Chapter 8

ECOLOGY OF ACTINORHIZAL PLANTS

J. O. DAWSON
Departments of Natural Resources and Environmental Sciences and Plant Biology, University of Illinois at Urbana-Champaign, IL, U.S.A.

1. IMPORTANCE OF ACTINORHIZAL PLANTS

The actinorhizal plants that are nodulated by the symbiotic, N_2-fixing actinomycete *Frankia* include 25 genera in 8 families of angiospermous plants (Table 1). Actinorhizal plant families, together with all legumes and the rhizobially-nodulated genus *Parasponia*, have been placed in the rosid clade, which contains plants with a predisposition to nodular symbiosis with diazotrophs (Soltis *et al.,* 1995). The symbiotic organ is a coralloid-root nodule formed on primary roots infected by *Frankia*. Many important ecological interactions, patterns, and functions of actinorhizal plants are specifically associated with this N_2-fixing symbiosis but, in this chapter, only the symbiosis-related ecological features will be discussed.

Fixed nitrogen (fixed-N) availability is commonly limiting to primary productivity and other processes of ecosystems throughout the world. Nitrogen fixation by actinorhizal plants is a major source of fixed-N in diverse and widespread terrestrial ecosystems, including forests, bogs, swamps, coastal dunes, landslides, glacial deposits, riparian zones, shrub lands, prairies, and deserts (Dawson, 1986). Actinorhizal plants play important roles in wild-land ecosystem function and are used in land reclamation, range management, forestry, agro-forestry, and horticulture. It is probable that actinorhizal plants contribute a high proportion of the total amount of N fixed globally, primarily in wild or extensively managed lands. Dixon and Wheeler (1986) estimated that the contribution of actinorhizal plants to terrestrial global nitrogen fixation could be as great as 25% of the total.

Estimated rates of actinorhizal nitrogen fixation are comparable to those of legumes. Nitrogen-fixation rates vary widely within and among actinorhizal

K. Pawlowski and W. E. Newton (eds.), Nitrogen-fixing Actinorhizal Symbioses, 199–234.

Table 1. Actinorhizal plant taxa, species number, number of species known to be nodulated, and native distribution.

Family	Genus	Number of species	Known nodulated	Native distribution
Betulaceae	*Alnus*	47	47	Europe, Asia, N. America, Andes Mtns.
Casuarinaceae	*Allocasuarina*	59	54	Australia
	Casuarina	18	18	Australia, Tropical Asia, S-W Pacific
	Ceuthostoma	2	2	Oceania
	Gymnostoma	18	18	Australia, New Caledonia, Sumatra
Coriariaceae	*Coriaria*	16	16	Mediterranean, Asia, New Zealand, N America
Datiscaceae	*Datisca*	2	2	Asia, N America, Europe
Elaeagnaceae	*Elaeagnus*	45	35	Europe, Asia, N America
	Hippophae	3	2	Europe, Asia
	Shepherdia	3	2	N America
Myricaceae	*Comptonia*	1	1	N America
	Myrica	60	28	All continents except Australia
Rhamnaceae	*Adolphia*	1	1	N America
	Ceanothus	55	31	N America
	Colletia	17	4	S America
	Discaria	10	5	S America, Australia, New Zealand
	Kentrothamnus	2	2	S America
	Talguenea	1	1	S America
	Trevoa	6	2	S America
Rosaceae	*Cercocarpus*	20	4	Mexico, S-W United States
	Chamaebatia	2	1	Sierra Nevada Mtns.
	Cowania	25	1	Mexico, S-W United States
	Dryas	3	1	Arctic
	Purshia	4	2	W-N America

Adapted from Baker and Schwintzer (1990), Bond (1983), Dawson (1986), Huguet (2003), and Swensen (1996).

species depending on methodology used as well as ecological and genetic factors. Thus, it follows that a wide range of values has been reported for the major taxa. Estimates of the annual N_2-fixation rate for: (i) *Alnus rubra* (red alder trees) range from 22 to over 300kg/ha (summarized in Hibbs and Cromack, 1990); (ii) *Ceanothus velutinus* (snowbrush ceanothus) range from 24-101 kg/ha (McNabb and Cromack, 1985; Rose and Youngberg, 1981); and (iii) *Myrica faya* in Hawaii is 18kg/ha (Vitousek and Walker, 1989). Actual N_2-fixation values for *Casuarina equisetifolia* range from 15-94kg $ha^{-1}y^{-1}$ (summarized by Dommergues, 1997).

Much of what is known about actinorhizal associations has been derived from controlled studies in laboratories, growth chambers, and greenhouses. The physiology and biochemistry of both actinorhizal and other N_2-fixing systems is relatively well characterized, whereas our knowledge of both the amounts of N_2 fixed and the ecological controls of nitrogen fixation in nature remains less developed (Vitousek *et al.,* 2002). There are considerable difficulties inherent in ecological studies of actinorhizal plants, particularly the subterranean ecology. These difficulties include: (i) precisely estimating annual rates of fixation; (ii) dealing with the great complexity and heterogeneity of soil and associated ecosystems; and (iii) the necessity of excavating actinorhizal nodules, often in rocky soil and at considerable depth.

The host plants in actinorhizal symbioses are all perennial and all, but those of the genus *Datisca*, are trees or shrubs. Perennial plants have complex seasonal, annual, and multi-year reproductive, growth, and nutritional cycles, all of which renders them less amenable to study than annual plants. Reproductive capacity and related aspects of physiological maturity may require years to develop in trees, and the results of controlled studies that employ seedlings cannot be directly applied to mature plants.

Despite the difficulties associated with actinorhizal studies in general and ecological approaches in particular, greater ecological understanding is a prerequisite for managing and domesticating actinorhizal plants and their communities. Ecological inference in controlled experiments is strengthened by introducing some of the ecological complexities of nature, such as the multiple variables encountered in the field. However, there is no substitute for studying the actinorhizal symbioses *in situ*.

In nature, interactions occur among actinorhizal organisms and a dynamic range of biotic, physical and chemical agents. Such complexity is the norm and not the exception. A complex of environmental factors determines the actual survival, growth, nodulation, and nitrogen fixation of the symbiotic partners at any given site on the Earth's surface. So, ecological studies of actinorhizal plants, ranging from the global to the micro-ecosystem scale, are important and necessary complements to molecular and organism studies.

Major anthropogenic and natural changes in the environment have the potential to impact both the occurrence of actinorhizal plant and critical functions locally and globally. Evidence for climatic and human impact on regional actinorhizal plant occurrence and abundance has been obtained from the archaeologically rich Patacancha Valley in the mountains of southern Peru (Chepstow-Lusty *et al.,* 1998). An 8-meter core taken from a small, dry lake revealed an enormous increase in pollen of *Alnus acuminata* (Andean alder) occurring *ca*. A.D. 1000. At about this time, a warming trend reversed a 900-year period of cooler temperatures, affording a climate more suitable for Andean alder. Evidence from the core suggests that the cool period was accompanied by decreased pre-Incan farming, but continued degradation and erosion of the soil.

At the time of warming, the Inca took over the valley and the signature of soil erosion in the core dropped dramatically. The Inca employed soil-sparing techniques, such as terraces and irrigation canals, and both seeds and pollen of maize and other crops appeared in sediments and dated archaeological finds of the period. As the Incan population quadrupled and stabilized, the occurrence of alder pollen persisted, even as the growing population used alder trees for firewood, roof beams, and door lintels. The increase in population was accompanied by increased agricultural activity, but apparently with reduced soil erosion, which suggests a system for conserving alder trees that would have stabilized soil on steep slopes.

Speculation that Andean alders were used in agro-forestry practices, possibly even in association with terraces where they could both stabilize and improve the soil, is not unreasonable. Contemporary indigenous peoples of Central America still use alders, intermingled with corn, on steep slopes to stabilize soil and to increase fertility by lopping off branches and scattering them as a green manure. Additional evidence for alder management by the Inca appeared in historical records shortly after the Spanish conquest in the early 1500s. Chroniclers among the conquistadors wrote that the Inca had a strong tradition of tree planting. Alder cultivation was overseen by the emperor, who punished both illegal woodcutters and those who burned the trees by putting them to death (Krajick, 1998).

After the Spanish conquest, terraces, canals and tree plantings were abandoned, and alder stands gradually disappeared. Today, alder is restricted to a few remote ravines in the valley. These observations illustrate how changes in climate and civilization might have shifted the distribution to higher elevations and greatly increased the regional occurrence and use of an actinorhizal tree. In the city of Manizales, in Colombia, Andean alders are currently planted and protected in the municipal watershed to guarantee a clean and seasonally reliable source of water.

Today, humankind faces the probability of new, widespread, anthropogenic changes in the global environment as well as cyclical and episodic changes that occur on a global scale. According to geological patterns, massive volcanic eruptions with drastic global cooling, ice ages, asteroid collisions, and warming cycles are inevitable in the long term. More immediately, increasing atmospheric carbon-dioxide levels, fixed-N pollution of air and water, global warming, ozone pollution, acid rain, and other problems associated with industrial and agricultural activity are increasing. Plants, animals, and microorganisms cross geographic barriers routinely as global travel, commerce, and transport continue to increase. Introductions and escapes of biotic organisms into new territories, which lack the co-evolved checks and balances, can result in disease epidemics, harmful insect infestations, population explosions of animal pests that disrupt balanced ecosystems, and the invasion of biotic communities by exotic plants, including several actinorhizal species that could displace natives.

Ultimately, such continent hopping by living organisms can result in the extinction of native species and the loss of biological diversity. In the context of dynamic change, the occurrence, distribution, abundance, biotic associations, genetic makeup, and ecological function of actinorhizal plants will change accordingly. Understanding the ecological basis for change can facilitate effective and sustainable management of these important actinorhizal plant resources.

2. OCCURRENCE AND DISTRIBUTION OF ACTINORHIZAL PLANT TAXA AND THEIR MICROSYMBIONTS

2.1. Global Distribution of Actinorhizal Plant Taxa

Most actinorhizal plant species occur in boreal and temperate ecosystems of both the Northern and Southern Hemispheres. A widespread representative of an actinorhizal plant genus with many species of cold and temperate climates in the Northern Hemisphere is *Alnus* (alder). The genus *Alnus* dates back in the fossil record to the Upper Cretaceous, 70-80 million years ago. A basal position on the alder phylogenetic tree, a slow evolutionary rate, and morphologically primitive characteristics indicate that the subgenus Alnaster, represented by the widespread subspecies of *Alnus viridis* (green alder) that occurs in cold climates and at the margins of glaciers, is closest to the ancestral form of the alders (Navarro *et al.,* 2003). Northeast Asia is the probable center of origin for this genus because this region has the greatest number of alder species and the highest level of alder endemism (Murai, 1964; Navarro *et al.,* 2003).

A. viridis ssp. *sinuata*, *Shepherdia Canadensis*, and *Dryas drummondii* are post-glacial primary successional plants in the Northern Hemisphere (Kohls *et al.,* 2003), whereas *Coriaria* plays a similar role on post-glacial deposits in the mountains of New Zealand in the Southern Hemisphere. A sudden cooling started about 12,700 years ago, in the midst of the last global warming cycle, and lasted 1,300 years. This cold period, known as the Younger Dryas, is named for the pollen of this actinorhizal tundra plant that turned up in a lakebed in Denmark when it should not have. Two earlier sudden cold periods, known as the oldest Dryas (13,800 years ago) and the older Dryas (13,450 years ago), have also been revealed by sudden increases in the pollen of this actinorhizal plant at more southerly locations (Roberts, 1998). *Shepherdia* in Canada and *Hippophae* in Scandinavia were apparently post-glacial precursors of boreal forests at the end of the last Quaternary glaciation together with *Alnus* on both continents (Baker and Miller, 1980; Silvester, 1977). *Myrica gale* is widespread in bogs of boreal regions in the Northern Hemisphere, whereas *Alnus*, *Shepherdia*, and *Hippophaë* are actinorhizal plant genera present in boreal and cool temperate regions of the Northern Hemisphere today.

Many *Casuarina* and *Allocasuarina* species occur in warm temperate regions of Australia in a variety of habitats. Unlike most taxa of actinorhizal plants with distributions restricted to higher latitudes and altitudes, some *Casuarina* and all *Gymnostoma* spp. are endemic to tropical forests in the south-west Pacific region. The tropical genus *Gymnostoma* may represent an ancestral stock of the family Casuarinaceae. This genus was widespread in Gondwanaland at least by the beginning of the Tertiary. It predates *Casuarina* and *Allocasuarina* in the fossil record and is least specialized with the simplest morphology. It has small chromosomes with the lowest chromosome number (n = 8), and represents the only actinorhizal family of Gondwanian origin with generic differentiation occurring in early Tertiary times (Barlow, 1983). The genus *Gymnostoma* may have diverged into *Casuarina* and *Allocasuarina* species having both tropical and temperate adaptations. All other actinorhizal plant families are of northern Laurasian origin.

In the tropics, some genera of actinorhizal plants occur at high elevations with cool moist climates that are similar to those of temperate regions. Examples include: the *Myrica* belt around Mount Kilimanjaro in Africa; *Myrica* in the mountains of the Canary Islands, the Caribbean and Central America; and the alders (*Alnus* spp.) of the highlands of tropical regions in South America and Asia.

In cooler regions of the globe, actinorhizal plants, with the exceptions noted previously, are the predominant tree and shrub forms of N_2-fixers. In the tropics, tree legumes replace actinorhizal plants as the predominant N_2-fixing trees and shrubs. The reasons for this dichotomy are unknown. Many late-successional tropical forests contain canopy legumes capable of N_2 fixation, whereas N_2-fixers are absent from most late-successional temperate and boreal forests. Vitousek *et al.* (2002) suggest that the relatively high fixed-N availability in lowland tropical rainforests could permit legumes to maintain a fixed-N-demanding lifestyle (McKey, 1994) without the necessity of expending considerable energy to fix atmospheric N_2. Conversely, in temperate and boreal forests, early-successional actinorhizal species are typically replaced by tree species that are less demanding of available fixed-N, perhaps at some threshold level below that necessary to support actinorhizal plants without costly N_2 fixation.

In warm-temperate transitional zones between the tropics and temperate regions, such as the monsoonal arid regions of Australia and North America, both leguminous and actinorhizal species occur together. In the Sonoran Desert and other semiarid regions of western North America, shrubby species of *Cercocarpus, Cowania,* and *Ceanothus* occur together with a variety of woody legumes, including *Prosopis* and *Acacia*. In the canyons of desert mountains, the cool moist sky islands that are separated by deserts and grasslands, *Alnus oblongifolia* (Arizona alder), a relatively tall tree of the riparian zone, occurs along perennial streams extending out into the upper Sonoran Desert.

Similarly in Australia, species of *Acacia* and other tree legumes occur in scrublands and forests of arid and semiarid zones together with the casuarinas. *Allocasuarina* spp. are prominent together with shrubby legumes in drier regions, including the central and northern desert monsoonal regions, whereas larger *Casuarina* species occur primarily along streams, in coastal areas, and in the moist tropical rainforests of northeastern Australia (Midgley *et al.,* 1983). *Allocasuarina decaisneana* is striking for its large size among trees of the desert of central Australia. It is found in sandy soils and has a system of deeply penetrating roots, most likely allowing it to access permanent water in deep soil strata.

Among the actinorhizal genera, *Myrica*, in the family Myricaceae, has the widest geographic distribution. They are small trees or shrubs common to nearly all major landmasses and with species occurring from tropical to cold regions. According to morphological features, fossil and pollen records, and a recent molecular study based on the *rbcL* gene (summarized in Huguet, 2003), the family Myricaceae is considered to be the most ancient actinorhizal family with *Myrica* appearing at least as early as 85-83 million years before the present.

Apart from the Casuarinaceae and Myricaceae, actinorhizal plants seem to be restricted to cold and temperate climates, perhaps reflecting a global trend of continental cooling during their evolutionary development. They seem to be distributed accordingly within either the Northern or Southern Hemispheres. There is hemispheric separation of cold- and temperate-climate actinorhizal plants because of the tropical equatorial barrier to temperate-plant migration.

How did actinorhizal plants of Laurasian origin reach the Southern Hemisphere? An example of a Laurasian actinorhizal genus that occurs on both sides of the equator is *Coriaria*. *Coriaria* species are distributed in four separate areas in the world; the most conspicuous disjunct distribution in flowering plants. The phylogenetic relationships of 12 *Coriaria* species collected from the representative disjunct areas were inferred by comparing the combined data set of *rbcL* and *matK* (maturase K) genes (Hasebe *et al.,* 1998). The divergence time between the Eurasian species and other species distributed in the Southern Hemisphere was estimated as between 63 and 59 million years ago, using *rbcL* and *matK* molecular clocks, respectively. These results do not support the hypotheses that continental drift created the disjunct distribution pattern by separating earlier contiguous populations of ancestral *Coriaria*. Rather they suggest that the distribution pattern was formed by geographical migrations and separations in the Cenozoic. Other actinorhizal plant genera and their geographic distributions are included in Table 1.

2.2. Global Distribution of Frankia *Taxa*

Infective *Frankia* are known to occur on all continents, except Antarctica, and on many islands, but vary spatially (Paschke *et al.,* 1994; Simonet *et al.,* 1999) and temporally (Wollum *et al.,* 1968) in occurrence. Nodulation of an actinorhizal plant may not occur either because *Frankia* is absent or, more likely, because specific strains, which are able to nodulate a given host, are not present in a soil or are unable to nodulate a host under existing soil conditions.

Within their native ranges, most actinorhizal plant species nodulate with *Frankia* strains capable of symbiotic nitrogen fixation, although nodulation with ineffective strains also occurs in nature (Wolters *et al.,* 1997). Soil near actinorhizal hosts generally has greater nodulation capacity than surrounding soils (Jeong and Myrold, 2001; Smolander, 1990; Zimpfer *et al.,* 1999). However, infective *Frankia* can be found in a variety of soils both within and outside the immediate influence of actinorhizal hosts as well as outside of the native range of actinorhizal plant species (Burleigh and Dawson, 1994; Lawrence *et al.,* 1967; Maunuksela *et al.,* 1999, 2000; Paschke and Dawson, 1992a; Zimpfer *et al.,* 1997). *Frankia* able to nodulate *Alnus*, *Myrica*, *Dryas* and *Elaeagnus* are found widespread outside the native range of their host plants, suggesting that they have the capacity to persist as a saprophyte (Kohls *et al.,* 1994; Maunuksela *et al.,* 1999; 2000; Nickel *et al.,* 1999; 2000).

The presence of plant species of genera, which are not actinorhizal but that are closely related to actinorhizal genera, can increase *Frankia*-nodulation capacity of soil for actual host species (Gauthier *et al.,* 2000; Paschke and Dawson, 1992b; Smolander *et al.,* 1990). Increased rhizosphere soil-nodulation capacity of actinorhizal hosts and some other closely related plant species suggests the release of compounds that stimulate *Frankia* growth or infectious capacity or both (Zimpfer *et al.,* 2002; 2003). A compound from roots of an actinorhizal plant has been shown to stimulate *Frankia* spore germination (Krumholz *et al.,* 2003). In order to determine the distribution of *Frankia* in nature, they must first be retrieved from soil or nodules and described to allow differentiation among genotypes. Since the first confirmed isolation of the actinomycete genus *Frankia* (Callaham *et al.,* 1978), a variety of methods have been used to obtain a coherent classification of this bacterial symbiont.

Frankia can be difficult, or impossible in the case of some actinorhizal host genera, to isolate in pure culture from nodules. There are few reports of successful isolations of *Frankia* from soil (Baker and O'Keefe, 1984). Furthermore, not all cultures have been derived from single spores and some isolates could represent multiple strains. Many "atypical" strains, isolated from nodules but incapable of reinfecting a host, have been catalogued, perhaps as a result of the mistaken isolation of rhizosphere *Frankia* from inadequately sterilized nodule surfaces (Huguet *et al.,* 2001; see also chapter 2 in this volume). If the actinomycetous partner cannot be identified or isolated, the descriptive and predictive value of ecological inference is weak.

Methods for describing and comparing *Frankia* strains include soluble protein patterns, isoenzyme patterns, fatty acids, serology, DNA-DNA relatedness, and genome and plasmid restriction analyses. Recently, ecological studies of the symbiotic interactions between *Frankia* and its hosts have benefited from advances in molecular biology, which allow identification of nodular microsymbionts without first obtaining isolates (Hahn *et al.,* 1999; see also chapter 2 in this volume). Thus, ecological and distributional features can be studied with respect to specific rather than generic *Frankia*. Nodules are usually collected in the field, but *Frankia* in a soil are extracted, using actinorhizal bait plants to capture individuals from infective populations.

The earliest studies indicated that *Frankia* strains are heterogeneous and clustered within two main phylogenetic groups: the *Alnus-Myrica-Casuarina* group and the Elaeagnaceae group (reviewed by Hahn *et al.,* 1999). Strains that infect *Myrica* spp. often grouped with *Alnus*-infective strains. Additional work (Navarro *et al.,* 1997) placed *Gymnostoma-Frankia* in the *Elaeagnu*s group. Clawson *et al.* (1998) and Hahn *et al.* (1999) defined a third "*Dryas* group", which encompassed *Coriaria*, *Datisca*, *Ceanothus*, and the actinorhizal plants in the family Rosaceae (Table 2; see also chapter 4 in this volume).

Maggia and Bousquet (1994) have proposed that host-plant evolution has proceeded toward more specificity and, therefore, the most primitive actinorhizal plants, such as *Myrica* and *Gymnostoma*, should be able to host the broadest ranges of *Frankia* genotypes. Navarro *et al.* (1997) disagree and suggest that *Gymnostoma* and *Casuarina-Allocasuarina* constitute two divergent plant lineages associated with two divergent Frankia lineages. Simonet *et al.* (1999) have suggested that *Casuarina* species from Australia are less specific and have a broader host range than more recently evolved *Allocasuarina* spp. They further suggest that the more

Table 2. Dominant phylogenetic groups of Frankia *naturally associated with host genera.*

		Phylogenetic groups of *Frankia*		
Family	Genus	"Alnus"	"Dryas"	"Elaeagnus"
Betulaceae	*Alnus*	X		
Casuarinaceae	*Allocasuarina*	X		
	Casuarina	X		
	Ceuthostoma			?
	Gymnostoma			X
Coriariaceae	*Coriaria*		X	
Datiscaceae	*Datisca*		X	
Elaeagnaceae	*Elaeagnus*			X
	Hippophae			X
	Shepherdia			X
Myricaceae	*Comptonia*	X		
	Myrica	X		
Rhamnaceae	*Adolphia*		?	
	Ceanothus		X	
	Colletia			X
	Discaria			X
	Kentrothamnus			X
	Retanilla			X
	Talguenea			X
	Trevoa			X
Rosaceae	*Cercocarpus*		X	
	Chamaebatia		X	
	Cowania		X	
	Dryas		X	
	Purshia		X	

Adapted from Clawson et al. (1998), Hahn et al. (1999), Navarro et al. (1997), and Torrey (1990).

recently divergent and less specialized species of *Casuarina*, such as *Casuarina equisetifolia*, likewise symbiose with less-specialized *Frankia* strains possessing greater saprophytic abilities.

Myrica spp. have been described as promiscuous because some species, such as *M. pensylvanica* and *M. californica,* can host a diversity of nodular *Frankia* in the field that is sufficient to allow them to serve as a reservoir host for *Frankia* strains that infect plants from other actinorhizal families (Clawson *et al.,* 1999; Huguet, 2003). Other species of Myricaceae, such as *M. gale*, are not promiscuous in the field and have greater field specificity for microsymbionts (Huguet *et al.,* 2001). Actinorhizal plants in the family Elaeagnaceae have also been described as promiscuous and species, such as *Shepherdia canadensis*, can support diverse nodular *Frankia* (Huguet *et al.,* 2001).

It is noteworthy that green alder has been found nodulated in New Zealand (Benecke, 1969) and *Alnus nepalensis* (Himalayan alder) nodulates spontaneously in Canberra, Australia; in both cases, separated by thousands of miles and isolated by oceans from their native ranges. In contrast, *Casuarina* and *Allocasuarina* spp. from the same region do not nodulate in New Zealand, in other places outside their native ranges, or indeed even in some soils within their native ranges, unless the appropriate *Frankia* strains have been inoculated or otherwise introduced (Simonet *et al.,* 1999).

A diversity of *Frankia* genotypes able to nodulate *Casuarina* and *Allocasuarina* plant spp. seem to occur naturally only in soils within the host-genus native range (Simonet *et al.,* 1999). Elsewhere, exotic casuarinas must be inoculated in order to nodulate when planted in new locations (Diem and Dommergues, 1990). It has been postulated that the source of nodular *Frankia* in older introduced casuarinas that occur outside their native range may have been nodules and soils from seedlings planted by seafarers beginning at least 4 centuries before the present (Simonet *et al.,* 1999). Virtually all of the nodular *Frankia* in casuarinas found outside of Australia are of one phylogenetic group, which is also the only Casuarinaceae group of *Frankia* to successfully yield isolates, all of which suggests greater saprophytic capacity. In one Jamaican location where casuarinas have been introduced, this same group of *Casuarina*-infective *Frankia* is highly localized in soil around host trees. This may be a result of substances released from the host that increase infective *Frankia* populations in soil (Zimpfer *et al.,* 2002; 2003).

As noted previously, *Myrica, Alnus, Dryas,* and *Elaeagnus* spp. nodulate readily either outside their native ranges or with soils and nodule inocula obtained from locations distant from their native ranges (Kohls *et al.,* 1994; Paschke *et al.,* 1994). The explanation generally offered for this capacity is that the host species are promiscuous and can consort symbiotically with a heterogeneous mix of widespread microsymbiont genotypes. However, their ability to nodulate as newcomers in soils far from their place of origin could also be a result of: (i) the widespread occurrence of certain genotypes that include genera within their specific host-compatibility ranges; (ii) a greater dispersal capacity of their compatible microsymbionts; (iii) microsymbionts that possess greater competitive or saprophytic capabilities; or (iv) *Frankia* that are supported in the rhizospheres of many non-actinorhizal plants.

Some *Frankia* strains might be more readily dispersed over long distances because of differences in the amount of sporulation, spore longevity, spore resistance to harsh environmental conditions, or greater potential for wind dispersal. Additionally, the ability of spores to survive in soil particles, which are ingested by or adhering to animals, and to survive in the digestive tracts of soil arthropods and the birds and other animals that consume them, might magnify their dispersal. Likewise, there could be both biotic and abiotic agents in different soil types and conditions that influence nodulation and host/microsymbiont specificity.

Our knowledge of global distribution patterns of *Frankia* is far from complete. Even though we know that *Frankia*, which are able to infect a given host, may or may not occur in soils outside of native-host influence and range, we cannot yet comprehensively characterize non-infective *Frankia* soil populations. It also remains to be determined whether the lack of infectivity of soil-born *Frankia* in bioassays might be due to either genetic incompatibility or a number of environmental triggers that alter the physiological capacity of the microsymbiont and its plant partner to nodulate. Direct determination of the nature, dynamics and distribution of *Frankia* populations in soil and the range and occurrence of non-infective *Frankia* strains would allow more detailed determination of the range of *Frankia* groups globally and regionally.

2.3. Niches of Actinorhizal Plant Species

A common ecological niche for actinorhizal plants is where soil nitrogen availability is low. Low levels of available combined-N can critically limit the establishment and growth of plants unable to fix N_2 symbiotically. In such a niche, actinorhizal and other plants possessing symbiotic associations with N_2-fixing bacteria are often favored. Available combined-N is severely limiting to plant growth in soils low in organic matter, the major pool of soil N.

Examples of low-N soils include recent volcanic deposits, landslide areas on steep slopes, sand and gravel deposits of rivers and streams, sand dunes, beaches, recent glacial deposits, and eroded soils. Combined-N can also be limited in availability in grasslands, where an extensive system of fine roots competes strongly with the roots of other plants for water and nutrients. Many arid ecosystems are N-limited due both to extreme moisture and temperature conditions that inhibit organic matter mineralization and to patchy accumulation of wind-distributed soil particles containing nitrogenous substances. Wetlands may be N-limited because of slow mineralization of organic-N. In wetlands, low rates of N mineralization can result from low O_2 concentrations, sub-optimal temperatures for mineralization of N, and loss of both organic and mineral forms of N from soil through leaching, dilution, and transport by water. Furthermore, NO_3^- present in soil solution can be lost to plants as a result of denitrification in anaerobic strata and in flooded soils.

The early stage of plant succession following a disturbance, which creates a N-limited situation, is a common temporal niche for actinorhizal plants. In common with other pioneer plant species, actinorhizal plants are generally light demanding and do not persist under the closed canopies of later successional forest species (Côté *et al.,* 1988). Combined-N accumulates in soil with the shedding and decomposition of actinorhizal plant roots and litter. At a later point in successional development, the threshold of available soil-N, which allows other plants to become established, is crossed and other plants eventually displace the actinorhizal colonizers.

Alnus rubra in western North America and *A. glutinosa* (European alder) in Europe occur in riparian zones, such as gravel and sand bars, as well as in N-poor wetlands by virtue of their abilities both to fix N_2 and to tolerate flooding. *A. rubra* also occurs on landslides, volcanic mudflows, and after fire in the cool wet temperate regions of the Pacific Northwest of North America. These plants are light demanding and colonize disturbed sites. Species of *Alnus*, *Shepherdia*, *Hippophaë* and *Dryas* probably played a major role in soil formation immediately following the retreat of continental ice sheets over vast areas of Europe, Asia, and North America

(Baker and Miller 1980; Bond, 1983; Silvester, 1977), just as some of them do today as primary successional plants of recent glacial deposits (Kohls *et al.,* 2003).

Some actinorhizal plants of savannas, grasslands, chaparral, and shrublands differ from early successional actinorhizal plant species by persisting as stable components of plant communities. The ability to fix N_2 in these ecosystems probably allows the plant, *e.g.*, *Ceanothus* species in open grasslands and chaparral, to compete favorably with plants more efficient at either acquiring or using scarce available soil N. *Ceanothus americanus* (New Jersey Tea) occurs as a stable plant component of tallgrass prairies and oak savannas of central North America. This species has a massive main root and reproduces readily after fire from seeds and sprouts. These prairie and savanna ecosystems accumulate abundant organic-N in soil, but competition for nutrients and water by grasses, together with droughts that reduce N mineralization, may limit the availability of N to other plants. These examples illustrate typical niches and similar patterns of niche differentiation occur for other actinorhizal plant species globally.

2.4. Dispersal of Actinorhizal Plants and Frankia

The dispersal mechanisms of actinorhizal plants vary among taxa, and generally involve the movement of seeds. Alders and casuarinas produce many small winged seeds that are dispersed by wind and water. The rosaceous *Cercocarpus*, *Cowania*, and *Dryas* species have achenes with a persistent feathery style that facilitates wind dispersal. Actinorhizal plants in the Coriariaceae, Elaeagnaceae, and Myricaceae families produce seeds in fruits, which for some species of actinorhizal plants are ingested and the seeds likely dispersed by birds and mammals (Martin *et al.,* 1961). In the Rhamnaceae, *Ceanothus* species produce seeds in capsules. On drying, these capsules rupture in such a way as to forcefully eject the seeds a short distance. None of these mechanisms is either unusual or unique to actinorhizal plants.

Unlike host-plant dispersal methods, the mechanisms of dispersal of *Frankia* propagules have not been empirically established. Infective *Frankia* are present in newly deposited glacial till and young sand dunes prior to colonization by host plants (Kohls *et al.,* 2003; Young *et al.,* 1992). The likely mechanisms for *Frankia* dispersal are wind (anemochoric dispersal), water (hydrochoric dispersal), and biological vectors (zoochoric dispersal).

Frankia occurs in soils that lack host plants (Huss-Danell and Frej, 1986; Paschke and Dawson, 1992a; Zimpfer *et al.,* 1997) and on fresh substrates newly colonized by actinorhizal plants (Lawrence *et al.,* 1967; Schramm, 1966), which indicates that propagules of *Frankia* are mobile in nature. *Frankia* produces spores that are able to withstand desiccation (Burleigh and Torrey, 1990) for at least 18 months (Righetti and Munns, 1981; Tortosa and Cusato, 1991).

One possible mode of *Frankia* colonization of new habitats is anemochoric dispersal. However, unlike fungi, *Frankia* does not produce aerial sporangia for dispersal of spores and has a slow growth rate relative to soil saprobes. Aerial dispersal of *Frankia* would occur most readily where wind erosion and wind transport of soil particles containing *Frankia* propagules are prevalent. These conditions are widespread in arid regions, such as those of Australia, where there are many endemic *Casuarina* and *Allocasuarina* spp., as well as in glacial regions during the winter, when silt deposits are exposed with the recession of summer meltwaters. Of potential importance here is the induction of melanin by tyrosine, which is a prevalent trait of *Frankia* isolates from *Casuarina* spp. is (Lai, 1996). This induced trait, which is accompanied by increased sporulation in some strains, may afford a means of protection of airborne spores or hyphal particles of Casuarinaceae-infective *Frankia* from ultraviolet radiation.

Hydrochoric dispersal over long distances might be possible in riparian or lake ecosystems. Su and Lin (1989) speculated that *Frankia* moves in stream water in Asia; Huss-Danell *et al.* (1997) demonstrated that the action of streams, waves and changing water levels transport infective *Frankia* attached to superficial sediment particles in Alaska and Sweden; and Arveby and Huss-Danell (1988) suggested that *Frankia* moves laterally in the soil solution of peat.

Zoochoric dispersal is also very likely. McIlveen and Cole (1976) found that organisms that transport soil can act as dispersal vectors for spores of Endogonaceae fungi and it is probable that organisms, including humans, which move soil, may also passively transport *Frankia*. The transcontinental shipping of domestic livestock with soil particles adhering to fur and within folds of tissue is an example. Furthermore, the introduction of European sheep to New Zealand may have introduced *Frankia* from Europe to this isolated island nation.

Casuarina-infective *Frankia* can pass through the digestive tracts of earthworms, which undoubtedly disperse *Frankia* vertically together with large volumes of soil (Reddell and Spain, 1991). Birds, many of which consume earthworms and other soil invertebrates, also ingest large soil particles that function as grit for grinding food in their gizzards. Some bird species also transport mud that contains infective *Frankia* for nest construction as well as soil invertebrates as a food source for nestlings (Paschke and Dawson, 1993). *Frankia* spores are known to survive and maintain the ability to infect host plants after passage through the digestive tracts of birds (Burleigh and Dawson, 1995).

Infective *Frankia* is present in soils of tropical lowland forests of Costa Rica, which lack known actinorhizal hosts (Paschke and Dawson, 1992a). Central America is a funnel for the migratory routes of many bird species moving annually between North and South America. If birds are a major dispersal agent for *Frankia* and, if *Frankia* strains have some degree of saprophytic capacity, then the presence of *Frankia* in soils of regions where migratory birds congregate, but that lack host plants, would be easily explainable. Such findings provide support for the proposal that migratory bird species transport *Frankia* over long distances.

The possibility of codispersal of actinorhizal hosts and their *Frankia* microsymbionts exists for actinorhizal genera, such as *Myrica* and *Elaeagnus*, which have avian-dispersed seeds (Martin *et al.,* 1961). Birds tend to defecate when leaving perches in trees and shrubs, thus possibly more frequently inoculating soils near actinorhizal plants that are attractive as food sources and provide perches. Mammals that consume actinorhizal fruit and seeds could likewise codisperse the microsymbiont through soil adhering to their fur and other external tissues (Martin *et al.,* 1961). Seafaring traders, who transported *Casuarina* seedlings in soil for planting in distant lands, may also have introduced both soil and nodular *Frankia* throughout the tropical and warm temperate regions of the world (Simonet *et al.,* 1999).

What we know for certain about the distribution of actinorhizal plants and *Frankia* indicates the likelihood of many undiscovered interrelationships among actinorhizal partners and a variety of biotic and abiotic dispersal agents.

3. ECOLOGICAL FACTORS INFLUENCING INFECTIVE *FRANKIA* POPULATIONS

Many factors affect the abundance of soil-borne *Frankia* that are able to infect their host plants. Samples from uniform soils exhibit considerable variation in infective units of *Frankia* (Oremus, 1980; Zimpfer *et al.,* 1997; 1999), perhaps because of patchy favorable soil microhabitats, the localized release of *Frankia* from degrading nodules, localized stimulation of infective capacity by release of compounds from host plants and related genera, or the localized deposition by dispersal agents. Intense agricultural cultivation diminishes *Frankia*-nodulation capacity of soils (Paschke *et al.,* 1994; Zimpfer *et al.,* 1997), whereas the presence of actinorhizal host plants is a major factor in promoting *Frankia* presence and abundance in the rhizosphere (Zimpfer *et al.,* 1999), possibly as the result of compounds released from the host plants.

Research on *Frankia* ecology and physiology has some practical barriers not encountered in the parallel symbiosis between legumes and rhizobial bacteria. *Frankia* isolates grow slowly relative to rhizobial bacteria, and their filamentous nature precludes standard plate counts for enumeration. Furthermore, colonies of *Frankia* grown in liquid culture form coherent masses that cannot be directly assayed using photospectrometry, however, new methods to amend *Frankia* cultures to produce dispersed cells in liquid culture have recently been published (Harriott and Bourret, 2003). Despite this improvement in methodology, ecophysiological studies of the response of *Frankia* to physical and chemical variables *in vitro* remain more laborious than those of many other bacteria. Additionally, methods for genetic transformation of *Frankia* still remain elusive, so that strains with markers are not available for ecological studies in the field. Nonetheless, much has been learned about the ecology of this important microsymbiont despite these inherent difficulties.

3.1. Biotic Factors Influencing Frankia *in Soil*

As described in the preceeding section, zoochoric dispersal is probably a major ecological determinant of the occurrence of infective *Frankia* populations. Several studies have indicated that the presence of *Frankia* in young soils is not dependent on host-plant presence. In soils of the barrier islands off the coast of Virginia, colonization by *Frankia*, which is able to infect actinorhizal *Myrica cerifera,* occurs prior to colonization by host plants (Young *et al.,* 1992). On the islands of Java

(Becking, 1970) and Hawaii (Vitousek and Walker, 1989; Burleigh and Dawson, 1994), *Myrica* is nodulated on volcanic soils. On the island of Jamaica, one-year-old reclaimed bauxite mines harbored low levels of *M. cerifera*-infective *Frankia* (Zimpfer *et al.,* 1997). In Alaska's Glacier Bay, *Dryas drummondii* and *Alnus viridis ssp. sinuata* (Sitka alder) nodulate readily on newly-formed glacial soils (Lawrence *et al.,* 1967), indicating the early presence in glacial deposits of infectious *Frankia*.

Host plants apparently release compounds that increase the numbers of infective *Frankia* in soil (Krumholz *et al.,* 2003; Zimpfer *et al.,* 1999; 2002; 2003). Most actinorhizal plants are nodulated in their native habitats and *Frankia* is usually found in abundance in soils beneath nodulated host plants (Arveby and Huss-Danell, 1988; Oremus, 1980; Smolander, 1990; Van Dijk, 1979; Zimpfer *et al.,* 1999). However, some actinorhizal genera are often sparsely nodulated or not nodulated at all even within their native habitats (Lawrie *et al.,* 1982) and, therefore, probably contribute little to either host-plant success or the overall accumulation of soil nitrogen. In addition, there are numerous reports of *Frankia* in soils lacking actinorhizal host plants (Bermudez de Castro *et al.,* 1976; Dawson and Klemp, 1987; Huss-Danell and Frej, 1986; Paschke and Dawson, 1992b; Rodríguez-Barrueco, 1968; Smolander, 1990; Smolander and Sundman, 1987; Young *et al.*, 1992; Zimpfer *et al.,* 1997), providing indirect evidence that *Frankia* can grow and survive in soils in the absence of symbiotic hosts.

Compounds present in *Casuarina cunninghamiana* cladode tissue extracts increase the nodulation capacity of soil containing naturally occurring *Frankia*, whereas the addition of organic-binding agents to the host rhizosphere decreases nodulation (Zimpfer *et al.,* 2002). The increase in nodulation capacity caused by compounds released into plant rhizospheres may result from one or more of many possibilities, including: (i) the stimulation of *Frankia* growth; (ii) the stimulation of "helper" bacteria; (iii) the inhibition of competitors of *Frankia*; (iv) the enhancement of signaling by molecules important in the nodulation process; and (v) the stimulation of root-hair curling or fine-root production (Benoit and Berry, 1997; Gauthier *et al.,* 2000; Kapulnik *et al.,* 1987; Knowlton *et al.,* 1980). In addition, *Alnus* and *Elaeagnus* root extracts increase *Frankia* spore germination (Krumholz *et al.,* 2003). Further, *Alnus*-infective *Frankia* can grow as saprophytes in incubated soils and the addition of *Alnus* leaves to incubated soils can increase both growth and infectivity of *Alnus*-infective strains of *Frankia* (Nickel *et al.,* 1999; 2000).

Important compounds that may influence the interaction between plants and soil microorganisms include the phenolics in plant tissue (Harborne, 1973) and soils (Li *et al.,* 1970; Shindo *et al.,* 1978; Whitehead, 1964; Whitehead *et al.,* 1983). Various phenolics significantly stimulate or inhibit the *in vitro* growth of *Frankia* strains and also alter their morphological development (Perradin *et al.*, 1983; Vogel and Dawson, 1986). An isolate of *Frankia* from *A. viridis* ssp *crispa*, which has a high level of the phenolics, pinosylvin and pinosylvin methyl ether, to discourage browsing by arctic hares (Clausen *et al.,* 1987), was least impaired by a variety of plant phenolics tested *in vitro*. This result hints at the possibility of both intracellular and rhizosphere influences of plant phenolics on the growth, physiology, and morphology of *Frankia*.

The growth in pure culture of *Casuarina*-associated *Frankia* can be promoted with various fatty acids (Selim *et al.*, 1996). Flavonoids also influence the actinorhizal symbiosis (Benoit and Berry, 1997; Laplaze *et al.,* 1999). Because *Casuarina* trees are infected intracellularly following root-hair curling, which is induced by an unknown *Frankia* signal (Laplaze *et al.,* 1999), this and other similarities with the infection process in legumes has led to the hypothesis that flavonoids act as actinorhizal plant signals, that activate the production of *Frankia* root hair-deforming factor (Prin and Rougier, 1987; Van Ghelue *et al.,* 1997). Thus, it is plausible that actinorhizal plant hosts and their relatives produce compounds that either mediate host/symbiont signaling, stimulate *Frankia* growth, or otherwise promote nodulation (Benoit and Berry, 1997; Gauthier *et al.,* 2000; Kapulnik *et al.,* 1987; Knowlton *et al.,* 1980).

"Helper" bacteria are known that increase axenic nodulation of *Alnus* plants (Knowlton *et al.,* 1980), maybe by inducing root-hair curling and possible amelioration of rhizosphere pH (Knowlton and Dawson, 1983). The presence of soil biota can synergistically increase *Frankia* nodulation of a *Casuarina* host (Zimpfer *et al.,* 2003). Other soil organisms, therefore, seem to play a critical ecological role in enhancing the actinorhizal nodulation process in nature, however, the organisms and processes underlying this enhancement remain to be fully elucidated.

Frankia's ability to proliferate in *A. glutinosa* rhizospheres (Vergnaud *et al.,* 1985) and its localization near *C. cunninghamiana* (Zimpfer *et al.,* 1999) suggest that it may be favored in host rhizospheres. *Frankia* nodulation capacity is increased in the rhizosphere of *Betula pendula* Roth., a member of the same family as *Alnus* spp. (Smolander *et al.*, 1990). Similarly, *Frankia* nodulation is stimulated in soil beneath *Betula nigra* (Paschke and Dawson, 1992a). Using *Gymnostoma poissonianum* as a bait plant, soil from the rhizosphere of a non-nodulated endemic rhamnaceous species, *Alphitonia neocaledonica,* was shown to harbor more infective *Frankia* than the rhizospheres of either *Pinus caribea* or bare soil (Gauthier *et al.*, 2000). Evidently, stimulation of *Frankia*'s infectious capacity is independent of the regulation of host specificity. In addition, different populations of *Frankia* were found in alder capture plants depending on whether the soil originated from birch, pine, or spruce stands (Maunuksela *et al.*, 1999). It may be that non-actinorhizal genera able to stimulate *Frankia* had symbiotic ancestors, but have since lost the genetic capacity to symbiose (Simonet *et al.,* 1999).

Non-actinorhizal plants that stimulate the infectious capacity of rhizosphere *Frankia* may derive some benefit from a rhizospheric association with *Frankia*, or may represent plant clade members with a predisposition to the evolution of actinorhizal symbioses. Whatever the reason, the ability of non-actinorhizal plants to stimulate *Frankia* infectivity has important ecological consequences for the maintenance of actinorhizal microsymbionts in the absence of specific hosts. What is more, this situation raises many questions about the evolutionary and functional significance of rhizosphere *Frankia*.

In sympatric associations of *M. gale, Alnus incana* ssp. *Rugosa*, and *Shepherdia canadensis* in a Lake Michigan sand dune ecosystem in Wisconsin, USA, the likelihood of host-plant nodulation by soil-borne *Frankia* was increased by the presence of actinorhizal plants in general, but not the presence of specific host plants (Huguet, 2003). Infectious *Frankia* was detected in 82% of 120 plots located in sand dune communities at one study location, even though only 14% of the plots supported actinorhizal host plant species. Infective *Frankia* were present in soils of young dunes prior to the establishment of any actinorhizal hosts. Submerged soils had no infectious capacity whatsoever, whereas soils with greater *in-situ* moisture contents or subject to intermittent saturation had low infectious capacities overall.

In the same study, PCR-RFLP of the 16S-23S IGS indicated that *Frankia* nodular strains resulting from soil inoculations were in distinct host-specific groups (Huguet, 2003), which showed greater diversity among *Shepherdia*-infective strains than among the closely related *Alnus*-*Myrica* strains. Further, ecological patterns of nodulation corresponded with genetic differences of groups of host-specific nodular strains. All *Shepherdia*-infective *Frankia* were more abundant in soil samples from drier earlier-successional sites, whereas *Alnus*- and *Myrica*-infective *Frankia* strains were more abundant in moister soils of later-successional communities.

There were two strongly divergent phylogenetic groups of *Shepherdia*-infective *Frankia*. One group was dominant in soils of the early stages of sand dune formation and succession, where nodulation capacity was greatest, whereas the other group dominated elsewhere in later successional stages particularly near *Shepherdia* host plants (Huguet, 2003). These results suggest that soil-borne infective *Frankia* genotypes occurring together in the same sand dune soils are not only host-specific, but also are specifically associated with different ecological conditions in their infective state. They also indicate that host specificity is a dominant determinant of symbiotic partnerships in nature, even where host plants occur in close proximity in the same ecosystem. However, ecological factors can influence both the amount of nodulation in different host-specific *Frankia* groups and the infectivity of different strains within a host-specificity group.

Allelopathy can also influence nodulation and growth of actinorhizal partners. Actinorhizal plants have been successfully interplanted as N_2-fixing nurse trees with valuable black walnut trees (Friedrich and Dawson, 1984). Native Arizona alder (*A. oblongifolia*) and Arizona walnut (*Juglans major*) occur together in isolated stretches of riparian forest in Arizona as do various other combinations of walnut species and actinorhizal plants in forests and plantations throughout the world. Walnut's allelochemical, juglone (Dawson and Seymour, 1983), is a respiration inhibitor that was probably evolved as a chemical defense against insect herbivory, but it is also toxic in varying degrees to plants and microorganisms. Both *Frankia* strains and actinorhizal plants are inhibited by juglone, but both host species and microsymbiont strains vary in their response to this allelochemical (Dawson and Seymour, 1983; Neave and Dawson, 1989). This exemplifies just one of many such possible biotic agents with the capacity to alter the composition and growth of associated *Frankia* and actinorhizal plants.

3.2. Abiotic Factors

Abiotic factors that influence infective populations of *Frankia* in soil include time, moisture, pH, aeration, temperature, inorganic chemicals, clay content, organic matter, salinity, and movement by water and wind. For example, the nodulation of *C. velutinus* Dougl. is inversely proportional to the length of time that this shrub has been absent from a site (Wollum *et al.,* 1968). This light-demanding actinorhizal shrub colonizes coniferous forests in the Pacific Northwest of North America after either forest fires or logging. It is typically replaced over a 50-year period by either Douglas fir or mixed conifers in forest succession. In forest stands up to 100 years old, *Ceanothus* plants, which were established from soil seed banks after harvesting, nodulated well. As the age of the stands, which were harvested, increased to 350 years, nodulation declined. Thus *Ceanothus*-infective *Frankia* can apparently persist in soil for many years in the absence of a host. Dry storage of a soil that contained *Frankia* did not change its infective capacity after drying after 3.5 years (Zimpfer *et al.,* 1997). Whatever the means, *Frankia* can persist in an infective form in soils over a long period of time.

Myrold and Huss-Danell (1994) found that a soil's nodulation units changed seasonally, even though the number of genomic units obtained by PCR amplification of *Frankia*-specific DNA sequences remained constant in the same soil. This result is evidence that the physiological response of soil-borne *Frankia* to environmental stimuli is a primary determinant of infectious capacity.

Frankia infectious capacity increases with moisture availability in soils (Dawson *et al.,* 1989; Righetti *et al.,* 1986), although infective *Frankia* is not found in permanently submerged soils (Huss-Danell *et al.,* 1997). Waterlogged soils are generally deficient in O_2 and inhibit activity of free-living microaerophiles, such as *Frankia*. Some *Frankia* strains of wetlands cannot fix N_2 and *Alnus glutinosa* bearing these ineffective bacteria are stunted and have yellow leaves, characteristic of combined-N deficiency (van Dijk and Sluimer-Stolk, 1990). Ineffective *Frankia* strains are widely distributed in waterlogged soils that support *Alnus* spp. and can reach high densities at some sites (Wolters *et al.,* 1997). The actinomycete can survive desiccation both as spores and as hyphae that are induced to form cellular trehalose as an osmotic protectant (Burleigh and Dawson, 1994).

The nodulation of actinorhizal plants is inhibited in acid soils (Bond, 1951; Dixon and Wheeler, 1983; Griffiths and McCormick, 1984; Hensley and Carpenter, 1984) and growth studies of various *Frankia* strains have established a variety of optimal pH values, which are generally neutral to slightly acidic (Lechevalier and Lechevalier, 1990). Even so, infective *Frankia* occur in strongly acidic soils due either to saprophytic growth of acid-tolerant *Frankia* strains or to the persistence of spores of acid-sensitive strains (Faure-Renaud *et al.,* 1986). However, under natural conditions, in which soluble Al^{3+} is inherent to acid soils worldwide (Martin, 1988), Al^{3+} may partially overcome the inhibitory effects of low pH, possibly owing to cell-surface potential phenomena (Igual and Dawson, 1999). Thus, the classification of *Frankia* strains as either acid-tolerant or acid-sensitive, based solely on *in-vitro* growth capacity at low pH, is probably flawed and the inhibition of nodulation of actinorhizal plants in acid soils may not be explainable solely on the basis of low pH.

Frankia colonies in soil apparently behave as a free-living microaerophiles, requiring O_2 for growth and, possibly, N_2 fixation. So, not surprisingly, the greatest *Frankia* infectivity occurs in soil types and strata affording the least resistance to O_2

diffusion (Dawson *et al.,* 1989). Infectious capacity is usually greatest near the soil surface provided that moisture and other microhabitat requirements for *Frankia* are adequate (Paschke and Dawson, 1992b). Infectious capacity may be greater in deeper strata in sandy and other coarse-textured soils, which have greater macropore space to facilitate O_2 diffusion (Dawson *et al.,* 1989). It is possible that O_2 demands are greatest at high temperatures, which induce higher rates of respiration, and when other stresses induce rapid physiological responses, which are typically accompanied by higher rates of respiration. Insofar as free-living *Frankia* in soil might be able to fix N_2, its adaptive O_2-control system, which consists of multiple monolyaers of lipids located in the vesicle, is able to protect the nitrogenase enzyme from O_2 (Silvester and Harris, 1990; see chapter 5 in this volume).

The optimal temperature range for nodulation of *C. velutinus* from western North America is 22-26°C (Wollum and Youngberg, 1969). The optimal temperature range for nodulation of most other actinorhizal host-microsymbiont combinations is unknown. For a given pair of actinorhizal symbionts, the intersection of optimal temperature ranges in soil for growth of the microsymbiont and primary root development of the host plant would likely be the optimal range of temperatures for nodulation. Most isolated strains of *Frankia* grow fastest in culture at temperatures between 25°C and 35°C (Lechevalier and Lechevalier, 1990). The optimal temperatures for growth and development in soil of roots of actinorhizal plant species undoubtedly vary widely according to climate but are virtually unknown in detail. Among *Frankia* isolates, those derived from nodules of Casuarinaceae spp. generally have higher optimal culture temperatures than isolates from cooler temperate regions, perhaps reflecting adaptations to warmer climates in their places of origin. It would be reasonable to assume that Casuarinaceae host species would likewise be adapted to optimize root growth at higher soil temperatures. Freezing of soil containing *Frankia* for several years at –20°C did not alter the structure of nodulating *Frankia* populations, but did decrease the nodulation capacity of frozen soil (Maunuksela *et al.,* 2000).

Frankia strains interact with inorganic chemicals in soil in many ways likely to influence their free-living populations. For example, some strains produce siderophores in iron-limited situations to aid in iron uptake (Boyer *et al.,* 1999). The trace element molybdenum is an essential micronutrient for nitrogenase function in *Frankia,* and must be present in soils in order for symbiotic nitrogen fixation to occur (Hewitt and Bond, 1961). Martin *et al.* (2003) found, in red alder stands of varying ages, that a decline in nodulation capacity was most closely related to lower pH and higher nitrate concentrations. As alder litter and sloughed roots increase organic nitrogen in the soil, mineralization of the N-rich tissue also increases and results in higher nitrate concentrations and lower pH. The authors speculated that low pH resulting from nitrification and the stimulation by nitrate of competing soil bacteria may decrease *Frankia* populations in soil.

Other inorganic chemicals are potentially toxic to *Frankia* in soil ecosystems, particularly pollutants resulting from mining and industrial activity. *A. glutinosa* and *A. rubra* nodulation are reduced by cadmium in soils (Hensley and Carpenter, 1987). *Casuarina*-infective *Frankia* was able to grow and nodulate host plants at high nominal Al concentrations of 800µM (279µM monomeric Al) (Igual *et al.,* 1997). At the same time, soluble Al^{3+} overcomes the inhibitory effects of low pH on *Frankia* growth (see earlier in this section).

Nickel is required for hydrogenase synthesis and function in *Frankia* and amounts of this element greater than those typically found in soils results in greater nodulation and N_2 fixation in *A. glutinosa*. However, even higher Ni^{2+} levels apparently become toxic to the host plant before they become toxic to *Frankia* (Wheeler *et al.,* 2001).

Twelve *Frankia* strains were sensitive to low concentrations (<0.5mM) of Ag^{+}, AsO_2^{-}, Cd^{2+}, SbO_2^{--}, and Ni^{2+} (Richards *et al.* 2002). Most of the strains were less sensitive to Pb^{2+} (6-8mM), CrO_4^{2-} (1.0-1.75mM), AsO_4^{3-} (>50mM), and SeO_2^{2-} (1.5-3.5mM). Although most strains were sensitive to 0.1mM Cu^{2+}, four strains were resistant to elevated levels of Cu^{2+} (2-5mM and concentrations as high as 20mM). The mechanism of SeO_2^{2-} resistance may involve reduction of soluble selenite to insoluble elemental selenium, whereas Pb^{2+} resistance and Cu^{2+} resistance may involve sequestration or binding mechanisms. Some *Frankia* strains, therefore, have the capacity to resist heavy metal toxicity in soil.

Frankia infectious capacity increases with the amount of clay in substrates (Smolander *et al.,* 1988; Zimpfer *et al.,* 2002). Clay and organic matter particles in soil can increase its water-holding capacity and the amount of clay or organic matter in soil correlates positively with *Frankia* infectious capacity (Burleigh and Dawson, 1994; Dawson *et al.,* 1989; Righetti *et al.,* 1986; Smolander *et al.,* 1988). In Jamaica for example, a moist, fertile soil with a substantial clay component and neutral pH appeared particularly supportive of *Myrica*-infective *Frankia* (Zimpfer *et al.,* 1997).

Frankia strains isolated from *Casuarina* spp. are more tolerant of high concentrations of NaCl (approaching that of seawater) than isolates from North American hosts not normally exposed to sodic soils (Dawson and Gibson, 1987). *C. equisetifolia, M. pensylvanica*, and *Hippophaë rhamnoides* all nodulate in coastal soils. Infective populations of *Frankia* are present in soils of coastal sand dunes occupied by *M. pensylvanica* in Virginia, USA (Young *et al.,* 1992). This natural variability in salt tolerance of *Frankia* strains could be exploited to produce inocula for use with salt-tolerant host plants to increase the productivity of saline soils.

Soil organic material most likely supports the saprophytic grpwth of *Frankia* and its physiological status in soils. Evidence for this suggestion comes from enzymes secreted and the metabolic activities of *Frankia* strains. *Frankia* strains secrete proteinases, aminopeptidases, and proteasome-like corpuscles (Benoit *et al.,* 1992; Girgis and Schwenke, 1993; Muller *et al.,* 1991), all of which can degrade proteins in soil organic matter to give amino acids that could be used as N sources by *Frankia* (Zhang and Benson, 1992). Extracellular pectinases and cellulases could enable *Frankia* to break down complex organic substances into simple organic substrates to support growth in soil (Safo-Sampath and Torrey, 1988; Seguin and Lalonde, 1989). Also, some *Frankia* spp. are capable of metabolizing complex phospholipids (Girgis and Schwenke, 1993).

In addition to supplying nutrients, organic matter also improves the water-holding capacity of soils and supports beneficial as well as antagonistic microorganisms. *Frankia*'s ability to produce antibiotics could enhance the ability of this slow-growing actinomycete to compete as a saprophyte with faster-growing soil microorganisms (Haansu *et al.,* 2001).

At a given time during their growth and infective phases, any one factor might be the overriding determinant of growth and nodulation capacity of *Frankia*. Undoubtedly, however, many of the biotic and abiotic factors above would typically interact in a complex manner to influence nodulation.

4. ECOLOGY OF ACTINORHIZAL PLANTS

4.1. Ecophysiology

4.1.1. Light Responses

Light intensity and duration are important determinants of the occurrence and function of actinorhizal plants. Actinorhizal plants tend to be light demanding and many function physiologically as early successional shade-intolerant species (Côté *et al.,* 1988), which typically can take photosynthetic advantage of high light intensity. Other actinorhizal plants occur as stable components of ecosystems that feature scattered trees and shrubs in stands kept open to light penetration by either aridity or fires. Their intolerance of shade generally precludes their persistence or regeneration in the understory of dense forest stands. Evolutionary pressure for this type of photosynthetic adaptation could have arisen from the typically open nature of its disturbed or N-limited niches plus the considerable energy requirements for nitrogen fixation.

Because actinorhizal plants are capable of symbiotic nitrogen fixation, they can maintain high leaf-N levels that are associated with high rates of photosynthesis (Krueger and Ruth, 1968). Several species of alder, including *A. glutinosa*, retain green foliage longer than other temperate deciduous trees and do not resorb foliar N as efficiently as other temperate deciduous species in the autumn (Côté and Dawson, 1986). This prolonged leaf and nitrogen retention is associated with prolonged photosynthesis of *A. glutinosa* and probably other alders (Neave and Dawson, 1989). Prolonged autumnal photosynthesis may give alders a competitive growth advantage over other temperate deciduous species. It may also provide photosynthate for seasonally high rates of nodule growth, for maintenance of high rates of nitrogen fixation observed for alders in early autumn (Kaelke and Dawson, 2003), and for the energy demands for enzymatic processes inducing dormancy as well as the needs for carbohydrate reserves to maintain dormant tissue over the winter. Apparently, temperature, not photoperiod, eventually decreases photosynthesis and induces senescence in alder leaves (Côté and Dawson, 1986), illustrating an important difference in light response of alders in comparison with temperate deciduous trees that initiate foliar autumnal senescence processes in precise synchrony with day-length changes.

Photosynthesis is the primary driver of symbiotic nitrogen fixation (Gordon and Wheeler, 1978) and it is likely that the greatest gains in actinorhizal nitrogen fixation can be obtained by genetic manipulation of the host to increase photosynthetic capacity rather than through selection or alteration of the microsymbiont alone (Dawson and Gordon, 1979). Because whole-plant photosynthesis rates are linearly correlated with whole-plant nitrogenase activities in alder (Gordon and Wheeler, 1978), the search for strains of *Frankia* and host genotypes more efficient at nitrogen fixation might practically be evaluated on the basis of the ratio of nitrogenase activity to photosynthesis on a whole-plant basis.

The impact of elevated carbon-dioxide levels on plants has been the focus of many recent studies that were initiated in response to the alarm over the increasing levels of atmospheric carbon dioxide from the widespread use of fossil fuels. Vogel and Curtis (1995) found with *A. glutinosa* that a doubling of ambient CO_2 in open-top chambers for 160 days in the field resulted in a 16% greater rate of CO_2 fixation, a 50% increase in specific leaf N, and a 46% increase in specific nitrogenase activity of detached nodules relative to controls at ambient levels of atmospheric CO_2. Unlike other plants, actinorhizal trees and shrubs on N-limited soils can maintain high net CO_2 assimilation with minimal negative adjustment of photosynthetic capacity following prolonged exposure to elevated CO_2.

4.1.2. Moisture

Actinorhizal plants have a broad range of ecological amplitudes with respect to soil moisture. Species such as *Cowania mexicana* and *Purshia tridentata* occur in semi-arid temperate shrub ecosystems in North America and are able to tolerate drought. Dalton and Zobel (1977) found that the xerophytic shrub *P. tridentata* can tolerate severe water stress, but nitrogenase activity of nodulated plants declines sharply under these conditions and requires 12 days of watering to recover. In contrast, the wetland tree *A. glutinosa* is much more sensitive to water stress (Seiler and Johnson, 1984). *A. incana* ssp. *incana* (grey alder), an alder of Europe that occurs on drier soils than those occupied by *A. glutinosa*, exhibited declines in nitrogenase activity with decreasing water potentials from -0.6 to -1.4 Mpa (Sundström and Huss-Danell, 1987). Stomatal closure occurred with water stress and there was a strong relation between stomatal closure, which reduces photosynthesis, and the inhibition of nitrogenase activity. *A. viridis* ssp. *crispa, M. pennsylavanica*, *M. cerifera*, *Ceanothus* spp., and *Comptonia peregrina* occur on dry soils in North America and apparently have adaptations to drought.

The *Casuarina* spp. are riparian or coastal species in nature, but can develop a long tap root to exploit deeper sources of soil moisture (Yadav, 1983) and are commonly planted in uplands worldwide in tropical, subtropical, and warm temperate regions. The *Allocasuarina* spp. are associated with more arid environments in Australia and are more drought tolerant than *Casuarina* spp. (Subbarao and Rodríguez-Barrueco, 1995). Actinorhizal plants of riparian zones and wetlands have physiological and morphological adaptations that enable them to tolerate flooding and soil waterlogging. Wetland *M. gale* and riparian *Casuarina* spp. produce negatively geotropic “nodule roots” under waterlogged soil conditions. These serve as alternate O_2-delivery pathways to enable sufficient respiratory activity in nodules to meet the energy requirements of N_2 fixation under low soil O_2 conditions (Silvester *et al.,* 1988a; Wheeler *et al.,* 1979).

A. glutinosa and *A. rubra* are flood tolerant alders that develop nodule lenticels when flooded (Silvester *et al.,* 1988b; Tjepkema, 1978). In response to flooding, *Alnus rubra* forms hypertrophied nodule lenticels with subsequent full restoration of nitrogenase activity after 50 days, when there was a 10-fold increase in intercellular space around nodule cells containing *Frankia* vesicles (Batzli and Dawson, 1999). *A. incana* ssp. *rugosa* is a wetland alder that is not actually tolerant of soil waterlogging, but that inhabits the margins of wetlands with a stable water level where it maintains a superficial root system, thus avoiding the hypoxia of waterlogged soils (Kaelke and Dawson, 2003).

Soil moisture may also influence the occurrence of actinorhizal plants because the germinants may be particularly sensitive to desiccation, such as for *A. glutinosa* (McVean, 1956), which limits their occurrence to seasonally moist sites.

4.1.3. Fire and Heat

Species of the actinorhizal plant genus *Ceanothus* in chaparral vegetation of California have leaves that are coated with flammable resins. The leaves and branches of these plants are small, adding to their flammability. Thus, wildfires pass quickly with limited heating of soil, which prevents damage to seeds and roots important in supporting growth of sprouts after fire. *Ceanothus* roots are specially adapted to enable the plant to grow in areas that were recently burned. The plants' ability to produce heat-resistant seeds that remain dormant yet viable in ground litter for long periods of time contributes to the ability of *Ceanothus* to recover quickly following fire (Keeley, 1989). Fire also insures germination under open light conditions and releases nutrient to support post-fire growth. *Comptonia peregrina* seeds respond to a heat signal generated by direct sunlight, so stimulating germination under open conditions created by a disturbance that simultaneously assures the high light levels necessary to establish the seedlings (Dow and Schwintzer, 1999).

4.1.4. Seasonality

Actinorhizal nodules are perennial structures that become dormant along with the whole plant during the winter in temperate and boreal host species. During the winter, there are few symbiotic nodule vesicles, no detectable nitrogenase activity, reduced numbers of hyphae, and, in some instances, an increase in spores (Schwintzer *et al.,* 1982; Wheeler *et al.,* 1983). To maintain viability in the dormant state, nodules and other root tissues increase reserve carbohydrates prior to dormancy, which subsequently decrease over winter, perhaps by supporting the metabolism of the overwintering nodules (Wheeler and Bowes, 1974; Wheeler *et al.,* 1983). Trehalose and glycogen produced by *Frankia* may be reserve carbohydrates for overwintering in the nodular microsymbiont (Benson and Eveleigh, 1979; Lopez *et al.,* 1984).

An unusual aspect of actinorhizal alder physiology is a delay in foliar senescence in the autumn, associated with prolonged photosynthesis (Neave *et al.,* 1989) and a lack of resorption of foliar N prior to leaf drop. In contrast, the net resorption and conservation of foliar phosphorus, carbohydrates, and other nutrients during alder leaf senescence is apparently equal in efficiency to that of other temperate deciduous trees and shrubs (Côté and Dawson, 1986). One might speculate that there would be little evolutionary selection pressure for efficient resorption and reuse of foliar N in a N_2-fixing tree, however, the lack of resorption of foliar N leads to a consequent drop of leaves with high concentrations of N. This accelerated shedding of internal N by alder accelerates the rate at which recently fixed N is added to soil relative to the expected rate if alder resorbed and recycled N similarly to other temperate deciduous trees.

4.1.5. Plant Nutrients

Of the essential mineral nutrients, molybdenum, cobalt, phosphorus, and nitrogen are of special interest with respect to nitrogen fixation in actinorhizal plants. Molybdenum is required for nitrogenase and root nodules of *A. glutinosa* have a nodular Mo concentration 6-times greater than roots (Becking, 1970). Cobalt is the only mineral element exclusively essential for N_2-fixing plants because the microsymbionts require it for vitamin B_{12} production (Dixon and Wheeler, 1983).

Nitrogen-fixing plants have a relatively high demand for phosphorus because of the high energy demands for nitrogen fixation and, hence, for the phosphorus compounds important in biochemical energetics (Huss-Danell, 1990). Phosphorus deficiency limits nodulation and nitrogen fixation in actinorhizal plants (Diem and Dommergues, 1990). *Casuarina* windbreaks on infertile coastal sand dunes in southeast China become phosphorus limited after litter harvesting for fuel, resulting in loss of vigor and increased disease problems in subsequent plantings. The presence of mycorrhizae on nodulated alders in a tripartite symbiosis improves phosphate uptake by *A. viridis* ssp. *viridis* (Mejstrik and Benecke, 1969). In a tripartite association between the ectomycorrhizal fungus *Alpova diplophloeus*, *Frankia*, and *Alnus tenuifolia*, apparently the ectomycorrhizal partner increased the solubilization of ground basalt, enhancing mineral acquisition and consequent growth and nitrogen fixation of the host plant (Yamanaka *et al.,* 2003).

Combined-N at concentrations necessary for optimal plant growth not only inhibits nodulation but also inhibits nitrogen fixation in nodules already formed. These two inhibitory influences of combined-N occur concurrently under both field and laboratory conditions (Bond and Mackintosh, 1975; Ingsted, 1980; Rodríguez-Barrueco *et al.,* 1970). Theoretically, it is energetically more efficient for actinorhizal plants to take up combined-N in the form of NH_4^+ or NO_3^- from the soil solution than to fix N_2 symbiotically, hence the observed self-imposed inhibition of nodulation and nitrogen fixation in an early successional actinorhizal alder forest.

It is possible that the uptake and metabolism of NO_3^- complement nitrogen fixation seasonally by supplying N to plants during the autumn in temperate regions. Nitrate reductase activity has a lower optimal temperature than that of nitrogenase activity. At lower temperatures typically occurring in temperate-region soils during autumn, *A. glutinosa* nodules switch the expenditure of energy on nitrogenase activity to nitrate reductase activity, yielding temporally distinct and complementary mechanisms for N acquisition in an actinorhizal system (Vogel and Dawson, 1986).

Alder forest soils typically increase in acidity, total N, and organic matter content while decreasing in cation-exchange capacity with increasing stand age (Bormann *et al.,* 1994). With bacterial mineralization of its litter and sloughed roots, alder releases NH_4^+ into the soil, which is then converted to NO_3^- under aerobic conditions, sometimes at very high rates (Paschke *et al.,* 1989). The latter process of nitrification is the cause of acidification in alder soils (Van Miegrot and Cole, 1985). Increases in NO_3^- concentration and lower pH values were most closely associated with a decline in nodulation capacities of soils from red alder stands of different ages in the Pacific Northwest of North America (Martin *et al.,* 2003). This observation suggests that alder, *via* feedback mechanisms triggered by high NO_3^- levels, switches from nitrogen fixation to the more efficient uptake of mineral-N in response to its own enrichment of soil-N fertility. Acidification displaces nutrient cations from soil exchange sites by protons, followed by leaching that decreases fertility. With red alder, successional coniferous species replace the red alder and act as base recyclers (Van Miegrot and Cole, 1985), counteracting the decrease in fertility resulting from acidification.

One anthropogenic change evident today is an increase in atmospheric N pollution, which creates the potential for widespread increases in background levels of N in soil when delivered in precipitation. Such a change could decrease the biological diversity of plants and associated animals (Tilman *et al.,* 1996). Does this mean that fixed-N provided to terrestrial ecosytems by actinorhizal plants add to the threat to biodiversity? Biological diversity with respect to nitrogen fertility would predictably depend on patches with a wide range of available combined-N levels to support organisms with differing requirements for combined-N. An increase in soil-N resulting from atmospheric pollution would tend to first homogenize and eliminate patches at the low end of N-fertility levels together with their adapted organisms. However the N-rich patches associated with actinorhizal plants would remain important to organisms dependent upon high levels of N-fertility and hence would support biological diversity, barring an extreme increase in soil-N levels due to pollution.

It is a mistake to assume that actinorhizal plants are ecologically important only for the amount of nitrogen fixed in a given terrestrial ecosystem. Where the total or relative amount of nitrogen fixed by actinorhizal plants is unknown or relatively small, it can nonetheless be sufficient to allow hosts to establish competitively and assume other critical roles in the functioning of their particular terrestrial ecosystems. This could be the case for actinorhizal plants of sand dune ecosystems, deserts, grasslands and savannas.

Salt concentration can also differentially influence the survival and symbiotic performance of actinorhizal host plants (El-Lakany and Luard, 1982). In Australia, three *Casuarina* species occur along rivers according to their respective salt tolerances (Subbarao and Rodríguez-Barrueco, 1995). *C. cunninghamiana* occurs along freshwater portions of rivers, *C. glauca* occurs near brackish water and in lower reaches of rivers influenced by tidal surges, whereas *C. equisetifolia* occurs in coastal areas directly influenced by sea spray and incursions of seawater (Midgley *et al.,* 1983). At the boundaries between these zones of salinity, hybrid forms of these tree species are found.

In addition to the factors influencing the ecology and physiology of host plants summarized above, symbiotic performance can be compromised by the absence of appropriate mycorrhizal and N_2-fixing symbionts (Diem and Gauthier, 1982; Reddell *et al.,* 1988), low soil temperatures (Reddell *et al.,* 1985), deficiencies of other nutrients, and low soil pH (Bond, 1957; Coyne, 1973). Climate change, soil pollutants, human-altered fire regimes, increased ozone levels, sulfur pollution and acid rain, pests, diseases, soil erosion, hydrological changes, volcanic activity, desertification, logging, plantation establishment, and many other factors too numerous to list impact the occurrence, health, ecological and physiological function of actinorhizal species. The identification and study of ecological and physiological responses of actinorhizal plants to environmental change will be important in optimizing human stewardship of these valuable natural resources.

4.2. Interactions among Actinorhizal and Associated Plant Species

The preponderant open N-limited niches for actinorhizal plants as proscribed by their light-demanding nature and capacity to fix N_2 symbiotically have been described. The dynamics in time and space that create and eliminate these niches involves competition for plant resources. In many cases, actinorhizal plants ultimately facilitate the establishment and growth of other plant species that replace them in predictable patterns of plant succession. In other cases, the introduction of exotic actinorhizal plants can displace other plant species and alter ecological processes, so creating novel ecosystems and successional processes.

Crocker and Major were among the first to note a correlation between nutrient accumulation associated with early primary successional stands of *A. viridis* ssp. *sinuata* at Glacier Bay, Alaska, and the development of subsequent forest communities. However, the relative timing and importance of fixed-N supplied to associated plants by actinorhizal N_2 fixers and the balance of their facilitative *versus* their competitive influences over glacial chronosequences has seldom been determined precisely in ecological studies. Direct evidence for facilitation of associated plants by provision of fixed-N during primary succession is lacking at Glacier Bay and other areas of recent glacial deposits (Walker, 1993).

Using δN^{15} values in a chronosequence of deglaciation at Glacier Bay, Kohls *et al.* (2003) found that the amount of N supplied to willow trees by actinorhizal nitrogen fixation increased over a 40-year period to an equilibrium value, suggesting that fixed N_2 was the dominant source of N at that time. This time coincided with the occurrence of dense thickets of alder, which undoubtedly provided competitive pressure on the establishment of other plants. But, at the earliest stages of primary succession, actinorhizal nitrogen fixation was not the major source of N in associated plants; it would obviously not be advantageous to a N_2-fixing plant, which was establishing itself on a nutrient-limited site, to leak any substantial amount of the N fixed at the expenditure of considerable energy. Of the actinorhizal plants, *Shepherdia* depended most, *Dryas* least, and *Alnus* at an intermediate level on atmospheric nitrogen fixation. Further, the small colony-forming *Dryas* shrub seemed to decrease its reliance on N_2 fixation over time and with increased levels of available soil-N. It appears then that actinorhizal species may not be as important as fixed-N sources during the earliest stages of primary succession as once thought and that other sources of combined-N can be important during early primary succession (Vitousek and Walker, 1989). Even so, actinorhizal plants are ultimately a dominant source of N available to plants during a prominent phase of primary succession on glacial deposits at Glacier Bay.

To capture the benefits of nitrogen fixation in either balanced natural stands or inter-plantings of actinorhizal trees or shrubs with companion trees, it is important that the growth rate and density of the N_2-fixing tree or shrub not exceed that of associated trees (Dawson, 1990). In the Pacific Northwest of North America, *A. rubra* and *C. velutinus* are vigorous colonizers of logged and burned areas of Douglas-fir forest. Although they provide benefits from nitrogen fixation, a high density of colonizing alder or snowbrush slows the regeneration of the desired Douglas fir. Similarly, where the growth rate or density of a companion tree in plantations exceeds that of the actinorhizal nurse plant, or where allelopathy or other competitive influences inhibit the actinorhizal plant, the actinorhizal plant will be displaced, so causing the loss of both a supply of fixed-N and all other inter-planting benefits that were afforded the companion crop. If the companion crop is shade loving, such as shade-coffee, the growth rate and stature of the N_2-fixing shade planting are less important than its density and ultimate crown structure in providing optimal light conditions to the shade-requiring companion crop.

The impact of the introduction of exotic actinorhizal plants on terrestrial ecosystems can be dramatic, especially because of the competitive advantage afforded them by their ability to fix N_2 symbiotically (Richardson *et al.,* 2000). The best documented example of a major impact of an actinorhizal exotic on ecosystem composition, structure and function is that of the invasion of *M. faya* from the Canary Islands on volcanic deposits in Hawaii Volcanoes National Park (Vitousek and Walker, 1989). Dense stands of this exotic plant have established themselves in a novel community that greatly increases combined-N inputs from symbiotic fixation. Similarly, in south Florida, plantings of *Casuarina* species have given rise to widely naturalized offspring that displace native vegetation, particularly on sandy soils and near wetlands in this low-elevation peninsula. Frequent hurricanes disperse its small seeds over great distances and the exposure of bare soil by the uprooting of other trees creates an ideal seedbed and open site for its establishment in native plant communities. Elsewhere, exotic *Elaeagnus umbellata* and *Elaeagnus angustifolia* bear fruit, which is consumed by birds that disperse the plants widely. *E. umbellata* is a common weed of the eastern U.S.A., whereas *E. angustifolia* is a common invader of valuable riparian forests in semi-arid regions of western North America.

4.3. Actinorhizal Plant Interactions with Heterotrophic Organisms and Humans

There are some interactive relatiohships with heterotrophic organisms that are particular to actinorhizal plants and that relate to their N_2-fixing capacity. A few examples are given to illustrate the general nature of these unique relationships.

Spittlebugs (Homoptera: Cercopoidea) are common insects that feed on xylem sap. Many show a preference for N_2-fixing plants that afford an abundant and reliable supply of xylem-borne organic-N compounds (Thompson, 1994). At least 20 species have a primary association with actinorhizal *Alnus*, *Casuarina*, *Ceanothus, Comptonia, Elaeagnus*, and *Myrica* hosts (Thompson, 1999).

In plantations of *A. acuminata* in the cloud forest regions of Colombia, the avian fauna is more diverse and representative of native forest birds than those of plantations of either *Eucalyptus* or *Pinus* spp. Most common are native birds that feed on soil invertebrates. Most likely, the high rates of soil-N accretion associated with Andean alder plantations (Carlson and Dawson, 1985) increase the biomass of soil invertebrates, thus making a situation attractive to these birds.

Nitrogen-fixing plants are beneficial and attractive to herbivores because of their abundant tissue nitrogen. So, N_2-fixing trees typically invest resources in chemical and other defenses against herbivory (Haukioja, 1991), with phenolics being a one such deterrant. As mentioned above, *A. viridis* ssp. *crispa* produces phenolic pinosylvin methyl ether to discourage browsing by voracious arctic hares (Bryant *et al.,* 1983). Similarly, the wide-ranging actinorhizal shrub, *P. tridentata* (bitterbrush), which is the most important browse species for wildlife and livestock in the intermountain west of North America, has populations with varying phenolic contents, particularly in response to browsing (Paschke, 1997). Alder root tissue is also high in phenolics, and alders have been observed to decrease the incidence of fungal root pathogens in associated Douglas fir (Li *et al.,* 1970; 1972).

The cold streams that support trout and salmon, particularly the headwaters of rivers where anadromous salmon spawn, are often infertile. It is possible that alders commonly and abundantly associated with these freshwater streams are important sources of combined-N for the planktonic organisms at the beginning of the food chain for salmonids and their fry (Goldman, 1961).

The management and use of actinorhizal plants by humans has been documented extensively in several reviews (Dawson, 1990; 1992; Diem and Dommergues, 1990; Dixon and Wheeler, 1986; Gordon and Dawson, 1979; Hibbs and Kromack, 1990; Hibbs *et al.,* 1994; Huss-Danell, 1997; Paschke, 1997; Schwencke and Carú, 2001; Silvester, 1977; Wheeler and Miller, 1990). The potential for domestication and use of actinorhizal plants for food, medicine, soil improvement, bioremediation, soil stabilization, aesthetic plantings, agro forestry, wood, fiber and chemicals remains largely untapped. Although actinorhizal plants in general are not highly susceptible to pests or diseases (Dommergues, 1997), their use in large-scale monocultures on poor sites and under stressful conditions increases the likelihood of serious damage by insects and diseases. Some actinorhizal plants, such as Elaeagnaceae genera including *Hippophaë,* are receiving renewed attention because they produce high concentrations of antioxidant compounds, such as flavonols, which are important in human nutrition (Rösch *et al.,* 2003).

Human encroachment on the wild lands, where actinorhizal plants are key functional ecosystem components, continues unabated and accelerates with the growth of human populations and increasing standards of living. It is primarily through an increased understanding of both the importance of actinorhizal plants to ecosystem function and to our natural heritage, and their capacity to provide valuable products and by-products that they will be effectively managed, preserved, and used sustainably.

REFERENCES

Arveby, A. S., and Huss-Danell, K. (1988). Presence and dispersal of infective *Frankia* in peat and meadow soils in Sweden. *Biol. Fertil. Soils*, *6*, 39-44.

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., and O'Keefe, D. (1984). A modified sucrose fractionation procedure for the isolation of frankiae from actinorhizal root nodules and soil samples. *Plant Soil*, *78*, 23-28.

Baker, D. D., and Schwintzer, C. W. (1990). Introduction. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 3-13). New York, NY: Academic Press.

Barlow, B. A. (1983). Casuarinas, a taxonomic and biogeographic review. In S. J. Midgley, J. W. Turnbull, and R. D. Johnston (Eds.), *Casuarina ecology, management* and *utilization* (pp. 10-18). Melbourne, Australia: CSIRO.

Batzli, J. M., and Dawson, J. O. (1999). Development of flood-induced lenticels in red alder nodules prior to the restoration of nitrogenase activity. *Can. J. Bot.*, *77*, 1373-1377.

Becking, J. H. (1970). Frankiaceae, fam. Nov. (Actinomycetales) with one new combination and six new species of the genus *Frankia* Brunchorst 1886. *Int. J. Syst. Bacteriol.*, *20*, 201-220.

Benecke, U. (1969). Symbionts of alder nodules in New Zealand. *Plant Soil*, *30*, 149-149.

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

Benoit, P., Müller, A., Diem, H. G., and Schwenke, J. (1992). High-molecular-mass multi-catalytic proteinase complexes produced by the nitrogen-fixing actinomycete *Frankia* strain BR. *J. Bacteriol.*, *174*, 1495-1504.

Benson, D. R., and Eveleigh, D. E. (1979). Nitrogen fixing homogenates of *Myrica pensylvanica* (bayberry) non-legume root nodules. *Soil Biol. Biochem.*, *11*, 331-334.

Bermudez de Castro, F., Miguel, C., and Rodriguez-Barrueco, C. (1976). A study of the capacity of soil to induce nodules of *Alnus glutinosa* (L.) Gaertn. and *Myrica gale* L. with special reference to the specificity of the endophytes. *Ann. Microbiol.*, *127A*, 307-315.

Bond, G. (1951). The fixation of nitrogen associated with the root nodules of *Myrica gale* L., with special reference to its pH relation and ecological significance. *Ann. Bot.*, *15*, 447-459.

Bond, G. (1957). The development and significance of the root nodules of *Casuarina*. *Ann. Bot.*, *21*, 373-380.

Bond, G. (1983). Taxonomy and distribution of non-legume nitrogen-fixation systems. In J. C. Gordon and C. T. Wheeler (Eds.), *Biolgical nitrogen fixation in forest ecosystems: Foundations* and *applications* (pp. 55-87). The Hague, The Netherlands: Martinus Nijhoff/Dr. W. Junk.

Bond, G., and Mackintosh, A. H. (1975). Effect of nitrate nitrogen on the nodule symbioses of *Coriaria* and *Hippophae*. *P. Roy. Soc. Lond. B Bio., 190*, 199–209.

Bormann, B. T., Cromack, K., and Russel, W. O. (1994). Influences of red alder on soils andlong-term ecosystem productivity. In D. E. Hibbs, D. S. DeBell and R. F. Tarrant (Eds.), *The biology* and *management of red alders* (pp. 47-56). Corvallis, OR: Oregon State University Press.

Boyer, G. L., Kane, S. A., Alexander, J. A., and D. B. Aronson. (1999). Siderophore formation in iron-limited cultures of *Frankia* sp. 52065 and *Frankia* sp. CeSI5. *Can. J. Bot.*, ***77***, 1316-1320.

Bryant, J. P., Wieland, G. D., Reichardt, P. B., Lewis, V. E., and McCarthy, M. C. (1983). Pinosylvin methyl ether deters snowshoe hare feeding on green alder. *Science*, *222*, 1023-1025.

Burleigh, S. H., and Dawson, J. O. (1994). Occurrence of *Myrica*-nodulating *Frankia* in Hawaiian volcanic soils. *Plant Soil*, *164*, 283-289.

Burleigh, S. H., and Dawson, J. O. (1995). Spores of *Frankia* strain HFPCcI3 nodulate *Casuarina equisetifolia* after passage through the digestive tracts of captive parakeets (*Melopsittacus undulatus*). *Can. J. Bot.*, *75*, 1527-1530.

Burleigh, S., and Torrey, J. G. (1990). Effectiveness of different *Frankia* cell types as inocula for the actinorhizal plant *Casuarina*. *App. Environ. Microbiol.*, *56,* 2565-2567.

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.

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.

Chepstow-Lusty, A. J., Bennett, K. D., Fjeldsa, J., Kendall, A., Galliano, W., and Tupayachi Herrera, A. (1998). Tracing 4,000 years of environmental history in the Cuzco area, Peru, from the pollen record. *Mt. Res. Dev.*, *18*, 159-172.

Clausen, T. P., Reichardt, P. B., and Bryant, J. P. (1987). Pinosylvin and pinosylvin methyl ether as feeding deterrents in green alder. *J. Chem. Ecol.*, *12*, 2117-132.

Clawson, M. L., Carú, M., and Benson, D. R. (1998). Diversity of *Frankia* strains of root nodules of plants from the families Elaeagnaceae and Rhamnaceae. *Appl. Environ. Microbiol.*, *64*, 3539-3543.

Clawson, M. L., Gawronski, J., and Benson, D. R. (1999). Dominance of *Frankia* strains instands of *Alnus incana* subsp. *rugosa* and *Myrica pensylvanica*. *Can. J. Bot.*, *77*, 1203-1207.

Côté, B., Carlson, R. W., and Dawson, J. O. (1988). Leaf photosynthetic characteristics of seedlings of actinorhizal *Alnus* ssp. and *Elaeagnus* spp. *Photosynth. Res.*, *16*, 211-218.

Côté, B., and Dawson, J. O. (1986). Autumnal changes in total nitrogen, salt-extractedproteins, and amino acids in leaves and adjacent bark of black alder, eastern cottonwood, and white basswood. *Physiol. Plant.*, *67*, 102-108.

Coyne, P. D. (1973). (Ph.D. thesis, Australian National University, Canberra, Australia.). Summarized by Torrey, J. G. *Casuarina*: Actinorhizal nitrogen-fixing tree of the tropics. In *Biological nitrogen fixation technology for tropical agriculture* (pp. 427-439). Cali, Colombia: Centro Internacional de Agricultura Tropical.

Dalton, D. A., and Zobel, D. B. (1977). Ecological aspects of nitrogen fixation by *Purshia tridentata*. *Plant Soil*, *48*, 57-80.

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

Dawson, J. O. (1990). Interactions among actinorhizal and associated species. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 288-316). New York, NY: Academic Press.

Dawson, J. O. (1992). Nitrogen fixation in forestry and agroforestry. In F. B. Metting, Jr. (Ed.), *Soil microbial ecology* (pp. 227-253). Basel, Switzerland: Marcel Decker.

Dawson, J. O., and Gibson, A. H. (1987). Sensitivity to sodium chloride of selected *Frankia* isolates from *Casuarina*, *Allocasuarina* and North American host plants. *Physiol. Plant.*, *70*, 272-278.

Dawson, J. O., and Gordon, J. C. (1979). Nitrogen fixation in relation to photosynthesis in *Alnus glutinosa*. *Bot. Gaz.*, *140*, S70-S75.

Dawson, J. O., and Klemp, M. T. (1987). Variation in the capacity of black alder to nodulate in central Illinois soils. In R. L. Hay, F. W. Woods, and H. DeSelm (Eds.), *Sixth central hardwood forest conference* (pp. 255-260). Knoxville, TN: Department of Forestry, Wildlife, and Fisheries, University of Tennessee.

Dawson, J. O., Kowalski, D. G., and Dart, P. J. (1989). Variation with soil depth, topographicposition and host species in the capacity of soils from an Australian locale to nodulate *Casuarina* and *Allocasuarina* seedlings. *Plant Soil*, *118*, 1-13.

Dawson, J. O., and Seymour, P. E. (1983). Effects of juglone concentration on growth *in vitro* of *Frankia* ArI3 and *Rhizobium japonicum* strain 71. *J. Chem. Ecol.*, *9,* 1175-1183.

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.), T*he biology of* Frankia and *actinorhizal plants* (pp. 317-342). New York, NY: Academic Press.

Diem, H. G., and Gauthier, D. (1982). Effet de l'infection endomycorrhizienne (*Glomus mosseae*) sur la nodulation et la croissance de *Casuarina equisetifolia*. *C.R. Acad. Sci. III -Vie 294*, 215-218.

Dixon, R. O. D., and Wheeler, C. T. (1983). Biochemical, physiological, and environmental aspects of symbiotic nitrogen fixation. In J. C. Gordon and C. T. Wheeler (Eds.), *Biological nitrogen fixation in forest ecosystems: foundations* and *applications* (pp. 107-171). The Hague, The Netherlands: Nijhoff/Dr. W. Junk.

Dixon, R. O. D., and Wheeler, C. T. (1986). *Nitrogen fixation in plants*. New York, NY: Chapman and Hall.

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

Dow, M. A., and Schwintzer, C. R. (1999). Seed germination, seedling emergence, and seed bank ecology of sweet-fern (*Comptonia peregrina* (L.) Coult.). *Can. J. Bot.*, *77*, 1378-1386.

El-Lakany, M. H., and Luard, J. E. (1982). Comparative salt tolerance of selected *Casuarina* species. *Austr. Forest Res.*, *13*, 11-20.

Faure-Raynaud, M., Bonnefoy-Poirier, M. A., and Moiroud, A. (1986). Influence de pH acides sur la viabilité d'isolats de *Frankia*. *Plant Soil*, *96*, 347-358.

Friedrich, J. M., and Dawson, J. O. (1984). Soil nitrogen concentration and *Juglans nigra* growth in mixed plots with nitrogen-fixing *Alnus*, *Elaeagnus*, *Lespedeza*, and *Robinia* species. *Can. J. Forest Res.*, *14*, 864-868.

Gauthier, D., Jaffre, T., and Prin, Y. (2000). Abundance of *Frankia* from *Gymnostoma* spp.in the rhizosphere of *Alphitonia neocaledonica*, a non-nodulated Rhamnaceae endemic to New Caledonia. *Eur. J. Soil Biol.*, *36*, 169-175.

Girgis, M. G. Z., and Schwencke, J. (1993). Differentiation of *Frankia* by their electrophoretic patterns of intracellular esterases and aminopeptidases. *J. Gen. Microbiol.*, *139,* 2225-2232.

Goldman, C. R. (1961). The contribution of alder trees (*Alnus tenuifolia*) to the primary productivity of Castle Lake, California. *Ecol.*, *42*, 282-288.

Gordon, J. C., and Dawson, J. O. (1979). Potential uses of nitrogen-fixing trees and shrubs in commercial forestry. *Bot. Gaz.*, *140*, 88-90.

Gordon, J. C., and Wheeler, C. T. (1978). Whole plant studies on photosynthesis and acetylene reduction in *Alnus glutinosa*. *New Phytol.*, *80*, 179-186.

Griffiths, A. P., and McCormick, L. H. (1984). Effects of soil acidity on nodulation of *Alnus glutinosa* and viability of *Frankia*. *Plant Soil*, *79*, 429-434.

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

Hahn, D., Nickel, A., and Dawson, J. O. (1999). Assessing *Frankia* populations in plants and soil using molecular methods. *FEMS Microbiol. Ecol., 29*, 215-227.

Harborne, J. (1973). *Phytochemical methods*. London, UK: Chapman and Hall.

Harriott, O. T., and Bourret, A. (2003). Improving dispersed growth of *Frankia* using Carbopol. *Plant Soil*, *254*, 69-74.

Hasebe, M., Kofuji, R., Shindo, S., Hiwatashi, Y., Kobayashi-Arakawa, S., *et al.* (1998). Division of speciation mechanisms II: Annual report 1998. Japanese National Institute for Basic Biology. Retrieved October 10, 2003 from http://www.nibb.ac.jp/annual_report/1998/24.html.

Haukioja, E. (1991). Cyclic fluctuations in density: Interactions between a defoliator and its host tree. *Acta Oecol.*, *12*, 77-88.

Hensley, D. L., and Carpenter, P. L. (1984). Effect of lime additions to acid strip-mine spoil on survival, growth and nitrogen fixation (acetylene reduction) of several woody legume and actinomycete-nodulated species. *Plant Soil*, *79*, 353-367.

Hensley, D. L., and Carpenter, P. L. (1987). The effect of cadmium on growth and acetylene reduction (N_2 fixation) by *Alnus glutinosa*. *Hort. Science*, *22*, 69-70.

Hewitt, E. J., and Bond, G. (1961). Molybdenum and the fixation of nitrogen in *Casuarina* and *Alnus* root nodules. *Plant Soil*, *14*, 159-175.

Hibbs, D. E., and Cromack, Jr., K. (1990). Actinorhizal plants in Pacific Northwest forests. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 343-363). New York, NY: Academic Press.

Hibbs, D. E., DeBell, D. S., and Tarrant, R. F. (Eds.) (1994). *The biology and management of red alder*. Corvallis, OR: Oregon State University Press.

Huguet, V. (2003). Spécificité d'association du couple symbiotique *Frankia*/*Myrica* (Ph.D. thesis, Université Claude Bernard Lyon I, France).

Huguet, V., Batzli, J. M., Zimpfer, J. F., Normand, P., Dawson, J. O., and Fernandez, M. P. (2001). Diversity and specificity of *Frankia* strains in nodules of sympatric *Myrica gale*, *Alnus incana*, and *Shepherdia canadensis* determined by *rrs* gene polymorphism. *Appl. Environ. Microbiol.*, *67*, 2116-2122.

Huss-Danell, K. (1990). The physiology of actinorhizal nodules. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 129-156). New York, NY: Academic Press.

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

Huss-Danell, K., and Frej, A. (1986). Distribution of *Frankia* in soils from forests and afforestation sites in northern Sweden. *Plant Soil*, *90*, 407-418.

Huss-Danell, K., Uliassi, D., and Renberg, I. (1997). River and lake sediments as sources of infective *Frankia* (*Alnus*). *Plant Soil*, 197, 35-39.

Igual, J. M., and Dawson, J. O. (1999). Stimulatory effects of aluminum on *in vitro* growth of *Frankia*. *Can. J. Bot.*, *77*, 1321-1326.

Igual, J. M., Rodríguez-Barrueco, C., and Cervantes, E. (1997). The effects of aluminum on nodulation and symbiotic nitrogen fixation in *Casuarina cunninghamiana* Miq. *Plant Soil*, *190*, 41-46.

Ingsted, T. (1980). Growth, nutrition and nitrogen fixation in grey alder at varied rate of nitrogen addition. *Physiol. Plant.*, *50*, 353-364.

Jeong, S. C., and Myrold, D. D. (2001). Population size and diversity of *Frankia* in soils of *Ceanothus velutinus* and Douglas-fir stands. *Soil Biol. Biochem., 33*, 931- 941.

Kaelke, C. M., and Dawson, J. O. (2003). Seasonal flooding regimes influence survival, nitrogen fixation, and the partitioning of nitrogen and biomass in *Alnus incana* ssp. *rugosa*. *Plant Soil*, *254*, 167-177.

Kapulnik, Y., Joseph, C. M., and Phillips, D. A. (1987). Flavone limitations to root nodulation and symbiotic nitrogen fixation in alfalfa. *Plant Physiol.*, *84*, 1193-1196.

Keeley, S. C. (1989). The California chaparral: Paradigms reexamined. *Natural History Museum of Los Angeles County, CA, Science Series* Number 34.

Knowlton, S., Berry, A., and Torrey, J. G. (1980). Evidence that associated soil bacteria may influence root hair infection of actinorhizal plants by *Frankia*. *Can. J. Microbiol.*, *26*, 971-977.

Knowlton, S., and Dawson, J. O. (1983). Effects of *Pseudomonas cepacia* and cultural factors on the nodulation of *Alnus rubra* roots by *Frankia*. *Can. J. Bot.*, *61*, 2877-2882.

Kohls, S. J., Baker, D. D., van Kessel, C., and Dawson, J. O. (2003). An assessment of soil enrichment by actinorhizal N_2 fixation using $\delta^{15}N$ values in a chronosequence of deglaciation at Glacier Bay, Alaska. *Plant Soil*, *254*, 11-17.

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

Krajick, K. (1998). Green farming by the Incas? *Science*, *281*, 323.

Krueger, K. W., and Ruth, R. H. (1968). Photosynthesis of red alder, Douglas-fir, Sitka spruce, and western hemlock seedlings. In *Biology of alder: Proceedings of a symposium; 1967 April 14-15*. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Krumholz, G. D., Chval, M. S., McBride, M. J., and Tisa, L. S. (2003). Germination and physiological properties of *Frankia* spores. In P. Normand, K. Pawlowski, and J. O. Dawson (Eds.), Frankia *symbiosis* (pp. 57-68). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Lai, Chia-Chin. (1996). The effect of tyrosine on pigmentation, growth, and infectivity of *Frankia* (M.Sc. thesis, University of Illinois at Urbana-Champaign).

Laplaze, L., Gherbi, H., Frutz, T., Pawlowski, K., Franche, C., *et al.* (1999). Flavan-containing cells delimit *Frankia*-infected compartments in *Casuarina glauca* nodules. *Plant Physiol.*, *121*, 113-122.

Lawrence, D. B., Schoenike, R. E., Quispel, A., and Bond, G. (1967). The role of *Dryas drummondii* in vegetation development following ice recession at Glacier Bay, Alaska, with special reference to its nitrogen fixation by root nodules. *J. Ecol.*, *55*, 793-813.

Lawrie, A. C. (1982). Field nodulation in nine species of *Casuarina* in Victoria. *Aust. J. Bot.*, *30*, 447-460.

Lechevalier, M. P., and Lechevalier, H. A. (1990). Systematics, isolation, and culture of *Frankia*. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 35-60). New York, NY: Academic Press.

Li, C. Y., Lu, K., Trappe, J., and Bollen, W. (1970). Separation of phenolic compounds in alkali hydrolysates of forest soil by thin-layer chromatography. *Can. J. Soil Sci.*, *50*, 458-460.

Li, C. Y., Trappe, J. M., and Bollen, W. B. (1972). *Poria weirii*-inhibiting and other phenolic compounds in roots of red alder and Douglas fir. *Microbios*, *5*, 65-68.

Lopez, M. F., Fontaine, M. S., and Torrey, J. G. (1984). Levels of trehalose and glycogen in *Frankia* sp. HFPArl3 (Actinomycetates). *Can. J. Microbiol.*, *30*, 746-752.

Maggia, L., and Bousquet, J. (1994). Molecular phylogeny of the actinorhizal Hamamelidae and relationships with host promiscuity towards *Frankia*. *Mol. Ecol.*, *3*, 459-467.

Martin, A. C., Zim, H. S., and Nelson, A. L. (1961). *American wildlife & plants: A guide to wildlife food habitats*. New York, NY: Dover Publications.

Martin, K. J., Posavatz, N. J., and Myrold, D. D. (2003). Nodulation potential of soils from red alder stands covering a wide age range. *Plant Soil*, *254*, 187-192.

Martin, R. B. (1988). Bioinorganic chemistry of aluminum. In H. Sigel and A. Sigel (Eds.), *Metal ions in biological systems: Aluminum* and *its role in biology* (pp. 1-57). New York, NY: Marcel Dekker.

Maunuksela, L., Hahn, D., and Haahtela, K. (2000). Effect of freezing of soils on nodulation capacities of total and specific *Frankia* populations. *Symbiosis*, *29*, 107-120.

Maunuksela, L. Zepp, K., Koivula, T., Zeyer, J., Haahtela, K., and Hahn, D. (1999). Analysis of *Frankia* populations in three soils devoid of actinorhizal plants. *FEMS Microbiol. Ecol.*, *28*, 11-21.

McIlveen, W. D., and Cole, H. (1976). Spore dispersal of Endogonaceae by worms, ants, wasps, and birds. *Can. J. Bot.*, *54*, 1486-1489.

McKey, D. (1994). Legumes and nitrogen: The evolutionary ecology of a nitrogen-demanding lifestyle. In J. I. Sprent and D. McKey (Eds.), *Advances in legume systematics: Part 5 – the nitrogen factor* (pp. 211-228). Kew, UK: Royal Botanic Gardens.

McNabb, D. H., and Cromack, K. (1985). Dinitrogen fixation by a mature *Ceanothus velutinus* (Dougl.) stand in the Western Oregon Cascades. *Can. J. Bot.*, *29*, 1014-1021.

McVean, D. N. (1956). Ecology of *Alnus glutinosa* (L.) Gaertn. III. Seedling establishment. *J. Ecol.*, *44*, 195-218.

Mejstrik, V., and Benecke, U. (1969). The ectotrophic mycorrhizas of *Alnus viridis* (Chaix) DC and their significance in respect to phosphorous uptake. *New Phytol.*, 68, 141-149.

Midgley, S. J., Turnbull, J. W., and Johnson, R. D. (1983). Casuarina *ecology, management and utilization*. Melbourne, Austrralia: CSIRO.

Müller, A., Benoit, P., Diem, H. G., and Schwencke, J. (1991). Age-dependent changes in extracellular protein, aminopeptidase activities in *Frankia*. *J. Gen. Microbiol.*, *137*, 2787-2796.

Murai, S. (1964). Phytotaxonomical and geobotanical studies on genus *Alnus* in Japan (III).Taxonomy of whole world species and distribution of each section. *Bull. Gov. Forest Exp. Sta.* (Tokyo, Japan), *171*, 1-107.

Myrold, D. D., and Huss-Danell, K. (1994). Population dynamics of *Alnus*-infective *Frankia* in a forest soil with and without host trees. *Soil Biol. Biochem.*, *26*, 533-540.

Navarro, E., Bousquet, J., Moiroud, A., Munive, A., Piou, D., and Normand, P. (2003). Molecular phylogeny of *Alnus* (Betulaceae), inferred from nuclear ribosomal DNA ITS sequences. *Plant Soil*, *254*, 207-217.

Navarro, E., Nalin, R., Gauthier, D., and Normand, P. (1997). The nodular microsymbionts of *Gymnostoma* spp. are *Elaeagnus*-infective *Frankia* strains. *Appl. Environ. Microbiol.*, *63*, 1610-1616.

Neave, I. A., and Dawson, J. O. (1989). Juglone reduces growth, nitrogenase activity, and root respiration of actinorhizal black alder seedlings. *J. Chem. Ecol.*, *15*, 1823-1836.

Neave, I. A., Dawson, J. O., and DeLucia, E. H. (1989). Autumnal photosynthesis is extended in nitrogen-fixing European black alder compared with white basswood: Possible adaptive significance. *Can. J. Forest Res.*, *19*, 12-17.

Nickel, A. (2000). Population dynamics of *Frankia* in soil (Ph.D. thesis, Swiss Technical University (ETH), Zürich, Switzerland).

Nickel, A., Hahn, D., Zepp, K., and Zeyer, J. (1999). *In situ* analysis of introduced *Frankia* populations in root nodules obtained on *Alnus glutinosa* grown under different water availability. *Can. J. Bot.*, *77*, 1231-1238.

Oremus, P. A. I. (1980). Occurrence and infective potential of the endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in coastal sand dune areas. *Plant Soil*, *56*, 123-139.

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

Paschke, M. W., and Dawson, J. O. (1992a). The occurrence of *Frankia* in tropical forest soils of Costa Rica. *Plant Soil*, *142*, 63-67.

Paschke, M. W., and Dawson, J. O. (1992*b*). *Frankia* abundance in soils beneath *Betual nigra* and other non-actinorhizal woody plants. *Acta Oecol.*, *13*, 407-415.

Paschke, M. W., and Dawson, J. O. (1993). Avian dispersal of *Frankia*. *Can. J. Bot.*, *71*, 1128-1131.

Paschke, M. W., Dawson, J. O., and Condon, B.M. (1994). *Frankia* in prairie, forest, and cultivated soils of central Illinois, U.S.A. *Pedobiologia*, *38*, 546-551.

Paschke, M. W., Dawson, J. O., and David, M. B. (1989). Soil nitrogen mineralization in mixed plantations of black walnut with actinorhizal autumn olive or black alder. *Plant Soil*, *118*, 33-42.

Perradin, Y., Mottet, M., and LaLonde, M. (1983). Influence of phenolics on *in vitro* growth of *Frankia* strains. *Can. J. Bot.*, *61*, 2807-2814.

Prin, Y., and Rougier, M. (1987). Preinfection events in the establishment of the *Alnus-Frankia* symbiosis: Study of the root hair deformation step. *Plant Physiol.*, *6*, 99-106.

Reddell, P., Bowen, G. D., and Robson, A. D. (1985). The effects of soil temperature on plant growth, nodulation and nitrogen fixation in *Casuarina cunninghamiana*. Miq. *New Phytol.*, *101*, 441-450.

Reddell, P., Rosbrook, P. A., Bowen, G. D., and Gwaze, D. (1988). Growth responses in *Casuarina cunninghamiana* plantings to inoculation with *Frankia*. *Plant Soil*, *108*, 79-86.

Reddell, P., and Spain, A. V. (1991). Earthworms as vectors of viable propagules of mycorrhizal fungi. *Soil Biol. Biochem.*, *23*, 767-774.

Richards, J. W., Krumholz, G. D., Chval, M. S., and Tisa, L. S. (2002). Heavy metal resistance patterns of *Frankia* strains. *Appl. Environ. Microbiol.*, *68*, 923-927.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J., and Rejmanek, M. (2000). Plant invasions - the role of mutualisms. *Biol. Rev.*, *75,* 65-93.

Righetti, T. L., Chard, C. H., and Backhause, R. A. (1986). Soil and environmental factors related to nodulation in *Cowania* and *Purshia*. *Plant Soil*, *91*, 147-160.

Righetti, T. L., and Munns, D. N. (1981). Soil factors limiting nodulation and nitrogen fixation in *Purshia*. In J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains, and R. C. Huffaker (Eds.), *Genetic engineering of symbiotic nitrogen fixation* and *conservation of fixed nitrogen* (pp. 395-407). New York, NY: Plenum Press.

Roberts, N. (1998). *The Holocene: An environmental history* (Second Edition). Oxford, UK: Blackwell.

Rodriguez-Barrueco, C. (1968). The occurrence of the root nodule endophytes of *Alnus glutinosa* and *Myrica gale* in soils. *J. Gen. Microbiol.*, *52*, 189-194.

Rodriguez-Barrueco, C., Mackintosh, A. H., and Bond, G. (1970). Some effects of combined nitrogen on the nodule symbioses of *Casuarina* and *Ceanothus*. *Plant Soil*, *33*, 129-139.

Rösch, D., Bergmann, M., Knorr, D., and Kroh, L. W. (2003). Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agr. Food Chem.*, *51*, 4233-4239.

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

Safo-Sampath, S., and Torrey, J. G. (1988). Polysaccharide-hydrolysing enzymes of *Frankia* (Actinomycetales). *Plant Soil*, *112*, 89-97.

Seguin, A., and Lalonde, M. (1989). Detection of pectolytic activity and *pel* homologous sequences in *Frankia*. *Plant Soil*, *118*, 221-229.

Schramm, J. R. (1966). Plant colonization studies on black wastes from anthracite mining in Pennsylvania. *T. Am. Philos. Soc.*, *56*, 1-194.

Schwencke, J., and Carú, M. (2001). Advances in actinorhizal symbiosis: Host plant-*Frankia* interactions, biology, and applications in arid land reclamation. A review. *Arid Land Res. Manag.*, *15*, 285-327.

Schwintzer, C. R., Berry, A. M., and Disney, L. D. (1982). Seasonal pattern of root nodule growth, endophyte morphology, nitrogen activity and shoot development in *Myrica gale*. *Can. J. Bot.*, *60*, 746-757.

Seiler, J. R., and Johnson, J. D. (1984). Growth and acetylene reduction of black alder seedlings in response to water stress. *Can. J. Forest Res.*, *14*, 477-480.

Selim, S., Delacour, S., and Schwencke, J. (1996). Specific long-chain fatty acids promote optimal growth of *Frankia*: Accumulation and intracellular distribution of palmitic and propionic acid. *Arch. Microbiol.*, *165*, 252-257.

Shindo, H., Ohta, S., and Kuwatsuka, S. (1978). Behavior of phenolic substances in the decaying process of plants. IX. Distribution of phenolic acids in soils of rice paddy fields and forests. *Soil Sci. Plant Nutr.*, *24*, 233-243.

Silvester, W. B. (1977). Dinitrogen fixation by plant associations excluding legumes. In R. Hardy and W. Silvester (Eds.), *A treatise of dinitrogen fixation* (Vol. 4, pp. 141-190). New York, NY: Academic Press.

Silvester, W. B., and Harris, S. L. (1990). Oxygen regulation and hemoglobin. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 157-176). New York, NY: Academic Press.

Silvester, W. B., Whitbeck, J., Silvester, J. K., and Torrey, J. G. (1988a). Growth, nodule morphology, and nitrogenase activity of *Myrica gale* with roots grown at various oxygen levels. *Can. J. Bot.*, *66*, 1762-1771.

Silvester, W. B., Silvester, J. K., and Torrey, J. G. (1988b). Adaptation of nitrogenase to varying oxygen tension in root nodules of *Alnus incana* ssp. *rugosa*. *Can. J. Bot.*, *66*, 1772-1779.

Simonet, P., Navarro, E., Rouvier, C., Reddell, P., Zimpfer, J., *et al.* (1999). Co-evolution between *Frankia* populations and host plants in the family Casuarinaceae and consequent patterns of global dispersal. *Env. Microbiol.*, *1*, 525-533.

Smolander, A. (1990). *Frankia* populations under different tree species – with a special emphasis on soils under *Betula pendula*. *Plant Soil*, *121*, 1-10.

Smolander, A., Rönkkö, R., Nurmiaho-Lassila, E.-L., and Haahtela, K. (1990). Growth of *Frankia* in the rhizosphere of *Betula pendula*, a nonhost tree species. *Can. J. Microbiol.*, *36*, 649-656.

Smolander, A., and Sundman, V. (1987). *Frankia* in acid soils of forests devoid of actinorhizal plants. *Physiol. Plant.*, *70*, 297-303.

Smolander, A., van Dijk, C., and Sundman, V. (1988). Survival of *Frankia* strains introduced into soil. *Plant Soil*, *106*, 65-72.

Soltis, D. E., Soltis, P. S., Morgan, D. R., Swensen, S. M., Mullin, B. C., *et al.* (1995). Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proc. Natl. Acad. Sci. U.S.A.*, *92*, 2647-2651.

Su, H., and Lin, L.-P. (1989). The dissemination of endophytes and existence of endomycorrhiza on the rhizoplane of *Alnus formosana*. *J. Chin. Agric. Chem. Soc.*, *27*, 513-523.

Subbarao, N. S., and Rodríguez-Barrueco, C. (1995). *Casuarinas*. Lebanon, NH: Science Publishers.

Sundström, K.-R., and Huss-Danell, K. (1987). Effects of water stress on nitrogenase activity in *Alnus incana*. *Physiol. Plant.*, *70*, 342-348.

Swensen, S. M. (1996). The evolution of actinorhizal symbioses: Evidence for multiple origins of the symbiotic association. *Am. J. Bot.*, *83*, 1503-1512.

Thompson, V. (1994). Spittlebug indicators of nitrogen-fixing plants. *Ecol. Entomol.*, *19*, 391-398.

Thompson, V. (1999). Spittlebugs associated with actinorhizal host plants. *Can. J. Bot.*, *77*, 1387-1390.

Tilman, D., Wedin, D., and Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, *379*, 718.

Tjepkema, J. (1978). The role of oxygen diffusion from the shoots and nodule roots in nitrogen fixation by root nodules of *Myrica gale*. *Can. J. Bot.*, *56*, 1365-1371.

Torrey, J. G. (1990). Cross-inoculation groups within *Frankia* and host-endosymbiont associations. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia *and actinorhizal plants* (pp. 83-106). New York, NY: Academic Press.

Tortosa, R. D., and Cusato, M. (1991). Effective nodulation of rhamnaceous actinorhizal plants induced by air dry soils. *Plant Soil*, *131*, 229-233.

van Dijk, C. (1979). Endophyte distribution in the soil. In J. C. Gordon, C. T. Wheeler, and D. A. Perry (Eds.), *Symbiotic nitrogen fixation in the management of temperate forests* (pp. 84-94). Corvalis, OR: Oregon State University Press.

van Dijk, C., and Sluimer-Stolk, A. (1990). An ineffective strain type of *Frankia* in the soil of natural stands of *Alnus glutinosa* (L.) Gaertn. *Plant Soil*, *127*, 107-121.

Van Ghelue, M., Lovaas, E., Ringo, E., and Solheim, B. (1997). Early interactions between *Alnus glutinosa* and *Frankia* strain ArI3: Production and specificity of root hair deformation factor(s). *Physiol. Plant.*, *99*, 579-587.

Van Miegroet, H., and Cole, D. W. (1985). Acidification sources in red alder and Douglas-fir soils: Importance of nitrification. *Soil Sci. Soc. Am. J.*, *49*, 1274- 1279.

Vergnaud, L., Chaboud, A., Prin, Y., and Rougier, M. (1985). Preinfection events in the establishment of *Alnus-Frankia* symbiosis: Development of a spot inoculation technique. *Plant Soil*, *87*, 67-68.

Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., *et al.* (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry*, *57/58*, 1-45.

Vitousek, P. M., and Walker, L. R. (1989). Biological invasion by *Myrica faya* in Hawaii: Plant demography, nitrogen fixation, and ecosystem effects. *Ecol. Monogr.*, *59*, 247-265.

Vogel, C. S., and Curtis, P. S. (1995). Leaf gas and nitrogen dynamics of N_2-fixing field-grown *Alnus glutinosa* under elevated atmospheric CO_2. *Glob. Change Biol.*, *1*, 55-61.

Vogel, C., and Dawson, J. O. (1986). *In vitro* growth of five *Frankia* isolates in the presence of four phenolic acids. *Soil Biol. Biochem.*, *18*, 227-231.

Walker, L. R. (1993). Nitrogen fixers and species replacements in primary succession. In J. Miles and D. W. H. Walton (Eds.), *Primary succession on l*and (pp. 249-272). London, UK: Blackwell Scientific.

Wheeler, C. T., and Bowes, B. G. (1974). Effects of light and darkness on nitrogen fixation by root nodules of *Alnus glutinosa* in relation to their cytology. *Z. Pflanzenphysiol.*, *71*, 71-75.

Wheeler, C. T., Henson, I. E., and McLaughlin, M. E. (1979). Hormones in plants bearing actinomycete nodules. *Bot. Gaz.* (Chicago), Suppl., *140*, S52-S57.

Wheeler, C. T., Hughes, L. T., Oldroyd, J., and Pulford, I. D. (2001). Effects of nickel on *Frankia* and its symbiosis with *Alnus glutinosa* (L) Gaertn. *Plant Soil*, *231*, 81-90.

Wheeler, C. T., and Miller, I. M. (1990). Current and potential uses of actinorhizal plants in Europe. In C. R. Schwintzer and J. D. Tjepkema (Eds.), *The biology of* Frankia and *actinorhizal plants* (pp. 365-389). New York, NY: Academic Press.

Wheeler, C. T., Watts, S. H., and Hillman, J. R. (1983). Changes in carbohydrates and nitrogenase compounds in the root nodules of *Alnus glutinosa* in relation to dormancy. *New Phytol.*, *95*, 209-218.

Whitehead, D. (1964). Identification of *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acid in soils. *Nature*, *202*, 417-419.

Whitehead, D., Dibb, H., and Hartley, R. (1983). Bound phenolic compounds in water extracts of soils, plant roots and leaf litter. *Soil Biol. Biochem.*, *15*, 133-136.

Wollum, C. T., and Youngberg, A. G. (1969). Effect of soil temperatures on nodulation of *Ceanothus velutinus* Dougl. *Proc. Soil Sci. Soc. of America*, *33*, 801-803.

Wollum, C. T., Youngberg, A. G., and Chichester, F. W. (1968). Relation of previous timber stand age to nodulation of *Ceanothus velutinus*. *Forest Sci.*, *14*, 114-118.

Wolters, D. J., Akkermans, A. D. L., and Van Dijk, C. (1997). Ineffective *Frankia* strains in wet stands of *Alnus glutinosa* L. Gaertn. in the Netherlands. *Soil Biol. Biochem.*, *29*, 1702-1712.

Yadav, J. S. P. (1983). Soil limitations for successful establishment and growth of *Casuarina* plantations. In S. J. Midgley, J. W. Turnbull, and R. D. Johnston (Eds.), *Casuarina ecology, management* and *utilization* (pp. 138-157). Melbourne, Austrralia: CSIRO.

Yamanaka, T., Li, C.-Y., Bormann, B. T., and Okabe, H. (2003). Tripartite associations in alder: Effects of *Frankia* and *Alpova diplophloeus* on the growth, nitrogen fixation and mineral acquisition of *Alnus tenuifolia*. *Plant Soil*, *254*, 179-186.

Young, D. R., Sande, E., and Perters, G. A. (1992). Spatial relationships of *Frankia* and *Myrica cerifera* on a Virginia, U.S.A., barrier island. *Symbiosis*, *112*, 209-220.

Zhang, X., and Benson, D. R. (1992). Utilization of amino acids by *Frankia* strain CpI1. *Arch. Microbiol.*, *158*, 256-261.

Zimpfer, J. F., Kaelke, C. M., Smyth, C. A., Hahn, D., and Dawson, J. O. (2003). *Frankia* inoculation, soil biota, and host tissue amendment influence *Casuarina* nodulation capacity of a tropical soil. *Plant Soil*, *254*, 1-10.

Zimpfer, J. F., Kennedy, G. J., Smyth, C. A., Hamelin, J., Navarro, E., and Dawson, J. O. (1999). Localization of *Casuarina*-infective *Frankia* near *Casuarina cunninghamiana* trees in Jamaica. *Can. J. Bot.*, *77*, 1248-1256.

Zimpfer, J. F., McCarty, B., Kaelke, C. M., Mulongwe, L., Igual, J. M., Smyth, C. A., and Dawson, J. O. (2002). *Casuarina cunninghamiana* cladode extracts increase the *Frankia* infectious capacity of a tropical soil. *Symbiosis*, *33*, 73-90.

Zimpfer, J. F., Smyth, C. A., and Dawson, J. O. (1997). The capacity of Jamaican mine spoils, agricultural and forest soils to nodulate *Myrica cerifera*, *Leucaena leucocephala* and *Casuarina cunninghamiana*. *Physiol. Plant.*, *99,* 664-672.