TECHNICAL NOTES
U.S. DEPARTMENT OF AGRICULTURE BERKELEY, CALIFORNIA SOIL CONSERVATION SERVICE

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NITROGEN FIXATION AND LEGUME INOCULATION

The attached Technical Note prepared by J. W. Turelle, Regional Agronomist of the Technical Service Center in Portland, entitled "Symbiotic Nitrogen Fixation and Inoculation of Most Legumes in Western Region," has application in the State of California. It is being issued as prepared, realizing that not all species will be used in every location. As it is to be used as reference material, rather than for distribution, this should not detract from its value.

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State Soil Conservationist

Attachment

TECHNICAL NOTES

U.S. DEPARTMENT OF AGRICULTURE — SOIL CONSERVATION SERVICE
WEST REGIONAL TECHNICAL SERVICE CENTER - PORTLAND, OREGON

CONSERVATION AGRONOMY NOTE NO. 22 — February, 19[illegible]
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SYMBIOTIC NITROGEN FIXATION AND INOCULATION OF MOST LEGUMES IN WESTERN REGION

Most soils lose nitrogen by erosion, crop removal, drainage water, nitrate leaching, and presumably nitrogen gas and nitrous oxides. The nitrogen content of soils must, therefore, be increased to compensate for these losses. Among the processes for restoring nitrogen in soils is the use of legume inoculants. This is the most important natural process for increasing the nitrogen content of soils. Legume inoculations are essential in establishing grasses and legumes for conservation uses such as controlling soil losses, runoff, and converting cropland for pastures, hay and recreation.

Other than the addition of a few leguminous species by Dr. Hafenrichter and me, the material below is from an Arizona Agronomy Note prepared by Leon W. Kimberlin, Conservation Agronomist, SCS, Phoenix.

All of the following species, of course, are not adapted or used in each of the Western Region states. It is suggested this material be localized in each work unit by deleting the species not used.

Legumes perform more efficiently when properly inoculated. Through an effective partnership of plants and bacteria, legumes can produce better yields and play a more satisfactory role in soil maintenance and improvement.

Some facts concerning the why and how of legume inoculation are outlined as follows:

Importance of Legume Bacteria in Nitrogen Transformation

1. There are 35,000 tons of elemental nitrogen in the air above each acre of land; in a form usable by plants, this would have a value of $7,000,000 per acre at current fertilizer prices.

2. Elemental nitrogen (an inert gas) must combine with other elements to be useful to plants. It can be changed to a plant-available form by lightning, electrical arc processes, or soil micro-organisms. When nitrogen from the air is changed to a form which can be used by plants it is said to be "fixed."

3. Symbiotic Nitrogen Fixation.
Most legumes and some non-legumes benefit from a symbiotic or partnership relation with certain bacteria which are capable of changing atmospheric nitrogen to forms which the plant can use. The bacteria use carbohydrates from the host plant for energy and depend upon the host plant for certain minerals. In turn the bacteria supply the host with nitrogen in a usable form. Bacteria of the genus Rhizobium are capable of infecting legumes, causing nodules to form on the plant roots, and through symbiotic activity, fix nitrogen which can be used by the plant.

The amounts of nitrogen fixed, has been estimated as follows:

Legume	Average Amount of Nitrogen Fixed Per Acre (pounds)
Alfalfa	194
Ladino clover	179
Lupines	151
Sweetclover	119
Alsike clover	119
Red clover	114
Kudzu	107
Legumes in pastures	106
White clover	103
Lentils	103
Sourclover	98
Crimson clover	94
Cowpeas	90
Lespedezas (annual)	85
Fenugreek	82
Vetch	80
Bur-clover	78
Peas	72
Velvetbeans	67
Garbanzo	66
Soybeans	58
Winter peas	50
Peanuts	42
Beans	40

Approximately one-third of the above amounts of nitrogen is contained in the root system of the plant.

Some nitrogen is made available to companion crops as in the case of legume-grass pastures. Grasses benefit from nitrogen released by legume nodules.

How Rhizobium Bacteria and Legumes Work Together

1. Newly seeded legume germinates.

2. The root begins to develop and exerts a chemical attraction for the bacteria.

3. The bacteria secretes a substance which causes the root hair to curl and grow toward the bacteria.

4. The bacteria penetrates the root hair and infects the plant root.

5. Infection of the plant root causes enlargement of plant cells forming a nodule.

6. The bacteria multiply and live in the nodule. They draw upon the plant and certain minerals for energy and furnish nitrogen compounds to the plant.

7. Most Nitrogen is Fixed:

   (a) In daylight when the plant is photo-synthesizing at a moderate rate. (Excessive rates of photo-synthesis retard nitrogen fixation)

   (b) At temperatures of 54° - 83° F.

   (c) Under conditions of good soil moisture and aeration.

   (d) When soil nitrate levels are relatively low.

Effects of Soil Fertility and Fertilizer on Inoculation and Symbiotic Nitrogen Fixation

1. The presence of Nitrates:

   (a) Counteracts deformation of the root hair thus limiting entrance of the bacteria and reducing numbers of nodules.

   (b) Limits the effectiveness of nodules already formed by reducing the volume of bacterial tissue.

   (c) When the soil nitrate levels are high, the legumes use soil nitrates in greater amounts--the bacteria fixes less nitrogen.

2. Other Nutrient Elements:

   A nutrient regime favorable to the growth of the host plant is generally desirable for the bacteria. Molybdenum is essential for the bacteria to fix nitrogen.

Why Should Legumes be Inoculated at Time of Seeding?

1. Legume plants are incapable of forming nodules and using nitrogen from the air without the aid of nodule bacteria classified in the genus Rhizobium.

2. A field may not have previously grown the legume and inoculating bacteria may not be present in the soil.

3. Although the field may have previously grown the legume, the strains of inoculating bacteria in the soil may not be best for the plant.

Selecting the Correct Inoculant

1. Different species of Rhizobium bacteria are needed for various legumes. Rhizobium bacteria have been classified into groups which are capable of inoculating various legumes. These groups and the legumes which they can inoculate are listed as follows:

Common Name	Scientific Name
ALFALFA GROUP (Rhizobium meliloti)	
Alfalfa	Medicago sativa
Buttonclover	M. orbicularis
California bur-clover	M. denticulata
Spotted bur-clover	M. arabica
Black medic	M. lupulina
Snail bur-clover	M. scutellata
Tubercle bur-clover	M. tuberculata
Little bur-clover	M. minima
Tifton bur-clover	M. rigidula
Yellow alfalfa	M. falcata
White sweetclover	Melilotus alba
Hubam sweetclover	M. alba annua
Yellow sweetclover	M. officinalis
Bitterclover (sourclover)	M. indica
Fenugreek	Trigonella foenumgraceum

Common Name	Scientific Name
CLOVER GROUP (Rhizobium trifolii)	
Alsike clover	Trifolium hybridum
Crimson clover	T. incarnatum
Hop clover	T. agrarium
Small hop clover	T. dubium
Large hop clover	T. procumbens
Rabbitfoot clover	T. arvense
Red clover	T. pratense
White clover	T. repens
Ladino clover	T. repens (giganteum)
Sub clover	T. subterraneum
Strawberry clover	T. fragiferum
Berseem clover	T. alexandrinum
Cluster clover	T. glomeratum
Zigzag clover	T. medium
Ball clover	T. nigrescens
Persian clover	T. resupinatum
Carolina clover	T. carolinianum
Rose clover	T. hirtum
Buffalo clover	T. reflexum
Hungarian clover	T. pannonicum
Seaside clover	T. wormskjoldii
Lappa clover	T. lappaceum
Bigflower clover	T. michelianum
Puff clover	T. fucatum
PEA AND VETCH GROUP (Rhizobium leguminosarum)	
Field pea	Pisum arvense
Garden pea	P. sativum
Austrian winter pea	P. sativum (var. arvense)
Common vetch	Vicia sativa
Hairy vetch	V. villosa
Horsebean	V. faba
Narrowleaf vetch	V. angustifolia
Purple vetch	V. atropurpurea
Monantha vetch	V. articulata
Lana woollypod vetch	V. dasycarpa
Bird vetch	V. cracca
Bramble vetch	V. tenuifolia
Sweet pea	Lathyrus odoratus
Rough pea	L. hirsutus
Tangier pea	L. tingitanus
Flat pea	L. sylvestris
Lentil	Lens culinaris (esculenta)

Common Name	Scientific Name
COWPEA GROUP (Rhizobium sp.)	
Cowpea	Vigna sinensis
Asparagus-bean	V. sesquipedalis
Common lespedeza	Lespedeza striata
Korean lespedeza	L. stipulacea
Sericea lespedeza	L. cuneata
Slender bushclover	L. virginica
Striped crotalaria	Crotalaria mucronata
Sunn crotalaria	C. juncea
Winged crotalaria	C. sagittalis
Florida beggarweed	Desmodium tortuosum
Tick trefoil	D. illinoense
Hoary tickclover	D. canescens
Kudzu	Pueraria thunbergiana
Alyceclover	Alysicarpus vaginalis
(No common name)	Erythrina indica
Pigeonpea	Cajanus cajan (indicus)
Guar	Cyamopsis tetragonoloba
Jackbean	Canavalia ensiformis
Peanut	Arachis hypogaea
Velvetbean	Stizolobium deeringianum
Lima bean	Phaseolus lunatus (macrocarpus)
Adzuki bean	P. angularis
Mat bean	P. aconitifolius
Mung bean	P. aureus
Tepary bean	P. acutifolius var. latifolius
Partridge-pea	Chamaecrista fasciculata
Acacia	Acacia linifolia
Kangaroo-thorn	A. armata
Wild-indigo	Baptisia tinctoria
Hairy indigo	Indigofera hirsuta
BEAN GROUP (Rhizobium phaseoli)	
Garden bean, kidney bean, Navy bean, pinto bean	Phaseolus vulgaris
Scarlet Runner bean	P. coccineus (multiflorus)
LUPINE GROUP (Rhizobium lupini)	
Blue lupine	Lupinus angustifolius
Yellow lupine	L. luteus
White lupine	L. albus
Washington lupine	L. polyphyllus
Sundial	L. perennis
Texas bluebonnet	L. subcarnosus
Serradella	Ornithopus sativus

Common Name	Scientific Name
SOYBEAN GROUP (Rhizobium japonicum)	
Soybeans	Glycine soja
Wild soybeans	Glycine javonica
SPECIFIC STRAIN GROUP (Rhizobium sp.)	
Birdsfoot trefoil	Lotus corniculatus
Big trefoil	L. uliginosus
Foxtail dalea	Dalea alopecuroides
Black locust	Robinia pseudoacacia
Trailing wild bean	Strophostyles helvola
Hemp sesbania	Sesbania exaltata
Kura clover	Trifolium ambiguum
Sanfoin	Onobrychis vulgaris (sativus)
Crown vetch	Coronilla varia
Siberian pea-shrub	Caragana arborescens
Garbanzo	Cicer arietinum
Cicer milkvetch	Astragalus cicer
Leadplant	Amorpha canescens

2. Variations or strains of bacteria have different abilities to inoculate legumes and to fix nitrogen.

   (a) Some strains may form nodules readily but may be good or poor as nitrogen fixers. Some may be parasitic without benefit to the plant.

   (b) Nodules formed by poor strains are usually small, round, white in color, and scattered over the root system of the plant. Nodules formed by a good strain are usually fewer in number, larger, somewhat elongated, pink, and located near the main roots.

   (c) Strains may vary in effectiveness after successive passage through host plants.

   (d) Many of the strains found in their natural habitat, either in the soil or in nodules, are of the poor type.

3. Selected Strains of Inoculant are Easily Obtained:

   Inoculant is commercially available for all commonly grown legumes and may be purchased from most seed suppliers. Laboratory cultures of highly effective strains of bacteria are mixed with a carrier of moist humus, agar, or liquid for storage, transportation, and use by farmers.

Seed which has been pre-inoculated with the appropriate strain of bacteria is also available from some seed suppliers.

Costs of inoculant cultures will average about 50 cents per bushel of seed. Pre-inoculated seed usually costs about $2.50 per hundred weight more than seed which has not been inoculated.

To Achieve Successful Legume Inoculation.

1. When inoculating seeds on the farm:

   (a) Purchase the correct strain of bacterial culture. Check the expiration date on the container.

   (b) Keep the inoculant in a cool dark place until used. Avoid exposure to temperatures greatly in excess of 50° F. for extended periods.

   (c) At time of seeding - mix the bacterial culture with the seed. Use skim milk diluted with water, or a water and sugar solution to wet the seed immediately prior to mixing. This will cause the bacteria to stick to the seed and will supply nutrients for the bacteria.

   (d) Mix small quantities at one time; a drill box full or whatever amount can be used in a short time (2 hours or less). Mix thoroughly to assure that inoculant adheres to all seeds.

   (e) Keep the inoculated seed in the shade to avoid high temperatures, and drying before seeding.

   (f) Double inoculate all seed which has been chemically treated.

   (g) Make seedings in moist soil whenever possible.

2. When Using Pre-Inoculated Seed:

   (a) Be sure that seed has been stored at temperatures of 50° F. or less, and has not been exposed to higher temperatures for extended periods of time.

   (b) Re-inoculate seed if the expiration date on the tag has expired or if temperature limitations have exceeded those shown on the tag.

3. Avoid Excessive Application of Nitrogen.

Application of nitrogen fertilizers is generally detrimental to bacterial nodulation. However, on very infertile soils, nitrogen applied in limited amounts at seeding time may be beneficial.

Avoid direct contact of chemical fertilizers with the inoculated seed.

Nitrogen applied after stand establishment is detrimental to the effective activity of the nitrogen fixing bacteria.

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