Winter is coming: exploring seasonal changes in photoprotective pigments to better understand cold acclimation in *Rhododendron minus*

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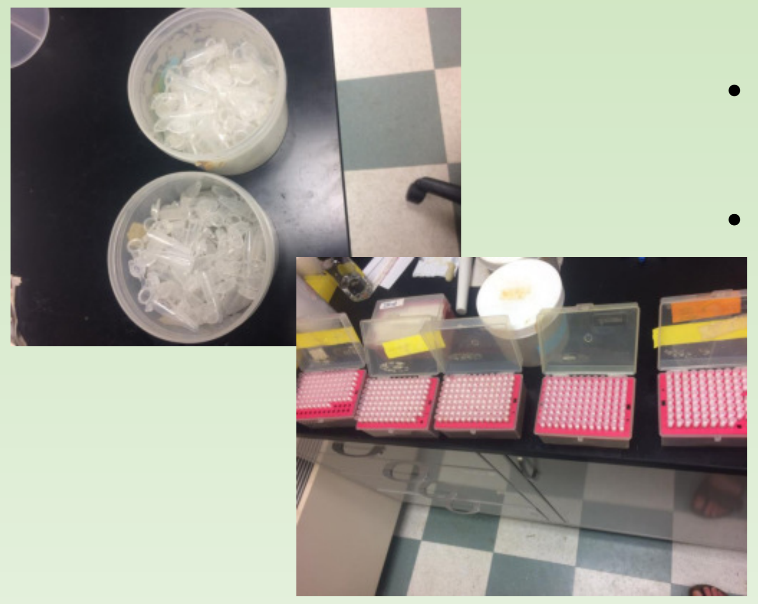
Objectives and background

Focus

- How do photoprotective pigments of *Rhododendron minus* differ across seasonal and geographical gradients?

Objectives

- Better understand physiological changes plants undergo to acclimate to seasonal changes in metabolic and photosynthetic rates
  - Population-specific changes may provide insight to whether acclimation is genetic or environmentally driven
  - Genes x Environment
- Conduct sampling in a sustainable, eco-friendly manner



- Approximately 500 pipette tips were rinsed in acetone and autoclaved
- Approximately 250 microcentrifuge tubes were washed and autoclaved for reuse

Xanthophyll cycle

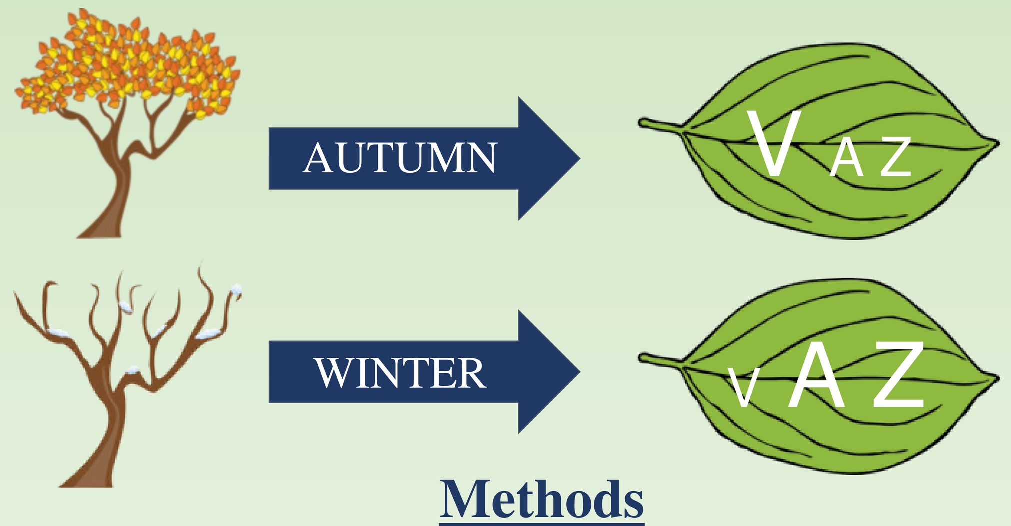
- The xanthophyll cycle mitigates the likelihood of photoinhibition by facilitating the de-excitation of excited chlorophyll and dissipating excess absorbed light energy as heat.
- Under low-stress circumstances, the xanthophyll cycle is largely comprised of violaxanthin.
- Abiotic stress may trigger the conversion of violaxanthin to antheraxanthin and then to zeaxanthin: an antioxidant capable of de-exciting excited chlorophyll - this pigment-type is associated with photoprotective qualities.

Photoinhibition

- Photons absorbed in excess of photosynthetic use enable the production of harmful oxygen species
- Harmful oxygen species (ROS) deactivate PSII, reducing the plant's photosynthetic capacity. Plants become more vulnerable to this phenomenon during winter months as metabolic rates decrease.

Hypotheses

- Larger levels of antheraxanthin and zeaxanthin and larger overall pigment pools will be observed during winter months in *R. minus* native to colder climates.
  - This increase would provide better photoprotection during winter months, where plant metabolism and light-utilization rates are low.
- Violaxanthin levels are expected to be greater in autumn samples compared to winter samples.
  - This hypothesis is based on the idea that autumn plants are in the process of winter acclimation although a fully protected state has not yet been reached - maybe these plants are trying to maximize photosynthetic output during autumn-warm spells?



Methods

- *R. minus* seeds were wild collected from six locations (Fig. 1)

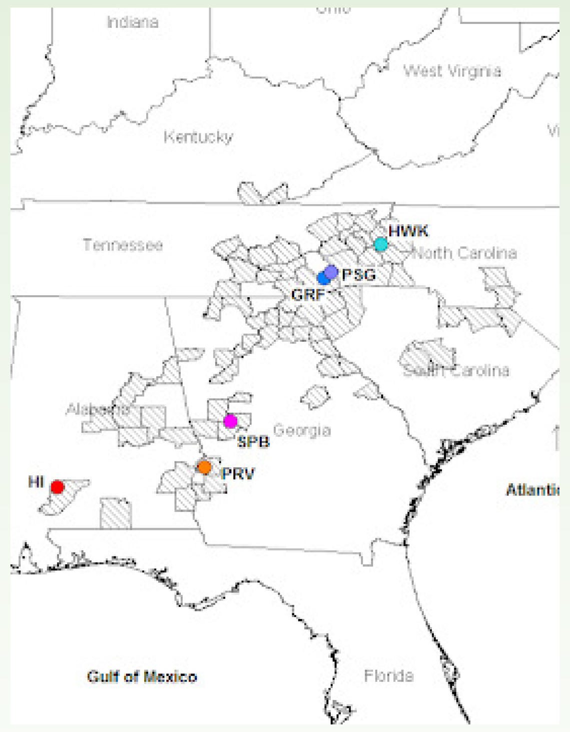


Fig 1. (left) depicts the geographical locations where R. minus seeds were wild collected:

- Haines Island Park, Monroe Co. AL (HI), Tmin = 1.3°C
- Providence Canyon State Park, Stewart Co. GA (PRV), Tmin = 1.6°C
- Sprewell Bluff Wildlife Mgt. Area, Upson Co./Talbot Co. GA (SPB), Tmin = 0.3°C
- Graveyard Fields, Blue Ridge Parkway, Haywood Co, NC (GRF), Tmin = -7°C
- Mt. Pisgah, Blue Ridge Parkway, Haywood/Buncombe Co, NC (PSG), Tmin = -5.7°C
- Hawksbill Mountain (Linville Gorge Wilderness NC) Burke Co. NC (HWK), Tmin = -6.1°C

- Twelve plants per population were grown in the lath house at the Ellen Corning Long and T. Dixon Long Center for Plant and Environmental Science in Kirtland, OH 44094.
- Two leaf punches (0.38cm^2)
  - Predawn (PD) and midday (MD)
  - Summer, short days, cold temperatures and freezing
    - Analyzed 2-3 plants per population from the last two time points
  - Pigment quantified using an HPLC calibrated with pigment standards.

Key findings

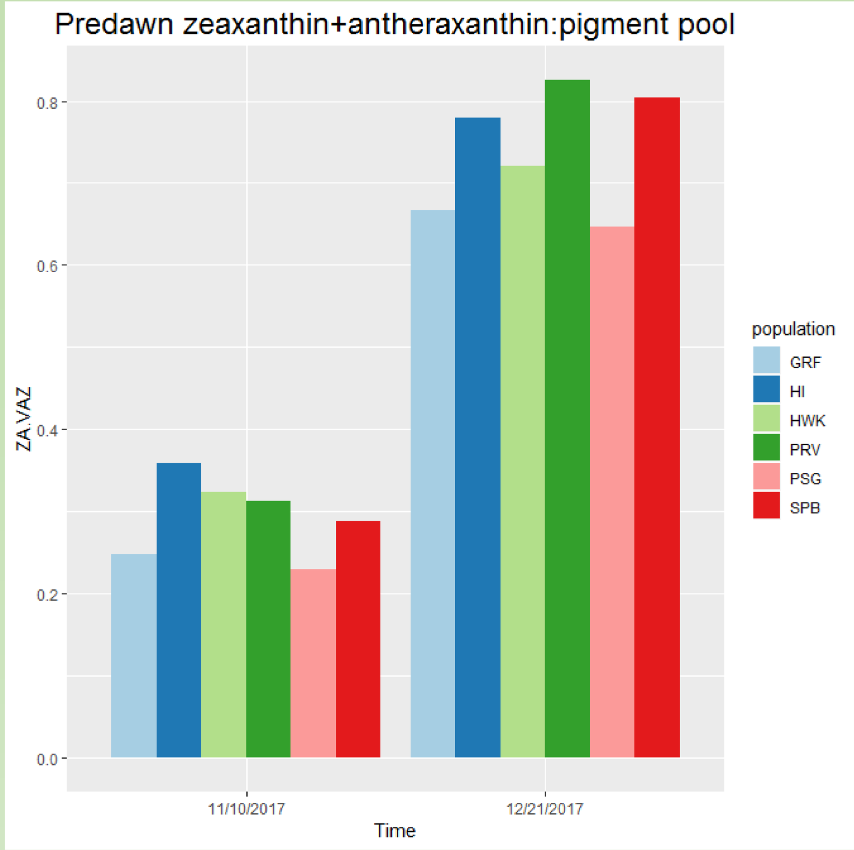


Figure 2. Note the drastic increase in antioxidants across a seasonal gradient. Changes in ZA:VAZ are statistically significant across time but not populations (p = 5.41e-14).

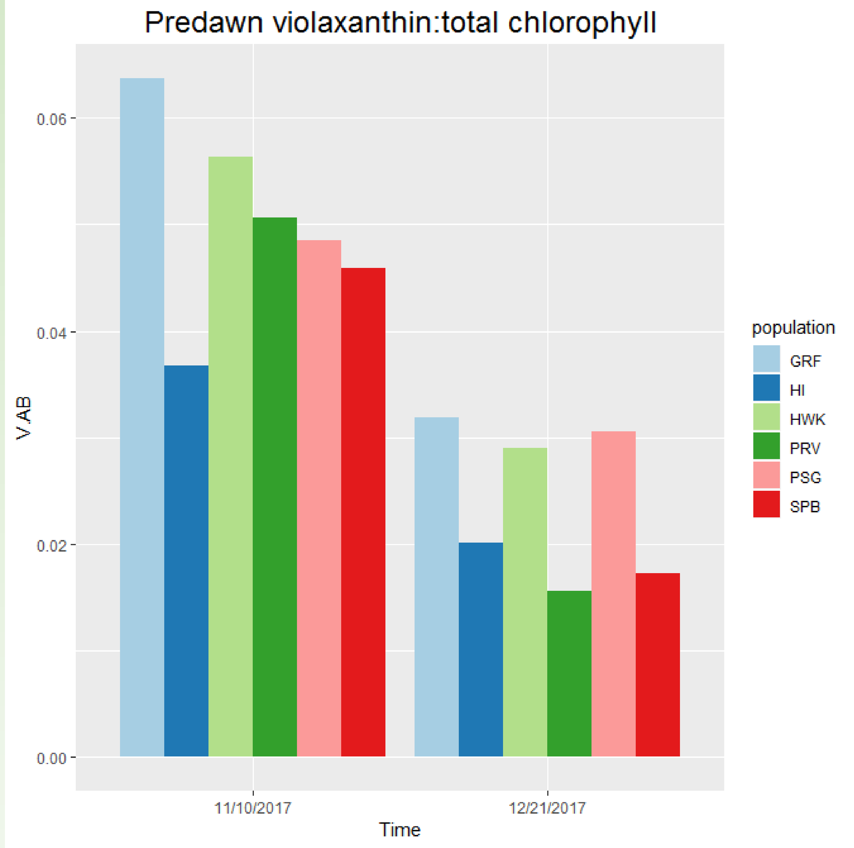


Figure 3. Note the decrease in Violaxanthin, the pigment associated with low-stress conditions. Changes in V:AB at predawn are statistically different across a seasonal gradient (p = 8.92e-07)

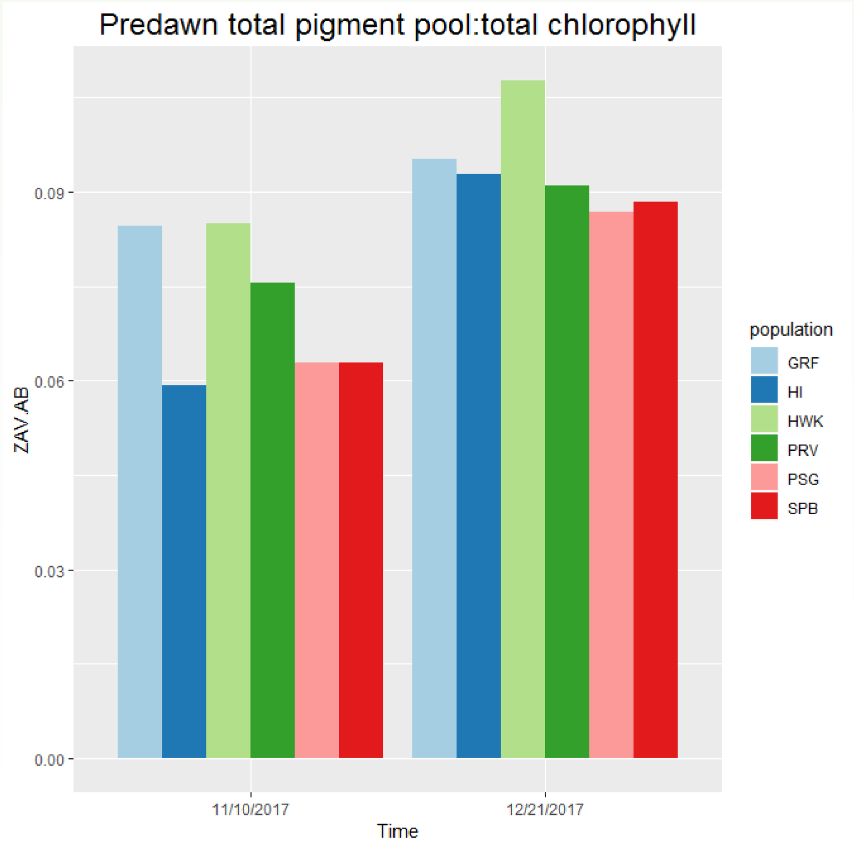


Figure 4. Note an increase in total pigment pool at predawn, increasing the plant's photoprotective capacity. This increase is significant across a geographical and seasonal gradient (p = 2.93e-05, p = 0.0172 respectively). G x E is also statistically significant (p=0.0215).

Key findings

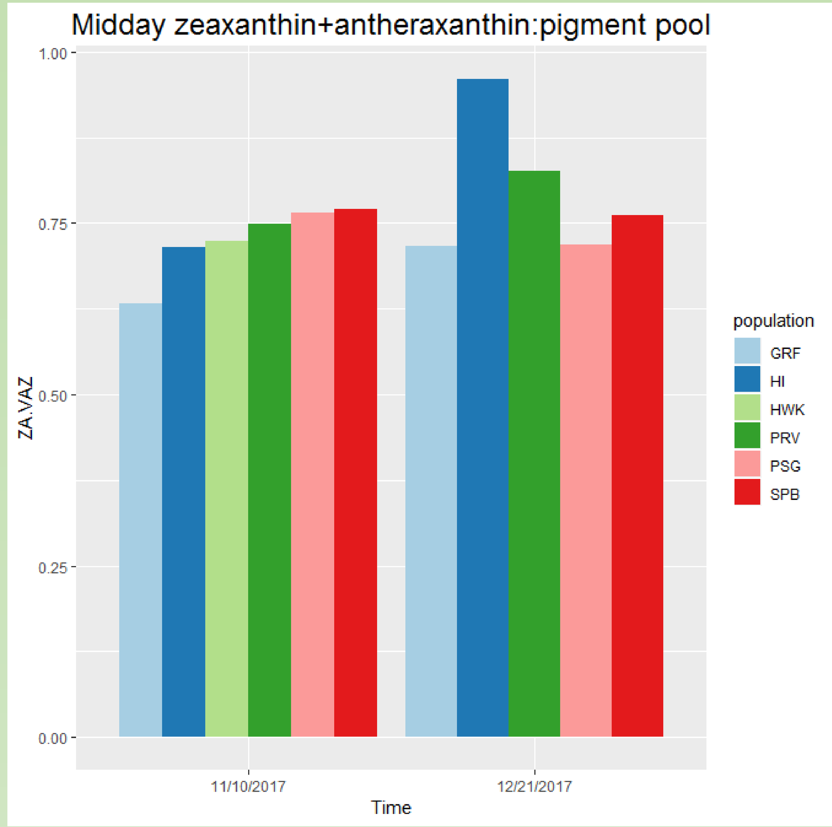


Figure 5. Changes in ZA:VAZ are statistically different across populations, but not times, indicating that native climate influences retention of ZA at midday periods (p = 0.496).

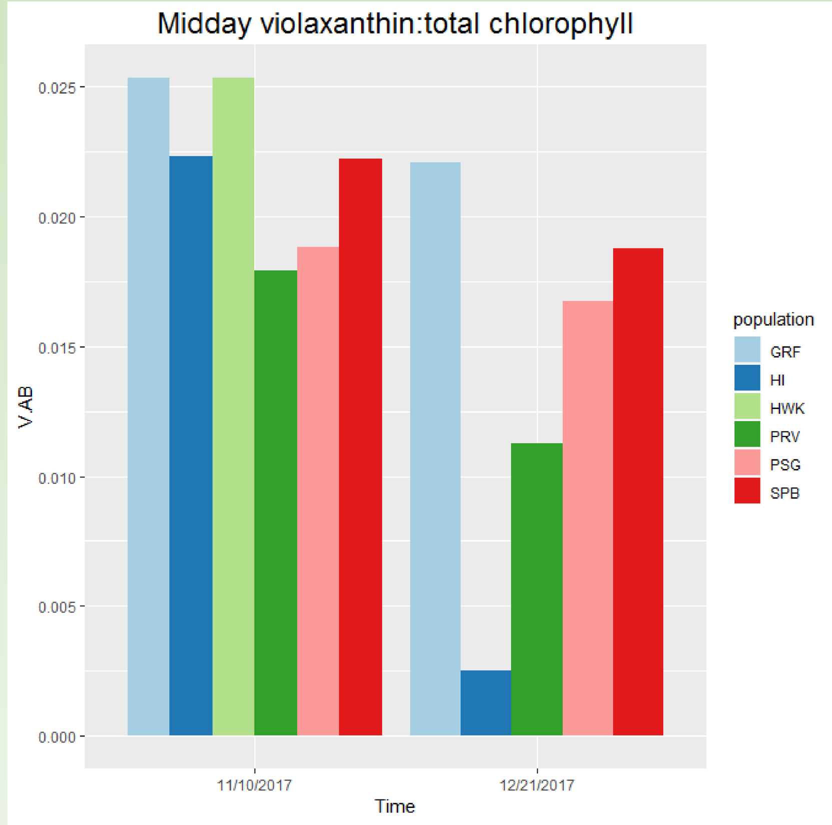


Figure 6. Note the decrease in Violaxanthin (low-stress pigment). Changes in V:AB at midday are statistically significant across time and population (p=0.00553, p=0.00390 respectively).

Further observations and acknowledgements

- Time (seasonal changes in abiotic factors) had a bigger impact on determining the levels of photoprotective pigments than population (native climate).

	Midday			Predawn		
	population	time	population:time	population	time	population:time
Chlorophyll B	ns	ns	ns	*	ns	ns
Chlorophyll A	ns	ns	ns	ns	ns	ns
AB	ns	ns	ns	ns	ns	ns
Neoxanthin:AB	ns	*	ns	ns	ns	ns
Violaxanthin:AB	**	**	ns	ns	***	ns
Antheraxanthin:AB	ns	ns	ns	ns	***	ns
Lutein:AB	ns	ns	ns	ns	ns	ns
Beta Carotene:AB	ns	ns	ns	ns	ns	ns
Zeaxanthin:AB	ns	ns	ns	ns	***	ns
ZA.AB	ns	ns	ns	ns	***	ns
ZAV.AB	ns	ns	ns	ns	**	ns
Z.VAZ	*	ns	ns	ns	**	ns
ZA.VAZ	ns	ns	ns	ns	***	ns
V.VAZ	ns	ns	ns	ns	***	ns
VAZ	ns	ns	ns	***	*	*
	*=0.5, **=0.01, ***=0.001					

Thank you Juliana Medeiros for the amazing opportunity to contribute to your ongoing research, and Charlotte Hewins for teaching me the methodology involved with this project. I would also like to thank the Holden Forests and Gardens for funding my internship and providing housing - my experience would not be possible without your generosity.