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The Impacts of Drought Stress and *Phytophthora cinnamomi* Infection on Short-Term Water Relations in Two Year-Old *Eucalyptus obliqua*

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Abstract: The effects of drought stress, *Phytophthora cinnamomi* infection and their interaction on water relations and growth were examined for 28 days on two year-old potted trees of *Eucalyptus obliqua* (L'Hér.). There were significant effects of drought stress on plant photosynthesis, stomatal conductance, biomass accumulation, plant water potential at turgor loss point and the bulk modulus of elasticity. *E. obliqua* was successfully infected but the trees showed only mild symptoms. Infection with *P. cinnamomi* led to a significant reduction in the root biomass and root-to-shoot ratio in well-watered and droughted plants but did not impact water relations. There was no observable cumulative effect of drought and *P. cinnamomi* infection. There are multiple potential reasons why *P. cinnamomi* infection did not lead to drought-like symptoms in *E. obliqua*, including short experimental duration, delayed infection symptoms, potential resistance of *E. obliqua* and a possible lower aggressiveness of the *P. cinnamomi* strain. Hence, our results indicate that *P. cinnamomi* infection will not always lead to immediate short-term symptoms, and that plants that are mildly symptomatic respond very similar to drought stress compared to non-infected trees.

Keywords: drought; *Phytophthora*; *Eucalyptus*; dieback; plant water relations

Citation: Umami, M.; Parker, L.M.; Arndt, S.K. The Impacts of Drought Stress and *Phytophthora cinnamomi* Infection on Short-Term Water Relations in Two Year-Old *Eucalyptus obliqua*. *Forests* **2021**, *12*, 109. https://doi.org/10.3390/f12020109

Received: 24 November 2020
Accepted: 16 January 2021
Published: 20 January 2021

1. Introduction

Drought stress is one of the major factors leading to the degradation of forests worldwide [1–4]. Biotic agents such as pathogens can also contribute to tree mortality and potentially exacerbate the impact of drought stress [5]. The presence of plant pathogens has been correlated to tree dieback in a range of forest ecosystems [6,7]. Furthermore, the interaction between drought and disease could influence deterioration of tree health status [8,9].

Phytophthora species are some of the most invasive fungal pathogens in the world [10–12]. They have been associated with tree decline in many ecosystems, including native forests and urban environments in a large variety of tree species across the world [13–20]. In Australia, *Phytophthora* has been strongly linked to many cases of tree dieback in both native and urban ecosystems [21,22], in Victoria [23], Northern Queensland [24] and Western Australia [25,26]. *Phytophthora* has a wide range of host plants, including many Australian native plant species [27], e.g., members of the genus *Eucalyptus* [20,21]. This pathogen is considered as a major threat to the Australian biodiversity under the Environmental Protection and Biodiversity Conservation Act 1999 [28,29].

The major stress of *Phytophthora* infection is associated with the impairment of the plants root system [24]. Zoospores are attracted to root exudates, germinate in the root cap cell zone, and develop mycelium in the cortical cells, phloem and xylem of the infected fine roots [27,30,31]. The infection then leads to the formation of necrotic lesions and eventually damages the roots, leading to inefficiency of water and nutrient uptake [28]. Accordingly, the infected host plants could develop symptoms similar to those under drought stress,

including leaf chlorosis, canopy dieback and the development of epicormic shoots and mortality [19,21].

In natural ecosystems, especially in Mediterranean ecosystems, *Phytophthora* infections often occur in wet and warm winters. The infected plants are subsequently exposed to hot and dry summers, and the compromised root system may by unable to supply the plants with sufficient water [30]. However, there is conflicting evidence if *Phytophthora*-infected plants actually experience water deficit, similar to those under drought-induced stress. It has been reported that *Phytophthora* infection leads to the presence of drought stress symptoms due to the inefficiency of water and nutrient uptake and thus causes water deficit in plants [15,27]. This has been confirmed in some studies where significant differences in water potential, stomatal conductance, and photosynthesis occurred following *P. cinnamomi* infection [32,33]. However, other studies did not find a significant effect of *P. cinnamomi* infection on plant water relations [34–36]. Furthermore, it has been suggested that drought events might worsen the impacts of *Phytophthora* infection on plants by lowering the plant resistance [28] or increasing the inoculum production and infection rate [37,38]. Consequently, drought events that frequently occur in Australia potentially exacerbate tree decline due the drought-induced water deficit on infected plants. The effects of climate change might also result in changes in the distribution of forest pathogens such as *Phytophthora* with some areas becoming more or less likely to support the species depending on temperature and water availability [39]. Nevertheless, there is insufficient evidence that the *Phytophthora* infection leads to more severe symptoms in a plant that initially has undergone water deficits during a drought event. Since the symptoms of *Phytophthora* infection resemble drought symptoms, it is often challenging to identify *Phytophthora* infection in the field [13]. As a consequence, the pathogens' contribution to tree mortality is often overlooked, thus highlighting the importance of understanding the relationship between drought stress and *Phytophthora* infection on tree mortality.

This study investigated the effects of drought, *P. cinnamomi* infection, and the interaction of both factors on the water relations and gas exchange in *Eucalyptus obliqua.* Three hypotheses were examined: (1) Drought stress significantly decreases plant water potential, gas exchange, and biomass accumulation due to limited soil water availability; (2) *P. cinnamomi* infection results in plant water deficit which leads to drought stress symptoms described above; (3) The interaction between drought stress and *P. cinnamomi* infection will increase drought stress symptoms due to the additive impacts of both factors on limiting water uptake through the roots.

2. Materials and Methods

2.1. Plant Material

Eucalyptus obliqua (L'Her.) seedlings for this study were sourced as tube stock from a commercial nursery (Bushland Flora, Mt. Evelyn, VI, Australia) in winter and grown in a shade house at the Burnley campus of The University of Melbourne, Victoria, Australia. In October 2018, the four months-old seedlings were about 0.3 m tall and transplanted into 9-L pots containing a mix of 50:30:20 medium, comprising pine bark (3–5 mm), expanded coir (fine grade: 0–6 mm), and coarse horticultural sand in 6-L pots. In addition, 4 kg Macracote Coloniser fertiliser plus (Fertool, Dandenong, VIC, Australia), consisting of 8–9 moths, N, P, K (15, 13, 9), and trace elements were added as the supply of macro and micronutrients. The plants were grown for a further four months in an open area in the nursery and watered once daily to soil capacity before the start of the experiment. In February 2019, the plants were transferred to a rainout shelter with open side walls where the experiment took place and were between 1.2–1.4 m tall at the start of the experiment.

2.2. Experimental Design

The experiment was conducted in a completely randomized design with two main factors; Drought and Disease (pathogen inoculation with *P. cinnamomi*). The combination of the two factors resulted in four treatments: *Control (Con)* (well-watered and non-inoculated),

Drought (Dro) (deficit irrigation and non-inoculated), *Disease (Phy)* (well-watered and inoculated), and combination of *Drought and Disease* (*DroPhy*) (deficit irrigation and inoculated). Due to an uneven number of trees, we allocated six replicate trees for *Con* and seven replicates for each of other three treatments (*Dro*, *Phy*, and *DroPhy*).

During the experiment, two pots of the same disease factor were placed in 60-L black plastic containers to prevent cross contamination between inoculated and non-inoculated plants. The pots were placed on a raised mesh within the plastic containers so that pots could drain freely and no cross contamination between the pots in the same container would occur. The trays were distributed randomly in the experimental area to eliminate bias due to potential differences in microclimatic conditions in the rainout shelter. The climatic conditions under the rainout shelter were similar to the outside conditions, with small reductions (5%–10%) of the level of photosynthetic active radiation due to the plastic cover of the shelter (GroTuff greenhouse plastic 180UM, Sage Horticultural, Hallam, VIC, Australia) and no rainfall.

2.3. Inoculation with Phytophthora and Re-Isolation

In December 2018, we conducted a preliminary study using four pots of *E. obliqua* seedlings to determine the time required for *P. cinnamomi* to infect the seedlings roots. The *P. cinnamomi* inoculum was isolated from soil samples collected from field sites in Eastfield Park, Maroondah Melbourne, where tree decline and the presence of *P. cinnamomi* had been observed. *P. cinnamomi* was isolated from soil by baiting using *Eucalyptus sieberi* cotyledons floated on water on the soil [40]. After five days the cotyledons were then removed from the water and placed on potato dextrose agar (PDA) plates [41]. The agar plates were stored in a cool environment to provide suitable environment for *Phytophthora* to grow for ten days [10].

The seedlings were inoculated with *P. cinnamomi* by pouring 100 mL of the *Phytophthora* inoculum solution into each pot. The suspension was prepared by suspending half of a 90 mm diameter PDA-cultured inoculum in 1000 mL water. The solution was stored in dark and cool environment for five days to promote the growth of active sporangia [42]. Four weeks after inoculation, the infection of roots was evaluated by collecting roots from plants in infected pots with a small cork borer and plating of root samples in PDA medium. Prior to the plating, the root samples were cleaned under distilled running water and placed in distilled water in a shallow container. After 24 h, the roots were sterilized using 70% ethanol for 20 sec and rinsed in demineralized water prior to the plating to suppress the presence of contaminants [43]. The *P. cinnamomi* species was evaluated with microscopy and identified based on and the colony growth pattern on PDA after 5–7 days, as described and illustrated in [10] and its morphological characteristic was examined as described and illustrated in [44].

In the preliminary study, *Phytophthora* was successfully re-isolated from the roots four weeks after inoculation. Dieback symptoms such as leaf chlorosis and wilting were also observed, and symptoms gradually worsened until the end of the trial. However, no tree mortality occurred during the experiment. Results of this trial were used to estimate the time for *Phytophthora* inoculation prior to the main experiment.

In late February 2019, plants subjected to Phy and DroPhy treatments were inoculated using the same method outlined above. Four weeks after inoculation, however, there were no visible symptoms of the infection on aboveground organs of the infected plants.

In March 2019, all disease treatment plants were re-inoculated using a second *P. cinnamomi* inoculum sourced from infected soil samples taken from field where *P. cinnamomi* symptoms were observed. *P. cinnamomi* in the soil was again baited by using fresh *Rhododendron spp.* leaves [45] sourced from the Burnley gardens. The infected leaf was cut into 5 mm × 5 mm pieces, placed in PDA media and grown for 7 d [10]. The inoculum was made of 90 mm diameter section of the pure culture of *P. cinnamomi* diluted in 1400 mL of deionized sterile water. Each pot was given 100 mL of liquid inoculum and in addition five pieces of 0.5 mm × 0.5 mm block of agar containing *P. cinnamomi* mycelium.

The *P. cinnamomi* was re-isolated from roots as described above after 14 d confirming successful inoculation.

2.4. Watering

Before the start of the study, all plants were hand watered to field capacity in the afternoon every two days. A week before the experiment, the weights of every pot were recorded before and after watering to estimate daily plant water use to determine the level of irrigation for Dro and DroPhy treatments. The irrigation requirements were calculated based on the average daily water use of each plant, which was gradually decreased by 10% every week for the plants in both drought treatments. Plants in the Dro and DroPhy treatments showed signs of rapid drought stress after the first observation. To prevent excessive plant stress, the irrigation level was increased from 80% of the estimated daily evapotranspiration to 80% of saturated pot weight. Subsequently, the irrigation requirements for the drought treatment were calculated based on the soil water capacity instead of daily evapotranspiration. The irrigation level of drought treatment was decreased by 10% every week (Figure 1), whereas the well-watered plants received water at field capacity (3L) at the same time.

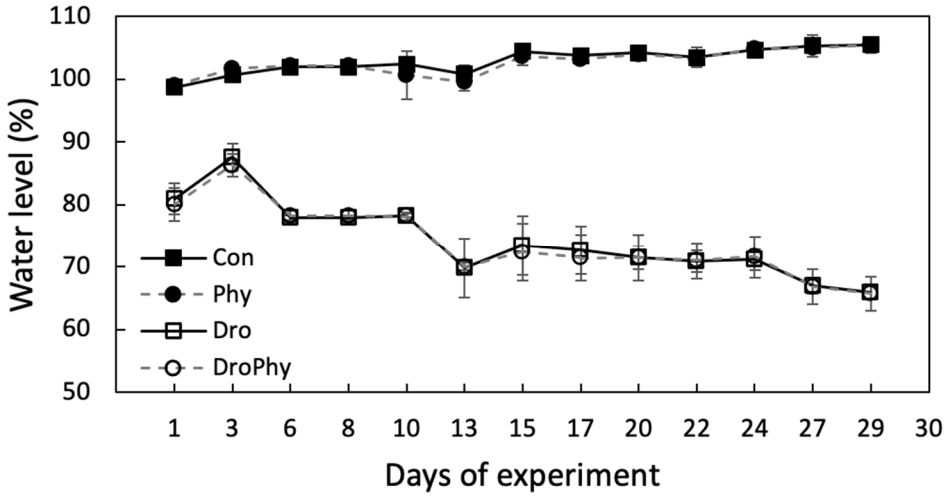

Figure 1. Relative pot weight as a percentage of the initial pot weight of *E. obliqua* seedlings subjected to Control (Con), Drought (Dro), *Phytophthora* (Phy), and Drought-*Phytophthora* (DroPhy) treatments. Bars indicate ± standard errors of the mean values. Where no error bars are visible, the values are smaller than the size of the symbol.

2.5. Data Collection

2.5.1. Water Relations

Pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential of all trees were measured once a week using a pressure chamber (3000 Series Plant Water Status Console, Soilmoisture Equipment Corp. Goleta, CA, USA) [46]. A fully expanded leaf with long petiole was cut from each tree, Ψ_{pd} samples were collected 30 min before sunrise and Ψ_{md} samples were collected at 13:00 h. Leaf samples were stored in ziplocked polyethylene bags inside a non-transparent box with an icepack to prevent water loss due to transpiration during the transportation from the nursery to the laboratory [47]. The samples were measured within one hour of collection.

2.5.2. Gas Exchange

Gas exchange of leaves was measured with an infrared gas analyzer (LI6400, Licor, Lincoln, NE, USA) once every week between 10:00 and 13:00 h on the same day as the water relations measurements on fully expanded leaves of each plant with two to three replicates per plant. This method was non-destructive to the leaves. Irradiance was set

at 1800 µmol m^{-2} s^{-1}, block temperature was set at 25 °C, and air flow rate through the chamber was 400 mL min^{-1}. Readings were taken after steady state was achieved, usually after three minutes for each measurement. Light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) were used as the key measurements for gas exchange.

2.5.3. Pressure-Volume (PV) Curves

Pressure volume (PV) curves were determined using the same pressure chamber described above. Two fully expanded leaves were collected from each plant at 08:00 h. The samples were rehydrated via the petiole in 50 mL Sarstedt tubes filled with 15 mL of distilled water inside a non-transparent box for about three hours. This is usually a sufficient amount of time to achieve rehydration of leaves of eucalypts [48]. One leaf sample per plant was used in the PV analysis. The leaf was weighed to determine its fresh weight and then the water potential was determined in the pressure chamber. The leaf was then allowed to dry out on a bench at room temperature and the process of leaf weighing and water potential measurement was repeated. Each leaf was measured approximately 12–15 times until 4–5 water potential measurements were collected that were more negative than the water potential at turgor loss point. Upon completion of the measurements, the leaf was oven-dried at 80 °C until constant weight and the dry weight obtained. The data were then processed to determine the osmotic potential at full turgor (π_{100}), water potential at Turgor Loss Point (Ψ_{TLP}), relative water content at turgor loss point (RWC_{TLP}), apoplastic water fraction (R_a), and bulk modulus of elasticity (ε) based on Schulte and Hinckley [49] using an Excel spreadsheet downloaded from Landflux webpage: http://landflux.org/Tools.php.

2.5.4. Final Harvest Biomass Assessment

All trees were harvested after four weeks. Leaves, branches, and stems were separated and stored in a weighed paper bag and oven-dried to constant weight at 80 °C. Roots were separated from potting mix in a root washing bay, bagged and also dried at 80 °C. The dry weight of each sample was recorded. Above ground biomass was determined from the combined dry weights of leaves, branches, and the stem (g). Below ground biomass was equal to the dry weight of root samples (g). The root-to-shoot ratio was calculated as the ratio of above ground biomass to below ground biomass.

2.6. Statistical Analysis

All data were analysed using one-way analysis of variance (ANOVA) to examine the effect of the treatments. Fisher's least significant difference (LSD) test was performed to determine the significant differences ($p \leq 0.05$) between treatments using Minitab version 17 statistical software (Minitab, LLC, State College, PA, USA).

3. Results

3.1. Phenotypic Symptoms of Drought and Pathogen

Well-watered plants had no drought-like symptoms (leaf necrosis or chlorosis, leaf wilting, leaf rolling) after four weeks, regardless of inoculation (Phy or Con). Leaf chlorosis and wilting were observed in the droughted plants; however, there were no differences between inoculated (DroPhy) and non-inoculated (Dro) treatments. Seedlings in both well-watered treatments (Phy and Con) had healthier-looking leaves that were greener in colour compared to the plants in both drought treatments.

Root necroses was not visible in any of the inoculated plants (Phy and DroPhy). Control plants appeared to have more vigorously growing roots compared to the other treatments and had more fine roots with a brighter colour. No visual differences were observed for roots of plants in drought (Dro) and drought-*Phytophthora* (DroPhy) treatments.

Despite the absence of root necroses, *P. cinnamomi* was re-isolated from most of the root samples that were collected randomly from the infected plants, indicating that the inoculation treatments were successful. In addition, *P. cinnamomi* was not recovered from

the root samples of non-inoculated plants (Con and Dro) indicating there was no cross-contamination between inoculation and no-inoculation treatments.

3.2. Water Relations

The pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) water potentials of the *E. obliqua* indicated that there were significant effects of the drought treatments but no effects of *P. cinnamomi* inoculation treatments. Both Ψ_{pd} and Ψ_{md} were statistically different between well-watered and drought treatments by the end of the experiment, regardless of *Phytophthora* inoculation (Figure 2).

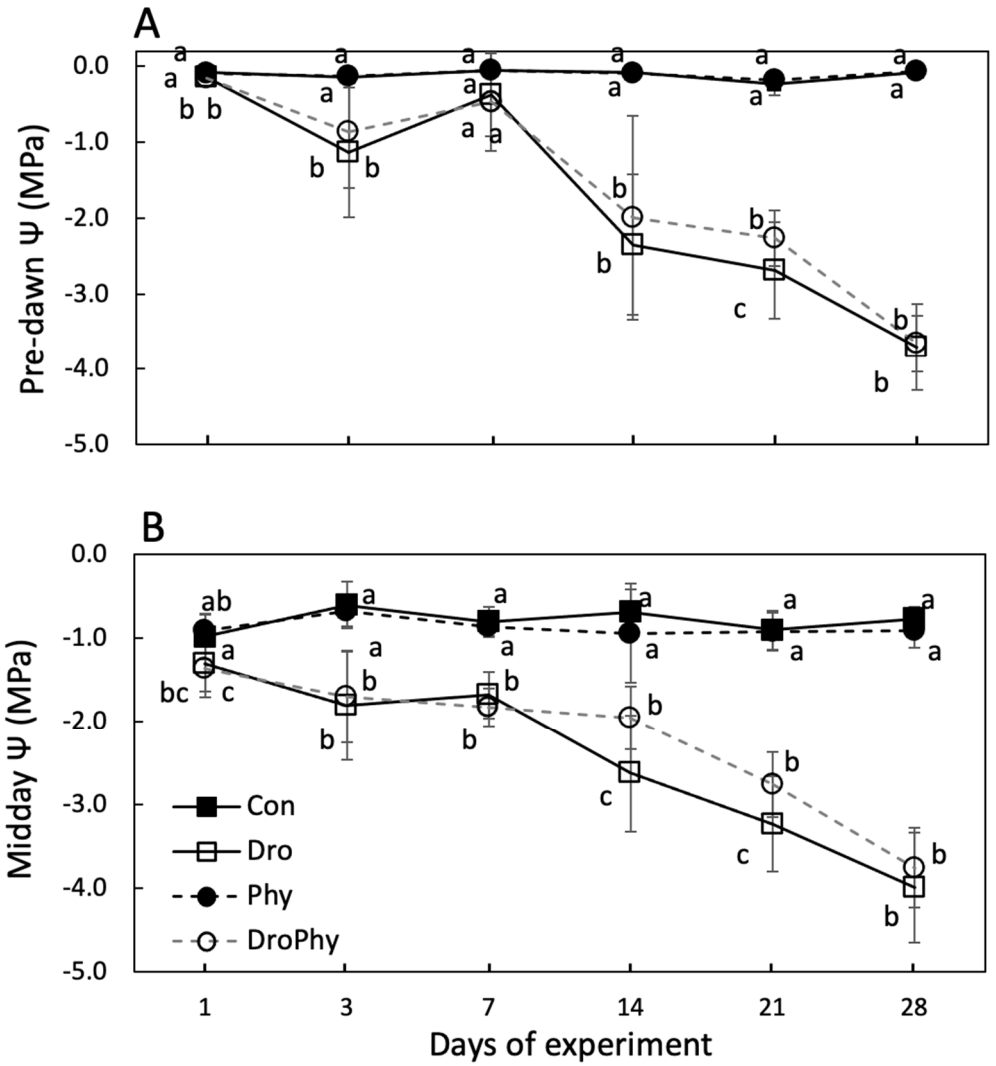

Figure 2. (**A**) Pre-dawn (Ψ_{pd}) and (**B**) midday (Ψ_{md}) water potentials (MPa) of E. obliqua seedlings subjected to Control (Con), Drought (Dro), *Phytophthora* (Phy), and Drought-*Phytophthora* (DroPhy) treatments for five weeks. Error bars indicate ± standard error of the mean values; where no error bars are visible, the values are smaller than the symbol. Means with different letters are significantly different at $p \leq 0.05$, after ANOVA and LSD test.

Both the Ψ_{pd} and Ψ_{md} of well-watered (Con and Phy) plants were relatively constant, with Ψ_{pd} ranging from −0.05 to −0.24 MPa and Ψ_{md} ranging from −0.60 to −0.99 MPa. There were no significant differences between Con and Phy treatments by the end of the experiment. The Ψ_{pd} and Ψ_{md} of plants in drought treatments (Dro and DroPhy) gradually decreased, and by the end of the experiment Ψ_{pd} were around −3.70 MPa and Ψ_{md} ranged from −3.76 to −3.99 MPa. There were no statistically significant differences between Dro and DroPhy plants. On a few occasions, however, significant differences between Dro and

DroPhy occurred as on the fifth observation of Ψ_{pd} and fourth and fifth observations of Ψ_{md}, which were primarily driven by few individual plants with lower water potential in the Dro treatment.

3.3. Gas Exchange

Light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) was significantly reduced in both drought treatments (Figure 3). The g_s of well-watered treatments (Con and Phy) showed some fluctuation and ranged from 0.14 to 0.38 mol H_2O m^{-2} s^{-1}, but there were no statistically significant differences between Con and Phy treatments. The g_s of the two drought treatments (Dro and DroPhy) were always lower compared to the well-watered treatments, and decreased considerably with increasing drought. In the last three weeks of the experiment stomata were almost completely closed in both drought treatments. There were no significant differences for g_s between Dro and DroPhy treatments (Figure 3).

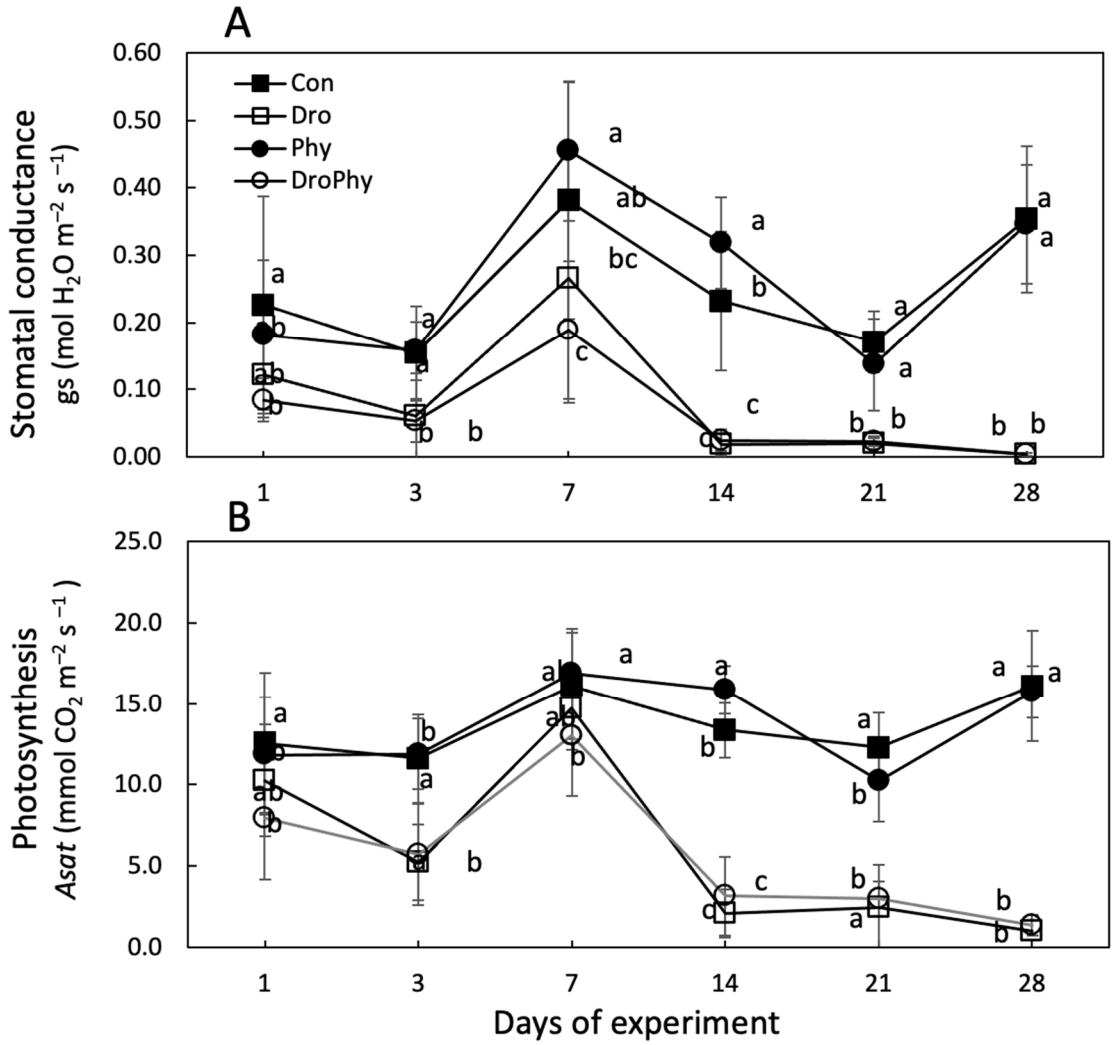

Figure 3. (**A**) Stomatal conductance (g_s) and (**B**) light-saturated photosynthesis rate (A_{sat}) of *E. obliqua* seedlings subjected to Control (Con), Drought (Dro), *Phytophthora* (Phy), and Drought- *Phythophthora* (DroPhy) after ANOVA and LSD test.

The light saturated photosynthesis (A_{sat}) of well-watered treatments (Con and Phy) fluctuated and ranged from 10 to 17 mmol CO_2 m^{-2} s^{-1}. There were no significant differences between the Con and Phy treatments at the end of the experiment. The A_{sat} of the two drought treatments (Dro and DroPhy) significantly decreased on the fourth observation and at the end of the experiment where A_{sat} was close to zero (around 1 mmol

CO_2 m^{-2} s^{-1}). There were no significant differences between Dro and DroPhy treatments (Figure 3).

3.4. Biomass

The aboveground biomass of *E. obliqua* plants subjected drought treatments (Dro and DroPhy) was significantly lower than both well-watered treatment plants (Con and Phy) (Figure 4). However, inoculation had no significant effect on aboveground biomass in well-watered or droughted plants.

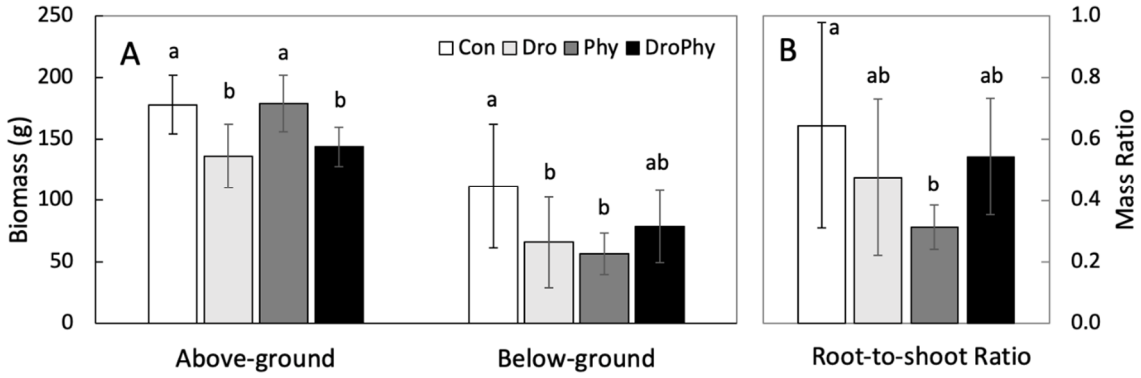

Figure 4. (**A**) Final above- and belowground biomass and (**B**) root-to-shoot ratio of *E. obliqua* seedlings subjected to Control (Con), Drought (Dro), *Phytophthora* (Phy), and Drought-*Phytophthora* (DroPhy) treatments. Error bars indicate ± standard error of the mean values. Means with different letters are significantly different at $p \leq 0.05$, after ANOVA and LSD test.

There were significant differences in below-ground biomass with well-watered (Con) plants having a greater root biomass than all other treatments, and well-watered inoculated (Phy) plants having significantly lower root biomass compared to the control treatment (Figure 4). Phy plants also had the lowest root:shoot ratio and there were significant differences between Phy and Con, but no differences between Phy, Dro, and DroPhy.

3.5. Pressure-Volume (PV) Analysis

Pressure-volume (PV) analysis showed no significant differences between treatments of the osmotic potential at full turgor (π_{100}) or leaf relative water content at turgor loss point (RWC_{TLP}) (Table 1). However, plants in the two drought treatments (Dro and DroPhy) had significantly lower water potential at turgor loss point (TLP) and decreased bulk modulus of elasticity (ε) compared to both well-watered treatments (Con and Phy) (Table 1).

Table 1. Osmotic potential at full turgor (π_{100}), water potential at turgor loss point (Ψ_{TLP}), relative water content at turgor loss (RCW_{TLP}) and bulk modulus of elasticity (ε) for *Eucalyptus obliqua* trees under well-watered control conditions or subjected to drought and *Phytophthora* infection. Data are means with standard error (n = 6). Treatments with the same letter do not differ significantly at $p \leq 0.05$, after ANOVA and LSD test.

Treatment	π_{100}	Ψ_{TLP}	RWC_{TLP}	ε
Con	−1.48 ± 0.05	−1.63 ± 0.05 ^{a}	0.91 ± 0.01	16.60 ± 2.25 ^{ab}
Phy	−1.52 ± 0.13	−1.67 ± 0.09 ^{a}	0.91 ± 0.01	20.47 ± 7.65 ^{a}
Dro	−1.75 ± 0.26	−2.18 ± 0.60 ^{b}	0.87 ± 0.07	12.14 ±4.77 ^{b}
DroPhy	−1.65 ± 0.13	−1.93 ± 0.35 ^{b}	0.87 ± 0.03	12.38 ± 4.96 ^{b}

4. Discussion

The first hypothesis was confirmed as drought stress significantly decrease plant water potential (Figure 2), stomatal conductance (Figure 3A), photosynthesis (Figure 3B) and above-ground biomass (Figure 4A) in both drought treatment plants. Moreover, we observed drought symptoms such as leaf wilting and chlorosis, consistent with previous

studies [50–52]. Limited soil water availability can lead to plant water deficit and therefore, substantially reduce plant water potential in most plant species [53]. Furthermore, many plants species, including *Eucalyptus* species, tend to close their stomata to reduce water loss following the onset of initial drought stress, which leads to a decrease in photosynthesis and ultimately limits plant growth [52].

Drought stress also affected longer-term water relation traits in *E. obliqua* as both Ψ_{TLP} and ε were reduced in plants of both drought treatments compared to plants in the well-watered treatments (Table 1). This decrease of the Ψ_{TLP} of plants subjected to drought supports previous studies, as many plants species under drought stress adjust their turgor loss point to maintain physiological activity with declining leaf water content [54,55]. This adjustment is often achieved by osmotic adjustment, or the increase in leaf solutes, which in turn leads to a decrease in the π_{100} which is seen as the main mechanism of turgor maintenance [56]. However, while the π_{100} was lower in both the drought treatments (Table 1) the change was not significant compared to the well-watered treatments, indicating that leaf solute accumulation alone did not lead to turgor maintenance. This is unusual because osmotic adjustment is a common response of *Eucalyptus* species to drought stress [56,57]. Instead, turgor maintenance was achieved by means of elastic adjustment. The cell walls of the *E. obliqua* leaves became more elastic in plants under drought stress, which is indicated by the significantly lower ε in both the Dro and DroPhy treatment plants (Table 1). These plants also had a lower (but not significantly different) relative water content at turgor loss point. Hence, more elastic cell walls allowed for more water to be lost before turgor was lost. Elastic adjustment is not often observed, but is recognized as a mechanism for turgor maintenance under water deficit conditions [58].

The second hypothesis was that *P. cinnamomi* infection would result in water deficit through reduced root water uptake, which then would lead to drought stress symptoms. However, our results did not confirm this hypothesis. *P. cinnamomi* infection often results in root necroses, which eventually damages the root system, and reduces plant water uptake [59,60]. The reduction of plant water uptake could cause water deficit in the plant and thus potentially decreases plant water potential and stomatal conductance [61,62]. This can also limit plant growth and often leads to the development of drought-like symptoms [19,21,60]. In this experiment, *P. cinnamomi* infection did not lead to root necroses and the development of drought-like symptoms. In addition, it did not significantly affect plant water relations (Figure 2, Table 1), photosynthesis (Figure 3B), stomatal conductance (Figure 3A), aboveground biomass (Figure 4A), and other drought tolerance traits. Nevertheless, *P. cinnamomi* infection significantly reduced average root biomass in well-watered inoculated plants (Phy) compared to control, and the root:shoot ratio of Phy was the lowest among all treatments (Figure 4B). In other studies, *Phytophthora* infection decreased root biomass by inhibiting the growth of new fine roots [35,61,62]. The absence of primary or secondary symptoms of *Phytophthora* inoculation, such as root necrotic and drought-like symptoms, is in contrast to previous studies [19,21,60,63]. However, our results are similar to Turco et al. [36], who observed no symptoms in *Quercus ilex* and *Q. cerris* that were inoculated by *P. cinnamomi* in a full irrigation treatment, although root necrosis was eventually observed after 11 weeks. The occurrence of secondary symptoms following the *P. cinnamomi* infection might be latent depending on the pathogen aggressiveness, host susceptibility and environmental condition [22,61]. A time lag of 6 to 18 months can occur during *Phytophthora* infection until drought-like symptoms are observed in field studies [22,35,61].

The decrease of plant water potential in some species following *P. cinnamomi* infection often causes severe root damage which limits plant water uptake to the point that the remaining roots are insufficient to meet plant water demand [60,64]. Similarly, the reduction in root mass could have contributed to the reduction of stomatal conductance, which tends to lower photosynthesis and eventually limits carbon gain for plant growth, as the trees adjusted their water balance following the reduction in plant water uptake by avoiding water loss from transpiration [59,61,62]. Some studies also reported that

although *P. cinnamomi* infection did not significantly affect plant water potential, the root loss following the infection is more likely to reduce stomatal conductance as it is more sensitive to root loss compared to water potential [59,60]. Additionally, *Phytophthora* infection can affect the cytokinin and phenolics which can be responsible for controlling stomata closure [65]. In our experiment, severe root damage was not observed in the infected plants, although lower root biomass was observed in the infected trees with full irrigation, suggesting that *P. cinnamomi* did not cause water deficit in *E. obliqua* as the plants still had sufficient roots to meet their water demand over the experiment period. These results are consistent with some previous studies such as Maurel et al. [61] and Turco et al. [36] which reported that *Phytophthora* infection does not always cause plant water deficit. Maurel et al. [61] suggested that *Phytophthora* infection will potentially lower plant water relations if more than 90% of the plants roots are damaged. This conclusion was also confirmed by Crombie et al. [66] that showed plant water potential was not affected until more than 80% of roots were removed.

Other factors could have contributed to the lack of effects of *P. cinnamomi* infection on plant water relations in this experiment including the relative short duration of the experiment, resistance of plants to the pathogen, delayed response of the plants to the infection and unexpected adaptation to the stress. A lack of effects of *P. cinnamomi* on plant water potential despite the development of root necrosis can be caused by high tolerance of the trees to the infection and delayed response of the trees to the infection [36]. This was previously observed in species with higher resistance to *P. cinnamomi* [67]. *P. cinnamomi* infection can affect resistant species; however, the progression of the symptoms is slower than in more susceptible species [68]. Differences in infection symptoms can be caused by differences in the defense response between susceptible and resistant species to *P. cinnamomi* [69].

The third hypothesis was that the interaction between drought stress and *P. cinnamomi* infection would increase the drought stress symptoms in plants due to the additive impacts of both factors on limiting plant water uptake. However, we observed no additional effects of *P. cinnamomi* infection under drought treatment, indicating that there were no cumulative effects of the interaction of both factors. It has been suggested that drought conditions could ameliorate the effect of *Phytophthora* on plants [64,68]. Soil drought can limit *Phytophthora* growth, which can inhibit the build-up of the inoculum and thus also reduce the rate of *Phytophthora* infection [70]. This was reported for *Eucalyptus marginata* where drought was impacted the growth of *P. cinnamomic* [71]. Consequently, more significant effects of *Phytophthora* infection were mostly observed in wet soils compare to dry soils [62,72,73]. Similarly, greater root loss was observed in infected plants with full irrigation compared to deficit irrigation [62]. Weste and Ruppin [70] also suggest that ecosystem devastation caused by *Phytophthora* was greater in areas with frequent waterlogging and poor drainage. In our experiment, the soil of the plants was kept moist after inoculation, thus favoring *P. cinnamomi* growth. But the soil in the drought treatments was very dry. The effects of *P. cinnamomi* infection on root conditions were more apparent in the inoculated plants with full irrigation rather than the droughted plants. Although plant water potential, photosynthesis, stomatal conductance, and above ground biomass accumulation of the plants in the DroPhy treatment were similar to those in the drought treatment, this was likely due to limited soil water availability rather than *P. cinnamomi* infection.

The absence of effects of *Phytophthora* infection on *E. obliqua* water relations, gas exchange and biomass accumulation in this study could also be due to other factors: First, it could be related to the low pathogenicity of the *Phytophthora* species used for inoculation. Zentmyer and Guillemet [42] reported that more than 300 *P. cinnamomi* isolates were distributed in the world and that each of them has different pathogenicity. However, we did not examine the strain and pathogenicity of *P. cinnamomi* used and its aggressiveness is unknown. This strain was isolated from soil in parks in Maroondah, Melbourne, where many heavily declined and dead eucalypt trees are present, including *E. obliqua*. The pathogen was isolated from the soil, was present in roots of eucalypts in the parks, and

it led to canopy decline and tree death, so it has had some pathogenicity. Several other *Phytophthora* were also isolated from the reserves and future investigations that examine the pathogenicity of each of these *Phytophthora* would help to understand its aggressiveness and the species that are susceptible [74]. It is also possible that *P. cinnamomi* may have lost its virulence after subculturing *in vitro*.

Second, it is also possible that this population of *E. obliqua* has a greater resistance to *P. cinnamomi*. A study by Stukely and Crane [75] demonstrated that some resistant trees were discovered among 16 *E. marginata* provenances, which are known as one of the most susceptible *Eucalyptus* species to *P. cinnamomi*. This *Phytophthora* resistance was strongly controlled by genetic factors, and the resistance of provenances was based on the mortality rate and lesion length following stem inoculation [75]. Since resistant trees have a lower probability of being severely affected by *P. cinnamomi* infection [67], future studies on intra-species variation of susceptibility to *P. cinnamomi* would also be useful. Should this particular *E. obliqua* provenance be more resistant to *P. cinnamomi*, it could be selected for breeding for rehabilitation projects in *P. cinnamomi* declined areas.

Third, it is also possible that our experimental period was too short, as *P. cinnamomi* could take a longer time to affect the trees [22,35,61]. The effects of *Phytophthora* infection can develop slowly, depending on the aggressiveness of the pathogen, the plant condition and the growth condition. In other studies, it could take one to two years of observation before the impacts of *P. cinnamomi* infection on plants were apparent [35,60]. Accordingly, a longer duration of the experiment is recommended for future studies.

5. Conclusions

In this study *P. cinnamomi* infection did not affect plant water relations, gas exchange and above ground biomass despite its effects on root biomass, and there were no cumulative effects with drought stress. While *Phytophthora* infection of roots was confirmed by the re-isolation from the root samples it is possible that the effects of the infection on plant physiology were delayed or other factors, including pathogen aggressiveness and plant condition could have contributed. As *P. cinnamomi* is a water-based pathogen, the drought conditions in our experiment could have reduced its pathogenicity. However, it is also possible that the links between drought stress symptoms and *Phytophthora* infection are not as common as previously proposed.

Author Contributions: S.K.A. conceived and designed the study, participated in the collecting of data, supervised data analysis and wrote and edited the manuscript; M.U. designed the study, collected the data, performed the data analysis and wrote the manuscript; L.M.P. collected data, performed some analyses and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Maroondah City Council as part of research into tree health in urban green spaces in Maroondah City Council.

Acknowledgments: The authors are very grateful for the support by Rowan Berry, Brett Hough and Sascha Andrusiak of the Burnley nursery in assisting with the preparation and maintenance of plants.

Conflicts of Interest: The authors declare no conflict of interest.

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