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Tiakitanga Pūtaiao Aotearoa

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Topic 1.1 — Identification of native and important exotic host species susceptibility to Myrtle Rust, including variability within species

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Contents

1 Executive summary 2

2 Recommendations 3

3 Introduction 3

4 Materials and methods 4

5 Results 6

5.1 Identification of resistance in Mānuka 6

5.2 Mānuka Families 7

5.3 Mānuka Plants 7

5.4 Pōhutukawa, Ramarama and Rohutu 9

5.5 Provenance of Mānuka families 9

6 Discussion 9

7 Acknowledgments 11

8 References 11

1 Executive summary

There is resistance to the pandemic biotype of *Austropuccinia psidii,* the causal pathogen of Myrtle Rust in New Zealand mānuka (*Leptospermum scoparium*). This resistance is evidenced by three phenotypes observed on plants, grown from New Zealand sourced seed, which had been artificially inoculated with fungal spores and maintained in conditions favourable for fungal spore germination, infection and development of disease symptoms. The first two symptoms are not visible on the leaf, or on the main or lateral stems of the plants. The third symptom observed were small yellow flecks or necrotic spots on the leaves consistent with a hyper-sensitive defence response. New Zealand mānuka is the first species tested in this disease assessment protocol to have shown significant stem infection. Whilst, the genetic basis of resistance and susceptibility is unknown, the analysis suggests that the three forms of resistance have limited genetic linkage, as leaf resistant-stem resistant, leaf susceptible-stem resistant, leaf-resistant-stem susceptible and leaf susceptible-stem susceptible phenotypes were all observed.

The first two forms of resistance (no symptoms of infection) are present in most of the seed families sourced from different geographic regions of the North Island: 90% of families contained plants that showed the leaf resistance and 98% of the families contained plants that showed the stem resistance mechanism. The L2 putative hypersensitive resistance is only present in 29% of the families tested to date. However, the combination of either of the leaf resistances with stem resistance was limited: only 18% of the plants assessed to date showed this double resistance phenotype, although this trait was widespread and was present in 80% of the families. Thus, while these resistance mechanisms are present in many different seed families, 82% of the plants grown from seed from those families had limited (i.e. leaf susceptible-stem resistant, leaf resistant-stem susceptible) or no (i.e. leaf susceptible-stem susceptible) resistance to this invasive pathogen.

In contrast, there was essentially no resistance (0.17% of plants tested) to *A. psidii* in plants grown from seed of 31 pōhutukawa families, and no resistant plants were found in the three ramarama (*Lophomyrtus bullata*) families and one rohutu (*Lophomyrtus obcordata*) family assessed to date.

These results are from artificial inoculation assessment trials and their applicability to the New Zealand natural estates are not yet known. Factors such as inoculum load, humidity, temperature and species density will be important to the rate at which this pathogen both spreads and causes ecological damage. These initial results are important as they reveal that New Zealand mānuka is susceptible to stem infection, that two of the three resistance mechanisms appear to be widely distributed in the plant gene pool but appear to have limited genetic linkage, that the number of individual plants with both stem and leaf resistance is limited, and there is a provenance effect as plants grown from seed collected from mother plants growing in the east of the North Island provided almost 50% of the leaf-stem infection-resistant plants despite only contributing 33% of the plants to this initial study.

2 Recommendations

1. Continue to assess the resistance of New Zealand sourced Myrtaceous seed (ongoing research supported by the MBIE Catalyst C11X1607 Myrtle rust: a significant threat to Australasia and the Pacific programme).
2. Understand the genetic and molecular and biochemical basis of the leaf and stem resistances in mānuka, to explore potential novel control options (this researchwas originally carried out in the MBIE Endeavour programme Beyond Myrtle Rust; however, the Science Challenge is now significantly larger with three (putatively independent) forms of resistance to investigate).
3. Investigate the genetic linkage between the resistance mechanisms in mānuka and other plant traits such as flowering time and duration, and methylglyoxal production.
4. Urgently, preserve the genetic diversity of New Zealand Myrtaceae via appropriate germplasm storage systems

3 Introduction

Response or management options to address the ecological consequences of the incursion of *Austropuccinia psidii*, the causal pathogen of myrtle rust in New Zealand at local and landscape levels requires an understanding of the level of susceptibility of the Myrtaceae species present in New Zealand to this pathogen. Worldwide, the Myrtaceae family is comprised of 5950 species in 132 genera (Christenhusz and Byng 2016). Buys et al. (2014) noted that New Zealand currently has over 100 Myrtaceae species in 43 genera. After the 2014 revision of *Kunzea* and noting that the *Leptospermum scoparium* aggregrate needs critical re-examination (de Lange and Rolfe 2010, de Lange 2014) New Zealand has 27 native species in six genera (*Kunzea, Leptospermum, Lophomyrtus, Metrosideros, Neomyrtus* and *Syzygium*).

In April (Raoul Island) and May (Kerikeri) of 2017, *A. psidii* was found infecting mature and seedling pōhutukawa (*Metrosideros kermadacensis* and *Metrosideros sp.)* plants. There are nine strains of *A. psidii* currently recognised and some have been grouped into biotypes (Stewart et al. 2018). The incursion into New Zealand was by the pandemic biotype of the pathogen: this biotype has an extensive (~450 species) host range and has been reported from Hawaii, Australia, Singapore, Indonesia and New Caledonia (McTaggart et al. 2019). There are published reports of the reaction of native New Zealand plant species to the pandemic biotype of *A. psidii* from other countries (i.e. *Metrosideros excelsa* in Hawaii (https://dlnr.hawaii.gov/hisc/files/2013/05/FY14-DOFAW-HISC-Rust-Surveillance-FINAL-REPORT.pdf (accessed 4th June 2019)) and *Leptospermum scoparium* in Australia (Sandhu and Park 2013)). However, the source of seed or plants was often not stated and thus the provenance of these plants was unclear, reducing the value of these observations in understanding the potential effect of myrtle rust disease in New Zealand.

A. psidii has been present in Australia since 2010. A significant component of the science response to this pathogen has been assessing the disease response of artificially inoculated plants grown from seed (Pegg et al. 2014, 2018; Sandhu and Park 2013) using a modification of the Junghans scale originally developed in Brazil (Junghans et al. 2003). Two key outcomes from this research were the identification of resistance in native Australian species and the identification that provenance (the locale where seeds were collected from) had a significant effect on the number of individual plants that were resistant in a species. Seed collected from plants growing in drier regions generally resulted in more resistant individuals in a population (Lee et al. 2014, Pegg et al. 2014, Freeman et al. 2018).

A research programme supported by an **MBIE Catalyst grant C11X1607 Myrtle rust: a significant threat to Australasia and the Pacific** commenced in June 2017 to assess the resistance/susceptibility of plants grown from seed of New Zealand native Myrtaceae species collected from provenances throughout the country at the Queensland Department of Agriculture and Fisheries (QDAF) facilities in Brisbane, Australia. In December 2017, additional investment via **MPI RFP 18608 Topic 1.1 Identification of native and important exotic host species susceptibility to myrtle rust, including variability within species** allowed for the expansion of this effort and the potential to include economically important non-native species (e.g. Eucalyptus and *Acca sellowiana* (Feijoa)) in this assessment programme. Unfortunately, because of the incursion of *A. psidii* into New Zealand, the Australian Federal Government Department of Agriculture and Water Resources (DAWR) suspended imports of Myrtaceae seed into Australia whilst the import conditions were reviewed, delaying this research by 8 months. As a result of this delay, only limited results from four New Zealand Myrtaceae species are available for this report.

Seed was collected from each individual plant and was not mixed or bulked with seed from other plants. Thus, each 'mother plant' provided a seed family: a collection of seeds that originated from one tree and, therefore, had a shared genetic base from that tree. The source of the pollen for each seed was unknown (potentially selfed from the mother plant, or open pollinated from one or more sources), thus the origin of the remaining genetics of each seed is unknown.

4 Materials and methods

Seeds from New Zealand native Myrtaceous species were collected from locations around the country and stored at Scion, Rotorua, or The New Zealand Institute for Plant and Food Research Limited, Palmerston North. These seeds were collected under a range of agreements with local mana whenua and landowners in particular by staff from the **MBIE Endeavour grant C09X1608 Building resilience and provenance into an authentic Māori honey industry** or under concession.

The seed was collected from each individual plant and was not mixed with seed from other plants. Thus each 'mother plant' provided a seed family: a collection of seeds that originated from one tree and therefore had a shared genetic base from that tree. The source of the pollen for each seed was unknown (potentially selfed from the mother plant, or open pollinated from one or more sources), thus origin of the remaining genetics of each seed is unknown.

A permit to import conditionally non-prohibited goods, 0002144655, was issued on 27 March 2018. This permit applied to *Kunzea* spp*., Leptospermum* spp*., Lophomyrtus* spp*., Metrosideros bartlettii, M. carminea, M. colensoi, M. diffusa, M. excelsa, M. fulgens, M. kermadecensis, M. perforata, M. robusta, M. umbellata* and *Syzygium spp. Neomyrtus* spp. and any non-native species were not covered by this permit. As per the permit conditions, seeds were treated with a fungicide with Mancozeb as the active constituent.

Table 1. Details of exported seed to Australia under the conditions prescribed under Australian Government Permit 0002144655, a permit to import conditionally non-prohibited goods issued under the Biosecurity Act 2015 Section 179 (1).

Date	Species	No. of Families
May-18	*Leptospermum scoparium* (mānuka)	100
Aug-18	*Leptospermum scoparium* (mānuka)	30
	Leptospermum scoparium incanum (mānuka kahikātoa)	1
	Lophomyrtus bullata (ramarama)	3
	Lophomyrtus obcodata (rohutu)	1
	Kunzea linearis (kanuka)	5
	Kunzea robusta (kanuka)	25
	Metrosiderios excelsa (pōhutukawa)	32
	Syzgium maire (swamp maire)	1
Nov-18	*Leptospermum scoparium* (mānuka)	300
Dec-18	*Lophomyrtus obcodata* (rohutu)	15
	Metrosideros diffusa (white rata)	35
	Leptospermum scoparium (mānuka)	586
Jan-19	*Metrosideros umbellate*	16
	Leptospermum scoparium (mānuka)	4
	Metrosideros fulgens	6
Feb-19	*Leptospermum scoparium* (mānuka)	29
Mar-19	*Syzgium maire*	1
Total		**1190**

The seeds were germinated in a secure Queensland Government glasshouse and when sufficiently mature a trial was established using a randomised incomplete block design with at least a single plant from each family treatment in each replicate. As a fungal infection positive control, seedlings from three provenances of *Melaleuca quinquenervia* (broad-leaved paperbark tree) were included. These *M. quinquenervia* seed lots have previously been tested (Pegg et al. 2018) and have a range of susceptibility levels within the populations. Plants were shipped to QDAF Brisbane where the seedlings were inoculated using a fine mist spray of a suspension of 1 x 10^5 fungal spores/ml applied to ensure leaf and stem surfaces of the seedlings were coated but runoff of the spore suspension was avoided.

Plants were monitored for progression of disease symptoms and were visually assessed 20 days post-inoculation using two scales described in Table 1 and Table 2. The leaf scale was modified from that of Junghans et al. (2003) and was applied to foliage symptoms only.

Table 2. Leaf disease visual assessment scale

Scale	Leaf visual symptoms
L1	No symptoms or the presence of flecking (yellow/clear) evident
L2	Presence of a hypersensitive reaction (HR) with fleck or necrosis
L3	Small pustules, <0.8 mm diameter, with one or two uredinia
L4	Medium pustules, 0.8–1.6 mm diameter with about 12 uredinia
L5	Large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots

Whilst monitoring disease symptom development, variability in stem infection levels (number of lesions and size of lesions) between species became obvious. This variability could not be captured using the modified Junghans method, so an additional stem assessment scale was developed.

Table 3. Stem disease visual assessment scale

Scale	Stem visual symptoms
S1	No evidence of infection on stems (includes main and lateral)
S2	Pustule present on stems but no lesion
S3	1–2 stem lesions; average length <5 mm
S4	1–2 stem lesions; average length ≥5 mm
S5	>2 but <5 stem lesions; average length <5 mm
S6	>2 but <5 stem lesions; average length ≥ 5mm
S7	≥5 stem lesions; average length <5mm
S8	≥5 stem lesions; average length ≥5mm

Figure 1. *Austropuccinia psidii* pustules on the main and lateral stems of a New Zealand mānuka plant (left and centre) and stem distortion as a result of fungal infection (centre and right). Photos: Grant Smith.

Each individual plant in the trial was scored for both leaf and stem infection using the above scales. Plants with L1, L2 or S1 ratings are considered to be resistant: plants with any rating in the range of L3-L5 or S2-S8 are considered susceptible in this report.

5 Results

5.1 Identification of resistance in mānuka

Plants from 97 mānuka seed families have been assessed and analysed to date. Only data from families that had 10 or more plants in the trial were analysed; thus 17 families were excluded from the analysis. Results from the remaining 1252 plants in 80 families were analysed. Three types of resistance phenotype were identified in the mānuka seed families:

L1 No symptoms or the presence of flecking (yellow/clear) evident
L2 Presence of a hypersensitive reaction (HR) with fleck or necrosis
S1 No evidence of infection on stems (includes main and lateral).

L1 and S1 with no symptoms or evidence of infection may be constitutive (pre-formed) resistances, whilst L2 may be a reactive hypersensitive response that results in death of the infected cell resulting in a coloured fleck or small necrotic region on the leaf. These initial interpretations require more investigation to understand the molecular and biochemical basis of the resistance phenotypes.

5.2 Mānuka families

Most of the 80 families contained resistant plants: 72 families contained one or more L1 resistant plants, 23 families contained one or more L2 resistant plants, whilst 78 families contained one or more S1 resistant plants. Many families contained plants with both L1 and S1 resistances: the 194 individual L1-S1 resistant plants were distributed across 63 of the 80 families, with the number of L1-S1 plants per family varying from one to 12. The 36 L2 plants were from 23 families, ranging from one to four plants per family; all of these 23 families also produced plants with an S1 phenotype. However, only 30 L2-S1 plants were present in 19 of these 23 families.

5.3 Mānuka plants

Whilst leaf and or stem resistance was widespread at the family level, the number of individual plants showing either or both resistances was limited. Overall, 31% of the individual plants had L1 or L2 resistance, while 31% of the individual plants showed S1 resistance. However, only 18% of the resistant plants showed L1-S1 or L2-S1 resistance. In contrast, 13% of plants with L1 or L2 resistant leaf phenotype were susceptible to stem infection (S2-S8), and the same percentage (13%) of plants with S1 stem resistance were susceptible to leaf infection (L3-L5) (Table 4). Most of the plants in this trial (56%) were susceptible to both leaf and stem infection. Infection of flowers and stem dieback as a result of fungal infection were also noted in this trial (Figure 2).

Figure 2. Fungal pustules on a mānuka flower (left) and stem dieback (centre and right) of a New Zealand mānuka plant after infection by *Austropuccinia psidii*. Photos: Geoff Pegg.

Table 4. Summary of 1252 mānuka plants with resistance and susceptible phenotypes to leaf and stem infection by the pandemic biotype of *Austropuccinia psidii.*

Phenotype	Organ/ Phenotype	Plants
Resistant	Leaf and Stem ((L1 or L2) and S1)	224 (18%)
Resistant and susceptible	Leaf (L1 or L2) and Stem (S2-S8)	167 (13%)
	Stem (S1) and Leaf (L3-L5)	167 (13%)
Susceptible	Leaf (L3-5) and Stem (S2-8)	694 (56%)

5.4 Pōhutukawa, ramarama and rohutu

Only one pōhutukawa plant was resistant to *A. psidii* of the 570 plants tested from 31 families, and no resistant plants were found in the three ramarama (*Lophomyrtus bullata*) families and one rohutu (*Lophomyrtus obcordata*) family assessed to date.

5.5 Provenance of mānuka families

The origin of the seed influenced the number of resistant ((L1 or L2) and S1) plants. Seeds were collected from four geographic regions: North (land), West (Waikato), East (Gisborne and inland) and East Cape in the North Island. Noting that the number of families and plants from the West is low and more material should be tested, the East region, which provided a third of the plants tested, contributed 47% of L1 or L2 and S1 resistant plants found in this trial (Table 5).

Table 5. Geographic origin of the 80 mānuka families and 1252 plants and contribution of resistance phenotypes by percent.

Region	Families	Plants	L1 & L2	S1	L1 & L2 and S1
North	41	44	31	47	35
West	6	6	3	7	5
East	33	32	50	34	47
East Cape	20	18	16	12	13

6 Discussion

There is resistance to the pandemic biotype of *A. psidii* in New Zealand mānuka. This resistance is evidenced by three resistant phenotypes: two leaf (L1 and L2) and one stem (S1) resistance. For effective plant-level field resistance either L1 or L2 combined with S1 would be required; only 18% of the plants (irrespective of family) showed either a L1-S1 or L2-S1 phenotype. This is the first species for which significant stem infection has been noted in the artificially inoculated disease assessment trial. Whilst stem infection may be an artefact of the inoculation method and trial conditions optimised from fungal spore germination and subsequent infection, mānuka is the first species tested that has shown substantial stem infection that required the development of a new stem-specific disease assessment scale. Significant stem infection can result in stem tip dieback and stem distortion (Figure 2) that may result in significant architecture changes to plants that survive repeated infection. The consequences of stem tip death may result in the initiation of new lateral stems with this new growth (flush) presenting ideal tissue for ongoing infection and inoculum production by the pathogen.

At the family level, there is significant distribution of resistance to this pathogen biotype with one or more resistant (L1-S1 or L2-S1) plants in 73 of the 80 families. However, at the plant level only 18% of the individual plants showed both resistance combinations, while 26% of the plants showed either leaf resistance-stem susceptibility or leaf susceptibility-stem resistance to the pathogen. Further, over half (56%) of the mānuka plants tested were both susceptible to leaf and stem infection. All families, irrespective of the number of L1, L2 or S1 plants in the family, contained susceptible plants. All these results suggest that the genetic linkage between leaf and stem resistance in mānuka is limited.

The definitions of resistance and susceptibility used for this initial analysis may have resulted in some Mānuka plants that may be tolerant to infection being classified as susceptible. The fungal spores for artificial inoculation are produced from infected *Syzygium jambos* (rose-apple). In Australia, this species appears to be relatively tolerant to repeated infection: the species becomes heavily infected but generally does not die, like many other species. However, tree death is not the only measure of effect, with changes in phenotypic characteristics (e.g. loss of apical dominance as a result of tip death resulting in changes to plant architecture) and reduced fecundity as a result of flower and fruiting body infection may also affect species prevalence in the natural estate. There is currently a limited understanding of the correlation of glasshouse results (under conditions controlled to optimise infection) with field infection rates, and disease effect under field conditions in the short and long term. Thus, potentially, plants rated L3 or S3-S5 may be tolerant to infection in the field, particularly should inoculum pressures be lower. The consequences of the different severity levels and type of stem infection (main stem v. branches) are also unknown in relation to dieback and flower bud formation and survival. Likewise, the consequences on growth, plant architecture, flowering, fecundity and viable seed production of repeated infection of tolerant plants is unknown. A few plants in this trial produced flowers: like the flowers of other species that have been tested these flowers where susceptible (Figure 2) and flower/flower bud death was observed. Under field conditions, a number of these effects are unlikely to become obvious in the short term and may also be very different on mature plants in comparison to seedlings. As reported in Australia, the effect on *Leptospermum* species is greatest following a disturbance event with epicormic regrowth and seedlings showing higher levels of infection and dieback than trees in undisturbed sites (Pegg et al. 2018). The direct and indirect effect on flowering of *Leptospermum* species in Australia has not been studied in any detail.

The high level of total (56%) and partial (26%) susceptibility to *A. psidii* found in plants grown from seed collected in the North Island presents a significant challenge to long-term management and response as the consequences of the fungal incursion become fully evident to New Zealand's native and introduced Myrtaceous flora. The very low level of findings of infected mānuka plants (3 of 19,808 at September 2018) during surveillance surveys (https://www.mpi.govt.nz/dmsdocument/31599-myrtle-rust-newsletter-september-2018) had been interpreted to suggest this species is largely resistant to infection. These results clearly demonstrate that there is significant susceptibility in this important ecological and economic species. The finding of only one resistant pōhutukawa plant in 570 plants from 31 families is of further concern as it suggests that susceptibility in this iconic taonga species to *A. psidii* infection is very common and dominant.

7 Acknowledgements

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