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Plain English Summary

European blackberry (*Rubus fruticosus* agg.) is an important invader of southern Australia pastures and natural ecosystems. The goal of this project was to explore new avenues for blackberry biocontrol. The project primarily focused on determining if the blackberry decline syndrome, observed in south-west Western Australia over the last 10 years, could be manipulated and developed as an effective and safe biocontrol tool. Results from glasshouse experiments revealed that *Phytophthora pseudocryptogea*, but not *Phytophthora bilorbang*, could kill or adversely affect different species of blackberry, when plants were exposed to fortnightly 72-h simulated flooding treatments. In host-specificity tests, *P. pseudocryptogea* did not significantly affect pasture species, but killed or considerably reduced growth of several native species, including many in the *Acacia* and *Eucalyptus* genera. These results were the basis for the decision not to proceed with field trials. Since *Phytophthora* species were found not to be a viable option for blackberry biocontrol, the project undertook a preliminary investigation into the field host-range of the stem-boring sawfly, *Phylloecus faunus* (=*Hartigia albomaculata*), identified in the 1970s in Europe as a potential biocontrol agent for blackberry. Field surveys conducted in mainland western Europe and the UK only found *P. faunus* on *Rubus ulmifolius*, which belongs to *R. fruticosus* agg., and not on the closely-related species *Rosa canina*, at sites where the two species were sympatric. A range of possible options as the next steps towards blackberry biocontrol in Australia are presented in this report.

Executive summary

European blackberry refers to a group of taxa within the *Rubus fruticosus* aggregate that are important invaders of southern Australia pastures and natural ecosystems where annual rainfall exceeds 700 mm. In 2006, annual loss of production and cost of control of blackberry in agriculture was estimated to be between $95.1 million and $102.8 million. The search for a biological control (biocontrol) solution for blackberry began in the 1970s with extensive field surveys in Europe, the native range. Only one biocontrol agent, the leaf-rust fungus *Phragmidium violaceum*, that does not pose a risk to cultivated brambleberries and native *Rubus* species has been introduced in Australia. The rust fungus has from time to time been reported to cause severe disease of blackberry that reduces growth, but only in some areas of the weed's range. The goal of this project was to explore new avenues for the biocontrol of blackberry.

The project primarily explored if the blackberry decline syndrome, observed in south-west Western Australia over the last 10 years, could be manipulated and developed as an effective and safe biocontrol tool. Based on previous studies, *Phytophthora bilorbang* and *Phytophthora pseudocryptogea* were believed to have a strong involvement in blackberry decline when exposed to temporary inundation. The project also performed a small feasibility study to determine whether further research is warranted into the stem-boring sawfly *Phylloecus faunus* (formerly known as *Hartigia albomaculata*), identified in the 1970s in Europe as a potential biocontrol agent for blackberry.

The objectives of the project were to:

1. Determine the potential of *Phytophthora* species, as an inundative biolcontrol tool for blackberry by conducting pathogenicity and host-specificity glasshouse tests and, if promising, evaluating efficacy in field trials.
2. If *Phytophthora* species are not promising, investigate an alternate option for biocontrol of blackberry.
3. Make recommendations for next steps in the biological control of blackberry.

Following an analysis of the relevant literature and consultation with Murdoch University partners, who are experts on *Phytophthora* species, a soil application technique was selected as the most appropriate for experimental work. Inoculum production of *Phytophthora* on pearl barley or millet was deemed unsuitable because the high nutrient content of the grains encouraged development of saprophytic fungi in the soil that potentially outcompeted *Phytophthora*. A vermiculite-based substrate was thus selected to produce *Phytophthora* inoculum for all glasshouse experiments. *Phytophthora* was shown to survive in fresh, colonised vermiculite-based substrate stored at 4 and ~22 °C for a period of up to 12 months, but not when stored at –20 °C. The project also demonstrated that it is possible to produce inoculum on either vermiculite or sugarcane mulch-based substrate contained in large breathable polypropylene bags with filters, widely used in mushroom spawn production. This system would have been ideal to produce large quantities of inoculum required to undertake field trials.

An initial series of experiments with blackberry was conducted using isolates of *P. bilorbang* that had been in storage at Murdoch University for some years. All experiments were inconclusive – overall

inoculated blackberry plants were similar in size and vigour to control (non-inoculated) plants. Further, by the end of the experiments a similar number of inoculated and control plants had died. In light of concerns that these isolates had lost pathogenicity during storage, new samples of blackberry from sites where the decline has been observed in Western Australia were collected and processed to recover new isolates. Two dominant species were recovered – *P. bilorbang* and *P. pseudocryptogea.*

A new experiment with a revised methodology was performed using isolates of both *Phytophthora* species. Results revealed that *P. pseudocryptogea*, but not *P. bilorbang*, was pathogenic towards blackberry. *Phytophthora pseudocryptogea* thus became the focus of subsequent research. Additional experiments demonstrated that *P. pseudocryptogea* killed or adversely affected blackberry only when inoculated plants were exposed to fortnightly 72-h flooding treatments and that doubling the amount of inoculum used did not make a difference to the effects of *P. pseudocryptogea* on blackberry.

A series of host-specificity tests were conducted to determine the effects of *P. pseudocryptogea* on different blackberry taxa and 47 non-target pasture and native plant species. *Phytophthora pseudocryptogea* severely affected accessions/clones of three blackberry species, *R. anglocandicans*, *R. ulmifolius* and *R. leucostachys*, but not those of *R. laciniatus* and *R. polyanthemus* and one other clone of *R. leucostachys* propagated with the cane tip-rooting technique. Another experiment using plants of these taxa propagated from seed would be necessary to confirm if these taxa are resistant to *P. pseudocryptogea*.

Other trials demonstrated that all pasture species tested, except white clover (*Trifolium repens*) in one trial, were not significantly affected by *P. pseudocryptogea*. The resistant pasture species were lucerne (*Medicago sativa*), perennial ryegrass (*Lolium perenne*), fescue (*Festuca arundinacea*), common wallaby grass (*Austrodanthonia caespitosa*), windmill grass (*Chloris truncata*), cocksfoot (*Dactylis glomerata*), microlaena (*Microlaena stipoides*), phalaris (*Phalaris aquatica*) and sub-clover (*Trifolium subterraneum*). *Phytophthora pseudocryptogea* however, was found to be able to kill or considerably reduce the foliage biomass of several native species. Species significantly affected by *P. pseudocryptogea* in one or both trials were: *Acacia aneara*, *Acacia dealbata*, *Acacia disparrima* ssp. *disparrima*, *Acacia doratoxylon*, *Acacia implexa*, *Acacia irrorata* ssp. *irrorata*, *Acacia longifolia* ssp. *sophorae*, *Acacia melanoxylon*, *Acacia pravissima*, *Acacia rubida*, *Brachychiton populneus*, *Callistemon citrinus*, *Callistemon linearifolius*, *Eucalyptus cladocalyx*, *Eucalyptus dives*, *Eucalyptus globulus* ssp. *biscostata*, *Eucalyptus globulus* ssp. *globulus*, *Eucalyptus macrophyllum* var. *macrophyllum*, *Eucalyptus pauciflora* ssp. *niphophila*, *Eucalyptus sieberi*, *Eucalyptus viminalis* and *Leptospermum lanigerum.* These results were the basis for the decision not to proceed with field trials.

Since *Phytophthora* species were found not to be a viable option for the biocontrol of blackberry, a preliminary investigation was made into an alternative option – the stem-boring sawfly, *P. faunus*. Field surveys of species within *R. fruticosus* agg. and closely-related *Rosa* species were conducted in mainland western Europe and the UK. Of the 80 insect specimens collected from *Rubus* and *Rosa* canes, 75% could be identified to at least family level using DNA barcoding. *Phylloecus faunus* was only found on *Rubus ulmifolius*, which belongs to *R. fruticosus* agg., and not on *Rosa canina* at sites where the two species were sympatric. The sawfly did not appear to prefer a particular habitat type.

It was found in blackberry stands occurring in open fields, along watercourses and in forest environments. The investigation also identified another promising candidate agent, a jewel beetle (*Agrilus solieri*), that bores into blackberry crowns and the base of the canes and appears to be restricted to *Rubus*.

This report outlines a range of possible options that could be explored as the next steps in the biocontrol of blackberry in Australia. There are no guarantees however, that investments in these options would generate effective and safe management solutions for blackberry applicable at the landscape scale. The main challenge for biocontrol is that invasive taxa of blackberry are closely-related to several native and commercial species within the Rosaceae family. It is thus difficult to find insects and pathogens that are sufficiently host-specific for introduction to Australia. Emphasising this challenge and stating that biocontrol is highly unlikely to be a 'silver bullet' management tool for blackberry in Australia, is a first step in managing expectations of stakeholders and encouraging them to use other currently available and effective methods (primarily herbicides) to control blackberry.

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Table of contents

1 Project rationale

European blackberry (*Rubus fruticosus* aggregate[1]; hereafter referred to as blackberry) is a thorny invasive shrub that grows primarily in southern areas of Australia where annual rainfall exceeds 700 mm. It readily invades land along watercourses, competing against native plants and pasture, and preventing access to the water by native fauna and livestock, and for recreational activities. In 2006, blackberry was estimated to occupy approximately 8.8 million hectares in Australia, with annual loss of production and cost of control in agriculture of between $95.1 million and $102.8 million (Page and Lacey 2006). The search for a biological control (biocontrol) solution for blackberry began in the 1970s with extensive field surveys in Europe, the native range (Morin and Evans 2012). The most promising biocontrol agent, the rust fungus *Phragmidium violaceum*, was shown to be highly specific and one isolate, F15, was authorised for release in Australia in 1991 (Bruzzese and Lane 1996). It is noteworthy that an unauthorised introduction of this fungus had already occurred in Australia in 1984 (Marks et al. 1984). The rust fungus was reported to be effective in slowing the vegetative spread of blackberry and reducing its competitiveness against other species only at some locations with optimal conditions for disease development[2] (Mahr and Bruzzese 1998). Pathogenicity studies later identified that some blackberry taxa were not infected by the rust population existing in Australia at the time (Evans et al. 2005). To address this issue, eight additional isolates of the fungus, which together could infect all species/genotypes identified within *R. fruticosus* agg.*,* were sourced from the native range and released in Australia in 2004 (Morin et al. 2011). Molecular screening of rust samples from release sites two years after their introduction, showed that alleles of the additional isolates had been incorporated into the existing population of the fungus (D. Gomez, unpublished data). It was hoped that the introduction of genetic material from the additional isolates would contribute to the co-evolution of the fungus with its blackberry host and the enhancement of biocontrol in Australia. Since then, the rust fungus has from time to time been reported to develop severe disease symptoms on blackberry in some areas, but this has primarily been attributed to wet and cool conditions in spring and summer.

Unexplained dead and diseased blackberry (*Rubus anglocandicans*) plants were discovered in 2006-07 at sites along the Warren River and Donnelly River in the south-west of Western Australia. This became known as the blackberry decline syndrome (Aghighi et al. 2014) (Fig. 1). While extensive death of blackberry plants were observed at these sites, none of the native plants in the areas were affected. Several *Phytophthora* species were recovered from declining blackberry plants, with two species, *P. bilorbang* and *P. pseudocryptogea* (formerly referred to as *P. cryptogea*) found to be the most pathogenic in glasshouse trials (Aghighi et al. 2015). These species were thus believed to have a strong involvement in blackberry decline, when exposed to temporary inundation that creates conducive conditions for infection (Aghighi et al. 2014). This initial research suggested that there may be prospects to exploit the blackberry decline syndrome as a biocontrol management tool for blackberry infesting riparian zones.

The sawfly *Phylloecus faunus* (formerly known as *Hartigia albomaculata*) was identified in 1977 as one of the natural enemies that should be investigated for biocontrol of blackberry in Australia (Bruzzese 1982). Throughout Mediterranean Europe, the sawfly oviposits into succulent primocanes and larvae tunnel into the pith, leading to cane collapse and dieback when levels of attack are high. Preliminary host-specificity tests under laboratory conditions performed in the late 1970s on 35 non-target plant species showed that larvae were able to feed on a number of cultivated brambleberries

[1] *Rubus fruticosus* aggregate comprises several taxa including for example, *R. anglocandicans*, *R. laciniatus*, *R. leucostachys*, *R. polyanthemus* and *R. ulmifolius* (Evans et al. 2007).

[2] Annual rainfall >750 mm, abundant summer rainfall and average maximum daily temperatures in January of 20°C (Pigott et al. 2003).

(thorny longanberry, youngberry, boysenberry, lawtonberry) and garden rose varieties. No attack however, was recorded on raspberry (*Rubus idaeus*) or four Australian native *Rubus* species (Bruzzese 1982). However, incidental surveys conducted in 2004 in southern France revealed that the sawfly was never found on closely related species of blackberry (*Rubus caesius*, *Rosa canina* and *Rosa rubiginosa*) (Sagliocco and Bruzzese 2004). This raised the possibility that results from preliminary host testing conducted 35 years ago may have been influenced by laboratory procedures used.

Figure 1. Site on the Warren River, Western Australia, where signs of blackberry decline were first observed in 2006, with the entire blackberry population dead by 2008 after winter floods (Photos courtesy of Paul Yeoh, CSIRO).

The goal of this project was to explore new avenues for the biocontrol of blackberry, which remains an important weed of the livestock industry and natural ecosystems in areas that are sub-optimal for development of the leaf-rust fungus introduced for biocontrol. The project primarily focused on better understanding the blackberry decline syndrome observed in Western Australia and exploring ways on how it could be manipulated, including assessing the susceptibility of different blackberry taxa and pasture and native species to the primary *Phytophothora* species responsible for the decline. The project also performed surveys in the native range of blackberry to determine the field host-range of the stem-boring sawfly by sampling *Rubus* species and closely-related species in the Rosaceae family in order to determine whether further research into the biocontrol potential of the sawfly is warranted.

2 Project objectives

2.1 Original objectives (executed agreement – March 2016)

By 1 September, 2018

1. Determine the potential of the fungus *Phytophthora bilorbang* as an inundative biological control tool for blackberry by developing prototype systems for its production and application, conducting host-specificity tests and evaluating its efficacy in field trials over two years.
2. If promising, devise a plan for future large-scale delivery of the fungus to land holders affected by blackberry. If not promising, make recommendations for next steps in the biological control of blackberry.

2.2 Modified objectives (executed variation – November 2017)

In light of challenges encountered during the project, the original objectives were modified, as follow, to make them less prescriptive and to include key decision points that occurred.

By 1 September, 2018

1. Determine the potential of *Phytophthora* species, as an inundative biological control tool for blackberry by conducting pathogenicity and host-specificity glasshouse tests and, if promising, evaluating efficacy in field trials.
2. If *Phytophthora* species are not promising, investigate an alternate option for biocontrol of blackberry.
3. Make recommendations for next steps in the biological control of blackberry.

3 Method and project locations

3.1 Blackberry decline

3.1.1 Plant propagation

Plants used in all experiments were watered regularly and fertilised fortnightly.

Blackberry propagation from seed

Rubus anglocandicans – used in all experiments unless otherwise specified – and *Rubus ulmifolius* were propagated from seed extracted from fruits collected at sites along the Warren River in Western Australia in 2014 and 2015, respectively. The seeds were surface sterilised in a bleach solution and rinsed well with sterile water. The endocarp (hard coat) of each seed was then removed with a scalpel under a dissecting microscope and the seed placed on the surface of water agar contained in Petri dishes. Plates were placed in a controlled-environment room set at 23 °C with a 12-h photoperiod. Once seeds had germinated and developed cotyledons, seedlings were planted into small pots containing pasteurised, washed river sand or potting mix, depending on the experiments, and pots were placed in the controlled-environment room. Small plants (before first compound leaf had developed) were transplanted into the same soil medium contained in larger pots (10-cm, 12-cm or 15-cm diam.) and placed in a glasshouse.

Blackberry propagation from cane tip-rooting

Blackberry taxa from the plant collection that has been maintained at CSIRO for many years were propagated by placing the tip of canes (5-6 cm long) into small pots containing potting mix in the glasshouse. The tip-rooted plants were separated from the mother plants a few weeks later once the cane tips had produced roots and either a shoot was growing from the roots or a portion of the original cane had a bud or small shoot. Plants were transplanted into potting mix in 12-cm diam. plastic pots and grown for approximately another month before use in an experiment.

Non-target plant species propagation

Seed of non-target plant species were obtained from commercial providers. They were treated as recommended to stimulate germination wherever necessary. Seed were planted in vermiculite-perlite mix, sand or potting mix contained in small pots placed in the controlled-environment room at 23 °C (12 h photoperiod) or directly in the glasshouse. Once seedlings had emerged they were transplanted into potting mix contained in 12-cm diam. plastic pots and placed in the glasshouse. A 50-mL centrifuge vial was inserted in the potting mix of each pot.

3.1.2 Plant measurements

Above-ground biomass (foliage) of living plants was harvested at the end of experiments when an unambiguous treatment(s) effect was observed. For each plant, stems were cut at soil surface and the entire foliage placed in a large paper bag. Bags were placed in an oven at 70°C for at least 3 days before dry weight was measured.

Below-ground biomass was also harvested for living blackberry plants grown in sand in a few experiments. For each plant, the sand was washed off from roots by immersing them in water. The volume of roots was measured using the water displacement method, before the root mass was placed in a paper bag, dried as above and dry weight measured.

3.1.3 *Phytophthora* isolates

Original isolates

Four isolates of *P. bilorbang* that had been used in the PhD studies of Sonia Aghighi and had been in storage for a few years were obtained from collaborators at Murdoch University – isolates no. 92, 142, 143 and 262 (Aghighi et al. 2015). The isolates were first reinvigorated with the 'green apple' technique utilised with many *Phytophthora* species. This involves inserting small plugs of agar colonised by the *Phytophthora* isolate approximately 20 mm deep into a surface sterilised apple (cv. Granny Smith) and reisolating in pure culture the isolate from the lesion that developed after incubation at 25 °C with a 14-h photoperiod (Crone et al. 2013) (Fig. 2). In laboratory tests, all recovered isolates produced the typical structures of *Phytophthora*; oospores, sporangia and zoospores. Once this was confirmed, a series of initial trials were sequentially initiated.

Field collection of new isolates

In mid-January 2016, we began to be concerned about the pathogenicity of the original isolates used in initial experiments, and decided to source new isolates from blackberry at sites where the decline had been observed in south-west Western Australia. Blackberry plants were dug up from the field in May 2016 and brought back to the laboratory for processing

Rhizosphere soil and root samples were baited twice with juvenile leaves of species commonly used to recover *Phytophthora* species (Aghighi et al. 2012). After 3–10 days, baits with brownish lesions were blotted dry, and the lesions cut into 2–5 mm sections and plated onto a *Phytophthora* selective medium contained in Petri dishes. Plates were incubated in the dark at 20 °C and checked regularly for *Phytophthora* colonies, which were then subcultured. DNA was extracted from each culture and

the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA amplified by PCR using DC6 and ITS4 primers and sequenced to confirm identification (Aghighi et al. 2012).

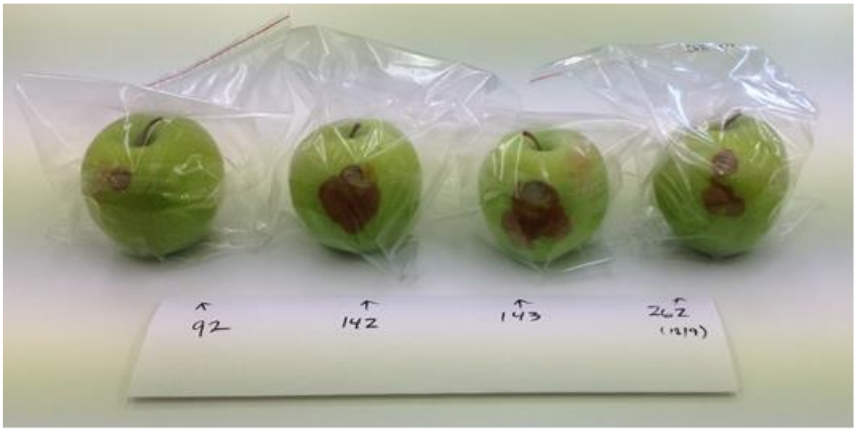

Figure 2. Reinvigoration of the stored isolates of *Phytophthora bilorbang* from Sonia Aghighi PhD studies using the 'green apple' technique.

3.1.4 Mass-production of inoculum

Standard production method

The standard method to produce *Phytophthora* inoculum for experiments involved a vermiculite-based substrate supplemented with millet and V8 broth. Erlenmeyer flasks with cotton plugs (500 mL), each containing 250 mL vermiculite (by volume), 2.5 g millet seed and 150 mL V8 broth (120 mL V8 juice, 480 mL distilled water and 2 g $CaCO_3$, pH adjusted to 7), were autoclaved twice over two consecutive days at 121 °C for 20 min. The vermiculite was separately autoclaved for approx. 1 h before use in the preparation of the medium. Five 10-mm diameter agar disks cut from the margin of 6–8 day-old cultures of the *Phytophthora* isolates grown on V8 agar plates were added to each flask. Non-colonised agar disks were added to control flasks. The flasks were incubated for 8–10 wks (unless stated otherwise) at 20 °C in the dark, and shaken weekly to ensure even colonisation of the substrate.

Production on different grains – original isolates of *P. bilorbang*

Pearl barley, millet, white and brown rice, were tested for inoculum production of *P. bilorbang* using the original isolates. Each 250-mL Erlenmeyer flasks with a cotton plug containing 20 g grain and 20 mL distilled water was autoclaved twice as above. All flasks were inoculated with *P. bilorbang* isolate no. 262 (as above except that only three agar disks per flask were used) and incubated for 4 wks (similar conditions as above). The isolate grew on all substrates, but white and brown rice were found to be unsuitable because of excessive stickiness leading to clumping of the substrate. These two substrates were thus not included in subsequent experiments.

The first experiment involved blackberry plants grown in sand contained in 10-cm diam. plastic pots into which one 50-mL plastic centrifuge vial was inserted per pot. Plants at the 5–6 leaf stage were inoculated with either pearl barley or millet colonised by *P. bilorbang* isolates no. 92, 142, 143 or 262, at a dosage of 10-11 g per hole/pot. Non-colonised pearl barley or millet was applied to control plants. Four replicates per substrate–isolate combination treatment were used. Plants were subjected to five 72-h flooding treatments, at approx. 2, 4, 6, 8, and 10 wks after inoculation. Plants were assessed at 5 months after inoculation.

The second experiment was similar to the previous one, except that pearl barley grains were washed or not under running tap water for 10 min. before the medium was prepared and autoclaved (same

as above). Each pot was inoculated with washed or non-washed pearl barley colonised by *P. bilorbang* isolate no. 262 (incubated for 17 days under similar conditions as above) at a dosage of 20 g per hole/pot. Non-colonised pearl barley substrate (washed or unwashed) was applied to other pots. Only sand was placed in the hole left once the centrifuge vial was removed for control plants. Four or six replicates per treatment combination were used. Plants were subjected to three 72-h flooding treatments, at 3 days, 5 and 8 wks after inoculation. Plants were assessed at 4 months after inoculation.

Production in bags – *P. pseudocryptogea*

Breathable polypropylene bags (dim. 45 X 32 cm, two filters; Unicorn, Plano TX, USA), each containing 1,500 mL vermiculite or shredded sugarcane mulch (by volume) with 15 g millet seed and 900 mL V8 broth, were autoclaved twice over two consecutive days at 121 °C for 20 min (Fig. 3). Half the bags were inoculated with a 5 mL mycelial suspension from a V8 broth shake culture of *P. pseudocryptogea* isolate RD2A (100 mL V8 broth contained in 250-mL Erlenmeyer flask inoculated with 1 mL of a mycelial suspension made from a 1-wk old colony growing on PDA and placed for 1 wk on a shaker in a controlled environment room at 20 °C with a 12-h photoperiod). The remaining half of the bags were not inoculated. The bags were placed in an incubator at 20 °C in the dark for 8 wks and shaken weekly before use.

Blackberry plants (*R. anglocandicans*; WA accession) grown using the cane tip-rooting method and *Medicago sativa* (lucerne) and *Lolium perenne* (perennial ryegrass) plants, grown from seed were transplanted at the 3–5 leaf stage into potting mix contained in 12-cm diam. plastic pots and placed in the glasshouse. Two 50-mL plastic centrifuge vials were inserted in the potting mix of each pot. Plants were inoculated 7 days after transplantation with the vermiculite or sugarcane mulch-based substrate colonised or not colonised by *P. pseudocryptogea* at a dosage of 10 g per hole (total of 20 g per pot). Three or four replicates for blackberry and five replicates for the other two species per treatment combination were used. Plants were subjected to four 72-h flooding treatments, at 1, 3, 5 and 7 wks after inoculation. Foliage of plants was harvested at 9 wks after inoculation and processed as described above.

Figure 3. Breathable polypropylene bags with filters used to produce inoculum of *Phytophthora pseudocryptogea*. Left – sugarcane mulch-based substrate; Right – vermiculite-based substrate.

3.1.5 Viability of inoculum

Vermiculite-based substrate colonised by *P. bilorbang* isolate no. 262 was dried using two methods: i) fast drying by placing a thin layer of substrate in a surface sterilised tray in a biosafety cabinet for 3 days, ii) slow drying by removing cotton plug from each Erlenmeyer flask containing the substrate and leaving opened flasks in a biosafety cabinet for 7 days. The viability of *P. bilorbang* in the dried inoculum was then compared with that of fresh inoculum by plating sub-samples onto half-strength potato dextrose agar (PDA) contained in Petri dishes. No colony of *P. bilorbang* grew from any particles of substrate dried using either the fast or slow-drying methods. In light of this, the shelf-life assessment trial was set up using fresh vermiculite-based substrate colonised by *P. bilorbang*. The colonised substrate (5-wk after inoculation with *P. bilorbang*) was stored in sterile MacCartney vials in the dark under three different conditions: room temperature (~22 °C), freezer (–20 °C) and fridge (4 °C). The viability of *P. bilorbang* in the inoculum stored at the different temperatures was assessed at 5 wks, and 5 and 12 months, using the plating method described above.

3.1.6 Application technique

Standard application method

The standard application method involved applying solid substrate colonised by *Phytophthora* within the root zone of plants (Fig. 4). To ensure that plant roots would not be disturbed during application, one or two 15-mL or 50-mL centrifuge vials (depending on the experiment) were inserted in the soil medium of each pot when plants were transplanted. The vials were subsequently removed at the time of inoculation and *Phytophthora* inoculum placed into the holes. Each hole containing inoculum was covered with soil medium. Plants were then subjected to a few sequential periods of simulated flooding conditions by placing pots into buckets of water (up to the soil surface).

Effect of different dosages – original isolates of *P. bilorbang*

The first experiment involved blackberry plants grown in sand contained in 10-cm diam. plastic pots into which one 50-mL plastic centrifuge vial was inserted per pot. Plants at the 4–5 leaf stage were inoculated with vermiculite-based substrate colonised by *P. bilorbang* isolate no. 262 (incubated for 4 wks under similar conditions as above), applied at a dosage of 5, 10 or 15 g per hole/pot. Only sand was placed in the hole left once the centrifuge vial was removed for control plants. Eight replicates per dosage treatment were used. Plants were subjected to five 72-h flooding treatments, at 4, 6, 8, 10 and 12 wks after inoculation. Plants were assessed at 6 months after inoculation.

The second experiment was similar to the previous one, except that blackberry plants were grown in 15-cm diam. plastic pots into which two 50-mL plastic centrifuge vials were inserted per pot. Plants at the 5–6 leaf stage were inoculated with vermiculite-based substrate colonised by *P. bilorbang* isolate no. 262 (incubated for 6 ½ wks under similar conditions as above), applied at a dosage of 25 g per hole (total of 50 g per pot). Non-colonised substrate was applied to control plants. Fifteen replicates per treatment were used. Plants were subjected to three 72-h flooding treatments, at 1, 4 and 6 wks after inoculation. Plants were assessed at 4 months after inoculation.

Figure 4. Standard application method used in experiments, which involved applying solid substrate colonised by *Phytophthora* within the root zone of plants and subjecting plants to periods of flooding conditions by placing pots into buckets of water. Centrifuge vials were inserted in the soil medium of each pot at the time of transplanting and the inoculum placed into the hole left behind once the vials were removed at the beginning of the experiments.

Effect of different dosages – *P. pseudocryptogea*

Plants of two blackberry species (*R. anglocandicans* and *R. ulmifolius*) grown from seed were transplanted at the 3–4 leaf stage into potting mix contained in 12-cm diam. plastic pots and placed in the glasshouse. One or two 50-mL plastic centrifuge vials were inserted in the potting mix of each pot. Plants were inoculated 4 days later with the vermiculite-based substrate colonised or not colonised by *P. pseudocryptogea* isolate RD2A at a dosage of 20 or 40 g per pot (20 g per hole). Five replicates per treatment combination were used. Plants were subjected to four 72-h flooding treatments, at 1 day and, 2, 4 and 6 wks after inoculation. Foliage of plants was harvested at 8 wks after inoculation and processed as described above.

Effect of flooding – *P. pseudocryptogea*

Plants of two blackberry species (*R. anglocandicans* and *R. ulmifolius*), *Medicago sativa, Lolium perenne* and *Festuca arundinacea* (fescue) grown from seed were transplanted at the 2–3 leaf stage into potting mix contained in 12-cm diam. plastic pot and placed in the glasshouse. One 50-mL plastic centrifuge vials were inserted in the potting mix of each pot. Plants were inoculated 13 days later with vermiculite-based substrate colonised or not colonised by *P. pseudocryptogea* isolate RD2A at a dosage of 20 g per pot. Half the plants were subjected to four 72-h flooding treatments, at 2 days and, 2, 4 and 6 wks after inoculation, and the remaining plants were not exposed to any flooding. Foliage of plants was harvested at 8 wks after inoculation and processed as described above.

3.1.7 Pathogenicity of *Phytophthora* species on blackberry

Effect of the four original isolates of *P. bilorbang*

This experiment involved blackberry plants grown in sand contained in 15-cm diam. plastic pots into which two 15-mL plastic centrifuge vials were inserted per pot. Plants were at slightly different leaf stages at the time of inoculation (3–4, 5–6, 6–7 or 7–8 leaves). Each pot was inoculated with vermiculite-based substrate colonised by *P. bilorbang* isolates no. 92, 142, 143 or 262 (incubated for 5 wks under similar conditions as above), at a dosage of 10 g per hole (20 g per pot). Non-colonised substrate was applied to control plants. Three replicates per age–isolate treatment were used, except for age 7–8 leaves where two replicates were used. Plants were subjected to a 48-h flooding treatment at 2 and 5 wks after inoculation, followed with a 72-h flooding treatment at 8 and 12 wks after inoculation. Plants were assessed at 6 months after inoculation.

Effect of *P. bilorbang* and *P. pseudocryptogea* – Aghighi's methods

The methods used in glasshouse pot experiments performed by S. Aghighi as part of her PhD studies involved exposing inoculated and non-inoculated blackberry plants to a maximum of five 48 to 72-h flooding treatments over a 6 ½ month period (Aghighi et al. 2015).

Blackberry plants grown from seed were transplanted at the 3–5 leaf stage into river sand contained in 15-cm diam. plastic pots and placed in the glasshouse. Two 15-mL plastic centrifuge vials were inserted in the potting mix of each pot. Plants were inoculated 1 wk later with vermiculite-based substrate colonised by a new isolate of *P. bilorbang* (Cross2BA) or *P. pseudocryptogea* (RD2A) at a dosage of 20 g per hole (40 g per pot), or with 20 g inoculum of each species per pot. Non-colonised substrate was applied to control plants at the same dosage. Eight replicates per treatment combination were used. Plants were not subjected to any flooding treatment or subjected to three flooding treatments (24-h flooding at 1 wk after inoculation and two 72-h flooding at 4 and 8 wks after inoculation) or five flooding treatments (24-h flooding at 1 wk after inoculation and four 72-h flooding at 4, 8, 12 and 18 wks after inoculation). Foliage and roots of plants were harvested at 22 wks after inoculation and processed as described above. The experiment was performed twice using the same methodology.

Effect of *P. bilorbang* and *P. pseudocryptogea* – Revised methods

This glasshouse experiment used methods revised based on results of previous experiments with *P. pseudocryptogea* that tested the effect of different dosages of inoculum and fortnightly flooding treatments.

Blackberry plants grown from seed and of two different ages (2–3 and 4–5 leaf stage) were transplanted into river sand contained in 12-cm diam. plastic pots and placed in the glasshouse. One 50-mL plastic centrifuge vial was inserted in the potting mix of each pot. Plants were inoculated 1 wk later with vermiculite-based substrate colonised by a new isolate of *P. bilorbang* (Cross2BA) or *P. pseudocryptogea* (RD2A) at a dosage of 20 g per pot. Non-colonised substrate was applied to control plants at the same dosage. Ten replicates per treatment combination were used. Plants were subjected to four 72-h flooding treatments, at 1, 3, 4 and 7 wks after inoculation. Foliage and roots of plants were harvested at 9 wks after inoculation and processed as described above. The experiment was performed twice using the same methodology.

3.1.8 Host-specificity tests of *P. pseudocryptogea* on different blackberries and non-target species

Effect on different blackberries

Plants of *R. anglocandicans* and *R. ulmifolius* grown from seed (5–6 leaf-stage), and of various accessions/clones of *R. anglocandicans*, *R. laciniatus* and *R. leucostachys* grown using the cane tip-rooting method, were transplanted into potting mix contained in 12-cm diam. plastic pot and placed in the glasshouse. Two 50-mL plastic centrifuge vials were inserted in the potting mix of each pot. Plants were inoculated 10 days later with vermiculite-based substrate colonised or not colonised by *P. pseudocryptogea* isolate RD2A at a dosage of 20 g per hole (40 g per pot). Up to five replicates per treatment combination were used. Plants were subjected to four 72-h flooding treatments, at 1, 3, 4 and 7 wks after inoculation. Foliage of plants was harvested at 9 wks after inoculation and processed as described above.

Effect on non-target species

A series of sequential trials were performed to evaluate the effect of *P. pseudocryptogea* on a range of pasture and native plant species that could co-occur with blackberry in the field. In each trial, blackberry (*R. anglocandicans*) was used as a reference species. Each non-target species was tested in two different trials, unless indicated otherwise. Plants at the 3–7 leaf stage were transplanted into potting mix in 12-cm diam. plastic pot and transferred to the glasshouse. One 50-ml plastic centrifuge vial was inserted in the potting mix of each pot. Plants were inoculated 5–7 days later with vermiculite-based substrate colonised or not colonised by *P. pseudocryptogea* isolate RD2A at a dosage of 20 g per hole/pot. Five replicates (unless indicated otherwise) per treatment combination were used. Plants were subjected to four 72-h flooding treatments, at 1, 3, 4 and 7 wks after inoculation. Foliage of plants was harvested at 9 wks after inoculation and processed as described above.

3.1.9 Analyses

A completely randomized design was used in all experiments. Data from all experiments, except the host-specificity test trials, were analysed separately with a one-way, two-way or factorial ANOVA with the statistical package R (release 3.4.4) (R Development Core Team 2018). Wherever the underlying assumptions (residuals normally distributed, unbiased and homoscedastic) were not met for the data, log natural or square root transformation was applied. Differences between means were established using the post-hoc comparison of least-square means (Tukey-adjusted comparisons) in R.

A F-test was first performed on each species included in the host-specificity tests to determine if the variances of the two populations (inoculated and control plants) were equal or not. Depending on results of F-test, a two-sample t-test assuming equal or unequal variances was performed on each species.

3.2 Sawfly

3.2.1 Field surveys

Colleagues from the Centre for Agriculture and Biosciences International (CABI) UK were first approached to gauge their views on the likelihood of finding the stem-boring sawfly (*P. faunus*; formerly *H. albomaculata*) in the United Kingdom. England is the region where *R. anglocandicans*, the most widespread taxon of *R. fruticosus* agg. in Australia, most likely originated according to genetic studies (Evans and Weber 2003). Concerns however, were raised following consultation with

expert taxonomists who stated that the likelihood of finding the sawfly in the UK would be low (Appendix 1).

A series of field surveys to sample blackberry and other closely-related species were thus primarily conducted across Western Europe between January and May 2018 (Fig. 5). Sampling was done at this time to collect *P. faunus* immature stages present within plant stems (i.e. larvae, pupae or newly-eclosed adults). Mediterranean Europe is the native area of *P. faunus*, consequently, eight sites were sampled across France (mainland and Corsica) (Fig. 6), one site in Italy (Sardinia) and 12 sites in Portugal. Surveys were also conducted near London, UK (four sites) to see if *P. faunus* could be detected in *Rubus* species occurring there. Where possible, sites that had both *Rubus* and *Rosa canina* present were sampled, but at other sites, only one species was present (Table 1). Details of every site are provided in Appendix 2.

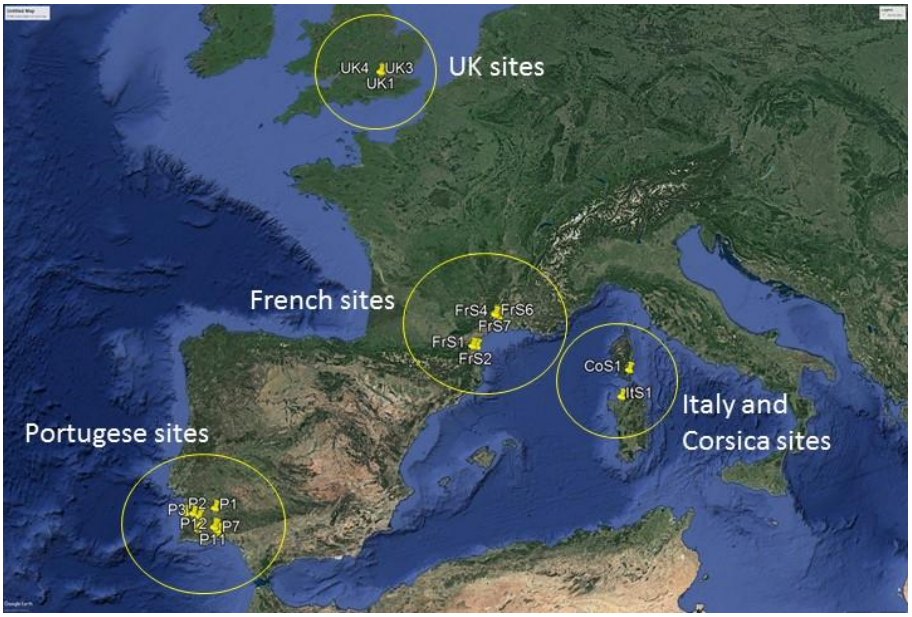

Figure 5. Survey sites for *Phylloecus faunus* in Western Europe and the United Kingdom. Not all sites are shown because of some have the same or very close coordinates.

Unfortunately, no investigation could be conducted in Spain as originally intended because "specimen collecting permits" that had been applied for several weeks before the travel, had not yet been issued by the Spanish government at the time the field survey was scheduled.

Opportunistic surveys were also conducted in March 2018 in western Morocco while CSIRO staff were present in the region for another project. A large part of the western Morocco was surveyed (from Marrakech to Essaouira, Safi, El Jadida, Berrchid and Marrakech), but unfortunately no *Rubus* was observed during the trip. The surveyed region was possibly located too far South in Morocco to observe high population of *Rubus* sp. Pastoral farming in this region is very important and grazing by goats and sheep could also be one the reasons for the absence (or the very low density) of *Rubus*.

For collections made in January and February 2018, three patches of *Rubus* species were selected at each site, and within these patches, 10 primocanes were collected (30 primocanes per site). Each cane was cut in 15 cm sections and samples of the same patch were pooled together in one paper bag. For *Rosa* species, 30 canes of the year (i.e. plants that grew up last spring) were haphazardly collected across the site. When it was not possible to collect young plants (canes of the year), young

canes from older plants were collected. The samples were processed as previously described for *Rubus* species.

Figure 6. Examples of sites where samples were collected as part of field surveys for *Phylloecus faunus*. Site numbers indicated in top left corner of each photograph.

Table 1. Total number of *Rubus* and *Rosa canina* populations sampled per region.

	Rubus sp.	*Rosa canina*
France (mainland)	8	6
Corsica	1	0
Italy	1	1
Portugal	11	4
UK	4	0

Back at the laboratory, the content of each bag was placed in a plastic box (1.5 L) in a controlled environment room, where samples were exposed to natural variations of temperatures (always > 10 °C) supplemented by an artificial 12-h photoperiod until 31 March 2018 and then to a 16-h photoperiod. Boxes are checked regularly for emergence of insects. Emerging insects were collected and stored in 96% ethanol. In early June, every 15 cm sections of canes were dissected to check for the presence of non-emerged insects.

For collections made between March and May 2018, the same method as above was used, except that canes were dissected directly in the field or laboratory following sampling. All insect stages (eggs, larvae, pupae and adults) found in canes were collected and stored in 96% ethanol.

3.2.2 Identification

For all specimens, a "rapid" identification (to family, subfamily or genus) was performed. Adult insects emerged from canes were identified morphologically to confirm that they were *P. faunus* (see Appendix 3 for description of the species). However, because of the larval stage and/or the bad conditions of some specimens (damaged during dissection, dried larva, etc.), every identification was considered tentative and required further investigation using molecular techniques. Hence, the preserved specimens were transported to Australia where DNA barcoding was undertaken by Agriculture Victoria (DEDJTR) insect taxonomists.

Images of all insect specimens were obtained using the automontage Leica LAS software on a M205 Leica microscope, prior to DNA extraction. DNA was extracted from ethanol preserved adult and larval insects using DNeasy Blood and Tissue extraction kit (Qiagen), following the manufacturers protocol. A single leg was removed from the adult insects, while larval specimens were processed using a non-destructive overnight digest method (Blacket, unpublished), which allows DNA to be extracted while preserving the insect voucher specimen for future morphological examination.

PCR amplifications of the standard DNA barcoding region of Cytochrome Oxidase I (COI) were performed using universal primers LCO1490 / HCO2198 (Folmer *et al.* 1994), on an ABI Veriti thermal cycler, with a PCR annealing temperature of 50^{o}C. Amplicons of the COI gene were sequenced using a commercial provider (Macrogen, Korea). Identifications of all specimens were performed through querying the BOLD reference sequence database (http://www.boldsystems.org).

4 Results

4.1 Blackberry decline

4.1.1 *Phytophthora* isolates – field collection

Blackberry samples, including roots and rhizosphere soil, were collected from three sites in south-west Western Australia where the decline has been observed:

- Rory Dean (on Donnelly River): One of the first sites where extensive and rapid blackberry decline occurred 7–8 years ago. Only a few blackberry plants present at the time of collection. Small plants that looked healthy and had no obvious crown or root damage were sampled (Fig. 7A).
- Pozzi Road (on Wilgarrup that feeds Warren River): Site where blackberry decline was observed in early 2013 and where the population crashed after big floods in 2013/14. Small plants that looked healthy and had no obvious crown or root damage were sampled. Large

plants that looked unhealthy, with signs of decline (large dead stems, small live stems, red crown/roots) were sampled.

- Cross Road (near Nyamup town site on Wilgarrup that feeds Warren River): Site where first signs of potential blackberry decline were observed in 2013 and where extensive decline is currently occurring. Large crowns with a small unhealthy cane growing from them were sampled. Roots and crowns were red, a sign of disease (Fig. 7B).

Several isolates of different *Phytophothora* species were recovered from samples (Table 2).

Figure 7. Rory Dean (**A**) and Cross Road (**B**) sites in May 2016 when blackberry samples were collected to recover new *Phytophthora* isolates for the project.

Table 2. Oomycetes species recovered from blackberry samples collected in May 2016 at three sites in south-west Western Australia where the decline has been observed (star indicates that the species had previously been recovered during S. Aghighi's PhD studies).

Site name	Isolate ID	Species
Rory Dean	RD-3A	*Phytophthora pseudocryptogea* * [1]
	RD-2A	*Phytophthora pseudocryptogea* *
	RD-1B	*Phytophthora pseudocryptogea* *
	RD-2	*Phytophthora pseudocryptogea* *
Pozzi Road	Pozzi-3	*Phytophthora gregata*
	Pozzi-4A	*Phytophthora thermophila* *
	Pozzi-4B	*Phytophthora inundata* *
Cross Road	Cross-2C	*Phytopythium. aff. litorale*
	Cross-2BA	*Phytophthora bilorbang* *
	Cross-2B.c	*Phytophthora bilorbang* *
	Cross-2B.b1	*Phytophthora bilorbang* *
	Cross-2B.b2	*Phytophthora bilorbang* *
	Cross-2B.d	*Phytophthora bilorbang* *

[1] Referred to as *P. cryptogea* in Aghighi's study.

4.1.2 Mass-production of inoculum

Production on different grains – original isolates of *P. bilorbang*

The first experiment performed to test the effect of the four original isolates of *P. bilorbang* applied as pearl barley or millet seed based inoculum on blackberry was inconclusive because all control plants had died by the end of the experiment (Fig. 8). It is noteworthy that in the couple of weeks following plant inoculation, saprophytic fungal growth on the sand surface of pots was observed.

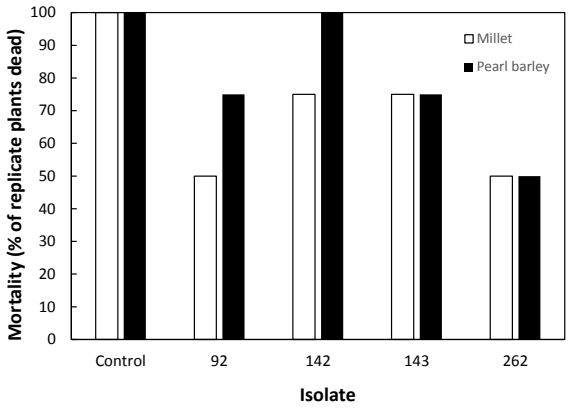

Figure 8. Percentage of replicate blackberry plants for each treatment and control that were dead at 5 months after inoculation with pearly barley or millet seed colonised or not by the four original isolates of *Phytophthora bilorbang*.

The second experiment performed to test the effect of one of the original isolates of *P. bilorbang* applied as pearl barley based inoculum prepared with washed or non-washed grains, was also inconclusive as several non-inoculated plants, where non-colonised substrate or no substrate was added to the sand, died (Fig. 9). Saprophytic fungal growth on the sand surface was also observed in most pots that contained pearl barley, washed or non-washed prior to autoclaving during inoculum preparation.

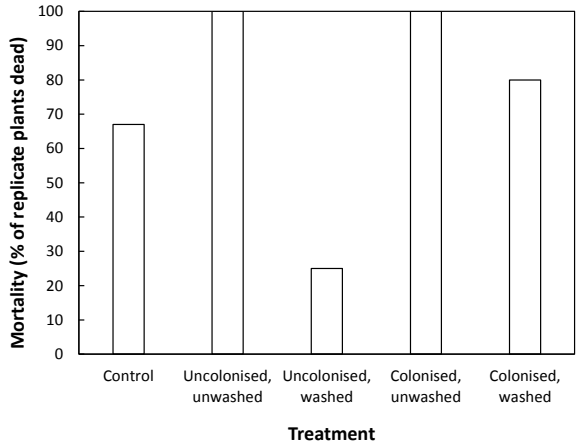

Figure 9. Percentage of replicate blackberry plants for each treatment and control that were dead at 4 months after inoculation with washed or non-washed pearly barley colonised or not by one of the original isolates of *Phytophthora bilorbang*.

Production in bags – *P. pseudocryptogea*

The experiment showed that the type of substrate used to produce inoculum of *P. pseudocryptogea* in breathable polypropylene bags did not significantly influence its effect on plants (p = 0.12) (Fig. 10). *Phytophthora pseudocryptogea* produced on either vermiculite or sugarcane mulch based

substrates, significantly reduced the foliage biomass of blackberry plants ($p < 0.001$) compared to non-inoculated control plants. The other plant species included in the experiment however, were not significantly affected by *P. pseudocryptogea*, irrespective of the substrates used to produce inoculum.

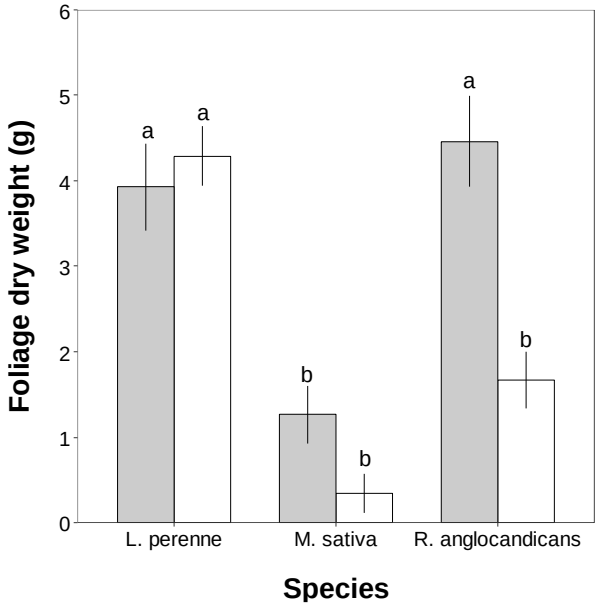

Figure 10. Foliage dry weight of plants of three different species (*Lolium perenne*, *Medicago sativa* and *Rubus anglocandicans*) inoculated with vermiculite or sugarcane mulch based substrates, colonised (open bars) or non-colonised (grey bars) by *Phytophthora pseudocryptogea.* Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter are not significantly different (alpha = 0.05, Tukey-adjusted). Significant effects of inoculation treatment ($p < 0.001$) and species ($p < 0.001$), but no significant effect of substrate ($p = 0.12$). Only the interaction between species and inoculation treatment was significant ($p < 0.001$).

4.1.3 Viability of inoculum

Five weeks after the commencement of the shelf-life assessment, *P. bilorbang* grew from all particles of the colonised vermiculite-based substrate stored at room temperature or in the fridge after plating on agar. In contrast, no colony of *P. bilorbang* grew from any particles of the colonised substrate stored in the freezer at –20 °C. Similar results were obtained after 5 and 12 months of storage, although a few colonies of bacterial and fungal contaminants were also observed growing from the substrate.

4.1.4 Application technique

Effect of different dosages – original isolates of *P. bilorbang*

The experiment performed to test the effect of one of the original isolates of *P. bilorbang* applied at different dosages of colonised vermiculite-based substrate on blackberry was inconclusive because several of the control plants had died by the end of the experiment (Fig. 11).

In the second experiment, 15 blackberry plants were inoculated with a very large dosage (50 g per pot) of vermiculite-based substrate colonised by one of the original isolates of *P. bilorbang* and compared to 15 control plants in which non-colonised substrate was applied. By the end of the experiment at 4 months after inoculation, no conclusion could be drawn because one of the control plants had died and only two plants inoculated with *P. bilorbang* were wilting.

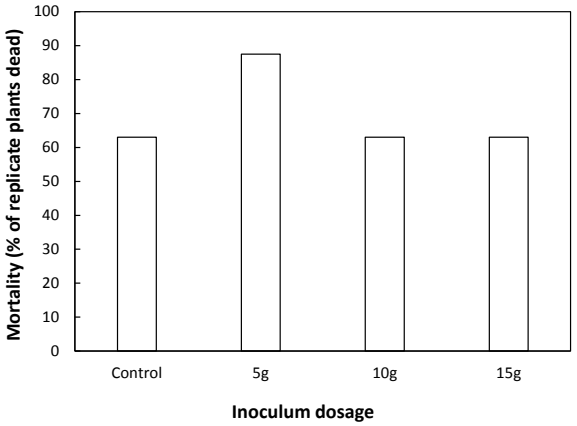

Figure 11. Percentage of replicate blackberry plants for each treatment and control that were dead at 6 months after inoculation with different dosages of vermiculite-based substrate colonised by one of the original isolates of *Phytophthora bilorbang*.

Effect of different dosages – *P. pseudocryptogea*

This experiment showed that there was no significant difference in the effect of *P. pseudocryptogea* on blackberry plants of either *R. anglocandicans* or *R. ulmifolius* ($p = 0.71$), when applied at a dosage of 20 or 40 g of colonised vermiculite-based substrate per pot ($p = 0.86$) (Fig. 12). There was however, a significant difference between inoculated and control plants ($p < 0.001$).

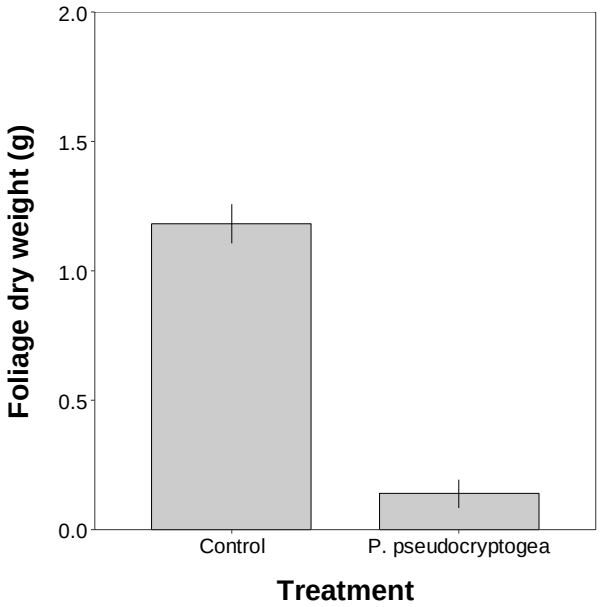

Figure 12. Foliage dry weight of two species of blackberry (*Rubus anglocandicans* and *Rubus ulmifolius*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora pseudocryptogea*, incorporated into the soil at a dosage of 20 or 40 g per pot. Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. A significant effect of inoculation treatment ($p < 0.001$), but no significant effect of species ($p = 0.71$) and dosage of colonised or non-colonised substrate applied ($p = 0.86$), and no significant interaction between any of the factors ($p > 0.1$).

Effect of flooding – *P. pseudocryptogea*

In this experiment, *P. pseudocryptogea* had a significant effect on either *R. anglocandicans* or *R. ulmifolius* only when plants were subjected to fortnightly 72-h flooding treatments ($p < 0.001$) (Fig. 13). There was no significant difference in response between the two blackberry species ($p = 0.45$). Exposure to flooding did not affect control, non-inoculated plants, as there was no significant difference between control plants subjected or not to flooding ($p < 0.001$). Inoculated plants not subjected to flooding were not significantly different to control plants.

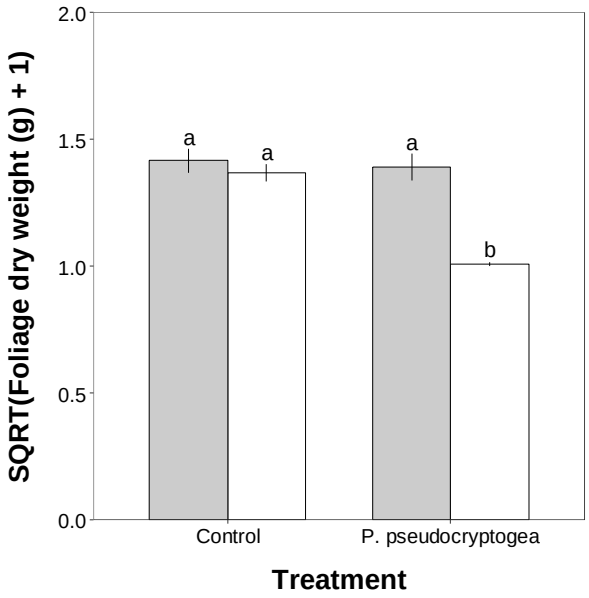

Figure 13. Square root-transformed foliage dry weight of two species of blackberry (*Rubus anglocandicans* and *Rubus ulmifolius*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora pseudocryptogea*, and exposed to no flooding (grey bars) or fortnightly flooding treatments (open bars). Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter are not significantly different (alpha = 0.05, Tukey-adjusted). A significant effect of inoculation treatment ($p < 0.001$) and flooding ($p < 0.001$), but no significant effect of species ($p = 0.45$). Only the interaction between flooding and inoculation treatment was significant ($p < 0.001$).

4.1.5 Pathogenicity of *Phytophthora* species on blackberry

Effect of the four original isolates of *P. bilorbang*

Similarly to other experiments that involved the original isolates of *P. bilorbang*, this experiment performed to test the effect of the four isolates on blackberry plants at different leaf-stage at the time of inoculation was inconclusive. Several of the control and treated plants were dead by the end of the experiment at 6 months after inoculation (Fig. 14).

Effect of *P. bilorbang* and *P. pseudocryptogea* – Aghighi's methods

A significant effect of inoculation treatment was detected only for foliage dry weight in the first trial of this experiment ($p < 0.001$) (Fig. 15). However, the significant interaction between flooding and inoculation treatments ($p = 0.02$) in this trial makes it difficult to interpret results. There was no significant effect of inoculation treatment on root dry weight (no difference between least-square means, alpha = 0.05, Tukey-adjusted) and root volume ($p = 0.10$), and only a significant effect of flooding for root dry weight ($p = 0.005$; root volume $p = 0.15$). Plants not subjected to any flooding had the highest root dry weight.

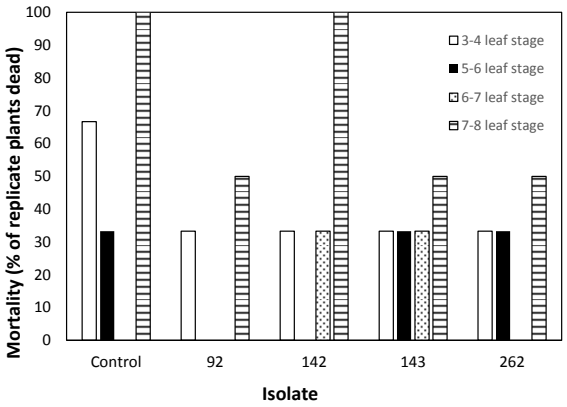

Figure 14. Percentage of replicate blackberry plants for each treatment and control that were dead at 6 months after inoculation with vermiculite-based substrate colonised or not by the four original isolates of *Phytophthora bilorbang*. The leaf stage of plants at the time of inoculation in each treatment and control is indicated.

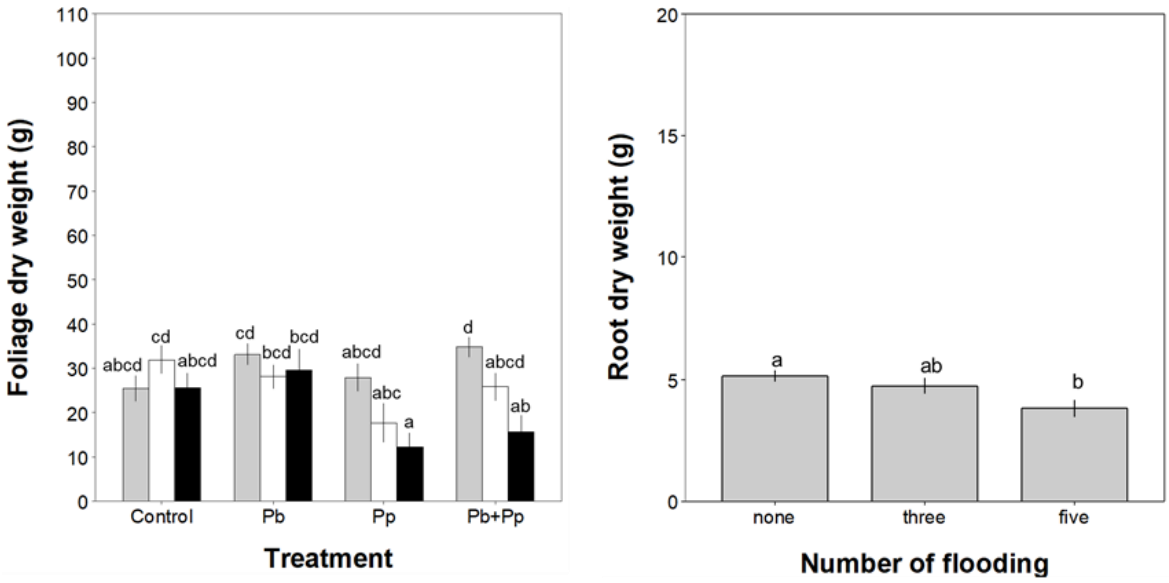

Figure 15. First trial: Foliage and root dry weight of blackberry (*Rubus anglocandicans*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora bilorbang* alone (Pb), *Phytophthora pseudocryptogea* alone (Pp) or *P. bilorbang* and *P. pseudocryptogea* combined (Pb+Pp), and exposed to none (grey bars), three (open bars) or five (solid bars) flooding treatments over 22 wks (5 ½ months). Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter in each graph are not significantly different (alpha = 0.05, Tukey-adjusted). For foliage dry weight, significant effects of inoculation treatment ($p < 0.001$) and flooding ($p < 0.001$), and a significant interaction between flooding and inoculation treatment ($p = 0.02$). For root dry weight, significant effects of flooding ($p = 0.005$) and inoculation treatment ($p = 0.03$), although no difference between least-square means of the latter (alpha = 0.05, Tukey-adjusted). No significant interaction between flooding and inoculation treatment ($p = 0.15$).

In the second trial of the experiment, there was a significant effect of flooding on foliage (p = 0.002) and root dry weight (p = 0.001) and root volume (p = 0.04), but no significant effect of inoculation treatment on any of the dependent variables (foliage dry weight p = 0.57; root dry weight p = 0.47; root volume p = 0.33) (Fig. 16). There was also no significant interaction between flooding and inoculation treatment (foliage dry weight p = 0.45; root dry weight w p = 0.67; root volume p = 0.24). Overall plants not subjected to any flooding had a higher foliage and root dry weight, and root volume.

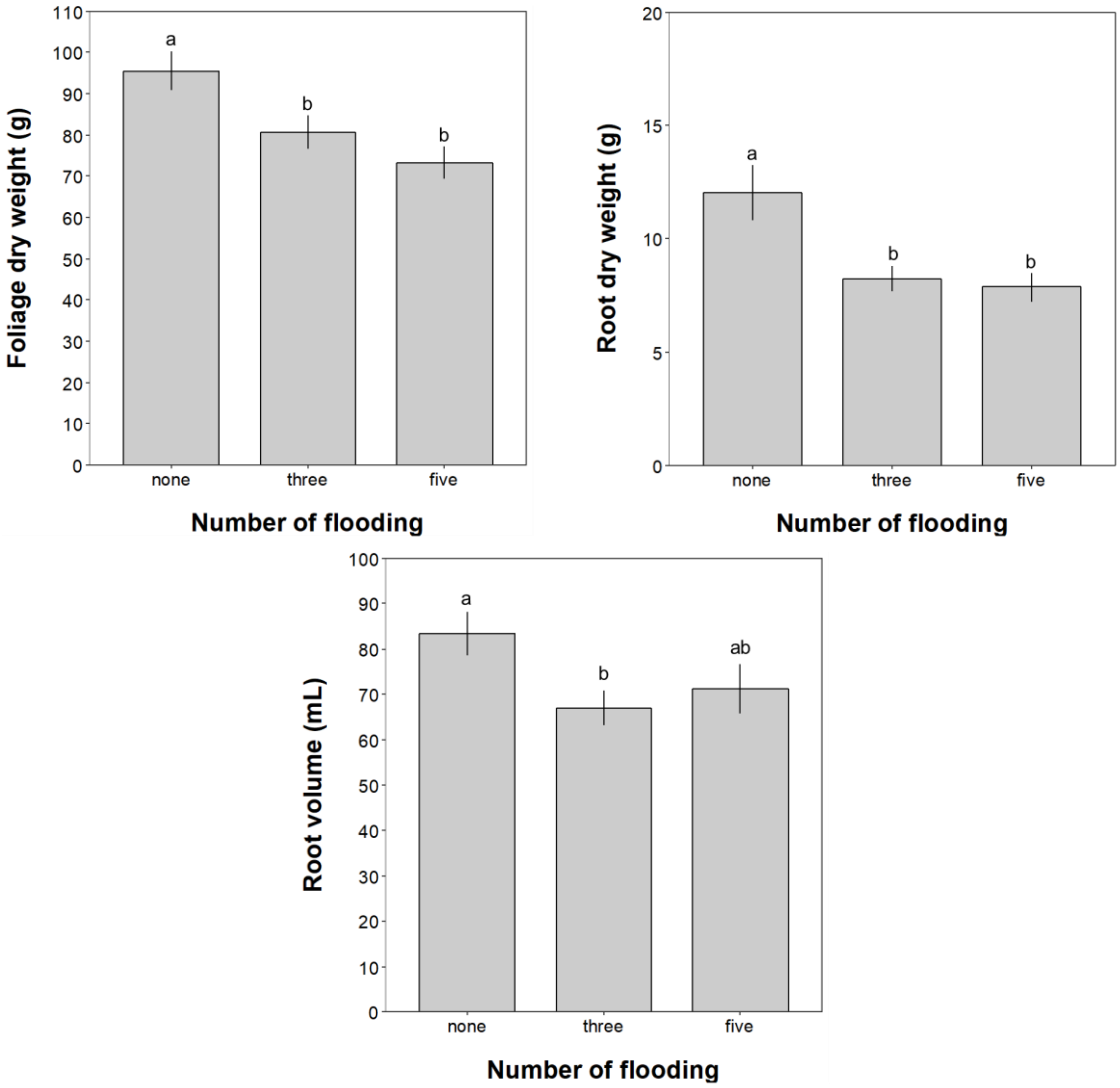

Figure 16. Second trial: Foliage and root dry weight, and root volume of blackberry (*Rubus anglocandicans*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora bilorbang* alone (Pb), *Phytophthora pseudocryptogea* alone (Pp) or *P. bilorbang* and *P. pseudocryptogea* combined (Pb+Pp), and exposed to none (grey bars), three (open bars) or five (solid bars) flooding treatments over 22 wks (5 ½ months). Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter in each graph are not significantly different (alpha = 0.05, Tukey-adjusted). For all dependent variables, a significant effect of flooding (foliage dw and root dw p < 0.01; root vol p = 0.04), but no significant effect of inoculation treatment (foliage dw p = 0.57; root dw p = 0.47; root vol p = 0.33) and no significant interaction between flooding and inoculation treatment (foliage dw p = 0.45; root dw p = 0.67; root vol p = 0.24).

Effect of *P. bilorbang* and *P. pseudocryptogea* – Revised methods

In the first trial of the experiment, both plant size and inoculation treatment were significant for foliage dry weight ($p < 0.001$), and root dry weight and volume ($p < 0.01$), but the interaction between these two factors for all dependent variables was not significant (foliage dw $p = 0.08$; root dw $p = 0.33$; root vol $p = 0.87$) (Fig. 17). Separate ANOVAs performed within each size level, since the interaction was not significant, revealed that there was only a significant effect of inoculation treatment for foliage dry weight in large plants ($p < 0.001$). In contrast, there was a significant effect of inoculation treatment for all dependent variables in small plants (foliage dw and root vol $p < 0.001$; root dw $p = 0.02$). Overall, plants inoculated with *P. pseudocryptogea* had the smallest foliage and root dry weight, and root volume. Plants inoculated with *P. bilorbang* were not significantly different to control plants.

A significant effect of plant size and inoculation treatment was detected for all dependent variables in the second trial of this experiment ($p < 0.001$) (Fig. 18). There was however, a significant interaction between plant size and inoculation treatment in this trial (foliage dw $p = 0.002$; root dw $p = 0.003$; root vol $p = 0.02$). When inoculation was performed on large plants, *P. pseudocryptogea* significantly reduced foliage and root biomass of plants, compared to the control and to plants inoculated with *P. bilorgang*. On the other hand, there was no significant difference between inoculation treatments at the end of the trial for blackberry plants that were smaller at the time of inoculation.

4.1.6 Host-specificity tests of *P. pseudocryptogea* on different blackberries and non-target species

Effect on different blackberries

Phytophthora pseudocryptogea significantly reduced (alpha = 0.05) foliage dry weight of the two species of blackberries grown from seeds, *R. anglocandicans* and *R. ulmifolius*, as well as two accessions of *R. anglocandicans* propagated via cane tip-rooting (Fig. 19). None of the other blackberry taxa propagated by cane tip-rooting (*R. polyanthemus* clone 961107, *R. laciniatus* clone KE1, *R. leucostachys* clone 972101, *R. leucostachys* clone EB9) were significantly affected by *P. pseudocryptogea*. It is noteworthy that there was considerable variation in foliage dry weight for these taxa, as illustrated with the large standard error bars associated with the means.

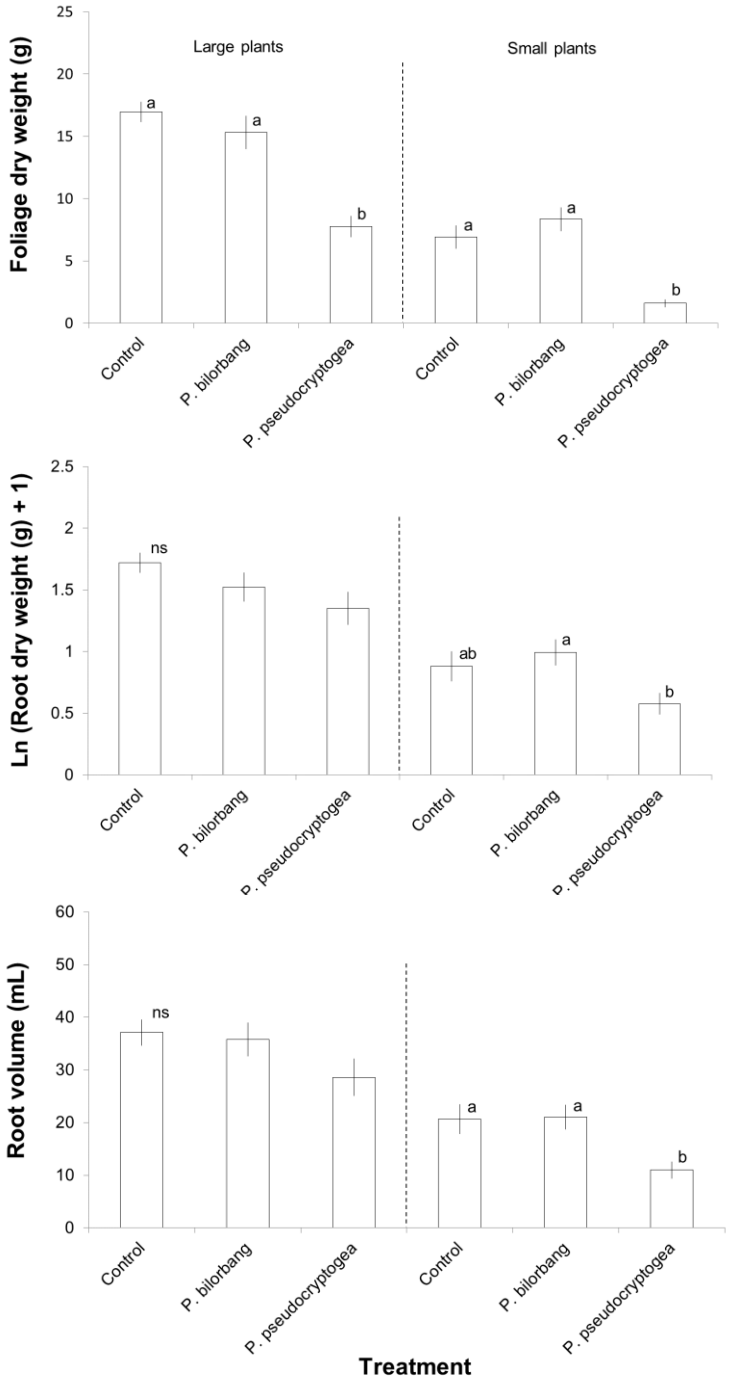

Figure 17. First trial: Foliage dry weight, log-transformed root dry weight and root volume of large and small blackberry (*Rubus anglocandicans*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora bilorbang* or *Phytophthora pseudocryptogea*. For all dependent variables, there was a significant effect of plant size (all $p < 0.001$) and inoculation treatment (foliage dw $p < 0.001$; root dw and vol $p < 0.01$), but no significant interaction between plant size and inoculation treatment (foliage dw $p = 0.08$; root dw $p = 0.33$; root vol $p = 0.87$). Since there was no significant interaction, separate ANOVAs within each size level were performed to explore the effects of one variable while the other stays constant. Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter within each plant size are not significantly different (alpha = 0.05, Tukey-adjusted). A significant effect of inoculation treatment only for foliage dry weight in large plants ($p < 0.001$), and for all dependent variables in small plants (foliage dw and root vol $p < 0.001$; root dw $p = 0.02$).

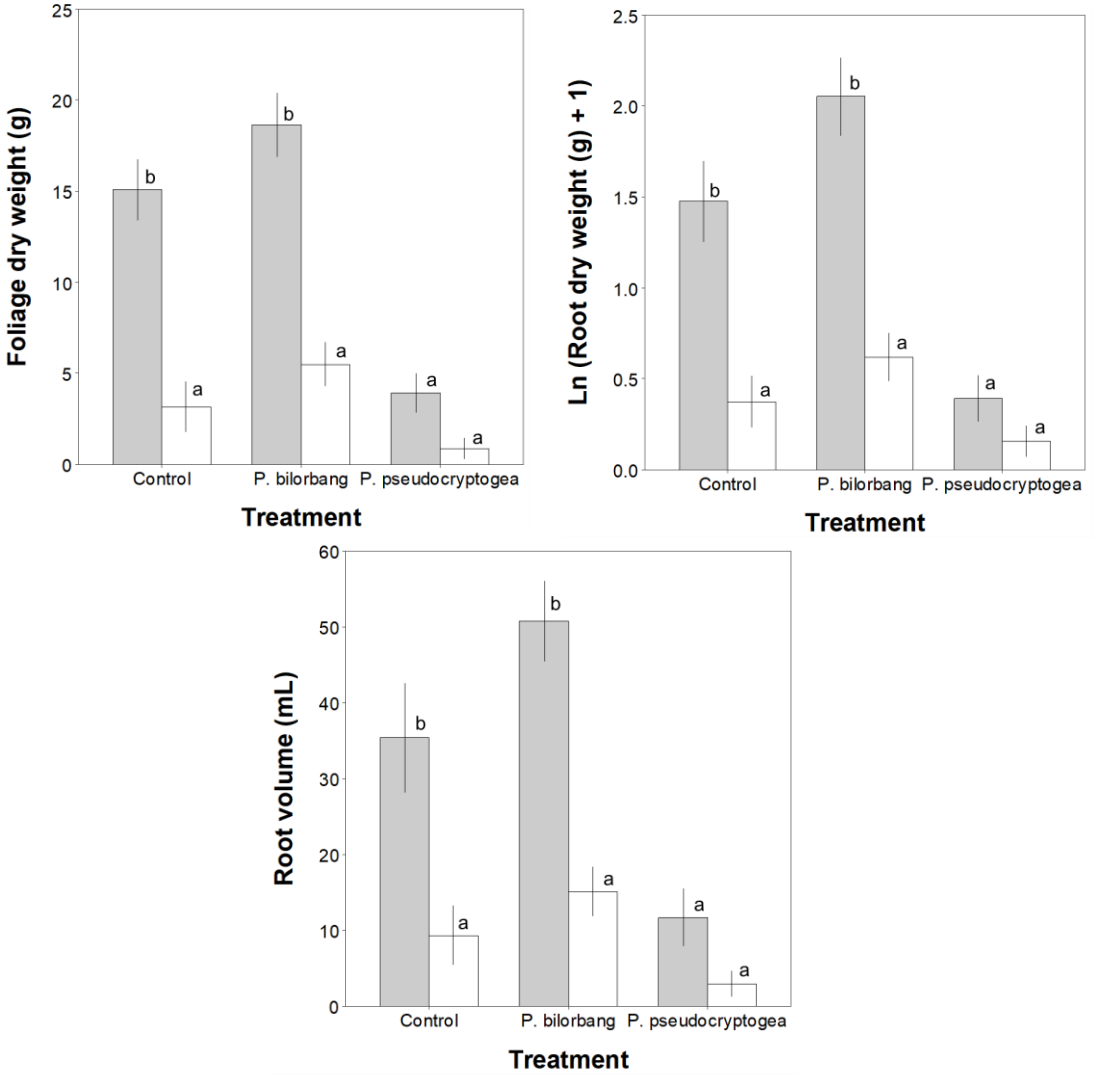

Figure 18. Second trial: Foliage dry weight, log-transformed root dry weight and root volume of large (grey bars) and small (open bars) blackberry (*Rubus anglocandicans*) plants inoculated with non-colonised substrate (control) or substrate colonised by *Phytophthora bilorbang* or *Phytophthora pseudocryptogea*. Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. Groups sharing the same letter are not significantly different (alpha = 0.05, Tukey-adjusted). For all dependent variables, there was a significant effect of plant size and inoculation treatment ($p < 0.001$) and a significant interaction between size and inoculation treatment (foliage dw $p = 0.002$; root dw $p = 0.003$; root vol $p = 0.02$).

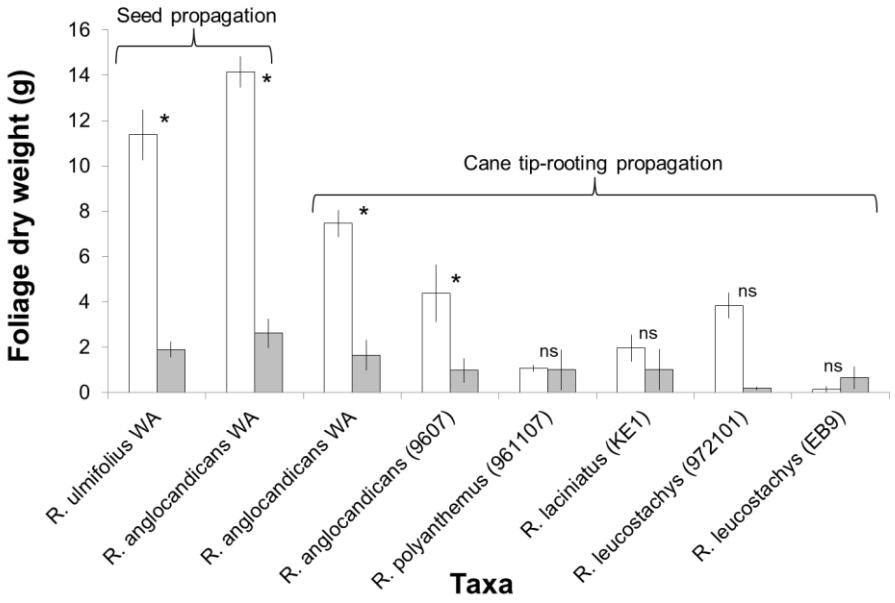

Figure 19. Foliage dry weight of different blackberry taxa plants inoculated with non-colonised substrate (control; open bars) or substrate colonised by *Phytophthora pseudocryptogea* (inoculated; grey bars). Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. A star symbol above bars of a taxon indicates that the control and inoculated treatment are significantly different according to two-sample t=test (alpha = 0.05). Non-significant differences are indicated with 'ns'.

Effect on non-target species

Thirteen separate trials were conducted to determine the effect of *P. pseudocryptogea* on a range of pasture and native plant species. Each species was tested in two different trials, except for *Dodonaea viscosa* ssp. *angustissima*, *Eucalyptus macrophyllum* var. *macrophyllum* and *Melaleuca preissiana*. *Rubus anglocandicans* was used as a reference species in each trial, as well as *R. ulmifolius* in two of the initial trials. Means of foliage dry weight data, including standard errors, are presented in a series of graphs in Appendix 4[3]. To facilitate comparison of results between trials, the difference between the foliage dry weight means of control and inoculated plants of each species in each trial was expressed as 'Percent of control'. This was done by designating the means of the control as the base and giving it the value of 100 and then expressing the means of the inoculated treatment as a percentage of the control.

Phytophthora pseudocryptogea significantly affected blackberry, *R. anglocandicans*, in 11 of the 13 trials performed (Fig. 20). Large variation in foliage dry weight of control and inoculated blackberry plants two of the trials explains why no significant effect of *P. pseudocryptogea* was detected (Appendix 4). Several of the non-target species tested were significantly affected by *P. pseudocryptogea* in one or both trials: *Acacia aneara*, *Acacia dealbata*, *Acacia disparrima* ssp. *disparrima*, *Acacia doratoxylon*, *Acacia implexa*, *Acacia irrorata* ssp. *irrorata*, *Acacia longifolia* ssp. *sophorae*, *Acacia melanoxylon*, *Acacia pravissima*, *Acacia rubida*, *Brachychiton populneus*, *Callistemon citrinus*, *Callistemon linearifolius*, *Eucalyptus cladocalyx*, *Eucalyptus dives*, *Eucalyptus globulus* ssp. *biscostata*, *Eucalyptus globulus* ssp. *globulus*, *Eucalyptus macrophyllum* var. *macrophyllum*, *Eucalyptus pauciflora* ssp. *niphophila*, *Eucalyptus sieberi*, *Eucalyptus viminalis*, *Leptospermum lanigerum* and *Trifolium repens* (Fig. 21). All pasture species, except *T. repens* in one

[3] Trial no 3: only four replicates for control *Microlaena stipoides* and for control and inoculated *Chloris truncata*. Trial no 4: only four replicates for control *M. stipoides*.

trial, were not significantly affected by *P. pseudocryptogea*. None of the four *Melaleuca* species tested were affected. Control, non-inoculated plants of some *Acacia* and *Eucalyptus* species, as well as the two *Brachychiton* species tested did not grow well under the fortnightly flooding regime they were subjected to (Appendix 4).

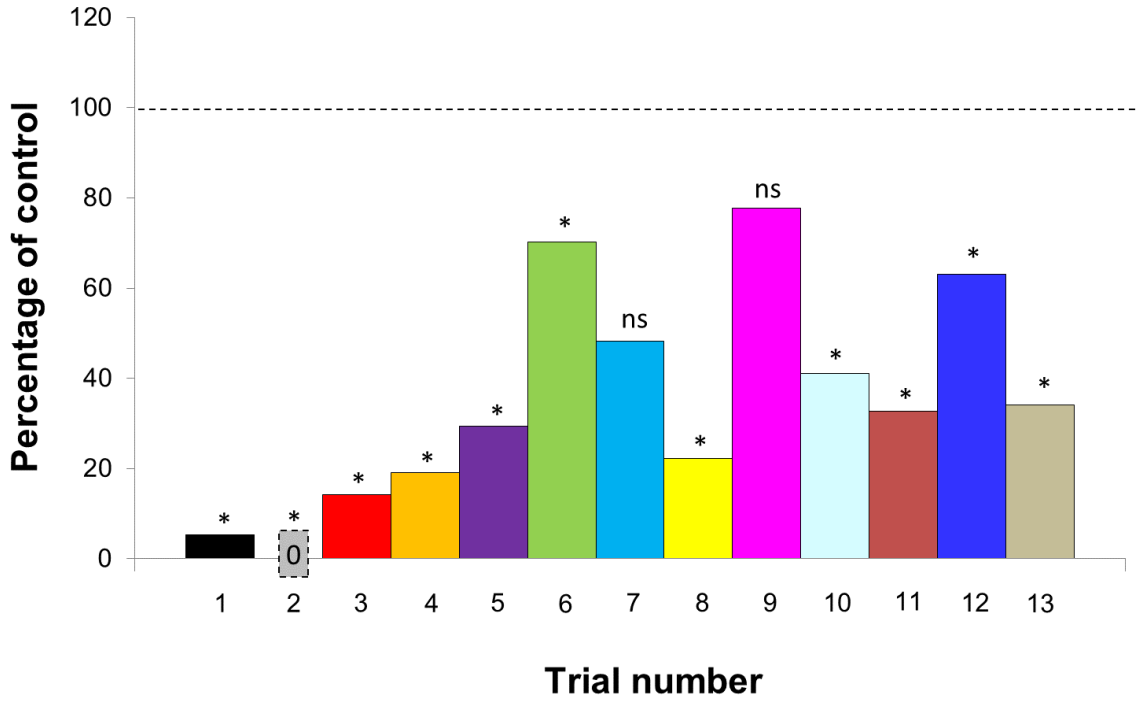

Figure 20. Difference between the foliage dry weight of control and inoculated blackberry (*Rubus anglocandicans*) plants, expressed as 'Percent of control', for each trial performed (see Appendix 4 for actual means and standard errors). Bar heights represent the percentage for the groups and colours correspond to the number of the different trials performed. A star symbol above bars indicates that the control and inoculated treatment in that trial are significantly different according to a two-sample t=test of the means (alpha = 0.05). Non-significant differences are indicated with 'ns'.

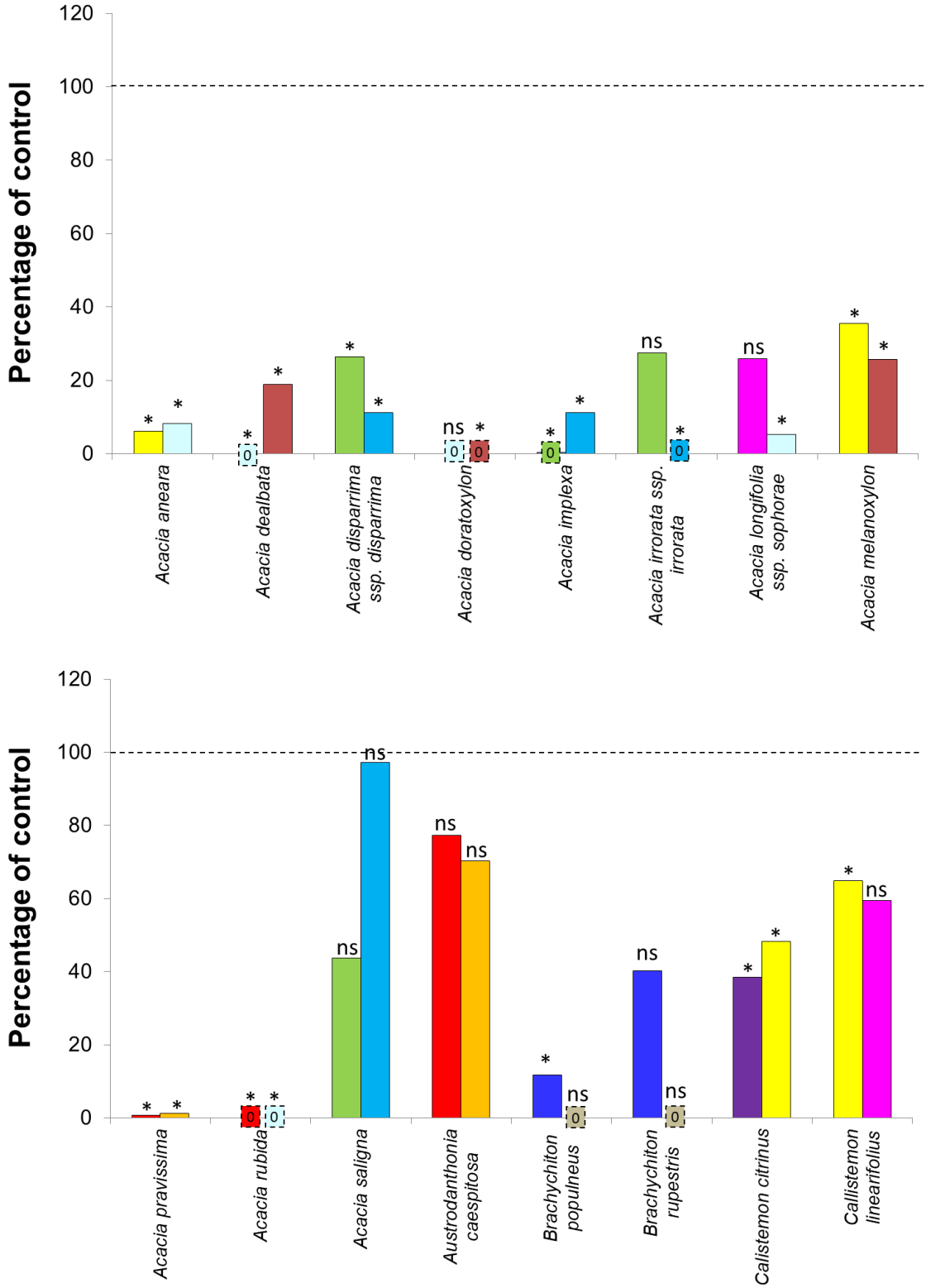

Figure 21. Difference, expressed as 'Percent of control', between the foliage dry weight of control and inoculated plants of each non-target species tested (see Appendix 4 for actual means and standard errors). Bar colour correspond to trial number, as indicated in Fig. 20. Bar heights represent the percentage for the groups. A 'X' indicates that a second trial was not performed. A zero in a broken lined square indicates that all inoculated plants were dead at the end of the trial. A star symbol above bars indicates that the control and inoculated treatment in that trial are significantly different according to a two-sample t-test of the means (alpha = 0.05). Non-significant differences are indicated with 'ns'.

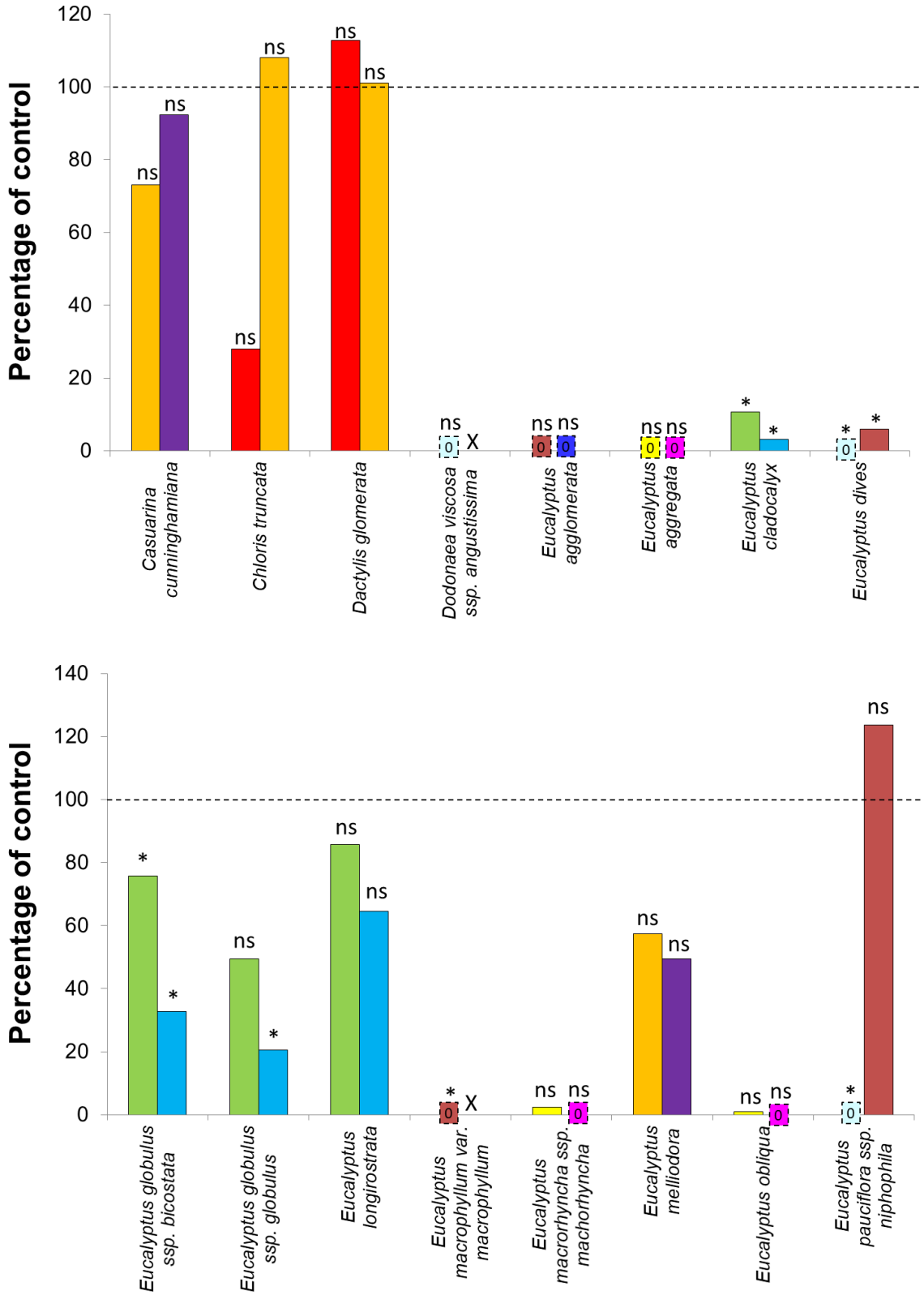

Figure 21. Continued.

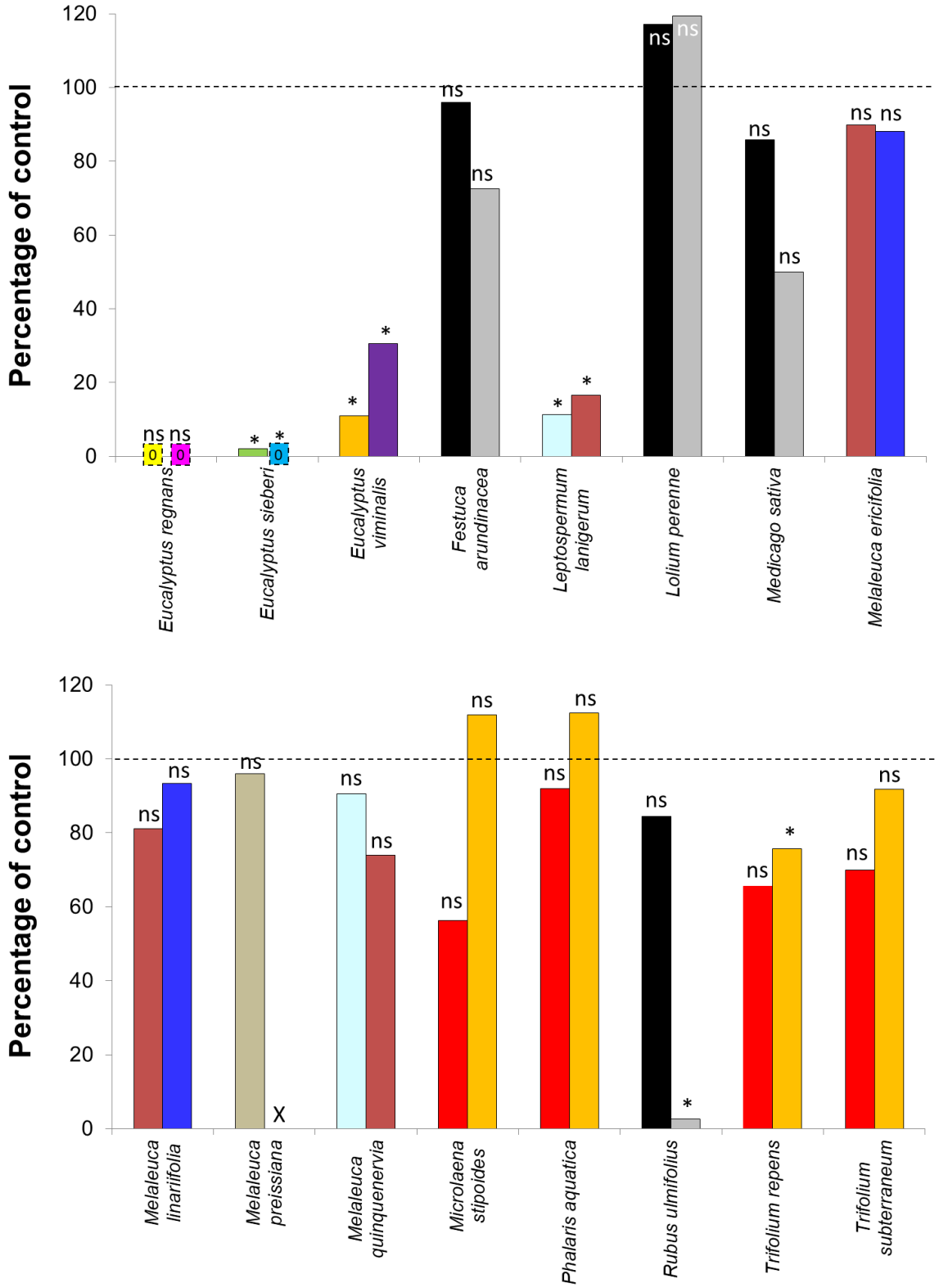

Figure 21. Continued.

4.2 Sawfly

4.2.1 Field surveys

A total of 904 canes were sampled – 543 from *Rubus ulmifolius* at 16 sites in mainland France and Corsica, Portugal, and Sardinia, Italy; 120 from *R. fruticosus* agg. at 4 sites in the UK; 241 from *Rosa canina* at 11 sites in France, Italy and Portugal. Plant samples were collected from most sites and have been placed in a herbarium collection at the CSIRO European laboratory. A combined total of 80 insect specimens were collected from the canes of the *Rubus* and *Rosa* species sampled. Internal stem damage was observed at every collection site for *Rubus* but for *Rosa,* only five sites had signs of stem-boring damage (Fig. 22).

Figure 22. Internal stem damage caused by an unidentified cane-boring insect in *Rosa canina* and by a *Phylloecus* sp. in *Rubus* sp.

***Rubus* insects**

Sawfly, *Phylloecus faunus*. A total of 18 *P. faunus* specimens were collected from 4 of the 7 French mainland sites (FrS3 (3 specimens), FrS5 (1 specimen) and FrS7 (5 specimens)), and from 4 of the 12 Portugese sites (P1 (3 specimens), P2 (2 specimens), P5 (2 specimens) and P7 (2 specimens)) (Fig. 23). Based on results of DNA sequencing, there appears to be two separate genetic lineages: one originating from France and one from Portugal (Fig. 24). Specimens were not detected at any of the sites sampled in Sardinia, Corsica or the UK. Only seven adults of *P. faunus* were obtained, with the majority of specimens being late instar larvae and pupae from within the tunneled stems, as was to be expected at this time of year (Fig. 23) (Bruzzese 1982).

Habitat preferences. Based on the habitats surveyed in Western Europe, *P. faunus* did not appear to prefer any particular habitat type. Sawflies were recovered from sites ranging from: (i) open sites in fields, along fence lines and roadside verges (P2, P5, P7, FrS5); (ii) semi-shaded sites of mixed under and overstory vegetation, particularly along watercourses (P1, FrS3); and (iii) a forest environment, consisting of Aleppo pines mixed with *Fraxinus* sp. (FrS7).

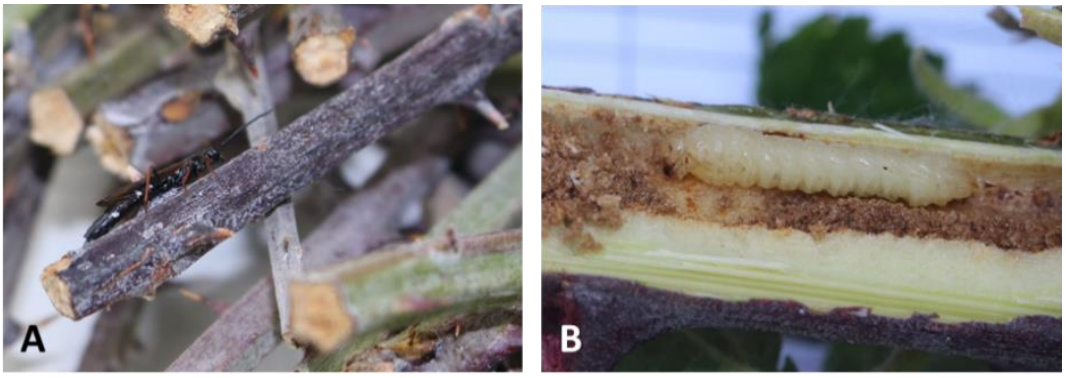

Figure 23. *Phylloecus* sp. (**A**) Newly emerged adult on *Rubus*; (**B**) Larva within its tunnel in *Rubus*. Photos: Vincent Lesieur.

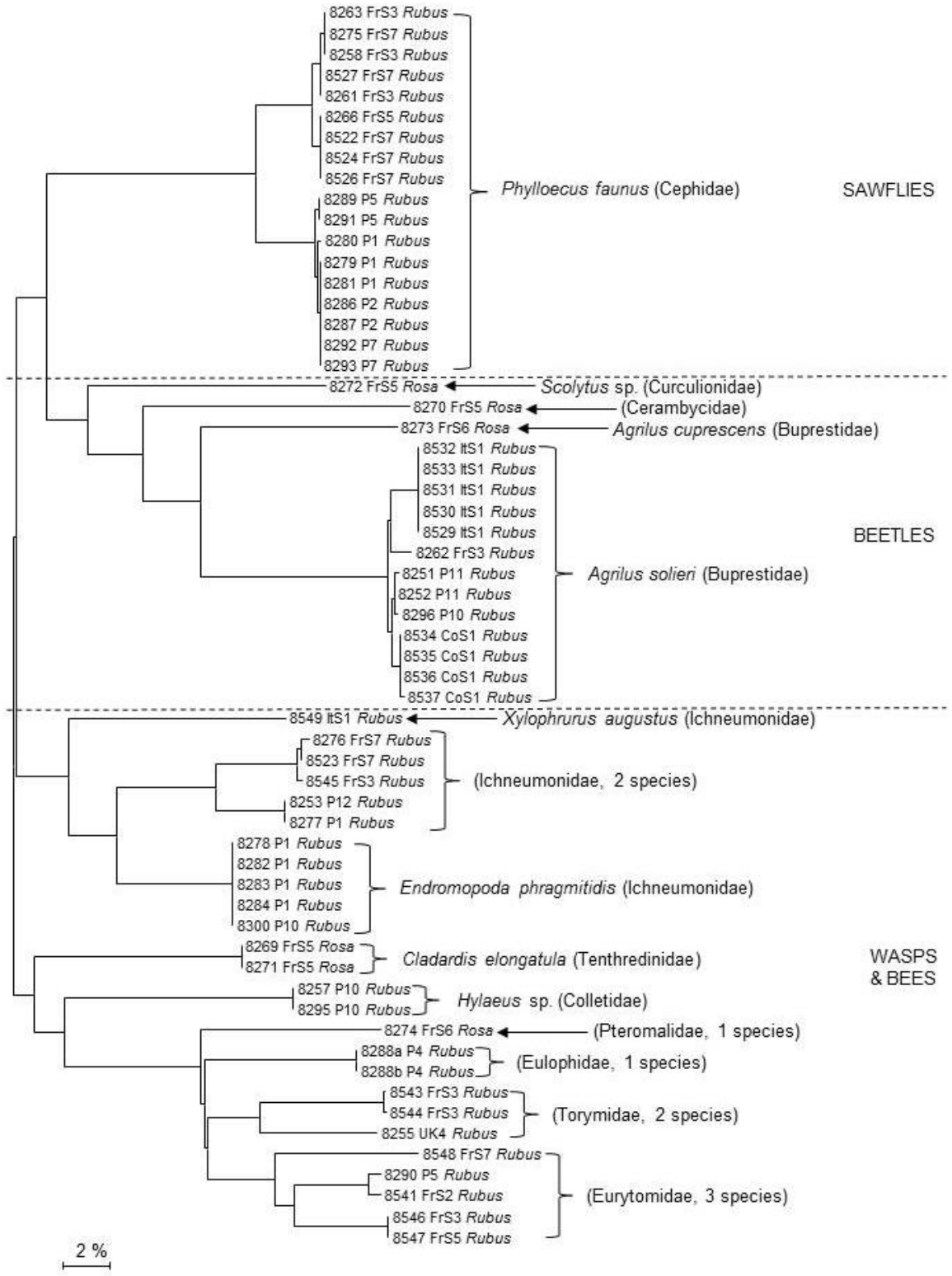

Figure 24. Neighbour-joining tree of COI DNA sequences (>500 b.p.) of insect specimens collected from *Rubus* and *Rosa* sp. stems in France (mainland and Corsica), Portugal, Italy (Sardinia) and the UK. See Appendices 2 and 5.

Beetles. Larvae of a species of jewel beetle (Buprestidae) were also found within tunnels, causing similar damage to that of *P. faunus* (Fig. 25). The DNA barcoding identified these as *Agrilus solieri* Gory & Laporte, 1837 (Fig. 24) and were collected from *Rubus* in mainland France (one site), Sardinia (one site), Corsica (one site) and Portugal (two sites).

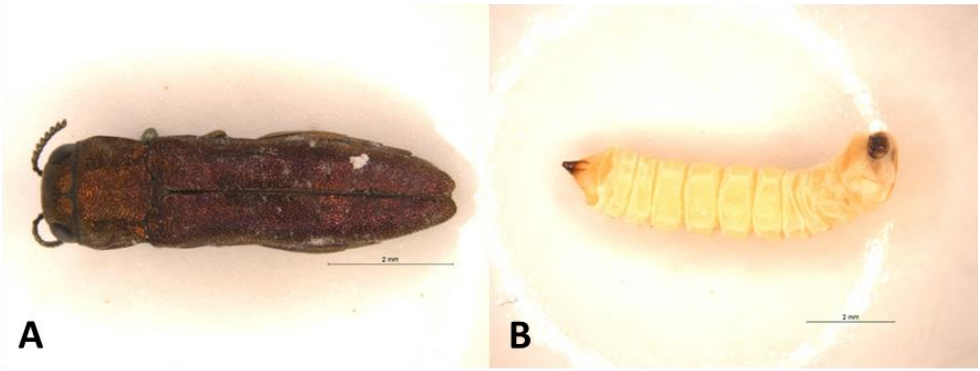

Figure 25. *Agrilus solieri* (Buprestidae). (**A**) Newly emerged adult on *Rubus* from France; (**B**) Larva dissected from within a tunnel in *Rubus* collected in Corsica. Photos: Raelene Kwong.

Wasps and bees. The other insects which emerged or were dissected from *Rubus* were largely parasitic wasps of *P. faunus* and/or *A. solieri* (Fig. 26). The parasitism rate was high and several species were obtained from the following families: Eulophidae (one species), Pteromalidae (one species) and Ichneumonidae (four species). Many of these species have already been identified as parasitoids of *P. faunus* (Bruzesse 1982). One species of solitary bee, *Hyalaeus* sp. (Colletidae) was found in Portugal utilising the empty tunnels as brooding sites.

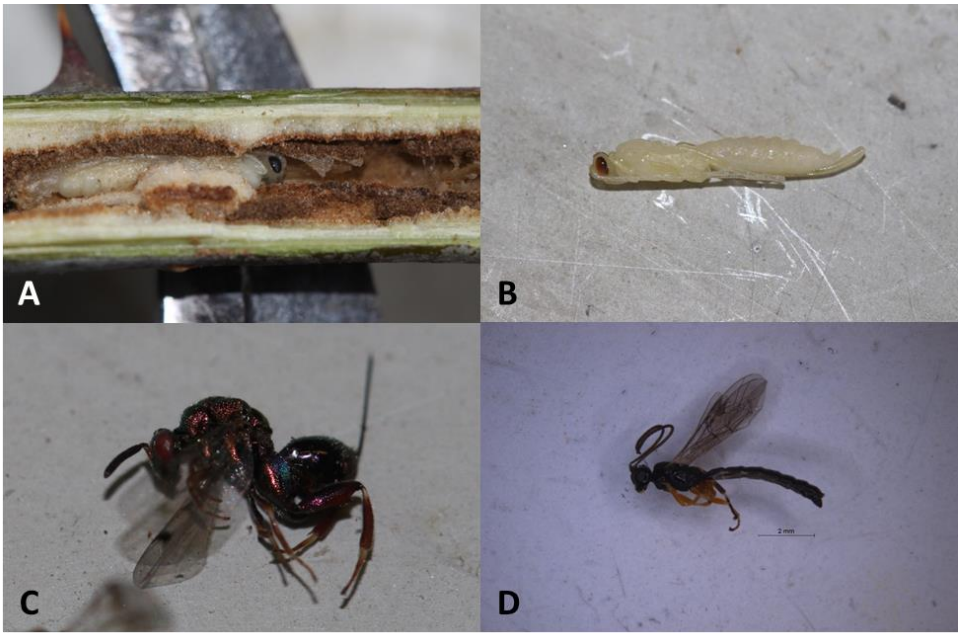

Figure 26. (**A-B**). Parasitoid pupa (Hymenoptera: Ichneumonidae) inside a *Rubus* cane at Montferrier sur Lez, France (FrS7), (**C**) Torymidae species emerged from *Rubus* collected at Saint Clément de Rivière, France (FrS3), (**D**) Adult *Endromopoda phragmitidis* (Ichneumonidae) dissected from *Rubus* in Portugal (P). Photos: A-C. Vincent Lesieur; D. Raelene Kwong.

Two Torymidae species (Fig. 26c) were collected from *Rubus,* one species was from France and the second species was dissected from stem galls on *Rubus* at Silwood Park, UK (UK4, Fig. 27a,b). Stem galls were also present on *Rubus* at one site in Portugal (P9, Fig. 27c). As these could not be identified to species using DNA barcoding, it is uncertain as to whether these insects are parasitoids or phytophagous. Many are parasitoids on gall-forming insects and some are phytophagous species (Grissell 1995). Similarly, three species of Eurytomid wasps were collected from *Rubus* canes in France (FrS2, FrS3, FrS5, FrS7) and Portugal (P5), but without knowing their precise identities it is difficult to know if they are parasitoids or phytophagous. The larvae of many species are known to feed in stems, seeds or galls.

Figure 27. Stem galls on *Rubus* at Silwood Park, UK (site UK4) (**A-B**), and Sero das Covas, Potugal (site P9) (**C**).

***Rosa canina* insects**

Sawfly. The rose shoot sawfly (*Cladardis elongatula* Klug) (Hymenoptera: Tenthredinidae) was collected from *R. canina* in France (FrS5).

Beetles. Internal stem damage caused by cane-boring insects was observed on *R. canina* in France (FrS5, FrS6) and Portugal (P9, P10, P11), but only specimens were found at the French sites. Three species of boring beetles were identified: *Agrilus cuprescens* (Buprestidae) (FrS6), one species of *Scolytus* weevil (Curculionidae) (FrS5) and a longhorn beetle (Cerambycidae) (FrS5).

Wasps. Compared to *Rubus*, only one parasitic wasp (Pteromalidae) was collected from *R. canina,* collected in France at Montferrier sur Lez (FrS6).

4.2.2 Identification

Of the 80 insect specimens collected from *Rubus* and *Rosa* canes, 75% were successfully sequenced, with specimen identifications shown in Table 3 and Figure 24 (also see Appendix 5).

All specimens of *Phylloecus* were collected exclusively from *Rubus* and were all identified as *Phylloecus faunus* (following Liston and Prous 2014, who have synonymised *P. faunus*, *H. albomaculata* and *H. helleri*). Four species of boring beetles were identified: two species of jewel beetles, *Agrilus solieri* (in *Rubus*) and *A. cuprescens*, one *Scolytus* weevil and a longhorn beetle (the latter three species all in *Rosa*). The other specimens identified included a species of bee, *Hylaeus* sp. (in *Rubus*), and the rose shoot sawfly, *Cladardis elongatula* (in *Rosa*) as well as many species belonging to five families of mostly parasitic wasps.

Table 3. Summary of identified insect specimens collected from *Rubus* and *Rosa canina* in France (mainland and Corsica), Portugal, Italy (Sardinia) and the UK.

	Rubus	***Rosa canina***
Cane-boring insects		
Sawflies	*Phylloecus faunus* (Cephidae)	*Cladardis elongatula* (Tenthredinidae)
Beetles	*Agrilus solieri* (Buprestidae)	*Agrilus cuprescens* (Buprestidae) Unidentified longhorn beetle (Cerambycidae) *Scolytus* sp. (Curculionidae)
Galling wasps / parasitoids	Torymidae (2 species) Eurytomidae (3 species)	
Parasitoids	Ichneumonidae: *Xylophrurus augustus* *Endromopoda phragmitidis* Unidentified (2 species) Eulophidae (1 species)	Pteromalidae (1 species)
Opportunists	*Hylaeus* sp. (solitary bee, Colletidae)	

5 Discussion

5.1 Blackberry decline

The blackberry decline syndrome in south-west Western Australia was first observed in 2006 and was investigated as part of the PhD studies of Sonia Aghighi at Murdoch University (Aghighi 2013). It was logical to build on this previous work for the project and focus on *P. bilorbang*, a novel species with a host-range unknown at that stage (Aghighi et al. 2012). We acquired four of the original *P. bilorbang* isolates, stored at Murdoch University since the completion of Aghighi's PhD, and set up an initial series of experiments with blackberry. Following Aghighi's methodology, we ran glasshouse experiments for several months and exposed plants to a few simulated flooding treatments during that period. *Phytophthora* species are Oomycetes, commonly refer to as water moulds that depend on water for sporulation, dispersal and infection (Hansen et al. 2012).

Following an analysis of the relevant literature and consultation with Murdoch University partners, who are experts on *Phytophthora* species, a soil application technique was selected as the most appropriate for experimental work. The initial experiments tested the suitability of different grains as substrate to produce inoculum of *P. bilorbang*. Within a couple of weeks, saprophytic fungal growth was observed on the sand surface of pots in which pearl barley or millet colonised by *P. bilorbang* had been used. This indicated that these grains contained too much nutrients, which encouraged development of common saprophytic fungi with potential to outcompete *P. bilorbang*. Based on these observations, the standard vermiculite-based substrate was selected to produce *Phytophthora* inoculum for subsequent glasshouse experiments.

Other experiments compared the effect of the four original isolates of *P. bilorbang* on blackberry at different leaf-stage at the time of inoculation and different amounts of vermiculite-based substrate colonised with one of the isolates. All experiments with the original *P. bilorbang* isolates were inconclusive – overall inoculated blackberry plants were similar in size and vigour to control (non-inoculated) plants. Further, by the end of the experiments a similar number of inoculated and control plants had died. While simulated flooding can be deleterious to plant growth, we were expecting to see a major reduction in the growth of plants inoculated with substrate colonised by the *P. bilorgang* isolates, and higher mortality than in the control plants. This was not the case and we became concerned that the isolates used had lost their pathogenicity during storage.

New samples of blackberry from sites where the decline has been observed in Western Australia were thus collected and processed to recover new isolates of *Phytophthora* species. Two dominant species were recovered – *P. bilorbang* and *P. pseudocryptogea.* The latter species was recently identified as a distinct phylogenetic lineage within the species complex *P. cryptogea* (Safaiefarahani et al. 2015), which was found associated with blackberry decline during Aghighi's PhD studies (Aghighi et al. 2014, 2015). Both *P. bilorbang* and *P. pseudocryptogea* have been detected in soil samples, using a metabarcoding sequencing approach, collected at sites in New South Wales, Victoria, Tasmania and Western Australia (Burgess et al. 2017).

We then decided to perform experiments using both *Phytophthora* species. Aghighi et al. (2015) had hypothesised that the blackberry decline syndrome could be caused by a combination of biotic and abiotic factors – *Phytophthora* species combined with temporary flooding conditions. A first experiment involving a new isolate of each *Phytophthora* species and different numbers of flooding treatments was performed twice using methods similar to those of Aghighi et al. (2015). Both trials of this experiment were inconclusive because overall there was no difference between control plants and plants inoculated with either of the two *Phytophthora* species or a combination of the two. This was a major setback that required us to revise the methodology used. Preliminary trials

indicated that more frequent flooding treatments would provide optimal conditions for disease development and severe debilitation of blackberry plants within a couple of months.

With the revised methods, a new series of experiments was conducted in which we found that:

- *P. pseudocryptogea*, but not *P. bilorbang*, was pathogenic towards blackberry
- *P. pseudocryptogea* could kill or adversely affect blackberry only when inoculated plants were exposed to four fortnightly 72-h flooding treatments
- doubling the amount of inoculum used did not make a difference to the effects of *P. pseudocryptogea* on blackberry
- *P. pseudocryptogea* grown on vermiculite or shredded sugarcane mulch-based substrates was equally effective at killing or adversely affecting blackberry

Demonstrating that inoculum of *P. pseudocryptogea* could be produced in large breathable polypropylene bags with filters, widely used in mushroom spawn production, was a promising development. The large quantities of inoculum that would have been required to undertake a minimum of six field trials could have been effectively mass-produced using cheap sugarcane mulch substrate contained in such bags.

The revised experimental methods were used in all host-specificity tests performed with different blackberry taxa and non-target pasture and native species. We had access to seed of only two blackberry species, *R. anglocandicans* and *R. ulmifolius*, and thus had to propagate other *Rubus* species and clones through cane tip-rooting using plants CSIRO has been maintaining for years. This propagation method was successful, but did not produce plants as uniform as those generated from seed. The considerable variation in those plants may explain why there was no significant difference between the control and inoculated treatment for four taxa tested (*R. polyanthemus* clone 961107, *R. laciniatus* clone KE1, *R. leucostachys* clone 972101, *R. leucostachys* clone EB9). Another experiment using plants of these taxa propagated from seed would be necessary to confirm if they are resistant to *P. pseudocryptogea*.

The first two trials performed to test the specificity of *P. pseudocryptogea* on non-target species involved three commonly occurring pasture species, lucerne (*M. sativa*), perennial ryegrass (*L. perenne*) and fescue (*F. arundinacea*). The fact that none of these pasture species were significantly affected by *P. pseudocryptogea* was encouraging. Indeed the other seven pasture species tested in subsequent trials, except white clover (*T. repens*) in one trial, were also found not to be significantly affected by *P. pseudocryptogea*. These trials, however, demonstrated that *P. pseudocryptogea* could kill or considerably reduce the foliage biomass of several *Acacia*, *Callistemon* and *Eucalyptus* species. These results were the basis for the decision not to proceed with field trials.

The methods of the trials, especially the use of young plants and fortnightly exposure to 72-h of simulated flooding, would have provided highly conducive conditions for development of *P. pseudocryptogea* on roots of the non-target species. Without such conditions however, *P. pseudocryptogea* would likely not have had a detectable effect on blackberry (*R. anglocandicans*), used as a reference species in the trials. It is noteworthy that in 2 of the 13 trials performed, the difference in foliage biomass between control and inoculated blackberry plants was found not to be significant. This was most likely due to large variation between plants in each group rather than an indication that blackberry escaped or tolerated infection by *P. pseudocryptogea.* Some of the native species tested across both trials (*Brachychiton rupestris*, *E. aggregata*, *E. agglomerata*, *E. macrorhyncha* ssp. *macrorhyncha*, *E. obliqua* and *E. regnans*) were particularly sensitive to the flooding regime they were exposed to, irrespective of the presence or not of *P. pseudocryptogea*. Both control and inoculated plants did not grow well or even died and foliage dry weights were thus not significantly different.

While *P. cryptogea* sensu lato is known to have a wide host range (Erwin and Ribeiro 1996), *P. pseudocryptogea* had only been previously recovered from roots of dying *Banksia cirsioides*, *Xanthorrhoea preissii* and *Isopogon buxifolius* in Australia and of *Solanum melongena* in Iran (Safaiefarahani et al. 2015). This project has contributed considerable additional knowledge on the host-range of this *Phytophthora* species, albeit under artificial experimental conditions. More isolations of *P. pseudocryptogea* from dying native plants in the field will be required to determine if it plays an important role in natural ecosystems in Australia.

Once it became evident that *Phytophthora* species were not a viable option for the biocontrol of blackberry, a preliminary investigation was made into an alternative option – the stem-boring sawfly, *P. faunus*. This insect belongs to the Cephoidea, a small superfamily commonly referred to as stem sawflies. It is found throughout Mediterranean Europe, particularly France, Spain, Portugal and Morocco. It is univoltine and parthenogenetic (development of an egg without fertilisation) and its attack is restricted to first-year canes (primocanes) (Bruzzese 1982).

5.2 Sawfly

Phylloecus faunus could be introduced to Australia for biocontrol if it was shown to only attack invasive blackberry taxa, and not develop on cultivated brambleberries and other species in the Rosaceae family present in Australia. Activities as part of the project gathered more information on the field host-range of this insect in order to better assess its potential for biocontrol.

While additional work is required to confirm the field host-range of *P. faunus*, based on this initial sampling performed, this stem-boring sawfly seems to be restricted to *Rubus*. The sawfly (larva or adult) was not found on the closely-related species, *Rosa canina* at any of the sites surveyed, include those where the two species were sympatric. Testing would be required to determine if cultivated blackberry taxa, as well as *Rubus* spp. native to Australia and other species in the Rosaceae family can be attacked by the sawfly.

The investigations for *P. faunus* in Europe also provided new data on the distribution of the sawfly. This species is reported to be distributed in Western Europe including Spain, France (mainland and Corsica), Switzerland, Morocco and Crete (Chevin 1993, Liston et al. 2015, Schedl 1987). To our knowledge, its finding in Portugal could be the first observation of the species in this country. Furthermore, DNA barcoding indicated that *P. faunus* specimens collected in Portugal represent a separate genetic lineage to those sampled in southern France (Fig. 24).

Phylloecus faunus affects plants by causing cane die-back, which decreases daughter plant production from cane apices and seed production. This sawfly could aid in reducing the canopy cover and rate of expansion of existing stands of blackberry if was introduced to Australia for biological control. It is important to note that *P. faunus* populations are adversely affected by a large guild of parasitoids in the native range. Hence, the release of *P. faunus* into Australia without its natural enemies would likely result in higher level of impacts on blackberry than that observed in the native range.

The jewel beetle *Agrilus solieri* was also found causing similar damage to that of *P. faunus* on *Rubus* at several sites. It was observed boring into crowns and the base of canes and appeared to be restricted to *Rubus*. Additional surveys would be required to confirm if it is restricted to *Rubus* spp.

5.3 Lessons learnt and key messages

Lesson 1
The original design of the project that included undertaking field trials was overly ambitious. It was not envisaged that difficulties would be encountered in reproducing results from glasshouse experiments performed as part of Sonia Aghighi PhD studies. Once new isolates were obtained and a refined methodology was developed, we were able to show that one of the *Phytophthora* species could kill or severely affect the growth of blackberry. Before this was achieved, we could not proceed with host-specificity testing.

Lesson 2
There are many land managers that have unrealistic expectations of what weed biocontrol can deliver. Blackberry is a major weed affecting both the livestock industry and natural ecosystems. If not managed readily, blackberry bushes become very large and, difficult and costly to control with herbicides. In those situations, land managers are discouraged and their hopes for a biocontrol 'silver bullet' increase. At every opportunities available, research providers in this field must reinforce the message that biocontrol on its own is unlikely to meet expectations and promote integrated weed management.

Lesson 3
The project greatly benefited from the understanding and flexibility of all partners, including MLA, Department of Agriculture and Water Resources (DAWR) and Murray Local Land Services (LLS), that provided financial support. There were delays in achieving some of the KPIs by the scheduled dates because of problems in repeating results from the previous study. Then when we discovered that the selected *Phytophthora* species adversely affected the first two *Acacia* species tested, we had to cancel field trials as it would have been irresponsible to introduce this pathogen to new areas. The funding partners agreed with this recommendation and accepted the proposal to expand glasshouse testing to comprehensively assess the host-range of the *Phytophthora* species and to undertake a small feasibility study on the prospects of using the stem-boring sawfly for blackberry biocontrol in lieu of field trials.

Lesson 4
The project invested considerable time and effort in making sure that experimental procedures were rigorous before embarking in comprehensive testing. It had to make sure that the most pathogenic *Phytophthora* species on blackberry was selected and that experimental conditions were conducive for blackberry decline to be reproduced. Taking such precautions ensured that we can have confidence in results of host-specificity tests on non-target species. Thoroughly understanding the pathosystem underpinned the decision not to proceed to field trials – it would have been irresponsible to decide otherwise.

5.4 Recommendations

There are a range of possible options that could be explored as the next steps in the biocontrol of blackberry in Australia, but there are no guarantee that investments in these options would generate effective and safe management solutions for blackberry applicable at the landscape scale.

1- Based on results obtained in this project on the two dominant *Phytophthora* species found associated with the blackberry decline in Western Australia, they have limited prospects for use as biocontrol agents. While *P. bilorbang* may play a role in the blackberry decline syndrome in the field, its pathogenicity towards blackberry could not be demonstrated in this project, even under highly conducive conditions for plant infection. In contrast, *P.*

pseudocryptogea was highly pathogenic on blackberry, but also on a range of non-target species in glasshouse host-specificity tests. These non-target effects may never be observed in the field, but knowing that they can occur under optimal conditions would make large-scale redistribution of these *Phytophthora* species a contentious venture.

2- The sawfly *P. faunus* was only found in Europe on *Rubus ulmifolius*, which is known to be invasive in Australia, and not on *Rosa canina*, including where the two species were growing together. This provided some supporting evidence that the field host-range of the sawfly is limited to the genus *Rubus*. Absence of the sawfly in *R. canina* samples however, could simply be due to chance or low populations of the insect at sites. Therefore, to fully assess the host-range of the sawfly, host-specificity tests under field conditions in Europe would be required. In parallel, investigations into the jewel beetle, *Agrilus solieri* and unidentified gall-inducing organisms found on *Rubus* during field surveys, through literature reviews and more surveys, would be necessary to determine if these have any potential for biocontrol of blackberry.

3- The purple blotch fungus, *Septocyta ruborum*, which is widespread on wild and commercial cultivars of blackberry (*R. fruticosus* agg.) in Europe, causes lesions on canes, which can develop into cankers that girdle the cane. It also occurs in the USA and New Zealand on commercial brambleberries. It has been proposed as a possible candidate biocontrol agent for blackberry, on the basis that isolates which only infect invasive blackberry would be found (Adair et al. 2012). Chances of finding an isolate specific towards invasive blackberry however, would realistically be very low. Further there is already a pathogen in Australia, the cane anthracnose fungus *Elsinoë veneta* that causes similar lesions on canes and do not appear to have a significant impact on blackberry infestations.

4- Proposing to introduce to Australia a new biocontrol agent for blackberry that also causes some damage on closely-related cultivated brambleberries would be contentious. Biocontrol agents are typically not approved for release in Australia by the authorities if results from host-specificity tests indicate that non-target damage could occur. The Commonwealth Government however, could agree for the Biological Control Act 1984 to be used to address conflicts over the potential release of a new biocontrol agent for blackberry. In such a situation, a technical analysis would need to be produced to show that the harm caused by invasive blackberry clearly exceeds the potential harm to cultivated brambleberries arising from releasing the agent. Enactment of the Act is a complex and costly process because it requires public enquiries. The Act however, has rarely been used and thus remains unchallenged legally with regards to the protection it offers to agencies undertaking releases (Palmer et al. 2010).

5- Boosting populations of natural enemies of blackberry that already exist in Australia through regular supplemental releases (augmentative biocontrol approach) is an option that could be considered. Natural enemies recorded on invasive blackberry in Australia comprise the sawfly *Priophorus morio*, the cane anthracnose fungus mentioned above, the Red Berry Disease mite (*Acalitus essigi*), two leaf-spot fungi (*Sphaerulina westendorpii* [formerly *Septoria rubi*] and *Cercospora rubi*), the cane and leaf-rust fungus *Kuehneola uredinis* and the leaf-rust fungus *P. violaceum* (Morin and Evans 2012, Scott et al. 2008). This approach however, would require considerable up-front investments to develop effective systems to mass-produce or mass-collect the natural enemies and identify the techniques and ideal conditions for making the supplemental releases. Continued annual investments would be required to maintain high populations of the natural enemies at release sites to achieve a significant reduction of blackberry infestations.

6 Project Achievements

The blackberry project outputs and KPIs used in this section were extracted from the executed variation of the contract in November 2017.

The project achievements against each of the outputs it was committed to deliver on are:

Output 3(a) develop a prototype mass-production system and assess viability of fungal material[4]

After testing different grains and a vermiculite-based substrate to mass-produce inoculum of the *Phytophthora* species, the project selected the latter as the most suitable for subsequent glasshouse experiments. Mass-production in large breathable polypropylene bags with filters was successful either using the vermiculite-based substrate or shredded sugarcane mulch. (see section 4.1.2)

The project demonstrated that *Phytophthora* can survive in fresh, colonised vermiculite-based substrate stored at 4 and ~22 °C, but not at –20 °C, for 5 wks, 5 and 12 months. Bacterial and fungal contaminants were present in the inoculum after 12 months of storage and therefore viability assessments after 18 and 24 months were not performed. (see section 4.1.3)

Output 3(b) experimentally test different application techniques for the fungus on blackberry plants

An efficient protocol to produce standardised young blackberry plants from seed was developed and used throughout the project. Following an analysis of the relevant literature and consultation with colleagues at Murdoch University who are experts on *Phytophthora* species, a soil application technique was selected as the most appropriate for this system. The project tested different dosages of inoculum and demonstrated the importance of subjecting inoculated blackberry plants to regular simulated flooding conditions to reproduce the decline syndrome. (see section 4.1.4)

Output 3(c) conduct at least two suitable field farm-based trial sites in partnership with stakeholders in each of ACT/NSW, Victoria and WA OR Conduct a scoping study on the prospect of the sawfly *Hartiga albomaculata* for blackberry biocontrol

Farm-based field trials were cancelled once it was discovered that some of the non-target plant species (e.g. *Acacia*) tested were adversely affected by the selected *Phytophthora* species. After consultation with MLA, we then initiated field surveys in Europe, the native range of blackberry, to gain a better understanding of the host-range of the stem-boring sawfly *P. faunus* (formerly known as *H. albomaculata*), previously identified as a possible candidate for blackberry biocontrol. At the time of writing this report, the sawfly had not emerged from samples collected during the first surveys conducted and results from the last survey in April were not yet available. (see section 4.2.1)

Output 3(d) perform host-specificity testing of the fungus on different blackberries and non-target plant species

The selected *Phytophthora* species was tested on seven blackberry taxa (different species and/or clones) propagated by seed or cane tip-rooting using the robust experimental methodology developed as part of the project. Plants from three of the seven taxa were significantly affected.

[4] Note that *Phytophthora* is classified as an Oomycete (water mould). Oomycetes are fungus-like eukaryotic microorganisms that are distinct from the true fungi.

There was a lot of variation between plants propagated by cane tip-rooting, and consequently the difference between control and treated plants for four blackberry taxa was found to be non-significant.

The *Phytophthora* species was also tested on 45 non-target plant species (12 of these species are being tested for the first time as part of on-going trials). So far, 15 native species in the genera *Acacia*, *Callistemon* and *Eucalyptus* have been significantly affected by the *Phytophthora* species. All pasture species tests, except *Trifolium repens* in one trial, were not significantly affected (see section 4.1.6)

Output 3(e) if results (Outputs 3(a) to 3(d)) indicate that the fungus may be a successful control agent for blackberry, prepare a plan for large-scale delivery of the agent to land holders. If the fungus is not a candidate agent, then make recommendations for next steps in the biological control of blackberry

Results from glasshouse experiments showed that the selected *Phytophthora* species could pose a risk to non-target plants species associated with blackberry in the field if it was to be redistributed on a large-scale. A series of possible options for the next steps in the biocontrol of blackberry in Australia have been outlined in section 5.3. There are however, no guarantees that investments in these options would generate effective and safe management solutions for blackberry applicable at the landscape scale.

Output 3(f) Deliver a report analysing all results for biocontrol of blackberry, including outcomes from investigating potential biocontrol agents.

This report.

Since project KPIs were tightly linked to each of the planned outputs, achievements against each KPI can be found in the list above.

KPI 1.7 – Report on mass-production system and assessment of the viability of fungal material after 1 month
See Output 3(a)
KPI 2.4 – Advise the MLA of application techniques identified and assessment of the viability of fungal material after 6 months
See Output 3(b) and 3(a)
KPI 3.6 – Selected field trial locations and initiation of trials
See Output 3(c)
KPI 3.7 – Report on assessment of fungal viability tests after 12 months provided to MLA
See Output 3(a)
KPI 4.5 –Advice of initial results of pathogenicity tests on blackberries; Initiate host-specificity testing and advice of initial results
See Output 3(d)
KPI 5.4 – Report to the MLA on results of pathogenicity and host-specificity testing of biocontrol agents on blackberries and update on activities of sawfly as a potential agent
See Output 3(d) and 3(c)
KPI 6.5 – Report on all results obtained during the project for potential biocontrol agents for blackberry
See Output 3(f)
KPI 6.6 – Plan for next steps in biological control of blackberry

See Output 3(e)

Comments on the extent to which activities undertaken achieved the project objectives (as per executed variation – Nov. 2017) are provided against each objectives below.

1. Determine the potential of *Phytophthora* species, as an inundative biological control tool for blackberry by conducting pathogenicity and host-specificity glasshouse tests and, if promising, evaluating efficacy in field trials.

The project performed a large number of glasshouse experiments to gather the necessary data to comprehensively assess the potential of *Phytophthora* species, found to be associated with blackberry decline in south-west Western Australia, for blackberry biocontrol in Australia. Despite initial problems encountered with the original *Phytophthora* isolates (*P. bilorbang*) obtained from Murdoch University, the project was able to achieve this objective by i) recollecting new isolates from the field, ii) demonstrating that one of the *Phytophthora* species (*P. pseudocryptogea*) can be highly damaging to blackberry regularly exposed to flooding conditions and iii) establishing that several native plant species can also be adversely affected. These results justified not going ahead with field trials.

2. If *Phytophthora* species are not promising, investigate an alternate option for biocontrol of blackberry.

The project initiated a small activity in late 2017, to gather information from the field in the native range on the host-range of the stem-boring sawfly identified as a possible candidate for blackberry biocontrol in the 1970s. This work will assist in deciding whether further investment in this potential insect agent is warranted.

3. Make recommendations for next steps in the biological control of blackberry.

The project has outlined a range of options that could be considered in section 5.3 of this report.

6.1 Contribution to project expectations

Since no biocontrol agent were released or redistributed as part of the blackberry project, it only contributed to the achievement of a few of the expected outcomes for the whole project:

a) Greatly increase the on-farm populations of 8 weed biocontrol agents
n/a

b) Reduce weed competition and herbicide use across more than 25 million ha
n/a

c) Reduce the densities of the six target weeds across northern and southern Australia
This project was unable to have impact on reducing densities of blackberry. It could not proceed to test the *Phytophthora* species in field trials due to results from glasshouse experiments that showed potential risk to some non-target plant species.

d) Increase long-term annual yield and reduce annual weed control costs
n/a

e) Improve agricultural natural resource management nationally

Researchers involved with the project (L. Morin and R. Kwong) had the opportunities to participate in community forums in Victoria where blackberry management and the prospects of developing new biocontrol options were discussed. The main challenge for biocontrol is that invasive taxa of blackberry are closely-related to several native and commercial species within the Rosaceae family. It is thus difficult to find insects and pathogens that are sufficiently host-specific for introduction to Australia. Outlining this challenge and stating that biocontrol is highly unlikely to be a 'silver bullet' management tool for blackberry in Australia, was a first step in managing expectations of stakeholders and encouraging them to use other currently available and effective methods (primarily herbicides) to control blackberry.

f) Inform producers of weed management options
The website established for the project (https://research.csiro.au/blackberry/) comprises a background page that includes information on how blackberry is currently managed in Australia, with a link to the national *Blackberry Control Manual.*

g) Establish a new collaborative national approach to weed biocontrol
The whole project is testimony to a new collaborative national approach to weed biocontrol. Development of the proposal included all research providers involved in weed biocontrol in Australia. The leaders of each component of the whole project met several times face-to-face or via conference calls to discuss progress and share insights.

6.2 Contribution to Rural Profit R&D programme objectives

While the project did not result in the release and redistribution of a new biocontrol agent for blackberry, it contributed to the achievement of the overall programme objective in other ways. The contributions the project made are stated below each component of the Rural R&D for Profit programme.

Program objective: To realise significant productivity and profitability improvements for primary producers, through:

- generating knowledge, technologies, products or processes that benefit primary producers

  The project generated important knowledge to assess prospects of manipulating the blackberry decline syndrome observed in the field in south-west Western Australia for blackberry biocontrol across the nation. It developed a robust methodology to test the effect of *Phytophthora* species, found associated with blackberry decline in the field, on blackberry and non-target plant species. The reliable experimental system utilised young plants propagated from seed and *Phytophthora* inoculum produced on a vermiculite-based substrate applied to the root zone of plants. It also involved exposing plants to 72-h of simulated flooding fortnightly to provide the necessary conditions for *Phytophthora* to infect and reproduce. The project also demonstrated that it is possible to mass-produce *Phytophthora* inoculum on vermiculite or sugarcane mulch based substrate contained in breathable polypropylene bags with filters, widely used in mushroom spawn production. This method would have been ideal to produce the large quantities of inoculum required for field trials, if the project had proceed with those.

  The discovery of the blackberry decline syndrome 10 years ago was exciting for primary producers, because it offered a possible novel avenue for blackberry biocontrol. This project demonstrated that while it could potentially be manipulated and used on blackberry infestations that are regularly exposed to inundation, there would be a considerable risk that

some native species would also be adversely affected, especially at the recruitment stage. Primary producers may not all be concerned about the conservation of native species, but considering broader society concerns it would be irresponsible to go ahead and widely spread the *Phytophthora* species found to be a key contributor to the decline syndrome.

- strengthening pathways to extend the results of rural R&D, including understanding the barriers to adoption

  Through face-to-face engagements with communities in Victoria concerned about blackberry, the project attempted to manage as best as possible their expectations with regards to biocontrol and future options. The project also used a dedicated website to provide background information on blackberry management, including biocontrol, and communicate key findings as it progressed.

- establishing and fostering industry and research collaborations that form the basis for ongoing innovation and growth of Australian agriculture.

  The project was based on research collaborations between CSIRO, Murdoch University and Agriculture Victoria.

7 Collaboration

The component of the project focusing on blackberry decline was based on a collaboration between CSIRO and Murdoch University. This collaboration began years before this project, during initial investigations of the blackberry decline syndrome in Western Australia, which involved a PhD student (Sonia Aghighi) and researchers from Murdoch University (Professor Giles Hardy and Associate Professor Treena Burgess), and a Perth-based researcher (Dr John Scott) and technical officer (Paul Yeoh) from CSIRO. The CSIRO-Murdoch University collaboration was continued for this specific project. Murdoch University played an important role in the project by reisolating *Phytophthora* species from blackberry samples collected by CSIRO staff at sites where the decline syndrome had been observed. Without these new isolates the project would have had to be discontinued in 2016. The agreement between CSIRO and Murdoch University was terminated when field trials had to be cancelled in light of results obtained during initial host-specificity tests. Murdoch University was to contribute to the molecular analysis of soil and root samples collected at each field trial sites. Nonetheless, the collaboration between the two research providers will continue beyond the duration of the project because we intend to work together to publish results in a peer-review scientific journal.

The collaboration with Agriculture Victoria (DEDJTR) was initially established for the implementation of field trials in Victoria. When field trials were cancelled, Agriculture Victoria proposed to undertake a small feasibility study on the prospects of using the stem-boring sawfly that occurs on blackberry in the native range, for biocontrol in Australia. The first option considered was to engage CABI to undertake field surveys in the UK, but this was ruled out when the likelihood of finding the sawfly in the UK was deemed too low. The responsibility for undertaking field surveys on mainland Europe was then given to entomologist staff based at the CSIRO European Laboratory in Montpellier, France. Agriculture Victoria however, continued to oversee this aspect of the project.

The project is grateful for the financial support provided by Murray LLS. A collaboration with staff from Murray LLS was envisaged at the onset to assist the project with identifying suitable sites for field trials in that region and setting up the trials. With the cancellation of field trials, this on-ground

collaboration did not eventuate, but nonetheless Murray LLS retained its interest in the project and were regularly updated on progress.

8 Extension and adoption activities

Different avenues were taken to inform weed researchers and land managers of the project and its outcomes.

Conference presentations

Keynote address entitled *Pathogens for weed biological control: a solid past towards a rosy future?* at the 20th Australasian Weeds Conference in Perth (11–15 September 2016), which included a component on this project (L. Morin, CSIRO).

Poster presentation entitled *Could Phytophthora species associated with declining populations of invasive European blackberry be used for biological control?* to be presented at the 21st Australasian Weeds Conference in Sydney (9–12 September 2018) (L. Morin).

Presentations at community meetings

Oral presentations entitled *Pathogens for blackberry biocontrol*, including details about this project (L. Morin) and *Invertebrate options for the biological control of blackberry* (R. Kwong, Agriculture Victoria), at the community forum *Managing Crown Land Boundaries* held at Cudgewa, Victoria on 18 August 2016 (Hosted by the Victorian Blackberry Taskforce; ~ 60 attendees) (L. Morin, CSIRO).

Oral presentation entitled *Blackberry: Biocontrol Prospects for a Prickly Problem* at the community forum *Blackberry Control Forum* held at Rutherglen, Victoria on 16 May 2017 (Hosted by the Mitta to Murray Blackberry Action Group; ~ 70 attendees) (R. Kwong).

> At this Forum, Raelene Kwong gave an update on the research being undertaken on *Phytophthora* species for biocontrol of blackberry and indicated that expressions of interest would be sought from interested community members following results of glasshouse experiments. The Victorian Blackberry Task Force and the Mitta to Murray Blackberry Action Group both agreed to coordinate the selection of suitable trial sites in Victoria. However, following unfavourable results, plans for field trials had to be cancelled.

Oral presentation entitled *Blackberry: Biocontrol Prospects for a Prickly Problem* at the Mitta Valley Landcare Group Annual General Meeting at Eskdale, Victoria 19 August 2017 (43 attendees) (R. Kwong).

Oral presentation entitled *Blackberry: Biocontrol Prospects for a Prickly Problem* at the Mitta 2 Murray Blackberry Action Group Annual General Meeting at Tallangatta, Victoria on 8 November 2017 (12 attendees) (R. Kwong).

Written communications

Contribution to an article entitled *Biocontrol – a weapon in the fight against weeds*, published in MLA Feedback magazine, August/September 2016 edition (http://www.mla.com.au/news-and-events/publications/feedback-magazine/).

A website on the project (https://research.csiro.au/blackberry/) to increase general awareness of the project and keep stakeholders informed of progress.

9 Financial Statement

Provided with draft Final Report.

9.1 Unexpended funds

None.

9.2 Project partners

Partners who have provided cash and in-kind support to the project:

Partner	Cash or in-kind
Agriculture Victoria [DEDJTR]	Cash – $6,353 in 2016/17 and $6,526 in 2017/18 collected by MLA.
Murray Local Land Services	Cash – $25K per annum for three years collected by MLA.
Murray LLS did not contribute any in-kind during the course of the project because field trials were cancelled. They were to contribute to the setting-up, execution and monitoring of trials in their region.	In-kind pledged was $20K per annum for three years. This in-kind to the project has been provided by CSIRO*.
Murdoch University contributed in-kind only at the start of the project in 2015/16. This in-kind contribution comprised infrastructure costs and time of Dr Giles Hardy and Dr Treena Burgess (< 0.05FTE combined) who participated in discussion to review results and experimental methods with the project leader and supervised the technical officer during the re-isolation *Phytophthora* species from field samples taken at blackberry decline sites in Western Australia and their molecular identification. Murdoch University was to contribute to the molecular analysis of soil and root samples collected at each field trial sites. In light of the cancellation of field trials, the agreement between CSIRO and Murdoch University was terminated.	In-kind – $21,015 for 2015/16 Other in-kind pledged was $21,015 per annum for 2016/17 and 2017/18. This in-kind to the project has been provided by CSIRO*.

* The actual in-kind contribution (in the form of overheads[5]) that CSIRO has made across the life of the project now totalled $241,930, a significant increase from the figure provided in the original project proposal because of the additional glasshouse experimental work undertaken. It is noteworthy that only $131,494 was recognised as CSIRO in-kind in the contract with MLA. Consequently the in-kind pledged by Murray LLS over 3

[5] Overheads = Indirect and infrastructure costs (including office space, computers, laboratory space, expertise from professional communicators, administration and depreciation) calculated using CSIRO data on the actual cost of research relative to the staff time allocated to the project.

years ($60K) and by Murdoch University in 2016/17 and 2017/18 ($42,030), which will not be provided by these partners, can be provided by CSIRO considering that not all our overheads were included in the contract.

9.3 Additional Funds

Additional funds could be used to support host-specificity tests with the stem-boring sawfly, *P. faunus* under field conditions in Europe, and further investigations into the jewel beetle, *Agrilus solieri* and unidentified gall-inducing organisms found on *Rubus* during field surveys. Funding of approx. $40K could support initial testing with the sawfly and additional field surveys to determine the potential of the other insect natural enemies found on *Rubus* as part of this project.

10 Attachments

10.1 Project, media and communications material and intellectual property

Abstracts

Abstract-Blackberry biocontrol-AWC Sydney-Final.docx

Morin-Abstract-20th Australasian Weeds Conference-21 Mar 2016.docx

Powerpoint presentations

Kwong blackberry biocontrol invertebrates_Cudgewa forum.pdf

Kwong_blackberry talk_190817 compressed.pdf

Kwong_blackberry talk_160517 and 081117 compressed.pdf

Morin-Cudgewa forum-18 Aug 2016 compressed.pdf

Morin-Perth conference-13 Sept 2016 compressed.pdf

Photographs and captions

Credit for all photographs provided below: CSIRO

Collection site-Phytophthora bilorbang.JPG
Site (Cross Road - near Nyamup town site on Wilgarrup that feeds the Warren River, Western Australia) where a new isolate of *Phytophthora bilorbang* was recovered from diseased blackberry collected in May 2016 as part of the project. This site is where the first signs of blackberry decline were observed in 2013 and where extensive decline was still occurring in 2016.

Collection site-Phytophthora pseudocryptogea.JPG
Site (Rory Dean – on the Donnelly River, Western Australia) where a new isolate of *Phytophthora pseudocryptogea* was recovered from diseased blackberry collected in May 2016 as part of the project. This is one of the first sites where extensive and rapid blackberry decline occurred about 10 years ago. Only a few blackberry plants were present at the time of collection.

Inoculation with Phytophthora via soil.JPG

For all glasshouse experiments, inoculum of the *Phytophthora* species was produced in a vermiculite-based substrate and applied in the root zone of plants growing in sand or in potting mix.

Pathogenicity glasshouse experiments-harvesting.JPG
The pathogenicity of the *Phytophthora* species was tested on blackberry plants in glasshouse experiments. At the end of experiments, above-ground biomass of plants was harvested and dried to compare inoculated and control treatments.

Host-specificity glasshouse experiments -flooding event.JPG
The specificity of *Phytophthora pseudocryptogea*, the species found to have the greatest adverse impact on blackberry in previous experiments, was tested on a range of non-target plant species in glasshouse experiments. The methodology involved exposing control and inoculated plants to fortnightly simulated flooding treatments by placing pots in buckets containing water for 3 days.

Host-specificity glasshouse experiments -cleaning up.JPG
Host-specificity glasshouse experiments performed in the project were quite large and time-consuming, especially because of the methodology that required exposing all plants to fortnightly simulated flooding treatments.

10.2 Equipment and assets

No equipment or assets created or acquired during the period covered by the project.

10.3 Staffing levels

2015/16
0.25 FTE Project Leader and Research Scientist (1 staff); 0.3 FTE Technical Officer (1 staff)

2016/17
0.25 FTE Project Leader and Research Scientist (1 staff); 0.6 FTE Technical Officer (3 staff)

2017/18
0.35 FTE Project Leader and Research Scientist (1 staff); 0.89 FTE Technical Officer (3 staff)

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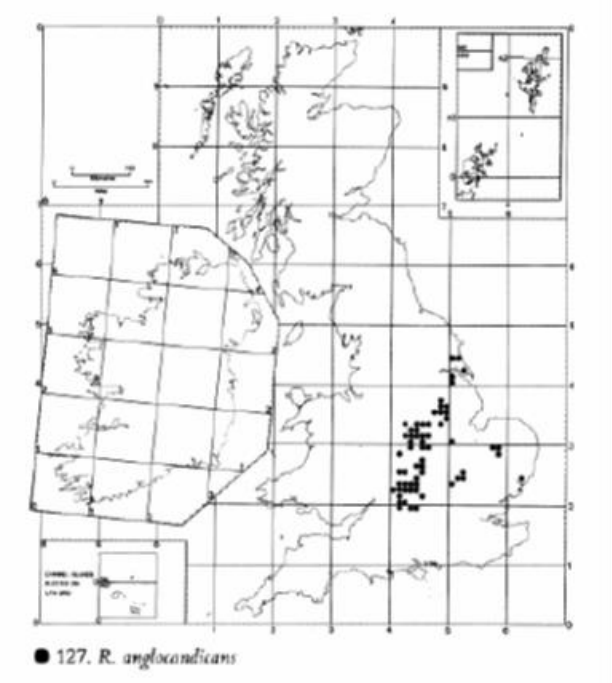
Appendix 1. Summary of the assessment performed by CABI colleagues on the likelihood of finding the stem-boring sawfly *Phylloecus faunus* in the UK (Nov. 2017).

The most recent UK records of *Janus femoratus* are specimens collected from the edge of Windsor Forest in 2010 and deposited at RHS, Wisley. There is only one other fairly recent record of this taxon from Basingstoke (1989, location of specimen unknown), with all other UK records older than 50 years (https://records.nbnatlas.org/occurrences/search?q=lsid:NBNSYS0000013251#tab_recordsView). Since *J. femoratus* had falsely been regarded as a synonym of *Phylloecus faunus* (which in turn is the revised name of *Hartigia albomaculata*) for a considerable period of time we thought it worthwhile to double-check the identity of these specimens. We have now done so with the help of the original collector of the specimens, Andrew Halstead, an expert in sawfly taxonomy. The specimens were confirmed as *J. femoratus*, which is a species in the UK only associated with oak (*Quercus*)!!

The occurrence of *Phylloecus faunus/Hartigia albomaculata* in the UK remains far from certain, with the type specimen recorded in 1837 near London remaining up until now the only reliable record of this species. If indeed the species is currently only present in continental Europe and *R. anglocandicans* is a species endemic to the UK then it seems unlikely that both species have co-evolved.

In the UK, *R. anglocandicans* has been recorded primarily along the Jurassic outcrop from Yorkshire to the Cotswolds (see map below from E. S. Edees and A. Newton, 1988, Brambles of the British Isles). It may still be worthwhile to conduct a survey of specialised invertebrates (or pathogens?) within the native range of *R. anglocandicans*. Considering the short evolutionary time window during which the *Rubus* host has evolved after the last ice age it is unlikely that we will discover herbivores host specific to this *Rubus* taxon alone. There is still the chance though to discover insect populations better adapted to the biology of *R. anglocandicans* compared to other *Rubus* species. There are certainly some sawfly species in the UK which are confined to *Rubus*. For example, *Hartigia/Phylloecus nigra* another very rare species on *Rubus* overlaps in distribution with *R. anglocandicans* although this seems to be a species potentially preferring *R. idaeus* as a host in the UK.



Appendix 2. Details of survey sites for *Phylloecus faunus* in Western Europe and the United Kingdom.

Site ID	Date	Country	Locality	Latitude	Longitude	Altitude	Field characteristics	Species sampled	Nb. canes sampled	Comments
FrS1	31/01/2018	France	Cases de Péne	42.780519	2.781823	52	Open field, old vineyard no longer cultivated. The site is located in the hills close to a small river. Presence of many brambles but also some *Rosa canina.*	*Rubus* sp. *Rosa canina*	36 30	30 primocanes individualized
FrS1 Riverside	31/01/2018	France	Cases de Péne	42.780519	2.781823	52	Same site as previously mentioned but very close to the river (~ 5 m).	*Rubus* sp.	30	From 1 big patch
FrS2	31/01/2018	France	Claira	42.750378	2.953007	10	Open field, heathland surrounding by a peach orchard (50 - 100 m). The plot is located 200 m from a river.	*Rubus sp.*	30	3 patches with 10 primocanes
FrS3	14/02/2018	France	Saint Clément de Rivière	43.715275	3.849264	67	A parcel close to the Lez river and just before scrubland and Aleppo pines forest. Only 2 plants of *Rosa canina.*	*Rubus sp.* *Rosa canina*	31 20	3 patches with 10 primocanes 20 branches from 2 plants (no young canes have been observed on the site, only 2 plants, 10 "young" branches collected from those plants).
FrS4	14/02/2018	France	Saint Gély du Fesc	43.665753	3.829322	117	Open field, wet heathland (kind of swamp, at least at the date of collection, with many herbaceous plants). Mixed stands of *Rubus* and *Rosa canina* (*R. canina* abundant on that site).	*Rubus sp.* *Rosa canina*	30 30	3 patches with 10 primocanes 30 young canes (canes of the year)
P1	08/04/2018	Portugal	Serpa	37.956273°	7.593007°	153	*Rubus ulmifolius* growing amongst *Arundo donax* along a creek.	*Rubus sp.*	30	23% (n=7) canes damaged. Sawfly specimens collected.
P2	09/04/2018	Portugal	Ourique	37.649683°	8.229892°	196	Open site. *R. ulmifolius* patch ~ 50 m long growing along fence line on roadside. Sawfly damage and specimens collected.	*Rubus sp.*	31	19% (n=6) canes damaged. Sawfly specimens collected
P3	09/04/2018	Portugal	Colos	37.735512°	8.452153°	144	Open site along watercourse and driveway. *R. ulmifolius* patch ~ 50 m long.	*Rubus sp.*	30	3% (n=1) cane damaged but no specimens found.

Site ID	Date	Country	Locality	Latitude	Longitude	Altitude	Field characteristics	Species sampled	Nb. canes sampled	Comments
P4	09/04/2018	Portugal	Vila Nova de Milfontes	37.733145°	8.774067°	41	Open field with *R. ulmifolius* patch ~ 100 m long.	*Rubus sp.*	30	13 (n=4) canes damaged but only one specimen (which looks like a cluster of eggs or small larvae) was collected.
P5	10/04/2018	Portugal	Messines	37.247720	8.255863	132	Two *R. ulmifolius* patches (20 x 20 m) either side of roadside beneath highway bridge.	*Rubus sp.*	30	20% (n=6) canes damaged. Specimens collected.
P6	11/04/2018	Portugal	Altura	37.185207	7.479903	9	Long *R. ulmifolius* patch ~ 300 m long growing along fence line of roadside, open field.	*Rubus sp.*	30	3% (n=1) cane damaged. No specimens found.
P7	11/04/2018	Portugal	Figueirais	37.262734	7.481264	11	Open site, *R. ulmifolius* patch ~ 100 m long growing along a ditch in an old quince orchard.	*Rubus sp.*	31	13% (n=4) canes damaged. Two pupae specimens collected.
P8	12/04/2018	Portugal	near Sero das Covas	37.340073	8.187429	337	One small *Rosa canina* bush growing on roadside.	*Rosa canina*	3	No damage.
P9	12/04/2018	Portugal	near Sero das Covas	37.348066	8.183809	384	One patch of mixed *R. ulmifolius* and *Rosa canina* ~ 10 m long. Open site growing on roadside on the side of a hill.	*Rubus sp.*	12	17% (n=2) canes with damage. No sawfly specimens found in *R. ulmifolius* but large galls found on the canes.
								Rosa canina	30	3% (n=1) cane *Rosa canina* with damage. One "blob" found in *Rosa canina* but could not be identified.
P10	12/04/2018	Portugal	Azinhal	37.319218	8.231724	150	Small *Rosa* patch growing amongst *R. ulmifolius* patch ~ 150 m long on a creek. Generally open with some willows interspersed.	*Rubus sp.*	30	27% (n=8) canes damaged with larvae and parasitized larvae collected.
								Rosa canina	30	10% (n=3) canes with signs of tunneling but no specimens found.

Site ID	Date	Country	Locality	Latitude	Longitude	Altitude	Field characteristics	Species sampled	Nb. canes sampled	Comments
P11	13/04/2018	Portugal	Cerro da Cruz (Fortes)	37.352069	7.613098	57	Open site growing on river with two small patches of *R. ulmifolius* and *Rosa canina* (each partch c.a. 2 x 2 m) growing 5 m apart.	*Rubus sp.*	1	1 cane sampled and two larvae found in tunnel.
								Rosa canina	12	100% (n=12) canes with tunneling but no signs of sawfly larvae/pupae present. An adult hoverfly (possibly a stem-boring species) observed on the stem but not collected.
P12	13/04/2018	Portugal	Cerro da Cruz (Fortes)	37.352924	7.612399	50	Open *R. ulmifolius* site growing on river approx. 50 m away from *Rosa canina* patch.	*Rubus sp.*	11	64% (n=7) canes with damage. One pre-pupa collected.
UK1	16/04/2018	UK	CABI, Egham, UK grounds	51.420573	0.568564	0	*Rubus fruiticosus* patches growing in an open field and under elm trees, with cane tunneling.	*Rubus sp.*	30	Some insect cane damage towards terminal ends causing deformation at nodes possibly caused by a galling insect. 2 canes with tip galling damage, no canes with sawfly damage.
UK2	16/04/2018	UK	Windsor Gardens Deer Park	51.451087	0.595060	41	*Rubus fruiticosus* patches growing beneath elm groves.	*Rubus sp.*	30	Tunnelling found in one stems. Parasitised pre-pupa collected.
UK3	17/04/2018	UK	Silwood Park grounds	51.409947	0.649627	62	Open grassy field, *Rubus fruiticosus* patch 3 x 15 m.	*Rubus sp.*	30	No canes with signs of sawfly damage.
UK4	17/04/2018	UK	Silwood Park grounds	51.413442	0.645689	51	*Rubus fruiticosus* patch (10 x 5 m) growing in small clearing in elm grove.	*Rubus sp.*	30	Galls found on stems. One dissected and a small larva found inside. No canes with signs of sawfly damage.
ItS1	25/04/2018	Italy	Osilo	40.718041	8.652760	360	Forest environment in a mountainous landscape.	*Rubus sp.*	30	3 patches with 11 primocanes; 8 and 11 primocanes
								Rosa canina	10	10 branches from 1 plant

Site ID	Date	Country	Locality	Latitude	Longitude	Altitude	Field characteristics	Species sampled	Nb. canes sampled	Comments
CoS1	26/04/2018	France	Figari	41.489085	9.127542	50	Small parcel of *Rubus* collected in a little village in a forest environment. Small population.	*Rubus sp.*	30	2 patches collected
FrS5	07/05/2018	France	Mauguio	43.604159	3.963069	13	Open field in an agricultural environment, collection carried out alongside a water channel. Site close to the one describe in the Bruzesse's paper. Both *Rubus* and *Rosa canina* are present in the field.	*Rubus sp.*	30	3 patches with 10 primocanes
								Rosa canina	30	Mix of young branches and canes of the year
FrS6	26/03/2018	France	Montferrier sur Lez - CSIRO	43.685588	3.874677	85	Open field in dry grassland.	*Rosa canina*	36	Mix of young branches and canes of the year from 10 plants
FrS7	07/05/2018	France	Montferrier sur Lez - CBGP	43.677012	3.872277	62	Forest environment (Aleppo pines mixed with other tree species, especially *Fraxinus* sp.). Big population of *Rubus* and few *Rosa canina*.	*Rubus sp.*	30	3 patches with 10 primocanes
								Rosa canina	10	10 branches from 1 plants

Appendix 3. Information on the stem-boring sawfly *Phylloecus faunus*.

Taxonomy

Class: Insecta
Order: Hymenoptera
Superfamily: Cephoideae
Family: Cephidae
Genus: *Phylloecus faunus* Newman, 1838

The following is an excerpt from "Sawfly taxa (Hymenoptera, Symphyta) described by Edward Newman and Charles Healy". Refer to Liston and Prous (2014) for references given within the text.

Description

= *Phylloecus faunus* Newman, 1838: 485-486; ♀♂; type locality: "in the vicinity of London". Note: faunus is a noun; the name of a Roman deity.

= *Cephus helleri* Taschenberg, 1871: 305-306; ♀; type locality: Insula Lesina [Island of Hvar, Croatia]. **syn. n.**

= *Cephus albo-maculatus* J.P.E.F.Stein, 1876

= *Hartigia albomaculata* Stein, 1876

= *Macrocephus fumipennis* var. *picticeps* Strand, 1910

= *Phyllaecus rubi* Perris, 1873

Type material examined.

Phylloecus faunus . Lectotype (hereby designated) ♀, Figs 7-12. "[handwritten] *Phylloecus faunus*, Newm. [printed] Det. in Coll. Ent. Club, Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[handwritten] Faunus Newm."; "[red] Lectotype Phylloecus faunus Newman, 1838 des. A. Liston 2013"; "*Hartigia faunus* (Newman, 1838) det. A. Liston 2013". Condition: missing most of right antennal flagellum, most tarsi except right middle and rear; abdomen after tergum 5 glued to specimen.

Figures 7–12. *Phylloecus faunus* Newman, 1838; lectotype. **7** dorsal **8** abdomen, dorsoapical **9** head, frontal **10** head, dorsal **11** abdomen, lateroapical **12** labels (Source: "Zookeys-398-083-g002". Via OpenMedia - https://species-id.net/o/index.php?title=Image:Zookeys-398-083-g002.jpg#/media/File:Zookeys-398-083-g002.jpg

Newman refers to a syntype series of three specimens of *Phylloecus faunus*: "Two specimens of this insect have been taken by Mr. Ingall, and one by Mr. Stephens". The single specimen examined agrees well with the brief description. Most taxonomic works and catalogues (e.g. Konow 1905a ; Taeger et al. 2010) have until now placed *Phylloecus faunus* as a synonym of *Janus cynosbati* (Linnaeus, 1758), although it should have been apparent from several characters described or discussed by Newman (1838) , that these are not conspecific. The mistaken synonymy was possibly first published by Kirby (1882).

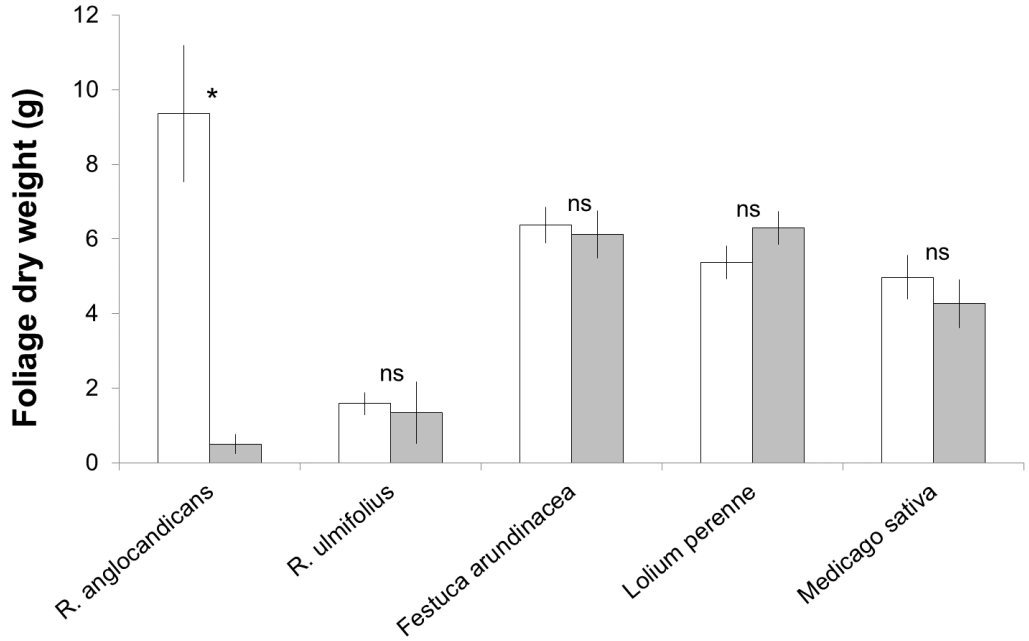
Although the name *faunus* has not to the best of our knowledge been used as valid after 1899, neither has the name helleri been sufficiently used (in 21 publications by 27 authors including co-authors) as valid in the last fifty years to satisfy the conditions of Article 23.9 (reversal of precedence) of the International Code of Zoological Nomenclature (ICZN 1999). A list of these references is available from us on request. The lectotype of *Phylloecus faunus* agrees in all important points with the characterisation of *Hartigia helleri* by Jansen (1998). Quinlan (1970) identified a second female specimen in the Natural History Museum, London, which should be regarded as a paralectotype of *Phylloecus faunus* , as *Hartigia albomaculatus* [sic!], noted that it bore a label “faunas” [presumably in reality faunus] and mentioned that no reliable information is available on where it was caught. One might doubt the reliability of Newman’s statement that the types of *Phylloecus faunus* were collected around London, because under its synonyms *Hartigia albomaculata* and *Hartigia helleri* no evidence for the presence of this species in the British Isles has been published, and because neither of the two type specimens still in existence bears any explicit label data referring to the collection locality. However, an occurrence in the London area, at least historically, seems not unlikely. Chevin (1993) presented several records from northern France, under the name *Hartigia albomaculata* , and later (Chevin and Chevin 2007) recorded *Hartigia helleri* from the Département de la Manche, not far from the Channel coast. It is concluded that *Phylloecus faunus* should be used as the valid name of the species referred to in recent years first as *Hartigia albomaculata* (or *Hartigia albomaculatus*, misspelling) and latterly as *Hartigia helleri*, and that after weighing up the evidence, the type locality of *Phylloecus faunus* can be accepted as being in the area of London.

Reference

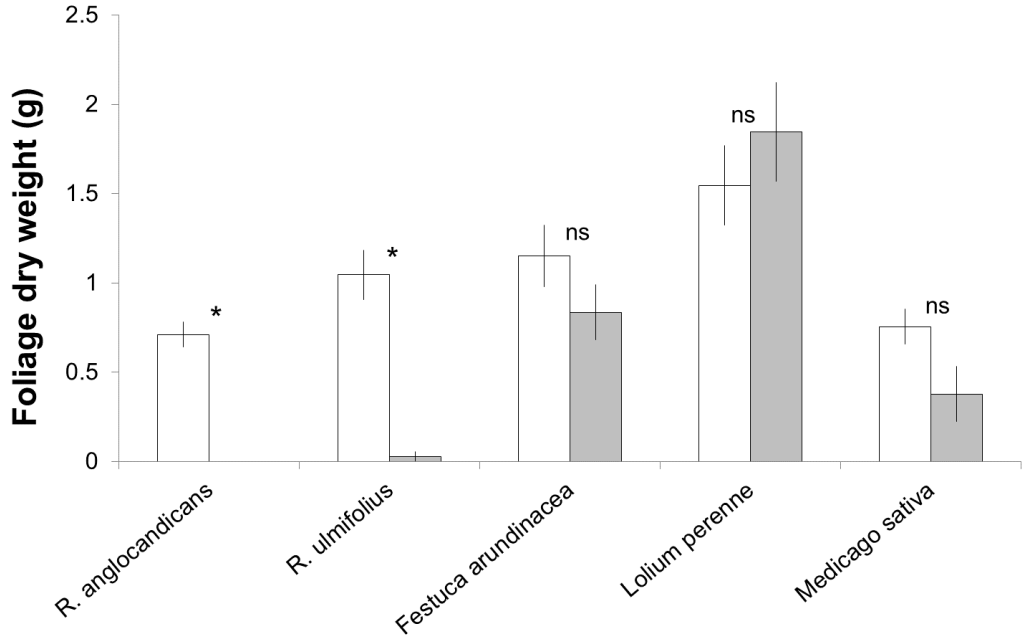
Liston, AD., Prous, M. (2014): Sawfly taxa (Hymenoptera, Symphyta) described by Edward Newman and Charles Healy. ZooKeys 398: 83-98, http://dx.doi.org/10.3897/zookeys.398.6595 (accessed 30 March 2018)

Appendix 4. Foliage dry weight of different non-target plant species inoculated with non-colonised substrate (control; open bars) or substrate colonised by *Phytophthora pseudocryptogea* (inoculated; grey bars) across a series of trials. Blackberry (*R. anglocandicans*) was used as a reference species in each trial. Bar heights represent means for groups, and error bars indicate 2× standard errors of the mean. A star symbol above bars of a taxon indicates that the control and inoculated treatment are significantly different according to two-sample t-test (alpha = 0.05). Non-significant differences are indicated with 'ns'.

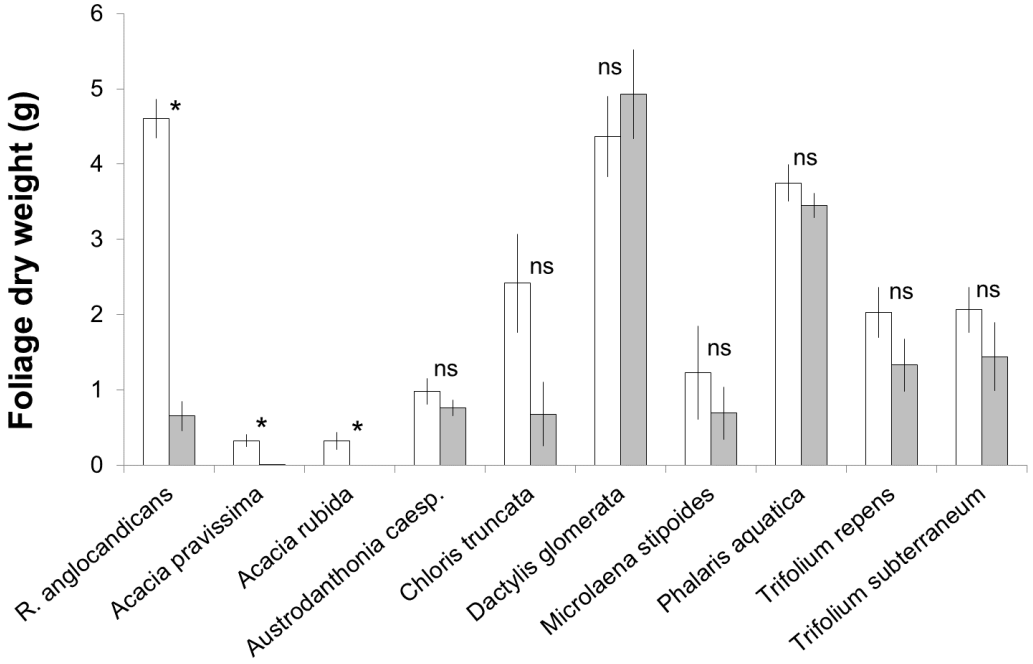
Trial 1



Trial 2



Trial 3



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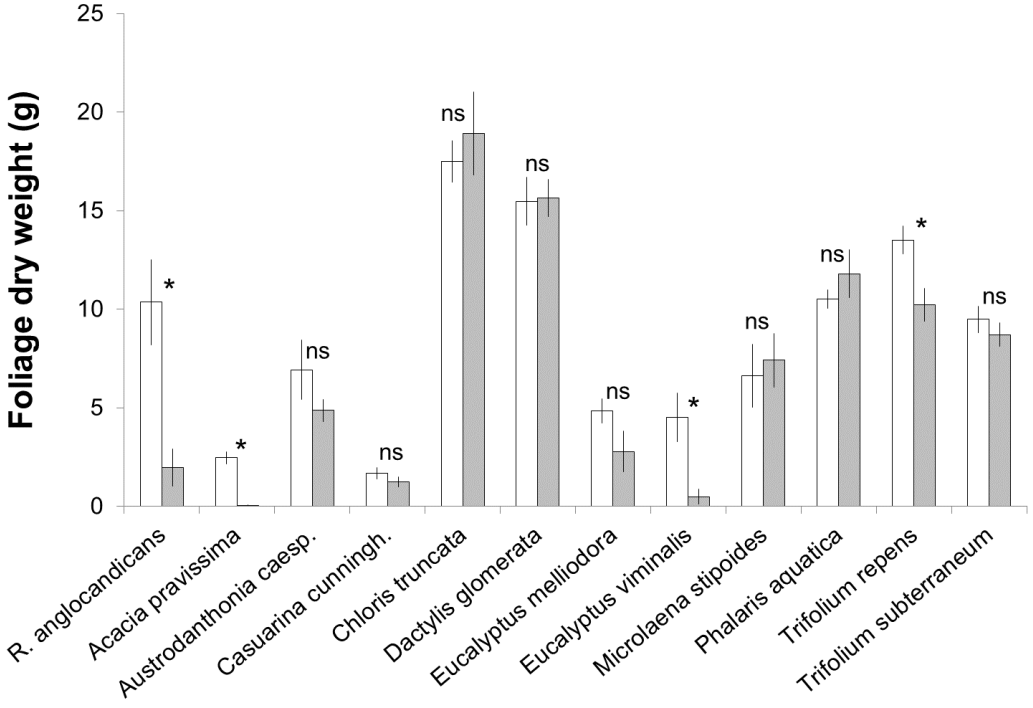
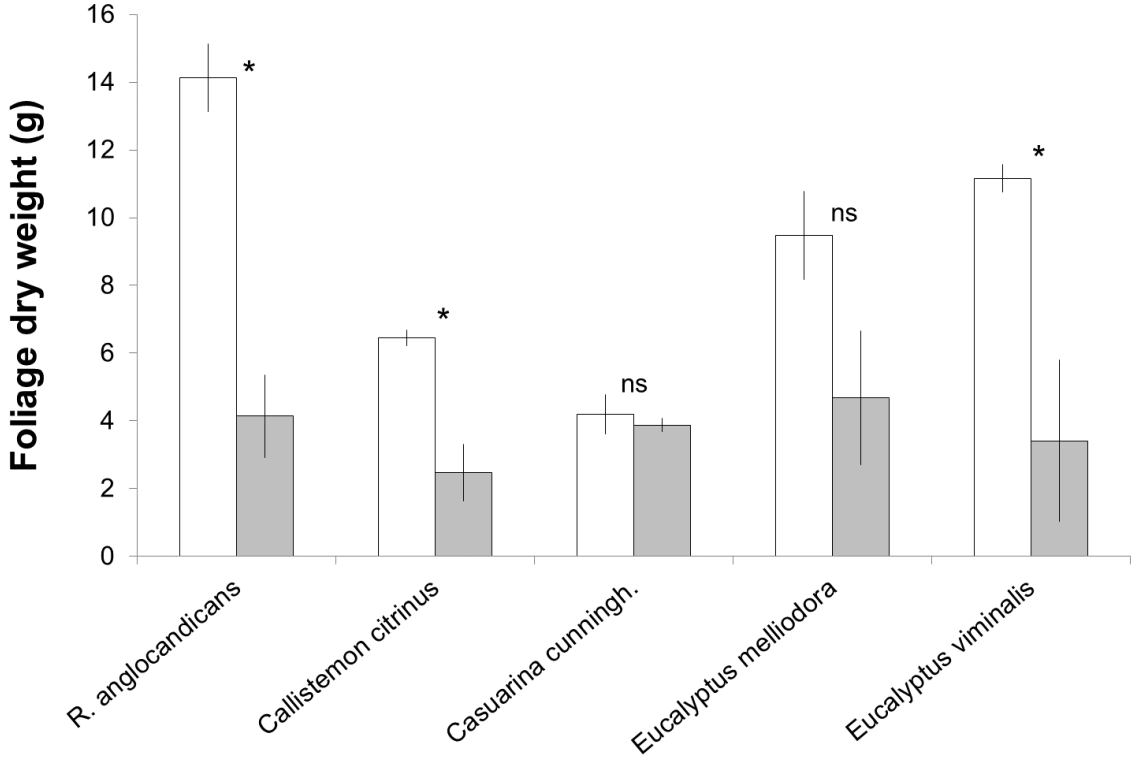
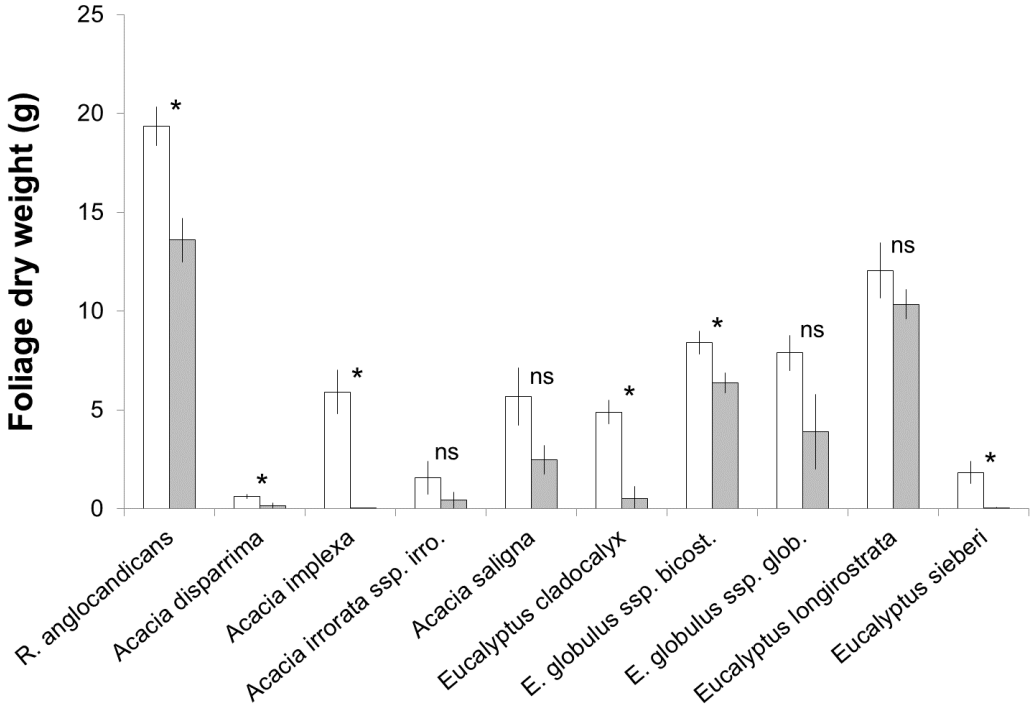


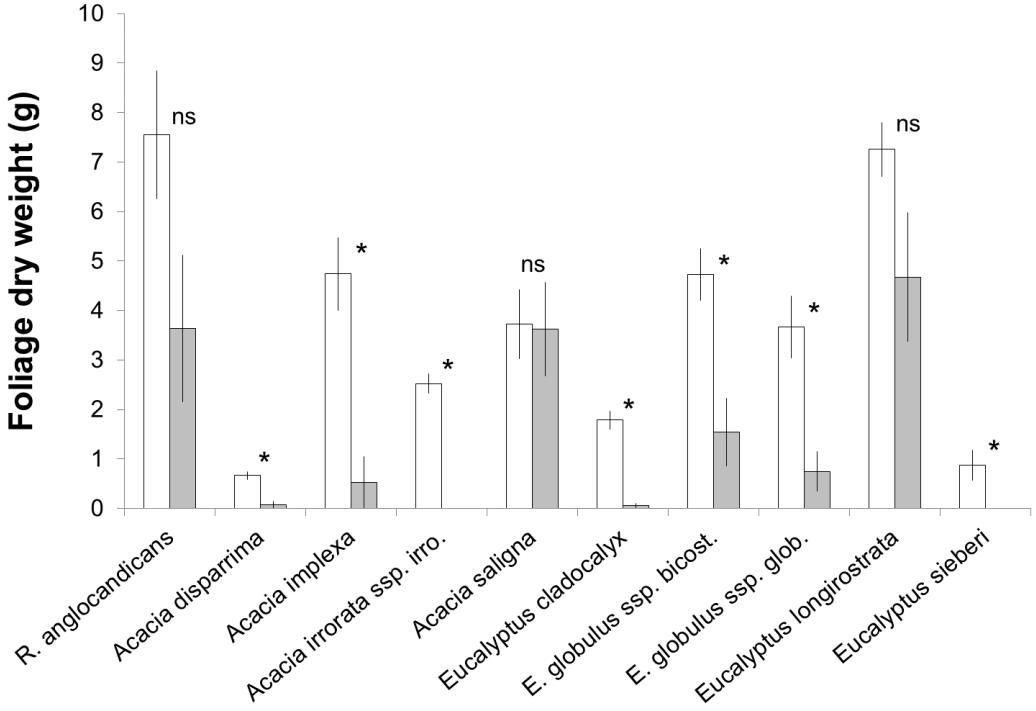
Trial 5



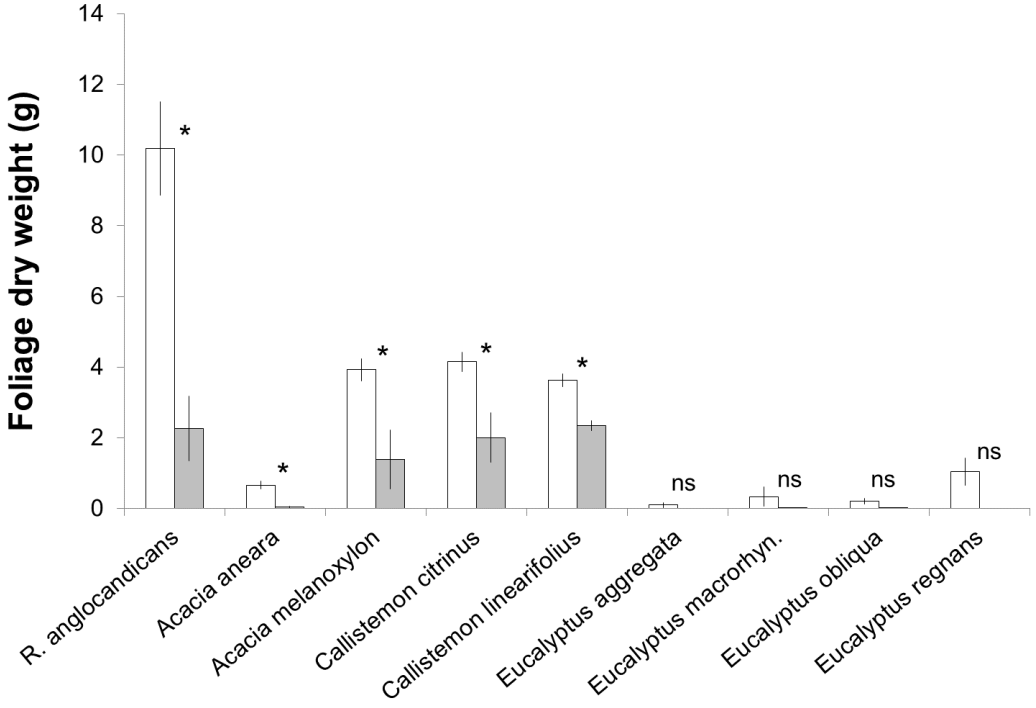
Trial 6



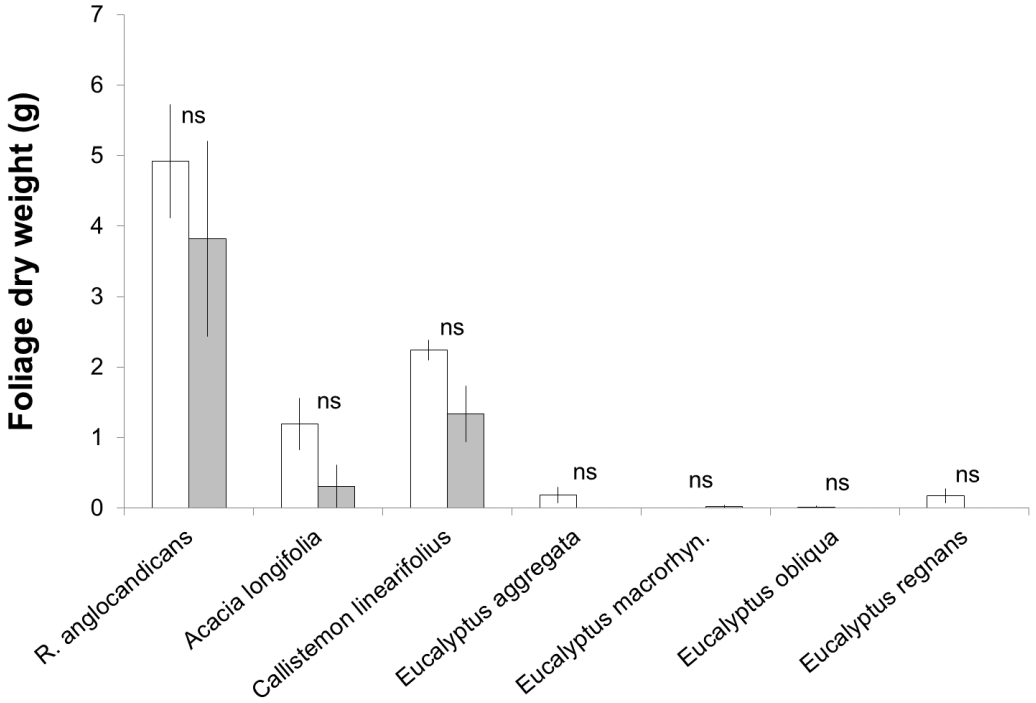
Trial 7



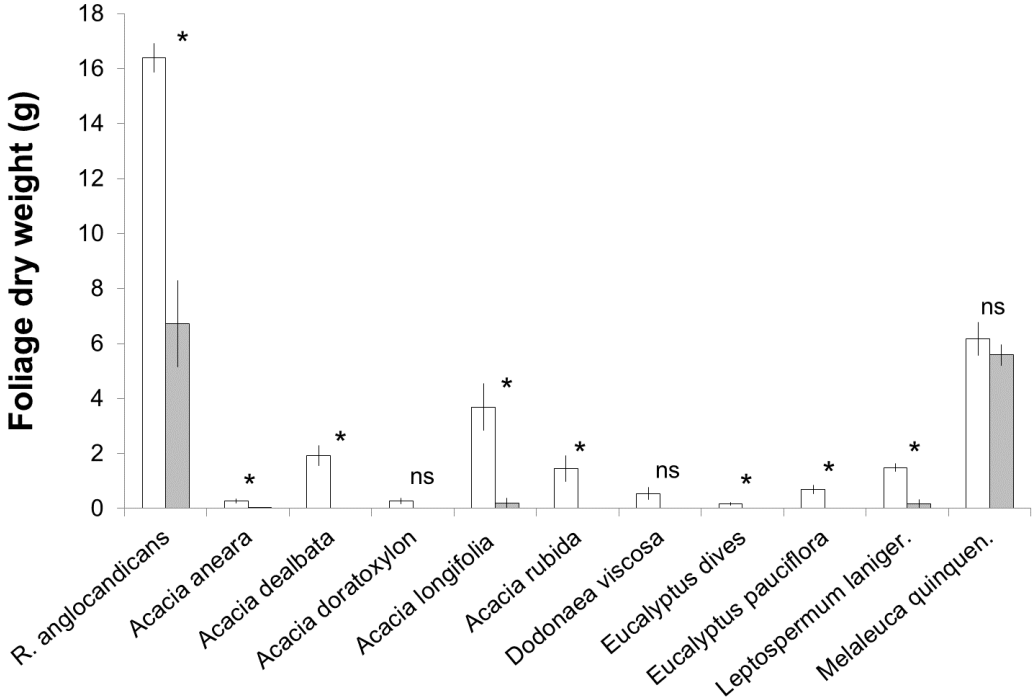
Trial 8



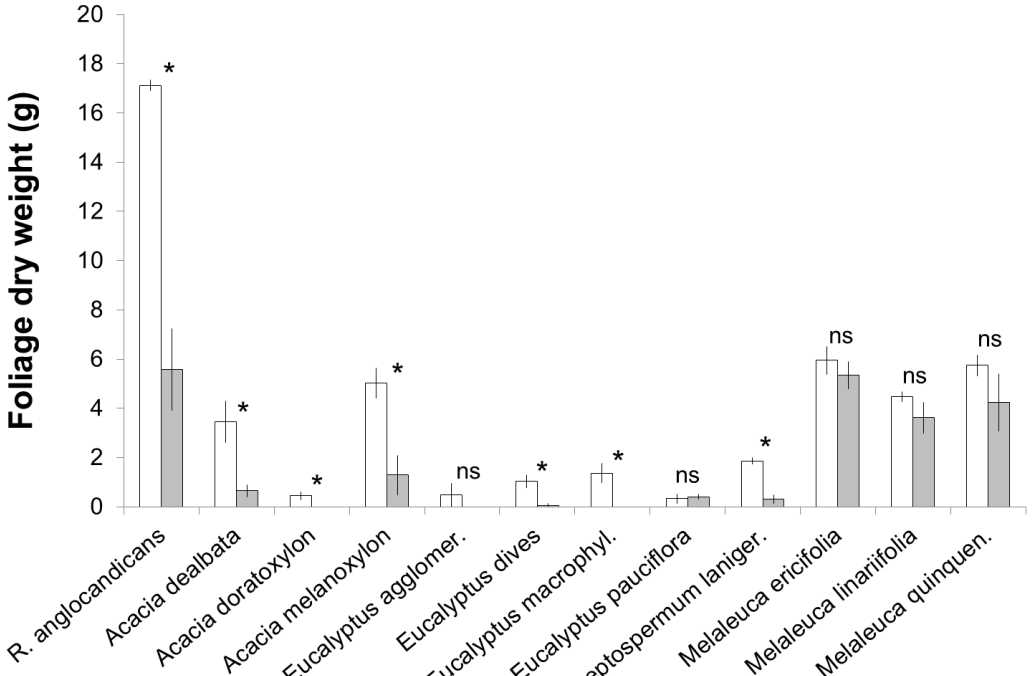
Trial 9



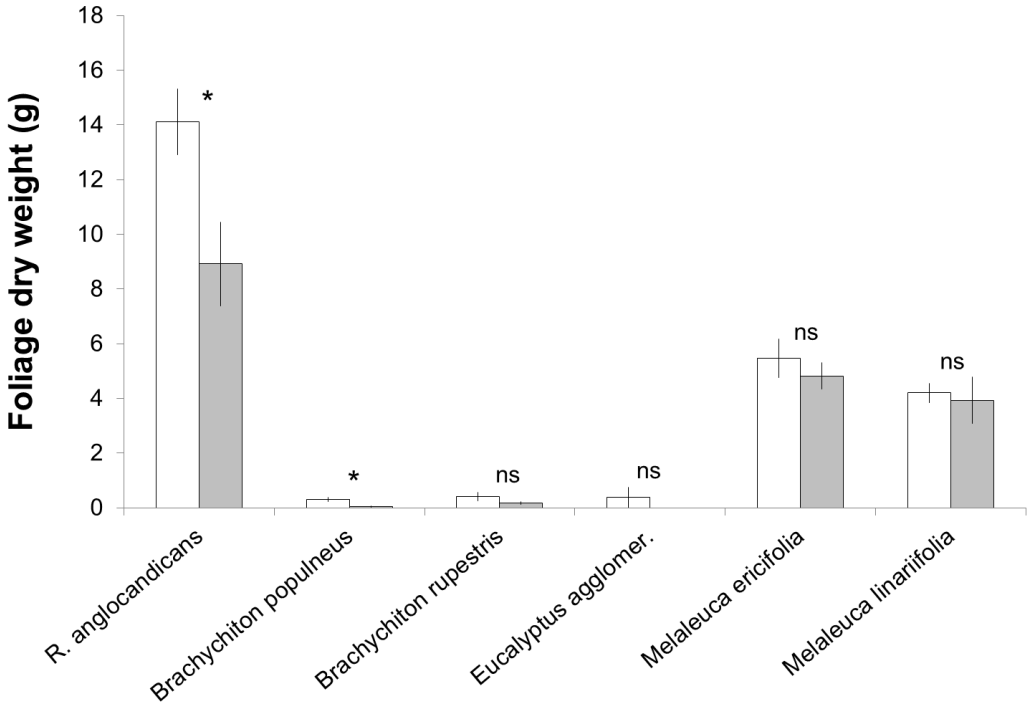
Trial 10



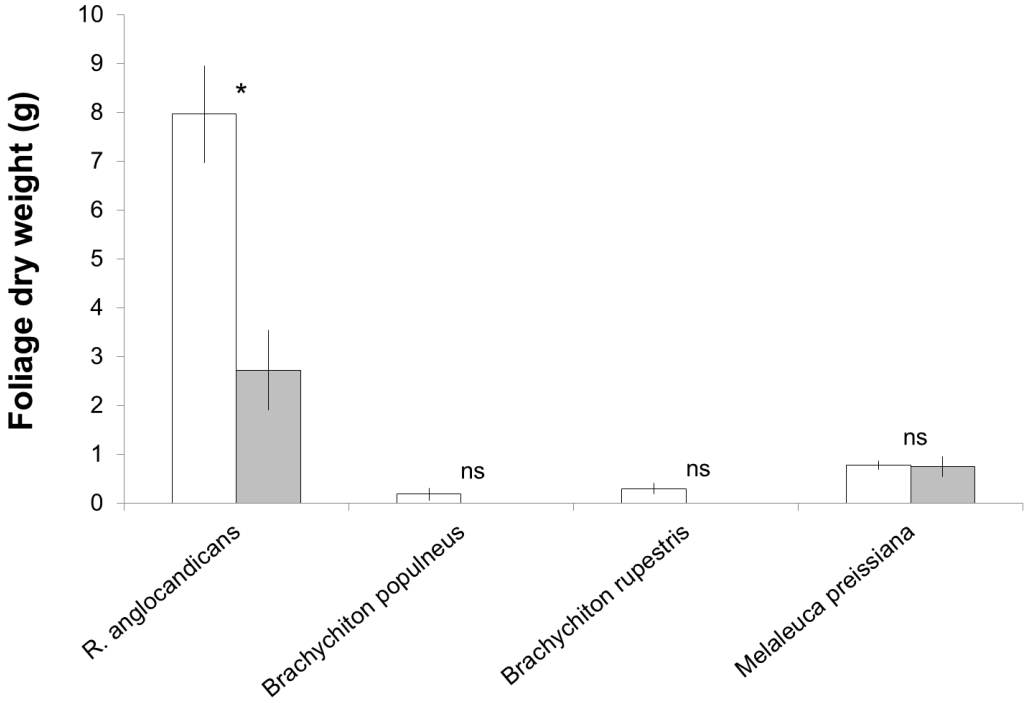
Trial 11



Trial 12



Trial 13


Foliage dry weight (g)
10
9
8
7
6
5
4
3
2
1
0
*
ns
ns
ns
R. anglocandicans
Brachychiton populneus
Brachychiton rupestris
Melaleuca preissiana

Appendix 5. DNA barcoding identification results for insect specimens. "Best match" refers to the closest match on the BOLD database (http://www.boldsystems.org), queried 3rd July 2018.

VAITC	Location	Host	Best Match Name	%	Family	Group	Notes
8258	FrS3	Rubus	*Hartigia helleri*	99.1	Cephidae	Sawfly	Current name = *P. faunus*
8261	FrS3	Rubus	*Hartigia helleri*	99.2	Cephidae	Sawfly	Current name = *P. faunus*
8263	FrS3	Rubus	*Hartigia helleri*	99.1	Cephidae	Sawfly	Current name = *P. faunus*
8266	FrS5	Rubus	*Hartigia helleri*	99.2	Cephidae	Sawfly	Current name = *P. faunus*
8275	FrS7	Rubus	*Hartigia helleri*	99.1	Cephidae	Sawfly	Current name = *P. faunus*
8522	FrS7	Rubus	*Hartigia helleri*	99.25	Cephidae	Sawfly	Current name = *P. faunus*
8524	FrS7	Rubus	*Hartigia helleri*	99.25	Cephidae	Sawfly	Current name = *P. faunus*
8526	FrS7	Rubus	*Hartigia helleri*	99.25	Cephidae	Sawfly	Current name = *P. faunus*
8527	FrS7	Rubus	*Hartigia helleri*	99.25	Cephidae	Sawfly	Current name = *P. faunus*
8279	P1	Rubus	*Phylloecus faunus*	97.93	Cephidae	Sawfly	Current name = *P. faunus*
8280	P1	Rubus	*Phylloecus faunus*	97.74	Cephidae	Sawfly	Current name = *P. faunus*
8281	P1	Rubus	*Phylloecus faunus*	97.93	Cephidae	Sawfly	Current name = *P. faunus*
8286	P2	Rubus	*Phylloecus faunus*	97.93	Cephidae	Sawfly	Current name = *P. faunus*
8287	P2	Rubus	*Phylloecus faunus*	97.93	Cephidae	Sawfly	Current name = *P. faunus*
8289	P5	Rubus	*Phylloecus faunus*	97.55	Cephidae	Sawfly	Current name = *P. faunus*
8291	P5	Rubus	*Phylloecus faunus*	97.55	Cephidae	Sawfly	Current name = *P. faunus*
8292	P7	Rubus	*Phylloecus faunus*	97.93	Cephidae	Sawfly	Current name = *P. faunus*
8293	P7	Rubus	*Phylloecus faunus*	97.86	Cephidae	Sawfly	Current name = *P. faunus*
8269	FrS5	Rosa	*Cladardis elongatula*	99.8	Tenthredinidae	Sawfly	Rose shoot sawfly
8271	FrS5	Rosa	*Cladardis elongatula*	99.8	Tenthredinidae	Sawfly	Rose shoot sawfly
8273	FrS6	Rosa	*Agrilus cuprescens*	100	Buprestidae	Beetle	Jewel beetle
8251	P11	Rubus	*Agrilus solieri*	99.1	Buprestidae	Beetle	Jewel beetle
8252	P11	Rubus	*Agrilus solieri*	99.25	Buprestidae	Beetle	Jewel beetle
8262	FrS3	Rubus	*Agrilus solieri*	98.3	Buprestidae	Beetle	Jewel beetle
8296	P10	Rubus	*Agrilus solieri*	99.06	Buprestidae	Beetle	Jewel beetle
8529	ItS1	Rubus	*Agrilus solieri*	98.12	Buprestidae	Beetle	Jewel beetle
8530	ItS1	Rubus	*Agrilus solieri*	98.12	Buprestidae	Beetle	Jewel beetle
8531	ItS1	Rubus	*Agrilus solieri*	98.12	Buprestidae	Beetle	Jewel beetle
8532	ItS1	Rubus	*Agrilus solieri*	98.12	Buprestidae	Beetle	Jewel beetle
8533	ItS1	Rubus	*Agrilus solieri*	98.12	Buprestidae	Beetle	Jewel beetle
8534	CoS1	Rubus	*Agrilus solieri*	99.62	Buprestidae	Beetle	Jewel beetle
8535	CoS1	Rubus	*Agrilus solieri*	99.62	Buprestidae	Beetle	Jewel beetle
8536	CoS1	Rubus	*Agrilus solieri*	99.62	Buprestidae	Beetle	Jewel beetle
8537	CoS1	Rubus	*Agrilus solieri*	99.44	Buprestidae	Beetle	Jewel beetle
8270	FrS5	Rosa	No named match	98.49	Cerambycidae	Beetle	Longhorn beetle
8272	FrS5	Rosa	*Scolytus* sp.	91.89	Curculionidae	Beetle	Bark beetle
8295	P10	Rubus	*Hylaeus* sp.	93.7	Colletidae	Bee	Plasterer bee
8257	P10	Rubus	*Hylaeus* sp.	93.55	Colletidae	Bee	Plasterer bee
8274	FrS6	Rosa	*Tetrastichus*	97.79	Eulophidae	Wasp	Parsitoid wasp
8547	FrS5	Rubus	Eurytomidae sp.	99.81	Eurytomidae	Wasp	Parasitoid? or Stem feeding?
8290	P5	Rubus	No named match	92.52	Eurytomidae	Wasp	Parasitoid? or Stem feeding?
8541	FrS2	Rubus	No named match	92.96	Eurytomidae	Wasp	Parasitoid? or Stem feeding?
8546	FrS3	Rubus	No named match	99.81	Eurytomidae	Wasp	Parasitoid? or Stem feeding?
8548	FrS7	Rubus	No named match	91.58	Eurytomidae	Wasp	Parasitoid? or Stem feeding?

VAITC	Location	Host	Best Match Name	%	Family	Group	Notes
8278	P1	Rubus	*Endromopoda phragmitidis*	100	Ichneumonidae	Wasp	Parsitoid wasp
8282	P1	Rubus	*Endromopoda phragmitidis*	100	Ichneumonidae	Wasp	Parsitoid wasp
8283	P1	Rubus	*Endromopoda phragmitidis*	100	Ichneumonidae	Wasp	Parsitoid wasp
8284	P1	Rubus	*Endromopoda phragmitidis*	100	Ichneumonidae	Wasp	Parsitoid wasp
8300	P10	Rubus	*Endromopoda phragmitidis*	100	Ichneumonidae	Wasp	Parsitoid wasp
8549	ItS1	Rubus	*Xylophrurus augustus*	97.84	Ichneumonidae	Wasp	Parsitoid wasp
8253	P12	Rubus	No named match	89.04	Ichneumonidae	Wasp	Parsitoid wasp
8276	FrS7	Rubus	No named match	88.26	Ichneumonidae	Wasp	Parsitoid wasp
8277	P1	Rubus	No named match	88.64	Ichneumonidae	Wasp	Parsitoid wasp
8523	FrS7	Rubus	No named match	88.64	Ichneumonidae	Wasp	Parsitoid wasp
8545	FrS3	Rubus	No named match	88.82	Ichneumonidae	Wasp	Parsitoid wasp
8288a	P4	Rubus	No named match	92.34	Pteromalidae	Wasp	Parsitoid wasp
8288b	P4	Rubus	No named match	92.34	Pteromalidae	Wasp	Parsitoid wasp
8255	UK4	Rubus	*Torymus rubi*	97.39	Torymidae	Wasp	Parasitoid? or Gall feeding?
8543	FrS3	Rubus	No named match	92.29	Torymidae	Wasp	Parasitoid? or Stem feeding?
8544	FrS3	Rubus	No named match	92.29	Torymidae	Wasp	Parasitoid? or Stem feeding?