

## Sampling

We took measurements between August 22 and October 3, 2023 in and around the tree diversity experiment IDENT-Montréal (August 22-September 20) and at the Jardin botanique de Montréal (September 28-October 3). The fall of 2023 was generally warm, and anecdotally, many plants initiated leaf senescence fairly late. Although we sampled some deciduous plants as late as early October, we managed to avoid trees with visible signs of leaf senescence, although we acknowledge that leaf aging and senescence may occur before they are readily visible to the eye. For evergreen species, we did not specifically keep track of the age of the needles we measured, but in all cases they were towards the accessible outer portions of their respective branches.

Within IDENT-Montréal, we sampled trees that were planted as part of the experimental design, which included both native and exotic European species, as well as some trees that were planted on the experiment's boundaries and a few incidental species in the understory or surrounding areas. The sampled plants included deciduous broadleaf, deciduous needleleaf (*Larix*), and evergreen needleleaf (or scaleleaf in the case of *Thuja occidentalis*) tree species. They also included two exotic, incidental shrubs (*Rhamnus cathartica* and *Frangula alnus*) and one agricultural herbaceous species (corn or *Zea mays*) growing in surrounding fields.

Within the Jardin botanique de Montréal, we sampled in two separate areas. The first was the Jardin Leslie-Hancock, where we sampled horticultural *Rhododendron* species or cultivars—all evergreen broadleaf shrubs. The second was the arboretum, where we sampled native and exotic deciduous broadleaf trees.

At IDENT-Montréal and at the arboretum, we cut branches (or individual long leaves, for *Z. mays*) using clippers or pole pruners and recut them under water at least an inch above the original cut, generally within around two minutes. We usually initiated gas exchange measurements within 20 minutes after recutting. In the Jardin Leslie-Hancock, we took gas exchange measurements while the leaves were attached to the whole plant. We generally sampled trees and leaves without visible signs of herbivory or ongoing pathogen infection; however, a small number of our sampled leaves did have some past herbivory damage. The plants had not been subjected to any experimental stress, and 2023 was not considered to be an exceptionally stressful year.

Leaves were classified informally as 'sun' or 'shade' leaves relative to what was typical for the species and site. For example, we considered certain *Betula papyrifera* leaves to be shade leaves in a relative sense, although the species tended to be dominant in mixtures and rarely had deep or dense enough crowns to undergo much self-shading. It is possible that their 'shade' leaves received more sun than other species' sun leaves, although we did our best to sample the most sun-exposed and shaded leaves of each species.

## Gas exchange and chlorophyll fluorescence

We measured gas exchange and chlorophyll fluorescence on each sample in the field using an LI-6400 XT (LI-COR Biosciences, Lincoln, NE, USA) equipped with a 2 cm<sup>2</sup> leaf chamber fluorometer (6400-40, LI-COR Biosciences, Lincoln, NE, USA). Leaf photosynthesis under saturating light ( $A_{\text{sat}}$ ) was measured by exposing leaves to a photosynthetic photon flux density (PPFD) of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , for a duration that typically lasted 1 to 2 minutes. In some shade leaves with low  $A_{\text{sat}}$ , PPFD was lowered to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to prevent photoinhibition. Stomatal conductance ( $g_s$ ) was recorded at the same time as  $A_{\text{sat}}$ . At all times, CO<sub>2</sub> concentration entering the leaf chamber was maintained at 415 ppm, while chamber temperature and air humidity were allowed to vary with ambient conditions. Leaves that had unusually low or high internal CO<sub>2</sub> concentration ( $C_i$ ) were discarded. At the end, we removed one C3 sample with very low estimated  $C_i$  and one with very high (above ambient) estimated  $C_i$  as they represented either leaves with closed stomata or potential errors.

Electron transport rate (ETR) was recorded immediately after measuring  $A_{\text{sat}}$  by exposing leaves to a brief strong flash of light that saturated photosystem II. The instrument recorded the maximum fluorescence ( $F'_m$ ) that resulted from this saturating flash and steady-state fluorescence ( $F_s$ ) under continuous actinic light. We assumed that the proportion of total quanta absorbed by photosystems was about 0.875, and that the proportion of these absorbed by photosystem II was 0.5, except for *Zea mays* for which the constant was assumed to be 0.4. Following, we measured leaf dark respiration ( $R_d$ ) by fully turning off the actinic light and allowing leaf CO<sub>2</sub> exchange rates to stabilize, which typically took 3 to 5 mins.

Most of our species are expected to only have abaxial stomata, but some (e.g. *Zea mays*) have stomata on both sides, while others (many needleleaf conifers) have more complicated leaf morphologies and stomatal placement. We did not alter the stomatal ratio parameter in the LI-COR system from the default of 0.5—twice as much conductance from one side as the other—which is recommended when the true stomatal ratio is not known. This assumption may have some effect on the estimation of stomatal conductance and internal CO<sub>2</sub> concentration via the boundary layer correction.

Between each leaf measurement, we collected a reading of assimilation with no leaf in the chamber. In cases where the assimilation readings differed from 0 due to instrument drift, we performed a corresponding correction to leaf measurements of  $A_{\text{sat}}$  and  $R_d$ .

To correct for leaves with areas lower than the chamber area (i.e., 2 cm<sup>2</sup>), we used a fine-point permanent marker to delineate the area of the leaf which was exposed to the inside of the leaf chamber. A picture of that area was then processed using color thresholds in ImageJ (Schneider et al. 2012) and all calculations of gas exchange were then adjusted to the actual leaf area.

## Reflectance spectra

We measured reflectance spectra immediately after gas exchange measurements using a CI-710 SpectraVue leaf spectrometer (CID Bio-Science, Camas, WA, USA), which has a manufacturer-specified spectral range of 400-950 nm, although the output data files show greater range in both directions. Before measuring each sample, we performed the calibration routine as recommended using the manufacturer-provided white reflectance panel.

For broadleaf and scale leaf species, we then measured spectra of the adaxial side of each leaf used for gas exchange measurements, placing a piece of cardstock painted with highly absorbing black acrylic paint behind the leaf as a background. For needleleaf species, we assembled mats of leaves—generally not the exact same needles used for gas exchange measurements, but from an adjacent twig—and measured their spectra with the same black-painted cardstock background. While we aimed to minimize gaps or overlaps between needles, there are likely to have remained some, especially for species with short or curved needles. Although needleleaf species varied in morphology, in cases where needles were flattened we aimed to capture the adaxial side. We took replicate measurements of each leaf sample—broadleaf and needleleaf—alike, with more replicates in general for needleleaf mats and for larger and more variable broadleaf samples.

In cases of error during measurements or (for needleleaf samples) when we obtained extremely low (<35%) or high (>80%) peak reflectance, we marked the measurements for exclusion. However, it is worth noting that even some of our broadleaf *Rhododendron* samples had nearly 80% measured peak reflectance. Other than samples marked for exclusion in the field, we excluded two measured spectra that we identified as unusual or anomalous while inspecting the data. We ended up with between three and six good replicate measurements per sample. We resampled all spectra to 1 nm nominal resolution and trimmed them to the 400-900 nm range, since measurements outside this range seemed prone to noise.

## **Traits**

We sealed leaves in a bag with a damp piece of paper towel and placed them into a dark cooler for transportation to the lab. We weighed them after ca. 24 hours in a refrigerator for fresh rehydrated mass. After weighing, we scanned each leaf sample on a flatbed scanner. (For needleleaf species, this sample was again a subsample of needles taken from near those measured for gas exchange.) We estimated the area from scans by using color thresholds in ImageJ (Schneider et al. 2012). After scanning, leaves were placed in a drying oven at 60 °C for at least 72 hours and weighed them again for dry mass. We calculated leaf dry matter content (LDMC, g g<sup>-1</sup>) as dry mass divided by fresh mass, and leaf mass per area (LMA, g cm<sup>-2</sup>) as dry mass divided by area.