Title: Droughting results in lower than genomically predicted susceptibility to myrtle rust in *Melaleuca quinquenervia*

Author: Karina Guo[1,2], Glenda M. Wardle[2], Karanjeet Sandhu[2], Geoff Pegg[3], Jason G. Bragg[1]

1 Research Centre for Ecosystem Resilience, Botanic Gardens of Sydney, New South Wales, Australia

2 School of Life and Environmental Sciences, The University of Sydney, New South Wales, Australia

3 Department of Primary Industries, Queensland, Australia

Contact: jason.bragg@botanicgardens.nsw.gov.au, karina.guo@botanicgardens.nsw.gov.au

Abstract:

- Ecological restoration often involves founding or supplementing plant populations. To support long-term resilience of these populations, we aim to promote their capacity to respond to known and unknown challenges of climate and disease. In this study, we set out to inform restoration by characterising the interacting effects of climate and disease stress on a foundation tree species.
- *Melaleuca quinquenervia* is a widespread ecological foundation species that form the canopy of extensive wetland ecosystems. It exhibits varying susceptibility to the disease myrtle rust (*Austropuccinia psidii*). In this study we conduct a glasshouse experiment to observe resistance to rust following drought treatments. We compare this post-drought rust resistance to Genomically Estimated Breeding Value for rust resistance for the same individuals (*N* = 671). Six drought treatments were applied, with all combinations of three drought severities (low, moderate, and high) and two drought lengths (33 and 62 days).
- Our drought treatments resulted in plants exhibiting higher than expected resistance (reduced susceptibility) to myrtle rust. This drought induced resistance, however, rapidly degraded when water replete conditions were restored. Seedlings from resistant maternal lines stayed relatively resistant across drought treatments.
- This study illustrates the benefits of understanding the resistance of seed lots used for restoration. Under forecasts of increased drought severity and lengths, resistant seed lots are expected to maintain resistance under varying drought conditions. We therefore recommend the incorporation of resistant seed lots in the design of restoration projects to improve the resilience of populations in the long-term.

Keywords: *Austropuccinia psidii*, climate change, disease, drought, genomic prediction, Melaleuca, myrtle rust, targeted genotyping

Introduction:

Restoration of degraded habitats is increasingly important in the protection and promotion of biodiversity. However, restoration activities are expensive, in terms of the allocation of time and other resources (Andres et al., 2024). It is therefore important that restoration actions are effective in the long-term and build ecosystems that will be resilient to future events, such as disease incursions or to climatic changes (Bragg et al., 2022; Prober et al., 2019).

When plant populations are restored, seeds can be sourced to achieve various goals. These goals include the promotion of genetic diversity (Lesica & Allendorf, 1999; Rossetto et al., 2019), adaptation to predicted future climate conditions (Onley et al., 2021; Prober et al., 2019; Ray et al., 2022), and selection of desired traits such as disease resistance (Ray et al., 2022). These objectives can be prioritised using a range of strategies. For instance, seed sourcing can be guided by genetics with adjustments made to match expected future climate conditions (Capblancq et al., 2020; Prober et al., 2016; Rossetto et al., 2019). However, such approaches must consider the interaction between environmental stressors and other important traits, such as disease susceptibility. Thus, to promote population health and resilience, there is a need to understand the relationship between important climate stressors and disease resistance trait.

Climate change, urbanisation, and changed water use have all contributed to altered hydrological regimes in wetlands (Prober et al., 2019; Saintilan et al., 2019; Salimi et al., 2021). Future changes in mean temperature and precipitation are predicted (Nazeri Tahroudi, 2025), bringing the possibility of droughts that are both prolonged and more intense. Drought stress can independently reduce plant growth or survival and may also interact synergistically with disease pressure by altering a plant's susceptibility (Choudhary & Senthil-Kumar, 2024; Desprez-Loustau et al., 2006; Ghanbary et al., 2017).

Novel plant diseases can have devastating impacts on plant populations and ecosystems (Jaureguiberry et al., 2022; Ristaino et al., 2021). The disease myrtle rust, and its effects on Australian plants and forests, is an important example. Myrtle rust is caused by a fungal pathogen, *Austropuccinia psidii* (G. Winter) Beenken, which has a known host range of more than 350 species of Myrtaceae (Sandhu & Park, 2013). Australia possesses a rich Myrtaceae floral composition, since the incursion of the disease in 2010 (Carnegie & Cooper, 2011) it has been documented to severely affect the dozens of moderate to highly susceptible species (Carnegie et al., 2016; Makinson, 2018; Pegg et al., 2017; Roderick et al., 2022). As such, recommended actions focus on supporting susceptible species and impacted ecosystems by introducing genetically resistant individuals through restoration (Chen et al., 2025; Chock, 2020; Guo et al., 2026; Makinson et al., 2018; Makinson et al., 2020).

Drought stress can influence the susceptibility of plants to pathogens in complex ways. For example, drought stress can lead to greater susceptibility (Desprez-Loustau, et al. 2006; O'Hara 2016; Parades et al., 2023; Vannini et al., 2009), reduced susceptibility (Roussin-Léveillée et al., 2024) or minor to no effects on susceptibility (Shishkoff & Bruckart, 1996). This depends on the degree of stress that is experienced (Dudney et al. 2021; Parades et

al., 2023) and the pathogen involved (Oliva et al., 2014; Velásquez et al., 2018). A positive relationship between drought stress and disease, where high drought stress results in increased disease susceptibility, may occur due to impaired cellular functioning and reduced defensive mechanisms (Desprez-Loustau et al., 2006). Conversely, a negative relationship is often observed in mildly drought stressed plants (Roussin-Léveillée et al., 2024). This may be due to overlap in functional responses to drought and pathogen infection, leading to deployment of defences prior to disease exposure (Choudhary & Senthil-Kumar, 2024; Nejat & Mantri, 2017), or increased secondary metabolite production instead of shoot growth and leaf area (Adams et al., 2015; Fahad et al., 2017). Myrtle rust is an obligate biotrophic pathogen, deriving all nutrients from live new shoot material. As drought reduces growth rates and alters biomass allocations (Adams et al. 2013), we hypothesise that drought stress simultaneously would reduce rust susceptibility.

Here, we examine the relationship between drought stress and myrtle rust resistance. Particularly we investigate experimentally whether: 1) there is a correlation between disease resistance and the severity and duration of drought stress, and 2) the maternal line of a seedling and its collection location influence drought tolerance (observed osmotic conductance) and post-drought disease resistance response. The relationship between drought stress and myrtle rust resistance can take the form of three scenarios. Scenario 1 (Fig. 1) depicts a negative relationship between drought severity and length with myrtle rust resistance, where individuals undergoing greater drought stress will express higher levels of susceptibility. Scenario 2 (Fig. 1) represents no correlation, where increased drought stress has no relationship to changes in myrtle rust resistance. Scenario 3 (Fig. 1) conversely depicts a positive relationship between increased drought stress and myrtle rust resistance, possibly due to the biotrophic nature of the pathogen.

For this study we focus on the tree *Melaleuca quinquenervia* (Cav.) S. T. Blake*.,* which is a foundation plant species in wetlands across east coast Australia (Benson & McDougall, 1998; Grover & Slater, 1994) and often used for restoration (Baumann & Hewitt, 2023). This species also has a growing set of molecular resources for examining resistance to myrtle rust (Guo et al., 2026).

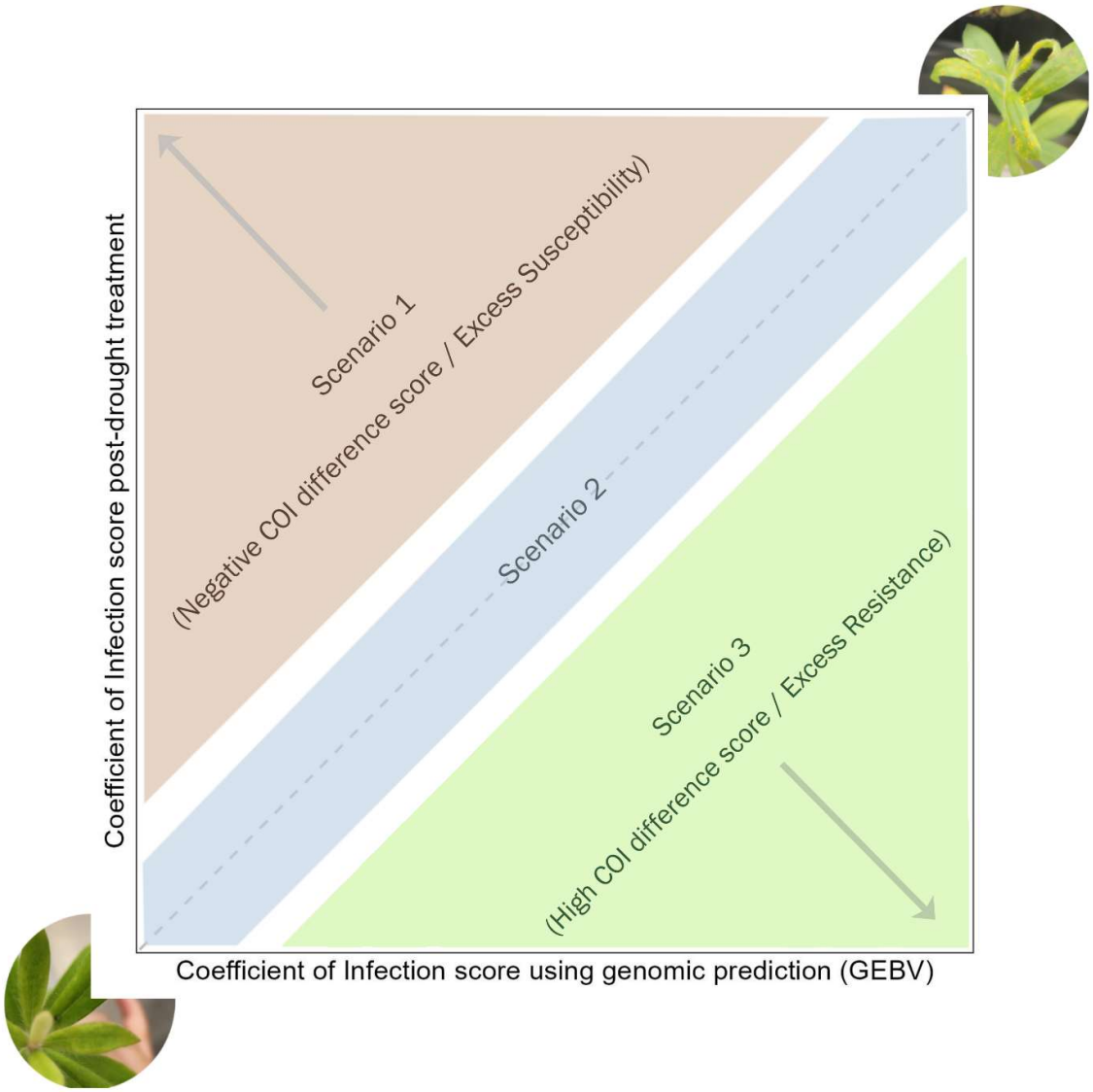


Figure 1. Conceptual diagram of post-drought disease susceptibility compared to baseline susceptibility scores (derived from Genomically Estimated Breeding Values). This represents the relationship between the expected level of disease susceptibility of an individual and the post-drought disease susceptibility for the same individual (y-axis, droughted score). Smaller scores represent lower susceptibility (and greater levels of resistance) on both axes.
Hypothesis 1 represents an impaired disease resistance, where droughted individuals express higher levels of infection than expected according to GEBV. Hypothesis 2 represents a one-to-one correlation between both scores, indicating that drought stress has no effect on expected resistance level. Hypothesis 3 is the converse, where droughting imparts a higher-than-expected disease resistance.

Methods:

Study species

The broad-leaved melaleuca, *M. quinquenervia*, is a widespread tree impacted by the disease myrtle rust (Pegg et al., 2025). It is a foundation species, forming the dominant canopy of wetlands across the eastern coast of Australia (Benson & McDougall, 1998; Grover & Slater, 1994). It is widely used for restoration of degraded wetland sites and as an important street tree. Myrtle rust is seen to impact *M. quinquenervia*, with only ~40% of individuals being resistant to the 'Pandemic' strain of the disease present in the continent (Guo et al., 2026; Pegg et al., 2018). This variation in resistance, including among maternal lines, provides a foundation that allows us to explore the interaction between drought impacts and myrtle rust resistance.

Maternal line sources

In a previous study, seeds of *M. quinquenervia* were collected from trees (*N* = 197) across NSW and assays were performed for myrtle rust susceptibility on seedlings of each maternal tree (Guo et al., 2026). Maternal line rust susceptibility scores were calculated by taking the mean Coefficient of Infection (COI) score of seedlings within each line, scored in the previous study. From these maternal lines, also referred to as seed lots, a subset of twelve were selected for this experiment: three seed lots classified as 'high resistance', four as 'middle resistance', and five as 'low resistance'. Seed lots were selected according to their mean seedling COI scores and collection location. Fewer higher resistance seed lots were selected to ensure a similar distribution across the range of susceptibility. This is as Guo et al. (2026) observed less variation among seedlings in high resistance seed lots than lower resistance seed lots (Table S2). Seed lots were also selected across NSW to ensure results were spatially representative, preventing the clustering of similar resistance levels at a single location (Fig. S2). Furthermore, the spatial distribution of seed lots ensured impacts of population structure across the landscape was minimised, however we note that across the geographic range of the seed lots used in this study, there are no evidence for strong genetic differentiation or population structure (Guo et al. 2026). Two seed lots of the 'middle resistance' category did not germinate successfully and were removed from the experiment.

Drought experimental design

The experimental drought treatment was conducted in a greenhouse with an ambient temperature of 25 – 28ºC and humidity of 50 – 70%. For germination, seeds were sowed onto a growth medium (1:2 coir peat to sand) in germination boxes and misted regularly to

maintain moisture. Seedlings were grown until at least two sets of true leaves were observed (3 months old). Once grown to sufficient size, seedlings were transplanted to forestry tubes (50w x 50d x 120h mm), with pots altered specifically for the drought protocol as detailed below. Forestry tubes were filled with 120 (+/- 5) g of substrate with no fertiliser addition (1:1 mixture of 0 – 8 mm pine bark, 2 – 5 mm pine bark, coir peat, coarse sand) and doused with anti-fungal mixture (1:600 Agri-Fos® 600 to water). After one week of establishment, seedlings were fertilised with soluble fertiliser to encourage growth (PowerFeed Dynamic Fertiliser & Soil Conditioner All Purpose Including Natives at half the manufacture's recommended concentration). They were then left to establish for a total of 3 months under optimal conditions to grow to a size resembling tube stock available in commercial nurseries. Once established, plants were randomly assigned to each drought length and severity treatment combinations through blocks in the drought apparatus below.

The drought experiment was set up in accordance with the protocol outlined in Marchin et al., (2020) (Fig. S3), with drought severity levels in this experiment selected using data from previous pilot studies and to balance between mortality over the drought length and the summer droughts experienced in wetlands (McJannet, 2008). The droughting protocol described in Marchin et al., (2020) is as follows. Forestry tubes had their bases manually removed, and fitted with fine nylon mesh (20 µm, Allied Filter Fabrics, Berkeley Vale, NSW). Commercial porous foams (Oasis IDEAL Floral Foam Maxlife brick; Smithers-Oasis, Kent, OH, USA) of varying heights were placed into tubs with 5 cm deep water baths that were maintained throughout the experiment period. Two individuals were placed on each floral foam block, with each water basin possessing six floral foam blocks in total (two floral foam blocks per drought severity). Floral foam blocks of 5 cm, 11.5 cm, and 23 cm, were used to represent the levels of drought severity (low, moderate, high respectively), resulting in simulating water depths from soil surface of 15 cm, 21.5 cm, and 33 cm. This resulted in each water basin (blocks) with twelve individuals at four per drought severity. Each block was randomised, once at the start of the droughting experiment, and again at 32 days. In total, a subset of plants was subject to drought conditions for 32 days and 62 days, with durations selected for operational feasibility. For simplicity, these will be referred to as 30 and 60 day drought lengths. This experiment utilised a fully crossed factorial design with the drought severity, drought length, and maternal lines. To account for anticipated mortality, each treatment was initiated with 10 replicates supplemented by 5 additional replicates per drought severity level, yielding an initial total of 750 samples. This drought treatment was conducted in the summer month, from dates of 1st January 2025 to 3rd March 2025.

To confirm the physiological effects of the drought treatments the rate of water vapor diffusion through the stomata, quantified as stomatal conductance (g_{sw}), was measured.

Measurements were recorded at 15, 30, and 60 days using a LICOR LI-600 Porometer (Licor Inc., Lincoln, Nebraska, U.S.A.), with lower g_{sw} values reflecting heightened water stress (Buckley, 2019). All measurements were conducted at the same location within the glasshouse, and between 08:00 – 13:00. Pilot trials indicated this was the best time of day for consistent measurements within an individual plant, and for avoiding stomatal closure induced by higher relative humidity and temperatures in the afternoon. For each individual, 3 to 5 measurements were taken in succession, and the median measurement was used for analysis. Due to time constraints, only a subset of individuals was measured and randomly selected within each combination of treatment factors (maternal line, drought severity, drought length) (total N = 123, minimum N = 2 per combination of levels). Readings of g_{sw} below -0.05 were deemed errors and removed prior to analyses (N = 25 out of 937).

Similarly, for a randomly selected representative of each factor level combination, individual plants in pots were weighed using a standard commercial digital scale (1 g) on a fortnightly basis from the establishment period to the end of the experiment. Mortality of individuals occurred during the experiment. In total, 683 individuals were scored post-droughting across both drought lengths, with the smallest number of repeats per treatment of 6.

Inoculation

After the 30 day and 60 day drought treatments, plants were artificially inoculated at the Plant Breeding Institute (Cobbitty, New South Wales) to determine their post-drought myrtle rust susceptibility status (Coefficient of Infection scores (COI)). Myrtle rust resistance assays followed a set of procedures described previously by Sandhu and Park 2013, with modifications noted below. Briefly, 20 mg of *Austropuccinia psidii* urediniospores were collected from a pre-inoculated susceptible *Syzygium jambos* plant ('Pandemic' strain, isolate ID: Au_3, PBI culture no. 622 and accession 115012). Seedlings were inoculated with an *A. psidii* urediniospores suspension (2 mg of urediniospores/1 mL of light mineral oil; Univar Solvent Naphtha L 100). The inoculations were performed using an airbrush connected to a motorised compressor. After atomisation of the rust suspension, the door of the inoculation room was kept closed for 5 minutes to allow urediniospores to settle on the leaves. Seedlings were then moved to a dark incubation chamber for 24 hours at 20°C and misted constantly to maintain >95% relative humidity. Following incubation, the seedlings were moved to a naturally lit microclimate room maintained at 22 ± 2°C for 14 days, prior to being scored for rust infection and host response, as below.

Reinoculation

To determine a water-replete rust resistance score and stable levels of high resistance, individuals were reinoculated using the same process as above after pruning to remove infected material. These included individuals showing no to low rust responses, and a random subset of selected individuals across all drought levels (minimum *N = 56* per drought severity and length combination). For the former group of individuals, these were reinoculated to ensure that highly resistant individuals were not 'escapees' of the inoculation and maintained their resistance. For the latter group of individuals, the inoculation procedure involves placing the seedlings on hydrated capillary mats during the two weeks of post incubation period (Sandhu & Park, 2013). This imparts a water replete condition, representing a drought relaxation treatment. By examining the direction and magnitude of the shift in COI scores (observed resistance) in individuals during reinoculation, we could then deduce the direction of transition post-relaxation of drought stress. For this analysis, to avoid artificially bolstering our results with 'escapees' we removed all individuals with an initial COI score of '0' (repeated without removal at Fig. S5).

Rust Scoring

COI scores were measured following a protocol described by Sandhu and Park (2013) and Guo et al. (2026). Briefly, the protocol records infection types observed (presence of active spores, flecking, chlorosis, and/or necrosis) and a coverage score. This data is then used to calculate a single numerical COI for each seedling, based on the modified Cobb's scale (Peterson et al., 1948) (Supplementary Information). This score provides a measure of susceptibility, with higher scores representing higher susceptibility observed in the individual. COI values were transformed (fourth root) to better meet normality assumptions for statistical testing (Guo et al., 2026).

Sampling and Genotyping

Genomic prediction was used to characterise an individual's baseline or expected myrtle rust susceptibility without the drought treatment (Fig. 1). This genomic prediction model was developed using rust susceptibility phenotypes from *M. quinquenervia* seedlings that were grown under water replete conditions (Guo et al. 2026).

The previous study identified molecular markers associated with resistance to myrtle rust in *M. quinquenervia* and produced an accurate genomic prediction model for this phenotype. In this present study, we genotyped all individuals, filtering out poorly sequenced loci and samples, and generated a predicted value of myrtle rust resistance (Genomic Estimated Breeding Value of COI, hereafter GEBV COI) which is used as a baseline or expected score for rust susceptibility in the absence of the drought treatment.

Samples in this study were genotyped using a high-throughput targeted genotyping method called DArTag, performed as a service by Diversity Arrays Technology Ltd. (DArT; Canberra, Australia) (2,550 SNPs). Fresh leaf tissue samples were frozen overnight at -80°C then freeze-dried prior to genotyping. The genotyping SNP panel targeted loci from different categories: myrtle rust resistance associated SNPs (1,050 SNPs, as described in Guo et al. 2026) and background SNPs selected randomly throughout the *M. quinquenervia* genome (Chen et al., 2023) (1,500 SNPs).

To perform the genomic prediction for the characterisation of an individual's base myrtle rust resistance without the drought treatment we follow methods detailed in Guo et al. (2026). Briefly, we used a genomic prediction model (genomic best linear unbiased prediction (gBLUP), implemented using the R package GAPIT v3.5 (Wang & Zhang, 2021) built with phenotype training datasets described by Guo et al. (2026). Prior to prediction, the genetic dataset was filtered for poorly sequenced loci (those missing more than 60% of genotypes across all individuals across all individuals for that locus). Poorly sequenced individuals (those missing more than 90% of genotypes across all loci) and individuals with high PEV (>0.5) (Wang & Zhang, 2021) were removed prior to analysis (final *N* = 683*)*. This genomic prediction approach subsequently produces a Genomic Estimated Breeding Values of forth root transformed COI (GEBV COI) for each analysed sample. Using the GEBV COI, a response variable was calculated for each individual to quantify departure from expected resistance levels (Eqn. 1), with post-drought observed values also transformed by fourth root to reflect the transformations conducted in the genomic prediction model.

$$Coefficient\ of\ Infection\ (COI)\ difference\ =\ GEBV\ COI\ -Post\ drought\ COI \qquad \textit{(Eqn. 1)}$$

Analysis

To investigate the effect of drought treatments on the fourth root transformed post-drought COI for each individual, we conducted an analysis of variance (ANOVA) using R (R Core Team, 2025). This model was fit with the drought severity, drought length and the maternal line of the seedling as additive effects (Eqn. 2) (See Table S3 for initial testing of the interaction between drought severity and the maternal line, with drought length as a covariate). This was then repeated with the COI difference score (Eqn. 1).

$$Post\ drought\ COI\ or\ COI\ difference \sim (Drought\ severity) + (Drought\ length)\ + (Maternal\ Line) \qquad \textit{(Eqn. 2)}$$

The procedure of artificial inoculation with *A. psidii* entailed a period of water replenishment (Sandhu & Park, 2013), resulting in relaxation of the drought effects. We then conducted

another round of artificial inoculation on the same individual's, producing a water replete COI score to compare against their droughted COI score. A paired t-test was used to test for differences between post-drought COI scores and water replete COI score. Both scores were transformed by the fourth root to better meet the assumption of normality.

To investigate local adaptation to drought by maternal lines, we used a linear model to test whether osmotic conductance (g_{sw}) was associated with MAP of the maternal line's collection location, or by an interaction with MAP to the drought severity treatment. Drought length was included as a main effect (Eqn. 3). This was repeated for the transformed post-drought COI score and COI difference (Eqn. 1).

$$Median\ g_{sw}\ or\ COI\ difference\ \ or\ Post\ drought\ COI \sim (Drought\ severity) * (MAP)\ + (Drought\ length) \quad \textit{(Eqn. 3)}$$

Results:

Data overview

Post-drought fourth root transformed COI scores across all individuals and drought treatments ranged from 0 to 3.08 (untransformed values were 0 to 90 respectively), with a maternal line mean of 1.37 and 2.17 fourth root transformed (untransformed values of 3.57 to 22.0). Water replete COI scores across all individuals covered the same range as post-drought COI scores. Reinoculation of individuals found 52 – 55% of individuals maintaining highly resistant statuses across both artificial inoculation events (maintenance of a COI score of 0). Genomically predicted GEBV COI scores were on average higher than post-drought COI scores for the same individuals, and ranged from 0.49 to 3.05 (forth root transformed for normality) (Fig. 2).

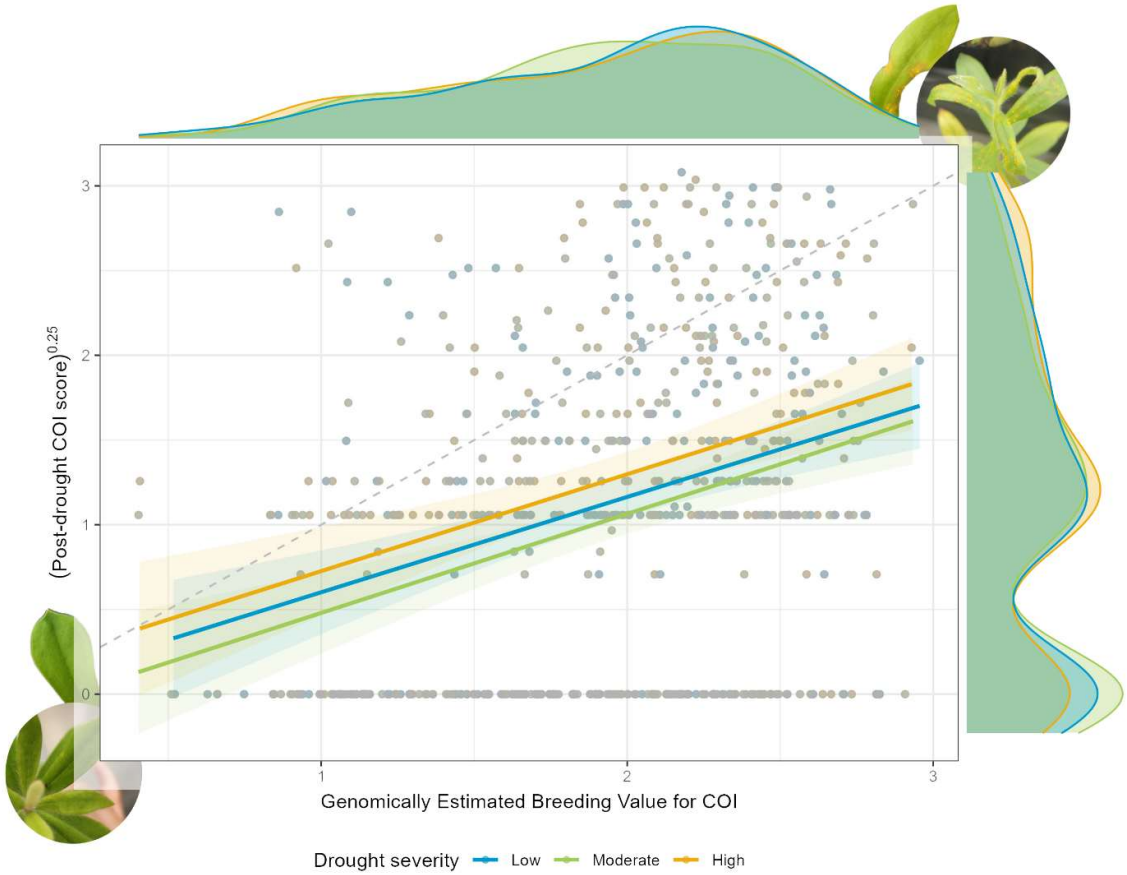
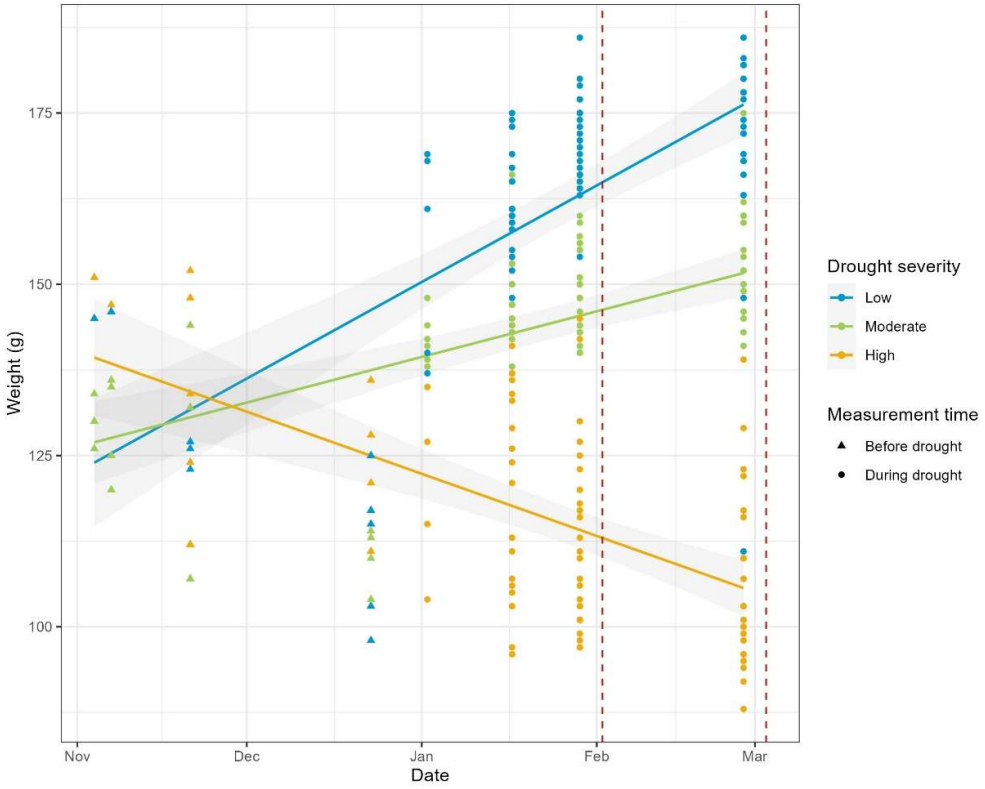


Figure 2. Post-drought Coefficient of Infection (COI) compared to Genomically Estimated Breeding Value (GEBV, expected COI). Lower COI scores represent higher levels of resistance. Each datapoint represents a unique individual. Sample sizes of each treatment are, low drought severity N = *235, moderate drought severity* N = *238, high drought severity* N = *197. The dashed*

line in grey represents the one-to-one association between the two values (Hypothesis 2), with datapoints above the dashed line representing individuals expressing higher susceptibility (larger COI) than expected (Hypothesis 1) and vice versa (Hypothesis 3). Linear model correlation between COI scores have been coloured and grouped by the individual's drought severity treatment. Marginal density plots provide a visual aid to the distribution of COI scores on each axis, coloured by drought severity treatment.

Experimental drought treatments affected weight and g_{sw} measurements

Total weights of plants were significantly greater for low and moderate drought severity treatments than high drought severity treatments (ANOVA, $F_{(2,244)} = 11.936$, $p << 0.05$, Fig. 3a). Conversely, prior to drought conditions, the baseline total weights showed no significant differences across the treatment groups ($F_{(2,36)} = 0.23$, $p = 0.796$, Fig. 3a). Similarly, osmotic conductance was significantly different across plants of different drought severities (ANOVA, $F\text{-}val_{(2,56)} = 6.841$, $p\text{-}val < 0.05$ at Day 30 g_{sw} measurements, $F\text{-}val_{(2,28)} = 3.186$, $p\text{-}val = 0.057$ at Day 60 g_{sw} measurements, Fig. 3b)



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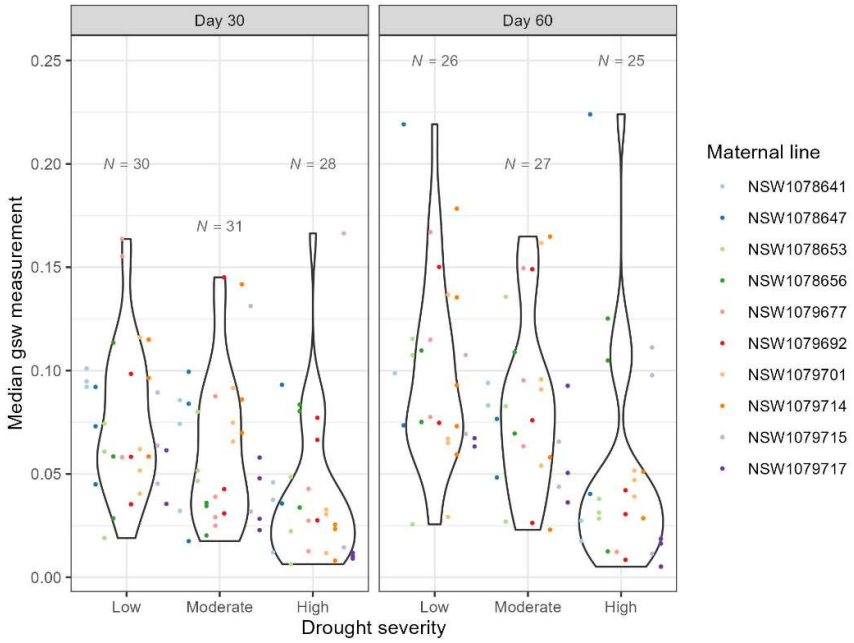


b)

Figure 3. a) Weight of individuals in pots measured across time. Each individual is coloured by their drought severity. Data points depicted with a triangle are measured prior to imparting the drought treatment on the individuals. Vertical lines are placed at Day 30 and Day 60 treatment's inoculation date. b) Osmotic conductance by drought length (30 and 60 days) and drought severity (low, moderate, high). Multiple measurements were made per individual during the porometer readings. Each datapoint represents an individual, coloured by their maternal line. Also grouped by maternal line in Fig. S4.

Plants under long and severe drought had greater resistance than expected

Post-drought COI scores were significantly correlated to the drought severity and the drought length treatment imparted (Eqn. 2, ANOVA, $p < 0.05$, $p << 0.05$ respectively, Table S4). Similarly, the post-drought COI scores were observed to be lower than their corresponding GEBV COI (Fig 2), indicating a higher than expected disease resistance (henceforth also referred to as excess resistance for brevity). Both drought severity and drought length were significant predictors to this excess resistance observed (Fig. 5, Eqn. 2, ANOVA, $p < 0.05$, $p << 0.05$ respectively, Table S5a). When testing post-hoc, individuals under high drought severity were shown to be significantly different to the moderate drought severity group (Tukey HSD, $p < 0.05$, *95% C.I.* = [0.0344, 0.446], Table S5b). *S*imilarly, excess resistance scores were greater for plants droughted for 60 days than those droughted for 30 days (Tukey HSD, $p << 0.05$, *95% C.I.* = [0.125, 0.402], Table S6b).

The maternal line for each seedling was also observed to have a significant effect on both the excess resistance observed and the post-drought COI (Eqn. 2, ANOVA, $p << 0.05$, $p <<$

0.05, Table S5, Table S4). We observed seedlings from resistant maternal lines (NSW1078643 and NSW1078641) to remain relatively resistant regardless of drought treatments conducted. When conducting post-hoc tests between the resistant maternal lines against the remaining maternal lines, we found the post-drought COI scores to be significantly different in 13 out of 15 maternal line comparison pairs (Fig. 4, Table S6).

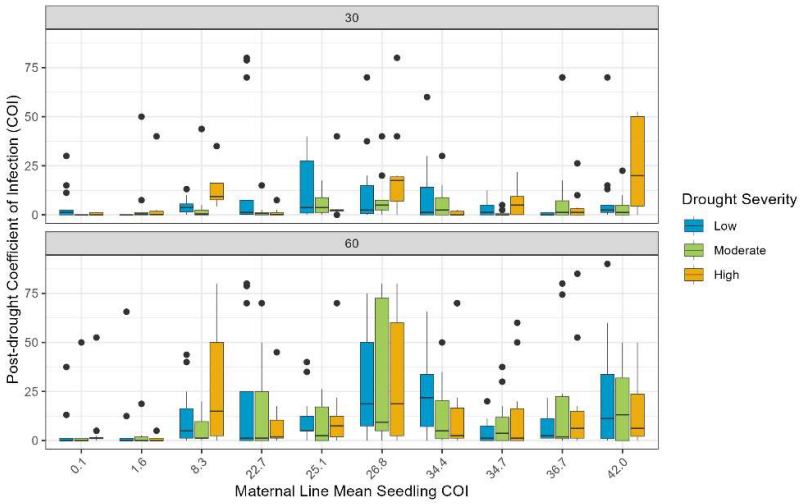


Figure 4. Plot of Coefficient of Infection (y-axis) by their maternal line (x-axis). The x-axis has been labelled with their maternal line's mean seedling COI value from Guo et al. (2026). Higher COI scores represent higher levels of susceptibility. From left-to-right this represents maternal lines NSW1078653, NSW1078641, NSW1079715, NSW1079692, NSW1078647, NSW1079677, NSW1079701, NSW1078656, NSW1079717 (Table S2). Box plots have been coloured by drought severity, with blue corresponding to low drought severity, green to moderate drought severity group, and orange to high drought severity. The graph has been faceted into their drought length treatments, 30 and 60 days.

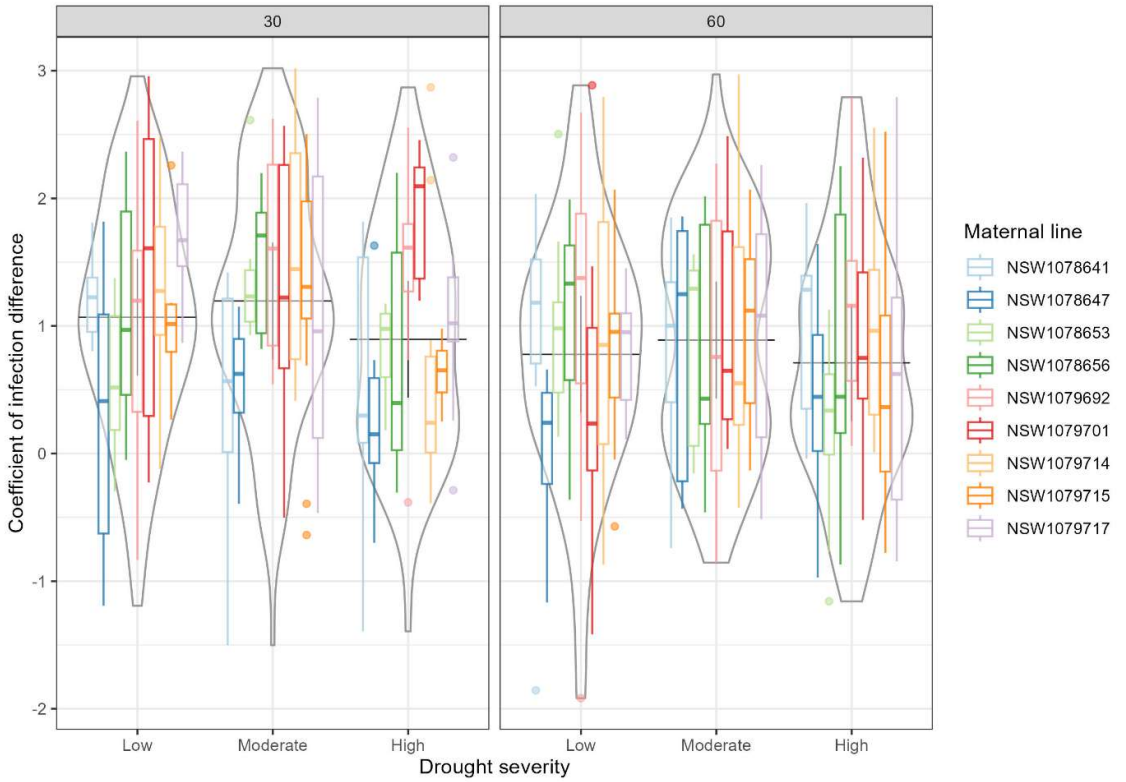


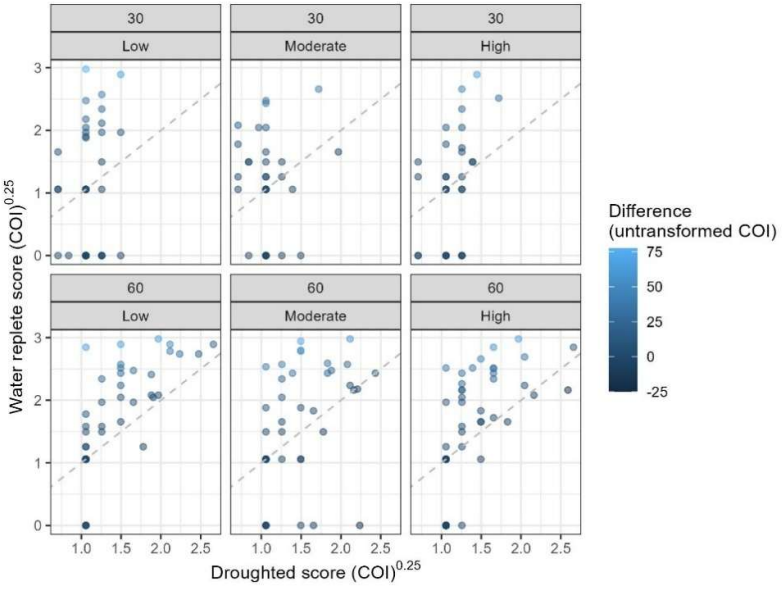
Figure 5. Difference in values between genomically predicted value (GEBV) COI and post-drought COI scores (fourth root transformed) across drought treatments. Larger difference values represent a higher resistance than expected. Each boxplot represents a maternal line and is assigned to their corresponding drought length (30, 60 days) and drought severity (low, moderate, high). Violin plots represent the overall shape of the dataset, with solid grey horizontal line representing the overall mean for the drought treatment. The graph has been faceted into their drought length treatments, 30 and 60 days.

Reinoculation

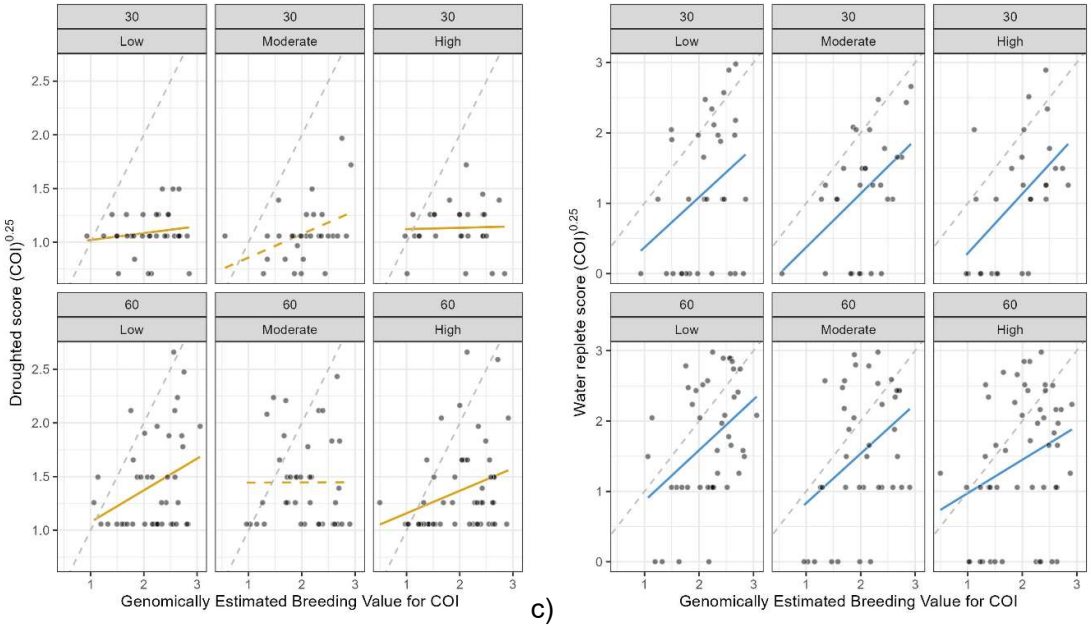
The artificial inoculation procedure induces a water replete status to the individuals during the two weeks of post incubation (Sandhu & Park, 2013). Reinoculation of individuals with an initial COI of 0 resulted in 52% of the 30 day drought length individuals and 55% of the 60 day set remaining highly resistant (COI score of 0). This represents a mix of 'escapees' of the artificial inoculation treatment and a departure from the excess resistance offered by the drought treatment from the individuals tested. We explore this further in the Discussion.

To investigate the effect of drought relaxation on rust resistance, we removed individuals with an initial COI score of 0 for the following analyses (inclusion of individuals with initial score of 0 available at Fig. S5). Individuals were observed to exhibit higher COI scores (higher susceptibility) than their post-drought excess resistance status (Fig. 6a). When in

comparison to the GEBV COI scores, the post-drought COI scores were significantly lower (excess resistance observed) (Fig. 6b). This post-drought COI score was significantly different from the water replete inoculation COI score for the same individuals (paired t-test, $t_{(234)}$ = 1.970, p = 0.05) which more closely resembled the GEBV COI scores (Fig. 6c).



a)



b) c)

Figure 6. Comparison of post-drought myrtle rust resistance scores (Coefficient of Infection, COI), water replete rust resistance scores (COI), and Genomically Estimated Breeding Value of COI (GEBV COI). Higher COI scores represent higher levels of susceptibility. Individuals with an initial inoculation score of '0', were removed as they potentially represented 'escapees' of an initial inoculation rather than the desired water relaxation effect. Grey dashed lines in all plots

represent a one-to-one correlation between the respective x- and y-axes. Post-drought rust resistance scores and water replete scores have been transformed by fourth root for normality. Each data point represents an individual. Plots are faceted by their drought severity and length experienced. a) Individuals inoculated with myrtle rust after droughting (x-axis) and after water replete conditions (y-axis). Data points are coloured by their untransformed COI score. b) Comparison of post-drought COI score to expected rust resistance scores (GEBV COI). The orange line represents the linear correlation between both values, which presents to be shallower than the one-to-one slope in grey. c) Comparison of water-replete COI score to expected rust resistance scores (GEBV COI), faceted by their drought severity and length experienced. The blue line represents the linear correlation between both values, which presents to be similar to the one-to-one slope in grey.

Maternal line climate and drought tolerance

The experiment was conducted using seed lots collected across a climatic gradient spanning the latitude of NSW, Australia (Table. S2, Fig. S2). We aimed to test the climate (MAP) of the maternal line was associated with the effects of drought severity on rust resistance. MAP was not found to be a significant main effect on median g_{sw} (ANOVA, $F_{(1,160)} = 0.058$, $p >> 0.05$, Table S7) nor as a significant interaction with drought severity (Eqn. 3, $F_{(2,160)} = 1.588$, $p > 0.05$, Table S7). Similarly, when testing on the post-drought COI score MAP did not have significant interaction with drought severity (ANOVA, $F_{(2,636)} = 0.127$, $p >> 0.05$, Table S9), or when testing on excess resistance (ANOVA, $F_{(2,636)} = 0.087$, $p >> 0.05$, Table S8). MAP however was a statistically significant main effect with both ($p << 0.05$ for both, Table S8, Table S9).

Discussion:

Restored forest populations will have a substantial role in building ecosystems that are designed for long-term resilience. However, to do so, they will need to withstand future changes in climate and urban use, which are expected to shift hydrological regimes across large areas of the globe (Gedney et al., 2024; Prober et al., 2019; Salimi et al., 2021). To build resilience against these changes, the interacting effects of other known stressors must be considered. *Melaleuca quinquenervia* is ecologically, a foundation species that is impacted by myrtle rust, a destructive plant disease caused by the fungus *A. psidii*. In this study we observe excess resistance (reduced susceptibility) to myrtle rust in seedlings under drought stress conditions. This excess resistance relaxed when drought treatments were suspended and plants were water-replete. By better characterising the impact of drought on myrtle rust resistance, this study provides applicable information for restoration and

reforestation projects. In turn, this will help promote the resilience of restored ecosystems and carbon sequestration projects.

Drought stress and disease susceptibility

Relationships between drought stress and fungal disease susceptibility vary across different host-pathogen systems (Oliva et al., 2020). In general, our study found that relatively severe drought conditions resulted in higher levels of resistance (Fig. 1). This was especially the case for moderately droughted individuals, similar to observations in other biotrophic fungal disease systems (Hsu et al., 2013; Illouz-Eliaz et al., 2025). This potentially arises from a decrease in shoot production (Adams et al., 2013), which is required by obligate biotrophs (Oliva et al. 2020), and an increase in the production of secondary metabolites under resource limited conditions (Fahad et al., 2017).

This elevated resistance observed in our droughted plants was lost rapidly when the plants returned to water replete conditions. In natural environments, this may occur when significant rainfall follows a period of drought (Qing et al., 2023). This results in higher relative humidity which creates ideal conditions for *A. psidii* infection (Narouei-Khandan et al., 2020) and stimulates the flushing out of new susceptible tissue. In this study, we found the drought relaxation times to be relatively short, which is also seen in other pathogen systems (Sadhukhan et al., 2022). We note that in this study, the disease resistance score after drought relaxation was measured at a single time point after the return to water replete conditions. Therefore, the length of time taken to reverse the excess resistance afforded by the drought treatments may be potentially shorter. In natural environments, myrtle rust impacts on plants are also likely to influence the impacts of future drought stress experienced, increasing the risk of mortality. For instance, diseases have been observed to reduce root growth and carbohydrate reserves (Schmittgen et al., 2015), leading to lower drought tolerance. Furthermore, myrtle rust has been observed to shift stomatal dynamics and reduce leaf gas exchange (Gonçalves et al., 2023). Under these impairments, the reduced transpiration can further weaken host individuals and make them more susceptible to mortality from drought stress (Oliva et al., 2014).

More broadly, drought affects potential host plants and other abiotic conditions in many ways that could affect disease resistance and susceptibility (Simler et al., 2019). For myrtle rust, the increased risk of fire due to drought is an important example (Dutra et al., 2026). Fire has been observed to result in increased mortality in *M. quinquenervia* as the regrowth of individual plants is highly susceptible to *A. psidii* (Pegg et al., 2025) and thus, can further predispose susceptible individuals to higher chances of mortality.

Experimental methods: progress and caveats

This study used a genomic prediction model to generate 'expected' scores of myrtle rust resistance for plants used in the experiment. The genomic prediction model was based on myrtle rust susceptibility phenotypes from a previous experiment (Guo et al. 2026) where plants had been grown under water replete conditions. This provided a way to calculate a score for the excess resistance observed for each plant under the drought treatments and thus allow a comparison to resistance observed under water replete conditions. We note that under water replete conditions in this experiment, predicted scores were strongly corroborated with the observed scores (Fig. 6c). This suggests that this approach might be a useful experimental tool in studies of resistance in relation to different kinds of conditions or stressors.

Our study aimed to provide information to help manage the impacts of this invasive disease in-field. However, we note the caveat that our experimental greenhouse conditions differs to a field site. We attempted to approximate field conditions by using bottom-up watering methods, mimicking wetland systems. We used drought severities that were informed by empirically measured water table depths (McJannet, 2008), and drought lengths that were informed by the soil moisture zone measurements from the Australian Water Outlook (Frost et al. 2021, Supplementary Information).

Finally, we note that our observations of the relaxation in resistance at the end of droughting were likely conservative. When performing analyses, we removed individuals that showed no sign of infection (COI score of 0) in the initial inoculation following drought. We did this because we could not exclude the possibility that these plants 'escaped' exposure to the pathogen during inoculation. Our analysis therefore focused on plants that initially showed infection and considered how their level of infection (COI) changed between drought and water replete conditions. However, there is reason to believe that droughted plants also had an elevated propensity to remain uninfected. In this experiment, plants that were resistant in the initial (droughted) inoculation were retested, and 52 – 55% remained resistant in the second (water replete) inoculation. However, in a previous experiment (Guo et al. 2026), plants that were inoculated initially under water replete conditions were also retested, and 81% remained resistant in a second test. That is, when plants are retested under similar conditions, a much smaller fraction of 'escapes' (19%) are expected than were observed here (44 – 48%), suggesting that droughting resulted in an elevated fraction of plants that did not become infected.

Diverse, resistant, and climatically adjusted seed lots for restoration

The interaction between drought stress and host susceptibility to *A. psidii* is complex. However, one important theme that emerged from our study was that seed lots that were most resistant to myrtle rust maintained their resistance under the drought treatments. Conversely, across moderately and highly susceptible seed lots, large variations in post-drought rust resistance were observed in the seedlings. This highlights the benefits of prioritising resistant seed lots in restoration.

We also investigated whether the mean annual precipitation (MAP) of seed collection sites was associated with drought tolerance, and in turn, the myrtle rust resistance of seedlings. Most notably, there was no evidence that post-drought infection (COI) was influenced by interacting effects of MAP and drought severity. This may be because there was a lack of local adaptation to drought among the experimental seed lots. However, we note that we deliberately chose seed lots that were contrasted for resistance to myrtle rust, not drought tolerance, so it is possible that variation in drought tolerance would exert a larger effect among a different group of seedlings.

Conclusions

This project examines interactions between drought stress and resistance to an invasive pathogen in a wetland foundation tree species. These stressors will have ongoing effects on restored populations for decades, and it is therefore useful to characterise their relationship to inform the design of populations for long-term resilience. Our results show that it would be useful to prioritise seeds and seedlings from maternal lines that show high levels of rust resistance, while simultaneously sourcing them from sites matched to future climates, and in ways that bolster population genetic diversity. In light of the complexity of interactions between myrtle rust and abiotic stressors, it would also be highly valuable to monitor the health of *Melaleuca* forests, especially locations recently restored with seedlings which are more vulnerable to the disease.

Author Contributions

Karina Guo, Jason Bragg, Geoff Pegg conceptualised the idea. Karina Guo, Jason Bragg, Glenda Wardle worked on the experiment design. Karina Guo conducted the drought experiment and artificial inoculation and rust response scoring. Karanjeet Sandhu assisted with the host-pathogen interaction study including artificial inoculations and rust response scoring. Karina Guo, Jason Bragg analysed the data and wrote the first and final draft manuscript. All authors have read and approved of the final manuscript. All researchers included are based in the study region. We sought out stakeholder feedback to finalise the

selection of the abiotic stressor tested in this study. This ensured the findings remain highly applicable to practitioners within the field.

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Conflict of Interest

All authors declare that there is no conflict of interests.

Data availability statement

Scripts and data for the figures in this publication are available in a Zenodo repository at https://doi.org/10.5281/zenodo.20986749

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748

Supplementary

Protocol for scoring

Protocol for scoring was conducted similar to Guo et al. 2026 and have been republished here for ease of access.

Scoring is completed by giving each individual a numerical score representing the maximum percentage coverage affecting a leaf on the individual plant (0-100, with 100 being full coverage). A second score of the rust response is then given to each plant. For images and further information, see Sandhu & Park, 2013. This score can be used to determine a multiplicative factor (Table S1), which is used in Eqn. 1, to calculate the Coefficient of Infection (COI) for an individual plant.

Table S1. Immune type response score and their multiplicative factor.

Rust response	Description	Multiplicative factor
HR	Highly resistant	0
R	Resistant	0.25
MRMS	Moderately resistant to moderately susceptible	0.5
MS	Moderately susceptible	0.75
S	Susceptible	0.875
HS	Highly susceptible	1

Equation S1. Using both the coverage score and the immune type score to calculate the Coefficient of Infection for a seedling

$$Coefficient\ of\ infection\ = (Coverage\ score)\ *\ (Immune\ type's\ multiplicative\ factor)$$

a) b)

c) d)

Figure S1. Examples of varying rust responses and coverages, with higher COI scores representing greater susceptibilities. a) R Individual with flecking and chlorosis, rated as 50 coverage score corresponding to a COI of 12.5. b) MRMS Individual with chlorotic, necrotic spots and one highly restricted pustule, rated as 30 coverage score corresponding to a Coefficient of Infection (COI) of 15. c) S Individual with some necrosis, restricted to fully developed pustules, rated as 30 coverage score

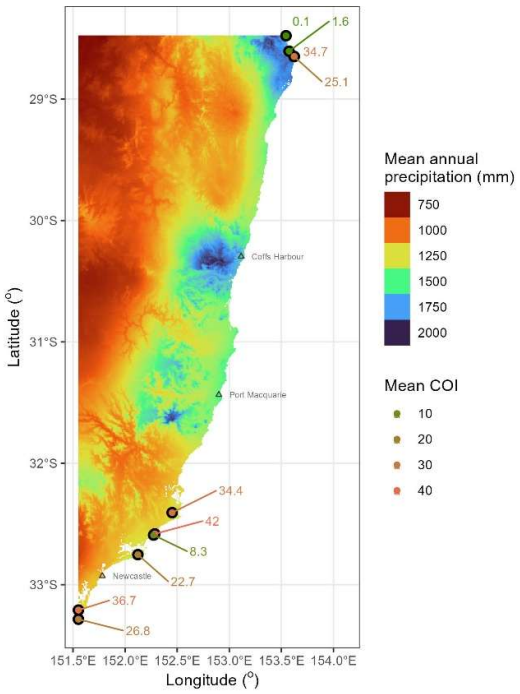
Determining drought length

The selection of drought lengths was conducted by taking the Australian Water Outlook's measurement of root zone soil moisture for NSW between 2020 to 2024 (Frost et al. 2021). A threshold of 10% was selected and the number of consecutive days below this threshold was calculated. A maximum of 57 days below this threshold was decided and tested in an initial pilot study for insurance against excessive mortality.

Supplementary figures

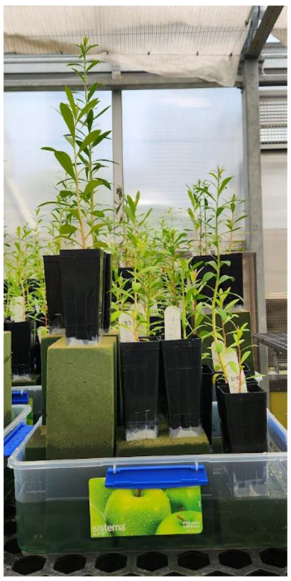
Table S2. Characteristics of the maternal lines/seed lots from which the seedlings were grown from. Where higher mean COI represent more susceptible individuals.

Maternal Line UID	Latitude of collection	Longitude of collection	Number of seedlings phenotyped in Guo et al. 2026	Mean COI of seedlings phenotyped in Guo et al. 2026	Standard deviation of seedlings phenotyped in Guo et al. 2026	Susceptibility category
NSW1079717	-33.20924	151.5524	3	36.66667	30.5505	High
NSW1079714	-32.58144	152.2852	6	42	22.62742	High
NSW1079701	-32.40648	152.4517	10	34.4	18.0259	High
NSW1079677	-33.28459	151.5522	10	26.75	22.79285	High
NSW1078656	-28.64853	153.6242	6	34.66667	35.85898	High
NSW1078647	-28.64924	153.6220	9	25.11111	30.02268	Moderate
NSW1079692	-32.75248	152.1226	3	22.66667	18.61003	Moderate
NSW1078653	-28.47786	153.5446	9	0.1111	0.3333	Low
NSW1079715	-32.59141	152.2735	8	8.28125	10.78643	Low
NSW1078641	-28.60517	153.5740	9	1.5556	2.4552	Low



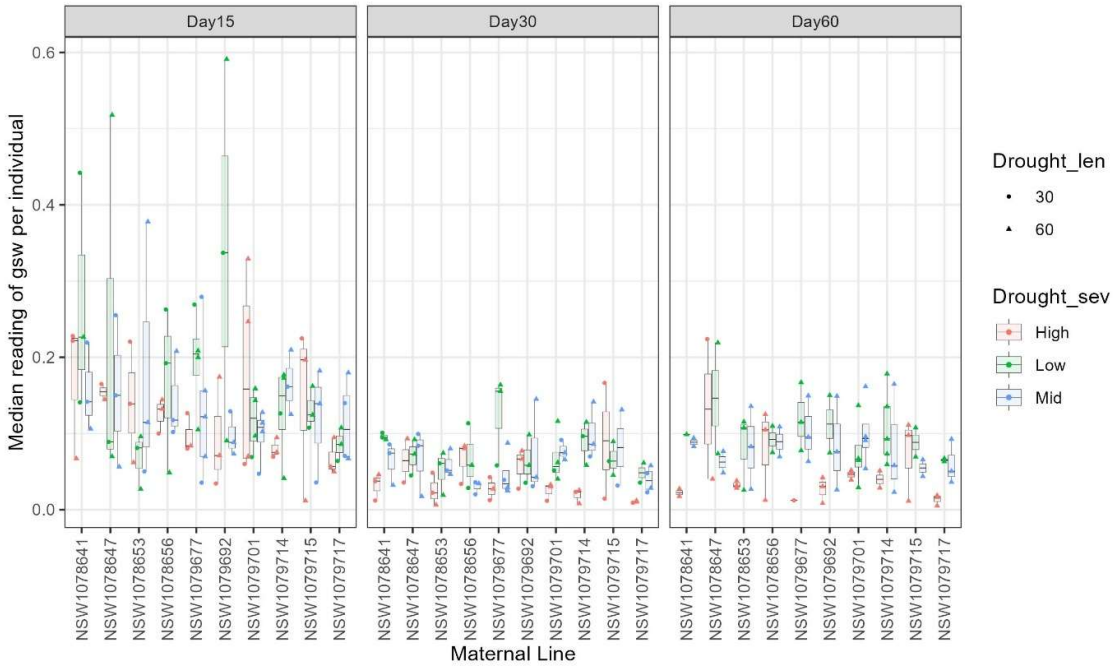
782

783 *Figure S2. Seed lots used for experiments. Map is coloured by the mean annual precipitation*
784 *(mm) and data points are coloured and annotated by their mean seedling's Coefficient of*
785 *Infection from Guo et al. 2026.*



786

787 *Figure S3. Experimental set up. Floral foams of different heights are placed into a tub. Water is*
788 *added to the box and is taken up by the floral foam to provide different levels of water availability*
789 *to the plants, imparting different levels of drought stress.*



790

791 *Figure S4. Distribution of the g_{sw} readings across the different drought treatments. The plot has*
792 *been faceted by the day the measurements were conducted. The x-axis for each facet plot*
793 *represents a maternal line. The y-axis is the median reading out of the multiple repeat readings*
794 *for each individual on the day. Triangle data points represent individuals designated to have a 60*
795 *day total drought length. Circle data points represent 30 day total drought length. Both data*
796 *points and box plots have been coloured by their designated drought severity treatment.*

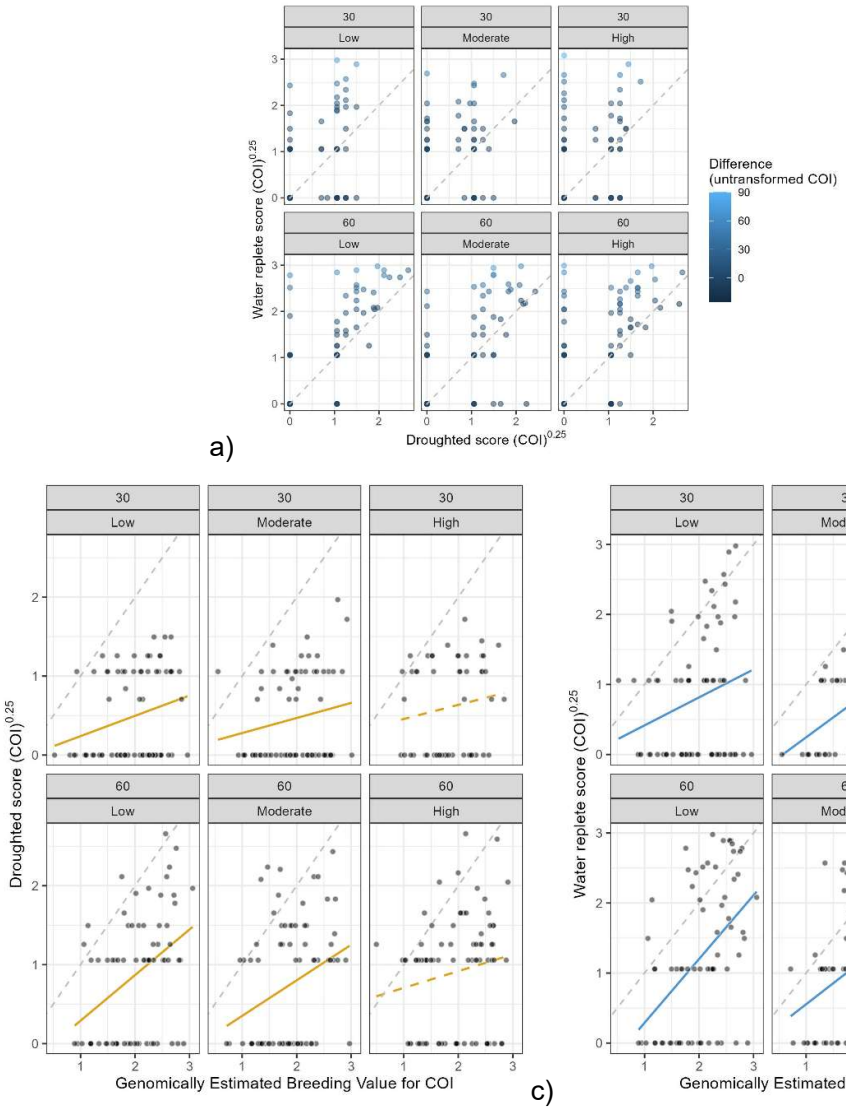


Figure S5. Comparison of post-drought myrtle rust resistance scores (Coefficient of Infection, COI), water replete rust resistance scores (COI), and Genomically Estimated Breeding Value of COI (GEBV). Higher COI scores represent higher levels of susceptibility. Possible 'escapees' (first inoculation COI score equal to 0) from the first inoculation remain in this dataset. Grey dashed lines in all plots represent a one-to-one correlation between the respective x- and y-axes. Post-drought rust resistance scores and water replete scores have been transformed by fourth root for normality and clarity. Each data point represents an individual's COI a) Individuals reinoculated with myrtle rust after droughting (x-axis) and after water replete conditions (y-axis). Data points are coloured by their untransformed COI score. b) Comparison of post-drought COI score to GEBV, faceted by their drought severity and length experienced. The orange line represents the linear correlation between both values, which exhibits a slope shallower than the one-to-one slope in grey. c) Comparison of water-replete COI score to GEBV, faceted by their

drought severity and length experienced. The blue line represents the linear correlation between both values, which exhibits an on average slope similar than the one-to-one slope in grey.

Table S3. *Summary of results for the linear model with the maternal line interacting with drought severity. Formula = COI difference ~ Drought severity * Maternal Line + Drought length*

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Maternal Line	9	34.1	3.784	4.768	3.71E-06
Drought severity	2	6.3	3.158	3.979	1.92E-02
Drought length	1	10.6	10.593	13.348	2.81E-04
Maternal Line : Drought severity	18	17.1	0.95	1.197	2.6E-01
Residuals	612	485.7	0.794		

Table S4. *Summary of results for the linear model with the maternal line interacting with drought severity. Formula = (Post-drought COI)$^{0.25}$ ~ Drought severity + Maternal Line + Drought length*

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Drought severity	2	5.8	2.922	3.907	2.06E-02
Drought length	1	12.8	12.83	17.156	3.91E-05
Maternal Line	9	81.9	9.104	12.173	< 2E-16
Residuals	630	471.1	0.748		

Table S5. *Summary of results for the a) linear model b) and the post-hoc test (Tukey's HSD) with the maternal line interacting with drought severity. Formula = COI difference ~ Drought severity + Maternal Line + Drought length*

a)

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Maternal Line	9	34.1	3.784	4.742	4.02E-06
Drought severity	2	6.3	3.158	3.956	1.96E-02
Drought length	1	10.6	10.593	13.273	2.91E-04

Residuals	630	502.8	0.798		

821

Test	Comparison	Difference	Lower	Upper	P-value adjusted
Drought length	Day 60 - Day 30	0.282	0.148	0.416	4.06E-05
Drought severity	Low-Mid	0.122	-0.069	0.314	2.91E-01
	High-Mid	0.237	0.037	0.436	1.50E-02
	High-Low	0.114	-0.085	0.314	3.70E-01
Maternal Line	NSW1078653-NSW1078641	0.065	-0.426	0.557	1.00E+00

822 b)

Test	Comparison	Difference	Lower	Upper	P-value adjusted
Drought length	Day 30 - Day 60	0.264	0.125	0.402	2.00E-04
Drought severity	Low-High	0.153	-0.054	0.359	1.92E-01
	Moderate-High	0.240	0.034	0.446	1.73E-02
	Moderate -Low	0.088	-0.110	0.285	5.49E-01
Maternal Line	NSW1079677-NSW1078647	0.165	-0.343	0.673	9.90E-01
	NSW1078653-NSW1078647	0.386	-0.124	0.895	3.26E-01
	NSW1078641-NSW1078647	0.451	-0.065	0.967	1.47E-01
	NSW1079715-NSW1078647	0.499	-0.017	1.014	6.80E-02
	NSW1078656-NSW1078647	0.589	0.087	1.092	8.06E-03
	NSW1079714-NSW1078647	0.620	0.116	1.125	4.07E-03
	NSW1079717-NSW1078647	0.625	0.117	1.133	4.07E-03
	NSW1079701-NSW1078647	0.719	0.203	1.235	4.77E-04
	NSW1079692-NSW1078647	0.766	0.269	1.263	5.58E-05
	NSW1078653-NSW1079677	0.220	-0.279	0.720	9.27E-01
	NSW1078641-NSW1079677	0.286	-0.220	0.791	7.39E-01
	NSW1079715-NSW1079677	0.333	-0.172	0.839	5.33E-01

	NSW1078656-NSW1079677	0.424	-0.068	0.916	1.61E-01
	NSW1079714-NSW1079677	0.455	-0.039	0.949	1.00E-01
	NSW1079717-NSW1079677	0.460	-0.038	0.957	9.87E-02
	NSW1079701-NSW1079677	0.554	0.048	1.059	1.92E-02
	NSW1079692-NSW1079677	0.601	0.114	1.088	3.90E-03
	NSW1078641-NSW1078653	0.065	-0.442	0.573	1.00E+00
	NSW1079715-NSW1078653	0.113	-0.395	0.621	9.99E-01
	NSW1078656-NSW1078653	0.204	-0.290	0.698	9.51E-01
	NSW1079714-NSW1078653	0.235	-0.261	0.731	8.91E-01
	NSW1079717-NSW1078653	0.239	-0.260	0.739	8.84E-01
	NSW1079701-NSW1078653	0.333	-0.174	0.841	5.38E-01
	NSW1079692-NSW1078653	0.381	-0.108	0.869	2.86E-01
	NSW1079715-NSW1078641	0.048	-0.466	0.561	1.00E+00
	NSW1078656-NSW1078641	0.138	-0.362	0.639	9.97E-01
	NSW1079714-NSW1078641	0.169	-0.333	0.671	9.87E-01
	NSW1079717-NSW1078641	0.174	-0.332	0.680	9.85E-01
	NSW1079701-NSW1078641	0.268	-0.246	0.782	8.19E-01
	NSW1079692-NSW1078641	0.315	-0.180	0.810	5.85E-01
	NSW1078656-NSW1079715	0.091	-0.409	0.591	1.00E+00
	NSW1079714-NSW1079715	0.122	-0.380	0.624	9.99E-01
	NSW1079717-NSW1079715	0.126	-0.379	0.632	9.99E-01
	NSW1079701-NSW1079715	0.220	-0.293	0.734	9.38E-01
	NSW1079692-NSW1079715	0.268	-0.228	0.763	7.87E-01

	NSW1079714-NSW1078656	0.031	-0.457	0.519	1.00E+00
	NSW1079717-NSW1078656	0.035	-0.457	0.528	1.00E+00
	NSW1079701-NSW1078656	0.130	-0.371	0.630	9.98E-01
	NSW1079692-NSW1078656	0.177	-0.305	0.658	9.77E-01
	NSW1079717-NSW1079714	0.005	-0.489	0.498	1.00E+00
	NSW1079701-NSW1079714	0.099	-0.403	0.601	1.00E+00
	NSW1079692-NSW1079714	0.146	-0.337	0.629	9.94E-01
	NSW1079701-NSW1079717	0.094	-0.412	0.600	1.00E+00
	NSW1079692-NSW1079717	0.141	-0.346	0.628	9.96E-01
	NSW1079692-NSW1079701	0.047	-0.448	0.542	1.00E+00

Table S6. *Summary of results of the a) linear model and the b) post-hoc test (Tukey HSD).*

Formula = (post-drought COI)$^{0.25}$ ~ Drought severity + Drought length + Maternal line

a)

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Maternal Line	9	81.9	9.104	12.173	< 2.0E-16
Drought severity	2	5.8	2.922	3.907	0.0206
Drought length	1	12.8	12.830	17.156	3.91E-05
Residuals	630	471.1	0.748		

b)

Test	Comparison	Difference	Lower	Upper	P-value adjusted
Drought length	Day 60 - Day 30	0.282	0.148	0.416	4.06E-05
Drought severity	Low-Moderate	0.122	-0.069	0.314	2.91E-01
	High-Moderate	0.237	0.037	0.436	1.50E-02
	High-Low	0.114	-0.085	0.314	3.70E-01

Maternal Line	NSW1078653-NSW1078641	0.065	-0.426	0.557	1.00E+00
	NSW1078656-NSW1078641	0.421	-0.063	0.906	1.51E-01
	NSW1079692-NSW1078641	0.498	0.018	0.977	3.46E-02
	NSW1079717-NSW1078641	0.600	0.111	1.090	4.27E-03
	NSW1079701-NSW1078641	0.661	0.164	1.158	1.16E-03
	NSW1078647-NSW1078641	0.810	0.311	1.309	1.53E-05
	NSW1079715-NSW1078641	0.861	0.364	1.358	2.50E-06
	NSW1079714-NSW1078641	0.944	0.458	1.430	1.00E-07
	NSW1079677-NSW1078641	1.206	0.716	1.695	0.00E+00
	NSW1078656-NSW1078653	0.356	-0.122	0.834	3.50E-01
	NSW1079692-NSW1078653	0.432	-0.041	0.905	1.08E-01
	NSW1079717-NSW1078653	0.535	0.051	1.018	1.70E-02
	NSW1079701-NSW1078653	0.595	0.104	1.087	5.11E-03
	NSW1078647-NSW1078653	0.744	0.251	1.238	9.03E-05
	NSW1079715-NSW1078653	0.796	0.304	1.287	1.61E-05
	NSW1079714-NSW1078653	0.878	0.398	1.358	4.00E-07
	NSW1079677-NSW1078653	1.140	0.657	1.624	0.00E+00
	NSW1079692-NSW1078656	0.076	-0.390	0.542	1.00E+00
	NSW1079717-NSW1078656	0.179	-0.297	0.655	9.73E-01
	NSW1079701-NSW1078656	0.239	-0.245	0.724	8.62E-01
	NSW1078647-NSW1078656	0.388	-0.098	0.875	2.52E-01
	NSW1079715-NSW1078656	0.439	-0.045	0.924	1.13E-01
	NSW1079714-NSW1078656	0.522	0.050	0.995	1.73E-02

	NSW1079677-NSW1078656	0.784	0.308	1.261	1.03E-05
	NSW1079717-NSW1079692	0.103	-0.369	0.574	1.00E+00
	NSW1079701-NSW1079692	0.163	-0.316	0.643	9.86E-01
	NSW1078647-NSW1079692	0.312	-0.169	0.794	5.57E-01
	NSW1079715-NSW1079692	0.363	-0.116	0.843	3.23E-01
	NSW1079714-NSW1079692	0.446	-0.022	0.914	7.61E-02
	NSW1079677-NSW1079692	0.708	0.237	1.180	9.90E-05
	NSW1079701-NSW1079717	0.060	-0.429	0.550	1.00E+00
	NSW1078647-NSW1079717	0.209	-0.282	0.701	9.41E-01
	NSW1079715-NSW1079717	0.261	-0.229	0.750	8.01E-01
	NSW1079714-NSW1079717	0.343	-0.135	0.821	4.03E-01
	NSW1079677-NSW1079717	0.605	0.124	1.087	2.93E-03
	NSW1078647-NSW1079701	0.149	-0.350	0.648	9.95E-01
	NSW1079715-NSW1079701	0.200	-0.297	0.697	9.58E-01
	NSW1079714-NSW1079701	0.283	-0.203	0.769	7.03E-01
	NSW1079677-NSW1079701	0.545	0.056	1.034	1.58E-02
	NSW1079715-NSW1078647	0.051	-0.448	0.550	1.00E+00
	NSW1079714-NSW1078647	0.134	-0.354	0.622	9.97E-01
	NSW1079677-NSW1078647	0.396	-0.096	0.888	2.40E-01
	NSW1079714-NSW1079715	0.083	-0.403	0.569	1.00E+00
	NSW1079677-NSW1079715	0.345	-0.145	0.834	4.33E-01
	NSW1079677-NSW1079714	0.262	-0.216	0.740	7.72E-01

Table S7. *Summary of results for the linear model using the mean annual precipitation* (MAP) *of the maternal line's collection location interacting with drought severity. Formula = Median gsw ~ Drought severity * MAP + Drought length*

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
MAP	1	0.0001	0.000097	0.058	8.09E-01
Drought severity	2	0.04996	0.02498	15.114	9.72E-07
Drought length	1	0.00626	0.006258	3.786	5.34E-02
MAP : Drought severity	2	0.00525	0.002624	1.588	2.08E-01
Residuals	160	0.26444	0.001653		

Table S8. *Summary of results for the linear model using the mean annual precipitation* (MAP) *of the maternal line's collection location interacting with drought severity. Formula = COI difference ~ Drought severity * MAP + Drought length*

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
MAP	1	6.5	6.543	7.854	5.23E-03
Drought severity	2	6.1	3.049	3.66	2.63E-02
Drought length	1	11.1	11.137	13.368	2.77E-04
MAP : Drought severity	2	0.1	0.073	0.087	9.16E-01
Residuals	636	529.8	0.833		

Table S9. *Summary of results for the linear model using the mean annual precipitation* (MAP) *of the maternal line's collection location interacting with drought severity. Formula = (Post-drought COI)$^{0.25}$ ~ Drought severity * MAP + Drought length*

	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Drought severity	2	5.800	2.922	3.56	0.0289
MAP	1	31.300	31.343	38.2	1.12E-09
Drought length	1	13.000	13.022	15.9	7.51E-05
MAP : Drought severity	2	0.200	0.104	0.127	0.8807

Residuals	636	521.3	0.820		