Nodulation of Common and Endangered Legumes by Symbiotic Nitrogen-Fixing Bacteria Present in Native Illinois Prairie Soils

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Introduction

The legume-*Rhizobium* symbiosis is one of the most interesting and important plant-bacteria interactions in nature (1). Legumes are a diverse group that includes such economically important crop plants as soybeans and alfalfa and such native prairie plants as lead plant and prairie clover (2). Bacterial species in the genera *Rhizobium* or *Bradyrhizobium* (collectively referred to as rhizobia) are normal components of most soils and can infect the roots of leguminous plants. Infection of a given legume is highly specific, requiring a specific rhizobial species, and leads to the formation of root nodules that are capable of nitrogen fixation. In nodules, bacterial symbiots provide the plant with reduced nitrogen in the form ammonia while receiving carbon and energy from the host plant (3). Thus, symbiotic nitrogen fixation by nodulated leguminous plants is a metabolic process that is vital to both agricultural and native terrestrial ecosystems. However, to date, most research has centered on the legume-*Rhizobium* symbiosis in crop plants and its overall influence on crop yields.

Little, if any, information is available on the types of rhizobia that nodulate prairie legumes or the potential impact that these symbiotic nitrogen-fixing bacteria may have on the growth, reproduction, and competitiveness of common and endangered legumes in their native habitats. Research proposed in this project is aimed at studying the metabolic potentials and host-specificity of rhizobia isolated from the nodules of common and endangered leguminous prairie plants in Illinois.

Astragalus crassicarpus, an endangered plant species in Illinois, occurs in dry rocky prairies, glades, gravel prairies, open woods, and blufftops. This species was thought to be extirpated from Illinois until it was discovered in 1987 along a limestone bluff in Jersey County. This species was also rediscovered in Macoupin County. Neither population is protected (4). The reason why *Astragalus crassicarpus* is endangered in Illinois is presently unknown. This study will focus on the symbiosis between *Astragalus* crassicarpus and rhizobia, and how this relationship might impact the population size of *Astragalus crassicarpus*.

For example, the rhizobia that infect the roots of *A. crassicarpus* may require special environmental conditions for growth and survival. Or, neighboring plants and their associated microbial populations that are needed to infect *Astragalus crassicarpus* are rare. Also, the environment may prevent the *Astragalus crassicarpus* - rhizobia symbiosis, and the plant only survives in areas where nutrients are available (e.g., when supplemented with fertilizer in runoff from agricultural fields). Hopefully this study will help shed some light on the nature of the interactions between rhizobia and *Astragalus crassicarpus* as well as other common and endangered prairie legumes.

Objectives

- To screen various bait soils collected from different various prairie field sites for rhizobia that can infect and nodulate the roots of endangered and common prairie legumes
- To determine the frequency of root nodulation of trap host species by microorganisms present in prairie bait soils
- To isolate and identify the rhizobia present in the nodules of *Astragalus crassicarpus* and common prairie legumes used as trap host species

Materials and Methods

Field Sites, Bait Soil Collection, and Sources of Leguminous Seeds. The main prairie field site for the collection of plants, soil, and seeds was located at Beaver Dam State Park. Soil was also collected from two other prairies sites within Illinois: Green River Conservation Area and Loxa Prairie. Soil samples were collected, transferred to a plastic container, sealed, transported to the laboratory., and were stored at 4°C in the dark until subjected to analysis**.** Seeds of *Astragalus crassicarpus* were collected from Beaver Dam State Park. Seeds of *Amorpha canescens*, *Lespedeza virginica*, and *Petalostemum candidum* were purchased from Prairie Moon Nursery (Winona, MN).

Seed Preparation and Germination. Seed surfaces were sterilized then rinsed three times in sterile water, then scarred by nicking them with a sterile scalpel. Pots were sterilized in a bleach solution, allowed to dry, then filled 3/4 full with potting soil. Three seeds for a given plant species were added to a pot; 5 pots were used per plant species per bait soil type. Pots were watered with deionized water every other day, and N-containing 1/4 strength Hoagland's Nutrient Solution was added once a week. Four weeks after germination, pots were watered with N-free Hoagland's Nutrient Solution and was used once per week for two weeks or until the bait soil was applied.

Bait Soil Studies. A slurry of each prairie soil was made by mixing 10 g of soil in deionized water and applied to each pot at the rate of 5% of the volume of the pot. Plants were watered daily, and, once per week for four weeks, received the N-free Hoagland's Nutrient Solution. After four weeks, plants were removed from potting soil. Root systems were examined for the presence of nodules as well as the extent of root nodulation.

Isolation and Identification of Rhizobia from Root Nodules. Nodules were removed from the plant roots, rinsed with tap water, and surface sterilized. Nodules were transferred to a sterile saline solution and crushed with a sterile glass rod. Material was then streaked onto yeast-extract mannitol agar (YEMA) plates and incubated at 250C. Colonies presumed to be rhizobia were restreaked on YEMA plates to check for purity.

Results

Field Studies. Root systems collected from *Astragalus crassicarpus* and common prairie legumes from several areas at the field site at Beaver Dam State Park did not possess nodules.

Preliminary Greenhouse Studies with Bait Soil from Beaver Dam State Park. When *Astragalus crassicarpus* was inoculated with soil collected from Beaver Dam State Park, 14 of the 15 plants that were inoculated developed nodules on their root system (data not shown).

Greenhouse Studies with Bait Soil from Different Prairies and Different Trap Host Plants.
When *Astragalus crassicarpus* was inoculated with soil collected from different prairie sites, only microorganisms present in the Beaver Dam State Park were able to produce nodules on/in the roots of this endangered leguminous plant (Table 1). Microbes present in Beaver Dam State Park were also able to nodulate *Petalostemum candidum* though nodulation was limited to one plant (Table 1). Microbial populations in Loxa and Green River Conservation Area soils failed to produce nodules on *Astragalus crassicarpus* or on any of the more common prairie legumes (*Amorpha canescens*, *Lespedeza virginica*, and *Petalostemum candidum*) (Table 1).

Microbiological Studies. Nine isolates were obtained from the nodules of *Astragalus crassicarpus* and one isolate from the nodules of *Petalostemum candidum*. Based on growth characteristics on YEMA plates, these isolates have been presumptively identified as rhizobia.

Table 1. Nodulation of Endangered(*Astragalus crassicarpus*) and Common (*Amorpha canescens*, *Lespedeza virginica*, and *Petalostemum candidum*) Prairie Legumes with Soil from Different Prairies

Prairie Legume (Trap Host Species)	Prairie Soil (Bait Soil)	Number of Pots Containing Nodulated Plants (total number of pots examined)	Number of Nodulated Plants (total number of plants examined)	Average Number of Nodules Per Nodulated Plant
Astragalus crassicarpus	Control	0 (4)	0 (6)	NA[a]
	Beaver Dam	4 (5)	8 (11)	5
	Loxa	0 (4)	0 (7)	NA
	Green River	0 (4)	0 (5)	NA
Amorpha canescens	Control	0 (4)	0 (4)	NA
	Beaver Dam	0 (4)	0 (5)	NA
	Loxa	0 (3)	0 (4)	NA
	Green River	-[b]	-	-
Lespedeza virginica	Control	-	-	-
	Beaver Dam	-	-	-
	Loxa	0 (1)	0 (1)	0 (1)
	Green River	-	-	-
Petalostemum candidum	Control	0 (4)	0 (7)	NA
	Beaver Dam	1 (3)	1 (3)	4
	Loxa	0 (4)	0 (6)	NA
	Green River	0 (5)	0 (6)	NA

a NA, not applicable. None of the plants were nodulated.
b No data was obtained because of high plant mortality. In these experiments, seeds germinated but none of these plants survived the duration of the experiment because of high temperatures which often occurred in the greenhouse during the Spring (2001) months.

Conclusions

- Microbial populations in soils from Beaver Dam State Park were competent in the nodulation of the endangered legume *Astragalus crassicarpus*.
- Soils from prairies at Loxa and Green River Conservation Area failed to produce nodules on *Astragalus crassicarpus*; both of these sites are not inhabited by *Astragalus crassicarpus*.
- Microbial symbionts in prairie soils from Beaver Dam State Park, Loxa, and Green River Conservation Area were generally deficient in their ability to nodulate common prairie legumes (*Amorpha canescens*, *Lespedeza virginica,* and *Petalostemum candidum*).
- Understanding prairie legume-rhizobial symbioses and how these symbioses impact the distribution of leguminous plant populations, especially endangered legumes, will help us to define strategies in the future for the conservation of our native prairie plant populations.

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