Digital Imaging for Measuring Phenotypic Traits in *Polygonum*

Jolie Villegas, Sonia Sultan, and Ellen Woods

Department of Biology, Wesleyan University, Middletown, CT 06459

Introduction

An organism's growth and development is shaped by both genetic and environmental factors, which include current and parental conditions. In plants, these environmental inputs are manifested through phenotypic changes in both aboveground and belowground tissues. While shoot systems respond primarily to light, root systems are strongly influenced by soil moisture and nutrients. We have developed two digital imaging methods to precisely examine these aspects of plant phenotypes as influenced by experimental parent and offspring environments. One is a non-destructive technique to measure the total area of the plant's leaf canopy; the other uses scans to visualize and quantify complex root systems.

This summer, the Sultan Lab is studying two *Polygonum* species (Figs. 1-2) in two large-scale greenhouse experiments. These species are common plants that serve as model organisms for studying individual plasticity. Both species are non-native annuals, but only *P. cespitosum* is invasive.

Fig. 1: *P. persicaria* field population (with Robin Waterman BA '19 / MA '20)

Fig. 2: *P. cespitosum* field population

Acknowledgements

- Robin Waterman (BA '19, MA '20) for developing the root scanning technique.
- Sultan Lab members for carrying out the experiments in CT.
- Andy Tan ('21) for the greenhouse photos.
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Methods A: Measuring Total Canopy Area in P. *persicaria* (sun/shade)

Part of a larger project funded by the Templeton Foundation, this study examines how inherited effects of a parental environment (either sun or shade) influence how a developing individual responds to its own environment (either sun or shade).

I. Growing Experimental Seedlings

- Each flat contains 24 *P. persicaria* seedlings planted in a mix of soil, sand, and vermiculite.
- All seedlings within a flat have the same genotype and their parents were grown in the same environmental conditions (either sun or shade).

Fig. 3: Labeled seedling flats in the Wesleyan Research Greenhouse. Flats closer to the camera are grown in the sun, while others are hidden and growing under the dark shade tent towards the top left-hand corner of the image.

II. Taking RAW Photographic Images

- 6-8 days after emergence, experimental seedlings are ready for the stage of photo capture.
- Cohorts of six flats are photographed using the same vantage point and location.

Fig. 4: Lab member and COE summer research fellow Andy Tan '21 photographing a cohort with a Nikon D750 in the greenhouse.

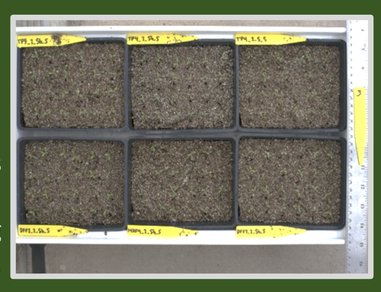


Fig. 5: Each flat in the cohort is labeled with its genotype, parental environment, and offspring environment (in this cohort, sun).

III. Color Editing: Selective Desaturation

- Each cohort is divided into six images, one for each flat, during image processing.
- Adobe Camera Raw is used to enhance the green color in the plant leaves.
- Adobe Photoshop is used to desaturate the vermiculite and soil for more accurate color thresholding in Step IV.

Fig. 6: A single flat from Fig. 5 ready to be edited in Camera Raw.

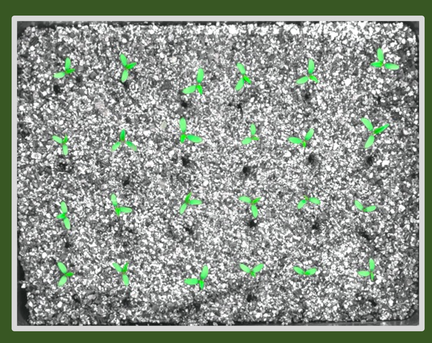


Fig. 7: A color edited image of Fig. 6, with enhanced green hues and a desaturated background.

IV. Image Binarization

- Color edited images are color thresholded using Adobe Photoshop.
- Green pixels become black and all other colors become white, creating a binary image.

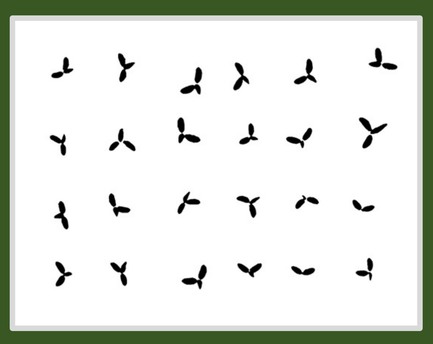
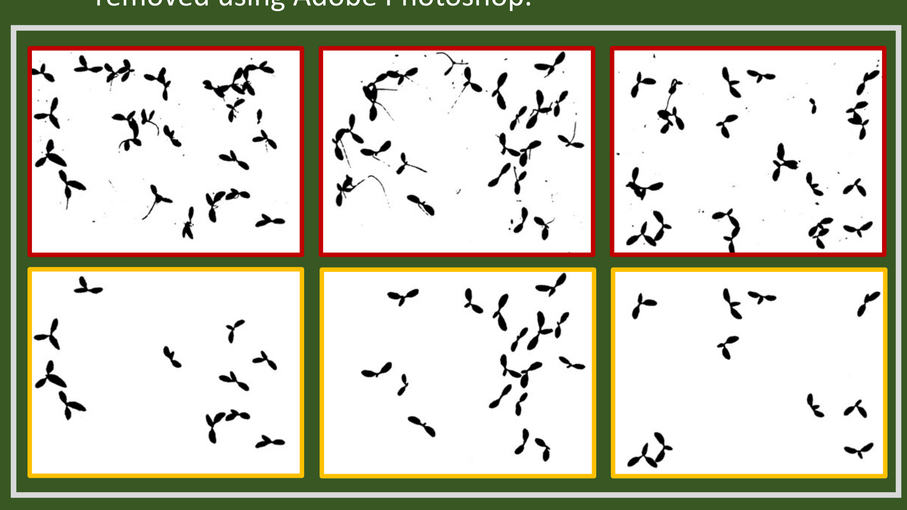


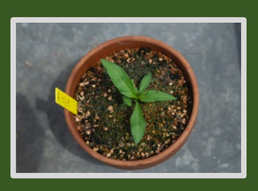
Fig. 8: A binarized version of Fig. 7 with all 24 seedlings visible and no overlap or noise.

- Rarely do images appear as clean as Fig. 8 – seedlings often overlap, grow outside the image frame or are oriented sideways.
- Vermiculite can also appear on the surface of the leaves, altering their shape after binarization.
- Most photos have noise in the background from vermiculite or plant stems that were not fully desaturated during the editing process.
- All of these prevent us from obtaining an accurate canopy area for that plant, so any problematic seedlings and noise are removed using Adobe Photoshop.

Fig. 9:
- Red borders = binarized images with overlapping, cropped, or sideways seedlings and background noise.
- Yellow borders = the same image as the one above it but cleaned to remove noise and problematic seedlings.



- Once all images of flats taken at a time point are binarized and cleaned, they are analyzed for mean canopy area. Higher pixel count indicates larger plant canopy area.



Figs. 10 & 11: *P. persicaria*.

- This method can also be used to measure canopy area of larger plants as growing seedlings are transplanted to individual containers (Figs. 10-11).

Methods B: Estimated Root Length in P. *cespitosum* (drought stress)

This study tests whether parental drought stress mediates heritable phenotypic effects by comparing root systems of seedlings grown in dry conditions.

I. Preparing *P. cespitosum* Roots

- Seedlings were germinated and then raised individually in a growth chamber for 22 days with limited water to maintain dry soil.

Fig. 12: Labeled seedlings ready for growing in their individual containers. Parents either come from dry or moist environments.

- At harvest, roots were separated from shoots, carefully washed, and preserved in alcohol for later study.

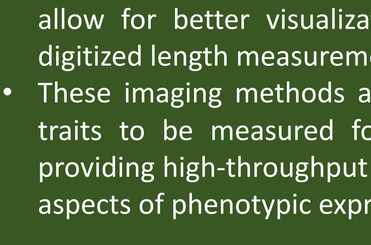
II. Scanning Roots

Fig. 13: A root from one plant with unopened root samples in the background.

- Each root system is spread out across a 15cm petri dish so that as much as possible of its complex branching network is visible.
- Roots are scanned using an Epson Perfection V800 Photo Scanner, and files are exported to be thresholded.

Fig. 14: A completed root scan.

III. Image Binarization



- Using similar methods as binarizing images for canopy area, the image is cleaned and converted to black and white.

Fig. 15: A binarized and cleaned version of Fig. 14.

Further Applications

In ongoing grant-funded experiments, the lab will use these imaging methods to track phenotypes throughout the plant life-cycle and match these developmental changes to transcriptome data collected at several timepoints.

Summary

- Digital imaging for plant canopy area is a non-destructive method to measure an individual *Polygonum* plant at different points in its life.
- Although only possible post-harvest, root scans provide exact measurements of root length that allow for better visualization and more accurate digitized length measurement than other methods.
- These imaging methods allow whole-plant growth traits to be measured for numerous individuals, providing high-throughput phenotyping for complex aspects of phenotypic expression.