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Survey of the incidence of chestnut rot in south-eastern Australia

L. A. Shuttleworth · E. C. Y. Liew · D. I. Guest

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Abstract Chestnut rot of *Castanea sativa* (European chestnut) and *C. crenata* (Japanese chestnut) × *C. sativa* hybrids is a significant problem facing the Australian chestnut industry. It affects the chestnut kernel, symptoms occur as pale, medium and dark brown lesions occurring on the endosperm and embryo. Twenty-two orchards in Victoria and New South Wales were surveyed in 2008, and 21 of these were again surveyed in 2009. All 22 orchards were affected by chestnut rot. Incidence at individual orchards up to 72 % was found in 2008, and 35 % in 2009. Incidence varied widely between orchards and within individual orchards between the 2 years. All varieties were affected, including Decoppi Marone, Purton's Pride and Red Spanish, displaying examples of both high incidence (>1 %) and industry acceptable incidence (0–1 %) depending on orchard and year. In 2008 and 2009, surveys of Sydney Markets showed incidence of >1 % (2008: varieties Decoppi Marone, Purton's Pride; and in 2009: varieties Purton's Pride, Red Spanish). The early December flowering Red Spanish had significantly higher average incidence than the early to mid December flowering Decoppi Marone and Purton's Pride, suggesting that the timing of flowering may be an important factor affecting incidence. There was a mild positive correlation between orchard incidence and December rainfall of the previous year (r^2=0.4), indicating rainfall during the chestnut flowering period had a mild effect on incidence.

L. A. Shuttleworth · D. I. Guest (✉)
Faculty of Agriculture and Environment,
The University of Sydney,
1 Central Avenue, Australian Technology Park,
Eveleigh, NSW 2015, Australia
e-mail: david.guest@sydney.edu.au

L. A. Shuttleworth (✉) · E. C. Y. Liew
The Royal Botanic Gardens and Domain Trust,
Mrs Macquaries Rd,
Sydney, NSW 2000, Australia
e-mail: lucas.shuttleworth@sydney.edu.au

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Introduction

The Australian chestnut industry is relatively small but increasing in production annually. In 2007, fresh chestnut production for 2010 was projected as 1,880 metric tonnes for the Australian domestic market and 120 metric tonnes for the export market (Horticulture Australia Limited 2007). The estimated value of the industry in 2010 was $13M ($12.2M p.a domestically and $0.78M p.a. in export) based on the price of $6.50 per kg of fresh unpeeled chestnuts (Horticulture Australia Limited 2007). Chestnuts are grown in cooler areas of Australia such as North-East Victoria (VIC) (where 80 % of the Australian crop is grown), the Dandenong Ranges (VIC), Gippsland (VIC), the Macedon Ranges (VIC), the Blue Mountains (New South Wales [NSW]), Central-West NSW, the North Coast of NSW, and the South Coast of NSW (McLaren 1999). South Australia, Tasmania and Western Australia also grow small quantities (McLaren 1999). The main species grown in Australia are *Castanea sativa* (European chestnut) and hybrids of *C. crenata* × *C. sativa* (Japanese chestnut × European chestnut).

Chestnut rot is a significant problem facing the Australian chestnut industry (Anderson 1993; Washington et al. 1993, 1997, 1998, 1999; Ogilvy 1998; Smith and Ogilvy 2008; Shuttleworth et al. 2010, 2012a, b; Shuttleworth 2012). Chestnut rot is also a significant problem in many other countries including Chile (Grau and France 1999), China (Wang et al. 2000; Wu et al. 2001; Zhu et al. 2009), France (Baudry and Robin 1996; Breisch 2008), India (Puttoo et al. 1988), Italy (Gentile et al. 2009, 2010; Longa et al. 2012; Visentin et al. 2012), New Zealand (Wadia et al. 2000; Osmonalieva et al. 2001; Smith and Ogilvy 2008), Switzerland (Jermini et al. 2006; Sieber et al. 2007), and the United States of America (Wright 1960; McCarter et al.

1980; Donis-Gonzalez 2008; Donis-Gonzalez et al. 2010). Healthy chestnut kernels have a creamy light yellow appearance with very firm endosperm and embryo tissue (Fig. 1a). Chestnut rot symptoms occur as pale, medium and dark brown lesions on the endosperm and embryo of the chestnut (Fig. 1b). The disease is often not visible externally, proving a challenge for growers and consumers alike. Symptoms mainly occur post-harvest (Rutter et al. 1991; Shuttleworth et al. 2010, 2012a, b; Shuttleworth 2012); however observations by Australian growers indicate chestnuts can be affected while still attached to the tree (Shuttleworth 2012).

Chestnut rot has previously been reported to be increased by rainfall during flowering (Ogilvy 1998). Ogilvy (1998) also reported that chestnut flowers have 'critical periods' of receptiveness to airborne ascospores stated as days 8–17 of flowering. Subsequently the disease was found to be caused through a floral infection by overwintered ascospores released from dead burrs on the orchard floor (Smith and Ogilvy 2008; Shuttleworth 2012; Shuttleworth et al. 2012a, b). Varieties in Australia flower over a 2 ½week period from early to mid December. Red Spanish for example, flowers in early December and Decoppi Marone and Purton's Pride both flower in early to mid December. Therefore they will likely have different critical periods and timing of receptivity to ascospores. A previous report ranked the early flowering Early Marone as most susceptible to the disease, whilst Purton's Pride and Red Spanish were reported as least susceptible to the disease (Rinaudo et al. 2009).

Recent studies have identified the main causal organism of chestnut rot in Australia and New Zealand as the endophyte anamorph *Phomopsis castanea* (Anderson 1993; Washington et al. 1993, 1997, 1998, 1999; Wadia et al. 2000; Osmonalieva et al. 2001). Additionally, two teleomorphs have been described in Australia overwintered on dead burrs, and their

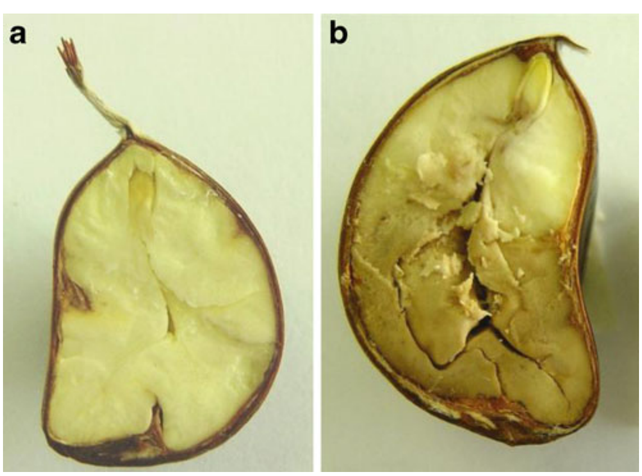

Fig. 1 **a** A dissected healthy chestnut collected in the 2008 orchard survey. **b** A dissected chestnut with medium brown chestnut rot symptoms collected in the 2008 orchard survey

anamorphs reported associated with rotten chestnuts: *Diaporthe castaneti* (anamorph: *Phomopsis castanea*) and the informally described *Gnomonia pascoe* prov. nom. (anamorph: *Discula pascoe* prov. nom) (Smith and Ogilvy 2008). *Discula pascoe* prov. nom. has also been reported as associated with rotten chestnuts and as an endophyte of *C. sativa* in Italy (Gentile et al. 2009, 2010). ITS sequences from isolates associated with this Italian study were deposited in GenBank as *Gnomoniopsis* sp. (Tamietti et al. 2010). Species of *Gnomoniopsis* are documented in Australia, India, Italy and New Zealand as infecting *C. crenata*, *C. sativa* and *Castanea* spp. as endophytes, pathogens and saprobes (Sogonov et al. 2008; Tamietti et al. 2010; Vettraino et al. 2011; Dar and Rai 2011; Shuttleworth et al. 2012a, b; Shuttleworth 2012). They are also reported in Italy associated with galls caused by the chestnut gall wasp, *Dryocosmus kuriphilus* (Magro et al. 2010). In Australia, Shuttleworth et al. (2012a, b) and Shuttleworth (2012) found the *Gnomoniopsis smithogilvyi* anamorph as mainly associated with chestnut rot, as an endophyte isolated from asymptomatic chestnut flowers, leaves, stems and developing fruit, and the *G. smithogilvyi* teleomorph as a saprobe overwintered on dead burrs on the orchard floor.

Very recently Visentin et al. (2012) reported a new species, *Gnomoniopsis castanea*, as the causal agent of nut rot of chestnut based on morphology and DNA sequences from the ITS (Tamietti et al. 2010) and tef1-α loci. The ITS phylogeny by Shuttleworth et al. (2012a) grouped all the isolates from Tamietti et al. (2010), which included the ex-type culture of *G. castanea* (GenBank accession HM142946), and a New Zealand isolate associated with the Smith and Ogilvy (2008) study reported as *G. pascoe* prov. nom (GenBank accession HM142948) in the same lineage as *G. smithogilvyi* (GenBank accession JQ910642). A phylogeny using ITS and tef1-α in combination with a morphological analysis by Shuttleworth (2012) provided evidence that *G. smithogilvyi*, *G. castanea* and *G. pascoe* prov. nom. are all one species, *G. smithogilvyi*.

In Australia, chestnut industry standards require a tolerance of incidence at markets to be less than 1 % (Rinaudo et al. 2009). However, a survey of Melbourne Wholesale Fruit, Vegetable and Flower Market have showed incidence of up to 40 % (Anderson 1993). Based on 40 % incidence, this represents potential losses to the industry of approximately $5.2M p.a. on the basis of 2010 production figures (HAL 2007).

To establish the scope and extent of chestnut rot in south-eastern Australia comprehensive surveys of both orchards and markets became imperative. The pattern between incidence and rainfall during the flowering in the previous year reported by Ogilvy (1998) and Smith and Ogilvy (2008) also required further investigation.

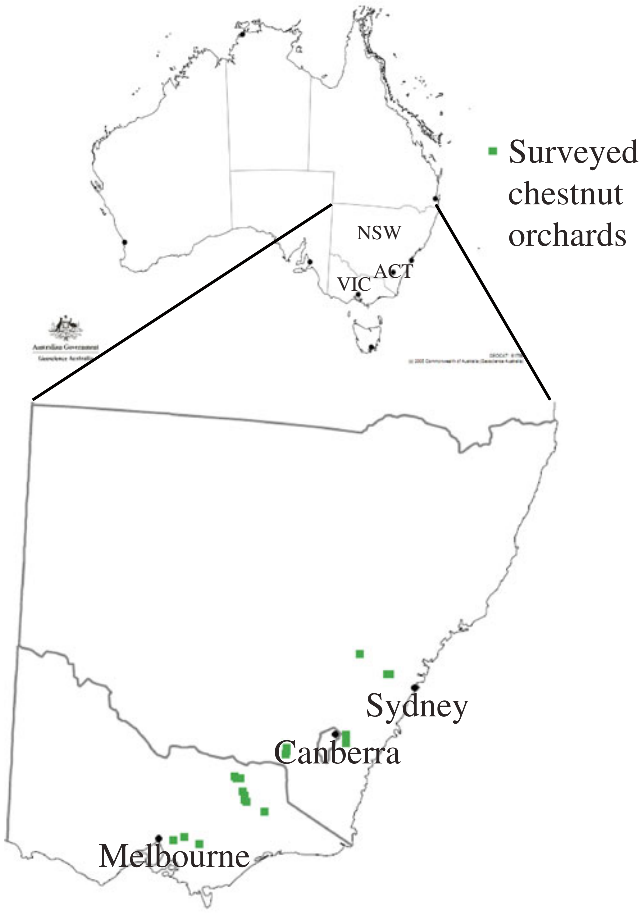

Fig. 2 Location of the chestnut orchards in south-eastern Australia surveyed for chestnut rot in 2008 and 2009 (template map sourced from Geoscience Australia 2006)

The specific aims of this study were to survey the incidence of chestnut rot at orchards in NSW and VIC; to survey Sydney Markets for chestnut rot; to elucidate the incidence of commercial chestnut varieties Decoppi Marone, Purton's Pride and Red Spanish; to investigate the possible effects of the timing of the chestnut flowering period in December (early or early to mid December) on incidence the following year; and the possible effect of rainfall during the flowering period on incidence the following year.

Materials and methods

Orchard surveys

Twenty-two chestnut orchards were selected ensuring good representation of the main production areas of the Australian chestnut industry: 14 in VIC and 8 in NSW. All orchards were surveyed in April/May 2008, and 21 were surveyed in April/May 2009 (Fig. 2, Table 1). A hierarchical survey strategy was used. Three-hundred chestnuts per orchard were randomly surveyed from each orchard. Fifty to 100 chestnuts per variety were surveyed depending on the number of varieties present (e.g. if an orchard had 3 varieties, 100 chestnuts per variety were surveyed, 3 trees per variety, 33-34 chestnuts per arbitrarily selected tree).

A range of varieties were surveyed with an emphasis on the more common commercial varieties, including Decoppi Marone (DM), Purton's Pride (PP) and Red Spanish (RS). An additional ten varieties were surveyed depending on availability in the orchard, including April Gold (AG), Buffalo Queen (BQ), Colossal (C), Early Marone (EM), King of the Valley (KV), Manjimup Mahogony (MM), Mullion Range (MR), New Zealand (NZ), Sassafras Red (SR) and Tarhoa Tops (TT) (Table 1). All of the varieties surveyed gave a representation of chestnuts being sold commercially, locally and direct to consumers visiting chestnut orchards for the 'pick your own' method. After collection, chestnuts were dissected longitudinally from stylar end to hilum and visually assessed for chestnut rot. If symptoms were present the chestnut was classified as rotten as severity of the disease has been found to increase rapidly once the chestnut is infected (Anderson 1993; Washington et al. 1993, 1997). Rotten chestnuts were counted as a percentage of the total chestnuts sampled, expressed as chestnut rot incidence (%). In this study chestnut rot incidence was considered high if >1 %, and acceptable if between 0-1 %.

The effects of flowering period (early or early to mid December), variety (Decoppi Marone, Purton's Pride, Red Spanish) and December rainfall of the previous year on 2008 and 2009 chestnut rot incidence (%) were analysed with multiple linear regression using the statistical package GenStat 14th edition (Payne et al. 2011). The correlation of December rainfall of the previous year and incidence was also analysed in GenStat 14th edition. The number of December rainfall observations used in this study was 43 (22 from 2007, 21 from 2008), sourced from the Bureau of Meterology weather station closest to each orchard (BOM 2011). There was a trend in the residuals plot when performing the multiple linear regression analysis on the raw incidence data, therefore the data was square-root transformed before analysis.

Market surveys

Sydney Markets, located in Flemington, NSW was surveyed for chestnut rot in July 2008 and July 2009. Sydney Markets is the largest fresh fruit and vegetable wholesale market in Australia (www.sydneymarkets.com.au). The chestnuts surveyed in the market surveys were sourced from some of the orchards sampled in the orchard surveys. Three-hundred chestnuts were sampled per orchard, per variety available. Fifteen-hundred chestnuts were surveyed in 2008 from 5 orchards (Bright, VIC, Fumina, VIC, Wandiligong, VIC, Batlow, NSW, Hoskinstown, NSW), 900 chestnuts were surveyed from

Table 1 Details of chestnut orchards in south-eastern Australia surveyed in 2008 and 2009

ABS region and state	Orchard number	Town	GPS co-ordinates (datum:WGS84)	Varieties surveyed	
				2008	2009
Ovens-Murray VIC	1	Stanley	36° 24.816′S 146° 47.179′E	DM, PP, RS, BQ, C, EM	DM, PP, RS, BQ
Ovens-Murray VIC	2	Stanley	36° 24.874′S 146° 46.719′E	DM, PP	DM, PP, RS
Ovens-Murray VIC	3	Silver Creek	36° 22.272′S 146° 42.359′E	DM, PP, RS	DM, PP, RS
Ovens-Murray VIC	4	Beechworth	36° 24.145′S 146° 39.493′E	DM, PP	Not sampled.
Ovens-Murray VIC	5	Bright	36° 42.529′S 146° 58.555′E	DM, PP	DM, PP
Ovens-Murray VIC	6	Bright	36° 43.068′S 146° 55.877′E	DM, KV	DM, KV
Ovens-Murray VIC	7	Porepunkah	36° 43.361′S 146° 53.475′E	PP, RS, WW	PP, RS, WW
Ovens-Murray VIC	8	Wandiligong	36° 48.082′S 146° 58.669′E	BQ, PP, RS	PP, RS
Ovens-Murray VIC	9	Wandiligong	36° 48.993′S 146° 57.978′E	DM, PP, RS, WW	DM, PP, RS, WW
Ovens-Murray VIC	10	Harrietville	36° 54.008′S 147° 3.997′E	PP, RS, AG, EM	PP, RS, AG, EM
East Gippsland VIC	11	Benambra	36° 50.263′S 147° 41.757′E	DM, PP, AG, BQ, EM, KV	PP, AG, EM, KV
Yarra Ranges VIC	12	Olinda	37° 51.909′S 145° 23.565′E	DM, PP, RS	DM, PP, RS
Gippsland VIC	13	Fumina	37° 54.065′S 146° 6.430′E	DM, PP, RS	DM, PP
East Melbourne VIC	14	Yarra Junction	37° 51.538′S 145° 41.874′E	PP, RS	PP
Murray-Murrumbidgee NSW	15	Tumbarumba	35° 40.437′S 148° 2.402′E	DM, PP, RS	DM, PP, RS
Murray-Murrumbidgee NSW	16	Tumbarumba	35° 41.761′S 148° 2.367′E	PP, KE	RS, KE
Murray-Murrumbidgee NSW	17	Batlow	35° 32.431′S 148° 7.692′E	DM, PP, RS, MR	DM, PP
South-Eastern NSW	18	Hoskinstown	35° 24.758′S 149° 26.039′E	DM, PP, RS, AG	DM, PP, RS
South-Eastern NSW	19	Bungendore	35° 14.147′S 149° 24.25′E	DM, PP	DM, PP
Blue Mountains NSW	20	Mount Wilson	33° 30.307′S 150° 22.998′E	PP, RS, MM, V	PP, RS
Blue Mountains NSW	21	Mount Irvine	33° 29.129′S 150° 27.693′E	PP, NZ, SR, TT	PP
Central-West NSW	22	Mullion Creek	33° 7.744′S 149° 9.013′E	DM, PP, RS	DM, PP, RS

Varieties surveyed include the commercially important Decoppi Marone (DM), Purton's Pride (PP), and Red Spanish (RS), with additional varieties including April Gold (AG), Buffalo Queen (BQ), Colossal (C), Early Marone (EM), King of the Valley (KV), Manjimup Mahogony (MM), Mullion Range (MR), New Zealand (NZ), Sassafras Red (SR) and Tarhoa Tops (TT). Regional classifications were sourced from the Australian Bureau of Statistics (ABS 2001)

2 orchards in 2009 (Batlow, NSW, Hoskinstown, NSW). The difference in sample number between years was due to the number of orchards from which chestnuts were available. Chestnuts were dissected longitudinally and

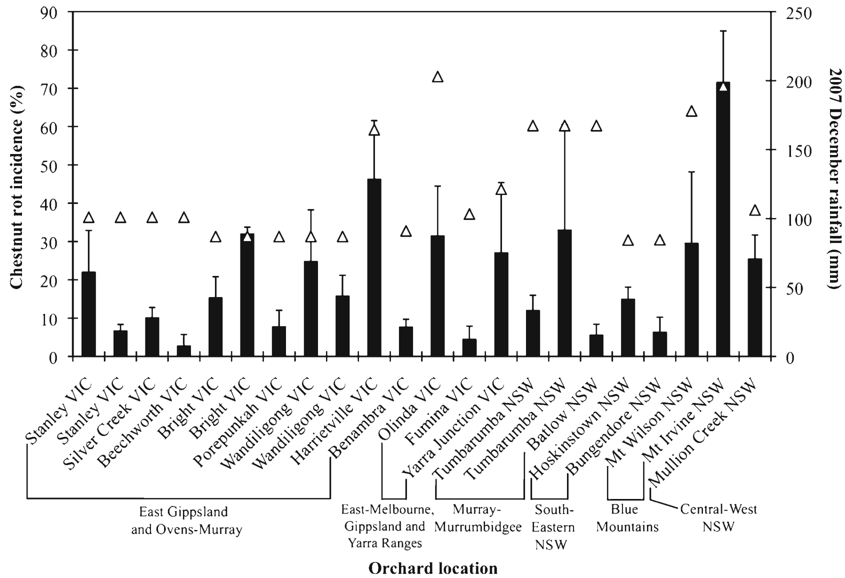

Fig. 3 Orchard chestnut rot incidence of orchards surveyed in VIC and NSW in 2008. *Error bars* indicate the standard error of the mean of chestnut rot incidence. The rainfall in December 2007 is indicated by *triangles*

visually inspected and chestnut rot incidence (%) determined as per the orchard surveys.

Results

Orchard surveys

Gnomoniopsis smithogilvyi was the organism most often isolated from rotten chestnut kernels in the orchard surveys. All orchards were affected by chestnut rot in both 2008 and 2009. Incidence varied widely between orchards and within orchards between the 2 years surveyed (Figs. 3 and 4). In 2008, the orchard with the highest incidence was Mt Irvine, NSW (72 %), while the lowest was Beechworth, VIC (3 %). In 2009, the orchard with the highest incidence was Mt Wilson, NSW (35 %), while the lowest was Batlow, NSW (1 %). Incidence was higher in 2008 than in 2009 for 17 of the 21 orchards with 2 years of data. Only 4 of the 22 orchards surveyed in 2008 and 3 surveyed in 2009 had incidence within the industry recommendation of 0–1 %.

All varieties surveyed in 2008 and 2009 were affected by chestnut rot. High incidence (>1 %) was observed in the varieties Decoppi Marone, Purton's Pride and Red Spanish (Table 2). In 2008 Purton's Pride had the highest incidence at 73 %, and in 2009 Red Spanish the highest at 43 %. Decoppi Marone and Purton's Pride had the lowest incidence in 2008 (0 %), while Purton's Pride had the lowest incidence in 2009 (0 %). Red Spanish had no examples of 0–1 % in either 2008 or 2009, while Purton's Pride was the only variety that had incidence of 0 % for both 2008 and 2009 (Olinda, VIC). There was great variation in the range of incidence for most of the

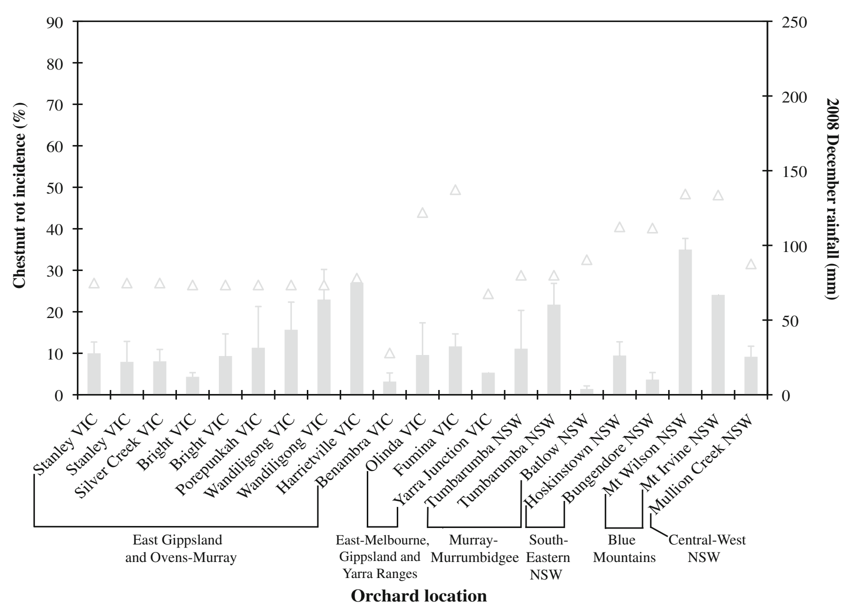

Fig. 4 Orchard chestnut rot incidence of orchards surveyed in VIC and NSW in 2009. *Error bars* on columns indicate the standard error of the mean. The rainfall in December 2008 is indicated by *triangles*

Table 2 Mean orchard chestnut rot incidence of three varieties from orchards surveyed in 2008 and 2009, Decoppi Marone, Purton's Pride and Red Spanish

Variety	2008 mean and range of chestnut rot incidence (%)	2009 mean and range of chestnut rot incidence (%)
Decoppi Marone	(0-) 10 (−28)	(2-) 8 (−27)
Purton's Pride	(0-) 10 (−73)	(0-) 9 (−38)
Red Spanish	(4-) 23 (−70)	(3-) 19 (−43)

varieties surveyed in both years. The variety with the largest range in 2008 was Purton's Pride (0–73 %) and in 2009, Red Spanish (3–43 %) (Table 2). The early December flowering variety Red Spanish had a higher average incidence (21 %) than the early to mid December flowering Decoppi Marone (10 %) and Purton's Pride (11 %) (Fig. 5). The multiple linear regression analysis showed that there was a significant effect of flowering period and December rainfall of the previous year on incidence (F pr=0.009 and 0.001). There was no significant effect of variety on incidence (F pr=0.645). No significant interactions were found between flowering period, December rainfall of the previous year, or variety. There was a mild correlation between December rainfall of the previous year and incidence (r^2=0.4).

Market surveys

Gnomoniopsis smithogilvyi was the organism most often isolated from rotten chestnut kernels in the market surveys. All varieties surveyed at Sydney Markets had incidence of >1 % (Table 3). In 2008 the highest incidence was Purton's Pride from Fumina, VIC (10 %), while the lowest was

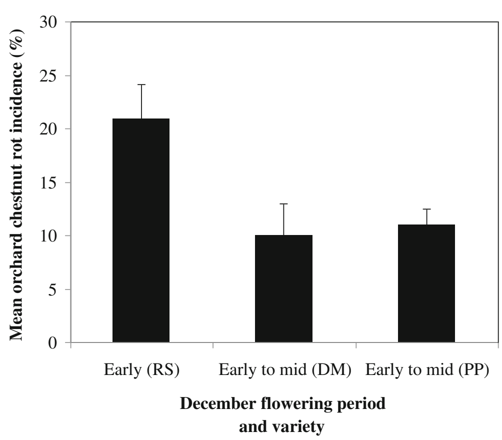

Fig. 5 Mean orchard chestnut rot incidence of 3 chestnut varieties and 2 flowering periods, Red Spanish (RS) (early December flowering), Decoppi Marone (DM) (early to mid December flowering), and Purton's Pride (PP) (early to mid December flowering). Data was collected in both 2008 and 2009, Red Spanish n=25, Decoppi Marone n=28, Purton's Pride n=40. *Error bars* indicate standard error of the mean

Purton's Pride from Bright, VIC (2 %). In 2009, the highest incidence was Red Spanish from Tumbarumba, NSW (9 %), while the lowest was Purton's Pride from Tumbarumba, NSW (4 %). In 2008, chestnuts from 3 of the 5 orchards increased in incidence from orchard to market, 1 decreased and 1 remained unchanged. In 2009, chestnuts from 2 of the 3 orchards increased in incidence from orchard to market and 1 decreased substantially (30 % to 9 %).

Discussion

In both 2008 and 2009 *Gnomoniopsis smithogilvyi* was the organism most frequently isolated from rotten chestnut kernels in the orchard and market surveys. All of the surveyed orchards and the market samples were affected by chestnut rot in both years indicating the disease continues to be a significant problem for the Australian chestnut industry. The orchard surveys showed incidence varied widely between orchards in both years, and within individual orchards between the 2 years. Incidence at Sydney Markets in 2008 and 2009 was above the industry threshold of 0–1 % for all the market samples, again indicating chestnut rot is a significant problem for the industry. Five out of the eight chestnut samples recorded from field to market in 2008 and 2009 showed an increase in incidence. This is evidence that chestnut rot develops further in storage, suggesting a result of infected chestnuts further developing symptoms once they are placed in storage.

The highly variable incidence observed from year to year and between orchards is most probably due to biotic and abiotic factors occurring in the micro-climate of each orchard. Shuttleworth (2012) discovered evidence that chestnut rot is caused by a floral infection in summer by ascospores of *G. smithogilvyi* released from dead burrs on the orchard floor. After the floral infection Shuttleworth (2012) also isolated *G. smithogilvyi* as an endophyte from chestnut flowers (in summer), leaves (summer, autumn), stems (summer, autumn, winter) and developing fruit (summer, autumn). A key biotic factor and essential part of the infection process is therefore the release of ascospores from dead burrs on the orchard floor and the receptivity of chestnut flowers to the ascospores when they are present in the orchard atmosphere. Ogilvy (1998) reported that the critical period for receptivity of chestnut flowers to spores was days 8–17 of flowering. In Australia, chestnut trees flower over an approximately 2 ½week period in December. Flower receptivity is therefore affected by the time of flowering of each variety, with each variety having a different critical period. All varieties were affected by the disease in the current study, indicating they are all susceptible under conducive conditions. However, the early flowering variety, Red Spanish, had significantly higher incidence in both 2008 and 2009 than the early to mid December flowering Decoppi Marone and Purton's Pride, showing the timing of flowering was an important factor

Table 3 Chestnut rot incidence at Sydney Markets sampled in July 2008 and 2009

Orchard location	Variety	Market chestnut rot incidence (%)		Orchard chestnut rot incidence (%)	
		2008	2009	2008	2009
Bright VIC	Purton's Pride	2	NA	7	NA
Wandiligong VIC	Purton's Pride	5	NA	3	NA
Fumina VIC	Purton's Pride	10	NA	3	NA
Tumbarumba NSW	Red Spanish	NA	9	NA	30
Tumbarumba NSW	Purton's Pride	NA	4	NA	2
Batlow NSW	Decoppi Marone	9	NA	7	NA
Batlow NSW	Purton's Pride	NA	6	NA	1
Hoskinstown NSW	Decoppi Marone	4	NA	4	NA

Orchard chestnut rot incidence is included from the same orchards for comparison. $n=300$ chestnuts per orchard per variety

NA not available

affecting incidence. The higher incidence of Red Spanish was likely to be due to a combination of ascospores being released in early December and the critical period of the variety coinciding with this period. A previous study has reported an early flowering variety, Early Marone to be more susceptible to chestnut rot, while Purton's Pride and Red Spanish were reported as least susceptible (Rinaudo et al. 2009). Interestingly, this report found that Red Spanish was least susceptible to the disease and therefore is in conflict with the current study. This finding by Rinaudo et al. (2009) was likely an observation of the variable incidence that has been found to occur in chestnut varieties from year to year.

Another biotic factor potentially affecting incidence is arthropod transmission of ascospores. Insects such as ants, bees, beetles and earwigs are common on chestnut flowers and on dead burrs in Australia during the flowering period. They provide a direct mode of transmission of ascospores from infected burrs to flowers, and also between flowers in the canopy (Fig. 6a, b).

Some of the abiotic factors potentially affecting incidence include December rainfall in the previous year, wind patterns, temperature, and rain splash of ascospores. As mentioned, Ogilvy (1998) reported a link between rainfall during flowering and increased incidence the following year. The current study found rainfall during December of the previous year was a significant factor affecting incidence. The orchard with the second highest December rainfall in 2007 (Mt Irvine, NSW) had 12 times higher incidence in 2008 than the orchard with the (equal) lowest December rainfall in 2007 (Bungendore, NSW). However there was only a mild positive correlation found between incidence and December rainfall ($r^2=0.4$). This mild correlation is likely due to the high variation in incidence observed between orchards, and within individual orchards between the 2 years surveyed.

The timing of ascospore release and flowering of host plants have previously been reported as closely coinciding (Tate 1979), which indicates that there is an intricate host–

Fig. 6 **a** Chestnut flowers being pollinated by European honey bees (*Apis mellifera*). Bees are potential transmitters of *G. smithogilvyi* ascospores. **b** A lady-beetle (*Coccinellidae*) walking on a dead infected chestnut burr are also potential transmitters of *G. smithogilvyi* ascospores

pathogen–environment relationship associated with floral infections. The effects of temperature and wind patterns on incidence were not analysed in this study, as measurements were not recorded in the field and comprehensive data was not available from the Bureau of Meterology. However, these environmental factors have previously been reported as factors affecting the ascospore release of other species of Gnomoniaceae. The optimum temperature for ascospore release of species in the Gnomoniaceae include 22–24 °C for *Apiognomonia quercina*, the causal agent of oak anthracnose (Ragazzi et al. 2007), and 18 °C for *Apioplagiostoma populi*, the causal agent of bronze leaf disease of *Populus* (Smith et al. 2002). In December 2008, the temperature range and mean of Orange, NSW (the closest weather station to the surveyed orchard in Mullion Creek) was (12.1 °C) 22.8 °C (27.4 °C) (BOM 2011). These values coincide with the temperatures of maximum ascospore release of *A. quercina* and *A. populi*, and are likely to be within the optimum temperature range of other species in the Gnomoniaceae including *G. smithogilvyi*. When temperatures are within this range ascospores are more likely to be released and the potential of floral infection is increased. Temperatures during the season are also likely to affect the timing of flowering of host plants (Post et al. 2008; Tooke and Battey 2010). Warm weather, particularly in the months leading up to flowering, can advance the timing of flowering and conversely if there is cold weather it can delay flowering. Changes in temperature may also advance or slow the timing of ascospore release, which would increase the probability of flowers becoming infected.

The presence of wind is critical for the dispersal of spores of fungal pathogens on a global scale (Brown and Hovmøller 2002). Wind is reported as a mode of dispersal of members of the Gnomoniaceae including *Anisogramma anomala*, the causal agent of eastern filbert blight on European hazelnut (Heckert 2011), *Apiognomonia erythrostoma*, the causal agent of cherry leaf scorch (Chalkley et al. 2012) and *Ophiognomonia clavigignenti-juglandacearum* the cause of butternut canker (Tisserat and Kuntz 1983; Broders and Boland 2011). Ascospores of *G. smithogilvyi* have been found to be wind dispersed and can therefore be mobilised to the orchard canopy where they can potentially infect chestnut flowers (Shuttleworth 2012). It is currently unknown how far ascospores of *G. smithogilvyi* can travel in the air from their source of origin. The distance ascospores can travel has particular implications for orchards that closely neighbour others, which is prevalent in North-East VIC.

Rain splash of spores is another factor suggested as contributing to chestnut rot (Ogilvy 1998). It is documented as a mode of ascospore transmission with pathogenic species in the Gnomoniaceae, such as *O. clavigignenti-juglandacearum* (Tisserat and Kuntz 1983; Broders and Boland 2011) and *Sirococcus tsugae*, the causal agent of Sirococcus shoot blight of western hemlock, mountain hemlock and sitka spruce (Holsten et al. 2008).

Smith and Ogilvy (2008) found infection of chestnut flowers occurs during the flowering period in December, coinciding with ascospore release from dead burrs on the orchard floor. There are many other examples of flower infecting pathogens that are responsible for economically significant crop losses and quarantine issues worldwide (Ngugi and Scherm 2006). These include *Claviceps purpurea* on rye (ergot of rye) (Tudzynski and Scheffer 2004), *Erwinia amylovora* on apple and pear (fire blight) (Pusey and Curry 2004), *Gibberella zeae* on wheat (fusarium head blight) (Shaner 2003) and *Telletia indica* on wheat (karnal bunt) (Bonde et al. 1997). Spores of fungal pathogens are reported to infect flowers through various methods including direct penetration of the ovary wall (Schönbeck 1966), infection via the gynoecial (stigma-style) pathway (Ngugi and Scherm 2004; Tudzynski and Scheffer 2004), the nectaries (Ngugi and Scherm 2006), and direct and passive infection of pollen grains (Stelfox et al. 1978; Huang and Kokko 1985; Huang et al. 1997). It is currently unknown how ascospores of *G. smithogilvyi* gain access to the internal floral parts of the chestnut flowers, whether by active or passive entry and whether they can be transported on or in pollen grains.

Another important question regarding the infection of chestnut flowers is the period that ascospores can remain viable in the orchard. Ascospores from infected burrs kept dry and at 23 °C in the laboratory at the Botanic Gardens and Domain Trust were still viable and grew in culture 3 years after collection from the field. This demonstrates that ascospores of *G. smithogilvyi* are resilient and provide a potentially long lasting reservoir of infection.

There are a number of field and post-harvest treatments that may reduce incidence of chestnut rot. Targeting burrs as a source of primary inoculum is the key to any chestnut rot management program. Potential options for targeting burrs include removal of burrs, placement of a thick layer of organic mulch on top of the infected burrs, and growing a thick vegetative groundcover to create a physical barrier to ascospores being released into the orchard atmosphere. The use of biocontrols such as *Trichoderma* spp. and *Gliocladium virens* have been suggested as potential control measures to spray on infected burrs (Shuttleworth 2012). More research needs to be completed to determine if these treatments are effective in reducing the incidence of chestnut rot.

These surveys have documented the scope and extent of chestnut rot in south-eastern Australia. They show chestnut rot continues to be a significant and widespread problem for the Australian chestnut industry. Further research work is needed to continue reducing the incidence of the disease and to enable growers to make more effective disease management decisions. Areas for future investigation include the effects of removing infected burrs from the orchard floor, the use of organic amendments to block ascospore movement, measuring the distance that ascospores can travel in air and potentially infect chestnut flowers, the role that arthropods play in transmission and dispersal, and the effectiveness of biocontrols on reducing chestnut rot.

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