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Soybean Inoculation: Understanding the Soil and Plant Mechanisms Involved (Part one of a two-part series)

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This NebGuide explains how soybean inoculation occurs and which environmental conditions impact nitrogen fixation.

Nitrogen gas (N₂) comprises nearly 80 percent of total atmospheric gases, yet most organisms are unable to use N₂ as a source of nitrogen. Legumes, such as soybean, are able to capture atmospheric nitrogen and utilize it through the process of nitrogen fixation. This NebGuide is part one of a two-part series on soybean inoculation. Here, we will investigate how soybean inoculation occurs and which environmental conditions impact nitrogen fixation.

Introduction

The nitrogen fixation process depends on a successful symbiotic relationship between a bacteria species and a host plant. Soybean acts as the host plant and recognizes *Bradyrhizobium japonicum* when it is placed nearby in the soil. Other nitrogen fixing plants recognize different bacteria species. For example, *Rhizobium meliloti* can only fix nitrogen within an alfalfa crop. Therefore, it is not possible to use inoculant products across most crops. Soybean inoculated with *B. japonicum* will form structures called nodules on the plant's roots. These nodules house the bacteria. Atmospheric nitrogen (N₂) is converted by *B. japonicum* to ammonia (NH₃) which the soybean plant can use.

How much nitrogen can *B. japonicum* convert?

The amount of nitrogen fixed by *B. japonicum* varies throughout the season based on the plant's growth stage and its demand for nitrogen. Following emergence and up to 20 days afterward, soybean seedlings may appear nitrogen deficient. This yellowing can occur due to a time lag between when nitrogen stored in the seed is exhausted and active nitrogen fixation begins. This deficiency should not hinder overall plant development or final soybean yield.

Soybean uses large amounts of nitrogen throughout the growing season, with requirements ranging up to 315 pounds

per acre.¹ A substantial amount of this is required during the reproductive stages of R1 (initial flowering) to R5 (beginning seed set). Pod fill (R3 and R4) requires the greatest amount of nitrogen and *B. japonicum* respond by converting approximately 3.0 lbs of nitrogen per acre per day. At 50 to 75 days after emergence (approximately R2 to R5), the soybean plant derives 60 to 70 percent of its needed nitrogen through the process of nitrogen fixation.

How do nodules form?

Nodules develop on soybean roots following a form of “communication” between the host root and bacteria. The process begins in the root with the production and release of compounds (isoflavonoids) which are recognized by the bacteria. The *B. japonicum* respond by expressing “nod factors” (lipochitooligosaccharides) which eventually lead to root hair modification allowing the *B. japonicum* to enter. After the bacteria have invaded the root hair, they multiply rapidly. Nodules, which individually house thousands of *B. japonicum* cells, can be visible within one week after the time of infection. Nodule quantity as well as nodule color are two primary factors considered when determining if nodulation is adequate. In good conditions, a well-nodulated soybean plant should have five to seven nodules on the tap root two weeks after emergence or twelve nodules per inch of tap root at flowering (R1). To evaluate nodule performance, cut nodules in half. Nodules that are actively fixing nitrogen will be colored pink to bright red, while nodules that are white or green are ineffective or have not become active yet.

Once nodules form, they can capture atmospheric nitrogen (N₂) and convert it to ammonia (NH₃). Ammonia proceeds through a series of biochemical conversions within the nodule, resulting in nitrogen-rich compounds called ureides. Ureides are the major form of nitrogen exported from soybean nodules and transported to the above-ground plant for use.

¹Nutrient Management for Agronomic Crops in Nebraska, 2000. University of Nebraska–Lincoln Extension EC155 (p. 125).

What soil factors affect *B. japonicum* production and efficiency?

The soil environment can impact the ability of *B. japonicum* to infect soybean root hairs, develop nodules and fix nitrogen. A suitable environment for *B. japonicum* is obtained when multiple soil factors are positive for bacterial growth and productivity. Soil characteristics that need to be considered in terms of *B. japonicum* survival are pH, temperature, texture, water content and residual nitrogen levels. The optimal soil pH is between six and seven. A soil pH significantly outside this range (less than five or greater than eight) is detrimental because it disrupts the communication process that needs to occur for root hair infection, thereby limiting nodule development. Soil pH also affects the amount of nitrogen fixed. For example, in a very acidic soil (pH = 4.4), nitrogen fixation can be reduced up to 30 percent.

Extreme root temperature can have a negative impact on the ability of *B. japonicum* to communicate with the soybean plant and infect the roots. Optimum root temperatures for nitrogen fixation and soybean growth range from 77° to 86°F. In this temperature range, the bacteria can begin actively fixing nitrogen within seven days of forming nodules. When soils are cooler than this, which is common through the spring in the Midwest, both nodule formation and nitrogen fixation will be slowed. Producers often plant soybean in May. On average, soils in south central Nebraska reach 60°F by May 15 (see Figure 1).

A continually cool root zone temperature can significantly delay the onset of nitrogen fixation compared to an optimum soil temperature (see Figure 2). For example, researchers found that soil held at 60°F did not begin fixing nitrogen until 31 days after a soil at 77°F had started.

Sandy soils are most detrimental to supporting *B. japonicum*

populations long-term. In research with extremely sandy soil (95 percent sand), the survivorship of multiple bacteria species was reduced compared to soil that had clay mixed with the sand. Lower soil water holding capacity and wider

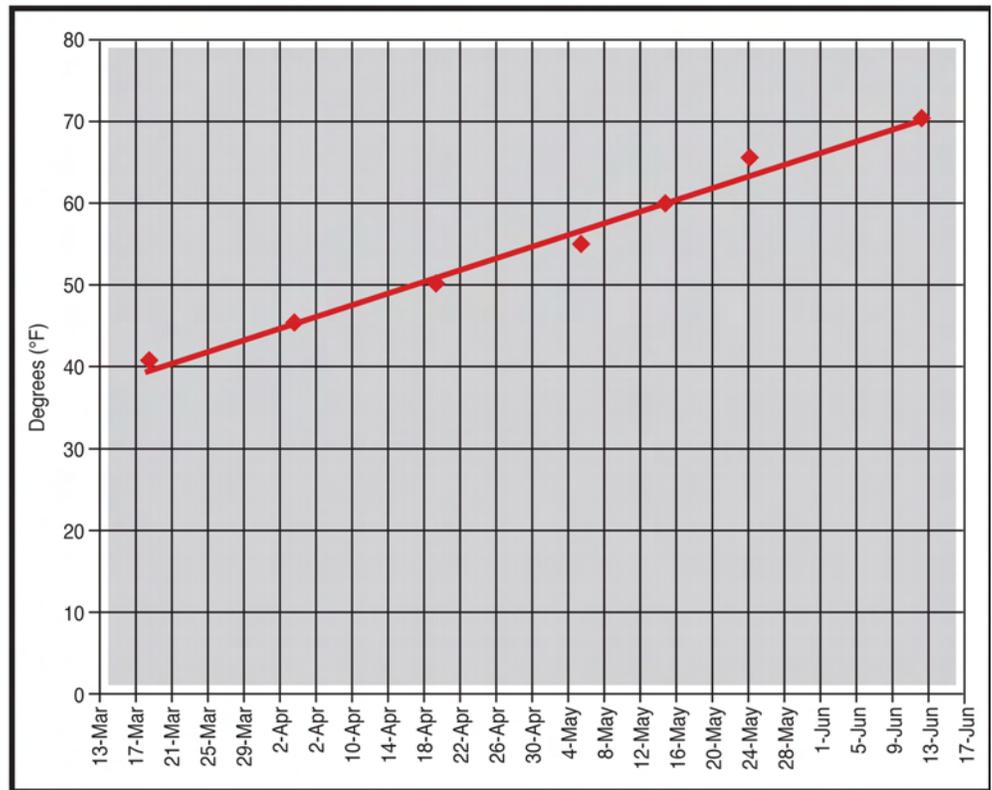


Figure 1. Soil temperature (4-inch depth) in south central Nebraska, 10 year average (1987-1996). Reference: *Soil Temperatures and Spring Planting Dates*. Meyer and Dutcher. University of Nebraska–Lincoln Extension NebGuide G1362.

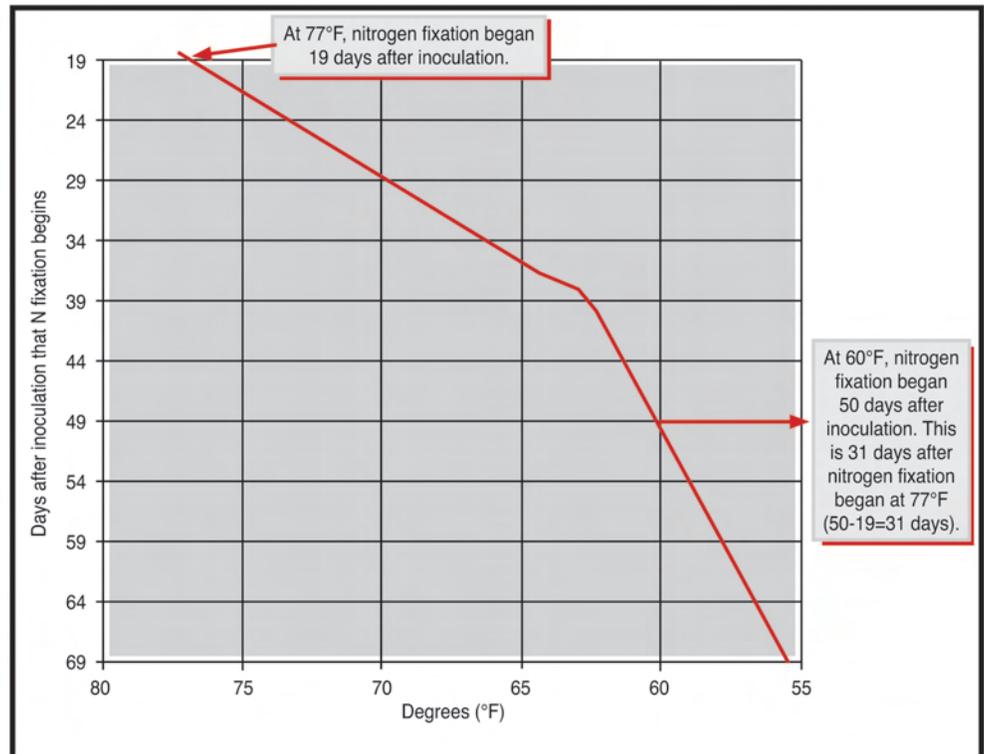


Figure 2. Cool soil temperatures delay nitrogen fixation. Reference: Zhang, Lynch, and Smith. *Impact of low root temperatures in soybean [Glycine Max. (L.) Merr.] on nodulation and nitrogen fixation*. 1995. Environmental and Experimental Botany.

fluctuations in soil moisture are the primary factors reducing bacterial populations in sandy soils. Yet, research is limited in defining a suitable range of soil moisture for *B. japonicum*. Survival of *B. japonicum*, as well as their ability to fix nitrogen, is reduced as soil dries. Initially, as soils dry (especially if soil moisture is below 50 percent of field capacity) the total bacteria count decreases. Normal wetting and drying of soils is expected, though, and if fields are under irrigation or suitable rain-fed conditions, the soil moisture should not substantially limit bacteria survivorship or productivity. Excess soil water is also detrimental to *B. japonicum* as this reduces the amount of oxygen in the soil which is necessary for survival. If a field is flooded for seven days, nitrogen fixation can be inhibited up to 55 percent.

Nitrogen fixation is one of the most water-sensitive processes within soybean. Nodules have a porous surface yet are able to resist some water loss through alterations in their structure. In certain environmental conditions, such as severe drought or flooding, the plant will increase its proportion of nitrogen derived from fertilizer sources compared to the amount fixed by *B. japonicum*. This preference shift occurs due to differences in the amount of energy expended by the soybean plant. The plant must allocate a greater amount of energy to fix atmospheric nitrogen than what is required to use soil residual nitrogen or nitrogen fertilizer.

The effect of residual nitrogen in the soil cannot be over-emphasized as this plays a significant role in determining the amount of nodules formed as well as the amount of nitrogen fixed throughout the season. Although *B. japonicum* are efficient producers of nitrogen, the soybean still acts as a host

and is depleted of energy resources by the bacteria. Therefore, if soil residual nitrogen is high, we would not expect to see as many nodules on the roots as in a soil environment with deficient or normal nitrogen levels.

Nitrogen is highly mobile in the soybean plant, and when the plant is exposed to nitrogen fertilizer, the productivity and growth of nodules decreases within a day. This impact becomes greater as the rate of nitrogen fertilizer increases. *B. japonicum* responds to the demands of the above-ground plant by increasing or decreasing nitrogen fixation, depending on how much nitrogen is used by the plant. The bacteria's ability to compensate for the nitrogen status of the plant and soil is important in understanding why applying nitrogen fertilizer is detrimental to nodule formation and nitrogen fixation. In a few instances, yield increases have been observed when nitrogen fertilizer is applied during the growing season; however, in fields without severe water stress, a yield response is not expected from an application of nitrogen fertilizer, even during the plant's reproductive stages.

Please refer to G1622, *Soybean Inoculation: Applying the Facts to Your Fields* (part two of this two-part series). This NebGuide details research conducted in Nebraska on soybean inoculants and provides a method to determine if inoculation is needed in your fields.

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