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Exploration of RNAi vaccines as a novel control for Myrtle Rust in critically endangered Australian taxa

Final Progress Report (PBSF034)

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Contents

1. Executive Summary

Myrtle rust is a highly invasive fungal disease caused by the exotic rust fungus *Austropuccinia psidii.* Since its detection in New South Wales in 2010, myrtle rust has spread rapidly and is now established in Queensland, Victoria, Tasmania and the Northern Territory, with recent incursions in Western Australia and on Lord Howe Island. Myrtle rust infects young, actively growing and emerging leaves, buds, flowers, green stems, fruit and shoots, and has been recorded on more than 480 species across 57 genera of Myrtaceae (Beresford *et al.*, 2020). Symptoms range in severity from leaf spots, blight, and dieback, to whole-tree malformation and mortality in highly susceptible species. Myrtle rust is threatening whole ecosystems as well as industries relying on Myrtaceae, such as native forestry, cut flower, essential oils, honey and native foods.

Management of myrtle rust in nurseries and plantations predominantly relies on the use of clean planting material, fungicide treatments and the selection of resistant genotypes. However, these control strategies are not feasible for natural environments. Fungicides, while effective in preventing infections, are costly and force growers to forego their organic accreditations, resulting in significant revenue losses. Additionally, most fungicides are broad-spectrum and their use can impact the environment through loss of beneficial microbes, insects, and other organisms. A new solution which is both effective, as well as environmentally, culturally, and economically responsible, is therefore urgently needed. Indeed Objective 4.3 of the Myrtle Rust Action Plan (Makinson *et al.* 2020) is to explore methods for resistance and control.

The aim of this project was to explore RNA interference (RNAi) vaccines as a novel control for myrtle rust in natural populations of *Decaspermum humile* and *Syzygium hodgkinsoniae* (both Very High priority species, Objective 4.3.5 Myrtle Rust Action Plan). RNAi vaccines are non-toxic, non-GM, pathogen-specific, environmentally friendly biological alternatives to chemical pesticides. The approach involves applying pathogen-specific double-stranded RNA (dsRNA) to knock down pathogen genes and is proven against a range of plant pests and diseases, including soybean rust and switchgrass rust (Yin *et al.* 2015; Hu *et al.* 2020).

We have established artificial leaf assays, as well as detached leaf and whole plant bioassays in *Syzygium jambos, Syzygium hodgkinsoniae* and *Decaspermum humile*. In work that has now been published (Degnan *et al.* 2023), we demonstrated dsRNA uptake by *Austropuccinia psidii* and identified a number of *A. psidii*-specific dsRNAs that inhibit *A. psidii* urediniospore germination leading to a reduction in disease on detached leaves and whole plants. In *PBSF042*, we are investigating dsRNA uptake and movement by host plants, longevity of dsRNA-induced protection, and performance of the vaccines under field conditions.

2. Introduction

Austropuccinia psidii is the causal agent of the biosecurity threat myrtle rust and infects plants of the family Myrtaceae. Due to the severity of symptoms, wide host range, and the foundational role of Myrtaceae species in Australian ecosystems, *A. psidii* is considered an environmentally and economically important pathogen. Current controls of rusts, including systemic fungicides and deployment of resistant genotypes, are limited by cost and potential downstream or off-target effects in natural environments. The Myrtle Rust Action Plan (Makinson *et al.* 2020), developed in response to the environmental impact of myrtle rust in Australia, prioritises research on innovative controls that are effective, and environmentally, culturally and economically responsible. The aim of this study was to explore the potential of RNA interference (RNAi) vaccines as a control for myrtle rust, in line with Action 4.3.5 of the Myrtle Rust Action Plan, 'Explore novel Myrtle Rust controls through reviews and scoping studies'.

RNAi is a highly conserved form of post-transcriptional gene silencing found in eukaryotes that is triggered by double-stranded RNA (dsRNA). RNAi can be manipulated to defend plants against a variety of plant pathogens, including fungi (Nowara *et al.*, 2010). Until recently, RNAi-mediated plant protection was achieved through genetic modification (GM) of host plants to produce pathogen-specific dsRNA in a process known as host-induced gene silencing (HIGS) (Nunes & Dean, 2012; Koch *et al.*, 2013; Zotti *et al.* 2017). However, HIGS requires stable transformation of host plants, which has proven difficult in many crop varieties and almost all non-agricultural species (McLoughlin *et al.*, 2018). As a GM approach, HIGS is met with resistance from consumers, and does not present a solution that allows growers to effectively control disease while keeping their organic accreditations (Machado *et al.*, 2018). However, protection can also be conferred through a non-GM approach known as spray induced gene silencing (SIGS), where pathogen specific dsRNA is applied topically to the plant, usually by spraying (Koch *et al.*, 2016; Mitter *et al.*, 2017). This is the approach we are harnessing with RNAi vaccines. It is free from the stringent regulation and corresponding costs subject to genetically modified organisms (GMOs) and has proven successful against viruses, fungi and insects (Wang *et al.* 2016; Koch *et al.* 2013; Mamta *et al.* 2017; Mitter *et al.* 2017).

3. Aim

To explore RNAi vaccines as a novel control for myrtle rust in natural populations of *Decaspermum humile* and *Syzygium hodgkinsoniae* (both Very High priority species, Objective 4.3.5 Myrtle Rust Action Plan).

4. Methods and Results

4.1 dsRNA either associates externally or is internalised by urediniospores

To determine whether *A. psidii* spores can take up dsRNA from the environment, urediniospores and teliospores were incubated with *in vitro*-synthesised Cy3-labelled *green fluorescent protein (GFP)* dsRNA on water agar or glass slides, and then viewed under a confocal fluorescence microscope. Micrococcal nuclease (MNase) was used to remove excess external Cy3-dsRNA as in Qiao et al. (2021). Germinating urediniospores displayed Cy3 fluorescence 4–8 h post dsRNA application, indicating that dsRNA is either internalised or associates with the surface of the spore, which may protect it from MNase activity (Figures 1a and b). Germinating urediniospores of *A. psidii* only displayed Cy3 fluorescence in their germ tubes (Figure 1a and 1b). Teliospores were fluorescent before MNase treatment (Figure 1a) but not after (Figure 1c), indicating that dsRNA was not internalised or externally associated.

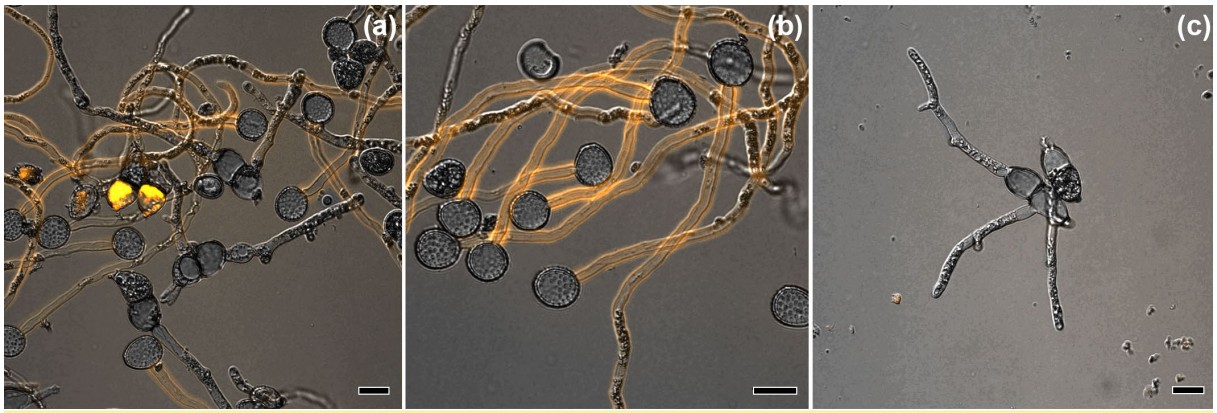

Figure 1 Double-stranded RNA (dsRNA) either associates externally or is taken up by *Austropuccinia psidii* urediniospores but not teliospores. Spores were incubated with 1 µg of Cy3-labelled *green fluorescent protein* dsRNA on 1% water agar for 8 h and were then treated with 75 U of micrococcal nuclease and Cy3 fluorescence observed using a Zeiss LSM700 Confocal Microscope. *A. psidii* urediniospores and teliospores are shown before **(a)** and after **(b and c)** micrococcal nuclease treatment. **(a)** is a mixture of *A. psidii* urediniospores and teliospores, **(b)** is *A. psidii* urediniospores and **(c)** is *A. psidii* teliospores. Scale bars represent 20 µm.

4.2 Target gene selection and double-stranded RNA synthesis

Ten *Austropuccinia psidii* genes were selected as targets for RNAi. Seven were chosen based on previous studies in wheat, soybean and barley (Yin *et al.* 2015; Koch *et al.* 2016; Panware *et al.* 2018; Hu *et al.* 2020), and three for their essential cellular function and specificity at species rank as barcoding genes. Double stranded RNA (dsRNA) targeting these genes was synthesised by *in vitro* transcription (NEB HiScribe kit) using *A. psidii* cDNA as a template. Two dsRNA molecules of different lengths (240 and 700 base pairs (bp)) were synthesised for the *28S* gene (*28S-1 and 28S-2,* respectively) to determine whether dsRNA length had any effect on RNAi however the other dsRNAs were all between 200 and 400 bp.

4.3 Urediniospore germination

Germination assays were used to establish optimal germination conditions of urediniospores. We found that *A. psidii* spores germinated optimally in the dark at 16°C with 80% relative humidity (Figure 2). Germination rate increased significantly between 8°C and 11°C (p=0.0022) and decreased significantly between 20°C and 21.5°C (p=0.0003), with only 10.2% spores germinating at 21.5°C. Therefore, spores were germinated at 16–18°C in all future assays.

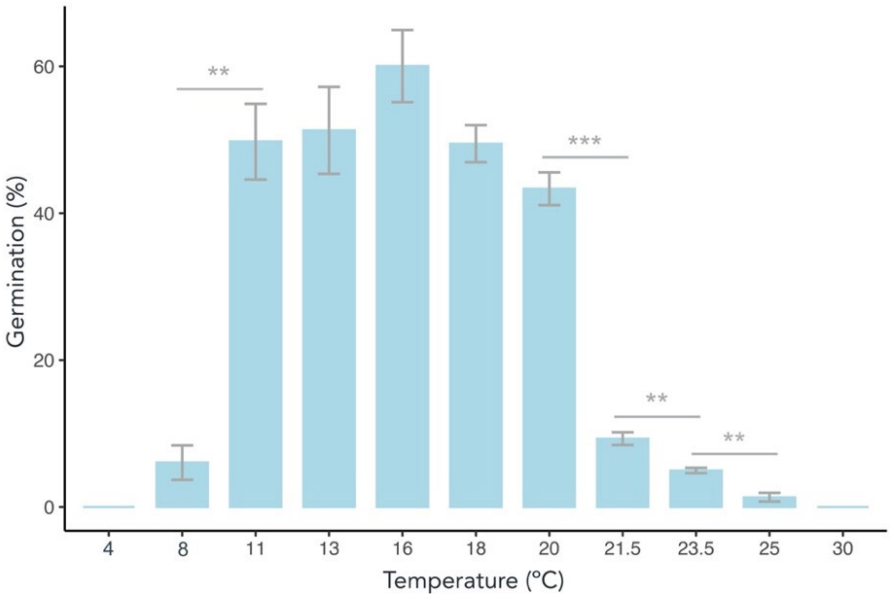

Figure 2. *Austropuccinia psidii* spores germinate optimally at 16°C. Bar plot representing mean (*n*=3) germination rate of *A. psidii* spores on water agar at 4°C, 8°C, 11°C, 13°C, 16°C, 18°C, 20°C, 21.5°C, 23.5°C, 25°C, and 30°C. Germination was assessed 24 h post-inoculation. All treatments were in the dark with 80% relative humidity. Significance between temperatures is represented by grey asterixis (*= <0.04, **= <0.004, ***= <0.0004 (student's t-test)). Bars represent standard error of the mean. 100 spores were assessed for germination at each temperature, the experiment was repeated three times with three different spore batches.

4.4 Detached leaf assays

A detached-leaf assay (Figure 3) was developed using *Syzygium jambos* leaves to determine whether the synthesised dsRNA molecules reduced disease symptoms. *Green Fluorescent Protein (GFP)* dsRNA was included as a non-specific control to make sure that any differences in pustule numbers were a result of *A. psidii*-specific dsRNA, and not a non-specific effect of the dsRNA itself.

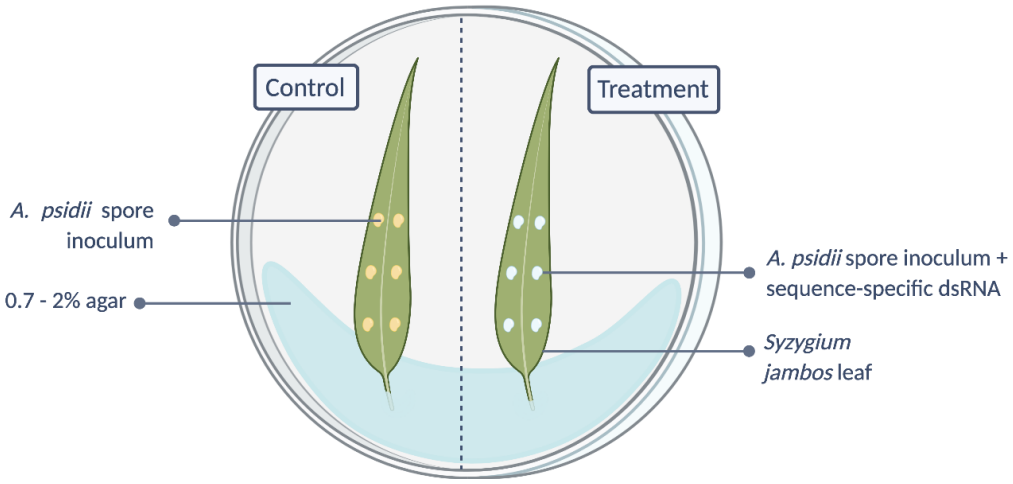

Figure 3. *In vitro* detached leaf topical RNAi assay with *Austropuccinia psidii* inoculum on *Syzygium jambos* leaves. *S. jambos* leaves were positioned with their petioles in 0.7–2% agar at the base of the Petri dish. 5 µL droplets of control and treatment solution were pipetted onto control and treatment leaves respectively. Control solutions contained 10 µL of $1x10^6$ uredinospores/mL in 0.05% Tween. Treatment solutions additionally contained 1 µg of *A. psidii*-specific dsRNA. The same number of droplets were pipetted onto control and treatment leaves. This illustration was created with the web application, Biorender.com.

A. psidii-specific dsRNA significantly reduced the number of *A. psidii* pustules per *S. jambos* leaf in 7 out of 11 treatments when added to the inoculum (Figure 4). Significant reductions in pustule number were observed in treatments targeting *transcription elongation factor (EF1-α/TEF), MAP-kinase (MAPK), 28S-1, β-tubulin (B-TUB), acetyl-CoA acyltransferase (ATC), the glycine cleavage system H protein (GCS-H*), *cytochrome P450* (*CYP450),* as well as haustorial gene *H01215*. Reductions in pustule number were not significant in treatments targeting haustorial genes *H01136* and *H12890*, housekeeping gene *28S-2,* or *GFP*. These results showed that topical application of *A. psidii*-specific dsRNA reduces the number of *A. psidii* pustules (a key myrtle rust symptom) on *S. jambos.*

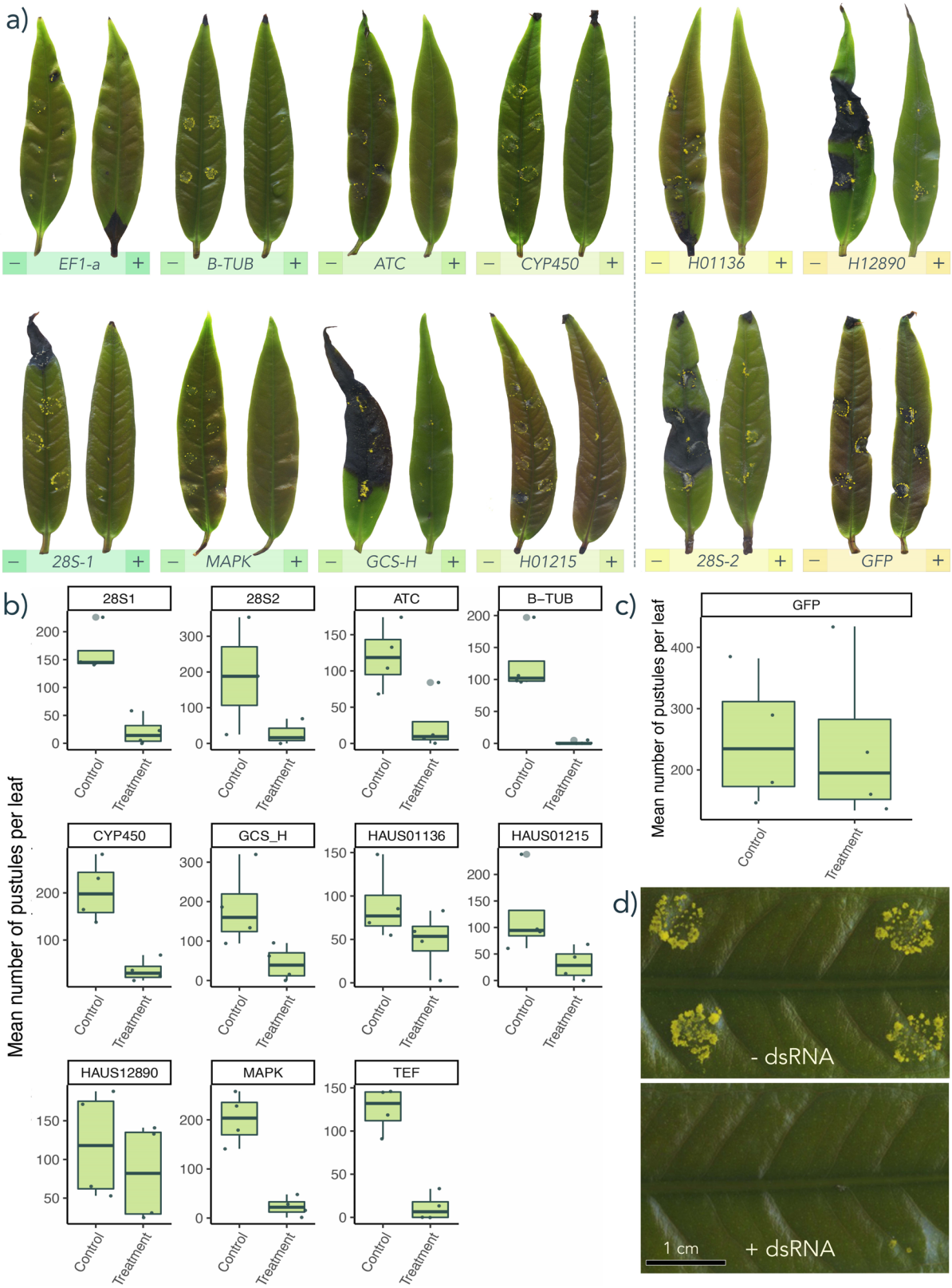

Figure 4 Exogenous application of *Austropuccinia psidii*-specific double-stranded RNA (dsRNA) decreases incidence of myrtle rust (MR) on *Syzygium jambos* in detached-leaf assays. *S. jambos* leaves (*n=4*) infected with *A. psidii* and treated with 11 *A. psidii*-specific dsRNAs were assessed two weeks post-inoculation. Treatments consisted of 1×10^6 *A. psidii* spores/mL in sterile distilled water containing 0.05% Tween 20 mixed with 100 ng/µL dsRNA. −dsRNA (control) was *A. psidii* spores in sterile distilled water and 0.05% Tween 20. *Green fluorescent*

protein (GFP) dsRNA was included as a non-specific control. **(a)** Boxplot with superimposed scatter representing mean number of *A. psidii* pustules per *S. jambos* leaf (n=3) treated with 11 *A. psidii*-specific dsRNAs. Significance as compared to the control (−dsRNA) is represented by grey asterixis (* = <0.05, ** = <0.01, *** = <0.001 (Student's t-test)). This figure was made in R 4.0.3 (R Core Team, 2020). **(b)** Photo comparisons of all dsRNA treatments and controls on *S. jambos* leaves. For each gene target a control (-) and treatment (+) leaf is shown. Each treatment leaf had a respective control leaf from the sample plant, and of the same age, size, and growth stage to ensure accurate comparison. Colours from green to orange represent most to least significant reduction in spore number from control to treatment, respectively. All treatments to the left of the dotted line (ribosomal RNA *28S*-1 *(28S*-1*), ß-tubulin (ß-TUB), translation elongation factor 1-alpha (EF1-A), mitogen activated protein kinase (MAPK), acetyl CoA-transferase (ATC), glycine cleavage system-H (GCS-H), cytochrome P450 (CYP450),* haustorial target *H01215*) resulted in a significant reduction in pustule number compared to control leaves. All treatments to the right of the dotted line (haustorial target *H01136, ribosomal RNA 28S*-2 *(28S*-2*),* haustorial target *H12890)* did not result in a significant reduction in pustule number from control to treatment leaves. **(c)** Boxplot with superimposed scatter representing *GFP* (non-specific control)) mean number of *A. psidii* pustules per *S. jambos* leaf (n=3) treated with 11 *A. psidii*-specific dsRNAs. Significance as compared to the control (−dsRNA) is represented by grey asterixis (* = <0.05, ** = <0.01, *** = <0.001 (Student's t-test)). This figure was made in R 4.0.3 (R Core Team, 2020). **(d)** Close-up photo comparison of control (*A. psidii* only) and treatment (*A. psidii* + *ß-TUB* dsRNA) on *S. jambos* leaves*,* taken two weeks post-infection.

4.5 In planta dsRNA assays

In planta assays with mature plants of *S. jambos* were used to confirm that RNAi-mediated control of myrtle rust was also effective and consistent on whole plants. Due to availability of *S. jambos* plants, *A. psidii* spores, and the large amount of dsRNA required, only two dsRNA treatments were assessed in whole plant assays. *EF1-α* and *B-TUB* were chosen based on their efficacy in detached leaf assays, and the effects of these treatments on *A. psidii* urediniospores in artificial leaf assays.

When mixed with the *A. psidii* inocula, both *EF1-α* and *B-TUB* dsRNA treatments resulted in a significant decrease in diseased tissue compared to the −dsRNA control (25.4% disease coverage, Figure 5). Two weeks after inoculation, plants treated with *B-TUB* dsRNA showed no myrtle rust symptoms (Figure 5). The decrease in mean lesion size on *EF1α*-treated plants was not significant compared to the −dsRNA control (Figure 5).

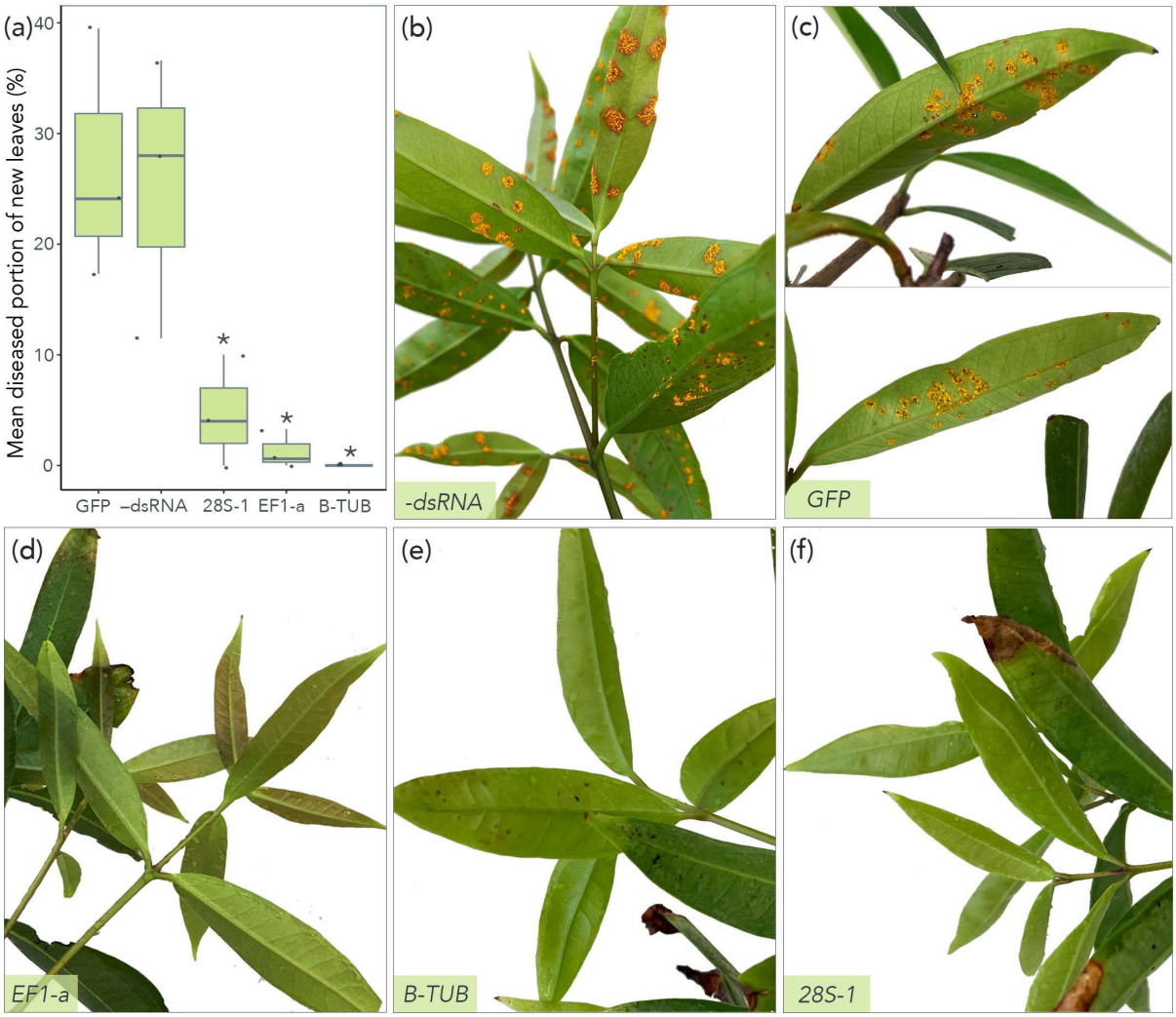

Figure 5 Exogenous application of *Austropuccinia psidii*-specific double-stranded RNA (dsRNA) decreases incidence of myrtle rust on *Syzygium jambos* in whole plant assays. *S. jambos* plants were infected with *A. psidii* and spray treated with three *A. psidii*-specific dsRNAs. Treatments contained 1×10^6 *A. psidii* urediniospores/mL mixed with 1 µg/10 µL of either *ß-tubulin (ß-TUB)*, *translation elongation factor 1-alpha (EF1-A),* or ribosomal *28S* RNA *(28S-1) A. psidii*-specific dsRNA. Control (–dsRNA) is *A. psidii* urediniospores in 0.05% Tween 20. *Green fluorescent protein* (*GFP*) dsRNA was included as a non-specific control. **(a)** Bar plot of diseased tissue as mean (*n*=3 biological replicates) percent (%) coverage of leaf. Each biological replicate represents a separate plant and includes all young (susceptible) growth in the disease assessment (usually four to eight leaves). Bars represent standard error of the mean. Significance (as compared to the −dsRNA control group) is represented by asterisks (* = <0.05, ** = <0.01, *** = <0.001 (Student's t-test)). Disease area was measured using the Leaf Doctor application (Pethybridge and Nelson, 2015). This figure was made in R 4.0.3 (R Core Team, 2020). **(b, c, d, e, f)** Photo comparison of *S. jambos* plants inoculated with *A. psidii* (**(b)** –dsRNA control) and treated with **(c)** *GFP*, **(d)** *EF1-A,* **(e)** *ß-TUB,* or **(f)** *28S* dsRNA. Plants were photographed two weeks post-inoculation.

When challenged with *A. psidii* infection six days post-dsRNA application, *EF1a*-treated plants exhibited 19.7% disease coverage, as compared to just 1.3% in assays where dsRNA and *A. psidii* inoculum were applied simultaneously (Figure 6). This was no longer a significant reduction in diseased tissue compared to the control. However, *B-TUB*-treated plants maintained a reasonably low disease coverage (6.3%), still a significant reduction from −dsRNA

control plants. Additionally, a significant reduction in mean lesion size was observed in both *EF1-1* and *B-TUB* treatment groups.

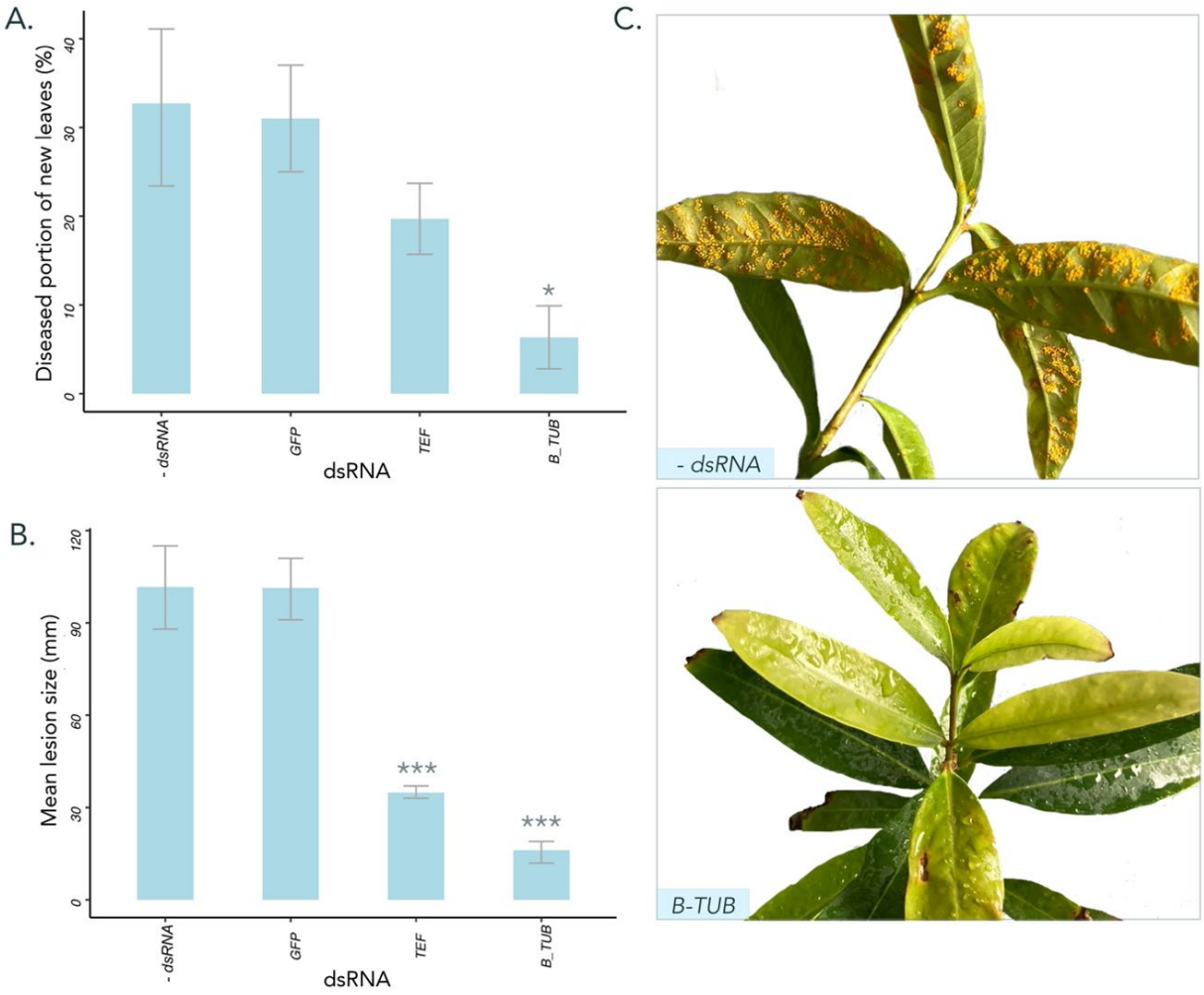

Figure 6. Exogenous application of *Austropuccinia psidii*-specific dsRNA provides significant protection against myrtle rust for up to 6 days post-application. *Syzygium jambos* leaves (*n=3*) were sprayed with dsRNA and challenged with *A. psidii* 6 days later. *GFP* dsRNA was included as a non-specific control. **(a)** Bar plot of diseased tissue as mean (*n*=3) percent (%) coverage of leaf. Each biological replicate includes all young (susceptible) growth in the disease assessment (usually four to eight leaves). Measurements were taken automatically using the Leaf Doctor application (Pethybridge & Nelson, 2015). For each biological replicate, all new (susceptible) growth was included in the disease assessment (usually four to eight leaves). Significance is represented by grey asterixis. *= <0.04, **= <0.004, ***= <0.0004 (Student's t-test). Bars represent standard error of the mean. **(b)** Barplot of mean (*n=3*) *A. psidii* lesion size with 10 lesions measured per biological replicate (or as many lesions as present if less than 10). Lesions were randomly selected on youngest leaves of each plant. Both treatments resulted in a significant reduction in mean lesion size. Bars represent standard error of the mean. Each biological replicate is also an independent experiment carried out on separate dates with different *S. jambos* plants, *A. psidii* spore batches, and different dsRNA preparations. Significance is represented by grey asterixis. *= <0.04, **= <0.004, ***= <0.0004 (Student's t-test). Bars represent standard error of the mean. **(c)** Comparison of *S. jambos* +/- *B-TUB* dsRNA. −dsRNA control plants showed severe myrtle rust symptoms while dsRNA-treated plants showed significantly reduced symptoms. Photos were taken two weeks post-inoculation.

These results show that topical RNAi-mediated protection against *A. psidii* is effective *in planta*, and that results on a whole-plant scale are consistent with those observed in detached leaf assays. However, in protective assays where plants were challenged with *A. psidii* six days post-dsRNA application, topical RNAi was not as effective in reducing myrtle rust symptoms as in assays where dsRNA and *A. psidii* inocula were applied simultaneously.

4.6 Artificial leaf assays

In vitro artificial leaf assays were set up to study spore germination and the early stages of infection using polyvinylpyrrolidone (PVP)-treated polydimethylsiloxane (PDMS) leaf mimicking substrate kindly provided by Dr Grant Smith (Plant and Food Research, New Zealand). Due to limited availability of resources, we chose to study the most effective detached leaf dsRNA treatments *(TEF, 28S-1, B-TUB,* and *MAPK)* in this assay. The 24 h germination rate of *A. psidii* spores on artificial leaves decreased significantly compared to the −dsRNA control (78% germination), when *MAPK, TEF, 28S-1* and *B-TUB* dsRNA was added (Figure 7A). Germination rate was high in *GFP* and −dsRNA control groups (75 and 78%, respectively) and germ tubes commonly formed appressoria and infection pegs, with visible cytoplasmic streaming (Figures 7A and 7C). Reasonable germination rates were observed in *A. psidii* spores treated with *B-TUB* (43%) and *TEF* (23%) dsRNA, but germ tubes were withered, and no cytoplasmic streaming, appressoria, or infection pegs were present (Figures 7A and 7C). Germination rate was low in spores treated with *28S-1* (9%) and *MAPK* (7%) dsRNA and any germ tubes present were short, straight, and terminating without the presence of appressoria (Figures 7A and 7C). These results indicated that dsRNA can be taken up within 24 hours by *A. psidii* urediniospores to initiate an RNAi response.

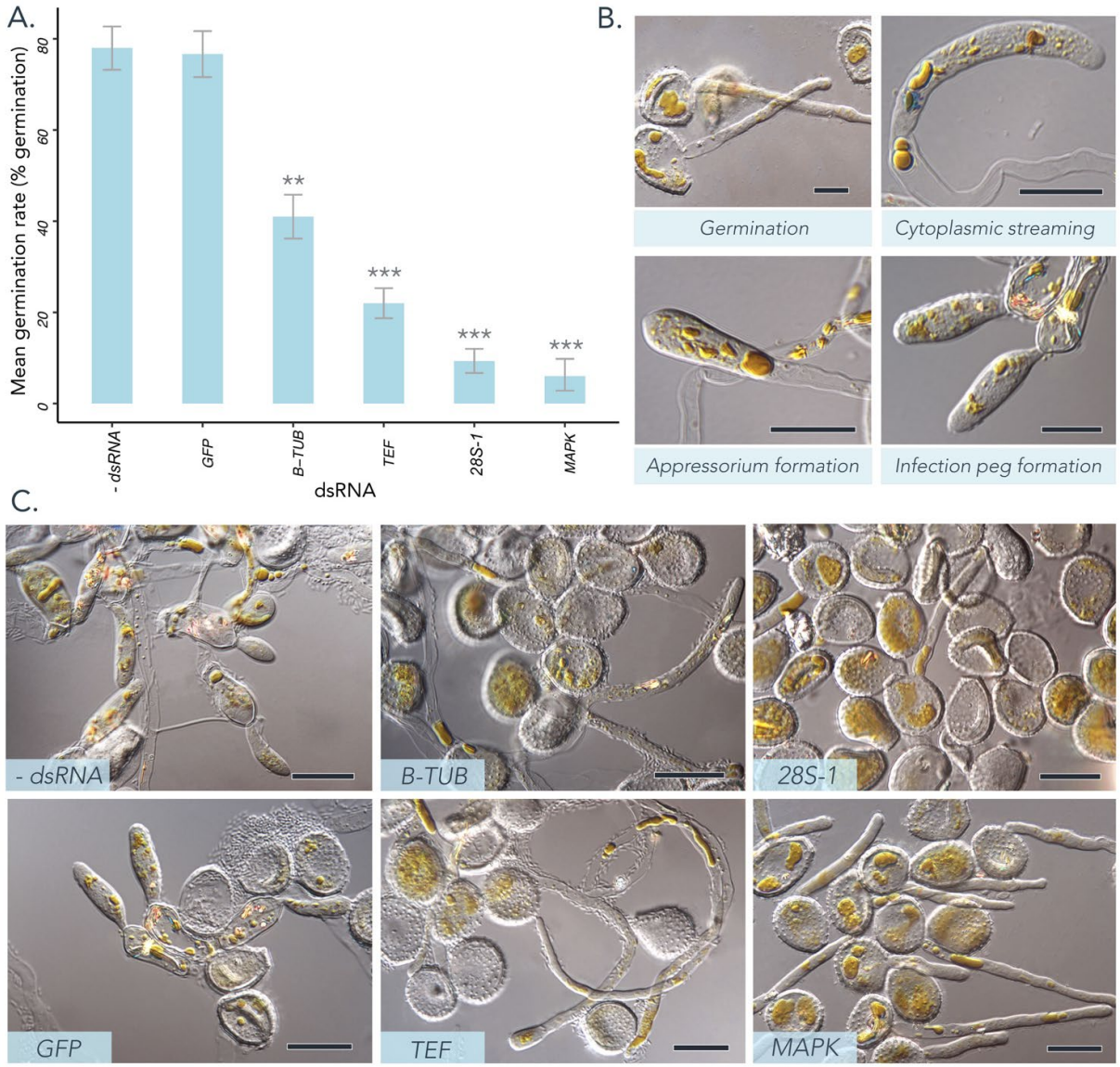

Figure 7. Austropuccinia psidii dsRNA targeting essential genes significantly alters growth of A. psidii urediniospores on polyvinylpyrrolidone (PVP)-treated polydimethylsiloxane leaf mimicking substrate. **(a)** Bar plot representing mean germination rate (n=3) (as % germination) of *A. psidii* spores +/- dsRNA on PVP-treated leaf mimicking substrate. Significance (as compared to the −dsRNA control group) is represented by grey asterixis (*= <0.04, **= <0.004, ***= <0.0004 (Student's t-test)). Bars represent standard error. 100 spores were counted per replicate, and counts were repeated three times on separate substrate/ spore regions. **(b)** *A. psidii* spores on PVP-treated substrate at various stages of germination, ranging from germinated to advanced infection peg development at 20× magnification. Scale bars represent 10 µm. **(c)** Comparison of *A. psidii* germ-tube morphology between control and treatment groups on PVP-treated substrate at 20× magnification. Scale bars represent 20 µm. In control and treatment groups, germination rate was high and most germinated spores had progressed to appressoria, and infection peg development. In *B-TUB* and *TEF* treatments, some spores had germinated but germ tubes were withered, cytoplasmic streaming rare, and early-stage appressoria observed only in a very small number of cases. In *28S* and *MAPK* treatments germination rate was very low. In spores that had germinated, germ-tubes were stunted, and there was no cytoplasmic streaming or appressoria development. Photos were taken 24 h post-inoculation.

4.7 dsRNA protection assays in *D. humile* and *S. hodgkinsoniae*

Double-stranded RNA protection assays were set up on *Syzygium hodgkinsoniae* and *Decaspermum humile* to determine whether dsRNA was also effective on native plants. *S. hodgkinsoniae* leaves developed pustules (Figure 8) similar to those seen on *S. jambos* and could be scored easily, however, in *D. humile* pustules developed predominantly on young stems (Figure 9), which made disease scoring difficult. There was a high level of variability even within the control plants making it difficult to draw any conclusions from the assays.

Figure 8 Myrtle rust symptoms on *Syzygium hodgkinsoniae.* Photos were taken 2 weeks post-inoculation.

Figure 9 Myrtle rust symptoms on *Decaspermum humile.* Photos were taken 2 weeks post-inoculation.

5. Achievements, Impacts and Outcomes

This research illustrates the exciting potential of exogenous dsRNA as a novel control of myrtle rust. Our results show that dsRNA can reduce myrtle rust disease incidence on detached *S. jambos* leaves and whole plants up to six days post dsRNA application, with artificial leaf assays showing that dsRNA can inhibit appressoria and germ tube development within 24 hours. Myrtle rust infection assays were established for *Syzygium hodgkinsoniae* and *Decaspermum humile* and protection assays on these host plants is ongoing as part of *PBSF042.*

Project achievements/impacts/outcomes include:

- Completion of Honours project 'RNA interference-mediated control of the plant-pathogenic rust fungus, *Austropuccinia psidii*' by Rebecca Degnan who received First Class Honours

- *Molecular Plant Pathology* publication which was featured on the cover of the journal:

  Degnan R M, McTaggart A R, Shuey L S, Pame L J S, Smith G R, Gardiner D M, Nock V, Soffe R, Sale S, Garrill A, Carroll B J, Mitter N and Sawyer A (2023) Exogenous double-stranded RNA inhibits the infection physiology of rust fungi to reduce symptoms in planta. *Molecular Plant Pathology*, 24(3):191-207.

- Back from the Brink Film

  Season 3 Episode 4 – Angle-stemmed Myrtle, Dec 16 2020
  https://youtu.be/G1EHXW-YkdA

- Back from the Brink Podcast

  Episode 9 – The race against the rust, Dec 21 2020
  https://backfromthebrink.libsyn.com/e09-the-race-against-the-rust

- Presentation of the work at the National Myrtle Rust Symposium, Ballina 23-25 March 2021

- Presentation of the work in the Beyond Myrtle Rust Webinar Series, 12 May 2021

  https://www.youtube.com/watch?v=QWAmhzif0TU

  https://www.landcareresearch.co.nz/discover-our-research/biosecurity/ecosystem-resilience/beyond-myrtle-rust/webinar-series/

- New collaboration with Australian Native Products, who are interested in the potential of RNA vaccines to protect their lemon myrtle trees from myrtle rust
- New collaboration with The Australian Tea Tree Industry Association who are interested in the potential of RNA vaccines to protect the tea tree industry from myrtle rust if another more aggressive strain arrives in Australia, or if there are changes in the current pathogen population.
- New collaboration with Scion, New Zealand, who are interested in the potential of RNA vaccines to treat myrtle rust and *Phytophthora* pine diseases
- Discussions with the New Zealand Department of Conservation regarding changes to New Zealand's RNAi legislation
- Presentation of the work at the APEC High Level Policy Dialogue on Agricultural Biotechnology 2021 Plenary Meeting, Online, August 2021
- Presentation of the work at the Australasian Plant Pathology Society Queensland Seminar Series, Online, February 2022
- Presentation of the work at the Plant Biosecurity Research Symposium, Adelaide 2022
- Presentation of the work at the ComBio Conference, Melbourne 2022
- Presentation of the work at the TropAg Conference, Brisbane, October 2022
- Presentation of the work at the European Molecular Biology Laboratory (EMBL) PhD Symposium, Heidelberg, Germany, December 2022
- Presentation of the work at the 4th International Congress on Biological Invasions, Christchurch, New Zealand, May 2023
- Presentation of the work at the Australasian Myrtle Rust Conference, Sydney June 2023

6. Future work

Future work will include repeating the *Syzygium hodgkinsoniae* and *Decaspermum humile* dsRNA protection assays with larger numbers of plants, testing dsRNA uptake and movement in plants, and testing the longevity and systemic nature of protection. This is already in progress as part of *PBSF042.*

7. References

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