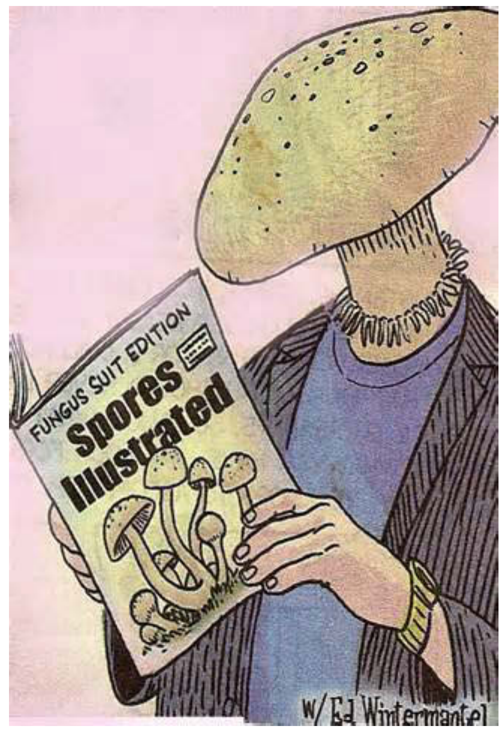
A FUNGUS AMONG US.

DATING IN THE DARK: SOME MICROBIAL ASSOCIATES OF NATIVE PLANTS

OR

DOES YOUR PLANTING NEED A FIX; N & P THAT IS?

Revised 13 July 2013
Dədicated to all those konsultants who kant spel mycorhizal or innoculant. May palletized mycorhizal innoculants fall on your head.



Most multicellular plant life on earth is symbiotic with soil microorganisms. There is great diversity in the populations of rhizosphere microorganisms. Mycorrhizae, endophytes, rhizobia, & actinorhizae are largely mutualistic, although the line between mutualism & parasitism is fine. Beneficial, or mutualistic microorganisms increase the host plant's tolerance to drought, toxic metals, disease, heat, herbivory, &/or promote growth & nutrient acquisition. The influence of rhizosphere microorganisms on plant growth & competitive ability is considerable & the interaction between roots & microorganisms result in positive & negative impacts on plant productivity.

TYPES OF SYMBIOSES

SPECIES 2 \ SPECIES 1		+	0	-
	+	Mutualism	Commensalism	Parasitism
	0	Commensalism	Neutralism	Amensalism
	-	Parasitism	Amensalism	Antagonism

Symbiosis is currently used to refer to only mutualistic associations, where both the host & symbiont benefit.

The restoration of native plant communities occurs on sites that are degraded or deeply disturbed.

MYCORRHIZAL FUNGI

DEFINE MYCORRHIZA

Mycorrhiza, plural *mycorrhizae,* mycorrhizas, mycorrhiza. n. Literally mushroom-root, from post-classical Latin & scientific Latin *myco-*, from ancient Greek μύκης*, mykes,* mushroom, fungus; & post-classical Latin & scientific Latin *rhizo-,* & its etymon ancient Greek ῥιζο-, *rhizo,* combining form of ῥίζα, *rhiza*, root, probably ultimately from the same Indo-European base as root & wort; after German *Mycorhiza* (AB Frank 1885, in *Ber. der Deutsch. Bot. Ges.* **3** 129; now *Mykorrhiza*) (oed).

Mycorrhizae are symbiotic fungi that exist as thread-like hyphae in the soil that grow upon or within the roots of a healthy host plant & extend a network into the soil. The hyphae gather water, phosphorus, & nitrogen for the host & receive carbon (sugar) from the host. The hyphal net may extend to another mycorrhiza or another host. The net allows native plants to share water & nutrients. (The name is properly used to indicate the symbiotic state, not the fungi or its spores. The following usage needs corrected!!)

Not to minimize the importance of mycorrhizae, but it is commonly miss-stated that 95 percent of the world's plant species (or 90% of plants) are mycorrhizal, but the truth is "about 95 percent of the world's present species of vascular plants belong to families that are characteristically mycorrhizal…." (Trappe 1977). A more recent survey of mycorrhizal research found that about tested taxa, 80% & 92% of the species & families are mycorrhizal. (44) (Yet many authors agree almost all native plants are mycorrhizal.)

Almost all individual plant species have not been adequately examined as hosts. Many species have been studied only as a very few individuals, or from a limited number of localities. The data on many families often represents data from a single species of that family. Experience with some extensively studied species has shown "that the more a usually mycorrhizal species is examined, the more it will be found to occasionally lack mycorrhizae. Similarly, the more a nonmycorrhizal species … is studied, the more it will be found to occasionally form mycorrhizae" (Trappe 1987).

Summary of Mycorrhizal Status of 3617 species & 263 families (44)

Group	% Myco Species	% Myco Families
Angiosperms	85	94
Gymnosperms	all, most obligate	
Pteridophytes	52	93
Bryophytes	46	71

Mycorrhizae are thought to have been vital in enhancing the primitive root systems of the first land plants. Mycorrhizae predate the evolution of roots & originally colonized the above- & below-ground plant axes, with AM having been the first "roots". Chemical signaling systems evolved in order for plants & fungi to recognize suitable partners. Arbuscles of 400 million year old AM are known from the Lower Devonian Rhynie chert (Remy et al 1994). Glomeromycetes spores & hyphae have been found in 460 million year old Ordovician fossils. The ancestral type was glomeromycetous mycorrhizae (originally called zygomycotous) & ascomycetous & basidiomycetous mycorrhizae belong to the derived types. Glomeromycetous fungi have relatively low host specificity, while ascomycetous & basidiomycetous mycorrhizal fungi have much higher host specificity. Land plants evolved from obligate mycorrhizal to facultatively mycorrhizal & some finally to nonmycorrhizal. (Trappe 1987) A species is considered as obligately mycorrhizal if it is always found with mycorrhiza, while a species is considered as being facultatively mycorrhizal if it is found to form mycorrhizae in one habitat but not in another (44). Nonmycorrhizal plants are defined as those plants that normally exclude mycorrhizal fungi from their young, healthy roots (4.4). Mycorrhizal fungi may also invade older, non-receptive roots of a host & live as an endophyte.

Benefits/services

Buffering effect against abiotic stresses, including increased plant resistance to drought, salinity, heavy metals, pollution, & mineral nutrient depletion.

Increased water, P, & N uptake. Promote plant growth while reducing fertilizer requirement

Increases root system by 100X, with up to 2000X surface area.

Larger & more active root systems increase fertilizer efficiency & plant resistance to high salts, drought, & pH.

Develops a root/soil community of beneficial bacteria, nematodes, springtails, earthworms, &c., & inhibits herbivores & pathogens.

The improved overall health of roots & plant renders diseases less damaging. Increased plant resistance against biotic stresses while reducing phytochemical input, disease, nematodes. Protecting against root pathogens

Stabilizing soil aggregates, in preventing erosion, & in alleviating plant stress caused by biotic & abiotic factors (Smith & Read 2008).

Increase plant/soil adherence & soil stability (binding action & improvement of soil structure) Secretion of 'glomalin' into the soil. Improved soil structure. Increased soil stability & water retention.

Modification of plant metabolism & physiology. Bioregulation of plant development & increase in plant quality for human health.

Mycorrhizae Types

Types of Mycorrhizae Along Two Structural Gradients (after Allen 1991)

The two types that occur on the most economically important crops are endomycorrhizae & ectomycorrhizae. The other minor types are intermediate in structure.

No penetration of cortical cells		Enclosed Root
↓	Ectomycorrhizae (ECM)	↑
↓	E-strain	↑
↓	Arbutoid (ABM)	↑
↓	Ectendomycorrhizae (EEM)	↑
↓	Ericoid (ERM)	↑
↓	Monotropoid (MTM)	↑
↓	Orchid (ORM)	↑
↓	Endomycorrhizae	
↓	Vesicular-Arbuscular (AM)	↑
Penetration of Cortical cells		↑ Open Root

Endomycorrhizae (AM) are the predominant types & are the ancestral types of mycorrhizae in land plants. Endomycorrhizae occur in the majority of vascular land plants & in early diverging bryophytes suggesting mycorrhizae originated with the first land plants, & AM symbiosis is the normal, original state for land plants. (AM, VA, or VAM?)

The only genes thus far discovered controlling mycorrhizal symbioses are all for AM development. One gene has been found in mosses & other early land plants (Wang & Qiu unpublished data). There might be a general genetic program in plants controlling plant-fungal interaction that was likely established at the beginning of land plant evolution (Harrison 1999). This program has been inherited by most? land plants & is responsible for the predominance of AM symbiosis. Most herbaceous plants & some temperate trees used in restoration are AM.

Ectomycorrhizae.

Ectomycorrhizae (ECM) occur on a variety of unrelated plants, indicating ECM evolved from AM several times through parallel evolution. (*Pinaceae, Orchidaceae, Nyctaginaceae, Polygonaceae, Ericaceae, Aquifoliaceae, Salicaceae, Fagaceae, Betulaceae, Cistaceae, Dipterocarpaceae, Myrtaceae, & Melastomataceae*.) ECM probably originated with the rise of the *Fagales* & *Pinaceae* in the Cretaceous. Land plants & ECM then coevolved contributing to their mutual diversity. ECM plants dominate some landscapes especially coniferous & deciduous forests. Homobadisiomycetous fungi? (Wang & Qiu 2006) Mycoheterotrophy & the nonmycorrhizal condition also evolved many times in land plants through parallel evolution. List types derived from ECM.

Hartig Net, growth between the cells

ECM plants also arose in rosid branch of the eudicots, which also includes many AM plant families. Plant families that associate with N-fixing actinorhizal nodules are also concentrated in the rosids (of which all except one genus are also ECM). Native oaks & pines are ECM.

Landscape products include ECM *Lacarria laccata, Pisolithis tinctorius, Rhizopogon amylpogon, R. fulvigleba, R. rubescens, R. villosuli, Scleroderma cepa*, & *S. citrinum* (Bio Organics).

ERICACEOUS MYCORRHIZAE.

Ericaceous mycorrhizae are ascomycetous or basidiomycetous fungi. Arbutoid (ABM), Monotropoid (MTM), & Ericoid (ERM) are probably derived from ECM (Brundett 2002 in (44)). (ABM, MTM, & ERM occur in *Ericaceae*, but ERM also occur with *Diapensiaceae,* a close relative of *Ericaceae*.) Ericaceous mycorrhizae have many traits in common with ECM, but display a high degree of intracellular penetration. Ectendomycorrhizae (EEM) are probably a transitional stage between ECM & the ericaceous mycorrhizae as ECM & EEM are found only in the basal lineages of *Ericaceae, Monotropoideae,* & *Arbutoideae.* (EEM is also found in *Pinaceae, Araucariaceae, Ericaceae, Salicaceae, Fabaceae, Betulaceae,* & *Cistaceae*.) (Wang & Qiu 2006)

"One interesting phenomenon is that the fungal strains isolated from these three types of mycorrhizas (ABM, MTM, & ERM) can also form ECM with other plant species (reviewed by Smith & Read 1997). This observation strongly suggests a close relationship between ECM & the ericaceous mycorrhizas. An interesting question that arises is why the same fungi cannot penetrate into root cells of ectomycorrhizal hosts yet are able to do so with ericaceous plants. One answer could be that host plants play an important role in controlling development of different types of mycorrhizas. " (Wang & Qiu 2006) (As the host does in determining the shape of rhizobial nodules.)

ORCHID MYCORRHIZAE.

Orchid mycorrhizae (ORM) are mostly basidiomycetous fungi & with few exceptions, are restricted to *Orchidaceae.* A very similar type of mycorrhiza is known from subterranean nonphotosynthetic liverwort *Cryptothallus mirabilis* (Ligrone et al 1993; Bidartondo et al 2003, in 44) & the monocot *Thismia* sp. (4.5 in 44) ORM is likely to be a highly specialized ECM. Some species of *Epidendroidea*, an early diverging lineage in *Orchidaceae* have ECM. (*Thismia americana,* known only from natural areas in the early 1900's in the south side of Chicago, is a subterranean, non-photosynthetic plant (verify photosynthetic status).)

There is no evidence the ORM fungus receive any benefit from the orchid, & represent a mycoheterotrophic relationship.

MYCOHETEROTROPHY THE EARTH BEGAN TO COOL, THE AUTOTROPHS BEGAN TO DROOL.

Mycoheterotrophy (MTH) is the process by which plants, either wholly or partially achlorophyllous, sustain a heterotrophic metabolism via a fungal partner that provides a source of carbon & mineral nutrients (Smith & Read 2008). MHT plants are considered parasites of their fungal hosts, which are often, but not always, associated with the roots of a fully autotrophic plant. The associated plant is the carbon source while the mycorrhizae provide mineral nutrients.

MTHs are often obligate MTH, with stunted root systems, degenerated vascular tissue, little or no photosynthetic ability, & dust-like seeds with no food reserves. MHT are often but not exclusively associated with patches of ectomycorrhizal plants. Seedlings attach to an existing net, Mixotrophy.

MTH evolved independently many times from AM, ECM, & ECM-derived types of mycorrhizae. The fungi include basidiomycetous & ascomycetous ECM, & *Glomeromycota* arbuscular mycorrhizae. MTH plants are very host specific. They associate with both saprotrophic fungi which obtain carbon from dead plant matter & mycorrhizae that obtain carbon from host-plant photosynthate (Leake 2005).

MTH plants include liverworts, ferns, & angiosperm monocots (*Orchidales*), & dicots (*Monotropideae*). Orchids are MTH for part of their life cycles during establishment, but then most, but not all become photosynthetic & join in mutualistic symbiosis with mycorrhizae.

MTH plants are known from the following families: *Aneuraceae, Podocarpaceae, Petrosaviaceae, Burmanniaceae, Triuridiaceae, Corsiaceae, Orchidaceae, Ericaceae (Monotropoideae), Gentianaceae,* & *Polygalaceae*.

AM, VAM, & VA?

Arbuscular mycorrhizal fungi (AM) or vesicular-arbuscular mycorrhizal fungi (VAM or VA). All endomycorrhizae form arbuscles, but not all form vesicles, hence the AM vs VAM. AM is the current acceptable verbiage. Exploitative endomycorrhizae colonizing *Gentianaceae* do not form arbuscles, so are they called VM?

In AM, the fungal hyphae penetrate the cell wall & often form arbuscles (branched root structures inside cells) & some also form vesicles (oil storage organs). In ECM, the hyphae form an intercellular meshwork in the root epidermis & cortex (a Hartig net), & a sheath around the root develops from this net. (Parallel inter- & intracellular penetrations exist with legumes & rhizobia. *Vide infra* ca pp 16-17.) AM are not visible to the naked eye, & colonized root are not discernable. (*Spores may approach visibility*?)

Angiosperms evolved in the early Cretaceous. The AM state is predominate in angiosperms & its near ubiquity is evidence that AM was the original state. 82% of modern angiosperms are AM. (Trappe 1987, Harley & Harley 1987) In most natural ecosystems, most dominant plants are mycorrhizal.

AM fungi are members of the zygomycete order *Glomales* in the genera *Glomus, Acaulospora, Scutellospora, Gigaspora, Paraglomus*, & *Archaeospora* (Morton & Redecker 2001).

AM fungi primitive due to: 1) relatively simple spores, 2) lack of sexual reproduction, 3) relative few species, & 4) association with wide diversity of plants. Difficult to define individuals & species.

AM are incapable of growth without plants

Glomus intraradices is a naturally occurring, widely distributed species that is the constituent or a main constituent of commercial inoculants. Other commercial species include *Glomus clarum, G. deserticola, G. etunicatum, G. microaggregatum,* & *G. mosseae* (Musselman personal communication). Others found for sale include *G. aggregatum, G. monosporus, Gigaspora margarita,* & *Paraglomus brasilianum.*

NON-MYCORRHIZAL PLANTS (NM)

Most are herbaceous, generally are most abundant in harsh plant habitats, such as extremely wet, saline, or arid soils. NM plants are thought to have evolved about 100 million years ago.

Predominantly NM plant families include the *Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Cyperaceae, Juncaceae*, & *Polygonaceae*. Many aquatic plants have lost their AM due to root reduction & habitat factors. Some families have AM species that either never completely lost the capacity for mycorrhizal formation, or they have re-acquired it. (Brundett 2002)

SPECIFICITY & SIGNALING.

As early land plants & fungi joined to form mycorrhizae, chemical-signaling systems evolved in order for plants & fungi to recognize suitable partners.

AM fungi are attracted to young roots by soluble or volatile exudates such as secondary metabolites like flavonoids. The initial penetration takes place in a zone where the exodermis is developing where the fungi may be attracted by phenolics involved in suberin synthesis. Initial colonization is similar with roots lacking an exodermis & do not produce these chemical signals. (AM may be descended from saprophytic fungi & inherited their ability to chemically breakdown plant cell walls.)

NEED SNAPPY TITLE HERE

Prairie restoration & native landscaping are primarily concerned with endomycorrhizae, or vesicular arbuscular mycorrhizae (AM). (*Establishing native trees & shrubs will need both endo- & ectomycorrhizal inoculants*) AM hyphae are analogous to the "roots" of a mushroom, but they do not develop above ground fruiting bodies like mushrooms. They form large spores underground. (*Ectomycorrhiza form mushrooms & puffballs.)* This type of fungus has a mutualistic relationship with much of our herbaceous native flora. The fungi increase the volume of soil available to the root system for the nutrient & water uptake, by one estimate a 700% increase in soil volume. In return, up to 20% of the photosynthate is used by the mycorrhizae. (*There can be over 100 meters of hyphae per cubic centimeter of soil (Parniske 2008*), *or 30 - 50 meters per gram, aka 8.46 - 14.1 miles per pound (Cavagnaro et al 2005, Allen 1991).)* They may also protect the roots from certain diseases,

In an unfragmented landscape, AM are present in most undisturbed (*in situ*) soils. In disturbed soils, including most chemo-dependent, Corn Belt soils, mycorrhizae may be absent or present in low enough numbers that plant growth is limited. (*AM in some corn fields have been known to respond to continual fertilization by becoming parasitic on corn roots*. (Source this)) AM fungi survive in the soil as resting spores. When active, they receive all their food from host plant roots. It is not likely they receive much, if any, organic nutrients from the soil. They do not exist as free-living, independent organisms. (National Academy of Sciences 1979) AM cannot be cultured without the host.

AM have very little host specificity. Some strains may function better in certain soil types than others. In nature, AM thrive in grasslands, deserts, & tropical forests, especially in soils low in organic matter & P.

The restoration industry's awareness & utilization of AM is approximately where commercial prairie restoration was 45 years ago. Our hearts are in the right place, but our heads are where the sun don't shine. And out of the sun is where properly installed inoculants should be!

Describe the inoculant product.

The spores of AM are highly resistant & can live away from host roots for many years. The product has at least a 2-year shelf life.

Types of propagules? Spores, vesicles, & root segments. Propagule per unit?

Hydroseeding minimum inocula per X times 1000 gallons. Caltrans sponsored study suggests 30 lbs per 3000 gallons minimum.

Compost is commonly applied in lieu of topsoil in urban areas where production is abundant at landfills. Compost may have mycorrhizae if the mix contained plant roots. Other composts may have high salt content or high nutrient content that inhibits mycorrhizal growth. Some mushroom compost may be high in fungicides & insecticides that also inhibit mycorrhizae. (*The good old buy network said let the mushroom compost age (or leach) a year before using it.*)

Mycorrhizal inoculants are an important tool for restoration. Mycorrhizae are helpful on extreme sites, such as road cuts, fresh construction, landfills, & sterile "urban topsoil" that has been stockpiled to death. Mycorrhizae are absolutely necessary for some native species. Some plant species are so dependent on mycorrhizae, they starve without them. A good AM population is undoubtedly the key to Gentians in Dr. Betz's stages of prairie establishment.

However, mycorrhizae are not the "ecological pixie dust" some claim. Some common C4 prairie grasses are said to be obligate mycorrhizal, meaning they will not prosper, flower, fruit, or live long without mycorrhizae (or fertilizer). Nevertheless, millions of seeded acres & millions of greenhouse plants of these species have been produced without any inoculants. Some of these necessary endomycorrhizae are apparently ubiquitous, at some density in all but the worst soils.

In an un-fragmented, non-developed, rural landscape, mycorrhizae move readily by animals, wind, & water, but not so in urban areas dominated by roofs, pavements, & toxic turf deserts. The AM spores are large & not readily blown around, but they are blown around with dust & dirt during wind erosion events. (VA mycorrhizae have less effective means of long-distance dispersal than other types of mycorrhiza (Trappe 1987).) The more fragmented & disturbed the landscape, the greater becomes the need to inoculate. The longer the soil has been stockpiled, the greater the need to inoculate. The more urgently beneficial plant growth is needed, as in erosion control, the greater the need to inoculate.

As a suggested rule of thumb, the living, *in situ,* agricultural soils west & south of the Chicago collar counties may not absolutely require mycorrhizal inocula, but their use is still recommended. From World War II through the 1960s, agriculture mechanized & artificial fertilizers came into use. Old fashion small grains, legume forage, & corn crop rotation fell out of practice to the extent most soils have had only corn & soybeans, or just corn grown for decades, via chemo-dependent management systems. Few farms are managed to maintain healthy soil microbes. High rates of fertilizers inhibit mycorrhizae. Some beneficial fungi may be present, but they are in densities too low & at distances too far to be effective or to allow the formation of mycorrhizae in reasonable time. The longer agricultural or turf monocultures are grown, the more depauperate the mycorrhizae become, in numbers & diversity. Continuous cornfields are begging to be inoculated.

Nevertheless, a chemically & toxin assaulted agricultural soil does respond & benefit from inoculation. The cost of adding a seed box inoculant at this stage is literally chump change. Disturbed, reconstructed soils should always receive seed box treatments at 4X-5X the manufacturer's recommended rate. Inoculants are not effective in & should not be used in the restoration of perpetually saturated soil communities.

Mycorrhizal inoculants are needed whenever the natural inocula is not present in adequate numbers to provide timely benefits. Soils that have been fumigated always require inoculation. Road cuts, mine sites, land fills, highly eroded soils, saline or alkaline soils, nutrient poor & droughty soils, & rebuilt construction site soils always need mycorrhizal inoculation. Plants grown in soilless medium should also be inoculated.

The 3-5 year prairie development from seed & the depauperate mycorrhizae corollary.

Native plant species are mycorrhizal, many obligate.

The mycorrhizal spores in ag &/or urban soils are too few & far between to colonize native seedlings.

In 3-5 years, mycorrhizal populations have recovered enough to host a prairie, as soil structure returns. Conservative species begin.

FROM A TYPICAL SET OF NATIVE SEEDING SPECIFICATIONS.

"Mycorrhizal inoculant shall be palletized (sic) & mixed with the fine seeds before installation. The inoculant shall contain the fungal species *Glomus intraradices* in palletized (sic) form. (1.5 lb per acre)." Why would anyone hire a consultant who does not know a pellet from a pallet? Why would anyone distribute job specifications with glaring errors? Pellet/pallet aside, pelleted inoculants are made for & mixed with large seeded species or chemically coated seeds, like lima beans & peanuts, or fungicide coated soybeans. Pelleted inoculants are not used with or mixed with fine seeds! Duh! "Palletized" inoculants are mixed with great big, humongous seeds & placed in the great big, humongous seed box on the great big, humongous seed drill. Nonetheless, this specification is better written than most.

Alternately, many job specifications are written requiring 50-60 pounds of inoculant per acre, with a material & installation cost potentially rivaling & exceeding the seed cost (up to $60 per pound). (*The consultant making the recommendation usually sells the inoculant, or their wholly-owned subsidiary sister company right across the hall that sells &/or installs the product*.) There is seldom a material specification adequately defining the inoculant & almost never a prescribed method of installing the inoculant. Careful shopping will reveal economical products that do not require an extra trip across the field, such as the "pelletized" seed box treatment in the above paragraph.

INSTALLING THE PRODUCT

Enough product must be applied to allow fungus propagules to come into direct contact with roots. Multiple colonization sites are best to establish mycorrhizal benefits as soon as possible.

Incorporation? Necessary placement immediately adjacent to the root.

Do not apply mycorrhizae with fungicides. Soil fumigants will kill mycorrhizae.

Existing stands of plants are difficult to colonize. Inoculum suspensions may be injected or applied as soil drench into the root system.

MYCORRHIZAL INOCULANT SOURCES

AgriEnergy Resources. http://agrienergy.net/ 21417 1950E Street, Princeton, Illinois 61356, 815.872.1190, refer to Myco Seed Treat, http://www.agrienergy.net/docs/renewablelabels/label-10-mst-042210.pdf or their current equivalent. Ask for Ken Musselman. info@agrienergy.net .

AgBio Inc., http://www.agbio-inc.com/ 9915 Raleigh Street, Westminster, Colorado, 877.268.2020. Refer to AgBio-Endos, http://www.agbio-inc.com/agbio-endos.html or their current equivalent. agbio@agbio-inc.com

ConservaSeed, Rio Vista, California. Endonet.

Mycorrhizal Applications Inc. (MAI) http://www.mycorrhizae.com/ PO Box 1029, Grants pass, OR 97528, physical address 810 NW E ST, Grants Pass, OR 97526. Toll Free 866.476.7800, Phone 541.476.3985, fax 541.476.1581 Biogrow.

Reforestation Technologies International (RTI) 800.RTI.GROW, 831.424.1494, Salinas , California. AM 120.

Rocky Mountain Bio Products http://www.rockymtnbioproducts.com/index.htm 10801 E. 54th Avenue, Denver Colorado. 303.696.8964. 888.696.8960. Fax 303.696.0620. info@rockymtnbioproducts.com

"MYCO SEED TREAT (MST) is formulated to be used as a dry seed treatment. It should be thoroughly mixed with the seed at planting time at a rate of 8 ounces per 100# of seed on small seeds (such as clover & grass seed) & 4 ounces per 100# of seed on larger seeds (such as corn & soybeans). It is available year round. It can be applied spring, summer or fall. It would not be ideal for dormant seeding, as we want actively growing roots present within a short time of being applied in the soil. The mycorrhizal organisms present in MST are *Glomus mosseae, Glomus intraradices, Glomus etunicatum, Glomus clarum, Glomus deserticola,* & *Glomus microaggregatum."*

Current pricing as of April 30th, 2013

1# pouch	$41.00 each	treats 200-400 pounds of seed
5# bucket	$33.40 per #	treats 1000-2000 pounds of seed
10# bucket	$29.30 per #	treats 2000-8000 pounds of seed
30# bucket	$27.90 per #	treats 6000-12000 pounds of seed

(Ken Musselman, AgriEnergy Resources, personal communication)

THE MOOCHES

Mycorrhizae are essential to establishing hemiparasitic forbs, including *Comandra* & the many hemispheric species of the recently redefined *Orobanchaceae.* (*Many were part of a broadly defined Scrophulariaceae*.) These plants must be established with or near a known host or with known associates. (See C12 Snapdragons in UPURC on the website or DVD.) The connection between host & hemiparasite is through adventitious roots working with mycorrhizal fungi to form *haustoria*. Orchids depend on mycorrhizal symbionts (in at least one stage of their development), as do saprophytic forbs.

Mycorrhizae are also crucial in "keystone species" such as *Pedicularis canadensis* reducing the dominance of C4 grasses in diverse remnants.

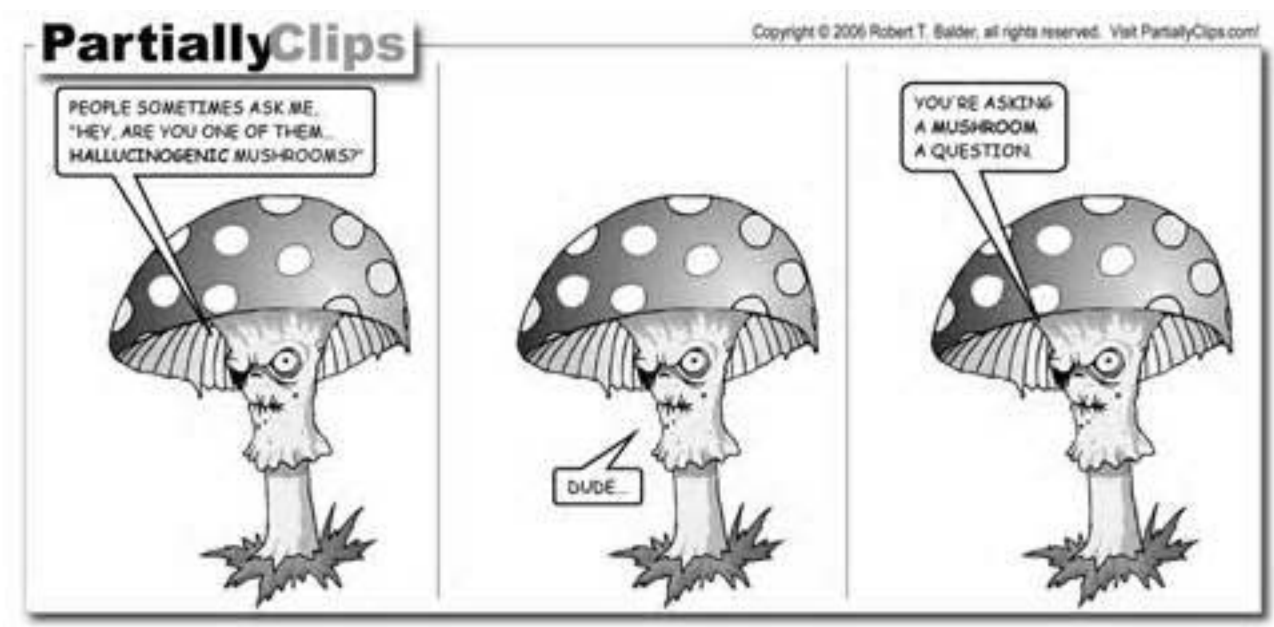
TRIUNE.

To establish a healthy legume population, legumes must be inoculated with both rhizobia & mycorrhiza, which work in concert. The increased P uptake from the mycorrhiza helps the increased assimilation of N fixation in rhizobial root nodules, especially in low phosphate soils (Smith & Read 1997)

Virtually all rhizobial legumes & actinorhizal plants are mycorrhizal, predominately endomycorrhizal. (*Lupines are the only known legume genus to be naturally amycorrhizal (Sprent 2001).*) (Actinorhizal species are predominately woody plants & are ectomycorrhizal, except *Ceanothus* which is AM.) All herbaceous legumes are V A mycorrhizal, but leguminous trees are predominately VA-mycorrhizal, & occasionally ectomycorrhizal, & may lack rhizobium nodules. Potentially, a leguminous tree may be "infected" with rhizobia & ecto- & endomycorrhizal fungi (& an endophyte?). Some species of *Caesalpinioidea* may be predominately ectomycorrhizal (Meyer 1973, Redhead 1980). (*Gleditsia*, *Gymnocladus* (& *Cercis*?) in the *Caesalpinioidea* do not nodulate, apparently relying on mycorrhizal uptake of N.)

More arbuscules & nodules form at 64.4ºF (18°C) compared with 71.6ºF (22°C). Few nodules form at 71.6ºF (22°C), & almost no nodules form at 78.8ºF (26°C). (Saito et al 2007 & Kanamori et al 2006 in Parniske 2008) Get monthly statewide soil temps from State climatologist's office. *Potential Critical Impact upon seeding dates here, dude.*

ASIDE FOR MORE THOUGHT. *Alliaria petiolata,* GARLIC MUSTARD, destroys mycorrhizae (or mycorrhizal DNA) (source). Savanna & woodland restoration seedings in areas where this species has been growing must be reinforced by properly installed endo- & ectomycorrhizal inoculants. Cf comment under leghemoglobin in Legume section.



ENDOPHYTES

COMING SOON! FUNGAL ENDOPHYTES! BUT WAIT… IF YOU CALL NOW, WE WILL DOUBLE YOUR ORDER FOR FREE!

MOVE THIS SECTION TO THE END OF FRANKIA

"All plants in natural ecosystems are thought to be symbiotic with mycorrhizal &/or endophytic fungi (Petrini 1996; Brundrett 2006)" (Rodriguez & Redman 2008).

Endophytes live entirely within the host tissue & emerge during host senescence.

Mostly *Ascomycetes* & some *Basidiomycetes.*

Endophytes can be subdivided into four classes based on host range, colonization pattern, transmission, & ecological function.

Endophytic benefits to hosts include tolerance to herbivory, heat, salt, disease, & drought, & increased below- & above-ground biomass.

Mycorrhizal fungi may invade older, non-receptive roots & live as an endophyte.

ENDOPHYTIC TALL FESCUE & NATIVE GRASS CONVERSION.

WARM SEASON GRASS NOTES

Back to top.

A mushroom goes into a bar & sits down to order a drink. The bartender walks over & says, "I'm sorry sir, but we don't serve your kind here."

The mushroom sits back & asks, "Why not? I'm a fun guy (fungi)!"

Symbiotic Diazotrophs

Legumes, Rhizobial Bacteria, & Inoculation

- Define Legumes.
    - The Alternative.
  - When you're number 2, you try harder.
  - The Value of Inoculated Legumes
    - Caveats.
    - Sidebar. The Noble Dandelion.
- Inoculants
  - The need For Inoculants.
  - Ground Rules.
  - Specificity & The Inoculation/Joining Process.
    - Sidebar. Hypothetically Speaking.
  - infection threads
  - The Good, the Bad, & the Ugly.
- Rhizobia Sources
- Role of legumes in prairies & restorations.
  - Restorations.
  - Erosion Control.
- Inoculating the seed.
  - Hulling the seed.
    - sidebar. Hulled or Unhulled.
  - Pretreatments.
    - Scarification.
    - Stratification.
    - sidebar. Hard or Dormant?
    - sidebar. The Hull You Say!
  - Applying powder on the seed & planting the seeds.
    - Premixing Additives.
    - Sticker Solution Method/Homemade Stickers.
    - Extenders/Homemade Extenders.
  - Mixing.
- Planting Season.
    - Dormant Seeding.
- Planting.
  - Drilling.
  - Hydroseeding.
- Alternate Strategies of Establishing a Healthy Rhizobia Population.
- Development, Timing & Evaluation.
- Why Did It Fail?
- Rescue/Supplemental Inoculation.
- POST-PLANTING Management.
- Introduced Alien Legumes.
- In the best of all possible worlds (with apolpgies to Voltaire or Leonard Bernstein; highly optimistic yet undone specs):

DEFINE LEGUMES.

Rhizobium plural *rhizobia* n. From scientific Latin *Rhizobium*, genus name (B. Frank 1889, in *Ber. d. Deutsch. Bot. Ges.* 8 338) post-classical Latin & scientific Latin *rhizo-,* & its etymon ancient Greek ῥιζο-, *rhizo,* combining form of ῥίζα, *rhiza*, root, probably ultimately from the same Indo-European base as root & wort; & *-bium,* post-classical Latin *bio-*, from ancient Greek βίο-, *bio-,* combining form of βίος, *bios*, life, course or way of living, as distinct from ζωή, *zoe,* 'animal life, organic life'. "Any bacterium of the genus *Rhizobium* (family *Rhizobiaceae*), comprising aerobic, Gram-negative, typically rod-shaped bacteria which invade the root hairs of leguminous plants & induce the formation of root nodules, in which the bacteria live as symbionts & fix atmospheric nitrogen, making it utilizable by the host plant; (in form *Rhizobium*) the genus itself. Also (loosely): any bacterium that performs a similar function." (oed)

The composition of prairie classically begins with the *mirepoix* of grasses, composites, & legumes. The *Leguminosae (Fabaceae)* is a family with three subfamilies, or is treated as three closely related families, as in *Caesalpiniaceae, Papilionaceae,* & *Mimosaceae,* or *Caesalpinioideae, Papilionoideae* (*Faboidea*), & *Mimosoideae*. It is a cosmopolitan family of trees, shrubs, lianas, vines, & herbs of about 730 (650) genera & 20,000 (15,000, 18,000, or 19,000) species, worldwide; in North America (FNA 2013 forth coming) & Illinois (46 genera & 131 species). Legumes are the 3rd largest family of Angiosperms, following composites & orchids. Legumes are second only to grasses in the production of food.

Common elements of legumes: pods (or loment), hard coated, long-lived seeds, mostly pinnate leaves, 5-merous flowers, & symbiosis with nitrogen fixing bacteria, hence tissues high in N, seeds high in protein from the N; endo- & ectomycorrhizae.

Nutritional importance. Legumes are rich in high quality protein, high in phosphorus & calcium, & a good source of vitamins, especially A & D. Nutritious & economical forage & browse. Legume hay averages almost 2x the protein of grass hay. Secondary metabolites. Legumes are also some of the most poisonous plants in the world. In prairie, A legume plant is a high N, high protein tidbit in a low protein (grass) menu. Fore gut (cow) versus hindgut (horse) processors. Secondary metabolites, *Astragalus, Oxytropis,* but a little loco is better than dead.

Nutritious seeds & fruits (fleshy "fruit" of *Astragalus crassicarpus* & related spp.). Woody but internally sweet pods of *Gleditsia* & *Gymnocladus.*

Pollinators, nectar, larval hosts, & arthropod production & game bird chicks. Many species are important nectar sources for honey production & other insects. Some, such as *Chamaecrista fasciculata,* have extrafloral nectaries at the base of leaf stalks that produce nectar before the plant flowers.

Leguminous trees are important sources of firewood in some areas of the world. (Botanical name) MESQUITE of the American Southwest, produces beautiful hardwood furniture. Native Americans used *Robinia pseudoacacia* as a bow wood. *Gymnocladus* seeds were used as counting pieces for gaming by Native Americans. Many species have medicinal properties, with some currently sold in herbal preparations.

Most leguminous plants have a symbiotic relationship with nodule-forming, nitrogen-fixing bacteria called rhizobia. Rhizobia are microscopic, single-celled bacterial organisms. Legumes cannot fix atmospheric N_2 without rhizobia. Rhizobia cannot fix N_2 unless they are inside a legume root nodule (*stem nodules also exist*). Some rhizobia have threadlike flagella that allow them to propel themselves in water films in soil & plant roots. Rhizobia move one-sixteenth to one-quarter of an inch per year.

Rhizobia are living vegetative cells. Unlike mycorrhizal fungi, they do not form spores. They must have food (carbon), mineral nutrients, oxygen, & water, a temperature of 59º-86ºF (15º-30ºC), & pH 6.0-7.5. Excess heat, desiccation, acidic or alkaline soils, toxins in fertilizer, fungicides, & insecticides, or heavy metals kill rhizobia. (*pH tolerances vary with rhizobia strains*.) Inoculated legume seeds are a perishable product, somewhat like milk & egg salad sandwiches. A quart of milk & a couple of egg salad sandwiches left on the dash of your pickup on a 95º day aren't very good for much, neither are inoculants or inoculated legume seeds.

Nodules are rare on the *Senna* family, more common on the *Mimosa* family, & very common on the bean family. In northern Illinois, the *Mimosa* group, or *Mimosoideae,* are rare native plants, ie, *Desmanthus* & *Schrankia.* The *Senna* group, or *Caesalpinioidea* are more common, & the most common & numerous are the bean group or *Faboidea*.

The first species, *Rhizobium leguminosarum,* was identified 1889. All species were originally classified as *Rhizobium,* but they have been split into *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium,* & *Sinorhizobium*, 6 (7, 12) genera & 38-40, or 50 (90 Drew et al 2012) species. They are generally referred to as *Rhizobia* or rhizobia for convenience. 10 new rhizobia species are discovered every year.

Estimates range that 10% of species to "most" of the legume species are nodulating. These rhizobial bacteria convert atmospheric dinitrogen gas (N_2) into ammonia (NH_3), which is converted into amino acids & amines usable by the legume plants. In turn, the plant provides shelter, water, & carbon-compound nutrients (principally dicarboxylic acids malate & succinate) to the bacteria.

Ironically, the air we breath is almost 80% N_2, but available N is second only to water as the limiting factor in all plant-based ecosystems. Atmospheric nitrogen, N_2, is chemically very stable. The N_2 molecules are bound by three electrical charges that are quite difficult to break.

THE ALTERNATIVE.

Manufactured nitrogen fertilizer is the alternative to legume-based N. Manufactured N is what fueled the Green Revolution. But, we are running out of it.

The manufacturing process of artificial N fertilizer requires very high temperatures & pressures & considerable hydrocarbon energy input.

N fertilizers are manufactured from natural gas. One ton of anhydrous ammonium requires 33,500 cubic feet of methane. The Claude-Haber process, using a metal catalyst, combines atmospheric N & methane at 900ºF & 200-1000 (500) atmospheres pressure to produce anhydrous ammonium. Hydrocarbon fuels are also the energy source used to reach this high temperature & pressure. Urea & ammonium nitrate are manufactured from anhydrous ammonium in further energy demanding processes.

The N_2 molecule is held together by three shared electrons, which, as noted above, requires tremendous energy to break apart. In nature, these electrochemical bonds are dramatically broken by lightening, providing small amounts of biologically available N during thunderstorms. In legumes, the meek & timid enzyme nitrogenase breaks these N_2 bonds combining each N atom with 3 H, creating ammonia ($NH_3 + H^+ \rightarrow NH_4^+$), or alternately converting ammonia into amino acids & amines, forms usable by plants, all at about 70ºF & one atmosphere of pressure, & all in your own back yard or garden.

The formula for the reaction in nitrogen-fixing bacteria is: $N_2 + 8H^+ + 8e^- \rightarrow 2\ NH_3 + H_2$.

WHEN YOU'RE NUMBER 2, YOU TRY HARDER (AVIS). Next to photosynthesis, nitrogen fixation is the second most important plant process in the world. Next to water, available soil nitrogen is the most growth-limiting factor. Legumes are key to both.

Rhizobia can live in the soil in the absence of their host, as saprophytes feeding on dead & decaying organic matter. This trait is known as "saprophytic competence" & is exemplified by rhizobia of annuals such as *Chamaecrista* & *Strophostyles.* Rhizobia can live on or near the rhizospheres of non-leguminous plants such as WINTER WHEAT (& CANADIAN RYE? check Graham). (*Nodules are relatively short lived (50-60 days determinate nodules) while indeterminate nodules may also senesce at the end of the growing season, & both break down releasing their nutrients & rhizobia to form a new generation of nodules at the first flush of growth in spring? So in essence, even perennial legumes have cyclical, seasonal partners, where rhizobia survive part time as free-living soil organisms.*) In the continued absence of a host, their population progressively declines, & the strains tend to mutate to non-beneficial strains. (Herried 2013, Graham year?, Drew et al 2012)

Types of nodules, longevity of types, life spans vs latitude, arctic species perennial?.

In native ecosystems & agricultural production, N is the limiting factor of plant growth. Synthetic N fertilizers feed half the people on earth but they are derived from natural gas. They are grossly inefficient & environmentally detrimental. As fossil fuels increase in cost & scarcity, the importance of low cost, biological nitrogen becomes more important every day.

As we create native landscapes & restorations, we must attempt to foster plant communities that persisted for millennia with no inputs & no loss of vigor or diversity. Prairies were low N systems, with a rapid turn around cycle of N_2 from atmosphere to legume to soil to plant to fire to atmosphere. (*Prairies also received useable N during lightening storms.)* Nitrogen was the limiting growth factor. Native legumes & rhizobial bacteria provided the limiting nutrient that allowed the tremendous annually biomass production of the tallgrass prairie.

(*Analogy to tropical rainforest destruction, prairie was North America's single greatest ecosystem loss. Unsequestering of millions of tons of carbon from soil organic matter & the cessation of annual above-ground carbon growth-cycles dumped in the atmosphere at the end of the Little Ice Age?*) (Studies show that the organic matter in Saskatchewan prairie soils supplied the N needs of crops for 30 years as the N was released from the break down of humus. "When the prairie sod was broken the amount of nitrogen released during a fallow period was more than enough to grow a crop. The straw could even have been removed for use elsewhere & enough nitrogen would have been produced from just the humus to satisfy an average crop." (C.A. Campbell & W. Souster, Can. J. Soil Sci. Volume 62:651 (1982) in http://www.agriculture.gov.sk.ca/Default.aspx?DN=4b50acd7-fb26-49a9-a31c-829f38598d7e

THE VALUE OF INOCULATED LEGUMES in agriculture, landscaping, erosion control, & restoration.

Inoculated, agricultural legumes fix 20-300 (416) lbs N per acre per year. A species may produce from 50 to 90% of the N required for growth (*alfalfa up to 100%*). (*Agricultural legumes are planted at much higher rates than native legumes*.) Even with the removal of high N seeds or foliage, these functional legumes increase soil N as an economical & ecologically sound alternative to manufactured N fertilizers. With high levels of N, legumes provide nutritious, high protein forage for grazers & browsers & high protein seeds for humans & other animals.

100-200 species of legumes cultivated; in North America they are grain legumes, forage legumes, & erosion control species. There is a positive correlation between dry matter production & bacterial nitrogen fixing capacity. Biennial & perennial forage legumes fix more N than annual grain legumes.

30 days are required from seedling emergence to nodule formation & N_2-fixation. During this time, the developing ag crop will need 15 lb/ac. (*Apply 20-30 lb/ac away from the plants has been suggested.)* 35 lb/ac inhibits nodule formation with little fixation occurring above 55 lb/ac.

Rhizobial N-fixation is highly efficient. Fixation is directly related to carbohydrate flow from the leaves, therefore it is directly proportional to & fully synchronized with growth rate. Plants must expend considerable energy to bring manufactured N through cell membranes, with more energy required to change the N into a form that can be metabolized. Combined with leaching, denitrifying bacteria, &c., synthetic N fertilizer is only 20-50% efficient.

Perennial forage legumes provide greater benefits than annual legumes. They have larger, deep root systems, longer growth periods, & a greater capacity for N-fixation. Deep-rooted perennial legumes take up phosphorus from subsoil, eventually enriching the topsoil. The increased readily decomposable or "active" soil organic matter & microbial life also improves soil structure by aiding the formation of soil aggregates & forming more soil pores. The soil becomes more friable, less erosive, easier to till, & holds more water. Deep-rooted perennials such as alfalfa, will improve internal soil drainage.

The benefits of inoculation may not manifest in increased yields. The benefits may be evident only in increased N/protein levels of seeds & plant residues. High N/protein seeds & forage are valuable in livestock. The high N/protein stubble & high N soil levels are of value in a crop rotation.

Annual legumes in a rotation can significantly increase the yield of subsequent crops. Called the rotation effect, the benefits are attributed to improved physical, chemical, & biological characteristics of the soil, & the reduced duration & severity of diseases & insect pests. Annual legumes can also be used as green manures, yielding the greatest benefits from legume crops grown from inoculated seed.

CAVEATS.

Commercially available agricultural & erosion control legume seeds may be coated with fungicides or insecticides. Many of these chemicals can be toxic to rhizobia. Consult the pesticide label for compatibility information. Pesticide coated legume seeds should not be direct inoculated. A granular inoculant should be drilled into the seed furrow. If you cannot use a granular inoculant, use 2-4X the recommended rate of powdered inoculant. On particularly poor sites, 10 X the recommended amount of inoculant are not unprecedented.

Inoculants or inoculated seeds should not be exposed to fertilizer. The soil solution around fertilizer granule often has a low pH & high ion concentration that may kill the rhizobia, seed or seedling. If you must seed with a fertilizer, use 2-4X the recommended rate of powdered inoculant. (48)

In all non-agricultural contexts, there exists a necessity (or an obligation?) of designing stable, self-sustaining, plant communities that mimic the rudiments of natural systems.

Landscaping with minimalist lawns of grasses & legumes, with species-rich meadows where lawns are not required. Grass-legume lawns & roadsides are self-sustaining, fertilizer-free, & pollinator friendly (beware invasive legumes). Very few activities justify a turf monoculture. Nothing justifies fertilizers & pesticides on lawns. We have the opportunity to implement self-fertilizing lawns & roadside plantings if we get past our misguided sense of aesthetics & our self-congratulatory sense-of-accomplishment after spending 4 hours on a riding mower.

Your neighbor on his/her self-propelled mower is robbing your grandchild of OXYGEN. Leonard, grab your inhaler.

SIDEBAR. The noble dandelion is highly desirable in the lawn. When it is in bloom, it is the only time your lawn has any visual interest or aesthetic value. Dandelions are self-renewing, self-maintaining, & free as long as someone in the neighborhood mows like a fool. Dandelions provide valuable early season nectar & pollen for bees, & they are larval hosts for moths. The mature seeds provide valuable songbird food. The thick, fleshy, contractile root opens up compacted soils, brings deep nutrients to the surface where shallow-rooted plants may use them, adds deep soil organic matter as it decays, & when decayed, provides literal funnels for rain to penetrate deeply into the subsoil. The leaves are edible in salads or cooked & are high in vitamins & minerals, the flowers are the source of dandelion wine, & the ground, roasted root makes an *ersatz* coffee. The root was an ingredient of root beer. If you add a legume to the yard you have the grass, composite, legume triad of the prairie. &, the more you mow, the more you dandelions you have. Mowing promoted forbs. Dandelions are lawn forbs. Dandelions are proof positive mowing encourages forbs.

Native plantings & restorations are often planted in dead, rebuilt urban soils, stockpiled construction site soils, or farmed-to-death, worn-out agricultural soils that mandate the soil organisms must be restored. Erosion control plantings are also in poor soils or subsoils & receive zero to minimal input after final contract acceptance, that is, until slope failure. We must design these projects with rhizobia & mycorrhizae to be healthy, self-sustaining, self-sufficient, plant communities for the longest possible timespan, with a mix of fibrous & tap-rooted herbaceous species & fibrous & tap-rooted woodies. This includes the proper suite of native plants & their microbial complements to maintain adequate fertility levels. Nitrogen & phosphorus levels must foster a healthy microbial community.

Fancy plant spreadsheets do not stop erosion. Plant lists don't stop erosion. Fancy coefficients do not stop erosion. Plants selected for the right reasons stop erosion.

INOCULANTS

THE NEED FOR INOCULANTS. TEENAGE MUTANT NINJA RHIZOBES.

Prior to the breakup of the Tallgrass Prairie, native legume species distributions, with their soil microbes, were continuous & contiguous. Species & genetics moved like a population of amoebae across the landscape, each with their own agenda. Rhizobia got around, even at 0.06" to 0.25" per year. But, what's time to a bacterium? With 99.9% of Illinois prairies destroyed, native legumes & soil microbes are bound to remnants & well-implemented restorations. When the native plants were destroyed, the rhizobia populations plummeted without a proper host. The rhizobial remnant populations no longer move. Native legumes have not grown in northern Illinois soils since the 1830s. They must be restored along with the plant species.

Many soils contain resident rhizobia, especially agricultural soils where a legume has been grown within the past few years. They are called indigenous, endemic, or "native" rhizobia regardless of their origin (though some times "native" means "native"). The existing rhizobia are typically present in numbers too low to effectively nodulate a crop or the strains produce ineffective nodules. Within 5 years, the beneficial pea & bean rhizobia population declines by 100 fold, but pea rhizobia have been found in pea fields that had not had an inoculated crop in

25 years (Vessey 2003). Unless the same legume crop grew in a field in the last 2-3 years, you must inoculate. Even when adequate levels of rhizobia are to be expected, benefits will occur one-third to one-half of the time. Feeling lucky?

When rhizobia grow without a host they become free-living saprophytes instead of symbionts, & they become "weedy". Soil rhizobial populations have a high degree of genetic diversity & often lack genetic similarity to the original symbiotic strain. Those with better saprophytic traits & poor nodulation abilities prosper, while the symbiotic strains incapable of extended saprophytic existence dwindle. The genetic changes in soil rhizobia result in populations that are inferior at N-fixation, & that produce lower yields in crops compared to commercial inoculants. (Vessey 2003) Genetic instability & mutation are also significant obstacles in the development of a marketable rhizobial strain.

Ground Rules Better Section Title Needed

Agricultural inoculants are available commonly as powder & granular (pelleted), with liquid & frozen concentrates being available for special purposes. Granular inoculants are used with large seeded or fragile-seeded legumes (peanuts) or chemical- or pesticide-coated seeds & are 2-3 times the cost of powders, but they flow through planters & are more desiccation resistant. Some other formulations have stickers or extenders added. Granular inoculants are designed for application in the furrow with the seed. Agricultural legumes may be coated with chemicals or pesticides that are usually toxic to rhizobia. Chemically-coated or pesticide-coated seeds must be used with granular or pelleted inoculant. Native legume strains are limited to powder-based products, with special strains custom-cultured.

Powdered inoculants are peat-based, black or occasionally brown, & contain approximately 200,000,000 to 1,000,000,000 rhizobia per gram. Inoculants must be stored in a cool area away from desiccation or heat, preferably refrigerated. *Rhizobia* will die if exposed to air, heat, light, or if dried out (below 35% relative humidity?).

An inoculant package should be labeled with the type of legume, the quantity of seed treatable (or acreage to be covered), & the expiration date. Not all inoculants are packaged for legal resale, nor can a bag be legally be divided & resold. Most nurseries provide inocula with seed at no additional cost, or offer preinoculated seed. If you are buying an inoculant that is not in the manufacturer's original packaging, the transaction may bend a law or two. Nevertheless, it would be a great injustice to restoration if this service were discontinued.

Cross-Inoculation Groups.

Specificity & The Inoculation/Joining Process. Legume hosts have typical relationships with rhizobial symbionts. Non-beneficial rhizobia may be detrimental to the host & act as a parasite (parasitism). A non-beneficial rhizobia may be of little impact to the host (commensalism). Or, a strain may nodulate & fix N_2, with the legume host & rhizobial symbiont may both benefiting (mutualism). Legumes do not produce nodules except in the presence of rhizobia.

The inoculant species must match the legume species or not. A rhizobia species may successfully inoculate more than one legume species. It is also possible that more than one species of rhizobia will beneficially colonize a legume species. One strain is quite promiscuous & is known to nodulate over 100 species. A strain may also nodulate a legume but not fix N.

There is also evidence there is some regional variations in host-symbionts preferences. Wide-ranging species of legumes will have several symbionts across its range. For example, a variety of peas originating in Afghanistan will have different rhizobia than a strain that originated in the Caucasus Mountains (find source, confirm locations). N_2 fixation may also vary with the symbiont genotype (Drew et al 2013).

After the properly inoculated seeds are properly planted, the rhizobia feed on organic matter & multiply greatly in the soil. As the legume hosts germinates & develops root hairs, the host & symbiont communicate, & the bacteria start to invade.

Rhizobia & host recognize each other by chemical signals. As the legume seed germinates or the perennial plant begins its spring flush of growth, root hairs form, *& if, & only if,* the plant detects low N supply in the soil, the root exudes a flavonoid. The flavonoid activates the nodulation gene in the rhizobia. The rhizobia respond with the Nod Factor, or lipo-chitooligosaccharide (or lco or ???). Nodulation can then begin.

During the chemical signaling, there is a degree of selection. Legume species put out different types of flavonoids, with species of rhizobia responding differently to specific flavonoids. Different strains of rhizobia produce different Nod factors with minor chemical structures that distinguish which plants can be nodulated. (Graham 2004) Some rhizobia that are capable of joining more than one host can make several Nod factors (Schultze et al 1992).

Native prairie legumes form nodules by two methods. Some nodules are altered root hairs that have deformed & grown around rhizobia. This is the most common method, & is called intracellular infection. Rhizobia attach to a young root hair, which curls around the bacteria. The attachment area softens allowing up to 20 rhizobia to enter & move toward the root cortex. They move inside a plant-derived infection thread to modified cells in the root cortex. In the cortex, the rhizobia are released into modified cells & are surrounded by a plant-derived membrane, with nodule formation around the membrane. (Graham et al 2004)

The second method is called "crack entry", where the bacteria penetrate between cells through cracks produced by lateral root emergence. The rhizobia move inside a plant-derived infection thread. The rhizobial "infection" trigger cell division in the cortex of the root where the nodule appears. In both methods, the infection thread & membrane protect the rhizobia from the plant defense responses

The rhizobia in the nodules differentiate into bacteroids (find a real definition) &, with the aid of the enzyme nitrogenase as a catalyst, fix atmospheric nitrogen N_2, into ammonia, ($NH_3 + H^+ \rightarrow NH_4^+$), or alternately into amino acids & amines, forms usable by plants.

In spite the chemical screening processes, ineffective nodulations still occur. There are records of a legume plant being nodulated by over 10 different rhizobia strains (Drew et al 2013). There may be more than one rhizobia strain within a nodule. As the root hairs & root cracks soften, they allow up to 20 nearby rhizobia into the root. The 20 rhizobia may be from more than one strain.

HYPOTHETICALLY SPEAKING. Ineffective nodulations are quite common. Some hypotheses exist as to how the legume copes with parasitic symbionts. The hypotheses are very similar, & seem to say the same thing, but have been given different names by their authors. The PARTNER CHOICE HYPOTHESIS proposes plants use chemical signaling to eliminate ineffective rhizobia. Some plants such as soybeans, reduce the reproduction of ineffective rhizobia by reducing their oxygen supply (Kiers et al 2003). Lupines have also been shown to provide fewer resources to nodules containing inefficient rhizobia, limiting their reproduction (Simms et al 2006). The SANCTIONS HYPOTHESIS suggests the plants minimize resources allocated to inefficient rhizobia. Sanctions against nodules that fix less nitrogen might take the form of reduced nodule growth, early nodule death, decreased carbon supply to nodules, or reduced oxygen supply to nodules. (source this)

The legume plant can foster occupancy of its nodules by the more effective strains from within the rhizobial community. Plant may also increase number of nodules to compensate for inefficiency. (Drew et al 2013)

INFECTION THREADS

THE GOOD, THE BAD, & THE UGLY. Green, green-white, white, tan, or brown nodules are ineffective nodules that are not fixing N. Actively N-fixing nodules are bright pink or beefsteak colored when exposed to air, from leghemoglobin. (*Both hemoglobin & leghemoglobin are used in the literature.)* Leghemoglobin is similar to hemoglobin in blood carrying oxygen to help regulate the nitrogenase molecules. Brownish-pink nodules are senescing, formerly-productive nodules. Green nodules are dying, formerly-productive nodules, with the green color from a breakdown product of leghemoglobin. White nodules lack leghemoglobin & never contributed to the common good, & as total parasites would make good administrators. (*Alliaria* screws up hemoglobin in animals; does it do so to legumes? Cf *Alliaria* effect on mycorrhizae.)

RHIZOBIA SOURCES

Becker Underwood Inc. 1.800.892.2013 Native inocula are available where they overlap with agricultural inocula (such as EL, H). They also offer a single blend said to work for all prairie legumes. Check for granular/pelleted.

INTX Microbials, LLC, 200 West Seymor St., Kentland, Indiana, 47951, 219.474.5510, fax 219.474.3700. Pleasant, easy to work with, good service.

Nitragin Inc. 1.800.558.1003 (They prefer to sell to mass marketers, & have forgotten their long standing relationship with the little guy in the 1970-80s (see Joe Burton 1970).)

Native legume inoculants have a very low market share, low profit margin, are often custom cultured, & have suffered from loss of some lab strains over time, & the loss of at least some corporate altruism. Many inoculant manufacturers are unlikely to provide adequate research needed to provide high quality inoculants for native legumes. Like horseshoes, rolle-bolle, bocce ball, hand grenades, & thermo-nuclear warfare, close is good enough.

The special strains are generally custom-cultured. Allow 2 or more weeks when ordering them.

ROLE OF LEGUMES IN PRAIRIES & RESTORATIONS.

Legumes & other similar nitrogen fixing plants are a critical part of the grassland nitrogen cycle, providing a constantly renewing source of N. Midwestern native grasslands are typically low soil nitrogen systems. Our atmosphere is 79% N, but it is chemically unavailable to plants. N available for plant growth comes from decomposing soil organic matter, thunderstorm rains, free-living N-fixing soil bacteria, &c. Plants utilize the N from the soil, & when grasslands burn, the N in the burning duff is released into the atmosphere, & soon, a low N soil level steady state is maintained. Native legumes with productive rhizobial nodules in this environment have a competitive edge in the low N prairie & they flourish.

Prairie legumes are idealized as magnificent, living chemical factories that selflessly provide the whole system with free nitrogen. Normally, an individual legume produces less N than it consumes, let alone munificently dishing out three squares a day for its neighbors. Legumes prefer to use available soil N, as nodulation is an expensive process for the plant. The legumes' N fixation is inversely proportional to the level of soil N. In remnant prairies, the soil N levels were low, with N production high. In planted prairies, soil N levels are usually high enough (30 total lbs per acre) to inhibit nodulation & N fixation. Legumes are favored by N shortages. Planting native legumes on rich, former agricultural soils will result in limited nodulation & N fixation. Shading of legumes by tall grasses will also diminish N fixation.

Nevertheless, the symbiotic nitrogen becomes available to neighboring plants in due time. Most prairie legumes are herbaceous & die back to the ground each fall. Shrubby species behave as herbs when burned or grazed. Native prairie herbaceous plants typically have 30% of their root systems senesce & regenerate each year. As the legumes' leaves, stems, roots, & nodules decompose, the organic nitrogen is released into the soil & is mineralized into forms available for uptake by other plants. (Legume leaves have a low carbon to nitrogen (C:N) ratio & will quickly degrade in the fall.)

Nodules on perennial legumes form in the spring with the first flush of growth. Legumes will have more than one generation of nodules per year. Nodules have a lifespan of 50-60 days after formation. They senesce & decompose, releasing the rhizobia that become available for nodule formation. The N-rich nodule tissue decomposes, releasing the N into the soil. (Graham et al 2004, Herried 2013)

Add discussion of indeterminate nodules that grow throughout the season. Lobed, coralloid appearance, growing from a meristem-like region. (*Do indeterminate nodules live for a whole growing season versus determinate at 50-60 days*?) Base of an indeterminate nodule may be green, but the tip is pink & presumably fixing N_2. The legume determines the type of nodule, not the rhizobia.

In some cases, mycorrhizal connections between legumes & grasses allow the direct use of nitrogen by the grasses (Burton 1970). Erdmann (1967) notes there is some exchange of N in a mixed clover-grass field. Some exudation of N by roots has been noted (source?). During the growth of grain legumes, considerable amounts of nitrogen are leaked from roots into the soil (48).

RESTORATIONS. Inoculating the seeds of native legumes will give your seedlings a boost by providing some of their own nutrients, especially when planting in poor quality, low nutrient, constructed urban soils of commercial restoration. In rebuilt urban soils, it is imperative rhizobia be used in concert with mycorrhizae. At a minimum, specify that legumes be inoculated with both inoculants, if you do not inoculate the whole seed mix with mycorrhizae.

Taproots, deep soil organic matter, soil permeability, subsoil moisture, channels for future roots. In is the third or later year, native perennial legumes produce enough above- & below-ground biomass to initiate contributing to the nutrient needs of the planting.

Inoculation is also recommended when planting *in situ* agricultural soils. The prairies of northern Illinois were broken starting in the 1830s after the Blackhawk War. Native legumes have not grown in some of these soils in 180 years. It is important to re-establish the correct, beneficial rhizobia in these restoration sites. Old fashion crop rotation has fallen out of practice to the extent most soils have had only corn & soybeans grown for decades. Few farms are managed to maintain healthy soil microbes.

Agricultural soils with a history of soybeans, clover, alfalfa, &c, may have resident rhizobia already present. Once established, rhizobia will persist for several years between crops. Rhizobia populations may be high enough for 2 or 3 to 5 years that reinoculation for an agricultural legume is not needed (Herried 2013). These resident agricultural bacteria may aggressively colonize the newly seeded native legumes. The aggressive rhizobia may be present in numbers too low to optimally inoculate agricultural legumes, but in numbers high enough to be ineffective symbionts on native legumes. Established strains are usually more competitive than an introduced strain for inoculation sites on new plants. Alfalfa strains have been recorded moving into neighboring fields. (Linderman & Glover 2003) They may have hitchhiked on dirt clods on farm equipment, as rhizobia move 0.0625-0.25 inch a year in the soil (intxllc.com, Herried 2013). Suggest increasing normal inocula rate here.

High levels of soil N, even seasonally high, can inhibit nodulation & N fixation in existing nodules. If there are low levels of soil N left from corn or soybean crops, plant growth & photosynthesis may be enhanced, improving nodulation & N fixing. Inhibiting affects start when the N level in the top 12 inches of soil exceeds 30 lbs per acre (or soil N plus fertilizer N equals or exceeds 30 lbs per acre). The best results from legume inoculation are obtained on low to average fertility soils.

Soil acidity is damaging to the growth of legumes & also adversely effects rhizobial nodulation & N-fixation. At pH of 6 & lower, heavy metals such as aluminum & manganese can cause toxicity affecting legumes.

EROSION CONTROL. Legumes are useful as part of an erosion control seed mix but they must be paired with fibrous rooted grasses. Pure stands of legumes are not suited for erosion control. Most perennial species are taprooted which, in a pure stand, allows soil erosion to occur under the mat of legume surface vegetation. The severe erosion under stands of CROWN VETCH was a favorite topic of Corliss 'Jock" Ingels of the LaFayette Home Nursery. Legume leaves have a low carbon to nitrogen (C:N) ratio & will quickly degrade in the fall leaving less cover than grasses through the winter.

In compensating for low nutrient soils, complete fertilizers should never be allowed to come in direct contact with inoculated seeds. Phosphates are not as harmful as nitrogen or potassium (Erdmann 1967). Fertilizers must be applied in an operation separate from inoculated seeding.

Plant experts cite native legumes as inferior to introduced forage species for erosion control plantings, but that natives are definitely superior for conservation, wildlife, & pollinator plantings. (Any legume by itself is not suited for erosion control.) Native legumes are by comparison 1) more expensive, 2) slower to establish, 3) higher in hard seed requiring scarification, 4) more sensitive to proper inoculation, & 5) low to medium in nitrogen fixation capacity, & 6) compatible only with non-rhizomatous fine fescues, short warm season grasses or wild ryes. (Salon & Miller 2012) Some species are also subject to availability problems. Native legumes are seldom planted at the density of forage legumes, nor are they often planted in monocultures (ca 40,000+ plants per acre), nor has their performance been evaluated in these situations.

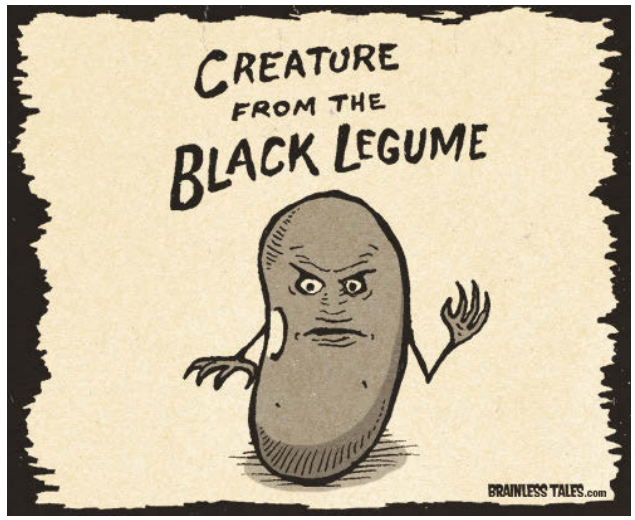
INOCULATING THE SEED.

HULLING. Seed of several genera of legumes are harvested with some floral remains intact. Some are called loments, as in *Desmodium, Hylodesmum,* or *Lespedeza*. Others are enclosed in floral parts as in *Amorpha* & *Dalea* (*Petalostemum*), or in small, single-seeded legumes such as *Psoralea (add new name equivalents),* & *Baptisia tinctoria*. These small legume seeds should be hulled, which results in higher seed counts, causes some scarification of the seed coat, & results in more reliable establishment in the greenhouse or field. The species with single-seeded pods should have the pods removed. Spring planted legumes should always be hulled.

PRETREATMENTS.

Hulled seeds of many native species may have additional impediments to germination. The seeds have protective adaptations that prevent germination at a time inhospitable to seedling development or that prevent the germination of an entire cohort at one time. These characteristics are valuable survival traits in a wild plant. These adaptations include seed coats that are impermeable to water vapor & other gases & embryos that are not fully developed.

Those of you that have bought properly tested & labeled legume seeds may have noticed legume viability is noted as germination plus hard seed, not dormant seed. When testing a legume seed, the dormant seed is considered hard seed & is listed separately as "hard seed "on the seed tag (Salon & Miller 2012). Any legume species seeds will be properly labeled as germination plus hard or dormant, but very, very seldom does a seed lab use hard & dormant in test results. In our 4300+ recorded seed tests, we have seen one lot so labeled.



SCARIFICATION.

Hard seeds are seeds that have seed coats that are largely impermeable to moisture, thus inhibiting germination. Hard seed coats are an evolutionary survival device designed to prevent the germination of an entire cohort at one time during a bad establishment year, with generational carryover in a short- to mid-term soil seed bank. Over time, weather, microorganisms, & soil chemicals weaken the seed coat & allow germination. Hard seed is also an advantage when germination can occur after the initial flush of weed pressure has passed. Hardseededness occurs in legumes, mallows, geraniums, smartweeds, &c.

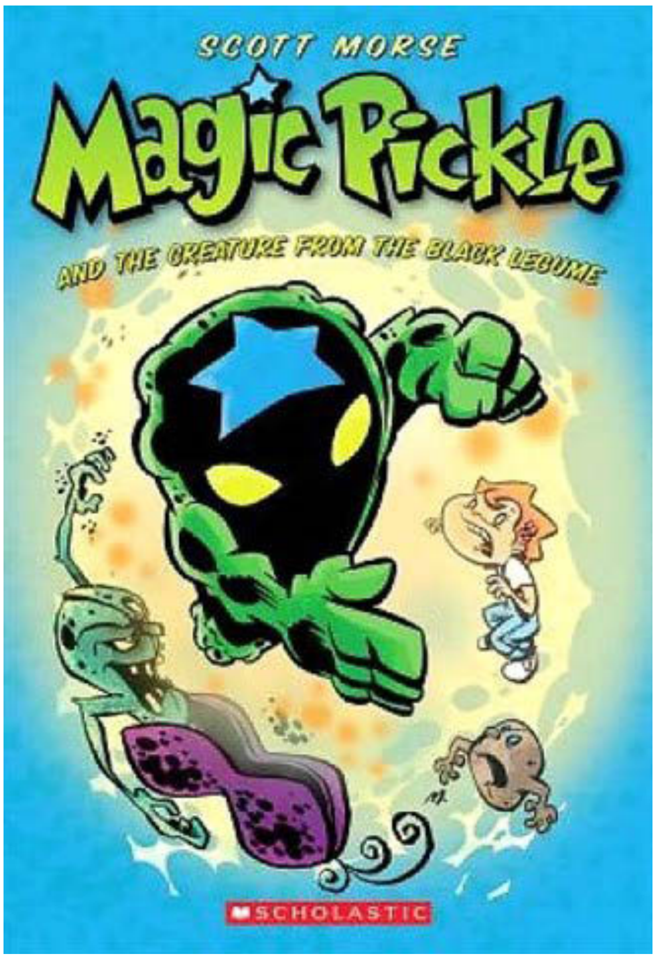
Commercial forage & turf legumes have had hardseededness bred to a minimum. Native legume seeds are still wild type where hardseededness is a desirable survival trait. Scarification may help overcome this impediment to establishment.

Hard seed coats may require scarification. Legumes that have had the pod or floral remains removed are generally effectively scarified during hulling. Dormant seeded legumes do not need scarification. Spring seeded legumes are best scarified. Small batches can be hand sanded by rubbing the seed between two pieces of sand paper until the seed coat is visually rough looking. Hot water treatment is also used to scarify hard seeds. Larger lots can be treated with a commercial scarifier, skillfully hammermilled, or acid scarified.

Some authorities may recommend avoiding legumes seed lots with excessive % of hard seeds. With limited production of most ecotypes of native species, this generally is not an option. It is also recommended that if the hard seed is 40-50% or more, the lot should be scarified. Scarified seed must be planted during the current growing season, as its viability will quickly diminish in storage. Dormant seeded legume seed should not be scarified, nor should legume seeds planted after August 15th.

STRATIFICATION.

Some species may also require cold moist stratification after hulling & scarification. Cold moist stratification allows ………………….. Seeds are mixed with a moist, sterile, inert medium such as sand or peat moss & placed in a refrigerator at 34-38ºF for 30 to 60 days. Stratification times, when known, are listed in Beano's section of UPUR C in the website or on the DVD. Some species may require 120 to 300 days.



THE HULL YOU SAY!

Many job specs call for de-hulled seed. Hull is a transitive verb meaning to remove the outer coating of a seed, & the prefix de- means to undo, or reverse, so de-hulled means to undo the removal of the outer coat, or a complete nonsense word, & it is open to interpretation, therefore unfit for use in job specifications. By the nomenclature of the Association of Official Seed Analysts (AOSA, whose rules are specified to be followed by seed law in most states, including Illinois) seeds are either hulled or unhulled, never dehulled. Don't be a dumb bunny. Genesis offers hulled seed that has had the loment or dried floral remains removed.

APPLYING POWDER ON THE SEED & PLANTING THE SEEDS.

And when you checked the manual
You kept in side the case
It said put it in a cool dry place.
Cool Dry Place, Travelling Wilburys, Volume 1

Inoculant powder needs to be stored in a refrigerator or a cool dry place, between 40 & 77ºF. Rhizobia are killed by heat over 95ºF (35ºC). They should be transported to the jobsite in a cooler with coldpacks. While in the field, keep inoculants out of direct sunlight & never store inoculant powder in an overheated vehicle, on the dashboard of a vehicle, or in the back of a pickup, van, or tool trailer.

Most rhizobial inoculants are completely organic. As a common sense precaution, avoid prolonged or repeated skin contact & inhalation. A dust mask, safety glasses, & protective gloves are recommended when applying humus inoculants. The recommended precautions are the same as for handling potting soils.

PREMIXING ADDITIVES

STICKER SOLUTION METHOD/HOMEMADE STICKERS.

Wet the seed with a 1:4 solution of sugar or corn syrup or a commercial sticking agent (*At last a sensible use for high fructose corn syrup*).

1). Make a sugar solution of 1 cup sugar to 1 quart of warm water. Slightly moisten the seeds with a small amount of water or sticker & mix with the inoculant. Use only a small amount & do not get the seeds too wet. Pour the seed inoculant mixture back & forth between 2 clean dishpans or 5 gallon buckets. The seeds should be covered with small black specks. Keep the seed out of sunlight & plant immediately. (*The following is optional*) If the seed cannot be planted immediately, place the seed in a plastic bag, add a moistened paper towel & close tightly. Protect from sunlight & keep cool. (after Lindermann & Glover 2003)

2). “Sticking agents include 10% (in water) solutions of: powdered milk, corn syrup, or sugar. Calf milk replacer should not be used as it contains antibiotics that will kill *Rhizobia*. Do not use any compounds that are highly acidic or basic. For best results, follow the instructions provided on the package.” (McDermott et al nd)

3). “This is the most effective method because it `glues' the inoculant to the seed. The adhesive material or inoculant sticker also serves to feed the rhizobia & to protect them from drying conditions on the seed. Several sticker products are available commercially from the companies that manufacture inoculants. Suitable stickers can also be prepared on the farm by making a 10% solution of corn syrup, table sugar or honey in water (Table 10). Powdered milk is also an effective adhesive agent, however, do not use milk replacer for livestock that contains antibiotics.” (48)

Table 10. Effect of Adhesive Agents on yield of King Grain Line X005 Soybeans.

Adhesive Agent	Nodules per Plant	Plant Yield (mg)
Uninoculated	0	350
Water	39	779
Nitracoat	109	911
Nutrigum	109	961
Pelgel	89	754
Gum Arabic	105	1013
Carboxymethyl cellulose	103	1127
Wallpaper glue (if non-toxic)	128	1226
Sugar	83	751
Corn Syrup	88	964
Honey	94	4
Powdered milk	96	1081
Evaporated milk	78	970

Source: MS Elegba & RJ Rennie, Can J Soil Sci., Volume 64:631-636 (1984) in (48)

EXTENDERS

Extenders are products designed to extend the viability of the rhizobia on the seed. Extenders are also applied before the seed & inoculant are mixed. Few commercial extenders are intended for use with native legumes, but extenders are occasionally specified in plantings. List INTX extender product here. Homemade extenders are a lot like homemade stickers.

HOMEMADE EXTENDERS.

1). Use of an adhesive, such as 10% solution of sirup* or a commercial preparation used to stick the inoculant to the seed may extend the life of the bacteria in dry soil for 2-3 weeks. An experiment on alfalfa in dry soils in North Carolina with a 10% sirup solution versus water alone as stickers yielded 1915 lbs versus 1040 lbs of alfalfa. (Erdman 1967) (*sugar water or molasses)

MIXING SEEDS & INOCULANT

Powdered inoculants are the most commonly used in restoration. They must be applied to the seed in a manner that the inoculant is placed immediately adjacent to the germinating seed. The old on the farm practice was dry application where the powder was spread on top of the seed in the seed box then stirred with a stick. This method is inefficient & wasted most of the powder. Two other methods are commonly used to apply the inoculant.

The water-slurry or slurry method:

1) "The inoculant is suspended in water & then mixed thoroughly with the seed until each seed is coated uniformly with the inoculant powder. Although water application is preferable to applying dry powder to the seed, it is not as effective as the sticker solution method" (48)

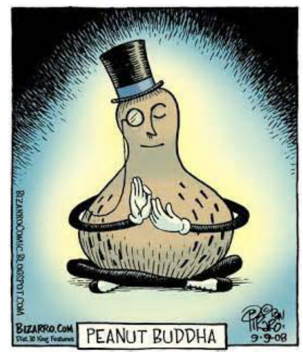
2) "The optimum method for applying N-DURE is by the slurry method. Dampen the seed with non-chlorinated, clean, cool water at a rate of 8.5 oz of water per 50 lbs of seed. Add appropriate amount of inoculant (2.5 oz / 50 lbs of seed) & water, seed, & inoculant thoroughly until the seed is uniformly coated. This method should be done in a container outside the planter box. Allow 1-3 minutes for the mixture to dry then plant as soon as possible. N-DURE can also be applied directly onto the seed. Mix seed & inoculant thoroughly until seed is uniformly coated. Layering seed & inoculant will aide in this process. Applying the inoculant dry is also recommended for seed that is pretreated with fungicide. However, maximum seed adhesion will not be obtained by applying this product dry. **For soils that have never been host to this specific legume, apply N-DURE at 1.5-2.0 X the suggested rate, or use N-DURE with a liquid inoculant product from INTX Microbials, IIC.**

Open package only when ready for use: plant within 24 hours of application" (N-DURE (Special Strains) Specimen Label)

3). "Seed of forages & other small-seed legumes should be placed in a large container (cement mixer, tub, pails,) & sufficient sticker applied to slightly wet all seeds. Then half of the required amount of inoculant powder should be sprinkled on the seeds while mixing until the seeds are uniformly coated. To eliminate the need for spreading out & drying the inoculated seed & to avoid clogging of the seeder, the other half of the required amount of inoculant powder is then added to the partially inoculated seed & mixed thoroughly in the container. The fully inoculated seed can then be planted with normal seeding equipment. This type of seed inoculation may be done one to two days before the actual seeding date but only if the inoculated seeds can be stored in a cool place. However, prompt seeding of freshly inoculated seed is preferable. Detailed instructions are given on inoculant & sticker package labels." (48)

4). Burton (1967) recommends adding a small amount of water to the inoculant to form a slurry, & then thoroughly mix the seed with the slurry. Moistening the seed first & then applying the powder is an alternative.

Seeds should be inoculated immediately before the seed is planted to 1) protect the inocula from drying out, 2) to keep the inocula from falling off the seed, & 3) to protect the inocula from dying. Inoculated seed must be kept cool, moist, & out of direct sunlight. Authorities recommended seed be reinoculated after 3-12 hours, or if properly protected & stored, the seed can be planted the next day. If inoculated seeds are to be stored for a few hours or overnight, place the seeds back in the original seed bag, add a moistened paper towel & close tightly. Alternately, seed should be planted within 48 hours or be re-inoculated (McDermott et al nd, Erdman 1967).



Recommended times until reinoculation is recommended.

3 hrs

12 hrs

24 hrs INTX, Drew et al 2013,

"Seeds can be stored for several hours or overnight" McDermott et al nd.

1-2 days (48)

48 hrs McDermott et al nd, Erdman 1967

Caltrans (2006) had? a 90 day window in their specs, but was trying to change to 7-10 days. 5ºC storage with controlled humidity.

If planting on a hot day, or into dry soil, or the inoculant is old or has been stored questionably, or the soil is stockpiled, re-applied topsoil, apply 2X to 10X the recommended inoculant. Additional inoculant will not harm the seed or the soil environment. Use of an adhesive, such as 10% solution of sugar/syrup or a commercial sticker preparation may extend the life of the bacteria in dry soil for 2-3 weeks.

PLANTING SEASON

Some legumes are sold preinoculated. *Caveat emptor.* Legally, they should be planted within 1 year of the date on the inoculant package (*not the date of inoculation*) or reinoculated. Improper storage conditions by the vendor may have damaged or killed inocula.

Pelleted seed can be reinoculated by using a small amount of vegetable oil as a sticker.

Legumes are slower to establish in fall seedings than cool season grasses. They should be seeded 2-4 weeks earlier than grasses as a late summer seeding (July 15th to August 1^{st,} or dates which are 2-4 weeks earlier than IDOT's, if indeed IDOT's planting dates are referenced to an adequate growing season/killing frost date, plus compensating for global warming & a longer frost free period). This timing allows better establishment & greater tolerance to freeze-thaw cycles. (Salon & Miller 2012) It would also allow more time for rhizobia attachment. ***IDOT CLASS 3 MUST HAVE A DIFFERENT SEEDING SEASON.***

Stock Seed Farms recommends seeding legumes & cool season grasses from August 15th to September 15th (Stock Seed Farms July 2013 Newsletter).

DORMANT SEEDING

Dormant seeding is seeding when soil temperatures are low enough to preclude any germination, typically 40ºF & below. Native forbs, including legumes, benefit from dormant seeding. Many species require a cold moist period to break dormancy. Many prefer to germinate in the cool soils of spring, & when dormant seeded they are in place at the proper time to germinate. Many benefit from having gone through their moisture induction period & are ready to germinate at the earliest opportunity. Last, dormant seedings have the benefit of spring rains for better establishment.

Dormant seeded legumes should be drilled with a granulated rhizobia at a date late enough to minimize the number of freeze-thaw cycles the inoculant must endure. The plantings should also be mulched, netted, or blanketed to minimize freeze-thaw, which creates a cache-22-situation, as the mulch, blanket, &c, will also minimize the diurnal temperature swings that promote germination.

There is no evidence that the applied inoculant will be alive in the spring. Each freeze-thaw cycle is fatal to 12% of the rhizobia. With 6 freeze-thaw cycles, theoretically 54% of the rhizobia are dead.

PLANTING

DRILLING.

HYDROSEEDING.

Seeds planted by hydroseeding should have 4X (minimum) the recommended inoculant added to the tank mix plus 4X a mycorrhizal seed-box product such as Myco Seedtreat. One-quarter to one-third of the mulch should be applied with the seed & inoculant. The remaining mulch shall be immediately applied in a second pass with the hydroseeder. A small amount of hydroseeding liming agent is sometimes added to adjust the slurry pH when seeding legumes. Never mix fertilizer or pesticides with the legume inoculant slurry. Complete fertilizers should never be allowed to come in direct contact with inoculated seeds. Phosphates are not as harmful as nitrogen or potassium (Erdmann 1967). add water pH.

A two-step broadcast-hydroseed method has been suggested. Inoculated legumes are kept separate from the remainder of the mix & are dry broadcast. The remainder of the seed & mulch are then applied over the top. (Caltrans 2006) This method relies on the hydromulch to protect the inoculant from UV light & desiccation.

Minimum mycorrhizal inocula per X times 1000 gallons.   Caltrans sponsored study 30 lbs per 3000 gallons.

DESIGN LEGUME COMPONENTS around rhizobia hosts? Don't think it is possible? Too many specials.

ALTERNATE STRATEGIES OF ESTABLISHING A HEALTHY RHIZOBIA POPULATION.

It has not been established that inoculating fall planted legume seeds results in successful nodulation. Use of rhizobia in fall seedings is a matter choice (*& hope*); it is a lot like peeing your pants in a dark suit; it gives you a warm feeling, but nobody notices. It is also more efficacious to broadcast fall seedings on prepared soil or into soybean stubble. In this case, it would be beneficial to inoculate Roundup Ready® Soybeans with native inocula when planting the soybean crop in the spring, & dormant seed native legumes.

It is also possible to establish rhizobial populations by inoculating winter wheat, which is typically planted September & later. (The nasty, phytotoxic Cereal Rye will germinate one whole week later than Winter Wheat. Big Hairy Deal!) Burton (1970) recommends inoculation a companion seed when planting chemically-treated legume seeds.

Erdmann (1967) noted that some farmers preferred to mix seed & inoculant dry in the seed box. This method was 20-50% as effective as wetting the seed then inoculant (Erdmann 1967).

Inoculant applied over the top of seed in the planter is very inefficient, but it is better than a stick in the eye. From Erdmann's observations, it seems possible to use 2-5 X the inoculant & dry mix the seed & inoculant. It is best to drill inoculated legume seeds in spring, rather than broadcasting the treated seed. Drilling the seed places the inocula out of the direct sunlight, where the inocula are sheltered until the legume germinates & the bacteria attach.

DEVELOPMENT, TIMING & EVALUATION.

Small nodules should be evident 2-3 weeks (Herried 2013), 5-6 days (Graham et al 2004), or 1 week (Graham 2005) after legume emergence. N fixation may begin 13-21 days after emergence (Graham et al 2004). Saskatchewan D.O.A. cites 30 days are required from seedling emergence to nodule formation & N_2-fixation. Evaluation of the nodulation efficacy begins at 4-6 weeks after emergence. A successful inoculation is determined by the number, vigor, color, & mass of the nodules. Deep, dark green vegetation is also an indicator of success. So is a state employee on a mower heading towards the planting.

To evaluate nodulation & fixation, carefully dig several typical plants from various parts of the field. Wash the soil from the roots & examine the crown region for nodules. Slice open several nodules from each plant. Effective nodules are pink to beef steak-red. The number of nodules & rate of N-fixation will increase with time & normally peaks just before the legume blooms. (48)

There is often a pattern to the distribution of nodules on an inoculated legume plant. There is a cluster of large, vigorous, pink nodules near the top of the taproot or main roots. These nodules contain the inoculant strain. Further out on the laterals, are smaller, less vigorous, white, grey, or tan nodules that were formed by "wild" rhizobia strains persisting in the soil.

WHY DID IT FAIL?

Potential N-fixation is closely linked to plant growth factors. Any management practice, stress, or condition that affects plant growth will affect nodulation & N-fixation.

1). The wrong inoculant was used.

2). The inoculant was of poor quality or dead. Inoculant quality is monitored in Australia & Canada, but there is no formal mechanism for monitoring quality in the United States (McDermott et al nd).

3). Planting into dry soil allows inoculant to die. Plant early in the year to take advantage of spring rains.

4). Inoculated seed was planted on top of the ground or too shallowly & exposed the rhizobia to UV light, killing 99% the first day.

5). Lack of rain after planting inoculated seed. Inoculants on seeds at or near the soil surface will die in 2-3 weeks without rain. Plant early in the year to take advantage of spring rains.

6). Soil temperatures reached 68ºF (20ºC) for a few days right after planting. High soil temperatures kill many rhizobia, decreasing nodulation. (48)

7). Soils are too acidic. This is seldom a problem in northern Illinois with soils derived from dolomitic glacial drift. Rhizobia do poorly below 6.0 & stop growing at 5.5 (5.2). pH below 5.5-5.0 can kill rhizobia. Soils with pH below 6 have low molybdenum availability. MO is essential for N-fixation.

8). Soils with a pH 7.8 (7.5) or higher.

9). Soils are too sandy or too low in organic matter, & inoculant desiccated or starved.

10). Low levels of phosphorus, potassium, boron, manganese, & iron. Low levels of molybdenum.

11). Excessive N in the soil. In high N-fertility soils, little or no nodulation or N fixation will occur. Legumes did not produce flavonoids, &c.

12). Soils ponded or became flooded. Rhizobia are aerobic organisms.

13). Soils have been treated with a fumigant or harsh insecticide. Insecticides, including DDT, disrupt the host-symbiont chemical signaling, causing nodulation failure. (Fox et al 2007)

14). The inoculant died because too much time elapsed between sowing the inoculated seed & germination, as in dormant seeding with germination the next spring.

The drought & extreme heat of 2012 may have impacted rhizobia population levels. Rhizobia survive best in moist soils with ambient temperatures of 40-80ºF. The drought of 2012 resulted in the top 6 inches of many midwestern soils to become extremely hot & dry. Either condition is fatal to rhizobia, but together it's a TKO. (Watters 2012) The drought necessitated the inoculation of all agricultural legumes in 2013, regardless of the previous crop history. Any native legume seeding from fall 2011-summer 2012 would probably benefit from a rescue inoculation or replanting. In our area, existing populations of deep-rooted, tap-rooted species such as *Amorpha canescens, Baptisia leucantha, Dalea candida* & *Dalea purpurea* held up well under this stress.

RESCUE/SUPPLEMENTAL INOCULATION. If the inoculation has failed on lands where success is likely, a planting can be reinoculated by watering with a solution containing a peat-based inoculant, followed by additional watering to help the *Rhizobia* percolate into the soil (McDermott et al nd). Hydroseeders can be used for this option (Graham 2005).

Inoculants on small seeds at or near the soil surface will die in several weeks (2-3) with dry winds & no rain. Supplemental inoculation "may be done by mixing a legume inoculant with cottonseed meal or wheat middlings or even sand, & broadcasting the mixture over the soil immediately before or after a rain." (Erdman 1967)

ALTERNATELY. "It is extremely difficult to rectify a nodulation failure after sowing. The best option would be to over-sow a granular product as soon as possible in close proximity to the original sowing furrow. Responses will decline with time, as mature roots are less likely to form nodules." (Drew et al 2012)

POST-PLANTING MANAGEMENT.

A low soil N environment must be maintained. Native legume plantings should be burned after their 2nd full growing season. The burning will invigorate the legumes. Charate from the ash may stimulate germination of any remaining hard/dormant seed. The burn will also lower the N balance of the planting by volatizing the N in the duff, removing it from the system, & stimulating more nodulation & N fixing. Mowing & removing the clippings will emulate several benefits of controlled burning, including lowering the available N. Accessible areas that cannot be burned should be mowed & baled. Do not do nothing!

INTRODUCED ALIEN LEGUMES.

Most species of introduced legumes that are used for forage or erosion control have shown invasive or noxious weed characteristics & encroach upon natural areas. These include *Coronilla varia (Securigera?)* CROWN VETCH, *Lathyrus sylvestris* FLAT PEA, *L. latifolius* PERENNIAL PEA, & *Melilotus officinalis* YELLOW SWEETCLOVER. Many of these erosion control & forage crops are now included in legislated noxious weed lists & cannot be legally planted. These & other species should never be used in combination with native grasses or wildflowers. They will out compete the natives. In the past, it was common to see mitigation projects in the northern USA with native seed mixes that included introduced forage legumes.

These species are easily spread by contaminated equipment, especially seeding equipment & mowers. Drills, broadcasters, drop seeders, mowers, & tractors should be vacuumed &/or blown clean with an air compressor. Two *Crown Vetch* seeds in a Brillion, left uncontrolled in a roadside planting, will become a nightmare.

IN THE BEST OF ALL POSSIBLE WORLDS (HIGHLY OPTIMISTIC YET UNDONE SPECS):

A POTENTIAL SEED SPECIFICATION. "All seed shall be cold dry stratified. Spring planted legumes & other hard seeded taxa shall be scarified.

BROADCAST & OR DRILL SEEDING. Legumes shall be separate from the forb mixture until after inoculation. The remainder of the seed mixture shall be blended by the vender & ratios of various species shall be guaranteed by the vendor in writing as specified. Legumes shall be inoculated with proper rhizobia immediately prior to planting (3-12(48) hours or less). The legumes shall be blended with the remainder of the seed mix immediately before planting. The contractor shall not inoculate more seed than can be planted in one day. Inoculated seed mixes must be placed in the shade (air conditioned trailer) & kept below 95ºF(??)"

HYDROSEEDING. "Total mix blended by the supplier. 4X rhizobial inoculant, 4X mycoseed treat added directly to the tank, plus 25-33% mulch with seed. Remaining 75-67% mulch applied immediately. Fertilizers or pesticides shall not be applied native hydroseeded areas."

Minimum inocula per X times 1000 gallons. Caltrans sponsored study 30 lbs per 3000 gallons.

NEED APPROPRIATE HEADING HERE

It is common for an upland prairie seed mix to contain 3 to 5 or more species of legumes. BMPs dictate each legume species should be inoculated individually with the appropriate strain. This needs to be detailed in planting specifications.

Keeping the seed & the appropriate inoculant in immediate proximity is key. Whenever possible, every legume species must be individually inoculated with the appropriate rhizobia strain, mixed with the remainder of the seed mix, & installed with a drill immediately.

5 LB PER ACRE METHOD

It is all to common to see a specification for 5 lbs of rhizobia applied per acre, but the specification states no compositional breakdown & no specified method of applying the inoculant to the seed, no installation method for applying the inoculant to the soil, & no method of installing inoculated seed. & people pay good money for these specifications.

Applying bulk rhizobia, or a rhizobial cocktail, when drilling a diverse mix of legumes may result in legume seedlings potentially mixed "infections" inefficient species that may act as parasites, producing little or no nitrogen, of no benefit to the plant. This is not a wise method. Indeed, one size does not fit all. (Must compare this to Burton's personal communications & go that way) (It may also be possible legumes have defenses against ineffective rhizobia.)

For decades, the Midwest natives industry has planted native grasses alone or native grasses with forbs. We have observed several, successful, low & tall stature grass plantings slowly deteriorate & degrade into weeds. (This is usually attributed to insufficient controlled burns.) It is time that we rethink the forbs/no forbs mentality. We need to plant basic grass mixes with native legumes. These native grass plantings accumulate more top growth than is decomposed every year. The N compounds in the duff oxidize (denitrification?) into N_2 into the atmosphere, creating a low N soil environment?

Thinking ahead, create a composite of C3 & C4 low to mid grasses, 1-2 native legumes each seeded at 1-2 lbs each per acre, plus appropriate mycorrhizae & rhizobia. Implement a management regime that fosters a low soil N environment. Nitrogen fertilizers are not used, clippings are removed when mowed, & plantings are regularly burned until legumes prevail.

Parallels of native plantings & roadside turf.

In an all turf grass/native grass planting, there are no mechanisms to maintain fertility levels; the planting is designed to fail. In an all turf grass/native grass planting, there are too many open niches that will be occupied by weeds; they are designed to fail. We must redesign IDOT roadside turfs with mixes of grasses & native legumes with lower grass rates for non-critical areas.

For Successful Legume Inoculation-

Use the right inoculant for the legume.
Keep commercial culture in cool, dark place until used.
Follow directions & mix culture well with seed.
Plant seed within 48 hours after they are inoculated, or reinoculated.
Inoculate in all cases of doubt & always on new land.
Prepare a good, well-fertilized, moist seedbed: after
planting small seeds, cultipack the soil.

(Erdmann 1967)

Back to top.

LEGUME HOST & PARTNERS.

SPECIES NOTES

Amorpha canescens.

Amorpha is related to *Petalostemum*. *Amorpha* special 1 (Nitragin). N-Dure *Amorpha* (INTX).

In a planting, *A. canescens* was nodulated with the same rhizobia as *Dalea*. Species is said to be specific in its requirement. *Mesorhizobium amorphae.* (Graham et al 2004)

Amorpha fruticosa.
Special Strain (INTX).

Amphicarpa.
Amphicarpa special 1 (Nitragin).

Apios.
?

Astragalus canadensis.
Astragalus special 1 (Nitragin). N-Dure *Astragalus* (INTX).
Mesorhizobium huakii. Indeterminate nodules, elongated with a distinct meristemic region. (Graham et al 2004)

Astragalus crassicarpus.
Special Strain (INTX)

Baptisia.
Type EL (Burton 1970). *Baptisia* special 1 (Nitragin). N-Dure *Baptisia* (INTX).

Baptisia australis.
Type EL (Burton 1970).

Baptisia bracteata.
Type EL (Burton 1970). Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX).

Baptisia leucantha (alba).
Type EL (Burton 1970). Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX).

Baptisia tinctoria.
Type EL (Burton 1970).

Chamaecrista fasciculata.
Cowpea. *Bradyrhizobium spp. (Chamaecrista).* Special (Burton 1970). Special, (Type EL) (Nitragin). N-Dure *Cassia* (INTX). Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX).
Formerly part of broadly defined *Cassia,* which has been divided into *Cassia, Senna,* & *Chamaecrista*. Usually, only *Chamaecrista* nodulates.

Crotalaria sagittalis.
Type EL (Burton 1970). Type EL (Nitragin). N-Dure Cowpea, Peanut, *Bradyrhizobium sp*. *Vigna* (INTX)

Dalea.
Said to be specific in its symbiont requirement. (Graham et al 2004)

Dalea alopecuroides.
Special (Burton 1970).

Dalea candida.
Special (Burton 1970).

Dalea purpurea.
Indeterminate nodules, elongated with a distinct meristemic region. Special (Burton 1970).

Desmanthus
Type EL (Burton 1970). Type EL (Spec.1) (Nitragin). N-Dure *Desmanthus* (INTX)

Desmodium.
Cowpea. Type EL (Nitragin). N-Dure *Desmodium* (INTX).

Desmodium canadense.
Bradyrhizobium spp. (Desmodium). Type EL (Burton 1970). Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX). Determinate nodules.

Desmodium canescens
Type EL (Burton 1970).

Desmodium spp.
Bradyrhizobium sp. Vigna (INTX).

Gleditsia triacanthos.
No nodulates, no effective symbiosis.

Glycyrrhiza
Glycyrrhiza spec 1 EL (Nitragin). Special Strain (INTX).

Gymnocladus dioica.
No nodulates, no effective symbiosis.

Hylodesmum (*Desmodium)*
Type EL? EL (Nitragin).

Lathyrus
Type C or EL (Nitragin). Introduced species use *Rhizobium leguminosarum* biovar *viceae* (INTX). Wild Canadian prairie strains will nodulate adjacent fields of fababean, lentils, & peas (Vessey 2003).

Lathyrus japonicus, ochroleucus, venosus & several introduced species.
Type C (Burton 1970).

Lespedeza capitata.
Type EL (Burton 1970). Cowpea. *Bradyrhizobium spp. (Lespedeza).* Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX).

Lespedeza capitata, hirta, leptostachya.

Type EL (Nitragin). N-Dure Cowpea, Peanut (INTX).

Lespedeza hirta
Type EL (Burton 1970).

Lespedeza leptostachya.
Type EL (Burton 1970).

Lespedeza sp. Slender Bushclover
Peanut, Cowpea; *Bradyrhizobium sp. Vigna* (INTX). 7 lespedeza species use this strain, others not listed (INTX).

Lotus americanus. ? (*Hosackia americana*)
Special (Burton 1970). Special 1, EL (Nitragin). (*L. corniculatus Mesorhizobium loti*; INTX dude)

Lupinus.
Type H (Burton 1970). Type H, EL (Nitragin). N-Dure Lupine (INTX).

Lupinus perennis
Type H (Burton 1970). Lupine, *Bradyrhizobium sp. (Lupine)*, as do 16 other species (INTX).

Mimosa.
Special 1 EL (Nitragin).

Orbexilum (*Psoralea).*
Special? EL (Nitragin).

Oxytropis.
Special1, EL (Nitragin).

Oxytropis lambertii
Special (Burton 1970).

Pediomelum (*Psoralea).*
Special? EL (Nitragin).

Petalostemum.
Type F (or Special) M,F EL (Nitragin). N-Dure *Petalostemum* (INTX).

Psoralea.
Special (Burton 1970). Special EL (Nitragin).

Psoralea esculenta.
Special Strain (INTX).

Psoralidium (*Psoralea).*
Special EL (Nitragin).

Robinia hispida & pseudoacacia.
Robinia spec 1 EL (Nitragin). Special (INTX)

Schrankia.
Schrankia Special 1 EL (Nitragin).

Senna (*Cassia).* *Senna* is generally said to be non-nodulating.
Special EL (Nitragin).

Senna marilandica. *Senna* is generally said to be non-nodulating.
Special (Burton 1970).

Strophostyles.
Strophostyles Spec 1 EL (Nitragin).

Strophostyles helvola
Special (Burton 1970).

Strophostyles leiosperma
Special (Burton 1970).

Tephrosia.
Special (Burton 1970). *Tephrosia* spec 1 EL (Nitragin). N-Dure *Tephrosia* (INTX).

Thermopsis ?

Vicia.
Type C, EL (Nitragin). Wild Canadian prairie strains will nodulate adjacent fields of fababean, lentils, & peas (Vessey 2003).

Vicia americana.
Type C (Burton 1970). Vicia Spec. 5 EL (Nitragin). Pea, Lentil, Vetch, *Rhizobium leguminosarum* biovar *viciae* (INTX).

Vicia caroliniana.
Type C (Burton 1970).

Wisteria ?????

Data after Burton 1972, Burton et al 1977, Smith et al 1988, &c.

STRAINS

Alfalfa group: *R. meliloti* for alfalfa & sweetclover.

Clover group: *R. trifolii* for red clover, white clover & alsike clover.

Cowpea miscellany. Promiscuous, slow-growing, bradyrhizobia. Colonizes *Desmodium canadense, Chamaecrista fasciculata, & Lespedeza capitata.* “Commonly nodulated without artificial inoculation” (Burton 1967). The Cowpea miscellany is commonly nodulated without artificial inoculation.

Sainfoin. *R. spp*. (special strains).

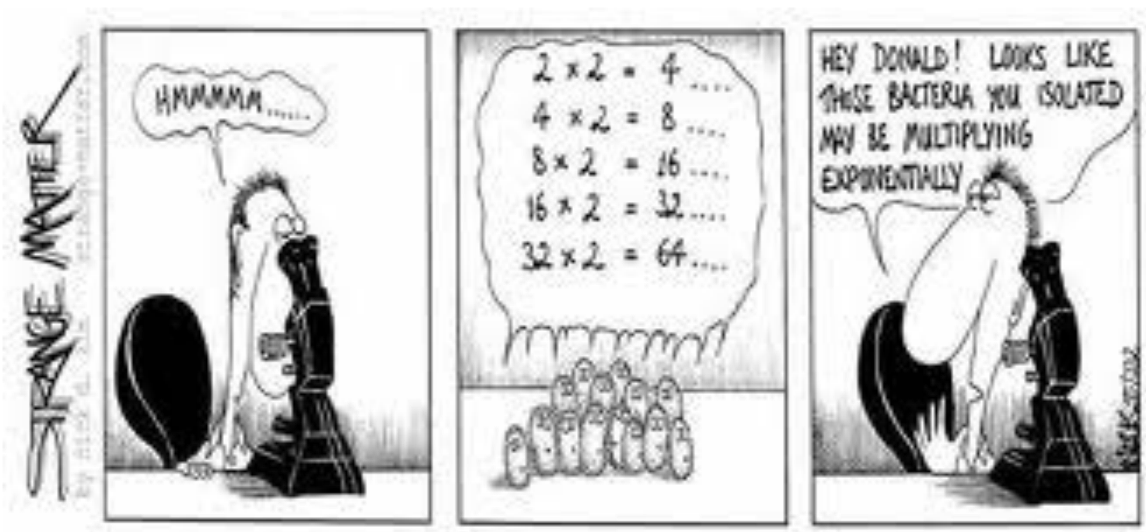
Birdsfoot trefoil. *R. loti.*

Pea & vetch group. *R. leguminosarum* for lentil, pea, flatpea & common vetch.

Bean group. *R. phaseoli* for field & garden beans.

Lupin group. *R. lupini* for white, yellow & blue lupins.

Fababean. *R. leguminosarum* (special strains).



[Back to top.](#)

ACTINORHIZA, FRANKIA, & CEANOTHUS.

The alder, whose fat shadow nourisheth --
Each plant set neere him long flourisheth.
William Browne 1613

Actinorhizal symbiosis is analogous to rhizobium-legume symbiosis, but it is thought to be less evolutionarily advanced. This opinion may reflect the current knowledge (or lack there of) of actinorhizal symbiosis. (Chanway et al 1993)

Frankia is a nitrogen-fixing actinomycetes that produces N2-fixing nodules on certain nonleguminous plants. They were originally believed to be eukaryotic fungi, but were found to be filamentous bacteria. *Frankia* is facultative symbiotic. Actinorhizal plants are mostly trees & shrubs, most are ECM, but *Ceanothus* is AM. Actinorhizal (=Actinomycete) symbiosis.

Frankia associates with *Betulaceae, Elaeaganaceae*, *Rhamnaceae, Myricaceae,* & some *Rosaceae* (8 families, 7 orders, 24 (25) genera, & 194± (170) species), mostly perennial dicot shrubs & trees, including *Alnus* ALDER, *Comptonia, Elaeagnus* AUTUMN OLIVE*, Myrica* BAYBERRY, *Sheperdia, Purshia,* & *Dryas* (Clawson et al 1998). (*Datisca* has herbaceous shoots.) These plants commonly grow in marginally fertile soils, low in nitrogen, phosphate & other minerals, often serving as pioneering species. These actinorhizal plants are known from arctic tundra, coastal dunes, riparian areas, freshly exposed glacial tills, forests, chaparrals & xeric sites, & alpine areas. They are useful in reforestation & pyrodenitrification. (Benson & Sylvester 1993).

Types of mycorrhizae associated with actinorhizae-nodulated plants.

Family	Genus	Mycorrhizae type
Betulaceae	*Alnus*	EC, VA
Casuarinaceae	*Casuarina*	EC, VA
Myricaceae	*Myrica*	EC, VA, Endo
	Comptonia	EC, VA
Elaeagnaceae	*Elaeagnus*	EC, VA
	Hippophaë	EC, VA
	Sheperdia	EC, VA
Rhamnaceae	*Ceanothus*	VA
	Colletia	VA
	Discaria	VA
	Trevoa	
	Talguenea	
	Kentrothamnus	
Datiscaceae	*Datisca*	VA
Rosaceae	*Purshia*	EC, VA
	Dryas	EC, VA
	Cercocarpus	EC, VA
	Rubus	
	Chamaebatia	
	Cowania	
Coriariaceae	*Coriaria*	EC, VA

(after & mycorrhizal types per Gardner (1986), who compiled from Trappe (1979) & Daft et al (1985)).

Is there a relationship between actinorhizal & ECM in the *Rosales*?

1st cultured in 1978.

Alnus, Comptonia, Sheperdia, & six other genera have been successfully inoculated with crushed *Frankia* nodule suspensions (Benson & Sylvester 1993). Crushed nodule suspensions may contain several *Frankia* strains &/or microbial contaminants, yielding mixed results. Pure culture of *Frankia* have been used to infect the following groups: 1) *Alnus* & *Myrica*; 2) *Casuarina* & *Myrica*; 3) *Elaeaganus* & *Hippophaë*; & 4) only *Elaeaganaceae* only (Baker 1987). There is a degree of infection specificity with actinorhizal plants, but it is nothing like the specificity in legumes (Richards 1987).

"*Frankia* cells enter symbiosis by root hair infection (for *Alnus, Casuarina, Comptonia*, & *Myrica* species [51, 63, 264]) or by a process of intercellular penetration of root epidermis &

cortex (for *Ceanothus, Elaeganus*, & *Sheperdia* species [146,161,203]) (Benson & Sylvester 1993). *Frankia* forms root nodules containing symbiotic N-fixing bacteria. The *Frankia* nodules are perennial, external to the vascular system, & are generally free of leghemoglobin, where as legume nodules are generally annual, internal to the vascular system, & contain leghemoglobin. (Chanway et al 1991)

Some *Frankia* sporulate readily in root nodules. Those nodules that contain numerous spores are designated sp(+) & those that produce relative few spores are sp(-). Crushed nodule suspensions made with sp(+) endophyte are more successful than those from sp(-).

Ceanothus

Contrary to some Midwest native nursery seed lists, *Ceanothus* is not a legume, & does not respond to commercial rhizobia inoculants. *Ceanothus* is in the *Rhamnaceae* or Buckthorn family, a genus of 50-60 species of evergreen & deciduous shrubs or small trees confined to North America. The greatest diversity is in western USA (California), with 2 species ranging into eastern USA, *Ceanothus americanus* & *C herbaceous.*

Ceanothus is symbiotic with *Frankia*, a nitrogen-fixing actinomycetes. *Frankia* grow as long filaments & appear more like fungi than bacteria (Drew et al 2012). Very little is known about the symbiotic relationship, in part because no one has isolated a culture that has re-infected another plant.

Ceanothus should always be seeded with mycorrhizal inoculants, as virtually all actinorhizal plants are mycorrhizal. (Most actinorhizal plants are ectomycorrhizal, but *Ceanothus* species are endomycorrhizal.) On a stewardship level, you might consider "borrowing" some soil from a restoration where *Ceanothus* is nodulated & flourishing & inoculating the soil at your site. Use a soil-sampling probe to minimize the disturbance, & refill the holes with quality, weed free, toxin- & pathogen-free local topsoil. Keep the liberated soil cool & dark in a cooler & immediately incorporate into the topsoil of your planting site. Add endomycorrhizal inoculant if you have it. Spot seed scarified, boiled, & stratified *Ceanothus* seeds or install plugs in the inoculated area. Alternately, mix the donor soil with mycorrhizal-inoculated greenhouse soil & bump *Ceanothus* plugs in the mixed soil, grow on & plant out.

In some cases, soil-based preparations are more infective than crushed nodules (Bond 1974). Strand & Laetsch (1977) successfully inoculated Ceanothus integerrimus seedlings with soil from around the roots of nodulated plants, where crushed nodule preparations were non-infective.

Or (not for the meek & timid) Crushed Nodules Suspensions. *Ceanothus* plants have been inoculated from suspensions of *Frankia* obtained from crushed nodules. Remove some *Frankia* colonized roots from a donor site, mash said roots/nodules & threads, & mix with properly boiled & stratified seed or inoculate greenhouse plants. Establish your personal *Frankia* donor site.

Other Frankia Friendly Folk

Not all species of the 21 genera have been examined for nodules, so generic nodulation characteristics have not been determined. "Thus it may well be that Trappe's postulation was correct but it must be remembered that even to date not all the nodulated species within each genera have been investigated & only a few plants of each species have been sampled." (Gardner 1986)

Betulaceae; A single genus nodulates; fungi live in loose association with *Betula* roots, fix N(?), but do not nodulate; *Frankia* may be present under *Betula* in greater numbers than under *Alnus.*

Casuarinaceae; monogeneric,
Coriariaceae. monogeneric,
Elaeagnaceae; nodulation is characteristic of the family;
Myricaceae; nodulation is characteristic of the family;

Frankia is named after Albert Bernard Frank who described mycorrhizae on the roots Alnus in 1888. Frank "coined" the word mycorrhiza in 1885.
&c,
Other nitrogen-fixing bacteria are known. *Bradyrhizobium* is capable of nodulating *Parasponia andersonii* Planch., a member of the elm family. Several nitrogen-fixing cyanobacteria, *Nostoc*, are associated with aquatic ferns, & *Cycas* & *Gunneras*. Free-living diazotrophs are often found in the rhizosphere & in the intercellular spaces of several plants including rice & sugarcane.

Back to top.

The world's alive beneath our feet :
We feel the pulse of nature beat :
Cantycroft, in Weeds & Wild Flowers, Mowry Bell, 1908.

MINIGLOSSARY.

achlorophyllous

aerenchyma Air spaces within plant organs. These can form between cells, or in the case of large spaces result from cell death. They often form continuous channels along the length or organs such as the root. The main role of aerenchyma is to provide gas exchange to cells in waterlogged soils (Armstrong 1979).

adventitious roots A root which arises from a stem.

apex The root tip which is covered by a root cap (covering sheath) & secretes mucilage (water soluble polysaccharides which adhere to the root).

amensalism n. (Latin from the table)

anastomosis A hyphal fusion with a cytoplasmic connection.

antagonism n. () "Inhibition of or interference with the action of a substance or organism (as a salt, microbe, mould, &c) by another substance or organism" (oed).

apical meristem The zone of dividing cells at the root apex which give rise to new cells in a growing root. Actively growing roots have gradients of maturing tissues away from the apical meristem.

apoplast The cell wall space inside living plants is collectively known as the apoplast.

appressorium pl. appressoria n. Hyphal swellings between two adjacent root epidermal cells. These are sites where hyphae first penetrate root cells by exerting pressure &/or enzymatic activity. A flattened, hyphal organ that facilitates the penetration of cells or tissues of other organisms.

arbuscules n. These are intricately branched "haustoria" that form within root cortex cells that look like little trees (Gallaud 1905). Arbuscules are formed by repeated dichotomous branching & reductions in hyphal width from an initial trunk hypha that ends in a proliferation of very fine branch hyphae. They are considered to be the major site of exchange with the host plant. Old arbuscules collapse progressively until only the trunk remains. Collapsed arbuscules are sometimes called peletons.

Ascomycetes, ascomycetous n, adj. (from *ascus,* modern Latin, from Greek ἀσκός, *askos,* bag, sac, & post-classical Latin *Mycetes* (in K. P. J. Sprengel *Systema Vegetabilium* (1827) (ed. 16) IV. 376), from ancient Greek μύκητες, *myketes,* plural of μύκης, *mykes,* mushroom, fungus.) Cup & flask fungi.

aseptate Not containing septae.

aseptate hyphae These are hyphae which are without cross walls (coenocytic hyphae). Cross walls may form as hyphae age.

associative adj. Description of an association between dissimilar living organisms in which intimate physical contact or attachment is lacking but mutual benefits are realized eg PGPR.

autotroph n. (post-classical Latin *auto*- & its etymon ancient Greek αὐτο-, αὐτός, *auto-, autos*, self, one's own, by oneself, independently, cf Hellenistic Greek αὐτότροφος, *autotrophos,* bringing one's own provisions) "Of an organism: self-nourishing; capable of synthesizing organic compounds from simple inorganic molecules (such as carbon dioxide); not dependent upon organic compounds as a source of energy. Opposed to heterotrophic adj." (oed)

auxiliary bodies These structures, which are also called external vesicles /or accessory bodies, are clustered swellings on external hyphae. These are often ornamented by spines or knobs & are characteristic of *Scutellospora* & *Gigaspora*. These apparently do not function as propagules.

bacteroid adj. n. (from modern Latin, from Greek βακτήριον*, bakterion,* diminutive of βάκτρον, *baktron, stick*, staff, & classical & post-classical Latin -*oīdēs* & its etymon ancient Greek -οειδής, *oeides*, from -o- , representing either the stem vowel of the preceding element or a connective, & -ειδής, *eides,* having the form or likeness of, like, from εἶδος, *eidos,* form; *bacterioid* would be a better form.) (1) "Of the nature of, or allied to, the bacteria." (2) "A micro-organism of bacterial character; *spec.* one found *(a)* in the root-nodules of leguminous plants, *(b)* in the body of certain insects." (oed) "1887 Philos. Trans. (Royal Soc.) 178 B. 552 The gemmules or 'bacteroids' [in the Leguminosæ]. 1898 R. T. Hewlett Man. Bacteriol. 26 If the roots of a pea, bean, or vetch be examined, numerous little nodules will be found upon them; on examining these microscopically small irregular bodies are found to be present, which have been termed bacteroids." (in oed)

"Legume root nodule bacterium that has morphologically & physiologically differentiated into a cell that is capable of symbiotic nitrogen fixation within the nodule" (Chanway et al 1991).

Basidiomycetes, basidiomycetous   n. adj. (from modern Latin, from Greek βάσις, *basis,* base, & -ιδιον, *-idion,* diminutive ending; & post-classical Latin *Mycetes* (in K. P. J. Sprengel *Systema Vegetabilium* (1827) (ed. 16) IV. 376), from ancient Greek μύκητες, *myketes,* plural of μύκης, *mykes,* mushroom, fungus.) A large number of Basidiomycetes are ECM.

brown roots, suberized roots, &c.   These additional terms are sometimes use to designate old roots, woody roots, or roots with a *suberized exodermis*. These general terms are misleading & should not be used.

calcium spiking   A sharp periodic increase in calcium concentration around the nucleus of symbiotically stimulated root cells.

capillaroid roots   A type of root cluster produced by rushes (*Restionaceae*) with exceptionally dense coverings of long root hairs (Lamont 1982).

Casparian band   A specialized cell wall structure where *suberin* is deposited in a radial band. Cells with these structures are arranged in one or two cylinders within roots to form the *endodermis* & *exodermis*. These bands are thought to provide a barrier to *apoplastic* transport of solutes (Esau 1977, Clarkson & Robards 1975, Peterson 1988).

cell   The basic component of plant organs, consisting of cytoplasm, organelles, vacuoles, &c, bounded by a plasma membrane.

cell wall   Structure located outside the plasma membrane of most plant cells. It is primarily made of structural carbohydrates such as cellulose. Cell walls provide mechanical support & space for *apoplastic* transport of substances. They often contain secondary metabolites, *suberin* or *lignin*.

cluster roots   A specific type of root clusters, where closely-spaced lateral roots are densely covered with root hairs. These occur in particular dicotyledon families (Proteaceae, Fabaceae, &c). Cluster roots function by secretion of organic acids to solubilize mineral nutrients from infertile soils (Lambers et al 2006).

coarse roots   The "distributive" root system comprised of lower order roots, which is responsible for mechanical support & the transport of substances between fine roots & the shoot.

coenocytic   Multiple nuclei within the same cell.

colony   Hyphal colonization of a root resulting from one external hypha (these may arise from several adjacent entry points). These are often called infection units.

commensalism   n. (medieval Latin *commensālis*, from *com-* together with, & *mensa* table, *mensālis,* belonging to the table) "Applied to animals or plants which live as tenants of others (distinguished from *parasitic*). 1881 J. Lubbock in Nature No. 618. 405. Schwendener proposed, in 1869, the..theory..that lichens are not autonomous organisms, but commensal associations of a fungus parasitic on an alga." (oed)

conjugation Transfer of DNA from a bacterial donor cell to a recipient cell involving indirect cellular contact (Chanway et al 1991).

cortex The cell layers occurring between the epidermis & stele. Cortex cells typically have a large central vacuole used to store solutes & are the site of arbuscule formation in AM associations.

crystals Specialized root cells may contain crystals, along with mucilage, or other substances in their vacuole.

dauciform roots Swollen lateral roots produced by sedges (Cyperaceae) that are densely covered in root hairs (Lamont 1974). These are much thicker than normal lateral roots & spindle shaped (tapering), i.e. "carrot-shaped". They have a similar role to cluster roots in nutrient uptake (Shane et al 2004).

dikarya A subkingdom of fungi including the phyla *Ascomycota* & *Basidiomycota*, both of which in general produce dikaryons.

dikaryon The state in certain fungi in which each compartment of a hypha contains two nuclei, each derived from a different parent.

dichotomous branching A symmetrical branching pattern which occurs when two branches arise simultaneously from the tip of a hyphae, plant or fungus organ. Divergent branches grow at the same rate.
dichotomous branching This is a distinctive form of branching of the ECM short roots of some Gymnosperm trees (e.g. *Pinus* species). These bifurcations result in two equal branches & may result in a cluster of branches with an even number of root tips.

ectomycorrhiza A symbiotic association between a fungus & a plant root characterized by formation of a relatively thick mantle by fungal hyphae & penetration of mycelial strands inward between cortical cells & outward into the soil (Chanway et al 1991).

endodermis A cortex cell layer found in all roots, next to the vascular cylinder. The cell walls contain a *Casparian band* & may develop *suberin lamellae* (Esau 1965, Clarkson & Robards 1975).

endomycorrhiza A symbiotic association between a fungus & plant root characterized by extensive inter- & intracellular penetration by fungal hyphae, but with no mantle formation, & by mycelial (Chanway et al 1991).

endophyte n. (Greek ἔνδον*, endon,* within, & φυτόν, *phyton*, plant) "Bot. †(a) (see quot. 1835); (b) a plant growing inside another, an internal fungus. 1835 J. Lindley Introd. Bot. (1848) I. 21 A division..separates, in trees, the bark from the internal part, or endophyte as he [Count de Tristan] terms it. 1867 J. Hogg Microscope (ed. 6) ii. i. 293

Endophytes..originate from germs which penetrate healthy plants & develop a mycelium." (oed)

epidermis The outermost layer of cells of the root, in direct contact with the soil. As the soil-root interface, the epidermis is an important site for nutrient uptake & the initiation of mycorrhizal associations.

eukaryotic

exodermis The hypodermis is called an exodermis if its cell walls contain a *Casparian band* & these cells often also have *suberin lamellae* (Peterson 1988). The exodermis is thought to reduce root permeability (to apoplastic flow) & increase resistance to pathogenic organisms, water loss, &c.

exploitative Coiling VAM of myco-heterotrophic plants, usually without arbuscules.

exudates Root exudates are defined as substance released into the substrate by healthy & intact plant roots (Rovira 1969). These include water, sugars, amino acids, &c.

feeder roots, fine roots The fine, higher order lateral roots that are thought to be responsible for most nutrient & water uptake, as well as mycorrhiza formation.

first order lateral roots Roots that arise from the seminal root or adventitious roots.

flavonoid n. (from flavone, from German *flavon* (von Kostanecki & Tambor 1895, in *Berichte der Deutsch. Chem. Ges*. 28 2302), from flav- (in flavo- comb. form, ultimately Latin *flavus,* yellow), & -one suffix used in chemistry forming the names of organic compounds derived from other compounds & -oid) A flavone. "a. A colourless crystalline tricyclic compound, $C_{15}H_{10}O_2$; 2-phenylbenzo-1, 4-pyrone. b. Any of the derivatives of this compound, many of which are plant pigments; a flavonoid. "1949 Jrnl. Pharmacol. & Exper. Therapeutics 95 399. At a recent symposium on vitamin P it was suggested..that the generic term 'Flavonoids' be used to refer to the flavonols, flavanones & related compounds. 1970, Watsonia 8 168. Flavonoids may have important physiological functions & are not simply waste products of metabolism." (oed)

fruit bodies These are also called sporocarps, basidiocarps, ascocarps, mushrooms, truffles, &c. They are relatively large reproductive structures formed by Basidiomycetes or Ascomycetes which form sexual basidiospores or ascospores respectively. These develop from primordia produced by the mycelial system.

Hartig net n. An intercellular meshwork in the root epidermis & cortex formed by ECM.
Hartig net Labyrinthine network of specialized fungus hyphae, with frequent branching (or wall ingrowths) that forms a layer between the walls of adjacent root epidermal or cortex cells. This is considered to be the major site of nutrient exchange between the fungus & host plant.

haustorium The structure (usually a modified root) connecting a parasite to its host(s). Haustoria physically penetrate the host & function by connecting its vascular tissue to the parasite.

hemiparasite A parasite that is photosynthetic & may have roots, but obtains water & nutrients from one or more host plants. Most have haustoria that connect to xylem, but some also obtain photosynthates from the host phloem (e.g. dwarf mistletoes). Cf holoparasite.

heterorhizy Root system with distinct long & short elements, resulting from reduced longitudinal growth by fine laterals.

heterotroph

holomycotrophs

holoparasite A nonphotosynthetic parasite that obtains all the water & nutrients required for sustenance from a host plant. These have connections with both the xylem & phloem of the host.

hypodermis The layer of cells below the epidermis is called a hypodermis if it is not *suberized* (Peterson 1988).

intercellular space The spaces outside the root cells, often in the cortex at the junction of cells. These form longitudinal air channels in many roots, which can be seen by observing whole-living roots mounted in water. Air channels provide conduits for gas transport in waterlogged soils (Armstrong 1979) & influence AM formation.

internal hyphae, intraradical hyphae Hyphae which grow within the cortex of a root to form a colony & later develop arbuscules & vesicles. These comprise the body (thallus) of a fungus in the root.

intercellular hyphae Hyphae which grow between the walls of adjacent root cells. These are in the root apoplast -- the zone outside the cytoplasm of cells.

intracellular hyphae Hyphae which grow within root cells. These penetrate the walls of cells & grow within them, but are separated from the cytoplasm by the plasma membrane.

lateral roots Any root which grows from another root.

lignin A cell wall type that is impregnated by phenolic compounds. These walls are often considerably thickened to strengthen plant organs. Xylem cells & fibres are typically lignified, but other cells in the stele or cortex can have lignified walls.

long roots The lateral roots which bear ECM short roots. These often undergo early secondary growth.

mantle layers of fungal hyphae covering the root surface.

metacutinization This is the modification of dormant root tips by *suberization* of one or more root cap cell layers (Romberger 1963). Inactive roots of many perennial plants develop a metacutinized apex, which functions as an extension of the *exodermis*, presumably for protection from adverse soil factors (Brundrett *et al.* 1990).

microfilament Strong, but flexible, linear polymer of actin subunits & component of the cytoskeleton.

middle lamella A cell wall zone rich in the carbohydrate pectin connecting adjacent cells.

mistletoe Epiphytic hemiparasite attached to tree branches by haustoria.

mucigel Gelatinous material secreted by plant roots which is composed of simple & complex carbohydrates (Chanway et al 1991).

mucilage High molecular weigh, poorly diffusible substances actively secreted by root epidermal & root cap cells (Rougier & Chaboud 1985). These primarily consist of carbohydrates, but also may contain sloughed cells, enzymes, phenolic compounds, &c.

mutualism n. (classical Latin *mūtuus,* borrowed, corresponding, reciprocal) "The relationship existing between two organisms of different species which contribute mutually to each other's well-being; an instance of this. By some writers applied spec. to a relationship that is essential for the survival or reproduction of one or both of the organisms involved; by others used to denote a relationship that is not essential to either. Cf symbiosis." (oed)

mycelial strands & rhizomorphs Interwoven hyphae that function as transport conduits & spread the association. Rhizomorphs contain specialized types of hyphae.

mycoheterotroph, mycoheterotrophic (post-classical Latin & scientific Latin *myco-,* from ancient Greek μύκης, *mykes,* mushroom, fungus; the combining form of Greek ἕτερος, *heteros*, the other of two, other, different; a formative of many scientific & other terms, & German -*troph*, forming adjectives, from ancient Greek -τροφος, -*trophos,* combining form, meaning "that nourishes") expand this. Obtains carbon sources from a fungal symbiont.

neutralism

nitrogenase n. An enzyme complex that catalyzes the reduction of molecular nitrogen in the nitrogen-fixation process of bacteria. "Any of a class of complex enzymes (typically containing iron & molybdenum atoms) which bring about the conversion of molecular nitrogen into ammonium ions, as the first step in biological nitrogen fixation" (oed).

Nod factors The bacterial symbionts of legumes (rhizobia) produce signaling molecules named Nod factors. They consist of an N-acetylglucosamine backbone that carries various strain-specific decorations including a lipid side chain.

obligate biotroph An organism that is unable to complete a reproductive cycle in the absence of a living host.

obligate parasite A plant that must attach to a host to complete its life cycle (some grow inside the host). All holoparasites are obligate, but some hemiparasites are not.

other spores   Small asexual spores (conidia) which function as propagules, may be produced by some mycorrhizal fungi.

parallel evolution   n.

parasite   n. (classical Latin *parasītus* (also *parasīta*, feminine) A person who lives at another's expense, from ancient Greek παράσιτος *parasitos,* a person who eats at the table of another, a person who lives at another's expense & repays him or her with flattery, a person who dines with a superior officer, a priest who is permitted meals at the public expense, from παρα- para-, by the side of, beside, & σῖτος, *sitos,* food) "An organism that lives on, in, or with an organism of another species, obtaining food, shelter, or other benefit; (now) *spec.* one that obtains nutrients at the expense of the host organism, which it may directly or indirectly harm. The term *parasite* originally included (& is still sometimes used for) animals & plants that are now considered to be commensals, mutualists, epiphytes, or saprophytes, as well as birds or other animals that habitually steal food from, or use the nests of, other species." (oed)

parasitism   n. () The condition of living as a parasite on, in, or with a host organism; parasitic quality or habit.

passage cells, short cells   Small exodermal cells that remain unsuberized that are surrounded by longer *suberized* cells (long cells). In many plants, long & short cells alternate in a uniform pattern (called a dimorphic exodermis).

periderm   The bark layer formed on the surface of roots or branches by secondary growth. Walls of periderm cells are strengthened by *suberin* & *lignin* deposits, which reduce their permeability & susceptibility to microbial activity & adverse soil conditions.

PGPR   Plant Growth-Promoting Rhizobacteria compromise a group of naturally occurring soil bacteria that colonize root systems & stimulate plant growth (Chanway et al 1991).

pinnate branching   Also called sympodial branching, is unequal branching of mycorrhizal lateral roots with perpendicular side branches.

phi thickenings   These are localized deposits of lignified wall material which form a thickened ring in cortex cell walls (von Guttenberg 1968).

primary growth   The initial growth of a plant organ caused directly by cell division in its apical meristem & cell enlargement in subapical regions.

rhizoplane   The surface of the root & habitat for organisms which live in contact with the root. 2). The root surface (Chanway et al 1991).

rhizosheath   n. Soil which tightly adheres to the rhizoplane (Chanway et al 1991).

rhizosphere The zone surrounding roots where soil properties & microbial populations are influenced by root exudates. 2). The region of soil surrounding plant roots & influenced by their metabolism (Chanway et al 1991).

root clusters Dense aggregations of lateral roots such as cluster roots & capillaroid roots (Lambers et al 2006).

root hair Narrow cylindrical hair-like cell extension of an epidermal cell on the root surface. These may be long or short & provide a dense or sparse root covering. Root hairs increase root contact with the soil & are thought to have a role in water & nutrient uptake.

sclerotia Storage structures produced in soil by some fungi, comprised of compact fungal tissue, which is often highly melanized.

second & third order laterals, &c. Roots which arise from first order laterals which in turn may produce third order laterals, & so on. Higher order laterals may be categorized as feeder roots or fine roots (see below).

secondary growth New growth activity which begins from mature cells in a plant organ. This normally results from radial enlargement of an organ by a new lateral meristem.

secondary metabolites Plant cells & cell walls often contain secondary metabolites (substances not required for metabolism). Phenolic compounds, including tannins (dark brown pigments) are especially common, but many other chemicals, including alkaloids, terpenes, flavonoids, &c, accumulate in roots of particular plant species. These may color the root & result in uv-induced autofluorescence.

secondary roots, woody roots Roots, which develop a *periderm* & additional vascular tissue due to secondary growth. These would normally have a much longer lifespan than feeder roots & will not contain mycorrhizas if secondary growth has resulted in *cortex* loss.

seminal root A root initiated by a germinating seed.

short roots These are roots with ECM with reduced apical growth & more frequent branching, resulting in heterorhizy.

soil hyphae These are also known as extraradical or external hyphae & are the filamentous structures which comprise the fungal thallus (body) in the soil. They acquire nutrients, propagate the association, & produce spores & other structures. AM fungi produce thick "runner" or "distributive" hyphae as well as thin, highly branched "absorptive" hyphae.
soil hyphae These are also known as extraradical or external hyphae, mycelia or the fungus thallus. They extend outwards from the fungal mantle into soil to initiate mycorrhizal associations, acquire soil nutrients, &c.

spores These are swollen structures with one or more subtending hyphae that form in the soil or in roots. Spores usually develop thick walls, which often have more than one layer. They can function as propagules. Spores of AM fungi are sometimes called chlamydospores or azygospores.

sporocarps Aggregations of spores into groups, which may contain specialized hyphae & can be encased in an outer layer (peridium). Soil particles may be included in the spore mass. This term can be misleading, as the sporocarps produced by most Glomeromycotan fungi are small & relatively unorganized structures compared to those produced by larger fungi. 2). A hard, usually globose multicellular fruiting body that contains spores e g mushrooms (Chanway et al 1991).

stele, vascular cylinder The zone internal to the endodermis which contains specialized tissue responsible for the transport of water & minerals to the shoot (xylem) or organic nutrients, such as photosynthetically fixed carbon, (phloem). Additional layers of xylem & phloem form radially during secondary growth & lateral root initiation also occurs in this zone. Xylem cells develop *lignified* walls & are dead when mature.

sticker n. Substance used in inoculation to ensure that the rhizobia adhere to the seed during planting. They range from milk or sugar solutions used in simple seed inoculation, to stronger adhesives (40% gum arabic, 5% methyl ethyl cellulose) used in pelleting seed. (rrl)

suberin n. (classical Latin *sūber* cork oak, the bark of this, of unknown origin. *Quercus suber* is the Cork Oak of the Mediterranean) This is a hydrophobic material, containing lipids & phenolics, which impregnates the cell walls of specialized cells (Kolattukudy 1984). Suberin is thought to prevent the passage of water & other materials in the *apoplast*.

"*Bot. & Chem.* Originally: cork; the outer bark or periderm of the cork oak. In later use: a waxy material forming the outer layer of the periderm in parts of certain other plants, comprised of tightly packed suberized cells." (oed)

suberin lamellae These are concentric layers of suberin deposited on the inner surface of cell walls & considered to function as barriers to microbial & solute penetration. These are most often found in *endodermal* or *exodermal* cells.

symbiotic adj. Description of a close association between two dissimilar living organisms often involving physical attachment & generally assumed to be mutually beneficial e g nitrogen-fixing root-nodule bacteria (Chanway et al 1991).

symplast The space inside living plant cells is collectively known as the symplast. The cytoplasm of adjacent plant cells is often connected by channels through the cell wall (plasmodesmata).

transduction n. Transfer of a DNA fragment from a bacterial donor cell to a recipient cell mediated by a bacterial virus (Chanway et al 1991).

transformation n. Uptake of a DNA fragment by a bacterial recipient cell from growth medium which had been previously released by a donor cell (Chanway et al 1991).

vesicles Intercalary (-o-) or terminal (-o) hyphal swellings formed on internal hyphae within the root cortex. These may form within or between cells. Vesicles accumulate lipids & may develop thick wall layers in older roots. The production & structure of vesicles varies between different genera of Glomeromycotan fungi. They are oil storage organs which may also function as propagules.

zygomycete

Mostly after Brundett 2008, Chanway et al 1991, Parniske 2008.

Back to top.

SELECTED SOURCES:

(1) EB Allen, JC Chambers, KF Connor, MF Allen, & RW Brown, 1987, Natural…………..

(1.1) Michael F Allen 1991, The Ecology of Mycorrhizae, Cambridge University Press.

(2) ON Allen, 1949, Inoculate Legumes It Pays, Bulletin 484, March 1949, Agricultural Experiment Station, University of Wisconsin, Madison.

(3) David R Benson & Warwick B Silvester, 1993, Biology of *Frankia* Strains, Actinomycete Symbionts of Actinorhizal Plants. Microbiological Reviews, June 1993, 293-319.

(3.1) G. Bond, 1974, Root-nodule symbioses with actinomycetes-like organisms. In, “The Biology of nitrogen Fixation” (A. Quispel, ed.) pp. 342-378. Elsevier-Holland Pub., Amsterdam.

(4) Jim Beuerlein & Harold Watters 2012, Survival of Soybean Rhizobia Cells in Soil http://corn.osu.edu/newsletters/2012/2012-26/survival-of-soybean-rhizobia-cells-in-soil

(4.4) Mark C Brundrett, 1991, Mycorrhizas in Natural Ecosystems, Advances in Ecological Research Vol 21:171-313.

(4.5) Mark C Brundett 2002. http://www.ffp.csiro.au/research/mycorrhiza/

(4.55) Mark C Brundrett, 2002, Coevolution of roots & mycorrhizas of land plants, New Phytologist, (2002) 154: 275-304.

(4.6) Mark C Brundrett, 2008. *Mycorrhizal Associations: The Web Resource.* Access starting 8/11/13. http://mycorrhizas.info/index.html

(5) Joseph C Burton, 1970, Nodulation & Symbiotic Nitrogen Fixation by Prairie Legumes, reprinted from The Second Midwest Prairie Conference, Madison Wisconsin, September 18-20, 1970.

(6) JC Burton, RL Curley, & CJ Martinez, 1977, Rhizobia Inoculants For Various Leguminous Species, Nitragin Informational Bulletin, No. 101, 4/15/1977.

(7) Joseph C Burton, 1967, Rhizobium Culture & Use, in Henry J. Peppler, Microbial Technology, Universal Foods Corporation, Milwaukee, Wisconsin, Reinhold Publishing Corporation, New York.

(8) Joseph C Burton, 1979 & 1980, Personal Communications.

(9) Caltrans (better authorship here?), 2006, Legume Seed Inoculation for Highway Planting in California;
http://www.dot.ca.gov/hq/LandArch/research/docs/final_seed_innoculation_2006.pdf

(10) CA Campbell & W Souster, Can. J. Soil Sci. Volume 62:651 (1982) in http://www.agriculture.gov.sk.ca/Default.aspx?DN=4b50acd7-fb26-49a9-a31c-829f38598d7e

(11) TR Cavagnaro, FA Smith, SE Smith, I Jakobsen, 2005, Functional diversity in arbuscular mycorrhizas: Exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell Environ 28:642–650.

(11.1) CP Chanway, R Turkington, & FB Holl, 1991, Ecological Implications of Specificity between Plants & Rhizosphere Micro-organisms. Advances in Ecological Research Vol 21. pp 121-169.

(12) RF Denison, 2000, "Legume sanctions & the evolution of symbiotic cooperation by rhizobia". American Naturalist 156: 567–576.

(13) Elizabeth Drew, David Herridge, Ross Ballard, Graham O'Hara, Rosalind Deaker, Mathew Denton, Ron Yates, Greg Gemell, Elizabeth Hartley, Lori Phillips, Nikki Seymor, John Howieson, & Neil Ballard, 2012, Inoculating Legumes: A Practical Guide. Grains Research 7 Development Council, Kingston, Australia.

(14) Lewis W Erdman, 1967, Legume Inoculation: What It Is- What It Does, Farmers' Bulletin No. 2003, U.S. Department of Agriculture. U.S. Government Printing Office.

(14.1) Isobel C Gardner, 1986, Mycorrhizae of actinorhizal plants, MIRCEN Journal, 1986, 2, 147-160.

(15) Fox, J.E., Gulledge, J., Engelhaupt, E., Burow, M.E., and McLachlan, J.A. 2007. "Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants". PNAS. 104(24):10282-10287.

(16) Peter H Graham 2005, Practices & Issues in the Inoculation or prairie Legumes Used in Revegetation & Restoration, Ecological Restoration, 23:3 September 2005. pp 187-195.
(17) Peter H Graham 2008

(18) Peter H Graham, Becki Tlusty, & Elena Beyhaut, 2004, Inoculated Legumes & Revegetation/Roadside Plantings, Minnesota Department of Transportation Report MN/RC - 2004-32, http://www.lrrb.org/PDF/200432.pdf

(18.1) JL Harley & EL Harley, 1987. A check-list of mycorrhiza in the British flora. New Phytologist 105(2): 1–102.

(19) MJ Harrison, 1999, Molecular & cellular aspects of the arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 50:361–389

(20) Layne Herried, INTX LLC, (2013) Personal Communication.

(21)

(22) Brent N Kaiser*, Kate L Gridley, Joanne Ngaire Brady, Thomas Phillips & Stephen D Tyerman, 2005, The Role of Molybdenum in Agricultural Plant Production, Annals of Botany 96: 745-754, http://aob.oxfordjournals.org/content/96/5/745.full.pdf

(23) ET Kiers, RA Rousseau, SA West, & RF Denison, 2003. Host sanctions & the legume–rhizobium mutualism. Nature 425: 79-81

(24) Joe Lara, 2005, Well Rooted for Conservation, Land & Water Magazine, May/June 56-60.

(24.5) J Leake, 2005, "Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs & debunking the 'saprophytic' plant myth" Mycologist, Volume 19. p. 113-122. (http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B7XMS-4R10WNH-4&_user=571676&_coverDate=08%2F31%2F2005&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1707263361&_rerunOrigin=scholar.google&_acct=C000029040&_version=1&_urlVersion=0&_userid=571676&md5=88ca37bff8307df5ea6de40e64990e99&searchtype=a)

(25) WC Linderman & CR Glover, 2003, Inoculation of Legumes. NMSU Cooperative Extension Service Guide A-130.

(26) Timothy R McDermott, Ronald H Lockerman, S Dennis Cash & Deb Solum, Legume Inoculation (MT 9619), no date, http://animalrangeextension.montana.edu/articles/forage/General/LegumeInoculationMTGuide.pdf

(27) FH Meyer, 1973, Mycorrhizae in native & man-made forests. In Ectomycorrhizae. Their Ecology & Physiology, Eds. G.C. Marks & T.T. Kozlowski, pp. 79-105. New York, Academic Press Inc.

(28) RM Miller, CR Smith, JD Jastrow, & JD Bever, 1999, Mycorrhizal status of the genus *Carex* (*Cyperaceae*), American Journal of Botany 86:547-553.

(28.1) JB Morton & D Redecker, 2001. Two new families of *Glomales, Archaeosporaceae* & *Paraglomaceae,* with two new genera *Archaeospora* & *Paraglomus,* based on concordant molecular & morphological characters. Mycologia 93: 181–195.

(28.9) Ken Mussleman, AgriEnergy Resources, (2013) Personal Communication.

(29) National Academy of Sciences, 1979, Microbial Processes: Promising Technologies for Developing Countries; Washington D.C. http://www.nap.edu/openbook.php?record_id=9544&page=R1

(30) Martin Parniske, 2008, Arbuscular mycorrhiza: the mother of plant root endosymbiosis, Microbiology, Volume 6, October 2008, pp763-775.

(31.0) D Redecker, R. Kodner, LE Graham, 2000, Glomalean fungi from the Ordovician. Science 289:1920–1921.

(30.1) Winfried Remy, Thomas N Taylor, Hagen Hass, & Hans Kerp, 1994, Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA. Vol. 91, pp 11841-11843, December 1994.

(30.2) BN Richards 1987, The Microbiology of Terrestrial Ecosystems. John Wiley, new York.

(31) Nancy J Ritchie & David D Myrold, 1998, Geographic Distribution & Genetic Diversity of *Ceanothus*-infective *Frankia* Strains, Applied & Environmental Microbiology, April, 1999, Vol 65, no 4 pp 1378-1383.

(32) Rusty Rodriguez & Regina Redman, 2008, More than 400 million years of evolution & some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. Journal of Experimental Botany Volume 59, Issue 5 pp 1109-1114.

(33) Gene R Safir, ed, 1987, Ecophysiology of VA mycorrhizal plants, CRC Press, Boca Raton, Florida.

(34) Paul R Salon & Chris F. Miller, A Guide to: Conservation Plantings on Critical Areas for the Northeast USDA, NRCS, Big Flats Plant Materials Center, Corning, NY. http://plant-materials.nrcs.usda.gov/nypmc/

(35) M Schultze, B Quiclet-Sire, E Kondorosi, H Virelizier, JN Glushak, G Endre, SD Gero, & A Kondorosi, 1992, "*Rhizobium meliloti* Produces a Family of Sulfated Lipo-Oligosaccharides Exhibiting Different Degrees of Plant Host Specificity. 1992. *Proceedings of the National Academy of Sciences of the United States of America*, Volume 89. pp 192-196.

(36) Simms et al, 2006. An empirical test of partner choice mechanisms in a wild legume-rhizobium interaction. Proc. Roy. Soc. B 273:77-81.

(37) SE Smith & DJ Read, 1997, Mycorrhizal Symbiosis, 2nd Edition, Academic Press, San Diego.

(37.5) SE Smith & DJ Read. 2008, "Mycorrhizal symbiosis, Third Edition". Elsevier Ltd. Chapter 13: Mycorrhizas in acholorophyllous plants (mycoheterotrophs). p. 458-507.

(37.55) Janet I Sprent, 2001, Nodulation in Legumes, Kew, UK; Royal Botanic Gardens.

(37.6) Janet I Sprent, 2008, Evolution & Diversity of Legume Symbiosis, pp 1-22; in M.J. Dilworth et al (eds) Nitrogen-fixing Leguminous Symbiosis; Springer, Dordecht, The Netherlands.

(38) Janet I Sprent, 2009, Legume Nodulation: a Global Perspective, Wiley-Blackwell, Singapore.

(39) Janet I Sprent

(39.5) R Strand & WM Laetsch, 1977, Cell & endophyte structure of the nitrogen-fixing root nodule of Ceanothus integerrimus H. & A. I. Fine structure of the nodule & its endosymbiont. *Protoplasma* 93, 165-178.

(39.6) John G Torrey, 1990, Cross-Inoculation Groups within Frankia & Host-Endosymbiont Associations, pp 86-106, Christa R Schwintzer & John Tjepkema, 1990, The Biology of Frankia & Actinorhizal Plants, Academic Press, San Diego.

(40) JM Trappe, 1977, Selection of fungi for ectomycorrhizal inoculation in nurseries, Ann. Rev. Phytopathol., 15, 203.

(41) JM Trappe, 1987, Phylogenetic & Ecological Aspects of Mycotrophy in the Angiosperms from an evolutionary standpoint, in Gene R. Safir, ed., 1987, Ecophysiology of VA mycorrhizal plants, CRC Press, Boca Raton, Florida.

(42)

(43) Kevin Vessey, 2003, The benefits of inoculating prairie legume crops (Part 1 & Part 2), http://www.umanitoba.ca/afs/fiw/030828.html http://www.umanitoba.ca/afs/fiw/030904.html

(44) B Wang & YL Qiu, 2006, Phylogenetic distribution & evolution of mycorrhizas in land plants. Mycorrhiza (2006) 16: 299–363. e-mail: ylqiu@umich.edu

(45) Wetzel & van der Valk, 1995, Vesicular-arbuscular mycorrhizae in prairie pothole wetland vegetation in Iowa & North Dakota. Can J Bot 74:883-890.

(46) Charlie T Wilbury, Jr., Lefty Wilbury, Lucky Wilbury, Nelson Wilbury, & Otis Wilbury, aka The Traveling Wilburys, 1988, Traveling Wilburys Vol. 1. *Store It In A Cool Dry Place.*

(47) GWT Wilson, CW Rice, MC Rillig, A Springer, DC Hartnett, (2009) Soil aggregation & carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461.

(48) Editors: BJ Green, VO Biederbeck; Authors: VO Biederbeck, HA Bjorge, SA Brandt, JL Henry, GE Hultgreen, GA Kielly, AE Slindard, 2013, Soil Improvements With Legumes - Agriculture.
http://www.agriculture.gov.sk.ca/Default.aspx?DN=4b50acd7-fb26-49a9-a31c-829f38598d7e

(49) http://www.jokes4us.com/miscellaneousjokes/foodjokes/mushroomjokes.html

(50) http://www.rhizobium.umn.edu/.

(51) http://teachline.ls.huji.ac.il/72346/Nitrogen/Rhizobium-legumeassociation.htm

Back to top.

A mushroom went into a bar & saw some algae at a table. He went up to one & said, "You're lookin' all gal (algal) to me." She looked him over & said "And you look like a fun guy (fungi)." And they took a liken (lichen) to each other.

May the spores be with you.

notes

IDOT & ISTHA must reach a new level in design & maintenance of roadsides. Self-sustaining plantings. All upland Class 4 mixes must have a legume component at 40,000+ plants per acre. The species must not be an aggressive forage legume, ie the next kudzu. All mixes must have both mycorrhizal & rhizobial components.

Fertilizer specs shall be rewritten to foster mycorrhizae & rhizobia. Low N, moderate P. Installation dates must foster legume development & rhizobia maintenance.

Do determinate & indeterminate nodules have different life spans? I have read some conflicting info, as in 50-60 days with multiple generations per growing season for determinate nodules versus a growing season for indeterminate nodules?

LÉ GUME FÉRTILITÈ; PLURAL LÉS GUMES

Molybdenum (Mo) is an essential micronutrient for all plants, but especially legumes, enabling N metabolism & synthesis of protein. Mo is a component of the enzymes nitrate reductase (NR) & nitrogenase. Rhizobia bacteroids require 10X the Mo than the legume requires. (Kaiser et al)

Insert page 8?. One subunit of nitrogenase is the MoFe protein involved in the reduction of N_2 to NH_3. (Kaiser et al)

insert page 8?. Fixed nitrogen is exported as either amides (glutamine & asparagine) or ureides (allantoin & allantoic acid), which are initially derived from the oxidative breakdown of purines. The fixed N is mobilized & exported by molybdoenzyme XDH. (Kaiser et al 2005).