GARDEN CLIMATE EFFECTS ON ENDOPHYTE ABUNDANCE AND MORPHOLOGY OF *POPULUS FREMONTII* (FREMONT COTTONWOODS) POPULATIONS FROM ACROSS THEIR TEMPERATURE RANGE

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ABSTRACT

GARDEN CLIMATE EFFECTS ON ENDOPHYTE ABUNDANCE AND MORPHOLOGY OF *POPULUS FREMONTII* (FREMONT COTTONWOOD) POPULATIONS FROM ACROSS THEIR TEMPERATURE RANGE

SCOTT BAKER

Premise of the study: Fungal endophytes are symbionts that reside asymptomatically in plant tissues and have relationships that can range from mutualistic to parasitic. These fungi can have profound impacts on plant fitness, evolution, and communities as well as on the structure of the broader phytosphere community. It is known that biotic and abiotic factors affect the endophyte community and abundance; however, few studies have looked at the effect of source population and site simultaneously. We investigated three main questions: (1) Is endophyte abundance in *Populus fremontii* (Fremont cottonwood) affected by population source climate, garden climate, and their interaction? (2) Are quantitative tree traits linked to endophyte abundance? (3) Do population source climate, garden climate, and their interaction have an effect on quantitative tree traits in *P. fremontii*? We also present preliminary data on endophyte community composition across the three gardens.

Methods: Endophytes were isolated from twigs of replicated *P. fremontii* populations in three common gardens across an elevational and temperature gradient and characterized with DNA sequencing. Twig length and diameter and leaf mass were also quantified.

Key results: 1) Fungal endophyte abundance varied by garden climate and population source climate, but there was no significant population by garden interaction. Garden climate had the greatest effect with cottonwoods from all source populations showing dramatically reduced endophyte abundance in the coldest garden. Interestingly, cottonwoods from the coldest

populations had the highest endophyte abundance in all three gardens. 2) Shoot length and dried leaf mass were associated with endophyte abundance in Fremont cottonwoods while twig diameter was not. 3) Twig diameter was only affected by population source climate; however, shoot length and dried leaf mass were significantly affected by population source climate, garden climate, and their interaction. 4) The Warm and Hot gardens were dominated by endophytes of the genus *Alternaria*, while the Cold garden was dominated by members of the genus *Valsa*. *Conclusions:* These results suggest that even a modest change in temperature can greatly influence fungal endophyte abundance and host plant morphology; however, endophyte abundance and the plant traits we measured were not tightly linked and in the opposite direction of our prediction. Given that climate change is affecting temperatures across the globe, the relationship between host plant and endophyte abundance and community may be crucial to conservation efforts for foundation species, but difficult to predict from plant traits.

Key words: Endophyte, class III endophyte, Fremont cottonwood, abundance, climate, plant response.

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INTRODUCTION

In response to climate change plant species are forced to acclimate, adapt, or migrate lest they face total extinction (Etterson and Shaw 2001a; Aitken et al. 2008). For slow migrating species, like long-lived trees, symbiotic relationships with endophytes may provide fitness benefits that are critical to species persistence as predicted rates of evolutionary responses may be slower than the predicted rates of climate change (Etterson and Shaw 2001).

Endophytes are a broad classification of microbial symbionts, including both bacteria and fungi, that live asymptomatically in plant tissue. Unlike mycorrhizal associations, endophytes live within the host cells and only produce external structures during host senescence (Herre et al. 2005). Endophytes can provide a myriad of benefits like disease resistance, increased nutrient uptake, increased water use efficiency, heavy metal resistance, decreased herbivory, and heat/cold tolerance, to the host plant in exchange for sugars, a place to live, and/or reciprocated fitness benefits (Redman et al. 2002; Rodriguez et al. 2008); however, these relationships can become parasitic when the host becomes too stressed (Krabel et al. 2013). Benefits can be habitat adapted (HA) like resistance to soil salinity and pH and temperature tolerance, to nonhabitat adapted benefits like drought tolerance (Rodriguez et al. 2008). Leaves and twigs of trees are frequently colonized by Class 3 endophytes (Rodriguez et al. 2009) which are known for their high diversity (Arnold et al. 2000; Gamboa and Bayman 2001). Class 3 endophytes are spread through hyphal fragmentation and spores (sexual or asexual) that are dispersed by wind, rain, or herbivory (Rodriguez et al. 2009). These propagules readily and rapidly infect plants (Arnold and Herre 2003); however, these infections are characterized by limited local tissue colonization and low biomass of the endophyte (Rodriguez et al. 2009). Due to the lack of extensive tissue colonization the infections are transitory (Rodriguez et al. 2009). Despite their diversity and

transitory nature, Class 3 endophytes can have significant impacts on forest trees. Common taxa of Class 3 endophytes also can be latent pathogens, potentially causing damage to their hosts under stressful conditions (Carroll 1988; Rodriguez et al. 2009).

In order to understand how fungal endophytes influence forest tree responses to the environment, it is important to determine the factors that influence their distribution. Endophyte community composition and diversity are affected by abiotic conditions like temperature and precipitation (Arnold & Lutzoni 2007; Zimmerman & Vitousek 2012; Whitaker et al. 2018) as well as biotic conditions such as host specificity, host and endophyte genetics, and interspecific interactions with other microbes (Lau et al. 2013; Bálint et al. 2013; Bailey et al. 2015; Harrison et al. 2018; U'Ren et al. 2019). Presently, there is a dearth of knowledge of how environmental factors and host genetics interact to affect endophyte communities. The only study to our knowledge is Bálint et al. (2015) which found that there were significant interactions between host genotype and temperature in balsam poplar (*Populus balsamifera*).

This study aims to address this lack of knowledge by using a common garden experiment involving replicated gardens at three elevations to investigate the twig endophyte community of a riparian foundation species: Fremont cottonwood (*Populus fremontii*). Common gardens are useful for studying local adaptation patterns to investigate adaptive variation in a population for single traits, and multiple traits across a wide climate gradient because they can remove the confounding effects of the local environment (Clausen et al. 1940; Kawecki & Ebert 2004; de Villemereuil et al. 2016; Cooper et al. 2019; Blasini et al. 2020). Fremont cottonwood is widespread along riparian corridors across the western U.S. (Ikeda et al. 2017) and is a driver of riparian community structure and ecosystem processes (Whitham et al. 2006). Specifically, we looked at three main questions: (1) Is endophyte abundance affected by population source

climate, garden climate, and their interaction? (2) Are quantitative tree traits linked to endophyte abundance? (3) Do population source climate, garden climate, and their interaction have an effect on quantitative tree traits in *Populus fremontii*?

METHODS

Common Garden Design:

Three common gardens (Cold, Warm, Hot) were set up across an elevational and temperature gradient (Table 1) in October 2014. The garden designated as 'Cold' was located on Dugout Ranch near Canyonlands National Park, Utah (N 32.8498, 114.4928; 1581m elevation). The ranch is maintained by The Nature Conservancy's Canyonlands Research center and sits along the Colorado River. This garden was the coldest of the three common gardens with a mean temperature gradient from -3.2°C to 24.6°C (Table 1). The 'Warm' garden was located on former cropland in central Arizona next to the Agua Fria River at Horseshoe Ranch (N 34.2567, 112.0661; 988m elevation). The garden is maintained by the Arizona Game and Fish Department. Its mean temperature gradient falls between the climatic extremes of the other two gardens (7.6°C to 28.5°C). The 'Hot' garden was located in Yuma, Arizona near Mittry Lake (N 32.8498, 114.4928; 49m elevation). The garden is maintained by the Bureau of Land Management. This garden has the highest mean temperature gradient 12.7°C to 33.8°C.

The gardens were populated with rooted cuttings of *Populus fremontii*. The cuttings were taken from 16 populations that span the climatic range of the Sonoran Desert Ecoregion (Ikeda et al. 2017) (Table 1). Cuttings were taken during the 2013-2014 winter at random from 12 trees, representing different genotypes, per source population. Cuttings were taken from low-hanging branches of disease-free trees greater than 20m apart to avoid gathering clones within a population. Cuttings were rooted in the Northern Arizona University greenhouse for four months. Cuttings were transplanted into the common gardens after they reached approximately 0.3m tall.

The three common gardens each had four replicated blocks of 16 population level plots. Every population plot consisted of 64 trees with three to six replicates of the 12 genotypes giving individual gardens a total of 4096 trees. The trees were planted with 1.85m spacing in the cardinal directions. To discourage ungulate grazing a 2.5m fence was erected around the garden perimeters.

The Warm and Cold gardens (Auga Fria and Canyonlands, respectively) were drip irrigated during the growing season (i.e. >0°C) with approximately five gallons of water per tree, three times per week. The Hot garden (Yuma) was flood irrigated every two weeks between March and October with one-acre foot of water. Outside Mar-Oct, the Hot garden is flood irrigated only once per month with the same volume of water.

We sampled seven of the 16 source populations which collectively represent the greatest range in source population mean annual temperature (10.7- 22.6°C) and have an elevational gradient from 26 to 1920m. The three source populations (SCT-MEX, LBW-BIL, CCR-COL) designated as 'Hot' have a mean annual temperature (MAT) greater than 22°C (Table 1). The two 'Cold' defined source populations (KKH-OPI, JLA-JAK) have a mean annual temperature lower than 12.5°C (Table 1). The two 'Warm' labelled source populations have mean annual temperatures which fall between the two other source populations (15°C to 17.4°C) (Table 1).

Sample collection:

Twigs with three years of growth, as indicated by bud scale scars, were collected from the common garden sites from May to July in 2019 matching phenological timing in the gardens. A single representative twig from the main stem or trunk was taken from each tree. A resprouting twig was selected in cases where the tree exhibited severe dieback resulting in some twigs being

only one to two years of age. Twigs were kept inside a cooler in the field. Afterwards they were transferred to a refrigerator until further processing within one to two days. Twigs were measured for length, mid-twig diameter, leaf surface area, dried leaf mass, number of leaves, and bud scars.

Culturing:

Twigs were chosen for culturing as it has been found that Fremont cottonwood leaves harbor a small amount of foliar endophytes (Wilson 1995; Lau et al. 2013). Twigs were surface sterilized with 70% ethanol (EtOH) for 2.5 mins then in 50% bleach for 2.5 mins before being rinsed twice in sterile DI water and plated onto potato dextrose agar (PDA). Plates were split into quadrants and a total of four, 1 cm length twig segments per placed on each plate. Fungi that emerged were subcultured based on culture morphology by cutting agar plugs from the leading edge of hyphal growth and plating onto fresh PDA plates .

DNA Extraction, PCR, and Sequencing:

DNA extraction protocol was adapted from Mayjonade et al. (2017) for use with fungal endophytes by reducing the centrifugation gravities from 12000g to 8000g.

The extracted DNA was amplified using primers “ITS1F-xt” and “ITS4-fun” which target the ITS1 and ITS2 sequences. DreamTaq DNA polymerase was used for the PCR. The PCR was run on a Veriti™ 96 well thermal cycler. The bead cleanup protocol for the PCR products was adapted from Rohland & Reich (2012). The cleaned PCR products were cycle sequenced with BigDye v3.1 using the following parameters: initial denaturation at 95°C for two minutes;

denaturation at 95°C for 10 secs; annealing at 50°C for 10 secs; extension at 60°C for 2 mins; infinite hold at 10°C. Denaturation, annealing, and extension were repeated for 60 cycles.

After cycle sequencing, the samples were cleaned with the above adapted protocol in preparation for Sanger sequencing. Sequencing was done on a ABI 3730xl DNA Analyzer in NAU's Environmental Genetics and Genomics facility as described by Lamit et al. (2014).

Sequences were trimmed using the Whitehead Institutes for Biomedical Research's Staden pregap4 (v1.6-r) and aligned and combined with Gap4 (v4.11.2-r) into a single sequence (Bonfield 1995). Sequences were then compared to the Nucleotide collection database from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) and the UNITE database v8.3 (https://unite.ut.ee/). The set operational taxonomic unit was set at a 95% sequence similarity as in U'Ren et al. (2019).

Functional Analysis:

A general linear model (glm) was fitted with Garden climate and Population source climate as fixed effects with shoot length, twig diameter, and dried leaf mass as dependent variables in IBM SPSS statistics v.28. The glm had two factors (Garden climate and Population source climate) with three levels. A two-way multivariate analysis of variance was performed followed by Tukey's Honestly Significant Difference (HSD) Test to examine differences between groups. Then a univariate analysis of variance was performed to investigate the interaction between Garden climate and Population source climate. This was followed by Tukey's B Test to elucidate how isolation differed by Garden Climate and Population Source Climate.

RESULTS

Endophyte Abundance:

Endophyte abundance, defined as the percent of twigs yielding culturable endophytes, was affected by garden climate and population source climate, but the interaction was not significant. Garden had the largest effect with percent isolation highest for all cottonwood populations in the Warm garden and lowest in the Cold garden ($F_{2, 216} = 160.656$, $p = <0.001$) (Fig. 1). Population source climate also had a significant effect with percent isolation highest in the Cold population and no significant difference between the Warm and Hot populations ($F_{2, 216} = 7.671$, $p = <0.001$) (Fig. 1). The interaction between garden climate and population source climate did not significantly affect endophyte abundance ($F_{4, 216} = 2.151$, $p = 0.076$).

Shoot length and dried leaf mass were associated with endophyte abundance. Shoot length had the strongest association ($R^2 = 0.121$, $F_{1, 216} = 29.673$, $p = <0.001$). Dried leaf mass was also associated ($R^2 = 0.029$, $F_{1, 216} = 6.364$, $p = 0.012$). Twig diameter did not have a significant association with endophyte abundance ($R^2 = 0.002$, $F_{1, 216} = 0.379$, $p = 0.539$)

Cottonwood Quantitative Traits:

Cottonwood shoot length was affected by garden climate, population source climate, and their interaction. Garden climate had the largest effect with shoot length being longest for all populations in the Cold garden except for the Cold population which was longest in the Hot garden ($F_{2, 216} = 30.537$, $p = <0.001$)(Fig. 2). Plants in the Warm and Hot garden did not have significantly different shoot lengths from each other, but both were significantly different from plants in the Cold garden (Fig. 2). Population source climate had a significant effect on shoot length with the Hot populations being significantly different from the others ($F_{2, 216} = 4.900$, $p =$

0.008)(Fig. 2). For shoot length, the interaction had a greater effect than population source climate ($F_{4, 216} = 14.809$, $p = <0.001$).

Twig diameter was only affected by population source climate ($F_{2, 216} = 8.395$, $p = <0.001$). The average twig diameter of the Cold population was significantly different from the Warm and Hot populations (Fig. 3).

Dried leaf mass was affected by garden climate, population source climate, and their interaction. Population source climate had the greatest effect with dried leaf mass greatest in the Cold population across all gardens ($F_{2, 216} = 8.395$, $p = <0.001$) (Fig. 4). The Cold population was also significantly different from the Warm and Hot populations. Garden climate also had an effect ($F_{2, 216} = 3.549$; $p = 0.031$). Dried leaf mass in the Warm garden was not significantly different from the Cold or Hot gardens; however, both significantly different from each other (Fig. 4). The interaction had the least effect on dried leaf mass ($F_{4, 216} = 2.730$, $p = 0.030$).

Community Data:

A total of 49 sequences were included in this study from across all populations and gardens. The genus *Alternaria* was found for 33 of the 49 sequences. *Alternaria* dominated the Warm and Hot gardens accounting for more than 70% of the relative abundance in each (Fig. 5). The species *Valsa sordida* was reported for nine of 49 sequences. *Alternaria* and *V. sordida* each account for more than 42% of the relative abundance in the Cold garden (Fig. 5). The species *Cytospora salicinia* was found for two of 49 sequences and was not found in the Warm garden. *Cytospora* is the anamorphic (asexual) genus of the teleomorph (sexual) genus *Valsa* (Wang et al. 2015). The club moss species *Huperzia serrata* was found for 2 of 49 sequences and was likely a result of contamination as both sequences were returned from the same run despite being from

different gardens. *Cladosporium perangustum*, *Peyronellaea glomerata*, and *Tricharina cretea* each account for one of 49 sequences and come from the Hot, Warm, and Cold gardens, respectively (Fig. 5).

DISCUSSION

Cottonwood Traits:

Morphological variation in response to climatic changes are well known phenomena that can result from phenotypic plasticity or local adaptation of a population (Kroon et al. 2005; Cooper et al. 2019). These differences in morphology are caused by differing resource allocation in response to different biotic or abiotic stressors (Kleiman & Aarssen 2007; Wang et al. 2019; Blasini et al. 2020). We report differences in Fremont cottonwood morphology within populations across the three gardens. In general, all populations grew longer shoots and had a greater number of leaves in the Cold garden (except for the Cold source population). This suggests that either greater photosynthetic capabilities are needed in the Cold garden to meet the same energy production as the Warm or Hot gardens or transpirational stress from high temperatures in the Warm and Hot gardens reduced the number of leaves. It is likely that both of these explanations account for some portion of the variance as the Cold garden has a shorter growing season and leaf number decreases as the mean annual temperature in the gardens increases. Sampling of specific leaf area (SLA) may help to elucidate these effects as leaves adapted for hot climates typically have lower SLA and leaf size which reduces heat stress through transpiration (Wang et al. 2019). Blasini et al. (2020) reports that populations from low-elevations (our Hot and Warm populations) in the Warm garden have a smaller leaf area compared to those from high-elevation (our Cold populations) despite the low-elevation populations having greater SLA.

Endophyte Abundance:

Garden climate had the largest effect on endophyte abundance. Although all three gardens had significant differences in endophyte abundance the Cold garden had a distinctively low percent isolation (Fig. 1). These results support findings in previous studies which show that class III endophyte abundance follows a latitudinal gradient (Arnold and Lutzoni 2007). Class III endophytes are reliant on horizontal transmission meaning that infections must come from an environmental pool of hyphal fragments and spores (Arnold & Herre 2003; Saikkonen 2007). This suggests that a great number of class III endophytes are unable to withstand cold temperatures *ex planta*. This is supported by findings in Bálint et al. (2015) which reported a significantly lower endophyte abundance in balsam poplar clones grown in cold environments compared to hot. In addition, the host-endophyte symbiosis has been shown to modulate thermotolerance in the host and the endophyte (Redman et al. 2002). This difference may also be explained by a decreased need for endophytes for pathogen defense given that abiotic stress, like low temperatures, can increase concentrations of resistance hormones and pathogen resistance in other plants (Bergelson & Purrington 1996; Pedranzani et al. 2007; Gaudet et al. 2011); however, most species found in the preliminary sequencing data are reported to be pathogens (Fig. 5; see Community section below).

Local adaptation to environmental factors can lead to genetic variation among populations (Kawacki & Ebert 2004; Rua et al. 2016; Cooper et al. 2019). In our study, trees from different population source climates differed in endophyte abundance. In particular, populations coming from a cold source climate had greater percent isolation than trees from Warm or Hot source climates, regardless of garden location. Other studies have observed that host genetics can influence endophyte abundance (Pan & May 2009; Lau et al. 2013; Bálint et al. 2013; Bailey et al. 2005; Bálint et al. 2015; Harrison et al. 2018), but the mechanisms for these

differences are not always known. Differences among source populations in endophyte abundance may be due to differences in leaf phytochemistry as condensed tannins (a secondary plant metabolite with antifungal properties (Holeski et al. 2009)) was shown to vary by genotype in the closely related Narrowleaf cottonwood (*Populus angustifolia*) (Lamit et al. 2014), Fremont cottonwood, and their hybrids within a shared study site (Bailey et al. 2005). Bailey et al. (2005) reported condensed tannins to be strongly negatively associated with the endophyte abundance, but Lamit et al. (2014) did not find any significant association. This difference between studies may be due to the greater genetic variation between the three cross types in Bailey et al. (2005) when compared to lower intraspecific variation within only *P. angustifolia* in Lamit et al. (2014). Further sampling is recommended to resolve these conflicting results as condensed tannin concentration was not measured in our system.

Endophyte abundance can be affected by plant size (Elamo et al. 1999; Harrison et al. 2018) and age (Bernstein & Carroll 1977; Stone 1987; Wilson 2000). Generally, plant size increases with age. Plant age was largely held constant in the common gardens we sampled as they were planted using cuttings collected at the same time and in the same way. Possible variation in endophyte abundance due to age was further minimized because we sampled twigs with no less than four bud scars (i.e. three years of age) unless tree mortality necessitated sampling resprouts. Larger plant size represents a larger target for endophyte infection as well as providing more inhabitable space and resources. Despite this, we found a significant negative association between shoot length and endophyte abundance as well as no association between twig diameter and abundance. Our findings conflict with Lamit et al. (2014) who report that twig diameter was positively associated with endophyte abundance while shoot length had no significant association in *P. angustifolia*. This may be due to plant growth inhibition by

endophytes (Faeth & Sullivan 2003) or interspecific interactions or competition between endophytes preventing infection (Harrison et al. 2018). Also, *P. fremontii* in our study generally produced the longest twigs in the coldest garden, where endophyte abundance was dramatically reduced, likely due to abiotic factors.

Community:

Although sequencing was not robust enough to determine statistical differences in community composition, observations may still be made from the preliminary data. Of the 47 fungal sequences only one did not have a pathogenic association: *Tricharina cretea*. Since *T. cretea* was only recently identified by Vu et al. (2019) there is less than a paucity of research on its ecological function; however, other species from the genus *Tricharina* have been found to be mycorrhizal or endophytic with antimicrobial qualities (Yang & Wilcox 1984; Park et al. 2017). The genus *Alternaria* can be found almost globally and is a well-known pathogen of economically important fruit-producing plants like jujubes, apples, and tomatoes (Hu et al. 2017; Wei et al. 2016; Yang et al. 2017; Kwaśna et al. 2021); however, members of the genus *Alternaria* can also be beneficial, promoting growth (Lindblom et al. 2018) and producing secondary metabolites that have anti-microbial actions (Park et al. 2020). The genera *Valsa* and *Cytospora*, and more specifically the species *Valsa sordida*, are well known as pathogens of apples and poplars that causes cankers (Worrall et al. 2008; Xie et al. 2009; Wang et al. 2015; Wang et al. 2020; Kwaśna et al. 2021). The genus *Cladosporium* is widespread with a near global distribution that contains saprobes as well as plant pathogens (Braun et al. 2003; Amirmijani et al. 2014; Hassan et al. 2021; Kwaśna et al. 2021); thus, it may act either as a latent saprotroph or pathogen. The genus *Peyronelleae* is associated with plant disease (Castillejo et al.

2020). More sequencing, accompanied by functional assays, need to be performed in order to relate the presence of these fungi to the performance of the trees in each of our common gardens.

CONCLUSION

Our results show that Garden climate and Population source influence the abundance of fungal endophytes in Fremont cottonwood. The colder temperatures found in the Cold garden may limit the number of possible endophytes in the local hyphal fragment and spore pool reducing infection frequency. As temperatures warm it is possible that an increased number of OTUs will be recovered from the site. Differences between populations may be explained by variation in condensed tannin concentration. Further sampling of trees within the gardens at the genotypic level and sampling of different variables like SLA and condensed tannin concentrations are needed to fully explain these differences. In addition, further sequencing of the culturable fungal endophytes retrieved may expose a relationship between endophyte community composition and plant performance which may impact conservation and forest management practices.

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TABLES

Table 1. Common garden and population source environmental information.

Garden	Climate Type	Latitude	Longitude	Elevation (m)	MAT (°C)	MWMT (°C)	MCMT (°C)	MAP (mm)
Canyonlands, Dugout Ranch, Utah	Cold	38.0925	109.5878	1581	10.7	24.6	-3.2	225
Agua Fria River, Horseshoe Ranch, Arizona	Warm	34.2567	112.0661	988	17.2	28.5	7.6	440
Mittry Lake, Yuma, Arizona	Hot	32.8498	114.4928	49	22.8	33.8	12.7	93
Population Source								
Keams Canyon	Cold	35.81152	-110.17	1920	10.7	23	-1.3	258
Jack Rabbit, Little CO	Cold	34.96	-110.436	1507	12.3	25.3	-0.7	212
Agua Fria, Horseshoe	Warm	34.25671	-112.066	988	17.4	28.7	8	440
Willow Creek, Kingman	Warm	35.143	-113.543	1126	15	26.6	5	243
San Luis, Colorado	Hot	32.52702	-114.804	26	22.1	32.9	12.4	88
Bill Williams, Colorado	Hot	34.27606	-114.059	143	22.3	34.6	10.9	137
Cibola, Colorado	Hot	33.3621	-114.698	70	22.6	33.9	12.2	97

FIGURES

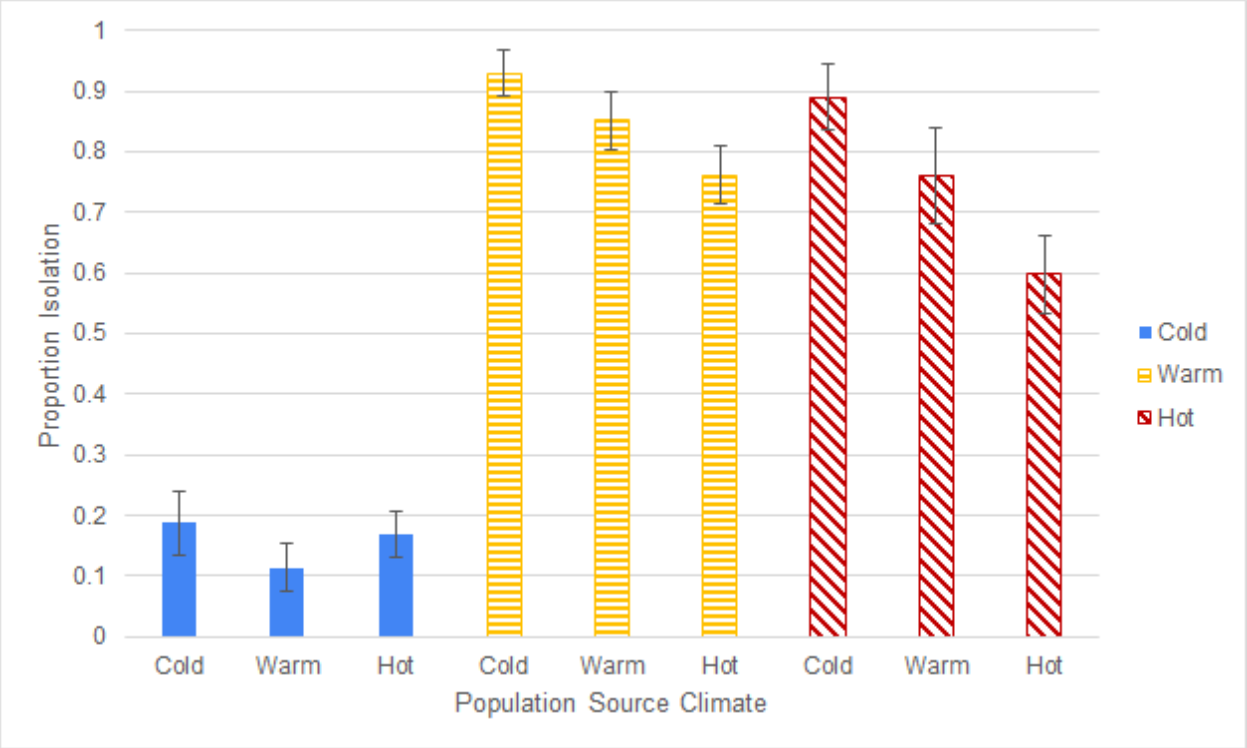

Figure 1. Proportion isolation of fungal endophytes from *Populus fremontii* by population source climate and garden climate.

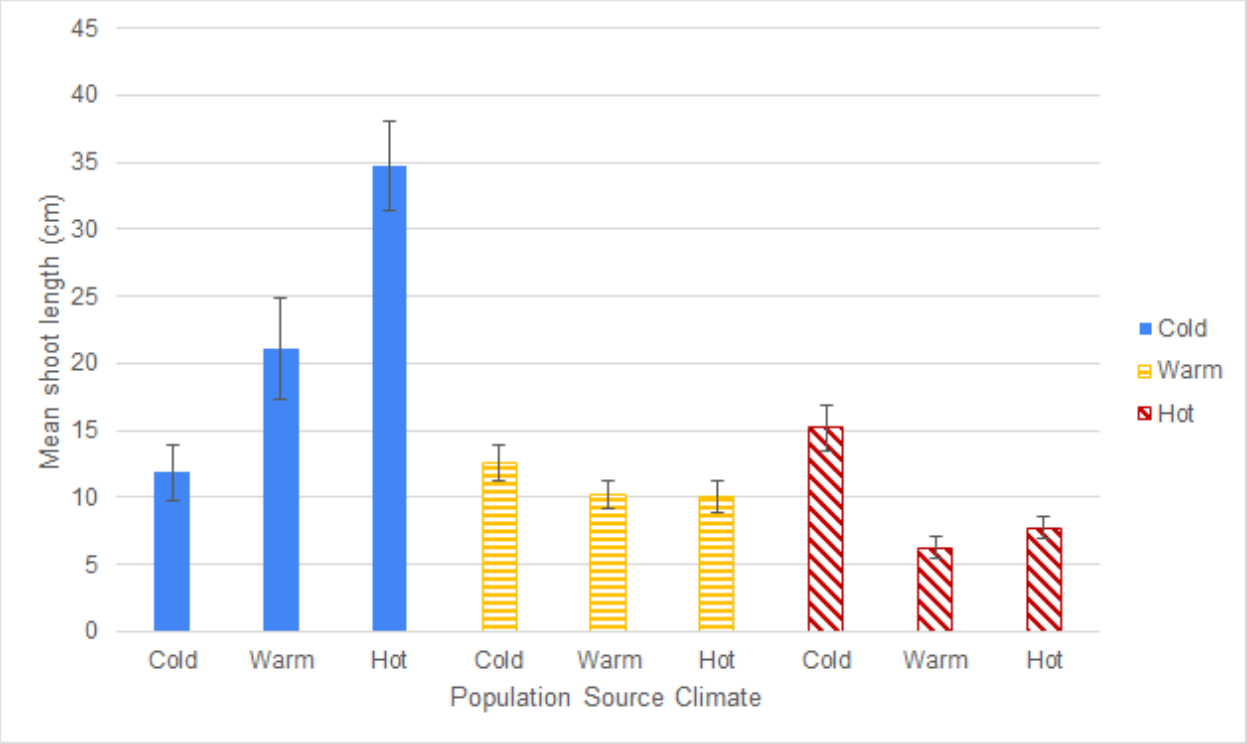

Figure 2. Lengths of shoots in centimeters (cm) in *Populus fremontii* from different population source climates in Cold, Warm, and Hot common gardens.

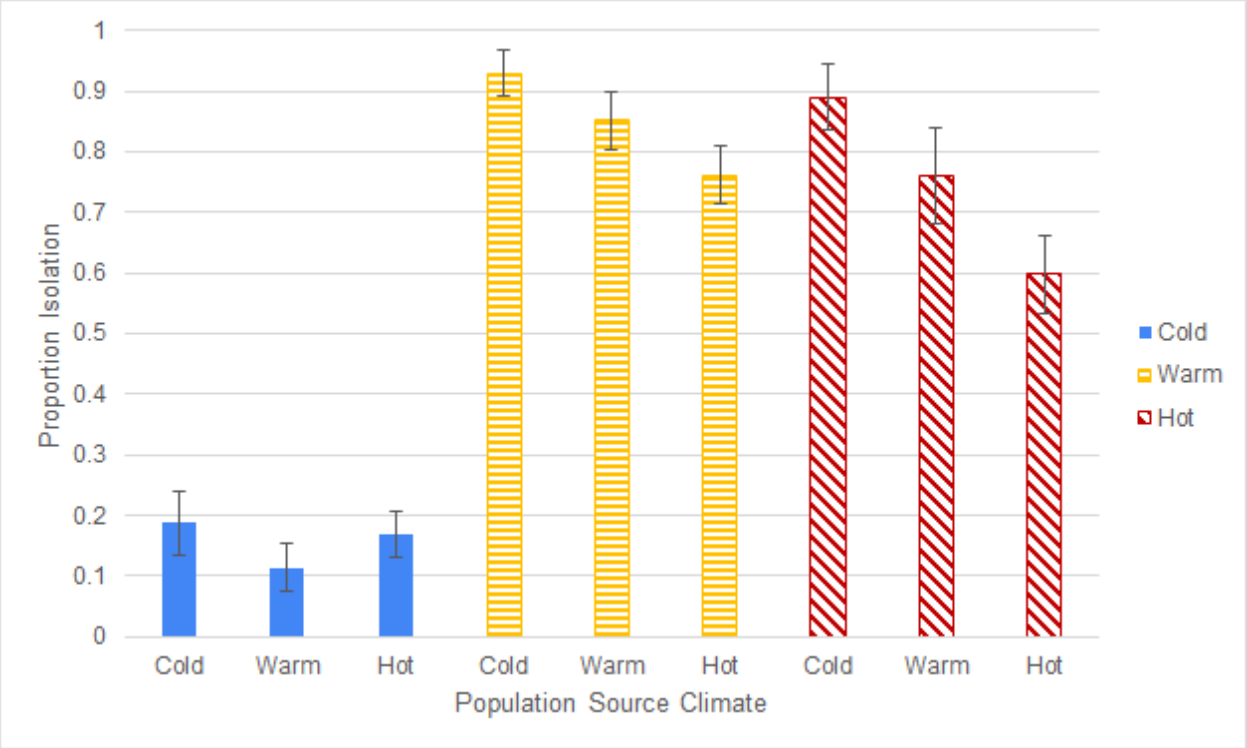

Figure 3. Twig diameter in centimeters (cm) in *Populus fremontii* from different population source climates in Cold, Warm, and Hot common gardens.

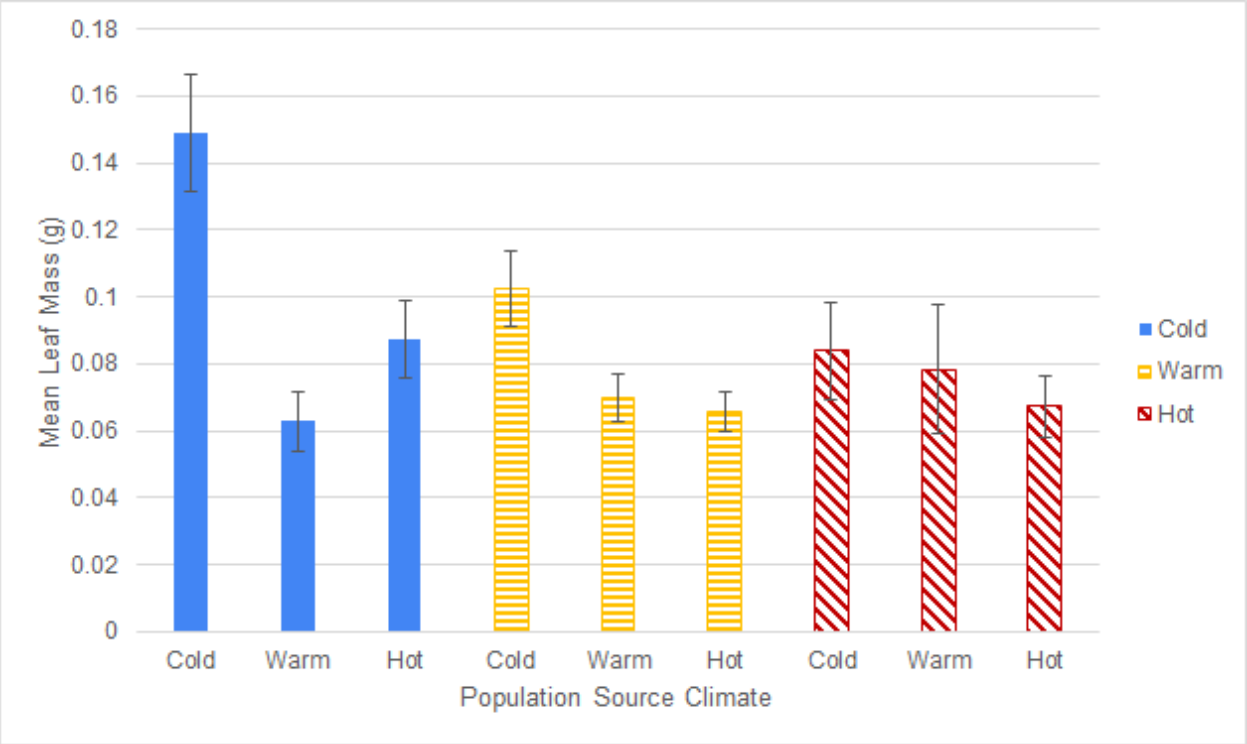

Figure 4. Dried leaf mass in grams (g) of *Populus fremontii* from different population source climates in Cold, Warm, and Hot common gardens.

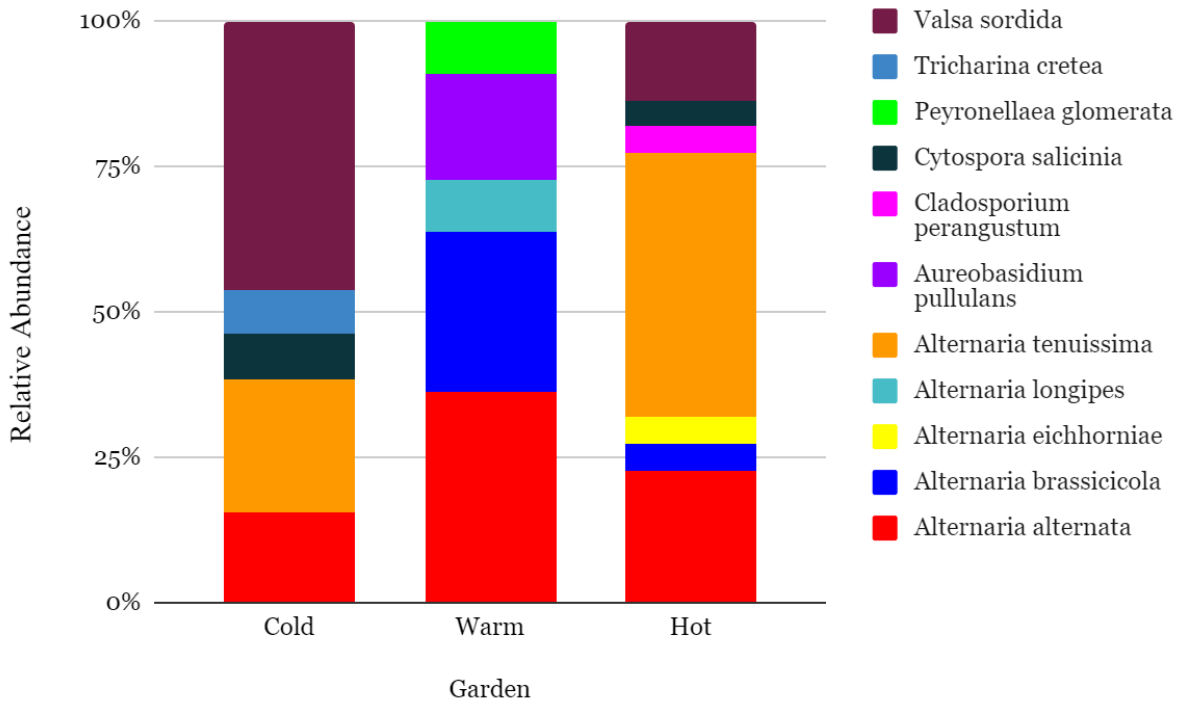

Figure 5. Relative abundance of fungal endophytes cultured from *Populus fremontii* twigs from common gardens with different climates.

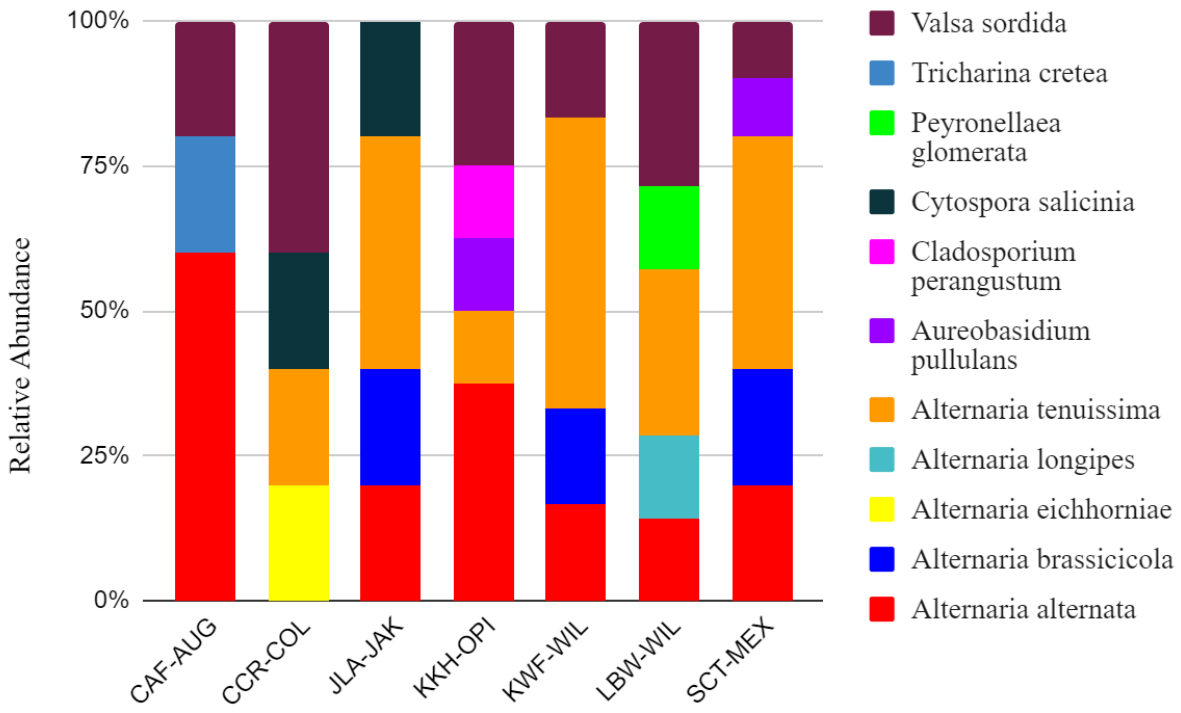

Figure 6. Relative abundance of fungal endophytes cultured from *Populus fremontii* twigs from source populations with different climates.