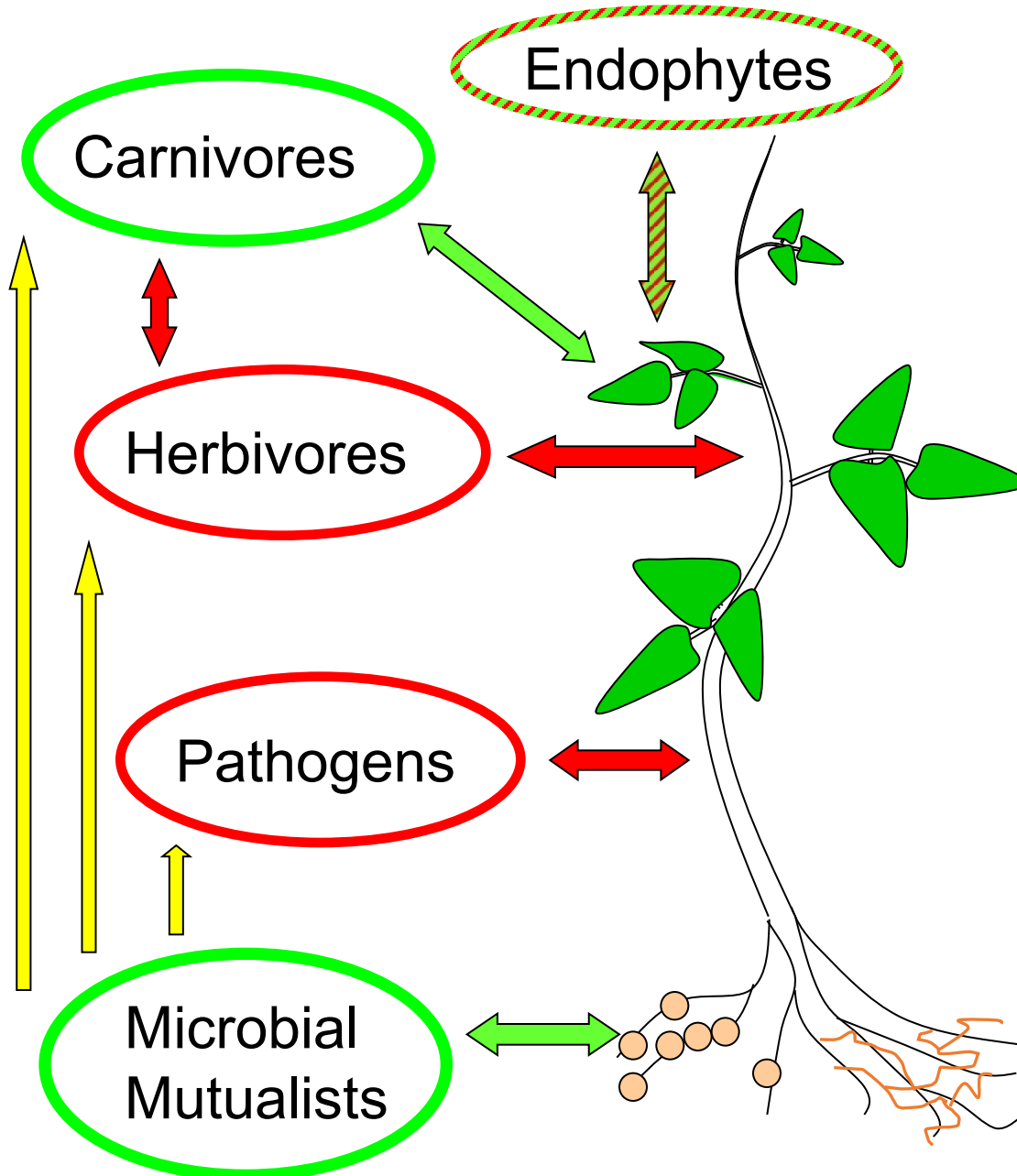


The Red Alder Nodule Microbiome ...How Little Do We Know

Daniel J. Ballhorn





Chemical defenses

1. Direct defenses

- Toxins/Inhibitors
- Bad tasting compounds



2. Indirect defenses

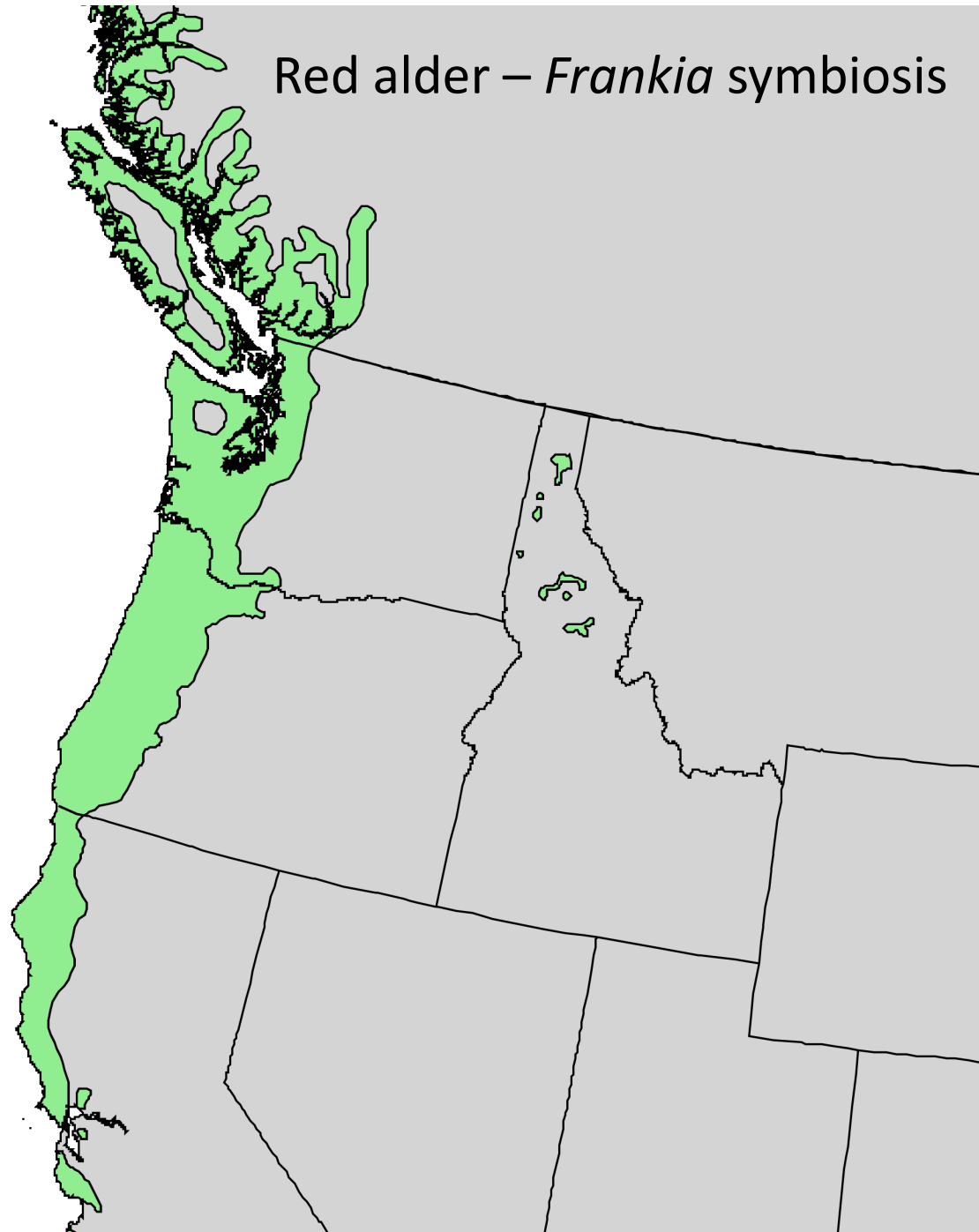
- Volatile organic compounds (VOCs)
- Extrafloral nectar (EFN)



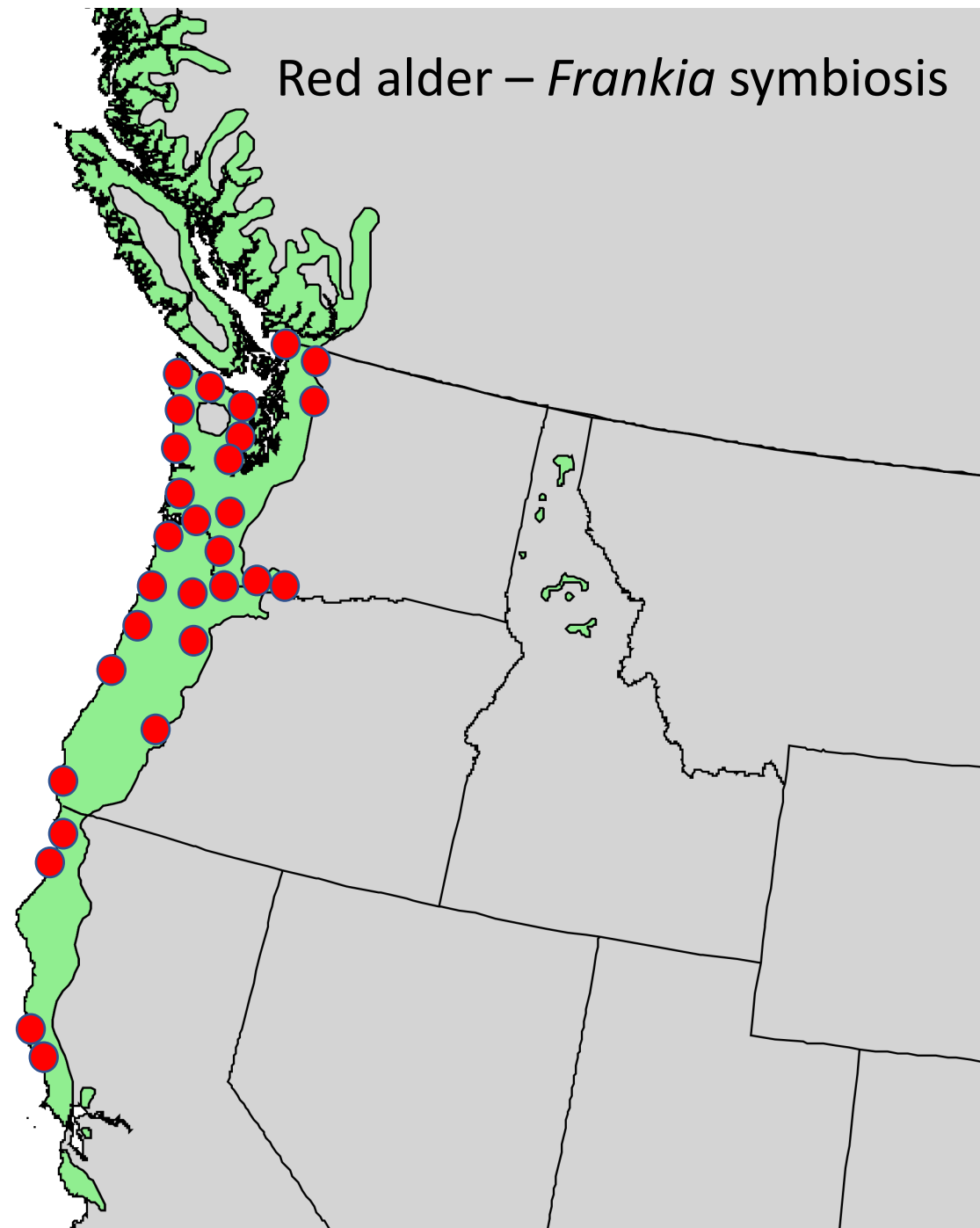
Plant nutritive compounds

- Proteins
- Amino acid composition
- Digestible carbohydrates

Red alder – *Frankia* symbiosis



Red alder – *Frankia* symbiosis

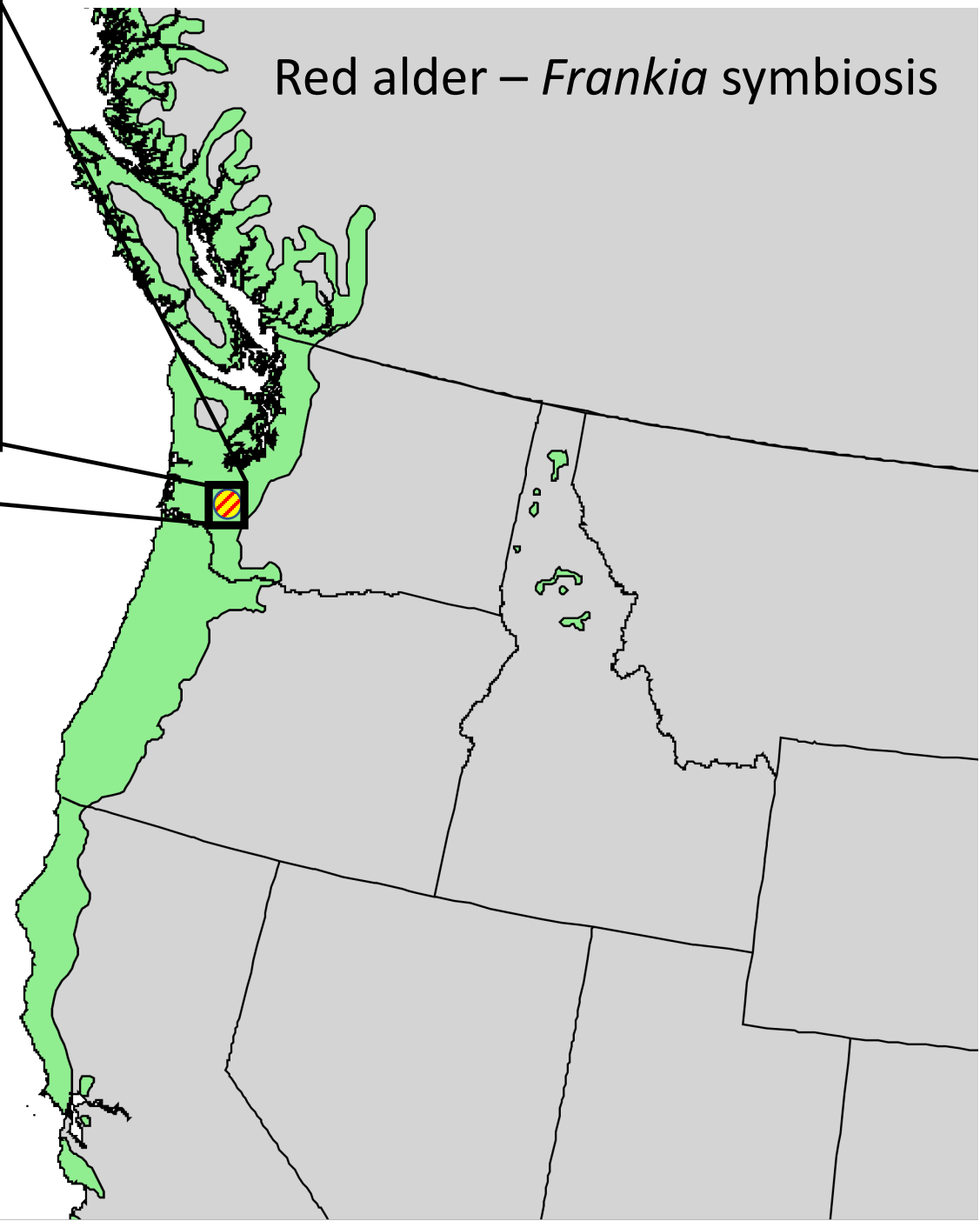


Frankia Strain(s)

- B1 (mature trees)



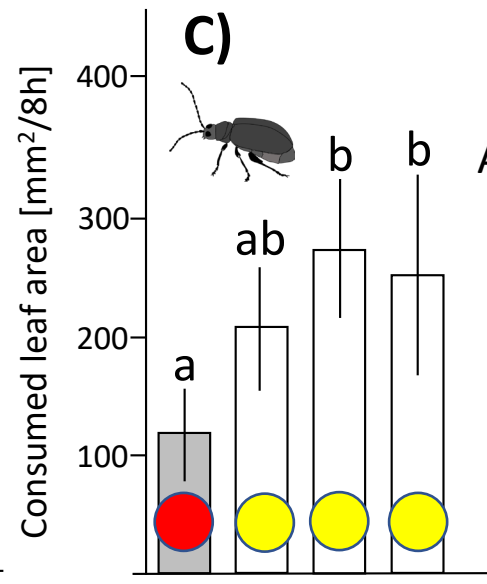
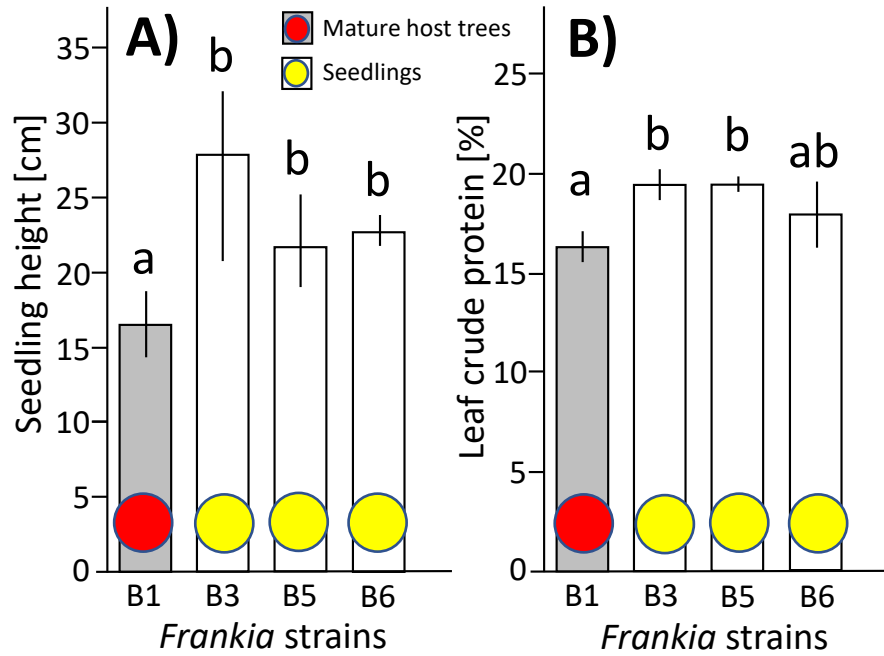
Red alder – *Frankia* symbiosis



Frankia Strain(s)

- B1 (mature trees)
- B1 – B18... (seedlings)





Alder flea beetle (*Altica ambiens*)



Red or chocolate arion (*Arion rufus*)

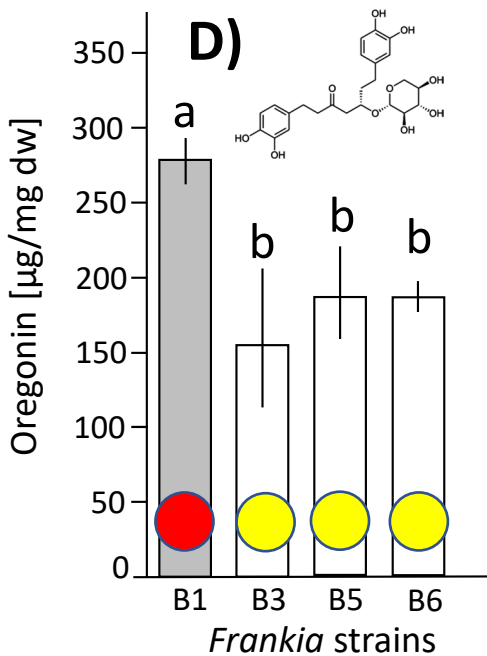
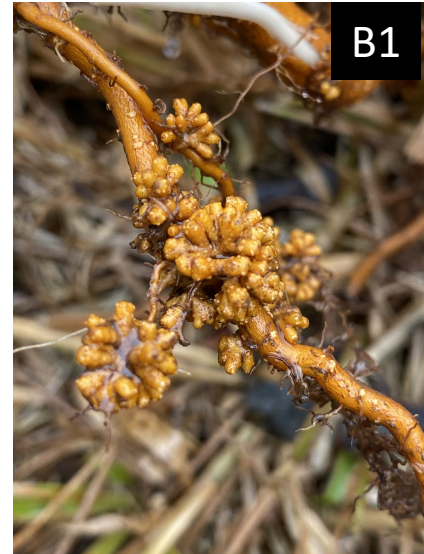


Figure 2. Frankia-mediated effects on plant growth, leaf chemistry and herbivory. Red alder seedlings were inoculated with the dominant *Frankia* strain found in mature red alders (B1) as well as a panel of diverse and less host-specific strains found in red and Sitka alder seedlings (B3-5). Seedlings (grown from seeds collected from one mother plant) inoculated with the B1 strain showed **significantly reduced height growth, leaf protein content, and herbivory** by alder leaf beetles (*A. ambiens*) than B3,B5-6 inoculated seedlings, while leaf **oregonin concentration was significantly increased** [according to post-hoc Test (Tukey's $P < 0.05$) after one-way ANOVA; values are means \pm SD, N=10 seedlings per treatment].



Oregonin
Diarylheptanoid Xyloside
 ((5S)-1,7-bis(3,4-dihydroxyphenyl)-5-(β-D-xylopyranosyloxy)-heptan-3-one)

Take-home Messages

Findings suggest that different *Frankia* strains have variable effects on their host's leaf chemistry, susceptibility to herbivores, and growth.

- 1) How do different *Frankia* strains affect chemical leaf traits and aboveground herbivory?
- 2) What are the drivers resulting in the “disappearance” of seemingly high-performing *Frankia* strains in the course of host development?
- 3) What are the ecological costs and benefits of different *Frankia* strains in nature and do plant-herbivore interactions influence the diversity of *Frankia*-alder associations?


<https://doi.org/10.1002/mbo3.1424>



Cover caption: The cover image is based on the Research Article *Root nodules of red alder (Alnus rubra) and Sitka alder (Alnus viridis ssp. sinuata) are inhabited by taxonomically diverse cultivable microbial endophytes* by Robyn Dove et al., <https://doi.org/10.1002/mbo3.1422>. Image credit to Felisha Pooler.

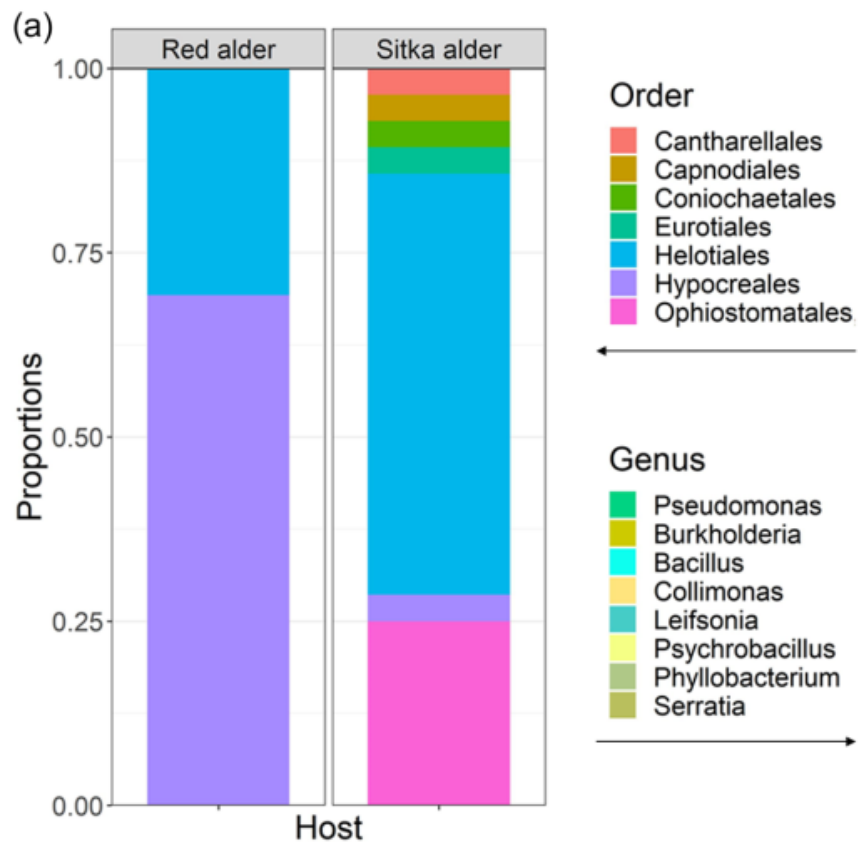
ORIGINAL ARTICLE |  Open Access |  

Root nodules of red alder (*Alnus rubra*) and Sitka alder (*Alnus viridis ssp. sinuata*) are inhabited by taxonomically diverse cultivable microbial endophytes

Robyn Dove , Emily R. Wolfe, Nathan U. Stewart, Abigail Coleman, Sara Herrejon Chavez, Daniel J. Ballhorn

<https://doi.org/10.1002/mbo3.1422>

Fungal endophytes



Bacterial endophytes

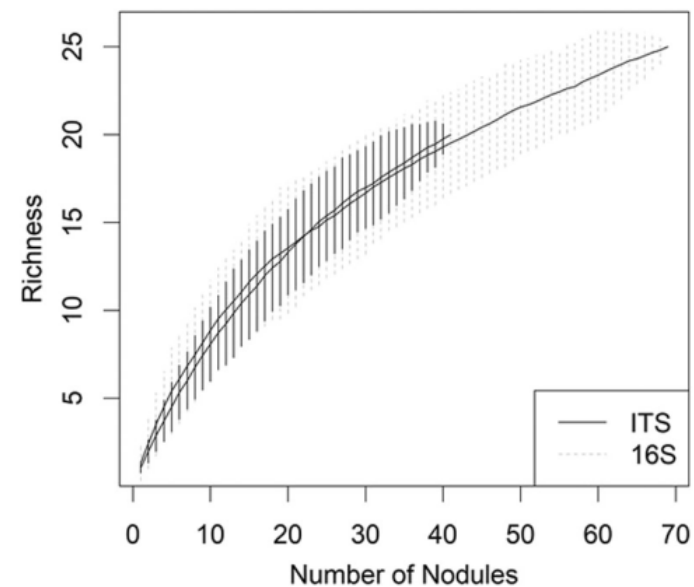
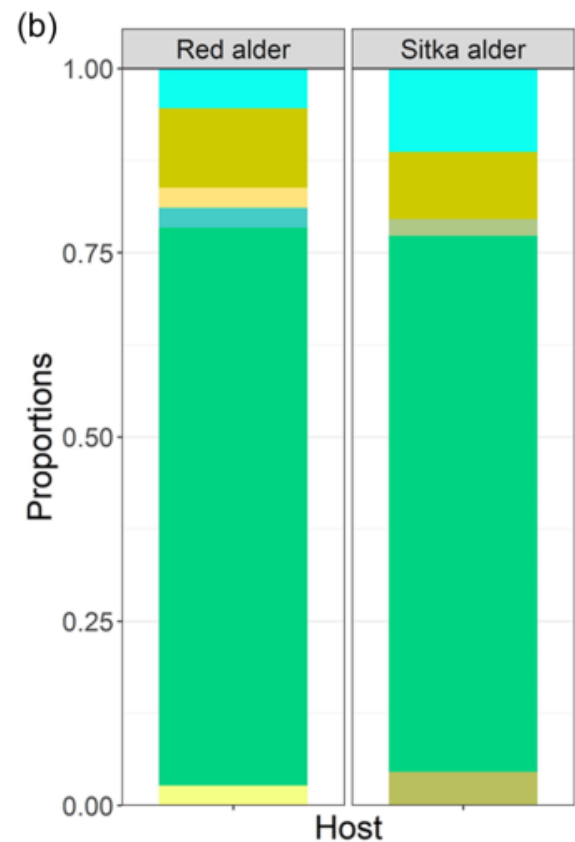


FIGURE 3 Bacterial (16 S) and fungal (ITS) species richness as a function of number of nodules sampled. Nodules ($n = 70$) were collected from sympatric populations of two alder host species (*Alnus rubra* and *A. viridis*) in a successional environment at Mount St. Helens (Lawetlat'la). Shaded areas represent 95% confidence intervals.

FIGURE 2 Abundance plots of fungal (a) and bacterial (b) endophytes isolated from root nodules collected in the field, Mount St. Helens (Lawetlat'la), WA. For (A) $n = 13$ red alder root nodules and $n = 28$ Sitka alder root nodules, for (B) $n = 31$ red alder nodules and $n = 37$ Sitka alder root nodules.

Fungal endophytes

Frankia (B1)
+ BE444

Frankia (B1) - only

TABLE 1 Fungal operational taxonomic units and their hosts (R = red alder, S = Sitka alder).

| OTU | Phylum | Order | Family | Genus | Species | Host species |
|-----|------------|-----------------|------------------|---------------------|----------------------|--------------|
| 1 | Ascomycota | Ophiostomatales | Ophiostomataceae | <i>Sporothrix</i> | <i>guttuliformis</i> | S |
| 2 | Ascomycota | Helotiales | Vibrissaceae | | | S |
| 3 | Ascomycota | Hypocreales | Nectriaceae | <i>Ilyonectria</i> | <i>destructans</i> | R |
| 4 | Ascomycota | Helotiales | Incertae sedis | | | R, S |
| 5 | Ascomycota | Hypocreales | Nectriaceae | <i>Gibberella</i> | sp. | R |
| 6 | Ascomycota | Helotiales | Nectriaceae | Unclassified | | R |
| 7 | Ascomycota | Helotiales | Helotiaceae | <i>Fontanospora</i> | sp. | R, S |
| 8 | Ascomycota | Helotiales | Unclassified | | | R, S |

CZECH MYCOL. 50 (1), 1997

Fontanospora fusiramosa sp. nov., a hyphomycete from live tree roots and from stream foam

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²Department of Biological Sciences, University of Exeter, Exeter EX4 4PS, U. K.

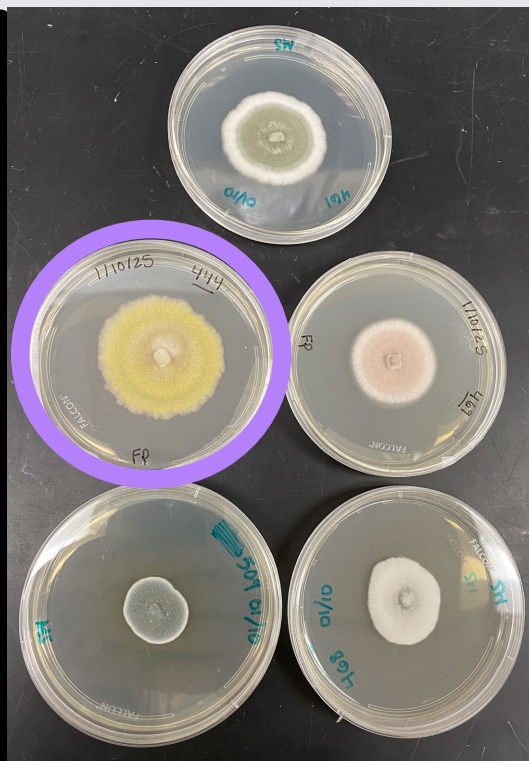
³Instituto Mediterráneo de Estudios Avanzados de las Islas Baleares (CSIC/UIB), Univ. Baleares, 07071 Palma de Mallorca, Spain

⁴Department of Biology, Mount Allison University, Sackville, N. B., EOA 3C0, Canada

Marvanová L., Fisher P. J., Descals E. and Bärlocher F. (1997): *Fontanospora fusiramosa* sp. nov., a hyphomycete from live tree roots and from foam.— Czech Mycol. 50: 3–11

Fontanospora fusiramosa is described from *Alnus* roots and from stream foam. It is based on isolates from the U. K., Canada and the Czech Republic.

Key words: *Fontanospora*, aquatic hyphomycetes, endophytes, streams.



Fungal endophytes

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| 4 | Ascomycota | Helotiales | Incertae sedis | | | R, S |
| 5 | Ascomycota | Hypocreales | Nectriaceae | <i>Gibberella</i> | sp. | R |
| 6 | Ascomycota | Helotiales | Nectriaceae | Unclassified | | R |
| 7 | Ascomycota | Helotiales | Helotiaceae | <i>Fontanospora</i> | sp. | R, S |
| 8 | Ascomycota | Helotiales | Unclassified | | | R, S |

Bacterial endophytes

TABLE 2 Bacterial operational taxonomic units and their hosts (R = red alder, S = Sitka alder).

| OTU | Phylum | Genus | Species | Host species |
|-----|----------------|------------------------|---------|--------------|
| 1 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 2 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 3 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 4 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 5 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 6 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 7 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 8 | Firmicutes | <i>Bacillus</i> | sp. | R, S |
| 9 | Proteobacteria | <i>Burkholderia</i> | sp. | R, S |
| 10 | Proteobacteria | <i>Burkholderia</i> | sp. | R, S |
| 11 | Firmicutes | <i>Bacillus</i> | sp. | S |
| 12 | Proteobacteria | <i>Pseudomonas</i> | sp. | S |
| 13 | Proteobacteria | <i>Collimonas</i> | sp. | R |
| 14 | Proteobacteria | <i>Pseudomonas</i> | sp. | R, S |
| 15 | Proteobacteria | <i>Pseudomonas</i> | sp. | R |
| 16 | Proteobacteria | <i>Pseudomonas</i> | sp. | R |
| 17 | Actinobacteria | <i>Leifsonia</i> | sp. | R |
| 18 | Proteobacteria | <i>Pseudomonas</i> | sp. | S |
| 19 | Firmicutes | <i>Psychrobacillus</i> | sp. | R |
| 20 | Proteobacteria | <i>Pseudomonas</i> | sp. | R |
| 21 | Firmicutes | <i>Bacillus</i> | sp. | S |
| 22 | Proteobacteria | <i>Phyllobacterium</i> | sp. | S |
| 23 | Proteobacteria | <i>Serratia</i> | sp. | S |
| 24 | Proteobacteria | <i>Burkholderia</i> | sp. | R |
| 25 | Proteobacteria | <i>Serratia</i> | sp. | S |

CZECH MYCOL. 50 (1), 1997

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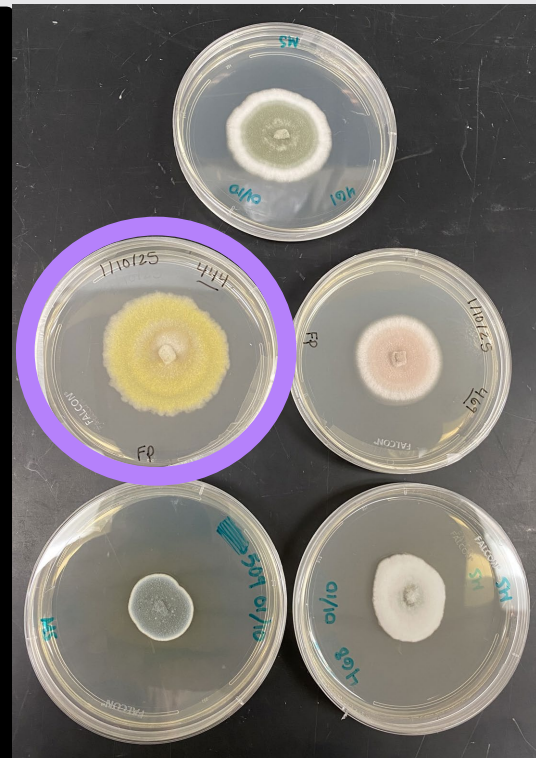
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Frankia (B1) + Pseudomonas (BP16)

Frankia (B1) - only



Bacterial endophytes

TABLE 2 Bacterial operational taxonomic units and their hosts (R = red alder, S = Sitka alder).

| OTU | Phylum | Genus | Species | Host species |
|-----|----------------|-----------------|---------|--------------|
| 1 | Proteobacteria | Pseudomonas | sp. | R, S |
| 2 | Proteobacteria | Pseudomonas | sp. | R, S |
| 3 | Proteobacteria | Pseudomonas | sp. | R, S |
| 4 | Proteobacteria | Pseudomonas | sp. | R, S |
| 5 | Proteobacteria | Pseudomonas | sp. | R, S |
| 6 | Proteobacteria | Pseudomonas | sp. | R, S |
| 7 | Proteobacteria | Pseudomonas | sp. | R, S |
| 8 | Firmicutes | Bacillus | sp. | R, S |
| 9 | Proteobacteria | Burkholderia | sp. | R, S |
| 10 | Proteobacteria | Burkholderia | sp. | R, S |
| 11 | Firmicutes | Bacillus | sp. | S |
| 12 | Proteobacteria | Pseudomonas | sp. | S |
| 13 | Proteobacteria | Collimonas | sp. | R |
| 14 | Proteobacteria | Pseudomonas | sp. | R, S |
| 15 | Proteobacteria | Pseudomonas | sp. | R |
| 16 | Proteobacteria | Pseudomonas | sp. | R |
| 17 | Actinobacteria | Leifsonia | sp. | R |
| 18 | Proteobacteria | Pseudomonas | sp. | S |
| 19 | Firmicutes | Psychrobacillus | sp. | R |
| 20 | Proteobacteria | Pseudomonas | sp. | R |
| 21 | Firmicutes | Bacillus | sp. | S |
| 22 | Proteobacteria | Phyllobacterium | sp. | S |
| 23 | Proteobacteria | Serratia | sp. | S |
| 24 | Proteobacteria | Burkholderia | sp. | R |
| 25 | Proteobacteria | Serratia | sp. | S |

Take-home Messages II

Root nodules of red and Sitka alder harbor diverse endophyte communities of non-*Frankia* symbionts including both bacteria and fungi

1) Preliminary experiments with fungal endophyte BE444 suggest stress-tolerance enhancing effects when added to *Frankia*-only nodulated trees.

2) *Pseudomonas* and *Burkholderia* species are particularly abundant in root nodules; some strains show growth promoting effects in alder seedlings when co-inoculated with *Frankia* (B1 strain).

3) Studying tree genotype x microbiome effects on host tree performance and adaptation will be critical moving forward.



Thank You!

