On tripartite *Frankia*–mycorrhizal associations in the Myricaceae

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Actinomycorrhizal symbiosis was studied in *Comptonia peregrina* (L.) Coult. and in *Myrica gale* L., both of the Myricaceae. Root nodules were common in all *Comptonia* plants in their natural habitats and in pot cultures under greenhouse conditions. Spontaneous actinorhizal infection under greenhouse conditions differed in two native forest soils. Spontaneous mycorrhizal infection in *C. peregrina* and in *M. gale* was found neither in natural habitats nor in plants growing in native forest soils under greenhouse conditions. *Comptonia peregrina* and *M. gale* inoculated with the actinomycete *Frankia*, with the vesicular–arbuscular mycorrhizal fungus *Glomus intraradices*, or with *Frankia* and *G. intraradices* together were infected by *Frankia* only. The nodulated plants were significantly larger compared with unnodulated plants, and their root systems showed acetylene reduction in a bioassay for nitrogenase activity. Uninfected *Comptonia* plants that grew in a forest soil under greenhouse conditions developed well when watered with a complete mineral nutrient solution or with a solution that lacked phosphorus, but degenerated when watered with a solution that lacked combined nitrogen or with deionized water. *Comptonia peregrina* and *M. gale* formed cluster roots that resembled proteoid roots in the Proteaceae. In *Comptonia*, cluster-root formation in sand culture was common in nodulated plants as well as in unnodulated plants. In *M. gale*, cluster-root formation was common in nodulated plants and infrequent in unnodulated plants.

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Les auteurs ont étudié la symbiose actinorhizienne chez *Comptonia peregrina* (L.) Coult. et chez *Myrica gale* L., espèces appartenant à deux genres de Myricacées. En milieux naturels aussi bien que cultivés en pots dans une serre, tous les plants de comptonie portent des nodules racinaires. La colonisation actinorhizienne spontanée sous des conditions de serre s'est avérée différente dans deux sols forestiers. Il n'y a pas eu de colonisation mycorhizienne spontanée chez *C. peregrina* ni chez *M. gale* que ce soit en nature ou en serre, dans les deux sols forestiers. L'inoculation de *C. peregrina* et de *M. gale* avec l'actinomycète *Frankia* ou avec le champignon endomycorhizien à vésiculaire–arbusculaire *Glomus intraradices* ou encore avec à la fois le *Frankia* et le *G. intraradices* n'a permis que la formation de nodules actinorhiziens. Les plants nodulés étaient significativement plus gros que les plants non nodulés, et leurs systèmes racinaires ont réduit l'acétylène dans un test biologique pour déceler l'activité nitrogénasique. Des plants de comptonie poussant en serre sur un sol forestier se sont développés normalement lorsqu'ils ont été irrigués avec une solution minérale complète ou avec une solution sans phosphore, mais ils ont dégénéré lorsqu'ils ont reçu une solution sans azote ou bien de l'eau désionisée. Le *C. peregrina* et la *M. gale* forment des racines en amas qui ressemblent aux racine protéoïdes des Protéacées. Chez la comptonie cultivée sur sable, les racines en amas sont courantes sur les plants nodulés aussi bien que non-nodulés. Chez la myrique, la formation des racines en amas est commune sur les plants nodulés mais peu fréquente sur les plants non-nodulés.

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Introduction

A multisymbiosis located in the roots, among plants and microorganisms capable of fixing dinitrogen or absorbing phosphorus from the soil, may be a great advantage to the host plant. Such plants may develop well in environments in which nitrogen and phosphorus sources for plant nutrition are limited. Tripartite symbiosis is very common in the Leguminosae in which plants associate with dinitorgen-fixing bacteria of the Rhizobiaceae (Barea and Azcon-Aguilar 1983), and also with species of ectomycorrhizal or vesicular–arbuscular (VA) endomycorrhizal fungi that facilitate the uptake of phosphorus by host plants (Harley and Smith 1983).

Tripartite symbiosis in woody species in other plant families with the dinitrogen-fixing actinomycete *Frankia* and mycorrhizal fungi is far less studied (Gardner 1986; Rose 1980), although it has been shown that such multiple symbiosis may make a significant contribution to the growth of the host (Gardner et al. 1984; Rose and Youngberg 1981).

Comptonia peregrina (L.) Coult. and *Myrica gale* L. are actinorhizal plants of the Myricaceae that are effectively nodulated by *Frankia* (Bowes et al. 1977; Callaham et al. 1979; Schwintzer et al. 1982). The mycorrhizal symbiosis was previously reported to be ectomycorrhizal for *Comptonia* (Harley and Smith 1983; Rose 1980) and VA endomycorrhizal for *M. gale* (Harley and Harley 1987; Rose 1980).

Both species inhabit nutrient-deficient environments. *Comptonia peregrina* is widespread in disturbed places, mainly on poor sandy soils along roadsides and in new clearings in forests of northeastern North America (Del Tredici 1977). *Myrica gale* is a common plant that occurs in stands along riverbanks and freshwater ponds in the temperate region of the northern hemisphere.

The purpose of this study was to determine the role of tripartite symbiosis in these genera and to examine the relationships between the host plant and the microorganisms that are involved in the multiple symbiosis.

Materials and methods

Field observations

Frequent sampling of *C. peregrina* and *M. gale* plants growing in the field in natural habitats was made during the course of these studies to determine the occurrence of mycorrhizal associations. Root systems of about 100 plants were examined from the field (see Table 1). Whole root systems were processed for microscopic examination following the method of Phillips and Hayman (1970) as described below. Because of discoloration and pigment production in

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TABLE 1. Sites of field collections of plant species examined microscopically for mycorrhizal associations

	Location
Comptonia peregrina	
November 18, 1986	Harvard Pond, adjacent to gravel pit
May 28, 1987	Route 32A, south of Petersham
July 7, 1987	Route 2, Baldwinville exit
August 1, 1987	Fay lot, Harvard Forest, Petersham
October 7, 1987	1- to 2-year-old seedlings; Prospect Hill road near Fox Run, Phillipston
October 1988	Quabbin Reservoir, gate 35
October 1988	Gravel pit, Harvard Pond
Myrica gale	
August 10, 1987	Border of Harvard Pond, pond site
August 10, 1987	Border of Harvard Pond, causeway site
November 4, 1987	Harvard Pond, pond site
October 1988	Near outlet dam, Harvard Pond

field-collected roots, samples were left for prolonged periods (10–15 days) at room temperature with frequent changes of dilute KOH solution to clear the roots before examination. Five to 10 specimens were sampled at each site. Pressed-dried plants were retained for confirmation of species identification.

Seed germination

Seeds of *C. peregrina* and *M. gale* were collected from wild plants around Harvard Forest and were germinated in sand in a controlled environment chamber. For *Comptonia*, seed treatment involved scarification and presoak in 100 ppm gibberellic acid for 24 h (Del Tredici and Torrey 1976). Seeds of *M. gale* were stratified for 2 months at 4°C.

Growth of plants

Seedlings were transplanted 3 weeks after germination to 90-mL plastic pots and grown in the greenhouse in semicontrolled conditions (approximately 16 h light at approximately 20°C). Three separate experiments (*a*–*c*) are reported. Plants were watered daily with deionized water in expts. *a* and *c* and also once a week with a modified one-quarter strength Hoagland solution without nitrogen in expt. *c*. In four treatments in expt. *b*, plants were watered every second day with one of the following: (*i*) complete mineral nutrient solution that contained 0.4 mM KNO_3, 0.4 mM $Ca(NO_3)_2$, 0.15 mM $MgSO_4$, 0.13 mM NaH_2PO_4, and minor elements at one-tenth the concentration in modified Hoagland solution (Epstein 1972); (*ii*) mineral nutrient solution without NaH_2PO_4; (*iii*) mineral nutrient solution without KNO_3 and $Ca(NO_3)_2$, but with KCl, $CaCl_2$, and KH_2PO_4; or (*iv*) deionized water. At harvest time, the shoots were dried in a 75°C oven for 48 h and were weighed, and the roots were inspected for infection with *Frankia* and with mycorrhizal fungi. In expt. (*c*), root dry weight was estimated from their fresh weight according to the ratio of fresh weight to dry weight determined in 10 other specimens in which the fresh and dried roots were weighed.

Examination of infection in root systems

In expts. *a* and *c* the presence of actinorhizal nodules was recorded for each plant. In expt. *c*, nitrogenase activity was determined using the acetylene-reduction bioassay (cf. VandenBosch and Torrey 1984) on whole plants prior to harvest. The number of actinorhizal nodules per plant was counted, and the nodules were dried in 75°C for 48 h and were weighed. At the end of each experiment, the whole root system of each plant was cleared and stained (Phillips and Hayman 1970) after removing the nodules to assess mycorrhizal infection under a microscope with ×16, ×100, and ×250 magnification. *Trifolium subterraneum*, *Zea mays*, *Sorghum bicolor*, and *C. peregrina* were used as host plants in pot cultures to recover and identify vesicular–arbuscular mycorrhizal (VAM) fungal spores from local soils.

Soils

Two local soils were used in expt. *a*, one local soil in expt. *b*, and pure commercial sand in expt. *c*. A 1:3 mixture by volume of local soil with sand was used for recovery and identification of VAM fungal spores from different host plants that grew in pot culture for 9 months. The local soils were collected in November from two undisturbed sites in hardwood and in conifer forests in the Harvard Forest, central Massachusetts and were labelled soils H and C, respectively. These are shallow sandy-loam glacial-till soils, moderately to well drained, and laid over granite bedrock. The pH of soils H and C is 3.9 and 4.5, respectively, and both are rich with organic matter. The soils were dug from 5–40 cm under the raw humus layer and were stored in dark plastic bags at room temperature. Experiments with soils began shortly after field collection of the soil and for a period up to 1 year after original collection.

Recovery of VAM fungal spores from the soil

Spores were recovered from the soil using centrifugation in 40% sucrose that followed wet sieving (Daniels and Skipper 1982). The double procedure minimized problems with organic debris. Spores then were rinsed in tap water and were identified under a light microscope (Schenck and Perez 1987).

Inoculation with Frankia *and with VAM fungal spores*

For inoculation with *Frankia*, 0.9 mL of packed cell volume of *Frankia* strain HFPArI3 (catalog No. HFP013103) was rinsed twice with distilled water. The filaments were homogenized and suspended in 30 mL distilled water. Roots of 3-week-old *C. peregrina* and *M. gale* seedlings were immersed in the suspension for 15 min and transplanted to pots of sand. Spores of the VAM fungus *Glomus intraradices* Schenck and Smith were recovered from a commercial stock obtained from Nutrilink Nurseries (Salt Lake City) by a sieving and decanting technique (Daniels and Skipper 1982), rinsed with distilled water, and placed along the lower half of the root system. Each root system was inoculated with 100 spores. The same procedure was carried out with 20 plants of *T. subterraneum* and *Z. mays* to confirm the infectivity of the spores. In treatments inoculated with both microorganisms, *Frankia* inoculation preceded *Glomus* inoculation.

Results

Observations on infection in Comptonia *and* Myrica *roots that grew in their natural environments*

The root systems of both species, including seedlings and mature plants that were excavated from their natural habitats (see Table 1), were examined for the presence of actinomycorrhiza repeatedly during the growing season between May and November over a 2-year period. These roots were always found to have actinorhizal nodules. Although quite a few different fungal species were found on the root surfaces and in the root cortex, none of them produced any typical mycorrhizal structures. The roots were fine and branched, and in some cases they showed high frequency of lateral root formation periodically along the root length.

Spontaneous infection of Comptonia *by* Frankia *and by mycorrhizal fungi in local soils*

In expt. *a*, 3-week-old *Comptonia* seedlings were planted in pots with native, untreated soils C or H. The complete root systems of five seedlings in each soil were examined weekly after the 3rd week. Actinorhizal nodules were found first in a few plants in soils C and H 5 and 6 weeks, respectively, after the seedlings were transplanted to these soils (Table 2). After 8 weeks and on to the 10th week, four of five plants were nodulated in soil C, and only a few more were nodulated in soil H until the 18th week. None of the 95 plants was infected by any mycorrhizal fungus, although they often were infected by other nonmycorrhizal fungi. Nodulated plants had green leaves and

TABLE 2. Number of *Comptonia* seedlings that had actinorhizal nodules and mycorrhizal roots in hardwood and conifer forest soils in different ages from transplanting to the soil (expt. *a*)

	Conifer forest soil		Hardwood forest soil	
Age (d)	Actinorhizal plants	Mycorrhizal plants	Actinorhizal plants	Mycorrhizal plants
28	0	0	0	0
35	2	0	0	0
42	0	0	2	0
49	1	0	0	0
56	4	0	0	0
63	4	0	1	0
70	4	0	0	0
77			3	0
84			1	0
91			2	0
98			0	0
125			1	0

NOTE: For this experiment, there were five replicates per age group.

were larger than unnodulated plants. Most of the leaves of unnodulated plants turned from green to yellow–purple.

Effect of mineral nutrient solution on growth of uninfected Comptonia *plants*

In expt. *b*, uninfected *Comptonia* seedlings were grown in native soil H and were watered with one of the following: (*i*) a complete mineral solution; (*ii*) a minus-phosphorus mineral solution; (*iii*) a minus-nitrogen mineral solution; or (*iv*) deionized water. There were seven replicates each per treatments *i* and *ii* and six replicates each per treatments *iii* and *iv*. The plants were harvested after 75 days. No infection was found in the roots except for one plant that spontaneously nodulated in the minus-nitrogen treatment. This plant was excluded from the results.

The mean values for the number of leaves per plant, the height of the shoot, and the dry weight of the shoot were significantly greater in treatments in which the plants were watered with a complete mineral solution or with a minus-phosphorus solution than in treatments watered with a minus-nitrogen solution or with deionized water (Table 3). There was no significant difference between watering treatments using a complete mineral nutrient solution or minus-phosphorus solution and treatments using a minus-nitrogen solution or deionized water (Table 3).

Effect of inoculation with Frankia *and with* Glomus intraradices *on the growth of* C. peregrina *and* M. gale

In expt. *c*, seedlings of *Comptonia* and *Myrica* grown in pure washed river sand were inoculated with one of the following: (*i*) *Frankia*; (*ii*) *Glomus*; (*iii*) *Frankia* and *Glomus* together; or (*iv*) they were not inoculated. There were 15 replicates per treatment, except 12 *Comptonia* replicates in treatment *i* and 13 *Myrica* replicates in treatment *ii*. Acetylene-reduction bioassays were carried out on whole plants prior to harvest at 124 days after inoculation. The mean values of nitrogenase activity measured as acetylene reduction, the number of actinorhizal nodules per plant, the dry weight of nodules, roots and shoots per plant, and the presence of mycorrhiza and cluster roots are summarized in Table 4.

In both species, plants that were inoculated with *Frankia* or with *Frankia* and *Glomus* did not differ significantly from each other. Both groups were nodulated effectively and grew well. None of the plants inoculated with *Glomus*, with or without *Frankia*, was infected with *Glomus*, although the inoculum was found to be fully infective with subclover and with sweet corn. Plants inoculated with *Glomus* or uninoculated controls were not significantly different in growth at the end of the experiment. Similar relationships were obtained for *Alnus firma* that grew under the same conditions in the same time and place (S. S. Tzean, personal communication). Means of dry weight of roots and shoots in treatments in which both hosts were inoculated with *Frankia*, with or without *Glomus*, were significantly greater than means of plants that were not infected by *Frankia*. The *Frankia*-infected plants developed nodules that were active in acetylene reduction.

Unexpectedly, both plant species in all the treatments developed cluster roots that resembled proteoid roots (Lamont et al. 1984). In *Comptonia*, cluster roots were observed in most of the plants in each treatment, while in *Myrica* cluster roots were present in all the nodulated plants but in very few specimens in treatments with uninfected plants.

Observations on infection in pot clusters with Comptonia, *subclover, sweet corn, sorghum, and* Myrica

Comptonia plants that grew in pot cultures and in sand mixed with soils H or C for 9 months were not infected by any mycorrhizal fungi, but were spontaneously infected by *Frankia* and formed actinorhizal nodules. The nodulated plants were often infected by fungi different in their morphological structures from any mycorrhizal types. Recovery of VAM fungal spores from 9-month-old pot cultures of *Comptonia*, subclover, sweet corn, and sorghum with five replicates for each host in each soil mixture showed that except for *Comptonia* all the pots contained plenty of fresh VAM fungal spores, the majority of which belonged to *Acaulospora* spp., probably *A. mellea*, and a few to *Glomus* spp.

A 2-year-old *Comptonia* plant that previously had been inoculated with *G. intraradices* and grown in a pot with Turface (soil amendment from International Minerals and Chemical Corp., Mundelein, IL) was found to have no mycorrhizal infection, although two other species of weeds from the Compositae and the Oxalidaceae, which grew occasionally in the same pot, were infected by VAM fungi.

Five species in the genus *Myrica*, viz. *M. californica*, *M. cerifera*, *M. gale*, *M. parvifolia*, and *M. rubra*, that grew in the Turface under greenhouse conditions for about 5 years in Petersham were examined for the presence of infection. The potted plants had received routine watering with occasional inorganic nutrient supplement. All the plants were nodulated and had cluster roots, and none was mycorrhizal.

Discussion

Tripartite symbiosis may be of great advantage to plants inhabiting nutrient-poor soils like those around Harvard Forest. However, no evidence was found for its existence in *C. peregrina* and in *M. gale* from the Myricaceae.

The results of this study support previous work (Schwintzer et al. 1982; Torrey and Callaham 1978; VandenBosch and Torrey 1984) that show the dependence of *Comptonia* and *Myrica* on the actinomycete *Frankia* when growing without the addition of mineral nitrogen in the growth medium. It has been observed that all *Frankia*-infected plants that grew under greenhouse conditions, in local soils, or in soils mixed with sand without nitrogen fertilizer developed well, while plants

TABLE 3. Effect of a complete mineral solution (CMS), minus-phosphorus solution, minus-nitrogen solution, and deionized water (H_2O) on the growth of *Comptonia* seedlings in hardwood soil (expt. *b*)

Mineral solution	Height of shoot ±SE (mm)	No. of leaves per shoot ±SE	Dry wt. of shoot ±SE (mg)
CMS	97.0±6.1*a*	15.0±0.5*a*	157.0±19.6*a*
Minus phosphorus	93.3±6.0*a*	17.3±0.6*a*	193.4±27.8*a*
Minus nitrogen	52.4±3.8*b*	11.7±0.6*b*	70.7±6.8*b*
H_2O	39.1±3.0*b*	10.1±0.1*b*	44.4±7.0*b*

NOTE: There were seven replicates for each of the first and second treatments and six replicates for each of the third and fourth treatments. Plants were measured 75 days after initiation of the experiment. SE from analysis of variance (Snedecor 1956) is for each parameter separately. Different letters within a column denote significant difference ($P < 0.05$).

TABLE 4. Effect of inoculation with *Frankia* sp. and *Glomus intraradices* on growth and nitrogen fixation of *Comptonia peregrina* and *Myrica gale* in sand cultures (expt. *c*)

Treatment	Dry wt. of shoots ±SE (mg)	Dry wt. of roots ±SE (mg)*	Presence of mycorrhiza (+, −)	No. of nodules per root system ±SE	Dry wt. of nodules per root system ±SE (mg)	Mole C_2H_2 reduced per plant per hour ±SE
Comptonia peregrina						
Frankia	451.7±77.4*a*	364.9±42.5*a*	—	2.6±0.5*a*	28.1±6.0*a*	0.158±0.017*a*
Glomus	35.6±2.6*b*	91.9±7.2*b*	—	0*b*	0*b*	0*b*
Frankia and *Glomus*	217.5±35.7*a*	240.3±36.4*a*	—	3.9±0.7*a*	16.1±3.3*a*	0.117±0.018*a*
Control	34.4±4.1*b*	95.9±9.0*b*	—	0*b*	0*b*	0*b*
Myrica gale						
Frankia	92.6±13.4*a*	174.7±26.5*a*	—	3.1±0.5*a*	8.0±3.0*a*	0.159±0.028*a*
Glomus	8.3±0.7*b*	18.5±2.5*b*	—	0*b*	0*b*	0*b*
Frankia and *Glomus*	96.7±15.9*a*	150.7±26.5*a*	—	5.2±0.9*a*	9.0±1.6*a*	0.184±0.036*a*
Control	9.4±0.7*b*	25.1±3.2*b*	—	0*b*	0*b*	0*b*

NOTE: There were 15 replicates per treatment, except 12 *Comptonia* replicates inoculated with only *Frankia* and 13 *Myrica* replicates inoculated with only *Glomus*. Measurements were made 124 days after inoculation. SE from analysis of variance (Snedecor 1956) is for each parameter separately. Different letters within a column denote significant difference ($P < 0.05$).
*Dry weight of root was calculated from fresh weight.

that were not infected with *Frankia* and were not fertilized with mineral nitrogen solution were smaller, their leaves were yellow−purple−green, and they degenerated with age. Nodulation caused by spontaneous infection was first observed 5−6 weeks after *Comptonia* seedlings were transplanted to local soils in the greenhouse. The difference in infection rate between the two soils may reflect the abundance of *Frankia* propagules in each particular site. These soils were sampled about 200 m from the closest *Comptonia* stands for soil C, about 6000 m from the closest *Comptonia* stand in soil H, and a few metres from actinorhizal *Alnus incana* ssp. *rugosa* in soil H. Estimation of the number of *Frankia* infective propagules in these soils, using the most probable number method, confirmed the ratio between infection rate achieved for both soils (unpublished data).

There was no evidence for the presence of mycorrhiza of any type in *Comptonia* and in *Myrica* in any of the experiments or observations that were made. These examinations included several hundreds of seedlings and mature plants of *Comptonia* and about two dozen mature plants of *Myrica* that grew in their natural environments or under greenhouse condition within local soils or within local soils mixed with sand. None of the 58 seedlings of *Comptonia* and *Myrica* inoculated with the mycorrhizal fungus *Glomus intraradices* formed VA endomycorrhiza.

Harley and Smith (1983) placed *Comptonia* and *Myrica* in the list of genera reported to contain at least one species in which ectomycorrhiza has been described. Since the genus *Comptonia* has only one species, *C. peregrina*, endemic to North America, their data differ from the present observations, although the latter were collected from forest soils where ectomycorrhizal plants and ectomycorrhizal inoculum prevail. Rose (1980) reported on ectomycorrhiza in two specimens of *Comptonia* that grew in mine refuse and tailings in Massachusetts. We could find no support in our study for her observations. Malloch and Malloch (1981) reported observing only 1 root sample out of 30, examined in *Comptonia* collected in the boreal forest region of northeastern Ontario, that showed possible endomycorrhizal infection.

The mycorrhizal status of *M. gale* is recorded in the update list of Harley and Harley (1987) as "having VA in better drained sites but not very wet" and also "having no mycorrhiza in wet soil conditions." Twenty-eight *Myrica* seedlings that grew in well-drained sand and were inoculated with *G. intraradices* never formed VA endomycorrhiza. Rose (1980) found VA endomycorrhiza in five specimens in each of four species in the genus *Myrica* that were collected in their natural habitats: *M. californica*, *M. cerifera*, *M. gale*, and *M. pensylvanica*. The first two species were also recorded by her to be ectomycorrizal. It is not clear from her report whether VA endomycorrhizal fungi and ectomycorrhizal fungi inhabited the same specimen, or whether the VAM fungal spores

that were recovered from the rhizosphere around the host plants were attached to them.

Koske and Halvorson (1981) reported that *M. pensylvanica* plants that grow on a barrier sand dune in Rhode Island were associated primarily with the VA mycorrhizal species *Gigaspora gigantea* and *Acaulospora scrobiculata*, with less frequent association with *Glomus* spp.

Our studies suggest that *Comptonia* and *Myrica* do not form mycorrhiza in our local soils, or do so rarely. The failure to establish mycorrhizal symbiosis by inoculating *Comptonia* and *Myrica* with spores of mycorrhizal fungus cannot be the result of inoculum quality, as this inoculum infected well both subclover and sweet corn.

It may be questioned whether *G. intraradices* is compatible with host species of the Myricaceae, although according to Harley and Smith (1983) VAM fungi tend to have wide host ranges. Many different considerations of soil characteristics may determine infection capacity.

The results for uninfected *Comptonia* seedlings that developed well in the soil while being watered with a complete mineral solution or with minus-phosphorus solution show that phosphorus absorption from soil is well sustained without having mycorrhizal infection. This case is unlike that of subclover and white clover that grew in the same soil and responded significantly to phosphorus fertilizer by increasing the dry weight of shoots 2–18 times (unpublished data). Uninfected *Comptonia* seedlings that were watered with nutrient solution that lacked nitrogen did not differ from deficient seedlings that were not fertilized. Hence, these results support those that show the important role of *Frankia* in nitrogen nutrition of *Comptonia*, that otherwise cannot support itself with sufficient nitrogen from the soil.

The development of cluster roots in *C. peregrina*, *M. californica*, *M. cerifera*, *M. gale*, *M. parvifolia*, and *M. rubra* and their similarity in appearance to proteoid roots in the Proteaceae suggests that they may have a similar function in phosphorus absorption in the Myricaceae (Lamont 1981). This suggestion is supported by the results from the previous experiment, showing the ability of nonmycorrhizal *Comptonia* plants to develop well in the soil when watered with minus-phosphorus solution. Unfortunately, cluster roots in *Comptonia* were first observed in sand culture, where they were easy to handle, and their presence in earlier experiments within soils had been ignored. If cluster roots in the Myricaceae help to support the plant by overcoming phosphorus deficiency in the soil, they may be considered to be a different mechanism from mycorrhizal symbiosis for phosphorus absorption from the soil.

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