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by John Doran, Dave Lea and David Bush

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Foreword

This research indicates that it may be possible to select cultivars of *Backhousia citriodora* (lemon myrtle) with greater myrtle rust resistance than the moderately to highly susceptible cultivars that are currently in use by the native food, essential oil and horticultural industries in Australia.

It is well recognised that different research approaches will be required to address problems faced by both industries and the environment as we respond to the management of myrtle rust. The presence of the gene-pool planting of lemon myrtle at Beerburrum, in South-east Queensland where myrtle rust is currently prevalent provided an ideal opportunity to observe, monitor and select for the variable responses of this important native food species to myrtle rust. This research has provided specific insights into resistance breeding within the species and has also provided learnings in relation to other disease management options for this crop.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report is an addition to RIRDC's diverse range of over 2100 research publications and it forms part of our Essential Oils and Plant Extracts R&D program. RIRDC's vision for this program is of a profitable and sustainable industry producing essential oils and plant extracts of the quality and content that meets customers' evolving demands.

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Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

Thanks are due to Alan House, Grant White and other former staff of the Queensland Forestry Research Institute, Gympie who were involved in establishing the Beerburrum *Backhousia* genebank. Stephen Midgley former leader of the research program in CSIRO Forestry and Forest Products including the Australian Tree Seed Centre approved the funding that supported the range wide seed collections of the species. Reg Lockyer and his wife are thanked for directing one of us (Dave Lea) to the interesting Silver Valley population and striking cuttings of the ten clones included in the genebank. We acknowledge the recent assistance of Mick White, Senior Ranger of DERM (Maleny), for firebreak slashing, in obtaining permits to burn heaps of cut stems and facilitating access to the site. Ken Old, formerly of CSIRO, is acknowledged for helpful comments on the report and particularly for providing details of this internationally important disease. David Lee of Agri-Science Queensland is thanked also for his cooperation in allowing these assessments to take place.

Abbreviations

ATSC	Australian Tree Seed Centre of CSIRO Plant Industry
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEEDI	Queensland Department of Employment, Economic Development and Innovation (now Agri-Science Queensland - Department of Agriculture Fisheries and Forestry)
DERM	Queensland Department of Environment and Resource Management

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Executive Summary

What the report is about

This research indicates that it may be possible to select cultivars of *Backhousia citriodora* (lemon myrtle) with greater myrtle rust resistance than the moderately to highly susceptible cultivars that are currently in use by the native food, essential oil and horticultural industries in Australia. This should reduce the need for growers to use fungicides to control the disease.

Who is the report targeted at?

This report is targeted at the Australian native food, essential oil and horticultural industries that utilise *Backhousia citriodora* as a source of citral-rich leaves and as an ornamental.

Where are the relevant industries located in Australia?

The Australian native food industry is the industry most likely to benefit most from this research. northern Queensland and north eastern New South Wales are the main locations where lemon myrtle leaf is produced. The largest planting (1.2 million plants) is at Palm Grove near Proserpine in northern Queensland complemented by multiple smaller plantings (± 10 000 plants) in northern New South Wales and south eastern Queensland (RIRDC 2012). Accurate production figures are unavailable. RIRDC (2012) estimates a total farm gate value at between $7 million and $23 million for dried leaf and essential oil.

Background

Backhousia citriodora (lemon myrtle) is a small- to tall-sized tree (2 to 30 m) occurring naturally in several, small disjunct populations in Queensland. It is grown in plantations for its leaves that contain a citral-rich essential oil. The leaves are used commercially for production of lemon-flavoured herbal teas, culinary herbs or as a source of oil that is used as a food flavouring and in personal care products. It is also used as an ornamental shrub in the nursery trade. Cultivars of *B. citriodora* currently in use for these purposes are moderately to highly susceptible to an internationally important disease of plants of the family Myrtaceae that is new to Australia, *Puccinia psidii* sensu lato (s.l.) (syn *Uredo rangelii*) or myrtle rust as it is commonly called in Australia and Guava rust elsewhere. Myrtle rust is jeopardising the future of this species in the organic native food industry. Myrtle rust frequently forms purplish lesions with abundant spores on young leaves and shoots which may die-back as a result of rust attack. Rust affected leaves of *B. citriodora* are unsuitable for its main uses and the application of chemical sprays to control the disease is unsustainable in a market which demands a clean green product. The native food industry, therefore, is in urgent need of rust-resistant cultivars of *B. citriodora* if this part of the industry is to prosper into the future.

Aims/objectives

The principal aim of this project was to identify if there was any heritable myrtle rust (*Puccinia psidii sensu lato* (*s.l.*) *syn Uredo rangelii*) resistance in a 1995-96 genepool planting of *B. citriodora* (lemon myrtle) seedlots and clones at Beerburrum in south eastern Queensland. Any rust resistant plant types (phenotypes) found in the trial were to be marked during the final project assessment in May 2012.

Methods used

A genepool planting of *B. citriodora* provenances, families and clones established near Beerburrum in south eastern Queensland in 1995-96 by CSIRO Forestry and Forest Products (now part of CSIRO Plant Industry) and Queensland Forestry Research Institute (now part of Agri-Science Queensland) provided the genetic resources for this project. Populations from throughout the known natural range of the species in south eastern and northern Queensland were represented.

This project commenced in November 2011, two months after coppicing, with the relabelling of the treatments and a preliminary assessment of the level of rust attack on each of the 1500 plants present. Rust was observed on 30% of plants but at low levels and it was deemed by the authors to be too early to reliably rate rust susceptibility of the coppicing plants at this stage. The second and final visit to the site under this project was in May 2012, eight months after coppicing. Plant height was in the range of 1-2 m with each plant carrying many new 'soft' shoots with some carrying abundant rust spores. A subjective assessment was undertaken during 2-6 May 2012 to gauge the incidence and relative severity of rust attack (eight category scoring system) on individual plants in the Beerburrum trial. The presence or absence of stem rust was also noted and the vigour of the coppice regrowth was assessed as either vigorous, average or slow.

Results/key findings

Families of northern Queensland origins had, on average, higher levels of rust attack than southern Queensland sources. The clones from the atypical, prostrate form from Silver Valley, the most northern population present, were the exception at the time of this assessment. Ramets of all clones were attacked but only at a very low level. The clones were of compact shape which would facilitate their being maintained as a hedge through mechanical harvesting.

Variation in incidence and severity of rust attack was noted between and within families within provenances providing opportunities for selecting the most resistant phenotypes. Family 1010 from Woondum in southern Queensland had the largest number of progeny (19) showing no signs of rust attack at this time. Several of these plants and plants from other families that had low rust scores combined with vigorous coppice growth have been flagged for future work.

If a number of these selections maintain their resistance to myrtle rust in further trials on other sites then the most vigorous of them will be candidates for release to industry to replace the moderate to highly susceptible cultivars in current use.

Implications for relevant stakeholders for:

Identification and release of rust resistant cultivars of *B. citriodora* will be a boon for the Australian native food industry utilising this species. It will also be a positive for the nursery trade and essential oil producers.

The identification in this myrtaceous species of some inherent resistance to myrtle rust offers hope that similar resistance will be found in other Australian species that are presently under heavy attack.

Recommendations

It is recommended that in a follow-on project: (i) the preliminary rust-resistant selections in the Beerburrum trial of *B. citriodora* seedlots and clones be vegetatively propagated into separate breeding populations ; (ii) that the trial be coppiced and assessed again to affirm the level of resistance recorded in the first assessment. This should be undertaken in collaboration in the first instance with the Australian native food industry, once intellectual property arrangements are in place with the trial custodians, CSIRO ATSC and Agri-Science Queensland. These new breeding populations will form the basis for further studies of rust resistance in this species and be the prime source of rust-resistant cultivars for the Australian native food, essential oil and horticultural industries using *B. citriodora*.

Introduction

Backhousia citriodora (lemon myrtle) is a shrub to tall-sized tree, 2 m to 30 m in height and 10 cm to 60 cm in stem diameter at breast height, with persistent rough, grey to light brown bark and a bole that is frequently buttressed at the base. Tree habit varies greatly throughout its range from a mallee on exposed sites to a forest tree on soils that are fertile and moist but also well drained. Crown break is usually at about half tree height. Lemon myrtle is a Queensland rainforest species that occurs naturally in several, small disjunct populations over a wide geographical area (Figure 1). Altitude varies from 40 m at Eumundi in the south to 700 m at Lead Creek, Silver Valley in the north of the species' range. The Silver Valley population is interesting morphologically in that a prostrate, creeper-form of the species predominates in this relatively arid environment with only the occasional plant attaining mallee form to 6 m. Clearly this is a population at the boundaries of the species' environmental range. The species is presently well protected in Queensland National Parks or in State Forests and Reserves, although some populations were lost to clearing and particularly in southeast Queensland in earlier times.

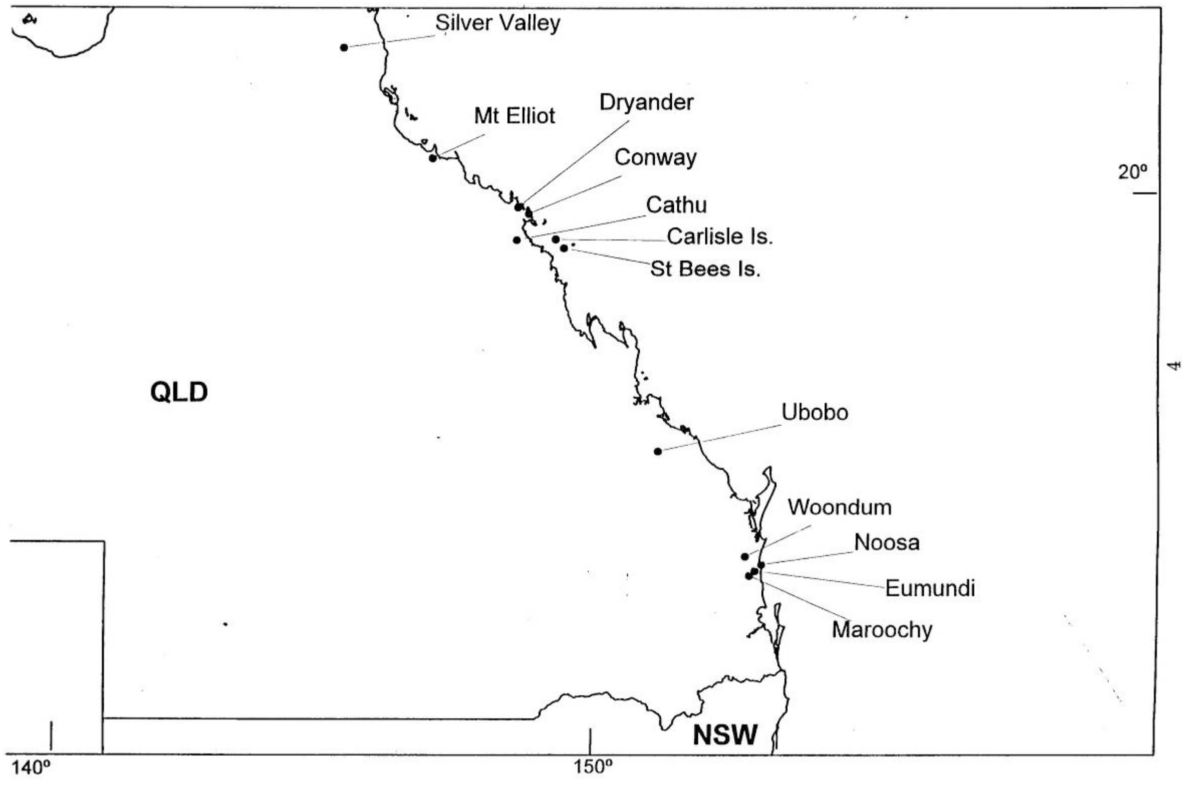


Figure 1: **Sites in Queensland where *Backhousia citriodora* occurs naturally. All these sites were sampled for seed and clones (Silver Valley) in the early 1990s and propagules established in the Beerburrum genebank in 1995-96. All provenances except Ubobo were represented in the genebank when monitored for myrtle rust attack in 2011-12 (this project).**

The leaves of lemon myrtle are rich in a lemon-scented essential oil. Two lemon-smelling chemotypes with oils that comprise of mainly citronellal or citral (neral and geranial) have been found in the species (Brophy *et al*. 1995; Doran *et al*. 2001). The citral type is by far the most common while the citronellal form is of no commercial value at present. The stop-start history of the commercial exploitation of the citral-rich *B. citriodora* leaves is documented by Archer (2004). Since the early 1990s plantations have been the source of leaves used in the production of lemon-flavoured herbal teas, culinary herbs or essential oil that is used as a food flavouring and in personal care products. Presently northern Queensland and north eastern New South Wales are the main locations where lemon myrtle leaf is produced. The largest planting (1.2 million plants) is at Palm Grove near Proserpine in northern Queensland complemented by multiple smaller plantings (± 10 000 plants) in northern New South Wales and south eastern Queensland (RIRDC in press). Accurate production figures are unavailable. RIRDC (2012) estimates a total farm gate value at between $7 million and $23 million for dried leaf and essential oil. The species is also a popular ornamental marketed by the nursery trade.

Cultivars of *B. citriodora* currently in use for these purposes are moderately to highly susceptible to an exotic rust fungus of Australian plants of the family Myrtaceae, *Puccinia psidii* sensu lato (s.l.) (syn *Uredo rangelii*) (Carnegie and Cooper 2011). It has been referred to commonly as myrtle rust in Australia. Myrtle rust is a member of the eucalyptus/guava rust (*Puccinia psidii* sensu stricto) complex which has caused substantial mortality and economic losses amongst young (< 3 m tall) *Eucalyptus* plantations in parts of Brazil (Tommerup *et al*. 2003). Guava or eucalyptus rust, the disease's common names outside of Australia, has spread from Brazil to other parts of South and Central America, the Caribbean, China, California, Florida where it is acting as a biological control agent of *Melaleuca quinquenervia* in the Everglades, Hawaii and Japan (Invasive Species Council 2011, Morin *et al*. 2012). Australia could well be added to this list once the identity of myrtle rust is confirmed beyond doubt.

Myrtle rust in Australia was first observed in April 2010 on nursery stock in the Central Coast of New South Wales. The origins of the disease are not known. It has since spread south to Victoria and north to Queensland where it is common in the southeast and has been reported as occurring in central and northern parts of the state including in the wet tropics south and north of Cairns. Myrtle rust has a wide host range and, as Australia has an abundant resource of Myrtaceous plants to the east of the Great Dividing Range in the climatic zone most suitable for the disease; further sightings of disease outbreaks in new areas are anticipated. Facilitating the diseases' spread are the robustness of its numerous microscopic rust spores (longevity of 10 to 90 days depending on conditions), their ease of transport on the wind or by animals (e.g. bees, flying-foxes, birds), humans and on goods and on the many available host plants (Invasive Species Council 2011).

The young leaves and shoots of seedlings, the outer growing tips of the crowns of saplings and in some cases adult trees (e.g. *Melaleuca quinquenervia*) and coppice from stumps or damaged trees are most vulnerable to attack by myrtle rust. The rust causes spots or lesions on young leaves and shoots that spread and develop masses of yellow powdery spores. The rust can also infect floral buds and young fruit depending on the host. Infected leaves become curled and distorted and severe infection can cause shoots to die causing these plants to become stunted after repeated infections. In the worst cases, death of the whole plant can occur. The potential negative impacts of this newly introduced disease on Australian plants and ecosystems are extremely serious. For any one plant species, the extent of the damage will depend in part on severity of infection and the extent of any inherent resistance to the disease. CSIRO in collaboration with partner agencies in Australia, Brazil and South Africa have carried out guava/myrtle rust host screening in glasshouse trials since the early 2000s and ranked the susceptibility to the rust of a wide range of Australian myrtaceous plant genera at the species, provenance and individual plant level (e.g. Tommerup *et al*. 2003; Zauza *et al*. 2010; Morin *et al.* 2012). This work indicated that there could be some level of resistance present in many species. For example, Zauza *et al.* (2010) screened 102 seedlots of species of *Corymbia*, *Eucalyptus* and *Melaleuca* by artificially infecting seedlings with *P. psidii* race UFV-02. Large differences in resistance to the rust were detected between species, between provenances within species and between seedlots of the same provenance. Zauza and co-workers stressed, however, that only one pathotype of *P. psidii* was tested and that species or individuals classed as resistant may still produce lesions bearing spores and thus facilitate the spread of the disease. Brazilian plantations now use eucalypt clones that are genetically resistant to Guava rust. However, pathogens can outpace resistance-breeding programs through sexual recombination or mutation and a pathotype of *P. psidii* has occurred in Brazil that is now infecting some of the previously resistant clones (Graça *et al.* 2011). In the case of myrtle rust only one strain of the fungus is present in Australia at this time and quarantine agencies are keen to try to prevent other strains from entering the country (Invasive Species Council 2011).

Myrtle rust is jeopardising the future of *B. citriodora* in the organic native food industry. Rust affected leaves of *B. citriodora* are unsuitable for its main uses and the application of chemical sprays to control the disease is unsustainable. The native food industry, therefore, is in urgent need of rust-resistant cultivars *B. citriodora* to replace the limited number of rust susceptible cultivars presently used if this part of the industry is to prosper into the future.

Objectives

The principal aim of the project was to identify if there is any heritable resistance to myrtle rust (*Puccinia psidii* sensu lato (s.l.), syn *Uredo rangelii*) in a 1995-96 genepool planting of *Backhousia citriodora* (lemon myrtle) seedlots and clones at Beerburrum in south eastern Queensland. Any rust resistant plant types (phenotypes) will be marked in the trial during the final assessment in May 2012. It is proposed that, if rust-resistance is found in the taxa, these selections be vegetatively propagated into separate breeding populations in a follow-on project. These populations will form the basis for further studies of rust resistance in this species and be the prime source of rust-resistant cultivars for release to the native food and other industries using *Backhousia citriodora*.

Methodology

Plant Materials

This research took place in a genepool planting of *Backhousia citriodora* provenances, families and clones established near Beerburrum in south eastern Queensland in 1995-96 by CSIRO Forestry and Forest Products (now part of CSIRO Plant Industry) and Queensland Forestry Research Institute (now part of Agri-Science Queensland). Maps of the planting are given in Appendix 1. *Backhousia citriodora* takes up 18 rows of the planting with one row (row 5) dedicated to *Syzygium anisatum* (syn *B. anisata*). The *B. citriodora* rows consist of plant material propagated mainly from seed, but also comprising ten clones from one remote population in Silver Valley. Most of the known natural range (16 families from four south eastern Queensland provenances [521 plants surviving], forty-one families from six northern Queensland provenances [229 plants surviving] and ten clones [175 plants surviving] from one far northern Queensland population) of the species is represented in the trial. Seven (rows 7 to 13) rows (567 plants surviving) and plots of 4 families (14 plants surviving) include plants that are not currently identified by their provenance, although a search of Agri-Science Queensland files is currently underway to hopefully provide information on their origins (D. Lee pers comm.). Figure 1 gives the geographic location of the provenances represented in the Beerburrum planting which range from Maroochy in the south to Silver Valley in the north and include families from two Whitsunday Islands (Carlisle Island and Saint Bees Island). One family from Ubobo was planted but there were no surviving plants of this provenance in 2012.

The planting had been left untended by the collaborators for several years because of lack of interest in the genetic material it contains. By 2011 the trees were about 4 m tall and suffering the competitive effects of close spacing (1 m between rows and 0.5 m between plants within rows) and lack of tending. RIRDC were made aware of the availability of the planting in 2011 and recognised its potential importance in studying variation in susceptibility to naturally invading myrtle rust spores in this species. They funded separately the cutting back of the plants by one of us (Dave Lea) to about a 30-50 cm stump height in September 2011.

Methods

This project commenced in November 2011 with a six day visit to the site by the authors to identify and label families and to undertake a preliminary assessment of the level of rust attack on each individual plant. Each of the 1500 plants present on the site was inspected for evidence of myrtle rust attack and rated using a four point scoring system (not given here). At this point, two months from coppicing, most of the stools were just developing new coppice shoots and leaves and these proved impossible to score. The usually abundant new and mature leaves on branches below the coppice cut were used. Rust spores were observed on plants (446 plants or 30% of population infected) throughout the trial but nowhere was the rust ubiquitous and appeared confined to only a few leaves per plant at this time. It was deemed to be too early to reliably rate rust susceptibility of the coppicing plants at this stage.

The next visit to the site was in May 2012, eight months after the plants in the trial were cut back and coppiced. Plant height was in the range of 1-2 m with each plant carrying many new 'soft' shoots (Figure 2). The preceding summer and start to autumn had been wet and humid so conditions were assumed to be ideal for rust attack. Plants throughout the trial were first inspected for rust attack which was plentiful and a scoring system developed (see Table 1) to indicate the incidence and relative severity of rust attack on individual plants. During 2-6 May each plant in the Beerburrum trial was inspected and scored. The vigour of the coppice regrowth was also assessed as either vigorous, average or slow. The trial was visited again by one of us (D. Lea) during the week commencing 20 May 2012 and further observations made and photographs taken.

Figure 2: **General views of the Beerburrum *Backhousia citriodora* genebank planting in May 2012, eight months after coppicing**

Table 1: Scoring system used to categorise the incidence and relative severity of rust attack on individual plants

Disease Score	Description	Images
1	No visible symptoms	
2	Purplish spots or blotches only	
3	Purplish spots or blotches with very sparse sporulation	 
4	Purplish spots or blotches with very obvious sporulation at 5 levels:	
4/1	One or few sporulated lesions on one or very few leaves (low level of infection)	 
4/2	Multiple sporulated lesions on several leaves (low level of infection)	 

Disease Score	Description	Images
4/3	Multiple sporulated lesions on many leaves and shoots (moderate level of infection)	
4/4	Abundant sporulated lesions on growing tips and shoots (high level of infection)	
4/5	Abundant sporulated lesions on growing tips and shoots with dead, dying and deformed leaves and shoots (high level of infection)	
St	Sporulation on stems of flush growth and petioles. Usually observed on plants with moderate to severe (4/3, 4/4 or 4/5) sporulation on their leaves	

Statistical analysis

The trial was established as a contiguous, rectangular gene-pool planting. There was no formal statistical design applying to the overall planting, though two parts of the block planting were established as stand-alone randomised-complete-block designs: the first four rows which comprise 6-tree row plots of 16 south eastern Queensland families replicated 4 times and the unidentified plants in rows 7 to 13 that are planted in four replications (see Appendix 1). Despite this limitation, plots of families of various sizes ranging from 1-39 trees were established as line plots and families were replicated three times on average. It was possible, therefore to 'post-block' the planting by overlaying incomplete blocks allowing a mixed-model analysis making use of the entire dataset.

Traits

Disease scores were analysed as two separate traits. The leaf disease scores were converted to an eight-point scale, which resulted in an approximately normally distributed trait. Stem disease, which was measured as present or absent on each plant, was summarised as plot mean proportion of affected trees. The growth rank was analysed both as a response variate and as a covariate with the leaf disease score.

Restricted maximum likelihood analysis of variance in each measured trait was carried out using a general linear mixed-model of the form:

[Eq. 1] $$y = \mathbf{X}b + \mathbf{Z}u + e$$

where y is the vector of observations on n traits, b and u are vectors of fixed and random effects respectively, $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices for fixed and random model terms and e is a vector of random residual terms. Variants of this model were applied as follows:

1. *Assessment of disease variation among taxa*

An analysis of variation of each trait at the taxon (i.e. provenance and clone) level was carried out using Genstat 13 (VSN International, Hemel Hempstead). The vector *b* (Eq. 1) contained a sub-vector for the fixed taxon effect, while *u* contained sub-vectors for the random row, column, plot and residual effects. For the leaf and stem rust traits, the growth variable and its interaction with taxon were included as fixed-effect covariates.

2. *Variation among clones within Silver Valley*

This analysis included the clonal material only. Vector *b* (Eq. 1) contained sub-vectors for the fixed effect of clone, and *u* contained sub-vectors for the random effects of plots rows and columns. For the leaf and stem rust traits, the growth variable and its interaction with clone were included as fixed-effect covariates.

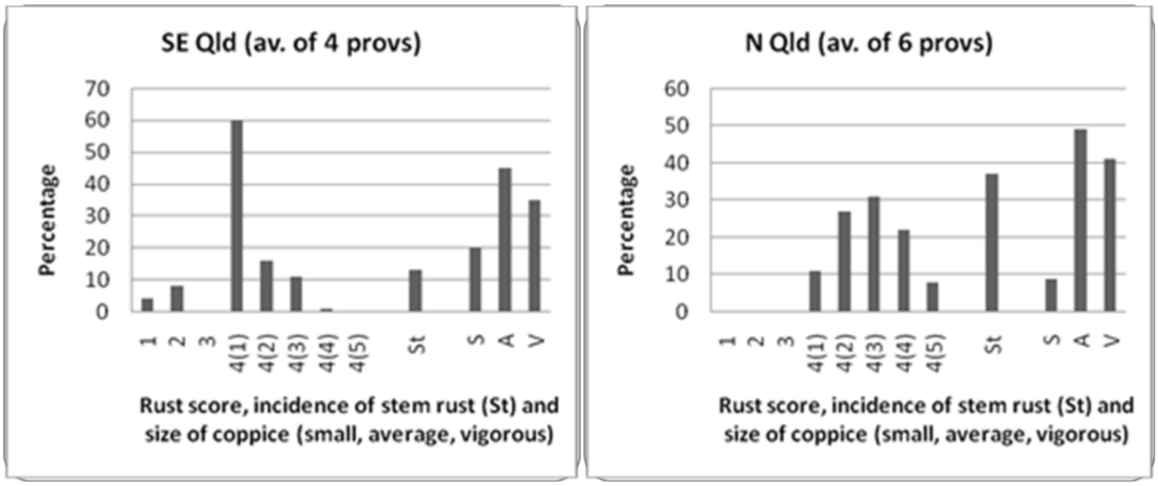
3. *Assessment of disease variation among families-within-provenances*

An analysis of variation of each trait at the family-within-provenance level, excluding the clonal entries, was carried out. The vector *b* (Eq. 1) contained a sub-vector for the fixed provenance effect, while *u* contained sub-vectors for the random family-with-provenance, row, column, plot and residual effects. For the leaf and stem rust traits, the growth variable and its interaction with family-within-provenance were included as fixed-effect covariates.

Results

Number of families (or clones) and individual plants representing each provenance and the percentage of plants in each category for rust score (see Table 1), incidence of stem rust (St) and vigour of coppice growth (vigorous [V], average [A], slow [S]) as assessed on 2-6 May 2012 are given in Table 2.

This raw data shows a substantial difference between regions of occurrence (south eastern vs northern Queensland) in incidence and severity of rust attack amongst the plants grown from seed (Figure 3 A & B). Of the 521 plants assessed of confirmed south eastern Queensland origins, four percent (9 plants) had clean leaves with no visible signs of rust attack (score: 1) and a further 11 percent (42 plants) had a low incidence of purplish blotches of their leaves with no spores evident (score: 2). All 229 plants of northern Queensland seedlots showed some level of spores present so none were placed in these categories (neither Score 1 or 2). There was also a major difference between regions in severity of attack. Sixty-one percent of plants of northern Queensland origins had moderate to high levels of infection (score: 4/3 and above) compared to only twelve percent of plants from south eastern Queensland. Thirty-seven percent of plants of northern Queensland origins had spores on shoots and petioles compared to thirteen percent for southern Queensland origins. In terms of vigour of growth of the coppices, plants of northern Queensland origins tended to be more vigorous than southern origins but presumably this will change over time due to the effects of more severe rust attack.



A B

Figure 3: **Average of percentage of plants by rust score, incidence of stem rust (St) and size of coppice (small [S], average [A] and vigorous [V]) for four south eastern Queensland provenances of *Backhousia citriodora* (A) compared to averages for the six northern Queensland provenances (B) propagated from seed.**

Table 2: **Number of families (or clones) and individual plants representing each provenance and the percentage of plants in each category for rust score (see Table 1), incidence of stem rust (St) and vigour of coppice growth (vigorous [V], average [A], slow [S]) as assessed on 2-6 May 2012.**

Provenance	No of fams	Plant nos	1	2	3	4(1)	4(2)	4(3)	4(4)	4(5)	St	S	A	V
NQld clones														
Silver Valley	10	175		2		70	13	15			9	3	91	6
NQld families														
Mt Elliot	4	43				7	19	23	42	9	14	12	86	2
Dryander	5	51				12	25	33	20	10	37	10	43	47
Conway	13	64			2	9	38	37	8	6	53	6	33	61
Cathu	2	12						33	42	25	25	8	50	42
Carlisle Island	11	36				8	20	36	28	8	53	6	39	55
St Bees Island	5	7				30	40	17	13		13	17	57	26
Totals/Averages	40	229/			0.4	11	27	31	22	8	37	9	49	41
SE Qld families														
Woondum	10	216	9	11		69	6	5	0.5		4	28	49	23
Noosa	2	44		18	2	57	23				5	22	39	39
S. Maroochy	3	26		12		38	31	15	4		8	62	23	15
Eumundi	6	235		3		55	21	18	3		23	8	46	46
Totals/Averages	21	521/	4	8	0.2	60	16	11	1		13	20	45	35
Unkown origin		581	0.2	6		86	6	1	0.3		4	10	49	41
Grand Tots/Av		1506/	2	5	0.1	64	13	11	4	1	12	12	53	35

Differences among provenances and clones

Significant differences (p<0.001) among provenances and clones were found for leaf rust and vigour traits, but not stem rust (p=0.17) (Figures 4-6). Figure 4 shows that the most northern provenances (Mt Elliott, Dryander and Conway) had more severe leaf infection than both those further south and the northern Silver Valley clones. Figure 5 shows that stem infection was generally lower in the southern provenances than the northern, though there were no significant differences among provenances and clones. Grouping the provenances into northern and southern regions of provenance (divided between St Bees Island and Woondum) and re-running the provenance and clone-level analysis for stem rust confirmed the significant difference between northern and southern regions (p=0.003) with predicted means of 22.6% and 11.1% for northern and southern respectively.

There were minor differences in vigour among provenances, with the S. Maroochy being the least vigorous (Figure 6). Mt Elliot was less vigorous than the rest of the northern group. Vigour, and its interaction with provenance/clone were also added as covariates to the analyses of the leaf and stem rust traits. In neither case was vigour a significant covariate, but for both leaf and stem rust, the provenance-by-vigour interaction was significant (p=0.002 and p=0.03 respectively).

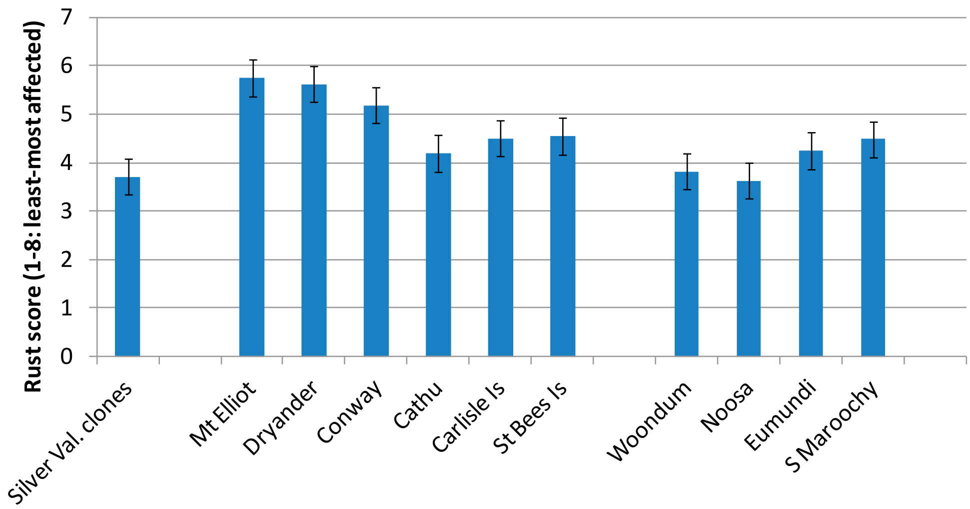


Figure 4: **Leaf rust index – provenance and clone means (northern-most to southern-most, left to right) with average standard errors of difference**

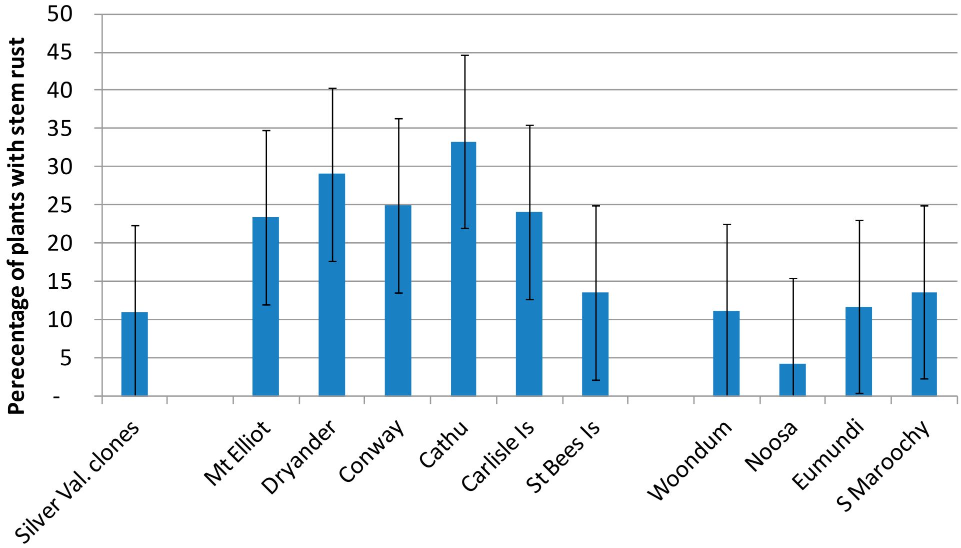


Figure 5: **Stem rust index (% of plants with visible stem rust) – provenance and clone means (northern-most to southern-most, left to right) with average standard errors of difference**

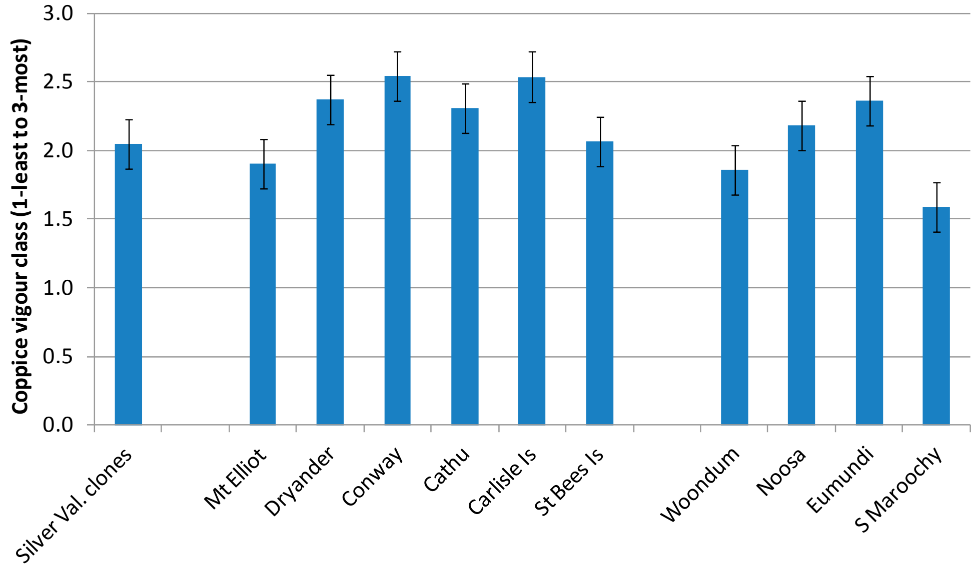


Figure 6: **Plant vigour index – provenance and clone means (northern-most to southern-most, left to right) with average standard errors of difference**

Difference among Silver Valley clones

There was no significant difference for the rust traits among the Silver Valley clones. There was a significant difference ($p<0.001$) in the vigour trait, with clone RL4 being the least vigorous (predicted mean 1.74) and clone RL3 being the most (predicted mean 2.4). The vigour-by-rust index interaction terms were not significant.

The leaves of the clones with spores present were invariably at the base of ramets where the leaves had been amongst grass growing unchecked between the rows of plants. This combined with the wet conditions preceding the assessment may have promoted rust attack on otherwise resistant leaves. The clones appeared different morphologically to their seedling counterparts. Although of healthy appearance, they were generally shorter by up to 0.5 m to adjacent seedlings and the leaves were firmer possibly due to their greater physiological age (Figure 7).

Figure 7: Compact growth form of ramets of ten Silver Valley clones of *Backhousia citriodora* with the taller growth of seed-derived plants evident behind the assessor. The tall grass growth between the rows of plants throughout the trial was cut before the assessment.

Family-within-provenance and plant-within-family variation

Family-within-provenance variance component estimates for the traits were generally of low precision, with standard errors being as large as the component estimates for leaf rust and vigour, and substantially larger than the component for stem rust. Small samples of families within provenances in many cases will have contributed to the lack of precision.

Variation between plants-within-family in rust incidence and severity of attack was most clearly expressed in Woondum family 1010 (Figure 8) which was planted in plots throughout the trial. Nineteen plants (17%) in this family of 109 plants had clean leaves with no visible signs of rust attack (score: 1). It was the only family from Woondum and, in fact the only family in the entire trial, that produced plants that appeared immune to myrtle rust attack at the time of this assessment.

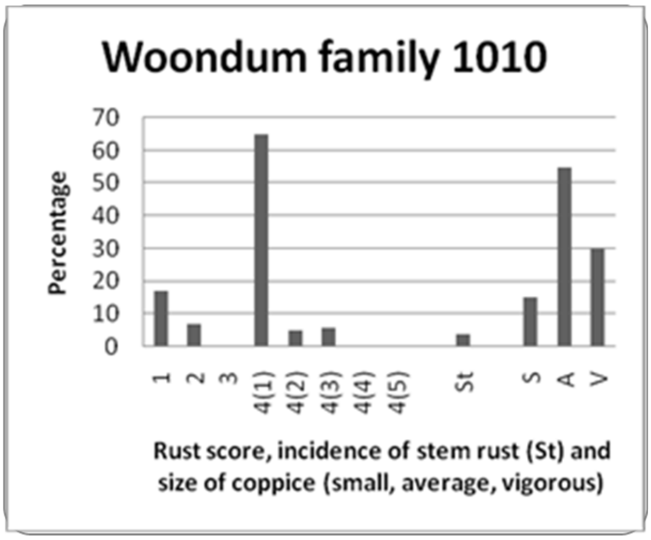


Figure 8: **Variation between plants of Woondum family 1010 in rust score, incidence of stem rust (St) and size of coppice (small [S], average [A] and vigorous [V])**

Identity of unlabelled plants in rows seven to thirteen

The identities of families (567 plants) in rows seven to thirteen were not given in the available documentation at the time of assessment. These rows provided many of the trees selected for further work (Appendix 2) as they displayed a low level of rust attack in combination with vigorous coppice growth. The graph of their average rust scores (Figure 9) strongly suggests that they are families of southern Queensland origins. A search of archival records is underway to try and trace the origins of this material.

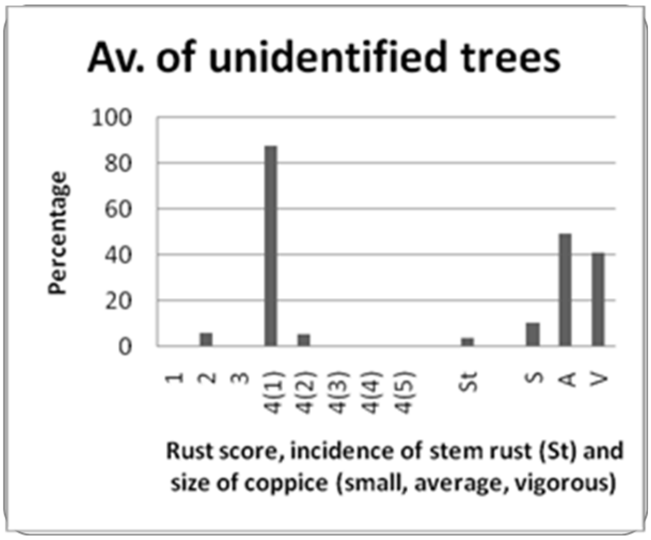


Figure 9: **Average rust score, incidence of stem rust (St) and size of coppice (small [S], average [A] and vigorous [V]) for the trees in rows 7 to 13 of unknown identity**

Selection of trees for further rust screening

Trees with a low level of rust attack and good vigour and deemed by the assessors to warrant further observation for rust resistance were marked with flagging tape as the assessment progressed. These trees are identified in Appendix 2.

Discussion

The trial appears to offer opportunities to select amongst the provenances and families present for greater rust resistance than the cultivars presently in use by industries utilising *B. citriodora*. Some of the phenotypes marked for further work, and particularly those in Woondum family 1010, appeared to be immune to the disease at his stage while others were subject to a very low level of attack. Caution is warranted, however, because there was evidence of increased rust attack during the brief visit to the trial in late May, three weeks following the main assessment. As we know little about the seasonal patterns of rust attack at this stage, the propagation out of the trial and further testing of the apparent resistant phenotypes on a range of sites is recommended before release of any clones to industry. The uncertainty about the future survival of the trial, established as it is on a site now earmarked for railway realignment, adds further justification for the early propagation and transfer of rust resistant phenotypes to other sites representative of where *B. citriodora* is being planted.

It is also recommended that the natural population at Woondum in south eastern Queensland be sampled again for seed and new, family-identified breeding populations be established on multiple sites to screen for rust resistance. This will be particularly important if further strains of myrtle rust develop, as is likely the case (K. Old pers comm. 2012), potentially leading the rust strains that are more aggressive than the existing strain. If resistance genes are identified in *B. citriodora* by the research planned at the Australian National University, then DNA analysis techniques can be applied to this material to speed up the selection of new rust resistant genotypes.

The leaves of the Silver Creek clones appear stiff and more mature than the leaves on other northern Queensland provenances propagated from seed. The advanced physiological age of the leaves of the clones might account for their apparent greater rust resistance. Old (pers. comm. cited in Cannon 2011) reports that, while most rusts rely on penetrating leaves through their stomata, Eucalyptus rust penetrates through the cuticle. Thus, as the cuticle thickens with leaf age, the rusts' ability to create an infection is reduced. Most of the rust infection of the Silver Creek clones at Beerburrum was on the lower leaves that were growing amongst grass thus increasing wetness and humidity. The top of clonal explants had a very low level of attack. This combined with the low stature and bushiness of the explants may present opportunities for them to be maintained as a hedge employing a different form of mechanical harvesting than is presently employed in the *B. citriodora* native food industry.

Implications

Identification and release of rust resistant cultivars of *B. citriodora* will be a boon for the Australian native food industry utilising this species. It will also be a positive for the nursery trade and essential oil producers. This will not be a one-off task. The industry will need to gear up for an on-going program of screening of plants of broad genetic base for rust resistance and regular release of new clones. Clone deployment, both in space and time, will also be of importance to maintain an equilibrium between presence in plantations of resistant plants and the variation in pathogenity of the rust (K. Old pers comm. 2012).

The identification in this myrtaceous species of some inherent resistance to myrtle rust offers hope that similar resistance will be found in other Australian species that are presently under heavy attack.

Recommendations

The following recommendations flow from this research:

1. Seek as a matter of urgency clarity from Queensland authorities as to their timetable for development of the Beerburrum site. This will determine the urgency or otherwise for action on the following recommendations pertaining to the plant materials at Beerburrum.

2. Identify a range of new trial sites representative of where *B. citriodora* is grown commercially and where collaborators (private and government) are interested in participating in establishing, maintaining and monitoring clones and seedlots for rust resistance.

3. Propagate vegetatively the most promising, rust resistant selections from the Beerburrum trial into new clonal breeding populations where they will be intensively screened for rust resistance, vigour of growth and oil quality. Some of the most susceptible selections should also be propagated vegetatively and incorporated in the design as disease 'spreaders' to ensure that the rust is uniformly present in the new trials. Three activities should be undertaken as prerequisites to 3: (a) formal agreement should be sought from the custodians of the Beerburrum genebank, CSIRO PI and Agri-Science Queensland, documenting the terms and conditions of access to the plants the trial contains; (b) the Beerburrum selections should be assessed again for their rust resistance before propagation to ensure that the early May 2012 scores have been maintained; and (c) the genebank should be coppiced and fertilised in spring of 2012 to stimulate vigorous coppice growth suitable for propagating as stem cuttings.

4. Commission new seed collections from natural stands. The first priority is to collect as many families as possible in Woondum provenance of *B. citriodora*. These seedlots should be propagated and established in progeny trials to screen for rust resistance. The progeny trials would be carried out most efficiently in parallel with the clonal trials proposed in 3 above. As a second objective, collect seed from as many individual plants as practical throughout the species range to allow establishment of a replacement for the Beerburrum gene-pool planting. This new ex-situ conservation planting of the species is particularly important in light of the susceptibility of the far northern provenances of the species to myrtle rust.

5. Maintain the Beerburrum genebank and continue to regularly monitor rust attack on families throughout the planting. This will provide baseline data on seasonal and yearly variation in rust attack and its long-term effect on families of varying susceptibility to myrtle rust. The genebank contains seedlots from throughout the species natural range. It will serve as an important resource of known genetic material for seeking out rust resistance genes in *B. citriodora* and could be used for a wide range of other research activities. This will depend on the continuing availability of the trial as determined in 1 above.

Appendices

Appendix 1: Maps of the layout of families in the Beerburrum *Backhousia citriodora* genebank. Although mapped in sections as material was planted, the trial is, in fact, one contiguous block.

SE QLD *B.citriodora*
Planted January 1995
(6 tree line plots per family @ 0.5m spacing)

HOOP HEDGES

1010	1026	1029	1025	R
1031	1016	1030	1013	E
1028	1009	1023	1012	P
1027	1011	1014	1024	1
1030	1012	1013	1029	R
1010	1014	1023	1025	E
1024	1031	1026	1011	P
1009	1016	1027	1028	2
1025	1029	1012	1014	R
1026	1009	1027	1013	E
1030	1031	1023	1016	P
1028	1024	1011	1010	3
1010	1012	1024	1027	R
1031	1028	1014	1030	E
1025	1009	1026	1029	P
1023	1013	1011	1016	4
ROW 1	ROW 2	ROW 3	ROW 4	

NQ 1st Collection
Planted August 1995
(6 tree line plots per family @ 0.5m spacing)

B. anisata	*B. citriodora*	
1072(4)/1071(2)	1379(4)/1337(2)	
1071	1343	
1068	1331	
1069	1356	
1067	1330	
1067	1343	
1069	1343	
1069	1331	
1068	1330	1337(2)/1353(4)
1068	1343	1355
1067	1343	1330
1068	1330	1342
1067	1356	1329
1069	1356	1330
1071(2)/1070(4)	1342	1331
1072	1381(2)/1352(4)	1341(2)/1344(1)/1345(3)
ROW 5	ROW 6	ROW 7

NQ 2nd Collection *B. citriodora*
Planted October 1997
(trees @ 0.5m spacings; family i.d. x no. planted)

			1468 - 2			
			1454 - 4	1025 - 1		
			1459 - 1	***4 - 4		
			1028 - 3	1455 - 9		
1023 - 2			1461 - 1	1455 - 3		
RL 2 - 20			1462 - 1	1491 - 1		
RL 1 - 15			1452 - 2	1474 - 3	1465 - 1	
			1016 - 2	1027 - 5	1023 - 3	
			1012 - 1	1473 - 1	1012 - 2	
	1009 - 4	1016 - 5	1023 - 3	1471 - 9	1010 - 21	
	RL 8 - 8	1027 - 39	1014 - 1	1424 - 1	1010 - 28	
	RL 7 - 30	DL1325-1	1468 - 5	1428 - 5	1470 - 4	
	RL 6 - 9	DL1236-3	1029 - 4	1431 - 10	1470 - 5	
	RL 5 - 23	DL1227-3	1464 - 1	1430 - 8	1010 - 19	
	RL 4 - 19	RL10 - 33	1466 - 2	1433 - 5	1452 - 4	
	RL 3 - 6	RL 9 - 15	1458 - 2	1462 - 8	1451 - 2	
			1010 - 6	1461 - 9	1450 - 2	
			1011 - 2	1467 - [illegible]	1449 - 4	
			1015 - 2	1453 - 2	1447 - 3	
			1025 - 13	1457 - 1		
			1014 -2	1456 - 1		DL1486-1
			1027 - 10	1458 - 2		DL1424-6
			1025 - 5	1458 - 5		DL1430-9
			1016 - 9	1454 - 4		DL1431-8
			1027 -16			1010 - 38
ROW 13	ROW 14	ROW 15	ROW 16	ROW 17	ROW 18	ROW 19

B. citriodora Planted December 1995
(trees @ 0.5m spacings; refer spreadsheet for clonal/family i.d.)

Rep 1 Trees 49 - 98	Rep 1 Trees 1 - 98	Rep 2 Trees 3 - 98	Rep 3 Trees 55 - 98	Rep 3 Trees 1 - 98	Rep 4 Trees 9 - 98	
		Rep 1 Trees1-2	Rep 2 Trees 1 - 54		Rep 3 Trees1-8	Rep 4 Trees 1 - 60
ROW 7	ROW 8	ROW 9	ROW 10	ROW 11	ROW 12	ROW 13

B. citriodora (Set 10/11/91)
Planted February 1998
(trees @ 0.5m spacings; family i.d. x no. planted)

DL1227-1
DL1325-1
DL1227-3
DL1325-2
DL1227-1
RL9 - 4
DL1227-1
DL1323-1
DL1325-1
ROW 19

Appendix 2: Location and details of trees tentatively selected for further screening for their apparent rust resistance and good vigour (to be used in the field in conjunction with the booking sheets)

Row (Rep)	Family	Tree	Score	Row (Rep)	Family	Tree	Score
2 (1)	1009	3	2/1/0/A	11	-	37	2/1/0/V
8	-	25	2/1/0/A	11	-	70	4/1/0/V
8	-	32	2/1/0/A	11	-	72	4/1/0/V
8	-	44	4/1/0/V	12	-	8	4/1/0/V
8	-	50	1/0/0/A	12	-	37	4/1/0/V
8	-	78	2/1/0/V	12	-	41	2/1/0/V
8	-	90	4/1/0/Vv	17	1027	2	4/1/0/V
9	-	28 & 29	4/1/0/AV	18	1450	1	4/1/0/V
9	-	48	2/1/0/A	18	1010	2	4/1/0/A
9	-	51	2/1/0/V	18	1010	16	1/0/0/V
9	-	73	4/1/0/V	18	1010	17	1/0/0/V
9	-	92	4/1/0/V	18	1010	18	1/0/0/V
10	-	3	2/1/0/V	18	1010	2	1/0/0/A
10	-	5	2/1/0/V	18	1010	20	4/1/0/V
10	-	21	2/1/0/A	19	1010	5	1/0/0/A
10	-	30	4/1/0/V	19	1010	16	1/0/0/A
10	-	45	4/1/0/V	19	1010	17	4/1/0/V
10	-	73	4/1/0/A	19	1010	20	1/0/0/A
10	-	77	4/1/0/A	19	1010	28	1/0/0/A
10	-	81	4/1/0/V	19	1010	30	1/0/0/A
11	-	1	2/1/0/A	13	RL 1, 2	selected	ramets
11	-	11	2/1/0/V	14	RL 3,4	selected	ramets
11	-	16	4/1/0/V	14	RL 5,6	selected	ramets
11	-	19	4/1/0/V	14	RL 7,8	selected	ramets
11	-	30	4/1/0/V	15	RL 9,10	selected	ramets

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Assessing Myrtle Rust in a Lemon Myrtle Provenance Trial

By John Doran, Dave Lea and David Bush

Pub. No. 12/098

Lemon myrtle is one of several native food species under threat from myrtle rust, a well know fungal disease of international importance first found in Australia in April 2010.

This research indicates that it may be possible to select cultivars of *Backhousia citriodora* (lemon myrtle) with greater myrtle rust resistance than the moderately to highly susceptible cultivars that are currently in use by the native food, essential oil and horticultural industries in Australia.

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