Impact of mycorrhizal fungi on ostrich fern *Matteuccia struthiopteris* resilience to drought and heat stress

Ellie Churchill and Ben Keegan -- NR 103 Signature Assignment -- April 2021

Background:

The fern family carries much importance to the ecosystems they inhabit. They provide shelter to small animals and for species that live underneath them. They also protect from toxins and as a result of that become a sort of bioindicator for the well being of their ecosystem (Sierra Club, 2018). More specifically, silver maple and ostrich fern forests are coveted because of their rarity and their agricultural potential, with such few rocks and high annual flooding these forests were like gold mines for agricultural farmers in the 1700-1800s (Thompson, 2019). As a result of this most of them were lost to development, and because they are threatened, so are the unique ecosystems and species that inhabit them. Development of agricultural land is not the only thing harming these ecosystems: extreme drought and extreme heat are lethal for unprotected ecosystems. The average temperature in Vermont has gone up over a degree in the last 15 years, (Climate Central, 2016). Luckily, studies show that having a mycorrhizal fungi network, which are present in some of these ecosystems, increases that ecosystems resilience to these obstacles of climate change, (Simard, 2009). Our research would contribute to conservation efforts for these special riverine ecosystems by understanding what role mycorrhizal fungi networks play in the resilience of ostrich fern forests in the face or heat and drought stress from climate change. This data will support conservation efforts by provide new tools for increasing forest resilience.

Prediction: Ostrich ferns inoculated with mycorrhizal fungi will be more resilient to heat and drought stress than those grown without mycorrhizal fungi.

Null hypothesis: There will be no difference in height of shoots, length of roots, dry weight, or relative water content between ferns frown with and without mycorrhizal fungi.

Figure 1 (Jadrane et al., 2021)

Table 1. Root length (RL), Shoot height (SH), Shoot fresh weight (SFW), Root fresh weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW) and Relative water content (RWC) in non-mycorrhizal (NM) and inoculated (AMF) carob seedlings. Mean values ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 0.05 by Tukey test. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

Water regime (% FC)	AMF inoculation	RL (cm)	SH (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
100%	NM	44 ± 0.58 d	6 ± 0.10 bc	1.02 ± 0.01 c	0.76 ± 0.02 bc	0.62 ± 0.01 de	0.31 ± 0.01 b
	AMF	63.67 ± 0.67 a	9.57 ± 0.09 a	2.13 ± 0.26 a	1.17 ± 0.05 a	1.49 ± 0.13 a	0.69 ± 0.06 a
75%	NM	43.33 ± 0.67 d	5.7 ± 0.06 cd	1.04 ± 0.03 c	0.68 ± 0.02 bcd	0.64 ± 0.01 de	0.27 ± 0.01 b
	AMF	64.67 ± 0.33 a	9.1 ± 0.12 a	1.96 ± 0.15 a	0.92 ± 0.1 ab	1.26 ± 0.05 ab	0.64 ± 0.01 a
50%	NM	48.33 ± 0.88 c	5.2 ± 0.06 de	1.07 ± 0.09 c	0.54 ± 0.03 cd	0.58 ± 0.01 de	0.18 ± 0.01 b
	AMF	54.67 ± 0.88 b	6.43 ± 0.24 b	1.81 ± 0.02 ab	0.93 ± 0.04 ab	1.05 ± 0.03 bc	0.65 ± 0.06 a
25%	NM	50.33 ± 0.88 c	4.93 ± 0.09 e	0.9 ± 0.04 c	0.44 ± 0.02 d	0.48 ± 0.03 e	0.16 ± 0.01 b
	AMF	52.67 ± 0.88 bc	6.13 ± 0.12 bc	1.26 ± 0.06 bc	1.053 ± 0.02 a	0.86 ± 0.08 cd	0.58 ± 0.03 a
AMF inoculatin (AMF)		555.02***	750.23***	93.27**	109.97***	192.09***	310.61***
Field Capacity (FC)		7.03**	171.50***	7.18***	7.19**	15.62***	5.92**

Figure 2 (Climate Central, 2016)

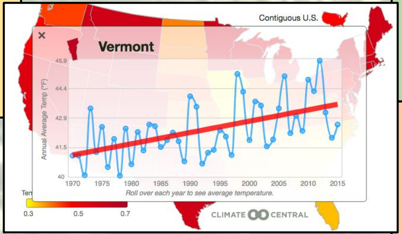
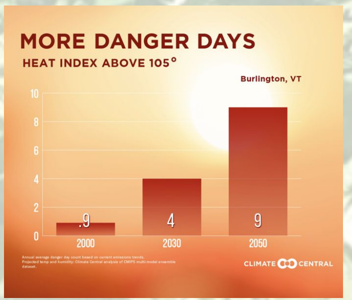


Figure 3 (Climate Central, 2016)



Experimental Design:

1. Fiddlehead crowns will be randomly selected from riverine Ostrich Fern forests in Vermont. All will be planted in equally sized pots with identical soil mix, which will have been autoclaved to ensure no fungal contamination. Half of the pots will be randomly chosen to be inoculated with mycorrhizal fungi by placing 5g of freshly harvested mycorrhizal fungi roots into the pots during planting. The pots will be kept in the same greenhouse and watered daily for four weeks. After four weeks, the pots will be randomly separated into 4 groups.

   Heat group: increase temperature and keep watering the same: Temperature (day°F/night°F) increases from 75/60 to 90/70
   Heat and drought group: increase temperature and decrease water: Temperature (day°F/night°F) increases from 75/60 to 90/70 and watering decreases 10% each week
   Drought group: keep temperature the same and decrease water. Watering decreases 10% each week
   Control group: Keep temperature and water the same

2. Each group will consist of 10 fungi-treated and 10 non-treated pots (80 total pots). The experimental conditions will be maintained for 8 weeks.
3. After 8 weeks, the ferns will be harvested and the length and dry weight of the roots and shoots will be measured. The resilience of the plants will be compared using data from height of shoots, length of roots, dry weight, and relative water content. These criteria were chosen because as seen in Figure 1, Jadrane et al. used similar criteria to measure resilience of carob seedlings to drought stress when inoculated with mycorrhizal fungi (Jadrane et al., 2021).
4. Relative water content determined by: Relative water content will be determined using this formula: RWC = 100 × [(FreshWeight-DryWeight)/(TurgidWeight-DryWeight)] (Talaat & Shawky, 2014). The turgid weight will be found by fully submerging the plants in water in the dark for 24 hours at 40°F," (Jadrane et al., 2021).

Intended Analysis: The results from the mycorrhizal and non-mycorrhizal treatments under each heat/drought stress condition will be compared to determine whether there is a significant difference in response variables (height of shoots, length of roots, dry weight, and relative water content) in response to change in predictor variables ferns grown with or without mycorrhizal fungi).

Because the independent variable is categorical (mycorrhizal vs non-mycorrhizal treatments) and the dependent variables are continuous (height, length, dry weight, water content), we will use T-tests to compare each treatment group across the 6 variables. The T-tests will give a p-value. If the p-values are less than 0.05, we will reject the null hypothesis that there is no difference between the treatment groups.

Because this is a lab study, not a field study, the scope of this inference can be applied to lab grown ostrich ferns, and may also be able to be extended to ostrich ferns growing naturally in riverine forests in Vermont, where the samples came from. It will hopefully help inform whether mycorrhizal fungi networks confer resilience to ostrich fern forest ecosystems in the context of global climate change.

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