Healthy trees, healthy future — Enabling technologies to combat *Phytophthora* diseases

SCION — FORESTS ▪ PRODUCTS ▪ INNOVATION

Date: September 2016
Reference: HTHF-TN001

Technical Note

Development of an approach to investigate the effects of RNC-induced defoliation on photosynthesis and carbon assimilation

Author: Mireia Gomez-Gallego

Corresponding authors: Martin Bader, Peter Scott, Sebastian Leuzinger, Nari Williams

Summary: We established two simultaneous artificial defoliation experiments, one for Douglas-fir, and another for radiata pine. These data show an actual compensation in the photosynthesis activity of the remaining needles in radiata pine grafts in the three weeks after treatment. However, these measurements will be repeated across time to determine the duration of the upregulation, and to what extent the carbon assimilation rate can be maintained.

One of the most important effects of *Phytophthora pluvialis* and *Phytophthora kernoviae* on their hosts, radiata pine and Douglas-fir, is their impact on photosynthesis, because they are foliar pathogens. The infection of the needles is assumed to reduce the photosynthetic rate. Further, the eventual needle cast reduces total needle area, presumably reducing the total carbon uptake of the tree. However, the remaining needles could compensate for the loss in needle area by upregulating the photosynthetic activity. To analyse to what extent that compensation takes place, we set up an artificial defoliation experiment.
With this experiment we aim to answer these research questions:

- How does the decrease in net CO2 assimilation rate at the needle level and the subsequent defoliation translate into a hypothetical decrease in net CO2 assimilation at the canopy level?
- How does the decrease in net CO2 canopy assimilation affect the pattern of carbon allocation to growth and storage in the different tissues of the tree?

1.1 This Project

We established two simultaneous artificial defoliation experiments, one for Douglas-fir, and another for radiata pine.
In June and July, we optimized the procedure for the non-structural carbohydrates (NSC) sampling and performed the different pre-trial measurements for biomass assessment, photosynthesis and NSC.
In August, we established the defoliation treatments. In September, we measured the photosynthesis parameters of the remaining needles. Unfortunately, the Imaging PAM machine to assess the chlorophyll fluorescence parameters broke down, and it has been sent to Germany to be fixed. Therefore, the measurements have not been completed yet, but we can present the results from the measurements that we managed to do.

1.1.1 Experimental design and defoliation treatments

This experiment consists of an artificial defoliation treatment during two consecutive years, to assess the effect of multiple defoliation events.
For radiata pine, the starting plant material was: 40 grafts of the clone A, and 35 grafts of the clone B. Five plants of each clone were assigned for initial destructive measurements which include biomass and NSC sampling of stem and roots. We randomized the control and defoliation treatments in the space among the rest of the plants.
For Douglas-fir, the starting plant material consists of 75 open pollinated plants from the South Island to minimize the Swiss needle cast (SNC) pathogen. Thirteen plants were assigned for initial destructive measurements which include biomass and NSC sampling of stem and roots. We randomized the control and defoliation treatments in the space among the rest of the plants.

supported by forestgrowers commodity levy

FOREST OWNERS ASSOCIATION

This programme is also funded by Scion and the Ministry of Business, Innovation and Employment, New Zealand.

1.1.2 NSC sampling procedure

Assessing the NSC concentration in needles, stem and roots is important to understand the way carbon is allocated within the plant when it is affected by a defoliation event. Those measurements together with the biomass assessment and growth quantification will shed some light on how the plant deals with RNC infection and how its productivity is affected.

Stem and root NSC analysis implies a destructive sampling, which will coincide with the biomass destructive sampling. That sampling will be done before treatments in the first year and the second year, and at the end of the experiment.

The needle analysis for NSC is not destructive, and can be performed several times across the year.

For stem sampling, disc sections from the stem above the graft have been taken, and immediately frozen in liquid nitrogen. Root sampling required removing the soil and mycorrhiza from the roots before cutting and freezing them. The grinding of the material was successfully performed by using tubes containing 2 mm glass and zirconium oxide beads in a homogenizer.

Needles were similarly frozen and ground using 5 ml tubes with stainless steel balls in a Genogrinder.

The resulting powder has to be analysed using a new technique based on NIR spectrometry, and calibrating the procedure with chemistry analyses.

1.1.3 Pre-trial measurements

The pre-trial measurements for growth assessment consisted of diameter, height and intermodal distance measurements. These measurements were complemented with the biomass assessment in a subsample which consisted of the harvesting of the assigned plants, sorting out the roots, stem, branches and corresponding needles. We determined the total needle area with the aid of a leaf area meter. Afterwards, the material was dried in an oven at 70º C during 48 hours, then weighed. With these data we aim to establish allometric relationships to determine an easy way to assess needle area and biomass by the measurement of other parameters.

For the photosynthesis pre-assessment, determined the saturating light level for the plants in our experiment by determining the light curves of 5 radiata pine grafts of each clone.

1.2 Key Results

Pre-assessment of photosynthesis behaviour

The saturating light level is around 600 µmol/m^2/s in radiata pine (Fig. 1a), and around 400 µmol/m^2/s in Douglas-fir (Fig. 1b). Those levels of light are low and can be explained by the fact that the plants have been mostly grown under shelter to avoid pathogen infection which may alter the NSC. Those levels will be used in the measurement of electron transport rate and ACi curves to analyse the photosynthetic activity.

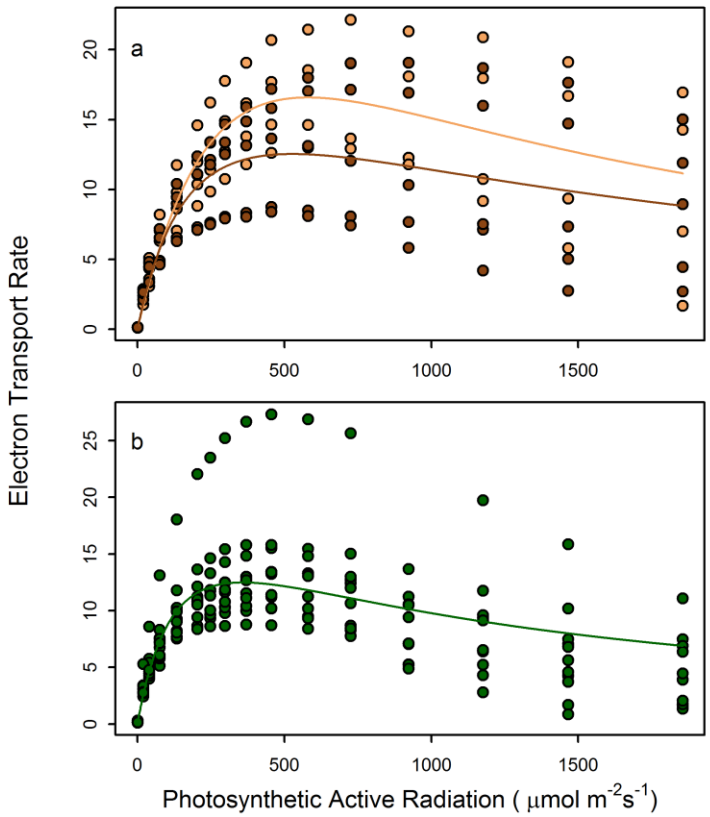

Figure 1. Light responses of electron transport rate in 1-year-old needles of (a) radiata pine grafts for clone A (light brown) and clone B (dark brown), and (b) Douglas fir (green). Each curve corresponds to one plant.

We performed time series measurements to decide which time of the day is the best to do the future measurements. We measured the net photosynthetic rate, the electron transport rate and the stomatal conductance every hour from 8 am to 2 pm. We determined that between 8 am to 12 pm to perform the measurements was suitable, as there were no differences between hourly measurements for any of the variables over that period.

Photosynthetic behaviour of the remaining needles three weeks after treatment

We only have preliminary results for radiata pine. We measured the ACi curves in a subsample of 40 grafts (10 plants per treatment combination: two clones x control/defoliation). The ACi curves analyse the response of the net photosynthetic rate (A) for different levels of intracellular CO_2 concentration at a saturated light level (as analysed previously for both species). Three parts can be distinguished in an ACi curve, with the three correspondent parameters. In the initial slope, photosynthetic activity depends on the carboxylation rate of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and maximum of this carboxylation rate can be defined (V_{cmax}). Secondly, the photosynthetic rate reaches a plateau defined by the maximal rate of electron transport (J_{max}). Finally, the photosynthetic rate can decrease limited by the triose-phosphate utilisation (TPU-limitation). We analysed the effect of the treatment on these three parameters. In Figure 2, the upregulation of the remaining needles can be observed. There is a significant difference in the ACi curve for defoliated and non-defoliated plants and there is a significant difference between clones (data not shown).

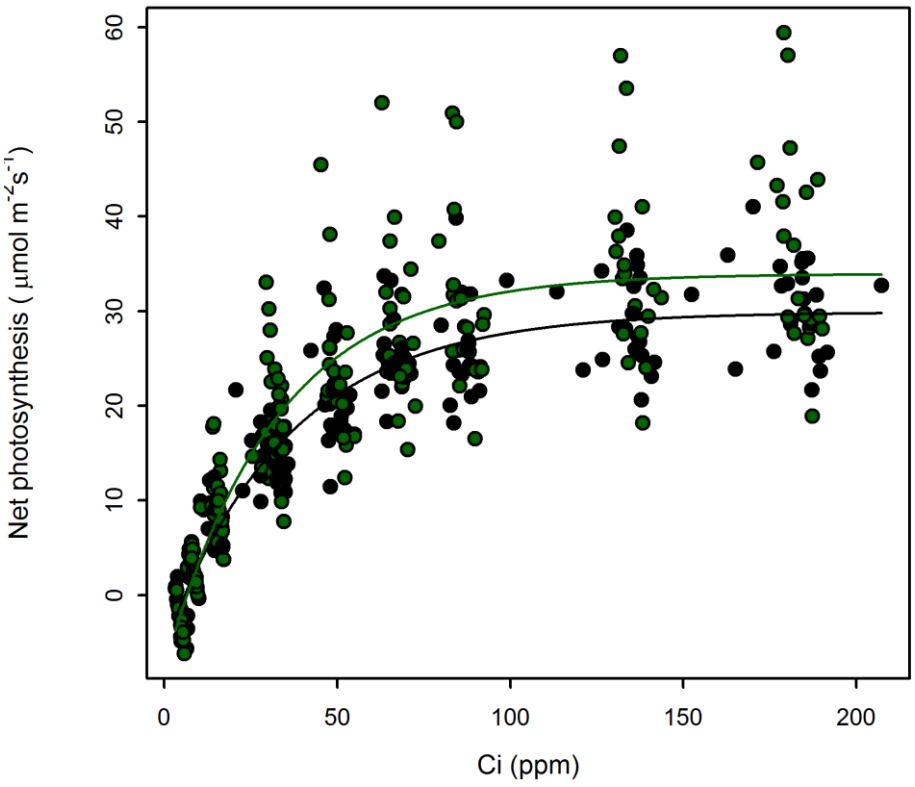

Figure 2. Response of light-saturated net photosynthesis to intercellular CO_2 concentration in upper-canopy remaining needles of 40 radiata pine grafts. Dark green dots correspond to plants that have been defoliated in 75% of the bottom canopy, simulating RNC disease. Black dots correspond to control plants. n = 40.

1.3 Final Conclusions and Implications of Results

These data show an actual compensation in the photosynthesis activity of the remaining needles in radiata pine grafts in the short term, three weeks after treatment. The plant tries to compensate for the loss of needle area and the associated decrease in carbon assimilation. However, these measurements will be repeated across time to determine the duration of the upregulation, and to what extent the carbon assimilation rate can be maintained. Moreover, other informative chlorophyll fluorescence parameters will be measured to complement these results, once the Imaging PAM machine is repaired. Similarly, these measurements will be performed on the Douglas-fir plants.

1.4 Further Work

The experiment will last two consecutive years to assess the recovery of the defoliation events in terms of photosynthetic activity and carbon allocation.

1.5 Acknowledgments

The authors are indebted to the Forest Owners Association and Ministry of Business, Innovation and Employment who provided the funding for this work. Further, the authors would like to thank S. Ellis for assistance with defoliation treatments and biomass measurements.