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Bradyrhizobium japonicum Inoculants Have Little Effect in Kentucky's Soybean-Corn Rotation

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Crop Management—Briefs

Core Ideas

- *Bradyrhizobium japonicum* soybean inoculants did not increase seed yield and number of root nodules.
- Differences were detected for higher leaf N concentration in the R6 growth stage, but this did not result in increased seed yield.
- Increasing the rate of *B. japonicum* soybean inoculants did not increase the seed yield nor the number of root nodules.

Soybean (*Glycine max* [L.] Merrill) is an important row crop in Kentucky. In recent years intensive agronomic management has increased, specifically the use of *Bradyrhizobium japonicum* soybean inoculant at planting (University of Kentucky Cooperative Extension Service, 2017). *B. japonicum* is a bacteria that has a symbiotic relationship with the soybean root to fix nitrogen (N) from the atmosphere for plant use, which is essential for producing high yielding soybean (Bhuvaneswari et al., 1980). Typically, *B. japonicum* seed inoculants are an inexpensive input ($3 to 4/acre) that producers use to guarantee adequate N availability and uptake and yield potential. However, claims that twice or three times the labeled rate produce greater root nodulation and N uptake have increased the interest and cost of soybean inoculants. The objective of this study was to determine the effect of different rates of commercially available *B. japonicum* inoculants on full season and double crop soybeans for seed yield, number of root nodules, and N concentration of soybean leaves.

Field trials were conducted at the University of Kentucky Research and Education Center in Princeton, KY (37°6′ N, 87°52′ W) and Spindletop Research Farm in Lexington, KY (38°7′ N, 84°29′ W) in 2016. A full season and double crop trial were evaluated on three soil types: a well-drained Crider silt loam (Typic Paledalf) and moderately well-drained Zanesville silt loam (Oxyaquic Fraguidalf) at Princeton and a well-drained Maury silt loam (Typic Paleudalf) at Lexington. Both trials examined five treatments: America's Best Inoculant at the recommended (1×; 12.5 fl oz/cwt) and twice the recommended rate (2×; 25 fl oz/cwt); Optimize XC at the recommended (1×; 2.8 fl oz/cwt) and twice the recommended rate (2×; 5.6 fl oz/cwt); and a non-inoculated control. Treatments were arranged as a randomized complete block design with four replications. *B. japonicum* inoculants were applied directly to untreated soybean seed (DuPont Pioneer [Johnston, IA] 94PY23) according to the labels. Soybean seeds were planted on 15-inch row spacing at 140,000 seeds per acre. The previous crop for the full season trial was no-till corn (*Zea maize* L.), while the double crop trials were planted into no-till winter wheat (*Triticum aestivum* L.) stubble, which was preceded by corn. At Princeton, two seven-row plots were planted for each treatment to provide one yield plot and one plot for destructive sampling of root nodules number and leaf tissue analysis. The Lexington location had one six-row plot per treatment; the middle

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Abbreviations: AUGCC, area under green cover curve; IPM, integrated pest management.

Conversions: For unit conversions relevant to this article, see Table A.

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Table A. Useful conversions.

To convert Column 1 to Column 2, multiply by	Column 1 Suggested Unit	Column 2 SI Unit
Length		
2.54	inch	centimeter, cm (10^{-2} m)
Area		
0.405	acre	hectare, ha
Volume		
2.96×10^{-2}	ounce (liquid), oz	liter, L (10^{-3} m^3)
Mass		
4.54×10^{-1}	hundredweight (short), cwt	kilogram, kg
Yield and Rate		
67.19	60-lb bushel per acre, bu/acre	kilogram per hectare, kg/ha

four rows were harvested for seed yield, while the outer two rows were used for destructive sampling. The full season trial was planted in late May, while the double crop trial was planted in late June. Both locations were managed according to University of Kentucky's recommendation for soil fertility and weed management. Root nodule number and leaf N concentration were measured from three plants at V3, V6, and R6 growth stages at both locations, while Princeton also had an R1 measurement. Roots were gently washed with water to remove soil, nodules were counted, and leaf tissue was dried prior to analysis for leaf N concentration (Water's Agricultural Laboratory, Owensboro, KY). Seed was harvested in October. Seed yields were adjusted to 13% grain moisture concentration.

The full season and double crop trials were analyzed separately using SAS v9.4 (SAS Inst. Inc., Cary, NC) with PROC GLIMMIX. Treatment was considered a fixed effect, while replication and location were considered random effects. Least Square Means (LSMeans) were calculated in PROC GLIMMIX and separated with the lines option.

Seed yield and number of root nodules were similar ($P \geq 0.05$) among the non-inoculated control and the inoculated treatments for the full season (Table 1) and double crop (Table 2) trials. Results show low full season seed yields, due to the Zanesville locations (29 bu/ac), which has lower soil quality and prolonged periods of soil saturation during the growing season. The full season Crider location yielded 55 bu/ac and the Lexington location yielded 62 bu/ac (data not shown). Even though there were differences between locations for overall yield, there were no differences among the inoculant treatments for seed yield. Seed yield findings corroborated previous studies where fields with a recent history of soybean throughout the Midwest (De Bruin et al., 2010; Mason et al., 2016) and mid-South (Golden et al., 2016) did not observe a yield increase from the added inoculant. Similarly, number of root nodules were not different among the treatments, which agrees with previous findings that rhizobia within the soil produces adequate nodulation for maximum yield (Caldwell and Vest, 1970).

Differences were detected for leaf N concentration at R6 growth stage in the double crop trial ($P = 0.022$) (Table 2). It

Table 1. Soybean seed yield, number of root nodules, and leaf N concentration for the full season *Bradyrhizobium japonicum* inoculant trials at Princeton and Lexington, Kentucky, in 2016.

Treatment	Seed yield†	Root nodules V3	Root nodules V6	Root nodules R1‡	Root nodules R6	Leaf N concentration V3	Leaf N concentration V6	Leaf N concentration R1	Leaf N concentration R6
	bu/ac	no.				%			
Non-inoculated control	49.2	15.9	49.9	66.8	149.1	4.0	4.2	3.6	4.0
America's Best Inoculant 1× rate§	48.6	16.3	45.6	82.3	149.3	4.0	4.4	3.6	4.3
America's Best Inoculant 2× rate	49.6	16.9	42.2	73.4	140.9	4.2	4.5	3.7	4.3
Optimize XC 1× rate	48.4	19.1	40.5	79.4	136.3	3.9	4.3	3.6	3.9
Optimize XC 2× rate	48.6	18.3	40.8	70.4	157.6	4.0	4.3	3.8	3.8
P value	0.964	0.285	0.305	0.212	0.521	0.484	0.330	0.893	0.166
R^2	0.002	0.029	0.049	0.026	0.013	0.020	0.107	0.181	0.023
CV	33.370	42.040	34.058	47.912	42.601	12.091	6.422	10.682	32.240
Root MSE	16.425	7.112	16.135	35.675	65.068	0.488	0.279	0.391	1.305

† Seed yields were adjusted to 13% moisture concentration.

‡ R1 data were only collected from Princeton locations.

§ ABI, America's Best Inoculant (Van Wert, OH), Optimize seed inoculant (Novozymes BioAg, Saskatoon, Canada). Mention of a brand name does not constitute an endorsement.

Table 2. Soybean seed yield, number of root nodules, and leaf N concentration for the double crop *Bradyrhizobium japonicum* inoculant trials at Princeton and Lexington, Kentucky, in 2016.

Treatment	Seed yield†	Root nodules: Growth stages V3	V6	R1‡	R6	Leaf N concentration: Growth stages V3	V6	R1	R6§
	bu/ac	no.				%			
Non-inoculated control	46.8	26.0	49.8	38.9	44.8	4.5	4.1	4.3	3.7 B
America's Best Inoculant 1× rate¶	48.7	27.9	42.9	41.8	40.4	4.5	4.2	4.2	4.3 A
America's Best Inoculant 2× rate	47.6	26.4	42.3	44.4	45.4	4.6	4.1	4.4	4.4 A
Optimize XC 1× rate	46.3	27.4	41.9	37.3	41.1	4.3	4.1	4.4	4.3 A
Optimize XC 2× rate	46.5	31.1	40.2	39.0	40.0	4.4	4.1	4.8	3.9 AB
P vlaue	0.777	0.248	0.093	0.263	0.446	0.054	0.873	0.3932	0.022
R^2	0.007	0.032	0.047	0.033	0.007	0.083	0.011	0.108	0.149
CV	20.247	36.848	35.544	34.422	58.404	7.585	9.793	13.938	16.047
Root MSE	9.857	10.042	15.037	13.858	21.094	0.338	0.402	0.613	0.662

† Seed yields were adjusted to 13% moisture concentration.

‡ R1 data were only collected from Princeton locations.

§ *P* value significant at an alpha of 0.05. Means with the same letter are not significantly different across treatments.

¶ America's Best Inoculant (Van Wert, OH), Optimize seed inoculant (Novozymes BioAg, Saskatoon, Canada). Mention of a brand name does not constitute an endorsement.

is possible that this increased leaf N concentration could be attributed to differences in photosynthetic activity of the soybean leaves. Buttery et al. (1981) found that during pod fill (R5-R7), leaf N concentration was positively correlated with apparent photosynthesis and ultimately seed yield. Despite a 20% increase in leaf N concentrations for three of the inoculated treatments, these higher concentrations did not result in increased seed yield.

Seed yield and number of root nodules were similar for the non-inoculated control and *B. japonicum* inoculants regardless of application rate. These results support the University of Kentucky's recommendations that soybean seed inoculant is not necessary for fields with a history of soybean production in the past three to five years unless there were previous problems with soybean root nodulation or if the field remained saturated for extended periods of time. Although annual application of inoculants may seem like an inexpensive input at the recommended rate, they can ultimately be a considerable cost, especially when applied at higher than recommended rates to large areas without a yield increase.

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