A Nitrogen Fixation: The Story of the *Frankia* Symbiosis

Peter Del Tredici

Some of the plants we scorn as weeds perform important biological functions. By adding nitrogen to impoverished soils, nonleguminous nitrogen-fixing trees and shrubs play a key role in the process of forest succession.

It's a great irony of the botanical world that plant growth is often limited by the availability of nitrogen in the soil when almost eighty percent of the atmosphere is composed of dinitrogen gas (N_2). The explanation lies in the chemical stability of nitrogen gas. Before atmospheric nitrogen can be used by plants, it must be "fixed," that is, split and combined with other chemical elements. This process requires a large input of energy and can occur either biologically, within the cells of various bacteria, or chemically, in fertilizer factories or during lightning storms.

Among all living organisms, only bacteria have evolved the complex biochemical mechanisms required for nitrogen fixation. All "higher" plants and animals that are said to fix nitrogen are really only the symbiotic partners of the bacteria that do the actual work. Among plants, the cultivated legumes (peas, beans, peanuts, etc.) are the best-known nitrogen-fixers, but many plant families besides the Leguminosae can also fix nitrogen. On a worldwide scale, these nonlegumes, as they are somewhat negatively called, fix as much nitrogen as legumes, but for a variety of historical reasons they have been relatively neglected by scientists.

Quite a few of these nitrogen-fixing nonlegumes are native to North America and are mostly found in impoverished, sandy soils low in nitrogen. The most common are alder (*Alnus* sp.) in wet, open land; bayberry (*Myrica pensylvanica* in the North, *M. cerifera* in the South) on the seashore and on exposed sandy soils back from the coast; sweet fern *(Comptonia peregrina)* on exposed, dry, sandy soils; sweet gale *(Myrica gale)* in swamps; and New Jersey tea *(Ceanothus americanus)*

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This flat of southern bayberry seedlings (Myrica cerifera) *was supposedly grown both without nitrogen and without the* Frankia *bacteria. One of the seedlings, however, did manage to form* Frankia*-induced root nodules, producing a clearcut advantage over its siblings.*

in open, wooded sites. On the west coast of North America, various *Ceanothus* and *Alnus* species are the most common nonlegume nitrogen-fixers. In the arid mountains of the West, buffalo berry *(Shepherdia canadensis)*, bitterbrush *(Purshia tridentata)*, and the mountain mahoganies (*Cercocarpus* spp.) are important. In southern Florida, the introduced and somewhat weedy Australian pine (*Casuarina* spp.) is important for stabilizing beaches, and throughout the Midwest and East Coast the autumn and Russian olives *(Elaeagnus umbellata* and *E. angustifolia)* have been widely planted along highway embankments. All of these plants thrive in poor soils where little else grows. Their ability to fix nitrogen is a significant factor in their survival under conditions that would be inhospitable to ordinary plants.

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A Frankia-*induced nodule on a root of sweet fern,* Comptonia peregrina. *Note the upwardly growing roots emanating from the lobes of the nodule.*

In legumes and nonlegumes alike, the actual fixation of nitrogen is done by bacteria living inside the roots of the host plant. It is a classic example of a mutually beneficial symbiosis: the plant provides the bacteria with sugars and a variety of minerals, and the bacteria provide the host with a usable supply of nitrogen. In the case of the legumes, the bacterium may be one of several different species of the genus *Rhizobium*, a rod-shaped bacterium found naturally in most soils and available commercially in most seed catalogs as legume inoculant. In the nonlegumes, the bacterium involved is an actinomycete, or filamentous bacterium, in the genus *Frankia*. Unlike the rhizobia, which exist as discrete cells, the actinomycetes grow in long chains of cells similar to fungal hyphae, but much smaller. All of the plants infected by *Frankia*, with one exception, are trees and shrubs, whereas among the legumes both annual herbs and trees may be infected by rhizobia.

The microorganisms enter the plant through the root hairs and grow in the cells of the roots, stimulating them to grow and divide. Basically, the bacteria, by producing large quantities of the hormones that normally occur in very small concentrations in the plant, force the root cells to proliferate much faster than normal, causing multi-lobed swellings, or nodules, to form wherever the bacteria have penetrated. Among the actinomycete-nodulated plants, properly referred to as actinorhizal plants, these nodules have a well-defined structure and a rather striking appearance, being densely branched and more or less spherical. The nodules are perennial and increase in size each year, eventually becoming over an inch in diameter. In some plants, such as alder, the individual lobes that make up the nodule are very tight and compact. In others, such as sweet fern, each lobe of the nodule grows out into an upwardly growing root creating a sort of witch's broom effect.

These nodules are the site of nitrogen fixation. Both *Rhizobium* and *Frankia* bacteria possess special enzymes, nitrogenases, that allow them to transform the nitrogen gas in the air into ammonium which, in turn, is converted into amino acids. Because the reaction can only occur in a low oxygen environment, the process is often dependent on hemoglobin compounds found in the nodules, which are virtually iden-

Several old nodules of the root system of the southern bayberry, Myrica cerifera, *growing on the sand dunes along the outer banks of North Carolina. The root, with its attached nodules, was exposed by the wind, which in this area never seems to stop blowing.*

tical to those found in the red blood cells of animals. By binding with oxygen, the hemoglobin in the nodule helps to create the microenvironment that the nitrogenase enzyme requires. It is interesting to note that when actinorhizal plants are grown in water culture, the young, succulent nodules are often pink in color, due to the presence of hemoglobin.

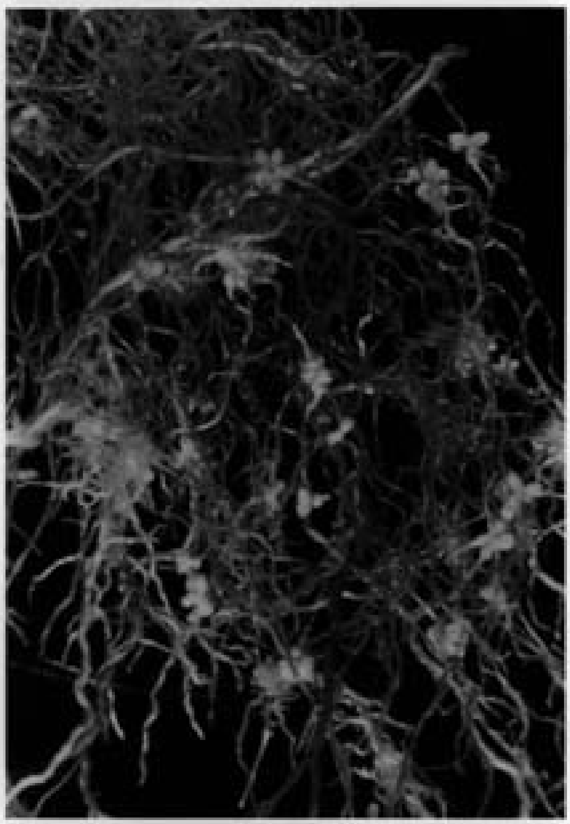
Work on actinorhizal plants took a giant step forward in 1978 when a research team that included Dale Callaham, currently of the University of Massachusetts at Amherst, the late Professor J. G. Torrey of the Harvard Forest in Petersham, Massachusetts, and the present author, successfully isolated and grew, independent of its host plant, the slow-growing *Frankia* bacteria responsible for fixing nitrogen in sweet fern *(Comptonia peregrina)*. This was the first time that the bacteria from any actinorhizal plant of the nonlegumes had been cultivated independently, and it marked the end of nearly seventy years of unsuccessful attempts to isolate the organism responsible for nitrogen fixation from an actinomycete-nodulated plant. In contrast, the faster-growing *Rhizobium* bacterium was first isolated in pure culture ninety years earlier, in 1888. This disparity is the main reason why the actinorhizal symbiosis is not nearly as well understood as the *Rhizobium* association.

In general, actinorhizal plants are sunloving pioneers in early successional stages of revegetation of the north and south temperate regions (with the exception of *Casuarina* and *Myrica* species in the tropics). They do best on sandy or swampy soils where nitrogen is scarce and their ability to extract it from the air is a distinct advantage. Usually they are not found in shady, forested situations or on rich farmland, where they would lose their competitive advantage. Most nitrogen-fixing legumes, on the other hand, are tropical and subtropical herbs that have migrated north and become important in agricultural environments.

It is primarily because the legumes are involved with food production that they have attracted the lion's share of scientific attention, but this situation is changing rapidly. People are becoming aware that the potential value of actinorhizal plants is of equal importance, if not equal conspicuousness, to the legumes. Experiments have been conducted by forestry managers in which actinorhizal plants are grown in conjunction with various economically desirable trees: red alder and *Ceanothus* with Douglas fir on the West Coast, alder with poplar for pulp on the East Coast, and *Elaeagnus* with black walnut in the Midwest. In all cases, the experiments resulted in richer soil and faster growth rates in the desired tree species. In the Northeast, sweet fern, bayberry, and *Elaeagnus* are used extensively for stabilizing roadside bankings and revegetating traumatically disturbed ground.

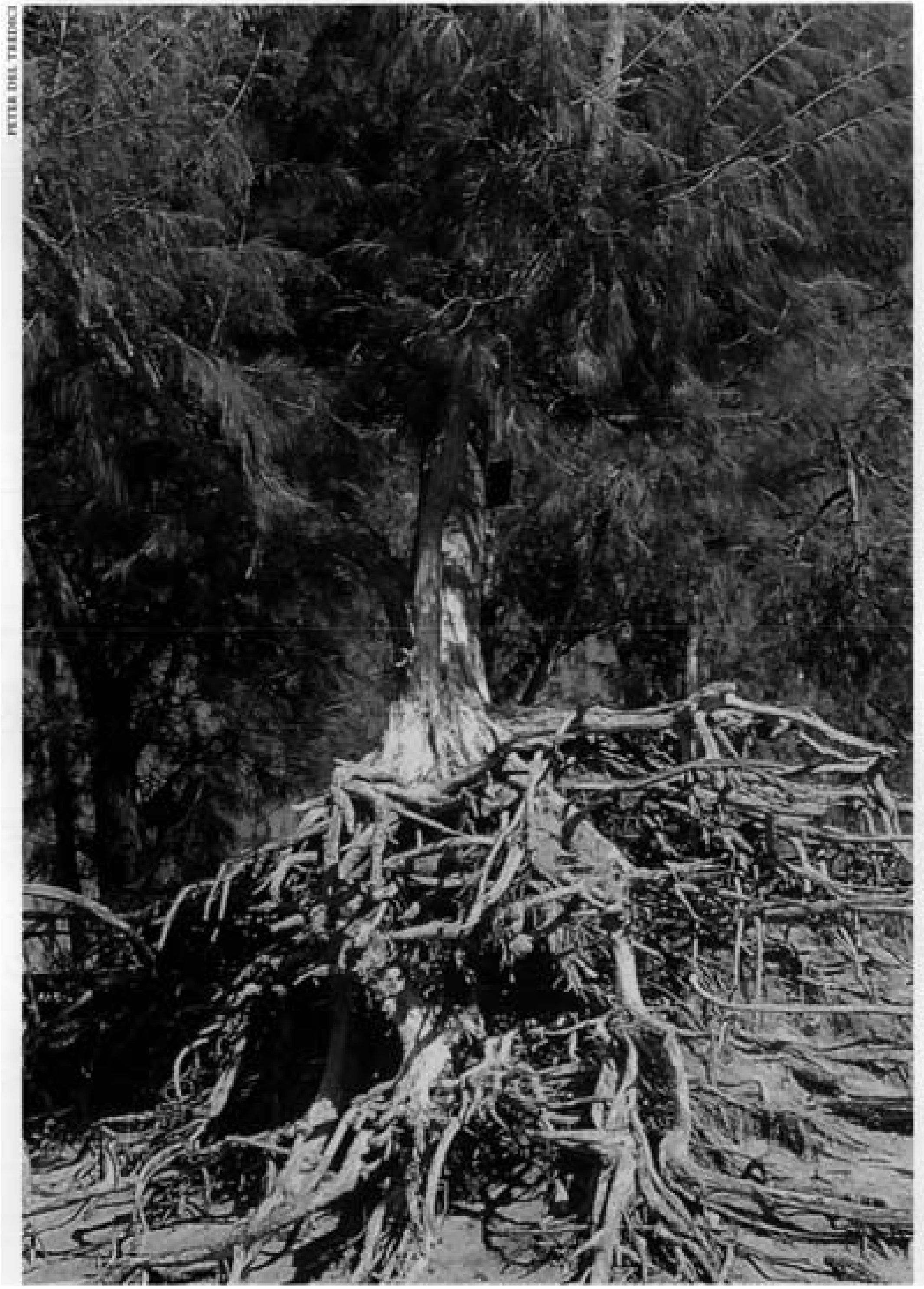
Actinorhizal plants have a much longer history of human use in Europe and Asia than they do in North America. In Japan, the Asian species of *Myrica* and *Alnus* are grown in association with various conifers to improve the soil and stop erosion, while along the northern coast of Europe and the British Isles, the sea buckthorn, *Hippophae rhamnoides*, is cultivated for the purpose of stabilizing shorelines, as well as for its edible fruit. Throughout the tropics, the genus *Casuarina* is not only important in stopping seashore erosion, but is also an important source of fuel and timber in areas that otherwise produce very little. The future looks bright for the actinorhizal plants, especially in the context of forestry and habitat restoration, as land managers move from experimentation into implementation.

The fact that actinorhizal plants grow where little else can makes them particularly useful for covering bare ground. Apart from this functional recommendation, however, many of these plants are aesthetically pleasing as well. Various *Ceanothus* species, which are widely grown in mild climates, are covered in springtime with blue, pink, or white flowers. Sweet fern, which is fast growing and small, is perfect for any situation with full sun and sandy soil.



The root system of Comptonia peregrina *four weeks after innoculation with a pure culture of* Frankia *bacteria. The bacteria stimulated the development of over fifty-five nodules on this ten-week-old seedling.*

Bayberry is an attractive midsize shrub that keeps its leaves longer than most plants and is covered for most of the winter with waxy gray berries (the source of bayberry candles). In addition, bayberry is highly salt tolerant and performs equally well near the seashore or on highway embankments where road salt applied during the winter tends to accumulate. The arborescent alders have traditionally been thought of only in terms of land reclamation, but recently commercial nurseries have begun to recognize their ornamental potential. Finally, the autumn and Russian olives, which were widely planted in the 1970s, have a beautiful silver-gray foliage and fruits that birds love to feed on. Unfortunately, the birds love the fruits so much they have dispersed the plant well beyond its initial area of cultivation. As



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The Australian pine, Casuarina equisetifolia, *growing on the beach at Haena Point on the north coast of Kauai, Hawaii. Incessant wave action has exposed the massive, layered root system of the tree. No doubt this species' ability to fix atmospheric nitrogen is primarily responsible for its ability to survive the harsh conditions.*

a result of this weedy tendency, the olives are no longer recommended for large-scale erosion-control plantings.

Measurements of nitrogen fixation in actinorhizal plants taken over extended periods of time have shown that pure stands of alder bush are capable of fixing up to 280 pounds of nitrogen per acre per year. This is much greater than the amount of nitrogen fixed by soybeans (90 pounds per acre per year) or peas (66 pounds per acre per year), but comparable to that fixed by alfalfa. Most of the nitrogen fixed by the actinorhizal plants enters the nutrient cycle through the decomposition of fallen leaves, twigs, branches, and fine roots. This process is much slower than that which occurs in agricultural situations, where leguminous cover crops are plowed into the soil at the end of a single growing season.

It is worth keeping in mind that the greatest degree of nitrogen fixation, in legumes and actinorhizal plants alike, occurs when soil levels of nitrogen are relatively low. High levels of nitrogen, applied as fertilizer, tend to reduce bacterial activity. What this means is that the plant and the bacteria work best together when conditions are worst: the symbiosis is most effective when it becomes most necessary. To put it another way, the nitrogen-fixation symbiosis is a dynamic interaction between two independent organisms that is entered into when ecological conditions are such that neither partner could survive long without it.

The silvery gray foliage of the Russian olive, Elaeagnus angustifolia, *can be very striking in the landscape, as shown here at the Montreal Botanical Garden.*

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Peter Del Tredici is Director of Living Collections at the Arnold Arboretum.