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Evaluation of Bradyrhizobium formulations on performance of soybean grown on soil without a long-term history of the crop

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JA conceived project, supervised work, wrote manuscript. KM carried out experiment, collected data, wrote dissertation.

ABSTRACT

Farmers have raised concerns about the inability of some commercial soybean inoculants to elicit effective nodulation. Nodulation failure has been attributed to, among others, high temperatures, soil acidity and type of inoculant used. This work examined the influence of two *Bradyrhizobium japonicum* inoculant formulations on soybean grown on soil without a soybean cropping history for about eight years. The experiment was conducted in a controlled environment facility at the University of KwaZulu-Natal. The treatments were two levels of a liquid formulation of the *Bradyrhizobium* WB 74 initially consisting of 2.6×10^9 colony forming units ml-1 and one level of a powder formulation containing a minimum of 6.5×10^8 live cells g^{-1}. The results showed that applying the two formulations of *B. japonicum* to seed, enhanced soybean nodule number, leaf number, nutrient concentration and uptake compared to the control treatment, and both powder and liquid formulations enhanced yield components to the same extent. Although the low concentration of the liquid formulation was less effective in increasing nodule number, other responses it elicited were comparable to the high concentration of the liquid and powder formulations. We conclude that small differences may exist between commonly available commercial inoculants of Bradyrhizobium WB 74 but they are effective in promoting nodulation and growth of soybean, and that reported major nodulation failures may be attributed to management factors other than inoculant formulation *per se*.

Key words: *Bradyrhizobium japonicum, Glycine max*, inoculation, N_2 fixation, nodulation

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INTRODUCTION

Soybean (*Glycine max*. (L.) Merr.) is an important source of oil and protein and is also beneficial for inclusion in cropping systems because of its nitrogen fixing ability, resulting in low N fertilizer requirements. One way to improve the crop's ability to fix nitrogen is via improved nodulation by inoculation using appropriate rhizobia (Hassen et al., 2014). Seeds are inoculated because rhizobia that infect soybean may not occur naturally in areas not previously cropped with soybean and the relevant rhizobia have not coevolved with the crop.

Soybean is not indigenous to South Africa and since commercial production started in the 1960s (Strijdom, 1998), several rhizobia strains have been introduced with varying degrees of success at improving nodulation (Jansen van Rensberg et al., 1976; Jansen van Rensberg and Strijdom, 1985). Currently, the strain W74 developed from strain CB 1809 is the sole recommended rhizobium for South Africa (Botha et al., 2004; Sivparsad et al., 2016). There are different inoculant formulations of this strain available on the market usually as powder, liquid or granular forms (Stephens and Rask, 2000; Sivparsad et al., 2016). Inoculant formulations have carriers by which bacteria are made available to crops and the use of different carriers have resulted in different shelf-life and hence efficacy of the inoculant formulation (Balume et al., 2015).

Although the *Bradyrhizobium japonicum* strain WB 74 is the one solely recommended for soybean in South Africa, farmers choose different formulations according to inoculant availability and farmers' inoculation and farm management practices. The inoculants are applied directly to the seed or the soil prior to planting, with variable results (Thelen and Schulz, 2009). The efficacy of these formulations is reportedly dependent on the concentration of the WB 74 strain and adjuvants they contain (Sivparsad et al., 2016). Some concerns have been raised by farmers about nodulation failures in soybean which have been attributed to, among others, high temperatures, soil acidity and inoculant formulation (Hassen et al., 2014).

This study was carried out to examine the effect of *B. japonicum* WB 74 formulations applied directly to the seed, on soybean nodulation, shoot nutrient content and pod production in view of differences in carriers, adjuvants or concentration of bacteria in the formulations. The study also quantified differences in commercially available formulations based on manufacturers' recommendations with the view to examining farmers' concerns that nodulation failures may be attributed to inoculant formulation.

MATERIALS AND METHODS

Experimental and soil conditions

The study was conducted in a growth room at the controlled environment facility of the University of KwaZulu-Natal. Soybean was grown in soil without a soybean cropping history for eight years, collected from the Ukulinga Farm (29º40'S, 30º24'E, 806 m elevation) of the University. The growth room conditions were set at day/night temperatures of 28/20ºC, relative humidity of 60%, and photoperiod of 14 hours; light sources were fluorescent (731.3 Wm^{-2}) and incandescent (113.4 Wm^{-2}) lamps which gave a light intensity of 285.15$\mu molm^{-2}s^{-1}$.

Liquid (L) and powder formulations (S) of *B. japonicum* strain WB 74 were applied to seeds of two short season cultivars 6050R and 6162R obtained from Linkseed, Greytown, South Africa, and planted. The powder formulation contained a minimum of 6.5×10^8 live cells/g, while the liquid formulation contained 2.6×10^9 colony forming units ml^{-1}. The liquid formulation was applied at a low dosage (LL) of 48 µl plus 72 µl of water per 12 g of seeds and a high dosage (LH) of 48 µl per 12 g of seeds plus 48 µl of water, as suggested by the manufacturer. The powder formulation was applied at 48 mg plus 48 µl of water per 12 g of seeds after shaking to mix in a beaker. An un-inoculated control (N) was included. The experiment was therefore a factorial combination of two cultivars and four levels of inoculant formulation in a complete randomised block design with three replicates. Four seeds were planted in 3 L and 20 cm diameter pots with 3.67 kg soil at a depth of 5 cm and thinned to two seedlings after emergence. No nutrients were applied as a result of the soil analysis recommendations.

Sampling

The first shoot harvest was done at eight weeks after planting, i.e. at the R1 (first flower) stage. Leaves were cut and counted. The entire shoot was then dried in an oven at 70ºC for 48 h for dry weight measurements. The soil was washed from the roots avoiding damage or loss of nodules from the roots. Nodule number and shoot dry weight were taken.

Plant analysis

The plant shoot was then analysed for the nutrients N, P and K concentrations using standard procedures (Manson and Roberts, 2000). The samples were milled to pass through a 0.84 mm sieve and subsamples were dry-ashed overnight and taken up in 1 M HCl. Thereafter, P and K were determined using ICP spectrometry. Nitrogen was determined separately using the Automated Dumas dry combustion method (Matejovic, 1996) with a LECO CNS 2000 (Leco Corporation, Michigan, USA). Nutrient uptake was determined as a product of the nutrient concentration and shoot dry mass. At maturity, plants in the remaining pots were harvested and the number of pods per plant, pod length and mean seed mass were recorded.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using GenStat 14th edition (VSN international 2011) and treatment means were compared using the LSD at $p<0.05$ significance level.

RESULTS

Nodule and leaf numbers

The high concentration of the liquid formulation (LH) produced higher nodule numbers ($p<0.05$) compared to the powder formulation (S) while the un-inoculated treatments (N) produced the lowest numbers (Figure 1). Cultivar 6162 R had a higher ($p<0.05$) nodule number compared to 6050 R. The use of inoculants significantly enhanced leaf number ($p<0.001$) compared to the un-inoculated treatment. There were no significant differences attributable to formulation. No significant interactions were observed.

Shoot nutrient profile

Inoculated plants had significantly higher ($p < 0.05$) shoot N concentration and uptake than the controls (Table1). No significant differences were obtained between the formulations. Furthermore, there were no interactions between the formulations and cultivars.

Both powder and liquid formulations treatments gave higher K concentration values than un-inoculated treatments ($p<0.05$). The low concentration of the liquid formulation was only slightly lower than the normal concentrations of both formulations.

There were no significant differences between formulations in P uptake and only small differences between treatments in respect of P concentration although the two formulations had higher values than the controls. Also, there were no significant differences between cultivars nor interactions between formulations and cultivars.

Yield components

In practically all the yield components examined, the responses to the powder and liquid formulations were similar and they were superior to the un-inoculated controls (Table 2). No significant differences were observed between formulations with respect to seed mass, pod weight and pod length ($p<0.05$) (Table 2).

DISCUSSION

This study shows that seed inoculation enhances root nodulation to the benefit of soybean as yield components were increased with this practice. Similar responses have been widely reported for soybean (Asei et al., 2015; Balume et al., 2015; Blazinkov et al., 2015). In this study which focused on different formulations, however, little differences were observed between the liquid and powder formulations suggesting that the reported failure of

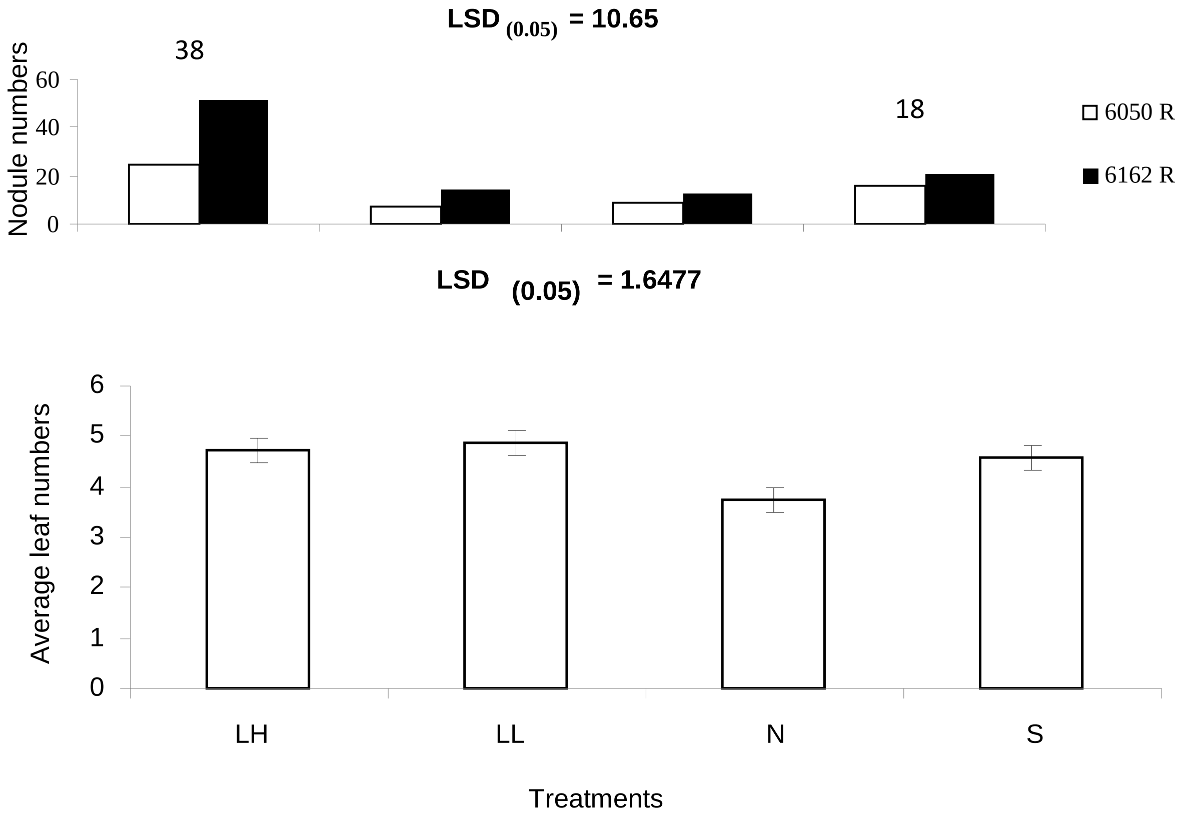

Figure 1. Responses of nodule number and leaf number at the R1 stage of soybean to inoculation with different formulations of *Bradyrhizobium* strain W 74 (LH = liquid high concentration, LL = liquid low concentration, N = un-inoculated, S = powder formulation)

Table 1. Nutrient concentration and uptake of soybean inoculated with different formulations of *Bradyrhizobium* strain W 74*.

	6050R	6162R	6050R	6162R	6050R	6152R	6050R	6162R	LSD
	LH	LH	LL	LL	N	N	S	S	
N (%)	3.75	3.80	3.73	3.26	2.93	2.78	3.58	3.83	0.507
Mean	3.77a		3.49a		2.85b		3.70a		0.718
N uptake (g plant^{-1})	0.81	0.76	0.75	0.67	0.49	0.43	0.58	0.78	0.095
Mean	0.78a		0.71a		0.46b		0.68a		0.135
K (%)	2.64	2.79	2.54	2.45	2.26	2.12	2.76	2.68	0.162
Mean	2.71a		2.49b		2.19c		2.72a		0.230
K uptake (g plant^{-1})	0.57	0.56	0.51	0.50	0.37	0.33	0.44	0.55	0.020
Mean	0.57a		0.51a		0.35b		0.50a		0.030
P (%)	0.24	0.33	0.26	0.26	0.22	0.21	0.24	0.27	0.030
Mean	0.29a		0.26a		0.22b		0.26a		0.030
P uptake (g plant^{-1})	0.05	0.07	0.05	0.05	0.04	0.03	0.04	0.06	0.020
Mean	0.06		0.05		0.04		0.05		NS

* Means followed by the same letter(s) are not significantly different at $p \leq 5\%$ level.
LH = liquid high concentration, LL = liquid low concentration, N = un-inoculated, S = powder formulation.

effective nodulation with inoculation by some farmers may be attributed to factors other than the inoculant formulation *per se.* Among these factors are inoculant handling and management (Larson, 2013; Balume et al., 2015). In a recent study that has reportedly shown differences between formulations (Sivparsad et al., 2016) the differences were attributed to variations in micro-organism content of the inoculant used rather than the formulation.

Table 2. Yield components of soybean inoculated with different formulations of *Bradyrhizobium* strain W 74.

Treatments	Pod number	Pod length (cm)	Tot Pod* weight (g)	Mass (10 seeds)$^{-1}$
6050 R N	18	3.4	11.59	2.08
6162 R N	23	2.2	10.26	2.33
Mean	21a	2.8a	10.92a	2.21b
6050 R S	34	3.93	25.08	3.37
6162 R S	32	4	16.77	2.24
Mean	33b	3.97b	20.92c	2.80a
6050 R LL	22	4.03	14.46	2.56
6162 R LL	32	3.83	18.43	2.52
Mean	27ab	3.93b	16.44b	2.54a
6050 R LH	35	4.3	22.27	2.66
6162 R LH	31	3.83	16.44	2.36
Mean	33b	4.08b	19.08c	2.472a
LSD	7.69	0.794	4.252	0.353

*Represents pod mass per plant in 20cm diameter pot. LH = liquid high concentration, LL = liquid low concentration, N = un-inoculated, S = powder formulation.
Means with the same letters in a column are not significantly different at p≤5% level.

In the current study, inoculation of seed directly with *B. japonicum* WB 74 affected yield components, through improved nodulation and nutrient uptake of soybean. Increased nutrient uptake with various inoculants have been reported although none actually focused on formulation (Tairo and Ndakidemi, 2014; Zoundji et al., 2015). The area of inoculant formulation has been sparsely studied in recent years.

Most of yield components in this study were significantly higher as a result of both powder and liquid formulation application compared to the controls. Although the powder formulation had fewer nodule numbers compared to the high concentration of the liquid formulation, our failure to separate effective and ineffective nodules for the two treatments makes it difficult to determine whether these differences were of significance in terms of the effectiveness of the nodules in contributing to nitrogen fixation. Large nodule numbers are not always equivalent to efficient nodulation (Tajima et al., 2007). Indeed, the lack of real differences in yield determinants in this study suggests that high nodule number may not necessarily translate to high yield because although the powder formulation had relatively low nodule numbers compared to the high concentration of the liquid formulation, it was as effective as the liquid formulation in promoting increases in yield components. Similarly, the lower level of the liquid formulation elicited good responses just as in the high level liquid and the powder formulations. Thus, even the lower concentration of the liquid formulation still had enough microbial levels to elicit good responses.

It has been reported that increased yield as a result of inoculant application is attributable to increases in concentration of rhizobia (Albareda et al., 2009) and differences in rhizobia strains (Zerpa et al., 2013, Ulzen et al., 2016) suggesting that inoculant concentration and strains in the inoculant may be more important than formulation. Also, in recent years studies on inoculants are mostly limited to single formulations but focus on supplementation of the selected formulation with fertilizer application (Abbasi et al., 2010; Muhammad, 2010; Zoundji et al., 2015) and micro-organism addition (Tran et al., 2007; Afzal et al., 2010) and these have resulted in improving effectiveness of inoculant formulations.

CONCLUSION

Inoculation of soybean with *B. japonicum* WB 74 enhanced nodulation and yield components irrespective of formulation. Thus, both liquid and powder formulations were effective and we conclude that although small differences may exist between commonly available commercial inoculants, they are effective in promoting nodulation and growth of soybean, and that reported major nodulation failures may be attributed to management factors other than inoculant formulation. Further studies need to be carried out on management of inoculants for consistent results with inoculant application by farmers.

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CONFLICT OF INTEREST

None.

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