Soybean Inoculation; its Science, Use and Performance

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The soybean is a legume who's seeds generally contains 37% to 45% protein by weight. Depending on the protein content, a bushel of soybeans will contain between three and four pounds of nitrogen. The production of a 60-bushel per acre crop requires in excess of 300 pounds of nitrogen. Some of the nitrogen comes from the oxidation of soil organic matter with the balance produced by bacteria residing in nodules on the plant's roots. Each legume species requires different bacteria to fix nitrogen from the air and produce ammonium for use by the plant.

The Inoculation Process

The bacterial strain of *Rhizobium* used by soybean is *Bradyrhizobium japonicum*, and the process by which it gains access to the plant is very complex, but fairly well understood. Soybean plants release chemical signals (flavanoids) into the soil as they germinate and the plants emerge. These signals are picked up by the rhizobia in the soil and in inoculation material, and produce a return signal "Nod factor signal" to the plant. This return signal results in the plants' roots preparing for infection by the bacteria. The Nod factor signal causes the root hairs to curl, trapping rhizobia that are present on the surface of the root. An infection thread develops, and the rhizobia multiply until they reach the inside of the root. The cells of the root also begin to divide and form a specialized structure called a nodule. The rhizobia continue multiplying inside the nodules increasing their size. The enzyme (nitrogenase) that fixes nitrogen from the air to form ammonium cannot function in the presence of oxygen. In order for nitrogen fixation to occur, the plant must produce Leghaemoglobin inside the nodule to absorb oxygen in the root to prevent it from interfering with the nitrogen fixation process. Since Leghaemoglobin is red, the inside of an actively fixing nodule is pink. Sugars produced in the leaves travel to the root system and move into the nodules to provide energy that the rhizobium uses to extract nitrogen from the air to make the nitrogen compounds which the plant then uses to produce protein. The better this process works, the greater the grain yield and amount of protein produced.

Both plant and bacteria genes govern the entire nitrogen fixation cycle. Stresses on the bacteria or the plant such as cold, flooding, drought, and low soil pH can all interfere with this process. Cold temperatures delay the recognition of both the plant and bacteria signals. The plants and bacteria have to produce more signals in order to begin nodulation, which delays the onset of nitrogen fixation. Once enough signal is received and nodules develop, ammonium is produced and provided to the crop.

Drought reduces the moisture in the soil that protects the bacteria while it lives on the seed surface after planting. A dry seedbed and dry seed will quickly draw moisture from the inoculation material causing the bacteria on the surface of the seed to dry and die. If enough bacteria die, there will be little nodule formation and insufficient ammonium production for a good yield. Other stresses, including low soil pH, seed treatment chemicals, and starter fertilizers, can also kill the bacteria or inhibit nodulation. In fact, soil nitrogen levels in excess of 30-50 lbs per acre can stop both nodulation and nitrogen fixation altogether.

Advances in inoculants

In 1995 we started a program at The Ohio State University to evaluate soybean inoculation materials and in 1996 we tested both the new sterile materials and the non-sterile materials that had been in use for many years. Averaged over five field tests the new sterile materials produced four times more yield increase

(0.6 vs 2.4 bushels per acre) than the non-sterile materials produced. After 1996 we discontinued testing the non-sterile materials due to their poor performance.

In recent years, inoculant manufacturers have focused their research and development efforts on finding ways to improve inoculants. One focus is improving strain selection and increasing the number of viable bacteria per gram of product. Combining strains of *Bradyrhizobium japonicum* that are most productive in different environments results in products that are productive over a wider range of environments. Other areas of focus have been on easing application through the development of liquids and improved dry products. Combining organisms that offer plant growth promotion hormones or disease control in conjunction with regular rhizobials is another new development. Other areas of interest are the biological signals that induce nodulation. Combining the signal compounds with inoculants also allows companies to speed up the inoculation process. The addition of "extenders" to inoculation materials allows the materials to be applied to seed up to thirty days or more before planting without loss of productivity if the seed is stored properly. The extender materials also extend the longevity of bacteria cells applied to seed that has been treated with fungicides and in some cases allows the combined application of fungicides and inoculation materials. High quality inoculants contain over 2 billion live bacteria per gram, and use at the recommended rate results in 800,000 to 1,400,000 bacterial cells per seed depending on seed size and quality of the inoculant material.

Caring for inoculation products
Rhizobia cells survive best at temperatures of 40 - 80 degrees F. Prior to application, inoculants should be stored in a cool place and out of direct sunlight. Packets exposed to sunlight during the planting season will overheat rapidly due to the greenhouse effect and all the bacteria can be killed in less than an hour of exposure. When transporting the inoculant to the field, try to keep it cool. The back of the cab and out of the sunlight is best, and under a parked truck in the cool shade when in the field. Sunlight is a problem of heat, not radiation, so keeping the inoculation material in its box will stop some heat absorption. Under normal conditions the shelf life of inoculation materials varies from a few months to two years depending on the formulation and additives in the product. Dry materials generally have shorter shelf lives than liquids, but some sterile products have a shelf life approaching that of liquids.

Applying inoculation materials
Before applying an inoculant, it is important to check for compatibility with chemical seed treatments, fungicides and insecticides. Chemical compatibility can vary with seed treatment brand. Anchor and Vitaflo 280 both contain the same active ingredients; Thiram and Vitavax; however, they react to inoculants very differently. Compatibility is measured as inoculant survival on untreated seed *vs* treated seed. Seed treatment compatibility is influenced by specific formulations of both the fungicide and the inoculation material and by application method, e.g. slurry application *vs* sequential and/or simultaneous applications. Inoculants with an "extender type" additive have significantly improved on-seed planting intervals.

Seed inoculated with a product not having an "extender material" should be planted as soon as possible after treatment (12 hours or less) so the bacterial cells will remain moist and survive long enough to infect soybean roots following germination. Seed inoculated with a product having an "extender" can be held up to 30 days before planting depending on the product and which fungicides may be present. Always check the inoculation label for fungicide compatibility and the recommended planting interval. Most inoculation companies are working to increase compatibility with fungicides to give producers more management flexibility in the use of inoculation materials. It is notable that some fungicides, such as Apron-Maxx RFC (Rhizobia Friendly Concentrate) are designed for lower Rhizobia Toxicity. When applying a fungicide or using fungicide treated seed, be sure the fungicide has dried before applying inoculation material to the seed. Some inoculation products may be mixed with some fungicides and applied to the seed together, and many of the good liquid inoculants allow a 4-hour tank mix with fungicides so they can be simultaneously applied from the same tank. For specific product information refer to the product label. Most companies provide compatibility information on their websites. www.agribiotics.com, www.americasbestinoculant.com, www.beckerunderwood.com, www.nitragin.com, www.philombios.com, www.percisionlab.com, www.tracechemicals.com, www.intxmicrobials.com,

When loading a drill or planter using an auger; liquid or dry inoculation materials should be added to the seed as it enters the auger for thorough application. While over-application of a dry inoculant should not be toxic to seed, as it can with chemical seed treatments, it increases the cost and may interfere with seed flow through the planter or drill. Seed inoculated at the recommended rate with peat inoculation materials will appear untreated from a distance. Twenty to forty small specks of inoculation material on each seed are adequate. Over-application of liquid products is also costly and may cause bridging in the seed box. Calibration of the application system is a must if the proper rate of inoculation material is to be applied. First determine the delivery rate of seed through the auger by weighing the amount of seed passing through the auger in 30 seconds. Then calculate the amount of inoculation material needed for that amount of seed and set the inoculation applicator to apply that amount of inoculation material in 30 seconds.

Inoculation test results

The Ohio State University soybean inoculation trials have been conducted in fields where one would not expect a yield increase from inoculation because the fields were in a corn-soybean crop rotation, had excellent surface and subsurface drainage, good fertility and an adequate soil pH. Our thought was that if inoculation was profitable in fields where yield increases should be minimal, then inoculation would be even more profitable in most other fields.

During the past eleven years we have conducted 64 field trials containing from four to over twenty inoculation products or treatments. In nine of these trials the soil was very hot and dry following planting and there was no yield increase due to inoculation. In other tests the fields became saturated for several days following planting which also resulted in no yield increase, so very dry or wet soil conditions interfere with the establishment of nodules. One year we had to move a test site at the last minute and ended up in a field that had been in continuous corn for eight years. The field was well drained, had good fertility, an organic matter content of about 1.5 percent, but a low pH. The yield increase due to inoculation at that site ranged from eight to eleven bushel per acre depending on the inoculation product. This trial indicates how valuable inoculation can be when there are few Rhizobium bacteria in the soil and the soil organic matter content is low.

The average yield increase from 64 Ohio trials is 1.94 bu/ac and has produced a profit of about 300 percent when beans were worth $6.00 per bushel and when the inoculation material costs $3.00 per acre**.** For most inoculation products, a one half bushel per acre yield increase is profitable. Yield increases of two to seven bushel per acre have been common in productive producer fields where the seed was inoculated properly and planted into moist soil and in a timely manner.

North Dakota State University tested 15 to 39 soybean inoculation materials yearly during 1999 through 2005 at their Carrington Research Extension Center. The average yield increase from those 155 product evaluations is 2.7 bu/ac, with a yield change due to inoculation ranged from -0.5 bu/ac in 2005 to 8.2 bu/ac in 2003. Michigan State University conducted soybean inoculation trials in 2003 and 2004 containing 12 and 13 treatments, respectively. The average yield increase for the six test sites in 2003 and five test sites in 2004 was 1.33 bu/ac and 0.72 bu/ac, respectively. Purdue

University has tested inoculation products for eleven years and reported average yield increases up to 2.4 bu/ac in some years and a long-term average yield increase of 1.0 bu/ac. In 2004, Iowa State University evaluated 12 inoculation materials at two locations. The effect of inoculation for the two sites combined ranged from –2.4 bu/ac to 4.4 bu/ac.

Over the long term, soybean inoculation appears to be profitable throughout the Midwest. Although yield increases are small in many fields with a history of soybeans, the cost to inoculate the seed is also small and the practice is profitable over the long term. As products continue to improve, the profitability from their use will also increase.

The Future

There have been major improvements in inoculation materials and in their performance during the past ten years. Soybean inoculation is a practice that more producers would use to increase their profits if its use were more convenient. The largest deterrent to inoculating soybeans is the calibrating of application equipment and taking time to treat the seed during the busy planting season. Custom application of inoculation materials by seed suppliers assures the proper application rate and releases the producer from the burden of in-field treatment. The ability to apply some inoculation products to seed well before planting offers a great convenience to both the crop producer and his seed supplier. Shifting the inoculation application function to seed processors will increase the use of inoculation as well as the material’s performance, and allow producers to concentrate on timely planting and would be a “win” for crop producers, seed processors, and inoculation companies.