P.O. Box 1760, Carman, MB
Canada R0G 0J0
T 204.745.6488
F 204.745.6213
@MbPulseGrowers
www.manitobapulse.ca

# MPSG FINAL EXTENSION REPORT

PROJECT TITLE: Soybean Inoculant Strategies

PROJECT START DATE: May 1, 2014
PROJECT END DATE: December 31, 2016

## RESEARCHERS

| | |
|---|---|
| **LEAD** | Manitoba Pulse & Soybean Growers |
| **COLLABORATORS** | Dr. Yvonne Lawley, University of Manitoba<br>Scott Chalmers, Western Agricultural Diversification Centre<br>Craig Linde, Canada Manitoba Crop Diversification Centre<br>James Frey, Parkland Crop Diversification Foundation<br>Nirmal Hari, Prairies East Sustainable Agriculture Initiative |

## EXECUTIVE SUMMARY

Selection of the appropriate *Bradyrhizobium japonicum* inoculant formulation, rate and combination of products is dependent on field history, equipment available, cost of inoculant and environmental conditions. The objective of this study was to compare fourteen inoculant products, formulations, rates and combinations across a range of locations and field histories in Manitoba. Field experiments were conducted in 2014, 2015 and 2016 at Melita, Carberry, Carman, Roblin and Beausejour. Four of the site-years tested had a history of soybeans and five of the site-years had no history of soybeans. Site-years were combined and analyzed based on this cropping history.

Inoculation had important economic implications on fields with *no* history of soybean. Averaged across all site-years *without* a history of soybean, inoculant treatments increased number of nodules per plant by 20, yield by 15 bu $ac^{-1}$ and protein by 4.8% compared to the uninoculated control. On fields *with* a history of soybean, there was no difference in yield, number of nodules or seed protein between inoculant treatments and the uninoculated control. Regardless of field history, under the optimal seeding and plant establishment conditions encountered in this study, inoculant product, rate or combination did not have an effect on nodule number per plant or seed yield. There are several possible explanations for the lack of response to double inoculation in this trial which cannot always be guaranteed under field conditions. Therefore, MPSG recommends using a double inoculation strategy on fields with a limited history of soybean and a single inoculation strategy after at least two successfully nodulated soybean crops have been established on a particular piece of land. See MPSG's Soybean Fertility Factsheet for more details regarding inoculation recommendations.

The minimum number of nodules required to reach 90% of maximum yield was approximately ten nodules per plant at the R4 stage. The R1 stage, however, permits assessment of nodulation failure prior to the ideal window to apply rescue nitrogen fertilizer (R2-R3).

## *INTRODUCTION*

Soybeans are capable of creating 50-60% of their nitrogen (N) requirements through biological N fixation (Salvagiotti, et al. 2008.) The remainder of the required N is taken up from soil reserves. *Bradyrhizobium japonicum* is the soybean-specific bacteria which causes nodule development on roots and works symbiotically with the soybean to fix N within the nodules. This bacteria is not native to Canadian Prairie soils and thus must be introduced by using commercial inoculants. Once successfully inoculated soybean crops have been grown on a particular piece of land, populations of *B. japonicum* can build up and overwinter, providing sufficient inoculum for proceeding soybean crops.

There are many effective inoculant products available to soybean farmers in Manitoba. Selection of the appropriate formulation, rate and combination of products is dependent on the field history, equipment available, cost of inoculant and environmental conditions. Seed-applied liquid and peat-based products are generally cheap and can conveniently be applied to the seed prior to seeding. Granular inoculant applied in-furrow has been found to provide greater nodulation and higher yields compared to seed-applied inoculant on fields with no history of soybean (Muldoon, et al., 1980). Granular inoculants have also shown to be more resilient to environmental stress such as excess moisture (Hynes et al., 2001) and acidic soils (Rice et al., 2000) compared to seed-applied liquid formulations. However, granular inoculant is generally more expensive and must be applied in furrow, requiring an extra tank on the seed cart.

Some inoculant products are also formulated with additional molecules or living organisms which claim to improve early crop development, plant nutrition or the rate of nodulation. For example, both JumpStart® and TagTeam® contain a phosphate-solubilizing rhizopheric fungus, *Penicillium bilaii*. *P. bilaii* lives in the rhizosphere (soil immediately surrounding the root) and may increase soil phosphorus (P) availability and hence, plant uptake. This occurs through one of two mechanisms: the bacteria secreting organic acids that acidify the soil, solubilizing P or chelating P molecules, protecting P from precipitation or adsorption to soil. Nodulator® N/T is formulated with *Bacillus subtilis* a plant growth promoting rhizobacteria which may increase soybean growth and nodule formation resulting from co-inoculation with *B. japonicum*. Optimize® is formulated with the lipo-chitooligosaccharide (LCO) molecule. The process of nodule development requires both the plant root and *B. japonicum* bacteria to send and receive signals for the process to initiate. The bacteria migrate towards roots, attracted by root exudate (root to bacteria signals); these exudates cause the bacteria to produce proteins called Nod factors (LCOs). The LCO molecules (bacteria to plant signals) in Optimize® may hasten the process of nodule development.

For first and second-time soybean fields a “double inoculation” strategy is recommended to insure adequate populations are introduced to the soil, facilitating proper nodulation. Double inoculation refers to the use of two inoculant formulations or placement techniques. A common strategy for double inoculation is to use a seed-applied liquid inoculant in addition to an in-furrow granular product. Increasing the rate of inoculant may also effectively increase rhizobia levels in the soil and improve nodulation (Muldoon, et al., 1980), but multiple formulations or placements provides the added benefit of potential better survivability of the rhizobia.

Once several successfully nodulated soybean crops have been established on a particular piece of land, farmers may choose to use a more economical, single inoculation strategy. MPSG’s On-Farm Network found that double inoculation provided a significantly higher soybean yield compared to single inoculation at only two out of 25 trial sites in fields with at least two prior soybean crops. Similarly, in the upper Midwest United States a meta-analysis found that inoculation seldom increased yield or economic return compared to the untreated control on fields where soybeans had previously been produced (Bruin, et al., 2010).

The objective of this study was to compare inoculant products, formulations and rates across a range of locations and field histories in Manitoba. More specifically, the project aims to quantify the yield benefits of using 1) in-furrow granular 2) double inoculation, 3) 2X rate or 4) "enhanced" inoculant products compared to a standard seed-applied liquid inoculant (Cell-Tech® Liquid).

## METHODS

Field experiments were conducted in 2014, 2015 and 2016 at Melita, Carberry, Carman, Roblin and Beausejour, Manitoba. Field sites varied based on their cropping history: four sites had a history of soybean (Carman 2015, 2016, Carberry 2016 and Beausejour 2016) and five sites did not have a history of soybean (Melita 2014, 2015, 2016, Carberry 2015, and Roblin 2015). Fourteen inoculant strategies tested, i.e. different products, formulations, combinations and rates, and are listed in Table 1 and 2. A subset of 11 treatments were tested at Melita in 2014. Treatments were arranged as a randomized complete block design with four replicates at all sites except Beausejour in 2016, were there was only three replicates.

A complete list of site characteristics and field operations is listed in Table 5. NSC Reston soybeans were seeded at 210,000 seeds/ac on narrow row spacing into cereal stubble at all locations except for Melita in 2015, where the soybeans were seeded into flax stubble. The trial was seeded from late May to early June. Liquid inoculants were seed-applied and granular inoculants were applied in-furrow. No fungicide or insecticide seed treatments were used. Inoculant treatments were seeded in order of listing in Table 1 and 2. Seeding equipment was sanitized with bleach solution and an air hose after seeding the sixth, seventh, eighth, ninth and eleventh treatments. Weeds were controlled using pre-and post-emergence herbicides and supplementary phosphorus, potassium or sulphur fertilizer was applied as required. Soybeans were desiccated if necessary before direct harvesting using a plot combine.

Plant density was assessed at V1 and plants from four randomly selected, one meter rows were recorded and reported as plants per acre. The number of nodules per plant was assessed at both R1 and R4. Within each plot, ten randomly selected plants were dug up using a shovel and rinsed with water to wash off excess soil. Roots were generally then frozen and nodules counted at a later date. At R4, plant biomass was also measured by harvesting all above ground biomass from two, one meter rows. Biomass was dried at 60°C for two days and dry weight was reported in kilograms per hectare. Harvested grain was cleaned if necessary and grain moisture was recorded when clean samples were weighed. Reported grain weight was standardized to 14% moisture. Yield was analyzed as kilograms per hectare and converted to bushels per acre for reporting purposes. A subsample of grain from each plot was analyzed for seed oil and protein content and thousand kernel weight using a near-infrared reflectance grain analyzer (Foss NIR Systems, Inc., Laurel, MD, USA).

The Glimmix Procedure in SAS 9.4 was used to conduct the analysis of variance and orthogonal contrasts. Each measured variable was modelled with inoculant treatment and field history as a fixed effects and site-year and block as random effects. Because there was a significant interaction between treatment and field history, site-years were grouped based on field history and analyzed separately. Heterogeneous variance of the fixed effect was modelled only when it improved model fix as tested by chi test. The Univariate Procedure was used to test the normality of the data using the Shapiro-Wilk Statistic. Differences between treatment means using pre-planned contrasts were considered significant at $P<0.05$. The Regression and Non-Linear Procedures were used to analyze the relationship between number of nodules per plant and seed yield. Treatment means from individual site-years were used to develop these models. Linear, quadratic, exponential, linear broken-line and quadratic broken-line models were tested for model significance and best fit. A quadratic broken-line model was chosen based the best fit as determined by the lowest AIC value of all models tested.

## RESULTS

### *Fields with a History of Soybean*

Yield was, on average, slightly higher (449 kg $ha^{-1}$ or 6.7 bu $ac^{-1}$) on fields *with* a history of soybean compared to *no* history of soybean (Table 1, 2). There was no yield response to inoculant compared to the uninoculated check at the sites with a history of soybean (Table 3). In addition, there was no statistical difference in yield between individual inoculant strategies (Table 3). For example, there was no difference in seed yield between in-furrow granular inoculant compared to seed-applied liquid inoculant, nor was there a difference between single versus double inoculation treatments (Table 3). Similarly, there was no yield difference between 1X and 2X rates of liquid or granular inoculant (Table 3). In addition, 'enhanced' inoculant treatments did not result in higher yields compared to the standard *B. rhizobium* inoculant of equivalent formulation (Table 3).

The lack of yield response to inoculant on fields with a history of soybean is consistent with findings from the United States and Ontario. Bruin et al. (2010) reviewed studies from Indiana, Iowa, Minnesota, Nebraska and Wisconsin that tested 51 different inoculant products in 2000 to 2008 across 73 environments that all had a history of soybean. Of these 73 test sites, 63 showed no yield response to inoculant. Four sites showed a negative yield response (5-7% yield differene) and six sites showed a positive yield response (5-23% yield difference) to inoculant compared to the untreated control. This study also found that economic return was actually reduced by the small investment in inoculant and did not recommend the use of inoculants in fields with a history of soybean, regardless of price or ease of application. Similarly, in Ontario, failure to obtain a positive yield response to inoculant was documented in 1979 by Ernest and Hume at Ridgetown and Elora, where soybeans had been previously grown. A positive response to soybeans was only achieved at Woodstock, where soybeans had never been grown. MPSG's On-Farm Network is also currently investigating soybean response to single inoculation compared to no inoculant on fields with at least three previous years of soybeans. To date, none of the nineteen trial sites from 2016 and 2017 have shown a statistical yield response to single inoculation.

The lack of response to inoculant and amongst inoculant strategies at sites with a history of soybean was also reflected in the assessment of nodules conducted both at R1 and R4. The mean number of nodules per plant was 45 and 58 at R1 and R4 stages,(Table 1) respectively, and there were no statistical differences in nodule number across any treatments compared (Table 3). The average number of nodules per plant was notably higher at sites with a history of soybean than without a history (Table 1, 2)

There was also no response to inoculant or difference amongst inoculant treatments in protein or thousand kernel weight. There were statistical differences in plant density, biomass and oil content among some inoculant strategies; however, these differences are not understood and may be due to random variation.

***Fields with No History of Soybean***

As expected, there was a statistically and agronomically significant yield response to inoculant at field sites with *no* history of soybeans. On average, the uninoculated soybeans yielded 1725 kg $ha^{-1}$ or 25.6 bu $ac^{-1}$. Using an inoculant increased yield by an average of 1019 kg $ha^{-1}$ or 15.1 bu $ac^{-1}$. The difference in yield between the untreated control and inoculated soybeans can be explained by the increase nodules per plant recorded at the R4 stage with the use of inoculant. The mean number of nodules increased from less than two nodules on the uninoculated soybeans to 22.3 nodules per plant on inoculated soybeans.

Similar to the response seen on fields with a history of soybean, there were no differences in yield observed between any of the inoculant strategies (Table 4). There were some differences in nodule number per plant among inoculant treatments at R1, but the data reported is from a single site and these differences did not appear at R4 (Table 4), when the crop's nitrogen requirements are highest.

Although there was no yield or nodulation benefit to double inoculation in this trial, the recommendation to double inoculate soybeans when grown on fields with two or less soybean crops grown previously still stands. There are several possible explanations for the lack of response to double inoculation in this trial which cannot always be guaranteed under field conditions:

*Soybeans were seeded into ideal soil conditions.* These trials were all seeded in late May to early June, when soil conditions were relatively favourable for crop emergence and inoculum survival. Unfavourable soil conditions often encountered with earlier seeding dates may reduce the viability of inoculant. Therefore, using an in-furrow inoculant in addition to the seed applied inoculant may ensure adequate rhizobium populations are present in fields with low rhizobia populations.

*Inoculants were properly stored, handled and applied*. Inoculants should always been kept in a cool, dry environment, should not be frozen, used before the expiration date and opened only just before using. Ideally, seed treated with inoculant should be planted within the same day as inoculant application. Planting windows for seed-applied inoculants do vary and review of individual product labels is recommended.

*No compatibility issue with seed treatment*. Fungicide and/or insecticide seed treatments may affect the effectiveness of seed-applied inoculant; however, in this experiment seed treatment was not applied in an effort to standardized inoculant application and avoid potential differences in treatment compatibility. Be sure to review product labels for specific inoculant and seed treatment combination compatibility.

Seed quality was also markedly influenced by inoculation. Although mean protein and oil content was similar at sites without a history of soybean compared to sites with a history of soybean, inoculant increased protein by 4.8% and decreased oil by 1.9% compared to the uninoculated control at sites without a history of soybean. This large increase in seed N due to inoculation demonstrates the level of whole plant N sufficiency caused by proper nodulation. In addition, thousand kernel weight also increased by 12.5 g per 1000 seeds, which shows that the increase in yield due to inoculation can be attributed in part by an increase in individual seed weight.

***Soybean Yield and Nodules per Plant***

How many nodules should a soybean have to maximize yield? Regardless of the inoculant strategy and field history, success of the inoculant and nodulation should be assessed on every field, every year. Ideally, nodulation should be assessed at R1 to ensure the crop will have adequate N during critical growth stages (R4-R5) to maximize yield (Heard et al., 2014).

At R4 to R5, N fixation and N requirements for soybean have reached a maximum (Heard 2006). At this point, however, it is too late to conduct a rescue N application. In this study, most sites only recorded nodules numbers at R4 so the relationship between yield and nodules per plant was modelled using the data from the R4 stage. Results from this study found that an average of at least 10 nodules per plant was required to reach 90% of maximum yield potential (Figure 1).

## RELEVANCE TO FARMERS

Inoculation has important economic implications on fields with no history of soybean. Averaged across all site-years without a history of soybean, inoculant treatments increased number of nodules per plant by 20, yield by 15 bu $ac^{-1}$ and protein by 4.8% compared to the uninoculated control.

Regardless of field history, under the optimal seeding and plant establishment conditions encountered in this study, inoculant product, rate or combination did not have an effect on nodule number per plant or yield. MPSG recommends using a double inoculation strategy on fields with a limited history of soybean and a single inoculation strategy after at least two successfully nodulated soybean crops have been established on a particular piece of land. See MPSG's *Soybean Fertility Factsheet* for more details regarding inoculation recommendations.

The minimum number of nodules required to reach 90% of maximum yield was approximately ten nodules per plant at the R4 stage. Assessing nodulation at R1, however, permits assessment of nodulation failure prior to the ideal window to apply rescue nitrogen fertilizer (R2-R3).

## ACKNOWLEDGEMENTS

*Thank you to Scott Chalmers, Yvonne Lawley, Craig Linde, James Frey and Nirmal Hari for leading and executing the field studies and sharing your data.*

*Thank you to the many technicians and summer students at each collaborating institution for contributing to the success of this project.*

## REFERENCES

Bruin, J.L., et al. 2010. Probability of Yield Response to Inoculants in Fields with a History of Soybean. Crop Sci. 50:265-272.

Ernest, S. and D.J. Hume. 1979. Effects of inoculation and fertilizer N levels on $N_2$ fixation and yields of soybeans in Ontario. Can. J. Plant Sci. 59:1129-1137.

Heard, J. 2006. Nutrient uptake and partitioning by soybeans in Manitoba. Proc. Manitoba Agronomists Conference.

Heard, J., G. Bardella and K. Podolsky. 2014. Rescue nitrogen applications on poorly nodulated soybeans in Manitoba. 2014. Proc. Manitoba Agronomists Conference.

Hynes R.K., D.C. Jans, E. Bremer, N.Z. Lupwayi, W.A. Rice, G.W. Clayton, and M.M. Collins. 2001. Rhizobium population dynamics in the pea rhizosphere of rhizobial inoculant strain applied in different formulations. Can. J. Mircobiol. 47:595-600.

Muldoon, J.F., D.J. Hume and W.D. Beversdorf. 1980. Effects of seed- and soil-applied Rhizobium japonicum inoculants on soybean in Onatario. Can. J. Plant Sci. 60:399-409.

Rice, W. A., Clayton, G. W., Olsen, P. E. and Lupwayi, N. Z. 2000. Rhizobial inoculant formulations and soil pH influence field pea nodulation and nitrogen fixation. Can. J. Soil Sci. 80: 395–400.

Salvagiotti, F. K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, A. Dobermann. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A Review. Field Crop Research. 108:1-13.

## APPENDIX

**Table 1. Field operations and site characteristics.**

| | Melita | | | Carman | | Carberry | | Roblin | Beausejour |
|---|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| Field Operations | | | | | | | | | |
| Seeding Date | Jun-11 | Jun-05 | Jun-07 | May-27 | Jun-07 | Jun-04 | Jun-07 | May-25 | Jun-07 |
| Plant Density Assessment | - | Jun-15 | Jun-23 | Jun-17 | Jun-29 | - | Jul-15 | Jun-18 | N/A |
| Pre-Emergent Herbicide Application | Jun-12 | Jun-03 | Jun-06 | Jun-04 | N/A | - | Jun-06 | May-25 | N/A |
| In-Crop Herbicide Application | Jul-23 | Jun-15 & Jul-6 | Jun-22 | Jul-6 & Jul-30 | Jun-25 | - | Jun-30 | Jun-16 | - |
| Biomass Collection | Aug-25 | Aug-10 | Aug-04 | Jul-30 | Aug-11 & Aug-12 | - | Aug-10 | Aug-25 | Aug-11 |
| R1 Nodule Assessment | N/A | N/A | Jul-22 | Jul-10 | N/A | - | N/A | N/A | N/A |
| R4 Nodule Assessment | N/A | Aug-10 | Aug-02 | Jul-30 | Aug-12 to Aug-16 | - | Aug-10 | Aug-12 | Aug-11 |
| Desiccation Date | N/A | N/A | Sep-27 | - | N/A | - | N/A | Sep-29 | N/A |
| Harvest Date | Oct-14 | Oct-01 | Sep-26 | - | Oct-15 | Oct-19 | Oct-18 | Oct-14 | Oct-14 |
| Site Characteristics | | | | | | | | | |
| Previous Crop | Winter wheat | Flax | Winter wheat | Spring wheat | Spring wheat | - | Wheat | Wheat | Cereals |
| Row Spacing (in) | 9.5 | 9.5 | 9.5 | 7.5 | 7.5 | - | 12.0 | 9.5 | 8 |
| Soil pH (0-6”) | 7.4 | 7.3 | 7.6 | 5.2 | 6.0 | - | 6.3 | 6.8 | - |
| Soil Organic Matter % (0-6”) | 3.1 | 3.8 | 2.9 | 2.7 | 4.2 | - | 5.4 | 3.7 | - |
| NO3-N (0-24” lbs $ac^{-1}$) | 36 | 145 | 29 | 81 | 91 | - | 31 | 30 | - |
| PO4-P (0-6” ppm) | 4 | 7 | 3 | 7 | 20 | - | 13 | 9 | - |
| K2O (0-6” ppm) | 424 | 366 | field | 113 | 346 | - | 321 | 151 | - |
| SO4 (0-24” lbs $ac^{-1}$) | 59 | 317 | 264 | 40 | 52 | - | 37 | 50 | - |
| N fertilizer (lbs $N0_3^-$ $ac^{-1}$) | 16 | N/A | N/A | N/A | N/A | - | N/A | 16 | N/A |
| P fertilizer (lbs $P_2O_5$ $ac^{-1}$) | 23 | 61 | N/A | 60 | N/A | - | N/A | 35 | 27 |
| K fertilizer (lbs $K_20$ $ac^{-1}$) | 20 | N/A | N/A | N/A | N/A | - | N/A | 15 | N/A |
| S fertilizer (lbs $SO^{-4}$ $ac^{-1}$) | 14 | N/A | N/A | N/A | N/A | - | N/A | 10 | N/A |

"N/A" refers to not applicable information

"-" refers to missing information

| Treatment | Plants ac$^{-1}$ | R1 Nodules plant$^{-1†}$ | R4 Nodules plant$^{-1}$ | Biomass (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) | Protein % | Oil % | g 1000 seeds$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Untreated Control | 201982 | 44.5 | 60.7 | 3746 | 3078 | 35.18 | 17.07 | 135.02 |
| Cell-Tech® Liquid | 181513 | 48.4 | 60.6 | 3550 | 3170 | 35.27 | 17.15 | 136.42 |
| Cell-Tech® Liquid (2x rate) | 209340 | 46.8 | 57.7 | 3866 | 3126 | 35.27 | 17.04 | 135.36 |
| Cell-Tech® Liquid + Cell-Tech® Granular | 184722 | 41.0 | 56.7 | 3779 | 3084 | 35.08 | 17.03 | 135.18 |
| Cell-Tech® Granular | 193462 | 49.8 | 62.6 | 3465 | 3040 | 35.16 | 17.09 | 134.2 |
| Cell-Tech® Granular (2x rate) | 194790 | 46.3 | 57.1 | 3383 | 2989 | 35.33 | 17.03 | 135.27 |
| Cell-Tech® Liquid + JumpStart® | 214991 | 46.0 | 60.6 | 3518 | 3087 | 35.26 | 17.14 | 136.71 |
| Optimize® Liquid | 205394 | 45.6 | 60.3 | 3404 | 3071 | 35.36 | 16.93 | 134.62 |
| TagTeam® Granular | 199714 | 44.8 | 58.9 | 3598 | 2972 | 35.12 | 17.07 | 134.36 |
| Nodulator® Granular | 185717 | 42.6 | 61.1 | 3823 | 3173 | 35.16 | 17.12 | 134.14 |
| Nodulator® Granular (2x rate) | 215267 | 46.6 | 59.2 | 3959 | 3301 | 35.29 | 17.22 | 133.18 |
| Nodulator® N/T LQ | 211276 | 42.9 | 57.2 | 4303 | 3082 | 35.12 | 17.09 | 132.55 |
| Nodulator® N/T LQ (2x rate) | 201097 | 49.3 | 61.8 | 3640 | 3091 | 34.78 | 17.26 | 135.17 |
| Nodulator® N/T LQ + Nodulator® Granular | 196063 | 42.6 | 56.8 | 3756 | 3147 | 34.98 | 17.14 | 135.03 |
| Mean | 200685 | 45.1 | 57.8 | 3683 | 3123 | 35.11 | 17.10 | 134.80 |
| Coefficient of Variation (%) | 33.2 | 31.0 | 33.3 | 33.8 | 16.7 | 4.6 | 2.2 | 8.5 |
| Test of Fixed Effects (P>F) | 0.1613 | 0.6253 | 0.9376 | 0.2604 | 0.1729 | 0.5533 | 0.3119 | 0.9512 |

**Table 2.** Least squared means for plant density, nodules per plant, biomass, yield, protein, oil and thousand kernel weight for site-years **with a history of soybeans**

†only two site-years of data (Carman 2015, Carberry 2016)

**Table 3.** Least squared means for plant density, nodules per plant, biomass, yield, protein, oil and thousand kernel weight (TKW) for site-years **with no history of soybeans**

| Treatment | Plants ac$^{-1}$ | R1 Nodules plant$^{-1†}$ | R4 Nodules plant$^{-1}$ | Biomass (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) | Protein % | Oil % | g 1000 seeds$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Untreated Control | 149777 | 0.4 | 1.9 | 3574 | 1725 | 29.89 | 19.46 | 135.89 |
| Cell-Tech® Liquid | 160096 | 22.1 | 27.6 | 4601 | 2858 | 34.74 | 17.49 | 148.09 |
| Cell-Tech® Liquid (2x rate) | 157887 | 20.5 | 31.3 | 4688 | 3087 | 35.03 | 17.34 | 148.03 |
| Cell-Tech® Liquid + Cell-Tech® Granular | 152339 | 22.4 | 33.2 | 4662 | 2654 | 35.64 | 17.11 | 149.23 |
| Cell-Tech® Granular | 144919 | 7.6 | 27.6 | 4365 | 2848 | 35.51 | 17.19 | 149.92 |
| Cell-Tech® Granular (2x rate) | 146203 | 10.4 | 31.5 | 4401 | 2794 | 35.46 | 17.19 | 148.60 |
| Cell-Tech® Liquid + JumpStart® | 135008 | 18.4 | 23.2 | 4005 | 2566 | 34.76 | 17.53 | 145.79 |
| Optimize® Liquid | 164143 | 14.1 | 27.2 | 4691 | 2874 | 35.04 | 17.38 | 151.99 |
| TagTeam® Granular | 162603 | 10.1 | 18.7 | 3883 | 2594 | 34.49 | 17.65 | 149.06 |
| Nodulator® Granular | 168676 | 2.8 | 7.2 | 3481 | 2671 | 33.68 | 17.99 | 146.62 |
| Nodulator® Granular (2x rate) | 141686 | 4.8 | 12.6 | 3933 | 2651 | 34.46 | 17.69 | 147.55 |
| Nodulator® N/T LQ | 162591 | 3.4 | 15.7 | 4042 | 2688 | 34.02 | 17.80 | 145.10 |
| Nodulator® N/T LQ (2x rate) | 162317 | 5.3 | 14.5 | 3484 | 2595 | 33.76 | 18.01 | 151.14 |
| Nodulator® N/T LQ + Nodulator® Granular | 172032 | 7.3 | 19.2 | 4361 | 2788 | 34.63 | 17.63 | 147.84 |
| Mean | 156048 | 10.7 | 22.3 | 4076 | 2674 | 34.36 | 17.68 | 143.98 |
| Coefficient of Variation | 29.1 | 75.1 | 76.1 | 44.3 | 25.4 | 5.2 | 6.7 | 14.4 |
| Test of Fixed Effects | 0.5427 | <.0001 | <.0001 | 0.3591 | 0.0001 | <.0001 | 0.3119 | <.0001 |

†Only one site-year of data (Melita 2016)

**Table 4.** Orthogonal contrasts comparing the difference in plant density, nodules per plant, biomass, yield, protein, oil and thousand kernel weight (TKW) between select inoculant strategies, averaged across site-years **with a history of soybeans**. Means reported in columns is the difference in treatment means between treatment 1 minus treatment two for each measured variable.

| Treatment 1 (+) | Treatment 2 (-) | Plants ac$^{-1}$ | R1 Nodules plant$^{-1}$ | R4 Nodules plant$^{-1}$ | Biomass (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) | Protein % | Oil % | g 1000 seeds$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| All Inoculant Treatments | Untreated Control | -2494 | 1.1 | -1.5 | -51 | 25 | -0.01 | 0.03 | -0.23 |
| Cell-Tech® Liquid | Cell-Tech® Liquid (2x rate) | -27827* | 1.6 | 2.9 | -316 | 43 | 0.00 | 0.11 | 1.06 |
| Cell-Tech® Liquid | Cell-Tech® Granular | -11950 | -1.4 | -2.0 | 85 | 129 | 0.11 | 0.06 | 2.22 |
| Cell-Tech® Liquid | Cell-Tech® Liquid + Cell-Tech® Granular | -3209 | 7.3 | 4.0 | -229 | 86 | 0.18 | 0.12 | 1.23 |
| Cell-Tech® Granular | Cell-Tech® Granular (2x rate) | -1328 | 3.4 | 5.5 | 83 | 52 | -0.17 | 0.07 | -1.07 |
| Cell-Tech® Granular (2x rate) | Cell-Tech® Liquid + Cell-Tech® Granular | 10069 | 5.3 | 0.4 | -396 | -95 | 0.24 | -0.01 | 0.08 |
| Cell-Tech® Liquid | Optimize® Liquid | -23882 | 2.8 | 0.3 | 146 | 99 | -0.10 | 0.22* | 1.80 |
| Cell-Tech® Liquid | Cell-Tech® Liquid + JumpStart® | -33478* | 2.4 | 0.0 | 32 | 83 | 0.01 | 0.01 | -0.29 |
| Cell-Tech® Granular | TagTeam® Granular | -6251 | 5.0 | 3.8 | -132 | 68 | 0.04 | 0.03 | -0.16 |
| Cell-Tech® Liquid | Nodulator® N/T LQ | -29763* | 5.5 | 3.4 | -753* | 88 | 0.15 | 0.06 | 3.87 |
| Nodulator® N/T LQ | Nodulator® N/T LQ (2x rate) | 10179 | -6.4 | -4.6 | 663* | -9 | 0.33 | -0.17 | -2.62 |
| Nodulator® N/T LQ | Nodulator® N/T LQ + Nodulator® Granular | 15214 | 0.3 | 0.4 | 547 | -65 | 0.13 | -0.05 | -2.48 |
| Nodulator® Granular | Nodulator® Granular (2x rate) | -29550* | -4.0 | 1.8 | -136 | -128 | -0.13 | -0.10 | 0.96 |

* Difference between treatment means is statistically significant at P<0.05
†only two site-years of data (Carman 2015, Carberry 2016)

**Table 5.** Orthogonal contrasts comparing the difference in plant density, nodules per plant, biomass, yield, protein, oil and thousand kernel weight (TKW) between select inoculant strategies, averaged across site-years **with no history of soybeans**. Means reported in columns is the difference in treatment means between treatment 1 minus treatment two for each measured variable.

| Treatment 1 (+) | Treatment 2 (-) | Plants ac$^{-1}$ | R1 Nodules plant$^{-1}$† | R4 Nodules plant$^{-1}$ | Biomass (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) | Protein % | Oil % | g 1000 seeds$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| All Inoculant Treatments | Untreated Control | 6416 | 11.1* | 20.4* | 626 | 1019* | 4.82* | -1.92* | 12.50* |
| Cell-Tech® Liquid | Cell-Tech® Liquid (2x rate) | 2209 | 1.6 | -3.7 | -87 | -229 | -0.29 | 0.14 | 0.06 |
| Cell-Tech® Liquid | Cell-Tech® Granular | 15177 | 14.5* | 0.0 | 236 | 10 | -0.78 | 0.30 | -1.83 |
| Cell-Tech® Liquid | Cell-Tech® Liquid + Cell-Tech® Granular | 7757 | -0.3 | -5.6 | -61 | 204 | -0.91 | 0.38 | -1.14 |
| Cell-Tech® Granular | Cell-Tech® Granular (2x rate) | -1285 | -2.8 | -3.9 | -36 | 54 | 0.05 | -0.01 | 1.32 |
| Cell-Tech® Granular (2x rate) | Cell-Tech® Liquid + Cell-Tech® Granular | -6136 | -12.0* | -1.7 | -261 | 140 | -0.18 | 0.09 | -0.63 |
| Cell-Tech® Liquid | Optimize® Liquid | -4047 | 8.0* | 0.4 | -89 | -16 | -0.31 | 0.11 | -3.90 |
| Cell-Tech® Liquid | Cell-Tech® Liquid + JumpStart® | 25088 | 3.7 | 4.4 | 596 | 292 | -0.03 | -0.04 | 2.30 |
| Cell-Tech® Granular | TagTeam® Granular | -17685 | -2.5 | 8.9 | 482 | 254 | 1.03 | -0.46 | 0.86 |
| Cell-Tech® Liquid | Nodulator® N/T LQ | -2495 | 18.7* | 11.9* | 559 | 170 | 0.72 | -0.31 | 2.99 |
| Nodulator® N/T LQ | Nodulator® N/T LQ (2x rate) | 274 | -2.0 | 1.2 | 559 | 93 | 0.26 | -0.21 | -6.03 |
| Nodulator® N/T LQ | Nodulator® N/T LQ + Nodulator® Granular | -9441 | -3.9 | -3.6 | -318 | -100 | -0.61 | 0.17 | -2.74 |
| Nodulator® Granular | Nodulator® Granular (2x rate) | 26990 | -2.0 | -5.4 | -452 | 20 | -0.78 | 0.30 | -0.94 |

* Difference between treatment means is statistically significant at P<0.05
†only one site-year of data (Melita 2016)

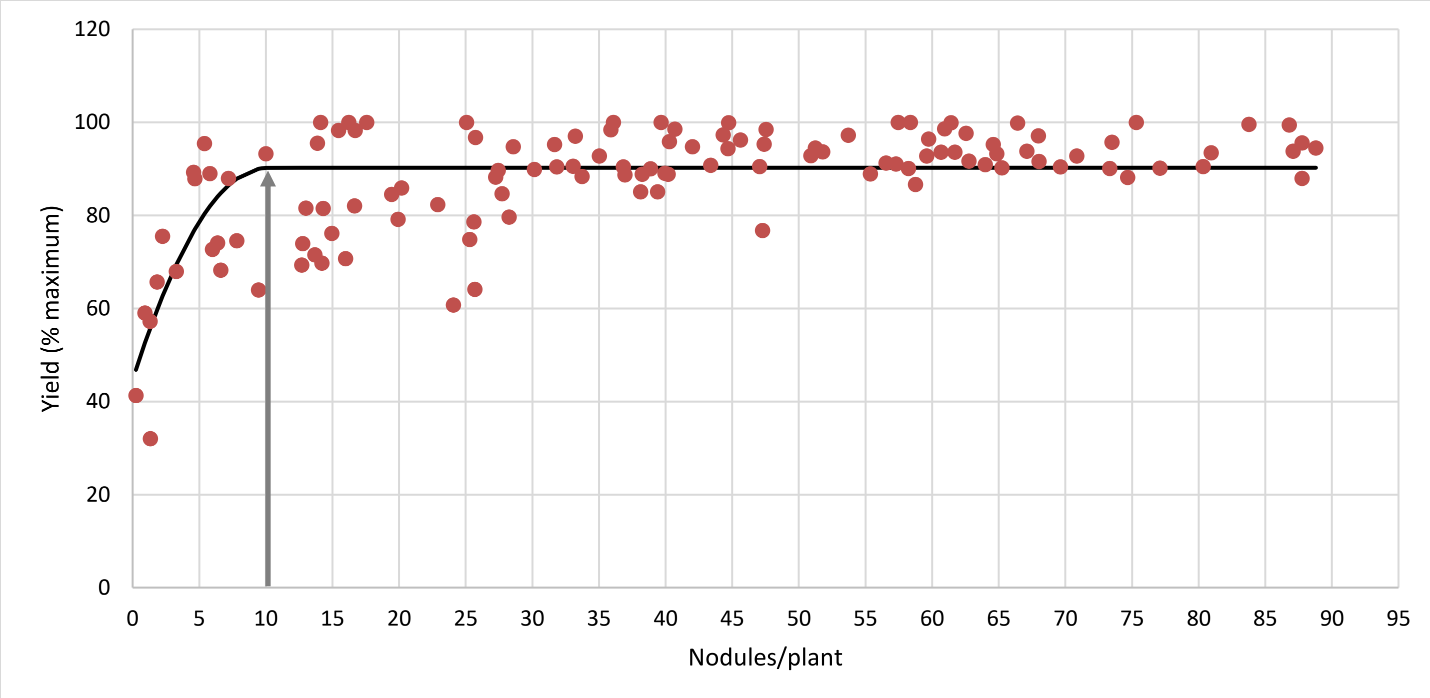


**Figure 1.** Relationship between number of nodules per soybean plant and relative yield.