Australian Native Plants Association (Australia)

Banksia Study Group Newsletter No. 25.

ISSN 1444-285X

Issue 25: Autumn 2020.

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IN THIS ISSUE

Banksia Study Group at Albany bi-annual ANPSA Conference.

The Banksia Study Group was well represented and well received at the ANSPA Conference in Albany in September 2019.We were thrilled to be able to showcase many of our beautiful S.W. W.A. banksias during the course of the conference. Thanks to the many conference patrons who we met at our study group display stand and for the wonderful compliments received. In a study group talk forum, we welcomed fifteen new members to the Banksia Study Group. We look forward to your contributions and banksia snippets.

Banksia Farm was honoured to contribute with the wildflower displays on the stage and named banksia displays at our stand. See pics below.

We also contributed displays for the Dryandra Study Group stand (Dryandras, of course!) Many banksia lovers who hadn't in the past had the opportunity to visit our unique complete banksia collection on our Banksia Farm property in Mount Barker did so with the Mount Barker day tour conference options.

Fifteen species; *candolleana, serrata X aemula, pilostylis, telmatiaea, grossa, petiolaris, incana var. incana, quercifolia, meisneri var. ascendens, repens, blechnifolia, gardneri var. gardneri, micrantha, ilicifolia and oreophila*.

Twelve species; *coccinea (orange & red), praemorsa (burgundy & yellow), speciosa, prionotes X menziesii, littoralis, grandis, aemula, menziesii (bronze, pale & dark pink), ericifolia var. micrantha, ericifolia var. ericifolia, ericifolia "giant candles' and conferta var. conferta.*

Nine species; *coccinea (orange & red), aquilonia (gold, lime & mauve), paludosa ssp paludosa, marginata, spinulosa var. spinulosa, media, integrifolia var. monticola, integrifolia var. integrifolia and ericifolia "limelight*".

Eight species*; solandri, laevigata ssp laevigata, caleyi (bud), lemanniana (bud), dryandroides (bud), attenuata (bud), spinulosa "birthday candles", spinulosa "stumpy gold".*

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ANPSA Conference Albany 2019.

I, Kevin, was delighted to be one of the speakers in the 'Grow' category of the conference and presented my talk 'Mastering Banksia Growing'. I also presented a small segment on the Banksia Study Group. Key points for discussion were; a local dieback project on B. *coccinea*, ongoing partnership development of the new banksia garden in the Australian National Botanic Gardens (ANBG) in Canberra, the continued discontent with amalgamation of dryandra and banksias and the possible new edition of 'Banksias' for 2020. The Wildflower Society of Western Australia (WSWA) conference organising committee, whom we were able to assist a little, did an amazing job considering they all reside in Perth. The conference was a huge success with a record attendance (342), a fascinating group of key note speakers, a terrific venue and the conference tours were extremely well planned and ran to schedule. Congratulations to all concerned.

Featured below are a few of the banksias seen on the ANPSA post-conference tour to Esperance.

B. violacea at Roe hill (Newdegate Road). Photo Kathy Collins.

B. violacea low coastal form Cave Point in FNP. (Fitzgerald National Park).

B. nutans var. nutans at Thistle Cove (immature follicles)
& B. lemanniana at West Mount Barren**.**

Beautiful new foliage, immature cones and inflorescences of *B. speciosa* at Lake Monjingup near Esperance.

Floriferous plant and immature cone of B*. speciosa* five years post bushfire at Lake Monjingup near Esperance.


B. repens at Duke of Orleans Bay. *H. victoriae* in Fitzgerald National Park.

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Banksia snippet No.1

John Heerey from Gwelup, W.A. recently sent pictures of an amazing *B. audax* plant growing roadside 68km east of Southern Cross W.A., near Kalgoorlie. This is the largest plant I've ever seen and the flowers are quite large as well. The plant is approximately 2.0m tall whereas they typically grow around 1.0m in height.

B. audax inflorescences and tall roadside plant east of Southern Cross (photos: John Heerey).

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Banksia snippet No.2

Colourful pictures of W.A. beachfront banksias.

B. *praemorsa* (yellow flower form) Short Beach, Bremer Bay.
(photos: Sophie Xiang).

B. *attenuata* (low shrub form), Two Peoples Bay, Albany. W.A. (K.Collins).

B. attenuata at Little Beach, Two Peoples Bay, Albany (Photo: Kevin Collins).

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Banksia snippet No.3

Whilst visiting Neil & Wendy Marriott's scenic property just south of Stawell, in view of the Grampian Mountains, we observed an interesting *B. caleyi* plant. It had leaves intermediate between *B. aculeata and B. caleyi* both of which he has growing nearby. Neither of these two species were in flower to compare. The odd-leafed *caleyi* had set seed and Neil kindly gave me a cone to grow some.

Subsequently, I planted 10 seeds and 8 germinated. Low & behold half have leaves similar to *caleyi* and the others more closely resemble *B. aculeata*. This confirmed my suspicion of it being a possible hybrid of the two.

These two plants do not grow anywhere near each other in the wild, however I had grown plants in Mount Barker 20 years ago with them growing side by side. I believe I may have provided Neil with seed taken from these plants when they later died.

Leaf upper surfaces. (*caleyi* – LHS, Hybrid? – centre, *aculeata* – RHS).
Leaf under surfaces.

Seedlings LHS & RHS from *caleyi* variant. CENTRE true *caleyi.*

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Banksia snippet No.4

I made a chance discovery of a hybrid of *B. baueri* & *B. oreophila* near East Mount Barren, Fitzgerald National Park 15 months ago. The flowers looked like *baueri* however the leaves were different. I have grown some seed (which more closely resemble *oreophila* in size and shape). The four seedlings are uniform with foliage more closely resembling *oreophila*.

I now have to be patient and wait for them to grow and flower. K. Collins.

Two plants on LHS are from mystery species (foliage is more like *oreophila*).
Plants on RHS are typical *baueri*.

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Banksia Saxicola germination

Robbie Blackhall Miles, from Llanberis in Wales, reports his findings with germinating *saxicola* seeds at 3 degrees C. Using his traditional method, he couldn't achieve any germination of *saxicola*, so he sowed the seed in moist vermiculite and kept them in the fridge at a constant 3 degrees C for the recommended 3 months. Interestingly, prior to end of the 3 months, 80% had germinated.

Robbie asks for feedback please-

*How have other members germinated their saxicola seed**?***

*How many established saxicola do you have in your garden**?***

*If you do have plants, how many of Grampians form and how many Wilson's Promontory-Sealers' Cove form**?***

Just by the way, look at this!

Potted *B. spinulosa var. neoanglica* flowering at Llanberis, Wales.
Robbie Blackhall Miles

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I have managed, at Banksia Farm, to grow Grampians form of *saxicola* (18 years ago) and, 3 years ago, some of each form without stratification. I have 5 Grampian and 4 Sealers' Cove plants in the collection. Kevin Collins.

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U.K. / Western Europe journey continues……

26th May 2018. National Botanic Gardens of Wales.

Ben & Robbie kindly arranged for the curator, James, to show us through the massive glass dome built into the hill-side. James and two trainees gave us an extensive tour pointing out the Australian Proteaceous species. These were in the Mediterranean section with plants from Australia, South Africa, Chile and other classified Mediterranean climatic zones.

Included in the garden were Isopogon *formosus* & *latifolius*, a few Hakeas including *drupaceae* and Dryandras *formosa* & *nervosa* (Bremer Bay form).

Banksias they had growing were *integrifolia, marginata, serrata, coccinea, candolleana, speciosa, baxteri, gardneri var. gardneri, oreophila* & *media.* The underlined species are ones that have produced seeds. Possibly thanks to bumble bees.

Their plants are on elevated beds with very good drainage and many had grown very well. Like most gardens a few have succumbed over the years. It was nice to see some rarely cultivated, in the northern hemisphere, species like *candolleana, gardneri* & *oreophila.*

Domed glass house set in hillside.

Left, James showing us through. Right, Kevin showing trainees *baxteri* buds.

Magnificent domed glasshouse – vents in roof – fans in lower walls.

B. media & *prionotes* in background.

Dryandra *formosa* with a few flowers.

Banksias *caleyi* and *media* (prostrate) in rocket pots in their nursery**.** K&K Collins.

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Notice – 'Banksias' second edition.

The book is in China and has been printed. We have recently received our advance copy. The printing is on schedule but the sea freight is possibly in jeopardy… hard to predict.

The scheduled delivery was to be Canberra by 16th April and Perth 23rd April. We now anticipate it may be in Australia sometime in May/June.

We had hoped to launch it whilst in Canberra for the garden opening but that function has now been postponed. We have subsequently cancelled planned talks for the Grampians and Bendigo branches and Celia Rosser's gallery due to COVID 19 lock downs. See below details of the new book.

Banksias: second edition.

Alex George, Kevin Collins and Kathy Collins

Banksias are Australia's most iconic plants after Eucalypts – known for thousands of years to Australians and – through writing and art – to many more who have never visited this land.

This extensively revised edition includes the most recently discovered Banksia, *vincentia*, with descriptions and illustrations of all 79 known species with many new photographs and updated species descriptions. It provides the history of their discovery, evolution, how to find and grow them and how they have inspired artists and artisans. With some 400 beautiful colour illustrations it is the comprehensive, up-to-date guide to these unique and fascinating plants.

Tables list which Banksias to grow for particular purposes and the three authors have between them, studied and grown all 79 species of Banksia, their combined experience totalling over a hundred years.

Selling Points

- The most comprehensive book ever published on one of Australia's most loved and iconic plants. Ideal for gardens requiring low water and low maintenance.
- Written by the leading authorities in the world on the genus Banksia covering identification, cultural and growing issues.
- **RRP: $69.95**

Hardback, 384 pages 233 x 152mm,
Over 400 illustrations in full colour
ISBN 9780992290030

Publication: April, 2020

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The Authors

Alex George has 60 years' experience as a botanist and editor, was author of *The Banksia Book* (1984) and has written 15 books and 200 botany papers. He has also discovered and named many new species.

Kevin Collins has pursued his passion for Banksias for 35 years, developing *Banksia Farm,* Mount Barker in Western Australia and growing all known species. He now works as a consultant, lectures, and assists Banksia research projects.

Kathy Collins assisted with *Banksia Farm*, is a keen environmentalist, established a Landcare Group and is a joint fellowship recipient with Kevin, from Land and Water Australia.

Book Orders

The authors are offering Banksia Study Group members a special discount price.

RRP: $69.95. Discounted signed copies $60.00.
Unsigned copies $55.00

Email Kevin and Kathy for online order-purchase form:

banksia@westnet.com.au.

Australian National Botanic Gardens
50th Anniversary Celebrations

Sorry for the delayed newsletter. We were holding off to post the planned 10-day Banksia Festival programme which included workshops, the new garden opening, a complete banksia cone collection display, a launch of the second edition of 'Banksias', grafting workshops and many other exciting activities.

Final planning was imminent when the COVID19 virus started taking hold in Australia.

The Festival and garden opening have been postponed and will hopefully be held sometime in the future.

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Financials and membership.

Bank balance as of 30.04.2020. $1,553.67.

(Plus $3,000.00 ANPSA loan held temporarily for 'Banksias' - 2nd edition).

Membership as of 30.03.2020. 91.

A big welcome to all new members, including some who have joined us from Karlo's very popular Banksia Lovers' facebook site.

Thank you, Karlo, for the amazing interest your site has generated with over 3,000 members.

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We would love to have any reports (short or long) of your failures and success in germination and growing of our wonderful Banksias. This helps in keeping the study of Banksias progressing. What has been flowering near you? What effect on banksias have you seen after the bushfires? Is there something growing/flowering out of season? Who is predating banksias near you? Let's hear from you.

Kathy & I wish members and their families all the very best in these unprecedented times. Keep safe and we hope banksias will be providing positivity during these periods of isolation and unfortunate job losses.

K & K Collins.

Appendix No. 1

Project report

This is an extra-long newsletter with the full project report provided by Meredith Spencer on the 'Investigation of Dieback Disease resistance within *Banksia coccinea*' below. The project, in Albany with the assistance of Murdoch university in Perth, investigated possible natural resistance to various dieback pathogens within B. *coccinea* focusing on Phytophthora *cinnamomi*.

Courtesy - Meredith & Jeremy Spencer, Giles Hardy, Sarah Barret & Kevin Collins.

Investigation of Dieback disease resistance within *Banksia coccinea*

(NLESG67457)

August 2019

Investigation of Dieback disease resistance within *Banksia coccinea.*

(NLESG67467)
Meredith Spencer, Great Southern Bio Logic
Jeremy Spencer, Great Southern Bio Logic
Giles Hardy, Murdoch University Centre for Phytophthora Science & Management
Sarah Barrett, Department of Biodiversity, Conservation & Attractions Parks and Wildlife Service
Kevin Collins, Banksia Farm
August, 2019

Record of Distribution

No. of copies	Report Ref	Report Status	Date	Prepared for:	Initials
1	Banksia coccinea investigation report-July 2019-V1	Final	14 August 2019	Australian Government	MS
1	Banksia coccinea investigation report-July 2019-V1	Final	14 August 2019	Great Southern Bio Logic	MS
1	Banksia coccinea investigation report-July 2019-V1	Final	14 August 2019	Murdoch University	MS
1	Banksia coccinea investigation report-July 2019-V1	Final	14 August 2019	Department of Biodiversity Conservation and Attractions	MS
1	Banksia coccinea investigation report-July 2019-V1	Final	14 August 2019	The Banksia Farm	MS

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EXECUTIVE SUMMARY

Phytophthora Dieback caused by the introduced plant pathogen *Phytophthora cinnamomi* is listed as a 'key threatening process' to Australia's biodiversity in the *Environmental Protection and Biodiversity Conservation Act* (1999). Many populations of the iconic *Banksia coccinea* have become locally extinct as a result of the disease. As *B. coccinea* is a dominant keystone species when present, its loss results in significant changes to plant community structure and also impacts dependent fauna species.

Observations of surviving *B. coccinea* on long infested, high impact Phytophthora Dieback sites prompted the question, 'are these individuals resistant to Phytophthora Dieback?'. To test this potential for resistance, seed from surviving plants was collected and propagated, and germinants were screened for resistance to *P. cinnamomi* and three other *Phytophthora* species. Seven hundred and thirty plants were successfully grown in individual containers from the seed of 26 parents. These were then screened against *P. cinnamomi, P. nicotianae, P. multivora and P. pseudocryptogea*. All but five plants from three parents were killed by *P. cinnamomi,* whilst 68/146, 118/146 and 144/146 plants survived following inoculation with *P. nicotianae, P. multivora, and P. pseudocryptogea*, respectively.

Phytophthora cinnamomi, P. nicotianae, P. multivora and likely *P. pseudocryptogea* are introduced to Australia; therefore, it is of interest to see some inherent resistance to these *Phytophthora* species occurring in *B. coccinea*. Consequently, opportunities exist to further screen these surviving individuals by allowing them to flower, conduct controlled pollinations, collect the subsequent seed and screen these for resistance to *Phytophthora* species.

The expectation is that we can enhance resistance through selection and this needs further detailed exploration. In addition, it would be expedient to look at opportunities to develop clonal methods of propagating *B. coccinea* to increase the numbers of plants that can be produced from resistant individuals for planting into infested Phytophthora Dieback sites to help restore habitat to conditions similar to those prior to infestation.

INTRODUCTION

Phytophthora Dieback is a plant disease caused by a group of soil-borne water moulds from the genus *Phytophthora*. There are over 140 species of *Phytophthora*; however, *P. cinnamomi* has the greatest impact in South Coast ecosystems. It affects approximately 40% of native plant species in Western Australia and can irreversibly alter plant communities, killing susceptible species, many of which are both iconic and fundamental to the ecosystems they support. Phytophthora Dieback caused by *Phytophthora cinnamomi* is listed with the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) as a 'key threatening process' to Australia's biodiversity. It has already had widespread, devastating impact on threatened species and communities in Western Australia and management options are extremely limited.

Development of management techniques to mitigate impact of Phytophthora Dieback on species and communities is fundamental to improving ecosystem health in disease impacted sites. The National Threat Abatement Plan for disease in natural ecosystems caused by *Phytophthora cinnamomi* (DotEE 2018a) and associated background paper (DotEE 2018b) identifies objectives and actions to mitigate the impact of *Phytophthora* on biodiversity assets. These include research to develop resistant lines of native plant species, through screening of priority species for resistance to the pathogen (Action 4.4).

Phytophthora Dieback susceptible proteaceous species dominate the South Coast ecosystems. The Scarlett Banksia, *Banksia coccinea* is an iconic component species of these ecosystems, including the EPBC Act listed *Proteaceae Dominated Kwongkan Heathlands Threatened Ecological Community* (Kwongkan TEC). This TEC occurs across approximately 1.2 million hectares of the region and is essential habitat for unique and diverse species. It includes over 50 plant and 18 fauna species listed as threatened, many of which are endemic to the area. *Banksia coccinea* is also a dominant component of the Western Australian State recognised *Banksia coccinea Shrubland/Eucalyptus staeri/Sheoak Open Woodland Priority Ecological Community* (*Banksia coccinea* PEC), which is considered concordant with the Kwongkan TEC.

Banksia coccinea is highly susceptible to *Phytophthora cinnamomi* and other *Phytophthora* species and has become locally extinct from many sites where *P. cinnamomi* is present. Working in partnership, Great Southern Bio Logic (GSBL), Murdoch University's Centre for Phytophthora Science and Management (CPSM), the Department of Biodiversity, Conservation and Attractions (DBCA) Parks and Wildlife South Coast region and The Banksia Farm received funding from the Australian Government's Environment Small Grant scheme (NLESG67467) to undertake an investigation of Phytophthora Dieback disease resistance within *Banksia coccinea*.

1 AIM

The project aimed to provide innovative, preliminary investigations into potential natural resistance within *Banksia coccinea* persisting in high impact Phytophthora Dieback disease infested vegetation. The study was to determine whether resistant individuals could be found amongst these so-called 'disease escapes' and, if so, provide the opportunity to develop breeding programs that could ultimately lead to the return of resistant *B. coccinea* lines to infested sites.

Long term aims of the research included providing a foundation for similar work on other susceptible species including threatened flora, informing recovery actions and potentially leading to re-introduction to wild populations.

A further aim was to provide knowledge, understanding, practical methodologies and techniques that could be easily adopted for use by the natural resource management community and others to inform and deliver improved on-ground management including environmental restoration, flora production and forestry.

2 METHODS

2.1 Field Collections

2.1.1 Seed

A high impact Phytophthora Dieback infested occurrence of Kwongkan TEC/*Banksia*

coccinea PEC at Gull Rock National Park, 25 km east of Albany town site, was surveyed for persisting *Banksia coccinea* individuals. Seed was collected separately from 32 individuals persisting over approximately 120 ha. Individual plant locations were recorded with handheld GPS. Field data for each individual plant and site was collected and included site reference, location, fire history, plant health, proximity to other *B. coccinea* and disease impact.

Seed was also collected from a planted individual persisting in infested soils at the Banksia Farm, Mount Barker.

2.1.2 *Phytophthora cinnamomi, P. multivora, P. nicotianae and P. pseudocryptogea* inoculum

Six soil and tissue samples were collected in accordance with the methods described in the *Phytophthora Dieback Interpreters Manual for lands managed by the Department (DBCA, 2015)* and forwarded to the CPSM to obtain local *Phytophthora cinnamomi* isolates for use in inoculation trials. Sample locations were recorded using a handheld GPS. *Phytophthora cinnamomi* was obtained from each of the six samples, and all were determined to be the A1 mating type.

In addition to one Gull Rock *P. cinnamomi* (CPSM18-281) isolate, individual isolates of *P. multivora* (TRH4), *P. nicotianae* (PAB10.104) and *P. pseudocryptogea* (VHSC16118) were obtained from the CPSM culture collection in preparation for inoculation. All three of these species have been isolated from dying native plant species in Kwongkan heath communities across the south-west of Western Australia.

2.2 Propagation

Seed was extracted through fire stimulation. A gas fuelled blow torch was used to burn cones which were then submerged in water for 20-30 minutes. Cones were allowed to sun dry and released seeds were collected. The process was repeated until all follicles opened. Seeds from each individual parent plant were kept in discreet groups for propagation. Propagation was undertaken immediately following extraction to enhance viability and germination success. All propagules were labelled according to parent seed tree.

2.3 Glasshouse Pathogenicity Trials

2.3.1 Inoculum Production

Briefly, an isolate of each *Phytophthora* species was grown on Campbells Vegetable 8 agar (V8A) for 7-10 days in the dark at 20°C, and then inoculated into a flask as follows. Four hundred mL of millet [*Panicum miliaceum*] seeds was placed into a 1000 mL Erlenmeyer flask, sealed with non-absorbent cotton wool and covered with aluminium foil. The flasks were autoclaved three times at 121°C for 20 minutes over three consecutive days, and then inoculated on the third day once the substrate had cooled. Inoculum per flask consisted of one Petri-dish (9 cm diameter) of V8 agar plugs colonized for 7-10 days by one of the *Phytophthora* species. Flasks were shaken and then placed in zip-lock plastic bags and incubated at a constant temperature (20°C) in the dark. The flasks were shaken every 3 days in the first two weeks to evenly spread the inoculum. Six-week old inoculum was used to inoculate the seedlings. Colonization of the inoculum was confirmed by plating 3 g sub-samples

onto NARPH, a *Phytophthora*-selective medium (Hüberli et al. 2000). These were incubated at room temperature and checked to ensure the viability of the inocula. The amount of inoculum used for all the trials was 2.5 mL (1% of the volume of container medium in the forestry tubes containing the *B. coccinea*).

2.3.2 Inoculation

A total of 730 *B. coccinea* 6 month-old seedlings growing in 70 ml Forestry tubes were divided into groups based on their parents. There were 26 parent plants and, depending on numbers, either 5 or 6 replicate plants per *Phytophthora* species and the control (non-inoculated) treatments (Table 1). There were 146 plants for each of the four *Phytophthora* species and control treatments. The plants were inoculated on the 1st April 2019 by placing a 5 mm diameter hole along one side of each container into the container substrate to a depth of 5cm, and placing 5mls (approximately 1% of volume of the container substrate in each container) of the millet seed inoculum into each hole. The hole was covered with container medium and the plants were immediately watered to container capacity (Plates 1a-c, Appendix A). The plants were then randomly placed on a bench in an evaporatively cooled glasshouse (Plate 2, Appendix A). For the first two days, the plants were watered to container capacity three times daily to stimulate sporangial production and zoospore release. Thereafter, the plants were watered daily and monitored for symptoms of disease caused by *Phytophthora*.

Since, the aim of the experiment was to identify plants resistant to the different *Phytophthora* species, only the time taken for plants to die post inoculation was recorded. The experiment was halted on the 14th of June 2019. At this time, the number of surviving plants in each of the *Phytophthora* treatments was counted.

Table 1. *Banksia coccinea* parents, number of seedlings per parent, and percentage survival of plants 75 days after inoculation with *Phytophthora cinnamomi* (P.c), *P. nicotianae* (P.n), *P. multivora* (P.m), and *P. pseudocryptogea* (P.p). No control plants died (data not shown).

Parents	Number of Seedlings per Treatment	P.c (%)	P. n (%)	P. m (%)	P. p (%)
LB1-1	6	0	16.6	50	100
LB1-2	6	0	100	83.3	100
LB1-4	5	0	60	100	100
LB2-1	5	0	40	60	100
LB2-2	6	50	16.6	83.3	100
LB3-1	5	0	80	60	100
LB3.2	6	0	83.3	66.6	100
LB4-1	6	0	33.3	83.3	100
LB4-2	6	0	83.3	83.3	100
LB4-3	5	20	40	100	100
LB5-1	6	16.6	50	83.3	100
LB5.2	6	0	50	66.6	100
LB5-3	6	0	50	66.6	100
LB5-6	6	0	100	66.6	100
LB6-1	5	0	60	100	100
LB6-2	6	0	16.6	100	100
LB6-3	6	0	50	83.3	100
LB6-5	6	0	0	100	100
LB7-1	6	0	33.3	83.3	100
GR1-1	5	0	40	80	100
GR1-2	5	0	80	80	20
GR1-3	5	0	80	80	100
GR2-1	5	0	0	80	20
GR2-2	6	0	16.6	100	100
GR3-1	5	0	40	80	100
BF1	6	0	0	83.3	100

3 RESULTS AND DISCUSSION

None of the control plants died during the trial. Inoculated plants began to show symptoms within the first week, typically showing foliar wilting frequently with black girdling lesions (Plates 3 a-b, Appendix A) at the collar of the plants. These symptoms were first observed on some of the plants from different parent trees by the 8th of April, or 8 days after inoculation with one of four *Phytophthora* species.

- *Phytophthora cinnamomi* killed plants from all parents except LB2-2, LB4.3 and LB5-1, with 50% (6/6), 20% (1/5) and 16.6% (1/6) (Figure 1a) of plants remaining alive, respectively.

- *Phytophthora nicotianae* was the next most pathogenic *Phytophthora* species, and killed individual plants from all parents except two (LB1-2 and LB5-6) (Figure 1b). It killed all plants from the parents LB6-5 and BF1.

- *Phytophthora multivora* killed plants from all parents except six (LB1-4, LB4-3, LB6-1, LB6-2, LB6-5, GR2-1) (Figure 1c). Interestingly, it did not kill all individuals from any one parent.

- *Phytophthora pseudocryptogea* only killed plants from two parents GR1-2 and GR2-1 with 80% survival for plants from each parent (Figure 1d), otherwise 100% of plants from each of the remaining parents remained alive at the end of the trial.

Clearly, there are considerable differences in the ability of *B. coccinea* to induce a resistant response to the different *Phytophthora* species assessed. Not unexpectedly, *P. cinnamomi* was the most pathogenic; with very few (5) remaining plants by the end of trial. However, in terms of parent plants sampled, there was some resistance within progeny from 11.5% of parents assessed. Lesioned stem material from 10% of plants which died per treatment were randomly selected and plated out onto NARPH, the *Phytophthora* selective medium, to confirm that death was caused by the *Phytophthora* species they were inoculated with.

Of interest is the evidence of resistance in the *B. coccinea* Gull Rock populations to the other three *Phytophthora* species. This is an important observation given that *P. multivora* and *P. nicotianae* are both introduced pathogens like *P. cinnamomi* to Australia, and both have broad host ranges in Australia and elsewhere in the world. The status of *P. pseudocryptogea* as an introduced or native pathogen is unclear, but it is likely to be introduced as it is found elsewhere in the world.

Phytophthora multivora is of particular concern as it is now more regularly isolated from urban and peri-urban bushland and parks than *P. cinnamomi* (Barber et al., 2013; Scott et al., 2009)*;* this is in contrast to 20 years ago, when *P. multivora* (part of the *P. citricola* species complex) was rarely isolated. *Phytophthora multivora,* unlike *P. cinnamomi,* appears to be a good saprophyte and is also active in calcareous soils. It is likely to become more widespread in the future and may possibly compete with *P. cinnamomi.* It is also being increasingly isolated from native bush away from urban areas including the south coast. *Phytophthora pseudocryptogea* has only recently been formally described (Safaiefarahani et al, 2016), and is part of the *P. cryptogea* species complex. It also has been isolated from other dying *Banksia species* along the south coast and it is interesting that it had little impact on *B. coccinea*.

Since this study demonstrated there is resistance within the *B. coccinea* populations to the different *Phytophthora* species, there is the opportunity to set up trials where the surviving plants are potted up into large pots or planted into field plots and allowed to flower. Once flowering occurs, normally at 3-4 years old, controlled cross-pollination can be attempted between the survivors from the different *Phytophthora* species and any seed produced collected and again screened as above. There is a good possibility that more individuals resistant to all the *Phytophthora* species including *P. cinnamomi may* be obtained.

It was interesting that all six soil/root samples collected contained only the A1 mating type of *P. cinnamomi.* In much of the south-west of Western Australia, the A2 mating type is most frequently isolated, and the A1 mating type tends to be rare. However, mating type tests are rarely conducted by the CPSM or other diagnostic laboratories including the DBCA Vegetation Health Service, and there may be more A1 mating types present than expected. It would be of interest to collect more isolates from dead and dying *B. coccinea* and to determine their mating types and whether there are differences in resistance of *B. coccinea* between the two mating types. There is some debate as to whether the A1 mating type of *P. cinnamomi* should be designated as a separate species (Frans Arentz, pers. comm).

In conclusion, this study has shown that there are likely some individuals resistant to *P. cinnamomi* in *B. coccinea* populations. However, a comparison of mortality rates in this study with those of Shearer et al. (2013) does not suggest a significantly lower mortality rate in individuals propagated from "disease escapes" and further work is required to confirm whether "disease escapes" are indeed more resistant. This would include collection of seed from a larger sample group of "disease escapes".

The study has also shown that there is potentially stronger resistance to the other introduced *Phytophthora* species screened. This demonstrates that further work should be conducted on additional screening studies, methods of clonal propagation, mechanisms of resistance and the inclusion of more than one *Phytophthora* species in future screening trials to understand if the presence of more than one species can break down resistance.

4 RECOMMENDATIONS

- Repot and re-inoculate all surviving plants with the same *Phytophthora* isolates they were inoculated with in the present study to confirm that they were not 'disease-escapes'. This is particularly important for the few plants that survived inoculation with *P. cinnamomi*. This should be undertaken prior to any further work on the surviving plants.

- Any surviving individuals should be either potted up or planted into field sites and allowed to flower. Controlled cross-pollination should then be conducted between the surviving plants and seed collected. Plants should then be screened as above for resistance to the *Phytophthora* species.

- Since this study indicates that there are likely to be resistant individuals present in the *B. coccinea* populations, seed should be collected from individuals persisting in infested areas as well as from the broader population for comparison, and from a larger number of parent plants for additional resistance screening to *P. cinnamomi* and other *Phytophthora* species.

- Conduct screening with an A2 *P. cinnamomi* isolate(s) in addition to the current A1 mating type isolate used. It is unlikely that there will be differences in pathogenicity, but given that A2 mating types are theoretically more prevalent than A1 mating types it would be expedient to confirm there are no differences between them with respect to pathogenicity and *B. coccinea* resistance.

- Include *P. crassamura* in future trials; this has been recently described, and is what was previously considered as *P. megasperma* and now known to be a species complex. What was *P. megasperma* and is now *P. crassamura* has been isolated from a range of dying species including *Banksia* along the south coast.

- Consideration should be given to researching different clonal propagation methods (cuttings, tissue culture) for *B. coccinea*. This will allow more rapid up-scaling in numbers of known resistant individuals compared to the development of seed orchards and associated problems with these.

- Consideration should be given to screening *Phytophthora* species other than *P. cinnamomi* to test whether phosphite has a variable impact on pathogenicity for different types of *Phytophthora spp*. *P. crassamura* should be included. Recent studies at Murdoch University show there is considerable variability of response to phosphite between *Phytophthora* species in vitro and in planta. This is relevant, given phosphite is used on some TECs/PECs, including those at Gull Rock National Park, to mitigate the impact of *P. cinnamomi*.

- Once adequate material is available, conduct molecular and biochemical studies to determine the mechanisms of resistance and compare these mechanisms between the different *Phytophthora* species.

- Include more than one *Phytophthora* species in future resistance screening work, as frequently more than one species can be isolated together. This is particularly true for *P. cinnamomi, P. multivora* and *P. nicotianae*.

5 ACKNOWLEDGEMENTS

We thank the Australian Government's Environment Small Grant scheme (NLESG67467) for funding this research.

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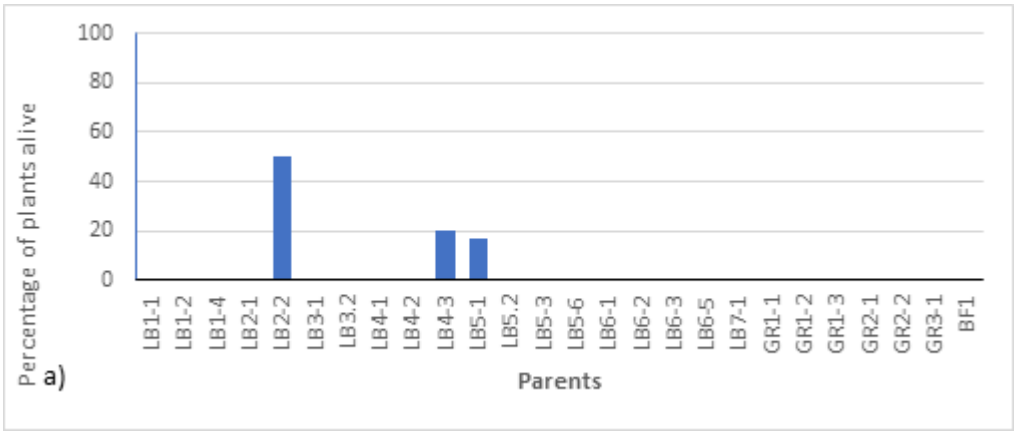

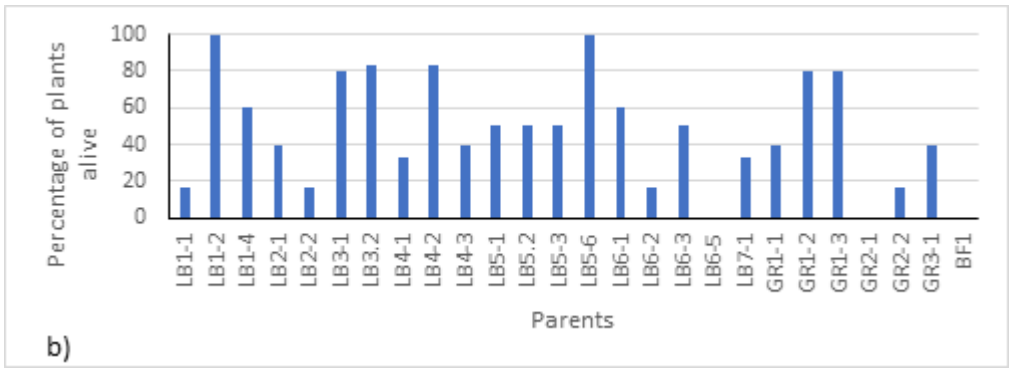

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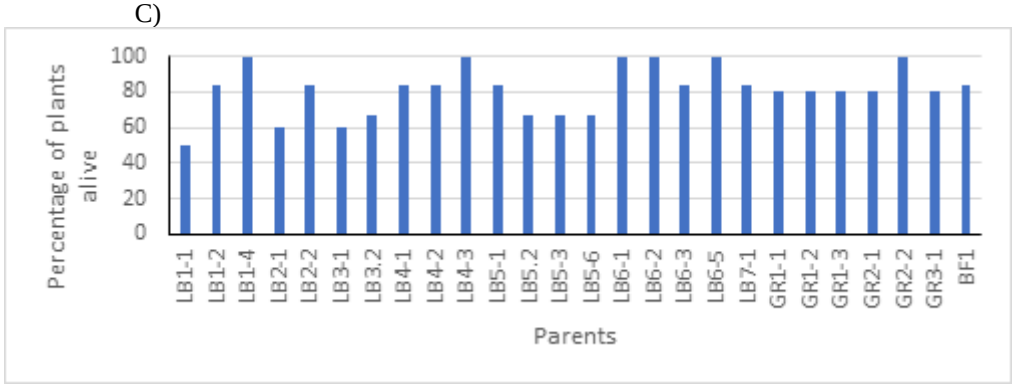

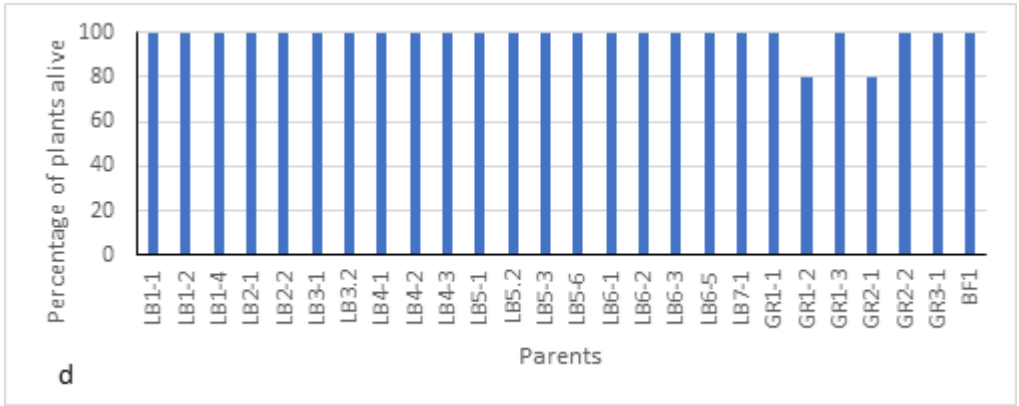

Figure 1 a-d: Percentage of plants from different *Banksia coccinea* parents remaining alive 75 days after soil infestation with (a) *Phytophthora cinnamomi*, (b) *P. nicotianae*, (c) *P. multivora*, and (d) *P. pseudocryptogea*.

Plate 1a. Decanting *Phytophthora* colonised millet seed inoculum from 500 mL flask.

Plate 1b: Tube used to create a hole in the container medium, and left for 10 days prior to inoculation to allow any root damage to heal prior to inserting *Phytophthora* colonised millet seed into the hole after removal of the tube

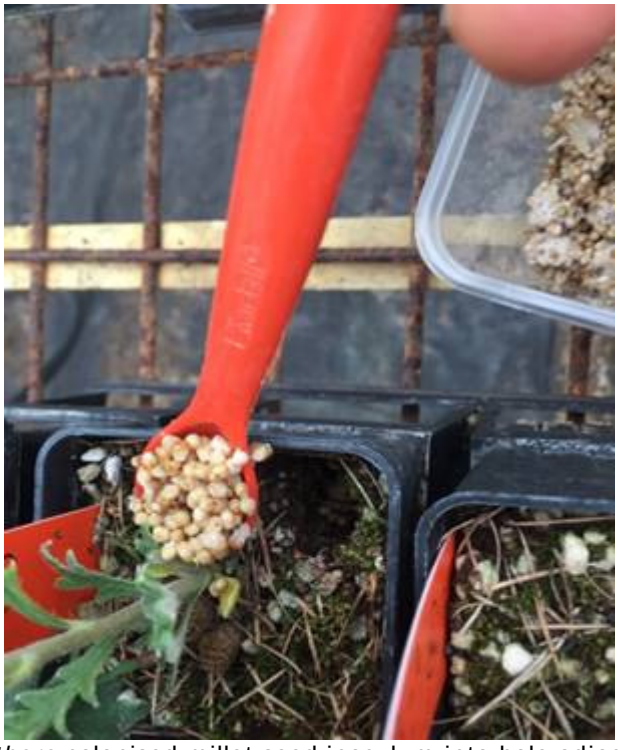

Plate 1c: Adding *Phytophthora* colonised millet seed inoculum into hole adjacent to *Banksia coccinea* plant

Plate 2: *Banksia coccinea* plants randomised on glasshouse bench immediately after inoculation

Plate 3a: Plant from of Banksia coccinea LB3-1 parent with black girdling lesion in the collar and up the stem (upper limit shown by blue arrow). Inoculated with *Phytophthora cinnamomi*

Plate 3b: Plant from of *Banksia coccinea* GR2-1 parent inoculated with *Phytophthora multivora* showing symptoms of yellowing, wilting and black girdling lesion in the collar