required for infection of host roots by zoospores. Disease is favoured by soil profiles that temporarily impede drainage. Very wet soils (a soil matric potential of 10kPa or less) lead to severe disease (Sterne *et al*., 1977).

- *Soil salinity*
  Soils with high salinity are favourable for root rot development.

- *Soil nutrient status*

Disease is more severe in coarse textured, infertile soils with low levels of organic matter, nitrogen and calcium.
High organic content, high nitrogen, high levels of exchangeable cations especially calcium and magnesium are associated with soil microflora known to suppress *P. cinnamomi* (Broadbent & Baker, 1975; Pegg *et al.,* 1982; Stirling *et al.,* 1992, Turchetti & Maresi, 2005).

Very good drainage is perhaps the most important criterion for a chestnut soil. The higher the rainfall and the probability of exceptional 'wets', the more important this becomes. A well-drained top soil may not be sufficient if there is a marked increase in clay with depth, an impermeable clay subsoil or hardpan. All these increase the potential for temporary soil saturation.

Even in the absence of *P. cinnamomi,* hypoxia (low oxygen concentration in the soil) and anoxia (lack of oxygen in the soil) that result from flooded soils will affect root tips. Hypoxic conditions caused by a few days of standing water will negatively impact physiological processes such as net carbon dioxide assimilation, stomatal conductance of water vapour, and transpiration and cause tree mortality (Schaffer *et al.,* 2013).

A water-saturated, oxygen-deficient soil stresses plants and predisposes them to infection by *P. cinnamomi* (Davison, 1994). Oxygen deprived roots leak greater amounts of soluble metabolites (Zentmyer & Richards, 1952) and ethanol which attracts zoospores (Allen & Newhook, 1973). *P. cinnamomi* is an aerobic organism and in a water-saturated soil zoospores will infect roots and crown near the soil surface where there is sufficient oxygen.

Therefore good internal drainage and aeration, as well as uniformity of structure, are needed to reduce the impact of the pathogen. This reduces the time that *P. cinnamomi* is in contact with free water which is required for host infection. Inadequately drained and aerated soils can be modified by mounding or ridging. The vertical drainage must be good enough to cope with exceptional rainfall events and reduce the period of soil saturation.

Calcium (gypsum) applied to soils can reduce *Phytophthora* root rot by increasing soil permeability, allowing soils to drain freely and thereby improving soil aeration. Messenger *et al.* (2000) found that gypsum added to

soil reduced the number of sporangia and zoospores, thus reducing disease pressure. An excessive amount of gypsum can displace K and Mg so there is a need to regularly monitor levels of all nutrients to maintain tree health. Calcium also stabilises membrane permeability in the host cells and prevents leakage of carbohydrates and amino acids which attract zoospores.

Biological control: mulches, composts, manures, suppressive soils

The benefits of mulches to suppress *Phytophthora* are well known (Broadbent & Baker, 1974; Pegg, 1977; Turney & Menge, 1994; Wolstenholme *et al.,* 1998; Downer *et al.,* 2001). Avocados generally respond positively to under-tree mulching and compost/manure application. However, it can be a costly operation. Care needs to be taken when transferring technology developed for a subtropical evergreen tree to a deciduous tree such as the chestnut. There may be beneficial responses, but growers will need to consider the cost/benefit relationship. This may involve choosing an appropriate and readily available material, and determining the time of application to suit local growing conditions.

The avocado tree evolved in soils with a high organic content and an abundant natural leaf litter mulch under trees. These provide a nutrient rich, well aerated substrate with a rich microbial community which will suppress *P. cinnamomi.* It also improves root growth by allowing roots to proliferate in an environment relatively free of the pathogen, and reduces plant stress by protecting roots from desiccation and large changes in temperature. As mulching and adding compost and manure has these additional benefits besides suppressing *P. cinnamomi,* this management strategy is increasing in popularity. It is also compatible with sustainability.

The most suitable mulch material is still debatable; choice will depend on the availability and expense of local materials. A mulch with a C:N ratio of 25:1 to 100:1 is recommended to avoid a serious nitrogen draw-down (e.g. hardwood or softwood sawdust have a C:N ratio of 400-500:1). Suitable mulches include composted pine or hardwood bark, aged hardwood chips and high fibre straws

(wheat, barley). Natural leaf fall is also effective but not as effective as woody mulches. The mulch should not be too moisture retentive and must not be allowed to accumulate around the base of the trunk as it will encourage crown infection and canker development. Mulching will alter irrigation and nutritional requirements of the tree.

In forests and avocado orchards, *P. cinnamomi* is known to be present but does not cause disease under conditions suitable for disease development. The Ashburner System (Broadbent & Baker, 1974), designed to maintain a healthy avocado orchard in *P. cinnamomi* infested soils, simulated a disease-suppressive rainforest soil by continually adding large amounts of plant residues from cover-cropping and mulching with straws, as well as chicken manure and calcium to improve soil health and stimulate the activity of indigenous suppressive microbes. If extensive applications of mulches are not made to these soils serious outbreaks of root rot occur in avocado orchards. The soil becomes conducive to disease development. A key difference between the healthy and sick orchards was the soil organic matter content and the biological activity of the disease-conducive and disease-suppressive topsoils. *P. cinnamomi* is a relatively poor saprophytic competitor and struggles to survive in soils that support an active and abundant microflora. In recent times the Ashburner System has been modified with the addition of coarse mulches (e.g. wood chip) to provide an oxygen-rich root environment and regular applications of gypsum. Light applications of chicken manure, broadcast on the mulch are also occasionally made.

Chicken manure can also be used as a preplant treatment. Planting sites are filled with chicken manure (as fresh as possible) which releases ammonia and organic acids which are toxic to *P. cinnamomi* but unfortunately also to plant roots. It is best incorporated months prior to planting to allow toxic levels of ammonia and chlorides to fall. The treatment also increases total biological activity and populations of antagonistic actinomycetes, pseudomonads, fungi and endospore forming bacteria (Broadbent & Baker, 1974; Aryantha *et al.,* 2000).

There is a lot of interest from growers in using organic amendments in their orchards. These include applying products such as 'compost tea', humic acid

and other biologically enriched mixtures as organic fertilisers and root growth promoters. Crowley (2008) states that standard organic mulches and composts are 'transformed *in situ* by soil microorganisms to the same end products that contribute to stable organic matter in the soil'.

It should be stressed that the addition of organic matter alone (phosphonate omitted) is usually insufficient to control *P. cinnamomi* where there is high disease pressure.

There is also interest in using antagonistic organisms to control root rot. Organisms such as *Trichoderma, Gliocladium, Bacillus, Pseudomonas* and *Streptomyces* may suppress disease in nurseries where there is a well-drained and aerated potting mix, and favourable temperatures and moisture levels for root growth. They are less effective in the field where soils and weather conditions are often quite unsuitable. Biological control is generally more effective using mulches, composts and manures that stimulate resident antagonists rather than simply adding beneficial microorganisms to poor soils.

Phosphonate* and tree phenology

(*Refer to Disclaimer at the end of this document)

Successful *Phytophthora* management in all horticultural tree crops requires an integrated approach using resistant rootstocks (where available), disease free nursery trees, improved drainage, mulching and appropriate applications of chemical.

It is important to protect trees and avoid the much more difficult task of rehabilitating trees that are already suffering from disease.

In the absence of highly resistant rootstocks, phosphonate (salts or esters of phosphonic acid) becomes a vital component of the integrated management system. Phosphonate is systemic and mobile in xylem and phloem, and injection of the chemical into tree trunks and/or foliar sprays has been very effective in controlling *Phytophthora* in tree crops such as avocado. There have

been some studies carried out in chestnuts (Gouveia *et al.,* 2010) but more work is needed.

The mode of action of phosphonates is considered to be a disruption of phosphorus metabolism in the organism which causes fungistasis and the consequent activation of the defence responses in the tree. Because they are translocated in the phloem they can be applied to any part of the plant and carried to all other plant parts according to source-sink relationships in the tree. During periods of high vegetative growth, during flowering and fruit development phosphonate applied as injections or sprays will not be transported to roots and trunk where it is required. The chemical will remain in the canopy. Hence, the timing of applications in relation to tree phenology will be of paramount importance in determining the distribution of phosphonate within the chestnut tree and subsequently the control of *Phytophthora cinnamomi.*

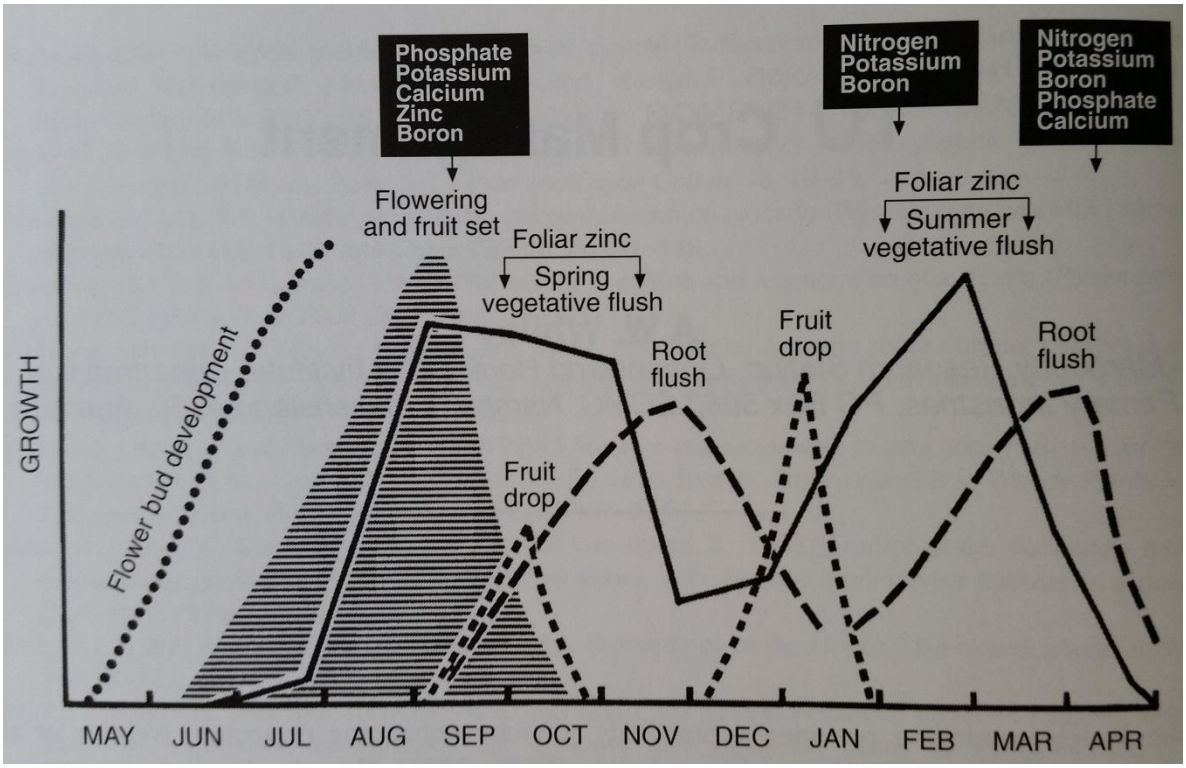
A survey of the literature has failed to locate a phenology model (Figure 1) for chestnuts growing in southern Australia. Phenology was used as the basis for the successful control of *Phytophthora* root rot in avocado and pineapple, where the timing of applications of injections and sprays was linked to growth events.

It is recommended that the industry determine the phenological activity of chestnut trees in the major growing area with particular reference to maximising phosphonate concentrations in the trunk and roots. Once the period of maximum root growth is established (this will be when root sink strength will be high and they will have resource priority), apply phosphonate injections and foliar sprays and monitor the distribution of phosphonate in roots and trunk. The amount of phosphonate applied by injections or sprays will need to be sufficient to give a concentration of phosphonate which will halt the pathogen in the trunk and roots. Anecdotal evidence suggests that the critical level needed to halt the pathogen will be >25ppm.

The avocado is an evergreen subtropical tree which has rhythmic growth. There are two growth flushes which alternate with periods of quiescence. Active root growth alternates with shoot growth. Avocado trees showing root

rot symptoms are injected twice per growing season when shoot growth has matured, whereas healthy trees are injected only once to achieve maximum and persistent levels of phosphonate in feeder roots. The latter injection is made after summer leaf and root flushing are complete and fruit are no longer a major sink, but before floral bud development.

High volume foliar sprays (0.5% a.i. mono-dipotassium phosphonate, pH adjusted to 7.2) applied without surfactants to thoroughly wet trees (>2500 litres/hectare) will supply the same root concentration as one injection. Four to six strategically timed sprays will give the same root phosphonate level in the roots as the injection. These are usually applied two weeks apart.



From: Whiley AW, 2002

Figure 1: Phenology model developed for avocado in the Australian subtropics. Fungicides are applied, either by trunk injection or foliar sprays, prior to major root flushes. Sick trees are injected twice a year – once after the spring flush has matured and again after the summer flush has hardened. Healthy trees are generally only treated after the summer flush either with one injection or three to four foliar sprays (2 to 4 weeks apart). Applications to healthy trees are varied depending on disease pressure and the results from root sampling and analysis.

Without access to a phenology model for chestnut the following statements are conjectural.

In deciduous trees, like the chestnut, root growth is thought to occur in the spring and autumn. Injections or sprays made in spring to chestnut after the main phase of extension growth may translocate some phosphonate to the roots but there will be competing sinks. Also the first root growth in deciduous trees is possibly made at the expense of reserve materials, and it may be receiving little from current photosynthesis. Maximum root growth may occur after nut maturity (there will no longer be other competing sinks for photoassimilate). This will be the ideal time to inject or apply foliar sprays. This must be done before leaves become senescent. This program is designed to protect trees and will not rejuvenate severely affected trees. They will require two injections (at spring flush maturity and at nut maturity) and sprays will be ineffective due to lack of leaves. Root levels of phosphonate should be monitored regularly.

In avocado, research has moved from curing severely affected trees to preventative management. To protect healthy trees growers either inject or apply foliar sprays in late autumn or early winter. Research indicated that a 0.1% spray (the original registered rate for spraying) was ineffective. Rates of 0.5% (8.3mL/L of 600 product) and 1.0% gave good protection, although the former was preferred because 1.0% was occasionally phytotoxic. In the study of Fleet & Dawson (1998) foliar sprays failed to improve health of trees, but it was not investigated as a preventative treatment on healthy trees with the pathogen present in the root zone.

Metalaxyl will also reduce the impact of *P. cinnamomi.* It is highly water soluble, moves readily in soils, is absorbed by roots and moves rapidly in the xylem. It will kill some but not all *Phytophthora* inoculum in the soil (phosphonate does not have any effect on the pathogen in the soil). Growers should consider using metalaxyl on young trees especially when planting in infested soils. It usually provides disease control for three months. In high organic matter soils its efficacy is reduced due to rapid biodegradation and this limits its use for treating large bearing trees.

As phosphonate* is such a key component of the integrated management strategy, I have included a summary of the key facts:

- Annual applications of phosphonate are usually required. Even though phosphonates persist well in plant tissue, sequential applications are required to maintain effective concentrations in plant tissue.

- Monitor phosphonate levels in roots by sampling and analysis. A commercial monitoring service is available in Queensland.

- Sink strength at the time of application is the dominant factor influencing the translocation of phosphonate within the tree. Monitor feeder root growth in the orchard; applications should coincide with periods of active growth.

- Foliar sprays (0.5% buffered to pH 7.2 and applied without surfactants) are only recommended for healthy canopies. High volume sprays are more effective than low volume applications.

- Three to four foliar sprays usually give the same level of phosphonate in the roots as one injection*.

- Inject sick trees, usually twice a year. Healthy trees generally need one injection, but this may vary depending on the disease pressure and results from root analyses*.

- Soil drenching with phosphonate is ineffective.

- Foliar applications at or near floral initiation or early anthesis may be detrimental to pollen germination and pollen tube growth in chestnut. In avocado a level of 2500 ppm phosphonate was phytotoxic to pollen germination, a level far in excess of that measured in plant tissue following standard commercial applications. Fairbanks *et al.* (1997, 1999) found that phosphonate sprays used to control *Phytophthora* in Jarrah forests of Western Australia significantly reduced pollen viability and germination as well as seed germination of a number of native species. The effect on phosphonate on the floral biology of chestnut is

unknown. If sprays or injections are made at spring flush maturity phosphonate is likely to accumulate in developing floral structures due to their sink strength.

- Isolates of *P. cinnamomi* with resistance to phosphonate have not been detected in the field. However, isolates do over time become less sensitive to the chemical, but despite this commercial applications of phosphonate still provide good disease control in the orchard.

The industry needs to be aware that some commercial formulations of phosphonate translocate better than others. The following (Table 1) are results from avocado trees which were injected with three different commercial products.

Product	A	B	C
Pre-treatment sample root level	53ppm	53ppm	34ppm
Root level after 1 month	66ppm	113ppm	121ppm
% increase	24.5%	113.2%	255.9%

Table 1: Phosphonate injection of avocado trees on 21/5/14 with root sampling on 19/6/14

Originally, all registered fungicides (e.g. Fos Ject) were based on phosphorous acid flake supplied by Albright and Wilson Limited, who manufactured their product by the hydrolysis of phosphorus trichloride. This is no longer available and the *Phytophthora* control products now contain a technical grade produced as a by-product of the manufacture of acid chlorides and carboxylic acids and phosphorus trichloride.

Pathogen–free nursery plants

Pathogen-free nurseries are key to the establishment of healthy orchards. *Phytophthora cinnamomi* is primarily dispersed in infested soil, water or infected planting material. The pathogen can be especially detrimental to nursery plants, as it will thrive in the nursery environment. Free water, high levels of humidity, favourable temperatures, and susceptible plant tissue

(young, rapidly growing, unsuberised roots) are always available for infection. Most nursery crops are monocultures with limited genetic diversity and they are extremely vulnerable to disease epidemics such as those caused by *Phytophthora cinnamomi.*

It was suggested by Fleet & Dawson (1998) that the industry consider having an accreditation scheme for chestnut nurseries. Accreditation is usually a voluntary process administered by industry where no one is obliged to join. Such an accreditation scheme (ANVAS) has been operated by the avocado industry since 1979. Nurseries must abide by ANVAS Guidelines and are inspected and tested for soilborne pathogens twice a year (Appendix 1).

Before considering such a scheme the industry needs to be aware of the life cycle of the pathogen and how it is disseminated and controlled.

Phytophthora cinnamomi is a soilborne pathogen that survives in the soil in infected roots as mycelium or chlamydospores. When favourable temperatures return (temporary flooding, soil temperatures >12°C) sporangia produce zoospores that swim through the soil or are spread by surface flowing water. Zoospores are attracted to plant roots by chemical stimulus as well as root generated electric fields. They encyst and produce germ tubes which infect root tips. If infection is successful another generation of sporangia and zoospores is produced on the host surface within three to five days, which will result in an explosive epidemic if environmental conditions are favourable.

In low-lying areas where the soil can become water-saturated and oxygen deficient, oxygen deprived roots leak greater amounts of soluble metabolites and ethanol which attract zoospores. Thus waterlogging predisposes plants to attack, and as *Phytophthora is* an aerobic organism, zoospores will infect roots near the surface where there is adequate oxygen.

The fundamental principle for disease control in nurseries is that it is better to avoid disease than have to apply controls after a disease outbreak. Excluding soilborne pathogens from production nurseries can be difficult at times, but where it is possible it is the most cost effective disease management strategy. It is perhaps easier for the production of container grown plants, but it is achievable for in-ground production. It may involve more intensive sampling

for soilborne pathogens. Infested sites may require fumigation with an approved fumigant. Long term success will then depend on effective quarantine of the site after treatment. The area will need to be well drained and perhaps include a raised bed system. All water from adjacent areas will need to be deflected from the site. The area needs to be fenced to prevent access to animals and vehicles. Access to the site should be limited to essential staff. They will need to walk through footbaths which are regularly cleaned and replenished. Before starting work they should wash their hands with soap and water or an approved hand-washing biocide. Good hygiene will be required in the nursery especially the use of irrigation water that is free of soilborne pathogens. Surface water supplies are nearly always contaminated and must be disinfested.

Fungicides to control soil-borne diseases in a nursery should only be used to limit spread of a disease outbreak. They can also be used to protect healthy plants at vulnerable times (e.g. when roots are damaged during repotting). They rarely eradicate a pathogen or eliminate disease, and should not be used to compensate for poor nursery hygiene. They generally only mask symptoms of a disease, the pathogen will still be present, and this will lead to serious problems when they are planted on the farm. The chemicals metalaxyl and phosphonate have been very effective in controlling *Phytophthora.* Metalaxyl is very water soluble, moves readily in soil and is absorbed by plant roots. It will kill some of the inoculum in the soil but not all. Phosphonates are xylem and phloem mobile. They do not have any effect on the inoculum in the soil. They act as a fungistat and activate the defence responses of the host plant.

Resistance

Host resistance is the best method of reducing root and trunk rot. There are three components of general resistance to *Phytophthora.*

1) resistance to penetration
2) restricted colonisation of the root
3) reduced sporulation of the pathogen on the host.

Resistance may not be absolute, particularly where disease pressure is high, and resistance must be combined with traditional methods of management. With *Phytophthora,* resistance is often a form of tolerance. Some rootstocks produce new feeder roots more quickly than susceptible rootstocks in the presence of the pathogen.

The selection for resistance in chestnut in forestry commenced in 1932. The American chestnut (*C. dentata)* is highly susceptible to *P. cinnamomi.* In France, Dufrenoy (1930) reported that Japanese chestnut (*C. crenata)* was resistant to *P. cinnamomi* and attributed this to the rapid production of phenolic compounds in cortical cells. In 1945, Crandall *et al.* reported that all available strains or selections of *C. crenata, C. mollissma, C. henryi,* and *C. seguinii* were highly resistant.

Selection and propagation studies are currently underway for resistance to *P. cinnamomi* and resistance to *P. cambivora* (Robin *et al.,* 2006; Miranda-Fontaina *et al.,* 2007) while a genomic screening effort to find molecular markers is ongoing (Olukolu *et al.,* 2012; Costa *et al.,* 2011; Santana *et al*., 1999).

In 2006, Robin *et al*. (2006), from France, Italy and Greece, commented that despite the economic importance of fungal pathogens, namely *Phytophthora cambivora*, to the European chestnut industry, genetic variation for disease resistance remains poorly understood. A major control measure for the future is the selection and breeding for resistance to reduce the impact of disease on new plantations and nursery stock.

In Europe (France, Italy, Greece, Spain, UK), it is speculated that domestication and widespread use of clonal varieties has resulted in loss of genetic diversity. A large research project was initiated out to determine the genetic variation in susceptibility to ink disease caused by *P. cambivora* and *P. cinnamomi* in chestnut populations and determine possible relationships to geographic origin and level of domestication (Robin *et al.*, 2006; Vannini & Vettraino*,* 2001). They found a large amount of genetic variation in resistance, suggesting that breeding for this trait might be an option for the future. In more recent work, Cuenca *et al.* (2010) collected 206 samples to establish

clones and assess for resistance to ink disease in parts of Spain. Of these 130 managed to establish. Only eight were shown to be resistant using a single test, however, only two clones were resistant using three different tests.

It has been reported that genetic resistance to *Phytophthora* spp. (*P. cinnamomi* and *P. cambivora*) occurs in the Asian chestnut species *C. crenata* and *C. mollissima* (Rutter *et al*., 1991. However, for European *C. sativa*, graft incompatibility between species has prevented use of the resistant Asian species as rootstocks. (Craddock & Bassi, 1999; Huang *et al.,* 1994; Santamour, 1988). Craddock & Bassi (1999) investigated the use of *Phytophthora*-resistant Euro-Japanese hybrid clones as rootstocks for four Italian 'Marrone' cultivar scions with mixed results in preliminary work with no further results since. In the USA, Jeffers *et al.* (2012) have found that *C. dentata* seedlings are consistently susceptible to inoculation with *P. cinnamomi*, starting to die 3 weeks after inoculation in the field. *C. mollissima*, on the other hand, has been consistently resistant and survived. They have been testing *C. dentata* and *C. mollissima* and hybrid seedlings since 2004 and will continue into the future as they search for resistance. More recently they have been evaluating transgenic plants which have been developed for resistance to chestnut blight (*Cryphonectria parasitica*). Over seven years they have tested 197 families, finding a total of 40 families with resistant plants but with mortality rates ranging from 68% in 2010 to 99% in 2006.

Olukolu *et al*. (2012) have investigated the genes responsible for resistance to *Phytophthora* root rot. They have identified two plausible genes which can ultimately be used as markers for breeding programs. The ultimate goal is to produce transgenic chestnut trees (*Castanea dentata*) using cloned candidate genes. This type of research is very specialised and, therefore, costly and infinite.

The industry should rely on research carried out overseas with the possibility of applying it in the future if possible, either by using techniques designed overseas or by importing already developed rootstocks if phytosanitary protocols and budget allow. At this stage, however, international research is moving very slowly and there is not a lot in the literature to suggest that work has progressed in the last five years.

Summary and Recommendations

This review presents important existing knowledge of the biology and epidemiology of *Phytophthora cinnamomi*, gives practical advice on how to integrate components of disease control and suggests promising avenues for research. An understanding of the basic biology of *Phytophthora* is essential when developing an integrated management program. The ability of the 'water mould' to increase inoculum density rapidly, is the basis for its success as a plant pathogen, and helps explain why effective control is rarely achieved by a single disease control measure. There is a need for a number of different approaches, several of which are sensible agronomic/horticultural practices, to be used in an integrated manner. Drainage control is fundamental to success. Shallow, poorly drained soils, containing a large number of host roots that are water-saturated for some time, provide ideal conditions for zoospore formation and dispersal, infection and predisposition to infection. Disease-free nursery trees are a key element in any horticultural enterprise. Besides preventing the spread of the pathogen to new areas, it is well understood that when nursery trees are destined for areas where pathogens are already present, disease control is increased greatly if plants are free of pathogens at time of establishment. High soil organic matter is a factor commonly implicated in *P. cinnamomi* suppression. It is a key factor in horticultural soils known to be suppressive to *Phytophthora* root rot. There are many examples where organic matter has been added to soils to minimise the impact of root rot and induce suppressive conditions in the soil. A major role of organic matter is related to microbial antagonism of the pathogen. The use of chemicals requires knowledge of optimum timing of sprays or injections, rates of application, and methods of application to be applied effectively. With phosphonates, timing of applications in relation to growth events (relative sink strength), maximises root phosphonate concentrations thus maximising protection. The planting of resistant rootstocks, is perhaps the most important type of control measure, but with many horticultural crops resistance is not always attainable. Resistant

trees will still benefit from well-drained and aerated soils and mulching to produce good soil health.

Although the review did in part concentrate on increasing the understanding of the biology of *P. cinnamomi*, its major role is to determine how this knowledge can be translated into practical control measures for the chestnut industry. The integrated control program mentioned in the review is largely derived from research undertaken for the Australian avocado and pineapple industries. Research findings were successfully adopted by these industries – adoption by industry is the ultimate test of research. Much of the research is also applicable to the chestnut industry and there is no need to try to reconstruct the wheel, but to be aware of crop and regional differences.

The following lines of investigation should be of value to chestnut industry and are worthy of industry support:

Nursery practices

All *Phytophthora* species are primarily dispersed in contaminated soil and water and less frequently in infected planting material. For this reason nursery practices designed to prevent the spread of the pathogen with planting material should focus on preventing infested soil and water from entering the nursery.

It is suggested that the chestnut industry initiate some form of industry self-regulated nursery accreditation scheme. The aim of this scheme would be to foster sound nursery practices for the production of pathogen tested, true-to-type chestnut trees. This may also include the accreditation of seed trees. Keith Bodman (2014) has recently reviewed the avocado nursery voluntary accreditation scheme (ANVAS) (HAL Project AV13020) and has proposed to partner this scheme with the Nursery Industry Accreditation Scheme, Australia (NIASA) system.

He suggests having 'a national self-regulated process for the development, continuing improvement, and adherence to guidelines for the production of pathogen tested, true-to-type avocado plants suitable to transplant'. Such a

scheme may require an enlistment process for chestnut nurseries some of which may already be NIASA accredited. Accredited nurseries should not be allowed to use chemicals to suppress pathogens. This will not eradicate a pathogen or eliminate disease and could lead to serious problems further down the supply chain.

Evaluation of high concentration foliar phosphonate applications*

Research for the avocado industry found that the most acceptable formulation for use by the industry was 0.5% mono-dipotassium phosphonate buffered to pH 7.2 and applied without surfactants. Sprays produced similar root phosphonate levels as the trunk injection treatment and gave similar protection against the disease.

As sink strength at the time of application is the dominant factor influencing the translocation of phosphonate in the tree, phenological activity of the chestnut tree needs to be identified. There is a need to know the timing of phenological development phases of roots, shoots, flowers and nuts to changing source and sink strengths. As phosphonate is required in the trunk and roots for *Phytophthora* control, root growth can be based on relatively superficial measurements by placing newspaper under a mulch layer. Root growth can then be determined by lifting the paper at regular intervals. Foliar sprays should be applied to coincide with root flushes. A phenology model will be more accurate if a trunk starch concentration curve is superimposed on the phenology model. The distribution and persistence of phosphonate applications can be monitored using root sampling and analysis to determine the most appropriate times for spraying or injecting.

Resistant rootstocks

Rootstocks may change the situation dramatically in the future. As plant improvement programs require a long term investment of funds and research effort, the industry should encourage collaboration with international programs that have identified sources of resistance to *Phytophthora*. Material can then be

introduced and screened to identify useful rootstocks. Meanwhile, there is a need to develop robust tests for disease resistance evaluation of local selections.

Focus on the implementation and adoption of technologies based on sound principles of integrated disease management

Be guided by what has been adopted by other industries. There is no need to reinvent the wheel.

References

Ada S, Ertan E, 2013. Histo-cytological study of the graft union of the Chestnut (*Castanea sativa* Mill.)/Oak (*Quercus vulcani* Boiss.). *Agriculture, Forestry and Fisheries* **2**, 110-115.

Allen RN, Newhook FJ, 1973. Chemotaxis of zoospores of *Phytophthora cinnamomi* to ethanol in capillaries of soil pore dimensions. *Transactions of the British Mycological Society* **61**, 287-302.

Aryantha IP, Cross R, Guest DI, 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. *Phytopathology* **90**, 775-782.

Broadbent P, Baker KF, 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Australian Journal of Agricultural Research* **25**, 121-137.

Broadbent P, Baker KF, 1975. Soils suppressive to *Phytophthora* root rot in eastern Australia. In: Bruehl GW, ed. *Biology and Control of Soil-borne Plant Pathogens.* Minnesota, USA: The American Phytopathological Society, 152-157.

Brown BN, 1976. *Phytophthora cinnamomi* associated with patch death in tropical rainforests in Queensland. *Australian Plant Pathology Newsletter* **5**, 1-4.

Costa R, Santos C, Tavares F, Machado H, Gomes-Laranjo J, Kubisiak T, Dana Nelson C, 2011. Mapping and transcriptomic approaches implemented for

understanding disease resistance to *Phytophthora cinnamomi* in *Castanea* sp. BMC Proceedings **5** (Suppl. 7), 018.

Craddock JH, Bassi G, 1999. Effect of clonally propagated interspecific hybrid chestnut rootstocks on short-term graft compatibility with four cultivars of Italian "Marrone". In: Salesses G, ed. *Proceedings of the Second International Chestnut Symposium*. Bordeaux, France. *Acta Horticulturae* **494**, 207-121.

Crandall BS, Gravatt GF, Ryan MM, 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. *Phytopathology* **35**, 162-180.

Crowley D, 2008. Managing soils for avocado production and root health. *California Avocado Society Yearbook* **90**, 107-130.

Cuenca B, Ocana L, Salinero C, Pintos C, Mansilla JP, Rial C, 2010. Selection of *Castanea sativa* Mill. for resistance to *Phytophthora cinnamomi*: micropropagation and testing of selected clones. *Acta Horticulturae* **866**, 111-119.

Davison EM, 1994. Role of the environment in dieback of jarrah. *Journal of the Royal Society of Western Australia* **77**, 123-126.

Downer AJ, Menge JA, Pond E, 2001. Effects of cellulytic enzymes on *Phytophthora cinnamomi. Phytopathology* **911**, 839-846.

Dufrenoy J, 1930. Control of the chestnut disease. *Annales des Epiphyties* **16**, 25-49.

Fairbanks MM, Hardy GE St J, McComb JA, 1997. The effect of the fungicide phosphite on pollen germination and fertility. *Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society.* Perth, Western Australia, 187.

Fairbanks MM, Hardy GE St J, McComb JA, 1999. Does phosphite affect pollination and seed viability in native plants? *Proceedings of the Ecological Society of Australia Inc.,* Freemantle, Western Australia, 57.

Fleet S, Dawson J, 1998. Control of *Phytophthora* with phosphorous acid. *HAL Report* (CH95002).

Gouveia E, Coelho V, Fonseca F, Nunes L, Monteiro L, 2010. Systemic immunity against soil borne *Phytophthora* and control of ink disease of chestnut by foliar spray of potassium phosphonate. *Acta Horticulturae* **866**, 449-453.

Huang H, Norton JD, Boyhan GE Abrahams BR, 1994. Graft compatibility among chestnut (*Castanea*) species. *Journal of the American Society of Horticultural Science* **119**, 1127-1132.

Jeffers SN, Meadows IM, James JB, Sisco PH, 2012. Resistance to *Phytophthora cinnamomi* among seedlings from backcross families of hybrid American chestnut. *Proceedings of the Fourth International Workshop on Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees.* General Technical Report PSW-GTR-240, Albany, CA, 194-195.

Klinac D, Seelye R, Knowles R, Nguyen H, 1999. Chestnut production and chestnut research in New Zealand. *Proceedings of the Second International Chestnut Symposium*. Bordeaux, France: *Acta Horticulturae* **494**, 43-48.

Messenger BJ, Menge JA, Pond E, 2000. Effects of gypsum on zoospores and sporangia of *Phytophthora cinnamomi* in field soil. *Plant Disease* **84**, 617-621.

Milburn M, Gravatt GI, 1932. Preliminary note on a *Phytophthora* root disease of chestnut. *Phytopathology* **22**, 977-978.

Miranda-Fontaina ME, Fernandez-Lopez J, Vettraino AM, Vannini A, 2007. Resistance of *Castanea* clones to *Phytophthora cinnamomi*: testing and genetic control. *Silvae Genetica* **56**, 11-22.

Olukolu BA, Nelson CD, Abbott AG, 2012. Mapping resistance to *Phytophthora cinnamomi* in chestnut (*Castanea* sp.). *Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees* **31**, 177.

Oraquzie NC, McNeil DL, Thomas MB, 1998. Examination of graft failure in New Zealand chestnut (*Castanea* spp.) selections. *Scientia Horticulturae* **76**, 89-103.

Pegg KG, Forsberg LI, Whiley AW, 1982. Avocado root rot. *Queensland Agricultural Journal* **108**, 162-168.

Pegg KG, 1977. Biological control of *Phytophthora cinnamomi* root rot of avocado and pineapple in Queensland. In: *Seminar Papers, Annual Conference Australian Nurserymen's Association Conference*, Hobart, 7-12.

Perlerou C, Tziros G, Vettraino AM, Diamandis S, 2010. *Phytophthora cryptogea* causing ink disease of *Castanea sativa* newly reported in Greece. Plant Pathology **59**, 799.

Prospero S, Vannini A, Vettraino AM, 2012. *Phytophthora* on *Castanea sativa* Mill. (sweet chestnut). *JKI Data Sheets* **6**, 13.

Robin C, Morel O, Vettraino AM, Perlerou C, Diamindis S, Vannini A, 2006. Genetic variation in susceptibility to *Phytophthora cambivora* in European chestnut (*Castanea sativa*). *Forest Ecology and Management* **226**, 199-207.

Rutter PA, Miller G, Payne JA, 1991. Chestnuts (*Castanea*). In: Moore JN, Ballington JRJr, eds. *Genetic Resources of Temperate Fruit and Nut Crops*. *Acta Horticulturae* **290**, 761-788.

Santamour FS Jr, 1988. Graft incompatibility related to cambial peroxidase isozymes in Chinese chestnut. *Journal of Environmental Horticulture* **6**, 33-39.

Santana C, Oliveira CM, Valdiviesso T, 1999. Molecular typing of rootstock hybrids (*Castanea sativa* x *Castanea crenata*) and Portugese *Castanea sativa* cultivars based on RAPD markers. *Proceedings of the Second International Chestnut Symposium*. Bordeaux, France. *Acta Horticulturae* **494**, 44.

Schaffer B, Gil PM, Mickelbart MV, Whiley AW, 2013. Ecophysiology. In: Schaffer B, Wolstenholme BN, Whiley AW, eds. *The Avocado: Botany, Production and Uses (2nd Edition),* CAB International, 168-2000.

Serdar U, Kose B, Yilmaz F, 2005. The structure of graft unions in European chestnut using different grafting methods. *Hortscience* **40**, 1474-1477.

Sterne RE, Zentmyer GA, Kaufman MR, 1977. The effect of matric and osmotic potential of soil on *Phytophthora* root disease of *Persea indica. Phytopathology* **67**, 1491-1494.

Stirling AM, Hayward AC, Pegg KG, 1992. Evaluation of the biological control potential of bacteria isolated from a soil suppressive to *Phytophthora cinnamomi. Australasian Plant Pathology* **21**, 133-142.

Turchetti T, Maresi G, 2005. Phytosanitary criteria for the protection of chestnut orchards and stands against chestnut blight and ink disease. *Acta Horticulturae* **693**, 521-528.

Turney J, Menge J, 1994. Root health: mulching to control root health in avocado and citrus. *California Avocado Society Circular* No. CAS94/2.

Uchida K, 1967. *Phytophthora* disease of chestnut. *Plant Protection* **21**, 383-387.

Vannini A, Vettraino AM, 2001. Ink disease in chestnuts: impact on the European chestnut. *Forest Snow and Landscape Research* **76**, 345-350.

Vettraino AM, Natili G, Anselmi N, Vannini A, 2001. Recovery and pathogenicity of *Phytophthora* species associated with a resurgence of ink disease in *Castanea sativa* in Italy. *Plant Pathology* **50**, 90-96.

Vettraino AM, Morel O, Perlerou C, Robin C, Diamindis S, Vannini A, 2005. Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink disease and crown decline. *European Journal of Plant Pathology* **111**, 169-80.

Vettraino AM, Franceschini S, Natili G, Pasganini R, Alicicco D, Vuono G, Vannini A, 2010. Integrated control protocol (ICP) of ink disease of chestnut in Central Italy: principles and future perspectives. *Acta Horticulturae* **866**, 425-430.

Whiley AW, 2002. Crop Management. In: Schaffer B, Wolstenholme BN, Whiley AW, eds. *The Avocado: Botany, Production and Uses (2nd Edition),* CAB International, 232.

Wicks TJ, Volle D, 1976. *Phytophthora* wilt of Chestnuts in South Australia. *Plant Disease Reporter* **60**, 700-702.

Wolstenholme BN, Moore-Gordon C, Ansermino SD, 1998. Some pros and cons of mulching avocado orchards. *South African Avocado Growers' Association Yearbook* **19**, 87-91.

Zentmyer GA, Richards SJ, 1952. Pathogenicity of *Phytophthora cinnamomi* to avocado trees, and the effect of irrigation on disease development. *Phytopathology* **42**, 35-37.

Acknowledgements

This project has been funded by HAL using the Chestnut Industry levy and matched funds from the Australian Government.

Dr Ken Pegg provided significant input and background knowledge to this project from his years of expertise working with the avocado and pineapple industries on *Phytophthora* diseases.

*Disclaimer

The information provided in this report is a recommendation only. Where fungicides or other chemicals are mentioned, they are meant as a guide only and are not an endorsement or otherwise of a particular company's product. You must check on the APVMA website that a particular product is registered for use on chestnuts and follow all label directions, including safety directions and warnings.

Appendix 1

AUSTRALIAN AVOCADO GROWERS' FEDERATION INC

The Executive Officer
P.O. Box 19
Brisbane Market,
Queensland 4106
Australia

Telephone: (07) 213 2444
Facsimile: (07) 213 2480

ANVAS

GUIDELINES

September 1996

– The Most Nutritious Fruit On Earth –
(The Guinness Book of Records)

The Avocado Nursery Voluntary Accreditation Scheme (ANVAS) Guidelines are formed by combining the previous ANVAS accreditation, ie nursery inspection and freedom from Phytophthora, with the use of virus-tested planting material through the Virus Tested Tree Registration Programme (VTTR).

The major benefit of the combination of the two operations is that trees produced from accredited nurseries will be free from Phytophthora and other fungal root pathogens and will be propagated from registered, true-to-type, virus indexed material. The result being growers will be able to purchase trees that are pathogen tested and true-to-type.

It is recognised that at the present time there is an insufficient range of virus tested varieties available to nurserymen. The Australian Avocado Growers' Federation (AAGF), in conjunction with State Departments of Agriculture and Primary Industries will be introducing imported varieties as well as local selections over the coming years. Growers requiring blocks of registered indexed trees must use ANVAS accredited plants propagated from sunblotch viroid indexed material.

Funding of the Scheme is by an annual levy of 10 cents per avocado tree. AAGF labels signifying that the trees are propagated from virus indexed material are available for 10 cents each.

It is hoped that nurserymen and growers will continue to take advantage of the previously successful programmes under the ANVAS guidelines.

Enquiries into the Scheme are welcome and should be addressed to:

ANVAS Registrar
PO Box 19
BRISBANE MARKETS QLD 4106

AVOCADO NURSERY VOLUNTARY ACCREDITATION SCHEME GUIDELINES

1. Aims of the Scheme

The Scheme shall foster:

1) sound nursery practices;

2) the use of virus-tested and registered sources of seed and budwood; and

3) the exclusion of soil-borne plant pathogens and root diseases.

2. Eligibility

Any avocado nurseryman or grower may apply for accreditation under the Scheme. Participation shall be voluntary provided participants agree to abide by this set of Guidelines.

3. Administration

The Scheme shall be administered by the Australian Avocado Growers' Federation (AAGF) and its affiliated bodies.

The AAGF shall consider applications for accreditation as required. All dealings between the nurseryman and the AAGF shall be confidential.

The AAGF may seek competent technical advice before reaching a decision on any application but shall reach its decision independently of that advice.

The Registrar of ANVAS shall publish a list of ANVAS accredited nurseries in each edition of Talking Avocados and when there is any doubt to the standing of an accreditation the nursery's name should be deleted from the list until accreditation is re-instated.

4. Application for Accreditation of Nurseries

Application shall be made on the prescribed form (Application for Membership) and may be made at any time of year to the Registrar.

Applicants will be advised when accreditation has been granted or of any reason accreditation cannot be granted or continued.

Accreditation of successful applications will in general be for one year, commencing 1 January, but shall be subject to periodic review by the AAGF during that year.

5. Privileges

A nurseryman whose nursery has been accredited under the Scheme may use the words "ANVAS Nursery" in advertising and the logo approved by the AAGF.

An accredited nursery is eligible to become an approved supplying nursery of the AAGF.

Accredited nurseries shall have a minimum of two inspections each year to monitor nursery hygiene and occurrence of plant diseases. These inspections shall be in late October/early November and late March/early April.

Accredited nurseries may send soil samples for biological assay at any time.

Accredited nurseries may use Virus Tested Tree Registration Programme labels on trees that are propagated from material provided under the Programme.

6. Responsibilities

Nurseryman

A nurseryman may not guarantee plants as being "disease-free" as a consequence of gaining accreditation under the Scheme, nor shall any warranty be implied by the AAGF, its affiliated bodies or co-operating organisation or personnel.

A nurseryman on request shall agree to officers of the relevant State Departments or the AAGF or employees of the AAGF entering the nursery to make inspections or to take samples for biological assay.

A nurseryman must complete the Nurseryman Declaration of the Technical Report.

A nurseryman must advise the DPI/Dept of Agriculture Inspector IMMEDIATELY if there is any suspicion that a pathogen is present or in the event of a positive result indicating presence of pathogens.

A nurseryman shall suspend all avocado orders to customers as ANVAS registered when accreditation has been suspended because of a soil-borne plant pathogen by an ANVAS designated Plant Pathologist in soil samples taken directly from plants in the nursery, until accreditation is restored.

A nurseryman must not use chemicals to suppress the incidence of plant pathogens in plants in the ANVAS facility, however this may be carried out in the dispatch area, provided that the dispatch area is in the opinion of the inspector clearly segregated from the ANVAS facility.

A nurseryman must aim to produce all trees from propagation material from the Virus Tested Tree Registration Programme.

Varieties Committee of AAGF

ANVAS accreditation of nurseries;
Development of protocol and procedures;
Revoking, suspending or discontinuing ANVAS accreditation;
Recommendations to AAGF
Varietal data; and
Any decision to require random tests to be conducted for presence of suppressive chemicals.

ANVAS Registrar

Advise DPI/Dept of Agriculture Inspector of protocol and procedures;
Request DPI/Dept of Agriculture Inspector to conduct inspections, sampling and where necessary investigations;
Advise Varieties Committee result of inspections, assays and investigations;
Communicate decisions of Varieties Committee to nurseries and block holders;
Bookkeeping; and
Maintain tree list data base and planting material register.

DPI or Dept of Agriculture Inspector

Conduct inspections and sampling in accordance with prescribed procedures contained in this protocol;
Conduct investigations to assist the nursery to isolate and eradicate pathogens from the nursery;
Provide technical advice to the Varieties Committee
Provide timely reports to the ANVAS Registrar; and
Supervise destruction of diseased trees.

Plant Pathologist, DPI

Provide assay for plant pathogens;
Advise Inspector and Registrar of assay results; and
Provide technical advice to the Varieties Committee.

7. Termination or Suspension of Accreditation

Accreditation shall terminate on 31 December but may be continuous if the annual application is successful.

Accreditation shall be suspended if, in the opinion of the AAGF:

a) satisfactory nursery hygiene is not being maintained;
b) plant health is unsatisfactory;
c) Phytophthora, Pythium, Verticillium or other soil-borne plant pathogen is detected by a qualified Plant Pathologist;
d) virus or viroid is detected by the indexing authority in nursery plants being sold as registered nursery trees;
e) the nurseryman does not abide by the schedule of fees;
f) rootstock and or varieties are misrepresented.
g) Pasteurised potting mix has not been used.

Accreditation shall be terminated if, in the opinion of the AAGF, the nurseryman has abused any privileges or neglected any responsibility of the Scheme.

8. Restoration of Accreditation

The AAGF may restore accreditation to a nurseryman upon meeting all the necessary guidelines.

9. Sampling Procedures for ANVAS Facilities

Procedures to be followed by DPI/Dept of Agriculture Inspectors for taking soil and root samples in ANVAS facilities are set out in the ANVAS Sampling Procedures.

10. Action on Obtaining a Positive Pathogen Result

The following action is to be taken in the event of a positive pathogen report being received by the inspector, irrespective of how the report is received:

a) A follow up inspection and resampling by DPI/Dept of Agriculture Inspector is to be conducted to confirm infection, and determine the extent of contamination and determine the likelihood of elimination.

b) If in the opinion of the Inspector the outbreak is widespread, a recommendation to revoke ANVAS Accreditation is to be sent to the ANVAS Registrar.

c) If further inspection and sampling indicates an isolated outbreak, the diseased trees are to be destroyed under supervision, and further testing conducted to ensure elimination of these pathogens from the ANVAS facility before ANVAS accreditation will be afforded.

11. The Use of Private Inspectors

Only DPI/Dept of Agriculture Inspectors appointed under the respective Plant Health Acts are authorised as the official inspectors of the scheme.

Nurseries are however free to employ private inspectors, pest scouts or consultants and carry out any sampling and testing as they desire, including assay for plant pathogens.

In the event of a positive result being obtained under any circumstances, the nursery is obligated to advise the DPI/Dept of Agriculture Inspector.

The DPI/Dept of Agriculture Inspector is to investigate all positive pathogen results as "highly suspicious", however the initial result is NOT to be regarded as official until a positive result is obtained from a sample obtained by the official inspector.

The DPI/Dept of Agriculture Inspector is to report the result of his investigation, including further testing, to the ANVAS Registrar.

The Registrar is to advise the Varieties Committee of the investigation and outcome.

AAGF is to decide on accreditation or suspension of accreditation.

12. Assay for Suppressive Chemicals

The Varieties Committee may require random assay for suppressive chemicals where the Committee is satisfied there is a likelihood that these chemicals are being used.

Such testing will be at the expense of AAGF.

In the event of a nursery being found to be using suppressive chemicals, the Varieties Committee may terminate ANVAS accreditation.

13. Penalties

Failure of disclosure on the Nursery Application may preclude accreditation. Failure of the nursery to fully comply with the protocol may also preclude accreditation.

Evidence of continued failure to comply with this protocol may result in the nursery not being ANVAS accredited in the future.

14. Block Registration

Application for registration of a candidate nuclear, multiplication or registered tree shall be made to the AAGF within (3) three calendar months of the date shown on delivery docket.

A candidate nuclear tree shall be assigned an in-test identification number consistent with the Australian Agricultural Council Accession List of Virus Tested Fruit Varieties and indexed in a glasshouse facility approved by the AAGF.

A candidate multiplication tree or candidate registered tree shall have an identification number which shall include the number or numbers of the foundation and/or nuclear source trees.

Registration of foundation, nuclear, multiplication and registered trees shall be continuous unless they are disqualified.

Foundation and nuclear trees will be indexed at least every five years and multiplication trees will be indexed at least every twenty years.

A nominee of the AAGF shall inspect all plantings of candidate trees to verify compliance with these regulations prior to registration or for the purposes of determining whether or not any registration shall be cancelled or amended. This will be on a twelve monthly basis for foundation, nuclear and multiplication trees.

15. Testing Procedures for Registered Trees

Testing procedures prescribed under these regulations shall be conducted in a manner approved by the AAGF.

Each indexing test shall involve a test for sunblotch infection either by a graft transmission test or a biochemical test.

A graft transmission test shall have a minimum of ten avocado seedlings grown from foundation or nuclear stock, and each indicator seedling shall be graft-inoculated with a minimum of two independent buds from the candidate tree. The graft-inoculated indicator plants shall be observed for a period of not less than two years, except where results show the candidate tree to be virus infected or inspections reveal the tree to be off-type.

A biochemical test shall be sufficiently sensitive to specifically detect one nanogram of avocado sunblotch viroid in one gram dry weight of leaf tissue.

An indexing authority may prescribe additional tests if seasonal conditions or other factors tend to obscure virus symptoms, when virus infection is suspected but not yet confirmed, or when symptoms may be masked in a particular variety.

The AAGF may, at the request of an indexing authority, permit the use of less than ten indicator seedlings per test, some other indicator plant, or some other indexing method.

The indexing authority shall provide a written report on the results of indexing tests before registration of a candidate tree is granted.

16. Planting and Management of Registered Blocks

An applicant will be responsible for the selection of candidate tree, the location of the planting, making application for registration, the proper maintenance of a planting and for maintaining the identity of all trees.

An applicant will make arrangements with the owner of the property on which the trees are located so that inspections and collections of propagation material for testing purposes can be done with the owner's consent.

No tree in this programme may be planted within fifteen metres of a non-registered avocado tree or any known host of avocado sunblotch viroid.

Cutting tools used on any tree in this programme shall be either restricted for that purpose or thoroughly cleaned with sodium hypochlorite.

17. Refusal or Cancellation of Registration

Registration may be refused or cancelled for any trees in this programme if:

a) fees prescribed under these regulations are not paid;

b) a tree, or any alternative host tree, within fifteen metres of it is found to be virus infected;

c) a tree is off type;

d) the identity of a tree becomes uncertain or has not been properly maintained;

e) a misrepresentation is made in relation to any matter under this programme; or

f) any other requirement of these regulations has not been met.

18. General Administration for Registered Blocks

Participation in this programme shall be voluntary and may be withdrawn at the option of the applicant. The applicant shall furnish any information requested and shall give his consent to the AAGF or any indexing authority to take propagation material for testing purposes.

Registration under this programme shall not imply any warranty or endorsement on the part of the AAGF, its affiliated bodies or any indexing authority.

Any claim for rights to plant patents or other forms of exclusive control over any tree shall be the prerogative of the applicant and the AAGF shall not by reason of registration under the programme be deemed to be supporting or hindering any such claim.

The AAGF shall prescribe forms of application and certificates of inspection and registration as may be required.

The AAGF shall from time to time, appoint a Registrar to administer all matters pertaining to these regulations and shall determine the period and terms of such appointments. The Registrar will maintain a register of trees in this programme, and record any other matters related to these regulations.

The AAGF shall prescribe a schedule of fees to finance administration, inspections and indexing costs of this programme but may waive payment of fees in special circumstances.

The AAGF shall prepare consecutively numbered labels which shall be used exclusively on trees eligible for registration under these regulations. These labels will be made available to supplying nurserymen selling nuclear or multiplication stock as seed, marcoted rootstocks, seedlings or grafted plants after pre-payment of fees.

An applicant may use an AAGF label as evidence to establish the origin of a candidate tree, providing the label is annotated by the supplying nurseryman with the appropriate registration number of the foundation or nuclear source trees.

An applicant who disagrees with a decision of the AAGF concerning any matter under these regulations may make a written submission to the AAGF for review. The decision of the AAGF will be final.

19. Administrative Forms

Listed below are the administrative forms prescribed under these regulations for the Registration section of ANVAS.

1. AAGF Label

Note: Address for information is:

ANVAS Registrar
PO Box 19
BRISBANE MARKET QLD 4106

2. Form 3 and 3a

Application for registration of a candidate nuclear tree, including a seedling avocado.

3. Form 5

Delivery Docket

4. Form 5a

Application for registration of candidate trees

DEFINITIONS

1. "AAGF" means the Australian Avocado Growers' Federation

2. "virus infected" means infected by a virus or viroid or having symptoms or behaviour characteristic of a virus disease, and "virus infection" shall have a similar meaning

3. "indexing" means testing a plant for virus or viroid infection by grafting tissue from it to avocado seedling indicator plants or by other means approved by the AAGF and "indexed" and "virus-tested" shall have similar meanings

4. "indexing authority" means a plant pathologist or similarly qualified person in charge of indexing at a particular location and whose name is included in the list of indexing authorities maintained by the AAGF

5. "off type" means different from the variety or selection listed on the application for registration

6. "candidate tree" means a tree for which registration under these regulations is being sought

7. "propagation material" means seed, scions, buds and marcots

8. "foundation tree" means a tree held at an Australian Fruit Variety Foundation approved station and "foundation stock" means propagation material derived from foundation trees

9. "nuclear tree" means a tree propagated from foundation stock or a candidate tree that has been registered to serve as a primary source of propagation material under these regulations, and "nuclear stock" means propagation material derived from nuclear trees

10. "multiplication tree" means a tree propagated from foundation or nuclear stock and registered in the programme

11. "registered tree" means a tree propagated from foundation, nuclear or multiplication stock and registered in the programme

12. "supplying nurseryman" means a nurseryman approved by the AAGF to sell registered trees

13. "registrar" means a person appointed by the AAGF to administer the Scheme

14. "ANVAS facility" means that part of a nursery in which ANVAS avocado plants are propagated, grown and hardened off. It may be the whole or part of a nursery. It may or may not include a dedicated dispatch area.

ANVAS FEE SCHEDULE

Fees will be charged for goods and services provided under the Programme. These will vary from time to time and participants will be advised when they are payable. The present fees (as at 1 September 1996) are as follows:

Nursery Inspection and Labels

1) Nurseries will be charged an annual levy of 10 cents per tree on projected sales of accredited trees for the year.

2) Tree labels will cost 10 cents each.

3) Nursery inspection for technical report, ie potting media samples, hygiene:

   Queensland - $33 per hour including travel

   New South Wales - $60.00

4) Pathologist examination - $30 per 500 g sample.

Virus Testing Procedures

(a) Inspection of Registered Blocks - $1.00 per tree

(b) Inspectors Fee (inc travel) - $33 per hour
(New South Wales - Inspectors fees unknown)

(c) Inspection of Multiplication and Nuclear Blocks - $1.00 per tree

(d) Indexing of Multiplication and Nuclear Trees - $24.00 per tree (**Note 1)

(e) Individual re-testing if required - $120.00 per tree to a maximum of $600 (**Note 1)

(f) Re-registration - $1.00 per tree

**Note 1 - Indexing costs - $120.00 per test. Each test comprises a pool of five samples (trees). In the event that a test returns a positive result the suspect sample is isolated and tested separately.

APPLICATION FOR MEMBERSHIP

AVOCADO NURSERY VOLUNTARY ACCREDITATION SCHEME

Administered by The Australian Avocado Growers' Federation and its affiliated bodies.

NAME OF
NURSERYMAN:.....

POSTAL ADDRESS:.....

LOCATION OF NURSERY IF NOT AS ABOVE:.....

.....

ESTIMATED NURSERY PRODUCTION FOR THE CALENDAR YEAR 19.....

IS.....AVOCADO PLANTS

(This estimate is CONFIDENTIAL information for administration purposes ONLY.)

I hereby apply for accreditation in the Avocado Nursery Voluntary Accreditation Scheme and agree to abide by its Guidelines.

Signed:.....(Nurseryman).....(Date)

10. Plants from nurseries not accredited under ANVAS excluded from ANVAS facility (yes/no).....

11. Suitable dust suppression (yes/no).....

12. Is water obtained from a deep well or bore **NOT** exposed to surface run off? (yes/no) .....

OR

Is water chlorinated and tested to ensure that it contains at least 2 mg/kg available chlorine at time of treatment (yes/no).....

13. Have the following fungicides active against Phytophthora been used in the nursery during the past 12 months: Fos-ject 200 (phosphorous acid), Aliette, Previcur, Ridomil, Terrazole or Le-San (yes/no).....

Nursery.....

Address.....

Signed:.....(Owner/Manager).....Date

B. INSPECTOR'S REPORT

1. Comments on items included in nurseryman's declaration -

.....

.....

.....

2. General tree health (inspect a minimum of 10 containers per bench and comment)

.....

.....

3. General Comments

.....

.....

.....

4. On the basis of this inspection, I recommend that this nursery should:

(i) be accepted for accreditation;

(ii) be rejected for accreditation;

(iii) be advised that accreditation cannot be given until the following modifications have been effected:.....

.....

.....

(iv) be considered for accreditation after further testing.

5. Projected sales of accredited trees for the year .....trees

Technical Officer's Name (Please Print).....

Signed:.....(Technical Officer).....Date

C. PATHOLOGIST'S REPORT

1. Assay for soil-borne pathogens:

Phytophthora.....Pythium.....Verticillium.....

Comments.....

.....

Signed:.....(Pathologist).....Date

ANVAS SAMPLING PROCEDURES

Objective:

1. The objective of the sampling procedure is to provide a representative sample of avocado roots and potting mix for *Phytophthora cinnamomi* testing.

2. The method involves identifying different "lots" to sample from each ANVAS nursery. A "lot" is defined as any number of avocado plants in the nursery which can readily be characterised as similar. For example those which are of different ages, or sources, or which can be identified as different by the nursery man should be classified as different lots with respect to sampling. Each "lot" should then be sampled as a separate sample.

Sample size

3. A representative sample must be taken. At least 10 sub-samples per 1,000 plants should be taken and incorporated into a single sample of 500 grams per sample.

A minimum of 2 samples and a maximum of 5 samples to be taken depending on nursery size.

What to sample

4. Any plants showing stress, wilting, fungal growth (mushrooms or toadstools in the potting mix) or excessive weeds which may lead one to expect incomplete potting mix sterilisation should be included in the sub-sample.

5. In the absence of any obvious stressed, wilting, fungal or weed contaminated plants, a representative sample needs to be taken of the entire "lot", ensuring at least 10 sub-samples per bench. Sub-samples from benches may be combined into one sample providing the contents are defined as the same "lot". A "lot" should be a minimum of 500 grams".

How to sample

6. The most appropriate tool to assist in sampling is an auger drill bit of approximately 20 mm diameter.

7. Plant bags should be sampled by inserting the auger bit at right angles to the side of the bag approximately 30 mm above the base or where one could expect to find roots.

8. The auger bit should be withdrawn, and the adhering potting mix and roots should be tapped into a clean polythene bag.

9. At least 2 such auger bits should be used to ensure that adequate drying of the ethanol occurs between each sub-sample.

10. All plant samples should be clearly identified. This can easily be done by tying red flagging tape around the plants sampled. This method provides an easy trace back in the event that additional testing is necessary.

Additional samples

11. Individual avocado plants showing stress such as wilting, dieback or chlorosis, or likely infection (mushrooms or toadstools growing in the potting mix) should also be sampled and the entire root system sent to the DPI Redlands Research Station for soil borne pathogen testing (phytophthora, pythium and verticillium).

Avoiding cross contamination

12. Extreme care must be taken to avoid cross contamination between sub-samples and samples. It is recommended that 70% ethanol be used to sterilise each auger between sub-samples and lots.

Sample identification

13. Samples should be clearly identified as ANVAS samples, and the following detail should appear both on the polythene bag and a label firmly attached to the polythene bag:

a. Nursery name

b. Shade house ID

c. Bench ID (This could be a combination of several benches)

d. Any other sample ID (This could be a variety or any other sample ID)

e. Date sampled

f. Officer who conducted the sampling

g. Officers phone no and fax no

Request for Diagnosis Form

14. The attached ANVAS Specimen Advice Note should also be completed in duplicate, and the original forwarded with the sample to the DPI Redlands Research Station.

Where to send ANVAS samples

15. Samples should be sent to:

Mr Keith Bodman
Extension Horticulturist
QDPI
Redlands Research Station
P O Box 327
CLEVELAND QLD 4163

Records to be maintained

16. Copies of the request together with all results and any other correspondence should be maintained on an ANVAS file.

E.N. Gall
Senior District Inspector
Plant Health Regulation
South East Region

Tel: (074) 412 211
Fax: (074) 412 235

01 September 1996

Mr Keith Bodman
QDPI
Redlands Research Station
P O Box 327
CLEVELAND QLD 4163

Reply to: .....
(Please print)
.....

.....

ANVAS SPECIMEN ADVICE NOTE

HOST/VARIETY: Avocado potting mix/root samples DATE: ___/___/___

SYMPTOMS INCLUDING SEVERITY:

.....

NURSERY NAME: .....

NURSERY ADDRESS: .....

DISTRICT: .....

COLLECTORS COMMENTS ON DISEASE OCCURRENCE:

.....
.....

ECONOMIC LOSS AND/OR IMPORTANCE: ANVAS sample.

INFORMATION REQUIRED: Assay for soil borne pathogens.

Signature of inspector: .....
Date received: ___/___/___ Date replied: ___/___/___
REPLY: Nursery Name: .....

Shade House ID	Bench ID	Sample ID	Result

Signature of Plant Pathologist: .....

.....