SYMBIOTIC N_2 - FIXATION BY FRANKIA:

ACTINORHIZAE ESTABLISHMENT ON DISTURBED LANDS[1]

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Nodules are common occurrences on roots of certain nonleguminous plants. The nodules are mutualistic symbioses (also called actinorhizae) between actinomycetes of the genus Frankia and plants from the Betulaceae, Casuarinaceae, Coriariaceae, Eleagnaceae, Rhamnaceae, Myricaceae and Rosaceae. Nodules formed on roots of these woody angiosperms fix nitrogen much like leguminous systems, and presumably this habit is crucial to their success in sites of low fertility. Reestablishment of actnorhizal plants of disturbed sites may be dependent on adequate soil moisture, low soil fertility and presence of appropriate Frankia.

INTRODUCTION

Nitrogen fixation by the Frankia bacteria-woody angiosperm mutualistic symbiosis (actinorhizae) is potentially useful in amending the low nitrogen status of reclaimed topsoil or spoils from strip mining operations. Actinorhizae are commonly found in several plant families, some of which are of interest in revegetation (Table 1). Among these families is the Rosaceae, of which 12 species in six genera have actinorhizal nodules (Nelson 1982). Next to the Compositae, members of the Rosaceae are possibly the most widely distributed shrubs in the western United States and form an integral component of browse on rangelands (McGinnies 1972).

The fixed nitrogen from actinorhizae is made available to the ecosystem on an extended time basis through leaf fall, nodular decomposition, and plant death (Torrey 1978). Nitrogen input depends on the frequency of nodulation, as well as the rate of fixation. There is little information on the occurrence of nodule-bearing roots in field data collected for some plant species (Nelson 1983; Righetti et al. 1983). Where information exists, it is often conflicting. Dalton and Zobel (1977) and Nelson (1982) indicate Purshia tridentata is only sparsely nodulated, whereas our own data (see text) indicates P. tridentata is well nodulated.

Nitrogen fixation rates of actinorhizal nonleguminous seedlings compare favorably to those of herbaceous legumes (Torrey 1978). Some species, such as Myrica gale, are capable of a wetland fixation rate of 34 kg N/ha/yr which is equivalent to over four times the amount of nitrogen derived from rainfall (Schwintzer 1979). Soil formation on Alaskan glacial moraines is accelerated if successional vegetation includes Alnus crispa which can have a fixation rate of 20 kg N/ha/yr (Bradshaw and Chadwick 1980). A. crispa is reported to fix up to 157 kg N/ha/yr on other sites, and Alnus rubra up to 300 kg N/ha/yr (Tarrant and Trappe, 1971). In arid portions of the western United States, the rates of nitrogen fixation by actinorhizal systems are probably less due to moisture restrictions. However, even small inputs of nitrogen may be important on N-deficient soils (Righetti et al. 1982).

Reclamation of disturbed lands using actinorhizal plants may require the presence of proper Frankia strains within the planted soil. Where soils are deficient in inoculum, there may be a desire to inoculate seed, seedling, or soil with the Frankia endophyte. With this perspective, the objectives of this paper are:

1. Review current literature on the actinomycete-woody angiosperm symbiosis.

2. Discuss aspects of the symbiosis which relate to disturbed land ecology.

3. Evaluate the possibility of artificially inoculating disturbed sites with Frankia.

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Table 1. Plants that form actinorhizae (root nodules) following *Frankia* infection, distribution and nature of sites occupied. (Updated from Bond 1976 and Torrey 1978).

Family	Genera	No. sp. nod./ total[2]	Geographic Distribution	Sites
Betulaceae	*Alnus*[1]	33/35[1]	Europe, Siberia, N. America, Japan, the Andes	poor soils, sand hills/dunes, glacial till, wet bogs, mine dumps, gravel, wasteland, volcanic ash
Casuarinaceae	*Casuarina*	24/45	Australia, tropical Asia, Pacific Islands, widely introduced elsewhere	sand dunes, salt marshes, tropical forests, nondesert areas
Coriariaceae	*Coriaria*	13/15	Mediterranean to Japan, New Zealand, Chile to Mexico	lowlands & subalpine, sandy or gravelly soils or clay
Elaeagnaceae	*Elaeagnus*	16/45	Asia, Europe, N. America	disturbed areas, sand dunes, poor soils, riparian sites
	Hippophae	1/3	Asia, Europe, from Himalayas to Artic Circle	sand dunes, coastal areas
	Shepherdia	3/3	N. America	sand soils, disturbed areas, riparian, under *Pinus* forests
Myricaceae	*Myrica*	26/35	many tropical, subtropical, & temperate regions, extending nearly to Artic Circle	acidic bogs, sand dunes, mine wastes
Rhamnaceae	*Ceanothus*	31/55	N. America, esp. western USA	dry forest & chaparral, subalpine zones
	Colletia	1/17	S. America	
	Discaria	2/10	the Andes, Brazil, Oceania	gravelly soils, arid zones
Rosaceae	*Cercocarpus*	4/20	western USA to Mexico	foothills, disturbed soils, rocky areas
	Chamaebatia	1/2	California	chaparral (Heisey et al., 1980)
	Cowania	1/4	Utah, Arizona	foothills, high plateaus
	Dryas	3/4-6	Alaska to Colorado in N. America, Europe, circumpolar	high altitude and latitude, variable soils
	Purshia	2/2	western N. America	disturbed soils on upland plains, foothills & under *Pinus* forests
	Rubus	1/250	Indonesia; N. America	sand, rock sites, bogs

[1] Total of 162 species in 16 genera in 7 families.
[2] Number of species nodulated/total number of species in genus.

LITERATURE REVIEW AND DISCUSSION

Physiology and Ultrastructure of *Frankia*

The endophyte of actinorhizae is an actinomycete of the genus, *Frankia*. The actinomycetes form filaments or hyphae resembling fungal hyphae but with dimensions - usually 0.5 to 1.0 µm - that more closely resemble bacteria (Alexander 1977).

Frankia hyphal tips are the sites of growth with the majority of filamental cells in a stationary growth phase (Bensen and Hanna 1983). Hyphal extension into the root hairs of woody dicots causes curling and deformation of the root hairs (Angulo et al. 1976; Torrey 1978). The hypha ramifies through the root hair until it penetrates the cortical cells of the root causing adjacent noninfected cells to begin mitosis (Angulo et al. 1976). The mitotic activity leads to the formation of an invasion site that eventually gives rise to the nodule (Callaham and Torrey 1977). Angulo et al. (1976) believe that the invasion site or primary nodule in *Alnus* is subsequently followed by mitotic division in the pericycle, wherein a lateral root primordium is formed. Extension of hyphae into the cells of the lateral root primordia causes the development of a true nodule primordium.

Nodular formation in the nonleguminous woody angiosperms tends to follow two structural types: *Alnus* or *Myrica* types. The former type has a pinkish, knoblike appearance, whereas the latter is in the shape of a determinate nodule root that has an upward growth pattern (Torrey 1978). The nodules may overgrow and envelop adjacent roots from neighboring plants (Angulo et al. 1976).

As ramification into the cortical cells occurs, the hypha is encapsulated with a polysaccharide layer that surrounds the endophyte during its existence (Torrey 1978; Lalonde 1979). At several weeks after infection, hyphae within the cortical cells form vesicles or terminal swellings at hyphal tips (Angulo et al. 1976). It is generally agreed that the vesicles are the sites of formation of the nitrogenase complex and nitrogen fixation (Tjepkema et al. 1981). In *Frankia* strains isolated from *Comptonia peregrina*, Tjepkema et al. (1981) observed that vesicles can occur only in nitrogen-free media. Vesicle formation may therefore be dependent on the endophytes need to provide reduced nitrogen for internal consumption. Excess fixed nitrogen beyond the endophytes metabolic needs can be transferred to the host plant in amide carriers. In alder, the citrulline amide carrier is translocated throughout the plant (Salisbury and Ross 1978).

The host plant contributes carbohydrates to the endophyte in exchange for fixed nitrogen. Dicarboxylic acids such as succinate and glutamate may be provided by the plant for metabolism by the *Frankia* endophyte (Akkermans et al. 1983). Other plant synthates such as vitamins may also be utilized by the symbiont.

A second nongrowing cell in addition to the vesicle is the spore. The spore is derived from sporangia and constitutes a resting stage (Akkermans et al. 1983). The spore or granule tends to occur after vesicle formation although concomitant formation between the two nongrowing cells has been observed (Angulo et al. 1976). Angulo et al. (1976) believe that death of the host cortical cell causes the polysaccharride layer to deteriorate thereby releasing the granules into the dead host cell. During periods when the endophyte is reduced throughout the nodule, the granules may constitute the only remaining viable part of the endophyte.

Growth of the *Frankia* Endophyte

The first frankiae endophytes were successfully isolated from a host plant in 1978 (Callaham, et al. 1978) Since that time numerous isolates have been taken from actinorhizal plants. The majority of these have been from *Alnus*. Only a handful of endophytes are available from the Rosaceae. The wide diversity in *Frankia* strains isolated suggests that consolidation of principles related to *Frankia* taxonomy and physiology is still forthcoming.

Strip Mining Aspects: Edaphic Influences

Trophic Relationships

The role of *Frankia* in the trophic structure of soil ecosystems is not well understood. *In vitro* growth culturing indicates that sources usable by *Frankia* are highly variable and include arabinose, fumaric acid, D-galactose, galacturonic acid, glucose, glycerol, malic acid, propri-onic acid, L-rhamnose, sodium acetate, starch, succinate acid, and even Tween 80 (Shipton and Burggraaf, 1983). Shipton and Burggraaf (1983) believe that *Frankia* may act as secondary colonizers requiring the established presence of other organisms and their metabolic byproducts. Since microbial activity, along with organic matter, may be drastically lowered following strip mining operations (Cundell 1977; Miller et al. 1979; Fresquez and Lindemann 1982), *Frankia* may have a lowered capacity to initiate nodulation on reclaimed lands.

Moisture Availability

During periods of moisture stress, actinomycetes can withstand greater stress than bacteria, thereby giving the actinomycetes a competitive advantage. Between -0.1 MPa and -0.6 MPa water potential, bacterial respiration will diminish rapidly with only minimal respiration at -2.0 MPa (Wilson and Griffin 1975). Some *Frankia* strains have been observed to grow minimally at potentials of -2.3 MPa (Shipton and Burggraaf 1983). This potential is well below that tolerated by many plants; however, the capacity of the frankiae to infect much below -0.7 MPa has been questioned by researchers (Shipton and Burggraaf 1982; Shipton and Burggraaf 1983). Infection has been observed as occurring only during periods of root elongation (Angulo et al. 1976; Torrey 1978). Restrictive dry periods may hamper *Frankia* growth and shorten periods during which roots can be infected. Righetti and Munns (1982) noted that nodulation of *Purshia* was less common on root samples gathered from dry locations.

The lack of available water also affects the nodule. Periods of drought cause the symbiont to decrease nitrogen fixation as well as formation of nodular material. Also, only part of the nodule decomposes during drought years or, possibly, during seasonal declines in precipitation (Nelson 1982). The role of *Frankia* spores may be to reinfect roots after dry periods and allow fixation to continue.

Temperature

Frankia exhibits maximal growth in *in vitro* cultures at about 30°C and is therefore probably mesophilic (Burggraaf and Shipton 1982, Shipton and Burggraaf 1983). The daily and seasonal patterns of heat flow within a soil may never approximate this optimal temperature, however. Shipton and Burggraaf (1983) found that some strains of *Frankia* from European and American plant species can grow rather rapidly at 18°C allowing growth during short seasons at suboptimal temperatures. Nitrogenase activity may also be affected by low soil temperatures. Hensley and Carpenter (1979) reported initiation of nodular nitrogenase activity at 8-11°C and maximal activity at 34-36°C for *Elaeagnus umbellata*.

The rate at which heat flux penetrates a soil depends on the temperature gradient and the thermal conductivity. The thermal conductivity is a function of porosity, moisture content, and organic matter (Rosenberg 1974). The extent to which these factors have been altered on disturbed lands may cause different nitrogen fixation rates than those encountered on undisturbed sites along with changes in patterns of *Frankia* growth in a soil.

Soil pH

Actinomycetes generally favor neutral to basic soils. Burggraff and Shipton (1982) reported optimal pHs of 6 to 8 for four *Frankia* strains. The influence of the microsite may allow *Frankia* infection in adverse pH conditions beyond those in media cultures (Shipton and Burggraaf 1983). *Pseudomonas cepacia* may interact with *Frankia* in the soil allowing root hair penetration under adverse pH levels by modification of the microsite (Knowlton and Dawson 1983).

Particulate Coal

Although coal particulates in mine soils have been considered to be biologically inert on western strip mines (Schafer et al. 1980), there can be associated physical changes in the soil along with biological effects resulting from coal's presence. Coal dust emissions may be significant near mining operations. A strip mine near Gillette, Wyoming was estimated to produce emissions of 125 tons/yr during coal removal (USGS 1977). Coal dust mixed with minesoil or spoil may contribute to higher cation exchange capacities and electrical conductivities while lowering pH levels (Schafer et al.1980), cause higher microsite temperatures (Iswaran et al. 1980, Nicholson and Bell 1981), and allow greater water infiltration (Nicholson and Bell 1981).

During snow melt or other precipitation events, a soil solution containing phenolics leached from coal particulates may arise. Phenolic types and phenolic levels can have a direct influence on *Frankia* growth. Perradin et al. (1983) found ferulic, o-coumaric and p-coumaric acids to be inhibitory toward *Frankia*, whereas benzoic and p-hydroxybenzoic acids increased vesicle formation. A soil solution bearing phenolics may be antagonistic to *Frankia* infection during periods of root elongation. Even high soil organic matter may be negatively correlated with development of nodules (Tables 2 and 3). Soil organic matter has numerous phenolic functions; however, the capacity to inhibit nodulation may lie in the ability of soil organic matter to release nitrogen. It should be noted that phenolics within the soil may inhibit other soil microorganisms more strongly than a symbiont microrganism as suggested by Iswaran et al. (1980).

Lignite coal can have high concentrations of molybdenum which may be released locally after mining (Stone et al. 1983). Because of high soil molybdenum levels and the lignite-molybdenum release, molybdenum will probably not be limiting for the Mo-Fe protein in the nitrogenase enzyme complex except possibly in special areas of reclamation.

Strip Mining Aspects: Reclamation Practices

Establishment of actinorhizal plants may not only require the inoculation of seeds/seedlings with *Frankia* cultures but also the retention of nodular nitrogen fixing activity past the time of infection. Both considerations need appraisal if symbiotic introduction is to be useful in reclamation.

Table 2.--Means (and standard deviations) of surface soil properties (to 30 cm depth) from root zones of nonnodulated *Cercocarpus montanus* (unpublished data, Williams and Munn).

	Nodulated (n = 12)	Nonnodulated (n = 20)	F-test
Organic matter (percentage)	0.61 (0.66)	2.94 (1.37)	4.25**
pH (in water)	7.98 (0.41)	8.17 (0.25)	2.83*
Electrical conductivity	0.44 (0.42)	0.38 (0.16)	6.00**
Extractable P (ppm)	0.68 (0.37)	1.66 (0.87)	5.43**
Extractable NO_3 (ppm)	0.59 (0.50)	2.81 (1.73)	11.92**
Extractable K (ppm)	51.62 (30.92)	111.00 (58.92)	3.63*

Table 3.--Means (and standard deviations) of surface soil properties (to 30 cm depth) from root zones of nodulated and nonnodulated *Purshia tridentata* (unpublished data, Williams and Munn).

	Nodulated (n = 5)	Nonnodulated (n = 9)	F-test
Organic matter (percentage)	0.68 (0.59)	3.73 (2.24)	14.74***
pH (in water)	7.23 (0.67)	7.15 (0.63)	1.13 N.S.
Electrical conductivity (mmhos/cm)	0.33 (0.13)	0.33 (0.14)	1.00 N.S.
Extractable P (ppm)	2.29 (1.47)	3.50 (2.09)	2.03 N.S.
Extractable NO_3 (ppm)	0.92 (0.76)	3.50 (2.35)	9.53**
Extractable K (ppm)	81.70 (60.48)	194.50 (86.0)	2.02 N.S.

*Significant at p = 0.05
**Significant at p = 0.01
***Significant at p = 0.001
N.S. = non-significant

Nitrogen Fertilization

Fertilization with nitrogen on reclaimed lands is possibly a barrier to nodule establishment. Aside from decomposition, the extent of nodulation is greater in soils low in nitrogen as compared to those where nitrogen is low or absent (Vlamis et al. 1964; Righetti and Munns 1982; Granhall et al. 1983, Tables 2 and 3). In *Rhizobium* - legume symbioses, the addition of fertilizer nitrogen causes a decline in translocated carbohydrate to the nodules and a rise in carbohydrate translocation to the roots (Small and Leonard 1969). The same effect may occur with the actinomycete symbiosis.

The value of the symbiosis to actinorhizal plants tends to be intermediate between fertilized and nonnodulated conditions. Fertilized, nonnodulated plants have greater vigor/biomass than unfertilized but nodulated plants which in turn have greater vigor/biomass than unfertilized, nonnodulated plants (Vlamis et al. 1964; Youngberg and Hu 1972; Granhall et al. 1983). Since nitrogen fertilization is necessary on some N-deficient disturbed lands or in conjunction with mulching (Berg 1980), fertilization is possibly unavoidable. Although fertilization can enhance actinorhizal plant establishment without resorting to symbiotic nitrogen fixation, the removal of the initial nitrogen fertilization subsidy may be detrimental to long-term plant survival. Cessation of fertilizing after plant establishment is required to maintain a balance of plant species diversity and prevent seasonal shifts in plant communities (Goetz et al. 1978; Doerr et al. 1983). On reclaimed lands, nitrate can be readily leached and possibly become unavailable within the rooting zones of plants (Houston and Hyder 1975). Symbiotic nitrogen fixation may provide a nitrogen source for actinorhizal plants past the fertilization period on lands with early successional stages and immature nutrient cycling.

Several approaches in negating fertilization's effects may be feasible. Varying the rate and timing of nitrogen application may allow the nodules to coexist in a fertilization program. Granhall et al. (1983) observed that single massive doses of nitrogen prevented nodulation in *Alnus incana* seedlings, whereas several light applications facilitated nodular retention and a low rate of nitrogenase activity. They also observed that the greatest growth was obtained with fertilized seedlings that exhibited the nitrogenase activity.

A second approach may be to delay the time of revegetation of actinorhizal plants. The seral stagnation period that occurs on reclaimed lands in the Northern Great Plains within 3-4 years after seeding may be due to microbial immobilization of available nitrogen (Schafer et al. 1980). These periods may provide a "window" for nodular establishment by such means as planting with nodulated seedlings. Scalping areas to remove litter accumulations and competitive plants may be necessary.

Supplemental Moisture

Infection of root hairs by *Frankia*, nodule death and decomposition are influenced by the soil moisture studies. Actinorhizal seedlings in greenhouse pots commonly nodulate when inoculated, whereas inoculated field seedlings often fail, the lack of available water apparently inhibits nodulation (Dalton and Zobel 1977). On some sites, water may never be available in sufficient quantities to allow significant nitrogen fixation (Righetti and Munns 1982).

Irrigation by flooding, sprinkling or drip irrigation may be useful in root nodule infection in *Frankia* as well as actinorhizal seedling establishment. If irrigation is used to supplement natural precipitation levels throughout the growing season, the periods for root elongation may be extended allowing additional time for infection. Prolonged irrigation beyond one season is probably not an option. Doerr et al. (1983) noted that prolonged irrigation caused an overdeveloped and unsustainable production of plant biomass to occur on plots in Colorado. They also concluded that two years of irrigation reduced the biomass of shrubs because of increased competition from grasses.

Topographic modifications may also be utilized in establishing nodules. Practices such as contour furrowing and pitting can increase soil water availability by retaining rainfall and snowmelt runoff, snowtrapping and increasing infiltration (Wight 1976). With relatively severe topographic modifications, such as artificially created basins, actinorhizal seedlings may be located within the depression to take advantage of moisture collection. Gross surface configuration, e.g., slope shaping, may be beneficial to nodulation by producing convergent landscapes where runoff can collect (Wollenhaupt and Richardson 1982).

Topsoiling.

Topsoil storage and topsoiling strategies may affect frankiae infective processes as seen in other topsoil-microbial interactions. Storage of topsoil can reduce mycorrhizal fungi (Rives et al. 1980). The lack of *Rhizobium* or the presence of rhizobial strains that are incapable of nitrogen fixation can hamper legume nodulation and fixation after reclamation has occurred (Bradshaw and Chadwick 1980). Naturally-induced infection of actinorhizal plants during reclamation may be impossible as a result of extended topsoil storage or adverse topsoil handling. DePuit (1984) noted that topsoiling strategies such as direct place topsoiling, multiple-lift topsoiling and supplemental top-dressing may be useful in retaining microbial activities. The same strategies may be beneficial to *Frankia* activity. Selective choice of spoil materials may also be advantageous. Nitrogen-deficient overburden placed on the surface of reclaimed lands may be less deleterious in sustaining nodulation than topsoils with high levels of nitrogen.

Seedlings Establishment/Inoculation

Righetti et al. (1982) believe inoculation will be necessary on sites where actinorhizal shrubs have not previously grown. They feel that the sparse nodulation present on some sites in xeric climates may be due to low *Frankia* populations. The time required for sufficient frankiae succession onto disturbed lands may be too long for utilization during reclamation. If *Frankia* is not a primary colonizer, as Shipton and Burggraaf (1983) believe, then the synchronization of seedling establishment and infection may not be possible without artificial inoculation.

The methodology for inoculation and inducing nodules on disturbed lands is still lacking in the literature. Inoculations of actinorhizal plants have been shown to be effective in establishing nodules (Righetti and Munns 1982; Granhall et al. 1983). The procedures have involved use of *Frankia*-bearing isolates. Problems associated with these methhods include lack of specificity in strains, slow microbial growth, and recalcitrant infection by some *Frankia* strains for particular actinorhizal plant species.

To assure infection, the actinomycete should be placed in proximity to the elongating roots of germinated seeds or seedlings. A possible field method for concomitantly seeding and inoculating actinorhizal plants may be the fluid-gel drilling technique. The technique has been applied with *Rhizobium* cultures and shown to increase nodulation (Hardaker and Hardwick 1978). Available sources of carbon such as propionic acid or acetic acid along with other growth media may possibly be added to the various gels to provide for nutritive requirements between the time of inoculation and seed germination. Mulch carriers, bearing Frankia and growth media, may also be an alternative.

Another approach to establishing frankae in the field may be the use of nodulated bare root or containerized transplant stock. Nodulated transplants have been suggested as a means of accelerating establishment of symbioses (Righetti et al. 1982). Inoculation with strains effective in nitrogen fixing (Righetti et al. 1982) or strains that are capable of interspecific infections (Lalonde 1979, Righetti and Munns 1980) may be possible. Nodule depth along the root axis may be manipulated by the placement depth of the inoculum.

SUMMARY/CONCLUSIONS

The practicality and economics of introducing symbionts onto disturbed lands has yet to be determined. Problems remain in evaluating the importance of the symbiosis to ecosystem functioning. Despite the theoretical benefits of adding symbiotically fixed nitrogen, there has been little study of its importance in plant succession on reclaimed lands when compared to nitrogen inputs of fertilizer.

Possibly the main value of nitrogen fixation to a plant may be as a passive, reliable source of nitrogen as opposed to the potentially variable soil nitrogen fluxes encountered in reclaimed lands. Low vigor of shrub species resulting in reduced diversity of plant communities has been a major problem for reclamationists. A number of these shrub species are actinorhizal and may be directly benefited by inoculation. Methodologies for inducing nodulation that are compatible with current reclamation practices should be researched.

Review of the literature indicates that low soil water and high soil nitrogen levels are potential obstacles in establishing viable nodules with adequate nitrogen fixation. The flexibility of reclamation technology under arid climates to alleviate drought restrictions on nodules is often limited. Nodule infection or eatablishment of nodulated seedlings may be enhanced by supplemental irrigation, but extended irrigation to prevent nodule death and decomposition is probably incompatible with current reclamation practices.

Similarly, potential heavy nitrogen fertilization on N-deficient disturbed soils may pose as a barrier period for actinorhizal activity until the nitrogen flux has passed beyond the rooting zone. The introduction of the Frankia symbiont or renodulated transplants may have to be timed in accordance with nitrogen fertilization and mineralization/immobilization or leaching patterns on reclaimed lands.

Incorporation of actinorhizal symbionts into reclamation planning may affect nutrient cylcing on reclaimed lands beyond that associated with nitrogen. Sustained use of legumes in agronomic situations can cause additional uptake of nutrients such as phosphorus, possibly leading to nutrient deficiencies. Since a number of plant species involved with actinorhizae are endomycorrhizal as well, a pansymbiotic or "tripartite" association has been suggested as a more appropriate approach to inoculation (Rose 1980). Some actinorrhizal plants have been observed to be endomycorrhizal as well as ectomycorrhizal (Williams, 1979). Research on the interaction between mycorrhizal associations and actinorhizal associations on a single plant host are almost totally lacking. Rose and Youngberg (1981) have examined the influence of actinorhizal and VA mycorrhizae on Ceanothus velutinus in a greenhouse environment, and Gardner et al. (1984) have examined the same interaction on Hippophae rhamnoides. However, work has not been reported for rosaceous plants and from sites in the field.

All DOI links of the cited references are at the end of the references.

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