Comparing methods and technologies for assessing vertical size distribution of American beech leaves (Fagus grandifolia Ehrh)

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Abstract
Leaves of American beech (*Fagus grandifolia* Ehrh) were collected in the winter of 2019, at 1.8 m, 3.7 m, and 5.5 m above the forest floor. Area was determined for each leaf to assess phenotypic trends between upper and lower canopy using a handheld laser area meter in benchtop mode, and from digital images using ImageJ software, the LeafByte App. Results reveal no significant differences in leaf areas obtained from the three methods. Trends between lower and upper canopies follow predicted large to small phenotypes. Results obtained from this study also demonstrate that *F. grandifolia* leaves offer acceptable opportunities for studying phenotypic differences in overwinter beech leaves.

Background
The American beech (*Fagus grandifolia* Ehrh) is a deciduous species that occurs throughout the eastern United States and Southeast Canada (USDA, 2019). This species grows 20–35 meters in height and is tolerant of a range of environmental conditions (USDA, 2019). Leaves of *F. grandifolia* are known to have a higher lignin content and thus carbon to nitrogen ratio than other collocated temperate deciduous species. This higher lignin content results in slower decomposition and likely longer attachment of the sentenced leaves to the tree after leaf fall than other temperate species (Melillo, Aber, & Muratore, 1982). Because *F. grandifolia* will hold their leaves longer into the winter months and because this species occurs over a wide geographical and ecological range, it is an ideal tree species for conducting comparative studies between sites or environmental conditions across seasons.

Objectives
The objectives for this study were two-fold; 1) to determine if the vertical size distribution of beech leaves remained consistent samples collected from multiple trees at similar heights. 2) To compare results from different area measurement techniques to assess which method would offer the widest access to analyzing leaf area.

Methods
- Ten to 12 beech leaves were collected from roughly 1.8 m, 3.7 m, and 5.5 m heights from 3 trees.
- Leaf Area Meter: Leaves were individually fed through a laser leaf area measurer (CID Biosciences Camas, WA) attached to a benchtop conveyor. Data were converted to CSV files and downloaded to a spreadsheet.
- ImageJ: Leaves were photographed using a smart phone camera and save onto a PC computer. Files were uploaded into ImageJ software. Leaf Image Analysis – Surface Area protocol (NIH Bethesda, MD).
- App Analysis: Because LeafByte relies on green leaves for analysis leaves from this study were converted to green in ImageJ. The converted image was loaded onto the LeafByte app and the area was determined directly in the app.
- Area data was analyzed using a Factorial ANOVA followed by the Tukey Unequal N test. Date were log-transformed to meet assumptions of homogeneity.

Results
- Results from a two-way ANOVA of transformed leaf area data revealed significant interactive effects between tree and height on single leaf area (ANOVA: (df 4, 87) F = 6.65, = p <0.01.) (Figure 2).
- When comparing aggregate leaf area date by height among the three methods we found the lower canopy were found to be significantly different from the upper canopy measurements (ANOVA: (df 4, 87) F = 6.65, = p <0.01.) (Figure 2).

Discussion/Conclusions/Next Steps
The trends between lower and upper canopy leaf area differences agreed with previous studies completed on other deciduous trees like Maple (Lei & Lechowicz, 1990, Seiwa, 1999). However, we found no statistically significant difference between leaves collected from the two upper sections of the canopy. This study is an excellent demonstration of phenotypic plasticity in overwintering leaves as well as an exploration of leaf area measurement technologies. By assessing the validity of publicly accessible tools for measuring leaf area, we make them more available for future citizen science initiatives.

References