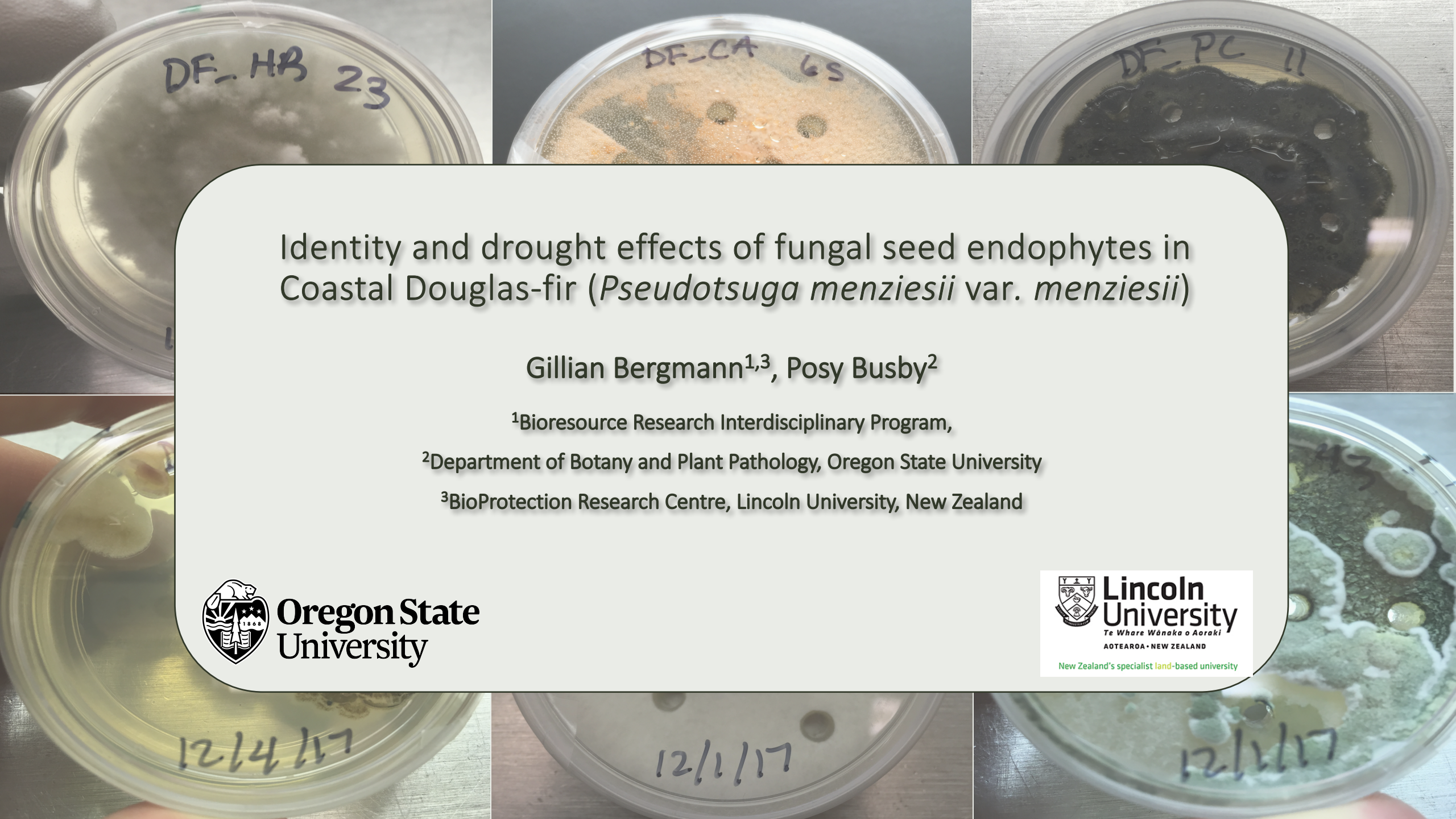

Identity and drought effects of fungal seed endophytes in Coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*)

Gillian Bergmann[1,3], Posy Busby[2]

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Oregon State University

Lincoln University
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Douglas-fir: An ecologically and economically important tree in the PNW and worldwide


Watt 2018


MacLaren 2009

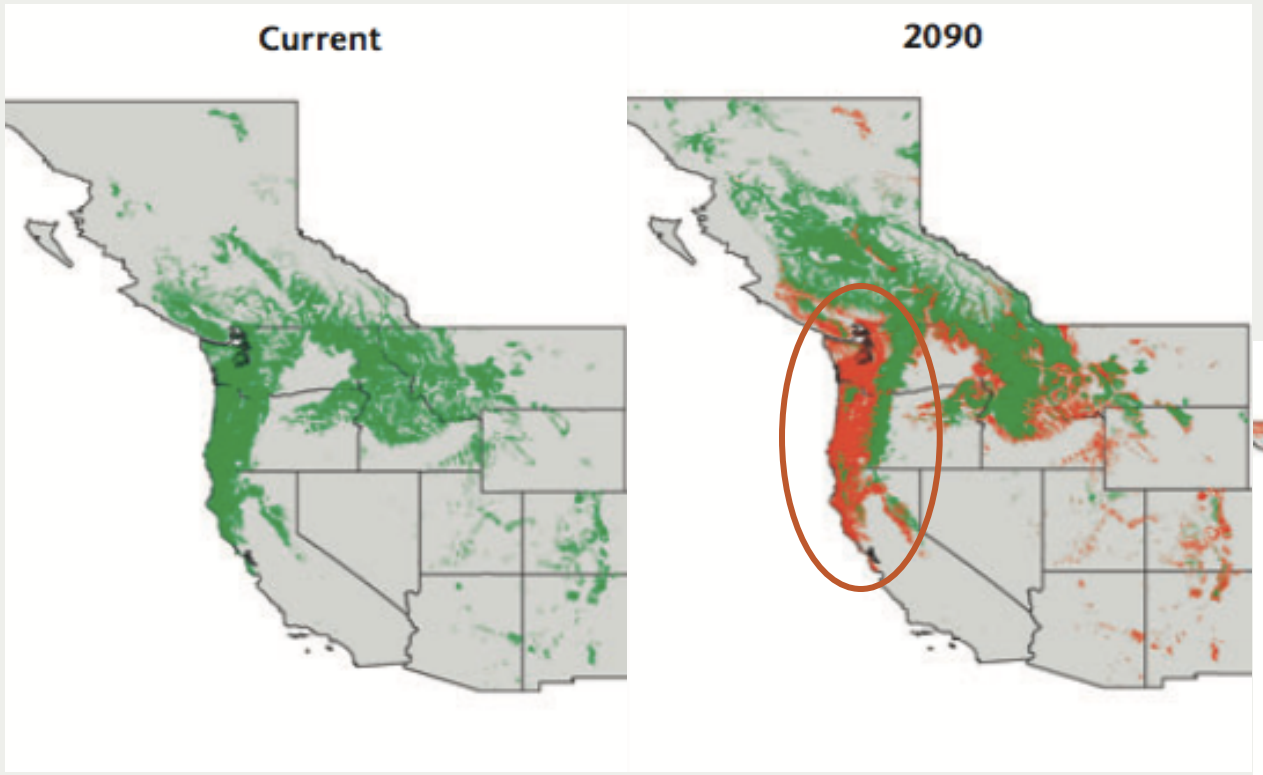
[Ferrell & Woodard 1966; MacLaren 2009; Stewart 2015; Uchytil 1991; Watts et al. 2017]

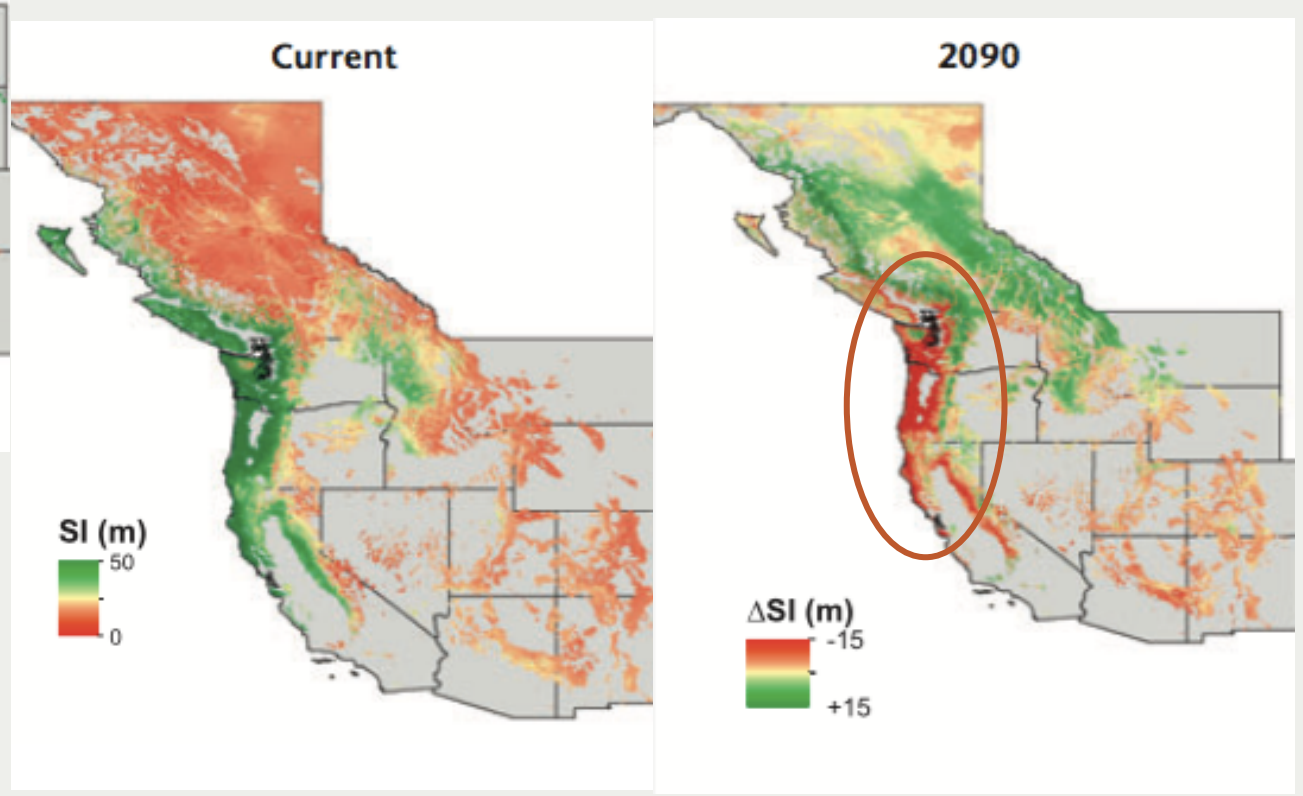

or 60 Years!

Drought events limit Douglas-fir growth and survival

[Pokorny 2018; Stewart 2015]

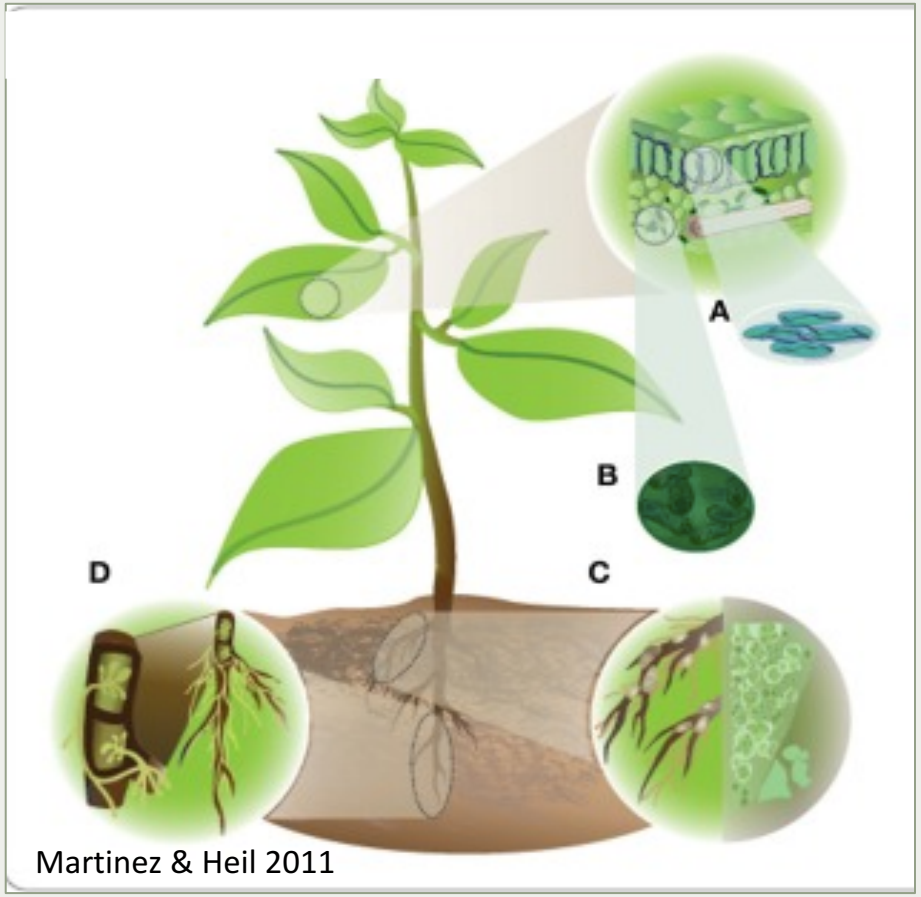
The climate crisis is predicted to change Douglas-fir range and productivity



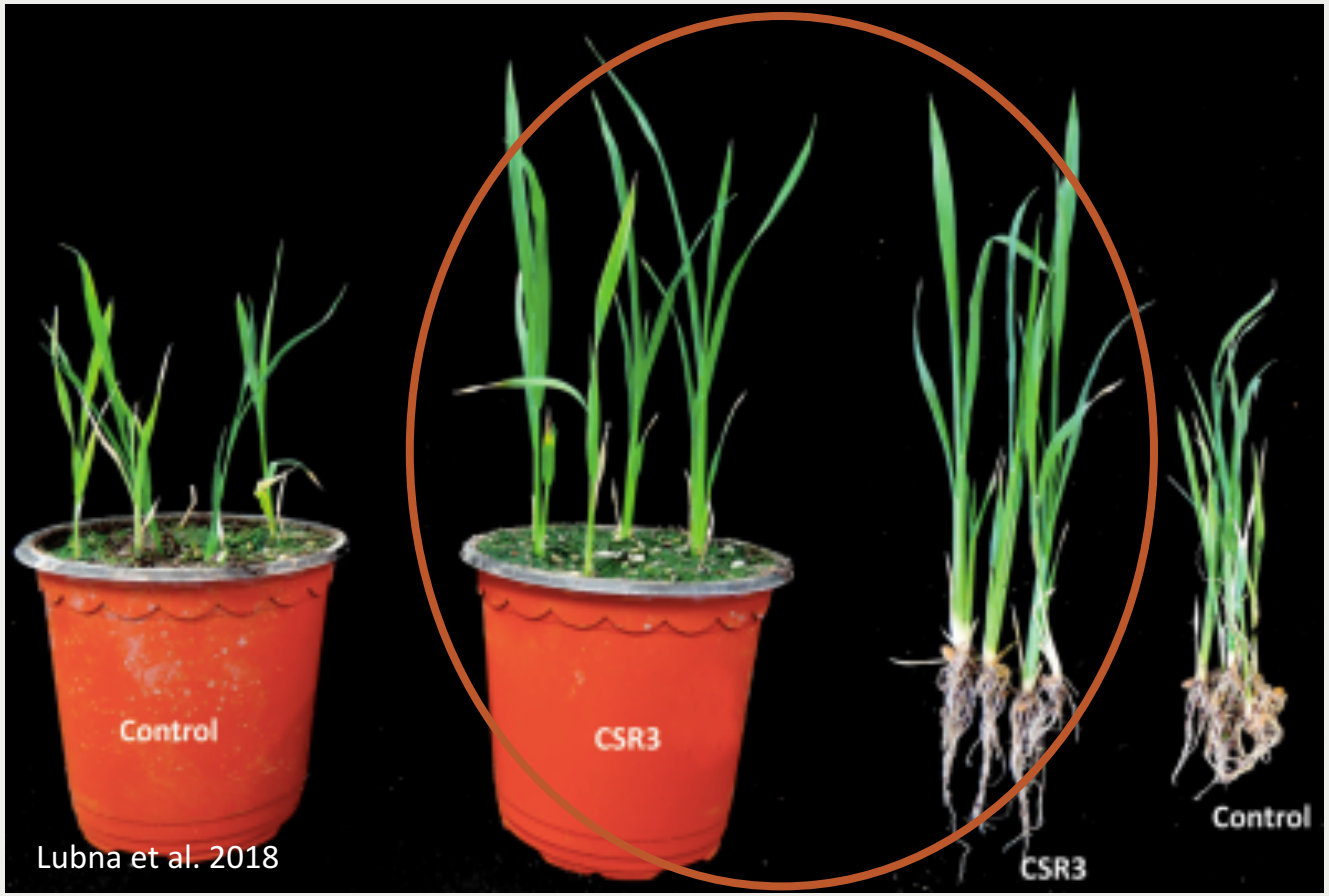


[Watts et al. 2015; Weiskittel et al. 2012]

Fungal endophytes can alter plant phenotypes and fitness



Martinez & Heil 2011



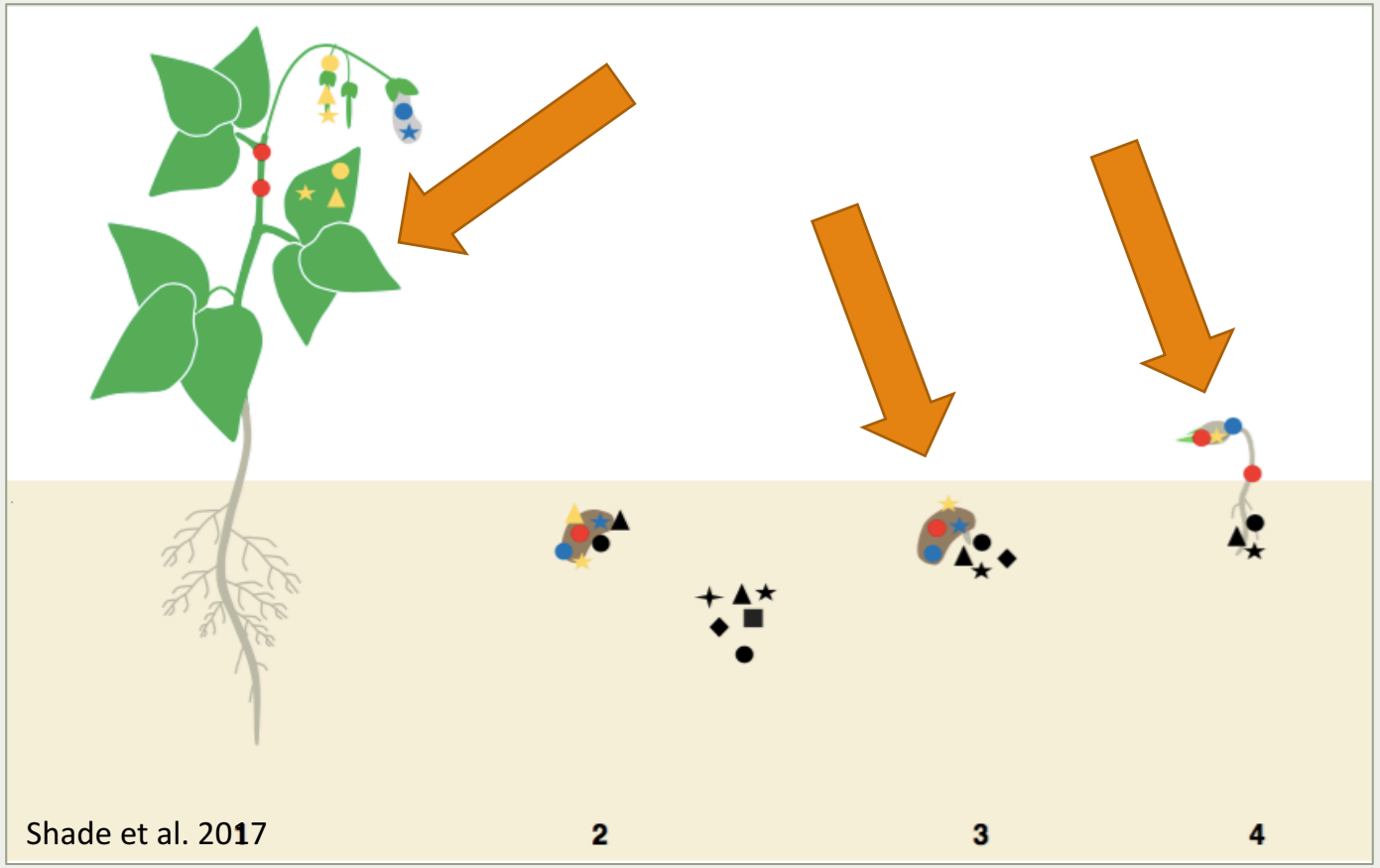
Lubna et al. 2018

[Busby et al. 2016; Rodriguez et al. 2009; Saikkonen et al. 1998]

Seeds and seedlings are a bottleneck in plant populations

[Fenner & Thompson 2005]

Seed endophytes as microbial tools for improved plant fitness



[Nelson 2018; Shade et al. 2017]

Current knowledge about Douglas-fir seed endophytes is limited

THE OCCURRENCE OF ENDOPHYTIC FUNGI IN DOUGLAS-FIR SEEDLINGS AND SEED

W. J. BLOOMBERG

Department of Forestry of Canada, Forest Research Laboratory, Victoria, British Columbia

Received October 12, 1965

Abstract

Segments taken from the roots and shoots of healthy 1-year-old seedlings of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) collected over a 2-year period from two British Columbia forest nurseries, were surface-sterilized, and incubated on various media. Fungi grew from segments of over 80% of the seedlings, the most frequent being *Fusarium oxysporum* Schlecht., *F. redolens* Wr., *Mycelium radicis atrovirens*, *Cylindrocarpon didymum* (Hart.) Wollenw., and *C. radicicola* Wollenw. Fungi were isolated more frequently from the shoot than from the root segments. Isolations from segments with the bark intact were more frequent than from segments that had the bark removed. Seedlings collected in the winter produced more fungi than those collected during the summer. Segments from diseased seedlings and seedlings grown under sterile conditions produced mycofloras which were different from each other and from that of healthy nursery-grown seedlings. Without exception, fungi grew from surface-sterilized seed placed on malt agar and samples from different seedlots had different floras. Stained sections clearly showed hyphae and chlamydospores well within the tissue of roots, shoots, and seed coat.

Research Questions

- **What fungi are present in douglas-fir seeds?**
- **Do some of these fungi confer drought tolerance in seedlings?**
- **Does seed region of origin impact which fungi are present and their effects?**

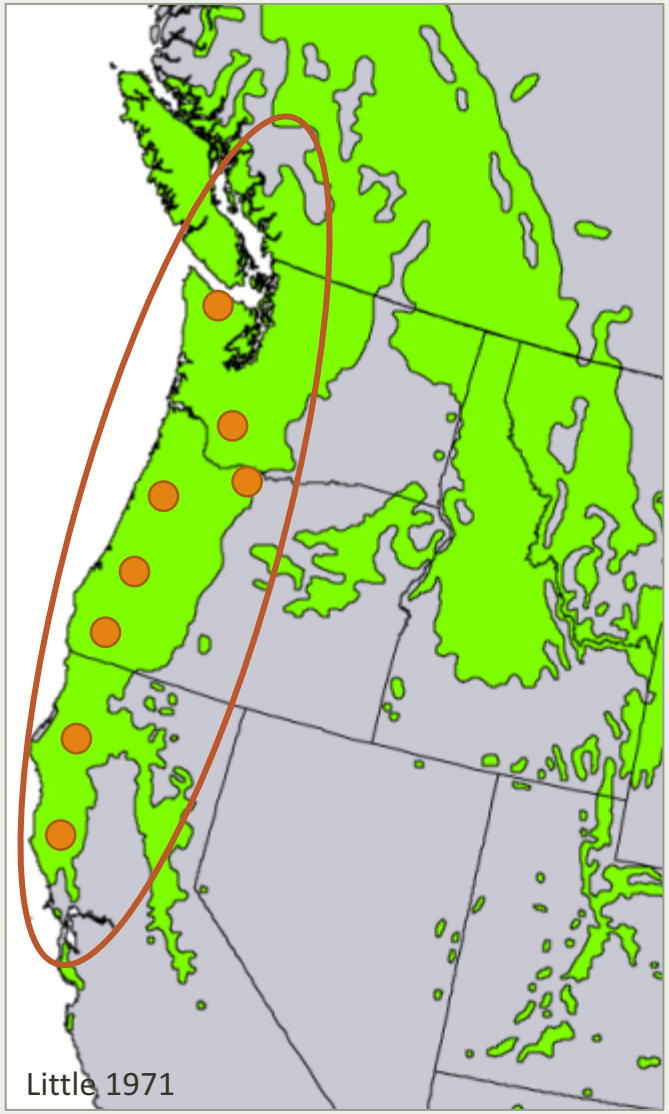
Part I: identifying fungal endophytes of Douglas-fir

Hypothesis for this observational study

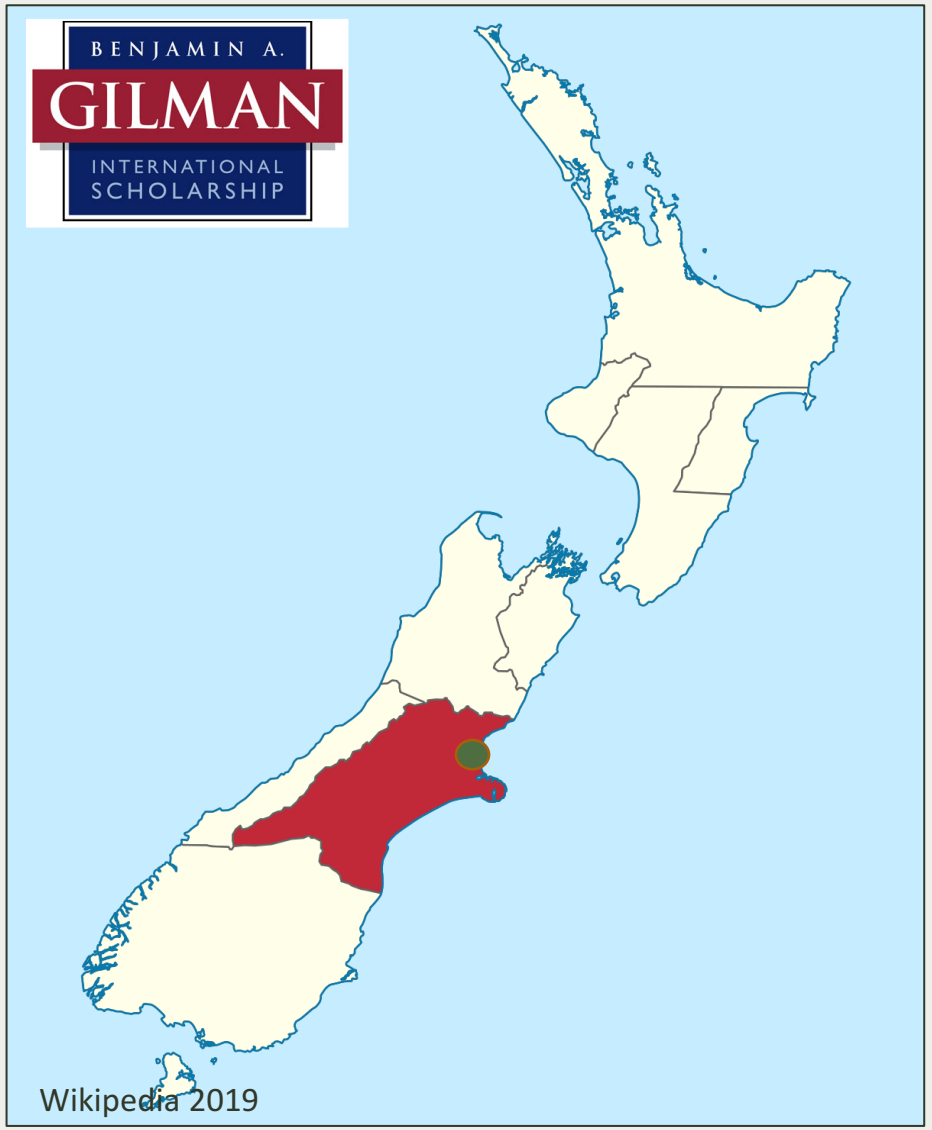
That endophytes present in seeds will differ based on seed region of origin

- Within native range and between native and introduced ranges

Selecting populations for seed collection

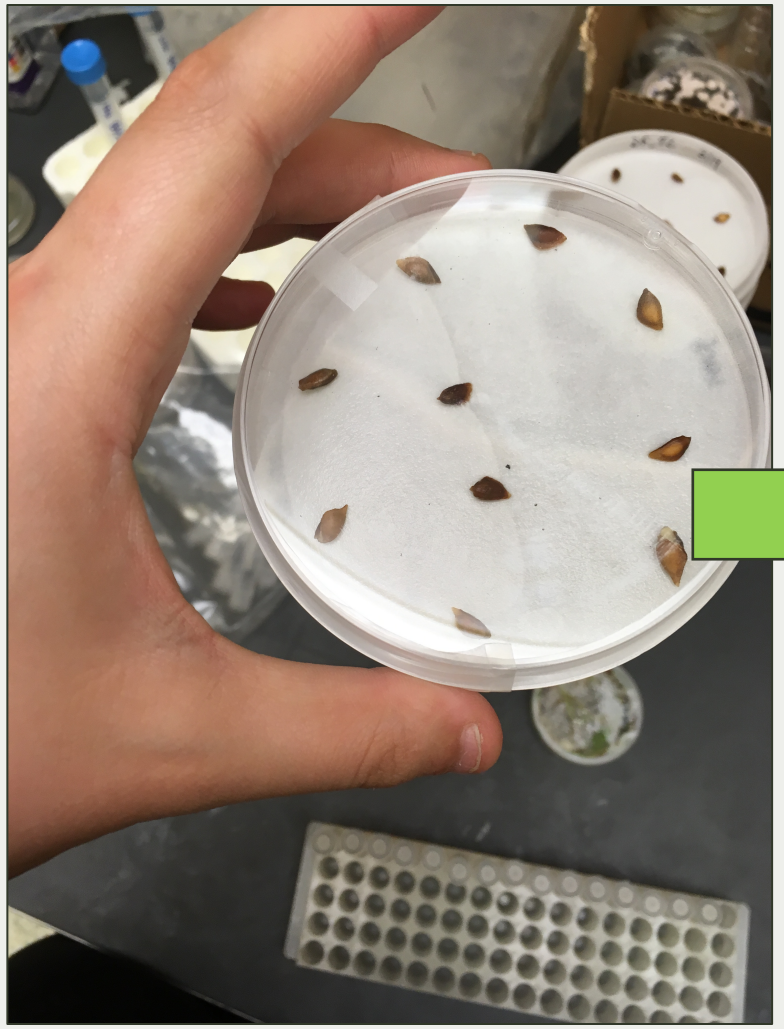


Little 1971

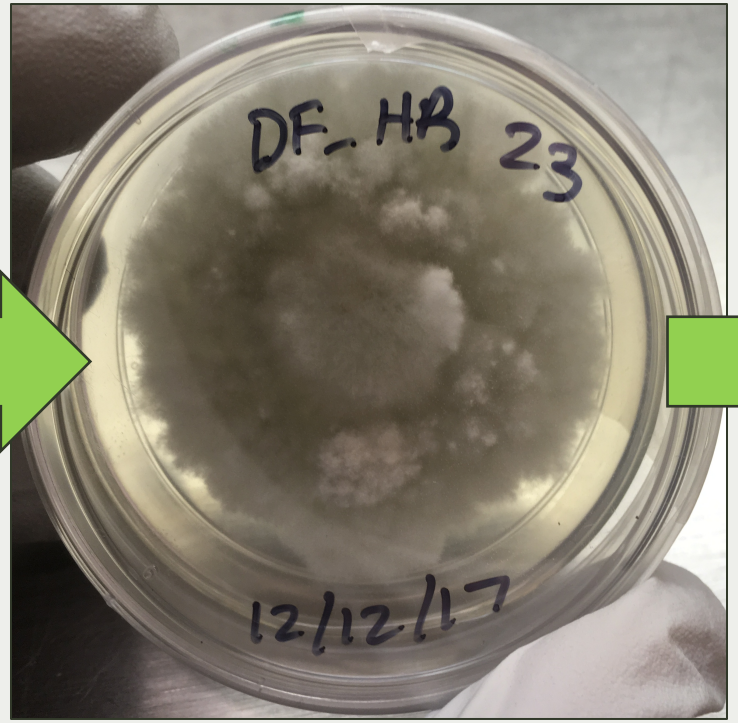


Wikipedia 2019

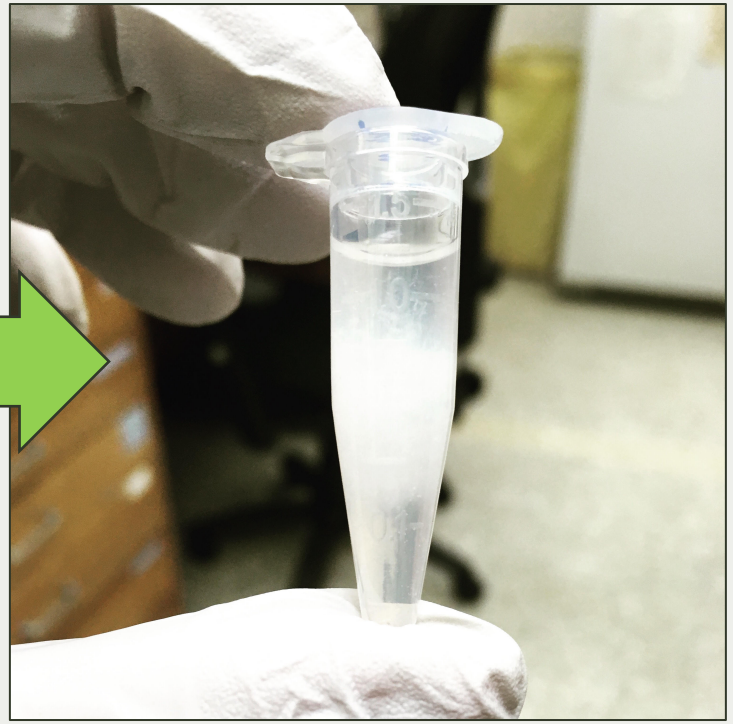
Preparing for endophyte identification



Seed stratification, sterilization and plating

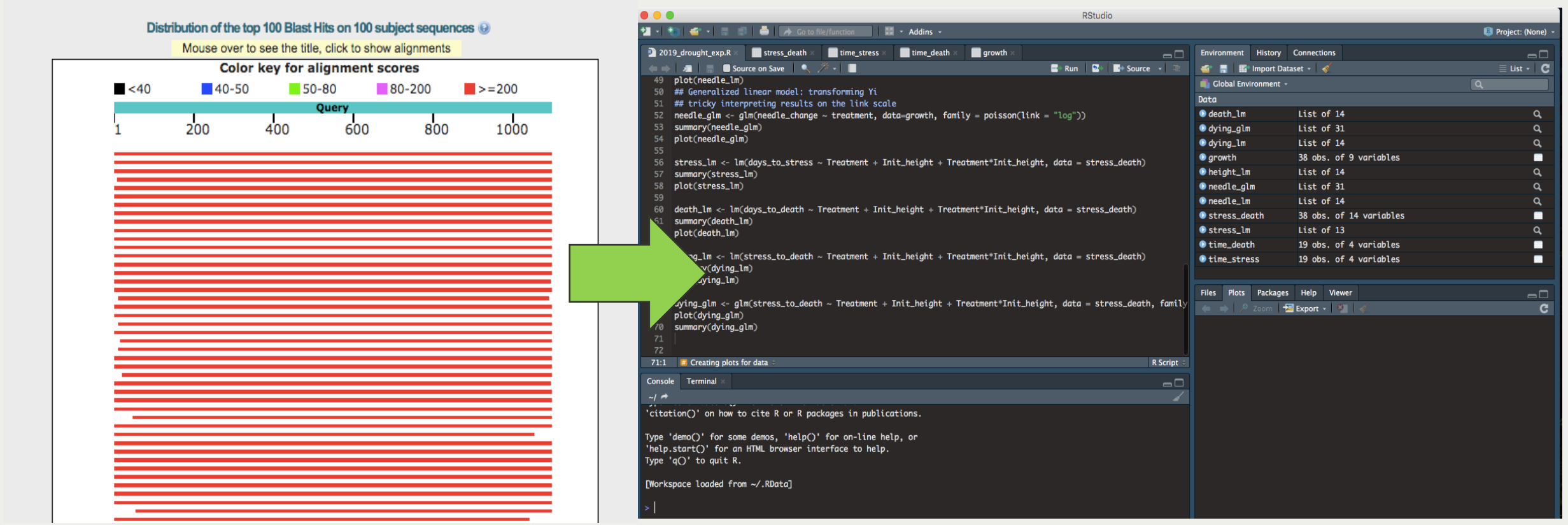


Endophyte isolation



DNA extraction and sequencing

Endophyte identification and analysis



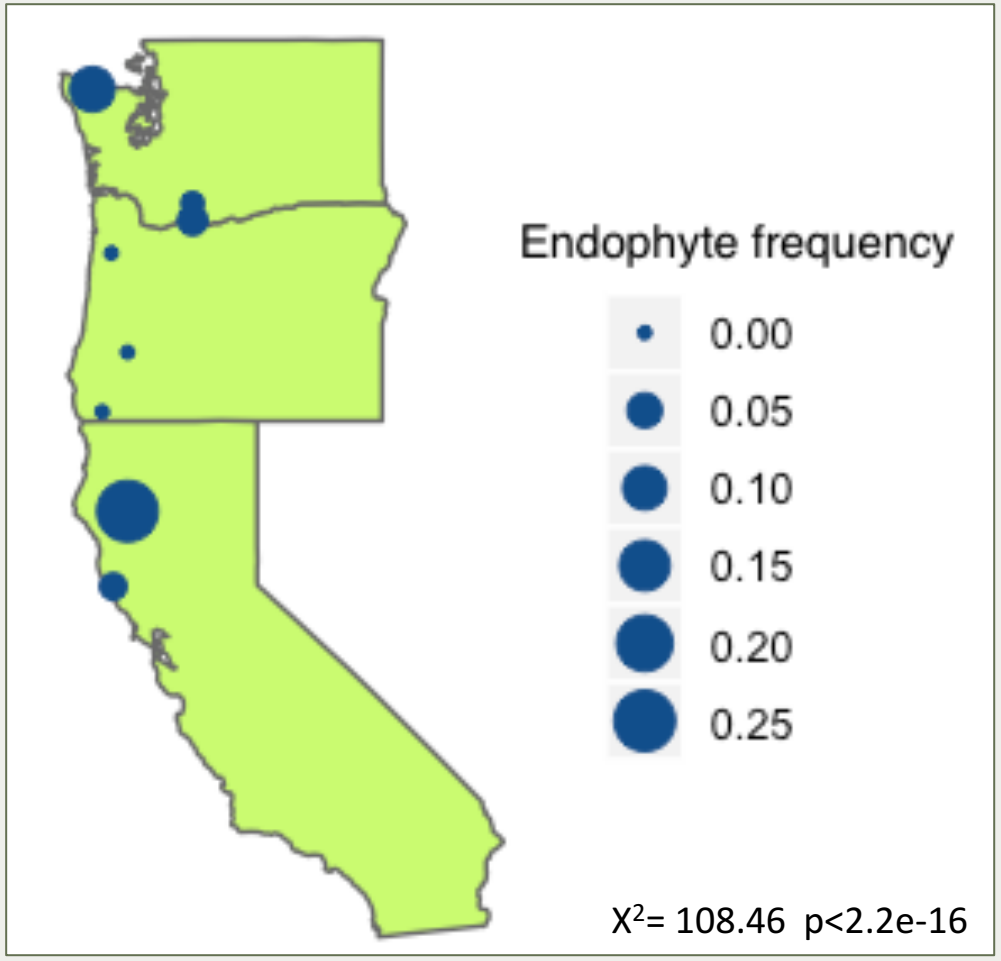
Endophyte identification with databases

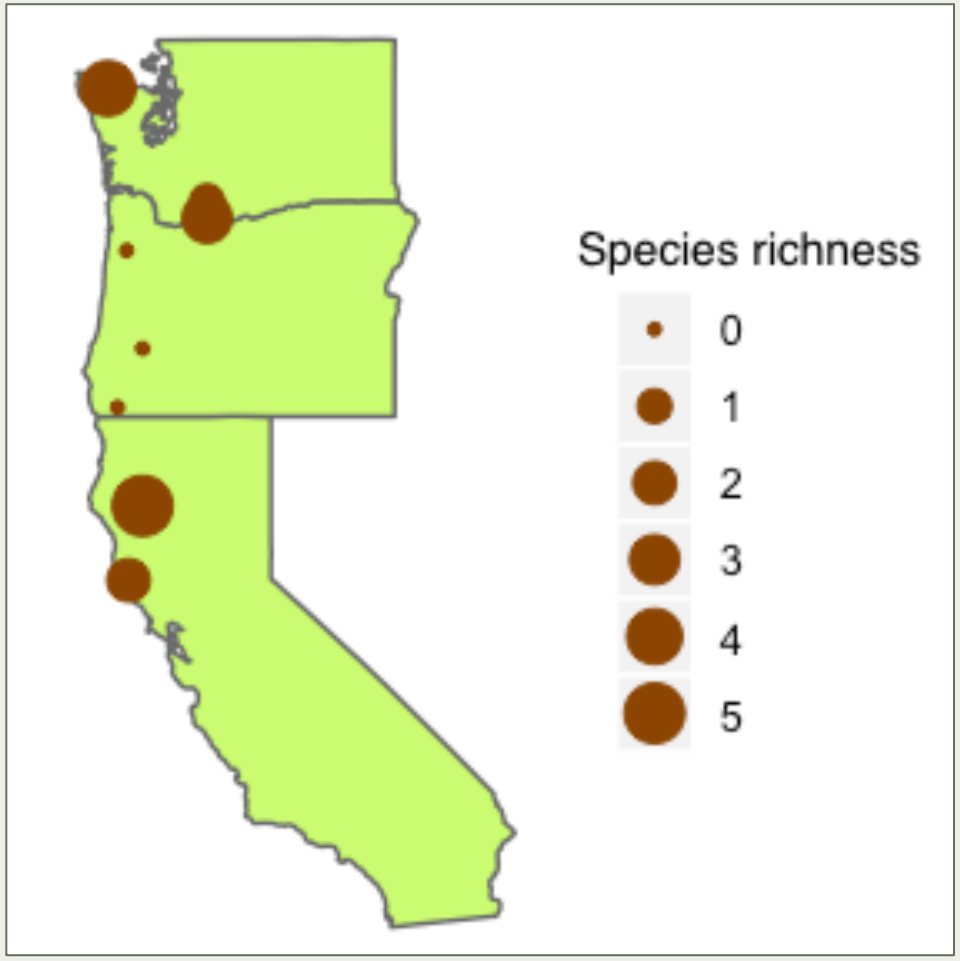
Analysis of endophyte frequency

Low richness and isolation frequency observed in seed microbiome

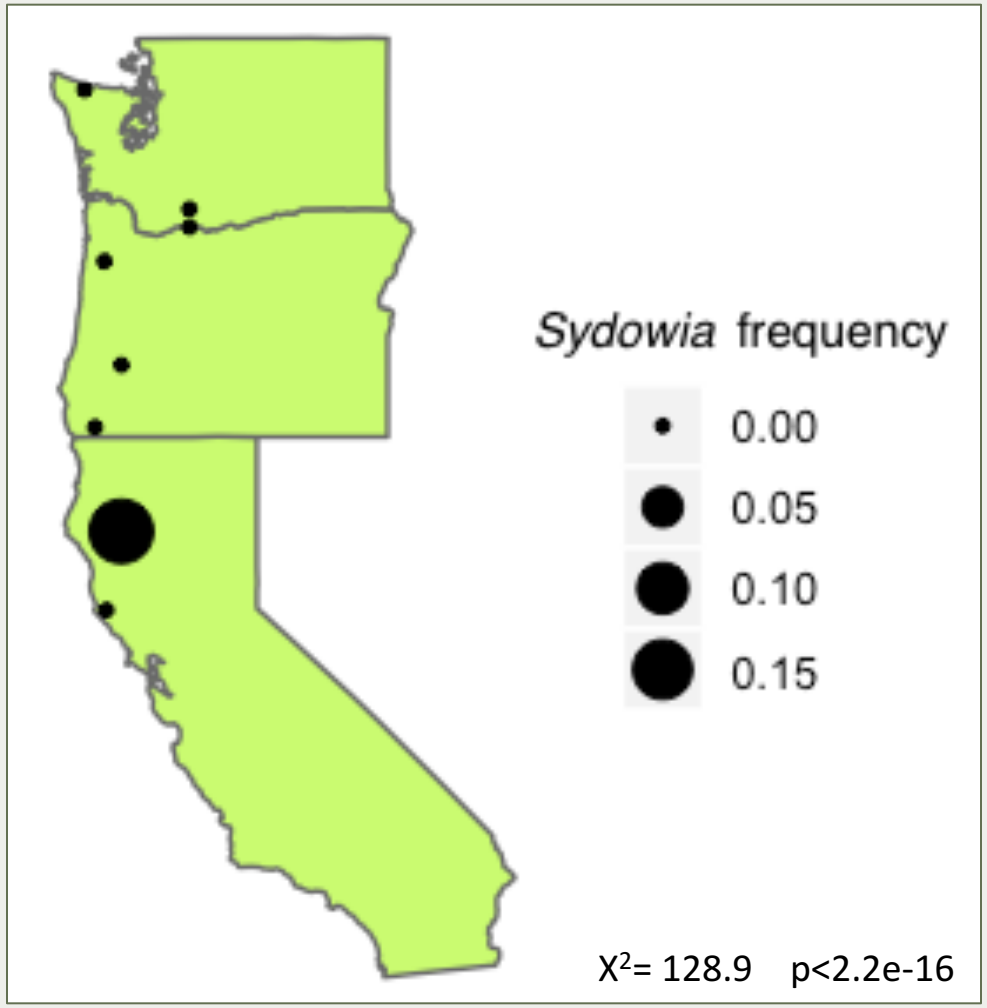
United States			New Zealand		
Taxon	Seed Source	Relative abundance	Taxon	Seed Source	Relative abundance
Trichoderma spp. (6)	CB, PC, HR, TL	44%	*Sydowia polyspora*	OR, WA	91%
Sydowia polyspora	PC	42%	*Rhizophaera kalkhoffii*	WA	2%
Unknown Rhytismataceae spp.	PC	6.98%	*Trichoderma harzianum*	OR	2%
Rutstroemia longipes	HR	2.33%	*Aspergillus pseudoglaucus*	OR	2%
Moellerodiscus pinicola	HR	2.33%			
Trichothecium roseum	CA	2.33%			
		100%			**100%**

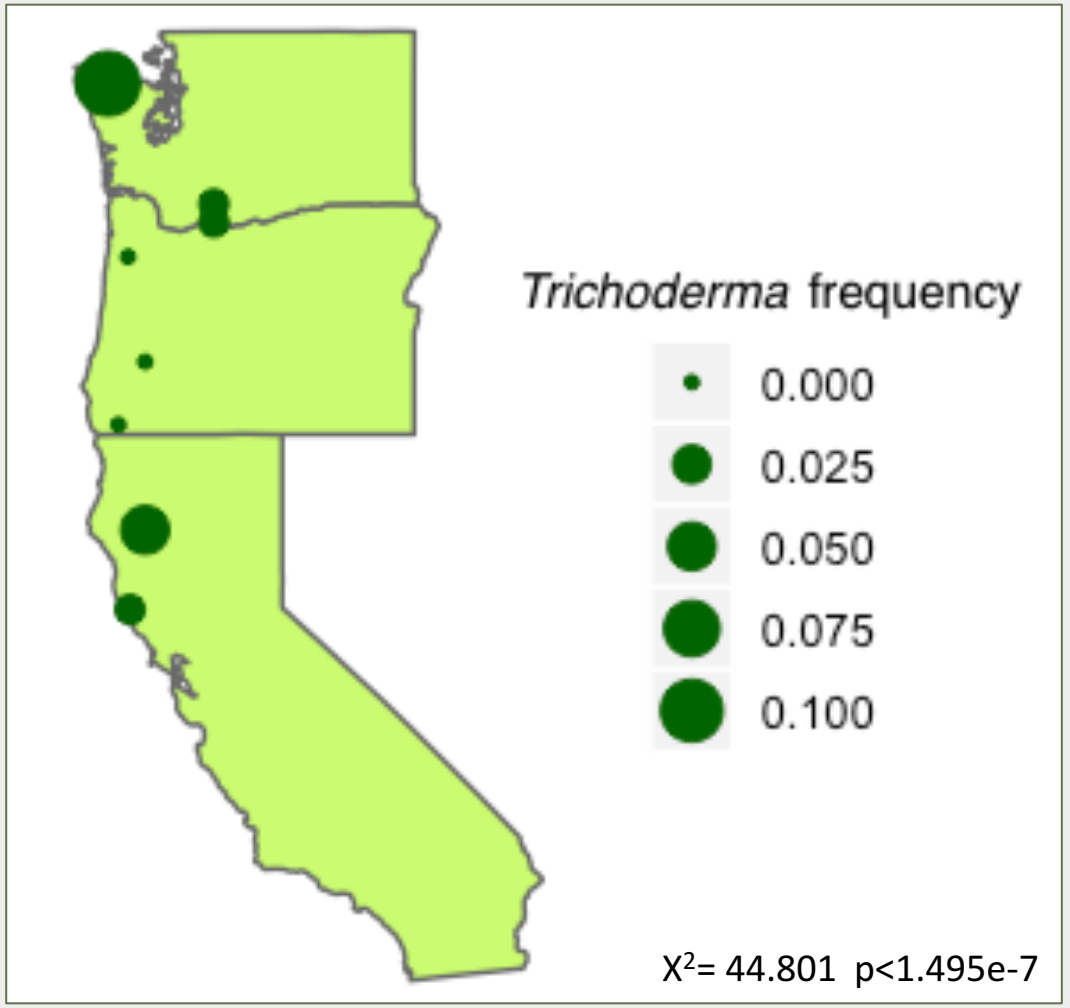
Analysis of United States populations showed an association between endophyte frequency and seed provenance



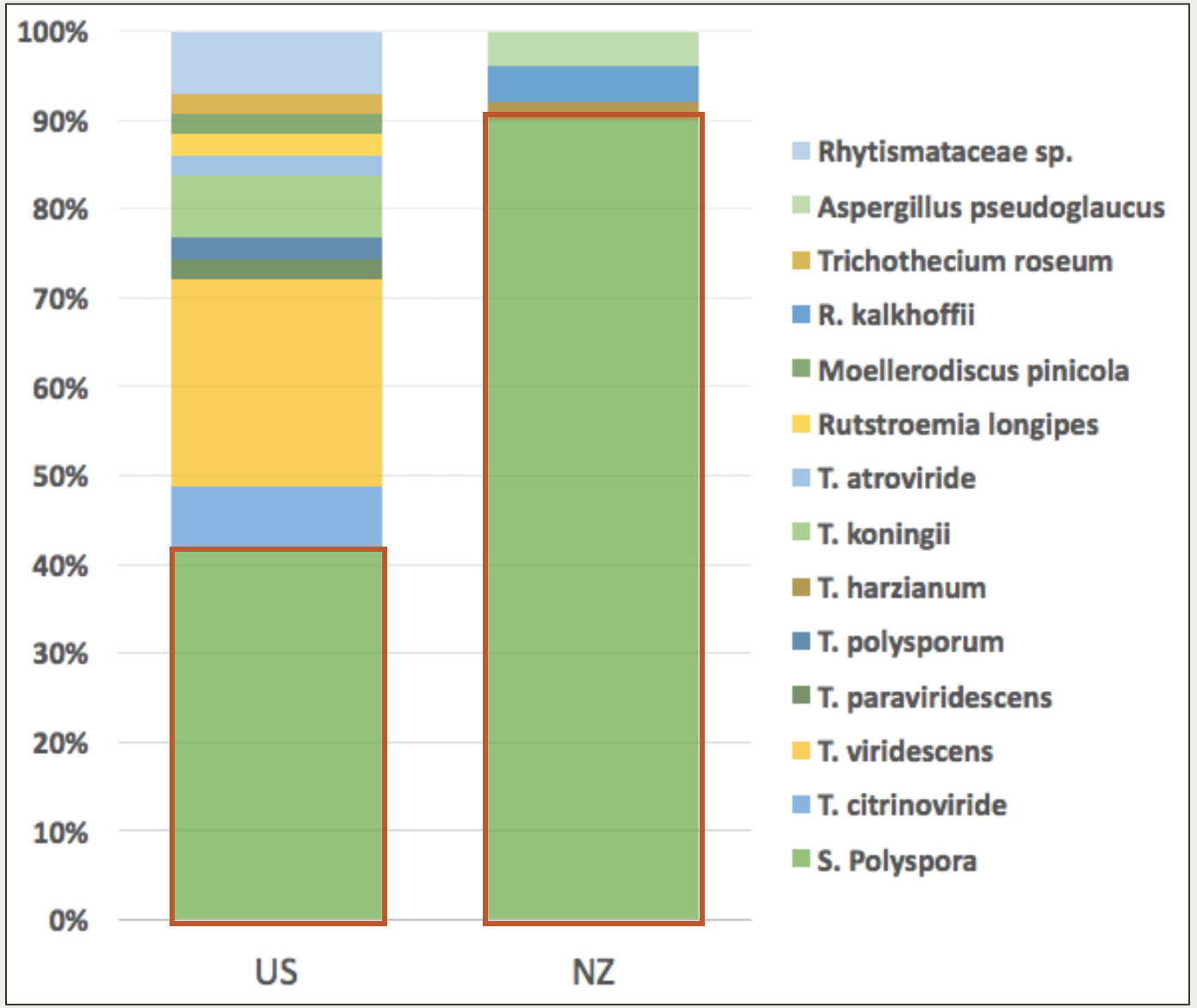


Analysis of United States populations showed associations of *Trichoderma* and *Sydowia* frequency with seed provenance

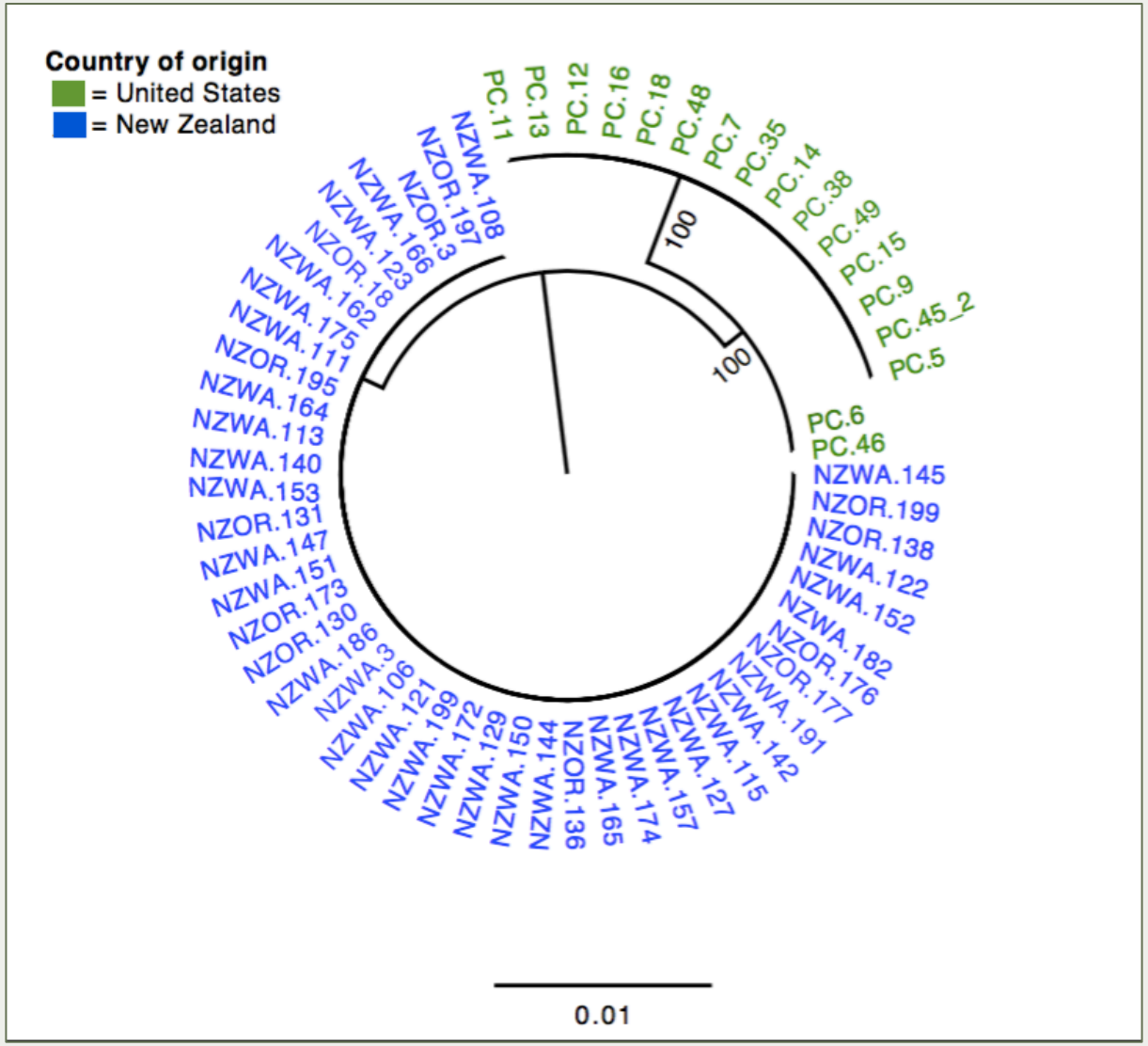




Qualitative comparison of US and New Zealand populations showed reduced diversity and *Sydowia polyspora* as a common species

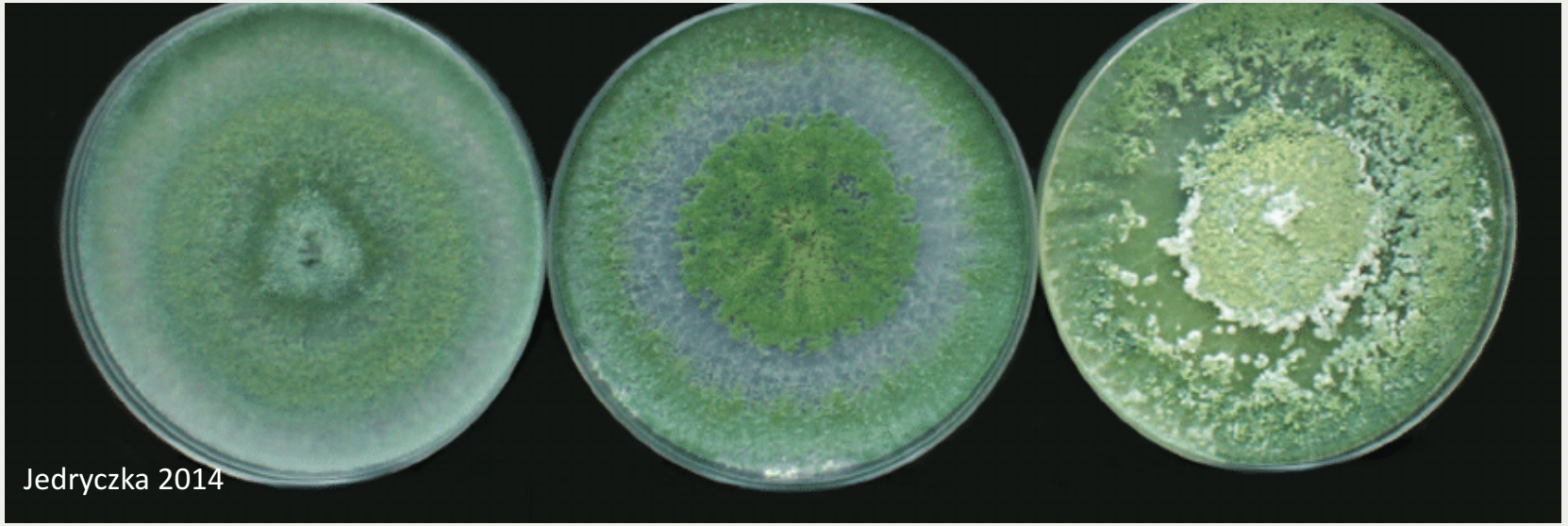


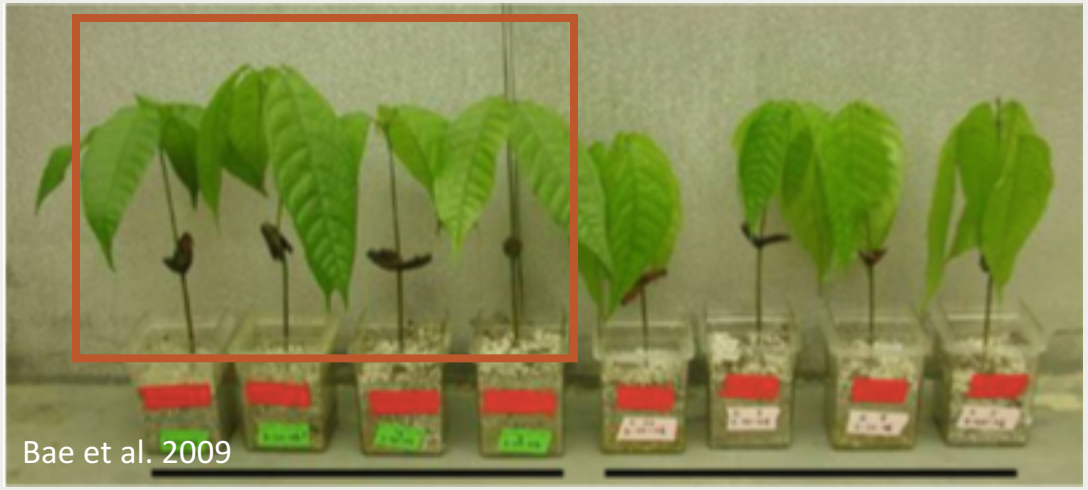
Qualitative comparison of US and New Zealand populations showed genetic differences in *Sydowia polyspora* isolates



Part II: Examining endophyte function

Selecting *Trichoderma* for endophyte inoculation





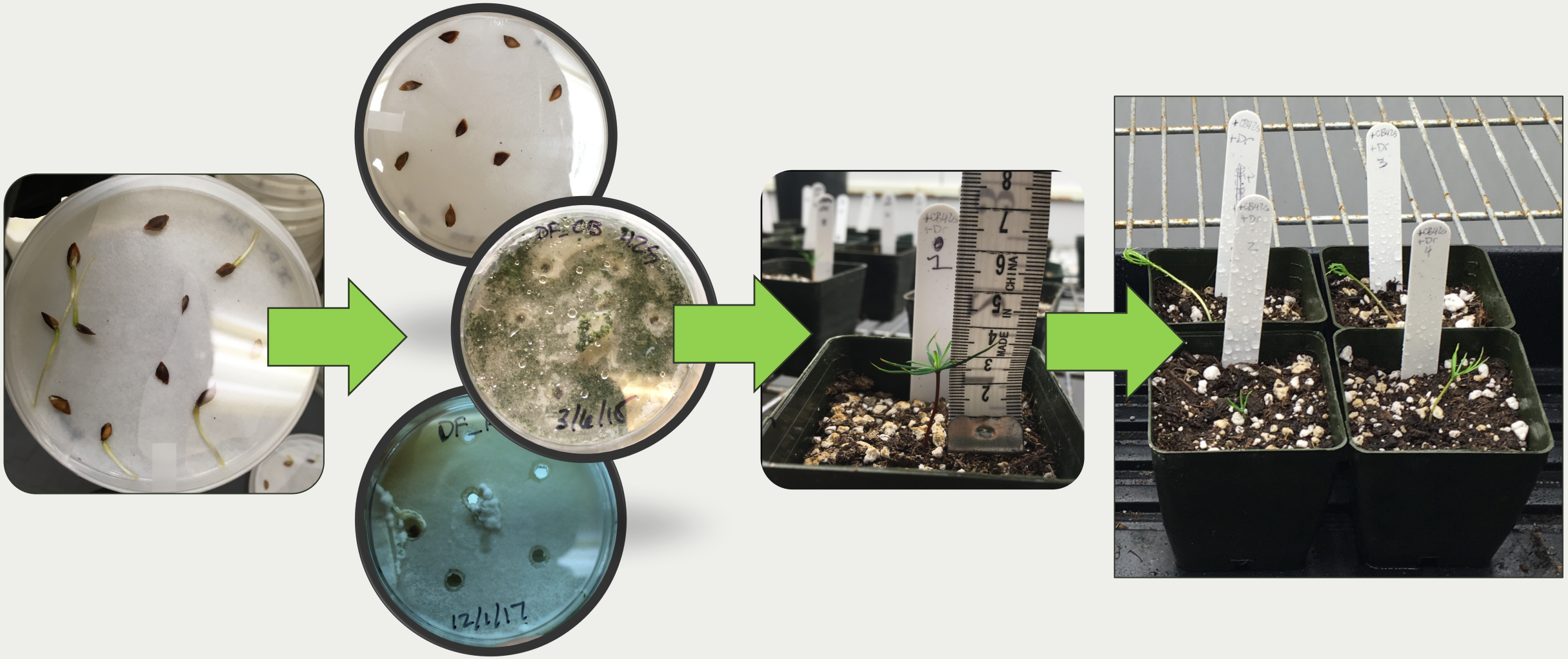
[Bae et al. 2009; Woo et al. 2014]

Hypotheses for this experiment

1. That the *Trichoderma* endophytes will lengthen survival time and improve growth under drought conditions
2. That the southern *Trichoderma* isolate, from a drier environment, will confer greater drought tolerance because of habitat-adapted symbiosis

[Rodriguez et al. 2008]

Experimental design for testing seedling drought tolerance



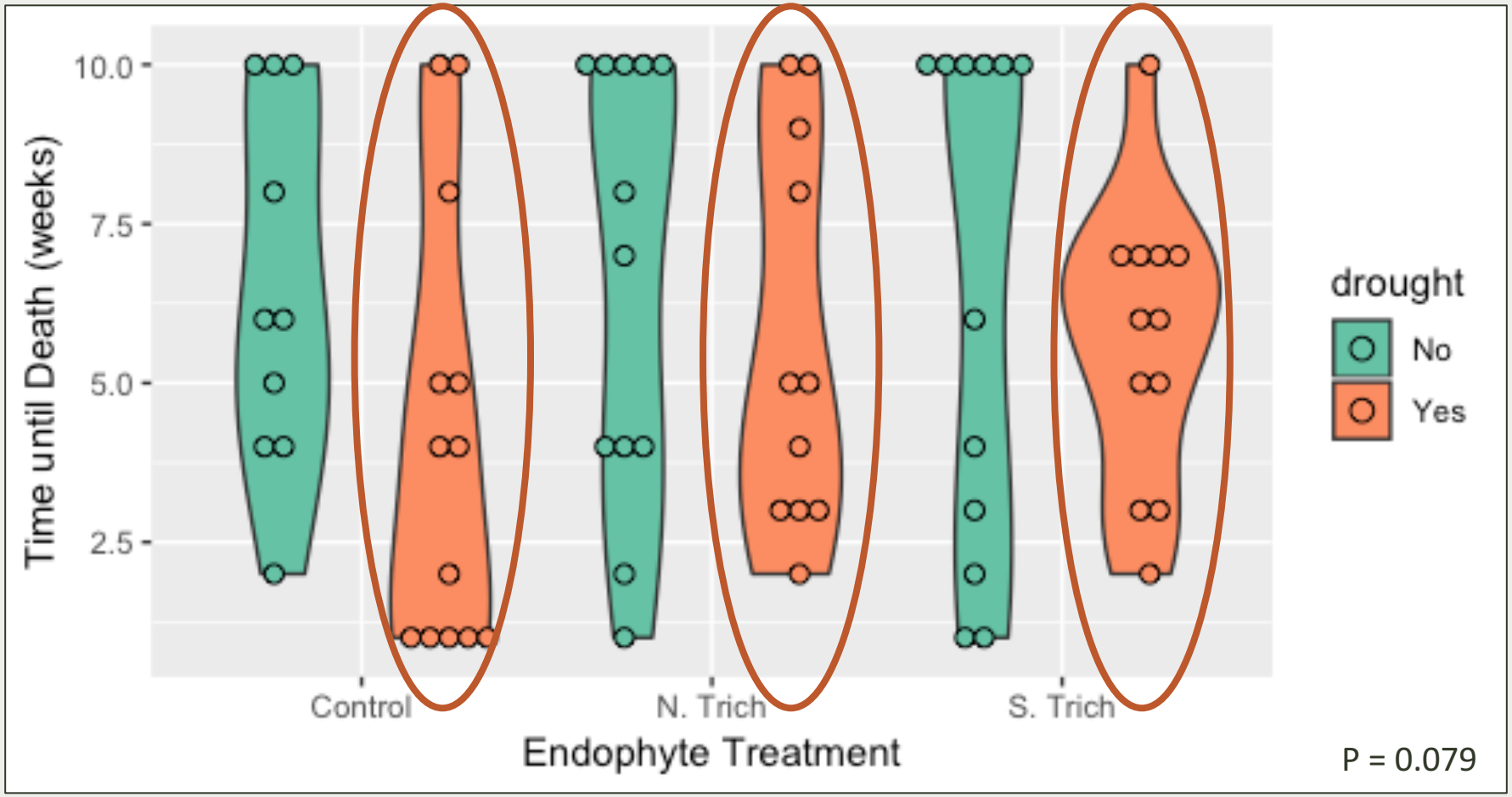
Germinate seedlings from DF_PC

Inoculate with endophyte treatments

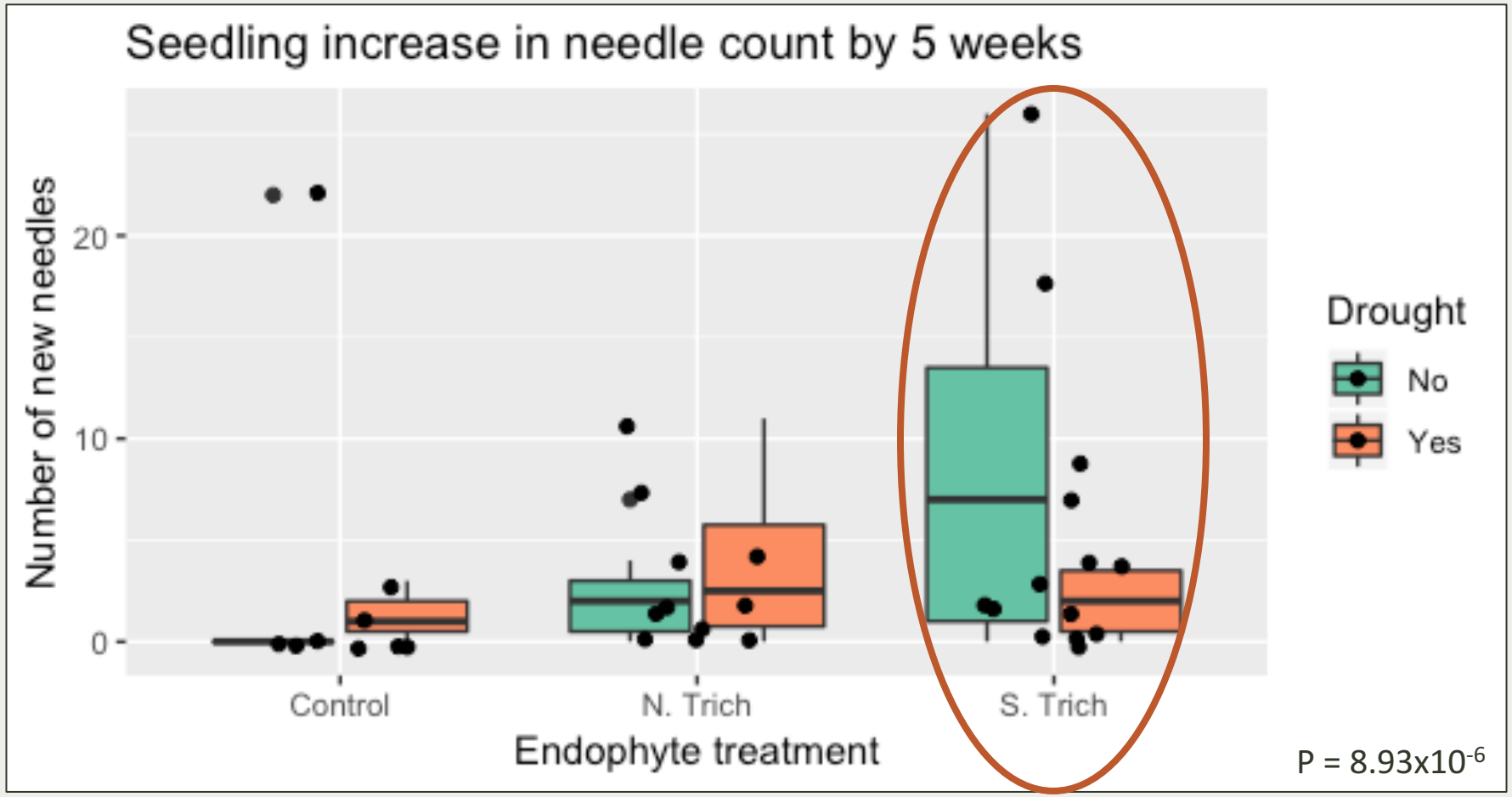
Establish seedlings in sterile soil

Apply drought and collect data

Survival analysis showed that drought treatment was the primary factor in seedling survival



Southern *Trichoderma* reduced needle growth under drought conditions



Conclusions from this work

- Low isolation frequency of endophytes and the hypothesized bottleneck in the seed microbiome
- Association between seed provenance and endophyte presence
    - NZ v. USA
- General trend of endophyte effects on seedling drought tolerance

Limitations in this project

Observational Study

- Opportunistic nature of sampling method
- Culture-based techniques
- Differences in methods between US and NZ

Experiment

- Sample size
- Checking for endophyte presence
- Applicability in the field

Further questioning and future work

- Exploring seed endophyte communities further with culture-independent methods
- Evaluating endophyte-mediated drought response in Douglas-fir on a larger scale

Metabarcoding.org 2019

Proseed NZ 2019

Acknowledgements

- Lab members: Ed Barge, Shikhar Hatwal, Devin Leopold, Kyle Gervers, Sabrina Heitmann, Lucas Nebert
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- BRR advisor: Wanda Crannell