Edaphic Characteristics of Nitrogen Fixing Nodulation (*Actinorhizae*) by *Cercocarpus montanus* Raf. and *Purshia tridentata* (Pursh) DC

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Abstract: Edaphology, excavation techniques, nodulation and some nitrogen fixation characteristics of mountain mahogany (*Cercocarpus montanus* Raf.) and antelope bitterbrush (*Purshia tridentata* (Pursh) DC) were examined principally in southeastern Wyoming environments. Although critical levels of soil nitrogen and soil organic matter were not identified, relatively high levels of nitrate nitrogen (above 3 ug/g) and soil organic matter (above 2 to 4 percent) inhibit nodulation, whereas low levels of nitrate (less than 1 ug/g) and soil organic matter (less than 1 percent) are conducive to nodule formation. Excavation of these nodulated Rosaceous plants in the field requires care and attention to detail. Strategies for excavation should involve allowing sufficient time and input of energy into the excavation process, and excavation of mature plants may take even weeks to accomplish. Field observations and subsequent greenhouse studies strongly suggest that the agents responsible for nodulation of these plants (member of the actinomycete genus *Frankia*) is either not present or is inactive in surface soils. The paradox of stressing plants via sampling and the expectation that collected data represents normal response is discussed.

Introduction

Cercocarpus montanus Raf. and *Purshia tridentata* (Pursh) DC, mountain mahogany and antelope bitterbrush, respectively, are common shrubs in Western North America, being found mostly in the foothills topographically between grasslands and forested environments. Sometimes both are in the understory of lower elevation coniferous forest. These two plants, both in the family Rosaceae, are important as browse for many wildlife species and as cover for others, and both may periodically occupy deep soils, but mostly they can be found in areas of shallow soil where the lithic contact is within a meter of the surface. Usually the geological substrata, which best host both species, are sedimentary materials with limestone and sandstone; claystones and siltstone are less favored.

That these two plants are adaptable to a diversity of fairly harsh environments may well be a function of the relatively large variety of mutualistic symbiotic microorganisms known to occupy the root systems of both plants. Both plants are known nitrogen fixers, and are infected by bacteria of the Actinomycetales genus *Frankia*. These bacteria elicit development of root cortical hypertrophies (nodules and often called actinorhizae) that function to biologically fix atmospheric nitrogen. Further, both plants can be infected by mycorrhizal fungi including arbuscular mycorrhizal fungi expressed as an endomycorrhizal morphology as well as by fungi that are manifest as an ectomycorrhizal morphology (Williams 1979). It is highly probable that the capacity of these plants to host such a variety of known beneficial root microorganisms results in these plants being somewhat independent of essential soil nitrogen because they can fix their own. They also can benefit from the enhanced uptake function of mycorrhizal roots, which includes uptake of critical nutrients such as phosphorus and zinc (Allen 1991) as well as uptake of water (Stahl and others 1998). Ectomycorrhizal plants are also known to have some protection against root pathogens (for example, Marx 1972).

Mountain mahogany and antelope bitterbrush (*C. montanus* and *P. tridentata*) are two species among perhaps a dozen or more species spread across five or six genera in the Rosaceae that fix nitrogen via association with *Frankia*. The genera *Chamaebatia*, *Cowania*, *Dryas*, and perhaps *Rubus* are also known for their nitrogen fixing habit (Peterson and others 1991). It is not very well known how much nitrogen is commonly fixed by these associations, although speculation is that it is low especially in highly arid environments (Dalton and Zobel 1977; Kummerow and others 1978). Further, there remains speculation that even though these rosaceous plants have nodules and fix nitrogen that such activity is not very important to supplying nitrogen needed

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by the plant. This general observation is based on observations by a number of researchers that these plants are not well nodulated or are often not nodulated in the field.

The objectives of this work are to (1) discuss soil properties that influence actinorhizae development on these two plants, (2) derive strategies for and show results of plant excavation to locate and quantify actinorhizae development on these two and similar plants, (3) report nodulation characteristics and nitrogen fixation activity for these species under various environmental conditions, and (4) discuss the uncertainty that arises from determining normal characteristics of plant activity when done destructively and where the plant is otherwise stressed to secure such characteristics.

Materials and Methods

Soil Properties That Influence Actinorhizae Development

Mountain mahogany and antelope bitterbrush have been excavated by the authors and associates since the mid-1970s mostly in southeastern and central Wyoming. In the early 1980s to about 1995, numerous plants were excavated and examined by excavation of the root system to a minimum depth of 30 cm to consolidated and immovable rock or to the extent of the root system. Plants of all ages were excavated; however, the majority of plants were less than 12 years of age. Here, excavation is used to mean that the entire root system of each plant was systematically unearthed, nodules counted, fresh and dry weights taken, and soils collected from nodule zones. If a plant bore no nodules, soil was still taken from that rooting zone of the plant where, from our experience, we would normally expect to find nodules. This zone was normally in the range of 30 to 60 cm from the soil surface. Numerous plants were taken from highly disturbed soils where top soil had been removed. We became aware early that such plants were much more likely to be nodulated than those on well developed soils.

Plants and soils were examined at approximately 50 sites throughout Wyoming. Bitterbrush was examined primarily in the Laramie Range, The Snowy Range foothills, the Wind River Range foothills, and the Absaroka Range foothills. Mountain mahogany samples were taken exclusively from the Laramie Range and the Snowy Range foothills.

Plants were examined visually for presence of nodules. Organic matter (Allison 1965), NO_3-N (Oien and Olsen 1969), pH (1:1 in water), available K (Bower and Wilcox 1965), available P (Wantanabe and Olsen 1965), and electrical conductivity (Bower and Wilcox 1965) were determined for each soil sample.

Strategies for Plant Excavation

Techniques—The tendency when looking for nodules on root systems is to take digging tools to the field and oftentimes indiscriminately and randomly sample from the soil around the base of the plant, relying mostly on chance that one will strike a cluster of nodules. This rather arbitrary method can be successful when nodules are needed for general examination or for a crude assay. It is particularly fruitful when looking for nodules on cultivated legumes, and can be successful when examining members of the genus *Alnus* in many riparian systems. However, when nodules are deep within soils, and when clusters are apparently rare, more methodical methods are used, and sufficient time must be allocated to fully excavate plant roots.

There are numerous problems encountered when quantitatively examining plant root systems. Actinomycete nodulated shrubs often have root systems that extend to considerable depth, the rooting medium may be obstreperous (for example, too clayey, too many large coarse fragments), roots of differing species make following the roots of the target individual difficult, roots of same species make following target individuals difficult, and plants can be in steep topographical positions that may defy examination.

We have used a variety of techniques to examine roots systems of shrubs. Herein we describe methodology for shrub excavation that can be used to quantitatively remove shrub root systems. The objectives and scale of research will dictate, to some degree, the intensity of excavation needed and the data that will be recorded. However, as the spatial and temporal development of plant roots are examined, as well as symbiotic associations between roots and a variety of mutualistic, pathogenic, and commensalic biota, roots systems will need to be more accurately and methodically excavated. There are a variety of reference materials that can be accessed that detail root excavation considerations and techniques. Two works from the archaeology literature are very much worth consideration especially for project level excavations (Joukowsky 1980; Roskams 2001).

Examination of Soil Taxa Associated With Nodulated Shrub Communities—During plant excavation to locate and quantify actinorhizae development, soils and plants were examined along a trenched transect that was made from a site occupied by mountain mahogany, through the boundary of the plant community, and into an associated soil type having no mountain mahogany and dominated primarily by grasses and forbs. The site was representative of a number of associations between mountain mahogany and adjacent vegetation and was located 10 km NNE of Laramie, WY, south of the mouth of Roger Canyon.

Soils and plants were examined also along a transect made at a site occupied by antelope bitterbrush, through the boundary of the antelope bitterbrush community, and into an associated soil type that was void of the shrub but dominated primarily by *Artemisia* sp. shrubs, grasses, and forbs. The transect was located 15 km ESE of Laramie, WY, adjacent to Blair Road. This transect was not trenched, but rather was examined by locating pits and observation of natural and manmade cuts that intersected the established transect.

The two transects indicated above were established primarily to determine if there were identifiable morphological features of soils associated with these two rosaceous shrub communities. Soils were classified in accordance with Soil Taxonomy (Soil Survey Staff 1999) and the Soil Survey of Albany County, WY (Reckner 1998).

Nodulation Characteristics and Nitrogen Fixation Activity

Actinorhizal Plants During Winter Months—Antelope bitterbrush and mountain mahogany were observed

occasionally during winter seasons between 1988 and 1998. Observation years were categorized as dry winters or wet winters where dry winters had very little surface snow accumulation and the soil profile was generally dry. Wet winters were those where there was considerable surface accumulation of snow.

During one wet winter, 1988, antelope bitterbrush individuals were excavated from a site along the Happy Jack Road on Pole Mountain near Laramie, WY. Soils here were weakly developed with some organic matter accumulations in the upper 20 to 30 cm over an undeveloped coarse subsoil. Nodules were found in the coarse subsoil. Three plants were excavated in February of that year—a 1-year-old plant on a recently disturbed roadcut and two 5-year-old plants on an adjacent, but undisturbed site. Nodules were taken and incubated in a 10 percent atmosphere of acetylene to ascertain nodule nitrogen fixation via analysis of ethylene (Upchurch 1987). Incubation chambers were 450 ml volume, incubation time was up to 1 hour, and was done at ambient soil temperatures in the field. Gas samples from the incubation chambers were stored in vacutainers prior to analysis. Subsamples of 100 microliters each were analyzed via gas chromatography (Upchurch 1987). Observations of antelope bitterbrush and mountain mahogany during several winter seasons showed that during dry winters, nodules were very hard to find and that when found, they had no visual signs of activity. Nodules were usually brown to black including nodule tips. During wet winters and especially under snow pack, nodules were easier to find and often gave the visual appearance of considerable activity, that is, nodule tips were white and apparently turgid.

Biological Assay of Soil Materials for Nodulation Capacity—The field portion of this study was done in SE Wyoming, in Laramie County. Seven sites were located in two separate areas. Six of these sites were inhabited by mountain mahogany, and one site that did not have this plant provided soil materials as a control. Pits were excavated on each of these sites to the depth of consolidated bedrock or 1.8 m, whichever was less. Five-kg soil samples were taken from each horizon of each site. These were split, with half used in a greenhouse bioassay and half used for soil analysis. Soil analysis included texture (hand-textured according to Thien 1979). Soils were taxonomically described in the field.

Seeds were collected from plants at four of the seven sites. Tails were removed and the seeds submerged in a 0.525-percent sodium hypochlorite solution for 20 minutes to control fungi. Sterile water-rinsed seeds were placed in glass containers in wetted sterile vermiculite and refrigerated at 2 °C for 60 days, and then at 12 °C for 10 days. This treatment resulted in 90-percent germination.

Autoclaved (121 °C at 0.1035 MPa for 20 minutes) pottery shards were placed in the bottoms of plastic 30.5-cm-diameter pots to expedite drainage. Pots had been soaked overnight in a 0.525-percent sodium hypochlorite solution. Each pot was half filled with autoclaved sand. Soil from each horizon of the seven sites was each mixed 1:1 (weight to weight) with autoclaved sand. The sand-soil mixture from each specific horizon was used to layer 5 cm over the sand in each pot. Over this an additional 5-cm layer of a 1:1 sand to perlite mixture was added. Across the seven field sites, 23 different horizons were collected. Because two pots of diluted soil material were derived from each horizon, a total of 46 pots resulted. Two control pots were also constructed, where a 1:1 sand to perlite mixture was substituted for the 1:1 sand to soil mixtures. Pots were thoroughly watered (to near field capacity) and five germinated mountain mahogany seeds planted in the surface 2 cm of each container and lightly covered with the sand to perlite mixture. All plants were placed in a greenhouse in late April and misted daily during the emergence and establishment period of the plants. Pots were randomly placed, on 45-cm centers, in the greenhouse and rerandomized monthly. Damping-off fungi were problematic, and resulted in necessary replacement of some seedlings. Pots were watered as needed. Temperatures in the greenhouse averaged 20 to 22 °C during daylight hours and 7 to 8 °C during night. Plants remained in this environment for 10 months.

At the conclusion of this time, whole plants were removed from the rooting media and observed for nodulation. Tops and roots were separated and weighed individually.

Results

Soil Properties That Influence Actinorhizal Development

For both mountain mahogany and antelope bitterbrush, soil organic matter and extractable nitrate nitrogen are strongly related to the capacity of these plants to host the nitrogen fixing symbiont and consequent formation of actinorhizae (see Peterson and others 1991; and especially White and Williams 1985). Nodulated mountain mahogany and antelope bitterbrush had, respectively, 0.61 (standard deviation of 0.66) percent organic matter and 0.68 (0.59) percent organic matter in root zones as compared to non-nodulated plants that were 2.94 (1.37) percent and 3.73 (2.24) percent. These differences were statistically significant between nodulated and non-nodulated populations (within species) at 0.01 and 0.001, respectively, for mountain mahogany and antelope bitterbrush. Extractable nitrate nitrogen showed similar trends and significance levels—0.59 (0.50) ug/g and 0.92 (0.76) ug/g for nodulated mountain mahogany and antelope bitterbrush, respectively; and 2.81 (1.73) and 3.50 (2.35), respectively, for non-nodulating plants. Differences were statistically significant at 0.01 levels for both comparisons within species. Other soil parameters (pH, electrical conductivity, extractable P, and extractable K) were all significantly different between nodulated and non-nodulated mountain mahogany, but were not significant for antelope bitterbrush. These findings suggest that nodulation of these rosaceous plants is controlled by edaphic factors such as organic matter and nitrate levels. Given the high level of resources that a plant must invest in the acquisition of nitrogen from atmospheric sources, it makes sense that these plants would not allocate such resources if nitrogen is available in mineral forms (for example, as nitrate) or in organic forms (for example, from soil organic matter) at levels above a basal level needed for survival. Artificial fertilization of antelope bitterbrush (Tiedeman 1983) seedlings in a greenhouse study showed a positive response to

N, P, and S, but in the field, established mature plants did not respond to fertilization with N, P, and S, although associated understory plants did. Tiedeman's work did not examine the influence of fertilization on nodule formation.

Strategies for Plant Excavation

Techniques—Standard, nonstructured digging usually results in holes that are roughly conical shaped with the flat surface of the cone represented by the original surface of the soil. This geometry is a result reflective of enthusiastic excavation early and at the surface where digging is easy. This activity wanes with time as the digger's energy reserves dissipate, and earthy substrates become more consolidated.

Our experiences have taught us to be somewhat and sometimes opportunistic when seeking observations of the belowground plant parts, especially of deeply rooted species like mountain mahogany and antelope bitterbrush. Cuts and trenches made by digging machinery can be excellent places to observe roots of these plant species. Often these can be accessed opportunistically when new roads or pipelines are being constructed. It is possible and often desirable to then work from the surface, progressing down the face of the cut, excavating specific intervals of the substrate sequentially to the depth of the roots or to the depth of the trench or cut. For mature mountain mahogany and antelope bitterbrush it is often convenient to stake off a distinct zone where the shrub stem is roughly in the middle of the zone. We often use a full square meter designation, where one side of the square follows the edge of the cut or trench.

As excavation proceeds, it is usually necessary to remove the aboveground portion of the plant. Some excavation can be done with the plant in place, but this can be awkward. An alternative is to divide the surface meter square into half-meter or quarter-meter sectors and excavate those to depth. In such cases, the aboveground plant parts can often be left in place for part of the excavation.

We have generally found it expeditious to stake out the area to be trenched and proceed as rapidly as possible with the trenching. Certainly root observations can be made, but it is also an option to ignore observations and extend the trench to the length, depth, and width desirable. One should be sure that the trench face, which will become the working and access plane to the root system of the target plant, is sufficiently far from the target so that root disturbance of that individual is minimized. Having a level indicator for such an excavation is of value to be sure the functional side of the trench is vertical.

Before the careful excavation of the root system begins, one should decide at what intervals soils should be removed. It is often desirable to remove the soil by pedogenic horizon. Sometimes this does not make sense if such horizons are very thin, and at other times it may be desirable to subdivide a particularly thick horizon. Intervals of 20 cm are often convenient and seem to represent a scale that fits with the size of these organisms.

In working with the first or top interval of the profile, one can work from the surface of the exposed face back into substratum to the extent of the predetermined boundaries of the excavation. As this is done, photographs or maps of roots and associated phenomena can be made. Nodules can be observed and collected or tested (for example, acetylene or ^{15}N incubations). It is best to completely remove a defined volume of soil before moving to the next. It is usually best also to sieve that material to catch missed roots and nodules using a box sieve of usually 60 by 60 cm construction with sieve openings of 1.25 cm. The substratum can be placed on the sieve and agitated to pass through. Roots and nodules will be caught on the sieve and recovered. The ease and success of such an operation is very much connected with the integrity of the substratum. Sand or gravelly material is often easy to excavate and passes the sieve quickly and efficiently. Decomposing granites can also work fairly well and can be usually easily crushed to pass the sieve. High clay content materials often require washing to remove roots as do materials containing silts.

Usually as an excavation progresses to deeper depths, coarse fragments tend to increase. This is especially true in soils inhabited by mountain mahogany and antelope bitterbrush. Very coarse fragments are often very common especially in subsoils. Further, roots often do not abate when they encounter consolidated rock. Roots may penetrate cracks, and we have often come across nodules in cracks. Roots may extend a meter or more into such materials. Nodules may be found wherever roots go.

Examination of Soil Taxa Associated With Nodulated Shrub Communities—Soils identified along the trench that intersected the mountain mahogany community were Typic Haplustolls, loamy-skeletal, frigid under both the grassland community adjacent to mountain mahogany and under the mountain mahogany community. The soil solum under both vegetation types consisted of noncalcareous A and B horizons containing approximately 15 percent course fragments overlying calcareous, skeletal C horizons. The soils in this association were more highly developed under the mountain mahogany than observed in other locations where mountain mahogany was examined. The major consideration at this site was a thin laminar petrocalcic horizon at the surface of the skeletal layer in the soil under the grassland community. The petrocalcic layer prevented root penetration into the lower, high course fragment containing C horizon. Under the mountain mahogany stand such a restrictive layer was not present, and roots penetrated to a considerable depth (to more than a meter). These soils developed in limestone colluvium.

In many cases, mountain mahogany grows in very small lenses of soil in cracks between rocks. From a taxonomic standpoint these materials are essentially unconsolidated rock. In the Rogers Canyon site it was noted that none of the mountain mahogany whose roots were exposed in the trench had nodules in the A horizon (the zone of highest organic matter content); however, numerous plants whose root systems extended well below this horizon were nodulated in subsoil horizons below the A horizon. These horizons were very low in soil organic matter (less than 0.5 percent). In other sites where plants were growing essentially in contact with consolidated rock, nodules were seldom found; however, complete excavation of the root systems of these plants was usually not possible.

In the antelope bitterbrush transect, the soils under the adjacent sagebrush grassland mixture were Typic Argiustolls, fine-loamy, on upland sites grading into Cumulic Haploustolls, fine-loamy, on sites (drainages) where there

was transport of soil material onto the site. Bitterbrush occupied Typic Torriorthents, loamy-skeletal, on the footslopes grading into Lithic Torriorthents, loamy-skeletal, and eventually to rock on steeper sites.

Antelope bitterbrush, as is usually the case with mountain mahogany, occupied sites of shallower rocky soil than associated vegetation (sagebrush and grasses). No nodules were found where plant roots were in contact with high organic matter soils (the A horizon). Nodules were found in subsoils below the A horizon, although even these on this site were sparse.

Nodulation Characteristics and Nitrogen Fixation Activity

Actinorhizal Plants During Winter Months—Nodules encountered during the 1988 winter season had visual signs (white tips, elastic and turgid) that suggested they were active. However, all plants displayed necrotic nodules (dark, shrunken, and brittle). Even the 1-year-old plant had necrotic nodules (0.08 g) associated with active nodules (0.05 g). The 5-year-old plants also had necrotic nodules (an average of 0.13 g per plant) as well as active nodules (average of 0.12 g dry weights 70 °C for 48 hours).

Ambient soil temperatures in the field and of the incubation chambers were just barely above the freezing point of water (about 2 °C). Acetylene reduction assay indicated that all nodules were inactive, even though some gave visual appearances of activity. Ethylene produced by these nodules was indistinguishable from background levels. Implications here are that soil temperatures were too cold and plants too inactive to provide photosynthate to accommodate nitrogen fixation. However, an argument could be made from the appearance of some nodules that they were poised to rapidly begin nitrogen fixation once conditions or temperature and photosynthate availability came more into functional ranges.

Biological Assay of Soil Materials for Nodulation Capacity—Essentially the sites examined came from two completely different parent materials. Sites 1, 2, and 3 were on igneous and metamorphic rocks in the western part of the county, whereas sites 4, 5, 6, and 7 were on sedimentary rocks in the northern part of the county (table 1). The sites were separated by approximately 40 m of elevation change and 51 km of lateral change. The soils were Haplustolls and Calciustolls on the upland, frigid sites (1, 2, and 3) and Haplustolls on the lower elevation, mesic sites (4, 5, and 6). Site 7 was a Torripsamment (Stevenson 2001).

Seedlings were susceptible to damping-off fungi. Although soil material was dispersed between two pots for each horizon tested, and a total of five seedlings were planted to each pot, approximately 80 percent of the plants were lost, although at least one plant survived in soil from each horizon tested (table 1). Plants harvested at the end of the 10-month period were observed for a variety of microbial manifestations on root systems, including the presence of nodules.

Roots were removed from the rooting media and stained using an acid fuchsin technique (Williams 1979). Ecto-mycorrhizal and endomycorrhizal (arbuscular fungi) were observed on all root systems. Infection density (mantled, swollen root tips for ectomycorrhizae and arbuscules, vesicles and characteristic hyphae for endomycorrhizae) was variable, ranging from light to very high in all horizons from sites 1 through 6. Infection was unrelated to horizon or depth below the surface. Mycorrhizal infections in roots exposed to the soil material collected at site 7, where mountain mahogany was absent from the flora, were present but the density was consistently light.

Very small nodules were observed on bioassay roots exposed to subsoils from sites 1, 2, and 4. No other nodules were observed.

After 10 months, most bioassay plants appeared stunted and had very low fresh-weight biomasses (less than 0.5 g). Large plants (greater than 1.0 g) were present, but were always in soil material derived from A horizons or A/C horizons. Some of these plants were more than 5.0 g fresh weight. Nodules were never observed, however, on any plants growing in A or A/C horizon materials. All nodules were on plants growing in subsoil material derived from C horizons. However, these nodulated plants were all small plants.

The data from table 1 suggests that there were factors present in the A horizons and sometimes the A/C horizons of most soils examined that allowed mountain mahogany to develop substantial growth. It is likely that these factors were higher soil fertility associated with higher soil organic matter in these horizons. However, sometimes a soil horizon tested would generate a large plant and a stunted plant (for example, site 1, horizon A generated plants of 5.64 and 0.39 g; see table 1). Genetic variability among plants could also account for these differences. Plants on materials derived from C horizons almost always were very small, although a few plants exceeded 0.5 grams and one plant exceeded 1.0 g in size. Table 1 shows these average differences; however, it does not show deviations from averages, many of which were as large as the mean.

Table 1 also suggests that whatever factor was the causative agent of nodule formation was found only in subsurface horizons (C material), but was inconsistent in those horizons or was incompatible with some test plants. It would be consistent with established theory that plants that became nodulated would be large and robust. Here we suspect that nodulated plants had not had sufficient time to respond to nodulation, or that the nodules were ineffective. Another season of growth may have resolved this issue, but this would have required that nodulated plants be identified as nodulated, a process that can only be done destructively.

Discussion

The work reported herein was conducted over several decades and focused on acquiring field data on nodulation of two Rosaceous shrubs, mountain mahogany, and antelope bitterbrush.

From field observations, it is hypothesized that soil properties influence nodule initiation on both plants. Available nitrogen, here as nitrate, and soil organic matter determine nodulation in both plants. Although critical levels of nitrogen and soil organic matter were not identified, relatively high levels of nitrate nitrogen (approximately 3 ug/g) and soil organic matter (2 to 4 percent) inhibited nodulation, whereas low levels of nitrate (less than 1 ug/g) and soil organic matter (less than 1 percent) were conducive to nodule formation. It is

Table 1—Sites, locations, soil descriptions, and results of bioassay for mountain mahogany soils from Laramie County, Wyoming. Soil material from each horizon was planted with mountain mahogany seedlings that were examined after 10 months of growth. A sand and perlite mixture (8) was planted as a control (see text).

Site	Horizon	Depth	Surviving plants	Nodulation[a]	Mycorrhizae[b]	Average plant fresh weight[c]
		- - - cm - - -				*- - - - - g - - - - -*
1. On calcareous igneous colluvium (41°11'5" N.; 105°10'48" W.; 2,128 m, 15 percent north slope). Native vegetation: *Achnatherum hymenoides*, *Yucca glauca*, *Cercocarpus montanus* (nodulated extensively).	A	0 to 8	2	None	Variable	3.01
	AC	8 to 30	2	None	From light	.21
	C1	30 to 61	2	Yes	to very	.08
	C2	61 to 91	3	Yes	high	.84
2. On mixed calcareous alluvium (41°11'5" N.; and 105°10'48"W., 2,134 m, 3 percent southwest slope). Native vegetation: *Agropyron* spp. and *C. montanus* (nodulated)	A	0 to 20	2	None	Variable	4.56
	Bw	20 to 46	1	None	From light	1.21
	C1	46 to 102	1	Yes	to very	.06
	C2	102 to 152	2	None	high	.40
3. On red sandstone over white sandstone (41°11'5" N.; 105°10'48" W., 2,164 m, 20 percent west slope). Native vegetation: *C. montanus* (no nodules). Cr is hard, white sandstone.	A	0 to 8	2	None	Variable	1.59
	AC	8 to 30	3	None	From light	.08
	C	30 to 56	2	None	to very	.05
	Cr	56+			high	
4. On very fine, noncalcareous sandstone (41°26'44" N., 104°40'22" W., 1,740 m, 20 percent north slope). Native vegetation: *Yucca glauca*, *Eriogonium* spp., *Phlox* spp., and nodulated *C. montanus*.	A	0 to 15	2	None	Variable	.73
	AC	15 to 51	2	None	From light	.25
	C1	51 to 122	1	Yes	to very	.41
	C2	122 to 183	1	None	high	.31
5. On semicalcareous sandstone (41°26'44" N., 04°40'22" W., 1,749 m, 30 percent west slope). Native vegetation: *Yucca glauca*, *Phlox* spp., and nodulated *C. montanus*. Cr is semi-calcareous sandstone .	A	0 to 8	2	None	Variable	.28
	AC	8 to 28	2	None	From light to very high	.42
6. On white sandstone (41°26'44" N.; 104°40'22" W., 1,750 m, 15 percent north slope). Native vegetation: *Bouteloua gracilis*, *Ribes* spp., *Gutierrezia* spp., and nodulated *C. montanus*. Cr is hard, calcareous sandstone.	A	0 to 8	2	None	Variable	.38
	AC	8 to 66	1	None	From	.24
	C	66 to 102	1	None	light to	.39
	Cr	102+			very high	
7. On slightly calcareous sandstone (41°26'44" N.; 104°40'22" W., 1,747 m, 10 percent southeast slope). Native vegetation: mainly *Bouteloua gracilis* and *Yucca glauca*. No *C. montanus*. Cr is hard, calcareous sandstone.	A	0 to 15	2	None	Light	.39
	AC	25 to 30	2	None	Infection	1.37
	C	30 to 122	1	None	only	.07
	Cr	122+				
8. Sand; perlite mixture.	Control		2	None	No infection	.06

[a] None, no nodules. Yes, nodules on at least one plant.
[b] Ectomycorrhizae and endomycorrhizae were observed. Data not shown.
[a] Fresh weight of whole plants.

quite likely that the inhibitory effects of soil organic matter and nitrate are closely related. Formation of nodules and supplying them with photosynthate require considerable energy inputs from the plant. It is likely that when sufficient available nitrogen is present that feedback mechanisms in the plant block nodule formation. The presence of soil organic matter, which can be viewed as a form of potential labile nitrogen, may also block nodule formation. It has also been demonstrated that phenolic compounds, which are common components of soil organic matter, often inhibit activity of *Frankia*, the microbial causative agents of nodulation (Perradin and others 1983).

Excavation of these nodulated Rosaceous plants in the field needs to be done with care and attention to detail. Strategies for excavation should involve allowing sufficient time and input of energy into the excavation process, and excavation of mature plants may take even weeks to accomplish.

Often recording accurate morphological data of roots and nodules in the field can be done with some accuracy even if the plant is being destroyed. Certainly the value of such data is less with time as roots die, turgidity is lessened, and colors are muted. However, to claim that a plant is responding normally under such extreme conditions of stress is unjustified. Certainly, physiological data such as nitrogen fixation activity, root uptake function, or photosynthesis cannot be assumed to occur at normal rates while the plant is being stressed during excavation. However, what roots do in soil and what they do in unconsolidated or consolidated rock are likely different. Nodules often and perhaps most likely on some plants (for example, those of this report) occur very deep below the soil surface. Mycorrhizal associations may also occur very deeply in substrates. This poses a dilemma of how to observe belowground biological phenomena in field settings so that physical, chemical, and biological characteristics can be taken that are undistorted by the stress imposed on the biological system. Conversely, if a plant is minimally sampled to minimize or even eliminate stress on that plant, then there is uncertainty that the physical, chemical, or biological observations being made are accurate.

Nodules observed and acetylene reduction made of bitterbrush during the winter months indicated that although nodules gave visual appearance of activity, physiologically they were not active in nitrogen fixation. Given the time of year (winter), cold soil temperatures, and absence of photosynthetic organs on the plant tops (leaves), it is not surprising that the physiological function of the nodules was nil. However, given that excavation of the plant was not a normal condition of these plants, did excavation-induced stress mask the physiological phenomena? Given the low temperatures and shortened day length, it seems unlikely that physiological response was being masked by plant stress. A case might be made that plant physiological response may be more unfettered by excavation-induced stress during these winter months than during any other time of the year.

Evidence from the study where mountain mahogany was grown in soil materials derived from pedogenic horizons complements the work done and reported above, and suggests that soil properties (nitrate and soil organic matter) inhibit nodulation. The work corroborates the observation that mountain mahogany does not nodulate when in the presence of soil materials having high organic matter. However, it provides a little evidence that active factor(s) that cause nodulation reside within subsurface horizons very low in soil organic matter. Members of the genus *Frankia* are very slow growing (Callaham and others 1978; Stowers 1987). The fact that they would exist deep in soils, under low carbon regimes and in zones of low microbial activity and competition, is consistent with what we know about their growth habit in controlled cultures. However, the results of this part of the study really suggest only a hypothesis, not currently adequately challenged: *Frankia*, responsible as the microbial agent in nodulation of Rosaceous shrubs, are active only in subsoils low in carbon and nitrogen.

A principle that has emerged from this particular work is that there is a question that physiological measurements represent normal plant response when the plant is being stressed by the sampling process. Simultaneously, there is a question that an adequate sample has been collected, especially of root parameters, if the stress due to sampling is minimized or eliminated. Paradoxically, accurate measurements belowground that are reflective of a normal plant, cannot be made because the plant must be stressed to make such measurements. If the relationship between carbon fixation and nitrogen fixation can be determined, then monitoring carbon fixation during the excavation of the plant can, at least theoretically, provide a means to correct nitrogen fixation levels.

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Germination and Seedling Establishment

Ipomopsis spicata ssp. *robruthii*

Illustration on the reverse side is by Walt Fertig. In: Wyoming Natural Diversity Database. 2000. Available at: http://www.uwyo.edu/wyndd (Accessed 12/18/03).