

Horticulture Innovation Australia

Final Report

Desktop analysis and literature review of chestnut rot

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Summary

Chestnuts are a rapidly growing industry in Australia, with production predicted to increase by 25% over the next five years. Chestnut rot is a significant problem facing the Australian chestnut industry. Symptoms of the disease occur as brown lesions on the kernel of the chestnut fruit. Surveys of orchards and markets in south-eastern Australia have recorded incidence of up to 72%. Access to pest and disease technology was identified as one of the key constraints to future industry growth in the Chestnut Industry Strategic Plan. The key priorities identified by the Australian chestnut industry include elucidation of the disease management methods available to chestnut growers, and analysis of the relative costs and benefits of these methods. The current gaps in knowledge for chestnut rot include the elucidation of disease management methods and the cost-benefit analyses of these methods. Laboratory and field trials are needed to establish if treatments such as burr and twig/branch removal, groundcovers, mulches, application of urea, fungicides and biological controls decrease the incidence of chestnut rot. The key to reducing chestnut rot is a disruption of the infection process. Infection of chestnut flowers and vegetative tissues by ascospores of *G. smithogilvyi* during the flowering period has been found as central to disease development. Therefore blocking the movement of the ascospores from the infected burrs and twigs/branches on the orchard floor up to the flowering trees is critical. Cultural management methods include the removal of burrs and twigs/branches from the orchard floor, mulching of burrs using a wood chipper, growing thick ground covers in the lead up and during the flowering period in December, application of mulches and organic amendments on top of dead burrs and twigs/branches. Chemical methods include application of urea on burrs and twigs to enhance breakdown and alter the Carbon/Nitrogen ratio to reduce fungal sporulation, and spraying chestnut flowers with fungicides to prevent infection by ascospores or reduce fungal growth after infection. Spraying flowers with fungicides needs to be tested before large-scale use as it may have deleterious effects on pollination and fruit set or cause phytotoxicity. Biological methods include spraying burrs and twigs with bio-controls such as *Trichoderma* spp. and *Gliocladium virens*. The disease management methods that are selected by an individual grower depend on many factors including the size of the orchard, the time a grower has to allocate to disease prevention and control, the financial capacity to purchase or hire machinery/casual labour to remove dead chestnut material from the orchard floor. This includes if the appropriate hire equipment is locally available, the capacity of the grower to use fungicides as a method of disease control (organic growers cannot use the majority of fungicides). Additionally, fungicides may become ineffective over time. It is likely best to use an integrated approach with a combination of methods incorporating the understanding of the infection process, host-pathogen interaction and disease cycle in combination with the available time and funding of the grower. An example of cost-effective low input level for control is to grow a thick groundcover from Oct-Nov in the lead up and during flowering in December, a medium input level example is to remove burr and twig/branch material from the orchard floor by manual raking, then grow a thick groundcover in the lead up to flowering. An example of the high input level is remove burr and twig/branch material from the orchard floor, either mechanically or by manual raking, apply urea to remaining burrs and branches/twigs on the orchard floor, grow a groundcover as in the lower input methods, then apply a protectant fungicide in the lead up to and/or during the flowering period (once the chemical type, effectiveness, timing, and application rates are established). The application of the discussed orchard management strategies will help reduce the current levels of chestnut rot incidence from up to 72%, down to the Australian agent and retailer threshold of 0-1%.

Keywords

Castanea, disease control, disease management, fungal pathogen.

Introduction

Chestnuts are a rapidly growing industry in Australia, with production predicted to increase by 25% over the next five years (CISP 2011). Chestnut rot is a significant problem facing the Australian chestnut industry. Symptoms of the disease occur as brown lesions on the kernel of the chestnut fruit. The disease usually occurs post-harvest and during storage, and is often not externally visible, providing a challenge for both growers and consumers. Australian agent and retailer thresholds for the disease are 0-1% (Rinaudo et al 2009), however surveys of orchards and markets in south-eastern Australia have recorded incidence of up to 72% (Anderson 1993; Shuttleworth 2013; Shuttleworth et al. 2012a). Estimates of losses to the disease in 2010 were \$5.2M (Shuttleworth 2013; Shuttleworth et al. 2012a). Access to pest and disease technology was identified as one of the key constraints to future industry growth in the Chestnut Industry Strategic Plan (CISP 2011).

Internationally, research has concentrated on developing post-harvest treatments to control chestnut rot. These include the use of cold storage in Greece, Italy, and Switzerland (Mencarelli 2001, Jermini et al. 2006, Vekiari et al. 2007), controlled atmosphere storage in Italy (Mencarelli 2001), hot-water treatments in Greece, Italy, Switzerland and the USA (Mignani & Vercesi 2003, Panagou et al. 2005, Rieger 2005, Jermini et al. 2006). In China, Greece, Italy, New Zealand and the USA, fungicide dips such as iodine, sodium hypochlorite, natamycin, paracetic acid and other organic acids are reported (Paglietta & Bounous 1979, Giacalone & Bounous 1993, Mencarelli 2001, Kader 2002, Mignani & Vercesi 2003, Panagou et al. 2005, Klinac 2006, Yang et al. 2006, Donis-Gonzalez 2008, Donis-Gonzalez et al. 2010). Several fungicides are registered in the USA for the control of various 'rots' in chestnuts including Propiconazole, Tebuconazole, Fluopyram + Tebuconazole, Fluopyram, and Boscalid + Pyraclostrobin (Peter Dal Santo, pers comm.). Biological treatments include *Bacillus subtilis* and *Trichoderma* spp. However, there are no published efficacy data for any of these treatments. Additionally, these fungicides and biologicals are not currently registered or approved for use in Australia. Registration would require a review of safety as well as efficacy, including and evaluation of the optimum timing and application rate, first in the laboratory, and then in field trials. Cost-benefit analyses have not been previously completed regarding any of these treatments.

Priorities of the Australian chestnut industry regarding chestnut rot

The key priorities identified by the Australian chestnut industry include:

- Elucidation of the disease management methods available to chestnut growers
- Analysis of the relative costs and benefits of these methods

Lessons from other pathogens and other tree crops

Advice has been sought from plant pathologist Dr. Olufemi Akinsanmi, and Professor André Drenth at the University of Queensland, who have developed management recommendations for husk spot of macadamia and *Alternaria* leaf blotch of apple. They report that orchard sanitation, such as the removal of residues that serve as a source of inoculum, is an effective cultural practice for both husk spot and *Alternaria* leaf blotch. With macadamia, they recommend integrated management combining cultural practices - removal of husk residue from the tree canopy – with strategically timed fungicide spray applications at 2-4 weeks interval (max 4 sprays). In apples they recommend post-harvest or pre-bud burst orchard clean up, fungicide spray applications to remove fungal structures in the tree canopy, breakdown of leaf residue using urea or mulch under the trees. A systematic approach is taken, which includes the understanding of the infection process, host-pathogen interaction and disease cycle, to determine which fungicides to use in field trials. First, they screen different fungicides at different concentrations against the pathogen in-vitro in the lab. Then any potential effective fungicides are selected for field trials. Based on their experience, they suggest investigations for chestnut rot management should include:

- Prevention of infection through removal/rapid breakdown of the main source of ascospores (burrs and twigs/branches)
- Timing spray applications to protect flowers from infection
- Spray applications to eradicate possible infection of burrs

Fungicides recommended for husk spot of macadamia include Carbendazim + copper, and for *Alternaria* leaf blotch they recommend Pyraclostrobin or Difenoconazole + copper. Due to restrictions or potential withdrawal of certain fungicides, the choice of fungicide is critical for the Australian chestnut industry.

Dr Oscar Villalta from the Victorian Department of Environment and Primary Industries (DEPI) has also been contacted regarding control measures used for brown rot of stonefruit. He said the main control measures used are cultural – growers remove mummified fruit and prune out cankers to reduce overwintering inoculum. However, these alone do not remove all of the inoculum, especially removal of wood cankers, as excessive pruning affects yield. Additionally Oscar said they are focusing on enhancing disease predictive tools to improve fungicide use and disease control. They are also investigating antagonistic biological control agents or nutrient based products to clean up the orchard but since the pathogen is not systemic, they do not recommend fungicide trunk injections. Details of the types of fungicides, timing and rates of application have not been provided at this stage.

Biological controls and antagonistic fungi such as *Gliocladium virens* and *Trichoderma* spp. (*T.*

harzianum, *T. parceramosum*, *T. uiride*) have been found to be antagonistic to the growth of *Cryphonectria parasitica* (the chestnut blight pathogen) both in chestnut twig inoculation experiments and in culture (Arisan-Atac et al. 1995), and to *Phytophthora cinnamomi* (a cause of ink disease, root rot, collar rot and bark-canker of chestnut) and *P. citricola* in culture (Chambers and Scott 1995). There is scope to test the effectiveness of these biocontrol agents on *G. smithogilvyi* both in the field and the laboratory. *Trichoderma* based products are available in Australia including 'Tri-D25' which is a mix of *Trichoderma harzianum* and *Trichoderma koningii* (Zadco 2011). The presence and activity of naturally occurring microbial antagonists could also be monitored following cultural interventions such as removal of burrs and twigs, mulching/addition of organic amendments on top of burrs and twigs, and groundcover establishment. The aim of these methods is to reduce the source of inoculum in the orchard through:

- physically remove the inoculum source from the orchard
- physically block the movement of ascospores that do survive (mulches/ground covers)
- biological: application of biological controls on dead burrs and twigs
- chemical: application of protectant fungicides to trees, and urea to dead burrs and twigs on the orchard floor

There is likely to be variation in the capacity of each control method to reduce disease incidence. The integration of several methods to reduce inoculum is likely to be more effective than using one method alone. Each method needs to be tested experimentally to determine their effectiveness at reducing incidence. The resources and capacity of each grower also need to be taken in to account when selecting the appropriate control methods.

Current gaps in knowledge

The current gaps in knowledge are directly related to the questions and priorities of the Australian chestnut industry. These include the elucidation of disease management methods and the cost-benefit analyses of these methods. Laboratory and field trials are needed to establish if treatments such as burr and twig/branch removal, groundcovers, mulches, application of urea, fungicides and biological controls decrease the incidence of chestnut rot. Peter Dal Santo, HAL's former pesticide minor use co-coordinator, confirmed that there are no fungicides registered in Australia for chestnut rot, and there are currently very few fungicides available via permit to the chestnut industry. Penthiopyrad (Fontelis) is currently

registered for almonds, chestnuts & filbert (hazelnuts) for brown rot (blossum blight) (*Monilinia* spp). Table 1 shows the fungicides currently permitted for use in Australia on chestnuts.

Table 1. Examples of fungicides currently permitted for use in Australia by the Australian Pesticides and Vet Medicines Authority (APVMA). Accessed at <https://portal.apvma.gov.au/permits>

Permit ID	Description	Date Issued	Expiry Date
PER11979	Foli-R-Fos (phosphorous acid) / Chestnuts / Phomopsis nut rot	22-Jun-10	30-Jun-15
PER12507	Tsunami or Adoxysan / Chestnuts / Surface moulds	16-Mar-11	30-Jun-16
PER13273	Copper present as Cupric hydroxide / Chestnuts / Chestnut Blight	01-Apr-12	31-Mar-17
PER13375	Scholar Fungicide (fludioxonil) / Chestnuts / Suppression of surface moulds	24-Dec-12	31-Oct-17
PER13640	Sodium Hypochlorite / Chestnut / Surface Moulds	01-Nov-12	31-Oct-17

Previous research completed with fungicides on chestnut in Australia

Washington et al. (1998) investigated the use of chemical controls on the incidence of chestnut rot in Australia. They found when fungicides were applied to cultures of *Phomopsis castanea* (now considered *Gnomoniopsis smithogilvyi*) *in-vitro* that benomyl, imazalil, prochloraz and propiconazole were most effective. Iprodione was found to be less effective, while chlorothalonil and phosphorous acid were ineffective. In field trials, benomyl, or phosphorous acid were applied to trees during the growing season and significantly reduced incidence during cold storage in 2 out of 5 trials, or 1 out of 3 trials respectively. In one trial, incidence was reduced from 42.6% in unsprayed trees to 23% in chestnuts from trees sprayed with benomyl or 23.6% in trees sprayed with phosphorous acid. Prochloraz sprays were not effective. Trunk injection with imazalil or phosphorous acid at flowering in December did not control the disease, however in one trial, phosphorous acid reduced chestnut rot levels in stored nuts from 51.1% in untreated trees to 41.8%. Imazalil treatments caused leaf scorching and in some cases limb death above the point of injection. Slight leaf scorching was reported associated with some phosphorous acid injections. Trunk injection has additional concerns such as the infection of the injection site by other pathogens, such as that observed with the chestnut blight incursion in North-East Victoria in 2010.

The key to reducing chestnut rot is a disruption of the infection process. Infection of chestnut flowers and vegetative tissues by ascospores of *G. smithogilvyi* during the flowering period was found as central to disease development (Shuttleworth 2013, Smith and Ogilvy 2008, Ogilvy 1998). Therefore blocking the movement of the ascospores from the infected burrs

and twigs/branches on the orchard floor up to the flowering trees is critical.

There are several methods that can be employed in the battle against chestnut rot. Below are some of the methods that can potentially be used with a cost-benefit analysis (CBA) for each method. It is likely best to use an integrated approach with a combination of the methods. For example removing the majority of the burr and twig/branch material from the orchard floor, applying urea or another fungicide to the remaining dead material, growing a thick groundcover in the months leading up to and during flowering in December.

Note: the suggested methods below still need to be scientifically tested to understand their full effectiveness. They are based on knowledge of the infection process but still need to be experimentally confirmed. This is particularly pertinent for methods that may have side-effects on pollination or fruit set, such as application of fungicides to trees

Cultural management methods to block the movement of ascospores include:

- Removal of burrs and twigs/branches from the orchard floor
- Mulching of burrs using a wood chipper
- Growing thick ground covers in the lead up and during the flowering period in December
- Application of mulches and organic amendments on top of dead burrs and twigs/branches

Chemical methods include:

- Application of chemicals e.g. urea on burrs and twigs to enhance breakdown and alter the Carbon/Nitrogen ratio to reduce fungal sporulation
- Spraying chestnut flowers with fungicides to prevent infection by ascospores or reduce fungal growth after an infection (this method needs to be tested before large scale use as it may have deleterious effects on pollination and fruit set)

Biological methods include:

- Spraying burrs and twigs with bio-controls such as *Trichoderma harzianum*, *Trichoderma koningii* (Zadco 2011) and *Gliocladium virens* (Arisan-Atac et al. 1995)

The desktop analysis and literature review will enable the Australian chestnut growers to elucidate the key methods for management and control of the disease, and to drive the future direction of chestnut rot research in Australia. The application of additional orchard management strategies will help reduce the current levels of chestnut rot incidence from up to 72%, down to the Australian agent and retailer threshold of 0-1%.

Methodology

A cost-benefit analysis (CBA) of the various control methods that could be used by growers to reduce the incidence of chestnut rot are provided. The CBA was based on a model 10 ha commercial orchard with 1,000 trees, trees being 10 years of age, with a tree spacing of 10 metres, 100 trees per hectare. Ten year-old trees are reported to produce between 6.5-63 kg per tree (Anagnostakis & Miller 2009). A figure of 30 kg per tree, 3 tonnes per hectare, 30 tonnes per whole orchard was selected. A price of \$2.50 per kg was also selected based on the 2014 price received by growers for Decoppi Marone in Victoria. Therefore the wholesale price for 30 tonnes of chestnuts is \$75,000. Based on the findings of Shuttleworth et al. (2012) up to 72% incidence was found at Australian orchards. This equates to potential losses up to \$54,000 per year for the example orchard. The orchard with 72% incidence in Shuttleworth et al. (2012) did not remove burrs from the orchard floor and did not apply any type of ascospore blocking strategy. The Australian agent and retailer incidence threshold of 1% is used in the analyses. Median incidence values are also included for reference. The CBA for each method is calculated in Australian dollars (AUD).

Cultural method - Raking burrs and twigs manually

Several of the NSW and VIC chestnut growers surveyed in the 2008/09 seasons had very stringent orchard hygiene practices and removed burrs and twigs from the orchard thoroughly. These orchards often had lower incidence than those that did not remove burrs. Table 2 shows the costs of burr and twig removal via manual raking. Labour calculated based on the MA000028 *Horticulture Award 2010* minimum wage [which incorporates all amendments up to and including 27 June 2014 (variation PR551831) (<https://extranet.deewr.gov.au>)]. For an adult range from classification level 1 = \$16.37 per hour to level 5 = \$19.07 per hour. Level 3 was selected for this analysis as a median value. One person is estimated to rake and dispose of burrs under a single tree in 15 mins (4 trees per hour).

Advantages

- No cost required for purchasing or hiring mechanical equipment
- Cheaper in the short term than purchasing a specialised mechanical harvester
- Contributes to the local economy through employing people to complete the job
- The surface of chestnuts will likely be shinier and less damaged compared to mechanical harvesting

Disadvantages

- May not be as cost effective long term as using a mechanical harvester to remove burrs
- Availability of labour may be inconsistent

Table 2. Cost of burr and twig removal by manual raking.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment 36%: median incidence 72%: high incidence	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$135,000 \$270,000
Losses with treatment 1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total Income without treatment 36%: median incidence 72%: high incidence	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$240,000 \$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment) Casual labour \$17.33 per hr @ 4 trees per hour, whole orchard = 250 hrs. with 1% incidence	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$371,250- \$21,662.50= \$349,587.50

Cultural method - Mechanical removal of burrs and twigs

There are several different types of chestnut harvesting machines including 'sweeper' types (Fig 1a) and vacuum types (Fig. 1b). Machinery could be purchased or hired, depending on the financial position of the grower, the size of the orchard, and availability of machinery for hire. A costing of the purchase or hire of mechanical chestnut harvesters is provided in tables 3 & 4.



a
Figure 1. Examples of specialised chestnut harvesters that could also remove burrs and twigs/branches from the orchard floor. **a.** FACMA® C160S model mechanical harvester. **b.** AMB Rousset Vacuum Harvester RA7.

Advantages

- Can use harvester to remove burrs while simultaneously harvesting chestnuts
- No need to hire casual labour for harvesting or burr/twig removal if using model such as the FACMA
- If the machine is operated by the grower as a harvester and a burr and twig remover, there are reduced labour costs involved for harvesting and for burr and twig removal
- Potential to hire chestnut harvesters would significantly reduce cost compared to purchasing one

Disadvantages

- If purchasing, the expense of the initial purchase
- May only be financially viable for larger scale commercial orchards. The Missouri Nut Growers Association (2014) reported that the cost of purchasing a mechanical harvester is only offset with production areas above 50 acres (20 hectares). This recommendation is outside of the size of the 10 hectare model orchard in this study
- Need good canopy management as low hanging limbs on trees will hinder movement of the machinery
- Mainly suited to firm, flat, smooth and debris free orchards. If the orchard is hilly and steep, the use of these machines may not be an option
- May leave a proportion of the burrs and twigs behind, may not be as effective as hand raking. This could be important if inoculum is incompletely eradicated.
- The additional costs of machine maintenance need to be considered

Table 3. Cost of mechanical removal of burrs with purchase of chestnut harvesting machinery in year 1. Based on FACMA®, Italy 2014. Price ranges used with permission.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36% median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72% high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income without treatment						
36% median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72% high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of machine model C160S \$37,858 with 1% incidence	\$74,250- \$37,858= \$36,392	\$74,250	\$74,250	\$74,250	\$74,250	\$333,392
Cost of machine model SEMEK 1000 \$92,246 with 1% incidence	\$74,250-\$92,246= \$-17,996	\$74,250	\$74,250	\$74,250	\$74,250	\$279,004

Table 4. Cost of mechanical removal of burrs with hired harvesting machinery. Harvest period was classed as 3 months. As a guide, hire fees are based on Bindoon Tractors Pty Ltd <http://www.bindoontractors.com.au/hire/hire.phtml> and Palmer Hire Ballarat, at a reduced rate for long term hire (\$1000 per week). Please note Bindoon Tractors Pty Ltd and Palmer Hire Ballarat do not offer chestnut harvesting machinery, and have only been used as a price guide.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36% median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72% high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income without treatment						
36% median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72% high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of machine hire for 12 weeks 12*\$1000=\$12000 with 1% incidence	\$74,250-\$12,000= \$62,250	\$74,250- \$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$371,250- \$60,000= \$311,250

Cultural method - Mulches and organic amendments

The application of mulch on top of the dead burrs and twigs/branches provides a physical barrier to prevent the movement of ascospores up to chestnut flowers. It can also stimulate soil microbial health and nutrient availability in the soil depending on the type of mulch or amendment used. The costs of mulch as a groundcover with hand raking are listed below (Table 5). The amount needed to cover most of the 10 ha orchard with a 10cm high layer is 1000 m³.

Advantages

- Adds nutrients to soil during decomposition, reducing need for additional chemical fertilisers
- Reduces erosion
- Improves soil health
- Creates a significant physical barrier to ascospores

Disadvantages

- High cost of mulch and labour
- Mulch may be vulnerable to being blown away by strong winds
- Risk that mulch could introduce weed seeds into the orchard, depending on the quality of the mulch and how well decomposed it is

Table 5. Costs of applying 10cm thick cover of mulch on top of burrs employing casual labour prices sourced from www.mulchnet.com

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total Income without treatment						
36%: median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72%: high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (income minus cost of treatment)						
Casual labour \$17.33 per hr @ 4 trees per hour, whole orchard = 250 hours.	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$21 662.50
Cost of 1000m ³ of mulch @ \$15/m ³	\$15 000	\$15 000	\$15 000	\$15 000	\$15 000	\$75 000
with 1% incidence	\$74,250-\$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$371,250- \$96,662.50= \$274,587.50

Cultural method - Growing a ground cover in the lead up to and during the flowering period

A thick ground cover can be encouraged naturally through a reduction or elimination of mowing and herbicide use. Water, if available, could be applied to the orchard floor in Oct-Nov to encourage the growth of the ground cover, however this could also have the added effect of encouraging sporulation of *G. smithogilvyi*. Inducing sporulation before flowering may be useful if there is a depletion of the ascospore source and decreased production of fresh ascospores. Adding water to infected burrs would also depend on the amount of rainfall in this period, and the availability of irrigation water. Seeds could also be sown under trees to encourage the groundcover. If leguminous species were grown, they would provide an additional source of nitrogen to the soil through nitrogen fixation. The purchase of seeds and sowing would be an additional cost. A costing table is not included for this method.

Advantages

- Can be used with little inputs, especially in higher rainfall areas or during high rainfall years
- Viable for smaller and larger orchards

Disadvantages

- May not be a viable option for growers in lower rainfall regions such as parts of South-Eastern NSW, or during dry years
- May encourage the establishment and growth of weed

Chemical method - Application of Pyraclostrobin as an example

Phosphorus acid is the only chemical registered for use in Australia on chestnuts for pre-harvest chestnut rot diseases for '*Phomopsis* nut rot' (considered in this review as the same disease as chestnut rot caused by *G. smithogilvyi*). Chemicals registered for use on chestnut trees in Australia are for chestnut blight (copper) or post-harvest treatments such as sodium hypochlorite, fludioxonil (phenylpyrrole group), Tsunami/Adoxysan (Hydrogen peroxide/Peroxyacetic acid). As an example Table 6 shows the cost of applying Pyraclostrobin as a potential spray to chestnut trees if and once it is registered. Pyraclostrobin is a fungicide recommended for *Alternaria* leaf blotch and is currently not registered in Australia for chestnuts. Any new fungicides will need to be registered before they can be used.

Advantages

- Lower cost of fungicide compared to purchasing mulch
- Can be applied by the grower, no need to hire casual labour for application

Disadvantages

- Effectiveness of most fungicides on reducing chestnut rot are not currently known. Washington et al. (1998) showed benomyl, and phosphorous acid had a significant effect on reducing 'Phomopsis nut rot' incidence (considered the same disease as chestnut rot caused by *G. smithogilvyi*) when applied to chestnut trees in the field. The effects of Phosphorous acid and benomyl on *G. smithogilvyi* need to be determined, as *Phomopsis* (now *Diaporthe* after the "one fungus, one name" nomenclature changes) and *Gnomoniopsis* both occur on *Castanea* spp. (Udayanga et al. 2011) and have both been reported as causal agents of chestnut rot
- Fungicides need to be registered and maximum residue limits established before they can be used on chestnut
- The potential effects on pollination and fruit development of the trees need to be determined
- Fungicides can be toxic to the person applying them, depending on the chemical, and may require the purchase of specialised protective clothing and equipment
- They can have deleterious effects on the environment and wildlife
- *G. smithogilvyi* may eventually develop resistance to fungicides
- Attention needs to be paid to chemical residues that may be found in chestnuts after fungicides are applied
- There can be strong consumer food safety concerns with the use of fungicides

Table 6. Cost of using the fungicide Pyraclostrobin as an example for the control of chestnut rot. It is assumed the grower has equipment to apply sprays. If not, there will be an additional cost with buying or hiring relevant machinery. Pyraclostrobin is a fungicide used for *Alternaria* leaf blotch of apple, and is not registered for use on chestnut. Prices of fungicide were sourced from www.Amazon.com and include the cost of product shipping.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment (depends on effectiveness of fungicide used)						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of Pyraclostrobin 3 L, 40ml/100L application rate. 3 L of the working solution is used per tree in this example. For 1000 trees 132ml/application is needed. Applied 4 times at weekly intervals.	\$559.70	\$0	\$559.70	\$0	\$559.70	\$1679.10
with 1% incidence	\$74,250- \$559.70= \$73,690.30	\$74,250	\$74,250- \$559.70= \$73,690.30	\$74,250	\$74,250- \$559.70= \$73,690.30	\$371,250- \$1679.10= \$369,570.90

Applying urea on burrs and twigs to reduce sporulation of *Gnomoniopsis smithogilvyi*

In apple crops, urea is applied in late autumn to dead plant material harbouring resting pathogen propagules on the orchard floor, to assist in their breakdown and to prevent sporulation of *Alternaria* leaf blotch of apple. Given the similar disease cycle, urea application to chestnut burrs and leaf litter in late autumn may disrupt the sporulation of *G. smithogilvyi*. Again the assumption is made that the grower has access to spray equipment. The cost of using urea is presented in Table 7. Like the other chemical treatments optimum timing and rate of application need to be experimentally determined. It is currently unknown what effect using urea on burrs and twigs on the orchard floor will have on incidence.

Advantages

- Cost of urea is lower compared to some of the alternative methods
- Provides a source of nitrogen
- Easy to apply if spray equipment is already owned by the grower

Disadvantages

- Urea acidifies the soil, so lime will likely need to be applied if it becomes too acidic, adding a further expense
- Regular pH testing of soil will be needed

Biological method - Spraying infected burrs and twigs with bio-controls

Biological controls such as such *Gliocladium virens* and *Trichoderma* spp. are reported and marketed as effective against plant diseases including chestnut blight (Arisan-Atac et al. 1995, Zadco 2011). Like the chemical controls, the effectiveness of biocontrols on *G. smithogilvyi* needs to be determined experimentally including rate and timing of applications. Table 8 shows the costing of using two applications per year to the model orchard, and most importantly total income from chestnuts when the incidence is reduced to 1%.

Advantages

- Marketed as safe to humans, animals, and plants
- Encourages diversity of microbial biomass
- Developers claim the product competes with pathogens and results in healthier plants.

Disadvantages

- Effectiveness of these biocontrols on *G. smithogilvyi* is currently unknown
- Higher cost than chemical methods

Table 7. Cost of applying urea to dead burrs and twigs on the orchard floor. Price of urea and application rate was sourced from Cooley et al. (2014).

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3750
Total Income without treatment						
36%: median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72%: high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of feed grade urea 46% nitrogen (mix a 5% solution in water) to cover 10 000m ² land area. \$49.4/ha x 2 applications/year	\$988.00	\$988.00	\$988.00	\$988.00	\$988.00	\$4940.00
with 1% incidence	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$371,250- \$4940= \$366,310

Table 8. Cost of using TRI-D25 leaf and soil inoculant (*Trichoderma harzianum* and *Trichoderma koningii*, Zadco 2011).

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3750
Total Income without treatment						
36%: median incidence	\$96,000	\$96,000	\$96,000	\$96,000	\$96,000	\$480,000
72%: high incidence	\$42,000	\$42,000	\$42,000	\$42,000	\$42,000	\$210,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
TRI-D25 (recommended rate is 2kg/ha). Amount used for the model orchard is 2 applications of 2kg.	\$5700.40	\$5700.40	\$5700.40	\$5700.40	\$5700.40	\$28 502
with 1% incidence	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$742,500- \$28,502= \$713,998

Outputs

The key output of this project is the desktop analysis and literature review. This document will enable the Australian chestnut growers and HIA to elucidate the key methods for management and control of the disease, and to drive the future direction of chestnut rot research in Australia. The application of additional orchard management strategies will help reduce the current levels of chestnut rot incidence from up to 72%, down to the Australian agent and retailer threshold of 0-1%. This document is a foundation for future field and laboratory experiments testing the various management methods.

Outcomes

As this is a desktop analysis and literature review, the economic, social and environmental impacts will be fully realised after the completion of experiments testing the various management methods. The final outcome will be the reduction of current levels of chestnut rot incidence from up to 72%, down to the Australian agent and retailer threshold of 0-1%.

Evaluation and Discussion

As this is a desktop analysis and literature review the effectiveness of the project will come later when field and laboratory experiments are completed testing the control methods. Feedback about the review will come after the chestnut growers read and consider the various ideas and decide how they want to proceed with further research projects. Future experimentally based projects that result from the current review will be invaluable to reducing the incidence of chestnut rot.

Recommendations

The disease management methods that are selected by an individual grower to reduce chestnut rot incidence depend on many factors including:

- The size of the orchard
- The time a grower has to allocate to disease prevention and control
- The financial capacity to purchase or hire machinery and casual labour to remove dead chestnut material from the orchard floor. This includes if the appropriate hire equipment is locally available
- Capacity of the grower to use fungicides as a method of disease control. Organic growers cannot use the majority of fungicides. Additionally, fungicides may become ineffective over time. Fungicides do seem to be one of the most cost effective options – if they work.

It is likely best to use an integrated approach with a combination of methods incorporating the understanding of the infection process, host-pathogen interaction and disease cycle in combination with the available time and funding of the grower. The suggested cost-effective integrated disease management options for reducing incidence are in table 9.

Table 9. Cost-effective options for reducing incidence.

Option	Input level	Activity
1	Low	Grow a thick groundcover from Oct-Nov in the lead up and during flowering in December
2	Medium	Remove burr and twig/branch material from the orchard floor by manual raking Grow a thick groundcover as described above
3	High	Remove burr and twig/branch material from the orchard floor, either mechanically or by manual raking Apply urea to remaining burrs and branches/twigs on the orchard floor Grow a thick groundcover as described above Apply a protectant fungicide in the lead up to and/or during the flowering period (once the chemical type, effectiveness, timing, and application rates are established)

The costing of option 1 will be negligible unless water and seed is applied to the orchard floor to encourage the growth of the groundcover. This will depend on the existing groundcover and the rainfall at each orchard. Option 2 is likely to be similar to the manual removal of burrs and branches/twigs ie. \$4332.50 per year (Table 2). The high input option 3 would be \$5880.20/year (Table 10). These are in comparison to the potential losses of \$54,000/year for the model orchard, using the 72% chestnut rot incidence figure.

Research priorities

The various control methods need to be tested experimentally, independently and in combinations, for effectiveness in the field and in the laboratory when possible, to determine their effects on reducing incidence. This will help elucidate if they are methods growers should use or not. This information will also enable the development of a series of Low/Medium/High management level options for farmers to adopt according to their individual circumstances.

The experiments that should be conducted and the determination of their effects on incidence the following year include:

- Removing burrs and twigs from the orchard floor vs not removing them
- Growing a ground cover in the months leading up to flowering vs not
- Testing the application of mulch over burrs and twigs vs not
- Observe the effects of applying urea on dead burrs and twigs on the orchard floor
- Determine the effect of spraying developing flowers with protectant fungicides
 - Elucidate appropriate spray concentration and timing. Fungicides tested should be those that have potential to be registered for chestnuts in Australia. Examples include Pyraclostrobin or Difenconazole + copper, and phosphoric acid.
 - Observe any deleterious effects of fungicides on pollination and nut set and general phytotoxicity on trees
 - Test effects of applying fungicides to cultures of *G. smithogilvyi* in the laboratory
- Determine the effect of spraying biocontrols on dead burrs and twigs on the orchard floor and elucidating which biocontrol is most effective at reducing incidence

Scientific Refereed Publications

No publications are attributed to this project.

Intellectual Property/Commercialisation

No commercial IP generated in this project.

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Appendices

Full length report. Milestone 190.
HIA Project CH13002.
Desktop Analysis and Literature Review of Chestnut Rot
Dr. Lucas Shuttleworth & Prof. David Guest
The University of Sydney, Faculty of Agriculture & Environment

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1. Background

Chestnuts are a rapidly growing industry in Australia, with production predicted to increase by 25% over the next five years (CISP 2011). Production in 2010 was 2,000 metric tonnes and the estimated value of the industry was \$13 million (Shuttleworth 2013; Shuttleworth et al. 2012a). Seventy per cent of the national crop is grown in large commercial groves in North-East Victoria e.g. Bright, Myrtleford, Buckland Valley, Harrietville, Beechworth and Stanley. The remaining production comes from the high country of Gippsland and the Macedon Ranges in Victoria, in the Central West, Blue Mountains, Snowy Mountains, South Coast, and Northern Tablelands of NSW, with smaller quantities grown in South Australia, Western Australia and Tasmania (McLaren 1999; Shuttleworth 2013). The main species grown in Australia are *Castanea sativa* and hybrids of *Castanea crenata* x *C. sativa* (Shuttleworth 2013; Shuttleworth et al. 2012a, 2012b).

Chestnut rot is a significant problem facing the Australian chestnut industry. Symptoms of the disease occur as brown lesions on the kernel of the chestnut fruit. The disease usually occurs post-harvest and during storage, and is often not externally visible, providing a challenge for both growers and consumers. Australian agent and retailer thresholds for the disease are 0-1% (Rinaudo et al 2009), however surveys of orchards and markets in south-eastern Australia have recorded incidences of up to 72% rot in nuts from individual orchards (Anderson 1993; Shuttleworth 2013; Shuttleworth et al. 2012a). Estimates of losses to the disease in 2010 were \$5.2M (Shuttleworth 2013; Shuttleworth et al. 2012a).

Previous studies had suggested that chestnut rot was caused by the asexual fungus *Phomopsis castanea*, although the mode of infection and disease cycle was found to be exclusively endophytic (Anderson 1993, Washington et al. 1999). More recent work by Shuttleworth et al. (2012a, 2012b) and Shuttleworth (2013) showed that chestnut rot is caused by an infection of chestnut flowers, leaves and stems in summer by ascospores of the fungus *Gnomoniopsis smithogilvyi*. Ascospores were found to be released from dead burrs and twigs/branches on the orchard floor. After infection of the flowers, leaves and stems, the fungus was found to infect asymptotically as an endophyte. The symptoms of chestnut rot develop during fruit maturity and while the chestnuts are in storage.

The PhD thesis of Lucas Shuttleworth, which has been provided to Chestnuts Australia Inc. (CAI), provides detailed information regarding the infection process and potential control methods (see Chapter 1.9, 1.9.3-1.9.5, 7.3). Research in Australia and New Zealand has focused on the pre-harvest prevention and control of chestnut rot (Anderson 1993, Washington et al. 1997, 1998, 1999, Ogilvy 1998, Rinaudo et al. 2009, Osmonalieva et al. 2001, Smith and Ogilvy 2008, Shuttleworth et al. 2012a, 2012b, Shuttleworth 2013), as well as post-harvest treatments, particularly cold storage, controlled atmosphere storage (CALM) and flotation disease grading (Klinac et al. 1999, Morris 2006a, 2006b). The use of infra-red technology to separate rotten chestnuts from healthy ones is also reported in China (Zhou et al. 2011).

Internationally, research has concentrated on developing post-harvest treatments to control chestnut rot. These include the use of cold storage in Greece, Italy, and Switzerland (Mencarelli

2001, Jermini et al. 2006, Vekiari et al. 2007), controlled atmosphere storage in Italy (Mencarelli 2001), hot-water treatments in Greece, Italy, Switzerland and the USA (Mignani & Vercesi 2003, Panagou et al. 2005, Rieger 2005, Jermini et al. 2006). In China, Greece, Italy, New Zealand and the USA, fungicide dips such as iodine, sodium hypochlorite, natamycin, paracetic acid and other organic acids are reported (Paglietta & Bounous 1979, Giacalone & Bounous 1993, Mencarelli 2001, Kader 2002, Mignani & Vercesi 2003, Panagou et al. 2005, Klinac 2006, Yang et al. 2006, Donis-Gonzalez 2008, Donis-Gonzalez et al. 2010). Several fungicides are registered in the USA for the control of various 'rots' in chestnuts including Propiconazole, Tebuconazole, Fluopyram + Tebuconazole, Fluopyram, and Boscalid + Pyraclostrobin (Peter Dal Santo, pers comm.). Biological treatments include *Bacillus subtilis* and *Trichoderma* spp.

However, there are no published efficacy data for any of these treatments. Additionally, these fungicides and biologicals are not currently registered or approved for use in Australia. Registration would require a review of safety as well as efficacy, including an evaluation of the optimum timing and application rate, first in the laboratory, and then in field trials. Cost-benefit analyses have not been previously completed regarding any of these treatments. Access to pest and disease technology was identified as one of the key constraints to future industry growth in the Chestnut Industry Strategic Plan (CISP 2011). The desktop analysis and literature review will enable the Australian chestnut growers to elucidate the key methods for management and control of the disease, and to drive the future direction of chestnut rot research in Australia. The application of additional orchard management strategies will help reduce the current levels of chestnut rot incidence from up to 72%, down to the Australian agent and retailer threshold of 0-1%.

2. Priorities of the Australian chestnut industry regarding chestnut rot

The key priorities identified by the Australian chestnut industry include:

- Elucidation of the disease management methods available to chestnut growers
- Analysis of the relative costs and benefits of these methods

3. Lessons from other pathogens and other tree crops

Advice has been sought from plant pathologist Dr. Olufemi Akinsanmi, and Professor André Drenth at the University of Queensland, who have developed management recommendations for husk spot of macadamia and *Alternaria* leaf blotch of apple. They report that orchard sanitation, such as the removal of residues that serve as a source of inoculum, is an effective cultural practice for both husk spot and *Alternaria* leaf blotch. With macadamia, they recommend integrated management combining cultural practices - removal of husk residue from the tree canopy - with strategically timed fungicide spray applications at 2-4 weeks interval (max 4

sprays). In apples they recommend post-harvest or pre-bud burst orchard clean up, fungicide spray applications to remove fungal structures in the tree canopy, breakdown of leaf residue using urea or mulch under the trees. A systematic approach is taken, which includes the understanding of the infection process, host-pathogen interaction and disease cycle, to determine which fungicides to use in field trials. First, they screen different fungicides at different concentrations against the pathogen in-vitro in the lab. Then any potential effective fungicides are selected for field trials. Based on their experience, they suggest investigations for chestnut rot management should include:

- Prevention of infection through removal/rapid breakdown of the main source of ascospores
- Timing spray applications to protect flowers from infection
- Spray applications to eradicate possible infection of burrs

Fungicides recommended for husk spot of macadamia include Carbendazim + copper, and for *Alternaria* leaf blotch they recommend Pyraclostrobin or Difenconazole + copper. Due to restrictions or potential withdrawal of certain fungicides, the choice of fungicide is critical for the Australian chestnut industry.

Dr Oscar Villalta from the Victorian Department of Environment and Primary Industries (DEPI) has also been contacted regarding control measures used for brown rot of stonefruit. He said the main control measures used are cultural – growers remove mummified fruit and prune out cankers to reduce overwintering inoculum. However, these alone do not remove all of the inoculum, especially removal of wood cankers, as excessive pruning affects yield. Additionally Oscar said they are focusing on enhancing disease predictive tools to improve fungicide use and disease control. They are also investigating antagonistic biological control agents or nutrient based products to clean up the orchard but since the pathogen is not systemic, they do not recommend fungicide trunk injections. Details of the types of fungicides, timing and rates of application have not been provided at this stage.

Biological controls and antagonistic fungi such as *Gliocladium virens* and *Trichoderma* spp. (*T. harzianum*, *T. parceramosum*, *T. uiride*) have been found to be antagonistic to the growth of *Cryphonectria parasitica* (the chestnut blight pathogen) both in chestnut twig inoculation experiments and in culture (Arisan-Atac et al. 1995), and to *Phytophthora cinnamomi* (a cause of ink disease, root rot, collar rot and bark-canker of chestnut) and *P. citricola* in culture (Chambers and Scott 1995). There is scope to test the effectiveness of these biocontrol agents on *G. smithogilvyi* both in the field and the laboratory. *Trichoderma* based products are available in Australia including 'Tri-D25' which is a mix of *Trichoderma harzianum* and *Trichoderma koningii* (Zadco 2011). The presence and activity of naturally occurring microbial antagonists could also be monitored following cultural interventions such as removal of burrs and twigs, mulching/addition of organic amendments on top of burrs and twigs, and groundcover establishment.

The aim of these methods is to reduce the source of inoculum in the orchard through:

- physical removal the inoculum source from the orchard
- block the movement of ascospores that do survive (mulches/ground covers)
- application of biological controls on dead burrs and twigs (biological)
- application of protectant fungicides to trees, and urea to dead burrs and twigs on the orchard floor (chemical)

There is likely to be variation in the capacity of each control method to reduce disease incidence. The integration of several methods to reduce inoculum is likely to be more effective than using one method alone. Each method needs to be tested experimentally to determine their effectiveness at reducing incidence. The resources and capacity of each grower also need to be taken in to account when selecting the appropriate control methods.

4. Current gaps in knowledge

The current gaps in knowledge are directly related to the questions and priorities of the Australian chestnut industry. These include the elucidation of disease management methods and the cost-benefit analyses of these methods. Laboratory and field trials are needed to establish if treatments such as burr and twig/branch removal, groundcovers, mulches, application of urea, fungicides and biological controls decrease the incidence of chestnut rot. Peter Dal Santo, HAL's former pesticide minor use co-coordinator, confirmed that there are no fungicides registered in Australia for chestnut rot, and there are currently very few fungicides available via permit to the chestnut industry. Penthiopyrad (Fontelis) is currently registered for almonds, chestnuts & filbert (hazelnuts) for brown rot (blossum blight) (*Monilinia* spp). Table 1 shows the fungicides currently permitted for use in Australia on chestnuts.

Table 1. Examples of fungicides currently permitted for use in Australia by the Australian Pesticides and Vet Medicines Authority (APVMA). Accessed at <https://portal.apvma.gov.au/permits>

Permit ID	Description	Date Issued	Expiry Date
PER11979	Foli-R-Fos (phosphorous acid) / Chestnuts / Phomopsis nut rot	22-Jun-10	30-Jun-15
PER12507	Tsunami or Adoxysan / Chestnuts / Surface moulds	16-Mar-11	30-Jun-16
PER13273	Copper present as Cupric hydroxide / Chestnuts / Chestnut Blight	01-Apr-12	31-Mar-17
PER13375	Scholar Fungicide (fludioxonil) / Chestnuts / Suppression of surface moulds	24-Dec-12	31-Oct-17
PER13640	Sodium Hypochlorite / Chestnut / Surface Moulds	01-Nov-12	31-Oct-17

5. Previous research completed with fungicides on chestnut in Australia

Washington et al. (1998) investigated the use of chemical controls on the incidence of chestnut rot in Australia. They found when fungicides were applied to cultures of *Phomopsis castanea* (now considered *Gnomoniopsis smithogilvyi*) *in-vitro* that benomyl, imazalil, prochloraz and propiconazole were most effective. Iprodione was found to be less effective, while chlorothalonil and phosphorous acid were ineffective. In field trials, benomyl, or phosphorous acid were applied to trees during the growing season and significantly reduced incidence during cold storage in 2 out of 5 trials, or 1 out of 3 trials respectively. In one trial, incidence was reduced from 42.6% in unsprayed trees to 23% in chestnuts from trees sprayed with benomyl or 23.6% in trees sprayed with phosphorous acid. Prochloraz sprays were not effective. Trunk injection with imazalil or phosphorous acid at flowering in December did not control the disease, however in one trial, phosphorous acid reduced chestnut rot levels in stored nuts from 51.1% in untreated trees to 41.8%. Imazalil treatments caused leaf scorching and in some cases limb death above the point of injection. Slight leaf scorching was reported associated with some phosphorous acid injections. Trunk injection has additional concerns such as the infection of the injection site by other pathogens, such as that observed with the chestnut blight incursion in North-East Victoria in 2010.

The key to reducing chestnut rot is a disruption of the infection process. Infection of chestnut flowers and vegetative tissues by ascospores of *G. smithogilvyi* during the flowering period was found as central to disease development (Shuttleworth 2013, Smith and Ogilvy 2008, Ogilvy 1998). Therefore blocking the movement of the ascospores from the infected burrs and twigs/branches on the orchard floor up to the flowering trees is critical.

There are several methods that can be employed in the battle against chestnut rot. Below are some of the methods that can potentially be used with a cost-benefit analysis (CBA) for each method. It is likely best to use an integrated approach with a combination of the methods. For example removing the majority of the burr and twig/branch material from the orchard floor, applying urea or another fungicide to the remaining dead material, growing a thick groundcover

in the months leading up to and during flowering in December.

Note: the suggested methods below still need to be scientifically tested to understand their full effectiveness. They are based on knowledge of the infection process but still need to be experimentally confirmed. This is particularly pertinent for methods that may have side-effects on pollination or fruit set, such as application of fungicides to trees

Cultural management methods to block the movement of ascospores include:

- Removal of burrs and twigs/branches from the orchard floor
- Mulching of burrs using a wood chipper
- Growing thick ground covers in the lead up and during the flowering period in December
- Application of mulches and organic amendments on top of dead burrs and twigs/branches

Chemical methods include:

- Application of chemicals e.g. urea on burrs and twigs to enhance breakdown and alter the Carbon/Nitrogen ratio to reduce fungal sporulation
- Spraying chestnut flowers with fungicides to prevent infection by ascospores or reduce fungal growth after an infection. (This method needs to be tested before large scale use as it may have deleterious effects on pollination and fruit set)

Biological methods include:

- Spraying burrs and twigs with bio-controls such as *Trichoderma harzianum*, *Trichoderma koningii* (Zadco 2011) and *Gliocladium virens* (Arisan-Atac et al. 1995)

6. Cost-benefit analysis (CBA) of disease management methods

The CBA was based on a model 10 ha commercial orchard with 1,000 trees, trees being 10 years of age, with a tree spacing of 10 metres, 100 trees per hectare. Ten year-old trees are reported to produce between 6.5-63 kg per tree (Anagnostakis & Miller 2009). A figure of 30 kg per tree, 3 tonnes per hectare, 30 tonnes per whole orchard was selected. A price of \$2.50 per kg was also selected based on the 2014 price received by growers for Decoppi Marone in Victoria. Therefore the wholesale price for 30 tonnes of chestnuts is \$75,000. Based on the findings of Shuttleworth et al. (2012) up to 72% incidence was found at Australian orchards. This equates to potential losses up to \$54,000 per year for the example orchard. The orchard with 72% incidence in Shuttleworth et al. (2012) did not remove burrs from the orchard floor and did not apply any type of ascospore blocking strategy. The Australian agent and retailer incidence threshold of 1% is used in the analyses. Median incidence values are also included for reference. The CBA for each method is calculated in Australian dollars (AUD).

6.1.1 Cultural method - Raking burrs and twigs manually

Several of the NSW and VIC chestnut growers surveyed in the 2008/09 seasons had very stringent orchard hygiene practices and removed burrs and twigs from the orchard thoroughly. These orchards often had lower incidence than those that did not remove burrs. Table 2 shows the costs of burr and twig removal via manual raking. Labour calculated based on the MA000028 *Horticulture Award 2010* minimum wage [which incorporates all amendments up to and including 27 June 2014 (variation PR551831) (<https://extranet.deewr.gov.au>)]. For an adult range from classification level 1 = \$16.37 per hour to level 5 = \$19.07 per hour. Level 3 was selected for this analysis as a median value. One person is estimated to rake and dispose of burrs under a single tree in 15 mins (4 trees per hour).

Advantages

- No cost required for purchasing or hiring mechanical equipment
- Cheaper in the short term than purchasing a specialised mechanical harvester
- Contributes to the local economy through employing people to complete the job
- The surface of chestnuts will likely be shinier and less damaged compared to mechanical harvesting

Disadvantages

- May not be as cost effective long term as using a mechanical harvester to remove burrs
- Availability of labour may be inconsistent

Table 2. Cost of burr and twig removal by manual raki

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment 36%: median incidence 72%: high incidence	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$135,000 \$270,000
Losses with treatment 1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total Income without treatment 36%: median incidence 72%: high incidence	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$240,000 \$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment) Casual labour \$17.33 per hr @ 4 trees per hour, whole orchard = 250 hrs. with 1% incidence	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$74,250- \$4332.50= \$70,667.50	\$371,250- \$21,662.50= \$349,587.50

6.1.2 Cultural method - Mechanical removal of burrs and twigs

There are several different types of chestnut harvesting machines including 'sweeper' types (Fig 1a) and vacuum types (Fig. 1b). Machinery could be purchased or hired, depending on the financial position of the grower, the size of the orchard, and availability of machinery for hire. A costing of the purchase or hire of mechanical chestnut harvesters is provided in tables 3 & 4.



a



b

Figure 1. Examples of specialised chestnut harvesters that could also remove burrs and twigs/branches from the orchard floor. **a.** FACMA® C160S model mechanical harvester. **b.** AMB Rousset Vacuum Harvester RA7.

Table 3. Cost of mechanical removal of burrs with purchase of chestnut harvesting machinery in year 1. Based on FACMA®, Italy 2014. Price ranges used with permission.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36% median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72% high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income without treatment						
36% median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72% high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of machine model C160S \$37,858 with 1% incidence	\$74,250- \$37,858= \$36,392	\$74,250	\$74,250	\$74,250	\$74,250	\$333,392
Cost of machine model SEMEK 1000 \$92,246 with 1% incidence	\$74,250-\$92,246= \$-17,996	\$74,250	\$74,250	\$74,250	\$74,250	\$279,004

Table 4. Cost of mechanical removal of burrs with hired harvesting machinery. Harvest period was classed as 3 months. As a guide, hire fees are based on Bindoon Tractors Pty Ltd <http://www.bindoontractors.com.au/hire/hire.phtml> and Palmer Hire Ballarat, at a reduced rate for long term hire (\$1000 per week). Please note Bindoon Tractors Pty Ltd and Palmer Hire Ballarat do not offer chestnut harvesting machinery, and have only been used as a price guide.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36% median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72% high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income without treatment						
36% median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72% high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of machine hire for 12 weeks 12*\$1000=\$12000 with 1% incidence	\$74,250-\$12,000= \$62,250	\$74,250- \$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$74,250-\$12,000= \$62,250	\$371,250- \$60,000= \$311,250

Advantages

- Can use harvester to remove burrs while simultaneously harvesting chestnuts
- No need to hire casual labour for harvesting or burr/twig removal if using model such as the FACMA
- If the machine is operated by the grower as a harvester and a burr and twig remover, there are reduced labour costs involved for harvesting and for burr and twig removal
- Potential to hire chestnut harvesters would significantly reduce cost compared to purchasing one

Disadvantages

- If purchasing, the expense of the initial purchase
- May only be financially viable for larger scale commercial orchards. The Missouri Nut Growers Association (2014) reported that the cost of purchasing a mechanical harvester is only offset with production areas above 50 acres (20 hectares). This recommendation is outside of the size of the 10 hectare model orchard in this study
- Need good canopy management as low hanging limbs on trees will hinder movement of the machinery
- Mainly suited to firm, flat, smooth and debris free orchards. If the orchard is hilly and steep, the use of these machines may not be an option
- May leave a proportion of the burrs and twigs behind, may not be as effective as hand raking. This could be important if inoculum is incompletely eradicated.
- The additional costs of machine maintenance need to be considered

6.1.3 Cultural method - Mulches and organic amendments

The application of mulch on top of the dead burrs and twigs/branches provides a physical barrier to prevent the movement of ascospores up to chestnut flowers. It can also stimulate soil microbial health and nutrient availability in the soil depending on the type of mulch or amendment used. The costs of mulch as a groundcover with hand raking are listed below (Table 5). The amount needed to cover most of the 10 ha orchard with a 10cm high layer is 1000 m³.

Table 5. Costs of applying 10cm thick cover of mulch on top of burrs employing casual labour prices sourced from www.mulchnet.com

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total Income without treatment						
36%: median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72%: high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (income minus cost of treatment)						
Casual labour \$17.33 per hr @ 4 trees per hour, whole orchard = 250 hours.	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$21 662.50
Cost of 1000m ³ of mulch @ \$15/m ³	\$15 000	\$15 000	\$15 000	\$15 000	\$15 000	\$75 000
with 1% incidence	\$74,250-\$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$74,250- \$19,332.50= \$54,917.50	\$371,250- \$96,662.50= \$274,587.50

Advantages

- Adds nutrients to soil during decomposition, reducing need for additional chemical fertilisers
- Reduces erosion
- Improves soil health
- Creates a significant physical barrier to ascospores

Disadvantages

- High cost of mulch and labour
- Mulch may be vulnerable to being blown away by strong winds
- Risk that mulch could introduce weed seeds into the orchard, depending on the quality of the mulch and how well decomposed it is

6.1.4 Cultural method - Growing a ground cover in the lead up to and during the flowering period

A thick ground cover can be encouraged naturally through a reduction or elimination of mowing and herbicide use. Water, if available, could be applied to the orchard floor in Oct-Nov to encourage the growth of the ground cover, however this could also have the added effect of encouraging sporulation of *G. smithogilvyi*. Inducing sporulation before flowering may be useful if there is a depletion of the ascospore source and low production of fresh ascospores. Adding water to infected burrs would also depend on the amount of rainfall in this period, and the availability of irrigation water. Seeds could also be sown under trees to encourage the groundcover. If leguminous species were grown, they would provide an additional source of nitrogen to the soil through nitrogen fixation. The purchase of seeds and sowing would be an additional cost. A costing table is not included for this method.

Advantages

- Can be used with little inputs, especially in higher rainfall areas or during high rainfall years
- Viable for smaller and larger orchards

Disadvantages

- May not be a viable option for growers in lower rainfall regions such as parts of South-Eastern NSW, or during dry years
- May encourage the establishment and growth of weed

6.1.5 Chemical method - Application of Pyraclostrobin as an example

Phosphorous acid is the only chemical registered for use in Australia on chestnuts for pre-harvest chestnut rot diseases for '*Phomopsis* nut rot' (considered in this review as the same disease as chestnut rot caused by *G. smithogilvyi*). Chemicals registered for use on chestnut trees in Australia are for chestnut blight (copper) or post-harvest treatments such as sodium hypochlorite, fludioxonil (phenylpyrrole group), Tsunami/Adoxysan (Hydrogen peroxide/Peroxyacetic acid). As an example Table 6 shows the cost of applying Pyraclostrobin as a potential spray to chestnut trees if and once it is registered. Pyraclostrobin is a fungicide recommended for *Alternaria* leaf blotch and is currently not registered in Australia for chestnuts. Any new fungicides will need to be registered before they can be used.

Advantages

- Lower cost of fungicide compared to purchasing mulch
- Can be applied by the grower, no need to hire casual labour for application

Disadvantages

- Effectiveness of most fungicides on reducing chestnut rot are not currently known. Washington et al. (1998) showed benomyl, and phosphorous acid had a significant effect on reducing '*Phomopsis* nut rot' incidence (considered the same disease as chestnut rot caused by *G. smithogilvyi*) when applied to chestnut trees in the field. The effects of Phosphorous acid and benomyl on *G. smithogilvyi* need to be determined, as *Phomopsis* (now *Diaporthe* after the "one fungus, one name" nomenclature changes) and *Gnomoniopsis* both occur on *Castanea* spp. (Udayanga et al. 2011) and have both been reported as causal agents of chestnut rot
- Fungicides need to be registered and maximum residue limits established before they can be used on chestnut
- The potential effects on pollination and fruit development of the trees need to be determined
- Fungicides can be toxic to the person applying them, depending on the chemical, and may require the purchase of specialised protective clothing and equipment
- They can have deleterious effects on the environment and wildlife
- *G. smithogilvyi* may eventually develop resistance to fungicides
- Attention needs to be paid to chemical residues that may be found in chestnuts after fungicides are applied
- There can be strong consumer food safety concerns with the use of fungicides

Table 6. Cost of using the fungicide Pyraclostrobin as an example for the control of chestnut rot. It is assumed the grower has equipment to apply sprays. If not, there will be an additional cost with buying or hiring relevant machinery. Pyraclostrobin is a fungicide used for *Alternaria* leaf blotch of apple, and is not registered for use on chestnut. Prices of fungicide were sourced from www.Amazon.com and include the cost of product shipping.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment (depends on effectiveness of fungicide used)						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of Pyraclostrobin 3 L, 40ml/100L application rate. 3 L of the working solution is used per tree in this example. For 1000 trees 132ml/application is needed. Applied 4 times at weekly intervals.	\$559.70	\$0	\$559.70	\$0	\$559.70	\$1679.10
with 1% incidence	\$74,250- \$559.70= \$73,690.30	\$74,250	\$74,250- \$559.70= \$73,690.30	\$74,250	\$74,250- \$559.70= \$73,690.30	\$371,250- \$1679.10= \$369,570.90

6.1.6 Applying urea on burrs and twigs to reduce sporulation of *Gnomoniopsis smithogilvyi*

In apple crops, urea is applied in late autumn to dead plant material harbouring resting pathogen propagules on the orchard floor, to assist in their breakdown and to prevent sporulation of *Alternaria* leaf blotch of apple. Given the similar disease cycle, urea application to chestnut burrs and leaf litter in late autumn may disrupt the sporulation of *G. smithogilvyi*. Again the assumption is made that the grower has access to spray equipment. The cost of using urea is presented in Table 7. Like the other chemical treatments optimum timing and rate of application need to be experimentally determined. It is currently unknown what effect using urea on burrs and twigs on the orchard floor will have on incidence.

Advantages

- Cost of urea is lower compared to some of the alternative methods
- Provides a source of nitrogen
- Easy to apply if spray equipment is already owned by the grower

Disadvantages

- Urea acidifies the soil, so lime will likely need to be applied if it becomes too acidic, adding a further expense
- Regular pH testing of soil will be needed

6.1.7 Biological method - Spraying infected burrs and twigs with bio-controls

Biological controls such as such *Gliocladium virens* and *Trichoderma* spp. are reported and marketed as effective against plant diseases including chestnut blight (Arisan-Atac et al. 1995, Zadco 2011). Like the chemical controls, the effectiveness of biocontrols on *G. smithogilvyi* needs to be determined experimentally including rate and timing of applications. Table 8 shows the costing of using two applications per year to the model orchard, and most importantly total income from chestnuts when the incidence is reduced to 1%.

Advantages

- Marketed as safe to humans, animals, and plants
- Encourages diversity of microbial biomass
- Developers claim the product competes with pathogens and results in healthier plants.

Disadvantages

- Effectiveness of these biocontrols on *G. smithogilvyi* is currently unknown
- Higher cost than chemical methods

Table 7. Cost of applying urea to dead burrs and twigs on the orchard floor. Price of urea and application rate was sourced from Cooley et al. (2014).

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3750
Total Income without treatment						
36%: median incidence	\$48,000	\$48,000	\$48,000	\$48,000	\$48,000	\$240,000
72%: high incidence	\$21,000	\$21,000	\$21,000	\$21,000	\$21,000	\$105,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
Cost of feed grade urea 46% nitrogen (mix a 5% solution in water) to cover 10 000m ² land area. \$49.4/ha x 2 applications/year	\$988.00	\$988.00	\$988.00	\$988.00	\$988.00	\$4940.00
with 1% incidence	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$74,250- \$988.00= \$73,262	\$371,250- \$4940= \$366,310

Table 8. Cost of using TRI-D25 leaf and soil inoculant (*Trichoderma harzianum* and *Trichoderma koningii*, Zadco 2011).

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment						
36%: median incidence	\$27,000	\$27,000	\$27,000	\$27,000	\$27,000	\$135,000
72%: high incidence	\$54,000	\$54,000	\$54,000	\$54,000	\$54,000	\$270,000
Losses with treatment						
1% incidence	\$750	\$750	\$750	\$750	\$750	\$3750
Total Income without treatment						
36%: median incidence	\$96,000	\$96,000	\$96,000	\$96,000	\$96,000	\$480,000
72%: high incidence	\$42,000	\$42,000	\$42,000	\$42,000	\$42,000	\$210,000
Total income with treatment (Income with 1% incidence minus cost of treatment)						
TRI-D25 (recommended rate is 2kg/ha). Amount used for the model orchard is 2 applications of 2kg.	\$5700.40	\$5700.40	\$5700.40	\$5700.40	\$5700.40	\$28 502
with 1% incidence	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$148,500- \$5700.40= \$142,799.60	\$742,500- \$28,502= \$713,998

7. General recommendations

The disease management methods that are selected by an individual grower depend on many factors including:

- The size of the orchard
- The time a grower is willing to allocate to disease prevention and control
- The financial capacity to purchase or hire machinery and casual labour to remove dead chestnut material from the orchard floor. This includes if the appropriate hire equipment is locally available
- Willingness of the grower to use fungicides as a method of disease control. Organic growers cannot use the majority of fungicides. Additionally, fungicides may become ineffective over time. Fungicides do seem to be one of the most cost effective options – if they work!

It is likely best to use an integrated approach with a combination of methods incorporating the understanding of the infection process, host-pathogen interaction and disease cycle in combination with the available time and funding of the grower. The suggested cost-effective integrated disease management options for reducing incidence are in table 9.

Table 9. Cost effective options for reducing incidence.

Option	Input level	Activity
1	Low	Grow a thick groundcover from Oct-Nov in the lead up and during flowering in December
2	Medium	Remove the majority of the burr and twig/branch material from the orchard floor by manual raking Grow a thick as described above
3	High	Remove the majority of the burr and twig/branch material from the orchard floor, either mechanically or by manual raking Grow a thick groundcover as described above Apply a protectant fungicide in the lead up to and/or during the flowering period (once the chemical type, effectiveness, timing, and application rates are established) Application of urea to remaining burrs and branches/twigs on the orchard floor

The costing of option 1 will be negligible unless water and seed is applied to the orchard floor to encourage the growth of the groundcover. This will depend on the existing groundcover and the rainfall at each orchard. Option 2 is likely to be similar to the manual removal of burrs and branches/twigs ie. \$4332.50 per year (Table 2). The high input option 3 would be \$5880.20/year (Table 10). These are in comparison to the potential losses of \$54,000/year for the model orchard, using the 72% chestnut rot incidence figure.

Table 10. Cost of option 3 including and growing a thick ground cover in the months leading up to and during flowering, manual burr and twig removal, use of a protectant fungicide on trees, and application of urea on dead burrs and twigs.

	Year 1	Year 2	Year 3	Year 4	Year 5	5 year total
Losses without treatment 36%: median incidence 72%: high incidence	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$27,000 \$54,000	\$135,000 \$270,000
Losses with treatment 1% incidence	\$750	\$750	\$750	\$750	\$750	\$3,750
Total Income without treatment 36%: median incidence 72%: high incidence	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$48,000 \$21,000	\$240,000 \$105,000
Total income with treatments (Income with 1% incidence minus cost of treatment)						
Burr and twig removal with casual labour (table 2)	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$4332.50	\$21 662.50
Apply a protectant fungicide, 4 times at weekly intervals (table 5)	\$559.70	\$0	\$559.70	\$0	\$559.70	\$1679.10
Apply urea to remaining burrs and twigs (table 6)	\$988.00	\$988.00	\$988.00	\$988.00	\$988.00	\$4940.00
Grow a thick groundcover between Oct-Dec, the months leading up to and during chestnut flowering.	\$0	\$0	\$0	\$0	\$0	\$0
with 1% incidence	\$74,250- \$5880.20= \$68,369.80	\$74,250- \$5320.50= \$68,929.50	\$74,250- \$5880.20= \$68,369.80	\$74,250- \$5320.50= \$68,929.50	\$74,250- \$5880.20= \$68,369.80	\$371,250 \$28,281.60= \$342,968.40

8. Research priorities

The various control methods need to be tested experimentally, independently and in combinations, for effectiveness in the field and in the laboratory when possible, to determine their effects on reducing incidence. This will help elucidate if they are methods growers should use or not. This information will also enable the development of a series of Low/Medium/High management level options for farmers to adopt according to their individual circumstances.

The experiments that should be conducted and the determination of their effects on incidence the following year include:

- Removing burrs and twigs from the orchard floor vs not removing them
- Growing a ground cover in the months leading up to flowering vs not
- Testing the application of mulch over burrs and twigs vs not
- Observe the effects of applying urea on dead burrs and twigs on the orchard floor
- Determine the effect of spraying developing flowers with protectant fungicides
 - Elucidate appropriate spray concentration and timing. Fungicides tested should be those that have potential to be registered for chestnuts in Australia. Examples include Pyraclostrobin or Difenconazole + copper, and phosphoric acid.
 - Observe any deleterious effects of fungicides on pollination and nut set and general phytotoxicity on trees
- Test effects of applying fungicides to cultures of *G. smithogilvyi* in the laboratory
- Determine the effect of spraying biocontrols on dead burrs and twigs on the orchard floor and elucidating which biocontrol is most effective at reducing incidence

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