Effect of Row spacing on *Bradyrhizobium japonicum* populations in Soybean fields

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Soybean is an agronomically important crop that was introduced into Manitoba in the early 1990's. In 1996, fewer than 800 acres of land were seeded with soybean, whereas in 2009, about 425,000 acres were seeded for with an estimated value of almost $120 million annually.

Soybean is a legume which forms a symbiotic relationship with the bacterium *Bradyrhizobium japonicum*. This symbiosis manifests itself as nodules formed on the roots of the soybean plants. A well nodulated plant can derive all the nitrogen necessary for growth from the bacteria.

A problem often encountered is having sufficient numbers of the correct Rhizobia species present as the seed is germinating so that effective nodules can develop in timely manner. This is usually circumvented by the application of inocula of Rhizobia either directly to the seed, or to the field at the time of planting.

Currently, the method for determining the amount of *B. japonicum* in the soil is dependant on culture methods that involve a "most probable number" (MPN) assay. The MPN test consists of taking samples of field soils and using them to inoculate surface sterilized soybean seed. The inoculum is also diluted up to million fold. After 3-4 weeks the plants are scored for the presence of nodules. Based on the number of plants that have developed nodules, a most probable number of *Rhizobium* can be determined.

With funding from Manitoba Pulse Growers Association we have been developing and optimizing a quantitative PCR test. Basically, the test involves extracting all the DNA from a small sample of soil and using specific primers that are designed to detect *B. japonicum*. If any *B. japonicum* DNA is present, it will be amplified, and the rate at which it is amplified can be used to determine the number of *B. japonicum* that are present in a sample. The entire assay, from extraction to enumeration, can be carried out within a day.

Development of a quantitative PCR test for *B. japonicum*. The development of the test involved the design of the specific primers as well as optimizing our ability to extract DNA from field soils. To date, our data clearly show that the PCR based assay can differentiate between Rhizobium species and that they could work in a controlled conditions. What we wanted to determine is how well this assay will work on real field soils. To determine if the assay could work on real samples, field soils were spiked with known amounts of *Bradyrhizobium japonicum*. The assay was able to detect 75 ± 25% (n=3) of the added bacteria and that we could detect down to 50-100 *B. japonicum*/assay. When the PCR assay results were compared to the MPN assay, the results compared favourably; $>10^6$ nodulating bacteria/gm soil (MPN) vs. 10^7 *B. japonicum* equivalents/gm (PCR).

Soybean fields planted with 30 inch row spacing support populations with a higher proportion of *B. japonicum*. Row spacing can affect overall soybean yields. It is obvious that more plants in a given area can affect the amount and the quality of the bean that is harvested. Whereas final yields and quality can be readily assessed, the effect row spacing has on the population of *B. japonicum* is unknown.

To address this, samples were taken from soybean fields that had either 15 or 30 inch row spacing. When the soil samples were extracted, we could typically isolate about 1-2 µgm of DNA (enough for about 10^9 bacterial cells). Our analysis determined that regardless whether the samples were from fields with15 or 30 inch row spacing, we could typically detect around 10^7 *B. japonicum* per gram of soil. When the data were analyzed with respect to what proportion of the population was made up of *B. japonicum*, it was found that *B. japonicum* accounted for 8% of the bacterial population taken from a field with 15 inch row spacing, whereas a field with 30 inch row spacing the proportion was only 4% (Figure 1). Since it is possible that this was due to the amount of inoculum that was originally used, a control experiment using defined conditions was carried out to determine if plant density affects the proportion of *B. japonicum* population. The results of these experiments were consistent with our field observations; the number of soybean plants positively affect the *B. japonicum* population.