SNCC Research Progress 2025: Screening Tolerance Advances & PCT Field Trials

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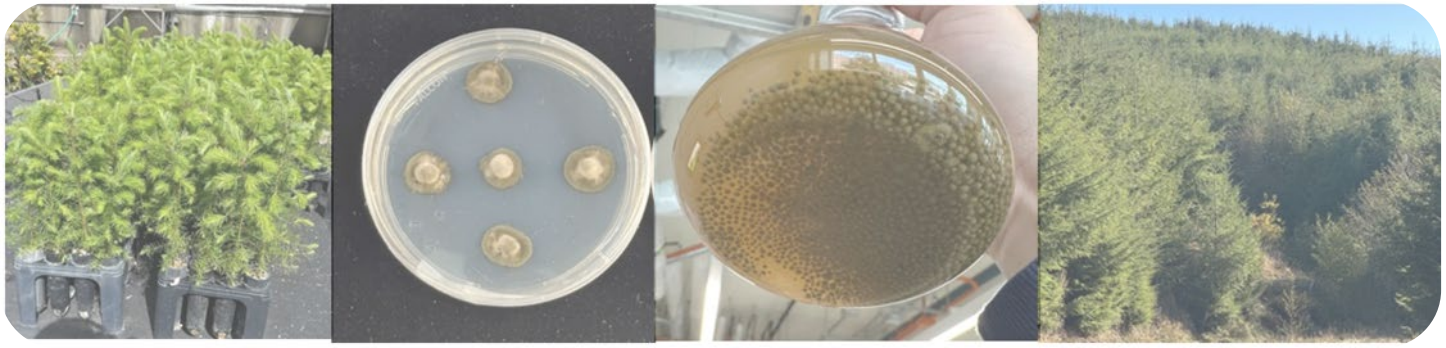
2025 SNCC Annual Meeting



Overview

- **Screening Tolerance:** Methodology and inoculation trials.
  - Inoculum production
  - Experiment trials
- **PCT Plot Network:** Field trial design and site selection.

SNCC Screening Tolerance

- Development of a SNC tolerance program, based on previously developed screening assays, to **identify Douglas-fir breeding populations with improved growth under controlled levels of disease pressure.**
  - **Phase I: Develop screening assay methods.**
  - Phase II: Collect additional data on top-ranked families.
  - Phase III: Outplant top-ranking families.

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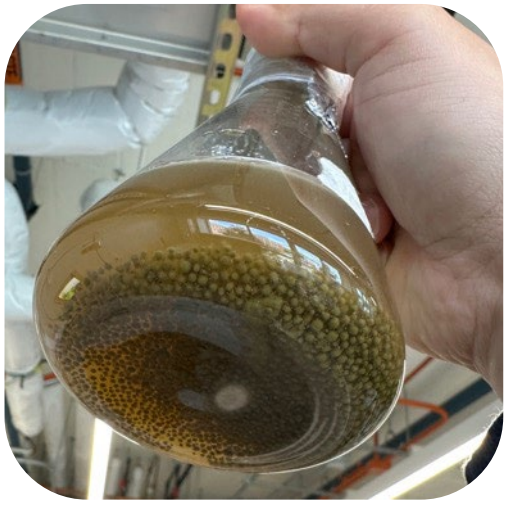
SNC Screening Tolerance

- **Phase I: Develop screening assay methods**
  - Inoculum production
  - Trial design
  - Inoculation
  - Plants maintenance
  - Sampling and QPCR detection



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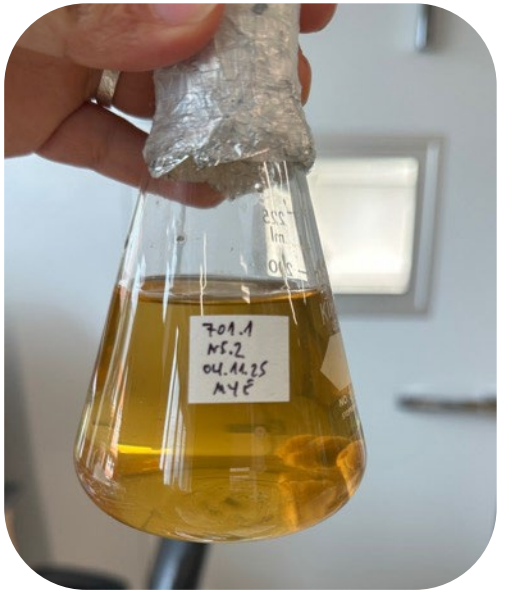
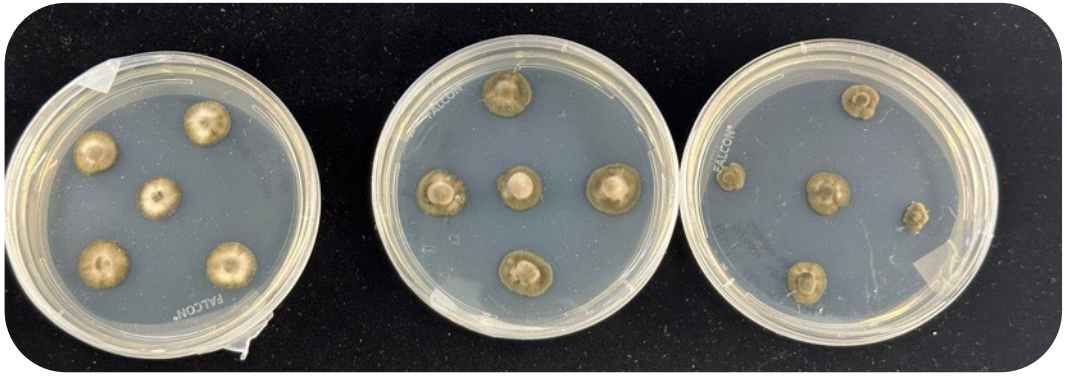
Inoculum production

- As many of you know, *N. gaeumannii* grows very slowly on standard culture media.
- A major challenge is producing large amounts of mycelium within a reasonable time.
- Traditional production relies on artificial solid or liquid media such as:
  – Malt Extract
  – PDA (Potato Dextrose Agar)
  – Other sugar- or carbohydrate-based formulations
- To address this limitation, we tested a new approach aimed at producing substantially larger amounts of mycelium in a shorter period of time.



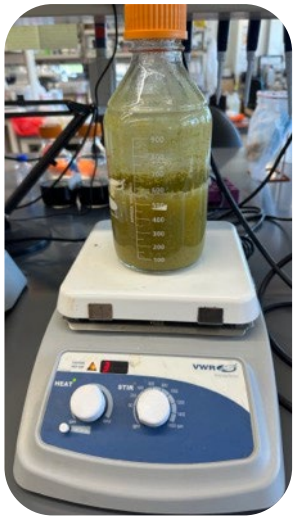


Inoculum production: Liquid media with Needles extract

Healthy needles

Grinding needles

Needle concentrated



Boiled and Yeast, glucose added and filter

Sterilizing

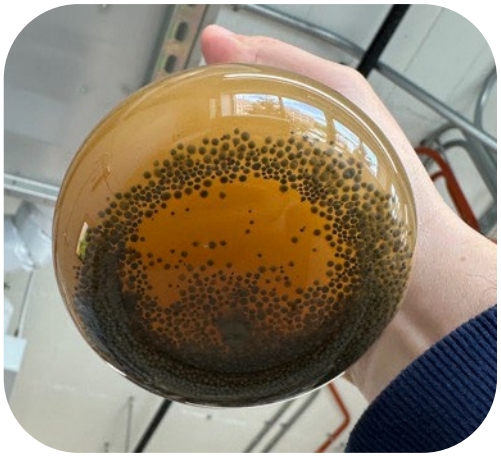
Transferring to flasks of 200 ml

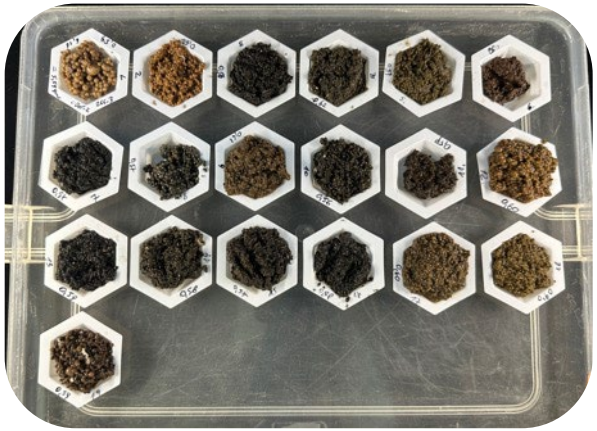
Incubated room 21 Tº and shaking by 140 rpm

Inoculum production: Malt Yeast Extract vs Pine Needle Yeast

- 53 days MYE
(Malt Yeast extract)

- 18 days PNY
(Pine Needle Yeast)





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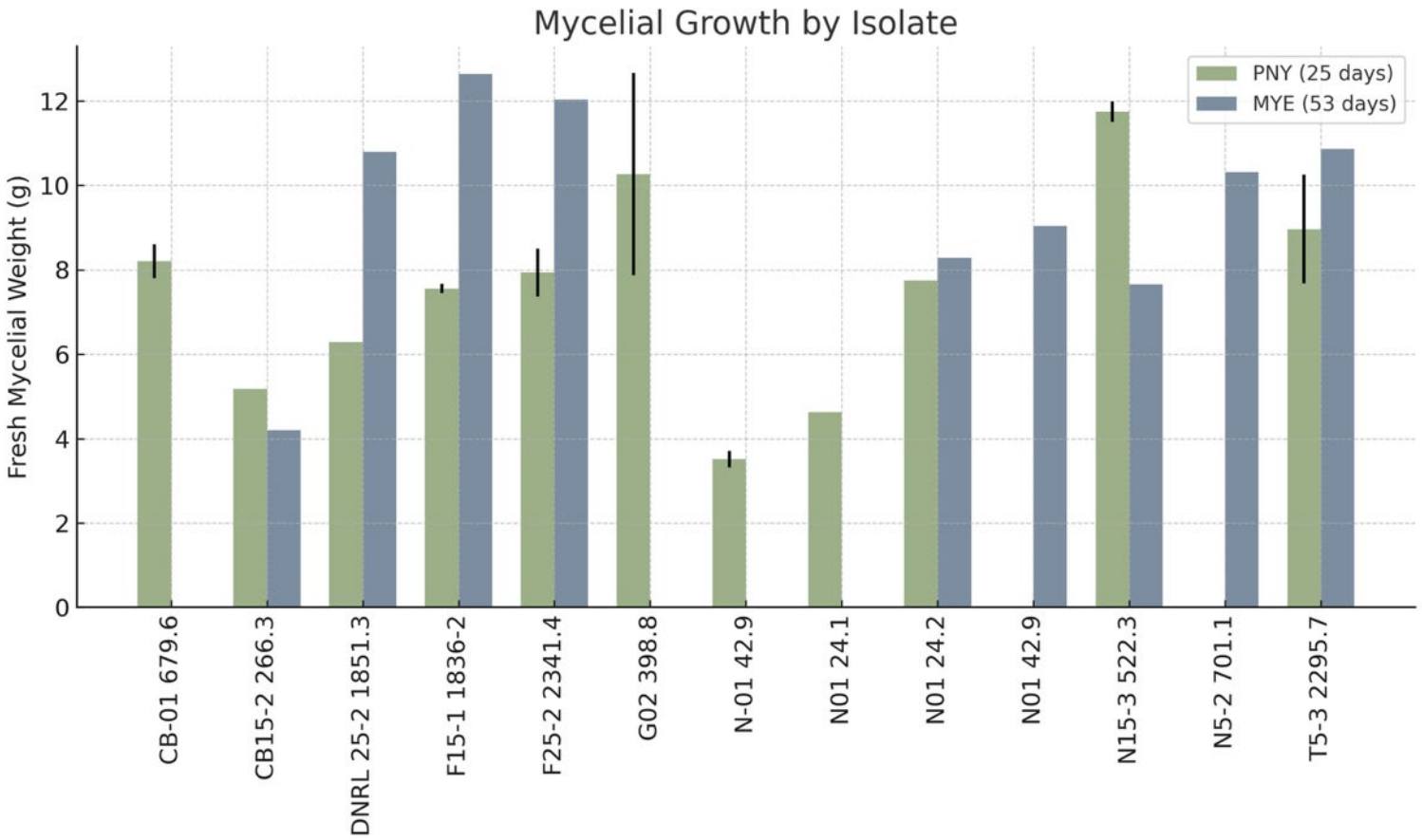
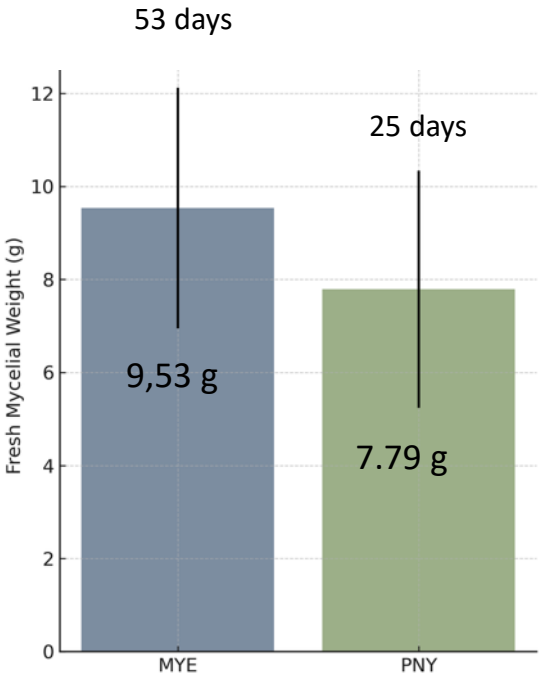
Inoculum production: MYE vs PNY



Inoculum production: MYE vs PNY



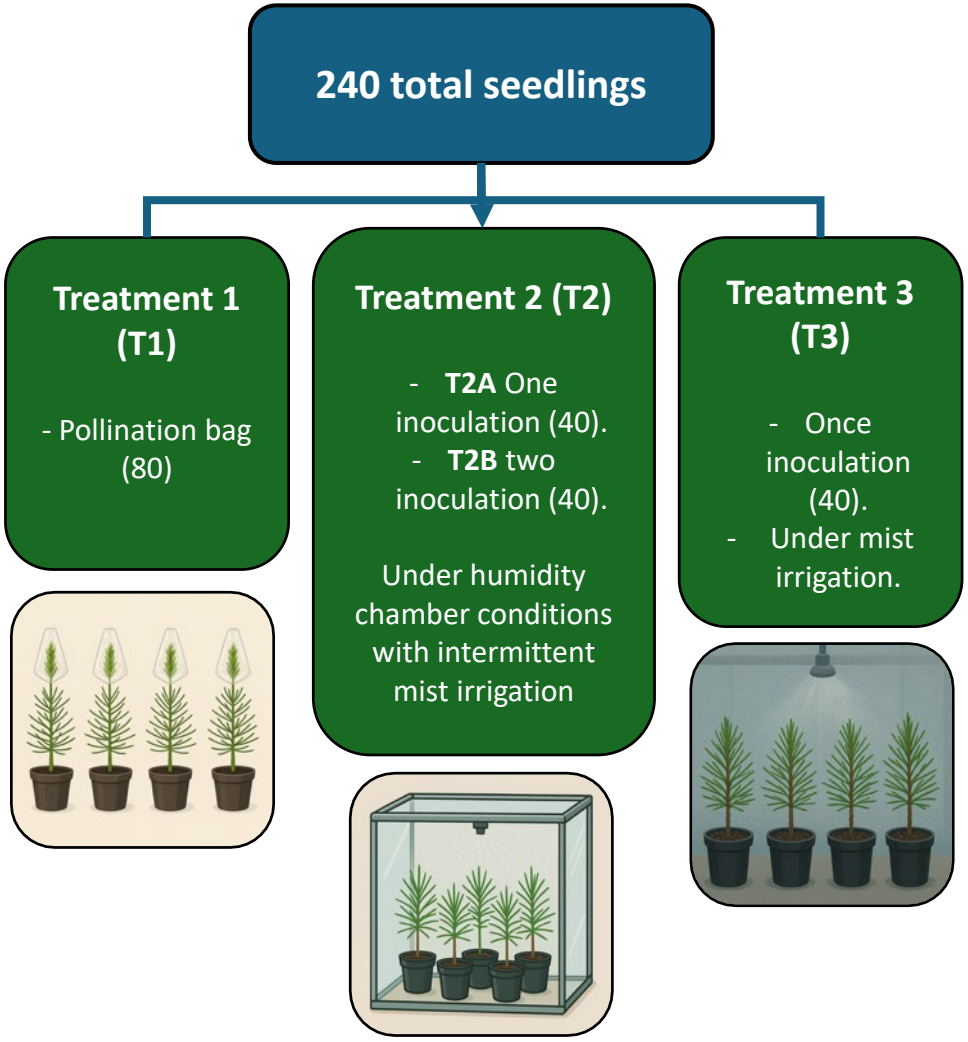
MYE (53 Days)

PNY (25 Days)

The PNY (Pine Needle Yeast) method yielded significantly higher biomass in a shorter time compared to traditional media.

Experimental Design

- 200 inoculated seedlings + 40 controls
- Sprayed fragmented mycelium (20gr/L)
- Three inoculation methods:
  - T1: Pollination bag
  - T2: Humidity chamber
    - T2A Single inoculation
    - T2B double inoculations
  - T3: Continuous mist system



Inoculation and Plant Maintenance

Treatment 1

Pollination bags

Treatment 2A, 2B

Humidity chamber under mist

Treatment 3

Under mist W/O Humidity chamber

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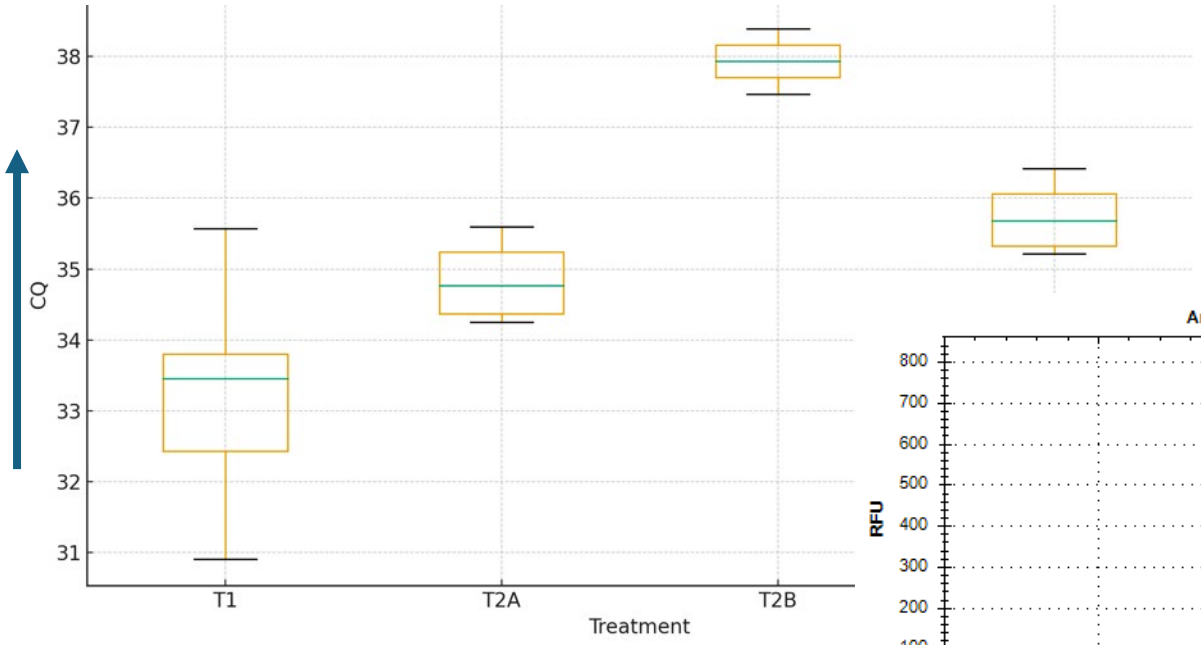
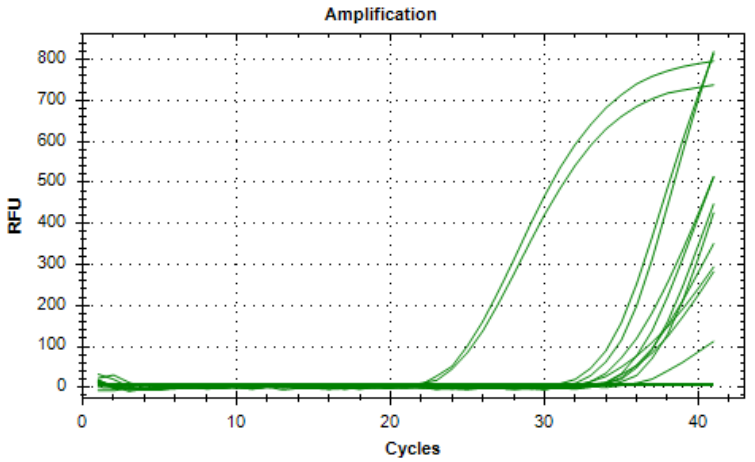
Sampling & DNA Workflow

Month 3: Needle sampling (4 needles each plant) → DNA extraction (OmegaBioTek M1130, CQLS) → qPCR detection of *N. gaeumannii*

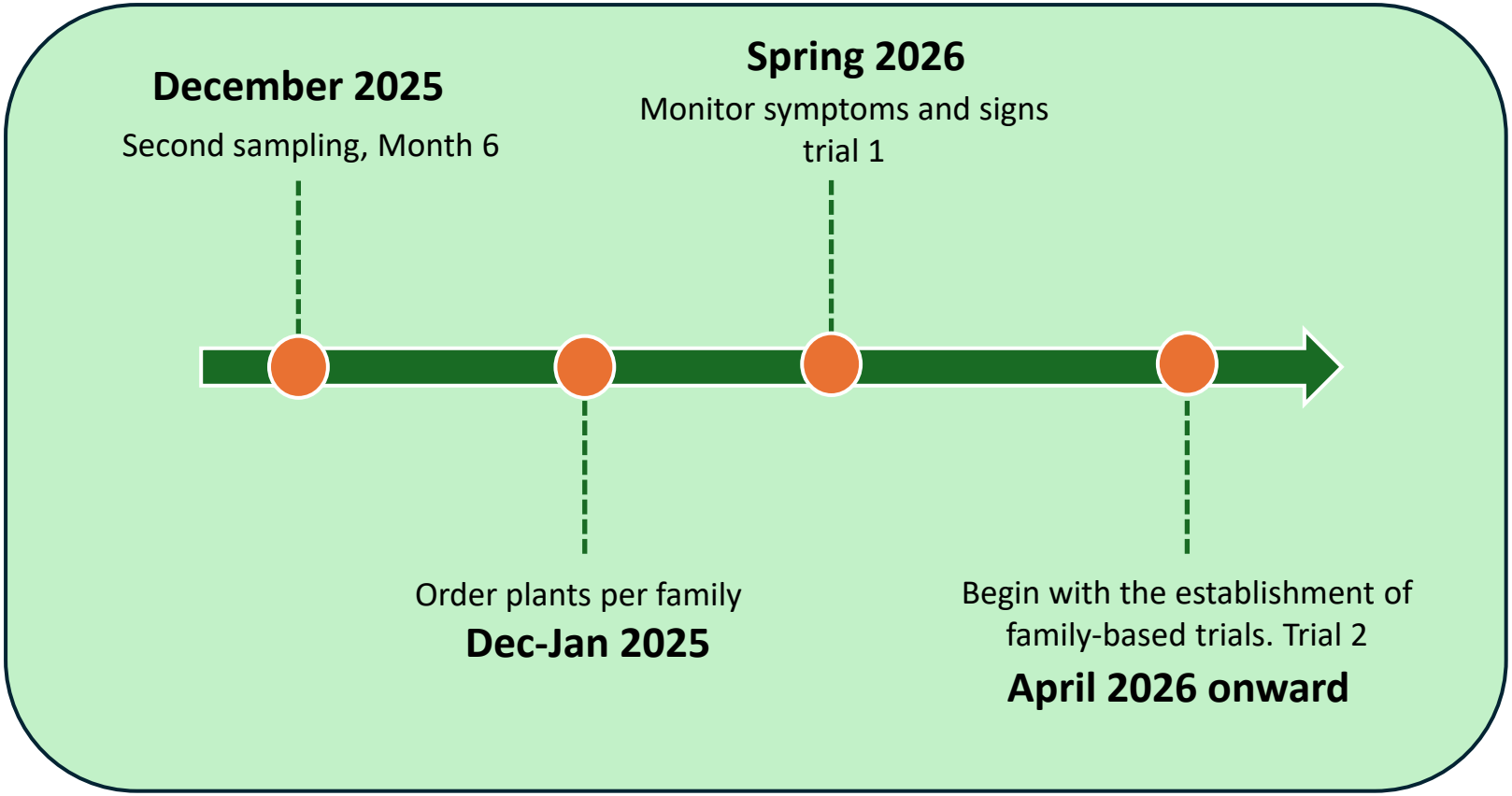
No SNC

Some SNC





Inoculation trial: Next steps



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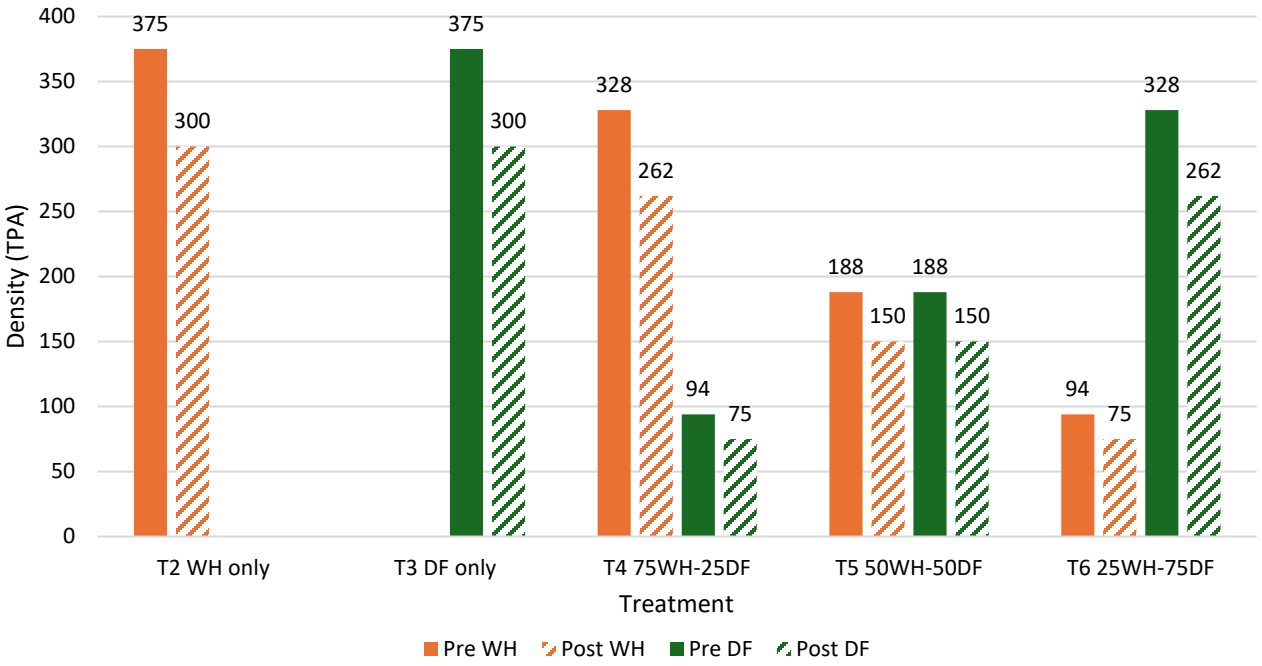
SNC PCT Plot Network

- **Research Question:** In high density mixed Douglas-fir/western hemlock stands where Swiss needle cast is present, **what is the best pre-commercial thinning prescription for maximizing volume production and financial benefit?**
- **Plot Characteristics**
  - Size: 0.1 ac + 20 ft buffer
  - Stand age: 8-13 years old
  - Density: 500+ TPA
  - Minimum total: 9 plantations (3 x SNC severity each)
- **SNC Severity Levels**
  - **Low** Foliage retention: ≥2.75 years
  - **Moderate** Foliage retention: 2-2.5 years
  - **High** Foliage retention: ≤1.75 year
- **Measurements**
  - Years: 0, 3, and 6 post-thinning
  - Variables: dbh, height, height to crown base
  - SNC: Foliage retention on 5 largest Douglas-fir.

Treatment	TPA Range	WH TPA	DF TPA
1. No thinning (control)	500+	-	-
2. Best tree to 300–350 TPA (WH only)	300–350	300-350	0
3. Best tree to 300–350 TPA (DF only)	300–350	0	300-350
4. Best tree to 300–350 TPA (75% WH / 25% DF)	300–350	262-265	75-88
5. Best tree to 300–350 TPA (50% WH / 50% DF)	300–350	150-175	150-175
6. Best tree to 300–350 TPA (25% WH / 75% DF)	300–350	75-88	262-265

SNC PCT Plot Network

- Pre-thinning = 25% more trees than post-thinning targets for each species. All thinning treatments must retain ≥450 TPA (WH + DF combined).
- We already have the Information of stands from all companies and we are organizing and scouting stands.



SNC PCT Plot Network

- To organize the data and establish the criteria, we created an Excel spreadsheet.
- By simply entering the density information for each species, we can determine what treatment can be established in each stand.

WH_TPA	DF_TPA	TOTAL_TPA	PCT_eligibility	T1_Control (TOTAL≥500)	T2_WH_only (WH≥375, TOTAL≥450)	T3_DF_only (DF≥375, TOTAL≥450)	T4_75WH/25DF (WH≥328, DF≥94, TOTAL≥450)	T5_50/50. (WH≥188, DF≥188, TOTAL≥450)	T6_25WH/75DF (WH≥94, DF≥328, TOTAL≥450)	Possible_Treatments
357	291	648	Eligible (≥500)	Yes	No	No	Yes	Yes	No	1, 4, 5
284	325	609	Eligible (≥500)	Yes	No	No	No	Yes	No	1, 5
186	505	691	Eligible (≥500)	Yes	No	Yes	No	No	Yes	1, 3, 6
500	379	879	Eligible (≥500)	Yes	Yes	Yes	Yes	Yes	Yes	1, 2, 3, 4, 5, 6
525	161	686	Eligible (≥500)	Yes	Yes	No	Yes	No	No	1, 2, 4
413	323	736	Eligible (≥500)	Yes	Yes	No	Yes	Yes	No	1, 2, 4, 5
660	105	765	Eligible (≥500)	Yes	Yes	No	Yes	No	No	1, 2, 4
659	261	920	Eligible (≥500)	Yes	Yes	No	Yes	Yes	No	1, 2, 4, 5
465	210	675	Eligible (≥500)	Yes	Yes	No	Yes	Yes	No	1, 2, 4, 5
315	336	651	Eligible (≥500)	Yes	No	No	No	Yes	Yes	1, 5, 6
236	408	644	Eligible (≥500)	Yes	No	Yes	No	Yes	Yes	1, 3, 5, 6
60	202	600	Eligible (≥500)	Yes	No	No	No	No	No	1
94	171	600	Eligible (≥500)	Yes	No	No	No	No	No	1
100	216	600	Eligible (≥500)	Yes	No	No	No	No	No	1
50	200	1000	Eligible (≥500)	Yes	No	No	No	No	No	1
100	200	1450	Eligible (≥500)	Yes	No	No	No	No	No	1
100	250	1150	Eligible (≥500)	Yes	No	No	No	No	No	1
100	250	700	Eligible (≥500)	Yes	No	No	No	No	No	1
100	250	850	Eligible (≥500)	Yes	No	No	No	No	No	1
71	384	454	Borderline (450–499)	No	No	Yes	No	No	No	3
42	350	392	<450	No	No	No	No	No	No	No treatment applies
388	46	434	<450	No	No	No	No	No	No	No treatment applies
40	354	394	<450	No	No	No	No	No	No	No treatment applies
92	335	428	<450	No	No	No	No	No	No	No treatment applies
145	298	442	<450	No	No	No	No	No	No	No treatment applies
117	349	465	Borderline (450–499)	No	No	No	No	No	Yes	6
425	41	466	Borderline (450–499)	No	Yes	No	No	No	No	2
118	269	387	<450	No	No	No	No	No	No	No treatment applies
74	349	424	<450	No	No	No	No	No	No	No treatment applies
185	302	487	Borderline (450–499)	No	No	No	No	No	No	No treatment applies
98	298	397	<450	No	No	No	No	No	No	No treatment applies
76	352	428	<450	No	No	No	No	No	No	No treatment applies
115	382	497	Borderline (450–499)	No	No	Yes	No	No	Yes	3, 6

Treatment	Number of Stands
T1 – Control (no thinning)	93
T2 – WH only	41
T3 – DF only	78
T4 – 75% WH / 25% DF	23
T5 – 50% / 50% DF	14
T6 – 25% WH / 75% DF	28

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SNC PCT Plot Network

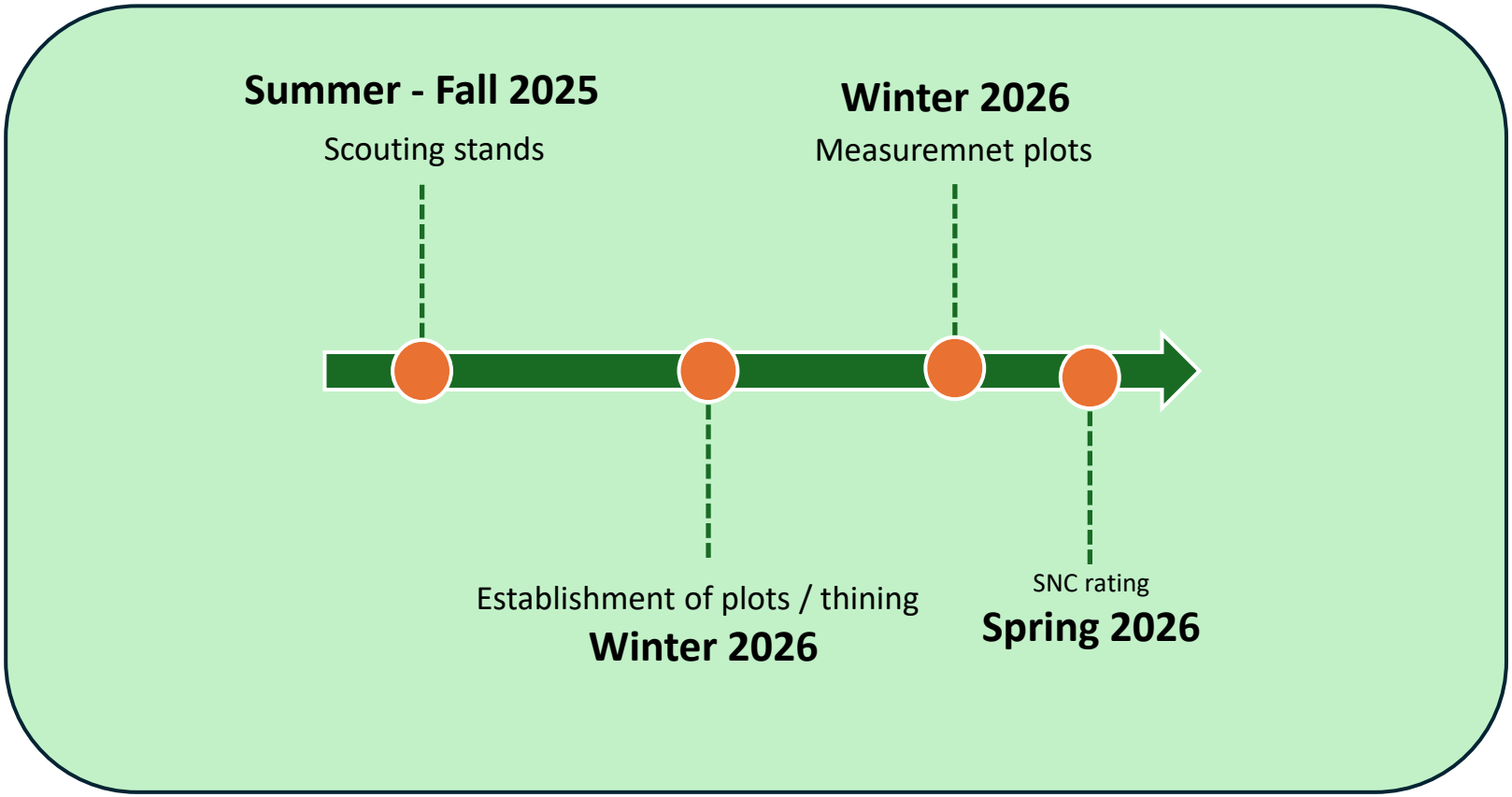
stands visited during June 2025

SNC PCT Network

Stands visited during October 2025

- Already visited 12 stands in two areas

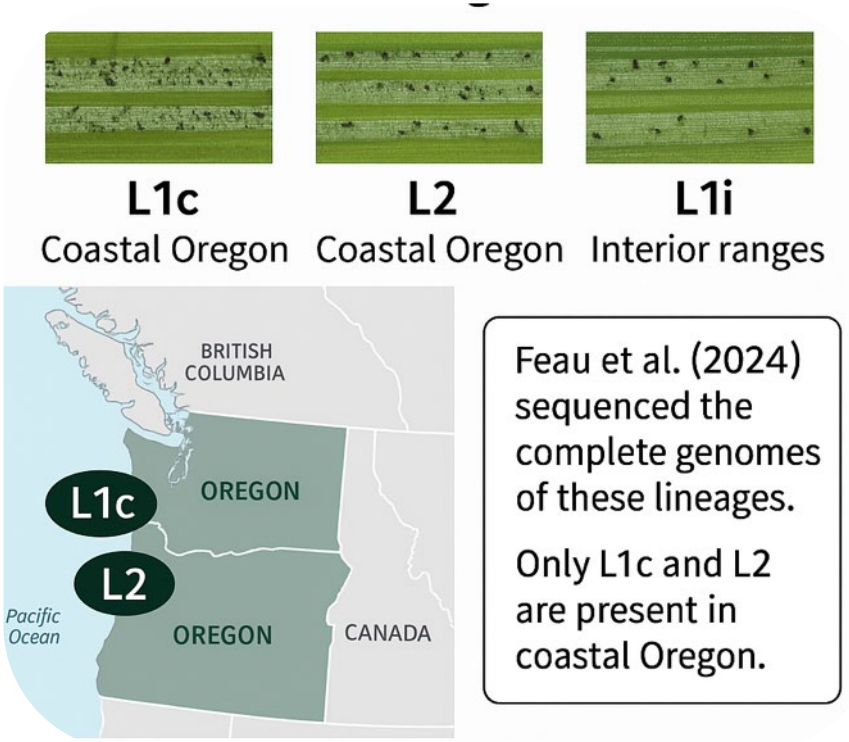
SNC PCT Network: Next steps



Population genomics

Last but not least

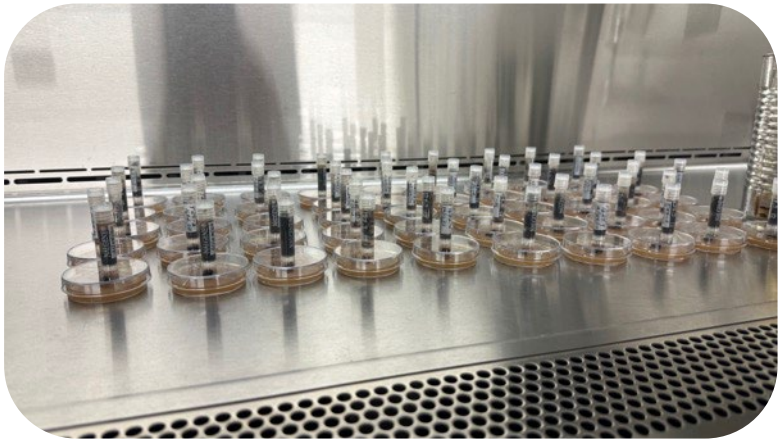
- **Previous work has identified two coastal lineages of *N. gaeumannii* (L1c and L2).**
  Our **expanded genomic sampling will clarify lineage boundaries, detect fine-scale substructure, and provide greater resolution of pathogen diversity across Oregon** — information that can improve disease risk assessment, guide management decisions, and support SNC tolerance screening.



Population genomics

- Reactivation and recovery of Patrick Bennett's isolate collection is underway.
- Approximately 800 isolates have been successfully subcultured and are actively growing.
- We are now ready to begin large-scale DNA extractions.

N	Box	Box Position	Isolate	Sub. Agar Media	Sub. Liquid Media	OBS
1	3	1	DNRS 25-1 1766.2	Yes	12/6/25	-
2	3	2	T15-1 846.6 LOW	Yes	12/6/25	-
3	3	3	T15-1 908.7 MID	Yes	12/6/25	-
4	3	4	T15-1 931.6	Yes	12/6/25	-
5	3	5	T5-1 908.1 LOW	Yes	12/6/25	-
6	3	6	DNRS 25-1 1766.3	Yes	12/6/25	-
7	3	7	T15-1 867.2	Yes	12/6/25	-
8	3	8	T5-1 908.1 MID	Yes	12/6/25	-
9	3	9	DNRS 25-1 1766.8	Yes	12/6/25	-
10	3	10	T5-1 908.3 MID	Yes	12/6/25	-
11	3	11	T5-1 844.5	Yes	12/6/25	-
12	3	12	T5-1 808.5 LOW	Yes	12/6/25	-
13	3	13	T5-1 931.1	Yes	12/6/25	-
14	3	14	T5-1 914.7	Yes	12/6/25	-
15	3	15	T5-1 908.6 MID	Yes	12/6/25	-
16	3	16	T5-1 931.2	Yes	12/6/25	-
17	3	17	T15-1 846.1 TOP	Yes	12/6/25	-
18	3	18	T5-1 913.6	Yes	12/6/25	-
19	3	19	T5-1 928.2	Yes	12/6/25	-
20	3	20	T25-2 1644.01 Low	Yes	06-16-25	-
21	3	21	T25-2 1644.08 Mid	Yes	06-16-25	-
22	3	22	T25-2 1644.08 Mid	Yes	06-16-25	-
23	3	23	T25-2 1646.01	Yes	06-16-25	-
24	3	24	T25-3 1672.01	Yes	06-16-25	-
25	3	25	T25-3 1693.07 Low	Yes	06-16-25	-
26	3	26	T25-3 1693.05 Low	Yes	06-16-25	-
27	3	27	T25-3 1691.06	Yes	06-16-25	-
28	3	28	T25-1 903.2 Low	Yes	06-16-25	-
29	3	29	T25-3 1693.08 Mid	Yes	06-16-25	-
30	3	30	T25-3 1693.07 Mid	Yes	06-16-25	-
31	3	31	T25-3 1693.04 Mid	Yes	06-16-25	-
32	3	32	T25-3 1693.01 Mid	Yes	06-16-25	-



Understanding fungal lineage diversity helps predict disease pressure, improve stand management, and support decisions for Douglas-fir breeding programs