

# Interactions between Root Symbionts, Root Pathogens and Actinorhizal Plants

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## ABSTRACT

Actinorhizal trees with nitrogen-fixing actinomycetes (*Frankia* sp.) as microsymbionts in root nodules play an important ecological role as pioneer plants on nitrogen-poor soils. Up to 200 perennial species, all trees and shrubs, distributed over about 20 genera, have been found to be nodulated with *Frankia* as the nodule symbiont. Some of those, e.g., *Alnus* spp. in temperate regions and *Casuarina* spp. in subtropical and tropical regions, have great potentials for biomass production and erosion control. The growth of such plants is largely dependent upon the presence of proper *Frankia* strains in the soil.

**Keywords:** Root Symbionts, Root Pathogens, Actinorhizal Plants

## I. INTRODUCTION

*Frankia* has been found in many types of soil, particularly in soils where its host plants have been grown previously (Fraga-Beddiar, 1987; Houwers and Akkermans, 1981; Rodriguez-Barrueco, 1968), inoculation of the plants with selected *Frankia* strains can give a positive response with respect to plant yield. Pot and field experiments have indicated that the effect of inoculation of plants with *Frankia* is dependent upon the environmental conditions, including the interaction with other soil microorganisms (van Dijk, 1984; Houwers and Akkermans, 1981; Maas et al., 1983; Oremus, 1980, Oremus and Otten, 1981). These results indicate that plant growth is often limited by factors other than N<sub>2</sub> fixation.

Each soil ecosystem comprises a large number of different types of organisms with a complex network of interactions. Tree growth is therefore affected by interaction with many different types of organisms. In soil, the roots are in close contact with pathogenic fungi, nematodes and insects, but also with symbiotic organisms, such as mycorrhizal fungi, rhizobacteria

and nodule-forming *Frankia*. Although the importance of such interactions is generally recognized in forestry, little attention has been paid to their effect on nitrogen-fixing actinorhizal plants. In the present paper, we will give an overview of the interactions between root symbionts, pathogens and actinorhizal plants, with special attention to *Alnus* and *Hippophae* spp.

## II. ROOT SYMBIONTS

### 2.1 *Frankia*

Actinorhizal plants bear several types of symbionts on their roots. So far, most attention has been paid to microorganisms which induce nitrogen-fixing root nodules (i.e., actinorhizas). Although it has been known for about a century that the microsymbionts in nodules of, e.g., *Elaeagnus*, *Alnus* and *Casuarina* are different from *Rhizobium* in leguminous nodules, pure cultures of the microsymbionts have only been available since 1978 (Callaham et al., 1978). These microbes are classified within the genus *Frankia*. *Frankia* is characterized by its hyphal growth type, analogous to many other actinomycetes. It forms typical intercalary and terminal sporangia and vesicles

at the tips of short side branches. These 2 structures are unique to the genus *Frankia* and can be used as morphological markers in the identification of the microbes, excluding, however, ineffective and non-infective strains.

After local colonization of the roots of actinorhizal plants, *Frankia* strains invade the plants either through deformed root hairs, e.g., in *Alnus* spp. or through intercellular spaces, as has been demonstrated in *Elaeagnus* sp. (Miller and Baker, 1986). These observations indicate that at least 2 types of invasions exist in actinorhizal plants.

Pure cultured *Frankia* strains can be classified into 3 groups, based on host specificity, viz. *Alnus*-compatible, *Elaeagnus*-compatible and *Casuarina*-compatible strains (Baker, 1987). The degree of nitrogen-fixing activity in the nodules varies with the host plant and the *Frankia* strain. Strains which are effective (i.e., N<sub>2</sub>-fixing) on its original host, may be ineffective (non-N<sub>2</sub>-fixing) on other hosts within the same cross-inoculation group. In addition to this host-induced ineffectivity, *Frankia* strains which lack nitrogenase have been found in soil (van Dijk and Sluimer-Stolk, 1984) and pure cultures of these ineffective strains have been described (Hahn et al., 1988; Hahn et al., 1989).

After initial invasion of the cortical cells, *Frankia* readily develops into an endosymbiont with vesicles at the hyphal tips. The form of these vesicles is largely determined by the host plant, and varies from spherical (e.g., in root nodules of *Alnus* and *Hippophae*, spp.) to club- or pearshaped (e.g., *Myrica* and *Comptonia* spp.). So far, *Frankia* strains usually form spherical vesicles in pure culture and no alternative forms have been observed in vitro.

Host-controlled morphogenesis has also been observed in the spore-formation of *Frankia*. So far, isolated *Frankia* strains are usually able to produce

sporangia, depending upon the medium. In the nodules, however, strains fail to form sporangia. Field studies by van Dijk have indicated the presence of 3 types of nodules in *Alnus glutinosa*, viz. spore-positive (i.e., spore-forming) types, spore-negative types, in which no spores are visible, and ineffective nodules, which contain nonnitrogen-fixing endophytes which are only present in the hyphal form (van Dijk, 1984; van Dijk and Sluimer-Stolk, 1984). Crossinoculation experiments by van Dijk have clearly demonstrated that this feature is dependent upon the type of strain and not on the plant. The occurrence of spore-positive nodules; has also been discovered recently in *A. incana*, *A. rugosa* and *Myrica gale* and the ecology of the strains has been investigated. Spatially distinct distribution patterns of the spore (+) and spore (-) types of nodules indicate that both strains have distinct ecological preferences. Chemical analysis of isolates of both types of *Frankia* strains indicates significant differences which permit taxonomic distinction between *Frankia alni* subspecies *Pommeri* (spore-negative) and subspecies *Vandijkii* (spore-positive) (Lalonde, 1988). Unfortunately, only very few spore-positive *Frankia* strains, if any, have been obtained in pure culture and the ability to sporulate within the nodules has not always been well documented. Over the last decennium, several thousand *Frankia* strains have been isolated. The results have been reported or summarized at the various conferences and workshops on *Frankia* and actinorhizal plants (Akkermans et al., 1984; Huss-Danell and Wheeler, 1987; Lalonde et al., 1985; Torrey and Tjepkema, 1983). Identification and characterization of these strains have been made on the basis of morphological features, host-specificity, nitrogen-fixing ability, protein pattern, lipid composition or DNA characteristics (Normand et al., 1988; Simonet et al., 1988; 1989). Promising techniques for identification have also been found in the analysis of unique sequences in the 16S rRNA (Hahn et al., 1989).

## 2.2 Endo- and Ectomycorrhizal Fungi

Both endo- and ectomycorrhizal fungi are known to occur in actinorrhizal plants and have a direct effect on the growth of the plants. In some soils, mycorrhizal fungi are highly abundant and may compete with Frankia for sites on the roots. The occurrence and role of actinorrhizal-mycorrhizal associations have recently been summarized by Daft et al (1985) and Gardner (1986). Some actinorrhizal plants, such as Hippophae, predominantly contain VA (endo)mycorrhizal fungi, while others, e.g., Alnus spp., contain both endo- and ectomycorrhizal fungi. Various findings indicate their role in the uptake of phosphate and their antagonistic effect on root pathogens. In soil low in both N and P, *Glomus fasciculatus* VA-mycorrhiza greatly stimulated the N<sub>2</sub>-fixing activity by Frankia on Hippophae (Gardner et al., 1984). Although most VA-endomycorrhizas are generally non-specific, recent observations by Fraga-Beddiar (1987) indicate the existence of host-specific types on *A. glutinosa* in acid soil. Ectomycorrhizas have been found in nature and the associations have been synthesized in vitro (reviewed by Gardner, 1986). Fraga-Beddiar (1987) observed that ectomycorrhizal fungi invade the roots of alder at a late stage, i.e., after initial infections by endomycorrhizal fungi and Frankia. Field and laboratory observations indicate that the genus *Alnus* may express strong specialization regarding its ectomycorrhizal fungal partners (Matsui, 1926, Neal et al., 1968; Mejsstrik and Benecke, 1969; Molina, 1979). Since *Alnus rubra* intermixed with Douglas fir results in a reduction of the population of the root pathogen fungus *Poria weirri*, it has been suggested that this is due to the presence of obligate mycorrhizal symbionts that antagonize the pathogen (Trappe, 1972). As will be shown later, various other explanations have been given to explain this phenomenon.

## 2.3 Rhizobacteria

In addition to Frankia and mycorrhizas, several other microorganisms have been suggested to influence the growth of the plants positively producing plant-

stimulating growth hormones and anti-microbial compounds. So far, specific root associations with rhizobacteria and actinorrhizal plants have been described only occasionally. Recently, Dobritsa and Sharaya (1986) isolated H<sub>2</sub>-consuming *Nocardia autotrophica* from the roots and nodules of *Alnus glutinosa* and proposed an interesting new type of tripartite interaction in which the H<sub>2</sub> formed by the nodules is recycled by *Nocardia*. It is likely that this kind of symbiosis is most effective with uptake hydrogenase-negative Frankia strains which are unable to recycle the H<sub>2</sub> produced by nitrogenase.

## III. ROOT PATHOGENS

Although pathogens have a significant effect on tree growth in managed forests, little attention has been given to their effect on actinorrhizal plants. Our basic knowledge of the plant-parasite relationship in natural ecosystems is therefore extremely limited. Several actinorrhizal plants, e.g., *Alnus*, *Hippophae* and *Casuarina* form monocultures as pioneer vegetation, which degenerate after a period of time. Our observations indicate that pathogens may be involved in this process, as will be described below. Reduction of pathogens can often yield greater economic profit than inoculation with Frankia alone, particularly when native Frankia populations are already present.

### 3.1 Fungi

Several fungi have been described to be pathogenic to the roots of actinorrhizal plants. *Pythium* spp. (oomycetes) which form zoospores is potent root killers that often occur in moist soils. Several *Penicillium* strains have been found to form mycorrhizal nodules on the roots of *Alnus glutinosa* (Capellano et al., 1987; van Dijk, 1984; van Dijk and Sluimer-Stolk, 1984). This interesting new type of association occurs in certain soils and may affect plant growth, either by competition for nutrients or by competition with Frankia for infection sites on the roots (van Dijk, 1984; van Dijk and Sluimer-Stolk,

1984). Nevertheless, little information is available on the physiology of this association.

Red alder (*Alnus rubra*) is resistant to infection by *Poria weirii*, one of the major root pathogens of conifers in western North America (Wallis and Reynolds, 1962; 1965). In addition to the involvement of specific ectomycorrhizas, as described above, this phenomenon has also been explained by competition for available nitrogen. Soils under red alder trees contain high levels of nitrate, which cannot be utilized by *Poria* as a nitrogen source (Li et al., 1968). Moreover, the presence of polyphenoloxidases in alder tissue which oxidizes adihydric phenol into fungitoxic compounds may explain the resistance to *Poria* (Li et al., 1968). It has been suggested that either *Alnus* or its root nodule symbiont, *Frankia*, exudes anti-fungal compounds which suppress *Poria* sp. Alder plants may contain various toxic compounds, including polyphenols and antibiotics. The exudation of bactericides by *Alnus glutinosa* has been reported (Seidel, 1972) and it has been applied in purification plants with polluted waste water from hospitals.

The anti-microbial effects of plant polyphenols have been reported and it is likely that this will largely explain these phenomena. In addition, it has been shown that some *Frankia* strains exude anti-fungal and anti-bacterial compounds under axenic conditions (Akkermans, unpublished). Their ecological role, however, is unknown. The influence of pathogenic fungi on the growth of *Hippophae* has been demonstrated by treatment of the soils with benomyl (against hyphomycetes) and propamocarb (against oomycetes) (Zoon, in preparation). The effect of these compounds on pathogenic fungi is dependent upon the soil type. In young sandy dune soils with *Hippophae* vegetation, addition of benomyl resulted in a 2-fold increase of nodule number/plant. In older soils (60-100 yr old dune area) with degenerating *Hippophae* vegetations, addition of benomyl resulted in a 10-fold increase in nodule number/plant. These

soils often contain *Cylindrocarpon* spp. and *Fusarium oxysporum* as the major rhizosphere and root fungi. Similar treatments of old soils (ca 200 yr) with degenerated *Hippophae* vegetations, had less effect, probably because other organisms had more effect on plant growth as will be shown below. The impact of *Pythium* (oomycetes) in some of the soils has been demonstrated by the addition of propamocarb (20 mg/kg dry soil). Other studies on the effect of benomyl application to soils had demonstrated that a reduction of the fungal population in the rhizosphere resulted in an increase in the population of actinomycetes in the rhizosphere. The positive effect of benomyl on nodulation might therefore be explained either by a direct stimulation of *Frankia* in the rhizosphere or indirectly by changing the microbial interactions in the rhizosphere (van Faassen, 1974). This needs further studies.

### 3.2 Nematodes

Field studies on nodulation of *Hippophae* in England (Stewart and Pearson, 1967) and The Netherlands (Akkermans, 1971; Oremus, 1980) indicated that shrubs in old dune areas were often badly nodulated and degenerated rapidly. Subsequent pot experiments have shown that soils under degenerated *Hippophae* shrubs contain plant parasitic nematodes which seriously affect the growth of *Hippophae* seedlings, in spite of the presence of *Frankia* (Oremus, 1980; Oremus and Otten, 1981; Maas et al., 1983; Zoon, in preparation).

Special attention in these studies was paid to the large plant parasitic nematode *Longidorus dunensis* (Brinkman et al.) which occurs in low numbers and the smaller plant parasite *Tylenchorynchus microphasmis* Loof, which occurs in much higher densities. Pot experiments in which *Tylenchorynchus* is added to the soil show damaging effects on the plants (Zoon, manuscript in preparation) similar to those seen in field studies. With increasing numbers of nematodes added per pot, the number of nodules

per unit of root length decreases. In addition, the total root length decreases. Chemical analysis of the plants shows a decreased P content and a slightly increased N content of the shoots. Since P uptake, in contrast to N uptake, is highly determined by the size and activity of the root system, the effect of nematodes on the size of the root system mainly results in reduced P uptake by the plant.

The effect of nematodes on plant growth and nodulation of Hippophae has also been demonstrated by the addition of oxamyl, a nematostatic compound, to soil samples. Addition of oxamyl to soil samples from both vigorous and degenerating Hippophae vegetations, generally containing *T. microphasmis*, significantly improves the number of nodules formed per seedling, indicating that nematode effects occur on most field sites. Field studies demonstrate that plant parasites increase in numbers in older Hippophae vegetations, while available soil phosphorus and total plant production decrease. It is tempting to suggest that nematodes play a significant role in the degeneration of the shrubs (Zoon, 1986).

#### IV. CONCLUSION

During the last decennia, foresters have gained much information on the growth of natural stands of economically important actinorrhizal plants, e.g., *Alnus* spp. Physiologists have gained information on the effect of abiotic factors on plant growth and soil microbiologists have recognized the role of root symbionts and pathogens in the growth of the plants. Combination of the knowledge obtained in different disciplines is needed in order to understand the complexity of the interactions between plants, microbes, small animals and their environment. This multidisciplinary approach will help us to improve wood production and will give new ways for controlling tree growth. The overview presented in this paper indicates that our information about root interactions is fragmentary and has to be improved in

the near future. The examples demonstrate that several root symbionts are host-specific, which opens up the opportunity to manipulate the system. Introduction of selected or even genetically engineered mycorrhizal fungi or *Frankia* can be used for biological control of root pathogens and for improvement of symbiotic nitrogen fixation in forestry.

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