NODULATION AND SYMBIOTIC NITROGEN FIXATION BY PRAIRIE LEGUMES

By

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ABSTRACT

The indigenous flora of the Midwest Prairies consists of a great number of leguminous plant species capable of working symbiotically with special bacteria called "rhizobia" in nodules on their roots and gathering free atmospheric nitrogen for their own growth and possibly for their non-leguminous associates which may otherwise be deprived of nitrogen.

The occurrences of various prairie legumes and their nitrogen-fixing abilities will be demonstrated in color slides. These include species of *Astragalus*, *Baptisia*, *Cassia*, *Desmodium*, *Lespedeza*, *Petalostemum*, *Strophostyles*, *Tephrosia*, and *Vicia*. Effective and ineffective nodulation and methods of sending nodules to the laboratory for isolation purposes will be reviewed.

The final part of the discussion concerns artificial inoculation of prairie legume seeds--the inoculants available and easy methods to effectively inoculate leguminous seeds.

Introduction

Nature endowed leguminous plants with a perhaps unique system of obtaining nitrogen. By working with special bacteria (rhizobia) in nodules on their roots, they can utilize the gaseous nitrogen of the atmosphere. This built-in nitrogen source makes legume growth independent of the soil supply. Perhaps because of this fact, leguminous plants were apparently well represented in the original prairie ecosystem (Fasset, 1939; Turner, 1959; Anderson, 1961).

In some instances, effectively nodulated leguminous species also provide nitrogen for companion grasses as a result of intermingling of root systems. However, root nodules provided no protection against the plow or shovel, and the leguminous species were lost along with other biotic constituents when the prairies were uprooted for other purposes.

Much interest has developed recently in restoring some of our tall-grass prairies to their natural beauty and resourcefulness as habitats for bird and animal life. However, as several workers (Schulenberg, Schramm, Bland, and others) pointed out in our first symposium, restoration is a long and arduous task. The prairie remnants are often the only source of propagating stock of adapted indigenous species, and plants or cuttings must be removed carefully and sparingly to avoid further destruction. Seeds can be collected from native plants but establishment is difficult because the environment is often very hostile to young seedlings. Special techniques of plant culture and prairie restoration are being developed (Bland, 1968; Kilburn, 1968; Landers et al, 1968; Ode, 1969; Schulenberg, 1968; Vocelka, 1968). Fortunately, many of the leguminous species in the biota of the native prairie produce seeds which can be collected and planted in new areas.

The ability of leguminous species to grow and compete with weeds and other natural enemies in the ecosystem often depends upon the presence of effective nodules on their roots which give them a nitrogen supply not available to other plants. But often the proper nodule bacteria are not present in our soils. To insure effective nodulation of new legume seedlings, it is often necessary to add the proper bacteria (rhizobia) to the seed or soil before planting, a process called "inoculation."

The Rhizobium: Legume Association

Early in the study of symbiotic nitrogen fixation, all nodule bacteria were considered one species capable of nodulating all leguminous plants. However, investigators soon learned that there were many kinds of rhizobia; those which nodulated certain species would not nodulate others. Furthermore, not all rhizobia capable of producing nodules on a particular host were beneficial to growth. Certain rhizobia produce nodules and live off their host without providing any nitrogen. Consequently, for maximum benefit to plant growth, rhizobia must be screened for their nitrogen-fixing capabilities in association with particular hosts. Effective symbiosis depends upon discreet matching of both symbionts.

The efficiency of a rhizobium:plant association can often be determined by examining nodulation of the root system. In contrast to effective nodules, which are generally large, few in number, pink to red inside, and located on the upper tap root, ineffective nodules are small, numerous, white in color, and scattered on the lateral roots.

Rhizobia and Inoculants

The rhizobia are microscopic, unicellular plants which multiply by binary fission. They exist only in the vegetative state and are highly susceptible to drying and temperatures in excess of 40° C. While the soil is their natural habitat, rhizobia are easily cultured in the laboratory when supplied with an energy source such as mannitol or sucrose, certain growth factors contained in yeast or plant extracts, mineral salts, and a minimal supply of oxygen.

In preparing inoculants, strains of rhizobia selected for their effectiveness on particular host plants are cultured to high populations in a liquid medium before being mixed with a heat-treated, finely-pulverized sedge peat which serves as a protective home or carrier for the bacteria until they are applied to seeds or soil. The inoculant--a moist, brownish-black powder teeming with millions of live rhizobia--is usually applied to leguminous seeds immediately before planting.

Effective inoculants for agriculturally important leguminous crops are available at most local seed stores but appropriate inoculants for most of the wild indigenous prairie legumes are more difficult to obtain. Nodulation data and inoculants required for most legumes of the prairie region are listed in Table 1.

The leguminous species listed are known to form nodules and work symbiotically in fixing atmospheric nitrogen. Practically all species have been grown in Nitragin greenhouses or growth chambers and tested for their abilities to symbiose effectively with various strains of nodule bacteria. Effective cultures of the microsymbiont are maintained in the Nitragin stock collection for preparation of the various special inoculants listed.

Inocula designated by letters are wide-spectrum inocula effective on agriculturally important crops and are available at most seed stores. The special inocula for individual legumes, designated by "Sp.", are prepared on request and available only from our Milwaukee laboratory.

Methods of Inoculating Seeds

Leguminous seeds do not generally provide a good habitat for rhizobia; they are applied to the seed simply because this is an easy, convenient way to implant the nodule bacteria into the soil or rhizosphere where the young seedling will develop. It is best to inoculate as close to planting time as is practical and to protect inoculated seed from hot, drying winds and direct sunshine until they are planted. When planted, inoculated seed should always be covered with soil or some type of mulch. Large numbers of live rhizobia on seeds favors early nodulation and stronger, more vigorous seedlings.

Leguminous seeds vary greatly in size, shape and physical characteristics. Inoculation methods must be adjusted to suit the seed. Normally, the seed is moistened with a small amount of water, skim milk, or sugar solution (6 to 8 ml. of liquid per kilogram of seed). The powdered inoculant, 7 or 8 g. per kilogram of seed, is then added and mixed thoroughly with the moistened seed. If the seeds are too wet, addition of more inoculant or a little dry soil is recommended. Unhulled seeds require quite a bit more liquid than hulled seed. A wide-mouth glass fruit jar with lid makes a good container for inoculating small lots of seed.

With low quality seeds, chemical seed treatment is sometimes necessary to obtain acceptable stands. This presents another problem. Most chemical seed protectants will kill nodule bacteria applied to seed but will not harm rhizobia in the soil. The inoculum can be diluted with any inert organic material or screened soil and applied directly to the soil rather than to the seed. Another alternative is to inoculate a companion seed not treated with a fungicide and which is included in the seed mixture.

In many instances leguminous seeds are planted in seed flats or jiffy pots before transplanting to the field. The inoculum can easily be added directly in the seed furrows along with the seed. No further inoculation is necessary when the seedlings are transplanted to the field.

Special Methods of Inoculating Under Adverse Conditions

Busy schedules and labor shortages sometimes make it necessary to plant leguminous seed in dry, hot soils in anticipation of an early rain. While seeds can maintain vitality during sustained drought periods, the nodule bacteria are less fortunate. A pelleting technique of inoculation has proven quite effective under such conditions. Larger inocula are used and made into a slurry. Four or five times the usual amount of inoculum (18 grams per kilogram of seed) is mixed with a gum arabic solution (43% gum arabic) and applied to the seed in a fruit or peanut butter jar. The wet seeds are then mixed quickly with a very fine calcium carbonate powder (0.5 kg. $CaCO_3$ to 1.0 kg. seed) which coats and enables separation of the seeds for easy planting. Rhizobia encased in the pellet can usually withstand the hazards of hot, dry soils for 2 to 3 weeks in sufficient numbers to bring about effective nodulation when rain comes. When soils are not highly acid, the seeds can be coated with alfalfa meal, pulverized wheat straw, additional powdered inoculant, or even dry screened soils. Excellent results are usually obtained.

Development of New Inoculants

The Nitragin collection of rhizobia comprises around 1,150 strains of rhizobia obtained from 171 species in 61 genera of the family Leguminosae. While this collection is probably one of the largest, it represents only a small percentage of the 12,000 species of leguminous plants. Undoubtedly there are species of legumes indigenous to the Midwest Prairie for which there is currently no effective inoculum or strains of rhizobia for preparing one. This problem can be solved by a cooperative effort.

When new species of legumes are discovered and they are effectively nodulated, we would greatly appreciate receiving nodules so that new strains of effective rhizobia can be isolated and supplied to others who may be interested in growing these particular leguminous species. The roots should be washed quickly and segments of roots with large, healthy nodules on them should be cut, wrapped in moistened paper, put into a plastic bag, and mailed immediately to our laboratory. The rhizobia in these nodules will be isolated, cultured, tested for nitrogen-fixing ability, and used in preparation of inocula for you and your colleagues. Many of the inocula listed in Table 1 were made possible by this cooperative approach. Provision of effective rhizobia for the wild leguminous species is not done for profit but as a service to all who are working with this wonderful family of plants which are important in so many ways. They not only provide abundant supplies of protein food for man but they feed the birds and they enhance the beauty of the countryside.

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TABLE I. Symbiotic properties and appropriate rhizobia inocula for leguminous species found in the tall-grass region of North America.

LEGUMINOUS SPECIES	NODULATES	EFFECTIVE SYMBIOSIS	INOCULUM
*Alysicarpus vaginalis DC (alyceclover)	Yes	Yes	EL
Amorpha L. (amorpha)			
canescens Pursh (leadplant)	Yes	Yes	Sp.
fruticosa L. (indigobush a.)	Yes	Yes	Sp.
*Anthyllis vulneraria L. (kidneyvetch)	Yes	Yes	K
Astragalus L. (milkvetch; poisonvetch)			
canadensis L. (Canada m.)	Yes	Yes	Sp.
*cicer L. (cicer m.)	Yes	Yes	Sp.
*pectinatus (Hook.) Dougl. (narrowleaf sp.	Yes	Yes	Sp.
Baptista Vent. (wild indigo)			
australis L. (blue w.)	Yes	Yes	EL
leucantha T. & G. (Atlantic w.)	Yes	Yes	EL
leucophaea Nutt. (plains w.)	Yes	Yes	EL
tinctoria L. (yellow w.)	Yes	Yes	EL
*Cajanus cajan Millsp. (pigeonpea)	Yes	Yes	EL
*Caragana arborescens Lam. (Siberian pea-shrub)	Yes	Yes	Sp.
Cassia L. (senna; partridgepea)			
fasciculata Michx. (showy p.)	Yes	Yes	Sp.
marilandica L. (wild s.)	Yes	Yes	Sp.
*Coronilla varia L. (crownvetch)	Yes	Yes	M
Crotalaria L. (crotalaria; rattlebox)			
sagittalis L. (arrow c.)	Yes	Yes	EL
Dalea alopecuroides Willd. (foxtail dalea)	Yes	Yes	Sp
Desmanthus Willd. (bundleflower)			
illinoensis (Michx.) MacM. (Illinois b.)	Yes	Yes	EL
Desmodium Desv. (tickclover)			
canadense (L.) DC. (Canada t.)	Yes	Yes	EL
canescens (L.) DC. (hoary t.)	Yes	Yes	EL
*Dolichos lablab L. (hyacinthbean)	Yes	Yes	EL
*Hedysarum coronarium L. (sulla; sweetvetch)	Yes	Yes	Sp.
Hosackia americana (Nutt.) Piper (prairie trefoil; deervetch)	Yes	Yes	Sp.
Lathyrus L. (peavine)			
*hirsutus L. (rough p.)	Yes	Yes	C
japonicus L. (maritime p.)	Yes	Yes	C
*latifolius L. (perennial p.)	Yes	Yes	C
ochroleucus Hook. (cream p.)	Yes	Yes	C
*odoratus L. (sweetpea)	Yes	Yes	C
*sylvestris L. (flat p.)	Yes	Yes	C
*tingitanus L. (tangierpea)	Yes	Yes	C
venosus Muhl. (veiny p.)	Yes	Yes	C
*Lens culinaris Medic. (lentil)	Yes	Yes	C
Lespedeza Michx. (lespedeza; bushclover)			
bicolor Turcz. (shrub l.)	Yes	Yes	Sp.
capitata Michx. (roundhead l.)	Yes	Yes	EL
*cuneata (Dumont) G. Don (sericea l.)	Yes	Yes	Sp.
*daurica (Laxm.) Schindl. var. schimadae (Masam.) M. & Hosok. (rush l.)	Yes	Yes	Sp.
*hedysaroides (Pall.) Kitagawa (bush l.)	Yes	Yes	Sp.
hirta (L.) Hornem. (hairy l.)	Yes	Yes	EL
leptostachya (L.) Engelm (slender-spiked l.)	Yes	Yes	EL
procumbens Michx. (trailing l.)	Yes	Yes	Sp.
repens (L.) Bart. (creeping l.)	Yes	Yes	Sp.
Lotus L. (trefoil; deervetch)			
*corniculatus L. (birdsfoot t.)	Yes	Yes	K
pedunculatus L. (wetland d.)	Yes	Yes	Sp.
*tenuis Waldst. and Dit. ex. Willd. (narrow leaf b.t.)	Yes	Yes	K
americanus (Nutt.) Bisch. (See Hosackia)			

TABLE I. (Continued)

LEGUMINOUS SPECIES	NODULATES	EFFECTIVE SYMBIOSIS	INOCULUM
Lupinus L. (lupine)			
*albus L. (white l.)	Yes	Yes	H
*angustifolius L. (blue l.)	Yes	Yes	H
*luteus L. (yellow l.)	Yes	Yes	H
perennis L. (perennial l.)	Yes	Yes	H
piattensis S. Wats. (Nebraska l.)	Yes	Yes	H
Medicago L. (medic; burclover; alfalfa)			
*arabica (L.) Huds. (spotted b.)	Yes	Yes	Sp.
*falcata L. (yellow a.)	Yes	Yes	A
* lupulina L.(black m.)	Yes	Yes	Sp.
*sativa L. (alfalfa)	Yes	Yes	A
Melilotus Mill. (sweetclover)			
*alba Desr. (white s.)	Yes	Yes	A
*officinalis (L.) Lam. (yellow s.)	Yes	Yes	A
Oxytropis lambertii Pursh (lambert crazyweed)	Yes	Yes	Sp.
Petalostemum Michx. (prairie clover)			
candidum (Willd.) Michx. (white p.)	Yes	Yes	Sp.
purpureum (Vent.) Rydb. (purple p.)	Yes	Yes	Sp.
Phaseolus L. (bean)			
*angularis Wight (adsuki b.)	Yes	Yes	EL
*aureus Roxb. (mung b.)	Yes	Yes	EL
*lunatus L. (lima b.)	Yes	Yes	Sp.
*vulgaris L. (common b.)	Yes	Yes	D
Pisum L. (pea)			
*arvense (L.) Poir. (field p.)	Yes	Yes	C
*sativum L. (garden p.)	Yes	Yes	C
Psoralea spp. L. (scurfpea)	Yes	Yes	Sp.
Robinia L. (locust)			
hispida (bristly l.)	Yes	Yes	Sp.
pseudo-scacia L. (black l.)	Yes	Yes	Sp.
Strophostyles Ell. (wildbean)			
helvola (L.) Ell. (trailing w.)	Yes	Yes	Sp.
pauciflora L. (leiosperma (T&G) Piper) (small w.)	Yes	Yes	Sp.
Tephrosia virginiana (L.) Pers. (goatstrue; Virginia tephrosia)	Yes	Yes	Sp.
Trifolium L. (clover)			
*hybridum L. (alsike c.)	Yes	Yes	B
*pratense L. (red c.)	Yes	Yes	B
*repens L. (white c., Dutch c.)	Yes	Yes	B
Vicia L. (vetch)			
americana Muhl. (American v.)	Yes	Yes	C
caroliniana Walt. (Carolina v.)	Yes	Yes	C
*cracca L. (bird v.)	Yes	Yes	Sp.
*dasycarpa Ten. (woollypod v.)	Yes	Yes	C
*faba L. (broadbean)	Yes	Yes	C
var. equina Pers. (V. faba L. subsp. major Alef.) (horsebean)			
*villosa Roth (hairy v.)	Yes	Yes	C
Gleditsia triacanthos L. (honeylocust)	No	No	--
Gymnocladus dioica (L.) K. Koch (Kentucky coffeetree)	No	No	--

*Species introduced from other continents.