Chapter 8

NITROGEN-FIXING TREES WITH ACTINORHIZA IN FORESTRY AND AGROFORESTRY

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1. INTRODUCTION

Nitrogen-fixing trees with actinorhiza (or actinorhizal trees) form a group that is a key component in many natural ecosystems, agro-ecosystems, and agroforestry systems in the world, and that provides an important source of fixed nitrogen in these ecosystems. The general characteristics of the actinorhizal symbiosis, including aspects of nodule formation, co-evolution of both partners, and nitrogen fixation rates at field level of some selected species, as well as mycorrhizal associations in actinorhizal trees, will be described. In addition, topics related to actinorhizal trees in agroforestry, plus the role these woody perennial components may play in agroforestry systems, will also be explored, with emphasis on two genera, Casuarina and Alnus, widely known and used in this kind of production systems. The experience of the Central America Fuelwood Project, the case of Alnus acuminata in tropical highlands, and other uses of actinorhizal trees are also covered. Just as actinorhizal trees open new horizons for research and applications aimed toward sustainability, this chapter attempts to make a modest complementary contribution to the knowledge generated in the last four decades.

Actinorhiza is the result of a symbiotic relationship between a special soil N_2-fixing actinomycete (filamentous bacteria of the order Actinomycetales of the genus Frankia) and fine plant roots; it is neither the actinomycete nor the root, but rather the structure or root nodule formed from these two partners. Because the result of the symbiotic association is a root nodule, the host plant is known as an actinomycete-nodulated plant or actinorhizal plant (Torrey and Tjepkema, 1979; Huss-Danell, 1997). Actinorhizal associations range from the arctic to the tropics and from the semi-desert to rainforest ecosystems. Actinorhizal plants can be found in forest, swamp, riparian, shrub, prairie, and desert ecosystems. In moving from warmer to colder climates, actinorhizal plants become more prevalent and seem to

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fill the niche dominated by woody legumes in the tropics (Dawson, 1986). The actinorhizal trees are much less numerous than the vast group of the nitrogen-fixing legume trees. Even so, they are an important source of fixed nitrogen in their ecosystems and can fix from 2 to 300 kg N ha^{-1} yr^{-1}.

Despite of the size of the group, actinorhizal trees are important because: (a) their nitrogen-fixing ability is not restricted to one family but it expands to eight families; (b) most of the actinorhizal trees are good and sometimes aggressive colonizers that are capable of regenerating poor soils or disturbed sites, which are rapidly increasing in many tropical countries; and (c) tropical actiinorhizal trees, especially Casuarina and Alnus, produce not only timber but also firewood and charcoal, sometimes also providing shelter to the cattle, and are used for the protection and recovery of degraded soils (Diem et al., 1984). Many actinorhizal plants are pioneering species, like Alnus, which grows in moist environments, or Myrica, which grows on landslides, eroded slopes, and mined areas, or Casuarinas, which have been identified as nitrogen-fixing trees for adverse sites (NRC, 1984).

This chapter will assess the realities and opportunities of actinorhizal trees in forestry and agroforestry, with an emphasis on tropical regions where the author has expertise and working experience.

2. GENERAL CHARACTERISTICS OF THE ACTINORHIZAL SYMBIOSIS

Actinorhizal trees are defined by their ability to form N_2-fixing root-nodule symbioses with the genus, Frankia. This association is typical for angiosperms but has not been reported in gymnosperms. Frankia is a Gram-positive, branched, septate, filamentous bacterium of the order Actinomycetales, which shows a complex pattern of differentiation in vitro and in the nodule (Berry, 1986). Frankia has also been defined as a diverse group of soil actinomycetes that have in common the formation of multi-locular sporangia, filamentous growth, and nitrogenase-containing vesicles that are enveloped in multi-laminated lipid envelopes (Benson and Silvester, 1993). Frankia infects a host plant to form a nodule on the root system. In the symbiosis, Frankia provides the plant with a source of fixed nitrogen and, in return, the plant provides the Frankia with a carbon source. Although some 40 attempts had been made earlier (Baker and Torrey, 1979), the first confirmed Frankia strain was isolated in 1978 from nodules of Comptonia, using an enzymatic method (Callaham et al., 1978). Other methods, e.g., sucrose-density-gradient centrifugation (Baker et al., 1979), serial dilution (Lalonde, 1979), selective incubation (Quispel and Burggraaf, 1981), and osmium-tetroxide treatment (Lalonde et al., 1981), have successfully produced Frankia in pure culture.

2.1. Host Specificity Groups.

Early on, compatibility groups of host plants and *Frankia* were thought to exist (Becking, 1974), however, later studies found that some isolates were capable of nodulating hosts from different “compatibility” groups (Lechevalier and Ruan, 1984) and this finding opened new horizons for research concerning host specificity

(Berry, 1986). These newer studies often apply molecular-biology tools to questions related to the genetics, ecology, and evolution of actinorhizal symbiotic systems. Molecular phylogeny groupings of host plants were correlated with morphological and anatomical features of actinorhizal nodules in the past, but host-plant phylogenies that are based on molecular data soon revealed different relationships among host plants than have previously been assumed (Berry, 1994; Swensen and Mullin, 1997a).

Three host-specificity groups, *Alnus*, *Elaeagnus*, and *Casuarina*, have been identified and the majority of *Frankia* strains studied fell into one of these three cluster groups. The agreement between the phenotypic clusters and the genospecies described previously shows that the grouping may reflect the taxonomic structure of the genus, *Frankia*. Dobritsa (1998) validated this concept with a study in which thirty-nine selected *Frankia* strains, which belonged to different genomic species, were clustered on the basis of their *in vitro* susceptibility to 17 antibiotics, pigment production and ability to nodulate plants of the genus *Alnus* and/or the family Elaeagnaceae or the family Casuarinaceae. The author found differentiating phenotypic characters for some clusters that may be useful for species definition.

Simultaneously, Huguet *et al.* (2000) carried out a study to identity *Frankia* strains from nodules of *Myrica gale*, *Alnus incana* subsp. *rugosa*, and *Shepherdia canadensis* growing in close proximity and also from nodules of two additional stands with *M. gale* as the sole actinorhizal component. Using a gene-polymorphism technique, they demonstrated that, at the first site, each host-tree species was nodulated by a different phylogenetic group of *Francia*. At the second site, they found that the *M. gale*-strains belonged to yet another group and were relatively low in diversity for a host genus considered promiscuous with respect to *Frankia* microsymbiont genotype. It should be remembered here that plants in the Myricaceae family are considered to be promiscuous hosts because several species are effectively nodulated by most isolated strains of *Frankia* in the greenhouse. This observation has led to the hypothesis that plants in the Myricaceae family have sufficient diversity to serve as a reservoir host for *Frankia* strains that infect plants from other actinorhizal families (Clawson and Benson, 1999).

These issues have contributed to the development of new hypotheses on the origin and evolution of actinorhizal symbiotic systems and have initiated the notion that nitrogen-fixing endosymbionts of actinorhizal plants can interact with a very broad range of unrelated host-plant genotypes (Kohls *et al.*, 1994). Furthermore, a model for actinorhizal specificity has been proposed that includes different degrees of specificity of host-symbiont interactions, ranging from fully compatible to incompatible, and also that actinorhizal plants undergo feedback regulation of symbiosis, involving at least two different consecutive signals that control root nodulation (Wall, 2000). Further, the use of heterologous probes in combination with nucleotide-sequence analysis have allowed a number of *nif* genes to be mapped on the *Frankia* chromosome and this work will ultimately contribute to our understanding of *nif*-gene regulation in *Frankia* (Mullin and Dobritsa, 1996).

Actinorhizal nodules are ontogenically related to lateral roots and may result from modification of the developmental pathway that leads to lateral-root formation

(Goetting-Minesky and Mullin, 1994). This type of root nodule is characterized by differentially expressed genes, which supports the idea of the distinctiveness of this new entity, called "Actinorhiza", a name that refers to both the filamentous bacteria and to the root location of nitrogen-fixing nodules (Wall, 2000).

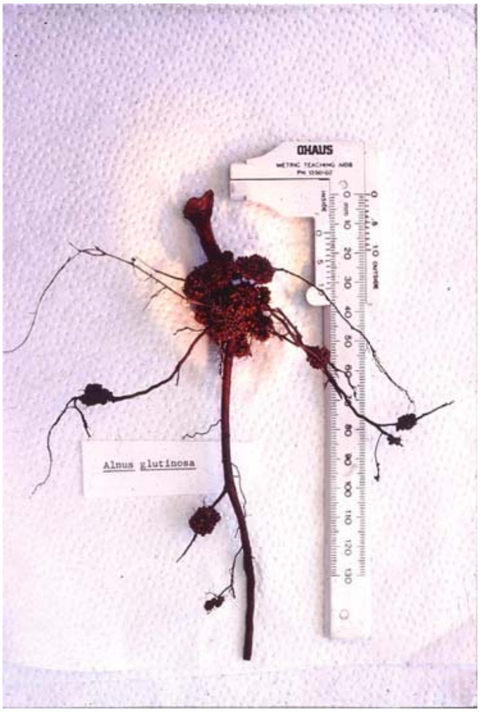

Figure 1. Root Nodules on Alnus glutinosa.
(Photo: R.O. Russo)

2.2. Infection Pathways.

Two pathways for root infection have been described for compatible *Frankia* interactions; they are either root-hair infection or intercellular penetration. The functional physiology of nodule morphogenesis has been reviewed by Berry (1994). As stated by this autor, nodules are initiated in actinorhizal plants either *via* root infection by *Frankia* or by intercellular invasion (Miller and Baker, 1985) and that the mode of infection is host-determined because single strains of *Frankia* have been shown to nodulate different hosts by different infection mechanisms (Miller and Baker, 1986). Premature stages of the developing infection embrace Esther deformations of the root-hair wall or, in the case of intracellular penetration, the release of host extracellular pectic polysaccharides (Liu and Berry, 1991). Host cortical-cell proliferation is followed by expansion and even hypertrophy of groups of cells infected by *Frankia*. The endosymbiont penetrates cortical cells after meristematic growth, probably during cell expansion. Within the host cells, *Frankia* proliferates and vesicles are formed, wherein nitrogen fixation takes place (except with the Casuarinaceae family). *Frankia* strains can be classified in two groups, those that form spores (sp+) within the nodules (spore-positive nodules) and those that do not (sp-) and so produce spore-negative nodules (Schwintzer, 1990). It appears that sp+ strains are less common than sp-.

The morphology of the vesicles is relatively constant in culture but, in symbiotic conditions when interacting with the host plant, it is modified to give a diverse array of vesicles shapes (Benson and Silvester, 1993). Another line of research involves the function of flavonoid compounds on the patterns of nodulation by *Frankia*, *e.g*., Benoit and Berry (1997) found that flavonoid-like compounds from seeds of red alder (*Alnus rubra*) influenced host nodulation. Independent of the infection mechanism, some authors support the concept that the opening (or closing) of the window of susceptibility for infection and nodule development in the growing root are signal-mediated processes (Wall, 2000), even though rhizosphere nodulation signals have not been as well characterized for actinorhizal plants as they have been for legumes. Recently, the steps in nodule formation for actinorhizal and rhizobial symbioses have been compared and phylogenetic aspects of nodulating both actinorhizal and legume plants described (Gualtieri and Bisseling, 2000).

2.3. External Factors That Impact Nodulation.

Many factors intervene in the process of nodulatin and nodule formation. As for legumes, fixed-nitrogen levels in the substrate inhibit both nodulation and N_2 fixation in actinorhizal plants (Huss-Danell, 1997). This effect had been observed in 1975 by Bond and Mackintosh, who reported that nitrate inhibited both nodule biomass and nitrogenase activity in *Hippophaë rhamnoides* and *Coriaria arborea* (Gentili and Huss-Danell, 2002). The same pattern was also observed by Arnone *et al.* (1994), who used a split-root system that allowed them to distinguish between local and systemic effects of fixed-N compounds. They found that nodulation was inhibited locally by nitrate in *Casuarina cunninghamiana*.

But fixed-N compounds are not the only impacting factor. Phosphorus compounds interact with fixed-N compounds and together affect nodulation. This interaction effect between ammonium nitrate and phosphate on nodulation by the intercellular infection was studied by Gentili and Huss-Danell (2002). They found that phosphate modifies the effects of fixed-nitrogen compounds on nodulation in split-root systems of *Hippophaë rhamnoides.* Further, the inhibition of nodulation by fixed-N compounds was systemic for both nodule number and nodule biomass and the high phosphate level had a systemic stimulation on nodule number and biomass. In this study, phosphate prevented the systemic inhibitoin by fixed-N compounds. The stimulation by phosphate was specific to nodulation and not simply mediated *via* plant growth. This study, however, did not allow the authors to make conclusions on the effect of both fixed-N and P compounds on N_2 fixation.

In addition to root nodulation, aerial nodulation has been observed in *Casuarina* by a number of authors. Prin *et al.* (1991a; 1991b) reported aerial nodules on the trunks of *Casuarina cunnimghamiana.* Then, Valdés and Cruz Cisneros (1996) described stem nodulation of *Casuarina* in Mexico and Bertalot and Mendoza (1998) observed aerial nodulation in *Casuarina equisetifolia* that was shading pastures in the inlands of the State of Sao Paulo in Brazil. The authors described them as stem protuberances, which contained actinorhizal nodules and adventitious roots that were 3-8 cm thick. They covered irregularly up to 20 percent of the trunk surface below breast height and tested positively by the acetylene-reduction assay.

2.4. Co-Evolution of the Symbionts.

Taking into consideration that two genomes, one from the host and one from the actinomycete, are involved in the host-endophyte interactions, this is an intricate symbiotic relationship. Many factors are involved in the actinorhizal symbiosis; these include host-plant physiology, host compatibility, host specificity in terms of both infectivity or affectivity, ability to promote host-plant growth, and interactions of the microsymbiont with other microorganisms inside or outside the host root. All these interactions have led to a coevolution process between the two genomes. Considerable evidence exists to support the hypothesis that coevolution has taken place through mutual interaction of host plants and *Frankia* populations in the soil to produce the most efficient symbiotic associations (Lie *et al.,* 1984). Several research groups interested in coevolution have studied *Frankia*, when associated with host plants of different genera and families, and have found some correspondence between the bacterial and plant phylogenies, which suggests coevolution (Benson *et al.,* 1996). Although an exploratory approach, based on genome interactions, is helpful in looking at this problem, the situation is much more complicated than it appears, mainly because the host-plant genome is more complex than that of the prokaryote. Studies on co-evolution between *Frankia* populations and host plants in the family Casuarinaceae were carried out by Simonet *et al.*, (1999), who deduced patterns of global dispersal.

The ecological significance of considering the evolution of both symbiotic partners is that the complete symbiotic unit can explore a wider environmental range. This is the situation for those actinorhizal trees that grow in: (i) poor, disturbed, or degraded soils; (ii) in nitrogen-poor sites, such as sandy soils, disturbed soils, and wet soils; or (iii) as pioneer vegetation at early stages of plant succession following disturbances, such as eruptions, flooding, landslides, and fires.

3. HOST BOTANICAL FAMILIES

Actinorhizal hosts described to date are trees and shrubs that, according to the classification scheme of Cronquist (1981), which is based primarily on the analysis of morphological characters, are distributed among four subclasses, Rosidae, Hamamelidae, Magnoliidae and Dilleniidae, in eight families of angiosperms that include 25 genera and over 200 plant species. This broad phylogenetic distribution of nodulated actinorhizal plants has created the notion that nitrogen-fixing endosymbionts, particularly those of actinorhizal plants, can interact with a very broad range of unrelated host-plant genotypes (Swensen and Mullin, 1997b). Several reviews on the biology and development of these actinorhizal associations have been published in recent years (Benson and Silvester, 1993; Berry, 1994; Schwintzer and Tjepkema, 1990). Not all genera in these families and not all species in the 25 genera fix atmospheric N_2. In certain environments, species of these genera may become more abundant and ecologically significant. According to Benson and Clawson (2000), nodulating families occur sporadically within the families; in the Casuarinaceae, Coriariaceae, Datiscaceae, Elaeagnaceae, and Myricaceae, all or most genera are nodulated, whereas nodulation occurs only occasionally in the Betulaceae, Rhamnaceae, and Rosaceae. Table 1 summarizes the taxonomy of the actinorhizal genera with their distribution.

4. NITROGEN FIXATION IN ACTINORHIZAL TREES

Measurement of N_2 fixation in actinorhizal trees (Table 2) is still a difficult task because no simple and accurate methods to determine annual rates are available. Conventionally, N_2 fixation has been measured by the acetylene-reduction assay, which is based on measuring the production of ethylene from acetylene, catalyzed by nitrogenase, with a gas chromatograph. The enzyme, within the N_2-fixing organisms, can also reduce a number of other compounds besides N_2 and acetylene. However, several other methods have been used, including N accretion, N difference, ^{15}N isotope dilution, and ^{15}N natural abundance. Each of the methods has its own assumptions and inaccuracies (Table 3).

Of these, the ^{15}N stable-isotope methods are currently considered to have the least drawbacks for the quantitative measurement of biological nitrogen fixation (BNF) (Warembourg, 1993). Their advantages are based on: (i) the capacity to measure BNF cumulatively over more than one growing season; (ii) the high degree of precision of stable isotopes; and (iii) the capability of assessing the relative efficiency of N_2 fixation, *i.e.*, the proportion of plant-N derived from fixation (N_{dff}). Stable-isotope methods are not without their limitations, however. Several assumptions, which primarily involve the selection of the reference plant, must be satisfied to ensure an accurate measurement of BNF. Reference plants are required in order to estimate the relative proportion of plant-N derived from fixation *versus* uptake of soil-N. They must have a similar rooting profile, a similar timing of N-uptake, and a similar internal isotopic discrimination to those of the N_2-fixing plants. Finding an appropriate reference plant is always difficult. In the case of actinorhizal plants, the N_{dff} determined by ^{15}N isotope-dilution methods averaged 87% for *Ceanothus velutinus* and 83% for *Purshia tridentata* (Busse, 2000a; 2000b). Other studies have reported values of 68 to 100 percent for *Alnus glutinosa* for N derived from fixation (Beaupied *et al*., 1990; Domenach *et al*., 1989) and 48-67% for *Casuarina equisetifolia* (Gauthier *et al*., 1985, Parrotta *et al*., 1994).

Table 1. Taxonomy of the Actinorhizal Genera with theirDistribution.

Subclass*	Family	Genus	Present distribution
Hamamelidae	Betulaceae	*Alnus*	Europe, N. America, Highlands of Central and South America, scattered in North Africa.
	Casuarinaceae	*Allocasuarina*	Australia, primarily S and SW
		Casuarina	Australia, troprical Asia, Pacific Islands.
		Ceuthostoma	Sumatra to New Caledonia, Fiji and Cape York Pen., Australia.
		Gymnostona	
	Myricaceae	*Comptonia*	Europe, Asia, North America.
		Myrica	Europe, Asia, North America.
Rosidae	Elaeagnaceae	*Elaeagnus*	North America.
		Hippophae	Widespread tropical, subtropical, temperate.
		Shepperdia	
	Rhamnaceae	*Adolphia*	Southern South America.
		Ceanothus	North America.
		Colletia	Temperate and tropical South America.
		Discaria	South America, New Zealand, Australia.
		Kenthrothamnus	Argentina, Bolivia.
		Retanilla	Southern Soth America.
		Talguena	Chile.
		Trevoa	Andes of South America.
	Rosaceae	*Cercocarpus*	West and S-W USA, Mexico.
		Chamaebatia	Sierra Nevada of West USA.
		Cowania	USA, México
		Dryas	Artic mountains, North Temperate Zone
		Purshia	North America.
		Rubus	North Temperate Zone
Magnolidae	Coriariaceae	*Coriaria*	Mediterranean, Japan, China, New Zealand, Chile, Mexico.
Dillenidae	Datiscaceae	*Datisca*	Mediterranean to Himalayas and Central Asia, S-W USA.

Classification according to Cronquist (1981), Brewbaker et al. (1990), and Benson and Clawson (2000).

Table 2. Rates of Symbiotic N_2 Fixation of Some Actinorhizal Trees and Shrubs at Field Level

Actinorhizal species	kg N fixed ha^{-1} yr^{-1}	References
Alnus acuminata (A. jorullensis)	279	Carlson and Dawson, 1985
Alnus glutinosa	40 – 53	Cote and Camire 1984; Hansen and Dawson 1982
Alnus rubra	85 – 320	Cole *et al.* 1978; Newton *et al.* 1968
Casuarina equisetifolia	12 – 110	Dommergues 1987 ; Gauthier *et al.* 1985 ; Diem and Dommergues 1990 ; Baker 1990a
Coriaria arborea	192	Dommergues, 1990
Ceanothus velutinus	4 -100	McNabb and Cromack 1983; Youngberg and Wollum 1976; Zavitkovski and Newton 1968; Busse 2000a; 2000b ^{15}N
Purshia tridentata	1	Busse 2000a; 2000b ^{15}N

Table 3. Comparison of Methods of Estimating Nitrogen Fixation.

After I. Watanabe (2000) lecture in Cantho University, Vietnam. Retrieved June 6, 2002, from http://www.asahi-net.or.jp/~it6i-wtnb/BNF.html#ch4

Method	Advantages	Disadvantages	Sensitivity
Total N balance	Simplest	Low sensitivity including other inputs.	Lowest
$^{15}N2$ incorporation	Most direct	Expensive, only for short period	High-moderate
Acetylene reduction	Simple, highly sensitive	Indirect, semi-quantitative	High
^{15}N dilution	Throughout growing season	Only N Fixation in plant Varies with reference plants	High-low
Natural abundance	Simple, no disturbance to system	Only slight difference in ^{15}N content	Low
Substrate addition	Difference in ^{15}N content is large	Change of ^{15}N in time and space in soil	Moderate

The rates of nitrogen fixation measurable by the acetylene-reduction assay (ARA) in nodules of actinorhizal genera, such as *Alnus* and *Eleagnus*, range between 10-90 μmol ethylene g^{-1} nodule fresh weight h^{-1} (Berry, 1994). Other values of ARA reported for 120-days-old *Alnus acuminata* seedlings, which were inoculated with a crushed nodule suspension, were between 32.5 and 86.4 μmol ethylene g^{-1} nodule fresh weight h^{-1} (Russo and Berlyn, 1989; see Figure 2) and are consistent with the above mentioned range. However, variability is very high and accuracy has been challenged because acetylene, in some cases, inhibits nitrogenase activity. In other cases, the ARA technique is used inappropriately. Furthermore, conversion from acetylene-reduction to N_2-fixation values often uses the routinely assumed conversion ratio of 3:1-4:1 (due to H_2 production under N_2), however, conversion rates can fluctuate depending on a number of factors and are not constant. Finally, to extrapolate laboratory or greenhouse values to field conditions in terms of kg N ha^{-1} yr^{-1} can be a significant source of error (Giller, 2001).

Figure 2. In Alnus acuminata *seedlings inoculated with* Frankia*, nodules appear two weeks after inoculation and thereafter the rate of growth and interval of appearance of leaves is modified. Nodulated seedlings (inoculated) at 50 days have higher total height (P<0.05), higher leaf length (P<0.05), higher shoot biomass (P<0.05), and lower root/shoot ratio (P<0.01) than non-inoculated seedlings (Personal observations).*
Left side: non-inoculated; right side: Frankia*-inoculated seedlings. (Photo: R.O. Russo)*

Environmental effects on N_2 fixation have been well described by Huss-Danell (1997). Both nodulation and N_2 fixation are influenced by environmental factors but, unlike legume nodules, actinorhizal nodules are not O_2 limited and Frankia itself appears able to protect nitrogenase from O_2 inactivation. Five major effects, which themselves are affected by other factors, were highlighted (Figure 3).

At the forest-stand level, the rates of symbiotic N_2 fixation reported for actinorhizal trees range between 12 and 320 kg N ha^{-1} yr^{-1} (Table 2). By comparison, asymbiotic N fixation by free-living soil prokaryotes typically contributes only 1 kg N ha^{-1} yr^{-1} or less in forest ecosystems (Jurgensen et al., 1992), whereas associative N_2 fixation, although controversial, has been suggested to fix up to 50 kg ha^{-1} yr^{-1} in the rhizosphere of conifer roots (Bormann et al., 1993).

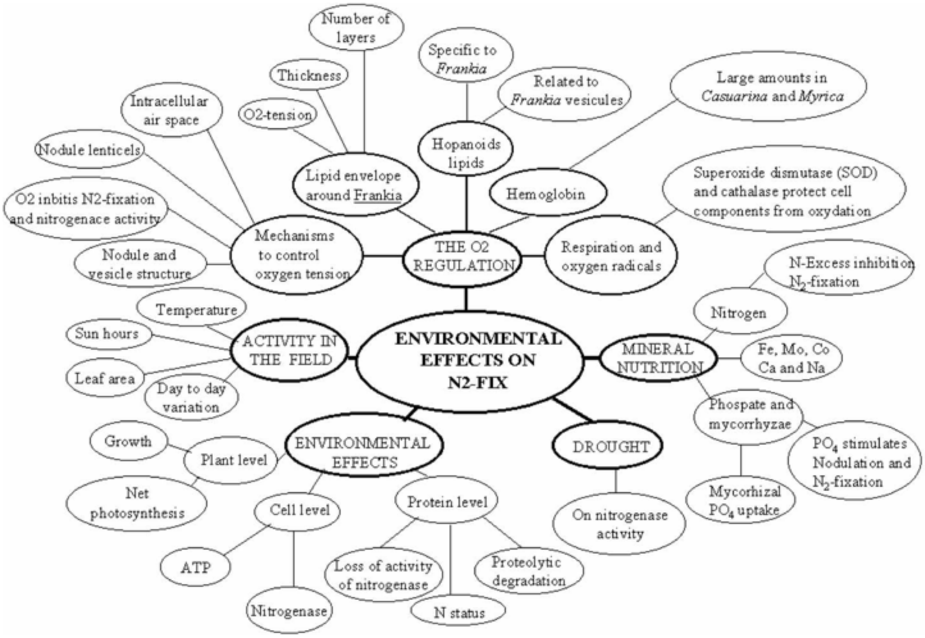

Figure 3. Concept Map of Environmental Effects on N_2 fixation by Actinorhizal Systems. Based on Huss-Danell (1997).

As Dommergues (1997) states, the nitrogen-fixing potential of a number of actinorhizal plants, e.g., Casuarina sp. and Alnus sp., is high but the amount of N actually fixed in the field is often low because the expression of this potential is limited by either unfavourable environmental conditions or improper management practices. In consequence, some strategies can be adopted to increase the input of fixed N into ecosystems by using management practices to optimize actinorhizal N_2 fixation. Actinorhizal plants should receive serious consideration as soil improvers in a number of situations and sites where they are not yet used. Furthermore, under-exploited actinorhizal trees and shrubs should be domesticated to exploit their ability to contribute to the rehabilitation of wasted lands and possibly to the phytoremediation of polluted sites (Baker, 1990a; 1990b).

5. MYCORRHIZAL ASSOCIATIONS WITH ACTINORHIZAL TREES

The existence of tripartite symbiotic associations among actinorhizal plants, *Frankia*, and mycorrhizal fungi has been reported for *Alnus* spp. (Rose and Trappe, 1980), for *Ceanothus velutinus* (Rose and Youngberg, 1981), for *Casuarina equisetifolia* (Gauthier *et al.*, 1983), for *Hippophae rhamnoides* (Gardner *et al.*, 1984), and for *Alnus acuminata* (Russo, 1989; 1992; see Figure 4). In these tripartite associations of tree-*Frankia*-mycorrhiza, the double symbiosis is established on the exchange of carbon, phosphate, and fixed-nitrogen between plant host and both bacterial and fungal microsymbionts. In addition, ectomycorrhizal associations of *Casuarina* have been reported (Warcup, 1980; Dell *et al.*, 1994) and the development and function of *Pisolithus* and *Scleroderma* ectomycorrhizas in *Casuarina* described. For *Alnus acuminata*, ectomycorrhizal associations with the basidiomycetes, *Phylloporus caballeroi* and *Gyrodon monticola*, have been reported (Singer and Gomez, 1984).

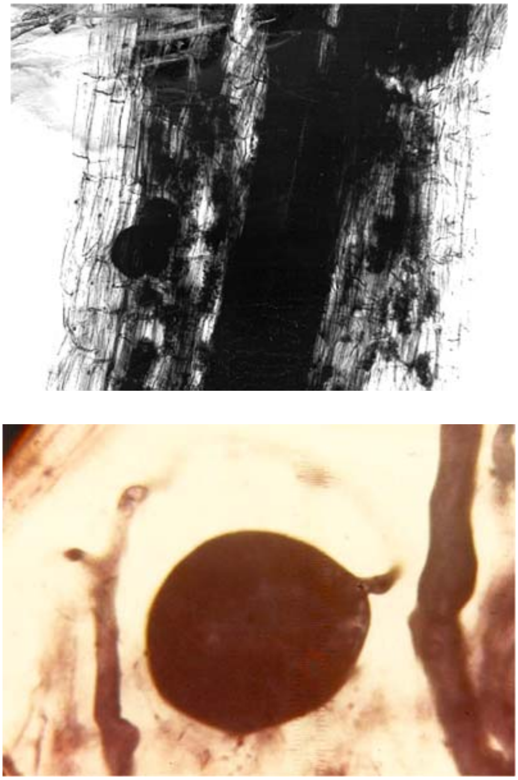

Figure 4. Arbuscular Mycorrhizas (AM) in Alnus acuminata *roots and* Glomus intraradices *internal spore in* Alnus acuminata *inoculated seedlings. (Photos: R.O. Russo)*

Several authors have reviewed the functional aspects of the relationship between the mycorrhizal and actinorhizal symbioses in non-legumes (*e.g.*, Barea and Azcón-Aguilar, 1983; Rodríguez-Barrueco, 1984; 1992; Gardner, 1986; Cervantes and Diem, 1997; Dommergues, 1997). Most of them agree that actinorhizal plants are predisposed to form both ecto- and endo-mycorrhizal symbiotic associations, either tripartite or tetrapartite, that are as essential for plant growth as the associations they form with *Frankia*. As expected, mycorrhizas on the same roots as *Frankia* stimulate both the development and the nitrogen-fixing activity of the actinorhizal symbiosis by improving mineral nutrition of the host plant, particularly, those aspects related to phosphorus uptake (Diem, 1997; Gentili and Huss-Danell, 2002; Yang, 1995). It remains to be proved whether the development of root nodules and endomycorrhizal symbioses involves processes and genes recruited from a common plant origin or whether each has its own unique properties (Gualtieri and Bisseling, 2000).

Early research done by Gauthier *et al.* (1983) with *Casuarina equisetifolia,* which were simultaneously inoculated with both *Frankia* and *Glomus mosseae*, found that total dry weight was more than 80% greater in doubly inoculated seedlings than in those inoculated with *Frankia* alone, whereas no differences between the control and the VAM-inoculated plants were found. All treatments used 10 μg P g^{-1} with one exception in which inoculation was by crushed nodules and 90 μg P g^{-1} was applied to the plants. These results have been validated for other actinorhizal species (Gardner *et al.*, 1984; Gardner, 1986; Chatarpaul *et al.*, 1989; Cervantes and Rodríguez-Barrueco, 1992).

The effect of arbuscular mycorrhizae (AM; *Glomus intraradices*) on both acetylene reduction and the growth response, with three different phosphorus levels (10, 50 and 100 μg g^{-1}), of *Alnus acuminata* seedlings, which were inoculated with *Frankia* strain ArI3 (originally isolated from *A. rubra* at Harvard Forest), was studied. Significant differences in acetylene reduction were observed after 120 days at the lowest P level (10 μg g^{-1}) between *Frankia*-inoculated and *Frankia*+AM-inoculated plants. In addition, significant differences in seedling growth at the middle P level (50 μg g^{-1}) were observed in favor of the doubly inoculated plants (Russo, 1989). The data suggest that the presence of AM favors acetylene reduction at lower P levels where the AM improves P uptake, but also can compete with the nodules as a carbohydrate sink at the middle levels.

Although the mycorrhizal symbiosis should promote plant growth in actinorhizal plants, certain aspects need to be considered. When nitrogen is a limiting factor, photosynthate allocation could be modified. With non-nodulated plants that are supplied with a growth-limiting level of combined-N, a higher proportion of dry mass is allocated to roots than in nodulated plants (Arnone and Gordon, 1990). The situation with *Frankia*-inoculated plants involves a different response, which can be related to the ability of the nodulated plants to get more fixed nitrogen than the amount supplied. Thus, the predictions that can be made from experimental studies in controlled conditions may not be realistic for all situations; they would be mainly applicable to growth conditions characterized by a sub-optimal nitrogen supply.

For *Casuarina*, the AM endophytes did not invade nodular cortical tissues (Khan, 1993), suggesting the presence of an exclusion mechanism that needs further study. Further, the highest endomycorrhizal infection occurred in nodulated specimens and a relationship was found between soil-moisture gradient and AM infection in *C. cunnimghamiana.* In this species, typical vesicles and arbuscules were found in roots from drier soils, however, there was a lack of arbuscules in relatively wet soils where large lipid-filled intracellular vesicles were present. Both mycorrhizal infection and AM spore number seem to be related to redox-potential;

they are lower at sites, such as swamps, water or sediments, with lower Eh values than in terrestrial soils with higher Eh values.

Recently, the genetic and molecular mechanisms of the development of N_2-fixing nodules (both *Rhizobium*- and *Frankia*-based) were compared with those of arbuscular mycorrhiza (Provorov *et al.*, 2002). The new primordium developing from root tissues is common for all known types of N_2-fixing nodules. However, their structure varies greatly with respect to: (i) tissue topology (the location of vascular bundles is peripherical in legumes or central in non-legumes); (ii) the position of the nodule primordium (in the inner or outer cortex in legumes and the pericycle in non-legumes); and (iii) the stability of the apical meristem (persistent in the indeterminate nodules, transient in the determinate ones). The origin of actinorhizal symbioses is suggested to be based on a set of pre-adaptations, *e.g.*, inter- and intra-cellular maintenance of symbionts, their control *via* defense-like reactions, and recognition of chitin-like molecules, many of which had been evolved in angiosperms during coevolution with arbuscular mycorrhizal fungi.

In addition to mycorrhizas, actinorhizal plants, including *Alnus* and *Casuarina,* are also able to form an unique type of root, called either "proteoid roots" or "cluster roots" in response to the detrimental effects of soil nutrient deficiencies. Proteoid roots, which are formed by clusters of closely spaced lateral rootlets of limited growth (Hurd and Schwintzer, 1996; Arahou and Diem, 1997), have been reported in 28 species from the Betulaceae, Casuarinaceae, Eleagnaceae, Leguminosae, Moraceae, and Myricaceae families, four of which are actinorhizal species (Dinkelaker *et al.*, 1995). Skene (1998) reviewed both the functional aspects and the ecological considerations of proteoid-root formation, emphasizing that the species with this kind of root formation can grow in soils with low nutrient availability. Watt and Evans (1999), who reviewed the physiology and development of proteoid roots, cite that auxin probably works alongside other hormones, such as ethylene and cytokinin, during proteoid-root development. In this way, P nutrition has been clearly implicated in the formation of proteoid roots, which decline as P availability to roots increases. By analogy, P may alter nodule formation and function in the *Casuarina-Frankia* symbiosis (Yang, 1995). Once developed, the proteoid roots: (i) mobilize mineral P that is bound to cations, such as Fe, Al, and Ca; (ii) extract P from organic layers in soil; (iii) obtain Fe and Mn from alkaline soils; and (iv) take up organic forms of N (Dinkelaker *et al.*, 1995).

Diem and Arahou (1996) visualize proteoid roots as a primary strategy of the Casuarinaceae to overcome soil nutrient deficiencies. They, thus, explain the ability of the family to grow in marginal soils by their adaptability to soil constraints. These authors also underline the likely role of proteoid roots as an alternative to mycorrhizas. These anatomical root structures improve the absorption of nutrients, other than N, from the soil, especially those needed for nitrogen fixation and growth (Diem *et al.*, 1999). Proteoid roots also occur for *Myrica gale*, an actinorhizal shrub that occurs in the higher latitudes of the Northern hemisphere and that dominates in waterlogged soils (Herdman *et al.*, 1999).

6. ACTINORHIZAL TREES IN AGROFORESTRY

Agroforestry is defined by the International Centre for Research in Agroforestry (ICRAF, 2002) as a dynamic, ecologically-based, natural-resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels. This definition is an up-dated response to current needs of social and environmental accountability. Previous definitions emphasized the fact that agroforestry systems are deliberate combinations of a woody perennial component with agricultural components (crops, pastures and/or livestock) interacting with each other at the same site as an integrated agroecosystem. During the 1980s, agroforestry systems were considered to be a solution for solving land-use problems in the tropics. Today, it is generally accepted that to be called agroforestry, a land-use practice must satisfy some minimum criteria. These are that the combination must be intentional or deliberate, interactions among components must be at least biological, and the components have to be integrated on the same unit of land. The goal and justification of agroforestry systems were to put emphasis on the positive interaction in order to obtain a higher total, a more diversified and/or more sustainable production from available resources than is possible with other forms of land-use under prevailing ecological, technological and socio-economic conditions (Nair, 1990).

Actinorhizal trees may play the role of the woody perennial in agroforestry systems. In fact, they have been used historically in agricultural systems in several ways (Baker, 1990). Probably one of the most known species of actinorhizal trees is *Casuarina,* which is used for firewood production in the tropics or as a component of a multipurpose agroforestry plantation or when large plantations are interplanted (Taungya system) with agricultural crops during the first few years of the rotation (Diem and Dommergues, 1990; Pinyopusarerk *et al.*, 1996). Other actinorhizal tree species have been used in agroforestry; these include: *Alnus*, which is cultivated as a primary crop for timber and pulpwood; *Elaeagnus spp.*, which is interplanted as a nurse plant for other more-valuable species; and *Elaeagnus, Shepherdia*, and *Purshia* spp, which are planted for soil reclamation (Kohls *et al.*, 1994; Paschke, 1997).

Several authors have listed a number of desirable characteristics of trees to be used in agroforestry. Basically, these traits are a function of the goals, production objectives, and needs of the user. A first consideration is compatibility with soil and local climate. Also, the tree should be a fast or moderately-fast-growing species that is either valuable for markets or biodiversity maintenance; it should be a nitrogen-fixing tree; it should have good shade characteristics appropriate to companion crops; and it should produce nuts or fruits that can have an acceptable local market. Additionally, it is desirable that the tree does not produce growth-inhibitory chemicals (allelochemicals), which would prevent some crops from growing near them, and that the trees should have minimal root competition (Beer, 1987; Hodge *et al.*, 1999; Perry, 1989, Werner and Müller, 1989).

Many of these traits are accommodated by *Casuarina* and *Alnus*, the main two genera of actinorhizal trees with uses and applications in agroforestry. Both genera have been reported as shade trees in various agroforestry combinations. However, as in field conditions, variation exists in *Frankia* strains in terms of infectiveness (the ability of a strain to nodulate a given host) and effectiveness (the relative capacity of the actinorhiza, once established, to fix N_2). A matter still to be answered in these agroforestry system is whether the level of nitrogen fixation achieved in actinorhizal nodules reflects the infectiveness and effectiveness of the *Frankia* strain or rather the inhibition (or promotion) by the level of N-fertility in the soil (Russo, 1990). This debate continues, even though it is known, under experimental conditions, that the combined-nitrogen level inhibits N_2 fixation (Arnone *et al.*, 1994; Huss-Danell, 1997). Dommergues (1997) states that the nitrogen-fixing potential of a number of actinorhizal plants, *e.g.* *Casuarina* sp. and *Alnus* sp., is high, but the amount of N_2 actually fixed in the field is often low because the expression of this potential is limited by either unfavourable environmental conditions or improper management practices. For instance, current N-fertilization in pastures associated with *Alnus acuminata,* as practiced by Costa Rican farmers (92 kg of ammonium nitrate every five weeks per hectare), may be depressing nodulation and nitrogen fixation (Russo, 1990).

7. THE GENUS *CASUARINA*

The genus *Casuarina* is one of the better known and disseminated actinorhizal trees. Today, *Casuarina* plantations are of large social and economic importance throughout the tropical regions of the world, given that there is a global trend towards greater reliance on plantations as a source of industrial wood. The global area planted with different *Casuarina* species reached 1.4 million ha around the world (Vercoe, 1993). Another more modest figure (Krishnapillay, 2000), based on unpublished data from D. Pandey, is 787 x 10^3 hectares in the tropical regions.

The world-wide interest in several species of the Casuarinaceae family has resulted in three international Casuarina Workshops. The First Workshop was held in Canberra, Australia, in 1981; the second in Cairo, Egypt, in 1990, and the third in Da Nang, Vietnam, in 1996. At the third workshop, thirty five papers, which covered genetics and tree breeding, reproductive biology, nitrogen fixation, silviculture, diseases, natural distribution and ecology, socio-economic aspects, and utilization, were presented (Pinyopusarerk *et al.*, 1996). *Casuarina equisetifoli*a has been planted extensively in the coastal areas of southern China for wood production and for stabilizing moving sands. There are currently about 300,000 ha of *Casuarina* plantations in the coastal areas of Guangdong, Hainan, Fujian, Zhejiang, and Guangxi Autonomous Region, bordering the South China Sea (Bai Jiayu and Zhong Chongu, 1996).

Bourke (1985) described an agroforestry farming system from the Papua New Guinea highlands (1400-2100m altitude), which was developed by village growers in about 1960 and has expanded rapidly since about 1970, where the tree component is *Casuarina oligodon* associated with numerous species of annual and perennial food crops (especially bananas) and arabica coffee. The system provides food, a cash crop, and timber for construction and fuel.

Another agroforestry experience involving two actinorhizal trees was in Guatemala, in two sites at the upper watershed area of the Achiguate River. Here, research plots were established in 1987 and included *Casuarina equisetifolia*, *Alnus acuminata*, and *Eucalyptus globulus*, all associated and non-associated to annual crops (maize and beans). After four years, it was reported that crop production decreased in all treatments where the crops were associated with trees, but the diminishing rates were less when the crop was associated with *Casuarina equisetifolia* when compared with the other tree species (Leiva and Borel, 1995). It is not clear whether the higher yields of the annual crops can be attributed to the relatively poorer growth of the *Casuarina* trees. No measurements of nitrogen fixation are available, although relatively high fixation rates have generally been reported for both actinorhizal species.

Erosion control, sand dune stabilization, and soil reclamation have been the most frequently mentioned environmental uses of *Casuarina* (NCR, 1984). More recently, in an extensive review on ecological restoration of land, with particular reference to the mining of metals and industrial minerals, Cooke and Johnson (2002) present an example of pioneer work done on the coast of Kenya on ecosystem restoration of exhausted quarries. They report that the land restoration is attributable to the establishment of 26 tree species and grasses, centered around *Casuarina equisetifoli*a. They also mention that this nitrogen-fixing tree is salt-tolerant and acted as the catalyst for ecosystem development, perhaps by favoring the increase of a millipede population (*Epilobus pulchripe*s). This arthropod fed on dry needles of *Casuarin*a, which when digested initiated humus formation and secondary colonization by plants and animals. Over 250 plant species now inhabit the quarry habitat, an achievement that was largely made without fertilizers or soil amendments. In Argentina, *Casuarina cunninghamiana* was planted along the river borders (in the delta of the Parana River) to protect the banks from erosion by waves (personal observation). In Colombia, *Casuarina equisetifolia* trees were planted along the borders of a drainage channel to protect the side slope in the "El Hatico" Farm in the Cauca River Valley. However, the Farm Manager decided to eliminate all the trees, some of them with diameters over 30 cm, after seeing that where *Casuarina* leaf litter was present "no other plants had grown under those trees" (Prof. Raúl Botero, personal communication). This field observation on allelopathy was validated by a study of Jadhav and Gaynar (1995) in which C*asuarina equisetifolia* leaf litter substances showed allelopathic effects on germination and seedling growth of rice and cowpea.

8. THE EXPERIENCE OF THE CENTRAL AMERICA FUELWOOD PROJECT

The genus *Casuarina* in America has experienced a high degree of deforestation and degradation of its natural environment, resulting in a scarcity of forest products, such as timber and fuelwood. As a possible solution, the development of agroforestry systems has become a priority in the region. Facing this challenge, The

Tropical Agricultural Research and Training Center (CATIE, 1991) responded by developing (1981-1984) a project in Central American, which is known as the Fuelwood and Alternative Energy Sources Project. In a second phase (1985-1990), the project was renamed as the Multi-purpose Tree Cultivation or Madeleña (timber and fuelwood) Project. In a third phase (1991-95), the project was committed to dissemination of multi-purpose tree cultivation (Belaunde and Rivas, 1993).

The Project became part of the Forestry Action Plan for Central America (PAFCA), under which a set of complementary projects were implemented in each Central American country. More than 100 forestry species were identified, both exotic and native, with good potential for widespread cultivation in the region. On-farm research methodologies were implemented and training was offered to technical staff of the different forestry institutions of the region. As a result, multi-purpose, fast-growing tree species began to be included in project research and demonstration sites. *Casuarina equisetifolia* was one of the successful species included in these trials due to its characteristics of fast growth, nitrogen-fixation capability and good fuelwood quality (CATIE, 1991). The initial growth results of the species in Costa Rica demonstration plots are summarized in Table 4.

Table 4. Growth of Casuarina equisetifolia *L. ex J.R. Forst. and G. Forst in Central America Country Plots. After CATIE,1991.*

Country	Site	Age (months)	Diameter		Height	
			Mean (cm)	MAI* (cm/yr)	Mean (m)	MAI (m/yr)
Guatemala	Amatitlán	244	14.60	0.72	15.50	0.76
	San Pedro	42	3.95	1.13	4.45	1.27
	La Conora	95	8.20	1.04	10.90	1.38
Honduras	F. Morazán	41	4.68	0.97	4.50	0.93
	V. Angeles	29	2.84	1.17	3.79	1.65
Costa Rica	Piedades S.	57	6.81	1.43	6.23	1.32
	Piedades N	53	7.81	1.77	6.68	1.51
	Sarchí	68	11.18	1.97	9.96	1.75
Panama	Coclé	39	2.30	0.71	2.60	0.80
	Coclé	35	2.40	0.82	300	1.03
Nicaragua	El Gurú	43	5.47	1.52	6.97	2.08
	Tipitapa	31	3.84	1.48	4.30	1.66
	UCA	58	9.69	2.01	7.80	1.61

* MAI = mean annual increment.

The best results were observed in Nicaragua, where height reached 2.08 meter per year, followed by those in Costa Rica and Honduras (Table 4). When the species was associated with other crops in agroforestry systems, growth in height was higher than reached in monoculture plots independently of tree density. In some cases, growth reached 2.8 m height in the first year. *Casuarina* was associated with beans and maize in Costa Rica and El Salvador. It was also planted both as a windbreak and associated with ornamental plants (*Dracaena* sp.).

9. THE CASE OF *ALNUS ACUMINATA* IN TROPICAL HIGHLANDS

Alnus acuminata is a fast-growing species valued for its wood, watershed protection, and soil improvement. Native from Mexico to Northern Argentina, it is known as: aliso (Mexico, Argentina, Colombia, Ecuador, and Peru); aile, ilite (Mexico); ramrám, lambdn (Guatemala, Costa Rica, and Peru); jaúl (Costa Rica); palo de lama (Guatemala); and cerezo and chaquiro (Colombia). Easily propagated either from seed or by natural regeneration, *A. acuminata* is a popular agroforestry species in its native range (Figures 5 and 6). It has been successfully introduced into southern Chile and southern New Zealand.

Figure 5. Alnus acuminata in a pasture in San Jerónimo, Costa Rica. Photo: R.O. Russo.

It grows in moist soil environments, usually along the banks of streams, rivers, ponds, and swamps, where it typically forms dense pure stands. It also associates with wet flood plains, or moist mountain slopes. It may be adapted to somewhat drier conditions, however, it is usually restricted to zones with extra soil moisture, such as cool tropical highlands and cool high-latitude regions with abundant rainfall where mist and cloud cover can be a source of fog-drip precipitation. In the tropical highlands of Central and South America, clouds and mist are important in supporting *Alnus acuminata* and grass, when associated, through the dry season. *Alnus acuminata* prefers deep, well-drained soils with high organic matter content, however, it is commonly found growing on shallow soils, such as landslides. Rojas *et al.* (1991) report that it will grow in soil with pH as low as 4.5.

Figure 6. Nodule of Alnus acuminata from San Jerónimo, Costa Rica. Photo: R.O. Russo.

Farmers in Costa Rica have grown *Alnus acuminata* in pastures and as a shade tree for coffee crops for more than 100 years. Trees are either regenerated naturally or planted from nursery stock at a spacing of 8-14 m (about 100 trees/ha). According to the farmers, one benefit of including trees in cattle pastures is greater milk production.

Alnus acuminata is also cultivated in plantations mainly in Colombia and Costa Rica, but in other countries as well. In Colombia, an initial spacing of 2.6 x 2.6 m (1,480 trees/ha) is common (Sicco Smit, 1971). In Costa Rica, an initial spacing of 3 x 3 m is preferred. At least two thinnings are recommended, the first after the third year and the second after 10-15 years, leaving 250-350 trees per hectare. Trees are harvested in rotations of about 20 years. Average annual wood production is 15-20 m^3 per hectare. According to Canet (1985), a stand of 30-year-old trees with a density of 35 trees/ha yielded 70 m^3/ha of timber, 18.3 ton/ha of dry fuelwood, and 3.6 ton/ha of leaves and fine branches. *Alnus acuminata* resprouts vigorously from the stump after cutting.

Another interesting experience in the eastern Himalayas is the *Alnus*-cardamom agroforestry system, where cardamom (*Amomum subulatum*), the most important perennial cash crop in the region, is cultivated predominantly under the shade of N_2-fixing *Alnus nepalensis* (Sharma *et al.,* 2000). The biomass, net primary productivity, energetics, and energy efficiencies of the system at different ages (from 5 to 40 years) were determined and the impact of stand age on the performance of mixtures *Alnus* and cardamom plantations was evaluated. The results showed that net primary productivity was lowest (7 t ha^{-1} $year^{-1}$) in the 40-

year-old stand and was more than three-times higher (22 t ha^{-1} $year^{-1}$) in the 15-year-old stand. The agronomic yield of large cardamom peaked between 15 and 20 years of age, whereas cardamom productivity doubled from the 5- to the 15-year-old stand and then decreased with plantation age to reach a minimum in the 40-year-old stand. The annual net energy fixation was highest (444 x 10^6 kJ ha^{-1} $year^{-1}$) in the 15-year-old stand, which was 1.4-times that of the 5-year-old stand and 2.9-times that of the 40-year-old stand. The results indicate that the younger plantations are more productive and also suggest that the beneficial association can last 20 years or longer if replanting is undertaken.

10. OTHER USES OF ACTINORHIZAL TREES

The environmental benefits provided by the actinorhizal trees also include soil reclamation and erosion control on steep slopes; other uses have been reported in the literature. For instance, *Alnus rubra* (Red alder) is considered the most important commercial hardwood of the Pacific Northwest of the United States (Leney *et al.*, 1978). Red alder also contains salicin, which chemically is closely related to acetylsalicylic acid (commonly known as aspirin), which is probably why Native Americans used various preparations for medicinal purposes. Native Americans also used the wood for various utensils (Arno and Hammerly, 1977).

Casuarina wood is known as the best firewood in the world with a calorific value greater than 20,000 kJ/kg, and also is excellent for charcoal making. The wood also has many other uses, including for poles, posts, roundwood for fencing, beams, roofing shingles, paneling, furniture, marine pilings, tool handles, and cabinets. The wood, however, is subject to cracking and splitting. *Casuarina equisetifolia* is used for paper pulp (CATIE, 1991; NRC, 1984). Additionally, some medicinal uses have been reported for *Casuarina equisetifolia.* According to Duke (1972), the fruit is mixed with powdered nutmeg to treat toothaches; ashes may be used to make soap; and the bark is rich in tannin and it is said to be antidysenteric and emmenagogic, and is also used in gargles for sore throat.

Many of the actinorhizal species of the New World, such as *Alnus rhombifolia* (white alder), *Cercocarpus betuloides* (birchleaf mountain-mahogany), *C. ledifolius* (Curlleaf mountain-mahogany), *Elaeagnus angustifolia* (Russian-olive), *E. umbellata* (autumn olive), *Myrica cerifera* (southern bayberry), *Purshia mexicana* var. *stansburiana* (Stansbury cliffrose), and *Sheperdia argentea* (silver buffaloberry), are important to wildlife such as deer, mule deer, elk, pronghorn, grizzly bear, a variety of birds and mammals, and also livestock (Davis, 1990; Sampson and Jespersen, 1963; USFS, 1937)

The development of an insect repellent made with an oil from *Myrica gale* has created a need for cultivation of the species. In addition to oil production, other compounds, such as pharmacologically active flavonoids, can also be obtained by extraction of the byproducts, so adding more value to the species. Because *M. gale* thrives on well-aerated acid peatland, it could become a valuable crop on lands of low agricultural value. *M. gale* could also be used either with other crops in agroforestry systems or combined with softwood forestry because those crops and trees would benefit from the soil-nitrogen enrichment due to the symbiotic association of *M. gale and Frankia* (Simpson *et al*., 1996).

The production of antibiotics is another potential use for *Frankia.* Some studies demonstrate that *Frankia* has the potential to inhibit growth of competing soil microbes by producing antibiotic compounds. Lang (1999) showed that *Frankia* strains, which were isolated from different *Casuarina* sp., produced metabolites active against Gram-negative bacteria. More recently, Haansuu *et al.* (2001) and Klika *et al.* (2001) isolated and described the biological activity of frankiamide, an antibiotic isolated from *Frankia.* This compound, also known as demethyl (C-11) cezomycin, showed strong activity against Gram-positive bacteria, particularly pathogenic *Clavibacter michiganensis* subsp. *Sepedonicus*, as well as against several plant pathogenic fungal strains (Haansuu, 2002).

11. CONCLUDING CONSIDERATIONS

Actinorhizal trees, in general, have practical importance because of their characteristic features, such as the environmental benefits they provide. These benefits include nitrogen fixation, erosion control, soil conservation, regeneration of degraded soils, climate-change mitigation through carbon sequestration, micro-climate regulation through a windbreak effect, pollination, and biodiversity and wild-life conservation.

With reference to the symbiosis itself, Huss-Danell (1997) remarks that: (i) *Frankia* actinorhiza shows host-determined variations the infection pathway, the morphology and the anatomy of nodules, and in the differentiation process of *Frankia* cells in the nodules; (ii) nitrogenase in *Frankia* is localized in vesicles, except for *Casuarina* and *Allocasuarina* nodules where only filaments (hyphae) are formed; (iii) both nodulation and nitrogen fixation are influenced by environmental factors; and (iv) unlike legume nodules, actinorhizal nodules are not O_2 limited and *Frankia* itself appears able to protect nitrogenase from O_2 inactivation. All these features are remarkable in themselves.

In the case of *Alnus* and *Casuarina* species, there are countless forestry and agroforestry applications. As agroforestry is a land-use system that involves the growing of crops and woody perennials on the same land unit in space or time, both genera may be useful as tree components in these systems. Many scientists agree that the actinorhizal-tree properties, which are based on both their nitrogen-fixation and soil-restoration capabilities, are real and not just conceptual.

Alnus and *Casuarina* are important to rural households throughout the tropics, providing a variety of products and services. Fast-growing *Casuarina* makes excellent fuelwood and charcoal. The deeply penetrating and wide spreading roots of *Casuarina* can reach moisture during dry seasons and also protect stream banks from erosion. Windbreaks of *Casuarina* planted in single or multiple rows on windward field boundaries help prevent soil desiccation and yield secondary tree products. *Casuarina* has been planted for dune stabilization in over 1,000,000 hectares in China. In addition to *Alnus* and *Casuarina*, species of *Eleagnus* and *Shepherdia* also have the potential to add large amounts of fixed N and C to soils and they should be given more consideration as soil-improvement tools. Nitrogen dynamics is favored by tree litter in agroforestry systems where actinorhizal trees (*Alnus* and *Casuarina*) are interplanted with agricultural crops. For instance, alder leaves decompose very rapidly and their nutrients are incorporated into the soil.

Research on nitrogen-fixing, actinorhizal trees has basically focused on the physiology of the endosymbiont. However, taking into consideration that sustainability is a desired property in current agricultural systems, research and development of agroforestry combinations, including actinorhizal trees, should receive more attention, especially as they relate to crop yield, the biomass yield, and the interaction of crops and trees. Additionally, depending on local needs and preferences, a variety of different planting schemes with actinorhizal trees can be utilized to yield a wide variety of products.

A nursery stock of actinorhizal species has been experimentally inoculated with *Frankia* and mycorrhizal fungi prior to planting in order to increase successful nodulation and mycorrhizal infection. However, this experiment has not been attempted on a large scale. Some authors question whether techniques for the mass-culturing of *Frankia* and the inoculation of rangelands might become a possible management tool for stimulating the vigor of actinorhizal shrub stands.

The study of the actinorhizal symbiosis is still in an early phase. Because of the abundance of wild actinorhizal plants and their importance in natural ecosystems, the group is gaining recognition as valuable trees and shrubs for diverse ecological roles in natural ecosystems, including food and cover for wildlife and livestock. Such is the case of many of the actinorhizal shrubs in the Rhamnaceae and Rosaceae families, which are adapted to survive on the harsh rangelands in the western regions of North America.

REFERENCES

Arahou, A., and Diem, H. G. (1997). Iron deficiency induces cluster (proteoid) root formation in *Casuarina glauca*. *Plant Soil*, *196*, 71–79.

Arno, S. F., and Hammerly, R. P. (1977). *Northwest trees* (222 p.). Seattle, WA: The Mountaineers.

Arnone, J. A., III., and Gordon, J. C. (1990). Effect of nodulation, nitrogen fixation and CO_2 enrichment on the physiology, growth and dry mass allocation of seedlings of *Alnus rubra* Bong. *New Phytol., 116*, 55-66.

Arnone, J. A., III., Kohls, S. J., and Baker, D. D. (1994). Nitrate effects on nodulation and nitrogenase activity of actinorhizal *Casuarina* studied in split-root systems. *Soil Biol. Biochem., 26*, 599–606.

Bai J., and Zhong C. (1996). Management of *Casuarina* plantations in China. In K. Pinyopusarerk, J. W. Turnbull and S. J. Midgley (Eds.), *Current research and development in Casuarinas. Proceedings of the Third International Casuarina Workshop*. (pp 196-199). Canberra, Australia: CSIRO Forestry and Forest Products.

Baker, D. (1990a). Actinorhizal plants: Underexploited trees and shrubs for forestry and agroforesty. *Nitrogen Fixing Tree Research Reports*, *8*, 3-7.

Baker, D. (1990b). Optimizing actinorhizal nitrogen fixation and assessing actual contribution under field conditions. In: Werner, D. and Müller, P. (Eds.), *Fast growing trees and nitrogen fixing trees.* (pp. 291-299). Stuttgart, Germany: G. Fischer Verlag.

Baker, D., and Torrey, J. G. (1979). The isolation and cultivation of actinomicetous root nodule endophytes. In J. C. Gordon, C. T. Wheeler, and D. A. Perrin (Eds.), *Symbiotic nitrogen fixation in the management of temperate forest.* (pp. 38-56). Corvallis, OR: Oregon State University.

Baker, D., Torrey, J. G., and Kidd, G. H. (1979). Isolation by sucrose density fractionation and cultivation *in vitro* of actinomycetes from nitrogen-fixing root nodule. *Nature*, *281,* 76-78.

Baker, D., and Miller, N.G. (1980). Ultrastructural evidence for the existence of actinorhizal symbioses in the late Pleistocene. *Can. J. Bot.*, *58*, 1612-1620.

Baker D. D., and Schwintzer C. R. (1990). Introduction. In D. D. Baker, J. D. Tjepkema, and C. R. Schwintzer (Eds), *The biology of* Frankia *and actinorhizal plants*. San Diego, CA: Academic Press, Inc.

Baker D. D., and Mullin B. C. (1992). Actinorhizal symbioses. In G. Stacey, R. H. Burns and H. J. Evans (Eds.). *Biological nitrogen fixation*. New York, NY: Routledge, Chapman and Hall.

Barea, J. M., and Azcón-Aguilar, C. (1983). Mycorrhizas and their significance in nodulating nitrogen-fixing plants. *Adv. Agron.*, *36*, 1-54.

Beaupied, H., Moiroud, A. Domenach, A. M. Kurdali, Fawaz, A., and Lensi, R. (1990). Ratio of fixed and assimilated nitrogen in a black alder (*Alnus glutinosa*) stand. *Can. J. Forest Res., 20*, 1116-1119.

Becking, J. H. (1974). Family III. Frankiaceae. In R. E. Buchanan, and N. E. Gibbon (Eds.), *Bergey's manual of determinative bacteriology*, 8th ed. (pp. 701-706). Baltimore, MD: The Williams and Wilkins Co.

Beer, J. (1987). Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. *Agroforestry Systems*, *5*, 3-13.

Benoit L. F., and Berry A. M. (1997). Flavonoid-like compounds from seeds of red alder *(Alnus rubr*a) influence host nodulation by *Frankia* (Actinomycetales). *Physiol. Plant., 99*, 588-593.

Benson, D. R., Stephens, D. W., Clawson, M. L., and Silvester, W. B. (1996). Amplification of 16S rRNA genes from *Frankia* strains in root nodules of *Ceanothus griseus, Coriaria arborea, Coriaria plumose, Discaria toumatou,* and *Purshia tridentata*. *Appl. Environ. Microbiol.*, *62*, 2904-2909.

Benson, D. R., and Silvester, W. B. (1993). Biology of *Frankia* strains, actinomycetes symbionts of actinorhizal plants. *Microbiol. Rev., 57*, 293–319.

Benson, D. R., and Clawson, M. L. (2000). Evolution of the actinorhizal plant symbiosis. In E. Triplett (Ed.), *Prokaryotic nitrogen fixation: A model system for the analysis of a biological process*. (pp. 207-224). Wymondham, UK: Horizon Scientific Press.

Berry, A. M. (1987). Cellular aspects of root nodule establishment in *Frankia* symbioses. In T. Kosuge, and E. W. Nester (Eds.), *Plant-microbe interactions: Molecular and genetics perpectives, Vol. 2*. (pp. 194-213). New York, NY: Macmillan Publishing Company.

Berry, A. M. (1994). Recent developments in the actinorhizal symbioses. *Plant Soil*, *161*, 135-145.

Berry A. M. (1998). Oxygen relations in *Frankia* and in *Actinorhizal* nodules. In C. Elmerich, A. Kodorosi, and W. E. Newton, (Eds.). *Biological nitrogen fixation for the 21st century*. (pp. 357-358). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Bertalot, M. J. A., and Mendoza, E. (1998). An observation of aerial nodulation of *Casuarina equisetifolia* in an agroforestry system in Brazil. *Forest, Farm, and Community Tree Research Reports*, *3*, 61-65.

Bino, B., and Kanua M. B. (1996) Growth performance, litter yield and nutrient turnover of *Casuarina oligodon* in the Highlands of Papua New Guinea. In K. Pinyopusarerk, J. W. Turnbull, and S. J. Midgley (Eds.), R*ecent* Casuarina *research and development: Proceedings of the Third International Casuarina Workshop*, Da Nang, Vienam. (pp 167-170). Canberra, Australia: Forestry and Forest Products, CSIRO.

Bourke, R.M. (1985). Food, coffee and casuarina: An agroforestry system from the Papua New Guinea highlands. *Agroforestry Systems*, *2*, 273-279.

Bourke, R. M. (1997). *Management of fallow species composition with tree planting in Papua New Guinea.* Canberra, Australia: Resource Management in Asia-Pacific Project, Division of Pacific and Asian History, Research School for Pacific and Asian Studies, The Australian National University.

Brewbaker, J. L., Willers, K. B., and Macklin, W. (1990). Nitrogen fixing trees; Validation and prioritization. *Nitrogen Fixing Tree Research Reports, 8,* 8-16.

Busse M. D. (2000a). Ecological significance of nitrogen fixation by actinorhizal shrubs in interior forests of California and Oregon. In R. F. Powers, D. L. Hauxwell, and G. M. Nakamura (Eds.), *Proceedings of the California Forest Soils Council conference on forest soils biology and forest management*, Sacramento, CA. Gen. Tech. Rep. PSW-GTR-178. (pp.23-41). Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture.

Busse M. D. (2000b). Suitability and use of the ^{15}N-isotope dilution method to estimate nitrogen fixation by actinorhizal shrubs. *Forest Ecology and Management*, *136*, 85-95.

Callaham, D., Del Tredici, P., and Torrey., J. G. (1978). Isolation and cultivation *in vitro* of the actinomycete causing root nodulation in *Comptonia*. *Science*, *199*, 899-902.

Canet, G. C. (1985). Caracteristicas del sistema silvo-pastoril jaúl (*Alnus acuminata*) con lechería de altura en Costa Rica. In R. Salazar (Ed.), *Técnicas de producción de leña en fincas pequenas. Actas de los simposios.* (pp. 241-249). Turrialba, Costa Rica: CATIE.

Carlson, P. J., and Dawson, J. O. (1985). Soil nitrogen changes, early growth, and response to soil internal drainage of a plantation of *Alnus jorullensis* in the Colombian highlands. *Turrialba*, *35*, 141-150.

Centro Agronómico Tropical de Investigación y Enseñanza – CATIE. (1991). *Casuarina: Casuarina equisetifolia L. Ex J.R. Forst and Forst., árbol de uso múltiple en América Central* (53 p.). Turrialba, Costa Rica: CATIE.

Cervantes, E., and Rodríguez-Barrueco, C. (1992). Relationships between the mycorrhizal and actinorhizal symbioses in non-legumes. *Methods in Microbiology*, *24*, 417-432.

Cervantes, E., and Rodriguez-Barrueco, C. (1994). Relationships between the mycorrhizal and actinorhizal symbioses in non-legumes. In J. R. Norris, D. Read, and A. K. Varma (Eds.), *Techniques for mycorrhizal research methods in microbiology*. (pp. 877-891). San Diego, CA: Academic Press, Inc.

Chatarpaul, L., Chakravarty, P., and Subramaniam, P. (1989). Studies in tetrapartite symbioses. I. *Role of ecto- and endomycorrhizal fungi and* Frankia *on the growth performance of* Alnus incana. *Plant Soil, 118*, 145-150.

Clawson, M. L., and Benson D. R. (1999). Natural diversity of *Frankia* strains in actinorhizal root nodules from promiscuous hosts in the family Myricaceae. *Appl. Environ. Microbiol.*, *65*, 4521–4527.

Cote, B., and Camire, C. (1984). Growth, nitrogen accumulation, and symbiotic dinitrogen fixation in pure and mixed plantings of hybrid poplar and black alder. *Plant Soil, 78*, 209-220.

Cronquist, A. (1981). *An integrated system of classification of flowering plants*. New York, NY: Columbia University Press.

Cruz-Cisneros, R., and Valdés, M. (1991). Actinorhizal root nodules on *Adolphia infesta* (H.B.K.) Meissner (Rhamnaceae). *Nitrogen Fixing Tree Res. Rep.*, *9*, 87-89.

Davis, J. N. (1990). General ecology, wildlife use, and management of the mountain mahoganies in the intermountain West. In: K. L. Johnson (Ed.), *The genus* Cercocarpus*, Proceedings of the 5th Utah Shrub Ecology Workshop* (pp. 1-13). Logan, UT: Utah State University, College of Natural Resources.

Dawson J. O. (1986). Actinorhizal plants: Their use in forestry and agriculture. *Outlook on Agriculture,* 15, 202-208.

Dell, B., Malajczuk, N., Bougher, N. L., and Thomson, G. (1994). Development and function of *Pisolithus* and *Scleroderma* ectomycorrhizas formed in vivo with *Allocasuarina*, *Casuarina* and *Eucalyptus*. *Mycorrhiza, 5*, 129-138.

Diem, H. G. (1997). Mycorrhizae of antinorhizal plants. *Acta Bot. Gall.*, *143*, 581-592.

Diem, H. G., and Dommergues, Y. R. (1990). Current and potential uses and management of *Casuarinaceae* in the tropics and subtropics. In C. R. Schwintzer, and J. D. Tjepkema (Eds.), *The biology of Frankia and actinorhizal plants*. New York, NY: Academic Press, Inc.

Diem, H. G., and Arahou, M. (1996). A review of cluster root formation: A primary strategy of Casuarinaceae to overcome soil nutrient deficiency. In K. Pinyopusarerk, J. W. Tyrnbull, and S. J. Midgley (Eds.), *Recent Casuarina research and development. Proceedings of the Third International Casuarina Workshop, Da Nang, Vietnam.* Canberra, Australia: CSIRO, Forestry and Forest Products.

Diem, H. G., Duhoux, E., Zaid, H., and Arahou, M. (1999). Cluster roots in *Casuarina*: Role and relationship to soil nutrient factors. *XVI International Botanical Congress, Abstracts,* 4236.

Dinkelaker, B., Hengeler, C., and Marschner, H. (1995). Distribution and function of proteoid roots and other root clusters. *Bot. Acta*, *108*, 183–200.

Dobritsa, S. V. (1998). Grouping of *Frankia* strains on the basis of susceptibility to antibiotics, pigment production and host specificity. *Int. J. Syst. Bacteriol., 48*, 1265–1275.

Domenach, A. M., Kurdali, F., and Bardin, R. (1989). Estimation of symbiotic dinitrogen fixation in alder forest by the method based on natural ^{15}N abundance**.** *Plant Soil, 118*, 51-59.

Dommergues, Y. R. (1987). The role of biological nitrogen fixation in agroforestry. In H. A. Steppler, and P. K. R. Nair (Eds.), *Agroforestry: A decade of development*. Nairobi, Kenya: ICRAF.

Dommergues, Y. R. (1990). *Casuarina equisetifolia*: An old-timer with a new future**.** *Nitrogen Fixing Tree Association Highlights*,. May 1990, 1.

Dommergues, Y. R. (1997). Contribution of actinorhizal plants to tropical soil productivity and rehabilitation. *Soil Biol. Biochem*., *29*, 931-941.

Doran, J. C., and Turnbull, J. W. (1997). *Australian trees and shrubs: Species for land rehabilitation and farm planting in the tropics.* Canberra, Australia: Australian Centre for International Agricultural Research, ACIAR.

Duke, J. A. (1972). *Isthmian ethnobotanical dictionary*. Fulton, MD: Published by the author. (Currently online as *Tico Ethnobotanical Dictionary* at http://www.ars-grin.gov/duke/dictionary/tico).

Gardner, I. C. (1986). Mycorrhizae of actinorhizal plants. *Mircen J. Appl. Microbiol. Biotechnol., 2*, 147-160.

Gardner, I. C., Clelland, D. M., and Scott, A. (1984). Mycorrhizal improvement in non-leguminous nitrogen fixing association with particular reference to *Hippophae rhamnoides* L. *Plant Soil, 78*, 188-200.

Gauthier, D., Diem, H. G., and Dommergues, Y. (1983). Preliminary results of research on *Frankia* and endomycorrhizae associated with *Casuarina equisetifolia*. In S. J. Midgeley, J. W. Turnbull and R. D. Johnson (Eds.), Casuarina *ecology, management and utilization*. (pp. 211-217). Melbourne, Australia: CSIRO.

Gauthier, D. L., Diem, H. G., and Dommergues Y. R. (1984). Tropical and subtropical actinorhizal plants *Pesquisa Agropecuaria Brasileira*, *19*, 119-136.

Gauthier D., Diem, H. G., Dommergues, Y. R., and Gantry, F. (1985). Assessment of N_2 fixation by *Casuarina equisetifolia* inoculated with *Frankia* ORS02001 using ^{15}N methods**.** *Soil Biol. Biochem.*, *17*, 375-379.

Gauthier, D., Jaffre, T., and Prin, Y. (1999). Occurrence of both *Casuarina*-infective and *Elaeagnus*-infective *Frankia* strains within actinorhizae of *Casuarina collina*, endemic to New Caledonia. *Eur. J. Soil Biol.*, *35*, 9-15.

Gauthier, D., Navarro, E., Rinaudo, G., Jourand, P., Jaffre, T., and Prin, Y. (1999). Isolation, characterisation (PCR-RFLP) and specificity of *Frankia* from eight *Gymnostoma* species endemic to New Caledonia. *Eur. J. Soil Biol.*, *9*, 199-205.

Gentili, F., and Huss-Danell, K. (2002). Phosphorus modifies the effects of nitrogen on nodulation in split-root systems of *Hippophaë rhamnoides. New Phytol., 153*, 53 –61.

Giller, K. (2001). Assessment of the role of N_2 fixation. In *Nitrogen fixation in tropical cropping systems, 2nd edit.* (pp. 71-92). Wallingford, UK: CABI *Publishing*.

Goetting-Minesky, M. P., and Mullin, B. C. (1994). Differential gene expression in an actinorhizal symbiosis: Evidence for a nodule-specific cysteine proteinase. *Proc. Natl. Acad. Sci. USA, 91*, 9891-9895.

Gualtieri, G., and Bisseling, T. (2000). The evolution of nodulation. *Plant Mol. Biol*., *421*, 81-94.

Haansuu, J. P. (2002). *Demethyl (C-11) cezomycin - a novel calcimycin antibiotic from the symbiotic, N_2-fixing actinomycete* Frankia. Academic Dissertation in General Microbiology. Helsinki, Finland: Graduate School in Microbiology, University of Helsinki.

Haansuu J. P., Klika, K. D., Söderholm, P. P., Ovcharenko, V. V., Pihlaja, K., Haahtela, K. K., and Vuorela, P. M. (2001). Isolation and biological activity of frankiamide. *J. Ind. Microbiol. Biotechnol*., *27*, 62-66.

Herdman, L., Skene, K. R., and Raven, J. A. (1999). Structural-functional relations in cluster roots of *Myrica gale* L. *XVI International Botanical Congress, Abstracts,* 4478.

Hodge, S., Garrett, H. E., and Bratton, J. (1999). Alley cropping: An agroforestry practice. *Agroforestry Notes*, *12*, 1-4.

Huguet, V., Batzli, J. M., Zimpfer, J. F., Normand, P., Dawson, J. O., and Fernandez, M. P. (2000). Diversity and specificity of *Frankia* strains in nodules of sympatric *Myrica gale*, *Alnus incana*, and *Shepherdia canadensis* determined by rrs gene polymorphism. *Plant Mol. Biol*., *42*, 181-194.

Hurd, T. M., and Schwintzer, C. R. (1996). Formation of cluster roots in *Alnus incana* ssp. *rugosa* and other *Alnus* species. *Can. J. Bot*., *74*, 1684–1686.

Hurd, T. M., and Schwintzer, C. R. (1997). Formation of cluster roots and mycorrhizal status of *Comptonia peregrina* and *Myrica pensylvanica* in Maine, U.S.A. *Physiol. Plant., 99*, 680-689.

Huss-Danell, K. (1997). Actinorhizal symbioses and their N_2 fixation. *New Phytol., 136*, 375-405.

International Center for Research in *Agroforestry* - ICRAF. (2002), Did you know? Agroforestry facts. ICRAF Website. Retrieved June 12, 2002, from http://www.icraf.cgiar.org/ag_facts/ag_facts.htm.

Jadhav, B. B., and Gaynar, D. G. (1995). Effect of *Casuarina equisetifolia* leaf litter leachates on germination and seedling growth of rice and cowpea. *Allelopathy J.*, *2*, 105-108.

Jurgensen, M. F., Graham, R. T., Larsen, M. J., and Harvey, A. E. (1992). Clear-cutting, woody residue removal, and nonsymbiotic nitrogen fixation in forest soils of the inland Pacific Northwest. *Can. J. Forest Res.*, *22*, 1172-1178.

Khan, A. G. (1993). Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. *Mycorrhiza, 3*, 31-38.

Klika, K. D., Haansuu, J. P., Ovcharenko, V. V., Haahtela, K. K., Vuorela, P. M., and Pihlaja, K. (2001). Frankiamide, a highly unusual macrocycle containing the imide and orthoamide functionalities from the symbiotic actinomycete *Frankia. J. Org. Chem.*, *66*, 4065-4068.

Kohls, S. J., Thimmapuram, J., Buschena, C. A., Paschke, M. W. and Dawson, J. O. (1994). Nodulation patterns of actinorhizal plants in the family Rosaceae. *Plant Soil*, *162*, 229-239.

Krishnapillay, B. (2000). Silviculture and management of teak plantations. *Unasylva*, *51*, 14-21.

Lalonde, M. (1979). A simple and rapid method for the isolation and cultivation *in vitro* and characterization of *Frankia* strains from *Alnus* root nodules. In J. C. Gordon, C. T. Wheeler, and D. A. Perrin (Eds.), *Symbiotic nitrogen fixation in the management of temperate forest.* (p. 1180). Corvallis, OR: Oregon State University.

Lalonde, M., Calvert, H. E., and Pine, S. (1981). Isolation and use of *Frankia* strains in actinorhizae formation. In A. H. Gibson and W. E. Newton (Eds.), *Current perspectives in nitrogen fixation*. (pp. 296-299). Canberra, Australia: Australian Academy of Sciences.

Lang, L. (1999). Inhibition of bacterial wilt growth by *Frankia* isolated from Casuarinaceae. *Forest Res.*, *12*, 47-52.

Leiva, J. M., and Borel, R. (1995). Evaluación de tres especies forestales en plantación pura y sistema taungya: Crecimiento de los árboles y producción de los cultivos. *Nitrogen Fixing Tree Research Reports*, Special Issue, 85-93.

Lechevalier, M. P., and Ruan, J. (1984). Physiology and chemical diversity of *Frankia* spp. isolated from nodules of *Comptonia peregrina* (L) Coul and *Ceanothus americanus* L. In A. D. L. Akkermann, D. D. Baker, K. Huss-Danell, and J. D. Tjepkema (Eds.), Frankia *symbiosis*. (pp. 15-22). The Hague, The Netherlands: Martinus Nijhoff/Dr. W. Junk.

Leney, L., Jackson, A., and Erickson, H. D. (1978). Properties of red alder (*Alnus rubra* Bong.) and its comparison to other hardwoods. In D. G. Briggs, D. S. DeBell and W. A. Atkinson (Edds.), *Utilization and management of alder: Proceedings of a symposium* (pp. 25-33) Ocean Shores, WA: U.S. Department of Agriculture, Forest Service.

Lie, T. A., Akkermans, A. D. L., and van Egeraat, A. W. S. M. (1984). Natural variation in symbiotic nitrogen fixing *Rhizobium* and *Frankia* spp. *Antonie van Leeuwenhoek*, *50*, 489-503.

Liu, Q., and Berry, A. M. (1991). Localization and characterization of pectin polysaccharides in roots and root nodules of *Ceanothus* spp. during intercellular infection by *Frankia*. *Protoplasma*, *163*, 93-101.

McNabb, D. H., and Cromack, K., Jr. (1983). Dinitrogen fixation by a mature *Ceanothus velutinus* (Dougl.) stand in the western Oregon Cascades. *Can. J. Microbiol., 29*, 1014-1021.

Miller I. M., and Baker D. D. (1985). The initiation, development and structure of root nodules in *Elaeagnus angustifolia* L. (Elaeagnaceae). *Protoplasma, 128*, 107-119.

Miller I. M., and Baker D. D. (1986). Nodulation of actinorhizal plants by *Frankia* strains capable of both root hair infection and intercellular penetration. *Protoplasma, 131*, 82-91.

Mullin, B. C., and Dobritsa, S. V. (1996). Molecular analysis of actinorhizal symbiotic systems: Progress to date. *Plant Soil*, *186*, 9-20.

Markham, J., and Chanway, C. P. (1998). *Alnus rubra* (Bong.) nodule spore type distribution in southwestern British Columbia. *Plant Ecol.*, *135*, 197.

Nair, P. K. R. (1990). *The prospects for agroforestry in the tropics.* Technical Paper Number 131. Washington, D.C.: The World Bank.

Newton, M., El Hassen, B. A., and Zavitkovski, J. (1968). Role of red alder in western Oregon forest succession. In J. M. Trappe et al. (Eds.), *Biology of alders.* (pp. 73-84). Portland, OR: U.S. Department of Agriculture.

Paschke, M. W., and Dawson, J. O. (1992). Avian dispersal of *Frankia. Can. J. Bot.*, *71*, 1128-1131.

Parrota, J. A., Baker, D. D., and Maurice, M. (1994). Application of ^{15}N-enrichment methodologies to estimate nitrogen fixation in *Casuarina equisetifolia***.** *Can. J. Forest Res.*, *24*, 201-207.

Paschke M. W. (1997). Actinorhizal plants in rangelands of the western United States. *Journal of Range Management*, *50*, 62-72.

Perry, T. O. (1989). Tree roots: Facts and fallacies. *Arnoldia (Magazine of the Arnold Arboretum)*, 49, 3-21.

Pinyopusarerk, K., Tyrnbull, J. W., and Midgley, S. J. (Eds.) (1996). *Recent* Casuarina *research and development. Proceedings of the Third International Casuarina Workshop. Da Nang, Vietnam.* (249p.). Canberra, Australia: CSIRO, Forestry and Forest Products.

Prin, Y., Duhoux, E., Diem, H. G., Roederer, Y., and Dommergues, Y. (1991a). Nitrogen-fixing aerial nodules on the trunks of *Casuarina cunninghamiana. Nitrogen Fixing Tree Research Reports*, *9*, 100-101.

Prin, Y., Duhoux, E., Diem, H. G., Roederer, Y., and Dommergues, Y. (1991b). Aerial nodules in *Casuarina cunninghamiana*. *Appl. Environ. Microbiol., 57*, 871-874.

Provorov, N. A., Borisov, A. Y., and Tikhonovich, I. A. (2002). Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscular mycorrhiza. *J. Theor. Biol., 214*, 215-232.

Quispel, A, and Burggraff, A. J. P. (1981). *Frankia* the diazotrophic endophyte from actinorhizas. In A. H. Gibson and W. E. Newton (Eds.), *Current perspectives in nitrogen fixation*. (pp. 229-236). Canberra, Australia: Australian Academy of Sciences.

Rose, S. L. (1980). Mycorrhizal associations of some actinomycete nodulated nitrogen-fixing plants. *Can. J. Bot.*, *58*, 1449-1454.

Rose, S. L., and Trappe, J. M. (1980). Three new endomycorrhizal *Glomus* spp. associated with actinorhizal shrubs. *Mycotaxon.*, *10*, 413-420.

Rose, S. L., and Youngberg, C. F. (1981). Tripartite associations in snowbrush (Ceanothus velutinus): Effect of vesicular-arbuscular mycorrhizae on growth, nodulation and nitrogen fixation. *Can. J. Bot.*, *59*, 34-39.

Russo, R. O. (1989). Evaluating *Alnus acuminata-Frankia* -mycorrhizae interactions. I. Acetylene reduction in seedlings inoculated with *Frankia* strain ArI3 and *Glomus intraradices* under three different phosphorus levels. *Plant Soil*, *118*, 151-155.

Russo, R. O. (1990). Evaluating *Alnus acuminata* as a component in agroforestry systems. *Agroforestry Systems*, *10*, 241-252.

Russo, R. O. (1995). *Alnus acuminata* ssp. *arguta* (Schectendal) Furlow: A valuable resource for neotropic Highlands. *Nitrogen Fixing Tree Research Reports*, Special Issue, 156-163.

Russo, R. O., and Berlyn, G. P. (1989). The effect of a new growth biostimulant on acetylene reduction in nodulated seedlings of *Alnus ascuminata. Abstracts of 12th North American Symbiotic Nitrogen Fixation Conference,* Ames, Iowa.

Russo, R. O., Gordon, J. C., and Berlyn, G. P. (1993). Evaluating *Alnus acuminata-Frankia*-mycorrhizae interactions. Growth response of *Alnus acuminata* seedlings to inoculation with *Frankia* strain ArI3 and *Glomus intraradices*, under three phosphorus levels**.** *J. Sustainable Forestry***,** *1*, 93-110.

Sampson, A. W., and Jespersen, B. S. (1963). *California range brushlands and browse plants.* (162 p.). Berkeley, CA: University of California, California Agricultural Experiment Station.

Schwintzer, C. R. (1990). Spore-positive and spore-negative nodules. In C. R. Schwintzer, and J. D. Tjepkema (Ed.), *The biology of* Frankia *and actinorhizal plants*. (pp. 177–193). New York, NY: Academic Press, Inc.

Schwintzer, C. R., and Tjepkema, J. D. (Eds.). (1990). *The biology of* Frankia *and actinorhizal plants*. (408 p.). New York, NY: Academic Press, Inc.

Sharma, G., Sharma, E., Sharma, R., and Singh, K. K. (2000). Performance of an age series of *Alnus*–Cardamom plantations in the Sikkim Himalaya: Productivity, energetics and efficiencies. *Ann. Bot.*, *89*, 261-272.

Sicco Smit, G. (1971). Notas silviculturales sobre *Alnus jorullensis* de Caldas, Colombia. *Turrialba, 21*, 83-88.

Simonet, P., Navarro, E., Rouvier, C., Reddell, P., Zimpfer, J., Dommergues, Y., *et al.* (1999). Co-evolution between *Frankia* populations and host plants in the family Casuarinaceae and consequent patterns of global dispersal. *Environ. Microbiol., 1*, 525-534.

Simpson, M. J. A., MacIntosh, D. F., Cloughley, J. B., and Stuart, A. E. (1996). Past, present and future utilisation of *Myrica gale* (Myricaceae). *Econ. Bot., 50*, 122-129.

Singer, R., and Gomez, L. D. (1984). The basidiomycetes of Costa Rica. III. The genus *Phylloporus* (Boletaceae). *Brenesia*, *22*, 163-181.

Skene, K. R. (1998). Cluster roots: Some ecological considerations. *J. Ecol.*, *86*, 1060–1064.

Swensen, S. M., and Mullin, B. C. (1997a). Phylogenetic relationships among actinorhizal plants: The impact of molecular systematics and implications for the evolution of actinorhizal symbioses. *Physiol. Plant., 99*, 565-573.

Swensen, S. M. and Mullin, B. C. (1997b). The impact of molecular systematics on hypotheses for the evolution of root nodule symbioses and implications for expanding symbioses to new host plant genera. *Plant Soil*, *194*, 185-192.

Tjepkema, J. D., Schwintzer, C. R., Burris, R. H., Johnson, G. V., and Silvester, W. B. (2000). Natural abundance of ^{15}N in actinorhizal plants and nodules. *Plant Soil*, *219*, 285-289.

Torrey, J. G. (1990). Cross-Inoculation Groups within *Frankia.* In D. D. Baker, J. D. Tjepkema, and C. R. Schwintzer (Eds.), *The biology of* Frankia *and actinorhizal plants*. San Diego, CA: Academic Press Inc.

Torrey, J. G., and Tjepkema, J. D. (1979). Symbiotic nitrogen fixation with actinomycete-nodulated planta. *Botanical Gazette*, *140*, supplement, i-ii.

U.S. Department of Agriculture, Forest Service - USFS. (1937). *Range plant handbook*. Washington, D.C.: U.S. Forest Service.

Valdés, M., and Cruz-Cisneros, R. (1996). Root and stem nodulation of *Casuarina* in Mexico. *Forest, Farm, and Community Tree Research Reports, 1*, 61-65.

Vercoe, T. K. (1993). Australian trees on tour: A review of the international use of Australian forest genetic resources. In *Proceedings of 15th Biennial Conference of the Institute of Foresters of Australia*. Canberra, Australia: CSIRO.

Warcup, J. H. (1980). Ecotomycorrhizal associations of Australian indigenous plants. *New Phytol., 85*, 531 –535.

Watt, M., and Evans J. R. (1999). Proteoid roots: Physiology and development. *Plant Physiol.*, *121*, 317–323.

Warembourg, F. R. (1993). Nitrogen fixation in soil and plant systems**.** In R. Knowles, and T. H. Blackburn (Eds.), *Nitrogen isotope techniques*. (pp. 127-156). New York, NY: Academic Press, Inc.

Watanabe, I. (2000). Lecture at Cantho University, Vietnam. Retrieved June 6, 2002, from http://www.asahi-net.or.jp/~it6i-wtnb/BNF.html#ch4.

Webster, S. R., Youngberg, C. T., and Wollum, A. G. (1967). Fixation of nitrogen by bitterbrush (*Purshia tridentata* (Pursh)D.C.). *Nature, 216*, 392-393.

Werner, D., and Müller, P. (Eds.) (1990). *Fast growing trees and nitrogen fixing trees* (pp. 3-8). Stuttgart, Germany: G. Fischer Verlag.

Yang, Y. (1995). The effect of phosphorus on nodule formation and function in the *Casuarina-Frankia* symbiosis. *Plant Soil*, *176*, 161-169.

Youngberg, C. T., and Wollum, A. G. (1976). Nitrogen accretion in developing *Ceanothus velutinus* stands. *Soil Sci. Soc. Am. J.*, *40*, 109-112.

Zavitkovski, J., and Newton, M. (1968). Ecological importance of snowbrush *Ceanothus velutinus* in the Oregon *Cascades. Ecology*, *49*, 1134-1145.

Zhong Chonglu (2000). A New Record on Casuarina Blister Bark Disease in Southern China. *NFT News Improvement and Culture of Nitrogen Fixing Trees*, *3*, 6-7.