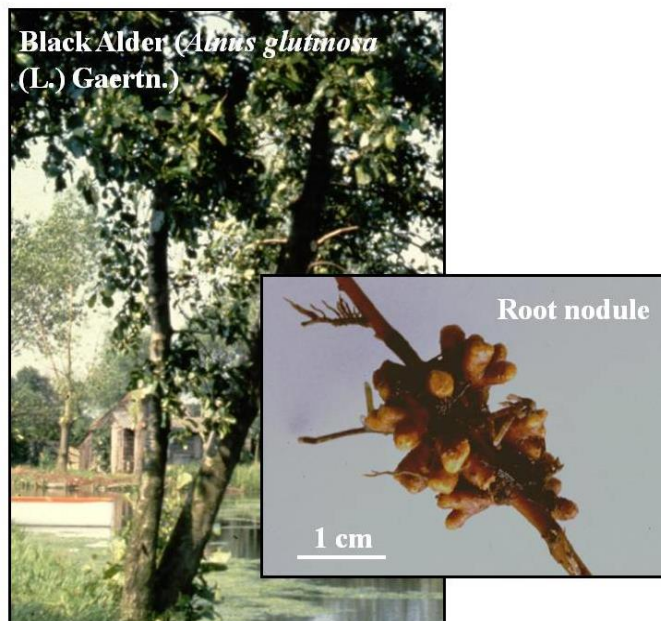


Frankia-actinorhizal plant symbiosis

Actinorhizal plants form root nodules in symbiosis with the nitrogen-fixing actinomycete *Frankia*, which enables them to grow on sites with restricted nitrogen availability. They are therefore successful pioneer plants that are increasingly recognized in forestry and agroforestry for reforestation and reclamation of poor soils, but also for commercial use as nurse trees in mixed plantations with valuable tree species, for the production of fuelwood, and as a source of timber themselves. The establishment and efficiency of the symbiosis between *Frankia* and actinorhizal plants such as *Alnus*, *Elaeagnus*, or *Casuarina* species is affected by environmental factors such as the soil pH, the soil matric potential, and the availability of elements such as nitrogen or phosphorus, but ultimately constrained by the genotypes of both partners of this symbiosis.

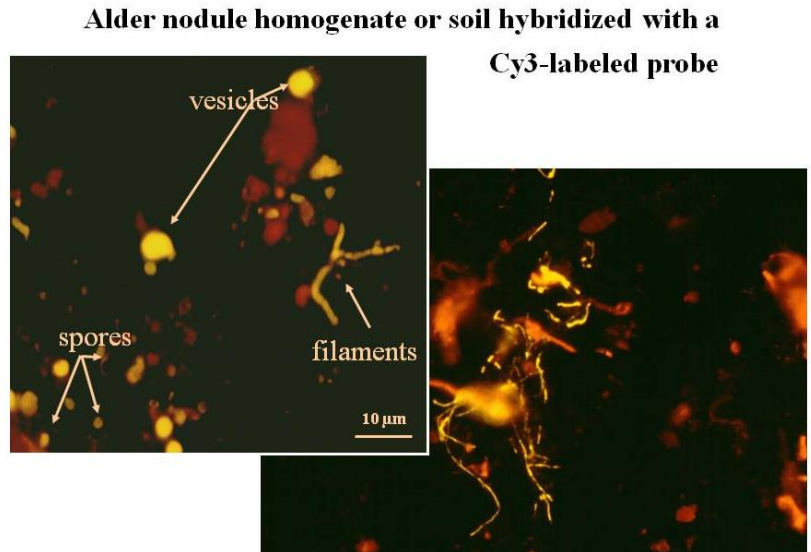


Effects of environmental conditions, plant species, and isolates of *Frankia* on the establishment of the symbiosis are relatively easy to assess under laboratory conditions and thus a considerable amount of information is available on isolates of *Frankia* and on their interaction with host plant species. Quantitative analyses of specific *Frankia* populations originating from soil and their interaction with plants and site conditions, however, are methodologically extremely challenging due to problems encountered with isolation and identification of populations, and consequently information on the occurrence and diversity of *Frankia* populations in soil is

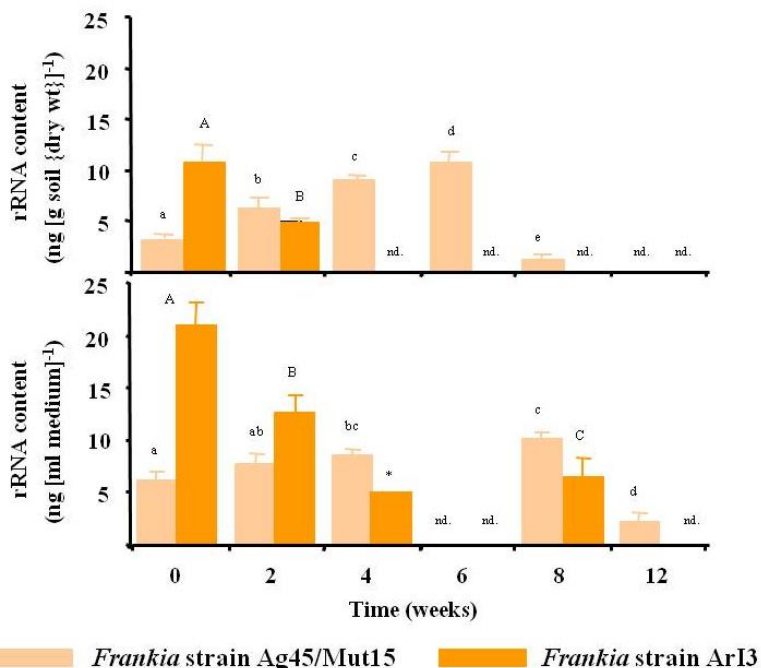
scarce.

Questions on the ecology of nitrogen-fixing members of the genus *Frankia* have been addressed for many years, starting with my M.S. thesis research in 1984/85 and nearly continuously studied until today. Initial studies on frankiae focused on the use of standard techniques in microbiology (including isolation), plant physiology and soil sciences applied in microcosm- and greenhouse studies. These studies were expanded to the application of molecular biological techniques in order to solve problems concerned with the isolation and identification of this recalcitrant microorganism. Identification attempts without isolation of the microorganism have been made after rRNA sequencing by oligonucleotide probes against isolated and immobilized rRNA, by PCR and by *in situ* hybridization targeting rRNA sequences in fixed bacteria. The latter studies included investigations on the applicability of different probes (e.g., oligonucleotides or *in vitro*

transcripts) for the *in situ* detection of *Frankia* cells in nodule homogenates and in soil. These studies enabled to address questions on the competition of *Frankia* populations for nodule formation at different water potentials, the fate of introduced strains in competition with indigenous populations, population shifts after environmental changes, etc.) without the drawbacks of isolation or the biases concerned with PCR detection.



Studies on *Frankia*-soil interactions initially focused on the diversity of frankiae in different soils. Diversity was analyzed in plant bioassays with subsequent identification of frankiae in nodules by *nifH* gene sequence analyses or by rep-PCR, or in *nifH* gene clone libraries from soil DNA extracts.



Bioassays using *Morella pensylvanica* as capture plants demonstrated large differences in nodule-forming frankiae in five soils from a broad geographic range, i.e. from sites in five continents (Africa, Europe, Asia, North America and South America), but a low diversity of nodule-forming *Frankia* populations within any of these soils. Meta-analysis displayed large differences in cluster assignments between sequences retrieved from nodules and from clone libraries generated from DNA from the respective soils, with assignments to the same

cluster only rarely encountered for individual soils. The potential of the host plant to select specific *Frankia* strains for root nodule formation was shown in bioassays with two *Morella*, three *Elaeagnus* and one *Shepherdia* species as capture plants inoculated with the same soil slurry. This study demonstrated that none of the plants captured the entire diversity of nodule-forming frankiae and that the distribution of *Frankia* populations and their abundance in nodules was

unique for each of the plant species. The diversity of *Frankia* populations in nodules retrieved by plant bioassays might therefore reflect preferences of the host plant rather than describe the diversity of frankiae in the soil analyzed.

Additional studies focused on the ability of *Frankia* strains to grow in the rhizosphere of a non actinorhizal plant, *Betula pendula*, in surrounding bulk soil and in soil amended with leaf litter. Growth responses were related to taxonomic position as determined by comparative sequence analysis of *nifH* gene fragments and of an actinomycetes-specific insertion in Domain III of the 23S rRNA gene. Phylogenetic analyses confirmed the basic classification of *Frankia* strains by host infection groups, and allowed a further differentiation of *Frankia* clusters within the *Alnus* host infection group. Except for *Casuarina*-infective *Frankia* strains, all other strains of the *Alnus* and the *Elaeagnus* host infection groups displayed growth in the rhizosphere of *B. pendula*, and none of them grew in the surrounding bulk soil that was characterized by very low organic matter content. Only a small number of strains that all belonged to a distinct phylogenetic cluster within the *Alnus* host infection group, grew in soil amended with ground leaf litter from *B. pendula*. These results demonstrate that saprotrophic growth of frankiae is a common trait for most members of the genus, and the supporting factors for growth (i.e. carbon utilization capabilities) varied with host infection group and phylogenetic affiliation of the strains.

Current projects include the development of *qPCR* assays for the quantification of natural and introduced frankiae in soils. A SYBR Green-based quantitative PCR (*qPCR*) that used either *nifH* or 23S rRNA gene sequences as target in DNA extracts from soil samples allowed us to quantify nitrogen-fixing members of the genus *Frankia* directly in soil samples. Quantification results in different mineral soils from temperate regions using both targets were comparable, with cell density estimates for frankiae of up to more than 10^6 cells [g soil {dry wt.}]⁻¹ depending on the soil. In contrast to the *nifH* gene, the 23S rRNA gene also provided target sequences that allowed us to distinguish between the *Alnus* and *Elaeagnus* host infection groups, and subgroups within the *Alnus* host infection group, including the *Casuarina*-infective strains.

Selected publications

1. **Vemulapally, S., Guerra, T.M., Hahn, D.** 2019. Localization of typical and atypical *Frankia* isolates from *Casuarina* sp. in nodules formed on *Casuarina equisetifolia*. *Plant and Soil* **435**, 385-393.
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