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Legumes May Be Symbiont-limited During Old-field Succession

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ABSTRACT.—We employed a bioassay using soybean seeds and native prairie bush clover (*Lespedeza capitata*) seeds to demonstrate that legumes may be rare throughout secondary succession in nitrogen-poor grasslands due to a lack of suitable rhizobia and consequently lower growth rates.

INTRODUCTION

In many systems (Vitousek and Howarth, 1991), including Cedar Creek Natural History Area (Tilman, 1984, 1987), nitrogen is the nutrient most limiting plant growth, and through succession soil nitrogen concentration increases (Gorham *et al.*, 1979; Inouye *et al.*, 1987). At low soil nitrogen concentrations, nitrogen-fixing legumes can be competitively superior to nonlegumes. However, studies at Cedar Creek have found that legumes are always rare and their abundance is unrelated to field age and soil nitrogen content (Inouye *et al.*, 1987). Currently there is no clear explanation for their rarity, although herbivores (Ritchie and Tilman, 1995), low relative growth rates (Tilman, 1982) and higher requirements for other nutrients compared to nonleguminous plants (Tilman, 1982) may have an effect. They may also be at a competitive disadvantage due to a lack of compatible rhizobia that limit the formation of nodules.

Rhizobia differ in their ability to infect different species of plants (Bergey *et al.*, 1984; Paul and Clark, 1989; Prescott *et al.*, 1996). After abandonment of a field previously planted with soybeans, there may initially be an abundance of rhizobia. Through time, the numbers of these rhizobia may decline if their corresponding symbiont is not present (Paul and Clark, 1989; Kucey and Hynes, 1989). Therefore, we hypothesized that the ability of soybeans to form nodules will decrease with time since last cultivation. Because rhizobia for native legumes may come from accumulation of rhizobia in the plant-root environment of colonizing legumes (Weaver *et al.*, 1971), we hypothesized that the number of nodules on roots of native prairie legume seedlings may increase with time since abandonment. In order to estimate the nodulation potential of legumes in fields of different successional ages, we used a bioassay employing *Lespedeza capitata* (bush clover, a common native prairie legume at Cedar Creek) seeds and soybean seeds.

MATERIALS AND METHODS

This work was performed with soils from 20 grassland fields at Cedar Creek Natural History Area which is located approximately 50 km N of Minneapolis, Minn. One field was last planted with potatoes, three with corn, one with oats, eleven with soybeans, four with rye and the crop history of one field is unknown (Inouye *et al.*, 1987). Field ages were 10, 19 (four fields), 21, 31, 35, 38, 39 (two fields), 44 (two fields), 45 (two fields), 46, 53 (three

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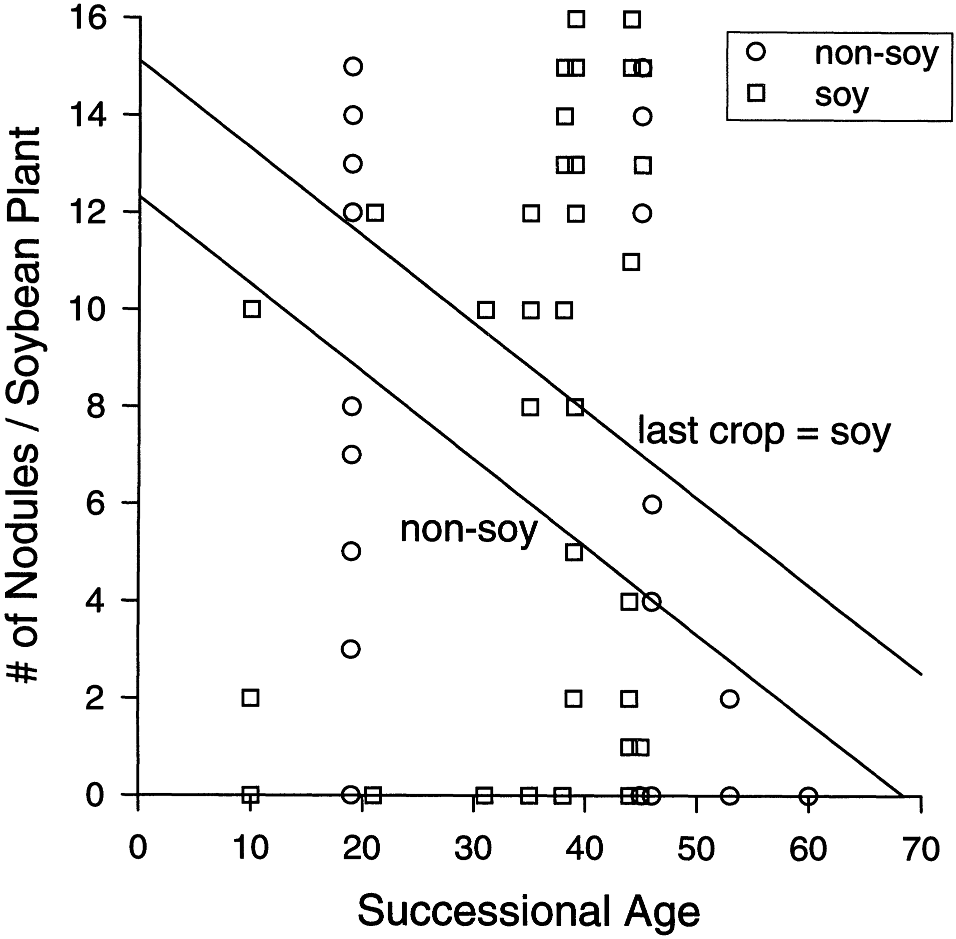

FIG. 1.—The number of root nodules per soybean plant versus the successional age (yr) of the soil in which they were grown. The lines on the planes at the rear of the figure are from a multiple regression with field age and last crop as predictors

fields) and 60 yr (Inouye *et al.*, 1987). All fields had sandy soils that belong to the same taxonomic group, a Typic Udipsamment (Grigal *et al.*, 1974; Johnson *et al.*, 1991).

In July 1996, five pairs of 2.5 cm × 30.5 cm soil cores were taken at 1.5 m intervals along an existing 40-m transect in each of the 20 fields (same as used by Inouye *et al.*, 1987). The two soil samples within each pair were mixed and split into two samples, one for a *Lespedeza* seed and one for a soybean seed (five of each species for each field). In order to limit the bias of nodule growth results, the sample location was first surveyed for the presence of legumes within 1 m. If legumes were present in the desired collection location, the sample was taken from 1 m away. Because legumes were rare in these fields, this eliminated the bias from sampling at a legume but may have biased our rhizobia abundance estimates to be slightly too low.

Before planting, the *Lespedeza* and soybean seeds were surface sterilized to reduce rhi-

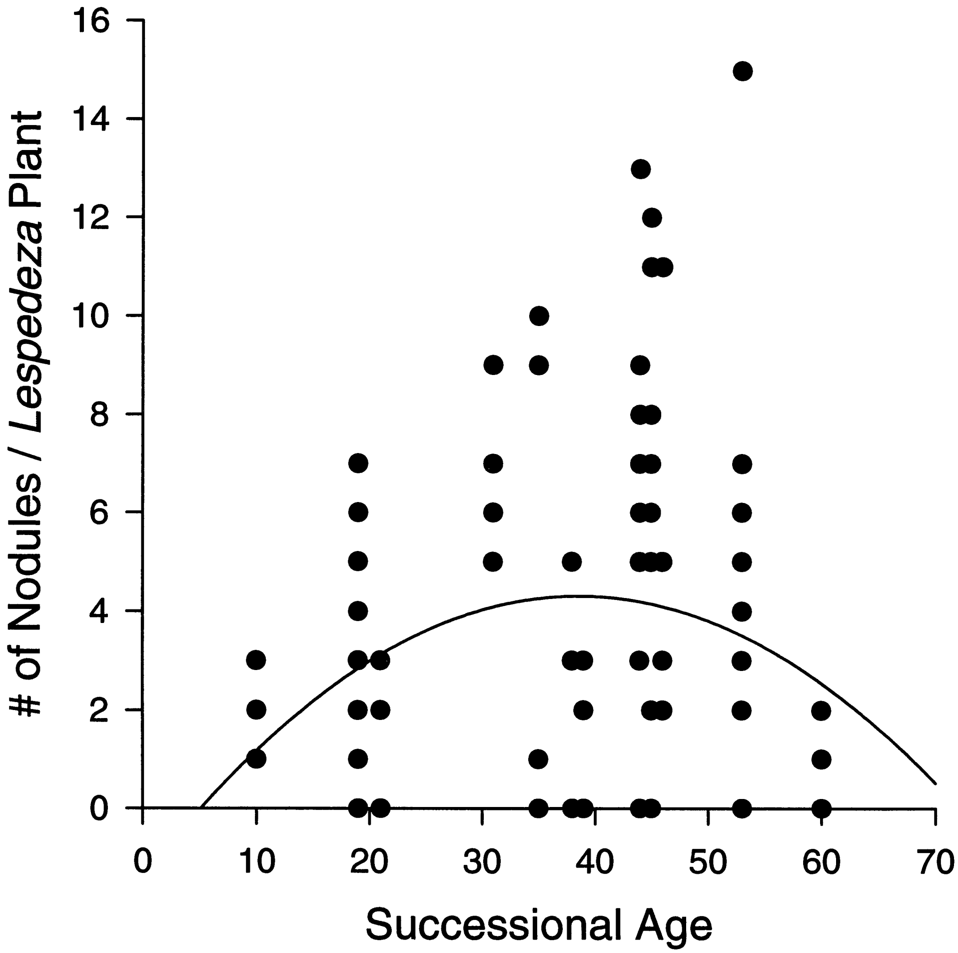

FIG. 2.—The number of root nodules per *Lespedeza* plant versus the successional age (yr) of the soil in which they were grown. The fitted curve is from a quadratic regression

zobia numbers from the seed surface, and scarified to increase the germination of the seeds using the sulfuric acid soak method (Somasegaran and Hoben, 1994). The seeds were then planted into "cone-tainers," each of which contained soil from a single core and a small plug of sterile potting soil to retain the soil samples. The soybean and *Lespedeza* seedlings were allowed to grow under standard greenhouse conditions for 30 and 60 days, respectively. There were up to four replant dates, arbitrarily chosen at 3-wk intervals, for those individuals that either failed to germinate or that died following germination.

Following the allotted growth period, we washed the roots of each plant and counted nodules on the entire root system of each plant. The plants were then dried and weighed.

All regressions were Ordinary Least Squares (OLS) regressions.

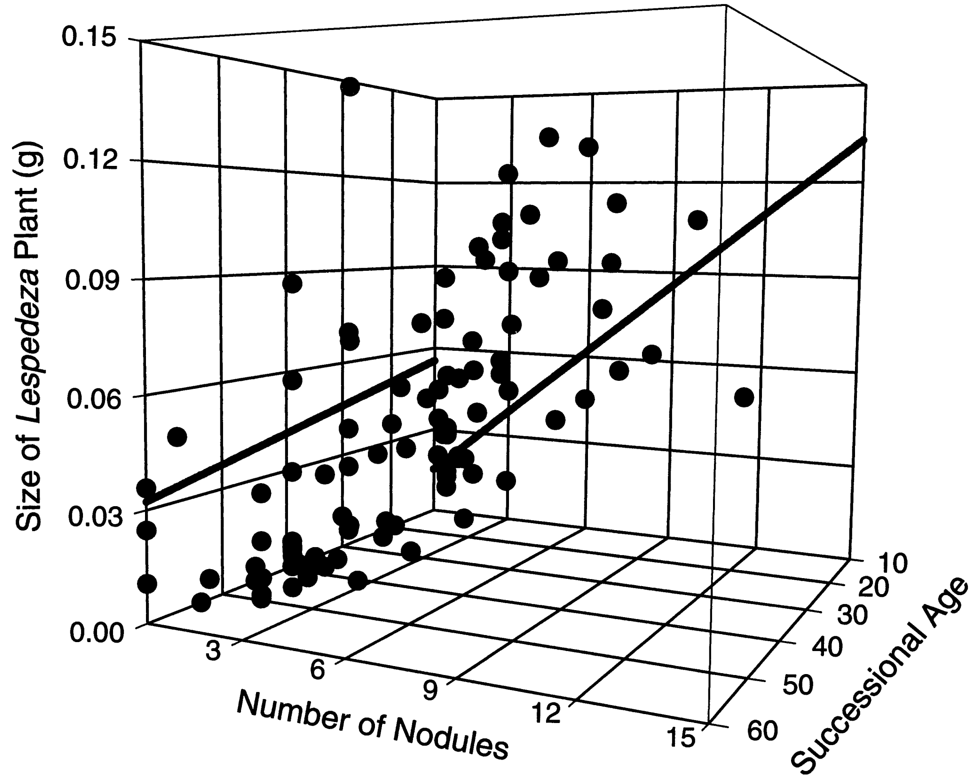

FIG. 3.—The sizes of *Lespedeza* plants with different numbers of root nodules and grown in soils of different successional ages (yr). The lines in the back planes show the conditional dependencies of *Lespedeza* plant size on numbers of nodules and successional age in a multiple regression that included both predictors

RESULTS

The number of nodules per soybean plant decreased significantly with field age (t-test: $P < 0.001$) and was significantly higher for fields last planted with soybeans (crop: 1 = soy, 0 = not soy; t-test: $P = 0.05$). (Soy # = 12.3 − 0.18 age + 2.79 crop, $P < 0.001$, $N = 100$, $R^2 = 0.16$, Fig. 1). Soybean plant size was significantly positively correlated with the number of nodules [Size (mg) = 185 + 22.6 Soy #, $P < 0.001$, $N = 100$, $R^2 = 0.50$].

The number of nodules per *Lespedeza* plant was significantly higher in intermediate age fields (Lesp # = −1.40 + 0.30 age − 0.0038 age^2, $N = 100$, $R^2 = 0.06$, P(age, age^2, regression) < 0.05, Fig. 2). *Lespedeza* nodule number was independent of the last crop planted in the fields ($P = 0.39$). *Lespedeza* plant size was significantly positively correlated with number of nodules (Size = 16.1 + 7.6 Lesp #, $P < 0.001$, $N = 100$, $R^2 = 0.45$). When nodule number and field age were both included in a multiple regression, *Lespedeza* plant size decreased significantly with age (t-test: $P < 0.05$) and increased significantly with nodule number (t-test: $P < 0.001$). [Size (mg) = 3.2 − 0.46 age + 7.9 Lesp #, $P < 0.001$, $N = 100$, $R^2 = 0.48$, Fig. 3].

DISCUSSION

We used nodule number as an indicator of the relative abundance of compatible rhizobia in the soil through succession. The number of nodules per soybean plant decreased significantly with field age and was significantly higher for those fields last planted with soybeans (Fig. 1). In younger fields, especially those previously planted with soybeans, one might expect a greater number of rhizobia compatible with soybean seedlings (*Bradyrhizobium japonicum*, Bergey *et al.*, 1984) than in older fields and those not last cultivated with soybeans. Our regression results suggest that only half as many of these soybean compatible rhizobia are left in the soil after 30–40 yr.

The opposite might be expected with *Lespedeza*, which are incompatible with soybean rhizobia (Bergey *et al.*, 1984); older fields would have more rhizobia compatible with *Lespedeza* (a promiscuous *Bradyrhizobium* species, Bergey *et al.*, 1984) due to their accumulation in the soil over time with the continued presence of the corresponding legume(s). We found a low number of nodules on plants grown in soil from young fields but also, surprisingly, found a low number of nodules in soils from old fields (Fig. 2). Such a slow increase might be expected when legumes are rare and occur by chance in different locations in different years. The low numbers in soils from older fields suggest that older fields may be poor environments for rhizobia or nodulation.

Controlling for the relationship between number of nodules and *Lespedeza* plant size, *Lespedeza* seedlings grown in soils from older fields were significantly smaller (Fig. 3). This may be due to several factors including lower inorganic nitrogen concentrations (Zak *et al.*, 1990), lower concentrations of micronutrients (Ritchie and Tilman, 1995), and/or increased occurrence of soil pathogens (Van der Putten *et al.*, 1993). These factors, together with the low abundances of compatible and effective rhizobia in early succession, may limit the rate of succession on nutrient-poor soils by limiting the abundance of nitrogen fixing plants (Connell and Slayter, 1977; Gorham *et al.*, 1979).

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