

# Influence of *Bradyrhizobium* Inoculation and Fungicide Seed Treatment on Development and Yield of Cowpea, Lablab, and Soybean

Rosemary Bulyaba\* and Andrew W. Lenssen

## Abstract

Cowpea [*Vigna unguiculata* (L.) Walp.] and lablab (*Lablab purpureus* L.) are grain legumes with limited testing for grain, forage, and cover crop use in the US Corn Belt. We conducted a 2-year field study to determine the influence of *Bradyrhizobium* spp. inoculation and fungicide seed treatment on development, nodulation, and yield of selected grain legumes. Soybean [*Glycine max* (L.) Merr.] was included as a well-understood control. The experimental design was a randomized complete block in a factorial of four grain legumes with and without *Bradyrhizobium* spp. inoculation and a fungicide seed treatment. The cultivars used in the experiment were soybean P92Y82, cowpeas CA46 and Top Crop, and lablab Rongai. The seed treatments were fungicide, mefenoxam + fludioxonil (ApronMaxx), and *Bradyrhizobium* spp. inoculant. The two experimental sites had four blocks each year, every block consisting of 16 plots with different crop–fungicide–*Bradyrhizobium* combinations. Each crop had four treatments: fungicide only, *Bradyrhizobium* spp. inoculant only, fungicide + inoculant, and a control (with no fungicide or inoculant). Cowpea plants had numerous functional nodules (pinkish or reddish on the inside) in the absence of *Bradyrhizobium* spp. inoculation, but lablab never produced any nodules without inoculation. We found no significant effects of *Bradyrhizobium* spp. inoculation and fungicide seed treatment on cowpea, lablab, or soybean grain yield.

**F**ungicide treatment and rhizobial seed inoculation are common practices prior to planting pulse crops, but reports vary widely for their impact on emergence, growth, development, and grain yield. Seed inoculation is done to provide sufficient numbers of viable and effective rhizobia that will induce fast colonization of the rhizosphere for rapid nodulation after seed germination and produce optimum yields at harvest (Catroux, 1991). Seed or soil inoculation in subsequent years may be unnecessary if the rhizobia become well established (van Kessel and Hartley, 2000). The

## Crop Management



### Core Ideas

- Cowpea did not require *Bradyrhizobium* inoculation for nodulation.
- Lablab required *Bradyrhizobium* inoculation for nodulation.
- Fungicide seed treatment did not compromise nodulation of cowpea or lablab.

Dep. of Agronomy, Iowa State Univ., Ames, IA 50011. Journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project number IOW05146, support by the Hatch Act and State of Iowa funds. \*Corresponding author (rbulyaba@iastate.edu).

Received 24 Jan. 2017.  
Accepted 13 Apr. 2017.

Published in Crop Forage Turfgrass Manage.  
Volume 3. doi:10.2134/cftm2017.01.0007

© 2017 American Society of Agronomy  
and Crop Science Society of America  
5585 Guilford Rd., Madison, WI 53711

All rights reserved.

Table A. Useful conversions.

To convert Column 1 to Column 2, multiply by	Column 1 Suggested Unit	Column 2 SI Unit
28.4	ounce (avdp), oz	gram, g
0.405	acre	hectare, ha
67.25	60-lb bushel per acre, bu/acre	kilogram per hectare, kg/ha
1.12	pound per acre, lb/acre	kilogram per hectare, kg/ha
1	parts per million, ppm	milligram per kilogram, mg/kg
5/9 (°F – 32)	Fahrenheit, °F	Celsius, °C

combination of fungicide and bacterial inoculant seed applications has also long been used to control root diseases in legumes and increase yield. Up to 40% yield increase was observed in soybean with *Bradyrhizobium japonicum* inoculation compared with noninoculated soybean in locations that had not been under soybean production in recent years (Schulz et al., 2005). Biological nitrogen fixation offers an inexpensive alternative to meet much of the nitrogen needs for pulse crop production. The combination of high levels of nitrogen fixation with better pest and disease management strategies from seed fungicide application may increase yield. It has also been documented that yields can be increased by seed inoculation compared with use of nitrogen fertilizer (Dobereiner et al., 1995). Farmers are often uncertain about the levels of indigenous rhizobia and their responsiveness to the legume crop; therefore, seed inoculation with rhizobia is used to ensure that maximum yield is attained (Kutcher et al., 2002). Entz (1998) reported that application of inoculant to seed at planting increased nodule number, which increased yield and aboveground biomass of cowpea, lablab, and soybean. Rhizobial inoculation of seeds may be done using single, double, or multiple strains (Daramola et al., 1994).

Pulse grain production can be decreased by root rot diseases (Muthomi et al., 2007), which are known to hinder seedling germination and cause postemergence damping-off, leading to poor crop stands, decreased root health, and low yields. Chemical seed treatment prior to planting is now a common practice to ameliorate the challenges above. However, the combination of rhizobial inoculation and fungicide seed treatment may be problematic regardless of the benefits of each. Schulz and Thelen (2008) reported that fungicide and rhizobium inoculant combinations increased soybean yield in situations where fungicide had no negative impact on the inoculant. They reported that rhizobial inoculation of seeds yielded an average yield increase of 1.3 bu/acre from 14 site-years that had previous soybean cropping history. With an average inoculant cost of \$3.4 acre<sup>-1</sup>, they determined that annual soybean seed inoculation with fungicide seed treatment was cost-effective to farmers. Bikrol et al. (2005) also reported that pretreatment of seeds with a fungicide had a positive role in biologically fixed nitrogen in soybean; grain protein content increased by 10% on average with fungicide seed treatment and inoculation (Schulz and Thelen, 2008). However, some research findings indicate negative effects of fungicide–rhizobium interaction on yield. Muthomi et al.

(2007) explained that sometimes seed-applied fungicide suppressed the effectiveness of the rhizobia bacteria in lablab and common bean (*Phaseolus vulgaris* L.). Fungicide seed treatment also limited soybean nodulation and subsequent grain yield (Campo and Hungria, 2000). Hashem et al. (1997) and Thombre et al. (1989) also reported that the compatibility of *Bradyrhizobium* and fungicides was uncertain as the tested fungicides differed in their effects on the survival and growth of rhizobium strains in peanuts (*Arachis hypogaea* L.) and soybean, respectively. The effect of fungicide on strains also depended on the strains and concentration of fungicide applied (Hashem et al., 1997). Dunigan et al. (1972) also reported that inhibition of rhizobia and nodulation by seed-applied fungicide in soybean arose from contact and concentration especially on primary roots although the permanent effects on nodulation of lateral roots were limited (Rennie and Dubetz, 1984). Similarly, findings by Bikrol et al. (2005) showed significant negative correlation between rhizobium inoculation–fungicide seed treatment and soybean plant protein content. Other grain legumes, including cowpea and lablab, may have similar problems.

Little is known about the productivity of cowpea or lablab in Iowa or the need for rhizobia inoculation or fungicide seed treatment. Consequently, we conducted a field study with the objectives of determining the influence of fungicide seed treatment and inoculation on nodulation, nitrogen fixation potential, and productivity of cowpea, lablab, and soybean in central Iowa.

## Experiment Locations and Site Descriptions

In 2013 and 2014, field experiments were conducted in Ames and Boone, IA, respectively. In 2013, the field site and trials were established at the Iowa State University Curtiss farm (42°00'18.0" N, 93°40'09.3" W). In 2014, the study was conducted at the Agricultural Engineering and Agronomy Research farm (42°1'18.76" N, 93°46'35.94" W). Both farms are drained by tiles and ditches due to slow natural drainage (Hofstrand, 2010). Soil samples were collected from 0 to 12 inches before planting and analyzed for pH, nitrate, available P and K (Mehlich-3), and organic matter at the Soil and Plant Analysis Laboratory, Iowa State University (Table 1). The predominant soils at the Curtiss farm were Canisteo clay loam (fine-loamy, mixed, superactive, calcareous, mesic

Table 1. Preplant soil test values for Story (2013) and Boone (2014) county experimental sites in Iowa.

Year	Depth	P	K	pH	OM†	NO <sub>3</sub>
		ppm			%	ppm
2013	0–6 inches	35	198	5.7	46,000	4
2013	6–12 inches	7	136	6.5	42,000	5
2014	0–6 inches	43	153	5.5	36,000	7
2014	6–12 inches	14	99	5.6	27,000	4

† OM, organic matter.

Typic Endoaquoll) (NCSS, 2015a) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) (NCSS, 2015b) (Table 1). Soils at the Agricultural Engineering and Agronomy Research farm were predominantly Canisteo silty clay loam (NCSS, 2015a) and Clarion loam (fine-loamy, mixed, superactive, mesic Typic Hapludoll) (NCSS, 2015c), Bemis moraine soils (NRCS, 2014) (Table 1). Both sites had been in a corn (*Zea mays* L.)–soybean rotation for several decades with no known production history of cowpea or lablab.

## Experimental Design and Treatments

The experiment was a randomized complete block design with a complete factorial of two seed treatments (fungicide and *Bradyrhizobium* spp. inoculation) and pulse crop. Fungicide treatment, mefenoxam + fludioxonil (ApronMaxx, Syngenta, Greensboro, NC), was applied to seed at rates of 0.06 oz a.i. of mefenoxam and 0.04 lb a.i. of fludioxonil per 100 lb of seed about 2 weeks before planting. N-DURE Premium (Verdesian Life Sciences, Cary, NC) liquid commercial inoculant was used in 2013 and EL type inoculant (peat-based) (INTX Microbials, LLC, Kendall, IN) was used in 2014 to inoculate seeds at labeled rates prior planting. Both inoculants are suitable for crops in our study. The two sites had four blocks each year, with every block consisting of 16 plots with different crop–fungicide–*Bradyrhizobium* combinations. Each crop had four treatments: fungicide only, *Bradyrhizobium* spp. inoculant only, fungicide + inoculant, and a control (with no fungicide or inoculant). The four pulse crops were two cowpea cultivars, California black-eyed pea, CA46 and purple hull pinkeye southern pea, Top Crop; lablab Rongai; and soybean cultivar P92Y82 (DuPont Pioneer, Johnston, IA). Individual plot size was 25 ft long by 10 ft wide with 2.5-ft row spacing. The seeding rate was 3 pure live seeds ft<sup>-2</sup> and plots were planted on 6 and 13 June, 2014 and 2013, respectively. The seeds were planted at a 1-inch depth using a Max-Emerge row unit (John Deere, Moline, IL).

## Site Management and Data Collection

Fertilizers were not applied at either site because soil test analysis indicated adequate amounts of nutrients recommended for soybean production in Iowa, including P and K among others (Mallarino et al., 2013). A preplant application of pendimethalin [N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] was applied at a rate of 1.8 qt/acre in 80 qt H<sub>2</sub>O acre<sup>-1</sup> in 2013 and 2014. Additional weed management

was mechanically accomplished by cultivation and hand-roguing. In 2013, a weed infestation later in the season in soybean was controlled with glyphosate [N-(phosphonomethyl) glycine] at 48 oz a.i. acre<sup>-1</sup> in 40 qt H<sub>2</sub>O acre<sup>-1</sup>. Lambda-cyhalothrin ([1α(S\*),3α(Z)]-(±)-cyano-(3-phenyloxyphenyl) methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate) was used to control Japanese beetles (*Popillia japonica*) in 2013 and leafhoppers (*Empoasca fabae*) in 2013 and 2014 early in the season. The latter was applied at 0.13 oz a.i. acre<sup>-1</sup> in 40 qt H<sub>2</sub>O acre<sup>-1</sup>. The experiment relied solely on natural rainfall.

Phenological stages were determined weekly from the two middle rows of each plot using a soybean staging system (Fehr et al., 1971). Stand counts were measured when plants were between V4 and V6 stages using a randomly placed 17.5-ft measuring rod along the length of each row. At R4, two plants were selected from each of the two outer rows of each plot and dug from the ground with enough soil to avoid losing nodules. These plants were soaked in water at ambient temperature, and roots were carefully washed to prevent nodule loss. The number of nodules per plant was counted and the taproot sliced longitudinally to visually assess any symptoms of root diseases. Ten representative nodules per plot were dissected to determine nodule color and the number of functional (pink/red) nodules recorded. Above-ground biomass was determined at R5.5, prior to leaf loss, by hand-clipping 40-inch row from each plot. Samples were placed in a forced-air oven at 140°F until dry, weighed on the Ohaus Explorer balance (Model E1B120, Parsippany, NJ), and ground using the MTD chipper shredder (Model 465, Cleveland, OH). At R8, the pulses were hand-harvested for subsequent determination of yield components. Pods were hand-harvested from 79 inches of row from the two central rows of each plot. The pods were counted as they were taken off each stem and the number of pods per acre calculated. Pods were then threshed with a stationary thresher (Model Almaco BT-14, Nevada, IA). Seeds were counted using a Seedburo 801 Count-A-Pak (Seedburo Equipment, Des Plaines, IL) seed counter. The seeds were weighed after drying in an oven overnight at 140°F. Crude protein, oil, and fiber analyses were determined for soybean grain using near-infrared spectroscopy (Kovalenko et al., 2006). A subsample of cowpea seed from each plot was ground to pass a 0.039-inch sieve with a cyclone mill and used for total nitrogen (N) determination using colorimetric procedures (LECO, St. Joseph, MI). Crude protein was calculated as total nitrogen (N × 6.25).

Data were analyzed by generalized linear mixed models PROC GLIMMIX using SAS v9.4 (SAS Institute Inc., Cary, NC). During analysis, the different sites each year were accounted for by confounding blocks with years. Blocks in the model were treated as random elements whereas fungicide, *Bradyrhizobium* spp., and crop factors were treated as fixed effects. The PDIFF procedure was used to test for differences among means when *F* tests were significant for main effects or their interactions. A 0.05 significance level threshold was used, although differences amongst treatments were reported at the 0.001 significance level.

## Weather Conditions

During the growing season, temperatures tended to be warmer than the presented averages at both experimental sites in 2013 and 2014 (Table 2). Precipitation during the growing season was lower in 2013 than in 2014. Precipitation in 2013 from May to October was 34% lower than the past 12-year average whereas that in 2014 was 20% greater than the 12-year average (Table 2). From June to October (planting to harvest time), 2014 received 75% more rainfall than 2013 (Table 2).

## Plant Stand Density and Height

The interaction of fungicide and *Bradyrhizobium* spp. seed treatments was not significant for any of the response variables measured in the study. However, the pulse crops differed significantly for all response variables measured (Table 3). Plant population at V6 growth stage varied significantly among the pulse crops (Table 3). Top Crop cowpea had the greatest stand density, followed by CA46. Soybean and lablab had lower stand density than cowpea CA46, and their (soybean and lablab) stand densities did not differ from one another. Stand density did not differ with fungicide or *Bradyrhizobium* spp. seed treatments (Table 3). Plant height at maturity (R8) differed by pulse crop, but the main effects of fungicide seed treatment and *Bradyrhizobium* spp. and their interactions were nonsignificant (Table 3). Cowpea CA46 was the tallest and Top Crop was the shortest at maturity. CA46 plants were 28 and 62% taller than soybean and Top Crop, respectively, at R8. Since lablab did not reach maturity, its plant height at R8 could not be determined.

## Nodulation Following Fungicide and *Bradyrhizobium* Application

Nodule number per plant differed among pulse crops. Soybean had the greatest number of nodules per plant followed by cowpeas CA46 and Top Crop, and lablab had the least. However, although lablab had the least, its nodules were visually larger compared with those from other pulse crops in the experiment. The interaction of fungicide and *Bradyrhizobium* spp. application on nodule number and functional nodules was not significant. However, the number of functional nodules per plant differed among soybean, lablab, and cowpeas CA46 and Top Crop. Soybean had the greatest number of functional nodules per plant followed by CA46 and Top Crop

Table 2. Twelve-year monthly average air temperature and total precipitation during the 2-year study.

Month	2013	2014	12-year total avg.
°F			
Mean air temperature			
Apr.	46.4	48.0	51.8
May	60.8	62.6	62.6
June	69.8	71.8	71.6
July	73.4	69.0	75.2
Aug.	73.0	71.6	73.4
Sept.	67.0	62.6	64.4
Oct.	51.0	51.8	51.8
Avg. air temperature (Apr.–Oct.)	63.1	62.5	64.4
inches			
Total precipitation			
April	5.83	4.76	3.86
May	7.09	4.25	5.35
June	1.02	8.86	4.80
July	1.02	2.87	4.53
August	1.18	5.83	5.12
September	1.18	5.43	3.35
October	2.52	4.69	2.56
Cumulative precipitation (Apr.–Oct.)	19.84	36.69	29.57

whereas lablab had the least. Soybean roots had 91, 48, and 19% more functional nodules than lablab and cowpeas Top Crop and CA46, respectively. The absence of significant differences in nodulation following seed application of fungicide and *Bradyrhizobium* spp. inoculum may be attributed to factors such as climate, soil environment, and the existence of indigenous bacterial strains in the soil (Wright and Lenssen, 2013). In line with the latter, both experimental sites had multiple decades of soybean production and therefore previously introduced competitive *Bradyrhizobium japonicum* strains were likely present. Many soils usually harbor native rhizobium strains of different competitiveness and compatibility even before seed inoculation (Daramola et al., 1994). The observed differences in nodule number by pulse crop may be attributed to the host plant as well as rhizobium strain. Amarger and Lobreau (1982) reported that the number of nodules formed varied by strain in a study on faba bean (*Vicia faba* L.). Bhuvaneswari et al. (1988) also reported that the efficiency of nodule initiation and development following seed inoculation was greatly influenced by the host species. Similar to findings from our work, they showed that *Bradyrhizobium japonicum* formed nodules with cowpea and soybean. Somasegaran and Hoben (1985) also explained that differences in nodule number by crop may be attributed to variances in the forms of bacteroids found in the nodules of different legumes. The authors explained that the plant largely determined the size, shape, and number of these bacteroids in each nodule. This could further explain why soybean had more nodules than the other three pulse legumes whereas lablab had the least but visually largest nodules compared with soybean and cowpeas CA46 and Top Crop.

Table 3. Effect of fungicide and *Bradyrhizobium* application on stand density, R8 height, nodule number, biomass, seed yield, yield components, and seed crude protein (CP) of soybean, lablab, cowpea CA46, and cowpea Top Crop in central Iowa (2013 and 2014).

Treatment	Plant population V6†	Height R8	Total nodules	Functional nodules	Biomass	Yield	Pods	Seeds/pod	Seed weight	Seeds/sq yd	Seed CP
	no./acre	inches	— no./plant —		lb/acre	bu/acre	no./yd2	no./pod	oz/seed × 10 <sup>-4</sup>	no./yd2	%
Fungicide											
Applied	99,148	43.3	21.7	17.6	4120	30.7	669	4.2	57	2040	27.4
Not applied	101,172	39.4	21.5	16.9	3982	30.3	663	4.2	56	2053	27.9
Rhizobium											
Applied	100,362	39.8	21.7	17.3	4058	30.1	656	4.1	57	1999	27.5
Not applied	101,172	42.9	21.6	17.2	4045	30.8	676	4.3	57	2093	27.7
Crop											
Soybean	84,984 †	42.5 b	37.2 a	28.5 a	4945 a	46.6 a	1415 a	2.1 b	61 a	3022 a	35.0 a
Lablab	87,008 c	—	2.5 c	2.5 d	3669 bc	—	—	—	—	—	—
Cowpea CA46	106,028 b	59.4 a	25.9 b	23.2 b	4059 b	21.0 b	258 c	5.2 a	57 b	1380 c	22.6 c
Cowpea Top Crop	120,597 a	22.4 c	21.0 b	14.8 c	3531 c	23.9 b	326 b	5.3 a	54 c	1737 b	25.3 b
<i>P</i> > <i>F</i>											
Significance											
Fungicide (F)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rhizobium (R)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × R	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Crop (C)	***	***	***	***	***	***	***	***	***	***	***
F × C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R × C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × R × C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*\*\* Significant at the 0.001 level of probability.

† V6, Vegetative stage 6; R8, Reproductive stage 8.

‡ Values followed by the same letter within a column are not significantly different at *P* < 0.05.

It is worth noting that nodule number is a less reliable indicator of strain effectiveness, hence the number of functional nodules was used to determine potential for nitrogen fixation. Since the number of functional nodules per plant was not significantly different with or without fungicide and *Bradyrhizobium* spp. seed treatments, this may imply that crops that did or did not receive these treatments had the same potential for nitrogen fixation. The reddish or pinkish color inside sliced nodules caused by leghemoglobin indicates that nitrogen fixation was ongoing (Flynn and Idowu, 2015). Although it is still uncommon for lablab to be grown as a grain legume in Iowa, the crop showed good potential as a forage or cover crop, but it is important to inoculate seed prior to planting. Cowpea, on the other hand, could be grown for grain, forage as well as cover crop but apparently does not require seed inoculation with *Bradyrhizobium* if soybean had been produced in the recent past.

### Effect of Fungicide and *Bradyrhizobium* on Grain Yield and Aboveground Biomass

Aboveground biomass differed by crop but seed application of fungicide or *Bradyrhizobium* spp. and their interaction did not influence aboveground biomass (Table 3). Soybean had

the greatest amount of aboveground biomass followed by CA46 and lablab whereas Top Crop had the least. Soybean had 18, 25, and 29% more aboveground biomass than CA46, lablab, and Top Crop, respectively. Although no literature was found to compare soybean biomass accumulation with that of lablab, greater aboveground biomass accumulation by soybean compared with cowpea in our study corresponds with Rao and Northup (2009), who also reported that soybean produced more biomass than cowpea for 3 years in their 4-year experimental study. The range of cowpea biomass in our study (3531 to 4059 lb/acre for Top Crop and CA46, respectively) also corresponds with previous studies by Agza et al. (2012), who reported dry matter accumulation yields of different cowpea genotypes ranging between 3319 and 4220 lb/acre. Additionally, Somasegaran and Hoben (1985) reported that aboveground biomass accumulation was a generally accepted criterion for nitrogen-fixing effectiveness alongside others such as nodule dry weight. This corresponds to findings from our study for soybean and CA46 aboveground biomass accumulation and their corresponding number of functional nodules per plant.

## Relationship Between Yield Components and Fungicide-*Bradyrhizobium* Seed Treatments

Significant interactions for fungicide and rhizobia, fungicide and crop, rhizobia and crop, and fungicide × rhizobia and crop were not detected for any yield and yield components measured in the study. However, differences were observed among crops. Yield data were collected for soybean, CA46, and Top Crop, but lablab did not reach physiological maturity before frost. Lablab Rongai used in our study is a short-day plant and flowers best with <11 hours of daylight. Central Iowa day length was between 16 to 12 h for 2013 and 2014 growing seasons, respectively. Lablab also has no frost tolerance (Shivashankar and Kulkarni, 1989). Grain yield differed significantly among the pulse crops; however, the effects of fungicide and *Bradyrhizobium* spp. seed application and interactions were not significant (Table 3). Soybean had greater grain yield than Top Crop and CA46, with 49 and 55% more yield than the two cowpeas, respectively. Top Crop and CA46 had similar grain yield. Pod density also differed among crops (Table 3). Soybean had more pods/acre than Top Crop and CA46. Soybean had 77 and 82% greater pod density than Top Crop and CA46, respectively whereas Top Crop had 21% more pods than CA46. The influence of seed fungicide application, seed rhizobium inoculation, and their interaction was not significant on pod density (Table 3). Individual seed weight differed among pulse crops (Table 3). The effect of fungicide and *Bradyrhizobium* spp. seed treatment showed no significant differences on individual seed weight. The weight of seeds from plots that received or did not receive fungicide and *Bradyrhizobium* spp. treatments was not significantly different. The number of seeds per pod differed significantly by crop, but the effects of fungicide and *Bradyrhizobium* spp. seed treatments and interactions were not significant (Table 3). Seed number per pod between Top Crop and CA46 was not statistically different but was significantly greater than that of soybean. Top Crop and CA46 had 60% more seeds per pod than soybean. Seed no./yd<sup>2</sup> differed by crop (Table 3). Soybeans produced the most seed per area while CA46 produced the least. Soybean plots had 54 and 43% more seeds than CA46 and Top Crop, respectively. The seed no./yd<sup>2</sup> did not differ with fungicide and *Bradyrhizobium* spp. seed application or their interaction. Additionally, seed crude protein was significantly different among crops (Table 3). Soybean seeds had the greatest crude protein concentration whereas CA46 had the lowest. Soybean seeds had 35 and 28% more crude protein than Top Crop and CA46, respectively. No differences in seed crude protein were observed with the combination of fungicide and *Bradyrhizobium* spp. seed treatments. Greater soybean seed crude protein at maturity compared with cowpea is to some extent explained by Awolumat (1983). He further reported that, although cowpeas accumulated nitrogen at a rate much faster than soybean during seed development,  $3.51 \times 10^{-5}$  oz (994 μg day<sup>-1</sup>) compared with  $1.66 \times 10^{-5}$  oz (473 μg day<sup>-1</sup>), respectively, biomass crude protein generally decreased with development time from 40% in early seed development stages

to 26% in mature cowpea and from 35 to 33% in soybeans. Greater yield for soybean than for cowpeas in this study may be attributed to the long-term and significant efforts in soybean breeding and research done in the US Corn Belt.

## Conclusions

Since we did not observe significant effects of fungicide and *Bradyrhizobium* spp. on grains and biomass yield of soybean, cowpea, and lablab grown at sites with a soybean production history, we believe that these legumes can perform well in absence of these treatments. The use of seed fungicide-rhizobia treatments may not significantly affect development of these three crops and may be an unnecessary cost. While these legumes have the potential to fix nitrogen, we would not recommend omission of seed fungicide treatment in areas with known soilborne disease outbreaks. Overall, an adapted soybean performed better than cowpeas CA46 and Top Crop, and lablab for nodulation, aboveground biomass accumulation, yield, and seed crude protein, likely due to soybean having better adaptation to the environment in Iowa from long-term plant breeding efforts.

## Acknowledgments

The authors are grateful for technical assistance from Danielle Wilson, Luke Hodnefield, and Roger Hintz in the field and the laboratory.

## References

- Agza, B., K. Binyam, Z. Solomon, A. Eskinde, and A. Ferede. 2012. Animal feed potential and adaptability of some cowpea (*Vigna unguiculata*) varieties in northwest lowlands of Ethiopia. *J. Agric. Res.* 11:478–483.
- Amarger, N., and J.P. Lobreau. 1982. Quantitative study of nodulation competitiveness in Rhizobium strains. *Appl. Environ. Microbiol.* 44:583–588.
- Awolumat, E. 1983. Accumulation and quality of storage protein in developing cowpea, mung bean and soya bean seeds. *J. Sci. Food Agric.* 34:1351–1357. doi:10.1002/jsfa.2740341206
- Bhuvaneswari, T.V., A.P. Lesniak, and W.D. Bauer. 1988. Efficiency of nodule initiation in cowpea and soybean. *Plant Physiol.* 86:1210–1215. doi:10.1104/pp.86.4.1210
- Bikrol, A., N. Saxena, and K. Singh. 2005. Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicide seed treatment. *Afr. J. Biotechnol.* 4:667–671. doi:10.5897/AJB2005.000-3122
- Campo, R.J., and M. Hungria. 2000. Compatibility use of fungicides in the treatment and inoculation of soybean seeds. EMBRAPA. <https://www.agencia.cnptia.embrapa.br/gestor/feijao-caupi/catalogo/REC000ftw5mcb02wyiv80kxlb36t7i9p34.html> (accessed 2 Jan. 2017).
- Catroux, G. 1991. Inoculant quality standards and controls in France. In: J.A. Thompson, editor, Expert consultation on legume inoculant production and quality control. FAO, Rome. p. 113–120.
- Daramola, D.S., S.K.A. Danso, and G. Hardarson. 1994. Nodulation, N<sub>2</sub> fixation and dry matter yield in soybean [*Glycine max* (L.) Merrill] inoculated with combinations of effective and ineffective *Bradyrhizobium japonicum* strains. *Soil Biol. Biochem.* 26:883–889. doi:10.1016/0038-0717(94)90304-2
- Dobereiner, J., S. Uguiaga, and R.M. Boddey. 1995. Alternatives for nitrogen nutrition of crops in tropical agriculture. *Fert. Res.* 42:339–346. doi:10.1007/BF00750526

- Dunigan, E.P., J.P. Frey, L.D. Allen, and A. McMahon. 1972. Herbicidal effects on the nodulation of *Glycine max* (L.) Merrill. *Agron. J.* 64:806–808. doi:10.2134/agronj1972.00021962006400060031x
- Entz, P. 1998. Managing crop inputs in pea production. In: 20th Annual Manitoba-North Dakota Zero Tillage Workshop, "Zero Tillage and Farming's Future," Minot, ND. 2–4 Feb. 1998. p. 51–55.
- Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybeans *Glycine max* (L.) Merrill. *Crop Sci.* 11:929–931. doi:10.2135/cropsci1971.0011183X001100060051x
- Flynn, R., and J. Idowu. 2015. Nitrogen fixation by legumes. College of Agric., Consumer and Environ. Sci., New Mexico State Univ. [http://aces.nmsu.edu/pubs/\\_a/A129/](http://aces.nmsu.edu/pubs/_a/A129/) (accessed 2 Jan. 2017).
- Hardarson, G., and G.D. Jones. 1979. The inheritance of preference for strains of *Rhizobium trifolii* by white clover (*Trifolium repens*). *Ann. Appl. Biol.* 92:329–333. doi:10.1111/j.1744-7348.1979.tb03881.x
- Hashem, F.M., S.A. Saleh, P.V. Berkum, and M. Voll. 1997. Survival of *Bradyrhizobium* sp. on fungicide-treated peanut (*Arachis hypogaea* L.) seed in relationship to plant growth and yield. *World J. Microbiol. Biotechnol.* 13:335–340. doi:10.1023/A:1018595310239
- Hofstrand, J. 2010. Economics of tile drainage. Iowa State Univ., Ext. and Outreach. <https://www.extension.iastate.edu/agdm/articles/hof/HofJuly10.html> (accessed 2 Jan. 2017).
- Kovalenko, I.V., G.R. Rippke, and C.R. Hurburgh. 2006. Determination of amino acid composition of soybeans (*Glycine max*) by near-infrared spectroscopy. *J. Agric. Food Chem.* 54:3485–3491. doi:10.1021/jf052570u
- Kutcher, H.R., G. Lafond, A.M. Johnston, P.R. Miller, K.S. Gill, and W.E. May. 2002. Rhizobium inoculant and seed-applied fungicide effects on field pea production. *Can. J. Plant Sci.* 82:645–661. doi:10.4141/P01-180
- Mallarino, A., J.E. Sawyer, and S.K. Barnhart. 2013. A general guide for crop nutrient and limestone recommendations in Iowa. PM 1688. Iowa State Univ., Ext. and Outreach, Ames.
- Muthomi, J.W., P.E. Otieno, G.N. Chemining'wa, J.H. Nderitu, and J.M. Wagacha. 2007. Effect of root rot pathogens and fungicide seed treatment on nodulation in food grain legumes. *J. Biol. Sci.* 7:1163–1170. doi:10.3923/jbs.2007.1163.1170
- National Cooperative Soil Survey (NCSS). 2015a. Canisteo series. USDA. [https://soilseries.sc.egov.usda.gov/OSD\\_Docs/C/CANISTEO.html](https://soilseries.sc.egov.usda.gov/OSD_Docs/C/CANISTEO.html) (accessed 8 Mar. 2017).
- National Cooperative Soil Survey (NCSS). 2015b. Nicollet series. USDA. [https://soilseries.sc.egov.usda.gov/OSD\\_Docs/N/NICOLLET.html](https://soilseries.sc.egov.usda.gov/OSD_Docs/N/NICOLLET.html) (accessed 8 Mar. 2017).
- National Cooperative Soil Survey (NCSS). 2015c. Clarion series. USDA. [https://soilseries.sc.egov.usda.gov/OSD\\_Docs/C/CLARION.html](https://soilseries.sc.egov.usda.gov/OSD_Docs/C/CLARION.html) (accessed 8 Mar. 2017).
- Natural Resources Conservation Service (NRCS). 2014. NRCS-MLRA 103 Soil survey project. USDA. <https://www.nrcs.usda.gov/wps/portal/nrcs/detail/mn/newsroom/?cid=stelprdb1242954> (accessed 8 Mar. 2017).
- Rao, S.C., and B.K. Northup. 2009. Capabilities of four novel warm-season legumes in the southern great plains: Biomass and forage quality. *Crop Sci.* 49:1096–1102. doi:10.2135/cropsci2008.08.0499
- Rennie, R.J., and S. Dubetz. 1984. Effect of fungicides and herbicides on nodulation and N<sub>2</sub> fixation in soybean fields lacking indigenous *Rhizobium japonicum*. *Agron. J.* 76:451–454. doi:10.2134/agronj1984.00021962007600030019x
- Schulz, T.J., and K.D. Thelen. 2008. Soybean seed inoculant and fungicidal seed treatment effects on soybean. *Crop Sci.* 48:1975–1983. doi:10.2135/cropsci2008.02.0108
- Schulz, T.J., K.D. Thelen, and D. Wang. 2005. The effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. ResearchGate. [https://www.researchgate.net/publication/237651647\\_The\\_Effect\\_of\\_Bradyrhizobium\\_japonicum\\_inoculant\\_on\\_soybean\\_growth\\_and\\_yield](https://www.researchgate.net/publication/237651647_The_Effect_of_Bradyrhizobium_japonicum_inoculant_on_soybean_growth_and_yield) (accessed 2 Jan. 2017).
- Shivashankar, G., and R.S. Kulkarni. 1989. *Lablab purpureus* (L.) Sweet. In: L.J.G. van der Maesen and S. Somaatmadja, editors, Plant resources of South-East Asia. No 1: Pulses. Pudoc/Prosea, Wageningen, Netherlands. p. 48–50.
- Somasegaran, P., and H.J. Hoben. 1985. Methods in legume-*Rhizobium* technology. Univ. of Hawaii, Nitrogen fixation in Trop. Agric. Legumes (NifTAL). Hawaii Inst. of Trop. Agric. and Human Resour., Honolulu.
- Thombre, P.A., B.P. Kurundkar, and B.R. Kawla. 1989. Effect of fungicidal seed treatments on nodulation and yield of soybean. *J. Oilseeds Res.* 6:353–356.
- van Kessel, C., and C. Hartley. 2000. Agricultural management of grain legumes: Has it led to an increase in nitrogen fixation? *Field Crops Res.* 65:165–181. doi:10.1016/S0378-4290(99)00085-4
- Wright, D., and A. Lenssen. 2013. Staging soybean development. Iowa State Univ. [http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1189&context=extension\\_ag\\_pubs](http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1189&context=extension_ag_pubs) (accessed 24 Jan. 2017).