Seedling trait differentiation and quantitative genetic analysis of *Impatiens capensis* ecotypes

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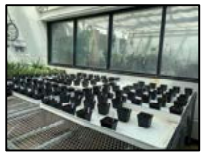
Introduction

Impatiens capensis is a weedy annual plant that grows contiguously from the Eastern U.S. to the Rocky Mountains through a range of soil conditions, from the moist habitats of the Eastern U.S. to very dry habitats in the West. This geographic range is possible through physiological and morphological adjustments in stomatal density, conductance, photosystem efficiency, and leaf growth (Bibee et al. 2011; Maruyama et al. 2016). Regional differences in these trait adjustments can be indicative of *Impatiens capensis* ecotypes in areas such as Pennsylvania and Colorado.

Previous work has examined maternal effects on physiology in the Pennsylvania ecotype, however studies have not yet examined seedling traits across multiple *Impatiens* ecotypes. Using Scanning Electron Microscopy (SEM), physiological, and morphological data, Colorado and Pennsylvania *Impatiens capensis* seedlings were analyzed for functional traits as well as genetic variance of photosystem efficiency, stomatal conductance, day of emergence, chlorophyll content index and height.

In addition to measuring and comparing traits, heritability calculations can be used to provide insight into the evolutionary potential of populations. With the changing climate, germination and seedling success could be affected by unpredictable spring weather patterns. Knowing the evolutionary potential of these ecotypes of *Impatiens capensis* seedlings could bolster our understanding of how this species will respond to climate change in regionally specific ways.

Maternal plants produced seeds for two experiments in this study. Plants were successfully inbred for four generations and inbred lines developed by single-seed descent were grown for at least 3-4 months in the Colorado College greenhouse.



Methods

Experiment 1

- Seeds from 44 lines (22 CO, 22 PA) were grown in the Colorado College greenhouse under stable conditions (20°C & 60% RH with a photoperiod of 14 hrs).
- After 13 days of growth, % photosystem efficiency (PE) measurements were taken on the largest cotyledon of each seedling with an EARS (NL) fluorometer (Fv/Fm), using a black background; after 16 days of growth, seedling height was measured (cm) from the soil surface to the apical meristem.
- To determine stomatal density, each cotyledon was observed in two different frames on the SEM. 66 cotyledons were fixed with 19% glutaraldehyde for two hours, put through 3 0.1M sodium cacodylate buffer washes, one two-hour wash of 1% OsO4, and 3 DI water washes, eight ethanol washes, critical point dried, and sputter-coated. In each frame the stomata were counted and converted to mm² density by dividing each plant's average number of stomata per frame by the average area of the frame (0.208mm²).
- Stomatal conductance measurements were taken on the maternal plants that provided seeds for this experiment; conductance was measured on newly expanded leaves between 10am & 3pm with a leaf porometer (Decagon Devices SC1).

Experiment 2

- Seeds from 35 lines (20 CO, 15 PA), were grown in the Colorado College growth chambers set at 20 degrees Celsius for 14 hours of light per day for 2 weeks and then grown in the greenhouse under stable conditions (20°C & 60% RH with a photoperiod of 14 hrs) for 2 weeks.
- Stomatal conductance was measured using a steady-state leaf porometer (Decagon Devices SC1). To do this, a leaf on the lowest node was chosen to measure, as it was the closet leaf to the cotyledons, similar to experiment 1. The same tools and methods in experiment 1 were used to measure height.
- Using a SPAD meter (Opti-Sciences), chlorophyll content index (CCI) were taken on a leaf of the second node.

Statistical Analyses

- All data were analyzed with JMP (vs. 4.0.4, SAS Institute) using one-way ANOVA to test if there are any significant differences in g_{ST}, PE, stomatal density, CCI, and height between Colorado and Pennsylvania populations.
- Quantitative genetic analysis was performed using g_{ST} data from seedlings grown in 2016 in identical greenhouse conditions and were similarly inbred (the maternal seed source for experiments 1 and 2) (Table 1).
- ANOVA was used to estimate genetic variance among lines for each population separately. Day of emergence, height, g_{ST}, and PE data from both experiments were used to estimate genetic variance. These variances were jack-knifed to test for differences in genetic variances between populations (Table 1).

Study Questions

1. Do *Impatiens capensis* seedlings from Pennsylvania and Colorado populations show differences in physiological and morphological traits such as stomatal density, stomatal conductance, photosystem efficiency, chlorophyll content index, and height?

2. Does phenotypic plasticity differ between Pennsylvania and Colorado varieties of *Impatiens capensis*?

Results

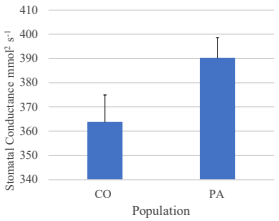


Figure 1. Average stomatal conductance (g_{ST}) for CO and PA seedlings. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=6.0699. df=1, P=0.0170]

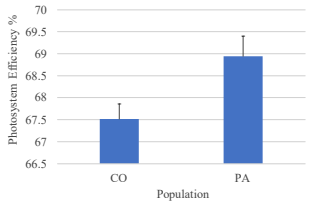


Figure 2. Average **%** Photosystem efficiencies for CO and PA seedlings. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=6.1899, df=1, P=0.0140]

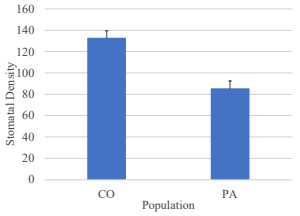


Figure 3. Average Stomatal Density per 0.208mm² for PA and CO seedlings. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=24.1513, df=1, P=0.<.0001]

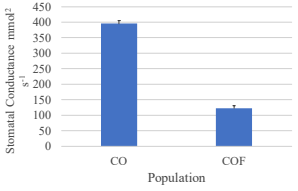


Figure 4. Average stomatal conductance (g_{ST}) for CO and Colorado field seedlings. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=440.5676, df=1, P=0.<.0001]

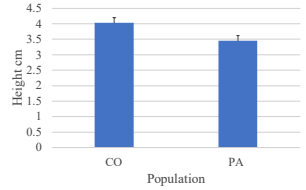


Figure 5. Average seedling height after 2 weeks of growth for CO and PA populations. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=6.0746, df=1, P=0.0152]

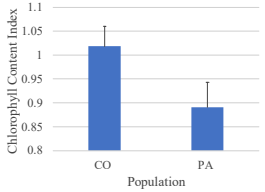


Figure 6. Average CCI for CO and PA seedlings. Mean and + SE bars shown. [One-way ANOVA Pop Effect: F=3.6192, df=1, P=0.06000]

Pop.	Trait	Vg	Ve	Heritability est.	F Ratio	Prob > F
CO	g_{ST}	16452.5*	44927.3	0.268	2.3607	0.0093
CO	PE	11.0227*	56.763	0.194	1.1348	0.339
CO	Day of emergence	12.0044*	24.5489	0.774	3.2034	0.0002
CO	Height	1.89929*	1.51836	0.5557	1.2509	0.2732
PA	g_{st}	8255.35	8023.99	0.507	1.0288	0.4471
PA	PE	12.4865	24.3222	0.5134	1.0079	0.4751
PA	Day of emergence	13.1872	9.1838	1.4359	2.3554	0.0191
PA	Height	4.77547	0.98023	0.8297	4.8718	0.0001

Table 1. Heritability estimates for stomatal conductance (g_{ST}), photosystem efficiency (PE), and day of cotyledon emergence from Colorado (CO) and Pennsylvania (PA) populations. F ratios and P-values are for ANOVA tests of genetic variance (i.e. line) significance. Genetic variance (Vg) and environmental variance (Ve) values were taken from mean squares (MS) of line and error in ANOVA tables. After jack-knifing each trait to generate sets of Vg values, the asterix indicates whether Vg is different between the populations at P≤ 0.05 with an ANOVA.

Literature Cited

Bibee, K., Shishido, K., Hathaway, R. P., & Heschel, M. S. (2011). Population Differentiation of *Impatiens capensis* (Balsaminaceae) at the Range Limit. *International Journal of Plant Sciences*, *172*(2), 211–219.

Maruyama, C., Goepfert, Z., Squires, K., Maclay, T., Teal-Sullivan, Q., & Heschel, M. S. (2016). Effects of population site and maternal drought on establishment physiology in *Impatiens capensis* meerb. (Balsaminaceae). *Rhodora*, *118*(973), 32–45.

Discussion

Functional Traits

- Colorado *Impatiens capensis* have an abundance of stomata but have low stomatal conductance when compared to Pennsylvania populations under moist conditions (Table 2; Fig. 1 and 3). This means CO *Impatiens* stomata are not always open and exchanging gas. In drought stressed conditions, CO seedlings can close stoma to retain water while still photosynthesizing (Fig 4). This figure shows how CO seedlings in the field had less stomatal conductance than CO seedlings in the greenhouse under stable conditions. Greater stomatal density allows for a greater range of transpiration rates in drier CO conditions. In essence, during large amounts of rainfall, CO seedlings can open all their stoma to uptake large amounts of water and during drought seedlings can close stoma to conserve water. PA seedlings are probably not as plastic in their water use due to lower stomatal density because PA has a more consistently wet environment than Colorado.
- There were significant differences in photosystem efficiency, indicating that PA seedlings have higher PE than CO (Fig. 2). Higher stomatal densities in CO populations may be a compensatory mechanism for this lower PE; i.e. CO cotyledons may have greater gas exchange capacity to compensate for less efficient photosystems. Moreover, CCI is marginally significant, making it possible for CO seedlings to compensate with increased CCI (Fig. 6).
- Day of seedling emergence varied between populations and genetic variation was significantly different (Table 1). This may be beneficial for the evolutionary trajectory of *Impatiens capensis* across all ecotypes. Moreover, equivalent establishment capabilities may be achieved with different combinations of stomatal density and PE.
- Early growth rates were significantly different (Fig. 5); CO seedlings were significantly taller than PA lines. The combination of increased stomatal density and CCI made the CO lines capable of faster growth, which could also be compensatory for having lower stomatal conductance and PE at 2 weeks of growth. Tall height can be advantageous during early growth stages for competition and acquiring nutrients.

CO Seedling Traits	VS	PA Seedling Traits
Stomatal Density	>	
Stomatal Conductance	<	
Photosystem Efficiency	<	
	>	Height
	=	Day of emergence
	>	Chlorophyll Content Index

Table 2. Summary of data analyses (one-way ANOVA) showing the differences between physiological and morphological traits of Colorado and Pennsylvania seedlings.

Quantitative Genetic Analysis

- Heritability and genetic variance were significantly different between populations (Table 1). This illustrates how variation between CO and PA populations could be due to natural selection. The CO population had generally lower seedling-trait heritability than the PA population; this difference might be due to past selection pressures, such as drought, decreasing genetic variability. Nonetheless, both populations had heritable seedling traits, indicating the potential for these populations to respond to future selection pressures like climate change.

Conclusions

Differences in physiological and morphological traits in *Impatiens capensis* seedlings from CO and PA are potentially caused by environmental selection pressures, such as water and light availability. The differences in evolutionary potential, such as lacking phenotypic plasticity, between these two regional ecotypes may have implications for the future of these populations as climate change becomes an even more predominant issue in these natural environments.

Acknowledgments

This research was made possible by funding from the Colorado College Hevey Fund and a Colorado College Summer Research Student Collaborative grant. We also appreciate the additional logistical support from Ali Keller and Donna Sison of the Colorado College Organismal Biology and Ecology Department.