



A non-native pathogen meets a native host: *Austropuccinia psidii* infection reduces photosynthesis and alters non-structural carbohydrates in seedlings of *Metrosideros excelsa*

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Received: 15 April 2024 / Accepted: 13 December 2024 / Published online: 24 January 2025
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Abstract

Key message *Austropuccinia psidii* infection and increase in diseased leaf area resulted in a reduction of photosynthesis, an upregulation of stomatal conductance, and an increase in leaf starch and sucrose content.

Abstract *Austropuccinia psidii* is a biotrophic rust pathogen that causes myrtle rust, affecting over 480 species in the Myrtaceae family. The development of chlorotic and necrotic leaf areas following *A. psidii* infection has been shown to affect leaf gas exchange. In this study, we quantified photosynthesis, stomatal conductance, and non-structural carbohydrates in seedlings of a long-lived tree, *Metrosideros excelsa* (pōhutukawa), following *A. psidii* infection in a glasshouse experiment (infected and control seedlings) conducted over 20 weeks. The diseased leaf area rose from 8% in week 2 to 95% in week 20 after *A. psidii* inoculation. The photosynthetic rate declined by over 90% within 6 weeks after inoculation and was associated with biochemical damage in CO₂ fixation. Stomatal conductance decreased over the first 4 weeks after inoculation and then increased. An increase in lesions and necrotic cells may inhibit stomatal regulation. Starch content was threefold higher in infected than control leaves 20 weeks after inoculation. Increased starch accumulation in the infected leaf area could be due to reduced export of newly fixed carbon from the infected leaves. Meanwhile, glucose + fructose content was 31% lower in infected leaves at the experiment's end, likely because of leaf necrosis. If the pathogen-induced damage and loss of leaves, reduction in photosynthesis and changes in non-structural carbohydrates shown in this study also occur in wild *M. excelsa* seedlings and reduces their biomass, this may in turn reduce their competitive ability in the primary successions that they currently often dominate.

Keywords Disease severity · Greenhouse experiments · Leaf chemistry · Leaf functions · Plant disease · Stomatal conductance

Introduction

Assessment of invasive species and their impacts is one of the challenges for biologists and ecologists in this century (Crowl et al. 2008; Guegan et al. 2023). The global spread of invasive pathogens results in the loss of primary productivity and biodiversity (Singh et al. 2023). Foliar fungal pathogens affect trees worldwide, reducing their growth. One of the most concerning pathogens is *Austropuccinia psidii*, a foliar biotrophic fungal pathogen (Berger et al. 2007). This globally invasive fungal pathogen is the causal agent of myrtle rust and has expanded beyond its original range of South and Central America rapidly over the last 50 years as far as Singapore, Australia, and New Zealand (Chock 2020). The urediniospores (asexual) spores of *A. psidii* mainly infect the young, actively growing tissue of plants in Myrtaceae, such

Communicated by R. Guy.

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as new leaves, floral buds, shoots, and fruits, causing distortion, defoliation, stunted growth, and even dieback (Glen et al. 2007; Watt et al. 2023; Zauza et al. 2015).

The *A. psidii* infection pathway begins with the leaf surface attachment and germination, followed by the formation of a specialized structure called an appressorium that punctures and penetrates the leaf cuticle epidermis of the host plant (Chock 2020). Following the penetration of host mesophyll cells, a haustorium forms to acquire nutrients (Chock 2020). The lower epidermis contains stomata through which CO₂ for photosynthesis enters the leaf, and O₂ and water vapor diffuse out of the leaf. Photosynthesis and the formation of carbon compounds are the central processes (i.e., primary metabolism) of plant growth and development.

The development of chlorotic and necrotic leaf areas following foliar pathogen infection reduces the number of chloroplasts in the mesophyll, leading to a decrease in the photosynthetic rates (Okorski et al. 2008; Scholes et al. 1994) and a reduction in stomatal conductance (Erickson et al. 2004; Gortari et al. 2019). For example, photosynthesis in poplar leaves infected by different poplar leaf rust species declined by up to 50% (Eberl et al. 2018; Gortari et al. 2019; Jiang et al. 2016), and infection by *A. psidii* reduced stomatal conductance in *Eucalyptus urophylla* leaves by 70% within 20 days (Alves et al. 2011). Photosynthesis and stomatal conductance in infected plants are correlated with disease severity (Alves et al. 2011; Hossain et al. 2019; Jiang et al. 2016).

Infection by foliar pathogens that inhibits photosynthesis, and stomatal regulation may also alter the dynamics of non-structural carbohydrate (NSC) production and accumulation (Berger et al. 2007; Voegelé and Mendgen 2011; Walters 2015). Non-structural carbohydrates, mainly consisting of water-soluble sugars (hereafter referred to as sugars) and starch, are an essential storage form of carbon in plants that provide substrate for primary and secondary plant metabolism (Hartmann and Trumbore 2016; Landhäusser et al. 2018). For example, lower sucrose, glucose, and fructose concentrations were measured in *Phaseolus vulgaris* L. leaves infected with the rust fungus *Uromyces phaseoli* var. *typica* L. race 3 compared to healthy leaves (Livne 1964). Sugar consumption by foliar fungal pathogens is also likely to affect the NSC content of infected leaves (Doidy et al. 2012; Voegelé and Mendgen 2011).

So far, studies on the impacts of *A. psidii* infection on leaf gas exchange of the host plants are limited in terms of species and time, typically less than 30 days following inoculation (e.g., Alves et al. 2011), which may be insufficient to quantify how the entire progression of the disease alters leaf gas exchange and NSC of long-lived host plants (Alves et al. 2011; McIntire et al. 2021). *Metrosideros excelsa* (pōhutukawa; Myrtaceae), one of New Zealand's 12 endemic *Metrosideros* species, is a locally dominant,

long-lived canopy tree found almost exclusively in coastal forests and coastal cliffs in the northern half of the North Island, New Zealand (Bergin and Hosking 2006). It plays an important ecological role by providing nesting sites for native birds; its flaky bark and dense leaf litter provide habitat for a wide range of host-specific indigenous insects, and its flowers are a seasonally important source of nectar for insects (Bylsma et al. 2014; Schmidt-Adam et al. 2000; Wardle et al. 2009). *Metrosideros excelsa* is highly susceptible to *A. psidii* under controlled environmental conditions and field experiments (Beresford et al. 2019). Myrtaceae species may respond differently to *A. psidii* infection according to their susceptibility and environmental conditions (Barrett et al. 2009; Fernandez-Winzer et al. 2020).

The aim of this study was to understand the potential effects of a novel pathogen (*A. psidii*) on leaf gas exchange and NSC of *M. excelsa* seedlings. We conducted a glasshouse experiment to (1) measure photosynthesis and stomatal conductance in *M. excelsa* seedlings over time following the inoculation of leaves with *A. psidii*, (2) quantify leaf NSC content, and (3) investigate the relationship between disease severity, photosynthesis, stomatal conductance, and NSC. The glasshouse experiment was run over 20 weeks to investigate pathogen–plant interactions that span the entire life cycle of the pathogen.

We hypothesized that (1) infection with *A. psidii* would reduce the photosynthetic rate and stomatal conductance of the infected leaves because of a reduction in the number of chloroplasts in the leaf tissue and/or disruption and damage of the leaf epidermis cells and (2) *A. psidii* infection would cause a decline of starch synthesis due to a decrease in the photosynthetic rate but lead to an increase in glucose + fructose content as a result of increased import and usage of stored sugars into infected leaves.

Materials and methods

Experimental design

Eighty-four *M. excelsa* seedlings, approximately 15 cm tall, were obtained from Scott Base Nurseries, Auckland, and transplanted into 1 L round plastic pots using a potting mix (Daltons 40 Garden Time Potting Mix). Seedlings were grown in a glasshouse under natural light and temperature conditions for 6 months until they were ~30 cm tall. They were then transferred into a glasshouse in a containment facility (PC2, Manaaki Whenua—Landcare Research, Auckland). We then randomly selected 42 plants, each group on a separate bench in the glasshouse, as plants inoculated with *A. psidii* and as non-inoculated plants (controls). Urediniospores of *A. psidii* were collected from infected plants in the wild, isolated, and cultivated on *Syzygium*

jambos, a highly susceptible species to *A. psidii*, to develop a spore source for the inoculation experiment. Urediniospores of *A. psidii* were collected from *Syzygium jambos* leaves using a mini-spore collector (GRA-201, Tallgrass Solutions) and stored in a freezer at -20°C for less than 2 months.

Metrosideros excelsa seedlings ($n=42$) were inoculated with a spore suspension (10^5 *A. psidii* urediniospores mL^{-1}) by spraying the lower and upper surfaces of all young leaves, following the procedure of Pegg et al. (2014). Inoculated plants were misted with water and covered immediately with black plastic sheets to maintain a high humidity level. They were kept in the dark at 19°C for the first 24 h. Inoculated and control plants were grown at 24°C for a 12 h light photoperiod and at 19°C for a 12 h dark photoperiod for the duration of the experiment.

The experiment ran over 20 weeks from the day seedlings were inoculated with *A. psidii*, until all infected plants had lost almost all infected young leaves (17 September 2020–4 February 2021). All seedlings (controls and infected plants) were watered, as necessary. To minimize cross-infection between inoculated and control plants, we kept the leaves dry by adding water to the trays the pots were sitting in. Control plants were assessed every few weeks for signs of infection. Disease severity assessment and leaf gas exchange measurements were done weekly until week 12 and in weeks 14, 16, and 20 after inoculation ($n=3$ plants per treatment and time point). Harvesting of plants for non-structural carbohydrate content in leaves took place in weeks 2, 4, 8, 12, 16, and 20 after inoculation ($n=6$ plants per treatment and time point).

Disease severity assessment

Disease severity was assessed on three infected leaves per inoculated plant and three inoculated plants per time point ($n=9$ leaves per treatment and time point). Three plants were randomly chosen each time. Leaf selection was random for each plant at nodes 2–3 from the top. In weeks 16 and 20, most infected plants had lost their leaves at nodes 2–3, and the assessment was done on three remaining infected leaves independent of the node. The evaluation started in week two after inoculation when the first visible symptoms were detected and was done weekly until week 20. Disease severity assessment included a separate evaluation of the diseased leaf area and lesion level on the same leaves.

Digital images of infected leaves were taken using a 12-megapixel rear-facing camera with an $f/1.8$ aperture (iPhone 8). Infected leaves were straightened to ensure a flat surface with no curling on the work surface and with a ruler for scale. The diseased leaf area was estimated using an imaging approach using the ImageJ software (version v1.53o) (Easlon and Bloom 2014). This approach allows precise estimation of the total leaf surface area and the leaf

area showing myrtle rust symptoms. A color threshold function delineated the diseased leaf areas from non-diseased leaf areas. The diseased leaf area was calculated as the ratio of the total leaf area covered with myrtle rust symptoms to the whole leaf area.

Lesion level assessment was conducted on the same infected leaves selected to monitor the diseased leaf area. Lesion level (on a scale of 1–5) was estimated visually based on the progression of myrtle rust symptoms in color and shape, such as yellow to brown pustules and chlorotic and necrotic spots on the leaf surface (Table 1).

Leaf gas exchange measurements

A portable photosynthesis system (CIRAS-2, PP Systems, PP Systems International, Inc., Amesbury, MA, USA) equipped with a detachable universal leaf cuvette (PLC-6, 1.7 cm^2) and a photosynthetically active radiation (PAR) sensor was used to measure net photosynthetic rate (P_n) and stomatal conductance (G_s). Leaf gas exchange measurements started 1 week after inoculation and were done weekly over 12 weeks, followed by weeks 14, 16, and 20 after inoculation to cover the entire disease progression. Measurements were carried out at a set temperature (19°C) and CO_2 concentration (~ 399 – 410 ppm) under saturating photosynthetic photon flux density ($\sim 1000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) reflecting the conditions in which the seedlings were grown.

Photosynthetic rate (P_n) and stomatal conductance (G_s) were measured on the same leaves used for disease severity assessment. The same selection procedure was used for control plants. Three plants were randomly selected at each time point. Three leaves at nodes 2–3 from the top were randomly chosen for each plant. The P_n and G_s measurements were conducted between 9 am and 12 pm. The measurement order was performed alternatively for each treatment to avoid temporal effects on leaf gas exchange.

Intracellular CO_2 concentration (C_i) and leaf transpiration rate (E) were also determined using the portable photosynthesis system. The instantaneous carboxylation efficiency was calculated as the ratio between P_n and C_i .

Leaf biomass and non-structural carbohydrate analysis

Three leaves from six plants per treatment were harvested to have sufficient material for subsequent NSC analysis at 2, 4, 8, 12, 16, 20, and 32 weeks after inoculation. Before harvesting, all urediniospores from erumpent pustules were removed from the upper and lower leaf surface into a capsule fitted to a mini-spore collector (GRA-201, Tallgrass Solutions). Leaf samples for biomass estimation were oven-dried at 60°C for 72 h and then weighed.

Table 1 Symptoms and rating scale of *Metrosideros excelsa* (plant host) leaves infected with *Austropuccinia psidii* (non-native pathogen)

Lesion level	1	2	3	4	5
Description	Slight infection: yellow lesions visible	Light infection: yellow lesions began partially to turn light brown	Moderate infection: all lesions turned light brown	Heavy infection: lesions were brown and partially necrotic, and leaves showed signs of distortion	Extreme infection: severe leaf necrosis, dryness and distortion
					

Leaf samples for NSC analysis were microwaved at 600 watts for 60 s, followed by oven-drying at 100 °C for an hour to deactivate enzymes and then oven-dried at 60 °C for 72 h to constant weight (Landhäusser et al. 2018). Leaves were ground into fine powder, and starch and soluble sugars (glucose, fructose, and sucrose) content (mg g^{-1} dry weight) was measured following the protocol described in Landhäusser et al. (2018). First, about 30 mg of the ground sample was weighted into a 2 mL screw-cap micro-centrifuge tube. Soluble sugars were extracted using 1.5 mL of 80% ethanol followed by heating at 90 °C for 10 min in a Thermo-Shaker (The Grant Bio PCMT Thermo-Shaker, Grant Instruments Ltd, UK). The heating was repeated three times. After heating, the sample solution was cooled down and centrifuged at 13,000 g for 1 min (Eppendorf Microcentrifuge 5424, Eppendorf AG, USA) to separate the supernatant and the pellet. The supernatant was transferred to a 2 mL screw-cap micro-centrifuge tube for sugar analysis. The whole pellet from the sugar extraction was used for starch analysis by digesting starch to glucose with 1 mL of α -amylase solution ($600 \text{ units mL}^{-1}$) at 85 °C for 30 min, followed by 0.5 mL of amyloglucosidase solution (12 units mL^{-1}) at 55 °C for 30 min. Glucose from starch digestion and glucose + fructose in the extracted supernatant were then added to hexokinase-glucose 6-phosphate dehydrogenase solution (GHK). At the same time, sucrose in the extracted supernatant was broken down by an invertase enzyme into glucose and fructose. The reaction with GHK reagent was performed in a 96-well transparent microplate, and absorbance was then measured at 340 nm wavelength by a microplate reader (The EnSpire Microplate Reader, PerkinElmer, Inc., UAS). Calculation

of NSCs content was done following Landhäusser et al. (2018).

Statistical analysis

Parameters were non-normally distributed when combining the control and inoculated group (Shapiro–Wilk test, $p < 0.05$). Normality was also tested separately for each group, showing that the control group's data were normally distributed ($p > 0.05$).

Leaf gas exchange parameters (photosynthesis and stomatal conductance), chemical parameters (non-structural carbohydrates) and disease severity data were analyzed using a linear mixed-effects model to assess the effect of treatment (control and inoculated), time (weeks after inoculation), and their interactions. The effect of disease severity (diseased leaf area and lesion level) on leaf gas exchange parameters and NSC was also assessed. Weeks after inoculation, treatment, diseased leaf area, and lesion level were used as fixed factors. Sampled plants and measurement times (date, time of measurement) were treated as random effects to account for multiple observations among individual leaves and plants between sampling dates. Post hoc comparisons using the pairwise Mann–Whitney U tests (significant at $p < 0.05$) were performed to determine the effect of treatments at each different time point. The p values were adjusted using the Bonferroni multiple-testing correction method.

Mixed-effects models were performed for (1) all individual response variables (single models), (2) containing variables without interactions (complex models), and (3) variables with their interactions (maximal models). To

compare models, Akaike's information criterion (AIC) value was calculated for each fitted model, with the lowest AIC value being considered as the highest explanatory power. Models in which the difference in AIC relative to AIC_{min} is < 2 can also be considered to have substantial support (Burnham et al. 1998). The mixed-effects model fits were conducted using the R packages lme4 (Bates et al. 2014) and lmerTest (Kuznetsova et al. 2017). We used the 'DHARMA' package to assess the residuals of the linear mixed-effects models. Post hoc analysis was performed using the effects function (effect()) to elucidate further the effect of interaction between diseased leaf area and the lesion level on leaf gas exchange if their significant interaction was found from fitted mixed-effects models.

To understand relationships between leaf gas exchange (Pn, Gs), non-structural carbohydrates (starch, fructose + glucose, and sucrose), disease severity, and lesion levels over the duration of the experiment, a principal components analysis (PCA) was conducted using the factoextra R package. Data were standardized using the prcomp() function. Disease severity and lesion level were used as categorical (qualitative) variables, while all other variables were set as continuous (quantitative) variables. The results were visualized using a biplot (fviz_pca_biplot() function) showing the samples (points) and variable information (vectors) over the first two PC axes. The median value of each variable for a given time point (weeks after inoculation) was used in the PCA.

All statistical analyses were performed using R (version 4.1.0) and RStudio (version 1.4.1717) (R Core Team 2021). We consider a p value ≤ 0.05 to be statistically significant.

Results

Disease severity

The first sign of infection was the appearance of bright yellow spots and/or orange–yellow lesions (uredinia) on both sides of the youngest leaves (only on the top leaf pairs 1–5) 2 weeks after inoculation (Table 1). Five weeks after inoculation, leaves started turning chlorotic, brown, and then necrotic, causing deformed leaves and defoliation (Table 1). The diseased leaf area rose from 8% in week 2 to 95% in week 20 after inoculation (Fig. 1a). The lesion level of all leaves measured was at scale one during the first 4 weeks and then increased in the following weeks and at scale five at the end of the experiment (Table 1).

Leaf gas exchange

Median leaf photosynthetic rates (Pn, at node two from the top) in control plants ranged from 7.7 to 9.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ over the 20 weeks of measurement (Fig. 1b, Supplementary Information Table S1). The median Pn of the control plants varied between time points, but differences were not significant (Table S1). A 28% reduction in Pn was observed 1 week after inoculation when visual disease symptoms were absent. Six weeks after inoculation, Pn in infected seedlings was 90% lower than in controls (Fig. 1b). From week 7 onwards, Pn was negative (indicating leaf respiration) when parts of the whole leaf tissue had become chlorotic and necrotic.

Control plants' median stomatal conductance (Gs) ranged from 73 to 101 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Fig. 1c, Table S1). Although the effect of *A. psidii* infection on Gs was highly significant (Table 2), pairwise comparisons showed that the difference between control and infected plants was not significant between week 6 and week 14 (Fig. 1c). The median Gs of infected leaves decreased by nearly 60% as early as 4 weeks after inoculation. However, Gs increased and started exceeding Gs of control leaves from week 10 post-inoculation (significantly higher at weeks 11, 16, and 20 after inoculation, Fig. 1c).

The median intercellular CO_2 concentration (Ci) of infected plants was up to 50% lower than in control plants (Fig. S1a). Significant differences in instantaneous carboxylation efficiency between control and infected plants were observed as early as 1 week after inoculation (Fig. S1b). Leaf transpiration in infected plants was significantly higher from week 1–6 following inoculation (Fig. S1c). At weeks 16 and 60 after inoculation, leaf transpiration in infected plants was significantly lower than in control plants (Fig. S1c).

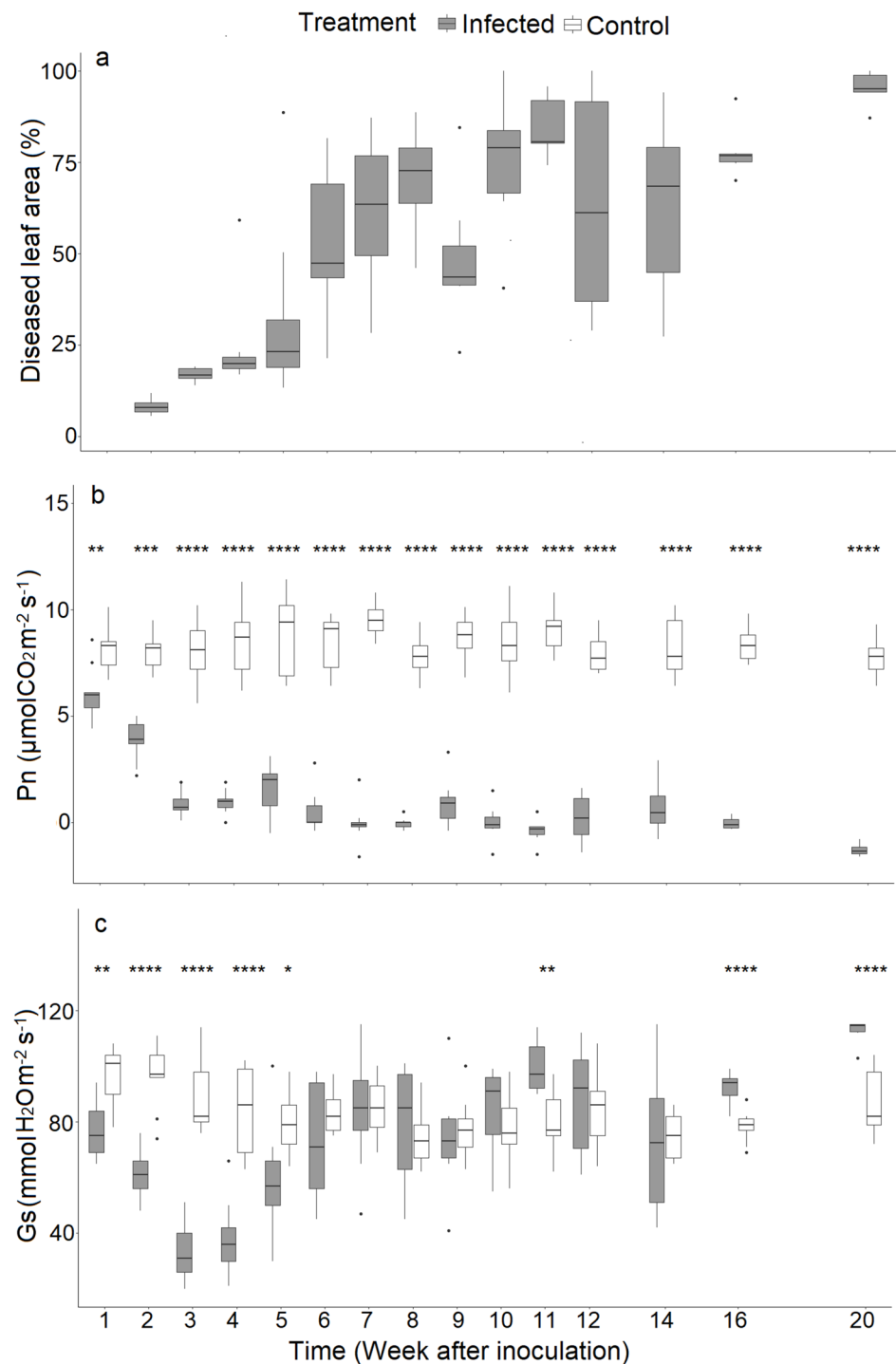
The fitted single mixed-effects model showed a stronger effect of diseased leaf area on Pn than lesion level, while maximal models revealed changes in Gs following *A. psidii* infection were affected by the interaction of diseased leaf area and lesion level (Table 2, Table S2). The increase in diseased leaf area was associated with a decrease in Gs at the early stage after inoculation (lesion level = 1). However, increased Gs was observed with increased lesion size and leaves turning brown, necrotic, and dry (Fig. 2c–d).

Leaf biomass and non-structural carbohydrates

The medium leaf biomass in control and infected plants ranged from 13–23 g and 10–17 g, respectively (Fig. S2). The medium leaf biomass in infected plants was significantly lower (50–60%) in weeks 16 and 20 post-inoculation compared to control plants (Fig. S2).

In control plants, medium leaf starch, glucose + fructose, and sucrose contents ranged from 8.2–15.1 mg g^{-1} ,

Fig. 1 **a** Diseased leaf area (DLA), **b** photosynthetic rate (Pn), and **c** stomatal conductance (Gs) of *Metrosideros excelsa* (plant host) between control and *Austropuccinia psidii* (non-native pathogen) inoculation (infected) treatment. Boxes represent the 25th and 75th percentile with a horizontal line at the median. Whiskers above and below the box indicate the largest and smallest observations falling within the 10th and 90th percentile. *Indicates a significant difference between infected and control plants at $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$



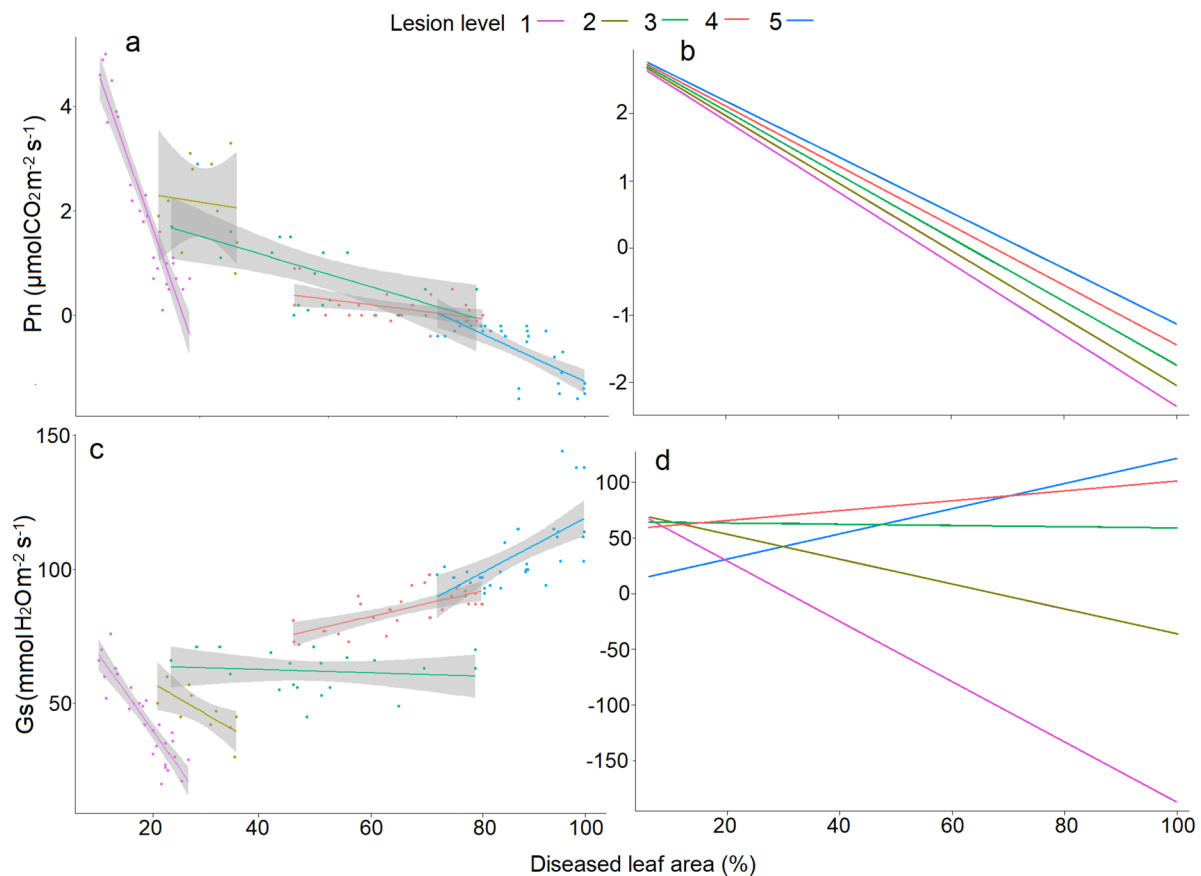
66.6–75.2 mg g^{-1} , and 19.9–30.7 mg g^{-1} , respectively (Table S1). The median leaf starch content of infected plants 2 weeks after inoculation was 38% lower than in control plants (Fig. 3a). However, from 8 weeks after inoculation onwards, the median starch content in infected plants increased and was threefold higher than the controls by week 20. Significantly higher (up to 39%) glucose + fructose content was measured in infected plants

2 to 12 weeks after inoculation (Fig. 3b). Glucose + fructose content in infected plants decreased from week twelve onwards. Still, differences between control and infected plants were not significant (Fig. 3b). The leaf sucrose content in infected plants was always greater than that of control plants (Fig. 3c).

Both diseased leaf area and lesion level significantly affected leaf starch and glucose + fructose content of A.

Table 2 Results from mixed-effects models to estimate the effect of *Austropuccinia psidii* (non-native pathogen) infection on *Metrosideros excelsa* (plant host) leaf gas exchange and leaf non-structuralcarbohydrate content. *WAI* weeks after inoculation, *DLA* diseased leaf area, *LL* lesion level

Models	Leaf gas exchange				Non-structural carbohydrate content (mg g ⁻¹)					
	Pn (μmol CO ₂ m ⁻² s ⁻¹)		Gs (mmol H ₂ O m ⁻² s ⁻¹)		Starch		Glucose + fructose		Sucrose	
	<i>p</i>	AIC	<i>p</i>	AIC	<i>p</i>	AIC	<i>p</i>	AIC	<i>p</i>	AIC
I Weeks after inoculation and treatment (infected and control)										
Maximal model										
WAI	0.739	836.2	0.026	2009.2	<0.0001	211.9	0.001	251.9	0.002	200.4
Treatment	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Treatment*WAI	<0.0001		<0.0001		<0.0001		<0.0001		0.227	
II Diseased leaf area and lesion level (infected only)										
Maximal model										
DLA	0.0005	253.6	0.115	863.1	0.016	132.7	0.032	152.2	0.113	128.8
LL	0.129		0.036		0.277		0.301		0.046	
DLA*LL	0.567		<0.0001		0.339		0.453		0.344	

**Fig. 2** Effect of diseased leaf area–lesion level interactions on photosynthesis (Pn) and stomatal conductance (Gs) of *Metrosideros excelsa* (plant host) inoculated with *Austropuccinia psidii* (non-native pathogen). **a** and **c** show the correlation lines from the raw data forPn (**a**) and Gs (**c**). **b** and **d** are predictor effects plots derived from the linear mixed-effects models for Pn (**b**) and Gs (**d**) at different lesion levels

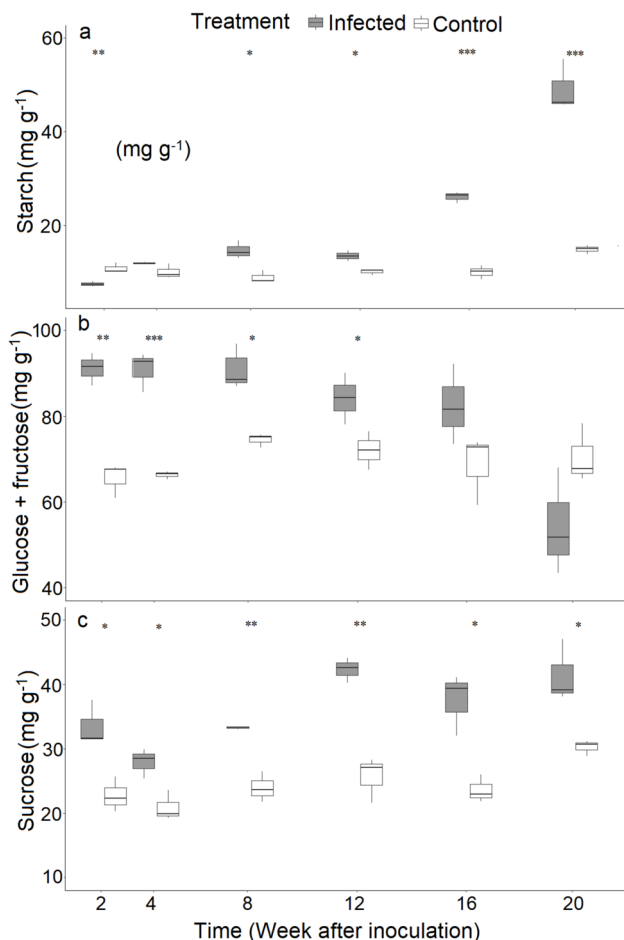


Fig. 3 **a** Starch, **b** glucose + fructose, and **c** sucrose content (mg g^{-1} dry weight) of *Metrosideros excelsa* (plant host) between control and *Austropuccinia psidii* (non-native pathogen) inoculation (infected) treatment from 2 to 20 weeks after inoculation. Boxes represent the 25th and 75th percentile with a horizontal line at the median. Whiskers above and below the box indicate the largest and smallest observations falling within the 10th and 90th percentile. *Indicates a significant difference between infected and control plants at $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

psidii-infected plants, but there were no significant effects on sucrose content (Table 2).

Relationship between non-structural carbohydrates and leaf gas exchange

The PC1 axis explained 72.5–92.8% of variances at 2, 12, and 20 weeks after inoculation, whereas PC2 explained 4.8–23% of the variance (Fig. 4). Two weeks after inoculation, Pn and Gs were positively related to starch content but were negatively associated with disease severity, lesion level, glucose + fructose and sucrose contents (Fig. 4a). There was a notable difference between 2 and 20 weeks after inoculation. By then, Gs was positively related to disease severity, lesion level, starch and sucrose contents, whereas

Pn was positively associated with glucose + fructose content (Fig. 4c).

Discussion

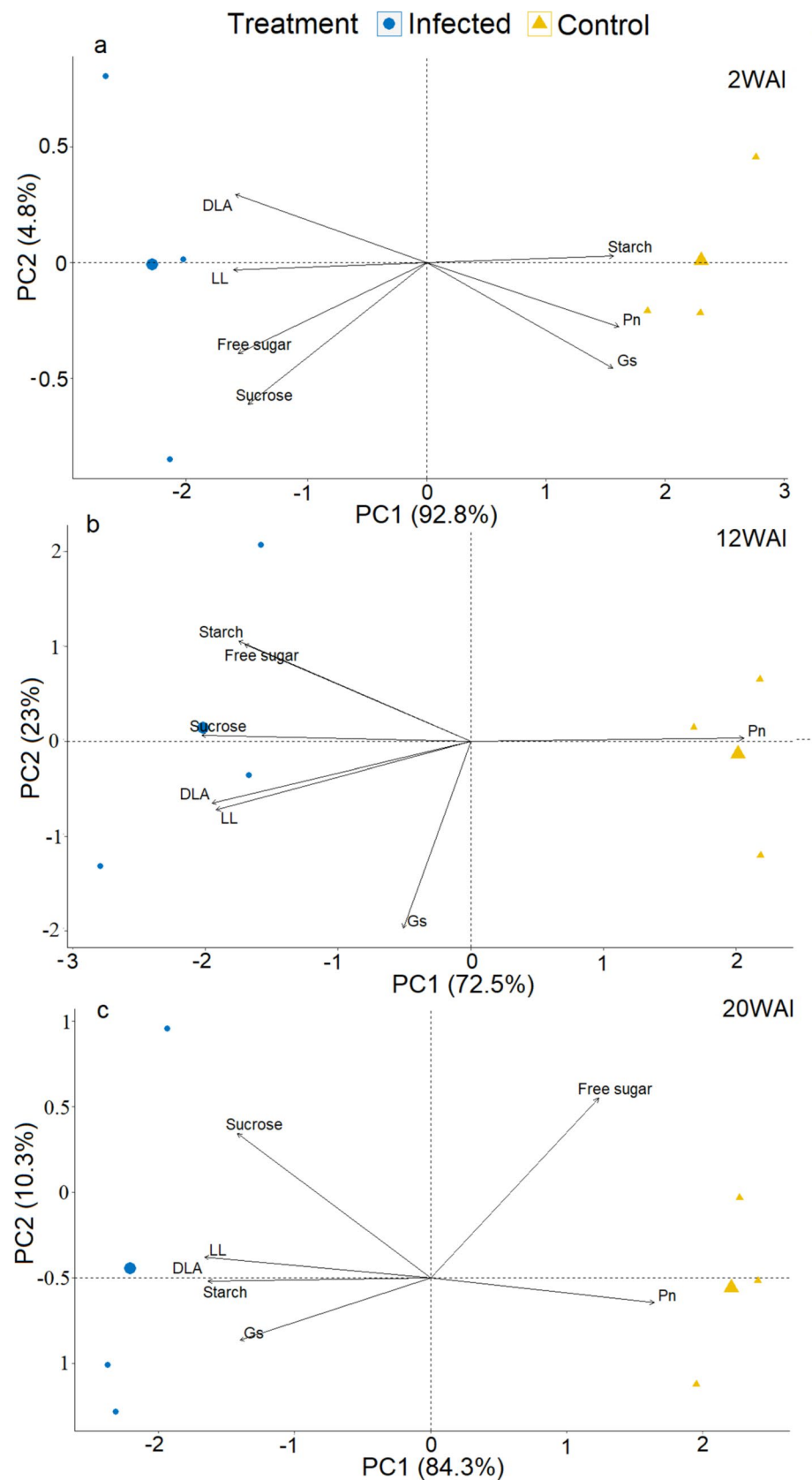
Leaf gas exchange

The 91% reduction in photosynthetic rate (Pn) in *M. excelsa* seedlings 3 weeks after *A. psidii* inoculation was at the upper end of Pn reduction found in previous studies. A 46% decrease in Pn was measured 20 days after *Eucalyptus urophylla* leaves were inoculated with *A. psidii* (Alves et al. 2011) and a 80% decrease in Pn in “Paluma” guava (*Psidium guajava*) leaves 20 days after *A. psidii* inoculation (Gonçalves et al. 2022). Although infected by the same foliar pathogen, *A. psidii*, differences in host susceptibility, tolerance, and resistance may partly explain the variation in the decline in Pn across host plants (Freeman et al. 2019; Pagán and García-Arenal 2018). Differences in disease severity and, in turn, Pn may also be related to inoculum concentration and spore germination rate. Myrtle rust disease severity across six Myrtaceae species increased as plants were exposed to increasing inoculum concentrations (ranging from 1×10^2 to 1×10^6 urediniospores ml^{-1}) (Ireland and Pegg 2020).

The decline in Pn caused by foliar pathogens has been attributed to changes in stomatal conductance and/or effects on the photochemical pathway (e.g., enzymes related with CO_2 fixation; Carretero et al. 2011). Higher intracellular CO_2 concentration (Ci) and lower instantaneous carboxylation efficiency in infected *M. excelsa* leaves than in control leaves suggests that Pn reduction is associated with biochemical limitations in carbon fixation. Higher Ci and lower instantaneous carboxylation efficiency have also been reported in *A. psidii*-infected *E. urophylla* (Alves et al. 2011) and *P. guajava* (Gonçalves et al. 2022). This may be related to a reduction in photosynthetic area (Chock 2020) and/or the inhibition of Rubisco or other photosynthetic enzyme activities in diseased tissue (Alves et al. 2011). In our study, a significant reduction in Pn and instantaneous carboxylation efficiency in infected *M. excelsa* leaves was found as early as the first week after inoculation when visual disease symptoms were absent. This is noteworthy because it shows a rapid effect on photosynthesis and, thus, primary host plant metabolism even during the early stages of penetration and intracellular growth of *A. psidii*.

The 20% decrease in Gs within the first week after infection without any apparent morphological leaf damage and the 40–50% decrease 4 weeks after infection in *M. excelsa* seedlings in this study is less than the 70% reduction in Gs of *E. urophylla* leaves 20 days after inoculation with *A. psidii* (Alves et al. 2011). Downregulation of Gs was also found

Fig. 4 Biplot of the first two dimensions (PC1 and PC2) of the principal component analysis with photosynthetic rate (Pn), stomatal conductance (Gs), starch, glucose + fructose, and sucrose content, and diseased leaf area (DLA) and lesion level (LL) of *Metrosideros excelsa* (plant host) between control and *Austropuccinia psidii* (non-native pathogen) inoculation (infected) treatment at **a** 2 weeks, **b** 12 and **c** 20 weeks after inoculation. The dots indicate median values per treatment. The larger blue circle and triangle represent the mean points of the control and infected plants (barycenters). The black arrows show the direction and magnitude of the variables



in poplar leaves (25–50%) within 10 days after inoculation by poplar rust species (*M. larici-populina* and *M. medusa*) (Eberl et al. 2018; Gortari et al. 2019). Reduced Gs in the early stage of infection could be due to physical blockage caused by pathogen sporulation (Mandal et al. 2009), colonization of fungal hyphae in the mesophyll intercellular spaces and the substomatal chamber disrupting the circulation of gas, thus reducing Gs (Gortari et al. 2019). The observed increase in the intercellular CO₂ concentration can also contribute to the reduction in stomatal conductance, as reported by Gonçalves et al. (2022, 2023).

We observed an increase in Gs and transpiration along with an increase in the size of the infected area, and these infected leaf areas turned chlorotic and necrotic 5 weeks after inoculation. Guimaraes and Stotz (2004) showed that the pathogen *Sclerotinia sclerotiorum* produced and accumulated oxalic acid within and around necrotic lesions, inhibiting the stomatal closure of infected leaves. Damage to the leaf epidermis along with an increase in temperature of infected leaf tissue (Grimmer et al. 2012) or changes in the physiological characteristics of the guard cells following sporulation has also been associated with the downregulation of stomata closure in fungal-infected leaves (Lindenthal et al. 2005). Increased transpiration rates have been associated with the host cuticle's rupture during the fungus's sporulation (Paul and Ayers 1984).

Non-structural carbohydrates

The observed decrease in starch content in *M. excelsa* leaves in the early stages of *A. psidii* infection may be associated with the reduction in photosynthesis, biochemical dysfunction in CO₂ fixation (Alves et al. 2011; Gortari et al. 2019), and/or destruction of chloroplasts (Scholes and Rolfe 1996). Higher starch content in infected *M. excelsa* leaves from week 4 until the end of the experiment was not expected given that less substrate for starch synthesis was produced as Pn was reduced. However, starch accumulation in infected leaf tissue has been reported in previous studies (Kim et al. 2009; Rios et al. 2017). This has been attributed to a down-regulation of the starch degradation pathway (Scheidig et al. 2002) and/or a reduction in the export of newly fixed carbon from the infected leaves (Livne and Daly 1966).

Glucose + fructose and sucrose in *A. psidii*-infected *M. excelsa* leaves was significantly greater than that of control leaves from 5 weeks after inoculation. Glucose + fructose and sucrose are photosynthetic assimilates; we expect their content to decrease along with a substantial reduction in Pn. The elevated amount of sugar content in *A. psidii*-infected *M. excelsa* leaves may partly be explained by the import of NSC from healthy leaves and other plant organs into infected leaves (Eberl et al. 2018; Livne 1964; Walters 2015). Higher glucose + fructose content in infected leaves could also be

due to the mobilization from the breakdown of starch into glucose + fructose in infected leaf areas driven by an increase in demand for sugars by the fungus during sporulation (Chou et al. 2000). While glucose + fructose production increases initially in response to stress, long-term stress depletes their concentrations (Hartmann and Trumbore 2016; Tomasella et al. 2019). This may explain the lower glucose + fructose content in infected *M. excelsa* leaves at the end of the experiment when infected leaves had a diseased leaf area close to 100% and all became dry and necrotic.

Conclusion

Our study provides new insights into the impact of a widely distributed biotrophic pathogen on leaf gas exchange and non-structural carbohydrates in *M. excelsa*. Our findings suggest that the reduction in photosynthesis in *A. psidii*-infected *M. excelsa* leaves was caused by biochemical damage and not by stomatal limitation. In the long term, reduction in photosynthesis, changes in non-structural carbohydrate content, and decrease in leaf biomass will likely decrease *M. excelsa* seedling biomass. It remains to be tested whether the disease severity and plant physiological responses observed in this study occur in wild *M. excelsa* seedlings. If it does, then this could have major implications for processes of primary successions in northern coastal New Zealand, including recent volcanic surfaces and landslides, which *M. excelsa* colonizes as seedlings and ultimately dominates as adult trees (Bylsma et al. 2014). If infection by *A. psidii* of leaves of wild *M. excelsa* seedlings reduces their photosynthesis and leaf biomass in primary successions, this could result in loss of their competitive ability alongside non-native tree species that increasingly colonize these sites, many of which are functionally different, including the fire-adapted conifer *Pinus radiata* (Bellingham et al. 2023). A loss of competitive ability of *M. excelsa* seedlings could in turn result in dominance of non-native trees during succession and, ultimately, long-term changes in plant communities and ecosystem processes (McCarthy et al. 2024).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00468-024-02593-8>.

Acknowledgements We thank Zane McGrath and Chantal Probst (both Manaaki Whenua—Landcare Research) who supported us in establishing glasshouse experiments in the PC2 laboratory at Manaaki Whenua—Landcare Research. We also would like to thank Natalia Abrego, and David Wackrow (The University of Auckland) for their assistance in the laboratory.

Author contributions H.N., P.J.B, M.P, L.S: conception and design; H.N, L.S.: methodology implementation, experiment execution, data collection; H.N.: data analysis; H.N., P.J.B, M.P., L.S: data interpretation; H.N.: original draft preparation; P.J.B, M.P, L.S: manuscript review and editing.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This research was funded through the Ministry of Business, Innovation and Employment's Beyond Myrtle Rust research program (C09X1806).

Data availability The data that support the findings of this study will be made available through Figshare (<https://doi.org/10.17608/k6.auckl.and.28092506>).

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

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