Phyllosphere fungal microbiome in Douglas-fir needles associated with *Nothophaeocryptopus gaeumannii*

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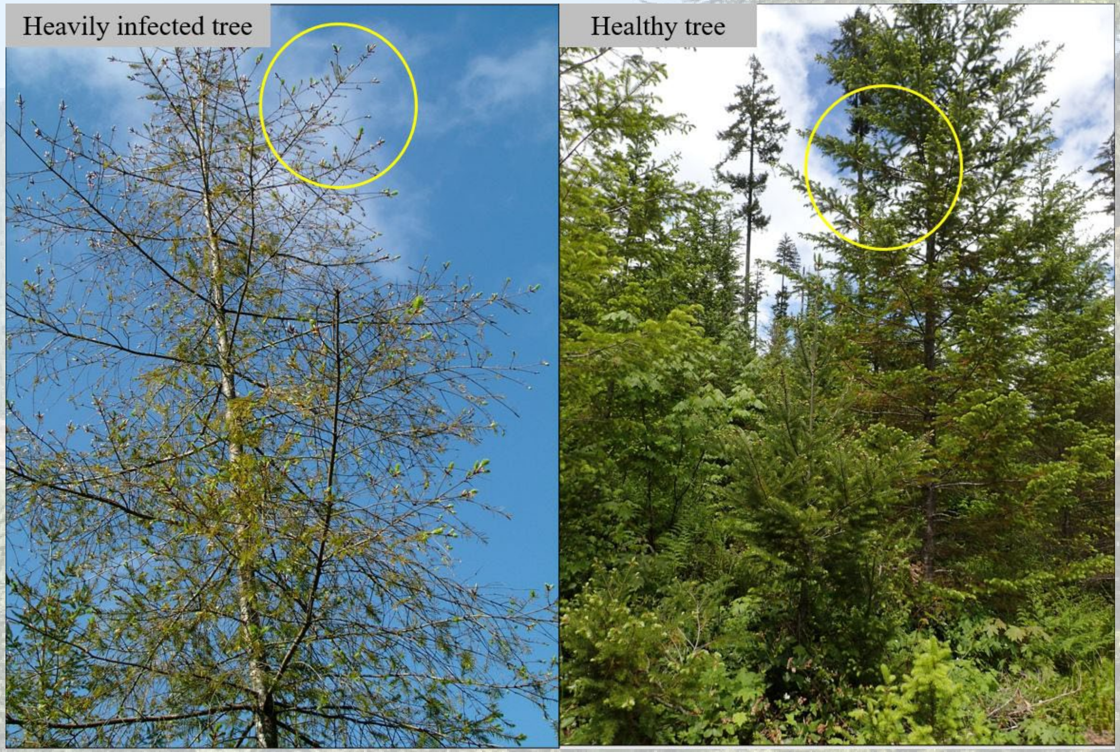
Oregon State University

USDA Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

Nothophaeocryptopus gaeumannii

- A native fungus to PNW, causes a foliage disease Swiss needle cast (SNC).
- The pseudothecia physically blocks stomates on needles, therefore reduces photosynthetic rate and Douglas-fir growth.



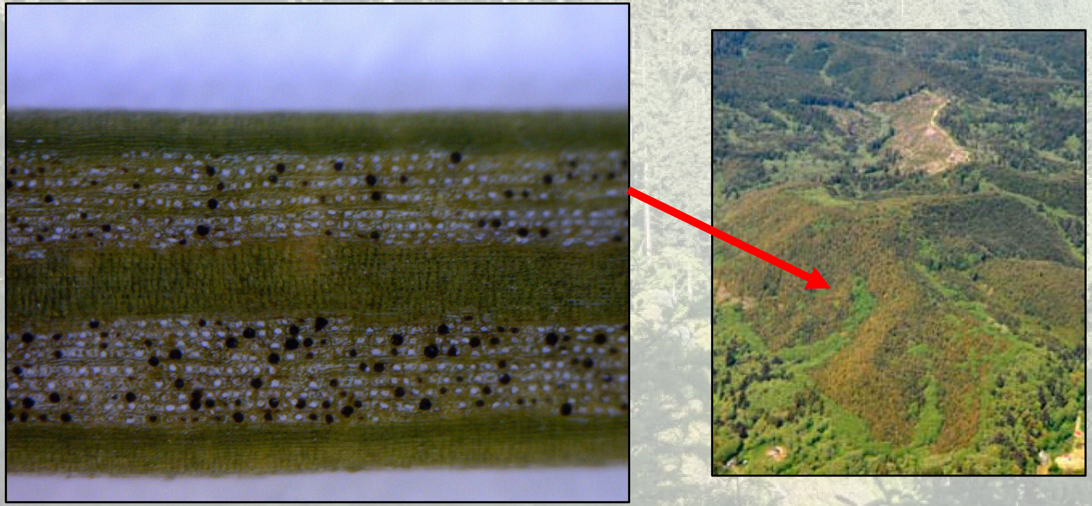
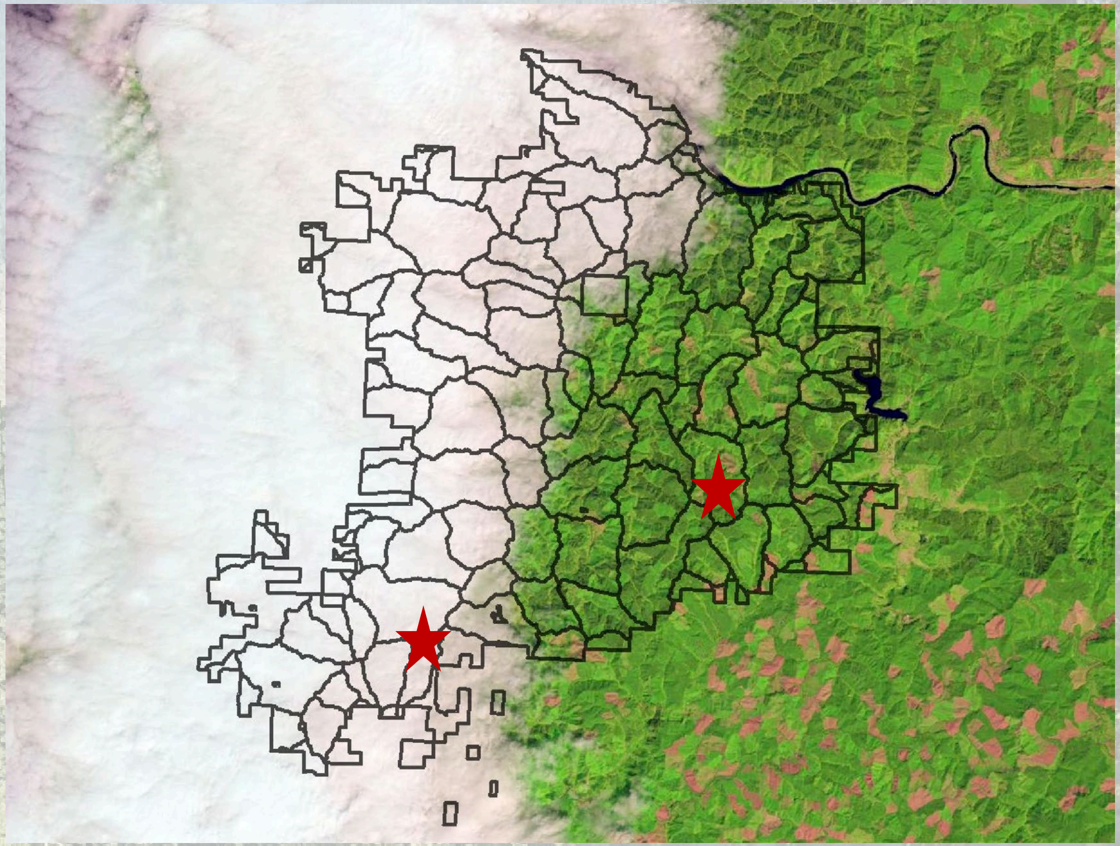


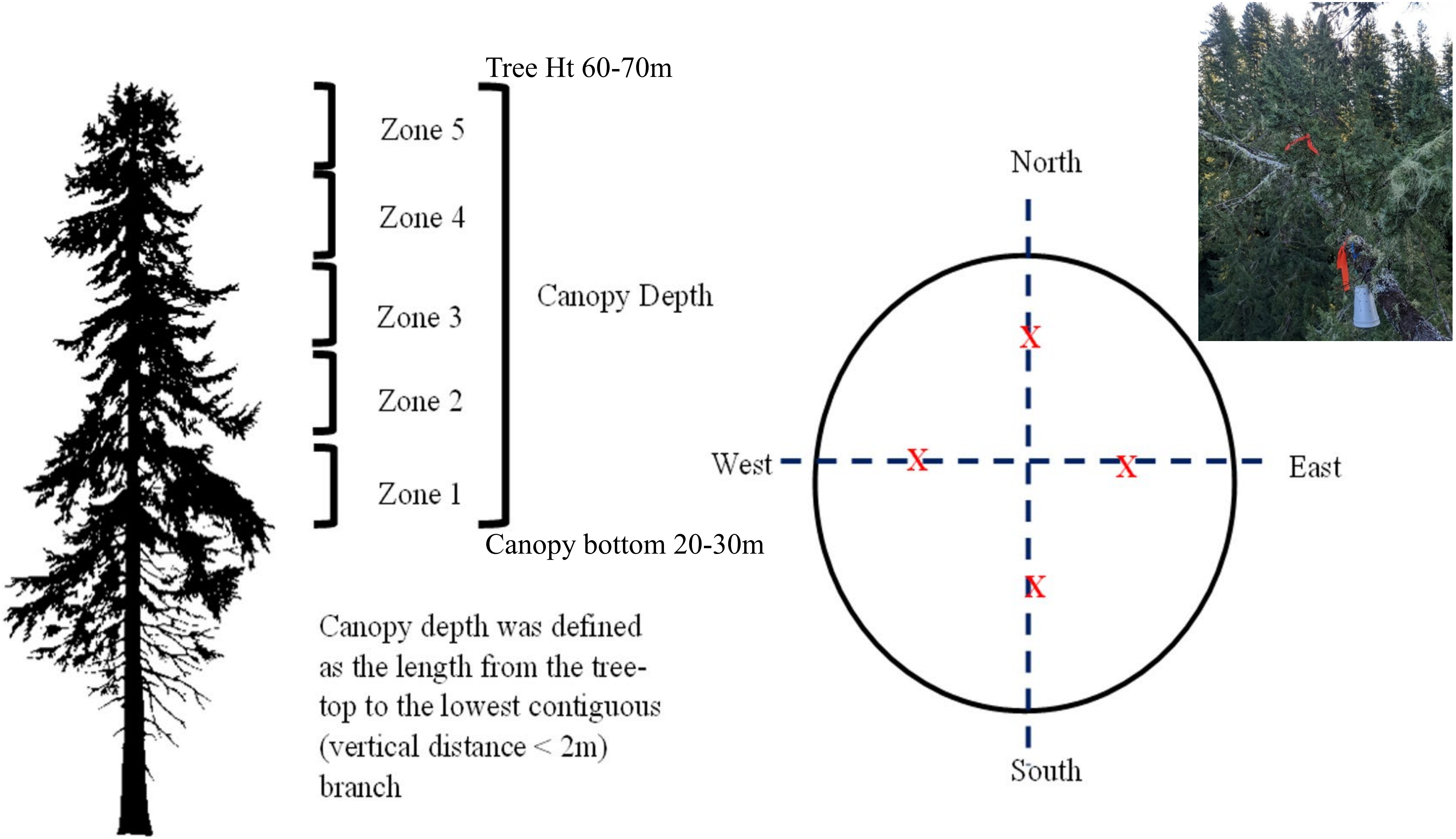
Photo from Swiss Needle Cast Cooperative: http://sncc.forestry.oregonstate.edu/

Research Questions

- *Nothophaeocryptopus gaeumannii* is an endophytic fungus that would mature and emerge in the spring while Douglas fir bud-breaking.
- Foliar microbiomes can be related to plant physiological traits such as stomatal conductance, also it is important for host resistance to diseases.
- So, do the foliar fungal communities relate to *Nothophaeocryptopus gaeumannii* occlusion therefore the SNC patterns? Can metabarcoding methods reveal more details about NOGA and other fungal communities?
- Do the fungal communities and NOGA differ among canopy locations due to macro- and micro-climates?

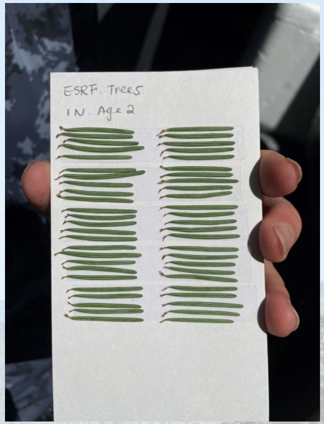
Elliott State Forest



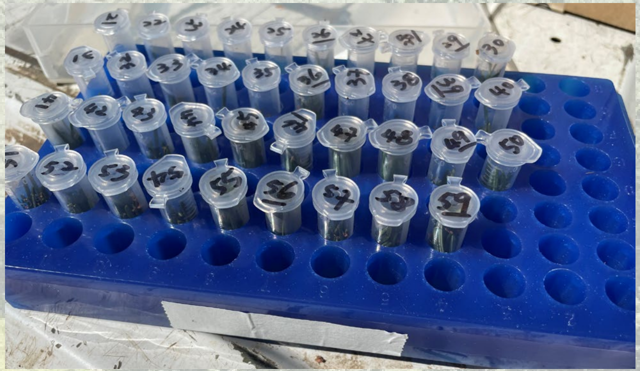


Canopy depth was defined as the length from the tree-top to the lowest contiguous (vertical distance < 2m) branch

Photo Credit: https://tidcf.nrcan.gc.ca/en/trees/factsheet/122



SNC samples: after evaluating the foliage retention, 50 needles were randomly selected from 3-year-old cohort, taped on an index card, and stored at -20˚C before reading. (June 2023)



Microbiome samples: lyophilized for 24 hours for drying, then stored at -80 ˚C before processing. (Nov 2022 & June 2023)

Visual examination of NOGA

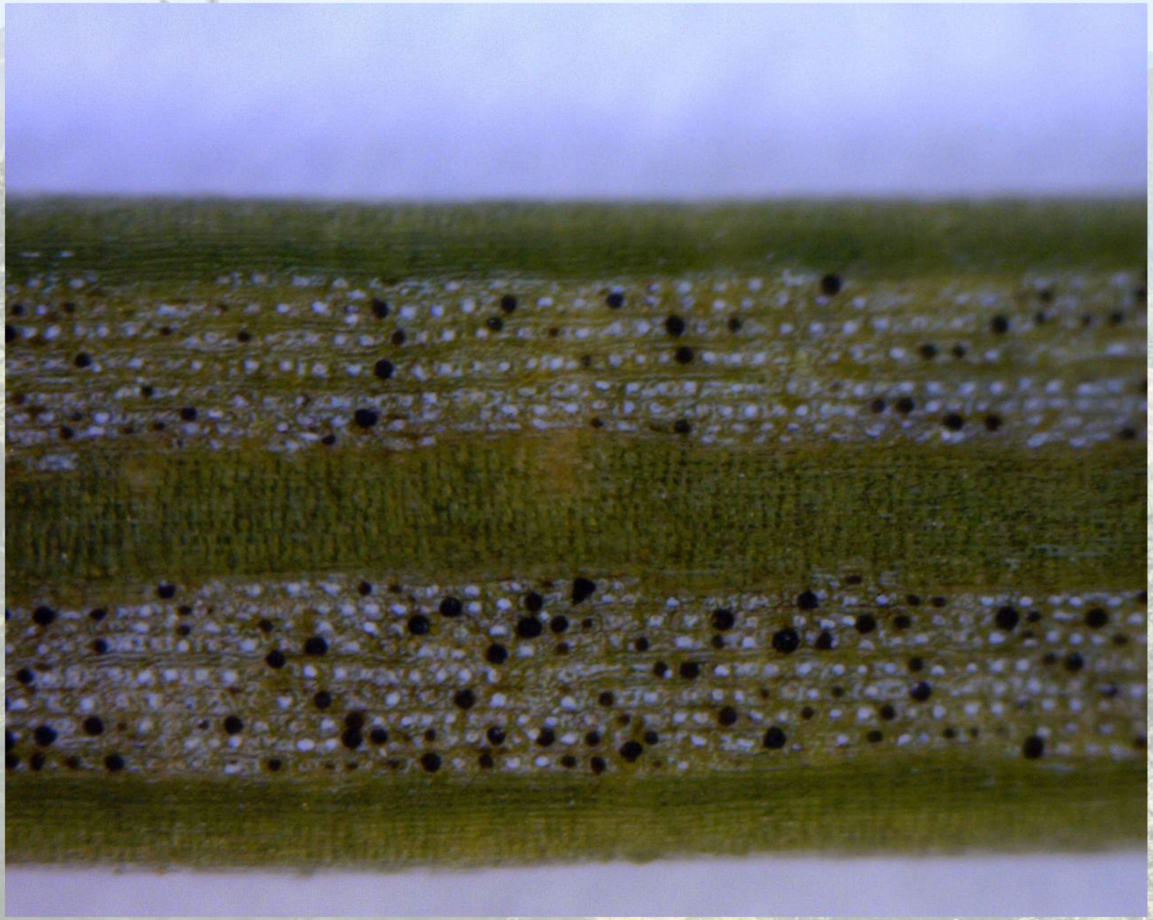
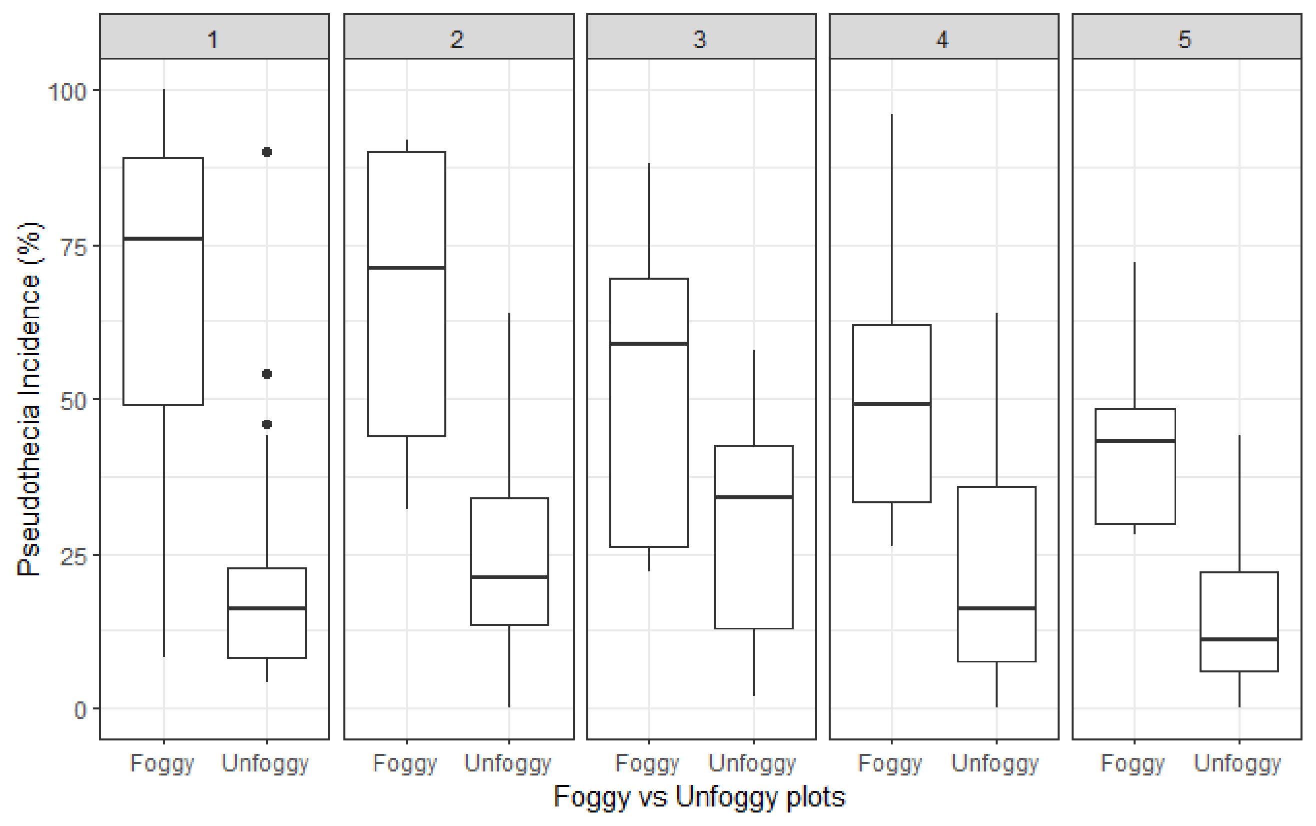
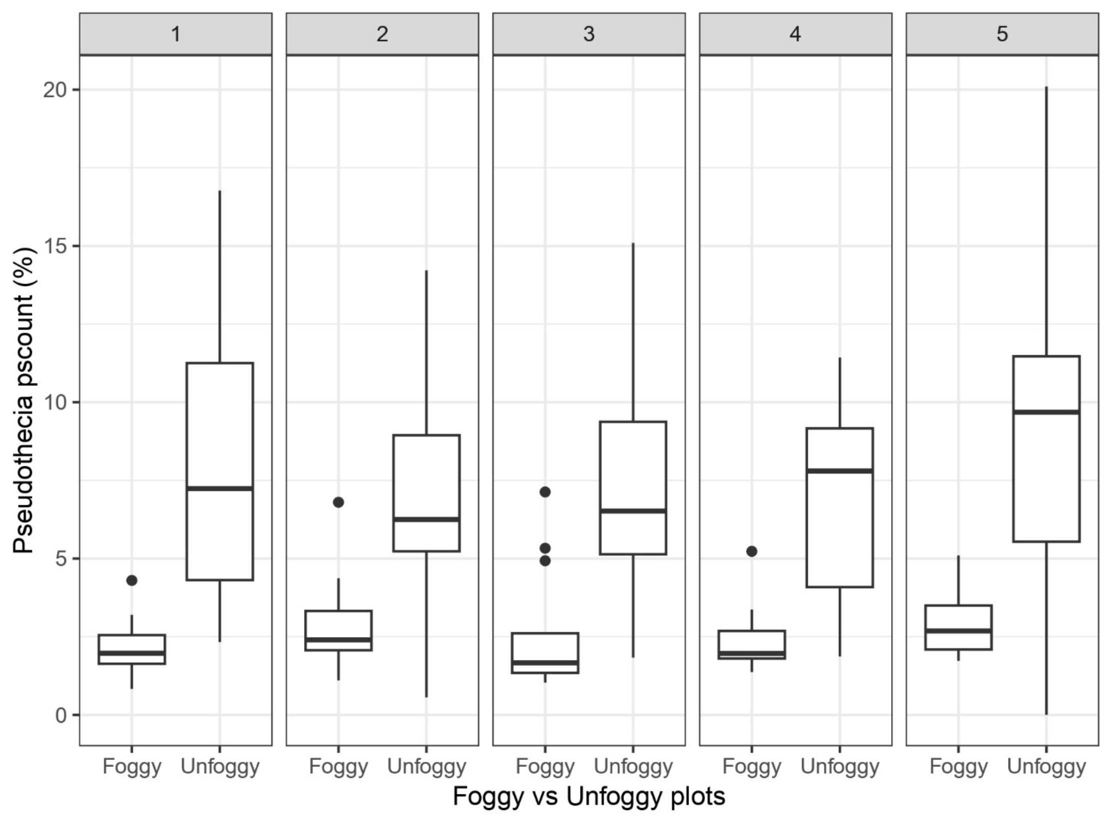


Photo credit: Swiss Needle Cast Cooperative http://sncc.forestry.oregonstate.edu/)

- Incidence = % of needles occluded by pseudothecia
- pscount = % of stomates occluded by pseudothecia
- SNC index = incidence *pscount *100 (%)

SNC incidence and pseudothecia density

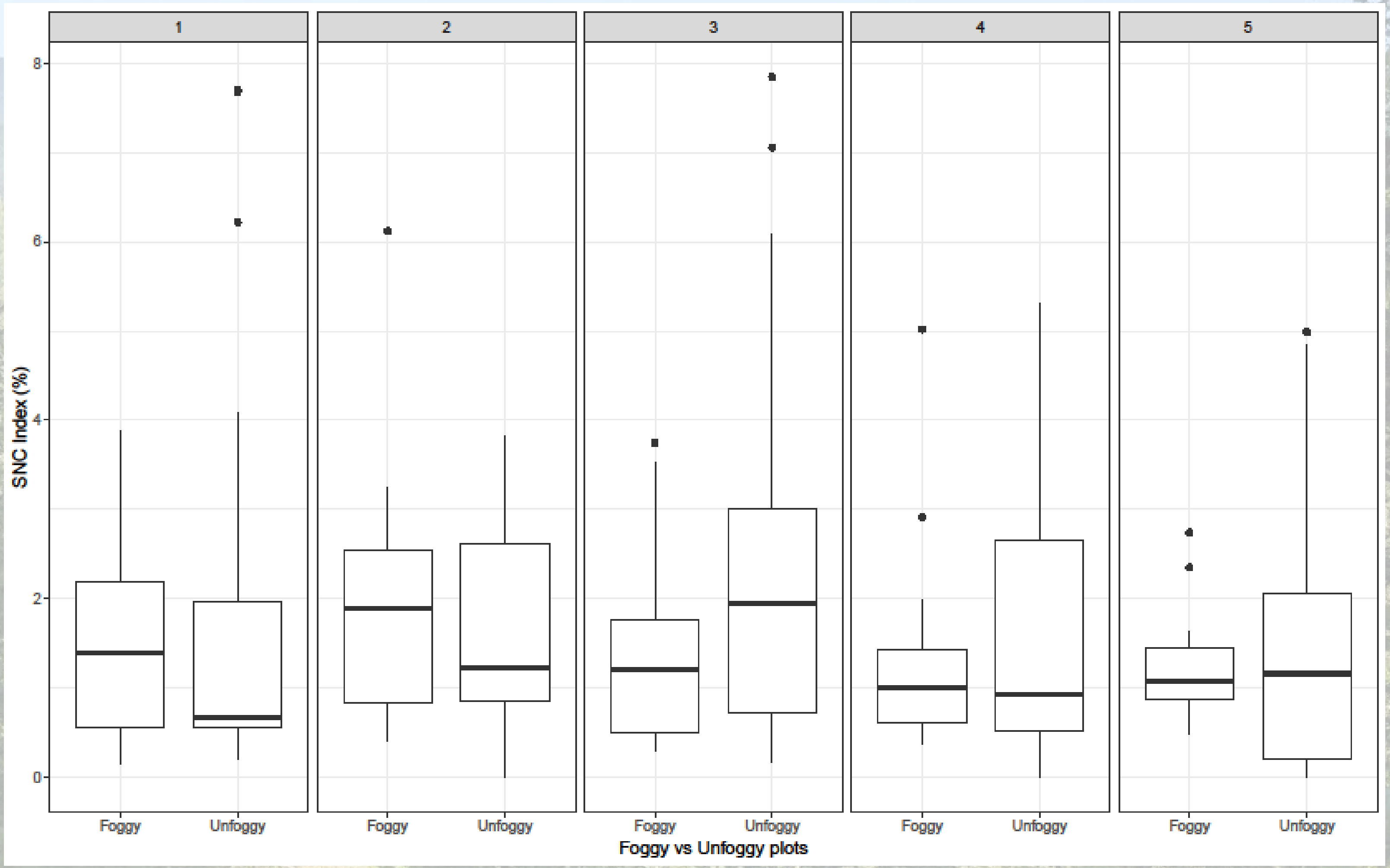




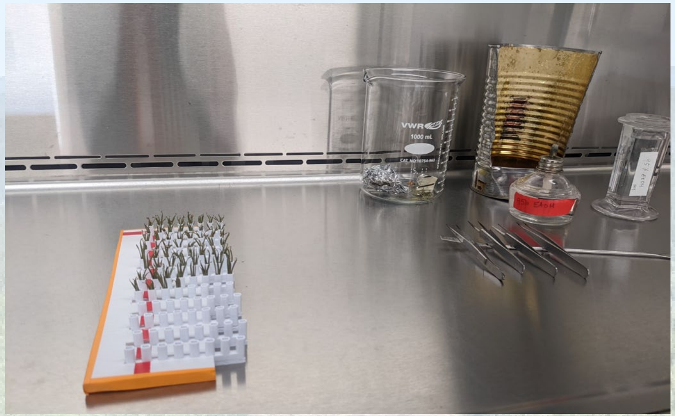
* Incidence = % of needles occluded by pseudothecia

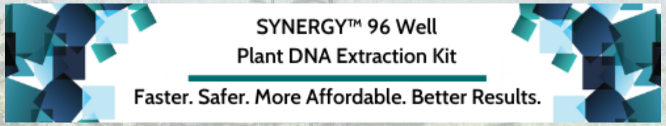
* pscount = % of stomates occluded by pseudothecia

SNC index



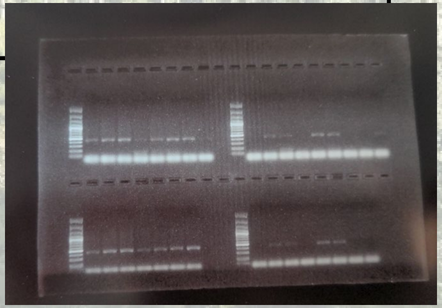
Microbiome sample processing



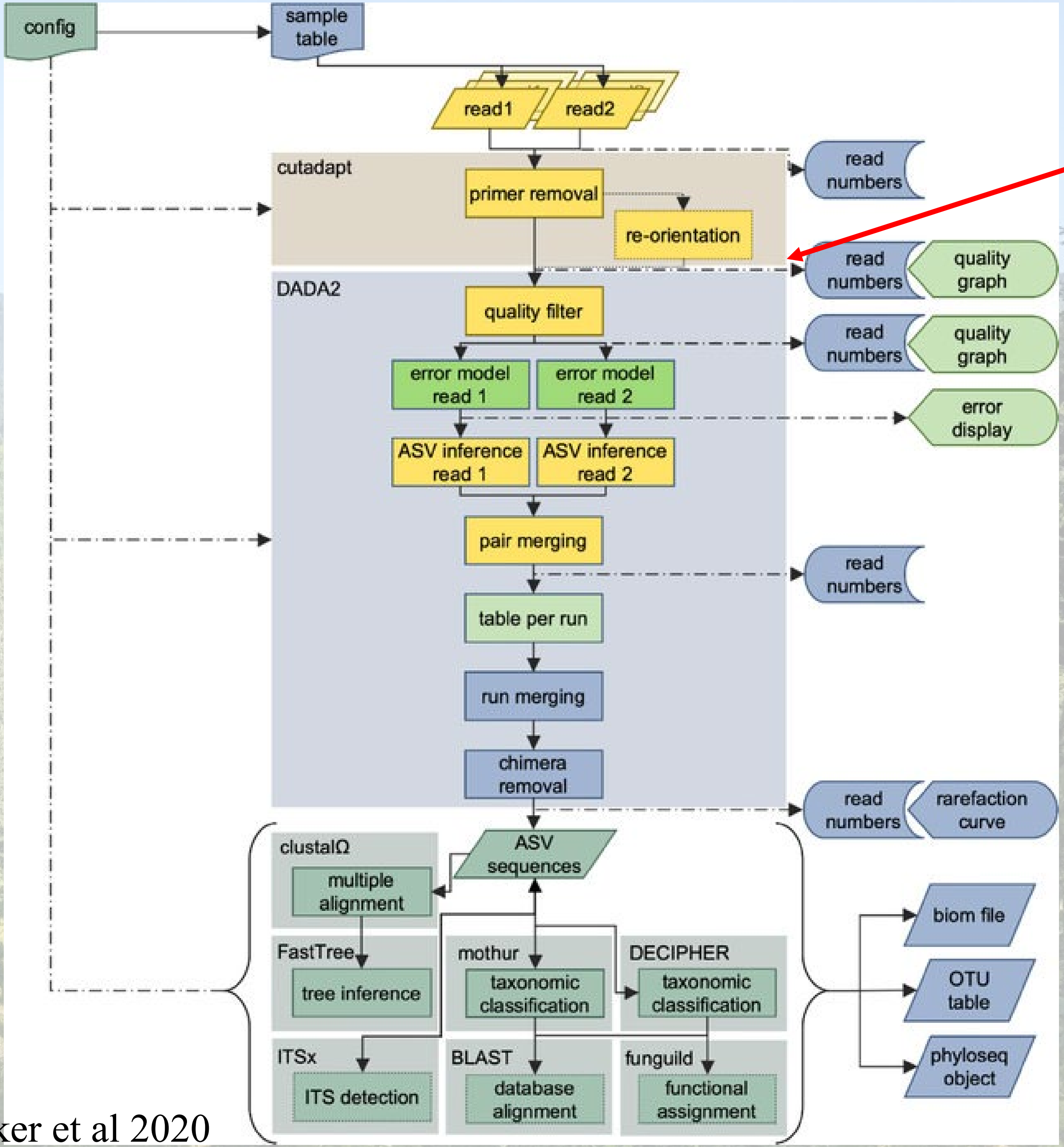


SYNERGY™ 96 Well
Plant DNA Extraction Kit
Faster. Safer. More Affordable. Better Results.

Two stage PCR applied. The ITS2 region was first amplified using the 5.85-Fun and ITS4-Fun primers, a 3-6 bp length heterogeneity spacer, and then followed by illumina adaptor sequences (Gervers et al 2022).



Conceptual workflow of Pipeline

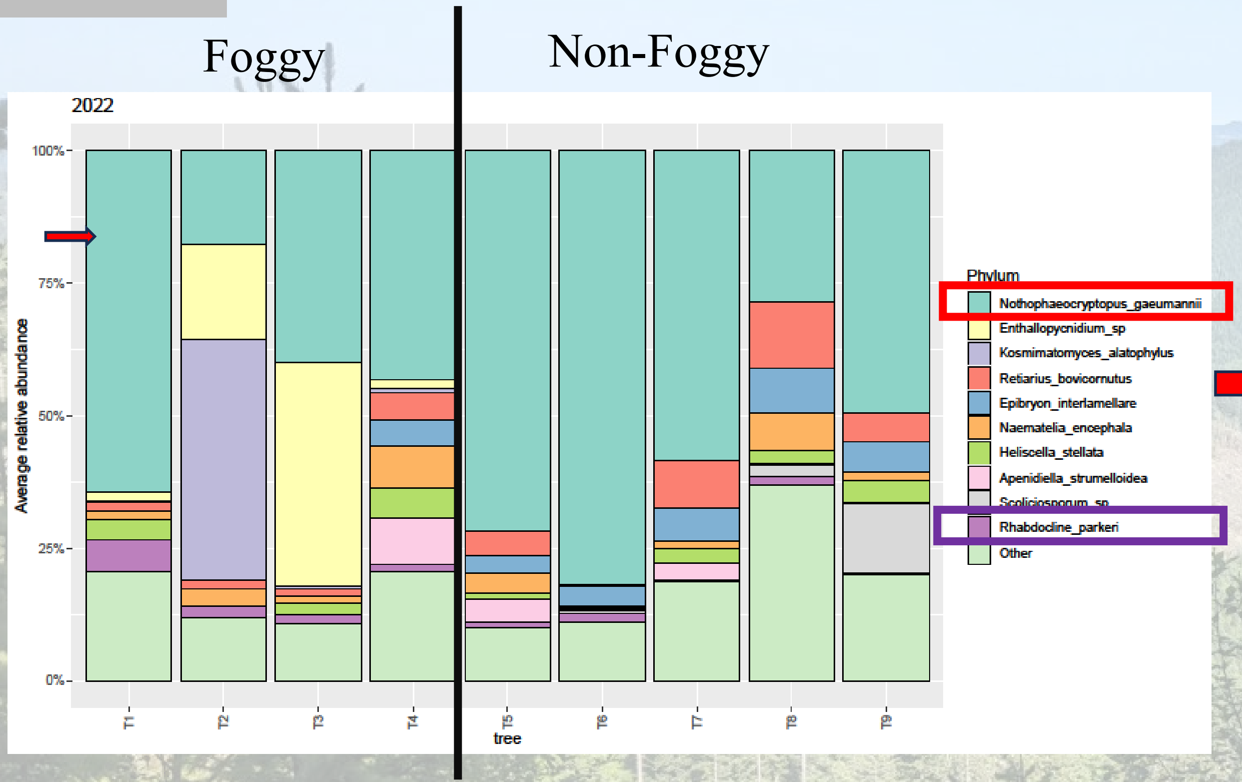


Remove host genes

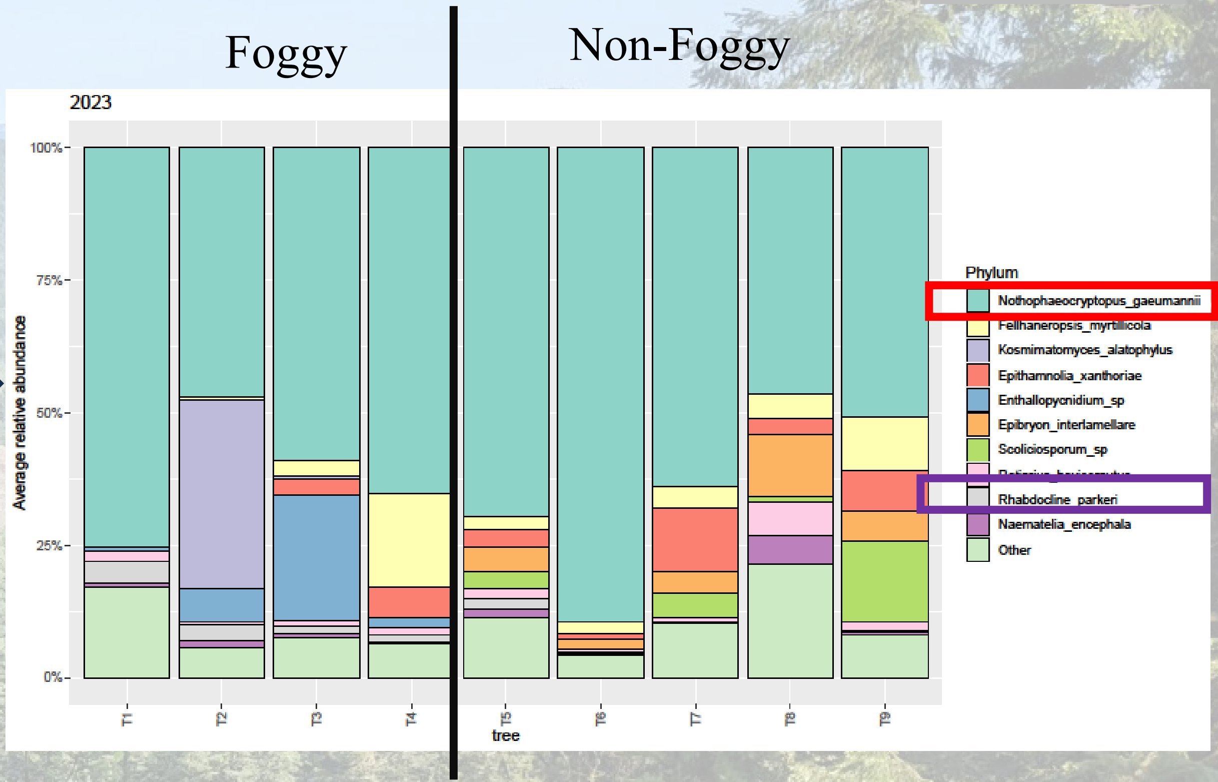
Weißbecker et al 2020

Phyllosphere microbiomes

Nov 2022

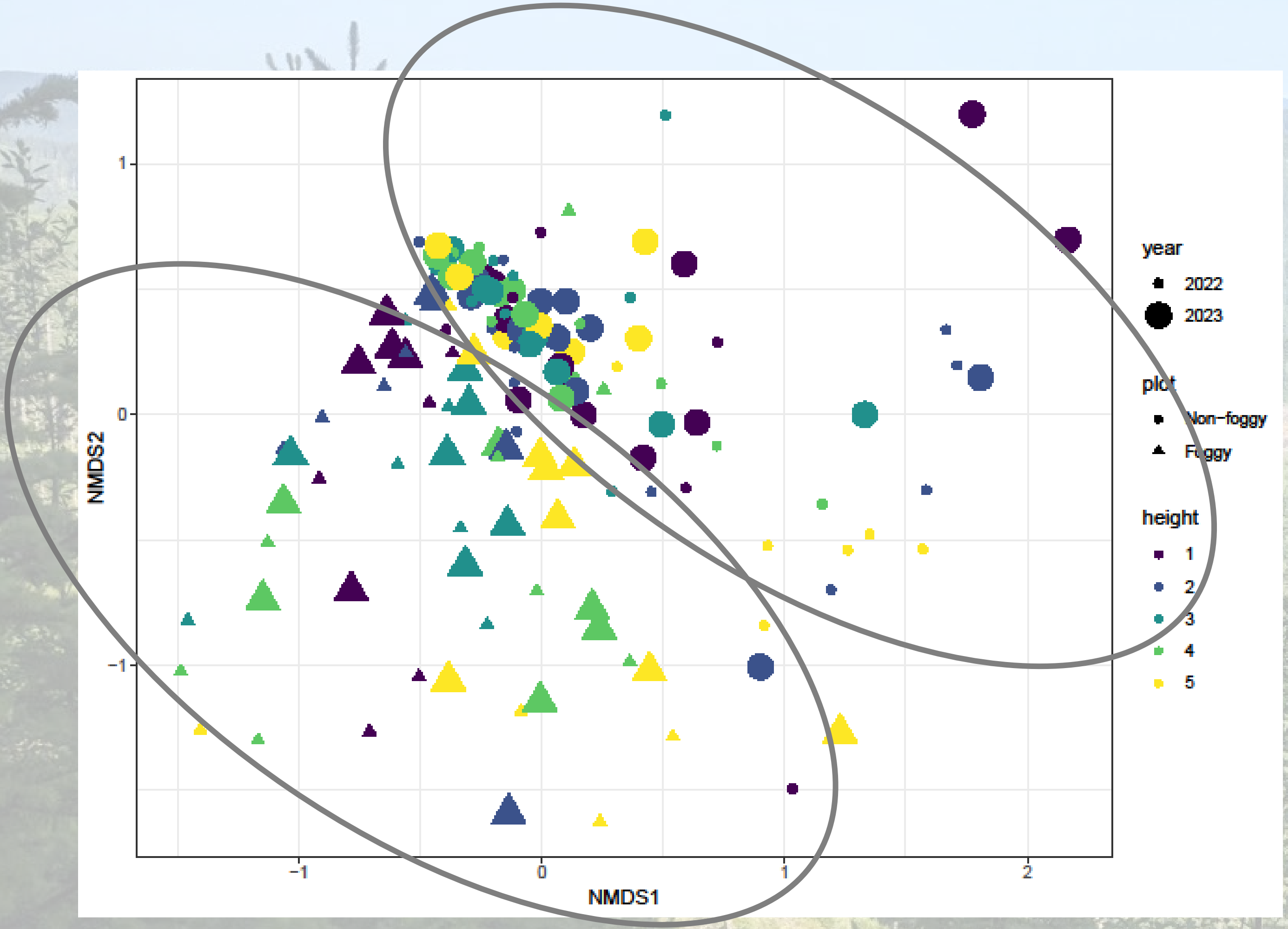


June 2023



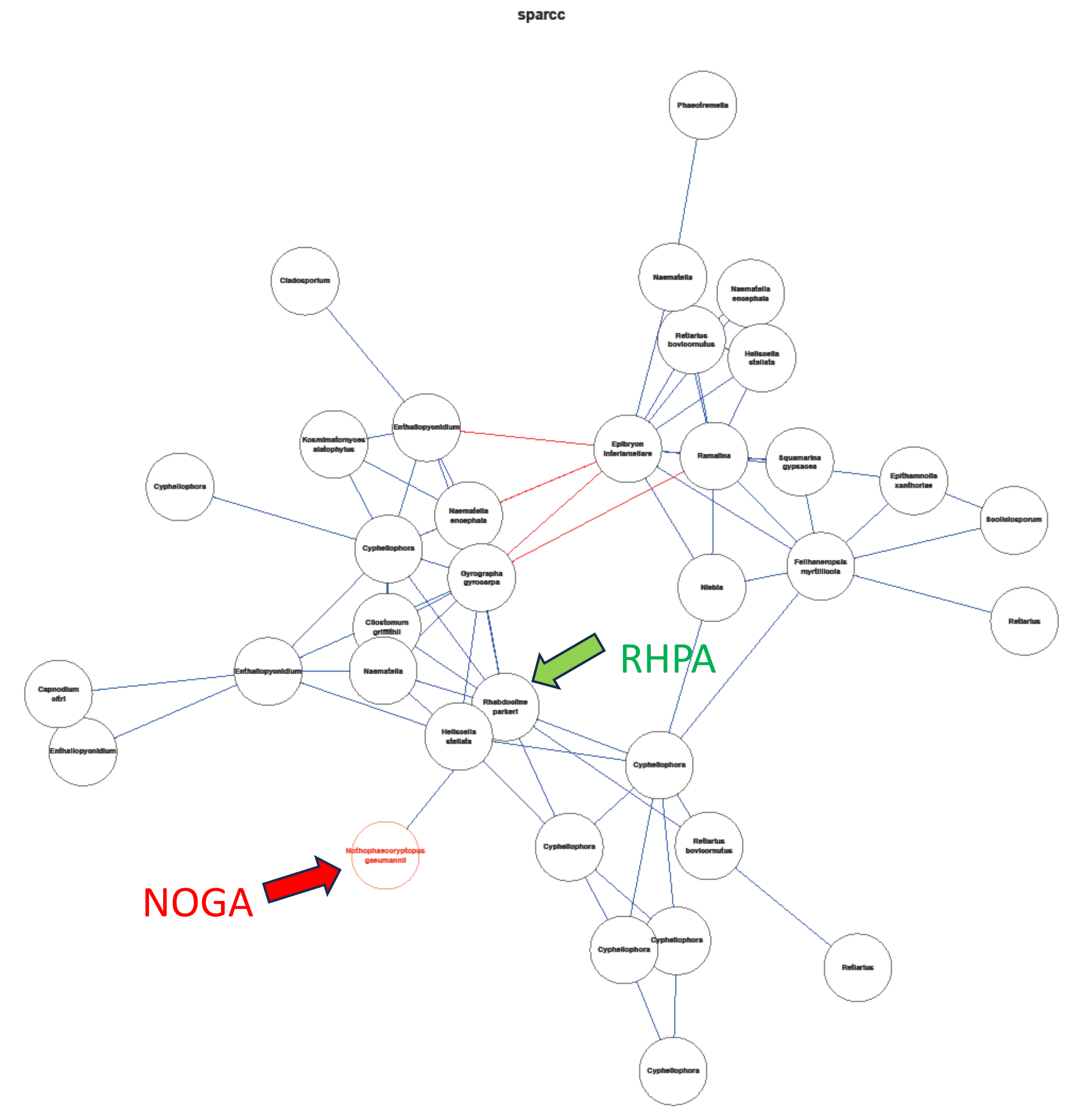
- Only use known species at Genus level. (1757 → 682 taxa)
- *N. gaeumannii* was abundant in most samples.
- From winter to spring: RA of *Nothophaeocryptopus gaeumannii* increasing.
- Other needle pathogens, such as *Rhabdocline* spp.
- Lichen associated species, like *Cliostomum griffithi, Scoliciosporum* spp, and *Usnea* spp.

Phyllosphere microbiomes



- PERMANOVA
- Foggy vs non-foggy plots, p = 0.0001.
- Sampling year 2022 vs 2023, p = 0.002.
- Canopy positions
  - Height differences (level 1-5), p = 0.002.
  - Directions (N, E, S, W), p = 0.12.

Correlations between species



- SpeciEasi::SparCC

- NOGA has some correction with *Rhabdocline parkeri* but not very strong, however, *Rhabdocline* is pretty central in the network

- NOGA is also (weakly) positively correlated with Niebla spp, which is a lichen-associated fungus.

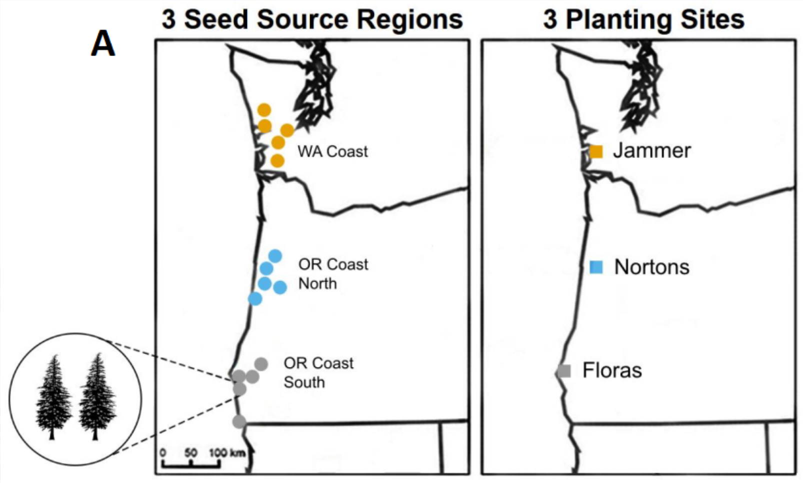
*** The threshold was low (Cor = 0.26)

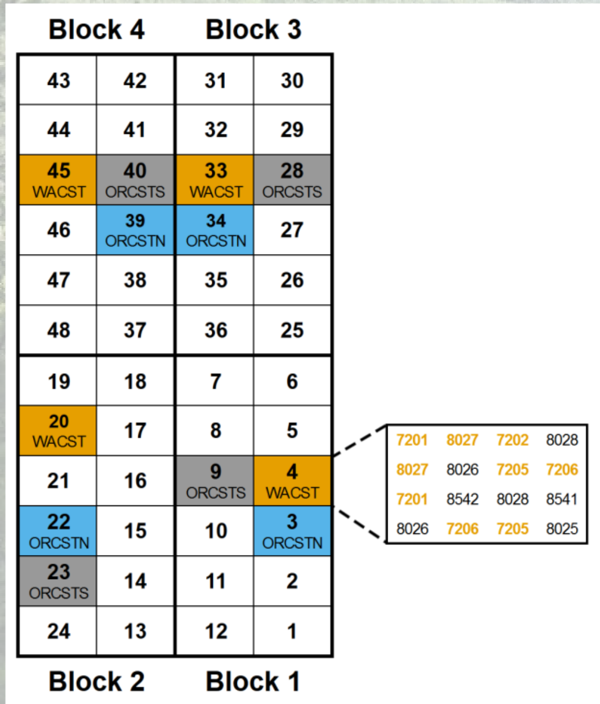
Applying to SNCC Plot Network

- Distance from Ocean (4*6 distance zones)
- Taking foliage samples for microbiome at panels 0&5 vs 25 to represent the “fog” and “non-fog” weather/area.

Photo from Swiss Needle Cast Cooperative: http://sncc.forestry.oregonstate.edu/

Douglas-fir Seed-Source Movement Trial





Block 4		Block 3	
43	42	31	30
44	41	32	29
45 WACST	40 ORCSTS	33 WACST	28 ORCSTS
46	39 ORCSTN	34 ORCSTN	27
47	38	35	26
48	37	36	25
19	18	7	6
20 WACST	17	8	5
21	16	9 ORCSTS	4 WACST
22 ORCSTN	15	10	3 ORCSTN
23 ORCSTS	14	11	2
24	13	12	1
Block 2		Block 1	

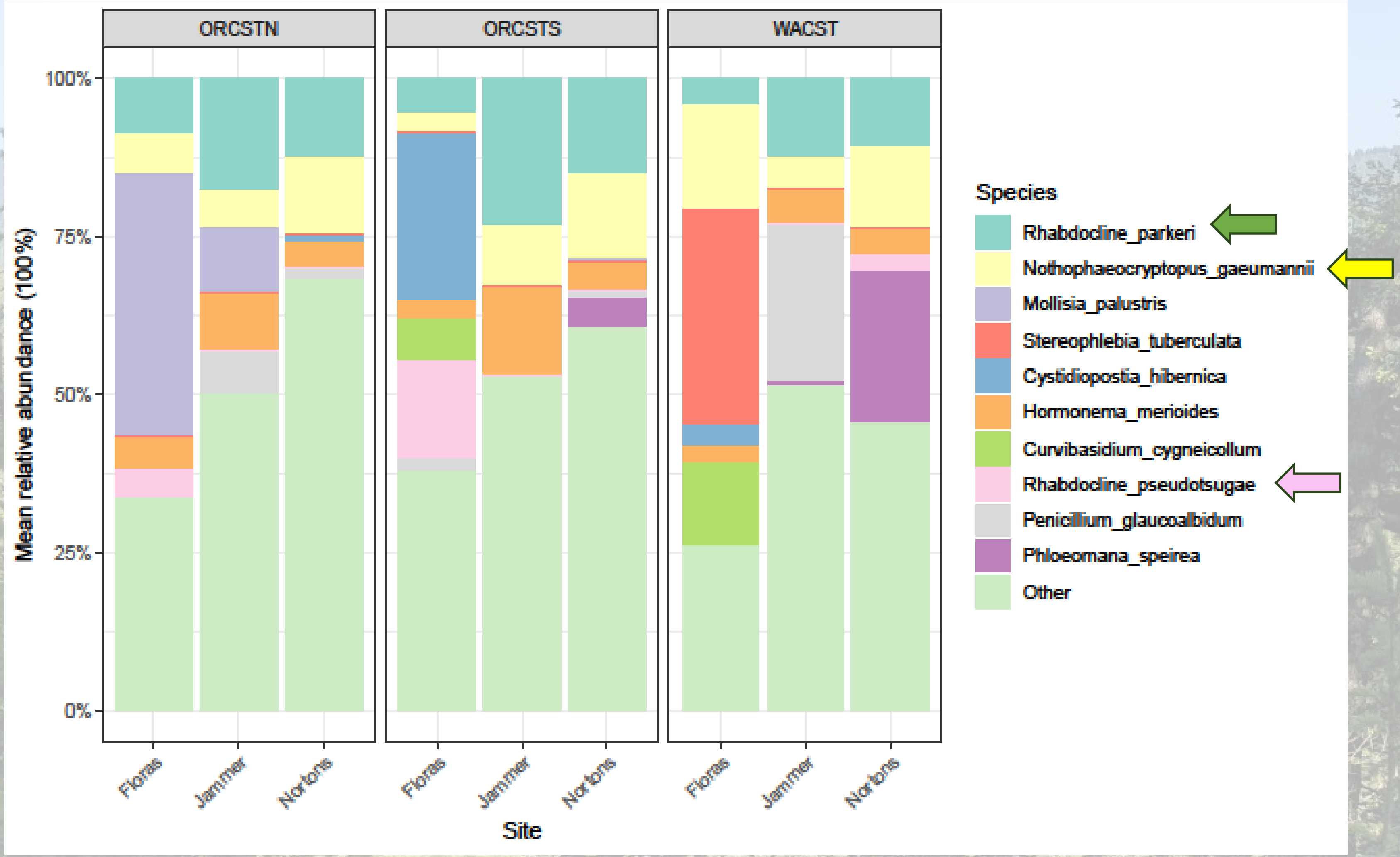
7201	8027	7202	8028
8027	8026	7205	7206
7201	8542	8028	8541
8026	7206	7205	8025

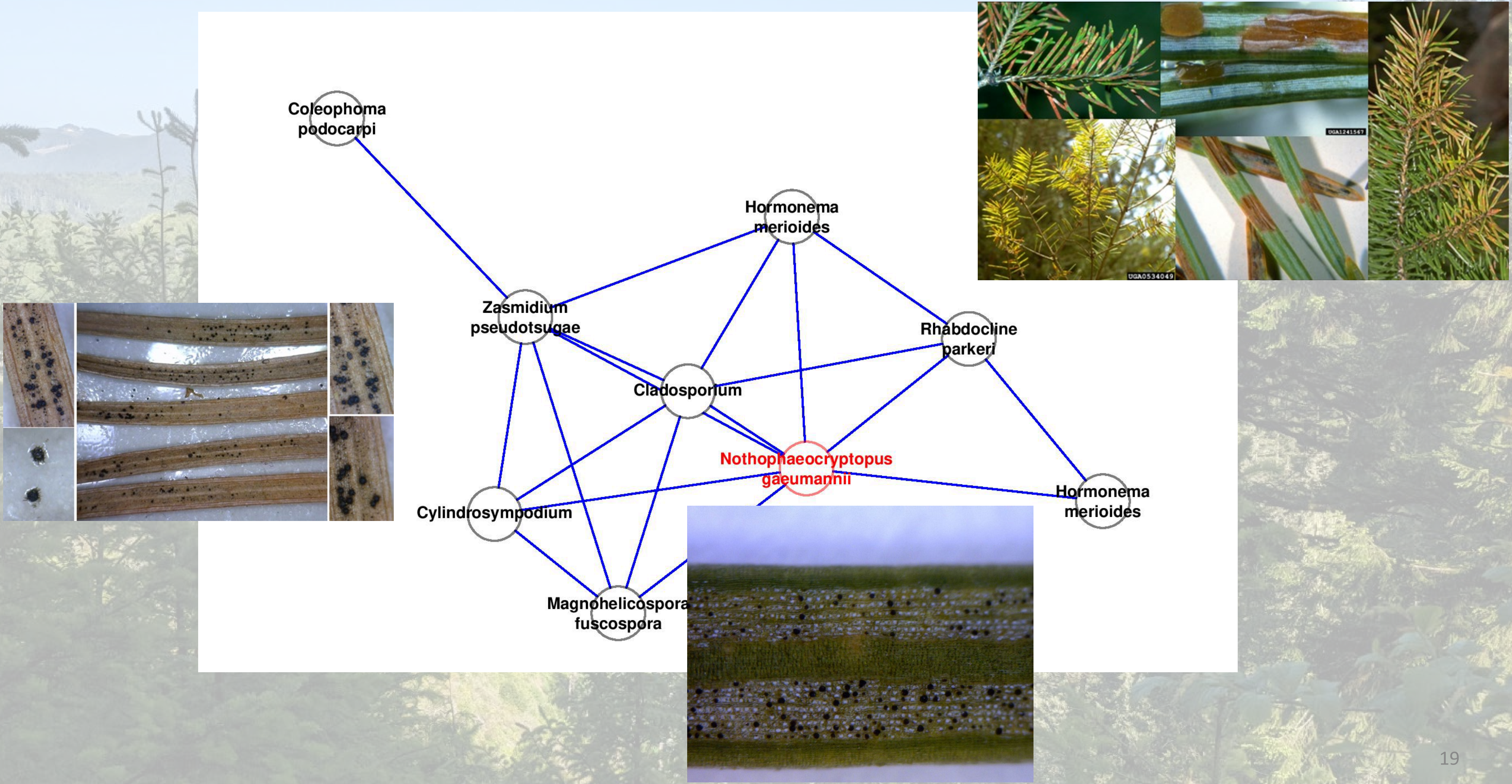
- Established in 2009 (Harrington and St. Clair 2017)

- Were the same gardens as Wilhelmi et al. 2017 regarding climate of seed source affects susceptibility of coastal Douglas-fir to foliage diseases.

- Shaken-fell needles, living green, surface-sterilized.

Photo credit: Lilah Gonen


ORCSTN
ORCSTS
WACST
100%
75%
50%
25%
0%
Mean relative abundance (100%)
Floras
Jammer
Nortons
Floras
Jammer
Nortons
Floras
Jammer
Nortons
Site
Species
Rhabdocline_parkeri
Nothophaeocryptopus_gaeumannii
Mollisia_palustris
Stereophlebia_tuberculata
Cystidiopostia_hibernica
Hormonema_merioides
Curvibasidium_cygneicollum
Rhabdocline_pseudotsugae
Penicillium_glaucoalbidum
Phloeomana_speirea
Other


Coleophoma podocarpi
Hormonema merioides
Zasmidium pseudotsugae
Rhabdocline parkeri
Cladosporium
Nothophaeocryptopus gaeumannii
Cylindrosympodium
Hormonema merioides
Magnohelicospora fuscospora
UGA1241567
UGA0534049

Questions?