Investigation into pre-determining factors for Bubbly Bark outbreaks in chestnut orchards

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Chestnut Growers of Australia Ltd

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For

The Chestnut Growers of Australia

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Purpose of the report:

Bubbly Bark has been evident in north-east Victorian chestnut orchards for over a decade. Initially it caused severe damage in a small number of orchards, but in recent years its occurrence has spread geographically. Some orchardists have reported losses of hundreds of trees in a single season. There have been numerous theories about its cause, but insufficient data to support any particular theory. The information about pre-disposing factors had been mainly anecdotal.

The aim of this project was to collect data from chestnut orchards to test for pre-disposing or common factors related to Bubbly Bark appearance.

Funding:

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✓	HAL contribution	- $ 10,475.
	TOTAL	- $ 22,500.

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MEDIA SUMMARY

The cause of the chestnut condition known as "Bubbly Bark" is still uncertain, although the Chestnut Growers of Australia (CGA) feel that a recent study has narrowed the possibilities.

Bubbly Bark in chestnuts has caused the death of hundreds of young chestnut trees in recent years. It is wide spread in north east Victoria, but also known in southern and northern New South Wales. The condition is very common in Chile and it is also known in New Zealand and the USA.

The early symptoms are a bubbling of the bark in late winter/early spring, often followed by a weak bud burst. Some trees may recover during the growing season, while many may die. In some orchards the death rate has reached nearly 50%, but in others it may be just 1-2%. Until the trees recover chestnut yield is reduced.

In a study jointly funded by Horticulture Australia Limited (HAL) and the CGA a number of orchards were monitored during the 2005/2006 growing season. Sites in Wandiligong, Eurobin, Beechworth and Stanley were included in the study. Plantation Development Services Pty Ltd from Bright was engaged by the CGA for the study, as well as two scientists from New Zealand who had previous experience with Bubbly Bark.

The affected trees are almost always young, grafted trees that usually only die above the graft. Seedling or non-grafted trees are seldom affected. Trees that reshoot and grow from below the graft may grow healthily for many years. No pathogens have been identified in affected trees, despite intensive testing. The levels of calcium and potassium, and possibly magnesium, manganese and aluminium may influence the presence of Bubbly Bark.

It appears that very wet, mild July-October periods cause a reaction in the tree that produces the bubbling of the bark. The process causing the bubbly effect is unknown, but it may be associated with the graft union of the tree. As yet, there is limited evidence available to support a particular hypothesis. If the tree is strong enough or the conditions improve the tree may regain strong vigour, but many trees die.

It is recommended that future efforts include identifying which varieties are more resistant to Bubbly Bark, which varieties are best suited as rootstock, and testing the theory that the graft union is a major factor in the cause of Bubbly Bark. However the search for a pathogen should continue, as should the research into the role of nutrition.

TECHNICAL SUMMARY

Bubbly Bark has been evident in north-east Victorian chestnut orchards for over a decade. Initially it caused severe damage in a small number of orchards, but in recent years its occurrence has spread geographically. Some orchardists have reported losses of hundreds of trees in a single season. There have been numerous theories about its cause, but insufficient data to support any particular theory. The information about pre-disposing factors had been mainly anecdotal.

This study involved the monitoring of thirteen orchards during the 2005/2006 growing season, some with Bubbly Bark and others without. Growers were surveyed about the management practices and history of each orchard. To determine whether there are pre-disposing factors the following data was collected from the monitoring sites:

- Previous land use.
- Rootstock variety.
- Source of planting stock.
- Pruning practice.
- Soil texture.
- Soil preparation for planting.
- Weed control methods.
- Herbicide use.
- Fungicide use.
- Nutrient analyses of foliage and soil.
- Use of irrigation.
- Slope.
- Aspect.
- Altitude.
- Presence of native forest, apples, olives and other crops, and
- Weather data from the Department of Sustainability's offices at Ovens, near Myrtleford was analysed for Bubbly Bark occurrence.

The cumulative losses reported by growers indicate that affected growers have lost about 3.4% of their total number of trees. The loss varies between growers from less than one per cent to about thirty percent. The monitoring plots indicate that the loss of trees in parts of affected orchards has been up to 48%, with losses averaging 22%. The rate of symptom display can be very high, but tree losses may be low. At one site 97% of trees were affected, but only 4% of the trees had died.

Many factors have been shown <u>not</u> to be correlated with Bubbly Bark incidence, including:

- Soil salinity,
- Soil nutrient levels of phosphorus, magnesium, potassium, sulphur, chloride, manganese, total nitrogen, copper, zinc, iron, boron, hydrogen, aluminium, calcium and sodium.
- Foliar nutrient levels of phosphorus, potassium, sulphur, chloride, manganese, total nitrogen, copper, zinc, iron, boron, aluminium, magnesium, molybdenum, calcium and sodium.
- Environmental factors including slope, aspect, elevation and adjacent vegetation.
- Management practices including irrigation, pruning, pruning intensity, weed control, use of glyphosate and previous land use.

Relevant observations include:

- Seedling trees rarely have Bubbly Bark; affected trees are almost invariably grafted trees.
- Many trees regrow from their base if the main trunk dies. It is rare for trees to reshoot above the graft. New shoots can grow in a healthy manner for over 15 years.
- Trees that are healthy in December do not contract the Bubbly Bark symptoms for the remainder of the growing season.
- Bark symptoms may be present in winter or early spring. Some of the affected trees may suffer bud death, while others may grow vigorously.
- It appears to affect all planted varieties to at least some extent.
- The age of trees affected is usually between two and ten years, and most commonly between two and six years. Older trees are infrequently affected.

Factors which may help solve the Bubbly Bark dilemma include:

- Bubbly Bark is most prevalent in spring when the July-October rainfall is high.
- Bubbly Bark is most prevalent in spring when the mean maximum temperatures for July-October are at their lowest.
- No pathogens have been found despite the study of over 20 Bubbly Bark infected trees being sent to five pathology laboratories over a period of 15 years.
- Calcium soil levels and soil pH are significantly higher under Bubbly Bark affected trees compared to non-affected trees.
- Potassium levels are significantly higher in Bubbly Bark affected trees compared to non-affected trees.

Combining these factors with the above observations it would appear that Bubbly Bark is a physiological response to certain environmental conditions, rather than a response to a pathogen.

To follow up the research on Bubbly Bark cause and management the following recommendations are made:

- Monitor a number of orchards to determine which are the more Bubbly Bark resistant varieties.
- Analyse rootstocks to test any differences to Bubbly Bark susceptibility.
- Continue with nutritional testing with particular emphasis on soil calcium and foliar potassium levels, but also including soil magnesium and foliar manganese, calcium and aluminium levels.
- Carry out a trial with young trees to test a range of moisture levels and if possible temperatures during July-October. These could be pot trials.
- Maintain a pathogen testing at least at a low level.
- Continue to monitor the monitoring sites established in 2005.

INTRODUCTION

Bubbly Bark was first noticed in north-east Victorian chestnut orchards during the late 1980s, and was first reported in 1993[1]. Initially it was of relatively minor significance, causing tree deaths or severe setbacks in tree health in several orchards, but on a relatively small scale across the industry. Periodic outbreaks were severe in some orchards and minor in others. In recent years its occurrence has spread geographically and its effect on chestnut orchards has been more significant. Some orchardists have reported losses of hundreds of trees in a single season.

Bubbly Bark is characterised by bubbling and softness of the bark; poor bud development or bud death; wilting and dying of branches, or the tree, usually above the graft. Observations relating to Bubbly Bark condition include:

- Seedling trees are rarely attacked.
- Many trees regrow from their base if the main trunk dies. It is rare for trees to reshoot above the graft. New shoots can grow in a healthy manner for at least 15 years.
- Trees that are healthy in December do not contract the Bubbly Bark symptoms for the remainder of the growing season.
- Bark symptoms may be present in winter or early spring. Some of the affected trees may suffer bud death, while others may grow vigorously.
- It appears to affect all planted varieties to at least some extent.
- A small borer is found in many trees affected.
- The age of trees affected is usually between two and ten years, and most commonly between two and six years. Older trees are infrequently affected.

Pathology tests in Melbourne during 1993 identified the pinhole borer beetle *Xyleborus perforans*, and two types of opportunistic wound pathogens – *Botryosphaeria obtusa* and *Schizophyllum commune*. *B. obtusa* is the more aggressive and can enter living trees. Subsequent Victorian tests have failed to identify any pathogens. New Zealand pathologists[2] have identified a number of pathogens, but none that are likely to cause tree death or extreme poor health.

The cause and pre-disposing factors for Bubbly Bark are unknown. There have been a number of hypotheses about its cause, but insufficient data to support any particular hypothesis. The information about pre-disposing factors had been mainly anecdotal. Bubbly Bark also occurs in New Zealand, Chile, and possibly North America[3]. Research in these countries has so far been unsuccessful in determining any cause for the condition.

The aims of this project were to determine if there are pre-disposing or common factors related to Bubbly Bark appearance, and to measure the impact of Bubbly Bark on individual orchards. The data collected included historical data relating to Bubbly Bark occurrence, regional weather data, and cultural and environmental data from selected chestnut orchards. Thirteen orchard sites and two nurseries were chosen to monitor Bubbly Bark at Wandiligong, Eurobin, Beechworth, Silver Creek and Stanley.

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[1] Borschmann 1993.
[2] Dance 1999.
[3] Chris Foster, Cascadia Chestnuts, Portland, Oregon USA. Personal communication.

MATERIALS AND METHODS

A number of orchards in north east Victoria were visited during Spring 2005 to gain an impression of the extent of Bubbly Bark, and the range of growing conditions where it was present and absent. Thirteen orchards were monitored, using seventeen monitoring sites, during late Spring 2005 to early Summer 2006. Two nurseries were also monitored. The monitoring sites covered the area from Wandiligong through Eurobin to Beechworth and Stanley (Map 1). Bubbly Bark symptoms varied from zero to very heavy.

To measure the impact of Bubbly Bark presence growers were queried about when it first appeared, its peak year and other significant years, and also the number of trees killed (Appendix 1). At each monitoring site trees were assessed for their current health on a scale of 1-8, and whether Bubbly Bark was present or not. A score of one indicated a tree in very good health, while a score of eight indicated that the tree was dead, at least from the graft up (Appendix 4). It was also noted whether the tree was a replacement tree or from the original planting. In assessing the impact of Bubbly Bark, trees which had been replaced, those which were dead and those which were missing, were assumed to be victims of Bubbly Bark if other causes were unknown. These assumptions may have over estimated the impact of Bubbly Bark on tree deaths.

From the above data the number of trees which were affected by Bubbly Bark (still showing symptoms or killed) was estimated, as was the number which were "very unhealthy" or dead. "Very unhealthy" trees were from categories 5-7.

To determine whether there are pre-disposing factors the following data was collected from the monitoring sites:

- Previous land use.
- Rootstock variety.
- Source of planting stock.
- Pruning practice.
- Soil texture.
- Soil preparation for planting.
- Weed control methods.
- Herbicide use.
- Fungicide use.
- Nutrient analyses of foliage and soil.
- Use of irrigation.
- Slope.
- Aspect.
- Altitude.
- Presence of native forest, apples, olives and other crops.

Weather data from the Department of Sustainability's offices at Ovens, near Myrtleford was analysed to test correlations between peak years of Bubbly Bark occurrence and weather. While the weather at Ovens does not represent weather at all monitoring sites, it was considered it would indicate relative changes in weather at the monitoring sites.

Foliage and soil samples were collected during 7th – 10th February 2006. Two or three leaves from about 20 trees were collected at each site. The target was for two leaves from 20 trees but the number of available trees sometimes dictated otherwise. The youngest fully expanded leaves from branches in the upper third of the crown were sampled. Soil samples were collected from under 20 trees (where possible) and combined. Sampling depth was from the surface of mineral earth to 15 cm. Samples were taken from midway between the trunk and the outer crown.

Analyses were completed by Farmright Technical Services, Kyabram, Victoria.

Pathogen testing on several samples selected from different sites was also conducted. In one case three young grafted tree were potted up and transported to a glasshouse at a testing site and monitored for several months.

RESULTS

Bubbly Bark Impact.
Loss reported by growers;
The cumulative losses reported by growers indicate that affected growers have lost about 3.4% of their total number of trees (Table 1). The loss varies between growers from less than one per cent to about thirty percent. For individual growers the loss of trees varies greatly between orchards.

Table 1. Cumulative tree deaths due to Bubbly Bark reported by growers.

Grower.	Total trees.	Tree Deaths.	Tree deaths as % of total trees.
1	1100	4	0.4
2	20000	500	2.5
3	2000	50	2.5
4	2000	2	0.1
5	600	15	2.5
6	1000	300	30.0
7	600	5	0.8
8	1050	90	8.6
9	2000	30	1.5
10	1000	60	6.0
Total	31350	1056	3.4

The monitoring plots indicate that the loss of trees in parts of affected orchards has been up to 48%, with losses averaging 22% (Table 2). The number of trees affected can be very high, but tree losses may be low. At Site 11, 97% of trees were affected, but only 4% of the trees had died. At the last inspection in January 2006 the trees were very healthy.

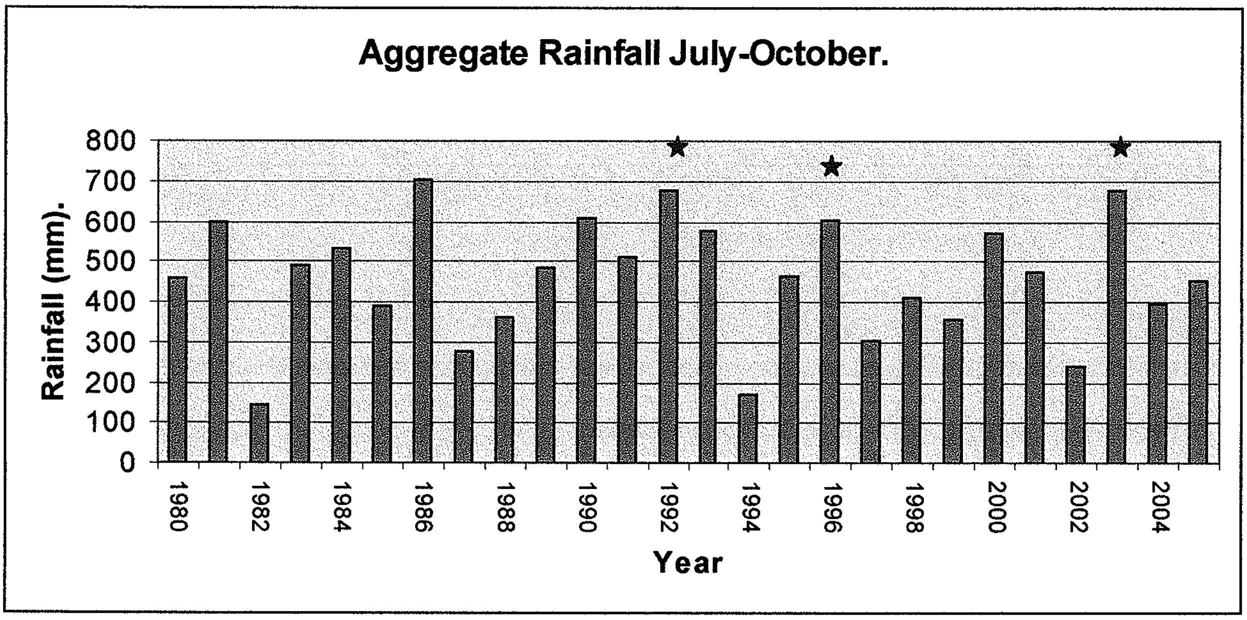
Table 2. Impact of Bubbly Bark on Chestnut trees at monitoring sites.

Monitoring site.	Total Trees Assessed.	Bubbly Bark present or Tree has been replaced.	% Affected	Very unhealthy or dead trees*.	% Very unhealthy or dead.
1	162	37	23	77	48
2	89	51	57	42	47
3	60	49	82	24	40
4	82	72	88	31	38
5	64	20	31	19	30
6	94	25	27	19	20
7	75	50	67	15	20
8	71	18	25	6	8
9	75	9	12	4	5
10	57	5	9	3	5
11	112	109	97	5	4
12	198	129	65	7	4
All	1139	574	50.4	252	22.1

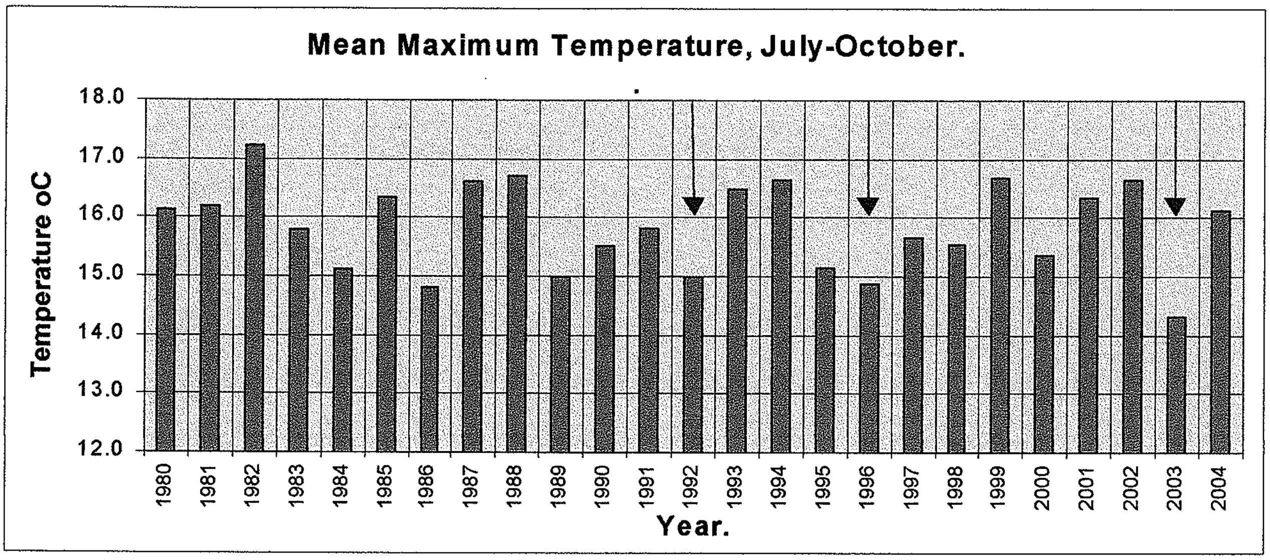
* Very unhealthy or dead = Tree Health categories 5-8 plus replaced or missing trees.

Pre-disposing factors
Weather:
The three most significant years for the incidence of Bubbly Bark have been 1992, 1996 and 2003. Based on the Department of Sustainability's Ovens weather records these three years have had the highest rainfall during July-October (Graph 1), and also the lowest mean maximum temperatures during July-October (Graph 2) since 1987. The chance of either the rainfall or the lowest mean maximum temperatures coinciding with the three most significant years for the incidence of Bubbly Bark between 1991 and 2005 is one in 455 (P<=0.002).

Graph 1. Rainfall for July-October, Ovens.



Graph 2. Mean Maximum Temperature July-October, Ovens.



The lowest minimum temperature, the lowest average minimum temperature, the number of frosts, the highest maximum temperature or the highest average maximum temperature do not correlate to the years of high Bubbly Bark incidence.

Therefore the mild, wet late winter/early spring periods coincide with high incidence of Bubbly Bark.

Soil Nutrition:

Correlation between Bubbly Bark incidence and soil nutrient levels:

The correlation between Bubbly Bark incidence and soil nutrient levels was tested using the regression tool in Microsoft Excel 97. Soil pH and salinity were also tested. For each nutrient or parameter measured, the level obtained was plotted against the incidence of Bubbly Bark and a regression was fitted. An R^2 value of 1 means that there is a perfect correlation between the parameter and the incidence of symptoms, while a value of 0 indicates that there is no correlation. F values are obtained from statistical tables and are used to indicate whether to accept or reject an hypothesis. Using this type of analysis there were no soil nutrients which had a significant ($P<=0.05$) correlation with Bubbly Bark incidence (Table 3). Salinity and soil pH also did not correlate with Bubbly Bark incidence. Salinity ranged between 0.05 and 0.14 dS/m (1:5 water), while pH ranged between 4.7 and 6.3 (1:5 water). The large standard deviation recorded for some nutrients indicates that there is a diverse range of soil conditions and/or fertiliser management between the monitoring sites.

Differences in soil nutrient levels:

The results of the soil analysis are shown in Table 4. The differences in soil nutrient levels under Bubbly Bark affected trees and non-affected trees were tested using a t-Test for paired samples. The paired samples were collected from the same monitoring sites under Bubbly Bark affected trees and under non-affected trees. The levels of calcium were significantly ($P=< 0.05$) higher in the soil from affected trees. The pH was also significantly higher in the soil from affected trees.

Table 3. Levels of, & Correlation between Soil parameters and Bubbly Bark incidence.

Analysis	Unit	R^2 value.	Significance level of F.	Mean of all samples.(n= 15)	Standard Deviation
Phosphorus (Olsen)	mg/kg.	0.104	0.64	20.23	17.19
Phosphorus (Colwell).	mg/kg.	0.003	0.80	57.06	17.19
Potassium (Colwell).	mg/kg.	0.009	0.67	301.19	141.42
Sulphur (KCL40).	mg/kg.	0.100	0.15	10.34	6.72
pH (1:5 water).		0.000	0.96	4.82	0.51
Salinity (EC, 1: water).	dS/m	0.006	0.72	0.08	0.03
Chloride.	mg/kg.	0.101	0.15	15.39	7.97
Manganese.	mg/kg.	0.112	0.13	23.75	21.72
Total Nitrogen.	%	0.012	0.62	0.23	0.05
Copper (DTPA).	mg/kg.	0.003	0.82	1.77	4.17
Zinc (DTPA).	mg/kg.	0.001	0.88	1.54	1.19
Iron (DTPA).	mg/kg.	0.005	0.76	86.04	36.70
Boron (HWS).	mg/kg.	0.032	0.42	0.51	0.18
Hydrogen (Exch).	meq/100 g.	0.024	0.49	0.14	0.11
Aluminium (Exch).	meq/100 g.	0.014	0.60	0.55	0.57
Calcium (Exch).	meq/100 g.	0.000	1.00	6.98	2.68
Magnesium (Exch).	meq/100 g.	0.173	0.054	1.48	0.71
Sodium (Exch).	meq/100 g.	0.000	0.95	0.07	0.05
Potassium (Exch).	meq/100 g.	0.061	0.27	0.71	0.35

Table 4. Soil nutrient levels in Bubbly Bark affected trees and non-affected trees.

Analysis	Unit	BB affected (n=8)		BB non-affected (n=8)		P(T<=t) 2Tail.	Signif-icance
		Mean	Std. Dev.	Mean	Std. Dev.		
Phosphorus (Olsen)	mg/kg.	16.44	12.77	19.05	14.77	0.371	n.s.
Phosphorus (Colwell).	mg/kg.	46.12	38.96	53.75	45.67	0.360	n.s.
Potassium (Colwell).	mg/kg.	296	180.7	314	179.5	0.565	n.s.
Sulphur (KCL40).	mg/kg.	10.76	9.07	10.01	6.37	0.668	n.s.
pH (1:5 water).		5.64	0.60	5.84	0.59	0.018	*
Salinity (EC, 1: water).	dS/m	0.076	0.029	0.072	0.012	0.675	n.s.
Chloride.	mg/kg.	15.62	9.62	15.00	10.34	0.608	n.s.
Manganese.	mg/kg.	26.36	23.93	26.9	22.45	0.719	n.s.
Total Nitrogen.	%	0.231	0.061	0.234	0.069	0.891	n.s.
Copper (DTPA).	mg/kg.	1.05	0.49	4.15	8.07	0.320	n.s.
Zinc (DTPA).	mg/kg.	1.60	1.45	2.10	1.49	0.254	n.s.
Iron (DTPA).	mg/kg.	84.7	36.95	85.3	33.19	0.817	n.s.
Boron (HWS).	mg/kg.	0.40	0.11	0.45	0.17	0.407	n.s.
Hydrogen (Exch).	meq/100 g.	0.132	0.14	0.106	0.13	0.157	n.s.
Aluminium (Exch).	meq/100 g.	0.516	0.63	0.318	0.50	0.184	n.s.
Calcium (Exch).	meq/100 g.	6.44	2.62	7.90	3.23	0.041	*
Magnesium (Exch).	meq/100 g.	1.21	0.53	1.44	0.71	0.089	n.s.
Sodium (Exch).	meq/100 g.	0.063	0.03	0.073	0.04	0.286	n.s.
Potassium (Exch).	meq/100 g.	0.695	0.42	0.736	0.44	0.553	n.s.

n.s. = not significant. * = significant at P<=0.05.

Foliar nutrients

Correlation between Bubbly Bark incidence and foliar nutrient levels:

The correlation between Bubbly Bark incidence and foliar nutrient levels was tested using the regression tool in Microsoft Excel 97 (Table 5). The nutrient levels were those for non-affected trees in a Bubbly Bark affected orchard. Using samples for non-affected trees showed the level of nutrients that are potentially available to healthy trees. The difference in nutrient levels between affected and non-affected trees is tested below (Table 6). There were no nutrients that showed any significant correlation with Bubbly Bark levels.

Difference in foliar nutrient levels:

The results of the foliar analysis are shown in Table 6. The difference in foliar nutrient levels in Bubbly Bark affected trees and non-affected trees were tested using a t-Test for paired samples. The paired samples were collected from the same monitoring site from Bubbly Bark affected trees and from non-affected trees. The levels of potassium were significantly (P=< 0.05) higher (1.01% vs 0.88 %) in the affected trees. The levels of manganese were also higher in the affected trees (1141 vs 778 mg/kg) but the differences were not significant.

Table 5. Correlation between Bubbly Bark incidence and Foliar nutrients.

Analysis	**Unit**	**R^2 value.**	**Significance level of F.**	**Statistical significance.**
Total nitrogen.	%	0.004	0.78	n.s.
Phosphorus.	%	0.022	0.51	n.s.
Potassium.	%	0.009	0.67	n.s.
Calcium.	%	0.299	0.18	n.s.
Magnesium.	%	0.001	0.92	n.s.
Sodium.	%	0.159	0.48	n.s.
Chloride.	%	0.022	0.51	n.s.
Manganese.	mg/kg	0.043	0.35	n.s.
Copper.	mg/kg	0.014	0.60	n.s.
Zinc.	mg/kg	0.088	0.70	n.s.
Iron.	mg/kg	0.007	0.71	n.s.
Boron.	mg/kg	0.029	0.45	n.s.
Sulphur.	%	0.053	0.30	n.s.
Molybdenum.	mg/kg	0.016	0.57	n.s.
Aluminium.	mg/kg	0.077	0.21	n.s.

n.s. = not significant at P=<0.05.

Table 6. Foliar nutrient levels in Bubbly Bark affected trees and non-affected trees.

Analysis	**Unit**	**Affected trees. (n=8)**		**Non-affected trees. (n=8)**		**P(T<=t).**	**Statistical significance.**
		Mean	Std. Dev.	Mean	Std. Dev.		
Total nitrogen.	%	2.66	0.401	2.62	0.399	0.46	n.s.
Phosphorus.	%	0.17	0.046	0.18	0.041	0.38	n.s.
Potassium.	%	1.01	0.312	0.88	0.270	0.009	*
Calcium.	%	1.30	0.500	1.15	0.408	0.07	n.s.
Magnesium.	%	0.38	0.058	0.42	0.120	0.29	n.s.
Sodium.	%	0.014	0.010	0.016	0.014	0.27	n.s.
Chloride.	%	0.18	0.034	0.17	0.025	0.50	n.s.
Manganese.	mg/kg	1141	1163	778	869	0.054	n.s.
Copper#.	mg/kg	6.68	0.904	6.38	0.821	0.31	n.s.
Zinc.	mg/kg	48.5	31.7	37.4	18.4	0.33	n.s.
Iron.	mg/kg	93.5	20.2	93.1	32.8	0.96	n.s.
Boron.	mg/kg	30.1	13.1	35.7	16.6	0.27	n.s.
Sulphur.	%	0.20	0.056	0.20	0.043	0.51	n.s.
Molybdenum.	mg/kg	0.03	0.014	0.025	0.008	0.28	n.s.
Aluminium.	mg/kg	290.8	160	331.2	199	0.08	n.s.

n.s. = not significant. * = significant at P<=0.05. .

One sample was not included due to an extremely high reading (202 compared to mean 6.68 mg/kg).

Environmental and Orchard management

Slope

Ground slope of monitored orchards varied between flat and 13°. There was no significant relationship between slope and Bubbly Bark incidence (R^2=0.203, F significance=0.43).

Elevation

Elevation of monitored orchards varied between 260 and 780 metres. There was no significant relationship between elevation and Bubbly Bark incidence (R^2=0.009, F significance=0.97).

Aspect

Aspect of monitored orchards included all of the eight major compass points except due south. There was no significant relationship between aspect and Bubbly Bark incidence (R^2=0.076, F significance=0.79).

Previous land use

The dominant land use prior to chestnuts was pasture, followed by tobacco and then wattle/blackberry mixture. Other uses included apples and Oregon plantations. Pasture featured in orchards with the high, medium and low incidence of Bubbly Bark. There were too few of other land uses to complete a statistical analysis, but on the basis of pasture it appears that previous land use is a most unlikely factor in Bubbly Bark incidence.

Pruning

Trees at all monitoring sites were pruned. Given that Bubbly Bark incidence varied from zero to 97% at these sites it appears that pruning is not a factor causing Bubbly Bark.

Pruning intensity
Pruning intensity was rated as low or moderate-high. Sites with low intensity pruning averaged 28% incidence while heavy pruning sites averaged 49% incidence. A T-test, assuming equal variance, showed that pruning intensity made no significant difference to Bubbly Bark incidence [P(T<=t) two tail 0.19].

Weedicide
Glyphosate was used at all monitored orchards on at least an annual basis. The use of glyphosate and weed control are therefore not factors contributing to Bubbly Bark incidence. A T-test, assuming equal variance, was used to test the difference of Bubbly Bark incidence in orchards with one or multiple applications of glyphosate per year. There was no significant difference in Bubbly Bark incidence with single or multiple applications [P(T<=t) two tail 0.89]. Sites with annual applications averaged 36% incidence of Bubbly Bark while sites with more frequent applications averaged 38% infection.

Adjacent vegetation
The proximity of olives, apples and eucalypt forest to the monitoring sites were noted or estimated. There were no significant correlation between distance to a particular crop or vegetation and Bubbly Bark incidence (R^2=0.087, F significance=0.74 for olives, R^2=0.218, F significance=0.42 for apples, and R^2=0.096, F significance=0.71 for eucalypt forest). Local olive growers indicated that they were unaware of any recent disease in olives in the region.

Rootstock
The rootstock(s) used at half the monitoring sites were either unknown or were mixtures of several. These unknowns did not allow for a statistical analysis of the relationship between rootstock and Bubbly Bark incidence. Menzies is the most commonly used known rootstock. Bubbly Bark incidence varied from very low through to very high in orchards with Menzies rootstock.

Irrigation
Irrigation is widely, but not universally used in chestnut orchards. A T-test, assuming equal variance, was used to test the difference of Bubbly Bark incidence in irrigated and non-irrigated orchards. Irrigation made no significant difference to Bubbly Bark incidence [P(T<=t) two tail 0.31].

Varieties
Most varieties appear to be susceptible to Bubbly Bark, at least to some extent. Of the varieties observed MP varieties appear to be least susceptible (Table 7), while Perdons Pride and Buffalo Queen appear to be the most susceptible

Table 7. Varietal susceptibility to Bubbly Bark.

Summary	Total trees observed.	% Affected by Bubbly Bark.	% Very sick or Dead
MP varieties	57	7	2
Colossal	11	9	9
Spanish Red	176	56	13
Di Coppi	349	72	14
Wandi Wonder	75	68	20
Bouche de Betizac	94	30	22
Perdons Pride	165	65	40
Buffalo Queen	91	40	53
Average	1018	57	22

Pathogen testing

Bubbly Bark affected trees were sent to the School of Forest and Ecosystem Science (University of Melbourne) laboratories at Heidelberg for observation and testing. The first tree was delivered on 22/10/2005 while the next three trees were delivered on 11/12/2005. The trees were tested for a range of pathogens and were observed for several months growing in the glasshouse. The trees continued to exhibit Bubbly Bark symptoms while they grew in the glass house but no pathogens were found. The Senior Pathologist concluded that "the symptoms may be caused by either an abiotic factor or a physiological factor of the grafted plant.

Table 8 provides a summary of pathogen testing for Bubbly Bark. There have been over twenty samples provided to five laboratories over a period of fifteen years. This has provided a range of entomologists and pathologists to study Bubbly Bark symptoms over time and from a wide geographical area. A pathogen specific to Bubbly Bark has not been identified. There have been a number of organisms identified, most have been considered as secondary invaders, but none have been consistently identified as likely pathogens. With this record it must be seriously questioned if there is a pathogen involved in the cause of Bubbly Bark.

Nursery monitoring

Two nurseries were monitored. Each was visited in December 2005 and January 2006.

At both nurseries there were many trees affected with "Bacterial Blast", a condition which causes leaves to curl and develop brown dry patches along the leaf. Some leaves shrivel, curl and turn light brown or dark brown to black. This condition did not appear to relate directly to the presence of Bubbly Bark.

At one nursery there were some trees with thick soft bark, a condition that could be a factor in Bubbly Bark. However the frequency was only about 2% in the nursery, and far below the frequencies recorded in the orchards. Seedlings and grafts were generally healthy.

At the second nursery there were seedlings exhibiting Bubbly Bark symptoms. There was bud rejection by seedlings, regrowth of buds from the base and sometimes from the stem, and the Bubbly Bark was apparent on some of the thicker stems. In December the proportion of healthy trees (from a sample of 400) was 22%. This had improved to 49% a month later.

Table 8. Summary of Pathogen Testing for Bubbly Bark.

Date	Laboratory	Submitted by	Results.
Early 1990's	Knoxfield (Vic).	John Pianegonda. Six samples.	No pathogens.
28/1/1993	Heidelberg (Vic).	Ray Borschmann.	Botryosphaeria obtusa (Black Rot) Schizophyllum commune (White Rot) Xyloborus perforans (Black borer)
16/12/2003	Knoxfield (Vic).	Darren Gasket.	No pathogens.
22/12/2003	Knoxfield (Vic).	Don Nightingale.	No pathogens.
22/10/2005	Heidelberg (Vic).	Ray Borschmann (ex Joe Sgambelloni)	No pathogens to date.
3/12/1999	Auckland, NZ.	Ray Knowles (?) Eight samples.	Shoot pathogens*: Botryosphaeria parva ++ Fusicoccum luteum + Pestalotiopsis maculans ++ Diaporthe perniciosa + Microsphaeropsis sp. + Phomopsis castaneae +++++ Root pathogens: Cylindrocarpon lucidem + Fusarium oxysporum +++ Phomopsis castaneae + Cylinrocladium florianum +++ Macrophomina phaseolina ++ Pythium sp +
20/12/2005	DPI Stanthorpe, Queensland.	Stephen Foster	No pathogens.
2/12/2005	Menangle, NSW.	Stephen Foster.	Pseudonomas spp. (at a low frequency)
8/11/2005	Menangle, NSW.	T&E Powell.	Hafnia alvei
11/12/2005	Heidelberg (Vic).	Ray Borschmann (ex Kim Brownhill).	Saprophytic fungi only – no pathogens.

* None of the shoot pathogens detected would be likely to cause extreme ill thrift and mortality.
What the shoot results tell us is that:

1. Sap flow has been weak (Botryosphaeria and Fusioccum), &
2. Tree thrift has allowed some pathogens to be aggressive (Diaporthe, Pestalotlopsis & Phomopsis).

Scale of ++ indicates relative frequency of isolation. + = trace, +++++ = consistent isolation.

DISCUSSION

The incidence of Bubbly Bark has become more intense and widespread in north east Victoria during the last decade. Its presence has resulted in cumulative death rates of about 3% over wide areas of the region, but in parts of some chestnut orchards the death rate has been up to 48%.

Many factors have been shown not to be correlated with Bubbly Bark incidence, including:
- Soil salinity,
- Soil nutrient levels of phosphorus, potassium, sulphur, magnesium, chloride, manganese, total nitrogen, copper, zinc, iron, boron, hydrogen, aluminium and sodium.
- Foliar nutrient levels of phosphorus, sulphur, chloride, manganese, total nitrogen, copper, zinc, iron, boron, aluminium, magnesium, molybdenum, calcium and sodium.
- Environmental factors including slope, aspect, elevation and adjacent vegetation.
- Management practices including irrigation, pruning, pruning intensity, weed control, use of glyphosate and previous land use.

Relevant observations include:
- Seedling trees rarely have Bubbly Bark; they are almost invariably grafted trees.
- Many trees regrow from their base if the main trunk dies. It is rare for trees to reshoot above the graft. New shoots can grow in a healthy manner for over 15 years.
- Trees that are healthy in December do not develop the Bubbly Bark symptoms for the remainder of the growing season.
- Bark symptoms may be present in winter or early spring. Some of the affected trees may suffer bud death, while others may grow vigorously.
- It appears to affect all planted varieties to at least some extent.
- The age of trees affected is usually between two and ten years, and most commonly between two and six years. Older trees are infrequently affected, but younger trees may be affected.

Factors which may help solve the Bubbly Bark dilemma include:
- Bubbly Bark is most prevalent in spring when the July-October rainfall is high.
- Bubbly Bark is most prevalent in spring when the mean maximum temperatures for July-October is at its lowest.
- No pathogens have been found despite the study of over 20 Bubbly Bark infected trees being sent to five pathology laboratories over a period of 15 years.
- Calcium soil levels and soil pH are significantly higher under Bubbly Bark affected trees compared to non-affected trees.
- Potassium levels are significantly higher in Bubbly Bark affected trees compared to non-affected trees.

Combining these factors with the above observations it would appear that Bubbly Bark is a physiological response to certain environmental conditions, rather than a response to a pathogen.

It is likely that wet, mild July-October periods cause some reaction in the tree that produces the bubbling of the bark. If the tree is strong enough or the conditions improve the tree regains at least some vigour.

Additional support for this hypothesis includes:

- The Senior Pathologist conclusion that the symptoms may be caused by either an abiotic factor or a physiological factor of the grafted plant.
- David Ogilvy (2005) being able to reproduce Bubbly Bark symptoms by applying copper and winter oil in early spring.
- The absence of Bubbly Bark until the late 1980's was possibly due to grafted trees not being planted in a reasonable quantity until about 1985, and 1986 being the only very wet July-October between 1981 and 1990.
- Trees in the nursery improving their health and vigour during December-January as the soil warms.

Experience in New Zealand supports the link between low maximum temperatures in July-October, but not so the high rainfall. Soils in New Zealand drain very easily and therefore water logging or high water content does not appear to be a factor.

TECHNOLOGY TRANSFER

This report will be presented to the Chestnut Growers of Australia for their consideration. Options to transfer the findings to their membership and other growers include:

- Publishing the findings in the Australian Nut Grower.
- Presenting the findings at a field day or the annual meeting of the CGA.

RECOMMENDATIONS

Scientific

To follow up the research on Bubbly Bark cause and management the following recommendations are made:

- Monitor a number of orchards to determine which are the more Bubbly Bark resistant varieties.
- Analyse rootstocks to test any differences to Bubbly Bark susceptibility.
- Carry out a trial with young trees to test a range of moisture levels and if possible temperatures during July-October. These could be pot trials.
- Continue with nutritional testing with particular emphasis on soil calcium and foliar potassium levels, but also including soil magnesium and foliar manganese, calcium and aluminium levels.
- Maintain a pathogen testing at least at a low level.
- Continue to monitor the monitoring sites established in 2005.

Industry

If Bubbly Bark is a physiological reaction to wet, mild conditions in late winter and spring, actions which improve soil drainage in the wet years may help to avoid or reduce Bubbly Bark. Monitor soil water levels in orchards in July-October and if necessary improve drainage. Avoid irrigating to excess, particularly early in the season.

Management practices such as pruning, weed control, fertilising and liming do not appear to affect Bubbly Bark. It appears to be reasonable to continue such current practices without predisposing the orchard to Bubbly Bark.

Monitoring the presence of Bubbly Bark will help build a database about the condition. Particular written note should be taken of the variety, rootstock and severity, with a number of sites being monitored over many years. Unaffected as well as affected sites should be included in the monitoring.

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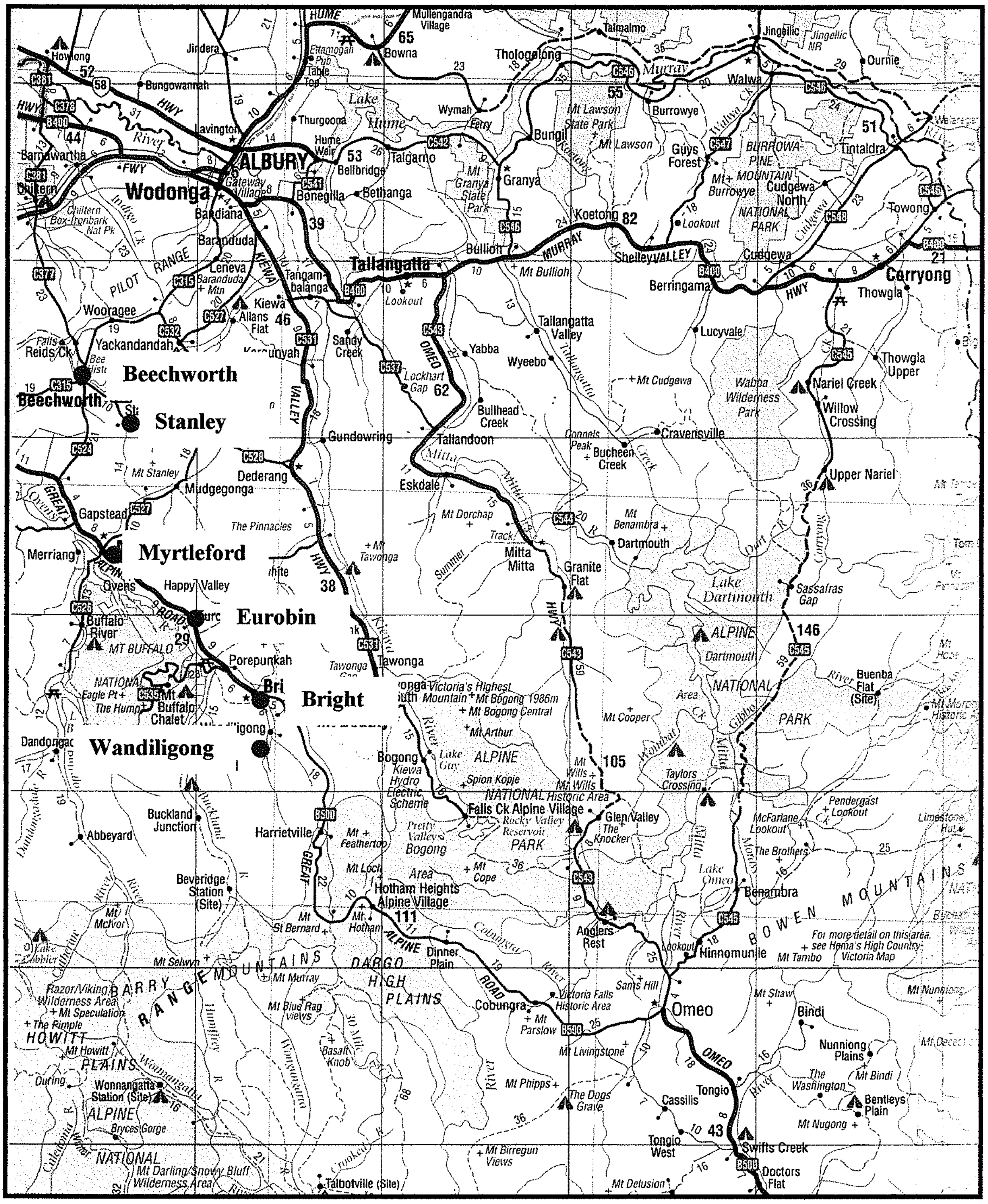
Map 1. Extent of Bubbly Bark in north east Victoria.

Map of north east Victoria showing locations of Stanley, Myrtleford, Wandiligong, Bright and Eurobin where Bubbly Bark is common.



Appendix 1. Survey of Bubbly Bark occurrence (for growers with monitoring sites).

CGA BUBBLY BARK PROJECT.	
	Date:
Bubbly Bark Occurrence.	
Grower.	
Address.	
Contacts.	
Orchard Location.	
Number of trees.	
Age range.	
Bubbly Bark occurences.	
First year noticed.	
No. trees affected.	
No. trees killed.	
Peak year.	
No. trees affected.	
No. trees killed.	
Other significant years.	
Is it getting more frequent?	
Is it more widespread?	
Growers comments (causes of BB, pre-disposing factors, control methods etc).	

Appendix 2. Monitoring site survey – orchard history.

CGA BUBBLY BARK PROJECT.		
		Date:
Orchard History.		
	Affected Orchard	Non-affected Orchard
Previous land use.		
Planting year.		
Tree variety.		
Rootstock variety.		
Source of planting stock.		
Tree size: Height.		
Diameter.		
Pruning practice.		
Intensity.		
Frequency.		
Sun scorch on trunks.	Present/Absent	Present/Absent
Soil:		
Parent rock.		
0-50 cm texture.		
50-100 cm texture.		
Drainage		
Soil preparation for planting.		
Weed control method.		
Herbicides used.		

Nutrition		Affected.		Non-affected.	
	(g/kg)	Foliage.	Soil.	Foliage.	Soil.
	Nitrogen				
	Sulphur				
	Phosphorus				
	Potassium				
	Calcium				
	Magnesium				
	Sodium				
	(mg/kg)				
	Aluminium				
	Iron				
	Manganese				
	Zinc				
	Copper				
	Boron				
			pH		pH
Fertiliser applied					
Fungicides used.					

Appendix 3. Monitoring site survey – Environmental data.

		Affected Trees	Non-affected Trees
Average rainfall.			
Irrigation - Type			
	Frequency		
	Volume/ha.		
Position in landscape.			
	Valley floor.		
	Break of slope.		
	Mid-slope.		
	High slope.		
	Ridge or plateau.		
Maximum slope (degrees).			
Aspect (degrees)			
Altitude.			
Closest native forest (km).			
Closest apples.			
Other crops.			

Appendix 4. Assessment of tree health and Bubbly Bark presence.

Scoring for Bubbly Bark presence and Crown Health.

1. Crown healthy. Health illustrated by vigorous growth of healthy, green shoots and dark green leaves.
2. Bubbly Bark symptoms on the bark. Leaves may or may not be healthy.
3. Foliage not completely healthy. Shoots not vigorous, leaves may be droopy, but tree not terminally ill.
4. Foliage a degree worse than 3. Crown is thinning.
5. Crown is notably sparse.
6. Crown is very sparse and unhealthy.
7. Tree almost dead. Few green shoots that are unlikely to recover.
8. Tree dead above the ground.