

# The effects of *Phoracantha*-induced dieback on photosynthesis, respiration and estimated leaf nitrogen in *Eucalyptus pauciflora*

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## Abstract

*Eucalyptus pauciflora* stands in Kosciuszko National Park are experiencing severe dieback events induced by *Phoracantha* beetles, threatening the habitat complexity of subalpine ecosystems. This study investigated the effects of *Phoracantha*-induced dieback on the maximum photosynthetic rate ( $A_{\max}$ ), average dark respiration ( $r_{\text{dark}}$ ) and nitrogen ( $N$ ) concentration in *E. pauciflora* leaves, and the relations between  $A_{\max}$ ,  $r_{\text{dark}}$  and estimated leaf  $N$  concentration. We collected leaf samples from a healthy stand in Charlotte Pass and a dieback-affected stand in Perisher and measured their rates of photosynthesis and dark respiration using a LI-COR 6400, and estimated leaf nitrogen using a SPAD. We found no difference in  $A_{\max}$  and  $r_{\text{dark}}$  between leaf categories, but epicormic leaves had significantly higher  $N$  content than healthy canopy leaves. In contrast to the literature, we found no correlation between  $A_{\max}$  and  $N$ . Our findings suggest that *E. pauciflora* may exhibit a plastic response in photosynthetic rate to drought or dieback stress, but that dieback may damage vascular tissue, limiting dark respiration. The high nitrogen content in epicormic leaves does not have a corresponding increase in photosynthetic rate. These results suggest the effects of *Phoracantha* attack on the vascular system and source-sink flows within a tree.

**Running Title:** Photosynthesis, respiration and nitrogen in dieback-affected snow gums.

**Key Words:** chlorophyll content, defoliation, source-sink flows, carbon flux

## Introduction

Recent dieback events of *Eucalyptus pauciflora* Sieber ex Sprengel, 1827 (snow gum) in the Australian Alps have been attributed to native wood-boring longicorn beetles in the genus *Phoracantha* Newman, 1840. Deep horizontal borer galleries have been observed in *E. pauciflora* trees afflicted with dieback, which appear to be associated with *Phoracantha* (Ward-Jones, 2020). These deep galleries cut off the phloem and xylem, leading to canopy decline and stem death, followed by widespread death of affected stands (Ward-Jones 2021). Significant outbreaks of *Phoracantha* have been associated with severely drought-

stressed forests when compared to healthy stands (Seaton *et al.* 2015), and these outbreaks will intensify as average temperatures increase and average rainfall decreases across southeastern Australia (Hughes 2003).

Snow gums are present throughout the alpine bioregion, and are the dominant tree species in the subalpine zone. Widespread mortality of snow gum stands will be detrimental to habitat complexity in the subalpine, affecting both animals and understory plants relying on snow gums, a keystone species, for shelter. Patches of snow gum in the alpine zone create islands of subalpine vegetation (Costin *et al.* 2000), providing a locally warmer site and shelter for flora and fauna to avoid freezing.

As dieback progresses, the canopy appears to also be affected: the leaf cuticles die and, eventually, the leaves turn brown and senesce (Ward-Jones, 2020). The removal of the leaf cuticle creates a positive feedback loop, as the leaf becomes susceptible to further damage from drought, extreme temperatures, UV radiation, pathogens and insect attack (Martin & Juniper 1970). Dieback events result in the disruption of water and nutrient supply to the photosynthetic tissue, and ultimately defoliation. When the canopy is damaged, both above and below-ground biomass is disrupted, creating an imbalance and shift in carbon dynamics. O'Grady *et al.* (2006) found strong positive relationships between foliage biomass and root biomass in *Eucalyptus globulus*, which suggests that if canopy biomass decreases due to defoliation, root shedding may occur in response. While effects of *Phoracantha* attack have been observed to result in canopy defoliation, ultimately the infestation begins at the stem. Currently, little is known about the effects of *Phoracantha* infestation on the photosynthetic and respiratory rates of *E. pauciflora*.

Photosynthesis is the process by which plants use light energy to transform carbon dioxide (CO<sub>2</sub>), water and minerals into oxygen and sugars for plant maintenance, growth and reproduction. Dark respiration is the process by which plants convert photosynthates, the products of photosynthesis, into energy (adenosine triphosphate – ATP) without the aid of light. Photosynthetic induction is the process by which leaves increase CO<sub>2</sub> uptake when transitioning from low to high light. A lag in efficiency occurs, as plants must regenerate substrate and activate enzymes involved in photosynthesis and carbon fixation, as well as open stomata, before photosynthesis can reach a steady state (Acevedo-Siaca *et al.* 2021).

Previous studies on photosynthetic rates of dieback-affected and defoliated trees have had mixed results. Bark beetle attack in *Pinus yunnanensis* decreased net photosynthesis (Liu *et al.* 2019), whereas the removal of *Eucalyptus nitens* lower canopy branches increased net CO<sub>2</sub> assimilation (Pinkard *et al.* 1998), which was attributed to the species' ability to up-regulate photosynthesis as a compensatory response. However, a meta-analysis of 100 studies on insect stem herbivory found that stem attack greatly increased branch mortality, although photosynthetic rate did not significantly differ between affected and non-affected stems (Stephens & Westoby 2015).

Factors that reduce photosynthesis can also reduce respiration. Stress events may result in greater respiration rates, as sink demand for carbon for repair increases (Pinkard *et al.* 2011). Water stress, a risk factor for *Phoracantha* attack, has been shown to increase respiration as organic solutes accumulate in leaves (Amthor 1989). Conversely, respiration may also decrease due to severe or chronic water stress (Evans 1975).

Nitrogen is an essential element for plant growth and maintenance, as well as for photosynthesis. Bark beetle attack in pines has been shown to increase nitrogen concentration in pine needles, due to premature needle fall (Morehouse *et al.* 2008). *Eucalyptus* leaves that regrow following insect leaf herbivory similarly have increased foliar nitrogen concentrations, particularly in epicormic leaves, which sprout as a response to stress (Marsh & Adams 1995).

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Current studies on wood-boring beetle attacks are largely centred around pine trees in timber plantations. The available studies on eucalypts have focused on defoliation via manual pruning of branches, and there are few studies examining the photosynthetic and respiratory response of eucalypts to attacks from wood-boring insects. *Phoracantha* attack begins in the trunk and moves outwards, and the destruction of the trunk causes defoliation by cutting off the flow of water and nutrients to the photosynthetic tissue, so it is possible that the responses of trees to this type of dieback are different from responses to pruning.

The goals of this study are to explore the effects of *Phoracantha*-induced dieback on photosynthesis and respiration rates in *E. pauciflora* leaves and to explore the interaction between estimated leaf nitrogen, photosynthesis and respiration. We expect the maximum photosynthetic rate to be higher in healthy trees, and the rate of respiration to be higher in dieback-affected trees, due to increased demand for carbon for repair. We expect estimated leaf nitrogen content to be highest in epicormic leaves, and lowest in healthy trees, and hypothesise that there will be a positive correlation between  $A_{\max}$  and estimated leaf nitrogen.

## Methods

### *Study area*

We sampled *E. pauciflora* branches from two sites: a healthy stand behind Charlotte Pass and a dieback-affected stand at Perisher Valley. Both the dieback canopy and epicormic samples were taken from Perisher. Elevations were similar at the two sites, 1,765m at Charlotte Pass and 1,720m at Perisher Valley. The data for the healthy and dieback-affected canopy leaves were collected on the same day as the Soil Plant Analysis Development (SPAD) measurements for the epicormic leaves (8 December 2021). The photosynthesis and dark respiration rates for the epicormic leaves were collected the day prior (7 December 2021).

### *Data collection*

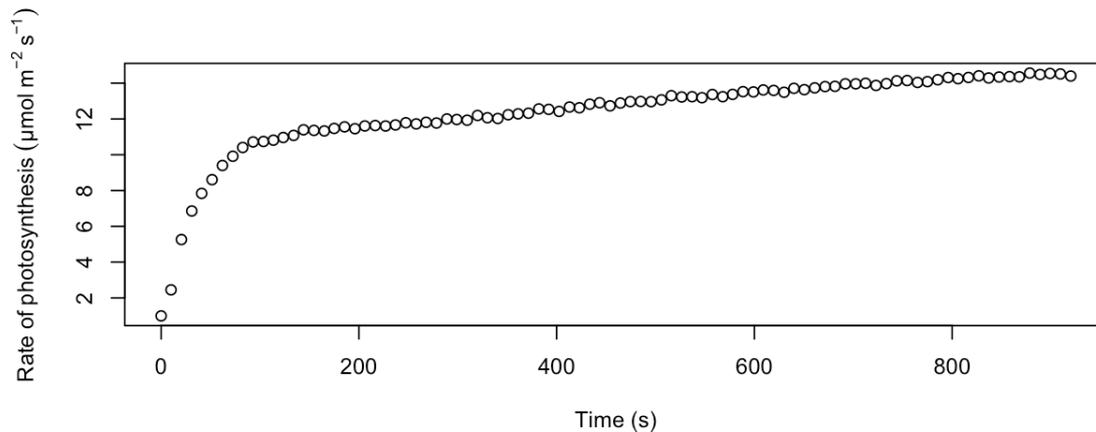
In total, we measured leaves from nine healthy branches and five mature branches from dieback-affected trees. The epicormic leaf sample size for the photosynthesis and dark respiration measurements is seven leaves, whereas the SPAD sample size is eight leaves.

We sampled branches that had non-juvenile leaves and stems and were covered by a canopy so that they were sizeable and did not suffer from edge effects. We did not sample branches that looked particularly unhealthy due to pathogens. We cut branches from the tree and then cut them again while in water to prevent embolisms. We collected whole branches, as opposed to just the leaves, as the LI-COR requires living tissue. The LI-COR 6400 measures rates of photosynthesis and respiration using two different cables, using an infrared gas analysis (IRGA) to measure the amount of CO<sub>2</sub> flowing in and out of the leaves (Evans & Santiago, 2014).

In the lab, the branches were covered with a black sheet for 30 minutes to acclimate them to the dark. One leaf from each branch was placed in the dark LI-COR (lamp switched off) for 3 minutes. After 3 minutes, three LI-COR measurements were logged every 30 seconds to measure the dark respiration rate.

We switched the LI-COR lamp on to low light (50  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 5 minutes, allowing the leaves to reach a steady state and avoid light shock. After 5 minutes, we turned the lamp up to full light (2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). We used an auto-log program to take a measurement every

5 seconds. This was left to run until the photosynthesis curve on the LI-COR display had plateaued, at which point we generated a light induction curve in the LI-COR (Figure 1).



**Figure 1:** A photosynthesis light induction curve measured by the LI-COR, beginning from when the lamp was switched on. The x-axis represents time in seconds, while the y-axis shows the rate of photosynthesis.

To test our hypothesis that foliar nitrogen was highest in epicormic leaves, we used a SPAD meter (Konica Minolta Sensing Inc.) to estimate leaf nitrogen content. The SPAD works by transmitting red light, which chlorophyll absorbs, and infrared light, which chlorophyll does not absorb. The ratio of the intensity of the wavelengths passing through the leaf is calculated, giving the density of chlorophyll, which can be used to estimate nitrogen content. The relationship between SPAD units and leaf nitrogen varies among species groups (Xiong *et al.* 2015), so this measurement is only an estimate. Three measurements were taken from three different leaves on the branch, and we calculated an average SPAD value per branch. We took the SPAD measurements for epicormic leaves while in the field, without detaching the branch.

### Data analysis

Our data analysis was conducted in RStudio version 2021.9.1.372 (RStudio Team, 2021). To test the hypothesis that the maximum photosynthetic assimilation rate was higher in healthy trees, we obtained the minimum ( $A_{\min}$ ) and maximum ( $A_{\max}$ ) photosynthetic assimilation rate values ( $A$ ).  $A_{\min}$  was the lowest  $A$  recorded, while  $A_{\max}$  was taken from the first  $A$  measurement in the photosynthesis curve plateau, where  $A$  values were within  $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  of the actual  $A$  maximum recorded for that leaf overall. We then calculated  $A_{90}$ , or 90% of the difference between  $A_{\max}$  and  $A_{\min}$  (i.e., 90% of the way from  $A_{\min}$  to  $A_{\max}$ ), and found the corresponding time ( $T_{90a}$ ) in the data where the photosynthetic rate first reached the  $A_{90}$  value.  $T_{90a}$  is the time taken, in seconds, for the photosynthetic rate to reach 90% of its maximum. We calculated this value to assess the efficiency of the samples in reaching their  $A_{\max}$ .

To test the hypothesis that the average dark respiration rate was higher in dieback-affected trees, we calculated the mean of the three measurements logged while the LI-COR lamp was off for each leaf. This gave us an average dark respiration rate ( $r_{\text{dark}}$ ) for each sample. In the LI-COR, respiration was measured as a negative photosynthetic rate, so we took the absolute value of these values to give a positive dark respiration rate.

To test our hypothesis that estimated leaf nitrogen will be positively correlated with  $A_{\max}$ , we fit a regression between SPAD and  $A_{\max}$ , as well as between  $r_{\text{dark}}$  and SPAD, and  $A_{\max}$  and  $r_{\text{dark}}$  to examine other possible relations. We have reported the Pearson correlation coefficients and the p-value, indicating the statistical confidence of the fitted line.

For statistical tests, we analysed  $A_{\max}$  and  $r_{\text{dark}}$  using analyses of variance (ANOVA) between the three leaf categories: healthy, dieback and epicormic. We used a two-sample t-test for the T90a data, as the epicormic leaf samples had not been left long enough in the LI-COR for a light induction curve to be generated. SPAD data were analysed using an ANOVA and a post-hoc Tukey test to identify where the significant difference occurred. The significance threshold we used in our testing was  $p < 0.05$ . We have used standard errors to represent the accuracy of the sample mean to the population mean.

## Results

### *Photosynthesis*

Healthy, mature leaves had an average  $A_{\max}$  of  $14.4 \pm 0.43 \mu\text{mol m}^{-2} \text{s}^{-1}$  and an average T90a of  $493 \pm 109 \text{s}$ . Mature leaves from dieback-affected trees had an average  $A_{\max}$  of  $11.8 \pm 1.93 \mu\text{mol m}^{-2} \text{s}^{-1}$  and an average T90a of  $356 \pm 86.8 \text{s}$ . Epicormic leaves from dieback-affected trees had an average  $A_{\max}$  of  $11.6 \pm 1.38 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Despite the range of values being narrower and higher in the healthy group (Figure 2), the differences are not statistically significant ( $p = 0.155$ ). This is contrary to our hypothesis that  $A_{\max}$  would be significantly higher in the healthy group.

### *Dark respiration*

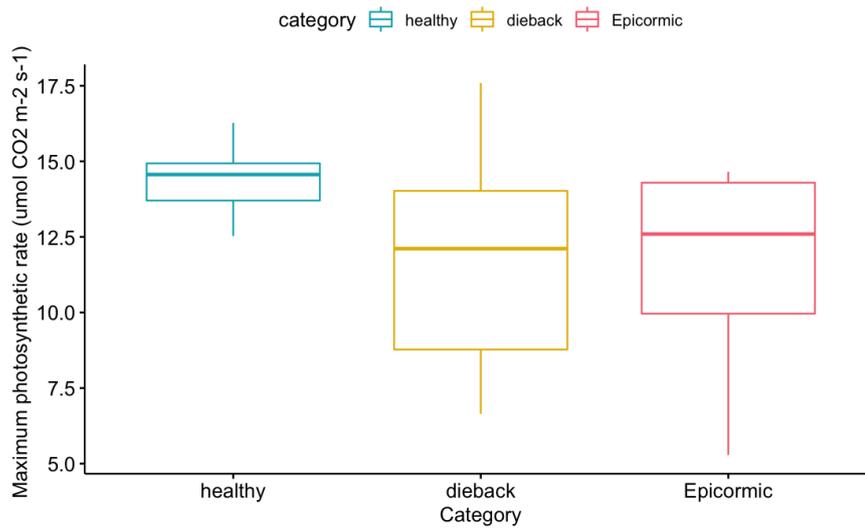
Contrary to our hypothesis, average dark respiration rates do not differ significantly ( $p = 0.12$ ) between the leaf categories, although the mean of the healthy group appears lower than that of the dieback group (Figure 3). The healthy, mature leaves have an average  $r_{\text{dark}}$  of  $2.67 \pm 0.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The mature leaves from dieback-affected trees have an average  $r_{\text{dark}}$  of  $3.41 \pm 0.28 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The epicormic leaves have an average  $r_{\text{dark}}$  of  $3.04 \pm 0.24 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

### *Estimated leaf nitrogen*

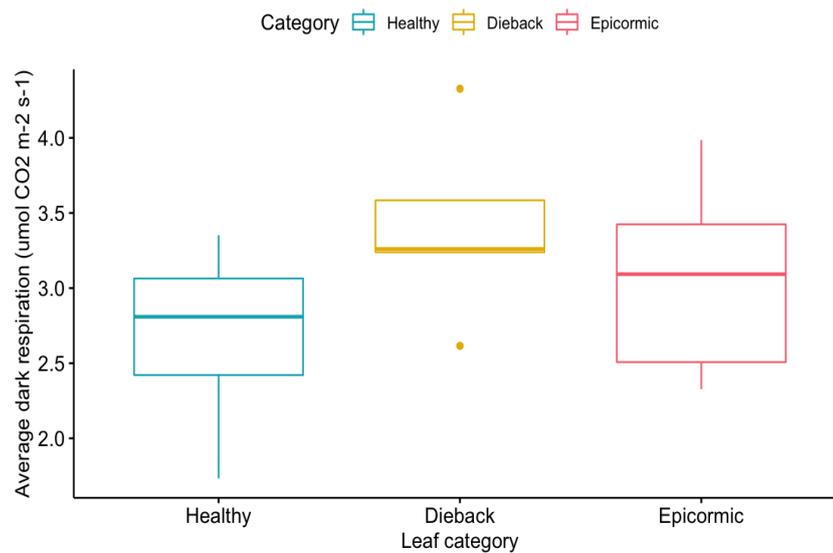
The mean SPAD value of the healthy leaves was  $67.2 \pm 0.79$  SPAD units. The mean SPAD value of the mature leaves from dieback-affected trees was  $68.6 \pm 1.14$  SPAD units. The epicormic leaf SPAD mean was  $74.5 \pm 2.26$  SPAD units. The post-hoc Tukey test found that there is no difference in SPAD units between the healthy and mature dieback groups ( $p = 0.84$ ). The epicormic group has a higher average and a broader range of values (Figure 4), though differences between the epicormic and mature dieback groups were not significant ( $p = 0.07$ ). However, the differences between the healthy and epicormic groups were significant ( $p = 0.007$ ). As expected, the epicormic group had a significantly higher average SPAD than the healthy group.

### *Linear regression*

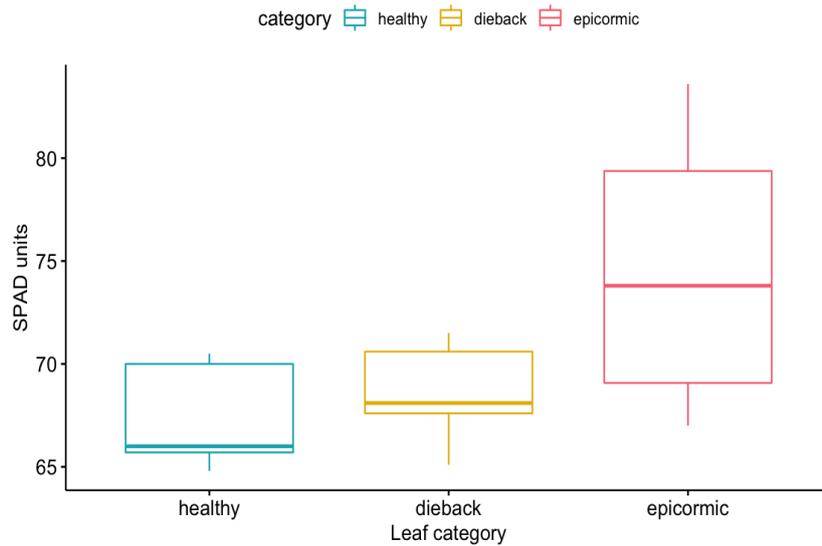
We modelled a linear regression between  $r_{\text{dark}}$  and  $A_{\max}$  (Figure 5a),  $A_{\max}$  and SPAD (Figure 5b), and  $r_{\text{dark}}$  and SPAD (Figure 5c). The  $r_{\text{dark}}$  and  $A_{\max}$  regression (Figure 5a) has a weak negative or negligible correlation ( $r = -0.13$ ) that does not reject the null hypothesis ( $p = 0.66$ ). The  $A_{\max}$  and SPAD regression (Figure 5b) has no or negligible correlation ( $r = -0.02$ ), and there is no statistically significant correlation ( $p = 0.95$ ). The  $r_{\text{dark}}$  and SPAD regression (Figure 5c) is moderately positively correlated ( $r = 0.58$ ), and the model is statistically significant ( $p = 0.029$ ).



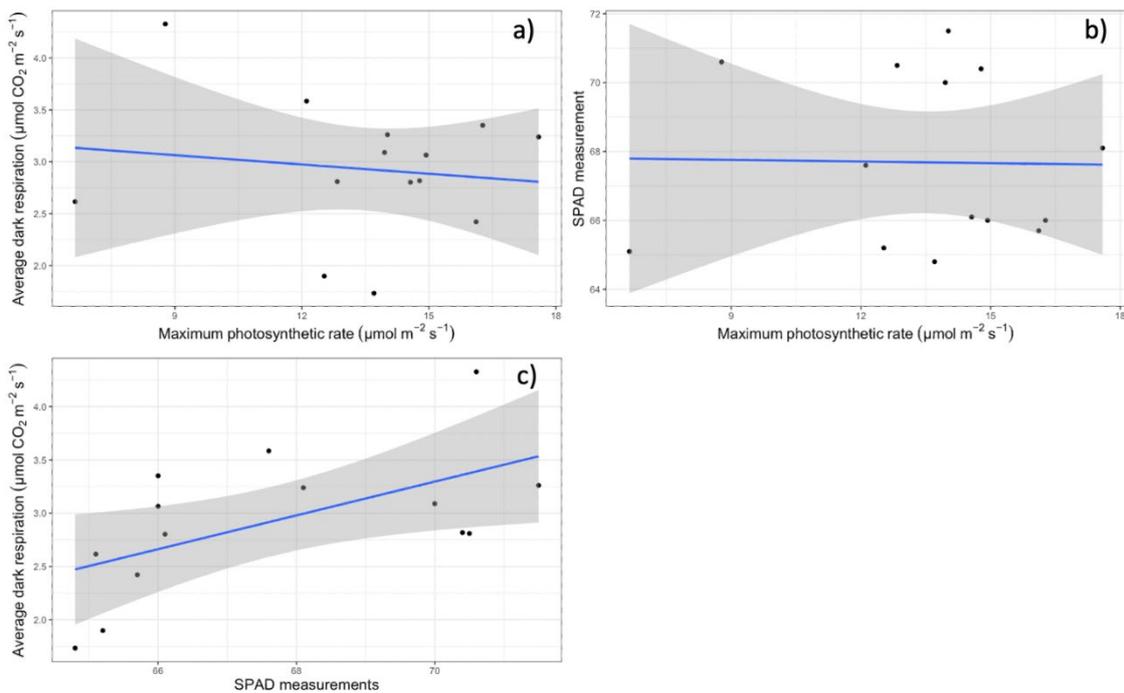
**Figure 2:** Boxplot of the maximum photosynthetic assimilation rates of healthy ( $n=9$ ), dieback ( $n=5$ ), and epicormic ( $n=7$ ) leaves, measured with a LI-COR 6400,  $p=0.155$  (ANOVA).



**Figure 3:** Boxplot of average dark respiration rates of healthy ( $n=9$ ), dieback ( $n=5$ ) and epicormic ( $n=7$ ) leaves, measured with a LI-COR 6400,  $p=0.12$  (ANOVA).



**Figure 4:** Boxplot of SPAD leaf nitrogen estimates of healthy ( $n=9$ ), dieback ( $n=5$ ) and epicormic ( $n=8$ ) leaves,  $p=0.008$  (ANOVA).



**Figure 5:** The relationships between (a) average dark respiration and maximum photosynthetic rate ( $r=-0.13$ ,  $p=0.66$ ), (b) maximum photosynthetic rate and SPAD ( $r=-0.02$ ,  $p=0.95$ ), and (c) average dark respiration and SPAD ( $r=0.58$ ,  $p=0.029$ ), analysed by linear regression in RStudio. The blue line represents the fitted linear regression line, and the grey area represents the 95% confidence interval.

## Discussion

Our study aimed to investigate the effects of dieback on the rates of photosynthesis, respiration and estimated leaf  $N$  content in snow gums. This was done by testing for differences in these rates and nitrogen content between healthy, dieback and epicormic leaves. We also investigated the relationships between photosynthesis, respiration and estimated leaf  $N$  using linear regressions.

### *Photosynthesis*

There was no significant difference in maximum photosynthetic rate ( $A_{\max}$ ) between the healthy, dieback (canopy) and epicormic leaf samples. The mean  $A_{\max}$  of the healthy samples was higher than the dieback and epicormic samples; however, this was not significant ( $p=0.16$ ) and we cannot reject the null hypothesis. This is contrary to our hypothesis that healthy trees would have a higher  $A_{\max}$ . The lack of a response in the photosynthetic rate to dieback could indicate the ability of snow gums to acclimatise or overcompensate in response to stress events, maintain carbon assimilation and support regrowth. This plastic response could be triggered by a combination of damage from boring beetles, and canopy loss and defoliation as a result of dieback. Existing studies on the effects of defoliation of eucalypts on photosynthesis have yielded similar results, where up-regulation and plasticity have resulted in an increased (Pinkard *et al.* 1998; Pinkard *et al.* 2011) or maintained  $A_{\max}$  (Pinkard *et al.* 2007). This result is also corroborated by Stephens & Westoby (2015), who found in their meta-analysis of 100 studies on insect stem herbivory that the photosynthetic rate did not differ significantly between affected and non-affected stems. It is also possible that, as photosynthesis occurs at the chloroplast level, there is a lag between the dieback stress event and a compensatory response of photosynthesis. Furthermore, we sampled leaves covered by the canopy to reduce edge effects. As these leaves are not exposed to direct sunlight, they have a lesser photosynthetic role. They would likely not have a higher  $A_{\max}$ , to begin with, and would not be as impacted by dieback. Future research should investigate the ability of *E. pauciflora* to regulate photosynthesis in response to dieback by measuring live samples over a longer period. This could assist our understanding of dieback by identifying a threshold of stress beyond which *E. pauciflora* can no longer regulate photosynthesis. Including canopy leaves exposed to direct sunlight would also be useful; however, analyses would have to take into account edge effects.

### *Dark respiration*

There was no difference in dark respiration ( $r_{\text{dark}}$ ) between the healthy, dieback and epicormic leaf samples ( $p=0.12$ ). This was contrary to our hypothesis that  $r_{\text{dark}}$  would be higher in leaves from dieback-affected trees, as sink demand for carbon for repair and maintenance would increase. The effects of wood-boring beetles on dark respiration rates have not been studied extensively. Pinkard *et al.* (2011) found that pruning defoliation treatments did not affect dark respiration, although there was a non-significant trend towards increasing  $r_{\text{dark}}$  following defoliation, except in low water treatments. It is possible that sink demand for carbon for regrowth had increased, but *Phoracantha* damage to the vascular system resulted in decreased transport of photosynthate and  $\text{CO}_2$  from source to sink, limiting the available inputs for cellular respiration. This could be tested by investigating the effects of wood-boring insects on the xylem and phloem, and their transport rates, to identify the extent to which the vascular system is damaged.

### *Estimated leaf nitrogen*

SPAD values were significantly ( $p<0.01$ ) higher in epicormic leaves than in healthy leaves. This supports our hypothesis, as we had predicted that epicormic leaves would have the highest estimated  $N$  concentration, and that healthy leaves would have the lowest. This is supported by Marsh & Adams (1995), who found that epicormic leaves have higher leaf  $N$  concentrations than canopy leaves. However, contrary to our hypothesis, there was no significant difference in estimated leaf  $N$  concentration between dieback and healthy canopy leaves ( $p=0.84$ ). A potential reason for this lack of difference is nitrogen partitioning, a process common in eucalypts where nitrogen is redistributed to areas of concern, including areas of new growth (Saur *et al.* 2000). Dieback-induced defoliation may have resulted in greater  $N$  content in the remaining canopy leaves, before being redistributed to the new epicormic shoots.

### Linear regressions

We found no correlation between  $A_{\max}$  and SPAD, or between  $A_{\max}$  and  $r_{\text{dark}}$ , but there was a significant ( $p < 0.05$ ) positive correlation between  $r_{\text{dark}}$  and SPAD. Our hypothesis that there would be a positive correlation between  $A_{\max}$  and SPAD, with SPAD as a proxy for chlorophyll and estimated leaf nitrogen content, was not supported. Studies have found a correlation between estimated leaf nitrogen and respiration in both subalpine and boreal trees (Ryan, 1995) and tropical trees (Rowland *et al.* 2016). The lack of a correlation between  $A_{\max}$  and  $r_{\text{dark}}$  has also been observed, as these rates are highly variable depending on environmental stresses, such as varying local temperature, drought and nutrient levels (Rowland *et al.* 2016). The lack of correlation between  $A_{\max}$  and SPAD ( $r = -0.02$ ,  $p = 0.95$ ) was unexpected, as SPAD measures chlorophyll density, a component of photosynthesis (Xiong *et al.*, 2015), and net photosynthesis has been directly positively correlated with leaf nitrogen content (Tjoelker *et al.* 2005; Medhurst & Beadle 2005). There was no significant difference in  $A_{\max}$  between healthy, dieback and epicormic leaves, although epicormic leaves had significantly higher nitrogen content than healthy leaves, indicating that increased nitrogen in the epicormic leaves did not affect their photosynthetic rates. Medhurst and Beadle (2005) found that where increased  $N$  has not led to an increase in  $A_{\max}$ , factors other than nitrogen availability were limiting  $A_{\max}$ . One such factor in this study could be prolonged periods of drought stress or legacy effects from prolonged water stress. Water availability is a limiting factor for photosynthesis, and Pinkard *et al.* (2011) found that water-stressed eucalypts had decreased  $A_{\max}$  regardless of defoliation or nitrogen treatment. It is possible that photosynthesis is limited in snow gums due to ongoing water stress across the region, resulting in no difference in  $A_{\max}$  in epicormic leaves despite having higher estimated leaf nitrogen. Future investigations could test water potential in healthy, dieback and epicormic leaves to identify differences in water stress between dieback-affected and unaffected stands, and correlating water potential to  $A_{\max}$ . This could identify a particular threshold of water stress at which *E. pauciflora* are at greatest risk of *Phoracantha* attack, which could then be used to find stands that are most vulnerable to dieback.

### Improvements and future directions

To test the effects of defoliation on photosynthesis, respiration and estimated leaf nitrogen more directly, we could have ranked the level of dieback on a scale, as well as quantified canopy loss more precisely using a densitometer. There is evidence of some plasticity in the rate of photosynthesis in eucalypts (Pinkard *et al.* 1998). Testing  $A_{\max}$  against dieback severity over a period of time could evaluate whether or not this plasticity exists in *E. pauciflora* and the extent to which it can maintain photosynthetic assimilation rates under stress. Broader research investigating water potential, and the conductivity of xylem and phloem in dieback-affected trees (Pérez-Harguindeguy *et al.* 2013), may provide valuable explanations for the results in this study.

Furthermore, a broader study incorporating both subspecies of snow gum occurring in Kosciuszko National Park, *E. pauciflora* subsp. *pauciflora* and *E. pauciflora* subsp. *Niphophila*, may identify differences between the subspecies in the responses of photosynthesis, respiration and estimated leaf nitrogen to dieback, as well as possible differences in water stress and xylem and phloem conductivity. This could also include samples from healthy and dieback-affected stands throughout the region to reduce the effect of confounding variables that may be present at a particular site.

Our methodology could have been improved with a larger sample size, with more even sample sizes between categories and measurements. We had nine healthy samples and five dieback samples, with seven epicormic samples measured with the LI-COR and eight epicormic samples measured with the SPAD. Our epicormic samples were also not paired, as the SPAD measurements were done using samples from the field that were not measured

with the LI-COR. It would be ideal to measure T90a in epicormic leaves as, although their  $A_{\max}$  was not higher, higher chlorophyll content in the leaves could enable them to reach their maximum photosynthetic rate more efficiently. These inconsistencies are in part due to the fact that two separate groups worked on this project with a handover, followed by editing of the methodology and scope of the study. Ideally, procedures would be consistent throughout the project.

## Conclusions

Despite damage to the vascular system, maximum photosynthetic rate and respiration do not appear to be affected by *Phoracantha*-induced dieback. Snow gums may have the ability to regulate photosynthesis to a certain extent, maintaining a level of carbon assimilation to compensate for damage and support regrowth. Hindering this is the damage *Phoracantha* inflict on the xylem and phloem, reducing the flow of inputs necessary for cellular respiration to occur in the sink, preventing maintenance and repair. While estimated leaf nitrogen content was higher in epicormic leaves, their maximum photosynthetic rate was not, indicating that photosynthesis is being limited by a factor other than nitrogen and chlorophyll content – likely water stress.

A positive feedback loop can be identified: drought stress increases the susceptibility of snow gums to *Phoracantha* attack, which damages the xylem, further reducing water availability for the tree. Low water availability would limit the rate of photosynthesis despite higher foliar nitrogen, while damage to the vascular system affecting the conductivity of the xylem and phloem would limit the rate of dark respiration. Future studies into how dieback affects water potential and xylem and phloem conductivity would be valuable.

*Phoracantha*-induced dieback appears to perturb the flows of nutrients, water and essential compounds from source to sink across *E. pauciflora*, preventing key maintenance processes from occurring. These findings highlight the damage that wood-boring beetles cause to essential systems and flows within a tree, beyond the visible damage they do to the trunk.

## Acknowledgements

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