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Inoculation Timing Effect on Biological Nitrogen Fixation and Soybean Productivity

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Abstract

Soybean [*Glycine max* (L.) Merr.], as other legume species, has the characteristic of fixing nitrogen (N) from the atmosphere via the biological N fixation (BNF) process. When a proper symbiosis relationship between soybeans and specific bacteria has been established, the plants can obtain up to 98% of the total N need. However, several factors can negatively affect BNF, impairing its contribution to nutrient demand and reducing crop productivity. In this scenario, additional inoculation could help the plant to overcome potential N gaps in BNF. Therefore, the goal of this project was to investigate if additional inoculation at different growth stages of the soybean growing season could increase nodulation, improve BNF (N contribution) and productivity of two varieties from maturity groups (MG) III and IV. To address this objective, different strategies for N supply were tested in a greenhouse and two field locations (Ashland Bottoms and Ottawa, KS) during the 2018 growing season. Trials were arranged in a complete randomized block design with four replications. The main outcomes of this study were that nodulation (total number of nodules per plant), plant dry biomass, relative abundance of ureide (RAU (%), indirect measurement of BNF), and productivity did not differ between inoculated treatments.

Keywords

Glycine max, inoculant, rhizobia

Authors

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Summary
Soybean [*Glycine max* (L.) Merr.], as other legume species, has the characteristic of fixing nitrogen (N) from the atmosphere via the biological N fixation (BNF) process. When a proper symbiosis relationship between soybeans and specific bacteria has been established, the plants can obtain up to 98% of the total N need. However, several factors can negatively affect BNF, impairing its contribution to nutrient demand and reducing crop productivity. In this scenario, additional inoculation could help the plant to overcome potential N gaps in BNF. Therefore, the goal of this project was to investigate if additional inoculation at different growth stages of the soybean growing season could increase nodulation, improve BNF (N contribution) and productivity of two varieties from maturity groups (MG) III and IV. To address this objective, different strategies for N supply were tested in a greenhouse and two field locations (Ashland Bottoms and Ottawa, KS) during the 2018 growing season. Trials were arranged in a complete randomized block design with four replications. The main outcomes of this study were that nodulation (total number of nodules per plant), plant dry biomass, relative abundance of ureide (RAU (%), indirect measurement of BNF), and productivity did not differ between inoculated treatments.

Introduction
Soybean (*Glycine max* L.) crop has a high content of oil and protein in the seeds. Argentina, Brazil, and the United States comprise 13, 33, and 35%, respectively, of the estimated global soybean production (USDA FAS, 2018). Soybean can establish symbiotic association with soil bacteria called rhizobia, obtaining on an average up to 50–60% of their needs through biological nitrogen (N) fixation (BNF). For high yielding soybean varieties, the gap between plant N demand and BNF supply becomes larger, and thus, more N might need to be potentially available from the soil to satisfy this demand. Symbiosis may fail for several factors such as stress, in the form of drought, excessive soil moisture, and high temperatures; soil pH; inadequate coverage of the seeds by the inoculum during inoculation; high soil inorganic N (ammonium and nitrate) levels; low soil phosphorus (P); and soil deficiency in molybdenum (needed for the formation and function of the nitrogenase enzymes) (Ciampitti et al., 2018).

Moretti et al. (2017) showed that even when initial nodulation is successful, additional spray inoculation at different soybean growth stages can promote nodulation, and the plant can overcome autoregulation of nodulation (AON) exerted by the host (Reid et al., 2011; Wang et al., 2014), resulting in improvement in grain yield. However, there is no information about the effect of additional inoculations in the US.

The main objective of this project was to investigate if additional inoculation at different stages of soybean growth can increase nodulation, improve BNF and productivity in two soybean maturity groups (MG).

Procedures

Site Characteristics

During the 2018 growing season, soybean N strategies were carried out in a greenhouse setting and in two field locations, Ashland, KS, (39.13N, -96.61 W) and Ottawa, KS (38.54 N, -95.24 W).

For the field studies, a soil initial characterization was done at 6-in. depth for the following soil chemical parameters: soil pH, P levels (Mehlich P), cation exchange capacity (CEC), organic matter (OM), calcium (Ca), magnesium (Mg), and potassium (K); and at 24-in. depth for nitrates ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4$). For the greenhouse study, substrate samples were collected in order to determine all soil chemical parameters (Table 1).

Experimental Design

For the greenhouse study, the plants were sown in pots with 1.6 gallons of volume, in a 70% of substrate Berger BM1 all-purpose and 30% of sand, mixed and steamed preparation; with 4 replications. For field studies, the plot size was 10-ft wide × 60-ft long with six replications. The greenhouse study was arranged in a split-plot randomized block design and field studies were arranged in a complete randomized block design.

Treatments

Five treatments were evaluated

1. non-inoculated (Control),
2. inoculation at sowing (Sowing),
3. inoculation at sowing + inoculation at V4 (Sowing + V4),
4. inoculation at sowing + inoculation at R1 (Sowing + R1),
5. non-inoculated but fertilized with 300 lb/a (Full-N).

Two soybean varieties were used, AG30X8 (MG 3.0) and AG45X6 (MG 4.5), both from Asgrow (Monsanto Company, Saint Louis, MO), with the Roundup Ready 2 Xtend events. The inoculant applied was VAULT HP plus integral (BASF, Ludwigshafen, RP, Germany). Additional inoculation mixed with a high volume of water, 4 inches away from the plants was applied toward the substrate or soil early in the morning to avoid losses due to high temperatures. The fertilizer N source was liquid urea ammonium nitrate (UAN), 28-0-0 (N-P-K) and was equally split into three applications: at planting, flowering (R1), and the beginning of seed filling (R5) following the plant N uptake curve for this crop. For field studies, herbicides and hand weeding were used to maintain no weed interference during the entire growing season; the target seeding rate was 140,000 seeds per acre. For the greenhouse experiment, the pots were sterilized. The irrigation system was disinfected (3 minutes with alcohol 80%, sodium hypochlorite (NaOCl) 5.25%, and distilled water) as seeds (2 minutes with ethanol 80%, followed by 1-minute bleach 1.25%, and washed very carefully 5 times with distilled water).

Measurements

Stand counts were performed measuring 5-ft sections per row, 4 rows in each plot, at the V4 stage (four fully developed trifoliate) in order to estimate final plant density in all replications (Table 2).

Measurements were nodule number and nodule dry weight, BNF by an indirect method - RAU (relative abundance of ureides, %), dry biomass accumulation and productivity (pod biomass for greenhouse and yield for field studies).

For the greenhouse, all measurements were at V4, V4+14 days, R1, R1+14 days, R6, and R7 growth stages.

For field studies, all measurements were at V4, R1, R1+14 days, R6, and R7 growth stages. Yield was collected from the central two rows (5 × 60 feet).

Weather Information

Irrigation was provided to the greenhouse pots in order to avoid water limitations. Precipitations were lower during the beginning of the growing season for field studies, being 26.7 (Ashland) and 25.6 (Ottawa) inches of rainfall during the growing season, 75% after R2 growth stage (full flowering).

Results

The total number of nodules per plant and nodule dry weight did not differ between inoculated treatments (Figure 1). Between maturity group (MGs), the nodulation followed a similar trend throughout the growing season. Field studies followed the same trend.

Regarding BNF, for the greenhouse study, the ureide-N concentration was similar across all the inoculated treatments being greater than the non-inoculated strategies (Full-N and control). The lower biomass production in the control is related to a lower N availability, which diminishes the N demand and consequently the BNF. This behavior was compensated by the N supply of the full-N strategy, allowing the plants to attain comparable biomass to the inoculated treatments (Figure 2).

Pod biomass in the greenhouse, as an indicator of the seed yield per plant, was not affected by soybean variety and presented lower values only for the control (Figure 3). In the field setting, in terms of yields, there were no significant differences across all treatments.

Preliminary Conclusions

- Additional inoculation at V4 or R1 growth stage did not improve soybean nodulation and BNF in greenhouse and field conditions.
- Soybean yield was not affected by the inoculation strategy under field conditions.

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Table 1. Chemical characteristics of substrate mix (greenhouse experiment, GH) and soil (field experiments, FD) at 6-in. and 24-in. depth, collected right before the onset of the experiment

Site	Depth	pH	P	K	Ca	Mg	Na	CEC [(a)]	NH_4	NO_3^-	SOM [(b)]
	inches		------------------------ *ppm*				------------------------	*meq_c/100g^{-1}*	------ *ppm*	------	*%*
GH	---	6.7	12.2	73	1363.5	303.4	28.9	9.7	2.8	6.8	5.1
FD	0 – 6	6.1	52.5	313	1972.8	204.6	15	17			2.2
Ashland	0 – 24								7.3	10.1	
FD	0 – 6	5.9	13.7	141	255.9	393.5	90	27.6			3.97
Ottawa	0 – 24								12.8	1.38	

P = phosphorus. K = potassium. Ca = calcium. Mg = magnesium. Na = sodium. CEC = cation exchange capacity. NH_4 = ammonium. NO_3- = nitrate. SOM = soil organic matter (loss on ignition).

Table 2. Final plant density (plants × 1000/a) per treatment in field experiments at Ottawa and Ashland, KS, during the 2018 growing season

Location	Maturity group	Control	Sowing	Sowing + V4	Sowing + R1	Full-N
Ottawa	3.0	122	130	124	130	120
	4.5	114	134	122	122	110
Ashland	3.0	117	120	118	118	115
	4.5	126	120	120	115	108

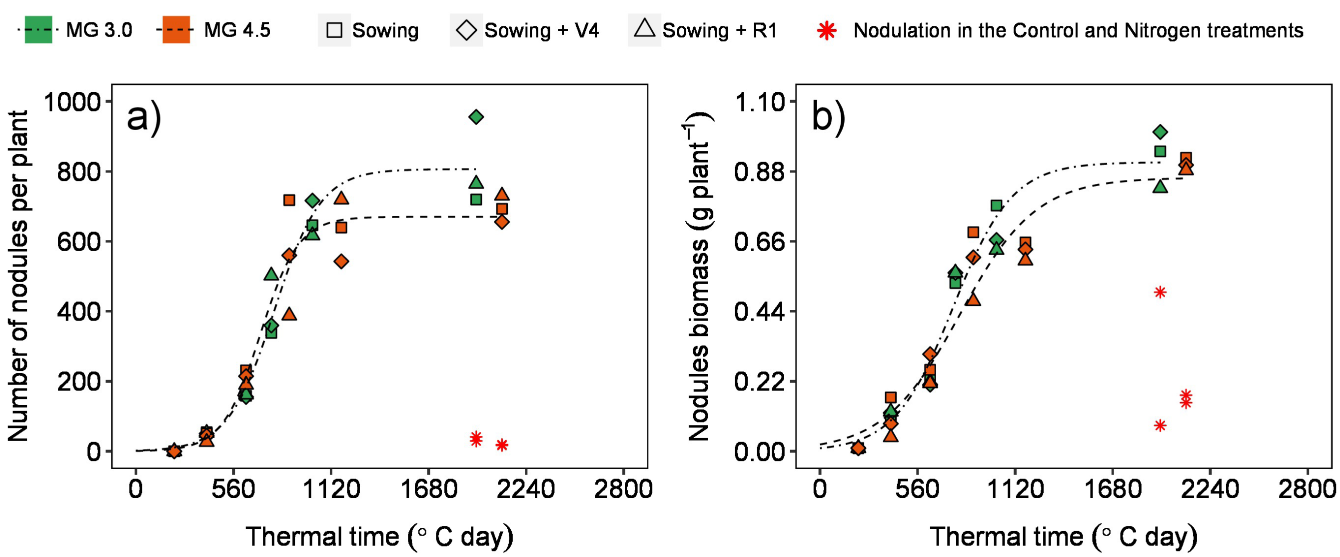

Figure 1. Number of nodules (a) and nodule dry biomass (b), in g/plant, for the inoculated treatments in soybean maturity group (MG) 3.0 and 4.5 in the greenhouse study. Points represent the least square means (lsmeans) from each model. A non-linear logistic function was fitted.

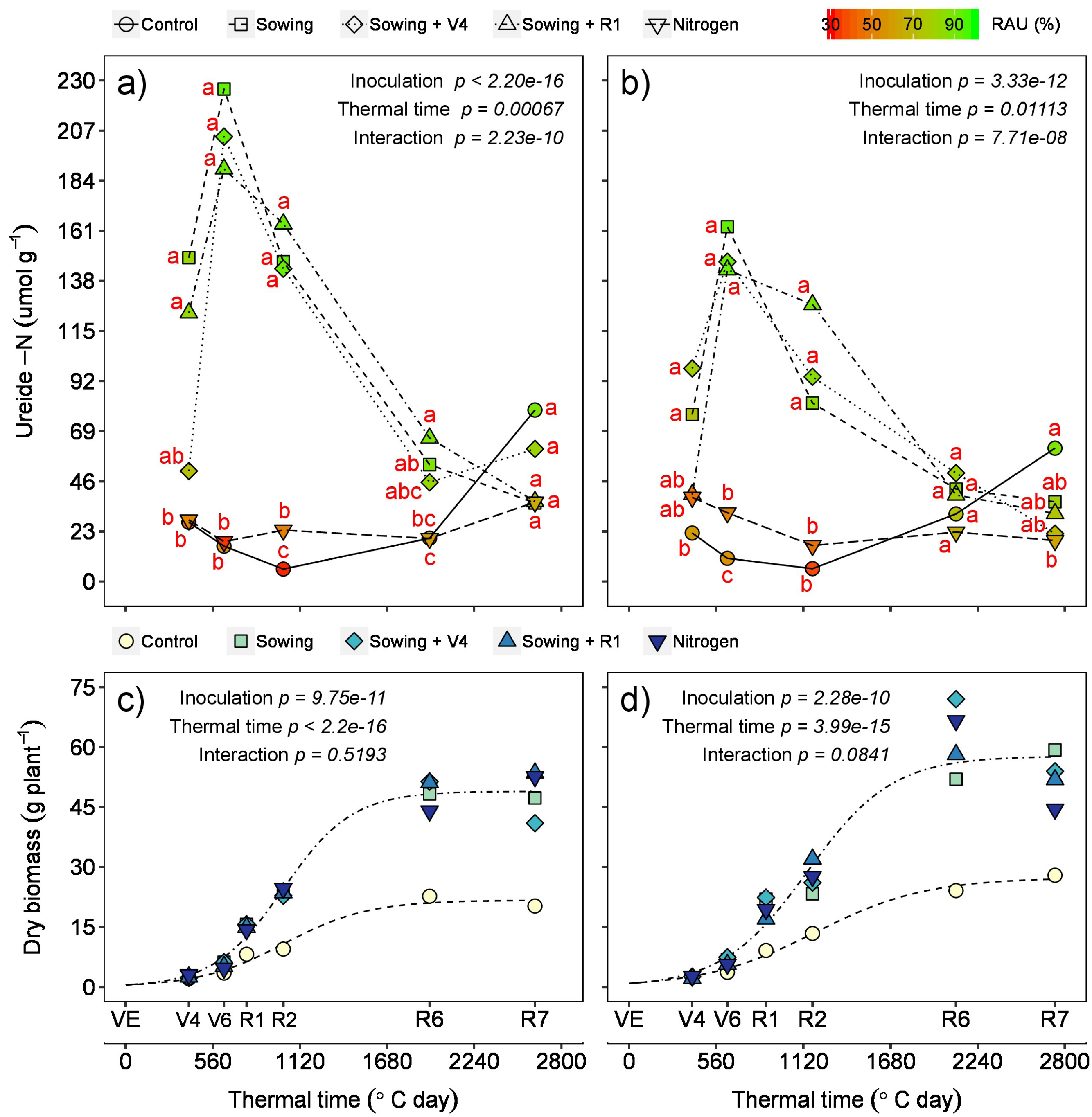

Figure 2. Ureide-N concentration, μmol/g, for MG 3.0 (a) and 4.5 (b). The color gradient is showing the Relative Abundance of Ureide-N (RAU, %). Points represent the least square means (lsmeans) from each model. Plant biomass accumulation, g/plant, for MG 3.0 (c) and 4.5 (d) is fitted by a non-linear logistic function. Letters are comparing inoculation strategies inside each sampling time by the Tukey test at 5% significance. Data from the greenhouse experiment.

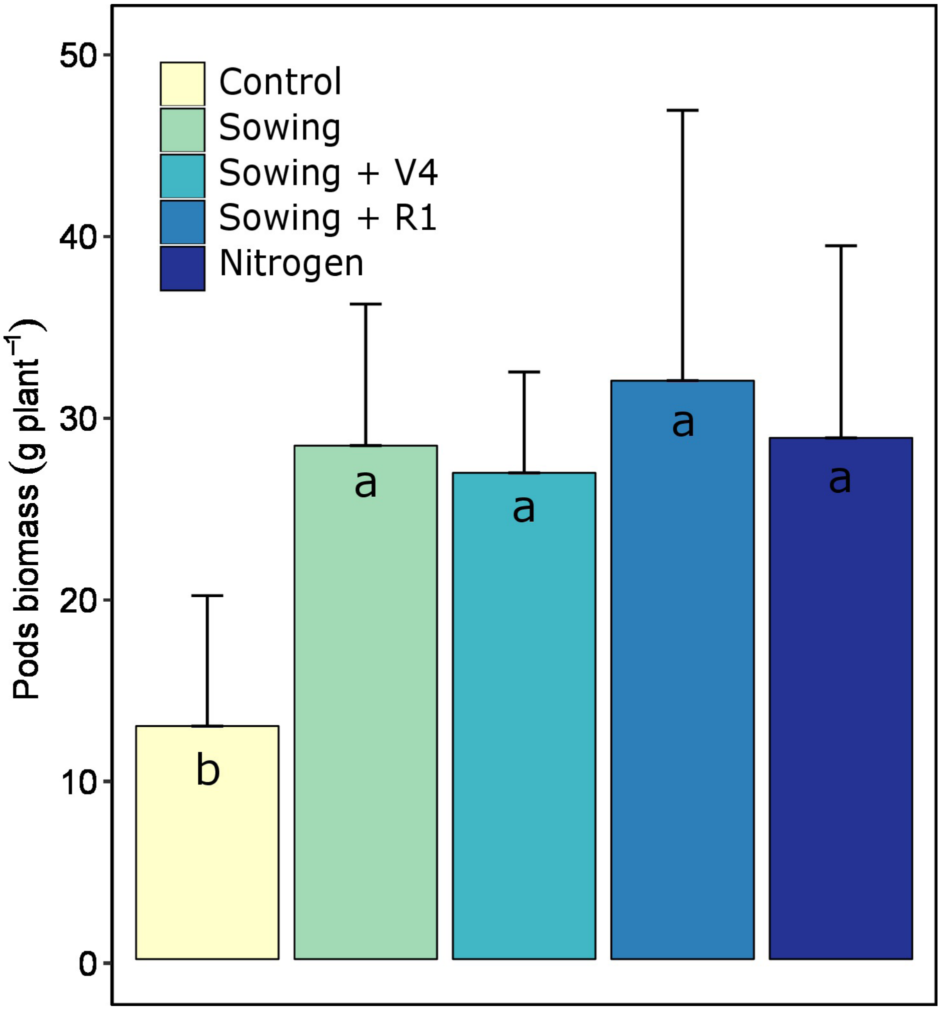

Figure 3. Pod dry biomass, g/plant, for the greenhouse study affected only by the inoculation strategy. Error bars are the standard deviation.